# **Operations Guide** Sequel<sup>®</sup> System: The SMRT<sup>®</sup> Sequencer



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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.

# 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**.

SM/ FLink	Name	Sequel Instrume	nt			
Network		APP	LY RESET			
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Notifications						
Updates						
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Quick Run						
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Name Instrument tab E

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**.

	INSTRUME	INT CONTROL	PRIMARY ANALYSIS CONTR	OL
SMRT Link	Boot Protocol	DHCP	STATIC	
Network	IP Address	10.7.40.254		
Transfer Scheme	Netmask	255.255.254.0		
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	Search	Nanofluidics.com		
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2				CLOSE
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				Primary Ana

- 4. At the **Connect to SMRT Link** option, fill out the required fields to connect the instrument to SMRT<sup>®</sup> 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.

instrument Name	Data directory		
SMRT Link	Lood CAT	scheme	srs
	LUCALSAT	id	local-sat
Network	PBI Collections Rsync rsyn	name	Local SAT
Transfer Scheme	PBI Collections Rsync+SSH srs (default)	destPath	/opt/pacbio/smrtsuite/current/userdata/data ro
Notifications	+ - DEFAULT	description	Transfer scheme to use when running on instru
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Updates		port	22
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			TEST SETTINGS SAVE
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- 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 Setting Timezones

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



Select Set Timezone

 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.



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



4. Then select OK.

# **Viewing Alarms**

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Shutdown	Sho	Faile	d Transfers	0	480	10,271	2.64
	2	-11-	152882_38E.,	Complete	480	15,650	3.16
	3	C01	102982_380	Complete	480	28,430	1.61
and the second	đ	001	PS_obl_5pM	Complete	840	14,816	3.75
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୍	ő	FRI	Govarts_otr	Complete	240	15,265	1.42
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rms: None 🎿 Updates: None							a LOCKE

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

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

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#### Instrument Restart

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ols >			1994 B	Star	ted 07.07.2017 17:0	Completed	07.10.2017 03:2
	241	Well	Sample	Status	Movie Time (min)	Read Length (bp)	Bases (Cb)
	1	A01	142882 3kb	Complete	480	18,271	2.84
	2	B01	152302_3kb	Complete	480	15,650	3,18
	з	C01	152382_3kb	Gomplete	480	29,433	1.61
	4	001	FS_ctrl_6pM	Complete	240	14,216	a.75
Contraction of the local division of the loc	5	E01	F8_m40_6	Complete	240	12,628	1.64
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	ž.	G01	Covaris m	Complete	240	13,488	3.16
Start	8	HOT	PS_otri_Am,	Complete	.240	11,988	0.26
Quick Run							

1. At the Main Menu, select Restart.

Select Restart

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

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Quick Run		(101	WellSample, 197	Resety	120		
						Alarms None	Status Idi
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#### Instrument Shutdown

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

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Tools	> 2011	Well	Sample	Statua	Movie Time (min) R	ead Length (bp)	Bases (Gb)
Restart	1	A01	142682 3kb	Complete	480	18,271	2.84
	2	B01	152992 3kb	Complete	480	16,650	3.18
	з	C01	152992_3kb	Complete	480	29,433	1.61
all the second	.4	1003	FS_ctrl_6pM	Complete	240	14,236	3.75
	5	E01	FS_m4C_6	Complete	240	12,628	1.64
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Chard	7	G01	Covaris, m	Complete	240	15,488	3.16
Start	8	H01	FS_ctri_Am	Complete	240	11,985	0.26
Quick Run							
					Alarn	ns None S	tatus Com

Select Shutdown

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

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	4 00	1 Wee0Sample_D01	Явасу	1.00	-	-
	0) EQ	1 WellSample B01	Ready	(120)	-	-
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	7 00	i Walisangaa GM	Beachy	120		24
Start	Ø (10	1 WellSample_H01	Авару	120	-	-
Quick Run						
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#### To Verify Instrument Software version

