TRANSFORMATION COMPETENTS CELL

MATERIALS

- Lb medium
- Competent cells
- Equipment
- Thermomixer
- Iced rack
- Shaker incubator

PROTOCOL

1.- Include all of the appropriate positive and negative controls.

2.- Add the transforming DNA (up to 25 ng per 50 ml of competent cells) in a volume not exceeding 5% of that of the competent cells. Swirl the tubes gently several times to mix their contents. Set up at least two control tubes for each transformation experiment, including a tube of competent bacteria that receives a known amount of a standard preparation of superhelical plasmid DNA and a tube of cells that receives no plasmid DNA at all. Store the tubes on ice for 30 min.

3.- Transfer the tubes to a rack placed in a preheated 42 °C circulating water bath. Store the tubes in the rack for exactly 90 sec. Do not shake the tubes. Heat shock is a crucial step. It is very important that the cells be raised to exactly the right temperature at the correct rate. The incubation times and temperatures given here have been worked out using Falcon 2059 tubes. Other types of tubes will not necessarily yield equivalent results.

4.- Rapidly transfer the tubes to an ice bath. Allow the cells to cool for 1-2 min.

5.- Add 800 ml of SOC medium to each tube. Warm the cultures to 37 °C in a water bath, and then transfer the tubes to a shaking incubator set at 37 °C. Incubate the cultures for 45 min to allow the bacteria to recover and to express the antibiotic resistance marker encoded by the plasmid. To maximize the efficiency of transformation, gently agitate (<225 cycles/minute) the cells during the recovery period.

6.- Transfer the appropriate volume (up to 200 ml per 90 mm plate) of transformed competent cells onto agar SOB medium containing 20 mM $MgSO_4$ and the appropriate antibiotic. When selecting for resistance to tetracycline, the entire transformation mixture may be spread on a single plate (or plated in top agar). In

this case, collect the bacteria by centrifuging for 20 sec at room temperature (RT) in a microfuge, and then gently resuspend the cell pellet in 100 ml of SOC medium by tapping the sides of the tube.

7.- Store the plates at RT until the liquid has been absorbed.

Invert the plates and incubate them at 37 °C. Transformed colonies should appear in 12-16 h.

References:

Hanahan, D. (1983). Studies on transformation of Escherichia coli with plasmids. *J Mol Biol* 166(4): 557-580.

Hanahan, D. (1985). Techniques for transformation of *E. coli*. *In DNA cloning: A Practical Approach* (ed. D.M. Glover), vol. 1 pp. 109-135. IRL Press, Oxford, United Kingdom.