



Protocol T-Vector System

	STANDARD REACTION	BACKGROUND CONTROL
5x Ligation buffer	2 μ l	2 μ l
T –Vector (50 ng)	1 μ l	1 μ l
PCR product	X μ l*	-----
T4 DNA Ligase (2 Weiss units/ μ l)	1 μ l	1 μ l
ATP (5 mM)	2 μ l	2 μ l
Deionized water to	a final volume of 10 μ l	a final volume of 10 μ l

1. Briefly centrifuge the T-vector and insert DNA tubes to collect contents at the bottom of the tube.
 2. Set up ligation reaction as described above
- * Molar ratio of PCR product: vector may optimization as described below
3. Mix the reactions by pipetting. Incubate the reaction 1 hour at room temperature.
 4. Alternatively, if the maximum number of transformants is required, incubate the reactions overnight at 40°C.