Restriction Digest

Introduction

The Restriction Digest protocol is used to cleave DNA at specific recognition sequences using restriction endonucleases. This procedure enables the generation of defined DNA fragments for downstream molecular biology applications, including cloning, plasmid verification, DNA mapping, and genetic engineering. This technique allows sequence-specific digestion of DNA by restriction enzymes under optimized reaction conditions.

Reference:
1. Restriction Digest Protocol | NEB

Materials

- 10X Buffer corresponding (standard or unique)
- Additives
  - Adenosine 5´-Triphosphate (ATP) (supplied when required)
  - rAlbumin (supplied when required)
  - DTT (supplied when required)
  - PaqCI Activator (supplied when required)
- DNA sample
- Nuclease-free Water

Procedure

Reaction Set-up

1. Set up the following reaction as follows on ice.

   Please note that enzymes should always be added last.
   Additive (such as DTT, Activator or ATP) may be required for certain enzymes.

Set-up list

	A	B	C
1	**COMPONENTS**	**50 µl REACTION**	**FINAL CONC. OR AMOUNT**
2	DNA	1 µg	
3	10X Buffer	5 µl	1X
4	Restriction Enzyme	1µl	
5	Nuclease-free Water	to 50 µl	Varies

Operation

2. Gently mix the reaction by pipetting up and down and microfuge briefly.

3. Incubate at 37°C for 1-2 hours.

4. Stop the reaction.

   If no further manipulation of DNA is required, use 10 µl per 50 µl reaction of stop solution containing EDTA.