Part No.:H-940-001884-00-02

User Manual

Version:3.0

MGIEasy Olink Library Circularization Kit

Leading Life Science Innovation

Research Use Only

Revision history

Version	Date	Description
2.0	May 13, 2024	Updated the formula to calculate the amount of ssDNA input when making DNB.
3.0	Aug 28,2024	Update the protocol to include the sequencing with APP-D primer

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

Search catalog number or product name to download instructions: www.mgi-tech.com/download/files.

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Contents

Chapter 1 Introduction

1.1 Application

This product is a kit of consumables to transfer the linear dsDNA libraries from Olink Explore HT library into the single-stranded circular DNA library which can be used for DNBSEQ-T7RS sequencing platform. This MGIEasy Olink Library Circularization Kit is intended for scientific research use only and cannot be used for clinical diagnosis.

1.2 Technology

This kit is used for the phosphorylation, circularization and linear digestion of the linear dsDNA library of Olink Explore HT. The products prepared by those procedures are dedicated for use with the MGI high-throughput (HT) sequencing platform.

1.3 Sample requirements

This kit is suitable for the linear dsDNA libraries generated from standard Olink Explore HT library preparation process only.

1.4 Warnings and precautions

- These products are used for scientific research use only. Please read this manual carefully before use.
- Do not swallow any samples or reagents. Avoid direct skin and eye contact with any samples and reagents. Rinse immediately with plenty of water and seek medical attention.
- All the samples and waste materials should be considered potentially infectious agents and must be disposed of according to applicable relevant laws and regulations.
- Do not use expired products.

Chapter 2 Kit components and user-supplied materials

2.1 Kit components

Table 1 MGIEasy Olink Library Circularization Kit (Box 1) Cat. No. : 940-001884-00

Component	Cap Color	Spec & quantity	Storage Temperature	Transportatio Temperature	Validity Period
App Splint Buffer		32 μL/ tube × 1 tube	-25 ℃ to -15 ℃	-80 ℃ to -15 ℃	10 months
T4 PNK		4 μL/ tube × 1 tube			
DNA rapid ligase		8 μL/ tube × 1 tube			
Ligation Buffer		32 μL/ tube × 1 tube			
Digestion Buffer		12 μL/ tube × 1 tube			
Digestion Enzyme		20.8 μL/ tube ×1 tube			
Digestion Stop Buffer		60 μL/ tube ×1 tube			
TE Buffer		400 μL/ tube ×1 tube			

Table 2 MGIEasy Olink Library Purification Kit (Box 2) Cat. No. : 940-001918-00

Component	Cap Color	Spec & quantity	Storage Temperature	Transportatio Temperature	Validity Period
Library purification beads		864 μL/ tube × 1 tube	2 ℃ to 8 ℃	2 ℃ to 8 ℃	10 months

2.2 User-supplied equipment, reagents, and consumables

Table 3 User-supplied equipment, reagents, and consumables

Type	Name	Recommended brand	Cat. No.
Equipment	Qubit fluorometer	Thermo Fisher	Q33226
	Mini centrifuge	-	-
	Vortex mixer	-	-
	Thermal Cycler	Bio-Rad	-
	Pipette	Eppendorf	-
	DynaMag Separation Magnets	Thermo Fisher	12321D
	-25 ℃ to -15 ℃ freezer	-	-
	2 ℃ to 8 ℃ refrigerator	-	-
Reagents	Qubit ssDNA Assay Kit	Thermo Fisher	Q10212
	Qubit dsDNA HS Assay Kit	Thermo Fisher	Q32851
	Absolute ethanol	-	-
	Nuclease-Free (NF) Water	Thermo Fisher	AM9932
	DNBSEQ-T7RS High-throughput Sequencing Set (FCL PE100)	CG	940-000838-00
	DNBSEQ High-throughput Sequencing Primer Kit (App-D) (Paired-End)	CG	940-000917-00
Consumables	Qubit Assay Tubes	Thermo Fisher	Q32856

	0.2 mL PCR 8-tube strip	-	-
	Ice bucket	-	-
	Sterile non-filtered pipette tips	AXYGEN	-

Chapter 3 Sample requirements

- The required sample type is linear dsDNA libraries generated from standard Olink Explore HT library preparation process, and the main band is approximately 150 bp.
- The concentration of library should be higher than 5 ng/ul, the total sample volume should be larger than 100 μL. (Since each reaction tube out of 8 tubes requires 60 ng of library, the total required amount of library should be larger than 480 ng.)

