



VERSION 2

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OPEN ACCESS

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**External link:**  
<https://www.neb.com/product/s/e7595-nebnext-ultra-ii-ligation-module#Protocols,%20Manuals%20&%20Usage>

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

**Created:** Jun 08, 2023

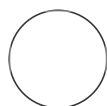
## NEBNext Ultra II Ligation Module (NEB # E7595) for NEBNext Ultra II FS DNA Module (NEB # E7810) V.2

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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](#)).

**PROTOCOL integer ID:**  
83069

**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.

### 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 2.1: Adaptor Dilution**


A	B	C
Input	Adaptor Dilution (Volume of adaptor: Total volume)	Working Adaptor Concentration
100 ng–500 ng	No Dilution	15 $\mu$ M
5 ng–99 ng	10-Fold (1:10)	1.5 $\mu$ M
less than 5 ng	25-Fold (1:25)	0.6 $\mu$ M


## MATERIALS

### MATERIALS


 NEBNext Adaptor for Illumina New England Biolabs Catalog #E7337 in Kits E7330, E7500, E771


 NEBNext Ligation Enhancer New England Biolabs Catalog #E7374


 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

 Ligation Enhancer New England Biolabs Catalog #E7374 in Kits E7370 or E7445

 NEBNext Adaptor for Illumina New England Biolabs

Materials that you may need that are not provided with this kit include:

Tris-HCL Buffer (pH 7.5)

10 mM NaCl

### BEFORE START INSTRUCTIONS

**Starting Material:** 100 pg–500 ng fragmented, end repaired and dA-Tailed DNA generated using the NEBNext Ultra II FS DNA Module ([NEB #E7810](#))

#### Note

**Caution:** If DNA input is < 100 ng, dilute the (red) NEBNext Adaptor for Illumina in 10 mM Tris-HCL, pH 7.5-8.0 with 10 mM NaCl as indicated in Table 2.1.

## DNA Ligation/End Prep

30m

- 1 Add the following components directly to the FS Reaction Mixture:

A	B
Component	Volume

A	B
FS Reaction Mixture	35 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	68.5 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 NEBNext Oligo kit options, which can be found at [www.neb.com/oligos](http://www.neb.com/oligos)



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



Ligation Enhancer New England Biolabs Catalog #E7374 in Kits E7370 or E7445



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. We do not recommend premixing 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 50 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 thermocycler **with the heated lid off**.

15m

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



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

#### Note

Note: Steps 4 and 5 are only required for use with NEBNext Adaptors. USER enzyme can be found in most NEBNext oligo kits, all options can be found on the [www.neb.com/oligos](http://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 protocols, manuals, and usage tab.

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

15m

6 DNA is now ready for size selection or cleanup.

#### Note

Note: Please see NEB #E7805/#E6177 manual for recommended size selection/cleanup and PCR amplification protocols.

#### Note

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