# Single-temperature Double Digest

## Introduction

This is the Double Digest Protocol with Standard Restriction Enzymes, using a common reaction and same incubation temperature for both enzymes.

More information from NEB can be found here.

Double Digests can be designed using NEB's Double Digest Finder.

See the NEBuffer Activity/Performance Chart with Restriction Enzymes for the incubation temperatures.

NEBcloner will help guide your reaction buffer selection when setting up double digests.

## Materials

- DNA **1 µg**
- NEBuffer
  - 1X
- NEB Restriction Enzymes
- Deionized Water

## Procedure

### Single Temperature DD Reaction

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

Table2

| | A | B |
|---|---|---|
| 1 | | **Reagent Volumes (µl)** |
| 2 | **Buffer (10x)** | 5 |
| 3 | **DNA *** | Input Volume for ng |
| 4 | **Restriction Enzyme #1 **** | 1 |
| 5 | **Restriction Enzyme #2 **** | 1 |
| 6 | **Deionized Water (µl)** | 48 |
| 7 | **Total Volume (µl)** | 50 |

* Recommended maximum of 1 µg of substrate per 10 units of enzyme.
** Restriction Enzymes should be added to the mixture last.

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.

01:00:00

Can be decreased to 5-15 minutes by using a Time-Saver™ Qualified Restriction Enzyme
See the NEBuffer Activity/Performance Chart with Restriction Enzymes for the incubation temperatures.