
PACBIO®

# Operations Guide

## Sequel® System:
## The SMRT® Sequencer

**For Research Use Only. Not for use in diagnostic procedures.**

P/N 101-055-100-06



Pacific Biosciences of California, Inc.
1305 O'Brien Dr.
Menlo Park, CA 94025
www.pacificbiosciences.com

## 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.

**DANGER! ELECTRICAL SHOCK AND LASER HAZARD**

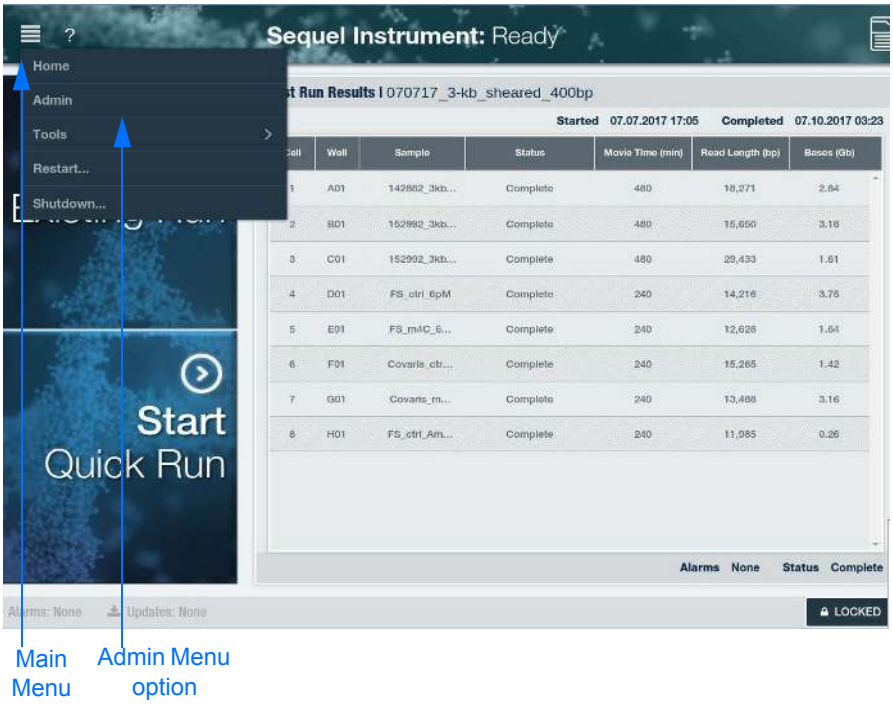
Installation, maintenance and repair are only allowed for authorized service personnel. Do **not** remove the panels of the Sequel System.

### Instrument LED Lights

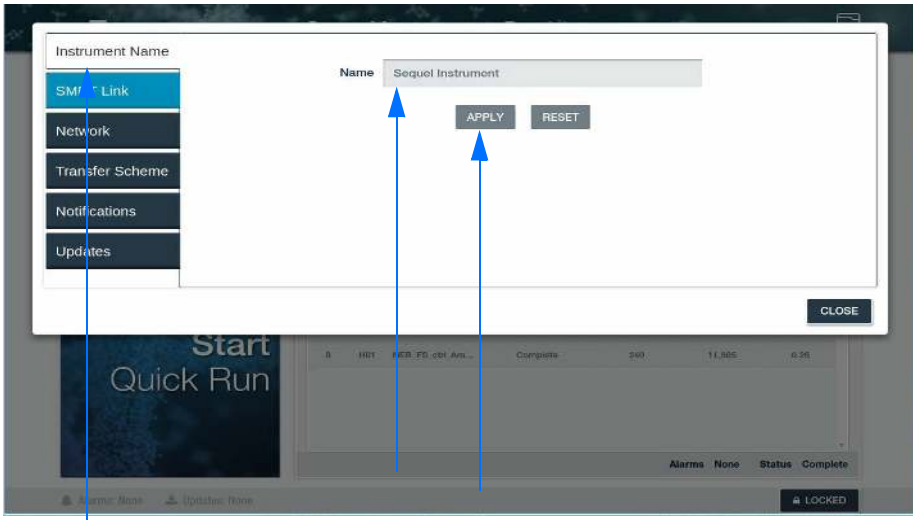
- After powering up, the light is initially **yellow** signifying that the instrument is not yet ready. This light will also display when the instrument is powering down or when the door is open. If the door is open, the light will remain yellow until the door is closed. After the door is closed, the light turns **green** (after about 5-10 seconds).
- Once the instrument is fully warmed up and ready for use, the light turns solid **green**. This signifies that the instrument is ready for sequencing.
- If the light is **flashing green**, then the system is busy.
- If there are any **critical** errors (e.g., the instrument failed to start up, failed to start diagnostics, failed to power down or if the door is forced open), then the light will be **flashing red**.

## Administrative Functions

1. Access the Administration Menu by selecting the Main Menu located at the top left hand corner of the screen and then selecting **Admin** from the drop-down menu option.

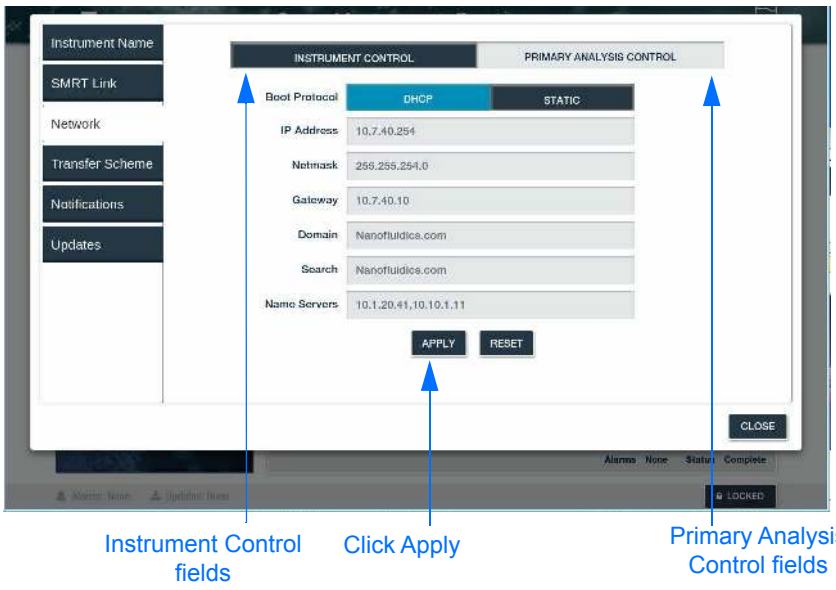


2. At the **Name Instrument** option, enter a name for the Instrument and click **APPLY**.

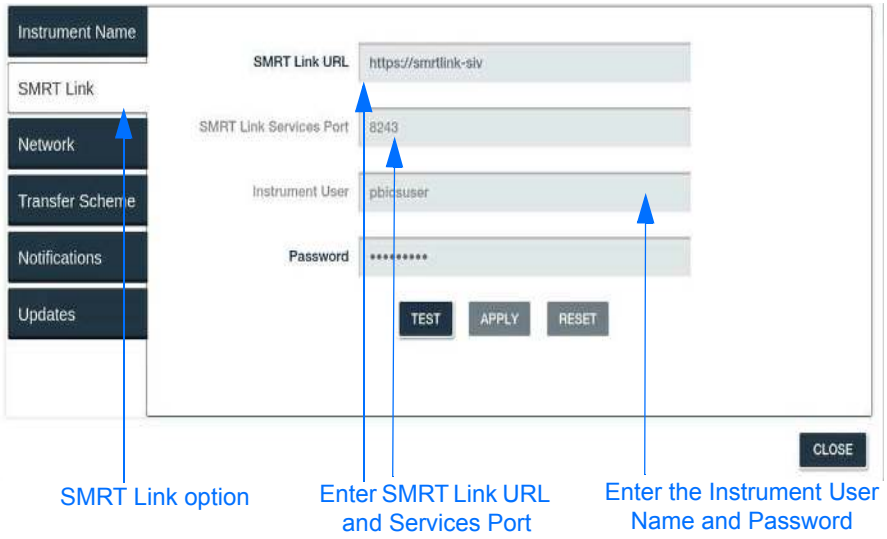



Name Instrument tab  Enter Instrument name and click Apply

3. At the **Network** menu option, connect the Instrument Control Computer and Primary Analysis Computer to the network by completing all the required fields shown below. This must be done for both Instrument Control and Primary Analysis Control. Note that all this information can also be obtained from your Network/IT administrator.
   a. Specify whether the Boot Protocol is **DHCP** (uses dynamic IP addresses) or **STATIC** (uses one IP address). Different fields display based on the protocol chosen. If DHCP is selected, the fields are auto-filled and read-only.
   b. If **Static** is selected, enter the information in the following fields:
      - **IP Address**: Fixed numeric IP address of the instrument.
      - **Netmask**: Filled in by Customer IT. Divides an IP address into subnets and specifies the network's available hosts.
      - **Gateway**: Filled in by Customer IT. A network point that acts as an entrance to another network.
      - **Domain**: Top-level customer domain name.
      - **Search**: Lower-level customer search domain name; accesses a subset of the full customer domain.
      - **Name servers (optional)**: Resolves the name of the individual instrument with its IP address.
      - Then click **APPLY.**




4. At the **Connect to SMRT Link** option, fill out the required fields to connect the instrument to SMRT® Link. Note that multiple instruments can be pointed to the same SMRT Link installation.
   c. Enter a web URL specifying where SMRT Link is installed on the network.
   d. Enter the **SMRT Link Services Port** number for the SMRT Link Services and **SMRT Link URL** in the appropriate fields (as shown below). Note that the SMRT Link URL and SMRT Link Services Port entries needed for connecting the instrument can be found on the SMRT Link server About page.
   e. Verify that the URL and Port are correct by clicking on **TEST**.
   f. If correct, click on **APPLY** to save the values. If the URL and Port did not work, click on **RESET** and try again.

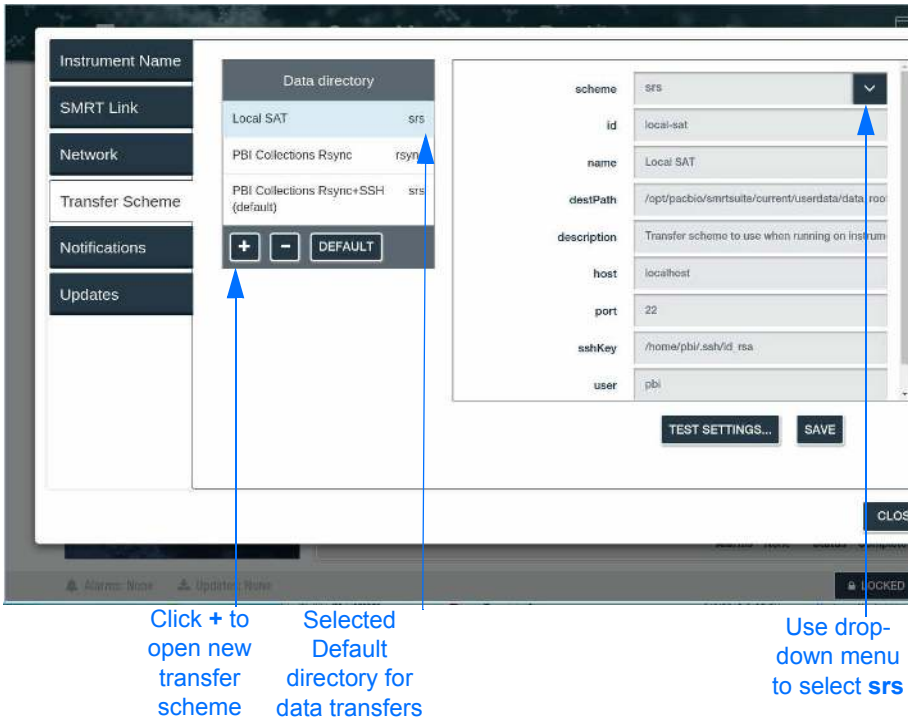


5. Use the **Transfer Scheme** option to configure transfer schemes (`rsync` or `srs`) to transfer data from the instrument to the local network. **Note**: `SMB` and `NFS` are **not** supported.

   **For srs transfer scheme:**

   This option provides the benefits of rsync (e.g., recovery from partial transfers) in addition to an encryption layer provided by SSH. An SSH public key is provided by PacBio personnel and must be manually installed on your SMRT Link server by your system administrator.

   a. In the **Data directory** dialog, click **+**. A new transfer scheme displays in the right-hand side of the tab, ready to be filled in.




   b. Select **srs** transfer scheme. The fields for that transfer scheme type will display.

   c. Enter information into the fields:

      - **id**: The transfer scheme id. User-specified text string. This can only contain A-z, 0-9 and "-" or "_". Example: `pbi-collections-test`.
      - **name**: User-specified text string that displays in the **Data directory** dialog to identify the transfer scheme.
      - **destPath**: Location or destination path to the network location (SMRT Link server) for storing data transferred from the instrument. For `srs`, this is the absolute path.
      - **description**: User-specified text string that describes the transfer scheme.

- **host**: The DNS name or IP address of the machine serving as the data transfer server. This may be the SMRT Link server or another computer on the network. The name of this system can be obtained from your system administrator.
- **port**: Defaults to port `873`.
- **sshKey**: The full path to the private key of the ssh key pair to be used for transfer authentication. The public key of this pair must have been installed on the transfer server specified in the **host** field. Unless otherwise directed, the value of this field should be `/home/pbi/.ssh/pbi.id_rsa`.
- **user**: Enter the name of the SMRT Analysis user. This could also be the LDAP user name, depending on the site. Your system administrator can help decide the user name.

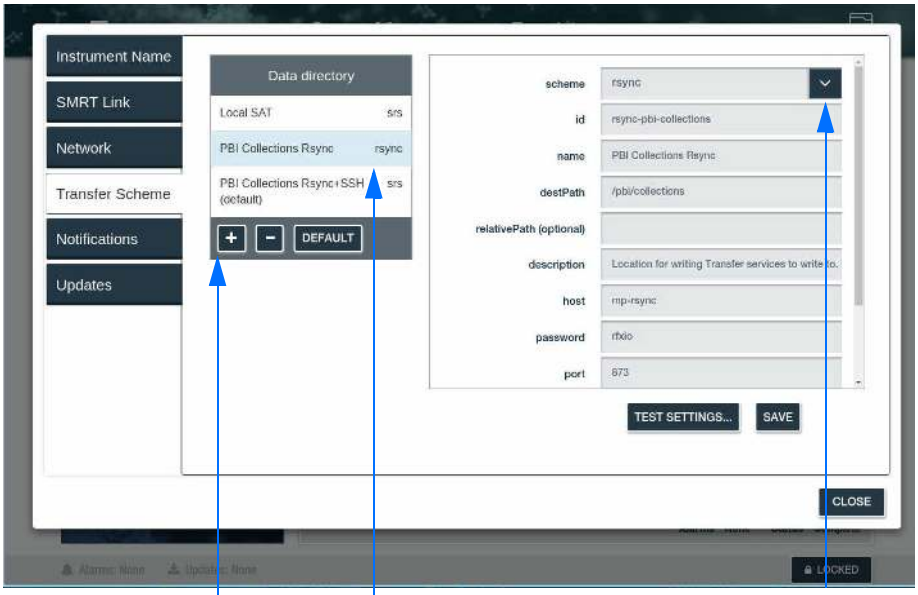
d. Click **SAVE**.

e. Click **TEST SETTINGS** to make sure that the configuration works correctly.
**Note**: You can save several different data directory locations but select one to be the default option. The default location will be applied to the current run. If you'd like to save the run to a different location, you must select that location and then select **DEFAULT**. The + and - can be used to add and delete transfer locations. Scrolling through the options will be enabled when there are several locations to choose from.

