

Biotechnology Education Infusion (BEI) Guide 2010

This Guide is a rich resource of information for teachers about biotechnology careers, funding opportunities, educational resources, professional opportunities, lab safety issues, bioethics, and laboratory activities including teacher preparation materials and student worksheets.

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This Guide is a rich resource of information for teachers about biotechnology careers, funding opportunities, educational resources, professional opportunities, lab safety issues, bioethics, and laboratory activities including teacher preparation materials and student worksheets.

I. NATIONAL SCIENCE FOUNDATION INFORMATION

- a. The BEI Guide was created as part of a National Science Foundation Advanced Technological Education grant program (DUE # 0757292). A link to the overview of the grant including goals, objectives, strategies and proposed outcomes can be found on the WSU Spokane CityLab website (<http://www.spokane.wsu.edu/researchoutreach/CityLab/biotech.html>).
- b. The National Science Foundation (NSF), an independent federal agency, was created in 1950 by Congress "to promote the progress of science; to advance the national health, prosperity, and welfare; to secure the national defense..." The NSF funds basic research and projects conducted by America's colleges and universities.
- c. The *Biotechnology Education Infusion (BEI) Project* was funded by an Advanced Technological Education grant from the NSF. An "A-Z list of funding opportunities" is available at <http://transcoder.usablenet.com/tt/www.nsf.gov/funding/azindex.jsp>. For more information, visit www.nsf.gov.

II. BIOTECHNOLOGY CAREERS EXPLORATION

- a. **ACTIVITY: Biotechnology Careers in Washington State using the *Biotechnology Industry Organization*.**
 - i. Go to <http://www.bio.org/> and click on *Member.BIO.org*.
 - ii. On the bar at the top of the page, click on *Member Directory*.
 - iii. Next click on "by US state" and find Washington State.
 - iv. Click on the website of a company of interest and look for the careers section on the website.
 - v. Find a scientific position (research and development, manufacturing and production, clinical research or quality control) or a nonscientific position (information systems, marketing and sales, regulatory affairs or administration/legal affairs) and click on view job (activity from *Biotechnology, Science for the New Millennium*. Paradigm Publishing, 2007. p.25):
 1. List the name of the company and describe the company.
 2. List the job title and location of the job.
 3. Name the education requirement for this position.

4. List the area of expertise desired.
5. Summarize the job responsibilities.
6. List the salary range if available.

b. ACTIVITY: Biotechnology Careers in Washington State using *BioSpace*.

- i. go to www.biospace.com and find Washington under the job search drop-down menu. Enter biotechnology for the job skill.
- ii. Find a scientific position (research and development, manufacturing and production, clinical research or quality control) or a nonscientific position (information systems, marketing and sales, regulatory affairs or administration/legal affairs) and click on view job.
 1. List the name of the company and describe the company.
 2. List the job title and location of the job.
 3. Name the education requirement for this position.
 4. List the area of expertise desired.
 5. Summarize the job responsibilities.
 6. List the salary range if available.

c. ACTIVITY: Plant Biotechnology Careers at Various Companies in the US

- i. Select a plant biotechnology company to research online. These may include DuPont, Inc, Dow AgroSciences, LLC, Mendel Biotechnology, Inc, Bayer CropScience, Shoffner Farms Research, Inc, etc. Answer the following questions (activity from *Biotechnology, Science for the New Millennium*. Paradigm Publishing, 2007. p.93):
 1. List the name of the company.
 2. List the main goals of the company.
 3. Where is the company located?
 4. List two important products and their uses.
 5. Name the education requirement for this position.
 6. List the area of expertise desired.
 7. Summarize the job responsibilities.
 8. List the salary range if available.

d. ACTIVITY: Biotechnology Careers at Various Companies in the United States.

- i. Choose one of the following biotechnology companies: Pfizer, Inc, Genentech, Inc, Chiron Corp, Amgen, Inc, Gilead Sciences, Inc, Baxter Healthcare Corp, Promega Corp, or Affymetrix, Inc
 1. List the name of the company and describe the company.
 2. List the job title and location of the job.
 3. Name the education requirement for this position.
 4. List the area of expertise desired.
 5. Summarize the job responsibilities.
 6. List the salary range if available.

e. ACTIVITY: Exploration of Biotechnology Careers in Washington State using *Washington Life Sciences*:

- i. Go to <http://www.washingtonlifescience.com> and click on “*Career*” on the top bar. View the job listings.
- ii. Find a scientific position (research and development, manufacturing and production, clinical research or quality control) or a nonscientific position (information systems, marketing and sales, regulatory affairs or administration/legal affairs) and click on a job of interest.
 1. List the name of the company and describe the company.
 2. List the job title and location of the job.
 3. Name the education requirement for this position.
 4. List the area of expertise desired.
 5. Summarize the job responsibilities.
 6. List the salary range if available.

f. Biotechnology Careers and Pathways

- i. Visit the *Washington Workforce Explorer* for current wages in biological technician fields at <http://www.workforceexplorer.com/cgi/dataanalysis/?PAGEID=4&SUBID=146>.
- ii. Visit the *Access Excellence Career Center* at <http://www.accessexcellence.org/RC/CC/#guides>. This site has a section on biotechnology with job descriptions and career profiles.
- iii. Visit the *North Carolina Biotechnology Center* and explore the various jobs in biotechnology at http://www.ncbiotech.org/resource_center/guide_to_biotechnology/what_jobs_exist_in_biotech/.
- iv. Visit the *North Carolina Biotechnology Center* and explore examples of biotechnology career pathways from North Carolina at http://www.ncbiotech.org/resource_center/guide_to_biotechnology/how_do_i_prepare_for_a_future_in_biotech/index.html
- v. The *California Society for Biomedical Research* has a brochure on careers and educational requirements at <http://www.ca-biomed.org/csbr/pdf/career.pdf>.
- vi. The *National Association of State Directors of Career and Technical Education* has a website that outlines career pathways and gives resources for helping students meet their career goals at www.careerclusters.org. There is a STEM brochure at <http://www.careerclusters.org/resources/ClusterDocuments/stemdocuments/brochure.pdf>.
- vii. Visit http://www.biotech.iastate.edu/biotech_info_series/bio2.html for information on careers in biotechnology.
- viii. A document called *Principles of Good Practice for Transfer and Articulation* may be viewed at <http://www.dhe.mo.gov/principlescredit.html>.

- ix. An example of a community college biotechnology program at Raritan Valley Community College can be found at <http://www.raritanval.edu/academics/biotech/index.html>. Another program is Southwestern College at <http://www.betsiproject.org/>.
- x. Another community college program offers a post-baccalaureate intensive certificate program in biotechnology. Visit <http://biotech.matcmadison.edu/>.
- xi. Accreditation guidelines may be viewed at www.abet.org.
- xii. Visit *Bellevue College Life Science Informatics* website for programs in bioinformatics <https://bellevuecollege.edu/informatics/>.
- xiii. NIH health and medical science career exploration can be found at <http://science.education.nih.gov/LifeWorks>

III. BIOTECHNOLOGY FUNDING RESOURCES FOR TEACHERS

- a. *Amgen Corporate Giving and Science Education*
http://wwwext.amgen.com/citizenship/science_education.html.
- b. *Intel grants* (<http://www.intel.com/community/washington/education.htm>).
- c. Visit the *Teachers Network* website at <http://www.teachersnetwork.org/> and click on the grants link. You will find links to science grants (http://www.teachersnetwork.org/grants/grants_science.htm) and technology grants (http://www.teachersnetwork.org/grants/grants_technology.htm).
- d. Visit the *Science Careers* funding directory from the journal *Science* at http://grantsnet.org/search/fund_dir.cfm. There are many opportunities for biotechnology.
- e. Visit www.grantwrangler.com to find grants for K-12.
- f. Visit the *MJ Murdock Charitable Trust* at <http://www.murdock-trust.org/> and click on the grants link.
- g. Visit Ellyn Daugherty's website (<http://ellyndaugherty.com/BiotechEd/>) and go to the link on *Bucks4Biotech* PowerPoint presentation for some great ideas for grant sources.
- h. Visit <http://www.ed.gov/fund/grants-apply.html?src=rt> for government grant opportunities.
- i. [Toyota TAPESTRY Grants for Science Teachers](#)
A total of 50 large grants of up to \$10,000 each, along with 20 to 30 mini-grants of up to \$2,500 each, will be awarded. Categories include environmental science, integrating literacy and science, and physical science.

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- j. [National Education Association Foundation](#) Accepting applications for \$2,000 and \$5,000 grants.
 - k. [National Council of Teachers of Mathematics](#) Several grants are available; deadlines vary with the grant program. Funds support professional development and content creation among other possibilities. There is also a "tips" link to help with writing proposals.
 - l. [National Science Foundation](#) Funding information and a searchable database, as well as guides for submission. Check the following link for grants for K-12 educators: http://www.nsf.gov/funding/education.jsp?fund_type=4
 - m. [US Department of Education](#) Grants generally have a short timeline, so keep checking for up-to-date funding information.
 - n. [Teachers Count](#) Use the right navigation bar to explore funding opportunities for professional development, the classroom, and more.
 - o. [Target Field Trip Grants](#) Grants of up to \$800 for trips to art museums, cultural events, civic experiences, or environmental sites.
 - p. [Washington Science Teachers Association](#) Regularly updates a list of funding resources.

IV. BIOTECHNOLOGY EQUIPMENT RESOURCES FOR TEACHERS

- a. *Science Education Partnership* (SEP, sponsored by Fred Hutchinson Cancer Research Center) kit loan program for teachers who have completed SEP training: (http://www.fhcr.org/science/education/educators/sep/kit_loan/index.html).
- b. *WSU Spokane City Lab*. Contact Sylvia Oliver (olivers@wsu.edu) for equipment and supply needs. (<http://www.spokane.wsu.edu/ResearchOutreach/CityLab/>).
- c. Find biotechnology companies in your area at www.bio.org and click on member directory and go to Washington State (<http://bio.org/members/biomembers.asp?list=WA>). Many companies donate excess supplies and equipment to local schools.

V. BIOTECHNOLOGY EDUCATIONAL RESOURCE WEBSITES

- a. Visit <http://learn.genetics.utah.edu/> and <http://teach.genetics.utah.edu/> for instructions, **student worksheets**, **answer keys** and overhead masters on a variety of topics, including stem cells, cloning, SNPs, pharmacogenomics, microarrays,

labs, epigenetics, heredity and traits, gene therapy and much more. There are also virtual labs on PCR, gel electrophoresis, DNA extraction and DNA microarrays.

- b. The *National Institutes of Health Office of Science Education* offers educational resources on a variety of topics (<http://science.education.nih.gov/home2.nsf/Educational%20Resources/Topics/>). There are also free copies of books, including one entitled *Inside the Cell*.
- c. The *Biotechnology Education Program* (<http://education.llnl.gov/bep/>) has **student worksheets** on a variety of biotechnology topics, including electrophoresis, micropipets, transformation and making solutions. There are also teacher lecture materials.
- d. Visit the *Genetics/Biotechnology Theme Pages* for links to various curricular resources and classroom activities relating to biotechnology at <http://www.cln.org/themes/genetics.html>.
- e. Visit the *Biotechnology Project* at MATC for teaching resources. For each activity, there is a **teacher protocol, student protocol and slide presentation**. The activities include DNA, bioremediation, the smallpox vaccine, a kit-free genetically modified organism lab and more. There are also resources for basic lab methods and protein purification and folding. The website is at <http://biotech.matcmadison.edu/>. Lisa Seidmen is one of the contacts on this website, the author of *Basic Laboratory Methods for Biotechnology*.
- f. Visit the *Tulsa Community College SeedBed* website at <http://www.tccbiotech.org/activities.html> for **classroom activities and worksheet**. There are activities involving restriction enzymes, proteomics, molecular forensics and more. **Student worksheets** are included.
- g. The *Federal Resources Educational Excellence* offer free teaching and learning resources in science at <http://www.free.ed.gov/>.
- h. Visit <http://publications.nigms.nih.gov/thenewgenetics/> for a free copy of *The New Genetics*. There are also interactive and accessible crossword puzzles.
- i. The *National Human Genome Research Institute* (www.genome.gov) offers educational resources, including transcripts and multimedia downloads on sequencing the genome, bioinformatics, genes, variation and human history and more. There are also vignettes and discussion material concerning ethical, legal and social implications of genetic knowledge (<http://www.genome.gov/25019880>). **Student worksheets** are available for some activities.
- j. There are a variety of educational materials from the *Human Genome Project Information* website at

- http://www.ornl.gov/sci/techresources/Human_Genome/education/education.shtml . There are downloadable primers on genomics, posters, **teaching modules** on bioinformatics, ethics, public policy, forensics, genetically modified organisms and more. There are teacher background information resources and **student lessons**.
- k. The *Northwest Association for Biomedical Research* has educational materials on ethics, HIV, stem cells and other lessons that include **student handouts** and **teacher guides** at (<http://www.nwabr.org/education/index.html>).
- l. *Access Excellence* at <http://www.accessexcellence.org/index.php> has resources relating to life sciences. There is an activities exchange site at <http://www.accessexcellence.org/AE/>. There is a lambda DNA fingerprinting simulation, DNA detectives, a DNA profiling activity and more in the Mystery Spot. A search under biotechnology in the activities exchange (<http://www.accessexcellence.org/AE/ATG/> yields over 14 different **activities**. There is also a biotech applied page at <http://www.accessexcellence.org/RC/AB/BA/#Anchor-23240>.
- m. Visit the *National Center for Biotechnology Information* at <http://www.ncbi.nlm.nih.gov/About/outreach/courses.html> for educational resources, including a BLAST information guide, an Entrez tutorial, Genes and Disease and more. The *Science Primer* (<http://www.ncbi.nlm.nih.gov/About/primer/index.html>) has links to bioinformatics, microarray technology, SNP's, molecular genetics and more.
- n. The *California Society for Biomedical Research* has downloadable **educational materials** at <http://www.ca-biomed.org/csbr/download.php>.
- o. *Genetics Education Partnership* from the University of Washington offers free instructional materials and **classroom activities** (<http://genetics-education-partnership.mbt.washington.edu/default.htm>).
- i. There are classroom activities for high schools and middle schools that include downloadable **student worksheets**
 1. High School: Sickle Cell Anemia, a Case Study (<http://genetics-education-partnership.mbt.washington.edu/class/high.htm>).
 2. Genetics at the Middle School Level: Toothpick Fish (<http://genetics-education-partnership.mbt.washington.edu/class/middle.htm>).
 - ii. The genetics education guide includes mapping of genetics concepts to state and national education standards.
 - iii. There are many instructional materials that have been reviewed for strengths and weaknesses for high and middle school students including a biotechnology manual and biotechnology projects for young scientists (<http://genetics-education-partnership.mbt.washington.edu/rev/revgrade.html#anchor412778>)

- p. *Biotechnology: Science for the New Millennium* by Ellyn Daugherty (<http://ellyndaugherty.com/BiotechEd/>). There are activities available online. Downloadable **biotechnology activities and labs** also at <http://ellyndaugherty.com/BiotechEd/coolthings.htm>.
- q. A protein folding game for students called *Fold It* is available online at <http://depts.washington.edu/bakerpg/>.
- r. *Seattle Biomedical Research Institute* has a *BioQuest Science Education Program* (<http://www.sbri.org/sci-ed/index.asp>) that offers teacher professional development, including **teacher and student packets** on tuberculosis, vaccines and malaria.
1. Tuberculosis curriculum (<http://www.sbriobioquest.org/content/tuberculosis-curriculum>)
 2. Vaccine curriculum (<http://www.sbriobioquest.org/content/vaccine-and-meningitis-curriculum>)
 3. Malaria curriculum (<http://www.sbriobioquest.org/content/malaria-curriculum>). This site also includes an animation featuring microarrays in vaccine development.

VI. BIOTECHNOLOGY PROFESSIONAL OPPORTUNITIES FOR STUDENTS AND TEACHERS

Biotechnology Science Fairs

- a. Student *BioExpo* in Seattle, WA coordinated through the Northwest Association for Biomedical Research (<http://www.nwabr.org/studentbiotech/default.html>)
- b. *Inland Northwest High School Science Symposium* at <http://academic.cahnrs.wsu.edu/images/Inland%20Northwest%20High%20School%20Science%20Symposium-2008-12.pdf>. Contact information is available on this website. For current information, contact Thomas Stralser at tstralser@cheneysd.org.

Biotechnology Professional Development for Teachers

- c. *Ellyn Daugherty's Biotechnology* workshops (<http://ellyndaugherty.com/BiotechEd/>)
- d. *Science Education Partnership* (SEP) sponsored by Fred Hutchinson Cancer Research Center (http://www.fhrc.org/science/education/educators/sep/about_sep/index.html).
- e. Visit the *CDC Science Ambassador Program* at <http://www.cdc.gov/excite/ScienceAmbassador/ScienceAmbassador.htm> for middle and high school science teachers to team up with the CDC and create epidemiologic-based lesson plans.

- f. Science on Tap is open to the public to hear scientists and researchers talk about the latest science and technology in a casual and informal setting in the Seattle area (<http://www.scienceontap.org/>)

Biotechnology Research Opportunities for High School Students in Washington State

- g. *WSU Spokane City Lab*. Contact Dr. Sylvia Oliver (olivers@wsu.edu) for available opportunities. (<http://www.spokane.wsu.edu/ResearchOutreach/CityLab/>).
- h. *Fred Hutchinson Cancer Research Center*, Seattle, WA
1. Hutch High, a half-day symposium for sophomore biology students in Seattle (<http://www.fhcrc.org/science/education/hs/index.html>).
 2. Summer training program, a 10 week research experience for high school students (<http://www.fhcrc.org/science/education/hs/index.html>).

Biotechnology/Science Outreach Resources for Students of Color and Young Women

- i. Seattle chapter of the Association for Women in Science (<http://www.seattleawis.org/>).
1. Women may apply for educational awards from the Association for Women in Science at (<http://www.awis.affiniscape.com/displaycommon.cfm?an=1&subarticlenbr=67>).
 2. Various programs are available in the Seattle area, including mentoring (<http://www.seattleawis.org/programs.htm>).
- j. Expanding Your Horizons in Science and Mathematic to encourage girls to pursue careers in science and math (<http://www.expandingyourhorizons.org/>)
- k. Washington Mathematics, Engineering, Science Achievement (MESA) (<http://www.washingtonmesa.org/>)

VII. IDEAS FOR ONLINE STUDENT ACTIVITIES

These activities were based on those from Ellyn Daughtery's book, *Science for the New Millennium*.(2007, Paradigm Publishing, Inc.).

- a. Visit the website, *Genomics and Its Impact on Science and Society: The Human Genome Project and Beyond*, at http://www.ornl.gov/sci/techresources/Human_Genome/publicat/primer2001/2.shtml and answer the following questions:
1. What are the goals of the Human Genome Project (HGP)?
 2. What was the origin of the HGP?
 3. Who were the people involved in launching the HGP?
 4. When was the HGP completed and how has its completion impacted research worldwide?
 5. List three social concerns arising from the new genetic information.

6. What future developments are now possible based on our understanding of the sequence of the human genome?
- b. Visit this *Genetech* website at <http://www.gene.com/gene/products/profiles/> and select a profile of a patient to learn how much a biotechnology product can improve a patient's life. Answer the following questions:
 1. List the name of the patient:
 2. List the name of the drug:
 3. Name the disease the patient experienced:
 4. Write a short summary of the story.
 5. If you worked at a biotechnology company, which disease would you like to target with a biotechnology product?
 6. Is there currently a biotechnology company working on a product for the disease you chose? Research this online and provide a reference.
 - c. Visit the *Public Broadcast System* (PBS) website at www.pbs.org/wgbh/harvest/exist/. Read the instructions for the survey. You will be asked seven times, "Based on what you now know, do you think we should raise genetically modified (GM) crops?" On a separate sheet of paper, write at least one sentence explaining the reason for your "yes" or "no" answers to the question each time you are presented with an argument. Your position may change based on the arguments. After you have taken your seventh position, make a final decision regarding your position. Write 3-5 sentences that summarize your position. Other viewpoints may be explored at www.pbs.org/wgbh/harvest/viewpoints/.
 - d. Search the *USDA* website (www.USDA.gov) for the FlavrSavr® tomato, the first genetically engineered food to the stores.
 1. Describe what was unique about this tomato.
 2. When was this tomato FDA approved?
 3. Is this tomato at the market? Why or why not?
 - e. Instruct students to visit the *Genentech Inc.* website www.gene.com/gene/pipeline/status/ and answer the following questions:
 1. Find a drug product in phase I of clinical trials.
 - a. Write the name of this drug.
 - b. List the potential applications of the drug.
 - c. In your own words write a description of the potential drug.
 - d. Describe what it means to be in phase I of clinical trials.
 2. Find a drug product in phase II of clinical trials.
 - a. Write the name of this drug.
 - b. List the potential applications of the drug.
 - c. In your own words write a description of the potential drug.
 - d. Describe what it means to be in phase II of clinical trials.
 3. Find a drug product in phase III of clinical trials.
 - a. Write the name of this drug.

- b. List the potential applications of the drug.
 - c. In your own words write a description of the potential drug.
 - d. Describe what it means to be in phase III of clinical trials.
 - e. Are there already approved uses for this drug and safety warnings?
- f. Visit the website www.sciencemuseum.org.uk/antenna/dolly/index.asp to see how Dolly was created.
1. Summarize how Dolly was cloned.
 2. What are telomeres?
 3. What is the significance of Dolly's telomeres?
 4. What was the cause of her Dolly's death?
- g. Visit the *California Society for Biomedical Research* at www.ca-biomed.org/csbr/students.php. Click on the students' arena. On the next page, click on the turquoise-colored circle to see the contribution of animals to research.
1. List two animals you chose to research.
 2. For each animal you chose, list three contributions of the animal.
- h. Pharmaceutical companies must be certain their products are properly identified and free of contaminants. The federal government has strict guidelines to ensure safe production. Companies are regularly checked for compliance. In groups of four students, produce a poster that illustrates one section of the *Code of Federal Regulations for Current Good Manufacturing Practices* (cGMP). Go to <http://www.fda.gov/AboutFDA/CentersOffices/cder/ucm095412.htm> and click on *Facts About Current Good Manufacturing Practices*. Select a section to create your poster. (Another idea may be to create a worksheet from the frequently asked questions sheet.)

VIII. ONLINE STUDENT ACTIVITIES AND ANIMATIONS RELATING TO BIOTECHNOLOGY

- a. A variety of virtual labs and animations are available at <http://learn.genetics.utah.edu/> and <http://teach.genetics.utah.edu/>.
- b. Students can analyze a DNA gel electrophoresis and run a **virtual lab** with *Virtual Lab: Agarose Gel Electrophoresis of Restriction Fragments* at <http://arbl.cvmb.colostate.edu/hbooks/genetics/biotech/gels/virgel.html>.
- c. Students can read about personal genomics (*The Scientific Foundation for Personal Genetics: Recommendations from a National Institutes of Health-Center for Disease Control and Prevention Multidisciplinary Workshop*, http://www.cdc.gov/genomics/resources/file/print/2009-08_personal_genomics_GIM.pdf), find out current uses of genomics in disease research and create their own family history file on the *CDC Public Health Genomics* website (<http://www.cdc.gov/genomics/famhistory/famhist.htm>).

