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NEBNext® Ultra™ II DNA PCR-free Library Prep Kit for Illumina®

NEB #E7410S/L, #E7415S/L 24/96 reactions

Version 1.0_9/21

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The Library Kit Includes

The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E7410S/#E7415S) and 96 reactions (NEB #E7410L/#E7415L). All reagents should be stored at –20°C. Colored bullets represent the color of the cap of the tube containing the reagent.

Package 1: Store at –20°C.

- (green) NEBNext Ultra II End Prep Enzyme Mix
- (green) NEBNext Ultra II End Prep Reaction Buffer
- (red) NEBNext Ultra II Ligation Master Mix
- (red) NEBNext Ligation Enhancer

Package 2: Store at room temperature. Do not freeze.

Supplied only with NEBNext Ultra II DNA PCR-free Library Prep with Sample Purification Beads, NEB #E7415.

NEBNext Sample Purification Beads

Required Materials Not Included

- 80% Ethanol (freshly prepared)
- Nuclease-free Water
- 0.2 ml thin wall PCR tubes
- DNA LoBind® Tubes (Eppendorf #022431021)
- NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 1) NEB #E7395
- Magnetic rack (NEB #S1515), magnetic plate (Alpaqua® cat. #A001322) or equivalent
- PCR machine
- Vortex
- Microcentrifuge
- Bioanalyzer®, TapeStation® (Agilent Technologies, Inc.) or similar instrument and consumables

For NEB #E7410 only:

- SPRIselect Reagent Kit (Beckman Coulter, Inc. #B23317) or AMPure® XP Beads (Beckman Coulter, Inc. #A63881)

Overview

The NEBNext Ultra II DNA PCR-free Library Prep Kit for Illumina contains the enzymes and buffers required to convert a broad range of input amounts of DNA into high quality libraries for next-generation sequencing on the Illumina platform without PCR amplification. The fast, user-friendly workflow also has minimal hands-on time.

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 indexed libraries on an 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.

Protocol

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 final library fragment size.

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

Starting Material: 250 ng–1 µg purified, genomic DNA sheared to 400 bp range. 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, samples can be diluted with 10 mM Tris-HCl, pH 8.0 or 0.1X TE.

1. NEBNext End Prep

1.1 Ensure that the Ultra II End Prep Reaction Buffer is completely thawed. If a precipitate is seen in the buffer, pipette up and down several times to break it up, and quickly vortex to mix. Place on ice until use.

1.2 Add the following components to a 0.2 ml thin wall PCR tube on ice:

COMPONENT	VOLUME PER ONE LIBRARY
Fragmented DNA	50 µl
● (green) NEBNext Ultra II End Prep Reaction Buffer	7 µl
● (green) NEBNext Ultra II End Prep Enzyme Mix	3 µl
Total Volume	60 µl

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

1.4. Place in a thermal cycler, with the heated lid set to 75°C, and run the following program:

30 minutes @ 20°C

30 minutes @ 65°C

Hold at 4°C

Proceed immediately to **Adaptor ligation** once the reaction temperature reaches 4°C.

2. Adaptor Ligation

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

COMPONENT	VOLUME
End Prep Reaction Mixture (Step 1.4)	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 (Unique Dual Index UMI Adaptors DNA Set 1, NEB #E7395). Please refer to the NEB #E7395 manual 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 at 4°C. Do not premix the adaptor with the Ligation Master Mix and Ligation Enhancer.

2.2. 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. **(Caution: The NEBNext Ultra II Ligation Master Mix is 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. Move immediately to Step 3 or place your sample at -20°C.

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

3. Size Selection of Adaptor-ligated DNA

Note: The volumes of NEBNext Sample Purification or SPRIselect Beads provided here are for use with the sample contained in the exact buffer at this step (93.5 µl; Step 2.1). These volumes may not work properly for a size selection at a different step in the workflow, or if this is a second size selection. For size selection of samples contained in different buffer conditions the volumes may need to be experimentally determined.

The following cleanup protocol is for libraries with ~ 350 bp or ~ 450 bp inserts only (Step 3.12). Size selection conditions were optimized with NEBNext Sample Purification Beads and SPRIselect beads. However, AMPure XP beads can be used following the same conditions. If using AMPure XP beads, please allow the beads to warm to room temperature for at least 30 minutes before use.

3.1. Bring the volume of the reaction to ~100 µl by adding 7 µl 0.1X TE (dilute 1X TE Buffer 1:10 with water).

3.2. Vortex NEBNext Sample Purification Beads or SPRIselect Beads to resuspend.

3.3. Add 50 µl of resuspended NEBNext Sample Purification Beads or SPRIselect beads to the 100 µl sample from Step 3.1. Mix well by pipetting up and down at least 10 times. Be careful to expel all of the liquid out of the tip during the last mix. Vortexing for 3–5 seconds on high can also be used. If centrifuging samples after mixing, be sure to stop the centrifugation before the beads start to settle out.

