Nanodigmbio

NadPrep EZ DNA Library Preparation Kit v2 User Manual V2.1

NadPrep EZ DNA Library Preparation Module v2
NadPrep Universal Adapter (SI) Module (for MGI)
NadPrep Universal Adapter (MDI) Module (for MGI)

Thoroughly Read This Manual Before Operation.
For Research Use Only.
Version2.1

Statement

For research use only. Not for use in diagnostic procedures.

This instruction is intended for use with the NadPrep EZ DNA Library Preparation Kit v2.

Revision History

Version	Data Released	Change
2.1	Nov 2023	Revise description of storage temperatures for kit and components.
2.0	Jan 2022	Upgrade from the v1 of library preparation module to v2.
1.2	Aug 2021	Add method of library construction of DNA fragments with a main distribution ranging from 300 bp to 350 bp.
1.1	Feb 2021	Add compatible Unique Dual Index Adapter Modules.
1.0	Jan 2021	Original version

Content

Announcement

Environment

- Temperature: 20-25°C; Humidity: 40-60%.
- The personal protective equipment should be equipped during the experiment period.

Temperature Control

- Avoid repeated freezing and thawing.
- Unless otherwise specified, the preparation of enzymatic reaction should be carried out on ice/cold metal bath.
- The fragmentase should be added at the end of reaction mix preparation to prevent its activity at room temperature.
- Make sure the thermal cycler reaches the designated temperature before loading any reaction tube in.

Avoid Cross-contamination

- Briefly centrifuge all the tubes to collect the contents to the bottom before opening the lids.
- Physically separate the laboratory space, equipment and consumables among different steps.
- Clean the lab area using 0.5% sodium hypochlorite once finishing the experiment.

Others

- Make sure the regents are fully thawed and thoroughly mixed prior to use.
- Unless otherwise specified, all the centrifuge steps are conducted at room temperature (20-25°C).
- Unless otherwise specified, all mixing steps listed as "mix thoroughly" should be performed by either vortexing for 10 sec or pipetting up and down for 10 times, and then briefly centrifuge to collect the contents.

Introduction

NadPrep EZ DNA Library Preparation Kit v2 is designed for preparation of high-quality sequencing libraries from double-stranded DNA (dsDNA) on MGI platforms. To simplify the experimental process, multiple processes were applied in one single step, including the fragmentation, end repair and adapter ligation. This A-T ligation-based kit applies to the whole genome sequencing with DNA input ranging from 5 to 500 ng, and is compatible with hybridization capture based targeted sequencing.

The kit contains magnetic beads for DNA purification, and is optimized on size selection with NadPrep Universal Adapter (SI) Module (for MGI) and NadPrep Universal Adapter (MDI) Module (for MGI). All the components have been verified by quality control with a stable and remarkable performance.

Kit Content

NadPrep EZ DNA Library Preparation Kit v2 consists of Library Preparation Module and Adapter Module, which can be used in multiple combinations as required.

NadPrep EZ DNA Library Preparation Module v2

Catalog#	Item	Package/Storage
1002601	NadPrep EZ DNA Library Preparation Module v2, 24 rxn	Box1 / -25 ~ -15°C Box2 / 2 ~ 8°C
1002602	NadPrep EZ DNA Library Preparation Module v2, 96 rxn	Box1 / -25 ~ -15°C Box2 / 2 ~ 8°C

Package#	Component	Volume 1002601 24 rxn	Volume 1002602 96 rxn	Storage
Box1	FERA Buffer	150 µL	575 µL	-25 ~ -15°C
	FERA Enzyme	150 µL	575 µL	-25 ~ -15°C
	Enhancer Buffer	250 µL	950 µL	-25 ~ -15°C
	Ligation Buffer	750 µL	2×1,500 µL	-25 ~ -15°C
	DNA Ligase	60 µL	230 µL	-25 ~ -15°C
	2X HiFi PCR Master Mix	720 µL	2×1,600 µL	-25 ~ -15°C
	Nuclease Free Water	2.5 mL	8 mL	2 ~ 8°C
	TE Solution	1 mL	4 mL	2 ~ 8°C
Box2	NadPrep SP Beads	4 mL	15 mL	2 ~ 8°C

NadPrep Universal Adapter Module (for MGI)

Universal Adapter Module with Single Index

Catalog#	Item	M-Adapter (SI) Volume	M-Index Primer Mix (SI)#	M-Index Primer Mix (SI) Volume	Storage
1003611	NadPrep Universal Adapter (SI) Module Set A1 (for MGI), 24 rxn	60 µL	1-12	12 X 15 µL（Tube）	-25 ~ -15°C
1003621	NadPrep Universal Adapter (SI) Module Set B1 (for MGI), 96 rxn	230 µL	1-24	24 X 25 µL（Tube）	-25 ~ -15°C
1003622	NadPrep Universal Adapter (SI) Module Set B2 (for MGI), 96 rxn	230 µL	25-48	24 X 25 µL（Tube）	-25 ~ -15°C
1003620	NadPrep Universal Adapter (SI) Module Set D1 (for MGI), 480 rxn	1,100 µL	1-96	96 X 30 µL（Plate）	-25 ~ -15°C

Universal Adapter Module with Unique Dual Index

Catalog#	Item	M-Adapter (MDI) Volume	M-Index Primer Mix (MDI)#	M-Index Primer Mix (MDI) Volume	Storage
1003711	NadPrep Universal Adapter (MDI) Module Set A1 (for MGI), 24 rxn	60 µL	1-12	12 X 15 µL（Tube）	-25 ~ -15°C
1003721	NadPrep Universal Adapter (MDI) Module Set B1 (for MGI), 96 rxn	230 µL	1-24	24 X 25 µL（Tube）	-25 ~ -15°C
1003722	NadPrep Universal Adapter (MDI) Module Set B2 (for MGI), 96 rxn	230 µL	25-48	24 X 25 µL（Tube）	-25 ~ -15°C
1003725	NadPrep Universal Adapter (MDI) Module Set B3 (for MGI), 96 rxn	230 µL	49-72	24 X 25 µL（Tube）	-25 ~ -15°C
1003726	NadPrep Universal Adapter (MDI) Module Set B4 (for MGI), 96 rxn	230 µL	73-96	24 X 25 µL（Tube）	-25 ~ -15°C
1003731	NadPrep Universal Adapter (MDI) Module Set D1 (for MGI), 480 rxn	1,100 µL	1-96	96 X 30 µL（Plate）	-25 ~ -15°C
1003732	NadPrep Universal Adapter (MDI) Module Set D2 (for MGI), 480 rxn	1,100 µL	97-192	96 X 30 µL（Plate）	-25 ~ -15°C
1003733	NadPrep Universal Adapter (MDI) Module Set D3 (for MGI), 480 rxn	1,100 µL	193-288	96 X 30 µL（Plate）	-25 ~ -15°C
1003734	NadPrep Universal Adapter (MDI) Module Set D4 (for MGI), 480 rxn	1,100 µL	289-384	96 X 30 µL（Plate）	-25 ~ -15°C

Note: The library requires single strand circularization before sequencing on the MGI platform. This instruction does not contain the library circularization.

