MGIEasy

Pa-SNPs Genotyping Kit User Manual

Cat. No.: 1000016270 (96 RXN)

Kit Version: V1.0

Manual Version: A1

For Research Use Only.

Revision History

Manual Version	Kit Version	Date	Description
A1	V1.0	Jan. 2021	Update contact information.
A0	V1.0	Oct. 2019	Initial release.

Note: Please download the latest version of the manual and use it with the corresponding kit.

Search manual by Cat. No. or product name from website:

https://en.mgi-tech.com/download/files.html

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Chapter 1 Product Description

1.1 Introduction

The MGIEasy Pa-SNPs Genotyping Kit was specifically designed for typing 2010 single nucleotide polymorphism (SNP) based on the MGI high-throughput sequencing platform series. The kit uses two-step multiplex PCR technology to amplify 2010 SNP sites in one tube. It is suitable for amplification of cell-free DNA of plasma and genomic DNA from various sample types, preparing circularized single strand DNA (ssCirDNA) for subsequent sequencing on BGISEQ/MGISEQ/DNBSEQ sequencers, and obtaining SNP typing by high-throughput sequencing. All reagents provided within this set have passed stringent quality control and functional verification procedures, ensuring performance stability and reproducibility.

1.2 Application

This library kit can be used for cell free DNA (cfDNA) from plasma and genomic DNA (gDNA) from blood, tissues, blood spot, saliva, buccal swabs, semen, hair, and nails.

1.3 Sequencing Platform Compatibility

Constructed libraries are compatible with:

MGISEQ-2000RS (SE50), DNBSEQ-G400RS (SE50)

MGISEQ-200RS (SE50), DNBSEQ-G50RS (SE50)

BGISEQ-500RS (SE50)

1.4 Library Prep Kit Contents

The MGIEasy Pa-SNPs Genotyping Kit contains 96 RXN split into 3 modules. Further information on Cat. No., Components and Specifications are listed in table 1.

Table 1 MGIEasy Pa-SNPs Genotyping Kit (96 RXN) (Cat. No.: 1000016270)

Modules & Cat. No.	Components	Cap Color	Spec & Quantity
MGIEasy Pa-SNPs Genotyping Kit (Box 1)	PCR Primer Pool	Blue	384 μL/tube× 1 tube
	PCR Primer A	Blue	192 μL/tube× 1 tube
	PCR Enzyme Mix	White	4800 μL/tube× 1 bottle
	PCR Clean Enzyme	Blue	96 μL/tube× 1 tube
	PCR Additive	Blue	96 μL/tube× 1 tube
	Splint Buffer	Purple	186 μL/tube× 1 tube
	DNA Rapid Ligase	Purple	8 μL/tube× 1 tube
	Digestion Buffer	White	23 μL/tube× 1 tube
	Digestion Enzyme	White	42 μL/tube× 1 tube
	Digestion Stop Buffer	White	120 μL/tube× 1 tube
MGIEasy Pa-SNPs Genotyping Kit (Box 2)	PCR Barcode Primer Mix (01-96)	/	5 μL/ wells× 96 wells
MGIEasy Pa-SNPs Genotyping Kit (Box 3)	DNA Clean Beads	White	4960 μL/tube × 3 tubes
	Short Fragment ssDNA Clean Beads	White	1440μL/tube× 1 tube
	Elution Buffer	White	4800 μL/tube × 2 tubes

1.5 Storage Conditions and Shelf Life

MGIEasy Pa-SNPs Genotyping Kit (Box 1)

- Storage Temperature: -25°C to -18°C.
- Production Date and Expiration Date: refer to the label.
- Transport Conditions: transported on dry ice.

MGIEasy Pa-SNPs Genotyping Kit (Box 2)

- Storage Temperature: -25°C to -18°C.
- Production Date and Expiration Date: refer to the label.
- Transport Conditions: transported on dry ice.

MGIEasy Pa-SNPs Genotyping Kit (Box 3)

- Storage Temperature: 2°C to 8°C.
- Production Date and Expiration Date: refer to the label.
- Transport Conditions: transported on ice bag or dry ice

Transport Conditions: transported on dry ice Performance of products is guaranteed until the expiration date, under appropriate transport, storage, and usage conditions.

