MDX219-B Lyophilized NGS Library Preparation Kit

Product Manual

1. KIT CONTENTS

Lyophilized NGS Library Preparation Reagents	Number of 8-Tube strips
End-Repair/A-Tailing Mix	1
Ligation Mix	1
Library Amplification Mix	1

2. STORAGE

The kit and its components can be stored at ambient temperature (20°C - 25°C).

3. SAFETY INFORMATION

When working with chemicals, always wear suitable personal protective equipment, including lab coat, gloves, and safety glasses.

4. PRODUCT SPECIFICATIONS

The Lyophilized NGS Library Preparation Kit is designed for Illumina® library preparation workflows. However, it can be optimized for other platforms, depending on the adapters used.

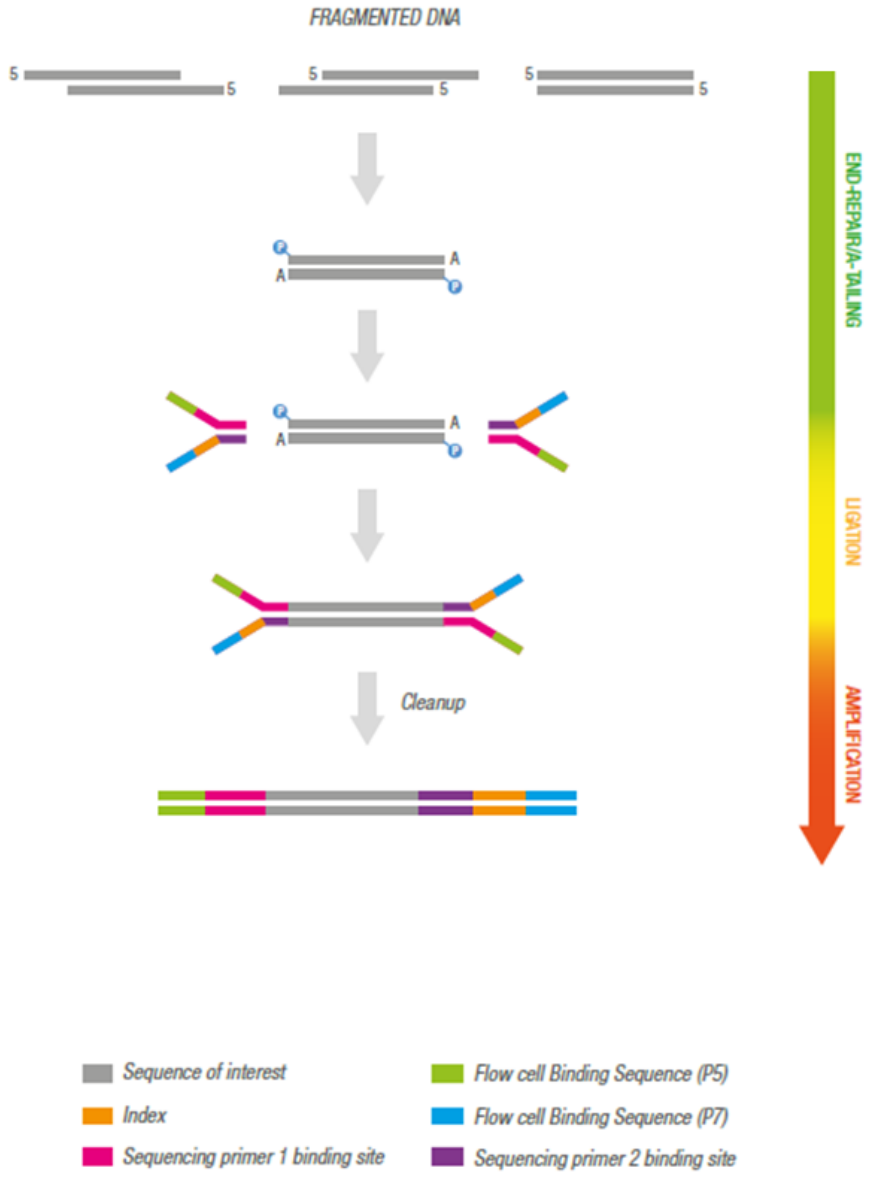

Fig. 1 Example of workflow for Illumina NGS Library Preparation

5. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY THE USER

The following additional items are required:

- Oligonucleotide adapters
- PCR primers for NGS library amplification compatible with the adapter system
- 10 mM Tris-HCl, pH 8.0
- Thermal cycler or heat block
- Equipment for the determination of DNA concentration such as Nanodrop™, Qubit™ or equivalent
- Equipment for the determination of DNA size distribution such as Tapestation™, Bioanalyzer or equivalent
- Reagents and equipment for the purification and size selection of DNA fragments such as Meridian NGS Clean and Select Beads or Beckman Coulter AMPure™ XP beads (with magnetic device)
- Molecular grade water

6. PROTOCOL

6.1.1 End-Repair and A-Tailing

1. Remove the End-Repair/A-Tailing Mix strip from the pouch.
2. For every library, dilute the desired amount of fragmented DNA into a final volume of 50 µL, to prepare the End-Repair/A-Tailing Mix as described in Table 1.

