· Part No.: H-020-001024-00 · Release date: November 2024
· Version: 1.0

Overview

This quick start guide covers the entire workflow from sample to library to analysis result, here are 4 general and important notes for automated experiments:

- Before each automated run, ensure that the automation script and PCR program have been properly installed in the corresponding control software.
- It is recommended to perform pre-clean or post-clean when initializing or closing the automated instruments. Please close the door of instrument before cleaning, and do not open it in the process to avoid harmful ultraviolet radiation.
- Remove the lid of tip boxes and sealing films of plates before running a script.
- Before placing the sample plate in the automated instrument, always label the serial number on the plate to avoid sample errors.

Getting started

Preparing MGI equipment and materials

Applicable sets/kits

Type	Name	Cat. No.	Quantity
Extraction kit	MGIEasy Magnetic Beads Genomic DNA Extraction Kit	940-000972-00	2
Library Preparation set	MGIEasy Large-scale PCR-Free FS Library Prep Set for Low-pass WGS (96RXN)	940-002162-00	2
Sequencing Kit	MGIEasy Dual Barcode Circularization Kit (16 RXN)	1000020570	1
	DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE150)	1000016952	2
	CPAS Barcode Primer 3 Reagent Kit	1000020834	2
Analysis Package	BOLT Low-pass WGS Software Analysis Package (96 reports)	970-000519-00	2

MGI equipment

Name	Cat. No.	Quantity
DNA sequencing Library Preparation System MGISP-100RS	900-000206-00	1
MGISP-960 High-throughput Automated Sample Preparation System-Custom Configuration 7-V5	900-000152-00	1
Genetic Sequencer DNBSEQ-G400RS	900-000170-00	1
Data Analysis Appliance MGI-ZTRON-LITE	900-000406-00	1
MegaBOLT Bioinformatics analysis accelerator	970-000085-00	1

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MGI consumables

Name	Cat. No.	Quantity
250 µL automated filter tips	1000000723	32 boxes
0.2 mL 96 well full-skirt PCR plate	091-000165-00	10 pcs
1.3 mL 96 Well U-bottom Deep-well Plate	1000004644	16 pcs
MGIDL-200H Portable DNB Loader	900-000218-00	2 pcs
V2L Gasket (Steril)	510-003139-00	2 pcs

User-supplied equipment and materials

Recommended instruments

Name	Brand	Cat. No.
Qubit® fluorometers	Thermo Fisher	Q33238
FLUOstar Omega	BMG LABTECH	/
Thermal Cycler	BIO-RAD	T100
Thermomixer	/	/
Vortex Mixer	/	/
Desktop centrifuge for tube	/	/
Desktop centrifuge for plate	/	/
Centrifuge for deep well plate	/	/

Recommended consumables

Name	Brand	Cat. No.
Axygen® Single Well Reagent Reservoir with 96-Bottom Troughs, High Profile, Sterile	AXYGEN	RES-SW96-HP-SI
UV-STAR MICROPLATE, 96 WELL, COC, F-BOTTOM (CHIMNEY WELL), CLEAR	greiner bio-one	655801
Axygen 0.5 mL Thin Wall PCR Tubes with Flat Cap, Clear, Nonsterile	AXYGEN	PCR-05-C
MicroAmp Clear Adhesive Film, 100 films	Thermo Scientific	4306311
Adhesive PCR Plate Foils	Thermo Scientific	AB0626

Recommended reagents

Name	Brand	Cat. No.
Qubit dsDNA BR Assay Kit, 500 assays	Invitrogen	Q32853
Qubit ssDNA Assay kit, 100 assays	Invitrogen	Q10212
Qubit dsDNA HS Assay kit, 500 assays	Invitrogen	Q32854
TE Buffer PH8.0, 500mL	Invitrogen	AM9849
Absolute ethanol, Analytically pure	/	/
Isopropanol, Analytically pure	/	/

Extracting genomic DNA

Preparing consumables

Prepare consumables for a workflow of automated extraction on MGISP-960RS according to the following table, and place them at room temperature until use.

Name	Brand	Cat. No.	Quantity
250 µL automated filter tips	MGI	1000000723	7 boxes
1.3 mL 96 Well U-bottom Deep-well Plate	MGI	1000004644	5 pcs
0.2 mL 96 well full-skirt PCR plate	MGI	091-000165-00	2 pcs

Preparing reagents

1. Take out all reagents from MGIEasy Magnetic Beads Genomic DNA Extraction Kit, place them at room temperature until use.
2. Add absolute ethanol into Buffer W1 and Buffer W2 according to the label and seal it until use.
3. Take out one 0.2 mL 96 well full-skirt PCR plate and four 1.3 mL 96 Well U-bottom Deep-well Plates, label each plate with component name according to the table below, and add corresponding reagent to each plate, respectively.

Reagent plate	Consumable	Component	Reagent volume/well
Magnetic Beads H	0.2 mL 96 well full-skirt PCR plate	Magnetic Beads H	20 µL
Buffer W1	1.3 mL 96 Well U-bottom Deep-well Plate	Buffer W1	400 µL
Buffer W2	1.3 mL 96 Well U-bottom Deep-well Plate	Buffer W2	800 µL
Buffer EB	1.3 mL 96 Well U-bottom Deep-well Plate	Buffer EB	110 µL
Isopropanol (user-supplied)	1.3 mL 96 Well U-bottom Deep-well Plate	Isopropanol	240 µL

Tips

Vortex and mix thoroughly the Magnetic Beads H before use. After the reagent is transferred to reagent plate, ensure that there are no bubbles at the bottom of plate and no liquid on the side wall.

Preparing samples

Tips

For animal tissue and blood sample, it is recommended that the collected samples be extracted within 24 h. For those cannot be extracted within 24 h, store them in a -25 ℃ to -15 ℃ freezer and avoid frequent freeze-thaw.

Preparing animal tissue sample

1. Prepare 10 mg to 20 mg of tissue sample, cut it into pieces the size of sesame, and add them to a 1.5 mL centrifuge tube.

2. Add 200 µL of Buffer LS and 20 µL of protease K to the tube, mix thoroughly with a vortex mixer, and instantly centrifuge the tube to collect liquid on the tube wall.
3. Place the centrifuge tubes on the thermomixer, set the incubation temperature to 65 ℃ , and speed 1000 rpm. Incubate the tubes for 20 min until the solution becomes clear, no visible turbidity after instant centrifuge, and no precipitation exist at the bottom of the tube.

