INSTRUCTION MANUAL

NEBNext® FS DNA Library Prep Kit for MGI®

NEB #E9620S/L

24/96 reactions

Version 1.0_06/24

Table of Contents

The Library Prep Kit Includes

The volumes provided are sufficient to prepare up to 24 reactions (NEB #E9620S) or up to 96 reactions (NEB #E9620L).

Store at –20°C

- (yellow) NEBNext FS Enzyme Mix
- (yellow) NEBNext FS Reaction Buffer
- (red) NEBNext Ligation Master Mix
- (red) NEBNext Ligation Enhancer
- (blue) NEBNext MSTC™ High Yield Master Mix
- (white) TE Buffer (1X)

Required Materials Not Included

- NEBNext Multiplex Oligos for MGI (Dual Index Primer Pairs Set 1) (#E9640)
- NEBNext Circularization Module for MGI (#E9635)
- SPRIselect™ Reagent Kit (Beckman Coulter®, Inc. #B23317) or AMPure® XP Beads (Beckman Coulter, Inc. #A63881)
- 80% Ethanol (freshly prepared)
- Nuclease-free Water
- DNA LoBind® Tubes (Eppendorf® #022431021)
- DNase-, RNase-free PCR strip tubes, for example TempAssure® PCR flex-free 8-tube strips (USA Scientific® #1402-4708)
- Magnetic rack/stand (NEB #S1515, Alpaqua® cat. #A001322, or equivalent)
- Vortex
- Thermal cycler
- Bioanalyzer® or TapeStation® (Agilent® Technologies, Inc.) and associated reagents and consumables

Overview

The NEBNext FS DNA Library Prep Kit for MGI contains the enzymes and buffers required to rapidly convert 0.1–500 ng of intact input DNA into high-quality libraries for sequencing on the MGI DNBSEQ® platforms. The fast, user-friendly workflow has minimal hands-on time.

Note: The FS Kit is incompatible with bisulfite conversion workflows and FFPE DNA.

This kit is used in conjunction with NEBNext Multiplex Oligos for MGI (Dual Index Primer Pairs Set 1) (#E9640) and libraries are circularized using the NEBNext Circularization Module for MGI (#E9635) before sequencing; both available separately.

Each kit component must pass rigorous quality control standards and, for each new lot, the entire set of reagents is functionally validated together by constructing and sequencing indexed libraries on an MGI sequencing platform.

Please refer to the product page on NEB.com for FAQs about this product.

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

Figure 1. Workflow demonstrating the use of NEBNext FS DNA Library Prep Kit for MGI

Protocol

Symbols

This is a point where you can safely stop and store the samples before proceeding to the next step in 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, such as the amount of input DNA.

●

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

Keep all enzymes and buffers on ice, unless otherwise indicated.

Starting Material: 0.1–500 ng intact DNA. If the DNA volume is less than 26 µl, add TE Buffer (1X) to a final volume of 26 µl. Alternatively, samples can be diluted with 10 mM Tris, pH 8.0, Low TE (10 mM Tris, 0.1 mM EDTA, pH 8.0), or H_2O.

1. NEBNext Fragmentation/End Prep

Note:

(1) Ensure that the FS Reaction Buffer is completely thawed. If a precipitate is seen in the buffer, pipette up and down several times to break it up, vortex to mix, and place on ice before use.

(2) It is important to vortex the FS enzyme mix before use for optimal performance.

(3) Fragmentation occurs during the 37°C incubation step in a time-dependent manner; see Appendix 1 for varying fragmentation times to alter library fragment size. The condition listed below in this section is for an average fragment size of 250 bp range.

1.1. Vortex the FS Enzyme Mix and Reaction Buffer 5-8 seconds before use and place on ice.

1.2. Add the following components to a sterile nuclease-free tube:

COMPONENT	VOLUME
Intact DNA (0.1–500 ng)	26 µl
○ (yellow) NEBNext FS Reaction Buffer	7 µl
○ (yellow) NEBNext FS Enzyme Mix	2 µl
Total Volume	**35 µl**

1.3. Vortex the reaction for 5 seconds and briefly spin down. A small number of bubbles in the reaction will not inhibit performance.

