Transformation

1. Thaw a tube of competent cells (100 µL) on ice, and use as soon as possible.
2. Pipette 50 ~ 100 ng DNA to the solution surface of competent cells.
3. Put the tube on ice for 15 min.
4. Heat shock: Put the tube at 42oC water bath for 2 min.
5. Put the tube in ice for 5 min.
6. Transfer the cells to a 1.5 mL microfuge tube
7. Add 1 ml LB broth.
8. Incubate the tube at 37oC shaker for 45 – 90 min with shaking (~ 250 rpm).
9. Spread dilution of cells onto the pre-warmed agar plate (with suitable antibiotics).
10. Incubate the agar plates upside down at 37 oC incubator overnight (12 – 18hrs).

Restriction digestion

1. Place the reagents on ice.
2. Set up the restriction digestion mixture as follow:

Reaction Volume	25 µl
DNA template	0.5 µg
10X Buffer (1-4, according to enzymes)	2.5
Enzyme	5U

3. Pipette the solution up and down to ensure all reagents are mixed well.
4. Place the reaction mixture at 37 oC incubation or dry bath for 2-4 hours.
5. Purify the DNA by PCR purification kit/gel extraction kit for downstream process.

Ligation

1. Place the reagents on ice.
2. Prepare the reaction mixture with the steps in the following table (recommended by NEB)

Total volume	20 µl
DNA (10-200 ng) + ddH_2O	17
10XT4 DNA Ligase Reaction Buffer	2
T4 DNA Ligase (400,000 units/mL)	1

3. Allow the ligation to take place a 22-25oC for 10 minutes.
4. 5 µl of the ligated sample should be used for agarose gel electrophoresis to confirm whether ligation has occurred (Optional)