Tips

To incubate 96 samples, 4 thermomixers that can accommodate 24 1.5 mL centrifuge tubes or a combination of thermomixers with equivalent throughput are required.

4. After incubation, take out a new 1.3 mL 96 Well U-bottom Deep-well Plate, label it as “sample + serial number”. Add 200 µL of Buffer LB to each well, and transfer 170 µL of the sample with lysis buffer.

Preparing blood sample

Take out a new 1.3 mL 96 Well U-bottom Deep-well Plate, add 140 µL blood sample, 20 µL protease K, and 210 µL Buffer LB into each well.

Initializing MGISP-960RS

Tips

The software version of MGISP-960RS should be V1.8.0.323 or above.

1. Turn on the MGISP-960RS and computer, double-click the icon to run the software.
2. Select **User** and **Real** mode and click **Login**.
3. Click **Initialize** on the top of main interface.
4. Click ☰ on the left of the interface and select **Clean** to open the **Clean** interface. Select **Pre-clean**, close the door, and click **Start**.

Performing automated gDNA extraction

1. Click ☰ on the left of the interface, open the **Run Wizard** interface, and set **Solution** and **Script** according to the table below:

Solution	JB-A22-011 Part B MGISP-960 Library_Prep_package_96RXN_RV1.0_SV1.0
Script	JB-A22-011 B1.MGIEasy Genomic DNA Extraction Prepacked Kit_SV1.0.py

Tips

Each run of the script completes extracting 96 samples, 192 samples require 2 runs.

2. Open the door of MGISP-960RS, place the consumables according to the figure and table below:

Tips

For the first run, place No. 1 plate at POS20, and in the second run, plate No.2 at same position.

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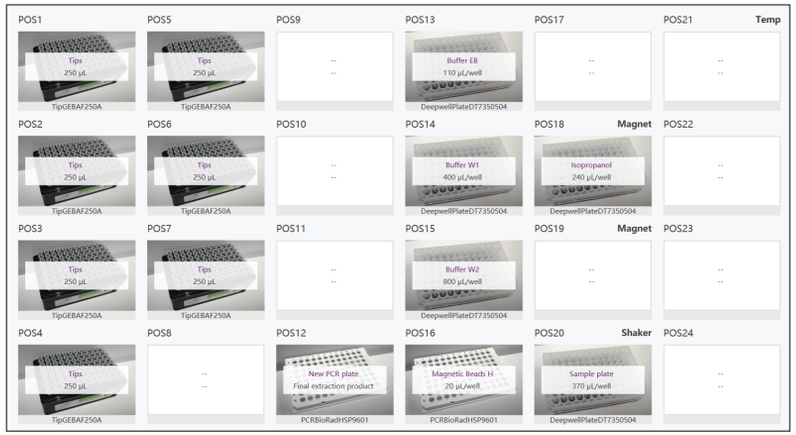

Name	Consumables	Position
New Tips	250 µL automated filter tips	POS1-POS7
New PCR plate	0.2 mL 96 well full-skirt PCR plate	POS12
Buffer EB	1.3 mL 96 Well U-bottom Deep-well Plate	POS13
Buffer W1	1.3 mL 96 Well U-bottom Deep-well Plate	POS14
Buffer W2	1.3 mL 96 Well U-bottom Deep-well Plate	POS15
Magnetic Beads H	250 µL automated filter tips	POS16
Isopropanol	1.3 mL 96 Well U-bottom Deep-well Plate	POS18
Sample plate	1.3 mL 96 Well U-bottom Deep-well Plate	POS20

3. Check the layout and close the door, click Run to start the extraction. The process takes about 2 h.
4. When the process is finished, take out the new PCR plate at POS12, label it with “Extraction product+ date + serial number”.
5. Empty the operation deck and discard the used deep-well plates, PCR plates and waste tips.
6. Repeat one complete process of sample preparation and automated extraction, to finish extraction of the second sample plate.

Tips

Clean the operation deck and tray with a dust-free paper moistened with 75% ethanol solution and perform a post-clean.

Quantifying and normalizing samples

Quantification

Preparing consumables

Name	Brand	Cat. No.	Quantity
250 µL automated filter tips	MGI	1000000723	7 boxes
UV-STAR PLATE, 96 WELL FLAT BOTTOM	Greiner bio-one	655801	3 pcs
Single Well Reagent Reservoir with 96-Bottom Troughs	AXYGEN	RES-SW96-HP	1 pcs
0.2 mL 96 well full-skirt PCR plate	MGI	091-000165-00	4 pcs

Preparing samples and reagents

Reagent	Quantity	Thaw Temperature
Extraction plate	2 plates	Thaw at room temperature
dsDNA Broad Range Assay Kit	1 kit of 500 assays	Thaw dsDNA BR Reagent away from direct sunlight and mix thoroughly before use.

Tips

We recommend using 200 µL of assay buffer per reaction to quantify samples.

1. Mix the extraction products (plate) thoroughly with a vortex mixer, and centrifuge to collect reagents to the bottom of the plates.
2. Take out dsDNA Broad Range Assay reagent and buffer, prepare quantification working solution for 300 reactions according to the table in the next step, mix them vigorously using a vortex mixer.

Reagent	Volume (mL)
dsDNA BR Buffer	59.7
dsDNA BR Reagent	0.3
Total volume	60

3. Transfer 4 mL of prepared quantification working solution to a new tube for later use in calibration standard quantification.

Tips

The prepared quantification working solution must be stored away from light.

Preparing sample quantification plate on MGISP-960RS

1. Click ☰ on the left of the interface, open the **Run Wizard** interface, and set **Solution** and **Script** according to the table below:

Solution	JB-A22-011 Part B MGISP-960 Library_Prep_Package_96RXN_RV1.0_SV1.0
Script	JB-A22-011 B2.gDNA Quantification.py

Tips

Each run of the script completes quantification solution preparation of 192 samples.

2. Before running the script, add 56 mL quantification working solution into the Single Well Reagent Reservoir with 96-Bottom Troughs.
3. Open the door of MGISP-960RS. Place the consumables according to the figure and the table below.

