

# The Joining of Competitors: The Dual Operation of the ABI 3730xl and GE MegaBACE4500 DNA Sequence Analyzers at the DOE Joint Genome Institute

Christopher Daum, Damon Tighe, Lena Philip, Danielle Mihalkanin, Cailyn Spurrell, Don Miller, Alex Copeland, Susan M. Lucas and the JGI Sequencing Team

## ABSTRACT

At the center of the Department of Energy's (DOE) Joint Genome Institute (JGI) Production Genomics Facility (PGF), lies a highly efficient and automated production line devoted to the generation of high-quality genomic DNA sequence. The JGI utilizes a dual platform of DNA sequence analyzers: Applied Biosystems 3730xl and GE Healthcare's MegaBACE4500. The operation of these high-throughput fluorescence based DNA sequence analyzers at the JGI will be assessed on the strengths and benefits of each platform, and instrument overviews of operational parameters and mechanical/component specifications will be supplied. In addition, instrument setups for production operation, operation schedules, loading, maintenance strategies, and the sequencing strategies for each platform will be discussed. Throughput numbers and sequencing quality results will be presented.

## INTRODUCTION

The DOE Joint Genome Institute (JGI) was created 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 (LLNL), and Los Alamos National Laboratory (LANL). In January 1999, high-throughput DNA sequencing began at the Production Genomics Facility (PGF) in Walnut Creek, California.

The Sequencing Department is at the heart of the PGF operations, and its aims are to generate high-quality sequence in a cost-efficient manner. The production sequencing process takes the DNA through library creation, sequencing prep, and finally to capillary sequencing & analysis. As genomics is a rapidly changing field, the PGF constantly adapts to take best advantage of new developments in technology. The current stable of sequencing machines is comprised of 70 Applied Biosystems 3730xl and 36 GE Healthcare MegaBACE4500 DNA Analyzers.

## DISCUSSION

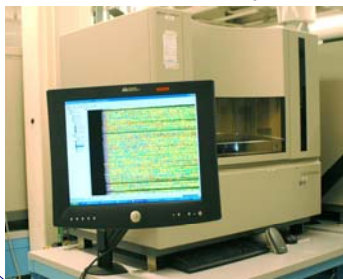
The MegaBACE DNA Analysis platform has been an indispensable component of the JGI production sequencing line for several years; with the inception of this capillary based platform in 1999, significant advances were made over the industry standard slab gel instruments of the time – in areas of automation, workflow, and operation. The current MB4500 platform offers much improved sample throughput with the ability to process 384 sample lanes in a single run and improved sequence quality over its predecessors with updated components.

In 2002 the ABI3730xl was released - this highly automated DNA analyzer was poised to shift the paradigm of high-throughput sequencing as the JGI and genomics facilities world-wide adopted this new platform; a new industry standard had been set.

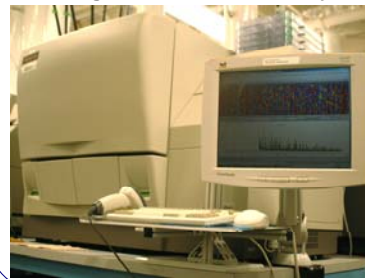
The JGI has chosen to utilize both the MB4500 - 36 instruments, and the ABI3730xl - 70 instruments. The highly automated ABI3730xl allows for 24hr-7day/week processing of sample plates, with only limited interaction by a small staff of Technicians who load/unload samples and change out reagents a few times per week. Alternatively, the 384-capillary array system of the MB4500 allows for high sample throughput on each sequencing run performed, however it requires manual interaction with a Technician to load each run.

The MB4500 uses an efficient high power solid-state laser, which mated to the scanning confocal optics system provides enhanced detection sensitivity for long reads; attaining on average 100bases more per lane (based upon JGI run parameters). Additionally, the MB capillary arrays and solid-state laser achieve much longer operational lifetimes when compared to the arrays and argon laser of the ABI. Although in JGI production, the ABI instruments have an operational uptime efficiency of 98.9% compared to only 96.2% for the MBs; downtime on both platforms is primarily due to instrument errors.

