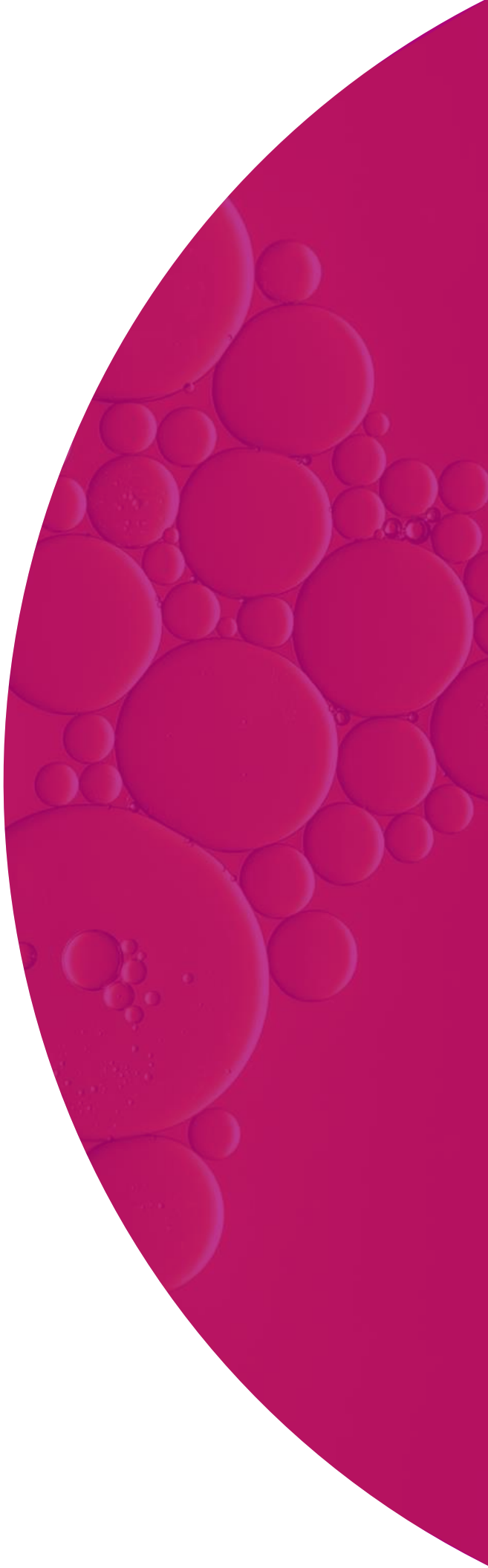
PacBio

Revio™ system

Operations guide



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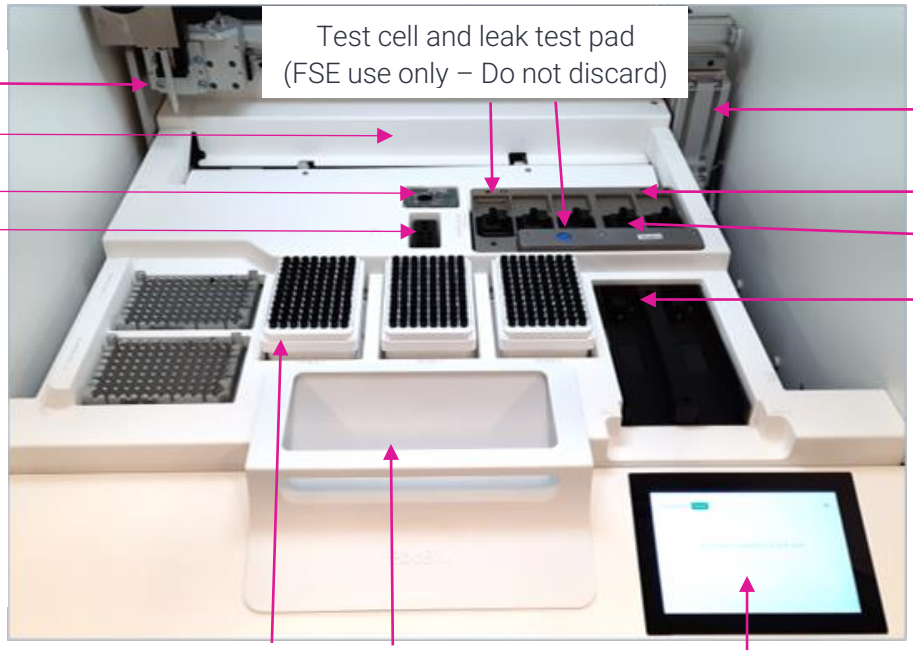
Introduction

