Ligation

Materials

- T4 DNA ligase buffer
- T4DNA Ligase
- Vector DNA ~ 50ng
- Insert DNA
- Nuclease free water

Procedure

1. Use NEB Biocalculator https://nebiocalculator.neb.com/#!/ligation to calculate the required insert DNA mass for a given amount of vector DNA (usually for a 20ul reaction the vector DNA is kept at 50ng.)

2. Make sure to have multiple ratios of Vector DNA: insert DNA (eg: 1:3, 1:5, 1:7) as the ideal ratio in the ligation mixture is not known.

3. An example reaction mix

Reagents	**Volume(ul)**
T4 DNA Ligase buffer 10X	2µL
Vector DNA (50ng)	50/nanodrop of vector(concentration)
Insert DNA	NEB calculator gives mass, divide mass by nanodrop result of the insert
Nuclease-free water	Complete to total 20µL
T4 DNA Ligase	1 µL

4. Do the reaction on ice and make sure to add T4 DNA ligase last.
5. Incubate overnight at 16°C followed by 30 min at room temperature the next day.
6. Heat inactivate at 65°C for 10 min.
7. Chill on ice and use this DNA sample to transform competent cells.