

Protocol 1-4: Ligation of DNA Fragments

General Protocol

- 1) Assemble the reaction mixture described below

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

- 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 μ l of the ligation reaction mixture for 100 μ l 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>