High-throughput Sequencing Set

User Manual

For use with: MGISEQ-2000 Version A0

Wuhan MGI Tech Co., Ltd.

High-throughput Sequencing Set

[Product Name]

High-throughput Sequencing Set

[Pack Size]

Applicable model	PN	Model	Specification
Genetic Sequencer (MGISEQ-2000)	1000017811	MGISEQ-2000 SE35	SE35 cycles/test, 1 test/set
	1000017812	MGISEQ-2000 SE50	SE50 cycles/test, 1 test/set
	1000017813	MGISEQ-2000 SE100	SE100 cycles/test, 1 test/set
	1000017814	MGISEQ-2000 PE50	PE50 cycles/test, 1 test/set
	1000017815	MGISEQ-2000 PE100	PE100 cycles/test, 1 test/set
	1000017816	MGISEQ-2000 PE150	PE150 cycles/test, 1 test/set

[Intended Use]

The sequencing set is a set of commonly used reagents for detecting the human genomic DNA libraries. It is used with the Genetic Sequencer to perform high-throughput sequencing and obtain sample sequence information. It provides universal reagents for the sequencing reaction system. The product is not intended for whole genome sequencing.

[Test Principle]

With the Combinatorial Probe-Anchor Synthesis (cPAS) technology, the sequencing set is to determine the base sequence carried by a DNA Nanoball (DNB) loaded on a sequencing flow cell. The test procedure is mainly divided into three parts, namely DNB preparation, DNB loading and sequencing. Specifically, the DNA libraries are cyclized using the reagents provided in the sequencing set, and then DNB is prepared by rolling circle amplification and loaded onto the sequencing flow cell. During the sequencing process, especially terminal modified bases are labeled as different fluorescent probes, DNA molecular anchors and fluorescent probes are incorporated on the DNBs, the high-resolution imaging system collects the optical signals, and then the sequence to be tested can be obtained after the optical signals are digitized.

[Main Components]

See the table below.

Table 1 Main Components of sequencing set for SE Sequencing

Package	Component name	Main ingredients	SE35 Spec & Quantity	SE50 Spec & Quantity	SE100 Spec & Quantity
Package 1	Sequencing flow cell (MGISEQ-2000)	Silicon slide	1 EA	1 EA	1 EA
Package 2	Cyclization Buffer	Magnesium acetate, potassium acetate, dithiothreitol, Tris(hydroxymethyl)aminomethane, and oligonucleotide	110 μL × 1	110 μL × 1	110 μL × 1
	Ligase	DNA ligase	5 μL × 1	5 μL × 1	5 μL × 1
	Low TE buffer	Tris(hydroxymethyl)aminomethane, hydrochloric acid, and ethylenediaminetetraacetic acid	300 μL × 1	300 μL × 1	300 μL × 1
	Make DNB Buffer	Ammonium sulfate, dithiothreitol, magnesium chloride, tris(hydroxymethyl)aminomethane, and oligonucleotide	100 μL × 1	100 μL × 1	100 μL × 1
	Make DNB Enzyme Mix I	Deoxyribonucleoside triphosphate (dNTP), ammonium sulfate, dithiothreitol, magnesium chloride, tris(hydroxymethyl)aminomethane, glycerol, and SSB.	200 μL × 1	200 μL × 1	200 μL × 1
	Make DNB Enzyme Mix II （LC）	Tris(hydroxymethyl)aminomethane, potassium chloride, ethylenediaminetetraacetic acid, DNB polymerase, and glycerol	25 μL × 1	25 μL × 1	25 μL × 1
	Stop DNB Reaction Buffer	Ethylenediaminetetraacetic acid, and molecular grade water	100 μL × 1	100 μL × 1	100 μL × 1
	DNB Load Buffer I	Disodium hydrogen phosphate, and molecular grade water	200 μL × 1	200 μL × 1	200 μL × 1
	DNB Load Buffer II	Potassium citrate, and citric acid	200 μL × 1	200 μL × 1	200 μL × 1
	dNTPs Mix	Tris(hydroxymethyl)aminomethane, hydrochloric acid, ethylenediaminetetraacetic acid, and chemically modified dNTPs	0.70 mL × 1	0.80 mL × 1	1.20 mL × 1