1.6 Equipment and Materials Required but not Provided

Table 2 Equipment and Materials Required but not Provided

Equipment	Vortex Mixer
	Desktop Centrifuge
	Pipets
	Thermocycler
	Magnetic rack DynaMagTM-2 (Thermo Fisher Scientific™, Cat. No. 12321D) or equivalent
	Qubit™ 3 Fluorometer (Thermo Fisher Scientific, Cat. No. Q33216)
	Agilent 2100 Bioanalyzer (Agilent Technologies™, Cat. No. G2939AA) / LabChip® GX, GXII, GX Touch (PerkinElmer), or Fragment Analyzer™ (Advanced Analytical)
Reagents	Nuclease free water (NF water) (Ambion, Cat. No. AM9937)
	100% Ethanol (Analytical Grade)
	1x TE Buffer，pH 8.0 (Ambion, Cat. No. AM9858)
	Qubit® ssDNA Assay Kit (Invitrogen, Cat. No. Q10212)
	Qubit® dsDNA HS Assay Kit (Invitrogen, Cat. No. Q32854)/ Quant-iT™ PicoGreen®
	Agilent DNA 1000 Kit (Agilent, Cat. No. 5067-1504)
Consumables	Pipette Tips
	1.5 mL centrifuge tubes (Axygen, Cat. No. MCT-150-C)
	0.2 mL PCR tubes (Axygen, Cat. No. PCR-02-C) or 96-well PCR plate (Axygen, Cat. No. PCR-96M2-HS-C)
	Qubit® Assay Tubes (Invitrogen, Cat. No. Q32856) or 0.5 mL Thin Wall PCR Tubes (Axygen, Cat. No. PCR-05-C)

1.7 Precautions and Warnings

- This product is for scientific research purposes only and is not intended for clinical diagnosis. Please read this manual carefully before use.
- Please familiarize yourself with the operation methods and precautions of the various instruments to be used before the experiment.
- The library preparation process can be adjusted and optimized based on experimental design, sample characteristics, sequencing applications, and equipment.
- Remove the reagents from storage beforehand, and prepare them for use: For enzymes, centrifuge briefly and place on ice until further use. For other reagents, first thaw at room temperature and invert several times to mix properly, then centrifuge briefly and place on ice until further use.
- To prevent cross-contamination, we recommend using filtered pipette tips. Use a new tip each time for pipetting different solutions.
- We recommend using thermocyclers with heated lids for reactions. Preheat to reaction temperature before use.

⚠ **Note: Improper handling of samples and reagents may contribute to aerosol contamination of PCR Products and may decrease the accuracy of results. Therefore, we recommend physically separating three working areas in the laboratory for PCR reaction preparation, PCR1 product cleanup and PCR2 product cleanup, respectively. Use designated equipment for each area and perform regular cleaning regularly to ensure a sterile working environment. (Use 0.5% Sodium Hypochlorite or 10% Bleach to clean working environment).**

- Avoid direct contact with skin and eyes. Do not swallow. If accidentally ingested, rinse immediately with plenty of water and seek medical attention.
- All samples and wastes should be disposed of in accordance with relevant regulations.
- If you have other questions, please contact MGI technical support MGI-service@mgi-tech.com

Chapter 2 Sample Preparation

2.1 Sample Requirements

2.1.1 DNA Type

This kit is applicable for DNA from various sample types, such as plasma, blood cells, blood spots, saliva, buccal swabs, semen, hair and nails.

2.1.2 DNA input

DNA input: volume ≤ 20.5 μL, recommended DNA concentration ≥ 0.4 ng / μL, total input 8-20 ng

⚠ **Note: For plasma samples, it is recommended to extract cfDNA from more than 1 mL of plasma, lower input may result in failed library construction or unreliable sequencing data.**

2.2 Sample Storage and Transport

Plasma samples are transported under dry ice and stored below -70 °C. Other types of samples should be stored in -20 ° C. Avoid repeated freezing and thawing.

Chapter 3 Library Construction Protocol

DNA library preparation by two-step PCR amplification, then circularization and digestion.

3.1 PCR1 Amplification

3.1.1 Transfer an appropriate amount of sample (recommended 8-20 ng) to a new 0.2 mL PCR tube and add Elution Buffer for a final volume of 20.5 μL. Place the tube(s) on ice.

