BGISEQ-500RS High-throughput Sequencing Set (stLFR) User Manual

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Manual Version: A0　　Kit Version：V1.0

【Product name】

BGISEQ-500RS High-throughput Sequencing Set (stLFR)

【Specification】

PE100+42, 242 cycles/kit

【Intended use】

This product is a universal kit for determination of stLFR library sequences on the BGISEQ-500RS Genetic Sequencer in a high-throughput manner. The kit is for Research Use Only (RUO) and not for clinical diagnostics.

【Principle of use】

This sequencing set utilizes DNBseq^{TM} technology. A sequencing run starts with the hybridization of a DNA anchor, then a fluorescent probe is attached to the DNA Nanoball (DNB) using combinatorial probe anchor sequencing (cPAS) chemistry. Finally, the high-resolution imaging system captures the fluorescent signal. After digital processing of the optical signal, the sequencer generates high quality and high accuracy sequencing information.

【Kit components】

This sequencing set consists of 4 modular kits (Kit I, II, III and IV):

Kites and storage conditions	Component	Specification and quantity
Kit Ⅰ BGISEQ-500RS DNB Make Load Reagent Kit (stLFR) Part. No: 1000012531	Molecular Grade Water	300 μL×1
	stLFR Make DNB Buffer	100 μL×1
	Make DNB Enzyme Mix Ⅲ	200 μL×1
	Make DNB Enzyme Mix Ⅳ	25 μL×1
	Stop DNB Reaction Buffer	100 μL×1
	DNB Load Buffer I	200 μL×1

(Storage: -25℃ to -15℃)	DNB Load Buffer II	200 μL×1
	stLFR Post Load Plate	1 EA
Kit Ⅱ BGISEQ-500RS High-throughput Sequencing Kit (stLFR) Part. No: 1000011542 (Storage: -25℃ to -15℃)	dNTPs Mix	1.25 mL×2
	dNTPs Mix II	1.25 mL×2
	Sequencing Enzyme Mix	4.90 mL×1
	MDA Enzyme Mix	0.60 mL×1
	MDA Reagent	3.50 mL×1
	Sequencing Reagent Cartridge I (stLFR)	1 EA
	Sequencing Reagent Cartridge II (stLFR) (PE100)	1 EA
Kit Ⅲ Sequencing Flow Cell Part. No: 1000005486 (Storage: 0-25℃)	Sequencing Flow Cell	1 EA
	Flow Cell Holder	1 EA
Kit Ⅳ Cartridges Cover Plate Part. No: 1000005055 (Storage: room temperature)	Cartridges Cover Plate	1 EA

Note: Mixed use of reagents from different batches is strictly prohibited.

【Key equipment and materials required but not provided in the kit】

Type	Item	Recommended brand	Catalog No.	Note
Equipment	Qubit® 3.0 Fluorometer	Thermofisher	Q33216	
	Electronic pipette	Labnet	FASTPETTEV-2	
	Pipette	Eppendorf	N/A	
	Centrifuge	Eppendorf	Centrifuge 5810	
	Mini centrifuge	N/A	N/A	
	Vortex	N/A	N/A	
	PCR machine	N/A	N/A	
	4℃ refrigerator	N/A	N/A	

	- 20℃ refrigerator	N/A	N/A	
Reagent	Qubit® ssDNA Assay Kit	INVITROGEN	Q10212	
	Qubit® dsDNA HS Assay Kit	INVITROGEN	Q32854	
Consumables	Sterile tips	AXYGEN	N/A	
	200 μL wide bore tips	AXYGEN	T-205-WB-C	
	Qubit Assay Tubes	INVITROGEN	Q32856	
	5 ml Serological pipet	CORNING	4487	
	100 ml Serological pipet	CORNING	4491	
	0.2 mL PCR tubes	AXYGEN	PCR-02-C	
	0.2 mL PCR 8-tube strip	AXYGEN	PCR-0208-C	
	1.5 mL microcentrifuge tubes	AXYGEN	MCT-150-C	
	Ice rack	N/A	N/A	Substitute: crushed ice

【Expiry Date】

See the product label for detail.

【Applicable instrument】

BGISEQ-500RS Genetic Sequencer

【Applicable environment】

The kit is applicable for stLFR libraries prepared with the stLFR Library Prep Kits manufactured by MGI Technology Co., Ltd.

Loader recipe: Sample load 2.0_LFR

Recipe: Chemistry_PE100_LFR _V3

Control Software: 1.2.0 and later versions.

【Sample requirements】

Library concentration ≥ 1.5 ng/μL.

