Bio-Link Discussion Group
January 2005—December 2005

Community college impact on the biotech workforce (January 2005)

I'm attempting to find publications and other references that point to the impact of community and technical colleges on the biotechnology workforce. For example, Elaine Johnson published an article in 2003 in the "Biochemistry and Molecular Biology Education" journal (www.bambed.org). Also, Hank Stern gave a presentation on this subject at the Community College Thread of the Biotechnology Industry Organization meeting, BiO 2004. Last year I read a similar article in a biotech trade journal, but I'm having trouble remembering where it was. I am hoping that this list serve will be an efficient way to assemble this list. Any input would be greatly appreciated. *Community College of Rhode Island* (#1199)

- This is a little dated, but the Journal of Industrial Microbiology published a special issue, Vol. 24, in 2000, http://www.springerlink.com/link.asp?id=b54bql4wf0pj that included many articles by community college biotech faculty. There was even an article by Todd and I. "Bioinformatics in the Biotechnology Classroom" J. of Industrial Microbiology and Biotechnology 24:314-318 (2000). Porter, S.G. and T.M. Smith. The journal is a little tricky to find since Nature sold it to Spinger-but I put the link above. Unfortunately, Springer requires a subscription to view the articles, but you can at least get the table of contents. *Geospiza, Inc.* (#1204)

Developing a basic high school biotech course for GISD (January 2005)

I would like to seed the idea of developing a Basic Biotechnology course for GISD (HS level). I am planning a meeting soon with school district officials, college and workforce program administrators (and others interested in the project). I would, greatly, appreciate your input and feedback on what elements I need to considerer to put this course together. I am in a two-year community college in Galveston, TX. We offer a two-year AAS in Biotechnology. I will be happy to send an informative program brochure upon request. *Galveston College* (#1208)

- A great resource for starting multilevel high school biotech courses is Ellyn Daugherty. She has published a textbook and lab manual. You can find her contact info and samples of her textbook at http://www.skipwagner.net/smbiotech/bioteched.htm. *San Jose State University* (#1209)
- I am a plant tissue culturist so you know my bias when it comes to biotech. Your program is probably more 'medical'. I would like to see info on your program and would give you some input if you want a plant component. Address; Carol M. Stiff 905 Champions Lufkin, TX 75901. *Kitchen Culture Kits, Inc.* (#1210)
- I have been working with the Virginia Department of Education on several high school courses. Biotechnology Foundations can be taught by technology, health science or agriculture teachers. Second-level courses include Bioengineering (taught by technology teachers), Biomedical Technology (taught by health science teachers), and Biotechnology Applications in Agriculture (taught by agriculture teachers). Visit the CTE resource center (http://www.cteresource.org/) and select the link "Task Lists and SCRs" to learn more about these courses. These are very new so I hope others will share their thoughts about the secondary level. *Old Dominion University* (#1213)

Program outcomes (March 2005)

I've been charge with creating measurable outcomes for a 2 year Biotechnology Program. I have created some but would like to compare with others, since we don't have a national organization

or accreditation that provides a gold standard. Would any Directors of similar programs be willing to share their own with me? Many of the students in other program at my college must successfully complete a regional or national exam in order to gain licensure. In this example, the measurable outcome is straight forward - the students will pass the national exam. (#1235)

- On the PDE website www.pde.state.pa.us if you just do a search in the box on the home page for Biotechnology Curriculum Frameworks - there is info. That might assist you to develop outcomes. (#1236)
- Would you be willing to share what you have created? Our college has just started doing student assessment for all programs. In the Biology & Chemistry Department, we have chosen to start with assessment of student's knowledge of safety procedures. However, we will would like to use some national Biotechnology standards in the future. *Delaware Technical & Community College* (#1238)
- We are developing a Life Sciences & Biotechnology section up here in Canada at Red River College in Winnipeg, Manitoba. I've copied some objectives for the QA/QC program and included them here for you. If you need more details you can contact me at my address below. 1. Interpret the requirements for testing of raw materials, in-process samples and finished product in accordance with pharmacopoeia compendia standards. 2. Perform a variety of Quality Control activities including developing of QC policies and Standard Operation Procedures, analyzing and archiving data, interpreting results. 3. Operate, validate and calibrate a variety of laboratory equipment used in the pharmaceutical industrial labs. 4. Assess instruments malfunction and troubleshoot analytical equipment failure in compliance with regulatory requirements. 5. Identify and analyze unexpected results during routine analyses and help to provide solutions based on scientific and regulatory considerations by implementing preventive action and corrective actions program. 6. Acquire a working knowledge of U.S. and Canadian GMP requirements to the manufacture of pharmaceuticals, blood products and natural health products. 7. Understand the concept of quality systems and compliance in the regulated industry and the role of quality assurance. 8. Understand the use of controlled documentation. 9. Review GLP, ISO 9000 and ISO 17025. 10. Prepare for and conduct internal and external audits and help prepare for a regulatory inspection. *Red River College* (#1240)

Fraction collector (April 2005)

We're looking to purchase a fraction collector for protein purification for our Biotechnology lab course. We'd appreciate any suggestions on models that people have been happy with. *Delaware Technical and Community College* (#1251)

- I have used the Frac 100 from Pharmacia (now call GE Health Care). If you look in the used equipment vendors, they may have it, brand new will cost ~ $3200.00 cdn. It is a very robust and reliable fraction collector, and I have collected fractions size up to 10ml, collection is based on time to achieve the desired volume per fraction. *Red River College* (#1252)
- I have been using ISCO units for years and years. (#1253)

Maxi preps for plasmids (April 2005)

We have been having trouble with maxi prepping plasmids lately - we get a yield but want a high enough concentration to use the plasmids for a restriction digest and DNA patterns mapping - we have been using Qiagen kits and in talking with another site doing similar attempts with same vendor, they also have had problems - anyone recommend another source of maxiprep kits where they have had success with high concentration yields? *Oklahoma City Community College* (#1262)

- Call me old fashioned, but we do a "kit less" alkaline lysis in our class. Yields are good, materials are cheap, and the students have to make up their own solutions. Chromosomal

DNA contamination can be a problem if they aren't careful, but we usually gel purify cut plasmids which seems to circumvent the issue. (#1267)

- We do the same (alkaline lysis) in our molecular techniques class and use the DNA for both restriction digests and cloning. It works well and the students learn what each reagent is for since they are all defined completely. It doesn't take very long (for minipreps - one three hour lab period) for a large scale prep (two three lab periods). (#1268)
- If you want to look into another kit, we have used the invitrogen kits with great success http://www.invitrogen.com/content.cfm?pageid=10577. I have also had great success with all kinds of RNA and DNA purification supplies from Sigma. We tend to go with Sigma because it is on our state contract, but I have been very happy with materials we use from them http://www.sigmaaldrich.com/B2B/Area_of_Interest/Life_Science/Molecular_Biology/\DNA_and_RNA_Purification/Plasmid.html. *Finger Lakes Community College* (#1269)
- We use Biorad and usually get yields of 0.5 ug/uL. (#1273)
- I've enclosed a couple of protocols (at the end of the document). Many more variations can be found on the web. Sue Ellen Chantler recently posted her protocol which is essentially the same, but written for a lab class and a little more student friendly. We start off with solution preparation with the students making up a variety of stock solutions that they can dilute for various working solutions (including this one). They then go into microbial cell growth and set up cultures for plasmid isolation (and genomic isolation in some cases). We use to do small cultures (10 ml, which we still do for boiling preps), but found that larger cultures (25-50) were easier for the students to handle and allowed for some inefficiency while still providing a usable yield (I don't have numbers as far as student yields from this procedure). IMHO kits are overused, especially in teaching labs where we hope to instruct students about what is happening and why we use certain reagents. That is hard to do with kits that have little documentation. Kits do have their place, and in a research environment where money is available they can accelerate the pace of research and reduce the variability that is sometimes found in home-grown procedures. Still, there is something to be said for the "old way". (#1277)

