· Part No.: H-020-001025-00
· Version: 1.0
Release date: November 2024

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 Genomic DNA Extraction Prepacked Kit (MGISP-NE384)	940-000974-00	4
Library Preparation set	MGIEasy Large-scale PCR-Free FS Library Prep Set for Low-pass WGS	940-002049-00	4
Sequencing Kit	MGIEasy Dual Barcode Circularization Kit (16 RXN)	1000020570	2
	DNBSEQ DNB Rapid Make Reagent Kit	1000028453	2
	DNBSEQ-T7RS High-throughput Sequencing Set (FCL PE150) V3.0	940-000268-00	4
	CPAS Barcode Primer 3 Reagent Kit	1000020834	4
Analysis Package	BOLT Low-pass WGS Software Analysis Package (96 reports)	970-000519-00	16

MGI equipment

Name	Cat. No.	Quantity
Automated Nucleic Acid Extractor MGISP-NE384RS	900-000358-00	1
DNA sequencing Library Preparation System MGISP-100RS	900-000206-00	1
MGISP-960RS High-throughput Automated Sample Preparation System-Configuration 5	900-000150-00	1
AlphaTool liquid handler	960-001486-00	2
MGIDL-T7RS DNB Loader	900-000134-00	1
Genetic Sequencer DNBSEQ-T7RS	900-000242-00	1
ZBOLT Pro Bioinformatics analysis accelerator	900-000460-00	1
Data Analysis Appliance MGI-ZTRON-LITE	900-000406-00	1

MGI consumables

Name	Cat. No.	Quantity
250 µL automated filter tips	1000000723	100 boxes
50 µL Transparent Automated Filter Tips	962-000077-00	16 boxes
200 µL Transparent Automated Filter Tips	962-000079-00	2 boxes
0.2 mL 96 well full-skirt PCR plate	091-000165-00	40 pcs
1.3 mL 96 Well U-bottom Deep-well Plate	1000004644	8 pcs
Break-away PCR Plate and Cover, 96-Well	100-000016-00	34 pcs
2 mL SC micro tube, PCR-PT	1000001553	8 tubes
0.5 mL SC micro tube, PCR-PT	1000001558	10 tubes
96-well tip comb	1000025661	16 pcs
24 Reagent Adaptor	962-000068-00	2 pcs
50 mL Reagent Reservoir	091-000488-00	2 pcs

User-supplied equipment and materials

Recommended equipment

Name	Brand	Cat. No.
Qubit fluorometers	Thermo Fisher	Q33238
FLUOstar Omega	BMG LABTECH	/
Thermal Cycler	BIO-RAD	T100
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, 500 mL	Invitrogen	AM9849
Absolute ethanol, Analytically pure	/	/
Isopropanol, Analytically pure	/	/

Extracting genomic DNA

Preparing consumables

Prepare consumables for a workflow of automated extraction on MGISP-NE384RS according to the following table, and place them at room temperature until use.

Name	Brand	Cat. No.	Quantity
96-well tips comb	MGI	1000025661	16 pcs

Preparing samples

Tips

For animal tissue and blood, it is recommended that the collected samples should 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.

1. Remove the prepacked plates from MGIEasy Genomic DNA Extraction Prepacked Kit (MGISP-NE384) (384 RXN). Centrifuge them in a plate centrifuge at 3000 rpm for 1 min to collect reagents to the bottom of the plates.
2. Proceed preparation based on the sample type:
   - Tissue sample: Add 10 mg to 20 mg of tissue sample into each well of the Buffer LS reagent plate.
   - Whole Blood sample: Add 200 µL of whole blood sample into a new 1.3 mL 96 deep-well plate, no need to add Buffer LS for blood sample.
3. Label the sample plates in the format of “Buffer LS + Proteinase K + Sample+ serial number of 1 to 16”.
4. Add 20 µL protease K to each sample well, and centrifuge at 3000 rpm for 1 min to collect reagents to the bottom of the plates.

Preparing reagents

1. Add absolute ethanol into Buffer W1 according to the label and seal it until use.
2. Add absolute ethanol into Buffer W2 according to the label and seal it until use.

Initializing MGISP-NE384RS

Tips

The software version of MGISP-NE384RS should be V1.5.0.126 or above.

1. Turn on the MGISP-NE384RS and computer, double-click the icon of MGISP-NE384 to run the software.
2. Select **User** in the username list, input the password *123456*, and set the mode to **Real**. Click **LOGIN**.
3. Click **Initialize** on the top of the interface to start initializing.
4. Select **Clean** in the main interface, close the door, and click **Start**. The default duration is 20 min. You can also set the time as required.

Performing automated gDNA extraction

1. Click **Workflow** in the main interface, set **Script** according to the table below:

Script	JB-A22-010 A1.MGIEasy_Genomic_DNA_Extraction_Prepacked_Kit_EN_SV1.0

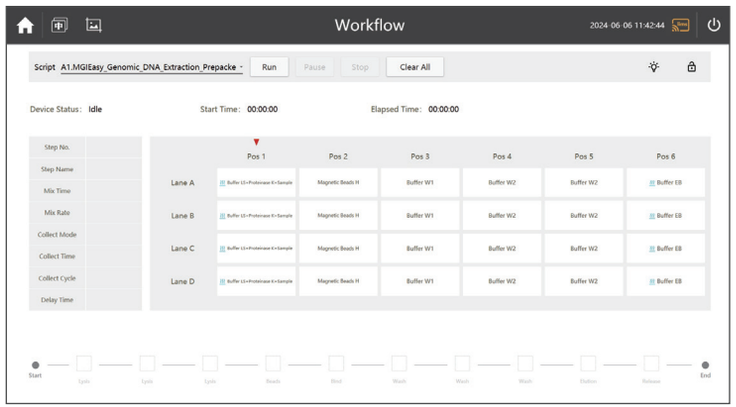
Tips

Each run of the script completes extracting 384 samples. 1536 samples require 4 runs.

2. Open the door of MGISP-NE384RS, place the samples, reagents, and consumables according to the figure and table below:

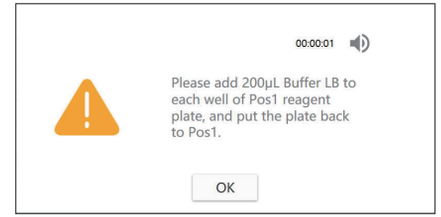
Tips

For the first run, place No.1 to No.4 Buffer LS plate on channels A, B, C, and D of Pos 1, and so on, until No. 13 to 16 is placed in fourth run.



Component	Position	Lane
Buffer LS + Proteinase K + Sample	Pos 1	A, B, C, D
Magnetic Beads H	Pos 2	A, B, C, D
Buffer W1	Pos 3	A, B, C, D
Buffer W2	Pos 4, Pos 5	A, B, C, D
Buffer EB	Pos 6	A, B, C, D

3. Place 96-well tips combs on 4 chanels of MGISP-NE384RS.
4. Click **Run**. Select the required lanes and tips comb in the pop-up window, click **OK** to start the program. The **Barcode** interface is displayed, click **Skip**. The automation experiment starts.
5. In around 20 min, the program step 2 ends, a prompt is displayed, as shown in the figure below. Take out 4 plates at Pos1, add 200 µL of Buffer LB to each sample well, and put back the plates to Pos 1. Click **OK** to continue the automation.



