MGIEasy

Signature Identification Library Prep Kit User Manual

Cat. No.: 1000020866 (576 RXN)

Kit Version: V2.0

Manual Version: A1

Revision History

Manual Version	Kit Version	Date	Description
A1	V2.0	Jan.2021	◆ Update contact information.
A0	V2.0	May. 2020	◆ Release. V2.0 kit version

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

Contents

Chapter 1 Product Description

1.1 Introduction

The MGIEasy Signature Identification Library Prep Kit V2.0 (Cat. No.: 1000020866) is a library preparation kit tailored to the MGI high-throughput sequencing platforms for human individual identification. Libraries can be prepared for MGI high-throughput sequencing platforms from dry blood spots or genomic DNA quickly by two-step PCR. Through optimized multiple PCR technology, sequencing library preparation for 131 STRs, 227 SNPs and mitochondrial hypervariable regions can be generated in a single reaction, which significantly improves the detection efficiency of various application scenarios in the forensic field. The dual-barcode technique used in this kit can increase sample throughput in a single run. All reagents provided within this kit have passed stringent quality control and functional verification procedures, ensuring performance stability and repeatability.

1.2 Application

This library prep kit is only suitable for the preparation of high-throughput sequencing libraries for human individual identification.

1.3 Sequencing Platform Compatibility

Constructed libraries are compatible with:

MGISEQ-2000RS (SE400+10+10);

DNBSEQ-G400RS (SE400+10+10)

1.4 Kit Contents

The MGIEasy Signature Identification Library Prep Kit V2.0 (576 RXN) split into 3 modules. Further information on Cat. No., Components and Specifications are listed in table 1.

Table 1 MGIEasy Signature Identification Library Prep Kit V2.0 (576 RXN) (Cat. No.: 1000020866)

Modules	Components	Cap Color	Spec & Quantity
MGIEasy Signature Identification Library Prep Kit V2.0（Box1 of 3）	PCR Primer Pool	Blue	1.3 mL/tube×3 tubes
	PCR Block	Blue	0.65 mL/tube×3 tubes
	T Buffer	Purple	30 μL/tube×1 tube
	S Enzyme	Purple	5 μL/ tube×1 tube
	Splint Buffer	Purple	28 μL/ tube×1 tube
	DNA Rapid Ligase	Purple	2.5 μL/ tube×1 tube
	Digestion Buffer	White	7 μL/ tube×1 tube
	Digestion Enzyme	White	13 μL/ tube×1 tube
	Digestion Stop Buffer	White	38 μL/ tube×1 tube
MGIEasy Signature Identification Library Prep Kit V2.0（Box2 of 3）	PCR Enzyme Mix	/	8.8 mL/tube×2 tubes
	PCR Dual Barcode Primer F (01-48)	/	32 μL/well×48 wells
	PCR Dual Barcode Primer R (01-96)	/	12 μL//well×96 wells
MGIEasy Signature Identification Library Prep Kit V2.0（Box3 of 3）	Clean Buffer	/	41 mL/ tube×2 tubes
	DNA Clean Beads	/	34 mL/ tube×1 tube
	TE Buffer	/	24 mL/ tube×1 tube

1.5 Storage Conditions and Shelf Life

MGIEasy Signature Identification Library Prep Kit V2.0 (Box 1 of 3)

- Storage Temperature: -25°C to -15°C.
- Production Date and Expiration Date: refer to the label.
- Transport Conditions: transported on dry ice.

MGIEasy Signature Identification Library Prep Kit V2.0 (Box 2 of 3)

- Storage Temperature: -25°C to -15°C.
- Production Date and Expiration Date: refer to the label.
- Transport Conditions: transported on dry ice.

MGIEasy Signature Identification Library Prep Kit V2.0 (Box 3 of 3)

- Storage Temperature: 2°C to 8°C.
- Production Date and Expiration Date: refer to the label.
- Transport Conditions: transported with ice packs.

* Please ensure that an abundance of dry ice remains after transportation.

* Please ensure that an abundance of ice in ice pack remains after transportation.

