Optimizing Restriction Endonuclease Reactions

Overview

There are several key factors to consider when setting up a restriction endonuclease digest. Using the proper amounts of DNA, enzyme and buffer components, in the correct reaction volume will allow you to achieve optimal digestion. By definition, 1 unit of restriction enzyme will completely digest 1 µg of substrate DNA in a 50 µl reaction in 60 minutes. This Enzyme : DNA : Reaction volume ratio can serve as a guide when designing reactions. However, most researchers follow the "typical" reaction conditions listed, in which a 5–10 fold over digestion is recommended to overcome variability in DNA source, quantity and purity. The following tips will help achieve maximal success in restriction endonuclease reactions.

A "Typical" Restriction Digest

Restriction Enzyme	10 units is sufficient, generally 1 µl is used
DNA	1 µg
10X NEBuffer	5 µl (1X)
Total Reaction Volume	50 µl
Incubation Time	1 hour*
Incubation Temperature	Enzyme dependent

* Can be decreased to 5-15 minutes by using a Time-Saver™ Qualified enzyme.

Enzyme

For additional information, please visit Restriction Enzyme Tips

- Keep on ice when not in the freezer
- Should be the last component added to reaction
- Mix components by pipetting the reaction mixture up and down, or by "flicking" the reaction tube. Follow with a quick ("touch") spin-down in a microcentrifuge. Do not vortex the reaction.
- In general, we recommend 5–10 units of enzyme per µg DNA, and 10–20 units for genomic DNA in a 1 hour digest.
- NEB has introduced a line of High-Fidelity (HF®) enzymes that provide added flexibility to reaction setup.
- Some restriction enzymes require more than one recognition site to cleave efficiently. These are designated with the “multi-site” icon 

. Please review recommendations on working with these enzymes.

DNA

- Should be free of contaminants such as phenol, chloroform, alcohol, EDTA, detergents or excessive salts. Extra wash steps during purification are recommended.

- Methylation of DNA can inhibit digestion with certain enzymes. For more information about methylation, Effect of CpG Methylation on Restriction Enzyme Cleavage and Dam and Dcm Methylases of *E.coli*

Buffer

- Use at a 1X concentration
- Supplement with rAlbumin, DTT or Activator to recommended concentration if required

Reaction Volume

- A 50 µl reaction volume is recommended for digestion of 1 µg of substrate
- Enzyme volume should not exceed 10% of the total reaction volume to prevent star activity due to excess glycerol
- Additives in the restriction enzyme storage buffer (e.g., glycerol, salt) as well as contaminants found in the substrate solution (e.g., salt, EDTA, or alcohol) can be problematic in smaller reaction volumes. The following guidelines can be used for techniques that require smaller reaction volumes.

	Restriction Enzyme*	**DNA**	**10X NEBuffer**
50 µl rxn	10 units	1 µg	5 µl

* Restriction Enzymes can be diluted using the recommended diluent buffer when smaller amounts are needed.

Incubation Time

- Incubation time is typically 1 hour
- Can often be decreased by using an excess of enzyme, or by using one of our Time-Saver Qualified enzymes.
- It is possible, with many enzymes, to use fewer units and digest for up to 16 hours. For more information, visit Extended Digests with Restriction Endonucleases.

Stopping a Reaction

If no further manipulation of DNA is required:

- Terminate with a stop solution (10 µl per 50 µl rxn) [1x: 2.5% Ficoll®-400, 10mM EDTA, 3.3mM Tris-Hcl, 0.08% SDS, 0.02% Dye 1, 0.001% Dye 2, pH 8.0@25°C] (e.g., NEB #B7024)

When further manipulation of DNA is required:

- Heat inactivation can be used for some enzymes
- Remove enzyme by using the Monarch® Spin PCR & DNA Cleanup Kit (5 µg) (NEB #T1130) or phenol/chloroform extraction.

Storage

- Storage at -20°C is recommended for most restriction enzymes. For a few enzymes, storage at -80°C is recommended for periods longer than 30 days. Please refer to the enzyme's technical data sheet or catalog entry for storage information.
- 10X NEBuffers should also be stored at -20°C

Stability

- All enzymes are assayed for activity every 4 months. The expiration date is found on the label.
- Exposure to temperatures above -20°C should be minimized whenever possible

Control Reactions

If you are having difficulty cleaving your DNA substrate, we recommend the following control reactions:

- Control DNA (DNA with multiple known sites for the enzyme, e.g. lambda or adenovirus-2 DNA) with restriction enzyme to test enzyme viability
- If the control DNA is cleaved and the experimental DNA resists cleavage, the two DNAs can be mixed to determine if an inhibitor is present in the experimental sample. If an inhibitor (often salt, EDTA or phenol) is present, the control DNA will not cut after mixing.