6. In around 15 min, the program step 3 ends, a prompt is displayed, as shown in the figure below. Take out 4 plates at Pos1, add 240 µL of isopropanol to each sample well, and put back the plates to Pos 1. Click **OK** to continue the automation.

7. After the program ends, immediately remove the Buffer EB plate (96-well square deep-well plate) from Pos 6, seal the plates, and label them as “Extraction product + date + serial number of 1 to 16”. Store them in 2 ℃ to 8 ℃ refrigerator.
8. Empty the operation deck. Transfer the 96-well tips combs and other consumables to the waste bag.
9. Repeat steps 2 to 8 until 4 runs are completed.

Tips

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

Quantifying and normalizing sample libraries

Preparing consumables

Name	Brand	Cat. No.	Quantity
250 µL automated filter tips	MGI	1000000723	20 boxes
UV-STAR PLATE, 96 WELL FLAT BOTTOM	Greiner bio-one	655801	17 pcs
Single Well Reagent Reservoir with 96-Bottom Troughs	AXYGEN	RES-SW96-HP	1 pcs
50 µL Transparent Automated Filter Tips	MGI	962-000077-00	16 boxes
200 µL Transparent Automated Filter Tips	MGI	962-000079-00	2 boxes
0.2 mL 96 well full-skirt PCR plate	MGI	091-000165-00	16 pcs
50 mL Reagent Reservoir	MGI	091-000488-00	2 pcs

Preparing samples and reagents

Name	Quantity	Requirement
Extraction products	16 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.

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Quantifying and normalizing sample

Tips

We recommend using 200 µL per reaction to quantify samples and using two dsDNA Broad Range Assay kit with 500 assays (250 mL Buffer available) to quantify 1536 samples.

1. Mix the extraction products (plate) thoroughly using 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 1800 reactions according to the table in the next step, mix them vigorously using a vortex mixer.
3. Transfer 4 mL of prepared quantification working solution to a new tube for later use in calibration standard quantification.

Reagent	Volume (mL)
dsDNA BR Buffer	358.2
dsDNA BR Reagent	1.8
Total	360

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 **MGISP-960** 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**.

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-A22-010 Part C MGISP-960 Library_Prep_384RXN_RV1.0_SV1.0
Script	JB-A22-010 C1.gDNA_Quantification_384RXN.py

Tips

Each run of the script completes preparing quantification solution for 384 samples, 1536 samples require 4 runs.

2. Add 89 mL of quantification working solution into the Single Well Reagent Reservoir with 96-Bottom Troughs.

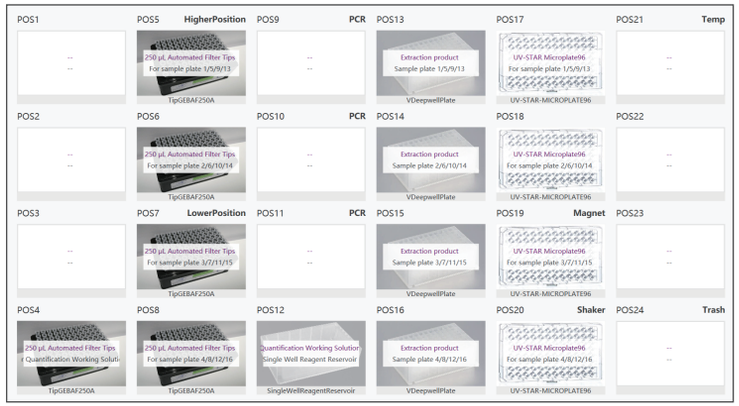
Tips

Before each script run, replace new solution for next script run.

3. Open the door of MGISP-960RS. Place the consumables according to the figure and the table below.

Tips

The extraction product plates need to be placed on POS13 to POS16 by ordinal, place No. 1 to No. 4 in the first run, and so on, until No.13 to No.16 are placed in the fourth run.



Name	Consumable	Position
New tips	250 µL automated filter tips	POS4 to POS8
Working Solution Buffer	Single Well Reagent Reservoir with 96-Bottom Troughs	POS12
Extraction products	96 Well Square Deep Well Plate	POS13 to POS16
New UV-STAR plate	UV-STAR PLATE, 96 WELL FLAT BOTTOM	POS17 to POS20

6. Close the door and click **Run** to start the automation.
7. In the pop-up window, select **4** in the drop-down list of plate number, click **Continue**.
8. After the process is completed, remove the UV-STAR plates at POS17 to POS20, label by serial number from 1 to 16, and transfer them to the FLUOstar Omega microplate reader.
9. Empty the operation deck and discard the used tips.
10. Seal the extraction plates with films, and store them in a 2 °C to 8 °C refrigerator.
11. Repeat running the script until all 4 runs are completed.
12. When all runs are finished, click ☰ on the left of the interface, open the Clean interface, 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.

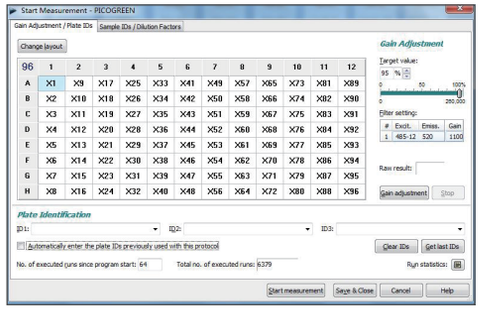
Tube	1	2	3	4	5	6	7	8
Add in	20 µL BR Standard 2	10 µL from tube 1	10 µL from tube 2	10 µL from tube 3	10 µL from tube 4	10 µL from tube 5	10 µL from tube 6	/
Nuclease-free H_2O	/	10 µL	10 µL	10 µL	10 µL	10 µL	10 µL	10 µL

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

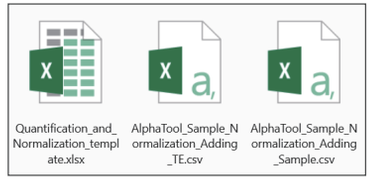
Quantifying dsDNA

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 in step 4.
3. For the same quantitative experiments, make sure **Gain** is set to the same value (recommended value: 1100).

