NEBNext® Quick Ligation Module

NEB #E6056S/L

20/100 reactions

Version 5.0_6/22

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The NEBNext Quick Ligation Module Includes

The volumes provided are sufficient for preparation of up to 20 reactions (NEB #E6056S) and 100 reactions (NEB #E6056L). All reagents should be stored at –20°C.

Quick T4 DNA Ligase

NEBNext Quick Ligation Reaction Buffer

The NEBNext Quick Ligation Module is Designed for use with the Following:

NEBNext End Repair Module (NEB #E6050)

NEBNext dA-Tailing Module (NEB #E6053)

NEBNext Q5® Hot Start HiFi PCR Master Mix (NEB #M0543)

NEBNext Oligo kit options can be found at neb.com/oligos

Alternatively, customer supplied adaptor and primers can be used, please see information in link below:
https://www.neb.com/faqs/2019/03/08/can-i-use-this-nebnext-kit-with-adaptors-and-primers-from-other-vendors-than-neb

Please note: This manual is not for use with UNIQUE DUAL INDEX UMI ADAPTORS.

Required Materials Not Included

- Thermal cycler
- AMPure® XP Beads (Beckman Coulter, Inc. #A63881) or SPRIselect® Reagent Kit (Beckman Coulter, Inc. #B23317)
- 10 mM Tris-HCl, pH 7.5–8.0 or 0.1 µM Tris-HCl, pH 8.0 (for adaptor dilution) or NEB #B1430
- Vortex Mixer
- Microcentrifuge
- DNase RNase free PCR strip tubes (USA Scientific® 1402-1708)
- Magnetic rack/stand (NEB #S1515, Alpaqua®, cat. #A001322 or equivalent)
- 10 mM Tris-HCl or 0.1X TE

Description

The NEBNext Quick Ligation Module has been optimized to ligate efficiently DNA adaptors compatible with Illumina sequencing to end-repaired, dA-tailed DNA fragments. The module is optimized for use with the NEBNext End Repair Module (NEB #E6050) and the NEBNext dA-Tailing Module (NEB #E6053) and is part of the original standard DNA library prep workflow, which is suitable for 1–5 µg of input DNA.

Each kit component must pass rigorous quality control standards, and for each new lot the entire set of reagents is functionally validated together by construction and sequencing of an indexed library on the Illumina sequencing platform.

For larger volume requirements, customized and bulk packaging is available by purchasing through the OEM/Bulks department at NEB. Please contact OEM@neb.com for further information.

Applications

DNA sample preparation

Ligation of DNA adaptors to blunt or dA-Tailed DNA

Ligation of blunt or dA-Tailed DNA and cloning vectors

Advantages

- Efficient – Ligates blunt DNA or dA-Tailed DNA
- Convenient – Reactions are provided in master mix format to reduce steps during DNA sample prep workflows
- Automation Friendly

Protocol for use with NEBNext Quick Ligation Module

Symbols

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.

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 type of DNA input.

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

1. Adaptor Ligation

1.1. Mix the following components in a sterile microfuge tube:

COMPONENT	VOLUME (µl) PER REACTION
• End Repaired, Blunt or dA-Tailed DNA	variable
• NEBNext Quick Ligation Reaction Buffer	10 µl
• DNA Adaptors (not provided please use adaptors appropriate to specific application)	variable
Quick T4 DNA Ligase	5 µl
Sterile H_2O	variable
Total Volume	50 µl

1.2. Incubate in a thermal cycler for 15 minutes at 20°C with the heated lid set to 30°C.

1.3. Add 3 µl of • (red) USER® Enzyme. Mix by pipetting up and down at least 10 times, and incubate at 37°C for 15 minutes.

Note: This step is only required for use with NEBNext Adaptors. USER enzyme can be found in the NEBNext Singleplex or Multiplex Oligos for Illumina (neb.com/oligos).

Note: If you need to stop at this point in the protocol, samples can be stored at –20°C. If not storing samples, proceed to sample purification.

2. Cleanup of Adaptor-ligated DNA

2.1. Vortex AMPure XP or SPRIselect Beads to resuspend.

2.2. Add 90 µl of resuspended AMPure XP or SPRIselect Beads to the ligation reaction (~53 µl). Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.

2.3. Incubate for up to ~5 minutes at room temperature.

2.4. Put the tube/PCR plate on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (~5 minutes), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain the DNA targets.