Table 1. Preparation of Input DNA Solution

Reagent	Quantity
Fragmented DNA	10 ng - 1 µg
Molecular grade water	up to 50 µL

3. Rehydrate the End-Repair/A-Tailing Mix using 50 µL of Input DNA Solution, keeping the reaction on ice. Flick the tube several times to ensure complete rehydration and spin down.
4. Incubate for **30 min at 37 °C, then 1 min at 72 °C**.
    a. **If a thermocycler is used, we recommend setting the heated lid at 85 °C.**
5. Cool down at 4 °C or transfer the reaction tube on ice.

6.2 Adapter Ligation

6.2.1 Preparation of Adapter Solution

1. Prepare an Adapter Solution by diluting adapter stocks in a 1 mM Tris-HCl (pH 8.0), 100 µM EDTA, 50 mM NaCl buffer according to Table 2.

Table 2. Indicative adapter concentration for various starting amounts of input DNA.

Input DNA Amount‡	Working Adapter Concentration
1 µg – 101 ng	15 µM
100 ng – 10 ng	1.5 µM

‡Users are advised to use this table as a guideline to optimize the Adapter:Insert molar ratio for DNA Input values different from the ones shown in this table.

NOTE: at this stage, select adapters with unique indexes for every sample sequenced in the same lane to support accurate demultiplexing.

6.2.2 Adapter Ligation Set-Up

1. Remove the Ligation Mix strip from the pouch and place it on ice.
2. Use 12.5 µL of Nuclease-free water to rehydrate the cake in the Ligation Mix tube. Flick the tube several times to ensure complete rehydration and spin down.
3. Add 2.5 µL of Adapter Solution to the rehydrated Ligation Mix cake (concentration as required, see table 2).
4. Transfer the 15 µL of Ligation Mix with Adapter Solution to the 50 µL of End-repaired reaction from section 6.1. Total volume is now 65 µL. Ensure optimal mixing by pipetting up and down 5 times.
5. Incubate for 15 min at 20 °C.
6. Proceed to post-ligation clean-up.

NOTE: we suggest using a minimum of 10 µL to rehydrate the ligation cake. The volume of adapters can be adjusted as needed, provided that the adapter concentration and the final ligation volume are maintained.

NOTE: we recommend performing the clean-up step immediately after ligation. However, if the user intends to stop after ligation without clean-up, it is suggested to inactivate the ligase by incubating the mix at 65 °C for 10 min, and then to store the adapter-ligated DNA overnight at -20°C. The clean-up step can be continued on the following day without affecting the quality or the yield of the library.

6.3 Post-ligation Clean-Up

This step is crucial to remove unligated adaptors and adaptor-dimers from the library.
Note: Equipment and reagents are not provided.

1. Allow AMPure XP beads to equilibrate at room temperature for at least 30 mins. Vortex beads thoroughly to ensure beads are homogenously suspended.

2. Perform a 1x bead-based clean-up by adding the appropriate volume of homogeneous AMPure XP beads, calculated based on the total ligation reaction volume, to each adaptor ligated DNA sample. Mix well by pipetting up and down at least 10 times. Incubate at room temperature for 5 mins.
3. Put the tube(s)/plate in the magnetic stand to capture the beads until the solution is clear (approximately 3-5 mins).
4. Keeping the tube(s)/plate in the magnetic stand, carefully remove and discard the supernatant without disturbing the beads.
5. Continue to keep the tube(s)/plate in the magnetic stand whilst adding 200 µL of 70% freshly prepared ethanol to each tube. **IMPORTANT**: Always use freshly prepared 70% ethanol.
6. Incubate the tube(s)/plate on the magnetic stand at room temperature for 1 min and remove the ethanol.
7. Repeat wash (step 5 to 6).
8. After the second wash, remove all residual ethanol without disturbing the beads. TIP: Use P20 or P10 pipettes and tips to aspirate small volumes of residual ethanol.
9. Leave the lids open and dry the beads at room temperature for 3-5 mins or until the residual ethanol has completely evaporated. **IMPORTANT**: Do not over-dry the beads as this will decrease yield. The bead pellet is dry when the appearance of the surface changes from shiny to matt.
10. Remove tube(s)/plate from the magnetic stand. Add **32 µL of dH_2O** to the bead pellet, mix well by pipetting up and down at least 10 times. Incubate for 5 mins at room temperature. Place tube(s)/plate back on magnetic stand for 2-3 mins or until the solution is clear.
11. Remove 30 µL of the supernatant and transfer to a fresh tube(s)/plate. Discard the beads.