3.1.2 Prepare PCR1 amplification mixture on ice (see Table 3).

Table 3 PCR1 Amplification Mixture

Components	Volume
PCR Enzyme Mix	25 μL
PCR Clean Enzyme	0.5 μL
PCR Primer Pool	4 μL
Total	29.5 μL

Note: Please mix the PCR Primer Pool thoroughly before use. Vortex 5-6 times, 3-5 s each time

3.1.3 Transfer 29.5 μL of the PCR1 amplification mixture to the PCR tube form step 3.1.1. Vortex three times (3s each) and centrifuge briefly to collect the solution at the bottom of the tube.

3.1.4 Place the PCR tube from step 3.1.3 into the thermocycler and run the program in Table 4.

Table 4 PCR1 Amplification Reaction Conditions

Temperature	Time	Cycles
Heated Lid	On	
37°C	5 min	1 Cycle
95°C	10 min	
95°C	15 s	12 Cycles *
60°C	5 min	
72°C	30 s	
72°C	2 min	1 Cycle
4°C	Hold	

* If the amount of DNA input is lower than recommended amount, increase the number of cycles by 1-2.

3.1.5 Centrifuge briefly to collect the solution at the bottom of the tube and transfer all of the solution

to a new 1.5 mL centrifuge tube.

3.2 Cleanup of PCR1 Product

⚠ **Note: Please read Appendix A carefully before you begin.**

3.2.1 Remove DNA Clean Beads from refrigerator and allow 30 minutes for the solution to come to room temperature. Vortex and mix thoroughly before use.

3.2.2 Transfer 80 μL of DNA Clean Beads to the centrifuge tube from step 3.1.5. Pipette up and down at least 10 times to mix thoroughly. Ensure that all of the liquid and beads are fully dispensed from the pipette tip into the tube before proceeding.

3.2.3 Incubate at room temperature for 5 minutes.

3.2.4 Centrifuge briefly and place the tube onto a Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.

3.2.5 Keep the tube on the Magnetic Separation Rack and add 200 μL freshly prepared 80% ethanol to wash the beads and the walls of the tube. Incubate for 30 seconds, then carefully remove and discard the supernatant.

3.2.6 Repeat step 3.2.5 once, remove all of the liquid from the tube without disrupting the beads. You may centrifuge briefly to collect any remaining liquid at the bottom of the centrifuge tube, separate the beads magnetically and then remove any remaining liquid using a small volume pipette.

3.2.7 Keep the centrifuge tube on the Magnetic Separation Rack with the lid open, and air-dry beads at room temperature until no wetness (reflectiveness) is observed but before the pellet begins to crack.

3.2.8 Remove the centrifuge tube from the Magnetic Separation Rack and add 18 μL of Elution Buffer to elute the DNA. Pipette up and down at least 10 times to mix thoroughly.

3.2.9 Incubate at room temperature for 5 minutes.

3.2.10 Centrifuge briefly and place the centrifuge tube back onto the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Transfer 16.5 μL of supernatant to a new 0.2 mL PCR tube.

✔ **Stopping Point: After cleanup, purified PCR1 Products can be stored at -20°C.**

3.3 PCR2 Amplification

Note: Please read Appendix B carefully before you begin.

3.3.1 Please refer to the instructions for PCR Barcode Primer Mix (see Appendix B). Add 5 μL of PCR Barcode Primer Mix (01-96) to the PCR tube from step 3.2.10.

3.3.2 Prepare PCR2 amplification mixture on ice (see Table 5).

Table 5 PCR2 Amplification Mixture

Component	volume
PCR Enzyme Mix	25 μL
PCR Clean Enzyme	0.5 μL
PCR Additive	1 μL
PCR Primer A	2 μL
Total	28.5 μL

Note: Please mix the PCR Primer A thoroughly before use, vortex 5-6 times, 3-5 s each time.

3.3.3 Transfer 28.5 μL of the PCR1 amplification mixture to the PCR tube form step 3.3.1. Vortex three times (3s each) and centrifuge briefly to collect the solution at the bottom of the tube..

3.3.4 Place the PCR tube from step 3.3.3 into the thermocycler and run the program in Table 6.

Table 6 PCR1 Amplification Reaction Conditions

Temperature	Time	Cycles
Heated Lid	On	
37°C	5 min	1 Cycle
95°C	10 min	
95°C	15 s	
60°C	5 min	16 Cycles
72°C	30 s	
72°C	2 min	1 Cycle
4°C	Hold	

3.3.5 Centrifuge briefly to collect the solution at the bottom of the tube and transfer all of the solution to a new 1.5 mL centrifuge tube

3.4 Cleanup of PCR2 Product

⚠ **Note: Please read Appendix A carefully before you begin.**

3.4.1 Remove DNA Clean Beads from refrigerator and allow 30 minutes for the solution to come to room temperature. Vortex and mix thoroughly before use.

