Bio-Link Discussion Group
January 2002—December 2002

Edvotek kits 333 and 334 (February 2002)

What is the consensus opinion of the Edvotek kits 333 and 334 for PV92 Alu and D1S80 VNTR PCR? We have had 100% failure rate so far. Are any of the kit components (i.e. either the reaction "bead" or the primer mix) reliable? *Santa Fe Community College* (#373)

- We usually do not have a problem. The one time we didn't see any produce, the lab aid had aliquotted out the Taq enzyme and it had undergone a freeze/thaw that rendered it less/ineffective. We're slated to do it again later this semester, will let you know if we have trouble. But we have been using it for about 3 years now successfully. *Montgomery College* (#374)
- I have used both of these kits the last two years in our Biotech I class and have gotten results both years - not all students get good results, but at least two or three of 6 groups have gotten good results *St. Louis Community College - Florissant Valley* (#375)
- I have used the two kits ever since they came out and have had great success rates. I use both of these in my Biotech III class. (#376)
- You can always purchase the components separately from different sources and it will be less expensive. *Mira Costa College* (#377)
- These kits consist of primer mixes and lyophilized beads (including buffer, magnesium chloride, dNTPs, and Taq DNA polymerase) for PCR of the PV92 and D1S80 loci, for use with genomic DNA extracted from buccal swabs. We have experienced 100% failure rate to date with both kits. Edvotek technical assistance told me the beads are the "Ready-to-go" beads sold by Pharmacia, and that the primer mix, described as being a very low concentration in dilute TE buffer, was the most likely source of failure. Most colleagues who kindly responded to my first inquiry to the Bio-Link discussion group thought the template DNA preparation was the most likely source of failure. However, this week my students showed that in fact it was the beads that were the source of failure. The buccal swab DNA preparations amplified as expected using the primer mixes for both kits when we used a different source of other reaction components (buffer, magnesium chloride, dNTPs, and Taq DNA polymerase). Both reactions worked well at all magnesium concentrations between 1.5 and 3.5 mM, with optimum at 2.5 mM. For those familiar with the protocols, we used an annealing temperature of 58 C so that we could run both reactions simultaneously, and we modified the Edvotek protocol by increasing the amount of time at the extension step to one minute. Thanks to everyone who responded to my first inquiry. Beware of those beads. *Santa Fe Community College* (#380)
- I have used the Ready - To - Go beads from Pharmacia for a few years in my undergraduate Molecular Biology Techniques course to amplify buccal cell DNA with great results until this year. This year I couldn't get it to work, so I went back to separate components for the class. I wonder if the company has changed something in the beads? *Washington State University* (#381)
- If Pat Ryan is still checking in to the group, it might be nice to find out if Carolina is experiencing similar problems. I use the Carolina kits, and last year I had no problems with the Ready-to-Go beads, but if there are problems with this year's lots, I might go to the individual components. *Finger Lakes Community College* (#382)

Southern blot kit failures (April 2002)

Next I'll share our failures with Southern blotting. We tried 1) the Edvotek kit #311, which utilizes pre-biotinylated DNA fragments which students simply transfer to Nytran and detect with SA-AP conjugate and NBT/BCIP substrate, and 2) the Carolina Biological Supply kit #21-1215, which utilizes a biotinylated probe and classical Southern blotting steps, also detected with SA-AP and NBT/BCIP. Both kits failed to produce visible bands. The background with the Edvotek kit was terribly bad. The background with the Carolina kit was not bad at all, but there were no visible bands even after quite prolonged incubation. Our only modification to the kit instructions was that we electroblotted, using Bio-Rad Mini-Protean modules, instead of capillary blotting. No bands remained visible by ethidium bromide staining of the gels after electrotransfer. Otherwise we followed the kit instructions with scrupulous attention to detail. Has anyone else gotten these kits to work, or is there a better Southern blot kit? Next week we will make our own probe by PCR biotinylation of the GFP gene using biotin-dCTP, and will use it to probe restriction digests of Bio-Rad's pGLO plasmid according to our own protocol. *Santa Fe Community College* (#389)

- We have great results with Southern blotting using biotin-avidin detection - not from a kit, though. The protocol is in the text Biotechnology DNA ----> Protein ISBN 0-07-241664-5. *St. Louis Community College - Florissant Valley* (#390)

Immunocyto kits (April 2002)

Does anyone know of an immunocyto chemistry kit, using fluorescent labeling? We're looking for a kit that includes the cells already fixed to slides and the antibodies also. It's the cells that we really need. *Madison Area Technical College* (#392)