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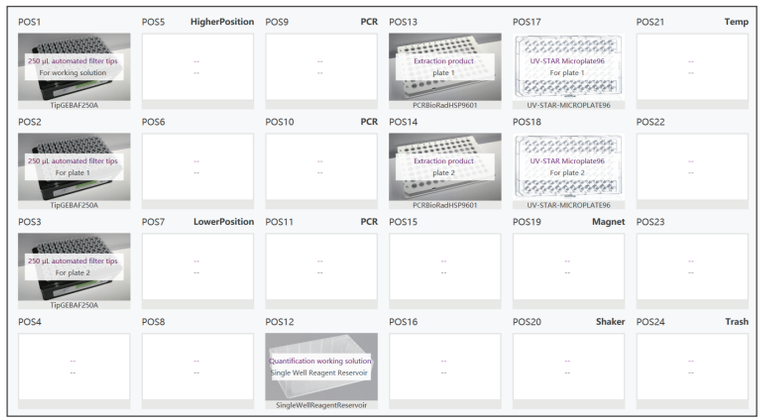


Name	Consumable	Position
New Tips	250 µL automated filter tips	POS1 to POS3
Working Solution Buffer	Single Well Reagent Reservoir with 96-Bottom Troughs	POS12
Extraction products	0.2 mL 96 well full-skirt PCR plate	POS13 to POS14
New UV-STAR plate	UV-STAR PLATE, 96 WELL FLAT BOTTOM	POS17 to POS18

4. Close the door and click **Run** to start the automation.
5. In the pop-up window, select **2** in the drop-down list of plate number, click **Continue**.
6. After the process is completed, remove the UV-STAR plates at POS17 to POS18, label by serial number from 1 to 2, and transfer them to the FLUOstar Omega microplate reader.
7. Remove the extraction product plates at POS13 to POS14, and seal with films, and store them in a 2 ℃ to 8 ℃ refrigerator.
8. Empty the operation deck and discard the used tips.
9. Click ☰ on the left of the interface, open the **Clean** interface and select **Post-clean**, close the door, and click **Start**.

Preparing calibration plate

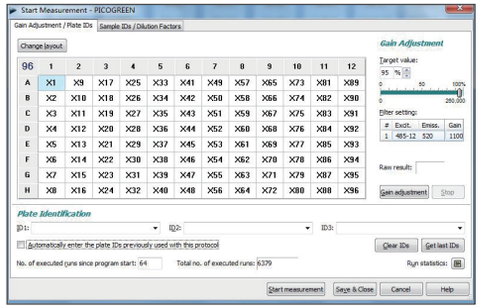
1. Dilute the calibration standards in an 8-strip tube according to the table below, and centrifuge after mixing.

Tube	1	2	3	4	5	6	7	8
Add in	20 µL BR Standard 2	10 µL from tube 1	10 µL from tube 2	10 µL from tube 3	10 µL from tube 4	10 µL from tube 5	10 µL from tube 6	/
Nuclease-free H_2O	/	10 µL	10 µL	10 µL	10 µL	10 µL	10 µL	10 µL

2. Take out a new UV-STAR plate, add 190 µL quantification working solution to the 8 wells in column 1, and add 10 µL of calibration standard to each well in the same order as for the 8-strip tubes, mix by pipetting. The calibration plate is ready.

Quantifying dsDNA

1. Turn on the FLUOstar Omega microplate reader and computer, double-click the icon **Omega** to run the software.
2. Select mode **Picogreen**, click **Change layout** to set all 96-well according to the figure below.
3. For the same quantitative experiments, make sure **Gain** is set to the same value (recommended value: 1100).
4. Put the calibration plate into the microplate reader, Click **Start measurement**.

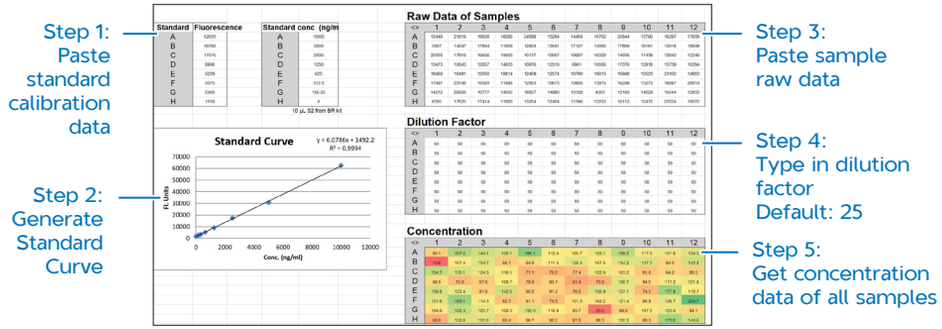


5. You can find three files in the support package which will be used in the normalization experiment. Open the file *Quantification_and_Normalization_template.xlsx*, sheet *Quantification*, copy the measured standard calibration data to corresponding area, you will get the standard curve automatically. Make 2 copies of the template file, label them by serial number for later use.

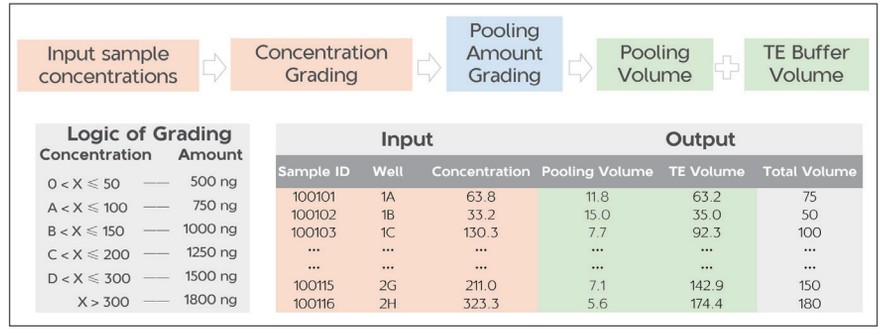
Tips

It is recommended that you prepare new calibration standards and update the standard curve every time a new quantification working solution is prepared, to minimize variables that affect quantification performance.

6. Put the prepared sample quantification plate into the microplate reader, click **Start measurement** to quantify the samples.
7. After the measurement ends, save the raw data and copy them to the *Quantification_and_Normalization_template.xlsx* copy of corresponding serial number. For more details, refer to the workflow in the figure below.



