ATTENTION! Put your freezer box (containing ampicillin and plasmid) in the freezer. Ampicillin should be protected from exposure to light.

Overview

There are two different types of transformation labs that SEP supports:

<table>
<thead>
<tr>
<th></th>
<th>Ampicillin resistant</th>
<th>X-Gal required</th>
<th>UV light needed</th>
<th>multiple colors</th>
</tr>
</thead>
<tbody>
<tr>
<td>pBLU</td>
<td>•</td>
<td>•</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pFLO</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
</tbody>
</table>

Safety Concerns and Sterile Technique

- Explain sterile or aseptic technique to your students. It is important when dealing with pathogenic or non-pathogenic microorganisms.
- Do not permit food or drink in the laboratory.
- Wash hands before and after each lab.

Lab Equipment Tip & Notes

These transformation labs require a great deal of preparation before the actual lab activities can begin. Please refer to the kit inventory list and go through the kit and equipment to make certain you have everything that is included in the kit. You will need to make solutions and aliquot other solutions for classroom use. You will need to pour plates days ahead of the lab. Further, some critical items are not supplied in the kit; e.g. microwave oven, ice, and 10% bleach or 70% ethanol (for disinfectant solutions).

Outline

<table>
<thead>
<tr>
<th>2-3 days before lab</th>
<th>Pour plates (2hrs/class)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day before lab</td>
<td>Set incubator to 37°C</td>
</tr>
<tr>
<td></td>
<td>Prep stock plates</td>
</tr>
<tr>
<td></td>
<td>Set out plates to reduce condensation</td>
</tr>
<tr>
<td>Tx Lab day 1</td>
<td>Obtain ice</td>
</tr>
<tr>
<td></td>
<td>Set water bath to 42°C</td>
</tr>
<tr>
<td></td>
<td>Aliquot LB broth, sterile water, plasmids, and CaCl₂</td>
</tr>
<tr>
<td></td>
<td>*Stopping point: after heat shock bacteria can be refrigerated until the next day</td>
</tr>
<tr>
<td>Tx Lab day 2</td>
<td>Remove plates from incubator</td>
</tr>
</tbody>
</table>
Freezer Box Inventory

**pBLU**

<table>
<thead>
<tr>
<th>Tube Label</th>
<th>Tube Contents</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>ampicillin 100 mg/ml</td>
<td>Thaw and mix before adding to agar.</td>
</tr>
<tr>
<td>2% X-gal</td>
<td>2% X-gal in DMF</td>
<td>Thaw and mix before adding to agar.</td>
</tr>
<tr>
<td>pBLU</td>
<td>pBLU® plasmid DNA 0.01 µg/µl</td>
<td>Thaw and mix before aliquoting for student use.</td>
</tr>
</tbody>
</table>

**pFLO**

<table>
<thead>
<tr>
<th>Tube Label</th>
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</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
<td>pFLO</td>
<td>pFLO® plasmid DNA 0.01 µg/µl</td>
<td>Thaw and mix before aliquoting for student use.</td>
</tr>
</tbody>
</table>

Stock Solutions & Ingredients

*Note: You will need to prepare and aliquot these for classroom use. Use sterile technique for all lab preparations!*

<table>
<thead>
<tr>
<th>Stock Solution</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Luria Bertani (LB) Agar</strong></td>
<td>An agar that is high in salt and yeast extract, which provide ideal growth requirements for lab strains of <em>Escherichia coli</em>. Supplied sterilized in bottles. In our kits, our bottles contain approximately 400 ml per bottle. Make calculations of additives or pouring quantities based on the volume in the bottle.</td>
</tr>
<tr>
<td><strong>Ampicillin</strong></td>
<td>An anti-microbial used to select for transformed <em>E. coli</em>. The provided ampicillin stock solution is 100 mg/ml. Use 1 ml ampicillin stock solution per 1 liter of LB agar.&lt;br&gt;• Heat sensitive - add after agar has cooled enough to pour!&lt;br&gt;• Light sensitive - keep agar plates covered with foil.</td>
</tr>
<tr>
<td><strong>Luria Bertani Broth (LB, or Luria Broth)</strong></td>
<td>A liquid broth that is high in salt and yeast extract, which provide ideal growth requirements for lab strains of <em>E. coli</em>. Supplied in a sterilized container.</td>
</tr>
<tr>
<td><strong>CaCl₂</strong></td>
<td>Calcium chloride solution necessary to render <em>E. coli</em> cells competent for transformation experiments. Supplied sterile.</td>
</tr>
<tr>
<td><strong>Plasmid (either pBLU® or pFLO®)</strong></td>
<td>pBLU® A plasmid that contains the LacZ gene and the Amp&lt;sup&gt;r&lt;/sup&gt; gene which confer the ability to hydrolyze lactose (or in this lab, X-gal) and to break down ampicillin, respectively.&lt;br&gt;pFLO® A plasmid that contains one of six different color fluorescent protein genes and the Amp&lt;sup&gt;r&lt;/sup&gt; gene which confer the to break down ampicillin.</td>
</tr>
<tr>
<td><strong>JM101 E. coli</strong></td>
<td>A laboratory strain of <em>Escherichia coli</em> that has been selected, based on its genotype, for several microbial and molecular biology teaching activities. We supply you with one slant of pure culture, which can be used to make 10-15 stock plates</td>
</tr>
</tbody>
</table>
Preparation for the Transformation Lab

