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NEBNext® Ultra™ II End Repair/dA-Tailing Module (NEB #E7546) V.1

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

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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**).

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

MATERIALS

NEBNext Ultra II End Prep Reaction Buffer **New England Biolabs Catalog #**E7647

NEBNext Ultra II End Prep Enzyme Mix **New England Biolabs Catalog #**E7646

Troubleshooting

Before start

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

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

3 Place in a thermocycler, with the heated lid set to ≥ 75°C, and run the following program: 1h

00:30:00 at 20 °C
00:30:00 at 65 °C
Hold at 4 °C

Note

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.