• Part No.: H-020-001063-00
• Version: 1.0
• Release date: Oct. 2024

Overview

The quick operation guide is intended to guide you to use DNBelab-D4RS Pa-SNPs Library Prep Set to perform paternity test.

The material information is as follows:

Type	Name	Remark
Equipment	DNBelab-D4RS Digital sample preparation system	Cat. No.: 900-000822-00
	DNBSEQ-G99RS Genetical Sequencer	Cat. No.: 900-000607-00
	Pipette (10 μL/20 μL/200 μL)	General laboratory brand
	Mini vortex mixer	General laboratory brand
	Mini centrifuge	General laboratory brand
Software	FGID Forensic DNA Analysis System	Cat. No.: FGI20230002
Reagent	DNBelab-D4RS Pa-SNPs Library Prep Set	Cat. No.: 940-002477-00
	DNBSEQ-G99RS High-throughput Sequencing Reagent Set (FCL SE100/PE50)	Cat. No.: 940-001268-00
Consumable	Filtered tip	General laboratory brand
	Tip	General laboratory brand
	0.2 mL PCR tube	General laboratory brand
	Wide-bore tip	BIOFOUNT, BI-200KX-H or AXYGEN T-205-WB-C

Preparing reagents

1. Take out magnetic beads (A567-Pa-BE) from DNBelab-D4RS Pa-SNPs Genotyping Kit (Box2) (Cat. No.: 940-002108-00) and equilibrate it to room temperature. Mix other reagents by using the mini vortex mixer, centrifuge them briefly and place at normal temperature for later use.
2. Take out all the reagents from DNBelab-D4RS Pa-SNPs Genotyping Kit (Box1) (Cat. No.: 940-002106-00), thaw them on ice, and tap the tube eight times (twice for C6-Pa-DNB 2). Centrifuge the reagents briefly and place them on ice for later use.
3. Use one of the following groups of BC:

Group	Reagent	Barcode list (1-128)	Group	Reagent	Barcode list (1-128)
1	B5-Pa-BC 1	1	3	B5-Pa-BC 9	9
	B6-Pa-BC 2	2		B6-Pa-BC 10	10
	B7-Pa-BC 3	3		B7-Pa-BC 11	11
	B8-Pa-BC 4	4		B8-Pa-BC 12	12
2	B5-Pa-BC 5	5	4	B5-Pa-BC 13	13
	B6-Pa-BC 6	6		B6-Pa-BC 14	14
	B7-Pa-BC 7	7		B7-Pa-BC 15	15
	B8-Pa-BC 8	8		B8-Pa-BC 16	16

CAUTION

- It is recommended to physically separate working areas in the laboratory. It is recommended to prepare samples and prepare sample preparation cartridge by using the filtered tips in the pre-PCR area and prepare DNBs by using the DNBelab-D4RS in the post-PCR area. By doing this, the contamination can be prevented and the accuracy of the results can be guaranteed.
- Do not place C6-Pa-DNB 2 at room temperature. Do not touch the tube wall for a prolonged time.
- Protect C7-Pa-QU away from light.

Preparing samples

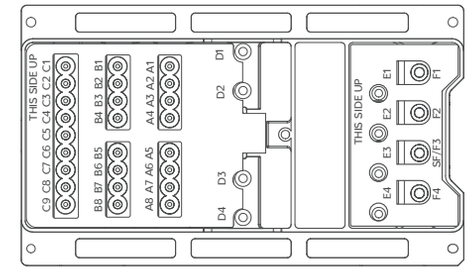
Tips

- It is recommended that the concentration of gDNA samples should be greater than or equal to 0.2 ng/μL and the concentration of cfDNA samples should be greater than or equal to 0.4 ng/μL.
- It is recommended to extract a fresh cfDNA before the library preparation.
- It is recommended to store the cfDNA at -20 ℃ or -80 ℃ and use it within a week. If the cfDNA is stored more than a week, it is recommended to re-extract the cfDNA by using the plasma stored at -80 ℃ or the fresh plasma.

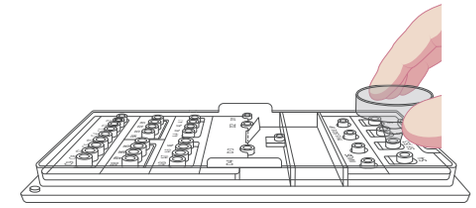
1. Separately aspirate and dispense 4 ng cfDNA of fetus and 2 ng gDNA of father and mother into three 0.2 mL PCR tubes, and add A123-Pa-EB until the total volume reaches 10 μL. Use a new tip when aspirating reagents every time.
2. Sample mixture: add 20 μL A567-Pa-BE into the 10 μL sample in the step 1. Mix the mixture three times by using the mini vortex mixer (3 seconds each), centrifuge the mixture briefly to collect the mixture at the bottom of the tube, and place it at room temperature for later use.

Filling the sample preparation cartridge

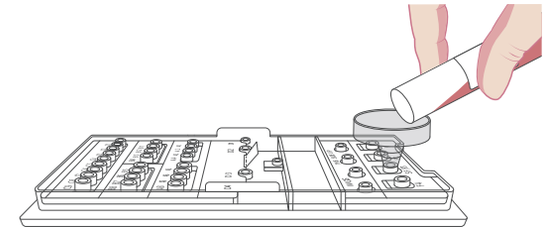
1. Place the instruction card onto the sample preparation cartridge.



2. Insert the funnel into well SF/F3.



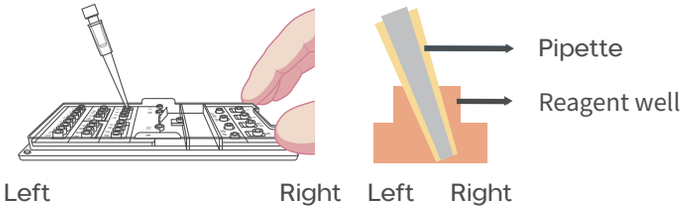
3. Uncap the sealing fluid tube, and empty the sealing fluid into the opening of the funnel at one time.



4. Remove the funnel when the sealing fluid has spread throughout the cartridge. Ensure that no bubbles exist in the cartridge before going to the next step.
5. Add the reagents into the cartridge according to the table below. Use new tips to add the reagents every time.

⚠ CAUTION

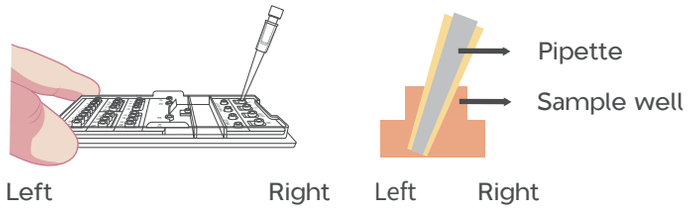
- The loading order for the reagent is well A, well B, and well C.
- Insert the tip vertically into the well, tilt it about 30° towards the intermediate reaction zone, then dispense the liquid.



