FRED HUTCHINSON CANCER RESEARCH CENTER
SCIENCE EDUCATION PARTNERSHIP

# *TEACHER'S GUIDE*
# *DNA LABS 1 & 2*

ATTENTION! Have you put the reagents that were in the freezer box in your freezer? Take care of all reagents so that they are stored properly for optimal lab use. Keep all enzymes on ice or in the freezer until ready to use.

**Overview**

1. These labs were written for SEP participants using the SEP Gel Electrophoresis Kits and supplies.
2. These DNA labs have been updated as of 2006. Please read through this guide and the labs before you get started.
3. DNA Labs 1 & 2, the Electrophoresis Exploration Lab, Measure for Measure Lab, and the Dye/Indicator Lab are all on a CD included in the kit notebook. Feel free to adapt the labs for your own use, but please credit SEP.
4. The DNA labs were written to be used sequentially, that is, DNA Lab 1 first, then DNA Lab 2.
5. Please, go through the kits and equipment to make certain you have everything that is included in the kit. You will need to make some solutions and aliquot other solutions for classroom use. Further, some critical items are not supplied in the kit; e.g. **microwave oven or hot plate, balances, ice, and deionized or distilled water for diluting the 50X TAE buffer. You will need to use your own resources for these items.**

**Lab Equipment Tips and Notes**

- The electrophoresis gel box lids crack easily and they are expensive to replace. Please encourage your students to be careful when using and cleaning the boxes. Store and repack carefully. When repacking, don't put anything on top of the gel boxes and make sure they are not crushed by the green crates' lids.
- We have provided pH paper to use in some of the gel box labs. This paper is very expensive. Please use the pH paper for the Electrophoresis Exploration Lab and/or once in either the Dye/Indicator or DNA Labs. It is not useful to measure the pH in every lab performed.
- For best results in pouring gels, let the hot agarose solution cool until it is hot, yet comfortable enough to hold the container. Less concentrated gels (0.7%) are less viscous when hot and can leak under the dams. Students can either measure out 25 ml to pour, or pour slowly and directly in between the dams to the height of the gel deck—which is 25 ml.
- For optimal results when running gels, use 100 V and a run time of 45 minutes or until the tracking dye bromophenol blue (the purple colored dye at this pH) is about half way down the gel. See other gel running tips in the Tips for Teachers section.
- When troubleshooting results from these labs, please refer to the DNA Science photos of gel results. This is included in the Gel Kit notebook in the DNA Science section.
- If the power supply beeps during the gel run, turn it down a notch. This is a safety mechanism (overcurrent alarm) and it may indicate that students are running gels at an excessive voltage and/or that the running buffer has been reused too many times.
- If you have difficulty with any of the equipment, please refer to the equipment manuals in the notebook, or call us at SEP 206.667.4487. We're happy to help!

**What's in the Freezer Box?**

| Tube label Top | Side | Tube Contents | Notes |
|---|---|---|---|
| Lab 1 | | | |
| λ | λ DNA 250 μg/ml | Uncut lambda DNA | Thaw and mix thoroughly before use |
| I | Marker I λ/EcoRI 250 μg/ml | Lambda DNA cut with EcoRI (precut DNA) | Thaw and mix thoroughly before use |
| II | Marker II λ/HindIII 250 μg/ml | Lambda DNA cut with HindIII (precut DNA) | Thaw and mix thoroughly before use |
| III | Marker III λ/EcoRI+HindIII 250 μg/ml | Lambda DNA cut with EcoRI and HindIII (precut DNA) | Thaw and mix thoroughly before use |
| Lab 2 | | | |
| EcoRI | EcoRI Use REact 3 | Restriction enzyme EcoRI | Keep on ice. Use with REact 3 buffer |
| HindIII | HindIII Use REact 2 | Restriction enzyme HindIII | Keep on ice. Use with REact 2 buffer |
| BamHI | BamHI Use REact 3 | Restriction enzyme BamHI | Keep on ice. Use with REact 3 buffer |
| REact 2 | REact 2 | 10X REact 2 Buffer | Use with HindIII reactions and with uncut λ DNA control |
| REact 3 | REact 3 | 10X REact 3 Buffer | Use with EcoRI and BamHI reactions |
| λ | λ DNA 250 μg/ml | Uncut lambda DNA | Thaw and mix thoroughly before use |
| II | Marker II λ/HindIII 250 μg/ml | Lambda DNA cut with HindIII (precut DNA) | Thaw and mix thoroughly before use |

Restriction enzyme activity: These enzymes are usually at > 10 U/μl in activity. One unit (U) of activity is defined as the amount of enzyme required to digest 1 μg of lambda DNA to completion in one hour in the preferred enzyme buffer at the optimal temperature for that enzyme (usually 37 °C).

