NEBNext® Fast DNA Library Prep Set for Ion Torrent™

NEB #E6270L

50 reactions

Version 9.1_8/20

Table of Contents

The Library Set Includes

The volumes provided are sufficient for preparation of up to 50 reactions (NEB #E6270L). All reagents should be stored at –20°C.

- (green) NEBNext End Repair Enzyme Mix
- (green) NEBNext End Repair Reaction Buffer
- (red) T4 DNA Ligase
- (red) *Bst* 2.0 WarmStart DNA Polymerase
- (red) T4 DNA Ligase Buffer for Ion Torrent
- (red) NEBNext DNA Library Adaptors for Ion Torrent
- (blue) NEBNext DNA Library Primers for Ion Torrent
- (blue) NEBNext Q5 Hot Start HiFi PCR Master Mix
- (white) TE Buffer (0.1X)

Required Materials Not Included

- 80% Ethanol (freshly prepared)
- Agencourt® AMPure® XP Beads (Beckman Coulter, Inc. #A63881)
- Size selection materials [E-Gel® (Life Technologies, Inc.) or AMPURE XP Beads etc.]
- NEBNext® Magnetic Separation Rack (NEB #S1515)

Applications

The NEBNext Fast DNA Library Prep Set for Ion Torrent contains enzymes and buffers in convenient master mix formulations that are ideally suited for sample preparation for next-generation sequencing on the Ion Torrent Sequencer (Life Technologies, Inc.). Each of these components must pass rigorous quality control standards and are lot controlled, both individually and as a set of reagents.

Lot Control: The lots provided in the NEBNext Fast DNA Library Prep Set for Ion Torrent are managed separately and are qualified by additional functional validation. Individual reagents undergo standard enzyme activity and quality control assays, and also meet stringent criteria in the additional quality controls listed on each individual component page.

Functionally Validated: Each set of reagents is functionally validated together through construction and sequencing of a genomic DNA library on a Ion Torrent PGM.

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.

Protocols

Symbols

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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.

●

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

Note: For use with the Ion Xpress™ Barcode Adaptors 1-16 Kit, a minimum of 100 ng starting material is recommended. Lower amounts may cause adaptor concatamerization

Starting Material: 10 ng –1 µg of Fragmented DNA.

1. End Repair of DNA Protocol

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

COMPONENT	VOLUME
Fragmented DNA	1-51 µl
● (green) NEBNext End Repair Reaction Buffer	6 µl
● (green) NEBNext End Repair Enzyme Mix	3 µl
Sterile H_2O	variable
Total Volume	60 µl

1.2. Incubate in a thermal cycler for 20 minutes at 25°C, followed by 10 minutes at 70°C, hold at 4°C.

1.3. Pulse spin the microfuge tube and return to ice.

2. Preparation of Adaptor Ligated DNA

2.1. ⚠

Add the following to the microfuge tube:

For 10 ng

COMPONENT	VOLUME
Sterile H_2O	18 µl
● (red) T4 DNA Ligase Buffer for Ion Torrent	10 µl
● (red) NEBNext DNA Library Adaptors for Ion Torrent	5 µl
● (red) *Bst* 2.0 WarmStart DNA Polymerase	1 µl
● (red) T4 DNA Ligase	6 µl
Total Volume	40 µl

For 100 ng– 1 µg

COMPONENT	VOLUME
Sterile H_2O	3 µl
● (red) T4 DNA Ligase Buffer for Ion Torrent	10 µl
● (red) NEBNext DNA Library Adaptors for Ion Torrent	20 µl
● (red) *Bst* 2.0 WarmStart DNA Polymerase	1 µl
● (red) T4 DNA Ligase	6 µl
Total Volume	40 µl

2.2. The total volume in the microfuge tube should be 100 µl. Mix the contents by pipetting up and down several times.

2.3. Incubate in a thermal cycler for 15 minutes at 25°C, followed by 5 minutes at 65°C, hold at 4°C.

If performing size selection with beads, proceed directly to size selection using AMPure XP Beads (Section 3). If using E-Gel or agarose gel for size selection, proceed to Cleanup of Adaptor Ligated DNA (Section 4) before proceeding to gel-based size selection.

3. Size Selection Using AMPure XP Beads

For AMPure XP Bead-based Size Selection, expect size distributions in the range of 230–270 for 100 bp reads and 310–370 for 200 bp reads.

Table 1: Recommended Conditions for Dual Bead-based Size Selection

	INSERT SIZE (bp)	
BEAD:DNA RATIO*	100 bp	200 bp
1st Bead Selection	0.9X	0.7X
2nd Bead Selection	0.15X	0.15X

*Bead :DNA ratio is calculated based on the original volume of DNA solution.

