NEBNext® Ultra™ II DNA Library Prep Kit for Illumina®

for use with NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA)

NEB #E7645S/L, #E7103S/L

24/96 reactions

Version 2.0_5/23

Table of Contents

The Library Kit Includes

The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E7645S/#E7103S) and 96 reactions (NEB #E7645L/ #E7103L). 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
- (blue) NEBNext Ultra II Q5 Master Mix

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

Supplied only with NEBNext Ultra II DNA Library Prep with Sample Purification Beads, NEB #E7103.

NEBNext Sample Purification Beads

Required Materials Not Included

- 80% Ethanol (freshly prepared)
- Nuclease-free Water
- 0.1X TE (1 mM Tris-HCl, pH 8.0, 0.1 mM EDTA)
- DNA LoBind Tubes (Eppendorf #022431021)
- NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA). NEBNext Oligo Kit options can be found at www.neb.com/oligos
- Magnetic rack/stand
- PCR machine

For NEB #E7645 only:

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

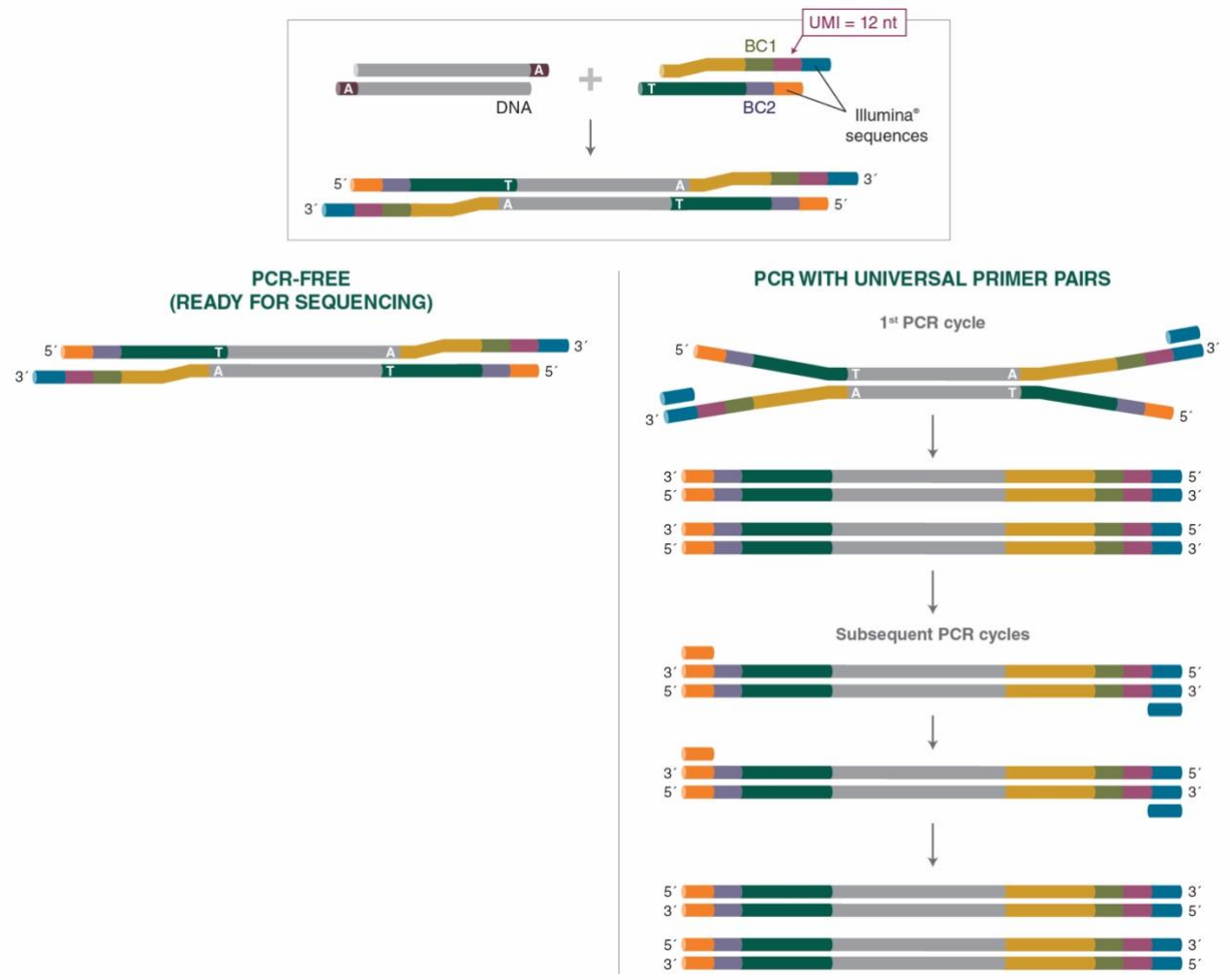
Overview

The NEBNext Ultra II DNA 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. 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 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 custom@neb.com for further information.