- d. There is an **RT-PCR animation** at http://www.bio.davidson.edu/courses/Immunology/Flash/RT_PCR.html and a PCR animation at <http://www.dnalc.org/ddnalc/resources/shockwave/pcranwhole.html>.
- e. Visit the Dolan DNA Learning Center Biology **Animation Library** Index at <http://www.dnalc.org/ddnalc/resources/animations.html> for animations on gene chips, transformation, cloning, microarrays, PCR, sequencing, electrophoresis, stem cell lines and more.
- f. Visit a *PBS* website at www.pbs.org/wgbh/aso/tryit/dna/# to do an activity on **DNA replication and protein synthesis**. There are also interactive activities on atom building and evolution at <http://www.pbs.org/wgbh/aso/tryit/>.
- g. Visit <http://www.hhmi.org/biointeractive/genomics/genechipdata/index.html> for a **microarray animation** plus a wide variety of other biology mini-lessons .
- h. Visit the *Facts About Current Good Manufacturing Practices* website (<http://www.fda.gov/Drugs/DevelopmentApprovalProcess/Manufacturing/ucm169105.htm>) and answer the following questions:
 - i. Give two reasons why it is important to adhere to the cGMPs at pharmaceutical companies.
 - ii. List two reasons why cGMPs are so important.
 - iii. How does the FDA determine if companies are in compliance?
 - iv. What are some consequences of cGMP violations?

IX. BIOTECHNOLOGY ONLINE RESOURCES

- a. www.ncbi.nlm.nih.gov/PubMed/
- b. www.bio-link.org is funded by the *NSF* to strengthen and expand biotechnology technician education.
 - i. Visit biotechnology in the news at <http://www.bio-link.org/newslist.htm>.
- c. *Genetic Engineering and Biotechnology News* at <http://www.genengnews.com/bestofweb/>.
- d. *Biotechnology Industry Organization*. www.bio.org
- e. *USDA office of Biotechnology* at <http://www.aphis.usda.gov/biotechnology/index.shtml>
- f. *National Agriculture Biotechnology Council* at <http://nabc.cals.cornell.edu/> .
- g. *Agricultural Biotechnology* at <http://agribiotech.info/>.
- h. *AgBiotechNet* at <http://www.cabi.org/agbiotechnet/> .

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- i. *BioSpace* at www.biospace.com
 - j. *National Science Foundation* at www.nsf.gov
 - k. *Biotechnology-Bioinformatics Discovery Project* at <http://www.occc.edu/bbdiscovery/>.
 - i. This includes suggested **course outlines** for biotechnology, **lab notebook keeping and grading rubric**, PCR without kits and more at http://www.occc.edu/bbdiscovery/documents/High%20School%20Biotech/main_page.htm.
 - ii. A PowerPoint presentation on *What Is Biotechnology* is available at http://www.occc.edu/bbdiscovery/PowerPoint/whatisbiot_files/frame.htm.
 - iii. There are also resources for **middle school teachers** at <http://www.occc.edu/bbdiscovery/documents/MSprebiot.html>. There are lab protocols.
 - l. *Bio-Rad Biotechnology Explorer Kits and Curriculum* at <http://www.bio-rad.com/evportal/evolutionPortal.portal?nfpb=true&pageLabel=verticalLandingPage&catID=1450>.
 - m. *Science for All Americans* from AAAS are recommendations for what all American citizens should know about science at <http://www.project2061.org/>.
 - n. The *National Science Teachers Association Learning Center* at <http://learningcenter.nsta.org/> has materials on biotechnology.
 - o. The *USDA Agricultural Research Service* website at <http://www.ars.usda.gov/main/main.htm> has spotlights with agricultural biotechnology applications.
 - p. For more information on bioinformatics, see the *National Center for Biotechnology Information* at <http://www.ncbi.nlm.nih.gov/About/primer/bioinformatics.html>.
 - q. Visit the *North Carolina Biotechnology Center Guide to Biotechnology* at http://www.ncbiotech.org/resource_center/guide_to_biotechnology/index.html. There are links for biotechnology products, careers and industries.
 - r. Visit the *Biotechnology Institute* at <http://www.biotechinstitute.org/>. There are links to careers in biotechnology and education resources with activities on extracting DNA from fruit.
 - s. The *DNA Age* is a series of articles and video clips from the *New York Times* at <http://topics.nytimes.com/topics/news/national/series/dnaage/index.html>.

t. **Biotechnology Books/Textbooks/Manuals**

- *Biotechnology: Science for the New Millennium* by Ellyn Daugherty. Paradigm Publishing, Inc., St. Paul, MN, 2007.
- *Biotechnology: Science for the New Millennium* Instructor's Guide by Ellyn Daugherty. Paradigm Publishing, Inc., St. Paul, MN, 2007.
- *Biotechnology: Science for the New Millennium* Course Planner by Ellyn Daugherty. Paradigm Publishing, Inc., St. Paul, MN, 2007.
- *Biotechnology Laboratory Manual* by Ellyn Daugherty. Paradigm Publishing, Inc., St. Paul, MN, 2007.
- *Basic Laboratory Methods for Biotechnology* by Lisa Seidmen and Cynthia Moore, Benjamin Cummings, 2009.
- *Shoestring Biotechnology: Budget Oriented High Quality Biotechnology Laboratories for Two-Year College and High School*, Biotechnology Institute and NABT. (http://www.biotechinstitute.org/resources/shoestring_biotech.html).

X. BIOTECHNOLOGY SAFETY

- a. Visit <http://biotech.matcmadison.edu/resources/methods/safety.htm> to view resources for teaching about safety.
- b. Record the location of the *Material Safety Data Sheet* (MSDS) for chemicals in your classroom. Your teacher will select chemicals to research. Record the following information for each chemical in a chart (from *Biotechnology: Science for the New Millennium*. pg. 93)
 1. molecular weight of the compound
 2. appearance (texture, color, and phase, etc)
 3. melting point
 4. solubility
 5. flammability
 6. action to take if it gets on skin
 7. action to take if it gets in eyes
 8. actions to take if it is ingested or inhaled
 9. other safety precautions

XI. BIOTECHNOLOGY MATH

- a. Visit the *Biotechnology Education Program* and click on the math link for a variety of activities at <http://education.llnl.gov/bep/math/math.html>. **Student worksheets** are included.
- b. Visit Ellyn Daugherty's website at <http://ellyndaugherty.com/BiotechEd/> and click on the presentation *5 Steps to Teach Solution Prep (5 Steps SolnPrep)*. There is easy to understand information on metric conversions, solutions, molarity and more.

- c. *Basic Laboratory Calculations for Biotechnology* by Lisa Seidman, Benjamin Cummings, 2008.
- d. pH calculator website at http://www.sensorex.com/support/education/pH_calculator.html. Visit this site to calculate the pH of a solution. You can type in the concentration, weight or volume of a chemical to find the pH. There is also a pH electrode technical education link at http://www.sensorex.com/support/education/pH_education.html.

XII. BIOETHICS

- A. Visit the *Northwest Association for Biomedical Research* at <http://www.nwabr.org/>. There is a complete ethics primer along with case studies, teacher-developed **lesson plans** and teacher-developed **action plans** under the education tab. Stem cells, HIV and other lessons are also provided.
- B. Read *Your Genes, Your Choices* by Catherine Baker, at this website: http://www.ornl.gov/sci/techresources/Human_Genome/publicat/genechoice/index.html. There are four examples of ethical issues concerning genomics. There are interesting ethical questions raised at the end of each story.
- C. Students may want to explore the ethics of cloning pets. Find a pro and con article and discuss the ethics of cloning pets chart (from *Biotechnology: Science for the New Millennium*. pg. 302)
- D. Investigate the story of the business *The Prodigene, Inc.* What mishap occurred at this business? You can visit <http://www.aphis.usda.gov/lpa/news/2002/11/prodigene.html> and http://www.historycommons.org/entity.jsp?entity=prodigene_1

XIII. ONLINE RESOURCES ON GENETICS

A. Online Genetics Education Resources listed on the National Human Genome Research Institute Website (<http://www.genome.gov/10000464>)

The following are resources from this site. .

1. Access Excellence

www.accessexcellence.org

A series of learning modules on multiple science and health topics, including biotech and genetics. Sponsored by the National Health Museum, a non-profit organization founded by former U.S. Surgeon General C. Everett Koop.

2. American Medical Association - Family History Tools

www.ama-assn.org/ama/pub/category/2380.html

Tools for gathering family history.

3. Current Topics in Genome Analysis 2010

<http://www.genome.gov/12514286>

A 15-lecture series covering the major areas of genomics.

- 4. Department of Genome Sciences Education Outreach Project**
chroma.mbt.washington.edu/outreach/outreach.html
Innovative programs that bring laboratory science and materials to K-12 students and teachers. Directed by the Department of Genome Sciences at the University of Washington in Seattle, Wash.
- 5. Diving into the Gene Pool**
www.exploratorium.edu/genepool/genepool_home.html
An online exhibition exploring genetics and the Human Genome Project from a variety of perspectives. Produced by the Exploratorium, San Francisco, Calif.
- 6. The DNA Files**
www.dnfiles.org/
A series of 14 one-hour public radio documentaries and related information.
- 7. DNA from the Beginning**
www.dnafb.org/dnafb
An animated primer on the basics of DNA, genes and heredity.
- 8. DNA Interactive**
www.dnai.org/index.htm
DNA and genome-related teaching guides and lesson builders, personalized Web pages, *My DNA*, student activities, more.
- 9. Dolan Learning Center**
www.dnalc.org/
Dolan's mission is to prepare students and families to thrive in the gene age, envisioning a day when all elementary students are exposed to principles of genetics and disease risk; when all high school students have the opportunity to do hands-on experiments with DNA; and when all families have access to genetic information they need to make informed health care choices. Includes an interactive DNA timeline.
- 10. Foundations of Classical Genetics**
www.esp.org/foundations/genetics/classical
Complete versions of classic genetics works written between 350 A.D. and 1932.
- 11. GeneTests**
www.genetests.org/
Information for health professionals about hundreds of genetic tests.
- 12. Genetic Science Learning Center**
gslc.genetics.utah.edu
From the Eccles Institute of Human Genetics at the University of Utah, a Web site created to help people understand how genetics affects their lives and society.
- 13. Genetics and Disease Prevention Information**
www.cdc.gov/genomics/default.htm2
Resources on genetics, including journals, reports and fact sheets. Also includes online multimedia presentations ranging from basic genetics to latest research.

-
- 14. Genetics and Molecular Medicine** (American Medical Association)
www.ama-assn.org/ama/pub/category/1799.html
Links to current articles and other resources
 - 15. Genetics and Your Practice**
www.marchofdimes.com/gyponline/index.bm2
A practical "how to" site on clinical genetics from the March of Dimes.
 - 16. Genetics at About.Com**
biology.about.com/cs/genetics/index.htm?terms=genetics
Genetics Web resources featured at About.Com, a homework help site.
 - 17. Genetics Education Center**
www.kumc.edu/gec
A comprehensive listing of genetics education resources, including networking sites, documentary films, lectures, booklets, activities, and programs. Compiled by the Genetics Education Center, University of Kansas Medical Center.
 - 18. Genetics Education Partnership**
genetics-education-partnership.mbt.washington.edu
Teacher instruction guides, curricula, classroom activities and suggested outreach activities on genetics. Produced by the Genetics Education Partnership, a coalition of Washington state teachers and genetics professionals committed to genetics teaching.
 - 19. Genetics Home Reference**
<http://ghr.nlm.nih.gov>
Provides consumer information about genetic conditions and the genes or chromosomes responsible for those conditions.
 - 20. Genetics in Clinical Practice: A Team Approach**
iml.dartmouth.edu/education/cme/Genetics/
Virtual Genetics Clinic.
 - 21. Genetics in Primary Care**
genes-r-us.uthscsa.edu/resources/genetics/primary_care.htm
Training program curriculum materials.
 - 22. Genetics Program for Nursing Faculty**
www.gpnf.org
Links to genetics resources of particular interest to nurses.
 - 23. Genetics Origins**
www.geneticorigins.org/geneticorigins
Provides biochemical methods and computer tools to allow students to use their own DNA "fingerprints" as a starting point in the study of human evolution.
 - 24. Genome Gateway**
www.nature.com/genomics
Comprehensive Web resource on genetic information. Hosted by Nature Publishing Company.
 - 25. Genome News Network** (The Center for the Advancement of Genomics)
www.genomenewsnetwork.org/index.php
Original articles and links

-
- 26. The Genomic Resource Centre**
www.who.int/genomics/en
From the World Health Organization, provides information and raises awareness on human genomics.
 - 27. The Genomic Revolution**
www.amnh.org/exhibitions/genomics/0_home/index.html
An online exhibit about genomics. Produced by the American Museum of Natural History, N.Y.
 - 28. The Human Genome**
www.ncbi.nlm.nih.gov/genome/guide/human/
Comprehensive one-stop genomic information center. Hosted by the National Center for Biotechnology Information (NCBI) of the National Library of Medicine (NLM).
 - 29. Human Genome Epidemiology Network (HuGENet)**
<http://www.cdc.gov/genomics/hugenet/default.htm>
Hosted by the Centers for Disease Control (CDC), an international collaboration for sharing population-based human genome epidemiologic information.
 - 30. Human Genome Project Education Resources**
www.ornl.gov/hgmis/education/education.html
An extensive collection of publications, teaching aids, and additional internet resources. Hosted by the Human Genome Program of the U.S. Department of Energy.
 - 31. infoGENETICS**
www.infogenetics.org/
Clinical practice tools.
 - 32. Information for Genetics Professionals**
www.kumc.edu/gec/geneinfo.html
Educational, clinical, and research resources.
 - 33. MendelWeb**
www.mendelweb.org/
Mendel's papers in English and German and related materials.
 - 34. National Coalition for Health Professional Education in Genetics**
www.nchpeg.org/
Core competencies in genetics and reviews of education programs.
 - 35. National Library of Medicine: PubMed**
www.ncbi.nlm.nih.gov/PubMed
Basic search engine for biomedical research, including research and commentary regarding clinical research ethics and regulations.
 - 36. The New Genetics: A Resource for Students and Teachers**
www4.umdj.edu/camlbweb/teachgen.html
Links to genetic education resources.
 - 37. Online Mendelian Inheritance in Man (OMIM)**
<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>
Information about human genes and disease.

38. Scitable

www.nature.com/scitable

A free science library and personal learning tool brought to you by Nature Publishing Group, the world's leading publisher of science. Scitable currently concentrates on genetics.

39. Tokyo Medical University Genetics Study Group

www.tokyo-med.ac.jp/genet/index-e.htm

The Tokyo Medical University Genetics Study Group developed this animated site to help provide better visual understanding of how chromosome abnormalities occur.

40. Understanding Gene Testing

www.accessexcellence.org/AE/AEPC/NIH/index.html

An informative, illustrated tutorial on genes and genetic testing. Hosted by the National Cancer Institute.

41. What's a Genome?

http://www.genomenetwork.org/resources/whats_a_genome/Chp1_1_1.shtml

An informative overview of genomics presented by the Genome News Network. Topics include: What's a Genome?, What's Genome Sequencing? and What's a Genome Map?

42. Your Genes Your Health

www.ygyh.org

A multimedia guide to genetics disorders.

43. Your Genome

www.yourgenome.org

Produced by the Wellcome Trust Sanger Institute, Your Genome provides an introduction to the main concepts of DNA, genes & genomes, focusing on basic questions such as "What is a genome?" and "What are genes?" There is also an introduction to the Human Genome Project and much more.

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Using a Digital Micropipette (<http://education.llnl.gov/bep/science/science.html>)**USING THE DIGITAL MICROPIPETTE , Student Worksheet****Introduction**

Molecular biologists and genetic engineers work with such small quantities that special tools had to be developed to ensure accurate measurements. One of these tools is the **digital micropipette**. For biotechnology, it is as common a tool as a graduated cylinder. Without such precision, genetic engineering would be severely hampered.

Objectives

- * The student will correctly use the micropipette.
- * The student will mix liquids in a microcentrifuge tube.
- * The student will load a gel.
- * The student will convert measurements between the units microliters (μl) and milliliters (ml).

Materials

1. Digital micropipette
2. Box of micropipette tips
3. Tubes of food coloring
4. Waste container
5. Tubes of H₂O and loading dye
6. An agarose gel (2%)
7. Parafilm
8. Large culture bowl

PROCEDURE**Part A: Measurement**

You already know how to measure volume in milliliters (ml). This investigation will teach you how to use the micropipette so that you will be able to carry out future labs involving more critical measurements using a special unit called the microliter (μl). Below are some equivalents for these units. Fill in the blanks below with the correct values.

1ml = 1/1000 liter _____ml = 1 liter

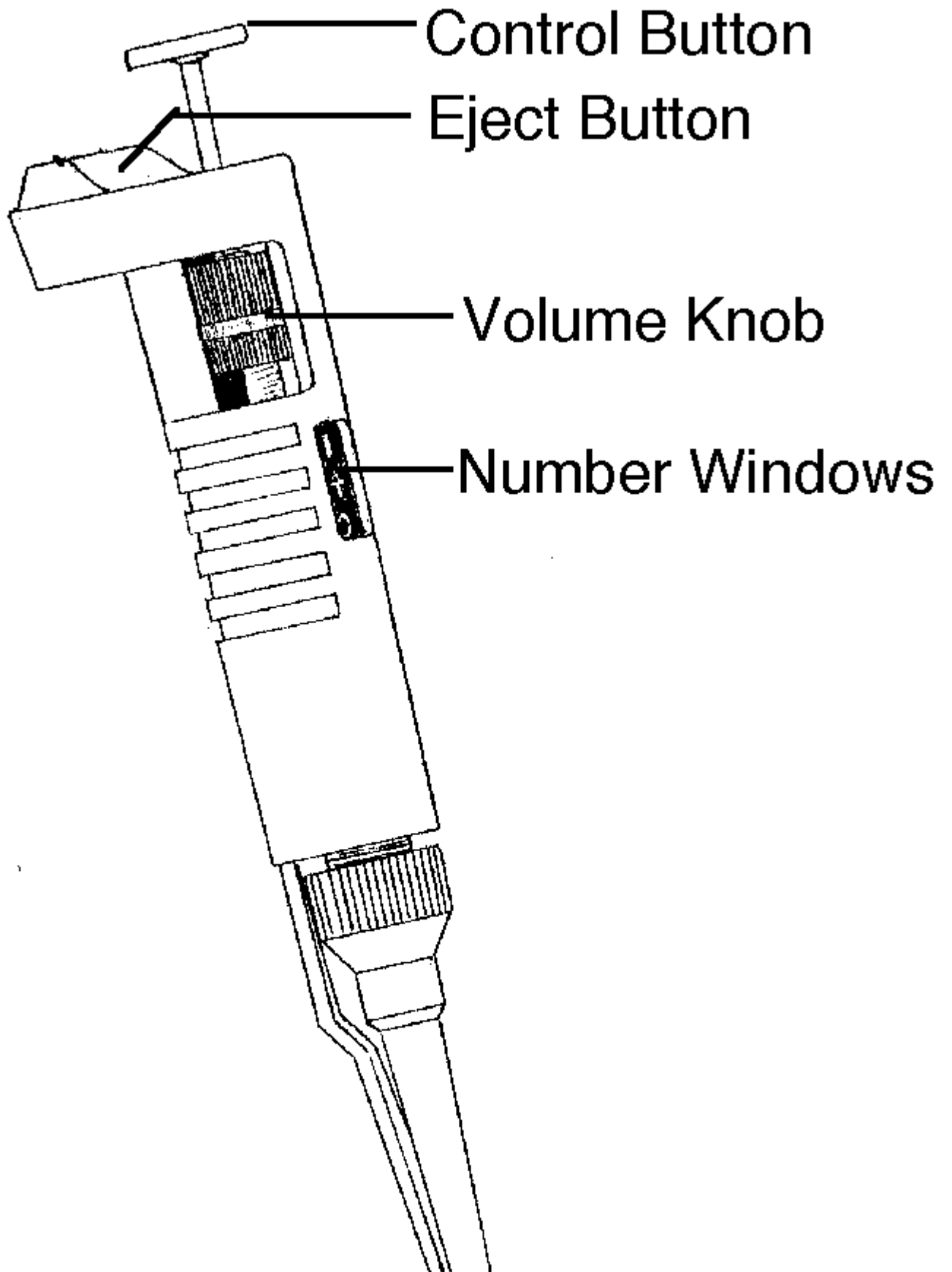
1 μl = 1,000,000 liter _____ μl = 1 liter

1,000 μl = 1ml _____ml = 1 μl

Part B: Some DON'TS

1. Do not use the micropipette without a disposable tip in place. Moisture may be drawn up inside the pipettor damaging the internal piston.

2. Do not lay either a liquid laden micropipette or a pipettor with an empty wet tip down. Moisture can run back inside causing damage to the piston.
3. Do not allow the control button to snap back when drawing up a sample. Keep your thumb on the control button allowing it to return gradually.



Part C: How to Dial the Volume

1. Different micropipettes use different ways to adjust to a specific volume. Have your teacher show you how to dial a volume on the pipettes you will be using for this particular lab.
2. Seeing what you are doing is very important. So always hold the micropipette in a vertical position at eye level when measuring a liquid.

Part D: Drawing Up a Sample

1. Practice pushing the yellow control knob. You should feel two stops. The first stop provides you with the dialed volume on the window. You use this stop when drawing up the desired volume. The second stop will release the desired volume. You push the plunger all the way when releasing the liquid.
2. Push the pipettor firmly into one of the disposable tips in the tip box.
3. Push down on the yellow control button with your thumb to the first stop and hold the button in that position. Dip the pipette tip into the liquid to be withdrawn (never dip into the liquid deeper than the top of the tip) and **slowly** raise the control button to draw up the liquid into the tip.

Part E: Dispensing the Measured Liquid

1. Insert the tip into a tube and slowly push the control button down to the first stop, wait a second. Now push the control button down to the second stop to blow out the final amount of liquid. **Keep the button fully depressed**, and while lifting the micropipette from the liquid drag the tip along the side of the tube. Now release the control button by slowly raising your thumb. DONT let the button snap back!
2. Now dispose of the used tip by placing it in the waste container. Hold the micropipette over the waste container and depress the eject button (raised blue area next to the control button) with your index finger. The tip will pop off the end of the pipettor.

Part F: Practice Withdrawing and Dispensing

1. Dial the micropipette to 10.0 μl . Each partner will withdraw three samples from the tube labeled H_2O and release them into a second tube following all the steps above except do not eject the tip until you have finished transferring your three samples.
2. Repeat the practice for 12 μl , 5 μl , and 19 μl .

Part G: Loading a Gel

1. In a culture bowl submerged under water is an agar gel with two rows of small rectangular wells (pockets for holding a small amount of liquid). For practice, you and your partner are going to load the wells with ordinary food coloring. Be sure you don't push the tip into the gel. YES, use both hands and support your elbows on the lab table top.
2. Use the scheme below for loading your gel. Begin by loading the end row of wells then move to the middle row. Load all the red lanes first, green second and finally yellow. You only need to change the tip when you switch to a different color. This saves time and tips.

Lane	Color	Volume
1	Red	8.2 μ l
2	Green	6.8 μ l
3	Yellow	9.3 μ l
4	Red	4.7 μ l
5	Green	2.5 μ l
6	Yellow	7.5 μ l
7	Red	3.9 μ l
8	Green	11.2 μ l

Part H: Extra Practice

1. Cut a piece of wax paper or parafilm. Lay it flat on the desk.
2. Adjust the micropipette to 12 μ l.
3. Using only one food color, write your name in 12 μ l drops on the plastic paper.
4. Repeat until the drops are equally spaced and your name can be clearly read.

Analysis

1. How precisely can a digital micropipette measure volume?
2. When should you use a new disposable tip?
3. What is the purpose of the first stop on the control button?
5. What is the purpose of the second stop on the control button?
6. Why should you use the mixing technique before drawing up a sample from a stock solution?

