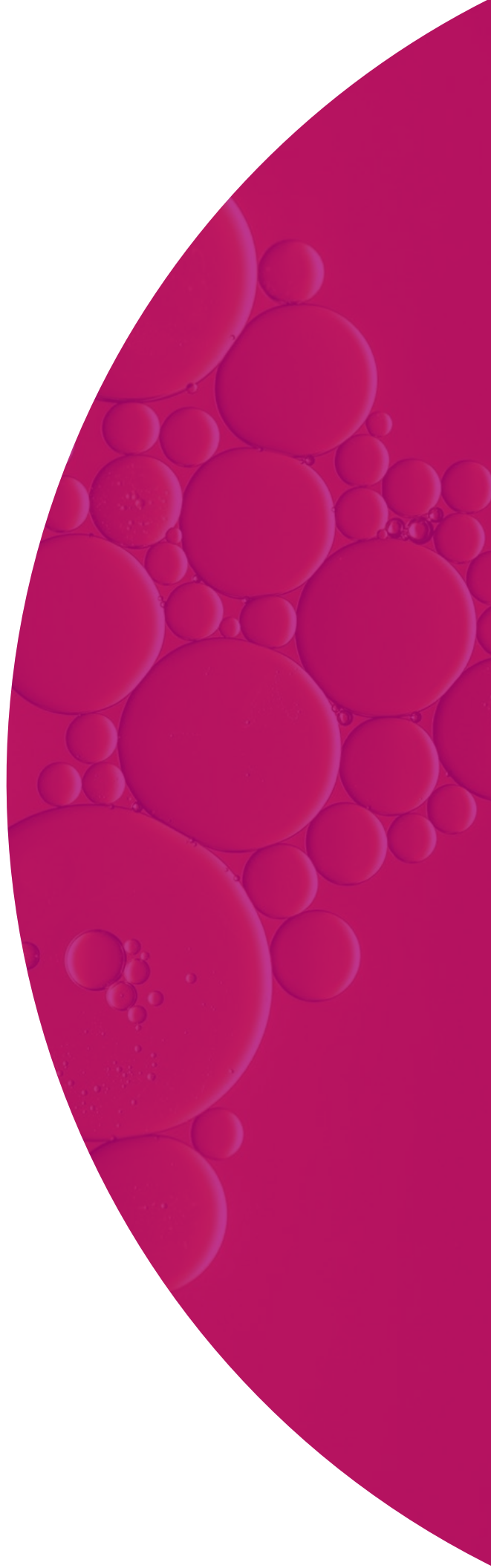




# Revio<sup>®</sup> system

Operations guide for ICS v13.5



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P/N 103-920-300 REV 01

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# Introduction

This guide describes the use and operation of the Revio system when installed with Instrument Control Software (ICS) version 13.5, which enables single use SMRT® Cell or multi-use SMRT® Cell - Nx sequencing.

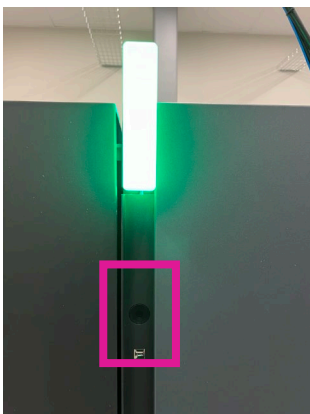
For information on instrument settings, sample setup, run design, user management, and Admin users, see the *SMRT® Link user guide corresponding to your installed version of SMRT Link (version 26.1 or greater)*.

## External system overview



1. Status light
  - Displays the various states of the instrument
2. Front panel display
  - Not a touchscreen interface
  - Used for monitoring sequencing runs and multi-use SMRT Cell – Nx status
3. Door
  - Door is opened / closed via button to allow access to work deck for loading / unloading consumables
4. Door open/close button
5. Logo light
  - Confirms instrument is powered on
6. Power button

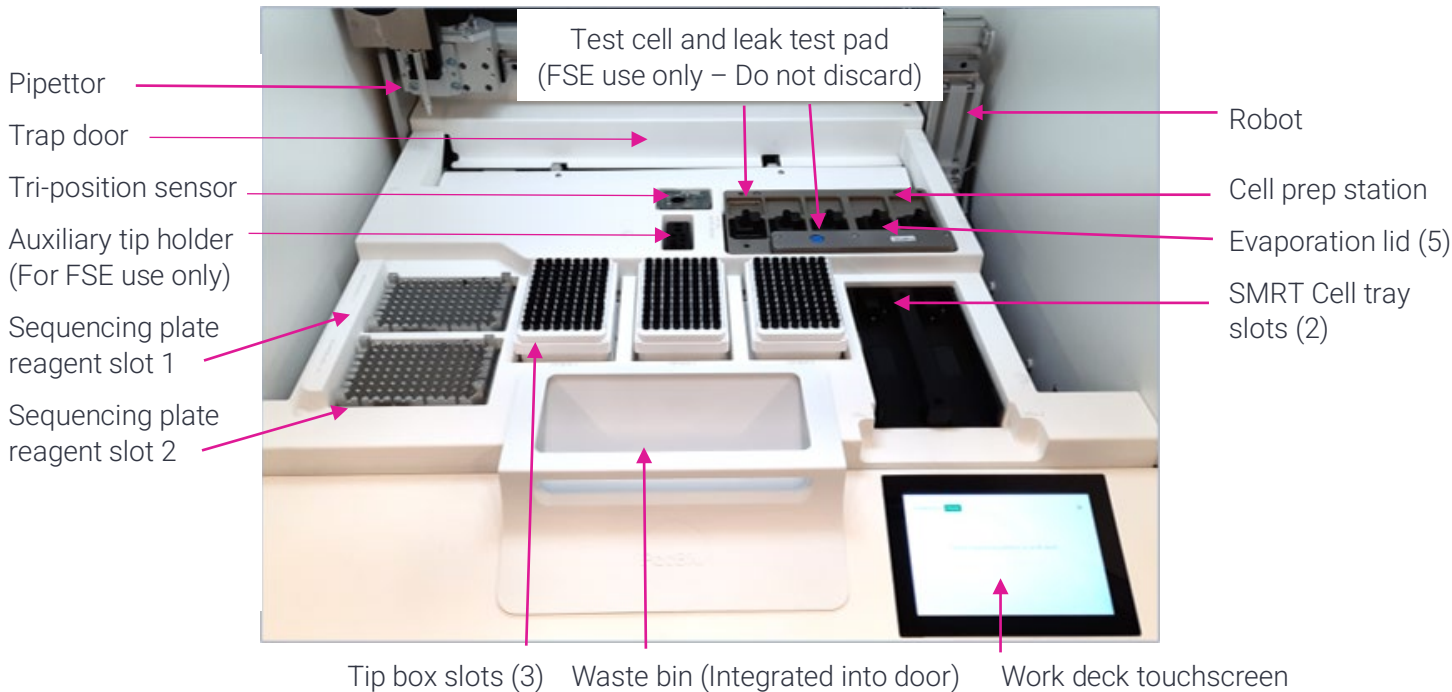
To power on the instrument, press the green illuminated button located on the right-side panel of the instrument. Note that it may take a few minutes for the instrument to power up.