Figure 1. Workflow demonstrating the use of NEBNext Ultra II DNA Library Prep Kit for Illumina for use with NEBNext Multiplex Oligos (Unique Dual Index UMI Adaptors DNA).



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 amount of input DNA.

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

Starting Material: 500 pg–1 µg fragmented DNA. 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. Add the following components to a sterile nuclease-free tube:

COMPONENT	VOLUME
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**

1.2. 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.

1.3. 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

If necessary, samples can be stored at –20°C; however, a slight loss in yield (~20%) may be observed. We recommend continuing with adaptor ligation before stopping.

2. Adaptor Ligation

2.1 Determine whether adaptor dilution is necessary.

If DNA input is ≤ 100 ng, dilute the NEBNext UMI Adaptors for Illumina using the UMI Dilution Buffer provided in the kit as indicated in Table 2.1.

Table 2.1: Adaptor Dilution

INPUT	ADAPTOR DILUTION (VOLUME OF ADAPTOR : TOTAL VOLUME)	WORKING ADAPTOR CONCENTRATION
1 µg–101 ng	No Dilution	20 µM
100 ng–5 ng	10-Fold (1:10)	2 µM
less than 5 ng	50-Fold (1:50)	0.4 µM

Note: The appropriate adaptor dilution for your sample input and type may need to be optimized experimentally. The dilutions provided here are a general starting point. Excess adaptor should be removed prior to PCR enrichment.

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

COMPONENT	VOLUME
End Prep Reaction Mixture (Step 1.3 in Section 1)	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**

* NEBNext Oligos must be purchased separately from the library prep kit. For oligo purchasing options refer to "Required Materials Not Included" section (page 1). Please refer to corresponding oligo 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 @ 4°C. We do not recommend adding adaptor to a premix in the Adaptor Ligation Step.

2.3. 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 very 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).**

2.4. Incubate at 20°C for 15 minutes in a thermal cycler with the heated lid off.

Samples can be stored overnight at –20°C.

3. Size Selection or Cleanup of Adaptor-ligated DNA

If the starting material is > 50 ng, follow the protocol for size selection in Section 3A. For input ≤ 50 ng, size selection is not recommended to maintain library complexity. Follow the protocol for cleanup without size selection in Section 3B.

3A. Size Selection of Adaptor-ligated DNA

Note: The following section is for size selection of the ligation reaction. The volumes of SPRIselect or NEBNext Sample Purification Beads provided here are for use with the sample contained in the exact buffer at this step. AMPure XP Beads can be used as well. If using AMPure XP Beads, allow the beads to warm to room temperature for at least 30 minutes before use. These bead volumes may not work properly for a cleanup at a different step in the workflow, or if this is a second cleanup at this step. For cleanups of samples contained in different buffer conditions, the volumes may need to be experimentally determined.

The following size selection protocol is for libraries with 150 bp inserts only. For libraries with different size fragment inserts, refer to the table below for the appropriate volumes of beads to be added. The size selection protocol is based on starting volume of 93.5 µl.

To select a different insert size than 150 bp, please use the volumes in this table:

Table 2.3.1. Recommended Conditions for Bead Based Size Selection

LIBRARY PARAMETER	APPROXIMATE INSERT SIZE	150 bp	200 bp	250-350 bp	350-450 bp	450-650 bp
	Approx. Final Library Size (insert + adaptor)	300 bp	350 bp	400 bp	500 bp	600-800 bp
BEAD VOLUME TO BE ADDED (µl)	1st Bead Addition	40	30	25	20	15
	2nd Bead Addition	20	15	10	10	10

3A.1. Vortex SPRIselect or NEBNext Sample Purification Beads to resuspend.

3A.2. Add 40 µl (~ 0.4X) of resuspended beads to the 93.5 µl ligation reaction. 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.

3A.3. Incubate samples on bench top for 5 minutes at room temperature.

3A.4. 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.

3A.5. After 5 minutes (or when the solution is clear), carefully transfer the supernatant containing your DNA to a new tube **(Caution: do not discard the supernatant)**. Discard the beads that contain the unwanted large fragments.

