Transformation of Plasmid cDNA into E. Coli

Take an aliquot of E. coli cells out of the –80 freezer and allow them to thaw on ice. Do not use your hands to thaw! And, do not return any cells back to -80 since after thawing they lose their efficiency.

Pipette 50 µl of cells into vial. Avoid pipetting up and down as this may shear cells.

Swirl 5ul of ligated cDNA into the cells. If you are transforming a purified plasmid vector alone, usually 0.5 µl or ~5 µl of a 1/50 dilution should be enough.

Let the stand on ice for 30 min.

Heat shock at 42 degrees for 45 sec.

Immediately place on ice and let stand for 2 min.

Add 500 µl LB and let stand at 37 degrees for 1 hr. This will allow the resistance gene to be expressed before plating.

Spin the cells down and remove 400 µl of the supernatant.

Re-suspend the cells in the remaining 100 µl by pipetting up and down and plate onto 100 X 20 mm LBamp (if ampicillin is the selection antibiotic) plate. Use 100 EtOH % and plastic cell spreader (or use plating beads).

Place upside down in a 37 degrees incubator and leave overnight.
**LB amp Plates**

Autoclave LB amp agar.

Melt on ~ half power in microwave and pour 50 ml into falcon. Let the agar cool to below 50 degrees before you add ampicillin. Add 50 µl of 100 mg/ml into the 50 ml LB agar. 50 ml should give you 3 plates. Let the plate solidify in the hood and then turn them over (may need to have them open to help dry).