## Ligation

1. Set up on ice. Molar ratio of vector to insert is 1:3.
2. Prepare the whole calculation before adding things to microcentrifuge tube.
3. Add autoclave dd$H_2O$ (to make the volume up to 20µl).
4. Add 2µl 10X T4 DNA Ligase Buffer .
5. Add 50ng Vector DNA (e.g 3kb).
6. Add 50ng Insert DNA (e.g 1kb).
7. Add 1µl T4 DNA ligase.
8. Mix everything by pipetting up and down.
9. For cohesive (sticky) ends, incubate at 16 $^oC$ overnight or 2 hours in room temperature.
10. For blunt ends or single base overhangs, incubate at 16 $^oC$ overnight or 2 hours in room temperature.
11. Chill on ice and transform 1-5µl of the reaction mixture into 50µl competent cells.