# Plasmid Ligation & Transformation

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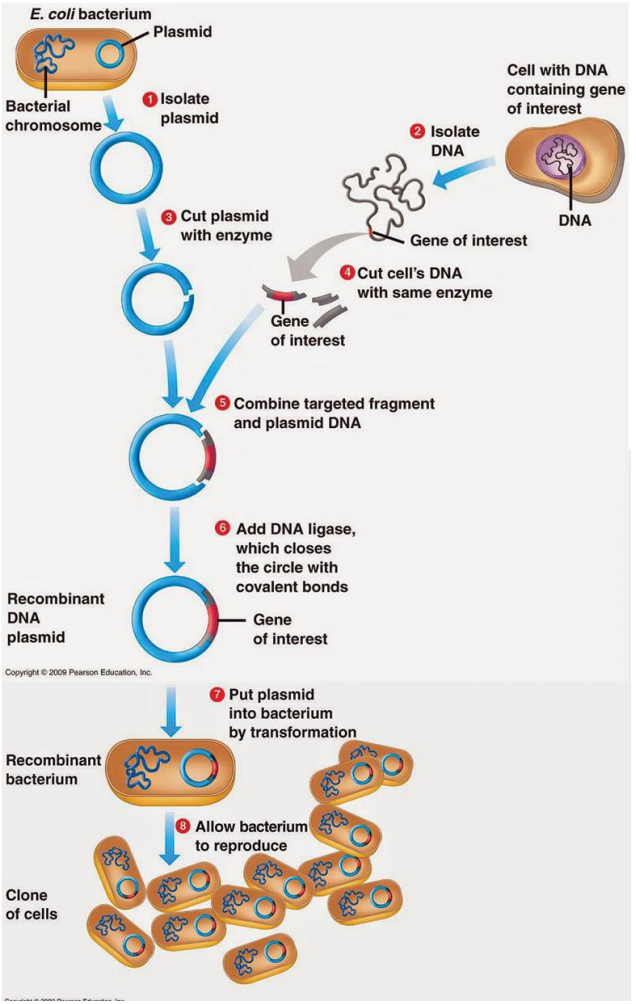
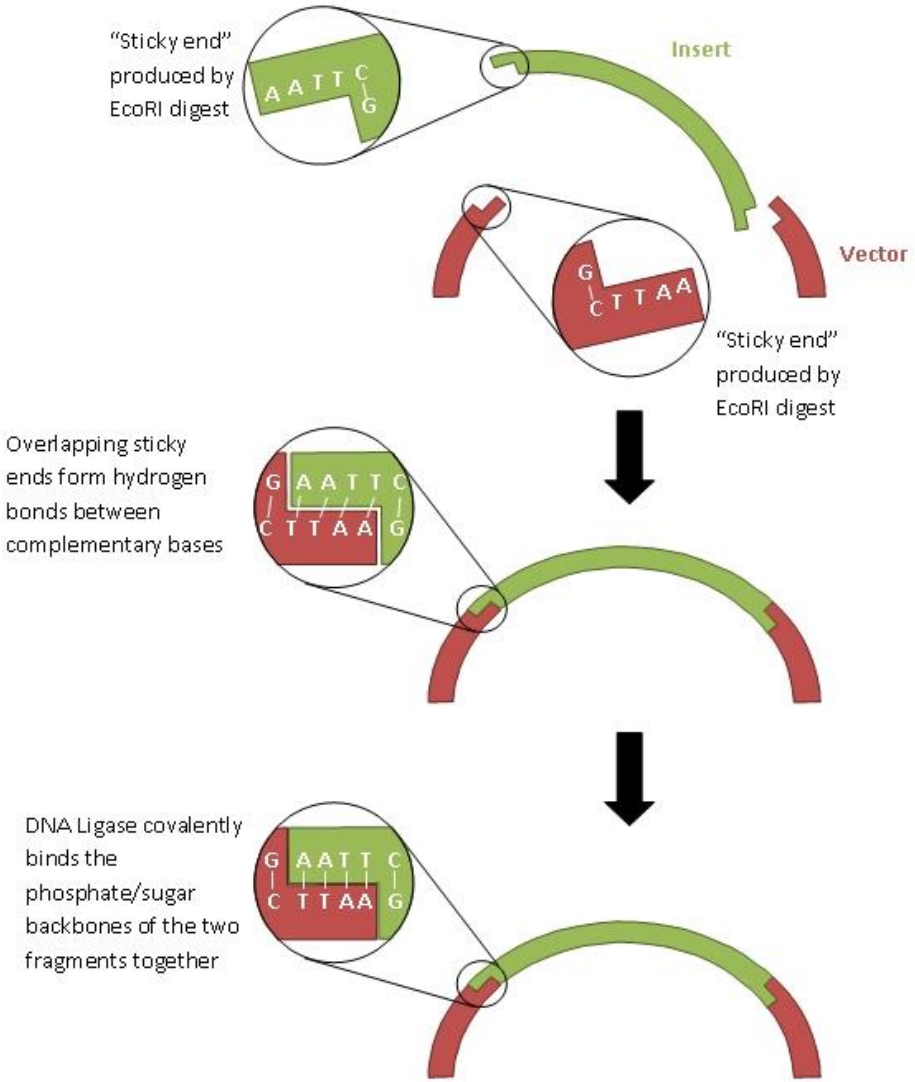
# What we will do?

