

# NEBNext<sup>®</sup> Fast DNA Fragmentation & Library Prep Set for Ion Torrent<sup>™</sup>

NEB #E6285L

50 reactions

Version 9.1\_8/20

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## 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 DNA Fragmentation Master Mix
- (green) NEBNext DNA Fragmentation 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
- (white) TE Buffer (0.1X)
- (white) NEBNext Stop Buffer
- (blue) NEBNext Q5 Hot Start HiFi PCR Master Mix
- (green) Magnesium Chloride (MgCl<sub>2</sub>) (200 mM)

## Required Materials Not Included

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

## Applications

The NEBNext Fast DNA Fragmentation & 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 Fragmentation & 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 an 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



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™ (Life Technologies, Inc.) Barcode Adaptors 1-16 Kit, a minimum of 100 ng starting material is recommended. Lower amounts may cause adaptor concatamerization.

**DNA quality and composition can affect fragmentation. Be sure to use the highest quality DNA.**

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

### 1. Fragmentation and End Repair of DNA Protocol



For GC Rich content, add 1 µl of MgCl<sub>2</sub> to the reaction. If fragmentation is insufficient, add additional 10 mM (1 µl) of MgCl<sub>2</sub> to reach desired fragment size. See Figure 1, page 6, for typical fragment size distribution.

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

COMPONENT	VOLUME
DNA	1-15.5 µl
● (green) NEBNext DNA Fragmentation Reaction Buffer	2 µl
Sterile H <sub>2</sub> O	variable
Total Volume	18.5 µl

1.2. Vortex for 3 seconds, pulse spin and place on ice.

1.3. Vortex the vial of NEBNext DNA Fragmentation Master Mix for 3 seconds and pulse spin to collect liquid from the sides of the tube ● (green).

1.4. Add 1.5 µl of ● (green) NEBNext DNA Fragmentation Master Mix to the microfuge tube, vortex for 3 seconds and pulse spin.

**Note:** For DNA with an AT content ≥ 70%, add 0.75 µl of ● (green) NEBNext Fragmentation Master Mix and 0.75 µl sterile H<sub>2</sub>O.

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

1.6. Pulse spin the microfuge tube and place on ice.

## 2. Preparation of Adaptor Ligated DNA

### 2.1.

Add the following to the microfuge tube:

#### For 10 ng

COMPONENT	VOLUME
● (red) T4 DNA Ligase Buffer for Ion Torrent	4 µl
Sterile H <sub>2</sub> O	9 µl
● (red) NEBNext DNA Library Adaptors for Ion Torrent	2 µl
● (red) <i>Bst</i> 2.0 WarmStart DNA Polymerase	1 µl
● (red) T4 DNA Ligase	4 µl
Total Volume	20 µl

#### For 100 ng– 1 µg

COMPONENT	VOLUME
● (red) T4 DNA Ligase Buffer for Ion Torrent	4 µl
Sterile H <sub>2</sub> O	1 µl
● (red) NEBNext DNA Library Adaptors for Ion Torrent	10 µl
● (red) <i>Bst</i> 2.0 WarmStart DNA Polymerase	1 µl
● (red) T4 DNA Ligase	4 µl
Total Volume	20 µl

- 2.2. The total volume in the microfuge tube should be 40 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.
- 2.4. Add 5 µl ○ (white) Stop Buffer, vortex and pulse-spin.



**If performing bead based size selection, 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**

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

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



**The following protocol is for size selecting 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 Table 1.**

**AMPure XP Bead-based Dual Bead Size Selection for 100 bp inserts.**

**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 55  $\mu\text{l}$  0.1X TE to the adaptor ligated DNA from Section 2, Step 2.4 to bring the total volume to 100  $\mu\text{l}$ .
- 3.2. Add 90  $\mu\text{l}$  (0.9X) resuspended AMPure XP Beads to 100  $\mu\text{l}$  DNA solution. Mix well on a vortex mixer or by pipetting up and down at least 10 times.
- 3.3. Incubate for 5 minutes at room temperature.
- 3.4. 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 3.4) to the beads. Unwanted smaller fragment sizes will not bind to the beads.

- 3.5. Add 15  $\mu\text{l}$  (0.15X) resuspended AMPure XP Beads to the supernatant, mix well and incubate for 5 minutes at room temperature.
- 3.6. Put the tube on a magnetic rack to separate beads from supernatant. After the solution is clear (approximately 5 minutes), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets (**Caution: do not discard beads**).
- 3.7. Add 200  $\mu\text{l}$  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.
- 3.8. Repeat Step 3.7 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.
- 3.9. 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.**

- 3.10. Remove the tube from the magnet. Elute DNA target from beads into 42  $\mu\text{l}$  sterile 0.1X TE. Mix well on a vortex mixer or by pipetting up and down, incubate for 2 minutes at room temperature.
- 3.11. Put the tube in a magnetic rack until the solution is clear. Transfer approximately 40  $\mu\text{l}$  of the supernatant to a clean tube.
- 3.12. Proceed to PCR Amplification of Adaptor Ligated DNA (Section 5).

