Bioc 463A
Bacterial Luciferase Experiment.
2003-2004

<u>Overall Protocol:</u>

Tues.

1. Isolate plasmid DNA from lux(+) cells.
2. Perform EcoRI, HindIII, and EcoRI + HindIII digest on plasmid DNA. DNA from digest will be frozen until Thursday.
3. Perform transformation reaction on competent Xl-1 Blue strain of E. coli. Dilutions of transformation will be plated out on two LB + Amp plates.

Wed.

1. You will have to come in on Wed. and pick colonies off of your LB + Amp plates and make luciferase patch plates (LB+Amp).

Thursday.

1. We run 1% agarose electrophoresis gels containing the restriction enzyme digests from Tuesday.
2. After the gel has run, you will determine the size of the restriction fragments using a lambda DNA-Bst E II cut ladder.

For all procedures given below we will need to use sterile techniques. Your pipette tip boxes will be sterilized and you should be careful to not leave the top of the boxes open for longer than necessary. Also BioHazard bags will placed in strategic locations to dispose of any material that has come into contact with E. coli. Please be careful to properly dispose of ALL contaminated material.

<u>Mini-Prep</u>

The purpose of the mini-prep is to purify small quantities of pure plasmid DNA from lysed E. coli cells, that also contains chromosomal DNA as well as a variety of RNAs. We will follow the instructions included in the Qiagen mini-prep kit, that will be handed out in class. You will obtain 50 uL of the plasmid (pJHD500) containing the luxA and luxB. **Be careful to NOT throw away your plasmid DNA!!!**

<u>Restriction Enzyme Digest.</u>

1. Reaction Volumes:
   - 1 uL 10x buffer
   - 2 uL restriction enzyme (single cut) or 1 uL EACH for two enzyme cut
   - 3 uL plasmid DNA
   - 4 uL H_2O
2. Mix reagents gently.
3. Digest plasmid DNA at 37°C for 60 minutes.
4. Digest will be frozen until Thursday.

E. coli Transformation with Plasmid DNA.

Whenever you perform a transformation, it is advisable to always perform a control experiment, where water is used instead of plasmid DNA in the transformation reaction. Cells "transformed" with water should not have any resistance to antibiotic and therefore should not grow on agar plates containing antibiotic. Because we have very limited amounts of competent cells, we will regrettably omit the control in this experiment.

1. Thaw two tubes of 50 uL competent XL-1 cells on ice. **DO NOT LET WARM UP OUT OF ICE!!!.** Simultaneously, place one 15 mL conical tube on ice.
2. Add 1 uL of plasmid DNA as a drop to the side of the 15 mL conical tube. Make sure you can see the DNA drop.
3. Add 25 uL of XL-1 Blue cells immediately above the DNA drop, washing the DNA and cells into the bottom of the conical tube. Flick the tube to mix, shake the cells and DNA to the bottom conical tube. Place on ice for **30** minutes.
4. Heat shock cells for 45 sec at 42°C in the water bath.
5. Immediately return the cells to ice for 2 min.
6. Add 500 uL of STERILE SOC (no antibiotic) media to each tube, gently mix. Grow on shaker for 60 minutes at 37°C.
7. Plate out transformed cells in 15 uL and 50 uL aliquots on LB + Amp plates. Grow cells overnight at 37°C.
8. On Wednesday, come in and check your colonies. Transfer single colonies from either plate to a single LB + Amp plate in a patch pattern (we will demonstrate this technique in class). Patching the colonies in this manner will allow us to induce lux expression in the cells growing on the plates then be able to observe the bioluminescence that occurs upon addition of the aldehyde substrate.

Agarose Gel Electrophoresis.

We will prepare 40 mL of a 1% (W/V) agarose solution.

1. Weigh out agarose and place in 125 mL flask.
2. Add 40 mL of TBE (Tris-Borate-EDTA) buffer.
3. Heat in microwave for a total of 45 sec using 15 sec burst. At the end of each 15 sec, gently swirl the solution to avoid boiling over.
4. Allow to cool till the flask can be held in your hand without pain.
5. Add 1 uL of Ethidium Bromide (EtBr). **CAUTION: EtBr is a very potent mutagen. Be very careful handling and report any spills. Wear gloves. ALL EtBr waste (buffers, gels, gloves, etc.) must be disposed of in EtBr waste bag.**
6. Pour agarose into gel cassette and immediately insert comb.
7. Allow gel to solidify at room temp.
8. Prepare DNA samples for electrophoresis.
    - 10 uL of digested DNA.
    - 2 uL of loading buffer.
9. Prepare Lambda marker.
    - 5 uL of pre-digested (BstEII) and prestained Lambda marker needs to be heated at 60°C for 3 minutes prior to loading on gel.

10. Run gels at 75 V until the marker band is near the bottom of the gel. Turn off and disconnect cassette from power supply.
11. WEARING GLOVES, remove the gel from the cassette and visualize the bands on a UV-black lite box. **CAUTION: THESE GEL BOXES EMIT UV-RADITION THAT WILL SERIOUSLY HARM YOUR RETINA. OVER EXPOSURE WILL ALSO CAUSE BURNING OF THE SKIN. YOU MUST WEAR A UV ABSORBING FACE SHIELD WHEN LOOKING AT YOUR GELS AND MAKE SURE NO UNPROTECTED PERSONNEL ARE NEARBY.**
12. Dispose of the agarose gel in the EtBr bucket and wash off the gel lite box with water and a Kimwipe. Dispose of the Kimwipe in the EtBr bucket.