· Part No.: H-020-000962-00 · Release date: April, 2024
· Version: 1.0

Overview

Applicable reagent set/kit

Name	Cat. No.	Brand
ATOPlex MTB Library Preparation Set (96 RXN)	940-001601-00	MGI
ATOPlex MTB Library Preparation Set (576 RXN)	940-001599-00	
DNBSEQ-G99RS High-throughput Sequencing Set	940-001269-00	
DNBSEQ OneStep DNB Make Reagent Kit V4.0	940-001654-00	

Applicable device and software

Model	Cat. No.	Brand
DNBSEQ-G99ARS	900-000609-00	MGI
MTB-Explorer software	970-000385-00	

Preparing MTB sample libraries

Sample requirement

The reagent kit is applicable to detecting the genome DNA in the mycobacterium tuberculosis culture-isolate samples or the microbial community of mixed samples, such as saliva or sputum.

- For the genome DNA extrated from the bacteria culture, it is recommended that the concentration of the DNA sample is greater than or equals to 0.1 ng/μL and the mass of the input sample is 1 ng to 10 ng.
- For the genome DNA extracted from the mixed microbial sample, it is recommended that the conentration of the DNA sample is greater than or equals to 1 ng/μL and the mass of the input sample is 1 ng to 10 ng.

  The input mass is determined according to the actual application requirements, but the input volume of the DNA sample should be less than or equal to 9.5 μL.

First PCR amplification

⚠ WARNING

- Prepare the PCR reaction mixture I in the pre-PCR area. Perform the first PCR reaction and prepare the PCR reaction mixture II in the PCR area.
- To avoid cross-contanmination, it is recommended to use filtered tips.
- Perform the purification immediately after PCR reactions. Do not place the PCR product in the thermal cycler overnight.

1. Take out the following reagents from the corresponding reagent kits. Thaw them at room temperature, mix them with the vortex mixer, centrifuge them briefly, and place them on ice until use.

Reagent	Reagent kit
MTB PCR primer Pool	ATOPlex MTB Panel
Spike-in Control PCR Primer Pool	ATOPlex Multiplex PCR Spike-in Control

2. Take out the following reagents from the corresponding reagent kit. Invert or tap the bottom of the tubes to mix the liquids, centrifuge them, and place them on ice until use.

Reagent	Reagent kit
PCR Enzyme Mix	ATOPlex DNA Multiplex PCR Amplification Module
PCR Clean Enzyme	

· Part No.: H-020-000962-00 · Release date: April, 2024
· Version: 1.0

3. Based on the number of desired reactions, prepare the PCR reaction mixture I according to the following table:

Reagent	Volume (μL)
PCR Enzyme Mix	12.5
PCR Clean Enzyme	0.5
MTB PCR primer Pool	2
Spike-in Control PCR Primer Pool	0.5
Total volume	15.5

4. Aspirate the DNA sample to be detected or the control (9.5 μL Spike-in Control PCR Primer Pool used according to your needs), dispense it into a new 0.2 mL PCR tube, and add TE Buffer into the PCR tube until the total volume reaches 9.5 μL. Aspirate 15.5 μL of the prepared PCR reaction mixture I with the pipette and dispense it into the PCR tube. Mix the mixture with the vortex mixer for 3 times (3 seconds each) and centrifuge the mixture to collect it at the bottom of the tube.
5. Place the PCR tube into the thermal cycler, and perform the first PCR reaction according to the following table:

Temperature	Time	Cycles
105 ℃ (heated lid)	On	/
37 ℃	5 min	1
95 ℃	5 min	
95 ℃	20 s	20
62 ℃	1 min	
58 ℃	1 min	
72 ℃	30 s	
72 ℃	1 min	1
4 ℃	Hold	/

