Bacterial Transformation, pH, and Making Solutions
2007-08 INS Winter Quarter – Lab 4

Goals: This lab will introduce you to techniques used applicable to research in molecular biology. You will learn how to transfer plasmid DNA into E. coli and subsequently select for bacteria with your plasmid of interest. You will also learn how to use a pH meter and make solutions for future plasmid mini-preparations.

PART I: Bacterial Transformation

Bacteria are powerful tools in conducting molecular biology experiments. *Escherichia coli* has become the workhorse for a wide range of laboratory techniques. A single cell can amplify and maintain a unique foreign DNA, and cells can be quickly grown to very high density in liquid phase, (~10^9 bacteria/ml in less that 24 hours.)

The outside DNA we are using as a transforming factor is a plasmid. Plasmids are circular double strand DNAs, usually of rather small size (~2-10 Kbp) incorporating a few genes. A plasmid needs an origin of replication, which is a sequence of bases recognized as a correct starting point by the cell's replication enzymes. Depending on the origin of replication, the plasmid may have from 1-2 to 500 copies per bacterial cell. Many plasmids carry one or more genes for resistance factors; this is a common route by which antibiotic resistance is transferred.

Because the transformation event may have very low probability, we want to have a mechanism for finding these rare events. The host bacteria that we will use today, *E.coli* DH5α, are susceptible to the antibiotic ampicillin. This antibiotic is in the penicillin family and selectively blocks the enzymes involved in cell wall biosynthesis and modification. The plasmid we will use contains the amp^r gene. The amp^r gene encodes the enzyme beta-lactamase, which inactivates ampicillin by hydrolyzing a specific bond in the antibiotic. By selecting the resulting mixture of the transformation by growth on an effective concentration of ampicillin, only cells that have gained the plasmid will be able to grow. This general strategy of a planned search that will reveal a desired genetic event is referred to as a screen.

Most recipes for plasmid transformation have developed by trial and error, and are very specific for different species of bacteria. Cells that have been treated to be more suited for transformation are referred to as competent cells. For *E.coli*, some common features in procedures to make competent cells are incubating in cold calcium chloride solutions, and a heat shock step in which the bacteria and the DNA are briefly (1-2 minutes) exposed to higher than normal temperature (perhaps up to 44 ° C.) Cells collected fairly early in the rapid growth phase usually seem to work better than older cells that are not actively growing. The quality of competent cells is judged by the number of transformed bacteria (determined by counting colonies of the correct phenotype) per µg of DNA. If you have little DNA or need to sample many colonies looking for a rare event, you want cells with a high transformation efficiency. It is possible to reach efficiencies of 10^6 to 10^{10} transformed colonies for 1 µg of DNA.

Methods:

1. Place 250 µl of ice-cold 50 mM calcium chloride solution into a sterile microfuge tube. Keep all materials on ice unless directed otherwise.
2. Using a sterile loop transfer a loop full of cells from several distinct colonies into your cold calcium chloride solution and using your loop gently suspend the cells.
3. Split this cell suspension into 2 – 100 µl portions in separate tubes.
4. Add 250 ng DNA (10 µl) to one sample and 10 µl sterile water to the other. Both additions should be ice-cold.
5. Incubate on ice for 15 minutes.
6. Transfer the tubes to –80 °C and incubate for 20 minutes. We will do this step by collecting all tubes and moving them to the –80 °C in one group.
7. Thaw and heat shock by placing the tubes in a 37 °C bath for 80 seconds.
8. Add 900 µl ice-cold TSB/ 0.2% glucose. (TSB= Tryptic Soy Broth) to each tube.
9. Incubate 45 minutes at 37 °C. During this incubation step you can prepare your dilution tubes and label your Petri dishes for the remaining steps. Also, you can move on to PART II.

10. Prepare the following dilutions of your two mixes: (Each bullet on the list represents a Petri dish that you will inoculate.)

For the DNA containing mix:

- You will plate 100 μl of the original transformation mix on an +amp plate.
- Prepare a 1:10 dilution (100 μl in 900 μl TSB broth) and plate 100 μl on a +amp agar plate.
- Prepare a $1:10^{4}$ dilution (10 μl transformation mix + 990 μl TSB, mix, then using a clean tip and tube take 10 μl of this first dilution +990 μl TSB to produce the final dilution. Plate 100 μl of this mixture on a NO amp agar plate.

For the transformation mix with no DNA you will make 2 platings.

- 100 μl will be placed without dilution on a + amp agar plate.
- Repeat the $1:10^{4}$ dilution done above with this sample, and plate 100 μl of this solution on a NO amp agar plate.

