Sequencer maintenance

Daily maintenance

- Check whether the fan at the rear of the device is operational. If not, contact CG Technical Support.
- During sequencing, pay attention to error messages and check whether the relevant parts are functioning properly. Contact CG Technical Support if needed. For information about how to find error messages, refer to *Menu area on Page 22*, and *Log interface on Page 24*.
- Clean the interior of the reagent compartment before sequencing:

  1) Open the reagent compartment, open the sequencing cartridge compartment door and pull out the washing cartridge drawer.

     For information on powering the device off, refer to *(Optional) Powering the devices off on Page 93.*

  2) Clean the interior of the reagent compartment with a 75% ethanol wipe. Ensure that the surface is free of DNBs, reagents, blood, and saliva.

Weekly cleaning

Perform the following steps:

1. Power the device off and open the reagent compartment.

   For information on powering the device off, refer to *(Optional) Powering the devices off on Page 93.*

2. Clean the touch screen and exterior of the reagent compartment door, sequencing cartridge compartment door, and washing cartridge drawer with a 75% ethanol wipe. Ensure that the surface is free of DNBs, reagents, blood, and saliva.

Monthly maintenance

Maintaining the device

Perform the following steps:

1. Power the device off and open the reagent compartment.

   For information on powering the device off, refer to *(Optional) Powering the devices off on Page 93.*

2. Clean the surface of the device with a 75% ethanol wipe. Ensure that the surface is free of DNBs, reagents, blood, and saliva.

Maintaining the power supply

- When the device is not in use for seven days or longer, perform a wash manually according to *Performing a manual wash on the sequencer (~40 minutes) on Page 120*. Power the device off after the wash.

  For information on powering the device off, refer to *(Optional) Powering the devices off on Page 93.*
- Check whether the power cord and cables are in good condition regularly. Contact CG Technical Support if new cables are required.

Maintaining the software

If necessary, contact CG Technical Support to update and maintain the software.

Maintaining the pure water container

Perform the following maintenance every week:

1. Empty the pure water container.
2. Spray 75% ethanol onto the inner surface of the lid and the surface of the pure water tube, and then wipe them with a clean Kimwipes tissue.
3. Add sufficient laboratory-grade water into the pure water container, and reattach the lid.
4. Gently swirl the container until all inner walls are cleaned.
5. Empty the container.
6. Repeat steps 3 to 5 twice.

Replacing the waste container

The waste container is connected to the device through tubes. To avoid liquid leakage and biological hazard exposure, monitor the waste container status frequently. Dispose of the waste and waste container when the waste approaches the maximum volume.

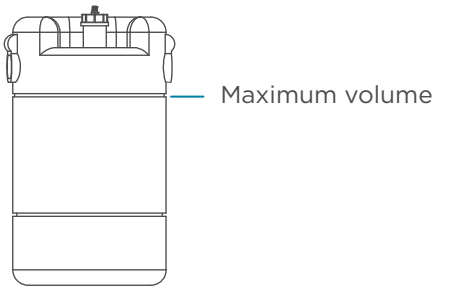

Figure 73 Maximum waste volume

Perform the following steps:

1. Wear protective equipment.
2. Remove the lid with tubes from the waste container, install a new lid with sealing gasket, and secure the lid until you hear a click.
3. Dispose of the waste and waste container.

DNB loader maintenance

Daily maintenance

Perform the following maintenance when the device is powered on:

- Check whether the fan of the device is operational. If not, contact CG Technical Support.
- During loading, pay attention to error messages and check whether the relevant parts are functioning properly. Contact CG Technical Support if needed. For information about how to find error messages, refer to *Log interface on Page 36*.

Weekly cleaning

Perform the following steps:

1. Power the device off and open the loading compartment door and flow cell compartment door.

2. Clean the touch screen, plate tray, and flow cell stage with a 75% ethanol wipe. Ensure that the surface is free of DNBs, reagents, blood, and saliva.

Monthly cleaning

Maintaining the device

Perform the following steps:

1. Power the device off.
2. Clean the surface of the device with a 75% ethanol wipe. Ensure that the surface is free of DNBs, reagents, blood, and saliva.

Maintaining the power supply

- When the device is not in use for seven days or longer, perform a maintenance wash, power the device off, and disconnect the power cord.
- Check whether the power cord and cables are connected correctly and in good condition before each use. Re-connect the cables if needed (ensure that the device is powered off), or contact CG Technical Support if new cables are required.

Annual maintenance

It is recommended that you calibrate and maintain critical components, such as the power of the laser, annually. For information on the service plan and preventative maintenance (PM), contact CG Technical Support.

Maintaining the flow cell stage

Perform cleaning and maintenance for the flow cell stage before use. Failure to do so may affect the attachment of the flow cell to the chuck.

Prepare the following tools and solutions to clean the flow cell stage:

- Washing flow cell
- Low-lint cloth
- Absolute ethanol
- Canned air duster

Perform the following steps:

1. Wear protective gloves.

2. Check for dust, debris, damage, or particulate matter on the back of the flow cell and the surface of the aluminum chuck of the flow cell stage.
3. If necessary, wipe the back of the flow cell or the surface of the aluminum chuck with a low-lint cloth moistened with absolute ethanol, and then let it air-dry.

   Do not wipe the inlet holes and vacuum attachment slot to prevent absolute ethanol from entering the holes and damaging the device.

4. Use a canned air duster to carefully blow particulate matter and dust from the surface of the silicon chip and aluminum chuck until they are clean.
5. Place the flow cell on the flow cell stage. Ensure that the flow cell and label are facing upward. Press the edges of the flow cell with your hands to ensure that it is securely seated.
6. Press the flow cell attachment button on the flow cell stage.

Maintaining the software

If necessary, contact CG Technical Support to update and maintain the software.

Storage and transportation

- Store the device according to the environment requirements in this guide.
- If you want to move or transport the device, contact CG Technical Support.

Disposal of the device

The service life of this device is seven years, which is determined by the simulated service life evaluation method. For the date of manufacture, refer to the label on the device. Perform the maintenance according to the requirements in this guide. Dispose of the end-of-life device according to local regulations. However, if it is confirmed that the device is still functioning safely and effectively after maintenance, continue to use the device.

08

FAQs

This chapter describes frequently asked questions about the reagents and sequencer.

If malfunctions occur during operation, the device alarms or a message is displayed on the screen. Follow the prompts to troubleshoot and solve the issue.

If the problem persists after you try the recommended actions, contact CG Technical Support.

Sequencer FAQs

Q: What should I do if the device does not power on after turning the power switch to the ON position?

Powering issues arise when the main power supply is in an abnormal condition, not connected to the main power supply/UPS, or if the UPS has run out of power.

Perform the following steps:

1. Check whether the main power supply and UPS is normal.
2. Ensure that the device is connected to the main power supply or UPS.

Q: What should I do if error messages appear when the control software is running?

Errors messages may appear when parameters are not set properly or if an error occurs in software-hardware communication.

Perform the following steps:

1. Perform a self-test in the maintenance interface. Check the record of the hardware that fails the self-test.
2. Check error messages in the log, and troubleshoot the problem according to on-screen instructions.
3. Restart the device.

Q: What should I do if temperature error messages and warnings related to the sequencing cartridge

compartment appear in the sequencing interface?

If the sequencer has been turned off after a long period of time, the sequencing cartridge compartment will be at room temperature. The sensor may detect that the sequencing cartridge compartment is exceeding the preset temperature. Issues may also occur when there is an error with the temperature control board.

Perform the following steps:

1. Let the sequencer run and let the sequencing cartridge compartment cool. The error message should disappear when the sequencing cartridge compartment is at operating temperature.
2. Restart the sequencer.

Q: What should I do if temperature error messages and warnings related to the LT (Laser Temperature) board appear in the sequencing interface?

Error messages may appear when the temperature of the LT board exceeds the preset limits and/or if there is an error with the temperature sensor error. It is recommended to record the warnings and the related logs of the sequencing run and contact CG Technical Support.

Q: What should I do if the waste level sensor alarm is activated?

The waste level sensor alarm may activate if the waste level exceeds the preset limit, the level sensor is not installed properly, and/or the level sensor is damaged. It is recommended to record the warning and the related logs of the sequencing run and contact CG Technical Support.

DNB loader FAQs

Q: What should I do if a message, indicating that the compartment door is opened, is displayed in the interface?

This message is displayed when the compartment door is open. The resolve this issue, ensure that the compartment door is closed.

Q: What should I do if the sampling needle bumps into the post-loading plate and bends during operation?

The sample needle may make contact with the post-loading plate if the position settings are incorrect. If this occurs, contact CG Technical Support for assistance.

Q: What should I do if bubbles are present in the fluidics lines of the flow cell?

Bubbles may be present in the fluidics lines of the flow cell when aspirating reagents.

To resolve the issue, perform the following steps:

1. After loading, press the flow cell attachment button to release the flow cell.
2. Check whether the sealing rings installed evenly and properly. If not, re-install the sealing ring.

Q: Why is the flow cell not attaching to the flow cell stage?

If the flow cell is not attaching to the flow cell stage on the loader, it may be due to the flow cell attachment button not being pressed. Any dust, debris, or damage that may be present on the flow cell stage and/or the flow cell can keep the flow cell from attaching.

To resolve the issue, perform the following steps:

1. Check whether the flow cell attachment button is pressed.
2. Check the flow cell stage for dust, debris, or damage. Clean the flow cell stage. For further details, refer to *Maintaining the flow cell stage on Page 125*.

Q: Why is liquid not passing through the fluidics lines of the flow cell?

When foreign particles are present on the sealing ring, or the sealing ring is damaged, liquid may not be able to pass through the fluidics lines. Foreign particles on the rear of the flow cell and blockages in fluidics line may also be present if liquid is unable to pass through the lines.

To resolve the issue, perform the following steps:

1. Check whether the sealing ring on the flow cell stage is intact or if any foreign particles are blocking the holes of the sealing ring.
2. Check whether there are any foreign particles on the rear of the flow cell or the surface of the flow cell stage. If particles are present, clean the flow cell stage. For details, refer to *Maintaining the flow cell stage on Page 125*.

Q: What should I do if the flow cell stage is leaking?

The flow cell stage may leak if:

- Sealing rings are not installed.
- Sealing rings are not correctly installed.
- There are foreign particles on the back side of the flow cell.
- The fluidics lines are blocked.

To resolve the issue, perform the following steps:

1. Check whether the sealing rings are installed.
2. Check whether the sealing rings on the flow cell stage are intact or if any foreign particles are blocking the holes of the sealing ring.
3. Check whether there are any foreign particles on the back side of the flow cell or the surface of the flow cell stage. If particles are present, clean the flow cell stage. For detail, refer to *Maintaining the flow cell stage on Page 125*.

Reagent FAQs

Q: What should I do if DNB concentration is low?

When DNB concentration is lower than that specified in *Table 24 on Page 63*, perform the following steps:

1. Check whether the DNB preparation kit has expired.
2. Check whether the library meets the requirements.
3. Make DNBs again. If the DNB concentration still does not meet the requirements after a new sample preparation, please contact CG Technical Support.

Q: What should I do if I forgot to add reagent into well No. 8 for PE sequencing run?

MDA Enzyme is required to make the second-strand template for PE sequencing. When preparing the Sequencing Reagent Cartridge, the appropriate amounts of MDA Enzyme Mix and MDA Reagent must be added to well No. 8. If MDA mixture was not added into well No. 8 before starting the sequencing run, this can be resolved by performing the following steps, as long as the sequencing run is in the sequencing phase of Read1:

1. Pause the run: Select the pause button ⏸ in the sequencing interface and select **Yes** when prompted.
2. Lift the needle:
    1) Select the stop button ■ and select **Yes** when prompted.
    2) Select **Finish**.
3. Fill well No. 8 of the Sequencing Reagent Cartridge:
    1) Open the reagent compartment door and take out the Sequencing Reagent Cartridge.
    2) Prepare the MDA mixture by adding the appropriate amount of MDA Enzyme Mix into the MDA Reagent tube.
    3) Mix thoroughly and transfer all solution into well No. 8. as described in *Preparing the Sequencing Reagent Cartridge on Page 79*.
    4) Insert the filled sequencing cartridge back into the sequencer.
4. Resume the run:
    1) Select **Sequence** > **Resume run** on the main interface.
    2) Clean the loaded flow cell with a canned air duster to ensure that no visible dust exists on the surface and back of the flow cell. Place the flow cell on the flow cell drive, and touch the flow cell drive control button to load the flow cell into the device.
    3) Select **Next** to review the parameters and ensure that all parameters are correct.
    4) Select **Start** > **Continue**.