SAFE STOPPING POINT: Adaptor-ligated DNA can be stored at -20°C up to 1 week.

6.4 Library Amplification (if required)

6.4.1 Primer Mix Preparation

Prepare the amplification Primer Mix by diluting the primers in nuclease-free water to a final concentration of 10 µM each. If needed, store at -20 °C and thaw on ice before use.

NOTE: The amplification primers should be compatible with the adapter system used.

6.4.2 PCR Set-Up

1. Prepare the Library Amplification Solution to be added to the tubes as described in Table 3.

Table 3. Library Amplification Solution

Reagent	Volumes per lyophilized cake
Purified adapter-ligated library from section 6.3	30 µL
Primer Mix (10 µM each) from section 6.4.1	5 µL
Nuclease-free water	15 µL
	50 µL (Total)

2. Remove the lyophilized Amplification Mix strip from the pouch and place it on ice.
3. Transfer 50 µL of Library Amplification Solution to rehydrate the cake in the Amplification Mix tube. Flick the tube several times to ensure complete rehydration and spin down.
4. Place the tube in a thermocycler and perform the PCR using the following cycling conditions:

Table 4. Cycling conditions

Temperature	Time	Cycles
98 °C	5 min	1
98 °C	30 sec	See Table 5
65 °C	2 min	
65 °C	4 min	1
4 °C	Hold	

NOTE: The following guidelines are based on amplification with Lyophilized NGS Library Preparation DNA polymerase and the primer mix (P5 and P7 primer sequences). Further optimisation of PCR cycle number may be required.

Table 5. Recommended number of PCR cycles best for standard library prep samples

Input DNA into end-repair reaction (Section 8.1)	Estimated number of PCR cycles
1 µg	1
100 ng	5 (10 for capture protocol)
50 ng	7 (11 for capture protocol)
10 ng	8 (13 for capture protocol)

6.5 Post-Amplification Clean-Up

1. Allow AMPure XP beads to equilibrate at room temperature for 30 minutes. Vortex the beads thoroughly to ensure they are homogeneously suspended.
2. Perform a 1.8 x bead-based clean-up by adding 90 µL of homogeneous AMPure XP beads to each sample. Mix well by pipetting up and down at least 10 times. Incubate at room temperature for 5 minutes.
3. Place the tube(s)/plate in the magnetic stand to capture the beads until the solution is clear (approximately 3-5 minutes).
4. Keeping the tube(s)/plate in the magnetic stand, carefully remove and discard the supernatant without disturbing the beads.
5. Continue to keep the tube(s)/plate in the magnetic stand while adding 200 µL of 70% freshly prepared ethanol to each tube/plate. **IMPORTANT:** Always use freshly prepared 70% ethanol.
6. Incubate the tube(s)/plate on the magnetic stand at room temperature for 1 minute, then remove the ethanol.
7. Repeat the wash (steps 5-6).
8. After the second wash, remove all residual ethanol without disturbing the beads. **TIP:** Use a P20 or P10 pipette and tips to aspirate small volumes of residual ethanol.
9. Leave the lids open and dry the beads at room temperature for 3-5 minutes, or until the residual ethanol has completely evaporated. **IMPORTANT:** Do not over-dry the beads, as this will decrease yield.
10. Remove the tube(s)/plate from the magnetic stand. Add 32 µL of 10 mM Tris-HCl, pH 8.0, to the bead pellet, and mix well by pipetting up and down at least 10 times. Incubate for 5 minutes at room temperature. Place on a magnetic stand for 2-3 minutes or until the solution is clear.
11. Remove 30 µL of the supernatant and transfer it to a fresh tube/plate. Discard the beads.

The purified, amplified libraries can be stored at 4 °C for up to two weeks or at -20 °C for longer periods of time.

The DNA library is ready for sequencing on an NGS platform compatible with the selected adapters and can be pooled if necessary. When loading the library into the sequencing machine, we recommend following the manufacturer's instructions.