Part No.: SOP-013-B01-128

Cat No.	Product model
1000022483	G400 SM FCS SE100
1000022484	G400 SM FCS PE100
1000022485	G400 SM FCS PE150

Universal Sequencing Reaction Kit

DNBSEQ-G400

User Manual

Version: 4.0

Leading Life Science Innovation

Address: Building 24, Stage 3.1, BioLake Accelerator, No.388, 2nd Gaoxin Road, East Lake High-Tech Development Zone, 430075, Wuhan, P.R.China
E-mail: MGI-service@mgi-tech.com
Website: www.mgi-tech.com

CE IVD

Wuhan MGI Tech Co., Ltd.

【About the user manual】

This user manual is applicable to Universal Sequencing Reaction Kit. The manual version is 4.0 and the kit version is V1.0.

This user manual and the information contained within are proprietary to Wuhan MGI Tech Co., Ltd. (hereinafter called MGI), and are intended solely for the contractual use of its customer in connection with the use of the product described herein and for no other purpose. Any person or organization cannot entirely or partially reprint, copy, revise, distribute or disclose to others the user manual without the prior written consent of MGI. Any unauthorized person should not use this user manual.

MGI does not make any promise of this user manual, including (but not limited to) any commercial special purpose and any reasonable implied guarantee. MGI has taken measures to guarantee the correctness of this user manual. However, MGI is not responsible for any missing parts in the instructions, and reserves the right to revise the instructions and the device, so as to improve the reliability, performance or design.

Figures in this user manual are all illustrations. The content might be slightly different from the kit, please refer to the kit purchased.

Unless otherwise informed, StandardMPS and CoolMPS sequencing reagents, and sequencers for use with such reagents are not available in Germany, Spain, UK, Hong Kong, Sweden, Belgium, Italy, Finland, Czech Republic, Switzerland, Portugal, Austria and Romania. No purchase orders for CoolMPS products will be accepted in the USA until after August 22, 2022. No purchase orders for StandardMPS products will be accepted in the USA until after January 1, 2023.

[Product name]

Universal Sequencing Reaction Kit

[Pack size]

Applicable sequencer	Cat. No.	Model	Specification
Genetic Sequencer (DNBSEQ-G400)	1000022483	G400 SM FCS SE100	1 Test/Kit
	1000022484	G400 SM FCS PE100	1 Test/Kit
	1000022485	G400 SM FCS PE150	1 Test/Kit

[Intended use]

This kit is a set of commonly used reagents and consumables to sequence the nucleic acid libraries. It is used with the Genetic Sequencer to perform high-throughput sequencing and obtain sample sequence information. The device is specially used with validated assays and analysis software for in vitro diagnostic purpose. The device is especially used by the personnel who has been trained professionally.

[Test principle]

With the Combinatorial Probe-Anchor Synthesis (cPAS) technology[1], the sequencing reaction kit 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 kit, 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]

Table 1 Main components of sequencing reaction kit for SE Sequencing

Component name	Main ingredients	G400 SM FCS SE100
Package I		
Rapid sequencing flow cell	Silicon slide	1 EA

Component name	Main ingredients	G400 SM FCS SE100
Package II		
Cyclization Buffer	Magnesium acetate tetrahydrate, potassium acetate, DTT, Tris(hydroxymethyl) aminomethane, and Ad153_Spint oligo	110 μL×1
Ligase	T4 DNA Ligase	5 μL×1
Low TE buffer	Tris(hydroxymethyl) aminomethane, Tris HCl (Powder), and EDTA	300 μL×1
Make DNB Buffer	Ammonium sulfate, DTT, magnesium chloride, tris(hydroxymethyl) aminomethane, and make DNB primer	100 μL×1
Make DNB Enzyme Mix I	dNTP Solution Mix, ammonium sulfate, DTT, magnesium chloride, tris(hydroxymethyl) aminomethane, glycerol, and Tth SSB.	200 μL×1
Make DNB Enzyme Mix II (LC)	Tris(hydroxymethyl) aminomethane, EDTA, Phi29 DNA polymerase (LC), and glycerol	25 μL×1
Stop DNB Reaction Buffer	EDTA, and molecular grade water	100 μL×1
DNB Load Buffer I	PBS, and molecular grade water	200 μL×1

