Protocol for DNA Ligation (Thermo)
Protocol code: Lig

Materials

- T4 DNA Ligase
- T4 DNA Ligase Buffer (10x)
- DNA vector
- DNA Upstream
- DNA Downstream
- Nuclease free water
- Competent cells
- Thermoblock

Procedure

1. Prepare the reactions* in a PCR tube in ice following the next table:

Reagent	1 Reaction (20µL)
T4 DNA Ligase Buffer (10X) **	2µL
DNA Vector	3µL
Upstream DNA	1µL
Downstream DNA	1µL
Nuclease free water	12µL
T4 DNA Ligase	1µL

*For a higher precision in the ligation the NEBio Calculator can be used. (http://nebiocalculator.neb.com/#!/ligation), only if you have the DNA insert mass (ng) and length (pb) needed from the vector. It is recommended to use a 3:1 relation between the inserted DNA and the DNA vector.

** The T4 DNA Ligase Buffer should be thawed and resuspended at room temperature.

2. Mix gently using the micropipette.

3. Incubate overnight at room temperature
4. Inactivate the enzyme at 80 °C for 20 minutes in the thermoblock.
5. Keep the sample on ice and transform from 1µL to 5µL from the reaction in 50µL of competent cells.