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Restriction Digest -- CHEM 384/584

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The following is a "typical" restriction endonuclease reaction. Please see the "guidelines" tab below for the NEB tips on optimizing restriction digests.

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https://www.neb.com/protocols/2012/12/07/optimizing-restriction-endonuclease-reactions

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Guidelines for Optimizing Restriction Endonuclease Reactions

Enzyme

- Keep in the enzyme storage box (Cool Box) when not in the freezer . Try to not remove the enzyme tube from the box while pipetting. Aliquots of enzymes may be provided during class where many students are digesting DNA simultaneously.

- 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.
- High-Fidelity (HF®) enzymes provide added flexibility to reaction setup.

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 SAM (S-Adenosyl methionine) to the 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**
10 µl rxn**	1 unit	0.1 µg	1 µl
25 µl rxn	5 units	0.5 µg	2.5 µl
50 µl rxn	10 units	1 µg	5 µl

* Restriction Enzymes can be diluted using the recommended diluent buffer when smaller amounts are needed.
** 10 µl rxns should not be incubated for longer than 1 hour to avoid evaporation.

Incubation Time

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- 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
- Remove enzyme by using a spin column or phenol/chloroform extraction

Storage

- Storage at -20°C is recommended for most restriction enzymes. For a few enzymes, storage at-70°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 CutSmart Buffer 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.

1 Set up the following reaction (total reaction volume **50 µl**).

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Restriction Enzyme	10 units is sufficient, generally 1µl is used
DNA	1 µg
10X CutSmart Buffer	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 volume should not exceed 10% of the total reaction volume to prevent star activity due to excess glycerol.

A 50 µl reaction volume is recommended for digestion of 1 µg of substrate.

The enzyme should be the last component added to reaction

Keep Enzyme in the enzyme storage box while at the bench rather than removing it and placing it on ice.

2 Mix components by pipetting the reaction mixture up and down, or by "flicking" the reaction tube. **Do not vortex the reaction.**

3 Quick ("touch") spin-down in a microcentrifuge.

4 Incubate for 1 hour at the enzyme-specific appropriate temperature.
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See the NEB Activity/Performance Chart for the incubation temperatures.

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