This guide explains how to operate the Revio system. **For information on instrument settings, sample setup, run design, user management, and Admin users, see the *SMRT® Link (v12.0) user guide*.**

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
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

System work deck overview



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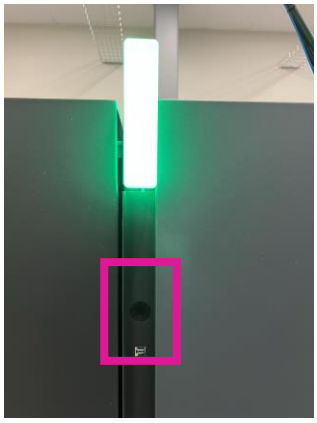
Powering up the instrument

To power up 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

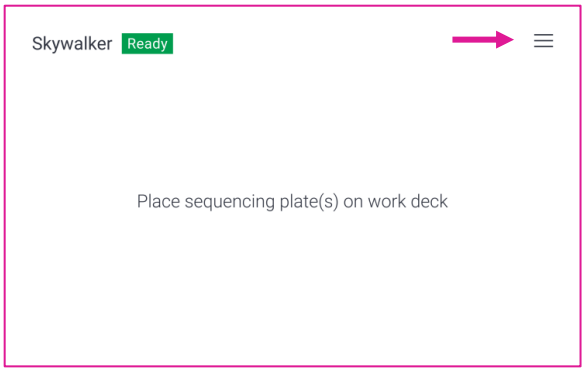
- White: Instrument is powering up, being updated, or powering down.
- Green: Instrument is idle and ready for a run to be loaded
- Blue: Instrument is sequencing (note: if status light is blue and door is unlocked, instrument can load the next run)
- Orange: Instrument error

DANGER! ELECTRICAL SHOCK AND LASER HAZARD

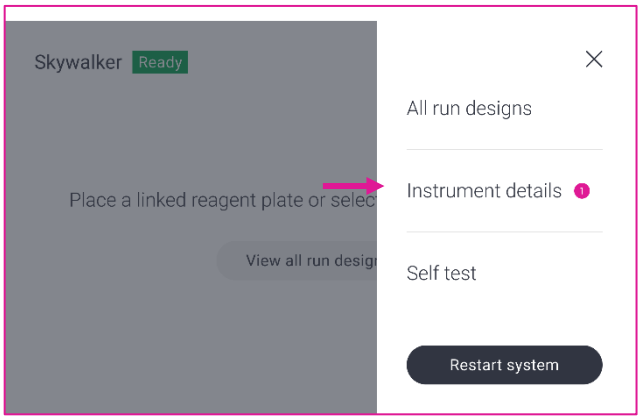
Installation, maintenance, and repair are only allowed for authorized service personnel. Do **not** remove the panels of the Revio systems. Please refer to *Guide – Revio system safety* for more information.

Verifying instrument software version

1. Select the **menu** icon on the work deck touch screen.



2. Select “Instrument details”:



A screen displaying the following will then appear:

- 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 systems:

Materials for sequencing

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

Prepare the Revio sequencing plate for sequencing

- Thaw the sequencing plate. Remove the sequencing plate from the mylar bag. You can locate the lot number, serial number and expiration dates on the sequencing plate label. The mylar bag also contains a QR code card. You can scan the QR code on the sequencing plate label by using a laptop or webcam camera, then clicking the Scan button. This automatically fills in the Lot, Serial and Expiry fields in SMRT Link.
- Place the sequencing plate in a room temperature (~22ºC) water bath covered with foil to protect the plate from light for 60 minutes.
- Remove the sequencing plate from the water bath and invert, tap, and observe the sequencing plate to look for any remaining frozen or precipitated materials. Repeat this 5 times to ensure sufficient observation. If wells remain frozen, return to the water bath for additional time.
- Once thawed, mix by vortexing at 1000 rpm for 1 minute.
- Spin down the sequencing plate for 1 minute at 150 rcf to ensure that the reagents are in the bottom of the plate.
- Before loading on the instrument, wipe any moisture or contaminants from the top of the sequencing plate.
- Note that the sample should have already been prepared and diluted according to instructions from SMRT Link Sample Setup.