## Instrument status light

- **White:** Instrument is powering up, being updated, or powering down
- **Green:** Instrument is idle and ready for a run to be loaded
- **Blue:** Instrument is sequencing (**note:** if status light is blue and door is unlocked, instrument can load the next run)
- **Orange:** Instrument error

# System work deck overview

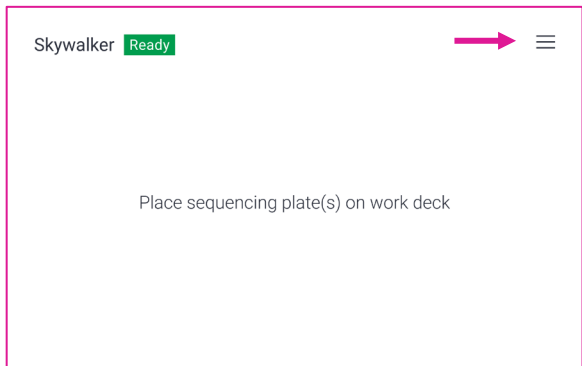


## DANGER! ELECTRICAL SHOCK AND LASER HAZARD

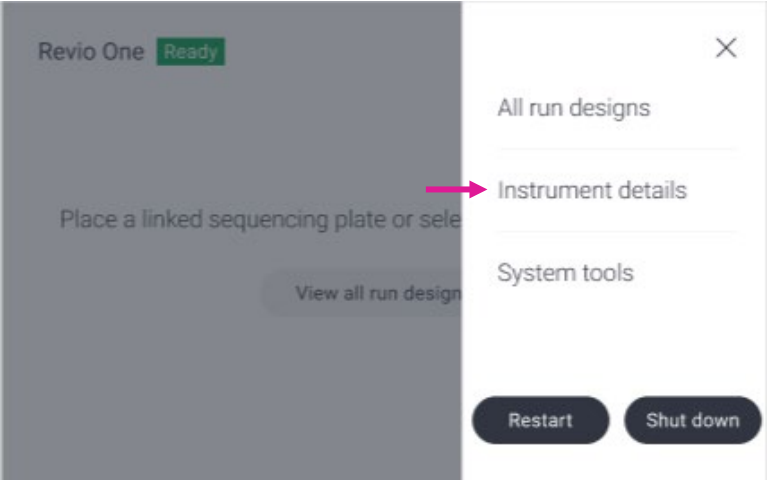
Installation, maintenance, and repair are only allowed for authorized service personnel. Do **not** remove the panels of the Revio systems. Please refer to *Guide – Revio system safety* for more information.

## Verifying instrument software version

1. Select the **menu** icon at the top right of the work deck touchscreen:



2. Select “Instrument details” from menu:



The **Instrument details** screen will display:

- Instrument name
- SMRT Link address
- Data path
- Software version
- Analysis version
- Chemistry version

# Preparing to load the instrument

The following materials and components are needed before sequencing on the Revio system:

## Materials for sequencing

| Materials                                            | Quantity | Source |
|------------------------------------------------------|----------|--------|
| Revio SMRT® Cell tray                                | 1 - 2    | PacBio |
| Revio sequencing plate (and accompanying foil strip) | 1 - 2    | PacBio |
| Sequencer pipette tips                               | 1-3      | PacBio |

Ensure the Revio sequencing plate(s) to be prepared are compatible with the Revio SMRT Cells to be used:

| Revio sequencing plate           | Revio SMRT Cell                       |
|----------------------------------|---------------------------------------|
| Revio SPRQ™ sequencing plate     | Revio SMRT Cell (single-use)          |
| Revio SPRQ sequencing plate - Nx | Revio SMRT Cell, Revio SMRT Cell – Nx |

The Revio SPRQ polymerase kit is used for both SPRQ and SPRQ-Nx sequencing.

## Prepare the Revio sequencing plate for sequencing

- Thaw the sequencing plate. Remove the sequencing plate from the mylar bag. Locate the lot number, serial number and expiration dates on the sequencing plate label. The mylar bag also contains a QR code card. Scan the QR code on the sequencing plate label by using a laptop or webcam camera, then click the Scan button. This automatically populates the Lot, Serial and Expiry fields in SMRT Link.
- Place the sequencing plate in a room temperature (~22°C) water bath covered with foil to protect the plate from light, for 60 minutes.

- Visually inspect the sequencing plate for any remaining frozen reagents. Remove the sequencing plate from the water bath, lift the plate to eye-level without inverting, and examine the plate from all angles to look for any remaining frozen or precipitated materials. If any reagents remain frozen, return to the water bath for an additional 15 minutes, protected from light.
- Once thawed, mix by vortexing/shaking at 1200 rpm for 1 minute.
- Spin the sequencing plate for 1 minute at 150 rcf to ensure all reagents are in the bottom of the wells.
- Before loading the plate on the Revio work deck, use a new KimWipe to wipe any moisture or contaminants from the top of the sequencing plate foil.
- **Note** the sample must already be prepared and diluted according to instructions in SMRT Link Sample Setup.
- **Important:** A standard sequencing plate supports 4 acquisitions and can be used for more than one run, when stored properly between uses (see below). Sample wells must be used sequentially. If well B01 is used in a first run, then well A01 cannot be used for any subsequent runs. The next run would have to start with well C01.

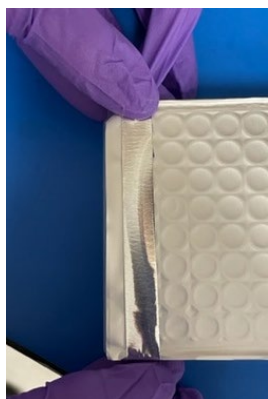
1. Pierce column 1 wells A, B, C, and D (even if not using all four wells).

- Using a pipettor, secure a 200  $\mu$ L tip.
- Press the pipette tip to the center of the well gently until the seal breaks. Touch the bottom of the well with the tip. Discard the tip.



Do not attempt to open the foil completely to the edge of the well by following the perimeter of the well with the tip.

2. Add the sample(s) to the respective well(s). To pipette accurately into the sequencing plate, dispense the sample against the middle of the well's side wall and avoid immersing the pipette tip in the sample during blow out.
3. Peel the adhesive foil strip (included with the Revio sequencing plate) from its backing. Use both hands (with gloves) to hover the seal over column 1 and firmly seal the pierced wells (being careful to not cover any wells in column 2). Do not attempt to peel and reseal seal.



**Note:** Only the foil strips included with the Revio sequencing plate should be used on the sequencing plate. Other seals may cause failures.

4. If using a sequencing plate for more than one run, and it has one layer of foil strip already:
  - Remove the sequencing plate from 4°C.
  - Do not remove the 1st foil strip.
  - Pierce the 1st layer of foil strip on the unused wells (wells B, C, or D) in column 1 and add samples to the respective wells as described above in steps 3-5.
  - Place the 2nd layer of foil strip directly over the 1st layer – DO NOT remove the 1st layer.
5. Spin the sequencing plate for 1 minute at 150 rcf.
6. Leave the plate on ice until ready to load on to the instrument.
7. After the sequencing run, if a part of the plate is unused:
  - Store the sequencing plate at 4°C and protected from light.
  - Revio sequencing plates can be stored at 4°C for up to two weeks after first use.

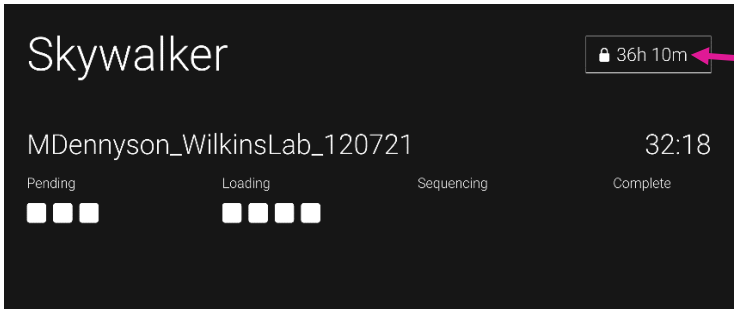
**Optional:** Cover the used wells (we recommend a removable glue plate seal - see below). Do not cover the unused wells as this may prevent piercing in the subsequent use.



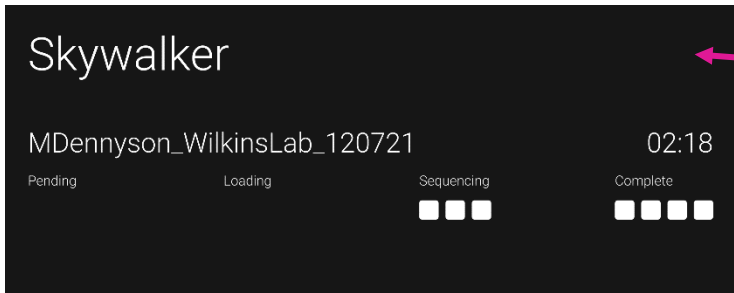
**Caution:** Only the foil strips included with the Revio sequencing plate should be used on the sequencing plate. Other seals may cause failures.