6. After the reaction, centrifuge the PCR tube, and proceed with the first product purification in the PCR tube.

First PCR product purification

1. Take out DNA Clean Beads from MGIEasy DNA Clean Beads in advance, place it at room temperature for 30 minutes, and mix it thoroughly with the vortex mixer.
2. Aspirate 32.5 μL DNA Clean Beads by using the pipette and dispense it into the first PCR reaction product. Gently pipette the mixture for at least 10 times until all beads are suspended in the liquid. Ensure that all the liquid and beads are dispensed into the PCR tube in the last pipetting.
3. Incubate the mixture at room temperature for 5 minutes.
4. Centrifuge the PCR tube briefly and place it on the magnetic rack for 2 to 5 minutes. When the liquid in the tube becomes clear, carefully aspirate and discard the supernatant by using the pipette.
5. Keep the tube on the magnetic rack, and add 160 μL of the freshly prepared 80% ethanol to wash the beads and tube wall. Gently pipette the mixture three times and keep the tube on the magnetic rack for 30 seconds. Carefully aspirate and discard the supernatant.
6. Repeat step 5. Aspirate the liquid in the tube as much as possible. If a small amount of liquid remains on the wall, briefly centrifuge the tube and place it on the magnetic rack until the liquid and beads separate. Use the pipette with a small volume range to remove all the liquids.
7. Keep the tube on the magnetic rack with the lid open. Let the beads air-dry until the surface of the beads has no reflection or cracking.

Tips

If the beads are over-dried (cracked), the yield will decline.

· Part No.: H-020-000962-00 · Release date: April, 2024
· Version: 1.0

8. Remove the tube from the magnetic rack, add 6 µL TE Buffer into it to elute DNA, and gently pipette the mixture at least 10 times until all beads are suspended in the liquid.
9. Incubate the mixture at room temperature for 5 minutes.
10. Briefly centrifuge the tube for later use.

Stoppoint

The purified PCR product can be stored in the -20 ℃ refrigerator for 7 days.

Second PCR amplification

Tips

The second PCR amplification is performed with DNA Clean Beads contained. Therefore, there is no need to perform magnetic absorption and transfer the supernatant.

WARNING

- Perform the second PCR reaction in the post-PCR area.
- Perform the second PCR product purification immediately after the second PCR reaction. Do not place the PCR product in the thermal cycler overnight.

1. Take out the following reagents from the corresponding reagent kits. Thaw them at room temperature, mix them with the vortex mixer, centraifuge them briefly, and place them on ice until use.

Reagent	Reagent kit
MTB PCR Block	ATOPlex MTB Panel
Spike-in Control PCR Block	ATOPlex Multiplex PCR Spike-in Control
ATOPlex Dual Barcode Primer	ATOPlex Dual Barcode Primer Module

2. Take out the following reagents from the corresponding reagent kit. Invert or tap the bottom of the tubes to mix the liquids, centrifuge them, and place them on ice until use.

Reagent	Reagent kit
PCR Enzyme Mix	ATOPlex DNA Multiplex PCR Amplification Module
PCR Additive	
PCR Clean Enzyme	

3. Perform one of the following operations:
   - If ATOPlex MTB Library Preparation Set (96 RXN) is used, aspirate 4 µL PCR Dual Barcode Primer Mix (01-96) and dispense it into to the first PCR purification product according to the appendix of *ATOPlex MTB Library Preparation Set User Manual*.
   - If ATOPlex MTB Library Preparation Set (576 RXN) is used, aspirate 2 µL Barcode 1 (01-96) and 2 µL Barcode 2 (01-48) from PCR Dual Barcode Primer Mix (48×96) and dispense them into the first PCR purification product according to the appendix of *ATOPlex MTB Library Preparation Set User Manual*.
4. Based on the number of desired reactions, prepare the PCR reaction mixtuire II according to the following table. Mix the mixture with the vortex mixer, centrifuge it briefly, and place it on ice until use.

