Digestion with NEBNext dsDNA Fragmentase (M0348) Version 2

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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.

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 immediately before use. Quick spin and place on ice.

NOTES
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Adequate mixing of NEBNext dsDNA Fragmentase is important for the success of this reaction.

Fragmentase is highly viscous. For reproducible results it is critical to pipette the same volume into each sample.

Step 2.

Combine the following components in a sterile PCR tube and vortex:

component	amount
DNA (5 ng–3 µg)	1–16 µl
10X Fragmentase Reaction Buffer v2	2 µl
Sterile Water	variable
Final Volume	**18 µl**

PROTOCOL

. M0348 Digestion Mixture

CONTACT: New England Biolabs

NOTES
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For tough digestions add 1 µl of $MgCl_2$ to the reaction. Additional $MgCl_2$ can be added if necessary.

Step 2.1.
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 2.2.
10X Fragmentase Reaction Buffer v2, **2 µl**

AMOUNT
2 µl Additional info:

NOTES
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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 2.3.
Sterile Water to **18 µl**

Step 3.

Add **2.0 µl** dsDNA Fragmentase and vortex mixture for 3 seconds.

AMOUNT
2 µl Additional info:

NOTES
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Adequate mixing of NEBNext dsDNA Fragmentase is important for the success of this reaction.

Fragmentase is highly viscous. For reproducible results it is critical to pipette the same volume into each sample.

Step 4.
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. The exact fragmentation time for a given sample type should be determined experimentally using a time course study.

Step 5.

Add **5 µl** of 0.5 M EDTA and vortex or mix to stop the reaction.

AMOUNT
5 µl Additional info:

NOTES
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Samples must be cleaned up before most downstream steps.

Step 6.

Clean up the fragmented DNA with column purification or using SPRI beads.

If using SPRI beads, it is recommended to dilute the sample 1:1 with water for easier handling of the

sample and faster collection of the beads to the magnet.

NOTES

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SPRI beads are available from Beckman Coulter: A63880, A63881, A63882 www.beckmancoulter.com

Bioanalyzer: Clean up the fragmented DNA prior to loading on a Bioanalyzer chip.

End Repair: Clean up the fragmented DNA then proceed with desired DNA end repair protocol.

Polyacrylamide Gel Analysis: Clean up the fragmented DNA prior to loading the samples on a PAGE gel.
Long Term Storage: Clean up the fragmented DNA prior to long term storage.

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.