# Starting a run with a sequencing plate linked to a Run Design

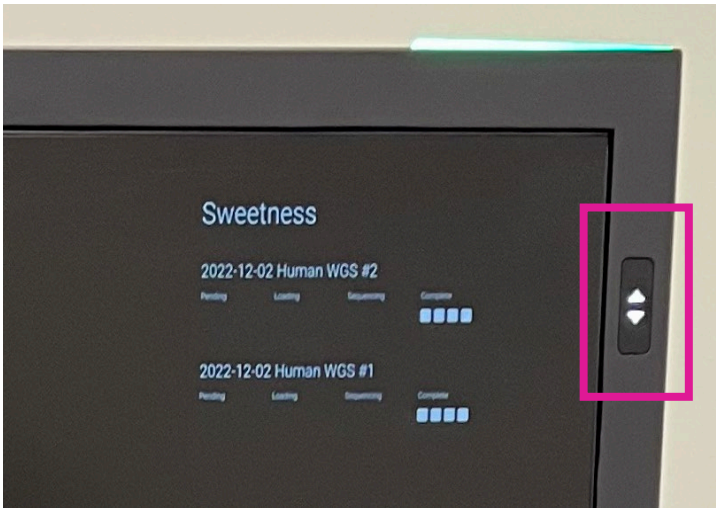
1. If the Revio front panel display is not showing the locked door icon, press the door button to open the instrument door and access the work deck.



Door locked, with remaining time before unlock

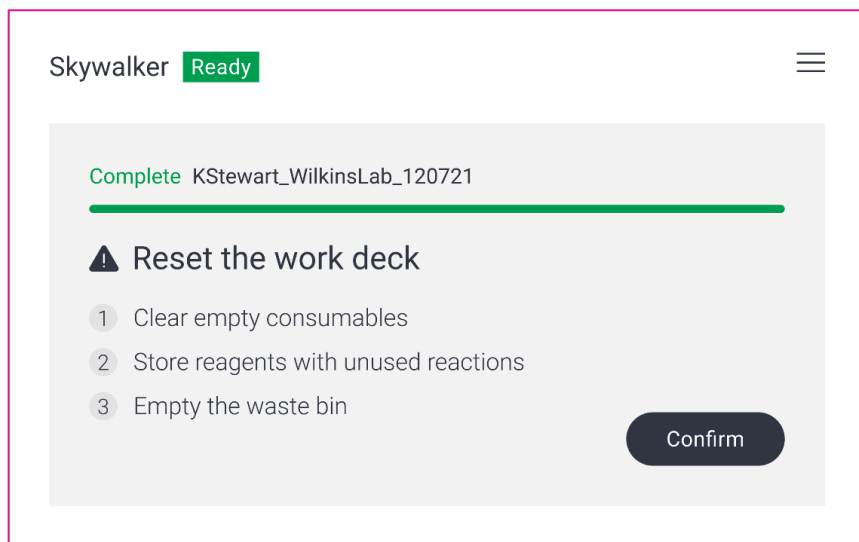


Door unlocked



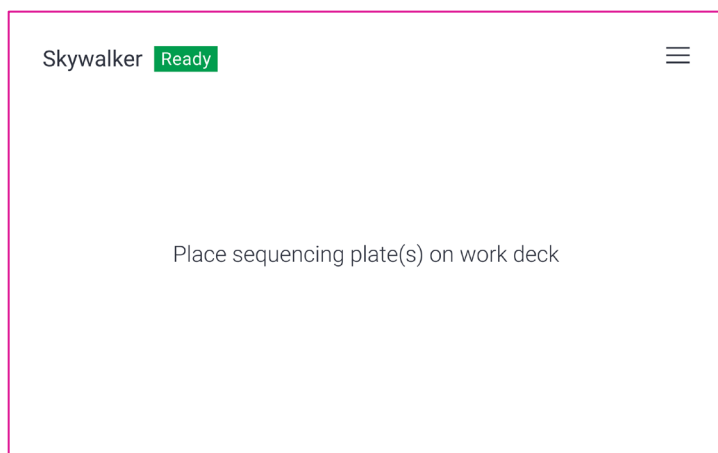
Door button

2. If a previous run has finished, the work deck touchscreen will display the following screen with instructions to reset the work deck. Complete the steps described on the touchscreen:



- After completing the steps to reset the work deck, press **Confirm**.

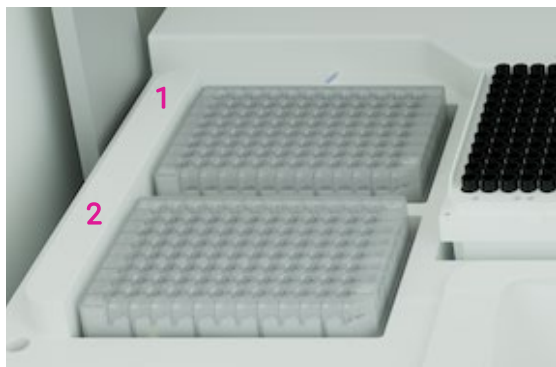
3. If the work deck was previously reset, the touchscreen will display the home screen with run setup instructions:



4. Place a **linked sequencing plate** in reagent slot position 1. Placing the plate will invoke the **Load all consumables** screen, displaying the linked run design information and required consumables.
- If there is condensation buildup on the reagent slot, use KimWipes to blot off the moisture. Then place the sequencing plate in sequencing plate position 1. Do not remove the foil from the reagent plate.
  - If the run requires 2 plates, place the second plate in sequencing plate position 2.

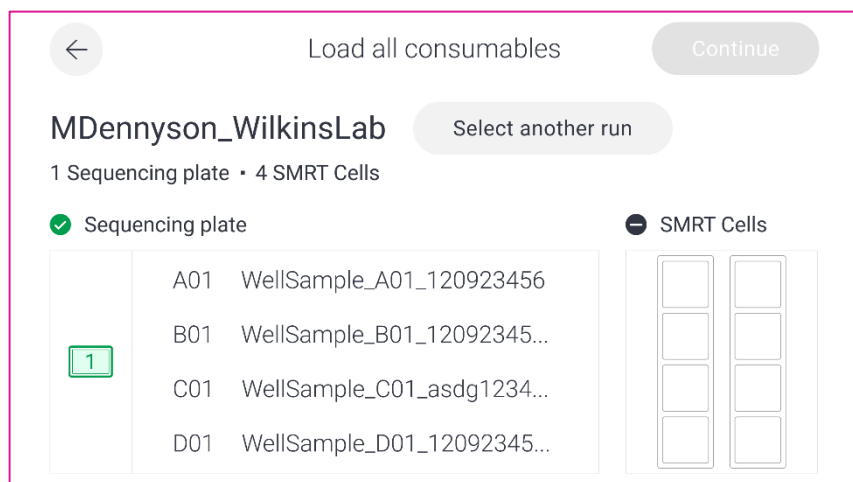
**Note:** The reagent plates are NFC-tagged for inventory tracking and Run Design selection.

**Important:** The sequencing plate should fit snug and level into the plate slot. If the plate is hit or disrupted while being placed into the plate slot, remove the plate, spin to ensure all reagents/samples are at the bottom of the wells, and then place the plate on the instrument deck. **It is critical that all reagents/samples are at the bottom of the wells during the run.**



- Per the instructions on the **Load all consumables** screen, place the required number of SMRT Cells into the appropriate SMRT Cell slots as shown below. When a sufficient number of SMRT Cells have been placed, a checkmark will appear next to the SMRT Cells icon displayed on the touchscreen.

In the example below, 4 SMRT Cells are required, 0 have been placed on the work deck.



- The SMRT Cell tray gently slides in and clicks into place. After clicking into place, the tray should pop back to a hard stop. If the tray sticks in the forward position, gently pull it back to the hard stop.

**Note:** The SMRT Cell trays are NFC-tagged for inventory tracking.



6. Replace any empty tip boxes on the work deck with full tip boxes by **removing the lids from the tip boxes** and placing the boxes in the tip box slots. Be sure to seat the boxes square and level to the work deck.