Equipment and Consumable

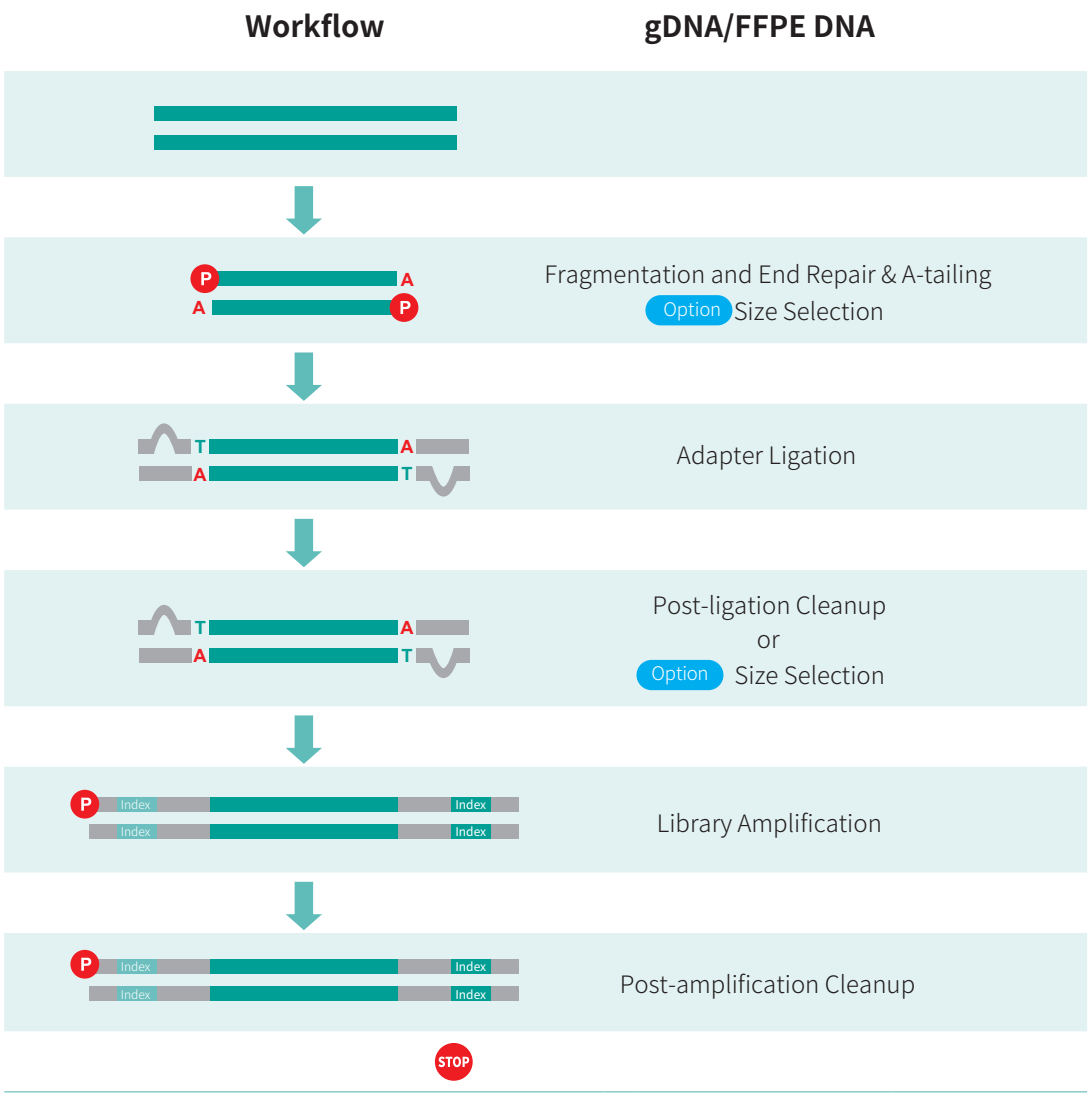
Equipment

Item	Description
Digital electrophoresis	Agilent 2100 Electrophoresis Bioanalyzer® system (Cat # G2939AA) Agilent 2200 TapeStation system (Cat # G2965AA) Bioptic Qsep100 capillary gel electrophoresis system or equivalent
Pipettor	General laboratory supplier
Thermal cycler	General laboratory supplier
Benchtop centrifuge	General laboratory supplier
Microcentrifuge	General laboratory supplier
Vortex mixer	General laboratory supplier
Magnet stand	Thermo Fisher DynaMag™ -2 Magnet（Cat # 12321D） Thermo Fisher DynaMag™ -96 Side Magnet（Cat # 12331D） BORTHEE-96 Side Magnet（Cat # MAG-96-11） or equivalent
Qubit fluorometer	Thermo Fisher Qubit™ 3.0 Fluorometer（Cat # Q33216） or equivalent
Timer	General laboratory supplier

Consumable

Item	Description
Absolute Ethanol	General laboratory supplier, analytical grade
0.2 mL PCR Tube	Axygen MAXYMum Recovery™ PCR Tubes, 0.2 mL flat cap（Cat # PCR-02-L-C） or equivalent

Workflow



NadPrep M-Adapter (MDI)

STOP Safe Stopping Point

Option Optional Step

*The schematic diagram takes the unique dual index (MDI) adapter as an example.

Protocol

Sample Requirement

Sample Quality: gDNA without serious degradation (tested by gel electrophoresis analysis); FFPE DNA with one clear strip with about 15 kb in size (refer to **Appendix 1: Grading Standard of FFPE DNA**).

Input Amount: 5-500 ng, > 0.5 ng/μL (quantified by Qubit™ 3.0 Fluorometer).

Sample Purification: High quality DNA input, with OD_{260}/OD_{280}=1.8-2.0 and OD_{260}/OD_{230}=2.0-2.5.

Note: EDTA inhibits the activity of fragment enzyme. If the extracted DNA sample contains EDTA, the sample must be pretreated according to Step 1, otherwise the enzymatic digestion effect will be affected.

Step 1: Sample Pretreatment

Before the experiment, please confirm the sample solvent:

1. If the DNA sample contains EDTA, 1.8X NadPrep SP Beads is recommended to be used for purification of the sample, and library preparation shall be conducted after the sample is eluted with Nuclease Free Water. If purification is not carried out, the final concentration of EDTA in the system shall be calculated according to the following table, or the DNA system shall be configured by adding corresponding volumes of Enhancer Buffer and Nuclease Free Water according to the sample volume containing EDTA (1 mM);
2. Sample pretreatment is not required for gDNA or FFPE DNA samples with purity meeting the requirements and without EDTA; this step can be skipped.

Example: the sample solvent contains 1 mM EDTA, and the sample concentration is 100 ng/μL, and 250 ng input DNA is used for fragmentation, then the final concentration of EDTA in the fragmented DNA system is calculated as 250 ng/100 (ng/μL)*1 mM/50 μL = 0.05 mM; or the volume of sample containing EDTA (1 mM) is calculated as 250 ng/100 (ng/μL) = 2.5 μL.

For samples containing EDTA, the method of configuring DNA system with Enhancer Buffer is as follows:

Final Concentration of EDTA in the System	Volume of Sample Containing EDTA (1 mM)	Volume of Enhancer Buffer	Volume of Nuclease Free Water
0.015-0.05 mM	0.75-2.5 μL	0.5 μL	37-38.75 μL
0.1 mM	5 μL	1.5 μL	33.5 μL
0.2 mM	10 μL	4 μL	26 μL
0.3 mM	15 μL	5 μL	20 μL
0.4 mM	20 μL	6 μL	14 μL
0.5 mM	25 μL	7 μL	8 μL
0.6 mM	30 μL	8 μL	2 μL

Note: When the final concentration of EDTA in the system is greater than 0.6 mM, excessive volume of Enhancer Buffer needs to be added, which may lead to the decline in data quality.

Step 2: Fragmentation and End Repair & A-tailing

1. Thaw FERA Buffer at room temperature. Mix thoroughly and centrifuge to collect the contents. Keep the tube on ice.
2. Thaw FERA Enzyme on ice. Briefly vortex and centrifuge to collect the contents.
3. Set up each reaction in a 0.2 mL PCR tube on ice as follows:

DNA System [1]	40 µL
FERA Enzyme	5 µL
FERA Buffer [2]	5 µL
Total	**50 µL**

Note [1]: If the input DNA is less than 40 µL, add Nuclease Free Water to a total volume of 40 µL. When the sample Input is ⩽ 10 ng, add 1 µL of Enhancer Buffer to obtain the expected fragment size. At this time, the input DNA should be ⩽ 39 µL.

Note [2]: When configuring FERA Enzyme and FERA as Mix for addition, pipette continuously for 3-5 times. Pipette slowly and carefully to ensure the expected volume.

4. Mix thoroughly and centrifuge to collect the contents.
5. Incubate the tube in the thermal cycler programmed as follows. Make sure the thermal cycler is stabilized at 25°C before loading.

25°C	See the following table for enzymatic digestion time
65°C	30 min
10°C	Hold

Note: The heated lid should be set at 70°C.