Tips If the Olink Explore HT library concentration is unknown, use the Qubit dsDNA HS Assay Kit to measure the concentration.

- Two sets of DNBSEQ-T7RS High Throughput Sequencing Set (FCL PE100) are required. It is necessary to use 8 tubes of reactions with the same conditions. Theoretically, the ssDNA produced by the 6 tubes reaction is enough for the subsequent experiment to make DNB. Taking experimental errors into account, 2 more tubes of reactions are required.

Chapter 4 Library circularization

4.1 Preparing reagents for library circularization

Perform the following steps :

1. Place the Olink Explore HT library on ice before use.
2. Take out the TE Buffer, App splint buffer, Digestion buffer, and Digestion stop buffer from the MGIEasy Olink Library Circularization kit (Box 1) and thaw the reagents at room temperature for 30 minutes.
3. Mix the thawed reagents by vortexing 5 sec, centrifuge briefly and place them on ice until use.

4.2 Library circularization

Tips The following steps cannot be paused midway, and the next step of the experiment must be continued until completing all steps in sections 4.2.1 through 4.2.5 .

4.2.1 Denaturation

Perform the following steps:

1. Take the App Splint Buffer out of Box 1 and thaw it at room temperature. Place the thawed buffer in an ice bucket. Add 60 ng of Olink Explore HT linear dsDNA library to a new 0.2 mL PCR tube. Repeat this step 8 times for a total of 8 tubes with libraries. The input volume can be calculated using the formula below.

The input volume of dsDNA libraries V (μL) = 60 ng / Concentration of dsDNA library (ng/μL)

Tips The following protocol describes the experiment process for 1 tube. The downstream sequencing process needs the product from 8 tubes; therefore, repeat the process 8 times.

2. Prepare phosphorylation as follows:

Table 4 Phosphorylation Reaction Mixture

Components	Volume (μL)
Olink Explore HT library	V

Components	Volume (μL)
App Splint Buffer	4
T4 PNK	0.5

3. Mix the Phosphorylation Reaction Mixture by vortexing for 5 sec and centrifuge briefly to collect the solution at the bottom of the tube.
4. Place the PCR tube into the thermal cycler and run the program with the following conditions:

Table 5 Phosphorylation Reaction Conditions

Temperature	Time
Heated lid (105 °C)	On
37 °C	10 min
95 °C	3 min
4 °C	Hold

5. When the reaction is completed, take the tube out and place it on ice immediately.

4.2.2 Circularization

Perform the following steps:

1. Add the 55.5 uL of reagents below to each PCR tube obtained from the last step. The total volume will be 60 μL:

Table 6 Circularization Reaction Mixture

Components	Volume (μL)
Ligation buffer	4
DNA rapid ligase	1
NF Water	50.5-V

2. Vortex the PCR tube 6 times (3 sec each). Centrifuge briefly to collect the solution at the bottom of the tube.
3. Place the PCR tube into the thermal cycler and run the program with following conditions:

Table 7 Circularization Reaction Conditions

Temperature	Time	Cycles
Heated lid (105℃)	On	/
95 ℃	10 s	/
95 ℃	30 s	4 Cycles
50 ℃	2 min	
37 ℃	20 min	
4 ℃	Hold	/

4. Transfer the PCR tube immediately to the ice when the reaction is completed and the temperature reaches 4 ℃.

4.2.3 Digestion

Perform the following steps :

