Genome Sequencer FLX Operator's Manual

October 2008

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Preface

About this Manual

The *Genome Sequencer FLX Operator's Manual*:

- provides a quick overview of all the parts of the Genome Sequencer FLX System
- describes the hardware and software components of the Genome Sequencer FLX Instrument
- tells how to maintain the Genome Sequencer FLX Instrument
- gives guidelines for troubleshooting instrument problems
- includes a glossary of terms that are unique to the Genome Sequencer FLX System

Important Note: October 2008 marks the first release of the new GS FLX Titanium series chemistry for the Genome Sequencer FLX System. For the time being, the system supports only the non-MID "General" (e.g. Shotgun) sequencing applications under the GS FLX Titanium chemistry. For Paired End or Amplicon sequencing, or for the preparation and sequencing of MID libraries of any type, users must continue to use the GS FLX standard series kits and procedures (last updated in December 2007).

Note, however, that samples prepared using kits and procedures from the GS FLX Titanium series are still sequenced on the same Genome Sequencer FLX Instrument. Also, the Genome Sequencer FLX Software version 2.0, associated with the October 2008 release, can process datasets generated with any of the Genome Sequencer's chemistries (GS 20, GS FLX standard, and GS FLX Titanium). Indeed, the software version 2.0 can co-process reads produced on any combination of Genome Sequencer System chemistries (and even "Sanger reads") within a given analysis, such as in an Assembly, a Mapping, or an Amplicon Variant Analysis Project.

The Genome Sequencer FLX Titanium series manuals are easily identified by their new cover graphics and distinctive tri-color stripes (reflecting the GS FLX Titanium kits packaging). All the methods, protocols and applications supported on the GS FLX standard chemistry will be enabled on the GS FLX Titanium chemistry in the near future.

Incompatible chemistries: The two different chemistries of the Genome Sequencer System (GS FLX standard and GS FLX Titanium) are completely incompatible with each other. For example, standard libraries cannot be amplified using the GS FLX Titanium series emPCR kits, and libraries prepared/amplified with GS FLX Titanium series kits cannot be sequenced on the standard PicoTiterPlate devices, or vice-versa. **It is crucially important that kits and procedures belonging to a single chemistry platform be used throughout the preparation, amplification and sequencing of a DNA sample.**

In this manual, the phrase "Genome Sequencer System" refers to whole system for DNA sequencing developed by 454 Life Sciences Corp., including the Genome Sequencer Instrument, all the kits for the preparation, amplification and sequencing of a DNA sample, the methods to use the kits as described in the Manuals and Guides, and the software provided to process and analyze the data from sequencing Runs. Likewise, "Genome Sequencer FLX System" refers to a Genome Sequencer System based on the Genome Sequencer FLX Instrument (as opposed to the Genome Sequencer 20 Instrument, which is now retired). Two versions of the Genome Sequencer FLX System have been released: the GS FLX standard series, last updated in December 2007, and the GS FLX Titanium series. 454 Life Sciences Corporation is a Roche company.

Revision History

Version	Instrument Version	Software Version	Revision Date
FLX.01 – USM-00025.A	GS FLX	1.1.01	December 2006
FLX.02 – USM-00025.B	GS FLX	1.1.02	June 2007
FLX.03 – USM-00028.A	GS FLX	1.1.03	December 2007
FLX.Ti.00 – USM-00045.A	GS FLX	2.0.00	October 2008

 Questions or comments regarding the contents of this manual can be directed to your Roche Representative or to the customer support address below:

454 Life Sciences Corporation
1 Commercial St.
Branford, CT
USA 06405

Related Publications

A full suite of publications are available that describe in detail the components and usage of the Genome Sequencer System:

- *Genome Sequencer FLX Operator's Manual (October 2008; this manual)* – describes the Genome Sequencer FLX Instrument and provides information on its operation, maintenance, and troubleshooting.
- *Genome Sequencer FLX Titanium Applications and Methods Manual*, including:
  - *GS FLX Titanium General Library Preparation Method Manual* – describes how to use the GS FLX Titanium General Library Preparation Kit to prepare a DNA library suitable for sequencing with the Genome Sequencer System, *e.g.* for shotgun sequencing.
  - *GS FLX Titanium emPCR Method Manual* – describes how to use the GS FLX Titanium emPCR and GS FLX Titanium emPCR Breaking Kits to clonally amplify the DNA fragments from an appropriately prepared DNA library, in a bead-immobilized form suitable for sequencing with the Genome Sequencer System.
  - *GS FLX Titanium Sequencing Method Manual* – describes how to use the Genome Sequencer FLX Instrument in conjunction with the GS FLX Titanium Sequencing Kit XLR70 and GS FLX Titanium PicoTiterPlate Kit 70×75 to determine the sequence of a properly prepared and amplified DNA library.
  - A Quick Guide version of each method is also included.
- *Genome Sequencer Data Analysis Software Manual* – describes the data processing and data analysis software used to transform raw data from a sequencing Run, or a set of sequencing Runs, into the final output of the Genome Sequencer FLX System (presented specifically from the point of view of the off-instrument version of the software).
- *Genome Sequencer System Site Preparation Guide (October 2008)* - describes how to set up an optimal environment for the successful operation of the Genome Sequencer System, including the laboratory space and the computer/networking infrastructure. Installation is performed by a Roche Representative.
- *GS LIMS Implementation Guide (October 2008)* - describes how to implement the LIMS lookup feature of the Genome Sequencer System. This will allow users to input sample information and Run settings to the Genome Sequencer FLX Instrument via a Laboratory Information Management System (LIMS). (This guide is only available online via the Customer Restricted Access portion of the Genome Sequencer FLX web site, www.genome-sequencing.com.).

Note also that some of the applications of the Genome Sequencer FLX standard System (December 2007) are not yet available for the GS FLX Titanium chemistry. These include the preparation and usage of Paired End and Amplicon libraries, and the usage of Multiplex Identifiers (MIDs). For these applications, the Genome Sequencer FLX standard System methods and kits must still be used. The December 2007 GS FLX manual set comprises the following:

- *Genome Sequencer FLX Operator's Manual (December 2007)*
- *Genome Sequencer FLX System Methods Manual,* including:
  - *GS FLX Shotgun DNA Library Preparation Method Manual*
  - *GS FLX Paired End DNA Library Preparation Method Manual*
  - *GS FLX Amplicon DNA Library Preparation Method Manual*
  - *GS FLX emPCR Method Manual*
  - *GS FLX Sequencing Method Manual*
  - A Quick Guide version of each method is also included.
- *Genome Sequencer FLX Data Analysis Software Manual*
- *Genome Sequencer System Site Preparation Guide*

Prior to and during instrument setup, your Roche Representative will also provide system support services, including a discussion of clean laboratory installation and procedures, sample handling/tracking, and data management. These services will help ensure your success with the Genome Sequencer System. Please contact your Roche Representative for more details. (See below for contact information.)

All Genome Sequencer Manuals, Guides and Bulletins are available three ways: in hardcopy form, on a CD from your Roche representative, or downloaded from the customer-restricted access area of **www.genome-sequencing.com**.

Assistance

If you have questions or experience problems with the Genome Sequencer System, please call, write, fax, or e-mail us.

When calling for assistance, be prepared to provide the serial number of your Genome Sequencer Instrument and/or lot number of the kit(s) you are using. The instrument's serial number is located on the label found on the back of the instrument cart.

If you are located in…	Please contact Roche Applied Science Technical Support via:	
USA or Canada	phone: 1-800-262-4911 (toll-free)	e-mail: **us.gssupport@roche.com**
Europe, Middle East, Asia Pacific, Mexico, South America or Africa	phone: +49-8856-60-6457 or toll-free +800SEQUENCE	e-mail: **service.sequencing@roche.com**
Japan	phone: +03-5443-5287	e-mail: **tokyo.biochemicals@roche.com**

Warranty

Information on warranty conditions are specified in the sales contract. Contact your Roche Representative for further information. Any unauthorized modification of the instrument entails invalidation of the guarantee and service contract.

Intended Use of the Genome Sequencer FLX Instrument

The Genome Sequencer FLX Instrument is designed to sequence clonally amplified deoxyribonucleic acid (DNA) fragments, which are derived from double-stranded sample DNA of various origins. The instrument can sequence *de novo* or re-sequence an average of 360 to 560 million bases per sequencing Run.

The Genome Sequencer FLX Instrument is intended for life science research applications and must be used exclusively by laboratory professionals who are trained in the correct and safe operation of this instrument and its accessories, are familiar with general laboratory techniques, and have studied the instructions for use of this instrument.

Notice to Purchaser

RESTRICTION ON USE: Purchaser is only authorized to use the Genome Sequencer Instrument with PicoTiterPlate devices supplied by 454 Life Sciences Corporation and in conformity with the operating procedures contained in the Genome Sequencer System manuals and guides.

Made in USA by 454 Life Sciences Corporation, Branford, CT, USA, a Roche company.

For life science research only. Not for use in diagnostic procedures.

Open Source Software

The Genome Sequencer FLX System of Roche Diagnostics Ltd. uses open source software. Among other things, the holders of the proprietary rights grant licenses under the terms of the GNU General Public License (GPL edition 2 or above), as well as under the GNU Lesser General Public License (LGPL).

This Genome Sequencer FLX Instrument was designed to be operated with the unmodified software, as shipped. The user assumes full responsibility for changing any part of the open source software, which excludes any liability of Roche Diagnostics Ltd. The following disclaimer shall be valid for all parts of the software that are liable to GPL:

This program is distributed *without any warranty*; without even the implied warranty of *merchantability or fitness for a particular purpose*. See the GNU General Public License for more details (www.gnu.org/copyleft/gpl.html).

Trademarks

454, 454 LIFE SCIENCES, 454 SEQUENCING, GS FLX TITANIUM, emPCR, PICOTITERPLATE, and PTP are trademarks of Roche.

Other brands or product names are trademarks of their respective holders.

Declaration of Conformity

The Genome Sequencer FLX Instrument has been manufactured and checked in accordance with all relevant safety standards prior to leaving the factory. The instrument has been approved for use by recognized testing institutions. Conformity to recognized standards is indicated by these symbols:

CE	The instrument meets the requirements stated in Council Directive 89/336/EEC relating to "Electromagnetic Compatibility" and Council Directive 73/23/EEC relating to "Low Voltage Equipment".
TÜV Rheinland of North America, Inc. C US	The instrument meets the requirements of NRTL standards UL61010, CAN/CSA C22.2 No. 61010-1.

The following symbols appear on the instrument:

CE	CE MARK	The CE mark on the instrument type plate expresses conformity with essential requirements of the directive relevant for this instrument.
TÜV Rheinland of North America, Inc. C US	cTUV MARKus	The cTUV MARKus on the instrument type plate expresses that the product meets U.S. and Canadian safety requirements for electrical equipment for measurement, control, and laboratory use.

Instrument Modifications: Any change or modifications made to the Genome Sequencer FLX Instrument, unless expressly approved in advance, in writing, by 454 Life Sciences Corporation, could void the warranty or maintenance agreement, either in part or in full.

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. However, this equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the present Operator's Manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case users will be required to correct the interference at their own expense.

Safety

Only trained personnel may use the Genome Sequencer FLX Instrument. It is essential that all users understand and observe the following safety information pertaining to installation and operation of the Genome Sequencer FLX Instrument. Please ensure that all safety information is accessible to every employee working with the Genome Sequencer FLX Instrument.

Normal Operation

During normal operation, no one should ever need to open any service panels on the Genome Sequencer FLX Instrument. If, during the operation of the instrument, any of the components requires inspection, please contact your Roche Representative.

Icons

Make sure to follow the precautionary statements presented in this manual. Such statements and other items of special interest are highlighted with the following icons:

Symbol	Heading	Description
	Warning	Indicates the possibility of severe or fatal injury to the user or other persons, or damage to a system component, if the precautions or instructions are not observed.
	Caution	Highlights information that is critical for optimal performance of the system. May also indicate that loss of data or invalid data could occur if the precautions or instructions are not observed.
	Information note	Identifies items of general interest and additional information about the topic or procedure being described.
▶▶▶		Table continued on next page.
■		End of table.

Specific Warnings

The following Warnings apply to the operation and regular (*i.e.* performed by the user) maintenance of the Genome Sequencer FLX Instrument:

- **Dangerous voltage:** Dangerous voltages exist within the Genome Sequencer FLX Instrument. Users should not remove any service panels on either the top unit or the cabinet of the Genome Sequencer FLX Instrument. Further:
  - If maintenance is being performed, do not touch any exposed PC boards or circuits.
  - Do not touch any electrical connections on the PC Boards or behind the camera.
  - Users should not attempt any maintenance or service procedures. Should the Genome Sequencer FLX Instrument require service, please contact your Roche Representative.

  Failure to heed these warnings could cause personnel to come in contact with voltages that could cause severe injury or death.

- **Delicate optical components:** The optics subsystem contains components that are very precise, delicate, and expensive. Exercise great care in handling and operating any component of the optics subsystem.

- **Constantly running fans:** The camera is air-cooled by a fan (at the back of the instrument) that runs continuously. Only trained and approved service personnel should disconnect or work on any of the camera subsystem components.
  - Never attempt to put your fingers into the air inlet/outlets in the instrument panels.
  - Never attempt to insert any instrument, like scissors or screwdrivers, into the inlet/outlets in the instrument panels.
  - If the fan stops or it sounds like something is caught in the fan, contact your Roche Representative immediately.

- **Camera face:** Always be extremely careful when working near the camera face. Never touch the camera face with anything other than lens paper. DO NOT USE KIMWIPES OR PAPER TOWELS TO CLEAN THE CAMERA FACE.

- **Liquid spills:** Take care of any spills near or around the camera immediately. Call your Roche Representative immediately if you suspect that fluid has gotten behind or underneath the camera.

- **Camera door:** Do not open or exert any pressure on the camera door while the camera and/or the fluidics are operating. Do not apply excess pressure to the camera face (beyond normal closing of the camera door). Do not disassemble the camera door. If service is required, contact your Roche Representative.

Specific Cautions

The following Cautions apply to the operation and regular (*i.e.* performed by the user) maintenance of the Genome Sequencer FLX Instrument:

- **Computer networking:** Connection to computer networks contains an inherent risk of infection by viruses and worms, as well as malicious targeted attacks through the network. You MUST protect and continually update the protection of any network to which you choose to connect the Genome Sequencer FLX Instrument or any data processing computer ('DataRig' or 'Cluster'). Precautionary measures should include installing a dedicated firewall to separate the instrument network from uncontrolled networks, as well as measures to ensure that the instrument network is (and will remain) free of malicious code. Failure to heed this warning may result in irreparable damage to your data and the Genome Sequencer FLX Instrument.

- **Fluidics subsystem:** Be careful when working with the fluidics subsystem. Specifically:
  - NEVER let the reagent inlet tubing lines of the sipper manifold (Sipper Tubes) touch any instrument surfaces.
  - Be careful not to touch the Sipper Tubes while performing normal procedures on the Genome Sequencer FLX Instrument.
  - Keep the sipper assembly in the lowered position with each Sipper Tube in its appropriate reagent container or Pre-wash Tube (unless changing reagents or Sipper Tubes).
  - Always empty the Reagents Cassette waste compartment before a Run, to avoid overflow spills.
  - Replace the Sipper Tubes before every Run. Do not disassemble any other reagent tubing or connectors of the fluidics subsystem.
  - Sipper Tube filters contain poly(vinyl chloride) (PVC); recycle these filters in accordance with local regulations.

Specifications of the Genome Sequencer FLX Instrument

General specifications of the Genome Sequencer FLX Instrument are summarized below.

Dimensions upper assembly	74.3 cm × 69.8 cm × 36.1 cm (29.25" × 27.5" × 14.2") (W × D × H), incl. monitor 82.5 cm (32.5") H
Dimensions lower assembly	75.2 cm × 90.8 cm × 92.7 cm (29.62" × 35.75" × 36.5") (W × D × H)
Weight	242 kg (532 lbs)
Power supply	120VAC (94VAC – 133VAC) 50/60Hz 1250VA 230VAC (187VAC – 264VAC) 50/60Hz 1250VA 100VAC (85VAC – 120VAC) 60Hz 1000VA (Japan) (Values in parentheses indicate operational voltage range.) (Les valeurs entre parenthèses correspondent à la marge d'opération.)
Noise level	< 65 dB(A)
Electromagnetic emission	Class A Important: This is a Class A device. In residential areas, this device may cause radio interference. The user should take the necessary precautions, if appropriate.
Electromagnetic immunity	This device meets the industrial immunity requirements with no degradation of performance (10V/m, Criterion A).

Genome Sequencer System Site Requirements

Ambient temperature	15ºC – 30ºC (59ºF – 86ºF) – air conditioning recommended
Ambient humidity	20% – 80% (non-condensing)
Ambient light	< 2000 lux

For detailed information on site requirements, see the *Genome Sequencer System Site Preparation Guide*.

