

Bio-Link Discussion Group

May 2000—December 2000

Summer recruiting activities (May 2000)

I am interested in hearing ideas that any of you have about what can be done for student recruitment in the summer months. I am trying to increase the number of students in my program so recruiting is very important. For example, have any of you sponsored summer workshops for high school teachers and found them to be good recruitment activities? *Des Moines Area Community College* (#7)

- We are hosting a Summer Educators workshop in August. The response has been very positive. Not only are we educating the educators but we use it as a marketing tool. Some of the high schools are calling to set-up articulation agreements. I think the Educators Conference is already paying-off. *Community College of Aurora, Colorado* (#8)
- I ran a summer program for teachers last summer. I want to reinforce your instincts - run the Bio-Rad pGLO experiments. These are EXCELLENT experiments. Also, if you do this, the teachers will then be prepared to be independent of you - they can just order the Bio-Rad kits (if they can get their administration to pony up the \$50 per experiment). Do run the transformation experiment AND the GFP protein isolation experiment that uses HIC chromatography. This gives the teachers a bit of work with DNA (which they often work with) and with protein (which they do not.) Remember, the DNA gets the glory but it's the protein that makes the money. I don't know how much time you have, or what your budget might be. If you have a bit of both, consider the BioRad gel filtration experiment (it's very quick but illustrates the principles) and the Bio-Rad restriction enzyme experiment. They have a new PCR experiment (which is the same Cold Spring Harbor ones that everyone does), but it's very expensive. In my seminar I also ran the Carolina Biological dye electrophoresis experiment first - and it was a success. I asked Bio-Rad and they did NOT supply the kits. I would be surprised if they do. I think that you should prepare to have to pay for them – they are about \$ 50 each. I've found Bio-Rad to be a high quality company - they get the kits to you quick and their materials seem to be well written. As you might be able to tell, I am sold on the pedagogic value of Green Fluorescent Protein - it's a remarkable protein. I use it as the model protein for my biotech students to grow up and isolate. *Solano College* (#11)
- We have done a few things and those that have worked best are: flyers describing the classes sent directly to industry as we have "drop-ins" from industry in addition to "program students" and informational "ad" in our class schedule which is mailed widely by the school. *Shoreline Community College* (#12)
- I offer two workshops on Biotechnology in summer. I have not seen any direct correlation to the teachers and my enrollment yet. I think the workshops will eventually have an impact but not over night. If you would like more information just let me know. *Amarillo College* (#16)
- I used to visit each semester the general biology, microbiology and general chemistry classes to recruit students since these courses are prerequisites for my biotech courses. However, nowadays my #1 recruiting strategy is to place each semester an advertisement in the Schedule of Classes (with a catchy illustration) with my phone # (actually home #). I get students at different stages in their academic progress. I invite prospective students to sit in on our biotech classes and join us on tours of biotech companies so they see the pre-service and vocational ends of the program. Our program has been around for almost 7 years so I also get a lot of word of mouth referrals from the industry (who recommends

personnel to enroll for cross training and ex students- many of whom are working in the industry as well). *San Diego City College* (#17)

HPLC (May 2000)

Can you recommend a protocol or source for a good experiment using HPLC for biotech students? *Oklahoma City Community College* (#22)

- When setting up my program's Chromatography course two years ago, I found Alltech's technical support division invaluable. Company info can be found at <http://www.alltechweb.com>. The experimental possibilities will depend on the type of column you have. They should be able to help you with sample chromatograms and sources of reagents. I use their test mixes extensively in teaching the basics of HPLC. At the end of the course, as a fun experiment, we have compared the results of a reverse phase chromatography of a store bought multivitamin to a sample chromatogram (#7332) provided by Alltech's tech support. Many of these chromatograms are featured in their product catalog. All of the pertinent run conditions are included. *Lakeland Community College* (#24)

Marketing your program (May 2000)

I just received some funds for biotech program marketing, and I wonder if any of you have any ideas on what are target groups for prospective biotech program students, how these groups can best be reached - what media to use, etc. - and any experience you've had with successful marketing for biotech programs. *Oklahoma City Community College* (#28)