It is important to replace empty tip boxes with full tip boxes only (and not half-full boxes).

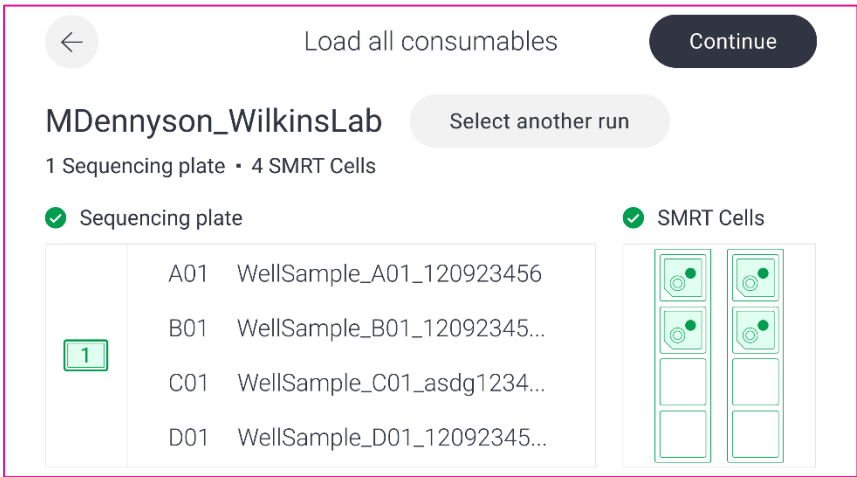


**Note:** Do **not** move or remove partial tip racks, because the software recalls the last position where it used a tip. However, if the system was powered off and on again, then the last known tip position will be lost. In this event, the system should be reloaded with 3 full tip boxes.

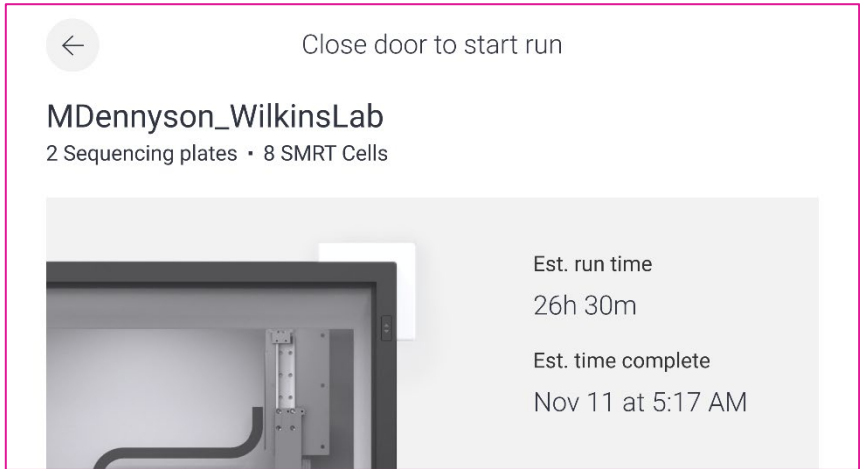
7. Empty the waste bin prior to starting a new run.
  - Pull the waste bin from the work deck using the built-in handle, and empty according to lab policy.
  - Return the waste bin to its proper location.



8. When all required consumables are placed on the work deck, the **Continue** button becomes active and illuminated. Press the **Continue** button to proceed.



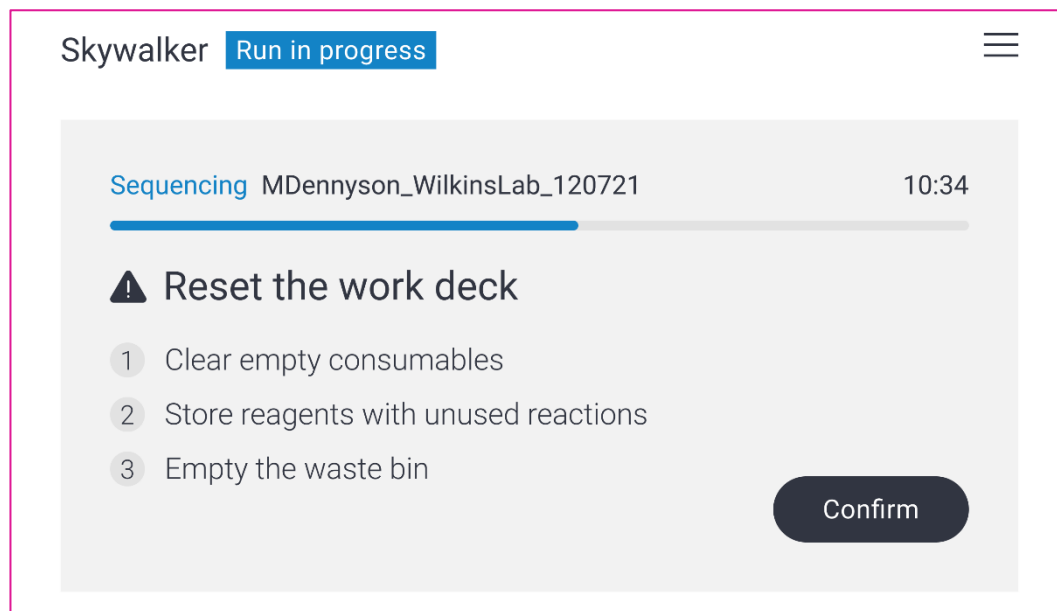
9. Review the run summary and close the instrument door to start the run.



10. If no prior run is currently in progress sequencing, the light tower will turn blue.

## Setting up a run while the instrument is sequencing

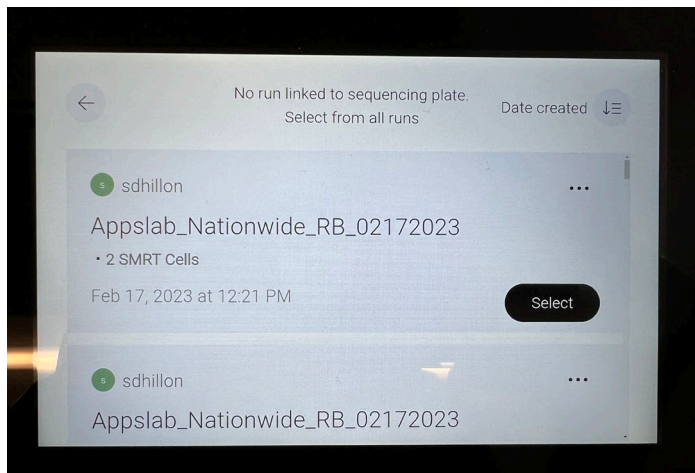
1. Prepare the sequencing plate as described above in section “**Prepare Revio sequencing plate for sequencing**”.
2. While the system door is unlocked, press the door button on the front of the system to open the instrument door and access the work deck. When a sequencing run is in progress, the work deck touchscreen will display a “Run in progress” screen with instructions to reset the work deck:



3. Follow the work deck touchscreen instructions to reset the work deck and press **Confirm**.
4. Place a linked sequencing plate in reagent slot position 1. This will invoke the **Load all consumables** screen with specific consumable loading instructions. Note the **Continue** button will not become functional until all required consumables are loaded on the work deck.
5. Load enough Revio SMRT Cells onto the instrument work deck to support the sequencing run, per the screen instructions.
6. Ensure all empty pipette tip boxes have been replaced with full tip boxes.
7. When all required consumables are placed on the work deck, the **Continue** button becomes active and illuminated. Press the **Continue** button to proceed.
8. Review the run summary and close the instrument door to start run.
9. The light tower color will be unchanged (blue).

