Transformation of *E. coli* Homemade Competent Cells

NB. This protocol differs from the protocol used with commercially made competent cells.

1. Defrost one 100 µl (or 200 µl if available) aliquot of *E. coli* competent cells per transformation, plus one extra as a control (use whole tube and do not re-freeze)
2. Add 1-5 µl of plasmid DNA (depending on concentration) to competent cells, mix by rolling eppendorf.
3. Incubate on ice for 40 minutes.
4. Heat shock in eppendorf at 42 °C for 2 minutes.
5. Incubate on ice for 5 minutes.
6. Add 0.7 ml of pre-warmed LB medium, incubate at 37 °C, 200-220 rpm for 60 minutes.
7. Spin down cells at 8 000 rpm for 5 minutes.
8. Remove 0.5 ml of the supernatant.
9. Re-suspend cells in remaining supernatant.
10. Plate out 200 µl on agar laced with 100 µg/ml ampicillin, 50 µg/ml kanamycin, 35 µg/ml chloramphenicol or 15 µg/ml tetracycline as appropriate.
11. Incubate at 37 °C overnight.

In the 1970's it was observed that *E. coli* cells that had been soaked in an ice-cold salt solution were more efficient in the uptake of DNA[1]. The cells are therefore incubated on ice for 40 minutes. During this time the DNA only binds to the cell walls. The actual movement of the DNA into the cells is initiated by briefly raising the temperature to 42 °C.

Agar is a gelling agent, an extract of certain species of red seaweed. It is present to provide a solid support for the bacteria to grow on. Ampicillin is an antibiotic and is present to provide a genetic marker for screening. Generally the vectors used (eg. pET-21) have an antibiotic resistance gene, Amp^R. This gene codes for a β-lactamase enzyme that modifies ampicillin to a form that is non-toxic to the host cell. Cells that have not been transformed will not show antibiotic resistance and therefore will not grow on the ampicillin laced plates. Addition of the LB medium and incubation time before plating, allows plasmid replication and enzyme expression to begin. This means when the cells are plated and encounter the antibiotic they will have already synthesised enough enzymes to be able to survive.

Luria Bertani media (LB)

Tryptone	10 g/l
Yeast extract	5 g/l
NaCl	10 g/l
Water	

LB is a non-specific growth medium for bacteria and contains a complex extract of biological material. Tryptone is the peptide formed by pancreatic digestion, so called because it is formed through the action of the enzyme trypsin, a serine protease. It is an organic source of nitrogen. Should be autoclaved in flasks that are ¼ full. This allows air to circulate during propagation, ie 5 ml volumes in 50 ml Falcon tubes, 40-50 ml volumes in 250 ml flasks and 250 ml volumes in 1 litre flasks.

LB-Agar plates

LB media	200 ml
Agar	3 g per 200 ml

Agar is added to the LB media before autoclaving. To pour plates the LB-agar must be heated in the microwave until liquid. Do not boil. Antibiotic is then added to the required concentration; generally antibiotic stock concentrations are 1 000 x concentrated. 200 ml of L-agar should pour into 7-8 petri dishs to a depth of ~½ cm.

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[1] Brown, T. A. *Gene Cloning: an introduction* Van Nostrand Reinhold, **Wokingham**, 1987.