NORTH CENTRAL COLLEGE
NAPERVILLE, ILLINOIS
Founded 1861

Biology Student Handbook

What you need to know

to succeed as a Biology major!

Revised 2015

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Acknowledgements

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Why a Biology Student Handbook?

The *Biology Student Handbook* was developed by the Biology Department as a guide for North Central College students majoring in the life sciences. Our goal is to put many of the things that all life-science majors need in one place! You will want to keep this handbook and use it throughout your four years here. It is especially useful for lab courses, and you should bring it to lab with you along with your lab manual.

The first section of the *Handbook* provides general information on being a biology or biochemistry major and using the resources that are available to you. It includes details on how to write lab reports, draw graphs and work with numbers. You are responsible for knowing this information, and you will need to refer to it frequently in nearly all of your biology courses. You should use the style and format standards outlined here for all lab reports and other written assignments unless you are specifically directed otherwise for a particular course.

The second section provides specific details on how to use laboratory equipment and carry out commonly used lab procedures. There are many techniques which you will use over and over again in many different courses, such as using a spectrophotometer, preparing a solution or running a gel, so it makes sense to have those in one place instead of repeating them in every course lab manual. (Of course each course will also use specific procedures and equipment that can't be covered here.) Many of the mistakes that students commonly make in lab can be avoided by carefully reading and applying the information in this section.

Is there something you needed to know that's not in the *Handbook*? Please let us know: this is a work in progress, and our goal is to give you the information you need. Additional resources can be found on the Biology Web page (www.noctrl.edu/biology) and the Biochemistry Web page (www.noctrl.edu/biochem).

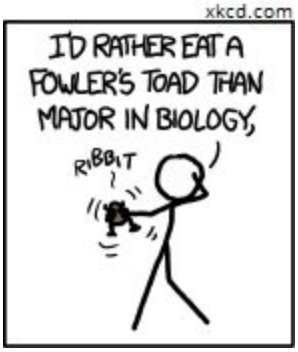
Studying Life Science @NCC

Life-science students at North Central College may be preparing for careers in research, medicine, education or industry. Studying life science usually means that you are a biology or biochemistry major. However, we also offer majors in radiation therapy or nuclear medicine technology, as well as minors in biology, neuroscience, bioinformatics and environmental studies.

Whichever path you choose, we recognize the importance of **broad experience** in all the main areas of biology, accomplished through introductory (BIO 151 and BIO 152) and "sophomore core" courses (BIO 251, 252 and 253). We also know you need to **focus in-depth** in areas that interest you most or that will best prepare you for your future: 300-level courses, usually taken by juniors and seniors, offer this specialization. Then, 400-level "capstone" courses immerse you in current research, independent projects and the **ethics and values** critical to a life-science career (e.g., ethical conduct of research, animal and human experimentation, authorship and funding). You will also be engaged in **research**: all biology and biochemistry majors learn how new knowledge is obtained by completing and presenting a research project. Almost all biology courses have labs where we emphasize the **skills** needed by a scientist, such as scientific writing, experimental design, data analysis and presentation skills. Thus, your North Central degree provides a solid foundation for a wide range of career options.

This section of the *Handbook* is intended to help you choose degree options and develop a plan for your study of the life sciences at North Central. It does not replace the College catalog, which gives official information on degree requirements, courses and policies. You should refer to your course catalog (the catalog in effect in your first year at North Central defines your graduation requirements unless you have requested a change to a later catalog) and meet regularly with your academic advisor to work out your individual program of study. The Biology (www.noctrl.edu/biology) and Biochemistry (www.noctrl.edu/biochem) Web pages also provide current information.

Degree Options



The two main degree paths are Biology and Biochemistry. A Biology degree can be tailored to a future involving any area of life science. Biochemistry, a program run jointly by the Biology and Chemistry faculty, is suitable for students with strong interest in cellular and molecular biology and biochemistry. The Radiation Therapy and Nuclear Medicine Technology programs are more specialized, focusing on meeting certification requirements for specific careers.

Students may choose to pursue either a Bachelor of Arts (BA) degree or a Bachelor of Science (BS) degree in either Biology or Biochemistry. The Biology BS degree provides the strong background in mathematics, chemistry and physics expected by graduate schools and employers as well as rigorous biology courses; this is the primary degree in Biology. The BA degree provides an alternative primarily for secondary education students and others whose academic program does not allow them to complete all of the courses for the BS. For biochemistry students, the BA degree provides appropriate preparation for graduate and professional schools; the BS allows students to gain even more depth of experience and expertise. The table below summarizes the Biology and Biochemistry degree requirements.

	Biology		**Biochemistry**	
	BA	**BS**	**BA**	**BS**
Introduction	BIO 151 & 152		BIO 151 & 152, CHM 141 & 142	
Sophomore core	BIO 251, 252 & 253		BIO 251 & 252, Organic chemistry, CHM 210	
Depth	≥ 2 300-level courses		BIO 360, BCM 365, CHM 340 or 341	
	34 total hrs in BIO	37 total hrs in BIO		3 advanced electives
Capstone	BIO 405, 416, 430, 440 or BCM 465		BCM 465	
Research	On- or off-campus research project and BIO/CHM 475			
Mathematics	MTH 141 or PSY 250	Calculus (MTH 152) Statistics (e.g., PSY 250)	Calculus (MTH 152)	Calculus (MTH 152) Statistics (e.g., PSY 250)
Support courses	CHM 141 & 142	CHM 141, 142 & 210 Organic chem, Physics	Physics	Physics

Planning Your Schedule

Sample four-year plans for life-science majors with various goals are available on the Schedule Planning page of the Biology and Biochemistry Web sites. The College catalog and the course schedule available in Merlin are key schedule-planning resources. Students are encouraged to frequently discuss career goals and how those objectives might be met with their academic advisors and with other faculty.

Preparing for a Medical or Health-Science Career

The Biology or Biochemistry major provides excellent preparation for medical school or for professional study in a variety of allied-health areas, including dentistry, veterinary medicine, physical therapy, physician assistant programs, optometry, pharmacy and occupational therapy. Generally, either a Biology or a Biochemistry major would be appropriate, and the choice of major depends on the student's interests as well as the specific coursework required for a particular professional program. North Central also offers degree programs in Radiation Therapy or Nuclear Medicine Technology (both requiring a year of courses and clinical training at Northwestern University) for students preparing for those specific careers.

Pre-health advisor Marguerite Degenhardt works with pre-health students on schools to apply to, admissions requirements, exam preparation and other aspects of preparing for professional study. Her assistance complements the role of the academic advisor in helping plan a program of study. In addition, the Pre-Health Organization is a student group whose activities provide information and assistance to pre-health students. Much more information on courses, exams, experience and other pre-health requirements can be found on the pre-health Web pages (for each subject area, click through to the "More Program Info" page). The table below very briefly summarizes key requirements for each pre-health area.

Area of Study	Required Coursework	Entrance Exam
Medicine	BIO 151 & 152; CHM 141, 142, 220, 221 & 222; BCM 365; PHY 141, 142 & 143; MTH 151 & 152 recommended: BIO 251, 252, 253, 302, 340 & 360	MCAT
Dentistry	BIO 151 & 152; CHM 141, 142, 220, 221 & 222; PHY 141, 142 & 143 some programs require: BIO 302 & 340; BCM 365; MTH 152 & 153	DAT
Nursing	BIO 147, 302, 340; CHM 141, 142, 220, 221 & 222; BCM 140; MTH 128 or 130; PSY 250	none for BS GRE for MS
Veterinary Medicine	BIO 151 & 152; CHM 141, 142, 220, 221 & 222; PHY 141, 142 & 143 may require: BIO 251, 302, 311 & 340; BCM 365, MTH 151 & 152	GRE or MCAT
Physician Assistant	BIO 151 & 152; CHM 141, 142, 220, 221 & 222 programs typically require: BIO 147, 302, & 340; BCM 365; PSY 250	GRE or MCAT
Physical Therapy	BIO 147, 151, 152 & 302; CHM 141 & 142; PHY 141, 142 & 143 may require: BIO 251, 340 or 360; PSY 250	GRE
Optometry	BIO 151 & 152; CHM 141, 142, 220, 221 & 222; PHY 141, 142 & 143, MTH 151 typically require: BIO 147, 302 & 340; MTH 152; BCM 365; PSY 250	OAT
Pharmacy	BIO 147, 151 & 152; CHM 141, 142, 205, 220, 221 & 222; PHY 141, 142 & 143; MTH 151 & 152 some programs require: BIO 302 & 340; PSY 250	PCAT
Occupational Therapy	BIO 147 & 302; PSY 250 some programs require: PHY 141, 142 & 143	GRE

Minors

Students can minor in biology by completing BIO 151, 152, 251, 252 and 253 and CHM 141. A 300-level biology course is recommended. A biology minor is a good way to prepare for a career in an interdisciplinary area involving biology. For example, a mathematics major with a biology minor would be a good combination if you want to study epidemiology or model ecological processes. An anthropology major with a biology minor would prepare you for graduate study in physical anthropology. Or a business major with a biology minor could be useful if you want to work on the administrative side of the pharmaceutical industry.

North Central also offers interdisciplinary minors related to the life sciences. The environmental studies minor allows students to examine environmental issues from multiple perspectives, including biological, political, sociological, literary and economic aspects. The bioinformatics minor links biology with computer science to learn computational techniques for dealing with genomic data and other complex biological datasets. The neuroscience minor bridges biology and psychology, dealing with the biological basis of behavior and cognition.

Minors of Potential Interest for Life-Science Students

- Bioinformatics
- Biology
- Chemistry
- Computer Science
- Environmental Studies
- Mathematics
- Neuroscience
- Physics
- Psychology

The table at right summarizes some minors of possible interest to life-science students. It is not necessary to have a minor, and having one will not necessarily make you a better candidate for a job or a graduate program. In deciding whether to add a minor, you should consider how a minor could improve your preparation for the specific career or field of study you want to pursue, and you should also think about how the additional coursework a minor requires will fit into your four-year plan. Further, there may be more than one approach to a particular goal. For example, someone interested in the biological side of psychology could be a biology major with a psychology minor, a psychology major with a biology minor or either a biology or psychology major with a neuroscience minor. Someone interested in computational biology could be a computer science major with a biology minor, a biology major with a computer science minor or either major with a bioinformatics minor.

Transfer Students

Transfer students complete the same requirements as any life-science major. The Registrar's office will evaluate the courses you took elsewhere and assign each course a North Central College equivalent. Some courses may not match up exactly with North Central courses, so they might be designated (for example) biology electives: these would count toward your total hours in biology but would not fulfill a specific course requirement. Transfer students seeking a Biology degree must complete 11 credit hours in biology here at North Central, and at least 7.5 of those hours must be at the 200 level or above. The Registrar's web page includes transfer guides for local community colleges and junior colleges that can help you see how your courses will transfer.

It is generally not true that a transfer student can "get all of the general education courses out of the way" at another school and then complete a science major at North Central in two years. In order to transfer as a junior and finish in two years, a transfer student would usually have to have taken 100- and 200-level biology and chemistry courses comparable to what is recommended below for North Central freshmen and sophomores. Additionally, it has generally been our experience that junior-college and community-college courses are not truly equivalent to North Central biology and chemistry courses. Those courses may cover a similar set of concepts but are unlikely to build the same skills in writing, experimental design, laboratory work or analysis. We advise incoming transfer students to work closely and proactively with biology faculty and their academic advisors to evaluate their readiness for courses and to develop an appropriately paced academic plan.

Research Experience

Research is how science is done: it's how the facts got in the textbook! Undergraduate research is nearly an absolute requirement for students who want to go on to graduate school or work as research technicians, and it is also very valuable for students whose careers will involve applying the research of others: medicine, the pharmaceutical industry, forensics and many other applied science areas. So, we believe that research experience is a crucial part of every Biology and Biochemistry student's education.

There are two components to the research experience required for your degree: doing the actual research, and presenting it to science faculty and students. There are several ways you can fulfill the research requirement; see the section "Doing Research" in this *Handbook* for more information. Biology and Biochemistry majors take BIO/CHM 475, our seminar course, three times. The first two times, the course is taken for zero credit, and students participate as audience members, observing seminars and asking questions. This will give you a good picture of what the possibilities and standards are for your own research project. The third time you take the course, you present the results of the research you have done and receive one hour of credit.

What could I do with a Biology or Biochemistry degree?

- Graduate study in preparation to direct research in an academic or industry lab and/or in the field. Possible areas of specialization include: biochemistry, botany, ecology, entomology, genetics, herpetology, marine biology, microbiology, molecular biology, physiology, virology, zoology, and many more. (Did you know that life-science graduate students pay no tuition and are paid a stipend usually in the area of $24,000/year?)
- Professional school programs to prepare for careers in medicine, dentistry, physical therapy, veterinary medicine, nursing, pharmacy, medical technology, physician assistant, occupational therapy, optometry or related fields
- Work as a technician in a research or biotech lab or in field research
- Public health, epidemiology or global health
- Education: teach junior-high or high-school science (with additional education coursework) or work in educational programs at zoos, parks, museums, etc.
- Genetic counseling
- Scientific writing or illustration
- Environmental monitoring, advocacy, policy, etc.
- Bioinformatics: application of computer technology to study of genomes, etc.
- Pharmaceutical research and development
- Forestry, fisheries, conservation and related areas
- Biomedical engineering or bioengineering
- Forensic medicine or other forensic careers
- Law firms specializing in biotechnology patent law or legal aspects of the medical fields
- Health care administration
- Government agencies such as the EPA, FDA, USDA or the Forest or Park Service
- Horticulture and landscaping
- Sales of pharmaceuticals or other biology-related products
- Many other possibilities…

Doing Biology

Science majors quickly discover that success requires active participation in their own education! The following essay was written by a senior biology major serving as a preceptor for a freshman-level course. It has many valuable suggestions about how to break out of the "Listen- to-Lecture, Cram-for-Exam" trap and start doing biology.

Yes, you CAN get good grades as a science major!

The first thing to know about science classes is that **you really do have to know all the material covered in lecture backward and forward, inside and out**. This might not have been necessary in high school, and perhaps it isn't even essential in some other college courses—but in science, it is! Your test scores can shock you if you're not prepared for this. But don't worry! Just prepare for it in advance.

The second thing to know is that if the information is not settled into your long-term memory, you will not remember things well enough to apply the info to new situations—and that's exactly what your science professors will ask you to do.

Two of the best things you can do for yourself are: (1) *want* to learn the material—find what's interesting to you about it even if it's not your favorite subject—and (2) start thinking of teachers as assistants who are helping you teach *yourself*. College requires autonomy. If you are a science major and your science classes seem too hard, consider whether you really, honestly want to study science. It gets harder from here—but more rewarding, too!

In class

Take complete notes. If it's a choice between getting everything down and understanding it the first time you hear it, just get everything down. It'll help you understand it more fully when you study it later on. Use abbreviations (b/c = because, w/ = with, fxn = function...). Include pictures in your notes. Put a question mark in the margin when you don't understand something—either something you want to ask about in class or something you need to put extra study time into later.

Don't just write what the professor writes on the board! What s/he *says* may be crucial to understanding. Don't rely on your memory. Even writing down a joke or a silly analogy might help you remember a key point.

Prepare in advance. Studies show you learn more when you have some idea of the subject matter *before* trying to learn it. Most professors give reading assignments in advance. Some put their lectures or outlines online or hand them out before class. Use these to help you get ready for the lecture, and you'll take better notes and remember the material better later.

Ask questions. If you have a question, other people probably do, too. They're more likely to be glad that someone asked than annoyed at the interruption. Remember the professor's goal is to help you understand...s/he wants you to ask questions!

Make connections. Relate lecture subjects to one another and to previous lectures. Take out your notes; have them handy in case you want to look something up.

Don't skip class. It's really not worth it! But if you're very sick and have to stay home, get the notes from someone else ASAP and talk to the professor about anything that's not clear.

After class

Keep up with the material. There is simply TOO much information to try to learn it all at the last minute. Most of us have tried it at some point—trust us on this one! We have to make an extra effort because we learn a semester's amount of material in 10 weeks at NCC.

Schedule your assignments and projects at the start of the term. Put everything down on a calendar (physical or electronic) so you always know when everything is due. Try a big one so you can see the whole term at a time. When lots of due dates pile up, that's a sign that you need to work ahead on some of them.

Get help. You want to be challenged, but you don't want to struggle needlessly! Get help before it gets to that point. One of the greatest things about studying science at NCC is that the professors are available to help you personally, and they want you to succeed just as much as you do!

Organizing your time

> There are 168 hours in a week. Of these 168 hours, you will be asleep for about 60, dressing and eating for about 20. If you take Saturday afternoon off for a hike, consider Sunday morning and afternoon as time off from studying, and have two four-hour dates a week, you have about 68 hours a week for schoolwork. If you are in class and laboratory for 20 hours, you still have 48 hours for study! It seems like a tremendous amount of time, doesn't it?–especially considering that you've taken off half of Saturday and most of Sunday. Just where does all the time go? A great deal of it is lost in ten-and twenty-minute idle discussions, time wasted during the twenty minutes while you wait before a class after you've needlessly spent another twenty minutes walking to the post office and back for a stamp you could have picked up just as easily on your way back from lunch, and so on. It is up to you whether you want to make good use of these numerous ten-, twenty-, or thirty-minute intervals. I'm not urging that you never take a minute off to enjoy life, but there is certainly little danger that you will use your time too efficiently. –– *S. Chapman*

School IS a full-time job! Studies show students who work may not do as well as students who don't. If you have a job, try to work as few hours as possible. Science classes genuinely require 15-20 hours a week of studying, and maybe more for researching and writing lab reports. Decide whether you really need to work to pay for school, or if you're just working for luxuries or to reduce your loans by a few hundred dollars. If you must work, consider an on-campus job; they're very flexible with our schedules. Scholarships and grants are available, too.

Know yourself. If you know you tend to get distracted in the evening, or sleepy after lunch, or disorganized after a while, take those things into account when setting up your study schedule. If you know you tend to read slowly, try getting up earlier in the morning, before the day gets too busy.

Beware of the phone. We all love our phones, but sometimes we let them take over our lives. If your phone is sitting next to you while you're "studying" and you keep texting your friend to see how her studying is going, checking to see if you got an e-mail, etc., then you're not studying.

Study tips

Study when you're rested and alert. For most, that's morning or late afternoon/early evening.

Take short breaks. Studies show you learn more when you take short breaks after 20-30 min. Also, study a lecture for 3-4 consecutive days, then take a break and come back to it. This helps move the info from short to long term memory.

Study actively. Remember you're not just studying in order to fulfill an assignment, you're studying to understand the material. Don't read or recopy your notes passively. The key is to hold yourself accountable for what you're studying—if you don't, you have no incentive to remember anything! After a study session, quiz yourself in as many ways as you can.

The textbook is a valuable friend! Read the text slowly enough that you really understand what you're reading. If you're not "getting" any of it, it's a waste of time. Go back and read something again if you need to. Don't be afraid of reading or learning more than you need to. When you "overlearn," you find that you understand the basic concepts—which are probably what you're supposed to learn—much better, because now you have a sense of them in a greater context. So skip ahead in the chapter, or continue reading into the next section if you have time.

Look at the chapter outline. Take note of the section titles. These are main points. Science texts aren't meant to be read the same way as novels. Be sure to look closely at the figures and read

their captions. Draw out illustrations and processes that are in your notes or in the text. Draw them out from end to beginning, or backward as well as forward, to make sure you understand them.

Get focused. Don't sabotage yourself with a distracting environment. Moving into a quiet room to study makes all the difference in being able to focus. Turn off your phone and the TV.

Take care of yourself. Think of sleep as a necessity, not a luxury. Even a little bit of sleep deprivation hurts your brain, because your whole body has to move into "survival mode," and it's harder to concentrate and remember things. Studies show that sleep is important for moving info from short- to long-term memory. Stay hydrated. We know now that even a 1-2% drop in body water affects your learning ability. Eat something before coming to class—it gives your brain the energy that it needs. BUT, don't have a lot of sugar: you need constant energy, not a sugar high followed by a coma.

Get exercise. Ever notice that cross-country runners tend to do well in their courses? They've disciplined themselves to get up early in the morning and run, and then they know they have to get their work done efficiently and get to bed at a decent hour. Exercise makes us both physically and mentally sharper and can do a lot to reduce stress.

Get help. If there's a problem in your personal life that's affecting your ability to focus and study, talk to someone: family, friends, RA, Hall Director, academic advisor, professor, doctor, psychologist—no one's saying college is a piece of cake, and they all want to help if they can.

Be disciplined. Don't equate working hard with being hard on yourself. Most worthwhile goals take real work. If you're a procrastinator, this is the time to start helping yourself be more effective at self-management. Discipline is the opposite of procrastination. When we're young, discipline is thought of as the same thing as punishment, but for an adult, discipline is a gift you give yourself, a way of showing respect for yourself, to actually do what you intend to do. You ARE capable of learning what you intend to learn, and getting those As—be sure you're not psychologically blocking yourself from it.

Doing Research

Science is not just facts in a textbook: more than 300,000 research papers are published annually in the United States alone, each of which contributes something new to our understanding! You can learn what we know *now* from textbooks, but that's not enough: a biologist needs to know *how* to learn more. That's why we teach you to learn from current scientific literature, and especially why we emphasize designing experiments, analyzing data and interpreting results: skills needed to generate *new* knowledge in the world of research. A highlight of your North Central education will be applying your knowledge and skills to your own independent research, seeking new knowledge.

As detailed in the section "Studying Life Science @NCC," an independent research project is a graduation requirement for both Biology and Biochemistry majors (whether B.S. or B.A.) and consists of two parts: (1) doing the actual research, and (2) presenting it in our interdepartmental seminar (BIO/CHM 475). This is a big part of your major, so begin planning early! We suggest participating in BIO/CHM 475 as an audience member in your first or second year so that you can see what other students are doing. There are several different ways that you might fulfill the research requirement:

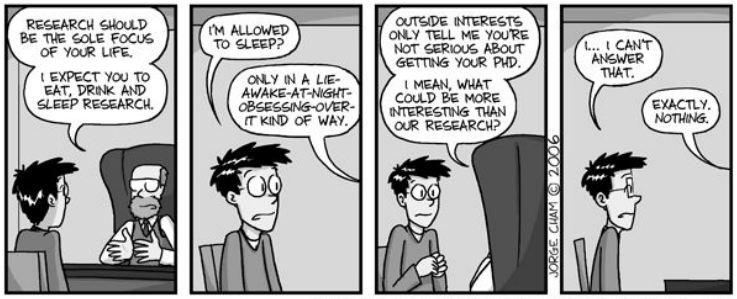
Collaborative research with a faculty mentor

One of the best ways to meet your research requirement is to get involved in the ongoing "real-world" research programs of the biology and chemistry faculty members. You'll contribute to actual, cutting-edge research, and you may be able to publish your results in a scientific journal or present them at a scientific meeting. You will also develop a relationship with a faculty research mentor who can help you understand how to design, carry out and interpret experiments and will encourage you to become more and more independent in doing so. When it comes time to move on to graduate school or a research job, a letter from a faculty member with whom you have worked closely on a collaborative research project carries a lot of weight.

- *Summer research.* Most students who want to do collaborative research with a faculty member participate in our summer research program. Summer research lasts eight weeks, usually beginning the Monday after commencement (the same timing as Summer term). Generally, students will have their own independent projects that fall within the "umbrella" of the faculty member's overall research goals and will gain increasing responsibility for directing their own projects as they gain experience and background knowledge.

  Summer research students (usually two per faculty member) are expected to work approximately 30 hours per week and are paid a stipend, currently $2500; volunteers may also be accepted when interest exceeds the number of available stipends. Grant-funded faculty may be able to accept more students or pay a larger stipend. Applications are accepted beginning early in Winter term, and successful applicants are usually notified at the beginning of Spring term. Students interested in summer research are encouraged to begin discussing projects and opportunities with prospective mentors prior to the application process. See the Research page on the Biology or Biochemistry Web site for an application.

- *Research during the academic year.* Often, students working during the academic year are summer researchers who are continuing their projects. However, it is also possible to start a project during the academic year. D-term can also be an opportunity to get started in a lab with a small project that might be expanded into subsequent Winter or Spring terms or into the summer. Students engaged in academic-year research should register for BIO 395, which can be taken for credit or for zero credit. Students interested in academic-year research should discuss availability and options with the faculty mentors they are interested in.



Most faculty members are happy to work with first-year or sophomore students, so don't feel that you need extensive experience in order to apply—the point is to *gain* experience! The most important factors are your interest in the project, self-motivation and perseverance.

Off-campus summer research programs

Summer programs are available at schools throughout the country and often pay travel and/or living expenses as well as stipends. Application deadlines are usually in January or February. Students should check with their academic advisors to ensure that the programs they are considering will meet the research requirement. See the Research page on the Biology or Biochemistry Web site or the college's Undergraduate Research page to find links to opportunities and search engines.

"Capstone" research courses

In these courses, the laboratory portion of the course is a short-term, independent research project. The results of this project can be presented in seminar to count for your research requirement; however, students going to graduate school or to a professional school where research experience is highly valued should elect to do additional research as discussed above. Current research courses are: Animal Behavior (BIO 405), Environmental Biology (BIO 416), Developmental Genetics (BIO 430), Infectious Disease (BIO 440) and Advanced Biochemistry (BCM 465). Please note that although other courses may have small independent projects, they do not count as research courses for the purposes of fulfilling your research requirement.

Additional opportunities

If you are doing an internship with a company, working or volunteering in a clinical setting or have a job that takes you into an environment with research opportunities, you might be able to work out something with your employer to allow you to do research as part of that experience. You should discuss this with your academic advisor and/or the department chair to ensure that what you plan to do will meet the research requirement. Projects which do not consist of genuine research which you are personally carrying out will not meet the requirement.

The College's Richter Grant program can provide funding for student-directed research projects which require travel, especially international travel. Additionally, some faculty are open to the idea of letting you conduct your own research project (that is, one which is not related to that faculty member's overall research goals) in their laboratories. Student-directed projects of this kind are not as easy as they sound, because without the help of a faculty member who is an expert in the area you are interested in, *you* have to become the expert and determine what has been done and what new work would be meaningful in that context. (In the real world of science, most new projects begin as offshoots of collaborative work you are already doing; it is rare to just think up a totally new research project and succeed in getting it funded and moving forward!) If you are interested in a project of this nature, be sure to consult with the faculty member you'd be working with and with your academic advisor well in advance to ensure that your proposed project will be acceptable.

Writing Lab Reports

Research results are useless if they're not disseminated to others, which is usually done by publishing the results in a scientific journal. Not only is this how you find out what others have learned, but it also gives other scientists the opportunity to evaluate, critique and validate your work. If you choose a career in research (in the lab, field, clinic or some other setting) you will be writing manuscripts for publication. (If you're lucky, the research you do as an undergraduate could get published, as well!) And even if you're not personally doing research, *any* career in science will require you to read and understand the scientific literature. So, in course labs, we ask you to develop your scientific writing skills by reporting your results in the style of a scientific manuscript for publication.

After preparing a manuscript, the scientist submits it to a journal editor, who then selects two or three other scientists who are experts in the same field to carefully review the paper. The reviewers examine each result and try to decide whether the experiment was conducted properly and reported clearly and whether the conclusions are justified by the results. They also evaluate the clarity and quality of the writing, whether related work done by others has been correctly cited, whether figures and tables are well-constructed, and so on. Based on this "peer review," the editor then decides whether to accept the paper, require revisions (the most common result) or reject the paper.

A formal lab report is written just as if it were an actual scientific paper reporting research results. (Your instructor will act as the reviewer in this case.) The remainder of this section of the *Handbook* will explain in detail the style that is to be used and provide examples. You should follow these guidelines precisely, just as you would the instructions given by a journal to which you were submitting an actual manuscript. In addition, it will be highly beneficial for you to look at actual journal articles from good journals like *Cell*, *Science*, *Nature* and others to see how real scientists write and try to emulate their style. These guidelines are to be strictly followed for all lab reports in Biology courses unless your instructor gives you specific directions for a specific assignment or course.

Overall Style and Format

Write in a formal, scientific style, but do not make your writing complex or dry. Good scientific writing is **clear**, **direct**, **concise** and **precise**. You should use appropriate scientific vocabulary, but not a lot of big words you don't really understand. It is not necessary to always use third person, and you shouldn't overuse passive voice. For example, "The concentration was measured by means of the spectrophotometer with which the researcher was provided and was determined to be 146 mg/ml" is wordy and awkward. Instead: "Using a spectrophotometer, we measured a concentration of 146 mg/ml." Assume that your reader is a competent scientist who is not intimately familiar with your research or methods.

Your lab report should be divided into six sections (in this order): Abstract, Introduction, Materials and Methods, Results, Discussion and References. Some journals change the names or order of the sections, but you will see sections very much like these in almost any journal article. Each section is discussed in detail below.

Title and Authors

Every paper needs a title, which should briefly give the subject of the paper. You can use an *informative* title, which gives the main conclusion ("Prolonged Water Deprivation Leads to Irreversible Dehydration in Plants") or an *indicative* one which just identifies the topic ("Effects of Water

Deprivation on House Plants"). Either way, it should be **specific**. "Water" or "Experiment 6" or even "Enzyme Activity" is too general.


Lipid Type Affects the Quality of Chocolate-Chip Cookies
Ernie Muppet and Cookie Monster*

The effect on chocolate-chip cookie quality of two different lipids was investigated, and butter was shown to result in significantly better cookie quality...

Below the title, list the authors. In a real paper, the first author is usually the one who did most of the work and the writing, middle authors contributed significantly to the work and the last author is the principal investigator in whose laboratory the work was done. The author who will be corresponding with the editor (and who is principally responsible for the content of the paper) is commonly indicated with an asterisk. You don't have to be this specific, and you don't need to include your instructor as an author, but you should include your name and the names of everyone in your lab group (get their names right: it's kind of rude to misspell the name of a colleague!). Place an asterisk after your name to indicate that you are the main author. Do not include an MLA-style name/date block.

Abstract

The abstract is a very short statement intended to: **summarize** your research, **situate** it in its context, and **signal** its importance (thanks to Martha Bohrer, English Dept., for that succinct description). The abstract is *not* an introduction; in fact, you should write the abstract *after* the rest of the paper, so that you know what the paper says about the results and conclusions and can summarize those sections. The abstract should be thorough enough that a reader who read only the abstract would still get the main idea of your paper, yet very concise: keep the abstract for a lab report under 100 words. Place the abstract after the title (it does not get a heading) and single-space it.

Introduction

The Introduction should catch your reader's interest (without being "cute"), explain why you did the experiments, and provide the background for understanding the rest of the paper. It should be understandable to any biology student, whether or not s/he has had the specific course you are taking. A "funnel" style is helpful in the Introduction: First, give a few lines of very **general background** in the area of your experiment. Then, spend a little longer talking about the **specific context** of your research: what is already known? Next, introduce the **rationale** for the experiments you did, including any major **hypotheses** that you started with, based on the context you gave. Finally, briefly outline what your experiments were. Don't include results in the Introduction, but be sure someone who read *only* the introduction would clearly understand what you were doing, as in this brief example:

> Smith and Jones (1923) previously showed that chemical reactions happen faster at higher temperatures. However, it is also clear that enzymes can be denatured when the temperature is high enough to break non-covalent bonds (Smythe et al., 1947). We investigated the effect of heat on the activity of chocolatase; we hypothesize that its activity will increase as temperature increases up to some optimum point and then decrease as the three-dimensional structure of the protein is affected. We expect this optimum temperature to be around 30 °C, the normal body temperature of the chocolate-munching skink (*Skinkus chocomunchii*) from which the enzyme was extracted.

Most of the introduction should be written in present tense. It's a little hard to get used to how verb tenses are used in scientific papers, but there is a definite style which is considered appropriate. As a rule of thumb, if you're writing about well-known facts and general information, use the present tense ("The sun *is* a big ball of gas."). If you're describing a particular result from someone else's experiments, use past tense ("Johnson and Smith *found* that the sun contains hydrogen.") When you're writing about your actual experiments and results, use the past tense ("These experiments *were designed* to identify gasses in the sun; we *found* that hydrogen is a major component.").

In the introduction (and throughout the paper), ***any* and *all* information which does not come *directly* from your own experiments needs to have an appropriate citation to identify its source** (the format is detailed in the "Finding and Citing Sources" section of this *Handbook*). This is how you give credit to those individuals whose work established the context for your own; not giving credit is plagiarism (see the "Scientific Integrity" section), a major form of scientific misconduct and subject to penalty as described in your course syllabus. All source material should be *paraphrased* in your own words: direct quotations (with or without quotation marks) are *not* appropriate in scientific writing. Even though the material is paraphrased rather than quoted, its source must always be identified by a

citation. Unless instructed otherwise, do not use your lab manual as a background source except for information that is specific to your lab and would not be published elsewhere.

After reading your introduction, the reader should have a clear understanding of (1) the overall question your experiments are intended to address, (2) why this question is important and interesting, and (3) specific hypotheses or questions (not all research is based on a hypothesis) you're investigating.

Materials and Methods

Materials and Methods is a *very brief* summary, written in past tense, of how the experiments were done, enough that a competent investigator should be able to repeat your work. However, Materials and Methods is *not* a step-by-step protocol! Usually, there is a subheading for each major technique used, and that technique is then described in one very short, terse, tight paragraph. Consider how to accomplish this in the briefest way possible. Do *not* give a list of materials or equipment, and do *not* write as if you were giving instructions. For example:

a typical first try at Materials and Methods:

We received seven test tubes, and we diluted our riboflavin solution by first pipetting 1 ml into a tube using a micropipettor and then using a 10-ml pipette to add 9 ml of water. Mix well. Then we pipetted 1 ml of this into the next tube and again added 9 ml of water and mixed. We repeated this process five more times for a total of seven dilutions: 1:10, 1:100, 1:1000, 1:10,000, 1:100,000, 1:1,000,000 and 1:1E07.

a big improvement:

Riboflavin was diluted to final concentrations of 500, 50, 5, 0.5, 0.05, 5×10^{-3} and 5×10^{-4} mg/ml.

When your procedure follows a previously published method (or the lab manual) closely, you can outline the procedure even more briefly and give a citation. For example, We measured protein concentration using the Lowry assay (Lowry, *et al*., 1951). If your method varies a little from what has been published, specify the differences: We measured protein concentration as described previously (Lowry *et al*., 1951), except that proteins were first precipitated with 10% trichloroacetic acid. **As with all other non-original material in the report, if you did not personally develop the method, it needs some kind of citation to identify its source**. Citing the lab manual as the source for a procedure is appropriate in this section if you don't know its original source.

Materials and Methods is also a good place to mention how you determined a rate (such as using a linear regression to generate a best-fit line) or show the equation you used to calculate a result. In a real scientific paper, you would not include "sample calculations," but in a student lab report it is sometimes useful to do so, to help your instructor decide if you interpreted the data correctly. Materials and Methods usually does not include figures but might include a table if you need to show a list of bacterial strains, PCR primers, reaction conditions, etc.

Results

The results section is the heart of your paper! This is where you should spend the most time and effort—in fact, your lab report will likely be much stronger if you take one simple step: **write Results *first***, then write the rest of the paper around it.

Results it is the most important section, because this is where you explain your actual findings. It is also where you tell the reader the "story" of your experiment: (1) what you did (not the method, but the design of the experiment), (2) *why* you did it, and (3) what happened. Results describes the experiments and their results *in words*, and each major point is also illustrated with a table, graph, photograph or other means of presenting data. You are walking the reader down a path so that he or she can see your data, understand the results and (hopefully) agree with what you think the data mean.

The Results section should be able to stand alone, so start it with a one- or two-sentence mini-introduction to recap your objective. Then, move on to telling the story of your first experiment. Do not start your Results section with a figure or table! Write about your experiment in paragraph form, directing your reader's attention to figures or tables where appropriate and describing what is in the figure or table. For example, We digested the plasmid with restriction enzymes (Figure 1) is not nearly as informative as We digested the plasmid with restriction enzymes (Figure 1) and found that it had only a single *Eco*RI site (lane 1 in Figure 1). Each major result should have at least a paragraph or two of text. If an experiment has only a very simple result, you don't have to make a table or graph for it—for example, it would be sufficient to say Based on the equation of our best-fit line (Figure 2), the

length of the plasmid was 3,600 base-pairs, rather than making a one-line table to report this single piece of data. Similarly, you would not say The five plasmids we examined had lengths of 3600, 4250, 8700, 1470 and 5400 base-pairs (Table 1)—since the numbers are in the table, just say We determined the lengths of the five plasmids (Table 1).

Although this is a "results" section, you do not want to just report your results: you should also analyze your data and explicitly interpret its meaning for the reader. In fact, you generally should not show raw data: if you have six replicate measurements, you do not have to give all six in a table but rather can show the average and a range or standard deviation in a graph. Remember that statistical analysis can be an important part of your results: use standard deviations, *t*-tests, ANOVA or other appropriate forms of analysis to demonstrate the significance of your results (see "Accuracy, Precision and Statistics" in this *Handbook*). Results is generally written in past tense. You may also want to break up the section with subheadings for each major result to help your reader follow the flow of the paper. Integrate your graphs and tables into your text (rather than placing them at the end of the paper), and put them close to the point where they're referenced for the first time.

Below is an example of a student who made the most of his/her data, describing and analyzing them well and presenting them effectively. Below that is a horrific example—even though both students have good data, the second example totally fails in making those data meaningful for the reader.

Differential rates of cookie disappearance. Fifty chocolate-chip cookies (see Materials and Methods for recipe details) were placed in each of two identical jars. Jar 1 was placed in the Science Division office, which is normally locked at night, while Jar 2 was left in a public hallway. Numbers of cookies remaining were measured daily for 10 days; the results are shown in Figure 1. Rates of disappearance were essentially linear, with a much steeper slope for Jar 2 (triangles and dashed line in Figure 1) than for Jar 1 (squares and solid line in Figure 1). No error bars are shown because this preliminary experiment was done only once. Rates of disappearance were calculated from the slopes of the line (see Table 1), and we found that Jar 2 had a rate of disappearance nearly twice that of Jar 1.

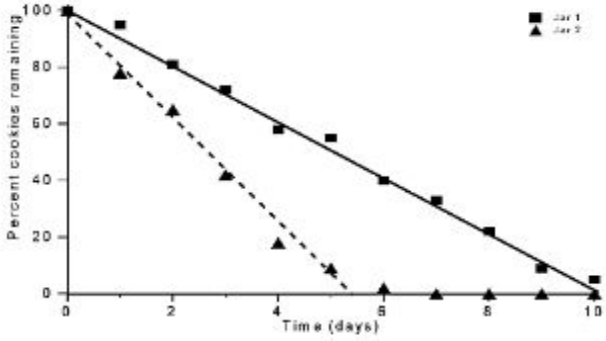


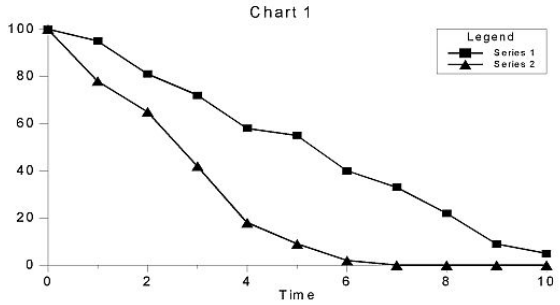
Table 1. Calculated Rates of Cookie Disappearance

Sample	Rate (cookies/day)[a]
Jar 1	5.00
Jar 2	9.09

[a]Rates were calculated by the least-squares method

Fig. 1. Reduction in cookie numbers over a 10-day period. Number of cookies remaining was measured for two jars (Jar 1, squares and solid line; Jar 2, triangles and dashed line) as a percentage of cookies present on Day 0.

When we did the cookie experiment, we saw that the cookies disappeared faster from the second jar (Figure 1).



Sometimes it's a little hard to decide what goes in Results and what goes in Discussion. Analyze and interpret your data in results, but save the big, general conclusions for Discussion. For example, The caffeine-treated mouse completed the maze 44 seconds faster than the control mouse is appropriate for Results, and so is We noticed in the first two experiments that brown mice completed

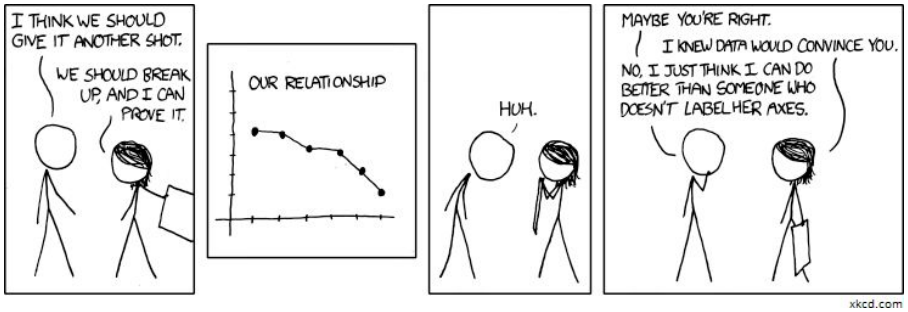
the maze an average of 12% faster than white mice, leading us to test specifically whether maze running ability correlates with the color of the mice. However, These results suggest that caffeine improves maze-running ability in mice is a general conclusion that belongs in Discussion.

Illustrations

Every illustration in a scientific paper is either a **figure** (graph, drawing, photograph, etc.) or a table (table of numerical data). Don't refer to an illustration as "Chart 1," "Graph 1," etc. Number your figures sequentially (Fig. 1, Fig. 2...) in the order that you discuss them, and number your tables separately (Table 1, Table 2...). If you have a couple of closely related graphs, you can make them panels of one figure (Figure 1A, Figure 1B...). Discuss *each* figure or panel *individually* in the text; never use something generic like "the results are shown in Figures 2-6."

Figures have a legend (caption) at the bottom which describes very briefly what's going on and identifies symbols. They do not have titles. Graphs and diagrams should be computer-drawn, and photographs should be cropped, processed (e.g., adjust contrast, sharpen) and computer-labeled. Be sure your graph presents your data appropriately; see the section "Graphing with Excel" for specific guidelines and tips. Do not settle for Excel's defaults: almost every aspect of an Excel graph can be configured, and as you can see in the example above, this makes a *lot* of difference. Pay attention to the scale of your graphs: two graphs showing related data should have the *same* scale to allow easy comparison. When appropriate, include error bars, standard deviations or other statistical measures.

Tables have a titles but do not have legends; any important details can be given in a footnote at the bottom of the table. Use a clean, clear format as shown in the example above. Think carefully about your data in deciding whether a graph or a table is the best way to present them: the goal is for the reader to grasp the meaning of the data clearly and quickly.



Discussion

Discussion is less important than Results, because it's not where the actual results are communicated, it is fun to read, because it explains what the research means. In this section, discuss the major findings, and draw conclusions, presenting your evidence for each conclusion by summarizing the relevant results briefly (without repeating the Results section). Explain *why* you think you got these results: what is going on biologically? Tell what you learned about the questions or hypotheses you laid out in the Introduction. You can also present new hypotheses based on your results, comment on possible future experiments, explain problems with the experiments and discuss possible sources of error. It is appropriate to use reference material (properly cited) in the Discussion section, especially to compare your results to those of others. Both past tense (when you're talking about your results) and present tense (when you're making a more general statement) can be used in Discussion. For example:

> The difference between the rates of disappearance of cookies in Jar 1 (office) and Jar 2 (hallway) is striking. Cookies disappeared nearly twice as fast (9.1 vs. 5.0 cookies/day; see Figure 1 and Table 1, above) from Jar 2. However, this experiment was done only once; hence, these data cannot be regarded as statistically significant. Based on this single experiment, we can tentatively hypothesize that increased disappearance results from the higher level of pedestrian traffic, particularly at night, in the hallway. This idea is consistent with a recent report (Smith and Jones, 1978) that at least twice as many people pass through the hallway than the office in a 24-hour period. However, we cannot exclude the possibility that there may have been differences in quality that made the cookies in Jar 2 more desirable, so the experiment should be repeated to control this variable and increase our sample size.

Every conclusion in Discussion must be carefully supported with evidence drawn from the data presented in Results. Don't assume that the reader understands how you came up with your conclusions: refer back to specific results and build your case explicitly, much the way a lawyer builds a case for a client's guilt or innocence in a closing argument. You can speculate about possible explanations for the results that remain to be tested, but any actual conclusions must be properly supported. Don't forget to discuss your controls specifically and show how they validated your experiment (or didn't!).