Component name	Main ingredients	G400 SM FCS SE100
DNB Load Buffer II	Potassium citrate tribasic monohydrate, and citric acid anhydrous	200 µL×1
dNTPs Mix	Tris(hydroxymethyl) aminomethane, HCl, EDTA, Hot dATP-Z1, Hot dGTP-ZB1, Hot dCTP-Z1 and Hot dTTP-Z1	0.90 mL×1
dNTPs Mix II	Tris(hydroxymethyl) aminomethane, HCl, EDTA, Cold dATP, Cold dGTP, Cold dCTP and Cold dTTP	1.70 mL×1
Sequencing Enzyme Mix	cPAS DNA Polymerase and glycerol	1.90 mL×1
Sequencing Reagent Cartridge	Tris(hydroxymethyl) aminomethane, potassium acetate, EDTA, magnesium sulfate solution volumetric, tween-20, HCl, ddATP, ddCTP, ddTTP, ddGTP, Ad153 Barcode Primer 1, cPAS DNA polymerase and glycerol	SE100×1
Micro Tube 0.5 mL (Empty)	PP plastic	1 EA
Transparent sealing film	PP plastic	2 EA

Table 2 Main components of sequencing reaction kit for PE Sequencing

Component name	Main ingredients	G400 SM FCS PE100	G400 SM FCS PE150
Package I			
Rapid sequencing flow cell	Silicon slide	1 EA	1 EA
Package II			
Cyclization Buffer	Magnesium acetate tetrahydrate, potassium acetate, DTT, tris(hydroxymethyl) aminomethane, and Ad153_Spint oligo	110 μL×1	110 μL×1
Ligase	T4 DNA Ligase	5 μL×1	5 μL×1
Low TE buffer	Tris(hydroxymethyl) aminomethane, Tris HCl (Powder), and EDTA	300 μL×1	300 μL×1
Make DNB Buffer	Ammonium sulfate, DTT, magnesium chloride, tris(hydroxymethyl) aminomethane, and make DNB primer	100 μL×1	100 μL×1
Make DNB Enzyme Mix I	dNTP solution mix, ammonium sulfate, DTT, magnesium chloride, tris(hydroxymethyl) aminomethane, glycerol, and Tth SSB	200 μL×1	200 μL×1

Component name	Main ingredients	G400 SM FCS PE100	G400 SM FCS PE150
Make DNB Enzyme Mix II (LC)	Tris(hydroxymethyl) aminomethane, EDTA, Phi29 DNA Polymerase (LC), and glycerol	25 μL×1	25 μL×1
Stop DNB Reaction Buffer	EDTA, and molecular grade water	100 μL×1	100 μL×1
DNB Load Buffer I	PBS, and molecular grade water	200 μL×1	200 μL×1
DNB Load Buffer II	Potassium citrate tribasic monohydrate, and citric acid anhydrous	200 μL×1	200 μL×1
dNTPs Mix	Tris(hydroxymethyl) aminomethane, HCl, EDTA, Hot dATP-Z1, Hot dGTP-ZB1, Hot dCTP-Z1 and Hot dTTP-Z1	1.50 mL×1	2.00 mL×1
dNTPs Mix II	Tris(hydroxymethyl) aminomethane, HCl, EDTA, Cold dATP, Cold dGTP, Cold dCTP and Cold dTTP	1.50 mL×2	2.00 mL×2
Sequencing Enzyme Mix	cPAS DNA polymerase and glycerol	3.10 mL×1	4.80 mL×1
MDA Enzyme Mix	phi29 DNA polymerase, and glycerol	0.60 mL×1	0.60 mL×1
MDA Reagent	dNTP solution mix, DTT, 100% DMSO, and sucrose	3.50 mL×1	3.50 mL×1

Component name	Main ingredients	G400 SM FCS PE100	G400 SM FCS PE150
Sequencing Reagent Cartridge	Tris(hydroxymethyl) aminomethane, potassium acetate, EDTA, magnesium sulfate solution volumetric, Tween-20, HCl, ddATP, ddCTP, ddTTP, ddGTP, Ad153 Barcode Primer 5, cPAS DNA polymerase and glycerol	PE100×1	PE150×1
Micro Tube 0.5 mL (Empty)	PP plastic	1 EA	1 EA
Transparent sealing film	PP plastic	2 EA	2 EA

Tips

- Mixed use of reagent components from different batches of kit is not recommended. Each sequencing reaction kit can be used only once.
- The components and packages are batched separately.
- Keep the components in the packages until use and do not take out them.