AMPure XP Bead-based Dual Bead Size Selection for 100 bp Inserts

The following size selection protocol is for libraries with 100 bp insert from a 100 µl volume. For libraries with a 200 bp insert please use the bead:DNA ratio listed in the chart above (Table 1).

1st Bead Selection to Remove Large Fragments: This step is used to bind the large, unwanted fragments to the beads. The supernatant will contain the desired fragments.

3.1. Add 90 µl (0.9X) 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 on a magnetic rack to separate the beads from the supernatant. After the solution is clear (about 5 minutes), carefully transfer the supernatant to a new tube (**Caution: do not discard the supernatant**). Discard beads that contain the large fragments.

2nd Bead Selection to Remove Small Fragments and to Bind DNA Target: This step will bind the desired fragment sizes (contained in the supernatant from Step 3.3) to the beads. Unwanted smaller fragment sizes will not bind to the beads.

3.4. Add 15 µl (0.15X) resuspended AMPure XP Beads to the supernatant, mix well and incubate for 5 minutes at room temperature.

3.5. Put the tube on a magnetic rack to separate beads from supernatant. After the solution is clear (approximately 3 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 80% freshly prepared ethanol to the tube 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 for a total of two washes. 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.

3.8. Keeping the tube on the magnetic rack, with the cap open, air dry the beads for up to 5 minutes.

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 from the magnet. Elute DNA target from beads into 42 µl sterile 0.1X TE. Mix well on a vortex mixer or by pipetting up and down, incubate for 2 minutes at room temperature.

3.10. Put the tube in a magnetic rack until the solution is clear, approximately 3 minutes. Transfer approximately 40 µl of the supernatant to a clean tube.

3.11. Proceed to PCR Amplification in Section 5.

4. Cleanup of Adaptor Ligated DNA

4.1. Add 180 µl (1.8X volume) of AMPure XP Beads to the sample and mix by pipetting up and down.

4.2. Incubate for 5 minutes at room temperature.

4.3. Pulse spin the tube and place in a magnetic rack for approximately 2–3 minutes until the beads have collected to the side of the tube and the solution is clear.

4.4. Carefully remove and discard the supernatant without disturbing the beads.

4.5. Keep the tube on the magnet and add 200 µl freshly prepared 80% ethanol. Incubate at room temperature, for 30 seconds, and carefully remove and discard the supernatant.

4.6. Repeat Step 4.5 once for a total of two washes. 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.

4.7. Keeping the tube in the magnetic rack, with the cap open, air dry the beads for up to 5 minutes at room temperature.

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.

4.8 Remove the tube from the magnet. Resuspend the beads in 25 µl of sterile 0.1X TE (volume may be adjusted for specific gel-based size selection protocol). Incubate for 2 minutes at room temperature.

4.9 Pulse-spin the tube and return to the magnet, until the beads have collected to the side of the tube and the solution is clear.

4.10. Transfer approximately 20 µl (or desired volume) of the supernatant to a clean tube, being careful not to transfer any beads.

5. PCR Amplification of Adaptor Ligated DNA

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A precipitate can form upon thawing of the NEBNext Hot Start HiFi PCR Master Mix. To ensure optimal performance, place the master mix at room temperature while performing size selection/cleanup of adaptor-ligated DNA. Once thawed, gently mix by inverting the tube several times.

5.1. ⚠

Mix the following components in a sterile microfuge tube:

For 10 ng – 100 ng

COMPONENT	VOLUME
Adaptor Ligated DNA	1–40 µl
● (blue) Primers	4 µl
Sterile H_2O	variable
● (blue) NEBNext Q5 Hot Start HiFi PCR Master Mix	50 µl
Total Volume	100 µl

For 1 µg

COMPONENT	VOLUME
Adaptor Ligated DNA	1–40 µl
● (blue) Primers	10 µl
Sterile H_2O	variable
● (blue) NEBNext Q5 Hot Start HiFi PCR Master Mix	50 µl
Total Volume	100 µl

PCR Cycling Conditions

STEP	TEMP	TIME
Initial Denaturation	98°C	30 seconds
4–12 Cycles	98°C	10 seconds
	58°C	30 seconds
	65°C	30 seconds
1 Cycle	65°C	5 minutes
Hold	4°C	∞

Cycling Suggestions:

DNA	CYCLES
10 ng	10–12
100 ng	6–8
1 µg	4–6

6. Cleanup of Amplified Library

6.1. Add 90 µl (0.9X volume) of AMPure XP Reagent to the sample and mix by pipetting up and down.

6.2. Incubate for 5 minutes at room temperature.

6.3. Pulse-spin the tube and place in a magnetic rack for approximately 3 minutes until the beads have collected to the wall of the tube and the solution is clear.

6.4. Carefully remove and discard the supernatant without disturbing the beads.

6.5. Keep the tube on the magnet and add 200 µl freshly prepared 80% ethanol. Incubate 30 seconds, and carefully remove and discard the supernatant.

