Genome Sequencer FLX System Software Manual, version 2.3

Part A: GS Sequencer and Other On-Instrument Software

October 2009

Genome Sequencer FLX System Software Manual
Software v. 2.3, October 2009

Part A

GS Sequencer and Other On-Instrument Applications

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1. ON-INSTRUMENT SOFTWARE OVERVIEW

The Genome Sequencer FLX On-instrument applications consist principally of the **GS Sequencer**, which operates on the Genome Sequencer FLX Instrument to capture the raw images of the PicoTiterPlate device during a sequencing Run, and the **GS Run Browser**, which allows the raw or processed data from the sequencing Run to be viewed. They can both be accessed by double-clicking on their application icons on the Genome Sequencer FLX Instrument computer desktop (Figure 1). **System Start** and **System Stop** icons are also available on the desktop to start and stop the Genome Sequencer FLX Instrument background processes. In addition, Run data processing can be performed on-instrument by embedding it a sequencing Run (or by launching the **GS Run Processor** software via the GS Run Browser), although only the first part of this, image processing, is normally carried out on the instrument. The GS Run Processor and associated software are described in Part B of this manual.

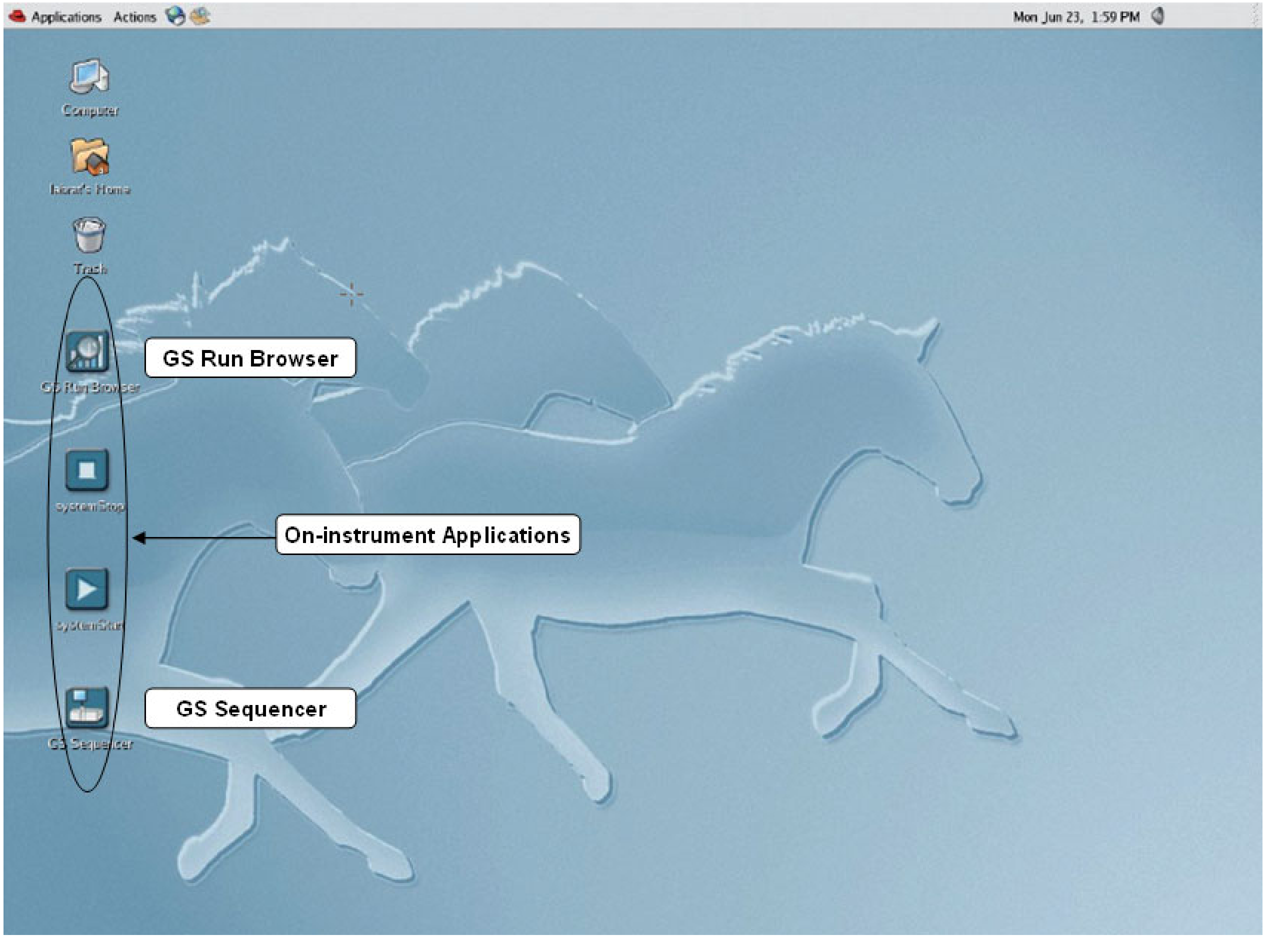


Figure 1: Genome Sequencer FLX Instrument Desktop

The **GS Sequencer** application serves several purposes:

- Setting up and launching sequencing Runs and other instrument procedures
- Genome Sequencer FLX Instrument monitoring
- sequencing Run data file management

- set up and management of instrument Operators and Run Groups

The main window of the GS Sequencer software has two tabs to carry out these functions, the Instrument and the Data tabs. In addition, the Start button of the main window evokes a “Run Wizard” that presents a series of questions through which the user sets up sequencing Runs and other procedures.

The **GS Run Browser** application serves two purposes on the Genome Sequencer FLX Instrument:

- viewing sequencing Run parameters and data sets
- launching data processing jobs using the GS Run Processor Manager tool (image processing, signal processing, and full processing)

The GS Run Browser window is divided into six tabs:

Overview
Wells
Signals
Reads
Control DNA
Filters

These four tabs are not available for data on which only image processing was carried out, or if you just view the Run data (R_ directory)

The **Overview** tab gives a summary of the sequencing Run, including the Run name, Operator name, number of regions and the number of cycles. It also provides a brief summary of the data processing results in the top right section. Directly below this information is a section where the GS Run Processor Manager tool can be used to launch data processing jobs.

The **Wells** tab is used to view the raw images from the PTP device taken during the sequencing Run, overlaid with processed well and region information (for signal-processed or full-processed datasets). The images can be viewed for each base flow and filtered for each well category.

The **Signals** tab summarizes and plots information on the distribution of raw well, filtered well or N-mer intensities for each base flow.

The **Reads** tab reports summary statistics and distributions of read length and read quality, for each region of the PTP device, for both library and control reads.

The **Control DNA** tab reports the accuracy results of the Control DNA fragments and allows access to the Control DNA fragment tri-flowgrams.

The **Filters** tab provides statistics on filtering results from the signal processing step, for each region of the PTP device, and for both library and Control DNA fragments.

The main functionalities of the GS Run Browser, to view sequencing Run and data processing results, are presented in detail in Part B, Section 3, of this manual. Part A of the manual focuses on the implementation and usage of the Genome Sequencer FLX on-instrument applications; this includes using the GS Run Browser application to launch processing jobs through the GS Run Processor Manager Tool, as discussed in Section 4.

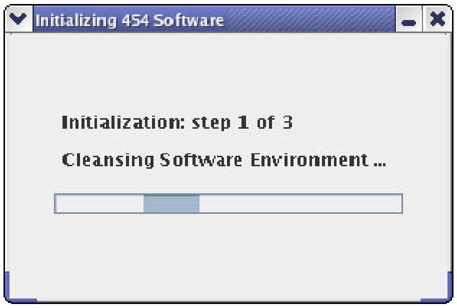
2. SYSTEM START AND SYSTEM STOP APPLICATIONS

The Genome Sequencer FLX Instrument is designed for continuous operation and under normal circumstances, sequencing Run Operators do not need to perform any restart or shutdown procedures. In the event that the instrument does need to be restarted or shutdown, the System Start and System Stop applications, accessed by the systemStart and systemStop icons on the Genome Sequencer FLX Instrument desktop, may be used (Figure 2).

Figure 2: System Start and System Stop application icons

The systemStart and systemStop icons launch shell scripts which control the instrument application background processes. The systemStart application is used to reinitiate these processes and the systemStop application is used to halt them.

The systemStart application can be launched by double-clicking on the desktop systemStart icon. This will initiate the background software processes in the proper sequence. A message box titled ‘Initializing 454 Software’ will report the progress of the initialization steps (**Error! Reference source not found.**). When the systemStart application has finished successfully, the message box will close and all on-instrument applications will become available. The instrument’s indicator light will go from red to solid yellow when the initialization steps are complete, indicating that the instrument is waiting for the next operator action.

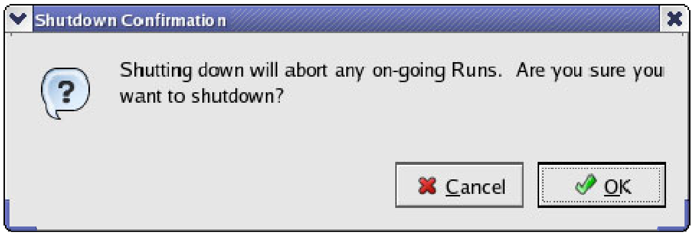


Initialization: step 1 of 3

Cleansing Software Environment ...

Figure 3: systemStart application progress message box

The systemStop application can be launched by double-clicking on the desktop systemStop icon. A Shutdown Confirmation window will appear (Figure 4). Clicking OK will initiate an orderly shutdown of the background instrument application processes. The instrument’s indicator light will go from solid yellow to red, indicating that the instrument application background processes are no longer active.



Shutting down will abort any on-going Runs. Are you sure you want to shutdown?

Figure 4: Shutdown Confirmation window for the systemStop application

- Both systemStart and systemStop applications, when launched, will shut down any active application processes. Any running procedures, such as sequencing Runs, will abort and no further data will be acquired.
- The systemStart and systemStop applications (or a user logging out event) will not shutdown the GS Sequencer or GS Run Browser GUIs. The GUIs remain functional even if the backend processes have been stopped. As mentioned above, there is normally no reason to stop the backend processes.

3. GS SEQUENCER

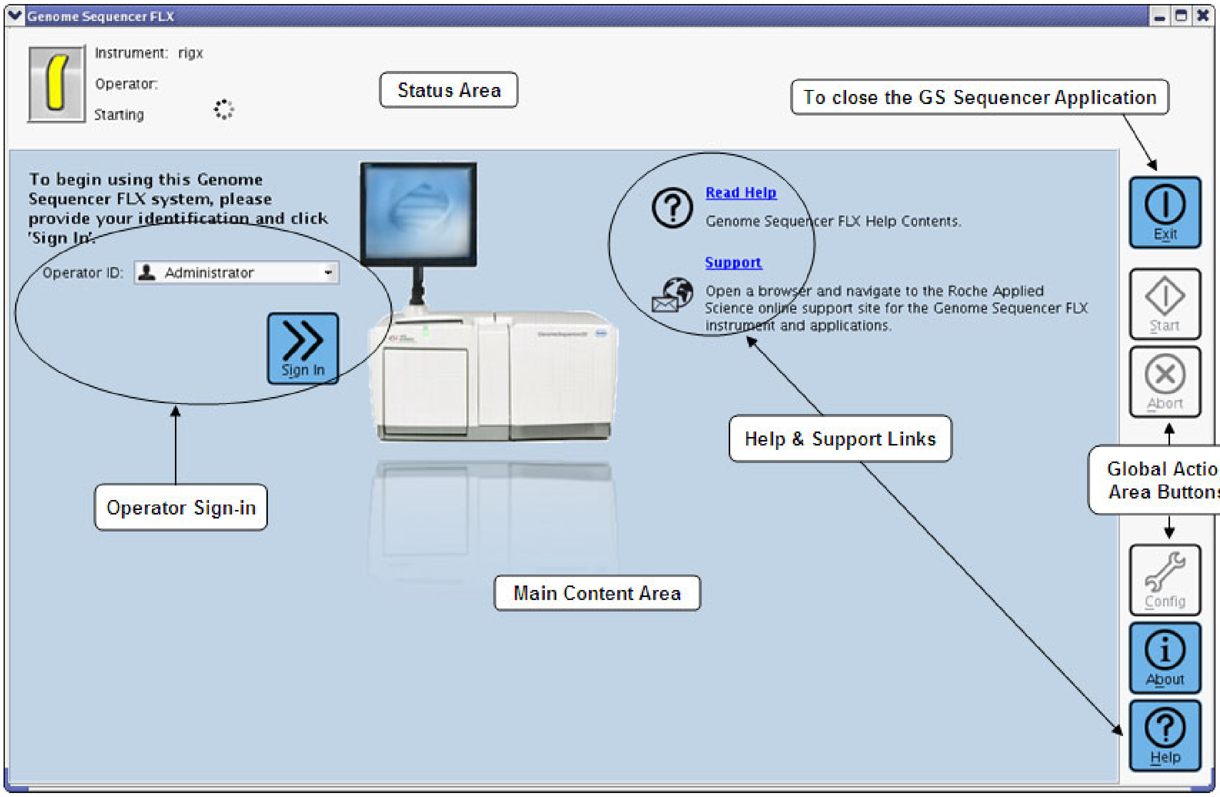
The GS Sequencer application can be launched by double clicking on its icon on the Genome Sequencer FLX Instrument desktop (Figure 5). The GS Sequencer application is used to set up and launch sequencing Runs, monitor the Genome Sequencer FLX Instrument components, and manage data sets.

Figure 5: The GS Sequencer icon on the desktop

The GS Sequencer application window has three areas; the Status Area (top), the Global Action Area (right), and the Main Content Area (center) (Figure 6).