1. Pierce column 1 wells A, B, C, and D (even if not using all four wells).
   - Using a pipettor, secure a 200 µL 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.

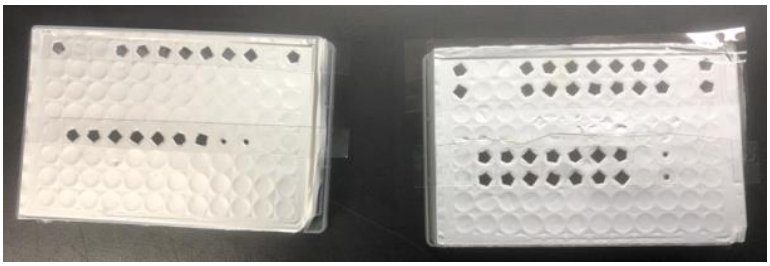
Do not attempt to open the foil completely to the edge of the well by following the perimeter of the well with the tip.

2. Discard the tip and add the sample to the respective wells.
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 well (being careful to not cover any wells in column 2). Do not attempt to peel and reseat 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 reusing a sequencing plate that has already been used and has one layer of foil strip:
   - Remove the sequencing plate from 4ºC.
   - Do not remove the 1st foil strip.
   - Pierce the 1st layer of foil strip on the unused wells (wells A, 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.
   - Do not use more than two strips per sequencing plate, each plate is limited to two uses.
5. Spin down 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 use, if a part of the plate is unused:
   - Store the sequencing plate at 4ºC and protected from light.
   - Revio sequencing plates can be stored at 4ºC 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

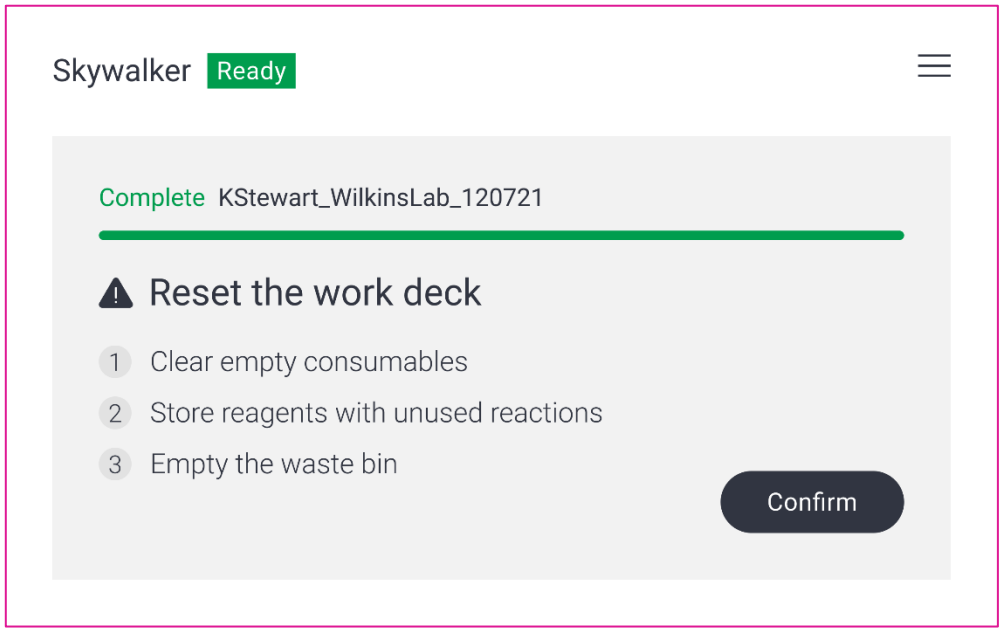
1. If the Revio front panel display is not showing the door locked icon, press the door button to open the instrument door and access the work deck.