Package	Component name	Main ingredients	SE35 Spec & Quantity	SE50 Spec & Quantity	SE100 Spec & Quantity
	dNTPs Mix II	Tris(hydroxymethyl)aminomethane, hydrochloric acid, ethylenediaminetetraacetic acid, and chemically modified dNTPs	0.60 mL × 1	0.70 mL × 1	1.00 mL × 1
	Sequencing Enzyme Mix	DNA polymerase and glycerol	1.40 mL × 1	1.60 mL × 1	2.30 mL × 1
	Sequencing Reagent Cartridge (MGISEQ-2000)	Tris(hydroxymethyl)aminomethane, sodium chloride, ethylenediaminetetraacetic acid, magnesium sulfate, Tween 20, hydrochloric acid, dideoxynucleoside triphosphate, sodium citrate, oligonucleotide, DNA polymerase, glycerol, and potassium chloride	SE35 × 1	SE50 × 1	SE100 × 1
	Micro Tube 0.5mL（Empty）	PP plastic	1	1	1

Table 2 Main Components of sequencing set for PE Sequencing

Package	Component name	Main ingredients	PE50 Spec & Quantity	PE100 Spec & Quantity	PE150 Spec & Quantity
Package 1	Sequencing flow cell (MGISEQ-2000)	Silicon slide	1 EA	1 EA	1 EA
Package 2	Cyclization Buffer	Magnesium acetate, potassium acetate, dithiothreitol, Tris(hydroxymethyl)aminomethane, and oligonucleotide	110 μL × 1	110 μL × 1	110 μL × 1
	Ligase	DNA ligase	5 μL × 1	5 μL × 1	5 μL × 1
	Low TE buffer	Tris(hydroxymethyl)aminomethane, hydrochloric acid, and ethylenediaminetetraacetic acid	300 μL × 1	300 μL × 1	300 μL × 1
	Make DNB Buffer	Ammonium sulfate, dithiothreitol, magnesium chloride, tris(hydroxymethyl)aminomethane, and oligonucleotide	100 μL × 1	100 μL × 1	100 μL × 1
	Make DNB Enzyme Mix I	Deoxyribonucleoside triphosphate (dNTP), ammonium sulfate, dithiothreitol, magnesium chloride, tris(hydroxymethyl)aminomethane, glycerol, and SSB.	200 μL × 1	200 μL × 1	200 μL × 1
	Make DNB Enzyme Mix II （LC）	Tris(hydroxymethyl)aminomethane, potassium chloride, ethylenediaminetetraacetic acid, DNB polymerase, and glycerol	25 μL × 1	25 μL × 1	25 μL × 1
	Stop DNB Reaction Buffer	Ethylenediaminetetraacetic acid, and molecular grade water	100 μL × 1	100 μL × 1	100 μL × 1
	DNB Load Buffer I	Disodium hydrogen phosphate, and molecular grade water	200 μL × 1	200 μL × 1	200 μL × 1
	DNB Load Buffer II	Potassium citrate, and citric acid	200 μL × 1	200 μL × 1	200 μL × 1
	dNTPs Mix	Tris(hydroxymethyl)aminomethane, hydrochloric acid, ethylenediaminetetraacetic acid, and chemically modified dNTPs	1.20 mL × 1	1.90 mL × 1	1.30 mL × 2
	dNTPs Mix II	Tris(hydroxymethyl)aminomethane, hydrochloric acid, ethylenediaminetetraacetic acid, and chemically modified dNTPs	1.00 mL × 1	1.60 mL × 1	1.15 mL × 2
	Sequencing Enzyme Mix	DNA polymerase and glycerol	2.30 mL × 1	3.60 mL × 1	4.80 mL × 1

