

1. Workflow



This procedure can also be used to perform a test sequencing Run, using only Control DNA Beads. This requires a GS FLX Titanium Control DNA Beads Kit, in addition to all the materials used for a standard Run. The only difference is the preparation of the DNA Beads. For details of the preparation of the Control DNA Beads, please see the Package Insert to the GS FLX Titanium Control DNA Beads Kit.

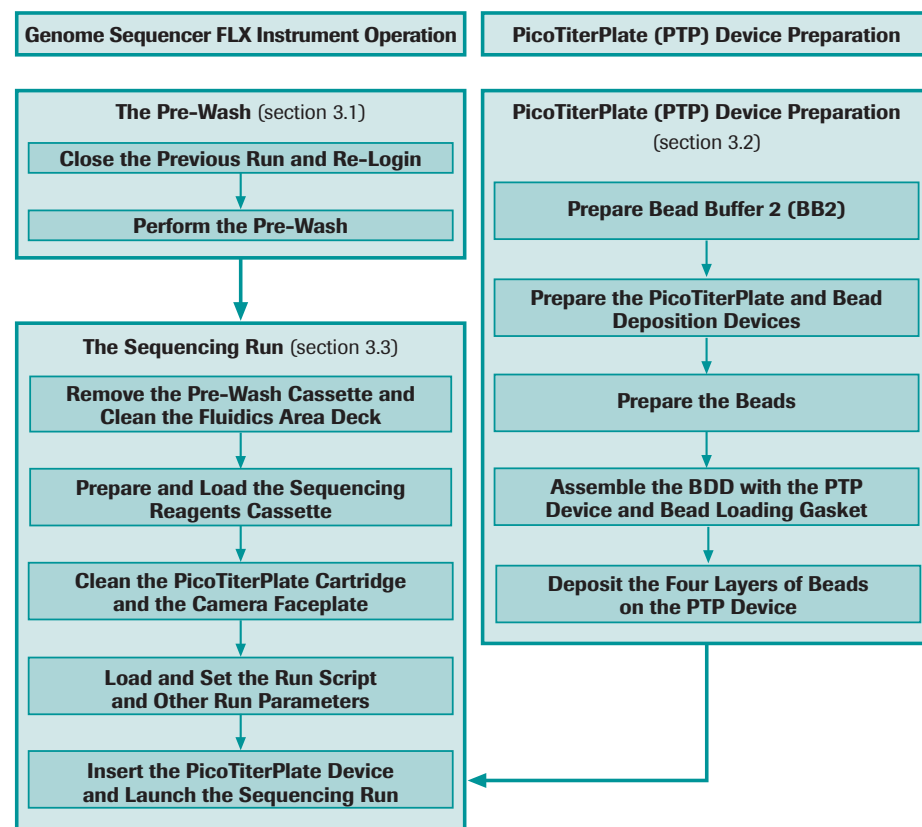


Figure 1: Workflow of a sequencing Run performed on the Genome Sequencer FLX Instrument

Note that instrument preparation and PicoTiterPlate device preparation are done in parallel such that the sequencing Run is initiated as soon as the PicoTiterPlate device is ready.

2. Before You Begin

2.1 What You Should Have Before Starting

2.1.1 Sample

The sample library being sequenced must have been prepared using the GS FLX Titanium methods and kits. The library will then have been amplified using one of the GS FLX Titanium emPCR Kits.

2.1.2 Required Genome Sequencer FLX System Equipment and Reagents

Refer to the customer-restricted 454 Web page at www.454.com/my454 for the lists of nonconsumables and material required but not provided.

2.1.3 GS Sequencing Kit

The GS FLX Titanium Sequencing Kit XLR70 is used in combination with the matching GS FLX Titanium PicoTiterPlate Kit 70 × 75. These kits provide reagents and components necessary for a single sequencing Run. Refer to the customer-restricted 454 Web page at www.454.com/my454 for a complete list of kits and reagents.

2.1.4 Choosing the Size and Number of PTP Regions for Your Sample

The GS FLX Titanium Sequencing and PicoTiterPlate Kits support various bead loading region configurations. The size and number of PTP regions depend on the sequencing throughput requirements of the experience, in total bases or total reads. To determine the proper gasket size, proceed as follows:

Use Table 1 to identify the size and number of bead loading regions best suited to yield the sequencing throughput for your DNA library sample. The Mbp throughput presented here is typical for good “Extra Long Read”, 200 cycles, sequencing Runs performed a good quality shotgun libraries. For example, column 5 shows the expected throughput of a full sequencing Run carried out with the longest run script available (200 cycles, generating an average read length of 350 to 450 nt), with all regions loaded.

Region Size	PTP Device Size	Regions per PTP Device	Bases/Region (Mbp)	Bases per Full PTP Device (Mbp)	Reads/Region (× 10 ³)
Large	70 × 75 mm	2	180 – 280	360 – 560	450 – 650
Medium	70 × 75 mm	4	60 – 110	240 – 440	160 – 250
Medium/Small	70 × 75 mm	8	30 – 55	240 – 440	80 – 120
Small	70 × 75 mm	16	10 – 20	160 – 320	25 – 40

Table 1: Gasket specification and throughput