Disposal of the Instrument

Dispose of the instrument according to local and/or labor regulations.

This product complies with the Waste Electrical and Electronic Equipment (WEEE) Directive (2002/96/EC) marking requirement. The affixed product label (see below) indicates that you must not discard this electrical/electronic product in domestic household waste.

Product Category: With reference to the equipment types in WEEE directive Annex 1, this product is classified as "Monitoring and Control instrumentation". Do not discard it in domestic household waste.

To return unwanted products, contact your Roche Representative (see "Assistance", above).

1. Introduction to the Genome Sequencer FLX System

The Genome Sequencer FLX System is an automated DNA sequencing system capable of preparing, amplifying and sequencing a library of DNA fragments in a massively parallel fashion. The system provides most of the components necessary for ultra-high throughput sequencing experiments, including the Genome Sequencer FLX Instrument and accessories; software to generate basecalls and interpret the raw reads; and reagent kits required for library construction, clonal amplification, and sequencing.

The *Genome Sequencer FLX Operator's Manual* provides a general overview of the Genome Sequencer FLX System, and then focuses on how to operate, maintain and troubleshoot the Genome Sequencer FLX Instrument. This section describes:

- Section 1.1: General Four-Step Workflow of the Genome Sequencer FLX System
- Section 1.2: Instrument Components
- Section 1.3: Overview of the On-Instrument Software Applications
- Section 1.4: Overview of a Sequencing Run
- Section 1.5: Overview of Data Processing in the Genome Sequencer FLX System

1.1 General Four-Step Workflow of the Genome Sequencer FLX System

Figure 1–1 shows an overview of the four-step workflow of the entire Genome Sequencer FLX System. Briefly, the four steps are:

1. **Preparing a DNA Library:** The DNA sample must be transformed into a library of DNA fragments appropriate for sequencing with the Genome Sequencer FLX System. The method for preparing this DNA library varies according to the type of sample and the objective of the experiment, but always includes the modification of each fragment to include special sequences that will be needed in later workflow steps.
2. **Amplifying the Library:** Fragments from the DNA library are immobilized onto microparticles (beads) with each bead carrying no more than one amplifiable DNA molecule. The entire bead-bound library is then emulsified with the amplification reagents, such that each bead is captured within its own microreactor for the amplification of a single (clonal) DNA fragment. Amplification is carried out in bulk, resulting in beads that are each covered with tens of millions of copies of a single DNA fragment; each bead contains a different fragment.
3. **Sequencing the Library:** After amplification, the DNA-carrying beads are loaded into the wells of a PicoTiterPlate device (PTP device) such that the wells contain no more than a single DNA bead. The loaded PTP device is then inserted into the Genome Sequencer FLX Instrument, and sequencing reagents are sequentially flowed over the plate. The Genome Sequencer FLX Instrument automatically performs and monitors the sequencing reactions in all the wells of the PTP device simultaneously.
4. **Analyzing the Data:** The raw output of a sequencing Run consists of a set of digital images (PIF files) from which the sequence of the DNA library fragments ("reads") can be determined and then subjected to sophisticated analysis, according to the type of library and the objective of the experiment.

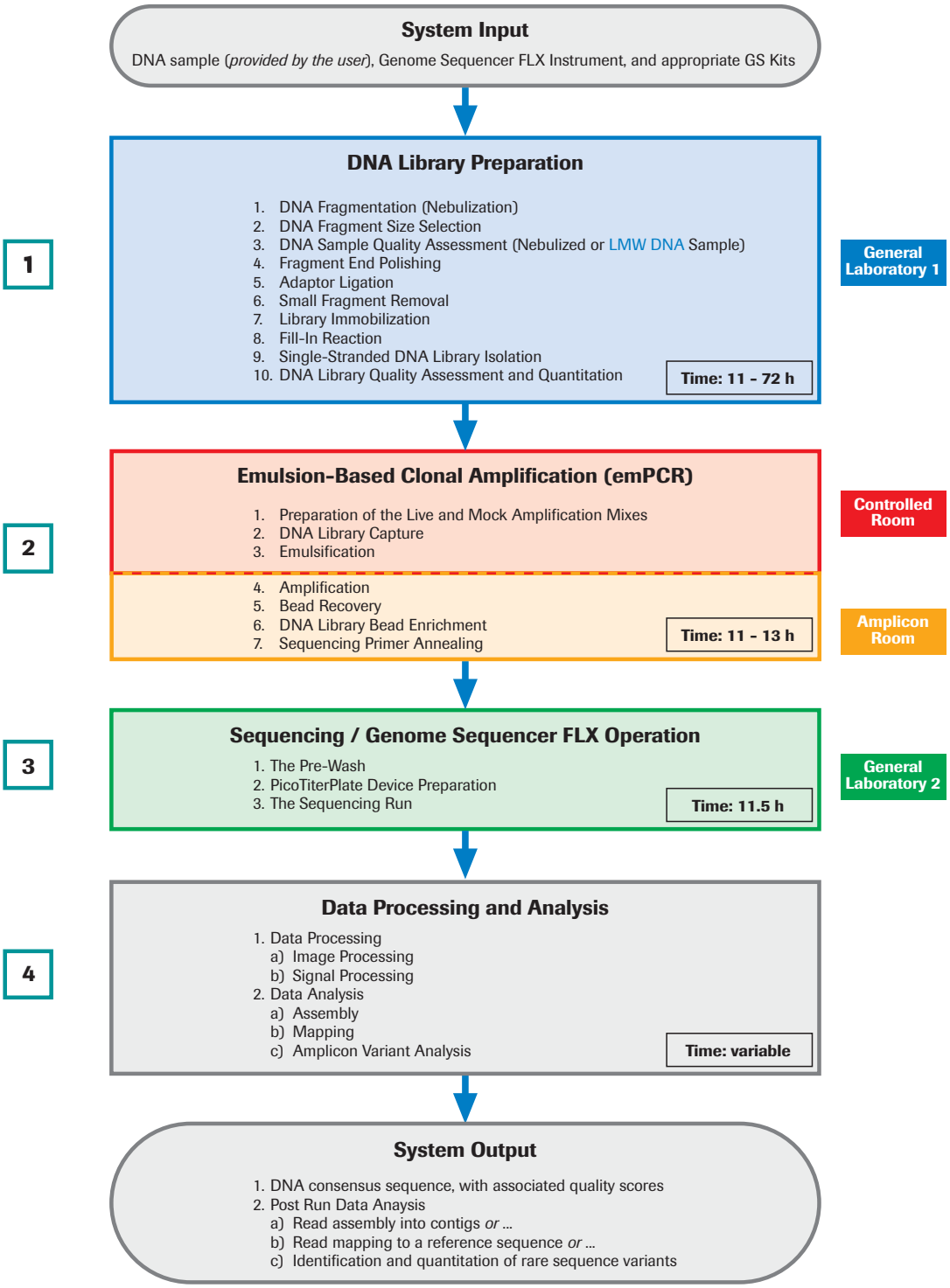


Figure 1–1: The general four-step workflow for the Genome Sequencer FLX System

1.2 Instrument Components

1.2.1 Hardware Components

1.2.1.1 Main Components of the Genome Sequencer FLX Instrument

The main components of the Genome Sequencer FLX Instrument are an optics subsystem and a fluidics subsystem which occupy the left- and right-hand sides of the instrument, respectively. Both systems are controlled by a computer subsystem, which is located in the cart (Figure 1–2).

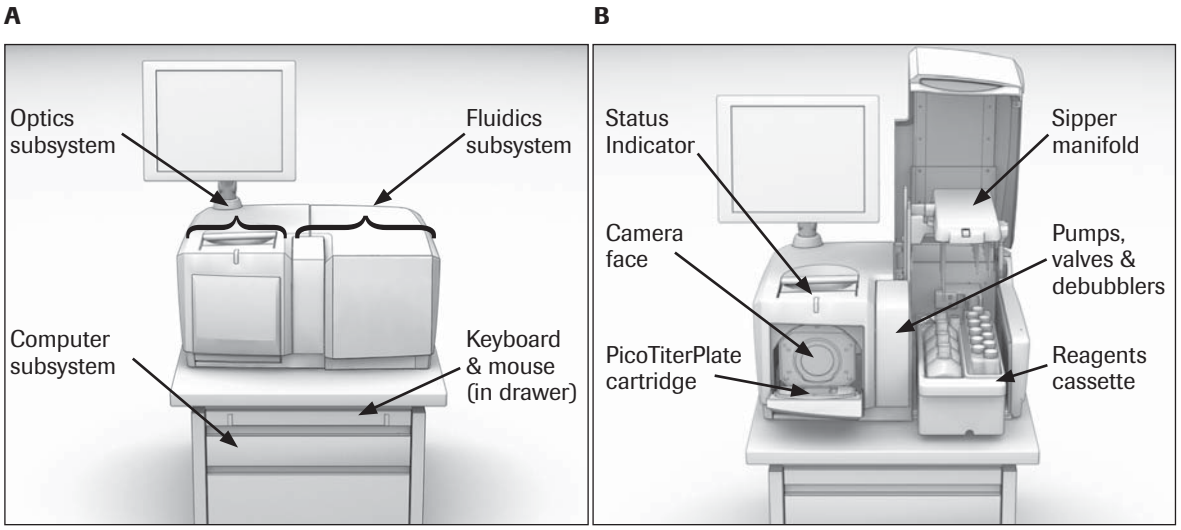


Figure 1–2: Main components of the Genome Sequencer FLX Instrument

(A) General view of the instrument with all doors closed, showing the location of the main subsystems (B) General view of the instrument with the camera door and fluidics door open, showing the sipper manifold in the raised position and the Reagents Cassette pulled out halfway

- The **fluidics subsystem** moves the sequencing reagents across the wells of a PicoTiterPlate device (PTP device), and then moves spent reagents from the PTP device to the waste receptacle. The fluidics subsystem includes a Reagents cassette, which holds the reagent containers and doubles as a waste container; a reagent sipper tube assembly and reagent tubing; a 16-position valve manifold; 2 main peristaltic pumps; 2 debubblers with secondary "purging" pumps; a heated PTP cartridge holder (in the camera door); and effluent tubing leading to the waste container (Figure 1–2**B**).
- The **optics subsystem** includes a cooled 16 megapixel CCD camera and a camera controller. The camera captures the light emitted from the wells of the PTP device during each step of the sequencing cycle, and sends the digital images to the computer subsystem for processing.
- The **computer subsystem** includes a keyboard, mouse, main CPU (with Linux operating system), and disk drive (all located in the cart), plus a post-mounted LCD monitor. The computer controls the other Instrument subsystems, and processes the digital images sent by the camera to extract the DNA sequence information.
- Another visible component of the instrument is a Status Indicator LED (above the camera door, Figure 1–2**B**), which shows at a glance the general status of the instrument (For details on the function of this LED, see Table 4–1 in section 4.2).
- Finally, the Genome Sequencer FLX Instrument includes an Uninterruptible Power Supply, which will keep the instrument operating normally for a few minutes in the event of a momentary power failure (or if the instrument is unplugged).

1.2.1.2 Separate Computer Cluster

The GS FLX Titanium cluster is offered as a convenient, cost-effective, plug-and-play solution for the data processing needs of sequencing Runs performed with the GS FLX Titanium chemistry. While optional, the cluster provides a turn-key method of processing the raw data from the GS FLX instrument at the same rate as the instrument can generate it. The cluster is matched to the GS FLX and allows for maximum sequencing throughput with minimal investment in Information Technology.

The GS FLX Titanium cluster offers the following added benefits:

- Multi processor architecture
- Optimized memory configuration for maximum performance
- High speed disk access optimized for GS FLX data processing tasks
- Installation and testing of all GS FLX applications and tools
- Data and power redundancy configured for improved reliability

1.2.1.3 How the Hardware Components Work

During a sequencing Run, a PTP device, containing the DNA being sequenced, constitutes the interface between the fluidics and optics subsystems (Figure 1–3). The side of the PTP device that is in contact with the fluidics subsystem contains microscopic (18.5 picoliter) wells in which the sequencing reactions take place. Each well is designed to contain a single, unique library bead carrying a clonally amplified DNA fragment. The bottom of each well is made of an optical fiber, which transmits light produced by the sequencing reaction across the thickness of the PTP device, to the camera (optics subsystem) and each well wall is lined with a metalized finish to reduce well-to-well crosstalk and signal interference.

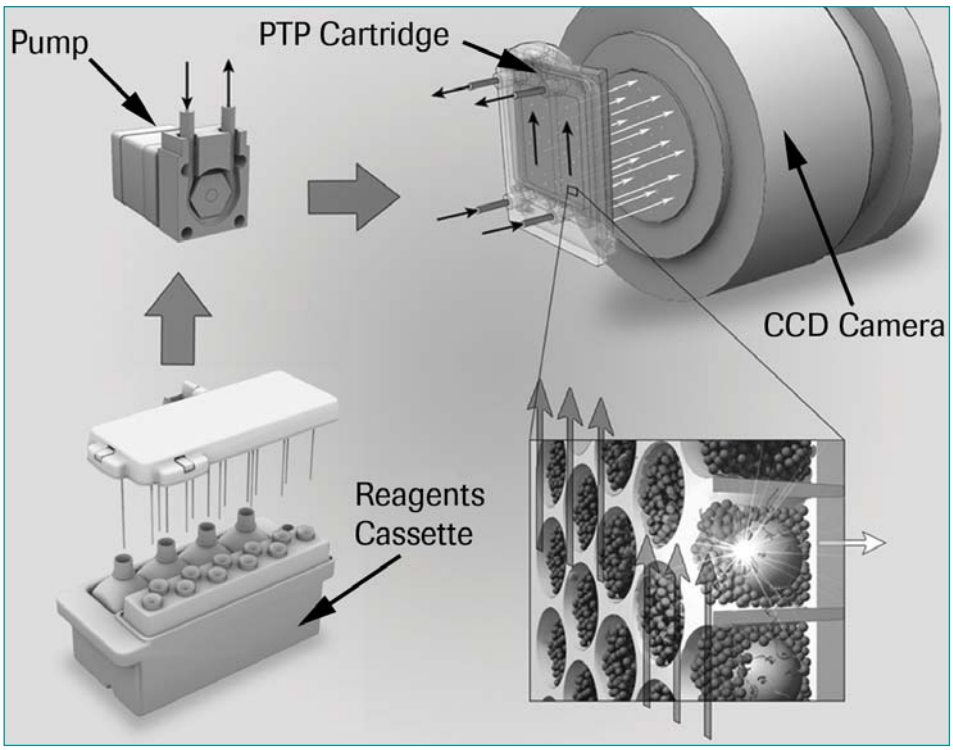


Figure 1–3: Schematic showing how the Genome Sequencer FLX Instrument works

Sequencing reagents are pumped from the Reagents cassette to the PicoTiterPlate (PTP) cartridge (reagent selection is done through a set of valves, not shown). Reagents flow across the surface of the PTP device into the reaction wells, while spent reagents flow back to the waste container (in the hollow Reagents cassette). The light generated by the sequencing reaction (white arrows) travels through the back of the PTP device (which is constructed of optical fibers), to reach the CCD camera. Inset: Each well contains no more than one large DNA bead (sample), which is surrounded by smaller beads that carry the enzymes required for the chemiluminescence reaction.

1.2.1.4 Accessories

The Genome Sequencer FLX Instrument comes with a set of accessories used to handle and process the biological (DNA) sample and the sequencing reagents (Table 1–1). The purpose and usage of these accessories are described elsewhere in this manual and in the relevant GS FLX Method Manuals.