3.4. Incubate samples for at least 5 minutes at room temperature.

3.5. Place the tube/plate on an appropriate magnetic stand to separate the beads from the supernatant. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing on the magnetic stand.

3.6. After 5 minutes (or when the solution is clear), carefully remove and discard the supernatant that contains unwanted DNA. Be careful not to disturb the beads that contain the desired DNA targets **(Caution: do not discard beads)**.

3.7. Add 200 µl of 80% freshly prepared ethanol to the tube/plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.

3.8. Repeat Step 3.7 once. Be sure to remove all visible liquid after the second wash. If necessary, briefly spin the tube/plate, place back on the magnet and remove traces of ethanol with a p10 pipette tip.

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

Caution: Do not overdry 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.

3.10. Remove the tube/plate from the magnetic stand. Elute the DNA target from the beads into 102 µl of 0.1X TE. Mix well on a vortex mixer or by pipetting up and down 10 times. Incubate for 2 minutes at room temperature. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing back on the magnetic stand.

3.11. Place the tube/plate on a magnetic stand. After 5 minutes (or when the solution is clear), transfer 100 µl to a new tube.

3.12. Add appropriate amounts of resuspended Sample Purification or SPRIselect Beads to the sample for the desired insert sizes. Mix well by pipetting up and down at least 10 times. Be careful to expel all of the liquid out of the tip during the last mix. Vortexing for 3-5 seconds on high can also be used. If centrifuging samples after mixing, be sure to stop the centrifugation before the beads start to settle out.

INSERT SIZE	BEADS VOLUME
350 bp	65 µl
450 bp	58 µl

3.13. Incubate samples for at least 5 minutes at room temperature.

3.14. Place the tube/plate on an appropriate magnetic stand to separate the beads from the supernatant. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing on the magnetic stand.

3.15. After 5 minutes (or when the solution is clear), carefully remove and discard the supernatant that contains unwanted DNA. Be careful not to disturb the beads that contain the desired DNA targets **(Caution: do not discard beads)**.

3.16. Add 200 µl of 80% freshly prepared ethanol to the tube/plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.

3.17. Repeat Step 3.16 once. Be sure to remove all visible liquid after the second wash. If necessary, briefly spin the tube/plate, place back on the magnet and remove traces of ethanol with a p10 pipette tip.

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

Caution: Do not overdry 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.

3.19. Remove the tube/plate from the magnetic stand. Elute the DNA target from the beads into 22 µl of 0.1X TE. Mix well on a vortex mixer or by pipetting up and down 10 times. Incubate for 2 minutes at room temperature. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing back on the magnetic stand.

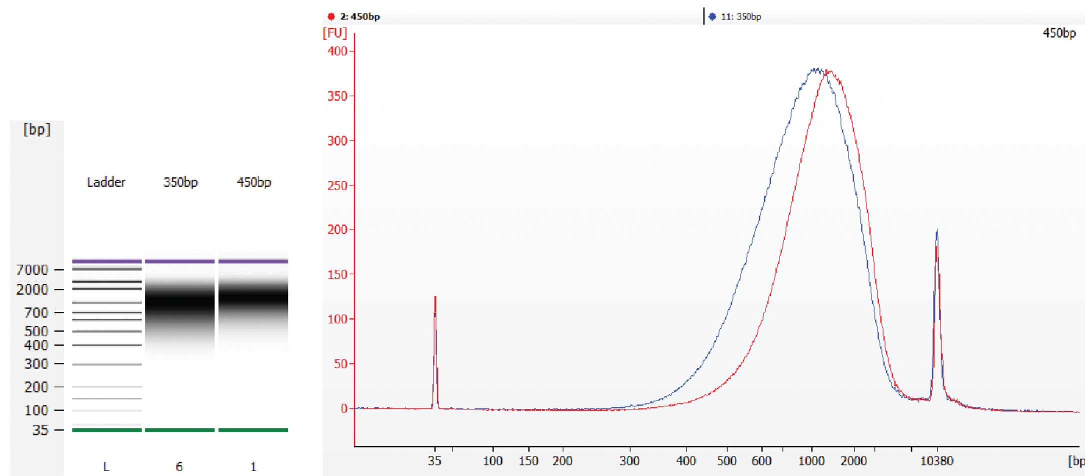
3.20. Place the tube/plate on a magnetic stand. After 5 minutes (or when the solution is clear), transfer 20 µl to a new tube.

3.21. Quantitate the library using qPCR (NEBNext Library Quant Kit for Illumina, NEB # E7630S/L).

3.22. Verify fragment size by checking the library size distribution on an Agilent Bioanalyzer or TapeStation. Run 1 µl library on a DNA High Sensitivity Chip (Bioanalyzer) or High Sensitivity D5000 ScreenTape® (TapeStation). See Figure 1 for an example.

