**Restriction Digestion and Analysis of Lambda DNA**

Student Group Directions- Preparing Enzymes

**Procedure**

1. YOU MUST KEEP THE ENZYMES ON ICE AT ALL TIMES. Before starting lab: Cut a Styrofoam cup to a height of around 8 cm. Fill with ice. Pick up a white ‘foamie’ and 7 clear Eppendorf micro tubes to have ready before doing the next steps. I advise you to label the tubes (see below) before you start.
2. Aliquot (micropipet) 5 ul of each of the 3 restriction enzymes into 3 labeled clear micro test tubes. Label the tubes: HindIII, Pstl, and EcoRI. Put the tubes in the foamie and keep on ice (at all times). Make sure you use the correct micropipetter for each sample (they have different ranges, so check carefully). Make sure to use a new tip every time. Never use one twice.
3. Aliquot 60 ul of restriction buffer into another clear tube. Label the tube ‘RB’. Place on ice.
4. Aliquot 25 ul of lambda DNA into another clear tube and label ‘lambda’. Place on ice.
5. **Students- skip this step and go to #6. THESE ARE TEACHER DIRECTIONS and NOT for the student (it’s a reminder to myself)**: Prepare HindIII lambda digest (DNA marker). Add 20 ul of sample loading dye to the stock tube (there’s only 1 for the entire class) containing the HindIII DNA marker. If possible, heat the marker to 65 degrees C for 5 minutes, then chill on ice- this results in better separation of the marker bands.
6. Get another clear tube and label ‘M’. Aliquot 15 ul of the HindIII lambda digest DNA standard marker (\* ask me if I remembered to put a loading dye in it) into the tube.
7. Get another clear tube and label ‘LD’. Aliquot 30 ul of the loading dye into the tube. *You likely can omit this step, if we use UV loading dye tomorrow. Ask me.*

**You may now prepare the samples for the gel loading we will do tomorrow.**

1. Go to student section in lab manual and follow the directions for Lesson 1, but see my handwritten notes. You will stop (for the day) with step 5 and we will let the tubes incubate overnight IN THE GREEN foams on the back table. LABEL YOUR FOAM so you know it is yours (you could put your foam on a labeled sheet of paper or try and write directly on the foam with a sharpie).
2. If time allows, pick up the colored eppendorfs you will need for tomorrow. Label each tube (see lab manual- Lesson 2) and put them in another green foam. Place on back table or on cart (ask). \*Make sure the tubes are closed.
3. Tomorrow, follow the lab directions EXCEPT YOU MUST ADD THIS: **as soon as you get to class, you will add 2 ul of UV dye** (there is only ONE stock tube for everyone) to each of the tubes and centrifuge the tubes before you begin loading.Also, you will load 11 ul to each well, **NOT** 10 ul.
4. **TIME WILL BE SHORT ON FRIDAY (pep rally). YOU MUST WORK QUICKLY!**
5. *There is a ‘quick guide’ also given as part of the directions, but I suggest you follow the lengthier directions and only use the quick guide as a reference.*