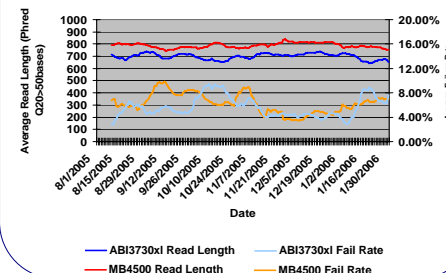
## AB 3730xl DNA Analyzer



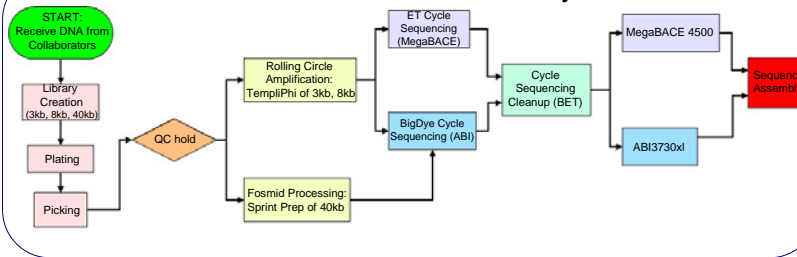
## GE MegaBACE 4500 DNA Analyzer



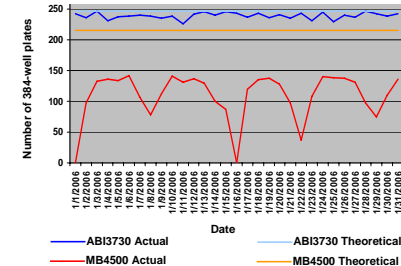
## Performance Comparison of ABI 3730xl & MegaBACE 4500 Platforms



## JGI Production Process Summary



## JGI Throughput Comparison of 36 MB4500s vs. 70 ABI3730s \*



## JGI Quick Reference – Operational Parameters & Specifications

Operational Parameter	ABI 3730xl Specification	MegaBACE 4500 Specification
Run Parameters	8.5kV for 79min	5.9kV for 215min
Injection Parameters	1.5kV for 15sec	2.0kV for 36sec
Operating Temperature	60°C	55°C
Sieving Matrix	POP-7 polymer	V2 LPA
Capillary Array Type	- 50 cm – 96 capillaries - 150 micron o.d. & 50 micron i.d.	- 70 cm – 384 capillaries - 200 micron o.d. & 75 micron i.d.
Sample Lane Throughput (based upon JGI Run parameters)	1322 samples/day	2304 samples/day
Laser power/type	25mW argon-ion	100mW solid state
Laser accessories (cooling)	External HVAC system with negative pressure hookup to draw air from system	None – no external Power Supply/Fan module or HVAC system required
Optical system – Excitation & Detection	In-capillary dual-side laser excitation & CCD camera detection	Scanning confocal optics for in-capillary laser excitation & detection by PMTs
Instrument accessories	- UPS for backup during power outages - PC \ Monitor \ Barcode Scanner	- UPS for backup during power outages - PC \ Monitor \ Barcode Scanner - High & Low pressure N <sub>2</sub> systems
Reagents/Materials Handling	- Automated sieving matrix delivery pump & on-board reagents for 2-day unattended operation. - Integrated auto-sampler & sample plate stacker (16 plate capacity)	- Technician loaded sieving matrix & reagents for each run performed. - Individually loaded sample plates by Technician
Sequencing Chemistry	BigDye v3	DYEnamic ET dye terminators
Instrument Software	Unified Data Collection v3.0	Instrument Control Manager v4.2
Basecalling Software	Sequencing Analysis v5.2 with 3730POP7LR basecaller	Sequence Analyzer v4.0 with Cimarron 3.12 basecaller

## CONCLUSIONS

The JGI has chosen to utilize both the MegaBACE 4500 and the ABI3730xl DNA sequencer analyzer instruments, and has found the running of these competing platforms to be beneficial to the production sequencing process. The highly automated ABI3730xl allows for 24hr-7day/week processing of sample plates, with only limited interaction. Alternatively, the 384-capillary array system of the MB4500 allows for high sample throughput during each sequencing run performed by a Technician, and its solid-state laser mated with the scanning confocal optics system provides enhanced detection sensitivity for longer reads. The MB platform also offers slightly reduced per lane cost when comparing the associated operational and sample preparation costs for each platform at the JGI; this reduced operational cost is primarily due to the long lifetimes achieved by the MB capillary arrays. Moreover, these two DNA sequencing platforms complement each other in the sequence they produce - thereby providing for better coverage when sequence derived from both platforms are used in genomic project assemblies at the JGI.