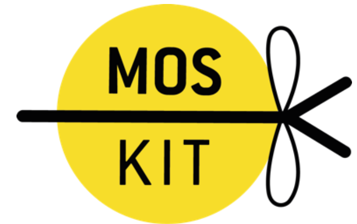
iGEM Pasteur Paris 2016

Protocols



DEPHOSPHORYLATION

Aim: To reduce vector self-ligation and favor that of vector to insert instead.
The presence of a free phosphate group at one end of the linear vector DNA after a restriction enzyme digestion will lead to self ligation, because it is intra-molecular.

Materials:

- Molecular biology equipment: 37 °C and 65 °C water baths, pipette set, ice bucket
- (Recombinant Shrimp alkaline phosphatase) rSAP Reaction Buffer (10X)
- rSAP
- DNA
- Nuclease-free water

Protocol:

1. Digest vector with the appropriate restriction enzymes. Enzymes that leave non-compatible ends are ideal as they prevent vector self-ligation as well.

2. Dephosphorylation is necessary to prevent self-ligation. NEB offers three products for dephosphorylation of DNA:
   - rSAP (NEB #M0371) is heat-inactivable phosphatases -> It works in all NEBuffers, but rSAP requires that the reaction be supplemented with Zn^{2+}

Dephosphorylation of 5' ends of DNA using rSAP:

- rSAP Reaction Buffer (10X) : 2 µl
- DNA ≥ 1 pmol of DNA ends (about 1 µl of 3 kb plasmid at 100 ng/ µl)
- 1 µl rSAP (1 unit/ µl)
- Nuclease-free Water : to 20 µl
- Incubation : 37°C for 30 minutes
- Heat Inactivation : 65°C for 5 minutes

Note: Scale larger reaction volumes proportionally