Optimization of the Roche/454 & Illumina Production Sequencing Pipelines at the DOE Joint Genome Institute

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ABSTRACT

The U.S. Department of Energy (DOE) Joint Genome Institute's (JGI) Production Sequencing group is committed to the generation of high-quality genomic DNA sequence to support the mission areas of renewable energy generation, global carbon management, and environmental characterization and clean-up. Within the JGI's Production Sequencing group, a robust Roche Genome Sequencer and Illumina Genome Analyzer pipeline has been established. Optimization of these sequencer pipelines has been ongoing with the aim of continual process improvement of the laboratory workflow. These process improvement projects are being lead by the JGI's Process Optimization, Sequencing Technologies, Instrumentation & Engineering, and the core Roche/454 and Illumina/GA Production groups. Primary focus has been on improving the procedural ergonomics and the technician's operating environment, reducing manually intensive technician operations with tools and robotic automation, reducing associated production costs, and improving the overall process and generated sequence quality.

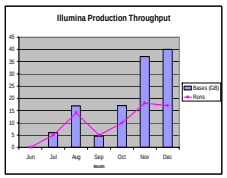
Introduction

The DOE Joint Genome Institute (JGI) was established in 1997 to unite the expertise and resources in genome mapping, DNA sequencing, technology development, and information sciences pioneered at the DOE genome centers of Lawrence Berkeley National Laboratory (LBNL), Lawrence Livermore National Laboratory (LLNS), and Los Alamos National Laboratory (LANL). In January 1999, high-throughput DNA sequencing began at the Production Genomics Facility (PGF) in Walnut Creek, California.

Platform	*Staff*	*Instruments*
454	9	8
Illumina	2	7

JGI Illumina Production Pipeline

In July 2008, Illumina transitioned to production with a single GAII and two full time operators. By August the production group moved to new lab and purchased two new GAII's. In November two additional GAII's and four paired end modules were delivered. With the additional instruments the Illumina group now has the capacity to run two paired end and six single read runs a week.



Illumina throughput of number bases collected and number of sequencing runs since moving to production July 2008

FY2009 Metrics to date	Avg. Base/Run	Capacity	Runs	Bases Collected
Standard Read	1.59Gb*	6 runs/wk	39	61.2Gb
Paired End	4.03Gb*	2 runs/wk	11	46.1Gb
Totals		8 runs/wk	50	107.3Gb

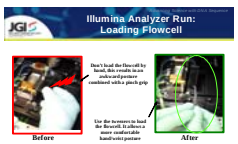
*Illumina progress towards DOE FY09 goals; *36 cycle runs*

JGI Illumina LIMS

In December 2008, a new LIMS system was released that included: a run statistic summary report, an improved flow cell setup page with an reanalysis request page, a workflow for registering runs that will reduce run folder naming errors, and reliable script for uploading run data to the database.

JGI Illumina Ergonomics

In an effort to ensure that production work is performed safely, best practice guides on how to perform lab work ergonomically were developed. An ergo points program was implemented to help determine the number and combination of work tasks that an operator can perform safely in a day. Currently, the JGI instrumentation group and Illumina are also working on improving the ergonomics of the GAII analyzer by creating quick disconnect reagent bottle adaptors as well as increasing the reagent tubing length for easier access.

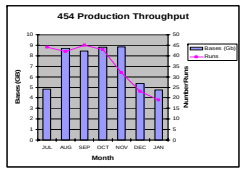


An example of an Illumina Best Practice Guide

Pictures of the quick disconnect bottle adaptors for the Illumina GAII Analyzer

JGI 454 Production Pipeline

In FY2008, the JGI 454 production group was able to scale up the number of sequencing runs by accomplishing three major milestones:



454 throughput of number bases collected & number of sequencing runs since moving into the new sequencing lab in July 2008

1. In July 2008, the JGI opened its new technology sequencing laboratories to house next generation instruments & production pipelines & operations.

2. In August, the number of 454 instruments in production increased from two to six.

FY2009 Metrics to date	Avg. Base/Run	Capacity	Runs	Bases Collected
Titanium	336Mb*	14 runs/wk	79	26.6Gb
FLX	77Mb**	2 runs/wk	43	3.3Gb
Totals			122	29.9Gb

*454 progress towards DOE FY09 goals; *2 & 4 region; **2,4, & 16 region*

3. The group has continued to grow through the year and now has 9 full-time staff. With the present staffing and planned process improvements, including automation, the group will have the capability to perform 20+ runs per week by the end of FY2009

JGI 454 Lab Ergonomics

When the new technology laboratories were designed, features such as advanced ergonomic workstations, equipment, and tools that allow the technicians to safely and efficiently prepare and sequence samples were incorporated.

Tube & Bottle Openers

There are a variety of openers for tubes & vials for operators to choose.

Ergonomic lab tools for uncapping bottles and tubes

Ergonomic lab bench

Work Space

Lab bench workspace is set up with height adjustable tables & features cutouts for optimal placement of supplies and arm/hand working heights.