Expected Insert Size	Recommended enzymatic digestion time
200 bp	30 min
250 bp	25 min
300 bp	20 min
350 bp	15 min

Note: The above enzymatic digestion time is applicable to blood gDNA samples without degradation. If the sample quality varies, the enzymatic digestion size may be different. It is suggested to adjust the fragmented enzymatic digestion time according to the reference time. The fragmented enzymatic digestion time shall not be less than 10 min, as insufficient enzymatic digestion time will affect the efficiency of library preparation.

6. Store the unused reagents at -25 ~ -15°C.

Option **Optional steps: For gDNA or FFPE DNA samples, fragments size can be selected according to downstream analysis requirements. For specific methods, see Appendix 2 1.1.**

Step 3: Adapter Ligation

1. Thaw Ligation Buffer at room temperature, mix thoroughly and centrifuge to collect the contents. Keep the tube on ice.

Note: The Ligation Buffer is viscous. Please pipette slowly and carefully to ensure the expected volume.

2. Thaw DNA Ligase on ice. Briefly vortex and centrifuge to collect the contents.

3. Remove the tube in **Step 2** from the thermal cycler and set up each reaction on ice as follows:

Products of **Step 2**	50 µL
NadPrep M-Adapter (SI)/NadPrep M-Adapter (DI) (as appropriate) [1]	2 µL
Ligation Buffer [2]	26 µL
DNA Ligase [2]	2 µL
Total	**80 µL**

Note [1]: Make sure to add NadPrep M-Adapter (SI)/NadPrep M-Adapter (DI) before other reagents to avoid the adapter self-ligation.

Note [2]: If working on multiple samples, prepare Ligation Master Mix in advance with Ligation Buffer and DNA Ligase.

The dilution ratio of NadPrep M-Adapter(SI)/NadPrep M-Adapter (DI) according to the amount of input DNA is recommended as follows:

Input DNA	Adapter Concentration	Dilution Ratio	Adapter Insert Molar Ratio
500 ng	15 µM	*	10:1-6:1
250 ng	15 µM	*	20:1-12:1
100 ng	15 µM	*	50:1-30:1
50 ng	15 µM	*	100:1-60:1
25 ng	15 µM	*	200:1-100:1
10 ng	15 µM	2.5	200:1-100:1
5 ng	15 µM	5	200:1-100:1

Note: When the input DNA ⩽ 10 ng, the adapter should be diluted prior to use. For the distribution of other fragments, the dilution ratio can be adjusted on the premise of ensuring the molar ratio.

4. Mix thoroughly and centrifuge to collect the contents.

5. Incubate the tube in the thermal cycler and run the Cycling Program III set as follows. Make sure the thermal cycler is stabilized at 20°C before loading.

20°C	15 min
4°C	Hold

Note: The heated lid should be left open.

6. Store the unused reagents at -25 ~ -15°C.

Step 4: Post-ligation Cleanup

Option **For gDNA or FFPE DNA samples, fragments can be screened according to downstream analysis requirements. For specific methods, see Appendix 2 1.2.**

1. Equilibrate the NadPrep SP Beads to room temperature for a minimum of 30 min. Thoroughly vortex the beads prior to use.
2. Add 40 µL of NadPrep SP Beads to the same tube from **Step 3**. Mix thoroughly and then incubate the tube at 25°C for 5-10 min.
3. Briefly centrifuge and then place the tube on the magnet stand until the solution is clear (5 min). Carefully remove and discard the supernatant without disturbing the beads.

Note: The incubation time should be adjusted according to the actual performance of various magnet stands to ensure that the solution is completely clarified.

4. Keep the tube on the magnet stand, add 150 µL of 80% freshly prepared ethanol without disturbing the beads. Incubate the tube on the magnet stand at room temperature for 30 sec. Carefully remove and discard the ethanol.
5. Repeat step 4 once.
6. Briefly centrifuge and then place the tube on the magnet stand. Carefully remove all residual ethanol without disturbing the beads by using a 10-µL pipette tip.
7. Open the lid, dry the beads on the magnet stand at room temperature for 5 min.

Note: Over-dried beads may be more difficult to suspend.

8. Remove the tube off the magnet stand. Add 20 µL of Nuclease Free Water into the tube with beads inside for **Step 5**.

Step 5: Library Amplification

1. Thaw 2X HiFi PCR Master Mix and NadPrep M-index Primer Mix (SI)/NadPrep M-index Primer Mix (MDI) on ice. Mix thoroughly and centrifuge to collect the contents.
2. Set up each library amplification reaction in a 0.2 mL PCR tube on ice as follows:

Purified post-ligation from **Step 4**	20 µL
2 X HiFi PCR Master Mix	25 µL
NadPrep M-Index Primer Mix (SI)/NadPrep M-Index Primer (MDI)	5 µL
Total	**50 µL**

3. Mix thoroughly and centrifuge to collect the contents.
4. Incubate the tube in the thermal cycler and run the Cycling Program IV set as follows, with the heated lid set at 105°C.

98°C	2 min	1 cycle
98°C	15 sec	Varies (refer to the following table)
60°C	30 sec	
72°C	30 sec	
72°C	2 min	1 cycle
4°C	Hold	-

Input DNA	Recommended PCR Cycles of NadPrep M-Adapter (SI)/ NadPrep M-Adapter (DI)	
	Library Yield ⩾ 500 ng	Library Yield ⩾ 1,000 ng
500 ng	3	3
250 ng	3	4
100 ng	4-5	5-6
50 ng	5-6	6-7
25 ng	6-7	7-8
10 ng	8-9	9-10
5 ng	9-10	10-11

Important notes:

The selection of PCR cycles shall take into account the following:

- If double-sided size selection has been performed, one or two additional cycles are necessary.
- The recommended cycles of DNA samples from different grades of FFPE is shown in Appendix 1: Grading Standard of FFPE Samples;
- If the library is constructed for hybridization capture-based targeted sequencing, the library yield should be twice as much as the capture input by adjusting the PCR cycles;
- To obtain 500 ng of library yield, the DNA input should be less than 250 ng.

Step 6: Post-amplification Cleanup

1. Add the corresponding volume of NadPrep SP Beads into the Library Amplification tube. Mix thoroughly and then incubate the tube at 25°C for 5-10 min.

Primer Mix Applied to Step 5	Volume of NadPrep SP Beads
NadPrep M-Index Primer Mix (SI)	60 µL
NadPrep M-Index Primer Mix (MDI)	50 µL

2. Briefly centrifuge and then place the tube on a magnet stand until the solution is clear (~5 min). Carefully remove and discard the supernatant without disturbing the beads.

Note: The incubation time should be adjusted according to the actual performance of various magnet stands to ensure that the solution is completely clarified.

3. Keeping the tube on the magnet stand, add 150 µL of freshly prepared 80% ethanol without disturbing the beads. Incubate the tube on the magnet stand at room temperature for 30 sec. Carefully remove and discard the ethanol.

4. Repeat step 3 once.

5. Briefly centrifuge and then place the tube on the magnet stand. Carefully remove all residual ethanol without disturbing the beads by using a 10-µL pipette tip.

6. Dry the beads on the magnet stand at room temperature for 5 min.

Note: Over-dried beads may be more difficult to suspend.

7. Remove the tube off the magnet stand. Thoroughly resuspend the beads in 20 µL of TE solution. Incubate the tube at 25°C for 5 min.

8. Briefly centrifuge and place the tube on the magnet stand. Incubate for 2 min until the solution is clear. Transfer the clear supernatant to a new 0.2 mL PCR tube. Avoid transfer of NadPrep SP Beads.

Safe Stopping Point: The DNA library can be stored at 2 ~ 8°C for one week or -25 ~ -15°C for longer.

Step 7: Library Evaluation

1. The Qubit™ fluorometer or qPCR is recommended for library quantification.
2. The Bioanalyzer® 2100 (Agilent) or Qsep 100 (Bioptic) is recommended for size distribution analysis.
3. The quality control standards include:
   a. No adapter dimers or free adapters.
   b. Relatively concentrated size distribution.
   c. Suitable for MGI platform in size.

