Ligation of DNA-fragments

Aim of the Experiment

This protocol can be used to connect DNA fragments with fitting overhangs or blunt ends through the reaction with a Ligase enzyme. One of the two (or multiple) DNA fragments needs to be dephosphorylated prior to ligation.

Materials

- DNA fragments of interest
- Ligase (i.e. Electro-Ligase, T4-DNA-Ligase)
- Accompanying buffer for Ligase enzyme
- Nuclease-free H_2O (nf H_2O, Carl Roth, Germany)
- Heat block

Procedure

1. Mix 20 fM of the insert DNA fragments with 60 fM of the backbone DNA fragment for a final volume of 20 μl
2. Add 2 μl of the Ligase accompanying buffer
3. Add 1 μl of the Ligase (for enzyme concentration of 5 U/μl)
4. Fill up to final volume with nf-water
5. Incubate reaction mix for 1 h at 37 °C or 16 °C overnight
6. Heat inactivate ligase at 65 °C for 15 minutes

Possible follow up protocols

The following protocols are the next steps of a possible cloning cycle after a restriction digest:

1. Transformation

2. Agarose gel electrophoresis

Ligation tips

If short time ligation at 37 °C for 1 hour does not work, try the overnight version, because of the higher effiency of the ligation rate over longer times.

For ligation reactions that do not function with the low molar DNA concentrations higher concentrations (up to 10x more) should be tried.