Restriction of DNA into Vector DNA (with T4 DNA Ligase)

Courtesy of Uté Kothe laboratory at the University of Lethbridge

1. In a 0.5 mL PCR tube prepare, in water, 5-10 µL mix of digested vector DNA (10/50-400 ng) and foreign DNA to be inserted.

Add in the following order:

10x Ligation buffer (vortex well)	2 µL
50% PEG 4000 solution (if using blunt end DNA)	2 µL
H_20	20 µL
SmaI (for blunt ends only)	0.2 µL
T4 DNA ligase	1-2 µL

1. Mildly vortex the tube and spin down in a microcentrifuge for 3-5 seconds.
2. Incubate the mixture overnight at 16°C or room temperature.
3. Inactivate T4 DNA Ligase by heating reaction mixture at 65°C for 10 minutes.
4. Use the mixture for transformation (2 µL for 20 µL competent cells).

Note: Use less than 100 ng DNA total in 20 µL ligation mixture.