Protocol 1-4: Ligation of DNA Fragments

General Protocol

1) Assemble the reaction mixture described below

10× ligation buffer	2 µl
vector DNA	50 ng
insert DNA	X ng (molor ratio of vector:insert =1:3)
T4 DNA Ligase (350U/µl)	1 µl
distilled water	up to 20 µl

2) Incubate the reaction mixture for 1-5 hours at 16°C for cohesive end DNA.
Incubate the reaction mixture for 1-24 hours at 16°C for blunt end DNA.

3) Transform E. coli competent cells directly using up to 10 µl of the ligation reaction mixture for 100 µl competent cells.

Tips

1) If good results are not obtained, the reaction can be extended. Incubation longer than 16 hours can be done at 4°C.

2) The ligation reaction mixture should not be used directly in electroporation.
For electroporation, ligated DNA should be ethanol precipitated and dissolved in a low Salt buffer such as TE buffer prior to use.

Reference

1) Sambrook J, Maniatis T, Fritsch EF. Molecular Cloning: a Laboratory Manual. cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 3rd ed., 2001.

2) Robert F. Weaver. Molecular Biology, McGrawHill, 4th edition, 2007

3) http://2009.igem.org/Team:Tsinghua/Protocol