3.4.2 Transfer 75 µL of DNA Clean Beads to the centrifuge tube from step 3.3.5. Pipette up and down at least 10 times to mix thoroughly. Ensure that all of the liquid and beads are fully dispensed from the pipette tip into the tube before proceeding.

3.4.3 Incubate at room temperature for 5 minutes.

3.4.4 Centrifuge briefly and place the tube onto a Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Carefully remove and discard the supernatant with a pipette.

3.4.5 Keep the tube on the Magnetic Separation Rack and add 200 µL freshly prepared 80% ethanol to wash the beads and the walls of the tube. Incubate for 30 seconds, then carefully remove and discard the supernatant.

3.4.6 Repeat step 3.4.5 once, remove all of the liquid from the tube without disrupting the beads. You may centrifuge briefly to collect any remaining liquid at the bottom of the centrifuge tube, separate the beads magnetically and then remove any remaining liquid using a small volume pipette.

3.4.7 Keep the centrifuge tube on the Magnetic Separation Rack with the lid open, and air-dry beads at room temperature until no wetness (reflectiveness) is observed but before the pellet begins to crack.

3.4.8 Remove the centrifuge tube from the Magnetic Separation Rack and add 25 µL of Elution Buffer to elute the DNA. Pipette up and down at least 10 times to mix thoroughly.

3.4.9 Incubate at room temperature for 5 minutes.

3.4.10 Centrifuge briefly and place the centrifuge tube back onto the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Transfer 23 µL of supernatant to a new 0.2 mL PCR tube.

✔ **Stopping Point: After cleanup, purified PCR2 Products can be stored at -20°C.**

3.5 Quality Control of PCR2 Product

3.5.1 Quantify the purified PCR2 products with dsDNA Fluorescence Assay Kits such as Qubit® dsDNA HS Assay Kit or Quant-iT PicoGreen® dsDNA Assay Kit. The required concentration of PCR2 products is ≥5 ng /μL.

3.5.2 Assess the fragment size distribution of purified PCR2 products with electrophoresis based equipment such as Bioanalyzer, Tapestation (Agilent Technologies), LabChip® GX, GXII, GX Touch (PerkinElmer), or Fragment Analyzer (Advanced Analytical). The final size distribution of purified PCR2 products should be 140-180 bp.

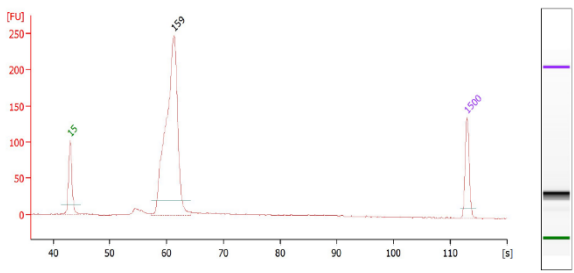

Figure 1 Agilent 2100 Bioanalyzer Fragment Size Distribution Results of the Purified PCR2 Product

3.5.3 After the library passes the quality metrics, pool the library according to the actual condition. The total amount of PCR2 products after pooling is 250 ng and the total volume is ≤48 μL.

⚠ **For example: There are N libraries that need to be mixed, and each sample library needs the same amount of sequencing data, then all libraries are mixed with the same mass, the pooling volume of a library (ng) = 250 ng/N, the pooling volume of a library (μL) = the pooling volume of a library (ng)/the concentration of a library (ng/μL).**

3.6 Denaturation

3.6.1 Transfer 250 ng multiplexed PCR2 product to a new 0.2 mL PCR tube. Add Elution Buffer for a total volume of 48 μL.

3.6.2 Place the 0.2 mL PCR tube from step 3.6.1 into the thermocycler and run the program in Table 7

Table 7 The Reaction Conditions of Denaturation

Temperature	Time
Heated Lid	On
95°C	3 min
95°C	Hold

3.6.3 When the reaction is complete, immediately place the 0.2 mL PCR tube on ice for 2 minutes, then centrifuge briefly.