8. Copy the concentration data into another sheet of **Normalization**. As the extracted DNA sample should be normalized to 10 ng/μL, the required pooling volume and TE buffer volume for sample normalization will appear in the output area. An example is shown below.



Logic of Grading

Concentration	Amount
0 < X ≤ 50	500 ng
A < X ≤ 100	750 ng
B < X ≤ 150	1000 ng
C < X ≤ 200	1250 ng
D < X ≤ 300	1500 ng
X > 300	1800 ng

Input			Output		
Sample ID	Well	Concentration	Pooling Volume	TE Volume	Total Volume
100101	1A	63.8	11.8	63.2	75
100102	1B	33.2	15.0	35.0	50
100103	1C	130.3	7.7	92.3	100
...	...	...	...	...	...
...	...	...	...	...	...
100115	2G	211.0	7.1	142.9	150
100116	2H	323.3	5.6	174.4	180

Tips

The excel file will grade the concentration and determine the amount of sample for normalization, and show output:

$$\text{Total volume} = \frac{\text{Graded amount}}{10}$$

$$\text{Pooling volume} = \frac{\text{Graded amount}}{\text{Concentration}}$$

$$\text{TE volume} = \text{Total volume} - \text{Pooling volume}$$

9. Make 2 copies of file *Sample_Normalization_Adding_Sample.csv*, and *Sample_Normalization_Adding_TE.csv*, respectively. You can fill these two types of files with the output data of pooling volume and TE volume, respectively.
10. Repeat measurement and data conversion until all plates have been quantified.

Normalizing samples on MGISP-100RS

Initializing MGISP-100RS

Tips

The software version of MGISP-100RS should be V1.9.3.476 or above.

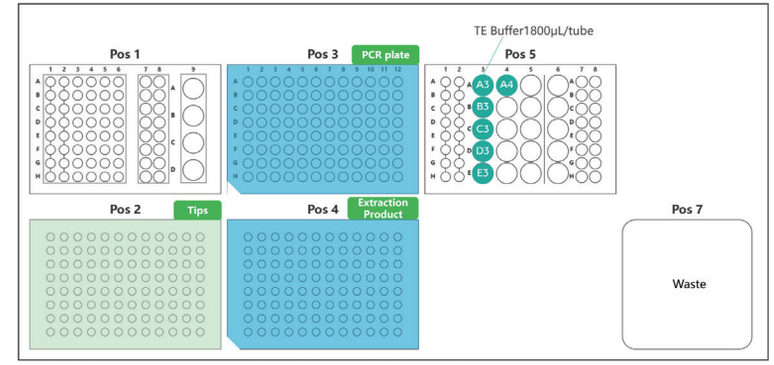
1. Turn on the MGISP-100RS and computer, double-click the icon of MGISP-100 to open the software.
2. Select **Real** mode and click **Create**. Click **User Entry** to open the main interface.
3. Click **Initialize** on the top of the interface to start initializing.
4. Click ☰ on the left of the interface, enter **Pre-post Clean** interface and select **Pre-clean**, close the door, and click **Start**.

Normalizing samples

1. Click ☰ on the left of the interface, open the **Run Wizard** interface, and set **Solution** and **Script** according to the table below:

Solution	JB-A22-011 Part A MGISP-100 Normalization_96RXN_RV1.0_SV1.0
Script	JB-A22-011 A1.Normalization_Adding_TE_SV1.0.py

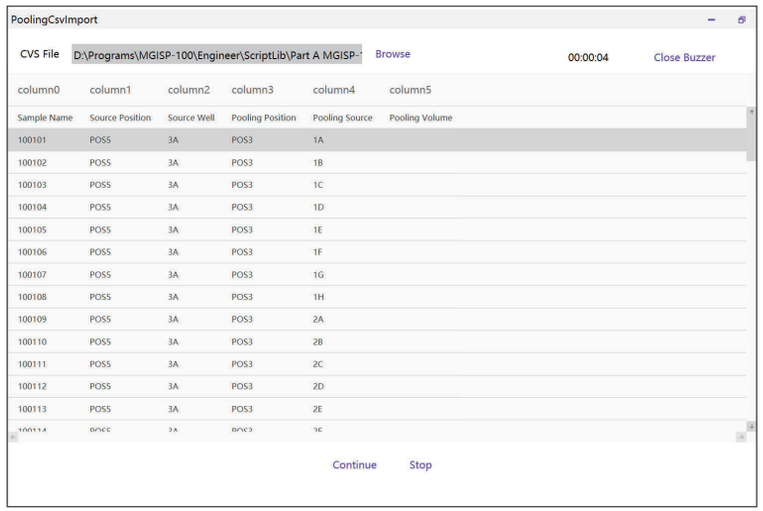
2. Open the door of MGISP-100RS, place the reagents and new consumables according to the figure and the table below.



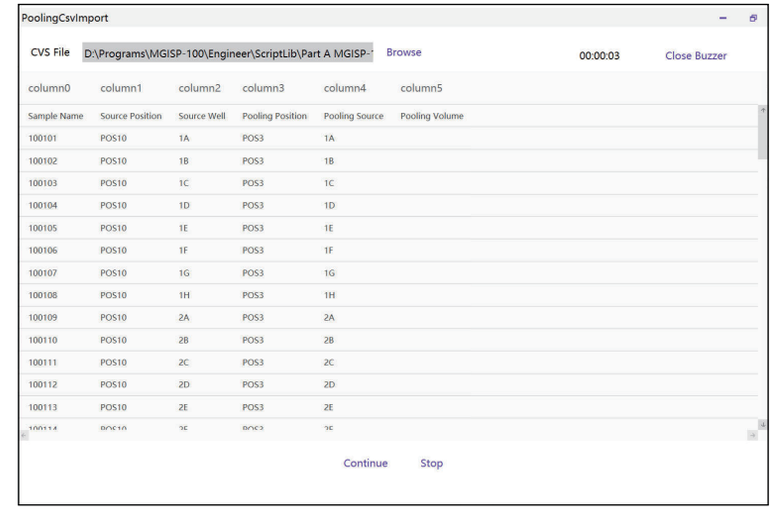
Name	Consumable	Position
New tips	250 μL automated filter tips	POS2
New PCR plate	0.2 mL 96 well full-skirt PCR plate	POS3
Extraction product	0.2 mL 96 well full-skirt PCR plate	POS4

Name	Consumable	Position
TE Buffer	2 mL SC microtubes, PCR-RT	POS5, A3, B3, C3, D3, E3 and A4, 6 tubes in total

3. Close the door, click **Run**. Click **Browse** in the pop-up window, select *A1.Sample Normalization Adding TE.csv*. The interface after importing is shown below. Click **Continue** to start the program.