**For rsync transfer scheme:**

This method can be used to transfer data by **rsync daemon**. This option maybe preferred by system administrator if an rsync server and daemon already exist on your network. Note that data transfer using this method is not encrypted.

a. In the **Data directory** dialog, click **+**. A new transfer scheme displays in the right-hand side of the tab, ready to be filled in.




b. Select `rsync` transfer scheme. The fields for that transfer scheme type will display.

c. Enter information into the fields:

- **id**: User-specified text string. The transfer scheme id. This can only contain A-z, 0-9 and “-” or “_”. Example: `pbi-collections-test`.
- **name**: User-specified text string that displays in the **Data directory** dialog to identify the transfer scheme.
- **destPath**: This is the file system location that contains all data transferred via rsync.
- **relativePath (optional)**: This optional path can be used to place run data in a specific sub-directory underneath the location specified in destPath (on a per instrument basis). A common value for this field is the instrument serial number or name. This field can contain only alphanumerics, “-“, “_”, and “/”. This field is not required, but is provided to allow separation of run data from different instruments. This allows for easier location of particular run data (when a user is browsing the file system).

- **description**: User-specified text string that describes the transfer scheme.
- **host**: The DNS name or IP address of the machine serving as the rsync server. This may be the SMRT Link server or another computer on the network. The name of this system may be obtained from your system administrator.
- **password**: The password used to authenticate the rsync user. Your system administrator (for rsync servers) can help decide the password.
- **port**: Defaults to port `873`.
- **rsyncModule**: This is the name of the rsync module configured on the rsync server. This module must share the path provided in the **destPath** field (from above). The value to be used can be obtained from your system administrator (responsible for setting up the rsync server). Note that you must scroll down in the window to see this field.
- **user**: The user name used to authenticate to the rsync server and module. Your system administrator (for rsync servers) can help decide the user name. Note that you must scroll down in the window to see this field.

d. Click **SAVE**.

e. Click **TEST SETTINGS** to make sure that the configuration works correctly.
**Note**: You can save several data directory locations but select one to be the default option. The default location will be applied to the current run. If you'd like to save the run to a different location, you must select that location and then select **DEFAULT**. The + and - can be used to add and delete transfer locations. Scrolling through the options will be enabled when there are several locations to choose from.

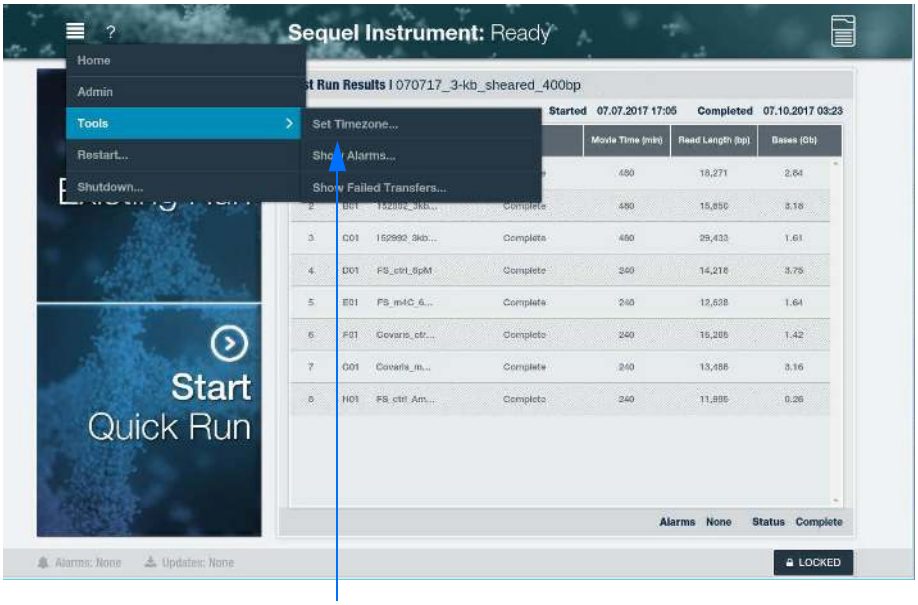
**To edit an existing transfer scheme**

1. In the **Data directory** dialog, scroll and select the transfer scheme to edit.
2. Edit fields and click **SAVE**. The edited transfer scheme displays in the **Data directory** dialog.
3. Click **TEST SETTINGS** to make sure that the configuration works correctly.

## Other Available Tools

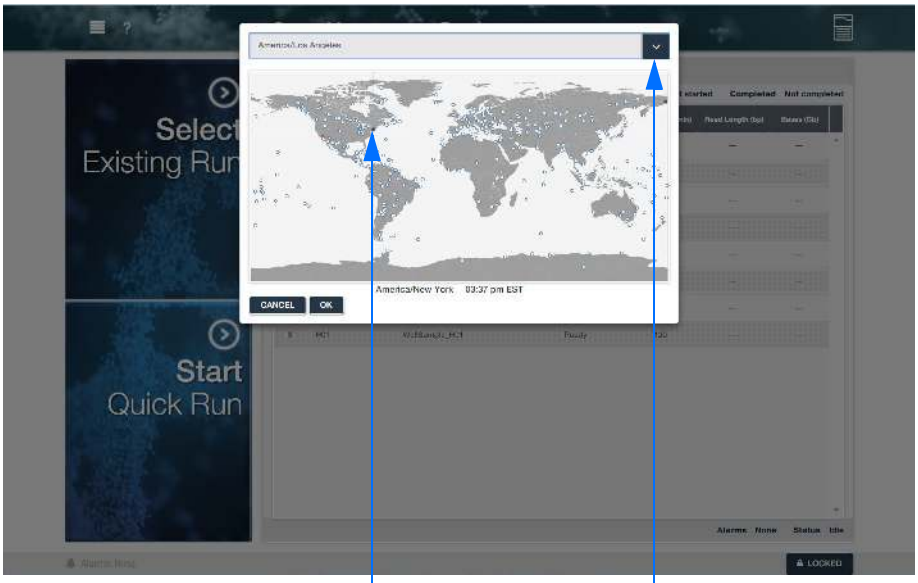
### Setting Timezones

1. From the Main Menu, select **Tools**, and then select **Set Timezone**.




Select Set Timezone

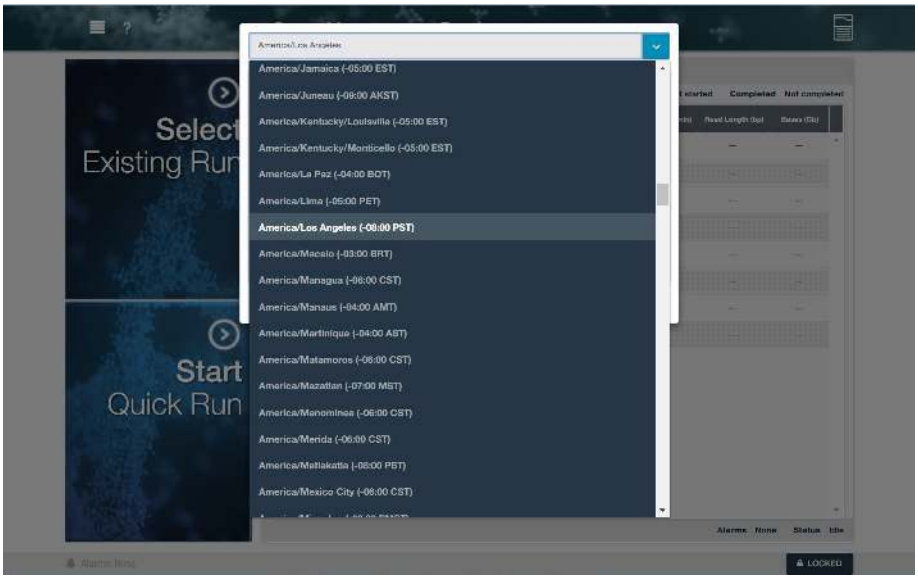
2. Select the desired timezone by choosing the appropriate location from the drop-down menu or selecting it directly from the map. When the selection is made directly on the map, the drop-down menu will be updated to further refine your location.




Select location directly on the map

Select arrow to view drop-down menu of locations

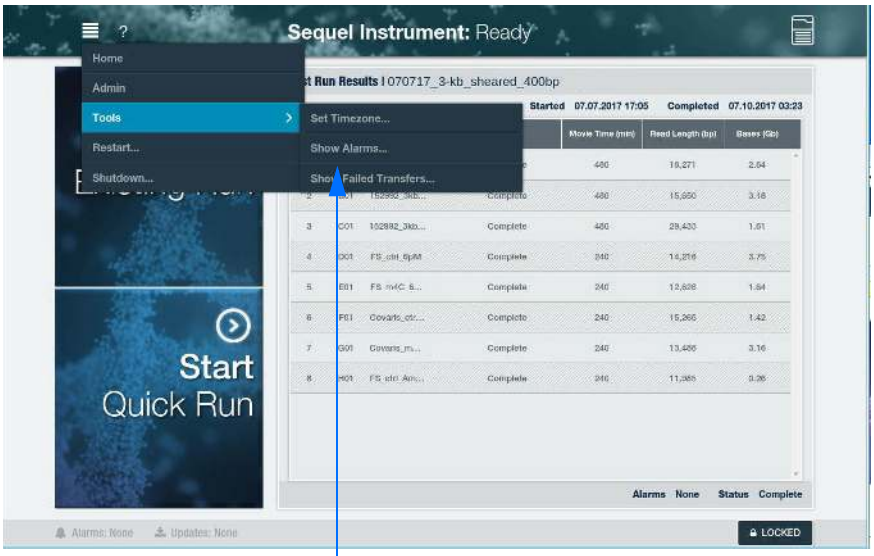
3. At the pop-up window, scroll down until you find the right location.




