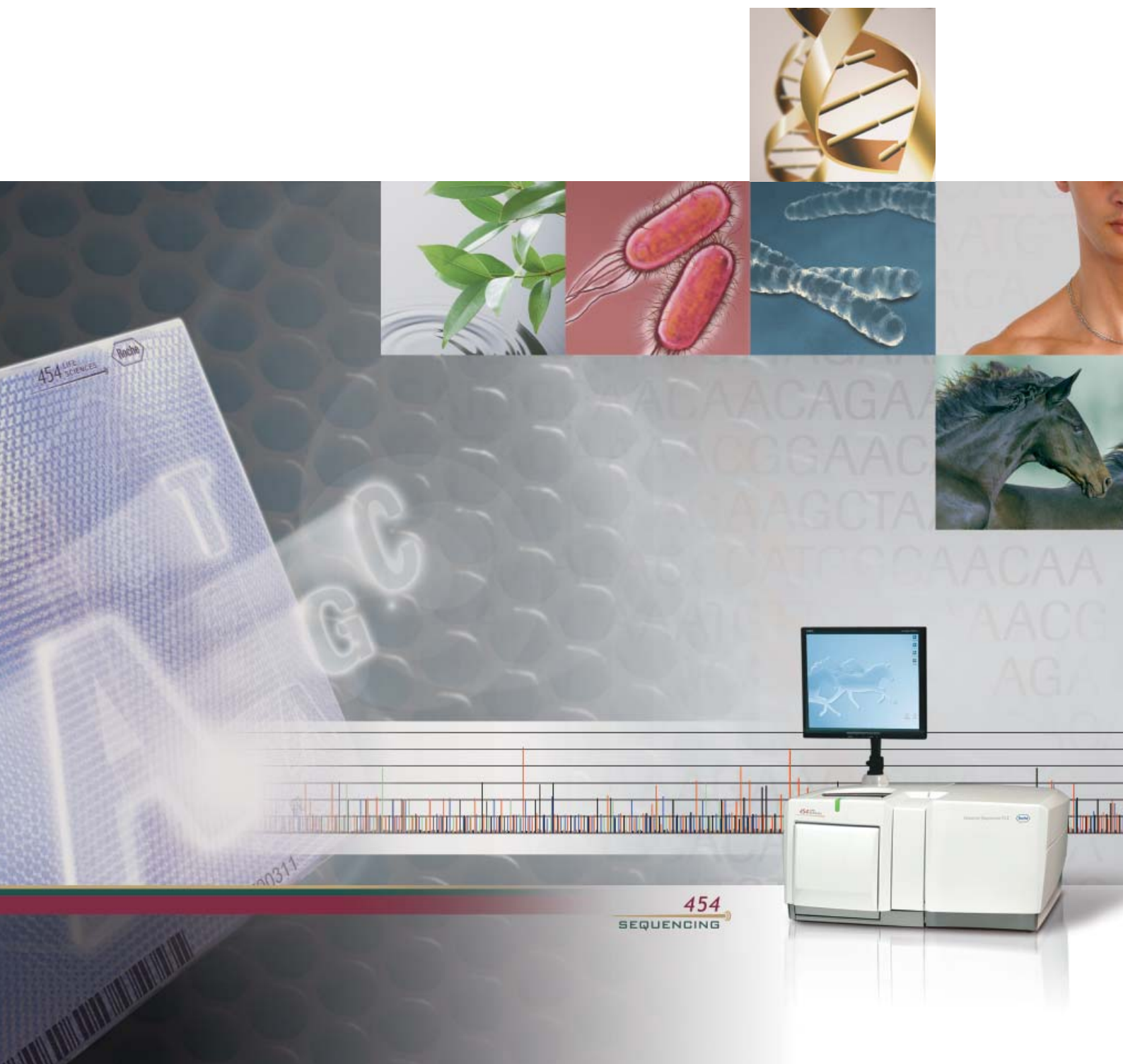


Genome Sequencer FLX Instrument Owner's Manual

October 2009



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1. Preface

1.1 About this Manual

The *Genome Sequencer FLX Instrument Owner's Manual* briefly describes the hardware and software components of the Genome Sequencer FLX Instrument, tells how to maintain the Genome Sequencer FLX Instrument, and provides guidelines for troubleshooting instrument problems.

1.2 Documentation Set

The documentation set describing the Genome Sequencer FLX System comprises the documents listed in Table 1. A brief description of each document is given below. At the time of publication, some of these are available in print and electronically (PDF) while others are available in electronic format only. Electronic documents can be downloaded from the 454 Life Sciences customer-restricted web page, at www.454.com/my454.




In the Genome Sequencer FLX System documentation, the phrase “Genome Sequencer System” refers to the 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.