Autoclave specifications (April 2005)

We're planning to buy the BioFlow 110 Fermentation system (3L vessel) and need to upgrade our autoclave. Any suggestions, including size specifications for a new autoclave? *Community College of Rhode Island* (#1282)

- We have recently purchased a Sanyo portable auto clave model MLS-3750, this unit runs off a single phase power 120 volts, and 20 amperes, so they will not plug into standard wall outlet which is 15 Amperes they need to be set up for 20 amperes wall socket outlets. This model comes with a 20 amperes twist lock plug. Some newly wire lab locations have this configuration; older labs have the older 20 amperes plug wall socket arrangement where one of the slots in the wall outlet is shaped like a sideways (T). It also requires 4.5 L of heater water. It was shipped with a configuration of two internal baskets for glassware. It has four cycles of operation with three pre-programmed programs for each program along with the option of creating your own. The four cycles are: Sterilize, Sterilize. keep warm, Melt keep warm, and Sterilize equipment. The internal chamber height for this model is 16.5 inches to the lid and additional 2 inches in the chamber cover once close, the chamber width is 13.5 inches. The complete external dimensions for this unit, it has a height of 30 inches and the width of 23.5 inches and the depth is 28 inches. The specifications from Sanyo operators manual are 370mm (diameter) X 410mm (depth) 465mm (depth including chamber cover). This unit was chosen for a couple of reasons one is ergonomically it was not as tall as other comparable units in this class of autoclave. Which should make it easier to put a bioreactor vessel into the chamber since

it is not as tall or deep it should be easier to be removed. Since you are not looking to lift the bioreactor vessel as high to place into the bioreactor chamber or pull it out of a deep chamber. The price was also a consideration in choosing the Sanyo autoclave. We have also a second portable unit a Hirayama; it has a chamber with the following dimensions; 26 inches high and width of 11.75 inches. The overall dimensions of the unit are 42.5 inches high by 17 inches width and the depth of 23 inches. The Hirayama also runs off single phase power, 120volts, and 20 amps. This has been a reliable unit over the years, and still is reliable. It is on the tall side to lift a bioreactor vessel into, since the chambers is 26 inches deep and narrow it makes it a little hard to place a 3L New Brunswick bioreactor into this chamber as well as pull the unit from the unit when the cycle is completed. Yamato makes a portable unit, seems like a good unit. The Market Forge Sterlimatic is a work horse, but it is not tall enough for the autoclaving of most Bioreactor vessels. *New Hampshire Community Technical College* (#1288)

Biotech incubator (April 2005)

Austin Community College is looking at creating an incubator for local companies and we want to find out if there are any colleges already doing this. Please let me know if I may provide any other information. *Austin Community College* (#1290)

- There are colleges that have done this in Canada, I will try to send there websites around....most in Quebec. We are in the early stages of developing one in Food Bioscience at this time. I also would be interested to see what others can share. We are looking at an incubator - bioscience company portal model but lots of strategies to consider. *Centre of Excellence in Agricultural and Biotechnological Sciences* (#1292)
- Madison Area Technical College in Madison WI has partnered with a local developer in the creation of an incubator facility T.E.C. The building is located across the street from our main campus in Madison WI and is an incubator for small startups. Of the 10 companies located in the facility, 3 are biotech companies. In addition to the small companies, local business development resources have a shared office for one-stop shop and on-site assistance for small business development. Madison Area Tech also has some offices in the incubator as well - our Business, Industry and Community Services, the Business Procurement Assistance Center, Office of Strategic Innovations, Office of Grants and Sponsored Projects and Center on New and Converging Technologies. The incubator is part of a business park with additional buildings going up. In the first building, we have two biotech companies and one working on biomass-fuel cell development. One of the companies is actually a "graduate" of the incubator. If you would like additional information about the MATC incubator and the process used to set up the partnership, Ed Clarke, VP of Strategic Innovation, was a leader in getting the incubator partnership set up. *Madison Area Technical College* (#1293)

Genetic engineering animations (May 2005)

Just found this...http://science.nhmccd.edu/biol/bio1int.htm. A superb collection of biology videos and animations for A-level and beyond. (#1308)

Microbiology lab manual (May 2005)

I will be a new faculty member in the fall at a small private college, and will be teaching introductory microbiology for the first time to undergraduate biology majors. Microbiology is somewhat outside my field (I'm an electrophysiologist). Do you have a recommendation for a good basic microbiology laboratory manual? In particular, one with a prep work section with recipes would be helpful (instructors do their own preps). Or, if you do not use a lab manual, what microbiology techniques do you consider important to learn? *North Carolina State University* (#1312)

- I have been teaching Microbiology to mostly pre-nursing students for about 8 years at Maui Community College. I like the lab book that goes with the Totora, Funk and Case textbook (Benjamin Cummings pub). The lab book is written by Johnson and Case. It is very comprehensive and comes with a book on how to make up all the reagents etc. If you want any more information like my syllabus as a guideline please feel free to email me. (#1313)
- I have been teaching microbiology (pre-nursing, pre-med, pre-dental, physician assistant, etc) for 15 years. I use the text: Foundations in Microbiology by Kathleen Talaro, 5th ed, by McGraw-Hill and am really pleased with it. I wrote my own lab manual several years ago after using Benson's (Short version) for ten years. I slant my lab towards health care and away from environmental, and we do a smidgen of molecular (transformation of E coli with pGreen, etc). Check out Benson lab manual (McGraw-Hill, I believe) before you decide...it also has lots of directions, recipes, and procedures. (#1315)
- I teach only a unit of HS microbiology, but find this unit the most exciting for most students. Feedback from students in college is that they are well prepared for micro. Some of the concepts covered in my advanced biology class:
    * Differential and selective medias- types and uses
    * Gram staining procedures and use
    * Benefits of bacteria
    * Food production and relation to bacteria (lots of discussion on the impact of large production farms and antibiotics in feed)
    * Food poisoning
    * Sexually transmitted infections
    * Dental caries
    * Criteria of an infection (agent, encounter, entry, spread, multiplication, damage, outcome)
    * Benefits and disadvantages of antibiotics

  I do my best to relate it to the students and their lives. Students stain, prepare and draw many slides of bacteria taken from cultured slants or petri dishes they have prepared (such as from a fomite or fingerprint). Most of the unit is lab oriented. (#1316)
- I used the Benson lab manual, but made modifications to make it easier for novice students. (#1321)
- I have used the Leboffe and Pierce "Photographic Atlas for the Microbiology Laboratory" as a reference in the classroom. The accompanying "Exercises for the Microbioloy Laboratory" has recipes in the back and good descriptions and illustrations of fundamental micro skills. (#1322)

Marketing community college graduates to industry (June 2005)

I am putting together talks to local economic development groups about community colleges and the biotech workforce. I'm pretty aware of what's happening here in Texas, but am looking for resources on biotech education and employment in other locations. Can you give me information regarding your grads and how they fit in with your particular employers? I'd like to hear about any statistics you care to share, also. *Montgomery College* (#1331)