Door locked

Door unlocked

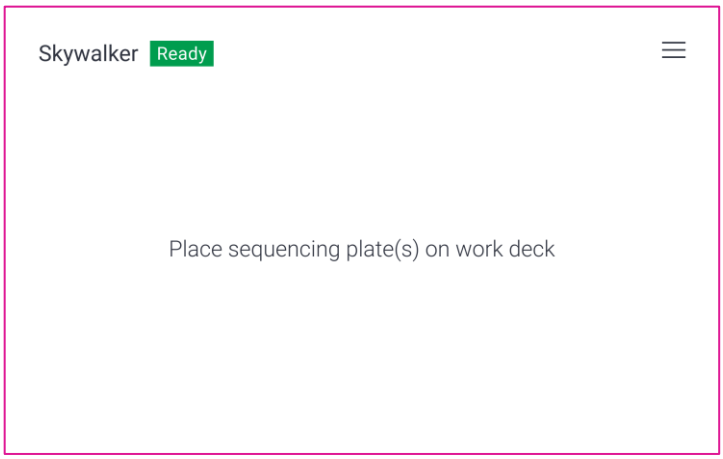
Door button

2. If a previous run has finished, the work deck touch screen will display the following screen with instructions on resetting the work deck:



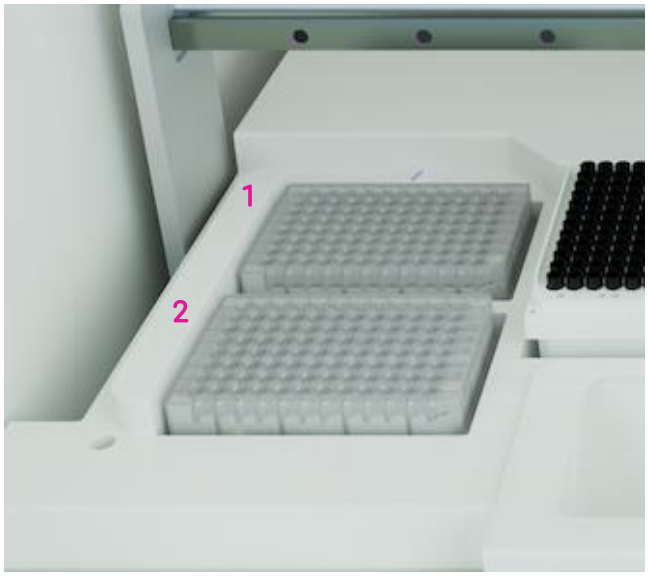
- After resetting the work deck, press confirm.

3. If the work deck was previously reset, the touch screen will display the home screen:



4. Place a linked sequencing plate in reagent slot position 1:
   - 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 jarred as it is put in place, remove the plate, and spin it down to ensure all reagents/samples are at the bottom of the wells and then return the plate to the instrument. It is important that all reagents/samples be at the bottom of the wells during the run.

5. Place the appropriate number of SMRT Cells into the appropriate SMRT Cell slots as shown below.
   - 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.

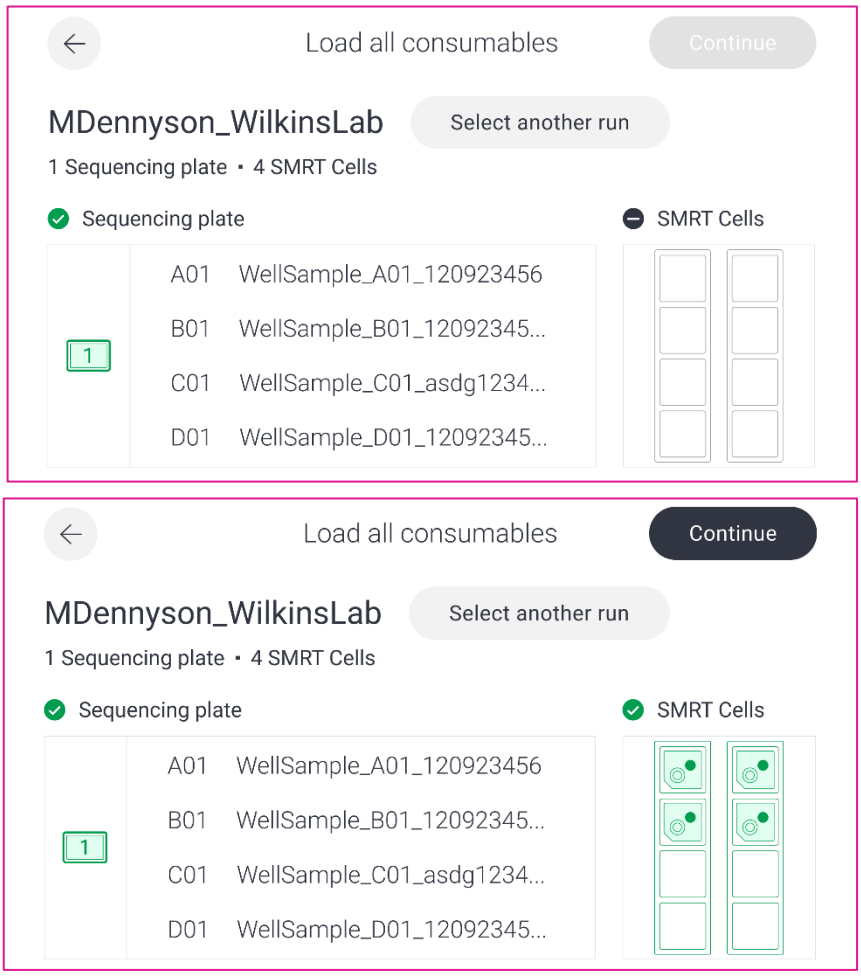
6. Replace any empty tip boxes on the work deck with full tip boxes by removing the lids from the tip boxes and placing the boxes in the tip box slots. Be sure to seat the boxes square and level to the work deck.

