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:

	10uL reactionsystem
Linearized vector DNA	around 100ng
Insert DNA (at 3:1 molar excess over vector):	variable
10x ligation buffer	1uL
T4 DNA Ligase	1uL
ddwater	Rest of volume

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:

1. The optima l insert/vector molar ratio is 3:1.
2. To minimize recircularization of the cloning vector, dephosphorylate linearized plasmid DNA with Alkaline Phospha tase(CIAP) prior to ligation. Heats inactivate the phospha tase or remove from the mixture after the dephosphorylation step.
3. DNA purity is an important factor for successful ligation. Plasmids should be purified using a method that will ensure isola tion of high quality DNA. Use only high quality agarose and fresh electrophoresis buffers for gel-purification of DNA fragments.