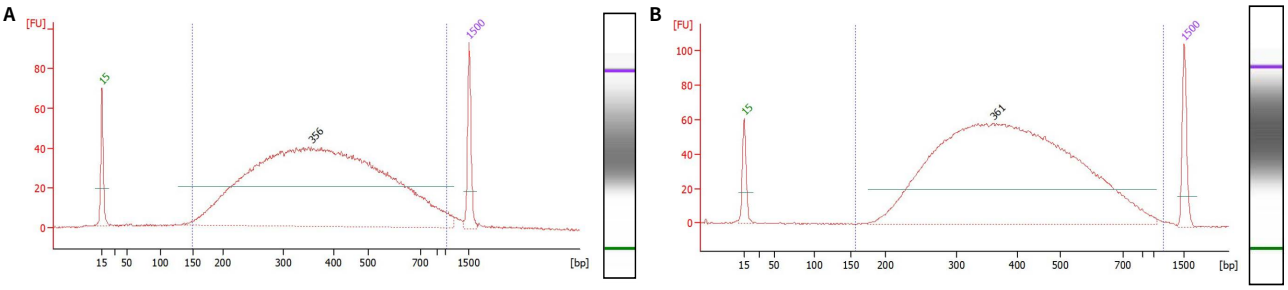


Fig 1. Example of size distribution of library. Use NadPrep EZ DNA Library Preparation Module V2 coupled with **A.** NadPrep Universal Adapter (SI) Module (for MGI) and **B.** NadPrep Universal Adapter (MDI) Module (for MGI) for size distribution of library (Agilent 2100, DNA 1000 Kit) after the library preparation of gDNA samples (with average insert fragment of 250 bp), respectively.

Step 8: Sequencing

Cyclize the DNA library with the circularization steps before loading on MGI platforms.
For targeted sequencing, please refer to the hybridization capture protocol (for MGI) from Nanodigmbio (www.nanodigmbio.com) and then perform the circularization steps.

Appendix 1: Grading Standard of FFPE DNA

FFPE DNA was classified into three grades. The corresponding experimental parameters were recommended as follows:

Grade	Grading Standard by Gel Electrophoresis	Recommended Input	Fragmentation Time (250 bp)	Recommended PCR Cycle (250 bp)
FFPE B^+	One clear and slight trailing strip with about 15 kb in size	⩾ 100 ng	Consistent with gDNA	Consistent with gDNA
FFPE B	One indistinct strip with about 15 kb in size, with medium diffusion	⩾ 100 ng	Reduce five minutes or more than gDNA	One or two extra cycles more than gDNA
FFPE C	Multiple strips ranging from 200 bp to 2,500 bp, with severe diffusion	⩾ 100 ng	Reduce ten minutes or more than gDNA	Two or three extra cycles more than gDNA
FFPE D	Stripes mainly ranging from 250 bp to 1,000 bp, with severe diffusion	⩾ 100 ng	Reduce fifteen minutes or more than gDNA	Three or four extra cycles more than gDNA

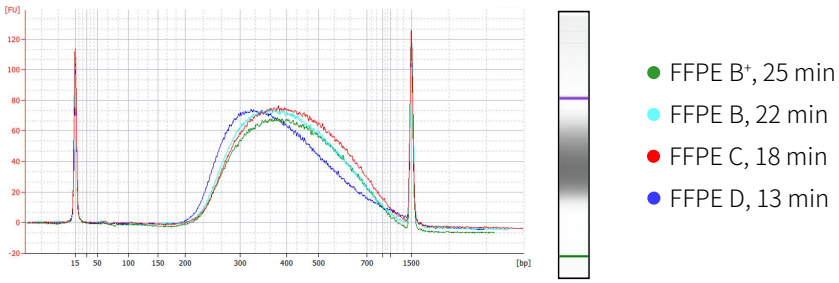


Fig 2. Example of size distribution of library. Use NadPrep EZ DNA Library Preparation Module v2 coupled with NadPrep Universal Adapter (MDI) Module (for MGI) for size distribution of library (Agilent 2100, DNA 1000 Kit) after the library preparation of FFPE samples (with average insert fragment of 250 bp).

Appendix 2: Size Selection

The NadPrep DNA Universal Library Preparation Kit v2 is compatible with a variety of size selection modes. Please choose the appropriate size selection modes according to the sample type, application direction, workflow and sequencing requirements.

Size Selection	Applicable Situation	Advantage	Disadvantage
Step 2 Option Double-sided size selection after fragmentation	Sufficient DNA input; Broad size distribution of DNA fragments after fragmentation	Narrow size distribution, high ligation efficiency and uniform sequencing data	Loss of sample
Step 4 Option Double-sided size selection after adapter ligation	Sufficient DNA input; Broad size distribution of libraries after adapter ligation	Narrow size distribution, Uniform sequencing data	Strict double-sized selection parameters based on library size

Note: Double-sided size selection during library construction can generate even size distribution and is therefore beneficial to data evenness after multiplexed capture and/or multiplexed sequencing. However, loss of sample is unavoidable due to extra handling steps. Please refer to the table to choose appropriate method.

1.1 Double-sided Size Selection after DNA Fragmentation (Optional Step after Step 2)

The volume of beads for double-sided size selection of fragmented product of **Step 2** are recommended as follows:

Important note:

- The average size of the fragmented DNA should coincide the predicted size, to avoid the aggravation of sample loss.

Expected DNA Size Distribution [1]	Sample Volume	Volume of NadPrep SP Beads (V1)	Volume of NadPrep SP Beads (V2)[2]
200 bp	50 µL	45 µL	50 µL
250 bp	50 µL	40 µL	50 µL
300 bp	50 µL	35 µL	50 µL
350 bp	50 µL	30 µL	50 µL

Note [1]: For WGS, it is recommended to select DNA fragments with a mean length of 300-350 bp. For targeted sequencing, it is recommended to select DNA fragments with a mean length of 200-250 bp.

Note [2]: The beads in V2 should be concentrated. Please refer to Appendix 1.1 step 3 as follows.

1. Equilibrate the NadPrep SP Beads to room temperature for a minimum of 30 min. Thoroughly vortex the beads for 45 sec at high-speed.
2. Transfer 50 µL of fragmented DNA in **Step 2 Fragmentation and End Repair & A-tailing** into a new 0.2 mL PCR tube. Add V1 volume of NadPrep SP Beads into the tube. Mix thoroughly and then incubate at 25°C for 5-10 min.

Note: If the fragmented DNA is less than 50 µL, add Nuclease Free Water to a total of 50 µL.

3. Prepare V2 volume of NadPrep SP Beads in a new 0.2 mL PCR tube. Briefly centrifuge and then place the tube on a magnet stand until the solution is clear (~5 min). Carefully remove and discard 40 µL of the supernatant without disturbing the beads. Remove the PCR tube off the magnet stand and mix the residues thoroughly.

Note: The incubation time should be adjusted according to the actual performance of various magnet stands to ensure that the solution is completely clarified.

4. Briefly centrifuge and then place the PCR tube in **Appendix 1.1** step 2. on the magnet stand until the solution is clear (~5 min). Transfer all the supernatant (50 µL+V1) into the PCR tube in **Appendix 1.1** step 3. Mix thoroughly and then incubate at 25°C for 5-10 min.

Note: Keep the supernatant (50 µL+V1) and discard the beads.

5. Briefly centrifuge and then place the tube on the magnet stand until the solution is clear (~5 min). Carefully remove and discard the supernatant without disturbing the beads.

6. Keeping the tube on the magnet stand, add 150 µL of freshly prepared 80% ethanol without disturbing the beads. Incubate the tube on the magnet stand at room temperature for 30 sec. Carefully remove and discard the ethanol solution.

7. Repeat step 6 once.

8. Briefly centrifuge and then place the tube on the magnet stand. Carefully remove all residual ethanol without disturbing the beads by using a 10-µL pipette tip.

9. Dry the beads on the magnet stand at room temperature for 5 min.

Note: Over-dried beads may be more difficult to suspend.

10. Remove the tube off the magnet stand. Thoroughly resuspend the beads in 43 µL of Nuclease Free Water. Incubate the tube at 25°C for 2 min.

