Advancing Science with DNA Sequence

Automated High-Throughput 384-well Fosmid Isolation and End-Sequencing Using Magnetic Beads and Reduced Terminator Cycling Sequencing Reaction Kit

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Abstract

High quality fosmid end-sequencing plays an important role in whole genome shotgun assembly. Accurate paired end information at the size of about 40 kb is crucial in building large genome scaffolds. We have developed an automated high-throughput fosmid DNA isolation and sequencing protocol using a magnetic bead prep (Agencourt) and terminator cycling sequencing. This method uses 384-well format plates from cell growth, DNA isolation to sequencer loading, significantly increases the throughput comparing to the method using 96-well format plates. Using Beckman's Biomek FX with dual pods but without stacker carousel, our throughput is 8 384-well plates in less than 2 hours per instrument. After the fosmid DNA is eluted, cycling sequencing was performed using reduced reagents and according to our standard production protocol. We are able to achieve a pass rate (Q20 > 50) of over 95% and average read length (Q20) over 650 bp. Next steps will be to utilize stacker carousels to double our throughput to 16 plates in same amount of time and to further reduce sequencing reagents while maintaining high quality.

Introduction

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 3 billion basepairs per month. The JGI sequences a variety of large and small genomes from DOE Microbial Genome Program and Community Sequencing Program. Fosmid end sequencing is an essential component of our whole genome shotgun sequencing strategy. Fosmid end sequencing results are used to build the assembly scaffold and to fill gaps and bridge contigs in the finishing process. Our current Whole Genome Shotgun sequencing strategy is to sequence 3kb and 8kb shotgun libraries to a combined 8x draft coverage and to sequence fosmid ends to 1x sequence coverage, which is equivalent to about 30x clone coverage.

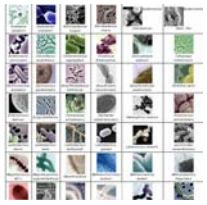
In September of 2004, JGI transitioned from Agencourt's CosMCPrep reagent kit to SprintPrep kit in 96-well format resulting in more consistent and high quality results. To double the coverage from fosmid end-sequencing to 1x sequence coverage in final assembly, JGI put efforts to increase to fosmid isolation throughput by using 384-well format on existing Beckman Biomek FX liquid handling system starting at the beginning of 2005.

Fosmid libraries are prepared by inserting 40kb DNA inserts into a pCC1FOS cloning vector (Epicentre) using blunt-ended ligation. The colonies are plated and picked (Genetix Qpix) into 384 well plates. The fosmid DNA isolation line has it's own dedicated equipment separated from the primary plasmid preparation line. Subsequent sequence chemistry steps and reaction cleanup steps utilize the same automation as the plasmid samples though separated fosmid protocols are used.

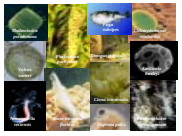
Fosmid sequencing reactions are loaded on ABI 3730xl sequencers. The JGI has 67 ABI sequencers running on a 24/7 schedule. We load approximately 240 384-well plates per day of which about 35 384-well plates are fosmid plates. The JGI also runs 35 GE MegaBACE 4500 sequencers on a 24/5 schedule and approximately 144 384-well plate per day are loaded.

WGS Sequencing Strategy

Microbes



Other species



Our results showed that even though SprintPrep was designed for DNA isolation of high copy number clones with small insert, it is feasible to use this method for fosmid isolation with appropriate modifications.

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Fosmid DNA Isolation Procedure Using SprintPrep

Cell culture growth
Ale in 384-well plate
18 hours 37°C, 85% humidity and 900 rpm

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One-step lysis and DNA binding
Add SprintPrep solution
Add isopropanol
Mix 30 times and incubate for 8 min
Incubate on magnet for 8 min
Wash with 70% ethanol for 5 times
Air dry at room temperature for 45 min

↓

Elution
RE1 with 0.0625% of Triton X-100

The process is straight forward and quick, there is no need to spin down the cells. All processes happen in one 384-well plate. SprintPrep solution is added with isopropanol directly to liquid cell culture. Mixing step is crucial to get uniform DNA yields. After the incubation on magnet, all the solution is discarded and the magnetic beads are washed with ethanol. Sufficient washing is also very important. Triton X-100 is used to facilitate the aspiration of the final DNA solution.