【Self-prepared equipment and materials】

Table 3 Self-prepared equipment and materials

Equipment&materials	Recommended brand	Catalog number
Qubit 3.0 fluorometer	Thermo Fisher	Q33216
Mini centrifuge	Major Laboratory Supplier (MLS)	/
Vortex mixer	MLS	/
Thermal cycler	Bio-Rad	/
Pipette	Eppendorf	/
200 μL Wide-bore pipette tips	AXYGEN	T-205-WB-C
100 μL Pipette tips	AXYGEN	/
Qubit ssDNA assay kit	Thermo Fisher	Q10212
0.2 mL PCR 8-strip tube	AXYGEN	/
1.5 mL tube	AXYGEN	MCT-150-C
2.0 mL cryotube	SARSTEDT	72.609.003
Ice box	/	/

[Storage & transportation condition and validity period]

Table 4 Storage & transportation condition and validity period

Package	Storage conditions		Transportation condition	Validity period
	Temperature	Humidity		
Package I	-25 ℃ to -15 ℃	20% RH to 80% RH	-80 ℃ to -15 ℃	8 months
Package II				

Tips For actual manufacturing date and use-by date, please refer to the labels.

[Applicable device]

Genetic Sequencer (DNBSEQ-G400), Wuhan MGI Tech Co., Ltd.

[Sample requirements]

- The total library requirement is no less than 1 pmol, and the library volume is no more than 48 μL.
- For the storage and preservation conditions of the sample, please refer to the requirements of the supporting clinical application kits.

[Test method]

1. Cyclization reaction

Perform the steps below:

1. Take out the Low TE buffer, Cyclization Buffer and Ligase from storage package II and place them on an ice box.
2. After thawing, mix reagents by using a vortex mixer for 5 seconds. Centrifuge briefly and place them on ice for use.

   Tips
   - Do not place Ligase at room temperature.
   - Avoid holding the tube for a prolonged time.

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

Formula: Conversion between pmol and ng of PCR product

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

Table 5 Cyclization reaction system 1

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

Tips If the volume of the pooled library is greater than 48 μL, please prepare a new library.

5. Mix the reaction system with a vortex mixer, centrifuge it for 5 seconds in a mini centrifuge.
6. Incubate the PCR tube on a thermal cycler at 95 ℃ for 5 minutes.
7. Immediately take the tube out after incubation and place it on ice for 2 minutes.
8. Add the following components to the above reaction system.

Table 6 Cyclization reaction system 2

Component	Volume(μL)
Cyclization Buffer	11.6
Ligase	0.5

9. Fully mix the reaction mixture by vortexing and briefly centrifuge by using a mini centrifuge.
10. Incubate the reaction mixture at 37 ℃ for 30 minutes. The reaction product can be used in the next reaction step or be placed in a refrigerator at -20 ℃ for storage.

2. Making DNB

Perform the steps below:

1. Take out the Make DNB Buffer, Make DNB Enzyme Mix I , Make DNB Enzyme Mix II (LC) and Stop DNB Reaction Buffer from storage package II and place them on an ice box.
2. After thawing, mix reagents by using a vortex mixer for 5 seconds. Centrifuge briefly and place them on ice for use.

   Tips
   - Do not place the Make DNB Enzyme Mix II (LC) at room temperature.
   - Avoid holding the tube for a prolonged time.