Package	Component name	Main ingredients	PE50 Spec & Quantity	PE100 Spec & Quantity	PE150 Spec & Quantity
	MDA Enzyme Mix	MDA polymerase, and glycerol	0.60 mL × 1	0.60 mL × 1	0.60 mL × 1
	MDA Reagent	Deoxyribonucleoside triphosphate, dithiothreitol, dimethyl sulfoxide, and sucrose.	3.50 mL × 1	3.50 mL × 1	3.50 mL × 1
	Sequencing Reagent Cartridge (MGISEQ-2000)	Tris(hydroxymethyl)aminomethane, sodium chloride, ethylenediaminetetraacetic acid, magnesium sulfate, Tween 20, hydrochloric acid, dideoxynucleoside triphosphate, sodium citrate, oligonucleotide, DNA polymerase, Glycerin, potassium chloride	PE50 ×1	PE100 × 1	PE150 × 1
	Micro Tube 0.5mL（Empty）	PP plastic	1	1	1

Note: Mixed use of reagent components from different batches is strictly prohibited. Each sequencing set can be used only once.

Main equipment and materials required but not provided:

Equipment: Qubit® fluorometer, mini centrifuge, vortex mixer, PCR machine, pipettes of various types, and transparent sealing film.

Materials: Qubit® ssDNA Assay Kit, tip with filter, 100 μL Wide-Bore Pipette Tips , 0.2 mL PCR tube, 0.5 mL cryotube, 1.5/2.0 mL centrifuge tube, and ice box.

[Storage conditions and shelf life]

Package 1 of the sequencing set is stored at room temperature (0-30 °C), and package 2 is stored at -25 °C - -15 °C. Both packages are valid for 8 months. See their labels for manufacturing date and expiration date.

[Applicable instrument]

Genetic Sequencer (MGISEQ-2000)

[Sample requirements]

1. Sample requirements: The total library requirement is ≥1 pmol, and the library volume is ≤48 μL.

2. Sample safety: All samples should be considered potentially infectious and should be handled in accordance with relevant national regulations.

[Test methods]

1. Cyclization reaction

1.1 Prepare reagents for cyclization reaction

Remove the Cyclization Buffer and ligase from storage and place them on an ice box. After thawing, mix reagents using a vortex mixer for 5 seconds. Centrifuge briefly and place them on ice until use.

⚠Note: Do not place ligase at room temperature and avoid holding the tube for a prolonged time.

1.2 According to the quantitative results of the DNA libraries, pool the libraries to be tested in a new 0.2mL PCR tube according to the barcode adaptor numbers, and then add about 1 pmol pooled DNA library (The actual volume can be taken according to the recommended volume of the kit. See formula 1 for conversion between pmol and ng) to a 0.2 mL tube according to the following system.

(Note: If the volume of the pooled library is greater than 48 μL, please prepare a new library).

$$1\ \text{pmol PCR product mass (ng)} = \frac{\text{Main-band DNA fragment (bp)}}{1000\ \text{bp}} \times 660\text{ng}$$

Formula 1 Conversion between pmol and ng of PCR product

Table 3 Cyclization Reaction System 1

Component	Volume (μL)
Pooled DNA library	V
Low TE buffer	48-V

1.3 Mix the reaction system with a vortex mixer, centrifuge it for 5s in a mini centrifuge, incubate the PCR tube on a PCR machine at 95 °C for 5 min. Immediately take the tube out after incubation and place it on ice for 2 min.

1.4 Add the following components to the above reaction system. Fully mix the reaction mixture by vortexing and briefly centrifuge using a mini centrifuge. Incubate the reaction mixture at 37 °C for 30 min. The reaction product may go to the next reaction step or be placed in a refrigerator at -20 °C for storage.

Table 4 Cyclization Reaction System 2

Component	Volume (μL)
Cyclization Buffer	11.6
Ligase	0.5

2. Make DNB

2.1 Prepare reagents for DNB making

Remove the Low TE buffer, Make DNB Buffer, Make DNB Enzyme Mix I , Make DNB Enzyme Mix II（LC）and Stop DNB Reaction Buffer from storage and place them on an ice box. After thawing, mix reagents using a vortex mixer for 5 seconds. Centrifuge briefly and place them on ice until use.

Note: Do not place the Make DNB Enzyme Mix II (LC) at room temperature and avoid holding the tube for a prolonged time.

