Title
Process Optimization in Production

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Process Optimization in Production

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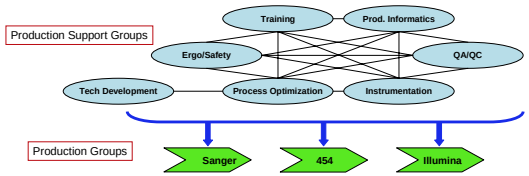
Missions, Interactions, and Projects

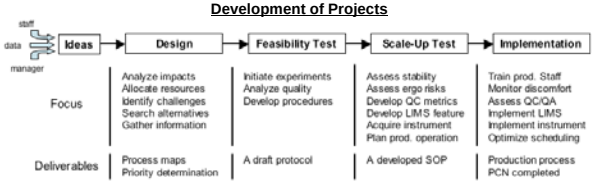
Missions

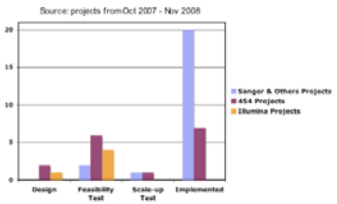
(1) Optimize production processes to increase efficiency, quality, and reduce cost;
(2) Serve as the pilot group to test out any new processes and ensure a smooth transition into the production operations;
(3) Develop new production pipelines to enhance the JGI sequencing capability

Interactions



Development of Projects





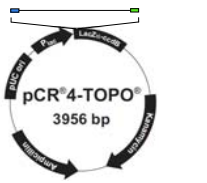
454: Quantitative PCR

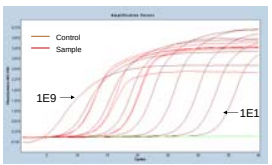
Goal of Project:

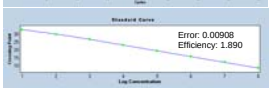
Develop a robust method to quantify libraries of the NexGen platforms. The qPCR offers the consistency and high sensitivity if measuring the adaptors-linked molecules in the libraries.

Project Progress:

We have construction a 454 qPCR copy number standard by cloning a 650 bp fragment flanked by the Titanium adaptors into the pCR4-TOPO vector. A series of diluted standards ranging from 10 to 10^9 molecules were used in the qPCR reaction to quantify the unknown library (red lines in the amplification curve).







Next Steps:

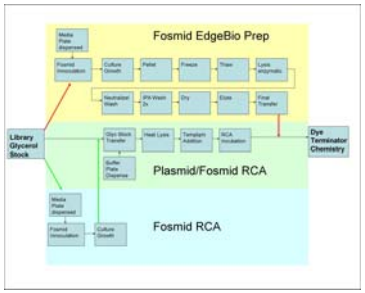
• Compare the Agilent chip readings with the qPCR readings in the ability to predict the percent bead enrichment

• Develop a qPCR copy number standard for the Illumina libraries

Sanger: Fosmid RCA

Goal of Project:

Streamline the production line by converting the sequencing of 40kb Fosmids from the Fosmid EdgeBio Prep to a process based on Rolling Circle Amplification (RCA) analogous to the plasmid line.



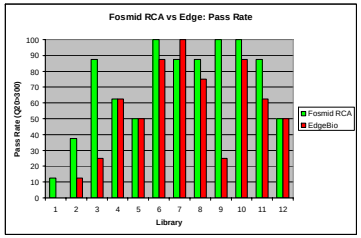
Project Process:

•Identify processes involved in viability of plasmid RCA method

•Processes optimized over large ranges using Fosmid templates

•Optimized processes combined into one continuous processes

•Further optimization of downstream Dye terminator chemistry in order to get more value back from the new line



Results of Project:

•Process streamlined

•Saving of ~$800,000 annually (3 full time employees, half the cost of Big Dye, no more EdgeBio kits)

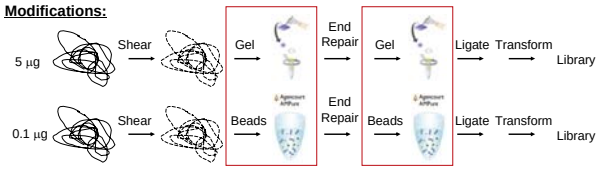
•Pass Rate (>90% on average) and Read Lengths (>620 on average) comparable or better than previous Fosmid EdgeBio Prep line