4. Then select **OK**.

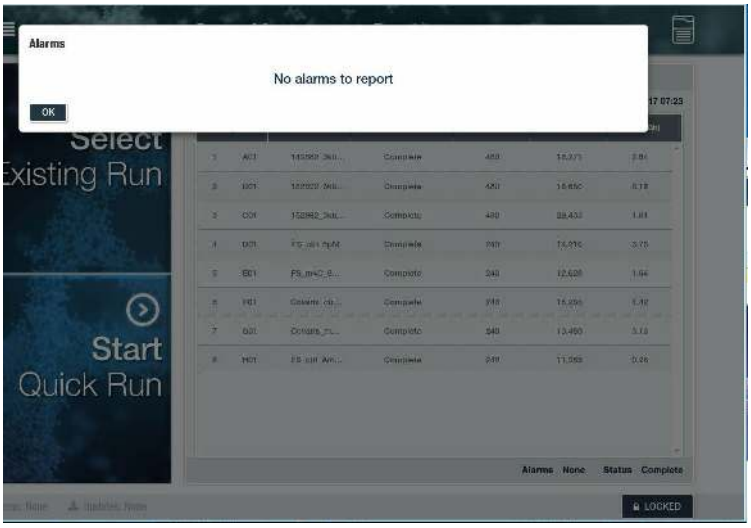
### Viewing Alarms

1. From the Main Menu, select **Tools**, and then select **Show Alarms**.



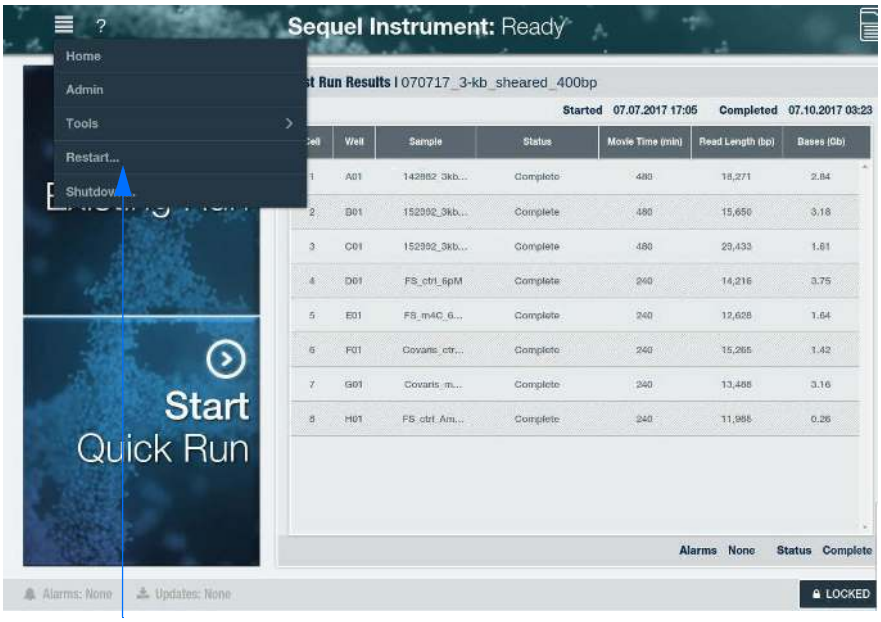

Select Show Alarms

2. An alarm pop-up window appears displaying system alarms, if any.



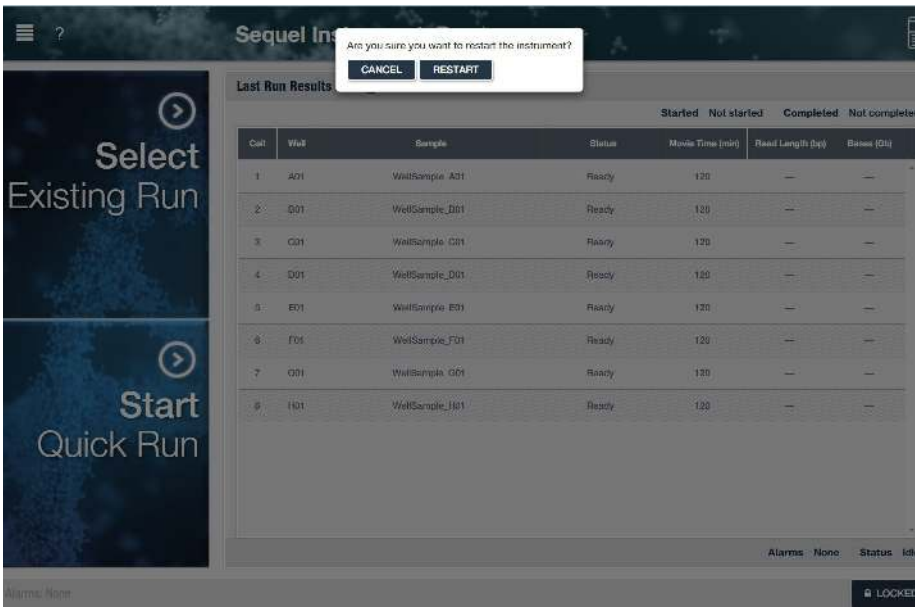
### Instrument Restart

1. At the Main Menu, select **Restart**.



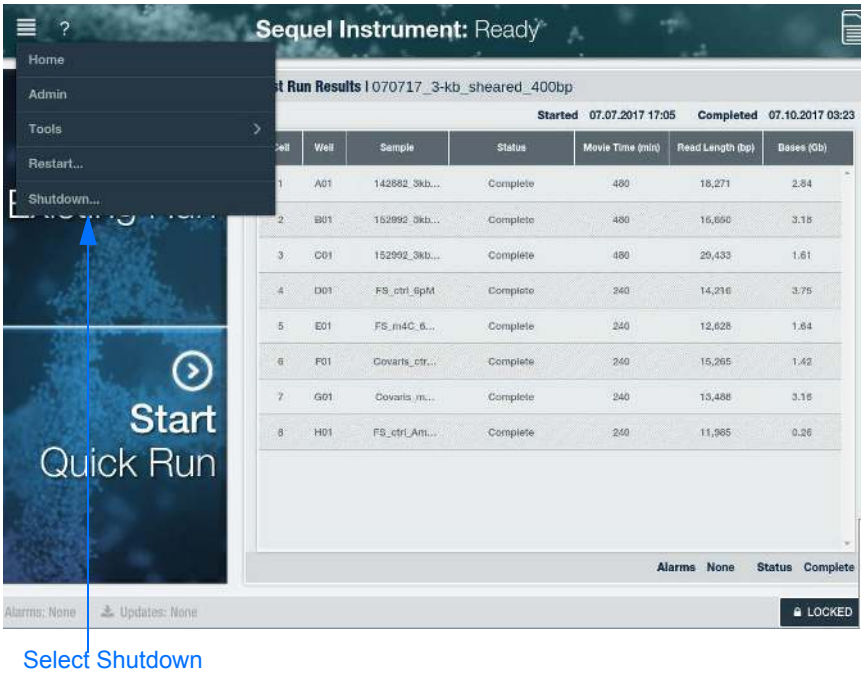

Select Restart

2. At the pop-up window, select **RESTART**.



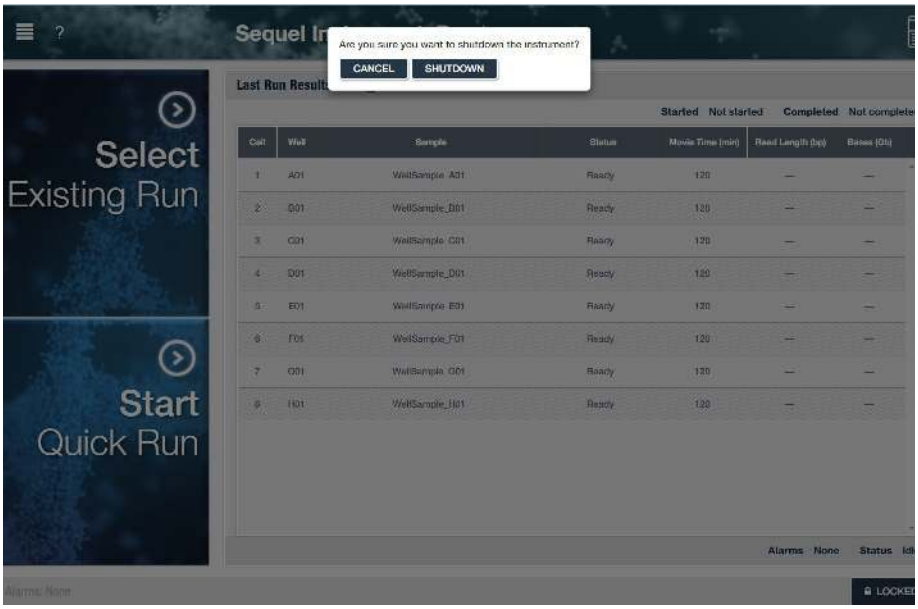
## Instrument Shutdown

1. At the Main Menu, select **Shutdown**. Note that it may take a few minutes for the instrument to shut down.



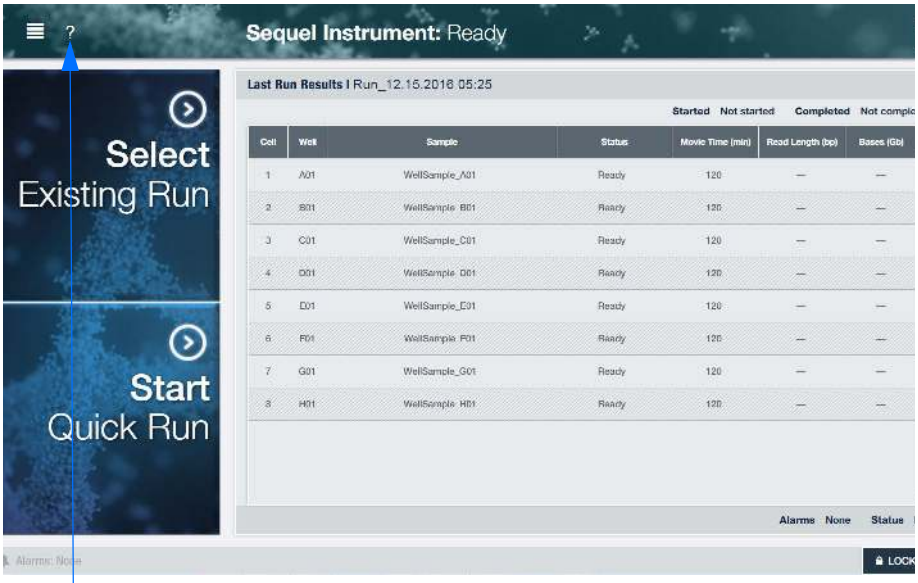

Select Shutdown

2. At the pop-up window, select **SHUTDOWN**.



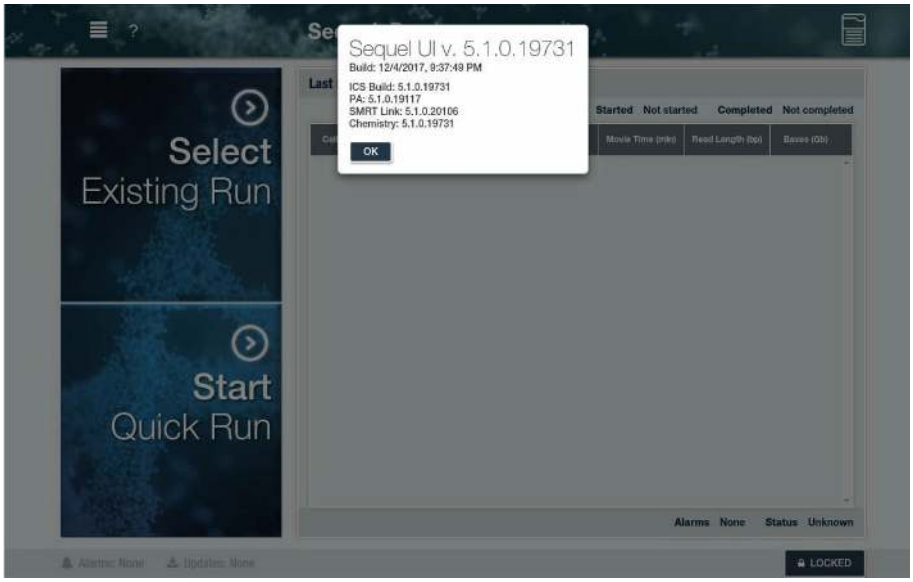
**To Verify Instrument Software version**

1. Select the question mark (**?**) next to the Main Menu option.




Select **?** mark

2. A pop-up window will show the following information:
   - Sequel User Interface software version and date
   - Instrument Control Software (ICS) version
   - Primary Analysis Software (PA) version
   - SMRT Link version
   - Chemistry version



## Loading the Instrument

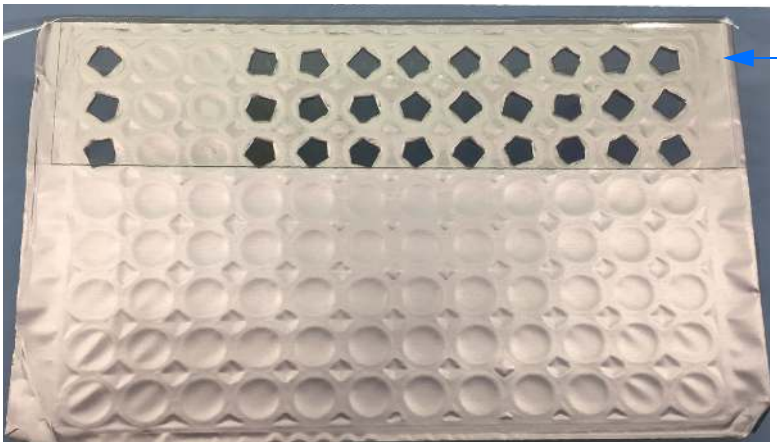
The following materials and components are needed before sequencing on the Sequel System:

- SMRT® Cells or SMRT® Cells LR
- Sequel® Sequencing Kit
- Sequel® SMRT® Cell Oil
- Tube Septa
- Sequel® Sample Plate Foil
- Sequel® Mixing Plate
- Sequel® Pipet Tips
- Sample Plate
- SealPlate® Adhesive Sealing Films for Microplates from E&K Scientific (recommended vendor)
- ALPS 50 V-Manual Heat Sealer (Thermo Fisher Scientific Catalog No. AB-1443)

### Prepare Reagents

Prepare the following reagents accordingly:

1. Remove the OS Enzyme from the sequencing package and place in ice until ready to use. When ready to use, spin down the tube for 5 seconds and replace the cap with a tube septum. Note that with Sequencing Plate version 3.0 or later, the OS Enzyme will be in the Sequencing Plate and no longer shipped in a separate tube. Therefore, this step will not be necessary.
2. Spin down the SMRT Cell Oil tube for 5 seconds and replace the cap with a tube septum.
3. When using the sequencing plate for the first time:
   - Remove the plate from the mylar bag
   - Place the plate in a room temperature (~22ºC) water bath covered with foil to protect the plate from light for 60 minutes.
   - Remove the plate from the water bath and invert, tap, and observe the 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 plate for 1 minute at 150 rcf to ensure that the reagents are in the bottom of the deep-well plate.
   - Before loading on the instrument, wipe any moisture or contaminants from the top of the plate.
4. After use, if a part of the plate is unused, ensure that the plate is kept at 4ºC and protected from light. Any accompanying unused OS enzyme should also be stored at 4ºC (note that with Sequencing Plate version 3.0 or later, the OS Enzyme will be in the Sequencing Plate and no longer shipped in a separate tube).
5. When using the sequencing plate for a second time:
   - Keep the plate at 4ºC and protected from light.
   - 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.




- Mix by vortexing at 1000 rpm for 1 minute.
- Spin down the plate for 1 minute at 150 rcf to ensure that the reagents are in the bottom of the deep-well plate.
- Remove the glue plate seal. It is important that the unused wells are not occluded by any foil seal other than that with which it is shipped.
- Before loading on the instrument, wipe any moisture or contaminants from the top of the plate.

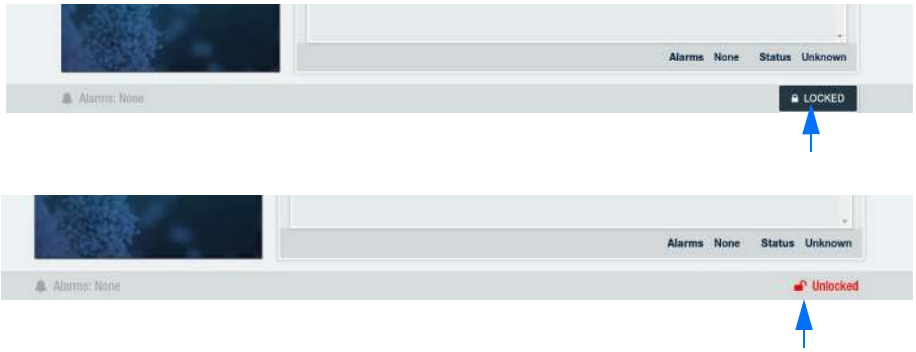
## Prepare the Sample Plate

1. Prepare samples for loading following the procedure outlined in SMRT Link Sample Setup.
2. Transfer samples to a 96-well plate (Eppendorf) and seal the plate with a heat seal foil for 2.5 seconds at 172ºC.
3. Spin down the sample plate for 1 minute at 150 rcf.
4. Leave on ice until ready to load on the instrument.

**Note**: Only the Sequel Sample Plate heat seal foil should be used on the sample plate. Other seals can cause run failures. Also, because the heat sealing necessarily compromises some of the structure of the sample plate, reusing sample plates is not recommended.

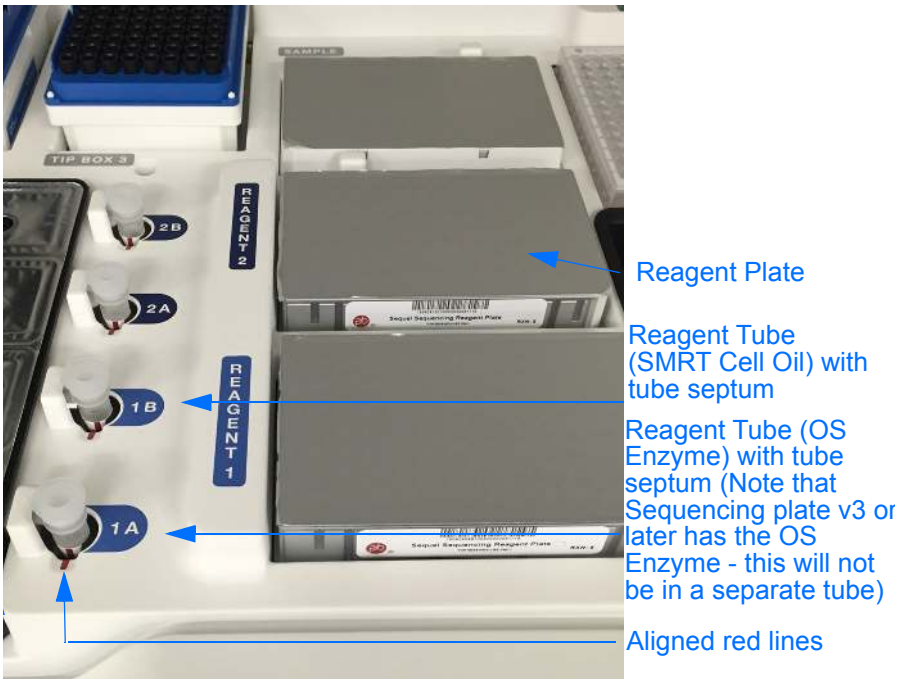
## Load the Reagent, Mixing and Sample Plates

Open the instrument door: On the Sequel ICS screen, select the **Locked** button at the bottom of the screen. The button name changes to **Unlocked**.