* 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	Plate sealing machine 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) Microplate Reader (BMG Labtech，FLUOstar Omega)
Reagents	Nuclease free water (NF water) (Ambion, Cat. No. AM9937) 2M NaOH solution 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® dsDNA Assay Kit (Invitrogen, Cat. No. P7589) 2800M Control DNA (PROMEGA, Cat. No. DD7101)
Consumables	aluminum sealing film (VITL, Cat. No. V901002) plastic sealing film 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) UV-Star 96-Well Microplates (GREINER BIO-ONE, Cat. No. 655801)

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.
- Instructions provided in this manual are intended for general use only and may require optimization for specific applications. We recommend adjusting according to the experimental design, sample types, sequencing application, and other 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.
- 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 two working areas in the laboratory for PCR reaction preparation and PCR product cleanup, respectively. Use designated equipment for each area and clean 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 Blood Cards

This library preparation kit is applicable for dry blood spots with common filter paper or FTA card (non-elute card is recommended) as substrates and is not applicable for blood card samples with substrates containing strong PCR inhibitors. Dry blood spot samples should have visible blood color on both sides, be fully dried, have no mildew, no serious degradation, and should be stored in a cool and dry place.

2.1.2 Genomic DNA

It is recommended to use genomic DNA with a good degree of integrity, the main band size of fragment ≥500bp, and the concentration ≥0.25 ng/μL. Genomic DNA samples should be stored at -25°C ~-15°C.

2.2 Sample Input

Depending on sample types, preparing libraries according to the input in the following table is recommended to achieve optimal results. Recommended inputs are shown in table 3 below.

Table 3 Recommended Sample Input

Sample Type	Input Range	Recommended Input	Recommended Concentration
Dry blood spots	1-1.2 mm disc	1.2 mm disc	/
Genomic DNA	0.25-10 ng	1 ng	≥0.25 ng/μL

Chapter 3 Library Preparation Protocol

3.1 Pretreatment of Blood Card Samples (Non-Elute Card) and The First PCR Reaction

⚠ **Note: The library preparation process of genomic DNA samples starts from step 3.2. The pretreatment process of elute blood card samples starts from step 3.3. The control groups with 2800M control DNA and NF water as templates are recommended for each library preparation.**

3.1.1 Use a puncher to take a 1.2 mm disc from the center of the dry blood spot for each sample and transfer it to the bottom of a 0.2 mL tube or 96-well plate.

3.1.2 Add 25 μL Clean Buffer and make sure the dry blood discs fall below the liquid level.

3.1.3 Incubate at 60°C for 10 min.

3.1.4 Take out the tube or 96-well plate and centrifuge briefly, ensuring that blood discs fall into the bottom of the 0.2 mL tube or 96-well plate. After rapid mixing with pipette 3-5 times, discard 20 μL supernatant. Add 95 μL Clean Buffer and rapid mixing with pipette 6-8 times (make sure the blood discs are thoroughly cleaned and bubbles should be avoided during the process), then discard 93.5 μL supernatant. The remaining (including blood disc and 6.5 μL supernatant) are the PCR reaction template. If the supernatant is still light red in color, discard all supernatant and then add 6.5 μL Clean Buffer.

3.1.5 Prepare first PCR reaction mixture on ice (see Table 4).

3.1.6 Transfer 18.5μL of the first PCR reaction mixture to the tube of step 3.1.4, after mix slowly with pipette 3-5 times, centrifuge briefly to ensure that the reaction mixture and blood discs are collected to the bottom of the tube.

3.1.7 Place the tube or 96-well plate from step 3.1.6 into the thermocycler and run the program in Table 5.

3.2 First PCR Reaction of Genomic DNA

3.2.1 Add 0.25-10 ng genomic DNA (1ng recommended input) to the PCR tube, and supplement TE Buffer to a volume of 6.5μL.

3.2.2 Prepare first PCR reaction mixture on ice (see Table 4).

3.2.3 Transfer 18.5μL of the first PCR reaction mixture to the tube from step 3.2.1, after mixing with pipette 3-5 times, centrifuge briefly to ensure that the reaction mixture is collected to the bottom of the tube.