- Avoid generating bubbles.

Name	Cap color	Volume	Well	Storage temperature
A123-Pa-EB		30 µL	A1, A2, A3	2 °C to 8 °C
A4-Pa-DNB 1		30 µL	A4	-25 °C to -15 °C
A567-Pa-BE		30 µL	A5, A6, A7	2 °C to 8 °C
B1-Pa-DNBB		15 µL	B1	-25 °C to -15 °C
B2-Pa-PCR 1		15 µL	B2	-25 °C to -15 °C
B4-Pa-ST		15 µL	B4	-25 °C to -15 °C
B5-Pa-BC 1		6 µL	B5	-25 °C to -15 °C
B5-Pa-BC 5				
B5-Pa-BC 9				
B5-Pa-BC 13				
B6-Pa-BC 2		6 µL	B6	-25 °C to -15 °C
B6-Pa-BC 6				
B6-Pa-BC 10				
B6-Pa-BC 14				
B7-Pa-BC 3		6 µL	B7	-25 °C to -15 °C
B7-Pa-BC 7				
B7-Pa-BC 11				
B7-Pa-BC 15				
B8-Pa-BC 4		6 µL	B8	-25 °C to -15 °C
B8-Pa-BC 8				
B8-Pa-BC 12				
B8-Pa-BC 16				
C6-Pa-DNB 2		6 µL	C6	-25 °C to -15 °C
C7-Pa-QU		6 µL	C7	2 °C to 8 °C

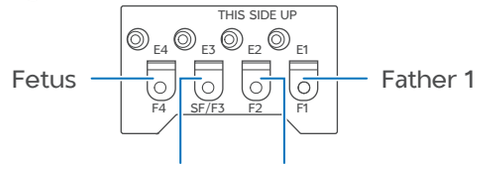
6. Separately add 30 µL of different sample mixtures into F1, F2, F3, and F4 wells.



Tips

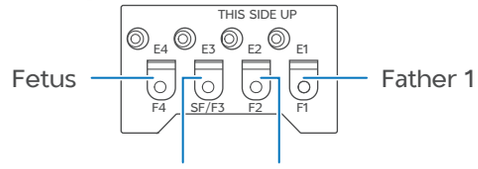
The order of samples mixtures in wells F1-F4 is father, mother, and fetus.

- If there are more than one father's sample, add the sample mixtures into F1 to F4 according to the figure below.



Mother 1 Father 2

- If there is only one father's sample, add the sample mixtures into F1 to F4 according to the figure below.



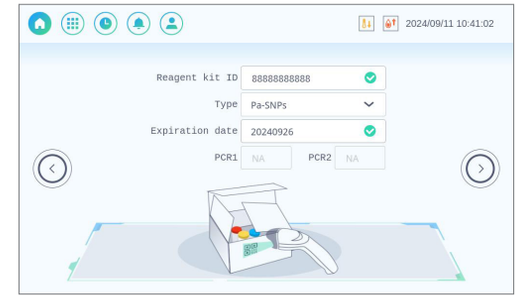
Fetus Mother 1

7. Ensure that all the reagents and samples have been added into preparation cartridge and covered by the sealing fluid.
8. Remove the instruction card.

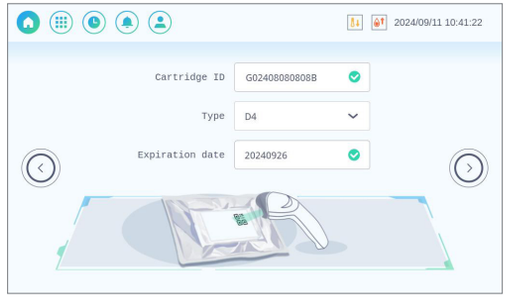
Making DNBs

1. Turn the power switch on the back of the DNBelab-D4RS Digital sample preparation system to the **ON** position. The device starts self-check.

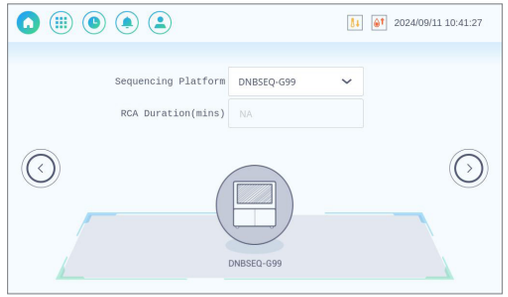
   After completion, the login interface is displayed.
2. Input the username (*admin*) and default password (*123456*) in the login interface and tap **Login** to go to the main interface.
3. Tap to start the workflow.
4. Scan the QR code on the packaging of the Prep set by using the scanner to input the information. After completion, tap to go to the next step.



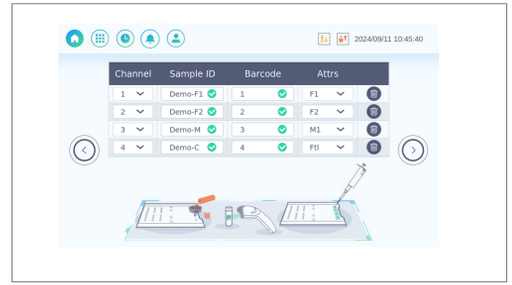
5. Scan the QR code on the package of the sample preparation cartridge by using the scanner to input the information. After completion, tap to go to the next step.



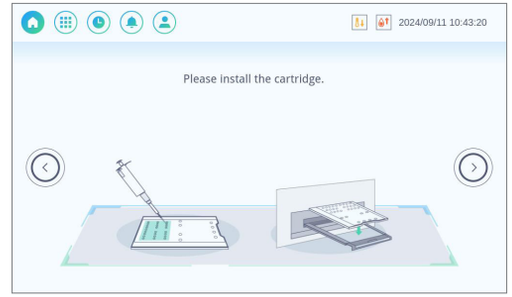
6. Tap the **Sequencing platform** list, and select **DNBSEQ-G99**. After completion, tap ⓘ to go to the next step.



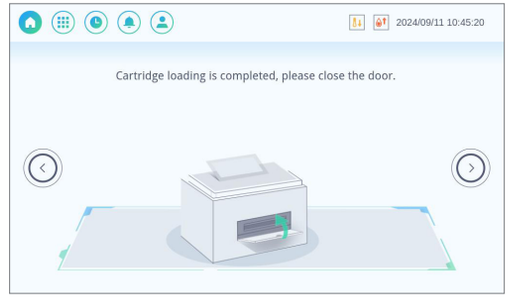
7. Fill in with sample name, barcode, and sample attribute. After completion, tap ⓘ to go to the next step, and tap ⓘ again to go to the next step. The compartment door and cartridge rack open automatically.