## Starting a run with a sequencing plate not linked to a Run Design

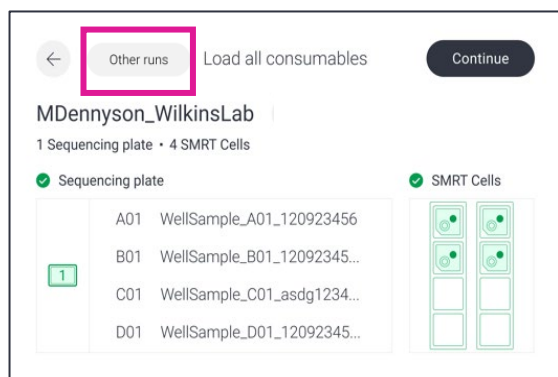
1. Prepare the sequencing plate as described above in section “**Prepare Revio sequencing plate for sequencing**”.
2. Place the unlinked sequencing plate(s) in reagent slot 1 (and slot 2, if needed) on the instrument work deck.
3. Select a Run Design from the list displayed on the work deck touchscreen:



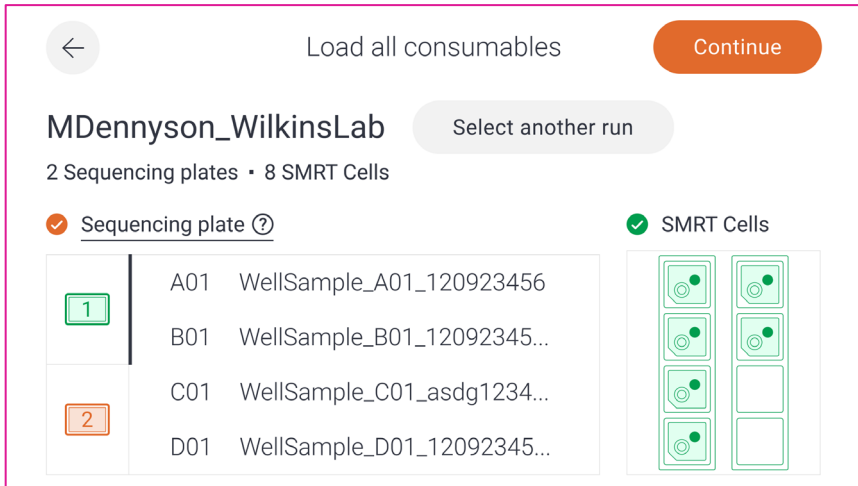
4. Proceed with run setup as described above in section “**Starting a run with a Sequencing plate linked to a Run Design**”.

## Starting a run with a linked sequencing plate using an alternative Run Design

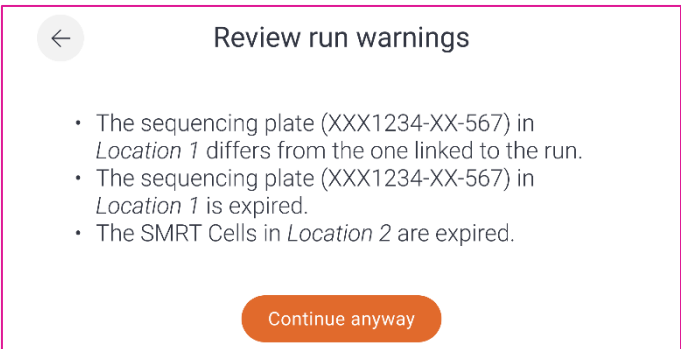
1. Prepare the sequencing plate as described above in section “**Prepare Revio sequencing plate for sequencing**”.
2. When loading a linked sequencing plate onto the instrument work deck, the linked Run Design will display on the work deck touchscreen. To choose an alternate run design for sequencing, select the **Other runs** button.



3. A list of other Run Designs available in SMRT Link will appear on the work deck touchscreen.
4. Scroll the Run Design list until the desired Run Design is found.
5. Press the **Select** button next to the desired Run Design.
6. The sequencing plate icon and **Continue** button will turn orange.



7. Proceed with run setup as described above in section “Starting a run with a Sequencing plate linked to a Run Design”.
8. Press **Continue** and review run warnings.



9. Press the **Continue anyway** button to proceed and start the run.

# Multi-use Revio SMRT Cell - Nx operation

Revio ICS v13.5 enables sequencing with multi-use Revio SMRT Cells - Nx, which support up to 3 sequencing acquisitions (uses) from one physical Revio SMRT Cell - Nx before it is retired to the instrument waste bin. Instructions for loading the required consumables to the work deck, Run Design selection, and preloading operations described above remain unchanged when using multi-use SMRT Cells - Nx and SPRQ-Nx sequencing plates.

This section describes additional operational and monitoring considerations when sequencing with multi-use SMRT Cell – Nx consumables.

## Example of a standard run setup using multi-use SMRT Cells - Nx

1. Prepare the SPRQ-Nx sequencing plates and load the samples for sequencing as described above in “Prepare the Revio sequencing plate for sequencing.”
2. On the work deck, place:
  - Sample-loaded SPRQ-Nx sequencing plate(s)
  - Full boxes of pipette tips
  - One or more multi-use SMRT Cell trays, per the requirements on the **Load all consumables** screen
3. Confirm on the touchscreen that the intended samples and consumables are loaded, then press **Continue**.

The instrument control software allocates the loaded samples to the available uses of each multi-use SMRT Cell placed on the work deck (up to three uses per multi-use SMRT Cell - Nx).

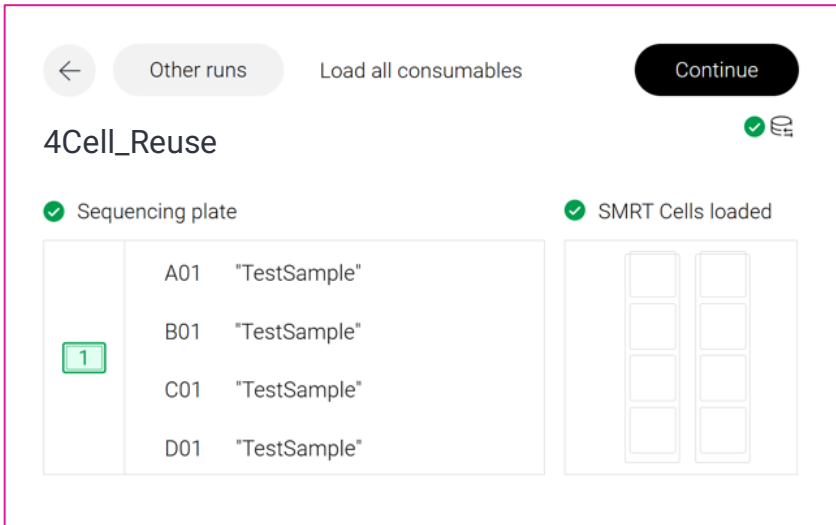
## Additional considerations

- There’s no benefit to placing extra SMRT Cells on the work deck beyond what is required to complete the run.
- In the event of a multi-use SMRT Cell - Nx failure mid-run, the samples to be sequenced will *not* be re-allocated to a different SMRT Cell. The sample currently loaded to the failed use will fail (depending on when the failure occurs), and any sample allocated to a remaining, not-yet-started use of that same SMRT Cell will be aborted. **Aborted samples remain untouched in the sequencing plate, and can be moved to a new sequencing plate for a new run.**
- In the event of an instrument failure while multi-use SMRT Cells are on the stages, the instrument control software continues to track remaining available SMRT Cell uses and the time remaining to load them.

