A NEW GENERATION COMPANY

SN Restriction Digestion Teaching Kit

Solution Based

Innovation Speaks

SN Restriction Digestion Teaching Kit

Cat no: SNLS-TK020

Duration of Experiment

Protocol: 2 hours Agarose Gel Electrophoresis: 1hour

Storage Instructions:

- The kit is stable for 12 months from the date of manufacture
- Store Lambda DNA, DNA Marker, Restriction Enzymes and the Buffers at-20^{O}C
- Store 6X Gel Loading Buffer at 2-8^{O}C
- Other kit contents can be stored at room temperature(15-25^{O}C)

For technical assistance please contact our technical Support:
Email: drmondal@snlifesciences.com / Phone: +91 63613 81129
www.snlifesciences.in

A NEW GENERATION COMPANY

INDEX

Aim:

Toper form restriction digestion of Lambda(λ) DNA using EcoRV enzymes.

Introduction:

In1978, the Nobel Prize for Medicine was awarded to Werner Arber, Daniel Nathans and Hamilton Smith for their discovery of restriction endonucleases, which led to the development of recombinant DNA technologies. The first practical use of restriction enzymes in science and medicine was the manipulation of E.coli bacteria to express recombinant human insulin for the treatment of diabetis. The restriction enzymes have been discovered in many different bacteria and other unicellular organisms. These restriction enzymes are able to scan along a length of DNA looking for a particular sequence of bases that they recognize.

Principle:

Restriction Digestion involves fragmenting DNA molecules into smaller pieces with special enzymes called Restriction Endonucleases commonly known as Restriction Enzymes (RE). Because of this property restriction enzymes are also known as molecular scissors. The restriction enzymes are named from the cellular strain from which they are isolated. Restriction enzymes recognize specific sequences in the double stranded DNA molecule and then cut the DNA to produce fragments, called restriction fragments. The target site or sequence which the restriction enzyme recognizes is generally from 4 to 6 base pairs, arranged in a palindromic sequence. Once it is located, the enzyme will attach to the DNA molecule and cut each strand of the double helix. The restriction enzyme will continue to do this along the full length of the DNA molecule which will then break into fragments. The size of these fragments is measured in base pairs or kilo base pairs (1000bases).

Common Restriction Enzymes:
Every restriction enzyme has unique target sites for digestion. Lambda DNA has multiple restriction sites for both EcoRV which result into several fragments of varying sizes.

Enzyme	Source	Recognition Sequence
EcoRV	Escherichia coli	5'-GAT|ATC-3' 3'-CTA|TAG-5'

- Construction of recombinant DNA molecules
- Mapping the locations of restriction sites in DNA
- Southern Blot Hybridization
- Construction of DNA Libraries

Kit Contents:
This kit demonstrates restriction digestion of Lambda DNA with EcoRV enzymes

Table1: Enlists the materials provided in this kit with their quantity and recommended storage

Sr.No.	Materials Provided	Quantity 10expts	Storage
1	Lambda DNA	0.120ml	- 20°C
2	DNA Marker	0.02ml	- 20°C
3	Agarose	3g	RT
4	Restriction Enzyme: EcoRV	0.005ml	- 20°C
6	10X Assay Buffer for EcoRV	0.025ml	- 20°C
7	5X TBE	100ml	RT
8	6XGel Loading dye	0.03ml	2-8°C
9	Polypropylene Tubes(0.5ml)	20Nos.	RT

Always give a quick spin before opening the vial as the liquid material may stick to the wall or to the cap of the vial Materials Required but Not Provided:
Glasswares: Measuring cylinder, Beaker
Reagents: Ethidium bromide(10mg/ml),Distilled water
Other requirements: Electrophores is apparatus, UV Transilluminator, Heating block or Water Bath, Vortex Mixer, Micropipettes, Tips, Adhesive tape, Crushed ice, Microwave/Hotplate/Burner

Storage:
SN Restriction Digestion Teaching Kit is stable for 12 months from the date of manufacture without showing any reduction in performance. On receipt, store the Restriction Enzymes, Assay Buffers, DNA Marker

and Control DNA at -20^{O}C. 6X Gel Loading dye should be stored at 2-8^{O}C. All other kit contents can be stored at room temperature (15-25^{O}C).
Important Instructions:

1. Read the entire procedure carefully before starting the experiment.
2. The restriction enzymes are temperature sensitive and should always be placed on ice during the experiment.
3. While performing the experiment place the assay buffers and restriction enzymes on ice.
4. Use fresh tip while adding different solution to the tube.
5. While preparing the reaction mixture the enzymes4should always be added at last.

