Biochemistry Lab

Competent Cell Transformation

General Protocol for Z Comp cells – any strain

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 purposefully cause cells to be 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. 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 form 1 x 10^4 to 1 x 10^7 cells per µg of added DNA.

E. coli bacteria are normally poisoned by the antibiotic ampicillin. Ampicillin acts to damage the membranes of *E. coli* by inhibiting the crosslinking of the bacterial membrane. This results in bacteria which are very structurally weak. In the hypotonic media in which these cells grow, the 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. The plasmid has an additional gene that codes for a protein, beta-lactamase, that is excreted by the cells and will hydrolyze the ampicillin in a localized area. Therefore by adding ampicillin, only bacteria that contain the plasmid will survive. We also need to be sure not to allow our transformed *E. coli* to become overgrown. If the colonies on the LB plates are large they will break down enough ampicillin so that surrounding bacteria (satellite colonies) will form that may not have the plasmid insert due to the lack of remaining antibiotic. Another commonly used antibiotic is kanamycin. This drug works by blocking protein synthesis at the mRNA level.

An Important Point When Transforming Cells – Depending on which strain of bacteria you are using (for simplicity we either use a strain to maintain DNA or a strain better suited for protein expression) and how they were made competent, there may be slight but important differences in using the cells for transformation. Therefore it is important to first check that you are using the proper protocol. DO NOT LET CELLS WARM UP! This will allow the holes in the cells to seal and your experiment will not work!

Procedure:

- Pre-warm agar bacteria plate with appropriate antibiotic at 37°C. Label the plate around the edge with your group name, the date, strain, and mutation as before.
- Thaw ON ICE, 100 µl Z-Comp cells
- Add 1-5 µl of plasmid DNA to a tube of thawed Z-Comp cells (still on ice), mix gently by tapping the side of the tube with your finger. Keep the cells on ice AS MUCH AS POSSIBLE. Keep the added volume of DNA less than 5% of the total volume.
- Incubate ON ICE for 5 min prior to plating.
- If using **Amp**, continue directly to the next step.
- If using **Kan** or other antibiotic, and outgrowth step is required:
    - add 400 µl of sterile SOC or ZymoBroth (for better performance)
    - incubate with shaking at 200-300 rpm for 1 hour
- Spread 50-100 µl of mixture onto the pre-warmed (37°C) culture plate containing appropriate antibiotic. Incubate upside-down at 37°C overnight.
- After 16-24 hours, wrap edge of plate with parafilm and store at 4°C.