meridian BIOSCIENCE®

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.

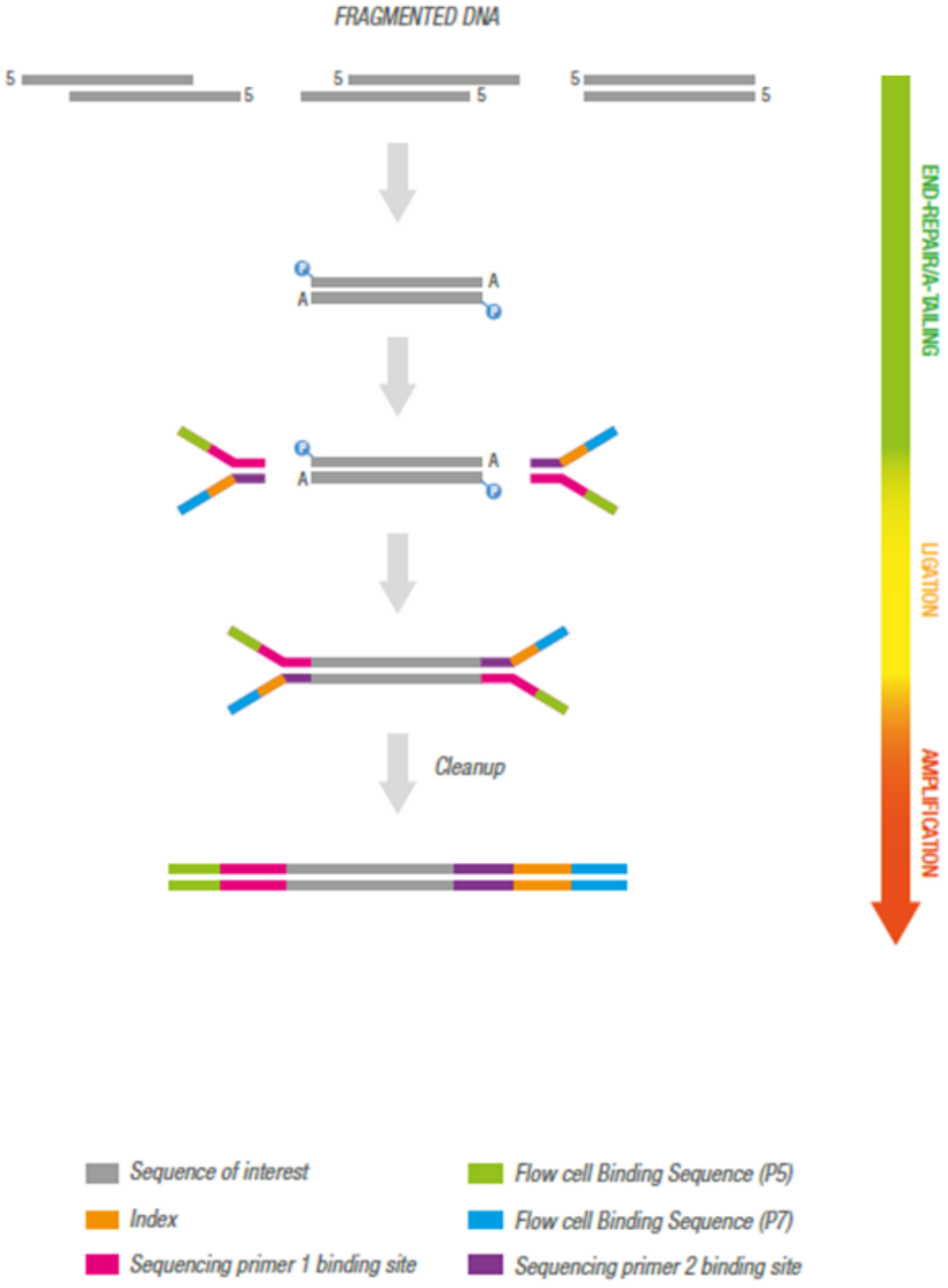

Fig. 1 Example of workflow for Illumina NGS Library Preparation

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
- 1 mM Tris-HCl, pH 8.0, 100 µM EDTA, 50 mM NaCl
- 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 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.
4. Incubate for 30 min at 20 °C, then 30 min at 65 °C.
   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.

6.2.2 Adapter Ligation Set-Up

1. Prepare the Adapter Ligation Solution to be added to the tubes according to Table 3.

Table 3. Preparation of Adapter Ligation Solution

Reagent	Volumes per lyophilized cake
Adapter Solution (concentration as required, see table 2)	2.5 µL
Nuclease-free water	12.5 µL
	15 µL (Total)

2. Remove the Ligation Mix strip from the pouch and place it on ice.
3. Use the Adapter Ligation Solution to rehydrate the cake in the Ligation Mix tube (15 µL for each tube).
4. Transfer the 15 µL of rehydrated Ligation Mix 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 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

Proceed with the clean-up procedure, according to the protocol of choice. Lyophilized NGS Library Preparation Kit is compatible with Meridian NGS Clean and Select Beads or Beckman Coulter AMPure™ XP beads.

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 4.

Table 4. 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. Ensure optimal mixing by pipetting up and down 5 times.
4. Place the tube in a thermocycler and perform the PCR using the following cycling conditions:

Table 5. Cycling conditions

Temperature	Time	Cycles
95 °C	5 min	1
95 °C	30 sec	See Table 6
65 °C	30 sec	
72 °C	2 min	
72 °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 6. 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	3-4
50 ng	5-6
10 ng	6-7

6.5 Post-Amplification Clean-Up

Proceed with the clean-up procedure, according to the protocol of choice. Lyophilized NGS Library Preparation Kit is compatible with Meridian NGS Clean and Select Beads or Beckman Coulter AMPure™ XP beads.