



Ligation

Time : 45 min

I. Principle

This is a molecular biology technique which allows to ligate a DNA fragment to another one or to a vector with an enzyme: T4 DNA ligase.

II. Material

- T4 DNA Ligase
- T4 DNA Ligase Buffer
- MilliQ water
- 0,2 mL PCR tube
- 10 and 20 μ L pipettes
- Digestion products

III. Method

- In a PCR tube, numbered, introduce :
 - 2 μ L of T4 DNA Ligase Buffer
 - 1 μ L of T4 DNA Ligase
 - 12 μ L of digested inserts
 - 4 μ L of digested vectors } (ratio 3 :1 \rightarrow 3 inserts for 1 vector)
 - 1 μ L of nuclease-free water
- Incubate at **15 minutes** at room temperature.
 - 1) Use 3 μ L for the transformation
 - 2) Incubate 17 μ L at **16°C overnight**
- **Measure** the concentration of the samples, **note it** and **conserve** the products on the « ligation » rack at **4°C**.