Protocol for ligation of insert DNA into plasmid vector DNA

Materials:

- DNA sample(s) in water or TE buffer
- 10x ligation buffer
- T4 DNA Ligase, 5 u/µl
- ddwater

Procedure:

1. Test the concentration of the DNA sample(s).
2. Pipet the following into a microfuge tube:

a. Linearized vector DNA: around 100ng

b. Insert DNA (at 3:1 molar excess over vector): variable

c. 10x ligation buffer: 1uL

d. T4 DNA Ligase: 1uL

e. ddwater: Rest of volume

Total volume: 10 uL

3. Vortex and spin briefly to collect drops.
4. Incubate the mixture at 16 degree for 60-120 min.
5. Use the ligation mixture for transformation.

Tips:

- Thoroughly mix the 10x ligation buffer before use.
- The optimal insert/vector molar ratio is 3:1.
- To minimize recircularization of the cloning vector, dephosphorylate linearized plasmid DNA with Alkaline Phosphatase(CIAP) prior to ligation. Heats inactivate the phosphatase or remove from the mixture after the dephosphorylation step.
- DNA purity is an important factor for successful ligation. Plasmids should be purified using a method that will ensure isolation of high quality DNA. Use only high quality agarose and fresh electrophoresis buffers for gel-purification of DNA fragments.