INSTRUCTION MANUAL

NEBNext® Circularization Module for MGI®

NEB #E9720

24 reactions

Version 1.0_06/24

Table of Contents

NEBNext Circularization Module for MGI Includes

The volumes provided are sufficient for preparation of up to 24 reactions. All reagents should be stored at –20°C. Colored bullets represent the color of the cap of the tube containing the reagent.

- (lilac) NEBNext Circularization Reaction Buffer
- (lilac) NEBNext Circularization Ligase
- (lilac) NEBNext Splint Oligo for MGI/Complete Genomics®
- (orange) NEBNext Exo Mix for MGI/Complete Genomics
- (white) TE Buffer (1X)

Required Materials Not Included

- 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)
- Magnetic rack /stand (NEB #S1515 or similar)
- Thermal Cycler
- Qubit® ssDNA Assay Kit (Thermo Fisher Scientific® #Q10212)

Overview

The NEBNext Circularization Module for MGI contains the enzymes and buffers required to circularize dsDNA MGI libraries for sequencing on a MGI platform. The fast, user-friendly workflow 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 constructing and sequencing an indexed library on the 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.

Protocol

Symbols

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

1. Library Pool Denaturation

1.1. Make 40 µl of a 25 nM (or equivalent of 1 pmol) linear library pool from the libraries prepared with a NEBNext Library Prep Kit for MGI.

Alternatively, the following formula can be used to calculate the Mass (ng) corresponding to 1 pmol of dsDNA sample with varying fragment sizes.

$$\text{Mass (ng)}_{\text{corresponding to 1 pmol PCR products}} = \frac{\text{DNA fragment size (bp)}}{\text{1,000 bp}} \times 660 \text{ ng}$$

1.2. Place in a thermocycler with the heated lid set to ≥ 105°C, and run the following program:
3 minutes at 95°C
Immediately place the denatured pool on ice for 2 minutes.

2. Splint Ligation

2.1. Add the following components directly to the denatured library pool on ice:

COMPONENT	VOLUME
Denatured pool	40 µl
• (lilac) NEBNext Splint Oligo for MGI /Complete Genomics	3 µl
• (lilac) NEBNext Circularization Reaction Buffer	5 µl
• (lilac) NEBNext Circularization Ligase	2 µl
Total Volume	**50 µl**

2.2. 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.

2.3. Place in a thermocycler with the heated lid set at ≥ 47°C, and run the following program:
15 minutes at 37°C
Hold at 4°C

3. Digestion

3.1. Add 2 µl of • (orange) NEBNext Exo Mix for MGI/Complete Genomics to the splint ligation reaction.

3.2. 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.

3.3. Place in a thermocycler, with the heated lid set at ≥47°C, and run the following program:
15 minutes at 37°C
Hold at 4°C

4. Cleanup of splint-ligated DNA circle

Note: The SPRIselect/AMPure XP Beads ratios recommended in this manual have been experimentally optimized for every step. Please adhere to these guidelines and not those recommended by other sources or for other kits. If using AMPure XP Beads, remove from 4°C and keep at room temperature for 30 minutes before use.

4.1. Vortex SPRIselect or AMPure XP Beads to resuspend.

4.2. Add **158 µl (3X) resuspended beads** to the Exo digest 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, stop the centrifugation before the beads start to settle out.

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

4.4. 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.

4.5. 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).**

4.6. Add 200 µl of freshly prepared 80% 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.

4.7. Repeat Step 4.6. 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.

4.8. 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.

4.9. Remove the tube/plate from the magnetic stand. Elute the DNA target from the beads by adding 22 µl of TE Buffer (1X).

4.10. Mix well by pipetting up and down 10 times, or using a vortex mixer. 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.

4.11 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.

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

4.13. Use Qubit ssDNA Assay to quantify the ssDNA circle concentration. A typical concentration range is 1–5 ng/µl. The concentration varies when different library preparation workflows are used.

Follow DNBSEQ® instructions to make DNA nanoballs (DNB) using single-strand circular DNA libraries. Circles can be stored at -20°C for two weeks.

We recommend using 80 fmol ssCircular DNA in each DNB making reaction when using NEBNext FS DNA Library Prep Kit for MGI (NEB #E705) and NEBNext RNA Library Prep Kit for MGI (NEB #E9710). 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.

Kit Components

NEB #E9720 Table of Components

NEB #	COMPONENT	VOLUME
E9623A	NEBNext Circularization Reaction Buffer	0.12 ml
E9624A	NEBNext Circularization Ligase	0.048 ml
E9626A	NEBNext Splint Oligo for MGI / Complete Genomics	0.072 ml
E9627A	NEBNext Exo Mix for MGI / Complete Genomics	0.048 ml
E9614A	TE Buffer (1X)	1.5 ml

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	6/24