USING THE DIGITAL MICROPIPET, TEACHER PREPARATION

Advanced Preparation

- Agar: Making up 2% agar in tap water can be done a day or two in advance if they are kept moist and cool. An agar solution should be heated almost to boiling to ensure that it is completely dissolved. Using a microwave is simple and quick (20 seconds on high for a 25 ml gel).
- Gels: You should have as many gel trays prepared as you have agar. Each gel requires 25 ml of agar. Line the gel trays inside the gel boxes and pour the agar into one side. If you are making a large batch of agar, premeasured 25 ml per gel tray using, for example, a 50 cc centrifuge tube. For the dye lab, the combs should be placed in the center of the tray.
- Micropipette tips: Be sure that all the micropipette tip boxes are filled. These tips do not have to be kept sterile so it's OK to handle them without gloves. This task is another one that can be done by your after school students or student service person. Single tips can also be placed in plastic trays at each station if you do not have tip boxes.
- Filling tubes: Fill microcentrifuge tubes (1.5 ml) with 1ml of food coloring. Add 100 μ l of glycerol to ensure that the dye samples sink to the bottom of the wells. Mix well. Dye sets can also be purchased through Sargent Welch (<http://sargentwelch.com/principles-and->

[practice-of-agarose-gel-electrophoresis-kit/p/IG0040829/](http://www.carolina.com/practice-of-agarose-gel-electrophoresis-kit/p/IG0040829/)), or Carolina Biological (<http://www.carolina.com/>).

- Preparation time: It takes about 1 hour to measure out and melt the agar. Preparation time for pouring gels should be 15-30 minutes. The time for marking and loading tubes should be 30-45 minutes. Total estimated prep time 2 hours.

Introduction

This exercise allows students to learn how to use a basic tool of molecular geneticists. They need this practice in order to be able to do the remaining laboratory exercises. It can be awkward for students to use these tools, especially ones that are operated with only one hand. They also should gain an appreciation for the small volume of material needed to conduct molecular genetic experiments.

Student Objectives

- The student will correctly use the micropipette.
- The student will mix liquids in a microcentrifuge tube.
- The student will load a gel.
- The student will convert measurements between the units microliters (μl) and milliliters (ml).

Class Time Needed

One 50-55 minute class period is required to do this exercise.

Materials

1. Digital micropipette
2. Box of micropipette tips
3. 2% agar gels
4. Tubes of food coloring
5. Empty microcentrifuge tubes (1.5 μl)
6. Waste container
7. Large finger bowl
8. Microcentrifuge tube rack
9. 1/2 inch masking tape

Recipes for Consumables

2% agar is made by melting 2 g powdered agar melted in 100 ml of water. Since these gels are only being used to learn the micropipet, they can be made with tap water and not distilled water. All other consumables are used as they come from the stock bottle.

Procedure

For the measurements portion, have the students read and then fill in the blanks. You can have students come to the front and explain how they got their answers or you can go about the room checking individual answers. Some time should be spent here but there is no need to over-emphasize this step.

Have the students read the "Don'ts", "Dialing", "Drawing Up" and "Dispensing" sections first. Then model all these steps before the students are allowed to actually begin the exercise at the "Withdrawing and Dispensing" step.

When practicing the "Loading a Gel" portion, seeing the wells in the gel is very difficult. The best thing is to place black paper under the finger bowl. To prevent the paper from getting wet, place it in a plastic liner or cover it with plastic wrap. If your lab counter tops are black in color, you can ignore this suggestion. When students are loading the gel, the most common mistake will be placing the micropipette tip too far into the well. The tip will actually be pushed into the gel, plugging it with agar and making it very difficult to expel the food coloring. You need to warn the students about this mistake many times. With practice, they will learn how deep to place the micropipette tip. Be sure that students load all red lanes before changing tips, then all green, then all yellow. This is done to save on tips. If you have an ample supply of tips, you can tell the students to eject the tip each time before drawing up and loading a new sample.

Disposal

- Agar can be placed directly in the garbage. It is probably best to put all the gels in a zip lock bag before placing them in the garbage.
- Micropipette tips can be washed in a soap solution, rinsed and dried for reuse with this lab.
- Tubes containing loading dye and food color can be saved for future use.

Background Information: Agarose Gel Electrophoresis

Agarose gel electrophoresis is a powerful and widely used method that separates molecules on the basis of electrical charge, size, and shape. The method is particularly useful in separating charged biologically important molecules such as DNA (deoxyribonucleic acids), RNA (ribonucleic acids), and proteins.

Agarose gel electrophoresis possesses great separating power, yet is very simple to perform. An agarose gel is made by boiling agarose powder in an acid/base controlled (buffered) solution. The solution is then cooled and poured into a mold where it becomes solid, like jello. A comb-shaped mold with square teeth is placed in the gel so that rectangular holes (wells) can be made when the agarose cools and gets solid. The gel is submerged in a buffered (acid-base controlled) chamber (gel box) containing two electrical contacts (“+” and “-“ electrodes).

Samples are prepared for electrophoresis by mixing them with a thick sugar solution. This makes the samples heavier than the buffer solution in the gel box. These samples are carefully loaded into the rectangular wells using a very expensive pipette (a micropipette or transfer pipette). The heavier samples sink through the buffer solution and settle into the rectangular wells – one sample in each well.

A power supply is connected to the electrophoresis gel box and direct current is applied to the samples. The buffer solution completes the circuit between the positive and negative electrodes. Charged molecules in the samples enter the agarose gel through the sides of the wells and move between the agarose molecules. Molecules with a negative charge (anions) move toward the red positive (+) electrode (the anode). Molecules with a positive charge (cations) move toward the negative black negative electrode (cathode). The higher voltage used, the faster the molecules travel. The buffer serves to make the water a better conductor of electricity and to control acid-base (pH) extremes. The pH is important to the charge and stability of many types of molecules.

Agarose is a very large sugar molecule found in certain kinds of marine algae. The agarose gel contains molecule sized pores, acting like molecular strainers. The pores in the gel control the speed that molecules can move. Smaller molecules move through the pores more easily than larger ones. Molecules can have the same molecular size (weight) and electrical charge, + or -, but different shapes. Molecules having a compact shape (eg a baseball versus a beach ball) can move more easily through the pores. Given two molecules of equal size (weight) and shape, the one with the greater electrical charge will move faster. Conditions of charge, size, and shape interact with one another depending on the structure and composition of the molecules, buffer conditions, gel thickness, and voltage.

Adapted from:

B. Schultz & N. Hutchison, Fred Hutchinson Cancer Research Center SEP Dye WN Sep 8, 1994
WSU Spokane CityLab, Washington State University Spokane, Spokane WA.
<http://www.spokane.wsu.edu/ResearchOutreach/CityLab/index.asp>

SEPARATION OF DYE MOLECULES USING AGAROSE GEL ELECTROPHORESIS

MATERIALS:

Electrophoresis apparatus and power supply
Agarose (0.2 gram in 2 ml Eppendorf tube – pre-measured)
125 ml Erlenmeyer flask
50 cc centrifuge tube for measuring the TAE buffer
1X TAE buffer
1 ml of a 1M solution of NaCl to be diluted in 125 ml distilled water.
micropipette (0-20 μ l)
micropipette tips
paper towels

METHOD:

1. To make a 0.8% agarose gel:

- Transfer the 0.2 grams of agarose into your 125ml Erlenmeyer flask.
- Measure **25 ml** 1X TAE Buffer using the 50 cc centrifuge tube and add to the agarose in the Erlenmeyer flask. Swirl gently to mix.
- Microwave for 20 seconds on High. Check to see if all the particles are dissolved and the solution is very hot. If not, heat again for no more than ten seconds.
- Bring to your station and **let cool until it is lukewarm to the touch**. To help the agarose cool, swirl it gently in the flask. If the agarose is too hot, it will leak out the ends of the red striped gel tray.
- While the gel is cooling, place the black dams in the gel box on both ends of the gel tray insert. **Be sure the flat side of the dams are against the gel tray insert**. The tops of the dams should be perfectly flat if they are put in correctly.
- Insert the **8-well comb** in the red striped tray in the **middle of the tray**.
- When the agarose has cooled to the point that you can hold the bottom of the Erlenmeyer flask comfortably, pour the 25 ml of agarose into the gel tray.
- Let the gel harden for at least 10 minutes. Do not move the tray while setting.
- Once the agarose is hardened, remove the two black dams.
- **Add 125 ml of a dilute salt solution (1 ml 1M NaCl diluted in 125 ml distilled water) – an amount sufficient to just cover the gel.**
- Carefully “wiggle” the comb back and forth to separate the comb from the gel and then lift *slowly and gently* out of the gel.

Background:

You are working as a laboratory technician in a major pharmaceutical company. It typically takes 10 years and one billion dollars (\$1,000,000,000) to bring a drug to market. The company is currently working on a new drug and is in its 5th year of product development. A drug testing department within the company needs a carrier molecule for additional testing. You have been given six dye samples to test using agarose gel electrophoresis, and the drug testing department needs a carrier molecule that is:

- 1. Negatively charged**
- 2. The smallest molecular weight (size)**
- 3. Pure (not a mixed dye sample)**

Adding the dye samples to the wells in the agarose gel:

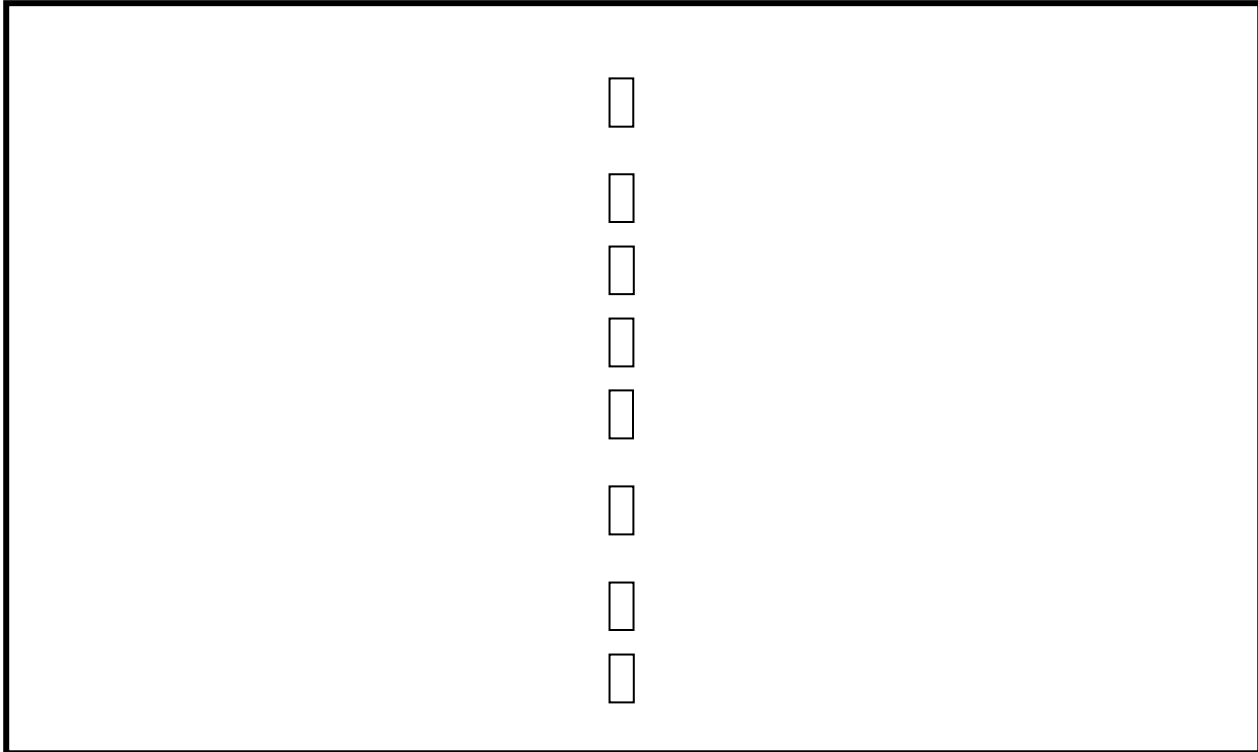
- Carefully pipette 10 μ l of each of the six dye samples into separate wells. Change pipette tips with each dye.
- One of the most common mistakes is not bracing arms or hands while pipetting. This may result in pushing the pipette through the bottom of the well, resulting in loss of the dye under the agarose, rather than in the well.
- Very small amounts of dye will often “float” out of the wells. These small amounts of dye will be diluted in the buffer and will not affect the running of the dyes that remain in the wells.
- Remember to write down the order in which you pipette the dyes.

Running the dye samples:

- After all dye samples are pipetted into the gel wells, close the lid, attach the power cords, and set the voltage range switch to approximately 100 volts.
- Run the gel for about 10 minutes or until you get good separation of all the dyes.

Recording the results:

- Turn the electrophoresis unit off and observe your results. Record your results on the back of this page by drawing where each of the dyes are in the gel.
 - Record the direction of travel (toward the cathode or anode) to determine what charge each of the different dye molecules has.
 - Record the distance each of the dye samples traveled to determine which has the highest molecular weight and which has the lowest molecular weight.
 - Determine whether or not there is a sample that was made from more than one dye.
- You can also have a permanent record of your results by doing a “blot” on a paper towel. Place the agarose gel onto several folded paper towels. Cover with several more paper towels and apply gentle pressure to the gel-paper towel stack. This will allow the dye to transfer out of the gel onto the paper towels, giving you a record of your results.



CATHODE (-)

ANODE (+)

1. Negatively charged sample(s)_____.
2. Positively charged sample(s)_____.
3. Smallest molecular weight_____.
4. Largest molecular weight_____.
5. Mixed dye sample (??)_____.

Dye sample to be sent to the drug testing department_____.

What is the purpose of the agarose gel?

Why did you add a dilute salt solution rather than distilled water to cover the gel?

**AGAROSE GEL ELECTROPHORESIS (DYE LAB)
INSTRUCTOR'S EQUIPMENT AND MATERIALS**

POWER SUPPLIES/GEL BOXES

For a classroom of 24 students, it is best to have **4 power supplies and 8 gel boxes:**

Gel electrophoresis boxes	Horizon 58 (Labreco)	\$400 each
Power Supplies	Model 250 VWR 27370-265	\$500 each

OR Carolina Biological set (<http://www.carolina.com/product/213620.do>)

Perfect for use in AP® Biology and the ABC CORD curriculum. Package I contains enough equipment for electrophoresis of 12 gels at one time. The laboratory manual, *DNA Science: A First Course*, gives complete instructions on molecular techniques for educators trained in science, but who lack a background in molecular biology.

Carolina package includes: (\$2,125)

- 3 Carolina™ Electrophoresis Power Supplies (item# 213672)
- 6 Carolina™ Gel Electrophoresis Chamber Sets (item# 213654)
- Disposable Wiretrol® II Micropipets Set (item# 211156)
- Mini-Pro Light Source (item# 216214)
- *DNA Science: A First Course* (item# 212209)

MICROPIPETTES:

16 adjustable volume micropipettes: (Rainin.com)

8 each PR-20	(2-20ul)	\$150 each with educational discount
8 each PR-200	(20-200ul)	\$150 each with educational discount

OR, you can use fixed volume pipettes at a greatly reduced price:

<http://www.dynalabcorp.com>

Item#: 4023-0010

Dynalon Item#: 240234-0010

MINIFIX MICROPIPETTE 10uL @ \$20 each.

- Fixed volume 10uL MiniFIX Micropipette
- Cost effective
- Ideal in schools for routine applications where speed and precision is required
- Color-coded button for easy volume identification
- Accommodates all common tips
- Good for approximately 5,000 pipettings

50 cc test tubes or graduated cylinders; 1 per station (CityLab can provide)

Distilled water (150 ml per station)

1X TAE (Tris Acetate EDTA buffer).

50X Research Organics	VWR 101110-714	\$175/4 L
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OR to make 50X TAE Buffer

242.0g Tri Base

57.1 ml glacial Acetic Acid

100 ml 0.5M EDTA (18.612 g EDTA in 100 ml dH₂O, pH to 8.0)

QS to 1 liter with dH₂O

NaCl Solution. 1M (58.44g/mole). Dissolve 58.44 g in 500 ml distilled water. Bring to 1000 ml in a 1 liter graduated cylinder. Mix well.

Each station will need **1 ml** prealiquoted in a 1.8 ml eppendorf tube OR provide each station with a bottle of 1M NaCl solution (approx 25 ml) and provide them with fixed 1000 ul micropipettes (DynaLab, \$20 ea).

Agarose (0.2 g per student team per gel)

EMD Omnipur VWR EM-2120 \$60/100 g

Average gel strength and standard melting and gelling ranges. Suitable for a wide range of nucleic acid and protein gel applications. DNase-, RNase-, and protease-free.

Dye samples: Prealiquote 1ml in 1.8ml microcentrifuge tubes of six samples including one sample that is a mix of two dyes of the same charge, but different size/weight. We typically use sulforhodamine and brilliant blue as the dye combination and give the students separate samples of sulforhodamine and brilliant blue.

CityLab can provide dyes samples

Can order through Carolina Science

Can use food coloring, but must dilute in glycerol to a final 10% glycerol concentration.

Can buy powdered dyes and dilute in 10% glycerol

Glycerol: VWR BDH1172-1LP \$30/L

*For this lab, the **agarose gel must be made in 1X TAE buffer**, but you can use a dilute salt solution (1 ml of a 1M NaCl solution in 125 ml distilled water) in place of buffer when running the gels.*

pH - ACIDS and BASES at <http://education.llnl.gov/bep/science/9/sPh.html>

pH - ACIDS and BASES, Student Worksheet

Introduction:

Many common substances are either acids or bases. Some acids, like stomach acid are necessary for our health, while others, like sulfuric acid are dangerous and can cause burns and other injuries. Baking soda is a common weak base used in our homes, while sodium hydroxide, a strong base, is hazardous to skin and eyes.

The easiest way to determine if a substance is acidic or a basic is to use a pH indicator. Indicators are organic molecules that change color in an acid or a base. When an indicator is placed on paper, it provides a fast way to determine if a substance has acidic or basic properties. The most common acid/base indicator paper is called litmus paper, so a litmus test is the first test used to determine acidic or basic properties. If the litmus paper does not change color, the substance is neutral,

How can we determine the strength of an acid or base? The strength of an acid or base is measured in pH which is the concentration of the hydrogen ion (H^+). A high pH indicates a strong base, while a low pH indicates a strong acid. A pH of seven indicates a neutral substance (like water).

Student Objectives:

- use litmus paper to test for an acid, base or neutral substance.
- use pH paper to test for the pH of a substance.
- explain the difference between test using litmus paper and pH paper.
- compile and evaluate the data in a class data table.

Materials:

Two pieces of litmus paper (one pink and one blue)

a 2" piece of pH paper

pH color chart

Procedure -1: Are You Acidic or Basic?

1. Take two pieces of litmus paper (1 pink, 1 blue) and place about one inch of each paper in your mouth.
2. Wait five seconds, then see if either changed color. You will determine whether your saliva is acidic or basic.

(Red to Blue means basic ; Blue to Red means acidic, no change mean neutral)

Are you acidic or basic? _____ Class totals Acidic _____ Basic _____

You will test either acidic, basic or neutral. The red litmus paper will turn slightly blue for a base. The blue litmus paper will turn slightly pink in an acid. If nothing happens, it is neutral. In order to determine how acidic or basic you are, you need to use pH paper, which changes color to indicate the pH. pH paper is treated with a broad range indicator that changes color with varying pH. (This pH value is an approximate value based on color comparison. More exact pH values are found using pH meters or by titration using acids and bases)

Procedure-2: How acid or basic are you?

1. Tear off a piece of pH paper about 1 in. long.
2. Place part of it in your mouth and wait about five seconds.
3. Remove the paper and compare the color with the pH color chart at your table.

(Do not let the paper dry because the colors change as the paper dries)

How acidic or basic are you? What was the pH? _____

Questions:

1. How many people tested acidic, basic or neutral with litmus paper?
Which group was larger?
2. Was there a pattern in the results for the whole class? If so, what was it?
3. What substances could affect the outcome of the litmus test? (food, drinks etc.)
4. What were the results with the pH paper? How many people were in each pH range?
5. Was there any pattern in the pH values for whole class? If so, what was it?

pH - ACIDS and BASES, Teacher Preparation

Advance Preparation:

pH paper is not pre-cut, so it will take at least five minutes to cut enough pH paper for one class. It should take ten minutes to assemble the other materials.

Introduction:

Acids and bases are common, everyday substances.

- Baking soda, sodium hydrogen carbonate (NaHCO_3) is a weak base.
- Soda-pop, carbonic acid (H_2CO_3) is a weak acid.
- Stomach acid (HCl) hydrochloric acid is a relatively strong acid.
- Ammonium hydroxide (NH_4OH) has a strong odor, but is not a strong base.
- Sulfuric acid (H_2SO_4) is a strong acid.
- Sodium Hydroxide (NaOH) is a strong base.

Strong acids and bases are hazardous to skin and eyes and can cause serious burns.

The indicators used to identify acids and bases, are large organic molecules that change color in acid or base. The most common acid/base indicator is litmus paper which changes color in the presence of any acid or base. Other indicators are specific and will change color only at a particular pH (H^+ ion concentration).

Once you have determined if a substance is an acid or a base, you need to determine how strong (or concentrated) it is. The simplest measure of the strength of an acid or base is the concentration of H^+ ion in the solution. The fastest way to determine pH is to use pH paper. It uses a broad range indicator to turn a series of colors from pH 1 to 14. The color change is then compared with a color chart to determine the pH. A high pH indicates a base, and a low pH indicates an acid. A pH near 7 indicates a neutral substance. Students should be aware that shampoos and soaps and most skin products are pH balanced near 7, to protect skin, hair and eyes.

Student Objectives:

The students will:

- use litmus paper to test for an acid, base or neutral substance.
- use pH paper to test for the pH of a substance.
- explain the difference between tests using litmus paper and pH paper.
- compile and evaluate the data in a class data table.

Materials:

1. Litmus paper, pink and blue (neutral is available too, but not necessary)
2. pH paper (1-14), cut into 2 " pieces
3. Color charts (one per lab group)

Procedure-1: Are you acidic or basic?

1. Students put a piece of both blue and red litmus paper into their mouths.
2. After five seconds, they take the papers out to see if either changed color. (Red to blue means a base; blue to red means an acid; no change means neutral)

Are you acidic or basic? _____ Class totals: Acidic _____ Basic _____ Their saliva is acidic or basic; men are usually basic more often than women.

Students will test either acidic, basic or neutral. The red litmus paper will turn slightly blue for a base. The blue litmus paper will turn slightly pink in an acid. If nothing happens, it is neutral. In order to determine how acidic or basic they are, students need to use pH paper, which changes color to indicate the pH. pH paper is treated with a broad range indicator that changes color with varying pH. This pH value is an approximate value based on color comparison. More exact pH values are found using pH meters or by titration using acids and bases.

Depending on how this lesson is used, you may want to demonstrate a pH meter, or an acid/base titration for the class.

Procedure-2: Are you acidic or basic?

1. Students place the end of a piece of pH paper into their mouths.
2. After five seconds, they remove it and compare the color of the paper with the pH color chart. (If the pH paper dries, the color comparison will be incorrect).

How acidic or basic are you? What was the pH _____?

Use a data table to compile the information for the class.

Questions:

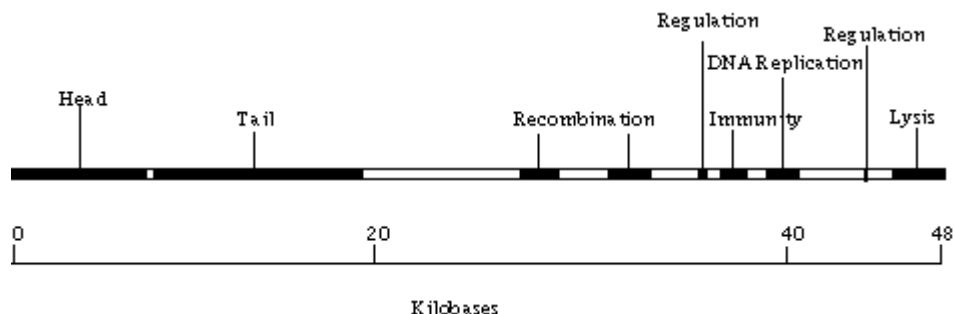
1. Explain the difference between the litmus test and the test using pH paper. Litmus paper tests for an acid or base. pH paper tests for the H^+ ion concentration and indicates the strength of the acid or base.
2. How many people tested acidic, basic or neutral with litmus paper? Which group was larger? The majority of the students will have saliva that is either acidic or basic, which helps with the breakdown of food in the mouth.
3. Was there a pattern in the results for the whole class? If so, what was it? The answers may lead to a discussion on whether gender influences acidic or basic properties.
4. What substances could affect the outcome of the litmus test? Answers may vary. Any acidic substance (soda pop, a pickle or salsa) could change the outcome. Any basic substance (an antacid, Alka-Seltzer or baking soda toothpaste) would also affect the outcome.
5. What were the results with the pH paper? How many people were in each pH range? It might be interesting to see if there is a gender component in this answer too.
6. Was there a pattern in the pH values for the whole class. If so, what was it? Answers will vary, it might be interesting to compile a series of class data tables to compare results and look for a trend.