Students often say their experiments "failed" or their data were "inconclusive." They sometimes "fish" for errors to explain a failed experiment, suggesting that they may have pipetted wrong or not read an instrument right even if there is no *evidence* for an error. This can lead to a lackluster discussion. Instead, realize that your experiment *did* produce a result and focus on *interpreting* that result based on the evidence you have. Think about your data *first* in terms of biological explanations: what might have happened in the cell, organism or DNA that explains your observations? If necessary, discuss problems with the experimental design or execution, but give evidence to support your claim; don't use wimpy generalizations like "our data resulted from human error." It's OK if your hypothesis was not supported: the purpose of a hypothesis is to be tested, and we can often learn more about what's really going on from unexpected results than from the outcome we predicted.

Citations and References

As noted above, you must give proper credit for *every* piece of information that is not your own idea or a result of your own experiment. Scientists get credit for the work they've done when it is cited by other scientists, and the code of scientific ethics which governs our work requires that proper credit be given. Thus, even the source of "well-known facts" must be identified by a citation: if you want to start your paper with "Most people love chocolate-chip cookies," then we need to know what study found this to be true. Sources could be primary scientific articles, review articles, scholarly books or textbooks, but must be scientific; don't use newspapers or popular-press publications except in the rare case where you need to establish what the non-scientific public thinks (maybe your paper refutes the popular belief that diet soda causes cancer, for example). Web sites are absolutely not acceptable sources unless you are specifically directed otherwise by your instructor. However, it does not matter whether you read a scientific article in a printed journal or as a PDF from the journal's Web site; published articles stored online are perfectly acceptable sources. Web sites that identify their sources can also lead you to good published articles.

When you use source material in your lab report, you use a **citation** to identify it within the text and then a **reference** in the reference list at the end of the paper to give the source information. The format for citations and references is detailed in the section "Finding and Citing Sources" in this *Handbook*, along with more information on where to get source material.

Polishing the Lab Report

A good scientist, doctor or other life-science professional must also be a good communicator: it doesn't matter how good your data are if your writing isn't equally good. Your lab report should be polished and professional—so much so that you would be willing to submit it for publication in a journal or for a scientific meeting. It should be computer-printed and double-spaced except for single-spaced figure legends and abstract. Draft the report in time to *rewrite* it: don't just proofread or edit, and for sure never turn in your first draft. Use your lab manual as well as the checklist that follows to ensure that the paper meets all of your instructor's expectations. There is no excuse for turning in a paper that doesn't use the correct reference format or doesn't meet the style guidelines in this section or doesn't include all the material your instructor asked for: these are the *minimum* requirements. If writing is a struggle for you, remember there is help available. Use the Writing Center, ask friends to read your paper critically, or ask your professor questions about a draft.

Use a scientific tone that is clear, direct and readable with precise, carefully chosen words. Remember that small details contribute significantly to the overall professionalism of your paper. Use standard symbols and abbreviations: such as μg, ml (not mL), h, min, mM, etc. If you use an abbreviation that would not be immediately recognized by any reader with a science background, spell it out the first time. Remember that genus and species names of organisms are always italicized (*Escherichia coli*, *Homo sapiens*); the genus name is capitalized, but the species name is not. Do not add superscripts, Greek letters, symbols and the like by hand: learn how to do this with your word processor. If you have last-minute corrections, re-print the paper rather than making handwritten

changes. Spell-check your paper, but remember that this will not catch punctuation, grammar and style errors (the program doesn't know whether you meant "affect" or "effect," "due" or "do"), so re-read carefully. Buy a good dictionary and a good guide to English style and use both frequently.

Academic Integrity and Plagiarism

You are expected to meet the highest standards of academic integrity in all aspects of your academic career. Standards for academic honesty and definitions of plagiarism are discussed in detail elsewhere in this *Handbook*. However, it might be useful here to briefly list some examples of unacceptable use of others' work that pertain specifically to lab reports:

- Using a graph or other figure prepared by another student in your lab report.
- Working with your partner to produce one graph or other figure which you then both use.
- Asking your partner or another student to let you read his or her lab report and then using what he or she has written as the basis for your own report. *Both* parties have violated the standards if the lender knows the borrower plans to use his/her work. Eliminate this temptation by making it a rule to work with your partner but to never show him or her your work.
- Working together with a partner on the writing of the lab report, so that two partners turn in identical or extremely similar reports. (Collaborating with your partner on understanding the experiment and analyzing the data is perfectly acceptable—in fact, encouraged—but each partner must do his or her own writing unless an instructor has specified that two partners may turn in a single lab report.)
- Failing to give credit (in the form of a properly formatted citation and reference) for any non-original facts or ideas used in the report—including facts that you might assume are "common knowledge" or that "I heard in class."
- Using data from another lab group or any data that you did not personally obtain, unless you have specific permission from your instructor to do so.
- Making up data, adjusting data to "come out right," leaving out pertinent data that doesn't fit your hypothesis or any other unethical manipulation of data.

Lab Report Checklist

Title/Abstract:

- ❏ Specific title (informative or indicative)
- ❏ All contributors listed as authors below the title; writer identified with an asterisk
- ❏ Abstract single-spaced; briefly summarizes entire paper, including results & conclusions

Introduction:

- ❏ Sufficient background for reader to understand the experiments
- ❏ Shows why the experiments are important and significant
- ❏ Includes research question or hypothesis and the rationale on which it is based
- ❏ All non-original facts and ideas identified with citations and references in proper format

Materials and Methods:

- ❏ Gives enough detail that the experiment could be repeated
- ❏ Concise, compact description in paragraph form, using full sentences (not a list of materials or a set of directions)
- ❏ Source(s) for procedures are properly cited and referenced

Results:

- ❏ Begins with a sentence or two of introduction; could stand alone
- ❏ "Tells the story" of the experiment
- ❏ Describes the results in words, not just figures
- ❏ Refers to tables and figures (by number) and describes each of them
- ❏ Includes analysis of the data, including appropriate statistical analysis
- ❏ Written in past tense

Discussion:

- ❏ Briefly recaps results
- ❏ Focuses on biological explanations for the results: why were these results obtained? What was going on in the cell or organism that led to these results?
- ❏ All conclusions are carefully supported by evidence
- ❏ Any specific problems are discussed, providing evidence and avoiding generalizations
- ❏ Controls are discussed carefully
- ❏ Hypotheses (from the Introduction) are evaluated
- ❏ Future directions, improvements to the experiments or new hypotheses are discussed
- ❏ All source material is properly cited and referenced

General:

- ❏ Graphs, tables and illustrations are clear, formatted properly and integrated into text
- ❏ Figures are numbered and have descriptive captions but no titles; tables are numbered separately and have titles but no captions
- ❏ Sources for all non-original material are properly identified by citations in the text as well as full references at the end of the report
- ❏ Style is clear, concise, formal and scientific yet clear and readable throughout
- ❏ Standard symbols, abbreviations, subscripts, superscripts, etc. have been used
- ❏ Report has been through at least two complete drafts with careful reading, editing and re-writing; final draft is carefully proofread and free from writing errors.

Sample Lab Report

The Effect of Cookie Type on Rate of Disappearance

Cookie Monster and Ernie Muppet*

Cookies disappear from cookie jars at different rates. This paper tests one factor that could play an important role in determining the rate of disappearance: the type of cookie in the jar. The type of cookies present in a jar was varied, and their disappearance measured under standard conditions. We found that chocolate-chip cookies had a higher rate of disappearance than coconut macaroons. We conclude that the type of cookie affects rate of disappearance and hypothesize that cookie preferences among local populations of *Homo sapiens* might be the determining factor.

In recent years, a number of researchers have reported the rapid, spontaneous disappearance of cookies that had been stored in jars (Keebler *et al*., 2002), especially when the jars were placed in publicly accessible locations (Monster, 2006). Furthermore, the rate of cookie disappearance is not constant from one experiment to the next (Oreo and Milk, 2006), suggesting that some as-yet unknown factor may differentially affect the rate of disappearance. We sought determine whether the type of cookie placed in the jar would affect the rate of disappearance. Because chocolate-chip cookies are known to be yummier than coconut macaroons (House, 1999), we hypothesized that yummier cookies would disappear faster.

Materials and Methods

For each trial, one jar containing approximately two dozen chocolate-chip cookies and one jar containing approximately two dozen coconut macaroons were placed on a table in a hallway of the North Central College Science Center where there was significant foot traffic. Jars were always set out at 8:00 a.m., and the number of cookies remaining was determined by manual counting every hour for 10 hours. The experiment was repeated three times.

Results

In order to determine whether cookie type influences the rate of cookie disappearance from jars in public locations, we compared the rate of disappearance of chocolate-chip cookies with that of coconut macaroons. When the two types of cookie were placed side-by-side and the number remaining was determined over a 10-hour period, we observed that the coconut macaroons disappeared more slowly. Very similar results were obtained in each of three trials. Average data for the three trials, reported as percentage of cookies remaining, are shown in Figure 1.

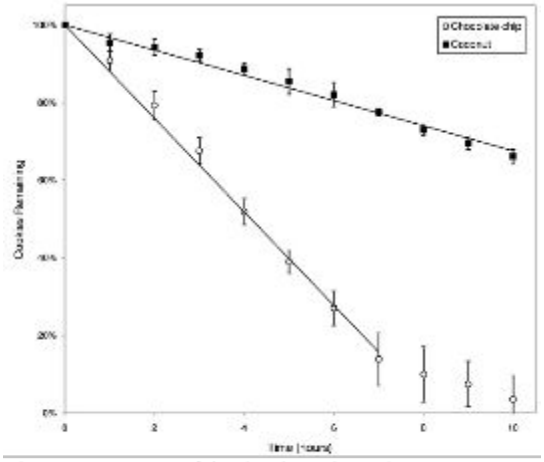


Figure 1. Disappearance of chocolate-chip cookies and coconut macaroons over a 10-hour period. Data are averages of three trials reported as percent of original cookies remaining. Error bars represent one standard deviation in each direction. Only the linear portions of the curves were considered in plotting best-fit lines.

We then determined the rate of disappearance for each trial of each kind of cookie using best-fit linear trendlines. The average rates (Table 1) indicate that chocolate-chip cookies disappeared about three times as fast as macaroons. We note, however, that for the chocolate-chip cookies, we observed that the rate of disappearance slowed after about seven hours in each trial; therefore, we calculated rate using only the data for the first seven hours in order to measure a linear rate of disappearance. We used Student's *t*-test (Student, 1908) to compare the rates and found the rate of disappearance of the chocolate-chip cookies to be significantly higher than the rate for the coconut macaroons ($p < 0.001$).

Table 1. Rates of Cookie Disappearance

Cookie type	Disappearance/hour[a]
Chocolate chip	10.5 ± 0.8 %
Coconut macaroon	3.4 ± 0.2 %

[a]Percentage of cookies disappearing per hour ± one standard deviation; N=3

Discussion

This experiment was designed to test the hypothesis that cookie disappearance rates would be affected by the type of cookie used. Specifically, we compared the rate of disappearance of chocolate-chip cookies to that of coconut macaroons. We found that chocolate-chip cookies disappeared nearly three times faster, a statistically significant difference which supports our hypothesis. This difference in disappearance rates was consistent across three trials, as shown by the relatively small standard deviations (Figure 1). We also observed a consistent slowdown in disappearance of the chocolate-chip cookies after seven hours. This phenomenon could be due to reduced foot traffic later in the day or could be due to the limited number of cookies remaining in the jar at this point. Increasing the duration of the experiment so that the number of macaroons remaining also approaches zero could provide further information.

Previous research (House, 1999) showed that chocolate-chip cookies are among the yummiest available, scoring on average 10 Yums (Ym) higher than coconut macaroons. Taken together with the data reported here, this suggests that their high rate of disappearance may be due to higher appeal to passing *Homo sapiens*. Future experiments will test this hypothesis by including a greater variety of cookies in the experiment, examining possible correlation between rates of disappearance and measured yumminess.

References

House, T. 1999. Quantitative measurement of taste value of various cookie types. J. Anal. Baking **45**:98-103.

Keebler, J., N. Abisco, and A. Baker. 2002. Spontaneous disappearance of cookies from jars. J. Cookie Res. **3**:104-113.

Monster, C. 2006. Cookie disappearance varies with location. Baking Sci. **23**:1114-1116.

Oreo, C., and W. Milk. 2006. Unexpected variation in cookie-disappearance experiments. J. Irr. Res. **16**:20046-20054.

Student. 1908. The probable error of a mean. Biometrika **6**:1-25.

Finding and Citing Sources

The Scientific Literature

The "scientific literature" refers to articles published in **journals**: scientific periodicals in which scientists report their research results. It is of great importance that you learn to use the scientific literature effectively, for two reasons: (1) This is where you find out what we already know—the background for a lab experiment, research project or term paper. (2) This is where new knowledge is published, so it's where you learn what's going on now, long before those ideas hit the textbook.

The scientific literature is **peer-reviewed**. To publish an article in a scientific journal, you submit a manuscript to an editor (also a working scientist), who then sends it out to 2-4 other scientists considered experts in your field. These expert reviewers read your manuscript carefully, scrutinize the data and decide whether the paper is scientifically sound, whether its conclusions are justified and whether it is a significant enough contribution to merit publication. This is an important means of quality control: a "scientific" report that is not peer-reviewed should be treated with suspicion!

Most scientists regarded the new streamlined peer-review process as "quite an improvement."

There are two main types of scientific article: **Primary** articles present actual research results and are written by the scientists who actually did the work. These detailed accounts of experiments include methods and data in the form of graphs, tables or photographs. **Review** articles are written by scientists, but do not report new data. They summarize recent research in a particular area and give perspective on where that area of research is going. Review articles are an excellent way to familiarize yourself with a topic and find (using the article's bibliography) relevant primary articles. You may also see **news** articles or short **commentaries** in scientific journals; these generally point out hot new research but do not give much detail; they usually refer you to primary articles.

There are thousands of scientific journals. Some, like *Science* or *Nature*, cover biology, chemistry, physics, astronomy and more. Others are specific to a particular field (e.g., *The Journal of Bacteriology*); still others are extremely narrow (e.g., *The Journal for ImmunoTherapy of Cancer*). And, journals are of different quality. *Cell*, *Science* and *Nature* are the most prestigious life-science journals, publishing only very important and exciting papers. Most high-quality work is published in second-tier journals with a strong reputation in a specific area, (e.g., *The Journal of Biological Chemistry* or *The Journal of Ecology*). Work with lesser impact might be published in a third-tier journal.

Nearly all scientific journals are now available online, and some are published only online. "Open access" journals allow free access to all their online articles. Other journals may provide open access six months or a year after an article is published, but some do not allow any access without a subscription or individual article purchase. Downloading a journal article as a PDF is the same as reading it in print, so online repositories of journal articles are perfectly acceptable sources. Web sites, however, are *not* peer-reviewed and therefore *not* considered part of the scientific literature (even if written by scientists or universities): they are *not* acceptable sources unless your instructor OKs them.

You do not always need journal articles; books written for scientific audiences are also reliable sources. Our library's book collection is a good way to get high-quality general background on a topic. There are also excellent magazines (e.g., *Scientific American*, *Natural History*) which present scientific topics at a level suitable for general readers.

Getting information from the scientific literature is a two-step process. First, identify articles of interest by searching databases that index many articles for keywords relevant to your topic (see "Searching Databases" below). Then, using the information from the database (e.g., the journal name, volume and pages), look up the actual article (see "Accessing Articles" below). The guidelines below will get you started; faculty and librarians can provide more help.

Searching Databases

The North Central College library provides access to many databases that can be used to search for articles. CardinalSearch on the library's home page (library.noctrl.edu) is a fast way to search

many databases at once, but you will often get better results by choosing a more specific database. From the home page, click Articles to see a drop-down list of available databases. Or, click Databases Alphabetically with Descriptions to learn more or More for additional options such as a subject listing.

It can be hard to decide where to search, especially because the library refers to several different kinds of resources as "databases." For example, Annual Reviews is listed as a database, but it is really an archive of articles from the several *Annual Reviews* journals with a search function to find articles only in these specific journals. ScienceDirect is an example of a database that allows you to search and retrieve more articles, but only from specific publishers that have signed on with that index, so again this may not be what you want. There are also really broad databases, like Academic Search Complete, that include both scientific and popular-press articles—again not what you want.

To cut through the confusion, search the databases that index the most scientific journals in your area of interest. You may need more specific databases to actually *access* an article, but you'll have the best chance of *finding* a useful article if you are looking at a database of scientific articles that does not limit itself to a few publishers. Two databases in particular will benefit you the most:

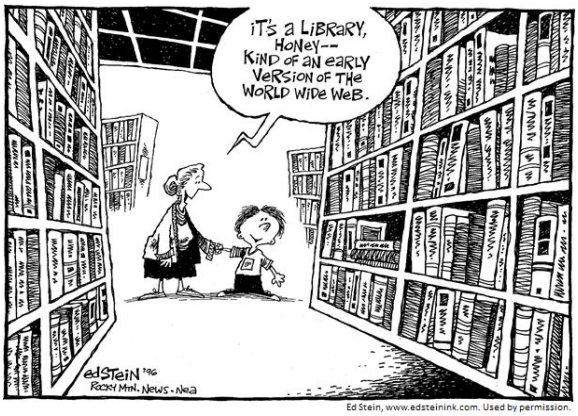
PubMed, maintained by the National Library of Medicine, indexes more than 5,600 journals relevant to biomedical science. PubMed is the best database for articles in areas with a connection to medicine: molecular biology, biochemistry, microbiology, physiology, cell biology, etc. However, it's *not* a good source for information in non-medical areas like ecology, evolution or botany. You can access PubMed via the library or directly at www.ncbi.nlm.nih.gov/pubmed.

Tips for searching PubMed:

- ❏ Searching for a single word finds the word anywhere in the article's citation: title, author, abstract, keywords, etc.
- ❏ To search for a combination of two words (*very* helpful in limiting your search results), join them with AND (must be capitalized), like cancer AND vaccine. You can also use OR: cancer AND (vaccine OR vaccination). Or, exclude a term with NOT: cancer AND vaccine NOT prostate.
- ❏ To search for a phrase, enclose it in quotation marks: "west nile" — but be careful with this one, as the search engine only looks for phrases which occur in its index.
- ❏ To search for an author, use the last name and initials with no space between them: Johnston SD
- ❏ To search for a word in the title of an article, use [title]: cancer [title] AND vaccine
- ❏ On the search results page are links to limit the results to review articles, limit by date, etc.

Biological Abstracts also indexes more than 5,000 journals but covers biology more broadly than PubMed. This would be your first resource if you are working in an area that is not closely connected to medical applications. Biological Abstracts requires a subscription, so you should access it via the library site. Our interface to Biological Abstracts includes fields where you can input multiple terms and limit the output in various ways to customize your search.

Obtaining Articles



Ed Stein, www.edsteinink.com. Used by permission.

Once you've found an article you're interested in, you need to get the article itself. Even though North Central is a small college, it is possible to obtain almost any article, but advance planning is needed to ensure you will be able to read it in time. *Don't* start looking for references right before a lab report or paper is due, *don't* ignore good references and pick weaker ones just because they're easier to access, and *don't* use an article as a reference if you've only read its abstract. Here are some ways to find your article:

- ❏ *Open access online*. These are easy: if the journal permits open public access, you can download the article from anywhere you can connect to the Internet. Download the PDF version, rather than the HTML version, so that you get the complete article with all of its figures, tables and formatting just as it appeared in print. PubMed and Biological Abstracts label free articles in their results, but check the journal's Web page anyway, as sometimes you may be pleasantly surprised to find out an unlabeled article is accessible.
- ❏ *Online via library subscription*. Our library's subscriptions provide access to many articles that aren't open access. Check our access by going to the library home page, clicking Articles and then

clicking Browse Journal, Magazine and Newspaper Holdings. Look up your journal and see whether the library subscribes to it or to a database which provides full-text access.

- ❏ *Print journals*. Our library still has a few journals in print or maybe on microfiche. You can determine if this is the case using the same journal lookup described above; if so, you can find it in the periodical collection on the lower level of the library.
- ❏ *Google Scholar*. Google provides this service (scholar.google.com) to search scholarly articles rather than the whole Web. It usually is not as good for finding articles as PubMed or Biological Abstracts, but once you've found one, try Google Scholar using its exact title as a search term. The results may include a hard-to-find free source (such as an individual researcher's Web site).
- ❏ *Interlibrary loan*. If none of these sources work, make an interlibrary loan request. On the library home page, click Articles, then More, then About Interlibrary Loan for articles. There will be a form to fill out with the details about the article you're requesting, and then the library will find it for you at some other library and get a PDF (usually) or FAXed copy within a couple of days.
- ❏ *Single-article purchase*. If you really, really, really need an article and there's not enough time for an interlibrary loan, many journal Web sites give you the option to purchase a single article for download. Prices are absurdly high (often $35 each), so consider this an absolute last resort.

Citing Sources in Your Writing

In scientific writing, source material is *paraphrased*, not quoted: read the article, think about its value for your paper, and express the ideas you learned in *your own* words. (Only quote in the very rare case where the author's *exact* words are essential to your point.) Use a **citation** to identify the source of the paraphrased material. *All* non-original material used in a paper or lab report—*everything* that is not your experimental results or your own original ideas—must be given proper credit.

The citation refers the reader to an entry in the reference list that gives complete information about the source. For each source, there will be one entry in the reference list and at least one (possibly several) citations in the text showing where that source was used. In life sciences, there is no one agreed-upon citation format: different journals have different style requirements. For consistency, we have adopted the author-date style for use in papers and lab reports within our department:

- ❏ Citations are placed in parentheses following the source material.
- ❏ For a single author, use the author's last name, a comma, and the year of publication: (Smith, 2015)
- ❏ If there are two authors, both are listed: (Brown and Green,, 2012)
- ❏ If there are three or more, use the first author's name and abbreviate the others with *et al.* (the abbreviation for *et alii*, Latin for "and others." Notice that *et al.* is italicized (because it's a foreign phrase) and that only the "*al*" has a period: (Johnson *et al*., 2014)
- ❏ If you have named the author in the text for some reason, the citation becomes just the date: As shown by Hershey and Chase (1952), viral genes are composed of DNA
- ❏ But you still need a full citation if you don't place it right after the name: As Hershey and Chase showed, viral genes are composed of DNA (Hershey and Chase, 1952)
- ❏ The citation is part of the sentence, so if it is at the end, place it before the period.
- ❏ If two or more citations are needed at the same place in the paper, separate them with a semicolon but put them both within one set of parentheses: (Jones *et al*., 1997; Smith, 2015)
- ❏ Never use double parentheses: We measured a protein concentration (using the Lowry method; Lowry *et al*., 1951) of 10 mg/ml – not: (using the Lowry method (Lowry *et al*., 1951))
- ❏ If two sources have the exact same authors and date, distinguish them with a and b: (Jones, 1999a)

Your reader should be able to easily identify what came from each source in your paper. But, don't go wild with citations, either: If a whole paragraph that is taken from one source, just put one citation at the end of the paragraph. Here is a sample paragraph with clear citations:

> It has long been known that plants are green (Smith, 1924). However, this green-ness has only more recently been studied in detail (for reviews, see MacIntosh and Brown, 2008; Oppenheimer, *et al*., 2013; Zales, 2011). Jones *et al*. (1993) concluded that the green color is due to the presence of chlorophyll, while others have suggested that another green molecule is responsible (Little, 2000; Peterson and Stevens, 1999). Two recent studies favor the chlorophyll model: in one, chlorophyll was found in green leaves from 147 plant species (Jacobs, *et al*., 2002a), while in the other, no chlorophyll could be detected in red, yellow or brown autumn leaves (Jacobs, *et al*., 2002b).

The Reference List

The reference list is at the end of the paper and gives full source information for each source cited in the text. The reference list contains *all* the sources cited in the text but *only* the sources cited in the text (not, for example, any sources you might have used for background reading but didn't wind up citing). Each source appears only once in the reference list, even if it is cited multiple times.

Authors are listed alphabetically by first author, in the *same order* as they are in the book or article, and with the *same initials* (so, John C. Smith appears as J. C. Smith, never just J. Smith). If all authors are the same for two or more references, order them by year (oldest to newest) and then alphabetically by title if necessary. Look carefully at the examples below and make your reference list match style *exactly*. *Exactly* means that every comma, period and space is in place—real scientific journals insist on meticulous attention to detail! *Do not* use other formats such as MLA or APA style.

Journal article (primary article or review):

Example: Jones, T. A., L. B. Little, and B. C. Smith. 1993. Identification of chlorophyll as the green pigment in plants. J. Plant Res. **126**:2224-2229.

Macintosh, A., and M. C. Brown. 2008. Recent insights into plant color. J. Fict. Sci. **4**:23-47.

Key points:
- Last names and initials (only) of all authors: no "*et al*.;" periods and spaces after initials
- First author is listed last-name-first, but all others are first-name-first
- Commas separate authors (including a comma before "and"); author list ends with a period
- Publication year is followed by a period; add a letter if references have same authors and date
- Complete article title; only the first word is capitalized (unless title contains a proper noun)
- Journal title is abbreviated (standard abbreviations are given in PubMed citations and usually on the title pages of articles); periods used only for abbreviated words (Res. but not Plant)
- Volume number is in boldface
- Issue number is not shown except in the rare case where page numbering restarts in each issue; if so, put the issue number in parentheses: Sci. Am. **24**(3):26-30
- Full page numbers (e.g., 2224-2229, not 2224-9) followed by a period

Book, where the entire book has the same author(s):

Example: Little, L. B. 2000. *Handbook of Plant Biochemistry*, pp. 12-17, 29. BioSci Publishing, Inc., New York.

Zales, X. Y. 2011. *Chlorophyll Chemistry*, pp. 146-154. Vanity Press, Inc., Chicago.

Key points:
- Author(s) and date same as for a journal article
- Title is italicized, has all important words capitalized and is followed by a comma
- Full pages used are listed, preceded by "pp." ("p." for a single page) and followed by a period
- Publisher's name (capitalized and followed by a comma), then city of publication and a period
- Do not specify an edition number (the publication date takes care of this)

Book, where individual sections have their own author(s):

Example: Jacobs, J. J., A. M. Morganstern, and C. B. Johnson. 2002. Chlorophyll content of plant species in the upper Midwest, pp. 575-580 in *Current Plant Physiology*, edited by B. G. Byrd, E. M. Uppet, and B. M. Uppet. Technical Press, San Diego.

Key points:
- List authors of the *section* first, same style as for a journal article, and publication date
- Give *section* title, using the same style as for a journal article
- Follow section title with a comma and full pages preceded by "pp." as for a book
- After the pages, add "in" and the *book* title, italicized and with important words capitalized
- The add a comma and "edited by" and then list the *book* authors (editors) in the normal style
- Publisher's name (capitalized and followed by a comma), then city of publication and a period

Course lab manual and *Biology Student Handbook*:

Example: Ruthig, G. R., and S. M. Alvarez-Clare. 2015. *Laboratory Guide for Biology 253*, pp. 26-31. North Central College, Naperville.

Johnston, S. D. 2015. *Biology Student Handbook*, pp. 56-60. North Central College, Naperville.

Key points:
- Unpublished, but treated like a book for reference purposes
- Use the current department chair as the author for the *Biology Student Handbook*
- Use *only* for methods unless you have specific permission from your course instructor

Preparing Scientific Presentations

You will often need to present your work to others, such as oral or poster presentations at scientific or medical conferences. We emphasize development of presentation skills, because those skills will greatly affect your ability to get a job, advance in your career or earn the respect of your colleagues.

General Ideas

The key to presenting scientific research is to clearly express why you did a particular experiment and what can be learned from the results. You will find it helpful to use the IF-THEN format: IF [hypothesis], THEN [predicted result]. For example, "IF the moon is made of green cheese, THEN chromatography of lunar surface material should show a high lipid content." This sets you up perfectly: your audience knows the hypothesis, how it can be tested and what results would support it. Then show the actual data and discuss whether it supports your hypothesis or points in a different direction: "Our analysis showed only silicon, so we now hypothesize that the moon is made of glass."

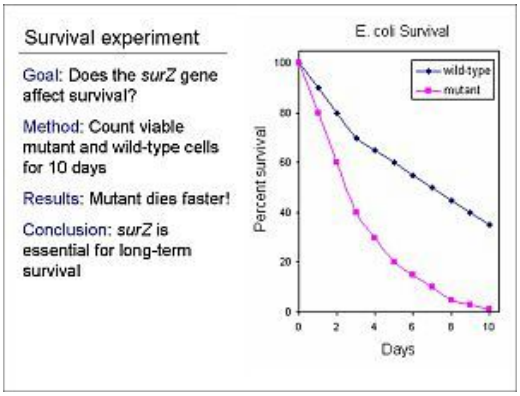
Be sure your own understanding of your material is clear and complete: the audience will be able to tell whether you genuinely know what you're talking about. Think about questions that might be asked and be sure you have good answers. If you get a question you don't know the answer to, say so: honesty is better than BS. If you don't understand the question, try restating it. Keep your presentation on a formal, professional level, and explain your work in clear, direct language. Don't try to sound smart by using fancy words you're not really comfortable with. Always treat your audience with respect; never be flippant, preachy, condescending or confrontational. Remember that when someone criticizes your data, experimental design or conclusion, s/he isn't attacking *you* personally but rather questioning the science: critical evaluation of results is how science works.

Oral Presentations

An oral presentation is a prepared talk illustrated with slides or other visual aids, usually followed by questions from the audience. In classes, you may present results of a lab project, talk about a group project or discuss a journal article in detail. You will present the results of your research in our BIO/CHM 475 seminar, and you might also present at a local conference (e.g., NCC's Rall Symposium), a national student meeting (e.g, NCUR) or a meeting of a professional society in your area of interest.

Planning and organizing. You will not have time to convey every detail of your research to the audience! Craft your presentation to (1) catch the audience's attention, (2) help them understand what you are doing and why it is important, and (3) get them excited about the results you're presenting and their significance and implications. Plan for your time limit: you should neither run over nor run short. Most people try to pack in too much, so draft an outline and then pare it down until you have only what you can talk about at a comfortable pace within the allotted time. This means you will need to rehearse your talk, out loud and with visual aids. Be sure to leave time for questions from the audience.

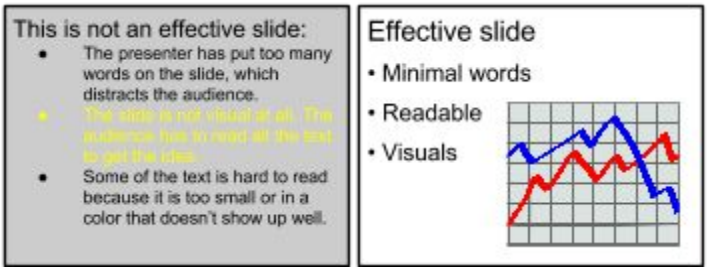
- **Know your audience**. In seminar, your audience includes first-years and seniors, chemists and biologists, students and faculty. That's very different from a focused session at a professional conference. Suit your presentation to the needs, background and experience of your audience.
- **The "big picture"**. In the first minutes of your talk, your audience should very clearly understand the big question your research addresses, your specific objectives and hypotheses, how you will accomplish your objectives and the significance of your research. Be sure these key points are clear before launching into detailed background.
- **"Package" each experiment**. A scientific paper must be in a rigid intro-methods-results-discussion format, but a presentation is more flexible. Your audience can't flip back to a previous page, so organize your presentation to help them. Think of each experiment as a package: Introduce its purpose, give background (like how a technique works), tell how the experiment was done, give the results and explain their meaning. Once you've delivered this package, go on to the next experiment. Summarize all the experiments once more at the end.



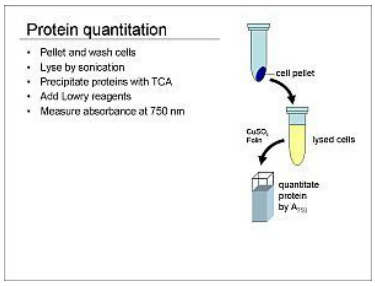
- **Show me the data!** Don't put results on one slide and text about the results on another. The audience wants to *see* what you're talking about, so put necessary text right on the result slide—or leave out the text completely and just show the results as you talk about them. Take the audience step-by-step through the actual data (graphs, gels, spectra, etc.) so that they can clearly see how your results lead to your conclusions. Include any statistical or other analysis you did.
- **Keep methods brief**. Listeners get lost in a long list of methods. Instead, list only the key steps of the method and tell *why* these steps were important. Anyone who wants to know details can ask.
- **Be persuasive**. As you design your presentation, ask yourself what you want to *convince* your audience of. A good scientific presentation builds a case for the conclusion you want your audience to believe. Don't worry about keeping them in suspense—it's perfectly OK to tell them the conclusion up front, then build up the evidence in support of it piece by piece.
- **Less is more**. Your audience does not have to hear about every experiment you ever did, including the one you totally screwed up. Present a limited number of ideas that really make your point.
- **Give credit**. Tell how your work fits into the context of what is already known. Show how others' work leads to or supports your hypotheses, or where your results disagree. Demonstrate your grasp of the scientific literature. And of course identify your sources: put a condensed reference (something like Smith *et al.,* J. Biol. Chem. **53**:11417 would be enough) in small type at the bottom of the slide where the source is used.

Visuals. Excellent visual aids are *essential*! Without them, the audience quickly gets lost. Your presentation should be as visual as possible: Show a graph, photo or diagram rather than a wordy explanation and replace paragraphs with bullet points or tables. Use graphics to add interest as well as to illustrate and explain. PowerPoint slides have become the staple of scientific presentations, but other visuals could also be used effectively, such as a carefully planned chalk-talk format.

- **Keep it simple**. No flashy backgrounds or fancy transitions. Use simple, clear fonts in colors and sizes that show up well against a plain background color. Use animation as a teaching tool, not for entertainment.
- **Minimize text**. If the slides are wordy, the audience will be reading instead of listening to you—and you'll be tempted to read instead of making eye contact. Use just a few key words to help your audience understand and remember.



- **Use pictures**. Wouldn't it be easier to explain that complicated experiment with a diagram? Take photos of your experiment, find images on the Web or draw your own diagrams with PowerPoint.
- **Show results clearly**. No one wants to squint at tiny numbers in a table when a nice, visual graph would show them better. Use the right graph for your data, label axes (don't forget units) and add titles, labels and arrows to graphs and photos to make your point.



- **Make it professional**. Small details affect how your audience reacts to your presentation. Proofread each slide carefully (in addition to spell-checking). Spend time formatting your graph for best effect. Learn how to insert subscripts, superscripts, and greek letters and to resize images without squashing. Use standard abbreviations, appropriate significant figures and scientific notation (1.3×10^4, not 1.311415E04).
- **Do a test drive**. See how your slides look when projected—preferably using the same computer that you will be using during your presentation. Watch for glitches caused by font differences, incompatible video formats, etc. (especially if you are translating between PC and Mac).

Speaking style. Mechanics (voice and style) make a big difference: an audience will be more impressed by a poised, enthusiastic, engaging speaker presenting mediocre data than by someone with great results but poor speaking skills.

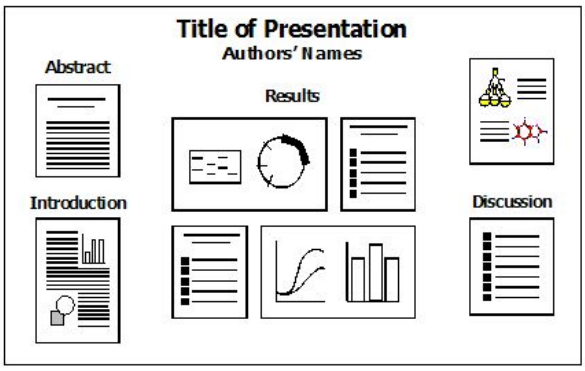
- **Know your stuff**. To engage your audience, you have to make eye contact and talk directly to them. Having notes is fine, but if you're reading directly from your slides or notes, your audience

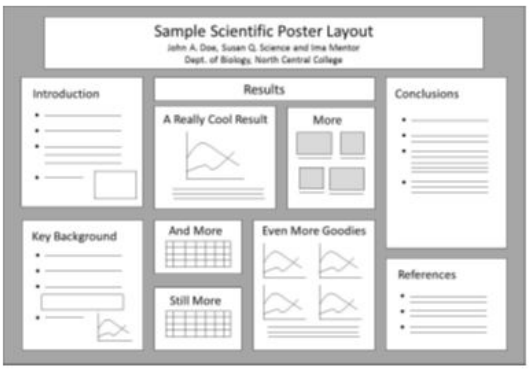
will get bored and think you don't know your material well. Practice your talk until you know it well, so you won't stumble or wonder what's coming next. This will also help you be less nervous.

- **Be natural**. You're talking to your colleagues, so speak as a fellow scientist explaining what you think are important results that need to be shared.
- **Be enthusiastic**. If you don't seem excited about the research, your audience won't be, either.
- **Make yourself heard**. If you tend to speak quietly, practice with a friend sitting in the back row and have him or her stop you every time you're not loud enough. Breathing from your diaphragm and pitching your voice a little lower than normal can help.
- **Slow down**. Most people speak too fast when they're a bit nervous.
- **Know the vocabulary**. If you misuse or mis-pronounce a key term, your audience automatically assumes you don't really know your material.
- **Avoid jargon**. You may use acronyms and slang in the lab, but clarity is the rule in a formal presentation: "I PCR-SOEd a 6His to PCM and screened on x-gal" will just confuse your audience.
- **Don't distract**. Don't stand on one foot, rock back and forth, shuffle through notes, fumble with an iPad or constantly sip from a water bottle.
- **Make your point**. Don't wave vaguely at your slide: point directly where you want your audience to look. A pointer or laser pointer is best, but if necessary just walk up to the slide and point. If you use a laser pointer, hold it still.
- **Dress nicely**. A suit and tie or dressy dress isn't necessary, but cutoffs and a torn shirt don't make much of an impression. Your dress helps let your audience know you're in charge.
- **Practice, practice, practice**. Don't just prepare the slides: prepare yourself. Go through the whole talk and figure out *how* you're going to say what you need to say. Practice in front of your mentor or some friends and ask them to be critical.

Poster presentations

A poster is a research "story" in a printed format. During a poster session, the presenter displays his/her poster and stands by it. Visitors commonly ask for a very brief overview of the work (2-3 minutes) and can then engage the presenter in one-on-one discussion. Poster sessions are common at scientific conferences, because they allow many people to present their work at the same time. At professional conferences, posters are the most common form of presentation for undergraduates, graduate students and post-docs. You might also prepare a poster for some of your courses, for our Summer Undergraduate Research Colloquium or as an option for the Rall Symposium or a student meeting like NCUR or $\beta\ \beta\ \beta$.





Designing the poster. In planning your poster, consider visual impact above all. The most important thing is to *show* your data in a form that others can clearly understand and judge. Good posters attract a potential reader's attention from across the room. They have large, readable pictures and a bare minimum of text in print that is easily readable from a distance. The flow of information should be clear and the most important information clearly emphasized. Posters are not limited to a strict introduction-methods-results-discussion format, and generally you should "package" the method, results and conclusion for each individual experiment as you would for an oral presentation.

- **Know your limits**. Each meeting establishes an allowable size for posters. For example, posters for the Rall Symposium are 48" wide by 36" high. *Before* starting on your poster, find out the size

restrictions. Then make a rough sketch to help you decide what you can present without overcrowding.

- **Title**. Be sure your title is large and clearly visible. Include your name, as well as the name of your faculty mentor and any others who worked on the project, and your institution.
- **Abstract**. For many meetings, you will have to submit an abstract as part of your application to attend; if so, include the abstract on your poster (it should be the same as the one you submitted).
- **Think visually**. Place related items together and frame them with blank space. Back up photos and text boxes with contrasting colors to make them stand out. Use headings such as "Introduction," "Methods," "Results" and "Conclusions" to identify the major parts of your presentation. Use titles to make your main points clear.
- **Keep text to a minimum**. Your audience will spend more time listening and talking to you than reading the actual words on the poster. Use illustrations and bullet points wherever possible.
- **Be professional**. Choose colors that are attractive but not distracting. Be sure your writing is clear, concise and precise. Use professional-looking headings, symbols, italics, sub- and superscripts, etc. Don't expect to put the poster together at the last minute; you can expect to spend 10-20 hours or even more putting together a high-quality presentation for a professional meeting.

Constructing the poster. A poster can be constructed by designing panels in PowerPoint, Word, Excel or other software, printing those panels and mounting them on poster board. Colored paper can be used to frame each panel. Be sure to use a paper cutter to get straight edges, and be certain your pieces are glued down securely to keep your data from crashing to the ground at an inopportune moment.

An increasingly popular alternative is to create your entire poster as a single PowerPoint slide and have it printed on a single sheet either by the North Central College print shop or by a commercial printing service (makesigns.com is a good one). This results in a very professional-looking poster, although it is a more expensive option (currently $2.75 per square foot from the NCC print shop). If you choose this option, below are some tips for constructing your poster. See also the Resource page of the Biology Web site.

- Open a new PowerPoint presentation and click Design | Page Setup to set the size of the slide to the size you want your poster to be. The largest slide PowerPoint can make is 56" × 56", so if you are making a very large poster, you'll have to scale it: for example, an 8' × 4' poster could be scaled to 4' × 2'.
- Use text boxes for the text of your poster. Choose sizes that will be clearly visible; remember that a 72-point font size will print 1" high. As a general rule, titles should be 108-144 points high, subheadings 48-60 points and body text 24-36 points.
- Use PowerPoint shapes to draw diagrams, frame panels and make headings; paste in graphs and photos.
- Proofread carefully; try printing the poster on 8.5" × 14" paper to get a good look at the whole picture and projecting it on a classroom projector to see how it will look at full size.
- Save a copy of your poster as a PDF and upload it to the NCC print shop using their online form or find out what formats a commercial printer will accept.

Presenting the poster. If you are traveling to your meeting, hand-carry your poster; don't trust it in checked baggage or try to mail it ahead. Dress professionally but don't over-dress; "business casual" would be appropriate for a presenter at most scientific meetings. Think in advance about what you will say to your audience. Although you're not making a formal oral presentation, it is very common for someone to ask you to "take them through the poster," and you will certainly get questions from your audience, as well. You should be prepared to walk someone briefly (3 min.) through the main points. Listen carefully to the comments your readers make; often, they may have very valuable ideas that can help shape your future research. Ask for business cards or e-mail addresses from people who seem to have particularly interesting ideas. For a professional conference, you may want to take along some print-outs of your poster reduced to 8.5" × 14" size to hand out to interested visitors.

Scientific Integrity

Science requires a high level of integrity and ethical behavior. Imagine what would happen if investigators in a clinical trial manipulated their data so that it looked like a drug was safe and effective for their own financial gain! Not everything you do in science will have life-or-death implications, but the same code of conduct applies to an introductory biology laboratory report.

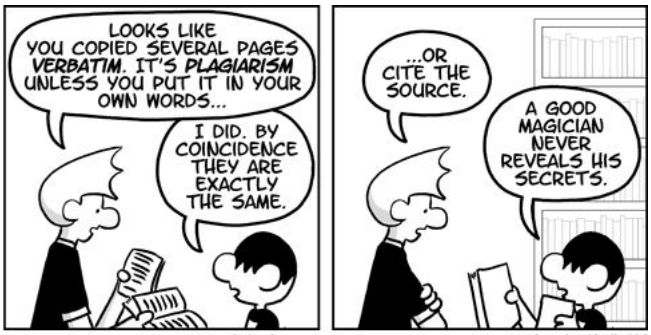
Scientific Ethics and Misconduct

There is no ethical contract that you sign when you become a scientist, but scientists agree that there are some basic ethical principles that should govern our conduct. Robert K. Merton articulated four key principles that are quite widely accepted:

- Scientific knowledge and understanding are "owned" collectively by all scientists, so scientists should be collaborative and treat one another as colleagues; science can only move forward when knowledge is shared rather than kept secret.
- Scientists should not be acting for their own benefit (e.g., for power, personal notoriety or financial gain) but for the greater good of science.
- Science is universal: scientific theories are based on impartial observation and experiment; thus, scientific investigation is open to anyone and the facts will not change with political conditions or the nationality, religion, race or culture of the experimenter.
- Scientists are skeptics: claims are accepted only after careful, critical investigation.

