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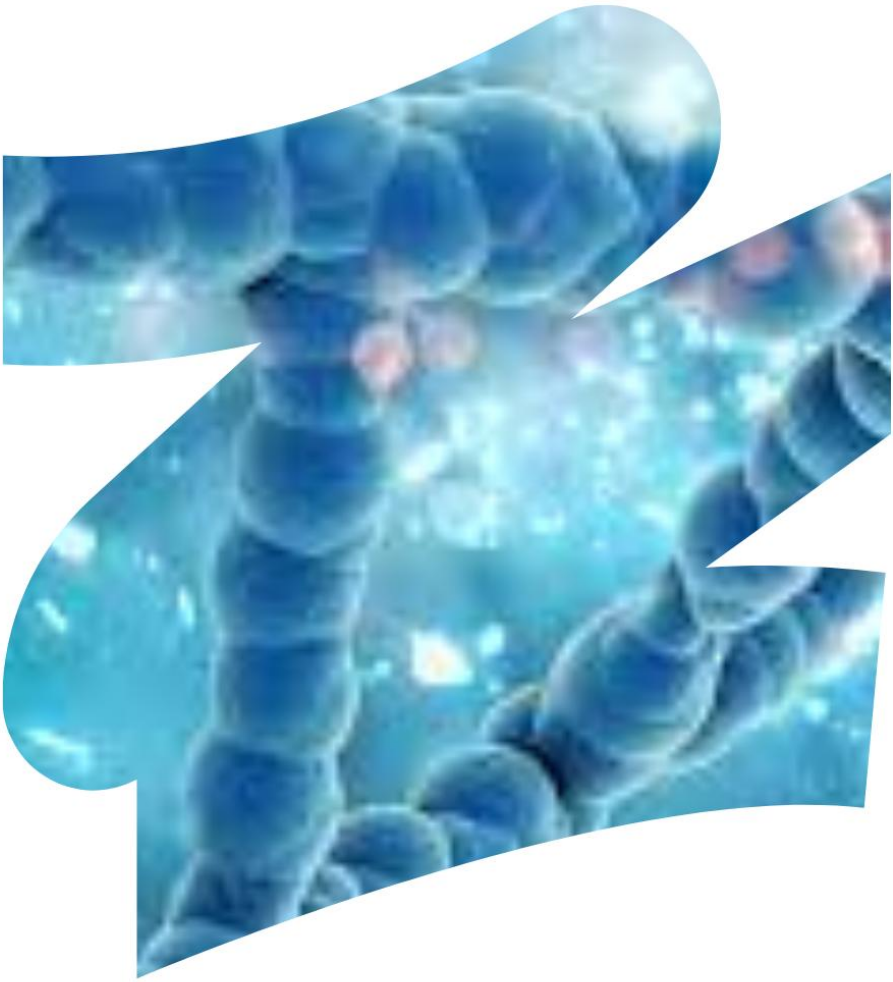

SN Ligation Teaching Kit

Solution Based

Innovation Speaks

SHANKARANARAYANA LIFE SCIENCES
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Ligation Teaching Kit

Cat no : SNLS - TK021

Number of experiments that can be performed:5/20

Duration of Experiment

Protocol: 3.5 hours Agarose Gel Electrophoresis: 1 hour

Storage Instructions:

- The kit is stable for 12 months from the date of manufacture
- Store DNA digest, T4 DNA Ligase and 10X Ligase Buffer at -20°C
- Store 6X Gel Loading Buffer at 2-8 °C
- Other kit contents can be stored at room temperature (15-25°C)

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Aim:

To perform ligation of DNA PstI digest and observing the efficiency of ligation reaction through agarose gel electrophoresis.

Introduction:

Two linear DNA molecule ends (either from the same or different molecules) can be joined together through a process called ligation. This process involves the formation of a covalent bond between two DNA fragments (having blunt or overhanging, complementary, 'sticky' ends) by the help of a specialized enzyme named as ligase. DNAligase forms a phosphodiester bond between the 3' hydroxyl of one nucleotide and the 5' phosphate of another. This process is the key player in constructing recombinant DNA molecule.

