Illumina Production Sequencing at DOE Joint Genome Institute – Workflow and Optimizations

Angela Tarver [1], Alison Fern [2], Matthew San Diego [1], Megan Kennedy [2], Matthew Zane [2], Christopher Daum [2], Christopher Hack [1], Eric Tang [1], Shweta Deshpande [1], Jan-Fang Cheng [1], Simon Roberts [1], Melanie Alexandre [1], Miranda Harmon-Smith [2], Susan Lucas [2]

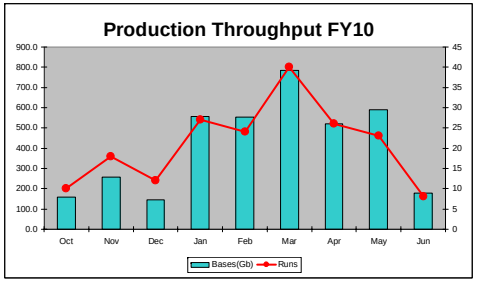
[1] Lawrence Berkeley National Laboratory, [2] Lawrence Livermore National Laboratory

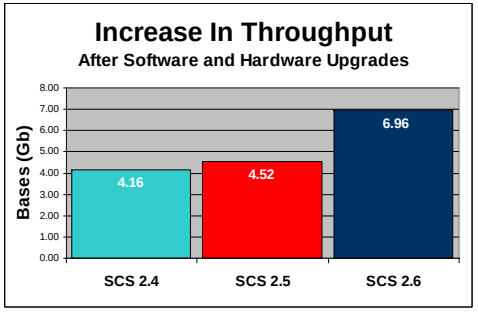
ABSTRACT The U.S. Department of Energy (DOE) Joint Genome Institute's (JGI) Production Sequencing group is committed to the generation of high-quality genomic DNA sequence to support the DOE mission areas of renewable energy generation, global carbon management, and environmental characterization and clean-up. Within the JGI's Production Sequencing group, the Illumina Genome Analyzer pipeline has been established as one of three sequencing platforms, along with Roche/454 and ABI/Sanger. Optimization of the Illumina pipeline has been ongoing with the aim of continual process improvement of the laboratory workflow. These process improvement projects are being led by the JGI's Process Optimization, Sequencing Technologies, Instrumentation & Engineering, and the New Technology Production groups. Primary focus has been on improving the procedural ergonomics and the technicians' operating environment, reducing manually intensive technician operations with different tools, reducing associated production costs, and improving the overall process and generated sequence quality. The U.S. DOE JGI was established in 1997 in Walnut Creek, CA, to unite the expertise and resources of five national laboratories— Lawrence Berkeley, Lawrence Livermore, Los Alamos, Oak Ridge, and Pacific Northwest – along with HudsonAlpha Institute for Biotechnology. JGI is operated by the University of California for the U.S. DOE.

Production

In October 2009, JGI production began ramping up Illumina sequencing, increasing the amount of certified operators from 2 to 5. In December 2009, the Illumina group introduced a weekend shift to increase efficiency and decrease instrument downtime. The Illumina group continues to work on improving quality and communication by instituting Run Report Checklists, and starting twice daily Instrument Status Reports among the Illumina operators across both shifts.

JGI Production Instruments		
12 GAIIx Analyzers	12 Paired-End Modules	6 cBot Cluster Stations





JGI Production FY10 Throughput to Date		
189 Runs	3.66 Tb	872 Projects

FY10 Throughput Events:

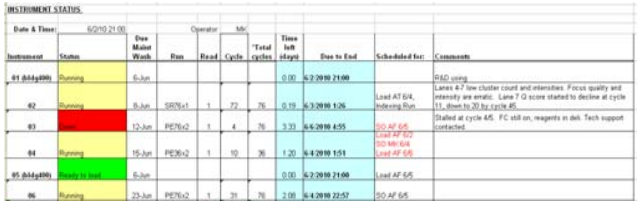
October: Upgraded to SCS v 2.5 software

November: Purchased 5 new GAIIx's and PEMIIx's; converted 5 PEMs to PEMIIx's

February: Purchased 3 cBots

March: Purchased 3 cBots, upgraded to SCS v 2.6 software and SBS v4 kits

Status Reports Currently, an operator from the AM shift and the PM shift both perform a walk-through of the Production lab, and create and email out an Instrument Status Report as shown below. This report shows what cycle a run is on and its approximate need for Read2 resynthesis reagents and run end time. This enables more efficient loading, and tracking of issues.