Load the Reagents onto the Instrument

1. If there is condensation buildup on the reagent slot, use KimWipes to blot off the moisture. Then place the reagent plate in the appropriate Reagent slot (do not remove the foil from the reagent plate). **Note**: The reagent plates are NFC-tagged for inventory tracking.




**Note**: The plate should fit snug and level into the reagent/sample 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.

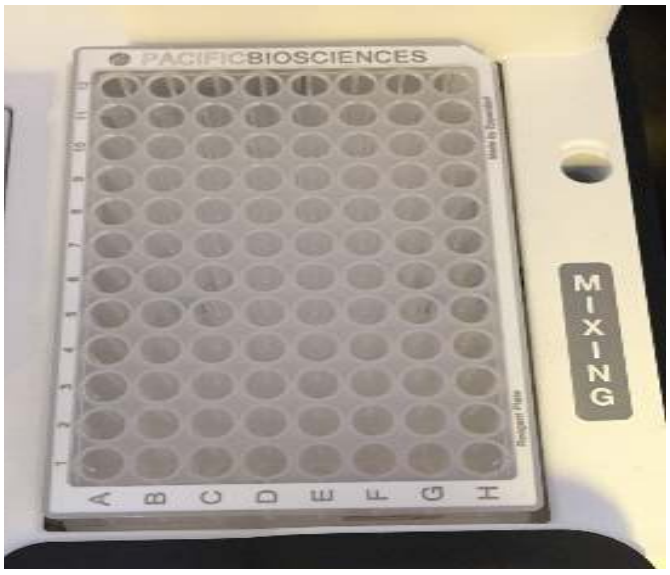
1. Place the OS Enzyme tube, with a tube septum, in either slot labeled **A** or **B**, matching the reagent plate location (**1** or **2**). Be sure to align the tube with the red line etched into the slot. Note that with Sequencing Plate version 3.0 or later, the OS Enzyme will be in the Sequencing Plate and no longer shipped in a separate tube. Therefore, this step will not be necessary.
2. Place the SMRT Cell Oil in the **other** slot matching the reagent plate location. Be sure to align the tube with the red line etched into the slot. **Note**: Both reagent tubes are NFC-tagged for inventory tracking - the software knows the position of each tube.
3. Ensure that the tubes are seated properly by pushing the tubes all the way down.


### Load the Sample and the Mixing Plate onto the Instrument

1. Place the sample plate in the slot labeled **Sample**. If there is condensation buildup on the sample plate slot, use KimWipes to blot off the moisture. Note that the plate **must** be heat sealed. See “Load the Reagent, Mixing and Sample Plates” on page 18.

**Note**: The plate should fit snug and level into the reagent/sample 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.


2. Place an empty unsealed Reagent plate (Eppendorf LoBind Deep-well plate) in the slot labeled **Mixing**. Note that this plate is not sealed.

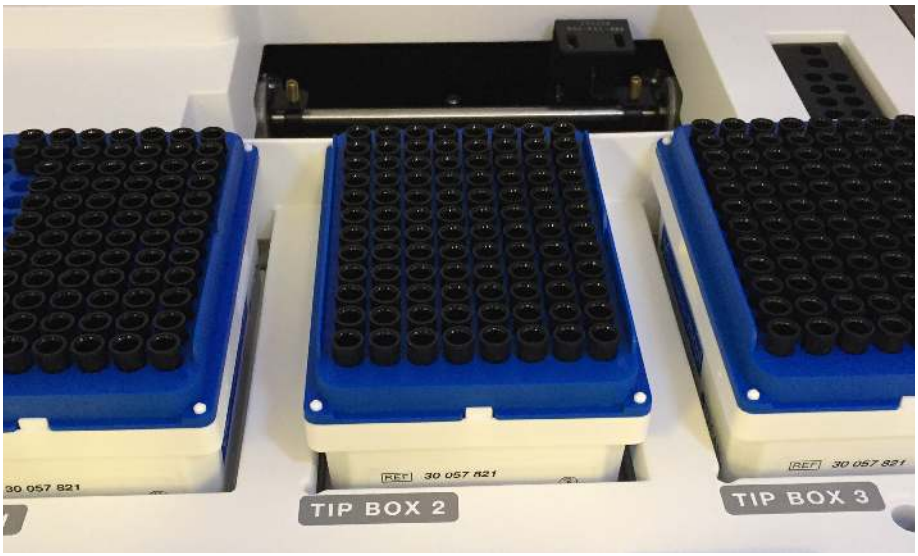


## Load the SMRT Cells or SMRT Cells LR and Tips onto the Instrument

1. Place the appropriate number of SMRT Cells or SMRT Cells LR 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.


2. Remove the lids from the tip boxes and place 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 unit was powered down and cycled back up, then the last known tip position will be lost. The unit should be reloaded with 3 full tip boxes.
   Also, replace any empty racks with full racks. The software will try (up to 96 times) to find the tips. If tips are not detected, the software

will not move to the next tip box. It is important to replace empty tip boxes with full tip boxes.

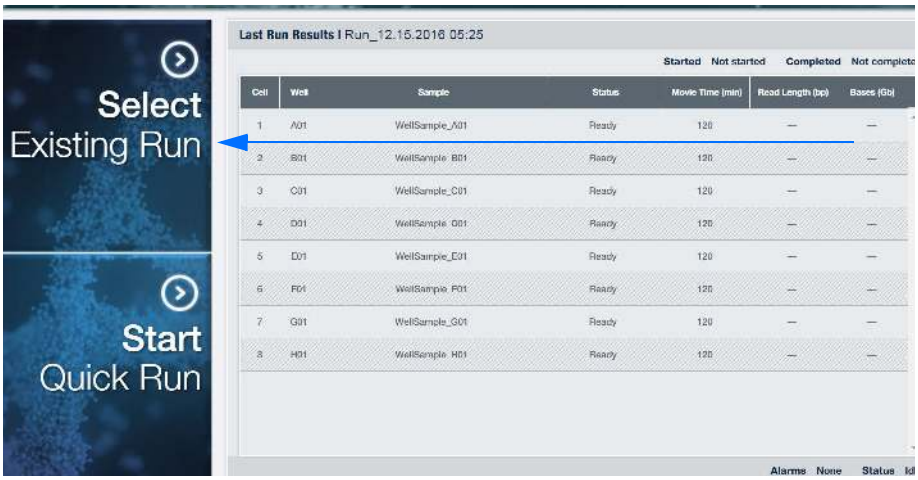



### Waste Bin and Nitrogen

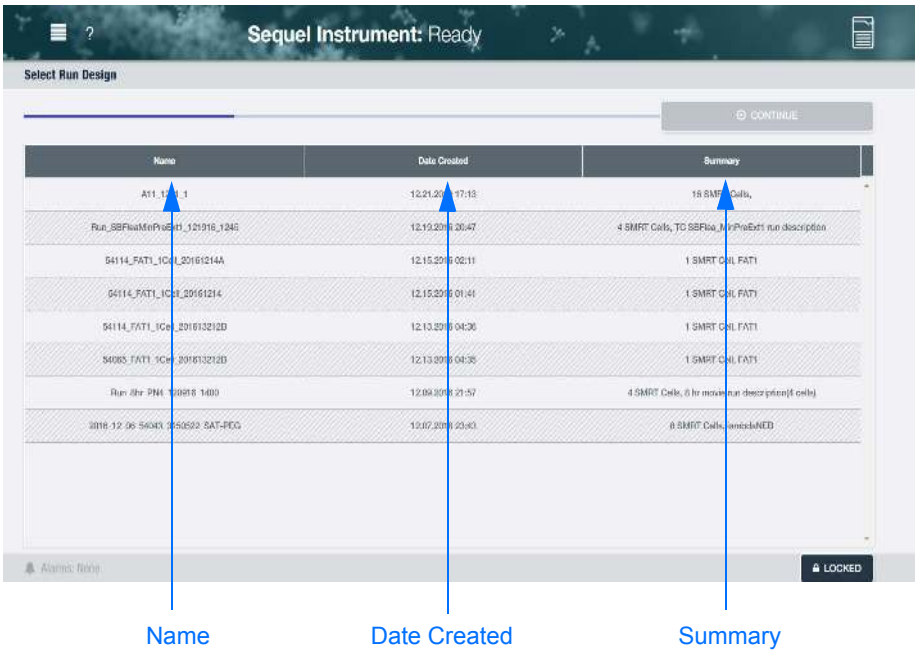

1. Verify that the Waste Bin is empty.
2. Locate the nitrogen tank (or source) connected to the instrument and verify that the supply pressure is at least 50 psi.

## Starting a Run Using an Existing Run

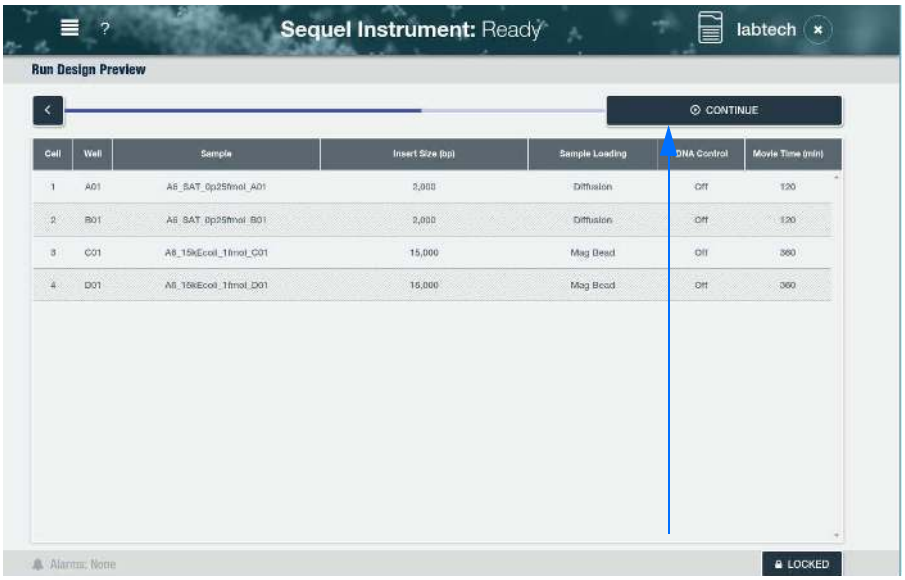
1. From the Home page, choose the **Select Existing Run** option.



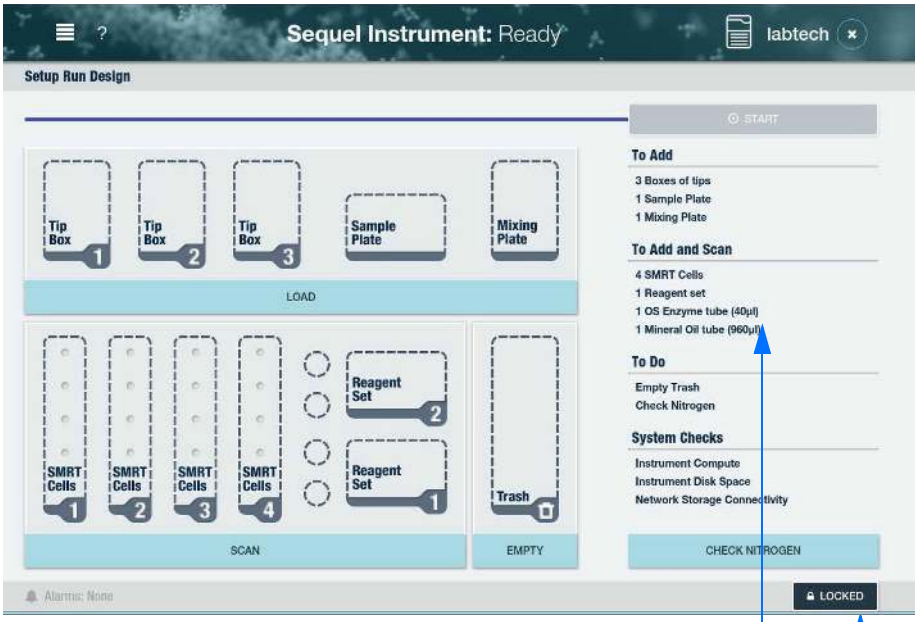

2. Select an Existing Run, created in the SMRT Link Run Design module **or** imported using a CSV file. Note that the run can be sorted by **Name**, **Date Created** or **Summary**. Select either of these headings to sort by that feature. Select them again to reverse-order the list.



3. Review the Run Design and select **CONTINUE** (at the top right-hand corner of the screen). It is important to note that the instrument can continuously run up to 96 hours (which includes time for all preparation steps and approximately 80 hours movie collection time). For example, the 96 hours would include running eight (8) SMRT Cells for 10 hours, 12 SMRT Cells for 6 hours, etc. You must verify that your network can accommodate the data transfer space for these runs. If all runs have successfully transfered off of the instrument, the instrument can store approximately 24 hours of run space.

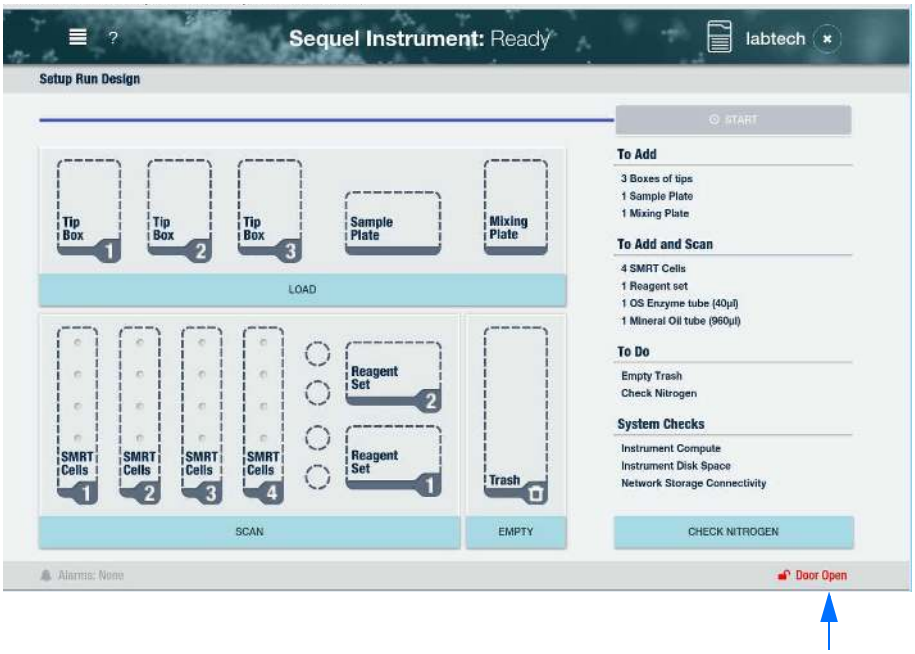



4. The Inventory page displays. If changes need to be made, unlock the door by selecting the **LOCKED** button at the bottom of the screen.