3A.6. Add 20 µl (0.2X) resuspended SPRIselect or NEBNext Sample Purification Beads to the supernatant and mix at least 10 times. Be careful to expel all of the liquid from the tip during the last mix. Then incubate samples on the bench top for 5 minutes at room temperature.

3A.7. 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.

3A.8. 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)**.

3A.9. 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.

3A.10. Repeat Step 3A.9 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.

3A.11. 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.

3A.12. Remove the tube/plate from the magnetic stand. Elute the DNA target from the beads into 22 µl of 0.1X TE.

3A.13. Mix well on a vortex mixer or by pipetting up and down 10 times. Incubate for at least 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.

3A.14. Place the tube/plate on a magnetic stand. After 5 minutes (or when the solution is clear), transfer 20 µl to a new tube.

Samples are ready for sequencing on the Illumina platform and can be stored at –20°C. We recommend qPCR based methods (NEBNext Library Quant Kit for Illumina, NEB #E7630) for quantification of PCR-free libraries.

Please proceed to Section 4 if PCR amplification is required.

3B. Cleanup of Adaptor-ligated DNA without Size Selection (for input ≤ 50 ng)

The following section is for cleanup of the ligation reaction. If your input DNA is > 100 ng, follow the size selection protocol in Section 3.A.

Note: The volumes of SPRIselect or NEBNext Sample Purification Beads provided here are for use with the sample contained in the exact buffer at this step. AMPure XP Beads can be used as well. If using AMPure XP Beads, allow the beads to warm to room temperature for at least 30 minutes before use. These bead volumes may not work properly for a cleanup at a different step in the workflow, or if this is a second cleanup at this step. For cleanups of samples contained in different buffer conditions, the volumes may need to be experimentally determined.

3B.1. Vortex SPRIselect or NEBNext Sample Purification Beads to resuspend.

3B.2. Add 65 µl (0.7X) **(Note: 75 µl for cell-free DNA)** resuspended beads to the Adaptor Ligation reaction. 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.

3B.3. Incubate samples on bench top for 5 minutes at room temperature.

3B.4. 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.

3B.5. After 5 minutes (or when the solution is clear), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets **(Caution: do not discard beads).**

3B.6. 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.

3B.7. Repeat Step 3B.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.

3B.8. 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 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.

3B.9. Remove the tube/plate from the magnetic stand. Elute the DNA target from the beads by adding 22 µl of 0.1X TE.

3B.10. Mix well by pipetting up and down 10 times, or on a vortex mixer. Incubate for at least 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.

3B.11. Place the tube/plate on the magnetic stand. After 5 minutes (or when the solution is clear), transfer 20 µl to a PCR tube.

Samples are ready for sequencing on the Illumina platform and can be stored at –20°C. We recommend qPCR based methods (NEBNext Library Quant Kit for Illumina, NEB #E7630) for quantification of PCR-free libraries.

Please proceed to Section 4 if PCR amplification is required.

4. PCR Enrichment of Adaptor-ligated DNA

4.1. PCR Amplification

4.1.1. Add the following components to a sterile strip tube:

COMPONENT	VOLUME
Adaptor Ligated DNA Fragments (Step 3A.14. or 3B.11.)	20 µl
• (blue) NEBNext Primer Mix*	5 µl
• (blue) NEBNext Ultra II Q5 Master Mix	25 µl
Total Volume	**50 µl**

* NEBNext Oligos must be purchased separately from the library prep kit. For oligo purchasing options refer to “Required Materials Not Included” section (page 1).

4.1.2. Set a 100 µl or 200 µl pipette to 40 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.

4.1.3. Place the tube on a thermal cycler and perform PCR amplification using the following PCR cycling conditions:

CYCLE STEP	TEMP	TIME	CYCLES
Initial Denaturation	98°C	30 seconds	1
Denaturation	98°C	10 seconds	3–15*
Annealing/Extension	65°C	75 seconds	
Final Extension	65°C	5 minutes	1
Hold	4°C	∞	

* The number of PCR cycles should be chosen based on input amount and sample type. Thus, samples prepared with a different method prior to library prep may require re-optimization of the number of PCR cycles. The number of cycles should be high enough to provide sufficient library fragments for a successful sequencing run, but low enough to avoid PCR artifacts and over-cycling (high molecular weight fragments on Bioanalyzer). The number of PCR cycles recommended in Table 4.1 are to be seen as a starting point to determine the number of PCR cycles best for standard library prep samples. Use Table 4.2 for applications requiring high library yields (~1 µg) such as target enrichment.

Table 4.1.