Q: How do I resume a sequencing run?

The sequencing run might be stopped due to some unexpected errors during the run, such as mechanical gripper operation failure, flow cell transfer failure, fluidics failure, and photographing failure. This stopped run may be continued after resolving the issues causing the run to stop.

Perform the following steps:

1. When the sequencing run is prematurely halted due to unexpected errors, the sequencer's interface display may resemble that of the figure below. Select **Finish** to end the stopped run.

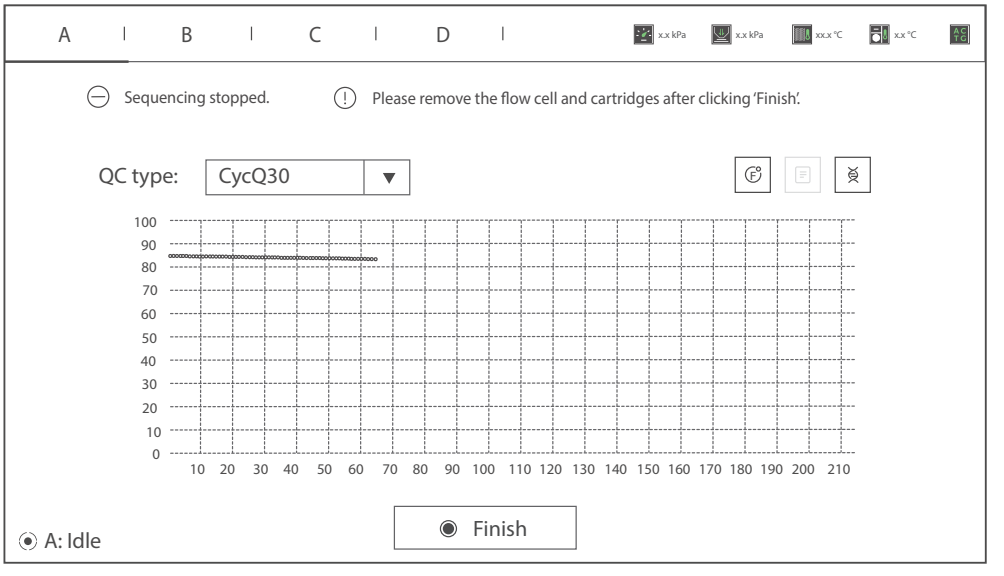

Figure 74 Interface of a stopped run

2. After resolving the issues that caused the run to stop, select **Sequence** > **Resume run** in the main interface.

   If the Sequencing Reagent Cartridge or Washing Cartridge is taken out for processing, ensure that the processed Sequencing Reagent Cartridge or Washing Cartridge is placed back in the corresponding compartment before resuming the sequencing run.

3. Re-load the flow cell:

   1) Dust the loaded flow cell of the interrupted sequencing run with a canned air duster. Ensure that no visible dust is present on the surface and back of the flow cell.
   2) Place the flow cell on the flow cell drive, and touch the flow cell drive control button to load the flow cell into the device.

4. Select **Next** to review the parameters and ensure that all information is correct.

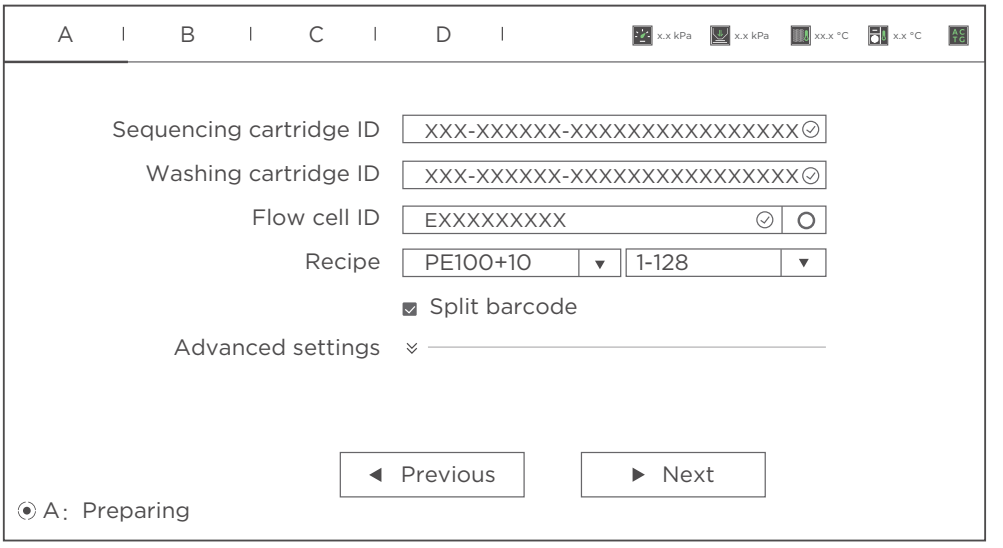

Figure 75 Cartridge ID, flow cell ID interface

5. Select **Start** > **Continue** to resume the sequencing run.

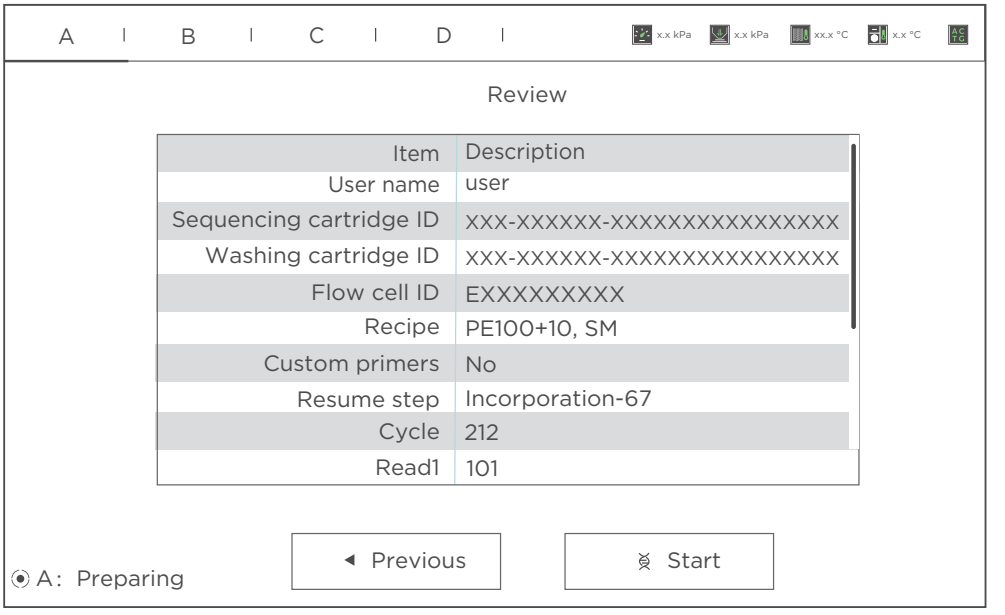

Figure 76 Sequencing parameter interface

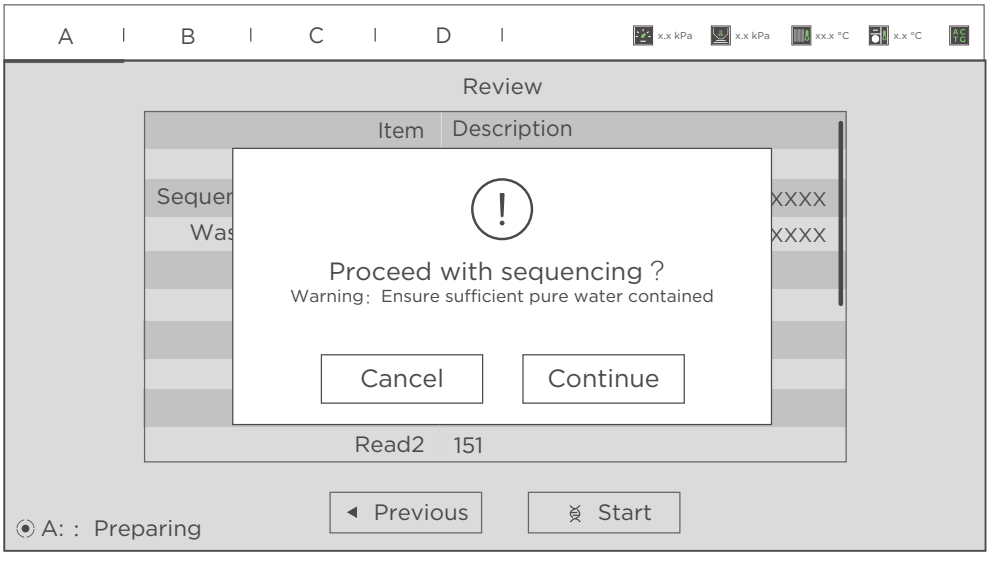

Figure 77 Continue run interface

Q: What rules should I follow if I need to store a reagent kit temporarily?

- If a kit has been thawed (including the dNTPs) but cannot be used within 24 hours, it can be frozen and thawed at most one time.

- If a kit has been thawed (including the dNTPs) but cannot be used immediately, store it at 2 °C to 8 °C. It is strongly recommended that you use it within 24 hours. A thawed kit that is stored at 2 °C to 8 °C may still be used within seven days, although it may compromise sequencing quality. It is not recommended that you use a kit that has been thawed and stored at 2 °C to 8 °C for more than seven days.
- If the dNTPs and Sequencing Enzyme Mix have been added into the cartridge, i.e. the cartridge has been prepared and the needles have punctured the seal but the cartridge cannot be used immediately, the cartridge must be covered with foil or plastic wrap. Store the kit at 2 °C to 8 °C and use it within 24 hours. Gently mix the reagents in the cartridge before use. When mixing, be careful not to spill any reagent from the needle holes to avoid reagent contamination.

Q: What should I do if abnormal negative pressure appears during flow cell attachment?

When the negative pressure value is shown in red, the negative pressure is abnormal. Perform the following steps:

1. Gently wipe the stage surface with a damp Kimwipes tissue and dust the stage with a canned air duster. Ensure that no dust is present on the flow cell stage.
2. Dust the back of the flow cell with a canned air duster to ensure no dust is present.
3. If the problem persists, please contact CG Technical Support.

Q: What should I do if bubbles appear in the flow cell?

Bubbles in DL-T7RS

- Check the rubber sealing ring to ensure that it is in the right position.
- Check the DNB loading plate to ensure that enough reagent is in each well.
- Replace the used flow cell and inspect the pump.
- If the problem persists, please contact CG Technical Support.

Bubbles in DNBSEQ-T7RS

- Check the water container to ensure that the water volume is sufficient.
- Ensure that the pure water tube goes through the handle.

  For information on placing the water tube, refer to *Preparing the pure water container on Page 45.*

- Check the reagent needles to ensure that they can immerse fully into the cartridges. Otherwise, restart the sequencing software.
- If the problem persists after a restart, please contact CG Technical Support.

Q: What should I do if pumping failure occurs during DNB loading and sequencing?

- Check if the pure water volume is sufficient.
- When error occurs on DL-T7RS and DNBSEQ-T7RS:
  - Remove the flow cell and check for is dust on the sealing gasket. Remove any dust with a canned air duster.
  - Place the flow cell by following the instructions and start the pump again.
- Check if the sampling needles are moving properly. If the sampling needles are not moving properly, restart the control software of the sequencer.
- If the problem persists, please contact CG Technical Support.

Q: What should I do if impurities appear in the original sequencing image?

If impurities appear, perform the following steps:

1. Perform a manual wash on DL-T7RS and DNBSEQ-T7RS.
2. If there is still no improvement after manual wash, prepare washing reagents again according to *Preparing washing reagents on Page 117*, and perform a manual wash again on DL-T7RS and DNBSEQ-T7RS.
3. If the problem persists, please contact CG Technical Support.