1. Prepare the Digestion Reaction Mixture on ice:

Table 8 Digestion Reaction Mixture

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

2. Transfer the above mixture into the PCR tube obtained from “Circularization ” on page 7. Vortex 6 times (3 sec each). Centrifuge briefly to collect the solution at the bottom of the tube.
3. Place the PCR tube into the thermal cycler and run the program with the following conditions:

Table 9 Digestion Reaction Conditions

Temperature	Time
Heated lid (45 ℃)	On
37 ℃	30 min
4 ℃	Hold

4. When the temperature reaches 4 ℃, take out the PCR tube immediately, and add 7.5 μL of Digestion Stop Buffer to the PCR tube, then centrifuge briefly to collect the solution at the bottom of the tube.

4.2.4 Purification of product after digestion

Perform the following steps:

1. Take the Library Purification Beads out of the MGIEasy Olink Library Purification kit (Box 2), and bring it to room temperature for 30 min. Vortex and mix thoroughly before use.

Tips Library Purification Beads must be vortexed and equilibrated to room temperature before use to ensure the recovery efficiency. Vortex to mix the beads thoroughly before each use.

2. Transfer 108 μL Library Purification Beads to the digestion product (generated from "Digestion" on page 8 and gently pipette the mixture up down at least 10 times to mix thoroughly. Ensure that all mixture and beads in the pipette tips are expelled from the tips.
3. Incubate the solution at room temperature for 10 min.
4. Centrifuge briefly and then place the 0.2 mL PCR tube onto a magnetic separation rack for 2 to 5 min until the mixture is clear. Carefully remove and discard the supernatant.

Tips
- Please allow enough time for the complete clarification of solution before remove the supernatant. The actual time may vary with different type of separation rack.
- Avoid touching the beads while pipetting. Volume of 2-3 μL of mixture can be left in the tube to avoid the contact between the beads and pipette tip. If tip touched the beads, please expel all of solution and beads back into the tube. Prestart the separation process.

5. Keep the 0.2 mL PCR tube on the magnet separation rack, and add 170 μL of freshly prepared 80% ethanol to rinse the beads and walls of the tube. Carefully remove and discard the supernatant.
6. Repeat step 5 and try to remove all of the liquid. If residual liquid remains on the tube wall, briefly centrifuge the tube and place it on the magnetic separation rack. Remove all of the mixture at the bottom of the tube by using a 10 μL pipette.
7. Keep the tube on the magnetic separation rack. Open the lid of the tube to air-dry the beads at room temperature until no wetness or glossiness is visible on the surface of the beads. Ensure that there is no cracking on the surface.

Tips
- Magnetic beads should be dried thoroughly at room temperature. Insufficient drying (glossiness on the surface of the beads) could result in

residual ethanol which affects the subsequent reaction. Excessive drying (cracks on the bead surface) could reduce the purification yield.

- It takes approximately 2 to 5 min, depending on your specific lab environment. Observe closely untill the pellet appears sufficiently dry with a matte appearance, and then continue to perform the elution step with TE Buffer.

8. Remove the PCR tube from the magnetic separation rack. Add 17 μL of TE Buffer to elute the DNA by using a pipette. Gently pipette the mixture up and down at least 10 times to ensure homogenous resuspension.
9. Incubate the solution at room temperature for 10 min.
10. Briefly centrifuge the PCR tube. Place the tube onto the magnetic separation rack for 2 to 5 min until the mixture is clear. Transfer the 15 μL of supernatant to a new 1.5 mL centrifuge tube.

Tips Avoid disturbing the beads when removing the supernatant. The residual beads could affect subsequent reaction. If the tip touches the beads, expel all of solution and beads back into the tube. Restart the separation process. After the supernatant is collected, approximately 2 μL of liquid will remain in the tube.

