Dephosphorylation

Aim of the experiment

This experiment can be used for dephosphorylation of 5 prime DNA ends. Dephosphorylation normally is performed for backbones in combination with the restriction. Dephosphorylation of one DNA part is needed for a functional ligation of backbone and insert.

NEB-Dephosphorylation Protocols and Tips

Materials

- Restriction digest reaction asset (see. Restriction digest protocol) ADD protocol URL When given
- 10 times Antarctic Phosphatase buffer (NEB, Germany)
- Antarctic Phosphatase (NEB, Germany)
- Thermocycler at 37 °C and 80 °C

Procedure

1. Add to the tube of an restriction digest was performed in:

Table 1: Dephosphorylation reaction mix

Volume (µl)	**Chemicals**
50	restriction digest asset
5.66	10x Antarctic phosphatase reaction buffer
1	Antarctic Phosphatase

2. Incubate at 37 °C for 1 hour
3. Heat inactivate Antarctic Phosphatase at 80 °C for 20 min

Possible follow up protocols

The following protocols are the next steps of a possible cloning cycle after a dephosphorylation reaction:

1. Ligation