- I have not seen an immunofluorescence kit on the market. I doubt that most schools would have access to an epifluorescent microscope. I have used an actin antibody that I order from Sigma (rabbit anti-actin, cat# A2066) in combination with a FITC conjugated goat anti-rabbit secondary. I have had spotty success. If your objective is to let the students get some experience with fluorescence microscopy, I may have a good alternative to finicky antibodies. Recently, I had the tissue culture class treat CV-1 cells in culture with various environmental stressors (0.5 mM hydrogen peroxide, 10mM CaCl2, 1mM Ethanol, 2% DMSO, etc., and of course untreated controls)and look for apoptotic cells by nuclear staining the next morning. The cells were seeded onto coverslips the day before the treatments. We looked for nuclear condensation using acridine orange and the same filter used for FITC and GFP on the scope. It worked great. We didn't have to worry about fixing the cells on the coverslips. We simply used a 1mg/ml stock solution as 1000X and placed the Acridine right in the growth media about 10 minutes prior to observation. Aspirate off the media, wash the cells once with HBS and place the coverslip cell side down into mounting media. The green glowing nuclear fragments of apoptotic cells were really dramatic. The peroxide treatment was the best at inducing apoptosis in our hands, probably followed by ethanol. *Lakeland Community College* (#394)

Plant tissue culture (May 2002)

I am trying to track down some sources for plant tissue culture and I have reference to Fralin Biotech and Toby Horn but the link to plant tissue culture does not work - does anyone know how to retrieve this material? *Oklahoma City Community College* (#411)

- Contact Dr. Carol Stiff at Kitchen culture Kits www.kitchenculturekit.com *Chula Vista High School* (#412)
- You might try emailing the new director at the Fralin Institute, Erin Dolan. We also offer a wide variety of plant tissue culturing kits and supplies here at Carolina. If you have questions you can contact the head of that department, George Isley. *Carolina Biological Supply Company* (#421)

High school biotech course (May 2002)

Do you know of states that officially accept a biotech high school course as a science credit for High school graduation - I would like to collect a list of which states officially recognize biotech as HS course. *Oklahoma City Community College* (#413)

- Ohio is in the process of developing Tech Prep Biotechnology in the high schools. Sinclair is one of 4 pilot sites (in the Miami Valley Tech Prep Consortium) in Ohio. We have been working with several high schools for the last year and are ready to launch the first class of high school juniors in Tech Prep Biotech next fall. There will be five high schools participating next year and 3 or 4 the following year. The students in the biotech track (in this consortium) still need to take general/physical science and biology as freshmen and sophomores and another science in their junior or senior year (human biology, chemistry, etc). They will earn some credit for two years of biotech courses, but biotech does not substitute for the basic science courses. So, Ohio recognizes the biotech courses, but not as substitutes for biology, chemistry, etc. *Sinclair Community College* (#414)
- I know there are at least three schools in Washington that have high school biotechnology courses and I think there's one in Utah. The Washington schools are: Juanita High School; Shorewood High School; Lynnwood High School. *Steve Scheidell Murray High School* (#415)
- George Cachianes at Lincoln High School in San Francisco has developed a high school biotechnology class. Ellyn Daugherty at San Mateo High School in San Mateo, CA has a full load of high school biotechnology classes. Gerri Horsma at Gunn High School has a biotechnology course. Andrew Pl Hill High School has a biotechnology academy (3200 Senter Road, San Jose, CA 95111 Berekley Biotech has a biotech academy (BBEI) Bill Thieman at Ventura College has a Tech Prep arrangement with a number of high schools. Judi Heitz teaches biotech in Southern California. (#416)
- Additionally in the bay area: Carolyn Abbott at Piedmont Hills HS has had a biotech course; Louise Handly at Overfelt HS has a biotech course; Mary Pat Slate at Wilcox HS has a biotech course; Jake Russo at Watsonville HS has a biotech course modeled after Ellyn Daugherty's; Mark Okuda at Silver Creek HS has a biotech course and also started a Forensics course this year; Geri Horsma's biotech course at Gunn HS is both Tech Prep and ROP. *San Jose State University* (#417)
- Contra Costa County Office of Education has a program in biotechnology that many of its schools in the county use as part of the Regional Occupational Program (ROP). *Diablo Valley College* (#418)
- I teach a 2 year biotechnology class, a three year program. The two year biotech class provides UC biology credit. *Chula Vista High School* (#419)
- Some school districts in New Hampshire accept biotech courses for science credit toward graduation. In NH, school districts are autonomous so it varies from district, but I would say the majority of schools in NH allow science credit for biotech courses. *Pease International Tradeport* (#420)
- I am the teacher of a biotech class at Winnacunnet HS in Hampton NH which also sends students to a biotech program at Seacoast School of Technology. I have also met some teachers from Utah that have a biotech program (Weber High School in Ogden Utah) beside the one at Murray HS. (#422)
- I teach a 3-year biotech program in NH at the Seacoast School of Technology in Exeter, NH. For Biotech I, students receive 1.5 high school credits in biology, 0.5 credits in health and 4 credits from the NHCTC for microbiology. Biotech II students receive 2 high school credits in advanced biology and 4 college credits in biology. Biotech III students will receive 3 college credits in internship experience; the high school credits they will receive are still in flux. *Seacoast School of Technology* (#423)