INSTRUMENT STATUS

Date & Time: 6/2/10 21:00 Operator: Mir

Instrument	Status	Due Maint Wash	Run	Read	Cycle	Total cycles	Time left (days)	Due to End	Scheduled for:	Comments
01 (bbfgd09)	Running	6-Jun					0.00	6/2/2010 21:00		R&D using
02	Running	8-Jun	SR76x1	1	72	76	0.19	6/3/2010 1:26	Load AT 6/4, Indexing Run	Lanes 4-7 low cluster count and intensities. Focus quality and intensity are erratic. Lane 7 Q score started to decline at cycle 11, down to 20 by cycle 85.
03	Down	12-Jun	PE76x2	1	4	76	3.33	6/6/2010 4:55	SO AF 6/5 Load AF 6/6 SO Mir 6/4 Load AF 6/8	Stalled at cycle 4/5. FC still on, reagents in deli. Tech support contacted.
04	Running	15-Jun	PE36x2	1	10	36	1.20	6/4/2010 1:51		
05 (bbfgd09)	Ready to load	6-Jun					0.00	6/2/2010 21:00	Load AF 6/5	
06	Running	23-Jun	PE76x2	1	31	76	2.08	6/4/2010 22:57	SO AF 6/5	

Ergonomics

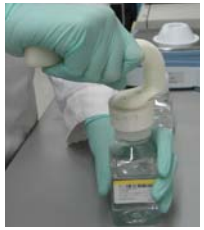
To continually improve safety and with the upgrade to cBot instruments, several tools and best practices were introduced in the past year.

Best Practices Guides for performing work ergonomically. Highlight and demonstrate neutral posture, using both hands to distribute the work and avoid awkward postures, and bringing work close to the operator. Created through collaboration among operators and JGI's ergonomist.

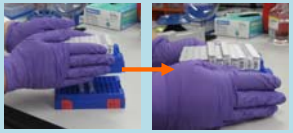
Rotating cBots "Lazy Susans" allow easy access for loading reagents and checking coolant levels on the back of the instrument. Eliminates awkward reaching.

Stylus Pen Reduces strain and prevents excessive force while using the touch screen. Increases accuracy of touch screen entries.

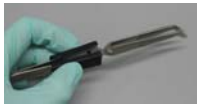
Waste Disposal A 96 well plate pressed into the reagent plate creates large drainage holes for efficiently disposing leftover reagent.



Reagent Bottle Decapper Designed in-house to reduce finger strain, this tool loosens buffer caps for easy opening.



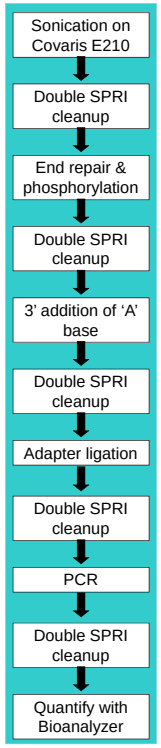
Reagent Defrosting Designed in-house, this tool pushes up strips 5 and 7, allowing them to be removed for faster thawing.



Tweezers Reverse action, plastic coated tweezers to easily and safely load flow cells.

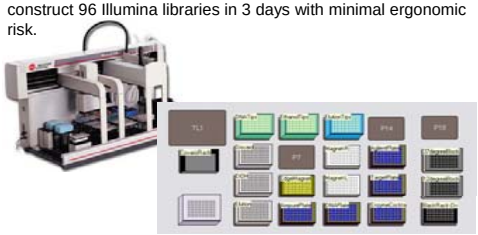
Developments

Automated Library Construction In response to the increasing demand for constructing Illumina libraries, a semi-automated process which enables us to construct 96 Illumina libraries in approximately 6-8 hours has been developed. With a few simple modifications, the library production efficiency has doubled. The modifications include the shearing of DNA with a Covaris E210, and the cleaning of enzymatic reactions and fragment size selection with SPRI beads and a magnetic plate holder. Recently, a BioMek FX robot has been programmed to carry out the library construction process.



Workflow for automated library construction

BioMek FX Robot The Beckman-Coulter Biomek FX robot is used to construct 96 Illumina libraries in parallel. This process automates the repetitive pipetting involved in library construction process and enables a single operator to construct 96 Illumina libraries in 3 days with minimal ergonomic risk.

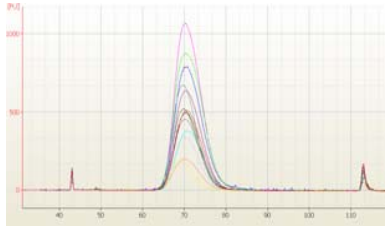


Deck Layout of the BioMek Robot

Illumina Library Quality The efficiency, quality and reproducibility of libraries created on BioMek are currently being optimized.

Library Quantification

Using the Agilent Bioanalyzer High Sensitivity DNA chip, the quantity of libraries was assessed. A sample is required to have a concentration of at least 10 nM. The graph below shows the Bioanalyzer traces of 13 libraries constructed by the BioMek FX robot.



Other Projects in Development:

- Testing of 95G and SCS 2.8/ RTA 1.8
- HiSeq 2000 installation
- Process Optimization is developing non-amplified library protocols and developing qPCR using Kapa reagents.
- Quality Control is developing an automated QC and developing Statistical Process Control (SPC) charts for monitoring instrument performance.

LLNL-POST-439651

This work was performed under the auspices of the US Department of Energy's Office of Science, Biological and Environmental Research Program, and by the University of California, Lawrence Berkeley National Laboratory under contract No. DE-AC02-05CH11231, Lawrence Livermore National Laboratory under Contract No. DE-AC52-07NA27344, and Los Alamos National Laboratory under contract No. DE-AC02-06NA25396.