11. Briefly centrifuge and then place the tube on the magnet stand. Incubate for 2 min until the supernatant is clear (collect 1 µL of supernatant for quantification). Transfer the supernatant (~40 µL) containing DNA to a new 0.2 mL PCR tube. Keep the tube on ice and directly perform **Step 3 Adapter Ligation**.

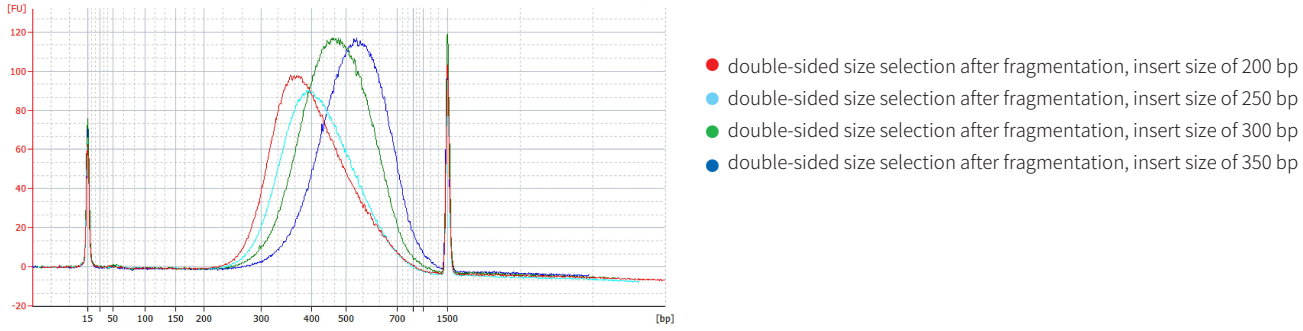


Fig 3. DNA size distribution with double-sided size selection after fragmentation (Covaris™) by using Agilent 2100 Analyzer.

1.2 Double-sided Size Selection after Adapter Ligation (Optional Step for Step 4)

The volume of beads for double-sided size selection of ligated product of **Step 3** are recommended as follows:

Important note:

- This step is not recommended for input DNA ⩽ 50 ng or DNA with low quality.

Expected DNA Size Distribution	Volume of NadPrep SP Beads (V1)	Volume of NadPrep SP Beads (V2)
200 bp	34 μL	32 μL
250 bp	26 μL	30 μL
300 bp	22 μL	28 μL
350 bp	20 μL	25 μL

1. Equilibrate the NadPrep SP Beads to room temperature for a minimum of 30 min. Thoroughly vortex the beads for 45 sec at high-speed.
2. Add V1 volume of NadPrep SP beads into the PCR tube of **Step 3 Adapter Ligation**. Mix thoroughly and then incubate at 25°C for 5-10 min.
3. Briefly centrifuge and then place the tube on the magnet stand until the solution is clear (~5 min). Carefully remove and discard the supernatant without disturbing the beads.

Note: The incubation time should be adjusted according to the actual performance of various magnet stands to ensure that the solution is completely clarified.

4. Keeping the tube on the magnet stand, add 150 μL of freshly prepared 80% ethanol solution without disturbing the beads. Incubate the tube on the magnet stand at room temperature for 30 sec. Carefully remove and discard the ethanol solution.
5. Repeat step 4 once.
6. Briefly centrifuge and then place the tube on the magnet stand. Carefully remove all residual ethanol solution without disturbing the beads by using a 10-μL pipette tip.
7. Dry the beads on the magnet stand at room temperature for 5 min.

Note: Over-dried beads may be more difficult to suspend.

8. Remove the tube off the magnet stand. Thoroughly resuspend the beads in 50 μL of Nuclease Free Water. Incubate the tube at 25°C for 2 min.
9. Add V2 volume of beads and then mix thoroughly. Incubate at 25°C for 5-10 min.
10. Briefly centrifuge and then place the tube on the magnet stand until the solution is clear (~5 min). Transfer all the supernatant (50 μL+V2) to a new 0.2 mL PCR tube.

Note: Keep the supernatant (50 μL+V2) and discard the beads.

11. Add 30 μL of NadPrep SP Beads to the supernatant (from Step 10). Mix thoroughly and incubate at 25°C for 5-10 min.
12. Briefly centrifuge and then place the tube on the magnet stand until the solution is clear (~5 min). Carefully remove and discard the supernatant without disturbing the beads.
13. Keeping the tube on the magnet stand, add 150 μL of freshly prepared 80% ethanol solution without disturbing the beads. Incubate the tube on the magnet stand at room temperature for 30 sec. Carefully remove and discard the ethanol solution.
14. Repeat step 13 once.

15. Briefly centrifuge and then place the tube on the magnet stand. Carefully remove all residual ethanol without disturbing the beads by using a 10-µL pipette tip.

16. Dry the beads on the magnet stand at room temperature for 5 min.

Note: Over-dried beads may be more difficult to suspend.

17. Remove the tube off the magnet stand. Add 20 µL of Nuclease Free Water into the tube with beads inside. Keep the tube on ice and directly perform **Step 5 Library amplification**.

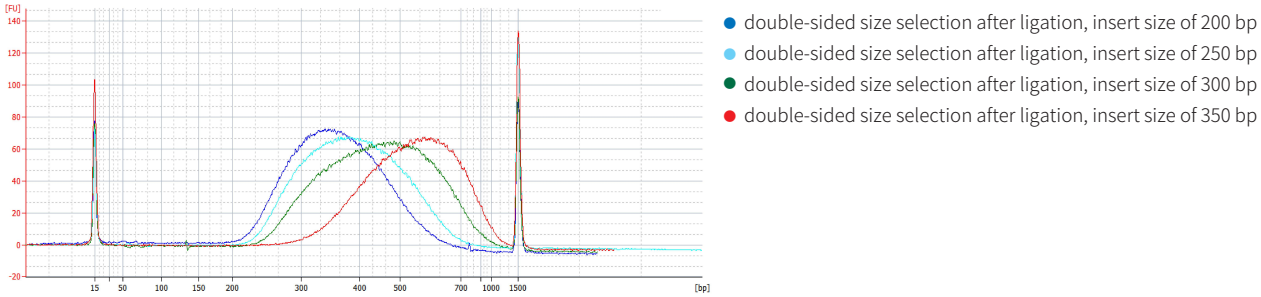


Fig 4. Library size distribution with double-sided size selection after ligation by using Agilent 2100 Analyzer.

Appendix 3: Index Information

The index sequences of NadPrep M-Index Primer Mix in NadPrep Universal Adapter (SI) Module (for MGI) are listed as follows:

Index# (96-well Plate Position)	Index Sequence
1 (A1)	TAGGTCCGAT
2 (B1)	GGACGGAATC
3 (C1)	CTTACTGCCG
4 (D1)	ACCTAATTGA
5 (E1)	TTCGTATCCG
6 (F1)	GGTAACGAGC
7 (G1)	CAACGTATAA
8 (H1)	ACGTCGCGTT
9 (A2)	TTCTGCTAGC
10 (B2)	AGGAAGATAG
11 (C2)	GCTCTTGCTT
12 (D2)	CAAGCACGCA
13 (E2)	CGGCAATCCG
14 (F2)	ATCAGGATTC
15 (G2)	TCATTCCAGA
16 (H2)	GATGCTGGAT
17 (A3)	GTGAGTGATG
18 (B3)	GAGTCAGCTG
19 (C3)	TGTCTGCGAA
20 (D3)	ATTGGTACAA
21 (E3)	CGATTGTGGT
22 (F3)	ACAGACTTCC
23 (G3)	TCCACACTCT
24 (H3)	CACCACAAGC
25 (A4)	TAGAGGACAA
26 (B4)	CCTAGCGAAT
27 (C4)	GTAGTCATCG
28 (D4)	GCTGAGCTGT
29 (E4)	AACCTAGATA
30 (F4)	TTGCCATCTC
31 (G4)	AGATCTTGCG
32 (H4)	CGCTATCGGC
33 (A5)	GCAACGATGG
34 (B5)	TAATCGTTCA
35 (C5)	GTTCGCTCTA
36 (D5)	TCTCACACAT
37 (E5)	CTGTTAGGAT
38 (F5)	CGCAGACGCG
39 (G5)	AAGGATCATC
40 (H5)	AGCGTTGAGC