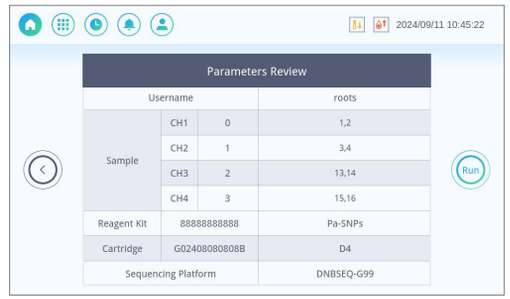
8. Place the prepared sample preparation cartridge onto the cartridge rack according to the on-screen instruction. After completion, tap ⓘ to go to the next step.



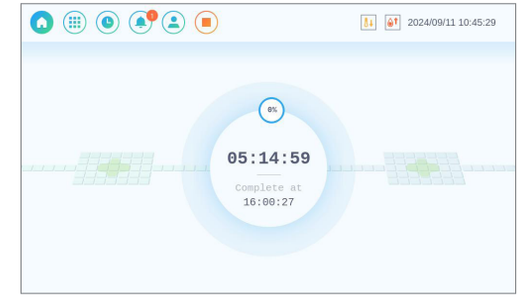
9. Ensure that the cartridge is installed. Tap **Yes**. The Cartridge loading interface is displayed, and the cartridge rack withdraws automatically.
10. When you are prompted that the cartridge loading is completed, manually close the compartment door. After completion, tap ⓘ to go to the next step.



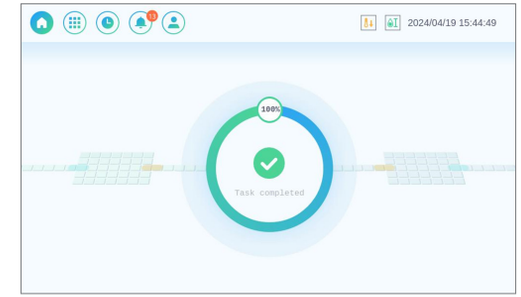
11. Check the information. Ensure that the information is correct, and tap (Run).



During running, the interface displays the remaining time and completion time.

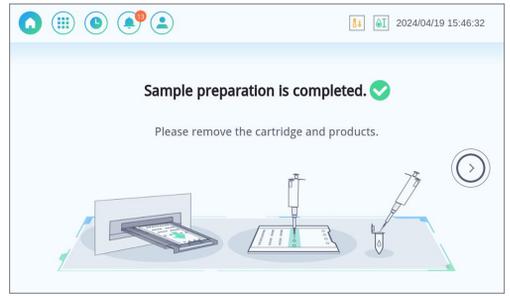


12. After library preparation is completed, tap (✓). A library preparation report is displayed.



The report displays the DNB concentration and recommended input volume. The DNB concentration should be greater than or equal to 8 ng/μL.

13. Tap ⟩ to go to the next step. The compartment door and cartridge rack open automatically.
14. Remove the sample preparation cartridge, and place it on a level and clean surface for later use.

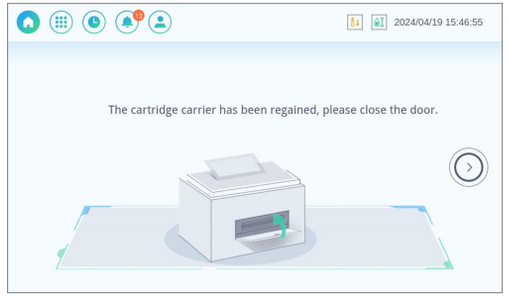


15. Aspirate DNBs:
    ① Adjust the pipette range to 50 μL.
    ② The wells D1 to D4 correspond to the well F1 to F4. Insert four wide-bore pipette tips vertically into the wells D1-D4 of the sample preparation cartridge, separately aspirate the DNBs from the wells, and transfer them into four new PCR tubes respectively. Ensure that the tips remain tightly closed when inserted into the wells.
    ③ Remove the upper oil by using new 200 μL or 10 μL sharp pipette tips until the transparent water phase (the DNB product) in the middle exposes.
    ④ Take out a piece of DNB separation paper and place it on the laboratory bench.
    ⑤ Adjust the pipette range to 10 μL, insert a new 10 μL sharp pipette tip vertically into the DNBs, and aspirate 10 μL DNBs. During aspirating, lower the tip when the DNB level declines. Avoid aspirating the oil phase. Use a new tip when aspirating the DNBs every time.
    ⑥ Transfer the DNBs onto the DNB separation paper. If there is no wet spot on the paper, proceed to step ⑦. If there is wet spot on the paper, transfer the DNBs to other clean position of the paper by using a new wide-bore tip until no obvious wet spot is left on the paper.
    ⑦ Aspirate the DNBs without oil and transfer them into four new PCR tubes (do not transfer them into one tube) by using four new 10 μL sharp pipette tip.
    ⑧ According to the mix volume on the interface of DNBelab-D4RS, aspirate the corresponding volume of DNBs, transfer them into a new 1.5 mL centrifuge tube, and gently mix the DNBs twice by using a new wide-bore pipette tip. The mixture volume should be greater than or equal to 7.5 μL. If the volume is less than 7.5 μL, increase the volume of DNBs in equal proportion. The DNB mixture and the remaining DNBs can be stored in a 4 ℃ refrigerator.

Tips

- If you fail to aspirate the DNBs, dispense the oil into F well, change a new wide-bore tip and aspirate again.
- The DNBs can be stored in 4 ℃ refrigerator for later use (use it within 48 hours).

16. Tap > **Yes**. The cartridge rack withdraws automatically.
17. After completion, manually close the compartment door. Tap to return to the main interface.



18. If you do not continue to use, tap > **Shut down**, and turn the power switch on the back of the device to the **OFF** position.

Performing sequencing

Preparing flow cell

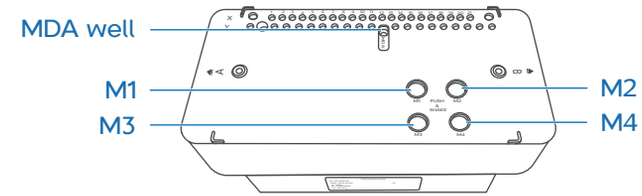
Place the flow cell at room temperature for 30 minutes to 24 hours before loading the DNBs.

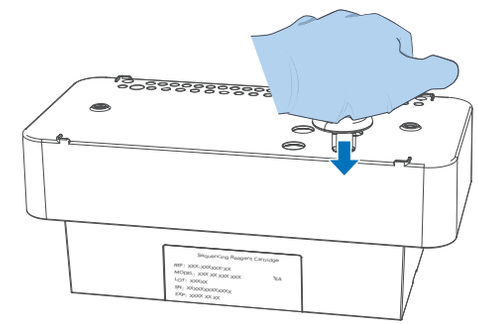
Tips

Do not open the package at this moment.

