INSTRUCTION MANUAL

NEBNext UltraShear® Long Read
NEB #E3430S/L

24/96 reactions
Version 1.0_1/26

Table of Contents

The Product Includes

The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E3430S) and 96 reactions (NEB #E3430L) for both manual and automated fragmentation setups. The NEBNext® Sample Purification Beads should be stored at room temperature. TE Buffer can be stored at 4°C after initial thawing for future use. All other reagents should be stored at –20°C.

- NEBNext UltraShear LR Enzyme Mix
- NEBNext UltraShear LR Reaction Buffer
- NEBNext UltraShear LR Supplement
- TE Buffer
- NEBNext Sample Purification Beads

Required Materials Not Included

- Any thin wall 0.2 ml PCR tube, for example TempAssure® PCR flex-free-8-tube strips (USA Scientific® #1402-4708)
- Magnetic rack (NEB #S1515), magnetic plate (Alpaqua® #A001322) or equivalent
- Thermal Cycler
- Vortex
- Microcentrifuge
- Agilent® Femto Pulse® (recommended), Bioanalyzer®, TapeStation®, Yourgene® Health LightBench® Discover or other fragment analyzer and associated consumables
- Qubit® and associated consumables
- 80% Ethanol

Where larger volumes, customized or bulk packaging are required, we encourage consultation with the Customized Solutions team at NEB. Please complete the NEB Custom Contact Form at www.neb.com/CustomContactForm to learn more.

Please read the FAQ section on NEB.com for additional information about this product.

Overview

NEBNext UltraShear Long Read contains reagents to enzymatically fragment genomic DNA (gDNA) prior to library preparation for long-read sequencing (e.g., Oxford Nanopore Technologies® and PacBio®). It serves as an alternative to mechanical fragmentation methods such as Covaris® gTUBE™. NEBNext UltraShear Long Read offers a fast, scalable, user and automation friendly workflow with minimal hands-on time.

Figure 1. NEBNext UltraShear Long Read workflow

The NEBNext UltraShear Long Read workflow accommodates a wide input range, from 250 ng to 5,000 ng of genomic DNA. The streamlined workflow can be completed in as little as 30 minutes. NEBNext UltraShear Long Read is automation friendly with minimal sample transfers, sample tracking flexibility and sufficient reagent overages to account for automation dead volumes. NEBNext UltraShear Long Read fragmentation is compatible with Oxford Nanopore Technologies and PacBio library preparation and sequencing.

Table 1. Examples of sequenced N50 read length and mean read length for high-quality gDNA when fragmented with NEBNext UltraShear Long Read

	FRAGMENTATION TIME AND TEMPERATURE						
	5 min. @ 30°C	**5 min. @ 37°C**	**10 min. @ 37°C**	**20 min. @ 37°C**	**30 min. @ 37°C**	**40 min. @ 37°C**	**50 min. @ 37°C**
Monarch® HMW (DQN=9.9)							
N50 read length (kb)	21.2 +/- 0.8	15.6 +/- 0.3	10.8 +/- 0.1	7.1 +/- 0.0	5.6 +/- 0.1	5.7 +/- 1.6	4.3 +/- 0.2
Mean read length (kb)	13.2 +/- 0.6	10.7 +/- 0.1	7.9 +/- 0.0	5.6 +/- 0.0	4.6 +/- 0.0	4.6 +/- 0.9	3.7 +/- 0.1
Monarch gDNA (DQN=9.2)							
N50 read length (kb)	18.4 +/- 0.4	14.4 +/- 0.0	9.7 +/- 0.1	6.8 +/- 0.0	5.8 +/- 0.2	4.8 +/- 0.1	4.5+/- 0.1
Mean read length (kb)	12.2 +/- 0.3	10.4 +/- 0.1	7.5 +/- 0.1	5.5 +/- 0.0	4.8 +/- 0.3	3.9 +/- 0.1	3.7 +/- 0.1
Salting Out (DQN=8.9)							
N50 read length (kb)	9.5 +/- 0.3	8.2 +/- 0.3	6.3 +/- 0.2	4.7 +/- 0.2	4.2 +/- 0.3	3.7+/- 0.0	3.7 +/- 0.1
Mean read length (kb)	7.2 +/- 0.1	6.4 +/- 0.3	5.1 +/- 0.1	3.9 +/- 0.1	3.4 +/- 0.2	2.9 +/- 0.0	2.9 +/- 0.0