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

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()	CI SI II		(0)_12.10.2010.00.20		Started Not started	Completed	Not comp
Select	Cell	Well	Sample	Status	Movie Time (min) R	ead Length (bp)	Bases (Gb)
	- 1	A01	WellSample_A01	Ready	120	-	-
Existing Run	2	801	WellSample BD1	Ready	120	-	-
	з	COL	WellSample_C01	Ready	120		-74
	4	001	WellSample D01	Bandy	120	-	-
Color August	5	E01	WellSample_E01	Ready	120	-	-
$\odot$	6	F01	WellSample F01	Biady	120	-	-
Start	7	G01	WellSample_G01	Ready	120	-	-
Start	8	H01	WellSample HD1	Ready	120	-	-
						Alama Nova	Status
10038						Alarms None	Stal

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<br/>InstrumentThe following materials and components are needed before<br/>sequencing on the Sequel System:

- SMRT<sup>®</sup> Cells or SMRT<sup>®</sup> Cells LR
- Sequel<sup>®</sup> Sequencing Kit
- Sequel<sup>®</sup> SMRT<sup>®</sup> Cell Oil
- Tube Septa
- Sequel<sup>®</sup> Sample Plate Foil
- Sequel<sup>®</sup> Mixing Plate
- Sequel<sup>®</sup> Pipet Tips
- Sample Plate
- SealPlate<sup>®</sup> 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:

- 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 (~22°C) 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 4°C and protected from light. Any accompanying unused OS enzyme should also be stored at 4°C (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 4°C 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.



Glue plate seal covering used wells

- 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 172°C.
- 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

 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.

- 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

 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 Deepwell 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

 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.

Cell         Weil         Sangle         Butue         Movie Time (min)         Read Langth (min)         Based (Bb)           1         A01         WeilSample_A01         Pready         120             2         BD1         WeilSample_BD1         Plandy         120             3         C01         WeilSample_CD1         Pleady         120             4         DD1         WeilSample_CD1         Pleady         120             5         DD1         WeilSample_CD1         Pleady         120             6         F04         WeilSample_CD1         Ready         120             7         G91         WeilSample_CD1         Ready         120             3         HD1         WeilSample_DD1         Ready         120		10			adirized Not sch	ted completed	Not complete
1         AD1         WellSample_A01         Pessly         120         -         -         -           2         801         WellSample_A01         Ready         190         - <th>lect 🦉</th> <th>¥/e8</th> <th>Sample</th> <th>Status</th> <th>Movie Time (min)</th> <th>Read Length (bp)</th> <th>Bases (Gb)</th>	lect 🦉	¥/e8	Sample	Status	Movie Time (min)	Read Length (bp)	Bases (Gb)
2         801         Weilfämple: B01         Paarty         190             3         C01         Weilfämple: 201         Ready         120             4         001         Weilfämple: 201         Ready         120             5         D01         Weilfämple: 201         Ready         120             6         R01         Weilfämple: 201         Ready         120             6         R01         Weilfämple: 201         Ready         120             7         O91         Weilfämple: 201         Ready         120             8         401         Weilfämple: A01         Ready         120	1	A01	WellSample_A01	Fleatly	120	-21	
3         C01         WellSample_C01         Ready         120             4         D01         WellSample_C01         Ready         128             5         D01         WellSample_C01         Ready         120             6         FD4         WellSample_C01         Ready         128             7         Q01         WellSample_C01         Ready         128             8         H01         WellSample_C01         Ready         120	2	B01	WellSample BD1	Ready	120	-	-
4         001         WeilSample_D01         Ready         120             5         D01         WeilSample_D01         Ready         120             6         RD1         WeilSample_D01         Ready         120             7         G01         WeilSample_D01         Ready         120             8         H01         WeilSample_H01         Ready         120	3	001	WellSample_C01	Ready	120	-	-
5         D/1         WellSample_E01         Resulty         120             6         FD1         WellSample_R01         Ready         128             7         G01         WellSample_R01         Ready         120             8         H01         WellSemple_R01         Ready         120	4	D01	WellSample 001	Ваясу	120		-
6         F01         Weißampie F01         Ready         120             7         G91         Weißampie_S01         Ready         120             8         H01         Weißampie_H01         Ready         120	5	E01	WellSample_E01	Ready	120	-	-
7         G01         WellSample_G01         Ready         120            3         H01         WellSample H01         Ready         125	6	FD1	WellSample, F01	Baady	120	-	-
3 H01 WeilSemple H01 Ready 120	7	G01	WellSample_G01	Fleady	120	-	-
	3	H01	WellSemple H01	Ready	120	-	-

 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.