- Status Area – contains the instrument name, the Operator ID, the status and status icon.
- Global Action Area – contains buttons for ‘Exit’, ‘Start’, ‘Abort’, ‘Config’, ‘About’, and ‘Help’.

- Main Content Area – initially contains the sign-in screen. After signing in, it contains the ‘Instrument’ and ‘Data’ tabs.
    - The Instrument tab is divided into an upper ‘Procedure’ area and a lower ‘Instrument Sensor Readings’ area (see Figure 7 and Section 3.3, below).
    - The Data tab contains tools to manage the data sets stored on the Genome Sequencer FLX Instrument (see Section 3.4).



Instrument: rigx
Operator:
Starting

To begin using this Genome Sequencer FLX system, please provide your identification and click 'Sign In'.

Operator ID: Administrator

Genome Sequencer FLX Help Contents.

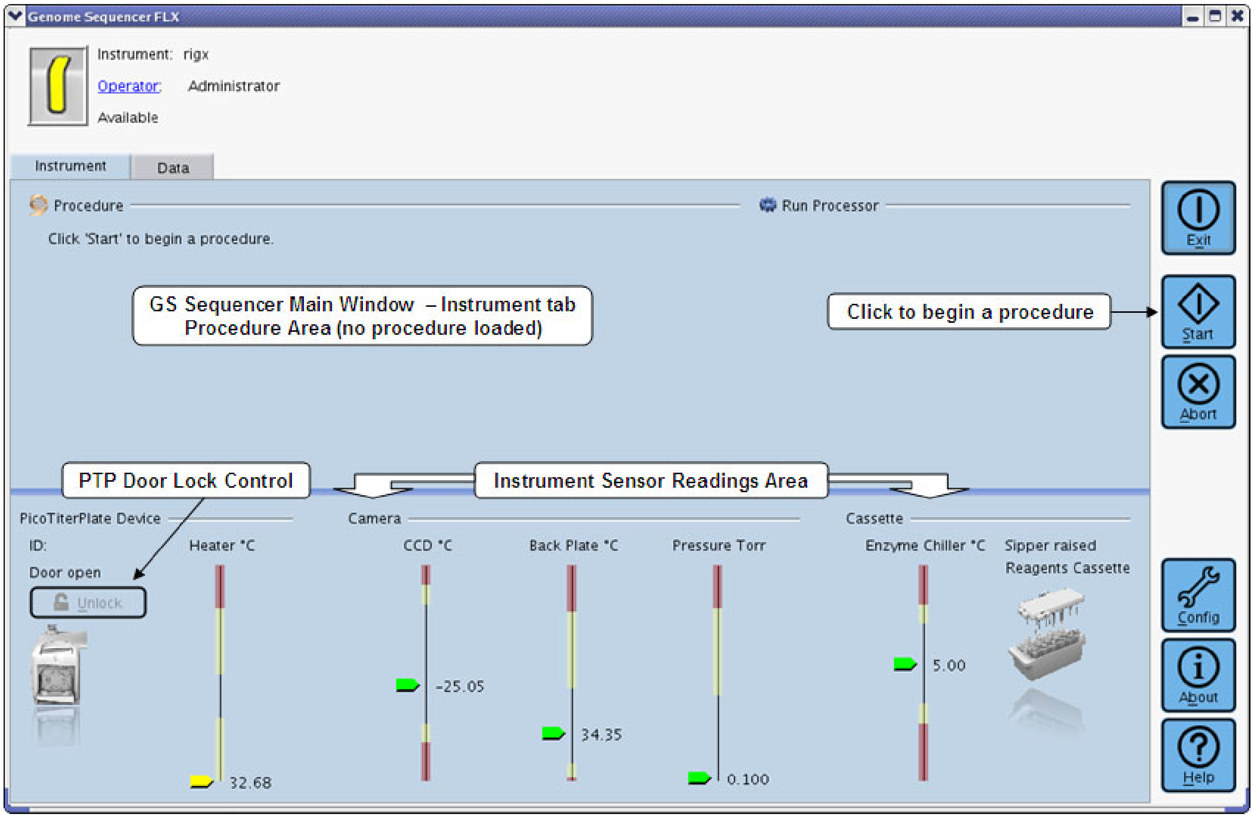
Open a browser and navigate to the Roche Applied Science online support site for the Genome Sequencer FLX instrument and applications.

Figure 6: The GS Sequencer Introduction and Sign-In Form

3.1 Signing In & Signing Out

An Operator signs in by selecting his or her ID from the Operator ID pulldown list and then clicking the ‘Sign in’ button (see Figure 6). Before an Operator signs in, the ‘Start’, “Abort’ and ‘Config’ buttons are inactive (grayed out). Once signed in, these buttons are active (blue), the Operator’s name is shown in the Status Area, and the field identifier ‘Operator’ becomes functional (blue and underlined). Clicking on the field identifier ‘Operator’ signs the current Operator out of the application and returns to the Sign in form.

Once an Operator has signed in, the ‘Instrument’ and ‘Data’ tabs are visible.



Instrument: rigx
Operator: Administrator
Available

Click 'Start' to begin a procedure.

GS Sequencer Main Window – Instrument tab Procedure Area (no procedure loaded)

Click to begin a procedure

PTP Door Lock Control

Instrument Sensor Readings Area

Figure 7: The GS Sequencer application window (after signing in)

3.2 Starting a Sequencing Run

The GS Sequencer application is used to setup, start and monitor procedures. Clicking the 'Start' button opens the Run Wizard, a set of questions through which instrument procedures, such as sequencing Runs, can be set up and launched. In the set up process, information is given about the type of procedure being started. The following section 'Before You Begin' specifies the information, decisions and general supplies needed before using the GS Sequencer application to setup and start an sequencing Run.

3.2.1 Before You Begin

There are three topics that need to be addressed before configuring and launching a sequencing Run using the GS Sequencer application Run Wizard (See the *Genome Sequencer FLX Instrument Owner's Manual*, Section 2.3.2, for more details):

- the Genome Sequencer FLX Instrument must be ready and available
- a data processing pipeline appropriate to the sequencing Run may be chosen (See *Section 8 – The GS Run Processor* of this manual for more details.)

- the sequencing Run lab materials must be available

In addition, you may want to set up lists of Operators and Run Groups via the ‘Config’ button (see Section 3.6.2). While the default installation contains a default Operator and Run Groups that allow you to sign-in and perform a sequencing Run directly, these features provide some convenience such as sending an e-mail alert to the Operator when a Run has completed.

The Genome Sequencer FLX Instrument is designed for continuous operation. Under normal circumstances, Operators launching sequencing Runs do not perform any shutdown procedures. All run scripts proceed autonomously after a sequencing Run is launched and each Run is completed by a fluidics maintenance wash after the full number of cycles of data have been collected. Sequencing Runs can take up to several hours to complete, and the used PTP device is left in place throughout the pre-wash of the next sequencing Run. The laboratory method details of launching a sequencing Run, including loading of the PTP device, the Reagents cassette, etc., are discussed in the *Sequencing Method Manual*.

3.2.2 Sequencing Run Set Up (Run Wizard)

Once the lab materials for the sequencing Run have been loaded into the Genome Sequencer FLX Instrument and the appropriate Run parameters and processing options have been chosen, the Genome Sequencer FLX Instrument is ready to begin a new sequencing Run. The set up is done in The Run Wizard. To launch the Run Wizard, click the ‘Start’ button in the Global Action area of the GS Sequencer application window. (Figure 8).

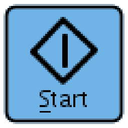
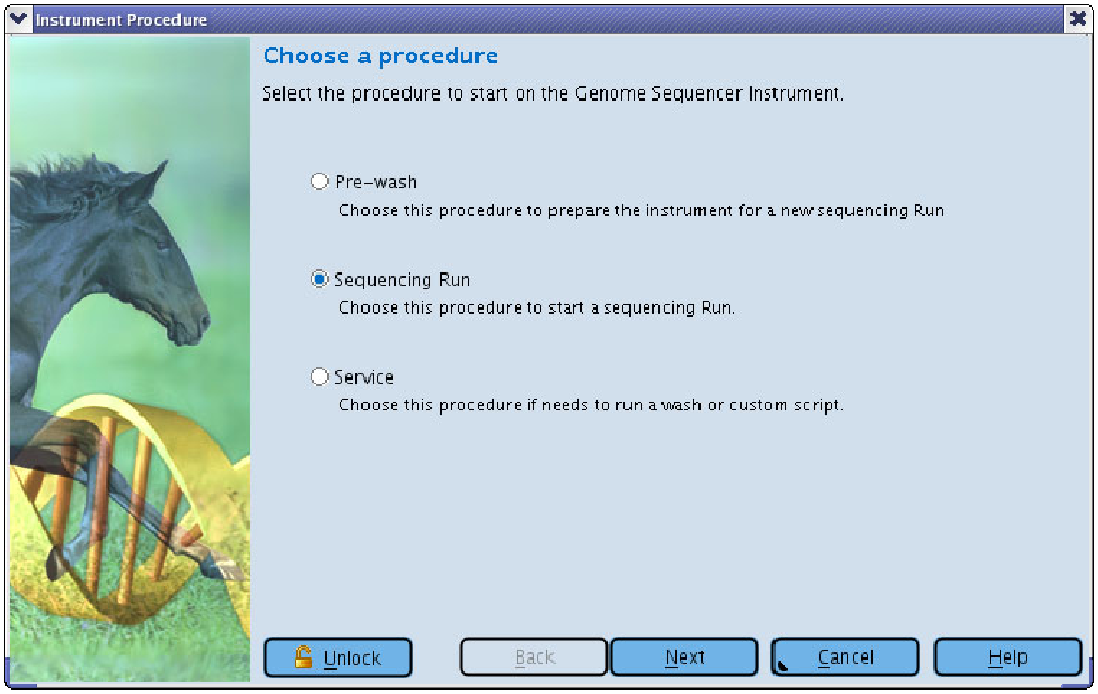


Figure 8: Start button

The Run Wizard will present a series of questions requesting information for the instrument procedure (a sequencing Run in this instance) that you are setting up. Each screen has a set of navigation buttons at the bottom (‘Back’, ‘Next’, ‘Cancel’, ‘Help’), allowing the user to modify the Run parameters as needed before starting. There is an additional ‘Door Unlock’ button in the event you need to open the camera door, on the instrument. After all the parameters have been selected (as shown in the following figures), the ‘Start’ button in the last Run Wizard window becomes enabled and can be clicked to launch the sequencing Run.

The succession of questions in the Run Wizard to set up a sequencing Run is described below. For procedures other than sequencing Runs, see Section 3.2.3.

1. **Choose a Procedure** (Figure 9) – Choose the procedure to run. The option listed second is the normal sequencing Run. A pre-wash must always be performed before a sequencing Run, and the last option is used to run any special wash or custom instrument script; these are described in Section 3.2.3.



Choose a procedure

Select the procedure to start on the Genome Sequencer Instrument.

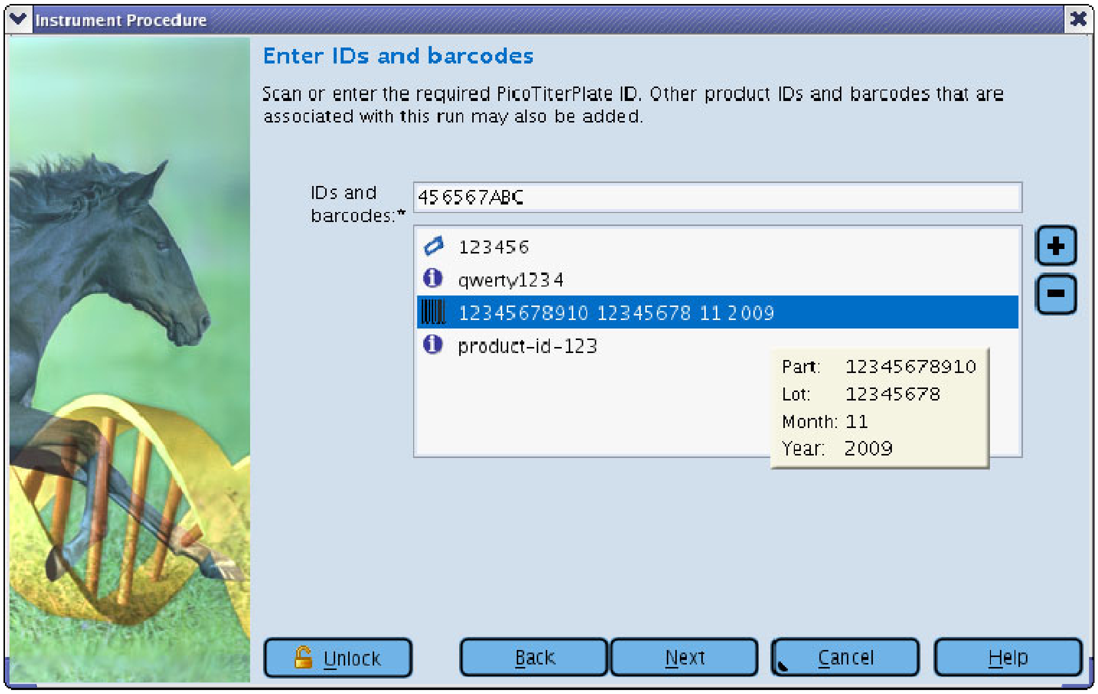
○ Pre–wash
Choose this procedure to prepare the instrument for a new sequencing Run

● Sequencing Run
Choose this procedure to start a sequencing Run.

○ Service
Choose this procedure if needs to run a wash or custom script.