gDNA was extracted from GM12878 cells (human) with three different extraction methods: Monarch HMW DNA Extraction Kit for Cells and Blood workflow (Monarch HMW; NEB #T3050), Monarch Spin gDNA Extraction Kit workflow (Monarch gDNA; NEB #T3010) and Salting out, which resulted in different levels of gDNA intactness (DQN 9.9 to 8.9 determined by Agilent Femto Pulse). 1,000 ng of GM12878 gDNA, from the different purification methods, was fragmented using NEBNext UltraShear Long Read workflow for 5 minutes at 30˚C or 5–50 minutes at 37˚C. Following fragmentation, 400 ng of fragmented DNA was used to generate libraries with Oxford Nanopore Technologies (ONT®) Native Barcoding Kit 96 V14 and sequenced on ONT PromethION® R.10.4.1. flow cells. The sequenced N50 and mean length (average of two technical replicates with error) are shown based on fragmentation time and intactness of extracted gDNA samples. The longer the fragmentation time the smaller the sequenced N50 and mean length. Incubation time may need to be optimized for individual samples to achieve desired N50 and mean read length based on sample quality.

NEBNext UltraShear Long Read Protocol

Symbols

This is a point where you can safely stop the protocol.

This caution sign signifies a step in the protocol that has multiple paths leading to the same end point but is dependent on a user variable, like the incubation time.

Starting Material: 250–5,000 ng high-quality, intact genomic DNA

1.1. DNA Preparation & Fragmentation

1.1.1   Ensure that the TE Buffer (1X TE; 10 mM Tris pH 8.0, 1 mM EDTA) is completely thawed and vortex briefly to mix.

Note: Can store TE Buffer at 4°C after initial thawing for future use.

Note: NEBNext UltraShear Long Read is compatible with gDNA samples eluted in common gDNA extraction kits' elution buffers, e.g., Monarch DNA Elution Buffer, Monarch gDNA Elution Buffer, Monarch gDNA Elution Buffer II, Qiagen® Buffer EB and Buffer AE, when used in place of TE Buffer. However, the fragmentation is slower in these elution buffers than in TE Buffer.

1.1.2.   On ice, combine high-quality genomic DNA (gDNA) with TE Buffer in a 0.2 ml, thin-wall PCR tube and make up the volume to 25 µl.

1.1.3.   Ensure that the NEBNext UltraShear LR Reaction Buffer is completely thawed, vortex briefly to mix, then spin down. Place on ice until use.

1.1.4.   Ensure that the NEBNext UltraShear LR Supplement is completely thawed and vortex briefly to mix, then spin down. Place on ice until use.

1.1.5.   Vortex the NEBNext UltraShear LR Enzyme Mix prior to use for 5–10 seconds at high-speed setting, then spin down and place on ice.

Note: It is important to vortex the enzyme mix prior to use for optimal performance.

1.1.6.   Add the following components to a 0.2 ml, thin-wall PCR tube on ice:

COMPONENT	VOLUME PER LIBRARY
gDNA combined with TE Buffer (Step 1.1.2.)	25 µl
NEBNext UltraShear LR Reaction Buffer	14 µl
NEBNext UltraShear LR Supplement	3 µl
NEBNext UltraShear LR Enzyme Mix	2 µl
Total Volume	**44 µl**

Note: A master mix can be made on ice; add components in the following order:

1.1.6a.   First, combine appropriate volume of NEBNext UltraShear LR Reaction Buffer and NEBNext UltraShear LR Supplement and mix by vortexing 5–10 seconds and briefly spin in a microcentrifuge.

1.1.6b.   Then add NEBNext UltraShear LR Enzyme Mix and vortex the master mix 5–10 seconds and briefly spin in a microcentrifuge.

1.1.6c.   Finally, add 19 µl of the master mix to gDNA prepared in Step 1.1.2. in 0.2 ml, thin-wall PCR tube on ice.

Note: The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E3430S) and 96 reactions (NEB #E3430L) for both manual and automated fragmentation setups.