4.2.5 Quality control of the purified product

Perform the following steps:

It is recommended that you use Qubit ssDNA Assay Kit to quantify the purification product. The concentration of ssDNA in each tube should be higher than 0.6 ng/μL. Collect the products from all PCR tubes into a 1.5 mL centrifuge tube, mix thoroughly, and then quantify it by Qubit ssDNA Assay Kit again. According to the quantified concentration, take an appropriate amount of circularized library for DNB preparation. The total input amount of circularized library is 50 ng.

Tips The circularization products of the 8 PCR tubes should be quantified separately. If the concentration of ssDNA in 1 PCR tube is significantly lower than that in the others, that tube should be discarded.

The circularization product in this step can be stored at -20 ℃.

4.2.6 Application

The purification product generated by this protocol can be used in making DNBs and subsequently used for sequencing on the DNBSEQ-T7RS.

Chapter 5 Making DNB

5.1 ssDNA used to prepare DNB for sequencing

Tips The following components in the DNBSEQ-T7RS High-Throughput Sequencing Set (FCL PE100) (PN: 940-000838-00) will not be used in this experiment.

Kit Name and Product Number	Components
DNBSEQ-T7RS DNB Make Reagent Kit Cat. No.: 940-000848-00	Make DNB Buffer
DNBSEQ-T7RS DNB Load Reagent Kit Cat. No.: 940-000844-00	DNB Load Buffer I
DNBSEQ-T7RS High-Throughput Sequencing Kit (FCL PE100) Cat. No.: 940-000837-00	MDA Reagent
	MDA Enzyme Mix

Tips The following components in the DNBSEQ High-throughput Sequencing Primer Kit (App-D) (Paired-End) (PN: 940-000917-00) will not be used in this experiment.

Kit Name and Product Number	Components
DNBSEQ High-throughput Sequencing Primer Kit (App-D) (Paired-End) (PN: 940-000917-00)	App-D Insert Primer 2
	App-D MDA Primer
	App-D Barcode Primer 2
	App-D Barcode Primer 3

This protocol can be used to prepare 1 reaction of DNB. For the standard Olink Explore library, it is recommended that you prepare 7 reactions of DNB simultaneously to meet the sequencing requirements for DNBSEQ-T7RS. To meet the subsequent analysis requirements, it is recommended that each Explore HT library be used with 2 sets of the DNBSEQ-T7RS High Throughput Sequencing Set (FCL PE100) (PN: 940-000838-00) for sequencing.

5.2 Making DNB

5.2.1 Preparing reagents for DNB making

Perform the steps below:

1. Vortexing the circularized libraries obtained in 4.2.6 Application for 5 seconds, briefly centrifuge and then place on ice for later use.
2. Take out the low TE buffer and Stop DNB Reaction Buffer from DNB preparing kit, and place them on ice for around 30 minutes.
3. Take out the Make DNB Enzyme Mix I from the DNB preparation kit ,and place it on ice for 30 min.
4. Take out the App-D insert primer 1 from the High Throughput Sequencing Primer Kit (App-D), and place it on ice for 30 min.
5. Take the App Make DNB Buffer out of High Throughput Sequencing Primer Kit (App-D) and thaw it at room temperature.
6. Mix the thawed reagents thoroughly by using a vortex mixer for 5 sec. Centrifuge them briefly and place them on ice until use.

5.2.2 Making DNB

Perform the following steps:

Tips The following steps use the preparation of 1 tube of DNB as an example, and 7 copies of DNB need to be prepared at the same time .

1. Take a 0.2 mL 8-strip tube or PCR tubes and prepare the reaction mix on ice using the mixture in the table below. Vortex the tube for 5 sec and centrifuge briefly and place it on ice until use.

The volume of ssDNA V (μL) = 6.75 ng / Concentration of ssDNA (ng/μL)

Table 10 Make DNB Reaction Mixture 1

Component	Volume (μL)
App Make DNB Buffer	20
ssDNA from 4.2.6	V
Low TE Buffer	20-V
Total Volume	40

2. Place the Mixture 1 into a thermal cycler and run the primer hybridization reaction with the following conditions:

Table 11 Primer Hybridization Reaction Conditions

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

Temperature	Time
40 ℃	1 min
4 ℃	Hold

3. Transfer the PCR tube immediately to the ice when the reaction is completed. Add 2 polymerase components according to the table below.