Quantity	Item Description
1	Data Cable CAT5E RJ-45M to RJ-45M
1	Power Cord, 6'7" unshielded 16AWG
1	PicoTiterPlate Cartridge 70×75 mm
1	PicoTiterPlate Cartridge 25×75 mm (with flow restriction device)
2	PicoTiterPlate Cartridge Bypass Tube
1	Camera Faceplate Guard
pair	emPCR Shaker Adaptors LV
1	70×75 Bead Deposition Device (2 large regions)
1	70×75 Bead Deposition Device (4 medium regions)
1	70×75 Bead Deposition Device (8 medium/small regions)
1	70×75 Bead Deposition Device (16 small regions)
1	25×75 Bead Deposition Device (1 medium region)
1	25×75 Bead Deposition Device (4 small regions)
1	Counterweight for Bead Deposition Devices
2	GS FLX Reagents Cassette
1	GS FLX Pre-wash Tube Holder
1	Nebulizer Holder
2	Swin-Lok Filter Holder
5	16 gauge blunt, flat tip needles
5	Plastic forceps
1	Zeiss wipes, box of 21

Table 1–1: Contents of the Genome Sequencer FLX Accessory set provided with the instrument

1.2.1.5 Other Hardware/Installation Components Not Supplied

In addition to the Genome Sequencer Instrument and accessories, some parts of the sample preparation or data processing procedures require additional equipment or installations, such as:

- Segregated laboratory areas for library preparation, for the preparation of library amplification reactions, and for the recovery of the amplified library
  - For details on the recommended installations, see the *Genome Sequencer System Site Preparation Guide*.
  - For more information on the recommended general laboratory workflow, see the various Genome Sequencer FLX System's *Method Manuals*.
- Various pieces of standard and specialized laboratory equipment
  - For details, see the relevant GS FLX *Method Manuals*.
- A Linux-based computer (termed a 'DataRig') or computational cluster loaded with the Genome Sequencer FLX System data processing and data analysis software. Depending on the desired sequencing throughput, none, some or all of the raw data processing can be performed on the instrument itself. The remainder of the processing can be performed on the 'DataRig' or cluster. Note, however, that performing all processing on-board the instrument could be extremely time consuming, especially for a sequencing Run performed using the GS FLX Titanium chemistry. In all cases, data analysis (Assembly, Mapping, or Amplicon Variant Analysis) must be performed on an independent (although not necessarily dedicated) Linux computer. For maximum throughput, a turn-key GS FLX Titanium computer cluster is offered that provides convenient data processing capabilities matched to the performance of the Genome Sequencer FLX Instrument and the GS FLX Titanium chemistry.
  - For more details on DataRig function and usage, see the *Genome Sequencer Data Analysis Software Manual*; for DataRig and computer cluster specifications, see the *Genome Sequencer System Site Preparation Guide*.

1.2.2 Software Components

The Genome Sequencer FLX System includes all the software required to perform sequencing experiments and fully process the resulting data. The functions of this software include:

- controlling the instrument for all types of Runs (pre-wash, sequencing, and maintenance)
- processing the raw images data into raw sequence reads
- assembling the raw reads into contigs and scaffolds
- mapping the raw reads to a reference sequence
- detecting, identifying and quantitating DNA Variants
- managing data files
- performing operations useful in maintenance and troubleshooting
- performing instrument self-diagnostic tests
- system administrative functions

1.2.3 General Consumables Not Supplied

Runs on the Genome Sequencer FLX Instrument require various consumables that are not supplied.

- For details, see the *GS FLX Titanium Sequencing Method Manual*.

1.3 Overview of the On-Instrument Software Applications

Individual functions of the Genome Sequencer FLX Instrument control software are packaged into distinct applications, each invoked when the Operator double-clicks the appropriate icon on the computer desktop (Figure 1–4).

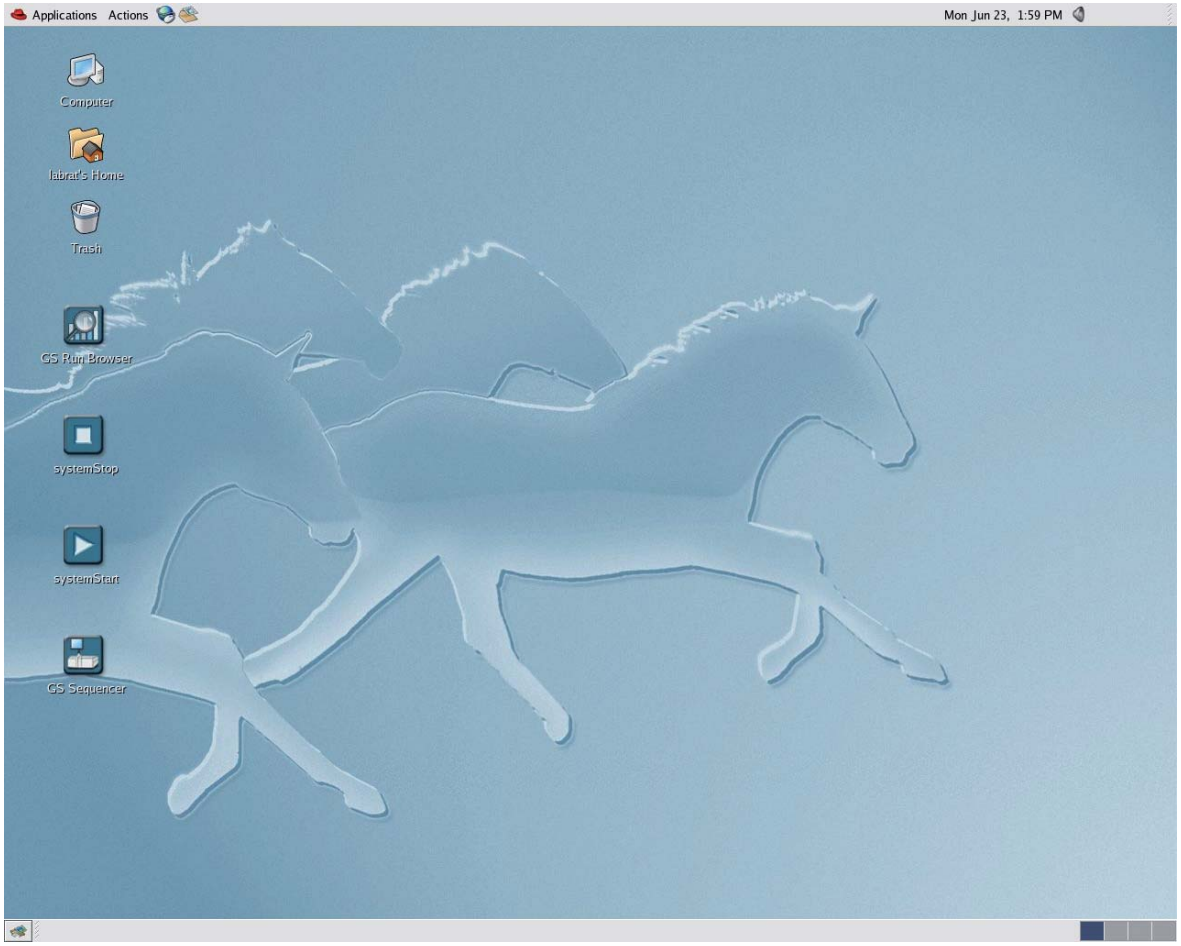


Figure 1–4: The Genome Sequencer FLX Instrument main screen, showing the Operator applications launch icons

During a Run, Operators interact with the instrument through the GS Sequencer application (see section 2.3 for details). Section 2 of this manual describes how to use all the available software features.

1.4 Overview of a Sequencing Run

1.4.1 Continuous Instrument Operation

The Genome Sequencer FLX Instrument is designed for continuous operation, except when it needs scheduled maintenance (as described in section 3.7 of this Manual). Under normal circumstances, Operators do not perform any shutdown procedures. All Run scripts proceed autonomously, and end with a fluidics maintenance wash, so the instrument is automatically prepared for the next Run. Therefore, the Operator will usually leave the instrument untouched after the Run script finishes. This includes leaving the used PTP device in place in the cartridge since the pre-wash, which must be carried out before each sequencing Run, requires that a used PTP device be in place (see the *GS FLX Titanium Sequencing Method Manual* for details).

For these reasons, in normal practice, the removal of the used PTP device and reagents, and the clean up of the instrument before the following Run, are the responsibility of the next Operator. This way, the hands-on task of performing a sequencing experiment on the Genome Sequencer FLX Instrument starts with "closing the previous Run" and ends as soon as the new sequencing Run script is launched.

If the Genome Sequencer FLX Instrument is turned off for any reason, it must be re-started using the *System Start* application. For more information, see section 2.1 in this manual.

1.4.2 Before You Begin a Sequencing Run

Before starting an experiment, make sure the following requirements are met.

1.4.2.1 State of the Instrument

As indicated above, the Genome Sequencer FLX Instrument is designed for continuous operation, so it is ready for use as soon as a Run completes. The Status Indicator LED located above the camera door shows at a glance what the Sequencer is doing (see Table 4–1). Before starting a new Run, therefore, make sure that the previous Run is complete and that the instrument is in the following condition:

- On the computer screen, the GS Sequencer application window is open.
  - For screenshots of the GS Sequencer application window, see section 2.3.
- The sipper manifold is in the lower position and the tips of all Sipper Tubes are submerged in their respective reagent containers, in the Reagents cassette.
  - For diagrams, see Figure 1–2 and section 3.1.
- The camera door is closed, with the spent PTP device and cartridge seal in place in the PTP cartridge.
  - For diagrams, see section 3.2.
- The Status Indicator LED is solid green.
  - If blinking yellow or solid red, see section 4 for troubleshooting information.

If the instrument is not in this state and the Operator cannot verify that it has been properly cleaned, the maintenance wash procedure, described in section 3.3, should be performed before a sequencing Run is started.

1.4.2.2 What You Should Already Have

Before starting the sequencing Run, make sure that the following are available:

- **Sample:** A properly prepared, clonally amplified, bead-immobilized DNA library [General or Shotgun (sstDNA) Paired End, or Amplicon library]
  - For more information, see the appropriate *GS FLX (Titanium) Library Preparation Method Manual* [for General Titanium, Shotgun (sstDNA), Paired End, or Amplicon libraries], and the *GS FLX (Titanium) emPCR Method Manual*.

    Only non-MID General DNA libraries are currently supported by the GS FLX Titanium chemistry. For preparing and sequencing Paired End or Amplicon libraries, or for MID libraries of any type, users **MUST** continue to use the standard Genome Sequencer FLX kits, manuals, and procedures (last updated in December 2007).

- **Run Script and Data Analysis File:** As part of the experiment set-up, an appropriate Run script and a Data Analysis scheme must be chosen.
  - For more information, see the *GS FLX (Titanium) Sequencing Method Manual*.
- **Non-consumable accessories for the instrument:** Table 1–1 lists the accessories supplied with the Genome Sequencer FLX Instrument. Most of them will be needed for the sequencing Run, including one of the PTP cartridges and one of the Bead Deposition Devices (as appropriate for the specific requirements of the experiment).
- **One GS Sequencing Kit and one GS PicoTiterPlate Kit:** These kits contain the reagents and other materials needed for a sequencing Run.
  - For more information on kit contents and their use, see the *GS FLX (Titanium) Sequencing Method Manual*.
- **Various laboratory equipment and supplies:** Various items needed to process the sample and load it on the PTP device.
  - For detailed information, see the *GS FLX (Titanium) Sequencing Method Manual*.

Run scripts and data analysis configuration files are provided by 454 Life Sciences, a Roche company, and come preinstalled on the Genome Sequencer FLX Instrument. Run scripts specify the whole sequence of reagent flows during the Run; while data analysis configuration files set parameters for how the Run data will be processed, yielding final sequencing file outputs.

Run scripts: All Run scripts are written by 454 Life Sciences, a Roche company. Users should never attempt to modify a Genome Sequencer FLX Instrument Run program. For more information on Run scripts, please contact your Roche Representative.

1.5 Overview of Data Processing in the Genome Sequencer FLX System

For a detailed description of data processing and data analysis in the Genome Sequencer FLX System, see the *Genome Sequencer Data Analysis Software Manual*.

The raw output of a sequencing Run is a set of digital images (PIF files) and associated meta-data files which are used to determine the sequence of the DNA library fragments ("reads"). The GS Run Processor application can be evoked on the Genome Sequencer FLX Instrument as part of the sequencing Run, or on a separate computer ('DataRig') following the Run. The processing consists of a series of automatic data correction steps that compensate for optical effects and chemical inefficiencies. The compensated reads are then passed through a series of tunable quality filters whereby low quality reads are identified and segregated. Finally, the high quality reads are subjected to an algorithm that converts the signal intensities to individual bases and quality scores. The raw data, intermediate processing results, meta-data and metrics data are stored in an archive format. From these composite files, a selection of reports and other artifacts (such as common FASTA files) about the sequencing Run can be generated.

The outputs from one or more sequencing Runs are then further analyzed (always on a separate computer and independently from the Runs) according to the type of library and the objective of the experiment:

- Reads from General (*e.g.* shotgun) libraries made from a high molecular weight DNA sample (*e.g.* genomic DNA) can be either assembled into a consensus sequence using the *GS De Novo* Assembler software, or mapped against a reference sequence using the GS Reference Mapper software. The final consensus sequence is output as a set of FASTA files, with an associated basecall quality score file. The system output also includes:
  - Standard Flowgram Format (SFF) files,
  - files to help visualize the sequence assembly or how the reads were mapped against the reference sequence (ACE format),
  - lists of differences between the consensus reads and the reference sequence(s), and
  - various metrics files.

  The sequencing output of General libraries made from low molecular weight DNA usually does not require processing beyond raw reads.

- Reads from Paired End libraries are normally analyzed in conjunction with those from a shotgun library (made from the same DNA sample), using the *GS De Novo* Assembler software: Paired End reads are used to order and orient the contigs generated by the shotgun sequencing Run(s). The output is similar to the one described above, but also contains contig scaffolding information (in AGP format).

- Reads from Amplicon libraries are analyzed completely differently. A special software application named the GS Amplicon Variant Analyzer aligns the reads against a defined target reference sequence. From the alignment, known and novel Variants are identified, quantitated, and then presented both in tabular form and in Variation Frequency Plots.

  - Amplicon libraries can also be made from pre-cloned DNA fragments of unknown sequence that have known flanking sequences, such as libraries of microRNA or small sequence tags. However, the Genome Sequencer System currently provides no special analysis software for such applications, so the system output would just be comprised of raw reads.
  - Only non-MID General DNA libraries are currently supported by the GS FLX Titanium chemistry. For preparing and sequencing Paired End or Amplicon libraries, or for MID libraries of any type, users **MUST** continue to use the standard Genome Sequencer FLX kits, manuals, and procedures (last updated in December 2007).

2. Genome Sequencer FLX Instrument Software Applications

Table 2–1 lists all the software applications available to Operators of the Genome Sequencer FLX Instrument. These applications are used to set up and control Runs, to manage the instrument and system, or to perform special satellite tasks. The various tasks supported by each application are described in the sections indicated.

ICON	APPLICATION	FUNCTION	See Section
	**System Start**	▶ Starts the Genome Sequencer FLX Instrument's background processes [a]	2.1
	**System Stop**	▶ Stops the Genome Sequencer FLX Instrument's background processes [b]	2.2
	**GS Sequencer**	▶ Set up and management of a Run ▶ Data management ▶ Instrument monitoring	2.3
	**GS Run Browser**	▶ Post-Run diagnostics ▶ User-friendly access to data metrics output	2.4

Table 2–1: Applications available to Operators of the Genome Sequencer FLX Instrument

[a] Normally, the Genome Sequencer FLX Instrument is not turned off. If it is turned off for any reason, it must be re-started using the System Startup application.

[b] Under normal circumstances, the Genome Sequencer FLX Instrument software should be left running at all times.

Advanced data analysis software is also provided with the Genome Sequencer FLX System. These applications are installed on a separate computer, or computing cluster, and used on the data resulting from one or more sequencing Runs; they are described in the *Genome Sequencer Data Analysis Software Manual*. This section focuses on the software applications that reside on the Genome Sequencer FLX Instrument itself.

/data disk partition: The /data partition should never be exported or otherwise made available on a network; it is necessary to ensure that the I/O system can be guaranteed to be available to the system while a Run is ongoing. Exporting the partition can result in a situation where an external user is accessing the drive and possibly performing an analysis, resulting in a lot of disk activity while the instrument is trying to perform its functions in a timely manner.

2.1 System Start: Starting the Genome Sequencer FLX Instrument Software

The System Start application initiates all the background processes for the Genome Sequencer FLX System software. It is launched by double-clicking the *systemStart* icon (Figure 2–1). This executes a shell script which controls the proper sequence of events for starting the software processes.

If System Start is launched while software is already running, it will shut down the active processes before re-initiating, and then automatically restart the software. After the System Start routine is complete, all sequencer operations will be available to the Operators when they start the GS Sequencer application.

1. Launch the System Start application by double-clicking the *systemStart* desktop icon (Figure 2–1).

Figure 2–1: The *systemStart* desktop icon, used to launch the System Start application

2. The "Initializing 454 Software" window will open (Figure 2–2), which automatically launches all the background processes necessary to perform sequencing experiments or other operations.

   ▶ When the system processes have fully started up, the "Initializing 454 Software" window will close automatically.

   Do NOT close the "Initializing 454 Software" window while this operation is in progress.

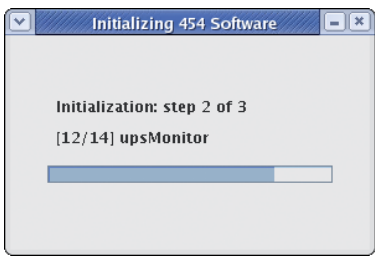


Figure 2–2: The "Initializing 454 Software" window of the System Start application.
The window is shown while the System Start application is launching the background processes.

