Molecular cloning and genetic engineering – Restriction Digest

Aim

Restriction digest can cut DNA into fragments at specific recognition sites.

Materials

DNA to be digested

NEB buffer 2

BSA

Digest Enzyme EcoRI

Digest Enzyme PstI

ddH_2O

Procedure

1. Add 250ng of DNA to be digested, and adjust with ddH2O for a total volume of 16ul.

2. Add 2.5ul of NEBuffer 2.

3. Add 0.5ul of BSA.

4. Add 0.5ul of EcoRI.

5. Add 0.5ul of PstI.

6. There should be a total volume of 20ul. Mix well and spin down briefly.

7. Incubate the restriction digest at 37℃ for 30min, and then 80C for 20min to heat kill the enzymes.

8. Run a portion of the digest on a gel (8ul, 100ng), to check that both plasmid backbone and part length are accurate.