Analyzing Precut DNA at <http://education.llnl.gov/bep/science/science.html>

ANALYZING PRE-CUT DNA, Student Worksheet

Introduction:

Lambda DNA comes from a virus called Phage Lambda. This virus is harmless to man and therefore makes an excellent and safe source of DNA. Below is a map of some of the genes found on Lambda.



Lambda is approximately 48,000 base pairs long. Since Lambda is a virus, it is able to express some of its genes by taking over the bacterial cell that it infects. In this investigation, you will observe the effects of three restriction enzymes on Lambda DNA. A restriction enzyme (also known as an endonuclease) will search for a specific sequence of base pairs. It will cut (chemically separate) a piece of DNA at that specific arrangement of base pairs. The DNA arrangement may appear many times, thereby providing the fragments that we are able to separate. When different restriction enzymes are used to cut a single strand of DNA such as the Lambda DNA, fragments of varying sizes are produced. These fragments can be separated by their size/shape through gel electrophoresis. DNA is negatively charged due to its phosphate backbone. Therefore, DNA will move toward the positive electrode (anode). Gel electrophoresis is based on the principle that the rate a molecule moves through a gel is determined by its size and/or shape and ability to move through pores in the gel. Thus, a small fragment will be able to move quickly, whereas a large fragment will move more slowly. By the same token, a DNA piece that is extended and very long may have more difficulty moving through a gel matrix than the same piece that is coiled upon itself. An analogy would be to equate this situation to your classroom in which all the desks have been randomly pushed together. An individual student can wind his/her way through the chair maze quickly and with little difficulty, whereas a string of students would require more time and have difficulty working their way through the maze.

The restriction enzymes used in this investigation are EcoR1, BamH1 and HindIII. You will use gel electrophoresis to separate the resulting DNA pieces. To help see the pieces, you will stain the gel with a chemical that will combine with the DNA causing it to take on a blue color. A permanent record of the gel can be made by photographing it with a Polaroid camera or photocopying it.

Student Objectives:

- * Identify restriction enzymes and their specificity.
- * Determine the number of restriction sites on Lambda DNA.

- * Visualize DNA pieces within a gel and effectively communicate this visualization.
- * Estimate the size of each DNA piece cut by a specific enzyme.

Materials:

1. Lambda DNA/BamH1 (0.2ug/10ul)
2. Lambda DNA/HindIII (0.2ug/10ul)
3. Lambda DNA/EcoR1 (0.2ug/10ul)
4. Uncut Lambda DNA (0.2 ug/10ul)
5. Gel tray, comb and box
6. Masking tape
7. (0.8%) Agarose
8. 1X TAE buffer
9. Micropipet(1-20ul)
10. Micropipet tips
11. Ice container
12. Millimeter ruler
13. Semi log graph paper
14. Zip lock bags
15. Gel staining dye
16. Microcentrifuge tube rack
17. 10X Loading dye
18. Power supply
19. Waste container
20. Permanent marker

Procedure:

Part A: Preparing an Agarose Gel

1. Prepare a gel tray by taping the ends with masking tape as instructed by your teacher.
2. Place the comb near one end of the tray (approximately $\frac{3}{4}$ of an inch) and pour melted agarose into the tray. (The agarose in this activity has a concentration of 0.8%)

Add 2-3 drops of gel stain to the melted agarose. Pour in just enough melted agarose to cover $\frac{1}{3}$ of the height of the comb teeth (about 25 ml) . Do not move or handle the gel tray until the gel has solidified (about 10 minutes or until it appears cloudy).

Part B: Mixing DNA and Loading Dye

1. Dial the digital micropipet to 2 μ l and transfer this amount of loading dye to each of the tubes marked B, E, H, and L in the microtube holder. Use the same tip for all tubes.
2. Now re-dial to 10 μ l, add a clean tip and transfer this amount of Lambda cut with BamH1 enzyme from the B tube on ice to the B tube in the microtube rack. Be sure to replace the B tube from the ice back onto the ice.
3. Repeat step 2 for the remaining tubes on ice. Be sure to use 10 μ l each time; change tip each time and place the tube from ice back on ice.
4. Place the four tubes from your microtube holder (these now have DNA and loading dye) into the microcentrifuge, being sure to space them evenly around the inside. Have your teacher check before spinning the tubes. Pulse spin the tubes (hold the button for a few seconds). This allows the DNA and loading dye to mix.

Part C: Separation by Electrophoresis

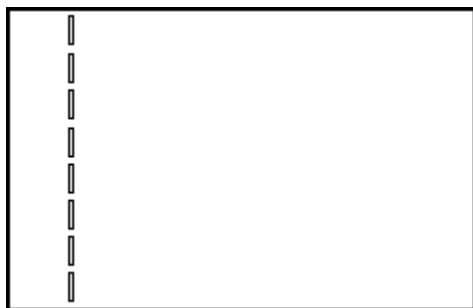
1. Gently remove the gel comb by lifting it straight up and out of the gel.
2. Remove the tape from the gel tray and place the tray with the gel into the gel box. Be sure to place the gel so that the wells are closest to the negative (black) electrode.
3. Fill the gel box with enough 1X TBE buffer to barely cover the gel.
4. Dial the micropipet to 12 μl and load the gel according to the following scheme. Be sure to change tips between EACH tube.

Lane	Tube
1	B
2	E
3	H
4	L

5. Place the lid on the gel box (remember black to black and red to red). Plug the gel box wires into the power supply (again black to black and red to red). Turn on the power supply and set it at the voltage specified by your teacher (approximately 110 volts). Let the gel run for approximately 30 minutes..

Data Collection:

1. Label the lanes according to the table above in section C4.
2. Label the positive and negative ends of the gel ().
3. Place your gel upon the light source provided and record the result on the diagram.



3. Linear DNA fragments migrate at rates inversely proportional to the \log_{10} of their base pair length. The table below gives the base pair sizes of the different DNA pieces from the HindIII restriction digest of the lambda DNA. Measure the distance in millimeters from the bottom of the well to the bottom of each band in the HindIII digest and place that measurement next to the base pair size that it matches.

Data Table

HINDIII (reference)	ECORI	BAMHI
Size of band of HindIII Lambda Phage restriction digest		
1	23,130	

2	9416
3	6557
4	4361
5	2322
6	2027

5. By using the known base pair sizes of the bands in the HindIII digest as reference points, we can estimate the other band sizes to it. The term given to these reference points is a **ladder**. We will use the ladder to estimate the base pair sizes of the EcoRI digest and the BamHI digest.
6. On semi-log graph paper, mark the distance (mm) on the x-axis (horizontal axis) and the base pair size on the y-axis (vertical axis). Plot and connect these points in a best-fit straight line.
7. Measure the distances migrated for each of the bands in the other two digests. Remember you need to be consistent: measure from the bottom of the well to the bottom of the band. Record each measured distance in the appropriate box in the data table.
8. Refer to the graph by moving along the x-axis to the designated distance; then move up until you reach your best-fit line. From this point on the best-fit line, find the corresponding height on the base pair size axis (y-axis). Read the size measurement indicated on the base pair size axis. Record the measurement in the appropriate box.

Analysis:

1. What do the bands on the gel diagram represent?
2. What is the connection between the restriction enzymes and these bands?
3. How many bands are in lane 4? What does this band represent?
4. Compare lane 4 with the other lanes. Do any of the other lanes have a band in the same position as lane 4? Why or why not?
5. Which lane has the smallest piece of DNA? How do you know?
6. a. How many pieces did HindIII produce by cutting Lambda DNA?
b. How many times did this enzyme cut the DNA?
7. a. How many pieces of DNA did the BamHI restriction digest produce?
b. How many times did this enzyme cut the DNA?
8. a. How many pieces of DNA did the ECORI restriction digest produce?
b. How many times did this enzyme cut the DNA?
9. To which electrode did the pieces of DNA move? Why?

Conclusion:

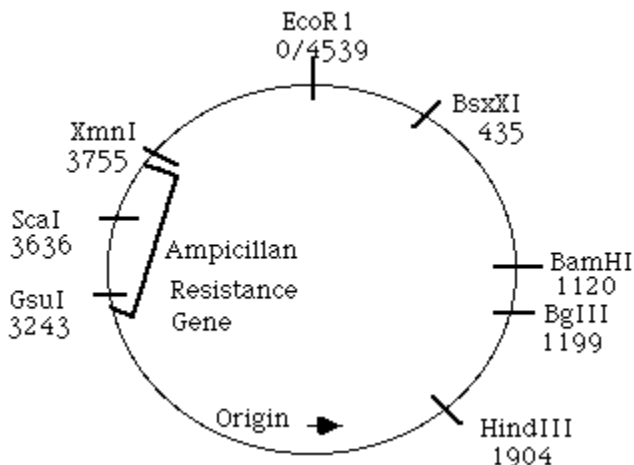
Remember that the three samples of DNA started out the same. Assuming that each sample was cut into pieces by the addition of three different restriction enzymes, how does a restriction digest give evidence that each enzyme cuts the DNA at different locations?

EXTENSION I

A plasmid is a small circular piece of DNA. Plasmids have restriction sites and usually carry a specific gene - often an antibiotic resistance gene. Since they are small, one restriction enzyme will usually find only one site for its specific cutting capabilities. The name of a plasmid contains the gene which it carries preceded by a "p"; for example, pAMP is the name for the plasmid that contains the gene for ampicillin resistance. The following is the plasmid map for pAMP. Notice that the map contains the location of the gene, the position of the origin (which is the spot at which replication begins), the names of the restriction enzymes, and their restriction sites designated by the base pair distance. Therefore, from a plasmid map a research scientist can determine which enzymes can most effectively be used to cut out the gene in order to transfer that gene to another plasmid. The scientist must be careful that **ALL** of the gene is removed but does not want unnecessary amounts of the plasmid DNA.

After viewing the plasmid, determine which enzymes would be best for cutting the ampicillin gene out of pAMP. In addition, determine the number of base pairs in the cut piece of plasmid.

Plasmid pAMP



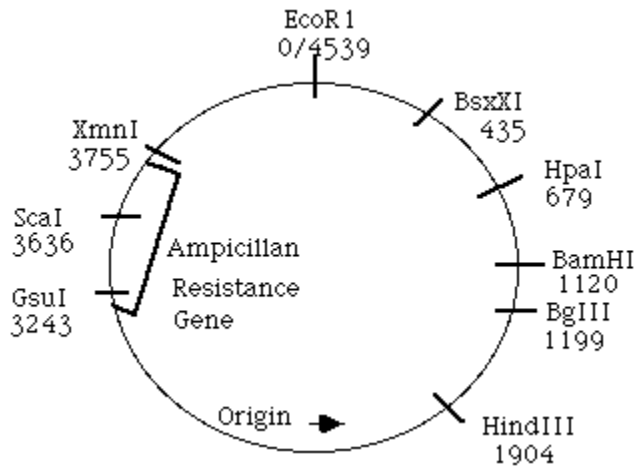
EXTENSION II

A research scientist will often want to combine the genes from two plasmids to make a new organism that contains the resistance to two antibiotics. When the scientist proceeds with this technique, the new organism must contain the complete genes and an origin in order for replication to occur.

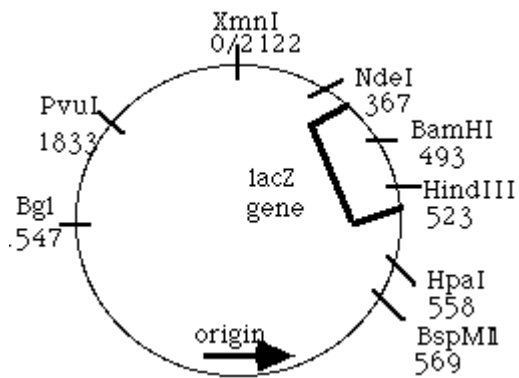
The plasmid maps for two different plasmids follow: pAMP and pLacZ. Select the restriction enzymes that a scientist would use to create the pAMP/LacZ.

Draw all possible plasmid combinations that could recombine including pAMP/LacZ and name them. Determine their sizes (# of base pairs).

Plasmid pAMP



Plasmid pLacZ



ANALYZING PRE-CUT DNA

Teacher Preparation

Advanced Preparation:

- Agarose: Making up 0.8% agarose (notice this is agarose and NOT agar) can be done at any time, even weeks in advance. The agarose must be made using FRESH 1X TAE buffer NOT water. It only needs to be melted NOT autoclaved. It can be stored in closed bottles at room temperature and melted by using a microwave oven or hot water bath. Be

sure to loosen the bottle's cap before heating. Using the microwave is much simpler and quicker.

- Gel Preparation by Teacher: The volume of 1X TAE buffer needed to make 20 gels is approximately 800 ml. You will need this volume times the number of classes doing this exercise to pour the gels. If you decide to pour the gels (the procedure has the students pour them), you should pour the gels a few days in advance of the lab date. When taping the ends of a gel tray, be sure that the masking tape is pressed firmly along the edges of the tray. This will prevent more leaks than trying to fold the tape under and attaching it to the bottom of the tray. Pouring the agarose should be done at approximately 60-65°C (**Caution Hot**). This temperature can be approximated by feel. If you can hold both hands around the bottle and not get a burning sensation, the agarose is at the correct temperature. However, for added protection, use hot gloves when pouring the gels. You should have as many gel trays prepared as you have agarose. Each gel requires 35-40 ml of agarose (depending on the size of the gel tray – some hold only 25 ml). Line the gel trays along the edge of a lab table and pour the agarose into one side. Pour until the agarose comes within 3/4 of an inch of the other end. Stop pouring and allow the agarose to flow to the end of the tray. This exercise requires that the gels have at least six wells and that the gel comb be placed within 3/4 of an inch of the end of the tray. The gels can be stored inside zip lock bags at room temperature. Pour one or two extra so students who make major mistakes can be handed a new one to complete the exercise. If you have highly motivated or advanced students, gel pouring might be a special project for them to do as an after school exercise.
- Gel Preparation by Students: This exercise gives the instructions for students to pour their own gels. In order to follow this procedure, you must have some method for melting the agarose ahead of time (a microwave is easiest) and a method for keeping the agarose at approximately 60° C (hot water bath) throughout the day. The number of water baths you use depends upon the amount of equipment available and how comfortable you are having them in the room. You can follow the above instructions for agarose preparation and determine how many bottles of agarose will be needed for the day. Gels can be stored in zip lock bags on the counter top for the student's use on the next day. Have students label their plastic bag and place their gel inside. The time needed to pour gels by an entire class is probably 20 minutes, therefore another activity needs to be planned for the remainder of the class period.
- Buffer: In addition to the 1X TBE buffer needed for the gels, you will need to make up approximately 500 ml for each electrophoresis chamber. Eight liters of 1X TBE buffer should be enough for 16 chambers. You can reuse the 1X TBE buffer from the chambers so long as it is not badly contaminated by any student team. If contaminated, discard and replace with fresh 1X TBE buffer. If you have highly motivated or advanced students, buffer dilution might be a special project for them to do as an after school exercise.
- Tip boxes: Be sure that all the micropipet tip boxes are filled. These tips need to be as clean as possible so filling the tip boxes should be done while wearing latex/vinyl gloves. This task is another one that can be done by your after school students or student service person.
- DNA Stain: * **Carolina Blu gel staining dye** – can use one kit for the entire classroom. FA-21-7300 Due to time restrictions, use the dye drops that stain the gel allowing students to see the DNA bands form as they move through the gel.

- **Preparation time:** Estimated time to make and melt agarose is 1 hour. Estimated time to pour gels (16 gels for a class of 32), if teacher chooses to pre-pour them, is 15-30 minutes. Time needed to dilute the DNA stain and transfer to staining trays is 20 minutes. Total estimated prep time 1.5 - 2 hours.

Introduction:

This exercise is designed to introduce the student to the function of restriction enzymes and gain an appreciation of their use as molecular tools when working with DNA. The students will also see the connection between gel electrophoresis and imaging the resulting DNA fragments of a restriction enzyme.

Student Objectives:

- Identify restriction enzymes and their specificity.
- Determine the number of restriction sites on Lambda DNA.
- Visualize DNA pieces within a gel and effectively communicate this visualization.
- Estimate the size of each DNA piece produced by each enzyme.

Class Time Needed:

Three 50-55 minute periods are needed to complete this exercise.

1. On day one the students will pour gels. Since this should take 20-30 minutes, you need to have an additional activity ready to fill the remainder of the period.
2. The second day is for loading the gels with cut Lambda DNA and separation by electrophoresis. Since loading the gel takes 10-15 minutes, you need to have an additional activity ready to fill in the remainder of the period.
3. The third day is for data analysis.
4. If you choose to pre-pour the gels, then this exercise only takes two periods.

Materials:

1. Lambda DNA/BamHI (diluted to 0.2ug/10ul)
2. 1X TAE buffer
3. Lambda DNA/EcoRI (diluted to 0.2 ug/10ul)
4. Gel tray, comb, electrophoresis chamber
5. Lambda DNA/HindIII (diluted to 0.2 ug/10ul)
6. 1/2 inch masking tape
7. Uncut Lambda (diluted to 0.2ug/10ul)
8. Micropipet (1-20µl)
9. Microcentrifuge tube rack
10. Loading dye (10X)
11. Box of micropipet tips
12. Power supply
13. 0.8% Agarose
14. Waste container
15. Staining trays
16. Gel Stain
17. Zip lock bags (sandwich size)
18. Permanent markers

19. Microcentrifuge tubes (1.5 ml)
20. Spatula
21. Millimeter ruler
22. Semilog graph paper

Recipes for Consumables:

0.8% agarose gels: 0.8 g of agarose powder melted in 99.2 ml of 1X TAE OR you can use the formula $C1V1 = C2V2$ to use any 2% agarose you may have left from other exercises. To make 100 ml of agarose at 0.8% from 2% agarose, use 40 ml of 2% agarose and dilute with 60 ml of 1X TAE. If you use this method, be sure to heat the 1X TAE so the melted agarose does not solidify when you pour the two solutions together.

Procedure:

Part A: Gel Preparation

Be sure your students have taped the edge of their gel tray properly and placed the comb approximately 3/4 of an inch from one end of the tray.

They can then pour the gel as directed in the student procedure (similar to the teacher directions above) or you can have them transfer 40 ml of melted agarose into a 50 ml beaker and then pour this amount into the tray.

Part B: Loading the Gel

Loading the gel should not be a problem. However, you may wish to quickly review the micropipet and its use. Special attention to which stop for drawing up and which stop for expelling are probably the most important items to review. Lane 1 is usually defined as the outermost well of the gel on the black electrode side of the electrophoresis chamber. Once this well is loaded, the students can then load the wells in sequence according to the procedure.

Closing the electrophoresis chamber and connecting it to the power supply deserves special attention. Be sure that the lid goes on black lead wire to black electrode and red lead wire to red electrode. Also, be sure that the electrophoresis chamber is positioned where you want it on the table. Once the chamber has been connected to the power supply and the power has been turned on, the student is NOT to touch, or handle the chamber. Check the lead wires from the lid of the chamber to be sure that they have been plugged into the correct receptacles of the power supply. Black to black and red to red and that the wires are plugged into receptacles that are next to each other. Once you have checked all of this at a lab station, you can turn on the power supply and start the experiment running. Set the power supply to approximately 100 volts and use this as the running voltage.

Separation of the Lambda DNA fragments can take up to 30 minutes. This means that the electrophoresis process from one period may continue into the next. This can pose an equipment problem. You can either increase the size of the student teams to four, therefore needing 1/2 the number of gels, or arrange to borrow extra equipment from another teacher. Regardless of the decision you make, you will have to set up each lab station such that chambers from the previous class will be running while the current class is loading gels.

Disposal

- These gels can be placed directly into the garbage. It is probably best to put all the gels into a zip lock bag before placing them in the garbage.
- Check with your math teachers to see if any of them intend to do the exercises that pertain to graphing the size of the DNA fragments versus their migration distances. You can save some of the better gels in zip lock bags so they can have them for their math classes.
- Be sure to save the 1XTBE from the electrophoresis chambers for future use.
- You can save all the DNA stain for future use so DON'T discard it.

TEACHER LECTURE NOTES:

Restriction of Lambda DNA & analyzing pre-cut DNA

One of the basic tools of modern biotechnology is gene splicing or recombinant DNA technology. The basic concept behind gene splicing is to remove a fragment of DNA from one organism and combine it with the DNA of another. The result would be the host organism implementing the instructions provided by the newly inserted DNA fragment. For example, certain plants can be given the genes for resistance to pests or disease.

The first step in recombinant DNA technology is to locate the specific gene of interest on a chromosome. A restriction enzyme is then used to cut (restrict) the targeted gene from the rest of the chromosome. This same enzyme will then be used to cut the DNA into which the fragment will be inserted.

The ability to cut (restriction) DNA predictably and precisely enables biotechnologists to readily manipulate and recombine DNA molecules. Restriction of DNA is used in recombinant DNA technology, DNA fingerprinting, DNA sequencing, and many other applications in the field of biotechnology. The restriction enzymes which are used to cut DNA are found to occur naturally in bacteria. They are in fact a means by which bacteria protect themselves from any foreign DNA which might invade the bacterial cell. Any foreign DNA encountering a restriction enzyme would be cut into fragments and rendered ineffective.

Each restriction enzyme is specific, and cuts at only a very specific sequence of the DNA molecule. For example, EcoRI will cut only at: GAATTC. If this specific

CTTAAG sequence occurs in more than one location on a DNA molecule, EcoRI will cut all of these sites. Therefore if a given piece of DNA (linear) is cut with a restriction enzyme, whose specific code is found at 5 different locations on the DNA molecule, the result will be 6 fragments of varying lengths. The length of each fragment would depend upon the location of restriction sites on the DNA molecule.

DNA which has been cut with restriction enzymes can be visualized using a process known as agarose gel electrophoresis. The term electrophoresis means *to carry with electricity*. Agarose gel electrophoresis separates DNA fragments by molecular weight. DNA fragments are loaded into an agar block, which is placed into a chamber filled with a liquid buffer solution. A direct current is passed between wire electrodes at each end of the chamber. DNA fragments are negatively charged, and when placed in an electric field will be drawn toward the positive pole and repelled by the negative pole. The matrix of the agarose gel acts like a sieve through which smaller DNA fragments can move more easily than larger ones. Therefore, the distance and rate at which DNA

fragments migrate through the gel is inversely proportional to its molecular weight. Over a period of time smaller fragments will have traveled further than larger ones. Fragments of the same size stay together and pool into discrete "bands".

DNA GEL ELECTROPHORESIS LAB INSTRUCTOR'S EQUIPMENT AND MATERIALS

For a classroom of 24 students, it is best to have **4 power supplies and 8 gel boxes:**

Gel electrophoresis boxes Horizon 58 (Labreco)	\$400 each
http://www.labreco.com/molecbio_electrophor.htm	
Power Supplies Model 250	\$500 each
VWR 27370-265 OR Labreco E11066016	

OR Carolina Biological set (<http://www.carolina.com/product/213620.do>)

Perfect for use in AP® Biology and the ABC CORD curriculum. Package I contains enough equipment for electrophoresis of 12 gels at one time. The laboratory manual, *DNA Science: A First Course*, gives complete instructions on molecular techniques for educators trained in science, but who lack a background in molecular biology.