2.2 System Stop: Turning Off the Genome Sequencer FLX Instrument Software

Under normal circumstances, the Genome Sequencer FLX Instrument software should be left running at all times. If necessary, however, the software can be shut down by running the System Stop application. If the GS Sequencer application is running, you must first exit it by clicking the Exit button found in the Global Action area (see section 2.3.1). Then, double-click the *systemStop* icon to execute a shell script which initiates an orderly shutdown of the background instrument software.

1. Launch the System Stop application by double-clicking the *systemStop* desktop icon (Figure 2–3).

Figure 2–3: The *systemStop* desktop icon, used to launch the System Stop application

2. A shell window will open (not shown), which stops all the background processes of the instrument.

   ▶ Once all the processes have been stopped, the window will close automatically.

   ▶ Running the System Stop application is the **only** way to shut down the Genome Sequencer FLX Instrument software, since all processes run in the background. Logging out will not shut down these processes.

   ▶ The System Stop application will NOT shut down the GS Sequencer GUI application. When exiting the system with the *systemStop* icon, the Operator should also shut down the GS Sequencer application.

   ▶ For procedures to prepare the instrument for long-term idle (more than 7 days), see section 3.5 in this manual.

   ▶ For procedures to carry out a complete shutdown of the Genome Sequencer FLX Instrument, see section 3.6.

2.3 GS Sequencer: Instrument Control User Interface Application

The GS Sequencer application is used to set up and carry out Runs, manage datasets, and monitor instrument health. The application is launched by double-clicking the *GS Sequencer* icon (Figure 2–4), which opens the GS Sequencer application.

Figure 2–4: The *GS Sequencer* icon, used to launch the GS Sequencer application

This section describes the GS Sequencer application from a structural point of view. For more details on how to use this application to set up pre-wash and sequencing Runs, see the *GS FLX (Titanium) Sequencing Method Manual*. For details on how to use this application to set up a maintenance wash Run, see section 3.3 in this manual.

2.3.1 The GS Sequencer Application Window

The GS Sequencer application window is divided into 3 major areas whose contents are listed in Table 2–2 (see also Figure 2–5, below):

Area Name	Location	Content
Status Area	Top	Overall status of the application and the instrument. This area displays the name of the instrument, the Operator currently logged in, and a status message indicating the state of the instrument. This area also contains a replica of the Status Indicator LED located above the camera door (see section 4.2). Finally, certain messages may appear in this area to notify the Operator of important information about the instrument. See also Trouble-shooting Guidelines, section 4.
Global Action Area	Right	Buttons used for general control of the instrument. ▶ **Exit** terminates the application. ▶ **Start** launches the Run Wizard which is used to configure a Run or perform other actions (wash). ▶ **Abort** aborts the current operation. ▶ **Config** opens the Configuration window used to add, delete, or modify Operators and Run Groups for the GS Sequencer software. ▶ **About** launches the splash screen which includes the current software version number. ▶ **Help** launches the online help pages. (Not currently enabled)
Action Area	Center	Contains tabs where instrument and Run information and status can be displayed. See section 2.3.3 for more details.

Table 2–2: Content of the 3 major areas of the GS Sequencer application window

2.3.2 Initial Functions

2.3.2.1 Logging In

When the application is launched, the Action area initially shows a login window; and in the Global Action area, the Start, Abort, and Config buttons are grayed out (Figure 2–5). Operators log in by selecting their identifier in the "Operator ID" drop down menu, and then clicking the Sign In button.

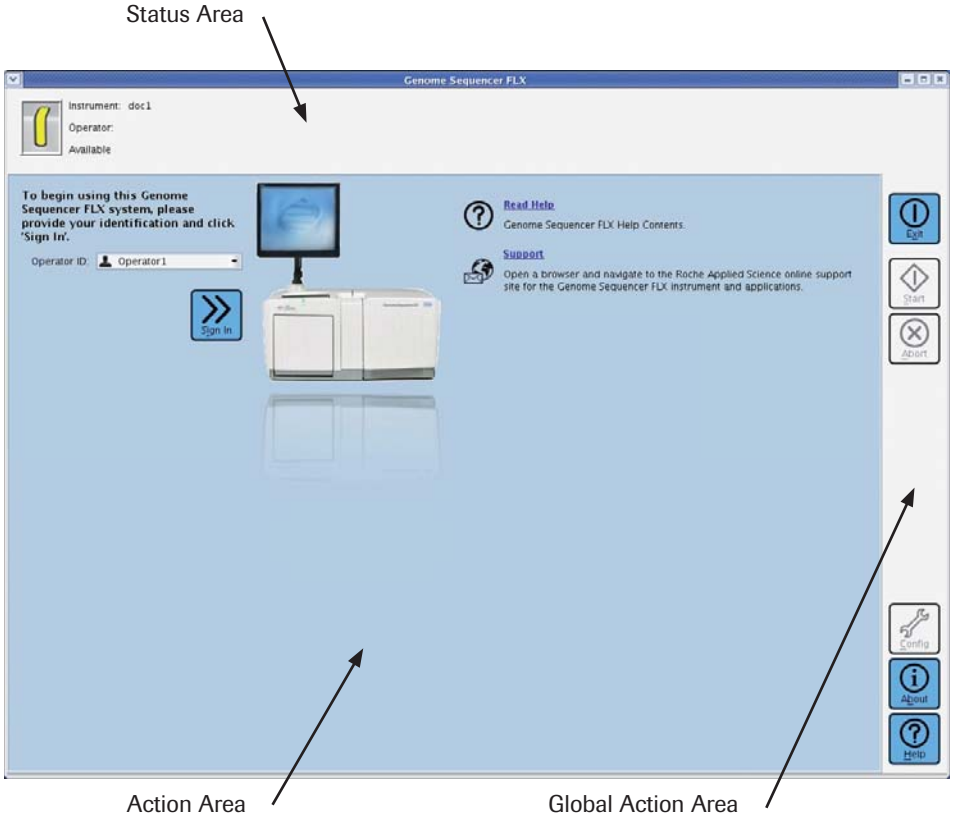


Figure 2–5: The Login window of the GS Sequencer application

Login has 4 main effects (Figure 2–6):

- The name of the logged in Operator is associated with sequencing Runs.
- The logged Operator is identified in the Status area. Also, the word "Operator" in the Status area becomes a functional tag which, when clicked, immediately logs the current Operator out of the system.
- A set of two tabs appears in the Action area: the Instrument tab, and the Data tab. The main functions of the GS Sequencer application are controlled from these tabs; these are described in more detail in section 2.3.3 of this manual and in the *GS FLX (Titanium) Sequencing Method Manual*.
- The Start, Abort, and Config buttons are enabled. See section 2.3.2.2, below, for the function and usage of the Config tools.

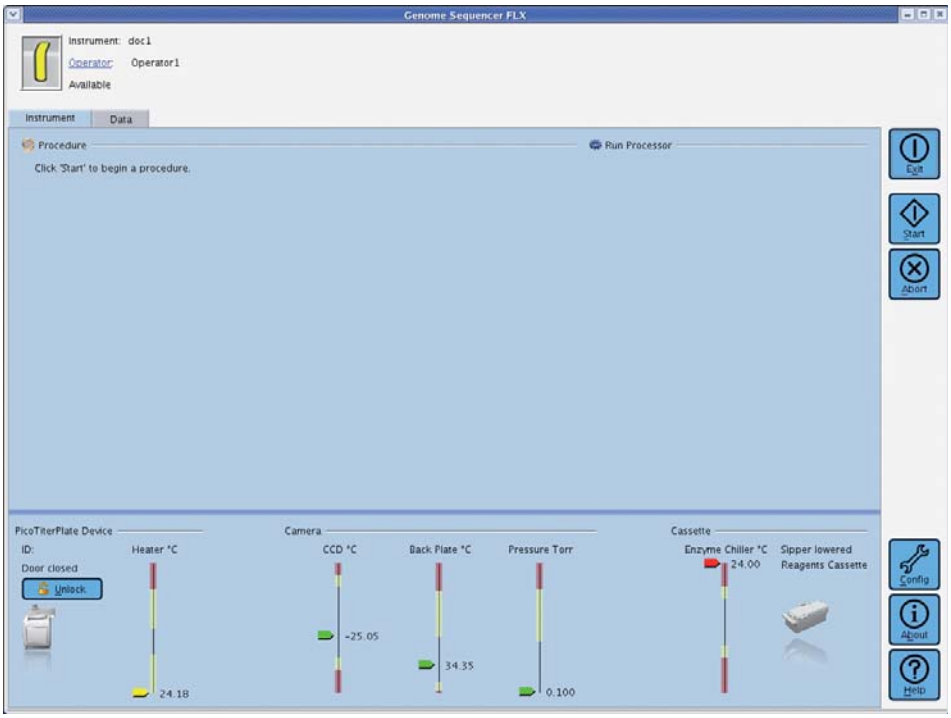


Figure 2–6: The GS Sequencer application window after an Operator logs in

2.3.2.2 The Config Button: Managing Operators and Run Groups

Clicking the Config Button of the GS Sequencer window's Global Action area evokes the Configuration window. This window contains two tabs used to add, delete, or modify entries identifying Operators and Run Groups for the sequencer instrument.

Logging in as an Operator is necessary to access the functions of the Genome Sequencer FLX Instrument. This allows the system to track the ownership of all tasks performed on the instrument. For example, the logged Operator's ID is included in all Run names. This also allows for an e-mail to be sent to the logged Operator upon completion of each sequencing Run.

To modify the list of Operators, do the following:

1. Click the Config Button, in the GS Sequencer window's Global Action area.
   ▶ The Configuration window will open.
2. Click the Operators tab to activate it (Figure 2–7).
3. Do one of the following:
   a. To Add an Operator entry, click the [+] button, and fill the appropriate fields on the right-hand part of the tab by either typing the correct values or (for the Run group) selecting the value from the drop down menu. Fields marked with an asterisk are required (ID and Last name).
   b. To Delete an Operator entry, select it from the list and click the [-] button.
   c. To Modify an Operator entry, select it from the list, and type or overtype the correct values in the fields on the right-hand part of the tab (or, for the Run group, select the value from the drop down menu). Fields marked with an asterisk are required.

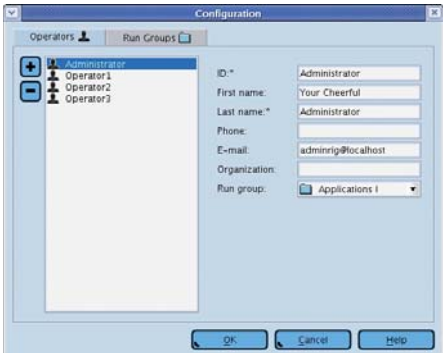


Figure 2–7: The Operators tab of the Configuration window

Run groups are used to associate different Runs with a single organization, or group, that uses the instrument. Run groups can also be used as a mechanism for automatically sending particular datasets to off-instrument analysis servers designated for use by particular groups.

To modify the list of Run groups, do the following:

1. Click the Config Button, in the GS Sequencer window's Global Action area.
   ▶ The Configuration window will open.
2. Click the Run groups tab to activate it (Figure 2–8).
3. Do one of the following:
   a. To Add a Run group entry, click the [+] Button, and type an appropriate name for it in the field on the right-hand part of the tab.
   b. To Delete a Run group entry, select it from the list and click the [-] button.
   c. To Modify a Run group entry, select it from the list, and type or overtype the correct name in the field on the right-hand part of the tab.

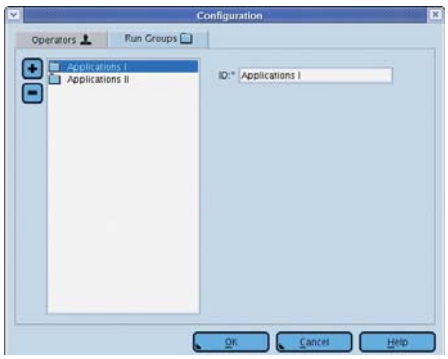


Figure 2–8: The Run groups tab of the Configuration window

2.3.2.3 The Start Button: Launching the Run Wizard

Clicking the Start button in the Global Action area of the application window (Figure 2–6) launches the Run Wizard. The Run Wizard consists of a series of windows that guide the Operator through the setup of a Run (the first Run Wizard window is shown in Figure 2–9). Setup procedures for pre-wash and Sequencing Runs, including those controlled by a Laboratory Information Management System (LIMS), are described in detail in the *GS FLX (Titanium) Sequencing Method Manual*.

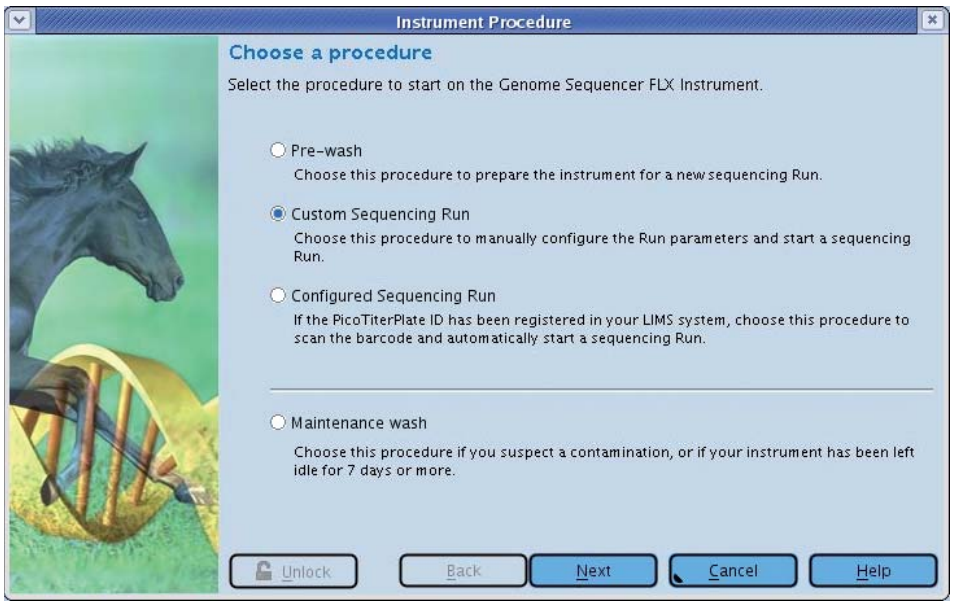


Figure 2–9: The Run Wizard opening window

2.3.3 The Action Area Tabs

2.3.3.1 The Instrument Tab

The Genome Sequencer FLX Instrument features a number of sensors that monitor the status of various functional parameters of the instrument. The values detected by these sensors are displayed in the lower part of Instrument tab (Figure 2–10), and include:

- PicoTiterPlate cartridge status
  - PTP Door Open/Closed (including a button to unlock the PTP door)
  - PTP Heater temperature
- Camera status
  - CCD temperature
  - Back-plate temperature
  - Pressure
- Cassette status
  - Enzyme chiller temperature
  - Sipper Raised/Lowered

An Unlock button is also available on each window of the Run Wizard. The PTP cartridge is voided to waste for about one minute each time the camera door is unlocked. Do not unlock the camera door unless prescribed by the operation you are carrying out.

During a Run, the Instrument tab also displays real-time information concerning the Run and the data processing (if active), in the upper part of the tab (Figure 2–10). Such information includes:

- In the Procedure area:
  - general settings of the Run or wash procedure
  - a log of all the informational and warning messages issued by the instrument during the Run
  - progress bars for both the reagent flow portion and the data processing portion of the Run
  - a thumbnail of the last image captured
- In the Run Processor area:
  - Various Run metrics and Run parameters including raw and keypass well counts.

This is described in detail in the *GS FLX (Titanium) Sequencing Method Manual.*

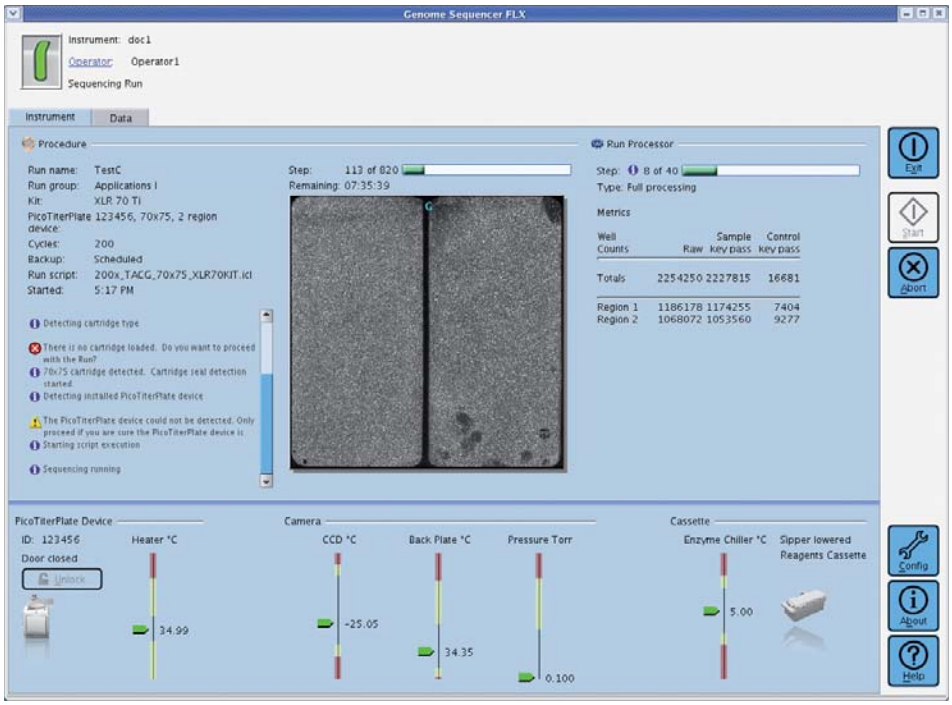


Figure 2–10: The Instrument Tab

2.3.3.2 The Data Tab

The Data tab (Figure 2–11) is used to manage the Run datasets stored on the Genome Sequencer FLX Instrument. Disk space is limited and will fill rapidly after a few runs. For example, on-instrument storage can only hold about five large GS FLX Titanium sequencing Runs (see Table 2:3 for a list of the approximate disk space requirements for a sample of sequencing Run configurations). When there is not enough disk space available for the next Run, old files must be deleted from the Genome Sequencer FLX Instrument before the new Run can begin.