		O CONTINUE
Nama	Date Created	Burnnary
ATT.12	12.21.29 17:13	18 SMF Cells,
Run_SBFloaMnPro <mark>5</mark> kb_121916_1246	12.19.2019 20:47	4 SMRT Cells, TC SERies, MinPreExt1 run description
54114_FAT1_1Ce1_20161214A	12.15.291 <mark>6</mark> 02:11	1 SMRT ONL FAT1
64114_FAT1_ICH_20161214	12,15:2010 01:41	1 SMRT CHL FATI
54114_FAT1_ICe_201613212D	12.13.2016.04:36	I SMRT CHL FATI
54065 FATT 1CP 2018132120	1213201004:35	1 SMPT CNI, FATI
Run Ahr PN4 120918 1400	12.09.3018 21:57	4 SMRT Cells, 8 hr movie nur description(4 cells)
ame 12 de 56083 3650522 247-PCC	19:07 300 ( 20:00)	Ø SMIT Galis, annickteD

3. Review the Run Design and select CONTINUE (at the top righthand 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.

<					© CONTI	NUE
Cell	Well	Sample	Insert Size (bp)	Sample Loading	DNA Control	Movie Time (min)
a	A01	A6_BAT_0p25fmol_A01	2,000	Diffusion	on	120
2	BOI	A6 BAT 0p25fmoi 801	2,000	Diffusion	on	120
3	001	A8_15kEcoll_1fmol_C01	15,000	Mag Bead	oli	360
4	001	All, 16kEcol, 1fmal, 201	15,000	Mag Bead	он	360

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.



		O START
	,	To Add
Tip Box 1 Box 2 Box 2	Sample Plate Plate	3 Boxes of tips 1 Sample Plate 1 Mixing Plate <b>To Add and Scan</b>
	[]	4 SMRT Cells 1 Reagent set 1 OS Enzyme tube (40µl) 1 Mincral tube (960µl) <b>To Do</b>
	Reagent Set	Empty Trash Check Nitrogen System Checks
SMRT Cells Cells Cells Cells Cells Cells	Reagent Set Trash	Instrument Compute Instrument Disk Space Network Storage Connectivity
SCAN	EMPTY	CHECK NITROGEN

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**.

				O START
				To Add 3 Boxes of tips 1 Sample Plate 1 Mixing Plate
Box	Box	Plate	Plate	To Add and Scan
(n (n		0AD		4 SMRT Cells 1 Reagent set 1 OS Enzyme tube (40µi) 1 Mineral Oli tube (950µi) <b>To Do</b>
0 0 0 0	e e e e	C Reagent Set		Empty Trash Check Nitrogen System Checks
SMRT Cells 1	SMRT Cells 3 4	C Reagent Set	Trash	Instrument Compute Instrument Disk Space Network Storage Connectivity
	SCAN		EMPTY	CHECK NITROGEN

 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.

					O START
Tip Box	Tip Box	Tip Box	Sample Plate	Mixing Plate	To Add 3 Boxes of tips 1 Sample Plate 1 Mixing Plate To Add and Scan
				· ()	4 SMRT Cells 1 Reagent set 1 OS Enzyme tube (40µl) 1 Mineral Oil tube (960µl) <b>To Do</b>
0 0		0 0 0 0	C Reagent		Empty Trash Check Nitrogen System Checks
SMRT Cells	SMRT Cells	MRT ells Cells	O Reagent Set	Trash	Instrument Compute Instrument Disk Space Network Storage Connectivity
		SCAN		EMPTY	CHECK NITROGEN