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Instructions for importing barcode

Preparing a barcode file

Ensure that the barcode file meets the following requirements:

- The barcode file to be imported should be named “barcode.csv”. In the imported directory, only one “barcode.csv” file is available.
- It is recommended that you use the “Notepad++” program to open the barcode file. Barcode ID and barcode sequences in the file should be separated by a comma.
- The barcode file should not contain blank lines or full-width characters. The barcode sequence should include no fewer than two bases.
- Barcode sequence should be unique, and barcode ID and barcode sequence should not be empty.
- Barcode sequences of a DualBarcode file should not contain any characters other than “A”, “T”, “C”, “G”, and “N”.
- Barcode sequences of a single barcode file should not contain any characters other than “A”, “T”, “C”, and “G”.
- Barcode name and mismatch number are mandatory for each barcode file.

Single barcode file

An example for single barcode file is shown in the figure below:

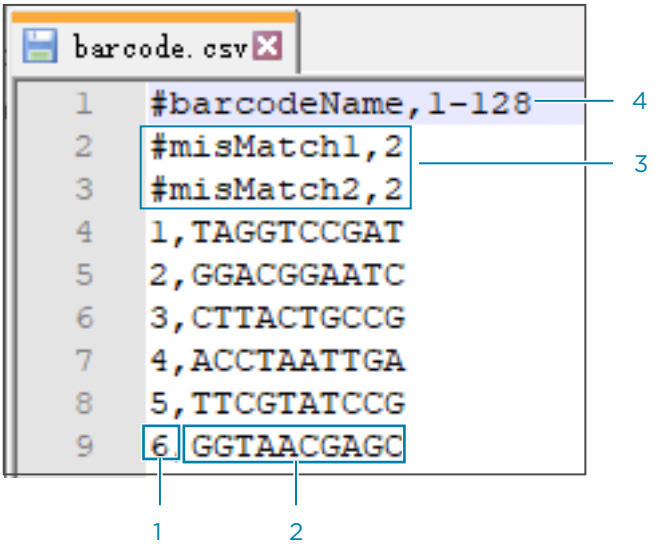

Figure 78 **Exemplary single barcode file**

No.	Name	No.	Name
1	Barcode ID	2	Barcode sequence
3	mismatch number	4	Barcode name

Single and DualBarcode file

Mixed barcode splitting (both single barcode and DualBarcode splitting) is supported in the following cases:

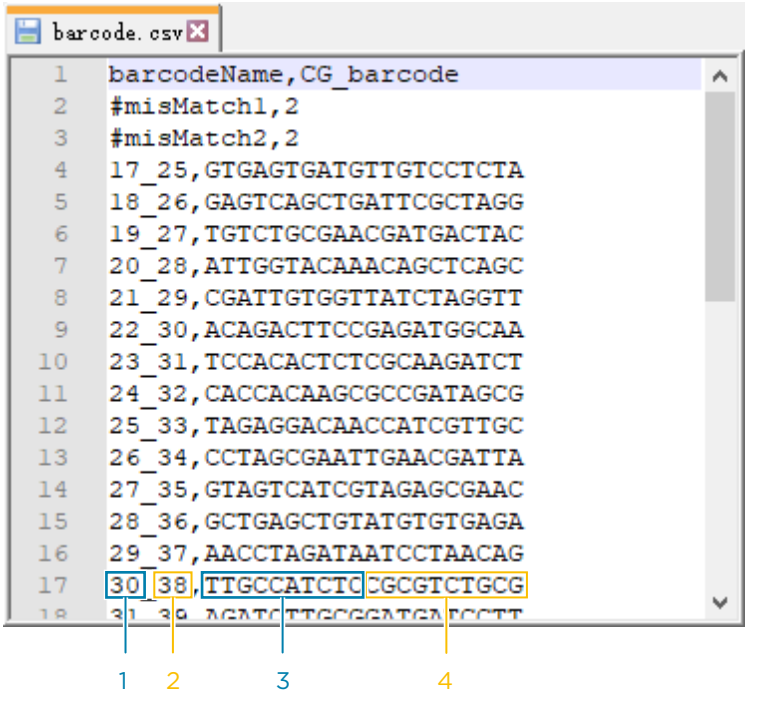

Figure 79 **Single and DualBarcode file1**

No.	Description
1	Corresponds to ID of **DualBarcode** in the **Customize a recipe** interface
2	Corresponds to ID of **Barcode** in the **Customize a recipe** interface
3	Corresponds to sequence of **DualBarcode** in the **Customize a recipe** interface
4	Corresponds to sequence of **Barcode** in the **Customize a recipe** interface

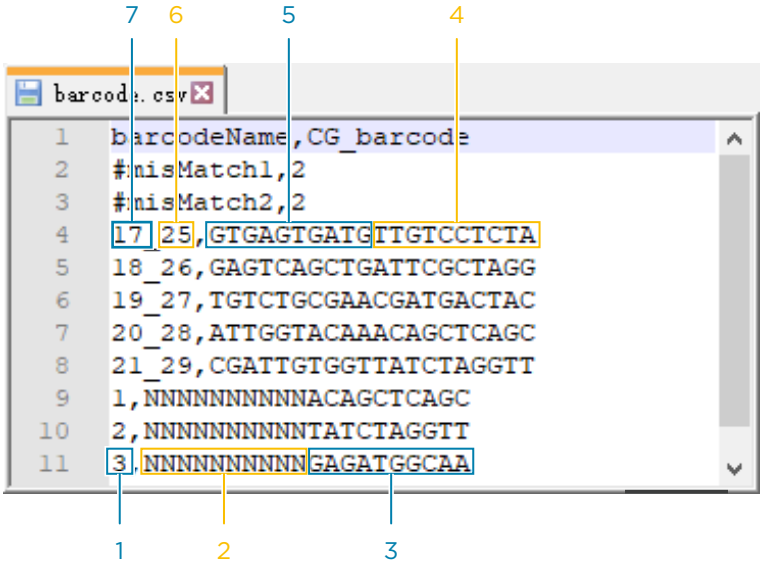

Figure 80 Single and DualBarcode file 2

No.	Description
1	Corresponds to ID of **Barcode** in the **Customize a recipe** interface
2	Placeholder
3	Corresponds to sequence of **Barcode** in the **Customize a recipe** interface
4	Corresponds to sequence of **Barcode** in the **Customize a recipe** interface
5	Corresponds to sequence of **DualBarcode** in the **Customize a recipe** interface
6	Corresponds to ID of **Barcode** in the **Customize a recipe** interface
7	Corresponds to ID of **DualBarcode** in the **Customize a recipe** interface

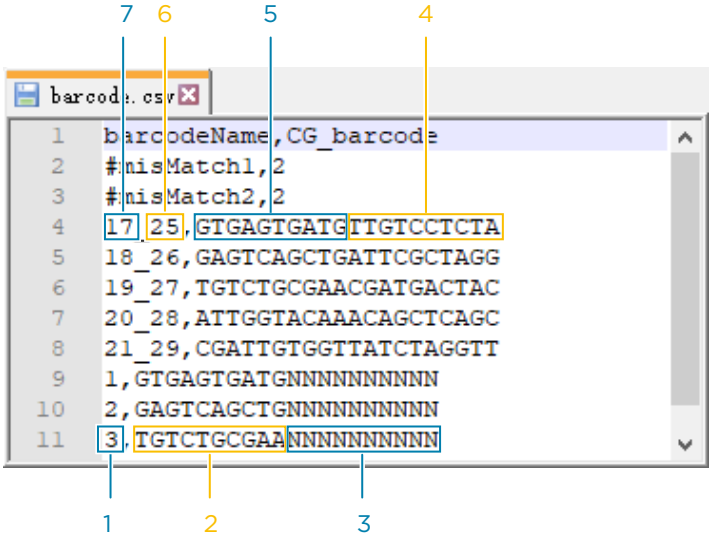

Figure 81 **Single and DualBarcode file 3**

No.	Description
1	Corresponds to ID of **Barcode** in the **Customize a recipe** interface
2	Corresponds to sequence of Barcode in the **Customize a recipe** interface
3	Placeholder
4	Corresponds to sequence of Barcode in the **Customize a recipe** interface
5	Corresponds to sequence of **DualBarcode** in the **Customize a recipe** interface
6	Corresponds to ID of **Barcode** in the **Customize a recipe** interface
7	Corresponds to ID of **DualBarcode** in the **Customize a recipe** interface

Importing a barcode file

Before use, it is recommended that you format the external storage device (for example, a USB storage drive).

Perform the following steps:

1. Obtain an external storage device (for example, a USB storage drive), and create a folder in the root directory of the storage device. Ensure that the folder name is in English. Copy the prepared "*barcode.csv*" file to the folder.
2. On the main interface, select **Sequence** > **New run**.

3. Select the second ▼ next to **Recipe**, and select **Import** to import barcodes to the device from the external storage device.

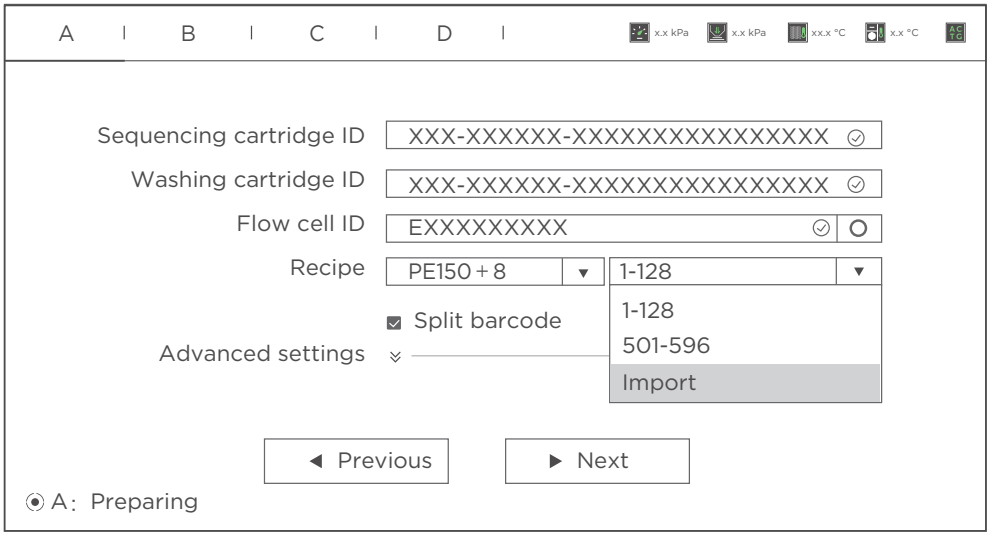

Figure 82 Barcode settings interface

4. Select **Split barcode** if needed.
5. Select **Next** and then **Previous** again to check whether the imported barcodes are displayed in the barcode list.

The same barcode only needs to be imported once.

Instructions for customizing a run

Introductions

This section provides instructions for customizing a sequencing run, which might be needed in the following situations:

- When read length(s) in Read2 and/or Read1 are not the same as those predefined in the Recipe list.
- For a single barcode sequencing run, the barcode sequences are customized.
- All DualBarcode sequencing runs:
  - There are four types of barcode splitting scenarios for DualBarcode sequencing: splitting only the barcode, splitting only the DualBarcode, splitting both the barcode and DualBarcode, and not splitting the barcode and DualBarcode.
  - In the case of DualBarcode sequencing but only one barcode splitting needed, the barcode file needs to be the same as that of splitting both the barcode and DualBarcode, do not delete the contents of the non-split barcode. That is, the base number of the barcode file needs to be consistent with that of the barcode and DualBarcode sequencing, otherwise it cannot be split.
- Dark reaction cycles are required in Read1 and/or Read2 sequencing.
- stLFR FCL PE100
- UMI (Unique Molecular Identifier) +UDI (Unique Dual Index)

Important interfaces for customizing a run

To enter the **Customize a recipe** interface, select the first ▼ next to **Recipe** and select **Customize a recipe** in the sequencing parameters interface.