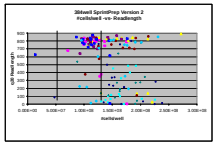
Major optimization required for following areas

Cell culture
- Cell count needs to be at 0.75 to 1.5 x 10^6 per well
  ATR orbital shaker set at 900 rpm
- Evaporation has to be minimized
  Humidity set at 85% in growth chamber

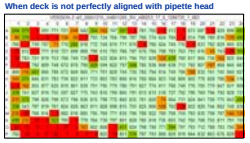
Biomek FX deck framing
- Disposable 384-well tips on pipette head may not be easily aligned with 384-well v-bottom plates on the FX deck, especially when magnetic beads pellet on alternative side in neighboring columns
- Less precise alignment will result in incomplete aspiration and bead loss

Mixing and washing
- Homogeneous mixture of cell culture and reagent
- Sufficient wash with ethanol

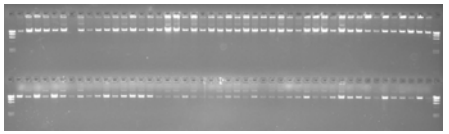
Elution
- Vigorous elution but not to disturb magnetic beads
- Low concentration of Triton X-100 is crucial

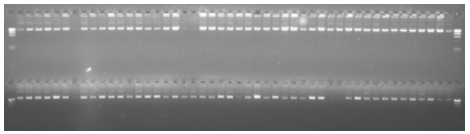


When deck is not perfectly aligned with pipette head



DNA isolation results





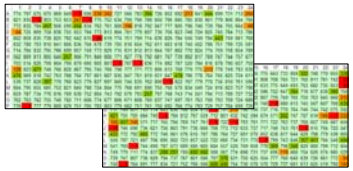
Sequencing method and results

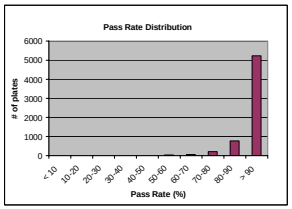
- 1/10 or 1/16 BigDye terminator reaction
  with or without DMSO
- 22% of fosmid DNA from SprintPrep product
- 6 ul total reaction volume
- 99 thermocycles
- Standard magnetic beads clean-up
- ABI 3730xl detection with modified run condition

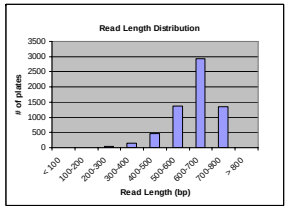
- Pass Rate (> 50 bp):
  96.2%
- Average Read length (all lanes):
  681 bp (Q20)
- Paired ends (>50 bp):
  85%

Sequencing results: trace view

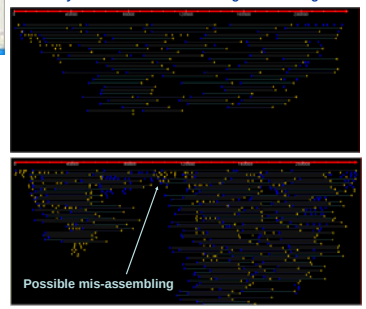
Sequencing results: plate view







Assembly view with fosmid reads aligned to contig



Current development

- More automation
  - Utilizing stackers and relaxing time constraint
- Higher throughput
  - From 40 plates to 64
- Reducing reagent usage
  - SprintPrep reagent
  - BigDye reagent

Acknowledgements

- Library Construction Group
  - Hope Tice, Eileen Dailin, and Doug Smith
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