2.2 Make DNB

2.2.1 Add the following components into a new 0.2 mL PCR tube:

Table 5 Make DNB Reaction 1

Component	Volume (μL)
DNA library	20
Make DNB Buffer	20

2.2.2 Mix gently by vortexing and centrifuge for 5 seconds using a mini centrifuge. Place the mix into a PCR machine and start the reaction. PCR machine settings are described in Table 6:

Table 6 DNB Reaction Condition 1

Temperature	Time
Heated lid (105 ℃)	On
95℃	1 min
65℃	1 min
40℃	1 min
4℃	Hold

2.2.3 Take the PCR tube out of the PCR machine when the temperature reaches 4℃. Centrifuge briefly for 5 s, place the tube on ice and add the following components:

Table 7 Make DNB Reaction 2

Component	Volume (μL)
Make DNB Enzyme Mix I	40
Make DNB Enzyme Mix II（LC）	4

2.2.4 ix gently by vortexing, centrifuge for 5 s using a mini centrifuge and place the tubes into the PCR machine for the next reaction. The conditions are shown in Table 8:

Table 8 DNB Reaction Condition 2

Temperature	Added volume (μL)
Heated lid (35 °C)	On
30℃	25 min
4℃	Hold

2.2.5 Add 20 μL Stop DNB Reaction Buffer immediately after the reaction enters cold hold at 4℃. Mix Gently using a wide bore pipette tip 5-8 times. Do not vortex or shake the tube. Store DNBs at 4℃ and perform sequencing within 48 hours.

⚠Note: It is very important to mix DNB gently using a wide bore pipette tip. Do not centrifuge, vortex, or shake the tube.

2.2.6 Quantify DNB: Quantify DNB: Take 2 μL of the product in 2.2.5 and use Qubit® ssDNA Assay Kit and Qubit® Fluorometer to quantify the DNB. Sequencing requires a minimum DNB concentration of 8 ng/μL. If the concentration is lower than 8 ng/μL, make a new DNB preparation. If the concentration exceeds 40 ng/μL, the DNB should be diluted to 20 ng/μL with DNB Load Buffer I before loading. Store DNBs at 4℃ before use.

3. Sequencing

3.1 Prepare reagents for DNB loading

Transfer 100 μL qualified DNB and 32 μL DNB Load Buffer II to a new 0.5 mL cryotube, then add 1 μL Make DNB Enzyme Mix II（LC）into the cryotube. Mix by gently pipetting 5-8 times using a wide bore tip (Do not centrifuge, vortex, or shake the tube). Prepare a fresh DNB loading mix before the sequencing run.

3.2 Sequencing reagent preparation

3.2.1 Remove the sequencing reagent cartridge, dNTPs Mix and dNTPs Mix II from storage and thaw them at room temperature. When the reagents are completed thawed, immediately place them in 4 ℃ refrigerator until use.

3.2.2 Take out the Sequencing Enzyme Mix and store at 4 ℃ before use.

3.2.3 Pierce the seal at the edge of well No.1 and No.2 to make a hole roughly 1 cm in diameter using 1 mL sterile tip.

3.2.4 Take a pipette with the appropriate volume range and add reagents to well No.1 according to the following table:

Table 9 dNTPs Mix Loading

Product model	Reagent name	Loading volume (mL)
MGISEQ-2000 SE35	dNTPs Mix	0.600
MGISEQ-2000 SE50	dNTPs Mix	0.700
MGISEQ-2000 SE100	dNTPs Mix	1.100
MGISEQ-2000 PE50	dNTPs Mix	1.100
MGISEQ-2000 PE100	dNTPs Mix	1.800
MGISEQ-2000 PE150	dNTPs Mix	2.400

3.2.5 Take a pipette with the appropriate volume range and add reagents to well No.1 according to the following table:

Table 10 dNTPs Mix II Loading

Product model	Reagent name	Loading volume (mL)
MGISEQ-2000 SE35	dNTPs Mix II	0.500
MGISEQ-2000 SE50	dNTPs Mix II	0.600
MGISEQ-2000 SE100	dNTPs Mix II	0.900
MGISEQ-2000 PE50	dNTPs Mix II	0.900
MGISEQ-2000 PE100	dNTPs Mix II	1.500
MGISEQ-2000 PE150	dNTPs Mix II	2.100