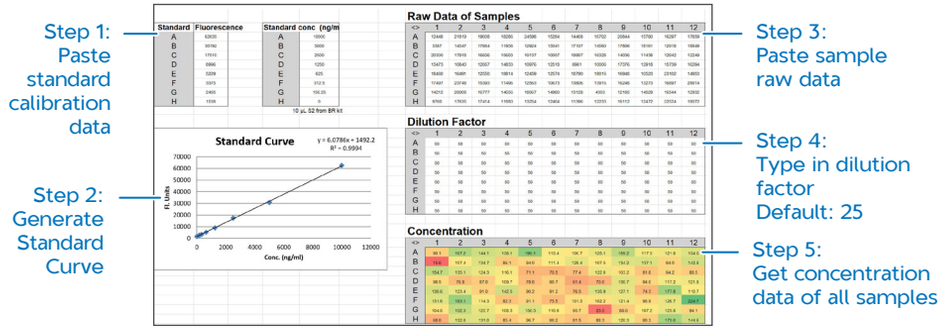
4. Put the calibration plate into the microplate reader. Click **Start measurement**.



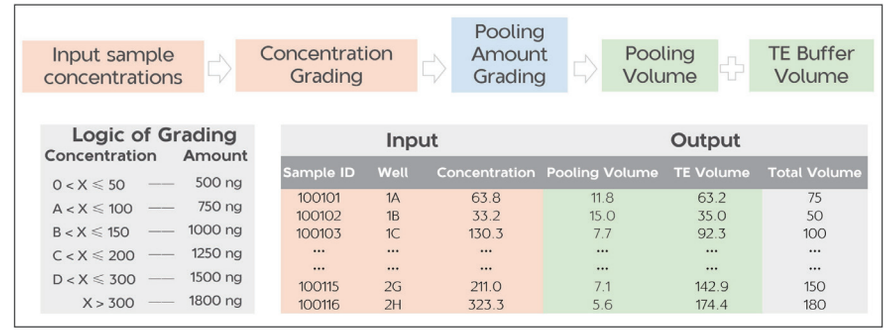
5. You can find three files in the support package which will be used in the normalization experiment. Open the file *Quantification_and_Normalization_template.xlsx*, sheet **Quantification**. Copy the measured standard calibration data to the corresponding area, and you will get the standard curve automatically. Make 16 copies of the template file and label them by serial number for later use.



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 apply them to [Raw data] zone of the *Quantification_and_Normalization_template.xlsx* copy with corresponding serial number. For more details, please follow 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, determine the amount of sample for normalization, and display the 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 16 copies of file *AlphaTool_Sample_Normalization_Adding_Sample.csv*, and *AlphaTool_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 are quantified.

Normalizing samples on AlphaTool

Tips

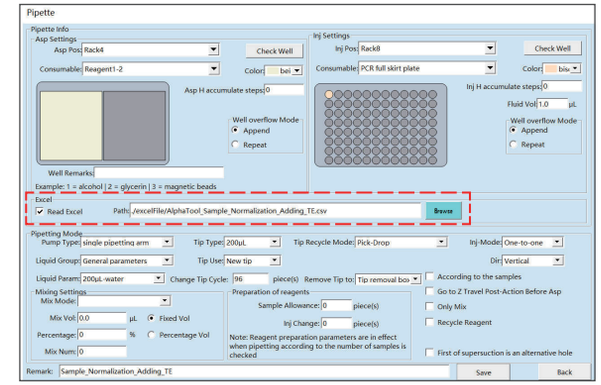
The software version of AlphaTool liquid handler should be V1.00.00.06 or above.

1. Turn on the AlphaTool and computer, double-click the icon **PipeRobot** to run the software.
2. Select **Admin** and click **Login**.
3. Click **Experiment** on the left of the interface, choose script **JB-A22-010 B1.AlphaTool_Sample_Normalization**.

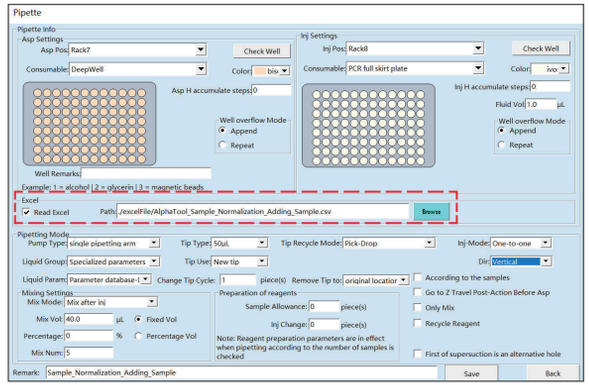
Tips

Each run of the script completes normalization of 96 samples, 1536 samples require two AlphaTools to run 8 times each.

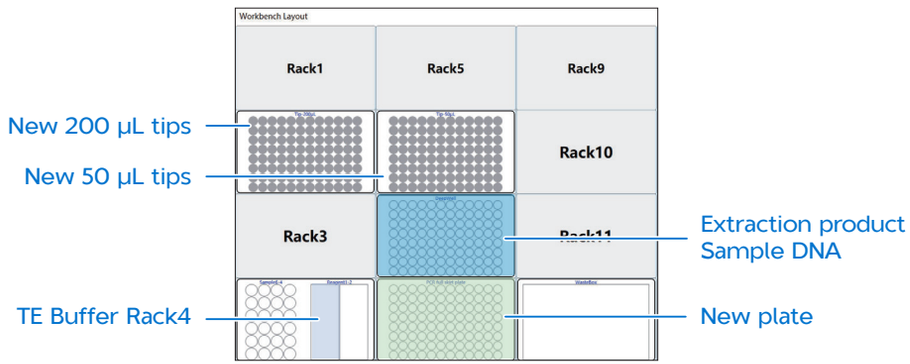
4. Double-click the script to open the **Experiment Editor** interface and double-click to open the action 1 **Sample_Normalization_Adding_TE**. Tick the box **Read Excel** and click **Browse** to select *AlphaTool_Sample_Normalization_Adding_TE.csv*. Click **Sure** and close the window.



5. Double-click the action 2 **Sample_Normalization_Adding_Sample**, tick the box **Read Excel**, and click **Browse** to select *AlphaTool_Sample_Normalization_Adding_Sample.csv*. Click **Sure** and close the window.



6. Click **Save** in the Experiment Editor interface, and then click **Back**.
7. Click **Main** on the left of the main interface to display the operation deck arrangement.
8. Place sample plate and consumables according to the figure and table below. Add 20 mL of TE buffer into the 50 mL Reagent Reservoir in Rack4.