Assessment of High GC Bias in 454 Titanium at JGI

Since the launch of 454 Titanium platform in October 2008, the JGI Technology Development group made a thorough assessment of the 454 Titanium platform that included looking at coverage in high GC regions. 454 FLX and Titanium platforms are compared in two high GC genomes:

Genome	Genome Size (MB)	GC of Genome (%)	454 System	Fold Coverage (X)	GC of Gaps (%)	Bases Not Covered (%)
I	3.6	72.08	FLX	22.61	73.62	0.43
			Titantium	50.68	78.54	6.23
II	4.3	71.89	FLX	27.23	67.14	0.31
			Titantium	39.15	77.47	6.20

Comparison of FLX and Titanium run performance of two high GC genomes.

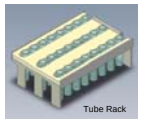
To alleviate the problem of low coverage in high GC regions observed in 454 Titanium, an optimized emulsion PCR protocol was developed at JGI. The optimized procedure significantanly improves the coverage in high GC regions. Applying the optimized method to high GC genomes, we observed at least a 21 fold improvement in base coverage over standard 454 Titanium procedure and a 4 fold improvement over 454 FLX protocol.

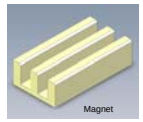
JGI 454-Titanium Enrichment Process Automation

The manual 454-Titanium Enrichment Process consists of a series of steps that require repetitive capping, centrifuging and pipetting of small sample tubes. It is a labor intensive operation that takes 2-3 hours to process 4 tubes.

All of the steps have been determined to be automatable and JGI has decided to achieve this goal through a collaboration with Hamilton Instruments.

The deck layout of the Hamilton STAR includes heating, cooling, rotating and centrifugation positions.





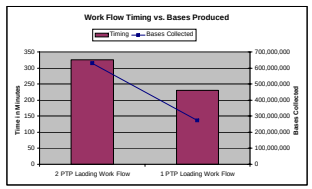
The custom 24 tube rack will interface with all deck positions: magnet, centrifuge and heating locations.

The Enrichment Instrument will combine Hamilton's STAR pipetting instrument, a SAIS Ixion centrifuge, Inheco heating elements and Lawrence Berkeley Laboratory's ability to produce custom magnets.

The resulting instrument will be capable of processing 24 samples in a single 2-3 hour run. A 6 fold increase in user productivity. Custom reservoirs may be developed to reduce tip waste.

JGI 454 Optimization of Loading Work Flow

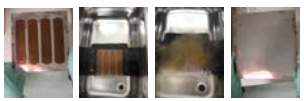
With recent ergonomic improvements to the 454 Titanium process an opportunity for optimization of the loading work flow presented itself. By loading 2 PTPs simultaneously, a single research technician can produce greater than twice as many bases with only 30% more effort.



Loading Work Flow (4-region run)	2 PTP Loading Work Flow 5 hr, 25 min	1 PTP Loading Work Flow 3 hr, 50 min
Register run, perform Pre-wash	60 min	45 min
Clean Bead Depo device		
Soak PTPs in BB2		
wash Packing, Enzme, and PPiase beads		
Break	15 min	15 min
Prepare bead layers and sequencing beads	60 min	30 min
Lunch	30 min	30 min
Add BIM to Sequencing beads and incubate, remove pre-wash cassette, begin preparation of reagent cassette	90 min	45 min
Break	15 min	15 min
Load all four layers onto PTPs, complete reagent cassette prep, clean sequencer optics	45 min	45 min
Load PTPs and start runs	10 min	5 min

Titanium PTP Reuse

A water bath sonication process has been developed at the JGI to clean sequenced PTPs. This allows a single PTP to be reused multiple times creating a cost savings of at least 66%.



Series of pictures showing a used Titanium PTP being cleaned via sonication for 1.5 minutes.

Enrichment Optimization

In our efforts to minimize ergonomic risk to operators for the enrichment process we have tested the reduction of the number of enhancing fluid washes after Enrichment and Sequencing Primer annealing. We reduced the number of washes from three to a single wash in both cases. We have also consolidated the number of tubes the sample beads are in coming into the process from emPCR. This effectively doubles the number of samples an operator can process in approximately the same time, while also affording some simplifications and increases in throughput in conjunction with our automation efforts. Further quality testing is underway before Production implementation.

Enrichment Work Flow (2 samples)	Optimized Work Flow Tube consolidation and reduced washes 1 hour	Standard Work Flow 2 Tubes/Sample and 3 washes 1 hour, 30 min
Melt to alkyne and neutralize	15 min	15 min
Anneal Enrichment Primer + Wash(es)	10 min	20 min
Enrichment Incubation + Washes	15 min	15 min
Anneal Sequencing Primer + Wash(es)	10 min	20 min
Bead Counts	10 min	10 min

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