Ligation protocol

1. Set up the following reaction in a microcentrifuge tube on ice.
   (T4 DNA Ligase should be added last. Note that the table shows a ligation using a molar ratio of 1:3 vector to insert.)*

COMPONENT	Volume
10X T4 DNA Ligase Buffer	2 µl
Vector DNA (50 ng)	
Insert DNA (**1:3 ratio**)	
T4 DNA Ligase	1 µl
MB H2O	to 20 µl
Total	**20 µl**

2. Gently mix the reaction by pipetting up and down and microfuge briefly.
3. Incubate for 2 hr at 30 °C, or 16 °C overnight.

*How to calculate a molar ration of 1:3

$$(insert\ amount[ng])\ X\ (plasmid\ length) = (plasmid\ amount[ng])\ X\ (insert\ length)$$

$$(insert\ amount[ng]) = \frac{(plasmid\ amount[ng])\ X\ (insert\ length)}{(plasmid\ length)}\ X3$$

Plasmid amount should always be 50 ng.