

## Performing ligation of DNA insert into vector DNA.

### Materials:

Linear Vector DNA

Insert DNA

10x T4 DNA Ligase buffer

T4 DNA Ligase

ddH<sub>2</sub>O

### Procedure:

- 1、 Add 2ul of digested plasmid backbone (25 ng)
- 2、 Add equimolar amount of EcoRI SpeI digested fragment
- 3、 Add equimolar amount of XbaI PstI digested fragment
- 4、 Add 1 ul T4 DNA ligase buffer. Note:
- 5、 Add 0.5 ul T4 DNA ligase.
- 6、 Add water to 10 ul.
- 7、 Ligate 16°C/30 min, heat kill 80°C/20 min.