4. When the program is finished, click the drop-down list of Script and select *JB-A22-011 A2.Normalization_Adding_Sample_SV1.0.py*. Click **Run** and another window will pop up. Click **Browse** to select *A2.Sample Normalization Adding Sample.csv* and click **Continue** to start the program.



5. After the program is finished, remove the new PCR plate at Pos 3, seal the plate with films, label it as "Normalized DNA + date + serial number of 1 to 2" and store it in a 2 °C to 8 °C refrigerator.
6. Remove the extraction product at Pos 4, seal the plate with adhesive PCR plate foils, and store it in a -25 °C to -15 °C freezer.
7. Replace new consumables, repeat step 2 to step 8 to proceed second plate normalization.
8. Empty the operation deck, discard the used consumables.

Tips

If no experiment will be conducted on the day, clean the operation deck and tray with a KimWipes tissue moistened with 75% ethanol solution, and perform a post-clean.

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Preparing fast PCR-FREE library

Tips

A complete run of the library preparation on MGISP-960RS includes three steps: fragmentation, adapter ligation and product pooling. Each run processes 96 samples, 2 complete runs are needed for 192 samples.

Preparing consumables

Name	Brand	Cat. No.	Quantity
250 µL automated filter tips	MGI	1000000723	8 boxes
0.2 mL 96 well full-skirt PCR plate	MGI	091-000165-00	6 pcs
1.3 mL 96 Well U-bottom Deep-well Plate	MGI	1000004644	2 pcs

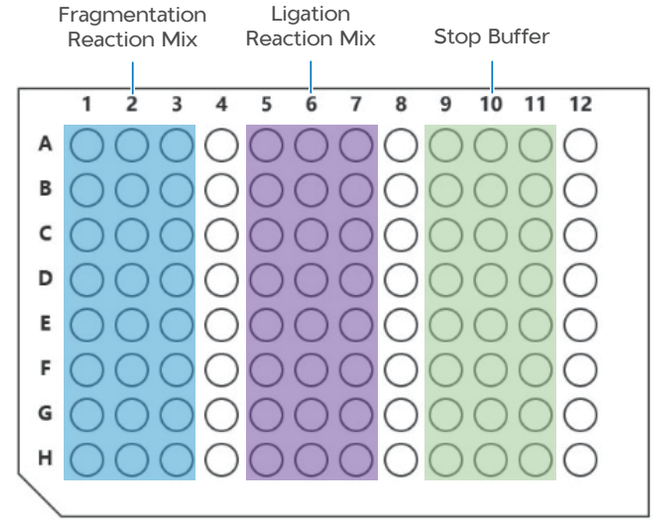
Preparing samples and reagents

1. Take out the normalized DNA (1 plate) from the refrigerator and thaw at room temperature. Mix the plates thoroughly with a vortex mixer, and centrifuge to collect reagents to the bottom of the plates.
2. Take out the following reagents from the -25 ℃ to -15 ℃ freezer 30 min before use and thaw them according to the table below:

Name	Component	Thaw condition
MGIEasy Large-scale PCR-Free FS Library Prep Module for Low-pass WGS (plate)	Library Prep Reagent	Ice box
MGIEasy UDB PF Adapter Kit A	UDB Adapters A	Room temperature

Tips

The Library Prep Reagent contains 3 components, as shown in the figure below.

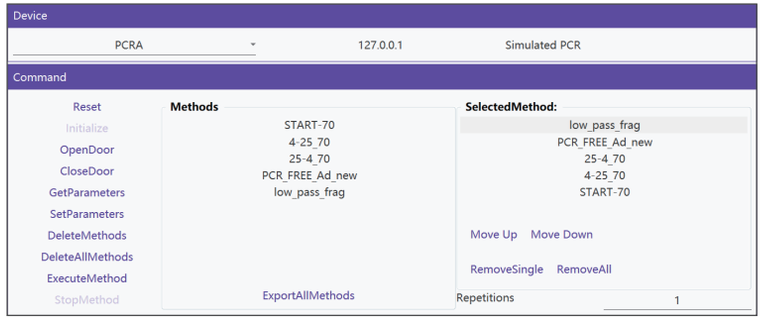


Preparing libraries on MGISP-960RS

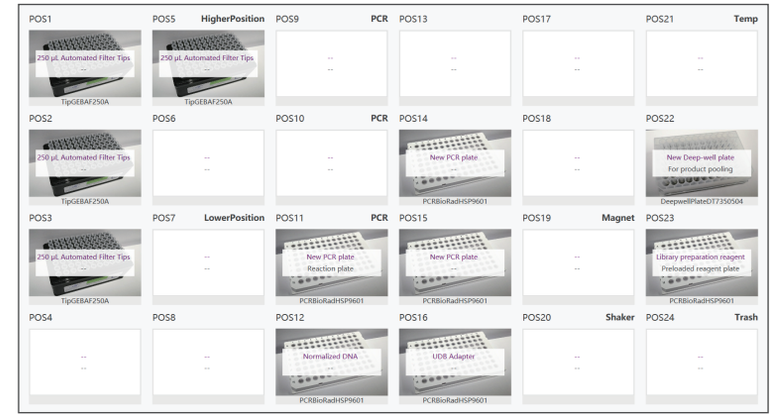
1. Click ☰ on the left of the interface, open the **Run Wizard** interface, and set **Solution** and **Script** according to the table below:

Solution	JB-A22-011 Part B MGISP-960 Library_Prep_package_96RXN_RV1.0_SV1.0
Script	JB-A22-011 B3.MGIEasy Large-scale PCR-FREE Library Prep_96RXN_SV1.0.py

2. Before running the script, perform the following steps to confirm that the PCR program is successfully installed: click ☰ to open the **PCR** interface, click the drop-down list of **Device**, select **PCRA**, and click **GetParameters**. Confirm that the PCR steps are shown as the figure below. If the interface is not displayed as shown in the figure below, re-install the PCR method by clicking **SetParameters** and import *Large-scale PCR-FREE FS Library Prep PCR Method_V1.0.xml*.