Scientific misconduct refers to inappropriate or unethical behavior by a scientist. Misconduct violates the relationship of trust and collegiality that should exist among scientists and thus is taken *very* seriously—a scientist guilty of misconduct will likely lose his/her job. Three major forms of scientific misconduct are generally agreed-upon by the scientific community:

- **Fabrication** of data means making up data or results. For example, if you didn't actually do a lab experiment but you wrote up a lab report as if you did, you'd be fabricating data.
- **Falsification** of data means manipulating the experiment or its results so that you are not accurately reporting the experiment. You'd be falsifying data if you "spiked" your tubes with something so that your experiment came out the way you wanted, if you recorded only the data that agreed with your hypothesis or if you manipulated an image to hide something you didn't want the reader to see.
- **Plagiarism** means using someone's words, ideas or results without giving credit. If you use a source in a lab report without citing it or use a figure that your lab partner made as if it were your own, then you are plagiarizing.



Importantly, honest mistakes are not misconduct. So, if you use a pipettor that is not calibrated correctly and get a wrong result, you have done nothing *ethically* wrong. It's important, however, to recognize the impact that errors can have in science and do your best to avoid sloppiness and use good experimental practices.

Responsible conduct of research (**RCR**) approaches integrity from the positive side: what are the characteristics of a responsible scientist, and how do responsible individuals engaged in research do their work? Scientists act responsibly when they conduct experiments and report findings honestly, accurately, efficiently, and objectively. They have responsibilities to their mentors, their fellow scientists, the greater scientific community and society at large. You will be encouraged throughout your time at North Central to participate in discussions of responsible conduct of research.

Academic Honesty at North Central College

All North Central College life-science students are expected to live up to the highest standards of scientific integrity and ethics. In the college setting, ethical behavior is governed by policies on academic honesty based on principles very similar to those outlined above. North Central College's policies are spelled out in the writing handbook given to each first-year student and available

electronically; they are also in the college catalog in a more abbreviated form. **Students are responsible for knowing and understanding College policies and can be penalized even if they do not know the policy or if the violation was unintentional**. Be sure you have thoroughly familiarized yourself with this essential information.

Just as cheating on a boyfriend/girlfriend destroys a healthy relationship, academic dishonesty violates the relationship of trust between you and your instructor, poisons the classroom environment and impedes learning. Thus, we as faculty members have to vigorously enforce the principles of scientific ethics and the academic integrity policies; we cannot let violations slide. Consequences (specified in individual course syllabi) may be serious, such as failing an assignment, exam or the course, and they will be imposed even if it's a first offense: we will not let you off with a warning! In serious cases, Academic Affairs can impose even more severe penalties, including dismissal from the College. We strongly urge you to embrace the highest standards of ethical conduct and academic integrity throughout your time at North Central and on into your future career.

We recognize that most students who cheat don't start out with the goal of being dishonest–usually, they've just gotten themselves into a bad situation and feel desperate. So, **if you feel desperate, please talk to us**. Go to your instructor, your academic advisor, the Academic Support office, the Dean of Students, or a counselor at Dyson Wellness Center. It may be difficult to face up to your problem, but once you have, you can turn things around and go forward. Don't dig yourself in deeper by adding a record of academic dishonesty to your troubles.

If you know someone is cheating or plagiarizing, you have a responsibility to notify your instructor. You could do this anonymously if you feel uncomfortable coming forward, or work through your academic advisor or another trusted third party. You are <u>not</u> doing your fellow student a favor by keeping quiet, and you are cheapening your own degree by allowing someone else to earn a grade s/he did not deserve.

Using Source Material Appropriately

In scientific writing, we generally paraphrase–rather than quote–source material. This means that you gain an understanding of the source and then express that understanding in your paper or lab report *in your own words*. Often, a plagiarism case results from a student not understanding how to paraphrase appropriately: *changing a word or two or rearranging a sentence is* <u>not</u> *paraphrase, it's plagiarism*. The examples below should help you see how to paraphrase properly.

original research article on chromosome structure in yeast:

> Deletion of any one or all three of the CAC genes is not lethal, indicating that there must be other activities in *S. cerevisiae* capable of chromatin assembly.

term paper #1:

> Loss of any one or all three of the CAC genes is not fatal, showing that there must be other activities in *S. cerevisiae* capable of chromatin assembly (Smith, 2015).
>
> ***Plagiarism!*** *This student used the original author's wording and structure, just changing a couple of words to synonyms.*

term paper #2:

> We know there must be other activities in *S. cerevisiae* capable of chromatin assembly, because deletion of any one or all three of the CAC genes is not lethal (Smith, 2015).
>
> ***Plagiarism!*** *This student just rearranged the original author's sentences.*

term paper #3:

> Losing the function of CAC1, CAC2 and/or CAC3 results in a yeast cell that is still viable, suggesting that there are at least two different mechanisms that yeast can use to assemble chromatin (Smith, 2015).
>
> ***Nice paraphrase!*** *This student understood the meaning of the original article and expressed it in his/her own words.*

term paper #4:

> "Deletion of any one or all three of the CAC genes is not lethal, indicating that there must be other activities in *S. cerevisiae* capable of chromatin assembly" (Smith, 2015).
>
> ***Wrong, but not plagiarism!*** *This student didn't know that scientists paraphrase instead of quoting, but at least it's not plagiarism.*

Examples of Academic Dishonesty

Below are some academic integrity problems that life-science students might encounter. All are cases of plagiarism or academic dishonesty that would have negative consequences for the student. Some may surprise you! Use this list to become well-informed about academic integrity in the college setting so that you don't wind up in a bad situation. If you have *any* question about whether something you are thinking of doing is appropriate or meets academic integrity standards, talk to your instructor or advisor. It is far better to change course and avoid a problem than to deal with the consequences.

- Bob ran out of time while writing a lab report and decided to skip adding citations and references.
- Alice knew she wasn't supposed to use an Internet source, so she didn't add a citation for that one.
- Stan thought that facts from the textbook or from lecture didn't require a citation.
- Mary missed lab, so she asked her lab partner for the data she would need for her lab report.
- Harold messed up the procedure, so he based his lab report on how the experiment should come out.
- After taking the exam with her early section, Cathy gave her friend in the later section some hints.
- Sam had a terrible week, so he looked at another student's quiz answers just this once.
- Violet knew her friend was peeking at her exam answers, but didn't say anything.
- Charlie felt clueless about how to write the lab report, so he asked Jenny if he could see hers.
- Jenny let Charlie see her lab report to get some ideas about how to write his.
- George called to ask his friend some questions about the exam, since it was take-home anyway.
- Deb and Barry were lab partners, so they sat down together to write their lab reports.
- Ina decided it would be faster to just read the abstracts of her sources.
- Lab partners Wayne and Lucy had the same data, so they made one graph and used it for both reports.
- Oscar slept in and missed lab, but he didn't want to lose the points, so he turned in a report anyway.

Research Ethics Training

All of the North Central College science faculty have active research programs in which they engage students; biology, chemistry and biochemistry students all carry out research projects as a graduation requirement. Educating students and faculty in the responsible conduct of research is therefore a priority for the College. Beginning Fall 2015, all faculty and students receiving research funding from NCC will be required to complete training offered through CITI. Modules to be completed deal with key topics in scientific integrity such as authorship, funding and plagiarism.

Students can create an account and complete the training at citiprogram.org; instructions can be found on the Resources page of the Biology Web site. You do not have to complete the Conflict of Interest module, and you do not need to complete the Animal Care and Use module or the Human Subjects research module unless you are instructed to do so by a professor or mentor in connection with a lab or research involving human or animal subjects (see the section "Working with Animal and Human Subjects" in this *Handbook*).

Accuracy and Precision

You will make many different measurements in lab and in research, and every measurement is subject to error. The error might be due to limitations of the instrument or to your skill in measuring—or it may simply be the chance fluctuations expected in all data. Since your conclusions depend on the measurements you made, it's important to know how good those measurements are.

Accuracy

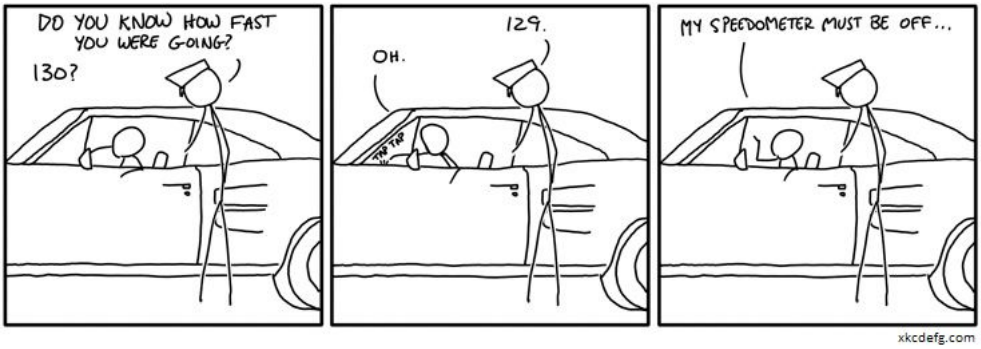
Accuracy refers to how close a measurement is to the "true" value. We usually determine a measurement's accuracy by comparing it with a known standard. Suppose you're using a micropipettor to measure 500 µl of water. How accurately can you measure this volume? To find out, you can just weigh the water: 1 ml of water has a mass of 1 g, and therefore 500 µl should have a volume of 0.5 g.

One way to express the accuracy is by calculating **percent error**: the difference between the measured value and the true value, expressed as a percentage of the true value: $\frac{|theoretical - actual|}{actual} \times 100$. So, if the water had a mass of 0.503 g, the percent error would be $\frac{|500-503|}{500} \times 100 = \frac{3}{500} \times 100 = 0.6\%$. Of course, then you might need to worry about the *precision* of your measurements (see below), so really you might want to make several measurements and calculate *average* percent error.

Accuracy also comes into play when you consider how well your measuring device can measure. For example, a graduated cylinder measures more accurately than a beaker, whose markings are merely approximate. (If you look carefully at the scale on a beaker, you'll often see something like ±5%, indicating that what looks like 100 ml could be anywhere from 95 ml to 105 ml.) If the mass of a chemical on a balance that gives four decimal places is 0.2564 g, you can feel confident about reporting a mass of 0.256 g, but if it's 0.256 on a three-place balance, you'd know you have less accuracy and would report 0.26. Likewise, you can confidently estimate length to the nearest 0.1 mm using an ordinary ruler, but two decimal places of accuracy would require a different measuring device.

Reporting Accuracy: Significant Figures

Always consider the accuracy of your measurements when recording and reporting your data. Scientists use **significant figures** to show how accurate a measurement is. In the three-place balance example above, giving the mass as 0.26 g—two significant figures—shows that the true value is between 0.255 and 0.265 g. Reporting 0.256 g from the four-place balance, similarly, shows that your measurement has greater accuracy: the true value is somewhere between 0.2555 and 0.2565 g.



Be sure to think about significant figures when recording data in your notebook or reporting data in a lab report, especially when you are making calculations. Suppose you dissolve your 0.2564 g of chemical in your 503 µl of water, and your calculator happily spits out a concentration of 0.5097415507 g/ml. If you just write that down, it means you know the true value is between 0.50974155065 and 0.50974155075 g/ml, which is absurd! You have three significant figures for the mass and two for the volume, so you should report 0.51 g/ml; *don't* just write down what you see on your calculator. Remember that significant figures apply to *every* number you use, so if you make a table, set the correct number of decimal places for each spreadsheet cell, and if you draw a graph, change the number of decimal places displayed for the axes or for the equation of a line.

Precision

Precision is how much variation there is when you make repeated measurements. Going back to our micropipettor example, suppose you measure 500 µl of water three separate times and weigh

the water each time. If the masses are 0.351, 0.353 and 0.350 grams, your measurements were not very *accurate* (because they're far from 500 µl), but they were very *precise*: the variation among the three measurements is small. This might suggest that your technique is good but your micropipettor may not be calibrated properly—whereas, on the other hand, imprecise measurements of 0.85, 0.32 and 0.55 g might suggest a problem in how you're using the micropipettor. Notice how accuracy and precision work together: very imprecise values like this second set make it very difficult to analyze accuracy.

Analyzing precision: average and standard deviation

In order to evaluate precision of data, we use statistical tools such as the average (or mean) and the standard deviation. The **mean** ($\bar{x}$) is simply the sum of all the measurements divided by the number of measurements. For the three measurements 0.25, 0.19 and 0.12 mg/ml, the mean concentration is 0.19 mg/ml. However, this doesn't tell us anything about the range of the data. To convey this information, we can also report the **standard deviation** (s). A large standard deviation tells you that the measurements are far apart: not very precise. The smaller the standard deviation, the less variation there was in the data.

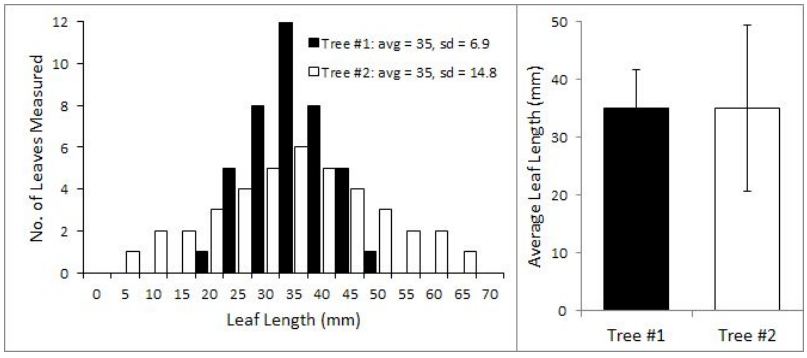
Standard deviation (s) is calculated by the formula at right and requires at least <u>three</u> measurements. For each measurement, the average of all the measurements (x_{avg}) is subtracted from the measurement itself (x_i), and the difference is squared. In other words, the "distance" from each measurement to the mean is calculated, and squaring takes care of any negatives. These results are added together, and the sum is divided by one less than the total number of measurements. The standard deviation is the square root of this number, so in rough terms, you can think of it as measuring the average distance from the measurements to the mean. Although it's a good idea to understand how standard deviation works, you do not need to memorize this formula or calculate standard deviation by hand: use your calculator or Excel.

$$s = \sqrt{\frac{\sum (x_i - x_{avg})^2}{n-1}}$$

One way to use standard deviation is to look at precision in **replicate** data: data resulting from measurements that are expected to be the same. In our micropipettor example, the micropipettor is expected to measure the same volume of water every time. So, if we pipette 500 µl three times and weigh the water from these replicate measurements, we expect to get 0.500 g each time. The standard deviation shows how much variation there is in the data, as in the table at right. Both pipettors gave similar average volumes. But, pipettor #1 had very high precision, as shown by the small standard deviation, 0.002g. The much larger standard deviation for pipettor #2 shows a lack of precision that could indicate a problem either with the measuring instrument or its use. Whenever we repeat an experiment, standard deviation show how similar the replicates are.

	pipettor 1	pipettor 2
	0.503 g	0.505 g
	0.499 g	0.527 g
	0.501 g	0.488 g
mean	0.501 g	0.507 g
std. dev.	0.002 g	0.020 g

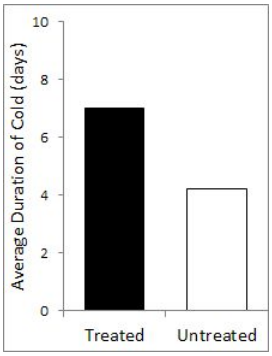
The graphs at right show another way standard deviation is useful. Suppose you're studying the influence of global warming on the size of tree leaves, so you measure the lengths of 40 different leaves from each of two trees. If you simply averaged the data, you'd get the same average for both trees, and the careless researcher might conclude that there is no difference between them. But, look at the left graph, which shows the results in the form of a **histogram** (the number of leaves from each tree between 0 and 5 mm in length, the number between 5 and 10, and so on). You can see that there is a much bigger range of leaf sizes for Tree #2. So, when you graph the average leaf size, as in the right graph, adding an **error bar** to show the standard deviation helps the researcher as well as the reader get the real picture of the data. Here, the error bar extends one standard deviation away from the mean in both directions. The much larger standard deviation for Tree #2 shows that the range of leaf sizes is greater for this tree. Providing standard deviations in the text or a table or showing them in a graph with error bars gives much more information about the data than if we only had the average.



Statistical Analysis

Hypothesis Testing and Statistical Significance

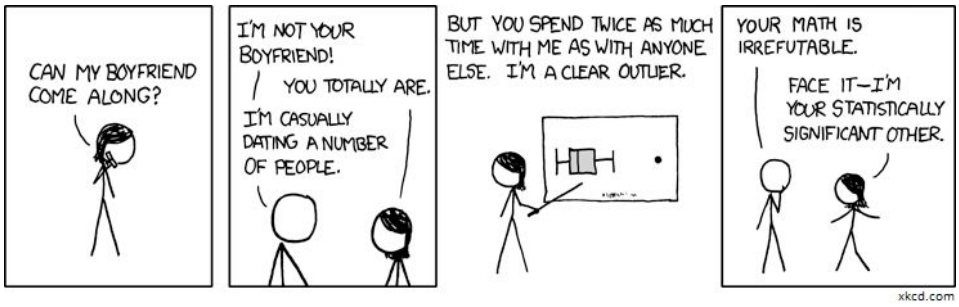
Science can be described as a process of developing and testing **hypotheses**: tentative, *testable* explanations for observed natural phenomena. A scientist may begin an experiment with a possible explanation in mind and design the experiment to test the hypothesis, or the hypothesis may emerge from initial results and is then tested. Either way, the ability to construct a good, testable hypothesis and design a good experiment to test it is an essential skill for any scientist.



In order to have confidence in the conclusion, we must ensure that the hypothesis is tested *rigorously*. This means using a statistical measure to ensure that our results mean what we think they mean. For example, the graph at right shows the results of testing a new drug thought to be useful in treating the common cold. Hey, this drug looks great, right? People who took the drug had colds that didn't last as long as they did for people who weren't treated.

...Or not. We're missing any information about the number of people tested, or any statistical analysis to determine whether the difference is **significant** or not. To say that a result is "significant" means that the likelihood of the same result occurring by chance is less than some established threshold value, often 5%. In this example, if only one or two people were tested, then it is very likely that the apparent difference is not significant: that is, the results are <u>not</u> different for the treated and untreated groups, even though they *look* different. However, if you knew that 1000 people were tested in each group, then this difference seems much more interesting.

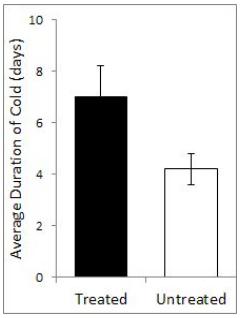
Statistical tests commonly used by biologists are discussed briefly below. To better understand statistical analysis, however, a statistics course (PSY 250 or MTH 341 and 342) is required for the Biology and Biochemistry BS degrees and strongly recommended for BA students. Ideally, take stats by the end of sophomore year so that it can help you with your data analysis in advanced courses.



Standard Deviation and 95% Confidence Limits

In the section, "Accuracy and Precision," standard deviation was discussed as a way to measure the precision of a set of measurements. Standard deviation can also be one straightforward way to evaluate statistical significance. For a set of measurements that fluctuate randomly around a mean, there is a 95% probability that the true value lies between two standard deviations below the mean and two standard deviations above the mean. So, if your mean is 35 and your standard deviation is 2, the chance that the true value is less than 31 or more than 39 is only 5%. A distance of two standard deviations from the mean in each direction is therefore referred to as the **95% confidence limit**.

Standard deviation doesn't directly measure whether the difference between two means is statistically significant or not. However, it is often used by scientists to suggest significance. Suppose our cold drug results are actually the means of large samples, and the error bars in the graph at right represent the 95% confidence limits (2 × standard deviation in each direction). Because these error bars don't overlap, there's a probability of <5% that the true value for either group lies within the confidence interval of the other. Thus, it's likely that this difference is statistically significant. However, a more rigorous determination of significance would require a more sophisticated statistical test such as a *t*-test.

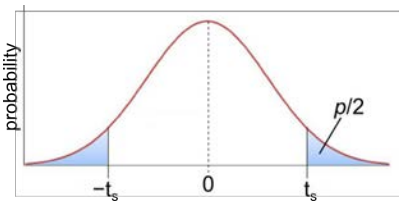


t-Test: Comparing Means

Whenever you have two sets of measurements, the **t-test** allows you to determine the probability that their means (averages) are different. Suppose, for example, you hypothesize that drinking coffee before an exam will improve exam scores; you might get the data shown in the table at right for a group of coffee drinkers and a control group who all take the same biology exam. You notice a two-point improvement in the coffee drinkers' scores vs. the control group—but is this a statistically significant difference?

	Exam scores:	
	Coffee	No coffee
	96	95
	87	82
	74	76
	72	70
	68	63
	54	54
mean	75.2	73.3

The *t*-test uses a value called the **t-statistic**, t_s, which essentially measures how different two means are, given the precision of those means. This value is calculated by: $\frac{\bar{x}_1 - \bar{x}_2}{S_{\bar{x}_1 - \bar{x}_2}}$ where $\bar{x}$ represents the two means and S represents the standard error (conceptually similar to the standard deviation). The figure at left shows the distribution of t_s values as the difference between two means gets farther from zero (identical means). If t_s is zero, the two means are the same. As t_s gets far from 0, the probability that the two means are the same decreases. This distribution was worked out by W. S. Gosset, who published his findings (to avoid reprisals from his employer) under the pseudonym "Student;" thus, the statistical test described here is known as **Student's t-test** (not "the student's *t*-test," as it is sometimes mis-identified).



To use the *t*-test, we usually take as our **null hypothesis** (H_0) the hypothesis that the two means are the same—that is, that our experimental treatment made no difference. In this example, the null hypothesis is that coffee drinkers got the same score on exams as the control group. The probability that this is true is the ***p*-value**, or probability value: $p = 0.95$ means there's a 95% probability that the "true" values of the two means are the same, given the precision of the data. We then set a **significance level**, α, which is the minimum p at which we will consider our hypothesis acceptable; usually, $\alpha = 0.05$.

In the figure above, p corresponds to the shaded areas: the area of the two "tails" of the curve that are farther away from zero than t_s in either the positive or negative direction. So, this is sometimes called a "two-tailed" *t*-test. If t_s is so far from zero—the means are so different from each other—that $p < \alpha$, then the null hypothesis has to be rejected. This is taken as evidence to support the original hypothesis, that the means are different.

You will likely use software to do a *t*-test rather than trying to calculate t_s and the p-value by hand. Excel can do a *t*-test, or you can also access the more sophisticated statistics package SPSS from any campus computer. Basic instructions for doing a *t*-test in Excel and in SPSS are given below.

Using Excel for a t-test. Set up your data in two columns representing the measurements for your two conditions as in the table above. From the Data ribbon, choose Data Analysis and then select *t*-test: Two-Sample Assuming Equal Variances from the menu of choices that appears. In the resulting dialog box, use the selection buttons and highlight the data for variable 1 (your first condition; here, coffee drinkers) and variable 2 (your second condition, no coffee in our example) so that the input boxes contain the range of cells that hold your data (don't include the labels). The null hypothesis is that there is no difference between the ranges, so set Hypothesized Mean Difference to zero. Alpha should already be set to 0.05; change it if necessary. Then, click Output Range and choose a cell where you want the results to appear. Click OK to do the analysis.

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	*Variable 2*
Mean	75.17	73.33
Variance	216.97	208.67
Observations	6.00	6.00
Pooled Variance	212.82	
Hypothesized Mean Difference	0.00	
df	10.00	
t Stat	0.22	
P(T<=t) one-tail	0.42	
t Critical one-tail	1.81	
P(T<=t) two-tail	0.83	
t Critical two-tail	2.23	

Your output should look like that shown at right. Notice that Excel calculates the mean and variance for each of your two data sets. Then it does the statistical analysis; most importantly, we see P (T<=t) two-tail: the p-value (probability that the two means are the same) is 0.83, far above the α cutoff of 0.05. Thus, we do not reject the null hypothesis: coffee drinkers and non-coffee drinkers got the *same* average score.

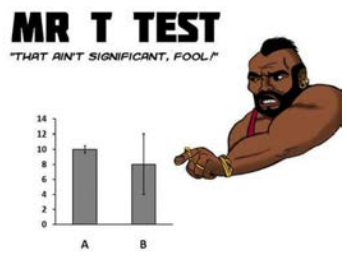
Using SPSS for a t-test. When you open SPSS, you'll see a window with two tabs at the bottom: Data View and Variable View. Enter or paste your data in the Data View tab. Unlike Excel, SPSS wants all the data in one column as shown below, not in side-by-side columns. SPSS does not like spaces, so

do not put a space in any of your terms. Now click the Variable View tab to label your two columns by putting names in the Name column. Use simple names with no spaces like Treatment and Score.

Coffee	96
Coffee	87
Coffee	74
Coffee	72
Coffee	68
Coffee	54
NoCoffee	95
NoCoffee	82
NoCoffee	76
NoCoffee	70
NoCoffee	63
NoCoffee	54

To run Student's *t*-test, click Analyze, choose Compare Means, then choose Independent-Samples T Test. Click on your dependent variable in the left box (Score in this example) to highlight it, and then click on the top arrow to move it into the Test Variable(s) box. Click on your independent variable (Treatment) to highlight it, then click on the lower arrow to move it into the Grouping Variable box. Next, click on the Define Groups button. Define Group 1 as Coffee and Group 2 as NoCoffee (you will need to type these in). Click Continue, then OK.

A new box will appear with the results of your *t*-test. The top box shows Group Statistics. This is a good place to see if you have entered all of your data correctly. You should see both of your groups in the Treatment column. In the N column you should see the correct number of replicates for each group. The Independent Samples box has the actual results of your test. There are three important numbers to look for in this box: df is your degrees of freedom. It is calculated by subtracting one from the number of replicates you have for each treatment, then adding those numbers together: in our example, (6-1)+(6-1) = 10. The next number is the *t*-value. The farther this number is from zero, the more likely that your two means are significantly different from one another. Finally the sig column is your *p*-value; $p < 0.05$ is usually considered significant.



Analysis of Variance (ANOVA)

Suppose you have not just two sets of measurements but many, and you want to compare their means. Couldn't you just use a *t*-test to compare each pair of measurements? To see why not, consider the probability that the *t*-test will reject the null hypothesis when the null hypothesis is in fact true. If $\alpha = 0.05$, then when $p = \alpha$, there's only a 5% chance that the difference between two means is due only to chance variation. But, there *is* a 5%, or 1 in 20, chance, and if we use the *t*-test repeatedly to make multiple comparisons on the same set of data, the likelihood of inaccurately rejecting a null hypothesis increases—to over 50% by the time we get to 8 *t*-tests. So, this is a poor way to look for significance among multiple independent samples. Instead, use the ANalysis Of VAriation (ANOVA) which can analyze variability across many samples in *one* test, eliminating the problem of multiple comparisons.

Suppose we want to examine the effect of several nutrients on productivity of tomato plants. In addition to an untreated control, we treat some plants with nitrogen-containing fertilizer (N), some with fertilizer containing phosphorous (P), some with fertilizer containing potassium (K) and a fifth group with balanced fertilizer containing all three (NPK). We measure the yield by weighing the fruit obtained from each plant in each group, shown at right.

Weight of fruit produced (kg) for each tomato plant

	Treatment				
	none	N	P	K	NPK
	13	8.5	6	8.5	16.5
	11	11	8	13	7
	8	9	9	9	10.5
	9.5	9	10	13	8
	11	12.5	9	10.5	14.5
	12	14	11	10.5	16
	14	10.5	15	8.5	12.5
	11	15	11.5	9.5	12
	10.5	14.5	11	11	11.5
	10	16	17	13	15
mean	11.0	12.0	10.8	10.7	12.4
s.d.	1.7	2.8	3.2	1.8	3.2

The null hypothesis is that none of the treatments have any effect, and that the means of all the groups differ only by chance variation. Notice that there is quite a bit of variation in the data, however, making it difficult to decide whether the differences in the means might be significant. ANOVA considers the variability **within** each group and also the variability **among** the means of the different groups and can determine whether the null hypothesis should be rejected for a given α. It does not, however, tell us *which* differences are significant, so if the overall null hypothesis is rejected, further statistical testing is required to determine which individual groups have significantly different means.

The first calculation in ANOVA is the **grand mean**, which is simply the average of all observations, regardless of treatment. In our example, we have 50 total measurements, so adding them all up and dividing by 50 gives a grand mean of 11.35. ANOVA then uses a calculation called the **sum of squares** as a measure of variation within and among groups. The sum of squares within groups, $\mathbf{SS_{within}}$, tells us about how much the observations in each group differ from the group's mean and is calculated

by: $\sum_i \sum_j (Y - \overline{Y})^2$ where i represents the groups, j represents the measurements in each group, Y represents a measurement and $\overline{Y}$ represents the average of measurements in the group. In our example, the calculation for the control group would start with $(13 - 11)^2 + (11 - 11)^2 + (8 - 11)^2$… and when completed for all 10 values of Y, this would give a sum of 26.5. The sums for the other four groups would be 69, 94.6, 30 and 94. These five sums are then added to give $SS_{within} = 314.2$.

We can then calculate the sum of squares *among* groups: $SS_{among} = n\sum_i (\overline{Y} - \overline{\overline{Y}})^2$, where n is the number of measurements in a group and $\overline{\overline{Y}}$ is the grand mean. With 10 measurements in each group in our example, $SS_{among} = 10((11 - 11.35)^2 + (12 - 11.35)^2 + (10.8 - 11.35)^2 + (10.7 - 11.35)^2 + (12.4 - 11.35)^2) = 24.0$.

Now we are ready to calculate the key value in ANOVA, the *F*-value, comparable to t_s in the *t*-test. If *F* is small, then it is likely that the difference between groups is due to chance variation, while a large *F* suggests that the means are actually different and the null hypothesis should be rejected. The equation at right shows how *F* is calculated. In this equation, i is the number of groups and n is the number of measurements per group. So for our sample data, F = (24/4) / (314.2/(10 × 4)) = 0.76.

$$F = \frac{\frac{SSamong}{i-1}}{\frac{SSwithin}{i(n-1)}}$$

As you saw for the *t*-test, the *F*-value by itself is not very meaningful unless we can associate it with a probability. Calculating the probability that a given variance occurs by chance is beyond the scope of this brief discussion of ANOVA, but either Excel or (better) SPSS can do this calculation.

Running ANOVA in Excel. Arrange the data in columns as shown in the table above, then choose Data Analysis from the Data ribbon, then select ANOVA: Single Factor from the resulting menu. Click the selection button and highlight your data to put it into the Input Range box, check the box below if your data include column labels. Set the desired Alpha, usually 0.05. Then click Output Range and use the selection button to highlight a cell where you want Excel to start the results table. Click OK to run the analysis. The results table for our sample data is shown at right.

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
none	10	110	11.0	2.94
N	10	120	12.0	7.67
P	10	107.5	10.8	10.51
K	10	106.5	10.7	3.34
NPK	10	123.5	12.4	10.45

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	23.95	4	5.99	0.86	0.50	2.58
Within Groups	314.18	45	6.98			
Total	338.13	49				

These results are conceptually similar to the *t*-test results. In the first table, the data are summarized, including sums and averages for the individual groups. Then, in the ANOVA table, we see the sum of squares for variation between (among) and within groups. The degrees of freedom (*df*) and mean squares (*MS*) values are explicitly shown; we didn't discuss them above but included them implicitly in our calculations. The *F*-value is then given (slightly different from ours due to rounding throughout the calculations). Importantly, Excel then gives the *p*-value of 0.5. As with the *t*-test, this is the probability of getting as much variance as we saw *given the null hypothesis that the means are <u>not</u> different*. Since this value is much higher than our α of 0.05, we would not reject the null hypothesis. Thus, in this particular experiment, it appears that fertilizer makes no difference in tomato yield.

Running ANOVA in SPSS. To run an ANOVA in SPSS, enter the data on the Data View tab and add names on the Variable View tab as shown for the *t*-test example above. Click Analyze, choose General Linear Model, and then click Univariate. Insert your dependent variable into the Dependent Variable box and your independent variable into the Fixed Factor box and click OK.

There are four important values to look for in the results of an ANOVA. Look for the box that says Test of Between-Subject Effects. Then look for the row that is labeled with the name of your independent variable. The *F* statistic is under the F column. The *p*-value will be under the sig column. There are two degrees of freedom to report with an ANOVA. The among-group degrees of freedom will be in the df column in the same row as your *p* and *F* values, and the within-group degrees of freedom will be in the df column in the Error row.

Regression: Correlation and Cause in Continuous Data

Regression is sometimes thought of as line-fitting, and indeed this is the same analysis that you're doing when you fit a trendline to some data on a graph in Excel. Regression is a way to work with data that are **continuous**, rather than grouped, such as a decrease in the number of viable cells over time or (in the example shown below) the effect of different concentrations of fertilizer on fruit

yield. In regression analysis, one variable is the **independent** variable, or the variable the experimenter manipulates (graphed on the x-axis when the data are shown graphically). The other is the **dependent** variable (graphed on the y-axis), the result measured as the independent variable changes. The goal of regression analysis is to test the hypothesis that changes in the independent variable *cause* changes in the dependent variable. In the example at right, the experimenter hypothesizes that increasing fertilizer application causes increased fruit yield.

Weight of fruit produced (kg) for each tomato plant

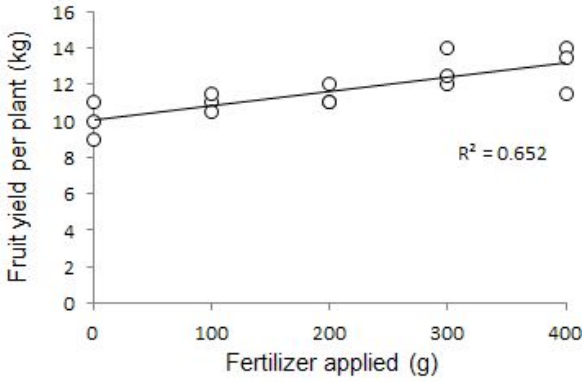
	Amount of fertilizer (g)				
	0	100	200	300	400
trial 1	11	11	11	12	14
trial 2	9	11.5	12	14	11.5
trial 3	10	10.5	11	12.5	13.5

The key statistic in regression analysis is r^2, the **coefficient of determination**. Given a set of continuous data, a measure of how well the two variables are correlated, r or the **correlation coefficient** can be calculated. The r^2 value is the square of r and can be thought of as the fraction of the change in the dependent variable that can be accounted for by the change in the independent variable. The r^2 value varies between 0 (no relationship between the two variables) and 1 (a perfect linear linear association between the two variables).

Linear regression (that is, regression assuming that the two variables have a straight-line relationship) is typically calculated by the **least squares** method; other methods are required to fit an exponential, logarithmic or other curve to the data. These calculations as well as how to use Excel's Data Analysis feature to do regression for different kinds of data are beyond the scope of this *Handbook*; instead, we'll focus on the more common use of Excel to calculate r^2 in connection with fitting a line. The table above actually won't give the desired result in Excel, because the program will assume that the three trials are three different data series that should be plotted separately; it will then fit a line to only one trial at a time. To overcome this problem, the data can be formatted as shown at right, with a single column of y values and a column with repeating x values. This produces the graph shown below.

	fertilizer (g)	fruit (kg)
trial 1	0	11.0
	100	11.0
	200	11.0
	300	12.0
	400	14.0
trial 2	0	9.0
	100	11.5
	200	12.0
	300	14.0
	400	11.5
trial 3	0	10.0
	100	10.5
	200	11.0
	300	12.5
	400	13.5

Fitting a best-fit line (Excel's "trendline") to these data (see "Graphing with Excel" in this *Handbook* for details on fitting lines to graphical data) gives the result shown at right. Optionally, the r^2 value can be shown on the graph. Here, $r^2 = 0.65$, so $r = \sqrt{0.65} = 0.81$. The correlation coefficient, r, expresses how well the data fit the linear model—in this case, reasonably well. The coefficient of determination, r^2, expresses the degree to which changes in the weight of fruit produced can be accounted for by changes in fertilizer application. We would say in this case that about 65% of the variation in fruit weight can be explained by fertilizer.



Categorical Data: χ^2 and the Test of Association

The **chi-squared** (χ^2) statistic is often used by biologists in testing hypotheses that are based on **categorical data**: data that fit into distinct groups (for example heads vs. tails, red vs. yellow vs. blue flowers, Democrats vs. Republicans). The χ^2 test is used to compare observed data with data expected based on a hypothesis.

Suppose, for example, you wanted to analyze the 2012 presidential election. Because people's identification with their political parties seems strong right now, you hypothesize that Democrats usually voted for Barack Obama and Republicans usually voted for Mitt Romney. You then survey 100 people and ask them their political party and how they voted in the election, with the results shown in the table at right. Mr. Obama has more Democrats in his column and Mr. Romney has more Republicans in his, but is this sufficient to say your hypothesis is supported? And in fact, what exactly does your hypothesis predict? If Republicans "usually" vote for Romney, does that mean 90% of the time? 80%?

Raw data from survey of voters

	Voted for:	
Party	Romney	Obama
Democrat	5	30
Republican	34	9
Independent	10	12

There's really no way to make a specific prediction for these data, so instead you can predict expected results based on a **null hypothesis** and test it against the actual results. In this case, the null hypothesis would be that there is no association between political party and voting (as if every voter just flipped a coin in the voting booth). This makes a specific prediction that we can test using the **test of association**: a measure of whether there is a statistically significant link between two variables (party and vote). The test of association is useful when you have no *a priori* expectation (an expectation you can determine before your experiment) about the frequencies of different results (you don't know what percentage of Democrats will vote for Obama), but you want to know whether there is a non-random association between the variables.

We have the observed values above; this is called a **contingency table** and shows our categorical data. Now we have to build the expected results based on our null hypothesis that party makes no difference. First, we need to add totals for each row and column, as shown at right. The **grand sum**, in the bottom right cell, is 100, the number of people you surveyed. This is a good place to check your math and be sure everything adds up. Now we can see that 49 of the 100 total voters chose Romney. If there were no association between party and vote, we would expect that 49% of Democrats, 49% of Republicans and 49% of independents chose Romney. So, the expected number of Democrats voting for Romney would be 35 (total number of Democrats) × 49 (total votes for Romney) / 100 (total votes) = 17.2. (You can also think of this as 49% of the 35 Democrats.) Or, more generally, the expected value for each cell is calculated by multiplying the total for that cell's row by the total for that cell's column and dividing by the grand sum:

Data from survey of voters with totals

	Voted for:		
Party	Romney	Obama	**Totals**
Democrat	5	30	35
Republican	34	9	43
Independent	10	12	22
Totals	49	51	100

Expected number of Democrats that voted for Romney: 35 × 49 / 100 = 17.2

Expected number of Republicans that voted for Romney: 43 × 49 / 100 = 21.1

Expected number of Independents that voted for Romney: 22 × 49 / 100 = 10.8

After calculating the expected values, we can construct the table shown at right. As an exercise, calculate the expected values for the Obama voters and then check your answers with the table. One great way to make sure that you did your math correctly is to make sure that the totals of your rows and the totals of your columns still add up to 100. Remember that these values are based on the null hypothesis, that there is no association between party and vote.

Expected results based on null hypothesis

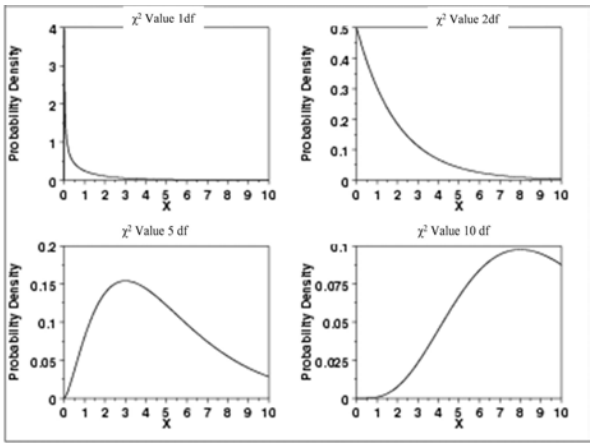
	Voted for:		
Party	Romney	Obama	**Totals**
Democrat	17.2	17.9	35
Republican	21.1	21.9	43
Independent	10.8	11.2	22
Totals	49	51	100

Now, we can determine the statistical likelihood that vote is associated with party using. The χ^2 value is basically a measure of how far apart the observed and expected data are and is calculated by the formula at right. For each item in our categorical data set, we subtract the expected value from the observed value, square the result and divide by the expected value. Then we sum the calculation for all the items to get χ^2.

$$\chi^2 = \sum\left(\frac{(observed - expected)^2}{expected}\right)$$

So, for Democrats voting for Romney, we observed 5 and expected 17.2; therefore, $(5 - 17.2)^2 / 17.2 = 8.7$. There are six total data points (three parties, two candidates), so we go through this six times and sum the results, which in this case adds up to $\chi^2 = 32.5$. This number measures the distance between the observed results and those expected from the null hypothesis. As χ^2 gets larger, our results are farther from the expected results. But at what point can we say with confidence that the observed and expected results are statistically different? To do that, we need to know about the probability of getting a χ^2 value this large, so we need to use the **χ^2 distribution** shown below.

This is a probability distribution: on the x-axis are χ^2 values, and on the y-axis is the probability (p) or likelihood that a given χ^2 value would occur if the hypothesis being tested were correct. Remember that we're testing the *null* hypothesis; you can then see that large χ^2 values have a low probability. Or, to put it another way, large χ^2 values will lead us to reject our null hypothesis.



Notice that the graphs are very different when the **degrees of freedom** (**df**) in the experiment change. More degrees of freedom make higher χ^2 values more likely. You can think of degrees of freedom as the number of ways that the results can vary. In a coin flip, there are two categories of data (heads and tails), but it can only be heads or tails, so there's only one degree of freedom for these two categories. If a voter didn't vote for Obama, then s/he voted for Romney (we didn't consider people who didn't vote at all), so that's one degree of freedom. But, there are three kinds of voters: if a voter isn't a Democrat, s/he could still be either a Republican or an Independent, so there are two degrees of freedom there. In general, to calculate the degrees of freedom in a contingency table, subtract one from the number of columns you have (2 − 1 = 1) and the number of rows that you have (3 − 1 = 2) and multiply these numbers (1 × 2 = 2). We have two degrees of freedom for the voting experiment.

The last thing we need is a cutoff value. High χ^2 values (on the right side of the graphs) represent results that are very unlikely if the null hypothesis is correct (party and vote are not associated). So, high χ^2 values indicate that we should reject the null hypothesis, supporting our hypothesis that party and vote *are* associated. But how high does χ^2 need to be to decide the variables are associated? In science we often use a 5% probability, or $p < 0.05$, as our cutoff point. If we get a χ^2 value for which there is not even a 5% probability, then the chance of getting our observed results given the null hypothesis is so small (less than one chance in 20) that we can safely reject the null hypothesis. If we set the cutoff, called the **alpha** (α) value, at 0.05, then for any *df* value, there is a **critical value** of χ^2 above which we reject the null hypothesis.

Critical values of the χ^2 statistic ($\alpha = 0.05$)

d.f.	critical χ^2
1	3.84
2	5.99
3	7.81
4	9.49
5	11.1
6	12.6
7	14.1

We can simplify the analysis by using a table of critical values for various degrees of freedom, shown at right. If χ^2 is higher than this critical value, we reject the null hypothesis. In our example, with $df = 2$, the critical value is 5.99, and our calculated χ^2 was 32.5, far above the critical value. Therefore, we can reject the null hypothesis. Remember that by rejecting the null hypothesis, we are actually supporting our original hypothesis (we have rejected the hypothesis of *no* association). Thus, shockingly, it seems that political party has an impact on whom voters are likely to choose for president.

Categorical Data: χ^2 and Goodness of Fit

A second way to use χ^2 to analyze categorical data is the **goodness of fit** test, which examines how well observed data fit the predictions of a hypothesis. The goodness of fit test is very commonly used in genetics, where the numbers of offspring of different phenotypes can be predicted based on hypothesized genotypes of their parents. The calculation of the χ^2 statistic is the same as for the test of the association; only the usage is different.

Goodness of fit could be used to investigate whether a coin is "weighted" so that it no longer comes up heads or tails randomly—a trick that could be used to gain an advantage in a coin flip. Imagine that you flipped a coin five times and the coin came up heads 4 times. Would you think that was strange? Maybe not, but what if you flipped the coin 50 times and it came up heads 40 times? At this point you'd be pretty sure that the coin was "weighted," but at what point could you be confident in this conclusion?

