Sequential Double Digest

Introduction

This is the Sequential Double Digest Protocol with Standard Restriction Enzymes. If there is no buffer in which the two enzymes exhibit > 50% activity, this sequential digest can be performed. More information from NEB can be found here. Double Digests can be designed using NEB's Double Digest Finder. NEBcloner will help guide your reaction buffer selection when setting up double digests. See the NEBuffer Activity/Performance Chart with Restriction Enzymes for the incubation temperatures.

Materials

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

Procedure

Sequential Double Digest

1. Set up the following reaction using the restriction endonuclease that has the lowest salt concentration in its recommended buffer (total reaction volume 50 µl).

2. Set up the following digest reaction on ice

	Volume (µl)	
Buffer (10x)	5	
DNA *	Input Volume for ng	
Restriction Enzyme #1 **	1	
Deionized Water (µl)	1	
Total Volume (µl)	#VALUE!	

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

- ** Restriction Enzyme, 10 units is sufficient, generally 1 µl is used

- ***The enzyme should be the last component added to reaction

3. Mix components by pipetting the reaction mixture up and down, or by "flicking" the reaction tube.

4. Quick ("touch") spin-down in a microcentrifuge. Do not vortex the reaction.

5. Incubate for 1 hour at the enzyme-specific appropriate temperature.

- 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.

6. Adjust the salt concentration of the reaction (using a small volume of a concentrated salt solution) to approximate the reaction conditions of the second restriction endonuclease.

- Alternatively, a spin column can be used to isolate the DNA prior to the second reaction.

7. Add the second enzyme.

- ** Restriction Enzyme #2, 10 units is sufficient, generally 1 µl is used

8. Mix components by pipetting the reaction mixture up and down, or by "flicking" the reaction tube.

9. Quick ("touch") spin-down in a microcentrifuge. Do not vortex the reaction.

10. Incubate for 1 hour at the enzyme-specific appropriate temperature.

- 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.