Kit Type	Number of Cycles	Disk Space
XLR70	200	30 Gb
	150	23 Gb
	100	16 Gb
LR70	100	15 Gb
	42	6 Gb
LR25	100	7 Gb
	42	4 Gb
SR70	42	6 Gb

Table 2–3: Amount of disk space used for a sample of Run configurations

All examples are for sequencing Runs using the bead loading gasket that allows the largest throughput: 2 large regions on the 70×75 mm PTP devices ("70" sequencing kits), and one medium region for the 25×75 mm PTP device ("25" sequencing kit). Disk space includes raw images plus processed data.

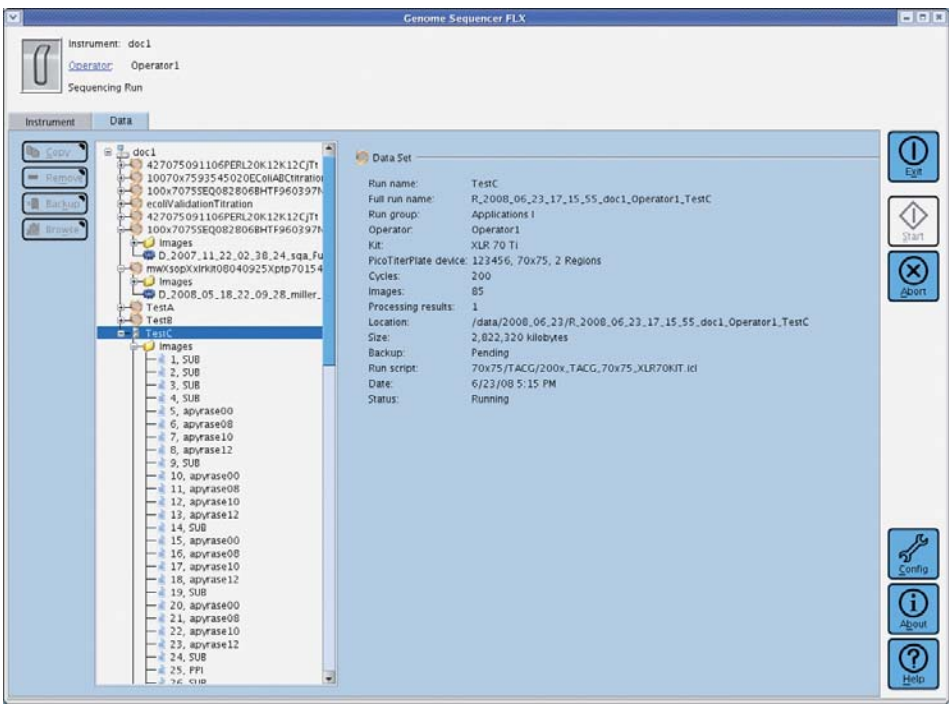


Figure 2–11: The Data tab

The operations available on the Data tab are listed and described in Table 2 4. To carry out any of these functions, select the item from the folder tree and click the appropriate button. These function can also be applied to multiple items at once: hold down the SHIFT or CTRL key while selecting the items of interest in the tree, and click the function button.

Button	Function
Copy	Copy selected data to another networked computer. This function applies only to Sequencing Run data sets (*e.g.* R_ directories). The entire content of the Run data set will be copied, including any Run Processor data sets (*e.g.* D_ directories) it may contain. However, you cannot copy individual Run Processor data sets.
Remove	Delete selected data from the Genome Sequencer FLX Instrument
Backup	Backup selected data from the Genome Sequencer FLX Instrument to another networked computer
Browse	View selected data in the GS Run Browser. Selecting a Sequencing Run data set (*e.g.* an R_ directory) will only show the Run and image information in the GS Run Browser; selecting a Run Processor data set (*e.g.* a D_ directory) will show the Run and images as well as all the well information and statistics.

Table 2–4: Dataset management functions available on the Data tab

- A back-up process that moves Run data to a permanent archive location can be automated by the System Administrator or by a Roche Representative. However, such automatic file transfers still leave a copy of all the files associated with the backed up Run on the Genome Sequencer FLX Instrument until they are deleted by an Operator.

- The Data tab indicates the backup status of every file listed: this will be either "None", "Pending", "Failed", or "Complete". If backup is pending, the file cannot be deleted. If backup has failed for some reason, the "Pending" status will continue to be displayed until the file has been backed up successfully. **If backup has been attempted and "Pending" is still displayed, please contact your System Administrator or your Roche Representative.**

- The Copy button opens a dialog box displaying a list of preconfigured network locations so the Operator can select the desired destination for the dataset. This list is specific for each Operator's site. Networked computers must be added to this menu list by qualified IT personnel.

  For more details, consult the *Genome Sequencer System Site Preparation Guide*.

2.4 GS Run Browser: Viewing Run Data and Reanalyzing Data

The GS Run Browser application allows the Operator to view and analyze the results of a sequencing Run, including graphic representations of various Run metrics. This software may be used to assess the general quality of a Run, and to troubleshoot problems encountered during the Run. The software can also be used to launch jobs to process intermediate results or reprocess completed data sets.

The application includes graphic interfaces for viewing:

- upper-level information about the Run
- the raw images from the Run
- the locations and status of all identified active wells, well density information for raw wells and keypass wells, and CAFIE correction data
- the raw or subtracted flowgram for any selected position(s) on the images; or fully processed flowgrams for all the data-generating wells detected during the Run
- raw signal statistics for the sample library or the Control DNA reads
- read length and quality statistics for the sample library or the Control DNA reads
- consensus flowgrams and accuracy metrics for the Control DNA sequence reads
- statistics on the results of the quality filtering algorithms

To launch the GS Run Browser, double-click the *GS Run Browser* icon located on the desktop (Figure 2–12). The browser can also be launched on a DataRig by using the appropriate Unix command.

Figure 2–12: The *GS Run Browser* desktop application launch icon

For complete details on the use of the GS Run Browser, see the *Genome Sequencer Data Analysis Software Manual.*

3. Instrument Maintenance

3.1 Sipper Tube Replacement

The reagent inlet tubing lines of the sipper manifold (Sipper Tubes) should be replaced before every Run (before the pre-wash), and any time they are bent or may have been contaminated. Possible sources of contamination of Sipper Tubes include someone touching any of the Sipper Tubes with an ungloved hand or a hand with a wet glove.

Fluidics contamination: To prevent the contamination of important fluidics components of the Genome Sequencer FLX Instrument and ensure quality results, always wear laboratory gloves while performing these procedures and change gloves as appropriate when gloves become soiled or contaminated during use.

Sipper manifold buttons: The sipper manifold has two buttons that control two different motions (Figure 3–1). The first button (A) is used to raise and lower the sipper manifold. The second button (B) is used to rotate the RAISED sipper manifold so the Sipper Tubes can be replaced, as described below. To rotate the sipper manifold, make sure to first raise it COMPLETELY using Button A (on the *short*, *front* side of the sipper manifold); then press button B (on the *longer*, *right* side of the manifold).

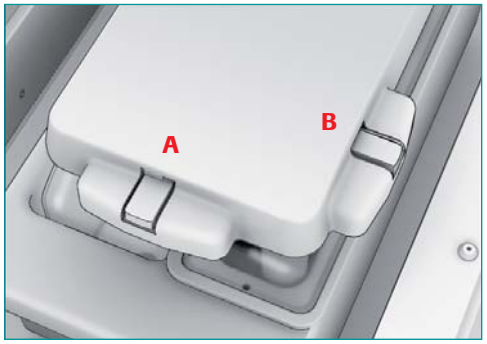


Figure 3–1: Sipper manifold buttons

- The "short" Sipper Tubes provided in the GS Sequencing Kits have an attached inline filter, to prevent any debris, precipitate, or other particulates that may be present in the concentrated reagents from entering the fluidics system of the Genome Sequencer FLX Instrument.
- Sipper Tube filters contain polyvinyl chloride (PVC) and should be recycled in accordance with local regulations.

1. Open the exterior fluidics door and raise the sipper manifold, using button **A** (on the *short*, *front* side; see Figure 3–2**A**) of the sipper manifold.
2. If present, slide out the Reagents cassette, toward the front of the instrument (Figure 3–2**B**).

A

B

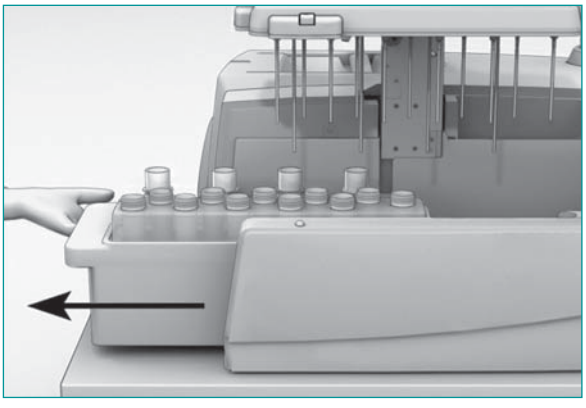


Figure 3–2: Removing the Reagents cassette
(A) Sipper manifold being raised, with the exterior fluidics door open (B) Sliding out the Reagents cassette, towards the front of the instrument

▶▶▶

3. Using the rotate button (button **B**), rotate the sipper manifold (Figure 3–3**A**). Be sure to hold the sipper manifold securely while rotating it, as it is counter-weighted. The underside of the sipper manifold holds 11 short, filtered Sipper Tubes on the right hand side and 4 longer Sipper Tubes on the left hand side (Figure 3–3**B**).

4. With your free hand, unscrew the Sipper Tube(s) you want to replace (Figure 3–3**C**). Discard the old Sipper Tube(s).

   The Sipper Tubes are Luer-Lok-type screws: turning in a clockwise motion tightens them, and turning in a counterclockwise motion loosens them.

5. Carefully install a new Sipper Tube by screwing it into an empty port in the sipper manifold. Make sure it is fully seated.

   Sipper Tube filters: Be careful with the filtered short Sipper Tubes; only finger-tighten to avoid breaking the filter. A broken or cracked filter would prevent suction of the reagent and cause a Run failure.

6. Repeat step 5 for all tubes that need replacement (*e.g.* a full set before every Run, or any suspected of contamination).

7. When all the Sipper Tube exchanges are complete, rotate the sipper manifold back to its normal horizontal position by pressing button **B** on the sipper manifold (Figure 3–3**D**).

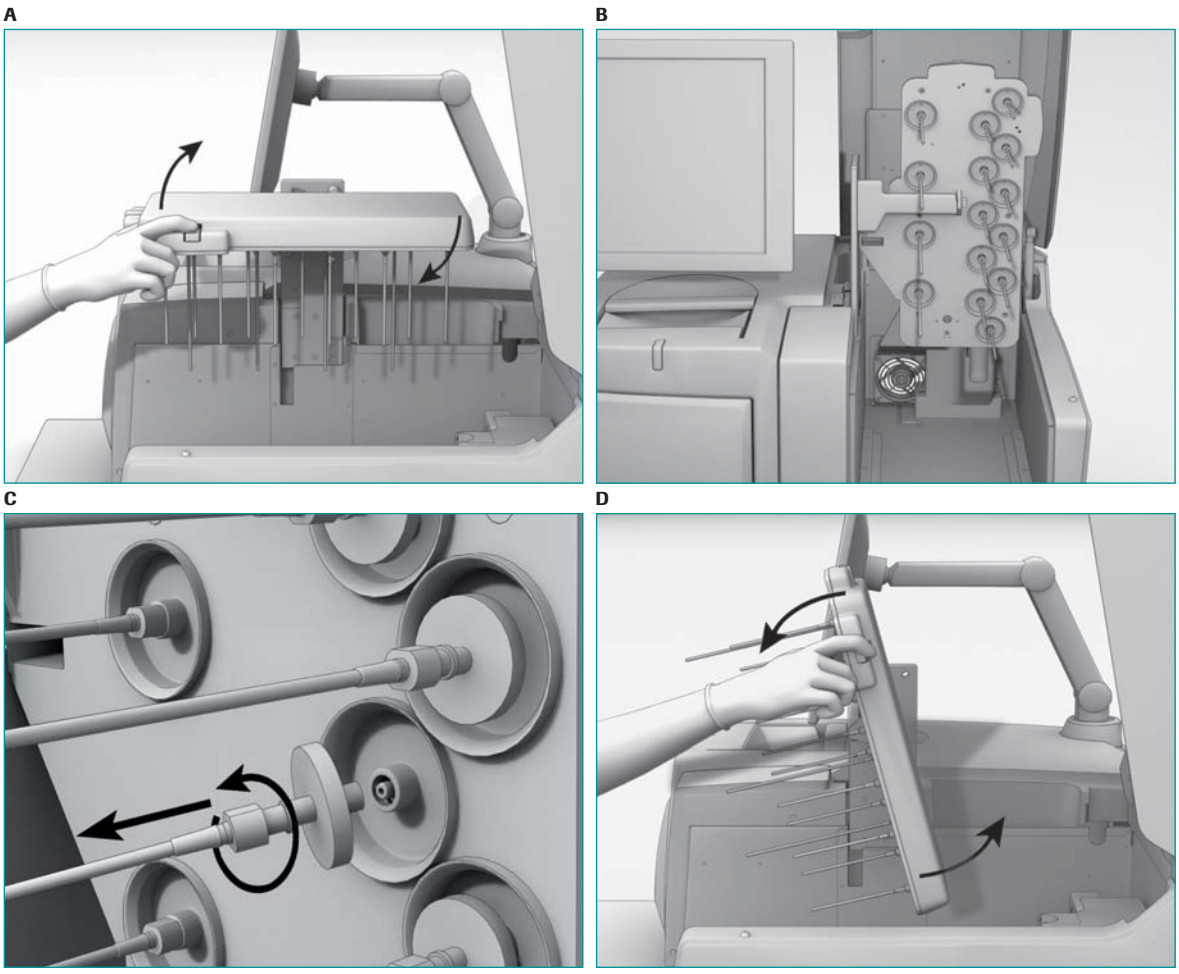


Figure 3–3: Replacing Sipper Tubes

(A) Rotating the sipper manifold to replace Sipper Tubes (B) Sipper manifold in the rotated position (C) Unscrewing a Sipper Tube from the sipper manifold (unscrew by turning counter-clockwise) (D) Rotating the sipper manifold back to its horizontal operation position, after replacing Sipper Tubes

8 Change gloves before proceeding further. If you replaced the set of Sipper Tubes as preparation for a sequencing Run, return to where you were in the procedure (see the *GS FLX (Titanium) Sequencing Method Manual*).

▶ If you replaced contaminated Sipper Tubes when you were not preparing for a Run, prepare a set of pre-wash reagent containers (or prepare a maintenance wash, depending on the level of contamination) and perform a separate pre-wash (or a maintenance wash Run followed by a pre-wash.)

For details on the pre-wash, see the *GS FLX (Titanium) Sequencing Method Manual*. For more information on a maintenance wash Run, see section 3.3, below.

3.2 Exchanging the PicoTiterPlate Cartridge in the Camera Door

The accessories kit of the Genome Sequencer FLX Instrument includes 2 PicoTiterPlate cartridges, one for each of the PTP device sizes supported by the GS FLX System, and a cartridge bypass tube. The function of the cartridge is to keep the PTP device firmly in place during a Run, between the fluidics subsystem and the optics subsystem of the Genome Sequencer FLX Instrument.

The cartridge is held in the camera door by a set of four latches. The cartridge features an articulated frame into which the PTP device is inserted; and a groove that defines the area(s) where fluids will flow, including the fluid inlet(s) and outlet(s) (Figure 3–4). The cartridge seal fits into this groove.