Remember that χ^2 is calculated based on observed and expected results using this formula above. In this case, the observed data are of course the coin flips that you counted: 40 heads and 10 tails. The expected data are the numbers that you would have expected to get based on some hypothesis. In the case of a coin, the null hypothesis would be that the coin is not weighted, so that if you flipped it 50 times, you would expect 25 heads and 25 tails. If you calculate χ^2 for these data, you should get 18, and there is of course just one degree of freedom (see above for discussion of degrees of freedom). You can then use the table of critical values to see if χ^2 exceeds the critical value or not. If it does, then the hypothesis that the coin is not weighted should be rejected; if not, then the data

support the unweighted hypothesis. Here, 18 is far above the critical value of 3.84 for $df = 1$, supporting the hypothesis that the coin is weighted!

Note, however, that this does not *prove* the hypothesis to be true—in fact, "prove" is a word that you should generally avoid using in science. A hypothesis may be well supported by experimental data—so well that eventually we come to believe the hypothesis is a fact—but it is always possible that an alternative hypothesis will eventually be developed that explains the observations even better.

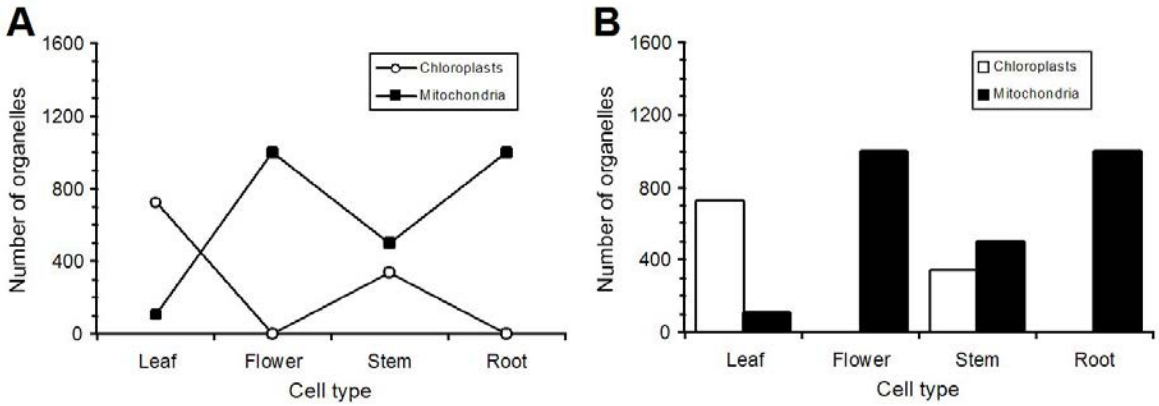
The goodness of fit test can be applied to many other questions, including those where there are more than two possibilities. Using the goodness of fit test merely requires categorical data and a hypothesis which makes specific predictions.

Graphing with Excel

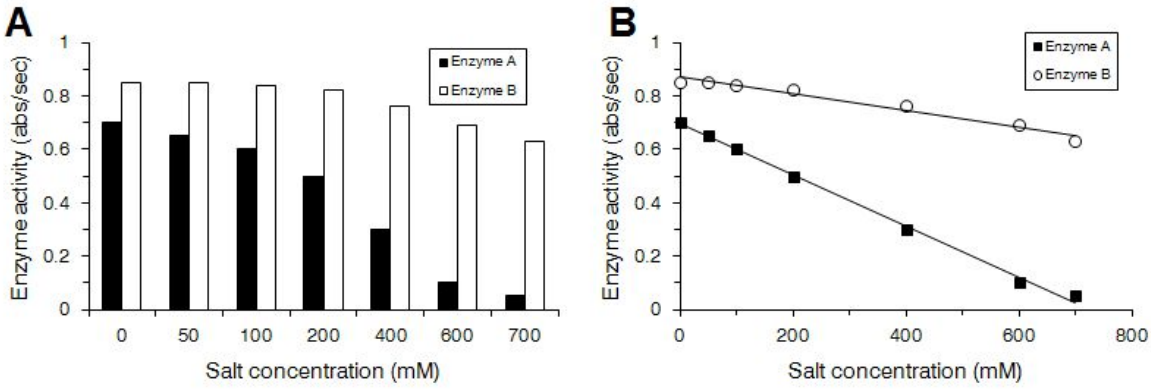
Well-constructed graphs are a very valuable way to convey scientific information: they can contain a large amount of information and quickly give a "picture" of an experiment. This section explains basic graphing with Excel (Microsoft's spreadsheet program), since it is available on all campus computers, and is written for Excel 2010. The same features exist in other versions but may be accessed differently.

What kind of graph should I draw?

Yes, it does matter! Most of the time, you will construct either a line graph or a bar graph. **Line graphs** (Excel calls these "scatter graphs") should always be used when you're showing a *mathematical relationship* between two sets of data. **Bar graphs** (Excel calls these "column graphs") should only be used when your data are in separate groups or *categories*.



For example, suppose you counted chloroplasts and mitochondria in plant tissues. A line graph, as in graph A above, looks OK at first glance, but actually makes no sense! The number of organelles does not change gradually from one tissue type to the other: each tissue has a distinct number. We call these **discrete** or **categorical** data. A bar graph (graph B above) shows differences between the different plant tissues clearly and demonstrates that each tissue represents a different data category.



On the other hand, if your data are continuous, such as the graphs of enzyme activity above, then the line graph (B) is much better than the bar graph (A). The line graph makes the distance between points *proportional*, whereas the categories are simply spaced evenly in the bar graph. Now we can clearly see the trends in the data, and we can use a best-fit line to describe those trends mathematically. The best-fit line in turn gives us even more information, showing a linear relationship.

Setting Up the Graph

The **independent variable** is plotted on the *x*-axis; this is usually what you change deliberately in the experiment. The result that you measure in response to that change is the **dependent variable** and goes on the *y*-axis. In the graph above, for example, the experimenter varied salt concentration (independent variable) and observed a resulting change in enzyme activity (dependent variable). This simple convention allows your reader to quickly understand what you are trying to show.

Preparing the Data

	A	B	C	D	E	F	G
1	**Cookie Preference Testing**						
2	BIO 540 lab, 22 Jun 2015						
3		Chocolate Chip			Peanut Butter		
4	Time (h)	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
5	0	25	28	24	30	28	31
6	1	22	26	22	29	26	30
7	2	19	22	20	29	26	29
8	3	16	19	17	28	26	28
9	4	12	15	13	27	25	27
10	5	9	11	10	25	25	26
11	6	6	9	6	24	24	25
12	7	3	6	2	23	22	24
13	8	2	5	1	22	20	23
14	9	1	4	1	21	19	22
15	10	0	3	0	20	18	21

In this section, we'll use sample data from an experiment to investigate cookie preferences. A researcher baked chocolate chip and peanut butter cookies and left them in the Science office, counting the cookies remaining each hour. The experiment was repeated three times, with the results shown at right. Notice that this researcher's clearly labeled data table will make sense when he comes back to it later or discusses it with a mentor or colleague.

A good graph should present the data to the reader as effectively as possible. It would be confusing and not at all effective to simply graph all these raw data, so how might the data be presented better? Since there are three trials, you can see that the researcher might want to average the data. But, notice that the *starting* number isn't the same in each case, so really these three trials aren't exact replicates and simply averaging the numbers would be misleading. This problem could be solved, however, by first converting the numbers to the *percent* remaining—so that all the trials start at 100%—and then averaging the percentage.

	I	J	K	L	M	N	O
1							
2	**Cookies remaining as percent of starting number:**						
3		Chocolate Chip			Peanut Butter		
4	Time (h)	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
5	0	100	100	100	100	100	100
6	1	88	93	92	97	93	97
7	2			83	97	93	94
8	3			71	93	93	90
9	4	48	54	54	90	89	87
10	5	36	39	42	83	89	84
11	6	24	32	25	80	86	81
12	7	12	21	8			
13	8	8	18	4			
14	9	4	14	4	70	68	71
15	10	0	11	0	67	64	68

=B5/B$5*100

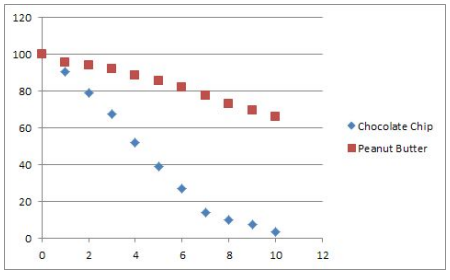
=E10/E$5*100

Excel can do a lot of the work for you, using **formulas** to manipulate numbers. The formula =B5/B$5*100 means: divide the number in cell B5 (25, the starting number of cookies in chocolate chip trial 1) by the number in cell B5 and multiply by 100 to get a percentage—in this case, 100%. The equal sign at the beginning lets Excel know you're typing a formula, not just some text. Notice that the second B$5 has a dollar sign in it. If you copy this formula down one row, the first B5 will automatically become B6. That's great, because now you want to find the percentage remaining after one hour. But, you don't want the *second* B5 to also become B6: you want to keep dividing by B5. The first B5 is a **relative reference** that will change, but B$5 is an **absolute reference**: when you copy it down one row, the 5 will not change. However, if you copy it over one *column*, the B will change to a C, which is also just what you want, to calculate percentages for trial 2. If you didn't want this to happen, you could write B5 and neither the row nor column would change. The table at right was made by simply copying this formula down and across so that now we have a table of percentages. The callout boxes show the formulas for two of the cells.

	Average % Remaining		Standard Deviation	
Time (h)	Chocolate Chip	Peanut Butter	Chocolate Chip	Peanut Butter
0	100	100	0.0	0.0
1	91	95	2.5	2.2
2	79	94	3.7	2.0
3	68	92	3.4	1.6
4	52	89	3.4	1.5
5	39	85	2.8	3.3
6	27	82	4.4	3.1
7	14	78	6.8	1.0
8	10	73	7.1	1.4
9	7	70	5.9	1.6
10	4	66	6.2	1.8

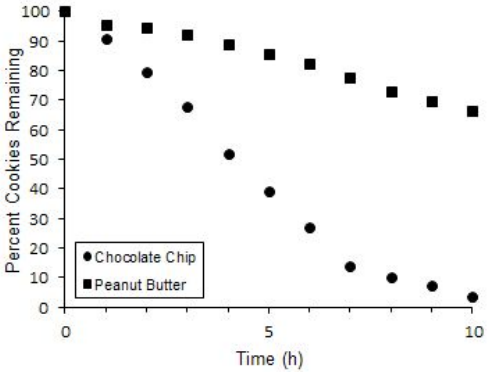
Another formula can be used to calculate averages. The percentages of cookies remaining for the three chocolate-chip trials at the start are in cells J5, K5 and L5. So, =AVERAGE(J5:L5) will average these three values. We should also tell the reader something about the precision of the data (See the section "Accuracy and Precision" in this *Handbook*), so let's determine the standard deviation of the three values as well: =STDEV(J5:L5). See the finished the table at right. Remember significant figures! Round your data appropriately by setting the number of decimal places (use the Number palette on the Home tab). Excel likes to graph data in columns, so this table is ready to graph.

Drawing the Graph



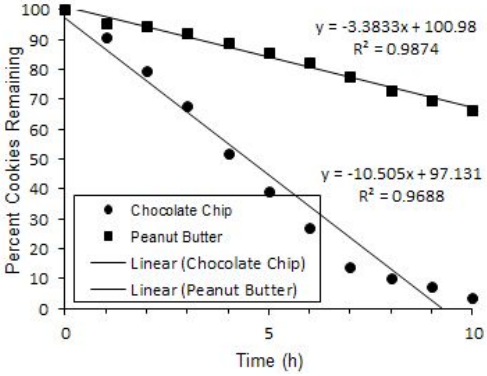
Excel refers to a graph as a "chart," so to draw a graph, highlight the data you want to graph (in this case, the first three columns from the table above), click the Insert menu, find the Charts palette and choose a Scatter graph with no connecting lines. (Most of the time, you'll want to add a best-fit line rather than a dot-to-dot line). You should get something like the graph at right. Excel tries to choose options for you, but you can see it didn't do a great job. Those colors won't look good when the graph is printed in black and white, there are no axis labels, and there are some distracting gridlines we

don't really need. Fortunately, everything is configurable. Double-click the data to change the symbols, use the Layout menu to add axis titles, then right-click them to change the font, select the gridlines and backspace them away and double-click the axes to change the scale. It takes only a minute or two to get the much better graph at right. You can configure almost every part of an Excel graph, so don't be shy about looking for options and making the graph come out the way *you* want it.

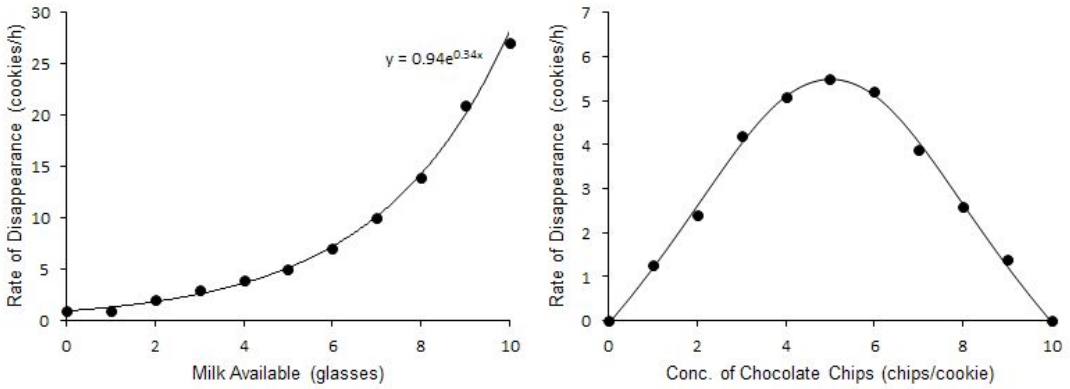


Drawing Best-Fit Lines

Notice that the cookies are disappearing at a nearly constant rate. Our graph would be even more informative if we used lines to show these trends in the data, and then we could in fact compare calculated rates of disappearance for each cookie type. To accomplish that, we need a **best-fit** line: a line that fits all of the data points as closely as possible. Excel can calculate the best-fit line if the data follow a linear, exponential or other simple mathematical relationship. Here, we see a linear trend, so by selecting each set of data and choosing a Trendline from the Analysis palette in the Layout menu, we can plot a linear best-fit as shown at right. Display the lines' equations by right-clicking each line and choosing Format Trendline.



The slopes of the lines give us the rates of cookie disappearance (notice the slopes are negative because the number of cookies is *de*creasing over time). Chocolate-chip cookies are being eaten at the rate of 10.5 cookies/hour, while peanut-butter cookies are being eaten at only 3.4 cookies/hour. Now we can be much more specific in talking about the data, telling the reader, for example, that chocolate-chip cookies are eaten at a rate nearly three times that of peanut-butter cookies. The r^2 value is also useful, as it tells you something about how well the best-fit line fits the data (see "Statistical Analysis" in this *Handbook* for more about r^2). A perfect fit would have $r^2 = 1$, while the worst possible fit would have $r^2 = 0$. If you fit a line to your data and get a terrible r^2 value, then your line doesn't fit and you should do something about it! If the data follow an exponential curve instead of a straight line, for example, choose an exponential best-fit line (left graph below). Or maybe there isn't a simple mathematical relationship that Excel can calculate automatically, like the right graph below. These data show a maximum, or optimum, value, and Excel can't fit that line. But it does include drawing tools so that you can draw your own best-fit line.

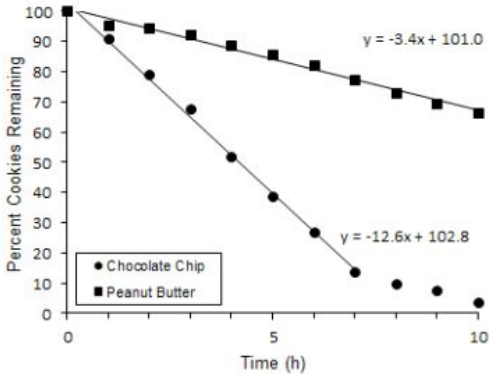


Hopefully you noticed some problems with the trendline graph above. Some are formatting problems that can be quickly corrected. We don't need the extra legend entries, so highlight and delete them. Then, look at the equations shown on the graph: there's no way we have five significant figures! Either right-click the trendline and format the label or just edit the label directly. It might also be wise to talk about the r^2 values in the text of the report rather than having them clutter the graph.

But there's a bigger problem here: even though both trendlines get a decent r^2 value, it's easy to see that the line for the chocolate-chip cookies doesn't truly fit the data. The rate of disappearance is higher at the beginning than at the end! Our linear trendline ignores this and just tries to fit all the

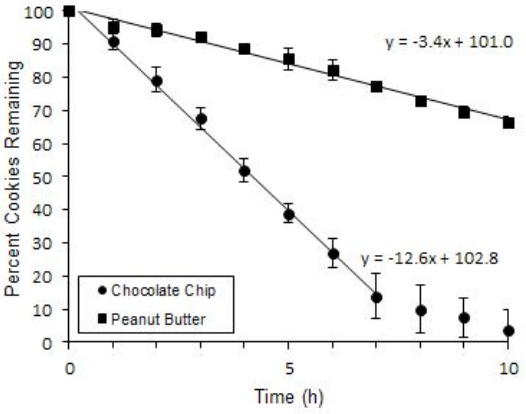
points. We could fix this by trying to fit a curve, rather than a straight line. But that would also make it very hard to compare the rates for the two cookie types. So it might be best to show a linear best-fit to *only* the linear portion of the data, explaining in the text that the rate decreases over time after a certain point (and maybe trying to explain why: perhaps no one wants to take the last few cookies. It would be great if Excel would let you choose the points you want the trendline to fit, but it doesn't. So the best way to do this is to add an extra column of data to the data table: a "dummy" column that contains a copy of the chocolate-chip data. Then, delete all the data that aren't linear (hours 8, 9 and 10). Add these data to the graph and fit the trendline to the "dummy" data instead of the real data. Finally, format the dummy data so they have no markers: the points will become invisible, leaving just the trendline. Voila! The finished graph is at right. Now we can see that the real rate for chocolate-chip cookies is in fact *more* than three times the rate seen for peanut-butter cookies.

	Average % Remaining		
Time (h)	Chocolate Chip	Peanut Butter	dummy
0	100	100	100
1	91	95	91
2	79	94	79
3	68	92	68
4	52	89	52
5	39	85	39
6	27	82	27
7	14	78	14
8	10	73	
9	7	70	
10	4	66	

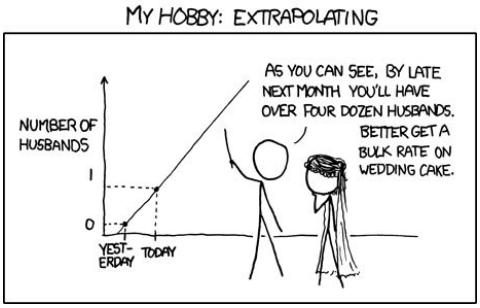


Error Bars

This graph still doesn't tell the reader anything about precision, or the repeatability of the experiment. The best way to show the range or amount of error or variation in the data is with **error bars**: lines which extend above and below the plotted point. Commonly, the error bars represent one standard deviation above and below the point, but they could also represent two standard deviations (the 95% confidence limit), another statistical measure such as the standard error of the mean, or even the whole range of the data from high to low.



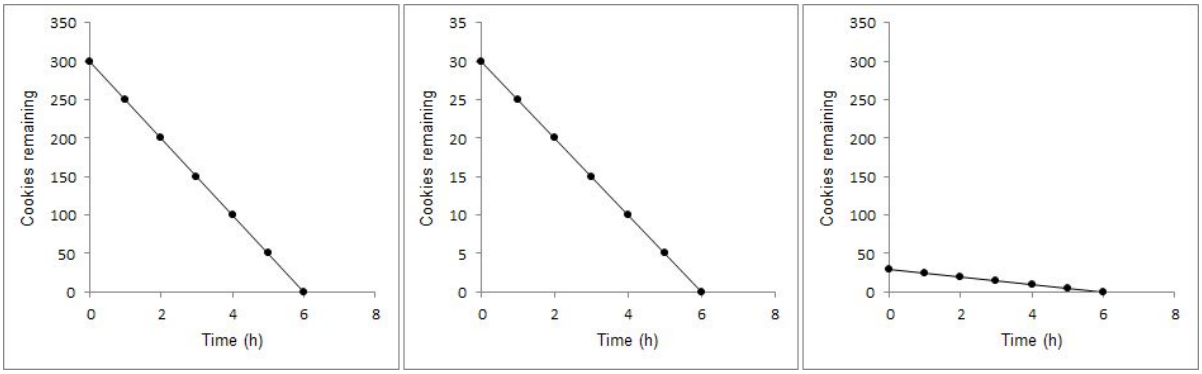
For our example, we'll use the standard deviation that is shown back in the original data table. To add error bars, click to highlight the data series, then click Error Bars on the Analysis palette of the Layout menu. It *looks* like you should choose Error Bars with Standard Deviation, but don't: this will result in every data point being given the *same* error bar, rather than showing the standard deviation for *each* point. Instead, choose More Error Bars Options and in the resulting dialog, be sure the radio button for Both positive and negative error bars is checked. Then click Custom at the bottom of the dialog and click the Specify Values button. Now click the icon at the end of the Positive Error Value box, which will let you choose values by selecting data in the spreadsheet. Highlight the entire column of standard deviation values for the selected data series (telling Excel to use the first value as the error for the first data point in the series, the second for the second data point, etc.) Click the icon again, and then repeat the process for the Negative Error Value box using the same standard deviation values (you want the error bars to be one standard deviation in each direction). Finally, repeat the process for the other data series. The graph now looks like the one at right. (Sometimes Excel also adds horizontal error bars, due to a bug in the program; if so, just select and delete them.) Again, this provides additional information to the reader and more for you to discuss in the text: Why do you think there was more precision in the data for chocolate-chip cookies than for peanut-butter cookies?



About Scales

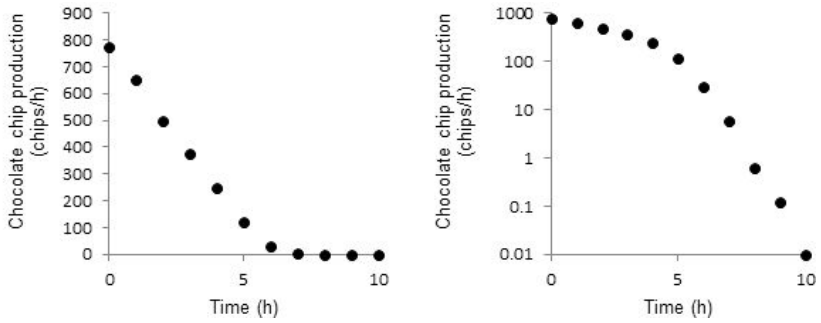
When you draw a graph, Excel tries to determine what scale should be used for the x- and y-axes, so that your data fill the whole graph. Often, this works OK, but it can create a problem when two graphs that you want your reader to compare don't have the same scale, as with the first two graphs below. These graphs make it look like cookies are disappearing at about the same rate, but look

at the scale on the y-axis! Oh, really, they're disappearing only very slowly from the second jar. You can solve this problem by putting both lines on one graph, or if you need to show two graphs, change the y-axis scales so that they're the same. Changing the scale on the second graph to match the first one, as shown in the rightmost graph below, allows the reader to see at a glance what's really going on.



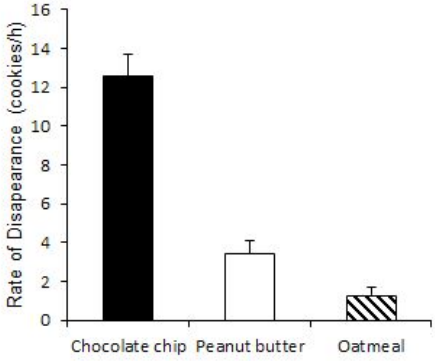
Another choice you can make is to plot your data on a **logarithmic scale**. This is very useful when you have a very wide range of data, like the data in the table at right. Plotted on a normal scale (left graph below), it looks like the last four points are zero, but they're not. Changing the y-axis to a logarithmic scale (right graph) shows this clearly. The logarithmic scale has powers of 10 spaced evenly along the axis: This has the same effect as if you took the $\log_{10}$ of all your y values. To convert to a logarithmic scale, just double-click the y-axis and check Logarithmic scale. You may also have to adjust where the x-axis is drawn.

Time (h)	Production
0	775
1	650
2	500
3	375
4	250
5	120
6	30
7	6
8	0.6
9	0.12
10	0.01



Bar Graphs

Most of the ideas above apply to bar graphs as well as to line graphs: labeling axes, formatting the graph, adding error bars, scaling appropriately, etc. You will not have to deal with trendlines or equations when drawing a bar graph, because bar graphs are used for categorical data. You should, however, think about how you will distinguish the different bars. Colored bars will often be hard to distinguish when converted to grayscale for printing. Even shades of gray may be hard to tell apart depending on the quality of the printer. It's best to use solid white bars, solid black bars, and patterns like diagonal stripes, as in the example at right. Sometimes, you don't need different colors: In this example, it would have worked just as well if all three bars were either black or white, because the labels below the bars are really enough to show what's going on.



⚠ Lab Safety

Biology laboratories have many potential hazards: flammable or toxic chemicals, pathogenic microbes and potentially injurious equipment. You will be working with considerable independence in a research-like environment, using the same tools and techniques as professional biologists. **Your safety will depend on taking hazards seriously and paying strict attention to proper laboratory practices**. You are responsible for handling chemicals, organisms and equipment safely in many different situations. You also need to carefully assess your ability to work safely and know when you need help. General guidelines for laboratory safety are discussed below; you will receive specific safety training and information as you learn to operate specific instruments or carry out specific protocols.

Key Safety Principles

North Central College's *Chemical Hygiene Plan* provides guidelines for laboratory safety, especially in chemical handling. Four key principles are outlined in that plan:

- **Minimizing exposure** of individuals to hazardous materials or situations
- **Appropriately assessing risk** in a situation and treating situations of unknown risk as hazardous
- **Providing information**, including both general safety rules and guidance for specific hazards
- **Engineering hazard control** by providing safety equipment and requiring personal protection

Think carefully about what you are doing at all times and put safety first. Assess your risk and determine necessary precautions, as well as whether you need help to complete a procedure safely.

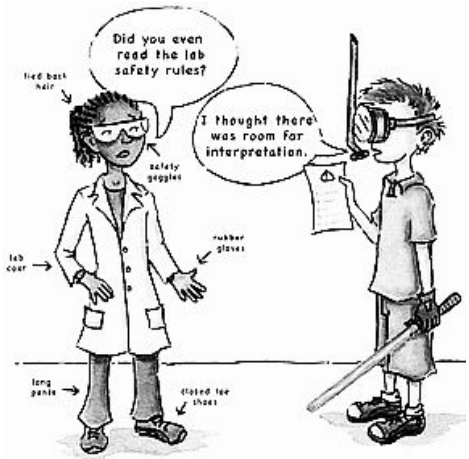
The Laboratory Environment

The laboratory itself must be a safe place where exposure to hazards is minimized for all. Students and faculty have responsibilities for maintaining a safe environment, including:

- ❏ Keeping walkways clear of coats or backpacks and ensuring accessibility of emergency equipment
- ❏ Knowing how and where to store the materials you are using and utilizing protective measure such as flammable/corrosive cabinets, chemical carriers or safety cans
- ❏ Keeping workspaces and common areas clean; disinfecting after working with microorganisms
- ❏ Using hoods and biological safety cabinets when appropriate to contain hazardous materials
- ❏ Being aware of the proximity and safety of others when using hazardous materials or equipment
- ❏ Knowing the exit routes to use in case of fire, tornado, chemical spill or other emergency
- ❏ Knowing the locations and use of emergency equipment, including eye washes, safety showers, fire extinguishers, first-aid kits, sinks, restrooms, water fountains

Personal Protection

Anyone working in a lab must take steps to protect himself/herself from hazards, including:



- ❏ Keeping <u>all</u> food and drink (even gum) outside the laboratory
- ❏ Wearing appropriate clothing: pants or skirts that are at least knee-length, shirts with sleeves and closed-toed shoes
- ❏ Keeping long hair tied back and avoiding dangling jewelry, hair ribbons, etc. that could get caught in equipment or flames
- ❏ Washing hands before and after lab
- ❏ Awareness that cell phones, notebooks, pens or other items may transfer chemicals or microbes from lab bench to hands, face or backpack
- ❏ Wearing a lab coat or apron and/or safety glasses or goggles when appropriate for the chemicals being used or procedures being performed, or as directed by the lab instructor
- ❏ Wearing appropriate gloves for handling hazardous materials
- ❏ Wearing a dust mask or respirator when working with hazardous chemical powders or aerosols
- ❏ Knowing how to find information on chemical safety in the *Chemical Hygiene Plan*

Working Safely

Good lab practices are essential to your safety and to the safety of those around you! Know how to use your equipment and materials and inform yourself about possible hazards. Pay attention to procedures and safety information found in lab manuals and this *Handbook*. Guidelines for working safely include:

- ❏ Performing only authorized experiments
- ❏ Keeping chemicals below eye level when pouring
- ❏ Using pipette bulbs or other appropriate devices for pipetting (never mouth-pipet!)
- ❏ Disposing of all lab materials according to specified procedures and minimizing chemical or contaminated waste whenever possible
- ❏ Ensuring that sufficient ventilation is available when working with any hazardous chemical
- ❏ Labeling all chemical containers and other lab materials with name or initials, contents and date
- ❏ Keeping all lab materials, equipment and chemicals within the lab
- ❏ Carefully observing any specific instructions given for the use and disposal of hazardous or toxic material, including animal tissue, microorganisms, chemicals, etc.
- ❏ Consulting with lab instructors when there is a question about proper procedures or handling

Animal and Human Experimentation

Experiments involving animal or human subjects are subject to special guidelines and review by campus committees. See the section "Working with Animal and Human Subjects" for more information.

Laboratory Access

Experiments don't often fit neatly into three-hour time frames: bacterial cultures need incubation, plants need time to grow, or animals may need to be observed over a long period. We therefore allow students to use labs outside of the scheduled time or after hours when necessary. Thus, there may be times when you and your lab partner are the only ones in lab, or even times when you work alone. The following guidelines will help to minimize the risks inherent in allowing expanded lab access:

- ❏ Limit laboratory access to students enrolled in a course or engaged in research, and work only in the labs that you have permission to access.
- ❏ Never work alone if your work includes hazardous materials, equipment or processes; ensure that someone else at least on the same floor and within hearing in this case.
- ❏ For course labs, instructors define what can appropriately be done on your own or after hours. Do not go beyond those limits. If you need to work outside of scheduled lab hours on something that wasn't specifically designated by your instructor or in your lab manual, consult your instructor first.
- ❏ If you are engaged in research, discuss with your instructor what parts of your work are safe for you to do alone or after hours.
- ❏ Never use unfamiliar chemicals or equipment when no faculty are present.
- ❏ Do not borrow chemicals or materials from areas other than the ones you are authorized to use without the consent of a faculty member or the lab manager.

Responding to Emergencies

- ❏ Any injuries must be reported to the instructor immediately, no matter how insignificant they seem.
- ❏ In case of an injury such as a cut, burn, or foreign material in the eye, the best immediate first-aid is to wash with running water for 15 minutes; use the safety shower or eye wash as appropriate.
- ❏ Report any chemical spill to your instructor. Clean up the spill as quickly as possible, but ***only*** if you know how to deal with what was spilled: some chemicals require specific precautions. See the Chemical Hygiene Plan for specific instructions for dealing with minor spills.
- ❏ Alert others to any danger, such as a major chemical spill. Close doors or take other steps to confine the incident and minimize exposure.
- ❏ Report any broken glassware or broken or malfunctioning equipment to your instructor.
- ❏ Call the Dyson Wellness Center (ext. 5550) for assistance with injuries, or dial 5911 for Campus Safety. In a life-threatening emergency, always call 911 immediately.

Biology Department Laboratory Usage Agreement

Each student enrolled in a Biology course will be required to sign a copy of this contract at the beginning of each laboratory course. Your signature on this page certifies that you have read and understood the departmental safety guidelines as outlined in the *Biology Student Handbook* and that you agree to comply with these guidelines, accepting your responsibility for your own safety and the safety of others as outlined in the handbook and below.

- I recognize the inherent risks of working in a biology laboratory, including flammable chemicals, toxins, potentially hazardous microorganisms and equipment that could cause injury if operated inappropriately. I agree to take necessary and appropriate precautions to reduce risk and to minimize my exposure and that of others to danger.
- I agree to maintain a clean and safe laboratory environment and to become familiar with safety practices, safety equipment and emergency procedures for each laboratory I work in.
- I agree to protect myself in accordance with departmental guidelines and the *Chemical Hygiene Plan*, including wearing appropriate clothing and footwear, using protective gear (e.g., eye protection, gloves, lab coats) as necessary, refraining from bringing any food or drink into any biology laboratory, washing hands and thoughtfully taking any other needed steps to minimize my exposure to hazardous materials or conditions.
- I accept my responsibility to utilize good laboratory practices, ensuring that I understand all laboratory procedures. I agree to use and dispose of chemicals and other hazardous materials according to specified guidelines and to ensure that I am operating all equipment safely and correctly.
- I will consult my laboratory instructor, another faculty member or the biology laboratory manager in the case of any uncertainty regarding the safe use of chemicals, materials or equipment.
- I will only access laboratories and use materials and equipment for which I have been granted permission and will not use materials or equipment for which I have not been properly trained.
- I will not work alone or after hours except as permitted by departmental guidelines.
- I will work with animals or human subjects only after receiving appropriate training and only in compliance with standards established by North Central's IACUC or IRB.
- I will report any injury, chemical spill, breakage or other incident as soon as possible to my laboratory instructor, research mentor or another faculty member and take appropriate steps to assist injured persons, contain the problem, warn others and (when it can be done safely) clean up.

Name (print): _____

Signature: _____

Date: _____

Working with Animal and Human Subjects

Work with vertebrate animals and especially with human subjects raises both safety and ethical concerns. North Central College seeks to comply with federal regulations intended to ensure that these experimental subjects are treated appropriately, and you as a student in a laboratory course or as a researcher will be subject to the same regulations.

Animal Research: IACUC

Every institution that is eligible for federal grant funds is required to establish an Institutional Animal Care and Use Committee (IACUC) to review proposed work done with vertebrate animals and ensure that the research complies with federal standards for ethical use and proper care of animals. The IACUC upholds key principles of experimental animal use laid out in the Policy on Humane Care and Use of Laboratory Animals published by the Public Health Service:

- Animals must be transported, cared for and used in accordance with the Animal Welfare Act
- Procedures involving animals should be designed and performed only upon careful consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society.
- Experimental animals should be of an appropriate species and quality and the minimum number required to obtain valid results. Alternative methods should be considered.
- Avoidance or minimization of discomfort, distress, and pain and use of sound scientific practices in animal experimentation is imperative. Anesthesia is required if pain is more than momentary.
- Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly killed at the end of the procedure.
- Animals' living conditions should be appropriate for their species and contribute to their health and comfort; housing, care and feeding of animals should be reviewed by a veterinarian.
- Investigators should be qualified and trained for conducting procedures on living animals.

If a course lab exercise requires animals, the instructor is responsible to obtain IACUC approval. A research mentor applies for IACUC approval of animal procedures used in his/her research lab, but individual researchers in the lab will require specified training. A student wishing to carry out an independent project involving animals will have to seek IACUC approval before starting the project. North Central College is currently transitioning from a more general Research Ethics Committee to a fully functioning IACUC; it is anticipated that detailed information and application instructions will be available on the Web as this process is completed.

Students involved in work with animals are required to complete the Animal Care and Use module of the CITI training program subscribed to by the College. (As described in the Scientific Integrity section of this *Handbook*, all students and faculty receiving research support from North Central College are required to complete the Responsible Conduct of Research module.) You can create an account and complete the training at citiprogram.org; instructions can be found on the Resources page of the Biology Web site.

Research with Human Subjects: IRB

At every institution eligible for federal grant funds, research with human subjects must be approved by an Institutional Review Board (IRB) charged with verifying that measures taken to protect these subjects comply with federal standards. The IRB reviews all research involving human subjects, not only biomedical research but also behavioral research or research involving surveys or questionnaires. Governing principles stem from the Belmont Report and the 1974 National Research Act and include:

- Respect for the subjects involved in the research. In practice, the two primary concerns resulting from this principle are: (a) Obtaining the informed consent of the subjects–to the extent possible, they must understand the risks and benefits of the research and the possible consequences of their participation. (b) Protecting those who cannot fully consent, such as children, prisoners and those of diminished capacity.
- Beneficence, or concern for the subjects' well-being. This involves minimizing risk to the participants and maximizing benefit (to society as well as the individual). The greater the risk

associated with the research, the greater its benefit must be in order to merit exposing subjects to potential harm.

- Justice: human subjects must be treated fairly, such as in the establishment of fair and egalitarian criteria for selecting subjects and for determining who will be exposed to what risk.

Any experimentation involving human subjects at North Central requires IRB approval as well as training of the students involved. Detailed information will be available on the Web as the current transition from a Research Ethics Committee to a functioning IRB is completed. Students working with human subjects are required to complete the Human Subjects Research module of the CITI training program subscribed to by the College. You can create an account and complete the training at citiprogram.org; instructions can be found on the Resources page of the Biology Web site.

Keeping a Lab Notebook

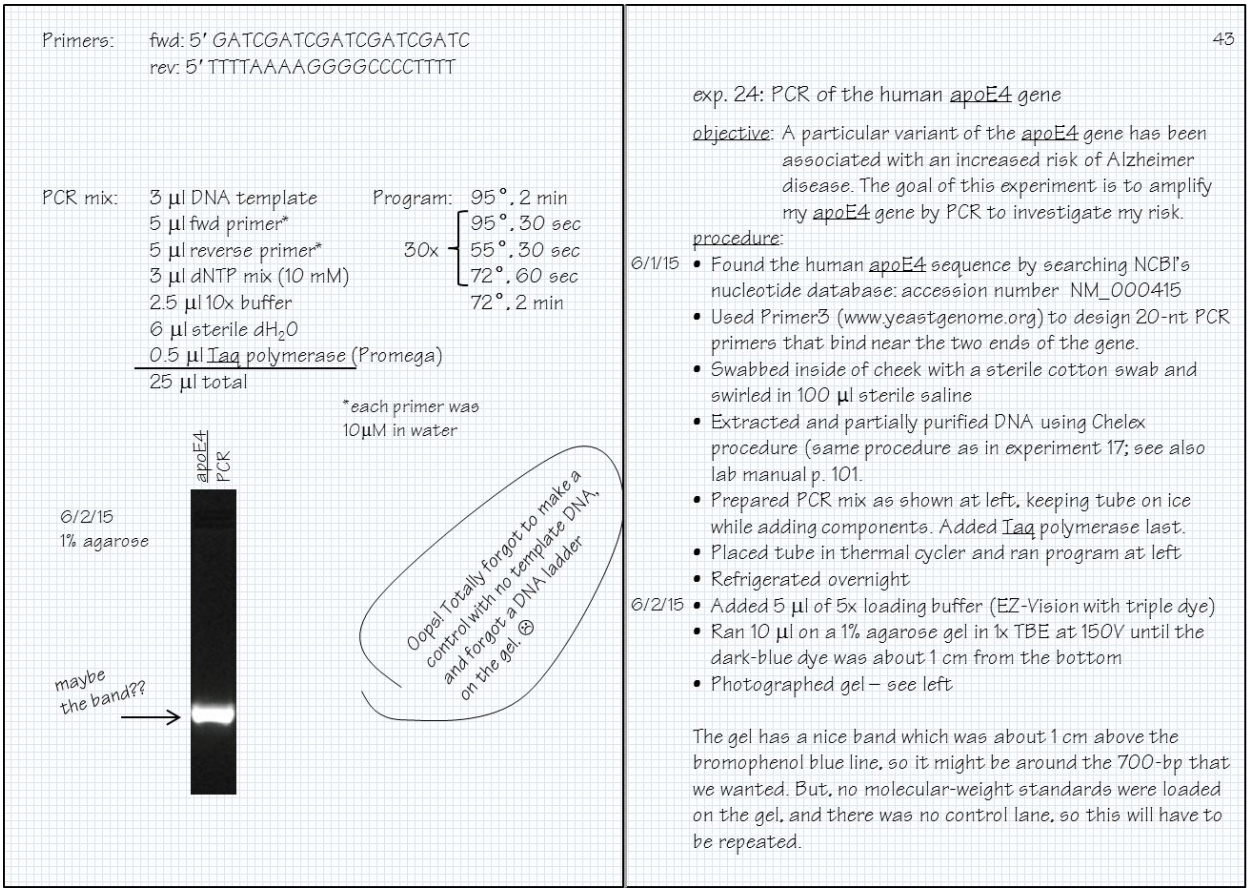
Keeping a laboratory notebook is not just a classroom exercise: notebooks are ***absolutely essential*** *to scientific research. Your notebook is the only record of what you actually did and is used in analyzing data, repeating experiments, communicating with colleagues, modifying protocols and establishing who did what when in case of a dispute.*

Organizing Your Notebook

You need a notebook for each lab course. Your notebook should be *bound* so that pages cannot be added or removed: this helps to show that it is a genuine record of your experiments. Do not use a spiral or looseleaf notebook, and do not use a carbon-copy notebook for biology courses. Your notebook should be quad ruled (that is, the pages have graph squares) so that you can easily make a table or sketch a graph. If you have a partially used notebook with enough space remaining for a new course, most instructors won't mind if you re-use it.

Put a table of contents on the first page of your notebook, and add each experiment and the page it starts on. If your notebook did not come with numbered pages, number them. Never remove pages: If you make an error, just draw a single line across the problem sentence, paragraph or page. Write legibly in ink, and record all of your data directly in your lab notebook. Do not record information somewhere else and re-copy it into your notebook later: your notebook is supposed to be a record of exactly what happened in lab. Neatness is not as important as completeness!

An instructor may ask you to set up your notebook in a particular way for a particular course. Otherwise, a good style is to start each experiment on a right-hand page with a title and objective. Then record your procedure, results, observations and thoughts on that page and the following right-hand pages. The left-hand pages are a good place to put pre-lab questions, recipes, calculations, notes, photos, etc. as shown in the example below.



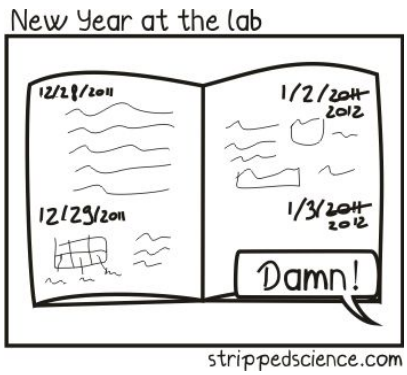
What should be in my notebook?

Your laboratory notebook is where you record all of the procedures, calculations, data and observations from your work in a lab–whether it's a lab course or a research lab. It should be legible

and complete enough that someone else could read it and repeat your experiment exactly the way you did it. When your partner wants to discuss some aspect of a lab report, you should be able to find the information you need to clarify the point in your notebook. When an experiment goes badly and your instructor asks exactly what you did, a look at your notebook should allow you to reply with confidence. You should record your data, do your calculations, make graphs, discuss the results, etc. in your notebook *before* you write your lab report—this will make it a lot easier to write a good lab report.

For every experiment you do, you should have an entry in your lab notebook. (If one experiment spans more than one lab session, it may be more convenient to record that as a single experiment.) Eac entry should include:

- A descriptive title (don't just copy the title from the lab manual; what do you think the experiment is about?), which should also be listed in the table of contents.
- A brief introduction stating the purpose or objective of the experiment. Avoid the simplistic "we will learn about yeast;" instead, consider what you expect to learn from your investigation, like "we will test the hypothesis that high temperatures slow sporulation in yeast."
- The date when each part of the experiment was done: if you come back to lab tomorrow to check plates, that gets a separate, dated entry.
- The procedure you followed.
- Observations and results (tables, graphs, photos, drawings but also verbal descriptions).
- Analysis of your data (calculations, averages, standard deviations, statistical analysis, etc.).
- The conclusions that you think you can draw from your results, ideas about what further data are needed, what you can't yet conclude, etc.