Note: The software remembers the last position where it used a tip, so it is important to not remove or move partial tip racks. However, if the system was powered down and cycled back up, then the last known tip position will be lost. The system should be reloaded with 3 full tip boxes. It is important to not replace empty tip boxes with other tip boxes that are only half full.

7. Empty the waste bin prior to starting a new run.

- Pull the waste bin from the work deck by using the built-in handle and empty according to lab policy.
- Return the waste bin to its proper location.

8. Once all consumables have been placed on the work deck, the **Continue** button becomes illuminated. Press the **Continue** button to proceed.

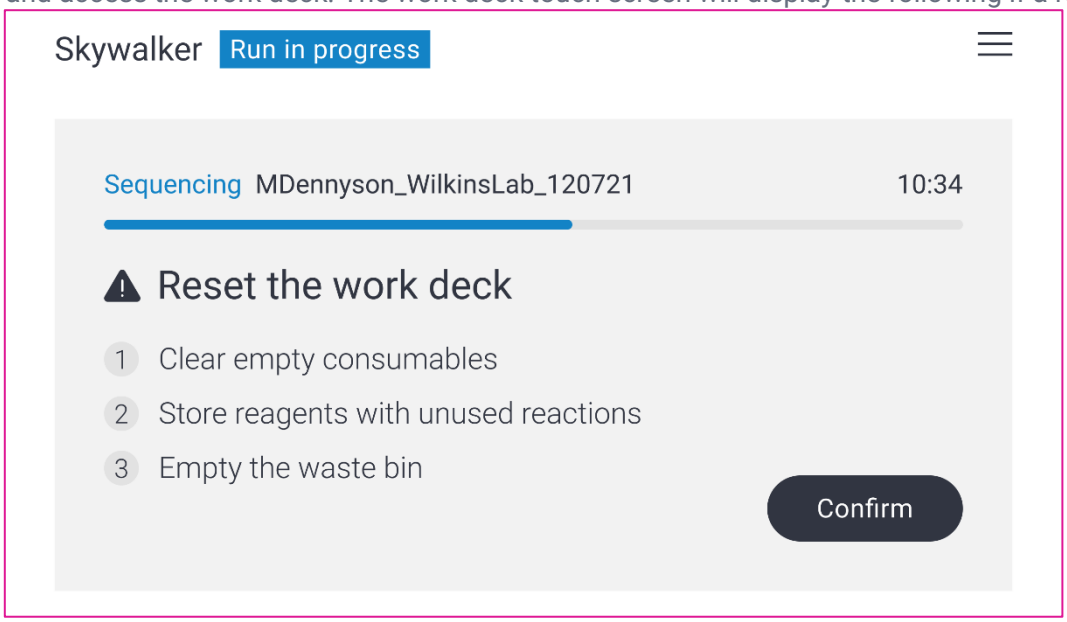


9. Review the run summary and close the instrument door to start the run.

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

Setting up a run while the instrument is sequencing

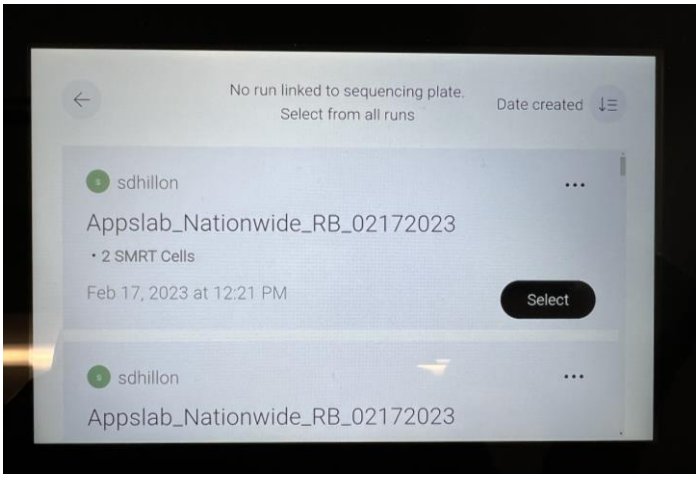
1. Prepare the sequencing plate as described above in section **"Prepare Revio sequencing plate for sequencing".**