Note: Make DNB input: 20 ng

The volume of library input (μL) =20 ng/C

C indicates the library concentration (ng/μL)

Safety Requirement: All samples are considered potentially infectious and should be handled in accordance with national regulations.

【Protocol of use】

1. DNB preparation

1.1 DNB preparation reagents

Remove the library, stLFR Make DNB Buffer, Make DNB Enzyme Mix III, Molecular Grade Water and Stop DNB Reaction Buffer from storage and place on ice for approximately 0.5 hour. Vortex for 5 seconds to mix and centrifuge briefly. Place on ice until use.

1.2 DNB preparation

a) Prepare the following mixture in 0.2 mL PCR tubes on ice:

Component	Volume (μL)
Library	V (20 ng)
stLFR Make DNB Buffer	16
Molecular Grade Water	32-16-V
Total volume	32

Vortex to mix, centrifuge the mixture for 5 seconds, and then place into the PCR machine with the following conditions:

Temperature	Time
Heated lid（105°C ）	On
95°C	3 min.
40°C	3 min.
4°C	Hold

Note: Qubit® dsDNA HS Assay Kit and Qubit® Fluorometer should be used to quantify the DNA library. Apply the concentration obtained to determine the input volume.

b) Remove Make DNB Enzyme IV from storage and place on ice. Centrifuge briefly and place back on ice until use.

Note: Do not place Make DNB Enzyme Mix Ⅳ at room temperature or hold the tube in hands for a prolonged time.

c) Remove PCR tubes from PCR machine when the temperature reaches 4°C, centrifuge for 5 seconds, and add the following reagents on ice:

Component	Volume (μL)
Make DNB Enzyme Mix Ⅲ	32
Make DNB Enzyme Mix Ⅳ	3.2
Total volume	67.2

Vortex to mix and then centrifuge for 5 seconds. Immediately place the mixture in PCR machine with the following conditions (set the heated lid to 35℃ or temperature closest to 35℃):

Temperature	Time
Heated lid（35°C）	On
30°C	30 min.
4°C	Hold

d) Immediately remove the PCR tubes from PCR machine when the temperature reaches 4 °C. Add 16 μL of Stop DNB Reaction Buffer and gently pipette up and down for 5 times to mix using wide bore tip. Do not centrifuge, vortex or vigorously pipette the mixture. Store at 4 °C and perform sequencing within 48 hours.

Note: Mix DNB using wide bore tips carefully. Do not centrifuge, vortex or pipette vigorously.

1.3 DNB Quantitation:

Quantitate DNB with Qubit® ssDNA Assay Kit and Qubit® Fluorometer. Sequencing requires the DNB concentration to be above 6 ng/μL. If the concentration is lower than 6 ng/μL, please repeat the Make DNB steps to prepare new DNB.

Note： As DNB is viscous, take 2 μL for Qubit® quantitation. If the concentration exceeds 40

ng/μL, the DNB need to be diluted to 20 ng/μL with DNB Load Buffer I for loading.

2. DNB loading and processing

2.1 Prepare Load DNB reagents

a) Thaw the stLFR Post Load Plate at room temperature (approximately 1 hour), vortex to mix and centrifuge briefly. Place at 4°C until use. Remove the DNB Load Buffer II from storage and vortex to mix. Centrifuge briefly and place on ice until use. Remove the Make DNB Enzyme Mix Ⅳ from storage. Centrifuge briefly and place on ice until use (It is recommended to take the reagent just before use).

b) Place the flow cell at room temperature until use.

2.2 DNB Loading

a) Place the flow cell: Check if both automatic sample loading system chuck and flow cell Holder are intact. Install the flow cell Holder on the flow cell stage and place the Sequencing flow cell on the flow cell Holder. Ensure the alignment holes at the back of the flow cell are aligned to the pins on the flow cell stage. Press the flow cell to ensure it is securely seated and held on the stage. The flow cell should not move in any direction once placed into the instrument.

b) Place the stLFR Post Load Plate: centrifuge the stLFR Post Load Plate briefly. Remove the sealing film and place the Post Load Plate on the designated position on the loading stage with reagent label facing outward.

c) Transfer all following solutions into the DNB to be loaded:

Component	Volume (μL)
DNB load buffer II	25.6
Make DNB enzyme mix Ⅳ	1

Gently pipette up and down for 5 times to mix using wide bore tip. Do not centrifuge, vortex or vigorously pipette the mixture. Place the mixture on the designated position of the loader. Select "Sample load 2.0_LFR" program and click "Start" to perform DNB Loading process.

Note: Do not move the flow cell during DNB loading procedure.

d) When loading is completed, incubate the flow cell at room temperature for 30 minutes. It is recommended to use the prepared flow cell for Sequencing as soon as possible. If not used in the same day, store the flow cell in a clean PE glove at 2-8℃ for use within 48 hours.

