LIGATION

Aim: To link an insert to its host plasmid before the transformation.
This step happens after the digestion of the insert and the plasmid with the same restriction sites enzymes, or compatible ends that may give illegitimate ligations.

1) T4 ligation

Materials:

- Molecular biology equipment: 37 °C and 65 °C water baths, pipette set, ice bucket, electrophoresis tank and power supply, imaging system
- 1.5 ml Eppendorfs
- T4 DNA ligase Buffer (10X)
- Vector DNA
- Insert DNA
- Nuclease-free water
- T4 DNA Ligase
- Topo cloning kit (Invitrogen, Thermofisher)

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 for the indicated DNA sizes.* Use NEBioCalculator to calculate molar ratios.

COMPONENT	20 µl REACTION
10X T4 DNA Ligase Buffer*	2 µl
Vector DNA (4 kb)	50 ng (0.020 pmol)
Insert DNA (1 kb)	37.5 ng (0.060 pmol)
Nuclease-free water	to 20 µl
T4 DNA Ligase	1 µl

The T4 DNA Ligase Buffer should be thawed.

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

3. For cohesive (sticky) ends, incubate at 16°C overnight or room temperature for 10 minutes.

4. 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 minutes ligation).

5. Heat inactivate at 65°C for 10 minutes.

6. Chill on ice and transform 1-5 µl of the reaction into 50 µl competent cells.

2) TOPO cloning ligation

https://www.thermofisher.com/content/dam/LifeTech/global/life-sciences/Cloning/pdfs/CO36609%20TOPO%20Cloning%20Brochure.pdf

Protocol:

This method uses the topoisomerase phophotransferase reaction to link a 5'end to another 3'OH. The Topo cloning vector reduces the complexity of trimolecular (Vector DNA, Insert, ligase) reaction to bimolecular (Vector-topoisomerase, Insert) . Use the following procedure to perform the TOPO Cloning reaction. Set up the TOPO Cloning reaction using the reagents in the order shown.

Reagent*	Volume
Fresh PCR product	0.5-4 µl
Salt Solution	1 µl
Water	Add to a total volume of 5 µl
TOPO vector	1 µl
Final volume	6 µl