

# Lawrence Berkeley National Laboratory

## Recent Work

### Title

Sanger, 454 and Illumina Production Lines

### Permalink

<https://escholarship.org/uc/item/6xd4d6sr>

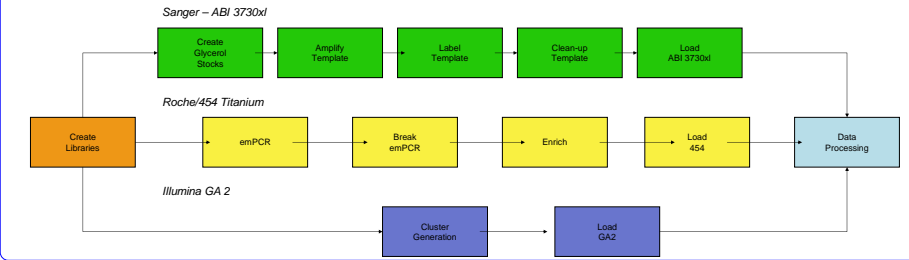
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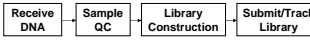
### Publication Date

2008-12-01

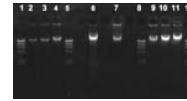
## Production Work Flow



## Cloning Technology Workflow



JGI has implemented a unique two-tier sample QC process. The collaborators are required to submit a QC gel on-line (as shown), which allows us to examine the quality and quantity of the DNA and approve or decline the shipping. JGI would also perform the sample QC process that includes a PFGE to examine the size range of the samples. This process has increased the success rate of library construction.

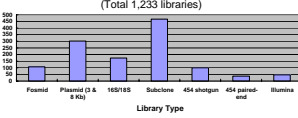


Lane 1: Marker 2 (Std)  
Lane 2: 15ng standard (Std)  
Lane 3: 30ng standard (Std)  
Lane 4: 60ng standard (Std)  
Lane 5: Marker 2 (Std)  
Lane 6: Organism #1 (Full loaded: 200ul total volume)  
Lane 7: Organism #2 (Full loaded: 200ul total volume)  
Lane 8: Marker 2 (Std)  
Lane 9: 15ng standard (Std)  
Lane 10: 30ng standard (Std)  
Lane 11: 60ng standard (Std)  
Lane 12: Marker 2 (Std)

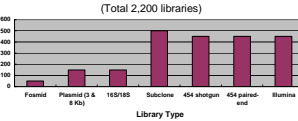
## FY08 Accomplishments

- Construct 1,233 libraries to support 3 sequencing platforms
- Complete the transition of Sanger library construction to 454 and Illumina library construction
- Establish a routine and reliable DNA sample QC and management process
- Complete library construction for 100 microbes selected for the GEBA pilot project
- Develop a freezer tracking system for DNA aliquots allocated for different types of libraries (collaboration with the Production Informatics Group)
- Develop several SOP's to enhance JGI's ability
  - To perform clone-free finishing tasks (bubble-PCR)
  - To clone trace amount of gDNA (AMPure bead)
  - To generate 16S/18S libraries
  - To count colonies using QPix
  - To generate Titanium paired-end libraries with consistent quality

## Libraries Constructed in FY08



## Libraries Projected for FY09

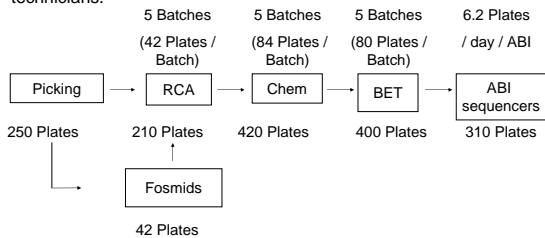


## Sanger

The Sanger Production Line, runs 2 shifts and 187 instruments, to sequence 20 Gbases per year with a staff of 24 lab technicians.

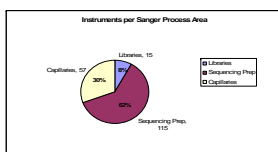
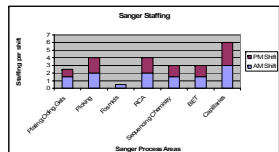


3730 ABI sequencers



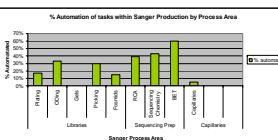
Each plate consists of 384 wells of a particular library needing to be sequenced. Each well on a plate corresponds to a lane, and each lane represents a sequence of base pairs.