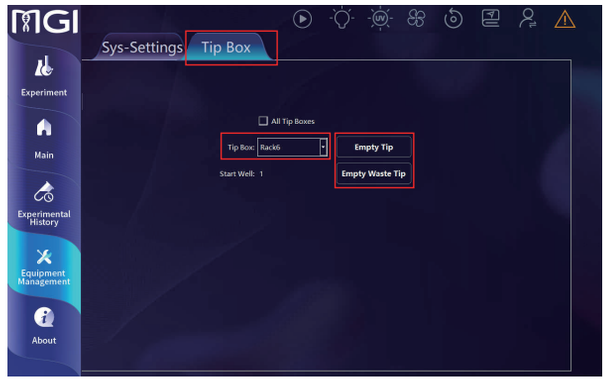
Name	Consumable	Position
New 200 µL tips	200 µL Transparent Automated Filter Tips	POS2
New 50 µL tips	50 µL Transparent Automated Filter Tips	POS3
Extraction product	96 Well Square Deep Well Plate	POS7
New plate	0.2 mL 96 well full-skirt PCR plate	POS8
TE Buffer	50 mL Reagent Reservoir	POS12

9. Click **Start** on top of the interface to start the program.
10. After the process is completed, remove the Normalized DNA plate on Rack8, seal the plate, label it as "Normalized DNA + date + serial number of 1 to 16" and store it in a 2 °C to 8 °C refrigerator.
11. Remove the extraction product plate on Rack7, seal the plate with adhesive PCR plate foils, and store it in a -25 °C to -15 °C freezer.
12. Replace the 50 µL tip box in Rack6, extraction product plate in Rack7, and new PCR plate in Rack8 on the operation deck.

Tips

The 200 µL tip box is sufficient for 8 runs and does not need to be replaced after each run.

13. Click **Equipment Management** on the left side of the main interface, and click **Needle**, select **Rack6** in drop-down list of **Tip box**, and click **Empty Waste Tip** to reset the tip box memory.



14. Repeat steps 4 to 13, until all rounds of normalization have been finished.
15. After the experiment is completed, clear up the operation deck and discard used tips to the waste area.
16. Click **Equipment Management** on the left side of the main interface, click **Needle**, tick the box of **All Tip Boxes**, and click **Empty Waste Tip** to reset all of the tip box memory.

Tips

If no experiment will be conducted on the day, clean the operation deck with 75% ethanol and close the instrument.

Preparing 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 384 samples, 1536 samples require 4 runs.

Preparing consumables

The consumables required to complete 4 runs of library preparation are as follows:

Name	Brand	Cat. No.	Quantity
250 µL automated filter tips	MGI	1000000723	72 boxes
0.2 mL 96 well full-skirt PCR plate	MGI	091-000165-00	20 pcs
1.3 mL 96 deep-well plate	MGI	1000004644	4 pcs

Tips

For a complete automated library preparation, the experimental operation is shown as follows:

Preparing samples and reagents

1. Take out the normalized DNA (4 plates) 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.

Tips

Take out the normalized DNA plates by ordinal. Use plates 1 to 4 for the first run, and so on, use plates 13 to 16 for the fourth run.

2. Take out the following reagents from the -25 ℃ to -15 ℃ freezer 30 min before use and thaw them according to the table below:

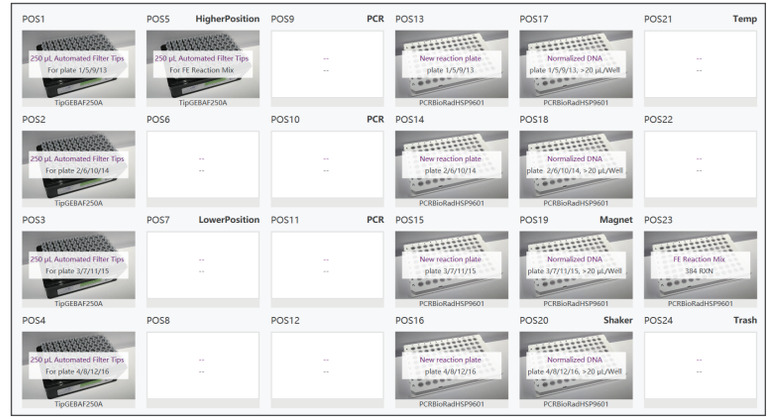
Name	Component	Thaw condition
MGIEasy Large-scale PCR-Free Library Prep Module for Low-pass WGS (Plate)	FE Reaction Mix	Ice box
	Ligation Reaction Mix	Ice box
	Stop Buffer	Room temperature
MGIEasy UDB PF Adapter Kit A	UDB Adapters A	Room temperature
MGIEasy UDB PF Adapter Kit B	UDB Adapters B	Room temperature
MGIEasy UDB PF Adapter Kit C	UDB Adapters C	Room temperature
MGIEasy UDB PF Adapter Kit D	UDB Adapters D	Room temperature

Performing fragmentation

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-A22-010 Part C MGISP-960 AgriHigh Low-pass WGS Solution_384RXN_RV1.0_SV1.0
Script	JB-A22-010 C2.MGIEasy_Large-scale_PCR-FREE_Library_Prep_384RXN_step1_FE_SV1.0.py

2. 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 to POS5
New reaction plates	0.2 mL 96 well full-skirt PCR plate	POS13 to POS16
Normalized DNA	0.2 mL 96 well full-skirt PCR plate	POS17 to POS20
FE Reaction Mix	0.2 mL 96 well full-skirt PCR plate	POS23

3. Close the door and click **Run** to start automatic pipetting.

Tips

There are two time points in the workflow where you can check the volume in the reaction plates by clicking **Pause**. After FE reaction mixture have been dispensed into reaction plates, the volume should be 7.5 µL per well. After Normalized DNA have been added to reaction plates, the volume should be 30 µL per well. If the reagent volume is insufficient, you can manually replenish it to the expected volume. After check, click **Resume** to continue the automation.

4. The mixture in the reaction plate is mixed by an automatic pipette. Each time a reaction plate is fully mixed, a window pops up to prompt you to remove the new reaction plate from the operation deck and seal the plate with adhesive PCR Plate Foil.
5. Place the reaction plate into a thermocycler and start a reaction according to the conditions in the table below:

Temperature	Time
80 °C (Heated lid)	On
30 °C	8.5 min
72 °C	20 min
4 °C	Hold

Tips

- During MGISP-960RS process, you can set thermocycler scheme in advance.
- For a 384 RXN reaction, you need 4 thermocyclers that fit the 0.2 mL 96-well full-skirt PCR plate.

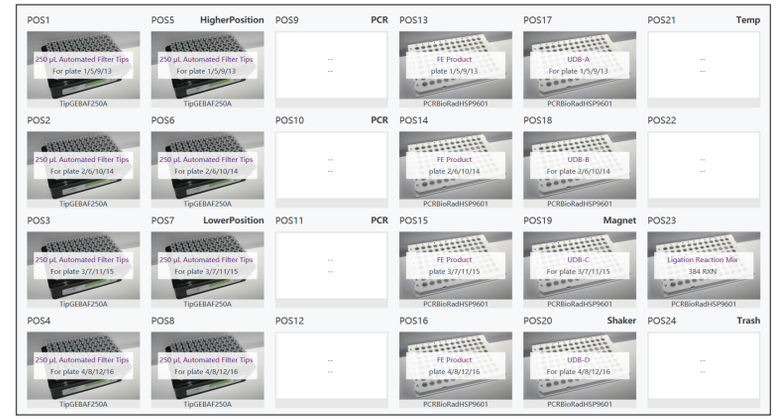
6. (Optional) If there is still DNA in the normalized DNA plate, you can seal the plates with adhesive PCR plate foils and store them in a -25 °C to -15 °C freezer as backup.
7. After the reaction is completed, remove the reaction plate from the thermocycler, label it as “FE product + serial number of 1 to 16”, and centrifuge the plate to collect the liquid exists on the inner wall.
8. Empty the operation deck of MGISP-960RS and discard the used consumables.