You will need a total of 5 Petri dishes: 3 TSB agar plates with ampicillin (50 μg/ml) and 2 TSB agar plates with no antibiotic.

11. Plate cells by adding 100 μl to a plate and using the hockey stick method to spread them. After spreading allow the bacteria to soak into the plate for 10 minutes.
12. Invert the plates and place them in the incubator. They will be removed tomorrow.
13. During open time next week you will count the number of colonies on each plate.
14. From the plate counts estimate the number of bacteria that were in your original transformation mix. Give the total number of transformants and the transformation efficiency in cfu (colony forming units) per μg DNA. What fraction of the total number of bacteria were transformed in this experiment?

PART II. Using a pH meter

The pH values are measured using an electronic pH meter. These meters contain at least two electrodes; the unit placed in solution contains both of these electrodes. One is a reference electrode. The second electrode is sensitive to hydrogen ion concentration. The most commonly used form of pH sensitive electrode is a glass electrode. This electrode allows the slow diffusion of cations through a slightly permeable glass surface. This slight permeability sets up an electrical potential that is dependent on hydrogen ion concentration. The meter then measures the electrical potential difference between the two electrodes and converts that into a pH value. It is difficult to measure absolute pH values more accurately than to within 0.1-0.3 pH units. However, these instruments can measure relative differences of pH between two solutions quite accurately-to within 0.001 pH unit; our instruments allow measurement to 0.01 pH unit. The relationship between voltage and pH, and the pH of the reference buffer both depend on temperature. There is a temperature compensation feature built into the meter. Before use, these instruments are calibrated using pH reference buffers of known pH. The lab staff will demonstrate this procedure during the lab. When you are ready to learn how to use the pH meter, we will come around to help your team.

PART III. Making Solutions

One of the most important aspects of research in molecular biology involves the making of solutions. It is very important that a solution is made correctly, having the right pH, concentration, and sterility. Today your team will follow a protocol to make one of three solutions used for isolating plasmid DNA from bacteria. The scanned version of the protocol is posted. We will designate each group of two students to make one of the three solutions.

Sometimes it may be more efficient to use molarity when calculating concentrations. A mole is defined as one gram molecular weight of an element or compound, and comprised of exactly 6.023 x 10^23 atoms or molecules (this is called Avagadro's number). The mass attributed to one mole of any element or compound is called its atomic weight (elements) or molecular weight, or formula weight for compounds. The number of moles of a given dry reagent can be calculated as: # of moles = weight (g)/ molecular weight (g)

Molarity is the unit used to describe the number of moles of a chemical or compounds in one liter (L) of solution. By this definition, a 1.0 Molar (1.0 M) solution is equivalent to one *formula weight* (FW = g/mole) of chemical dissolved in 1 liter (1.0 L) of solvent (usually water). Formula (or molecular) weight is always given on the label of a chemical bottle.

See the next page for calculation examples.

Molarity Calculation Examples:

Example 1: To prepare a liter of a simple molar solution from a dry reagent

Multiply the *formula weight* (or MW) by the desired molarity to determine how many grams of reagent to use:

Chemical FW = 194.3 g/mole; to make 0.15 M solution use

194.3 g/mole * 0.15 moles/L = 29.145 g/L

Example 2: To prepare a specific volume of a specific molar solution from a dry reagent

A chemical has a FW of 180 g/mole and you need 25 ml (0.025 L) of 0.15 M (M = moles/L) solution. How many grams of the chemical must be dissolved in 25 ml water to make this solution?

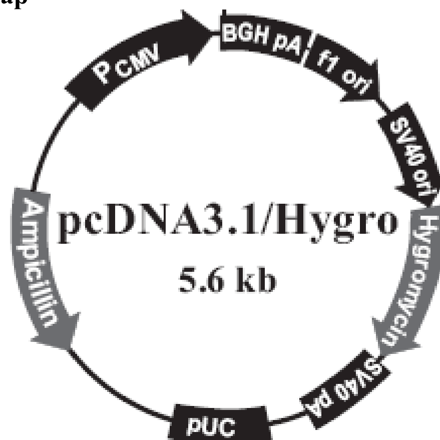
#grams/desired volume (L) = desired molarity (mole/L) * FW (g/mole)

by algrebraic rearrangement,
#grams = desired volume (L) * desired molarity (mole/L) * FW (g/mole)

#grams = 0.025 L * 0.15 mole/L * 180 g/mole

after cancelling the units,
#grams = 0.675 g

Appendix: pcDNA3.1 Hygro Plasmid Map



pGREEN is sold by Invitrogen