3. Click ☰ on the left of the interface, open the **Run Wizard** interface, and check the operation deck arrangement. Open the door of MGISP-960RS. Place the reagents and consumables according to the figure and the table below.



Name	Consumable	Position
New Tips	250 µL automated filter tips	POS1, 2, 3, 5
New PCR plate	0.2 mL 96 well full-skirt PCR plate	POS11, 14, 15
Normalized DNA	0.2 mL 96 well full-skirt PCR plate	POS12
UDB adapter	0.2 mL 96 well full-skirt PCR plate	POS16
New deep-well plate	1.3 mL 96 Well U-bottom Deep-well Plate	POS22
Library Prep Reagent	0.2 mL 96 well full-skirt PCR plate	POS23

4. Close the door and click **Run** to start the program.

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Tips

The automated program will perform fragmentation, adapter ligation, adding stop buffer and pooling product, where fragmentation and ligation are both performed in the PCR temperature control module.

5. After the process is completed, take out the new deep-well plate from operation deck POS22, use a pipette to transfer 120 µL of solution from each of the 8 wells in column 1 into a new 1.5 mL centrifuge tube, cap the tube, label it as "Pooling product + date + serial number", and store it in a 2 °C to 8 °C refrigerator. Discard the used deep-well plate.
6. Take out the Ligation Product plates from the operation deck POS11, seal the plates with adhesive PCR plate foils, and store them in a -25 °C to -15 °C freezer as backup. If there are still unused DNA in POS12 normalized DNA, you can also seal the plate and store in freezer as backup.
7. Empty the operation deck and discard used consumables.
8. Repeat the step 3 through step 7 to finish the library preparation of second sample plate. The final products are two pooling product in two 1.5 mL centrifuge tubes.

Tips

After 2 runs are completed, click ☰ on the left of the interface, open the **Clean** interface, and select **Post-clean**. Clean the operation deck with dust-free paper moistened with 75% ethanol, close the door, and click **Start**.

Making DNBs

Purifying ligation product

1. Take out DNA Clean Beads and TE Buffer from MGIEasy DNA Clean Beads kit and place them at room temperature for 30 min before use. Mix them thoroughly by using a vortex mixer.
2. Prepare 5 mL of 80% ethanol solution by using absolute ethanol and Milli-Q water.
3. Take out two tubes of pooling products from 2 °C to 8 °C refrigerator, centrifuge and mix thoroughly. Transfer 360 µL of pooling products from each tube into two new 1.5 mL centrifuge tubes, and label them by serial number, respectively.
4. Add 58.5 µL of DNA Clean Beads into each tube, mix thoroughly, centrifuge, and incubate at room temperature for 5 min.
5. Centrifuge the tubes briefly and place them on a magnetic rack for 2 min to 5 min until the liquid is clear. Pipette the supernatant from each tube into two new 1.5 mL tubes, respectively.

Tips

This step is to transfer the supernatant to new tubes, do not discard the supernatant. The tube with magnetic beads in the first round can be retained until the end of the experiment. Recycle DNA from the beads if needed.

6. Add 27 µL of DNA Clean Beads to each 1.5 mL tube with supernatant, vortex and centrifuge, and incubate at room temperature for 5 min.

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7. Centrifuge the 1.5 mL tubes briefly, place them at the magnetic rack for 2 min to 5 min until the liquid is clear, and then carefully remove and discard the supernatant. Add 160 µL of 80% ethanol solution to each tube to wash the beads and tube wall, wait for 30 s, carefully remove and discard the supernatant.
8. Repeat step 7. Try to remove all liquid from the tube. If some liquid remains on the tube wall, centrifuge the tube briefly and place it on the magnetic rack for separation. Remove all liquid by using a low-volume pipette. Open the tube cap and air-dry the beads at room temperature until no wetness or glossiness is visible on the beads' surface. There should be no visible cracking on the surface of the beads.
9. Remove the tubes from the magnetic rack and add 52 µL of TE Buffer to elute the DNA. Mix with a vortex mixer until all beads are suspended. Incubate the tubes at room temperature for 5 min.
10. Centrifuge the tubes briefly and place them on the magnetic rack for 2 min to 5 min until the liquid is clear. Carefully transfer 50 µL of the supernatant to a new 0.2 mL PCR tube.
11. Quantify purified products by using Qubit dsDNA HS Assay Kit or Quant-iT PicoGreen dsDNA Assay Kit. The product concentration should be no less than 1.2 ng/µL.

Circularizing library to single stranded DNA

Single strand circularization

1. Take out 1 kit of MGIEasy Dual Barcode Circularization Kit. Thaw the reagents according to the table below:

Reagent	Thaw condition
Dual Barcode Splint Buffer	Thaw at RT, mix thoroughly, centrifuge briefly, and place on ice.
Digestion Buffer	
Digestion Stop Buffer	
DNA Rapid Ligase	Flick and/or invert the tube gently, centrifuge briefly, and place on ice.
Digestion Enzyme	

2. Take out the two purified products and add 200 ng of each to two new 0.2 mL PCR tubes, add TE Buffer to 48 µL. If the product is lower than 200 ng, take 48 µL of the purified product.
3. Assume one purified product has a concentration of *X* ng/µL, prepare the normalized product according to the table below.

Reagent	Volume (µL)
Purified product	200/*X*
Low TE Buffer	48-(200/*X*)
Total (µL)	48

Tips

The minimum input amount of purified product per tube is 100 ng. In the range of 100 ng to 200 ng, the more input of purified product, the higher circularized library is yielded.