Genome Sequencer FLX Instrument Owner's Manual
Genome Sequencer FLX System Research Applications Guide
GS FLX Titanium Methods Manual (binder)
Rapid Library Preparation Method Manual
Paired End Library Preparation Method Manual – 20 kb and 8 kb Span
Paired End Library Preparation Method Manual – 3 kb Span
cDNA Rapid Library Preparation Method Manual
Amplicon Library Preparation Method Manual
emPCR Method Manual – Lib-L LV
emPCR Method Manual – Lib-L MV
emPCR Method Manual – Lib-L SV
emPCR Method Manual – Lib-A LV
emPCR Method Manual – Lib-A MV
emPCR Method Manual – Lib-A SV
Sequencing Method Manual
Genome Sequencer FLX System Software Manual
Genome Sequencer FLX System Site Preparation Guide
Genome Sequencer FLX System Administrator's Guide

Table 1: Components of the Genome Sequencer FLX System documentation set

- ▶ *Genome Sequencer FLX Instrument Owner's Manual* – describes the Genome Sequencer FLX Instrument and provides information on its operation, maintenance, and troubleshooting.
- ▶ *Genome Sequencer FLX System Research Applications Guide* – serves as a navigational tool to help the user choose the best methods to carry out experiments using the Genome Sequencer FLX System; also includes a general description of the Genome Sequencer FLX System as a whole, as well as a specific theory of operation of each of the methods that compose it.
- ▶ *GS FLX Titanium Methods Manual*, including:
 - ▶ *Rapid Library Preparation Method Manual* – describes how to use the GS FLX Titanium Rapid Library Preparation Kit to prepare a DNA library suitable for sequencing with the Genome Sequencer FLX System, e.g. for shotgun sequencing.
 - ▶ *Paired End Library Preparation Method Manual – 20 kb and 8 kb Span* – describes how to use the GS FLX Titanium Paired End Adaptor Set and other reagents to prepare a library of Paired End DNA fragments spanning long (20 kb) or medium (8 kb) distances, and suitable for sequencing with the Genome Sequencer FLX System.
 - ▶ *Paired End Library Preparation Method Manual – 3 kb Span* – describes how to use the GS FLX Titanium Paired End Adaptor Set and other reagents to prepare a library of Paired End DNA fragments spanning short (3 kb) distances, and suitable for sequencing with the Genome Sequencer FLX System.
 - ▶ *cDNA Rapid Library Preparation Method Manual* – describes how to use a cDNA preparation kit in conjunction with the GS FLX Titanium Rapid Library Preparation Kit to prepare a DNA library suitable for sequencing with the Genome Sequencer FLX System, for cDNA/transcriptome sequencing.
 - ▶ *Amplicon Library Preparation Method Manual* – describes how to prepare a library of amplicons suitable for sequencing with the Genome Sequencer FLX System, e.g. for sequence variant detection, identification and quantitation.
 - ▶ *emPCR Method Manual – Lib-L LV* – describes how to use the GS FLX Titanium LV emPCR Kit (Lib-L) and the GS FLX Titanium emPCR Breaking Kit, LV/MV, 12 pcs, to carry out a large volume preparation of clonally amplified DNA fragments from an appropriately prepared shotgun, Paired End, or cDNA library, in a bead-immobilized form suitable for sequencing with the Genome Sequencer FLX System.
 - ▶ *emPCR Method Manual – Lib-L MV* – describes how to use the GS FLX Titanium MV emPCR Kit (Lib-L) and the GS FLX Titanium emPCR Breaking Kit, LV/MV, 12 pcs, to carry out a medium volume preparation of clonally amplified DNA fragments from an appropriately prepared shotgun, Paired End, or cDNA library, in a bead-immobilized form suitable for sequencing with the Genome Sequencer FLX System.
 - ▶ *emPCR Method Manual – Lib-L SV* – describes how to use the GS FLX Titanium SV emPCR Kit (Lib-L) and the GS FLX Titanium emPCR Filters, SV, 64 pcs kit, to carry out a small volume preparation of clonally amplified DNA fragments from an appropriately prepared shotgun, Paired End, or cDNA library, in a bead-immobilized form suitable for sequencing with the Genome Sequencer FLX System.
 - ▶ *emPCR Method Manual – Lib-A LV* – describes how to use the GS FLX Titanium LV emPCR Kit (Lib-A) and the GS FLX Titanium emPCR Breaking Kit, LV/MV, 12 pcs, to carry out a large volume preparation of clonally amplified DNA fragments from an appropriately prepared Amplicon DNA library, in a bead-immobilized form suitable for sequencing with the Genome Sequencer FLX System.
 - ▶ *emPCR Method Manual – Lib-A MV* – describes how to use the GS FLX Titanium MV emPCR Kit (Lib-A) and the GS FLX Titanium emPCR Breaking Kit, LV/MV, 12 pcs, to carry out a medium volume preparation of clonally amplified DNA fragments from an appropriately prepared Amplicon DNA library, in a bead-immobilized form suitable for sequencing with the Genome Sequencer FLX System.