3.2.4 Place the tube or 96-well plate of step 3.2.3 into the thermocycler and run the program in Table 5.

3.3 Pretreatment of Blood Card Samples (Elute Card) and The First PCR Reaction

3.3.1 Use a puncher to take a 1.2 mm disc from the center of the dry blood spot for each sample and transfer it to the bottom of a 0.2 mL tube or 96-well plate.

3.3.2 Add 25 μL fresh 0.01M NaOH solution, centrifuge briefly after fully wetting the dry blood discs by mixing with pipette 3-5 times, make sure the dry blood discs fall below the liquid level.

3.3.3 Incubate at 98°C for 10 min.

3.3.4 Take out the tube and incubate at room temperature for 2 min, vortex for 3-5s at 4000rpm and centrifuge for 2 min, make sure the dry blood discs fall into the bottom of the tube or 96-well plate, the supernatant as the template of the first PCR. Transfer 1 μL supernatant carefully to a new tube and add TE Buffer up to a volume of 6.5 μL. Be careful not to aspirate the sediment.

3.3.5 Prepare first PCR reaction mixture on ice (see Table 4).

3.3.6 Transfer 18.5 μL of the first PCR reaction mixture to the tube from step 3.3.4, after mix with pipette 3-5 times, centrifuge briefly to ensure that the reaction mixture is collected to the bottom of the tube.

3.3.7 Place the tube or 96-well plate from step 3.3.6 into the thermocycler and run the program in Table 5.

Table 4 First PCR reaction mixture

Components	Volume
PCR Enzyme Mix	12.5 μL
PCR Primer Pool	6 μL
Total	18.5 μL

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

Table 5 First PCR Conditions

Temperature	Time	Cycles
105°C Heated Lid	on	
98°C	5 min	1 Cycle
98°C	15 s	14 Cycles
64°C	1 min	
60°C	1 min	
72°C	30 s	
72°C	2 min	1 Cycle
4°C	Hold	

Centrifuge briefly after first PCR and transfer 20 µL supernatant to a new tube or 96-well plate.

⚠ **Note: Stopping here is not recommended. Please continue with step 3.4. If it must be stopped, the first PCR product can be placed overnight in a -20°C refrigerator.**

3.4 Cleanup of First PCR Product and Second PCR

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

3.4.1 Take out DNA Clean Beads from the refrigerator and allow 30 min for the solution to come to room temperature. Vortex and mix thoroughly before use. Add 24 µL DNA clean beads to each transferred 20 µL of first PCR product.

3.4.2 Pipette up and down 8-10 times to mix thoroughly. Ensure that all 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 min.

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

3.4.5 Keep the tube or 96-well plate on the Magnetic Rack and add 100 µL freshly prepared 80% ethanol to wash the beads and the walls of the tube. Slowly mix by pipetting 3-5 times and then carefully remove and discard the supernatant.

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

3.4.7 Keep the tube or 96-well plate on the Magnetic 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 tube or 96-well plate from the Magnetic Rack and add 5.5 μL TE Buffer to elute the DNA. We should ensure that the beads are fully infiltrated and prevent the over-drying of the beads from causing PCR product loss.

3.4.9 Incubate at room temperature for 5 min.

3.4.10 Prepare the second PCR reaction mixture according to the table 6. Refer to the sequence of PCR dual barcode and assign them one by one to the samples, then them to the PCR tubes from step 3.4.9 respectively. Mix quickly by pipetting 5-8 times or vortex 3 times (3 s each time), then centrifuge briefly to collect the reaction mixture to the bottom of the tube.

Table 6 Second PCR reaction mixture

Components	Volume
PCR Enzyme Mix	12.5 μL
PCR Block	3 μL
PCR Dual Barcode Primer F	2 μL
PCR Dual Barcode Primer R	2 μL
Total	19.5 μL

⚠ **Note: The second PCR reaction was carried out with beads, don't place the tube back onto the Magnetic Rack or transfer the supernatant to a new tube.**

⚠ **Please mix the PCR Block thoroughly before use, vortex 5-6 times, 3-5 s each time.**

