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Digestion with NEBNext dsDNA Fragmentase (M0348) V.1

Forked from Digestion with NEBNext dsDNA Fragmentase (M0348)

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Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

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).

Materials

MATERIALS

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

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

Safety warnings

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

Before start

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 [M] 200 millimolar (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**

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

Note

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.

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

Note

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

Note

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.

Note

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 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.