Table 12 Make DNB Reaction Mixture 2

Component	Volume (μL)
Product mixture(from step 1)	40
Make DNB Enzyme Mix I	40
Make DNB Enzyme Mix II (LC)	4
Total Volume	84

4. Place the tubes into a thermal cycler for the next reaction. Run the program with the following conditions:

Table 13 Rolling Circle Replication Conditions

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

5. Add 20 μL of Stop DNB Reaction Buffer to the tube immediately when the temperature reaches 4 ℃. Mix gently by pipetting 5 to 8 times using a wide-bore, non-filtered pipette tip. Take 2 μL to measure the concentration and prepare sequencing on the DNBSEQ-T7RS.

Tips
- The concentration of DNB should be higher than 10 ng/μL.
- The concentration of the 7 tubes should be measured separately. If the concentration in 1 tube is significantly lower than that in the others, that tube should be discarded.

6. Transfer the DNB from 7 tubes to one 1.5 mL centrifuge tube, and mix gently by pipetting 10 times using a wide-bore, non-filtered pipette tip.

Chapter 6 Loading DNB

6.1 Preparing the post-loading plates and buffers

Perform the following steps:

Tips
- Two sets of DNBSEQ-T7RS High-Throughput Sequencing Kit (FCL PE100) are needed, but the following operations use one set as an example.
- The DNB loading system must be prepared right before use.

1. Take out the post-loading plate. Thaw it in a water bath at room temperature for 2 hr or thaw it in 2 ℃ to 8 ℃ refrigerator one day in advance. When the post-loading plate is thoroughly thawed, place it in the refrigerator at 2 ℃ to 8 ℃ until use.
2. Gently invert the post-loading plate to mix it 5 times and then centrifuge for 1 min before use.
3. Take the DNB Load Buffer II out of the DNBSEQ-T7RS DNB Load Reagent Kit. Thaw the reagents in a water bath at room temperature for approximately 30 min.
4. Mix the thawed reagents by using a vortex mixer for 5 sec. Centrifuge them briefly and place them on ice until use.
5. Take out the Make DNB Enzyme Mix II (LC) and allow it to thaw. Briefly centrifuge and place it on ice until use.
6. Take out the 0.5 mL microcentrifuge tube and place it on ice until use.

6.2 Preparing DNB loading mixture

Perform the following steps:

1. Mix 90 µL DNB Load Buffer II and 270 µL DNB in the 0.5 mL microcentrifuge tube gently by pipetting using a wide-bore, non-filtered pipette tip.
2. Add 1 µL Make DNB Enzyme II (LC), mix gently by pipetting 5 to 8 times using a wide-bore, non-filtered pipette tip and use it immediately.

6.3 Preparing the sequencing flow cell

Perform the following steps:

1. Take the flow cell out of the DNBSEQ-T7RS sequencing flow cell box.

2. Balance the flow cell at room temperature for at least 30 min, but no longer than 24 hr. Do not open the outer plastic package until use.
3. Take the flow cell out of the inner package; inspect the flow cell and confirm that it is intact. Clean the back of the flow cell using dust remover.
4. Align the positioning protrusions on the slide upwards with the positioning grooves on the slide platform, place the slide frame against the lower frame of the slide platform, and gently press down on the edge of the slide with both hands. Press the slide adsorption button on the loading platform to make it fully fit with the slide platform.