Index# (96-well Plate Position)	Index Sequence
41 (A6)	TTAGATGCAT
42 (B6)	GTCCAGAGCT
43 (C6)	CACGTGATAG
44 (D6)	CCACTAGTCC
45 (E6)	TGGACTTGGC
46 (F6)	GCTTGACAGG
47 (G6)	AAGACCTCTA
48 (H6)	AGTTGCCATA
49 (A7)	ATGTACGCAG
50 (B7)	TTAATGAGAT
51 (C7)	TGCGCCACTT
52 (D7)	CATTAAGGCC
53 (E7)	CCGCCTCAGA
54 (F7)	AATCGGCTCG
55 (G7)	GCCGGTTATC
56 (H7)	GGAATATTGA
57 (A8)	ATTCAACGGA
58 (B8)	AACTGTACTG
59 (C8)	GTACCTCAAT
60 (D8)	GACTTCTAAT
61 (E8)	TGAAGCGTTG
62 (F8)	CGTGCGATCC
63 (G8)	TCGGAAGGCA
64 (H8)	CCGATGTCGC
65 (A9)	ACTTAGAATG
66 (B9)	TCCAAGCCTG
67 (C9)	AGACGATGAT
68 (D9)	CTCACAAGAC
69 (E9)	CGTTCCTACT
70 (F9)	GTGGTTGTGA
71 (G9)	GAAGGCCTGC
72 (H9)	TAGCTTGCCA
73 (A10)	GACAATGCTC
74 (B10)	GCTAATCACA
75 (C10)	AGTCCATAGG
76 (D10)	CTATCGCCTA
77 (E10)	ATCGTGGTCT
78 (F10)	TGGCTAATAC
79 (G10)	CAGTGCAGAG
80 (H10)	TCAGGCTGGT

Index# (96-well Plate Position)	Index Sequence
81 (A11)	ATACTCACGC
82 (B11)	ATGCTCCGCG
83 (C11)	TGTGAACTTG
84 (D11)	GAGAGGTGCT
85 (E11)	TGCACTGTAA
86 (F11)	GCCTAGGCAA
87 (G11)	CCATCATAGC
88 (H11)	CATGGTAATT

Index# (96-well Plate Position)	Index Sequence
89 (A12)	CACCATGTCT
90 (B12)	ATATGTCTGG
91 (C12)	AAGGAAGCGT
92 (D12)	TCAAGACGTC
93 (E12)	CCGCTCAGTA
94 (F12)	GGTGTGTACA
95 (G12)	TTCACGTAAG
96 (H12)	GGTTCCACAC

Note: The index sequences are as same as the official index sequences on MGI platform.

The single-index sequences on MGI platform are designed to be balanced within each group. To achieve the best sequencing quality, it is recommended to use continuous indexes as indicated within one block in the table and involve at least 8 indexes for multiplexed sequencing.

The index sequences of NadPrep M-Index Primer Mix in NadPrep Universal Adapter (MDI) Module (for MGI) are listed as follows:

Index# (MDI)	Primer 1 Index	Primer 2 Index
1	CGTCGATGAC	TAACACGACG
2	ATATAAGGCG	TGTTCTCTTC
3	GATCGTGCTC	GAGTTCACAA
4	CAGTCTTCGG	CTGATGTCCT
5	AGAACGATCT	AGACAGTGGC
6	TTGGTGCATT	CTCACACTTA
7	GCCGTCATAA	GCCGGTAAGT
8	TCCAACCAGA	ACTGGAGGAG
9	GATAGCAAGA	CAACAGTAAC
10	ACCGTGCTTC	ATAACGCTCA
11	GCAGATGTAA	GATTGCGCCT
12	TGTTGGAGCG	CGGTGTTGGA
13	TTGTATCCAC	TTCCAACATC
14	CGCACAGATG	TGCGTCATGG
15	CAACTCTCGT	GCTATAGCTT
16	ATGCCATGCT	ACGGCTAGAG
17	AGGCAGCTTA	GTAGAGATTG
18	TAGCCTAGCG	TCGAGCGTTA
19	ATCACGTGCG	GATGAAGGAT
20	CGTTATGCGC	ACGAGGTACT
21	CAAGGATCGA	CTTCCTACAA
22	GTTGTCGTAT	CAATCTCGCG
23	GCAATCAATC	TGCCTATCGC
24	TCCTGACAAT	AGCTTCCAGC
49	TCACATTGCT	GATAGTAACG
50	AATGGCGCTC	TGAGTGGCTA
51	GTCTCAATGA	CCGTCATTAC
52	CGGATGCAAG	ATCCACCGGT
53	AAGCCTATTG	GCTTGTTCAG
54	CGCTACTGCA	AACAAGCACT
55	TCAAGAGCAT	TTGCCAGTGA
56	GTTGTGCAGC	CGAGTCAGTC
57	AGACAGGAAT	TCGCGATGTC
58	CCTTGCCGTA	AGTGACACCA
59	GTCATTACGG	GACATTCAAG
60	TAGGCATTCC	CTATCGGTGT
61	AGCCAGTAGG	CCGATGACGT
62	GTAAGTGTAC	TTATCTCGAG
63	TAGTCACGTT	AGCCGATACC
64	CCTGTCACCA	GATGACGTTA

Index# (MDI)	Primer 1 Index	Primer 2 Index
25	AGGTGCCTTA	GTGCTGTCAC
26	AAGAACCAAG	TCCGAGATGC
27	CATACATGAC	GAAGCTCTTA
28	TGCCTGGTGA	TTGAGTAAGG
29	TCTCGGAGTT	CAACACGAAT
30	GTCGTAGACT	ACCTCCGCTA
31	GCATATACCG	AGTATATGCG
32	CTAGCTTCGC	CGTTGACGCT
33	GACGTATCAA	CTAGCAGTTA
34	CCTGCTAGGA	GCATTCTCGC
35	CAACTTGGCG	GATCCAATAC
36	ATGCGACCTC	TTCGATGACG
37	GTTAAGGACG	CCGCATACTA
38	TCCAAGTTGT	TGGAGCCGAT
39	AGGTCCATTC	AGCTTGCGCT
40	TGATGCCAAT	AATAGGTAGG
41	CCTAAGAGTT	TATGCTGAGA
42	GATACTAGCT	CGGTCTCTGT
43	AAGGCATCTC	GTGTACGTCG
44	TGGCATCTGG	GTACTAAGAG
45	ACACTGGAGC	AGTGGATATC
46	GTATGCCACA	CAACTGCGCA
47	CTCTTAGTAG	ACCAAGACTC
48	TGCGGCTCAA	TCCAGCTCAT
65	ATCGTGGATG	GACGCGGTAT
66	TGGAGATCGA	ACGAGACGTC
67	CCTCACAGAT	CTACTCAAGA
68	GAATCTCTCC	TGTTATTCCG
69	GCAGACTGAC	CCGTCACTGA
70	CTCATTAACG	TGACGCAACT
71	TGGTGAGCTT	GTTGTTGCTC
72	AATCCGCTGA	AACAAGTGAG
73	TCGCATCAAC	TGGTTGGAAT
74	AGAACAGTGA	GAACGTTCGG
75	CATGTCTCCT	ATCGCACGCA
76	GTCTGGAGTG	CCTAACATTC
77	GAGGTCTGTG	CGTGTGGATG
78	CTATAGACGT	TTACGTCGGT
79	TGCAGTGACC	ACGAACATAC
80	ACTCCACTAA	GACTCATCCA

