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Bacterial Transformation
Protocol

Background Information – READ FIRST!

Transformation. 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 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 1×10^4 to 1×10^7 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, ß lactamase, that is excreted by the cells and in a local area will hydrolyze the ampicillin. 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 antiobiotic 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 us a strain to maintain DNA or a strain(s) 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!

Materials (see bacterial protocol for making media information)

42°C Water Bath	Incubator with Shaker	LB Plates with Antibiotic	Cell Spreader
Ice	Sterile SOC	Competent Cells	

Rapid Trans Protocol – 50 µl cells/tube (Active Motif)

- Add 1-5 µl DNA (1 – 50 ng of DNA) Mix by tapping and immediately place in the ice. Do not vortex.
- Incubate on ice for 30 min.
- Heat shock at 42°C for *exactly* 30 sec.
- Add 250 µl SOC
- Incubate in shaker (37oC) for 1 hr.
- Spread 50 – 200 µl on a prewarmed LB plate with antibiotic
- Allow plate to dry and incubate inverted at 37°C overnight.

Other Important Notes:

- If you are careful when thawing out cells they can be frozen one more time and used again, but no more than once
- Ice Ice Baby – keep the cells cold and thaw quickly in your hand. Keep the cells/tubes ***IN*** the ice not ***ON*** the ice.
- If possible make two plates – one with a small amount of transferred cells and another with a larger volume. That way you will ensure that you don't end up with a lawn.

Z-Comp Transformation Protocol

- Add 1-5µg DNA and gently mix
- Incubate on ice for 15-60min.
- Spread 50-100µl of cells on an appropriate plate warmed at 37° C
- Incubate the plate at 37° C

General Protocol for "homemade" cells.

- Add 1-10µg DNA to competent cells to 50–100 µl cells
- incubate on ice for 20'
- heat shock @ 42°C for exactly 2 min.
- incubate on ice for 5 min.
- Spread 50 – 200 µl on a prewarmed LB plate with antibiotic
- Allow plate to dry and incubate inverted at 37°C overnight.