Figure 9: GS Sequencer Run Wizard – 'Choose a procedure' window

2. **Enter IDs and barcodes** (Figure 10) This form requests you enter the product IDs or barcodes of materials that are associated with this Run. The only required ID is the six digit PTP ID found as a barcode stamped on the device. The list can only contain one PTP ID so additional six digits ID cannot be added to the list.
   a. If a barcode scanner is used the IDs will automatically be added to the list located below the entry field. If the ID is entered with the keyboard then press the 'enter' key or click the plus button to add the item to the list. Clicking the minus button will remove the selected product ID from the list.
   b. If a PTP ID lookup service (e.g., a LIMS) is available, the Run information associated with that ID will be applied. (See the Genome Sequencer FLX System Administrator's Guide for configuring the PTP ID lookup service.)
   c. The 'Next' button is enabled once a valid PTP ID is entered. If the Run parameters were applied using the PTP ID lookup service, the next page will be the Run Comments page (see step 9, below); otherwise the next page will ask for the Run name and Run Group (next step).



Enter IDs and barcodes

Scan or enter the required PicoTiterPlate ID. Other product IDs and barcodes that are associated with this run may also be added.

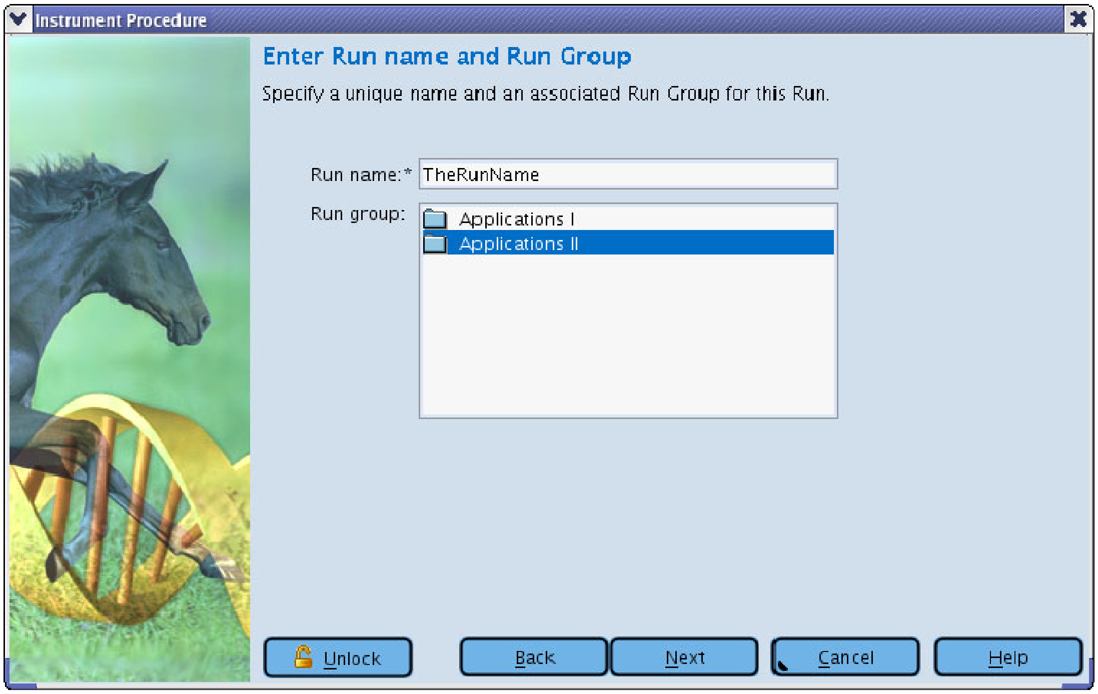
IDs and barcodes:* 456567ABC

123456
qwerty1234
12345678910 12345678 11 2009
product-id-123

Part: 12345678910
Lot: 12345678
Month: 11
Year: 2009

Figure 10: GS Sequencer Run Wizard – ‘Enter IDs and barcodes’ window

3. **Enter Run name and Run Group** (Figure 11) – Type in a Run name in the Run name entry field, and select a Run Group from the Run Group list. The Run name must contain only 0-9, a-z, underscore, or hyphen characters. This Run name will be part of the directory name used to store the sequencing Run results in the data file system; see step 9 below for more details on Run directory names.



Enter Run name and Run Group

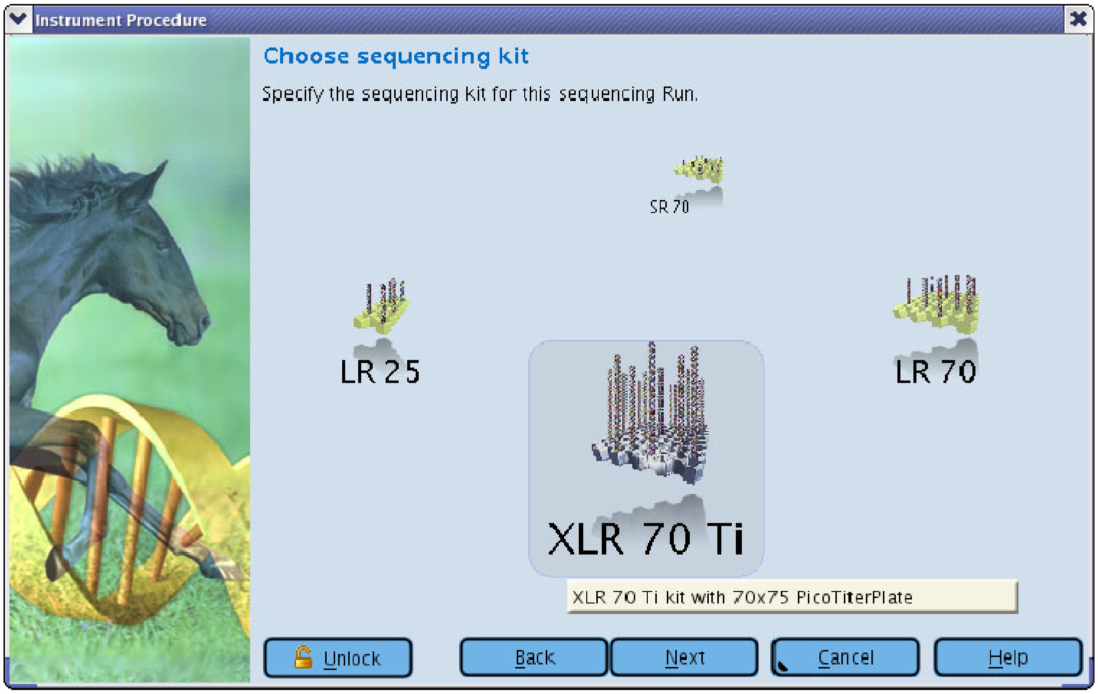
Specify a unique name and an associated Run Group for this Run.

Run name:* TheRunName

Run group:
Applications I
Applications II

Figure 11: GS Sequencer Run Wizard – ‘Enter Run name and Run Group’ window

4. **Choose sequencing kit** (Figure 12) – Choose the sequencing kit type that will be used for the sequencing Run.

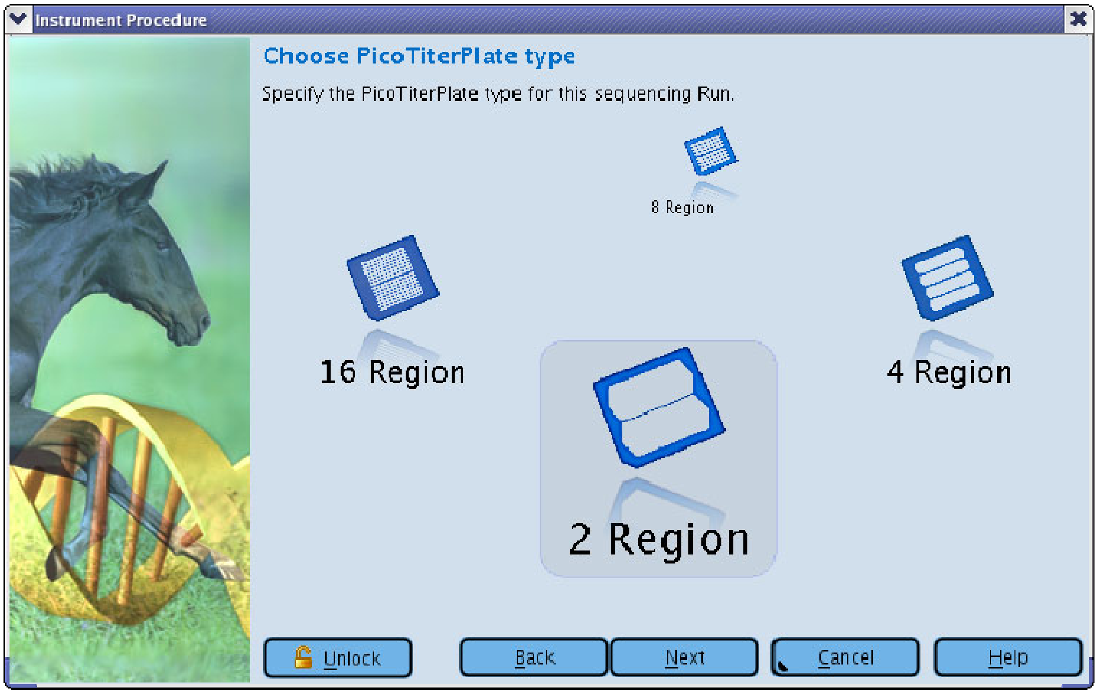


Specify the sequencing kit for this sequencing Run.

XLR 70 Ti kit with 70x75 PicoTiterPlate

Figure 12: GS Sequencer Run Wizard – 'Choose sequencing kit' window

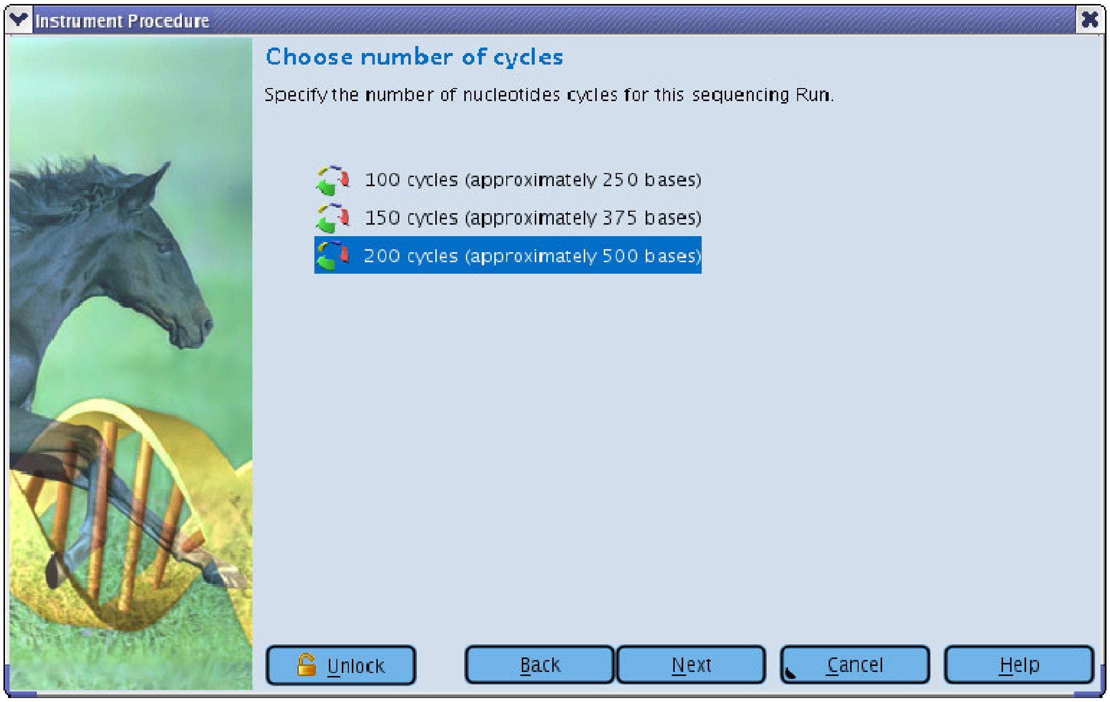
5. **Choose PicoTiterPlate type** (Figure 13) – Specify the bead loading gasket used to load the DNA sample onto the PTP device. The choices of gaskets presented match the sequencing Kit choice made in the previous step.



Specify the PicoTiterPlate type for this sequencing Run.

Figure 13: GS Sequencer Run Wizard – 'Choose PicoTiterPlate type' window

6. **Choose the number of cycles** (Figure 14) – Choose the number of cycles or nucleotide flows for the sequencing Run. The choices presented match the sequencing Kit choice made in the step 4. The greater the number of cycles chosen, the greater the final read length. Using the GS FLX Titanium chemistry:
    a. 100 cycles will produce reads of approximately 250 bases
    b. 150 cycles will produce reads of approximately 375 bases
    c. 200 cycles will produce reads of approximately 500 bases



Choose number of cycles

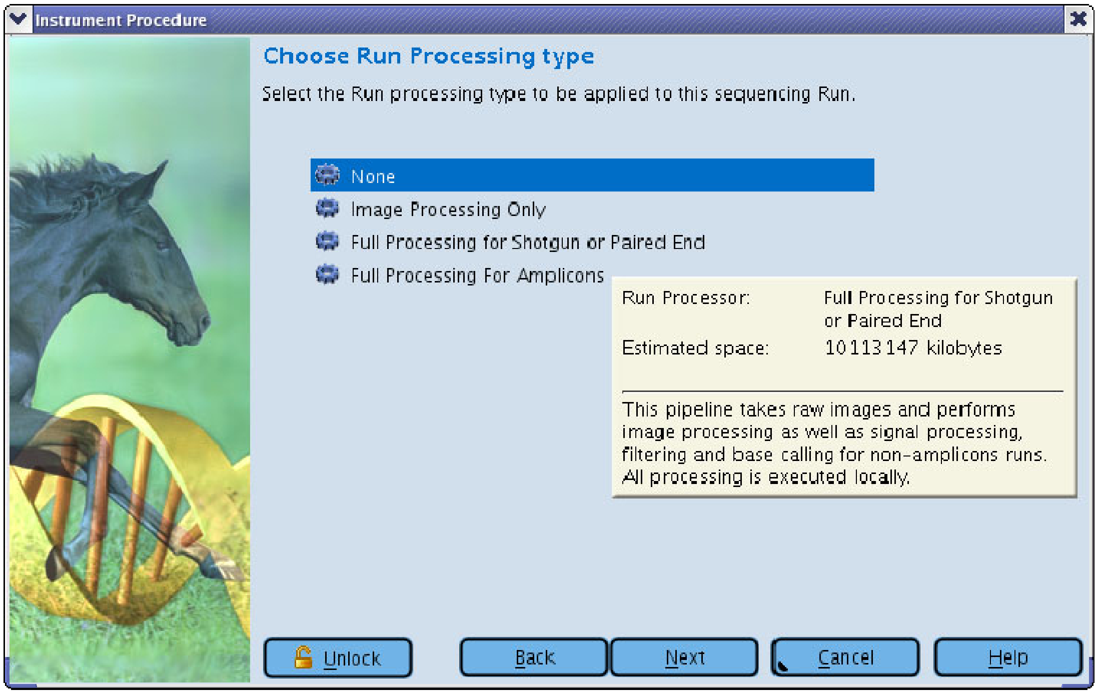
Specify the number of nucleotides cycles for this sequencing Run.

