T4 DNA POLYMERASE BLUNT-ENDING/FILL-IN REACTION FOR CLONING

Updated by Yi Shu 05/21/2017; edited by TCH

1) The restriction enzyme digested DNA should be precipitated with ethanol and washed with 70% ethanol twice;

2) Set up a 100µl reaction as the follows:

10 x NEB Buffer 2.1(NEB)	10.0
dNTPs (10mM each)	10.0
ddH_2O	79.0
Digested pDNA	dried
T4 DNA Polymerase (NEB)	1.0
	100.0 µl

3) **Incubate at 37°C for 15min ~ 30min**.

4) Precipitate blunt-end DNA with ethanol (see Ethanol Precipitation protocol) or directly load the reaction mix to 0.5% gel for purification.

NOTE: 1) T4 DNA polymerase exhibits both 3'- and 5'exonuclease activities so it can fill in or chew back either 3'- or 5'-protruding ends.
2) To avoid unnecessary chewing back, one must ensure a large excess of dNTPs in the reaction.
3) When two different restriction sites are blunt-ended and ligated, there is a 25% chance restoring either of the restriction sites.
4) For fill-in reactions only: T4 DNA Polymerase can be used in NEBuffers 1.1, 2.1, 3.1 and CutSmart® Buffer as well as NEBuffers 1-4 and T4 DNA Ligase Reaction Buffer.
5) For blunting reactions requiring removal of overhangs: T4 DNA Polymerase can be used in NEBuffers 1.1, 2.1, and CutSmart Buffer as well as NEBuffers 1, 2, and 4 and T4 DNA Ligase Reaction Buffer. NEBuffers 3.1 and 3 are not recommended when overhang removal is required.
6) Elevated temperatures, excessive amounts of enzyme, failure to supplement with dNTPs or long reaction times will result in

recessed ends due to the 3′→ 5′ exonuclease activity of the enzyme.