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Feb 23, 2022

Digestion with NEBNext dsDNA Fragmentase (M0348) V.3

New England Biolabs[1]

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dx.doi.org/10.17504/protocols.io.bccgistw

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NEBNext dsDNA Fragmentase is an enzyme-based reagent that shears DNA to produce fragments of the desired sizes in a time-dependent manner, for next generation sequencing library preparation protocols

- dsDNA Fragmentase provides random fragmentation, similar to mechanical methods (1,2).

DOI

dx.doi.org/10.17504/protocols.io.bccgistw

https://www.neb.com/protocols/0001/01/01/digestion-with-nebnext-dsdna-fragmentase-m0348

New England Biolabs 2022. Digestion with NEBNext dsDNA Fragmentase (M0348). **protocols.io**
https://dx.doi.org/10.17504/protocols.io.bccgistw
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M0348, dsDNA, Fragmentation, Fragmentase, Shear, DNA, NGS

protocol ,

Feb 09, 2020

Feb 23, 2022

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MATERIALS

NEBNext dsDNA Fragmentase - 250 rxns **New England Biolabs Catalog #M0348L**

NEBNext dsDNA Fragmentase - 50 rxns **New England Biolabs Catalog #M0348S**

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

Adequate mixing of NEBNext dsDNA Fragmentase is important for the success of this reaction. NEBNext dsDNA Fragmentase should be vortexed for **00:00:03 prior to use**.

For tough digestions, add **1 µL** of **200 Milimolar (mM) MgCl2** to the reaction. Additional $MgCl_2$ can be added if necessary.

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

1 Vortex NEBNext dsDNA Fragmentase for **00:00:03** , quick spin and place **On ice** .

2

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

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

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Add **2 µL dsDNA Fragmentase** and vortex mixture for **00:00:03** .

Fragmentase is very viscous and should be pipetted slowly. If the enzyme has been sitting for several minutes vortex it again before adding to the sample.

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Incubate at **37 °C** for the recommended times below to generate the desired fragment size:

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

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

To determine the exact incubation time for a given sample type, a time course study should be performed.

5 

Add **5 µL 0.5 M EDTA** to stop the reaction.

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 sterile water for easier handling of the sample and faster collection of the beads to the magnet.

SPRI beads are available from Beckman Coulter: A63880, A63881, A63882

For further analysis:

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

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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.

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