Reagent	Volume (µL)
PCR Enzyme Mix	12.5
PCR Clean Enzyme	0.5
PCR Additive	0.5
MTB PCR Block	1.0
Spike-in Control PCR Block	0.5
Total volume	15.0

· Part No.: H-020-000962-00 · Release date: April, 2024
· Version: 1.0

5. Apirate 15 μL of the PCR reaction mixtuire II and dispense it into the sample tube mentioned in step 3. Mix the mixture with the vortex mixer for 3 times (3 seconds each) and centrifuge the mixture to collect it at the bottom of the tube.
6. Place the sample tube into the thermal cycler, and perform the second PCR reaction according to the following table:

Temperature	Time	Cycles
105 ℃ (heated lid)	On	/
37 ℃	5 min	1
95 ℃	5 min	
95 ℃	20 s	15
62 ℃	1 min	
58 ℃	1 min	
72 ℃	30 s	
72 ℃	1 min	1
4 ℃	Hold	/

7. After the reaction, centrifuge the PCR tube, and proceed with the second product purification in the PCR tube.

Second PCR product purification

1. Take out DNA Clean Beads from MGIEasy DNA Clean Beads in advance, place it at room temperature for at least 30 minutes and mix it thoroughly with the vortex mixer.
2. Aspirate 25 μL DNA Clean Beads with the pipette and dispense it into the second PCR reaction product. Gently pipette the mixture at least 10 times until all beads are suspended in the liquid. Ensure that all the liquid and beads are dispensed into the PCR tube in the last pipetting.
3. Incubate the mixture at room temperature for 5 minutes.
4. Centrifuge the PCR tube briefly and place the tube on the magnetic rack for 2 to 5 minutes. When the liquid in the tube becomes clear, carefully aspirate and discard the supernatant by using the pipette.
5. Keep the tube on the magnetic rack, and add 160 μL of the freshly prepared 80% ethanol to wash the beads and tube wall. Gently pipette the mixture three times and keep the tube on the magnetic rack for 30 seconds. Carefully aspirate and discard the supernatant.
6. Repeat step 5. Aspirate the liquid in the tube as much as possible. If a small amount of liquid remains on the wall, briefly centrifuge the tube and wait until the liquid and beads separate. Use the pipette with a small volume range to remove all the liquids.
7. Keep the tube on the magnetic rack with the lid open. Let the beads air-dry until the surface of the beads has no reflection or cracking.

Tips

If the beads are over-dried (cracked), the yield will decline.

8. Take the tube from the magnetic rack, and add 25 μL TE Buffer to elute DNA. Gently pipette the mixture at least 10 times until all beads are suspended in the liquid.
9. Incubate the mixture at room temperature for 5 minutes.
10. Centrifuge the PCR tube briefly and place the centrifuge tube on the magnetic rack for 2 to 5 minutes until the liquid becomes clear. Transfer 23 μL of the supernatant with the pipete to a new PCR tube.
11. Quantify the second PCR purification product with the double-strand DNA quantification reagent. The concentration of the second PCR purification product should be greater than or equal to 10 ng/μL.

· Part No.: H-020-000962-00 · Release date: April, 2024
· Version: 1.0

Stoppoint

The second PCR purification product can be stored in the -20 ℃ refrigerator.

Making DNBs

1. Take out the following reagents from the corresponding set, and thaw them at room temperature. After thawing, mix them with the vortex mixer, centrifuge them, and place them on ice until use.

Reagent	Set
Low TE Buffer	DNBSEQ OneStep DNB Make Reagent Kit V4.0 (OS-Dual Barcode)
Make DNB Buffer (OS-DB-V4.0)	
Stop DNB Reaction Buffer	
Make DNB Enzyme Mix I (OS-V4.0)	

2. Mix the second PCR purification product (library) with equal mass. Aspirate 50 ng of the mixed PCR purification product, dispense it into a new 0.2 mL PCR tube, and prepare the make DNB reaction mixture I according to the following table:

Reagent	Volume (μL)
dsDNA libraries	V
Low TE Buffer	10 μL-V
Make DNB Buffer (OS-DB-V4.0)	10 μL
Total volume	20 μL

Tips

V=50/X and X indiactes the concentration (ng/μL) of the mixed dsDNA libraries.