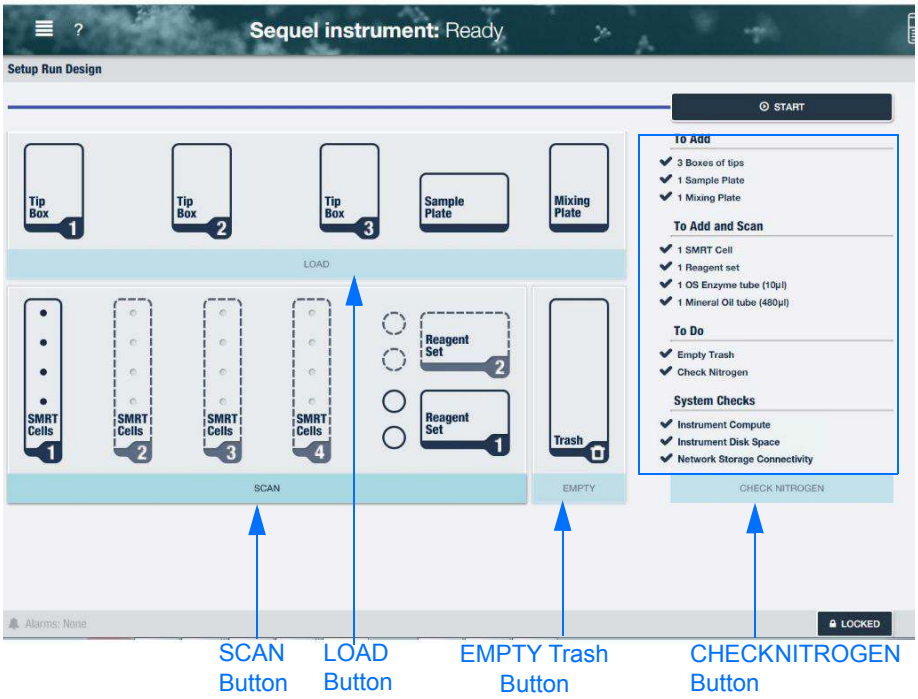



With Sequencing Plate v3.0 or later, the OS Enzyme tube option will not display


5. The **Unlocked** button displays the state of the unit as unlocked. It is now safe to open the door. Once the door is opened the UI will display **Door Open**.



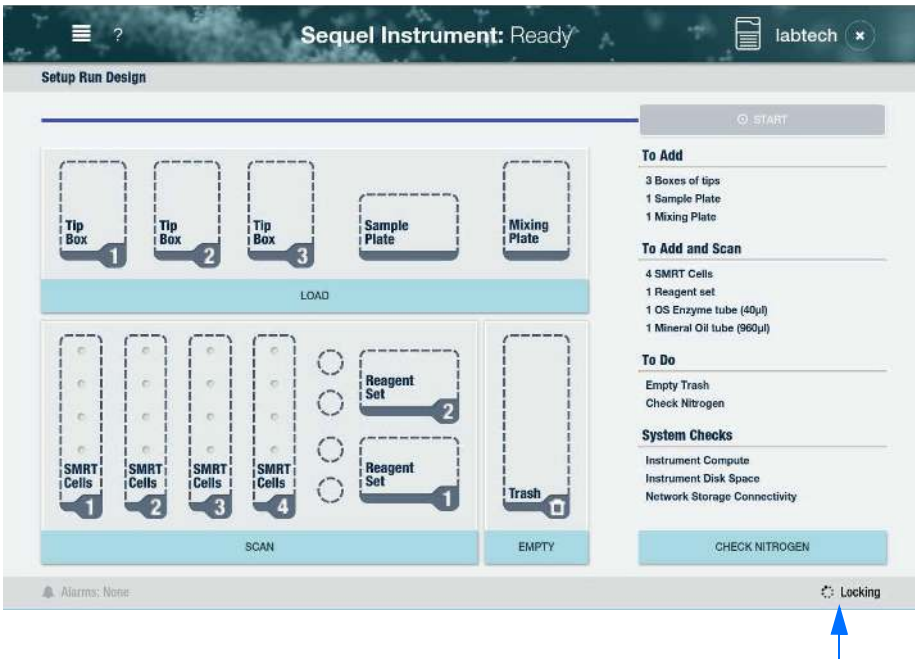

6. Verify the workdeck additions and selections using the list shown in the blue box below. Then check inventory by selecting **LOAD**, **SCAN**, **EMPTY** and **CHECK NITROGEN**. Note that these four buttons **must** be selected and the door closed to enable the **START** button.



Note that the System Checks verify the following:

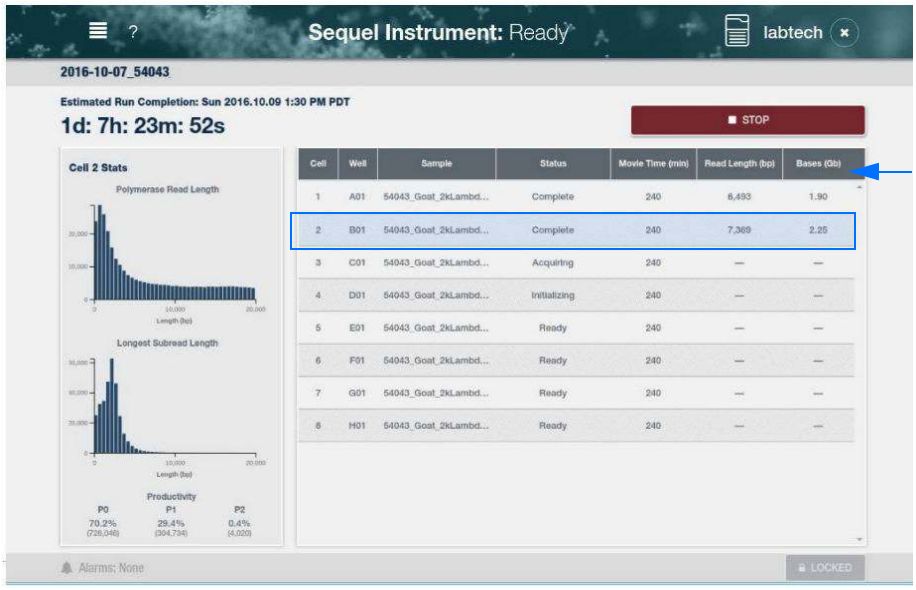
- **Instrument Compute**: Instrument systems and communications are functional.
- **Instrument Disk Space**: Verifies sufficient amount of instrument disc space to initiate a run.
- **Network Storage Connectivity**: Verifies that the transfer location is available and has read/write access (the initial run folder is written to prior to starting).

7. Close the door. The UI will display that the door is **Locking**.

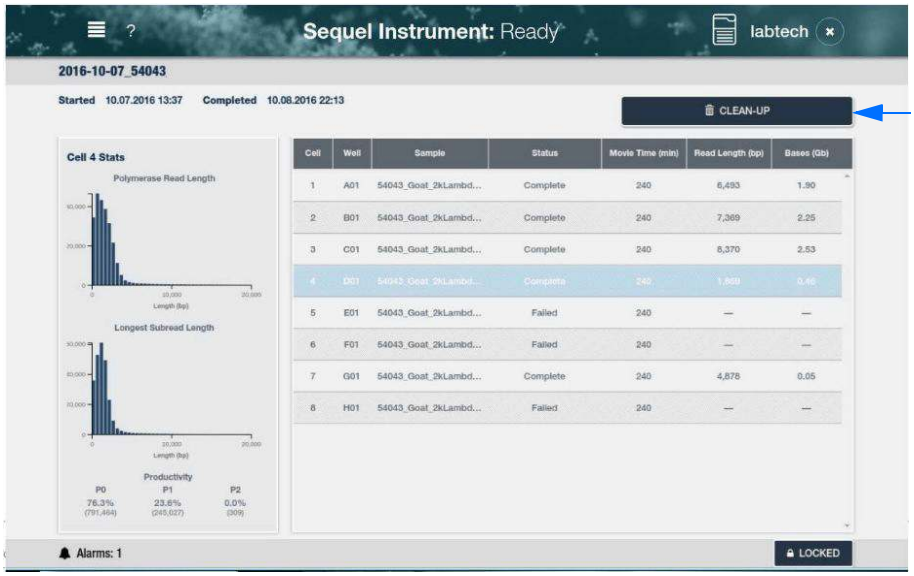



**Important**: Once the door is closed, **wait about 10 seconds,** until the instrument LED light turns green (the light is yellow while the door is open). The UI will display that the door is locked.

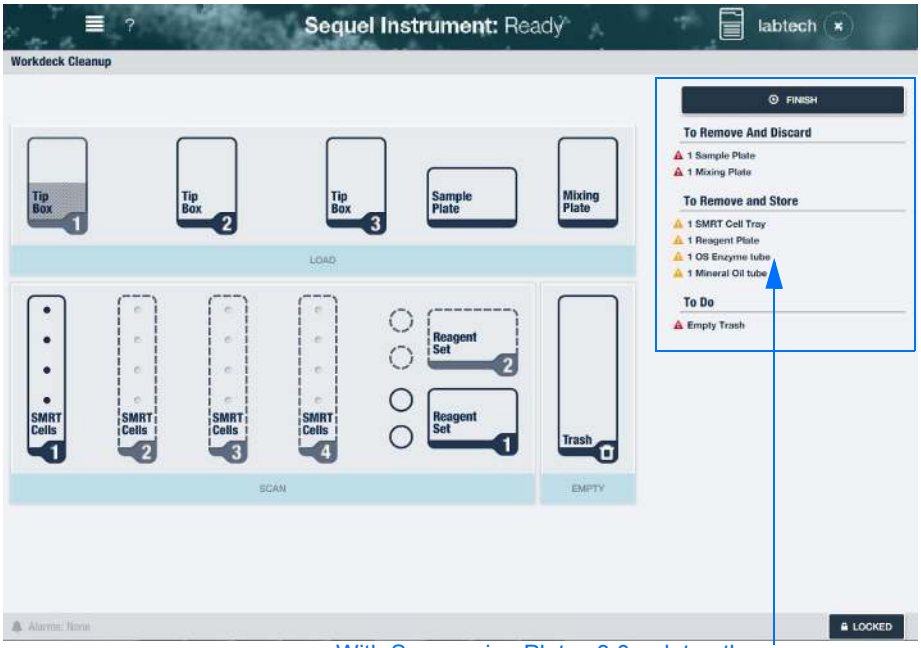
8. Select **START** to start the run. Note that several warning features may display. See Appendix A for a list of possible error messages and how to disposition them.
9. The screen displays estimated completion time, cell status and metrics.
10. After the run is complete, the status and metrics will display. During the run, you may select a cell to display its run metrics.




11. After the run, select **CLEAN-UP**, located at the top right-hand corner of the screen.




12. The Workdeck Cleanup screen provides guidance on what should be removed from the Workdeck. Note that it's important that anything that is removed be stored at 4ºC after removal.




With Sequencing Plate v3.0 or later, the OS Enzyme tube option will not display

13. Unlock the door by selecting the **Locked** button. Remove reagents and disposables according to the on-screen guidance.
14. Select **FINISH** (at the top right-hand corner of screen) to return to the Home screen. Once the run started, the stability of the sequencing plate and SMRT Oil is 100 hours and the OS Enzyme is 72 hours from that time.These reagents must be removed from the Workdeck and stored at 4°C for no longer than 72 hours or 100 hours, as indicated. Otherwise, they should be used for the next run.

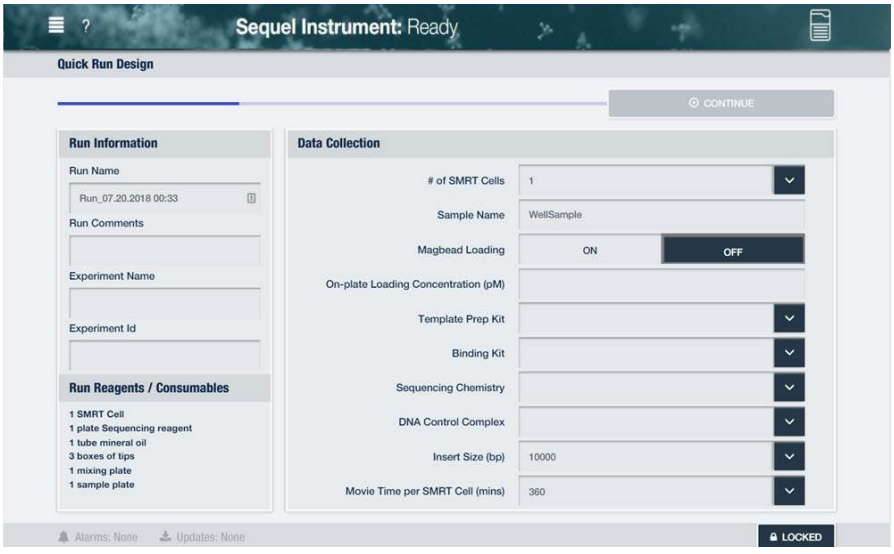
    Note that with Sequencing Plate version 3.0 or later, the OS Enzyme will be in the Sequencing Plate and no longer shipped in a separate tube. These reagents must be removed from the Workdeck and stored at 4°C for no longer than 72 hours. Otherwise, they should be used for the next run.

