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Title

Implementing Automated 384 Well Fosmid Prep at JGI

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Authors

Wilson, Steven

Chen, Feng

Jett, Jamie

et al.

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Implementing Automated 384 well Fosmid Prep at JGI

Steven Wilson, Feng Chen, Jamie Jett, Nancy Hammon, Duane Kubischta, Simon Roberts, Charles Reiter, Diana Lawrence and Paul Richardson

Overview:

High quality DNA sequence generated from fosmid inserts can aid in developing a scaffold for sequence assembly or sequencing difficult repeat regions of a genome. The Joint Genome Institute has implemented a 384 well automated fosmid prep that will increase the amount of fosmid DNA generated by the Sequencing Group and aid in assembling genomes at a faster rate.

Introduction:

Fosmid vectors host large fragments of DNA that can be inserted into *E. Coli* cells during the transformation stage of the sequencing process. These transformed bacteria are then spread onto 22cmx22cm bioassay trays that are picked on automated colony pickers. The bacteria in the 384 well destination plates, from the colony pickers, are then inoculated into deep well plates with enriched media by the PlatematePlus automated pipettor.

Automation Implementation:

JGI Technology Development, Technology Transfer and Instrumentation worked to remove operator variability and most of the manual steps from the fosmid process by moving it to automated instruments. Inoculation, Isolation and Resuspension have been automated on 2 platforms – the PlatematePlus (Figure 1) and Biomek FX (Figure 3) pipettors.



Figure 1: Fosmid inoculation instrument.

Hardware and Method Upgrades:

Changes to the Biomek FX included a new isolation method (utilizing both pipettors), a new computer to handle the demands of the dual pod method, new wash stations, a pump for emptying the waste container and holes in the deck.

The new method uses calls to a **Grip with Tips** command (Figure 5). This allows both pods to be used to both move plates and pipette reagents. This is the key to making the dual pod method work.

With both pods having the ability to pipette reagents, they both need to have access to all reagents (Figure 2). This requires that the reagents be in a central location and necessitated the drilling

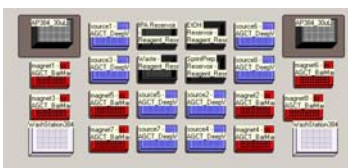


Figure 2: Isolation deck layout. Dual pod operation.



Figure 3: Biomek FX automated pipetting instrument. Dual Multi-channel pipette heads.

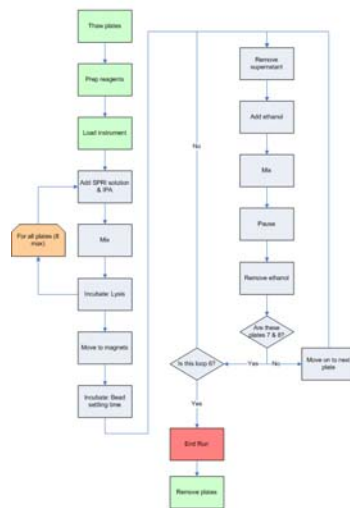


Figure 4: Process flowchart for isolation method. Shown for 1 pod.



Figure 5: Gripping with tips loaded.

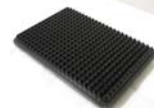


Figure 6: Custom wash station insert can be replaced virtually on the fly.



Figure 7: Custom wash station is easier to maintain and clean.



Figure 8: Previous resuspension line.

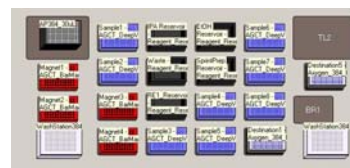


Figure 9: Resuspension deck layout. Single pod operation.

of holes in the deck of the Biomek. (Note the reagent tubes running beneath the deck to the recirculating pump in Figure 3.)

The wash stations on the Biomek are cleaned at least once per month due to the residue build up from the magnetic bead solution. The wash stations (Figures 6 & 7) were modified in order to improve maintenance capabilities. They are still made in 2 pieces like those provided with the instrument, but these wash stations are split into a base and insert configuration that allows the insert to be replaced without removing the base. This reduces the need to frame the wash station and prevents warping from sonication.

The ethanol waste pump (6 ethanol washes for 8 plates generated a lot of used ethanol) was implemented to reduce ergonomic concerns for the operators. A standard peristaltic pump is used to migrate the ethanol from the waste container to the flammable reagent container. The user only has to insert the output manifold into the flammable liquids container and activate the pump (instead of lifting the heavy waste container).

Resuspension:

The resuspension process has been moved from 4 manual Hydra pipettors to the Biomek FX (Figures 8 & 9). Only 1 pod is used for pipetting in this procedure, but the reagents remain on the deck because the tubing passes through the deck.

The resuspension procedure prevents more runs from being done on the Biomek FX (Figure 10). The manual resuspension line can be replaced with another automated pipettor to increase throughput.

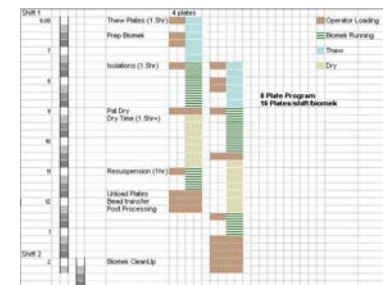


Figure 10: Operator shift flowchart. Shown for 1 Biomek.

Conclusions:

384 well fosmid automation has allowed the JGI to more than double its fosmid throughput from 14 plates per day to 32 plates per day (Figure 11).

Process optimization is ongoing as results are not yet consistent.



Figure 11: Daily throughput per machine for various fosmid processes.