- We are a new program and have been marketing anywhere we can, including our own students, high school students, regional life science industries and high school teachers. Our Chemtech program just finished an in-depth survey of past graduates of their 25 year program and found that an overwhelming majority of their students came from other life science classes on campus via active recruitment by their chemistry faculty. *St. Louis Community College* (#29)
- I would check with the Boston University City Lab biotech program for advice at www.bu.edu (#30)
- The best marketing tool I use for our R&D and bioprocessing technician training courses is actually free ads each semester in our college's Schedule of Classes. It is the single most effective "recruiting medium"- more successful than paid ads in the newspaper or even visits to the prerequisite classes. *San Diego City College* (#33)

Skills standards (May 2000)

Is there a set a biotech "skills standards" on the web somewhere? *St. Louis Community College - Florissant Valley* (#31)

- Go to www.nssb.org/ and on top bar, click on initiatives and standards, and on that page, click on skills standards and on that page, scroll down to bioscience - they have the two best sources out - from Gateway to the Future, and from National FFA agricultural biotech – both very similar. *Oklahoma City Community College* (#35)
- You can go to the National Skill Standards Board at www.nssb.org. From there you can download any of the skill standards. You may have to format them to make them look better. I also suggest that you contact the Education Development Corporation in Newton, Mass. They were responsible for the Bioscience Skill Standards. There are also agricultural biotechnology skill standards. *Middlesex Community College* (#36)
- I've a related question: is there a list of skills standards/curriculum coverage for skills and knowledge for TRANSFER students? I'm interested in overall curriculum standards for majors-level Biology, but especially for molecular biology and related techniques. Our students need competitive skills when they transfer if they are to have a chance of doing undergraduate research when they transfer, a necessity for almost all kinds of future

work. We're interested in ACADEMIC as opposed to purely VOCATIONAL biotechnology education. We're close to the San Francisco Bay area with its tremendous research focus, and want our students to have the flexibility of choosing between jobs AND advancement into higher degree programs, and of moving from one pathway to another. (#38)

- As far as I know, there are no common standards and no common curriculum for biology majors. This is a major problem in the ability of our students to transfer to the University of California, since every campus of UC teaches an entirely different curriculum and (until recently) they did not speak to one another. It's worse than that, since different faculty members on the same campus teach classes with an entirely different emphasis. For example, one faculty member at UC Berkeley, whose specialty is the control of gene expression in eukaryotic cells, teaches (I think it's) 6 weeks of control of gene expression in eukaryotic cells and includes many topics that most campuses only include in upper division molecular biology. The organismal classes are even more variable. The botanists fight to include more botany, physiologists fight to include more physiology, ecologists fight to include more ecology, and the winner(s) depend upon the campus. So there is no "standard curriculum," as far as I know. We find that our transfer students often tell us that they are better prepared in their upper division classes than their cohorts that took lower division at the university. I suppose that this shouldn't surprise us, that a university class taught to an amphitheater of 500 students without a lab or with a 3 hour per week lab with 500 students taught by a T.A. who's new to teaching doesn't prepare students as well as a class one tenth the size with a six hour per week lab taught by a veteran faculty member. At the same time the same universities are, because of their numbers, cutting back on their upper division labs. Therefore students graduate from these institutions with relatively few labs under their belt. It's probably for this reason that in almost every community college biotech class, more than 50% of the students will have a B.S. or higher. The community colleges are becoming a post-B.S. training ground for students to pick up additional and specific laboratory skills. In addition, most universities are hesitant to teach "biotech" instead of "molecular biology" because it sounds too "vocational" or "trade school." They ignore the harsh reality that, for the most part, it will take a PhD (or at least a Masters) to realistically enter research, so they are setting up their students who are finishing (only) with a B.S. and leaving them hanging out to dry. I have become more convinced in recent years that there is a role for A.A. or B.S. technicians in the high throughput screening efforts of the human genome project. But, the majority of jobs in a mature biotech company are still in the manufacturing end - exactly where our students are better prepared than their university trained counterparts. For example, at Genentech 80% of the employees are concerned with manufacturing and 20% with R and D. If a student wanted a job there, manufacturing (which until recently required a B.S.) is their better prospect. It's exactly in the Bay Area or Thousand Oaks, where the emphasis should be in the more "vocational" biotech. Having said that, most community college programs that have a manufacturing component also have a traditional "let's clone the gene" component too. Take a look at Sonia Wallman's (of New Hampshire Technical College) program on the web - you aren't going to find a graduate level course at any university that is as impressive. She has her students grow genetically engineered CHO cells, isolate the mRNA, reverse transcribe and clone it, express the protein, and isolate it. All of her protocols are on her website. Likewise, take a look at Sandy Porter's program at Seattle Central College. Other programs are as impressive (like Dave Singer's at San Diego City College where he brings in dozens of industry instructors to teach research techniques), but the New Hampshire and Seattle programs have the best web presence (and you can find the links through the bio-link.org website.) Take a look at the biotech skills standards - I think that you'll find that they do cover skills that would be applicable to the academic research lab - the skills that you are talking about. As for the skills and