Package includes: (\$2,125)

- 3 Carolina™ Electrophoresis Power Supplies (item# 213672)
- 6 Carolina™ Gel Electrophoresis Chamber Sets (item# 213654)
- Disposable Wiretrol® II Micropipets Set (item# 211156)
- Mini-Pro Light Source (item# 216214)
- *DNA Science: A First Course* (item# 212209)

Micropipettes:

16 adjustable volume micropipettes: Rainin
 8 each PR-20 (2-20µl) \$150 each (educational discount)
 This lab only requires 20 µl pipettes to aliquot 10 µl samples.

Microcentrifuge (to spin down samples containing the DNA, loading dye and enzyme)

VWR Galaxy Ministar 93000-196	\$250 ea
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1X TAE (Tris Acetate EDTA buffer)

50X Research Organics VWR 101110-714	\$175/4 L
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OR to make 50X TAE Buffer

242.0g Tri Base

57.1 ml glacial Acetic Acid

100 ml 0.5M EDTA (18.612 g EDTA in 100 ml dH₂O, pH to 8.0)

QS to 1 liter with dH₂O

Agarose (0.2 g per student team per gel)

EMD Omnipur VWR EM-2120 \$60/100 g

Average gel strength and standard melting and gelling ranges. Suitable for a wide range of nucleic acid and protein gel applications. DNase-, RNase-, and protease-free.

DNA samples:

Lambda DNA/HindIII, EcoR1, BamH1 cut Promega approx \$40/100 µg

Dilute to 0.2µg/10µl. The DNA is very stable and can last in the refrigerator for months after diluting with 1XTAE buffer.

Loading dye: Bromophenol blue in 30% glycerol – or any dye that runs faster through the gel than the precut DNA bands. Prealiquot 100 µl in 0.5 ml microcentrifuge tubes for each student team.

CarolinaBlu DNA stain – can use one kit for the entire classroom.

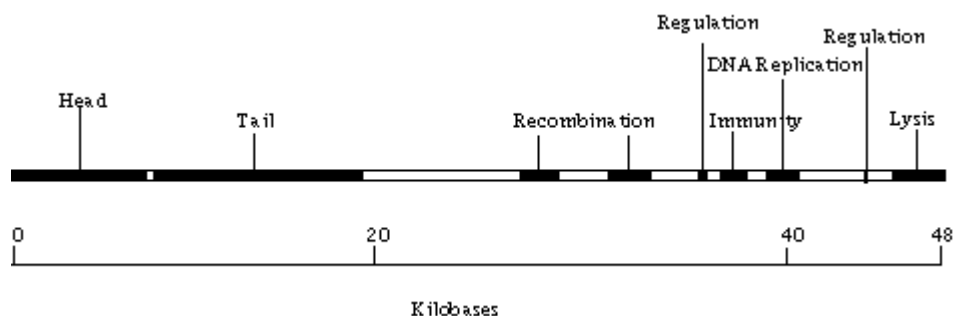
FA-21-7300 \$8.25 per kit.

RESTRICTION OF LAMBDA DNA <http://education.llnl.gov/bep/science/science.html> (with modifications)

RESTRICTION OF LAMBDA DNA, Student Worksheet

Introduction:

Lambda DNA comes from a virus called Phage Lambda. This virus is harmless to man and therefore makes an excellent source of DNA. Below is a map of some of the genes found on Lambda.



Lambda is approximately 48,000 base pairs long. Since Lambda is a virus, it is able to express some of its genes by taking over the bacterial cell that it infects. In this investigation, you will observe the effects of three restriction enzymes on Lambda DNA. A restriction enzyme (also known as an endonuclease) will search for a specific sequence of base pairs. It will cut (chemically separate) a piece of DNA at that specific arrangement of base pairs. The DNA arrangement may appear many times, thereby providing the fragments that we are able to separate. When different restriction enzymes are used to cut a single strand of DNA such as the Lambda DNA, fragments of varying sizes are produced since different restriction enzymes cut at different specific sequences of base pairs. These fragments can be separated by their size/shape through gel electrophoresis. DNA is negatively charged due to its phosphate backbone. Therefore, DNA will move toward the positive electrode (anode). Gel electrophoresis is based on the principle that the rate a molecule moves through a gel is determined by its size and/or shape and ability to move through the pores of the gel. Thus, a small fragment will be able to move quickly, whereas a large fragment will move more slowly. By the same token, a DNA piece that is extended and very long may have more difficulty moving through a gel matrix than the same piece that is coiled upon itself. An analogy would be to equate this situation to your classroom in which all the desks have been randomly pushed together. An individual student can wind his/her way through the chair maze quickly and with little difficulty, whereas a string of students would require more time and have difficulty working their way through the maze.

The restriction enzymes used in this investigation are EcoR1, BamH1 and HindIII. You will use gel electrophoresis to separate the resulting DNA pieces. To help see the pieces, you will stain the gel with a chemical that will combine with the DNA causing it to take on a blue color. A permanent record of the gel can be made by photographing it with a Polaroid camera or photocopying it on a photocopy machine.

Student Objectives:

- * The student will identify restriction enzymes and their specificity.
- * The student will determine the number of restriction sites on Lambda DNA.
- * The student will visualize DNA pieces within a gel and effectively communicate this visualization.
- * The student will estimate the size of each DNA piece cut by a specific enzyme.

Materials:

- | | |
|--|---------------------------------|
| 1. Lambda DNA (0.5µg/µl) | 10. Power supply |
| 2. (0.8%)Agarose | 11. Loading dye |
| 3. 1X TAE buffer | 12. 4 Microcentrifuge tubes |
| 4. Micropipet (1-20 µl) | 13. Micropipet tips |
| 5. Gel tray, comb and box | 14. Distilled water |
| 6. Microcentrifuge tube holder | 15. Permanent marker |
| 7. Masking tape | 16. Semi-log graph paper |
| 8. Enzymes-BamH1, EcoR1,
Hind III (10 units/µl) | 17. Millimeter ruler |
| 9. 2X Restriction buffer | 18. Uncut Lambda DNA (0.5ul/ul) |
| | 19. CarolinaBlu DNA Stain |

Procedure:**Part A: Restriction of Lambda**

1. Label 4 microcentrifuge tubes as follows:

B=BamH1 E=EcoR1

H=HindIII (-)=No enzyme

2. Use and mark the following table with checks as you add chemicals to each tube. Read down each column, adding the same chemical to all tubes. You only need one tip per chemical if you don't contaminate it by touching any other chemical in the tube. You can avoid contamination by placing each new chemical at a different spot on the side of the tube. Use a fresh tip for each different chemical. Remember to keep the enzyme tubes on ice and to add the enzyme last!!!

Tube	Lambda	Buffer	BamH1	EcoR1	HindIII	Water
B	6 ul	6 ul	1 ul			
E	6 ul	6 ul		1 ul		
H	6 ul	6 ul			1 ul	
(-)	6 ul	6 ul				1 ul

3. Seal each tube and place it into the microcentrifuge. Be sure to balance the centrifuge by spacing the tubes evenly inside. Pulse spin the centrifuge (push button for a few seconds) to make sure that the contents are mixed. Place the tubes in the microcentrifuge rack and incubate at 37° C

(either in a water bath or incubator) for at least two hours or leave them on the lab counter overnight.

Part B: Preparing an Agarose Gel

1. Prepare a gel tray by taping the ends with masking tape as instructed by your teacher.
2. Place the comb near one end of the tray (approximately $\frac{3}{4}$ of an inch) and pour melted agarose into the tray. (The agarose in this activity has a concentration of 0.8%.)
Add CarolinaBlu DNA Stain to the melted agarose. Pour in just enough melted agarose to cover $\frac{1}{3}$ of the height of the comb teeth. Do Not move or handle the gel tray until the gel has solidified (about 10 minutes or until it appears cloudy).

Part C: Separation by Electrophoresis

1. Gently remove the gel comb by lifting it straight up and out of the gel.
2. Remove the tape from the gel tray and place the tray with the gel into the gel box. Be sure to place the gel so that the wells are closest to the negative (black) electrode.
3. Fill the gel box with enough 1X TBE buffer to barely cover the gel.
4. Dial the micropipet to 2 μ l and add this amount of loading dye to each tube from the restriction digest in the above procedure. If you place the drop of dye along the side of the tube being careful not to touch the tube's contents with the pipet tip, you can load all tubes with the same tip. Place the tubes in the microcentrifuge and pulse spin for a few seconds. Remember to balance the centrifuge by spacing the tubes evenly inside the centrifuge.
5. Dial the micropipet to 14 μ l and load the gel according to the following scheme. Be sure to change tips between EACH tube.

Lane	Tube
1	H
2	B
3	E
4	(-)

5. Place the lid on the gel box (remember black to black and red to red). Plug the gel box wires into the power supply (again black to black and red to red). Turn on the power supply and set it at the voltage specified by your teacher. Let the gel run for approximately 90 minutes.

Data

1. Label the lanes according to the table above in section C4.
2. Label the positive and negative ends of the gel ().
3. Place your gel upon the light source provided and record the result on the diagram.



4. Linear DNA fragments migrate at rates inversely proportional to the \log_{10} of their base pair length. The table below gives the base pair sizes of the different DNA pieces from the HindIII restriction digest of the lambda virus. Measure the distance in millimeters from the bottom of the well to the bottom of each band in the HindIII digest and place that measurement next to the base pair size that it matches.

Data Table

Base Pair Banding Pattern of Lamba DNA cut with HindIII restriction enzyme:

1	23,130
2	9416
3	6557
4	4361
5	2322
6	2027

5. By using the known base pair sizes of the bands in the HindIII digest as reference points we can estimate the other band sizes. The term given to these reference points is a **ladder**.
6. We will use the ladder to estimate the base pair sizes of the EcoRI digest and the BamHI digest.
7. On semi-log graph paper, mark the distance (mm) on the x-axis (horizontal axis) and the base pair size on the y-axis (vertical axis). Plot and connect these points in a best-fit straight line.
8. Measure the distances migrated for each of the bands in the other two digests. Remember you need to be consistent: measure from the bottom of the well to the bottom of the band. Record each measured distance in the appropriate box in the data table.
9. Refer to the graph by moving along the x-axis to the designated distance; then move up until you reach your best-fit line. From this point on the best-fit line, find the corresponding height on the base pair size axis (y-axis). Read the size measurement indicated on the base pair size axis. Record the measurement in the appropriate box.

Analysis

1. What do the bands on the gel diagram represent?
2. What is the connection between the restriction enzymes and these bands?
3. How many bands are in lane 4? What does this band represent?
4. Compare lane 4 with the other lanes. Do any of the other lanes have a band in the same position as lane 4? Why or why not?
5. Which lane has the smallest piece of DNA? How do you know?

6. a. How many pieces did HindIII produce by cutting Lambda DNA?
b. How many times did this enzyme cut the DNA?
7. a. How many pieces of DNA did the BamHI restriction digest produce?
b. How many times did this enzyme cut the DNA?
8. a. How many pieces of DNA did the HindIII restriction digest produce?
b. How many times did this enzyme cut the DNA?
9. To which electrode did the pieces of DNA move? Why?

Conclusion:

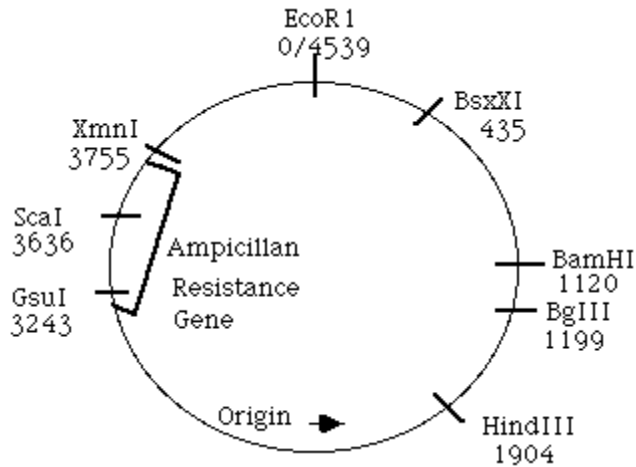
Remember that the three samples of DNA started out the same. Assuming that each sample was cut into pieces by the addition of three different restriction enzymes, how does a restriction digest give evidence that each enzyme cuts the DNA at different locations?

EXTENSION I

A plasmid is a small circular piece of DNA. Plasmids have restriction sites and usually carry a specific gene - often an antibiotic resistance gene. Since they are small, one restriction enzyme will usually only find one site for its specific cutting capabilities. The name of a plasmid contains the gene which it carries preceded by a "p": for example, pAMP is the name for the plasmid that contains the gene for ampicillin resistance. The following is the plasmid map for pAMP. Notice the map contains the location of the gene, the position of the origin (which is the spot at which replication begins), the names of the restriction enzymes, and their restriction sites designated by the base pair distance. Therefore, from a plasmid map a research scientist can determine which enzymes can most effectively be used to cut out the gene in order to transfer that gene to another plasmid. The scientist must be careful that **ALL** of the gene is removed but does not want unnecessary amounts of plasmid DNA.

After viewing the plasmid, determine which enzymes would be best for cutting the ampicillin gene out of pAMP. In addition, determine the size in number of base pairs of the cut piece of plasmid.

Plasmid pAMP



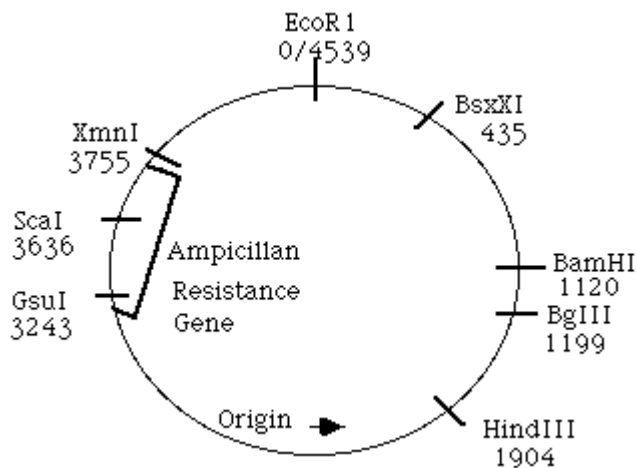
EXTENSION II

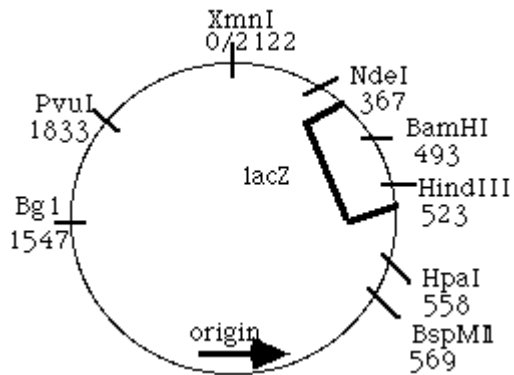
A research scientist will often want to combine the genes from two plasmids to make a new organism that contains the resistance to two antibiotics. When the scientist proceeds with this technique, the new organism must contain the complete genes and an origin in order for replication to occur.

The plasmid maps for two different plasmids follow: pAMP and pLacZ. Select the restriction enzymes that a scientist would use to create the pAMP/LacZ.

Draw all possible plasmid combinations that could recombine including pAMP/LacZ and name them. Determine their sizes.

Plasmid pAMP



Plasmid pLacZ**RESTRICTION OF LAMBDA DNA, TEACHER PREPARATION****Advanced Preparation**

- **Agarose**: Making up 0.8% agarose (notice this is agarose and NOT agar) can be done at any time, even weeks in advance. The agarose must be made using FRESH 1X TBE buffer, NOT water. It does not have to be autoclaved; only melted (clear not clouded). It can be stored in closed bottles at room temperature and melted by using a microwave oven or hot water bath. Be sure to loosen the cap before heating. Using the microwave is much simpler and quicker.
- **Gel Preparation by Teacher**: Determine the volume of 1X TAE buffer needed to make 20 gels. To pour the gels, you will need this volume of agarose times the number of classes doing this activity. If you decide to pour the gels (the procedure has the students pour them), you should pour the gels a few days in advance of the lab date. When taping the ends of a gel tray, be sure that the masking tape is pressed firmly along the edges of the tray. This will prevent leaks. Pouring the agarose should be done at approximately 60-65° C. This temperature can be approximated by feel. If you can hold both hands around the bottle and not get a burning sensation, the agarose is at the correct temperature. However, for added protection, you can use hot gloves when pouring the gels. You should have as many gel trays prepared as you have agarose. When the agarose is ready, add sufficient CarolinaBlu DNA Stain for all the gels (2-3 drops per gel). Pour to mix evenly.
- Line the gel trays along the edge of a lab table and pour the agarose into one side. Pour until the agarose comes within 3/4 of an inch of the empty end. Stop pouring and allow the agarose to flow to the end of the tray. This exercise requires that the gels have at least 6 wells and that the gel comb be placed within 3/4 of an inch of the end of the tray. The gels can be stored inside zip lock bags at room temperature. Pour one or two extra so students who make major mistakes can be handed a new one to complete the exercise. If you have highly motivated or advanced students, gel pouring might be a special project for them to do as an after school exercise.
- **Gel Preparation by Student**: This exercise gives the instructions for students to pour their own gels. In order to follow this procedure, you must have some method for melting the agarose ahead of time (a microwave is easiest) and a method for keeping the agarose at

approximately 60° C (hot water bath) throughout the day. The number of water baths you use depends upon the amount of equipment available and how comfortable you are having them in the room. You can follow the above instructions for agarose preparation and determine how many bottles of agarose will be needed for the day. Gels can be stored in zip lock bags on the counter top for the student's use on the next day. Have students label their plastic bag and place their gel inside.

- **Buffer:** In addition to the 1X TAE buffer needed for the gels, you will need to make up sufficient buffer for each electrophoresis chamber. You can reuse the 1X TAE buffer from the chambers so long as it is not badly contaminated by any student team. If contaminated, discard and replace with fresh 1X TAE buffer. If you have highly motivated or advanced students, buffer dilution might be a special project for them to do as an after school exercise.
- **Enzymes:** You will need to make up sets of enzyme for each student team by labeling microcentrifuge tubes (1.5 ml) as follows: B for BamH1; E for EcoR1; H for HindIII; and (-) for No enzyme. Transfer 15 µl of each enzyme (diluted to 10 units/µl) into appropriately labeled microcentrifuge tubes. Since the exercise only requires 1 µl per reaction, each set contains enough enzyme to supply a team of 2 students for up to seven periods. Enzyme sets need to be kept FROZEN until ready for use. On the day of the exercise you will need to have ice containers at each lab station to keep the enzyme cold throughout the day. Suggestions would be crushed ice in styrofoam containers or broken ice with water in empty pie tins or soup dishes. Pie tins and soup dishes work if the enzyme tubes are kept in the microcentrifuge tube racks so they don't turn on their side.
- **Tip boxes:** Be sure that all the micropipette tip boxes are filled. These tips need to be as clean as possible so filling the tip boxes should be done while wearing latex/vinyl gloves. This task is another one that can be done by your after school students or student service person.
- **DNA Stain:** CarolinaBlu DNA Stain added directly to melted agarose before pouring into the gel trays.
- **Preparation time:** If the teacher chooses to pour gels for the class, the time required for 1 class set is 15-30 minutes. Time needed to dilute the DNA stain and transfer to staining trays is 20 minutes. Time needed to label microcentrifuge tubes and transfer enzymes is 30-45 minutes. Total estimated prep time 2-3 hours.

Introduction:

This exercise is designed to introduce the student to the function of restriction enzymes and gain an appreciation of their use as molecular tools when working with DNA. The students will also see the connection between gel electrophoresis and imaging the result of the work of a restriction enzyme

Student Objectives:

- Identify restriction enzymes and their specificity.
- Determine the number of restriction sites on Lambda DNA.
- Visualize DNA pieces within a gel and effectively communicate this visualization.
- Estimate the size of each DNA piece produced by each enzyme.
-
-

Class Time Needed:

Three 50-55 minute periods are needed to complete this exercise

1. On the first day of this exercise students should complete pouring a gel and setting up the restriction of Lambda DNA.
2. The second day is for loading the gel with cut Lambda DNA and separation by electrophoresis. Since loading the gel with cut DNA takes only 10-15 minutes, you need to have an additional activity ready to fill in the remainder of the period on this day.
3. The third day is for data analysis.

Materials

1. BamHI enzyme
2. 1X TAE buffer
3. EcoRI enzyme
4. Gel tray, comb, electrophoresis chamber
5. HindIII enzyme
6. 1/2 masking tape
7. Uncut Lambda DNA
8. Micropipet (1-20 μ l)
9. Microcentrifuge tube rack
10. Loading dye (10X)
11. Box of micropipet tips
12. Power supply
13. 0.8% Agarose
14. Waste container
15. Staining trays
16. CarolinaBlu DNA Stain
17. Zip lock bags (sandwich size)
18. Permanent markers
19. Microcentrifuge tubes (1.5ml)
20. Spatula
21. Hot water baths
22. Ice baths
23. 2X Restriction buffer
24. Millimeter ruler
25. Semilog graph paper

Recipes for Consumables:

1X TAE (Tris Acetate EDTA buffer)

50X Research Organics VWR 101110-714 \$175/4 L

OR to make 50X TAE Buffer

242.0g Tri Base

57.1 ml glacial Acetic Acid

100 ml 0.5M EDTA (18.612 g EDTA in 100 ml dH₂O, pH to 8.0)

QS to 1 liter with dH₂O

0.8% agarose gels: 0.8 g of agarose powder melted in 99.2 ml of 1X TAE OR you can use the formula $C_1V_1 = C_2V_2$ to use any 2% agarose you may have left from other exercises. To make 100 ml of agarose at 0.8% from 2% agarose, use 40 ml of 2% agarose and dilute with 60 ml of 1X TAE. If you use this method, be sure to heat the 1X TAE so the melted agarose does not solidify when you pour the two solutions together.

DNA stain is made by diluting the stock solution 1 to 100. Dilute 10 ml of stock solution in 990 ml of distilled water.

Restriction buffer: The procedure is designed for this buffer to be at 2X concentration. If it is supplied at a higher concentration, use the formula $C_1V_1 = C_2V_2$ to dilute it to the 2X concentration. This MUST be done with DISTILLED WATER and JUST PRIOR to its use.

Procedure:

Part A: Gel Preparation

Be sure your students have taped the edge of their gel tray properly and placed the comb approximately 3/4 of an inch from one end of the tray. Prior to pouring, they will need to add several drops of CarolinaBlu DNA Stain to the agarose. It probably would be wise to have several sets of hot gloves in the room.

Part B: Loading the Gel

Loading the gel should not be a problem. However, you may wish to quickly review the micropipette and its use. Special attention should be paid to which stop to use for drawing up samples and which stop to use for expelling samples. Lane 1 is usually defined as the outermost well of the gel on the black electrode side of the electrophoresis chamber. Once this well is loaded, the students can then load the wells in sequence according to the procedure.

Closing the electrophoresis chamber and connecting it to the power supply deserves special attention. Be sure that the lid goes on black lead wire to black electrode and red lead wire to red electrode. Also, be sure that the electrophoresis chamber is positioned where you want it on the table. Once the chamber has been connected to the power supply and the power has been turned on, the student is NOT to touch or handle the chamber. Check the lead wires from the lid of the chamber to be sure that they have been plugged into the correct receptacles of the power supply. Black to black and red to red and that the wires are plugged into receptacles that are next to each other. Once you have checked all of this at a lab station, you can turn on the power supply and start the experiment running. Set the power supply to a constant 100 volts and use this as the running voltage.

Separation of the Lambda DNA fragments can take up to 30 minutes. This means that the electrophoresis process from one period might continue into the next. This can pose an equipment problem. You can either increase the size of the student teams to four, therefore needing 1/2 the number of gels, or arrange to borrow extra equipment from another teacher. Regardless of the decision you make, you will have to set up each lab station such that chambers from the previous class will be running while the current class is loading gels.

