**Competent cells formation :**

1. Pick a single bacterial colony from a plate that has been incubated for 16-20 hours at 37°C. Transfer the colony into 100 ml (LB-media ) in a 1-liter flask. Incubate the culture for 3 hours at 37°C with moderate shaking
2. Transfer the bacterial cells to sterile, disposable, ice-cold 50-ml polypropylene tubes. Cool the cultures to 0°C by storing the tubes on ice for 10 minutes.
3. Recover the cells by centrifugation at 2700*g* (4100 rpm) for 7 minutes at 4°C.
4. Decant the medium from the cell pellets. Stand the tubes in an inverted position on a pad of paper towels for 1 minute to allow the last traces of media to drain away
5. Resuspend each pellet by swirling or gentle vortexing in 10 ml of ice-cold .1MCaCl2 solution.
6. Recover the cells by centrifugation at 2700*g* (4100 rpm ) for 5 minutes at 4°C.
7. Decant the medium from the cell pellets. Stand the tubes in an inverted position on a pad of paper towels for 1 minute to allow the last traces of media to drain away.
8. Resuspend the pellet by swirling or gentle vortexing in 2 ml of ice-cold 0.1 M CaCl2, keep resuspend cells on ice for 5 min.
9. Recover the cells by centrifugation at 2700*g* (4100 rpm ) for 5 minutes at 4°C.
10. Resuspend each pellet by swirling or gentle vortexing in 2 ml of ice-cold .1MCaCl2 solution.
11. Freeze immediately at -80 °C

Restriction Digest for insert DNA and plasmid :

1. Combine the following in Eppendorf tube

*1μg DNA (25 ng)
1 μL of Restriction Enzyme
3μL 10x Buffer
25 μL dH2O (to bring total volume to 30 μL)*

1. Mix gently by pipetting.
2. Incubate tube at appropriate temperature (usually 37°C) for 1 hour.

Insert + Vector DNA Ligation

1.Combine the following in Eppendorf tube

25ng Vector DNA ( 4 μL )
 75ng Insert DNA ( 3 μL )
 10X Ligase Buffer (1μL/10μL reaction for 10X buffer )

 0.5 μL T4 DNA Ligase
 H20 to a total of 10μL ( 1.5 μL )
2. Incubate at room temperature for 2hr .

**Transformation**

1.Take competent cells out of -80°C and thaw on ice (approximately 20-30min). (Thaw the competent cells in your hand instead of on ice)

2. Take agar plates (containing the appropriate [antibiotic](http://www.addgene.org/mol_bio_reference/antibiotics/)) out of 4°C to warm up to room temperature or place in 37°C incubator

3. Mix 5μl of DNA (usually 10μg to 100μg) into 50μL of competent cells in a microcentrifuge or falcon tube. GENTLY mix by flicking the bottom of the tube with your finger a few times.

4. Place the competent cell/DNA mixture on ice for 20min.

5. Heat shock transformation tube by placing the bottom 1/2 to 2/3 of the tube into a 42°C water bath for 30-60 seconds (45sec is usually ideal, but this varies depending on the competent cells you are using).

6. Put the tubes back on ice for 2 min.

7. Add 500μl LB media (without antibiotic) and grow in 37°C shaking incubator for 30min.

Plate some or all of the transformation onto a 10cm [LB agar plate](http://www.addgene.org/plasmid_protocols/bacterial_plates/) containing the appropriate antibiotic.

9. Incubate plates at 37°C overnight.