curriculum required in a lower division Biology major's lab - this should be a priority for us all. If Northern Ireland can solve its problems, we should be able to get zoologists to agree with botanists, and ecologists to break bread with molecular biologists. Solano College (#39)

Protein extraction protocol (August 2000)

For our newly developed biotechnology course, we are looking for a reliable protein extraction protocol. We are trying to avoid relying too heavily on kits. Any ideas? College of the Canyons (#43)

- Lisa Seidman at MATC developed a protocol (several lab periods) for extraction of beta-galactosidase from E. coli - Sandra Porter from Seattle Comm. College has one also - contact them through BioLink regional center links. Also for shorter experiment, DNA Learning center developed extraction of GFP and the protocol is part of their Genomic Biology workshop handout. Oklahoma City Community College (#44)
- What do you want to extract and what do you want to do with the protein once extracted? Are you looking at column separation? Chula Vista High School (#45)
- There are several biotechnology lab manuals geared towards undergraduates on the market already. Check out those manuals for a protocol w/o kits. Prentice-Hall has a large one along with Ed-Votek. Naugatuck Valley Community College (#46)
- We are planning on extracting protein from an insect cell line and probably plant endosperm. We would like to do a colorimetric spectrophotometric analysis. College of the Canyons (#47)
- Are you transforming the insect cells first or are you looking for a native protein? If you want to transform, a plasmid with a GFP marker makes an easy and somewhat spectacular protein to purify on a hydrophobic column, but doesn't work well on a spec except if you are doing total protein with a lowery or something similar. Are you looking at total protein for some sort of enzyme assay? Look at invitrogen for plasmid with GFP for insect cells. Chula Vista High School (#50)
- I just got back from a week long workshop on GFP and I'm with Judi - it is the perfect model protein to isolate. I would suggest that you consider having students do a sequential isolation using different chromatography steps - gel filtration first, then ion exchange (DEAE), then hydrophobic interaction chromatography (GFP has hydrophobic patches and you can use octyl or phenyl). Have the students keep some at the end of each stage and then you can have the students run an SDS PAGE and an IEF of the different fractions to see the greater purity of isolation at each step and by the end you should have 98%+ purity. I have a His tagged GFP and if you would like, you could do affinity chromatography as well to get to 99%+. You can detect GFP with a fluorometer, of course, but it has a unique absorption at 397 nm that other proteins do not. I having had the trouble with A280 that Judi reports. I also bought Yellow Fluorescent Protein DNA (it's more green-yellow than yellow), Cyan Fluorescent Protein, the new Red Fluorescent Protein (from a different organism), and I have Blue FP. I bought the insect cell Invitrogen plasmid with the GFP gene, but it just came in this week and I haven't had time to play with it. I would suggest that you start with E coli first, unless you are sold on animal cells. Our students have a lot of trouble keeping the animal cell cultures from being contaminated. We separate the tasks into two separate classes - cell culture and protein recovery and purification. Both would be tough to work the bugs out of at the same time; it seems a bit ambitious to me. If you'd like a bit of this DNA, let me know. The reason that I like this system for teaching is that the students can see the protein by putting their solution up to a black light (long wavelength UV). So much of biotech is mixing one colorless solution with another - besides, glowing green is cool. By putting the different types of chromatography together, the students can see how proteins are

really isolated in industry to the 99.99% purity levels required by the FDA. Solano College (#51)