For example, in Oct 2008, the JGI sequenced 3,282,912 lanes, which corresponded to 2.2 billion Q20 bases, with an average read length of 711.4 base pairs, and a 94.6% pass rate.



### FY 2008 Sanger vs. New Technologies

Quarter	Sanger	Number of Sequencing Sites	454	Number of Sequencing Sites	Illumina	Number of Sequencing Sites
Q1 2008	4	55	3.2	-	-	-
Q2 2008	2.3	60	6.8	-	-	-
Q3 2008	5.0	60	8.8	6	20.0	-
Q4 2008	6.0	50	22.8	6	37.1	6
FY 2008 Total	20.3	-	45.17	-	64	-

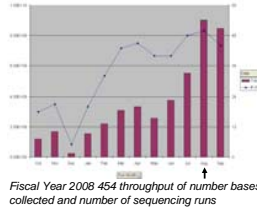


For Q4 of FY08, Sanger used 60 sequencers to generate 8.0 Gbases; while 454 used 6 instruments to generate 22.8 giga bases and Illumina used 5 machines to generate 37.1 giga bases.

[www.jgi.doe.gov/sequencing/statistics.html](http://www.jgi.doe.gov/sequencing/statistics.html)

## 454

In FY2008, the JGI 454 production group was able to scale up the number of sequencing runs as we progressed through the year by hitting three major milestones:



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

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

FY09 Current	FY09 Goal
76 Runs	512 Runs
18 Gb	128 Gb

	Mon	Tue	Wed	Thu	Fri
AM	em L	b/e b/e	em L	em b/e	em L
AM	em L	b/e b/e	em b/e	b/e L	em L
AM	em L	em b/e	em b/e	b/e L	em L
AM	em em	em L	em b/e	b/e L	em em
AM	em em	em L	em b/e	em L	em em
PM	e b/e	em L	em b/e	em b/e	b/e L
PM	b/e b/e	em b/e	em L	em b/e	b/e b/e
PM	b/e b/e	em b/e	b/e L	em b/e	b/e b/e
PM	b/e b/e	em b/e	b/e L	em em	b/e b/e

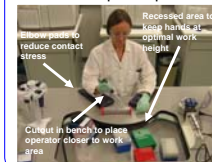
Sample weekly schedule of 454 tasks with the enrichment step automated.

### 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.

### Work Space

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



### Tube & Bottle Openers

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



## Illumina

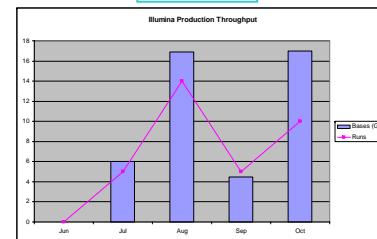


### Scheduling

Monday	Tuesday	Wednesday	Thursday	Friday
Cloning Tech Library Construction Day 1	Library Construction Day 2	Library Construction Day 1	Library Construction Day 2	Library Construction Day 1
Cloning Tech Library Construction Day 2	Library Construction Day 1	Library Construction Day 2	Library Construction Day 1	Library Construction Day 2
Production Cluster AM	Cluster AM	Cluster AM	Cluster AM	Cluster AM
Production Cluster PM	Cluster PM	Cluster PM	Cluster PM	Cluster PM

- ### Scheduling
- Total of 3 - 4 staff needed, 1 - 2 to generate libraries, 2 to generate flowcells and load them
  - Up to 16 libraries created a week by Cloning Tech
  - Production operators scheduled for two tasks daily
  - Two paired end runs a week
  - Six single read runs a week

### Throughput



### Throughput Events:

- July: Illumina moved to production
- August: Production moved to new lab in building 100, purchase two new GAIIs
- September: Reduced throughput due to lack of projects
- November: Production purchased two new GAIIs, 5 paired end modules

### STAFFING, INSTRUMENTS, & THROUGHPUT

