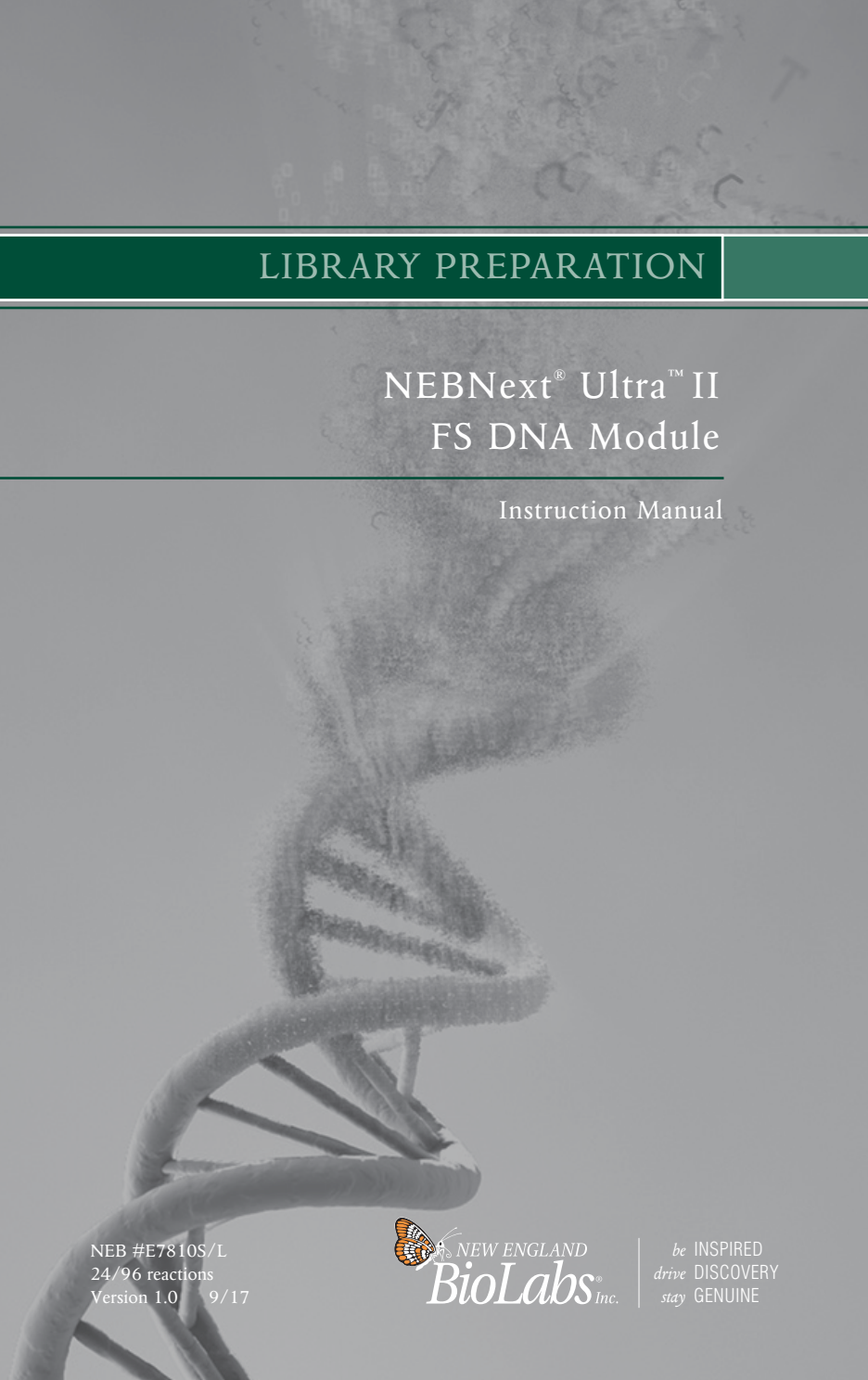
LIBRARY PREPARATION

NEBNext® Ultra™ II FS DNA Module

Instruction Manual



NEB #E7810S/L
24/96 reactions
Version 1.0 9/17

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drive DISCOVERY
stay GENUINE

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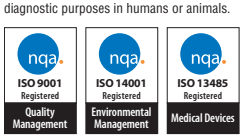

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The NEBNext Ultra II FS DNA Module Includes:

The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E7810S) and 96 reactions (NEB #E7810L). All reagents should be stored at –20°C. Colored bullets represent the color of the cap of the tube containing the reagent.

