Ligation

This protocol aims at ligating compatible, previously cut restriction sites on plasmids. Volumes of DNA are given for similar concentrations. You may want to adapt these volumes if your concentrations are significantly different. Enzymes and buffers were supplied by Fermentas.

Procedure

1. Standard ligation In an eppendorf tube, add the following solutions :

- 6 µL insert
- 4 µL vector
- 2 µL T4 DNA ligase buffer
- 6 µL water

Or 1. 3A ligation In an eppendorf tube, add the following solutions :

- 2 µL of each DNA solution
- 2 µL T4 DNA ligase buffer
- 10 µL water

2. Add 2µL of ligase.

3. Incubate at room temperature for 3h

4. Incubate at 70°C for 5 mn to inactivate the ligase.