You should include enough detail about what you did that another scientist, equipped only with your notebook, could precisely repeat your experiment. When you make a solution for the first time, include the recipe (if you make it again, just refer to the experiment where that recipe is written out). If you used a procedure from your lab manual, write the steps in your notebook the first time it is used (this can also help you organize your thoughts about what you'll be doing in lab). Don't assume you'll remember a detail—you won't. When in doubt, include more rather than less…the very fact you need for your lab report or presentation may be the one you forgot to write down! Include amounts, temperatures, times and other details that will help you recall exactly what you did and interpret your results later. However, it is not necessary to copy a whole set of instructions in sentence form from a lab manual or other source: write the procedure in a briefer form, maybe as a list of bullet points.

Record all your observations—even if you don't think they're important at the time. Think about all the things you might want to know later: what color were those colonies? what was the actual temperature of the incubator? did you make a mistake that might affect your results? how did you calculate the concentration of the solution? Write down questions you had or things you didn't understand. If something goes wrong, be sure to indicate what happened and why you think it happened. Make notes about how you want to modify the experiment next time. Include equations or other calculations you used, so that you can go back later and see if you calculated correctly. When appropriate, include Excel graphs, photographs, print-outs from instruments or other attachments. Tape or staple them in securely so that they're a permanent part of the notebook—no loose sheets.

Carefully label all data. If it's a photograph of a gel, label each lane with its contents and label the sizes of the molecular weight marker bands as well as any bands whose sizes you calculate. If it's a drawing of a cell, label it with the total magnification and label any parts of the cell that you can see. Don't hesitate to use underlined headings, cross-references ("continued on p. 24") or other helps to be sure you or another reader can find everything easily.

Measurements and Solutions

Measuring specific amounts of liquids and solids and making solutions of chemicals at precise concentrations are skills you will use frequently in Biology labs—important skills to develop, because the outcome of your experiment depends on how accurately you measure.

Metric measurements

All scientific measurements are made using the metric system. If you have not yet become comfortable with the metric system, one good guide can be found at www.dummies.com. The tables below provide some basic reference information.

Metric units of measure

length	meters	m
mass (weight)	grams	g
volume	liters	l
temperature	degrees Celsius	°C
atomic mass	Daltons	Da

Basic metric prefixes and symbols

Mega-	M	1,000,000×	10^{6}
kilo-	k	1,000×	10^{3}
(basic unit)		1×	10^{0}
centi-	c	0.01×	10^{-2}
milli-	m	0.001×	10^{-3}
micro-	μ	0.000001×	10^{-6}
nano-	n	0.000000001×	10^{-9}
pico-	p	0.000000000001×	10^{-12}

Temperature scale

water freezes	0 °C
water boils	100 °C
human body	37 °C
room temp	≅25 °C
refrigerator	4 °C
freezer	−20 °C

Conversions

1 in	2.54 cm
1 m	39.4 in
1 oz (mass)	28.3 g
1 kg	2.2 lb
1 oz (fluid)	29.6 ml
1 l	1.06 qt
1 Å	10^{-10} m
°C	$\frac{5}{9}$ (°F −32)
1 ml H_2O	1 g mass

How Should I Measure This?

Measuring mass. Mass ("weight") is measured with a **balance**. But different balances have different accuracies. Most digital balances in the department have readouts that show three decimal places. These could reasonably be used to weigh out as little as 10 mg (0.01 g) of a chemical. For more accuracy, there are some analytical balances that display four decimal places and could be used to weigh as little as 0.001 g. For a quick measurement that doesn't have to be so accurate (such as when weighing ingredients for growth media), there is also a small media balance that only shows one decimal place.

- ❏ Never weigh a chemical directly on the pan of the balance. Weigh it into a beaker, a plastic weigh boat (expensive, use paper when you can), or a piece of weighing paper (folding the paper in half diagonally will provide a "spout" for easy pouring).
- ❏ Place the boat or paper on the balance pan, press the "tare" or "zero" button, and wait for the balance to stabilize at zero before weighing the desired amount of the chemical.
- ❏ Close the doors or use the draft shield if you need accuracy better than 0.1 g or so.
- ❏ Don't cross-contaminate chemicals by using the same spatula without rinsing it (many people prefer not to use a spatula in a chemical bottle at all).
- ❏ After you're done, clean up the balance thoroughly: chemicals left on the balance will corrode the balance and might create a hazard for the next user. If necessary, find out how to remove the pan and clean beneath it.
- ❏ Turn the balance off when not in use.

Measuring volume. It's important to match the measuring device to the volume you want to measure. Rule of thumb: use the smallest measuring device that will still allow you to make only one

measurement. If you have to measure more than once, the error increases with each measurement. The table at right summarizes when to use the three most common tools for volume measurement.

Volume Needed	Appropriate Tool
1 µl to 1 ml	micropipettor
1 ml to 10 ml	pipet
> 10 ml	graduated cylinder

Of course, the volume of the measuring device must fit the volume you want to measure: for example, to measure 20 ml, you'd use a 50- or 100-ml graduated cylinder, never a 500-ml cylinder. Specific guides for pipets and micropipettors are given in separate sections of this *Handbook*. Remember to read the bottom of the meniscus when making a volume measurement in a cylinder or pipette. Lines on the sides of beakers and flasks are not accurate and should never be used for measuring volumes.

Making Solutions

Molar concentrations. Recall from your chemistry classes that a **mole** is equal to the formula weight of a molecule in grams. A concentration of one **molar** (1 **M**) is one mole dissolved in one liter of water. For example, the formula weight of salt, NaCl, is 58 (check the periodic table or the label on the bottle), so 58 grams of NaCl is one mole, and a 1M solution is 58 grams dissolved in one liter of water. Notice that the terms 'mole' and 'molar' are not interchangeable! We often use rather dilute solutions in biology laboratories, so you will frequently be thinking in terms of **millimolar** (**mM**), which is 1/1000 (10^{-3}) molar. For example, a 100 mM solution of NaCl is the same as 0.1 M, or 5.8 grams per liter (0.1 × 58).

Remember that a solid chemical will add some volume to the solution. So, you would not want to measure 1000 ml of water and add 58 grams of salt to it—the total volume would then be a little more than a liter. Instead:

- ❏ Weigh out the solid and transfer it to a beaker of appropriate size.
- ❏ Add about 80% of the final volume of water and stir to dissolve the solid.
- ❏ Pour the solution into a graduated cylinder of appropriate size.
- ❏ Add water carefully until the desired final volume is reached and mix.

Percentage concentrations. For a solid chemical, percentage solutions are based on weight (mass) per total volume (**w/v**). That is, a 1% solution of NaCl means 1 gram dissolved in a total volume of 100 ml. For a liquid chemical, a percentage solution is based on volume per total volume (**v/v**), so a 1% solution of ethanol means 1 ml of ethanol in a total volume of 100 ml. We can determine the percent concentration by simply dividing the mass of the solid in grams (or volume of liquid in ml) by the final volume of the solution in ml (since for water, 1 ml = 1 g) and multiplying by 100. So, to make a 0.85% solution of NaCl (the same salinity as your blood), we'd dissolve 8.5 grams in 1 l of water: 0.85 g / 1000 ml × 100 = 0.85 %.

Absolute concentrations. Sometimes, you'll be given the desired concentration of a solution directly: for example, a 100 mg/ml solution of an amino acid. This means just what it says: for every ml of solution, add 100 mg of the solid. Again, remember that you want to deal with total volume, so to make 100 ml of this solution, you'd weigh out 10 g of powder (100 mg/ml × 100 ml), dissolve it in water and then bring the final volume to 100 ml.

Making Dilutions

Very commonly, a biologist needs to dilute a solution to a desired concentration. For example, we often make a concentrated **stock solution** of some chemical and then use it to make other solutions at different concentrations. To accomplish this, we need to think about the **dilution factor**, which is just the volume of the thing you're diluting divided by the total volume. So, if you add 10 ml of ethanol (or a bacterial culture, or orange juice, or anything else) to 30 ml of water, giving a total volume of 40 ml, your dilution factor is 10/40 = ¼ = 0.25. Or, you could say this is a 1:4 dilution.

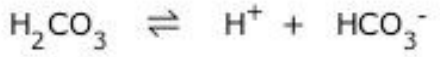
Now, realize that the dilution changes the concentration by the dilution factor. If you start with 100% ethanol and make a 1:4 dilution, you have 100 × ¼ = 25% ethanol. If you start with 2 M NaCl and make a 1:10 dilution, you have 2 × . = 0.2 M. If you start with 10^6 bacteria/ml of culture and make a 1/100 dilution, you have $10^6 \times 1/100 = 10^4$ bacteria/ml. It doesn't matter what units you are using, as long as they're concentration units: just multiply the old concentration by the dilution factor to get the new concentration.

If you're uncertain about how to set up a dilution problem, you can also use the familiar chemistry equation $C_1 V_1 = C_2 V_2$. Use C_1 as the concentration of the undiluted solution, V_1 as the volume of the undiluted solution, C_2 as the concentration after dilution and V_2 is the volume after dilution. Just be sure that the concentration and volume units are the same on both sides of the equation. For example, suppose you have a 5 M NaCl solution, but you need 100 ml of a 25 mM solution. First, make sure the units are the same: 5 M = 5000 mM, so let's make that C_1. The concentration we want, 25 mM, is C_2, and the volume we want, 100 ml, is V_2. Now we have 5000 V_1 = 25 × 100, or 5000 V_1 = 2500. Solving for V_1 gives V_1 = 2500/5000 = 0.5 ml. Therefore, 0.5 ml of the 5 M NaCl stock solution diluted to a total volume of 100 ml with water will give a new concentration of 25 mM.

Buffers: Controlling pH

Biologists almost always care about the pH of a solution, since that can have huge effects on the physiology of an organism or the activity of an enzyme. Recall from your Chemistry classes that pH is the measurement of the concentration of H^+ in a solution. Specifically, pH = -log[H^+]. Applying a little math, if a solution has a pH of 7.0, it has a concentration of H^+ of 10^{-7}M. More acidic solutions have a lower pH and a higher H^+ concentration. Conversely, more basic solutions have a pH higher than 7 and a lower H^+ concentration. There are no units for pH. Notice that a solution at pH 6 has a 10-fold higher H^+ concentration than one at pH 7!

All water-based solutions can be characterized by their pH. But most solutions quickly change pH if just a little H^+ is added or removed (notice that 10^{-7}M is a very small concentration - 100 nM!). Buffers are chemicals that we add to a solution to resist changes in pH. One common buffer is bicarbonate (HCO_3^-), due to the reaction shown at right. If extra H^+ ions are added to the solution, the bicarbonate picks them up and the reaction moves to the left, making carbonic acid (H_2CO_3). If H^+ ions are removed from the solution, then the reaction runs to the right and carbonic acid makes bicarbonate and frees additional H^+. Overall, the bicarbonate ion allows the solution to resist changes in pH.



Buffers are usually only effective across roughly two pH units. So we will use different chemicals as buffers, depending upon what pH we want our solution to be. The table below summarizes some of the most common chemicals used as biological buffers.

pH range	buffer
2.0 to 3.5	glycine
3.0 to 5.8	citrate
3.6 to 5.6	acetate
5.8 to 8.0	phosphate
7.2 to 9.0	Tris
8.6 to 10.6	glycine
9.2 to 10.6	carbonate

To make a buffered solution, begin just like you would for any other solution. Decide on the concentration (usually a molar concentration), calculate the amount of solid that you will need and dissolve it in about 80% of the final volume. Use a beaker with a stir bar and stir until fully dissolved. Then go to the pH meter. Put the beaker on the stir plate by the pH meter and let it begin stirring. Remove the cap from the probe that is attached to the pH meter and rinse it off with water from a squirt bottle. Place the probe into the stirring solution, taking care so that the stir bar doesn't strike the probe. Read the current pH of the solution. Assuming that it isn't at your target pH, add either acid (usually HCl) or base (usually NaOH) to get to the desired pH. Add just a drop at a time with continuous stirring until you get to the desired pH. Rinse off the probe with water from a squirt bottle and replace the cap. Pour your buffer into a graduated cylinder and add water until you get to your final volume (the water won't change the pH significantly).

Working with Microorganisms

Biologists frequently work with microorganisms such as bacteria and yeast. They may be directly studying the microorganisms, the bacteria or yeast may be making proteins or maintaining DNA needed for an experiment, or the microbes may even be grown as food for some other organism such as a nematode. Because these organisms are so small, we need specialized techniques to handle them.

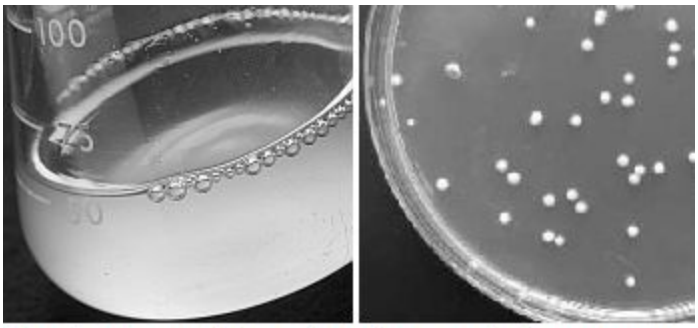
Aseptic Technique

In addition to ordinary lab safety precautions (see "Lab Safety" in this *Handbook*), working with microorganisms requires an extra layer of care which we refer to as **aseptic technique** or **sterile technique**. Some microorganisms can cause disease in humans, and some that are ordinarily harmless could possibly cause disease if ingested, injected or inhaled at high concentrations or by someone whose immune system is not full-strength. But another important reason for aseptic technique is simply that microorganisms are *everywhere*: in the air, on your skin, on every surface. Aseptic technique prevents your experiment from becoming contaminated by stray microorganisms as well as protecting yourself and others from the microorganisms you are working with. Always follow these guidelines when working with microorganisms:

- ❏ Treat all microorganisms as if they could cause disease.
- ❏ There is a wash bottle of disinfectant at your lab bench. Before and after lab, squirt some on your lab bench, use a damp sponge to spread it over the whole surface of the bench and let it dry. Rinse and wring out your sponge before putting it away.
- ❏ Wash your hands before and after lab.
- ❏ Be aware of what is sterile. Don't touch sterile pipettes or tips with your hands or let them contact any surface. Keep Petri dishes, tip boxes and bottles of sterile liquids closed when not in use.
- ❏ Pass the mouth of a bottle or tube of sterile liquid through a flame before and after using it.
- ❏ Keep all food and drink out of the lab and keep your cell phone put away.
- ❏ Know the procedures described below and carry them out exactly.
- ❏ Gloves are not needed for working with microorganisms if good aseptic technique is used.

Growing Microorganisms

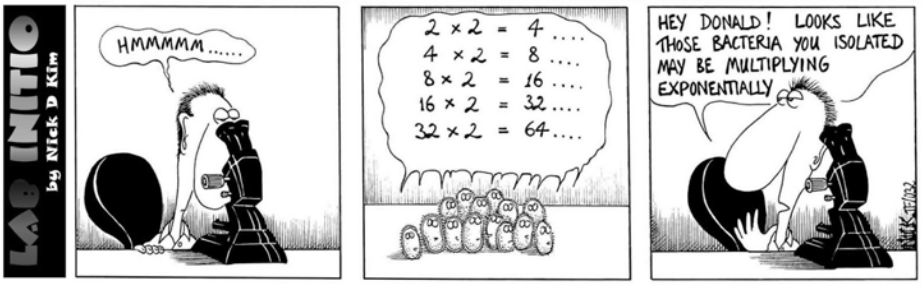
Bacteria and yeast require a **growth medium** containing nutrients. This may be a liquid **broth** in which the organisms grow in a culture tube or flask, or **agar** may be added as a solidifying agent for solid media poured into a **Petri dish** (or **plate**). In broth, the cells grow suspended in the medium, which becomes turbid (cloudy) with microbial growth as shown at right. On the surface of an agar plate, cells will stay in one place, dividing until enough cells (about 10^8 to 10^9) are present to form a visible **colony**.



Broth culture of bacteria Yeast colonies on agar

Many different growth media are available to meet requirements of specific organisms or to achieve a specific experimental goal. For general-purpose growth, **rich** media are commonly used: these contain organic molecules such as amino acids and nucleotides, allowing rapid growth. Luria-Bertani medium (**LB** medium) is a common rich medium for bacteria such as *Escherichia coli* (*E. coli*), while **YPD** medium (yeast extract, peptone and dextrose) is a good rich medium for yeast. For precise growth conditions, a **minimal** medium (such as **M9** for *E. coli*) contains only the minimal molecules the microbe needs. For example, *E. coli* can grow in a minimal medium containing only glucose and inorganic salts (sources of N, P, K, S, Mg, etc.), making its own amino acids, nucleotides and vitamins.

Environmental conditions for growth must also be considered, especially temperature and oxygen. Bacteria like *E. coli* that are adapted to live in warm-blooded organisms can be grown in an **incubator** at 37 °C (human body temperature), while other organisms may need lower temperatures, such as 30 °C for yeast. Broth cultures are usually incubated in a **shaker** to increase oxygen and speed up growth; however, some bacteria are killed by oxygen and require anaerobic conditions.



Growth media must be sterile (free of any microbial life) prior to use so that only the desired microorganisms grow. Normally, media are sterilized in the autoclave (see below) before using. Media containing heat-sensitive components may be sterilized by filtration.

Preparing Growth Media

Growth media are prepared either from a recipe listing ingredients (the *Difco Manual* available in the microbiology lab lists ingredients for hundreds of different media) or from a convenient mix purchased from a company. We usually have mixes available for the most common growth media. Solid media are prepared the same way as liquid broth, except that agar is added at a concentration of 15 g/l prior to autoclaving, and the resulting medium is poured into Petri dishes (see below). Some media mixes include agar and some do not, so be sure you know what you are working with.

Media should only be made in the media flasks found on the shelf in the autoclave room. Other flasks could contain detergents or chemical residue harmful to your cells. If you are planning to grow your microorganisms in a culture flask, you can prepare the media directly in that flask. If you are making a broth that will be used in culture tubes, dissolve it in a media flask first and then transfer it into square screw-cap bottles that you know are OK for media before autoclaving.

1. Weigh out the media mix or other ingredients. Use the designated balance for any powdery ingredients (LB mix, yeast extract, etc.) that could gum up the works of other balances.
2. Remember to include agar if you are making a solid medium (and not using a mix containing agar).
3. Pour the ingredients into a media flask of an appropriate size and add the desired volume of dH_2O (measured in a media cylinder). You usually don't have to worry about the volume of the powder.
4. Swirl the flask to get most of the powder off the bottom. You don't need a stir bar unless you plan to move the dissolved medium to a bottle: the ingredients will dissolve in the autoclave.
5. If you will be growing organisms directly in the flask, add a foam plug.
6. Cover the flask with foil and add a small piece of autoclave tape. Autoclave as directed below.
7. If you need to add antibiotics, amino acids, vitamins or other heat-sensitive components to your medium, cool the flask to 50 °C in the water bath adjacent to the autoclave before adding them. Turn on the bath when you start the autoclave, and it will be at 50 °C when your run is done.
8. Immediately after use (don't let agar harden!), rinse your flask thoroughly several times with tap water and then at least three times with dH_2O. Return it to its shelf. Media flasks never go through the dishwasher and should not be left in the dishwashing area or on the drying rack.
9. Turn off the 50 °C water bath if you used it.

Using the Autoclave

The autoclave works like a pressure cooker: it uses steam under pressure (15 psi) to raise the temperature above the boiling point, reaching 121 °C. After 15 minutes at this temperature, media, glassware or instruments are **sterile**: free of any living organisms, including highly resistant bacterial spores. We usually autoclave for 20 min. to ensure that everything in the autoclave reaches 121 °C and stays there for a full 15 min; you can increase the time if you have large volumes of media.

1. Check the jacket pressure gauge on the lower part of the autoclave: it must read at least 15 psi. If not, either someone recently turned the machine on and you need to wait for it to pressurize, or there is a problem: consult a faculty member for help.
2. Place the materials in the autoclave. Dry items (pipet tips, empty glassware, etc.) can be placed directly on the wire autoclave racks; liquids must always be on a tray.

If you are autoclaving any liquids, ensure that they are in containers filled no more than half full. When the pressure comes down at the end of the autoclave cycle, over-full containers can boil over and spill or crack the container. If you are using a screw-cap bottle, the cap must be loose: tighten it hand-tight and then back it off ¼ turn.

3. Be sure there is a piece of autoclave tape somewhere on the materials being autoclaved. The markings on the tape will turn dark when the autoclave reaches the correct temperature. If the tape fails to turn dark, something went wrong and your materials are not sterile.
4. Close the door and turn the wheel to extend the metal flanges to hold it shut under pressure. Keep turning until it is tight.
5. Choose the desired cycle by pressing the appropriate button.
6. Set the desired sterilizing time (usually 20 min) on the dial on the upper right. Set the drying time if using the dry cycle.
7. Press the start button. You should see an immediate increase in chamber pressure and a slower increase in temperature.
8. When the cycle is complete, a buzzer will sound. Check that the pressure gauge is at zero, then slowly open the door. A small amount of steam will escape.
9. Use autoclave gloves (be sure they're not wet!) to remove your items. Use caution, as they are extremely hot.
10. Clean up any spills from the autoclave trays or the bottom of the autoclave. Rinse the tray if it has agar or other media on it.

The fluid cycle must be used any time you are autoclaving liquids, even if you're also autoclaving dry materials. In this cycle, the pressure is released slowly after sterilizing, reducing boilovers. If all of your materials are dry, you may choose the fast cycle, which releases the pressure rapidly at the end, or the dry cycle, which releases the pressure fast and then puts the contents under vacuum for the amount of time you specify to help dry the items.

If you hear whistling or see steam escaping around the door, the door is not tight enough; tighten it quickly. A "pop" sound as the autoclave starts is normal.

Always double-check the pressure gauge even though you hear the buzzer. It would be extremely hazardous to open the door if there is still pressure in the chamber.

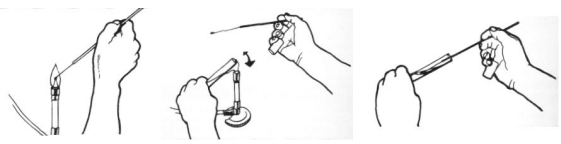
Pouring Plates

For most uses, we use disposable plastic Petri dishes which come in pre-sterilized stacks of 20 sealed in plastic bags. You need 25 ml of agar-containing medium per plate.

1. After autoclaving, cool your flask to 50 °C in the water bath (see above). At this temperature, the agar will still be liquid, but it won't be too hot to handle.
2. Add any heat-sensitive components such as antibiotics, amino acids or vitamins.
3. Swirl the flask thoroughly before use to ensure complete mixing, but try not to create a lot of bubbles. Melted agar is heavier than water, so if you don't mix thoroughly, your first few plates will have too little agar and your last few will have too much.
4. Spread out your plates on a benchtop and label them on the *bottom*, not the lids.
5. Open one plate at a time and pour in enough agar that the liquid will just cover the bottom on its own without swirling. This should be about 25 ml. If you look at the plate from the side, there is a line about halfway up the bottom half of the plate; you should have about this much medium.
6. Wash the media flask *immediately* as directed above, so that the agar won't harden in it. Use a brush to remove any agar residue if necessary.
7. Let the plates stand until they have hardened completely, usually about 15 min.
8. Dry your plates by placing them in a 37 °C incubator for 24 hours.
9. Put your plates back in the plastic sleeve they came in, tape the top shut, label with the type of medium and the date and store the plates in the refrigerator.
10. Do not return leftover empty plates: label and store them for your own future use.

Starting a Culture in Broth

1. A wire **loop** is used to move microorganisms from one place to another. **Flame** your loop to sterilize it by holding it vertically in a flame so that the entire wire gets red hot (right).
2. Let your loop cool in the air for several seconds (it should be cool enough that you don't hear a "hiss" when it touches the agar or sample), then pick up a small amount of your bacterial sample (a colony or a loopful of broth). Only a very tiny amount is needed.
3. Remove the cap from a broth culture tube and pass the top of the tube quickly through the burner flame to reduce the chance of contamination from the air. (Just a quick pass; don't let it get hot!) Try to hold the cap in your other hand as shown so that you don't have to set it down.

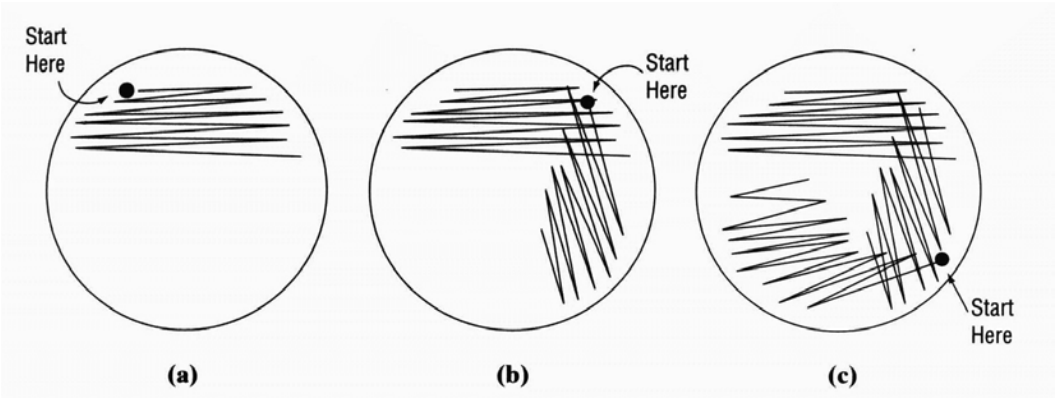


4. Dip the loop into the broth. Tip the tube so that the loop reaches the liquid without the non-sterile handle contacting the tube. Swish the loop around for a few seconds to dislodge the bacteria.
5. Pass the top of the tube quickly through the flame again and replace the cap. The whole procedure should happen fast to minimize the chance of any contamination.
6. Flame the loop again before setting it down.
7. You could also use a micropipettor and a sterile tip to transfer bacteria from one broth tube to another. Remember that only the bottom portion of the tip itself is sterile!

Making a Streak Plate

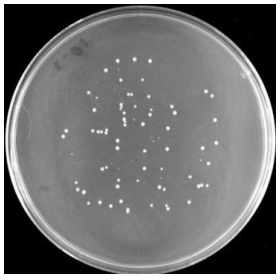
In an individual, isolated colony of bacteria or yeast on a plate, all the cells are genetically identical: they are all descendants of one original cell which landed in that spot. For this to happen, you have to spread out the starting cells on the plate so that they won't be too crowded. One way to do this is to use a **streak plate**: start with a concentrated sample and streak it out such that at the end of the streak, cells are far enough apart to form isolated colonies. On the streak plate shown at right, the first part of the streak (upper left) has solid growth where no colonies can be seen, but the last streaked region (upper right) has individual, isolated colonies.

1. Flame a wire loop and use it to pick up a colony or a loopful of a broth culture
2. Label your plate on the bottom (not the lid) with a Sharpie. Every plate should have the date, your initials and some kind of descriptive label written on it.
3. Gently streak the loop back and forth over a small area of the agar (¼ of the plate or less), as shown in figure (a) below. Make your streaks close together and hold your loop horizontally so that it glides over the agar and does not gouge into it.



4. Flame the loop again. From here on, the bacteria that you have already transferred to the plate will be diluted out so that single colonies can form.
5. Rotate the plate to make a second streak at right angles to the first. For **only** the first two or three sweeps of the loop, cross back into the first streak as shown in (b) above. Continue streaking *without* touching the first streak, spreading some bacteria from the first streak over a larger area.
6. Flame the loop again. Make a third streak as in (c) above, sweeping back into the second streak a couple of times and then spreading the material over as much of the untouched area as possible.
7. Flame the loop again before putting it away.
8. Incubate your plate upside-down (agar side up) at the desired growth temperature. This keeps condensation that may form on the lid from dripping onto your plate.

Making a Spread Plate



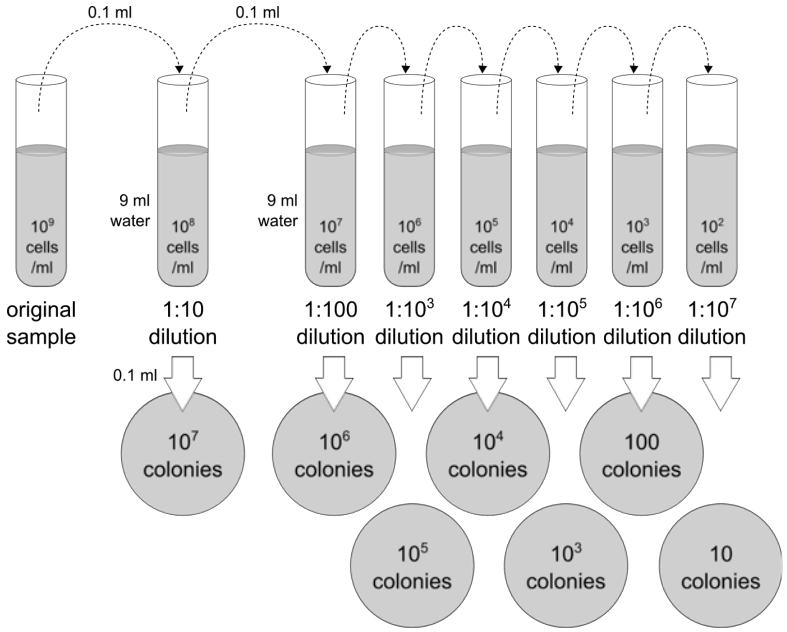
You could not count bacterial colonies from a streak plate, because they're all merged together in the heavy part of the streak. A **spread plate** (right) lets you see *all* the colonies that form from a (dilute) sample of bacteria.

1. Dip an L-shaped glass spreading rod (Dalley rod) into a jar of ethanol, then pass it through a burner flame. The ethanol will catch fire and burn off. Cool the rod in the air for a few seconds.
2. Use a pipettor to transfer the desired volume of bacterial culture to the surface of an agar plate.
3. Use the spreading rod to spread the liquid across the whole surface of the plate. A good technique is to make vertical or horizontal strokes from one side of the plate to the other while rotating the plate several times with your other hand.
4. Let any remaining liquid absorb into the agar.
5. Incubate your plate upside-down.

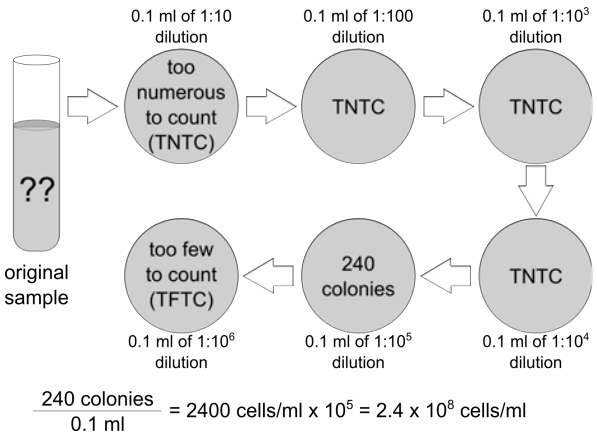
Counting Microorganisms: Viable Count

A spread plate could be used to determine the number of living microorganisms in a sample (a **viable count**), because each cell in the sample would form one colony, easily counted after incubation. But, this only works if the sample is dilute enough that individual colonies can be seen. Since cultures of microorganisms are typically very dense (a culture of *E. coli* grown overnight in a rich medium will have 10^9 to 10^{10} cells/ml), we use a **dilution series** to ensure that we can get a sample dilute enough to count colonies. A plate with between 30 and 300 colonies will give a statistically accurate count.

A dilution series starts with a dilution of the original bacterial sample; for example, 1 ml of sample plus 9 ml of diluent (typically 0.85% NaCl, which is isotonic to most cells; sometimes water or broth) gives a 1:10 dilution. Then, we make a dilution of this dilution—for example, diluting the 1:10 dilution by another 1:10 gives a 1:100 dilution relative to the original culture. This process continues until a dilution factor is reached which the experimenter is confident will contain a countable number of colonies. For example, as illustrated at right, if the original sample had 10^9 bacteria/ml, then a spread plate made with a 0.1-ml sample would have 10^8 cells—far too many to count their colonies. A 0.1-ml sample of a 1:10 dilution would have 10^7 cells, a 1:100 dilution would have 10^6, a 1:1000 ($1{:}10^3$) dilution would have 10^5 and so on. A $1{:}10^7$ dilution would have only 10 cells in 0.1 ml, too few for accuracy. So we would want to count the colonies in 0.1 ml of a 10^6 dilution: there should be 100.



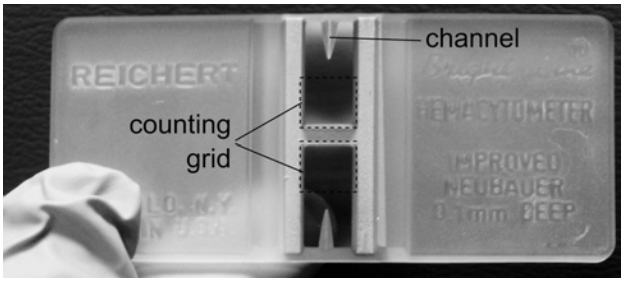
Of course, the experimenter doesn't know in advance how many cells are in the sample! So, spread plates are made from several different dilutions and whichever one gives between 30 and 300 colonies is used to calculate the number of cells in the original culture, as shown at right. The number of cells in the original culture is determined by simple math. Suppose we put 100 µl of a $1{:}10^5$ dilution of some sample on a plate and get 240 colonies. That means there would have been 2400 colonies in 1 ml of that dilution (100 µl = 0.1 ml). And, since it's a $1{:}10^5$ dilution, there are 10^5 times more bacteria in the original sample: $2400 \times 10^5 = 2.4 \times 10^8$ cells/ml. Concentrations of microorganisms are usually given as cells/ml or CFU (colony-forming units)/ml.



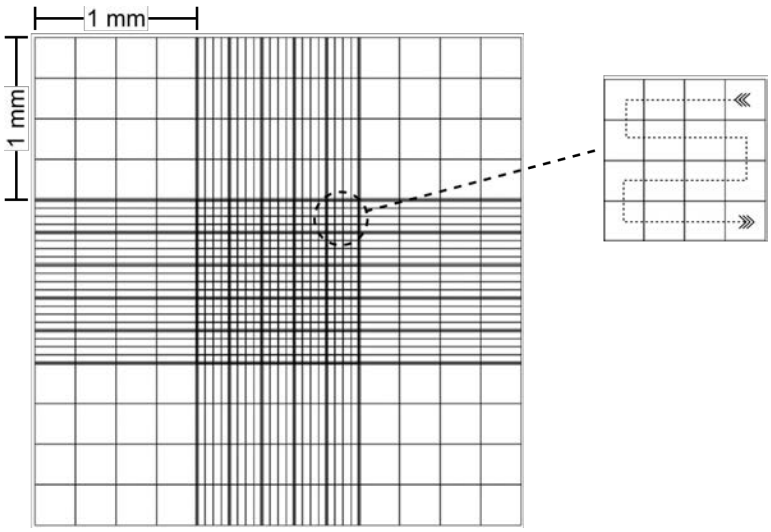
Counting Microorganisms: Using a Hemocytometer

Bacteria or yeast can also be counted using a microscope. This gives a **total count**—different from the viable count, because we can't tell by looking if the cell is alive (viable) or not: we're just counting *visible* cells. A **hemocytometer** gives a measured volume in which to count cells; it can also be used to count blood cells (hence its name) or any other kind of cells or small microscopic objects.

The hemocytometer (right) is a microscope slide with a grid precisely etched onto its surface. There are actually two grids on one hemocytometer, so that two samples could be loaded for counting at the same time.



1. Place a cover slip on the hemocytometer so that it covers the grids.
2. Transfer 10 µl of an appropriately diluted sample of the cells you want to count into the V-shaped channel. The liquid will be drawn under the cover slip by capillary action.
3. Place the hemocytometer on the microscope stage and focus on the grid at low power. ***The hemocytometer is thick so take extra care not to run the objective into the hemocytometer!***
4. You should see nine large squares (below), each of which is 1 mm on a side, or 1 mm^2 in area. The depth is 0.1 mm, so the volume of each large square is 0.1 mm^3 or 0.1 µl or 100 nl.
5. The corner squares are divided into 16 smaller squares each, and the remaining squares are divided into 25 smaller squares each. In the center square, each of the 25 smaller squares are further divided into 16 even smaller squares to allow for counting very small cells such as bacteria.



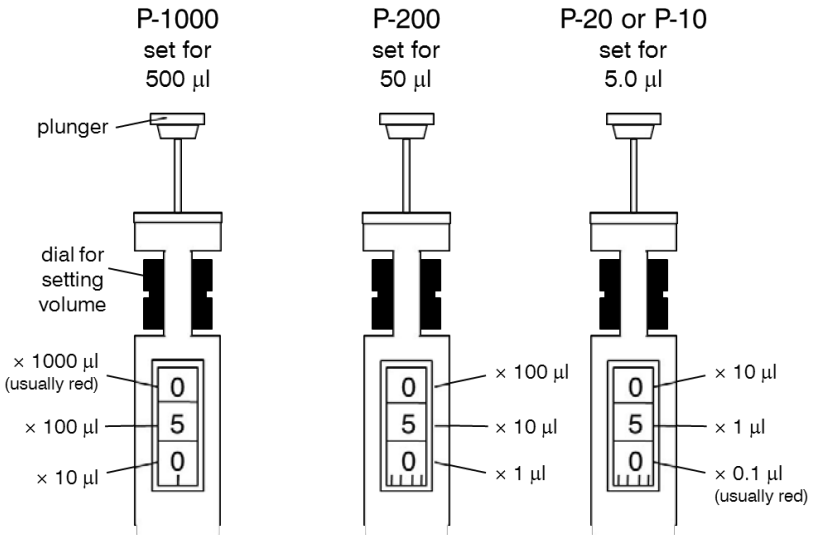
6. Decide which squares you want to use in your count. You should count at least 100 cells total and average the count of several squares for statistical accuracy. If you are counting large cells, you might want to use just the four large corner squares and the center square. For small cells, you might count several squares within the central square.
7. Center the square you want to use in the field and switch to the appropriate magnification for the cells you are counting.
8. Count cells in all the small squares within the square you have chosen systematically as shown above. If a cell touches one of the lines, count it as in the square if it touches the top or right line but out of the square if it touches the bottom or left line.
9. Divide the total number of cells you counted by the number of squares you counted and then divide by the volume of the square to get cells/ml. For example:
   - If you counted five large squares and got 196 cells, then you have 196/5 = 39.2 cells in each square. The volume of the square is 0.1 µl, so you have 39.2 cells/0.1 µl = 392 cells/µl = 3.9×10^5 cells/ml.
   - If you counted five squares within the central square and got 326 cells, then you have 326/5 = 65.2 cells in each square, or $65.2 \times 25 = 1630$ cells in the entire central square. Since its volume is 0.1 µl, you have 16,300 cells/µl or 1.6×10^7 cells/ml.
10. Remember to also account for any dilution of your original sample that you might have made before counting.

Using Micropipettors

For biology experiments, you will often need to measure volumes much less than 1 ml—in fact, as little as 1 microliter (μl), which is 1/1000 ml or 10^{-6} liter. This kind of precision requires a micropipettor.

Choosing a Micropipettor and Setting the Volume

Micropipettors only work accurately if you use them correctly! Each micropipettor is designed for accurate handling of a specific range of volumes. Our pipettors are labeled on top of the plunger button: P-20 (for pipetting 2–20 μl), P-200 (20–200 μl) or P-1000 (200–1000 μl). We also have P-10 pipettors which can pipette from 0.5 to 10 μl but are usually only used for very small volumes (2 μl or less). The volume is set using a dial which changes a digital readout on the front. The readout is different for each size of pipettor, so use the figure below to ensure you know how to read the pipettor you're using. You might want to copy of this image and tape it into your lab notebook for reference.



Testing Pipettor Accuracy

In order to measure accurately, (1) the pipettor must function correctly, and (2) you must be using it correctly. Both can be tested very simply: Water has a density of 1 g/ml (one ml weighs 1 gram), so you can simply pipette a specific volume of water and see if it has the correct mass! It's a good idea to do this to test your technique when you first start using micropipettors, and you should also do it anytime your pipettor seems to be behaving oddly or might not be giving the right volume. The seals inside need to be replaced periodically, and a malfunctioning pipettor can ruin an experiment.

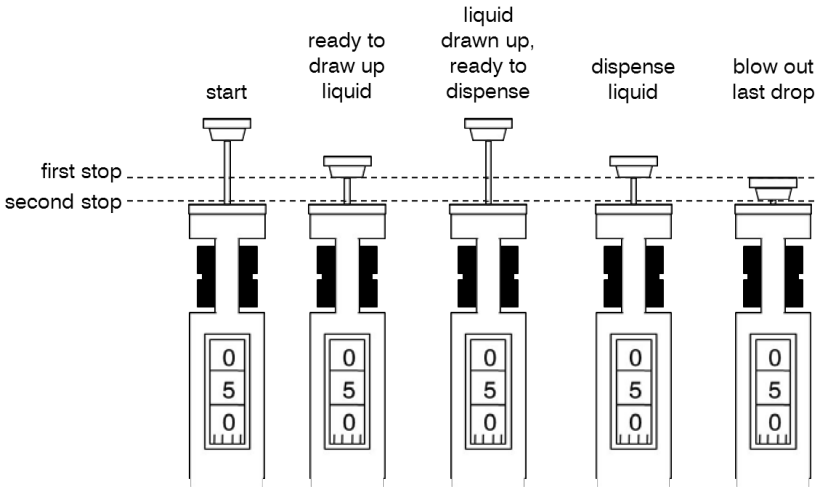
To test your pipettor, set it to the volume indicated in the table below, pipette water onto a piece of weighing paper and weigh it to determine the *actual* volume. Repeat the measurement at least three times (six is better). Then determine the accuracy of your pipettor by averaging the actual volumes and the precision by taking the standard deviation. The table below shows the expected accuracy and allowable error range for each type of micropipettor. If your pipettor is not measuring accurately, tell your instructor, who can either do the needed maintenance on the pipettor or help you see what you might be doing wrong. Do not just go on measuring inaccurately!

Micropipettor specifications			
Pipettor	Volume to test	Accuracy (avg. volume)	Precision (std. dev.)
P-1000	500	500 μl ± 4 μl	≤ 1 μl
P-200	100	100 μl ± 0.8 μl	≤ 0.12 μl
P-20	10	10 μl ± 0.15 μl	≤ 0.05 μl
P-10	5	5 μl ± 0.08 μl	≤ 0.03 μl

Using the Micropipettor

The micropipettor works basically like a syringe: you press down on the spring-loaded plunger to displace air from the tip and release the plunger to draw liquid in. As you push down on the plunger, you should be able to feel two "stops." You will feel the first stop as you press the plunger about ⅔ of the way down, but you can push past this stop and push the plunger to the very bottom. The first stop gives you the measured volume and is used to draw the liquid into the tip. The second stop is used only to "blow out" the very last of the liquid from the tip as shown below.

Be sure you can feel both "stops" and know their purpose. The most common reason for poor pipetting accuracy is that a student doesn't recognize the first stop.



Follow the steps below carefully for best accuracy when working with micropipettors. Even small details like how deeply the tip goes into the liquid can make a difference with these small volumes. If something goes wrong or your pipettor doesn't seem to be working, tell your instructor. Do not just throw it back in the drawer so that it affects someone else's experiment.

1. Set the dial for the desired volume by turning the black ring. If you're not sure how to read the dials, refresh your memory with the figure above.
2. Insert the end of the pipettor into a disposable plastic tip. N*ever let the barrel contact liquid directly*! Tell your instructor if liquid gets inside the barrel somehow.
3. Press the plunger down until you feel it stop (at the first stop: not all the way down!). Do this *before* you put the tip in the liquid.
4. Holding the pipettor vertically, insert the tip just below the surface of the solution (only 1-3 mm deep).
5. Release the plunger s-l-o-w-l-y. If you let it up too quickly, you'll get air bubbles that will affect your accuracy, and you might even suck liquid into the innards of the pipettor.
6. Take a look at the tip and see if the volume looks right. After a while, you'll have a good idea of what different volumes look like, and you'll recognize when there's a problem.
7. To dispense the liquid, press the plunger slowly back down to the first stop, pause, then press it all the way to the second stop to blow out the last drop.
8. Eject the tip into a tip bucket or the trash by pressing the white button behind the plunger.