PCR amplification of DNA of interest (DOI)
Purification of amplified POI
Restriction of amplified POI and plasmid
Purification of restricted DNAs
Ligation of POI and plasmid
Transformation of ligated DNA
DNA sequencing
Overexpression
Purification



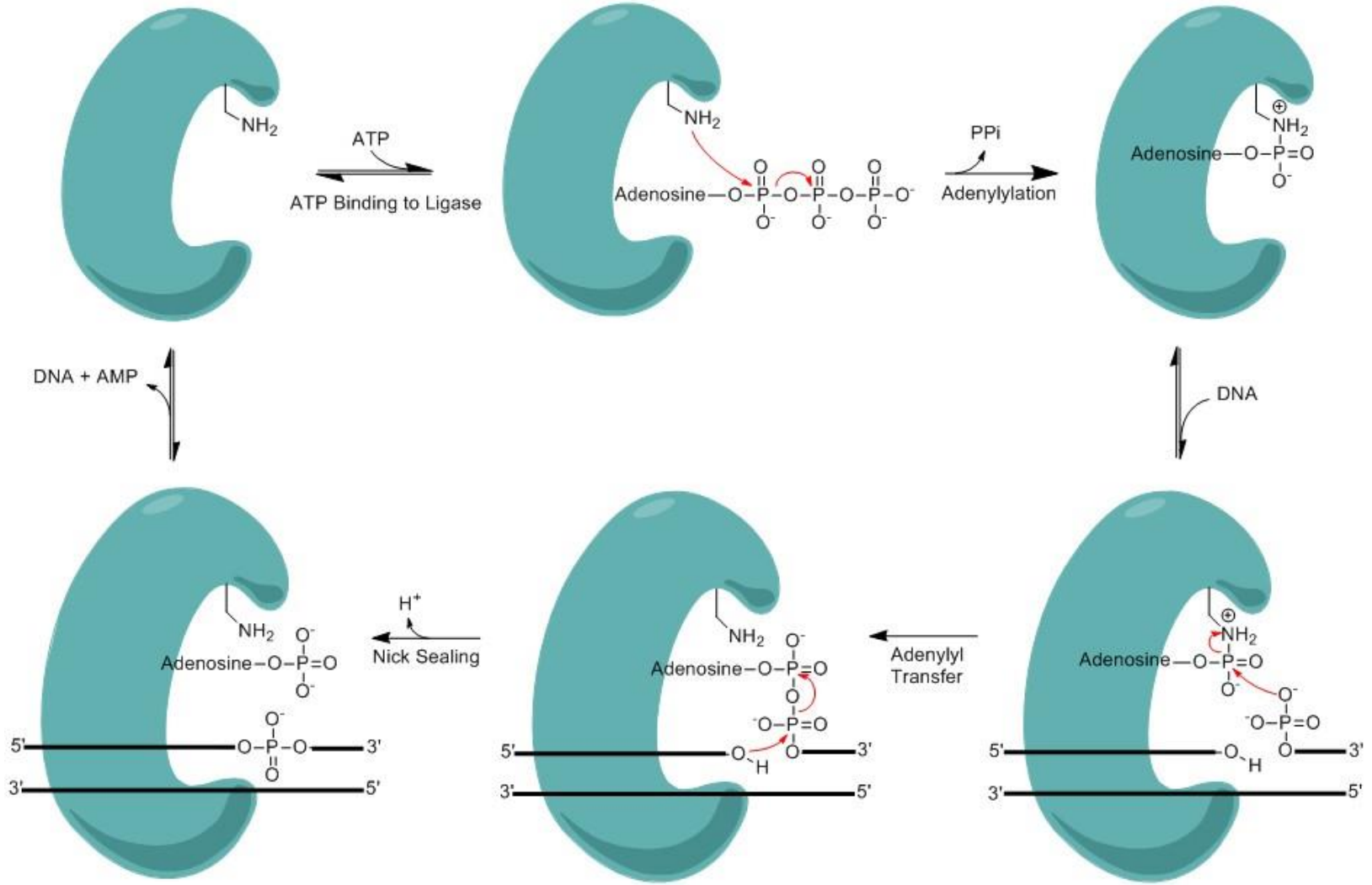
# DNA Ligation




Using DNA Ligase to Splice Together Sticky-Ended DNA Fragments

https://www.di.uq.edu.au/sparqligation

# DNA Ligation




http://easylifescienceworld.com/dna-ligase/

# DNA Ligation

Materials
DDW, T4 ligase ligation buffer, T4 ligase,
restricted DNAs (target of interest and plasmid)

1. Thaw digested DOI and plasmid and T4 ligase ligation buffer.
2. Prepare two new microtubes.
3. Data from last week, calculate relative amount of DOI to plasmid.
4. Add DOI and plasmid into a microtube. Insert ligation sample
   (Ratio of DOI and plasmid should be 3:1 ~ 5:1)
   Add DOI only into a microtube and DDW instead of plasmid (self ligation sample)
5. Add 1uL of T4 ligase ligation buffer.
6. Add DDW up to 9uL
7. Add 1uL of T4 ligase (total volume should be 10uL)
8. Close lid of microtube and centrifuge, vortex, centrifuge.
9. Incubate for 2h at 37℃

# DNA Ligation

**Standard reaction conditions**

When a restriction fragment as insert DNA is ligated to a vector DNA.

| | | |
|---|---|---|
| 10X T4 DNA Ligase buffer | | 2 µl |
| T4 DNA Ligase (400 units/µl) | For sticky ends | 1 µl |
| | For blunt ends | 2 µl |
| Vector DNA (50~400 ng/µl) | | 1 µl |
| Insert DNA (3X molar excess of vector DNA) | | X µl |
| Distilled water | | up to 20 µl |
| → Incubate at room temperature for 1hr. | | |