**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.

2016-10-07_54043							
Estimated Run Completion: Sun 2016.10.09 1d: 7h: 23m: 52s	1:30 PM P	DT				STOP	
Cell 2 Stats	Cell	Well	Sample	Status	Movie Time (min)	Read Length (bp)	Bases (Gb)
Polymerase Read Length	1	A01	54043_Goat_2kLambd	Complete	240	6,493	1.90
30,000 -	2	B01	54043_Gost_2kLambd	Complete	240	7,369	2.25
18.00-	3	C01	54043_Goat_2kLambd	Acquiring	240	-	-
	4	D01	54043_Goat_2kLambd	Initializing	240	-	-
Length (bg)	6	E01	54043_Goat_2kLambd	Ready	240	-	-
	6	F01	54043_Goat_2kLambd	Ready	240	-	-
10.00	7	G01	54043_Goat_2kLambd	Ready	240	-	-
	6	H01	64043_Goat_2kLambd	Ready	240		-
Length (ba)							

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

08.2016 22	:13				CLEAN-UP	
Cell	Woll	Sample	Status	Movie Time (min)	Read Length (bp)	Bases (Gb)
1	A01	54043_Goat_2kLambd	Complete	240	6,493	1.90
2	B01	54043_Goat_2kLambd	Complete	240	7,369	2.25
а	C01	54043_Goat_2kLambd	Complete	240	8,370	2.53
5	E01	54043_Goat_2kLambd	Failed	240	-	-
6	F01	54043_Goat_2kLambd	Falled	240	-	-
7	G01	54043_Goat_2kLambd	Complete	240	4,878	0.05
8	H01	54043_Goat_2kLambd	Falled	240	-	-
	08.2016 22	Cell         Well           1         A01           2         B01           3         Cell           4         D01           5         E01           6         F01           7         G01           8         H01	Cell         Veol         Sample           1         A01         54043_Goat_24Lambd           2         B01         54043_Goat_24Lambd           3         C01         54043_Goat_24Lambd           4         D01         54043_Goat_24Lambd           5         E01         54043_Goat_24Lambd           6         F01         54043_Goat_24Lambd           7         Q01         54043_Goat_24Lambd           6         H01         54043_Goat_24Lambd	Cell         Vell         Sample         Status           1         A01         54043_Goat_SkLambd         Complete           2         B01         54043_Goat_SkLambd         Complete           3         C01         54043_Goat_SkLambd         Complete           4         D01         54043_Goat_SkLambd         Complete           5         E01         54043_Goat_SkLambd         Failed           6         F01         54043_Goat_SkLambd         Failed           7         001         54043_Goat_SkLambd         Complete           6         H01         54043_Goat_SkLambd         Failed	Cell         Veidt         Sample         Status         Movis Time (min)           1         A01         54043_Goat_SLambd         Complete         240           2         B01         54043_Goat_SLambd         Complete         240           3         C01         54043_Goat_SLambd         Complete         240           4         D01         54043_Goat_SLambd         Complete         240           6         E01         54043_Goat_SLambd         Complete         240           6         F01         54043_Goat_SLambd         Failed         240           7         G01         54043_Goat_SLambd         Complete         240           6         H01         54043_Goat_SLambd         Failed         240           6         H01         54043_Goat_SLambd         Complete         240           6         H01         54043_Goat_SLambd         Failed         240	CLEAN-UP           Cedit         Wolf         Sample         Status         Morie Time (mh)         Read Length (op)           1         A01         54043_Goat_28Lambd         Complete         240         8,483           2         B01         54043_Goat_28Lambd         Complete         240         8,483           2         B01         54043_Goat_28Lambd         Complete         240         8,479           3         C01         54043_Goat_28Lambd         Complete         240         8,379           4         D07         16015_Coat_28Lambd         Complete         240            5         E01         54043_Goat_28Lambd         Failed         240            6         F01         54043_Goat_28Lambd         Failed         240            7         001         54043_Goat_28Lambd         Complete         240            6         H01         54043_Goat_28Lambd         Failed         240

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 4°C after removal.