Be very careful not to turn the ring too far. It should not move upward beyond the opening for your fingers. If you turn it too far, the adjusting screw will come loose, and the pipettor will have to be recalibrated. If this happens, be honest and inform your instructor—don't just leave the faulty pipettor to screw up another student's experiments!

There are three sizes of tips: large ones for the P-1000, medium ones for the P-200 and P-20, and very fine ones for the P-10. Be sure you are using the right tip with the right pipettor. The tips are usually sterile, so keep their boxes closed and don't let them touch any surfaces or your skin.

If by any chance you accidentally suck liquid into the pipettor, STOP, tell your instructor and ask for cleaning instructions. If you just pretend it didn't happen, the liquid can contaminate later samples and corrode the guts of the pipettor.

If you press the plunger after putting the tip in liquid, or if you put the tip too deeply into the liquid, or if you tilt the pipettor, you won't get good accuracy. Also, note that micropipettors are not accurate in thick or viscous liquids.

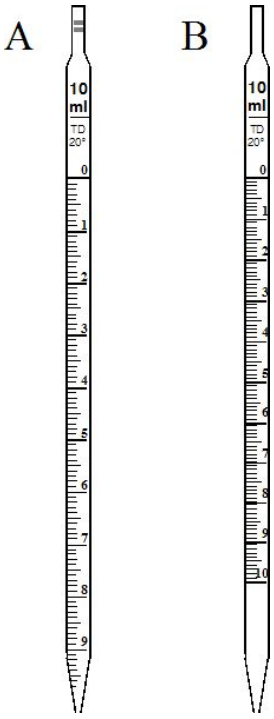
Measuring with Pipettes

Graduated pipettes made of glass or plastic are used for accurate measurement of liquid volumes. Use them for measuring volumes between 1 ml and 10 ml: For larger volumes, you would use a graduated cylinder, and for smaller volumes, a micropipettor is more convenient.

Several kinds of pipette fillers are available, from simple bulbs to fancy electric pumps. In Biology labs, you'll most often use a Pipet-Pump® filler which is both easy to use and reliable. This is a plastic tube which fits over the blunt end of the pipette and has a plunger sort of like a hypodermic syringe (left). To fill the pipette, insert its tip into the liquid and use the wheel to raise the plunger. Go slowly, so you don't get bubbles. Remember to read the bottom of the meniscus of the liquid to ensure an accurate measurement. To empty the pipette, simply press the plunger back down. Some Pipet-Pumps have a lever on the side which you can press to let in air and allow the pipette to empty. One problem you may have with the Pipet-Pump is how to blow out that very last drop from the pipette. Here's a hint: don't start with the plunger all the way down. Raise the plunger ½" or so before you put the tip in the liquid. That little extra distance will allow you to expel the last drop.

For your own safety, **never mouth pipette**—that is, never use your mouth to suck liquid into the pipette like a straw. This is a good way to fill your mouth with an undesirable chemical or a concentrated bacterial culture! Always use a pipette filler!

You will encounter two main types of pipettes in Biology labs (right). Most are the type called **serological pipettes**. These are graduated all the way to the tip (A). For example, if you look at a 10-ml serological pipette, there will be a line at the top marked zero. When you fill the pipette to this line, it contains 10 ml. Below this line, there are ten smaller, unmarked graduations, representing 0.1 ml each, and then a line marked 1 ml. Below that are more 0.1 ml graduations and marked lines for 2 ml, 3 ml, etc. The last marked line will be 9 ml, but there will be 0.1-ml marks below that and down on the tip to indicate that to dispense the full 10-ml volume, you have to go all the way to the tip.



The other type of pipette (B) is a **measuring pipette**. Notice that this one isn't graduated all the way to the tip, and there's actually a line for 10 ml. That means that if the pipette is filled to the zero line, it actually contains *more* than 10 ml, and to dispense 10 ml, you don't empty the pipette completely but instead just let out fluid until it reaches the 10-ml line.

In order to measure the volume you want, you may have to do a little mental math. Suppose you need 8 ml of water. You could fill the pipette all the way to the zero line and then dispense 8 ml by letting the water out until it reaches the 8-ml line. However, you then wind up with 2 ml extra in the pipette, and you have to either put it back (not a good idea if you're dealing with sterile solutions) or discard it. For a serological pipette, a better way would be to fill the pipette to the 2-ml line and dispense all the way to the tip: no waste. Some pipettes have a second scale that runs in the reverse direction to make this easier. You can use whichever scale is more convenient.

Some students seem to think that micropipettors are so much more accurate that they would rather measure 3 ml by using a 1-ml micropipettor three times than by using a 10-ml pipette. Not so! In fact, any time you have to make multiple measurements, accuracy decreases dramatically. Use the 10-ml pipette and just measure once. Also, think about efficiency: if you have to measure 10 1-ml volumes, it will be much faster to fill a 10-ml pipette and dispense 1 ml into each tube than to use a micropipettor 10 times.

Laboratory Materials and Citizenship

The biology laboratories, both teaching and research, are shared space, used by multiple courses and students. Each time a lab user sits down at the bench to work, he or she should find that bench and the lab as a whole in good shape, with all equipment working and in its place, glassware clean, supplies where they belong, etc. The life-science laboratory manager and student laboratory assistants ensure that the right materials are available for each lab, but it is the job of ***all*** *laboratory users—faculty, staff and students—to be good lab citizens and ensure that our laboratories are good working environments.*

Tips for Good Citizenship

- ❏ Leave your lab bench as you found it. Pay attention to where materials are located (in drawers, on the benchtop, in a common supply area, etc.) and return them there.
- ❏ Most labs have drawers or bins for individual lab pairs. Use *your* materials and don't take *anything* from someone else's drawer or bin without your instructor's permission.
- ❏ If something is running low or you are about to take the last one of something, notify your instructor or lab assistant. Don't just leave it for the next person to find.
- ❏ Refill wash bottles or replace other consumables if you know how to do so (find out how if not).
- ❏ Don't remove anything from your lab without permission—another lab may need it! If you do get permission to borrow something, be sure to return it promptly.
- ❏ Most lab supplies are organized in some way: chemicals are alphabetical, beakers are sorted by size, etc. Be observant! Recognize and respect the organization system.
- ❏ If you are working with sterile materials and think you might have contaminated something, let your instructor know. Don't let the next person find out the hard way.
- ❏ If you're working with bacteria, always wipe down your bench with disinfectant both before and after your lab. Rinse your sponge before putting it away.
- ❏ Label everything—never place an unlabeled tube or plate or bottle in a refrigerator or incubator or drawer. Unlabeled materials may be discarded without warning.
- ❏ Promptly remove materials from incubators, refrigerators, freezers or other common areas as soon as they are no longer needed. Check to see that you've cleaned up everything at the end of each course.
- ❏ If you need something or don't know how to use something, ask! Don't "just try it" or assume you know what you're doing. It only takes a minute to get help that could save an experiment.

Locations of Materials

Beakers, flasks, graduated cylinders, bottles	South end of back hall
Media flasks and cylinders (for growth media)	Shelves in SC224
Dry chemicals	Cabinet at south end of back hall
Liquid chemicals	Cabinet on north side of SC 222
Refrigerated/frozen chemicals	Refrigerator or freezer in SC222 unless course/lab specific
Flammable chemicals	Cabinet at back of SC222
Media ingredients	Cabinets in SC224
Petri dishes	Back hall behind SC222
Stir bars, spatulas, weighing paper	Drawer below balances in SC224 or near other balances
Centrifuge tubes and bottles, adaptors	Shelf above the centrifuge in SC222
Sterile, disposable 15/50-ml centrifuge tubes	Cabinet on north side of SC222
Pipette tips, microcentrifuge tubes	Cabinets on north side of SC222
Culture tubes (for growing bacteria/yeast)	Drawer on south side of SC222
Electrophoresis equipment	Cabinet on south side of SC222 or east side of SC216

Water

Tap water contains many minerals and salts; it is useful for handwashing and initial dishwashing but rarely used in biological experiments. These minerals and salts have been removed from **deionized water** (dH_2O), which is obtained from the reservoir in SC 224 (the autoclave room); this is used for most experiments as well as for a final rinse after dishwashing. Very high quality **Ultrapure water** ("Milli-Q water") is available in the biochemistry lab (SC 112) for experiments that require extremely low concentrations of trace elements. None of these water sources are sterile, so the water may contain bacteria, algae, spores, etc. If sterile water is required for an experiment, it must be autoclaved. Bottles of **sterile water** are usually labeled specifically. Think about the water you are using: water that has been sitting around the lab in a wash bottle will not be as clean as water fresh from the reservoir, and a bottle of sterile water opened and used by someone else may no longer be sterile.

Dishwashing

Dirty glassware or glassware with chemical residue can ruin an experiment. However, soap residue can also be problematic, especially for glassware in which living things are grown. So, the principle is to use the least harsh cleaning method that will result in glassware clean enough to use in experiments. Almost all glassware is rinsed with dH_2O after washing. We have a dishwasher that will do a thorough tap water rinse followed by a dH_2O rinse; use the dishwasher only with the specified detergent. Glassware that can go through the dishwasher can be left in the appropriate bins in SC224; for lab courses, your instructor may place bins for dirty dishes in your lab.

beakers, flasks, bottles	rinse; brush if needed to remove residue, place in bins for dishwashing
graduated cylinders	rinse with tap water (brush/soap only if needed), 3× dH_2O—no dishwashing
media flasks & cylinders	tap water rinse (no soap; brush if needed), then 3× dH_2O—no dishwashing
stir bars and spatulas	rinse 3× with dH_2O and return to drawer—no dishwashing
slip-cap culture tubes	disinfect (below), then discard tube; place cap in bins for dishwashing
screw-cap culture tubes	rinse well, brush if needed; place in bins for dishwashing
test tubes	rinse well, brush if needed; place in bins for dishwashing
agar plates	place in autoclave bags
microscope slides	discard cover slip in broken glass; wash with Bon Ami, rinse with dH_2O
glass pipettes	place in pipette jar for washing
plastic pipettes	discard in trash
caps and lids	separate from bottles
microcentrifuge tubes	discard in tip/tube buckets
pipette tips	discard in tip/tube buckets
electrophoresis equipment	rinse with dH_2O

Broken Glassware

All broken glassware should be discarded into a designated broken glass container. Full, closed containers can be left in the hallway with a sign indicating they are to be discarded. Notify your instructor if you break something, and let him/her know if there was a resulting spill of contaminated or hazardous material (e.g., bacterial culture, mercury from a broken thermometer) that will require special clean-up. Notify your instructor immediately of any injury; call 911 if the injury is serious.

Bacterial Cultures

Bacterial cultures should be disinfected before discarding. For broth tubes, add a squirt of 10% bleach, wait 5 min and then discard the liquid down the sink. Used agar plates are discarded in

autoclave bags. Don't put anything except plates in these bags unless you are specifically directed to do so! When the bag is full, tie the top, autoclave it (liquids cycle) and discard in the trash.

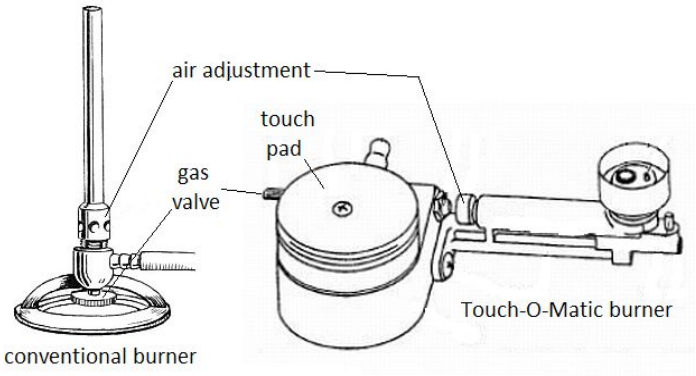
Balances and Weighing Supplies

Keep the balance area clean! Chemical or media residue gums up the balance. Brush the balance pan and the area around the balance immediately after use (exercise caution if the material being weighed is toxic or hazardous; don't brush this onto the floor or leave residue in the brush for others). Use a wet paper towel for more serious clean-up; rinse the balance pan with dH_2O if necessary. Turn off the balance after use to conserve power.

Plastic weigh boats work well for larger amounts of chemicals but are expensive; for small amounts, use a piece of weighing paper creased diagonally. Spatulas don't go through the dishwasher: when you're done with one, rinse, dry and return it. Avoid cross-contaminating chemicals: rinse and dry your spatula between two different chemicals you may be weighing. When possible (i.e., when the chemical will pour easily without raising too much dust), weigh chemicals without using a spatula.

Bunsen Burners

Two kinds of Bunsen burners are used in the building: the conventional type and Touch-O-Matic burners (right). For both types, turn on the gas fully (the handle of the gas valve should be parallel to the nozzle). Press and turn the touch plate on the Touch-O-Matic burner. Then use a striker to light the flame. You should only have to flick the striker once or twice—do not keep flicking it repeatedly, as this just wears down the flint. If the burner does not light right away, *turn off the gas* and see what the problem is. Usually, either someone has shut off the gas at the valve on the burner (open the valve) or the flint on the striker is worn down (ask your instructor for a replacement). After correcting the problem, try again.



The height of the flame as well as the amount of air mixing with the gas in the burner can be adjusted; you want a blue (not yellow) flame about 7-12 cm high. The Touch-O-Matic burners allow you to switch from full flame to a small "pilot light." With the flame lit, twist the touch pad until it pops out to switch to the pilot light. This is useful if you frequently need to flame a loop or the top of a tube (e.g., in working with bacteria), so that you don't have to keep shutting off your burner and re-lighting it.

Natural gas is a major laboratory hazard, so ensure that the gas is fully off when you have finished using a burner. Report any natural gas odor that might indicate a leak to your instructor.

Laboratory Access

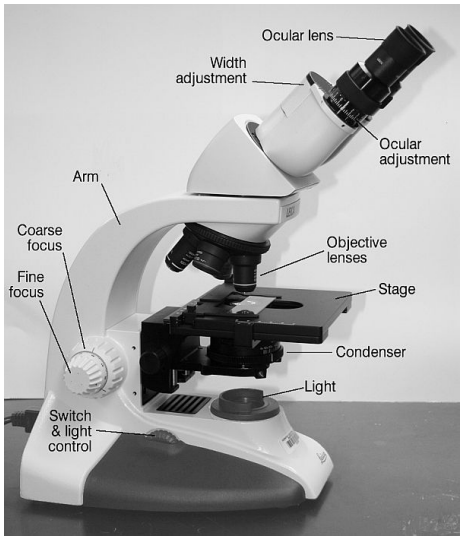
- ❏ During building hours (posted on the doors of the Science Center), your student ID will give you access to the building via the card reader on the south entrance.
- ❏ Except for evening laboratory courses, access to a laboratory after 6 p.m. requires permission.
- ❏ If you are taking a course which allows you to use a laboratory after hours, your instructor will include your name on a list accessible by Campus Safety. Call Campus Safety (637-5911 or 816-5447 for the on-call officer) for access to the designated laboratories (or to the building after building hours).
- ❏ Faculty members will add their research students to the access list as appropriate. Only research students can be issued laboratory keys; these students should not use their keys to give other students access to laboratories after hours.
- ❏ All after-hours laboratory use is subject to the guidelines described in the "Lab Safety" section of this *Handbook*. Instructors and mentors will designate types of experiments that can be done after hours. Students should never work with hazardous materials alone or after hours.
- ❏ All chemistry facilities are closed to student use from 5 p.m. to 8 p.m. There is no access, even by permission, after hours. Plan accordingly if you need these facilities.

Using Microscopes

Microscopy is a key biological technique: nearly every field of biology uses microscopes in one way or another. Using the microscope can be a very enjoyable experience or a very frustrating one; understanding how to use it well (plus a little practice) will make it a good experience.

Types of Microscopes

The microscopes you will use are **light microscopes**, meaning that they use lenses to focus light and produce an image of the object. Light microscopes can magnify an object about 1000×. Lenses that magnify more can be made, but the problem is insufficient **resolution** (ability to distinguish two objects close together). To magnify even more, an **electron microscope** focuses a beam of electrons rather than light.



To achieve a higher magnification than is possible with a single lens (like a magnifying glass), **compound microscopes** use two lenses. The object is first magnified by the **objective** lens, and that enlarged image is then further magnified by a second lens, the **eyepiece** or **ocular** lens. The total magnification is calculated by multiplying the magnification or "power" of the ocular lens by the magnification of the objective lens: a 40× (so if you're using a 10× objective, the total is 100×).

The biology department has two types of high-quality Leica microscopes for general use (right). These are binocular microscopes (two eyepieces, for ease of viewing) with 10× ocular lenses. The CME microscopes have three objective lenses: 4×, 10×, and 40×. For higher magnification, the DME microscopes add a fourth objective, a 100× oil immersion lens, and their 40× lenses can be used with phase-contrast. For lower-power views of larger specimens, you will use a dissecting microscope (stereoscope). The department also has fluorescence microscopes and a DIC microscope described later in this section.

Microscope Care

Please remember that microscopes are expen$ive and treat them with care:

- When moving the microscope, hold it by the arm and the base, using *both hands*.
- Plug in the microscope so that its cord is out of the way, so it won't get pulled off the table.
- Always start with a low-power (4× or 10×) objective lens in place and with the objective raised as far from the stage as possible.
- Use only lens paper—never a Kimwipe, Kleenex or paper towel—to clean the lenses. If cleaning beyond a simple wipe is required, ask your instructor about a safe cleaning solution.
- Put the microscope away with the lowest power lens in place. Be sure you haven't left a slide on the stage and that any oil has been removed from the oil-immersion lens with lens paper.
- Cover the microscope with its dust cover.

Basic Microscope Use

1. Plug in the microscope and turn on the light with the switch on the base. Set the illumination to its lowest setting.

I passed all the other courses that I took at my university, but I could never pass botany. This was because all botany students have to spend several hours a week in a laboratory looking through a microscope at plant cells, and I could never see through a microscope. I never once saw a cell through a microscope.

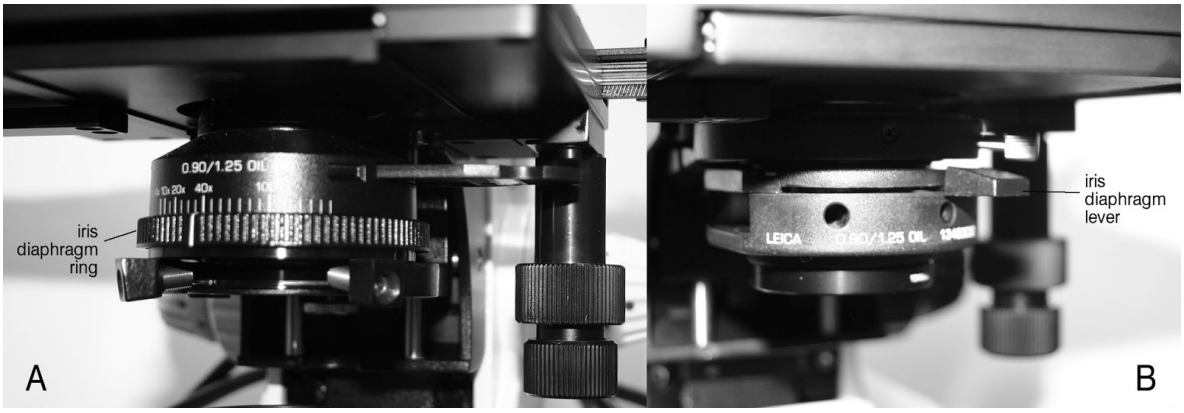
This used to enrage my instructor. He would wander around the laboratory pleased with the progress all the students were making in drawing the involved and, so I am told, interesting structure of flower cells, until he came to me. I would just be standing there. "I can't see anything," I would say. He would begin patiently enough, explaining how anybody can see through a microscope, but he would always end up in a fury, claiming that I could too see through a microscope but just pretended that I couldn't.

"Well," he said to me, cheerily, when we met in the first laboratory hour of the semester, "we're going to see cells this time, aren't we?" "Yes, sir," I said. "We'll try it," the professor said to me, grimly, "with every adjustment to the microscope known to man. As God is my witness, I'll arrange this glass so that you see cells through it or I'll give up teaching."

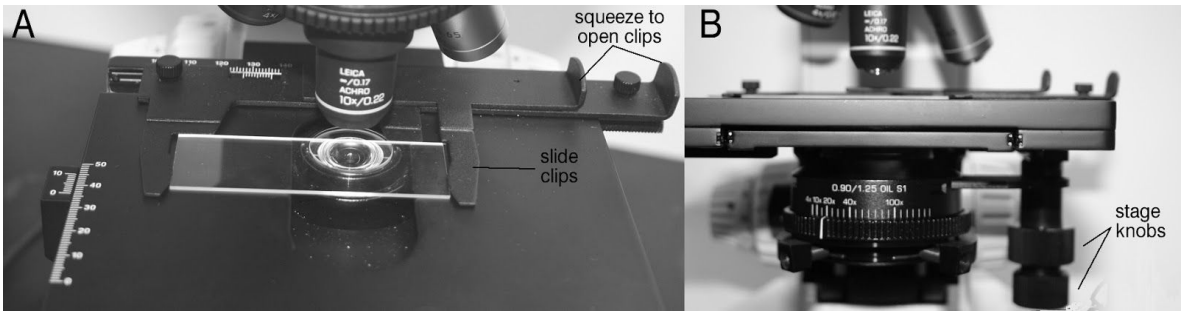
So we tried it with every adjustment of the microscope known to man. With only one of them did I see anything, and that time I saw to my pleasure and amazement, a variegated constellation of flecks, specks, and dots. These I hastily drew. The instructor, noting my activity, looked at my cell drawing. "What's that?" he demanded. "That's what I saw," I said. His head snapped up. "That's your eye!" he shouted. "You've fixed the lens so that it reflects! You've drawn your eye!"

--James Thurber, in *My Life and Hard Times*

2. Be sure the condenser is as far up as it can go. Open the iris diaphragm fully, as shown in the diagram below for the (A) DME microscope, which uses an adjustment ring, and (B) CME microscope, which uses an adjustment lever.



3. Be sure the 10× (or, if you are working with a very large specimen, 4×) objective is in place.
4. Open up the spring-loaded slide clip of the mechanical stage (see photo A below) and slip your slide into place. Be sure the slide is sitting flat on the stage and not caught on some part of the slide clips. Use the knobs under the stage (photo B below) to move the slide.



5. Looking from the side or front, ***not*** *through the lens*, use the coarse focus knob to bring the stage all the way to the top. For standard slides, the stage should stop before the slide hits the lens, but you need to watch to *make sure the slide does not hit the lens*, <u>especially</u> when using thick slides, well slides or hemocytometers.
6. Now, look through the 'scope and use only the <u>fine</u> focus knob to *slowly* move the stage down (<u>*away*</u> from the objective) until the specimen comes into focus. The microscope is set so that it should normally take no more than 1.5 full rotations of the fine focus knob to find the focus. If you don't see anything the first time, raise the stage again and start over, this time focusing more slowly.

> If your subject is nearly transparent, it can be hard to find the initial focus. A good trick is to move the slide so that the edge of the coverslip is visible and focus on that. This should be close to the right focus for your subject.

7. Adjust the width of the ocular lenses to match the width of your eyes. You should be able to look comfortably through both lenses and see a single image. Do not close one eye and try to look through one ocular–you will see *far* better if you learn to use both eyes.
8. If your vision is different in your two eyes (or if the microscope is adjusted for someone whose eyes were different), you can adjust the focus of the ocular lenses separately. First, close your left eye and use the fine focus knob to focus on the specimen. Then, close your right eye and use the ocular adjustment on the left lens to focus.
9. Adjust intensity of the light using the iris diaphragm.

> Light adjustment is critical! Too much light, and you'll wash out your image (especially if it's transparent). Too little, and you'll see nothing. Resolution is improved by using the smallest iris diaphragm setting that provides sufficient light. Try closing the iris until you get the sharpest image, then increasing the light intensity as necessary.

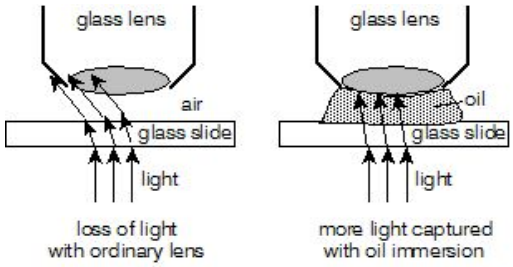
10. To change to a higher power, don't change the focus! Our objective lenses are **parfocal**, which means that once an object is in focus at low power, you can swing a higher-power objective into place and the focus should still be nearly perfect. You should only have to use the fine focus knob to make small corrections. If you lose the specimen, go back to a lower power and try again.
11. After changing lenses, adjust the iris diaphragm (higher power requires more light) and adjust the light intensity if needed, as well.

Which Lens Should I Use?

Choose an appropriate objective for what you are viewing. The lowest-power objective (4× or "**scanning**") is usually used only for very large specimens. The 10× objective ("**low power**") is good for getting an overview of the specimen, but won't show cells in detail. Usually you want to start your observations with the low-power lens because it's easy to focus. Using the 40× objective (called "**high-dry**"), cells can be seen clearly and some of their internal details can be observed; tiny organisms such as bacteria are also visible at this power. Detailed observations of bacteria or cellular structures require the 100× (**oil immersion**) objective (only found on the DME microscopes). Don't forget that the *total* magnification is the objective magnification multiplied by the ocular magnification—so if you're using the 40× objective, your magnification is 400×, *not* 40×!

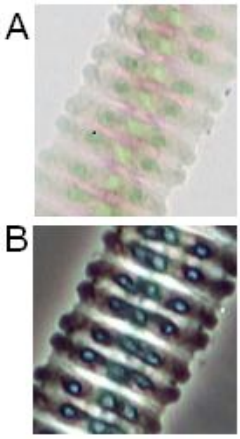
Using Oil Immersion (DME microscopes only)

At very high power, we can increase resolution by having the light travel through oil instead of through the air between the glass of the slide and the glass of the lens. Light is not bent as much when passing from glass to oil as from glass to air, as shown at right. The DME microscopes have a 100× oil immersion objective: this is the only lens that can be used with oil. It is very expensive and must be used with extra caution. **Do not get oil on any other lens, and be certain all oil is removed from the lens** (and elsewhere on the microscope) when you're finished.



1. Get your specimen in focus under the"high-dry" objective (40×).
2. Without changing the focus, swing the high-dry objective out of the way, but don't swing the oil immersion lens all the way into place yet.
3. Place one small drop of immersion oil on top of the cover slip, directly over where you see the light coming up from the condenser.
4. Slowly swing the oil immersion lens into place. It should slide into the oil drop, so that the oil fills the space between the lens and the slide.
5. Adjust the focus with the fine focus knob only. You will probably need to increase the light. Turn the knob slowly, as the focal point is very easy to miss.
6. After using the oil immersion lens, always wipe it clean (gently) with lens paper (never Kimwipes!).

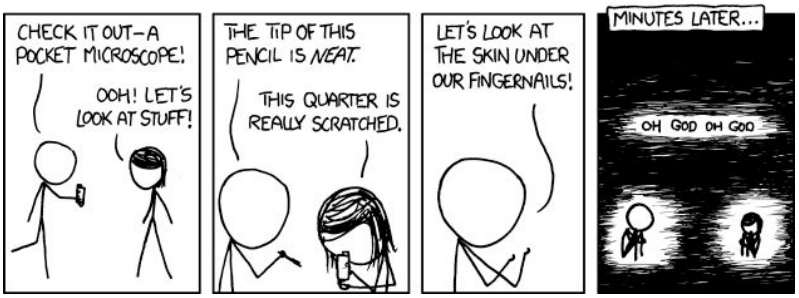
Using Phase-Contrast (DME microscopes only)



Most living, unstained cells are nearly colorless (see photo A at right). It's hard to see all their structural details because the contrast is low (makes you wonder how those early microscopists discovered the ER, Golgi, etc.!). **Phase-contrast** microscopy (photo B at right) can greatly increase the visibility of details in living cells. Cell structures refract (bend) light a little, but normally we can't see this. Phase-contrast microscopy passes the light through precisely aligned rings in the condenser and the objective lens. This makes refracted light take a longer path to your eye than light coming straight through the specimen, visibly changing the light intensity and making structures easier to distinguish.

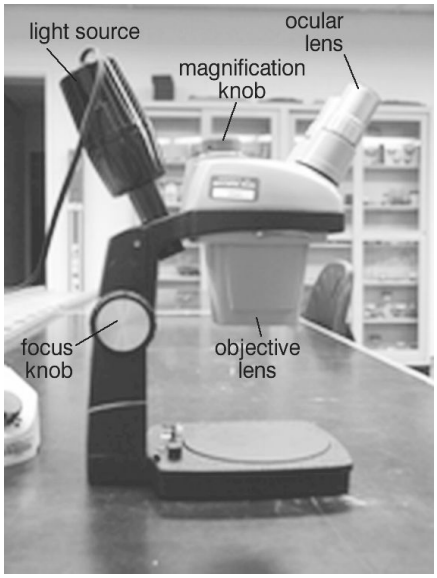
The DME microscopes have a 40× phase-contrast objective (*only* these lenses will work; don't try to use phase at another magnification). The condensers of these microscopes have a slider (shown at left) allowing you to quickly convert between normal and phase-contrast mode. You can thus easily see what illumination works best for a particular specimen.

1. Focus on your specimen and switch to the 40× objective as usual.
2. Push the phase slider gently in (toward the condenser) until it "clicks" into place. You should notice that the light intensity decreases dramatically.
3. Turn the light intensity control to its brightest setting.
4. Turn the iris diaphragm ring until the pointer lines up with the 40× mark on the condenser.
5. Observe your specimen; you should see significantly higher contrast.
6. Don't forget to slide the slider back out before you put the microscope away!



Using Dissecting Microscopes (Stereoscopes)

The dissecting microscope, also called a stereoscope (right), is the correct instrument when you want to view a large specimen like a whole animal or plant that could not be seen even at 40× magnification or that is too thick to fit on a slide. It also provides a much more 3D view. These are also compound microscopes (having both an ocular and objective lens). They have a "zoom" feature that allows you to change the objective magnification, resulting in a total magnification ranging from 5× to 40×, depending on the particular microscope you are using.

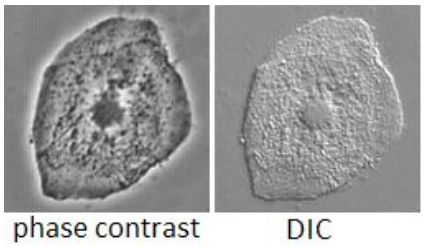


1. Determine what kind of light source your microscope uses. Some have a light source built into the stand, while others require a separate light source. The newer Leica scopes can illuminate the subject in several ways, including both transmitted light from below and reflected light from above.
2. Adjust the eyepieces to fit your eyes by moving them side-to-side until you see a single image.
3. Always start at a low power (0.5× or 1× objective) before increasing magnification. It is easier to find what you are looking for and focus on it at low power.
4. Place the specimen you want to view under the scope and focus with the focus knob.
5. Adjust the position of the specimen on the stage and the light to get the best view.

Remember that a thick specimen has many focus points which provide different views. Focus up and down to see the various focus points. Also, keep in mind that living critters move. To see them swimming in a solution, you will have to focus above the bottom of the container they are in—if you don't see anything, it's likely that you are focused on the bottom of the dish.

Using the DIC Microscope

Differential interference contrast (**DIC**) microscopy, or Nomarski microscopy, is a technique to increase contrast in unstained, transparent specimens such as living cells. It is conceptually similar to phase-contrast microscopy, but DIC splits a polarized light source into two parts which pass separately through the specimen and are then recombined before reaching the eye. This gives a sharper image than phase-contrast and allows the object's three-dimensional nature to be seen clearly, as shown in the two images of the same cell at right.



Using the Leica DM2500 DIC microscope (located in the darkroom) is not much different than the other compound microscopes in the department. However, this is a very expensive, high-precision instrument (a single objective lens can cost $4000), so please be sure you know what you are doing and treat it with extreme care. Your lab instructor will let you know if you need to use this microscope for a course lab; if you need it for your research or some other purpose, please discuss this use with your research mentor or another faculty member before using the microscope for the first time.

1. Uncover the Leica DM2500. Be sure to cover it when you are finished to protect it from dust.
2. Turn on the light with the power switch, on the front left-hand corner of the microscope base.
3. Lower the stage to its lowest point, using the focusing knob.
4. Swing the 10× objective into the front position (closest to you). Turn the silver ring on the condenser to one of the unmarked positions. The 10× objective does not have DIC or phase optics; it is just for finding your specimen and getting it in focus.
5. Place your slide on the stage, making sure it is secure with the stage clip. Now you can move the slide using the knobs hanging down from the stage.
6. While looking through the eyepiece, *slowly* raise the stage with the coarse focus (outer) knob until the slide comes into focus; use the fine focus (inner) knob to get a sharp image.
7. If you can't see anything—not even light—when you look in the eyepiece, the light might be going to the camera instead. On the left-hand side of the head of the microscope, near the eyepieces, is a metal rod: when it is pushed all the way in, light is going to the eyepieces, but when it is pulled out, light is directed to the camera.
8. The amount of light reaching the condenser can be adjusted with the dial next to the power switch. The diaphragm should be open far enough for the entire field to be illuminated, but you don't want to wash out your image.
9. The light passing through the condenser can be controlled with the iris diaphragm on the front of the condenser. Generally, a smaller aperture gives a sharper, higher-resolution image.

The microscope has three additional objectives: a 40× DIC objective, a 100× DIC objective, and a 100× phase-contrast objective (labeled Ph3 and identified by a green band). For DIC and phase-contrast illumination, the optics of the condenser and the objective must match: rotate the silver ring on the condenser until the correct label (40×, 100× or Ph3) is in front. Slide the iris diaphragm lever fully to the right when you switch to DIC or phase observations, and control the light intensity as needed with the dial on the base of the microscope.

1. To use the 40× DIC objective, swing it into place and set the condenser as described above.
2. Adjust the focus ***using only the fine-focus knob***. The lenses are parfocal, so your specimen should be almost in focus if it was focused at 10×. *Never* use coarse focus with the 40× or 100× objectives, to avoid damaging the objective. If you lose focus and can't get it back, go back to the 10× lens.
3. The 100× lenses are oil-immersion lenses and very expensive. **Read the section above on oil immersion before using these lenses**. Set the condenser as described above. Rotate the nosepiece so that the 100× lens you want to use, either phase-contrast (Ph3) or DIC, is positioned to swing into place but is not in place yet. Place a *small* drop of oil on your slide, right over where the light is coming up, and swing the lens into place. Remember that now you CANNOT go back to any other objective without cleaning the oil from the slide and the lens, using LENS PAPER ONLY (*never* Kimwipes or any other material). Adjust the focus ***using only the fine-focus knob***.

Most likely, you will want to capture digital images to document your microscopic observations. See the "Digital Photomicrography" section of this *Handbook* for more information on image capture. When you are finished with the DM2500, remove your slide, completely clean all oil off the 100× lenses (and anywhere else on the microscope it might happen to be), put the 10× objective in place and cover the microscope. If you have any difficulty using or cleaning the microscope, talk to a faculty member; do not leave the problem for the next person to discover.

Fluorescence Microscopy

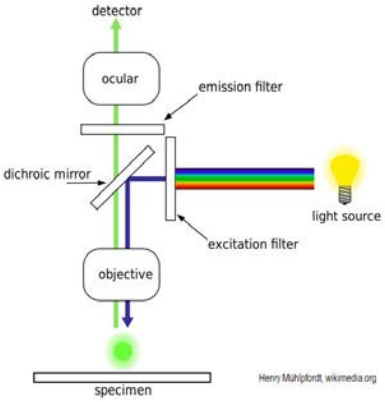
Fluorescence microscopy is currently a very popular technique; you can see many articles featuring images taken with a fluorescence microscope in current scientific journals. This technique allows for the visualization of specific structures or molecules that otherwise could not be seen

microscopically and can allow molecular processes to be investigated in a living organism or within a single living cell.

A molecule **fluoresces** because it contains atoms whose electrons are able to absorb energy from light of a particular wavelength (**excitation**) and then release a photon (usually at a longer wavelength) when they "relax" back to their ground state (**emission**). There are three general ways to make some part of a biological specimen fluoresce:

- A fluorescent molecule (or **fluorophore**) that binds to some cellular structure can be used like a dye or stain. For example, **DAPI** (4′,6-diamidino-2-phenylindole) is a molecule that binds DNA and fluoresces blue; it is often used to stain nuclei in a fluorescence image.
- Each type of **antibody** protein binds tightly to one specific antigen molecule; a rabbit or mouse can be used to make antibody specific for a protein of interest, and then the antibody can be linked to a fluorophore like **fluorescein** (**FITC**). Adding the antibody to a biological specimen will cause it to fluoresce wherever the antibody binds; this is called **immunofluorescence**.
- A fluorescent **reporter gene** can be fused to a gene or promoter, so that a fluorescent protein is made whenever and wherever that gene or promoter is activated. **Green fluorescent protein** (**GFP**) and its yellow (**YFP**) and red (**RFP**) derivatives are widely used reporters.

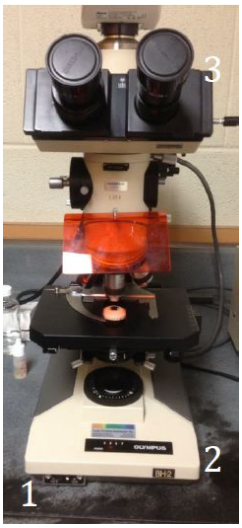
Instead of passing light through a specimen like a normal light microscope, a **fluorescence** (or **epifluorescence**) microscope directs light down onto the specimen from the top (see figure at right). This light has passed through a filter so that only light of the wavelength range appropriate for excitation reaches the specimen. The fluorophores will then absorb this light and emit photons of the emission wavelength. It is this emitted light that enters the objective and reaches your eye. (If the light passed through the specimen, you'd see the intense excitation light and not the faint emitted light.)



Fluorescence microscopes come with a variety of filters that can be used to obtain light of the correct excitation wavelength and to filter the emitted light that reaches your eye. To use a fluorescence microscope, you therefore need to know the excitation and emission wavelengths for the fluorescent molecule you are using and set the filters accordingly. The table at left gives excitation and emission wavelengths for some of the more common fluorophores.

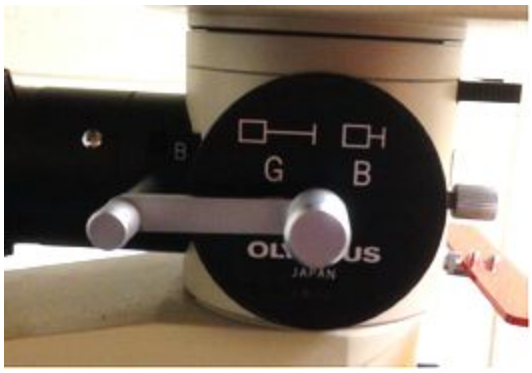
fluorophore	excitation	emission
DAPI	358 nm	461 nm
FITC	495 nm	518 nm
GFP	395 nm	509 nm
YFP	514 nm	527 nm
RFP	558 nm	583 nm
PI	490 nm	635 nm

The Biology Department has two fluorescence microscopes. The usage guide below applies specifically to the Olympus microscope, which currently (as of 2015) is the only one which has a camera to capture images (see the "Digital Photomicrography" section of this *Handbook*). The Nikon instrument is usually used for research and requires an SLR camera to be connected to it temporarily; discuss your needs with your instructor or research advisor prior to using this microscope.



1. Uncover the Olympus microscope (right). Be sure to cover it back up when you are finished to protect it from dust.
2. Turn on the microscope with the power switch, located on the bottom front left hand side of the microscope base (labeled 1 in the figure at right). Adjust the amount of light that is being transmitted with the sliding knob on the right-hand side of the base (labeled 2).
3. Lower the stage to its lowest point, using the focusing knob.
4. Swing the 10× objective into the front position (closest to you). Place your slide on the stage, making sure it is secure with the stage clip. Now you can move the slide using the knobs hanging down from the stage.
5. Remove the eyepiece covers (3 in the figure). You can slide the eyepieces closer together or farther apart depending on the distance between your own eyes. Make sure you to do this so that you are seeing one image with both eyes open. You do not want to have to close an eye in order to see the sample—this will give you a headache! While looking through the eyepiece, slowly raise the stage with the coarse focus (outer) knob until the slide comes into focus; use the fine focus (inner) knob to get a sharp image.

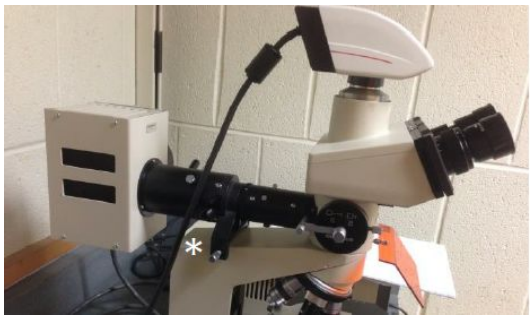
6. If you can't see anything (even light) when you look in the eyepiece, the light might be going to the camera instead. On the right-hand side of the head of the microscope, near the eyepieces, is a metal rod: when it is pushed all the way in (white stripe is visible), light is going to the eyepieces. If it is halfway out (green stripe is visible), the light is split between the eyepieces and the camera, so a specimen can be observed both on the screen and in the microscope. Pull it all the way out (red stripe is visible) to direct all the light to the camera.
7. This microscope has three additional objectives: 4× , 20×, and 100×. The 100× objective is an oil objective and must have oil on the slide. Read the section above on oil immersion before using this lens. Once you have put oil on the slide you should use ONLY the 100× objective. If you want to go back to a different objective you need to clean the oil off of your slide before proceeding.
8. If you want to use fluorescence, plug the mercury lamp into the wall to turn it on (the switch on its power supply is faulty). You must then unplug it to turn it off. It is important to make sure that the bulb inside actually turns on. In the back of the scope in the large square box is where the light should come on. If it doesn't after a few minutes, unplug and plug back in. Be careful when checking to see if the light is on, because it can get very hot!
9. We have the ability to excite our sample with any of several wavelengths of light. In order to select which wavelength to use, you need to switch the filter cube (see figure at right). In the figure, the Blue (488) and Green (594) cube is in place. When the slide is in, the sample will be excited with the blue light (B). When the slide is out, it will be excited with green light (G). We also have a filter cube with Blue and Orange and one with Ultraviolet and Violet.



10. A supplementary exciter filter can be inserted in the slot behind the filter cube to narrow the bandwidth of excitation light. This is not required but may improve your image. It usually makes the image dimmer but increases contrast by reducing autofluorescence and fading.
11. Above the cube is a barrier filter which filters the emitted light. Again, this is not required but can block unwanted fluorescence or refine the color of the image. The table below shows recommended filter combinations for several common fluorophores.

fluorophore	excitation filter	supplementary filter	barrier filter
GFP, YFP, FITC	Blue (B)	BP490	
FITC	Blue (B)	BP490	B-460 or G-520
RFP, TRITC	Green (G)	BP545	R-610
DAPI	Ultraviolet (U)	none	L-435

12. There is a slider (asterisk in the figure at right) in the light path between the mercury lamp and the body of the microscope. While setting up your slide, slide it all the way to the left to block the light from the mercury lamp, allowing you to use the normal, transmitted light. When your sample is in focus, slide the slider all the way to the right to open the path for the light of the selected wavelength to reach your objective (coming down from the top, as discussed above). Turn off the transmitted light and look carefully for fluorescence in your specimen. When you are finished, slide the slider back to the left.



Do not look directly at the high-intensity light coming from the objective! Extended periods of exposure could damage your vision. Keep the orange screen in place to block out this light.

13. If you want to take a picture of the image that you are seeing, slide the knob by the eyepiece all the way in to allow 100% of the light to go to the camera. Follow instructions in the "Digital Photomicrography" section of this *Handbook* to acquire the images using the Leica Application Suite software.

Digital Photomicrography

***Photomicrography**, or photomicroscopy, is the capturing of photographic images of microscopic objects. Today, almost all photomicrography is digital. Digital images can be captured from any of the Leica CME or DME microscopes using a Nikon SLR camera. The DIC microscope and the Olympus fluorescence microscope have dedicated Leica digital cameras.*

Using the Nikon SLR Camera

The Biology department has a Nikon SLR camera with an attachment designed to replace an eyepiece of any of our Leica DME or CME microscopes (right). Because using this camera requires removing an eyepiece, there is an opportunity for dust to get down the eyepiece tube or onto the back lens of the eyepiece or for the eyepiece to be scratched or broken. Please take extra care to protect your microscope throughout this process.