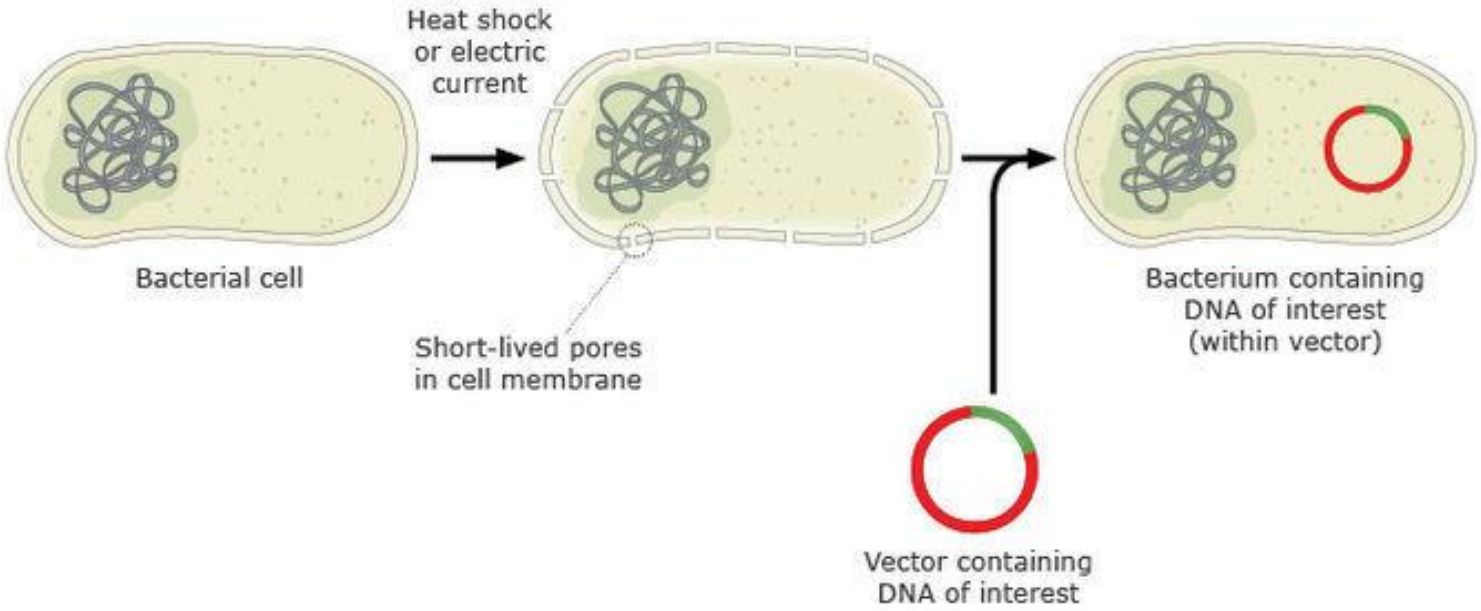
$$\frac{\text{ng of vector} \times \text{kb size of insert}}{\text{kb size of vector}} \times \text{molar ratio of } \frac{\text{insert}}{\text{vector}} = \text{ng of insert}$$

**Example:**

How much 0.5kb insert DNA should be added to a ligation in which 100ng of 3kb vector will be used? The desired vector:insert ratio will be 1:3.

$$\frac{100\text{ng vector} \times 0.5\text{kb insert}}{3\text{kb vector}} \times \frac{3}{1} = 50\text{ng insert}$$