- ▶ *emPCR Method Manual – Lib-A SV* – describes how to use the GS FLX Titanium SV emPCR Kit (Lib-A) and the GS FLX Titanium emPCR Filters, SV, 64 pcs kit, to carry out a small volume preparation of clonally amplified DNA fragments from an appropriately prepared Amplicon DNA library, in a bead-immobilized form suitable for sequencing with the Genome Sequencer FLX System.
 - ▶ *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.
 - ▶ *Genome Sequencer FLX System 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.
 - ▶ *Genome Sequencer FLX System Site Preparation Guide* – describes how to set up an optimal environment for the successful operation of the Genome Sequencer FLX System, including recommendations for the organization of the laboratory space. Installation of the Genome Sequencer FLX Instrument is performed by a Roche Representative.
 - ▶ *Genome Sequencer FLX System Administrator’s Guide* – describes how to set up the computing and networking environment for the successful operation of the Genome Sequencer FLX System.
-  The *GS FLX Titanium General Library Preparation Method Manual*, last published in April 2009, and the corresponding GS FLX Titanium General Library Preparation Kit are still available. However, the General library preparation method is now superseded by the Rapid library preparation method, which fulfils the same purposes.

1.3 Revision History

Revision Date	Instrument Version	Chemistry	Software Version
September 2005	GS 20	--	1.0.50
November 2005	GS 20	--	1.0.51
April 2006	GS 20	--	1.0.52
September 2006	GS 20	--	1.0.53
December 2006	GS FLX	Standard	1.1.01
June 2007	GS FLX	Standard	1.1.02
December 2007	GS FLX	Standard	1.1.03
October 2008	GS FLX	Titanium (Phase A)	2.0.00
April 2009	GS FLX	Titanium (Phase B)	2.0.01
October 2009	GS FLX	Titanium (Phase C)	2.3.00






Every effort has been made to ensure that all the information contained in the Genome Sequencer FLX System documentation was correct at the time of release. However, 454 Life Sciences Corporation and Roche Diagnostics GmbH reserve the right to make corrections, clarifications, updates, or any other changes deemed necessary, for any reason, without advance notice.

No part of the Genome Sequencer FLX System documentation may be reproduced or transmitted in any form or by any means, electronic or mechanical, for any purpose, without express written permission. 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

1.4 Icons

Various precautionary statements are presented in the Genome Sequencer System documentation. 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.
	Note	Identifies items of general interest and additional information about the topic or procedure being described.
		Table continued on next page.
		End of table.

All Genome Sequencer System Manuals, Guides and Bulletins are available for download in PDF format from the customer-restricted access area of www.454.com/my454.

1.5 Assistance

Prior to and during instrument setup, your Roche Representative will 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 FLX System. Please contact your Roche Representative for more details.

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



When calling for assistance, be prepared to provide the serial number of your Genome Sequencer FLX 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 (toll-free): 1-800-262-4911	e-mail: us.gssupport@roche.com
Europe, Middle East, Mexico, South America or Africa	phone: +49-8856-60-6457 or toll-free +800SEQUENCE	e-mail: service.sequencing@roche.com
Asia Pacific	Phone (toll-free): 800 820 0577 (China Mainland) 008 018 63123 (China Taiwan) 800 966 851 (China Hongkong) 800 852 3686 (Singapore) 180 002 4137(Australia) 080 088 2009 (New Zealand) 007 988 620647 (Korea)	e-mail: asc.support@roche.com
Japan	phone: +03-5443-5287	e-mail: tokyo.biochemicals@roche.com

1.6 Disclaimers

1.6.1 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.

1.6.2 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 or RNA 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.

1.6.3 Notice to Purchaser

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

1.6.4 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).

1.6.5 Trademarks

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

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


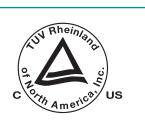
1.6.6 License Disclaimers

This product incorporates technology licensed from Promega Corporation that is protected under US Patents 5,583,024, 5,674,713 and 5,700,673.


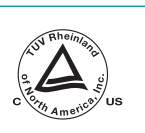
Restriction on use. As a condition of sale of this product, purchaser agrees not to use the product to perform less than 1,536 sequencing reactions on a sample or samples without changing the substrate. Failure to comply with this restriction will result in an infringement of patent rights and other intellectual property rights of seller or third parties and a breach of the terms of sale of this product.


1.6.7 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:

	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”.
	The instrument meets the requirements of NRTL standards UL61010, CAN/CSA C22.2 No. 61010-1.

The following symbols appear on the instrument:

	CE MARK	The CE mark on the instrument type plate expresses conformity with essential requirements of the directives “Electromagnetic Compatibility” and “Low Voltage Equipment” relevant for this instrument.
	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 Owner’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.

1.6.8 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.

1.6.8.1. 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.

1.6.8.2. 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 lodged 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.

1.6.8.3. 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.

1.7 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).