INPUT DNA IN THE END PREP REACTION	# OF CYCLES REQUIRED FOR STANDARD LIBRARY PREP: YIELD ~100 ng (30–100 nM)*
1 µg*	3
500 ng*	3
100 ng*	3
50 ng	3–4
10 ng	6–7
5 ng	7–8
1 ng	9–10
0.5 ng	10–11

* For best results, perform bead-based size selection when using these input quantities.

Table 4.2.

INPUT DNA IN THE END PREP REACTION	# OF CYCLES REQUIRED FOR TARGET ENRICHMENT LIBRARY PREP YIELD ~1 µg:
1 µg*	3–4*
500 ng*	4–5*
100 ng*	6–7*
50 ng	7–8
10 ng	9–10
5 ng	10–11
1 ng	12–13
0.5 ng	14–15

* Cycle number was determined for size-selected libraries.

4.1.4. Proceed to Cleanup of PCR Amplification in Section 5.

5. Cleanup of PCR Reaction

Note: The volumes of SPRIselect or NEBNext Sample Purification Beads provided here are for use with the sample contained in the exact buffer at this step. AMPure XP beads can be used as well. If using AMPure XP beads, allow the beads to warm to room temperature for at least 30 minutes before use. These volumes may not work properly for a cleanup at a different step in the workflow. For cleanups of samples contained in different buffer conditions, the volumes may need to be experimentally determined.

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

5.2. Add 40 µl (0.8X) **(Note: 50 µl for cell-free DNA)** resuspended beads to the PCR reaction. 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.

5.3. Incubate samples on bench top for 5 minutes at room temperature.

5.4. 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.

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

5.6. 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.

5.7. Repeat Step 5.6. 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.

5.8. 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 over-dry the beads. This may result in lower recovery of DNA. 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.

5.9. Remove the tube/plate from the magnetic stand. Elute the DNA target from the beads by adding 33 µl of 0.1X TE.

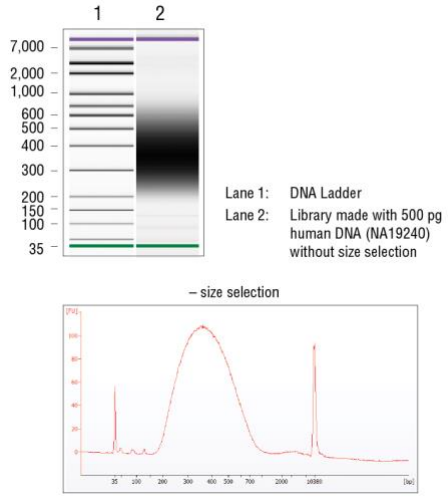
5.10. Mix well by pipetting up and down 10 times, or on a vortex mixer. Incubate for at least 2 minutes at room temperature. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing back on the magnetic stand.

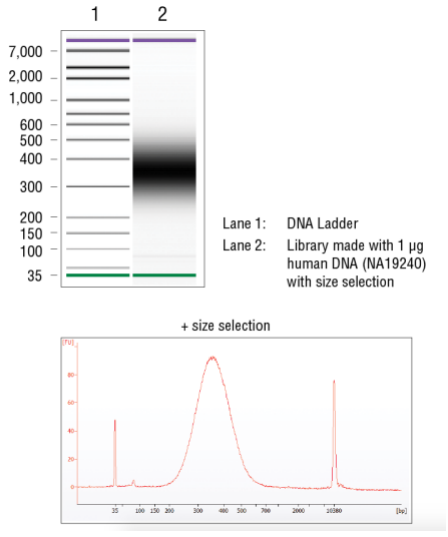
5.11. Place the tube/plate on the magnetic stand. After 5 minutes (or when the solution is clear), transfer 30 µl to a new tube and store at –20°C.

5.12 Check the size distribution on an Agilent Bioanalyzer High Sensitivity DNA chip. The sample may need to be diluted before loading.

Samples can be stored at –20°C.

Figure 5.1. Bioanalyzer traces representing typical size distribution of libraries prepared with human DNA (NA19240).