# Transformation



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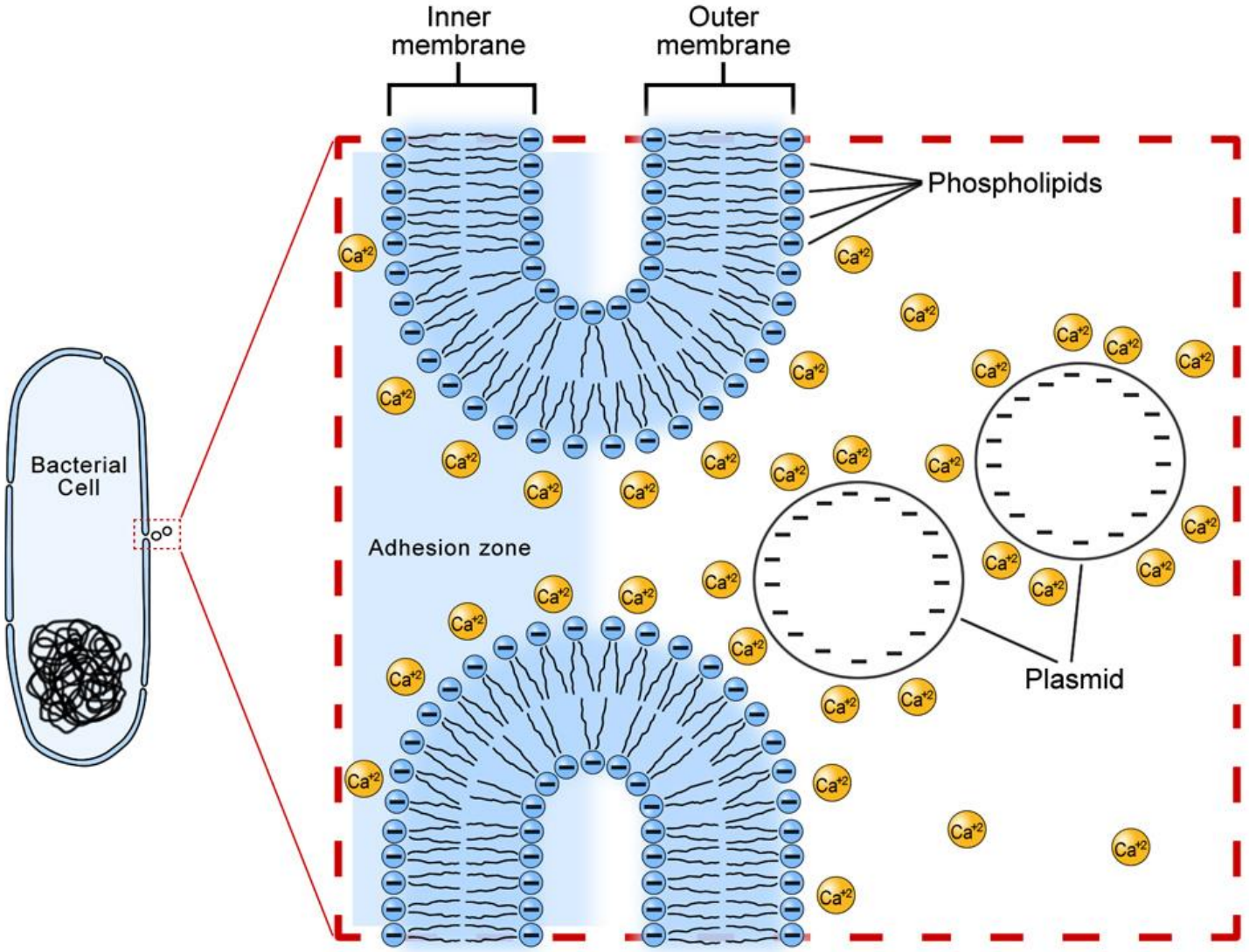