3. Mix the mixture with the vortex mixer, centrifuge it, and place it into the thermal cycler to start the reaction according to the following table:

Temperature	Time
105 ℃ (heated lid)	On
95 ℃	3 min
40 ℃	3 min
4 ℃	Hold

4. Take out Make DNB Enzyme Mix II (OS-V4.0) in DNBSEQ OneStep DNB Make Reagent Kit V4.0 (OS-Dual Barcode) from storage. Tap the bottom of the tube to mix the mixture thoroughly, centrifuge it and place it on ice until use.
5. After the reaction, add the following reagents into the make DNB reaction mixture I, mix the mixture, and centrifuge it to prepare the make DNB reaction mixture II:

Reagent	Volume (μL)
Make DNB Enzyme Mix I (OS-V4.0)	20
Make DNB Enzyme Mix II (LC)	1

6. Place the reaction mixture II (with a total volume of 41 μL) into the thermal cycler to start the reaction according to the following table:

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

7. After the reaction, add 10 μL Stop DNB Buffer immediately, and gently pipette the mxiture 5 to 8 times with a wide-bore tip.

Tips

- Do not pipette vigorously, or shake the tube.
- The DNB product can be stored at 4 ℃ for 48 hours.

8. Quantify the DNB product with the single-strand quantification reagent. The concentration of the DNB product should be greater than or euqal to 8 ng/μL. If it is lower than 8 ng/μL, prepare the DNB product again.

Sequncing and analyzing

For detailed instructions of the sequencer and analysis software, refer to relevant user manuals.

Inputting the DNB ID

1. Launch the Chrome browser, type the following IP address in the address bar, and press **Enter**:

   192.168.1.5

2. Input the authorized username (**lite**) and password (**lite123456**), and tap **Login** to go to the ZLIMS Home page.
3. Tap **Sequencing + Analysis** on the Home page. The New Sequencing + Analysis page is displayed.
4. Select **MTB-Explorer_ATOPlex** as the analysis product. Select **Importing the sample ID** and tap **New**.
5. Tap **Excel template** or **CSV template** to download the DNB sample template in *.xlsx* or *.csv* format in the Import Sequencing +Analysis dialog box.
6. Fill in the DNB template, and save the sample template in the designated path.

	A	B	C	D	E	F
1	Product Name(*)	DNB ID(*)	Barcode(*)	Sample ID(*)	Sample Nam	Sample Type(*)
2	MTB-Explorer_ATOPlex					DNA
3						

Tips

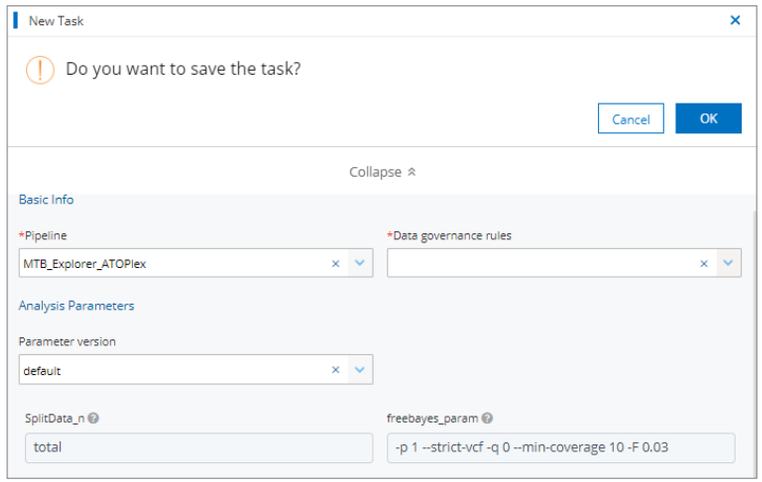
- A field with a red asterisk (*) is required, and other fields are optional.
- Cells in the Excel template cannot be merged, and no spaces or special characters should be input before or after the contents in each cell.
- Some fields include drop-down options, and other fields require manual input. The inputting method varies among the fields.
- **DNB ID**: The DNB ID should include both letters and numbers. It cannot be the same as the DNB ID that is already in ZLIMS.
- **Barcode**: When one sample ID corresponds to multiple barcodes, the multiple barcodes are separated by half-width commas (,). For multiple consecutive barcodes, a tilde (~) can be used.
- **Sample ID**: The sample ID should include both letters and numbers. It can be the same as the sample ID that is already in ZLIMS Lite. The same sample ID indicates the same sample. For the samples with both the same sample ID and sample type in the same batch, sequencing data of the samples will be merged for analysis.
- **Sample Name**: (Optional) Fill in the sample name according to the actual requirement.
- **Sample Type**: Only **DNA** can be selected.