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4. Place the tubes on the thermo cycler and start a reaction according to the table below:

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

5. Prepare the single strand circularization mixture in a 0.2 mL PCR tube according to the table below:

Reagent	Volume (μL)
Purified product	48
Dual Barcode Splint Buffer	11.5
DNA Rapid Ligase	0.5
Total (μL)	60

6. Vortex the tubes 3 to 6 times (3 sec each), and centrifuge briefly. Place them into a thermocycler. Run the program with the following conditions:

Temperature	Time
42°C (Heated lid)	On
37 °C	10 min
4 °C	Hold

7. When the program is completed, place the PCR tube(s) on ice, centrifuge briefly, and immediately proceed to the next step
8. Prepare the enzyme digestion mixture in a 0.2 mL PCR tube according to the table below:

Reagent	Volume (μL)
Circularization mixture	60
Digestion Buffer	1.4
Digestion Enzyme	2.6
Total (μL)	64

9. Vortex the tubes 3 to 6 times (3 sec each), and centrifuge briefly. Place them into a thermocycler. Run the program with the following conditions:

Temperature	Time
42°C (Heated lid)	On
37 °C	10 min
4 °C	Hold

10. When the program is completed, centrifuge the tube(s) briefly. Immediately add 7.5 μL of Digestion Stop Buffer to each sample tube. After instant centrifuge, transfer all solution into two new 1.5 mL centrifuge tubes.

Purifying circularization product

1. Add 130 μL of DNA Clean Beads into each tube of circularization product, mix thoroughly, centrifuge, and incubate at room temperature for 5 min.
2. Centrifuge the tubes briefly and place them on a magnetic rack for 2 min to 5 min until the liquid is clear. Carefully remove and discard the supernatant.

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3. Keep the PCR tubes on the magnetic rack, add 160 µL of 80% ethanol solution to each tube to wash the beads and tube wall, wait for 30 s, carefully remove and discard the supernatant.
4. Repeat step 4. Try to remove all liquid from the tube. If some liquid remains on the tube wall, centrifuge the tube briefly and place it on the magnetic rack for separation. Remove all liquid by using a low-volume pipette.
5. Keep the tubes on the magnetic rack. Open the tube cap and air-dry the beads at room temperature until no wetness or glossiness is visible on the beads' surface. There should be no visible cracking on the surface of the beads.

1. Remove the tubes from the magnetic rack and add 25 µL of TE Buffer to elute the DNA. Mix with a vortex mixer until all beads are suspended. Incubate the tubes at room temperature for 2 min to 5 min.

1. Centrifuge the tubes briefly and place them on the magnetic rack for 2 min to 5 min until the liquid is clear. Carefully transfer 24 µL of the supernatant to a new 0.2 mL PCR tube.
2. Quantify purified products by using Qubit ssDNA HS Assay Kit. The product concentration should be no less than 0.6 ng/µL.

Making DNBs for DNBSEQ-G400RS

1. Take out the DNB Make Reagent from DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE150). Thaw the reagents according to the table below:

Reagent	Thaw condition
Low-TE Buffer	Thaw at RT, mix thoroughly, centrifuge briefly, and place on ice.
Make DNB Buffer	
Stop DNB Reaction Buffer	
Make DNB Enzyme Mix I	Flick and/or invert the tube gently, centrifuge briefly, and place on ice.
Make DNB Enzyme Mix II (LC)	

Tips

Please confirm the DNB loading scheme. If you use a sequencer to load DNBs, you need to prepare 2 reactions of DNBs for each library. If you use the MGIDL-200H portable DNB loader to load DNBs, you only need to prepare 1 reaction of DNBs for each library.

2. Prepare the DNB making system in the new 0.2 mL PCR tube according to the table below, as one purified product with a concentration of *X* ng/µL, .

Reagent	Volume (µL)
Circularization product	16/Y
Low TE Buffer	20- (16/Y)
Make DNB Buffer	20

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3. Mix the DNB making system with vortex mixer and centrifuge briefly, place the tubes on the thermo cycler and start a reaction according to the table below:

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

4. When the reaction reaches to 4℃, take out the PCR tubes from the thermo cycler, centrifuge briefly, and add the reagents to the reaction tube on the ice box according to the table below:

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

5. Mix the reaction mixture tube with vortex mixer, centrifuge the tube briefly, and place the tubes on the thermo cycler instantly, and start making DNB reaction according to the table below:

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

Tips

It is recommended to preheat the heated lid to the required temperature before DNB reaction.

6. When the reaction reaches to 4 ℃, take out the PCR tubes from the thermo cycler, add 20 μL of Stop DNB Reaction Buffer to each tube, and pipette 8 times to mix the tube gently by using a wide-bore tip. Label the tube in the form of "DNB + date + serial number", and store the DNBs at 4 ℃ and use them within 48 h.

Tips

Mix DNBs gently by using a wide-bore tip. Do not vortex, shake or pipette vigorously.

7. Quantify the DNB products according to the instructions of Qubit ssDNA Assay Kit. The required concentration should be no less than 8 ng/μL.

Tips

If the DNB concentration is higher than 40 ng/μL, dilute the DNBs to around 20 ng/μL with DNB loading buffer I before use.

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Performing sequencing and analysis

Tips

The sequencing of 192 samples requires 2 sequencing runs. The following is guide for one run as an example.

Preparing for DNB loading

Take out DNBSEQ-G400RS flow cell from the -25 ℃ to -15 ℃ refrigerator and equilibrate them to room temperature for 1 h to 24 h.

Tips

Choose one of the following two methods of DNB loading based on the actual demand.

Loading DNBs by the sequencer

1. Take out the following reagents from the sequencing package and take out a new 0.5 mL SC tube. Add the following reagent according to the table below:

Reagent	Volume (mL)
DNB Load Buffer II	64
Make DNB Enzyme Mix II (LC)	2
DNB	200
Total volume	266

2. Mix the mixture by gently pipetting 8 times by using a wide-bore, non-filtered pipette tip. Place the mixture at 2 ℃ to 8 ℃ until use.