1.8 Genome Sequencer System Site Requirements

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

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

1.9 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).

2. The Genome Sequencer FLX Instrument and 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 Instrument Owner's Manual* provides a general description of the Genome Sequencer FLX Instrument, and information on how to operate, maintain and troubleshoot the instrument. For more information on the theory of operation of the Genome Sequencer FLX System, see the *Genome Sequencer FLX System Research Applications Guide*.

This section describes:

- ▶ Section 2.1: Instrument Components
- ▶ Section 2.2: Overview of the On-Instrument Software Applications
- ▶ Section 2.3: Overview of a Sequencing Run
- ▶ Section 2.4: Overview of Data Processing in the Genome Sequencer FLX System

2.1 Instrument Components

2.1.1 Hardware Components

2.1.1.1. 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).

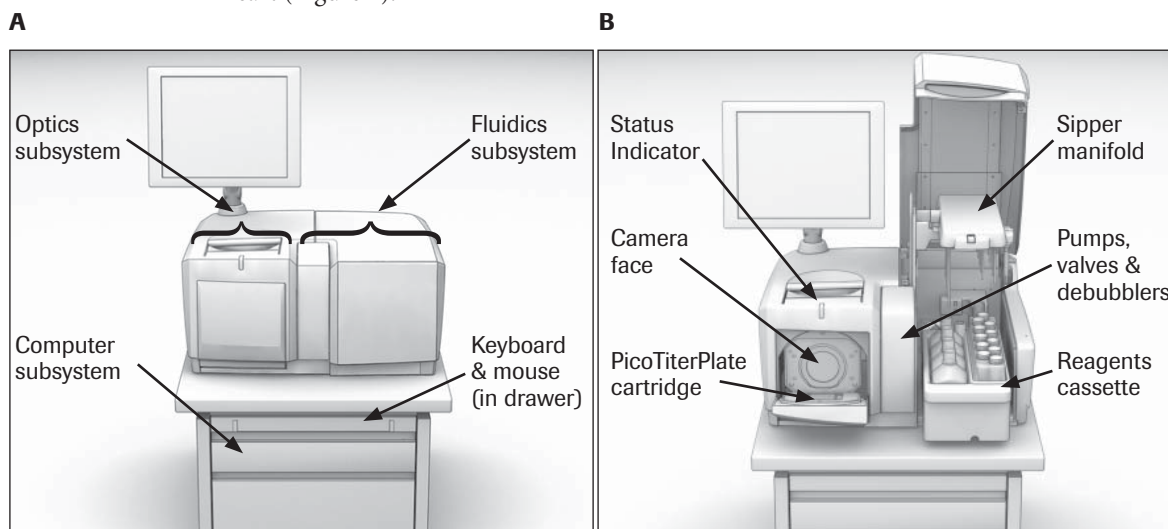


Figure 1: Main components of the Genome Sequencer FLX Instrument

(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 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 Genome Sequencer FLX 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 B), which shows at a glance the general status of the instrument (For details on the function of this LED, see Table 4 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).

2.1.1.2. 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 Genome Sequencer FLX Instrument at the same rate as the instrument can generate it. The cluster is matched to the Genome Sequencer FLX Instrument 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 Genome Sequencer FLX System data processing tasks
- ▶ Installation and testing of all Genome Sequencer FLX System applications and tools
- ▶ Data and power redundancy configured for improved reliability

2.1.1.3. 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 2). 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.

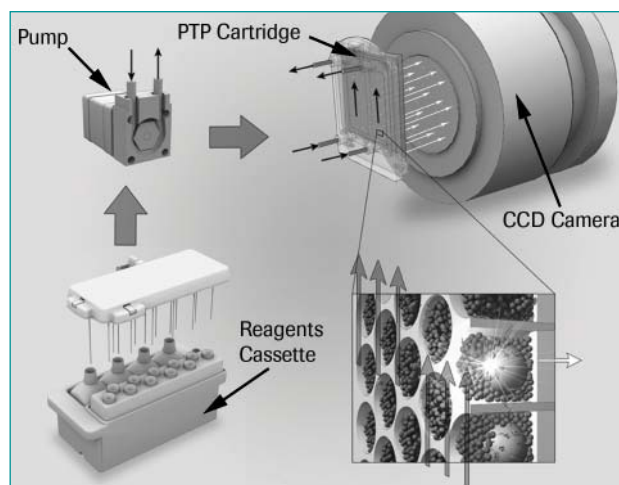


Figure 2: Schematic showing how the Genome Sequencer FLX Instrument works

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.