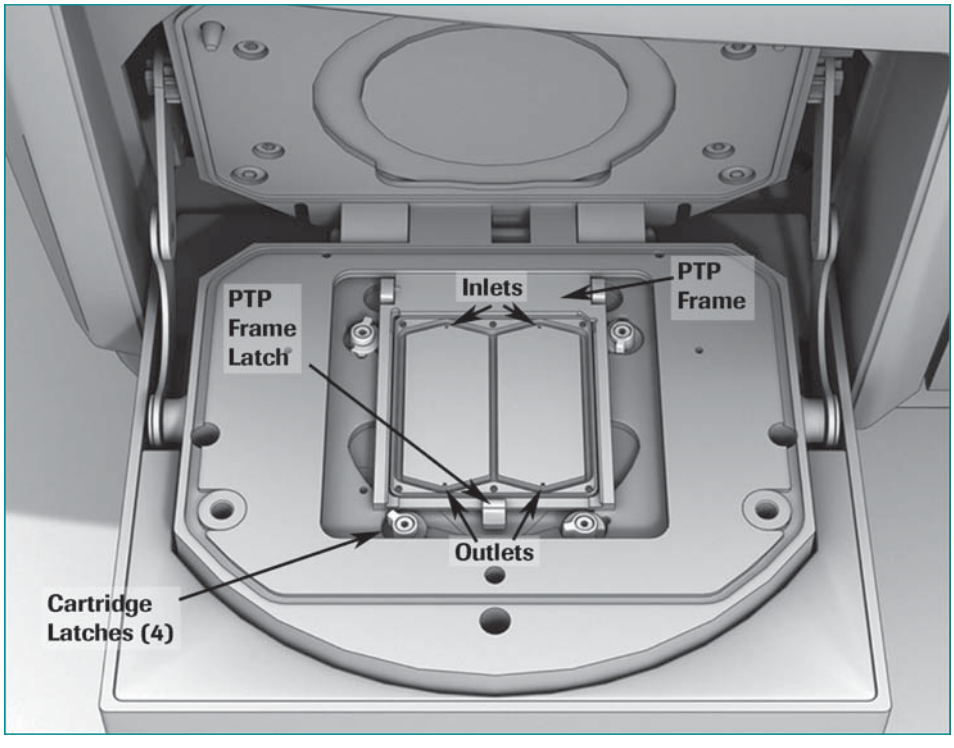


Figure 3–4: View of an installed PicoTiterPlate cartridge

A PTP cartridge (in this case, for the 70×75 mm PTP device) is shown in its proper place in the camera door, showing proper orientation of the cartridge relative to the camera door, as well as the PicoTiterPlate frame and cartridge latches

The cartridge is linked to the fluidics lines in the camera door through a set of 4 PEEK nuts that screw into ports in the back of the cartridge (see below). All 4 nuts are used for the 70×75 cartridge, which has two inlets and two outlets for two flow chambers (created by the 2× 30×60 mm PTP cartridge seal). With the 25×75 cartridge, however, the seal creates only one flow chamber, so this cartridge has only one inlet port and one outlet port. (Note that the number of flow chambers defined by the PicoTiterPlate cartridge seals is independent of the "bead loading regions" defined by the bead loading gaskets.) When the 25×75 cartridge is in place, therefore, the PEEK nuts of one inlet line and one outlet line are screwed into the cartridge, while the extra nuts are screwed into a bypass tube. The cartridge is not attached in any way to the optics subsystem; the PTP device in its cartridge is simply pressed against the camera faceplate when the camera door is closed.

If the cartridge currently in the instrument does not match the PTP device used for the next sequencing Run, it will have to be exchanged for the correct one. This section describes the cartridge exchange procedure.

1. Open the camera door.

2. Install the camera faceplate guard (from the accessories kit) on the camera face by hanging it on the metal faceplate seating pins that project from the camera faceplate holder (Figure 3–5**A**).

   ⚠ The camera faceplate is very fragile (and costly). It is very important to protect it with the camera faceplate guard while you replace the PTP cartridge.

3. Turn the four PTP cartridge latches to release the cartridge (Figure 3–5**B**).

A **B**

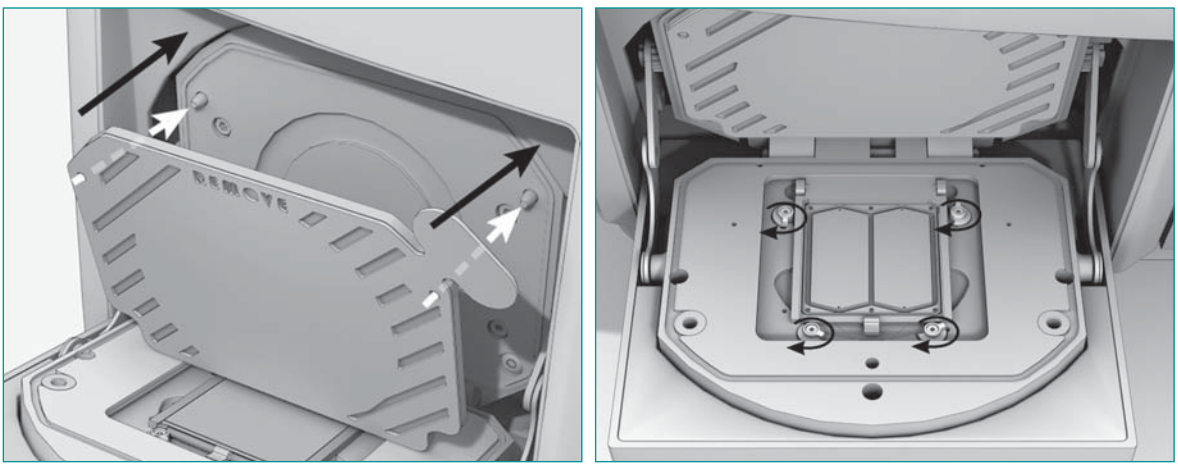


Figure 3–5: First steps in PTP cartridge exchange

(A) Installing the camera faceplate guard on the camera face by hanging it on the seating pins (B) Turning the cartridge latches to release the cartridge

❗ Be careful when you lift the PTP cartridge or otherwise handle the tubing used to connect the cartridge to the Genome Sequencer FLX Instrument, within the camera door. Pulling the tubing too hard or handling it roughly could damage it and degrade the performance of the instrument.

4. Gently lift the cartridge about 1.5 inches (3–4 cm) out of the camera door.

5. Rotate the cartridge slightly to see the PEEK nuts screwed into the back of the cartridge.
   - If you are replacing the 70×75 cartridge, two pairs of PEEK nuts will be attached, one pair of long nuts to the right and one pair of short nuts to the left (Figure 3–6**A**).
   - If you are replacing the 25×75 cartridge, only the long PEEK nuts will be attached to the cartridge (Figure 3–6**B**); note the flow restrictor at the outlet of the 25×75 PTP cartridge), and the shorter ones will be attached to the bypass tube (see below).
   - In both cases, the PEEK nuts at the fluidics inlet(s) of the PTP cartridge (bottom, away from you) are tan in color, and those at the outlet(s) (top, near you) are blue.

▶▶▶

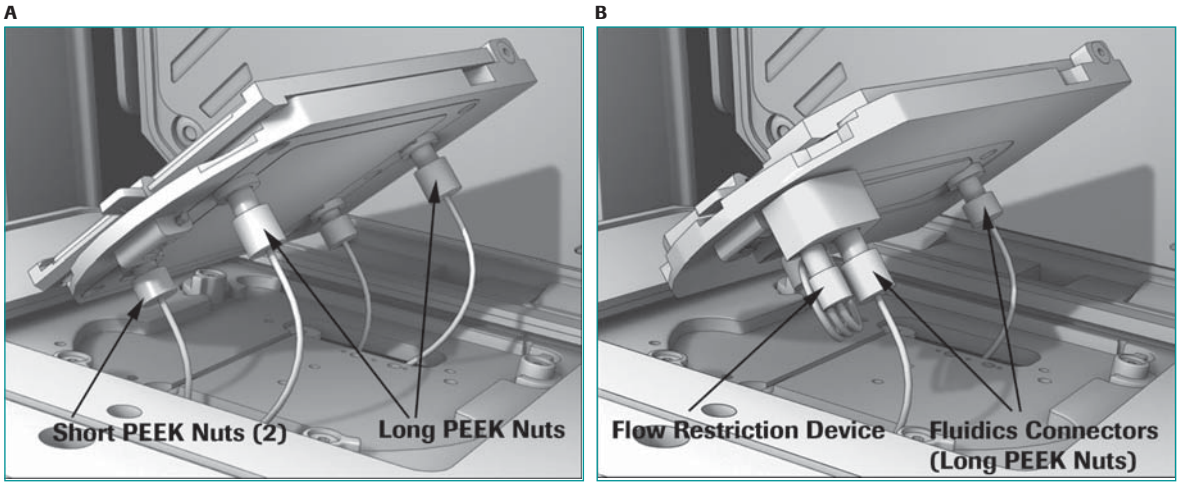


Figure 3–6: Pulling the PTP cartridge out of the camera door

This shows the PTP cartridge in the camera door, with the PEEK nuts (fluidics connectors) beneath the cartridge. (A) The 70×75 cartridge has both pairs of PEEK nuts connected. (B) The 25×75 cartridge uses only the long pair; the shorter pair is connected to the bypass tube (not shown here; see below). Note the flow restrictor at the outlet of the 25×75 PTP cartridge.

⚠ **Fragile PTP cartridge ceramic insert:** Use care when handling PTP cartridges. The ceramic insert is brittle and may crack if dropped. Always inspect the ceramic insert for cracks prior to running. To prevent leaks and ensure good performance, always replace a cracked cartridge.

6. Unscrew the inlet and outlet PEEK nuts from the cartridge, and set the cartridge aside until step 12 (below).

7. Install the cartridge needed for your Run:

   a. If you are changing to a 25×75 cartridge:

      I. Connect the short PEEK nuts to the cartridge bypass tube (if not already connected) (Figure 3–7**A**).

      II. Place the bypass tube into its slot in the camera door and press it down until it is held securely (Figure 3–7**B**; see also Note, below).

      III. Screw the long PEEK nuts into the fluidics ports in the back of the cartridge. On the upper, outlet side, the port is on the flow restrictor (see Figure 3 6**B**).

   b. If you are changing to a 70×75 cartridge:

      I. Disconnect the short PEEK nuts from the cartridge bypass tube (if not already disconnected).

      II. Store the bypass tube in the container with the other instrument cartridges.

      III. Screw the short PEEK nuts into the left-hand side fluidics ports in the back of the cartridge.

      IV. Screw the long PEEK nuts into the right-hand side fluidics ports.

▶▶▶

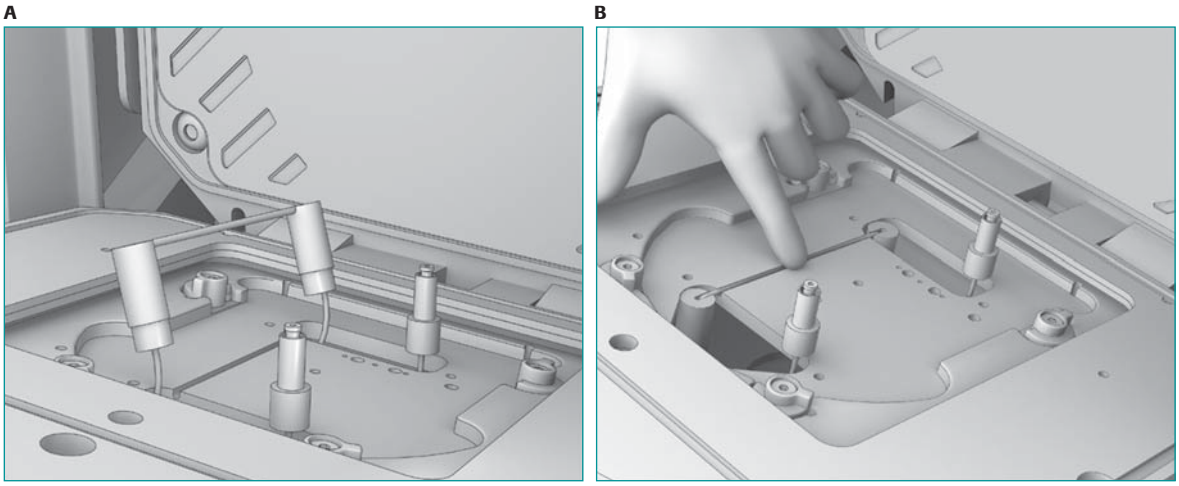


Figure 3–7: Bypass tube connection

A) The cartridge bypass tube lifted out of its slot in the camera door; this view shows the two disconnected long PEEK nuts and the two short PEEK nuts connected to the bypass tube. (B) Gently press the bypass tube into position, in its slot in the camera door.

The bypass tube is manufactured with a slight sideways curve (viewed from the top; see Figure 3–8), which helps secure it in place with a friction fit in its slot, in the camera door. With time and use, this curve may become less pronounced and the bypass tube may tend to pop out of its slot. If this happens, **gently** bend the bypass tube sideways to re-form its initial curve.

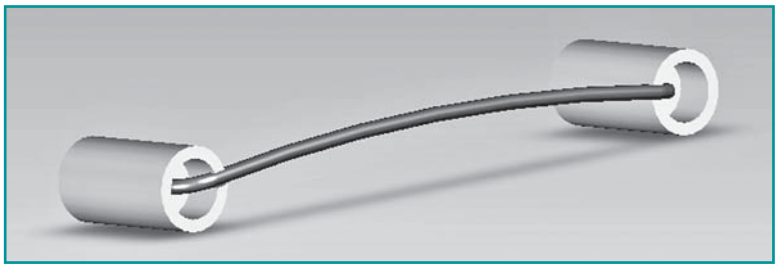


Figure 3–8: The PTP cartridge bypass tube, showing its curvature

8. Place the new cartridge down into the camera door.
9. Turn the cartridge latches to secure the cartridge in the camera door.
10. Wet a Kimwipe with 50% ethanol and wipe the surface of the newly installed cartridge. Allow the cartridge to air dry fully.
11. Remove the camera faceplate guard from the camera face plate and store it with the accessory items for the Genome Sequencer FLX Instrument.
12. Take the cartridge you just removed from the instrument (step 6 above) and:
    a. wash it with Sparkleen lab detergent, using a laboratory scrub brush;
    b. rinse it with fresh nanopure water;
    c. allow it to fully air dry;
    d. store it in the cartridge storage box.

Detergent: Do not use any cleaning reagents other than the one specified (Sparkleen) on the PicoTiterPlate cartridges. Other cleaning materials may react with the cartridge's surfaces and interfere with their proper function.

PTP frame seal: The PicoTiterPlate frame and frame latch can hold a PTP device securely in the cartridge. They do not, however, provide a liquid-tight seal. Do not run reagents through the cartridge until the camera door is closed.

3.3 Sterilization of the Fluidics Components ("Maintenance Wash Run")

The same GS FLX Maintenance Wash Kit and procedure are used whether working with the GS FLX standard chemistry or the GS FLX Titanium chemistry.

All sequencing Runs end with a maintenance wash that uses a combination of Bleach (GS FLX standard chemistry) or sodium chlorite (GS FLX Titanium chemistry) and Tween reagents. However, there are also other situations where a maintenance wash Run is either required or advisable. For example, if no sequencing Runs are performed on the instrument, you MUST perform a maintenance wash Run EVERY 7 DAYS that the instrument is idle. This will prevent contaminants (*e.g.* bacterial growth or biofilm) from building up in the fluidics.

- You do not need to perform this periodic wash as long as the instrument is used at least once a week for sequencing Runs, since all such Runs end with a maintenance wash step.
- Alternatively, if you know that the instrument will not be used for sequencing Runs for a long time, you may consider preparing the instrument for a long-term idle or a complete shut down, as described in sections 3.5 and 3.6 of this manual.
- If you think that the Genome Sequencer FLX Instrument's fluidics may require a more thorough maintenance wash prior to a sequencing experiment, you can perform a maintenance wash Run.

To perform a stand-alone maintenance wash Run, you must order a separate GS FLX Maintenance Wash Kit. The procedure for performing a maintenance wash Run is similar to a sequencing Run, as described in the *GS FLX (Titanium) Sequencing Method Manual*. An abridged form of that procedure is provided below.

PTP device and cartridge seal must be present: A used but intact PTP device and cartridge seal must be present in the cartridge to carry out a maintenance wash Run, or the fluidics will leak. Since the Genome Sequencer FLX Instrument is designed for continuous operation, the PTP device and seal from the previous Run are usually present when you prepare for a maintenance wash Run. If not, install a used but intact PTP device and seal in the PTP cartridge before carrying out the procedure below.

