Ligation

Ligate the purified insert and vector together.

5.1 Place 50 ng of digested vector, 5 μL of ligation buffer, and 1 μL of T4 ligase into a 500-μL
microfuge tube. The amount of insert to add is calculated from the following formula:

ng of insert=(2)(bp\ insert)(50ng linearized plasmid)/ (size of plasmid in bp).

Add water to increase the final volume to 10 μL.

5.2 Prepare both a positive ligation mixture that contains the digested vector and insert as well as a negative ligation mixture that contains only the digested vector. Add more water to the negative ligation mixture to prepare equal volumes.

5.3 Leave the ligation mixture at room temperature for 5 min, and then use it directly for transformation of E. coli competent cells, or store it by freezing until transformation.