7. Return to the Import Sequencing + Analysis dialog box, tap **Choose File**, select the completed DNB sample template, and tap **Upload**. After inputting the DNB sample information is completed, the New Sequencing + Analysis page returns to view.
8. Tap **Save** in the lower-right corner. The New Task page is displayed.
   - If you choose to use the default analysis parameters, tap **OK**.

- If you need to change one or more analysis parameters, tap **Click Expand to modify analysis parameters** to expand the analysis parameter area, change the analysis parameters, and then tap **OK**.

Tips

After changing the analysis parameters, do not tap **Collapse** to collapse the New Task page, but keep it in the expanded state. Otherwise, the analysis task will not apply the change.



Preparing the flow cell

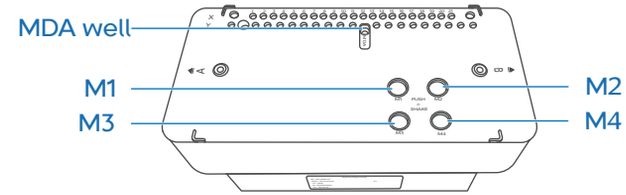
Take out the flow cell and place it at room temperature for at least 30 minutes, but not exceeding 24 hours.

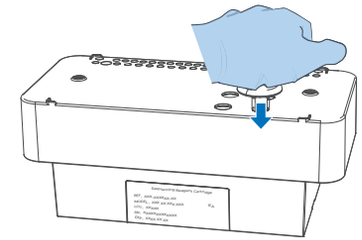
Tips

Do not open the package at this time.

Prepare the sequencing cartridge

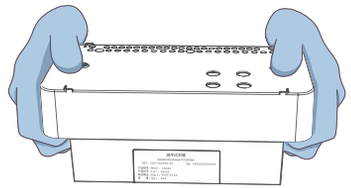
1. Take out the sequencing reagent cartridge and place it in water bath at room temperature for 4 hours, or pre-thaw it in the refrigerator at 2 ℃ to 8 ℃ one day ahead. Then, place the sequencing cartridge at 2 ℃ to 8 ℃ until use.
2. Invert the cratridge for 5 times. Tear off the package and wipe the condensate water on the package and wells with a dust-free cloth.
3. Align the puncher packed in the storage with the four wells on the cartridge. Press the puncher with a hand.





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.

· Part No.: H-020-000962-00 · Release date: April, 2024
· Version: 1.0



5. Pierce the MDA well on the cartridge with a clean 1 mL pipette tip.
6. Take out MDA Enzyme Mix and MDA Reagent in the DNBSEQ-G99RS High-throughput Sequencing Set form storage. Add 125 µL MDA Enzyme Mix to the MDA Reagent tube with the 200 µL pipette.
7. Invert the tube six times to mix the liquid.
8. Add all the mixture to the MDA well. The sequencing cartridge is ready for use.

