Biochemistry Lab

Bacterial Transformation Protocol

The uptake of exogenous DNA by cells that alters the phenotype or genetic trait of a cell is called transformation. For cells to uptake exogenous DNA they must first be made permeable so the DNA can enter the cells. This state is referred to as competency. In nature, some bacteria become competent due to environmental stresses. We can purposely cause cells to become competent by treatment with chloride salts of metal cations such as calcium, rubidium or magnesium and cold treatment. These changes affect the structure and permeability of the cell wall and membrane so that DNA can pass through. However, this renders the cells very fragile and they must be treated carefully while in this state. Competent cells vary in how well they take up DNA. We can express this The amount of cells transformed per 1 µg of DNA is called the **transformation efficiency**. Too little DNA can result in low transformation efficiencies, but too much DNA also inhibits the transformation process. Transformation efficiencies generally range from 1 x 10^4 to 1 x 10^7 transformed cells per µg of added DNA.

E. coli bacteria are normally poisoned by the antibiotic **ampicillin**. Ampicillin inhibits synthesis of the bacterial cell wall (in bacteria like *E. coli* , found between the inner and outer cell membranes), resulting in bacteria that are very structurally weak. In the hypotonic media in which these cells grow, cells exposed to ampicillin will swell and burst or not grow at all. For cells to survive, they must include a means to break down the ampicillin. If the colonies on the LB plates are large they will break down enough ampicillin so that bacteria without the plasmid will survive and form satellite colonies in the surrounding region of inactivated antibiotic. Another commonly used antibiotic is **kanamycin**. This drug blocks protein synthesis by binding irreversibly to the ribosome and preventing translation.

Most transformation protocols can be divided into these four major steps:

1. *Preincubation*: Cells are suspended in a solution of cations (such as Ca^{++}) and incubated at 0° C. The cations are thought to complex with the negatively-charged phosphates in membrane lipids in the bacterium. The low temperature congeals the cell membrane, stabilizing the distribution of charged phosphates and allowing them to be more effectively shielded by the cations.
2. *Incubation*: DNA is added, and the cell suspension is incubated further at 0° C. The cations again are thought to neutralize charged phosphates – this time in the DNA backbone – allowing DNA to adhere or at least be close to the *E. coli* cell membrane.
3. *Heat shock*: The cell-DNA mix is rapidly heated to 37°– 42° C for a short period and then returned to 0° C. The rapid temperature change creates a thermal imbalance on either side of the cell membrane, creating pores and a draft that sweeps the plasmid into a small percentage of cells.
4. *Recovery/Outgrowth*: Nutrient broth is added to the transformation mix which is then incubated at 37° C (ideally with shaking) before plating on selective media. Transformed cells recover from the treatment, amplify the transformed plasmid, and begin to express the plasmid's antibiotic resistance gene before encountering the antibiotic.

Antibiotic Selection and Outgrowth

Ampicillin (**Amp**) is a practical, inexpensive antibiotic for routine selection of transformed cells, although some prefer a sturdier version, **carbenicillin**. Amp is necessary, a recovery step can be omitted, although it is better to include one. When using other antibiotics, such as **Kanamycin** (**Kan**), a recovery step is required before plating transformed cells.

Satellite Colonies

If plates are incubated too long following a transformation (particularly when using an antibiotic like Amp), small 'satellite' colonies will appear that surround larger transformed colonies. In time, these can grow as large as the original transformed colonies, and can arise more quickly on plates with many transformants. These are non-transformed, nonresistant cells that grow in the 'antibiotic shadow' where Amp has been broken down by the large resistant transformed colonies (the Amp resistance protein is secreted by the cells). Initially, these colonies will be tiny, but will grow with time. Do not include tiny colonies (particularly surrounding a large colony) in your count of transformants. It is best to score transformation plates in the morning following transformation before satellite colonies appear or become large

DO NOT LET CELLS WARM UP! This will allow the holes in the cells to seal and your experiment will not work!

General Workflow / Protocol for traditional competent cells

- 20 - 100 µl of competent cells (ICE ICE ICE) into prechilled, labeled tubes.
- QUICKLY, mix 1-5 µl of plasmid DNA (1ng to 100 ng) and mix by tapping of "flicking" the tube with your finger.
- Incubate the mixture on ice for 20-30 min.
- Heat shock at 42° C for 45 sec, immediately place back in ice bucket and incubate for 2 min
- Add 250 µl of SOC and incubate at 37° C with shaking for 45 min. This is called the outgrowth step.
- Plate cells onto appropriate antibiotic containing LB Agar plates. Because it is difficult to predict the level of transfection (cell health, plasmid quality and quantity, overall technical handling and size of plasmid) it is best to spread two plates with a low volume (50 µl) and a high volume (200 µl) of outgrown cell culture.
- Spread with hockey stick, beads or inoculating loops. Ensure the entire culture is *evenly spread* over plate. You *are NOT trying to isolate streak in this procedure*. Give plates a few min for liquid to seep into agar.
- Incubate plates at 37° C overnight with the agar upside down.
- After ~12-16 hours, remove plates from incubator, seal with parafilm and store at 4°C upside-down.

Plasmid Preparation

- If the plasmid concentration is known, dilute plasmid with water (mol bio grade) to 0.05 mg/ml (aka 0.05 µg/µl).
- Use 1 µl of diluted DNA for each transformant. Calculate and record the mass (ng) of DNA used for the transformation.
- If the plasmid concentration is unknown, use 1 µl of DNA.
- Record the antibiotic resistance and other information on your plasmid in your notebook.

Tranformation

- Record the type of competent cells in the notebook - what cell strain, level of competent cells, if commercial name of company and information on the preparation of cell strain.
- For this class, you will conduct two transformants - one transformant plated for the appropriate antibody (two volumes - you decide how much for each) and the other will be divided equally onto an LB Agar plate without antibioitic and another LB plate with an incorrect antibiotic.

Notes:

SOB is Super Optimal Broth - a rich broth supporting cell growth that contains glucose. SOC is an SOB broth with glucose. Full name is Super Optimal broth with Catabolite repression. In this case, glucose serves to ensure the cells use glucose for metabolism leaving other energy sources (amino acids and lipids) for protein expression. Use of SOC will ensure the antibiotic selective protein can be expressed in transformed cells. Without SOC, much of the cell resources needed for protein expression will be used for metabolism instead

No transformants? Check the antibiotic resistance. Double check the DNA concentration and quality. Degraded or non-supercoiled plasmid will not transform as efficiently. If the amount of DNA used was high (0.1 to1 µg or higher), the transformation can be less efficient than if using a lower amount of cells. Also if new or not sure what is happening, use a positive control - a plasmid that works well, often supplied with commercial preparations.

Using Competent Cells. Newly thawed competent cells often come in larger than “single shot” volumes. To aliquot, pre-chill small tubes on ice and immediately after thawing the cells ON ICE, transfer 50 µl into the cells and immediately re-freeze. ICE ICE ICE – keep on ice and thaw only when ready

Mix and Go Cells – Zymo Research produces as competent cell product called “Mix & Go”. They report a higher transformation capacity and easier to use result. These cells do not need the ice incubation or heat shock. Unless using Kan, there is no need for an outgrowth step either. Using a traditional protocol shown above will still work with these cells.

- Add 1-5 µl plasmid DNA to thawed tube (50-100 µl) of Mix & Go cells on ice. Keep the volume of DNA less than 5% of the total volume.
- Spread the entire mixture onto a pre-warmed (37°C) culture plate and incubate as usual.