100 cycles (approximately 250 bases)
150 cycles (approximately 375 bases)
200 cycles (approximately 500 bases)

Figure 14: GS Sequencer Run Wizard – 'Choose number of cycles' window

7. **Choose Run Processing Type** (Figure 15) – There are four different options to launch a data processing job on the Genome Sequencer FLX Instrument:
   a. None: only the image capture is done on the Genome Sequencer FLX Instrument, and no processing of this data is carried out as part of the sequencing Run. Image and signal processing must be done off-instrument, after the Run.
   b. Image Processing Only: the image processing step but not the signal processing step of data processing is carried out as part of the sequencing Run. Signal processing must be done off-instrument, after the Run.
   c. Full Processing for Shotgun or Paired End: both the image and signal processing steps are performed on the instrument, as part of the sequencing Run. This option is appropriate for all library types except Amplicon libraries.
   d. Full Processing for Amplicons: both the image and signal processing steps are performed on the instrument, as part of the sequencing Run. This option is appropriate for Amplicon libraries only.

Data Processing Options are discussed in detail in Part B, Section 1 of this Manual.



Choose Run Processing type

Select the Run processing type to be applied to this sequencing Run.

Run Processor: Full Processing for Shotgun or Paired End

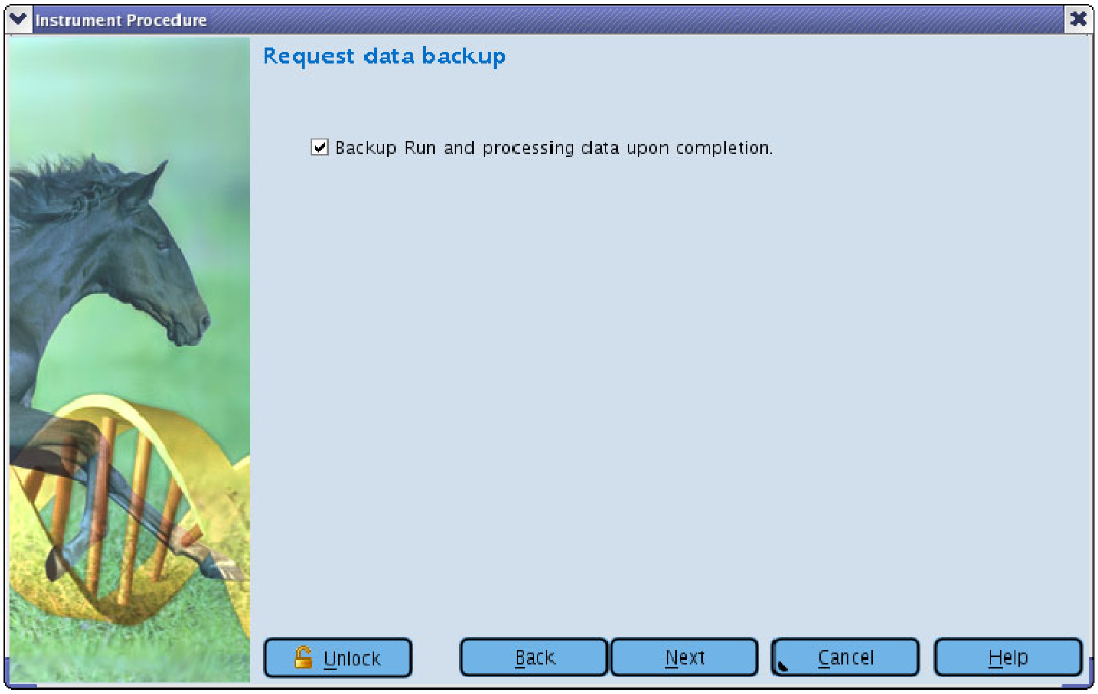
Estimated space: 10 113 147 kilobytes

This pipeline takes raw images and performs image processing as well as signal processing, filtering and base calling for non-amplicons runs. All processing is executed locally.

Figure 15: GS Sequencer Run Wizard – 'Choose Run Processing type' window

8. **Request data backup** (Figure 16) – If the 'Backup the Run and Processor data upon completion' checkbox is checked, the backupScript.sh script will be called at the end of the sequencing Run to archive the data to a data storage location specified in the script. (The backupScript.sh and data management are discussed in more detail in Part B, Section 5 of this manual.)
    a. The backup prompt can be configured in the gssequencer.xml configuration file. The default configuration is:
    ```
    <Backup>
        <Prompt>Backup Run and processing data upon completion.</Prompt>
        <Path>backupScript.sh</Path>
    </Backup>
    ```
    b. If the <Prompt></Prompt> element value is empty, this Run Wizard window will not appear and the backup will always occur at the end of the Run. If the <Path></Path> element value is empty, this Run Wizard window will not appear and the backup script will *not* run at the end of the Run.

In addition to the backupScript.sh script, the postAnalysisScript.sh will be called at the end of the data processing, and will perform any commands listed (see Part B, Section 5). For example, the postAnalysisScript.sh script can be modified to push the contents of a partially processed D_imageProcessingOnly directory to a DataRig (or cluster) and begin the signal processing on the designated computer.



☑ Backup Run and processing data upon completion.

Figure 16: GS Sequencer Run Wizard – 'Request data backup' window

9. Run comments (Figure 17) – This window allows operators to supply any comments about the Run. A tab is always present to assign comments to the Run. If the gasket used included 2 or more regions, additional tabs are displayed allowing comments to be associated with individual regions.

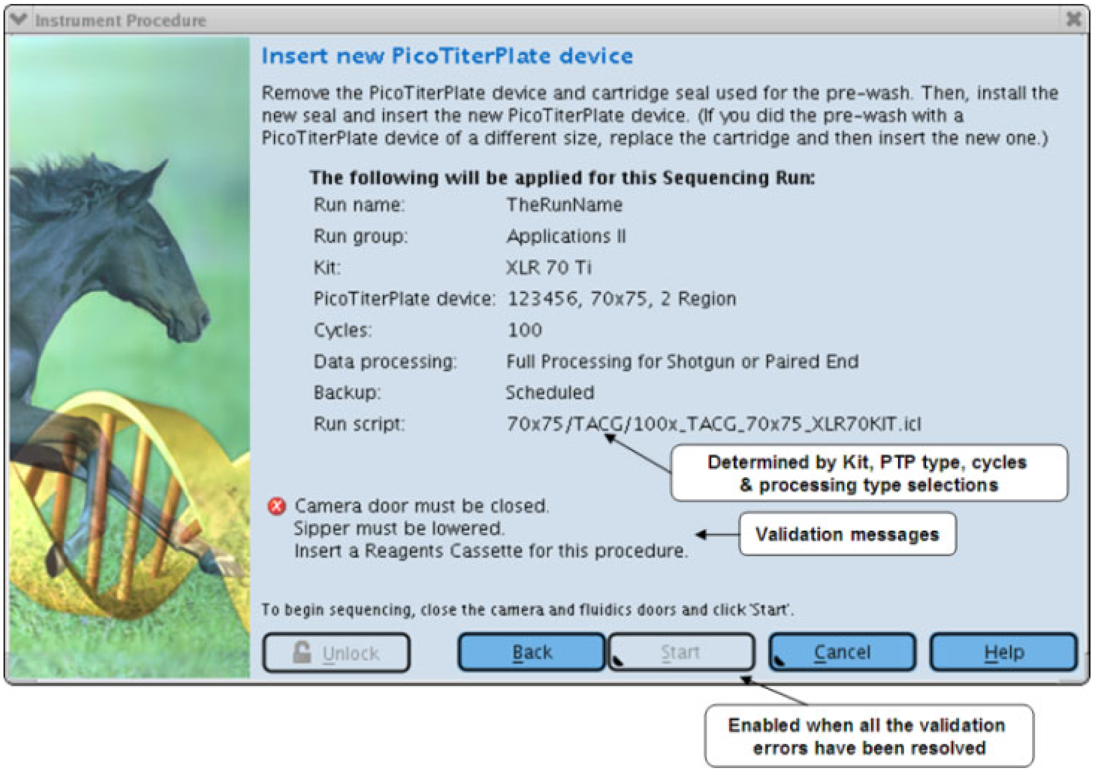
Enter the comments for this sequencing run. If the PicoTiterPlate device has multiple regions, comments may also be applied to the individual regions.

This is an Operator supplied comment for the run.

Individual comments for each region may also be added.

Figure 17: GS Sequencer Run Wizard – 'Run comments' window

10. **Insert new PicoTiterPlate Device** (Figure 18) – The final window of the Run Wizard provides a summary of the sequencing Run data entered and displays the name of the Run script (provided with the system) which will be used to carry out the Run. If any of the Run data is incorrect, the back button can be used to navigate to previous windows and incorrect it. Once all the necessary data has been entered and the PTP device has been inserted (as described in the *Sequencing Method Manual*), the 'Start' button is activated and the Genome Sequencer FLX Instrument is ready to begin a new sequencing Run. **Clicking on the Start button will initiate the sequencing Run.**



Insert new PicoTiterPlate device

Remove the PicoTiterPlate device and cartridge seal used for the pre-wash. Then, install the new seal and insert the new PicoTiterPlate device. (If you did the pre-wash with a PicoTiterPlate device of a different size, replace the cartridge and then insert the new one.)

The following will be applied for this Sequencing Run:

Run name:	TheRunName
Run group:	Applications II
Kit:	XLR 70 Ti
PicoTiterPlate device:	123456, 70x75, 2 Region
Cycles:	100
Data processing:	Full Processing for Shotgun or Paired End
Backup:	Scheduled
Run script:	70x75/TACG/100x_TACG_70x75_XLR70KIT.icl

Determined by Kit, PTP type, cycles & processing type selections

Camera door must be closed.
Sipper must be lowered.
Insert a Reagents Cassette for this procedure.

Validation messages

To begin sequencing, close the camera and fluidics doors and click 'Start'.

Enabled when all the validation errors have been resolved

Figure 18: GS Sequencer Run Wizard – 'Insert new PicoTiterPlate device' window

If the 'Start' or 'Next' button is not enabled in the Run Wizard, look for one or more validation messages above the buttons specifying the nature of the problem. Below is a list of the problems that may occur and how to resolve them:

Validation message	Cause	Resolution
Camera door must be closed.	The camera door sensor detected the camera door was not closed.	Close the camera door. If the door is already closed, contact your Roche Representative.

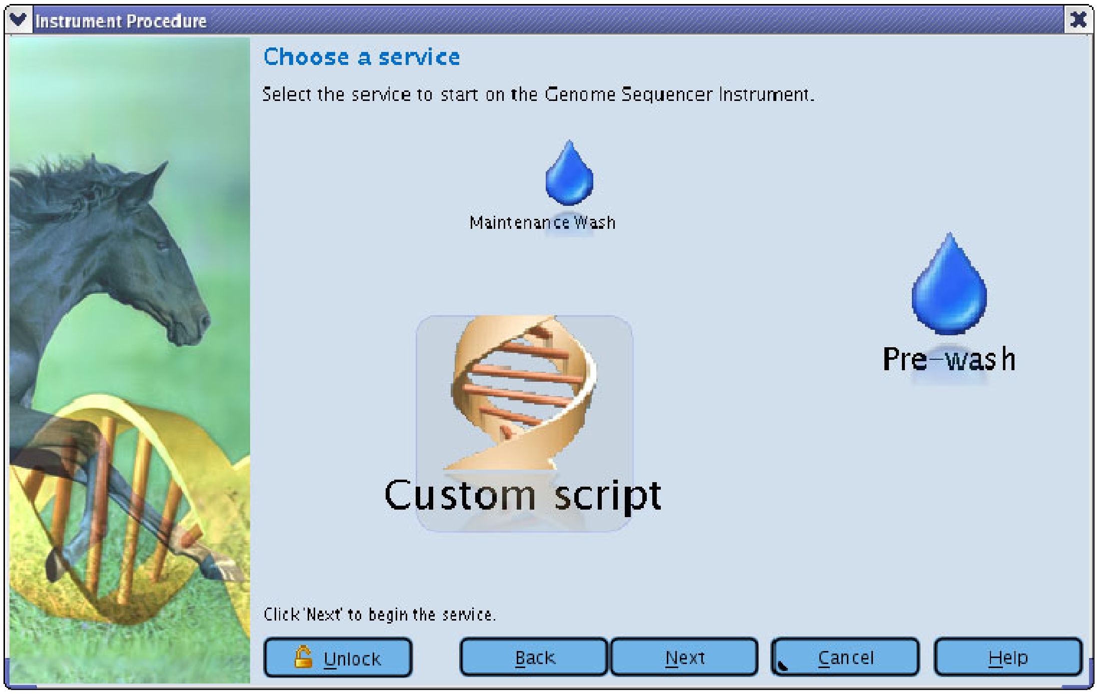
Validation message	Cause	Resolution
Insert a Cassette for this procedure. Or Insert a Pre-wash Cassette for this procedure. Or Insert a Reagents Cassette for this procedure.	There is a sensor that detects the presence of a cassette and its type. The sensor did not detect a correctly seated cassette.	For a sequencing Run or Maintenance Wash, insert a Reagent Cassette. For a Pre-wash procedure, ensure the Pre-wash cassette is inserted. If the correct cassette is already inserted contact your Roche Representative.
Sipper must be lowered.	There is a sensor that detects the position of the sipper. Before a procedure can start, the sipper must be lowered into the cassette.	Lower the sipper. If the sipper is already lowered contact your Roche Representative.
Run script is not defined. Or A valid instrument icl script file must be chosen. Or The instrument icl script file cannot be found. Make sure the file % exists.	In choosing a custom Run script, a path was supplied that did not point to a valid script. This can happened if you manually type in the path to the script instead of selecting it from the file chooser dialog.	Go to the custom script selection page (see Section 3.2.3) and verify that the script path is referring to a valid Run script .icl file at that location. Use the script file chooser button to ensure that the correct path is supplied.
A Run Group must be selected.	Normally a default Run Group is always selected. It is unusual if no Run Group is selected.	Go to the Run name and Run Group selection window and ensure that a Run Group is selected. If the Run Group list is empty, be sure that a list of Run Groups has been defined in the Configuration.
Unlocking camera door...	The camera door unlocking procedure takes a few moments to run and complete. Another procedure cannot be started until this completes.	Wait for the unlock procedure to complete. Once the next procedure is started, the door will relock.

