FRED HUTCHINSON CANCER RESEARCH CENTER
SCIENCE EDUCATION PARTNERSHIP

pFLO TRANSFORMATION KIT TEACHING TIPS & TROUBLE SHOOTING NOTES

Teaching Tips

- Transformation efficiency can be a difficult concept. Go through it with your students.
    - Use a creative demonstration to explain what transformation is and how it is done. See the kit notebook for one suggestion (Lesson 13 of An Introduction to Biotechnology: A Unit for Seventh and Eighth Grade Students). There are also several overheads included in the kit notebook that demonstrate the transformation process.

- The pre-employment interview is actually a type of pre-assessment for you to use. It is designed to fit with the Genes R Us Scenario. You can use this to assess your students' prior knowledge of transformation. It can be oral or written, before or after reading the scenario. You could also modify it and use it at the end of the lab.

- Demonstrate how to pick bacteria from a plate, and how to resuspend cells in $CaCl_2$ by repeated, slow pipetting.

- Go through a dry run of the ice⇒heat shock⇒ice steps.

- Remind students to label their culture tubes and plates so as not to confuse them with other groups' experiments.

- The first part of the transformation lab is designed for one 80-minute period; however, it can be divided up into two 50-minute periods. If you have 50-minute periods, follow these steps.
    - After the heat shock step, the tubes can be stored in the refrigerator overnight.
    - The next day, have students incubate the tubes at 37°C for 15 minutes so that the cells can recover before plating.
    - Spread bacteria on plates and incubate overnight at 37°C.

Discussion of Results and Trouble Shooting

- Encourage students to come up with their own explanations for problems before you offer a solution. Resist offering explanations until they have studied their results and made their own observations.

- Be prepared to explain the existence of *satellite colonies. (*These are different from the actual transformed colonies that the students should count.) These are tiny colonies appearing around a larger, central colony on an LB/amp plate. The large colony is an amp-resistant transformant; the smaller ones are usually not! They are living in an area where ampicillin was inactivated by β-lactamase secreted by the large colony.

- You may have other bacteria, yeast, or mold on your plates. Be prepared to discuss possible causes of contamination.

- Plates that were left in the incubator longer than the recommended incubation period allow for any contaminants to flourish. Even though the plates might be contaminated to begin with, the less than 24 hour incubation allows for the intended cells (the millions of *E.coli* plated!) to grow. Longer incubation times allow for the contaminants to appear.
- Fungal contaminants may have a filamentous appearance. Yeast colonies are often a dull, yellow color. Other bacteria may have grown as well; these could be different colors and shapes, depending on the type.

- If bacteria fail to grow, or if there was growth on plates where there should not have been---there are several variables that could cause these results.
  - Was the heat shock too harsh?
  - Was there ampicillin in all of the plates?
  - Were the bacteria dead on the stock plate?
  - Was there no ampicillin in the plates? Or was the ampicillin not working?
  - Were the plates labeled accurately?

  If the transformed bacteria do not grow on the LB/amp plates.
  - Were the cells made competent? Did you use $CaCl_2$?
  - Was the heat shock performed correctly?
  - Poor quality plasmid?

- Give students some possible experimental results and have students interpret sample data prior to interpreting their own. Have students suggest positive and negative controls. It's essential to emphasize the importance and role of controls to help interpretation of experimental results. Positive controls are samples included that you expect to work, that you expect to give you positive results. Positive controls help you check the effectiveness of your reagents. As much as possible, the treatment of your negative controls should match your experimental sample, ideally differing in only one respect (your variable). Negative controls are samples included which should <u>not</u> work, or show no effect, if everything goes as expected. A negative control helps to put results in context. For example, if bacteria transformed with water only (a negative control) form blue colonies, something is wrong and you need to explore what has happened. Until then you cannot trust your experimental results.

<u>Extension activities</u>

- Calculate the transformation efficiency, if students haven't already, and use it to quantify student-initiated experiments. (See the student lab sheets.) You may need to explain that ideally, transformation efficiencies achieved with the colony protocol used in this lab should be approximately 5×10^3 to 5×10^4 colonies per microgram of plasmid. Have students consider ways to improve efficiencies for commercial use.

- Discuss the challenges of antibiotic-resistant strains of bacteria, which are becoming prevalent; the appropriate use of antibiotics; and the challenges of developing new antibiotics.

- Collect samples from the classroom, homes, or outside and smear samples on LB plates (you could make some LB/amp plates too). Caution: some bacteria, molds, yeasts, and fungi, which you may collect, can be pathogenic. Be particularly careful handling these plates, do not culture for more than 3 days, and dispose of correctly and promptly.

- Calculate the number of bacteria in a colony, given that the generation time is typically 20 minutes and that the colony started from a single bacterium.

- Have students devise a way to mass produce a protein of their choice by searching gene sequence databases, researching possible vectors, and finding methods to design an experiment that their own biotech company could use.