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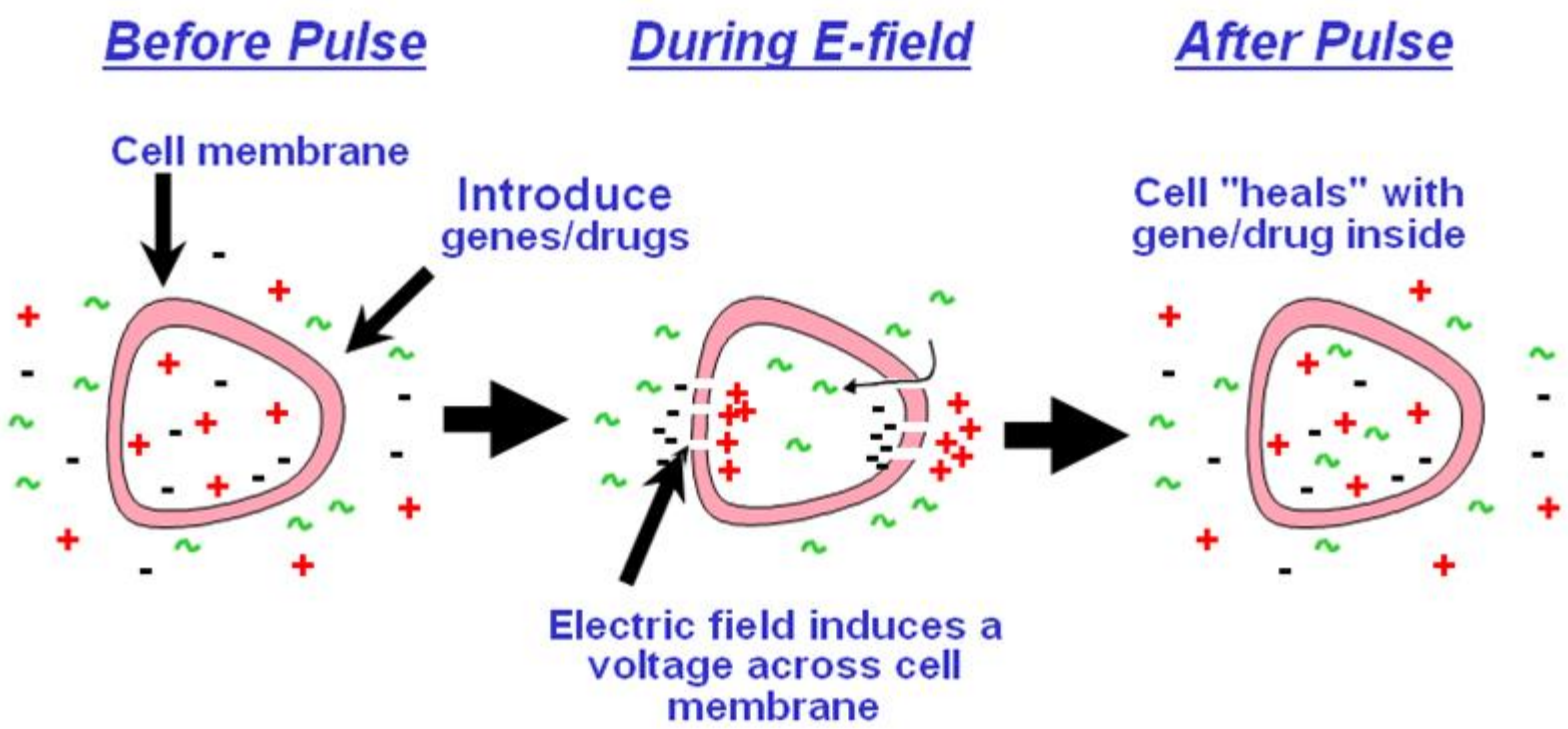


Electroporation

# Transformation

Materials
Competent cell (DH5a), ligated DNA, 42℃ heat-block, LB

1. Thaw competent cell in ice
2. Prepare two new microtubes.
3. Add 20uL of competent cell into microtubes.
4. Add 2uL of insert ligation sample and self ligation sample to each tube.
   (Generally, the ratio b/w cell and gene is 10:1)
5. Tapping and incubate for 10min in ice.
6. Move the microtube to 42℃ heat block and incubate for 30s.
7. After incubation, move the microtube to ice and incubate for 1min.
8. Add 800uL of LB medium.
9. Incubate for 30min at 37℃.
10. Prepare LB agar plate that contains appropriate antibiotics.
11. Spread the cell+gene solution in the LB agar plate with spreader.
12. Incubate for 12~18h at 37℃