Validation message	Cause	Resolution
Connection to the instrument services has failed. Or The instrument services failed to start. Or The instrument services have failed.	Communication to the backend services has failed or failed to start. These conditions are unusual since the Run Wizard cannot be accessed until the instrument is in an available state.	In rare conditions, the instrument backend service may fail to respond. To resolve the problem, exit the GS Sequencer application and restart the backend services with 'systemStart'. If the problem persists contact your Roche Representative.
The instrument services are starting...	The backend services have been restarted. This condition is unusual since the Run Wizard cannot be accessed until the instrument is in an available state.	This startup process takes a few minutes; wait for the backend to restart and return to the available state.
An unexpected instrument state is preventing the start of this procedure. Or A maintenance wash is unexpectedly running. Or A pre-wash is unexpectedly running. Or A sequencing Run is unexpectedly running.	The instrument is in an unexpected state. These conditions are unusual since the Run Wizard cannot be accessed until the instrument is in an available state.	Let any running process complete and perform a system restart. To restart, exit the GS Sequencer application and restart the backend services with 'systemStart'. If the problem persists contact your Roche Representative.
Connection to the data processor has failed	The data processing service is not running. This is a warning so a procedure can be started, but no data processing of a Run will occur.	Exit the GS Sequencer application. Run systemStart then start the GS Sequencer again. If the problem persists contact your Roche Representative.
There is not enough space available to complete this sequencing Run	The drive where $RIGDATA is located does not have enough free space to store the Run.	Free up some disk space on the drive by removing data sets that are no longer needed.

3.2.3 Other Procedure Types

Two other buttons are available on the first Run Wizard window ('Choose a procedure'; see Figure 9), besides the 'Sequencing Run' option described in the previous section:

- Pre-wash: this option starts a pre-wash of the instrument. A pre-wash must be carried out prior to a sequencing Run, using reagents and supplies provided in the Sequencing Kits as described in the *Sequencing Method Manual*.
- Service: the 'Service' option presents a page (Figure 19) where the following services can be selected:



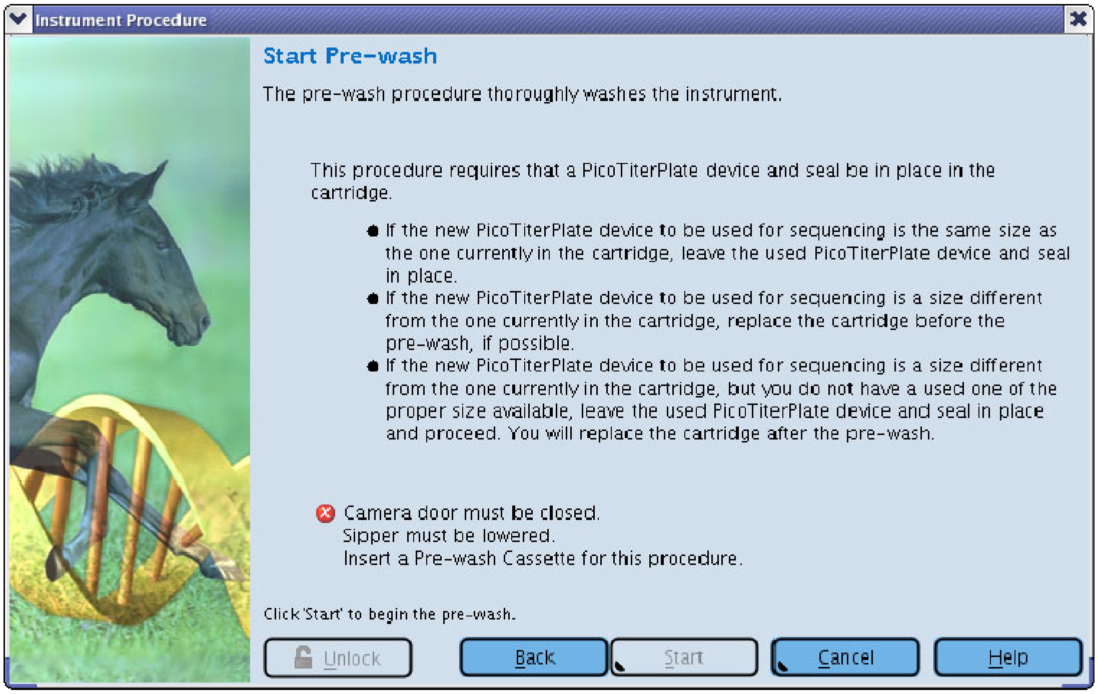
Choose a service

Select the service to start on the Genome Sequencer Instrument.

Click 'Next' to begin the service.

Figure 19: GS Sequencer Run Wizard – 'Choose a service' window

  - Pre-wash: this is the same as the Pre-wash button from the first Run Wizard window (see above). This functionality is reproduced here for convenience, during instrument service. Selecting this option from either location opens the 'Start Pre-wash' window of the Run Wizard (Figure 20).



Start Pre-wash

The pre-wash procedure thoroughly washes the instrument.

This procedure requires that a PicoTiterPlate device and seal be in place in the cartridge.

- If the new PicoTiterPlate device to be used for sequencing is the same size as the one currently in the cartridge, leave the used PicoTiterPlate device and seal in place.
- If the new PicoTiterPlate device to be used for sequencing is a size different from the one currently in the cartridge, replace the cartridge before the pre-wash, if possible.
- If the new PicoTiterPlate device to be used for sequencing is a size different from the one currently in the cartridge, but you do not have a used one of the proper size available, leave the used PicoTiterPlate device and seal in place and proceed. You will replace the cartridge after the pre-wash.

Camera door must be closed.
Sipper must be lowered.
Insert a Pre-wash Cassette for this procedure.

Click 'Start' to begin the pre-wash.

Figure 20: GS Sequencer Run Wizard – 'Start Pre-wash' window

- Maintenance Wash: this option presents an instruction window (Figure 21) similar to the one above, and the Start button is enabled when all instrument sensors report that the instrument is ready. Clicking 'Start' launches a Maintenance Wash. The Maintenance Wash procedure is described in the *Genome Sequencer FLX Instrument Owner's Manual*, and requires a GS FLX Maintenance Wash Kit, available separately.

Start Maintenance Wash

The maintenance wash procedure thoroughly cleans and rinses the instrument.

Please leave the existing, used, PicoTiterPlate device in place and keep the camera door closed. Before starting, insert the Reagents Cassette loaded with the Maintenance Wash reagents.

Click 'Start' to begin the maintenance wash.

Figure 21: GS Sequencer Run Wizard – 'Start Maintenance Wash' window

- Custom script: this option opens the ‘Select an instrument Run script’ window (Figure 22) where a special .icl file can be specified for the Run. Some of these script files come preinstalled on the instrument and are used by Roche Representatives during service of your Genome Sequencer FLX Instrument.

Specially designed instrument Run scripts may be specified here. For the standard sequencing kits this option should be avoided.

Instrument script:* /usr/local/rig/runScripts/Service/VerifyVolume.icl

Figure 22: GS Sequencer Run Wizard – ‘Select an instrument Run script’ window

3.2.4 Once a Procedure Has Started

After the ‘Start’ button at the end of the Run Wizard is clicked the Run Wizard window will close and the procedure can be monitored on the ‘Instrument’ tab of the GS Sequencer application main window. The Instrument tab is described in section 3.3.

It is recommended to monitor the beginning of a procedure, to make sure that everything is proceeding normally. Once the fluidics system starts, the instrument LED will show blinking-green. At this time it is safe to leave the instrument as the instrument advances through its procedure.

If the instrument encounters any problems during the initiation of a Run, a message describing the issue will appear at the top of the GS Sequencer application window, and Operator intervention will be required before the Run will continue. In most cases, the software will offer the choice to ‘Abort or Proceed with the Run’. In the event that an error occurs that prevents the Run from continuing or executing properly (error messages will be displayed), or if the instrument status is ‘Failure State’, the Run will not proceed. The instrument error messages are listed in the Troubleshooting section of the *Genome Sequencer FLX Instrument Owner’s Manual*, along with instructions on what to do in each case.

There is an ‘Abort’ button (Figure 23) in the Global Action area on the right side of the GS Sequencer application window that can be clicked to abort the sequencing Run (or other procedure) when needed. This will bring up a verification window to terminate the Run. There is no procedure for pausing and resuming a Run. If a Run is aborted, follow the abort with a pre-wash before proceeding with another sequencing Run launch.

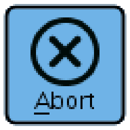
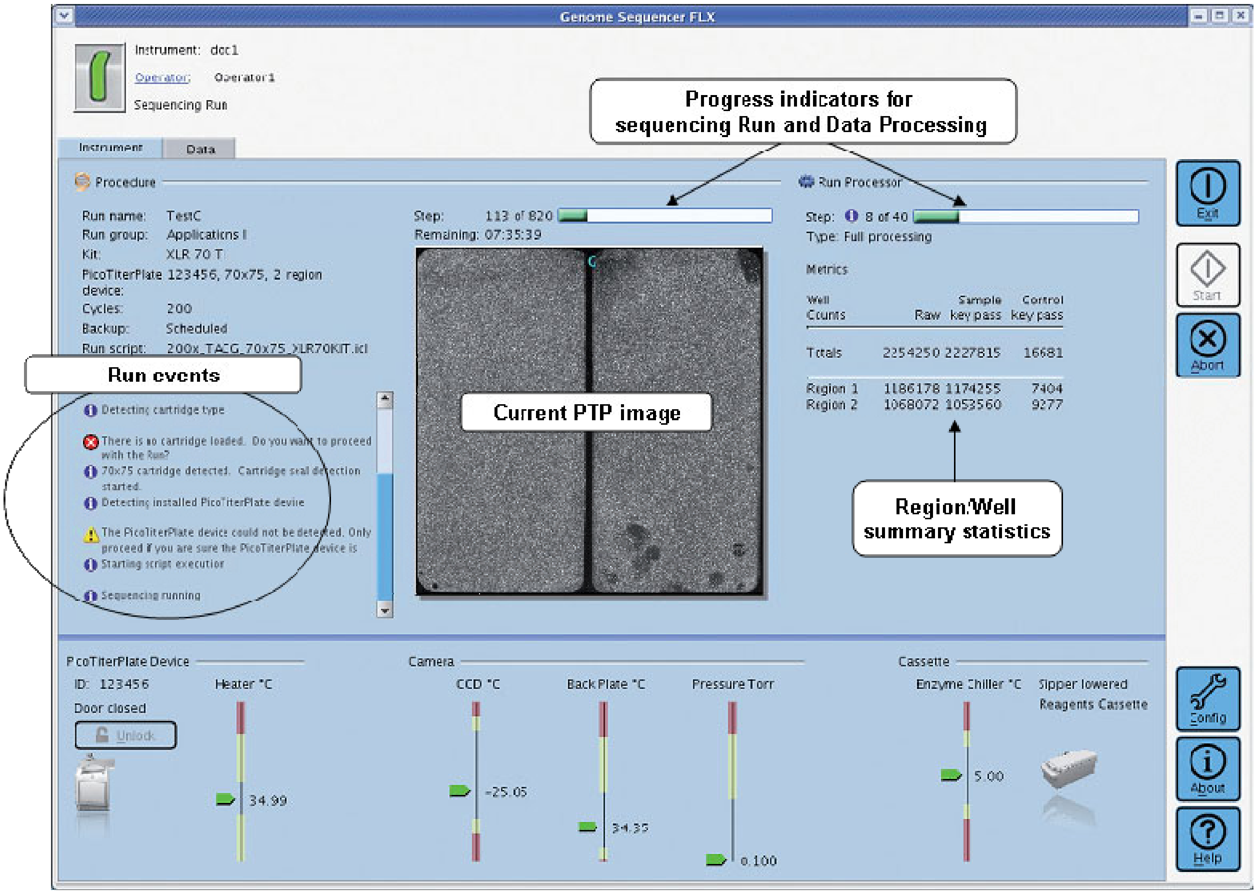


Figure 23: Abort button

3.3 Instrument Tab

The ‘Instrument’ tab of the GS Sequencer application is divided into an upper and a lower area (previously seen in Figure 7, above). Figure 24 shows how the Instrument tab looks when a Run is in progress.