3.7 Single Strand Circularization

3.7.1 Prepare the single strand circularization mixture in a new 0.2 mL PCR tube on ice (see Table 8).

Table 8 Single Strand Circularization Mixture

Component	volume
Splint Buffer	11.6 μL
DNA Rapid Ligase	0.5 μL
Total	12.1 μL

3.7.2 Pipette 12.1 μL of the single strand circularization mixture to the 0.2 mL PCR tube from step 3.6.3. Vortex 3 times (3s each), and centrifuge briefly to collect the solution at the bottom of the tube.

3.7.3 Place the PCR tube into the thermocycler and run the program in Table 9.

Table 9 The thermocycling program of single strand DNA circularization

Temperature	Time
Heated Lid	On
37°C	30 min
4°C	Hold

3.7.4 When the reaction is complete, immediately place the tube on ice for the next reaction.

3.8 Enzymatic Digestion

3.8.1 Prepare the following enzymatic digestion mixture (see Table 10) in a new 0.2 mL PCR tube on ice during the reaction in step 3.7.3.

Table 10 Enzymatic Digestion Mixture

Component	volume
Digestion Buffer	1.4 μL
Digestion Enzyme	2.6 μL
Total	4.0 μL

3.8.2 Transfer 4 μL enzymatic digestion mixture into the PCR tube from step 3.7.4. Vortex 3 times (3s each) and centrifuge briefly to collect the solution at the bottom of the tube..

3.8.3 Place the PCR tube from step 3.8.2 into the thermocycler and run the program in Table 11.

Table 11 Enzymatic Digestion Reaction Conditions

Temperature	Time
Heated Lid	On
37°C	30 min

3.8.4 Centrifuge briefly to collect the solution at the bottom of the tube.

3.8.5 Add 7.5 μL Digestion Stop Buffer into the PCR tube from step 3.8.4. Vortex 3 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube. Transfer all of the solution into a new 1.5 mL centrifuge tube.

3.9 Enzymatic Digestion Product Cleanup

⚠ **Note: Please read Appendix A carefully before you begin.**

3.9.1 Remove Short Fragment ssDNA Clean Beads from the refrigerator and allow 30 minutes for the solution to come to room temperature. Vortex and mix thoroughly before use.

3.9.2 Transfer 90 μL of Short Fragment ssDNA Clean Beads to the Enzymatic Digestion product from step 3.8.5. Gently pipette at least 10 times to mix thoroughly. Ensure that all of the solution and beads are fully dispensed from the tip into the tube.

3.9.3 Incubate at room temperature for 10 minutes.

3.9.4 Centrifuge briefly and place the 1.5 mL tube on the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Carefully remove and discard the supernatant using a pipette.

3.9.5 With the 1.5 mL tube on the Magnetic Separation Rack, add 200 μL of freshly prepared 80% ethanol to the tube without disturbing the beads. Incubate for 30 seconds. Carefully remove and discard the supernatant.

3.9.6 Repeat step 3.9.5 once. Remove all of the liquid from the tube without disrupting the beads. You may centrifuge briefly to collect any remaining liquid at the bottom, separate the beads magnetically, then remove any remaining liquid using a small volume pipette.

3.9.7 Keep the 1.5 mL centrifuge tube on the Magnetic Separation Rack with the lid open, and air-dry beads at room temperature until no wetness (reflectiveness) is observed but before the pellet begins to crack.

3.9.8 Remove the 1.5 mL centrifuge tube from the Magnetic Separation Rack and add 22 μL of Elution Buffer to elute the DNA. Gently pipette up and down at least 10 times to mix thoroughly or until the beads are fully resuspended.

3.9.9 Incubate at room temperature for 10 minutes.

3.9.10 Centrifuge briefly and place the centrifuge tube back onto the Magnetic Separation Rack for 2-5 minutes until the liquid becomes clear. Transfer 20 μL of supernatant to a new 1.5 mL centrifuge tube.

✔ **Stop point: Purified Enzymatic Digestion products can be stored at -20°C for one month.**

3.10 Quality Control of Enzymatic Digestion Product

Quantitate the purified Enzymatic Digestion product with Qubit® ssDNA Assay Kit. The final yield of the Enzymatic Digestion products should be ≥ 10 ng.