- P.S. If you use the GFP gene cloned into the pBAD plasmid (and this is the one that Bio-Rad uses), you can introduce students to the concept of the separation of cell culture (to increase cell numbers to maximum density) and induction (to generate the production of protein). The GFP in the pBAD plasmid is induced by arabinose (and it's L arabinose and NOT D arabinose - I learned this one the hard way). As it turns out, GFP does not seem to be that harmful to E coli and the arabinose can be added immediately when you start the cultures, but I don't - in order to illustrate the point. The Edvotek system uses GFP cloned behind a T7 promoter with the T7 polymerase gene cloned behind a lac promoter and induced by IPTG. Neither one of these seems to be very leaky. All of the E coli production in industry, as far as I know, uses genes that are induced only after the cell density is high. All of the yeast and cell culture systems that I can think of do this too, now that I think about it. So the students should learn about it. The insect GFP system that Judi talked about is constitutive and not inducible, as far as I know. Solano College (#52)
- So far I have only done the GFP transformation and isolated it with the hydrophobic interaction column kit from Biorad. (#53)
- It is constitutive but they have an inducible in the works for insect cells. Chula Vista High Biotech (#54)

HPLC query (October 2000)

We are a new biotech program and do have an HPLC apparatus, but as yet have not got a plan on how to use it with our students and how to get training to become familiar with using it. Does anyone have any suggestions on: source of workshops for faculty on HPLC and suggestions for labs for students on HPLC? Oklahoma City Community College (#64)

- We have a chemical instrumentation course that is a part of our Biotech program. The students learn to use the HPLC, GC, UV/Vis, Mass, and IR spectroscopy. The instructor is Skip Wiley and he is very creative in developing curriculum. I am sure that he will be able to offer you some helpful suggestions. Our students are very well trained at the conclusion of his course. Middlesex Community College (#65)
- Keep an eye on the vendors. Periodically Hewlett Packard will have a training session on HPLC. Solano College (#66)
- Waters also used to give very helpful seminars on request. (#67)

Sequencing DNA in the classroom (October 2000)

Is anyone out there teaching Sanger sequencing at the high school or undergraduate college levels - or do you know anyone who is? Are you actually sequencing in student laboratory sessions? If so, whose protocols are you using? The silver sequencing protocol (the Promega kit)? The Univ. of Washington High School Human Genome Project protocol (from Maureen Munn)? Alternatively, is anyone turning student-generated templates over to a core sequencing facility for automated sequencing or leading students through automated sequencing protocols and having them do it themselves? (#68)

- I doubt there's anyone sequencing DNA who doesn't use Sanger dideoxy sequencing. We've been doing this for about five years here at Seattle Central Community College, and as you noted below, Dr. Maureen Munn has been doing this for about 7-8 years, through the high school human genome program. Her web site has protocols and all sorts of info. It's at: <http://hshgp.genome.washington.edu/> We use Maureen's protocol. It doesn't require silver staining which I really like since silver staining creates toxic waste products which are a pain to dispose. Seattle Central Community College (#69)
- I had 2 years of HS seniors (12-16 per section) who decided to devote their senior research experience to sequencing. We used the USB sequenase kit or TaqTrack and had

much better results w/the sequenase kit. Students worked in teams and each adopted a gene for their independent contribution. We had 4 internet computers in the classroom (3 were generally functioning at any one time). It was tough to get things going w/90 minutes door-to-door on an alternate day cycle. It worked better when we met every day for 1 semester. Students learned to read and follow the original instructions that came with the kit. They had to work out the math, etc. We simply sequenced the M13 test sequence using biotinylated universal primer we ordered from LTI. We used calbiochem SA-AP and BCIP etc. We stored supplies that had to be frozen in a non-frost-free upright freezer that was fairly good at staying at -20C. For background, we used the UW Seattle (Maureen Munn's) wonderful manual and stratagene gel boxes and pre-poured gels. They worked just fine well past (>6 mo) their expiration date. We purchased a bio-rad power supply. Students practiced reading gels using old NIH sequencing autorads. Virginia Tech (#71)

