DNA Ligation

Protocol for DNA Ligation New England Biolabs

Materials:

- Digested plasmid
- Digested DNA fragments
- 10 X Buffer for T4 DNA ligase with 10 mM ATP NEB
- Hi-T4 DNA ligase NEB
- Nuclease Free Water (NFW)
- 50 µl Eppendorf tubes

Ligation:

1. Add the following volumes to an Eppendorf tube.

Reagent	Volume/mass
Digested Plasmid	3 µl
10 X Buffer for T4 DNA ligase with 10 mM ATP	1 µl
Hi-T4 DNA ligase NEB	1 µl
Digested gene	2 µl
NFW	Fill up to 10 µl
Total	10 µl

2. Incubate the ligation mix at 25°C for one hour. It can be incubated overnight if necessary.
3. Store at -20°C.
4. The ligation can be confirmed by Agarose gel electrophoresis compared to the empty plasmid without digestion as control.