Biotech application (July 2002)

Do you use an application for students wishing to enter your biotech program? Would you be willing to share it with me? (#439)

- The Sinclair program is a Tech Prep Program and thus the recruiting begins in the Miami Valley Tech Prep Consortium High Schools. Each school has its own local recruiting programs some of which use locally generated generic applications. I don't think that is what you are looking for. Sinclair students who begin the program at the Community College just sign up for the classes of course in a sequential order. No application is involved. (#441)
- We use the general MET College application since the BLCS program is partially MED (BUSM) and MET. The web site probably has the app: www.met.bu.edu. *Boston University School of Medicine* (#442)

Student internships (August 2002)

Our incoming Biotechnology Program students will do an internship during their last semester as a capstone course. We have a commitment from Delaware Biotechnology Institute, our partner in our ATE project, to line up the researchers/labs for the interns. Do any of you have internship guidelines, manual, or evaluation forms that you would be willing to share? *Delaware Technical & Community College* (#454)

- I have attached the forms and documents we use in our internship program. It has been very successful- every student that completed an internship went on to FT employment. One important caveat-in the device industry no one wanted to give students an "internship" in the classic sense, so all our students basically got jobs- either through a temp agency or they were already working in a device company. Students have to choose measurable competencies that result in some tangible accomplishment. We have our students give Power Point presentations at the end of the semester to an outside audience- their supervisors, other faculty, or our Biomedical Advisory Board. We had a dinner and the presentations as part of the semester Advisory Committee meeting. *Anoka-Ramsey Community College* (#455)

Used equipment (September 2002)

I have miraculously received a modest equipment budget for 2002-2003 and want to stretch our dollars. Does anyone have experience with purchasing used laboratory equipment? Can you recommend a reliable vendor (e.g. American Laboratory Traders)? We have a fairly laborious purchasing process that can take 2-3 weeks, so auctions seem to be out of the picture (auctions appear to want purchases done quickly). *Merced College* (#464)

- Did you check the used equipment page at my site? Go to: www.kitchenculturekit.com/usedequipment.htm. It is from Pioneer Hybrid and the contact name is on the site. *Kitchen Culture Kits, Inc.* (#466)

HPLC advice (September 2002)

We would like to upgrade the HPLC component of our chromatography course by purchasing a new/used HPLC which has a computer interface. Any ideas or suggestions on brands/models which work well for teaching? *Madison Area Technical College* (#467)

- We have two used Waters 625's that were donated from industry. The Millenium 32 software for four workstations was $20,000, so expensive, but they have about a 50% marketshare in industry so students are learning on what they will see in many of the labs. The software is not the simplest but there is a good tutorial. *Shoreline Community College* (#468)
- I'm not sure what is left but go to the used equipment page at my site and contact the person listed. If want you want is not on the page, he might be able to find it for you. www.kitchenculturekit.com/usedequipment.htm *Kitchen Culture Kits, Inc.* (#469)

Mitoch PCR (November 2002)

I have to admit I am getting close to being stumped - I have run mitoch. DNA with old and new primers, with PCR beads, and still getting no product - now I am wondering at my interpretation of the primer concentration to be used - can anyone look up their protocol for pcr of human mitoch. DNA and tell me what the FINAL concentration of primer is in a 25 microliter reaction using pcr beads - this has always worked like charm before. (#504)

