

Phosphorylation

1. Set-up the following reaction in a microcentrifuge tube on ice:

DNA	up to 300 pmol of 5' termini
T4 PNK Reaction Buffer (10X)	5 µl
ATP (10 mM)	5 µl
T4 PNK	1 µl (10 units)
Nuclease-free Water	up to 50 µl

Note: T4 PNK Reaction Buffer and ATP can be substituted with T4 ligase buffer.

2. Incubate at 37°C for 30 minutes.
3. Heat inactivate by incubating at 65°C for 20 minutes.
 - a. Note: Not needed if heat inactivating in Ligation step.