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

Table 7 Make DNB reaction 1

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

4. Mix gently by vortexing and centrifuge for 5 seconds by using a mini centrifuge.
5. Place the mix into a thermal cycler and start the reaction. Thermal cycler settings are described in the following table :

Table 8 DNB Reaction condition 1

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

6. Take the PCR tube out of the thermal cycler when the temperature reaches 4 ℃.
7. Centrifuge briefly for 5 seconds, place the tube on ice and add the following components:

Table 9 Make DNB reaction 2

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

8. Mix gently by vortexing, centrifuge for 5 seconds by using a mini centrifuge.
9. Place the tubes into the thermal cycler for the next reaction. The conditions are shown in the following table :

Table 10 DNB Reaction condition 2

Temperature	Time
Heated lid (35 ℃)	On
30 ℃	25 min
4 ℃	Hold

10. Add 20 μL Stop DNB Reaction Buffer immediately after the reaction becomes **Hold** at 4 ℃. Mix gently by using a wide bore pipette tip 5 to 8 times.

    Tips
    - It is very important to mix DNBs gently by using a wide bore pipette tip.
    - Do not centrifuge, vortex, or shake the tube.
    - Store DNBs at 4 ℃ and use it within 48 hours.

11. Quantify DNBs: Take 2 μL of the product of step 10 in *2 Making DNB on Page 12* and use Qubit ssDNA Assay Kit and Qubit 3.0 fluorometerr to quantify the DNBs.

    Tips Sequencing requires a minimum DNBs concentration of 8 ng/μL. If the concentration is lower than 8 ng/μL, make a new DNB preparation and use it within 48 hours.

12. If the concentration exceeds 40 ng/μL, the DNBs should be diluted to 20 ng/μL with DNB Load Buffer I before loading.

    Tips Make sure not to dilute it until use.

3. Sequencing

3.1 Preparing reagents for DNB loading

Perform the steps below:

1. Transfer 100 µL of qualified DNBs and 32 µL DNB Load Buffer II to a new 0.5 mL cryotube.
2. Add 1 µL Make DNB Enzyme Mix II (LC) into the cryotube.
3. Mix by gently pipetting 5 to 8 times using a wide bore tip.

Tips
- Prepare a fresh DNB loading mix before the sequencing run.
- Do not centrifuge, vortex, or shake the tube.

3.2 Preparing reagents for sequencing

Perform the steps below:

1. Take out the sequencing reagent cartridge, dNTPs Mix and dNTPs Mix II from storage package II and thaw them at room temperature.
2. When the reagents are completely thawed, immediately place them at 4 ℃ refrigerator for use.
3. Take out the Sequencing Enzyme Mix and store it at 4 ℃ before use.
4. Pierce the seal at the edge of well No.1 and No.2 to make a hole roughly 1 cm in diameter by using a 1 mL sterile tip.

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

Table 11 dNTPs Mix loading

Product model	Reagent name	Loading volume (mL)
G400 SM FCS SE100	dNTPs Mix	0.800
G400 SM FCS PE100	dNTPs Mix	1.400
G400 SM FCS PE150	dNTPs Mix	1.900

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

Table 12 dNTPs Mix II loading

Product model	Reagent name	Loading volume (mL)
G400 SM FCS SE100	dNTPs Mix II	1.600
G400 SM FCS PE100	dNTPs Mix II	2.800
G400 SM FCS PE150	dNTPs Mix II	3.800

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

Table 13 Sequencing Enzyme Mix loading

Product model	Reagent name	Loading volume for well No.1 (mL)	Loading volume for well No.2 (mL)
G400 SM FCS SE100	Sequencing Enzyme Mix	0.800	0.800
G400 SM FCS PE100	Sequencing Enzyme Mix	1.400	1.400
G400 SM FCS PE150	Sequencing Enzyme Mix	1.900	1.900

8. Seal the loading wells of well No.1 and No.2 with the transparent sealing film.

   Tips Do not cover the center of the well to avoid blocking the sampling needle.

9. Place the cartridge horizontally on the table, hold both sides of the cartridge with both hands, and move it clockwise 10 to 20 times, then counterclockwise 10 to 20 times. Ensure that the reagents are fully mixed.
10. Add 500 µL of MDA Enzyme Mix to the MDA Reagent tube with a 1 mL pipette.
11. Invert the tube 4 to 6 times to mix the reagents thoroughly,
12. Add the mixture to well No.15. When adding the mixture, make sure there are no bubbles at the bottom of the tube.

    Tips The steps 10 to 12 are only for PE sequencing.

