Automation of Fosmid Preps at the Joint Genome Institute

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The Joint Genome Institute

The Department of Energy Joint Genome Institute (www.jgi.doe.gov) in Walnut Creek, CA is a high throughput DNA sequencing facility with a current throughput of approximately 2.5 billion basepairs per month. The JGI sequences a variety of large and small genomes and Fosmid sequencing is an essential component of our whole genome shotgun sequencing strategy. Fosmids are used to build the assembly scaffold, fill sequence gaps, and bridge contigs in the sequence finishing process. Our current sequencing strategy is to shotgun sequence 3kb and 8kb libraries to 10x draft coverage and to sequence Fosmids to 0.5x sequence coverage. Several large genomes from 2004 include: Branchiostoma floridea (600 Mb), Emiliania huxleyi (220 Mb), Nematostella vectensis (340 Mb), and Xenopus tropicalis (1.2 Gb).

Fosmid Sequencing

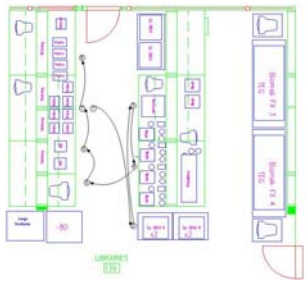
From January – October 2004 the JGI used Agencourt's CosMCPrep reagent kit to prepare Fosmid clones for sequencing. The JGI transitioned to Agencourt's SprintPrep reagent kit in mid September of 2004. Fosmid Libraries are prepared by inserting 40kb DNA inserts into a pCC1FOS cloning vector (Epicentre). The colonies are plated and picked (Genetix Qpix) into 384 well plates. The Fosmid DNA prep sample line has it's own dedicated equipment separate from the primary plasmid sequencing line. Subsequent sequence chemistry steps and sequencing utilize the same automation as the plasmid samples though separate Fosmid protocols are used.

Fosmid clones are sequenced on the ABI 3730xl sequencers. The JGI has 67 ABI sequencers running on a 24/7 schedule. JGI loads approximately 240 384-well plates per day on the ABI sequencers of which about 35 384-well plates are Fosmid plates. The JGI also runs 35 GE MegaBACE 4500 sequencers on a 24/5 schedule and approximately 144 384-well plates per day are loaded on the MegaBACE sequencers.

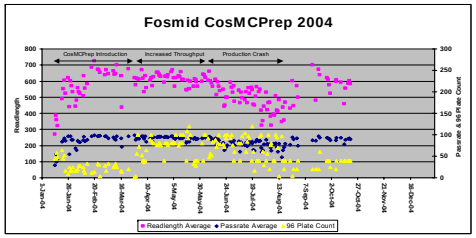
Fosmid Sequence Data

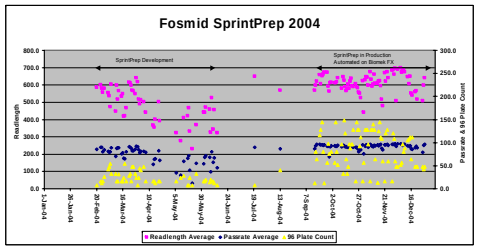
The JGI Fosmid readlength, passrate, and throughput data for the year 2004 is shown in the adjacent figures to the right. The CosMCPrep data shows the initial introduction of the CosMCPrep protocol into production followed by a period of good readlengths and passrates at higher throughput. The Fosmid line suffered a production crash starting in June where there was a significant decrease in readlengths and passrates. The causes of the crash were never fully identified.

Development of the SprintPrep began while the CosMCPrep was being implemented on a production scale. The SprintPrep data shows the initial development during the period of February – June. Full production implementation of SprintPrep, automated on the Biomek FX platform, began in mid September.



The production scale CosMCPrep protocol required numerous small instruments and a convoluted workflow to juggle multiple plate batches through the process.





Considerations for automation:

Routine automation was implemented for the inoculation steps. A Bio-tek uFill reagent dispenser with a Hudson Controls robot arm fill the 96-well destination plates with growth media. Glycerol stock cell cultures in 384 well plates are reformatted to the 96 well plates on a Platemate Plus Automated Pipettor. The destination glycerol stocks for the CosMCPrep were in deep well plates. The destination glycerol stocks for the SprintPrep are in shallow well Costar plates. Initially, overnight incubation was in Genemachine HiGro incubators. These were replaced in mid-2004 by 2 ATR Multitron shaking incubators.

The April – May CosMCPrep data shows that the CosMCPrep protocol can produce comparable readlength and pass rate results with respect to the current SprintPrep protocol. However there were several advantages to switching to the SprintPrep protocol.