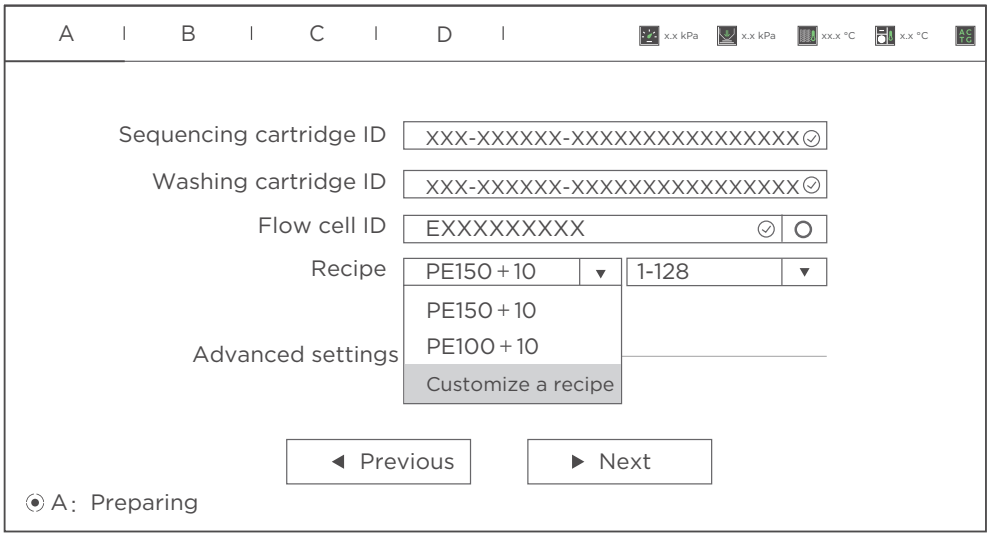

Figure 83 Selecting Customize a recipe

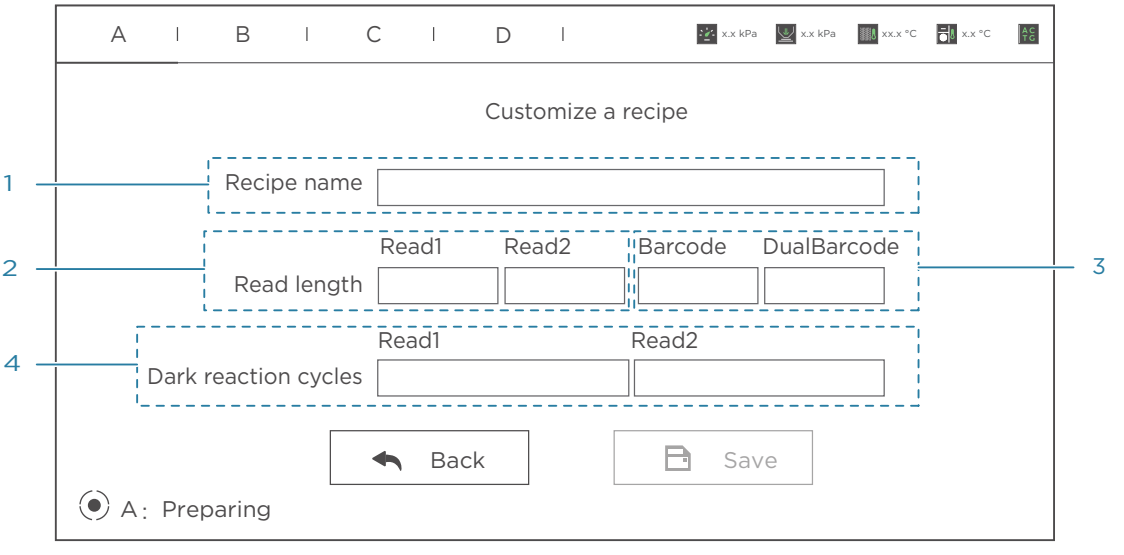

Figure 84 Customize a recipe interface

The following table describes control functions in the **Customize a recipe** interface:

No.	Item	Description
1	**Recipe name**	Name the customized recipe.
2	**Read1/Read2**	Customize Read1 and/or Read2 length for a sequencing run.
3	**Barcode/DualBarcode**	Customize Barcode length for a sequencing run. Customize DualBarcode length for a sequencing run.
4	**Dark reaction cycles**	Customize dark reaction range in Read1 and/or Read2.

The rules for filling in the **Customize a recipe** interface are as follows:

- When naming a sequencing recipe, use only letters, numbers, "+", "_" and "–".
- Because a previously named recipe will be saved in the recipe drop-down menu, duplicate name checking will be performed to ensure that each sequencing recipe name is unique (i.e., a new recipe name must not be the same as an existing recipe name).
- Enter numbers in the read length boxes of Read1, Read2, Barcode and DualBarcode.
- Multiple ranges of dark reaction cycles can be set in the Read1 and Read2 entry for "Dark reaction cycles". Use "," to separate the ranges. The dark reaction cycles of the ranges are presented in the format of "number" and "number-number".

Examples of customized run

- Before starting the customized run, confirm that the customized barcode files are already imported into the sequencer. If not, refer to *Instructions for importing barcode on Page 139* to import the customized barcode.
- Ensure that the total number of sequencing cycles including Read1, Read2, barcode, DualBarcode, and dark cycle is less than the maximum sequencing cycles for a given sequencing kit as defined in *Table 2 Sequencing cycle on Page 43*.
- The maximum read length for both Read1 and Read2 should not exceed that specified in the sequencing kit. For example: If PE150 is used, the maximum customized Read1 length and Read2 length should not exceed 150 bp.
- Dark reaction cycle: A sequencing cycle in which the chemical reaction is performed, but with no imaging. Therefore, the output FASTQ file will not contain the dark cycle information. For example: For FCL PE150 sequencing, if cycle 2-10 for Read1 are dark cycles, the total cycles in the FASTQ file for Read1 is 141.

You can refer to the following setting examples for your customized run.

1. Read1/Read2 lengths are not the same as those predefined in the Recipe list

Assumptions are as below:

- Sequencing run: PE150+10.
- Length of Read1: 120.
- Length of Read2: 140.
- Length of barcode: 10.
- Length of DualBarcode: 0.
- Split barcode: Yes.
- Total cycles = 120+140+10+2 = 272.
- Select a PE150 kit.

The Customize interface is set as follows:

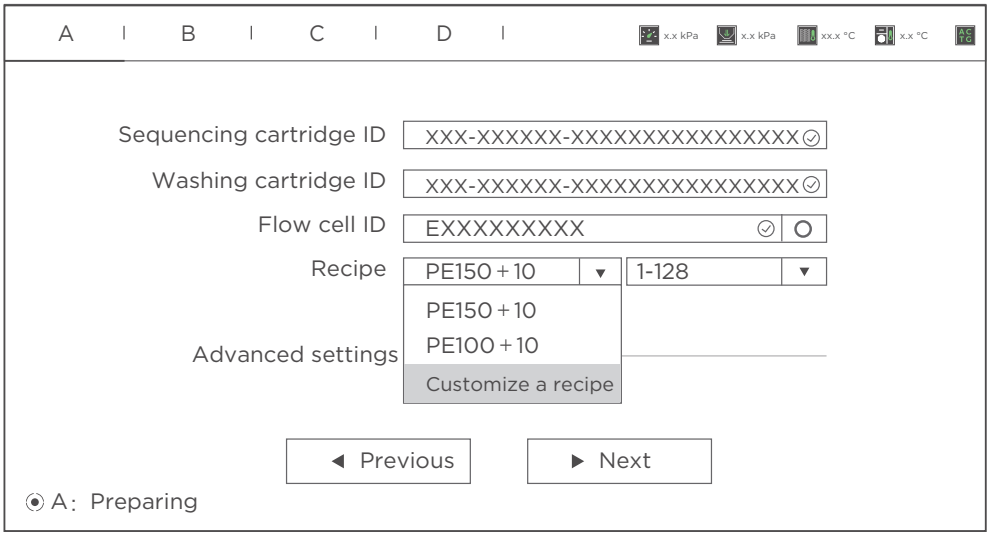

Figure 85 Selecting *Customize a recipe*

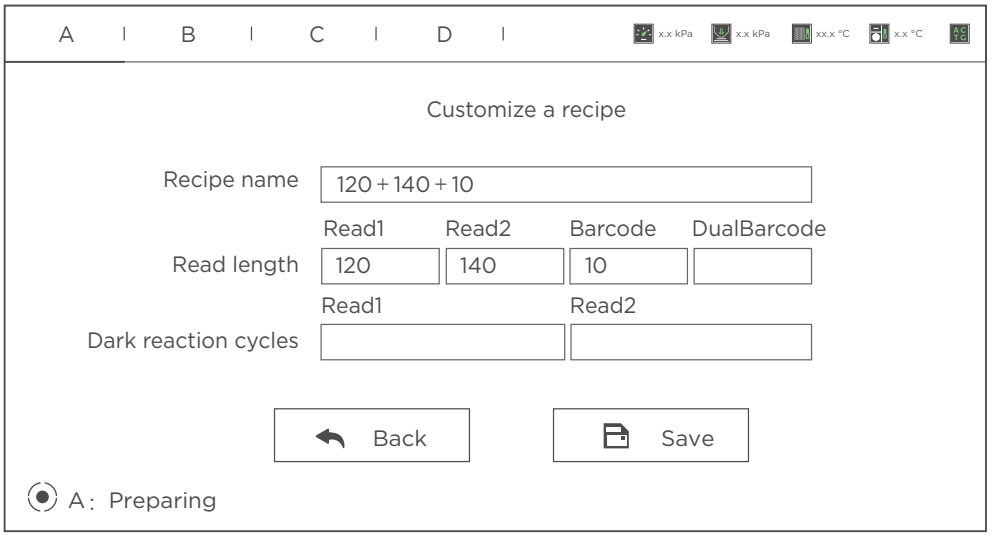

Figure 86 Configuring customized settings

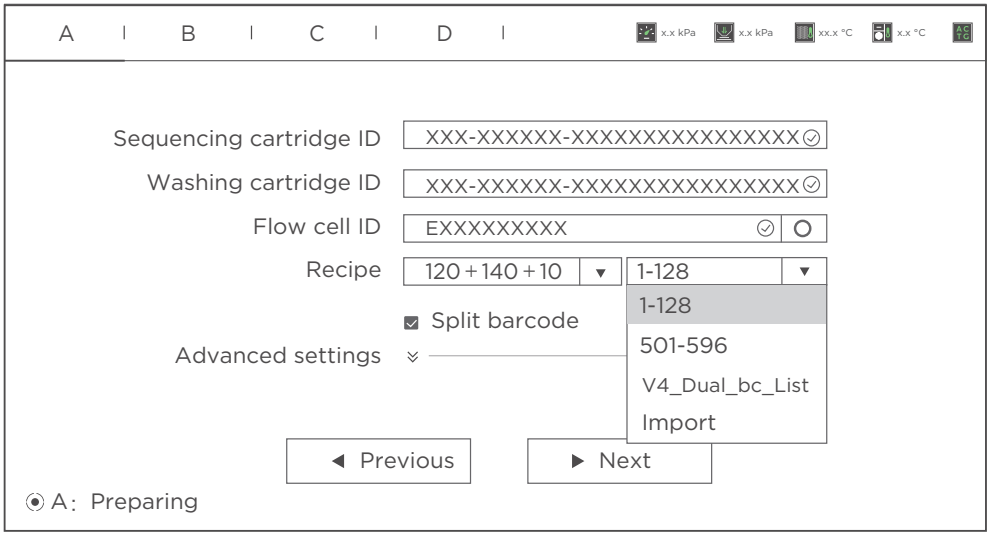

Figure 87 Selecting barcode file

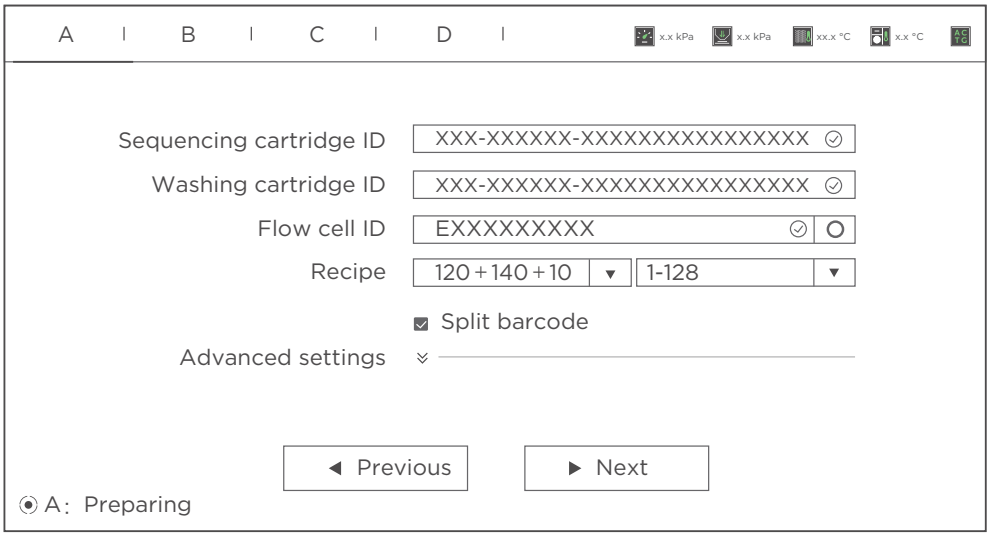

Figure 88 Checking barcode splitting

2. Length of the single barcode is not 10

Assumptions are as below:

- Sequencing run: PE150+8.
- Length of Read1: 150.
- Length of Read2: 150.
- Length of barcode: 8.
- Length of DualBarcode: 0.
- Split barcode: Yes.
- Total cycles = 150+150+8+2 = 310.
- Select a PE150 kit.