2. While the system door is unlocked, press the door button on the front of the system to open the instrument door and access the work deck. The work deck touch screen will display the following if a run is currently sequencing:



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

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

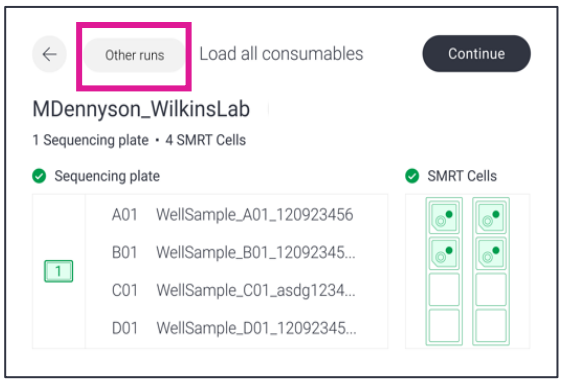
1. Prepare the sequencing plate as described above in section **“Prepare Revio sequencing plate for sequencing”.**
2. Place the unlinked sequencing plate in reagent slot 1 on the instrument work deck.
   - If starting a run with more than 4 SMRT Cells, place an additional sequencing plate in reagent slot 2.
3. Select a Run Design from the list using on the work deck touchscreen:



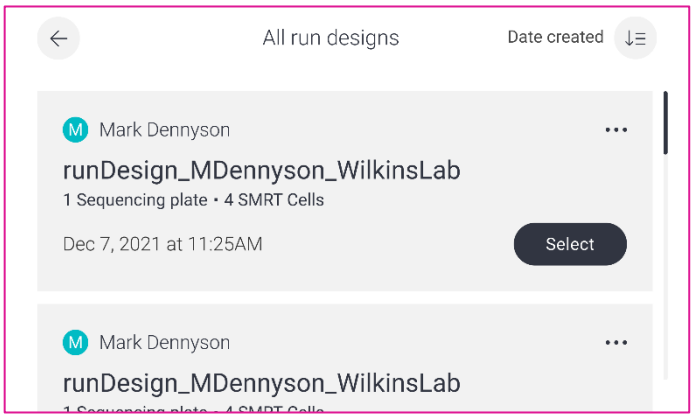
4. Proceed with run setup as described above in section **“Starting a run with a Sequencing plate linked to a Run Design”.**

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. After loading a linked sequencing plate onto the instrument work deck and the linked Run Design loads onto the work deck touchscreen, press the “Other runs” button.

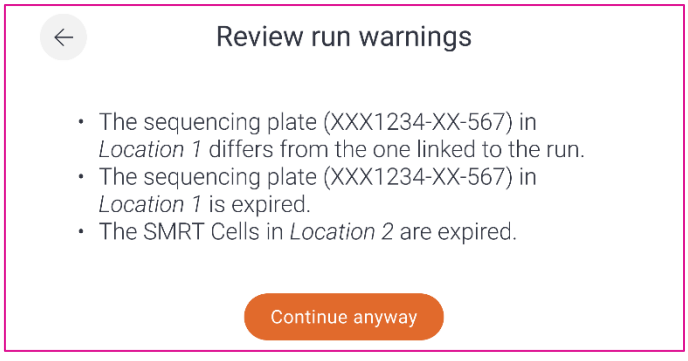


3. A list of other Run Designs will appear on the work deck touchscreen:



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.

