Protocol for Dephosphorylation of 5′-ends of DNA using Antarctic Phosphatase (NEB #M0289)

Overview

Protocols.io also provides an interactive version of this protocol where you can discover and share optimizations with the research community.

Protocol

1. Prepare a 20 µl reaction as follows:

DNA	1 pmol of DNA ends*
Antarctic Phosphatase Reaction Buffer (10X)	2 µl
Antarctic Phosphatase	5 units
H_2O, purified	to 20 µl**

2. Incubate at 37°C for 30 minutes.

3. Stop reaction by heat-inactivation at 80°C for 2 minutes.

** Note: 1 pmol of DNA ends is about 1 µg of a 3 kb plasmid.*
*** Scale larger reaction volumes proportionally.*

Dephosphorylation of 5′-ends of DNA in Restriction Enzyme Reaction

- The phosphatase can be added directly into the digestion reaction during or after DNA digestion
- Antarctic Phosphatase is active in all NEB restriction enzyme buffers only when supplemented with Antarctic Phosphatase Reaction Buffer, which provides Zn2+ required for enzyme activity
- The restriction enzyme should be heat inactivated at the same time as the phosphatase after digest and dephosphorylation
- If restriction enzyme cannot be heat inactivated, DNA purification is required before ligation