The **Customize a recipe** interface is set as follows:

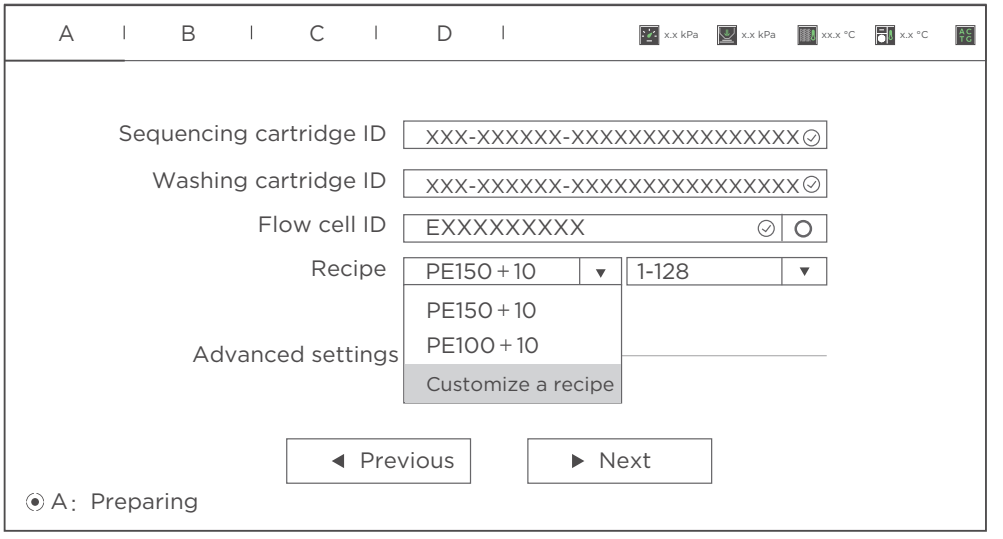

Figure 89 **Selecting *Customize a recipe***

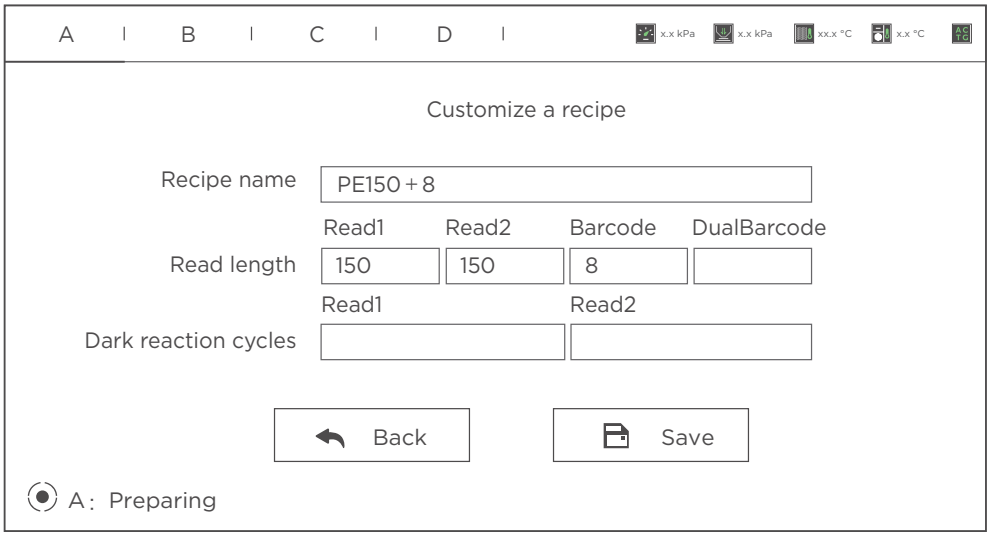

Figure 90 **Configuring customized settings**

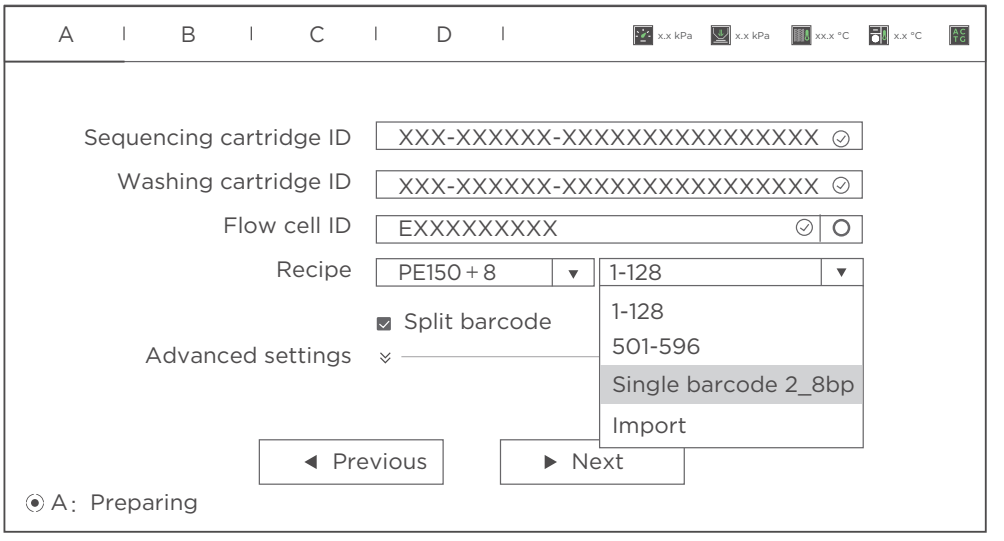

Figure 91 Selecting barcode file

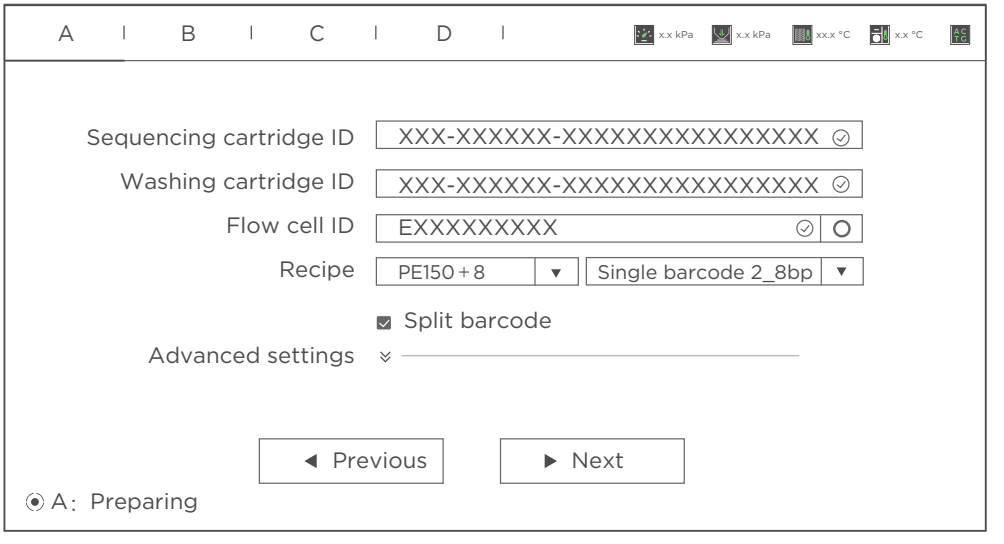

Figure 92 Checking barcode splitting

3. Different barcode lengths for DualBarcode sequencing

Assumptions are as below:

- Sequencing run: PE150+6+10.
- Length of Read1: 150.
- Length of Read2: 150.
- Length of barcode: 6.
- Length of DualBarcode: 10.

- Split barcode: Yes.
- Split DualBarcode: Yes.
- Total cycles = 150+150+6+10+2 = 318.
- Select a PE150 kit.

The **Customize a recipe** interface is set as follows:

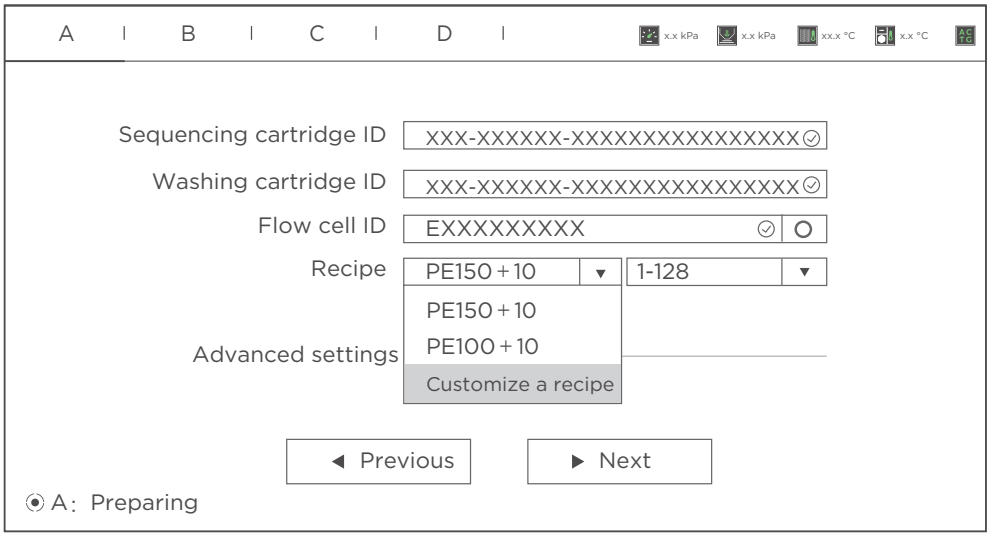

Figure 93 Selecting *Customize a recipe*

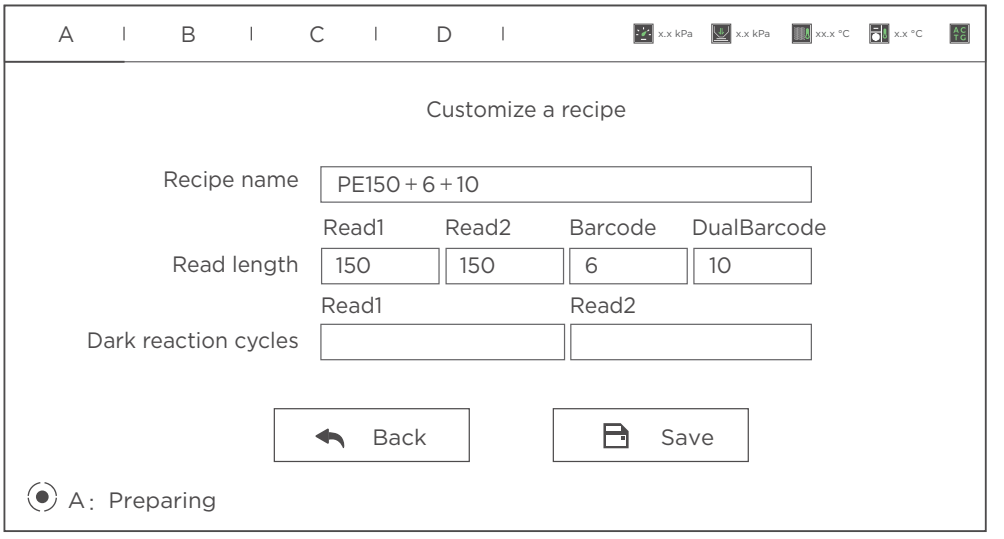

Figure 94 Configuring customized settings

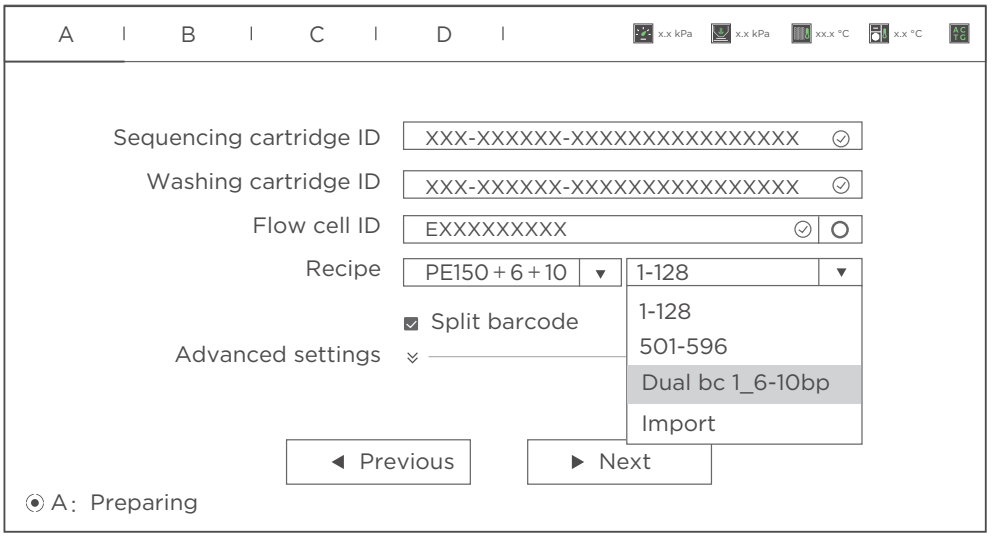

Figure 95 Selecting barcode file

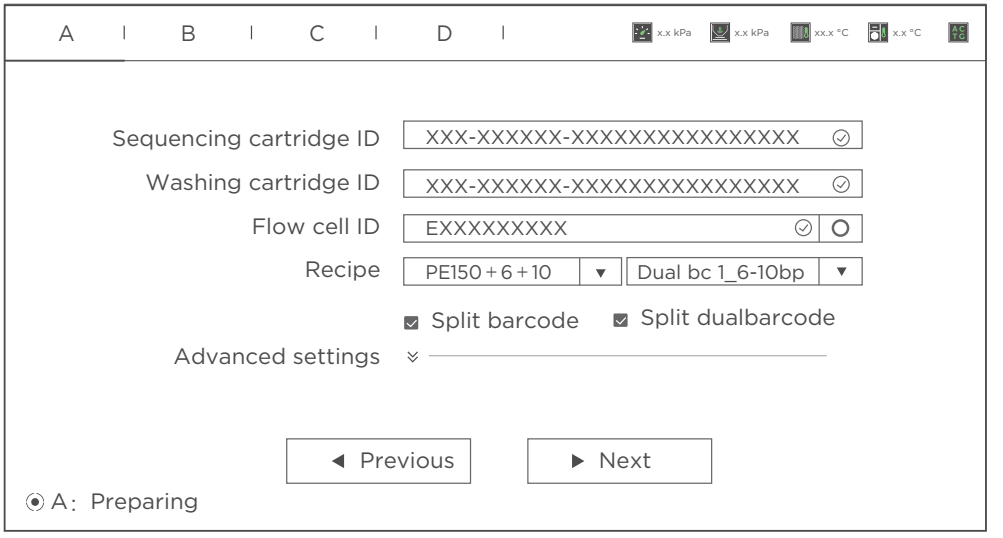

Figure 96 **Checking barcode splitting**

4. Dark reaction cycles are required in Read1 and/or Read2 sequencing

Assumptions are as below:

- Sequencing run: PE100+8+8.
- Length of Read1: 100.
- Length of Read2: 100.
- Length of barcode: 8.
- Length of DualBarcode: 8.
- Split barcode: Yes.
- Split DualBarcode: Yes.
- Dark cycles: From cycle-20 to cycle-30 and cycle-50 to cycle-60 in Read1 and cycle-16 to cycle-20 in Read2.
- Total cycles = 100+100+8+8 +2= 218.
- Select a PE100 kit.

The **Customize a recipe** interface is set as follows:

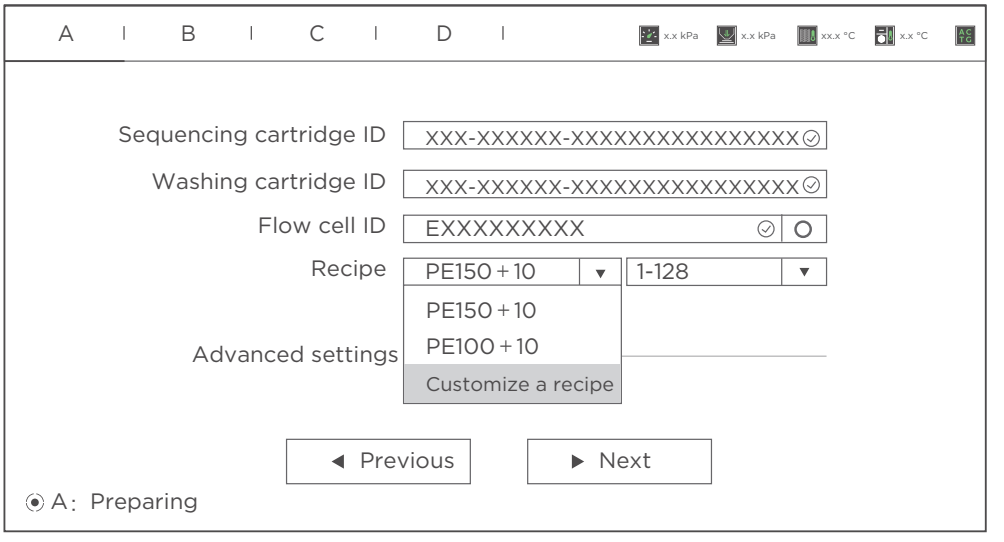

Figure 97 Selecting Customize a recipe

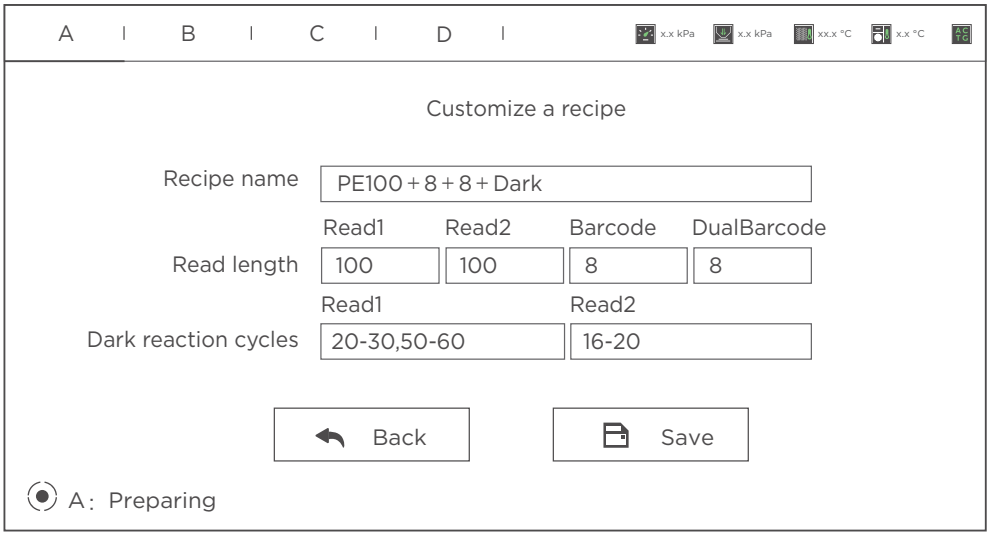

Figure 98 Configuring customized settings

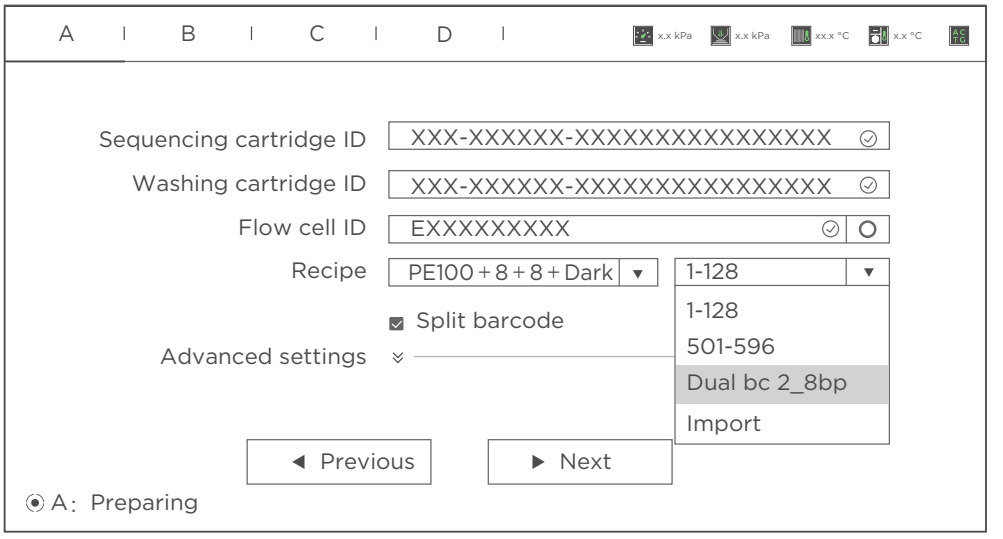

Figure 99 **Selecting barcode file**

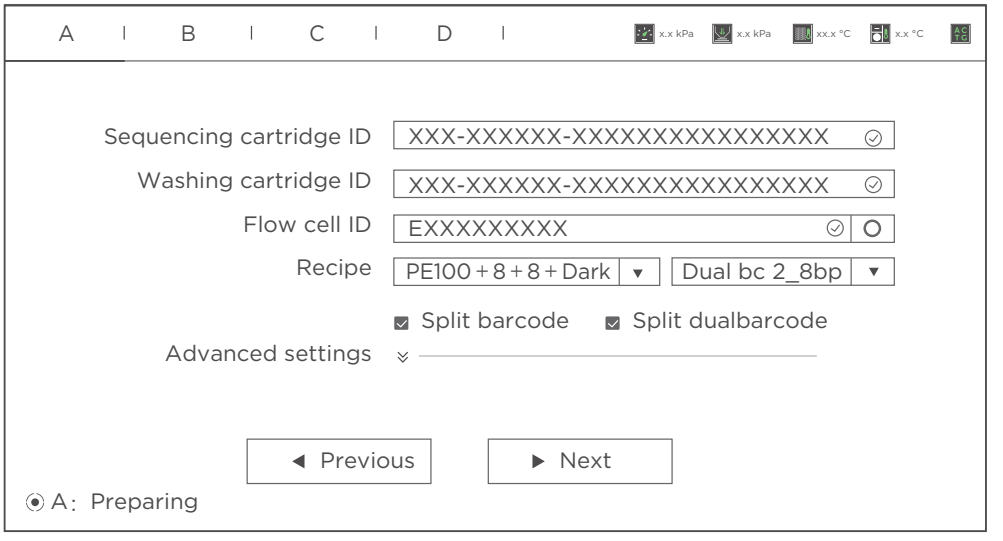

Figure 100 **Checking barcode splitting**

5. stLFR FCL PE100

Assumptions are as below:

- Sequencing run: stLFR FCL PE100.
- Length of Read1: 100.
- Length of Read2: 100.
- Length of barcode: 42.
- Length of DualBarcode: 10.

- Split barcode: Yes.
- Total cycles = 100+100+42+10 +2= 254.
- Select a stLFR PE100 kit.