81	GCGAAGTAGG	TGCCTGACTC
82	TGCCTAACCT	CATTATCGCT
83	AATGGTCTAC	GTAAGCGAGG
84	CTATCCGGTA	ACGGCATTAA
85	TACGCTTCAG	GACACTGCCA
86	CGGAGCATCT	CCGTAGCATG
87	GTACTAGATC	ATTCTATGGC
88	ACTTAGCGGA	TGAGGCATAT
89	ATCACTCCAT	ATACGCGCCA
90	GATCGCAGTG	TCTTAGTGAC
91	CCGGAATTCC	GAGGTACATT
92	TGATTGGAGA	CGCACTATGG
93	CACAAGGTCG	TGGCTCGCTT
94	TCTCGCAGGA	GTCACTTAAG
95	GTGGTATCAT	CAATAGAGGC
96	AGATCTCATC	ACTGGACTCA
97	CCGTTGTGAG	GCTCTGTGTC
98	AATGGTGAGT	TGGTGCGCAA
99	TGCAACCTTC	AACACACTGT
100	GTACCAACCA	CTAGATAACG
101	CCGATTCGAT	TAGTTGGTAC
102	ATTGCAGCCA	CGAGACAACT
103	GAATACTTGC	GTTCCACCGA
104	TGCCGGAATG	ACCAGTTGTG
105	ATGCGGATCC	CAAGCAGCAG
106	CCTTCATGAA	GCGAACTGGT
107	GACGATCATG	TGCTGTCTTA
108	TGAATCGCGT	ATTCTGAACC
109	TTCGTTCACA	CAACACCGAG
110	GAGACGGTTG	GTTGCAGATC
111	ACACAATGGT	TGGTGGTTGT
112	CGTTGCACAC	ACCATTACCA
113	AGAGTGCATG	GTCCGATTAC
114	GTTCGCATAC	CAGTCGGATT
115	TCGAATTCGT	TGTGATCGCG
116	CACTCAGGCA	ACAATCACGA
117	AGAAGTAGGA	TCGTGGAATT
118	CTTCCAGTTG	AATACAGCGC
119	GAGGTGCAAC	GTCCTCCTAA
120	TCCTACTCCT	CGAGATTGCG
121	TCCGACAGTT	CCGTAGTTGG
122	AGTAGTTACC	TTAGCTCGTT
123	GAATCGGTGG	GATCGCACAA
124	CTGCTACCAA	AGCATAGACC
125	GAGGCTGAAT	TCTAATCGCA
126	AGTAAGAGTC	AAGCTAACAC
127	TCCTGACTGA	CGAGCGTAGT
128	CTACTCTCCG	GTCTGCGTTG
129	CGAAGTACCG	GCGGATATTC
130	ACCGAATAGA	ATACCGCAAG
131	TAGTCGCTAT	TGTTGCGCGT
132	GTTCTCGGTC	CACATATGCA
133	AAGAGACACA	GCAACCTGAG
134	CCACTTGGAT	TAGTAGCACT
135	GTCTCCTTGC	AGCCTTATTC
136	TGTGAGACTG	CTTGGAGCGA
137	AGGATAGCCG	AGACGTGTTA
138	TCTGCTATAC	CCGGTGAGAT
139	GTCTACTGGT	GTCTCACAGC
140	CAACGGCATA	TATAACTCCG
141	GTACAGCGGA	GAAGTCCTTG
142	CGCATAGCAC	TCCAATGCCA
143	ACTGGTTATG	ATGTCGAGAC
144	TAGTCCATCT	CGTCGATAGT
145	ACCATACTCC	ACATGCGTAA
146	CGGTGTACAA	TGCAAGTAGC
147	GTTGCGGAGT	CTTCCAAGCT
148	TAACACTGTG	GAGGTTCCTG
149	CAAGTAACGG	CACGTTGCAG
150	TCGAGTGTAT	GTGAACAAGA
151	GTTCCGTACC	TGTTGGTGTC
152	AGCTACCGTA	ACACCACTCT
153	TCTAACGTGT	CGAGTTGAAT
154	AGGCCTCCAA	GACACCTTCC
155	GAATTGAACC	TTGCAACGTA
156	CTCGGATGTG	ACTTGGACGG
157	GTTCTAACTC	GTCATCGCGT
158	CCAACTCGCT	TAGGAATGCA
159	AGGTAGTAAG	CCTTGTCTAC
160	TACGGCGTGA	AGACCGAATG

161	CCATTGAATG	CGTAGATCGC
162	TTCAATCGAC	TAAGCGCGTG
163	AGGCGCTTCT	GTGCTTGTAT
164	GATGCAGCGA	ACCTACAACA
165	CGAGAATTCA	CCAGATGTGC
166	GCGTTCGCTT	GATTCGTGAG
167	TTCCGTAAGC	TTGCTCACCA
168	AATACGCGAG	AGCAGACATT
169	CGGTAATATC	TGGATCGGTC
170	ACAGCCGGAA	ATATAGCCGG
171	GTTCTGCTCG	CCTCCATTAT
172	TACAGTACGT	GACGGTAACA
173	CGTGGAGTTG	CAATTCCTCA
174	GTCTTCTCGA	GCGAGAAGAG
175	TCACCTCAAT	TTCCAGTAGT
176	AAGAAGAGCC	AGTGCTGCTC
177	TCTGATGGAA	ACTGGTGCAA
178	AGAACGTCCG	CGCCAGAATC
179	GTCTTCCATT	GTAATACTCG
180	CAGCGAATGC	TAGTCCTGGT
181	ACAAGCTCTA	ACTGGCTAAC
182	CTGCAGGAAG	CTACTGATGT
183	TGTTCTAGCT	GACTATGGCA
184	GACGTACTGC	TGGACACCTG
185	GTAAGGTGTT	TCTCACCAGG
186	AAGGCTAAGC	AACGTAGGCT
187	CGCTTCCTAA	CGATCTTCTC
188	TCTCAAGCCG	GTGAGGATAA
189	TAGGACCGTC	AGTGGATCAA
190	AGTCCTGTAA	TACATCAACG
191	CTAAGGAAGG	CTACCGCGTT
192	GCCTTATCCT	GCGTATGTGC
193	ACTCGCGGTA	ACAATCCGGC
194	CACGAACACT	TTGTGGTATG
195	GTAACTATGG	CGCCATGTAA
196	TGGTTGTCAC	GATGCAACCT
197	CGGTGAAGTC	CTACGCGAAG
198	GTAACTGCAT	AAGGTGATTC
199	TCTCTGTTGA	GCTTATTGCT
200	AACGACCACG	TGCACACCGA

201	GCGAATCAGC	ACTGGATCCG
202	AACTTCGGTA	GAGAACAGAT
203	TGTGGAATAG	TTACCTCAGA
204	CTACCGTCCT	CGCTTGGTTC
205	CCATAAGAGG	TCACCTCTCG
206	GTCGGTAGTC	ATCAGCGCTC
207	TGTCTGCCAT	CGGTAATGAA
208	AAGACCTTCA	GATGTGAAGT
209	TACACTCGGT	ACCAATCGTT
210	ATACAGGACG	TGTTCAGCAA
211	CGTGTCTCAC	CAGGTGTAGC
212	GCGTGAATTA	GTACGCATCG
213	GAACACTATC	AGTTGTCTGC
214	TCTGCGGTAA	CTAGAGGACA
215	AGGTTAAGCG	GCGACCTCAT
216	CTCAGTCCGT	TACCTAAGTG
217	CGCAATGAAC	GTGTTATCTC
218	TTGGCACTTA	TCAGACGTCG
219	GCATTCAGCG	AACCGGAAGT
220	AATCGGTCGT	CGTACTCGAA
221	CCTTGGTGCA	TGTGACTACA
222	GAGCATCCAT	ACACTTGCGG
223	AGAGTCGATC	CTGTGGCTAT
224	TTCACAATGG	GACACAAGTC
225	GTATCCTCAG	TCATACTGTC
226	TACCGAGTGT	GATACGGAGG
227	ACTGTGCACC	CGGCTAACCA
228	CGGAATAGTA	ATCGGTCTAT
229	GTGTCTACAG	GCCTCCTGTT
230	TACGTATACC	TATAGGACAC
231	AGTAAGCGGT	AGAGAACTGA
232	CCACGCGTTA	CTGCTTGACG
233	TAAGCTACCA	AACTGACCGT
234	AGCTGAGGTG	GTACTTATCC
235	CTTCTCCAGC	CGTACGTAAG
236	GCGAAGTTAT	TCGGACGGTA
237	GTGAATTGAC	AGAAGATGCA
238	TACCTAGACT	GTTCCGCTTC
239	AGATGCCTGG	CACTATGAGT
240	CCTGCGACTA	TCGGTCACAG

