

NEBNext® Ultra™ II Ligation Module

for use with NEBNext Multiplex Oligos for Illumina® (Unique Dual Index UMI Adaptors DNA Set 1, NEB #E7395)

NEB #E7595S/L

24/96 reactions

Version 2.0_7/23

Table of Contents

| | |
|--|-------------------|
| Section 1 | |
| Protocol for use with NEBNext Ultra II End Repair/dA-Tailing Module..... | 2 |
| Section 2 | |
| Protocol for use with NEBNext Ultra II FS DNA Module..... | 4 |
| Kit Components | 6 |
| Revision History | 6 |

The NEBNext Ultra II Ligation Module Includes

The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E7595S) and 96 reactions (NEB #E7595L). All reagents should be stored at -20°C . Colored bullets represent the color of the cap of the tube containing the reagent.

- (red) NEBNext Ultra II Ligation Master Mix
- (red) NEBNext Ligation Enhancer

The NEBNext Ultra II Ligation Module is Designed for use with the Following

NEBNext Multiplex Oligos for Illumina® (Unique Dual Index UMI Adaptors DNA Sets 1–4)

Please note: **a separate manual exists for non-indexed adaptors**. Please see neb.com/e7595 Protocols, Manuals and Usage Section.

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

NEBNext Ultra II FS DNA Module (NEB #E7810)

NEBNext Ultra II Q5® Master Mix (NEB #M0544)

Adaptor trimming sequences:

The NEBNext libraries for Illumina resemble TruSeq® libraries and can be trimmed similar to TruSeq:

AdaptorRead 1 AGATCGGAAGAGCACACGTCTGAACTCCAGTCA

AdaptorRead 2 AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT

Applications

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).

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 #E7546 or NEB #E7810 along with 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 Customized Solutions/OEM team at NEB. Please contact custom@neb.com for further information.

Section 1

Protocol for use with NEBNext Ultra II End Repair/dA-Tailing Module (NEB #E7546)

Symbols



This caution sign 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.



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.



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

Starting Material

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



If DNA input is ≤ 100 ng, dilute the NEBNext UMI Adaptors for Illumina in **ice cold** UMI Dilution Buffer provided in the kit as indicated in Table 1.1. and keep the diluted adaptor **on ice**.

Note: 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.

Note: Thaw the NEBNext UMI DNA Adaptor Plate on ice and briefly spin, if necessary. It is important to keep the adaptor cold at all times.

Table 1.1: Adaptor Dilution.

| INPUT | ADAPTOR DILUTION (VOLUME OF ADAPTOR: TOTAL VOLUME) | WORKING ADAPTOR CONCENTRATION |
|----------------|---|----------------------------------|
| 1 µg–101 ng | No Dilution | 20 µM |
| 100 ng–5 ng | 10-Fold (1:10) | 2 µM |
| less than 5 ng | 50-Fold (1:50) | 0.4 µM |

1.1. Add the following components directly to the End Prep Reaction Mixture:

| COMPONENT | VOLUME (µl) PER REACTION |
|--|-----------------------------|
| End Prep Reaction Mixture | 60 µl |
| NEBNext UMI Adaptors 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 |

* The NEBNext UMI adaptors are provided in NEBNext Multiplex Oligos for Illumina Set 1–4). Please refer to the manual for Unique Dual Index UMI Adaptor Sets 1–4 for valid barcode combinations.

** Mix the Ultra II Ligation Master Mix by pipetting up and down several times prior to adding to the reaction.

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 pre-mix the Ligation Master Mix, Ligation Enhancer and adaptor prior to use in the Adaptor Ligation Step.

- 1.2. Set a 100 μ l or 200 μ l pipette to 80 μ l 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.

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.

- 1.3. Incubate at 20°C for 15 minutes in a thermal cycler with the heated lid off.
- 1.4. DNA is now ready for size selection or cleanup.

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



Samples can be stored overnight at –20°C.

Section 2

Protocol for use with NEBNext Ultra II FS DNA Module (NEB #E7810)

Symbols



This caution sign 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.



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.

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

Starting Material

100 pg–500 ng fragmented, end repaired and dA-Tailed DNA generated using the NEBNext Ultra II FS DNA Module (NEB #E7810).



If DNA input is ≤ 100 ng, dilute the NEBNext UMI Adaptors for Illumina in **ice cold** UMI Dilution Buffer provided in the kit as indicated in Table 2.1. and keep the diluted adaptor **on ice**.

Note: 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.

| INPUT | ADAPTOR DILUTION (VOLUME OF ADAPTOR: TOTAL VOLUME) | WORKING ADAPTOR CONCENTRATION |
|----------------|---|----------------------------------|
| 100 ng–500 ng | No Dilution | 20 μ M |
| 5 ng–99 ng | 10-Fold (1:10) | 2 μ M |
| 1 ng–5 ng | 50-Fold (1:50) | 0.4 μ M |
| less than 1 ng | 100-Fold (1:100) | 0.2 μ M |

2.1. Thaw the UMI adaptor plate on ice and add the following components directly to the FS Reaction Mixture:

| COMPONENT | VOLUME (μ l) PER REACTION |
|--|-----------------------------------|
| FS Reaction Mixture | 35 μ l |
| NEBNext UMI Adaptors for Illumina* | 2.5 μ l |
| • (red) NEBNext Ultra II Ligation Master Mix** | 30 μ l |
| • (red) NEBNext Ligation Enhancer | 1 μ l |
| Total Volume | 68.5 μl |

* The NEBNext UMI adaptors are provided in NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 1–4). Please refer to the manual for Unique Dual Index UMI Adaptor Sets 1–4 for valid barcode combinations.

** Mix the Ultra II Ligation Master Mix by pipetting up and down several times prior to adding to the reaction.

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 pre-mix the Ligation Master Mix, Ligation Enhancer and adaptor prior to use in the Adaptor Ligation Step.

- 2.2. Set a 100 μ l or 200 μ l pipette to 50 μ l 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.

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.

- 2.3. Incubate at 20°C for 15 minutes in a thermal cycler with the heated lid off.

- 2.4. DNA is now ready for size selection or cleanup.

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



Samples can be stored overnight at –20°C.

Kit Components

Each set of reagents is functionally validated together with NEB #E7546 or NEB #E7810 along with NEB #M0544 to construct index libraries that are sequenced on an Illumina sequencing platform.

NEB #E7595S Table of Components

| NEB # | PRODUCT | VOLUME |
|--------|--------------------------------------|----------|
| E7648A | NEBNext Ultra II Ligation Master Mix | 0.72 ml |
| E7374A | NEBNext Ligation Enhancer | 0.024 ml |

NEB #E7595L Table of Components

| NEB # | PRODUCT | VOLUME |
|---------|--------------------------------------|--------------|
| E7648AA | NEBNext Ultra II Ligation Master Mix | 3 x 0.960 ml |
| E7374AA | NEBNext Ligation Enhancer | 0.096 ml |

Revision History

| REVISION # | DESCRIPTION | DATE |
|------------|---|------|
| 1.0 | N/A | 3/20 |
| 2.0 | Updated information about UMIs. Also updated table formatting and legal footnote. | 7/23 |

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be INSPIRED
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