Performing adapter ligation

1. In the **Run Wizard** interface, set **Script** according to the table below:

Script	JB-A22-010 C3.MGIEasy_Large-scale_PCR-FREE_Library_Prep_384RXN_step2_Lig_SV1.0.py

2. Place the reagents and new consumables according to the figure and the table below.



Name	Consumable	Position
New tips	250 µL automated filter tips	POS1 to POS8
FE Product	0.2 mL 96 well full-skirt PCR plate	POS13 to POS16
UDB adapters	0.2 mL 96 well full-skirt PCR plate	POS17 to POS20
Ligation Reaction Mix	0.2 mL 96 well full-skirt PCR plate	POS23

3. Close the door and click **Run** to start automatic pipetting.

Tips

Each well of the FE Product plate will be added with 3 μL UDB adapters and 17.5 μL Ligation Reaction Mix. The total volume should be 50.5 μL.

4. The mixture in the reaction plate is mixed by an automatic pipette. Each time a reaction plate is fully mixed, a window pops up to remind you to remove the plate from operation deck and seal with adhesive PCR Plate Foils.
5. Place the reaction plate on a thermocycler and start a reaction according to the conditions in the table below:

Temperature	Time
30 °C (Heated lid)	On
25 °C	15 min
4 °C	Hold

Tips

Set thermocycler scheme in advance or during MGISP-960RS process.

7. After the reaction is completed, take out the Ligation (Lig) product plates and mark with “Ligation product”, and immediately proceed to the next step to terminate the ligation reaction.

Adding stop buffer and pooling the product

1. In the **Run Wizard** interface, set **Script** according to the table below:

Script	B-A22-010 C4.MGIEasy_Large-scale_PCR-FREE_Library_Prep_384RXN_step3_stop_pooling_SV1.0.py

2. Place the reagents and new consumables according to the figure and the table below.

Name	Consumable	Position
New tips	250 μL automated filter tips	POS1 to POS5
Ligation Product	0.2 mL 96 well full-skirt PCR plate	POS13 to POS16
New PCR plate	0.2 mL 96 well full-skirt PCR plate	POS17
New deep-well plate	1.3 mL 96 deep-well plate	POS22
Stop Buffer	0.2 mL 96 well full-skirt PCR plate	POS23

3. Close the door and click **Run** to start automatic pipetting.

Tips

Each well of the Lig Product plate will be added 10 μL of Stop Buffer. The total volume should be 60.5 μL. The pooling step will be conducted with equal volume (20 μL). 384 samples will be pooled into 8 different mixtures, and each mixture should have a volume of 960 μL.

4. After the process is completed, take out the Pooling Product plate from operation deck POS22 and seal with sealing films. Label it as "Pooling product + date + serial number of 1 to 4". The product can be stored in a 2 °C to 8 °C refrigerator for a month.

Tips

You will obtain one pooling product plate every time a complete library preparation of 384RXN is finished, label the plate as the order of library preparation.

5. Take out the Ligation Product plates from the operation deck, seal the plates with adhesive PCR plate foils, and store them in a -25 °C to -15 °C freezer as backup.
6. Empty the operation deck and discard used consumables.

Tips

After all 4 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

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**.

Purifying ligation products

Preparing consumables for purification

Name	Brand	Cat. No.	Quantity
250 µL automated filter tips	MGI	1000000723	2 boxes
1.3 mL 96 deep-well plate	MGI	1000004644	2 pcs
Break-away 8 Strips PCR Tubes and Caps	MGI	100-000016-00	8 pcs
2 mL SC microtube, PCR-PT	MGI	1000001553	2 tubes
Axygen 0.5 mL Thin Wall PCR Tubes with Flat Cap, Clear, Nonsterile	AXYGEN	RES-SW96-HP	34 pcs

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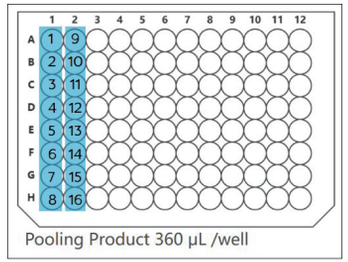
Preparing samples for purification

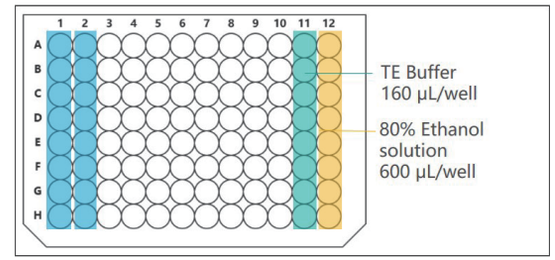
1. Take out the Pooling Product (4 plates) from the 2 °C to 8 °C refrigerator. Use a multichannel pipette to mix well the Pooling product.
2. Take out 2 new 1.3 mL 96 deep-well plates.
3. Transfer 360 µL of each product into the new 1.3 mL 96 deep-well plate, label the mark on the plate, such as 1-8, 9-16, and so on. Place the plates at room temperature for later use.



Pooling Product 360 µL /well

Preparing reagents for purification

1. Take out DNA Clean Beads and TE Buffer from MGIEasy DNA Clean Beads kit and put them at room temperature for 30 min before use. Mix them thoroughly by using a vortex mixer.
2. Prepare 25 mL of 80% ethanol solution by using absolute ethanol and Milli-Q water.
3. Take out 2 new 2 mL SC microtubes, add 1.8 mL of DNA Clean Beads into each tube, cap the tubes, and label them as “DNA Clean Beads”.
4. Take out two prepared pooling product plates, and add 160 µL of TE Buffer to wells in column 11 of each plate, add 600 µL of 80% ethanol solution to wells in column 12 of each plate. Label the plate as “Pooling product and purification reagent”.



Performing ligation product purification

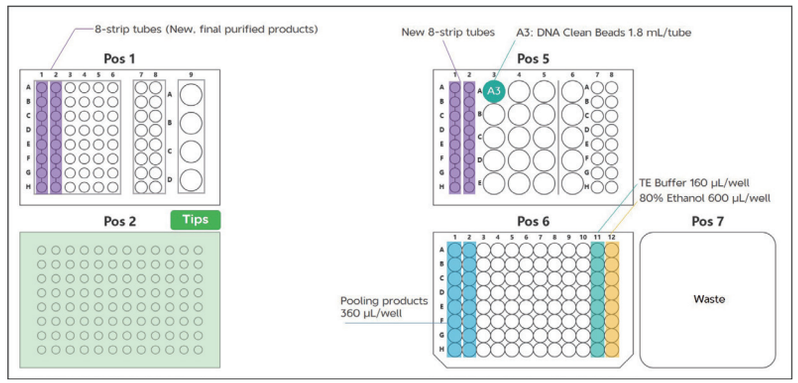
1. Click ☰ on the left of the interface, open the Run Wizard interface, and set **Solution** and **Script** according to the table below:

Solution	JB-A22-010 Part D MGISP-100 DNB_Making_RV1.0_SV1.0
Script	JB-A22-010 D1.Size_Selection_16RXN_SV1.0.py

Tips

Each run completes purification of 16 libraries, 32 libraries require two runs.