3. Sequencing

3.1 Prepare Sequencing Reagents

Thaw the stLFR Sequencing Reagent Cartridge I and stLFR Sequencing Reagent Cartridge II at room temperature (approximately 3 hours) and place at 4°C until use (alternatively, thaw the stLFR Sequencing Reagent Cartridge I and stLFR Reagent Cartridge II in a 4°C refrigerator overnight).

Thaw the dNTPs Mix and dNTPs Mix II at room temperature (approximately 1 hour) in advance and place at 4°C or on ice until use.

Place Sequencing Enzyme Mix on ice until use.

3.2 Prepare Reagent Cartridge

Note: Before using Sequencing Enzyme Mix, dNTPs Mix, and dNTPs Mix II, centrifuge briefly and pipette 5 times to mix. When adding solutions to corresponding wells, note not to contaminate the reagents.

a) Well No.5 (as shown in Figure 1)

Add the following solutions to well No.5 using a 1 mL pipette:

Component	Volume
Sequencing Enzyme Mix	2300 μL
dNTPs Mix	2300 μL

Pipette 10 times with 100 mL serological pipette to mix the solution in well No.5 thoroughly.

b) Well No.6 (as shown in Figure 1)

Add the following solutions to well No.6 using a 1 mL pipette:

Component	Volume
Sequencing Enzyme Mix	2300 μL
dNTPs Mix II	2300 μL

Pipette 10 times with 100 mL serological pipette to mix the solution in well No.6 thoroughly.

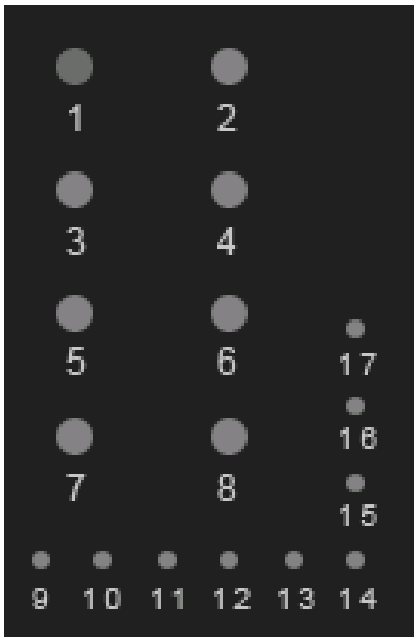

Figure 1 Well location on the Sequencing Reagent Cartridge

3.3 Sequencing

a) Start the sequencer: follow the BGISEQ-500RS User Manual to start the control program.

b) Perform the wash on BGISEQ-500RS: follow the BGISEQ-500RS User Manual to wash the fluidic pipelines with the flow cell for Wash. Refer to BGISEQ-500RS User Manual for details.

c) Assemble Reagent Cartridge: align the stLFR Sequencing Reagent Cartridge I and the stLFR Sequencing Reagent Cartridge II prepared in Step 3.2 according to the wells position. Assemble the two reagent cartridges using the Cartridges Cover Plate and placed the assembled cartridges into the reagent compartment of the sequencer.

d) Reagent Priming: Please follow the BGISEQ-500RS User Manual.

e) Remove the MDA Reagent and the MDA Enzyme Mix from -20°C storage. Vortex to mix and centrifuge briefly before adding 500 µL MDA Enzyme Mix to the MDA Reagent. Pipette with electronic pipette to mix the solution and then transfer the mixture to well No.14.

f) Load the flow cell and start sequencing. If the flow cell is stored at 4°C, equilibrate at room temperature for 15 min.

g) Select the read length

Select PE sequencing, enter “100” for read 1, enter “100” for read 2 and enter “42” for barcode read length. Do not select “Split barcode”.

【Testing results interpretation】

1. This set is sensitive to the following situations that might compromise the sequencing result:

a) Prolonged storage of the samples

b) Samples are contaminated by other DNA

2. Other factors affecting the sequencing results include: use of an expired reagent kit, poor accuracy of pipettes, high room temperature and failure to follow the user manual.

【Attention】

1. This product is restricted for research use only and cannot be used for clinical diagnosis. Please read this user manual carefully before use.

2. Ensure that you are familiar with all procedures, instrument operation and notes before you begin.

3. Avoid direct skin or eye contact with all samples and reagents and do not swallow the samples or the reagents. In case of direct contact or accidental ingestion, rinse with tap water and consult a doctor immediately.

4. All samples and waste should be treated and disposed in accordance with relevant laws and regulations.

【Manufacturer information】

Manufacturer name: MGI Tech Co., Ltd.

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