1.1.7.　Mix the reaction from Step 1.1.6. by setting a pipette to 30 µl and mix well by carefully pipetting up and down at least 10 times. On the final mix, be sure to expel all liquid from the pipette tip.

Note: Perform a brief spin in a microcentrifuge if needed.

Note: Do not vortex to mix.

⚠️ *NEBNext UltraShear Long Read is a time-dependent enzymatic fragmentation method. The shorter incubation times generate longer DNA fragments compared to longer incubation times.*

⚠️ *The gDNA can be fragmented at 37°C or 30°C with NEBNext UltraShear Long Read. Incubation at 30°C results in slower fragmentation and generates longer DNA fragments compared to 37°C. It is not recommended to fragment for extended times past 10 mins at 30°C as those fragmentation patterns match shorter incubation times at 37°C.*

1.1.8.　In a thermal cycler, **preheated** and with the heated lid set to 75°C, run the following program:

5–10* minutes at 30°C or 5–50* minutes at 37°C

15 minutes at 65°C

Hold at 4°C

* Please refer to Table 1. for suggestions on fragmentation time. Incubation time may need to be optimized for individual samples.

🛑 SAFE STOP

Safe Stopping Point: Samples can be stored overnight at 4°C.

1.2. Cleanup of fragmented DNA

The ratios recommended for NEBNext Sample Purification Beads in this manual have been experimentally optimized. Please adhere to these guidelines and not those recommended by other sources or for other kits.

1.2.1.　Vortex NEBNext Sample Purification Beads to resuspend.

1.2.2.　Prepare a dilution of NEBNext Sample Purification Beads in TE Buffer. Vortex immediately before use to resuspend the Sample Purification Beads.

For *R* number of reactions, add 44.5 µl x *R* of NEBNext Sample Purification Bead Volume to 82.5 µl x *R* of TE Buffer.

- For example, to prepare 3 reactions worth of diluted NEBNext Sample Purification Beads, add 44.5 µl x 3 reactions = 133.5 µl of NEBNext Sample Purification Beads to 82.5 µl x 3 reactions = 247.5 µl of TE Buffer.

Note: For consistent pipetting, we recommend making at least 2 reactions worth of the diluted NEBNext Sample Purification Beads, even if processing only one sample.

Table 1.2.2. Dilution of NEBNext Sample Purification Beads

NUMBER OF REACTIONS	NEBNEXT SAMPLE PURIFICATION BEAD VOLUME	TE BUFFER VOLUME	TOTAL VOLUME OF DILUTED NEBNEXT SAMPLE PURIFICATION BEADS
2 reactions	89 µl	165 µl	254 µl
8 reactions	356 µl	660 µl	1,016 µl
24 reactions	1,068 µl	1,980 µl	3,048 µl

Note: The diluted NEBNext Sample Purification Beads should be used immediately and leftovers discarded after use.

1.2.3.　Add 127 µl of diluted NEBNext Sample Purification Beads from Step 1.2.2. to each sample.

Note: Cleanups can be optimized to retain smaller sized fragments (< 3 kb); for example, use a 0.6X bead cleanup.

1.2.4.　Mix well by carefully pipetting up and down at least 10 times. On the final mix, be sure to expel all liquid from the pipette tip. If centrifuging samples after mixing, be sure to stop the centrifugation before the beads start to settle out.

Note: Do not vortex to mix.

1.2.5.　Incubate samples on the benchtop for at least 5 minutes at room temperature.

1.2.6.　Place the tubes/plate on an appropriate magnetic rack to separate the beads from the supernatant. If necessary, briefly spin the samples to collect the liquid from the sides of the tubes or plate wells before placing on the magnetic rack.

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

1.2.8.   Add 200 µl of freshly prepared 80% ethanol to the tubes while on the magnetic rack. 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.

1.2.9.   Repeat the wash once for a total of two washes.

1.2.10.   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.

1.2.11.   Air dry the beads for **up to 1 minute** while the tubes are on the magnetic rack with the lid open.

Caution: Do not over-dry the beads. This may result in lower recovery of DNA targets. 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.

