VERSION 2

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Protocol status: Working
We use this protocol and it's working

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NEBNext Ultra II FS DNA Module E7810 V.2

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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

Each module component must pass rigorous control standards, and for each new lot the entire set of reagents is functionally validated together with NEB #E7595 and NEB #M0544 to construct indexed libraries that are sequenced on an Illumina sequencing platform.
For larger volume requirements, customized and bulk packaging is available by purchasing through OEM & Custom Solutions at NEB. Please contact custom@neb.com for further information.

ATTACHMENTS

manual e7810 1.0 9:17.pdf

GUIDELINES

Safe Stop: This is a point where you can safely stop the protocol and store the samples prior to proceeding to the next step in the protocol.

Caution: Signifies a step in the protocol that has two paths leading to the same end point but is dependent on a user variable, like the amount of input DNA.

Color: A color listed before or after a reagent name indicates the cap color of the reagent to be added.

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®
NEBNext Oligo Kit options can be found at http://www.neb.com/oligos.
Alternatively, customer supplied adaptor and primers can be used; please see www.neb.com/faq-nonNEB-adaptos.

MATERIALS

MATERIALS

- TE Buffer (1X) **New England Biolabs Catalog #E7808**
- NEBNext Ultra II FS Reaction Buffer **New England Biolabs Catalog #E7807**
- NEBNext Ultra II FS Enzyme Mix **New England Biolabs Catalog #E7806**
- Vortex **Contributed by users**
- Microcentrifuge **Contributed by users**
- 0.2 ml thin wall PCR tubes **Contributed by users**
- PCR Machine **Contributed by users**

STEP MATERIALS

- NEBNext Ultra II FS Reaction Buffer **New England Biolabs Catalog #E7807**
- NEBNext Ultra II FS Enzyme Mix **New England Biolabs Catalog #E7806**
- NEBNext Ultra II FS Reaction Buffer **New England Biolabs Catalog #E7807**
- NEBNext Ultra II FS Enzyme Mix **New England Biolabs Catalog #E7806**

Materials that you may need that are not provided with this kit include:
0.2 ml thin wall PCR tubes
PCR Machine
Vortex
Microcentrifuge

BEFORE START INSTRUCTIONS

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 H_2O 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

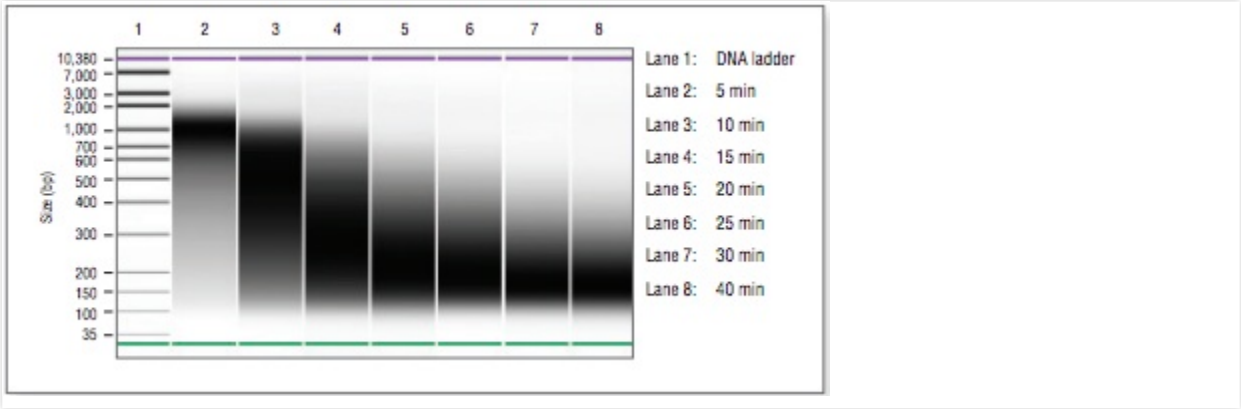
1h 50m 13s

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.

	A	B	C

	A	B	C
	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 mins.



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 **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 **New England Biolabs Catalog #E7806**

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

	A	B
	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 **New England Biolabs Catalog #E7807**

NEBNext Ultra II FS Enzyme Mix **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: 30m

5-30min @ 37 °C

00:30:00 **@** 65 °C

Hold @ 4 °C

Note

Safe Stop Point: 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.