## Starting a Run using “Quick Run”

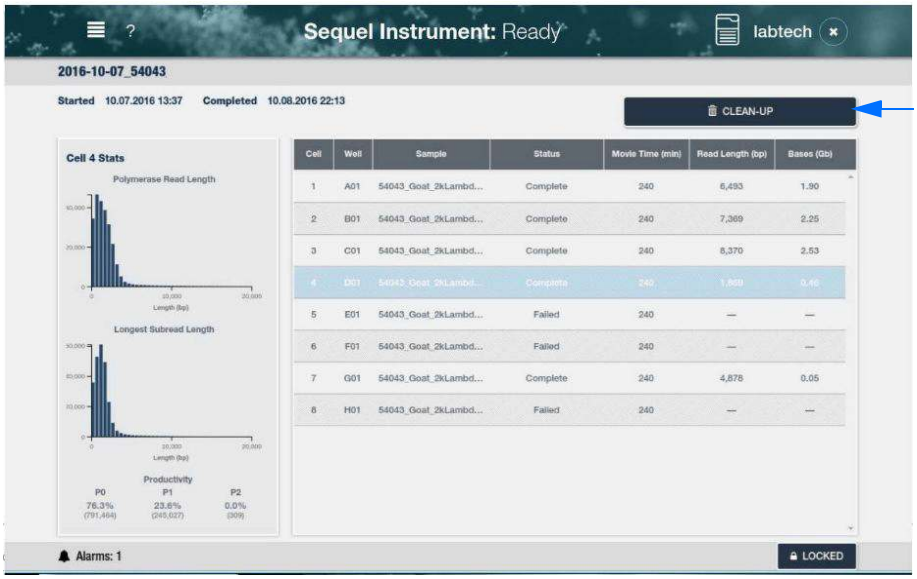
Runs can also be initiated using the **Quick Run** option.

**Note**: The information and run parameters entered using this feature apply to **all** samples within a run. Pacific Biosciences **highly recommends** using SMRT Link to **either** create a new Run Design or import a CSV file. All run constraints detailed in Using an Existing Run section above also applies to the Quick Run feature.

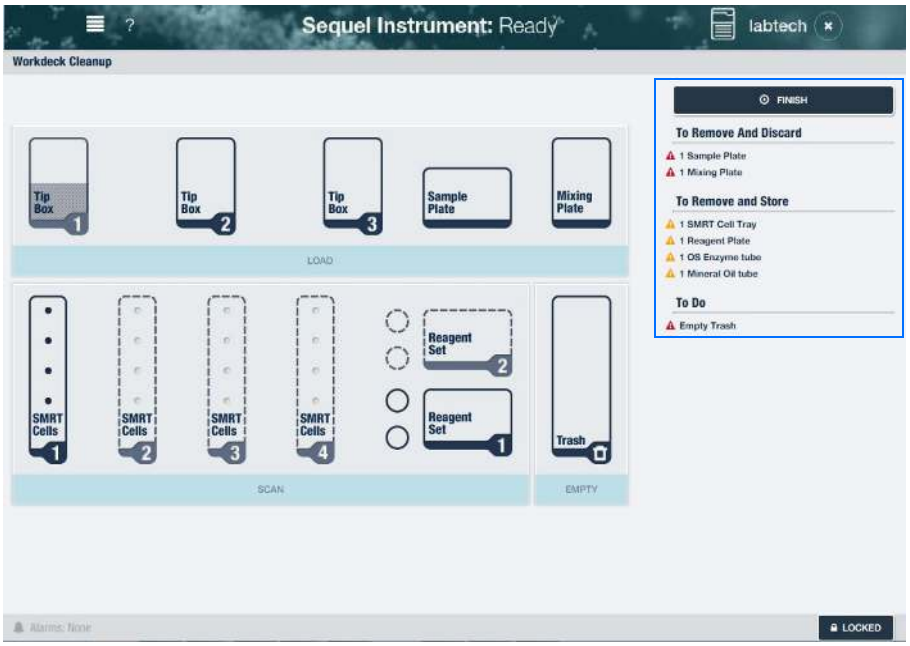
1. Select **Start Quick Run**.
2. Enter run information: (**Note**: Please **only** use alphanumeric characters in all text fields.)
   - **(Required)** Run Name
   - **(Optional)** Run Comments
   - **(Optional)** Experiment Name
   - **(Optional)** Experiment ID
3. Select the **number of SMRT Cells** to use in this run using the drop-down menu.
4. Enter a **Sample Name**. The information entered on this screen applies to **all** samples within a run. Currently, individual samples **cannot** be named.
5. Specify whether or not to use **MagBead Loading**. (**Off** specifies Diffusion Loading.) **Note**: Immobilization time **cannot** be customized using Quick Run. Diffusion samples will default to 1 or 2 hours (depending on the chemistry version you are using) and MagBead samples to 2 hour immobilization time.
6. Enter **On-plate Loading Concentration**.
7. Enter or scan, using a barcode scanner, the **Template Prep Kit** barcode.
8. Enter or scan, using a barcode scanner, the **Binding Kit** barcode.
9. Enter or scan, using a barcode scanner, the **Sequencing Plate** barcode.
10. Select the **Insert Size** (in base pairs) to use for this run.
11. Select the **Movie Time** (collection time) in minutes for **all** SMRT Cells in this run. Note that Movie Times greater than ten (10) hours require the use of SMRT Cell LR cells.



12. Select **CONTINUE**.
13. The Inventory page displays. Load the appropriate reagents and disposables onto the instrument.
14. Check inventory by selecting **LOAD**, **SCAN**, **EMPTY** and **CHECK NITROGEN**. The four buttons **must** be selected and the door closed to enable the **START** button. After closing the door, wait about 10 seconds, until the instrument LED light turns green.
15. Select **START** to start the run. Select **FINISH** (at the top right-hand corner of screen) to return to the Home screen. Once the run started, the stability of the sequencing plate and SMRT Oil is 100 hours and the OS Enzyme is 72 hours from that time.These reagents must be removed from the Workdeck and stored at 4°C for no longer than 72 hours or 100 hours, as indicated. Otherwise, they should be used for the next run.
    Note that with Sequencing Plate version 3.0 or later, the OS Enzyme will be in the Sequencing Plate and no longer shipped in a separate tube. These reagents must be removed from the Work-deck and stored at 4°C for no longer than 72 hours. Otherwise, they should be used for the next run.
16. After the run is finished, select **CLEAN-UP**.




17. The Workdeck Cleanup screen provides guidance on what should be removed from the Workdeck. Note that it's important that the reagents be stored at 4ºC.

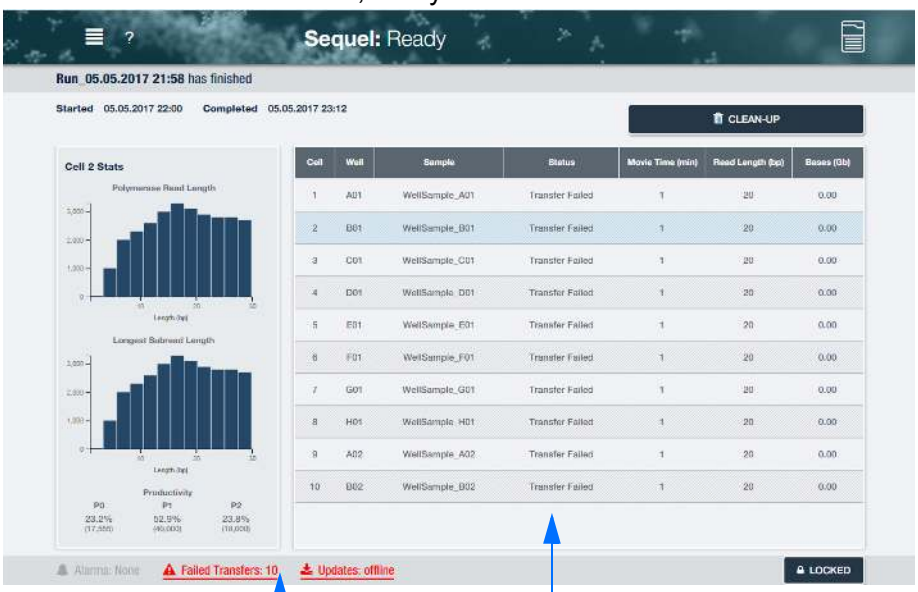



18. Unlock the door by selecting the **LOCKED** button. Select **FINISH** to return to the Home screen.

## Run Transfers

This feature displays any failures to transfer data from collections. Users can retry the transfers and receive feedback that retries are in progress. If successful retries are not possible, the capability to delete a failed transfer is available. You can also contact your IT Support to see if a network interruption caused the transfer failure or contact PacBio Tech Support for additional help.

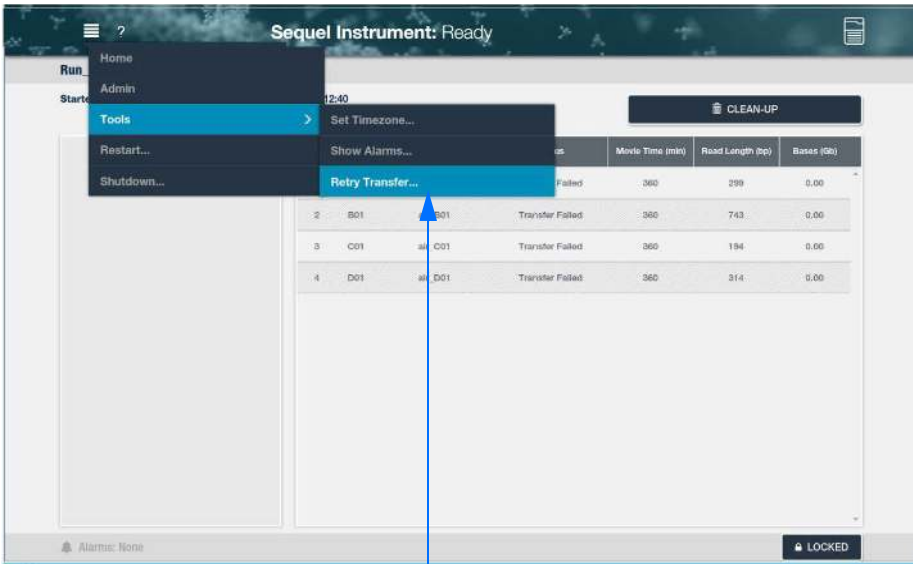
1. After a completed run, the following screen will show which Cell data was not transfered, if any.




The number of failed transfers

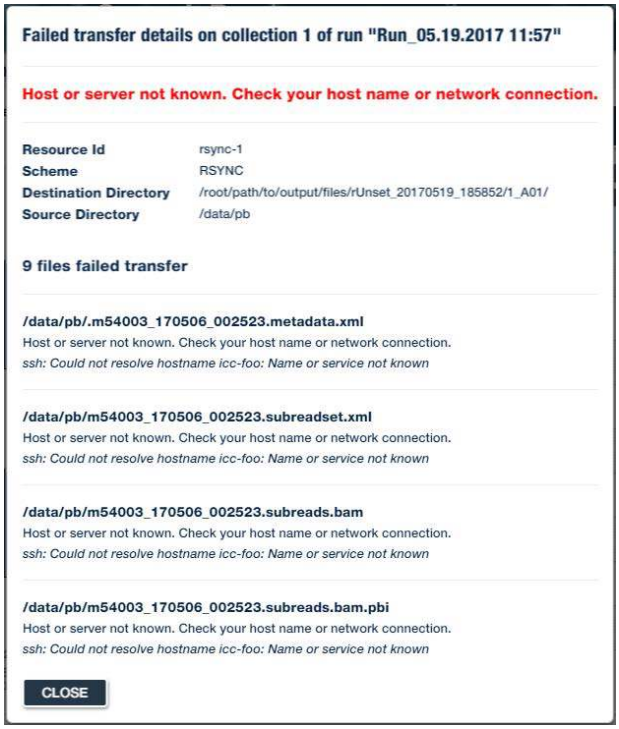
Failed transfers under Status column

2. If a Cell was not transferred, data transfer may be manually initiated. You can open the Failed Transfers Dialog box either by touching the Failed Transfers option shown above or from the Main Menu by selecting **Tools**, and then **Retry Transfer**. Note that a new run may be initiated during the transfer, however, if there is insufficient disk space to start the new run, then it will not proceed until there is sufficient space.



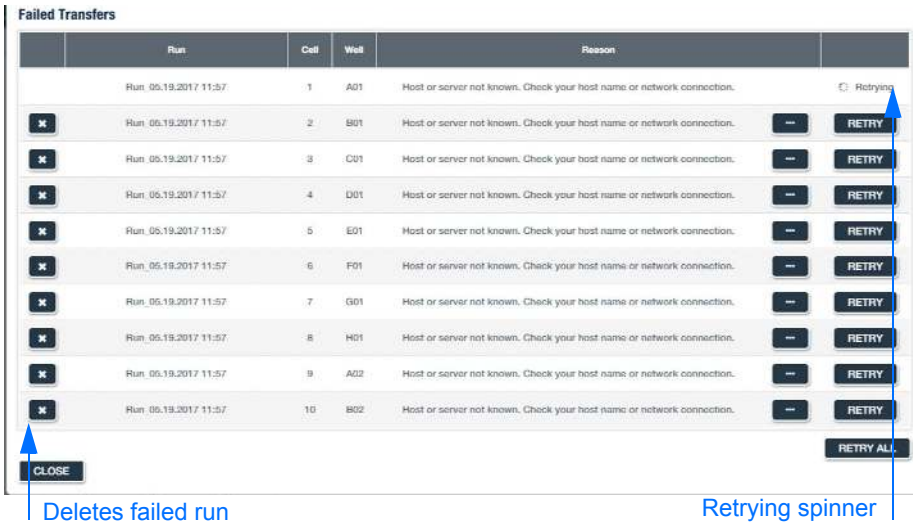

When selected, displays Failed Transfer details

3. The **Reason** column displays an explanation for the transfer failure. If all the file transfer failures are the same type, then the message displayed in the Reason field is the message for that type of file transfer failure. If there are multiple file transfer failure types, then the message in the Reason field will display the multiple transfer failure types. Note that the Reason column also shows a button with an ellipsis that, when selected, will open a Failed Transfer Details dialog box:



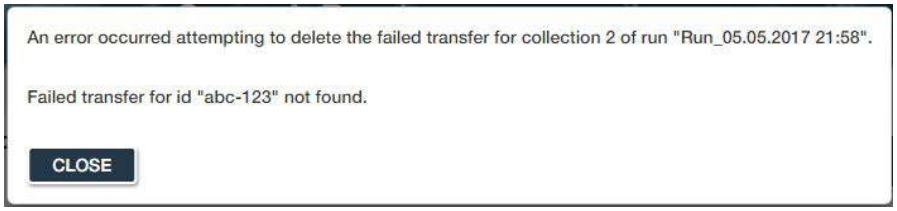

The red text displays the same message as the Reason field in the Failed Transfers dialog box. Below that is more information about the attempted transfer followed by more detailed information about each failed file transfer.

4. To retry a transfer, you can select the **RETRY** next to the specific file. The **RETRY** button will change to a **Retrying** spinner. To retry all the files, select **RETRY ALL** at the bottom of the RETRY column.




If the retried transfer attempt is successful, the failed transfer will disappear. If the retry attempt also fails, then the RETRY button will reappear with a new reason message, if any.