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



Name	Consumable	Position
New 8-strip tubes	Break-away 8 Strips PCR Tubes and Caps	POS1, Col 1, 2 POS5, Col 1, 2
New tips	250 µL automated filter tips	POS2
DNA Clean Beads	2 mL SC microtube, PCR-PT	POS5, A3
Pooling product and purification reagent	1.3 mL deep-well plate	POS6

3. Close the door, click **Run**.
4. After the process is completed, remove the purified products at Pos 1, Col 1 and Col 2 from the operation deck, cap the tubes, and label them as "Purified product + date", label each tube by serial number from 1 to 32. The volume should be 50 µL inside each tube.
5. Empty the operation deck, discard the used consumables.
6. Repeat steps 2 to 5 for the second run.
7. 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

Preparing consumables for circularization

1. Prepare consumables according to the following table,

Name	Brand	Cat. No.	Quantity
250 µL automated filter tips	MGI	1000000723	4 boxes
1.3 mL 96 deep-well plate	MGI	1000004644	2 pcs
0.2 mL 96 well full-skirt PCR plate	MGI	091-000165-00	2 pcs
Break-away 8 Strips PCR Tubes and Caps	MGI	100-000016-00	14 pcs
2 mL SC microtube, PCR-PT	MGI	1000001553	4 tubes
0.5 mL SC microtube, PCR-PT	MGI	1000001558	6 tubes
Axygen 0.5 mL Thin Wall PCR Tubes with Flat Cap, Clear, Nonsterile	AXYGEN	RES-SW96-HP	34 pcs

Preparing libraries for circularization

1. Take out 4 new 8-strip tubes and label each tube with serial numbers of 1-8, 9-16, 17-24 and 25-32. Take out the purified product and add 200 ng to the new 8-strip tube according to the serial number. If the yield of the post-ligation purified product is lower than 200 ng, take 48 µL of the purified product.

2. Assume one purified product has a concentration of *X* ng/μL, prepare the normalized product in the new 8-strip tubes 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.

Preparing circularization reagents

1. Take out 2 kits of MGIEasy Dual Barcode Circularization Kit (16 RXN), thaw the reagents according to the table below

Component	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 6 new 0.5 mL SC tubes and prepare the following three reagents in two batches. Be sure to transfer the reagents on an ice box, cover the tubes after preparation, vortex to mix, and then centrifuge briefly.

Label	Reagent	Volume (μL)
Circularization reaction mix	Dual Barcode Splint Buffer	220.8
	DNA Rapid Ligase	9.6
Digestion reaction mix	Digestion Buffer	27
	Digestion Enzyme	50
Digestion Stop Buffer	Digestion Stop Buffer	144

3. Take out 4 new 2 mL SC microtubes, add 1.5 mL of DNA Clean Beads into each tube, cap the tubes, and label them as “DNA Clean Beads”.
4. Take out 2 new 1.3 mL 96 deep-well plate, and add 160 μL of TE Buffer to wells in column 11 of each plate, add 600 μL of 80% ethanol solution to wells in column 12 of each plate. Label the plate as “Purification reagent for circularization”.

Circularizing library on MGISP-100RS

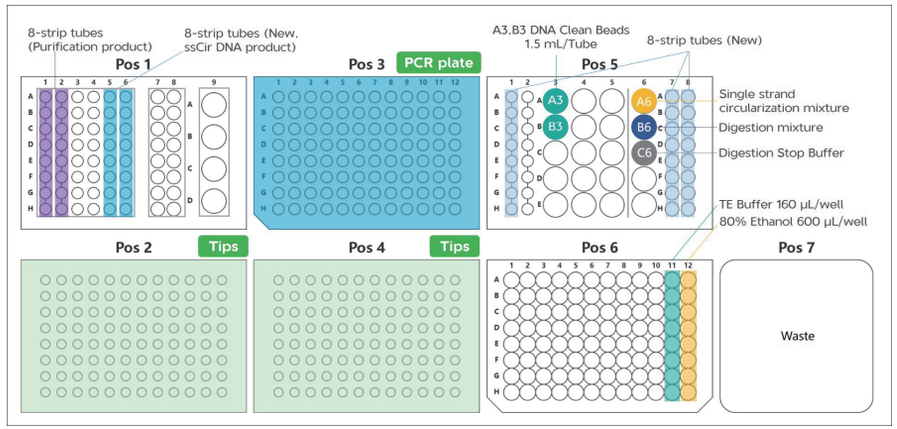
1. In the Run Wizard interface, set **Solution** and **Script** according to the table below:

Solution	JB-A22-010 Part D MGISP-100 DNB_Making_RV1.0_SV1.0
Script	JB-A22-010 D2.Circularization_Digestion_Purification_16RXN_SV1.0.py

Tips

Each run completes 16 circularized libraries, 32 libraries require two runs.

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



Name	Consumable	Position
Purification product	Break-away 8 Strips PCR Tubes and Caps	Pos1, Col 1, 2
New 8-strip tubes	Break-away 8 Strips PCR Tubes and Caps	Pos1, Col 5, 6 Pos 5, Col. 1, 7, 8
New tips	250 µL automated filter tips	Pos2, Pos4
New PCR plate	0.2 mL 96 well full-skirt PCR plate	Pos3
DNA Clean Beads	2 mL SC microtube	POS5, A3, B3
Circularization reaction mix	0.5 mL SC microtube	Pos 5, A6
Digestion reaction mix	0.5 mL SC microtube	Pos 5, B6
Digestion Stop Buffer	0.5 mL SC microtube	Pos 5, C6
Purification reagent for circularization	0.5 mL SC microtube	Pos5, C6

3. Close the door and click **Run**.
4. After the program ends, take out the DNBs in the 8-strip tube at POS1, Col 5 and 6, cap the tubes and label them as “Circularization product + date”, label each tube by serial number from 1 to 32, and store them in 4 °C refrigerator for later use.The volume of each tube of solution should be 23 µL.
5. Empty the operation deck and discard the used consumables.
6. Repeat steps 2 to 5 for the second run.
7. Quantify 32 circularization products according to the instructions of Qubit ssDNA Assay Kit. The required concentration should be no less than 0.6 ng/µL.