Progress indicators for sequencing Run and Data Processing

Run events

Current PTP image

Region:Well summary statistics

Instrument: dcc1
Operator: Operator1
Sequencing Run

Run name: TestC
Run group: Applications I
Kit: XLR 70 T
PicoTiterPlate device: 123456, 70x75, 2 region
Cycles: 200
Backup: Scheduled
Run script: 200x_TACG_70x75_XLR70KIT.icl

Detecting cartridge type
There is no cartridge loaded. Do you want to proceed with the Run?
70x75 cartridge detected. Cartridge seal detection started.
Detecting installed PicoTiterPlate device
The PicoTiterPlate device could not be detected. Only proceed if you are sure the PicoTiterPlate device is
Starting script execution
Sequencing running

Step: 113 of 820
Remaining: 07:35:39

Step: 8 of 40
Type: Full processing

Well Counts	Raw	Sample key pass	Control key pass
Totals	2254250	2227815	16681
Region 1	1186178	1174255	7404
Region 2	1068072	1053560	9277

ID: 123456
Door closed
Heater °C: 34.99
CCD °C: -25.05
Back Plate °C: 34.35
Pressure Torr: 0.100
Enzyme Chiller °C: 5.00
Sipper lowered
Reagents Cassette

Figure 24: The GS Sequencer application Instrument tab during a sequencing Run

In the upper panel of the ‘Instrument’ tab, the Procedure section contains the following real-time information on the sequencing Run:

- Run data – as entered in the Run Wizard (Run name, Run Group, Kit type, PTP ID & type, number of cycles, whether a backup of the data is scheduled, and the Run Script)
- Run launch time – time the Run was started
- Run events – a scrollable list of messages and warnings issued by the instrument during the sequencing Run

- A progress bar for the reagent flows (Step, '# steps completed' of '# total steps')

- A thumbnail of the last image captured during the Run

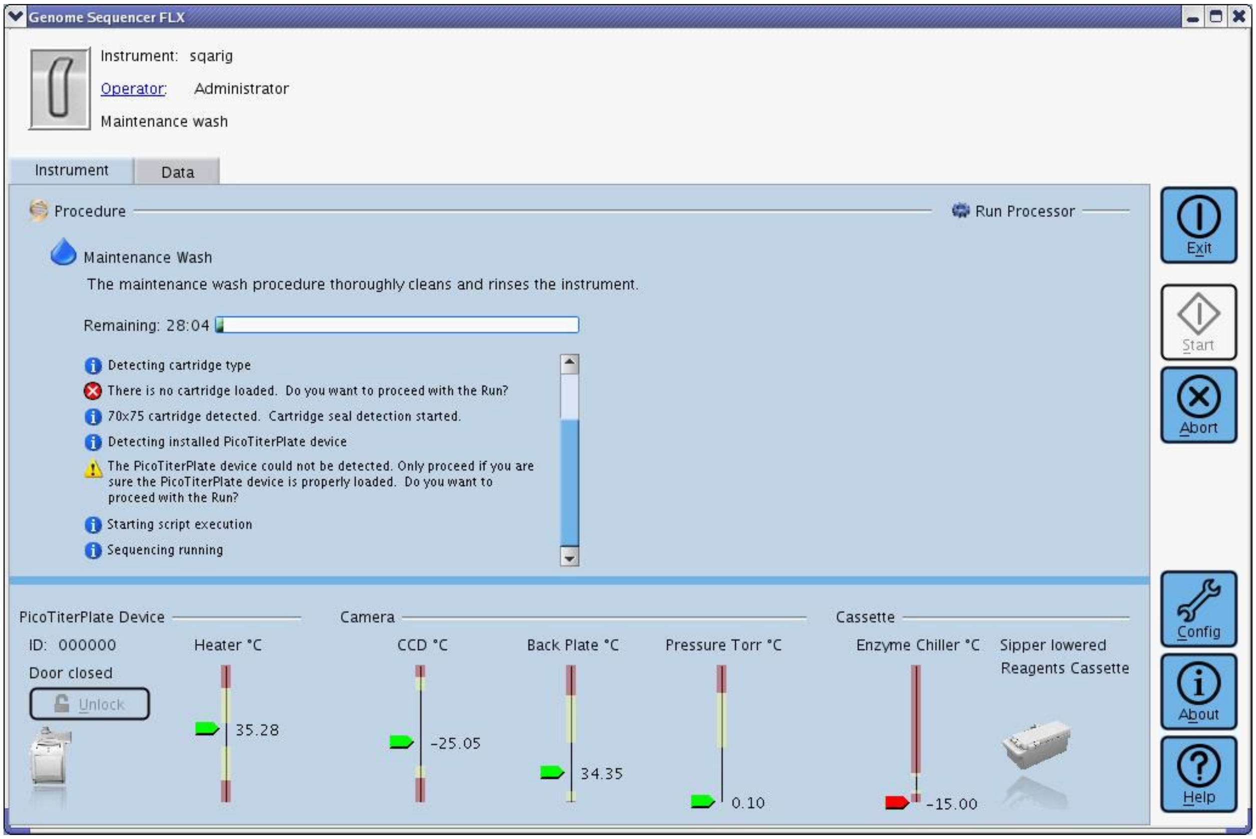
The Run Processor section on the right side of the Instrument tab contains the following information for the data processing in progress:

- A progress bar for the processing steps (Step, '# steps completed' of '# total steps') and an information icon that can be clicked to get more detailed information on the processing steps.
- The type of data processing that is being done on the Instrument
- Processing Metrics –Total raw and key pass wells, for all regions of the PTP device, for library as well as for control reads.

The lower area of the 'Instrument' tab displays the readings from the various sensors that monitor the status of the Genome Sequencer FLX Instrument components. The components and their status data are listed below:

- PicoTiterPlate (PTP) Device – holds the DNA sample
    - ID is the number detected by reading the data matrix on the PTP device. This may or may not be the actual number entered while setting up the instrument procedure.
    - Door Status – 'Door Closed', 'Door Open'
    - Door Lock/Unlock Button (same functionality also available in the Run Wizard)
    - PTP Heater Temperature indicator bar
- Camera – captures the images of the PTP device during the nucleotide flows
    - CCD Temperature indicator bar (°C)
    - Back Plate Temperature indicator bar (°C)
    - Camera Pressure indicator bar (Torr)
- Cassette – feeds reagents to the fluidics system through the sipper
    - Enzyme Chiller Temperature indicator bar (°C)
    - Sipper Status – 'Lowered', 'Raised'
    - Cassette type – 'Pre-wash', 'Reagent' or not present

For a wash procedure (pre-wash or maintenance wash) the 'Instrument' tab shows the progress of the procedure (Figure 25).



Instrument: sqarig
Operator: Administrator
Maintenance wash

Maintenance Wash
The maintenance wash procedure thoroughly cleans and rinses the instrument.

Remaining: 28:04

- Detecting cartridge type
- There is no cartridge loaded. Do you want to proceed with the Run?
- 70x75 cartridge detected. Cartridge seal detection started.
- Detecting installed PicoTiterPlate device
- The PicoTiterPlate device could not be detected. Only proceed if you are sure the PicoTiterPlate device is properly loaded. Do you want to proceed with the Run?
- Starting script execution
- Sequencing running

ID: 000000
Door closed

Heater °C	CCD °C	Back Plate °C	Pressure Torr °C	Enzyme Chiller °C
35.28	-25.05	34.35	0.10	-15.00

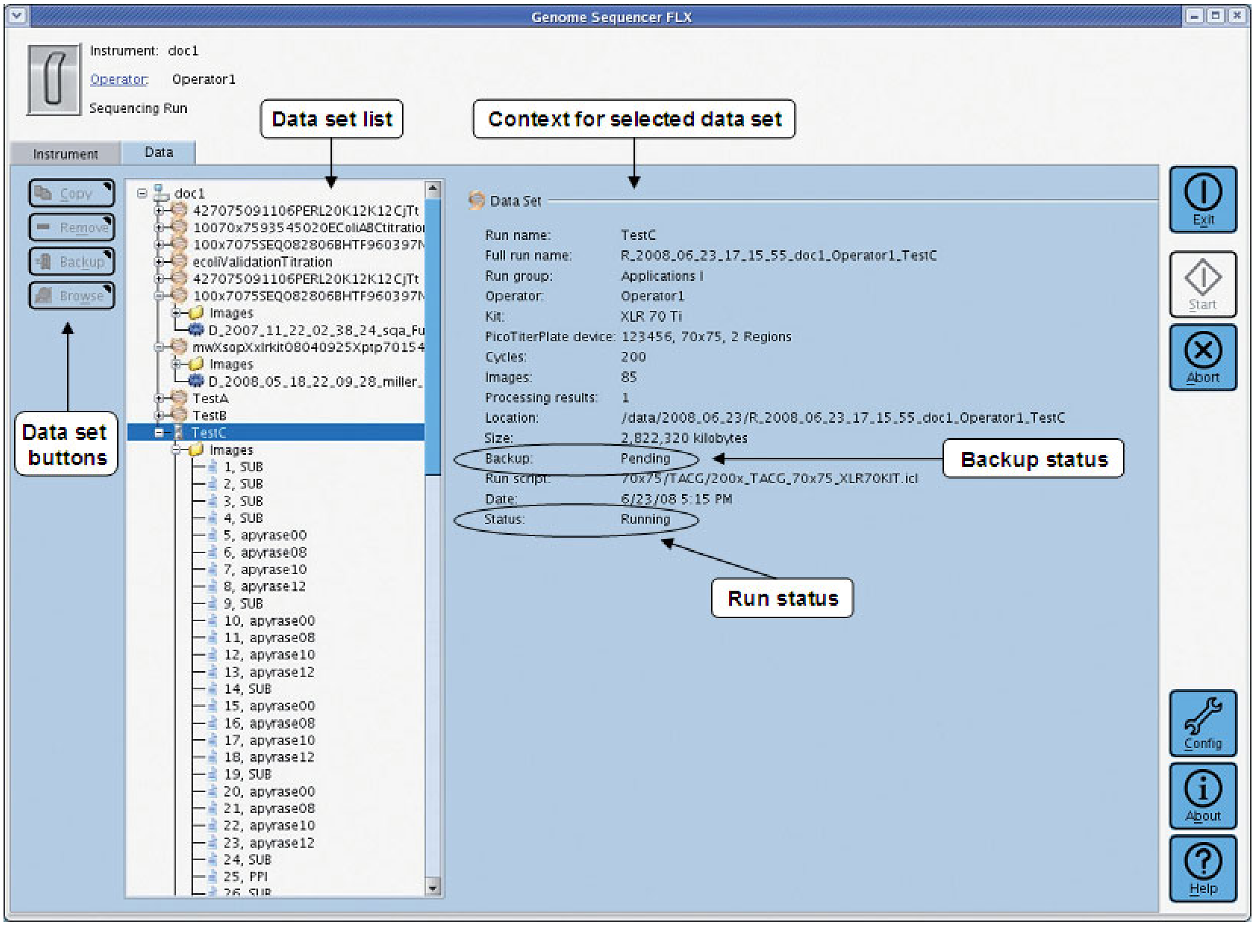
Sipper lowered
Reagents Cassette

Figure 25: The GS Sequencer application Instrument tab during a Maintenance Wash

3.4 Data Tab

The ‘Data’ tab is used to manage the sequencing Run data stored on the Genome Sequencer FLX Instrument. The components of the ‘Data’ tab include (Figure 26):

- four Data set action buttons on the left of the tab – ‘Copy’, ‘Remove’, ‘Backup’ and ‘Browse’
- a Data set list view (expandable to all sub-folders and files) in the center of the tab
- a ‘Data Set’ summary panel on the right of the tab, providing details and context for the selected data set.



Instrument: doc1
Operator: Operator1
Sequencing Run

Run name: TestC
Full run name: R_2008_06_23_17_15_55_doc1_Operator1_TestC
Run group: Applications I
Operator: Operator1
Kit: XLR 70 Ti
PicoTiterPlate device: 123456, 70x75, 2 Regions
Cycles: 200
Images: 85
Processing results: 1
Location: /data/2008_06_23/R_2008_06_23_17_15_55_doc1_Operator1_TestC
Size: 2,822,320 kilobytes
Backup: Pending
Run script: 70x75/TACG/200x_TACG_70x75_XLR70KIT.icl
Date: 6/23/08 5:15 PM
Status: Running

Data set list

Context for selected data set

Data set buttons

Backup status

Run status

Figure 26: The GS Sequencer application Data tab

The data set list allows the Operator to select a sequencing data set, by Run name, for further action and to see a list of all the folders and files that were created during the Run. The list can be sorted by name or date by right clicking on the list an selecting the appropriate sort. Once a data set is selected in the data set list, information and context about the Run are displayed to the right. In addition to the basic Run information (including the full Run name, also used to name the Run 'R_...' directory; see Section 3.5), this summary includes:

- the number of image files generated
- the number of data processing folders present ('D_ directories'; see the General Overview section of this manual)
- the location of the files, which is the absolute path of the sequencing Run folder on the Genome Sequencer FLX Instrument file system (/data/YYYY_MM_DD/R_...)
- the size of the data set (in kilobytes)
- the backup status [None, Pending, Failed, Complete]
- the Run script used (*.icl file)
- the date of the sequencing Run

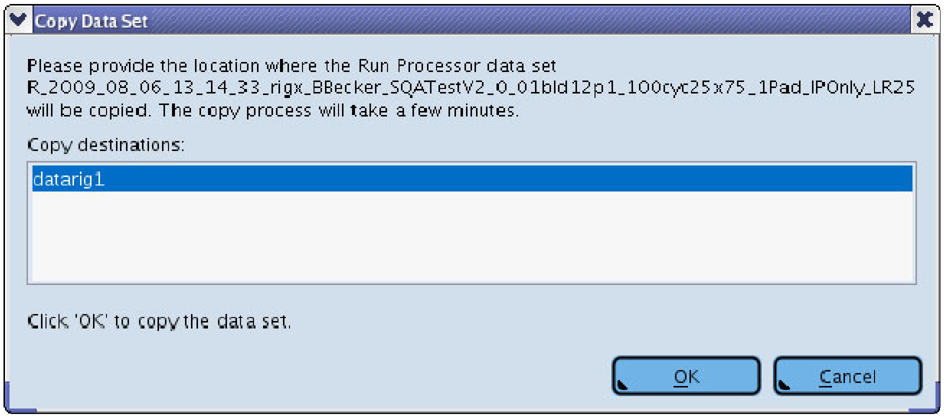
- the status of the sequencing Run [Running, Completed]

A new sequencing Run cannot be launched if the estimated disk space of the new Run is not available in the $RIGDATA directory.