2.5 Add 200 µl of 80% freshly prepared ethanol to the tube/PCR plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.

2.6 Repeat Step 2.5 once for a total of two washes.

2.7. Air dry beads for up to 5 minutes while the tube/PCR plate is on the magnetic stand with the lid open.

Caution: Do not over-dry the beads. This may result in lower recovery of DNA target. Elute the samples when the beads are still dark brown and glossy looking, but when all visible liquid has evaporated. When the beads turn lighter brown and start to crack they are too dry.

2.8 Remove the tube/plate from the magnet. Elute the DNA target by adding 105 µl of 10 mM Tris-HCl or 0.1 X TE to the beads for bead-based size selection.

Note: For size selection using E-Gel size select gels or standard 2% agarose gels, elute the DNA target at desired volume.

2.9 Mix well on a vortex mixer or by pipetting up and down 10 times and incubate for 2 minutes at room temperature.

2.10. Put the tube/PCR plate in the magnetic stand until the solution is clear. Transfer 100 µl of supernatant (or desired volume) to a new tube/well and proceed to bead based size selection.

Table 1.1: Recommended Conditions for Dual Bead-based Size Selection

INSERT SIZE	150 bp	200 bp	250 bp	300 bp	400 bp	500 bp	700 bp
Total Library Size (insert + adaptor)	270 bp	320 bp	370 bp	420 bp	530 bp	660 bp	820 bp
Bead: DNA ratio* 1st bead selection	0.9X	0.8X	0.7X	0.6X	0.55X	0.5X	0.45X
Bead: DNA ratio* 2nd bead selection	0.2X	0.2X	0.2X	0.2X	0.15X	0.15X	0.15X

3. Size Select Adaptor Ligated DNA Using AMPure XP Beads

The following size selection protocol is for libraries with 200 bp inserts only. For libraries with different size fragment inserts, please optimize bead: DNA ratio according to Table 1.1 above. Note: (X) refers to the original sample volume of 100 µl.

3.1. Add 80 µl (0.8X) resuspended AMPure XP Beads to 100 µl DNA solution. Mix well on a vortex mixer or by pipetting up and down at least 10 times.

3.2. Incubate for 5 minutes at room temperature.

3.3. Place the tube/PCR plate on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully transfer the supernatant to a new tube/well **(Caution: do not discard the supernatant)**. Discard beads that contain the large fragments.

3.4. Add 20 µl (0.2X) resuspended AMPure XP Beads to the supernatant, mix well and incubate for 5 minutes at room temperature.

3.5. Put the tube/PCR plate on an appropriate magnetic stand to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets **(Caution: do not discard beads)**.

3.6. Add 200 µl of freshly prepared 80% ethanol to the tube/PCR plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.

3.7. Repeat Step 3.6 once.

3.8. Air dry beads for up to 5 minutes while the tube/PCR plate is on the magnetic stand with the lid open.

Caution: Do not over-dry the beads. This may result in lower recovery of DNA target. Elute the samples when the beads are still dark brown and glossy looking, but when all visible liquid has evaporated. When the beads turn lighter brown and start to crack they are too dry.

3.9. Remove the tube/plate from the magnet. Elute the DNA target from the beads by adding 17 µl of 10 mM Tris-HCl or 0.1X TE.

3.10. Mix well on a vortex mixer or by pipetting up and down at least 10 times and incubate for ~2 minutes at room temperature.

3.11. Put the tube/PCR plate in the magnetic stand until the solution is clear. Without disturbing the bead pellet, carefully transfer 15 µl of the supernatant to a clean PCR tube and proceed to enrichment.

Kit Components

NEB #E6056S Table of Components

NEB #	PRODUCT	VOLUME
E6057A	Quick T4 DNA Ligase	0.1 ml
E6058A	NEBNext Quick Ligation Reaction Buffer	0.2 ml

NEB #E6056L Table of Components

NEB #	PRODUCT	VOLUME
E6057AA	Quick T4 DNA Ligase	0.5 ml
E6058AA	NEBNext Quick Ligation Reaction Buffer	1.0 ml

Revision History

REVISION #	DESCRIPTION	DATE
1.2		3/12
2.0	Create "Kit Component – Table of Components" for small and large size kits. Delete individual component information pages	4/18
3.0	Add "Designed for Use", "Materials not Included". Update the description text and the protocol.	1/19
4.0	New Format applied	9/19
5.0	Update protocol and required materials not included	6/22

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