Note: It is important to follow sterile or aseptic technique when preparing aliquots and pouring plates for this lab. Disinfect all work areas (table tops) before and after lab preparation. You can purchase a commercial disinfectant solution or make your own: use either a 10% bleach solution or a 70% ethanol solution. Label the solution for future reference.

Pouring Plates

1. Determine how many plates you will need:

\[
Total \# \text{ of LB plates needed} = \# \text{ of classes} \left( \frac{1 \text{ plate}}{1 \text{ group}} \times \frac{8 \text{ groups}}{1 \text{ class}} \right) + \frac{4 \text{ stock plates}}{1 \text{ class}} + \frac{2 \text{ extra plates}}{1 \text{ class}}
\]

\[
Total \# \text{ of LB/amp/Xgal plates needed} = \# \text{ of classes} \left( \frac{1 \text{ plate}}{1 \text{ group}} \times \frac{8 \text{ groups}}{1 \text{ class}} \right) + \frac{2 \text{ extra plates}}{1 \text{ class}}
\]

2. Check how much media you have. Each bottle of agar provided contains ~350 ml LB agar:

\[
Total \text{ ml of media required} = \text{ total \# of plates} \times 20 \text{ ml LB agar}
\]

3. Melt the LB Agar
   a. Loosen the cape
   b. Set microwave to medium-high power for 5 minutes. Pause if it starts to boil over.
   c. DO NOT TOUCH THE BOTTLE UNTIL IT IS FULLY MELTED. Otherwise the solid agar by “volcano” out the top.
   d. Hold agar in a 60°C water bath until ready to pour.

4. Clear off and wipe down a large, flat working surface with disinfectant.

5. Count the number of each type of plate you will need. Label the plates LB or LB/amp or LB/amp/X-gal by running a marker down the side of the plates designating color with added chemical. Note in your teacher notebook the date you poured the plates.

6. Pour LB plates
   a. Set out all the LB labelled plates. Keep the lids on until you are ready to pour.
   b. If you have a Bunsen burner or alcohol burner available, it is best to sterilize the mouth of the agar bottle before pouring.
   c. Holding the plate lid as you pour.
   d. Pour just enough agar to cover the bottom of the plate. Avoid creating bubbles.
   e. Replace the plate’s lid. Do not move until the agar is set.
7. **Pour LB/amp plates (for pFLO)**
   a. Make sure the agar has cooled to 60°C (warm to the touch but not solid). If too hot the ampicillin will degrade.
   b. Use 1 ml ampicillin stock per 1000 ml of LB agar (100 ug/ml). *For SEP bottles, 0.4 ml ampicillin per bottle of 400 ml LB agar.*
   c. Use the same pouring technique as in Step 6.

8. **Pour LB/amp/X-gal plates (for pBLU)**
   a. Make sure the agar has cooled to 60°C (warm to the touch but not solid). If too hot the ampicillin and X-gal will degrade.
   b. Use .8 ml X-gal per 1000 ml of LB agar (16 ug/ml). *For SEP bottles, use 0.32 ml of X-gal (2% stock) per bottle of 400 ml LB agar.*
   c. Use 1 ml ampicillin stock per 1000 ml of LB agar (100 ug/ml). *For SEP bottles, 0.4 ml ampicillin per bottle of 400 ml LB agar.*
   d. Use the same pouring technique as in Step 6.

9. Once the agar has solidified, store the plates upside down to reduce condensation. Cover the LB/amp or LB/amp/X-gal plates with foil to protect them from light.

10. Keep at room temperature overnight to allow excess moisture in the plates to dry.

11. Store at room temperature or in the refrigerator until needed.

### Making Stock Plates

*Make the stock plates one day before the lab to get reasonable sized colonies which are still in log-phase (i.e. rapid) growth. Make 3-4 stock plates per class.*

1. You will need 3-4 LB plates, sterile loops, and 1 *E. coli* (JM101) slant from SEP.
2. Set incubator at 37°C.
3. Using a sterile loop, gather a small amount of bacteria from the provide *E. coli* slant.
4. Steak the LB plate following the diagram. Make 4 zig-zag streaks on the plate using a new loop or toothpick each time. Drag the new loop or toothpick across the last streak to spread the bacteria. *This should create individual colonies by decreasing the density with each streak.*
5. Place upside down and Incubate overnight at 37°C.
6. The next morning you should have individual colonies in area 3 and 4.