Proper **exposure** is the key to good photomicrographs (or good photographs of any kind). In a digital camera, light entering through the lens strikes an array of sensors that electronically record the number and (if it's a color sensor) color of individual photons striking them. The density of the sensors determines the resolution of the camera: a 10-megapixel camera has 10 million individual sensors, each of which will contribute one pixel to the final image. Light striking the sensors is controlled in three ways:

- **Shutter speed**: Light can strike the sensor only as long as the shutter that blocks light from coming through the lens is open. Depending on the camera, shutter speed can be set from fractions of a second (like 1/1000 sec or even less) to many seconds. The longer the shutter is open, the more photons can strike the sensor to make an image of a dim object, but long exposures also mean the camera could move during the exposure and blur the picture.
- **Aperture (*f*-stop)**: A camera's lens has an iris diaphragm much like the one in your microscope that controls the size of the aperture, the opening through which light can pass. The *f*-stop measures this size: a small *f*-stop (e.g., 2.8) represents a *larger* aperture, while an *f*-stop of 11 or 13 represents a very small aperture. A larger aperture allows a dim object to be photographed but also gives a smaller **depth of field**: when a subject is in focus, less of the foreground and background in front of and behind that subject will be in focus.
- **Sensitivity**: Before the digital camera, different kinds of film had different sensitivities to light, measured with ISO numbers. Digital cameras can increase the sensitivity of their sensors by boosting the electrical signal. If you set a low ISO, like 100, it will take more light to produce the same exposure than at a high ISO like 1600. However, there will be more "noise" in your image at a high ISO.

An **SLR** (single-lens reflex) camera allows you to look through the lens itself to see exactly what the camera is seeing, wither in the viewfinder or on a digital display (press Lv) on our camera to get this "live view"). However, because we're replacing the lens with a microscope attachment, we can't control the *f*-stop (there's no lens and therefore no iris). You will want to control the exposure using the shutter speed and the sensitivity, being aware of their limitations.

1. Find an image you want to photograph and remove the left eyepiece from your DME or CME microscope. ***If the eyepiece doesn't slide out easily, then a set screw must be loosened: ask your instructor or the laboratory manager to do this for you, using an Allen wrench. Do <u>not</u> force the eyepiece.***
2. Stand the eyepiece on your benchtop so that it's resting on the rubber eye guard and the lens can't contact the surface. Put a Kimwipe over the open end to keep out dust.
3. Insert the camera's microscope adapter where the eyepiece was and turn the camera on.
4. Use the mode dial on the top right side of the camera body to put the camera in manual (M) mode.
5. The camera can't autofocus (no lens), so focus the image you see on the screen or in the viewfinder by using the microscope's focusing knobs.
6. Check the ISO sensitivity, displayed on the right side (if live view is off) or bottom right (if live view is on) of the camera display. ISO 1600 is a good place to start: a high-sensitivity setting that should

still be reasonably free of noise, but you can choose a higher or lower ISO if you wish by pressing the Menu button.

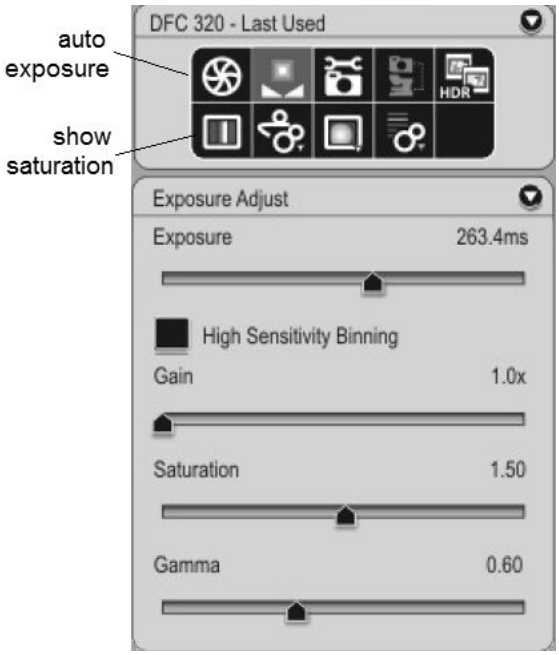
7. Use the dial at the top right of the back of the camera to set the shutter speed, which you can see in the middle (if live view is off) or bottom (if live view is on) of the display. A shutter speed less than about 1/50 sec will require you to be extremely careful not to shake the camera when pressing the shutter—you may want to set the self-timer if you need a long exposure.
8. Press the shutter button (silver button on the top right of the camera body) to capture the image. See if it's in focus, bright enough and shows what you want (remembering that you can crop it later to get exactly the view you need). If the image disappears before you're ready, use the playback button (▸) to get it back. Don't be distressed if it's not right—since it's digital, you can just adjust and take another.
9. When you are done capturing images, carefully return the eyepiece to your microscope.
10. Do not leave your images on the camera. You can either use the camera's USB adapter to transfer them to a computer, or you can remove the SD card from the camera and plug it into a computer's card reader. Then, delete them from the camera's card. And, unless it's *your* computer you've moved them to, move them to your own F: drive or a flash drive and delete them from the computer as well.
11. Your images will require processing after you take them. The field of view will look like a circle of light in a black box, and this isn't what you want for your lab report or notebook. Crop the image to show just the part of the field you're interested in and adjust brightness, contrast and sharpness as needed to make your image as informative as possible. IrfanView (www.irfanview.com) is a very versatile image processing program that is free and very easy to use; it's also available in a portable version that will run from your flash drive (portableapps.com).

Using the Leica Digital Microscope Cameras

The Leica DM2500 DIC microscope and Olympus fluorescence microscope have permanent Leica digital cameras. Both are connected to a dedicated computer in the darkroom equipped with Leica's LAS software which can capture images and can be used for image processing and analysis. Detailed instructions for analyzing images in LAS is beyond the scope of this *Handbook*, but the guidelines below will help you capture images and add size bars. For additional functions, you can find an LAS manual in the darkroom. See also the basics of image analysis with ImageJ elsewhere in this *Handbook*.

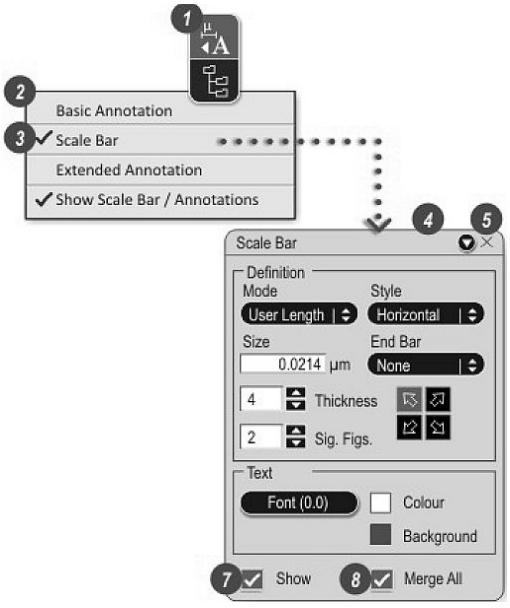
1. Log into the darkroom computer. You can use the username leica and password Leica2015 to log into the local computer only (not the network); you will then need a flash drive or SD card on which to store your files. If you need access to your own F: drive, you can also log into the network with your username and password; however, you may find that the LAS software will not transfer its calibration data to a new user.
2. Click on the application Leica Application Suite Framework V4 (be patient: : it takes a while to load).
3. Check to be sure the program is taking information from the correct camera and microscope. On the left-hand sidebar, click on Camera at the top. Just below that and above the icons there is the name of the camera that the program is currently taking data from: DFC295 for the DM2500 DIC microscope or DFC450C for the fluorescence microscope. If you need to change the camera, find the Input Options panel on the Camera tab, click on the drop-down menu under Camera and select the correct one.
4. Turn on the microscope and set it up as desired. Use the slider near the eyepieces to switch between passing the light up into the eyepieces or into the camera. When you have a specimen in view, click the Acquire tab in LAS if it's not already selected; you should see a live view on the computer screen.
5. You may have to adjust the fine focus slightly to get a sharp focus in the computer image (the focal distance for the camera and for your eye is just a little different). Notice there is a brief delay between when you make an adjustment and when the computer image updates, so make small, slow adjustments and wait for the image to "catch up."
6. You can adjust the brightness of the image using the light intensity control and iris diaphragm of the microscope, but you may also want to adjust what the camera is doing. The camera creates an image by integrating what it "sees" over some exposure time that you can set. Click to open the

Exposure Adjust panel (see illustration at right). You can set automatic exposure by clicking the icon that looks like a pinwheel; if you do this, you get a slider to adjust the brightness of the image. If you turn off automatic exposure, you get manual control of:



- ❏ exposure time (how long the camera integrates input)
- ❏ gain (higher gain boosts a weak signal, at the expense of adding noise)
- ❏ gamma (the relationship between the electrical signal from the camera and the colors you see; generally, higher gamma results in a brighter image)
- ❏ saturation (color intensity)

7. Although you want to choose exposure settings that allow you to see the dimmer parts of your image, you do not want your image to be saturated: if some of the sensors are maxed out with photons, then you lose information, because nothing changes if more photons hit them. Click the blue and red icon in the Exposure Adjust panel to see visually the effect of the current exposure settings: saturated areas of the image will be shown as red. If you see red areas, you may wish to decrease exposure time or adjust gain to eliminate saturation.
8. Record the exposure settings that work for your image in your notebook, as you will want to use the same ones for subsequent images of the same type.
9. When you are ready to capture the image, click Acquire at the bottom left corner of the window.
10. Now you are looking at the captured image rather than the live view. On the left hand side, you will see information about the image: name, objective magnification, exposure, format, location, size. You should rename the image to something meaningful to help you keep track of your images and then record in your notebook what the image is, how it's named and where you stored it.
11. You should add a scale bar to your image. LAS is calibrated to know the relationship between the actual size of the microscopic image and pixels in the captured image. Click the top icon on the right-hand side (#1 in the image at right) to open a menu of annotation options (2). Check Show Scale Bar/Annotations and then check Scale Bar (3) to open the Scale Bar panel.



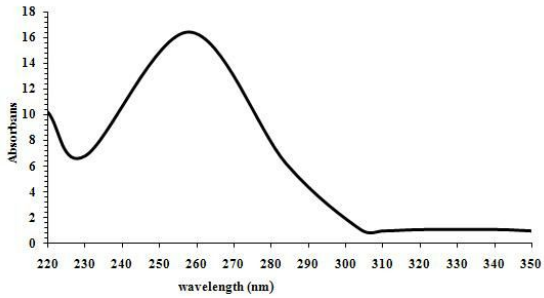
12. Be sure the orange Show box at the bottom (7) is checked. Then decide how long you want the scale bar to be: maybe 10 µm or 100 µm depending on the specimen and magnification. Use the arrows to position the scale bar in one of the four corners of the image or just drag it where you want it with the mouse.
13. You can also open the Basic Annotation panel from this same menu to add the date, file name or any text you wish to the image itself.
14. Click Merge All (8) to make the scale bar and any annotations a permanent part of the captured image (you can no longer move the scale bar or change its size after this step).
15. Save your image to your F: drive or to a flash drive or SD card by clicking the disk icon on the right-hand toolbar. Choose an appropriate file type: usually JPEG for a smaller, compressed file or TIFF for a large file that retains all image data (best if you think you may need it for publication or for more analysis).

Using Spectrophotometers

How a Spectrophotometer Works

Any colored solution absorbs some wavelengths of light and transmits others: blue food coloring, for example, absorbs most light that hits it, but wavelengths in the blue range pass through to the eye. Many molecules absorb some wavelengths of light: even colorless solutions may absorb invisible UV light. **Spectrophotometers** measure the **absorbance** of light by a solution.

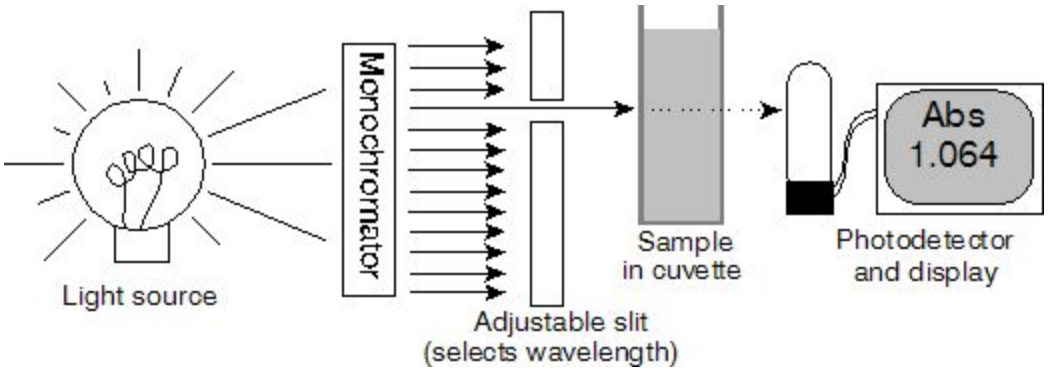
The spectrophotometer is a valuable analytical instrument, because the absorbance of a solution is proportional to the concentration of that solution (see below). So, measuring absorbance is an easy way to determine the concentration of any molecule that absorbs light. Or, the spectrophotometer can be used to determine a molecule's **absorption spectrum**: the wavelengths that particular molecule absorbs. For example, DNA has a peak absorbance at 260 nm as shown in its absorption spectrum at left. That peak is referred to as the λ_{max}. Any specific molecule has its own characteristic absorption spectrum—the absorption spectrum of any DNA molecule always has the same shape and the same λ_{max}.



We can use the spectrophotometer to quantitate all kinds of biological processes. For example, if an enzyme reaction produces or destroys a colored molecule, the spectrophotometer can measure how much is made or broken down, thus measuring the activity of the enzyme. Here are just a few ways that a spectrophotometer might be used in biology:

- ❏ calculate the concentration of DNA in a solution
- ❏ quantitate photosynthesis
- ❏ measure amounts of chlorophyll in a leaf
- ❏ determine the rate of an enzyme-catalyzed reaction
- ❏ measure protein concentration
- ❏ follow the growth of bacterial cells

A spectrophotometer passes light from a lamp through a **monochromator** (a prism or diffraction grating) that breaks it into individual wavelengths (diagram below). An adjustable **slit** allows only a single wavelength to reach the sample, which is placed in the light path in a transparent **cuvette**. On the other side of the cuvette, a photoelectric tube detects light that got through the sample. Strictly speaking, the spectrophotometer measures how much light is *transmitted*, not how much is absorbed, but absorbance is then calculated using the formula: $absorbance = log(\frac{1}{\%\ transmittance})$. Absorbance has no units, but specify the wavelength used when you report absorbance data.



The Biology department has computerized SmartSpec UV-VIS spectrophotometers from BioRad which use 1-cm square cuvettes and can measure absorbance of either visible or ultraviolet

light. There are also Spec-20 spectrophotometers which only measure absorbance of visible light (wavelengths above 340 nm) but can be used with square cuvettes, round cuvettes (these look like test tubes) or even culture tubes used to grow bacteria.

Using the BioRad Spectrophotometers for Single-Wavelength Measurements

Choose an appropriate cuvette for your experiment: either 3-ml or 1.5-ml ("semi-micro," which works with less sample). Disposable plastic cuvettes are used to measure absorbance of visible light (≥340 nm). Precise measurement of UV absorbance requires very expensive, fragile quartz cuvettes or else plastic cuvettes specifically designed to transmit light of lower wavelengths (they are less efficient than the quartz cuvettes but acceptable for most ordinary usage).

1. Turn on the unit using the rocker switch located on the back. Let it warm up about 15 minutes.
2. Press the λ key for single-wavelength measurement.
3. Press 1, to read absorbance at one wavelength, then ENTER.
4. Enter the wavelength that you wish to read.
5. Press ENTER to choose to not subtract a background setting.
6. Place your blank cuvette into the spectrophotometer.
7. Press the READ BLANK key and wait a moment while the zero is set. Then press the right arrow key to move on.
8. Remove your blank cuvette and insert a cuvette with your sample. Press the READ SAMPLE key. Record the absorbance.
9. Continue reading as many samples as you have.

Notice that the light path runs from front-to-back, so you want your cuvette oriented correctly. The cuvette needs to be at least half full, so that your solution is actually in the light path. Additionally, try not to touch the sides of the cuvettes where the light will pass through—you don't want to measure the absorbance of your fingerprints!

The job of the blank is to tell the spectrophotometer what "zero" is. If you're planning to measure a colored molecule dissolved in water, then the absorbance of plain water would represent "zero," and you should use water as your blank. However, you might be dissolving your molecule in a solution which is colored to start with. In that case, your blank should be the solvent with nothing added to it, so that the spectrophotometer will subtract out the color of the solvent itself.

Determining an Absorption Spectrum

In some cases, you won't know in advance the wavelength(s) absorbed by the solution you're interested in. In that case, the first step would be to measure its absorption spectrum and find out experimentally what wavelengths are absorbed best. The spectrophotometer has a program which will automatically try every wavelength and give you the absorbance at each.

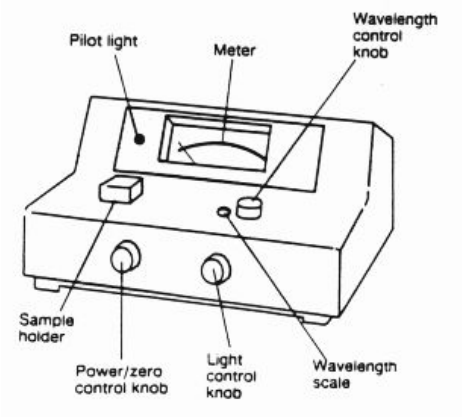
1. After the spectrophotometer warms up, press the SCAN key.
2. Enter the wavelengths that you wish to scan.
3. Press ENTER to choose to not subtract a background setting.
4. Press ENTER to select the 'slow' scan.
5. Put your blank cuvette in the spectrophotometer and press READ BLANK.
6. Insert your sample cuvette and press the READ SAMPLE key. It will take a few moments to scan.
7. Press the PRINT key to print your results. (You can press PRINT again to make a second copy for your partner.) The wavelength at which your sample absorbs best (max) is shown at the top.

Following Absorbance Over Time

In some cases, you will want to measure how the absorbance of your solution changes over time. This is particularly useful for enzyme assays where the concentration of a product that absorbs light increases over time. Rather than watching the clock and reading the absorbance repeatedly, the spectrophotometer can take time points automatically.

1. After the spectrophotometer warms up, press the KINETICS key.
2. Enter the wavelength that you wish to read.
3. Choose the *total* duration of the reaction. For example, if you want to collect the absorbance values every five seconds for one minute, enter 60 seconds as the duration.
4. Choose the interval between readings. In the example above, this would be five seconds.
5. Press ENTER to choose to not subtract a background setting.
6. Put your blank cuvette in the spectrophotometer and press READ BLANK.
7. Start the reaction, insert your cuvette and press READ SAMPLE.
8. When all data have been collected, press PRINT to print results.

Using the Spec-20 Spectrophotometer

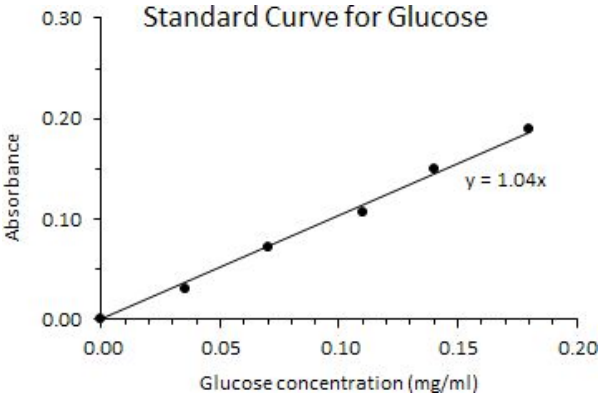


1. Turn on the spectrophotometer by turning the left knob clockwise until it clicks. Use this knob to adjust the pointer to zero on the *transmittance* scale (left end of the scale), or to set the digital display to zero in transmittance mode.
2. Let the spectrophotometer warm up for 15 minutes.
3. Use the large knob on top of the spectrophotometer to set the desired wavelength.
4. Re-adjust the zero point with the left knob if necessary.
5. Place a blank in the sample holder. Close the cover, or if you're using a tube that's too tall, cover the tube with something like a box to exclude stray light.
6. Use the right knob to adjust the reading to 100% transmittance (or zero on the absorbance scale). Remove the blank.
7. If you are using the digital spectrophotometer, use the Mode switch to choose absorbance or transmittance mode as needed.
8. Place your sample in the sample holder and read absorbance or transmittance.

Determining Concentration with a Standard Curve

The concentration of an unknown solution can be determined using a standard curve: a graph of absorbance vs. concentration for standard solutions whose concentrations are known. The equation of a best-fit line then relates absorbance to concentration and can be used to calculate the concentration of any solution of the same molecule whose absorbance falls within the range of the standard curve.

To make a standard curve, you need at least three or four different standard solutions. (You can make several standards by diluting one more concentrated solution.) Measure the absorbance of each standard and plot absorbance vs. concentration, as shown at right. Include the zero point. Draw a best-fit line to represent the relationship between absorbance and concentration. Use Excel or a good calculator to determine the equation of the line. Now, measure the absorbance of the unknown solution. In the equation $y = mx + b$, y is the absorbance, which you measured, so you want to solve for x, the concentration.



In order for this calculation to be accurate, the standard curve needs to be linear—due to the limitations of any spectrophotometer, only a certain range of standards will give a straight-line graph. The absorbance of the unknown must fall within the range of absorbances of the known solutions—if it doesn't, dilute the unknown or make different standards and try again. Finally, the measurements need to be done at the right wavelength: $\boldsymbol{\lambda}_{\mathbf{max}}$, or the wavelength at which the molecule absorbs best (which you can determine by measuring an absorption spectrum if necessary).

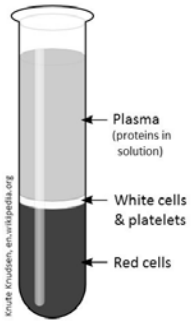
The Beers-Lambert Law

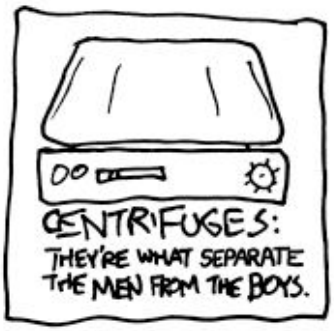
The Beers-Lambert law relates absorbance to concentration: $Abs = \varepsilon bc$, where c is the concentration, b is the length of the light path (always 1 cm for our spectrophotometers) and ε is a constant called the **extinction coefficient**. The extinction coefficient can be determined for any molecule by determining λ_{max} and drawing a standard curve, thus obtaining absorbance and concentration at several points. Using the Beers-Lambert equation, ε can be calculated for each point; ideally, ε would be the same at each point, but in reality there is likely to be experimental variation, so averaging would give the best estimate of the extinction coefficient. Once this constant is known, it can be used to quickly calculate the concentration of any solution of this molecule given its absorbance. Each molecule that absorbs light has its own characteristic extinction coefficient found at its λ_{max}.

Centrifugation

Introduction

Centrifugation is a means of separating components of a sample using centrifugal force. Particles with different densities will move through a liquid at different rates when pulled down by gravity; a centrifuge spins a tube at high speed to generate centrifugal force that may be many times the force of gravity, speeding up the separation process. For example, you might lyse (burst) some cells and then use a centrifuge to separate the heavier unbroken cells (which would form a pellet at the bottom of the tube) from the DNA, proteins and small molecules (which would remain in suspension or solution). As shown at right, centrifugation can be used to separate components of blood: heavier red blood cells will pellet first, then the lighter white blood cells and platelets will pellet on top of them, leaving plasma proteins in the remaining solution.

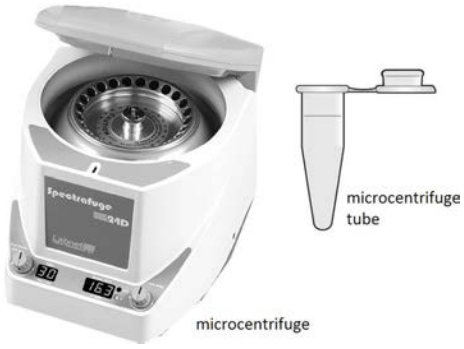




Centrifugation conditions are chosen based on what is to be separated. The longer a mixture is centrifuged and the faster it is spun (higher centrifugal force), the lighter the particles that can be pelleted. The relative centrifugal force (RCF) generated in a centrifuge is measured relative to g, the force of gravity, so a protocol might specify that a sample is to be centrifuged at 5,000 × g, for example. The RCF in a particular centrifuge depends on its speed (measured in revolutions per minute, or RPM) and also on the size and angle of the rotor that holds the centrifuge tubes. So, the spin speed that generates a given force is different for each rotor; the rotor manufacturer may provide a chart to determine this, or many centrifuges can do the calculation, allowing the operator to simply set the desired RCF.

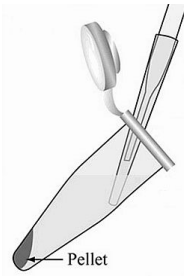
Using a Microcentrifuge

Small volumes can be centrifuged in a microcentrifuge (right). A microcentrifuge is generally used for simple separations, such as pelleting cells out of a solution. Samples are placed in disposable plastic microcentrifuge tubes (right); these tubes most commonly hold 1.5 ml of liquid but also come in 2-ml, 0.5-ml and 0.2-ml sizes. Plastic adaptors are used for the two smallest sizes so that they fit the holes in the rotor.



Microcentrifuges usually have only one rotor and may operate at a single, fixed speed (usually producing RCF of 10,000 to 13,000 × g) or may have a variable speed. Some allow RCF to be set directly, while some require you to know the speed you want in RPM. Most have a timer to set a specific length for the centrifuge run; often, there is also a button that can be held down for a brief spin (e.g., a 5 s spin to bring droplets of liquid to the bottom of a tube).

Place your tubes in the rotor so that the hinge of the tube faces outward. That way, even if you can't see a pellet, you will know which side of the tube it's on (right). **The rotor must always be balanced**: each tube must be directly across from another tube with the same weight (within 0.1 g). If you are spinning an odd number of tubes, balancer tubes containing various volumes of water are available to balance your rotor.



Some microcentrifuge rotors have a lid; if yours does, be sure the lid is in place. Then close the lid on the centrifuge itself, select the speed (or RCF) and time for the spin and start the centrifuge. For safety, be sure it has come to a complete stop before opening the lid (many microcentrifuges won't let you open the lid early).

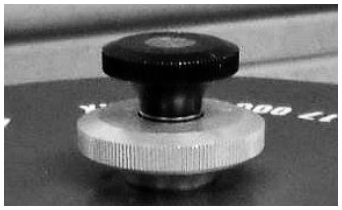
Using the High-Speed Centrifuge

For larger samples, higher-speed spins or more sophisticated separations, a high-speed Sorvall RC6+ centrifuge is available. There are two rotors, one (SA-600) which holds 50-ml centrifuge tubes (or 15-ml tubes, using adapters) and one which holds 250-ml centrifuge bottles. Your choice of tube depends on the volume of your sample, its contents, the RCF you plan to use and whether you need the

tube to be sterile. For example, a disposable 50-ml tube might be used for a very low-speed spin, but it will be crushed at higher RCF. Some tubes are autoclavable, and tubes also have different chemical resistances. You will find charts next to the centrifuge to help you choose. As a general rule, never use an organic solvent (e.g., acetone, ether) in a centrifuge tube unless you *know* the tube you have chosen is resistant to it. Ask a faculty member for help if you are uncertain.

You will find reuseable 50-ml open-top polypropylene tubes, 30-ml screw-cap tubes and 15-ml open-top tubes as well as autoclavable 250-ml polycarbonate bottles on a shelf above the centrifuge. Adapters are also found on this shelf. After using, rinse the tube several times with tap water (or scrub with a brush and a little detergent if it contains something like a sticky pellet) and three times with dH_2O, then return it to its shelf. Be sure all tape is removed. Centrifuge tubes and bottles should not be left for dishwashing. Never label a plastic centrifuge tube or bottle with a Sharpie; you may not be able to completely remove the markings even with ethanol.

Generally, tubes should be filled at least ⅔ full; tubes that have less liquid can crush at high speed (less liquid is OK at low speed, 5,000 × *g* or less). As for the microcentrifuge, **the centrifuge rotor must be balanced**. After filling your tubes, weigh them to ensure that tubes opposite each other have the same mass, within 0.1 g. (In most cases, you can add a little sterile dH_2O or appropriate buffer to a tube that is too light.) Load your tubes into the rotor and double-check that every tube is opposite a tube of the same mass—*imbalance at high speed could cause the rotor to come off the spindle, resulting in catastrophic damage*.



Each rotor has a lid, and the lid has two knobs on top (right). The outer knob tightens the lid onto the rotor, and the inner knob tightens the rotor onto the spindle of the centrifuge. **Both knobs must be tightened down before beginning the run!** If you have to use a tube (such as a 50-ml disposable centrifuge tube) that won't fit under the lid, you can leave the lid off and attach the rotor to the spindle with the **running nut** (right). Please be aware that the running nut is extremely expensive ($400); return it to its place immediately after each run so that it will not be lost. The lid or running nut should be as tight as you can get it with your fingers; notice that the threads are left-handed, so they tighten *counter-clockwise*, unlike most screw threads. This prevents them from loosening during the run.

Never, never attempt to run the centrifuge without either the lid or the running nut in place and properly tightened! If you have any question about your ability to use the centrifuge properly, check with a faculty member before starting the run.

> Recently, the centrifuge and rotor were badly damaged as a result of a student who failed to attach the rotor to the spindle. Fortunately, no one was hurt, but the repair bill was $16,000. Don't let anything like this happen to you.

Close the centrifuge lid and use the keypad to select the time, temperature and either speed or RCF for the run. Notice that the software will require you to choose the rotor you are using; this allows the RCF to be correctly calculated. Press the start key and listen for any unusual sounds that might indicate that the centrifuge is not properly balanced; *stop the run immediately* if anything seems amiss.

After your run (or after the last run, if you will be centrifuging several times or several people in a class are using the centrifuge), the rotor should be rinsed to prevent buildup of salts and particles that can eventually cause scratching and pitting. Remove the rotor from the centrifuge and rinse it with dH_2O, then turn it upside down on a paper towel to drain. Since most centrifuge runs are done at 4 °C, store rotors that are not in use in the refrigerator so they don't have to be cooled prior to a run.

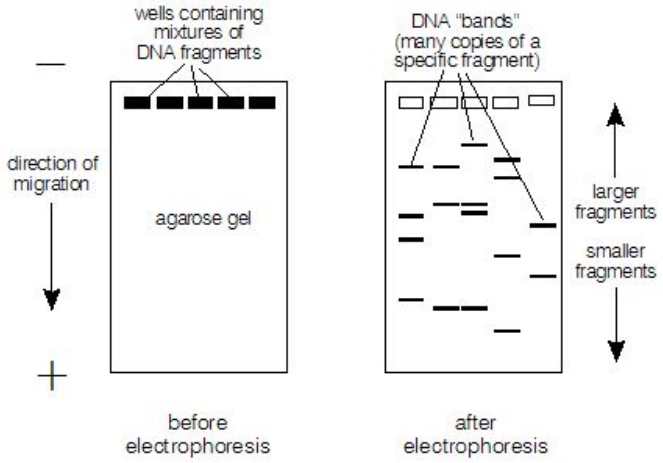
Gel Electrophoresis of DNA

Gel electrophoresis allows us to visualize DNA molecules and determine their length. It is used to map DNA molecules, isolate specific DNA fragments for cloning or even to diagnose genetic diseases.

Introduction to Gel Electrophoresis

In gel electrophoresis, DNA fragments move through a porous matrix made of **agarose**, a gelatin-like substance purified from seaweed. The agarose is melted like Jell-O® and then poured into a plastic tray and allowed to harden into a slab called a **gel**. A plastic comb inserted at one end while the gel is hardening forms **wells** where DNA samples can be placed. The DNA is mixed with a **loading buffer** that contains glycerol—this makes it heavier than water, so it will sink to the bottom of the well. The gel is then covered with a buffer solution that can carry electric current, and electrodes are placed at each end of the gel and connected to a power supply.

Because DNA is negatively charged (each nucleotide has a negatively charged phosphate attached to it), it will move toward the positive electrode. Larger molecules move through the agarose more slowly, while smaller ones can slip through the pores faster. So, the fragments wind up arranged in order according to size, with the smaller ones having moved farther toward the positive pole (right). DNA is not visible while the gel is running, so loading buffers also contain dyes that migrate down the gel and give an idea of how far the DNA has moved. The table below shows commonly used dyes; the loading buffer we use most often has all three of the dyes listed.



dye	color	apparent size
bromophenol blue	dark blue	300 bp
xylene cyanol	light blue	4000 bp
orange G	pink	50 bp

As the DNA migrates, the different fragments form **bands**; each band is composed of many identical copies of a particular-size piece of DNA (you can't do electrophoresis with one DNA molecule: you need millions or billions of identical molecules). The last step is to make the DNA bands visible, using a fluorescent molecule that inserts between the bases in the DNA helix. We use a commercial loading buffer called EZ-Vision which includes the fluorescent molecule, so the gel is already stained when it's done running. If your loading buffer does not include a fluorescent molecule, the gel must be soaked in an ethidium bromide solution after running it. Either way, the bands can be seen using ultraviolet light and photographed (see Using the GelLogic Image System in this *Handbook*).

Sample Preparation

Your DNA sample may be a plasmid, a PCR product, a segment of a chromosome, etc. Sizes of bands formed by circular molecules are difficult to interpret, because they may form open loops or be tightly coiled and migrate differently as a result. Circular DNAs, such as plasmids, are thus usually cut with restriction enzymes (see Restriction Enzyme Digestion in this *Handbook*) before electrophoresis.

The DNA sample must be mixed with loading buffer before loading it into the wells. Loading buffer is normally made at 6× concentration, so you need a volume equal to ⅙ the volume of the DNA sample. Add the appropriate volume of loading buffer to the DNA sample and mix well.

How to Pour and Run a Gel

1. For one gel, you will need a total of 30 ml of agarose solution. The concentration of agarose can be varied for use with DNA fragments of different sizes, as shown in the table at right. Most of the time, you will use 1% agarose. Weigh 0.3 g of agarose (or the appropriate amount for a different percentage gel) and pour it into a 125-ml flask.

agarose (%)	linear DNA fragments resolved	amount for 30-ml gel
0.5	1 kb to 30 kb	0.15 g
0.7	800 bp to 12 kb	0.21 g
1.0	500 bp to 10 kb	0.3 g
1.2	400 bp to 7 kb	0.36 g
1.5	200 bp to 3 kb	0.45 g

source: *Current Protocols in Molecular Biology*

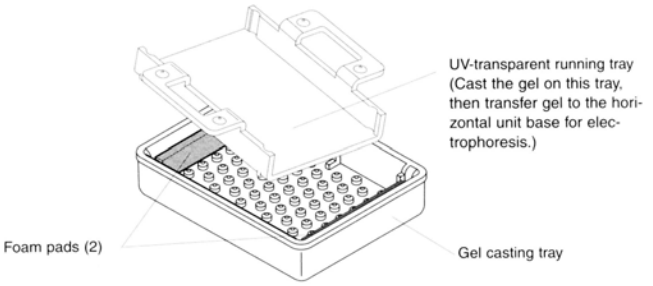
2. Prepare 300 ml of 1× TBE buffer by measuring 30 ml of 10× TBE stock into a 500-ml graduated cylinder, then filling the cylinder to the 300-ml mark with dH_2O (from the tap in the media room, not from a wash bottle, etc.).

TBE stands for Tris, boric acid and EDTA. The Tris and boric acid provide a buffer which allows electric current to flow through the gel and maintains an appropriate pH. EDTA binds to metal ions needed by enzymes that can break down DNA, so it helps protect your DNA while it is in the gel. The TBE stock solution is made up at 10 times the needed concentration (we call this 10× TBE), so we dilute it 1:10 for use at 1×.

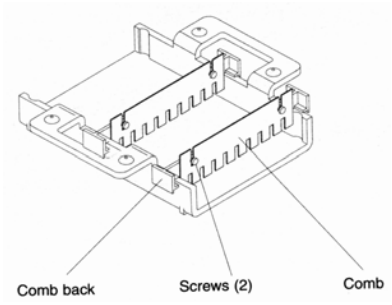
3. Cover the cylinder with a piece of parafilm (you only need a small piece—stretch it to get a good seal) and mix by inverting several times (never try to use a stir bar in a cylinder).
4. Pour 30 ml of the 1× TBE into the flask with your agarose. The remaining 1× TBE will be your **running buffer**, which carries the current from one electrode to the other.
5. Heat the agarose in the microwave on high for 15 seconds. Remove it (caution: hot!) and swirl the flask gently. See if the agarose has completely melted (no powder, no floating transparent flakes). If not, heat again for 10 seconds at a time until it all melts. Don't let it boil for more than a second or two but be sure that <u>all</u> the agarose is dissolved.

Do not overheat the solution! Agarose can become superheated and boil over very rapidly, ruining your experiment and potentially causing burns.

6. Allow the melted agarose to cool on the bench for two minutes while you set up the casting tray.
7. Insert a gel plate into a casting tray as shown at right. The gel plate has to be open on the ends to allow the current to flow through; the foam pads on the ends of the casting tray allow the gel to harden without leaking.
8. Use a bubble level to be sure the gel plate is flat; otherwise, you'll get a wedge-shaped gel.



9. The comb height should be set to leave just a little space between the bottom of the teeth and the bottom of the gel tray. Use a cardboard spacer to check its height.
10. Pour the entire 30 ml of melted agarose into the gel casting tray. Insert a clean comb (which will form the wells) near one end, and slide it up against the handles on the gel plate, as shown at right. (Two combs are shown in the figure, but you only need one. A second one could be added if you have lots of samples but don't need a lot of room to spread out multiple bands.)



11. Let the agarose harden for at least 15 minutes. You should notice that it becomes a little more opaque.
12. When the agarose has fully hardened, carefully remove the comb. Pull it straight up without tearing the wells.
13. Remove the gel plate, with the gel on it, from the casting tray and place it in the gel box.

The plastic gel box is filled with polyethylene glycol as a coolant and can be kept in the freezer prior to use for extra cooling if needed. This keeps the gel from melting from the heat of the electric current, particularly on a long run.

14. Add 1× TBE until the gel is completely submerged: if you look from the side, it should be covered by a few millimeters of liquid. The gel is now ready for use.
15. Move your gel close to the power supply so you won't have to move it once samples are loaded.
16. Use a micropipettor to transfer your DNA size standards to the first well. If you are using our standard 1-kb Plus ladder, you should need 10 µl.

You want the tip of your pipette just inside the well; the DNA will then fall to the bottom of the well as you release it slowly. You should be able to see the well clearly if you look straight down on it with the black benchtop underneath. Be careful not to poke your pipette through the bottom of the well, and also avoid dripping DNA anywhere but in the desired well. Don't release the plunger of the pipettor until you pull it out of the buffer, or it'll suck your DNA back up!

17. Use a micropipettor and a clean tip to transfer each of your DNA samples to a well. Keep track of which sample is in which well. Load 5-15 µl.
18. Place the lid on the gel box and carefully fit the power cords over the two electrodes. Be sure that the positive (red) electrode is at the bottom (non-well end) of the gel. Remember that DNA is

negatively charged and will migrate to the positive pole. Or, just remember "begin at black, run to red."

19. Connect the other ends of the power cords to a power supply, again being sure that the polarity is correct. Turn on the power and set the voltage to 150 V. It should take about 50-60 minutes to run at this voltage.

> You should see a large number of tiny bubbles forming along the wires at the negative pole. If not, something is wrong.

20. Typically, you would stop the gel when the bromophenol blue (dark blue) dye line is about 1 cm from the bottom of the gel. Stop it sooner if you need to see small DNA fragments or run it longer to resolve long ones. Turn off the power supply and disconnect the leads.
21. Carefully remove the gel tray and gel from the gel box. Don't let the gel slide off!
22. Use the GelLogic photodocumentation system (see Using the GelLogic Image System in this *Handbook*) to photograph your gel and to save an image in electronic format (for your notebook, lab report, poster, etc.) if desired.

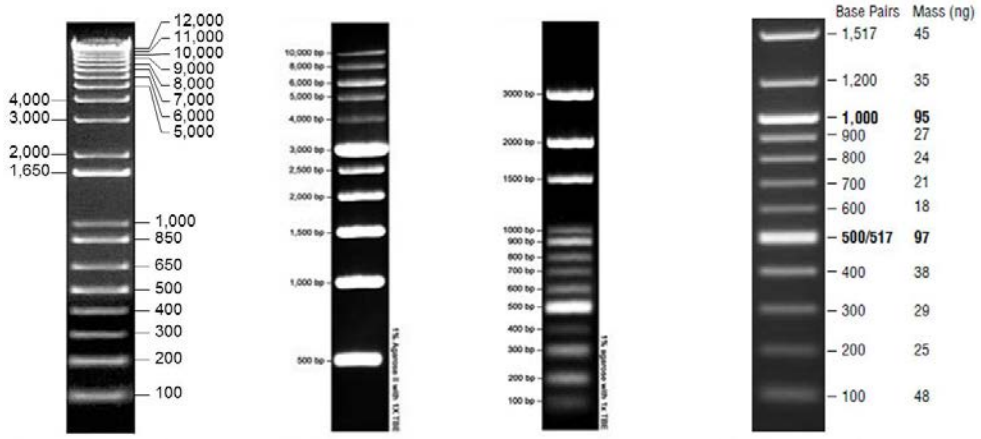
"Can you make it quick? My gel is running off."

23. When you have a good photo, dispose of the gel by wrapping it in a paper towel and discarding it in the trash. Use a wash bottle to rinse the UV light box with dH_2O and dry with a Kimwipe.
24. Pour the running buffer down the drain and rinse off the gel box, gel tray, comb and casting tray with dH_2O before putting them away.

Determining DNA Fragment Sizes

The length of a DNA molecule is measured by the number of nucleotide pairs, or **base-pairs** (abbreviated **bp**) that make it up. For a long molecule, we use **kilobase-pairs** (**kb**): 1 kb = 1000 bp. The distance that the molecule migrated in the gel is proportional to its length. So, the sizes of the DNA molecules in the bands on your gel can be determined by comparing the distance each band migrated with the distance migrated by DNA fragments of known length, called DNA size markers or a DNA "ladder"). A DNA gel should always include size markers in one lane; this will allow us to construct a standard curve to relate size to distance migrated.

There are many different DNA size markers available commercially. In Biology labs, you will usually use the 1-kb Plus DNA Ladder, produced by LifeTechnologies, Inc. (left photo below). However, for small DNA molecules (such as PCR products), you may want to use a 100-bp ladder, and other ladders may be used for specific purposes in some labs or in your research lab. Several ladders that you might encounter are shown below. Be sure you know what ladder you are using!



LifeTechnologies 1-kb Plus ladder | Amresco EZ-Vision 1-kb ladder | Amresco EZ-Vision 100-bp ladder | New England BioLabs 100-bp ladder

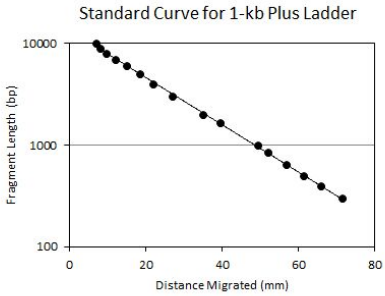
You will probably not be able to see *all* of the ladder bands in a real gel—small ones may run off the gel, while large one may be too close together to see clearly. Notice, however, that each ladder has some kind of "landmark," like a bright or distinctive band. For the 1-kb Plus ladder, the 2,000-bp and

1,650-bp bands are close together and well-separated from the others, with the lower one brighter than the upper. These are your landmarks: start here, then count up and down to identify all the bands that are clearly visible. Don't worry if you can't see every band.