- We have graduated 20 biotech students in the last year. 1 works as a lab tech for Gateway Community College, 5 work for the Translational Genomics Institute on various genomics projects such as prostate cancer, autism, etc., 3 work for the neurogenomics division of Barrow Neurological Institute studying Alzheimer's, Parkinson's, etc., 3 work for the Arizona Biodesign Institute at ASU; 2 in plant genomics, developing edible vaccines and 1 in endocrinology developing vasodilatation protein vectors such as Tat, or Sars spike, 4 have gone on to pursue M.S. and Ph.D. degrees at Arizona State, 1 works for GE health sciences developing SNP projects and QC, 1 works for Ribomed developing proprietary anthrax detection systems, 1 moved to Canada and has lost touch,

1 works for the Carl T hayden VA Medical Center in cardiovascular and diabetes research. 90% seeking jobs were employed full-time within 3 mos. Average salary is $27,674. Low is 25k, high is 35k. *Mesa Community College* (#1332)

- The best marketing to industry comes from the success of our students. Here in California we found that early on the industry was reluctant to hire community college graduates. Genentech prior to 1996 hired technicians solely from 4 year colleges. Then they started to hire 2 year college graduates and found that they worked out better in several ways. Hank Stern in manufacturing collected data and presented it at BIO - the most important finding was that this change in policy dramatically reduced turnover. Graduates from 4 year colleges tended to get trained, stay a year, and then move on to graduate or professional schools. Two year graduates stayed twice as long. They have since set a goal of hiring 1/3 of their workforce from 2 year schools, 1/3 from 4 year schools, and 1/3 from "other." (For example if you are a Peace Corps veteran I can almost guarantee you a job.) Of course, since 1/2 of our students in community college programs already have B.S. degrees, and there are new programs for training unemployed workers, we will be training part of the other categories as well. Try this link: http://www.sfgate.com/cgi-bin/article.cgi?f=/c/a/2005/03/14/BUGCIBO5O41.DTL&hw=poindexter&sn=002&sc=738. Here's some quotes from the SF Chronicle Article (March 14, 2005): "Carter said there may be some lingering preference among biotech firms to hire people with four-year degrees. But she said graduates of community college biotech programs are performing well enough to change that. Genentech, the second-largest biotech company in the world, changed its hiring policies two years ago after assessing its experience with community college students, said senior staffing manager Monica Poindexter. Genentech found that the community college graduates it hired stayed with their jobs longer than employees who had bachelor's degrees, who tend to move on sooner. The company now plans to hire one-third of new employees for its manufacturing operations from the community college pool, Poindexter said. Genentech, which aims to hire 1,500 people this year, also cooperates with Ohlone College in Fremont and Solano Community College in Fairfield on biotech programs." It was nice to finally get them to say these things to a source that we can quote. Take a look at the article on workforce training in Genetic Engineering News that was published two weeks ago (written by Dave Jensen). It describes the situation in California and especially in the Bay Area (that sells itself as "the birthplace of biotechnology.") Where it was important for us is that it put in print the conversations that had been taking place privately - that there will be a large expansion in the biotech workforce and that community college programs will play a major role in meeting this need. *Solano College* (#1333)
- Biotech is a new enough industry in Texas that we are fighting battles that you have been fighting (winning, I hope!) for years. I still have to convince our companies, one HR department at a time, to write job descriptions that don't REQUIRE at BS degree. One of the hardest nuts to crack has been the Houston Medical Center. They have a phenomenal number of jobs, but still hire AAS grads only on a "case by case exception" basis. Once I get them to try our grads, the success rate is phenomenal, but I'd like to try to break the barrier community wide. That's why I'm working on these talks to the larger Economic Development Organizations. *Montgomery College* (#1335)

Software for virtual labs in biotech (June 2005)

I would appreciate info on the availability of virtual lab software for electrophoresis, PCR and recombinant DNA. (#1346)

- The Virtual DNA Fingerprinting Laboratory software from UC Davis covers DNA extraction, gel electrophoresis, restriction enzymes, PCR, and southern blotting. Episodes may be played independently if you just need an overview of a particular technique.

Software is free and may be downloaded from: http://ppge.ucdavis.edu/Software/VDNA/vdna.htm. University of California (#1347)

- I don't know if you're interesting in using for lab software for doing experimental biology, too, but we have recently published our first CD-ROM that allows students to view and manipulate authentic molecular models of DNA. The CD contains:
  * molecular modeling software
  * several structures of DNA and DNA bound to drugs or proteins
  * a lab manual with worksheets and lots of hands-on activities (this can be purchased separately in a printed form).
  * animated tutorials that demonstrate how to use molecular modeling software for looking at structures

  And if you have an internet connection, many of the activities and files have links to internet resources. You may have even tried a demo version at one of the Bio-Link workshops or at BIO. The CD-ROM and book are entitled: Exploring DNA Structure. You can find out more at: http://www.ExploringDNAStructure.com and/or view an example of one of the tutorials on molecular structures at: http://www.geospiza.com/education/materials.html#tutorials. *Geospiza, Inc.* (#1348)
- Please let me know the purchase price of the CDROM and the book on Exploring DNA structure. (#1354)
- The CD-ROM is $29.95 and the book is $24.99. They can be ordered from our on-line store at: http://www.cafepress.com/geospiza. We also take school purchase orders and we offer discounts on bulk orders. I posted an on-line form for calculating costs (along with shipping and sales tax for some states) at our Geospiza Education store web site: http://www.cafepress.com/geospiza/669685. I also posted a PDF form that can be downloaded and used when submitting purchase orders. *Geospiza, Inc.* (#1355)

Industry outreach (July 2005)

How many of you work with industry partners that employ a dedicated full or part-time educational outreach specialist? If you do, I'd be interested in learning more about your experience with this. *Community College of Rhode Island* (#1359)

- I work with two educators on a regular basis. Gary Thull is with Pioneer Hi-Bred (DuPont) and he has curriculum materials available on the Pioneer website which is centered on Bt corn - a Pioneer product. The other person is Ric Devore with Integrated DNA Technologies. He is developing PCR based educational kits. I meet regularly with them since I'm lucky enough to have them in my neighborhood. We reciprocate with curriculum ideas and curriculum sharing and it has been a big help to me and my program. *Des Moines Area Community College* (#1360)
- You might be interested in the BTCI, an educational institute that was established by Promega Corporation in Madison, WI. They have a staff, labs, and a varied educational program for K-12 students, grad students, and scientists. Their address is www.btci.org. *Madison Area Technical College* (#1371)

Secondary agricultural biotech programs (July 2005)

Florida currently has a 3 year career/technical program for high school students in agricultural biotechnology. The first year is an overview of the agriculture industry, the second - an overview of biotech, and the third students can choose to specialize in a plant, animal, or environmental biotech course. Students can choose to take an individual research course their fourth year, or take the third year course in another area. The program is designed to prepare students for advanced training at a comm. college or higher education at a university. The state standards for this program are in need of an update. Having a minor in plant molecular biology and lab research experience from UF, I have been asked to be on a revision committee. I would appreciate some input on the types of skills we should be focusing on. The main question is, if

you had students articulating into your post-secondary programs coming from a secondary program such as this, what knowledge and skills would you want them to have? Suggestions for lab activities and curriculum resources would be great. I would appreciate hearing from any other secondary teachers with similar programs too. *John A. Ferguson Sr. High School* (#1364)

- You may want to check out the Washington State Skills Standards, "Phase II" articulation guide. The web site is: http://www.wa-skills.com/biotechbiomed.html. (#1365)

Workforce development site (July 2005)

