



## Molecular cloning and genetic engineering –

### Ligation

#### ● Aim

Performing ligation of DNA insert into vector DNA.

#### ● Materials

Linear Vector DNA

Insert DNA

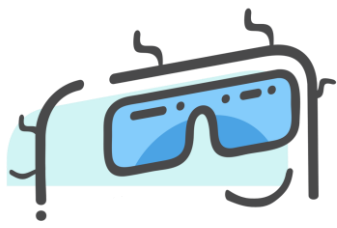
10x T4 DNA Ligase buffer

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.