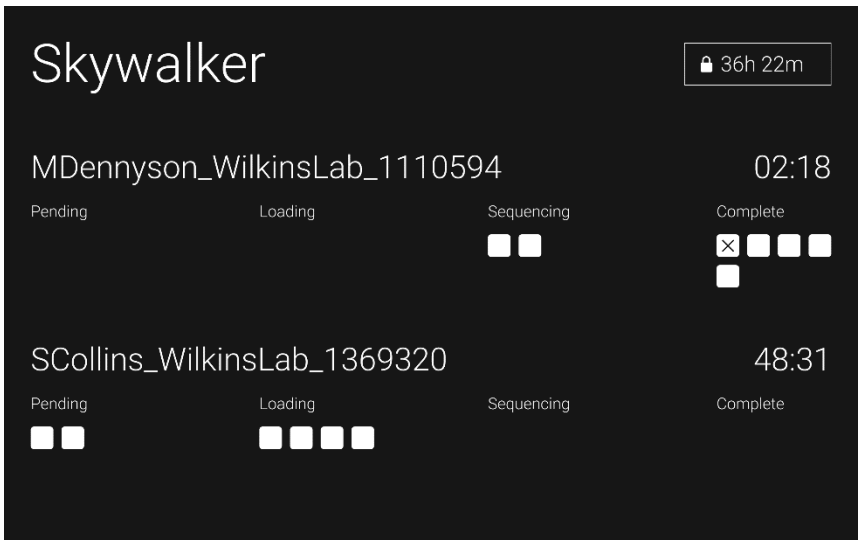


9. Press the **Continue anyway** button to proceed and finish run initiation.

Monitoring run progress from the front panel display

1. The front panel display will show run progress from a run currently sequencing as well as the previous run or a run which is queued to begin after the current run.
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 has been 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

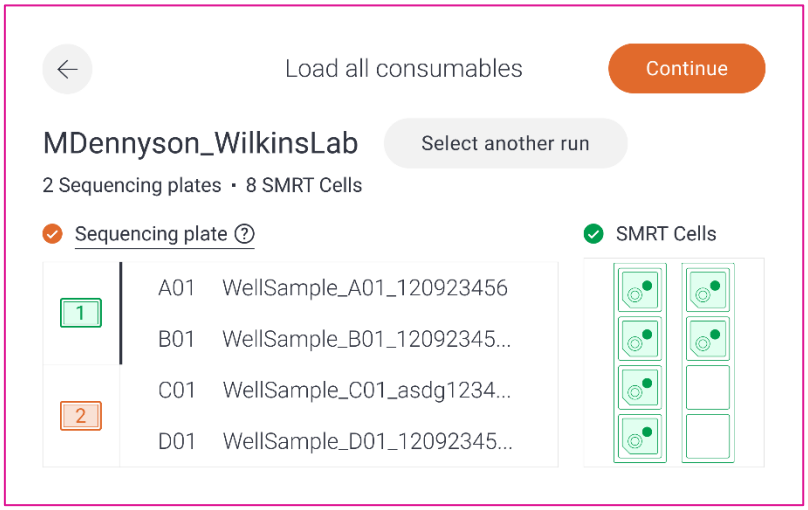
Did not complete seqencing due to an instrument error