### Clean Up

- **Soak used plates in a 10% bleach bath for at least 30 minutes before disposing in the garbage. Or, autoclave in a biohazard bag for 15 minutes at 121°C. Then, dispose in garage.** *Notify your custodian that the bacteria has been killed and is not longer biohazardous.*
- **If you would like to keep any plates to show, seal the plates with Parafilm and store upside down in refrigerator.**
- **Do not keep cultures growing in the 37°C incubator.** This will result in potentially pathogenic microbes to grow.
E. coli Background Information

The strain of E. coli we’re using is called JM101, after Joachim Messing and his colleagues; the people who originally developed it. This is the genotype of JM101 (expressed in shorthand):

\[ F' \text{ traD36 proA}^+ \text{ proB}^+ \text{ lacI}^q \text{D(lacZ)M15/ D(lac-proAB) glnV thi} \]

The key gene of interest for the pBLU transformation is \( \Delta(\text{lacZ}) \). The triangle, or delta, before \( \text{lacZ} \) means that the bacteria’s original \( B-\text{galactosidase} \) gene is gone. When we add a \( B-\text{galactosidase} \) gene through transformation with pBLU, we restore the bacteria’s original genotype and make them \( \text{lac}^+ \).

Important information about E. coli, pathogenicity, and antibiotic resistant strains of bacteria:

It is recommended that you give your students a little background about the commensal bacterium E. coli that resides in the human gastrointestinal tract. One of many natural and beneficial flora in humans, this commensal strain is harmless. There are pathogenic strains of E. coli, which are harmful to humans, and can cause severe illness, e.g. O157:H7, which is a strain that lives in bovine intestines. However, the E. coli cells used in this experiment are a laboratory variety, derived from the wild type strain K-12. Several lab strains from K-12 have been passed in vitro since the mid-1940s. They are not considered pathogenic. In fact, several experiments have been conducted to test whether these K-12 derived strains could colonize the human gut. So far this has not occurred, which is why K-12 derived strains (e.g. JM101, MM294, and HB101) are considered safe for laboratory use. One hypothesis for this non-pathogenicity phenomenon is that because these cells have lived in laboratory culture for so long, they have lost some of their original, wild type traits. In particular, these strains have lost the “O” antigen domain of the lipopolysaccharide that composes the outer membrane. The “O” antigen is thought to be necessary for infection in mammals.

Safe handling of any bacteria, regardless of pathogenicity, is a prerequisite for working in a shared laboratory environment. Even harmless bacteria can enter the body through cuts in the skin and later cause infections. Follow sterile technique and keep your students, and you, safe!

The results of a successful transformation experiment are cultures of antibiotic resistant strains of E. coli. After counting colonies and collecting the data necessary to complete the lab, these cultures should be destroyed and disposed of properly. These new strains of bacteria should not be released into the environment; after all, your students have genetically engineered new E. coli cells! However, it is important to note that these cultures probably would not survive in the wild, nor would they be able to pass on their antibiotic resistance to other bacteria. In this lab experiment, it was necessary to treat the cells chemically (\( \text{CaCl}_2 \)) and physically (heat shock) to allow the plasmid to be taken up by the cells. And even then, few cells actually take up the plasmid.

In the natural world of E. coli, plasmid DNA is passed from cell to cell via a pilus, which acts as a bridge between two cells. One cell acts as the donor, the other cell acts as the recipient. The plasmid DNA must also be able to “move” from one cell, through the pilus, to the other cell. The plasmid itself has the genes which, when expressed, allow it to be moved into the recipient cell. The plasmids, which are commonly used as “teaching plasmids”, (e.g. pFLO, pBLU, pAMP, and pKAN) have been genetically altered so they do not express the mobility protein required for transport.
Acknowledgement and References

We are indebted to Sciencebridge, University of California, San Diego for supplying SEP with the 6 fluorescent plasmids (pFLO), Suzanne Black (Inglemoor High School, Bothell WA), Carter Hoffman, Mark Hertle, SEP participants, and many others for field testing, comments, illustrations, and advice. References that contributed ideas and methods to this protocol include the Gene Connection v1.5 (1995), DNA Science (1990), Laboratory DNA Science, (1996), Working with Bacteria and DNA in Precollege Science Classrooms (1993), and the EduGen Amylase Kit. Please see the reference list for specific citations.

Working with Bacteria and DNA in Precollege Science Classrooms, 1989, by Toby Horn, is a comprehensive safety and appropriate use guide. (Out of print.)