Note that the **X** button deletes a failed transfer, and the associated collections and data, from the instrument so that they no longer display as failed transfers. A dialog box will ask to confirm the deletion. If an error occurs after confirming the delete request a dialog box will display the reason:



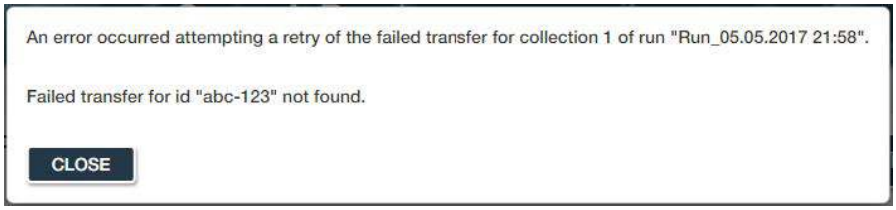

Also, the Failed Transfers dialog box can be closed while the retries are in progress. Closing the dialog box will not interrupt the transfers. Reopening the dialog box will continue to show the status of the failed transfers.

If the data transfer still fails, it will be saved for 72 hours on the instrument before being automatically deleted. Note that the 72 hours are counted only for working days (Monday through Friday). Weekends are not included. For example, if a run fails to transfer on a Saturday, the 72 hour time restriction will not start until Monday.

## Retry Errors

### Single Retry

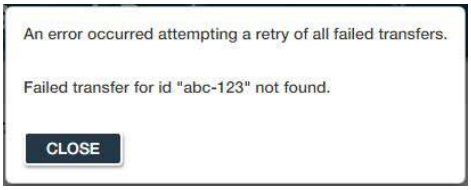
If an error occurs on the submission of a transfer RETRY request, the following dialog box will display. The first sentence will be common to all retry errors. The second sentence will be based on the particular issue that resulted in the error.




Note that a “Retry error” is an error on the retry **request**. It is not a failed transfer. A retry error is a failure in making the request, while a failed transfer is a successful request that later failed due to an issue.

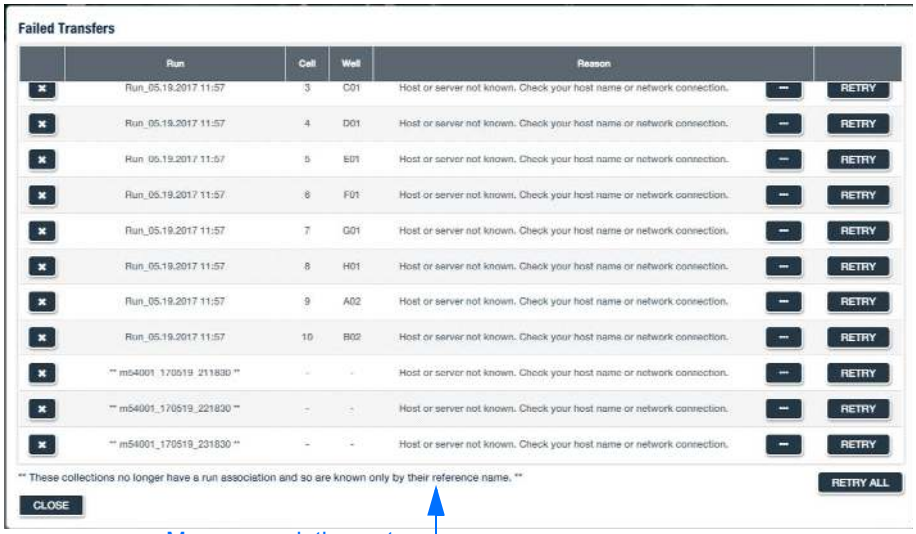
### Retry All

If an error occurs on the submission of a RETRY ALL request, the following dialog box will display.




## Orphaned Failed Transfers

These transfer failures can result when the Instrument Control Software no longer has the associated run information.This can happen if Instrument Control Software restarted before a failed transfer was resolved.



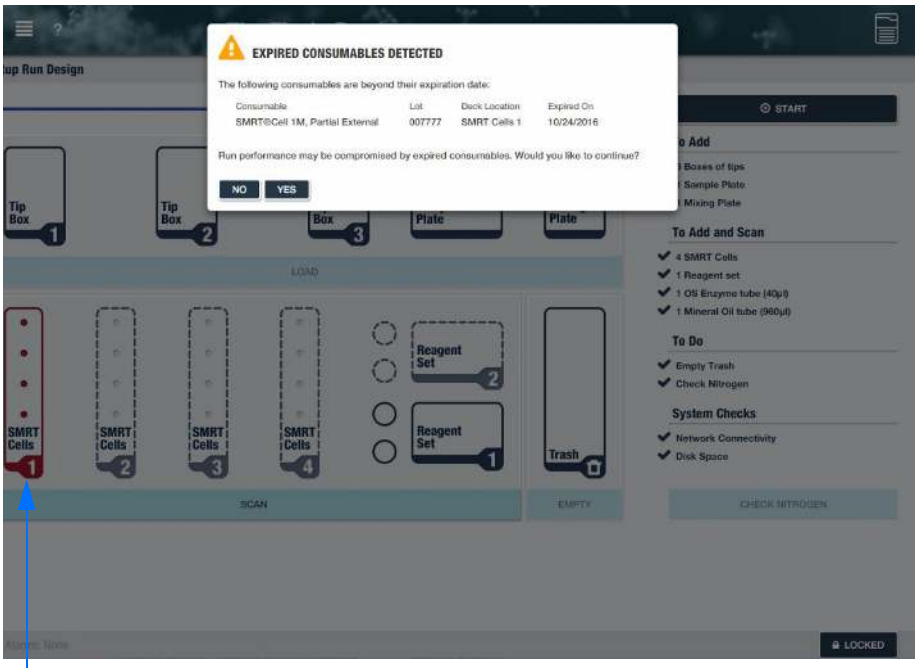

Message pointing out orphanded transfer

## Appendix A

This Appendix lists the possible notification warnings that may display after the Start button is selected.

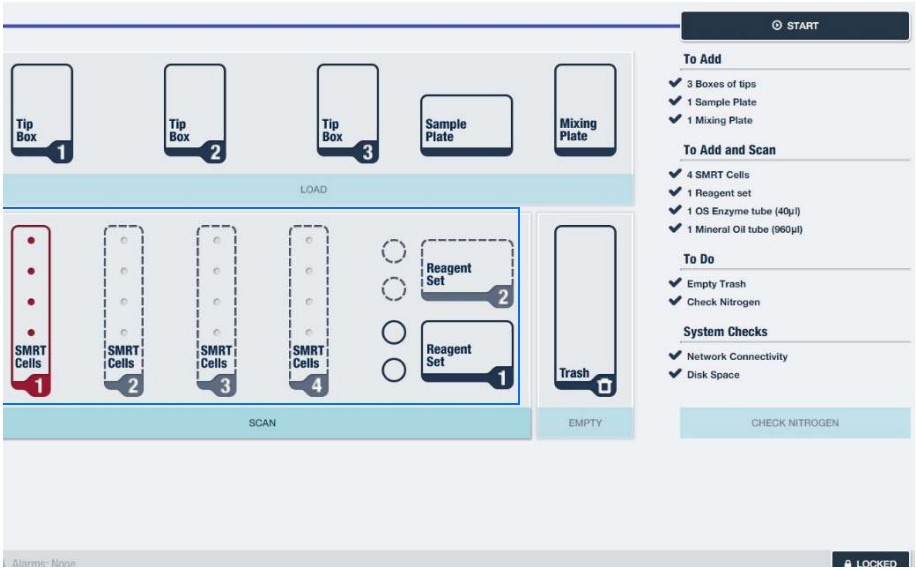
### Expired Consumables Detected: SMRT Cells

This notification warns that expired SMRT Cells were detected. The option to continue is allowed, once it is acknowledged that run performance may be compromised. If **NO** is selected, the expired SMRT Cells must be removed and replaced.

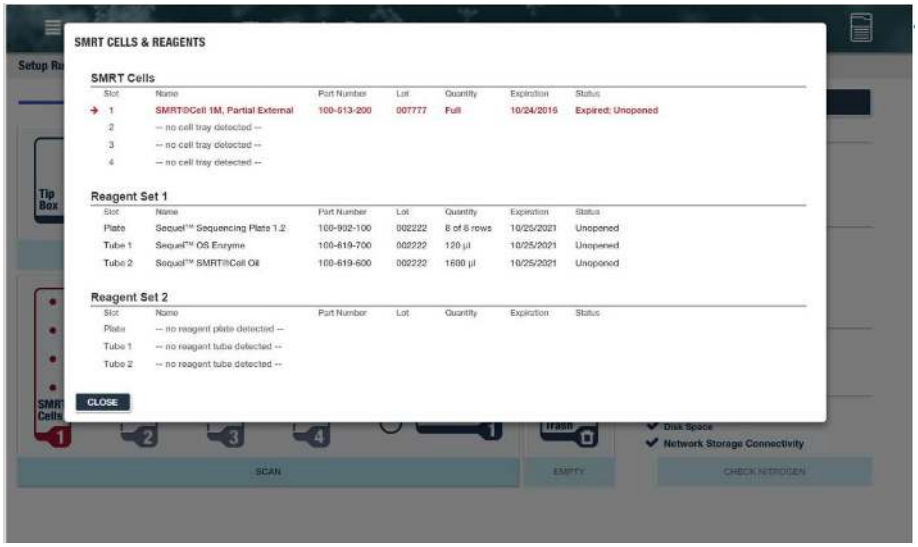



Location of expired SMRT Cells

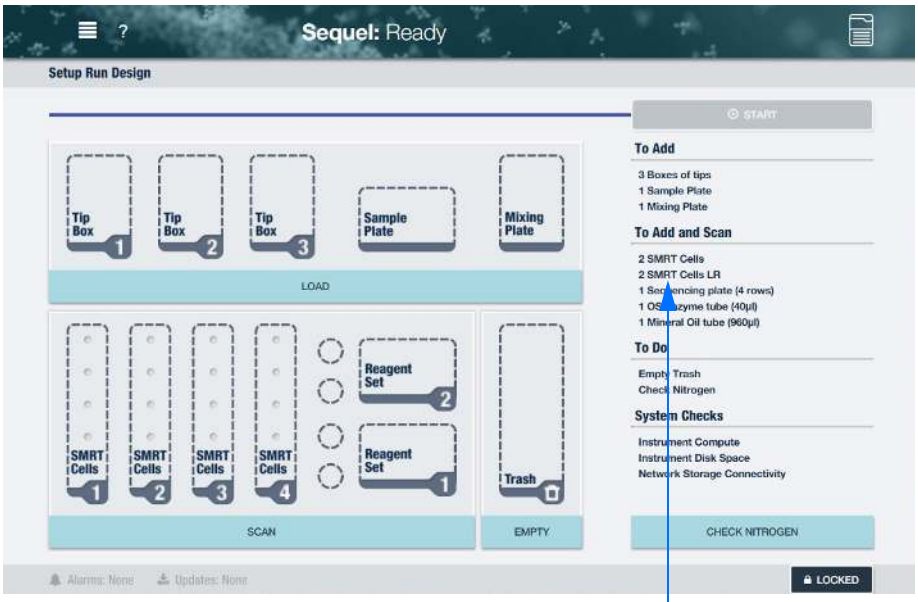
If **YES** is selected, the SMRT Cell tray will continue to display as red. Select any of the Reagent sets or SMRT Cells tray to see details on each item.



A detailed list of SMRT Cells & Reagents will display. The reagent you select will be highlighted with a red arrow for ease of location. Any expired item will be in red and will show its status. For example, the SMRT Cells here are “Expired” and “Unopened”.



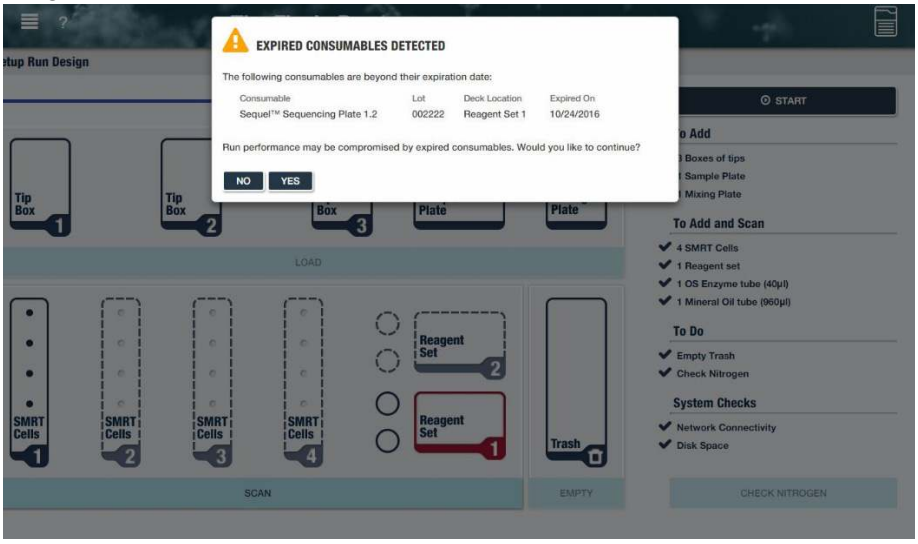

Note that a combined mix of SMRT Cells LR and SMRT Cells can also be used.




Combination of SMRT Cells and SMRT Cells LR

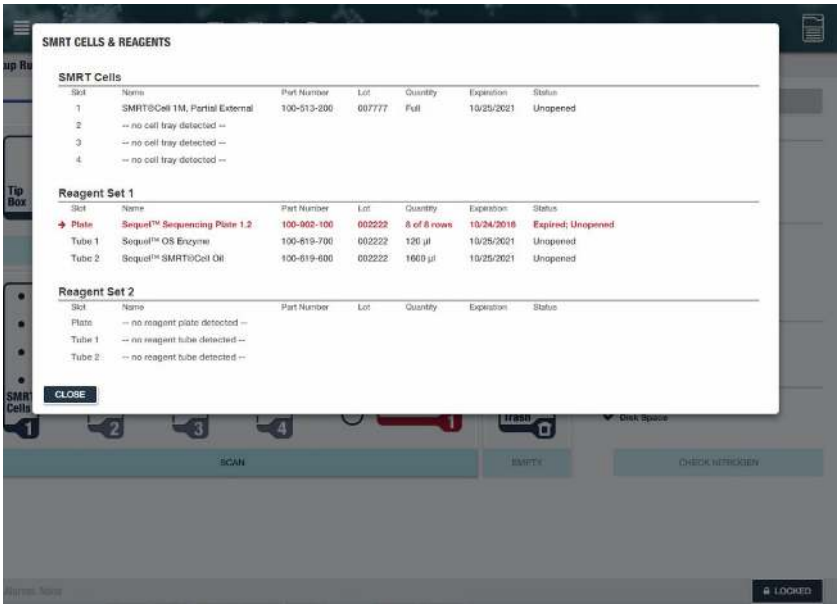
### Expired Consumables Detected: Reagents

This notification warns that expired reagents were detected. The option to continue is allowed, once it is acknowledged that run performance may be compromised. If **NO** is selected, the expired reagents must be removed and replaced.