3.3 Sequencing

Perform the steps below:

1. Start the Genetic Sequencer.
2. Load the DNB tube, sequencing flow cell and sequencing reagent cartridge.
3. Run the sequencing program following *DNBSEQ-G400 Genetic Sequencer User Manual*.

4. Data analysis

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

【Explanation of test results】

This kit cannot generate test results directly for in vitro diagnosis purpose. For the interpretation of test results, please refer to instructions for use of the applicable downstream molecular detection kit.

【Limitation of test method】

This product is only applicable to DNBSEQ-G400 Genetic Sequencer.

[Product performance indicators]

- 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 no less than 99%.

- 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).

- Inter-batch 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 should be no more than 5% (n=15).

- Performance result

Table 14 Accuracy and repeatability about intra-batch variation

/	G400 SM FCS SE100	G400 SM FCS PE100	G400 SM FCS PE150
Accuracy (%)	99.47	99.77	99.61
	99.44	99.74	99.56
	99.44	99.74	99.55
	99.46	99.75	99.57
	99.45	99.75	99.58
SD	0.01	0.01	0.02
CV(%)	0.013	0.012	0.023

Table 15 Accuracy and repeatability about Inter-batch variation

/	G400 SM FCS SE100	G400 SM FCS PE100	G400 SM FCS PE150
Accuracy Batch 1(%)	99.47	99.77	99.61
	99.44	99.74	99.56
	99.44	99.74	99.55
	99.46	99.75	99.57
	99.45	99.75	99.58
Accuracy Batch 2(%)	99.75	99.70	99.75
	99.74	99.66	99.71
	99.74	99.66	99.70
	99.75	99.68	99.72
	99.75	99.68	99.73
Accuracy Batch 3(%)	99.82	99.83	99.63
	99.81	99.81	99.58
	99.80	99.80	99.56
	99.81	99.82	99.59
	99.81	99.82	99.60
SD	0.16	0.06	0.07
CV(%)	0.162	0.060	0.071

[Warnings and precautions]

- This product is for in vitro diagnostic only.
- Please read the user manual carefully before use, master the operating method, and get familiar with the warnings and precautions. Failure to store and use kits under specified conditions may affect performance.
- The sequencing reaction kit is highly sensitive. The following conditions may affect the test results, and the effects should be precluded before testing.
  - Samples have been placed for too long.
  - Samples are contaminated with other nucleic acids.
  - Sample fragments are not uniform in size.
- Some operational errors may result in unsatisfactory test results, e.g., the sequencing reaction kit 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.
- Do not swallow any samples or 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.
- Samples and wastes are potentially infectious and should be disposed of in accordance with local regulations.
- Contact with the manufacturer for the safety data sheet (SDS).
- Routine laboratory precautions, proper laboratory practices and good laboratory hygiene are required.
- Any serious incident that has occurred in relation to the

device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

- It is especially used by the personnel who has been trained professionally.
- Do not use expired products.
- This product is for one sequencing run only and cannot be reused.

【 Reference 】

1. Dmitriy, K. et al. Comparative analysis of novel MGISEQ-2000 sequencing platform vs Illumina HiSeq 2500 for whole-genome sequencing. PLoS One. 2020 Mar 16;15(3):e0230301. doi: 10.1371.

[Manufacturer information]

Manufacturer	Wuhan MGI Tech Co.,Ltd.
Manufacturer Address	Building 24, Stage 3.1, BioLake Accelerator, No.388, 2nd Gaoxin Road, East Lake High-Tech Development Zone, 430075, Wuhan, P.R. China
E-mail	MGI-service@mgi-tech.cn
Website	www.mgi-tech.com

[European representative]

Name	Shanghai International Holding Corp. GmbH (Europe)
Address	Eiffestrasse 80, 20537 Hamburg, Germany

[Key symbols]

Symbol	Description
	Manufacturer
	Authorized representative in the European Community
	Catalogue number
	CE marking

Symbol	Description
	In vitro diagnostic medical device
	Consult user manual
	Keep dry
	Temperature limit
	Use-by date
	Batch code
	Keep away from sunlight
	Do not re-use

Symbol	Description
	Contains sufficient for <n> tests
	Serial number
	Unique device Identifier

[Latest revision of the user manual]

Version	Date	Description
4.0	September 2022	Update symbol description and explanation of test results