⚠ **Dual barcode is used in this kit for library preparation. There are two barcode primers: PCR Dual Barcode Primer F (containing 48 Barcodes) and PCR Dual Barcode Primer R (containing 96 Barcodes). Please read Appendix B in detail before use.**

3.4.11 Place the tube or 96-well plate from step 3.4.10 into the thermocycler and run the program in Table 7.

Table7 Second PCR Conditions

Temperature	Time	Cycles
105°C Heated Lid	on	
98°C	5 min	1 Cycle
98°C	15 s	16 Cycles
64°C	30 s	
60°C	30 s	
72°C	30 s	
72°C	2 min	1 Cycle
4°C	Hold	

3.4.12 Vortex and centrifuge briefly after second PCR, then transfer 20 μL PCR product to a new tube or 96-well plate.

3.5 Cleanup of Second PCR Product

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

3.5.1 Remove DNA Clean Beads from the refrigerator and allow 30 min for the solution to come to room temperature. Vortex and mix thoroughly before use. Transfer 22 μL DNA Clean Beads to the tube of step 3.4.12.

3.5.2 Pipette up and down 8-10 times to mix thoroughly. Ensure that all of the liquid and beads are fully dispensed from the pipette tip into the tube before proceeding.

3.5.3 Incubate at room temperature for 5 min.

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

3.5.5 Keep the tube or 96-well plate on the Magnetic Rack and add 100 μL freshly prepared 80% ethanol to wash the beads and the walls of the tube. Slowly mix by pipetting 3-5 times and then carefully remove and discard the supernatant.

3.5.6 Repeat step 3.5.5 once and remove all the liquid from the tube without disrupting the beads. You may centrifuge briefly to collect any remaining liquid at the bottom of the tube, separate the beads magnetically and then remove any remaining liquid using a small volume pipette.

3.5.7 Keep the tube or 96-well plate on the Magnetic 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.

3.5.8 Remove the tube or 96-well plate from the Magnetic Rack and add 23 μL of TE Buffer to elute the DNA. Pipette up and down at least 10 times to mix thoroughly.

3.5.9 Incubate at room temperature for 5 min.

3.5.10 Centrifuge briefly and place the tube or 96-well plate back onto the Magnetic Rack for 2-5 min until the liquid becomes clear. Transfer 21 μL of supernatant to a new tube or 96-well plate.

✔ **Stop Point: After clean, purified 2nd PCR Products can be stored at -20°C.**

3.6 Quality Control of Second PCR Product

Quantify the purified second PCR product with dsDNA Fluorescence Assay Kits such as Qubit® dsDNA HS Assay Kit or Quant-iT™ PicoGreen® dsDNA Assay Kit. The required concentration of second PCR products is ≥2 ng/μL. After the library passes the quality control metrics, pool the library according to the actual condition. The total amount of second PCR products after pooling is 500 ng and the total volume is ≤48 μL. (If the volume of the library is greater than 48 μL after pooling, it can be condensed to 48 μL by vacuum centrifugal concentrator, and the temperature should not exceed 45°C.)

⚠ **Note: There are N libraries that need to be mixed, and each library needs the same amount of sequencing data, then all libraries are mixed with the same mass. The pooling mass of a library (ng) = 500 ng/N, the pooling volume of a library (μL) = the pooling mass of a library (ng)/the concentration of a library (ng/μL). When the number of samples is very large, in order to reduce the aspirate error, X times of the mixed library scheme can be carried out, and 1/X is taken for the subsequent steps of circularization.**

Chapter 4 Circularization and digestion

4.1 Denaturation

4.1.1 Transfer 500 ng pooled second PCR products to a new 0.2 mL PCR tube. Add TE Buffer to a total volume of 48 μL.

4.1.2 Place the 0.2 mL PCR tube from step 4.1.1 into the thermocycler and run the program in Table 8.

Table 8 The Reaction Conditions of Denaturation

Temperature	Time
105°C Heated lid	On
95°C	5 min

4.1.3 When the reaction is complete, immediately place the 0.2 mL PCR tube on ice for 3 min, then centrifuge briefly.

4.2 Single Strand Circularization

4.2.1 Prepare the circularization pretreatment mixture in a new 0.2 mL PCR tube on ice (see Table 9)

Table 9 Circularization Pretreatment Mixture

Components	Volume
T Buffer	6 μL
S Enzyme	1 μL
Total	7 μL

4.2.2 Transfer 7 μL circularization pretreatment mixture to the 0.2 mL PCR tube from step 4.1.3 on ice. Vortex 3 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube.