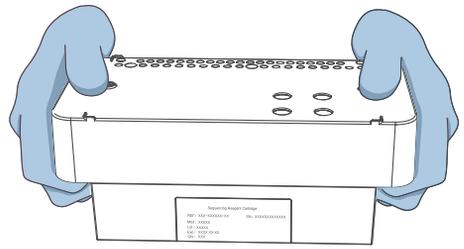
Preparing the sequencing cartridge

1. Take out the sequencing reagent cartridge and place it in water bath at room temperature for 2 hours, or pre-thaw it in the refrigerator at 2 ℃ to 8 ℃ one day ahead. Then, place the sequencing cartridge at 2 ℃ to 8 ℃ until use.
2. Invert the cartridge for 5 times. Tear off the package and wipe the condensate water on the package and wells with a dust-free cloth.
3. Use the Puncher to pierce the M1, M2, M3, M4 wells of the cartridge with the pre-mixed reagents.





4. Hold the A and B sides of the cartridge with both hands by following the marks A and B on the cartridge, and shake it vertically and horizontally for 10 to 20 times to mix the reagents thoroughly.



5. Pierce the MDA well on the cartridge with a clean 1 mL pipette tip.

Tips

The prepared cartridge can be placed at 4 ℃ until use and should be used within 24 hours.

Sequencing

1. Tap in the upper-right corner of the main interface of the control software of the sequencer. Enter the user name (*user*) and password (*123*). Tap **Login**. The main interface returns to view.
2. Tap **Sequence** in the A or B operation area in idle status. If you need to perform sequencing on both the stages A and B, tap **Sequence A&B**.
3. After tapping **Sequence**, perform one of the following operations:
   - If the waste container compartment door opens automatically, place an empty waste container into the compartment according to the on-screen prompts, and gently press the compartment door to close it. The system automatically starts the check before sequencing.
   - If the waste container compartment door does not open, the system automatically starts to check.
4. After the check is completed, tap **Next** to enter the sequencing parameters.

   Select **Sequencing only** and **No** in the **BBS** box by default. Input the DNB ID in the **DNB ID** box.
5. Select **SE50+10** in the **Recipe** box. Then select the barcode list **1-128** in the box next to the **Recipe** box. If there is no such recipe or the SoftwareVersion of the device has been updated to 1.7.0.674 or above from a previous version, delete the existing recipe and customize a recipe. For detailed operation, refer to the Q18 of *FAQ on Page 16*.
6. Select **Yes** for both **Split Barcode** and **Auto Wash** in the advanced settings.
7. Tap **Next**. The auto-sliding screen moves up automatically.
8. Push the sequencing cartridge into the reagent compartment. The system will automatically scan the cartridge ID and display it in the **Sequencing Cartridge ID** box.

Tips

If the automatic scanning fails, you can manually enter the ID.

9. Tap **Prime** and then **Yes** in the pop-up window. The system automatically starts priming.
10. After priming, the auto-sliding screen moves up automatically.

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• Version: 1.0
• Release date: Oct. 2024

Loading DNBs

1. Take out DNB Load Buffer II and TE Buffer place it on ice for 30 minutes until it thaws. Mix it thoroughly by using the vortex mixer, centrifuge it for 5 seconds, and place it on ice until use.
2. Mix 7 µL DNB load buffer II and 1 µL Make DNB Enzyme Mix II (LC), pipette gently to mix well to prepare as the DNB loading mix solution.
3. Add the DNB loading mix solution into the mixed DNBs prepared in *Making DNBs on Page 4* (the volume should be 7.5 to 21 µL) according to the table below. The total volume of the mixture should be greater than 10 µL.

	Volume (µL)	
Component	7.5 ≤ V < 21	V≥ 21
DNB loading mix solution	8×(V/21)	8
Mixed DNBs	V	21
Total volume	29×(V/21)	29

4. Gently mix the mixture for 5 to 8 times by using the wide-bore nonfiltered pipette tip. Place the mixture at 4 ℃ until use.
5. Take out the flow cell from the inner package and inspect it to ensure that the flow cell is intact.
6. Aspirate 10 µL of the DNB loading mixture by using the 200 µL, nonfiltered pipette tip and insert the tip into the inlet.

Tips

- The DNB loading mixture should be prepared freshly.
- Do not pipette vigorously, or shake the tube.

7. Fix the tip with one hand, press the tip ejector on the pipette to unload the tip with the other hand, and observe the liquid level in the tip:
   - If the liquid level drops automatically, the DNB loading mixture will automatically flow into the flow cell.
   - If the liquid level does not drop, perform the following steps:
     a. Leave the tip with DNB loading mixture in the inlet.
     b. Adjust the aspirate volume to 2 µL and take a new 200 µL non-filtered pipette tip.
     c. Hold the new empty pipette tip with one hand and gently insert it into the outlet while pressing the button down with the other hand.
     d. Gently release the button and remove the tip in the outlet after the liquid level of the tip at the inlet drops.
8. When the liquid level in the pipette tip stops dropping, remove the pipette tip in the inlet. Turn the flow cell upside down.

Loading flow cell and reviewing sequencing information

1. Insert the prepared flow cell into the flow cell compartment in the direction indicated by the arrow on the flow cell. The system will scan the flow cell ID automatically. If automatic scanning fails, you can manually input the ID.
2. Tap **Next**. The auto-sliding screen moves down automatically, and the review interface is displayed.

3. In the review interface, review all items in the review interface. After checking that all information is correct, tap **Sequence**, and then select **Yes** to start sequencing.

   The Sequencing interface of the control software displays real-time sequencing progress and step.
4. After the sequencing is completed, tap **Finish** to end the sequencing workflow. The auto-sliding screen automatically moves up and the waste container compartment door is automatically opened. Check the summaryReport, and ensure that the TotalReads(M) is greater than or equal to 80 M, the Q30(%) is greater than or equal to 90%, the SplitRate is greater than or equal to 90%.

Device maintenance

If **Auto Wash** is selected when setting the sequencing parameters, the device starts an automatic wash after the sequencing is completed. For details, refer to *DNBSEQ-G99RS High-throughput Sequencing Set User Manual*.