I just ran across this great website. The "in the news" and "publications" links are particularly interesting for those of us involved in Biotechnology workforce development. http://www.workforcestrategy.org/aboutus.html *Community College of Rhode Island* (#1374)

BLASTing update (August 2005)

BLASTing through the kingdom of life: This popular activity was updated this summer in response to student comments and teacher suggestions. Along with the BLAST for beginners tutorial, this activity is one of the most popular items on Geospiza's web site. Originally developed for the BIO 99 teachers' workshop, this activity is by several schools and in several venues from high school courses to workshops for researchers at the Lawrence Livermore National Laboratory. In this activity, students use blast to identify unknown sequences from a data set of 16 sequences. The 16 sequences were chosen from diverse organisms, representing everything from RNA viruses that infect yeast, to humans. This set was compiled from either cDNA sequences or other sequences without introns to minimize confusion. Further, every sequence in this set does code for some kind of protein that might be recognizable to students, such as amylase (an enzyme found in spit that breaks down starch) or DNA polymerase (makes DNA). In the recent update, I added an example sequence and answered all the questions for this sequence. This gives students an example strategy that can be used to complete the activity. The data set, worksheet, and answer key are available on-line at: http://www.geospiza.com/education/materials.html. The answer key is password-protected to limit access by students. If you wish to get the password, e-mail me with your name, position, and school name. I don't plan to use this info - I only want to check your e-mail address against the school info to verify that you're an instructor and not a student. BLASTing through the kingdom of life can be found at http://www.geospiza.com/education/materials.html#worksheets BLAST for beginners can be found at: http://www.geospiza.com/outreach/BLAST. *Geospiza, Inc.* (#1378)

Great link for plant tissue culture process (August 2005)

Just found this site which has a great series of photos of the tissue culture process. http://www.zvieli.co.il. *Kitchen Culture Kits, Inc.* (#1382)

Site directed mutagenesis GFP to BFP protocol (August 2005)

I am looking for a protocol for a student class project to covert GFP into the blue variant. I am aware that mutations in the following residues have the desired effect, but was looking for a more detailed protocol before I try and develop one on my own. Ser65 to Thr, Phe64 to Leu, Tyr66 to His, Tyr145 to Phe. (#1383)

- I've worked on this, but haven't perfected it. My suggestion, and what seems to work better, is to convert Blue Fluorescent Protein to Green. The Blue is much less obvious, and the green more so. The approach that I've been using has been to expose a solution of BFP gene-containing plasmid to a UV source and then using that plasmid solution to transform. *Solano College* (#1384)
- I used to do this sort of thing quite a bit long, long ago. There are at least two alternative methods that you can use that will work pretty well. And there used to be plenty of companies that sold kits with the materials for doing this. I think Stratagene used to sell a

kit for this and I would guess that Promega does too. METHOD 1: use PCR to make DNA fragments containing the mutation. This requires two restriction sites around the position that you wish to change, but the process is pretty easy. 1. First take a look at the GFP DNA sequence. Find restriction sites on both sides of the site that you plan to mutagenize. Order oligos (PCR primers). Your first oligo will look something like this:
5' XXXXXXX @ XXXXXXX $ XXXXXXXXXXXXXXX 3'
This oligo is about 30 bases long with the mutation (indicated by the $ sign) in the center. The @ sign shows the approximate position of the cut site. The cut site can be different places but cutting is more efficient if there are a few bases on the 5' side of the site since most restriction enzymes prefer to use double-stranded DNA as a substrate. The second oligo can be shorter (about 15 nts should be fine). Ideally, this should hybridize to a position within 1.5 kb of the mutation site. This oligo should also include a different restriction site to make the cloning easier.
5' XXXXXXX # XXXXXXXX 3'
The oligos will hybridize like this:
--------@--------$--------> 300-1500 bases
--------@--------
N----------#----------
--------@--------
N----------#----------
<---------#--------
2. Do PCR with the GFP plasmid and the two oligos. 3. Digest with the two restriction enzymes. 4. Digest the GFP plasmid with the same two enzymes. 5. If you want, you can purify the fragments. But since you can screen your products for color, it's simpler just to set up the ligation and transform. 6. Select for drug resistant bacteria. Screen for the blue fluorescent protein.
METHOD 2: use DNA replication 1. Clone the gene of interest into a phagemid, like Bluescript or pUC118. There are several versions of these. 2. Make single-stranded template DNA. I used the same procedure here that we generally used for DNA sequencing. This was before PCR so we made our own single-stranded DNA. Let me know if you need a more detailed procedure. 3. Order an oligo, about 30 nts long, with the desired change in the middle of the sequence. 4. Allow the oligo to anneal to the ssDNA template. 5. Add nts and DNA polymerase to finish synthesis. Add ligase. 6. Transform, select, and screen. *Geospiza, Inc.* (#1385)

- There is a third option to the two that Sandra proposed that is a little bit easier than the Kunkel method or megaprimer / cassette mutagenesis methods. That is to use a kit for inverse PCR; one that works very well is Stratagene QuikChange. You don't have to subclone your resulting PCR cassette fragment and you can do the mutagenesis in a double stranded plasmid without having to make single stranded DNA. I used this kit as part of a site-directed mutagenesis class that I taught. The method involves making two primers that overlap each other exactly, except that one goes in the 5' to 3' direction and the other in the 3' to 5' direction. The mutation of interest is exactly in the middle of both primers at the same sequence. You can make up to 6 base changes in one round as long as the maximum distance between them is 6 nt. Then, you copy the entire plasmid by PCR.

  ----------X---------->

  <---------X----------

  Because the primers overlap, the new plasmid copies will anneal to each other in a circle; it is stable enough to transform and the bacterial strain will repair the nick. It is not true PCR because only the original template is copied during each round (not the products), so there are fewer risks of unintended mutations. It is a fast procedure; one round of

PCR, digest, transform, and select your mutants. The original template is digested with a DpnI which chews up only methylated DNA, not your PCR product. That way you enrich for mutants. *University of the Cumberlands* (#1386)

Forensics for general bio (August 2005)

I would like to incorporate the topic of forensics into a general biology (non-majors) class for a "CSI week." Would you have any ideas for resources on content or classroom activities? *University of the Cumberlands* (#1388)