4.2.3 Place the PCR tube into the thermocycler and run the program in Table 10

Table 10 The Reaction Conditions of Circularization Pretreatment

Temperature	Time
45°C Heated lid	On
37°C	10 min
4°C	Hold

4.2.4 After the reaction is complete, immediately place the tube on ice for the next step.

4.2.5 Prepare the single strand circularization mixture in a new 0.2 mL PCR tube on ice (see Table 11).

Table 11 Single Strand Circularization Mixture

Components	Volume
Splint Buffer	5.6 μL
DNA Rapid Ligase	0.5 μL
Total	6.1 μL

4.2.6 Transfer 6.1 μL single strand circularization mixture to the 0.2 mL PCR tube from step 4.2.5 on ice. Vortex 3 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube.

4.2.7 Place the PCR tube into the thermocycler and run the program in Table 12.

Table 12 Reaction Conditions of Single Strand DNA Circularization

Temperature	Time
45°C Heated lid	On
37°C	30 min
4°C	Hold

4.2.8 After the reaction is complete, immediately place the tube on ice for the next step.

4.3 Enzymatic Digestion

4.3.1 Prepare the following enzymatic digestion mixture (see Table 13) in a new 0.2 mL PCR tube on ice during the reaction in step 4.2.7.

Table 13 Enzymatic Digestion Mixture

Components	Volume
Digestion Buffer	1.4 μL
Digestion Enzyme	2.6 μL
Total	4 μL

4.3.2 Transfer 4μL of enzymatic digestion mixture into the PCR tube from step 4.2.8. Vortex 3 times (3 s each) and centrifuge briefly to collect the solution at the bottom of the tube.

4.3.3 Place the PCR tube from step 4.3.2 into the thermocycler and run the program in Table 14.

Table 14 Enzymatic Digestion Reaction Conditions

Temperature	Time
45°C Heated lid	On
37°C	30 min

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

4.3.5 Add 7.5 µL Digestion Stop Buffer to the 0.2 mL PCR tube. 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.

4.4 Cleanup of Enzymatic Digestion Product

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

4.4.1 Take out DNA Clean Beads from the refrigerator and allow 30 min for the solution to come to room temperature. Vortex and mix thoroughly before use.

4.4.2 Transfer 170 µL of DNA Clean Beads to the Enzymatic Digestion product from step 4.3.5. Gently pipette at least 10 times to mix thoroughly. Ensure that all the solution and beads are fully dispensed from the tip into the tube.

4.4.3 Incubate at room temperature for 10 min.

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

4.4.5 With the 1.5 mL tube on the Magnetic Rack, add 500 µL of freshly prepared 80% ethanol to the tube without disturbing the beads. Incubate for 30 seconds. Carefully remove and discard the supernatant.

4.4.6 Repeat step 4.4.5 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, separate the beads magnetically, then remove any remaining liquid using a small volume pipette.

4.4.7 Keep the 1.5 mL centrifuge tube on the Magnetic Rack with the lid open, and air-dry beads at room temperature until no wetness (reflectiveness) is observed. Do not over-dry the beads (cracks can be observed on pellet).

4.4.8 Remove the 1.5 mL centrifuge tube from the Magnetic Rack and add 25 µL of TE Buffer to elute the DNA. Gently pipette up and down at least 10 times to mix thoroughly or until the beads are fully resuspended.

4.4.9 Incubate at room temperature for 5 min.

4.4.10 Centrifuge briefly and place the centrifuge tube back onto the Magnetic Rack for 2~5 min until the liquid becomes clear. Transfer 23 μL of supernatant to a new 1.5 mL centrifuge tube.