Disposal:

- Gels can be placed directly into the garbage. However, it is probably best to put all the gels into a zip lock bag before placing in the garbage.

- Check with your math teachers to see if any of them intend to do the exercises that pertain to graphing the size of the DNA fragments versus their migration distances. You can save some of the better gels in zip lock bags so they can have them for their math classes.
- Be sure to save the 1X TAE from the electrophoresis chambers for future use.
- You can save all the DNA stain for future use so DON'T discard it.
- Save all left over enzyme and uncut Lambda DNA. These need to be frozen and stored in a NON-DEFROSTING freezer. If you have a freezer with an automatic defrost cycle, DNA and enzymes can be stored by keeping them in the styrofoam container they were shipped in from the supply company. They can be saved frozen for up to 1 year.

TRANSFORMATION of E.coli (<http://education.llnl.gov/bep/science/science.html>)**TRANSFORMATION LAB, STUDENT WORKSHEET****Introduction**

In this lab you will put a small circular piece of DNA, called a plasmid, into a bacterium called Escherichia coli (E. coli for short). This process, in which a new piece of DNA is placed into an organism, is called transformation. It is a part of the new "genetic engineering" technology. Transformation can be used to introduce useful new genes from other species into a species that normally doesn't have those genes. For example, plants and animals can be made resistant to certain diseases by this process. Some transformation experiments have produced interesting and strange results. In one famous case, the gene that makes a firefly glow is put into a plant. This results in a plant that can glow in the dark! On a more practical basis we are looking to do transformations within a species for gene therapy in such areas as cystic fibrosis and muscular dystrophy.

In this experiment, the bacterium E. coli cannot normally grow in the presence of the antibiotic ampicillin, nor can it break down the sugar-like molecule "X-gal". However, the DNA plasmid pUC19, which will be put into the bacteria, contains two important genes. The first gene codes for resistance to the antibiotic ampicillin, and the second gene codes for an enzyme which can break down the X-gal. After undergoing transformation, the bacteria which successfully take in the new DNA will be identified by their ability to grow on ampicillin, and also by their ability to break down X-gal. Bacteria that can break down X-gal will turn a bright blue color, and so they will be especially easy to see.

Sterile Technique

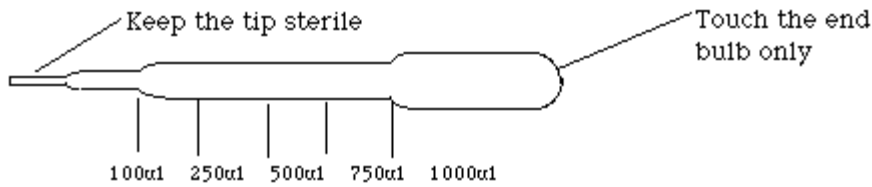
If you are not careful, other bacteria and even a fungus or two may grow on your petri plates and contaminate your experiment. Do not leave the petri plate lids off any more than you have to. If you are using a sterile tool, such as a pipette or plastic loop, do not touch the tip or allow the tip to touch ANYTHING except the designated bacteria, agar or sterile surfaces. Never assume a surface is sterile unless it has been specially prepared. If you make a mistake and you think the tool is no longer sterile, don't take chances. Get another tool.

Objectives

- * The student will define transformation.
- * The student will transform a bacterium.
- * The student will complete sterile technique.
- * The student will interpret and analyze experimental results using comparisons with controls.
- * The student will design an experiment using transformation technology.
- * The student will calculate transformation efficiency.

Materials

1. Sterile pipet. Note the measurements on the diagram. It's a little hard to see the numbers on the actual pipet.



2. Sterile plastic "inoculating loop". The loop end must remain sterile. Touch this instrument only on the handle.



3. Cultured E coli

- | | |
|---|-----------------------|
| 4. pUC19 plasmid | 10. test tube holder |
| 5. Calcium Chloride (50 mM) | 11. ice bucket |
| 6. 2 plain Luria Broth agar petri plates | 12. Luria Broth |
| 7. 2 Ampicillin Luria Broth agar petri plates | 13. Disposable gloves |
| 8. 2 X-gal/Ampicillin Luria Broth agar petri plates | 14. 42°C water bath |
| 9. 2, 15 ml sterile culture tubes | |
- Optional: Incubator

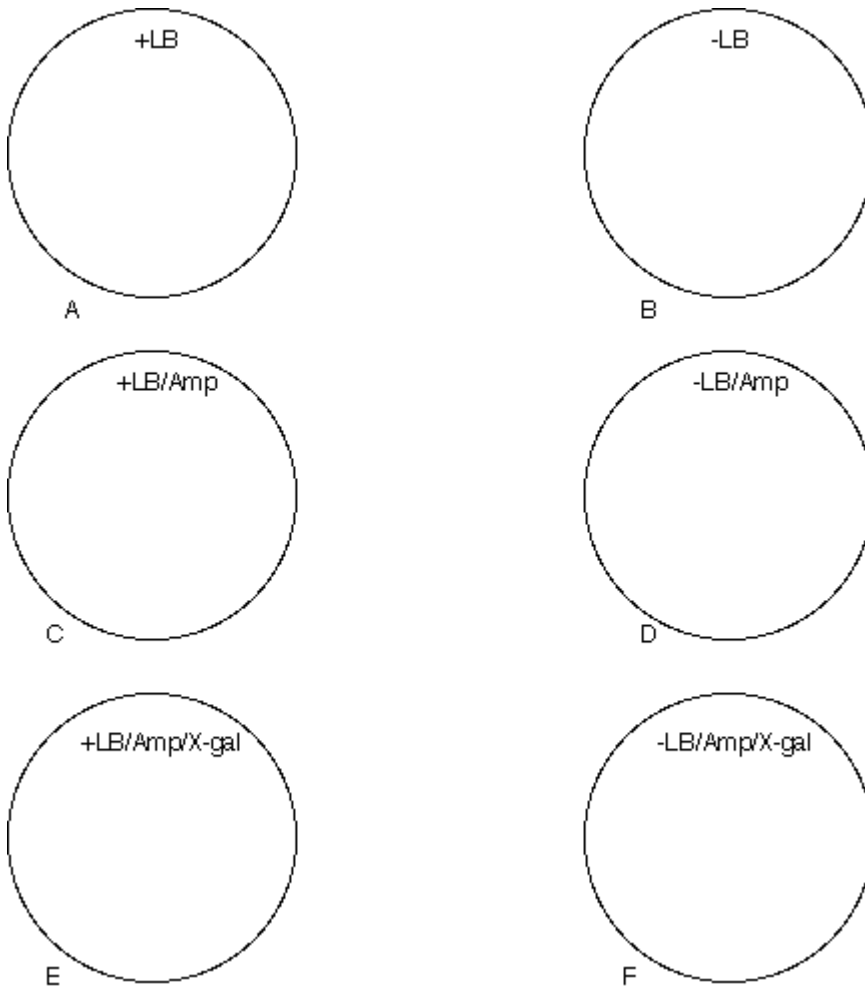
Procedure

1. First label one 15 ml test tube "+pUC" and the other "-pUC".
2. Use a sterile transfer pipet to add 250 μ l of ice cold calcium chloride to each of the two tubes. If you then put the pipet carefully back into the package it came from, being careful not to touch the tip on anything but the inside of the package, it should stay sterile.
3. Place both test tubes in the ice bucket.
4. Use a sterile plastic loop to transfer some of the E. coli bacteria from the starter plate culture to the -pUC tube. Be careful not to scrape up any of the agar along with the bacteria; slide the loop smoothly over the surface to pick up the bacteria. You don't need tons of bacteria, but get enough so that you can see a small clump sticking to the loop. When you put the loop into the calcium chloride solution in the tube, tap it against the tube wall so that the bacteria fall into the calcium chloride. Store the loop carefully on the lab bench **WITH THE TIP HANGING OVER THE EDGE SO IT DOESN'T TOUCH ANYTHING**. This will keep it sterile.
5. Now use the saved pipet to suck the suspension in and out a few times, so that the bacteria mix completely. No clumps of bacteria should be left in the tube. Place the pipette back into its wrapper.
6. Put the test tube back in the ice. Now take the +pUC tube and put some bacteria in it by following steps 4 and 5. You can use the same loop and the same pipet, as long as they have not touched anything which is not sterile.
7. Both tubes should now be on ice. Throw away the plastic loop AND the pipet.

8. Use a NEW sterile plastic loop to transfer a loopful of the pUC19 plasmid solution into the +pUC tube. Swish the loop in the tube to mix the DNA. Throw away the loop according to teacher instructions.
9. Return the +pUC tube to the ice. Leave both tubes on the ice for 15 minutes.
10. While the tubes are sitting on ice, label the six petri plates as follows:
Label one LB/Amp/X-gal plate "+" and the other one "-".
Label one LB/Amp plate "+" and the other one "-".
Label one LB plate "+" and the other one "-".
By the way, LB refers to the nutrient mixture that the bacteria grow on. Amp means the plate also contains the antibiotic ampicillin, and X-gal means it contains the sugar-like molecule X-gal.
11. Now that the test tubes have been on ice for 15 minutes, they need to be "heat shocked." This pulse will cause the cells to take up the DNA. To do this, remove the tubes from the ice and IMMEDIATELY put them in the 42⁰ C water bath for one and a half minutes. Watch the time carefully; don't let them sit longer in the hot water.
12. Then IMMEDIATELY put the tubes back in ice for 1 minute (at least).
13. Use a NEW sterile transfer pipet to add 250 µl of LB broth to each tube. Put the pipet back in the package carefully to keep it sterile. Gently tap the tubes to mix them. Set the tubes in a test tube rack for a 10 minute recovery.
14. Now you are ready to put the bacteria on your labeled plates. To do this, use the same sterile transfer pipet to take 100 µl from the -pUC tube and put it on the - LB. Use the same pipet to add another 100 µl from the -pUC tube onto the -LB/Amp plate. Then use the same pipet again to add 100µl from the -pUC tube to the -LB/Amp/X-gal plate. Tilt lid over the plate only as much as necessary and replace it. Now all three of your plates labeled "-" should have 100 µl of -pUC suspension on them. Put the pipet carefully back in the package to store it.
15. Next you have to spread the bacteria over the surface of the plates. You can do this by quickly moving a new sterile loop back and forth across the plate surface. Be careful not to dig it into the agar. It should slide over the surface as if it were an ice skater. Use the same loop for all three plates and in the same that you have done so far (LB, then LB/Amp, then LB/Amp/X-gal).
16. Now use the sterile transfer pipette (the same one previously used as long as it has not touched the agar or any other object) to take 100 µl of bacteria suspension from the +pUC tube and put it on the +LB plate. Then add 100 µl suspension to each of the other 2 plates (+LB/Amp and +LB/Amp/X-gal). You can use the same pipette for all three. Use the sterile loop to spread the suspension around on the three plates as described in step 15.
17. Tape the lids down on the plates (you can stack them all together). Put your name on the plates and place them UPSIDE DOWN in the 37⁰ C incubator until the next day (or at room temperature for several days.)

Data

1. Observe, draw and describe each of the six plates carefully and write down observations for each one. How much bacterial growth do you see on each, relatively speaking? What color are the bacteria? You may be able to count how many bacterial colonies (the spots you see) on each plate. This will depend on how many there are and how spread out they are.



Analysis

1. Why was one test tube labeled "+" and the other "-"?
2. Why do you suppose the CaCl_2 (Calcium Chloride) was added to the test tubes?
3. Why did you add the same bacteria to each tube?
4. Why did you add pUC19 (the plasmid or ring of DNA) to the "+pUC" test tube ONLY?
5. Why did you heat shock the bacteria?
6. Why did you add LB broth to each test tube? (HINT: What is a broth?)
7. Why do you suppose you needed to let the bacteria sit in the test tube racks for another 10 minutes after heat shocking and adding broth?
8. For each of the plates listed below describe what the label means and give a prediction of growth:

Plate	Description of Label	Growth/no Growth
"+LB"		
"-LB"		

"+LB/Amp"

"-LB/Amp"

"+LB/Amp/X-gal"

"-LB/Amp/X-gal"

9. Were you successful in transforming the bacteria? How do you know? Give possible explanations for ANY results that did not come out as expected. (Hint: review question #8 in procedure)

10. In this experiment, how many traits were placed into the bacteria? What are they and how are they expressed?

11. For each of the following pairs of plates, directly compare the results that you see. What does each pair of results tell you about the experiment?

A and B

A and C

A and E

B and F

B and D

C and D

C and E

E and F

Conclusions

Describe how a transformation can be performed and methods in which to test the success of a transformation.

Extended Activities

I. Experimental Design Activity

1. Design an experiment where the technique of transformation is used to solve a problem for society. You may use different cells than E. coli or even a virus. You may also use different genes or plasmids. If necessary, you may use a different medium than LB broth with ampicillin or X-gal. An example is that scientists have inserted normal genes into relatively undifferentiated cells of an individual with muscular dystrophy. These are grown in a test tube and injected into the muscles of a person with the affliction. The transformation has remained effective only for a few

months. Now design your own experiment using ideas from transformation technology. Make drawings if it enhances the description.

2. Analyze another person's or group's experiment. Are the steps, materials and equipment appropriate in their experimental design?

3. What kind of impact will this have on people, organisms, behavior and ecosystems? Including all positive and negative impacts, is the experimentation ethical, desirable and practical?

TRANSFORMATION, TEACHER PREPARATION

Advanced Preparation

- Luria-Bertani (LB) agar: LB agar can be made up at any time. It must be autoclaved and kept sterile in the refrigerator. Simply follow the directions on the bottle being sure that you pre-order an ample supply of distilled water. To avoid serious problems when re-melting the agar later, it is best to place only 350 ml of agar solution into a 500 ml bottle. Autoclaving (sterilizing) can be done using a pressure cooker at 15 psi for 20 minutes. It is simpler but more expensive to buy pre-made, sterile LB agar from any biological supply company.
- LB plates: Preparing the LB plates is the most demanding part of the preparation. Since each team requires 6 plates and there are 3 different types of plates per team and sterile conditions must be maintained, this needs to be done by the teacher OR students under very close supervision. This may be an opportunity to work closely with other department members or your best students as an after school or weekend project.
 1. Sterilizing plates: Empty glass petri dishes (15 mm X 100 mm) can be sterilized ahead of time by heating them in an oven at 400°F for 30 minutes (Caution Hot). Be sure to let them cool several hours before removing from the oven and handle only with hot gloves. If you can afford them, buy pre-sterilized plastic petri dishes from any biological supply company.
 2. Marking plates: Sterilized petri dishes should be marked on the bottom close to the edge. Each student team will have 2 plates labeled "LB"; 2 plates labeled "LB/AMP"; and 2 plates labeled "LB/AMP/X-gal". In addition to team sets of dishes, you will need one extra dish per lab station labeled LB. This dish will be sub-cultured with bacteria 2-3 days before the lab is done and will be the source of bacteria at that lab station for each student team throughout the day.
 3. Pouring plates: Since ampicillin and X-gal are both heat sensitive, it is essential that you have hot water baths capable of holding temperature at approximately 60°C. These can be made using pans or large beakers over hot plates with variable heat settings. The pre-sterilized agar must be melted. This can be done with a hot water bath or microwave oven. The temperature needed is 100°C and you must LOOSEN THE CAPS on the agar bottles before heating. When using a microwave, heat at the medium power setting and remove the caps, completely replacing them with sterile cotton or tissue. Once melted, the agar must cool to 60°C (this can take as long as 20-30 minutes). This temperature can be approximated by feel. If you can hold both hands around the bottle and not get a burning sensation, the agar is at the correct temperature. The agar can be placed

into the hot water bath and kept at this temperature for hours. There must be enough water in the bath to completely cover the agar in the bottles when they are placed in the bath. Once at this temperature, the Ampicillin and X-gal solutions can be added to the appropriately marked agar bottles. **DO NOT ADD AMPICILLIN OR X-GAL to agar above 65°C.**

4. When pouring the plates, place the sterilized, pre-labeled plates along the edges of your lab tables and lift the lids just enough to get the neck of the agar bottle into the space between the lid and the bottom of the plate. Pour enough agar (15 ml or less) to **BARELY** cover the bottom of the plate and replace the lid immediately. **BE SURE THE AGAR IS Poured INTO THE CORRECTLY LABELED PLATE.** To reduce the amount of condensation inside the plate, you can leave the lid propped at an angle (just the slightest opening) instead of placing it down flat on the bottom half of the petri dish. After the agar in the plates cools and solidifies, close the plates, turn them over and stack them. They can be stored in plastic bags in the refrigerator until lab day. Left over agar labeled LB can be saved in the refrigerator and re-melted for later use. Agar labeled AMP or AMP/X-gal **CAN NOT** be saved for future use. Re-melting this agar would destroy the AMP and X-gal molecules. Once you begin this preparation, you cannot leave until all the AMP, X-gal plates are poured. Be sure to plan plenty of time for this portion of the preparation; several hours are usually required. You might consider pouring LB plates one day and then the AMP and AMP/X-gal plates on another day.
- Sub-culturing bacteria: Two or three days before the activity is planned, you need to take the extra LB plates poured (the ones you determined would be the source of bacteria for each lab station) and streak them with E. coli bacteria.
 - Transformation Kit: The transformation kits need to be ordered 6 weeks in advance of their planned use. They can be ordered from Carolina Biological Supply Company catalog # F6-21-1146. Each kit comes with enough supplies and consumables for **SIX TEAMS**; you need to divide your classes accordingly. If the LB broth or calcium chloride comes in large containers, you will have to transfer these to 1.5 ml microcentrifuge tubes. Since most students have poor technique and don't measure very accurately with dropping pipets, you should make a double set of these tubes. This will prevent your having to try to transfer into these tubes during a class period.
 - Sterile technique: You should explain to your students the meaning of "sterile" and review those parts of the procedure that require using sterile technique. The procedure for this lab is written to save materials. In doing this, some sterile technique is sacrificed. If you have ample materials, instruct your students to discard all pre-sterilized pipets and loops after one use. You may choose to use a glass spreading rod for spreading bacteria rather than the sterile loop technique in the procedure. If so, be sure to place the ethyl alcohol (used to flame the glass rod) in petri dishes or beakers and keep it and the glass spreading rod away from the open flame of the Bunsen burner. You will have to demonstrate how to flame and use the glass spreading rod.
 - Preparation time: Estimated time for making LB agar and marking plates is 1.5 hours. Estimated time for sterilizing plates is 1 hour. Estimated time for pouring plates is 2-3 hours. Estimated time for sub-culturing bacteria is 10 minutes. Total estimated preparation time for 5 classes is 6 hours.

Introduction

This exercise allows students to learn the basic technique of inserting a piece of foreign DNA into a host organism. They will perform a transformation upon the bacteria *Escherichia coli* by inserting a plasmid containing two genes not normally found in the bacteria. By using pre-selected growth media, they can distinguish the bacteria cells that were transformed. This technique gives the students insight about one basic method used in genetic engineering.

Student Objectives

- The student will define transformation.
- The student will transform a bacterium.
- The student will complete sterile technique.
- The student will interpret and analyze experimental results using comparisons with controls.
- The student will design an experiment using transformation technology.
- The student will calculate transformation efficiency.

Class Time Needed

Four to five 50-55 minute periods are required to do this activity.

1. The first day is for transforming and plating bacteria. Some teachers may wish to spend 1 day just reviewing and practicing the procedure and the second day actually performing the lab.
2. The second day is for discussions, predictions and gathering the data.
3. The third day is for analysis of results and calculation of efficiency.
4. The fourth day is for designing another transformation experiment and discussing that design with the entire class.

Materials

1. Sterile plastic pipettes (4)
2. Sterile plastic loops (4)
3. *E. coli* culture
4. pUC 19 plasmid
5. 50 mM Calcium chloride
6. LB plates (2)
7. LB/AMP plates (2)
8. LB/AMP/X-gal plates (2)
9. 2 sterile culture tubes (15 ml)
10. Luria broth
11. Test tube holder
12. Ice bucket with crushed ice
13. 42°C water bath
14. Black permanent marker

15. OPTIONAL - INCUBATOR
16. 10% bleach solution
17. E. coli sub-cultured agar plates

Recipes for consumables

10% Bleach: Add 10 ml of bleach (household variety) to 90 ml of tap water.

50mM Calcium chloride: Add 50 ml of 1 M Calcium chloride to 950 ml of distilled water. Pre-rinse sterile filter with distilled water. Pass the Calcium chloride solution through the pre-rinsed filter. Transfer Calcium chloride to small, sterile tubes and store refrigerated until used.

Other consumables are mixed according to directions on the stock bottles or are provided in the correct concentrations by the vendor.

Procedure

The most important thing for students to understand is the idea of STERILE TECHNIQUE. If they think that they have contaminated a pipet or loop, it is essential that they discard that tool and get a new one. You should have an ample supply of sterile pipets and loops at each station. These can be placed in an empty beaker or jar.

The transfer of plasmid DNA from its stock tube to the pUC+ tube is crucial. Students must look carefully at the loop to see if there is a film of plasmid solution across the ring. This is similar to seeing a soapy film across a wire ring for blowing soap bubbles. If you prefer, you can have your students transfer 10 μ l of plasmid DNA with the micropipet. This will require extra materials and preparation if you choose this method.

It is essential that the students follow the directions regarding time. The pUC tubes are placed on ice for 15 minutes; the heat shock, for exactly 1.5 minutes; and the recovery in ice, for 2-3 minutes. These times are designed to maximize the number of transformants. The most critical part is getting the shocked tubes **BACK IN ICE IMMEDIATELY**.

Transferring bacterial suspension from the pUC tubes to the petri dishes requires some care. The bacteria will settle to the bottom, so be sure that students stir the suspension with the pipet before drawing it up. Also, make sure that they don't open the petri dish to the air when placing the bacteria on the agar or when spreading the bacteria across the agar surface.

Some teachers like to DRY RUN the procedure first to give ample time to explain sterile techniques, practice using the pipettes and loops and streaking and spreading bacteria on the agar's surface without completely removing the petri dish top. You will have to decide what is best for your students, based upon their lab experience and familiarity with these techniques.

Disposal

- All loops and pipettes can be placed in a 10% bleach solution for sterilization. It might be a good idea to have a shallow pan of this solution at every lab station. No matter what you choose, all used loops and pipettes should be collected for sterilization.
- Sterilize petri dishes by covering the agar with the 10% bleach solution. Let it stand for 10 minutes or more. Once sterilized, the agar can be placed in double zip lock bags and

placed in the garbage. Arrange to have your custodian pick these bags up the day you clean up.

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TRANSFORMATION TEACHER LECTURE NOTES

TRANSFORMATION

By 1926, the quest to determine the mechanism for genetic inheritance had reached the molecular level. Previous discoveries by Gregor Mendel, Walter Sutton, Thomas Hunt Morgan, and numerous other scientists had narrowed the search to the chromosomes located in the nucleus of most cells. But the question of what molecule was actually the genetic material had not been answered.

In 1928 Frederick Griffith, in a series of experiments with *Diplococcus pneumonia* (bacterium responsible for pneumonia), witnessed a miraculous transformation. During the course of his experiment, a living organism (bacteria) had changed in physical form.

The pneumococcus bacterium occurs naturally in two forms with distinctively different characteristics. The virulent (S-strain) form has a smooth polysaccharide capsule that is essential for infection. The nonvirulent (R-strain) lacks the polysaccharide capsule, giving it a rough appearance. Mice injected with S-strain of the pneumococcus bacteria die from pneumonic infection within a few days, while mice injected with the R-strain bacteria continue to live. Injection with heat-killed S-strain bacteria also results in the mice surviving.

Griffith was surprised to find in his experiments that mice injected with a mixture of heat-killed S-strain and live but nonvirulent R-strain produced lethal results. In fact, Griffith discovered living forms of the S-strain bacteria in the infected mice !

He hypothesize that the R-strain bacteria had somehow been transformed by the heat-killed S-strain bacteria. Some "transforming principle", transferred from the heat-killed S-strain, had enabled the R-strain to synthesize a smooth polysaccharide coat and become virulent.