- The following link will take you to the site for the Science in a Technical World series produced by the American Chemical Society, and published by WH Freeman. One of the series of 12 modular sets offered includes a Forensics set. Each set pertains to a particular "industry" and includes a video, CD, workbooks and notebooks.
  http://www.whfreeman.com/stw
  With respect to supplies:
  Forensics items:
  1) Crime Scene Process Kit; Sargent-Welch; WL5269-CSK; $74.99; many reusable items; www.sargentwelch.com
  2) Synthetic Blood, 250ml; Cynmar; 100+ tests; www.cynmar.com; 175-94730; $24.95; mimics crime scene blood with respect to LuminolÆ or Phenolphthalein tests.
  3) Simulated ABO Blood, 60 tests; Science Kit & Boreal; WW4565400; $35.50; www.sciencekit.com; allows for simplified cell counts, as well as typing, many reusable parts
  4) Refills for ABO blood typing; Science Kit & Boreal; WW4565401; $19.00
  5) Check the General Laboratory & Life Science Catalog from Wheaton Science Products for small, 1ml dropper bottles/vials to aliquot simulated blood and typing sera, which will allow faster access in large class sizes; www.wheatonsci.com. *Lakeland Community College* (#1389)
- Go to explorer.bio-rad.com and you will find a PV92 Informatics kit (for less that $200 ??). Or call Bio-Rad at 1800-4BIORAD. There you have enough for the entire “CSI week” and excite your students: DNA isolation from their own cells, PCR procedure, and then DNA amplification. I just received the brochure in the mail and thought of running this 3-lab session with my biotechnology students (Page 10). *Galveston College* (#1390)
- We do PV-92 and TPA to look for the Alu insert. I used to use the protocol from Dolan http://www.geneticorigins.org/geneticorigins/pv92/intro.html, but the pcr beads from Amersham became expensive, so we switched to a new lab. Biorad also has a kit for PV-92 (that was mentioned in a previous e-mail) and a new Crime Scene Investigator PCR Basics Kit that looks at STRs. Both work great, but can be expensive if you are doing them for a lot of students. Below is our protocol for TPA and I've attached a prep sheet as well for ordering and making up reagents. We get our primers from Sigma genosys (www.sigma-genosys.com; $0.30/base) for TPA (there is a small correlation to the TPA insert and cardiovascular disease, but I don't think it is statistically significant). The primers for PV-92 are on the Dolan website if you are concerned, but I have seen more variation in TPA. We have the students rinse their mouth with 10mL 0.9% (w/v) NaCl and expel it into a Dixie cup. They then use a sterile pipettor to fill a 1.7mL screw-top microcentrifuge container with their saliva. We spin this tube at 4000 rpm for 10 min to pellet their cheek cells. They pour off the supernatant in their Dixie cup and transfer the remaining cells into a 1.7mL microcentrifuge tube (again screw cap) containing 500uL of 10% chelex 100 (the lab techs prep this before the lab; you can also use instagene from Biorad. Instagene beads are smaller so they fit in most pipette tips). We then heat the cells to 99C for 10min to disrupt the cell membranes in a heat block (they have to be screw top or the pressure will cause them to pop open). Then they spin the Chelex and cell debris for 1 min at max speed (14k on ours) and then remove the supernatant

containing the DNA. I have them save this for other activities such as sequencing and store the left over at -70C. The students add 18uL of that DNA (above) to a supermix for PCR we get from Z-biomed (http://www.zbiomed.com/; $31/100rx). It is called 5x direct-load and contains everything including bromophenol blue and sucrose, so the students can add their pcr rx directly to a gel. The techs add in the primer to the 5X PCR rx and aliquot it to pcr tubes before the lab (it's on the prep sheet; 5uL rx mix, 1uL of 15uM primer 1+2, 25uL total rx volume), so all the students have to do is add their DNA to the supermix with primers and put in the thermocycler. We run the products on 2.5% agarose gels containing 2X concentrations of SYBR-Safe from invitrogen. It is similar to ethidium, but not considered hazardous so it doesn't need special handling. The other difference is we use SB buffer. It does not have as much resistance to current as TBE or TAE and so the gels can be run at 180V without melting or losing resolution and it takes ~ 1/2 the time. The sybr safe is visualized by UV and fluoresces green rather than orange. You can also stain with BioRad's bio-safe stain instead, but I found 1/2X works best. 100bp=no insert, 400bp=insert. We buy our DNA ladder from Bayou biolabs. It's about 1/2 as much as anywhere else. That's pretty much it. Hope that helps. If not, we have a FBI multiplex codis system we use looking at STRs, but we use polyacrylamide for that. I can send you the primer sequences and protocol if you like. (#1391)

- I suggest to give a look at the following web addresses: Crime Scene Investigation at http://www.criminologia.org/ (from the section "Links di criminologia"); National Criminal Justice Reference Service at http://www.ncjrs.org/; DNA Resource.com at http://www.dnaresource.com A web site for anyone who seeks information on the latest developments in forensic DNA policy; ISUMA - Canadian journal of policy research at http://www.isuma.net; Office of Justice Programs at http://www.ojp.usdoj.gov/; Forensic Science - Electronics and Electrical Engineering Laboratory - Office of Law Enforcement Standards at http://www.eeel.nist.gov/oles/forensics.html; Federal Bureau of Investigation at http://www.fbi.gov/ (search: forensic); Biobank The UK Biobank project will be the world's biggest resource for the study of the role of nature and nurture in health and disease at http://www.ukbiobank.ac.uk/; Nederlands Forensic Instituut - DNA: sporen naar de toekomst at http://www.dnasporen.nl/; American Paternity Center - DNA paternity testing to establish the father of a child at: http://www.paternity.us; an interesting question for forensic applications is the question of "chimeras" http://www.thetech.org/genetics/ask.php?id=23 *A Bureau for Biotechnology* (#1393)
- Great resources, everybody! Don't forget Carolina Biological kits though (less than $200 as well; http://www.carolina.com/). Check kits 21-1230 through 1232a for PV92 (allows you to extend from forensics into evolution and population genetics). Also, search Carolina's online product search engine for forensics and it will present you with a large variety of choices and ideas for various college and pre-college levels. *Dolan DNA Learning Center* (#1394)
- Here's a four-plex from the FBI CODIS that works quite nicely on 15% polyacrylamide gels. You can also run it on a 2.5% agarose gel, but it is hard to distinguish small differences in repeats. I have the rest of the 9 primer sets if you want to make it more like the real thing. Below is the Locus, Chromosome#, Repeat Structure, Repeat #, Genbank accession number, and Human genome database # when applicable. The primers for each locus are below as well.
  1) D7S820, 7, GATA, 5-15, G08616, n/a
  2) CSF1PO, 5, AGAT, 6-16, X1420, n/a
  3) Y-GATA-H4, Y, (TAGAATGGATAGATT A (GATG)pAA(TAGA)q *, G42676, AC011751
  4) HUMTH01, 11, TCAT, 3-14, D00269, n/a
  * Repeat numbers are not clearly defined

Locus, Forward primer, Reverse primer,
1) D7S820, 5'GTCATAGTTTAGAACGAACTAACG3',
5'CTGAGGTATCAAAAACTCAGAGG3'
2) CSF1PO, 5'CTGAGTCTGCCAAGGACTAGC3',
5'CACACACCACTGGCCATCTTC3'
3) Y-GATA-H4, 5'CCTAAGCAGAGATGTTGGTTTTC3',
5'CTGATGGTGAAGTAATGGAATTAG3'
4) HUMTH01, 5'GTGGGCTGAAAAGCTCCCGAT3',
5'CAAAGGGTATCTGGGCTCTGG3'
Annealing temp is 58C. 40 cycles 30 sec denature, anneal, extend, 10min autoextend. We use the same Z-biomed 5X PCR mix for this lab (as the Alu) and the same DNA from the Alu isolation. *Mesa Community College* (#1396)

Documentation (September 2005)

I'm an Instructor at Red River College in Winnipeg, Canada. We are in the pilot year of a Quality Assurance/Quality Control Program for the Pharmaceutical Industry. I'm about to do a module on Documentation in a GMP environment. Does anyone have instructional materials that they would be willing to share with examples of good and bad documentation practices that I can use in class? I have some (from the bio-link site) but need more and in the first year of the program I'm trying to build up a bank of instructional materials. I could use any type of manufacturing records, forms, or SOPs. *Red River College* (#1403)

- There are wonderful resources in "Basic Laboratory Methods for Biotechnology" by Lisa Seidman and Cynthia Moore. *Des Moines Area Community College* (#1405)
- You can find a lot of information of cGMP related with biological products (or biotech derived product) in CDER or CBER in: www.fda.gov/cder/ or www.fda.gov/cber/. (#1409)

High school text book for biotech (September 2005)

I am developing a biotech course to implement at my high school next fall. I would like suggestions on a text book or lab manual. In your opinions what are the most important concepts for them to learn in a high school course? My goal is for them to also develop and execute an independent research project during the year. (#1406)