✔ **Stop Point: Purified Enzymatic Digestion products can be stored at -20°C.**

4.5 Quality Control of Purified Enzymatic Digestion Product

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

Chapter 5 Sequencing

Transfer 10 ng of the purified Enzymatic Digestion products to make DNB. Please follow the protocol described in MGISEQ/DNBSEQ sequencing platform for DNB making and sequencing. This kit is suitable for the following sequencer and sequencing type:

MGISEQ-2000RS\ DNBSEQ-G400RS sequencing platform：SE400+10+10

⚠ **Note: Instrument control software version of ECR4.0 or above is required and barcode list file matched with this kit is used for splitting while dual barcode sequencing is performed with the library of this kit. Before sequencing, please check the software version of the sequencing instrument and import the barcode list of this kit.**

Chapter 6 Data Analysis

6.1 Data Analysis

Forensic Identification System (FIS) is a supporting software specially designed for MGIEasy Signature Identification Library Prep Kit V2.0. The software includes basic data QC, SNP analysis module, STR analysis module, MT analysis module, Ancestry and Phenotypic prediction modules, sample compare module and HTML report module. The entire process of sample input and report output is managed through the information management system zlims – MGI. At the same time, we also provide users with servers equipped with an FIS system. The system is simple to use, and the scripts are processed in parallel, which allows for fast, efficient, and accurate delivery.

6.2 Judgment of results

The software performs quality control in each analysis module and outputs corresponding quality control information to ensure the accuracy of the results. For detailed judgment standards, please refer to the corresponding FIS software manual.

6.3 Explanation of results

The output results include HTML report of overall data and HTML report of single sample. For detailed item explanations, please refer to the corresponding FIS software manual.

Appendix

Appendix A Magnetic Beads and Cleanup Procedures

For bead-based purification, we recommend using DNA 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 min 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 min. 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. 1-2 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 min 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.

- 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 PCR Dual Barcode Primer Strategies

- This kit contains a 96-well plate of PCR Dual Barcode Primer F (01-48) and a 96-well plate of PCR Dual Barcode Primer R (01-96). Each PCR Dual Barcode Primer F contains one corresponding Barcode (barcode2), and each PCR Dual Barcode Primer R also contains one corresponding Barcode (barcode1). Dual Barcode is developed for library preparation of a large number of samples and multiple sample sequencing. We selected the best adapter combination based on the principle of base balance. 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, the liquid should be concentrated at the bottom of the plate after centrifuge for 2min(2000rpm), and carefully tear off the sealing film. Pay attention to replace the tips during use to avoid contamination. After use, use plastic seal film to seal the plate (be careful not to use heat seal film), storage at -20°C.

PCR Dual Barcode Primer usage rules

- The layout of PCR Dual Barcode Primer F and PCR Dual Barcode Primer R are shown in figure 1a and figure 1b. Based on the principle of base balance, PCR Dual Barcode Primer F and PCR Dual Barcode Primer R must be used in specific groups. Please follow the instructions bellow to use in proper combination:

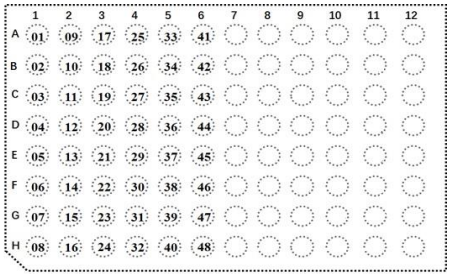

Figure 1a PCR Dual Barcode Primer F Layout

- 6 sets of 8 PCR Dual Barcode Primer F: 01-08、09-16、17-24、25-32、33-40、41-48.

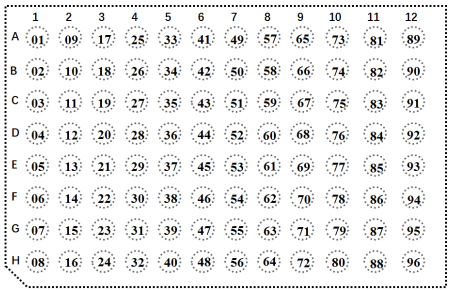

Figure 1b PCR Dual Barcode Primer R Layout

- 12 sets of 8 PCR Dual Barcode Primer R: 01-08,09-16,17-24,25-32, 33-40, 41-48, 49-56, 57-64, 65-72, 73-80, 81-88, 89-96.
- When the data requirements of each sample are the same, different sample numbers can refer to the recommended Dual Barcode combination scheme in Table 15.