The total size of the raw image data generated from a GS FLX Titanium sequencing Run is ~30GB. This amount of data can quickly fill up the Genome Sequencer FLX Instrument data partition. Hence it is very important to remove Run data from the instrument disk once it has been archived and transferred to an appropriate storage location. Before a Run can be started, the Run Wizard verifies if there is enough space for the Run and the selected data processing. If there is not enough space, the Run Wizard will show an error message and prevent initiation of a Run until more disk space is made available.

The action buttons on the left fulfill the following functions:

- Copy – Clicking the ‘Copy’ button for a selected sequencing Run data set (an R_... folder) copies the folder and its contents to a networked computer. A dialog box will open that displays a list of pre-configured network locations. The Operator can select the desired destination for the data set copy. (For details on configuration of networked computers, see the *Genome Sequencer FLX System Administrator’s Guide*.)



Please provide the location where the Run Processor data set R_2009_08_06_13_14_33_rigx_BBecker_SQATestV2_0_01bld12p1_100cyc25x75_1Pad_IPOnly_LR25 will be copied. The copy process will take a few minutes.

Copy destinations:

datarig1

Click 'OK' to copy the data set.

Figure 27: Copy Data Set dialog box

Individual GS Run Processor data files (D_... folders) cannot be copied using this function button.

- Remove – Clicking the ‘Remove’ button deletes the selected Run folder (R_...) from the Genome Sequencer FLX Instrument data file system. Removal of sequencing Run data sets is only allowed for files that have a backup status of ‘Complete’ or ‘None’.
- Backup – Clicking on the ‘Backup’ button for a selected sequencing Run data set (R_... folder) invokes the backupScript.sh script that archives the selected data set to a data storage location on the network. This is the same backup function as can be applied to a Run in advance, during Run set up (see section 3.2.2, step 8).

- Browse – Clicking the ‘Browse’ button launches the GS Run Browser for viewing the results of a sequencing Run. If a Run folder is selected (R_...), Run and image information is available. If a data processing folder is selected (D_...), signal intensities, read length distribution and filter metrics are also available. See Section 3 for an overview, and Part B, Section 3 for details on the GS Run Browser application.

- The Run data set is not removed from the Genome Sequencer FLX Instrument data file system by the ‘Copy’ or the ‘Backup’ buttons. Copy is used to transfer the data to an appropriate network location, e.g. for further analysis; while Backup is used for archival purposes. The two actions are distinct and separate. Copying a Run data set will not change its Backup status.
- The ‘Copy’, ‘Remove’ and ‘Backup’ functions can be applied to multiple data sets at the same time by selecting multiple data sets in the file list. To select multiple items, press and hold the control key and click on the list items of interest. To select an adjacent group of items on the list, click on the first item then press and hold the shift key and click on the last item of interest.

3.5 GS Sequencer Output

The files generated by the GS Sequencer application are called data sets and are placed in a Run directory (‘R_...’; see Note below), within the data partition of the instrument’s file system. The location of this data directory is set by the operating system environment variable $RIGDATA. The $RIGDATA setting is typically set to ‘/data’. The Run data sets include Run and image set information in the dataRunParams.parse file, and the set of raw images (*.pif format, in the ‘rawImages’ sub-folder). When data processing is configured as part of the sequencing Run, additional data processing data set files are output. These are described in the GS Run Processor section of this manual (Part B, Section 1). The output files from the GS Sequencer application are described below.

- dataRunParams.parse – This file is required in order to perform the image processing step of data processing. It contains all the parameters for the sequencing Run.
- runComments.xml – This file contains the Run and region comments that were entered by the Operator as part of the sequencing Run setup (see Section 3.2.2, step 9).

- imageLog.parse – This file is also required in order to perform the image processing step of data processing. It is a log file of all the images of the data set, including the reagent that was being flowed during the capture of each image.

- *.pif files - These files are required in order to perform the image processing step of data processing. Raw image files, showing photon counts for each pixel (in a rawImages sub-folder).

The following files are generated for troubleshooting purposes. While not strictly required for further analysis, it is recommended that these be archived with the raw data. You may be asked for these files if you seek help from Roche Customer Support (see Section 3.6.4) to resolve problems with a data set.

- ptpImage.pif
- runlog.parse
- aaLog.txt
- *.icl (name of the script used during the sequencing Run or other procedure)

Full Run name: Run directory names (R_ …) are formed by appending the Run name entered during Run set up (see section 3.2.2, step 3) to information provided by the system, in the following format:

R_yyyy_mm_dd_hh_min_sec_machineName_OperatorID_RunName

The 'R_' denotes this as a Run directory, 'yyyy_mm_dd_hh_min_sec' is the timestamp for the beginning of the Run, 'machineName' identifies the Genome Sequencer FLX Instrument on which the sequencing Run is performed, 'OperatorID' identifies the Operator of the sequencing Run, and the 'Run name' entered during Run set up.

Some useful information that may be included in the Run name may include the PTP device size and ID (bar code number), the sequencing kit lot #, the genome being sequenced, and a Run number (e.g., '70X75_123456_060209_ecoli_Run3'). Because all applications for the Genome Sequencer FLX System work under the Linux operating system, spaces should not be used in the run names – upper and lowercase letters and underscores are a good way to distinguish words.

If any data processing was carried out on this Run, a D_ directory is created within its R_ directory. Since a data set can be processed multiple times, an R_ directory can contain multiple D_ directories. See the General Overview section of this manual for more details.

3.6 Other Features and Functions

3.6.1 Instrument Status Area

The top area of the GS Sequencer application main window contains general information referred to as instrument status (e.g. see Figure 6 and Figure 7). Specifically, this includes four types of information:

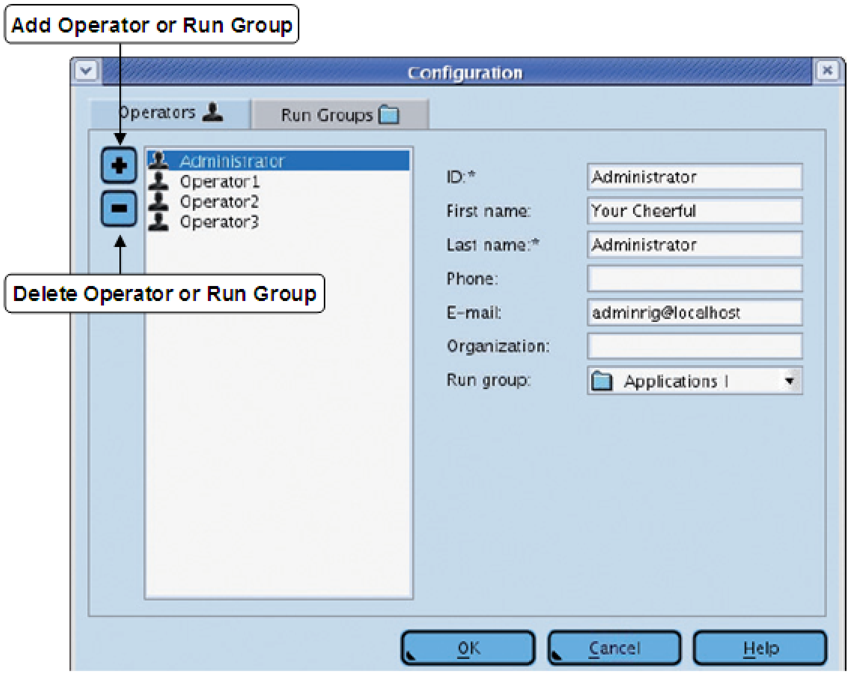
- name of this Genome Sequencer FLX Instrument
- Operator currently logged in
- a status message indicating the state of the instrument
- a replica of the Status Indicator LED located above the camera door ('Available' = green, 'Warning' = yellow, Unavailable' = red). For more details on the possible meanings of this indicator see the *Genome Sequencer FLX Instrument Owner's Manual*.

3.6.2 Configuration Button

Configuration allows Operators and Run Groups to be added to, modified in, or deleted from the GS Sequencer Operator and Run Group lists. Operator IDs and Run Groups are used to track ownership of all tasks performed on the Genome Sequencer FLX Instrument. These are included in the Run directory names, included as Run parameters, and are used to email the Operator upon completion of the sequencing Run. Clicking on the ‘Config’ button launches the configuration tool (Figure 28).

Figure 28: Configuration button

The Configuration dialog has two tabs; the ‘Operators’ tab and the ‘Run Groups’ tab. Each tab contains two sections; on the left is a list of current Operators or Run Groups, and on the right is an editable form associated with the selected list item. Fields marked with an asterisk are required for definition of a new Operator or Run Group. (Figure 29). A pair of buttons on the left of each tab (“+” and “-“) are used to add or delete and entry from the lists.


Add Operator or Run Group

Delete Operator or Run Group

ID:* Administrator
First name: Your Cheerful
Last name:* Administrator
Phone:
E-mail: adminrig@localhost
Organization:
Run group: Applications I

Figure 29: GS Sequencer Configuration Tools Window

- **To add a new Operator or Run Group**, click on the ‘+’ button on the left and edit the appropriate information fields on the right, then click the ‘OK’ button.
- **To edit an existing Operator or Run Group**, click on the entry in the left panel of the Operator or Run Group tab and edit the appropriate information fields on the right, then click the ‘OK’ button.

- **To assign a default Run Group to an Operator**, in the Operator tab click on the Operator entry in the left panel and then select the appropriate Run Group from the pull-down menu in the right panel and click the ‘OK’ button. This Run Group will be displayed by default for this Operator, in the Run Group field of the Run Wizard (see Section 3.2.2, step 3), but can be changed in the Run Wizard for a specific Run.

- **To delete an Operator or Run Group**, click on the entry in the left panel and check the fields on the right to be certain that it is the entry to be deleted, then click the ‘-’ button.

3.6.3 About and Help Buttons

The ‘About’ and ‘Help’ buttons are found in the Global Action Area, on the right hand side of the GS Sequencer application window. (Figure 30).

- The ‘About’ button will display information about the currently installed version of the GS Sequencer application (see Figure 31).
- The ‘Help’ button will provide instructions for where to find further information on the software, or will link you directly to a searchable version of the *Genome Sequencer FLX System Software Manual*.

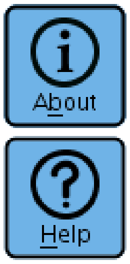
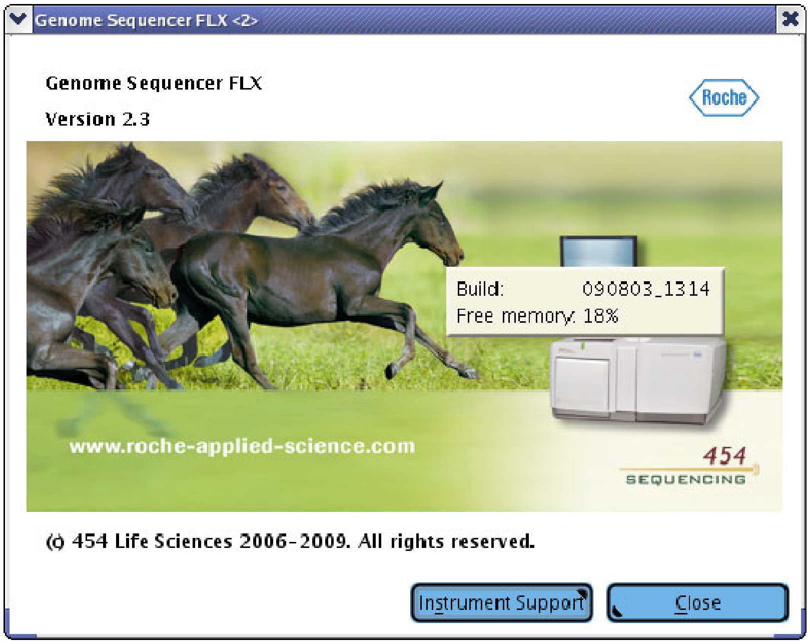


Figure 30: About and Help buttons

3.6.4 Support

If you are having problems with your instrument or with a sequencing Run data set, you can contact Roche Customer Support using the support feature found in the About dialog (Figure 31). Click ‘About’ and ‘Instrument Support’ to generate a support report.



Genome Sequencer FLX <2>

Genome Sequencer FLX
Version 2.3

Build: 090803_1314
Free memory: 18%

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Figure 31: GS Sequencer 'About' dialog

This will open the GS Support Tool application, which can be used to collect and conveniently package information that will help your Customer Support representative troubleshoot the problem. Usage of the GS Support Tool is described in Part B, section 4 of this manual, and in Technical Bulletin TCB-09007: Guide to Using the Support Tool. The TCB is available for download from the customer-restricted area of the www.454.com/my454 web site.