## Multi-use SMRT Cell - Nx use rules and monitoring

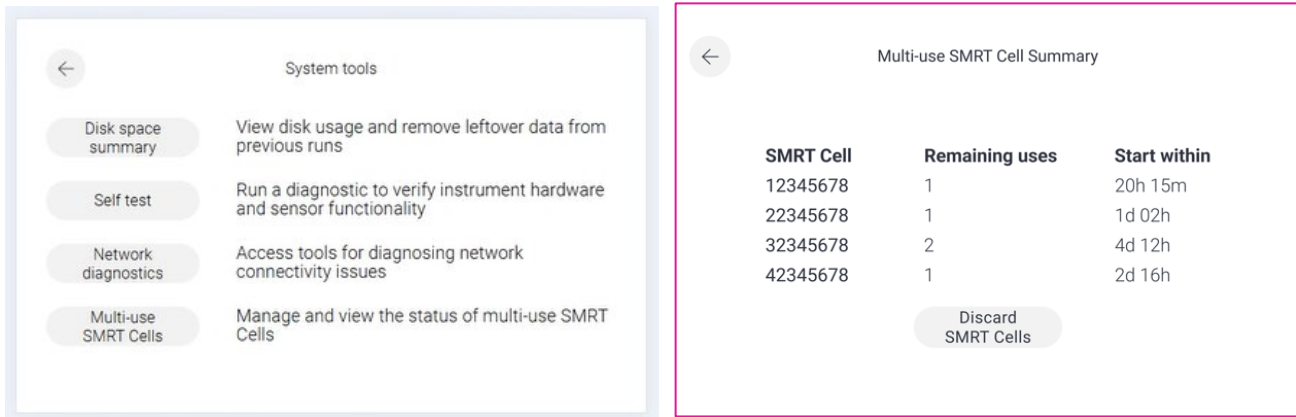
- Once a multi-use SMRT Cell is removed from its tray and placed on a stage, it is never returned to the work deck. It is used for sequencing up to three times and then moved to the waste bin, or it expires on the instrument and is moved to the waste bin. **The user has the option to discard remaining uses**, see below.
- Once removed from its tray, a software timer tracks the time remaining (108 hours, or 4.5 days) to *start* the final/third use of that multi-use SMRT Cell - Nx, regardless of the length of each movie acquisition.
- If the third use of the SMRT Cell – Nx is not started within 108 hours, it is marked Expired and moved to the instrument waste bin. There is no override option to use an expired multi-use SMRT Cell.

- When a user is loading a new run that will utilize the second and/or third uses of multi-use SMRT Cells - Nx that are already held on the stages below the work deck, the **Load all consumables** screen will display a green checkmark to indicate sufficient SMRT Cells are loaded:



## Multi-use SMRT Cell - Nx availability can be monitored in several ways:

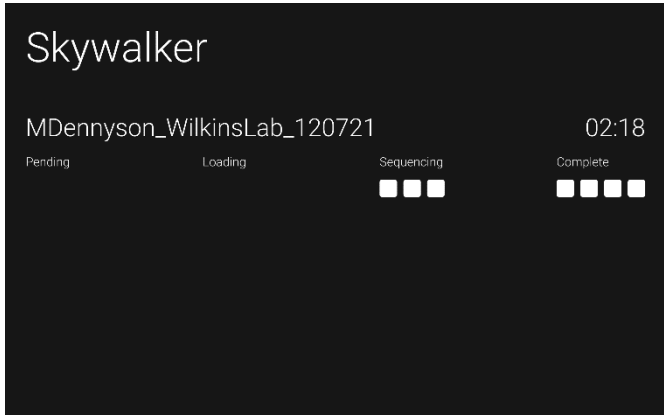
- The work deck touchscreen menu includes a “Multi-use SMRT Cells” option under **System tools**. This displays the SMRT Cell IDs on each stage, their remaining uses, and the remaining time to start the last use.



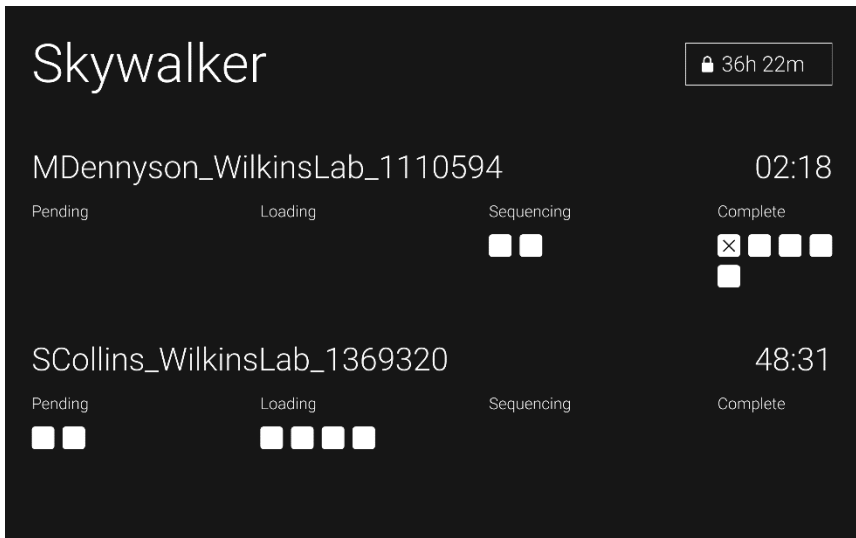
- The **Multi-use SMRT Cell Summary** screen includes a “Discard SMRT Cells” option. This will discard all remaining uses of **all** multi-use SMRT Cells on the instrument, once any in-progress runs complete.
- The front panel display (described below) also shows which stages hold multi-use SMRT Cells with uses available, and the time remaining to the next multi-use SMRT Cell expiration.
- Multi-use SMRT Cell status, including per-sample cell use and ID, is also available in SMRT Link. For more information please refer to the SMRT® Link user guide for your installed version.

# Monitoring run progress from the front panel display

1. The front panel display will show the run status of a run currently in progress, in addition to the previous run or a run which is queued to begin after the current run in progress.
2. Each SMRT Cell is represented by a white box on the front panel display.



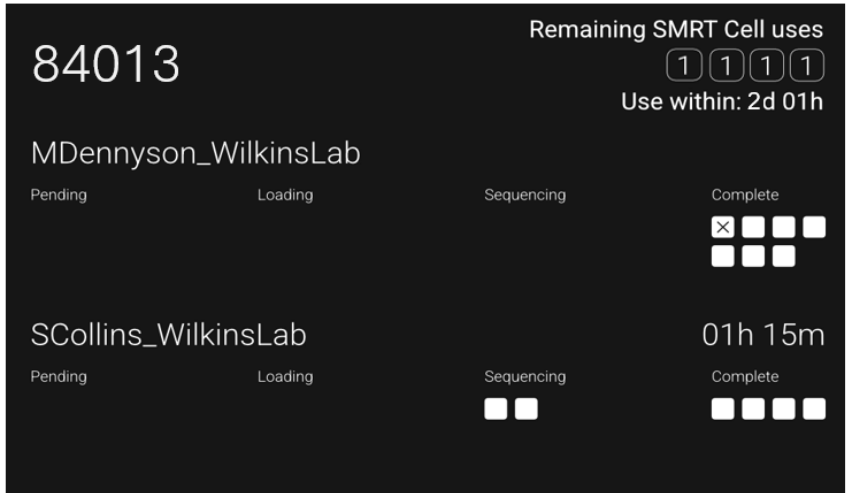
3. Users can track the progress of SMRT Cells as they move in the following series: **Pending -> Loading -> Sequencing -> Complete**
4. Each stage can be defined as follows:
  - Pending: SMRT Cells are waiting for system to be ready to perform SMRT Cell preparation.
  - Loading: Sample is going through immobilization or other pre-run workflows on the instrument.
  - Sequencing: Data is being acquired on SMRT Cell.
  - Complete: sequencing has completed.
5. If a subsequent run is loaded onto the system while the current run is still sequencing, the individual run status will appear as follows:



Further status of each individual SMRT Cell can be determined by the fill of the box representing the SMRT Cells in a run:

|  |                                                             |
|--|-------------------------------------------------------------|
|  | No error                                                    |
|  | Did not complete sequencing due to an instrument error      |
|  | Did not complete sequencing due to a manual run termination |

6. The top right corner of the front panel display also shows which stages hold multi-use SMRT Cells with uses available, and the time remaining to the next/soonest multi-use SMRT Cell expiration. Each square represents a multi-use SMRT Cell – Nx on a stage, and the number inside the square indicates the number of available uses remaining on that SMRT Cell. The “Use within” time indicates the time remaining before the next cell expiration. Further individual SMRT Cell-specific status and detail (uses remaining, time to expiration, error messages) can be found in SMRT Link.