6.6. Repeat Step 6.5 once for a total of two washes. 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.

6.7. Keeping the tube in the magnetic rack, with the cap open, air dry the beads for up to 5 minutes at room temperature.

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.

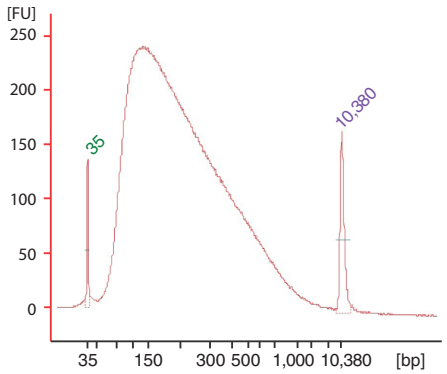
6.8. Remove the tube from the magnet. Resuspend the beads in 35 µl of 0.1X TE and incubate for 2 minutes at room temperature.

6.9. Pulse-spin the tube, return to the magnet until the beads have collected to the wall of the tube and solution is clear.

6.10. Transfer approximately 30 µl of supernatant to a fresh tube. Be careful not to transfer any beads.

6.11. Dilute 2-3 µl of the library 1:4 in 0.1X TE. Assess the library quality on a Bioanalyzer

Figure 1: Relative size distribution of Fragmented End Repaired DNA as seen using the Bioanalyzer® 2100 (Agilent Technologies, Inc.).



1 µg of E. coli DNA was fragmented and end repaired for 20 minutes at 25°C, followed by 10 minutes at 70°C.

Figure 2: Final Library Size distribution using E-Gel Size Selection.

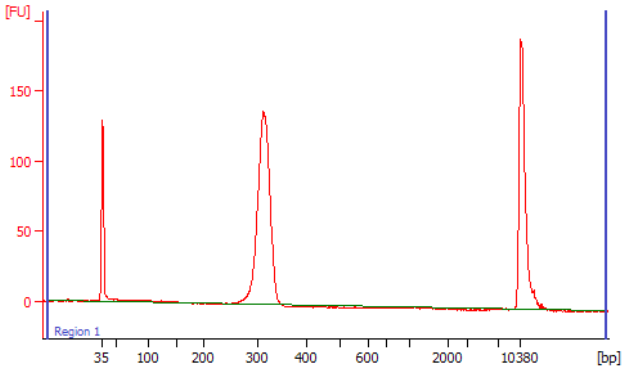
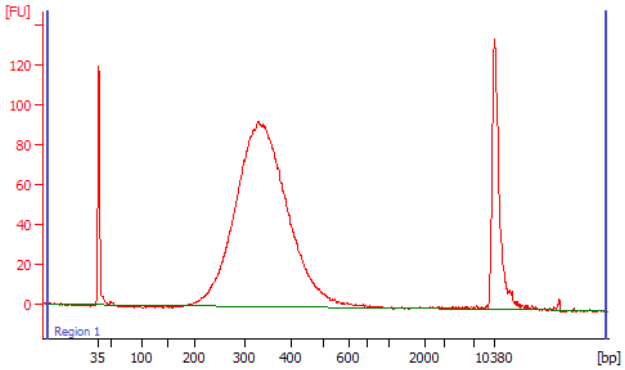


Figure 3: Final Library Size distribution using AMPure XP Beads.



Kit Components

NEB #E6270L Table of Components

NEB #	PRODUCT	VOLUME
E6271AA	NEBNext End Repair Enzyme Mix	0.150 ml
E6272AA	NEBNext End Repair Reaction Buffer	0.300 ml
E6275AA	T4 DNA Ligase	0.300 ml
E6277AA	*Bst* 2.0 WarmStart DNA Polymerase	0.05 ml
E6276AA	T4 DNA Ligase Buffer for Ion Torrent	0.5 ml
E6274AA	NEBNext DNA Library Adaptors for Ion Torrent	1 ml
E6291AA	NEBNext DNA Library Primers for Ion Torrent	0.500 ml
E6293AA	TE Buffer	3.3 ml
E6625AA	NEBNext Q5 Hot Start HiFi PCR Master Mix	2.4 ml

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	11/11
2.0		1/12
3.0		10/12
3.1		1/13
3.2		3/13
4.0		5/13
4.1		6/13
5.0	Include protocol for use with NEBNext Q5 Hot Start HiFi PCR Master Mix. Added 2 minute incubation after eluting DNA from AMPure beads.	1/15
6.0	Remove protocol for use with NEBNext High-Fidelity 2X PCR Master Mix	5/15
7.0	Create “Kit Component – Table of Components” for small and large size kits. Delete individual component information pages.	4/18
8.0	Discontinue small size kit.	6/18
9.0	New formatted manual. Update product license information	5/20
9.1	Add note before Section 5.1.	8/20

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