

Plasmid enzyme digestion and Gibson assembly

Reagents

- FastDigest buffer (10x) (Thermo Scientific Co., Ltd.)
- FastDigest™ enzymes (500 reaction) (Thermo Scientific Co., Ltd.)
- 800ng Plasmid (calculate the volume needed)
- ddH₂O (to make up the total volume to 50uL)
- Gel-lysis: guanidinium thiocyanate 3M, potassium acetate 0.375M, pH 5.0
- **Buffer W2**
- **Elution buffer**
- SynoFusion (40x reaction) (Synbio Tech Co., Ltd.)

A. Plasmid enzyme digestion

1. Prepare the enzyme digestion system in a PCR tube as the following order:
 - ___uL ddH₂O (volume calculated)
 - 5uL FastDigest buffer (10x)
 - FastDigest™ enzymes
 - ___uL Plasmid (800ng)
2. Finger flick to mix, then centrifuge briefly.
3. Place the reaction tubes in the Thermal Cycler.
4. Enzyme digestion will be run with the following programme:
 - 1 cycle of 37 °C, 30 min
 - 1 cycles of 80 °C, 5min
 - Hold at 4 °C

B. Digested plasmid purification and collection

1. Pipette 50uL enzyme digestion result into GeneClean column with 2mL collection tube, add 700uL Gel-lysis into each tube.
2. Centrifuge for 1min at 12,000rpm. After it finished, pour the solution back to the column and centrifuge for another 1min at 12,000rpm.
3. Discard the solution, add 700uL **Buffer W2** and centrifuge for 1min at 12,000rpm. After it finished, pour the solution back to the column and centrifuge for another 1min at 12,000rpm.
4. Discard the solution, centrifuge again at 12,000rpm for 2min.
5. Move the GeneClean column into 1.5 mL centrifuge tube, pipette 30uL **elution buffer** into the column, on the center of silica membrane.
6. Keep it still for 5mins, then centrifuge at 12,000 for 1min to collect the purified DNA.
7. Detect the purification result using detection gel electrophoresis.

C. Gibson assembly

1. Prepare the Gibson assembly system in a PCR tube as the following order:
 - 10uL SynoFusion
 - 6uL digested plasmid
 - 4uL DNA fragment
2. Place the reaction tubes in the Thermal Cycler.

3. Gibson assembly will be run with the following programme:
 - 1 cycle of 50 °C, 60 min
 - Hold at 4 °C