6.4 DNB Loading

Perform the following steps:

1. Start the MGIDL-T7RS program. Enter the user name and password then log into the main interface.
2. Select A/B in idle state:

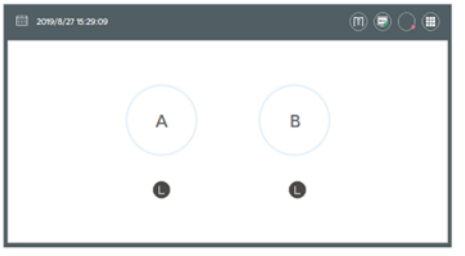

Figure 1 MGIDL-T7RS main interface

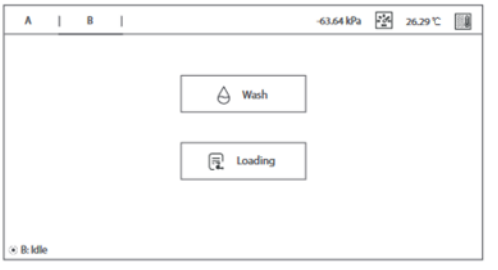

Figure 2 MGIDL-T7RS selection interface

3. Tap Loading to open the information input interface:

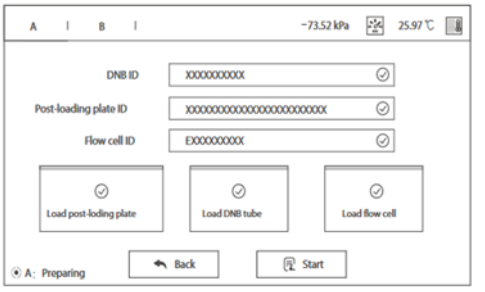

Figure 3 MGIDL-T7RS information input interface

4. Align the post-loading plate: Remove the seal of the post-loading plate, use a pipette to completely remove all of the reagent from well No.1 of the post-loading plate, then add 2 mL of App-D Insert primer 1 from High Throughput Sequencing Primer Kit (App-D).

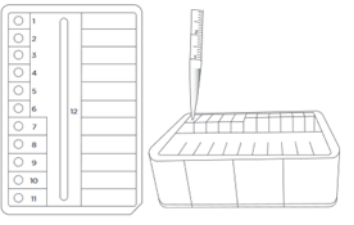

Figure 4 The well number of loading plate

5. Add 4 mL of 0.1 M NaOH into well No.11.
6. Place the prepared post-loading plate on the plate tray of MGIDL-T7RS.

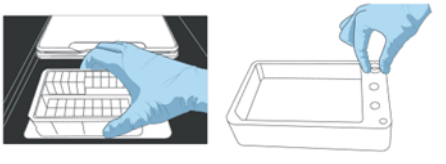

Figure 5 Post-loading plate placement diagram

7. Place the 0.5 mL microcentrifuge tube containing DNB loading mix into the DNB tube hole.
8. The operation to load sequencing slide please refer to *DNBSEQ-T7RS High-throughput Sequencing Set Instructions for Use.*
9. After loading is completed, put the slide into a resealable plastic bag and store it at 4 ℃.

Chapter 7 Preparation before sequencing

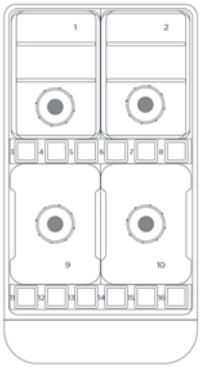

Figure 6 Sequencing cartridge wells

7.1 Preparing the sequencing cartridge

Perform the following steps:

1. Take the Sequencing Reagent Cartridge out of the High Throughput Sequencing Kit. Thaw the cartridge in a water bath at room temperature for 4 to 5 hr, or thaw it in 4 ℃ refrigerator one day in advance. After thoroughly thawing the cartridge, store it at 2 ℃ to 8 ℃ until use.
2. Take the dNTPs Mix V and dNTPs Mix II out of the sequencing kit and thaw them at room temperature. Centrifuge them briefly, and place them on ice until use.
3. Take the Sequencing Enzyme Mix out of the High Throughput Sequencing Kit and place it on ice until use.
4. Pierce the seal in the center of well No. 9 and No. 10 to make a hole approximately 2 cm in diameter by using a 1 mL sterile tip.
5. Add all dNTPs Mix V into well No. 9.
6. Add all dNTPs Mix II into well No. 10.
7. Add 2.76 mL Sequencing Enzyme Mix into well No. 9, add 2.76 mL Sequencing Enzyme Mix into well No. 10.
8. Seal the loading wells (well No. 9 and No. 10) with the transparent sealing film.
9. When applying the sealing film, rotate your fingers to press the sealing film at the lid. Ensure that the sticker is firm and free of air bubbles and that the reagent will not overflow from the sample hole.
10. Place the cartridge horizontally on the table, and hold both sides of the cartridge with both hands. Shake it vigorously clockwise 10 to 20 times, and then counterclockwise 10 to 20 times; ensure that reagents are fully mixed.

11. Take the seal of the loading wells out of the cartridge carefully after fully mixing.
12. Gently tap the cartridge on the bench to remove air bubbles from the reagents.

7.2 Washing cartridge and pure water container preparation

Refer to *DNBSEQ-T7RS High-throughput Sequencing Set Instructions for Use*.

Chapter 8 Sequencing

8.1 Sequencing parameter

Perform the following steps:

1. Align the sequencing cartridge, washing cartridge, and flow cell respectively with the RFID scanning area and the ID information will automatically display in the corresponding text box. If the scan fails, enter the ID information manually.

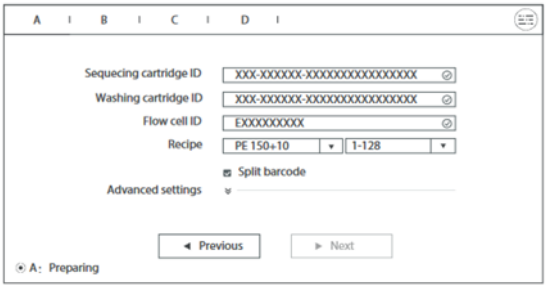

Figure 7 DNBSEQ-T7RS sequencing parameters

2. Tap ▾ next to **Recipe**. Select **Customize** in the drop-down menu to enter the interface, as shown in the figure below:

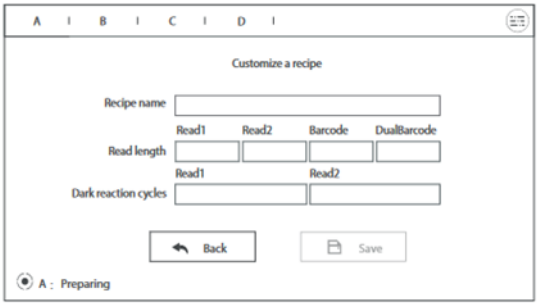

Figure 8 Customize a recipe

3. Add the sequencing parameters as follows:

- Enter the experiment name in the **Recipe name** field.
- Enter "66" in the **Read1 Read length** field. Skip the **Read2, Barcode**, and **DualBarcode Read length** fields.
- Enter "9-28, 37-58" in the **Read1 Dark reactioncycles** field. Skip the **Read2 Dark reactioncycles** field.

4. The protocol for starting sequencing and generating data is described in *DNBSEQ-T7RS High-throughput Sequencing Set Instructions for Use.*

5. The sequencing results (FASTQ files) are stored in "OutputFq" folder under "result", and they are ready for "NGS2Count" program for data quality control and downstream analysis.
6. The protocol for washing steps is described in *DNBSEQ-T7RS High-throughput Sequencing Set Instructions for Use*.

Appendix 1 Manufacturer information

Manufacturer	MGI Tech Co., Ltd.
Address	Main Building and Second floor of No.11 Building, Beishan Industrial Zone, Yantian District, Shenzhen. 518083, Guangdong, P.R. China
E-mail	MGI-service@mgi-tech.com
Website	www.mgi-tech.com

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