Oswald Avery, Colin McCleod, and Maclyn McCarty (1934-1944) at the Rockefeller Institute, building on Griffith's work, showed that only DNA could cause the transformation. They isolated a cell-free extract from the S-strain bacteria and were able to transform living R-strain into a culture containing both S-strain and R-strain cells. The purified extract contained Griffith's "transforming principle". Through biochemical testing, they showed it to be deoxyribonucleic acid (DNA).

Classroom Transformation

The transformation witnessed by Griffith is a random and rare event. Today it is possible to reproduce Griffith's transformation in the classroom in a more controlled and reliable process.

Requirements to complete the process include:

1. A host bacterium for gene insertion.
2. A plasmid, self-replicating vector, to carry the foreign gene into the host bacterium.
3. A means of selecting for host cells that have been transformed.

Genetic Engineering Science

TL 5-1

Classroom transformation employs *E. coli* as the recipient of genes coding for identifiable phenotypes. The genes are carried in a plasmid vector. Two common genes used in transformation are:

1. amp^r gene which codes for resistance to the antibiotic ampicillin.
2. lac^z gene which codes for beta-galactosidase, which breaks down the lactose analog X-gal to produce a visible blue product.

The classroom protocol uses a rapid method to render *E. coli* "competent" to uptake plasmid DNA containing the genes to be transformed. Although the exact mechanism of plasmid DNA by competent *E. coli* cells is unknown, it is believed that DNA molecules may pass through pores in the bacterial wall. Bacterial cells are treated at 0°C to crystallize the fluid membrane, preventing the electrostatic repulsion between the DNA and phospholipid membrane (both negatively charged). Freezing stabilizes the distribution of charged phosphates and addition of cations (Ca^{2+}) in a transformation buffer forms complexes with the exposed phosphate groups, shielding the negative charges. A plasmid molecule can then move through the pore. Heat shocking furthers the process by creating a thermal imbalance to facilitate movement of the DNA molecules.

Once plasmids have been introduced into the host cells, they must become established inside the *E. coli* cell. During this time the plasmid replicates and expresses the genes which will allow for selection of a transformed phenotype. Selection can be established by spreading bacteria onto a LB agar culture plate with ampicillin. Only transformed cells can survive in the medium, untransformed cells will fail to grow.

GEL QUANTITATION OF DNA (<http://education.llnl.gov/bep/science/science.html>)

GEL QUANTITATION OF DNA, Student Worksheet

Introduction

In this exercise you will be able to run a gel and determine the approximate amounts of DNA and the size of each DNA fragment. It is very important for researchers to not only determine the size of the DNA fragments that are generated by cutting the DNA with restriction endonucleases but also the amount of each of the fragments. There are multiple mechanisms for determining this information. One method is to measure the DNA with a spectrophotometer. This allows very accurate determination of DNA quantity. Another method that is equally effective though not as accurate is to use a known quantity of a DNA marker like Lambda cut with the restriction endonuclease Hind III. This generates known fragment sizes and if you start with a known quantity of the marker in your well you can calculate the amount of DNA in each band on the gel. After the gel is stained and photographed you can compare the known marker DNA bands to the unknown bands and estimate the amount of DNA and the size of each. It sounds complex but it is relatively easy.

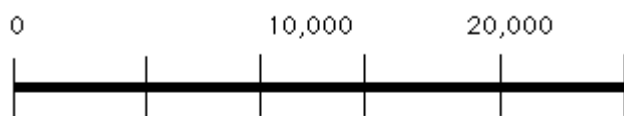
Objectives

- * The student will calculate the quantity of DNA produced in individual DNA fragments.
- *

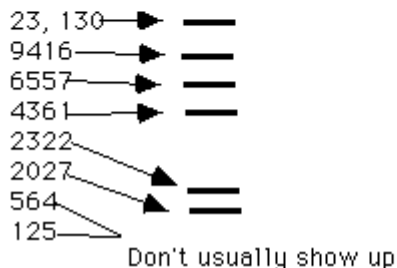
Protocol to Practice this technique

1. To be able to predict the amount of DNA in a particular DNA band on a gel there needs to be a control that is known. One common control is the Lambda viral DNA cut with the restriction endonuclease Hind III. It generates known bands. Below is an example of Lambda cut with Hind III.

Lambda



Lambda Hind III fragments



2. By knowing the size of each fragment you can calculate the percentage of the total Lambda virus DNA.

$$23,130 / 48502 = 48\%$$

$$9416 / 48502 = 19\%$$

$$6557 / 48502 = 13\%$$

$$4361 / 48502 = 9\%$$

$$2322 / 48502 = 5\%$$

$$2027 / 48502 = 4\%$$

$$564 / 48502 = 1\%$$

$$125 / 48502 = .2\%$$

3. By knowing how much DNA was placed into the well you can calculate the percentage of the total amount of DNA in each band.

If the concentration of DNA you were using was 0.1 ug/ul and you loaded 10 ul of the sample into the well, how much DNA did you load all together? How much DNA in each band? Show all work

Electrophoresis and Gel Quantitation, Teacher Preparation

Advanced Preparation

- Time: One day before lab; set up digest(15 minutes). Run gel; 1 hour
- The production of Pictures for students to practice on: Pictures can be produced by using various restriction enzymes on Lambda DNA or any other small source of DNA. If you use a large source you will get too many fragments and not much separation between fragments. One suggestion is to use Lambda cut with BamH1, Lambda cut with EcoR1, Lambda cut with Hind III. These enzymes are cheap and easy to obtain. Lambda is also inexpensive. If you have other sources of DNA and enzymes it will work just as well as long as the concentration is known. To produce the Lambda at a concentration of 0.1 microgram per microliter it is necessary to add 10 microliter (μ l) of DNA at 1microgram (μ g) / μ l, 10 μ l of 10x restriction buffer, 2 μ l of HindIII restriction enzyme at 1unit/ μ l concentration, and 78 μ l of double distilled water (sterile). Incubate overnight at room temperature. The next day place into a frost free freezer and freeze until ready to use or use before DNA is frozen.
- Follow the same procedure for the other DNA and enzymes by just substituting the other enzymes. This will produce enough for 10 samples each.
- To run gels (0.8 % gel) add 10 μ l of Lambda HindIII digest and one μ l of Loading dye. Draw up all eleven μ l of mixture and place into lane one.
- Repeat the procedures for the other digests and add them to the next two lanes.
- Add another Lambda HindIII to the last lane.
- Run gels at 74 volts for one hour.
- Stain with Ethidium Bromide for 5 minutes and rinse for 1 minute in water.
- Photograph gels

Introduction

This laboratory will enable students to develop an important laboratory skill. Gel quantitation is important for scientists working with DNA on a daily basis. If a restriction digest was carried out on a cosmid or other large piece of DNA and a particular sized fragment was desired, not only the size would be important, but the quantity of DNA in the fragment would also be important to estimate. A technician would know if they needed to cut more DNA in order to account for the loss of DNA in the gel purification protocol. This technique provides an estimation of the amount of DNA in a particular fragment. A more accurate method is to quantitate with a spectrophotometer over a range of wavelengths.

Student Objectives

- The student will correctly estimate the amount of DNA in a unknown band of DNA on a gel.
- The student will develop an experiment that would utilize gel quantitation as part of the laboratory.

Class time needed

One class period to complete initial estimations, one class period to do extensions.

Materials

1. Pictures for students to use: at least one per student
2. Rulers
3. Calculators

Recipes for making gels

0.8% agar is made by adding 0.8 grams of agarose and 99.2 grams of 1X TBE buffer. Microwave on medium for 1-2 minutes. DO NOT CLOSE. PUT PAPER TOWEL OVER TOP OR INTO TOP OF CONTAINER.

Procedures

- Having many other different samples for students to practice on is very helpful. If there are any laboratories near your school you could request some pictures to use in your class.
- Have students calculate the amount of all of the bands on a gel.
- This laboratory is nice to follow up with gel electrophoresis and graphing using semilog paper to calculate the size of DNA fragments.

ELECTROPHORESIS (<http://education.llnl.gov/bep/science/9/tElectro.html>)**Electrophoresis Student Worksheet:****Introduction**

Gel electrophoresis is the most widely used method in biology for separation and analysis of large molecules such as proteins and DNA. The goal of this exercise is to present the basic principles governing all varieties of electrophoretic technique.

Two basic pieces of equipment are required for electrophoresis: a DC Power Supply and a "box" with platinum electrodes to hold the buffer solution and the gel.

The Power Supply transforms the 110 Volt alternating current (AC) supplied by PG&E to an output of direct current (DC) of variable and regulated voltage. The simplest way to describe the power supply is to say that it pushes electrons out through a black wire called the **cathode** (labeled -) while simultaneously pulling electrons in through a red wire called the **anode** (labeled +). The output voltage (in VOLTS) indicates how much force is exerted to push and pull electrons. The output current (measured in AMPS) indicates how many electrons flow through the power supply in a given time.

Procedure**PART I:**

1. Make sure the power supply is turned off and unplugged.
2. Connect the lead wires from the power supply to the appropriate terminals on an empty electrophoresis box (red to red and black to black).
3. Turn the VOLTAGE to 50. This provides a very strong push to the electrons at the cathode.
4. Plug in the power supply. Turn it on.

What is the AMPS reading?

5. Turn the power supply off.

When you have nothing but air in the gel box, the electrical current is not able to pass from one electrode to the next. Air is not a good electrical conductor and electrons cannot flow from the cathode to the anode. At high voltage (higher than our power supplies can go) the resistance of the atmosphere would be overcome and an electrical spark would jump from the cathode to the anode.

PART II:

1. Turn off the power and unplug the gel box.
2. Fill each side in the electrophoresis box with 50 ml water (either distilled or tap). (The water should **not** flow over the table inside the box.)
3. Reconnect the box to the power supply and turn the power on.

What is the AMPS reading?

Look carefully at the platinum wire. Do you see bubbles forming on the wire?

4. Turn off the power supply.

Just as before, the current flow could not be measured even at the maximum voltage output because the connection between the electrodes is not made. Therefore, electricity cannot flow from the cathode to the anode. Remember, electrophoresis involves movement of positively and negatively charged ions through the buffer in the box and the movement of electrons through the wires and components of the power supply.

Part III:

1. Turn off the power and unplug the gel box.
2. Fill the gel box with approximately 300 ml of water (either distilled or tap). This time be sure that the water flows from one side of the box to the other.
3. Reconnect the gel box to the power supply and turn it on.

What is the AMPS reading?

Do you see bubbles forming on the platinum wires?

Analysis

1. Will electricity travel through air?
2. Will electricity travel through distilled water?
3. What are the good conductors in Parts I, II, and III?
4. What are the poor conductors in Parts I, II, and III?

PART IV:

1. Be sure the power supply is off and the box is disconnected from the power supply.
2. Fill the box with distilled water that contains NaCl (0.1 g/liter). (Be sure the salt water solution covers the table in the middle of the box.)
3. Connect the electrodes of the gel box to the power supply and plug in the power supply.
4. Turn on the power supply.

What is the VOLTAGE reading?

What is the AMPS reading?

5. Turn off the power supply.
6. Add a few drops of the red cabbage solution.
7. Turn on the power supply. Set the voltage at 100 V.

Analysis:

1. Carefully watch what happens in the box. Describe what happens.
2. Explain what is happening inside the box in terms of positive and negative charges.
3. Why is it important that the salt water solution connect both sides of the box in part IV?

Conclusion

Write three conclusions about electrical conductors.

How does electrophoresis work?

Extension

Reverse the electrodes. In other words, plug the red wire into the black hole on the power supply and plug the black wire into the red hole on the power supply. You have now reversed the charges. The red wire is now negatively charged and the black wire is positively charged. Add more red cabbage juice and turn on the power.

What do you observe?

How is this different from the results in Part III?

Explain how this difference can occur?

Electrophoresis Activity, Teacher preparation

(<http://education.llnl.gov/bep/science/9/tElectro.html>)

Advanced Preparation

1. Allow 1 - 2 hours for preparation.
2. Purchase 3 -4 gallons of distilled water to make the salt solution.
3. The teacher may choose to allow students to make their own pH indicator by boiling red cabbage in a beaker until the water turns a deep purple color. The teacher may also complete this process before class time.

Introduction

The process of electrophoresis is an important tool for molecular geneticists in separating DNA, proteins, and other molecules. The purpose of this activity is to allow students to become familiar with the gel box and the power supply.

Student Objectives

- The student will determine strong and weak electrical conductors.
- The student will determine how electrophoresis works.

Class Time Needed

If the students boil their own red cabbage, the activity will take 2 class periods. If the teacher boils the cabbage before the class, it will take 1 - 1 1/2 class periods.

Materials

1. Power Supply
2. Bottle for storing cabbage juice
3. Gel box
4. Beaker for boiling cabbage
5. Red cabbage
6. 0.1 g/liter NaCl

Recipes for Consumables Mix

0.1 g NaCl in 1 liter of distilled water.

Procedure

1. For each team of 2 students the teacher will need 1 gel box, one bottle cabbage juice, and 300 ml of salt solution.
2. For each pair of teams (4 students) the teacher will need 1 power supply.
3. Lecture on electrophoresis prior to activity.

Name:

Period:

Date:

Using PCR to Identify *E. coli* in the Spokane River Watershed

Introduction

While your goal is to investigate the water quality of the Spokane River, research almost always involves developing a technique that will work to address your question. In most science classes, the technique has been developed for you, and your job is simply to follow a protocol to make it work. For this project, you will be involved in developing the technique yourself. It will include: 1) finding primers that will amplify a genetic marker for *E. coli*, 2) extracting DNA from water samples and removing or neutralizing all materials that inhibit the PCR process, 3) developing the correct mix and concentration of ingredients for a success PCR amplification, 4) determining the correct temperature and time needed for each PCR cycle and correctly programming a thermocycle machine to do this, and 5) determining the best % agarose, buffer and voltage to use when electrophoresing the samples.

Day One: Prepare to run PCR reactions

1. Prepare bacterial controls
2. Dilute & aliquot stock solution of primers
3. Aliquot PCR ingredients
4. Prepare DNA stain and loading dye
5. Prepare electrophoresis buffer
6. Program thermocycler

Day Two: Run PCR reactions and prepare to run gels

1. Pour gels, and cover with buffer
2. Combine PCR reaction ingredients for each sample and control
3. Load and run thermocycler

Day Three: Run Gels

1. Remove amplified samples from thermocycler
2. Record and gel map and load samples
3. Electrophorese samples
4. Record gel results

DAY 1 IN DETAIL (Prepare to run PCR reactions)**1. Prepare Controls**

- ❑ Each group must prepare positive and negative controls to determine if an amplified PCR product is forming when it should.
- ❑ Each group must have a fresh culture of *E. coli* and *M. luteus*
- ❑ The following controls should be sufficient:
 - ❑ + control #1 – *E. coli* colony added to 500 µl of river water sample
 - ❑ + control #2 – *E. coli* colony added to 500 µl of river water sample that has been treated with Bio-Rad Instagene to help purify the sample and neutralize PCR inhibitors
 - ❑ – control #1 – *M. luteus* colony in 500 µl of dH₂O (could use river water and Instagene, but it is expensive)
 - ❑ – control #2 – 500 µl dH₂O with no bacteria added
- 1. Place 500 µl sterile dH₂O in a screw cap micro tube and use sterile technique to add 1 colony of *E. coli* to be treated with Instagene. For this sample, follow the steps on the Bio-Rad Instagene flyer for “DNA Preparation from Bacteria”.
- 2. Get 3 additional screw-cap tubes for the other controls. Label and add the appropriate ingredients to each.
- 3. Place each screw cap tube in a floating rack in boiling water for 5 minutes.
- 4. Store in the refrigerator overnight.

2. Dilute & aliquot stock solution of primers

Primer sequences (can order through IDTDNA.com)

Up 5'ccgatacgcgtccaatcagt 4–23

Down 5'acgcagaccgtaggccagat 868–887

From Chen J. and Griffiths M.W. 1998. PCR differentiation of *Escherichia coli* from other Gram-negative bacteria using primers derived from the nucleotidesequences flanking the gene encoding the universal stress protein. *Letters in Applied Microbiology* 27, 369-371.

- ❑ Get 2 student volunteers to dilute 10X primers for the class to use. Who will volunteer? _____ and _____
 1. Wipe down work table and pipets with bleach, get a box of sterile pipet tips that have cotton plugs, and put on a pair of fresh gloves.
 2. Label a clean microtube “1X forward primer”, and add 45 µl sterile dH₂O
 3. Add 5 µl of 10X forward primer to make a total volume of 50 µl 1X primer
 4. Repeat steps 1 & 2 with the reverse primer.

3. Aliquot PCR ingredients

- ❑ Each group will be running 4 PCR reactions. You will need to make a set of micro tubes with the ingredients to set up your own reactions so the filling process can be efficient.
- ❑ Wipe down work table and pipets with bleach, get a box of sterile pipet tips that have cotton plugs, and put on a pair of fresh gloves.
- ❑ Place all the tubes you will be using on ice. This can be done by pushing them into a dish of crushed ice, or by placing a rack with open bottoms on an ice pack.

- The following volumes should be sufficient for 4 PCR reactions:
 1. 4 μ l of 1X forward primer
 2. 4 μ l of 1X reverse primer
 3. 100 μ l of sterile dH₂O
 4. 2.5 μ l of each sample to be tested (these were prepared in step 1 as described above)
- Store your set of samples in the freezer

4. Prepare DNA stain and loading dye

- Get 2 student volunteers to prepare tracking dye for the class to use. . Who will volunteer? _____ and _____
 1. Wipe down work table and pipets with bleach, get a box of sterile pipet tips that have cotton plugs, and put on a pair of fresh gloves.
 2. Get a clean 10 ml graduated cylinder. Add the following ingredients:
 - i. 2 ml glycerol (stored in cupboard)
 - ii. 0.01 g bromophenol blue (stored in cupboard)
 - iii. 10 μ l SYBR Gold DNA stain (ask your teacher for this. It must be vortexed and centrifuged before opening, and returned to the freezer as soon as possible)
 3. Bring the volume up to the 10 ml mark by adding dH₂O with a plastic pipet.
 4. Use a plastic pipet to add 1 drop of 50X TBE buffer until it turns royal blue.
 5. Transfer to a screw-cap conical tube.
- Transfer 20 μ l into 8 different micro tubes. This will allow each group to have their own tube to work with tomorrow and increase our efficiency.

5. Prepare electrophoresis buffer

- Get 2 student volunteers to prepare 1X TAE buffer for the class to use. . Who will volunteer? _____ and _____
 1. Get stock 50X TAE buffer from cupboard.
 2. Calculate how much 50X buffer you will need and how much dH₂O you will need in order to make 1500 ml total volume.
 3. Have the teacher check your calculations before measuring.
 4. Use 2 clean graduated cylinders for measuring and a 1 gallon plastic water container for mixing.
 5. Label the gallon container if not already done for you.

6. Program thermocycler

- Get 2 student volunteers to prepare 1X TAE buffer for the class to use. . Who will volunteer? _____ and _____
- Follow the directions on the manual for the Edvocycler to program the steps described in the reference paper by Chen and Griffiths (1998) in Letters in Applied Microbiology vol. 27, pages 369-371. It must include the following steps:
 1. 94 °C for 5 minutes
 2. 94 °C for 2 minutes (denature)
 3. 70 °C for 1 minute (anneal)
 4. 72 °C for 1 minute (extend)
 5. Repeat steps 2-4 for 30 cycles

6. 72 °C for 5 minutes
7. 4 °C for a final holding temperature

DAY 2 IN DETAIL

1. Pour gels, and cover with buffer

- Get 2 student volunteers to prepare 1% agarose gel for the class to use. . Who will volunteer? _____ and _____
 1. 1 g agarose in 100 ml buffer is 1%, and you should make 300 ml.
 2. Weigh agarose powder (scale is in metal cabinet)
 3. Measure 1X TAE buffer in a graduated cylinder
 4. Combine them in a 500 ml glass bottle that is labeled
 5. Heat in microwave until all powder is dissolved
 6. Let cool until easily handled before pouring gels
- Every group must pour a gel and cover it with buffer. You are already practiced at doing this.

2. Every group must combine PCR reaction ingredients and get all samples loaded into the thermocycler

1. Wipe down work table and pipets with bleach, get a box of sterile pipet tips that have cotton plugs, and put on a pair of fresh gloves.
2. Get a 2ml thin-walled PCR tube containing a reaction bead for each sample. If you are running 4 samples, you will need 4 tubes with beads.
3. Get a container of crushed ice or place a rack on an ice pack. Keep all tubes on ice at all times.
4. Add the following PCR reaction ingredients to each tube, making sure to add the sample DNA last.
 - 1 µl of 1X forward primer
 - 1 µl of 1X reverse primer
 - 20.5 µl sterile dH₂O
 - 2.5 µl sample with DNA to be tested
 - _____ The total volume placed in each tube should be 25 µl
5. Keep prepared samples on ice until all groups have theirs prepared.
6. When all groups are ready, place labeled PCR reaction tubes into the thermocycler.
7. Close the lid and run the program.

DAY 3 IN DETAIL

1. Remove amplified samples from thermocycler
2. Add 5 µl SYBR Gold tracking dye to each PCR product tube
3. Record and gel map
4. Make sure gel is covered with 1X buffer
5. Load 25 µl of each sample into well
6. Electrophorese samples at 100 Volts until tracking dye is $\frac{3}{4}$ of the way across the gel.
7. Record gel results

D1S80: A Student DNA Fingerprint Activity

Revision #2 with BioRad “Instagene”

Please let us know if changes need to be made to this protocol!

Support information, D1S80 human population data, and laboratory procedures of other classroom D1S80 applications may be found online. “A Classroom Ready Protocol”, for example, has good background information. Please see the following website:

<http://www.garfield.k12.nj.us/ghs/Faculty/Janesak/DOCUMENTS/D1S80%20PCR.pdf>

I. “InstaGene Matrix” DNA Extraction

Supplies

1 ml pipettes

InstaGene Matrix (Cat No 732-6030EDU)

Clean 1.7 ml microfuge tubes or screw capped tubes

Sterile, flat, wood tooth picks

Sterile water

Clean forceps

Boiling water bath

Vortex

Centrifuge

Microfuge tube cap locks (or use screw-capped tubes)

Plastic floating rack for boiling tubes

Procedure: Day 1 on a 50 minute day

1. Vigorously scrape the inside of the cheek with a toothpick. Apply some pressure and scrape the cheek firmly 6-10 times with the round end of a flat toothpick. You should be able to see a small white film of cheek cells on the end of the toothpick. Do not draw blood.
2. Place the toothpick in a clean 1.7 ml microfuge tube or a screw-capped tube and break off the toothpick at the top so that it fits fully into the microfuge tube. Do not touch the toothpick where there might be cheek cells. The toothpick should fit completely, and move around inside the tube and the lid should shut firmly. Add 1 ml of sterile water.
3. Close the tube and vortex the sample for 10 seconds. Spin in a microcentrifuge for 45 seconds at full speed or for 5 minutes in a personal centrifuge (6 or 8 slots).
4. Remove the toothpick with clean forceps. Do not disturb the pellet of your cells. If you do disturb the pellet, spin it again. You should be able to see cheek cells in the bottom of the tube.
5. Remove 970 μ l of water without disturbing the pellet. Most of the water will be removed.
6. Vigorously shake the InstaGene Matrix solution to evenly disperse the beads. Immediately add 500 μ l of InstaGene Matrix to the sample.
7. Vortex for 10 seconds.
8. Incubate half submerged in a boiling water bath for 5 minutes. Use cap locks so that the tubes do not pop open if microfuge tubes are used rather than screw capped tubes.

9. Vortex for 10 seconds. Spin in a microcentrifuge for 3 minutes or 10 minutes in a personal centrifuge.
10. There will be 2 distinct layers visible in the reaction tube. The bottom layer contains the Chelex and cellular matter. The top aqueous layer contains the DNA. When you pipette out the DNA sample, do not disturb or pipette the Chelex layer.