Making DNBs for DNBSEQ-T7RS

Preparing consumables for making DNBs

1. Prepare consumables according to the following table,

Name	Brand	Cat. No.	Quantity
250 µL automated filter tips	MGI	1000000723	2 boxes
0.2 mL 96 well full-skirt PCR plate	MGI	091-000165-00	2 pcs
Break-away 8 Strips PCR Tubes and Caps	MGI	100-000016-00	8 pcs
2 mL SC microtube, PCR-PT	MGI	1000001553	2 tubes
0.5 mL SC microtube, PCR-PT	MGI	1000001558	4 tubes
Axygen 0.5 mL Thin Wall PCR Tubes with Flat Cap, Clear, Nonsterile	AXYGEN	RES-SW96-HP	34 pcs

Preparing reagents for making DNBs

1. Take out 2 kits of DNBSEQ DNB Rapid Make Reagent Kit, thaw the reagents under certain conditions according to the table below

Component	Thaw condition
Low TE Buffer	Room Temperature
Make DNB Buffer	Ice box
Make DNB Enzyme Mix II	Ice box
Make DNB Enzyme Mix II (LC)	Ice box
Stop DNB Reaction Buffer	Room Temperature

2. Take out 2 new 2 mL SC microtubes, transfer following thawed reagents into each tubes respectively, and label them as “Make DNB Enzyme Mix”, cap the tubes and put them on ice box until use.

Reagent	Volume (µL)
Make DNB Enzyme Mix II	800
Make DNB Enzyme Mix II (LC)	32
Total (µL)	832

3. Take out 4 new 0.5 mL SC microtubes, label 2 tubes with “Make DNB Buffer”, label 2 tubes with “Stop DNB Reaction Buffer”. Transfer the reagents of the volume listed in the following table into the tubes, cap the tubes, and place them at room temperature until use.

Reagent	Volume (µL)
Make DNB Buffer	384
Stop DNB Reaction Buffer	192

Preparing libraries

1. The products obtained from the purification step need to be normalized to the same mass. The recommended input amount of circularization product for DNB is 16 ng.
2. Assume one circularization product has a concentration of *Y* ng/µL, prepare the normalized product in a new 8-strip tube according to the table below.

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

3. Mix the 8-strip tube with a vortex mixer, centrifuge to collect any liquid exist on the inner wall to the bottom. Then put the tubes on ice until use.

Making DNBs on MGISP-100RS

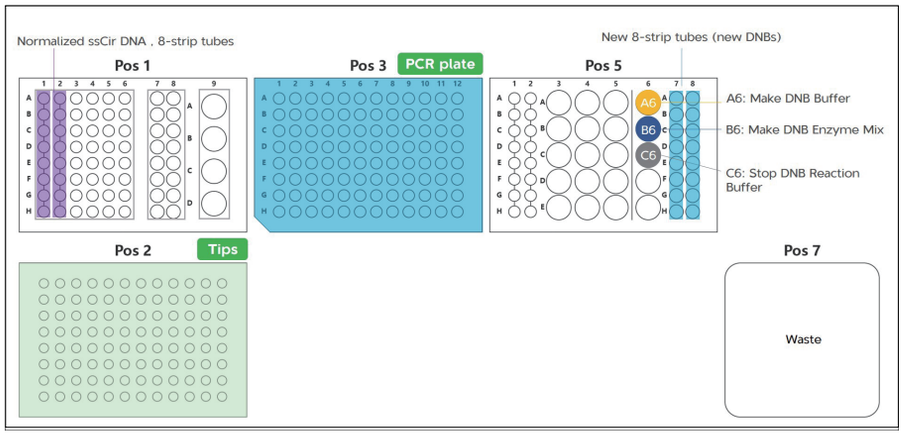
1. In the Run Wizard interface, set **Solution** and **Script** according to the table below:

Solution	JB-A22-010 Part D MGISP-100 DNB_Making_RV1.0_SV1.0
Script	JB-A22-010 D3.DNB_Making_for_DNBSEQ-T7_16RXN_SV1.0.py

Tips

Each run completes preparing DNBs for 16 libraries, 32 libraries require two runs.

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



Name	Consumable	Position
Normalized circularization products	8-strip tubes	Pos1, Col 1, 2
New 8-strip tubes	8-strip tubes	Pos5, Col 7, 8
New tips	250 µL automated filter tips	Pos2
New PCR plate	0.2 mL 96 well full-skirt PCR plate	Pos3
Make DNB Buffer	0.5 mL SC microtube	Pos5, A6
Make DNB Enzyme Mix	2 mL SC microtube	Pos5, B6
Stop DNB Reaction Buffer	0.5 mL SC microtube	Pos5, C6

3. Close the door and click **Run**.
4. After the program ends, take out the DNBs in the 8-strip tube at POS5, Col 7 and 8, cap the tubes and label them as “DNB + date”, label each tube by serial number from 1 to 32, and store them in 4 ℃ refrigerator for later use.
5. Empty the operation deck and discard the used consumables.
6. Repeat steps 2 to 5 for the second run.
7. Click ☰, open the **Pre-post Clean** interface and select **Post-clean**, clean the operation deck with dust-free paper moistened with 75% ethanol, close the door, and click **Start**.
8. Quantify 32 DNB products according to the instructions of Qubit ssDNA Assay Kit. The required concentration should be no less than 5 ng/µL.

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Performing sequencing and analysis

Tips

The sequencing of 1536 samples requires 4 sequencing runs. The following is guide for one run as an example.

Preparing DNB loading system

Pooling DNBs with equal amount

1. Prepare DNB pooling mixture from 8 DNBs in one 8-strip tube, ensuring that barcode corresponding to the library is not repeated.
2. Calculate the volume required for each DNB based on the DNB concentration, ensuring that they are pooled in equal amounts.

Tips

For T7 FCL PE150 sequencing, a total DNB volume of 300 µL is required.

3. Assume that the required amount for each sample is *Y* (400 ng to 600 ng as recommended) and the concentration of the DNBs are C_1, $C_2 \cdots C_8$ respectively.
   ① Calculate the relative volume for each DNB $V_n=Y/C_n$.
   ② Calculate the total relative volume $V=V_1+V_2+\cdots+V_8$.
   ③ Ensure the total volume V is larger than 300 µL. If V is less than 300 µL, you may increase the required amount of Y and recalculate.
4. Aspirate the DNBs with corresponding volume to a new 0.5 mL SC microtube and mix thoroughly.

Tips

It is important to use wide bore tips to gently pipette and mix DNB solution. Do not centrifuge, vortex or shake the tube.

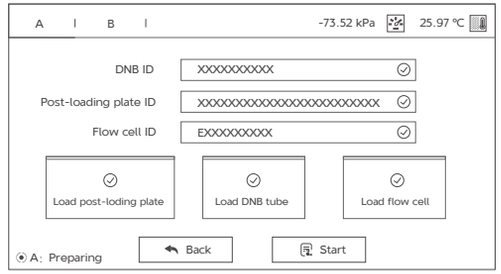
Preparing for DNB loading

1. Take the Rapid Post Load Plate and DNB Load Buffer IV out of the DNB Rapid Load Reagent Kit, thaw the Rapid Post Load Plate in a water bath at room temperature for 2 h, and thaw DNB Load Buffer IV at room temperature for approximately 30 min.
2. Take a new 0.5 mL SC microtube and add reagents according to the table below. Gently pipette the mixture 8 times.

