**Student Modifications: Analysis of Precut Lambda DNA**

***\*Make note of these modifications on your lab handout before you start lab (example: circle each step, below, on your sheet so you will remember to refer to this sheet)!***

1. **Page 25**: **Step #2**- Discard tips in bleach solution after **EACH & EVERY** use
2. **Step #3**: DO THIS BEFORE STEP #4! Micropipet 8-9 ul of UV dye into a clear Eppendorf tube (microtube). Centrifuge for 20-30 seconds (\*it must be balanced, so put an Eppendorf tube with water in it, directly opposite the tube in the centrifuge). Take the tube to your station.
3. **Step #4**- Pipet 2.0 ul **UV dye** (in clear microtube) into each tube (L, P, E, H). Discard tip after each sample is loaded. Centrifuge all 4 samples before loading the DNA into the wells.
4. **Page 27**: **Step #1**: **DO THIS BEFORE LOADING THE WELLS**! Put gel (in tray) in ELECTROPHORESIS BOX. Wells should be adjacent to the BLACK electrode. Gently, remove comb (pull it STRAIGHT up…don’t ‘wiggle’ it!). Cover your gel with buffer from the fridge (in Florence flask) until it reaches the fill line. There should be **NO DIMPLES** on your gel after you do this, so sometimes you have to go a little past the fill line. **Call me over** and have me make sure it’s okay before you load the DNA into the wells. Your wells should be deep enough to hold 10 ul in each. LOAD SAMPLES INTO THE MIDDLE 4 LANES (avoid lanes 1 and 6, if possible). **Step #6**- Use a funnel to pour the buffer back into the original Florence flask. Step #7- Label the plastic trays with your names (use labeling tape). While gels are running, CLEAN UP and complete the other assignment that I give you. We will not stain the gels after they run. We have 2 UV illuminators that all groups will have to share. YOU MUST PUT CLEAR PLASTIC WRAP OVER THE ILLUMINATOR SCREEN BEFORE PLACING YOUR GEL ON IT!! You must view your gels in the dark (stockroom). Take pictures! You may still need your gels tomorrow to measure fragment distances, so put gels back in plastic trays and either wrap with plastic wrap or but back in baggies. Put them in fridge before you leave. \*May want to squirt a little buffer on them (from wash bottles)
5. **Page 33 and 34** (Second box): We do not have gel support film (it wasn’t in the kit). Have someone in your group take a picture. Print (black and white is fine) and attach it to the 2nd box on p. 34. \*I will try and make a copy of what it should look like, also. REMIND ME!
6. **Page 34** (1st box): You will have to use your gel as a “template” for “tracing” for the 1st box on p. 34. You can use either clear plastic wrap or wax paper to place over your gel to trace the band patterns. Mark the wrap/ wax paper with a sharpie. \***I may have some clear acetate “transparencies” you can use. Ask me**! Measure your gel and the bands and, as accurately as possible, use the measurements to show the banding patterns & attach to the first box on p. 34.
7. **Page 35**: Use your gel to measure in mm.
8. **Page 36**: Use diagram shown earlier in the lab for Hind III. Determine Hind III fragment lengths and sizes first, then estimate the others, based on Hind III
9. Pick up semilog graph paper, if extra is needed. ***Use Hind III to make a standard curve*** (see example on p. 38) to determine fragment size of uncut Lambda, Pstl and EcoRI, based on distances traveled in relation to Hind III. **Use pencil, a ruler and BE NEAT**! Be sure to label each axis.