- Ellyn Daugherty just completed her book and it will be out by next fall. Ellyn has taught high school biotechnology (multiple years) for at least a decade and has distilled that experience into this book. There is a lab manual that is well integrated into the textbook. These have practical aspects of biotechnology (interviews with QA professionals and biomanufacturing specialists as well as researchers) and is published by EMC Paradigm. I would ask for a desk copy to review. Try this link: http://www.emcp.com/product_catalog/index.php?GroupID=170. *Solano College* (#1408)
- I recommend Ellyn Daugherty's new HS textbook and lab manual. I have several teachers, schools, and districts around the Northern CA Bay Area who are piloting Ellyn's labs and text. I believe the technical skills, the science behind them, and the applications are all important for them to build the foundation in HS for future learning at the CC or university level. You can reach Ellyn at <AEEDaugher@aol.com>. *San Jose State University* (#1411)

Introductory cell biology lab manual (September 2005)

I am looking for a recommendation for a new laboratory manual to use for an introductory cell biology lab for science majors. Most students will be second semester college freshman. Topics we would like to address are: proteins, DNA, enzymes, genetics, carbohydrates, respiration,

diffusion, photosynthesis. (Our current lab manual is from 1994 and thus a tad out of date). *University of the Cumberlands* (#1412)

- If you are interested in having part of your lab on the computer, I have a lab manual and a CD that can be used for part of the course. It's called "Exploring DNA Structure" and it involves using a molecular modeling program, Cn3D, to work with X-ray diffraction and NMR structures of DNA, and find all the different kinds of bonds and functional groups, determine which nucleotides pair with each other, and figure out how double-stranded DNA is put together, among other things. Altogether there ten computer lab activities with worksheets, over 70 structures on the CD, and animated tutorials to demonstrate what to do. The students don't need the printed manual since it's contained on the CD but sometimes they find it convenient to use. We tested it at Johns Hopkins University last year and this year they are using it in their introductory biology course. It's also been used in workshops at the Bio-Link fellows meeting, BIO 2005, and the Southwest Bio-Link workshop. More info is at: http://www.ExploringDNAStructure.com. *Geospiza, Inc.* (#1415)

Medicinal plant cultivation (October 2005)

Are there folks who cultivate medicinal plants for hobby? (#1419)

- There is much work going on with tissue culture of medicinal plants. You might check out the Home Tissue culture list - our hobbyists culture everything. Go to www.kitchenculturekit.com and click on "join a listserv". It's free to join and consists of a nice bunch of very knowledgeable people. Let me know if there is a particular plant you are interested in. There has been much in the literature lately on medicinal. *Kitchen Culture Kits, Inc.* (#1420)
- Yes, I do. Sonia Wallman 603-559-1581 (#1421)
- Thank you for the mail. Let me introduce myself, I am Dr. Srinivas K. Vellimedu, having done my PH.D in Biochemistry in India, Later went to Austria and did my Post Doc in Molecular Biology, Later returned back to India and presently work in a Biotech company making recombinant Vaccines and Biopharmaceuticals. I look after Production of these Molecules and also have number of research Project on Biotechnology. My interests are in growing plants especially Medicinal plants. I grow Amala (in India, I am not sure about the botanical name), Aswagandha (Indian Ginseng) and Brahmi (Memory) and certain other mints. I also produce Organic manure for these plants (vermiculture). I was just wondering what others are growing as medicinal plants. (#1422)

Nanotech (October 2005)

I have a student who is interested in preparing for a career in nanotechnology. Can anyone help direct me to a good advising resource for this field? The student prefers to remain in Rhode Island, at least initially and wants to be sure he is taking the best coursework to lead to this career. *Community College of Rhode Island* (#1425)

- This student may be interested in the Asnuntuck CC course in Enfield, CT (5 weeks/ 5days per week/ 8 hours per day=150 hours) in medical device production. That's as close as one comes to nanotechnology in these parts, I think. There is also a 4 year program that involves a consortium of universities (U. Lowell and UNH are two members of the consortium). (#1426)
- We have been looking at certificate programs to help students who are interested in the blossoming Houston nanotech industry. Our thought is to start with the biotech AAS and then design a 1 year certificate to add on the physics, material science and other courses. This would give our graduates another track to move into after entry-level employment in the biotech industry. Ideally the AAS + certificate courses would then articulate into a four year degree (biotech, nanotech, interdisciplinary science). Has anybody else worked on something like this? *Montgomery College* (#1427)

- <http://www.bu.edu/ame/research/areas/nano/> Not sure if he's looking for graduate degree, but BU has something. Not too far. *Boston University School of Medicine* (#1428)
- I've been working with our electronics dept chair and the TSTC bioinstrumentation dept chair, they have already worked out a plan that works for either a student in biotech or a student in electronics ---we can send you sequence we thought would work in Texas. *Austin Community College* (#1431)

Bioinformatics activity (November 2005)

I posted a new activity on our web site where students do a BLASTn search and determine where (and sometimes when) specific genes are expressed. The data set contains 30 sequences that are all expressed in a tissue specific manner. An example is given in the PDF handout. Students use BLASTn to identify their gene. Once students have identified their gene, they use a cool tool in UniGene to get a digital dot blot showing which tissues were looked at and whether or not any of the transcripts matched their sequence. The title is Head, Shoulders, Knees, and Toes. I posted a description of the activity at my blog: http://digitalbio.blogspot.com/2005/11/head-shoulders-knees-and-toes.html. The easiest way to get to the activity is to go straight to the data set: http://www.geospiza.com/outreach/BLAST/HSKTsequences.html. And if you want a password to access the answer key, write to me with your school and position and I will send it. *Geospiza, Inc.* (#1433)

Thermocycler recommendations (November 2005)

Our Biotech Program is interested in purchasing a new conventional thermocycler (for ~$7000, not real-time). We're currently using a PE 480 which still works fine. Some of the improvements we're hoping to make are - no mineral oil in the samples, easier programming, faster heating and cooling rates, and temperature monitoring. *Madison Area Technical College* (#1446)

- Bio-rad has two great machines that fits your needs, the mycycler (96 well and is gradient) and a new MJ minicycler (48 well, gradient). They are virtually student proof and super easy to use! I also think the mini may be upgraded to a 2-color real-time machine. I think EDU pricing (20% off) is $4800 for myCycler and $3500 for MJ mini. (#1447)
- I second the referral for a MyCycler from BioRad. We use this machine in our equipment loan program for local high schools and it has worked out very well. *UC Davis* (#1448)
- The ABI 2700 is great! 96 well, smaller footprint than the 9700 model, still has the top and bottom heating elements that many others don't have, programming simple enough even for a 3rd grader (my daughter programmed 2 units for me), lighter than the 9700, very durable (we have mobile PCR kits for schools), and dependable. *San Jose State University* (#1449)
- I highly recommend the Bio-Rad MyCycler. We have used many different cyclers for both conventional and real-time PCR. For the price, the MyCycler is very student friendly (easy to program and monitor), can handle individual tubes, strips and plates, and provides reliable block temperatures resulting in consistent data sets. It has a heated lit so no need for mineral oil overlays. *Monmouth University* (#1450)

Yeast GFP (November 2005)

A year or so ago I got a yeast strain from someone on this list-serve. I have lost the info that I got with the cultures on growth media and GFP extraction, as well we seem to have misplaced the culture. I would appreciate it if the person could get back to me. *Centre of Excellence in Agricultural and Biotechnological Sciences* (#1451)

