CHEM525 Experiment 5
Ligations Screens – aka "did it work?"

Purpose and Theory

Last week, we ligated our two pieces of DNA together and transformed *E. coli* with the ligation product. This week, our goal is to determine if the ligation reaction worked and, if it did, transform a different strain of *E. coli* with the pET28a::LDH plasmid. Next week, we will discuss the difference between these two bacteria strains and why one is optimized for DNA work and the other is optimized for producing protein.

Safety Precautions

- Ethidium bromide is a carcinogen. Always wear gloves when handling solutions containing this molecule. Make sure that gels containing ethidium bromide are put in appropriate waste container.
- Always wear glove, safety coat and goggles when in the lab.
- If a significant amount of any chemical is spilled, immediately seek the instructor for clean-up protocols.
- Never look directly into UV light – it can damage your eyes! Make sure the screen is up and look through the screen at your gel.

Equipment/Reagents Needed

1. Micropipettes and tips
2. Microcentrifuge
3. UV light box
4. Tabletop vortex
5. Miniprep kit.
6. 1X TBE buffer
7. Agarose
8. Electrophoresis chamber and power source.
9. 5X PCR master mix (keep on ice at all times)
10. Primers
11. MilliQ water
12. Inoculated *E. coli* cells

Procedure

1. Set up a 50 μL PCR reaction
    a. Split the reaction into 2 x 25 μL aliquots.
    b. Add ~0.5 μL of inoculated cell culture to each reaction (this is the template)
2. Start the PCR reaction using the same cycle as week 1.

To do while the PCR reaction is running:

3. Mini-prep the cell culture that you receive from the instructor.
4. Prepare a 30 mL 1% agarose gel. You'll be working with at least 2 samples.
5. Transform the mini-prepped cells into the BL21 (DE3) NiCo strain of *E. coli*. Use the same protocol that you did in the previous lab.