- For a superb animation that demonstrates DNA sequencing (and many other great teaching animations) go to Cold Spring Harbor's DNA Learning Center's website at: <http://www.dnalc.org/home.html>. These are the best animations (most are interactive) that I have come across to help students understand some very complex molecular biology concepts and techniques! There is also information about sequencing located at their DNA from the Beginning webpage. Carolina Biological Supply Company (#72)

Phage-typing (November 2000)

I am trying to gather information about phage-typing for a study I am doing. Can anyone help me with this or know of a good book or document which can help me with this? (#74)

- Cold Spring Harbor Lab Press has Phage Display: A Laboratory Manual, 738 pp ISBN: 0-87969-546-3 <http://www.cshl.org>. Lansing Community College (#76)

Plate-readers (November 2000)

I would like to purchase a plate-reader for 96-well plates for optical density measurements. Does any one know who are the leaders on this market? (#75)

- I suggest you talk to ABI. Their new PCR machines quantitative results via spectrophotometric measurements and they work in plate formats. (#77)
- Molecular Devices makes many types of plate readers. Their machines are easy to use, are interfaced with the computer and have worked well for me in an industry and academic setting. (#78)

Cuvettes (November 2000)

I'm trying to locate 50 ul cuvettes in either Methyl acrylate or optical glass for an enzyme kinetics lab. Currently, I can only find them in quartz. Does anyone have any ideas? I've tried all the big vendors; apparently they're not widely made. College of the Canyons (#79)

- I've never seen 50 ul cuvettes, but Pharmacia makes 100 ul cuvettes that actually work down to a 60 ul loading. They are made of something like quartz or Vycor and are about 10 mm x 10 mm x 5 mm high. (What wavelength do you need? 260nm?) If you find a cheap polymer cuvette of low volume, I'm interested, too. Check out ABI. Their new PCR machines quantitative products by spectroscopy - and since its PCR, the volume must be down at 50-25 ul. Yale University School of Medicine (#80)
- I would try VWR or Fisher Scientific. They both have electronic catalogs (or you could call your local representative). New Hampshire Community Technical College (#82)

Program review (November 2000)

Our Biomedical Advisory committee was inquiring about program review procedures and I wondered if BioLink has something we could use. They want info on the whole ball of wax-student satisfaction, student success in courses and in job placement, total numbers etc. We are

building a data base that will track all this, eventually. This means by the end of spring semester because that is when we will turn out our first graduates. What the committee really wants is a one page summary they can use to promote the programs with industry. If you could send me some examples of what your college does in this regard, I'd really appreciate it. Or just send me to your website! Anoka-Ramsey Community College (#81)

- Take a look (and perhaps submit your program's info) at the Bio-Link survey at <http://www.bio-link.org/survey.pdf>. You might find that helpful and perhaps you could tell us how it fits your needs (and where it doesn't fit your needs....what to add). New Hampshire Community Technical College (#83)

Bioinformatics program (November 2000)

Does anyone know of any community college that offers some sort of "bioinformatics" program? Oklahoma City Community College (#85)