- While we do not have a yeast strain expressing GFP, we do have excellent anti-GFP and anti-RFP antibodies in either polyclonal or monoclonal formats. (#1452)
- Do you also have cell cultures producing these antibodies? (#1453)

- No we do not. Our antibodies are available in purified form and many are conjugated to biotin, enzymes, fluoreschromes etc. We also may be able to make available to you purified GFP or RFP. Call me at 800-656-7625 to talk more if you wish. (#1454)

CSI forensics information (November 2005)

I am looking for lecture material and/or classroom activities to present to freshmen non-biology majors on forensics, perhaps related it to the type of evidence shown on CSI. Does anyone have ideas or resources I could explore? *University of the Cumberlands* (#1457)

- There are tons of great websites available. Here's a lab exercise from Dolan, but requires PCR, so may be lengthy: http://www.geneticorigins.org/geneticorigins/pv92/intro.html. You can also purchase pre-digested DNA from Carolina in their Electrophoresis and Forensics Kit (http://www.carolina.com/biotech/) or students can digest their own plasmid DNA in BioRad's Forensic DNA Fingerprinting Kit (www.bio-rad.com). The Carolina kit is very quick, and only requires basic electrophoresis equipment. This is probably best for your needs if you want to do some hands-on work. DNA Interactive (www.dnai.org) also has animations on how the FBI uses CODIS and 13 STR's to ID individuals: http://www.dnai.org/d/index.html *Mesa Community College* (#1458)
- As a guest speaker, I recently introduced a very short unit on biotechnology forensics to a non-science class of high school students. After talking briefly about DNA and DNA fingerprinting we headed to the lab to isolate DNA. I had no time to buy the standard wheat germ or strawberries at the store, so I'd decided to try some cranberries from my home freezer. It turns out they work great with the forensics theme because the blended cranberry mixture bears a striking resemblance to blood! You might consider adding to the mood by playing the musical CSI theme "You won't get Fooled Again" by the Who. There are several fruit DNA isolation protocols on the web. The one I used was at: http://gslc.genetics.utah.edu/units/activities/index.cfm *Community College of Rhode Island* (#1459)

Bioluminescent bacteria (November 2005)

I am looking for help in completing a lab investigating autoinduction in the Biotechnology on a Shoestring lab manual with my students. I am trying to obtain three different strains of Vibrio harveyi, and have looked at ATCC but they are very expensive and beyond my budget. Could anyone assist me in obtaining the three strains (BB721 lux+), (BB151 lux-), and (BB202B lux-)? *Germantown Academy* (#1461)

- I suggest that you contact Kathy Frame at the Biotechnology Institute. She recently told me that she has found a less expensive source for the strains you are looking for. Her tel. # is 703-248-8681 *Manchester Community College* (#1462)

Pestles and PCR query (December 2005)

We have been using the micro pestles to grind up corn and food samples to do GM PCR - those plastic pestles are expensive - does anyone know if we can "wash" and autoclave and validly use again for another pcr prep? PS - you might be interested - we have had success using just saline and chelex to prep the samples - simpler than most other methods - we have Bt and nonBt plants from seeds provided by Pioneer as our controls - we use 35s and Bt primer sets and PCR beads. (#1465)

- We use standard mortar and pestle and clean them with 10% bleach between uses to hydrolyze any left over DNA from the previous samples. It works quite well. We also use chelex extraction. *Mesa Community College* (#1466)
- We use the standard mortar and pestle also and clean real well. Also I ask the students to do the non-GM control first and later only work on the test sample. *Chandler Gilbert Community College* (#1467)

- Tell your students not to run test samples and waste their time unnecessarily it is not working well in our labs. (#1468)
- A very inexpensive mortar and pestle system can be made by using flamed "blue-tips" (1000 ul) micropipettor tips that are molded into a 1.5 ml microcentrifuge tube. I used this method successfully for years to homogenize insects & ticks. Using bunsen or Fisher burners or an alcohol lamp, briefly (the timing is critical) place the end of the "blue-tip" in the flame. When it just begins to melt (just catches fire), then jam it into the bottom of the 1.5 ml tube; let it cool without twisting. After a minute, you can twist to remove it. You will now have a sterile, molded pestle with a good long "handle". I teach this method to my students and we use it to homogenize Drosophila and crickets. Some additional points: 1. Don't leave the tip in the flame too long or you will have a big glob of melted plastic. The critical part of this protocol is to apply flame for the right amount of time. It takes some practice (it usually takes my students 5 or 6 tips to get the hand of it). 2. You want the tip to melt enough to close the hole at the end. 3. Avoid twisting the tip as you jam it into the microcentrifuge tube; after cooling simply twist the tip to remove. 4. Reject flamed tips that have rough edges, or strings of plastic as these tend to trap homogenate. *Merced College* (#1474)

Protocol for lysis buffer (December 2005)

Does anyone have a good protocol for the lysis buffer used in the Bio-rad Genes in a bottle DNA extraction from Human Cheek Cells? It doesn't really need to be that exact buffer of course, anything that works. (#1469)

- A quick answer, and some discussion: The university of Arizona has an excellent lesson guide which includes protocols for lysis buffer, and other reagents. I'm not sure if it's the same as Bio-Rad's, but it works, and the DNA which is extracted can be further manipulated. The site is: http://biology.arizona.edu/sciconn/lessons/vuturo/extraction/extractmaterials.htmlI love the concept of the Genes in a Bottle protocol - it is great for high school students. However, I have used basically the same protocol with simpler (and less expensive) reagents. I have even had separate groups of students make-up the reagents for the whole class and had beautiful results. This has only been used for precipitation, though the DNA may be clean enough for PCR. I wouldn't try a Southern with it. 1. Swish 10 mL of 0.9 % (w/v) NaCl solution and collect cells in a large test tube. 2. Add 2 mL of a 25% (v/v) solution of Ivory liquid hand soap (mostly SDS with some salt and EDTA, and not too much else). 3. Add 1mL of a 25% (w/v) solution of McCormick's meat tenderizer. (Papain is the protease). 4. Swirl or stir. 5. Leave overnight in a 60 deg. water bath and the cellular debris settles nicely to the bottom of the tube. 6. -20 deg. 95% EtOH works best, but we've gotten nice results with 75% isopropanol. I think all together the reagents cost about $10 at the local grocery store, and I have enough for several hundred students. *John A. Ferguson Sr. High School* (#1470)
- Thanks for the information. Our main objective is just to be able to see the DNA. Do you get a pretty good quantity with this? (#1471)
- We have never quantified it; however, it is plainly visible if done properly (the biggest problem seems to be students mixing the buffer and alcohol layers too much- a simple rocking like a metronome seems to work best). I also have the students cut off the narrow tip of a disposable transfer pipette and transfer to 10 mL test tubes, though you could order just the necklace beads from Bio-Rad, and it would work just as well. The best ones I've seen have been as good or better (visually speaking) as the picture in the Bio-Rad catalog. Truthfully, I prefer my students to use actual reagents, and I think Bio-Rad's kits are spectacular. But on a shoe-string budget, we have to improvise sometimes. *John A. Ferguson Sr. High School* (#1472)

Gathering data from bioluminescent bacteria (December 2005)

One of my biotech students wants to do research using bioluminescent bacteria to study water pollution. Her question to me was how to collect quantitative data and what tool should she use. Can any one on the listserve give me some advice on what direction this students should take? How does the level of a water pollutant affect the emittance of light from bioluminescent bacteria? How can you measure the level of light emitted from the bioluminescent bacteria? What instrument can you use to gather quantitative data? *Mesa Public Schools\Mesa High School* (#1473)

