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Recent Work

Title

Optimization of the Roche 454-Titanium GAllx 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/454 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. Primary focus has been on improving the procedural ergonomics and the technician's operating environment, reducing associated production costs, and improving the overall process and generated sequence quality. The foremost of these optimization projects, including automated 454 sample enrichment, improved 454 & Illumina sample library quantification via qPCR, and ergonomic tools, along with sequencing throughput strategies will be presented. 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.

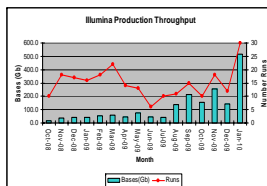
Introduction

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, Ca.

Platform	Staff	Instruments
454	13	8
Illumina	5	12

Illumina Production Pipeline

In Nov. 2009, JGI acquired 5 more GAIIx analyzers with paired-end modules which nearly doubled the Illumina throughput capacity. Between Oct 2009 and Jan 2010, four new operators were trained in Illumina. In Feb. 2010, two cBot cluster stations were validated and installed, as well as the new analysis Pipeline/OLB 1.6. Current throughput capacity is ten 2x76 cycle runs every two weeks.

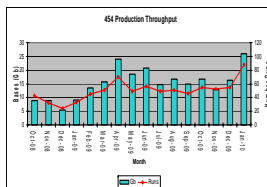


Illumina throughput of number bases collected & number of sequencing runs since Oct 2008

Month	Total Bases (Gb)					Totals (Gb)
	36 cycle	76 cycle	36 x 2 cycle	76 x 2 cycle	114x2 cycle	
October 2009	3.2	0.0	0.0	153.4	0.0	156.6
November 2009	9.4	43.8	0.0	204.2	0.0	257.4
December 2009	8.4	7.0	0.0	128.3	0.0	143.7
January 2010	7.0	0.0	30.1	478.1	58.4	573.6
Total per Run Type	28.0	50.8	30.1	964.0	58.4	1131.3
Average Bases per Run	2.8	10.2	10.0	18.5	29.2	

454 Production Pipeline

With FY2010 beginning, the projected throughput for the 454 platform had doubled from FY2009 totals. The platform was seeing another ramp and needed to make some automation and staffing adjustments once again. The process that posed the bottleneck worth tackling was enrichment. The enrichment robot was implemented in production in January 2010, which allowed the group to process 25-30 runs per week.



454 throughput of number bases collected & number of sequencing runs since Oct 2008

Month in FY09	Gb Goal	Gb Actual	# Run Goal	# Run Actual	Run Capacity
October	14	16.7	50	55	68
November	14	12.8	50	52	68
December	14	16.3	50	55	68
January	28	25.9	100	88	100

Ergonomics: Program and Philosophy

Employees at the JGI, whether working in an office, laboratory, or both are faced with repetitive and detail-oriented tasks daily. The staff, with management support, continuously seek to develop ergonomic and safety awareness, injury prevention and an education program to reduce ergonomic risk and repetitive strain injuries. As part of this effort, employees created an Ergonomics Working Group (EWG). Many employee-driven efforts have resulted from the EWG, including the Practice Workstation & Demo Room and Workstation Setup. The Demo Room contains many computer equipment options and items to customize one's workstation. These are available to test, borrow, and order. Workstation setups are now provided on an individual basis by a certified ergonomist. She discusses various options, equipment type and placement suitable for the user. Shared desks are now equipped with height-adjustable, programmable settings for multiple users.



Ergonomic Demo Room

Illumina Ergonomics

The JGI instrumentation group developed a quick disconnect reagent bottle adaptor for ease of loading and unloading reagents on the GAIIx analyzer.



Illumina Analyzer bottle adaptors



Bottle adaptors installed on the Illumina Analyzer

These bottle adaptors help the operator keep their hand, wrist and arm in a neutral position, making reagent changes more comfortable.

The Ovation pipette has also been implemented as an alternative to the traditional Rainin. The "palm up" orientation of the user's hand is drastically reduced.



Ovation Pipette

454 Ergonomics



The 'Lock Down' and 'Decapper' tools for removing caps of 50mL conical tubes.

The JGI instrumentation group engineered a clamping device used to secure reagent cassettes via a compressed air line. Additionally, a tool with a handle was developed to fit over caps for unscrewing purposes. The two used in conjunction eliminated the need to grip tubes and use excess force to remove caps.



Viaflo Pipette

The Viaflo pipette has been incorporated into the 454 procedures to reduce the number of manual pipette steps. The Viaflo allows operators to adjust pipetting volumes with a touch wheel and aspirate and dispense liquids with a touch of a button.

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 2 tubes.



View of tip racks, heating and cooling blocks, magnetic block and tube carriers

Most of the steps have recently been automated through a collaboration with Hamilton Instruments.



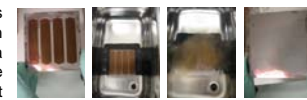
Hamilton deck overview showing the configuration of various sample stations

The resulting instrument is capable of processing 24 samples in a single 2-3 hour run. Currently we are processing 8 samples in less than 2 hours, a 4 fold increase in user productivity. Custom reservoirs and program modifications are being developed to reduce tip waste. A second robot is currently being validated to support projected increases in production.

In addition, with the initiation of best practices for robot use, combined with the substantial reduction in work effort now required for the enrichment process, we have substantially reduced the ergonomic risk for this process.

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.

qPCR for 454 & Illumina Library Quantification

Accurate quantification of prepared 454 and Illumina library fragments is critical to optimizing the quality and sequencing run output on both platforms. It's not enough to quantify all DNA present in a library prep, but to accurately quantify the amount of amplifiable fragments that will lead to successful Illumina cluster generations and 454 emPCR clonal amplifications. The JGI's Process Optimization group has developed custom qPCR methods to quantify 454 and Illumina libraries using both internal library standards and commercially available kits. JGI Production is currently targeting 300,000 clusters per tile for Illumina flowcells and 10-15% total yield from 454 emPCR reactions.

