Fosmid RCA Production Line at JGI

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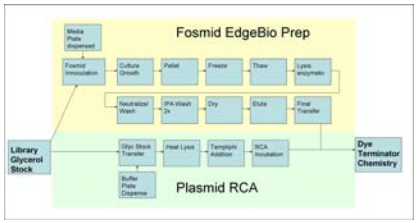
Overview:

Paired end high quality DNA sequence from Fosmids are critical in developing scaffolding for the assembly of genomes. Sequencing of Fosmids at the JGI has historically been an additional branch to the production line that cultured and isolated DNA instead of using Rolling Circle Amplification (RCA) like the plasmid production line to amplify template prior to dye terminator sequencing. The Joint Genome Institute has developed a method of sequencing Fosmids that utilizes RCA instead of time intensive and cost prohibitive isolation methods.

Goal of Project:

To streamline the production line by converting the sequencing of Fosmids to a RCA based method that will also save time, money and create a greater capacity for throughput.

Differences in sample amplification prior to Dye Terminator Chemistry for plasmids and fosmids.



Introduciton:

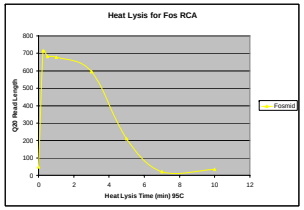
RCA has been used for over 5 years at the JGI to amplify plasmid DNA for sequencing. RCA is based upon the phi-29 polymerase which is active at room temperature, has strand displacement capabilities and is very good at amplifying circular DNA. The plasmid protocol does not produce useable sequence for fosmids and has been the reason so many alternative lines have been created at the JGI and other sequencing centers to handle the sequencing of fosmids.

Method:

Identify the key steps in the plasmid RCA process and test variations in these processes on fosmid clones

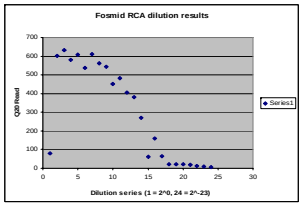
1) Lysis Conditions:

Plasmid RCA uses heat lysis, 95C for 5 minutes. Testing with fosmid clones the optimal lysis time is 10seconds. The correct lysis conditions are critical for Fosmid RCA to work.



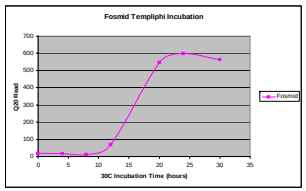
2) Glycerol Stock Dilution in Lysis Buffer:

Plasmid RCA uses a 1:2 dilution of glycerol stock to buffer. Testing with fosmid clones the optimal point appears to be 1:1.



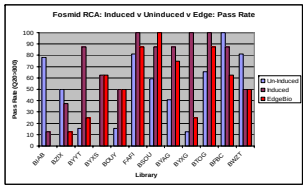
3) Incubation Time:

Plasmid RCA uses 20 hours and Fosmid RCA appears to reach the plateau at roughly 20 hours.



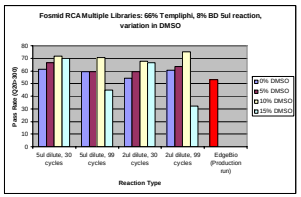
4) Induced Fosmid Clones:

Arabinose is added to increase the copy number of the vector about 5x. Testing shows that for most libraries induction greatly effects RCA performance.



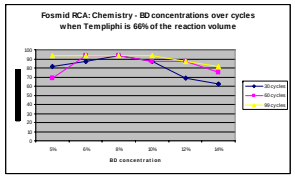
5) DMSO addition to RCA reaction:

DMSO is often used at the JGI to process high GC content genomes and can sometimes greatly effect the pass rate of a library. Using 12 libraries it was found that templiphi containing 10% DMSO by volume was the optimal condition



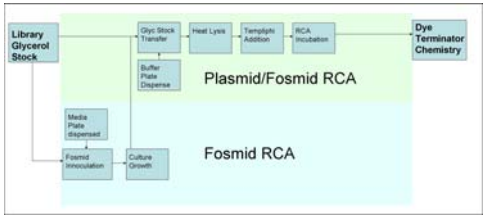
5) Chemistry Optimization – Big Dye and Cycles:

Matrixing Templiphi concentrations in the RCA reaction against Big Dye concentrations in the dye terminator sequencing and number of cycles the optimal conditions were found to be 66% of the RCA reaction should be templiphi, 8%Big Dye should be used in the chemistry reaction and cycled 30 times.



Introduction into Production Line:

The induction step is preserved because induced clones provide better Fosmid RCA performance. The rest of the process is analogous to plasmid RCA processing.



Savings:

The optimized chemistry for Fosmid RCA versus EdgeBio brings the cycling time down from 7.5 hours to 2.75 hours.

The cost of Big Dye is reduced by half.

Production Performance:

Approximately 200 384 well plates have been run in production using the Fosmid RCA protocol.

90% Pass Rate

620 Average Q20 Read Length

The Next Step – BAC RCA sequencing line:

Preliminary work with BAC's shows that clones in the size range of fosmids also sequence well using the above described protocol, minus the induction with arabinose. The process is currently being tested over a range of BAC sizes.

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