- How do you plan to use the mitochondrial DNA? For sequencing? Or for agarose gel electrophoresis? It's been a couple of years since I did this, but for agarose gels, we set up the PCR reactions so that the final concentration of each primer was 1.0 micromolar. I'm sure that's more primer than we needed, but we were using the PCR products for multiple things. One thing to consider too is that sometimes you can have problems with the beads. I spent a horrible winter break one year troubleshooting everything, only to find out that the beads were the problem component. (#505)
- I want to use it for electrophoresis and then send for sequencing to DNA learning center - thanks for the suggestion - I will try another lot of beads - we have several packages -and I was using a lower concentration of primers so will up the primer conc. (#506)
- Have you ever found that the PCR beads to the Alu kits (either from Edvotek and/or Carolina) can be a problem? I used to have just great results for our class Alu amplification from genomic DNA (buccal cell isolates) but during the past few semesters the results have been less than desirable. Initially I assumed the problem was strictly student technique but I am now questioning whether there may be some other inherent problems to the technique--although I remain unsure why the initial results a few semesters back were so good when it seems we did not change anything. *Santa Monica College* (#509)
- I wasn't using a kit. I think I ordered the Hot Start wax beads from Promega but I confess, I don't really remember for certain and it might have been another company. Your observations are pretty similar to what I and others have seen with PCR. Primers and nucleotides can go bad with repeated freezing and thawing. Enzymes, even heat tolerant polymerases, can lose activity over time, or suffer problems from temperature changes during transit or storage. And the only way to find the problem is to set up experiments that test each and every variable. I found the best approach was to aliquot PCR reagents as much as possible so that you avoid freezing and thawing anything. I also kept samples of reliable templates and PCR products so that I could have some positive controls. (#510)
- We have had horrible luck with either the Carolina kit or the Edvotek kit. It's our experience that the Bio-Rad kit (last I checked they have the old Cold Spring Harbor tpa-25 Alu still, even though CSH has dropped it for political reasons) works great. *Solano College* (#513)

African violet tissue culture (November 2002)

I'm starting a plant tissue culture course, and was planning to use African violet. As I began the ordering I notice Ward's "Plant Cloning Kit: African violet." Does anyone have any experience with this kit? If so please let me know what you think of it. (#544)

- I'm not familiar with Ward's kit but there is also one by Carolina Biological, The Cincinnati Zoo, and Kitchen Culture Kits. Websites are: www.carolina.com, email for the zoo and specific info (I'm making a website for them), www.kitchenculturekit.com. You can also buy the basic supplies directly from www.caissonlabs.com or www.phytotechlab.com. Email me your needs and I can advise. I am biased because I own KCK, work for Caisson, and am friends with Phytotech and the Zoo, and order much from Carolina, but I'm very open minded and a lousy business person (scientists usually

are). If you use the Ward's kit, would you report back to the list what you thought of it. *Kitchen Culture Kits, Inc.* (#545)

- I have tried to use both Waards and Carolina's plant TC kits, and the putative Edovek cloning kit. Kitchen culture kits work best, and have the best support. *Chula Vista High School* (#546)

Biological safety cabinets (November 2002)

Northampton community college and Lehigh Carbon community college are designing new biotechnology programs. We will be buying biological safety cabinets and were wondering if anybody from the group had any good recommendations on brand, type etc? We would like to buy something under 10,000 dollars. *Northampton Community College* (#548)

- The best hoods made are by Baker. The Baker Edgeguard laminar flow is the one I recommend for plant tissue culture. Baker also has units that are Class II and above. *Kitchen Culture Kits, Inc.* (#550)
- We just purchased a hood from KENDRO LABORATORY PRODUCTS with a unique feature that I think educators will like. The sides of the hood have large glass panels which students can look through when the operator is performing tasks. I know I have seen other hoods with this feature, but these are nice and big for viewing. This is an excellent student-friendly feature that I think everyone might be interested in. Kendro deals with Heraeus products and we have purchased CO2 incubators from them and have been very happy. I would say the cost would be in the $5-8K range for the hood (depending on accessories). *Finger Lakes Community College* (#551)

Request for updated info for website (December 2002)

Do you know of websites related to plant tissue culture including training and courses, biotechnology education, secondary teacher workshops, species specific pages on propagation, products of interest to tissue culturists, etc.? *Kitchen Culture Kits, Inc.* (#554)

- www.nsta.org
- www.TeachingScience.org
- www.biotechknowledge.monsanto.com
- www.biotechgoodtogrow.com
- www.whybiotech.com
- www.nabt.org
- www.biotech.icmb.utexas.edu
- www.biotechnologyyes.co.uk
- www.agron.iastate.edu/plantscience
- www.biology.arizona.edu
- www.ipd.anl.gov/biotech/
- www.bio.org
- www.accessexcellence.org
- www.fav.org/biotech
- www.biotechinstitute.org
- www.agriculture.tusk.edu/biotech/biotech.html
- www.bioworld.com
- www.ncfap.org
- www.attra.ncat.org
- www.acsh.org
- www.usda.gov/agencies/biotech/
- www.edugreen.teri.res.in/explore/bio/bio.htm *St. Louis Science Center* (#556)
- www.ibtreflab.com *ProGene Biomedical* (#558)
- www.biospace.com (#560)