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_2O

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℃/30 min, heat kill 80℃/20 min.