- 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 Work-deck 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 <b>Quick Run</b> option. <b>Note</b> : The information and run parameters entered using this feature apply to <b>all</b> samples within a run. Pacific Biosciences <b>highly</b> <b>recommends</b> using SMRT Link to <b>either</b> 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.
	<ol> <li>Select Start Quick Run.</li> <li>Enter run information: (Note: Please only use alphanumeric characters in all text fields.)         <ul> <li>(Required) Run Name</li> <li>(Optional) Run Comments</li> <li>(Optional) Experiment Name</li> </ul> </li> </ol>

- (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.

uick Run Design	der matument. Heady	- <u>A</u>	- F	
			O CONTINUE	
Run Information	Data Collection			
Run Name	# of SMRT Cells	1		~
Run_07.20.2018 00:33	Sample Name	WellSample		_
Run Comments				_
Experiment Name	Magbead Loading On-plate Loading Concentration (pM)	ON	OFF	
Experiment Id	Template Prep Kit	-		~
	Binding Kit			~
Run Reagents / Consumables	Sequencing Chemistry			~
1 SMRT Cell 1 plate Sequencing reagent	DNA Control Complex			~
3 boxes of tips 1 mixing plate	Insert Size (bp)	10000		~
1 sample plate	Movie Time per SMRT Cell (mins)	360		~

- 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**.

016-10-07_54043							
tarted 10.07.2016 13:37 Completed 10.	08.2016 22	:13				CLEAN-UP	
Cell 4 Stats	Cell	Well	Sample	Status	Movie Time (min)	Read Length (bp)	Bases (Gb)
Polymerase Read Length	1	A01	54043 Goat 2kLambd	Complete	240	6,493	1.90
NG. 000 -	2	B01	54043_Goat_2kLambd	Complete	240	7,369	2.25
20.000 -	a	C01	54043_Goat_2kLambd	Complete	240	8,370	2.53
e soloo soloo	5	E01	54043_Gost_2kLambd	Failed	240	-	-
SLOOD 7	6	F01	54043_Goat_2kLambd	Failed	240	-	-
m.000 -	7	G01	54043_Goat_2kLambd	Complete	240	4,878	0.05
10,000 -	8	H01	54043_Goat_2kLambd	Falled	240	-	-
9 20,000 20,000							
Productivity P0 P1 P2							

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 4°C.



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.

tarted 05.05.2017 22:00 Completed	05.05.2017 23	:12			- 1		
		0.0227007	15001000			- CLEAROF	-
Cell 2 Stats	Cell	Well	Sample	Blatus	Movie Time (min)	Read Length (bp)	Bases (Gb)
Polymerase Band Longth	1	PBA	WellSample_A01	Transfer Failed	1	20	0.00
	2	B01	WellSample_B01	Transfer Failed	.1	20	0.00
	а	C01	WellSample_C01	Transfer Failed	1	20	0.00
	4	D01	WellSample_D01	Transfer Failed	1	20	0.00
in in in in Length Day	5	E01	WellSample_E01	Transfer Falled	1	20	0.00
Longest Babrent Length	8	F01	WellSample_F01	Transfer Failed	1	20	0.00
	1	G01	WellSample_G01	Transfer Failed	1	20	0.00
100-	8	H01	WellSample H01	Transfer Falled	1	20	0.00
· <b></b>	3	A02	WellSample_A02	Transfer Failed	1	20	0.00
Length diej Productivity	10	802	WellSample_B02	Transfer Failed	1	20	0.00
P0 P1 P2 23.2% 02.9% 23.8%							

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.

Starte		12:40				-	€ CLEAN JIR	
Tools	>	Set Timezo	one				- CLEMPORT	
Restart		Show Alan	ms		s	Movie Time (min)	Read Length (bp)	Bases (Gb)
Shutdown		Retry Tran	ster		alled	360	290	0.00
	2	B01	801	Transfer F	Follod	360	743	0.00
	а	C01	air 001	Transfer F	allod	360	194	0.00
	- 14	DO1	80.5001	Transfer F	alled	360	314	0.00

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:

Host or server not kr	nown. Check your host name or network connection
Resource Id	rsync-1
Scheme	RSYNC
Destination Directory	/root/path/to/output/files/rUnset_20170519_185852/1_A01/
Source Directory	/data/pb
9 files failed transfe	,
/data/pb/.m54003_170	506_002523.metadata.xml
Host or server not known. C	Check your host name or network connection.
ssh: Could not resolve host	name icc-foo: Name or service not known
/data/pb/m54003_1705	06_002523.subreadset.xml
Host or server not known. C	Check your host name or network connection.
ssh: Could not resolve host	name icc-foo: Name or service not known
/data/pb/m54003_1705	06_002523.subreads.bam
Host or server not known. C	Check your host name or network connection.
ssh: Could not resolve host	name icc-foo: Name or service not known
/data/pb/m54003_1705	06_002523.subreads.bam.pbi
Host or server not known. C	Check your host name or network connection.

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.

 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.

	Run	Cell	Well	Reason	
	Run, 05.19.2017 11:57	3	A01	Host or server not known. Gheck your host name or network connection.	C Retryin
3	Run 05.19.2017 11:57	2	801	Host or server not known. Check your host name or network connection.	RETRY
	Run_05.19.2017 11:57	3	C01	Host or server not known. Check your bost name or network connection.	RETRY
	Run, 06.19.2017 11:57	4	001	Host or server not known. Check your host nume or network connection.	RETRY
	Run_06.19.2017 11:57	Б	E01	Most or server not known. Check your bost name or network connection.	RETRY
	Run_05.19.2017 11:57	6	F01	Host or server not known. Gheck your host name or network connection.	RETRY
	Run_06.19.2017 11:57	7	G01	Host or server not known. Check your bost name or network connection.	RETRY
	Run 06.19.2017 11:57	8	H01	Host or server not known. Gheck your host name or network connection.	RETRY
	Bun_06.19.2017 11:57	9	A(12	Host or server not known. Check your bost name or network connection.	RETRY
	Run 06.13.2017 11:57	10	802	Host or server not known. Check your host name or network connection.	RETRY

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:

An error occurred attempting to delete the failed transfer for collection 2 of run "Run_05.05.2017 21:58".
Failed transfer for id "abc-123" not found.
CLOSE

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.

An er	ror occurred attem	oting a retry of all f	ailed transfers
Faile	d transfer for id "ab	c-123" not found.	
CI	OSE		
-			

### 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.

	Run	Cell	Well	Reason
	Run_05.19.2017 11:57	3	C01	Host or server not known. Check your host name or network connection.
	Run_05.19.2017 11:57	4	001	Host or server not known. Check your host name or network consection.
1	Run 05.19.2017 11:57	5	ED1	Host or server not known. Check your host name or network connection.
l)	Run_05.19.2017 11:57	6	F01	Host or server not known. Check your host name or network connection.
l	Run_05.19.2017 11:57	7	G01	Host of server not known. Check your host name or network connection.
	Run_05.19.2017 11:57	8	H01	Host or server not known. Check your host name or network connection.
l.	Run_05.19.2017 11:57	9	A02	Host or server not known. Check your host name or network connection.
l.	Run_05.19.2017 11:57	10	802	Host or server not known. Check your host name or network connection.
l.	" m54001 170519 211830 "			Host or server not known. Check your host name or network consection.
l.			31	Host or server not known. Check your host name or network connection.
ļ	** m54001_170519_231830 **	9	( <b>1</b> 45)	Host or server not known. Check your host name or network connection.
e collect	ions no longer have a run associatio	n and so are	known or	ly by their reference name, **
SE				A

# **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.

				0.000
-	Run performance may be compron	nised by expired consumables. Wo	iuld you like to continue?	i Boxes of tips Sample Plate
Box	Box	Plate	Plate	To Add and Scan
		Reagent		4 SMRT Cells     1 Reagent set     1 OS Enzyme tube (40y)     t Mineral OI tube (90yi)     To Do
SMRT	RT SMRT	C Reagent		Empty Trash     Check Nitrogen     System Checks     Notwork Connectivity
	3 4		Trash	V Disk Space
	SCAN		EMPTY	CHECK SITROGEN

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".