Chapter 4 Sequencing

Transfer 5 ng of the Enzymatic Digestion products to a new 0.2 mL PCR tube and add Elution Buffer for a final volume of 20 μL

Please follow the protocol described in "BGISEQ/MGISEQ/DNBSEQ High-throughput Sequencing Set Instruction Manual" for DNB making and sequencing. The available sequencing kits including:

MGISEQ-2000RS\ DNBSEQ-G400RS sequencing platform

- MGISEQ-2000RS High-throughput Sequencing Set (SE50) Instruction Manual, PN: 1000012551
- DNBSEQ-G400RS High-throughput Sequencing Set (FCL SE50) Instruction Manual, PN: 1000016941

MGISEQ-200RS\ DNBSEQ-G50RS sequencing platform

- MGISEQ-200RS High-throughput Sequencing Set (SE50) Instruction Manual, PN: 1000004635.
- MGISEQ-G50RS High-throughput Sequencing Set (FCL SE50) Instruction Manual, PN: 1000016959.

BGISEQ-500RS sequencing platform

- BGISEQ-500RS High-throughput Sequencing Set (SE50) Instruction Manual, PN: 1000002072.

Appendix

Appendix A Magnetic Beads and Cleanup Procedures

For bead-based purification, we recommend using DNA Clean Beads and Short Fragment ssDNA Clean Beads included in the kit. If you choose Magnetic Beads from other sources, please optimize the cleanup conditions before getting started.

Before You Use

- To ensure capture efficiency of the Magnetic Beads, remove beads from 4°C refrigerator storage, and equilibrate at room temperature for 30 minutes before use. Vortex and mix thoroughly before use.
- Vortex or pipette up and down to ensure that the beads are thoroughly mixed before each use.
- The volume of the beads determines the lower limit of fragment size that can be purified.

Operation Notes

- If the sample volume decreases due to evaporation during incubation, add additional TE buffer to reach the designated volume before using the beads to purify. It ensures that the correct ratio for the beads is used.
- In the magnetic separation step, please allow the solution to become completely clear before removing the supernatant. This process takes approximately 2-3 minutes. Consider the different magnetic strength of your specific Separation Plate / Rack, and allow enough time for the solution to turn completely clear.
- Avoid touching the beads with pipette tips when pipetting. 2-3 μL of fluids can be left in the tube to avoid contact. In case of contact between the beads and the pipette tip, expel all of the solution and beads back into the tube and restart the separation process.
- Use freshly prepared 80% ethanol (at room temperature) to wash the beads. Keep the centrifuge tube on the Magnetic Separation Rack when washing. Do not shake or disturb the beads in any way.
- After the 2nd wash of beads with ethanol, try to remove all of the liquid within the tube. You may centrifuge briefly to collect any remaining liquid at the bottom, separate beads magnetically, and remove the remaining liquid by using a small volume pipette.
- After washing twice with ethanol, air-dry the beads at room temperature. Drying takes

approximately 2-5 minutes depending on your specific lab environment. Watch closely until the pellet appears sufficiently dry with a matte appearance, then continue to the elution step with TE Buffer.

- During the elution step, do not touch the beads with the pipette tips when removing the supernatant. Contamination in DNA by the beads may affect subsequent purification. Therefore, the total volume of TE buffer and the beads should be 2 μL more than the volume of the supernatant.
- Pay attention when opening/ closing the lids of centrifuge tubes on the Separation Rack. Strong vibrations may cause sample loss by spilling liquid or beads from the tubes. Secure the tubes before opening the lids.

Appendix B The Combination PCR Barcode Primer Mix Strategies

- The kit provides a 96-plate of PCR Barcode Primer Mix (01-96). Each PCR Barcode Primer Mix contains a corresponding Barcode. Barcodes were developed to meet requirements for batch processing library construction and Multiplex Sequencing. We selected the best adapter combination based on the principle of balancing base composition。For optimum performance, please refer to instructions in Appendix B.
- Please do not incubate above room temperature to avoid structural changes such as degradation, which might affect performance.
- Before use, please centrifuge to collect liquid to the bottom. Gently remove the adhesive film to prevent spillage and cross contamination; Mix the PCR Barcode Primer Mix. by pipetting up and down before you use. Remember to reseal the PCR Barcode Primer Mix. immediately after use. If the adhesive film is contaminated, discard and use a new PCR sealing film to reseal the 96-well plate.