Tips

- When adding the mixture to the MDA well, keep the pipette tip close to the concave side, tilt it to avoid generating bubbles or spilling to other wells.
- 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 flow cell A or B operation area in idle status. If you need to perform sequencing on both the flow cell 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 comparment 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 **Sequence & Transmission** as the workflow and **No** in the **BBS** box by default. Input the DNB ID in the **DNB ID** box.

Tips

Ensure that the DNB ID is consistent with *Inputting the DNB ID on page 6*.

5. Select **PE150+10+10** in the **Recipe** box. Then select the barcode list **ID576+48RXN** in the box next to the **Recipe** box. If there is no such barcode list, tap to import the barcode recipe. For details of the barcode list, contact the technical support.
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 **Reagent cartridge** 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.

· Part No.: H-020-000962-00 · Release date: April, 2024
· Version: 1.0

After priming, the auto-sliding screen moves up automatically.

Loading DNBs

1. Take out DNB Loading Buffer II and 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. Take out a new 0.5 mL tube, and prepare the DNB loading mixture according to the following table:

Component	Volume (μL)
DNB Load Buffer II	7.0
Make DNB Enzyme Mix II (LC)	1.0
DNBs	21.0
Total volume	29.0

3. Gently mix the mixture for 5 to 8 times by using the wide-bore non-filtered pipette tip. Place the mixture at 4 ℃ until use.
4. Take out the flow cell from the inner package and inspect it to ensure that the flow cell is intact.
5. Aspirate 10 μL of the DNB loading mixture by using the 200 μL, non-filtered pipette tip and insert the tip into the inlet (small hole near the edge). Press the tip ejector on the pipette to unload the tip with one hand. The DNB flow flows into the flow cell.

Tips

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

6. After ensuring that the DNB loading is completed, pull out the tip at the inlet. Keep the QR code on the flow cell facing upward and immediately transfer the flow cell to the sequencer.

Loading the flow cell and reviewing information

1. Install the prepared flow cell into the flow cell stage 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 **Sequencing**, and then select **Yes** to start sequencing.

   The Sequencing interface of the control software displays real-time sequencing progress and step. In addition, you can view sample status after refreshing the task management page of ZLIMS.
4. After the sequencing is completed, tap **Complete** to end the sequencing workflow. The auto-sliding screen automatically moves up and the waste container compartment door is automatically opened.

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*.

· Part No.: H-020-000962-00 · Release date: April, 2024
· Version: 1.0

Performing an analysis

After the sequencing is completed, the analysis software automatically starts the analysis.

You can tap any number in the **Task Status** area or tap **Task** on the left navigation bar on the Home page of ZLIMS to access the Task page and view the status and task progress.

Viewing and downloading the analysis report

1. Tap the number under **Report Today** on the Home page. The Analysis Report page is displayed.
   - The number 0 indicates that no report is generated on the current date, and the Analysis Report page displays all reports generated in ZLIMS Lite.
   - A number greater than 0 indicates the number of reports generated on the current date, and the Analysis Report page displays only these reports by default.
2. In the **Query** area, input a query term, and tap  to search for the to-be-viewed report.
3. Tap  in the **Report** column, or tap **Analysis** in the **Analysis Type** column, and then tap  on the pop-up page to view the single-sample report.
4. On the Analysis Report page, select rows of the to-be-downloaded reports.
5. Tap **Download Report**, and the selected reports are downloaded.
6. (Optional) Tap the in the **Result Path** column of the Analysis report page to go to the analysis result directory. Then, tap the **_en.html* file to view the summary report of a sample in a batch, or tap **Sub_Web** to view a singlesample report, or tap **Summary** to view all output results of the sample.
7. (Optinal) Tap the *All.Report.tar.gz* to download to the local directory the compression package that includes all analysis results of the target sample.

(Optinal) Powering off the device

1. Tap  and select **Shut down**. In the pop-up dialog box, select **Shut down**.
2. Turn the power switch to the  position.
3. Disconnect the power cord from the main power supply socket or UPS.