## Updated 8/21/2014 by MRS:

30µL Ligation reaction recipe:

22.2µL template (100ng DNA + pH2O)

2µL P1 working stock

2µL P2 working stock

3µL 10x buffer

0.8µL Ligase

1. Add samples and water to plate (takes several hours)

2. Add P1 adapters to plate

3. Create a working solution of the P2, buffer and Ligase

4. Add working solution to plate

* allowed rxn to occur on countertop at room temp for 1.5hr
* placed in thermocycler to heat kill (65˚ for 10 min then decrease of 2˚ every 90sec until room temp, hold at 4˚)

## 

## Setup

\*\*easiest to purchase T4 ligase at the concentration of 400,000ends/mL instead of 2,000,000 ends/mL (concentrated)

If T4 is concentrated (will say so on the sheet), make a 1x dilution of the T4 ligase buffer and then dilute T4 ligase to 400,000 ends/mL for the amount you need just for current day.

15 samples x 3µL per sample T4 ligase = 45µL - make a 50µL solution

4µL 10x buffer + 36µL pure H20 = 40µL 1x buffer

10µL T4 ligase + 40µL 1x buffer = 50µL T4 ligase at 400,000 ends/mL

## Our Recipe

## 100 ng DNA (100/qubit qty = vol to add)

## volume of H2O to bring volume up to 30µL

## 2µL P1 working stock (10 fold adapters)

## 2µL P2 working stock (10 fold adapters)

## 4µL 10x buffer

## 2µL ligase @ 400,000 ends/mL

## Gently mix the reaction by pipetting up and down and microfuge briefly.

## For cohesive (sticky) ends, incubate at 16°C overnight

## 

## Protocol

1. Set up the following reaction in a microcentrifuge tube on ice.
2. *(T4 DNA Ligase should be added last. Note that the table shows a ligation using a molar ratio of 1:3 vector to insert.)*
3. *\* The T4 DNA Ligase Buffer should be thawed and resuspended at room temperature.*
4. Gently mix the reaction by pipetting up and down and microfuge briefly.
5. For cohesive (sticky) ends, incubate at 16°C overnight or room temperature for 10 minutes.
6. For blunt ends or single base overhangs, incubate at 16°C overnight or room temperature for 2 hours*(alternatively, high concentration T4 DNA Ligase can be used in a 10 minute ligation)*.
7. Chill on ice and transform 1-5 μl of the reaction into 50 μl competent cells.