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NEBNext Ultra II Ligation Module (NEB # E7595) for NEBNext Ultra II End Repair/dA Tailing Module (NEB #E7546) V.2

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External link: https://www.neb.com/products/e7595-nebnext-ultra-ii-ligation-module#Protocols,%20Manuals%20&%20Usage_Manuals

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

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Abstract

This module is part of the Ultra™ II workflow, and is optimized for use with the NEBNext®Ultra II End Repair/dA-Tailing Module (NEB #**E7546**), for Illumina®-compatible library construction.

The NEBNext Ultra II Ligation Module is optimized for use with the NEBNext Ultra II End Repair/dA-Tailing Module (NEB #**E7546**) or the NEBNext Ultra II FS DNA Module (NEB #**E7810**).

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 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.

Adaptor Dilution Guidelines

The appropriate adaptor dilution for your sample input and type may need to be optimized experimentally. The dilutions provided here are a general starting point.

Table 1.1: Adaptor Dilution

	A	B	C
	Input	**Adaptor Dilution (Volume of adaptor: Total volume)**	**Working Adaptor Concentration**
	1 µg–101 ng	No Dilution	15 µM
	100 ng-5 ng	10-Fold (1:10)	1.5 µM
	less than 5 ng	25-Fold (1:25)	0.6 µM

Materials

MATERIALS

- Ligation Enhancer **New England Biolabs Catalog #**E7374 in Kits E7370 or E7445
- NEBNext Adaptor for Illumina **New England Biolabs Catalog #**E7337 in Kits E7335, E7500, E771
- NEBNext Ultra II Ligation Master Mix **New England Biolabs Catalog #**E7648
- USER Enzyme (Multiplex Oligos for Illumina) **New England Biolabs Catalog #**E7338

STEP MATERIALS

- USER Enzyme (Multiplex Oligos for Illumina) **New England Biolabs Catalog #**E7338
- NEBNext Ultra II Ligation Master Mix **New England Biolabs Catalog #**E7648
- NEBNext Ligation Enhancer **New England Biolabs Catalog #**E7374
- NEBNext Adaptor for Illumina **New England Biolabs**

Materials that may be needed that are not included in this kit:

Tris-HCL Buffer
10 mM NaCl

Protocol materials

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Troubleshooting

Before start

Starting Material:

500 pg–1 µg fragmented DNA that has been end repaired and dA-Tailed using the NEBNext End Repair/dA-Tailing Module (**NEB #E7546**).

Note

Caution: If DNA input is ≤ 100 ng, dilute the NEBNext Adaptor for Illumina in 10 mM Tris-HCl, pH 7.5-8.0 with with 10 mM NaCl as indicated in Table 1.1.

Ligation/End Prep

30m

1 Add the following products directly to the End Prep Reaction Mixture:

	A	B
	Component	**Volume (µl) Per Reaction**
	End Prep Reaction Mixture	60 µl
	(red) NEBNext Adaptor for Illumina**	2.5 µl
	(red) NEBNext Ultra II Ligation Master Mix*	30 µl
	(red) NEBNext Ligation Enhancer	1 µl
	Total Volume	**93.5 µl**

* Mix the Ultra II Ligation Master Mix by pipetting up and down several times prior to adding to the reaction.
** The NEBNext adaptor is provided in the NEBNext Oligo kit options, which can be found at **www.neb.com/oligos.**

NEBNext Ultra II Ligation Master Mix **New England Biolabs Catalog #**E7648

NEBNext Ligation Enhancer **New England Biolabs Catalog #**E7374

NEBNext Adaptor for Illumina **New England Biolabs**

Note

Note: The Ligation Master Mix and Ligation Enhancer can be mixed ahead of time and is stable for at least 8 hours @ 4°C. Do not premix the Ligation Master Mix, Ligation Enhancer and adaptor prior to use in the Adaptor Ligation Step.

2 Set a 100 µl or 200 µl pipette to 80 µl and then 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

Caution: The NEBNext Ultra II Ligation Master Mix is very viscous. Care should be taken to ensure adequate mixing of the ligation reaction, as incomplete mixing will result in reduced ligation efficiency. The presence of a small amount of bubbles will not interfere with performance.

3 Incubate at 20 °C for 00:15:00 in a thermal cycler **with the heated lid off**. 15m

4 Add 3 µl of (red) USER® Enzyme to the ligation mixture from Step 3.

USER Enzyme (Multiplex Oligos for Illumina) **New England Biolabs Catalog #**E7338

Note

Note: Steps 4 and 5 are only required for use with non-indexed NEBNext Adaptors. USER enzyme can be found in most NEBNext oligo kits, all options can be found on the **www.neb.com/oligos** page. If you are using the indexed UMI adaptor, USER is not needed. Please see corresponding manual for use with UMI on the E7395 product page under the protocols,manuals, and usage tab.

5 Mix well and incubate at 37 °C for 00:15:00 with the heated lid set to ≥ 47 °C . 15m

6 DNA is now ready for size selection or cleanup.

Note

Note: Please see NEB #E7645/#E7103 manual for recommended size selection/cleanup and PCR amplification protocols.

Note

Safe Stop Point: Samples can be stored overnight at –20°C.