⚠ **Note: To ensure optimal sequencing quality, it is recommended that at least 8 samples per lane, less than 8 samples may result in a lower split rate due to base imbalance. PCR Dual**

Barcode Primer F in each row must be used in conjunction with the PCR Dual Barcode Primer R of the same row (For example: Any PCR Dual Barcode Primer F in row A of PCR Dual Barcode Primer F can only be used with any PCR Dual Barcode Primer R in row A of PCR Dual Barcode Primer R), otherwise it couldn't decode the barcode and output the sample data.

Table 15 Usage rules of PCR Dual Barcode Primer F and PCR Dual Barcode Primer R

<table>
<tr><th>Number of samples/ lane</th><th>Usage (For example)</th></tr>
<tr><td>8</td><td>1. Take a set of 8 PCR Dual Barcode Primer F (such as 01-08), add 1 PCR Dual Barcode Primer F for each sample in equal volumes.
2. Take a set of 8 PCR Dual Barcode Primer R (such as 01-08), add 1 PCR Dual Barcode Primer R for each sample in equal volumes.</td></tr>
<tr><td>8n+x (1≤n<11, x=1-8, Total: 9-96)</td><td>Number of samples =8n+x， every 8 samples are divided into one group, and x is the number of ungrouped samples
1. For one 96-well plate of samples, use the same column of PCR Dual Barcode Primer F (For example, PCR Dual Barcode Primer F 01-08 was added to samples 1-8, and PCR Dual Barcode Primer F 01-08 was added to samples 9-16, and so on until 89-96). According to the number of x, a single PCR Dual Barcode Primer F from the PCR Dual Barcode Primer F set was added to each sample.
2.PCR Dual Barcode Primer R was added to each group using method of 8 samples/lane. According to the number of x, a single PCR Dual Barcode Primer R from the PCR Dual Barcode Primer R set was added to each sample. Note: PCR Dual Barcode Primer R was added for different groups.
Library preparation of 70 samples as an example, the PCR Dual Barcode combination of each sample is shown in the figure below:
<table>
<tr><th>70 Sample</th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th><th>7</th><th>8</th><th>9</th><th>10</th><th>11</th><th>12</th></tr>
<tr><td>A</td><td>R01-F01</td><td>R09-F01</td><td>R17-F01</td><td>R25-F01</td><td>R33-F01</td><td>R41-F01</td><td>R49-F01</td><td>R57-F01</td><td>R65-F01</td><td></td><td></td><td></td></tr>
<tr><td>B</td><td>R02-F02</td><td>R10-F02</td><td>R18-F02</td><td>R26-F02</td><td>R34-F02</td><td>R42-F02</td><td>R50-F02</td><td>R58-F02</td><td>R66-F02</td><td></td><td></td><td></td></tr>
<tr><td>C</td><td>R03-F03</td><td>R11-F03</td><td>R19-F03</td><td>R27-F03</td><td>R35-F03</td><td>R43-F03</td><td>R51-F03</td><td>R59-F03</td><td>R67-F03</td><td></td><td></td><td></td></tr>
<tr><td>D</td><td>R04-F04</td><td>R12-F04</td><td>R20-F04</td><td>R28-F04</td><td>R36-F04</td><td>R44-F04</td><td>R52-F04</td><td>R60-F04</td><td>R68-F04</td><td></td><td></td><td></td></tr>
<tr><td>E</td><td>R05-F05</td><td>R13-F05</td><td>R21-F05</td><td>R29-F05</td><td>R37-F05</td><td>R45-F05</td><td>R53-F05</td><td>R61-F05</td><td>R69-F05</td><td></td><td></td><td></td></tr>
<tr><td>F</td><td>R06-F06</td><td>R14-F06</td><td>R22-F06</td><td>R30-F06</td><td>R38-F06</td><td>R46-F06</td><td>R54-F06</td><td>R62-F06</td><td>R70-F06</td><td></td><td></td><td></td></tr>
<tr><td>G</td><td>R07-F07</td><td>R15-F07</td><td>R23-F07</td><td>R31-F07</td><td>R39-F07</td><td>R47-F07</td><td>R55-F07</td><td>R63-F07</td><td></td><td></td><td></td><td></td></tr>
<tr><td>H</td><td>R08-F08</td><td>R16-F08</td><td>R24-F08</td><td>R32-F08</td><td>R40-F08</td><td>R48-F08</td><td>R56-F08</td><td>R64-F08</td><td></td><td></td><td></td><td></td></tr>
</table>
</td></tr>
<tr><td>N * 96-well plate （N=1~6）</td><td>Use the above 8n+x method to add PCR Dual Barcode Primer F and PCR Dual Barcode Primer R. Note: PCR Dual Barcode Primer F with different columns should be added for different sample plates.
Library preparation of 2 plate samples as an example, the PCR Dual Barcode combination of</td></tr>
</table>