Creating and performing an analysis task

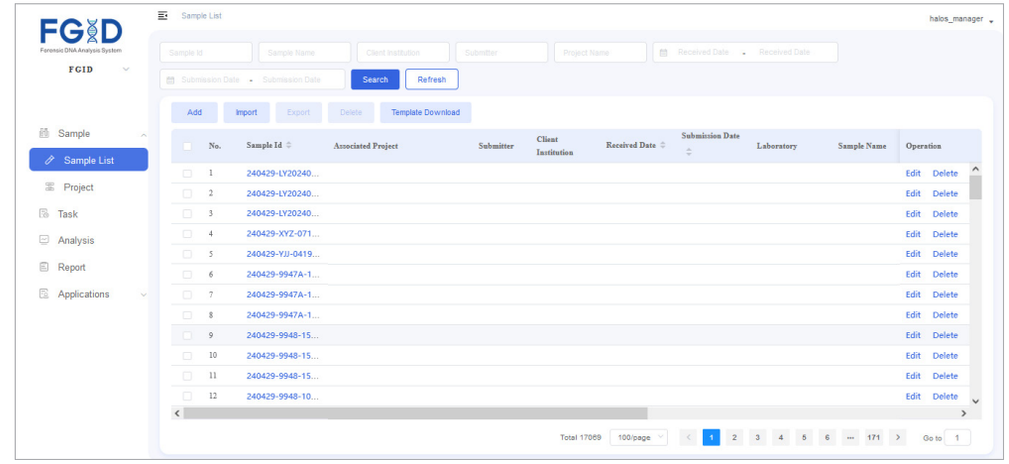
Selecting analysis product

1. Launch the Chrome browser, set the language to English, type the following IP address in the address bar, and press **Enter** to go to the login interface of the FGID:

   192.168.1.3:8080
2. Select the default username to go to the main interface.
3. Select **FGID** to go to the system.

Inputting the sample information

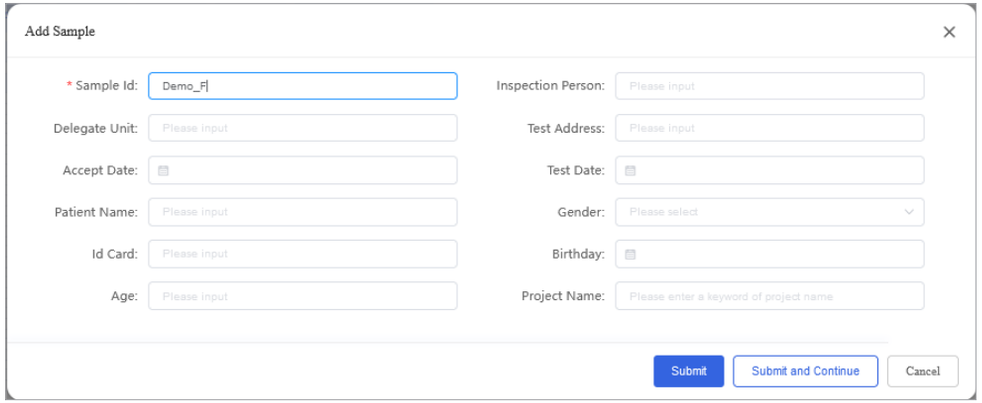
Click **Sample** and select **Sample List** to go to the sample list window.



Select one of the following methods to import sample information:

- Method 1: Manually inputting

  ① Click **Add** and input sample information in the Add Sample window.



② Click **Submit** or **Submit and Continue** to continue inputting sample.

- Method 2: Inputting information by Excel

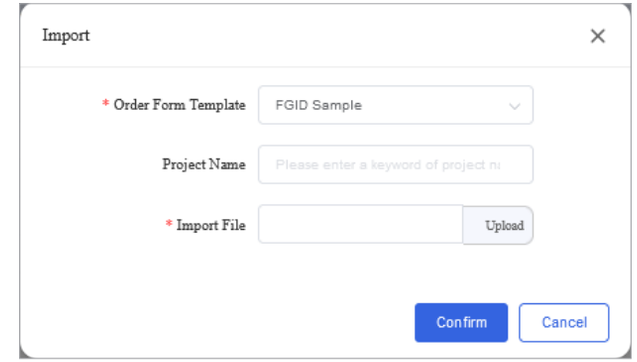
① Click **Template Download** to download the sample template.

② Input the information into the template and click **Save** after completion.

Tips

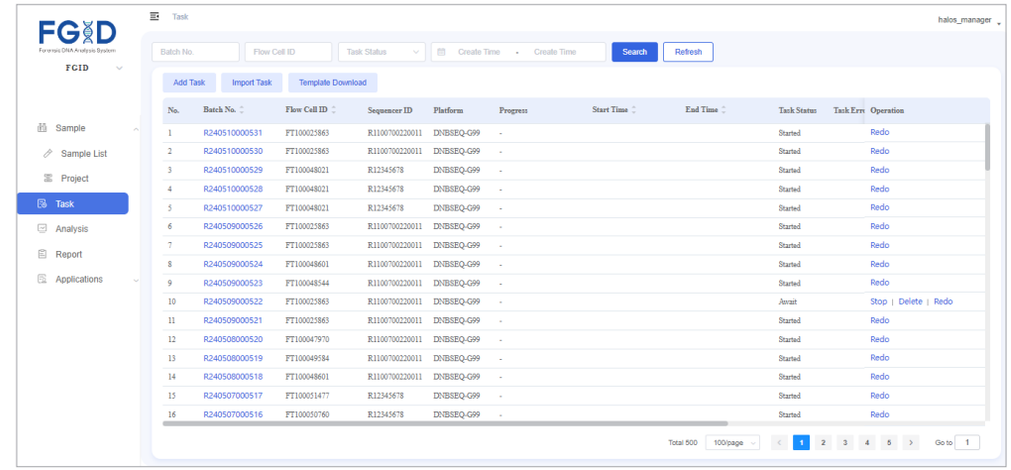
A field with a red asterisk (*) is required, and other fields are optional (Age≥1, the Id Card number should include less than or equal to 18 characters.).

③ Click **Import**, click Upload in the **Import** window, select the *.xlsx* document that has been filled in samples information, and click **Confirm**.



Creating an analysis task

Create an analysis task according to the following steps:



1. Click **Task** > **Template Download** to download the task template.
2. Input **Cutoff** in the H1 cell of the template.

3. Input the following information into the **FGID_task_template**:
   ① Input L01 in the **Lane ID** column.
   ② Input DNB ID in the **DNB ID** column and the DNB ID in an analysis task should be consistent.
   ③ Select **No** for **Is Dual Barcode** column.
   ④ Input barcode ID in the **barcode ID1** column and **leave barcode ID2** column blank.
   ⑤ Select **MGEasy Pa-SNPs Genotyping Kit** for the **Kit Name** column.
   ⑥ Input data amount for the sample (unit: M) in the **Cutoff** column. Input **5** for the parent sample which indicates that cut 5 M reads for analysis. Input **25** for fetal sample and input **None** if cutoff is not required.

Tips

- The sample ID should be consistent with the sample ID imported into the FGID system (including the upper- and lower-case letters), and one barcode can only correspond to one sample ID within a flow cell.
- It is recommended to submit one analysis task for one sample ID to avoid sample confusion during the analysis.

