NEBNext Ultra II FS DNA Module E7810

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ABSTRACT

The NEBNext Ultra II FS DNA Module contains the enzymes and buffers required to convert a broad range of input amounts of intact DNA into fragmented DNA with 5´ phosphorylated 3´ dA-tailed ends. The module is optimized for use with the NEBNext Ultra II Ligation Module (NEB #E7595) and with the NEBNext Ultra II Q5 Master Mix (NEB #M0544) if amplification is required. The fast, user-friendly workflow has minimal hands on time.

Note: The Ultra II FS Module is not compatible with bisulfite conversion workflows

PDF

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PROTOCOL STATUS

Working

GUIDELINES

The NEBNext Ultra II FS DNA Module is Designed for use with the Following:

NEBNext Ultra II Ligation Module (NEB #E7595)
NEBNext Ultra II Q5® Master Mix (NEB #M0544)
NEBNext Singleplex or Multiplex Oligos for Illumina®
(NEB #E7350, #E7335, #E7500, #E6609, #E7710, #E7730 or #E7600)

MATERIALS

NAME	CATALOG #	VENDOR
TE Buffer (1X)	E7808	New England Biolabs
NEBNext Ultra II FS Reaction Buffer	E7807	New England Biolabs
NEBNext Ultra II FS Enzyme Mix	E7806	New England Biolabs
Vortex	View	
Microcentrifuge	View	
0.2 ml thin wall PCR tubes	View	
PCR Machine	View	

STEPS MATERIALS

NAME	CATALOG #	VENDOR
NEBNext Ultra II FS Reaction Buffer	E7807	New England Biolabs

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BEFORE STARTING

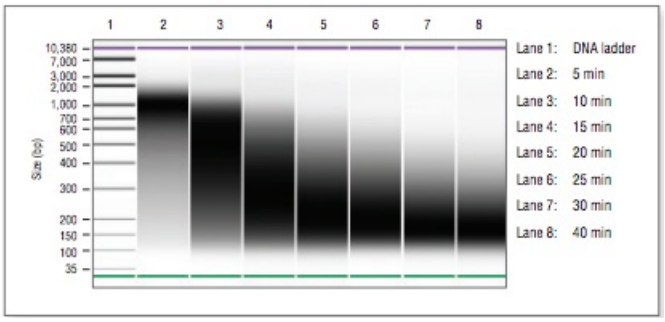
Starting Material: 100 pg–500 ng purified, genomic DNA. We recommend that the DNA be in 1X TE (10 mM Tris pH 8.0, 1 mM EDTA), however, 10 mM Tris pH 7.5–8, low EDTA TE or H2 O are also acceptable. If the input DNA is less than 26 µl, add TE (provided) to a final volume of 26 µl.

Fragmentation/End Prep

1 Fragmentation occurs during the 37°C incubation step. Use the chart below to determine the incubation time required to generate the desired fragment sizes. Incubation time may need to be optimized for individual samples. See Figure 1 for a typical fragmentation pattern.

Fragmentation Size	Incubation @ 37°C	Optimization
100 bp–250 bp	30 min	30–40 min
150 bp–350 bp	20 min	20–30 min
200 bp–450 bp	15 min	15–20 min
300 bp–700 bp	10 min	5–15 min
500 bp–1 kb	5 min	5–10 min

Figure 1: Example of size distribution on a Bioanalyzer®. Human DNA (NA19240) was fragmented for 5–40 min.



2 Ensure that the Ultra II FS Reaction Buffer is completely thawed. If a precipitate is seen in the buffer, pipette up and down several times to break it up, and quickly vortex to mix. Place on ice until use.

NEBNext Ultra II FS Reaction Buffer
by New England Biolabs
Catalog #: E7807

3 Vortex the Ultra II FS Enzyme Mix 5-8 seconds prior to use and place on ice.

NOTE

It is important to vortex the enzyme mix prior to use for optimal performance.

NEBNext Ultra II FS Enzyme Mix
by New England Biolabs
Catalog #: E7806

4 Add the following components to a 0.2 ml thin wall PCR tube on ice:

Component	Volume per One Library
DNA	26 µl
NEBNext Ultra II FS Reaction Buffer	7 µl
NEBNext Ultra II FS Enzyme Mix	2 µl
Total Volume	35 µl

NEBNext Ultra II FS Reaction Buffer
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Catalog #: E7807

NEBNext Ultra II FS Enzyme Mix
by New England Biolabs
Catalog #: E7806

5 Vortex the reaction for 00:00:05 and briefly spin in a microcentrifuge.

6 In a thermocylcer, with the heated lid set to 75°C, run the following program:

5–30 min @ 37°C
30 min @ 65°C
Hold @ 4°C

NOTE
If necessary, samples can be stores at –20°C; however, a slight loss in yield (~20%) may be observed. We recommend continuing with adaptor ligation using the NEBNext Ultra II Ligation Module (NEB #E7595) before stopping.