each sample is shown in the figure below:

Plate1	1	2	3	4	5	6	7	8	9	10	11	12
A	R01-F01	R09-F01	R17-F01	R25-F01	R33-F01	R41-F01	R49-F01	R57-F01	R65-F01	R73-F01	R81-F01	R89-F01
B	R02-F02	R10-F02	R18-F02	R26-F02	R34-F02	R42-F02	R50-F02	R58-F02	R66-F02	R74-F02	R82-F02	R90-F02
C	R03-F03	R11-F03	R19-F03	R27-F03	R35-F03	R43-F03	R51-F03	R59-F03	R67-F03	R75-F03	R83-F03	R91-F03
D	R04-F04	R12-F04	R20-F04	R28-F04	R36-F04	R44-F04	R52-F04	R60-F04	R68-F04	R76-F04	R84-F04	R92-F04
E	R05-F05	R13-F05	R21-F05	R29-F05	R37-F05	R45-F05	R53-F05	R61-F05	R69-F05	R77-F05	R85-F05	R93-F05
F	R06-F06	R14-F06	R22-F06	R30-F06	R38-F06	R46-F06	R54-F06	R62-F06	R70-F06	R78-F06	R86-F06	R94-F06
G	R07-F07	R15-F07	R23-F07	R31-F07	R39-F07	R47-F07	R55-F07	R63-F07	R71-F07	R79-F07	R87-F07	R95-F07
H	R08-F08	R16-F08	R24-F08	R32-F08	R40-F08	R48-F08	R56-F08	R64-F08	R72-F08	R80-F08	R88-F08	R96-F08

Plate2	1	2	3	4	5	6	7	8	9	10	11	12
A	R01-F09	R09-F09	R17-F09	R25-F09	R33-F09	R41-F09	R49-F09	R57-F09	R65-F09	R73-F09	R81-F09	R89-F09
B	R02-F10	R10-F10	R18-F10	R26-F10	R34-F10	R42-F10	R50-F10	R58-F10	R66-F10	R74-F10	R82-F10	R90-F10
C	R03-F11	R11-F11	R19-F11	R27-F11	R35-F11	R43-F11	R51-F11	R59-F11	R67-F11	R75-F11	R83-F11	R91-F11
D	R04-F12	R12-F12	R20-F12	R28-F12	R36-F12	R44-F12	R52-F12	R60-F12	R68-F12	R76-F12	R84-F12	R92-F12
E	R05-F13	R13-F13	R21-F13	R29-F13	R37-F13	R45-F13	R53-F13	R61-F13	R69-F13	R77-F13	R85-F13	R93-F13
F	R06-F14	R14-F14	R22-F14	R30-F14	R38-F14	R46-F14	R54-F14	R62-F14	R70-F14	R78-F14	R86-F14	R94-F14
G	R07-F15	R15-F15	R23-F15	R31-F15	R39-F15	R47-F15	R55-F15	R63-F15	R71-F15	R79-F15	R87-F15	R95-F15
H	R08-F16	R16-F16	R24-F16	R32-F16	R40-F16	R48-F16	R56-F16	R64-F16	R72-F16	R80-F16	R88-F16	R96-F16

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