	A	B	C	D	E	F	G	H
1	*Sample ID	*Lane ID	*DNB ID	*Is Double Barcode	*barcode ID1	barcode ID2 (If [Is Double Barcode] is yes, barcode ID2 is required)	*Kit Name	Cutoff
2	Demo_F	L01	20240430	No	1		MGIEasy Pa-SNPs Genotyping Kit	5
3	Demo_M	L01	20240430	No	2		MGIEasy Pa-SNPs Genotyping Kit	5
4	Demo_C	L01	20240430	No	3		MGIEasy Pa-SNPs Genotyping Kit	25
5								
6								
7								
8								

4. Click **Import Task** in the Task interface.
5. Select Sequencer ID, input Flow Cell ID, and click **Upload**.
6. Select the task list, click **Preview** to preview the imported task details. Ensure that the information is correct and click **Confirm**.

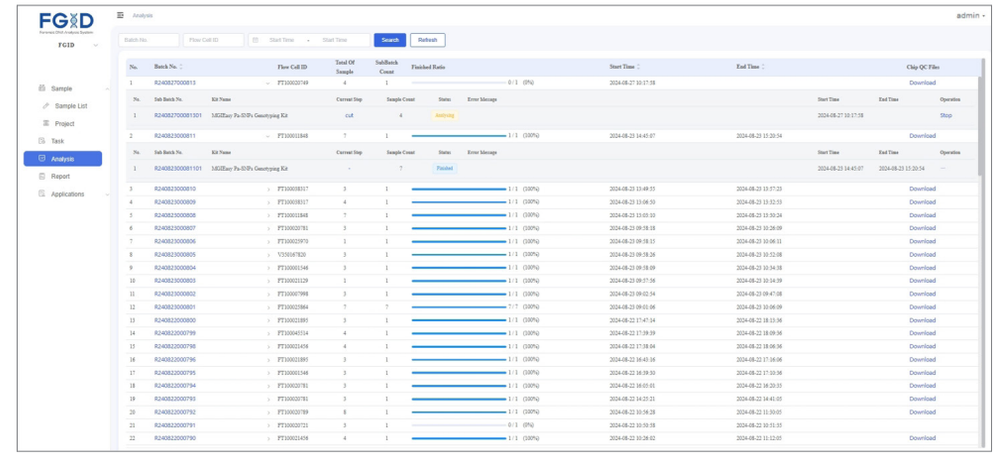
When the task status is in **Started**, the task is submitted successfully.

Starting analysis

After the sequencing is completed, the analysis system automatically starts the basic analysis task.

Viewing basic data analysis results

In the FGID system main interface, click **Analysis** to view the progress of basic data analysis.



1. Click ⟩ in front of **Flow Cell ID** to check the progress or error reason of this analysis task.
   - **Status** shows the current analysis task status. If it displays **Finished**, it indicates that basic data analysis is completed and you can proceed to the next step of paternity analysis.

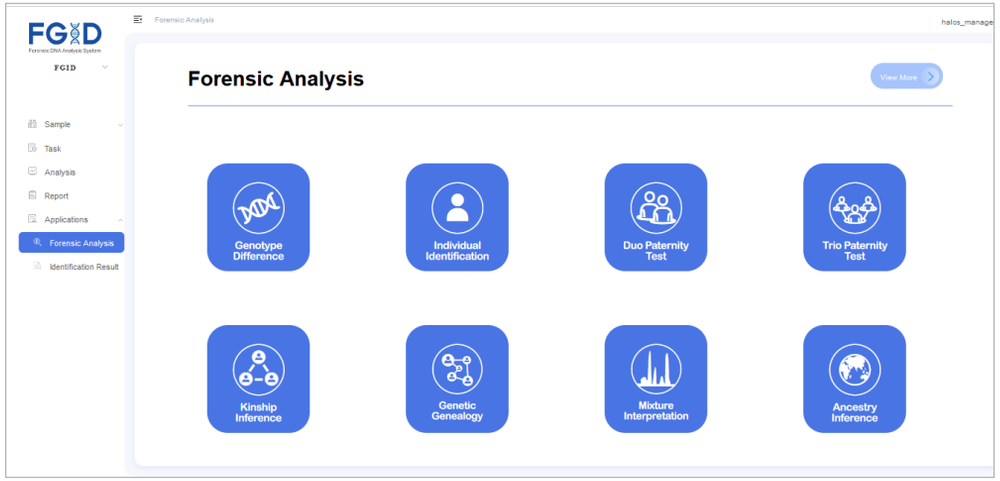
- **Current step** indicates the analysis step of the current task. Click the status below **Current** step to view the analysis flowchart and current progress.

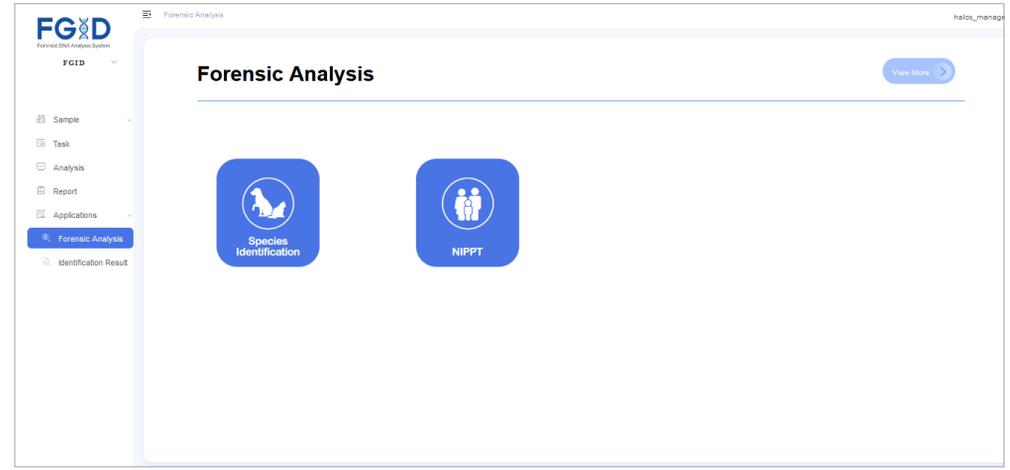
2. After the task is completed, click **Batch No.** to view the basic data analysis results for individual samples.

   Click **Export** to export the result files.