2.1.1.4. 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 2). The purpose and usage of these accessories are described elsewhere in this manual and in the relevant Genome Sequencer FLX System 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	Camera Faceplate Guard
pair	emPCR Shaker Adaptors LV
pair	emPCR Shaker Adaptors MV
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	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 2: Contents of the Genome Sequencer FLX Accessory set provided with the instrument

2.1.1.5. Other Hardware/Installation Components Not Supplied

In addition to the Genome Sequencer FLX 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 FLX 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 Titanium Method Manuals* and the list provided on the customer-restricted access area at www.454.com/my454.
- ▶ 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 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 FLX System Software Manual*; for DataRig and computer cluster specifications, see the *Genome Sequencer FLX System Administrator's Guide*.

2.1.2 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

2.1.3 General Consumables Not Supplied

Runs on the Genome Sequencer FLX Instrument require various consumables that are not supplied. For details, see the list of materials available on the customer-restricted access area at www.454.com/my454.

2.2 Overview of the On-Instrument Software Applications

Individual functions of the Genome Sequencer FLX Instrument control software are packaged into distinct software applications, each invoked when the operator double-clicks the appropriate icon on the computer desktop (Figure 3).

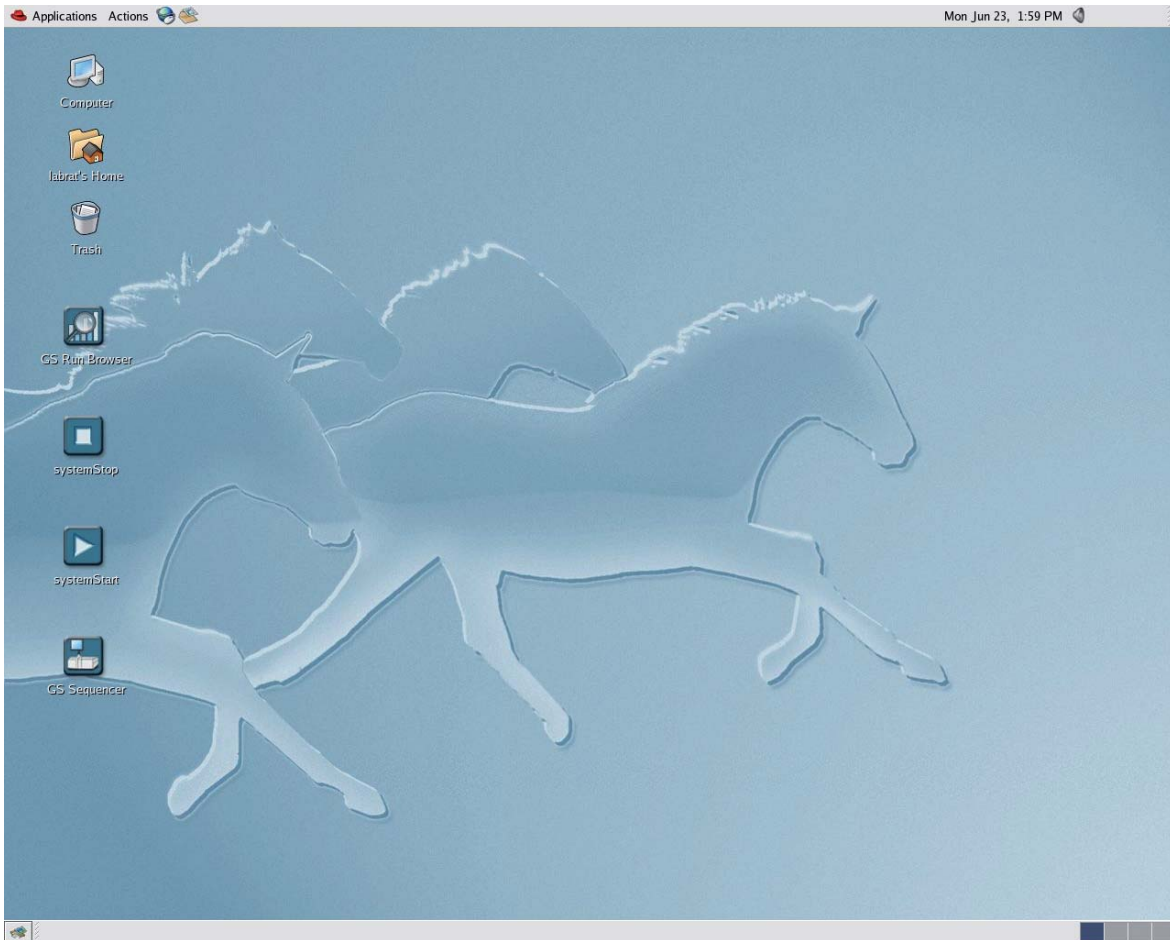


Figure 3: The Genome Sequencer FLX Instrument main screen, showing the operator applications launch icons

Table 3 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. All software applications are described in the *Genome Sequencer FLX System Software Manual*.





