LIGATION

Why to do this :

1. Insert one or several DNA segments into a vector

What you need :

1. Culture media : LB

- 10 g bactotrypton
- 5 g yeast extract
- 5 g NaCl
- 0,5 mL NaOH 10N
- Qsp 1 L

2. Antibiotics concentrations

Chloramphenicol (Cm) : 2 mg/mL
Tetracycline (Tet) : 1 mg/mL
Kanamycin (Kann) : 5 mg/mL
Ampicillin (Amp) : 10 mg/mL

⟶ 50 µL antibiotic / 5mL medium

3. Apparatus
4. Material

a) Digested vector plasmid (100 ng)
b) Insert DNA fragment (3:1 to 10:1 molar ratio over vector)
c) 10X T4 DNA Ligase Reaction Buffer 2 µl
d) T4 DNA Ligase 1 µl

How to do :

1. Preparation

a) Verify that the vector plasmid and the insert DNA fragment are completely digested by transform them into competent bacteria and spread them on agar plate containing the matching antibiotic.
b) Adjust the insert DNA fragment volume by calculating the matching mass:

$$M_{insert\ DNA} = 100ng * \frac{size\ of\ insert}{size\ of\ vector}$$

c) Make sure the total volume of vector DNA and insert DNA doesn't exceed 17µL.

2. Ligation

a) In an Eppendorf tube (tube 1), add the vector DNA, the insert DNA, the buffer ligase, the ligase. Adjust the total volume to 20 µL with sterile water. Redo the same thing in another tube (tube 2).

b) Take out 5µl content of tube 1 in another Eppendorf tube (tube 3). Adjust the volume to 10µl. Inactive immediately the ligase by thermic shock (10 min, 15°C). Keep the content for electrophoresis later.

c) Put the rest contents of tube 1 and tube 2 into incubation (between 1 to 3 hours, 22°C). Inactive the ligase in both tubes by thermic shock (10 min, 15°C).

d) Take out 5µl content of tube 1 in another Eppendorf tube (tube 4). Adjust the volume to 10µl. Keep the content for electrophoresis later.

e) Check the ligation by electrophoresis with tube 3 and 4.

3. Control

a) In an Eppendorf tube (tube 5), add the vector DNA, the buffer ligase, the ligase. Adjust the total volume to 20 µL with sterile water. This tube is designed to control the probability of the vector closing with the original segment.

b) In an Eppendorf tube (tube 6), add the vector DNA. Adjust the total volume to 20µL with sterile water. This tube design to control the digestion outcome.

4. Step 4

a) Transform the content of tube 2, 5 and 6 into competent bacteria.