SMILL CC	Martin	Doct Number	1.00	Contrille	Excitation	Show -
A 1	SMRTDCell 141 Radial External	100-513-200	007777	Euli	10/04/2016	Expired Depended
	- no cell tray detected	100-010-200		1.000	10-23-2010	Expense, empletere
<u>_</u>	and the set free states and					
4	- no cell tray detected					
Reagent	Set 1					
Exec	Marte	Part Number	Loi	Quantity	Expiration	Status
Plate	Sequel <sup>144</sup> Sequencing Plate 1.2	100-902-100	002222	8 of 8 rows	10/25/2021	Unopened
Tube 1	Sequel <sup>TM</sup> OS Enzyme	100-619-700	002222	120 µI	10/25/2021	Unopened
Tube 2	Sequel <sup>114</sup> SMRTINCell Oil	108-619-600	002222	1690 µl	10/25/2021	Unopened
Reagent :	Set 2					
Sict	Namo	Part Number	Lot	Quantity	Expiration	Status
Plate	- no reagent plate detected					
Tube 1	- no reagant tube detected					
Tube 2	- no reagent tube detected					
CLOSE						
1		-		_	1138	Disk Scote
5	2 3	4				V Network Storage Connectivit

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".

1	Sid	Norm PMDT/0/2-0 154 (Partial Followed)	Part Number	Lot	GuintPy	Expinition 10/08/09/11	Status
		ann coll has detected a	100-013-200	007777	1411	(weareve)	onuperies
0       = no. transmission         4       - no. containing vectored         Respend Sequencing Plate 1.2         100-06-107       000222       2.6 of 8 rows       8004/2000       800em         Plate       Sequent <sup>11</sup> Sequencing Plate 1.2       100-06-107       002222       1.0 in 10/25/2001       Respend Unoperined         Tube 2       Sequent <sup>11</sup> Sequencing Plate 1.2       100-06-10-00       002222       1.0 in 10/25/2001       Unoperined         Respend <sup>11</sup> Sequencing Plate 1.2       100-06-10-00       002222       1.0 in 10/25/2001       Unoperined         Tube 2       Sequent <sup>11</sup> Sequencing Plate 1.2       100-06-10-00       0022222       1.0 in 10/25/2001       Unoperined         Plate 1       - no respent blate detected -         Tube 2       - no respent blate detected         Tube 2       - no respent blate detected         CODE         2       2         2       - No.         <td colspan="</td> <td>2</td> <td>- no cell tray detected -</td> <td></td> <td></td> <td></td> <td></td> <td></td>	2	- no cell tray detected -					
State         Norm         Part Number         Lot         Ocurrently         Expension         Status           Plan         Singued <sup>119</sup> Sequencing Plant 1.2         100-062-100         602222         36 of annual         Status         Expension         Exp	4	no cell tray detected					
Bit         Neme         Part Number         Lot         Quartity         Expension         Status           Tube 1         Sequel <sup>16</sup> Seguencing Pater 1.2         100-681-00         00222         3 of 0 now         10242001         Expension         Status           Tube 1         Sequel <sup>16</sup> Seguencing Pater 1.2         100-681-0700         002222         126 µl         10252001         Unoperned           Tube 2         Sequel <sup>16</sup> SelfTiDCel 0k         100-619-000         002222         1660 µl         10252001         Unoperned           Station         Name         Part Number         Lot         Quartity         Expension         Status           Response 5         Part Number         Lot         Quartity         Expension         Status           Tube 2         - no reagent tube detected         Tube 2         - no reagent tube detected         Tube 2         - no reagent tube detected	Reagent	Set 1					
Parka         Sequell*Sequencing Point 1.2         100-602-00         002222         0.6 fa nows         102/42/016         Explicitly (Inopennel)           Tube 1         Sequell*Staff         SS Sequell*Staff         056         100-619-000         002222         126 µl         102/25/2011         Unopennel           Tube 2         Sequell*Staff         100-619-000         002222         126 µl         102/25/2011         Unopennel           Reagent Set 2         Part Number         Lot         Quarkity         Explicition         Balas           Tube 2         - no reagent bade detected         - <td>Skrt</td> <td>Name</td> <td>Part Number</td> <td>6.nt</td> <td>Guantity</td> <td>Experations</td> <td>Status</td>	Skrt	Name	Part Number	6.nt	Guantity	Experations	Status
Tube 1         Sequell® 05 Enzyme         100-619-700         002222         120 µl         102852021         Unopened           Stagent Set 2         Sequel® SMRTDGel 00         100-619-400         002222         1600 µl         102/25/201         Unopened           Stagent Set 2         Set Name         Part Number         Lot         QuarMey         Expension         Status           Flam         - no reagent tube detected -         -         -         Tube 2         - no reagent tube detected -           Tube 2         - no reagent tube detected -         -         -         Common Number 1         -	Plate	Sequel <sup>TM</sup> Sequencing Plate 1.2	100-902-100	002222	8 of 8 rows	10/24/2018	Expired; Unopened
Tube 2     Boguell* SMRTBOCH 00     100-019-000     02222     1000 µI     102/52/021     Unopened         Regent Set 2         Bot     Nome     Part Number     Lot     Quarkity     Expension     Balue         Tube 1     - no reagent tube detected -   Tube 2 - no reagent tube detected -          Loce         Loce </td <td>Tube 1</td> <td>Sequel<sup>tis</sup> OS Enzyme</td> <td>100-619-700</td> <td>002222</td> <td>120 µl</td> <td>10/25/2021</td> <td>Unopened</td>	Tube 1	Sequel <sup>tis</sup> OS Enzyme	100-619-700	002222	120 µl	10/25/2021	Unopened
Stot     Name     Plat Number     Lot     Quantify     Expension     Status       Plate     - no reagent tube detected -     Tube 2     - no reagent tube detected -	Tube 2	Sequel <sup>DL</sup> SMRTi0Cell Oil	100-619-600	002222	1600 µi	10/25/2021	Unopened
Skit Name Part Number Lot Quantity Expension Status Plate - no reagent table detected - Tube 2 - no reagent table detected - Tube 2 - no reagent table detected - CORE	Reagent	Set 2					
Plane - no reagent plate detected - Tabe 1 - no reagent blate detected - Tube 2 - no reagent blate detected - LOGE	Skol	Name	Part Number	Lot	Quantify	Experation	Status
Tube 1	Flate	- no reagent plate detected -					
Tube 2 no reagent tube detected	Tube 1	on reagent tube detected					
	Tube 2	- no reagent tube detected					
	K	2 -3 -	4		-0	Lis	Cink Space
SCAN ENVILS OVERSITE		BCAN					erts delick when