ICON	APPLICATION	FUNCTION
	System Start	Starts the Genome Sequencer FLX Instrument's background processes ^a
	System Stop	Stops the Genome Sequencer FLX Instrument's background processes ^b
	GS Sequencer	Set up and management of a Run Data management Instrument monitoring
	GS Run Browser	Post-Run diagnostics User-friendly access to data metrics output

Table 3: Applications available to Operators of the Genome Sequencer FLX Instrument

^a Normally, the Genome Sequencer FLX Instrument is not turned off (see Section 2.3.1). If it is turned off for any reason, it must be re-started using the System Start application.

^b Under normal circumstances, the Genome Sequencer FLX Instrument software should be left running at all times (see Section 2.3.1).



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 FLX System Software Manual*.



/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.

2.3 Overview of a Sequencing Run

2.3.1 Continuous Instrument Operation

The Genome Sequencer FLX Instrument is designed for continuous operation, except when it needs scheduled maintenance (as described in section 3.6 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 PicoTiterPlate device (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 *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.

2.3.2 Before You Begin a Sequencing Run

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

2.3.2.1. 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 instrument is doing (see Table 4). 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.
- ▶ 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 and section 3.1.
- ▶ The camera door is closed, with the spent PTP device and cartridge seal in place, in the PTP cartridge.
- ▶ 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 3.2, should be performed before a sequencing Run is started.

2.3.2.2. 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 (Rapid, Paired End, cDNA, or Amplicon library)
 - ▶ For more information, see the appropriate library preparation *Method Manual*, and the *emPCR Method Manual*.
 - ▶ **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 *Sequencing Method Manual*.
 - ▶ **Non-consumable accessories for the instrument:** Table 2 lists the accessories supplied with the Genome Sequencer FLX Instrument. Most of them will be needed for the sequencing Run, including one of the Bead Deposition Devices (as appropriate for the specific requirements of the experiment).
 - ▶ **One GS FLX Titanium Sequencing Kit XLR70 and one GS FLX Titanium PicoTiterPlate Kit 70 × 75:** These kits contain the reagents and other materials needed for a sequencing Run.
 - ▶ For more information on the use of these kits, see the *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 *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.


2.4 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 FLX System 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 Rapid libraries made from a high molecular weight DNA sample (e.g. shotgun libraries of 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 Rapid 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 reads. The output is similar to the one described above, but also contains contig scaffolding information (in AGP format).

- ▶ Reads from a cDNA library are used to get information from transcripts in a sample. They can be analyzed either using the GS *De Novo* Assembler software or the GS Reference Mapper software, depending on whether reference sequence (transcriptomic or genomic) information is available for the sample sequenced. Again, the output is similar to the one described above, but also contains specific information relevant to transcriptomics/cDNA sequencing experiments such as putative exon boundaries, transcripts (isoform) consensus sequences, etc.
 - ▶ 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.

3. Instrument General Procedures and Maintenance

This section describes certain general procedures you may have to carry out while working with your Genome Sequencer FLX Instrument, either in the course of normal operation or for the maintenance or testing of the instrument. A list of the items covered by the regular service of the instrument performed by a Roche Field Service Representative is also provided. The sub-sections are:

- ▶ Section 3.1: Sipper Tube Replacement
- ▶ Section 3.2: Sterilization of the Fluidics Components (“Maintenance Wash Run”)
- ▶ Section 3.3: Test Sequencing Run with Control DNA Beads
- ▶ Section 3.4: Leaving the Genome Sequencer FLX Instrument Idle for More Than 7 Days
- ▶ Section 3.5: Complete Instrument Shutdown and Instrument Start
- ▶ Section 3.6: Regular Service

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 4). 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 4: Sipper manifold buttons

- ▶ The “short” Sipper Tubes provided in the GS FLX Titanium Sequencing Kit XLR70 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 5 **A**) of the sipper manifold.
- 2 If present, slide out the Reagents cassette, toward the front of the instrument (Figure 5 **B**).

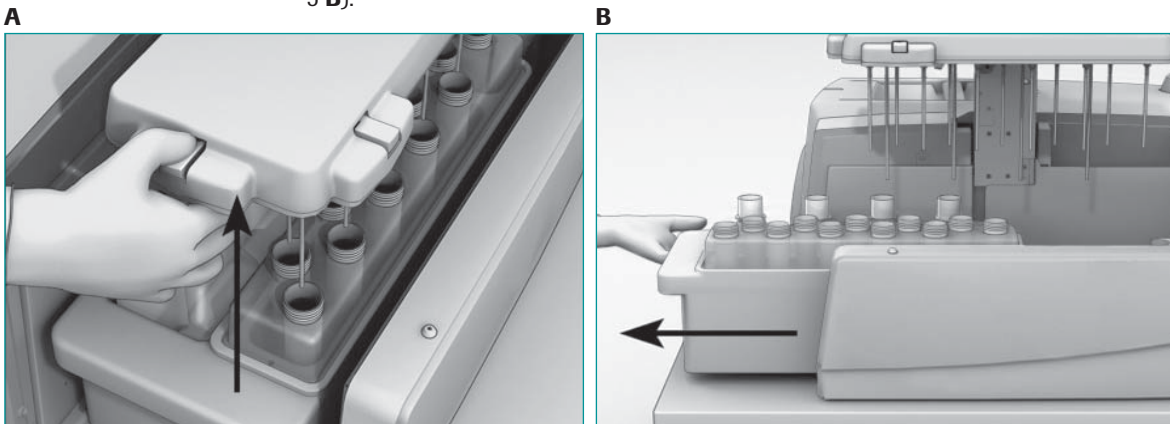




Figure 5: Removing the Reagents cassette

(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 6 **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 6 **B**).
- 4 With your free hand, unscrew the Sipper Tube(s) you want to replace (Figure 6 **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 6 **D**).

