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# Digestion with NEBNext dsDNA Fragmentase (M0348)

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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**Protocol Integer ID:** 100201

**Keywords:** M0348, dsDNA, Fragmentation, Fragmentase, Shear, DNA, NGS,

## 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.