Digestion with NEBNext dsDNA Fragmentase (M0348)

Protocols.io also provides an interactive version of this protocol where you can discover and share optimizations with the research community.

Introduction

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

For tough digestions, add 1 µl of 200 mM $MgCl_2$ 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.

Protocol

1. Vortex NEBNext dsDNA Fragmentase for 3 seconds, quick spin and place on ice.
2. Combine the following components in a sterile PCR tube and vortex:

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

3. Add 2.0 µl dsDNA Fragmentase and vortex the mixture for 3 seconds.
   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. To determine the exact incubation time for a given sample type, a time course study should be performed.

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

   *If starting material is 100 ng or less, incubation times should be increased by 10 minutes.
5. Add 5 µl of 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.

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.