SOP Name: T4 Blunting Reaction
Author: Marcia Pryce
Source(s): New England BioLabs (NEB)
Time Required: Overnight
Polymerase
Materials:

- T4 Polymerase buffer
- T4 Polymerase
- H_2O
- DNTP's
- Digested DNA

Procedure:

1. DNA should be dissolved in 1X reaction buffer* supplemented with 100 µM dNTPs.
2. Add 1 unit T4 DNA Polymerase per microgram DNA.
3. Incubate 15 minutes at 12°C.
4. Stop reaction by adding EDTA to a final concentration of 10 mM and heating to 75°C for 20 minutes (1,2).

Ligase
Materials:

COMPONENT	20 µl REACTION
T4 DNA Ligase Buffer (10X)*	2 µl
Vector DNA (4 kb)	50 ng (0.020 pmol)
Insert DNA (1 kb)	37.5 ng (0.060 pmol)
Nuclease-free water	to 20 µl
T4 DNA Ligase	1 µl

Procedure:

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

(T4 DNA Ligase should be added last. Note that the table shows a ligation using a molar ratio of 1:3 vector to insert for the indicated DNA sizes.) Use NEBioCalculator to calculate molar ratios. The T4 DNA Ligase Buffer should be thawed and resuspended at room temperature.

2. Gently mix the reaction by pipetting up and down and microfuge briefly.
3. For cohesive (sticky) ends, incubate at 16°C overnight or room temperature for 10 minutes.
4. For blunt ends or single base overhangs, incubate at 16°C overnight or room temperature for 2 hours (alternatively, high concentration T4 DNA Ligase can be used in a 10 minute ligation).
5. Heat inactivate at 65°C for 10 minutes.
6. Chill on ice and transform 1-5 µl of the reaction into 50 µl competent cells.