4. GS RUN BROWSER

This section focuses on the function of the GS Run Browser pertaining to launching data processing jobs on data sets, from the Genome Sequencer FLX Instrument. For details on the main GS Run Browser function, examining the details of a sequencing Run, see Part B, Section 3.

The GS Run Browser application allows an Operator to view and analyze the results of a sequencing Run as well as launch processing jobs via the GS Run Processor Manager.

4.1 Launching the GS Run Browser on the Genome Sequencer FLX Instrument

To open a data set in the GS Run Browser, do one of the following:

- From the Genome Sequencer FLX Instrument desktop double-click on the GS Run Browser icon (Figure 32 A). From the GS Run Browser application window, click on ‘Open a Data Set’ link under the Quick Start topic and use the file navigation window to select a data set (R_... or D_... folder), then click ‘OK’.
- From the GS Sequencer application ‘Data’ tab select a data set (R_... or D_... folder), then click on the ‘Browse’ button (Figure 32 B).

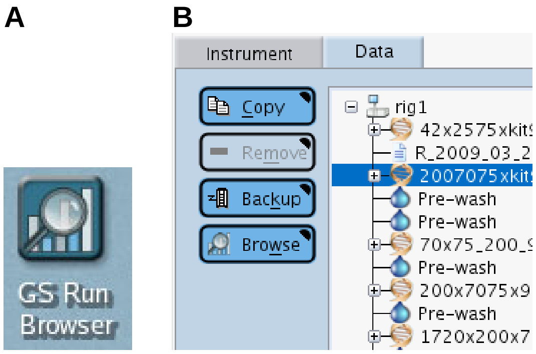
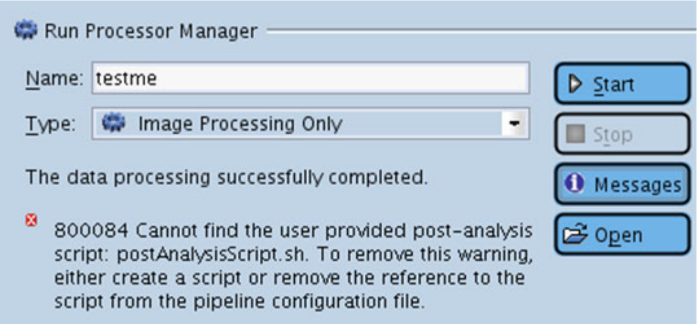


Figure 32: (A) GS Run Browser desktop icon. (B) ‘Browse’ button on the Data tab of the GS Sequencer application

4.2 Processing Run Data

Data processing jobs not configured as a part of the sequencing Run can be launched post-Run on the Genome Sequencer FLX Instrument, from the GS Run Browser. On the ‘Overview’ tab, locate the Run Processor Manager tool in the lower right-hand portion of the Overview tab. This tool can also be used to reprocess data sets that were processed previously; each round of processing will give rise to its own D_ directory.



Name: testme

Type: Image Processing Only

The data processing successfully completed.

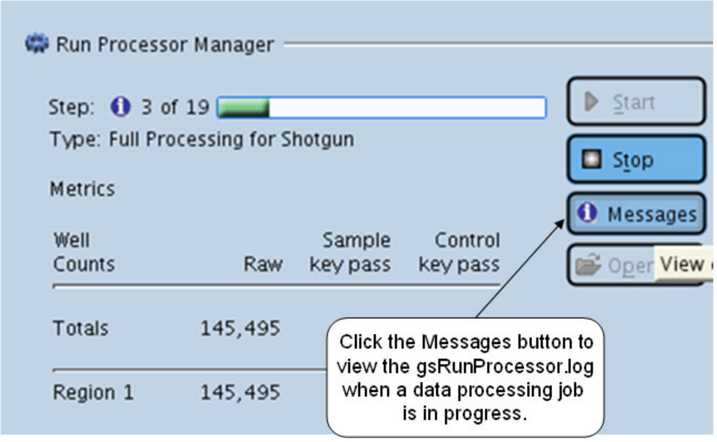
800084 Cannot find the user provided post-analysis script: postAnalysisScript.sh. To remove this warning, either create a script or remove the reference to the script from the pipeline configuration file.

Figure 33: GS Run Processor Manager Tool in the GS Run Browser Overview tab

To launch a data processing job:

- Type in a unique name for the processing job - This name will be appended to the data processing directory name (D_...) and the folder of processed results will be placed inside the sequencing Run folder (R_...) parallel to any existing data processing folders.
- Select the processing type. The options below are the standard choices shipped with the software, but your site may have additional options.
  - If you opened an R_ directory, the options presented will be:
    - Image Processing Only
    - Full Processing for Shotgun or Paired End
    - Full Processing for Amplicons.
  - If you opened a D_ directory, the options presented will be:
    - Signal Processing for Shotgun or Paired End
    - Signal Processing for Amplicons.
- Click the 'Start' button. There is a 'Stop' button if the processing job needs to be halted for any reason.

Once a job has been launched, the progress and statistics of the job is displayed, as shown on Figure 34.



Step: 3 of 19

Type: Full Processing for Shotgun

Metrics

Well Counts	Raw	Sample key pass	Control key pass
Totals	145,495		
Region 1	145,495		

Click the Messages button to view the gsRunProcessor.log when a data processing job is in progress.

Figure 34: GS Run Processor Manager Tool job-in-progress statistics

The gsRunProcessor.log file can be viewed in a separate pop-up window by clicking the ‘Messages’ button. The button is enabled for data processing jobs in progress or completed.

Once a processing job has been completed, the results can be loaded into the GS Run Browser for viewing by clicking the ‘Open’ button. See Part B, Section 3 for details on how to use the GS Run Browser to examine the results of a sequencing Run.

5. GS SEQUENCER APPENDICES

5.1 GS Sequencer Actions

5.1.1 Desktop Actions

Application	Action	location
systemStart	double click systemStart icon	Genome Sequencer FLX Instrument desktop
systemStop	double click systemStop icon	Genome Sequencer FLX Instrument desktop
GS Sequencer application	double click GS Sequencer icon	Genome Sequencer FLX Instrument desktop
GS Run Browser application	double click GS Run Browser icon	Genome Sequencer FLX Instrument desktop

5.1.2 GS Sequencer Application Actions

Intent	Action	location
Sign in	select Operator and click 'Sign in' button	GS Sequencer application sign in screen
Sign out	click on 'Operator' field identifier in the top status area	GS Sequencer application Status area
Configure	click on configuration button	GS Sequencer application Global Action area
Start Instrument Procedure (e.g. a sequencing Run)	click on the 'Start' button	GS Sequencer application Global Action area
Door unlock	Click 'Unlock' button	GS Sequencer application Instrument tab; GS Sequencer Run Wizard windows
Help	Click 'Help' button	GS Sequencer application Global Action area
About	Click 'About' button	GS Sequencer application Global Action area
Support	Click 'About' button; then, click 'Instrument Support' button	GS Sequencer application Global Action area
Exit	Click 'Exit' button	GS Sequencer application Global Action area

5.1.3 GS Sequencer Data Manager Actions

Intent	Action	location
Copy a data set from the Genome Sequencer FLX Instrument file system to a network destination	select data set in data set list, click the 'Copy' button, select network destination from the navigation window, click 'ok'	GS Sequencer application Data tab
Backup a data set from the Genome Sequencer FLX Instrument file system to an archive	select data set in data set list, click the 'Backup' button, select network archive destination from the navigation window, click 'ok'	GS Sequencer application Data tab
Remove a data set from the	select data set in data set list,	GS Sequencer application

Genome Sequencer FLX Instrument file system	check the data set backup status, if ‘Complete’, click the ‘Remove’ button, in confirmation window, click ‘ok’	Data tab
Browse a data set on the Genome Sequencer FLX Instrument file system	select data set in data set list, click the ‘Browse’ button	GS Sequencer application Data tab

5.1.4 GS Sequencer Configuration Actions

Intent	**Action**	**location**
Add a new Operator or Run Group	click on the ‘+’ button on the left and fill and in the required information fields on the right, click the ‘OK’ button	Operator or Run Group tab, GS Sequencer Configuration Tools
Edit an existing Operator or Run Group	click on the Operator or Run Group in the list and edit the appropriate information fields on the right, click the ‘OK’ button	Operator or Run Group tab, GS Sequencer Configuration Tools
Assign a default Run Group to an Operator	click on the Operator in the left list and select the appropriate Run Group from the pull-down menu in the right panel, click the ‘OK’ button	Operator tab, GS Sequencer Configuration Tools
Remove an Operator or Run Group	click on the Operator or Run Group item in the list and check the fields to verify it is the correct item to be removed, click the ‘-’ button	Operator or Run Group tab, Genome GS Configuration Tools

5.1.5 GS Run Browser Application

Intent	**Action**	**location**
View Run data	select data set by clicking the ‘Open a Data Set’ link to access a file navigation window or by clicking on the ‘Reopen Recent Data Set’ link to access a list of recently browsed data files	GS Run Browser entry page
Process Run Data	Type in a unique Run name in the Run Processor Manager Name field, select processing type, click the ‘start’ button	Run Processor Manager tool, GS Run Browser ‘Overview’ tab

5.2 Table of Figures

5.3 Glossary

C

Configuration; GS Sequencer application tool to manage Operators and Run Groups.

D

Data set; the set of files generated by Sequencing Run and Data Processing jobs. The Sequencing Run data set artifacts are located in the $RIGDATA directory under its R_ directory name. The data processing data set are stored under the D)_directories under their associated Run data set directories.

Data Processing Types; the series of digital images captured by the camera during the sequencing Run are processed to generate the sequence data in two steps: image processing and signal processing. Signal processing parameters differ for Amplicon libraries from other library types. The specification of which data processing step or steps to carry out on the Genome Sequencer FLX Instrument at the time of the sequencing Run and which type of library is being Run constitute the Data Processing type option. The choices are: None (no processing), Image Processing Only, Full Processing for Shotgun or Paired End, and Full Processing for Amplicon.

Data tab; GS Sequencer application tab lists the Run and Data processing data sets currently on the local instrument. There are an additional four actions to manage the data sets: copying (to a DataRig, a CPU for data analysis), removing data sets (that have been backed up), backup (to archive), and browsing sequencing Run data sets (launching the GS Run Browser). A context area to the right of the list displays summary information for the selected items in the data set list.

G

GS Run Browser; an application on the Genome Sequencer FLX Instrument or DataRig to view sequencing Run data and launch data processing jobs.

GS Run Processor Manager; tool in the GS Run Browser application to launch data processing jobs on the Genome Sequencer FLX Instrument or a DataRig.

GS Sequencer; an application used to set up and carry out sequencing Runs and other procedures, manage Operators and sequencing Run data sets, and monitor the instrument health.

Genome Sequencer FLX Instrument; an instrument to sequence DNA using the 454 Sequencing System. The instrument components include a PTP device for loading a bead-immobilized library of DNA fragments; a fiber optic bundle camera for capturing raw images of the PTP device during the sequencing Run; a fluidics system for delivering sequencing reagents to the wells of the PTP device during the Run; and a computer with software to control the Genome Sequencer FLX Instrument, carry out data processing, and store sequencing Run data sets.

Genome Sequencer FLX On-Instrument applications; systemStart, systemStop, GS Sequencer, GS Run Browser, GS Run Processor Manager (via the GS Run Browser)

I

Instrument Status Area; in the default application window of the GS Sequencer after login, contains Genome Sequencer FLX Instrument component sensor monitors. Components include; PTP Device & Loading Area, Camera, Reagent Cassette & Loading Area.

Instrument tab; GS Sequencer application tab, default screen after login, contains procedure summary information and Instrument status.

O

Operator; a person that uses the GS Sequencer application to configure and set up sequencing Runs, manage sequencing data sets, or browse sequencing Run data on the Genome Sequencer FLX Instrument.

Overview tab; GS Run Browser application tab, contains summary data of the sequencing Run, summary data of the processing results, if carried out, and the GS Run Processor Manager used to launch data processing or reprocessing jobs for the currently selected Run data set.

P

PicoTiterPlate (PTP) device; a device for loading a bead-immobilized library of DNA fragments for a sequencing Run on the Genome Sequencer FLX Instrument. Each device has a unique six digit ID stamped on its edge.

R

Raw image; the data captured during a sequencing Run from the Genome Sequencer FLX Instrument fiber optic bundle camera. Consists of images of the PTP device taken during each nucleotide flow, capturing the light released by the sequencing reaction in each well of the PTP device.

Run Wizard; an dialog launched from the GS Sequencer Start button to configure and launch a sequencing Run or other procedures. It consists of a series of questions used to provide the system with information about the Run. When all required Run information has been entered and the PTP device has been correctly loaded into the Genome Sequencer FLX Instrument, the sequencing Run can be launched by clicking on the ‘Start’ button in the last Run Wizard window. Required information: Procedure to carry out (Pre-wash, sequencing Run, or Service), PTP ID, Run name and Run Group, sequencing kit type (XLR70, LR70, SR70, or LR25), PTP device type (bead loading gasket; 2, 4, 8, or 16 regions), number of cycles to Run (cycles of 4 nucleotide flows; 100, 150, or 200), data processing type (see above), backup option.

S

Sequencing Run; the process of using a bead-immobilized DNA library loaded onto a PTP device, the 454 Sequencing method and the Genome Sequencer FLX Instrument to produce read data on a DNA sample from raw images of sequencing reactions as they occur on the PTP device.

systemStart; an application, located on the instrument desktop, used to start or reinitiate Genome Sequencer FLX Instrument operation background processes.

systemStop; an application, located on the instrument desktop, used to stop Genome Sequencer FLX Instrument operation background processes.

5.4 Index

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