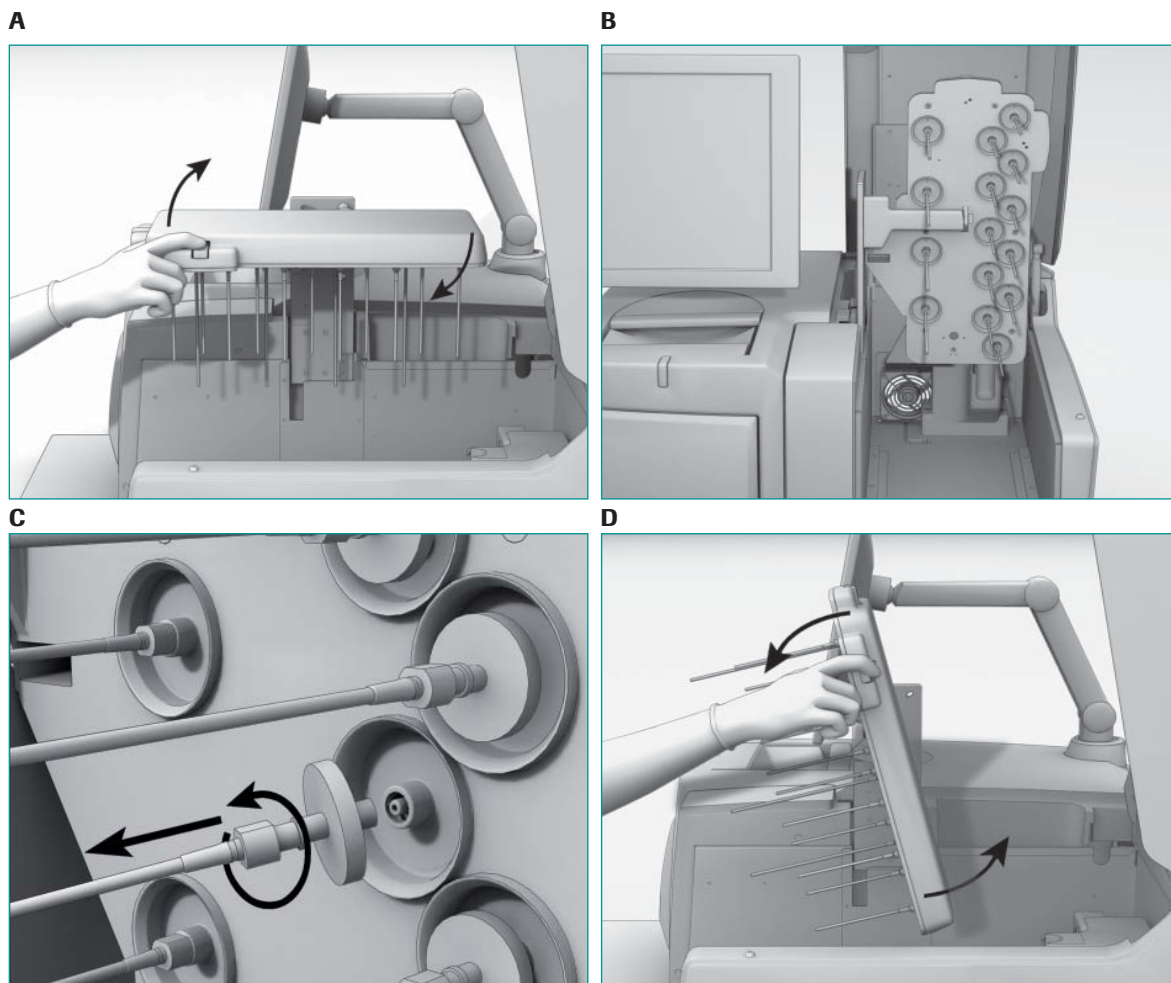


Figure 6: Replacing Sipper Tubes

(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 counterclockwise)

(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 *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 *Sequencing Method Manual*. For more information on a maintenance wash Run, see section 3.2, below.

3.2 Sterilization of the Fluidics Components (“Maintenance Wash Run”)

All sequencing Runs end with a maintenance wash that uses a combination of sodium chlorite 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 3.4 and 3.5 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 *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 Kit XLR70. The 4-tube “Maintenance Wash Buffers tray” should sit at the bottom of the left-hand side of the Cassette, as shown in Figure 7.

