Restriction Digestion of genomic DNA by use of Restriction enzymes e.g. Eco RI, Hind III, Bam H I and gel electrophoresis for visualization

By

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Key Topics

- Introduction
- Requirement
- Procedure
- Observation Profile
- Tips

Introduction

- Restriction digestion is a technique in which broken down (digested) large DNA molecules at specific sites into smaller pieces using the action of special enzymes is called restriction digestion.
- These special enzymes are known as restriction endonucleases, exonucleases, molecular scissors (restriction enzymes or REs).
- Werner Arber, Daniel Nathans and Hamilton Smith in 1978 received the Nobel Prize for their discovery of restriction endonucleases.
- The digestion is required in a number of applications like southern blotting, preparation of DNA libraries, DNA fingerprinting (RFLP, AFLP) etc.
- Restriction enzymes may be classified into four types based on their subunit structure, cofactor requirements, specificity of cleavage, and associated methylase activity.
- There are large numbers of restriction enzymes obtained from different bacteria, each of which recognizes unique restriction sites and cuts DNA differently.
- The recognition sequences usually vary between 4 and 8 nucleotides.

- Enzyme Activity: One unit of restriction enzyme activity is defined as the amount of enzyme required to produce a complete digest of 1 µg of DNA in 60 min at 37°C in a 50 µL reaction volume.
- Details of Restriction Enzymes which will be used:

Enzyme	Source	Recognition sequence	Cut
EcoRI	*Escherichia coli*	5'GAATTC 3'CTTAAG	5'---G ..... AATTC---3' 3'---CTTAA.....G---5'
BamHI	*Bacillus amyloliquefaciens*	5'GGATCC 3'CCTAGG	5'---G.....GATCC---3' 3'---CCTAG.....G---5
HindIII	*Haemophilus influenzae*	5'AAGCTT 3'TTCGAA	5'---A .....AGCTT---3' 3'---TTCGA .....A---5'

Equipment and accessories

- Glassware and Plastic ware
- Micro centrifuge tube
- Ice box and ice
- Dry Bath incubator
- Dispensers
- Micropipettes with autoclaved Micro tips

Chemicals and buffers

- Genomic DNA
- Restriction endonucleases (e.g. Eco RI, Hind III, BAM HI)
- Digestion buffer
- Autoclaved doubled distilled water

Buffer

- Restriction enzymes are provided with different buffers.
- These buffers have appropriate salts and sometimes include essential cofactors that are necessary for the enzyme to function.
- Major function of the buffer is to maintain pH of the reaction (usually, 8.0) and provide a favorable environment for the enzyme to function.
- Most companies provide about 4 different kinds of buffers. (called A, B, C, D, etc.)
- Occasionally **a** “unique buffer” for a particular enzyme which are provided as 10X buffer.

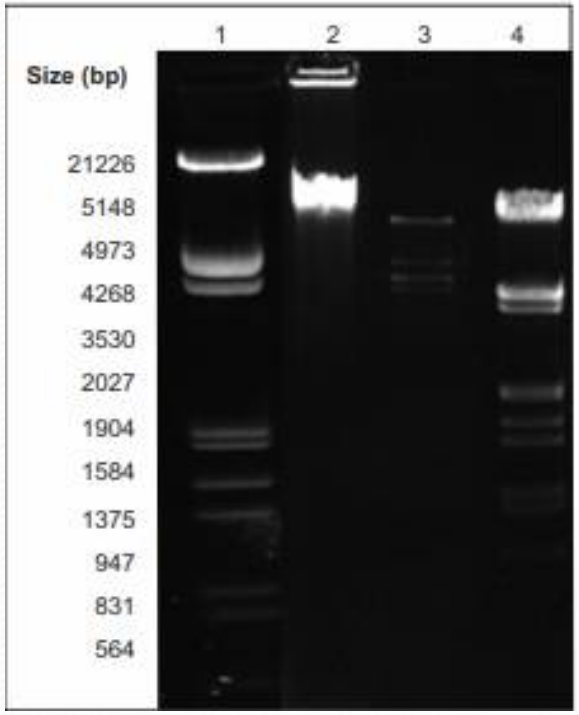
Procedure

- Components of Restriction Digestion Reaction Mixture given below (For a 30 µL reaction)
- Spin ⟶ Vortex ⟶ Spin
- This mixture is incubated at 37˚C for 3 hours.
- Spin ⟶ Vortex ⟶ Spin

S. No.	Components	Tube 1 EcoRI	Tube 2 Hind III	Tube 3 BAM HI	Tube 4 Hind III + EcoRI
1.	Milli-Q water	23 µL	23 µL	23 µL	22 µL
2.	gDNA (30 ng/µL)	3 µL	3 µL	3 µL	3 µL
3.	10X Buffer	3 µL (buffer D)	3 µL (unique buffer)	3 µL (buffer B)	3 µL (buffer D)
4.	Restriction enzyme (20 U/µL)	1 µL	1 µL	1 µL	1 µL + 1 µL

Visualization/Observation

- The digested DNA samples are visualized on agarose gel to check for digestibility.
- Undigested genomic DNA and a λ marker is also loaded for comparison.



Lane 1: l/EcoRI+Hind III digested ladder (Fermentas)
Lane 2: l DNA (undigested) (Fermentas)
Lane 3: l DNA digested with EcoRI (6 fr agments of which 4 are clearly visible)
Lane 4: l DNA digested with EcoRI and Hind III (11 fragments of which 9 are clearly visible here)

Tips

- All restriction enzymes should be stored at – 20°C.
- Restriction enzyme proteins are stored in glycerol. The enzyme should not be more than 1/10 of final volume of the reaction as 10% concentration of glycerol inhibits digestion and cause star activity. This leads to non-specific and aberrant cleavage of the DNA molecule.
- Compatible buffers should be used, while setting up a double digestion reaction.
- Enzyme and buffer should be kept on ice throughout the process.

Thanks