This sample is ready for PCR. Store at 4°C (refrigerator) temporarily or at -20°C (freezer) long term. To reuse the frozen sample, or if sample is mixed, vortex and repeat steps 9 and 10.

II. PCR Procedure: Day 2 on a 50 minute period

Supplies

D1S80 Primers 5 µM stocks	Thermal cycler
PCR tubes	Sterile water
20 µl micropipette and filtered tips	100 bp DNA ladder (Promega G210A)
Takara Premix <i>Taq</i> Hot Start Version (Cat. No. R028A)	

A master mix can be made for the students, containing everything but their own DNA. Students would pipette 40 µL of the PCR master mix into their own tubes and then add 10 µL of their DNA samples. Many procedures suggest using hot start PCR. This means using a special “Hot Start” *Taq* polymerase or adding standard *Taq* after the initial 95° denaturing step. It is easiest to use a Hot Start *Taq* even though it is more expensive than standard *Taq*. Hot Start *Taq* will allow you to avoid opening the PCR tubes at 95°C, which can lead to evaporation of the reaction mixture. Also, *Taq* can denature after exposure to high temperatures for long periods of time. (Takara’s Premix *Taq* Hot Start Version was used in this procedure, and is an excellent polymerase. Depending on the *Taq* that you purchase, the concentration (units/ micro liter) will vary. If your *Taq* comes at a different concentration, reduce or increase the water accordingly.)

PCR reagents should be thawed and stored on ice when out of the freezer. This is especially important for the DNA polymerase enzyme.

Primer stock solution will be a higher concentration than 5 µM. You should make a dilution to 5 µM dilution to follow this recipe. Primers can be ordered from IDTDNA.com. Directions for reconstituting primers can be found at their website.

You should also run a positive and a negative PCR control. A negative control is one with all reagents except DNA. This control verifies that no DNA contamination has occurred in any of the PCR reagent tubes. A positive control is one with all reagents and a DNA sample that you know can be amplified.

D1S80 Primers (primers can be purchased from the IDTDNA.com website)

Primer #1

5’ GAAACTGGCCTCCAAACACTGCCCGCCG 3’

Primer #2

5’ GTCTTGTTGGAGATGCACGTGCCCTTGC 3’

Thermal cycler Program

1 cycle- 95°C for 5 minutes

30 cycles- 95°C for 1 min**68°C for 1 min****72°C for 1 min**

1 cycle- 72 for 5 min

This reaction actually works for a range of annealing temperatures from 62°-68°C.

Reaction

When making a master mix, combine the ingredients in the order that they are listed. The order of the first 3 ingredients is not as important, although water should be added first and everything else should be pipetted into the water. After adding all of these reagents, mix them by pipetting gently up and down several times. Student should add their DNA after they have pipetted the master mix into their tubes. Store the mixture on ice until it is placed into the thermal cycler.

When setting up a master mix for a class, there will be 4 tubes per team. The 4 tubes are needed for 1) a positive control, 2) a negative control 3) student #1's cheek cell DNA and 4) student #2's cheek cell DNA. Multiply 4 tubes times the number of teams. For example, if there are 8 teams, you will need to set up 32 (4 x 8) reactions.

Stock solution	1 reaction	10 reaction	32 reactions	Final concentration
Sterile H ₂ O	10 µL	100 µL	320 µL	
D1S80 Primer #1 5 µM stock	2.5 µL	25 µL	80 µL	0.5 µM
D1S80 Primer #2 5 µM stock	2.5 µL	25 µL	80 µL	0.5 µM
DNA from Chelex extraction	10 µL	10 µL	10 µL	about 5 ng
Premix Taq Hot Start	25 µl	250 µl	800 µl	
	50 µL total			

III. Gel electrophoresis: Day 3 on a 50 minute period*Supplies*

Mini-Gel boxes with combs

1xTAE (40 mM Tris-Cl, 20 mM Glacial Acetic Acid, 1 mM EDTA) diluted from a stock solution

Molecular grade agarose

SYBR Gold loading dye

UV transilluminator

Shield and UV glasses

Vortex

Centrifuge

In one lane, you should use a DNA ladder such as the Promega Bench Top 100 bp DNA ladder (Promega, Catalog #G8292)

Prepare a 2% w/v Agarose gel in TAE Buffer

1. Volumes will differ depending on the size of your gel box. You want a gel about ¼ to ½ inch thick.
2. Weigh 0.7 g of Agarose
3. Prepare 35 mL of 1X TAE Buffer. Add 3.5 mL of 10X TAE Buffer to 31.5 mL of pure water in a 125 mL Erlenmeyer flask. The buffer needs to be at a final concentration of 1X.
4. Pour the agarose into the buffer and let sit with gentle shaking for 1 minute
5. Heat the agarose to dissolve it in a microwave oven, watching all the time to prevent boiling over! Be sure to boil the agarose for at least one minute to get it to become thoroughly dissolved, i.e., no clear particles swirling round in the solution
6. Let the dissolved agarose sit for 2 to 4 minutes.
7. Pour the agarose solution into the gel electrophoresis apparatus mold which has the gel comb in place.
8. When the gel is thoroughly set and grey looking, gently remove the comb.
9. Set the gel in the electrophoresis running configuration and add buffer to fill the anode and cathode reservoirs and to just cover the gel with 1XTAE.

Prepare Samples for Loading into the Gel

1. Add 2 µl of SYBR Gold to 4 microfuge tubes.
2. Place the needed solutions into the following 4 tubes:
 - a. Tube 1: 5 µl of Promega 100 bp DNA ladder (Cat#G2101)
 - b. Tube 2: 5 µl positive control PCR DNA
 - c. Tube 3: 5 µl negative control PCR DNA
 - d. Tube 4: 5 µl D1S80 PCR DNA*, student 1
 - e. Tube 5: 5µl D1S80 PCR DNA, student 2
2. Close the tubes and vortex them. Use the personal centrifuge to spin the liquid contents to the bottom of each tube.
3. Make a table in your notebook for each sample lane:
 - The sample lane number on the gel (make a diagram of the gel)
 - The corresponding DNA sample identification

Load the Samples Into the Corresponding Wells.

1. Carefully pipette each sample into its well, placing the pipette tip just into the well opening but not touching the well bottom.
2. Electrophorese the samples toward the Anode (+) (Red) electrode.
3. Use 100 volts and allow the dark blue dye to run to 2 cm from the bottom of the gel.
4. Visualize using a UV transilluminator. Be sure to use a shield and UV glasses.

SOLUTIONS (<http://education.lnl.gov/bep/science/science.html>)**SOLUTIONS WORKSHEET, Student worksheet****Introduction**

Molarity and molality of solutions are explained in most standard chemistry texts, and taught in most standard chemistry courses. In biology, however, solutions are more often written as a percentage rather than as a molarity or molality. (This keeps the biologist from having to add up molecular masses and doing any complex math.) Thus, while molarity and molality are often covered in a chemistry class, solutions as a percentage is not normally taught.

The concept is very straightforward. A 1% solution means there is 1 gram of solute dissolved in 99 grams of solvent. Thus, the solute makes up 1% of the total final mass of the solution.

As an example, if it is desired to prepare 50.0 grams of a 2.0% NaCl solution the following calculations would be made.

Total mass of solute and solvent = 50.0 grams

Mass of solute (NaCl) = 50.0 g X 2% = 1.0 g

Mass of solvent (water) = 50.0 g - 1.0 grams = 49.0 g

So the solution would be prepared by dissolving 1.0 gram of NaCl in 49.0 grams of water.

Student Objectives

- * Students will correctly determine the number of grams of solute and solvent given its percent of solution and the total final mass of the solution
 - * Students will review how to make solutions based on molarity and molality
 - * Students will review the concept of density
 - * Students will practice conversions from cm^3 to dm^3 .
- ($1000 \text{ cm}^3 = 1000 \text{ ml} = 1 \text{ dm}^3 = 1 \text{ liter}$)

Materials

Paper, pencil and calculator

Procedure

1. Answer the following, show all work.

Molarity is the number of _____ of solute divided by the number of _____ of solvent.

The abbreviation for molarity is .

Molality is the number of _____ of solute divided by the number of _____ of solvent.

The abbreviation for molality is .

% solution means the solvent is _____ of the total of the final solution.

Assume that all solutions are being dissolved in water.

Assume that the density of water is 1.0 g/cm³ and that the final density of all solutions is 1.0 g/cm³. (Thus, 1 cm³ of solution equals 1 gram of solution.)

1. How would you make 5.00 X 10² cm³ of a 4.0 M NaCl solution?
2. How would you make 1.00 X 10³ cm³ of a 5.5 m KBr solution?
3. How would you make 40.0 grams of a 5.0% KOH solution?
4. How would you make 40.0 cm³ of a 2.0 % Mg(OH)₂ solution?
5. How would you make 50.0 cm³ of a 0.025 M NaF solution?
6. How would you make 100.0 cm³ of a 0.50 m KCl solution?
7. How would you make 400.0 grams of a 7.0% Sr(OH)₂ solution?
8. How would you make 400.0 cm³ of a 4.0 % KNO₃ solution?
9. How would you make 4.0 grams of a 1.0% Na₂SO₄ solution?
10. How would you make 70.0 dm³ of a 25 % Ba(NO₃)₂ solution?
11. How would you make 17.0 grams of a 2.0% sucrose solution?
12. How would you make 0.250 dm³ of a 2.50 % Ca(NO₃)₂ solution?
13. How would you make 0.050 dm³ of a 1.0 % agarose?
14. How would you make 0.050 dm³ of a 2.0 % agarose?
15. How would you make 0.050 dm³ of a 3.0 % agarose?
16. How would you make 0.050 dm³ of a 4.0 % agarose?
17. How would you make 50.0 cm³ of a 1.0 % agarose?
18. How would you make 50.0 cm³ of a 2.0 % agarose?
19. How would you make 50.0 cm³ of a 3.0 % agarose?
20. How would you make 50.0 cm³ of a 4.0 % agarose?

The formula for an agarose monomer is C₁₂H₁₈O₉

21. What is the molecular mass of agarose?
22. Determine the molarity of a 1% agarose solution. (Hint: assume you are making 100 grams of total solution.)
23. Determine the molarity of a 2% agarose solution. (Hint: assume you are making 100 grams of total solution.)
24. Determine the molarity of a 3% agarose solution. (Hint: assume you are making 100 grams of total solution.)
25. Determine the molarity of a 4% agarose solution. (Hint: assume you are making 100 grams of total solution.)

Solutions Worksheet, Teacher Preparation

Advanced Preparation

Run off worksheets.

Introduction

Molarity and molality of solutions are explained in most standard chemistry texts, and taught in most standard chemistry courses. In biology, however, solutions are more often written as a percentage rather than as a molarity or molality. (This keeps the biologist from having to add up molecular masses and doing any complex math.) Thus, while molarity and molality are often covered in a chemistry class, solutions as a percentage is not normally taught.

The concept is very straightforward. A 1% solution means there is 1 gram of solute dissolved in 99 grams of solvent. Thus, the solute makes up 1% of the total final mass of the solution.

As an example, if it is desired to prepare 50.0 grams of a 2.0% NaCl solution the following calculations would be made.

Total mass of solute and solvent = 50.0 grams

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Mass of solvent (water) = 50.0 g - 1.0 grams = 49.0 g

So the solution would be prepared by dissolving 1.0 g of NaCl in 49.0 grams of water.

Student Objectives

- Students will correctly determine the number of grams of solute and solvent given its percent of solution and the total final mass of the solution
- Students will review how to make solutions based on molarity and molality
- Students will review the concept of density
- Students will practice conversions from cm³ to dm³. (1000 cm³ = 1000 ml = 1 dm³ = 1 liter.)

Class time needed

One 50-60 minute class period

Materials

Paper, pencil and calculator

Procedure

1. Review molarity and molality, then teach % solution.
2. Pass out worksheet
3. Correct the worksheet.

Problems With Solutions

Molarity is the number of Moles of solute divided by the number of liters or dm^3 of solvent. The abbreviation for molarity is M .

Molality is the number of moles of solute divided by the number of kg of solvent. The abbreviation for molality is m .

% solution means the solute is a given percentage of the total mass of the final solution.

Assume that all solutions are being dissolved in water. Assume that the density of water is 1.0 g/cm^3 and that the final density of all solutions is 1.0 g/cm^3 . (Thus, 1 cm^3 of solution equals 1 gram of solution.)

1. How would you make $5.00 \times 10^2 \text{ cm}^3$ of a 4.0 M NaCl solution? Dissolve 117 grams of NaCl in enough water to make 500.0 cm^3 of solution
2. How would you make $1.00 \times 10^3 \text{ cm}^3$ of a 5.5 m KBr solution? Dissolve 655 grams of NaCl in enough water to make 500.0 grams of solution
3. How would you make 40.0 grams of a 5.0% KOH solution? Dissolve 2.0 grams of KOH in 38.0 grams of water.
4. How would you make 40.0 cm^3 of a 2.0 % $\text{Mg}(\text{OH})_2$ solution? Dissolve 0.80 grams of $\text{Mg}(\text{OH})_2$ into 39.2 grams of water.
5. How would you make 50.0 cm^3 of a 0.025 M NaF solution? Dissolve 52 grams of NaCl in enough water to make 50.0 cm^3 of solution
6. How would you make 100.0 cm^3 of a 0.50 m KCl solution? Dissolve 3.7 grams KCl in 100.0 grams of water.
7. How would you make 400.0 grams of a 7.0% $\text{Sr}(\text{OH})_2$ solution? Dissolve 28 grams of $\text{Sr}(\text{OH})_2$ into 372 grams of water.
8. How would you make 400.0 cm^3 of a 4.0 % KNO_3 solution? Dissolve 16 grams of KNO_3 into 384 grams of water.
9. How would you make 4.0 grams of a 1.0% Na_2SO_4 solution? Dissolve 0.040 grams of Na_2SO_4 into 3.6 grams of water.
10. How would you make 70.0 dm^3 of a 25 % $\text{Ba}(\text{NO}_3)_2$ solution? Dissolve 17,500 grams of $\text{Ba}(\text{NO}_3)_2$ into 52,500 grams of water.
11. How would you make 17.0 grams of a 2.0% sucrose solution? Dissolve 0.34 grams of sucrose into 16.7 grams of water.
12. How would you make 0.250 dm^3 of a 2.50 % $\text{Ca}(\text{NO}_3)_2$ solution? Dissolve 6.25 grams of $\text{Ca}(\text{NO}_3)_2$ into 244 grams of water
13. How would you make 0.050 dm^3 of a 1.0 % agarose? Dissolve 0.50 grams of agarose into 49.5 grams of water.
14. How would you make 0.050 dm^3 of a 2.0 % agarose? Dissolve 1.0 grams of agarose into 49.0 grams of water.
15. How would you make 0.050 dm^3 of a 3.0 % agarose? Dissolve 1.5 grams of agarose into 48.5 grams of water.

-
16. How would you make 0.050 dm^3 of a 4.0 % agarose? Dissolve 2.0 grams of agarose into 50.0 grams of water.
17. How would you make 50.0 cm^3 of a 1.0 % agarose? Dissolve 0.50 grams of agarose into 49.5 grams of water.
18. How would you make 50.0 cm^3 of a 2.0 % agarose? Dissolve 1.0 grams of agarose into 49.0 grams of water.
19. How would you make 50.0 cm^3 of a 3.0 % agarose? Dissolve 1.5 grams of agarose into 48.5 grams of water.
20. How would you make 50.0 cm^3 of a 4.0 % agarose? Dissolve 2.0 grams of agarose into 50.0 grams of water.

The formula for an agarose monomer is $\text{C}_{12}\text{H}_{18}\text{O}_9$

21. What is the molecular mass of agarose? 306.30 g/mole
22. Determine the molarity of a 1% agarose solution. (Hint: assume you are making 100 grams of total solution.) 0.0326 M
23. Determine the molarity of a 2% agarose solution. (Hint: assume you are making 100 grams of total solution.) 0.0652 M
24. Determine the molarity of a 3% agarose solution. (Hint: assume you are making 100 grams of total solution.) 0.0979 M
25. Determine the molarity of a 4% agarose solution. (Hint: assume you are making 100 grams of total solution.) 0.131 M

Ethical, Legal, and Social Implications of Genetic Knowledge

(<http://www.genome.gov/25019880>)

Guidance for ELSI Vignettes at <http://www.genome.gov/25019884>. (NOTE: Several vignettes were copied from this site. There are many more)

Where and how should society respond to and integrate genetic information? The following vignettes provide an opportunity to explore this question in a variety of situations. They are designed to elicit an open-ended discussion. Everyone should be aware that there are no right or wrong answers.

As the discussion proceeds, the facilitator should remind the participants that genes are not "everything" and that an individual's health and behaviors are influenced by complex interactions between multiple environmental, social and genetic factors. There is a tendency for people to lose sight of the complexity of these non-genetic influences when discussing these issues. The facilitator should also keep in mind that different participants will approach the utilization or interpretation of genetic information in different ways and that each will bring to the discussion their own knowledge, perceptions and beliefs.

These vignettes raise some potentially sensitive issues (e.g., loss of a parent, the death penalty, discrimination, diseases such as breast cancer and Alzheimer's, etc.) Some discussants may have had personal experiences that will influence their responses to a particular vignette. Discussion facilitators should anticipate that such individuals might find these discussions sad, embarrassing and/or difficult and should be prepared to provide them with support.

Nature vs. Nurture in the Criminal Justice System

Background:

The pace of research into genetic factors that may influence how we think and act has increased drastically in the last few years. Some forms of mental illness have a strong hereditary component. For example, scientists are trying to determine how genetic factors make some people more susceptible to disorders like schizophrenia, depression and alcoholism. They also are exploring the contributions of genes to certain personality traits, like shyness and impulsiveness. Scientists currently believe that the vast majority of human behaviors and traits reflect a complex mix of genetics and the environment. It is unlikely that they will discover single genetic mutations that determine such characteristics as intelligence or that fully account for why some people become aggressive or violent.

Vignette:

It is 2010, and Joe Schmoe has been charged with assault. The physical evidence supporting his guilt is overwhelming and he pleads guilty. In preparation for his sentencing hearing, Joe's lawyer asks him to undergo a series of genetic tests to determine whether he carries any of four genetic mutations that have been associated in research literature with violent behavior. The tests, while controversial, show that Joe's DNA does, in fact, contain all four mutations. Based on these results, Joe's lawyer will argue that Joe should be sent to a psychiatric facility rather than to state prison. He claims that because Joe's genetic status predisposed him to this violent act, it would be unfair to sentence him as a criminal for behavior over which he had essentially no control.

Discussion Questions:

1. If you were the judge at Joe's sentencing hearing, how, if at all, would the results of this controversial genetic test influence your decision?
2. How would your decision be influenced if Joe had only 1 of the 4 mutations associated with violent behavior?
3. What would be your decision if Joe was shown to suffer from a mental illness such as schizophrenia? How come?
4. If Joe gets sent to prison and tries to get released on parole fifteen years later, should the fact that he may have a genetic predisposition to violent behavior be used to keep him in prison, even if his behavior has been consistently good during his incarceration?
5. In the future, should all newborn babies be screened to determine if they have genetic mutations that could be linked to violent behavior? How come?
6. What if a medication became available to treat people with these mutations?

Genetic Determinism: Endurance Athletes**Background:**

In 1999, scientists reported that a particular variant of the angiotensin–converting enzyme (ACE) gene was associated with superior physical endurance. The variant, known as the ACE insertion polymorphism, was found to be present in a higher proportion of elite endurance athletes, especially long distance runners, than in the general population.

Vignette:

Marathon University (MU) is offering full–tuition scholarships and a guaranteed spot on the varsity track team to high school sophomores who "pass" a genetic test. If the parents sign a consent form, University doctors will screen them for the ACE insertion polymorphism.

Discussion Questions:

1. Is it likely that a single gene determines physical endurance?
2. Are other genes likely to be important?
3. What other factors determine whether a child will grow up to be an elite endurance athlete?
4. Should parents be allowed to use genetic information to make decisions that will limit their children's choices in adulthood?
5. What else should MU do if it is concerned about the well being of the future student-athlete?

One for the Other**Vignette:**

Anna and Carlos' first child Vincent suffers from a rare form of cancer. Doctors explain to Anna and Carlos that chemotherapy could potentially cure Vincent's type of cancer, but there is no guarantee this mode of treatment would work in his particular case. Another option is a bone marrow transplant, which involves killing Vincent's cells that do not work correctly and replacing them with healthy cells from someone else. If successful, the bone marrow transplant could cure Vincent of the cancer. However, for this treatment to work, the donor's tissue type would have to be a very close match to Vincent's, which is very rare.

Anna and Carlos desperately want Vincent to have a bone marrow transplant, but neither of their tissue types are close enough to Vincent's to work. The doctor tells them they could have a second child, and, if this child's cells match Vincent's, the new baby could donate cells from its umbilical cord blood to complete a bone marrow transplant.

Anna and Carlos decide to go ahead and have a second child, whom they name Thomas. At the time of Thomas' birth, doctors collect the blood from his umbilical cord for possible use in Vincent's bone marrow transplant. The parents are overjoyed to learn that the baby's tissue is indeed a good match for Vincent's, but doctors caution that this treatment, while more successful than chemotherapy, still does not guarantee a cure. They caution that Vincent may need further tissue donations from Thomas later in life that require more invasive procedures, such as removal of bone marrow or organ donations, such as a kidney transplant.

Discussion Questions:

1. What do you think of Carlos and Anna's decision to have a second child to try and help Vincent?
2. What would you think if Vincent needs a second bone marrow donation when Thomas is 8, and Thomas decides he doesn't want to participate because he is afraid of the needles involved?

The Benefits of Research: Do We Share the Wealth?

Vignette:

Eva is a nurse working in Angola who has treated people infected by the Ebola virus for many years. Although most people who are infected with Ebola die quickly, she has been exposed frequently and has never gotten sick. Eva began to wonder whether there is something different about her that protects her from the virus. During a visit to the United States, Eva visits a local university where scientists are involved in Ebola research. She meets with one of the researchers and describes her experiences in Africa. She suggests that perhaps she is immune to the Ebola virus and offers to give a blood sample for the researcher's study. The researcher agrees, obtains informed consent, collects a blood sample, and thanks Eva for coming in. Informed consent is a legal process where a participant or patient knows all of the risks and costs involved in a study, treatment, or test. Informed consent must be given voluntarily. The researcher uses Eva's blood sample in his research and finds that she is immune to the virus.

Several years later, the researcher and her coworkers have used their knowledge about Eva's unusual cells to develop a new treatment for Ebola. The treatment works well and has few side effects. The press reports on the new treatment and it becomes widely used. Soon, the researchers

who developed the treatment are receiving a large amount of money, referred to as royalties, from selling the treatment to a drug company.

Meanwhile, Eva has heard about the success of the treatment that began with her blood sample. While she is proud to have played a role in saving lives, she also feels that she is entitled to a share of the profits. She again meets with the researcher; this time to plead her case for receiving some portion of the money. The researcher listens to Eva and politely explains that she is not entitled to share in the royalties. She says that the money only rewards those who contributed to creating the new treatment and supplying a blood sample is not enough of a contribution.

Eva leaves the meeting dissatisfied. She feels that she did more than just provide a blood sample because it was her idea that there was something different about her blood. In Eva's view, all of the researcher's work was based on her idea. She decides to discuss the matter with a lawyer and perhaps file a lawsuit seeking a share of the royalties.

Discussion questions:

Researchers often use tissue donated by volunteers to study and develop possible future treatments.

1. What information do you think these volunteers need to know and agree on before donating their tissue or blood?
2. Should researchers be allowed to use tissues obtained from hospitals that otherwise would be destroyed, such as blood samples?
 - a. If so, based on ethical reasons, should patients be notified that their tissues were used for research?
 - b. Based on legal reasons, should patients be notified that their tissues were used for research?