3.2.6 Take a pipette with the appropriate volume range and add reagents to well No.1 and No.2 according to the following table:

Table 11 Sequencing Enzyme Mix Loading

Product model	Reagent name	Loading volume for well No.1 (mL)	Loading volume for well No.2 (mL)
MGISEQ-2000 SE35	Sequencing Enzyme Mix	0.600	0.500
MGISEQ-2000 SE50	Sequencing Enzyme Mix	0.700	0.600
MGISEQ-2000 SE100	Sequencing Enzyme Mix	1.100	0.900
MGISEQ-2000 PE50	Sequencing Enzyme Mix	1.100	0.900
MGISEQ-2000 PE100	Sequencing Enzyme Mix	1.800	1.500
MGISEQ-2000 PE150	Sequencing Enzyme Mix	2.400	2.100

3.2.7 Seal the loading wells of well No.1 and No.2 with the transparent sealing film. Do not cover the center of the well to avoid blocking the sampling needle.

3.2.8 Place the cartridge horizontally on the table, hold both sides of the cartridge with both hands, and move it clockwise 10-20 times, then counterclockwise 10-20 times. Make sure that you see the vortex to ensure reagents are fully mixed.

3.2.9 Well No.15:

Note: The following instruction are only for PE sequencing!

Add 500 μL of MDA Enzyme Mix to the MDA Reagent tube with a 1 mL pipette. Invert the tube 4-6 times to mix the reagents thoroughly, then add the mixture to well No.15. When adding the mixture, make sure there are no bubbles at the bottom of the tube.

3.3 Sequencing

Start the Genetic Sequencer, load the DNB tube, sequencing flow cell and sequencing reagent cartridge, and run the sequencing program following the User Manual of Genetic Sequencer (MGISEQ-2000).

4. Data analysis

When sequencing is completed, the program will generate a standard sequence file.

[Explanation of test results]

1. The sequencing set is highly sensitive. The following conditions may affect the test results, and the effects should be precluded before testing.

a) Samples have been placed for too long.

b) Samples are contaminated with other nucleic acids.

c) Sample fragments are not uniform in size.

2. In addition, some operational errors may result in unsatisfactory test results, e.g. the sequencing set exceeds the expiration date, the pipette and loader are inaccurate, the room temperature is too high, and the test is not performed according to the test procedure specified in the User Manual.

3. Acceptable sequencing data should be judged along with clinical features and other test indicators to determine the test results.

[Limitation of test method]

This sequencing set is a qualitative in vitro diagnostic kit that does not have a quantitative function.

[Product performance indicators]

1. Accuracy

Test the enterprise reference Q, and the coincidence rate between the sequence information obtained by sequencing and the known reference sequence information should be ≥99%.

2. Repeatability

Repeat the test of the enterprise reference Q 5 times, and the CV value of the coincidence rate between the sequence information obtained by sequencing and the known reference sequence information is not more than 5% (n=5).

3. Inter-run variation

Test the enterprise reference Q 5 times using three different batches of kits respectively, and the CV value of the coincidence rate between the sequence information obtained by sequencing and the known sequence information is not more than 5% (n=15).

[Warnings and Precautions]

1. This product is for in vitro diagnosis only.

2. Please read the User Guide carefully before use, master the operating method, and get familiar with the warnings and precautions.

3. Do not swallow any samples and reagents. Avoid direct contact with skin and eyes. If this

happens, rinse them immediately with plenty of fresh water and seek medical advice in time.

4. Samples and wastes are potentially infectious and should be disposed of in accordance with local regulations.

[References]

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4. Drmanac, R. Nucleic acid analysis by random mixtures of non-overlapping fragments. US patent 7,901 891 (2006).
5. Lander, E. S. et al. Initial sequencing and analysis of the human genome. Nature 409, 860–921 (2001).
6. Drmanac, R. et al. Human genome sequencing using unchained base reads on self-assembling DNA nanoarrays. Science 327, 78–81 (2010).

[Basic Information]

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