Principle:

Recombinant DNA is made possible by two important enzymes, restriction enzymes and DNA ligase. Restriction enzymes "cut" DNA at a specific location and DNA ligase is used to "glue" two fragments of DNA together. DNA ligation is theprocess through which two DNA molecule ends from the same or different molecules are joined together. During this process a phosphodiester bond is formed between the 3' hydroxyl group of one fragment and the 5' phosphate ofanother. This ligation reaction is catalyzed by a DNA ligase enzyme which ligates DNA fragments having blunt or overhanging, complementary, ends. It is easier to ligate molecules with complementary sticky ends than blunt ends. The commonly used DNA ligases in nucleic acid research is T4 DNA ligase and E. coli DNA ligase. E. coli DNA ligase is morespecific for cohesive ends than T4 DNA ligase but can't be used for cloning purpose. T4 DNA ligase is the most versatile and commonly used ligase for DNA cloning. T4 DNA ligase is approximately 60000 dalton (60 kD) protein produced by Bacteriophage T4. This ATP dependent enzyme covalently joins blunt or compatible cohesive ends, as well as nicks in double-stranded DNA. A 5'-phosphoryl group is required for ligation to a 3'-hydroxyl group. Generally cohesive end ligation is carried out at lower temperature (12°C to 16°C) for the maintenance of a good balance between annealing of ends and activity of the enzyme. Blunt end

ligation can be carried out at 24°C as annealing of ends is not a factor. Due tothe lack of cohesive ends blunt end ligation is more complex compared to cohesive end ligation.

A typical ligation reaction requires the following components:

- Two or more fragments of DNA that have either blunt or compatible cohesive ends
- A buffer which contains ATP
- T4 DNA ligase

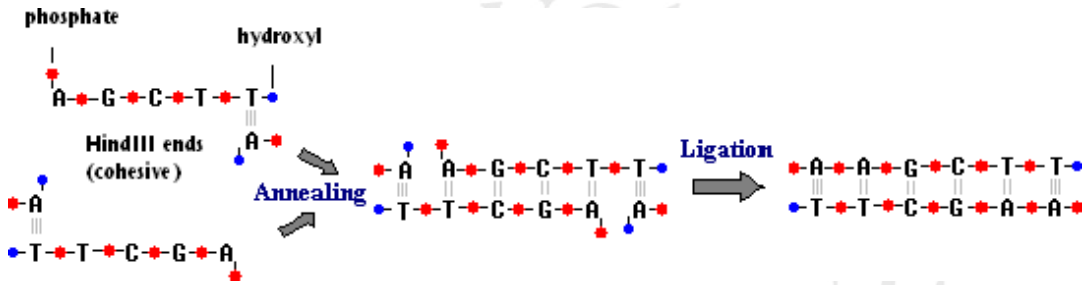

Fig 1: Schematic representation of a ligation reaction

Kit Contents:

This kit demonstrates the ligation of DNA- Pst1 digest using T4 DNA ligase

Table 1: Enlists the materials provided in this kit with their quantity and recommended storage

Sr. No.	Materials Provided	Quantity		Storage
		5 expts	20 expts	
1	DNA-PstI digest	0.035 ml	0.11 ml	-20°C
2	10X Ligase Assay Buffer	0.005 ml	0.02 ml	-20°C
3	T4 DNA Ligase	0.005 ml	0.02 ml	-20°C
4	Molecular Biology GradeWater (DEPC-treated)	0.015 ml	0.06ml	R T
5	Agarose	1.5 g	12 g	R T
6	5X TBE	15 ml	60 ml	R T
7	6X Gel Loading Buffer	0.01 ml	0.04ml	2-8°C
8	Polypropylene Tube (0.2 ml)	05 Nos.	20 Nos.	R T

Always give a quick spin before opening the vial as the liquid material may stick to the wall or to the cap of thevial

Materials Required but Not Provided:

Glasswares: Measuring cylinder, Beaker
Reagents: Ethidium bromide (10 mg/ml), Distilled water
Other requirements: Electrophoresis apparatus, UV Transilluminator, Water Bath, Micropipettes, Tips, Adhesive tape,Crushed ice, Microwave/ Hotplate/ Burner

Storage:

SN Ligation Teaching Kit is stable for 12 months from the date of manufacture without showing any reduction inperformance. On receipt, store the Lambda DNA - PstI digest, T4 DNA Ligase and 10X Ligase Assay Buffer at -20 ^{O}C. 6X Gel Loading Buffer should be stored at 2-8^{O}C. Other kit contents can be stored at room temperature (15-25^{O}C).