**Stock Solutions and Recipes for Solutions in the Kit (THESE ARE ALL SUPPLIED IN THE KIT—THE RECIPES ARE FOR YOUR INFORMATION)**

**STE** (Salt-Tris-EDTA) a buffered salt solution for storage of DNA
100mM NaCl
1mM EDTA
10mM Tris, pH 8.0

**Sample Loading Buffer** (concentrated-blue colored) contains Ficoll, to make the sample dense, and tracking dyes so that migration of fragments in a gel can be monitored.
15% Ficoll®
0.25% xylene cyanol
0.25% bromophenol blue
50mM EDTA

**Carolina Blu ™ DNA gel stain** This stain's ingredients are proprietary. Specific instructions for the use of this stain are in the lab handout in the kit notebook and as protocol sheets ("How to make a Carolina Blu™ Gel") in the kit.

- There are two different bottles of Carolina Blu™ in the kit. One is labeled #1, which is used to add to the running buffer and to the agarose gel solution once it is cooled enough to pour.
- The other bottle of Carolina Blu™ is labeled #2, which is used to stain the gel after running the DNA. Stain #2 can be reused.
  One Carolina Blu™ staining set should be enough to stain gels from approximately three classes. Reuse stain #2 throughout the staining process—save it in the Used stain container provided. You can use it up to three times.
- You need about 1μg of DNA per lane to see Carolina Blu™ stained bands.

**1X TAE** (Tris Acetate EDTA) Electrophoresis buffer, a.k.a. running buffer or gel buffer.
0.04M Tris-acetate
0.001M EDTA

This buffer may be reused several times if mixed well between runs. The kit contains 50X TAE stock. You will need to dilute it to make 1X. Measure out 20 ml of 50X TAE and add 980 ml of deionized or distilled water to a final volume of 1 liter. For eight gel boxes, you will need at least one liter of running buffer (8 x 125 ml=1 liter) plus the buffer needed to make the gels. So, make plenty of 1X so that you don't run out in the middle of a lab period.

Recipe to make your own 50X TAE
242 g Tris base
57.1 ml glacial acetic acid
100 ml 0.5M EDTA, pH 8.0
Bring volume to 1 liter with deionized or distilled water.

**Classroom Management**

- ❑ In order for these labs to run smoothly, predict and prevent any bottlenecks that may occur. If space allows, set up a few stations for weighing agarose, pouring buffer, and making solutions. Agarose gel solutions can be made days ahead of the actual lab day. Have students label their solutions and put them aside at room temperature. Add the Carolina Blu™ after they have melted the agarose and they are ready to pour gels.
- ❑ **SEP supplies you with stocks of all of the reagents you need---however, you need to aliquot these stocks for your classroom use.** Aliquot tubes of DNA, reaction buffers, enzymes, sterilized water, STE and loading buffer (dye) so that students can obtain what they need for their lab group. Depending on your class size, you can prevent bottlenecks by having these items accessible to as many groups who need them. You should have plenty of extra microcentrifuge tubes for aliquoting. Be certain to keep enzymes on ice!
- ❑ Place shared equipment, i.e. microcentrifuges, balances, light box---in areas that are easily accessible to the greatest number of students.
- ❑ For classroom discussion of results, try putting the gels on an overhead projector, or other multi-media device to project the image. You can also use a digital camera to photograph a gel that you can later project for the class.

**Suggested Stopping Points During the Labs:**

- **After pouring gels**. Prepare and pour gels ahead of time. Carefully slide them into a Ziploc® bag and add some 1X TAE buffer to maintain moisture and ion levels. Keep refrigerated until ready to use. Carefully slide the gel back into the gel deck and place in the gel box and continue with the gel loading steps.

- **After preparing pre-cut samples**. Have students prepare pre-cut samples and then freeze at -20°C until you're ready for the gel loading step. When you're ready: thaw samples, add loading buffer, mix, and load.

- **After setting up digests**. For the digest lab, you can digest samples overnight, spin them down and run the next day, or freeze them at -20°C until your ready for the gel loading step. If frozen, for best results, heat up the sample to 65°C (this helps detach those "cos" ends of the DNA, which may have stuck together during the cooling process) before adding loading buffer and loading.

- **After staining gels**. Run gels, stain in Carolina Blu™ (#2) destain a couple of washes then leave gels in a small volume of distilled water in the refrigerator overnight. You may lose some of the smallest bands, but overall the DNA will be more visible than during a rushed stain, destain, trace session.

- **After tracing bands in gel**. Have students trace DNA bands and record data in a table. Do the semi-log plot another day when all students have their data tables ready.

**References**

DNA SCIENCE: A First Course in Recombinant DNA Technology, 1990, Dave Micklos & Greg Freyer, Cold Spring Harbor Press

Gene Connection v1.5: 1994 San Mateo County Biotechnology Curriculum (No longer available—out of print)

A Sourcebook of Biotechnology Activities, 1990, NABT and North Carolina Biotechnology Centers(Out of print.)

Recombinant DNA and Biotechnology 2nd ed, 2001, Helen Kreuzer and Adrianne Massey, ASM Press.

## For an extensive list of teaching tips, please refer to Tips for Teachers