A production scale CosMCPrep sample prep line required numerous pieces of small equipment including: Bio-tek uFill dispensers, centrifuges, plate shakers, Multidrop dispensers, Hydra pipettors, and a Multimek pipettor. As shown in the figure to the left, the workflow for the operator was fairly complicated. The operator had a precisely timed routine to process multiple batches of plates with each batch in a different stage of the process. It was extremely labor intensive and there were numerous opportunities for error both by the machines and the operators. It was difficult to train staff to process samples for the complex workflow and timing requirements.

The SprintPrep protocol proved to be simpler in the number and type of processing steps. Many of the steps could be automated on the Biomek FX workstation. The few remaining manual steps are simple and well matched to the timing of the automated steps. SprintPrep has fewer individual reagents and the overall waste flow is smaller. The automated SprintPrep is much less labor intensive, less prone to errors, and easier to train operators. Operator training can be accomplished in less than a week.

Automated Fosmid DNA Prep

Plate inoculation instruments:

Bio-tek uFill dispenser/Hudson Plate Crane, Platemate Plus

Inoculation throughput:

Up to 36 384-well plates are inoculated into 144 96-well plates in an 8 hr shift. Destination plate filling is done in batches of 48 96-well plates and have a 40 minute run time on Bio-tek uFill/Hudson Plate Crane instrument. Inoculations are 6 ul transfers from 384-well plates reformatted to four 96-well plates. A batch of six 384-well plates can be processed in approximately 1 hr. 10 min. on the Platemate Plus.

SprintPrep instruments:

Two Biomek FX workstations are outfitted with: dual pods each with P200 96-channel pipet heads, reagent reservoirs, and 8 LBNL custom magnets each. Additional instruments used: 2 ovens, 1 Multidrop 384, 3 plate shakers, 4 Agencourt magnets, 4 Hydra automated pipettors, 4 additional LBNL custom magnets.

SprintPrep throughput:

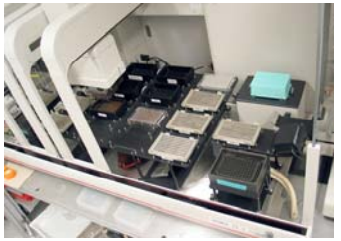
Four 96-well plates are processed on each of the 2 Biomeks in 40-45 minutes. The plates are processed on the Biomeks through the ethanol wash steps. Four plates from each machine are then manually processed through the following steps: ethanol drying, resuspension buffer addition, shaking, incubation, and reformatting into 384-well plates. This manual process takes 40-45 minutes. **Note:** 8 plates can be loaded on Biomek FX deck but only 4 plates are processed at a time. The first 4 plates on each machine are completed and removed while the Biomeks continue processing the remaining 4 plates on each machine. The automated and manual steps are both of 40-45 min duration resulting in a throughput of about 10-11 96-well plates per hour. However when setup time, takedown time, and other tasks are included an operator can process 12-16 384-well plates in an 8 hour shift. The JGI runs 2 shifts of SprintPrep processing.

Fosmid Protocol

- Inoculate 6 ul of glycerol stock into 150 ul growth media in 96-well Costar plate using Platemate
- Incubate 37C for 20 hours in ATR shaking incubators
- Biomek
  - Load cell culture in Costar plate (no magnet)
  - Add 69uL of SprintPrep bead solution
  - Add 73uL of IPA
  - Mix 36 times
  - Incubate off magnet 5 - 9 minutes
  - Incubate on LBNL custom magnet 8 - 14 minutes
  - Six 70% ethanol washes
  - Aspirate final ethanol from sample
  - Remove plate from Biomek
- Dry in oven for 9 minutes at 37C
- Dispense 30µl of RE1 with 0.0625% Triton-X
- Shake plate for 7 minutes
- Incubate for 5 minutes (off magnet)
- Place plate on Agencourt magnet for 5 minutes
- Reformat to 384-well plate, 25 ul transfer, using Hydra with LBNL custom magnet
- Processed plates are sent to sequence chemistry processing, cleanup, and sequencing on the ABI 3730 sequencing platform

The LBNL custom 96-well magnet has superior magnetic holding strength and draws beads into a ring pattern at the bottom of well

The Platemate Plus Automated Pipettor used for inoculating into 96-well plates from 384-well plates



The Biomek FX Workstation used for the first half of the Fosmid processing

Biomek FX Workstation deck detailed layout for Fosmid processing

Equipment layout for final manual Fosmid processing steps: ethanol drying, resuspension, incubation, and reformatting into 384-well plates.

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