1.4. Place in a thermocycler, with the heated lid set to ≥ 75°C, and run the following program:
15 minutes at 37°C
30 minutes at 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 < 1 ng, dilute the • (red) NEBNext Adaptor for MGI/Complete Genomics® (provided in NEB# E9640) *before setting up the ligation reaction using the provided, ice-cold NEBNext Adaptor Dilution Buffer for MGI/Complete Genomics. Keep the diluted adaptor on ice.*

INPUT	ADAPTOR DILUTION (VOLUME OF ADAPTOR: TOTAL VOLUME)
1–500 ng	No Dilution
Less than 1 ng	10-Fold (1:10)

*The NEBNext Adaptor for MGI/Complete Genomics and NEBNext Adaptor Dilution Buffer for MGI/Complete Genomics are provided in NEBNext Multiplex Oligos for MGI (Dual Index Primer Pairs Set 1, #E9640). The concentration of the Adaptor provided is 40 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.

2.2. Assemble the ligation reaction on ice by adding the following components, in the order given, to the fragmentation/end prep reaction from Step 1.4.:

COMPONENT	VOLUME
FS Reaction Mixture (Step 1.4.)	35 µl
• (red) NEBNext Adaptor for MGI/Complete Genomics*	5 µl
• (red) NEBNext Ligation Master Mix**	30 µl
• (red) NEBNext Ligation Enhancer	1 µl
Total Volume	**71 µl**

* The NEBNext Adaptor for MGI/Complete Genomics is provided in NEBNext Multiplex Oligos for MGI (Dual Index Primer Pairs Set 1, #E9640).

** Mix the Ligation Master Mix by pipetting up and down several times before adding to the reaction.

Note: Do not premix the NEBNext Ligation Master Mix and adaptor before use in the Adaptor Ligation Step.

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. We do not recommend adding adaptor to the premix in the Adaptor Ligation Step.

2.3. Set a 100 µl pipette to 60 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 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 number of bubbles will not interfere with performance.

2.4. Incubate in a thermocycler with the heated lid off and run the following program.
15 minutes at 20°C
Hold at 4°C

Samples can be stored at –20°C for up to 3 days.

3. Size Selection or Cleanup of Adaptor-ligated DNA

3A. Size Selection of Adaptor-ligated DNA (for input of 1–500 ng)

Note: The SPRIselect/AMPure XP Beads ratios recommended in this manual have been experimentally optimized for every step; this is critical since buffer compositions differ between steps and across protocols. Please adhere to these guidelines and not those recommended by other sources or for other kits. If using AMPure XP Beads, remove from 4ºC and keep at room temperature for 30 minutes before use.

The following size selection protocol is for libraries with 250 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 a starting volume of 103 µl.

Table 3.1 Recommended Conditions for Bead-Based Size Selection

Approximate Insert Size distribution	200 bp	250 bp	300–400 bp
1st Bead Addition (µl)	40	30	25
2nd Bead Addition (µl)	25	15	10

3A.1. Bring the reaction volume up to 103 µl by adding 32 µl 0.1X TE (dilute 1X TE Buffer 1:10 with water).

3A.2. Vortex SPRIselect or AMPure XP Beads to resuspend.

3A.3. Add **30 µl (~ 0.3X) of resuspended beads** to the 103 µl ligation reaction. Mix well by pipetting up and down at least 10 times. Be careful to expel all the liquid from 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.4. Incubate samples on bench top for at least 5 minutes at room temperature.

3A.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.

3A.6. After 5 minutes (or when the solution is clear), carefully transfer 130 µl supernatant containing your DNA to a new tube **(Caution: do not discard the supernatant)**. Discard the beads that contain the unwanted large fragments.

3A.7. Add **15 µl (0.15X) resuspended SPRIselect or AMPure XP 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.

3A.8. Incubate samples on the bench top for at least 5 minutes at room temperature.

3A.9. 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.10. 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.11. 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.12. Repeat Step 3A.11 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.13. 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 the 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.14. Remove the tube/plate from the magnetic stand. Elute the DNA target from the beads into 21 µl of 0.1X TE.

3A.15. Mix well on a vortex mixer or by pipetting up and down 10 times. Incubate for at least 2 minutes at room temperature. Transfer 19 µl of the bead slurry into a new tube for PCR. **Keep the beads and proceed to PCR amplification** (Don't put the solution on a magnetic stand to get the supernatant).

Samples in bead slurry can be stored at –20°C overnight without loss in yield.