Kit Components

NEB #E7645S 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	0.720 ml
E7374A	NEBNext Ligation Enhancer	0.024 ml
E7649A	NEBNext Ultra II Q5 Master Mix	0.6 ml

NEB #E7645L 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	2.88 ml
E7374AA	NEBNext Ligation Enhancer	0.096 ml
E7649AA	NEBNext Ultra II Q5 Master Mix	2.4 ml

NEB #E7103S 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	0.720 ml
E7374A	NEBNext Ligation Enhancer	0.024 ml
E7649A	NEBNext Ultra II Q5 Master Mix	0.6 ml
E7104S	NEBNext Sample Purification Beads	4 ml

NEB #E7103L 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	2.88 ml
E7374AA	NEBNext Ligation Enhancer	0.096 ml
E7649AA	NEBNext Ultra II Q5 Master Mix	2.4 ml
E7104L	NEBNext Sample Purification Beads	16 ml

Checklist

1. NEBNext End Prep

[_] 1.1. Add End Prep Reagents to 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 min 20°C, 30 min 65°C, Hold at 4°C)

2. Adaptor Ligation

[_] 2.1. Dilute UMI adaptors if necessary

[_] 2.2. Add Ligation reagents to sample:

[_] 2.5 µl UMI adaptor

[_] 30 µl Ligation Master Mix

[_] 1 µl Ligation Enhancer

[_] 2.3. Pipette mix 10 times with pipette set to 80 µl, quick spin

[_] 2.4 Incubate 15 min at 20°C (heated lid off)

3. Cleanup or Size Selection

3A. Size Selection of Adaptor-ligated DNA

[_] 3A.1. Vortex beads

[_] 3A.2. Add __ µl of beads to sample and mix by pipetting 10 times.

[_] 3A.3. Incubate 5 min

[_] 3A.4. Place tubes on magnet

[_] 3A.5. Wait 5 min then transfer the supernatant to a new tube (keep the supernatant)

[_] 3A.6. Add __ µl of beads to the supernatant and mix by pipetting 10 times. Incubate 5 min.

[_] 3A.7. Place tubes on magnet

[_] 3A.8. Wait 5 min then remove the supernatant (keep the beads)

[_] 3A.9. On magnet add 200 µl 80% ethanol, wait 30 seconds and remove

[_] 3A.10. Repeat Step 3A.9 once

[_] 3A.11. Air dry beads, do not overdry

[_] 3A.12. Off magnet add 22 µl 0.1X TE

[_] 3A.13. Mix by pipetting 10 times. Incubate 2 min.

[_] 3A.14. Place tubes on magnet. Wait 5 min and transfer 20 µl to a new tube

Skip to 4.1

3B. Cleanup of Adaptor-ligated DNA without Size Selection

[_] 3B.1. Add 65 µl of beads to sample and mix by pipetting 10 times.

[_] 3B.2. Incubate for 5 min

[_] 3B.3. Place tubes on magnet

[_] 3B.4. Wait 5 min and remove supernatant (keep the beads)

[_] 3B.5. On magnet add 200 µl 80% ethanol, wait 30 seconds and remove

[_] 3B.6. Repeat Step 3B.5 once

[_] 3B.7. Air dry beads, do not overdry

[_] 3B.8. Off magnet add 22 µl 0.1X TE

[_] 3B.9. Mix by pipetting 10 times. Incubate 2 min.

[_] 3B.10. Place tubes on magnet. Wait 5 min and transfer 20 µl to a new tube

4. PCR Enrichment of Adaptor-ligated DNA

4.1. PCR Amplification

[_] 4.1.1. Add PCR Reagents to sample

[_] 5 µl NEBNext Primer Mix

[_] 25 µl Q5 Master Mix

[_] 4.1.2. Pipette mix 10 times with pipette set to 40 µl, quick spin

[_] 4.1.3. Thermal cycle (Heated lid ≥ 103°C; 98°C 30 sec 3-15 cycles of 98°C for 10 sec and 65°C for 75 sec 65°C for 5 min, Hold at 4°C)

5. Cleanup of PCR Reaction

[_] 5.1. Vortex beads

[_] 5.2. Add 40 µl of beads to sample and mix by pipetting 10 times

[_] 5.3. Incubate for 5 min

[_] 5.4. Place tubes on magnet

[_] 5.5. Wait 5 min and remove supernatant (keep the beads)

[_] 5.6. On magnet add 200 µl 80% ethanol, wait 30 seconds and remove

[_] 5.7. Repeat Step 5.6 once

[_] 5.8. Air dry beads, do not overdry

[_] 5.9. Off magnet add 33 µl 10 mM Tris-HCl or 0.1X TE

[_] 5.10. Mix by pipetting 10 times. Incubate 2 min.

[_] 5.11. Place tubes on magnet. Wait 5 min and transfer 30 µl to a new tube

[_] 5.12. Check size distribution on Bioanalyzer

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	3/20
2.0	Updated to remove reference to specific UMI set. Also updated table formatting and legal footnote	