1. Replace all the Sipper Tubes as described in section 3.1.

2. Empty any waste fluid from the Reagents cassette, and load the two Reagent trays from the GS FLX Maintenance Wash Kit into the Reagents cassette: the 4-tube Maintenance Wash Buffers tray on the left-hand side and the 11-tube Maintenance Wash Reagents tray on the right-hand side, reflecting the "Buffer CB" and "Sequencing Reagents" of the GS FLX (Titanium) Sequencing Kits. The 4-tube "Maintenance Wash Buffers tray" should sit at the bottom of the left-hand side of the Cassette, as shown in Figure 3–9.

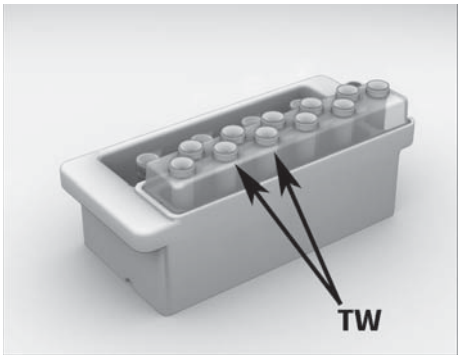


Figure 3–9: All reagents loaded into the Reagents cassette, for a maintenance wash Run

3. Remove the caps from all the reagent tubes.

4. Load the maintenance Reagents cassette into the instrument.

   Fluidics door must be completely open: Make sure that the exterior fluidics door is completely open and that the sipper manifold is completely raised before loading the Reagents cassette.

5. Lower the sipper manifold carefully, and close the exterior fluidics door.

   Sipper Tubes: Make sure that all the Sipper Tubes descend properly into the tubes. If any of the Sipper Tubes are bent due to missing the tube opening, raise the sipper manifold, remove the maintenance Reagents cassette, and follow the instructions provided in section 3.1 to replace the bent Sipper Tube(s).

6. Return to the instrument computer. A "Sequencing run complete" message may be displayed in the Status area of the GS Sequencer software main window. Click OK to continue.

7. If the GS Sequencer main window is not open, launch the GS Sequencer application by double-clicking the *GS Sequencer* desktop icon.

8. Click the Start button in the Global Action area.

   ▶ This opens the Run Wizard window: Choose a procedure.

   ▶ The Run Wizard has only this one window for maintenance wash Runs.

9. In the Run Wizard window, select the Maintenance wash option, and click the Next button.

   ▶ The maintenance wash Run will start automatically after a few moments, and proceed to completion (about 30 minutes) without any further user intervention.

3.4 Test Sequencing Run with Control DNA Beads

Under certain circumstances, your Genome Sequencer FLX Service Representative may ask you to perform a sequencing Run with GS FLX Control Beads to verify that your Sequencer is performing to specifications. This is the same test Run that is performed by the Service Representative at the time of installation of your instrument, and uses, as sample, a GS FLX Control Beads Kit, along with a GS LR70 Sequencing Kit and a GS PicoTiterPlate Kit (70×75).

The Pack Insert of the GS FLX Control Beads Kit describes in detail how to prepare the Control Beads for use in a test sequencing Run. Except for the use of the GS FLX Control Beads Kit, the procedure is otherwise identical to a normal sequencing Run, as described in the *GS FLX (Titanium) Sequencing Method Manual*.

Use of the GS FLX standard chemistry: The GS FLX Titanium Sequencing Kit XLR70 includes DNA Control Beads to spike into the sample DNA library beads and use as an internal control for sequencing Runs using the GS FLX Titanium chemistry. However, a separate Control DNA Beads Kit is not available at this time to suport the test sequencing Run procedure using the GS FLX Titanium chemistry. A sequencing Run performed using the GS FLX standard chemistry remains an appropriate test to determine the proper function of your Genome Sequencer FLX Instrument.

3.5 Leaving the Genome Sequencer FLX Instrument Idle for More Than 7 Days

Biofilm growth in idling instrument: The instrument clean up procedures described in this section should be performed if the instrument is not to be run for more than 7 days. If these procedures are not performed, biofilms may form in the fluidics subsystem of the Genome Sequencer FLX Instrument, causing subsequent sequencing Runs to fail and possibly requiring replacement of parts in the fluidics subsystem.

If these procedures are not performed, you must, at a minimum, carry out a maintenance wash Run every 7 days.

This procedure includes a maintenance wash Run (see section 3.3 in this manual), and therefore requires a GS FLX Maintenance Wash Kit. It uses procedures similar to those of a sequencing Run, which are described in detail in the *GS FLX (Titanium) Sequencing Method Manual*.

1. Perform a maintenance wash Run as described in section 3.3.
2. Perform a pre-wash according to the procedure described in the *GS FLX (Titanium) Sequencing Method Manual*.
3. Remove the Reagents cassette from the instrument and discard the used reagents, as described in the *GS FLX (Titanium) Sequencing Method Manual*.
4. Remove the Sipper Tubes from the sipper manifold (see section 3.1). Do NOT install new Sipper Tubes.

▶▶▶

5. Perform a mock pre-wash:
   a. Install the Reagents cassette with the GS FLX pre-wash tube holder in place, but do NOT fill it with Pre-wash Tubes and Pre-wash Buffer.
   b. Lower the sipper manifold.
   c. Run the pre-wash script flowing air through the system, following the procedure described in the *GS FLX (Titanium) Sequencing Method Manual*.

   Be sure to have a (used) PTP device and cartridge seal installed in the cartridge, since leftover pre-wash reagents will pass through the system.

6. Perform a second mock pre-wash, flowing air through the system.

7. When the second mock pre-wash is complete:
   a. Click OK.
   b. Close the GS Sequencer window by clicking the Exit button in the Global Action area.
   c. Close any other window that may be open.

8. Double-click the *systemStop* icon on the desktop, to launch the System Stop application (see section 2.2).

9. Click the Red Hat icon on the taskbar and select "Logout".
   ▶ This logs you out of the Genome Sequencer FLX System software.

10. Open the camera door, then:
    a. Remove the used PTP device and the cartridge seal.
    b. Gather all the PTP cartridges and clean them using a soft bristle brush and Sparkleen solution, being especially careful to remove any bead residues near the inlet and outlet ports or in the gasket channel.
    c. Rinse thoroughly with nanopure water and dry using a paper towel.
    d. Store them with the Genome Sequencer FLX Instrument.

11. Clean the camera face plate as described in the *GS FLX (Titanium) Sequencing Method Manual*.

12. Make sure there is not a cartridge in the camera door. Close the camera door.

13. Rinse the Reagents cassette with warm water and dry the exterior thoroughly.

14. Clean the Genome Sequencer FLX Instrument fluidics deck as described in the *GS FLX (Titanium) Sequencing Method Manual*, then:
    a. Return the Reagents cassette and GS FLX pre-wash tube holder to the fluidics deck area for storage.
    b. Close the exterior fluidics door.

15. When you have completed the clean-up procedures, leave the instrument idle:
    a. Leave the Genome Sequencer FLX Instrument on.
    b. Leave the main power cord plugged into the wall.
    c. Do not turn off the main power switch.
    d. Do not open or remove any panels from the instrument.
    e. Do not turn off the onboard computer.

■

- ▶ The next time you want to work on your Genome Sequencer FLX Instrument, you will need to run the System Start application to launch the Genome Sequencer System software (see section 2.1).
- ▶ Unplugging the Genome Sequencer FLX Instrument will not turn it off immediately. The instrument features a backup uninterruptible power supply (UPS) which allows it to function for a few minutes without external power. This will prevent loss of a Run if the power fails briefly. It may also allow you to temporarily unplug the instrument, *e.g.* to move it to another nearby location in your laboratory.
- ▶ Optionally, if the instrument is not to be used for an extended period, you may follow this clean up procedure with a complete instrument shutdown, as described in section 3.6, rather than leaving it idle.

3.6 Complete Instrument Shutdown and Instrument Start

During normal operation, the Genome Sequencer FLX Instrument should not be shut down. If a shutdown becomes necessary, however, or if you know that it will not be used for an extended period (weeks or more), carry out the procedures of section 3.5 with the following two differences:

1. In section 3.5, step 9, select "Turn Off Computer" instead of "Logout", in the Session menu.
   ▶ The computer will logoff the user and then shutdown.
2. When the screen goes black, flip the instrument's main power switch to the Off position (see Figure 3–11, below).

■

To turn on the instrument from this shutdown state:

1. Flip the instrument's main power switch to the On position.
2. Remove the server access panel (small front panel below the keyboard drawer; it just snaps off).
3. Turn on the on-instrument computer (round white button).
4. Wait for the computer to complete its boot-up process.
   ▶ A successful boot-up results in the login screen being displayed (Figure 3–10).

Figure 3–10: The screen used to log in the Instrument, after boot-up from the shutdown state

5. Login as user 'adminrig', password = 'adminrig'.
6. Run the System Start application to launch the Genome Sequencer System software (see section 2.1).
7. Proceed directly with any procedure you want to carry out on the instrument, *e.g.* sequence a DNA library as described in the *GS FLX Titanium Sequencing Method Manual*.

■

Instrument emergency shut down: During normal operation, the Genome Sequencer FLX Instrument should not be shut down. If a shutdown is nonetheless desired, it should be done by the orderly procedure described in this section.
In a **SEVERE EMERGENCY** (fire, flood, *etc.*), you can immediately cut off power to the system by using the **SYSTEM SHUTDOWN SWITCH** ("Arrêt du système") on the **RIGHT HAND SIDE OF THE INSTRUMENT** (Figure 3–11). Complete shut off of the instrument occurs approximately 5 seconds after you flip the switch, due to the powering down of the UPS.

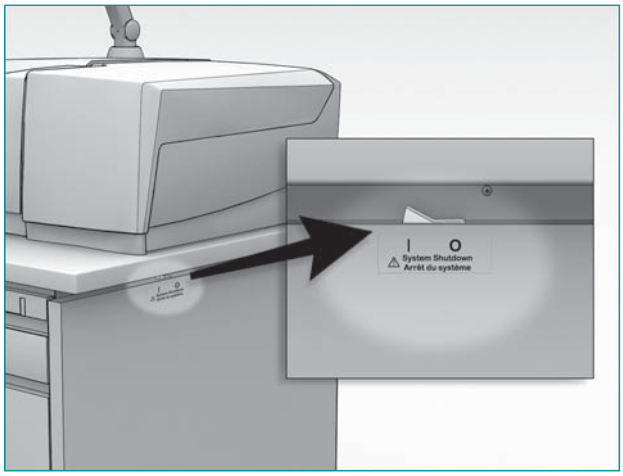


Figure 3–11: The System Shutdown switch of the Genome Sequencer FLX Instrument

3.7 Regular Service

The Genome Sequencer FLX Instrument should undergo scheduled preventative maintenance by GS FLX Service personnel. This maintenance is performed under a service contract. Scheduled maintenance typically occurs annually and might include:

- Cleaning the Genome Sequencer FLX Instrument
- Pump tubing line and rotor replacement
- Sequencer tubing lines and fitting verification/replacement
- Pump calibration
- Sipper assembly and valve manifold connection verification/adjustment
- Flow volume verification
- Equalization of camera quadrants
- Camera door verification/adjustment
- Camera vacuum monitoring/refresh
- Maintenance wash (if necessary)

4. Troubleshooting Guidelines

4.1 Basic Troubleshooting

	Problem	Solution
1	Leak at the camera door (after door has been closed)	▶ Make sure all PEEK nut fluidics connections are screwed correctly into the PTP cartridge and/or bypass tube. ▶ Make sure the cartridge seal and PTP device are in place and not damaged. ▶ If the problem cannot be solved, contact your Roche Representative for maintenance.
2	Software locks up and is not responding	Contact your Roche Representative for assistance.
3	Any software error message appears	See Section 4.2 for information on error messages.
4	Camera quadrant backgrounds appear to be significantly different from each other (DC offsets vary)	Contact your Roche Representative for maintenance.
5	Camera temperature too high	Ambient temperature is above 30°C (86°F) (or near 30ºC when the relative humidity is near 80%). Cool/dehumidify the room.
6	Run will not start after you choose Start in the last Run Wizard window	▶ Make sure the Sipper Tube manifold is completely lowered by checking its status in the Instrument tab. ▶ Make sure the camera door is closed. ▶ Make sure the Reagents cassette has been detected.

4.2 On-Screen Messages

The Genome Sequencer FLX Instrument is equipped with a variety of sensors to assist Operators and reduce errors during sequencing Runs. Operators can interact with the sensors, *i.e.* receive informational messages on the status of the instrument in three ways: through the sensor displays on the Instrument tab; through messages which appear in the Status area of the GS Sequencer application window; and through various status displays and progress bars which are available when a Run is in progress.

In addition, the Genome Sequencer FLX Instrument features a Status Indicator LED located above the camera door, which shows at a glance the current operating status of the instrument. (Table 4–1). In general, if the indicator is blinking, the instrument is progressing normally through its operating steps; if it is solid, the instrument is waiting for an action from the Operator.

Color	Blinking?	Instrument status
	Blinking	A Run is in progress, as directed by a Run script.
	Solid	A Run script has completed normally.
	Blinking	▶ The system is preparing to run a script, or ▶ The camera temperature rose above -21°C during a sequencing Run (but the Run is not interrupted, as this does not usually affect instrument performance), or ▶ Communication was lost between the server and the microcontroller during the execution of a Run.
	Solid	The system is waiting for an action (*e.g.* loading the Reagents cassette or the PicoTiterPlate cartridge) or for a response from the Operator.
	Blinking	▶ The software is not running, or ▶ If the software is running but the instrument is not currently executing a Run, it indicates loss of communication between the server and the microcontroller.
	Solid	A system error occurred, and Operator intervention is required. A message identifying the specific problem will appear in the Status Area of the GS Sequencer window. Please refer to this Troubleshooting section for more detailed information on error messages.

Table 4–1: Meanings of the colored Status Indicator LED, located above the camera door

When an error occurs, a message will appear in the Status area of the GS Sequencer application window indicating exactly what is wrong. The various system messages are listed in the tables below.

4.2.1 Normal Run Messages

Message	Definition
Instrument reset.	The instrument is ready for operation.
Instrument is initializing. Please wait.	When one of the monitored parameters is out of range, the system will wait until the parameter is in range. (The Start button will be disabled until all parameters are within their acceptable range.)
Instrument is dissipating excess charge.	At the beginning of all Run scripts, the camera dissipates the excess charge built up in the CCD before capturing the first image. This usually takes 15 seconds.
Detecting cartridge type.	At the beginning of all Run scripts, the system detects the PTP cartridge and compares it to the chosen script, to ensure that the cartridge and script selection are compatible.
Pre-wash process started.	The pre-wash has started.
Pre-wash complete.	The pre-wash is finished and the Operator must remove the pre-wash cassette before starting a sequencing Run.
Pre-wash operation completed. Quadrant equalization started.	Camera quadrant equalization (calibration of the camera) occurs automatically after every pre-wash operation.
The installed PicoTiterPlate device is valid for sequencing Run.	After the instrument reads the DataMatrix code, it verifies that the inserted PTP device is compatible with the script choice and PTP cartridge.
25x75 cartridge detected. Cartridge seal detection started.	After the PTP cartridge is detected, the cartridge seal detection starts.
70x75 cartridge detected. Cartridge seal detection started.	After the PTP cartridge is detected, the cartridge seal detection starts.
Priming sequence started with buffer priming.	The priming sequence (at the beginning of the sequencing Run) has started.
Priming complete. Sequence cycle started.	The priming sequence has finished and nucleotide cycling has begun.
User aborted run.	If a Run is aborted by the Operator, the system can be reset by raising the sipper manifold.
Sequencing complete. Disinfectant wash started.	Nucleotide cycling has finished and the maintenance wash has begun.
Sequencing run complete.	The sequencing Run (including the priming and the maintenance wash) has finished.
Run aborted	When the abort operation is selected and confirmed, the system indicates that the Run has been aborted.