Procedure:

1. Before starting the experiment, crush ice and place the vials containing Lambda DNA, Restriction Enzymes and Assay Buffers onto it.
2. In this experiment, Lambda DNA is digested with restriction enzymes; EcoRV.
3. Set up the reaction mixture as follows:

Reaction mixture (EcoRV digestion)

Lambda(λ)DNA	–12.0µl
10X Assay Buffer of EcoRV	– 2.5µl
EcoRV	–0.5µl
Total	**15µl**

4. After preparing the two reaction tubes, mix the components by gentle pipetting and tapping.
5. Incubate the tubes at 37°C for 2 hour.
6. After 2 hour incubation, immediately place the vials at room temperature (15-25^{O}C) for 10 minutes.
7. 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 10ml of 5X TBE Buffer to 90ml of sterile distilled water*. 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 and add 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

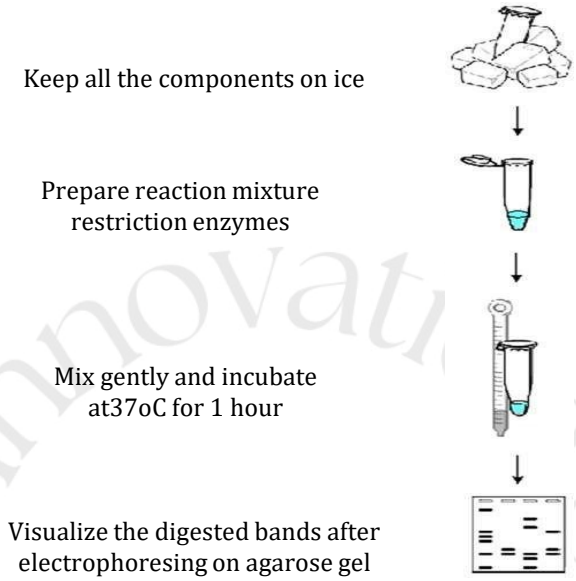
about 55-60^{O}C. Add 0.5µl Ethidium bromide (10mg/ml), mix well and pour the gel solution into the gel tray. Allow the gel to solidify for about 30 minutes at room temperature.

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

Loading of the DNA samples: Load 5µl of ready to use DNA Marker into the well. To prepare sample for electrophoresis, add 2µl of 6X gel loading dye to 15µl of digested product. Mix well by pipetting and load the samples into the well.

Electrophoresis: Connect the power cord to the electrophoretic power supply according to the conventions: Red-Anode and Black- Cathode. Electrophorese at 100-120 V and 90mA until dye markers have migrated an appropriate distance, depending on the size of DNA to be visualized.

Flowchart:



Observation and Result:
Perform Agarose Gel Electrophoresis. Visualize the DNA bands using UV Transilluminator.

Lanes 1 2

Lane1: DNA Marker
Lane2: λdna digested with EcoRV

Fig1: λ DNA digested with EcoRV

After running the digested samples on agarose gel, look for the digestion pattern of the two restriction enzymes. Compare the size of each fragment with that of the DNA marker.

Interpretation:

Restriction digestion patterns of lambda DNA obtained upon treatment with EcoRV are markedly different which demonstrates the fact that each restriction enzyme recognizes and cleaves only a specific base sequence unique to it. The size of the fragments can be determined by comparing with that of the DNA marker ran on the same gel.

Troubleshooting Guide:

S.No.	Problem	Possible Cause	Solutio n
1	Partial or no digestion	Insufficient incubation time	Incubate the samples for longer time at 37°C (120 minutes)
		Improper addition of restriction enzyme	Always add the restriction enzyme at the end of the reaction mixture and add appropriate amount 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
		Assay buffers inter mixed	Always add the specific buffer for the given enzyme i.e. 10X EcoRV assay buffer for EcoRV. Do not inter change the buffers and enzymes
		Degradation of restriction enzymes	Always place the vials containing restriction enzymes on ice as they are temperature sensitive
2	Star activity	Reaction mixture incubated for longer time than specified in the protocol	Do not exceed the incubation time beyond 2 hour
		Improper restriction enzyme addition	Add exact amount of restriction enzyme as per the procedure, avoid pipetting error
3	Improper resolution of bands on agarose gel	Gel not run for sufficient duration	Run the gel for longer period of time till the bands are separated properly

Technical Assistance:

SN we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance mail at www.snlifesciences.com

Shankaranarayana Life Sciences LLP
57/90,3rd Road, Bommasandra Industrial Area, 4th Phase, Bengaluru Urban, Karnataka-560099

SHANKARANARAYANA LIFE SCIENCES
A NEW GENERATION COMAPANY

Contact Us -

+91- 63613 81129 / 99005 05076
www.snlifesciences.in
sales@snlifesciences.com /drmondal@snlifesciences.com
No.57/90, 3rd Road, 4th Phase Bommasandra Industrial Area, Bengaluru - 560099

2025 - 2026

#startupindia DIPP Department of Industrial Policy & Promotion ISO STARTUP KARNATAKA birac MAKE IN INDIA