As long as the customize settings meet the following conditions: Read1 100 bp, Read2 100 bp, barcode 42 bp, and DualBarcode 10 bp, the interface of checking barcode splitting will automatically display only the **Split barcode** option. Checking this **Split barcode** actually means splitting the 10 bp DualBarcode.

The **Customize a recipe** interface is set as follows:

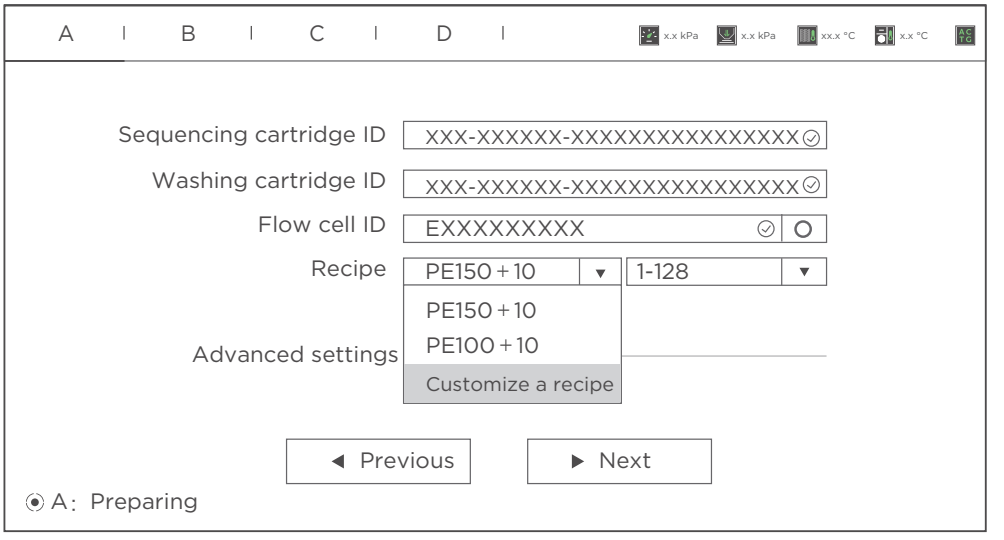

Figure 101 Selecting *Customize a recipe*

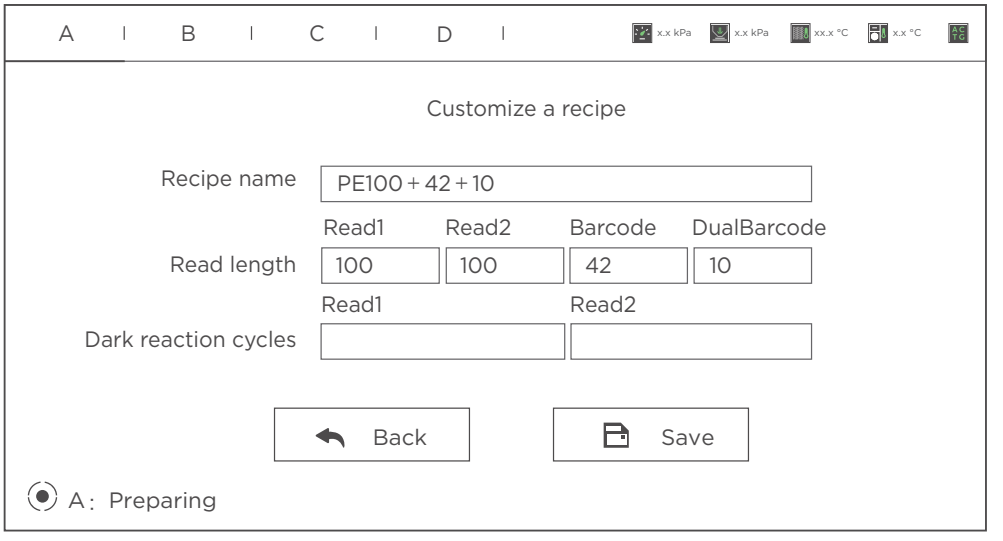

Figure 102 Configuring customized settings

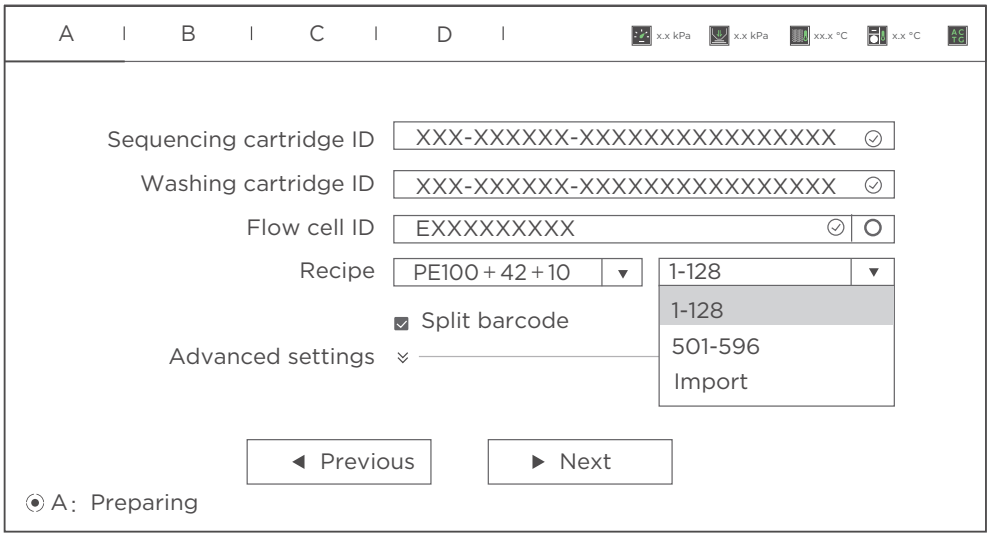

Figure 103 Selecting barcode file

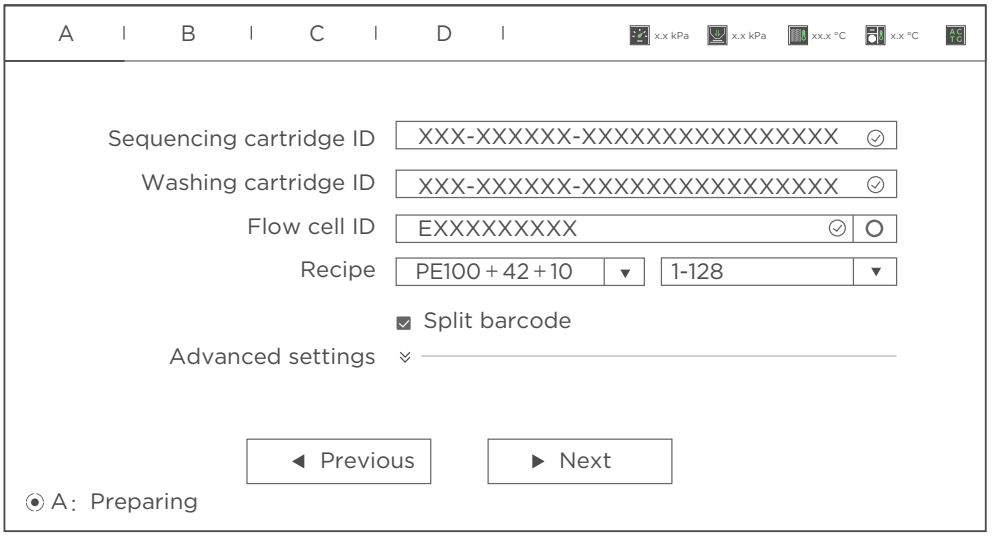

Figure 104 **Checking barcode splitting**

6. UMI+UDI

Assumptions are as below:

- Sequencing run: PE100+8+(8+9).
- Length of Read1: 100.
- Length of Read2: 100.
- Length of barcode: 8.
- Length of DualBarcode: 17.
- Split barcode: Yes.
- Split DualBarcode: Yes.
- Total cycles = 100+100+8+17+2 = 227.
- Select a PE100 kit.

The **Customize a recipe** interface is set as follows:

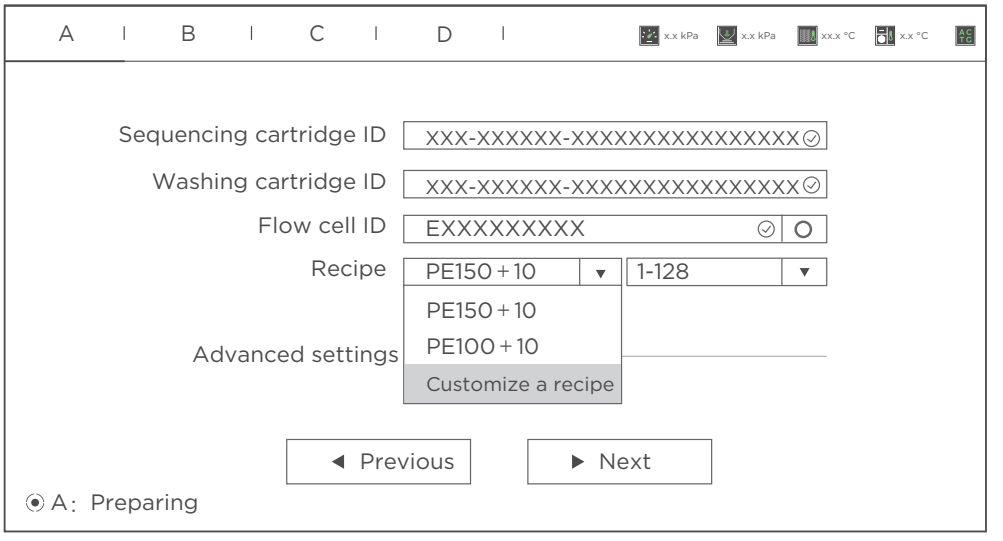

Figure 105 Selecting *Customize a recipe*

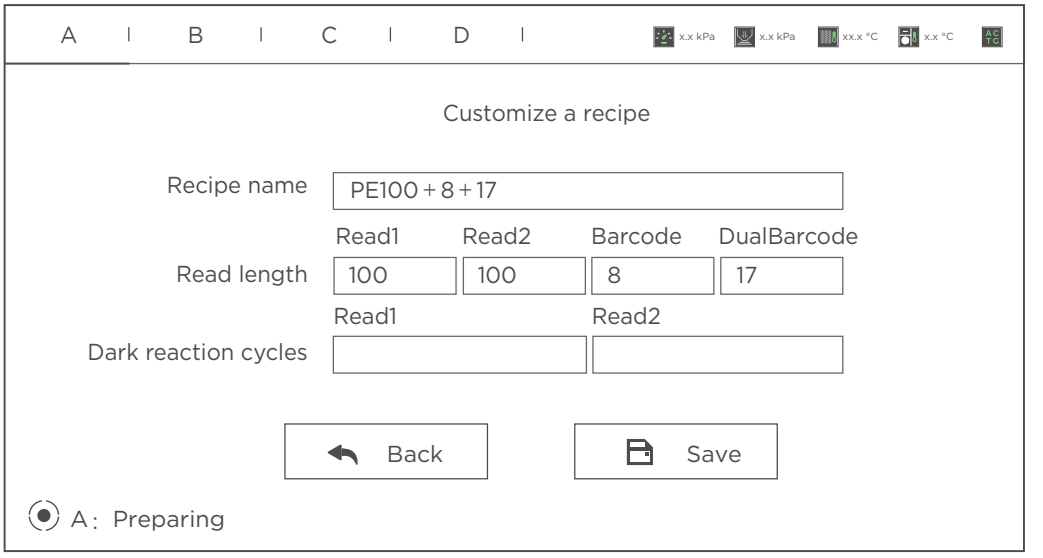

Figure 106 Configuring customized settings

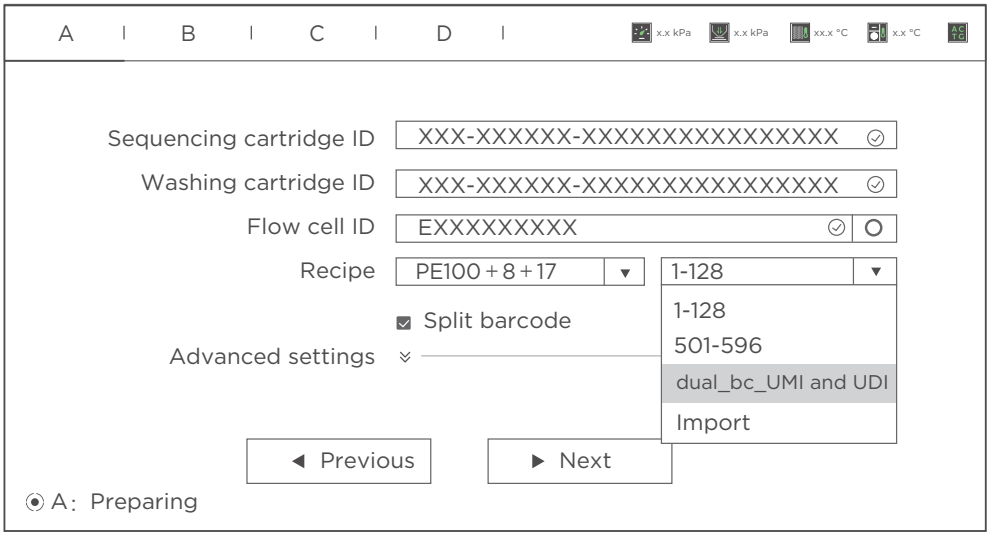

Figure 107 Selecting barcode file

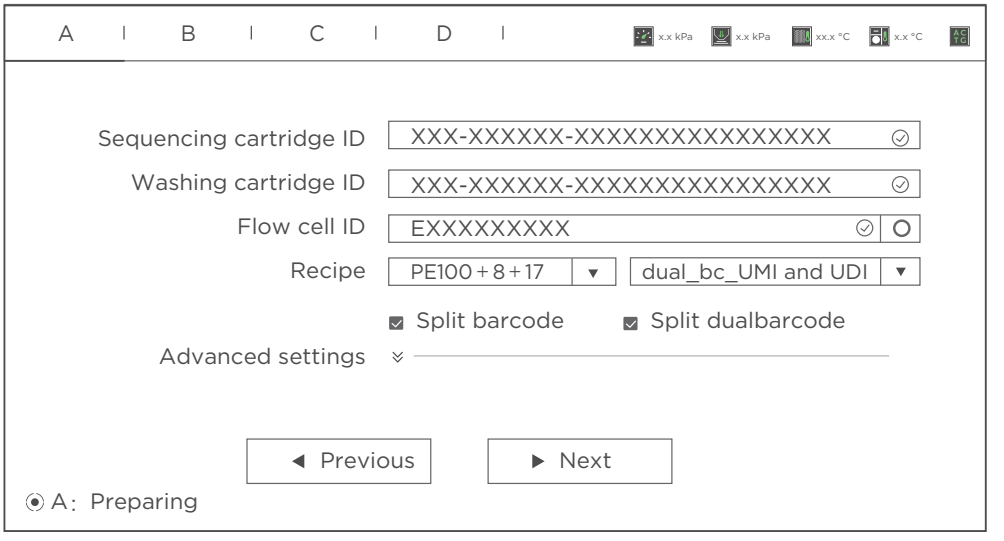

Figure 108 Checking barcode splitting

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Instructions for using Qubit to quantify the DNBs

- Working solution should be used within 0.5 hours after preparation.
- Avoid touching the wall of tapered detection tubes.
- Avoid introducing bubbles in detection tubes.

Perform the following steps:

1. Prepare the Qubit working solution by diluting the Qubit ssDNA Reagent 1:200 in Qubit ssDNA Buffer. Use a clean Qubit assay tube each time you prepare Qubit working solution. Do not mix the working solution in a glass container.

   The final volume in each tube must be 200 QL. Each standard tube requires 190 QL of Qubit working solution, and each sample tube requires anywhere from 180–199 QL of Qubit working solution.

   Prepare sufficient Qubit working solution to accommodate all standards and samples.

   For example: for 8 samples, prepare enough working solution for the samples and 2 standards. ~200 µL per tube in 10 tubes yields a total of 2 mL of working solution (10 µL of Qubit reagent plus 1990 µL of Qubit Buffer).
2. Add 190 µL of Qubit working solution to each of the tubes used for standards.
3. Add 10 µL of each Qubit standard to the appropriate tube and mix by vortexing 3 to 5 seconds. Be careful not to create bubbles.
4. Set up the required number of 0.5-mL tubes for standards and samples. The Qubit ssDNA Assay requires 2 standards.

   - Use only thin-wall, clear, 0.5-mL PCR tubes. Acceptable tubes include Qubit assay tubes (Cat. No. Q32856) or Axygen PCR-05-C tubes (Part No. 10011-830).
   - Number of Qubit test tubes needed are the number of samples plus 2 standards tubes. For example, if you have 3 samples, you will need 5 tubes.
5. Label the tube lids. Do not label the side of tube.
6. Prepare the solutions used for standards and sample tests according to the table below:

/	S1 (µL)	S2 (µL)	D1 (µL)	D2 (µL)	D3 (µL)
Working solution	190	190	198	198	198

/	S1 (µL)	S2 (µL)	D1 (µL)	D2 (µL)	D3 (µL)
S1 (0 ng/µL)	10	/	/	/	/
S2 (20 ng/µL)	/	10	/	/	/
Sample (µL)	/	/	2	2	2
Total volume	200	200	200	200	200

7. Mix the tubes by using a vortex mixer and centrifuge briefly for five seconds. Incubate at room temperature for two minutes.
8. Refer to the Qubit user manual for instructions on reading standards and samples. Follow the appropriate procedure for your instrument.

List of sequencing set components

Table 37 DNBSEQ-T7RS High-throughput Sequencing Set (FCL PE100)
Catalog number: 940-000838-00

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiration date
DNBSEQ-T7RS Sequencing Flow Cell (T7-2 FCL PE100) **Catalog number: 930-000076-00**					
Sequencing Flow Cell (T7-2 FCL)	/	1 EA	2 °C to 8 °C	2 °C to 8 °C	10 months
DNBSEQ-T7RS DNB Make Reagent Kit (FCL PE100) **Catalog number: 940-000848-00**					
Low TE Buffer		960 µL/tube×1 tube	-80 °C to -15 °C	-25 °C to -15 °C	12 months
Make DNB Buffer		400 µL/tube×1 tube			
Make DNB Enzyme Mix I		800 µL/tube×1 tube			
Make DNB Enzyme Mix II (LC)		80 µL/tube×1 tube			
Stop DNB Reaction Buffer		400 µL/tube×1 tube			
DNBSEQ-T7RS DNB Load Reagent Kit (FCL PE100) **Catalog number: 940-000844-00**					
DNB Load Buffer I		300 µL/tube×1 tube	-80 °C to -15 °C	-25 °C to -15 °C	12 months
DNB Load Buffer II		150 µL/tube×1 tube			
Micro Tube 0.5 mL (Empty)		1 tube			
DNB Load Plate (T7 FCL PE100)	/	1 EA			

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiration date
DNBSEQ-T7RS High-throughput Sequencing Kit (FCL PE100) Catalog number:940-000837-00					
dNTPs Mix II		8.28 mL / tube×1 tube	-80 °C to -15 °C	-25 °C to -15 °C	12 months
dNTPs Mix V		2.76 mL / tube×1 tube			
Sequencing Enzyme Mix		5.52 mL / tube×1 tube			
MDA Reagent		4.20 mL / tube×1 tube			
MDA Enzyme Mix		0.60 mL / tube×1 tube			
Sequencing Reagent Cartridge	/	1 EA			
Transparent Sealing film	/	2 sheets			
DNBSEQ-T7RS Cleaning Reagent Kit (FCL PE100) Catalog number: 940-000842-00					
Washing Cartridge	/	1 EA	below 40 °C	0 °C to 30 °C	12 months

Table 38 DNBSEQ-T7RS High-throughput Sequencing Set (FCL PE150)
Catalog number: 940-000836-00

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiry date
DNBSEQ-T7RS Sequencing Flow Cell (T7-2 FCL PE150) Catalog number: 930-000869-00					
Sequencing Flow Cell (T7-2 FCL)	/	1 EA	2 °C to 8 °C	2 °C to 8 °C	10 months
DNBSEQ-T7RS DNB Make Reagent Kit (FCL PE150) Catalog number: 940-000847-00					
Low TE Buffer		960 µL/tube×1 tube	-80 °C to -15 °C	-25 °C to -15 °C	12 months
Make DNB Buffer		400 µL/tube×1 tube			
Make DNB Rapid Enzyme Mix II		800 µL/tube×1 tube			
Make DNB Enzyme Mix II (LC)		80 µL/tube×1 tube			
Stop DNB Reaction Buffer		400 µL/tube×1 tube			
DNBSEQ-T7RS DNB Load Reagent Kit (FCL PE150) Catalog number: 940-000845-00					
DNB Load Buffer IV		200 µL/tube×1 tube	-80 °C to -15 °C	-25 °C to -15 °C	12 months
Micro Tube 0.5 mL (Empty)		1 tube			
DNB Load Plate (T7 FCL PE150)	/	1 EA			
DNBSEQ-T7RS High-throughput Sequencing Kit (FCL PE150) Catalog number: 940-000835-00					

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiry date
dNTPs Mix II		5.61 mL/tube×2 tube	-80 °C to -15 °C	-25 °C to -15 °C	12 months
dNTPs Mix V		3.74 mL/tube×1 tube			
Sequencing Enzyme Mix		7.48 mL/tube×1 tube			
MDA Reagent		4.20 mL/tube×1 tube			
MDA Enzyme Mix		0.60 mL/tube×1 tube			
Sequencing Reagent Cartridge	/	1 EA			
Transparent Sealing film	/	2 sheets			
DNBSEQ-T7RS Cleaning Reagent Kit (FCL PE150) **Catalog number: 940-000841-00**					
Washing Cartridge	/	1 EA	below 40 °C	0 °C to 30 °C	12 months

Table 39 DNBSEQ-T7RS High-throughput Sequencing Set (stLFR FCL PE100)
Catalog number: 940-000840-00

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiry date
DNBSEQ-T7RS Sequencing Flow Cell (stLFR FCL PE100) Catalog number: 930-000077-00					
Sequencing Flow Cell (T7 FCL)	/	1 EA	0 °C to 30 °C	0 °C to 30 °C	10 months
DNBSEQ-T7RS DNB Make Reagent Kit (stLFR FCL PE100) Catalog number: 940-000849-00					
Low TE Buffer		480 µL/tube×1 tube	-80 °C to -15 °C	-25 °C to -15 °C	12 months
stLFR Make DNB Buffer		160 µL/tube×1 tube			
Make DNB Enzyme Mix III		320 µL/tube×1 tube			
Make DNB Enzyme Mix IV		42 µL/tube×1 tube			
Stop DNB Reaction Buffer		200 µL/tube×1 tube			
DNBSEQ-T7RS DNB Load Reagent Kit (stLFR FCL PE100) Catalog number: 940-000846-00					
DNB Load Buffer I		500 µL/tube×1 tube	-80 °C to -15 °C	-25 °C to -15 °C	12 months
DNB Load Buffer II		500 µL/tube×1 tube			
Micro Tube 0.5 mL (Empty)		1 tube			
DNB Load Plate (T7 stLFR FCL PE100)	/	1 EA			
DNBSEQ-T7RS High-throughput Sequencing Kit (stLFR FCL PE100) Catalog number: 940-000839-00					

Component	Cap color	Spec & quantity	Transportation temperature	Storage temperature	Expiry date
dNTPs Mix II		4.90 mL/tube×3 tube	-80 °C to -15 °C	-25 °C to -15 °C	12 months
dNTPs Mix IV		5.40 mL/tube×1 tube			
Sequencing Enzyme Mix		5.15 mL/tube×2 tube			
MDA Reagent		4.20 mL/tube×1 tube			
MDA Enzyme Mix		0.60 mL/tube×1 tube			
Sequencing Reagent Cartridge	/	1 EA			
Transparent Sealing film	/	2 sheets			
DNBSEQ-T7RS Cleaning Reagent Kit (stLFR FCL PE100) Catalog number: 940-000843-00					
Washing Cartridge	/	1 EA	below 40 °C	0 °C to 30 °C	12 months