Digestion with NEBNext dsDNA Fragmentase (M0348)

Isabel Gautreau

Abstract

Protocol for digestion with NEBNext dsDNA Fragmentase (M0348)

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Guidelines

Adequate mixing of NEBNext dsDNA Fragmentase is important for the success of this reaction. NEBNext dsDNA Fragmentase should be vortexed for 3 seconds prior to use.

For tough digestions, add 1 µl of 200 mM MgCl2 to the reaction. Additional MgCl2 can be added if necessary.

The protocol listed below is for fragmentation of 5 ng–3 µg of DNA.

End Repair: Clean up the fragmented DNA (e.g. column purification, or using SPRI) then proceed with desired DNA end repair protocol.

Agarose Gel Size Selection/Analysis: Samples can be loaded directly on to an agarose gel. It is not necessary to clean up the reactions prior to loading.

Polyacrylamide Gel Analysis: Clean up the fragmented DNA (e.g. column purification) prior to loading the samples on a PAGE gel.

Long Term Storage: Clean up the fragmented DNA (e.g. column purifications, or SPRI Beads*) prior to long term storage.

*Note: If using SPRI Beads for sample purification, it is recommended to dilute the sample 1:1 with sterile water to allow for faster collection of beads to the magnet.

Before start

Adequate mixing of NEBNext dsDNA Fragmentase is important for the success of this reaction. NEBNext dsDNA Fragmentase should be vortexed for 3 seconds prior to use.

The protocol listed below is for fragmentation of 5 ng–3 µg of DNA.

Materials

NEBNext dsDNA Fragmentase - 50 rxns M0348S by New England Biolabs

Protocol

Step 1.
Vortex NEBNext dsDNA Fragmentase for 3 seconds

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This protocol is for fragmentation of 5 ng–3 µg of DNA.
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Adequate mixing of NEBNext dsDNA Fragmentase is important for the success of this reaction.

Step 2.
Quick spin and place on ice

Step 3.
Mix together the following components in a sterile PCR tube:

PROTOCOL

. M0348 Digestion Mixture

CONTACT: New England Biolabs

Step 3.1.
DNA (5 ng–3 µg), **1–16 µl**

NOTES
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If the starting material is 100 ng or less, incubation times should be increased by 10 minutes.

Step 3.2.
10X Fragmentase Reaction Buffer v2, **2 µl**

AMOUNT
2 µl Additional info:

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The Fragmentase Reaction Buffer v2 now contains BSA, eliminating the need to add it separately. The buffer also has increased Mg++ which improves the uniformity of fragmentation across different conditions.

Step 3.3.
Sterile Water to **18 µl**

Step 4.
Add **2.0 µl** dsDNA Fragmentase

AMOUNT
2 µl Additional info:

Step 5.
Vortex the mixture for 3 seconds

Step 6.
Incubate at 37°C for the recommended times below to generate the desired fragment size

Desired Fragment Size (bp)	Incubation Time (min)
50–200	25–35
200–1,000	15–25
1,000–2,000	10–15

NOTES
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If starting material is 100 ng or less, incubation times should be increased by 10 minutes.

Step 7.
Add **5 µl** of 0.5 M EDTA to stop the reaction.

AMOUNT
5 µl Additional info:

NOTES
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DNA fragments are ready for DNA end repair, size selection or analysis (see guidelines for more information).