PCR Barcode Primer Mix usage rules are as follows:

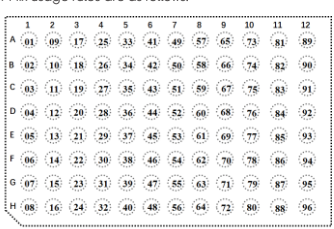

Figure 2 PCR Barcode Primer Mix Layout and Combination Instructions

Based on the principles of balancing base composition, PCR Barcode Primer Mix must be used in specific groups. Please follow the instructions bellow to use PCR Barcode Primer Mix in proper combination:

- 4 sets of 4 PCR Barcode Primer Mix: 01-04, 05-08, 09-12 and 13-16
- 8 set of 8 PCR Barcode Primer Mix: 17-24, 25-32, 33-40, 41-48, 49-56, 57-64, 65-72, 73-80, 81-88 和 89-96。

Assuming data output requirement is the same for all samples in a lane, please refer to the Table below to organize your PCR Barcode Primer Mix, combinations:

Table 12 PCR Barcode Primer Mix Instruction

Sample/lane	Instructions (Example)
1	Requires at least 1 set of PCR Barcode Primer Mix: 1、Take a set of 4 PCR Barcode Primer Mix. (01-04), mix equal volumes, then add to the sample Or (2.) Take a set of 8 PCR Barcode Primer Mix. (17-24), mix equal volumes, then add to the sample
2	Requires at least 1 set of PCR Barcode Primer Mix.: (1.) Take a set of 4 PCR Barcode Primer Mix. (01-04), mix equal volumes in pairs to obtain 2 mixtures of equal volume. Add 1 mixture to each sample. (e.g. Mix 01 & 02, then add to sample 1; Mix 03 & 04, then add to sample 2) Or (2.) Take a set of 8 PCR Barcode Primer Mix (17-24), mix equal volumes in groups of 4 to obtain 2 mixtures of equal volume. Add 1 mixture to each sample. (e.g. Mix 17-20, then add to sample 1; Mix 21-24, then add to sample 2)
3	Requires at least 2 sets of PCR Barcode Primer Mix: For sample 1&2, use the method for (2 samples/lane) above. For sample 3, use the method for (1 sample/lane) above. Note that you should use different PCR Barcode Primer Mix sets for sample 1,2 and 3.
4	Requires at least 1 set of PCR Barcode Primer Mix: (1.) Take a set of 4 PCR Barcode Primer Mix (01-04), add 1 PCR Barcode Primer Mix for each sample in equal volumes. (e.g. Respectively add Adapters 01, 02, 03, 04 to samples 1, 2, 3, 4.) Or (2.) Take a set of 8 PCR Barcode Primer Mix (17-24), mix equal volumes in pairs to obtain 4 mixtures of equal volume. Add 1 mixture to each sample. (e.g. Mix 17-18, 19-20, 21-22, 23-24, then add respectively to samples 1, 2, 3, 4.)
5	Requires at least 2 PCR Barcode Primer Mix sets: For samples 1-4, use the method for (4 samples/lane) above. For sample 5, use the method for (1 sample/lane) above. Note that you should use different PCR Barcode Primer Mix sets for sample 1-4 and 5.
6	Requires at least 2 PCR Barcode Primer Mix sets: For samples 1-4, use the method for (4 samples/lane) above. For sample 5-6, use the method for (2 sample/lane) above. Note that you should use different PCR Barcode Primer Mix sets for sample 1-4 and 5-6.

7	Requires all 3 PCR Barcode Primer Mix sets, follow these 3 steps: (1.) For samples 1-4, use the method for (4 samples/lane) above (2.) For samples 5-6, use the method for (2 samples/lane) above (3.) For sample 7, use the method for (1 sample/lane) above Note that you should use different PCR Barcode Primer Mix sets for sample 1-4, sample 5-6 and sample 7
8	Requires at least 1 set of PCR Barcode Primer Mix: (1.) Take a set of 8 PCR Barcode Primer Mix (17-24), add PCR Barcode Primer Mix for each sample in equal volumes.
8n+x (1≤n≤14, x=1-8, Total 9-96 samples)	For samples 8n+x, separate the samples into groups of 8, and use the method for (8 samples/lane) above. according to the value of x, use the methods above for 1-8 sample/lane accordingly. Remember to use separate PCR Barcode Primer Mix sets where appropriate. Note that you should use different PCR Barcode Primer Mix sets for steps

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Doc.#: B02-174