#### **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.

Slot	Name	Part Number	Lot	Quantity	Expiration	Status
1	SMRT@Cell 1M, Partial External	100-513-200	007777	Full	10/25/2021	Unopened
2	no cell tray detected					
3	no cell tray detected					
4	no cell tray detected					
Reagent	Set 1					
Slot	Name	Part Number	Lot	Quantity	Expiration	Status
> Plate	Sequel <sup>™</sup> Sequencing Plate 1.2	100-902-100	002222	3 of 8 rows	10/25/2021	Open 1 hour beyond usable limit
Tube 1	Sequel <sup>™</sup> OS Enzyme	100-619-700	002222	120 µl	10/25/2021	Unopened
Tube 2	Sequel <sup>™</sup> SMRT@Cell Oil	100-619-600	002222	1600 µl	10/25/2021	Unopened
Reagent	Set 2					
Slot	Name	Part Number	Lot	Quantity	Expiration	Status
Plate	no reagent plate detected					
Tube 1	no reagent tube detected					
Tube 2	no reagent tube detected					
CLOSE						
	2 3	4			Iras	Disk space
	SCAN	•			EN	IPTY CHECK NITR

Revision History	Version	Date
Updates throughout to reference SMRT Cells LR and switch out Binding Calculator for SMRT Link Sample Setup.	4	February 2018
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. Appendix: updates to screenshots.		
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