**pGLO Transformation II:**
**Plasmid DNA isolation**

Read pp. 421 – 426 and review Fig. 15.21 of Snustad & Simmons, fourth edition, for important background information.

**Week 2. Plasmid DNA isolation.**

You will now isolate the pGLO plasmid DNA from the bacteria. The trick is to isolate the plasmid (what we want) without isolating chromosomal DNA (what we don't want). This can be accomplished by performing the steps listed below. Following the DNA isolation, electrophoresis will be used to see if your isolation is successful. This will also allow you to verify the size of the plasmid and the presence of the GFP gene, since a plasmid without the gene will be smaller than a plasmid with a gene.

Each group will be given two bacterial cultures of unknown identity. The culture may be from a colony that has pGLO plasmid (that is, the plasmid with the GFP gene inserted in to it), or from a colony with the same plasmid but without the GFP gene inserted into it. Based on the results of the electrophoresis, you should be able to identify your unknown cultures. In addition, you should be able to estimate the size of the GFP gene that was inserted into the plasmid.

**Experimental Procedures.**

1. Transfer 1.5 ml of the overnight culture to a microcentrifuge tube and pellet cells at 10,000 RPM, 2 min.

2. Remove the supernatant completely and resuspend the cells in 300 µl STET buffer. Drag the tube across a rack approximately ten times. It is important that the cells are completely resuspended.

3. Add 25 µl lysozyme (10 mg/ml) and mix by inversion.

5. Immediately place tube in a boiling water bath and incubate 45 sec.

6. Spin 10,000 RPM, 5 min. The pellet contains cellular debris and chromosomal DNA; the supernatant contains plasmid DNA and RNA. Remove debris with a toothpick.

7. Add 350 µl isopropanol to the supernatant, mix well, and spin 10,000 rpm for 5 min.

8. Wash pellet once with 70% ethanol. Pellet DNA again.

9. Remove ethanol completely and let pellet dry for 10 min.

10. Resuspend DNA in 20 µl TE buffer; incubate 37°C for 10 min.
11. Remove 2 µl of the each DNA and combine with 8 µl restriction mix (0.2 µl Eco RI, 1 µl 10x buffer, 6.8 µl H2O). Mix well and incubate 37°C for 1 hour.

12. Analyze the digested DNAs on an agarose gel the results will be distributed later.

**Data Analysis.**

Provide some background information on transformation and its utility for molecular genetics. Because of time constraints, we were not able to construct a recombinant molecule, but in your report you might want to discuss briefly how this is done. As always, try to make a complete story, understandable to someone who is not so familiar with the techniques used. Include a picture of the gel (instructor will provide) as your data. Other data will be the number of colonies found on each of your plates. For the electrophoresis, identify each sample and draw a conclusion about the identity of the plasmid.

The following questions may be useful as you prepare our report:

- What is a plasmid? Why are they useful for molecular genetics?
- What is transformation and what role does it usually play in bacterial life cycles?
- What was the purpose of each step used in the transformation process?
- How many colonies were on each plate? Are these numbers consistent with what you expected?
- Were you able to verify the identity of each plasmid? If so, how did you do this?
- How did you separate the plasmid DNA form the bacterial chromosomal DNA?
- What is the size of the DNA fragment that includes the GFP gene?
- Explain the significance of digesting the DNA with EcoR I (very important!).