- (yellow) NEBNext Ultra II FS Enzyme Mix
- (yellow) NEBNext Ultra II FS Reaction Buffer

TE Buffer (1X)

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)

Required Materials Not Included:

0.2 ml thin wall PCR tubes

PCR Machine

Vortex

Microcentrifuge

Overview

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 quality 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 the OEM/Bulks department at NEB. Please contact OEM@neb.com for further information.

Protocol

Symbols

This caution sign signifies a step in the protocol that has multiple paths leading to the same end point but is dependent on a user variable, like the amount of input DNA.

Colored bullets indicate the cap color of the reagent to be added to a reaction.

Stopping points in the protocol.

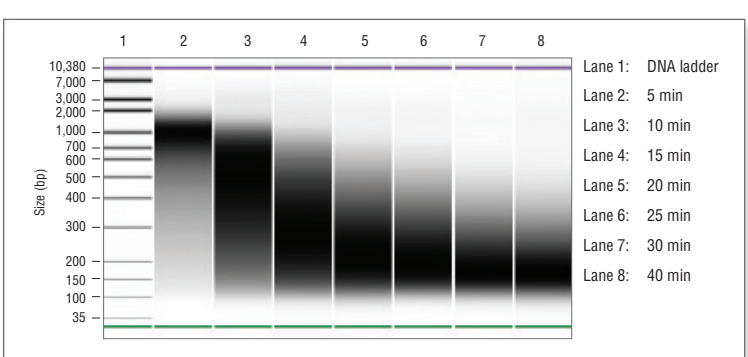
Starting Material: 100 pg–500 ng purified, genomic DNA. We recommend that the DNA be in 1X TE (10 mM Tris pH 8.0, 1 mM EDTA), however, 10 mM Tris pH 7.5–8, low EDTA TE or H_2O are also acceptable. If the input DNA is less than 26 µl, add TE (provided) to a final volume of 26 µl.

1.1. Fragmentation/End Prep

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



1.1.1. 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.

1.1.2. 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.

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

COMPONENT	VOLUME PER ONE LIBRARY
DNA	26 µl
(yellow) NEBNext Ultra II FS Reaction Buffer	7 µl
(yellow) NEBNext Ultra II FS Enzyme Mix	2 µl
Total Volume	35 µl

1.1.4. Vortex the reaction for 5 seconds and briefly spin in a microcentrifuge.

1.1.5. 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

If necessary, samples can be stored 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.

Kit Components

Each set of reagents is functionally validated and compared to the previous lot through construction of libraries using the minimum and maximum amount of Universal Human Reference Total RNA. The previous and current lots are sequenced together on the same Illumina flow cell and compared across various sequence metrics including individual transcript abundances, 5´→3´ transcript coverage, and fraction of reads mapping to the reference

NEB #E7810S Table of Components

NEB #	PRODUCT NAME	VOLUME
E7808A	TE Buffer (1X)	1.1 ml
E7807A	NEBNext Ultra II FS Reaction Buffer	0.168 ml
E7806A	NEBNext Ultra II FS Enzyme Mix	0.048 ml

NEB #E7810L Table of Components

NEB #	PRODUCT NAME	VOLUME
E7808AA	TE Buffer (1X)	4.3 ml
E7807AA	NEBNext Ultra II FS Reaction Buffer	0.672 ml
E7806AA	NEBNext Ultra II FS Enzyme Mix	0.192 ml

Revision History:

Revision #	Description
1.0	N/A

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