3B. Cleanup of Adaptor-ligated DNA without Size Selection (for input < 1 ng)

The following section is for the cleanup of the ligation reaction. If your input DNA is > 1 ng, follow the size selection protocol in Section 3A.

Note: The SPRIselect/AMPure XP Beads ratios recommended in this manual have been experimentally optimized for every step; this is critical since buffer compositions differ between steps and across protocols. Please adhere to these guidelines and not those recommended by other sources or for other kits. If using AMPure XP Beads, remove from 4ºC and keep at room temperature for 30 minutes before use.

3B.1. Vortex SPRIselect or AMPure XP Beads to resuspend.

3B.2. Add **50 µl (~ 0.7X) resuspended beads** to the Adaptor Ligation reaction. Mix well by pipetting up and down at least 10 times. Be careful to expel all the liquid from 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 at least 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. While in the magnetic stand, add 200 µl of 80% freshly prepared ethanol to the tube/ plate. 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 21 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. Transfer 19 µl of the bead slurry into a new tube for PCR**. Keep the beads and proceed to PCR amplification**. (Don't put the solution on a magnetic stand to get the supernatant)

Samples in bead slurry can be stored at –20°C overnight without loss in yield.

4. PCR Amplification

Note: Use NEBNext Multiplex Oligos for MGI (Dual Index Primer Pairs Set 1) and follow the index guidelines from the manual (#E9640).

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

COMPONENT	VOLUME
Adaptor Ligated DNA Fragments (Step 3A.15. or 3B.10. beads included)	19 µl
⚬ (white plate) NEBNext Dual Index Primer Pairs for MGI/Complete Genomics	6 µl
• (blue) NEBNext MSTC High Yield Master Mix	25 µl
Total Volume	**50 µl**

4.2. 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.

4.3. Place the tube on a thermocycler 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	5-15*
Annealing	60°C	30 seconds	
Extension	65°C	45 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 using a different method before library prep may require re-optimizing 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 is a starting point to determine the number of PCR cycles best for standard library prep samples.

Table 4.1.

INPUT DNA USED IN STEP 1.2.	# OF CYCLES REQUIRED FOR STANDARD LIBRARY PREP: YIELD ~1 PMOL** (20–60 nM)
100–500 ng*	5
10 ng*	9–10
1 ng*	11–12
0.1 ng	14–15

* These cycle recommendations are based on DNA library following the size selection protocol

** 1 pmol is the input requirement for a circularization reaction

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

5. Cleanup of PCR Reaction

Note: The SPRIselect/AMPure XP Beads ratios recommended in this manual have been experimentally optimized for every step; this is critical since buffer compositions differ between steps and across protocols. Please adhere to these guidelines and not those recommended by other sources or for other kits. If using AMPure XP Beads, remove from 4ºC and keep at room temperature for 30 minutes before use.

5.1. Vortex SPRIselect or AMPure XP Beads to resuspend.

5.2. Add **35 µl (0.7X) resuspended beads** to the PCR reaction. Mix well by pipetting up and down at least 10 times. Be careful to expel all the liquid from 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 at least 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 32 µl of 0.1X TE.

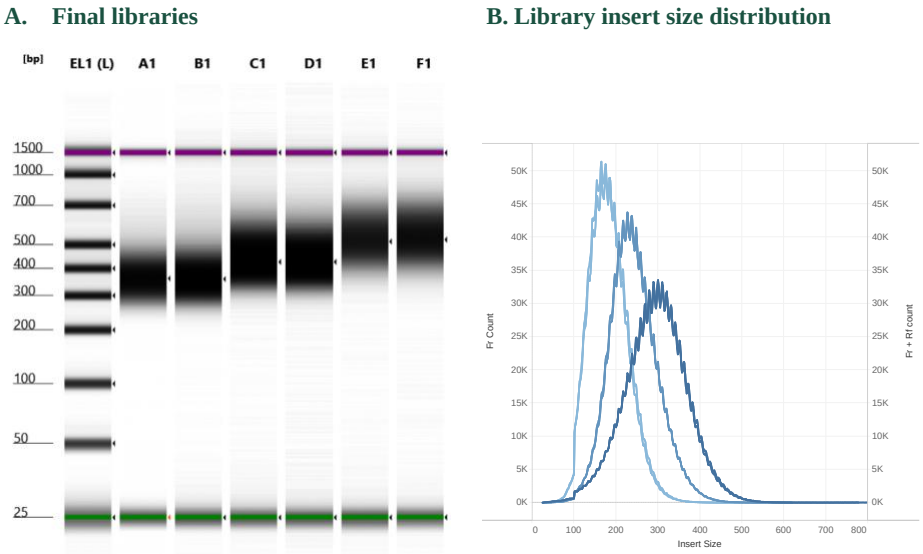
5.10. Mix well by pipetting up and down 10 times, or using a vortex mixer. Incubate for at least 2 minutes at room temperature. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing back on the magnetic stand.