4.2.2 Error Messages

Message	Definition
The detected cassette did not match the cassette specified by your script. Do you want to proceed with the Run?	The script chosen is for a different size PTP device and cartridge than the ones detected in the camera door. Change either the PTP device and cartridge or the script.
Camera door is open. Close camera door.	If the camera door is open after a Run has started, the Operator must close the door and start the Run again. Click the Start button to re-open the Run Wizard.
Sipper is up. Lower sipper assembly.	If the sipper manifold is in the raised position during Run setup, the Run will not commence until the sipper is lowered.
Camera door is open. Current operation is aborted.	If the camera door is opened during a Run, the system terminates the Run.
Sipper detected in the up position during run.	If the sipper manifold is raised during a Run, this warning is displayed, but the Run continues.
PicoTiterPlate device is not compatible with instrument configuration.	The PTP device is not compatible with the type of Reagents cassette or script chosen, or has the wrong DataMatrix code. Replace the PTP device.
Camera quadrants could not be equalized.	Camera failure. ▶ Restart the software using System Stop and then System Start. ▶ Open the GS Sequencer application and launch the Pre-wash script again. ▶ If the quadrant equalization fails again, call your Roche Representative.
DataMatrix code could not be read. Only continue if you are sure that you properly loaded the PicoTiterPlate device. Do you want to proceed with the Run?	▶ If you are sure that the PTP device is loaded, continue the Run. ▶ If not sure, verify that the PTP device is loaded properly, and then restart the Run.
DataMatrix code did not match the code entered in the initial Run Wizard. Do you want to proceed with the Run?	▶ If you are sure that the correct PTP device is loaded, continue the Run. ▶ If not sure, verify that the correct PTP device is loaded, and then restart the Run.
There is no cartridge loaded.	Cartridge detection failed. ▶ If you are sure that the cartridge is properly loaded, continue by selecting the size cartridge installed. ▶ If not sure, do not continue the Run.
The attempt to clear the CCD failed. Do you want to proceed with the Run?	Camera failure. Call your Roche Representative.
PicoTiterPlate heater temperature is out of range.	The set point temperature for the PTP cartridge has not yet been reached. If the temperature is out of range for more than 15 minutes, call your Roche Representative.

▶▶▶

Message	Definition
Enzyme chiller temperature is out of range.	The set point temperature for the enzyme chiller has not yet been reached. If the temperature is out of range for more than 15 minutes, call your Roche Representative.
CCD temperature is out of operational range.	The set point temperature for the CCD has not yet been reached. If the temperature is out of range for more than 30 minutes, call your Roche Representative.
CCD vacuum pressure is out of operational range.	If the vacuum pressure is out of range for more than 30 minutes, call your Roche Representative.
Camera backplate temperature is out of operational range.	If the temperature is out of range for more than 30 minutes, call your Roche Representative.
System is running on battery backup power.	If utility power is not restored, the instrument will automatically shutdown when the battery runs low.
System has switched back to utility power.	Utility power has been restored.
Instrument start-up failed.	This can occur when the CCD has not reached its operating temperature. Press OK to continue the start-up. If the camera temperature still does not reach operating temperature, call your Roche Representative.
Communication with camera subsystem failed.	Shutdown the instrument and then restart it. If the same error occurs, call your Roche Representative.
Script download to micro failed.	Restart the software, and setup your Run again. If the same error occurs, call your Roche Representative.
Script start on micro failed.	Restart the software, and setup your Run again. If the same error occurs, call your Roche Representative.
Critical system failure.	Call your Roche Representative.

5. Glossary

This section provides a brief explanation of some special terms, or terms that are unique to the Genome Sequencer FLX System.

Amplicon – In the GS FLX manuals, an Amplicon is a PCR product that is flanked with the appropriate 'Primer A' and 'Primer B' sequences, which make the PCR product ready for emulsion PCR and sequencing in the Genome Sequencer FLX System.

Apyrase – An enzyme that catalyzes the degradation of leftover dNTPs into dNMPs and ATP into AMP, without releasing PPi. During a wash step, it eliminates any nucleotides that might remain in the system. Note that Apyrase is not stable for long periods of time at room temperature, so it must be kept on ice until the start of the sequencing Run, and is refrigerated on the instrument.

CAFIE Correction – Software correction for CArry Forward and Incomplete Extension.

Sequencing by synthesis in the Genome Sequencer FLX System depends on the synchronized addition of each nucleotide to each of the tens of millions of individual identical (clonal) copies of the DNA library fragment present on each bead. However, this reaction is not 100% efficient. In particular, the presence of long homopolymers (runs of the same nucleotide) on the DNA template being sequenced in a given well can lead to artificially higher or lower signals for neighboring flows of the same nucleotide in that well. In each flow, some fragments will typically fail to extend, or may not be extended completely to the end of a homopolymer (incomplete extension). Furthermore, any nucleotides left in the wells after rinse steps may cause multiple nucleotides to be added to certain fragments during subsequent nucleotide flows (carry forward).

Both incomplete extension and carry forward will result in a signal that is out of phase with the majority of the fragments on the bead. It can also cause neighboring flows to produce artificially higher or lower signals in a given well.

For example, incomplete extension of the homopolymer sequence 'AAAAAA' will result in a somewhat lower 'A' incorporation signal than expected for this 'A' flow. The fragments that fell out of phase due to incomplete extension will not incorporate any more bases in subsequent T, G, or C flows, reducing signal in one or more of these flows. At the next 'A' flow, the fragments that were not completely extended in the previous cycle will now complete extension, adding this signal to the new 'A' flow. Longer homopolymers tend to generate more carry forward and incomplete extension events and have larger effects on subsequent flows.

The Genome Sequencer FLX System basecalling software includes algorithms that identify and correct for CArry Forward and Incomplete Extension; these are called CAFIE correction software (part of the "Signal Processing" portion of the GS Run Processor application).

See also Carry Forward and Incomplete Extension.

Carry Forward – Under certain conditions (*e.g.* incomplete washing of the PTP device at one or more steps during a sequencing Run, or contamination of one nucleotide solution with another), a nucleotide may be incorporated prematurely into certain DNA fragments in a well. Such premature incorporations will artificially increase the signal during the flow that gets the misincorporation. Furthermore, the fragments that "carried forward" will subsequently be sequenced out of phase.

See also CAFIE Correction and Incomplete Extension.

Control DNA– A known sequence of DNA introduced into the experiment in order to provide calibration for the processing software. "Control DNA" reads are distinguished from the sample DNA library reads by the key used on the Control DNA Beads, which is different from the key present on the Adaptors used to prepare the sample libraries. The GS FLX Titanium chemistry kits also contain a different set of Control DNA Beads than the GS FLX standard chemistry kits, and use a different key.

DataRig – A DataRig is a Linux-based computer dedicated to running Genome Sequencer FLX System data processing and data analysis software. Preferred specifications for a DataRig are given in the *Genome Sequencer System Site Preparation Guide*.

Flow – During a sequencing Run, nucleotides are flowed sequentially across the PTP device, one at a time, in the cyclical order 'TACG', as controlled by the Run script. When the flowed nucleotide is complementary to the next nucleotide (or homopolymer) on the DNA template in any given well, the polymerase extends the nascent DNA strand in that well. Addition of one or more nucleotide(s) releases a corresponding number of pyrophosphate (PPi) molecules. One molecule of ATP is synthesized for each molecule of PPi released, causing a flash of light (signal) whose intensity is proportional to the number of nucleotides incorporated.

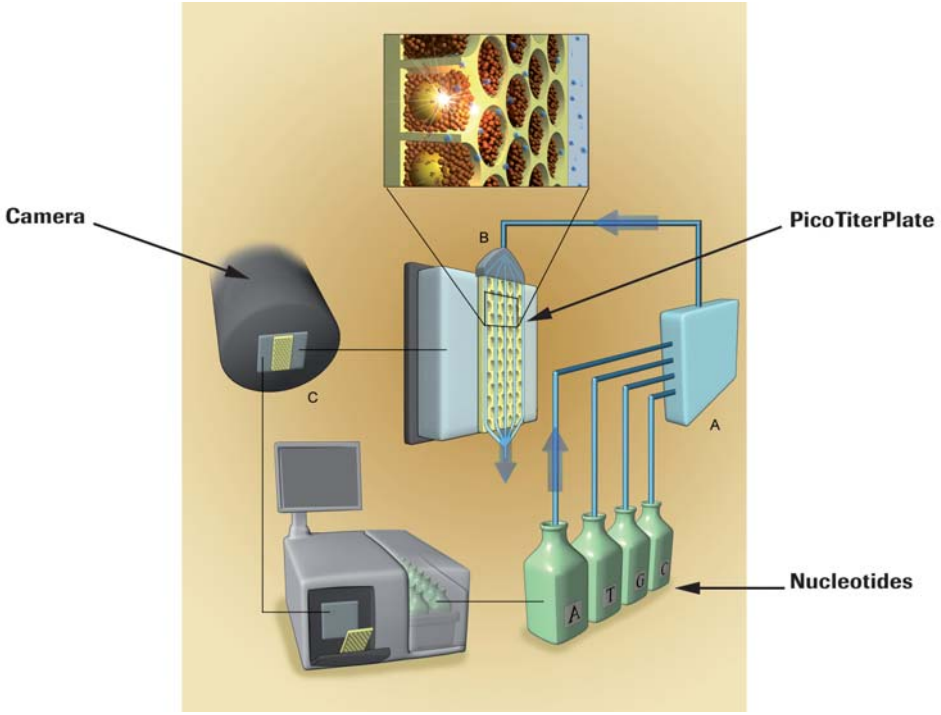


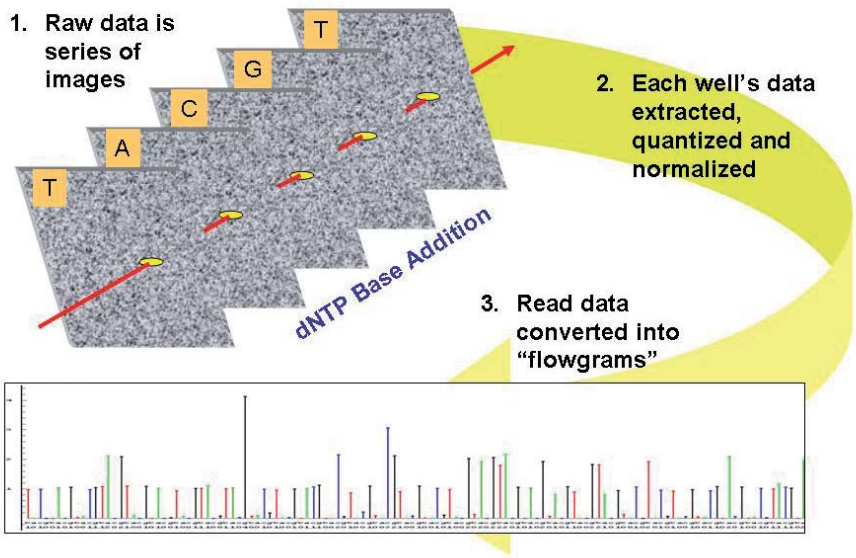
Figure 5–1: Schematic fluid path during a sequencing Run

For example, the flow order of the sequencing reagents for an "extra long reads" (XLR; GS FLX Titanium chemistry) Run is as follows:

1. ATP flow and wash (ATP will light up all the wells; establishes a positive signal)
2. 100 cycles of dNTP flows, with each nucleotide followed by washes
3. Another ATP flow and wash (to help monitor signal "drooping")
4. Another 100 cycles of dNTP flows (for a total of 200 dNTP flow cycles for the Run)
5. A third ATP flow (again, to monitor signal "drooping")

Signal intensity is measured in each well and recorded on each camera image; one image is captured at each nucleotide flow, and the complete set of images from a Run constitutes the sequencing data.

Flowgram – Data processing extracts information about the signal intensity in each well, over all flows. The signal intensity for each flow is plotted as a function of flow order, yielding a flowgram for the well. The signal intensity is proportional to the number of bases added (linear relationship): if no nucleotide is extended in that well during a flow, the signal will be very low (background); if one nucleotide is added, the signal will be similar in intensity to the key signal; if more than one nucleotide is added, the height of the signal will be correspondingly higher.



Flowspace – Data space corresponding to the signals generated at each nucleotide flow of the sequencing Run(s); as opposed to the nucleotide space, which corresponds to basecalls. The Genome Sequencer FLX Instrument captures the signal intensity of each nucleotide flow in each well during the sequencing Run. This allows data processing in Flowspace, using signal intensity of all nucleotide flows for that read over time.

A key advantage of working with Flowspace data is that it allows the averaging of flow signals (a continuous variable) at each nucleotide flow of the sequencing Run(s) rather than analysis of called bases (a discontinuous variable) at each nucleotide position. This signal averaging in Flowspace applies to read basecalling (GS Run Processor application) and consensus basecalling (GS *De Novo* Assembler, GS Reference Mapper, and GS Amplicon Variant Analyzer applications), improving the accuracy of the final basecalls and alignments. The added information available in Flowspace thus increases the power of the mapping and assembly algorithms.

Ghost Wells – In situations where the signal per base is very high, several non-DNA-containing wells (wells that contain only enzyme beads) that are adjacent to DNA-containing wells may be falsely identified as Keypass wells. This is especially likely to occur with Amplicon libraries, so the "Amplicon" Data Analysis pipeline includes a pair of screening algorithms to remove such ghost wells.

Incomplete Extension – In a sequencing Run, nucleotides are incorporated by a DNA polymerase during a succession of nucleotide flows, guided by a DNA template. During each round of synthesis, however, some templates on a bead fail to extend properly (incomplete extension).

As the number of nucleotides added during a single flow increases (long homopolymeric sequence), a higher percentage of templates on that bead fail to fully extend. In addition, the next time that a nucleotide is flowed, many of the incomplete templates will resume extension, thus sequencing 'out of phase' from the original cohort. This out of phase sequencing will lead to correspondingly higher signal intensity in the following flow, which can result in the 'overcall' of flows that follow long homopolymers. Incomplete extension can be measured and corrected; the Genome Sequencer FLX System software has been designed to recognize and compensate for incomplete extension, to improve basecalling accuracy.

See also CAFIE Correction and Carry Forward.

Key (or "key sequence") – The sequencing key is a known sequence of four nucleotides located immediately downstream from the sequencing primer. It is therefore the first to be sequenced in each well. The exact key sequence present in any given well is either (1) the key present on the Adaptors used to generate the sample DNA Library; or (2) the key present on the Control DNA Beads provided in the GS Sequencing Kits.

Sequencing keys always contain one each of the four nucleotides, so they always define single nucleotide incorporations for the first three extensions. (The fourth nucleotide of the key could be the first base of a longer homopolymer.) The key sequence serves as confirmation that the DNA sequence obtained from the well is valid, *i.e.* derived from the sample DNA library or from a Control DNA Bead. Also, the 3 single nucleotide incorporations from the key serve as a signal intensity reference for normalization and scaling.

Keypass Wells – Wells identified on the PTP device as containing a valid key sequence (a known sequence from the Adaptors used to prepare the sample DNA Library or from the Control DNA Beads). A keypass well is assumed to contain a legitimate DNA read. Keypass wells are the only wells that are further processed during downstream data processing.

Library – A library is a collection of DNA fragments representative of the entire DNA sample to be sequenced. Each library is created from user-supplied purified DNA. The Genome Sequencer FLX System supports 3 types of DNA Libraries:

- General DNA libraries (termed single-stranded template DNA (sstDNA) libraries in the GS FLX standard series literature) are for general purpose sequencing, *e.g.* shotgun sequencing of a genomic DNA sample, or sequencing of a collection of low molecular weight DNA species.
- Paired End libraries are used to order and orient contigs, *e.g.* those obtained by shotgun sequencing of an sstDNA library.
- Amplicon libraries are used to study discrete DNA regions, *e.g.* to identify and quantitate the prevalence of rare variants in a complex sample, by Ultra-Deep Sequencing.

In all libraries, each fragment to be sequenced is ligated to (or hybridized with) DNA Adaptors that carry the required sequencing key, as well as the amplification and sequencing primer sequences necessary for further processing. A prepared DNA library must be immobilized and amplified via the emulsion-based clonal amplification (emPCR) process before it can be sequenced with the Genome Sequencer FLX System.

Only non-MID General DNA libraries are currently supported by the GS FLX Titanium chemistry. For preparing and sequencing Paired End or Amplicon libraries, or for MID libraries of any type, users **MUST** continue to use the standard Genome Sequencer FLX kits, manuals, and procedures (last updated in December 2007).

MID – Multiplex Identifier. Short sequence that can be introduced immediately downstream from the Key sequence in all template of a DNA library (usually as part of the Adaptors used to prepare the library). MIDs can be recognized by the data analysis software of the Genome Sequencer FLX System and used to identify the library to which an individual read belongs. This feature allows multiple libraries tagged with different MIDs to be sequenced together, within an individual region of a PTP device.

Overcall – An overcall occurs when a homopolymer that is 'N' bases long is called as 'N+1' or greater.

PPi – Pyrophosphate. One molecule of PPi is liberated by the addition of each nucleotide during sequencing-by-synthesis, such as in the Genome Sequencer FLX System. In this system, the release of PPi is monitored following each nucleotide flow, to determine and quantitate nucleotide additions in each well of the PTP device.

Raw Wells – These are locations on the PicoTiterPlate device that the image processing software identifies as potentially having a DNA bead. A raw well is distinct from a keypass well in that the validity of that location has not yet been verified, and may just be optical spill-over from another well, camera noise or other image artifacts.

Signal per Base – The signal intensity in a given well that corresponds to the incorporation of a single nucleotide.

Singlet – Homopolymer of length 1, *i.e.* a nucleotide that is followed by a different nucleotide in the read sequence.

Undercall – An undercall occurs when a homopolymer that is 'N' bases long is called as 'N-1' or shorter.

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