Loading DNBs by MGIDL-200H Portable DNB Loader

1. For each flow cell, take out a new 0.5 mL SC tube and prepare DNB loading mixture according to the table below:

Reagent	Volume (mL)
DNB Load Buffer II	32
Make DNB Enzyme Mix II (LC)	1
DNB	100
Total volume	133

2. Mix the mixture by gently pipetting 8 times by using a wide-bore, non-filtered pipette tip.
3. Place a clean sealing gasket into the groove of MGIDL-200H Portable DNB Loader, align the holes of the flow cell with alignment pins of the device and place the flow cell on it, close the cover.
4. Place the loader on the bench with the back facing up, aspirate 30 µL of DNB loading mixture with a wide-bore, non-filtered pipette tip, and insert the tip into the fluidics inlet.
5. Eject the tip and DNBs automatically flow into the flow cell.
6. Lift up the device, but do not tilt it (keep it parallel to the bench), and verify that the DNBs flow through the flow cell.
7. Ensure that all DNBs flow into the flow cell. Hold the device and rotate the tip counterclockwise to remove it.
8. Repeat step 4 to 7 to load the DNBs for the rest of the lanes.
9. Place MGIDL-200H on the bench with the front facing up, wait 30 min for the DNB loading process. After that, open the cover, take out the flow cell, it is ready for sequencing.

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Preparing the sequencing reagent cartridges

1. Take out DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE150) from the -25 °C to -15 °C freezer. Take the Sequencing Reagent Cartridge out of the sequencing set and choose one of the following ways to thaw the cartridge:
   - Thaw it in a water bath at room temperature for 4 h to 5 h.
   - Thaw it in a 2 °C to 8 °C refrigerator 24 h in advance.
2. Take dNTPs Mix and dNTPs Mix II out of the sequencing kit, take 1 μM AD153 Barcode Primer 3 out of CPAS Barcode Primer 3 Reagent Kit, and thaw these reagents at room temperature.
3. After thawing, invert these reagents 6 times and place them on ice until use.
4. Add the reagents to the sequencing cartridge according to the table below. Make sure to pre-mix the reagents separately that need to be added into well No. 1, No. 2 and No. 15.

Name	Volume (mL)	Well
dNTPs Mix	2.4	No.1
Sequencing Enzyme Mix	2.4	
dNTPs Mix II	2.1	No.2
Sequencing Enzyme Mix	2.1	
MDA Enzyme Mix	0.5	No.15
MDA Reagent	3.5	
1 μM AD153 Barcode Primer 3	2.9	No.4

Tips

Take well No. 1 as an example, add dNTPs Mix and Sequencing enzyme Mix into a new 15 mL centrifuge tube, invert the tube 6 times, and then add the mixture into well No.1.

5. Seal well No.1 and No. 2 with sealing films. Shake the reagent cartridge clockwise 20 times and then counterclockwise 20 times to mix the reagents thoroughly, and then remove the sealing films.

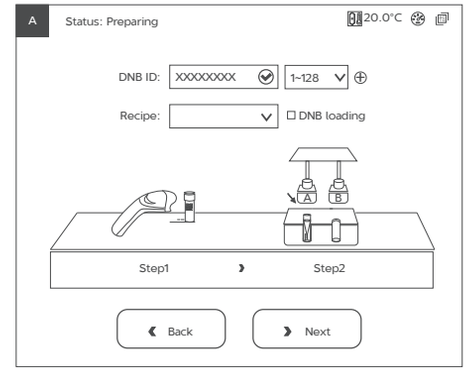
Performing sequencing using DNBSEQ-G400RS

1. Open the DNBSEQ-G400RS main interface, enter the username *user* and password *Password123*, and tap **Login**.

Tips

Check whether the remaining space of the storage drive is greater than 4.6 TB. If the remaining space is insufficient, clear history data by tapping **Device maintenance** > **Clear history data**.

2. In the main interface, tap **Sequence** to open the DNB ID entry interface.



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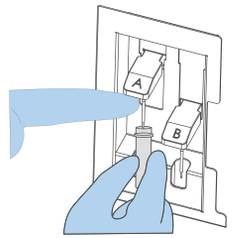
3. Tap the DNB ID box, enter the DNB ID manually. Select a proper barcode list from the list next to the DNB ID box.

Tips

The barcode list is provided by the technical support.

4. Tap the drop-down list of sequencing recipe and select **PE150+10+10**. If there is no corresponding barcode file in step 3, select **Customize** in the recipe list, and set related parameters in the customize page.
5. If you load DNBs by using the sequencer, perform the following steps:
   ① Open the reagent compartment door. Remove the cap of a 0.5 mL Microtube with DNB loading mixture, put the tube into the tube rack, slowly lower the DNB loading needle until the tip reaches the bottom of the tube.



   ② Tap the DNB loading box in the DNB ID entry interface.
6. After checking the recipe, tap **Next**.
7. Tap the sequencing cartridge box, manually enter the cartridge ID according to the SN printed on the cartridge label or use the barcode scanner to scan the cartridge barcode at the lower right corner of the sequencing reagent cartridge label.
8. Slide the prepared sequencing reagent cartridge into the compartment by following the direction printed on the cover until it stops. Close the reagent compartment door and tap **Next**.
9. Open the flow cell compartment door, Select the flow cell according to DNB loading method:
   - If you load DNBs by the sequencer, take the new flow cell out from the inner packaging and inspect to ensure that the flow cell is intact.
   - If you load DNBs by MGIDL-200H, take out the flow cell that is loaded with DNBs.
10. Tap the flow cell ID box, manually enter the ID or use the barcode scanner to scan in the information.
11. Hold the edge of the flow cell with both hands. Align the holes on the flow cell with the alignment pins on the flow cell stage. Gently slide the flow cell at an angle of 45° to the upper left corner to keep the flow cell aligned with the pins.
12. Press the flow cell attachment button. Press the left and right sides of the flow cell on the stage at the same time to ensure that the flow cell is properly seated on the stage.
13. Close the flow cell compartment door. Tap **Next**.
14. Check the information and tap **Start.** Tap **Yes** when prompted to begin sequencing.
15. When the sequencing is completed and the interface below appears, perform a wash. The device needs to be washed within 24 h after a PE sequencing. Refer to *DNBSEQ-G400RS High-throughput (Rapid) Sequencing Set Instructions For Use* to perform the post-run wash.

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Viewing sequencing results

1. In the sequencing interface of DNBSEQ-G400RS, tap  to view the first base report.
2. View the detailed sequencing report in the preset server directory.

Analyzing data using BOLT Low-pass WGS Software

Use BOLT Low-pass WGS Software (BOLT_Low-pass) to perform genotype imputation process on low-depth (0.1×-10×) whole genome sequencing data, and subsequent data analysis.

Tips

Refer to the BOLT Low-pass WGS Software manual for detailed analysis process.

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