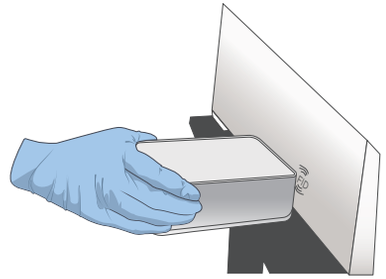
Adding order	Component	Volume (µL)
1	DNB	300
2	DNB Load Buffer IV	150

Loading DNBs on MGIDL-T7RS

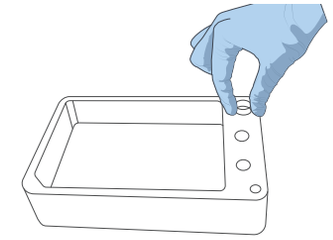
1. Take out the flow cell from the 2 °C to 8 °C refrigerator and equilibrate it to room temperature for 30 min to 24 h.
2. Start the MGIDL-T7RS program, enter the username and password, and then tap **Login** to open the main interface.
3. Tap **Loading** to open the information input interface. Enter the DNB ID.



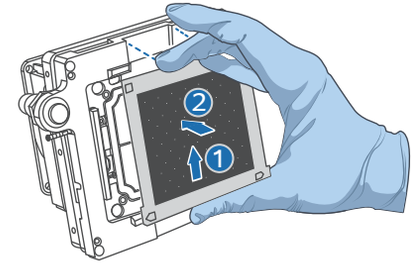
4. Align the post load plate to the RFID scanning area to identify the plate ID. Remove the outer package of the flow cell and identify the flow cell ID in the same way.

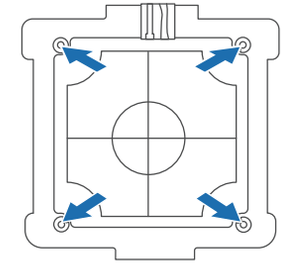


5. Remove the seal of the post load plate and add 4 mL of 0.1 M NaOH into well No.11.
6. Place the prepared post load plate on the plate tray.
7. Place the 0.5 mL microtube containing DNB loading mix into the DNB tube hole.



8. Hold both sides of the flow cell and install it onto the stage, press the attachment button to load the flow cell.





9. Tap **Start** and select **Yes**. The loading process takes 2 h.
10. After the loading is completed, press the attachment button and remove the loaded flow cell from the stage.
11. Install the washing flow cell onto the stage. Tap **Post-wash** to start MGIDL-T7RS wash.

Preparing the sequencing reagent

Preparing the sequencing reagent cartridges

1. Prepare the DNBSEQ-T7 High-throughput Sequencing Set (FCL PE150) V3.0.

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2. 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 ℃ to 8 ℃ refrigerator 24 h in advance.
3. Take the dNTPs Mix V 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.
4. After thawing, invert these reagents 6 times and place them on ice until use.
5. 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. 8, No. 9 and No. 10.

Name	Volume (mL)	Well
dNTPs Mix V	3.74	No.9
Sequencing enzyme Mix	3.74	
dNTPs Mix II	11.22	No.10
Sequencing enzyme Mix	3.74	
MDA Enzyme Mix	0.6	No.8
MDA Reagent	4.2	
1 μM AD153 Barcode Primer 3	3.5	No.3

Tips

Take well No. 9 as an example, add the dNTPs Mix V and Sequencing enzyme Mix into a new 15 mL centrifuge tube, invert the tube 6 times, and then add the mixture into well No.9.

6. Seal well No.9 and No. 10 with sealing films. Hold both sides of the sequencing cartridge on the table, shake it clockwise 20 times and then counterclockwise 20 times to mix the reagents thoroughly, and then remove the sealing films.

Preparing the washing cartridges

1. Take the Washing Cartridge out of the sequencing set, shake the cartridge clockwise 10 times and then counterclockwise 10 times.
2. Add 45 mL of 0.1 M NaOH into well No.2.

Preparing the pure water container

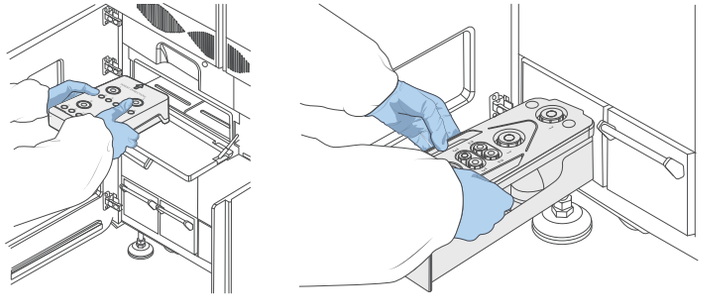
Fill the pure water container with laboratory-grade water. The pure water consumption for 4 flow cells of FCL PE150 is 18 L.

Performing sequencing using DNBSEQ-T7RS

1. Open the DNBSEQ-T7RS main interface, enter the username *research* and password *Admin123*, and click **Login**.
2. Place the sequencing cartridge and wash cartridge into the corresponding compartment, and then close the compartment door. The built-in RFID scanner will automatically identify the cartridge IDs and display them in the corresponding text box.

Tips

Enter the ID manually if the RFID scanner fails to identify the ID. Ensure that the ID is correct.

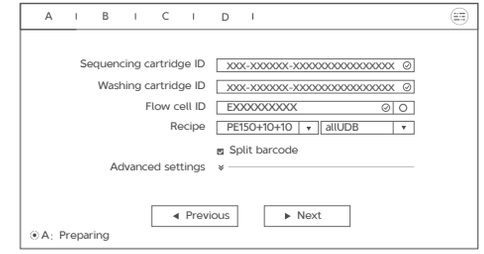


3. Choose the corresponding side in the main interface, tap **Sequencing**, and select **New run**,
4. Clean the loaded flow cell with a canned air duster to ensure that there is no visible dust on the surface and back of the flow cell. Put the flow cell on the flow cell drive and touch the flow cell drive control button to load the flow cell into the device. The built-in RFID scanner will automatically identify the flow cell ID and display it in the corresponding text box.

Tips

- When using the canned air duster to remove the dust on the back of the flow cell, avoid blowing air into the inlet holes.
- Enter the flow cell ID manually if the RFID scanner fails to identify the ID. Ensure that the ID is correct.

5. Choose the sequencing recipe **PE150+10+10**, select the barcode recipe **allUDB**, and tick **Split barcode**.



6. After confirming that all the information is correct, tap **Start**, and select **Yes**. It takes a few minutes for the sequencer to perform a self check. When the self check is complete, sequencing starts.

Viewing sequencing results

1. In the sequencing interface of DNBSEQ-T7RS, tap F5 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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