#### **4. Cleanup of Adaptor Ligated DNA**

- 4.1. Add 81  $\mu\text{l}$  (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  $\mu\text{l}$  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  $\mu\text{l}$  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  $\mu\text{l}$  (or desired volume) of the supernatant to a clean tube, being careful not to transfer any beads.
- 4.11. Proceed to PCR Amplification of Adaptor Ligated DNA (Section 5).

## 5. PCR Amplification of Adaptor Ligated DNA



A precipitate can form upon thawing of the NEBNext Q5 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 $\mu$ l
• (blue) Primers	4 $\mu$ l
Sterile H <sub>2</sub> O	variable
• (blue) NEBNext Q5 Hot Start HiFi PCR Master Mix	50 $\mu$ l
Total Volume	100 $\mu$ l

### For 1 $\mu$ g

COMPONENT	VOLUME
Adaptor Ligated DNA	1–40 $\mu$ l
• (blue) Primers	10 $\mu$ l
Sterile H <sub>2</sub> O	variable
• (blue) NEBNext Q5 Hot Start HiFi PCR Master Mix	50 $\mu$ l
Total Volume	100 $\mu$ l

### 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	$\infty$

### Cycling Suggestions:

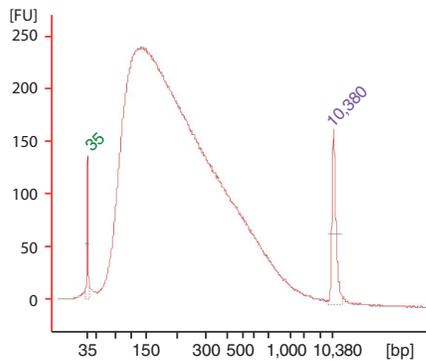
DNA	CYCLES
10 ng	10–12
100 ng	6–8
1 $\mu$ g	4–6

## 6. Cleanup of Amplified Library

- 6.1. Add 90  $\mu$ l (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 2–3 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.

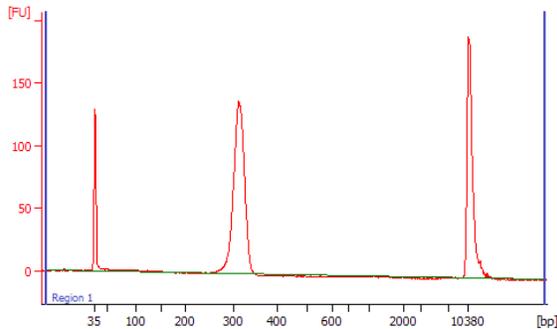
- 6.5. Keep the tube on the magnet and add 200  $\mu$ l freshly prepared 80% ethanol. Incubate at room temperature, for 30 seconds, and carefully remove and discard the supernatant.
- 6.6. Repeat Step 6.5 once for a total of two washes. Be sure to remove all visible liquid after the second wash. If necessary, 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.  
**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 25  $\mu$ l of 0.1X TE. Mix well on a vortexer or by pipetting up and down, and incubate for 2 minutes at room temperature.
- 6.9. Put the tube on the magnetic rack until the solution is clear. Transfer approximately 20  $\mu$ l to a clean tube.
- 6.10. Transfer approximately 20  $\mu$ l of supernatant to a fresh tube. Be careful not to transfer any beads.
- 6.11. Dilute 2–3  $\mu$ l 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.).**

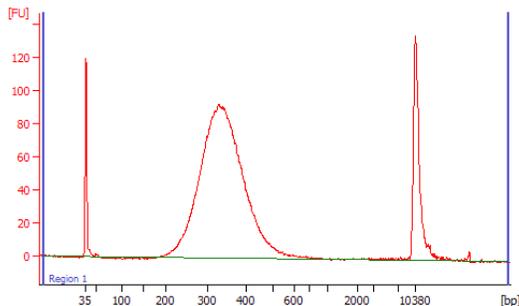


*1  $\mu$ g of E. coli DNA was fragmented and end repaired for 20 minutes at 25°C, followed by 10 minutes at 70°C.*

**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
E6286AA	NEBNext DNA Fragmentation Master Mix	0.100 ml
E6287AA	NEBNext DNA Fragmentation Reaction Buffer	0.100 ml
E6288AA	T4 DNA Ligase	0.2 ml
E6277AA	<i>Bst</i> 2.0 WarmStart DNA Polymerase	0.05 ml
E6296AA	T4 DNA Ligase Buffer for Ion Torrent	0.200 ml
E6290AA	NEBNext DNA Library Adaptors for Ion Torrent	0.500 ml
E6291AA	NEBNext DNA Library Primers for Ion Torrent	0.500 ml
E6299AA	TE Buffer	7.75 ml
E6294AA	NEBNext Stop Buffer	0.250 ml
E6625AA	NEBNext Q5 Hot Start HiFi PCR Master Mix	2.4 ml
E6295AA	Magnesium Chloride (MgCl <sub>2</sub> ) (200 mM)	0.25 ml

## Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	11/11
1.1		11/11
2.0		1/12
3.0		10/12
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
6.1	Adjusted the number of PCR cycles.	8/15
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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