Restriction Digest Protocol

Materials

- 10X NEBuffer (standard or unique)
- 6X Purple Load Dye (may be supplied)
- Additives if needed:
  - Adenosine 5´-Triphosphate (ATP) (supplied when required)
  - rAlbumin (supplied when required)
  - DTT (supplied when required)
  - PaqCI Activator (supplied when required)

Materials required but not supplied:

- DNA
- Nuclease-free Water (NEB #B1500)

Overview

Restriction enzyme digests require proper quantities of DNA, enzyme, and buffer, in an appropriate reaction volume ratio. For molecular cloning, the “typical” reaction conditions below use 5-10x over digestion to compensate for variabilities in DNA sample quality and quantity. Time-Saver™ qualified restriction enzymes and High-Fidelity (HF®) restriction enzymes perform under a broader range of recommended conditions. Critically, individual restriction endonuclease recommended reaction times and temperatures can vary significantly.

NEBcloner® tool is strongly recommended to design reactions for successful digests.

Additional information to optimize restriction enzyme digests can be found in our usage guidelines: Optimizing Restriction Endonuclease Reactions and Restriction Enzyme Tips.

Reaction set-up:

Set up the following reaction as follows on ice.

(Please note that enzymes should always be added last.)

COMPONENTS	50 µl REACTION	FINAL CONC. OR AMOUNT
DNA	1 µg	
10X NEBuffer**	5 µl	1X
Restriction Enzyme	1µl	
Nuclease-free Water	to 50 µl	Varies*

**We recommend 5-10 units of enzyme per ug of DNA. Adjust according to protocol at NEBcloner.neb.com. For convenience,1µl can be used.*

***Additive (such as DTT, Activator or ATP) may be required for certain enzymes.*

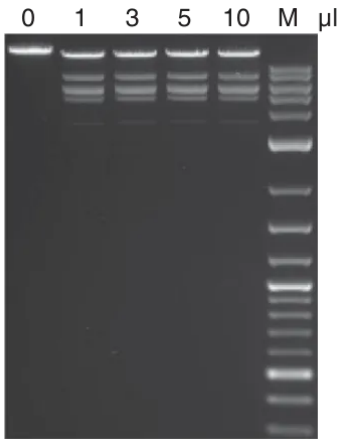
Digest protocol:

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

2. Incubate at temperature and time ranges recommended for the specific restriction endonuclease.

3. Stop the reaction.
   - If no further manipulation of DNA is required, use 10 µl per 50 µl reaction of stop solution containing EDTA,(e.g. Gel Loading Dye, Purple (6X), Gel Loading Dye, Purple (6X), no SDS, or Gel Loading Dye, Orange (6X))

Gel with restriction enzyme digested Lambda DNA



50 µl digestion reactions were set up using a range of amounts of EcoRI-HF® restriction enzyme, 1 µg of Lambda DNA, and the recommended reaction buffer, resulting in no star activity in overnight digests at 37°C, even when used at higher concentrations. Marker M is the 1 kb DNA Ladder (NEB# N3232). The Monarch Spin DNA Gel Extraction Kit (NEB #T1120) is recommended for DNA gel extraction and purification

General Guidelines

1. If further manipulation of DNA is required, use either heat inactivation at the appropriate temperature (65°C or 80°C) for 20 minutes (please note not all enzymes are inactivated by heat), phenol/chloroform extraction, or spin column purification (e.g.Monarch® Kits for DNA Cleanup) to remove the enzyme.

2. For highly concentrated DNA for downstream applications, use the Monarch Spin DNA Gel Extraction Kit (NEB #T1120) to attain high-quality DNA purification with low-elution volumes.

Related Resources

- Optimizing Restriction Endonuclease Reactions
- Restriction Enzyme Tips
- NEBcloner®
- NEBcutter®
- NEBioCalculator®
- REBASE®
- Double Digest Finder
- Enzyme Finder
- Competitor Cross-Reference Tools