Optimizing Restriction Endonuclease Reactions

- **Materials**
  - Restriction enzymes (EcoR1 and SpeI) (10 units is sufficient, generally 1µl is used)
  - DNA 1 ug
  - 10X NEBuffer (2.1) 5 uL (1X)
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- **Equipment**

Requirements:

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

Before you start

Enzyme: 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.

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

Process:

1. Set up the following reaction in an eppendorf tube

DNA	1 ug
10X NEBuffer (2.1)	5 uL
Restriction enzyme	1 uL
Water	

2. Mix components by pipetting the reaction mixture up and down, or by "flicking" the reaction tube.
3. Quick ("touch") spin-down in a microcentrifuge. Do not vortex the reaction.
4. Incubate for 1 hour at the enzyme-specific appropriate temperature.

Stopping a Reaction

If no further manipulation of DNA is required:

Terminate with a stop solution (10 µl per 50 µl rxn) [2.5% Ficoll®-400, 11 mM EDTA (pH 8.0), 3.3 mM Tris-HCl, 0.017% SDS, 0.015% bromophenol blue] (i.e., NEB #B7021)

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 product page for storage information. 10X NEBuffers should also be stored at -20°C

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