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 PCR tube and store at –20°C.

5.12 Check the size distribution on a TapeStation HSD1000 ScreenTape® or 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. Examples of libraries prepared with human DNA (NA12878).



(A)Libraries made from 100 ng human DNA (NA12878, Coriell Institute for Medical Research) in duplicate were size selected according to Table 3.1, PCR amplified and diluted 1:5, and run on a TapeStation HSD1000 ScreenTape.
(B) Graphical depiction of library sequenced insert size distribution as determined by Picard CollectInsertSizeMetrics tool. PE100 sequencing was done on DNBSEQ G400.

Follow NEBNext Circularization Module for MGI (#E9635) protocol to make single strand circular DNA libraries. Then follow DNBSEQ instructions to make DNA nanoballs (DNB) using single-strand circular DNA libraries (ssCircular DNA). We recommend using 80 fmol ssCircular DNA in each DNB making reaction when using NEBNext FS DNA Library Prep Kit for MGI (NEB #E9620). This has been tested on DNBSEQ-G400RS FCL PE100 reagent and DNBSEQ-G99RS with FCL PE150 reagent. For other DNBSEQ platforms and sequencing reagents, we recommend doubling the ssDNA input as suggested in the DNBSEQ instructions. For example, if the DNBSEQ instructions recommend 40 fmol ssDNA input for a 100 µl DNB reaction, we recommend doubling the ssDNA input to 80 fmol to prepare the 100 µl DNB reaction.

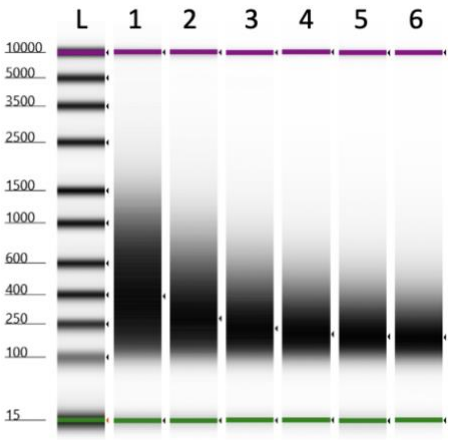
Appendix A

Recommendations for customized fragmentation times at 37°C for varying fragment size distributions.

Table A1. Time-dependent fragmentation pattern

FRAGMENTATION TIME (MINUTES)	FRAGMENT SIZE DISTRIBUTION (bp)
5	500 bp–1 kb
10	300–700 bp
15	200–450 bp
20	150–350 bp
30	100–250 bp

Figure A1. Example of size distribution on a Bioanalyzer. Human DNA (NA12878) was fragmented for 5-30 min.



Lane L: DNA ladder
Lane 1: 5 min
Lane 2: 10 min
Lane 3: 15 min
Lane 4: 20 min
Lane 5: 25 min
Lane 6: 30 min

Kit Components

NEB #E9620S Table of Components

NEB #	COMPONENT	VOLUME
E9616A	NEBNext FS Reaction Buffer	0.168 ml
E9617A	NEBNext FS Enzyme Mix	0.048 ml
E9613A	NEBNext Ligation Master Mix	0.72 ml
E7374A	NEBNext Ligation Enhancer	0.024 ml
E9615A	NEBNext MSTC High Yield Master Mix	0.6 ml
E9614A	TE Buffer (1X)	1.5 ml

NEB #E9620L Table of Components

NEB #	COMPONENT	VOLUME
E9616AA	NEBNext FS Reaction Buffer	0.672 ml
E9617AA	NEBNext FS Enzyme Mix	0.192 ml
E9613AA	NEBNext Ligation Master Mix	3 x 0.960 ml
E7374AA	NEBNext Ligation Enhancer	0.096 ml
E9615AA	NEBNext MSTC High Yield Master Mix	2 x 1.2 ml
E9614AA	TE Buffer (1X)	6 ml

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	6/24