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NEBNext® Ultra™ II End Repair/dA-Tailing Module (NEB #E7546)

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1 Works for me dx.doi.org/10.17504/protocols.io.4nngvde

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ABSTRACT

The NEBNext Ultra II End Repair/dA-Tailing Module is optimized to convert 500 pg-1 µg of fragmented DNA to repaired DNA having 5′ phosphorylated, 3′ dA-tailed ends.

This module is part of the Ultra™ II workflow, and is optimized for use with the NEBNext™ Ultra II Ligation Module (NEB #E7595), for Illumina®-compatible library construction.

This module is also compatible with some Oxford Nanopore MinION™workflows.

This module is designed for use with NEBNext Singleplex or Multiplex Oligos for Illumina (NEB #E7350, #E7335, #E7500, #E7600 or #E7535), NEBNext Ultra II Ligation Module (NEB #E7595), and NEBNext Ultra II Q5 Master Mix (NEB #M0544).

Kits that include reagents for every step in the Ultra II DNA library construction workflow are also available (NEBNext Ultra II DNA Library Prep Kit for Illumina (NEB #E7645) and NEBNext Ultra II DNA Library Prep with Sample Purification Beads (NEB #E7103).

EXTERNAL LINK

https://www.neb.com/-/media/catalog/datacards-or-manuals/manuale7546.pdf?rev=221a72d9f6624cfda4fc2fe760691f02

GUIDELINES

Safe Stop Point: 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 point.

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

MATERIALS

NAME	CATALOG #	VENDOR
NEBNext Ultra II End Prep Reaction Buffer	E7647	New England Biolabs
NEBNext Ultra II End Prep Enzyme Mix	E7646	New England Biolabs

BEFORE STARTING

Starting Material: 500 pg–1 µg fragmented DNA. We recommend that DNA be sheared in 1X TE. If the DNA volume post shearing is less than 50 µl, add 1X TE to a final volume of 50 µl. Alternatively, 10 mM Tris-HCl, pH 8.0 or 0.1X TE can be used.

NEBNext End Prep

1 Mix the following contents in a sterile nuclease-free tube:

Component	Volume
(green) NEBNext Ultra II End Prep Enzyme Mix	3 µl
(green) NEBNext Ultra II End Prep Reaction Buffer	7 µl
Fragmented DNA	50 µl

Total Volume	**60 µl**

2 Set a 100 µl or 200 µl pipette to 50 µl and then gently pipette the entire volume up and down at least 10 times to mix thoroughly. Perform a quick spin to collect all liquid from the sides of the tube.

Note: It is important to mix well. The presence of a small amount of bubbles will not interfere with performance.

1h

3 Place in a thermocycler, with the heated lid set to ≥ 75°C, and run the following program:

00:30:00 at **20 °C**

00:30:00 at **65 °C**

Hold at **4 °C**

Safe Stop Point: 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 before stopping.

4 Proceed directly to NEBNext Ultra II Ligation Module NEB #E7595.