Genetic Engineering laboratory protocol

Ligation of λ DNA /EcoR I digest using T4 DNA Ligase

Objectives:

1. To perform Ligation of λ DNA/EcoR I digest using T4 DNA Ligase.

Introduction:

The enzyme ligase, catalyzes the formation of phosphodiester bonds between juxtaposed 5 'phosphate and a 3' hydroxyl terminus of double stranded DNA. It can join double stranded DNA restriction fragments having either blunt ends or homologous cohesive ends. (Fig.1). In living organisms, ligases are essential enzymes with critical roles in DNA replication and repair. In laboratory, this reaction is used to join double stranded DNA fragments with blunt or cohesive ends to form recombinant DNA plasmids.

There are two types of DNA Ligases used in nucleic acid research, E. coli ligase and T4 DNA ligase. They differ in their requirement of energy source and in their ability to ligate blunt ends. T4 DNA ligase is approximately 60000 dalton (60 kD) protein produced by Bacteriophage T4 requiring ATP as the energy source. T4 ligase uses ATP, while E. coli ligase uses NAD. Another important difference is their ability to ligate blunt ends; under normal reaction conditions, only T4 DNA ligase will ligate blunt ends.

In this experiment we will use T4 DNA ligase to join EcoR I digested λ DNA sample.

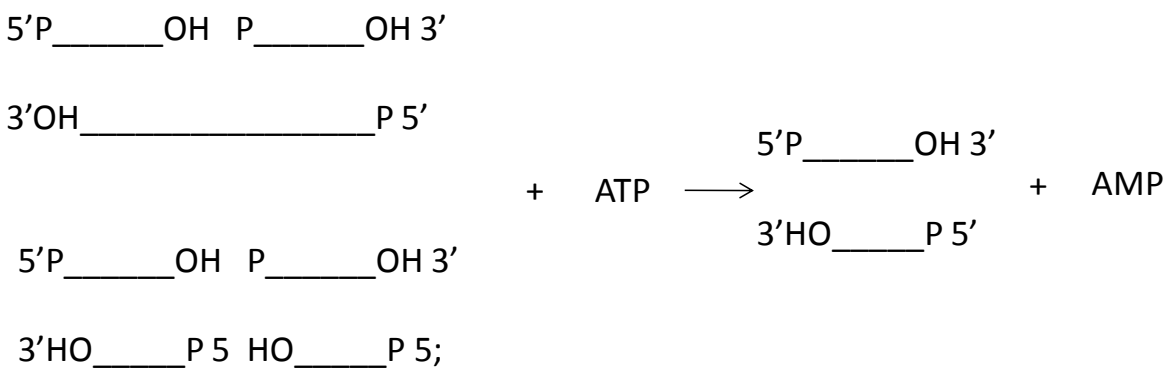

Fig 1.

Procedure: (Total volume of ligation reaction: 10 µl)

1. Thaw the ligase Assay Buffer and λ DNA/EcoR I Digest vials in ICE.
2. Place T4 DNA Ligase and a PCR tube on ice (or mini –cooler). Pipette 0.5 µL of ligase to the PCR tube, label as ligated sample.
   Note: Place the T4 DNA Ligase vial back in the freezer (immediately).
3. To the vial labeled as ligated sample, add 4.5 µL of λ DNA/EcoR I Digest.
4. Add 5 µL of 2X Ligase Assay Buffer and mix by tapping.
5. Incubate at 16 °C for 2 hours in a pre-set water bath for ligation.
6. Meanwhile, prepare 0.8% agarose gel for electrophoresis.
7. Add 2 µL of Gel Loading Buffer (6X) to the ligated sample at the end of 2 hours.
8. Pipette 4 µL λ DNA/EcoRI , 2 µl of Gel Loading Buffer and 4 µl of water into a vial, label it as **control.**
9. Load the ligated sample and control sample in the wells of the 0.8% agarose gel.
10. Electrophorese the samples at 90 V for 1 hours and visualize under a UV-Transilluminator.

Observation:

Examine the band(s) of ligated sample and compare it with control sample. Record your observations.

Interpretation:

From the gel, one can observe that the six double standed fragments formed by digestion of λDNA with *EcoR* I are ligated by T4 DNA Ligase to give a single band.