- Dr. Sandra Porter has included some wonderful bioinformatics instructional materials in her courses at Seattle Central. She has also published an article on Bioinformatics at Community Colleges in Journal of Industrial Microbiology & Biotechnology, vol 24, Number 5, May 2000. You can access her instructional materials by going through the Bio-Link website: www.bio-link.org. There may be a link to her site at Seattle Central. (#86)
- University College of Northeastern University (our part-time evening adult education unit) offers a 5 course graduate certificate in Bioinformatics Essentials. Information on this certificate can be found on our web site: www.neu.edu/cont-ed/bioinformatics/. We targeted mid-career scientists for this certificate and attracted 43 students in the Fall term - a popular and much needed program. We have a waiting list for the winter term. University College, Northeastern University (#87)
- I don't know of any community colleges that have bioinformatics programs, but there is one school in Texas that has a bachelor's degree in bioinformatics and several schools that offer a course or two in the subject (Keck Center for Computational Biology (<http://www-bioc.rice.edu>) in Houston). The most recent issue of THE SCIENTIST (http://www.the-scientist.com/yr2000/nov/index_001127.html) is also a great source of information. I would imagine that a community college program in this area would probably have one year of chemistry/biology/microbiology courses and at least one year of computer programming. We do include bioinformatics as part of the biotechnology program at Seattle Central and in a biotechnology course that we offer to non-science majors (Biotechnology and Society). We include it for two reasons. First, I think working with sequences gives students a better understanding of molecular biology and a better ability to comprehend how changes in a gene sequence are reflected in a protein. Second, we've found that our graduates use bioinformatics software on the job. For example: they do assemblies, they use BLAST to identify cDNAs and determine if they've cloned both ends, they design PCR primers, and they look at restriction maps. They also do lots of database work and graphing. So we have our biotechnology majors take a 3 credit course that covers graphing and bioinformatics (<http://www.seattlecentral.org/biotech/courses/CSC180/CSC180.html>) and we have them use bioinformatics in the biotechnology laboratory course. In that course, they use resources available free at NCBI (<http://www.ncbi.nlm.nih.gov>) to identify DNA fragments that they clone from E. coli and sequence. I described that in the article we recently published in the education issue of the Journal of Industrial Microbiology and Biotechnology (<http://www.stockton-press.co.uk/jim/>). Seattle Central Community College (#88)
- For those interested in introducing the concept of BIOINFORMATICS into your present lab, I recommend checking out Cold Spring Harbor's DNA Learning Center's website. They have a new web page called genetic origins that utilize bioinformatics. They also

have developed two PCR labs that go along with this site.

<http://vector.cshl.org/geneticorigins/> *Carolina Biological Supply Company* (#91)

Frying pan PCR (December 2000)

I am starting to plan a summer workshop for teachers and want to do lo-tech and hi-tech PCR - i.e. move from water bath to water bath vs. using thermal cycler. Can anyone suggest a robust and inexpensive system for doing this? One is the lambda system but the protocol I have amplifies a 1100 bp piece, rather on the large size, and I'm looking for something more foolproof. Any suggestions? Sources? *Oklahoma City Community College* (#94)

- Try this from the DNA learning center.
<http://vector.cshl.org/products/BiogeneratorManual.html> their Bio Generator. *Virginia Tech* (#95)
- Carolina Biological has a 'new' "Amplification of Lambda DNA by PCR" kit (21-1225; p. 325; \$120.00) that amplifies a 100 bp piece of DNA. This lab is designed to be an 'introductory' PCR lab. Students do a "time course" experiment whereby they amplify different samples for different numbers of cycles, i.e.; 0, (control), 5, 10, 15, & 20. Then they observe the various degrees of intensity for each band on electrophoresis gels. This lab is great for doing 'hand-cycling' which really drives home the concept of what is happening inside that 'magic' PCR machine. It would be an ideal lab to use to compare 'hand-cycling' with using a thermocycling machine. Another kit that could be used as a 'hand-cycling' vs. thermocycling machine would be the "Human Mitochondrial DNA Kit AT (21-1238; p. 325; \$155.00) that was developed in collaboration with the DNA Learning Center at Cold Spring Harbor Lab. This kit has tremendous extension opportunities by going to the DNA Learning Center's web site <<http://vector.cshl.org>>. Each individual teacher/student can get their individual DNA sequence of this 460bp piece of DNA to compare worldwide with other populations! *Carolina Biological Supply Company* (#96)
- I have a simple protocol that we regularly use with high school sophomores that goes something like this: 1. We use the Bluescript II SK- vector sold by Stratagene. You can find its sequence at Stratagene.com. (And I can lend you some to get started.) But I suggest the use of a small vector since less DNA is needed. A nanogram of this template should work well. 2. I designed primers to flank the multiple cloning region of the plasmid and produce a PCR product of about 700 bp's in length. (I can send you the primer sequences and/or a little primer.) By the way, you can choose other primers and make any length product you desire. 3. Students then do the PCR experiment and run 5 ul of the product out on a gel to test for DNA synthesis. You can always pull out aliquots (maybe 5-10 ul) after 10 and 20 cycles to demonstrate product formation. 4. Now I go further. (And I do that because I believe that one of the best reasons to teach PCR is that it gives teachers/students access to cheap DNA that they can make themselves.) At this point I have my students clean up the PCR product with a Qiagen kit. This little kit removes primers, enzyme, dNTP's, etc. and is a great little experiment in its own right. 5. Since we PCR'd thru a multiple cloning region in the plasmid - a region that contains a number of unique restriction sites - the door has now been kicked open to easily provide more data to prove that you made the correct product. Restriction enzyme analysis is introduced and the students take an aliquot of their PCR product and cut it in a restriction digest. From the sequence obtained at the Stratagene website, they can predict how long each of the two pieces of the cut should be. (The Stratagene site provides a restriction map.) Now they not only have the length of the original PCR product as proof of correct PCR synthesis but they have experimental evidence confirming the location of a known restriction site. Finally, please remember that water-bath PCR is a very robust technique that was used by many university researchers in the mid-1980's. It's slow but it works quite well. (#97)