1.2.12.   Remove the tubes from the magnetic rack. Elute the DNA targets from the beads by adding 14 µl of TE Buffer.

Note: Elution volume can be increased to larger volumes based on downstream application needs.

1.2.13.   Mix well by carefully pipetting up and down 10 times. Incubate for at least 1 minute at room temperature. If necessary, quickly spin the sample to collect the liquid from the sides of the tube before placing back on the magnetic rack.

Note: Do not vortex to mix.

1.2.14.   Place the tube on the magnetic rack.

1.2.15.   After the solution is clear (about 3 minutes), transfer 13 µl of the supernatant to a new PCR tube without disturbing the bead pellet.

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

1.3. DNA Fragmentation, Quantification and Library Prep

⚠ *The cleaned-up fragmented DNA can be analyzed by various fragment analyzers to determine size distribution.*

1.3.1.   Use an appropriately sized fragment analyzer (Agilent Femto Pulse, Bioanalyzer, TapeStation or Yourgene Health LightBench Discover) to determine the size distribution of the fragmented DNA.

1.3.2.   Use an Invitrogen® Qubit fluorometer to determine concentration of the fragmented DNA.

⚠ *The cleaned-up fragmented DNA can be used to generate libraries by various methods.*

1.3.3.   Generate long-read sequencing libraries (Oxford Nanopore Technologies or PacBio) or any downstream application following manufacturers' guidelines.

Figure 1.3. NEBNext UltraShear Long Read is a time-dependent enzymatic fragmentation method.
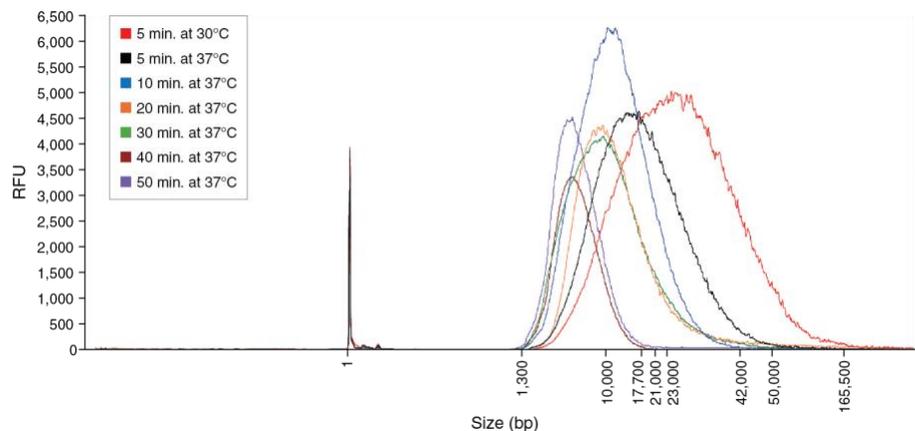
1,000 ng of gDNA (DQN = 9.9), extracted from GM12878 human cells using the Monarch HMW DNA Extraction Kit, was fragmented for 5 minutes at 30°C or 5 to 50 minutes at 37°C followed by 15 minutes at 65°C with NEBNext UltraShear Long Read. Agilent Femto Pulse trace (165 kb protocol) showing the time dependent shifts in fragmentation size and profile of gDNA fragmented using NEBNext UltraShear Long Read.

Components

NEB #E3430S Table of Components

NEB #	PRODUCT	VOLUME
E3431A	NEBNext UltraShear LR Enzyme Mix	0.068 ml
E3432A	NEBNext UltraShear LR Reaction Buffer	0.476 ml
E3433A	NEBNext UltraShear LR Supplement	0.102 ml
E3434A	TE Buffer	6.3 ml
E3435A	NEBNext Sample Purification Beads	2.67 ml

NEB #E3430L Table of Components

NEB #	PRODUCT	VOLUME
E3431AA	NEBNext UltraShear LR Enzyme Mix	0.22 ml
E3432AA	NEBNext UltraShear LR Reaction Buffer	2 x 0.778 ml
E3433AA	NEBNext UltraShear LR Supplement	0.333 ml
E3434AA	TE Buffer	22.5 ml
E3435AA	NEBNext Sample Purification Beads	9.79 ml

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	1/26

www.neb.com

NEW ENGLAND Biolabs®