To determine the sizes of the fragments in your gel, first measure the distance migrated for each band that you can identify in the ladder lane. Use a ruler and simply measure the distance from the well to the band, in mm. Measure from the bottom of the well to the bottom of the band. This has to be done separately for every gel, because no two gels will be absolutely identical (comb may be placed a little differently, run a little longer, etc.). Measure to the nearest tenth of a millimeter.

Put the distances migrated into one column of an Excel spreadsheet, and the fragment sizes into the next column. Have Excel graph fragment size (in bases, on the *y*-axis) vs. distance migrated (in mm, on the *x*-axis), but don't let it connect the dots. (See Graphing with Excel in this *Handbook* for help.) Give the *y*-axis a logarithmic scale. The points for your ladder should then form almost a straight line, as shown at right. Draw a best-fit line (*hint*: try an exponential trendline, which should come out linear in appearance because of the logarithmic scale). Now, the size of any unknown fragment in the gel can be calculated by using the equation of the best-fit line. But, be wise in how you use this equation: look carefully at your line and pay attention to how well it fits the data. If your data curve away from the line in some area, then you know that the equation will lead you to mis-size bands in that area.

Distance migrated (mm)	Fragment length (bp)
7.0	10000
8.0	9000
9.6	8000
12.1	7000
15.0	6000
18.5	5000
22.0	4000
27.0	3000
35.0	2000
39.5	1650
49.5	1000
52.0	850
57.0	650
61.4	500
66.1	400
71.5	300



Estimating Amount (Mass) of DNA

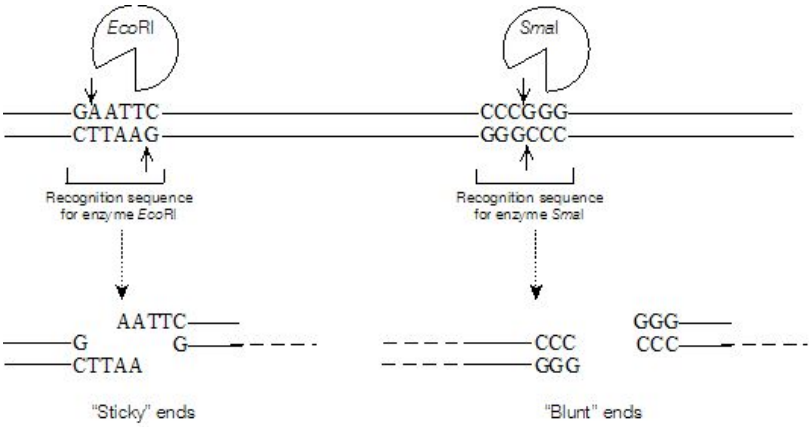
Some ladders can also be used to estimate the *amount* of DNA in a particular band. For example, in the 1-kb Plus ladder, the manufacturer tells us that the 1650-bp band contains 8% of the total mass of DNA. When we buy the ladder, we dilute it to 100 ng/µl in 1× loading buffer. So, if you use 10 µl of ladder in your marker lane, you have 1000 ng of DNA total, and the 1650-bp band should represent 1000 × 0.08 = 80 ng of DNA. (Obviously, you'll have to adjust this calculation if you load a different amount.) Now, you can either "eyeball" a band and get a rough idea of how much DNA it contains, or you use image analysis software such as ImageJ to quantitate the DNA in your band as compared to the 1650-bp ladder band.

✂Restriction Enzyme Digestion

Restriction endonucleases, *or* ***restriction enzymes***, *make double-stranded cuts in DNA molecules. In nature, they are made by bacteria and used to defend against foreign DNA (e.g., a virus). Because they cut at specific sequences, they are extremely valuable to molecular biologists and often used in cloning a gene or physically mapping DNA.*

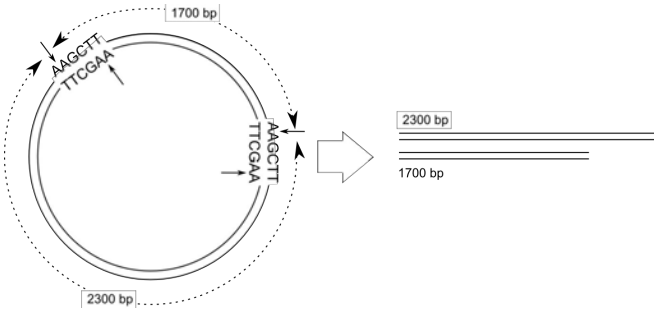
How Restriction Enzymes Work

Dozens of different restriction enzymes have been purified from different kinds of bacteria, and each one recognizes a specific nucleotide sequence in DNA and cuts the molecule at that **recognition sequence**. These sequences are usually 4-8 nucleotides long and are **palindromes**: sequences which are the same on the two DNA strands, reading from 5′ toward 3′. Because the sequence occurs on both strands, the enzyme can cut both strands of the DNA.



The DNA molecule shown in the figure at right contains the sequence 5′-GAATTC-3′, which is recognized by the enzyme *Eco*RI (an enzyme isolated from the bacterium *E. coli*). When *Eco*RI finds the sequence GAATTC in a DNA molecule, it cuts between the G and the first A. This is a palindrome, so *each* strand has a GAATTC here; the enzyme therefore cuts both. Notice that because it cuts off-center, each end left behind has a short, single-stranded region. We call these "**sticky ends**" because they can base-pair with a matching sticky end. A different enzyme, *Sma*I (from the bacterium *Serratia marcescens*) recognizes 5′-CCCGGG-3′ and cuts between the last C and the first G. The cut site is in the center of the recognition sequence, so this enzyme cuts both strands in the *same* place, leaving **blunt ends**.

Each enzyme will cut a particular DNA molecule only at the specific points where its recognition sequence occurs. The number of cut sites and their locations depend on the nucleotide sequence. For example, the plasmid shown at right happens to have two recognition sequences for the enzyme *Hin*dIII, which recognizes 5′-AAGCTT-3′. *Hin*dIII would cut the plasmid twice, producing two linear DNA fragments. The two recognition sites are 1700 base-pairs (bp) apart, so each 4000-bp circular plasmid is cut into two pieces: one is 1700 bp long, and the other is 2300—together, they add up to the original size of the plasmid.



Digesting DNA with a Restriction Enzyme

A restriction digest requires DNA, the enzyme and a buffer solution. The buffer solution contains a pH buffer, Mg^{2+} (a needed cofactor) and salts. It is supplied at 10× concentration, so you always add a volume equal to $^1/_{10}$ of the total volume of your digest (final concentration is then 1×).

Most of our enzymes are purchased from New England Biolabs (NEB). You can find a chart in your lab or detailed information on the NEB Web site (www.neb.com) which will specify which buffer your enzyme needs as well as the optimal incubation temperature (usually 37 °C). Different enzymes require different buffers, and the buffers produced by different companies are not interchangeable. Some companies provide "universal" buffers which can be used for many different enzymes. Be sure you know not only which enzyme you are using but what company it comes from, so that you can accurately determine its buffer requirements. Use special care if you are digesting your DNA with two enzymes at once: you will need a buffer that will allow *both* to cut efficiently.

A typical volume for a restriction digest is 20 µl, but you can increase this volume if you have a large amount of DNA. Restriction enzymes are measured in "units:" the amount of enzyme needed to digest 1 µg of DNA in one hour under optimal conditions. In order to ensure complete digestion, it's common to add 5-10 units of enzyme per µg of DNA. Or, as a rule of thumb, just use 0.5 µl or 1µl of enzyme for an ordinary-sized digest. Buffers are provided at 10× concentration, meaning that you want to add a buffer volume equal to 1/10 your total digest volume to dilute the buffer to 1×. After adding DNA, enzyme and buffer, the rest of the volume of your digest is sterile dH_2O.

Start by listing the components of your digest in your notebook. Figure out how much DNA you want to use, how much enzyme you will need, the kind of buffer and its volume and then how much water you will add. Then set up the digest itself, starting with the water (you can use one pipette tip to add the water to all your tubes), then the buffer (one pipette tip for every tube that uses the same buffer), then the DNA (new pipette tip for every tube). Use thin-walled 0.5-ml microcentrifuge tubes for your digests, as these transfer heat best.

Last of all, add the enzyme. Pull the cooler rack containing your enzyme tube out of the freezer. Be sure the tube stays in the rack as much as possible as you quickly measure out your enzyme (new tip every time) and add it to your tubes. Use a P-10 pipettor to increase accuracy and minimize waste if you are pipetting 1 µl or less. Get the enzyme back in the freezer ASAP.

IMPORTANT! Restriction enzymes are very sensitive to heat! Keep them in their cooler racks anytime you take them out of the freezer, and keep their time outside the freezer as short as possible. Never touch the part of the tube where the enzyme is with your warm fingers.

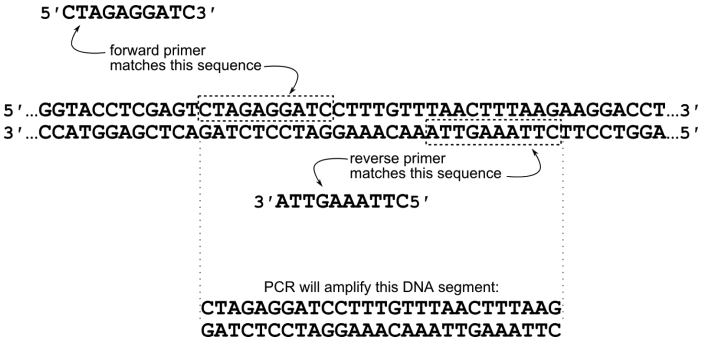
Incubate your digest at the desired temperature. Many manufacturers now designate most of their enzymes as "Fast Digest" or "Time-Saver Qualified," etc., meaning that one unit will digest 1 µg of DNA in 5-15 minutes (we suggest 15 min). Otherwise, a digest should be incubated for an hour.

⇆The Polymerase Chain Reaction (PCR)

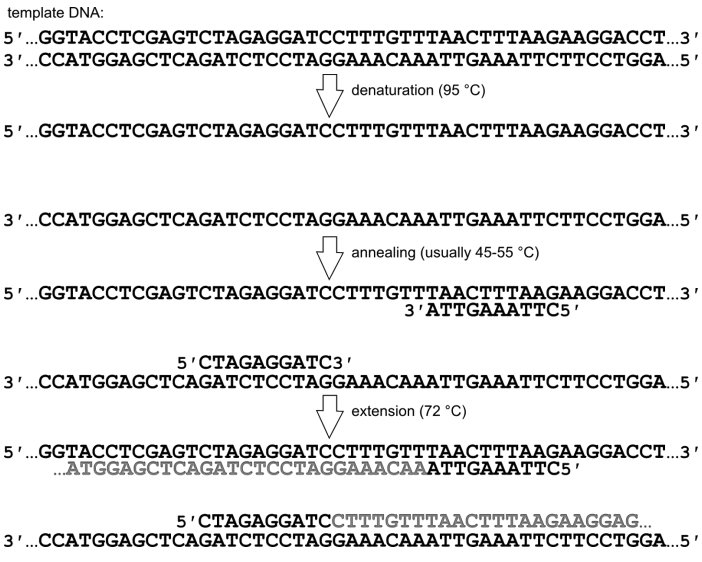
Background

The **polymerase chain reaction**, or **PCR**, is a technique that allows us to isolate a specific region from a complex DNA sample (such as a whole genome) and **amplify** (make many copies of) just that region. The product can be used for cloning, sequencing, genotyping or some other experiment. Invented by Kary Mullis in 1983, PCR quickly became one of the most important tools in the molecular biologist's toolbox and is now used routinely in everything from genetic engineering to ecological monitoring to forensics.

PCR is an application of DNA replication and in fact uses the replication enzyme **DNA polymerase**. Specifically, *Taq* DNA polymerase, isolated from the bacterium *Thermus aquaticus* (discovered in hot springs in Yellowstone National Park) is used, because it is heat-resistant. DNA polymerase requires a **primer**, a short stretch of nucleotides with a free 3′ end, to tell it where to start replicating. PCR controls where DNA replication happens by using two primers, one of which defines each end of the segment of DNA to be amplified as shown at right. Primers are made to order commercially, using an instrument which chemically joins nucleotides in a specified order. This means we have to know the sequence of the DNA in order to amplify it by PCR.



Amplification occurs because many **cycles** of replication are carried out, controlled by precisely controlling the temperature of the reaction in an instrument called a **thermal cycler**. The thermal cycler is programmed to initially heat the reaction tubes to 95 °C, a **denaturation** step that will separate the two DNA strands. It then cools down to the **annealing** temperature chosen for the primers used. During this annealing step, the primers base-pair with their matching DNA as shown at right. Notice that one primer binds to each strand of the DNA. Now the temperature is raised again to 72 °C, the temperature at which *Taq* polymerase works best: the **extension** step. *Taq* polymerase starts at the 3′ end of each primer and copies the DNA **template**.



Now the thermal cycler repeats the denaturation, annealing and extension steps over and over as shown in the figure on the next page—typically for 30 total cycles. With each cycle, more DNA molecules have their ends defined by the ends of the primers, so *Taq* polymerase can only copy up to that point. Thus, most of the DNA molecules produced will consist of exactly the region from where the 5′ end of the "forward" primer bound to where the 5′ end of the "reverse" primer bound. The first two of these are boxed in the figure. The number of amplified segments increases exponentially, so that after 30 cycles, we should have more than one billion amplified molecules, starting with only *one* single molecule of template DNA. (You can see how valuable this is for obtaining DNA from a forensic sample or an ancient dinosaur bone.)

Cold Spring Harbor Laboratory's DNA Learning Center is a great resource; see PCR animations, explanations and interviews at: www.dnalc.org/resources/animations/pcr.html.

A PCR reaction requires template DNA, primers, the four DNA nucleotides (dATP, dTTP, dCTP and dGTP; we call a mixture of these "dNTPs"), *Taq* polymerase, an appropriate buffer solution and $MgCl_2$ (which may be included in the buffer). Below, you will find directions for setting up a basic PCR

reaction and information about designing primers. These can be modified for a particular experiment (indeed, PCR can be used in many different ways) or if an enzyme purchased from a particular company needs slightly different conditions.

Setting Up a PCR Reaction

1. Before you start setting up the reaction, calculate in your notebook the *volumes* you will need for each component:
   - ❑ **Template DNA**: use 10-25 ng for a fairly simple template such as a plasmid or up to 50-125 ng for a complex template such as human genomic DNA
   - ❑ **Primers**: the final concentration in the PCR reaction should be 0.2 µM each. Usually, primers are shipped dry and then made up to a concentration of 100 µM (100 pmol/µl). You may need to dilute a small volume of the primers in order to get a concentration that will allow you to use a reasonable volume in your reaction.
   - ❑ **dNTPs** (mixture of the four DNA nucleotides): final concentration should be 0.2 mM of each nucleotide; typically, the purchased mixture contains 10 mM of each nucleotide, but check to be sure of your starting concentration.
   - ❑ **$MgCl_2$**: *Taq* DNA polymerase requires magnesium ions; usually 1.5-3.5 mM $MgCl_2$ is used. This may be included in the buffer or added separately, depending on the *Taq* supplier. The supplier will usually suggest a starting concentration, often about 3 mM.
   - ❑ **Buffer**: Depending on the *Taq* supplier, buffer may be provided at 5× concentration (meaning ⅕ of the reaction volume should be buffer) or 10× concentration (. of the volume should be buffer).
   - ❑ ***Taq* polymerase**: *Taq* is usually supplied at a concentration of about 5 units/µl (a "unit" is defined based on the amount of *Taq* needed to synthesize a given amount of DNA under standard conditions). Usually 2.5-5 units of *Taq* are used in most PCR reactions.
   - ❑ **Total volume** of the reaction should be either 25 or 50 µl. After calculating the volumes of the other components, determine how much water is needed to reach the desired total volume. Use filtered, autoclaved "PCR water" to ensure no contaminating DNA is present.
2. Consider controls you might need. It's a good idea to do a negative control without template DNA: any bands you see could be due to primer self-annealing or to contaminating DNA molecules. In some cases, you might also have a positive control, perhaps a template where you know your primers work.
3. Use 0.2-ml thin-walled microcentrifuge tubes for your reactions; these allow rapid heat transfer. Place your tubes on ice and keep them there until you are ready to start the reaction.
4. Add the components to your chilled tubes in this order: water, buffer, $MgCl_2$ (if needed), primers, dNTPs, template DNA, *Taq* polymerase.
5. If you are setting up many PCR reactions at once, you can prepare a "master mix" and aliquot it into each tube. Multiply the amount of water, buffer, $MgCl_2$, primers, dNTPs and *Taq* polymerase

you need by the number of tubes you want to set up and mix all these components in one tube, then aliquot the "master mix" to the reaction tubes and add template DNA to each.

Running a PCR Reaction

1. Determine your annealing temperature. The company that supplied the primers should have included their **melting temperatures (T_m)**—the temperature at which 50% of the primer would be base-paired with its target. The T_m of the two primers should be within 5° of each other, and the annealing temperature is usually set 5° below the lower T_m.
2. Determine your extension time. Standard *Taq* polymerase can synthesize DNA at a rate of about 1 kb (1000 bp) per minute, so a 500-bp PCR product would require an extension time of only 30 s, while a 2.5-kb product would require 2.5 min. Some *Taq* formulations work faster, so check the supplier's instructions to be sure.
3. Program the thermal cycler for the parameters you have decided upon. The following instructions apply to the Bio-Rad gradient cycler, but other cyclers work similarly.
   - ❏ Choose New from the menu and name your program by using the arrow keys to select letters.
   - ❏ Select a temperature for the heated lid. Heating the lid prevents the liquid from evaporating and condensing on top of the tube. The default temperature of 100 °C is usually fine.
   - ❏ Select the reaction volume you will use (usually 25 or 50 µl).
   - ❏ You should now see step #1 highlighted and have a choice of TEMP (single temperature) or GRAD (gradient of temperatures). Choose TEMP and enter 95° for an initial step to denature all the DNA. Set the time for this first step to 5 minutes by entering 5-0-0. This step will only be done once. OK the step when you have verified it is entered correctly.
   - ❏ Now, start programming the cycles of denaturing, annealing and elongating. You should see a choice of TEMP, GRAD, GOTO or END for step #2. You want this to be your repeated denaturing step; choose TEMP and set this step to heat to 95° for 30 s.
   - ❏ Set the annealing step to 30 s at the annealing temperature you have chosen.
   - ❏ Set the elongation step to the elongation time you have chosen and 72°.
   - ❏ At the next step (step #5), choose GOTO. This is how you get the cycles to repeat. You want to go back to your denaturing step (not the very first 5-minute step, but the 30 s at 95° step, step #2), so you should go to step #2 29 more times for a standard 30-cycle PCR reaction.

     If you need to try a range of temperatures (see Troubleshooting, below) or if you are doing reactions that need different temperatures, you can choose GRAD here and then pick the temperature you want for the front of the heating block and a higher temperature for the back of the heating block. The temperature of the heating block will increase from front to back by 1/7 of the difference between the two for each row.
   - ❏ After the 30 cycles, it is common to do one more extension step to allow the polymerase to finish up any unfinished ends. Set 5 or 10 minutes at 72° to accomplish this.
   - ❏ In case you don't get to your reaction as soon as it ends, you may want it to refrigerate the tubes until you come back for them. Set another TEMP step for 4°, but set the time to zero; this will show up as 4° "for ever," meaning that the temperature will stay at 4 ° until you continue manually.
   - ❏ Finally, choose END for the last step and save the program.
4. Place your tubes in the cycler (remember that if you've chosen a gradient step, the temperature varies from the low temperature you set at the front of the block to the high temperature at the back), choose your program from the menu and choose Run. The Screen button on the Bio-Rad thermal cycler will allow you to choose a display which shows what's happening currently or one which shows the time remaining.
5. When the program is complete, remove the tubes. Usually, you will use gel electrophoresis (discussed elsewhere in this *Handbook*) to analyze your results.

Troubleshooting a PCR Reaction

If your PCR reaction fails to give you the expected amplified DNA segment, the problem could be technical (a problem with the PCR reaction itself) or biological (maybe your DNA does not actually contain a match for your primer sequences). Here are some common problems with PCR that you might consider:

1. The most likely problem is the annealing temperature. Some primer/template combinations are very sensitive to temperature and may only work in a narrow range. Set up a few identical PCR

reactions and use the gradient feature of the Bio-Rad thermal cycler to test a range of temperatures, such as from 4° below your original annealing temperature to 4° above it.

2. Some primer/template combinations are very sensitive to the magnesium concentration. Try varying the concentration between 1 and 4 mM to see if this affects your results.
3. The quality of your template DNA can certainly affect your reaction; particularly if you are using fairly impure DNA extracted from tissue or an environmental sample, consider cleaning it up before using it as template, such as with one of the commercial DNA clean-up kits.
4. Even if you've used a computer program (see below) to choose primers that should theoretically work, they may not work in the real world. If you can't get a PCR product even after playing with some of these parameters, consider trying a different primer pair.

Designing PCR Primers

For a typical PCR reaction, primers are 20 nucleotides long and are an exact match to the template. A 20-nucleotide sequence is plenty long enough to ensure that there will be only one match in the template (unless, unluckily, your primer matches a repeated sequence!)—we would expect a given 20-nucleotide sequence to occur randomly only once in every $4^{20} = 1.1 \times 10^{12}$ bases. But, not just any 20-nucleotide sequence will work. For example, imagine a primer with the sequence AAAAAAAGGGGGGTTTTTTT. This primer could fold up so that the As paired with the Ts, making it pretty useless for binding template. We can use bioinformatics to find a primer that will not fold up and will not base-pair stably with its partner; there are many programs available to test this. Most will also ensure that the two primers have similar melting temperatures and similar content of Gs and Cs (which base-pair more tightly than As and Ts).

An easy-to-use primer design program that works well is the implementation of Web Primer at the *Saccharomyces* Genome Database: www.yeastgenome.org/cgi-bin/web-primer.

1. Enter your DNA sequence into the input box and choose PCR as the desired primer type.
2. On the next page, you will be asked if the PCR product needs to have *exactly* the same endpoints as the sequence you entered. You will get better primers if you choose NO here: Instead of entering exactly the DNA segment you want to amplify, include some additional bases (maybe 25 or 50) on each end so that the program can choose primers anywhere in this area. This gives the program more possible primers to choose from. (Of course, sometimes you need a segment with very specific endpoints.)
3. You will see boxes in which you can set the minimum and maximum length of the primers, their minimum and maximum T_m, and parameters to describe how much base-pairing within and between the primers is allowed. Generally you can start with the default settings unless you have some reason to change them.
4. Submit your choices and the program will attempt to choose possible primers; it will output the best pair as well as a table of other possibilities. It will let you know if the parameters you chose were too stringent (too few possibilities) or too relaxed (too many possibilities), and you can adjust them accordingly.

If you are amplifying a DNA segment so that you can clone it, you may want to add restriction sites to the ends of your primers that are not present in the template DNA. To do this, you would design 20-nucleotide primers as usual, but then add the restriction enzyme recognition sequence to the 5′ end of each primer. Then add four nucleotides, such as AAGG, to each end, because restriction enzymes don't like to cut at the very end of the DNA molecule. When your segment is amplified, the restriction sites will be part of the amplified DNA because they were part of the primer.

Using the GelLogic Image System

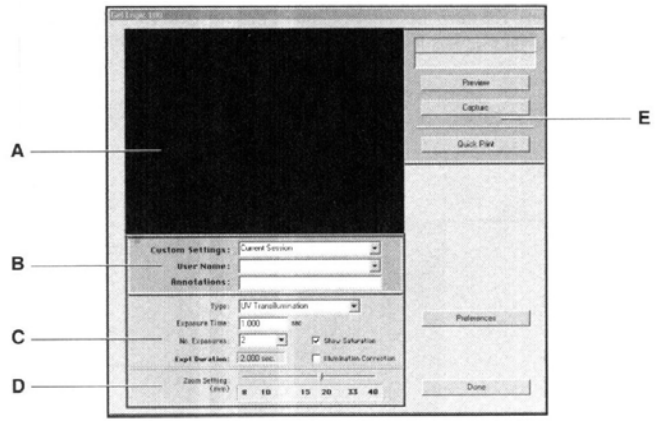
The GelLogic 100 System

We use a Kodak GelLogic 100 system (right) to document the results of gel electrophoresis. This system consists of a grayscale digital camera mounted in a cabinet (allowing us to control the light) and attached to a computer. It can capture a digital image of a DNA gel (using the UV light box at its base) or a protein gel (using white light from above, called **epi- illumination**). It can also be used to photograph agar plates or other small items. An image can be printed on the attached thermal printer or exported for further analysis (see "Image Analysis" in this *Handbook*).

The computer has a generic login to allow for quick image capture and printing: log in to the workstation only (not the network) with the username **science** and the password **lab**. Save your images to a flash drive or SD card if you use this option. Or, you can log in to your own account and save images to your F: drive. Please remember that this computer supports multiple users, so do not store images on the local hard drive.

Viewing and Printing a DNA Gel

1. Double-click the Kodak Gel Doc icon on the desktop.
2. When the application opens, there will be a button near the top left that says Capture GL-100. Click it; you should see the screen shown at right.
3. Slide open the door at the bottom of the cabinet and place your gel on the center of the light box. Your gel should fit nicely in the rectangle at the center of the black mat; if it is too large, there are additional black mats in the cabinet below.
4. Close the cabinet door and turn on the ultraviolet light. Since UV light is a mutagen and can seriously damage your eyes, be sure to have the door closed before turning on the light.
5. Use the drop-down box (area C in the illustration) to set Type to UV Transillumination.
6. Click the Preview button (E). The image of your gel should appear in the large box on the upper left (A).



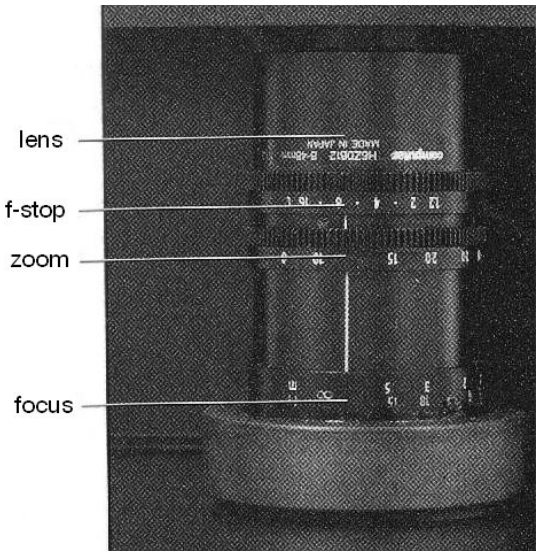
7. In most cases, the image should be in focus and you shouldn't need to zoom in or out. If you do need to make adjustments, see "Optimizing Your Image" below.
8. You will usually need to change the exposure time: the computer can gather light for anywhere from 0.001 to 16 seconds, allowing us to image of either very faint or very bright bands. Click the Show Saturation box (in area C), then adjust the exposure time so that you can see all your bands but none of them are colored red. Red indicates where the image is saturated: its brightness is beyond the sensitivity of the camera, so it's not displayed accurately.
9. Click Quick Print and the image will be printed on the thermal printer. If it's not rotated the right way, click the Preferences button to fix it.

Saving a Gel Image

1. View the image and adjust the exposure as explained above.
2. Click Capture. A new screen will appear with your gel image.
3. **Do <u>not</u> use Save or Save As to save the project as a .BIP file**. A software bug will prevent re-opening this file. Instead, choose Export Data from the File menu and then choose Image as the data to export.
4. Choose the file format for your image. The best choices are TIFF (very high quality but very large files) or JPEG (lower-quality, compressed image but fine for printing and most ordinary uses).
5. Select the location where you want your image to be stored (your F: drive or flash drive).

Optimizing Images

Usually, exposure time is all you have to adjust for ordinary gels. However, you can also adjust the focus, *f*-stop or zoom settings as needed. These adjustments are located on the camera itself (right). Remember there is a time delay (the length of the exposure time) before your adjustment will show up in the on-screen preview, so change the settings a little at a time and pause for the computer to catch up.



Focus: The camera's focus ring is the bottom one and is covered by a metal ring that prevents it from being turned accidentally. Lift up the protective ring and slowly turn the focus ring while looking at the image on the screen. If you have trouble getting a sharp focus, check the Focus Camera box while looking at a preview image. A rectangle will appear in the middle of the image and a numerical value will appear below the checkbox. Higher values indicate a sharper image in the rectangle. Manually adjust the focus ring to give the largest value.

***f*-stop**: The *f*-stop adjusts the **aperture** (opening that admits light into the camera), regulating the light reaching the lens. Adjusting the *f*-stop is done using the top ring on the camera and works much like adjusting exposure time. However, you can use a shorter exposure time with a lower *f*-stop value (larger aperture), and you can get a sharper image by increasing exposure time and *f*-stop (smaller aperture). Typically, use an *f*-stop between *f*4 and *f*6.

Zoom: The middle ring is used to increase or decrease the field of view of the camera. Ideally, the gel should fill the entire field of view, allowing the camera to capture the maximum amount of information about it (it captures 272 pixels/cm when fully zoomed in but only 46 pixels/cm when fully zoomed out). If you change the zoom setting, also change the Zoom Setting slider on the software's capture screen (D) to match the number on the zoom ring, so that the image is calibrated correctly.

Background subtraction: You will probably notice bright areas of your image where your gel was right over a UV tube. You may get a better image by subtracting the background, especially if your bands are very faint. Before capturing your image, click the Illumination Correction checkbox (C) on the capture screen. Capture your image normally. Then, you should see an illumination correction dialog box. Remove your gel from the cabinet and close the door without adjusting anything. Enter a name in the Output box and click Continue. The software will capture an image of the background and then "subtract" this background reference from your image. Save your image as a .BIP file, close it (don't close the program!), and then immediately re-open the file to see the corrected image.

Imaging a Protein Gel or an Agar Plate

The GelLogic system can also record images of protein gels, agar plates or other small items. Replace the black rubber mat on top of the UV light box with the white foam mat you will find in the cabinet below. Place your gel or plate on the white background. Do not turn on the UV light; rather, turn on the white epi-illumination light with the orange rocker switch on the upper left of the cabinet. Finally, slide the filter tray on the top right side of the cabinet all the way out; there is a colored filter used to enhance DNA gel images that we don't need for white light. Now, bring up the capture screen in the GelLogic software as you would for a DNA gel. Use the drop-down box (C) to change the Type from UV Transillumination to White Light Epi-Illumination. Preview and adjust exposure as usual.

Image Processing and Analysis

To crop or rotate your image or change brightness, contrast or sharpness, we recommend the freeware program **IrfanView** (www.irfanview.com). PowerPoint can also do basic image processing and is a good program to use for labeling images. For more sophisticated analysis of your image, such as to quantitate the amount of DNA or protein in a band, use the freeware program **ImageJ** (provided by the U.S. National Institutes of Health and available at imagej.nih.gov) as described in the section "Image Analysis" in this *Handbook*. Gel analysis can also be done using the GelLogic software, but this software runs only on the computer connected to the system and (as noted above) you cannot save your image as a .BIP project for later analysis.

Image Analysis

Digital images, ranging from microscopic photos to gel images to satellite imagery, allow for data analysis using image analysis software. This section is a guide to only some very basic image analysis: counting and measuring microscopic objects and quantitating bands on gels.

ImageJ

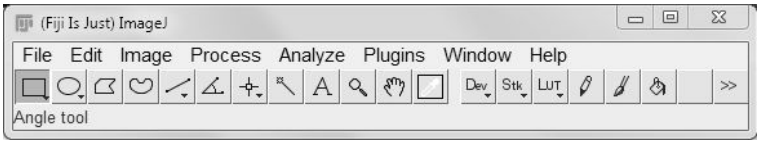
ImageJ (imagej.nih.gov)is a public-domain, open-source image analysis program developed at the National Institutes of Health. As such, it is free for anyone to use or even modify. You can find ImageJ installed on some computers in the Biology department, or you can install it on your own computer. However, since ImageJ requires installation (not permitted on campus computers) and requires Java to run (sometimes not installed or updated on a computer you may be using), you may find it more convenient to use **Fiji** (fiji.sc/Fiji), which bundles ImageJ, Java and some plugins into a portable software package. Fiji can be run from almost anywhere, including a flash drive, without installation or additional software.

ImageJ has many features for image processing and even more can be added by using plugins. Repetitive tasks can be automated with scripts; scripting is supported in several popular languages. Among ImageJ's uses are: cell counting, quantitating fluorescence (e.g., GFP or fluorescent antibody-labeled cells or structures), measurement of area, quantitating gel bands, converting images between formats, enhancing images, removing noise and many more. In this section, we will discuss using ImageJ to quantitate gel bands and count and measure microscopic objects. Tutorials for ImageJ are available from the NIH at rsb.info.nih.gov/ij/docs, and many other guides and tutorials readily available from the ImageJ wiki or other Internet sources.

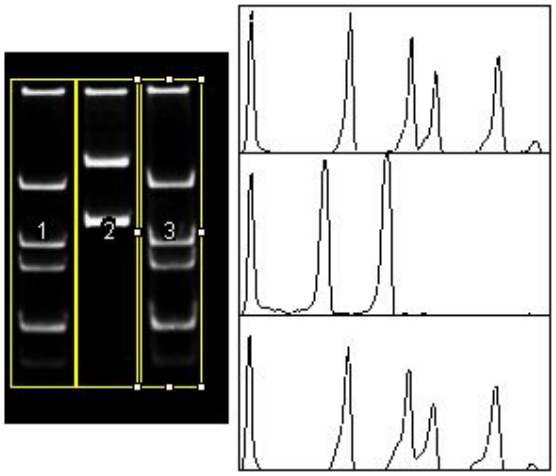
Quantitating Gel Bands with ImageJ

For some experiments, you may need to quantitate the amount of a plasmid fragment, PCR product or other DNA you have on a gel. ImageJ will allow you to determine the quantity of one band relative to another or to a band with a known amount of DNA such as a band in a DNA ladder.

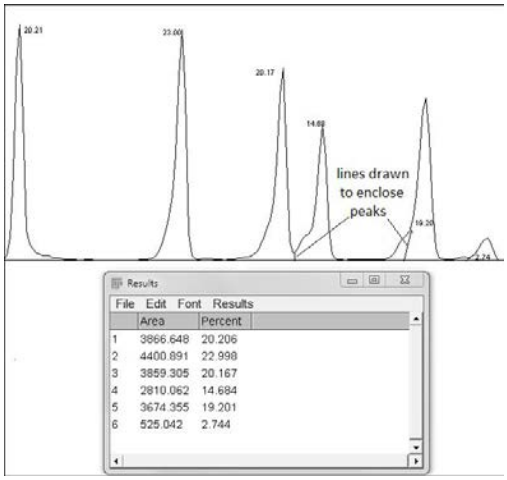
1. You will need an image of your gel saved as a JPEG, TIFF or similar image format. This could be an exported image from our GelLogic Image System (discussed in detail elsewhere in this *Handbook*), a Western blot image from the C-Digit instrument, a scan of a gel, etc. For best quantitation, ensure that your bands are not completely saturated (discussed in the "GelLogic" section).
2. Launch ImageJ; you should see the menu bar and toolbar shown at right. An image window and other windows will appear when you begin working with an image.



3. Open your gel image by choosing Open from the File menu (we'll use the shorthand File | Open to represent this).
4. If the image is not already a grayscale image, convert it by choosing Image | Type | 8-bit.
5. If your gel lanes run horizontally instead of vertically, use Image | Transform | Rotate 90 Degrees Right (or left) to make it vertical.
6. Click the rectangular selection tool (first tool in the toolbar) and use it to outline the leftmost lane that you want to analyze. Then choose Analyze | Gels | Select First Lane, or press 1. You should see your lane outlined with a 1 in the middle of it.
7. Click in the middle of the rectangle and drag to copy the rectangle over to the second lane. Center the rectangle over the lane, but don't worry about top-to-bottom alignment: ImageJ will take care of this automatically. Choose Analyze | Gels | Select Next Lane or press 2. Repeat for the remaining lanes you want to analyze. Your gel should look like the one at right.

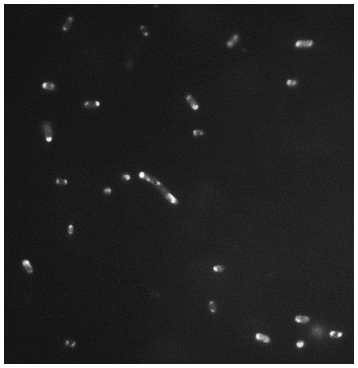


8. Now choose Analyze | Gels | Plot Lanes to draw plots of the pixel intensities that ImageJ finds as it scans down each marked lane from top to bottom (right). Each plot represents a lane (lane 1 is at the top), and each peak in the plot represents a band. If your gel image has white bands on a black background like the sample gel shown here, your peaks may initially look like "valleys;" if so, choose Analyze | Gels | Gel Analyzer Options and click the Invert peaks checkbox, then choose Analyze | Gels | Re-plot Lanes.



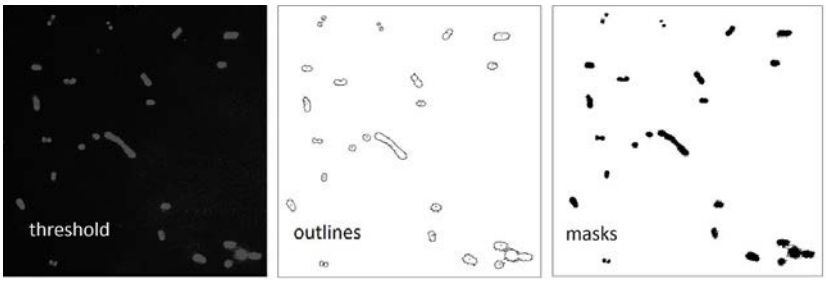
9. The area of each peak is proportional to the "brightness" of the band, which is in turn proportional to the amount of DNA (or protein, if it's a protein gel or Western blot) present. To measure the area, identify the peaks that represent bands you are interested in and use the straight-line tool to draw a baseline for each peak or to bring the peak down to the baseline (right). The peak must be a closed triangle in order for its area to be calculated; use good judgement to decide where the baseline goes, especially if there is some background "noise" in your gel.
10. Click with the magic wand tool inside each peak of interest. In the Results window, you'll see the area of each peak (arbitrary units) as you click inside it.
11. When you have highlighted all the peaks of interest, choose Analyze | Gels | Label Peaks to label each peak with the *percentage* of the total area in the lane represented by that peak. This will also add a Percent column in the results window.
12. Now you can work with the area data. For instance, looking at the two peaks close together in Lane 1, the first one (area of 3859) has 1.4× the area of the second one (area of 2810). So, if the upper band contains 5 ng of DNA, then the lower one contains 3.6 ng (5/1.4). The Results table can be transferred to a spreadsheet by pressing Ctrl+A (or Edit | Select All) and Ctrl+C (or Edit | Copy).

Counting Cells with ImageJ



Using the Analyze Particles method. The Analyze Particles function can be used to count cells in a microscope image (or this same technique could be applied to counting bacterial colonies from an image of a Petri dish or any similar application). The big problem is distinguishing what is to be counted from the background. Consider the sample image at right, which shows bacterial cells with fluorescent protein aggregates. You want to count all the cells, both long and short (there are 23), but you don't want to count the background noise (like the fuzzy blob in the lower right) or the individual aggregates. One way to do this is to convert the image to a two-color representation, setting **threshold** values to identify the objects you want.

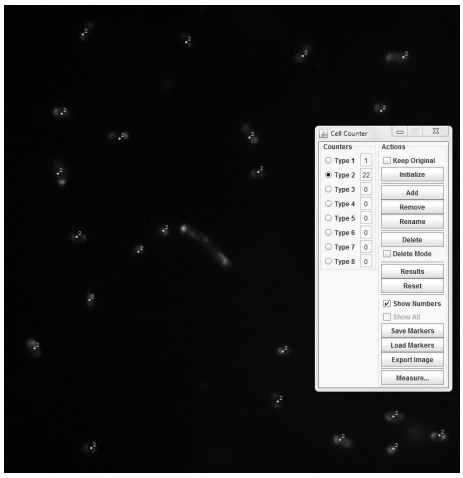
1. Open your image in ImageJ.
2. Use the image adjustment tools such as Image | Adjust | Brightness/Contrast to tweak your image so that the objects to be counted are as distinct from the background as possible.
3. Choose Image | Adjust | Threshold. You will see a different dialog box for a color image vs. a grayscale one, but both have sliders that allow you to choose the minimum and maximum brightness or color that an object can have in order to be counted. The display changes as you move these sliders, showing "countable" objects in red on a black background.
4. Adjust the sliders until the objects you are interested in are mostly red and the rest of the image is mostly black. Don't worry about really small speckles: filter them out later. The first image at right is a thresholded version of the original.



5. Now choose Analyze | Analyze Particles.

6. Set the Size (area) of objects to count; default is 0-Infinity (all objects).
7. Use the Circularity box to decide how circular an object has to be to get counted. If you only want round objects (maybe you're counting yeast cells), set values close to 1.0 (perfect circle). If you are counting rod-shaped bacteria, maybe a value closer to zero will help.
8. In the Show drop-down, choose Outlines to see each counted object outlined and numbered (middle image above), or Mask to show them as solid objects (right image above).
9. Check Display results and then click OK. You will see an image showing what was counted as well as a Results window with the area of each counted object.
10. For the sample image above, leaving Size at 0-Infinity resulted in counting a large number of tiny speckles. But, a look at the Results window shows that all of the cells of interest had an area less than 100 pixels^2. So, resetting the Size box to 100-Infinity gave the reasonable counts shown.
11. The Results window gives the areas of your cells, which you can then use to analyze sizes. Use Analyze | Set Measurements to add options such as x and y dimensions, perimeter, etc.
12. Analyze Particles is only as good as your image and your thresholding skill. Notice in this example that two cells in the upper left were counted as four because the program couldn't "see" the outlines of the cells well enough, and the bright blob in the lower right was counted because it wasn't different enough from a cell to be excluded by the threshold settings.

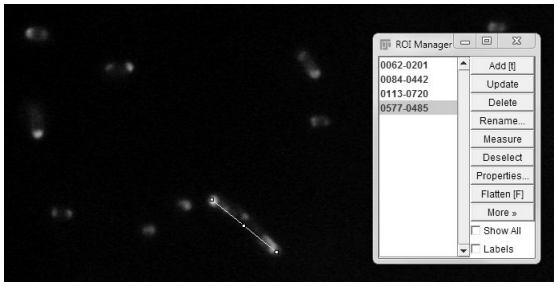
Using the Cell Counter method. ImageJ's Cell Counter plugin allows you to simply click on cells in an image to count them, and it allows you to use several separate counters to count different types of cells. In the image at right, the Type 1 counter was used to count elongated, filamentous cells (1) and Type 2 was used to count normal-length cells (22).



1. Open your image in ImageJ and choose Plugins | Analyze | Cell Counter. This plugin is installed in Fiji; if you are using the standard version of ImageJ, you may have to install it.
2. Click Initialize to get the plugin ready to run.
3. Click the radio button for a counter, and then simply start clicking cells. To change counters, click another radio button. Click Results to list counts for each counter.

Measuring Cell Length with ImageJ

Notice that Analyze Particles gives the length and width of the objects it finds, so this is one way to get the dimensions of cells or other objects in an image taken through the microscope. But, in some cases you may want to measure lengths that you define.



1. Open your image in ImageJ.
2. Click the straight-line selection tool in the toolbar.
3. Open the ROI ("region of interest") Manager by choosing Analyze | Tools | ROI Manager.
4. Draw a line the length of one of the cells, as shown at right. Then click Add to add this line to the list.
5. Draw a line the length of the next cell you want to measure. The first line will disappear, but the ROI Manager will remember it. Continue until you have added all the cells of interest.
6. Click Measure in the ROI Manager window. You will get a Results window with measurements for all the cells (note that Length is the very last column in the table; you may have to scroll to see it).
7. As always, these results can be copied to a spreadsheet. You could also used other tools to get, for example, the length, width and area of a rectangular or elliptical selection.

The measurements are in pixels, which is fine for *relative* measurements (e.g., how many cells are at least twice the average cell length?). To get actual sizes, you need a reference measurement that you can use to scale your length measurements. For an image captured with the Leica LAS software (see "Digital Photomicrography" in this *Handbook*), you can add a scale bar of known length to your image. Measure the scale bar in pixels, then use Analyze | Set Scale to enter the number of pixels and the actual length of the bar. Now, Results gives actual length in the units you chose.