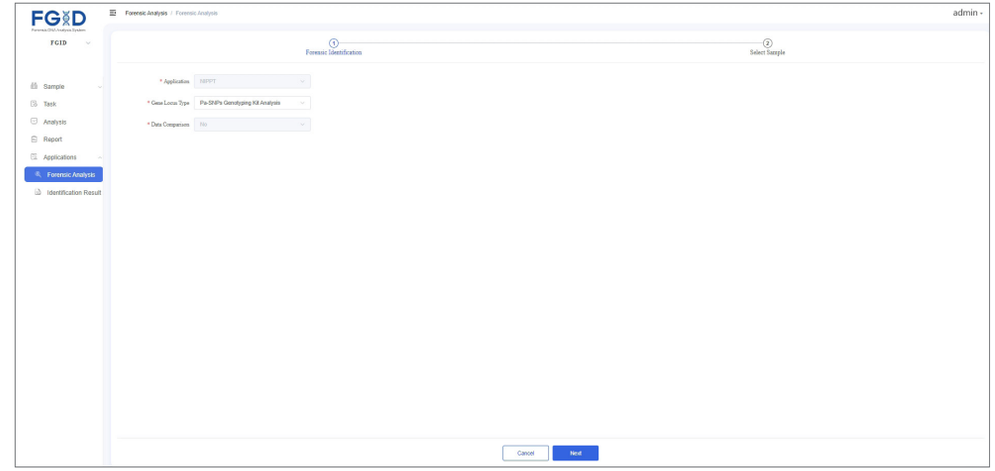
Submitting paternity analysis tasks

1. Click **FGID** > **Applications** > **Forensic analysis** > **View More** > **NIPPT** to go to the paternity analysis application.

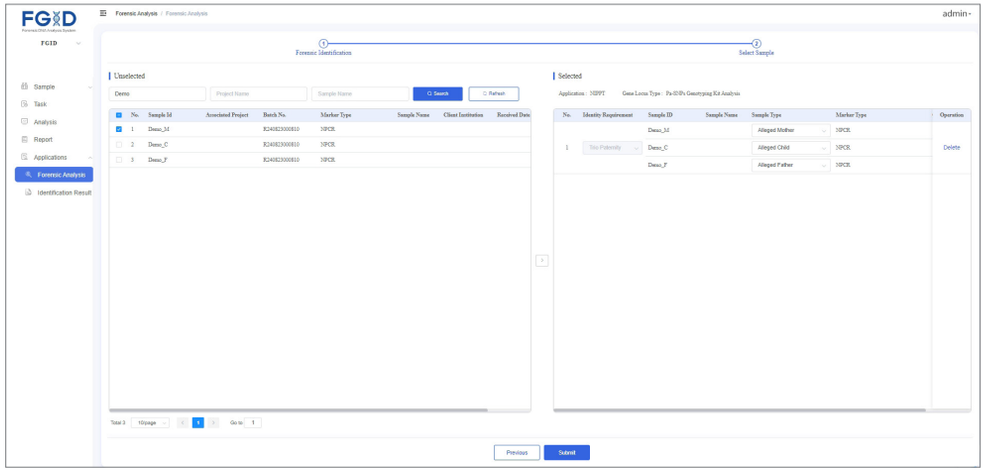




2. Select **Gene Locus Type** > **Pa-SNPs Genotyping Kit Analysis** parameters.



3. Click **Next** to go to the paternity analysis sample selection interface. Select samples which need to perform paternity analysis:



① Enter the corresponding sample information in the search bar for retrieval. Check the samples that need paternity analysis (ensure to check Sample ID and Batch No.), and click [›] to add them to the **Selected** list.

② Select the sample identity in the **Sample Type** column.

③ Click **Submit** to submit the paternity analysis task.

4. View paternity analysis results

① Click **FGID** > **Applications** > **Identification Result** to go to the analysis results interface.

② Click **Preview** to view the analysis report.

③ Click **Data Download** to download the paternity analysis result files.

The analysis result file is a compressed file that includes a report document in *.docx* format and a result folder. The result folder contains the following contents:

a. r0.paternity_results.txt: Indicates paternity analysis SNPs information, including parental SNP genotypes, fetal four-base depth, and PI results of each effective SNP.

b. r0.paternity_results.xlsx: The content is same as the r0.paternity_results.txt but in *.xlsx* format.

c. r1.result.txt: It is the paternity analysis result file, referenced in the Word report.

d. r2.analysis.xlsx: It includes paternity analysis indicators

Tips

- To ensure the reliability of the results, check the r2.analysis.xlsx in the result folder for each paternity analysis result to confirm if any indicators are abnormal. For more detailed information, refer to the Q16 of *FAQ on Page 16*.
- Paternity analysis results include Inclusion, Exclusion, Inconclusive, Inclusion-WrongMother, and WrongSample. If the result is WrongSample, it may indicate that the selected sample role identity is incorrect, and it is recommended to resubmit the paternity analysis task. For more detailed information, refer to the Q16 of *FAQ on Page 16*.

FAQ

Q1	What kind of laboratory environment is required for this experiment? How to maintain the experiment environment daily?
A	It is recommended to separately perform operations in the normal pre-PCR and post-PCR area and clean the laboratory regularly. Perform preparations in the pre-PCR area before preparing libraries to reduce aerosol contamination. If you cannot separately perform operations, perform preparations in a clean bench.

Q2	Are there any restrictions on the mother's samples?
A	• This product is only suitable for cfDNA samples extracted from plasma of 8 weeks and above gestation, with a fetal concentration greater than or equal to 2%. • This product is not suitable for twins or multiple pregnancy samples. • This product is not suitable for samples from mothers with tumor diseases, pre-eclampsia/eclampsia during pregnancy, or those who have received blood transfusions, bone marrow or organ transplants, or stem cell therapy.

Q3	Are there any restrictions on the father's samples?
A	The extracted DNA concentration must be ≥0.2 ng/μL.

Q4	Are there recommended kits for DNA extraction?
A	• For gDNA extraction, it is recommended to use the MGIEasy genomic DNA extraction kit (Brand: MGI, Cat. No.: 1000010524, suitable for saliva, blood, and oral swabs), and for trace sample genomic DNA extraction (Brand: TIANGEN, Cat. No.: DP316, suitable for samples with low DNA content like hair, semen, dry blood spots, chewing gum, and nails). • For cfDNA extraction, it is recommended to use the HiPure Circulating DNA Midi Kit (Brand: MAGEN, Cat. No.: D3182-03A).

Q5	Is the input amount for gDNA limited to 2 ng and cfDNA to 4 ng?
A	If the input amount is too large, it may lead to significant differences in sample output data amounts. If you need to increase the input amount for library preparation, it is recommended to perform sequencing for only one family on a single DNBSEQ-G99 FCL flow cell.

Q6	Can the sample preparation cartridge be inverted after loading the sealing liquid?
A	After loading the sealing liquid, the sample preparation cartridge should not be inverted or excessively tilted. Because this will cause the sealing liquid to leak, reduce the volume and fail to prepare library. The preparation cartridge cannot be used further. When transferring the sample preparation cartridge, keep it as horizontal as possible and do not tilt it.