- You can use a luminometer. We don't have one here, but ASU does. *Mesa Community College* (#1475)
- I just learned of an interesting technology that uses the Polaroid camera exposures to do bioluminescent assays for environmental problems like arsenic or mercury. My contact is Fernando Rubio at Abraxis. The modified camera was quite affordable- I think under $700.00. I am planning to test these products in the next few months and can report on them. Or, you can contact Mr. Rubio- he seemed very helpful. The level of light (technically) is controlled by using bacterial strains that have been engineered to turn on the luciferase gene when the promoter for the engineered gene is in contact with the heavy metal. There is a toxicology assay that directly uses the bacteria themselves (not engineered) and looks at LOSS of bioluminescence after exposure to toxins. Both are interesting methods. (#1477)

pH meters for training lab (December 2005)

I am looking to buy 3 or 4 pH meters for the training labs in the training labs in the Biomedical Lab and Clinical Sciences program here at BU (www.bu.edu/biotech, www.bu.edu/citylabacademy). Does anyone know of reliable, relatively inexpensive (in the $200- $250 range) pH meters? No bells or whistles are required! I'd be curious to know what others use in their teaching labs. *Boston University School of Medicine* (#1478)

- This may seem strange, but we mostly use the Hanna HI 98103 pen pH meters that are about $40. We also have 4 very expensive portable handhelds from VWR ($500/ea range), but I found the Hanna handhelds are just as accurate, practically indestructible, convenient to use, easy to calibrate, actually maintain their calibration longer, and are very cheap. I actually could afford one for each student pair for the cost of 1 VWR meter and they are so portable that the students can use them at their own lab benches. http://www.professionalequipment.com/xq/ASP/ProductID.1060/id.6/subID.263/qx. *Mesa Community College* (#1479)
- We have found that when it comes to quality equipment you get what you pay for. Bench top pH meters are typically in the $400+ dollar price range; this is what NHCTC uses for bench top meters. Hand held meters are not well suited for use in the teaching labs, to much of a chance of student damaging them. Even the portable meters are not well suited for use in the teaching labs; students will play balancing acts with attempting to hold the meter and probes in one hand, not a good situation. Smaller bench tops meters can be found if foot print is a critical issue with bench space concerns. The college has an older Alpha Medco EDT pH meter that has served them well for a lot of years. The college has recently started using the Orion line in the biology and biotechnology, the college is thinking of ordering the new Orion 3-star models, I have requested a PDF of the operation manual. You might want to request the PDF of the operations manual vs. the typical spec sheet that are typically on web sites or with sale brochures, this way you could review the operation prior to purchase it which may give you a better idea on the quality of the meter if the documentation is user friendly, the equipment should be. *New Hampshire Community Technical College* (#1480)

GMO food testing (December 2005)

I have a student that would like to design an experiment to test certain store-bought foods for GMOs. She is aware of test strips, such as those for Round Up Ready seeds that tell if a seed is genetically modified. She wants to know if the strips might also test a food, such as canned corn, for GMO. I thought it valid to let her experiment to answer the question. Has anyone worked with such experimentation in the past, and if so, did you find that it does or does not work? *Omaha North High School* (#1481)

- I have used the BIORAD GM kit (with PCR) to test for GM crops from corn chips, corn meal, cheetos etc. that we buy from our cafeteria for a couple of semesters. It works great. (#1483)
- BioRad actually has a kit (GMO Investigator PCR Kit) that supplies primers to amplify CaMV S35 promotor and the NOS terminator sequence which they say hits 85% of all commercially available GMOs. As long as you have a PCR machine it is easy. We used it this quarter and it works wonderfully well! *Seattle Central Community College* (#1484)
- The best source on this is Gary Thull - but my understanding is that the strips test for specific protein so the test strip for roundup ready would not test for corn that is modified for Bt. (#1485)
- AGDIA has eliza kits for most of these GM genes. (#1486)
- There is a GMO food detection lab posted on the Bio-Link web site, go to Curriculum Clearinghouse then Instructional Materials. The posting includes a wet lab, paper lab and additional resources for teachers. Madison Area Technical College (#1487)

Real time PCR (December 2005)

Can anyone recommend: 1. an affordable real time pcr machine and/or 2. teaching protocols for real time pcr? (#1488)

- We service and support used rt-pcr machines. We have several relatively inexpensive machines available at www.labcentraal.com. The ABI 7700 is a very nice machine. Many users think it is superior to what ABI currently offers. *Lab Central B.V.* (#1489)
- In my opinion, I would look for easy to use software. The software for the 7700 is dated and more difficult to use. Personally, I would not want to teach students on this machine. Plus it is relatively large. Of course, since service is just about to end on the 7700, the price would probably be relatively inexpensive which is good. But then you are left to fend for yourself with the service. Of what is currently available in the marketplace, the most popular choices are:
  Applied Biosystems 7300
  https://products.appliedbiosystems.com/ab/en/US/adirect/ab;jsessionid=Dbbpy7sXryJRL9JpgYNKfWD6h7phYbpKDrW9Gxqw3CmCtWVlTgwG!-359256942?cmd=catNavigate2&catID=601250&tab=DetailInfo
  Stratagene
  http://www.stratagene.com/products/displayProduct.aspx?pid=562

  Biorad
  http://www.biorad.com/B2B/BioRad/product/br_category.jsp?BV_SessionID=@@@@0125624846.1134238591@@@@&BV_EngineID=ccceaddghekhihhcfngcfkmdhkkdflm.0&loggedIn=false&lang=English&divName=Corporate&country=HQ&categoryPath=/Catalogs/Life%20Science%20Research/Amplification%20|%20Thermal%20Cyclers/Nucleic%20Acid%20Amplification%20and%20Detection/MyiQ%20Single-Color%20Real-Time%20PCR%20Detection%20System&catLevel=5&catOID=-24832&isPA=false&serviceLevel=Lit+Request

  Sorry for the long URL's, but I thought it might be helpful to link directly to the sites. Ok, disclaimer time, I do work as a sales representative for Applied Biosystems, I'm on this listserv since I used to be the lab technician for a Biotechnology program at a

community college. I've kept my membership and since this is a topic that I know quite a bit about, I felt inclined to respond. I would call each company and talk to the reps so that you could find out for yourself what you like/don't like about each machine. If I were selecting a machine, I would want one that gave me full real time capability, had a small footprint, was easy to use, and similar to what would be used in industry. For the links I provided, both the Applied Biosystems and Strategene have the ability to detect multiple colors. Of course, I would not recommend multiplexing any gene expression assays with the students. But I think it would be great to add a SNP assay to the course. With two colors, you could have one color be the wild type copy and one copy with the mutant. I know on the Applied Biosystems, it has this really neat graph that shows the clusters of the three populations: homozygotes for the wild type allele, heterozygotes, and homozygotes for the mutant. The Biorad machine I linked has the ability to run only one color, but I believe the price is lower than the rest and the footprint is nice. I would be happy to answer any follow up questions you have, either on the board or off the board. And I would do my best to remain impartial, I do not believe this board is a place for vendors to sell their wares (and I hope my post is not taken as such, because that is NOT the intention). (#1490)

Genes and skin color (December 2005)

Some of your students might be interested in the Science article that
was published on Friday, linking a mutation to white skin. I posted a short summary here:
http://digitalbio.blogspot.com/2005/12/white-people-are-mutants.html with links to the original abstracts at PubMed. *Geospiza, Inc.* (#1491)