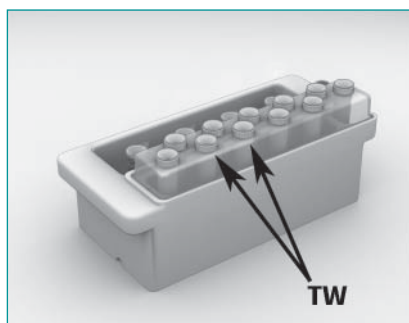





Figure 7: All reagents loaded into the Reagents cassette, for a maintenance wash Run

- 3 Remove the caps from all the reagent tubes.



- 4 Load the 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 reagent tubes. If any of the Sipper Tubes are bent due to missing the tube opening, raise the sipper manifold, remove the 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’s first window: Choose a procedure.
 - ▶ The Run Wizard has only two windows for maintenance wash Runs.
- 9 Select the “Service” Option and click the Next button. This opens the second Run Wizard window: Choose a Service.
- 10 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.
 -  Do not follow the Maintenance Wash Run by a pre-wash procedure unless you are preparing a sequencing Run. If the Genome Sequencer FLX Instrument is not being prepared for a sequencing Run, leave the previous reagents on the instrument. The maintenance wash reagents are automatically flushed through the lines at the end of each sequencing Run and should remain in place until the next Run.

3.3 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 Titanium 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 Titanium Control Beads Kit, along with a GS FLX Titanium Sequencing Kit XLR70 and a GS FLX Titanium PicoTiterPlate Kit 70 × 75.

The Pack Insert of the GS FLX Titanium 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 Titanium Control Beads Kit, the procedure is otherwise identical to a normal sequencing Run, as described in the *Sequencing Method Manual*.

3.4 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 3.2 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 *Sequencing Method Manual*.

- 1 Perform a maintenance wash Run as described in section 3.2.
- 2 Perform a pre-wash according to the procedure described in the *Sequencing Method Manual*.
- 3 Remove the Reagents cassette from the instrument and discard the used reagents, as described in the *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 *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 .
 - b. Close the GS Sequencer software 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.
- 9 Click the Red Hat icon on the taskbar and select "Logout".
 - ▶ This logs you out of the Genome Sequencer FLX System software.
- 10 Clean the PTP cartridge as follows:
 - a. Open the camera door.
 - b. Remove the used PTP device and the cartridge seal.
 - c. Clean the PTP cartridge 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.
 - d. Rinse thoroughly with nanopure water and dry using a paper towel.
 - e. Store with the Genome Sequencer FLX Instrument.
- 11 Clean the camera face plate as described in the *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 *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 FLX System software.
- ▶ 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 3.5, rather than leaving it idle.

3.5 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 3.4 with the following two differences:

- 1 In section 3.4, 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 9, 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 8).



Figure 8: 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 FLX System software.
- 7 Proceed directly with any procedure you want to carry out on the instrument, e.g. sequence a DNA library as described in the *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 9). Complete shut off of the instrument occurs approximately 5 seconds after you flip the switch, due to the powering down of the UPS.

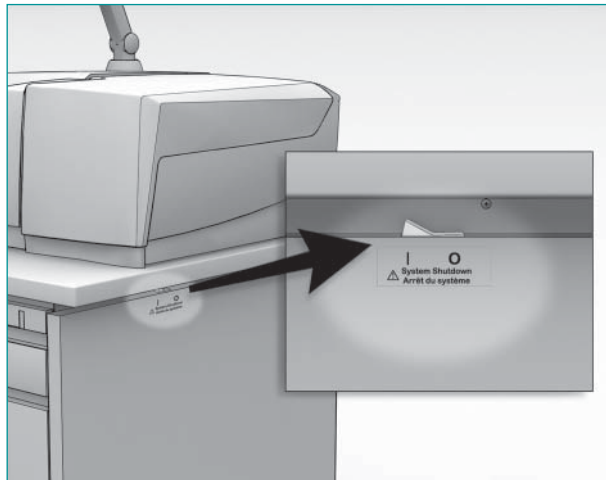


Figure 9: The System Shutdown switch of the Genome Sequencer FLX Instrument

3.6 Regular Service

The Genome Sequencer FLX Instrument should undergo scheduled preventative maintenance by Genome Sequencer 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 at the back of the PTP cartridge. 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 30°C 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 on the GS Sequencer application window: through the sensor displays on the Instrument tab; through messages which appear in the Status area; 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). 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 (<i>e.g.</i> 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, this 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: 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 about 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.
25 × 75 cartridge detected. Cartridge seal detection started.	After the PTP cartridge is detected, the cartridge seal detection starts.
70 × 75 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/Action
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 until you have verified that the cartridge is in place.
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.
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.



Message	Definition/Action
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.

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