TRANSFORMING COMPETENT CELLS

BEFORE STARTING:

- *Make sure the DNA is prepared before taking the competent cells from the -80°C freezer*
- *Preheat the heating block/water bath to 42.°C*
- *Take the SOC medium out of the freezer*
- *Make sure that the work bench is sterilized with ethanol*
- *Use a flame to create a sterile environment*

MATERIALS:

- Competent cells
- Plasmid DNA
- 300ul SOC medium
- Heating block/water bath
- Incubator
- LB agar plates with appropriate antibiotic

PROCEDURE:

□ Make sure the DNA is prepared before taking out the competent cells from the -80°C freezer

□ Place the competent cell on ice for about 5 minutes

□ Flick the tube to resuspend the cells

□ Add 10-20 ng of DNA to the cells

□ Pipette gently up and down

□ Put the cells back on ice for 20 minutes

□ Heat shock the bacteria at 42°C for 30-45 seconds

□ Place on ice for 10 minutes

□ Add 300 µl of SOC medium

□ Put into the incubator for 1h at 37°C and 200-250rpm

□ Pre-heat the agar plates for 30 in the incubator by placing them upside down and slightly open so that water can evaporate

□ Spread the sample evenly on the agarplates, let them stand for 5 minutes, then put them back into the incubator UPSIDE DOWN over night

ANTIBIOTICS

Commonly Used Antibiotics	Recommended Concentration
Ampicillin	100 µg/mL
Bleocin	5 µg/mL
Carbenicillin	100 µg/mL
Chloramphenicol	25 µg/mL
Coumermycin	25 µg/mL
Gentamycin	10 µg/mL
Kanamycin	50 µg/mL
Spectinomycin	50 µg/mL
Tetracycline	10 µg/mL