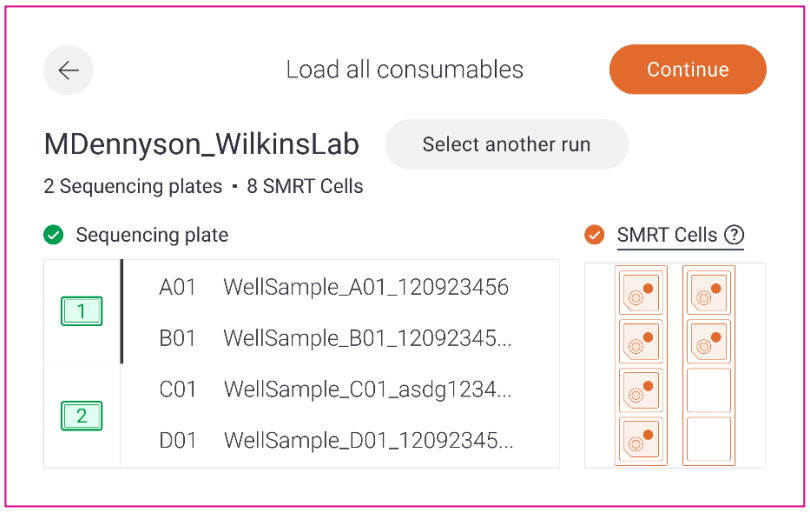
Did not complete sequencing due to a manual run termination

Work deck touchscreen warning

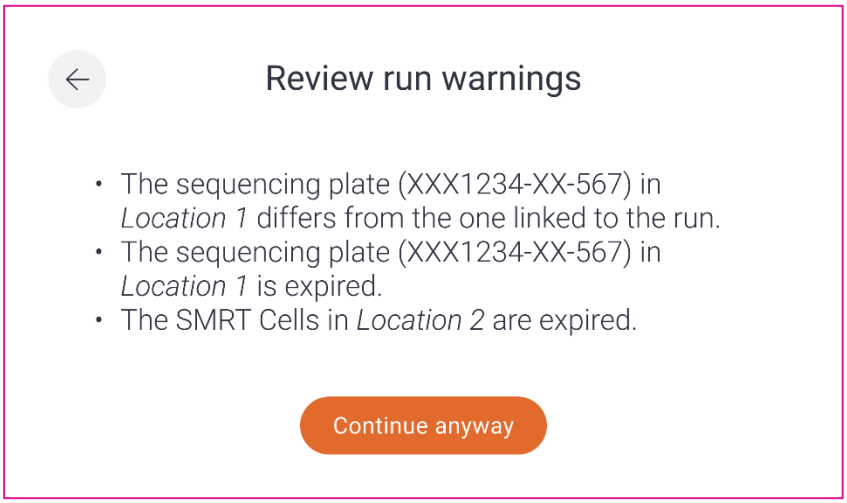
Expired consumables

1. Expired consumables can be used on the Revio system at the users 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 touch screen:
    - Expired consumables are indicated by an orange status color on the work deck touch screen.
    - The continue button is orange to indicate a warning.



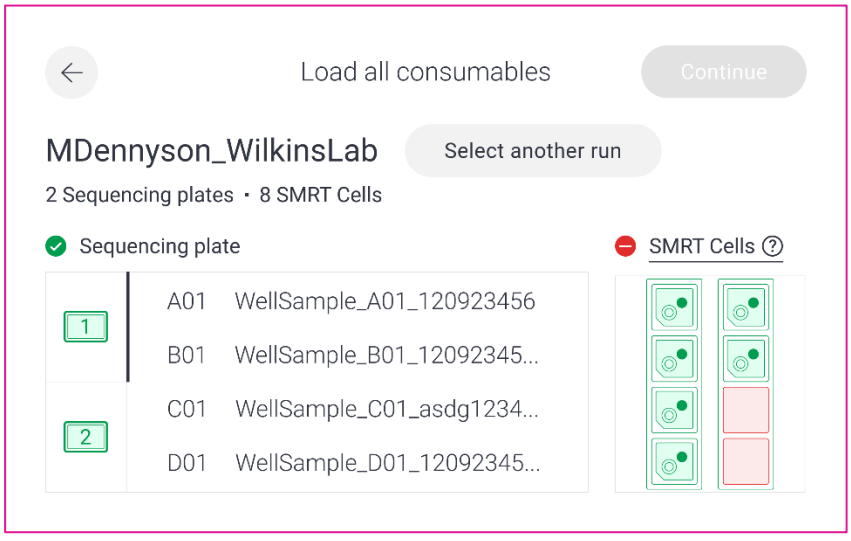


3. Upon pressing the continue button to proceed with starting the run, the user will be promted to acknowledge that expried reagents are being used:
    - Press continue to proceed to the run summary screen or the back arrow to replace consumables with non-expired consumables or cancel the run.



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 touch screen:
    - 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	March 2023