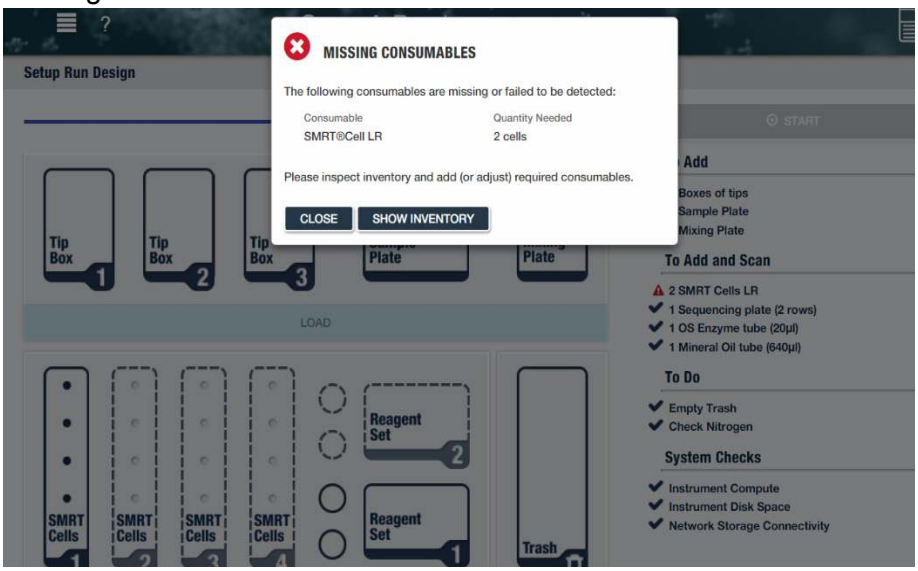

If **YES** is selected, the Reagent Set box will continue to display as red. Select any of the Reagent sets or SMRT Cells tray to see details of the inventory scan.

A detailed list of SMRT Cells & Reagents will display. The expired item will be in red and will show its status. For example, the Sequel Sequencing Plate here is “Expired“ and “Unopened”.



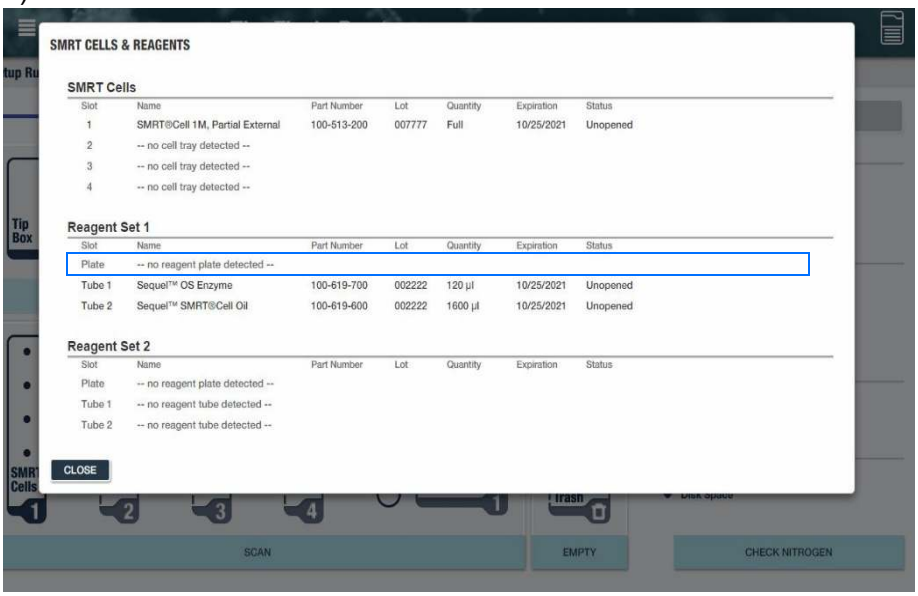
### Missing Consumables

This notification warns that there were missing consumables or that they were not detected. Select **Show Inventory** to see what is missing.



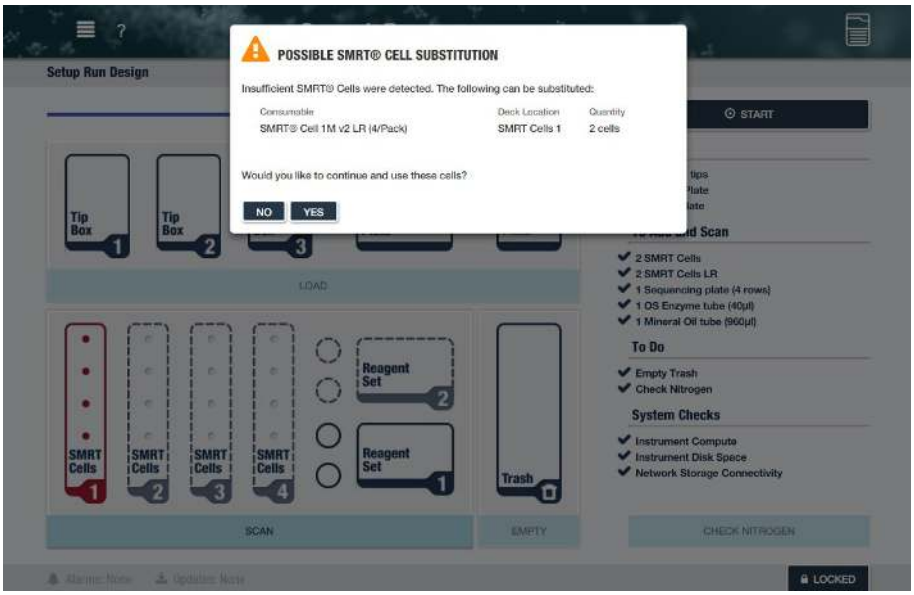

Note that if “Close” is selected, the UI will revert back to the loading screen. The item must be added and re-scanned.

The example below shows that the Reagent Plate (under Reagent Set 1) was not detected.



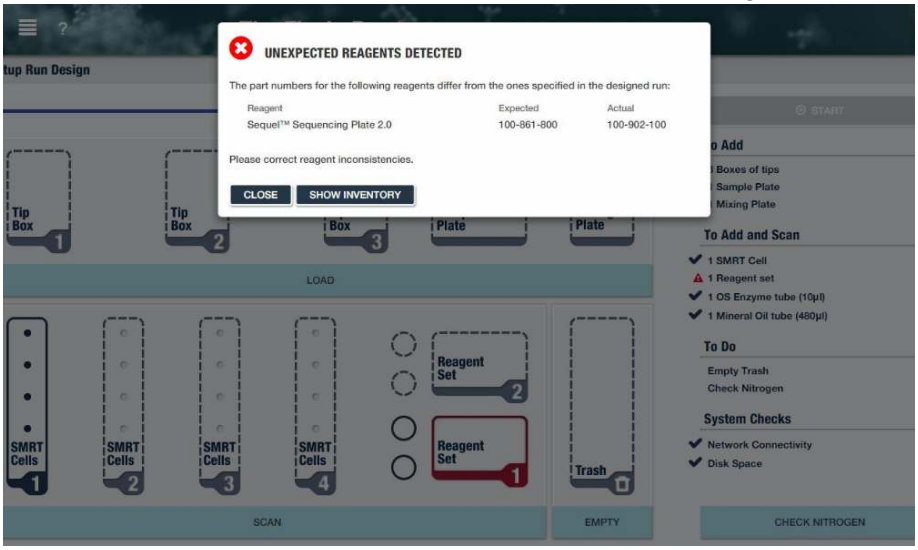
### Possible SMRT Cell Substitution

This notification points out that there is a possibility an incorrect SMRT Cell Tray was loaded. If there are insufficient SMRT Cells to complete the run, this prompt will request confirmation that the run should continue.

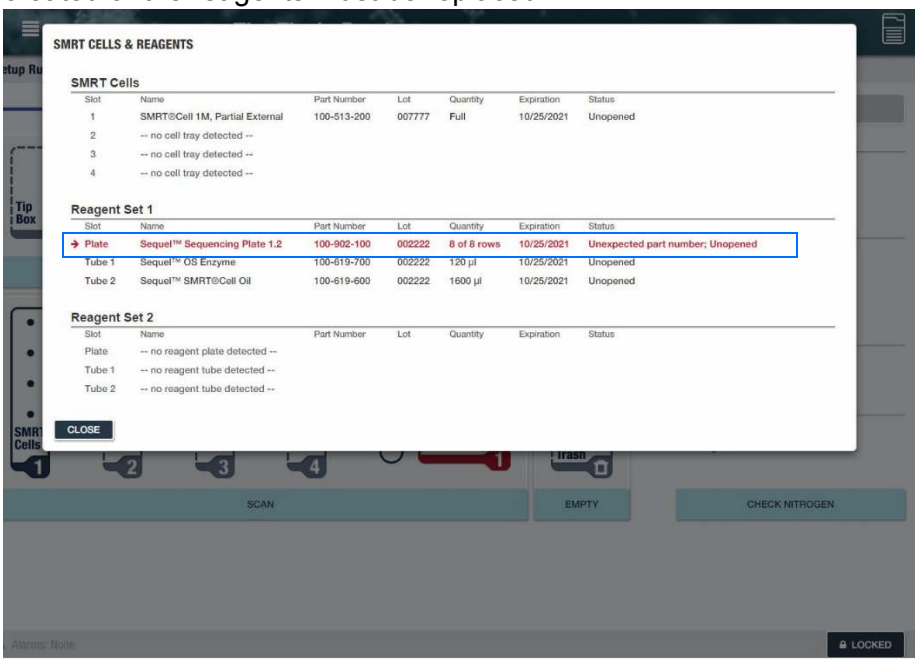



### Unexpected Reagent Detected

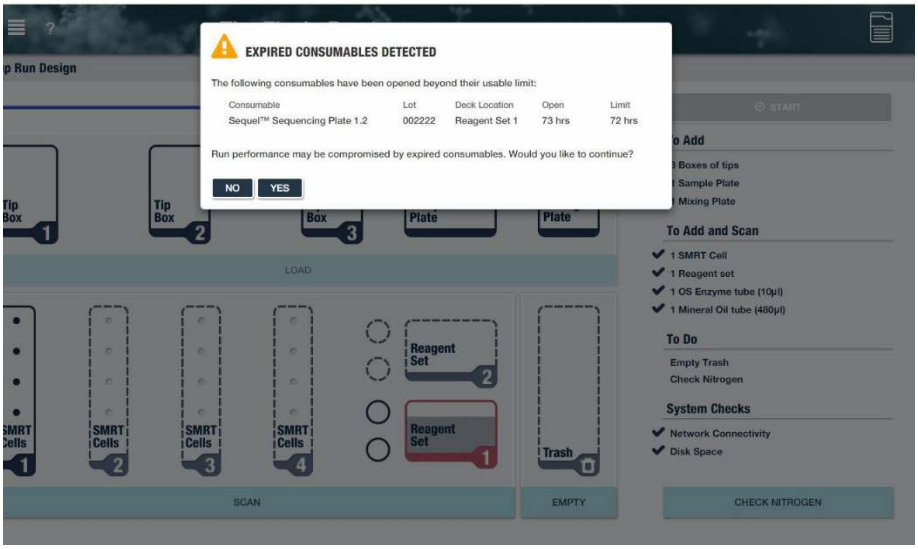
This notification shows that the part number of the reagent detected did not match the part number of the entered in Run Design.



Select **Show Inventory** to see exactly which item is causing the discrepancy. Note that the run cannot continue. A new run must be created or the reagents must be replaced.

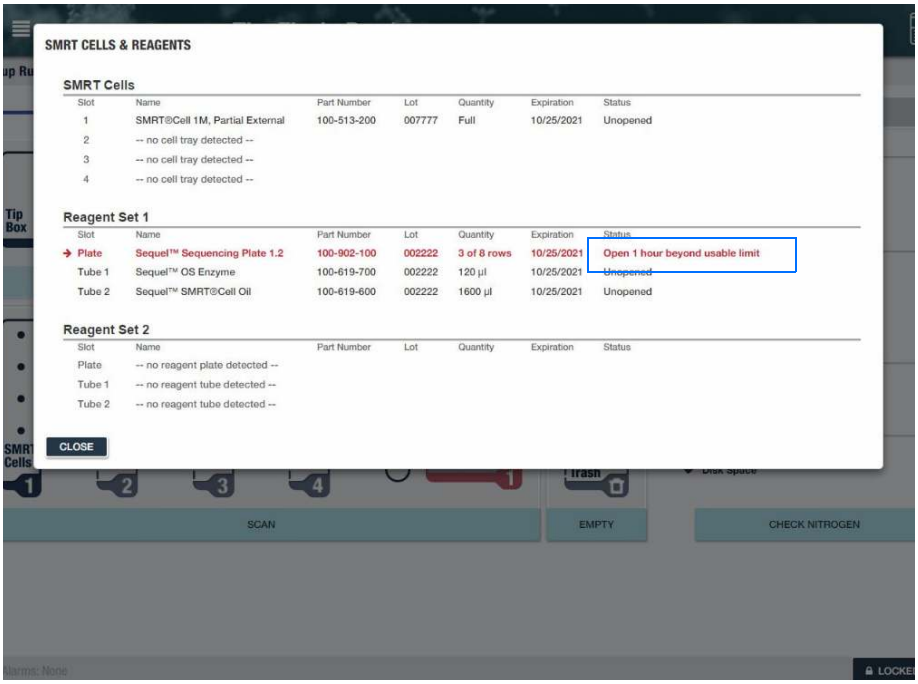



## Expired Consumables Detected: Consumable has been Open Beyond Usable Limit




The option to continue is allowed, once it is acknowledged that run performance may be compromised. Note that the amount of time past the expiration date will display to help with the determination to move forward. If **NO** is selected, the expired reagents must be removed and replaced.

If **YES** is selected, the length of expiration will be shown. Note that if several consumables have expired, they will all be shown here.




| Revision History | Version | Date |
|---|---|---|
| Updates throughout to reference SMRT Cells LR and switch out Binding Calculator for SMRT Link Sample Setup.<br>P16, step 3, bullet 3: Added “or precipitated” to frozen materials. P20, step 2: Updated to say “empty unsealed reagent plate.” P24, step 3: Updated movie collection time and example. P 28, step 12 and P31, 16: Removed reference to reagent “set”. Page 29 and 30, steps 14: Updated movie hours.<br>Appendix: updates to screenshots. | 4 | February 2018 |
| Edited to add that the OS Enzyme tube is no longer shipping separately and is now contained in the Sequencing Plate v3. Other minor updates and clarifications throughout document. | 5 | October 2018 |
| Fixed typo on page 16. Updated screenshot On page 30, updated screenshot to show On-plate Loading Concentration field. | 6 | October 2018 |