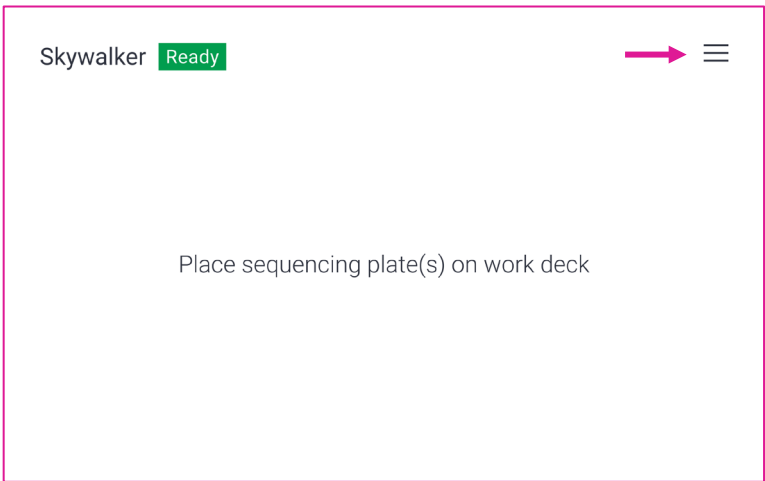


## Clear disk partitions

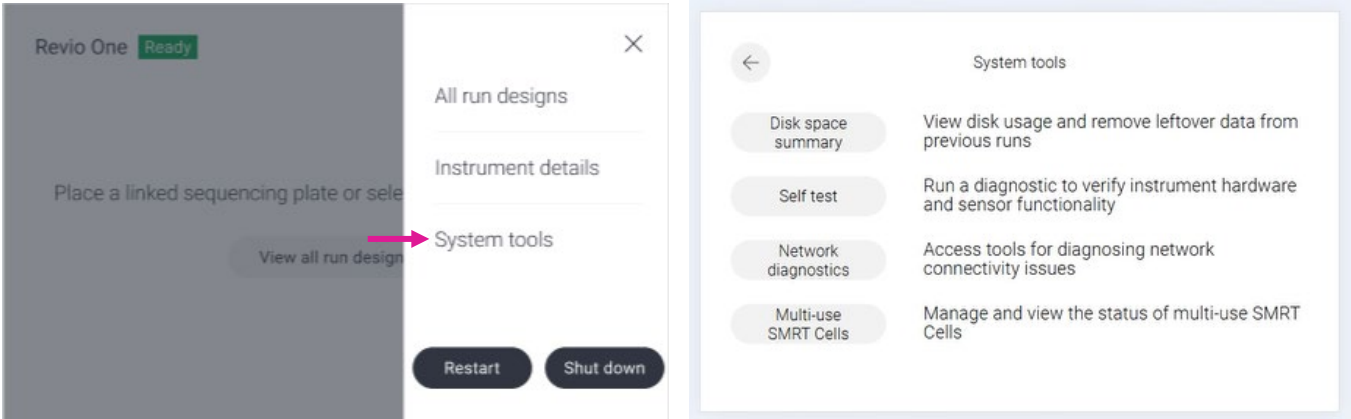
To restore an instrument with data files left behind from failed runs, disk partitions can be cleared using the disk clean up feature.

**Important:** Clearing disk partitions is irreversible. Contact your local field support team if unsure if disk data is still required.

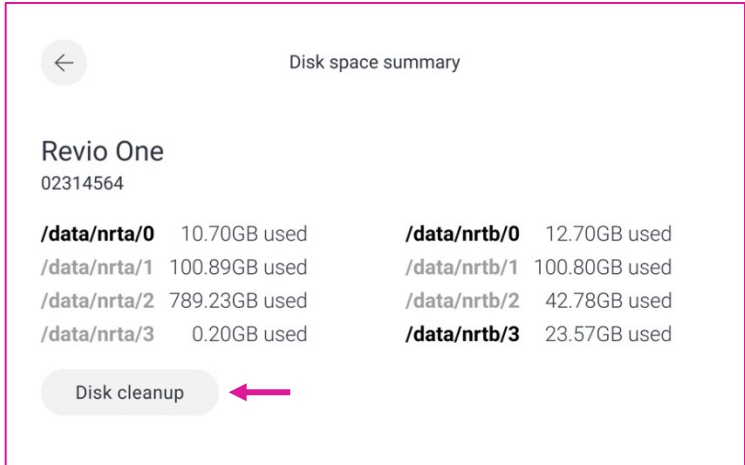
1. Open the instrument door.
2. Select the **menu** icon on the work deck touchscreen.



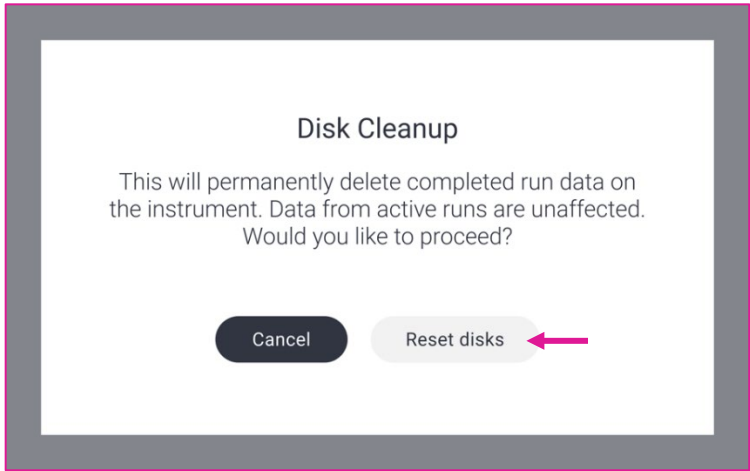
3. Select **System tools**, then **Disk space summary** from the menu options.



4. On the **Disk space summary** screen, if there is data to be cleaned up the disk cleanup button will be enabled.

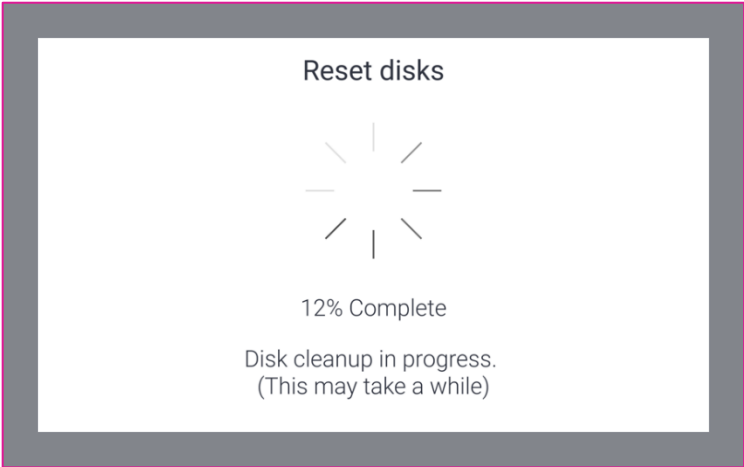


5. To perform a disk cleanup, touch the Disk cleanup button. A prompt will appear asking to confirm disk cleanup.

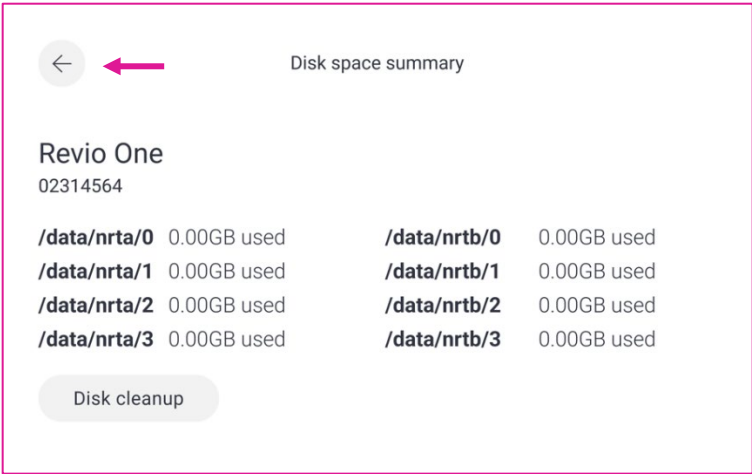


Select the **Reset disks** button to confirm.