Q7	What is the size range of the target amplification fragments of the experiment?
A	The target insert fragment size range of the experiment is 60 bp to 90 bp and the target amplification fragment size of 144 bp to 174 bp.

Q8	Can the library be sequenced with the NIPT library? How to do it?
A	They can be sequenced together, but use different groups of barcode, make DNBs separately, and mix the two kinds of DNBs in corresponding mass according to the required data amount.

Q9	Is it necessary to remove the upper oil phase?
A	The upper oil phase of the DNB should be removed. Otherwise, the residual oil will affect sequencing quality.

Q10	If the data come from other machine or the data are generated previously, they should be analyzed by using the server, and how to operate?
A	You can copy the fq files to the hard drive and transfer them to the server by using Filezilla. Perform the following steps: 1. Press down the server top cover, then lift it. 2. Insert the hard drive into the left USB port. 3. Double-click the Filezilla icon on the desktop to open the software, and click **File** > **Site Manager** in the upper-left corner, or directly click the site manager shortcut. Select the Upload account in **My Sites**, and click **Connect** to log in to the Uploader account. 4. Input **/mnt/das/Data** in the right remote site, and select the sequencer ID (which may not match the actual sequence ID). 5. Select the flow cell to upload in the left local site, with a folder directory of *flow cell number/L01*. 6. Select the flow cell number to upload, right-click the flow cell number, and select **Upload**. 7. In the right remote site under the uploaded sequencer ID directory, click **Info** > **Upload** go to the path, right-click in the blank area, and select **Create New File** to create a flag file named as **chip number_L01.txt**. 8. After uploading the fq file, you can submit the analysis task. **Tips** The flag file must be created; otherwise, the system will not start the analysis for that flow cell.

Q11	If automatic analysis fails after sequencing, how to restart the analysis?
A	1. Log in to the Uploader account. 2. Enter **/mnt/das/Data** in the right remote site, select the folder corresponding to the sequencer number, and check if the flow cell file exists. 3. If the corresponding flow cell file exists, check if the task submission form is correct, or go to the system interface, click **Task** and the Batch No. of the corresponding task number to confirm if the task submission information is correct. After confirming that the information is correct, click **Task**, then click **Redo** in Operation to restart the analysis. 4. If the corresponding flow cell file does not exist, refer to steps 3-8 in Q10 to upload the flow cell to the server.

Q12	What is the minimum data amount for a single family, and how many families can be sequenced on each G99 flow cell ?
A	A single family requires a minimum of 3M Reads for gDNA and 15M Reads for cfDNA. Up to 3 families of sample can be sequenced on each G99 flow cell.

Q13	What files are included in the exported basic data analysis results?
A	The exported basic data analysis results include infoQC.txt, r0.QualityControl.xlsx, r1.CompareSummary.xlsx, and the genotyping results and vcf files for each sample. • infoQC.txt: It refers to basic data analysis results for the samples, including MappedRate, TargetOnMap, Coverage ≥ 100X, Coverage ≥ 1000X, Uniformity (0.1), and other data quality control results. • r0.QualityControl.xlsx: The content is same as the infoQC.txt but in .xlsx format. • r1.CompareSummary.xlsx: It refers to the SNP comparison results between samples. When sample names contain "-", the first "-" is used as the group marker, and characters before "-" that are consistent are considered as the same group for SNP comparison. For example, for a-1-1, a-2-1, a_1-1, and a_1-2, a-1-1 and a-2-1 will be compared because they have "a-" which is regarded as the grouping marker. a_1-1 and a_1-2 will be compared because they have "a_1-" which is regarded as the grouping marker. • The folder named after the sample contains the genotyping results and vcf files.

Q14	What are the principles for selecting SNP points for analysis? What is the origin of the frequency information? How to replace it with local population allele frequencies?
A	The SNP frequencies used come from the 1000 Genomes Project which utilizes the global frequency data. Each selected SNP has an allele frequency higher than 0.35 with no linkage between SNPs. To replace it with local population frequencies, you can consult the technical support, provide the frequency information and let the technical support replace it.

Q15	Why are the basic data analysis results empty?
A	In this case, the sample may lack of fq file. Check the corresponding barcode of the sample in the submitted task. To view the task, check the task submission form, or click **Task** and the Batch No. of the corresponding task number to view the task submission information.

Q16	What are the determination indicators for paternity analysis results?
A	• Inclusion: The indicator is CPI > 1.0e+04 and Z-Score ≥ 3, indicating that the likelihood of the alleged father being related to the fetus is > 99.999999%. When the result is Inclusion, check if Err(SNPs) is less than or equal to 1.00%. If the value is greater than 1.00%, contamination may exist in the experiment, causing unreliable results, and library repreparation is recommended. • Exclusion: The indicator is CPI < 1.0e-04 and Z-Score < 10, indicating that the likelihood of the alleged father being related to the fetus is < 0.00000001%. • Inclusion-WrongMother: The indicator is CPI < 1.0e-04 and Z-Score ≥ 10, indicating that the mother of this family may not be the biological mother of the fetus. When the result is Inclusion-WrongMother, check sample concentration, library concentration, sample storage time, and conditions. • WrongSample: The indicator is Fetal(SNPs) ≥ 100% or Err(SNPs) ≥ 10%. When Fetal(SNPs) is greater than or equal to 100%, it may indicate that the father's sample was analyzed as the mother. When Err(SNPs) is greater than or equal to 10%, it may indicate that the father's sample was analyzed as the fetus. • Inconclusive Range: The indicator is CPI ≥ 1.0e-04 and ≤ 1.0e+04 and situations that cannot be determined as the above four results. The indicator results may be resulted from a low number of SNPs available for analysis due to contamination in the sample or poor sample quality. You can check if the SNP values in PI(SNPs) are low.

Q17	Why is the FGID_sample_template or FGID_task_template downloaded from the FGID system blank?
A	This may occur due to a caching delay in the system when downloading files. Please wait until the download is complete before opening the downloaded file.

Q18	How to create a new SE50+10 sequencing recipe?
A	If the device's Software Version has been updated from a lower version to 1.7.0.674 or above, and the ISW Version is V1.3.0.568 or above, you need to delete the original sequencing recipe and customize it. Perform the following steps: 1. Tap the  > **Settings** on the upper-right corner of the main interface G99 main interface, and select **Recipe** to go to the Sequencing recipe settings interface. In this interface, you can create, delete a sequencing recipe and adjust the display order of sequencing recipes. 2. Tap **Create** to go to the Create Recipe window. 3. Input **SE50+10** in the box behind the Recipe name, **50** in the box under the **Read1**, **0** in the box under **read2**, **10** in the box under **Dulbarcode**, and **0** in the box under **Barcode**. 4. Tap **Save**. The Recipe interface returns to view. 5. Tap **Close**. The main interface returns to view.