- Diana Bradner at MATC has been working on a wonderful experiment that uses RAPD primers for using PCR in a kind of forensic experiment. Kind of a spin off of Shoestring activities. Contact her to see if she has published or if she can send you the protocol. Students can take any kind of related plant material and see how closely related they are to each other. They can even take an unknown sample and identify it. The protocol uses the Amersham PCR beads and so is very simple for inexperienced students. DNA prep takes less than an hour. *Middlesex Community College* (#98)

Re: Breaking open cells (December 2000)

We are using new lab manual for proteins next semester, and lab protocol calls for disrupting *E. coli* cells (for extracting β -galactosidase) with sonicator - we don't have a sonicator and they cost about \$4000. Do you know if the cell lysis with lysozyme protocol will work just as well when goal is extracting and purifying β -galactosidase? It seems to me it would but I want to check in case there are hidden pitfalls. (#99)

- We routinely used to break open cells for isolating β galactosidase by using a mortar and pestle. (We never tried an enzymatic method.) The mortar and pestle worked fine, although the yield seems to have been a bit lower than with a sonicator. These directions for using mortar and pestle method are quoted from Jeffrey Miller, "Experiments in Molecular Genetics", Cold Spring Harbor Laboratory, 1972: "Centrifuge the cells (20 minutes at 8000 X g) weigh and freeze the pellets on parafilm (about 2 grams). All of the following operations are performed on ice or in a cold room unless otherwise indicated. After at least an hour at minus 20 degrees C, put the cells in a pre-chilled mortar and pestle and grind with 2.5 times the cell weight of alumina for 15-20 minutes. The sample will be very dry at first, gradually developing a smooth sheen and crackling as the cells break. Add 5 cell volumes of breaking buffer... and work this in for another 5 minutes. Pour this into a centrifuge tube and spin out the alumina and cell debris for 10 minutes at 10,000 X g. Remove the supernatant, record its volume and save an aliquot for assay (0.1 mL)." *Madison Area Technical College* (#100)
- I don't know about β gal, but many of the protocols for Green Fluorescent Protein isolation use several cycles of freeze-thaw (usually 3) to break open the cells. But using this gentle method, the yield is lower, but most of the genomic DNA and other gunk (a technical term, I know), is left behind. I noticed that the Bio-Rad GFP kit also used this (after a lysozyme treatment). It might be worth trying a run to see about yield. *Solano College* (#101)
- I'll bet lysozyme will work and there's yet another, much cheaper option than a sonicator. Look into nebulizing cells. This is routinely done to break up chromosomal DNA rather than sonication and works quite well. I think the Baylor Med School Genome Center site discusses its use. All you need is a little plastic nebulizer and some air pressure. Also how about freeze fracturing? (#104)

PCR on bone (December 2000)

I'm looking for a protocol for PCR on bone. This is for an anatomy/anthropology instructor. Also, how do identify the bone once it is amplified? (#102)

- GeneLex, a DNA testing company in Seattle, has a great web page with lots of abstracts from papers on isolating DNA from a variety of tissues, including bone. Their web site is www.genelex.com and the page with the abstracts is: <http://www.genelex.com/Genomicshtmls/forensicsabstracts.html> *Geospiza, Inc.* (#103)