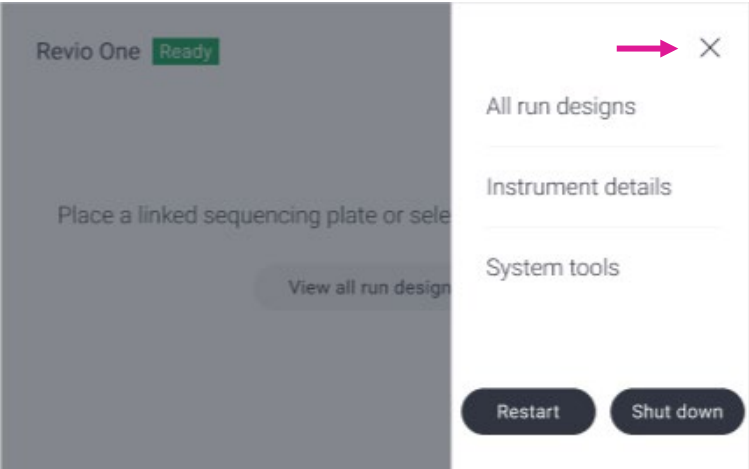
6. Once disk cleanup is confirmed, a progress screen will appear showing disk cleanup progress.



7. Once disk cleanup completes, the **Disk space summary** screen reappears. Touch the back arrow button to go back to the Menu screen.



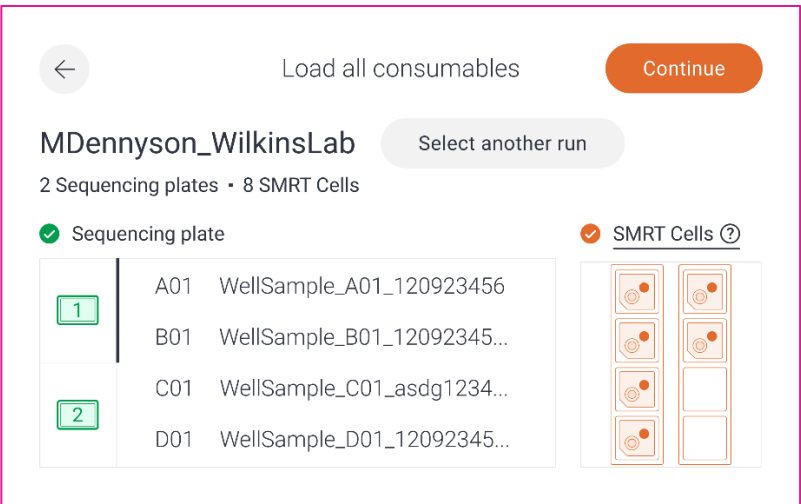
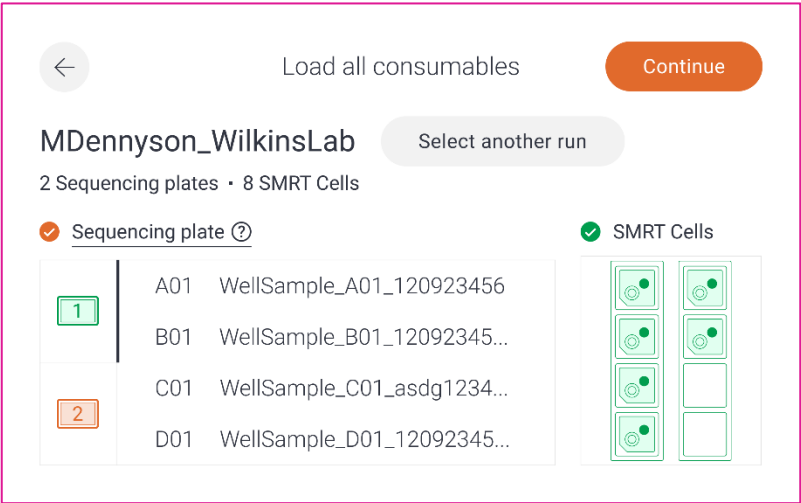
8. Close the menu and go back to the home by touching the X button in the top right of the Menu screen.



# Work deck touchscreen warnings

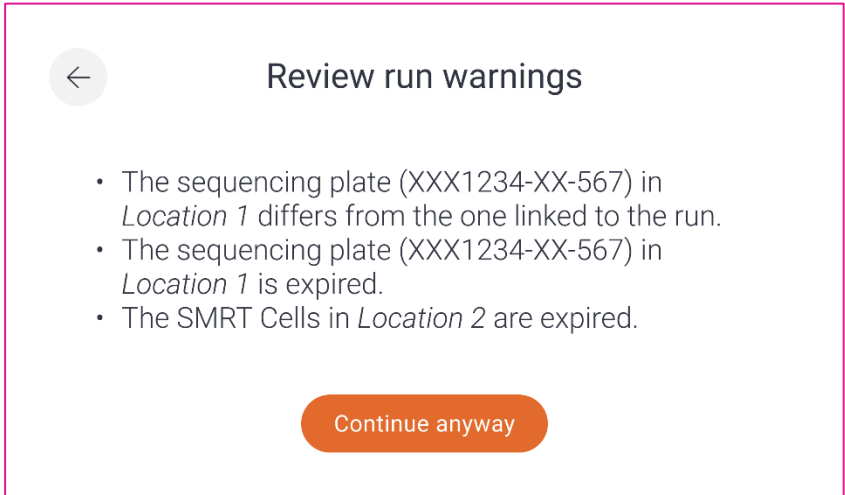
## Expired consumables

1. Some expired consumables (sequencing plates, single-use SMRT Cells) can be used on the Revio system at the user's own risk – there will be no guaranteed performance with expired consumables by PacBio.
2. If expired consumables are placed on the work deck, the respective consumable will appear orange on the work deck touchscreen:
  - Expired consumables are indicated by an orange status color on the work deck touchscreen.
  - The **Continue** button is orange to indicate a warning.



3. Upon pressing the **Continue** button to start the run, the user will be prompted to acknowledge that expired reagents will be used:

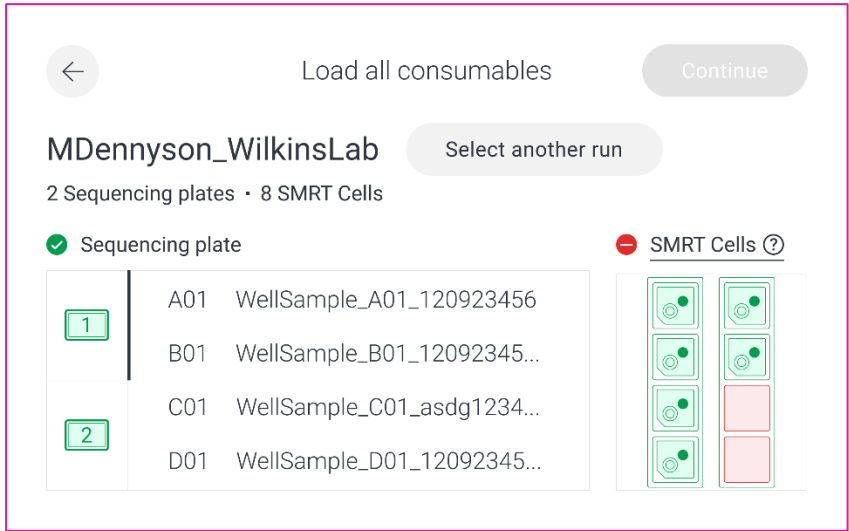
- Select **Continue anyway** to proceed to the run summary screen or the back arrow to replace consumables with non-expired consumables or cancel the run.



## Consumables with insufficient reactions

1. If consumables placed on the work deck do not have enough reactions to support the selected Run Design, the respective consumables will appear red on the work deck touchscreen:

- An example of an 8 SMRT Cell run with insufficient SMRT Cell quantity loaded on the work deck:



2. Consumables with sufficient reaction quantities to support the selected Run Design must be loaded on the work deck before proceeding to start the run.

| Revision history (description) | Version | Date           |
|--------------------------------|---------|----------------|
| Initial release.               | 01      | September 2026 |