DH5α Subcloning Efficiency Competent Cell Transformation  
(Standard Super-coiled Plasmid)

- Turn on hot block (37°C) and fill a few wells with water
- Turn on 37°C shaker to let it warm up
- Rapidly thaw 10 μL of DH5α Subcloning Efficiency cells by holding tube in your hand. Once thawed, immediately put tube on ice
- Add 0.5 ng of plasmid DNA to the thawed cells\(^1,2\)
- Tap the tube gently several times to mix
- Incubate the cells on ice for 30 minutes
- Heat shock the cells for 30 seconds at 37°C in hot block
- Incubate the cells on ice for another 2 minutes
- Add 1 mL of LB
- Shake the cells horizontally for 1 hour at 37°C
- Plate 200 μL of the cell dilution on an LB plate with the appropriate antibiotic
- Incubate the plate overnight (agar side up) at 37°C

**NOTES:**

1= The volume of the DNA added should not exceed 1 μL (10% the volume of the cells).

2= The cells are very fragile, do not pipette the solution repeatedly!