241	ATTGAGCACT	CCTATAATGC
242	TGAATTGTGC	AGCTACCAAT
243	GACCGATGTG	TTACGTGCCG
244	CCGTCCACAA	GAGGCGTGTA
245	ATAGGCGTTA	TCAAGTCGCC
246	TGCCATACGG	GTGTACACTA
247	CATTCGTGCC	CGCGTAGTGT
248	GCGATACAAT	AATCCGTAAG
249	TTAACGCGAC	TGCAGAATCC
250	CATGTTGTCA	ACTTAGGAGT
251	AGGCGATCTG	CTGGTCTCAA
252	GCCTACAAGT	GAACCTCGTG
253	GAGGTTAGTA	TGGCTACATA
254	AGTTCCGCCT	CCATGTAGAG
255	CCACAGCAAG	GTTGACGTCC
256	TTCAGATTGC	AACACGTCGT
257	CGGATACACT	TAACCACCTC
258	GACGATAGGA	ACGTGCTTAT
259	TCTCGGTCTG	GTCGTGGAGA
260	ATATCCGTAC	CGTAATAGCG
261	AGATAGCCTT	AACCGTGGTA
262	CTTACCATGC	GTTGTACTAC
263	TCGGTTGGAG	TCATCGAAGG
264	GACCGATACA	CGGAACTCCT
265	AACAACCTCA	AGTACAACTG
266	CGGCTGTAAC	CAAGTCTGCA
267	GTAGCAGGTG	GTGCAGCTAC
268	TCTTGTACGT	TCCTGTGAGT
269	AACAACTTGG	ACCATCCGAA
270	CCACCACCTA	CATTCGATTC
271	GTGGTGAGAC	GTAGGATAGG
272	TGTTGTGACT	TGGCATGCCT
273	CACGATGTGA	AGTCATGACA
274	TGGTCCAATT	TTGAGCCTGG
275	ATTAGGTGCC	CAAGTATCTC
276	GCACTACCAG	GCCTCGAGAT
277	AAGTGATCCA	CACTCGTAGC
278	GTACATCTTC	GCTGGTACCT
279	TCTGCCGGAT	AGGATCCTAA
280	CGCATGAAGG	TTACAAGGTG

281	CGCCTGTTGT	AGTTAGTCAC
282	GAGAATAGCA	CTCGTTGTCG
283	TTAGCACATC	GCGAGCCATA
284	ACTTGCGCAG	TAACCAAGGT
285	CGTGATTGGT	TAGCGGAGTG
286	ATCATGCTAC	AGCAACTAGA
287	GCATCCGATG	CTAGTTCTCT
288	TAGCGAACCA	GCTTCAGCAC
289	GATCCTCTCG	CATAATCCGG
290	TTGAAGGAAC	GTCCGCTGAT
291	CCATTCTGTA	AGATCAGTCC
292	AGCGGAACGT	TCGGTGAATA
293	TTCCACCTGG	CAAGCCTGGT
294	GCATCTTCTT	GTCCTTGCCA
295	AATGGAGAAC	TCTAGGATTG
296	CGGATGAGCA	AGGTAACAAC
297	CATGCCTATG	CTGGAGGTCT
298	GCGTGGACAA	AGCTCTACAA
299	AGCAATGGCT	GATATATGGC
300	TTACTACTGC	TCACGCCATG
301	AACGCCGTCA	TACCGCACAT
302	GCGCTGAATC	GCAGTACACG
303	CTAAGTTGGT	CGTACGGTTA
304	TGTTAACCAG	ATGTATTGGC
305	CGATACCTAG	CTGCCTTAGT
306	GCTATGACCT	GATGTGGTAC
307	TTCGCTGGTC	TCCTACAGCG
308	AAGCGATAGA	AGAAGACCTA
309	CGAGCACTGT	AGTCTAAGGA
310	GCTATTGATC	GAGGACGATC
311	TAGTACACAG	CCATGTTCAT
312	ATCCGGTGCA	TTCACGCTCG
313	GTTCATCGAG	GTACAATTCG
314	TACACATCGC	CATTCCGCAA
315	ACGTGCGACT	TGGATGCAGC
316	CGAGTGATTA	ACCGGTAGTT
317	CAGCTCCTCT	ATGCGTTCCG
318	GCAGATAAGC	CCAACACTAC
319	TGTTGATGAG	GACGTCGATA
320	ATCACGGCTA	TGTTAGAGGT

321	CCATACTCAG	TGGTTCGTCG
322	GTGAGTATTC	ACCGCACGTT
323	TGTGCGGACA	CTTAAGTCAC
324	AACCTACGGT	GAACGTAAGA
325	TGAACCGAAG	CTCCGCAAGT
326	ACCTGGCGTA	GATGTTGCAA
327	CAGCTATCCT	TGATAATGCG
328	GTTGATATGC	ACGACGCTTC
329	CCGCTTGTTG	CGGACGTTAA
330	TTCTGCTACA	ACTCAACGGC
331	AGAACAAGGT	GTATGCAATG
332	GATGAGCCAC	TACGTTGCCT
333	GCTCTATCCG	GCACTTCATG
334	TGAGCTAGTA	CTGGACAGGT
335	CTGTAGGAGC	TGTTGGTCCA
336	AACAGCCTAT	AACACAGTAC
337	GCGCATCGTT	TTCAAGGAGA
338	TTAAGCATCC	CCGTCTAGTG
339	CGCGTATCAG	AGAGGATCCT
340	AATTCGGAGA	GATCTCCTAC
341	AGCGAATGAT	ACCATGAGAC
342	CATCGTCCTG	TGTTCATCTG
343	GCGTTCAAGA	CTAGACCTGT
344	TTAACGGTCC	GAGCGTGACA
345	CGGATATCAA	TGCTGGATCT
346	GTACCGAAGC	AAGGCACAAC
347	TACTACCGTT	CTAATCTGGA
348	ACTGGTGTCG	GCTCATGCTG
349	CGTAATTCAG	GATCCTCAAC
350	GAAGCGGTCA	TGCAGCAGCA
351	TTCCGAAGGT	CTAGTGTTGG
352	ACGTTCCATC	ACGTAAGCTT

353	CTTGGTGACC	ACACTAGCCT
354	AGAACACTTG	TGTGAGCGAA
355	GCGCACACAA	CACTCCATGC
356	TACTTGTGGT	GTGAGTTATG
357	TTCAACAAGC	TACTCGGAAG
358	CATGCACGCT	ATTGGCCTGC
359	GCATGTGTTA	CGGCATTGTT
360	AGGCTGTCAG	GCAATAACCA
361	TTGTACGGCT	GTAGCGAGTT
362	CGAGTTAAGG	AACCGTTCGA
363	GATCCACTTA	TCTATCGTAC
364	ACCAGGTCAC	CGGTAACACG
365	TCCACGGTTC	GCAACTTGTC
366	ATATTCTCGG	ATGGAGACGG
367	CGGCGAAGAT	CGTCTAGTAA
368	GATGATCACA	TACTGCCACT
369	GACCTACTAT	GATCAACAAG
370	TGGAATACCA	ACATTCTTCC
371	ATTGCCGAGC	TTGGCTGCGT
372	CCATGGTGTG	CGCAGGAGTA
373	AGATTGTGCG	CGTGTATGTC
374	GATGAAGTGC	GTCTGTCAAG
375	TCGACTACAT	TCAACCGCCA
376	CTCCGCCATA	AAGCAGATGT
377	AGTTGCCGGA	GACAAGTGGT
378	CCAACTTAAC	CTGGTACACA
379	TAGGTGATCT	TGACCTGCTG
380	GTCCAAGCTG	ACTTGCATAC
381	TCCAAGCTCC	AGTGAGGTCA
382	AGGTTCGGTG	CTACTAAGGC
383	CTAGGTAAGA	GAGTCCTATT
384	GATCCATCAT	TCCAGTCCAG

The dual-index sequences on MGI platform are designed to be balanced within each group. To achieve the best sequencing quality, it is recommended to use continuous indexes as indicated within one block in the table and involve at least 8 indexes for multiplexed sequencing.