Protocol for Dephosphorylation of DNA 5′-ends using the Quick CIP in a Restriction Enzyme Reaction (NEB #M0525)

Overview

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

1. Digest 1–5 µg of plasmid DNA in a 20 µl reaction as follows:

DNA	>1 µl
Restriction Enzyme Buffer (10X)	2 µl
Restriction Endonuclease	1 µl
H_2O, purified	to 20 µl

Note: Scale larger reaction volumes proportionally.

2. Incubate at 37°C for 60 minutes or follow manufacturer’s recommendations.

3. Add 1 µl of Quick CIP for every 1 pmol of DNA ends (about 1 µg of a 3 kb plasmid) and incubate at 37°C for 10 minutes.

4. Stop reaction by heat-inactivation of Quick CIP and restriction enzyme (follow manufacturer's recommendations).

Note: The vector digest and dephosphorylation with Quick CIP can be performed in one reaction at the same time. If restriction enzyme cannot be heat-inactivated, DNA purification is required before ligation.