

University of Southampton iGEM 2009 Protocol:
Ligation using T4 DNA Ligase^[1]:

Ligation Protocol:

1. Prepare solutions on ice, in the order tabulated below^[2]:

Component	Amount/ μL
DNA Vector	8 μL
DNA Insert	(vector : insert – 3:1)
T4 DNA ligase buffer	1
T4 DNA ligase	1

NB. To calculate the respective volumes of vector and insert required per ligation, use the following calculation (bold figures need to be inserted manually):

	Vector	Insert
Ratio	r_v	r_i
Size of fragment (bp)	l_v	l_i
DNA Concentration ($\text{ng } \mu\text{L}^{-1}$)	c_v	c_i
MW (g mol^{-1})	$w_v = 660 * r_v * l_v$	$w_i = 660 * r_i * l_i$
Mass (ng)	$m_v = w_v * 3 * 10^{-5}$	$m_i = w_i * 3 * 10^{-5}$
Volume (μL)	$v_v = m_v / c_v$	$v_i = m_i / c_i$
Volume to use in 8 μL total DNA (double for 16 μL)	$x_v = v_v * [8 / (v_v + v_i)]$	$x_i = v_i * [8 / (v_v + v_i)]$

1. For each set of vector : insert ligations, a control reaction should be set up. This consists of replacing the insert with nuclease-free water, with all other reaction components remaining the same; this is known as the vector alone control.
2. Briefly centrifuge all PCR tubes then incubate the samples at room temperature for 2 hours.
3. Post-incubation, the solutions were heated at 65°C for 10 minutes.
4. Samples can be stored at -20 °C until further use.

[1] NEB T4 DNA ligase product technical reference

[2] Maximising Ligation Efficiencies, NEB (<http://www.neb.com> accessed July 2009)