Important Instructions:

1. Read the complete protocol thoroughly before beginning the experiment to ensure proper understanding and execution.
2. Keep T4 DNA ligase and 10X ligase buffer on ice at all times during the experiment, as they are temperature-sensitive reagents.
3. Thaw the ligase buffer on ice and return it to -20 °C immediately after use to maintain its stability.
4. Use fresh pipette tips for each reagent to avoid cross-contamination.
5. Add the T4 DNA ligase last when assembling the reaction mixture to preserve its activity.

Procedure:

1. Before starting the experiment, crush ice and place the vials containing Lambda DNA- *PstI* digest, 10X ligasebuffer and T4 DNA ligase onto it.
2. In this experiment Lambda DNA- *PstI* digest is ligated with T4 DNA ligase.
3. Set up the reaction mixture as follows:

- DNA- *PstI* digest	– 5.0 µl
- 10X Ligase Assay Buffer	– 1.0 µl
- Molecular Biology Grade Water	- 3.0 µl
- T4 DNA Ligase	– 1.0 µl
Total	**10 µl**

4. After preparing the reaction tube, mix the components by gentle pipetting and tapping.
5. Incubate the tubes at 16^{O}C waterbath for 3 hours.
6. After incubation run the samples on agarose gel as given below.

Agarose Gel Electrophoresis:

Preparation of 0.5X TBE: To prepare 100 ml of 0.5X TBE buffer add 10 ml of 5 X TBE Buffer to 90 ml of sterile distilledwater*. Mix well before use.

Preparation of agarose gel: To prepare 30 ml of 1% agarose gel, measure 0.3 g agarose in a glass beaker or flask andadd 30 ml of 0.5X TBE buffer. Heat the mixture on a microwave or hot plate or burner by swirling the glass beaker/flask occasionally, until agarose dissolves completely (Ensure that the lid of the flask is loose to avoid buildup of pressure). Allow the solution to cool to about 55-

60^{O}C. Add 0.5 µl Ethidium bromide (10 mg/ml), mix well and pour the gel solutioninto the gel tray.

Allow the gel to solidify for about 30 minutes at room temperature.

NOTE: Ethidium bromide is a powerful mutagen and is very toxic. Appropriate safety precautions should be taken bywearing latex gloves; however, use of nitrile gloves is recommended.

Loading of the DNA samples: To prepare sample for electrophoresis, take 1µl of 6X gel loading buffer and 5 µl of DNA- *PstI* digest in a tube, mix well by pipetting and load the sample into the first well. Add 2 µl of 6X gel loading buffer to the ligation mix, mix well by pipetting and load the sample into the next well.

Electrophoresis: Connect power cord to the electrophoretic power supply according to the conventions: Red-Anode andBlack- Cathode. Electrophorese at 100-120 V and 90 mA until dye markers have migrated an appropriate distance.

*Molecular biology grade water is recommended

Flowchart:

Keep all the components on ice

Prepare reaction mixture for the ligation reaction

Mix gently and incubate at 16oC for 3 hours

Visualize the ligated band after electrophoresing on agarose g

Observation and Result:

Perform Agarose Gel Electrophoresis. Visualize the DNA bands (ligated as well as unligated) using UV Transilluminator.

Lane 1: DNA- *PstI* digest (unligated)Lane 2: Ligated sample

Fig 2: gel image of ligation reaction observed after Agarose Gel Electrophoresis

After running the ligated and unligated samples on agarose gel, check the bands of both the sample and compare the band pattern of two samples.

Interpretation:

After running the ligated and unligated samples on agarose gel, one can observe that the seven double strandedfragments formed by digestion of DNA with *PstI* are ligated by T4 DNA ligase to give a single band.

Troubleshooting Guide:

Sl.No.	Problem	Possible Cause	Solution
1	Partial or no ligation	Ligation temperature not maintained properly	Incubate the samples at 16°C
		Improper addition of enzyme and buffer	Add appropriate amount of enzyme and buffer as given in the protocol
		Components of the reaction mixture not mixed properly	Ensure that all the components are thoroughly mixed by gentle pipetting after preparing the reaction mixture
		Degradation of T4 DNA ligase or buffer	Always place the vials containing ligase and buffer on ice as they are temperature sensitive

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