Safe Stopping Point: It is safe to store the library at -20°C.

Figure 1: Examples of Ultra II DNA PCR-free libraries on a Bioanalyzer. The PCR-free libraries migrate slower due to the single strand regions of the adaptors, thus appearing significantly larger than the actual fragment sizes.



Kit Components

NEB #E7410S Table of Components

NEB #	PRODUCT	VOLUME
E7646A	NEBNext Ultra II End Prep Enzyme Mix	0.072 ml
E7647A	NEBNext Ultra II End Prep Reaction Buffer	0.168 ml
E7648A	NEBNext Ultra II Ligation Master Mix	0.72 ml
E7374A	NEBNext Ligation Enhancer	0.024 ml

NEB #E7410L Table of Components

NEB #	PRODUCT	VOLUME
E7646AA	NEBNext Ultra II End Prep Enzyme Mix	0.288 ml
E7647AA	NEBNext Ultra II End Prep Reaction Buffer	0.672 ml
E7648AA	NEBNext Ultra II Ligation Master Mix	3 x 0.960
E7374AA	NEBNext Ligation Enhancer	0.096 ml

NEB #E7415S Table of Components

NEB #	PRODUCT	VOLUME
E7646A	NEBNext Ultra II End Prep Enzyme Mix	0.072 ml
E7647A	NEBNext Ultra II End Prep Reaction Buffer	0.168 ml
E7648A	NEBNext Ultra II Ligation Master Mix	0.72 ml
E7374A	NEBNext Ligation Enhancer	0.024 ml
E7104S	NEBNext Sample Purification Beads	4 ml

NEB #E7415L Table of Components

NEB #	PRODUCT	VOLUME
E7646AA	NEBNext Ultra II End Prep Enzyme Mix	0.288 ml
E7647AA	NEBNext Ultra II End Prep Reaction Buffer	0.672 ml
E7648AA	NEBNext Ultra II Ligation Master Mix	3 x 0.960
E7374AA	NEBNext Ligation Enhancer	0.096 ml
E7104L	NEBNext Sample Purification Beads	4 x 4 ml

Checklist:

1. NEBNext End Prep

[_] 1.1. Add End Prep Reagents to 50 µl of fragmented DNA sample:

- [_] 7 µl End Prep Reaction Buffer
- [_] 3 µl End Prep Enzyme Mix

[_] 1.2. Pipette mix 10 times with pipette set to 50 µl, quick spin

[_] 1.3. Thermal cycle (Heated lid @ 75°C; 30 minutes @ 20°C, 30 minutes @ 65°C, Hold at 4°C)

2. Adaptor Ligation

[_] 2.1. Add Ligation reagents to sample:

- [_] 2.5 µl NEBNext UMI adaptor
- [_] 30 µl Ultra II Ligation Master Mix
- [_] 1 µl Ligation Enhancer

[_] 2.2. Pipette mix 10 times with pipette set to 80 µl, quick spin

[_] 2.3. Incubate 15 minutes at 20°C (heated lid off)

3. Cleanup of Adaptor-ligated DNA

[_] 3.1. Add 7 µl 0.1X TE to sample

[_] 3.2. Vortex beads

[_] 3.3. Add 50 µl of beads to sample and mix by pipetting 10 times

[_] 3.4. Incubate 5 min at room temperature

[_] 3.5. Place tubes on magnet

[_] 3.6. Wait 5 min then remove and discard the supernatant (keep the beads)

[_] 3.7. On magnet add 200 µl 80% ethanol, wait 30 seconds and remove

[_] 3.8. Repeat Step 3.7. once. Remove all visible liquid

[_] 3.9. Air dry the beads for up to 5 minutes on the magnetic stand

[_] 3.10. Elute DNA from the beads into 102 µl of 0.1X TE off the magnetic stand and mix by pipetting 10 times. Incubate 2 min

[_] 3.11. Place the tube/plate on a magnetic stand. After 5 minutes (or when the solution is clear), transfer 100 µl to a new tube.

[_] 3.12. Add 65 µl (350 bp insert) or 58 µl (450 bp insert) of beads to the supernatant and mix by pipetting 10 times.

[_] 3.13. Incubate 5 min at room temperature

[_] 3.14. Place tubes on magnet

[_] 3.15. Wait 5 min then remove and discard the supernatant (keep the beads)

[_] 3.16. On magnet add 200 µl 80% ethanol, wait 30 seconds and remove

[_] 3.17. Repeat Step 3.16. once. Remove all visible liquid

[_] 3.18. Air dry the beads for up to 5 minutes on the magnetic stand

[_] 3.19. Elute DNA from the beads into 22 µl of 0.1X TE off the magnetic stand and mix by pipetting 10 times. Incubate 2 min

[_] 3.20. Place tubes on magnet

[_] 3.21. Wait 2 min then transfer 20 µl supernatant to a new tube

[_] 3.22. Store at -20°C

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	9/21

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