Sanger: Cloning Trace Amount of DNA

Goal of Project:

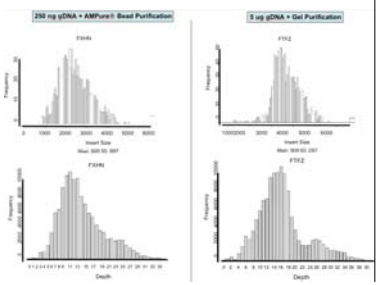
Enable the construction of Sanger libraries with trace amount of DNA sample. The projects that would be benefited from this include any projects that could not produce ug scale DNA such as environmental samples, organelle genomes like chloroplast and mitochondrial genomes.

Modifications:



Results:

The test was done on a chloroplast genome of a flowering plant, Brighamia insignis. We can now generate libraries from 100 ng DNA with good cloning efficiency. The quality of the sequences generated is comparable to the gel purification method.



Library name	Starting material (ng)	Colony Count	Single transformation (CFUs)	# of possible 384's at 60%
FXHH	100	25	5000	7.81
FXHN	250	127	25400	39.69
FXHO	500	640	128000	200.00
FXHP	750	498	99600	155.63
FXHS	1000	1460	292000	456.25

454: Barcode and High Throughput Library Construction

Goal of Project:

Enable high throughput construction of 454 Titanium libraries. Two project goals are (1) uniquely identify each library by the use of a 10-base sequence tag ("barcode"); (2) construct 96 shotgun or fosmid libraries in parallel using an automated platform.

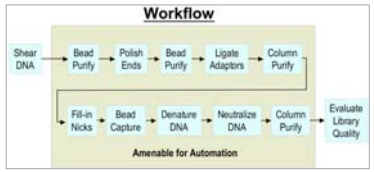
Project Progress:

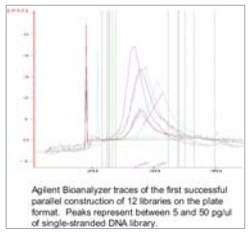
• Designed unique "barcodes"

• Developed a workflow for parallel library construction and successfully constructed 12 libraries

BARCODE SEQUENCE DESIGN REQUIREMENTS

- Oligo length: 10 nucleotides (1,048,576 possible sequences)
- No consecutive same bases (78,732 sequences)
- 40-60% GC content (64,472 sequences)
- No more than 2 di-nt or tri-nt repeats (62,072 sequences)
- Adapter sequences differ by at least 4 bases (270 sequences)





Agilent Bioanalyzer traces of the first successful parallel construction of 12 libraries on the plate format. Peaks represent between 5 and 50 pg/ul of single-stranded DNA library.

Next Steps:

• Pool libraries in equimolar ratios and perform emulsion-PCR and sequencing.

• Test software capabilities to ensure successful separation of libraries by "barcode" sequence tags.

• Develop automated platform in 96-well format to replace the manual pipetting steps.

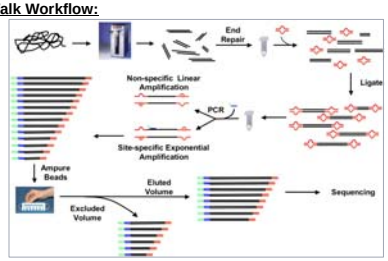
Finishing: Bubble PCR

Goal of Project:

Develop a clone-free approach to perform the finishing tasks. As the Sanger sequencing is being replaced by the next generation sequencing reads, the ability of closing gaps in the draft genomes without clones is crucial to generate finished genomes.

Bubble PCR (bPCR) Primer Walk Workflow:

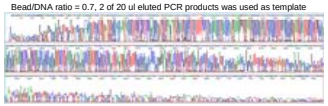
The DNA of interest is randomly sheared to 3 or 8kb fragments. After end repair, bubble adapters (red) are ligated to the DNA ends. After cleanup, bPCR reactions are set up with a site-specific primer (blue) with M13 tails (green). Most DNA fragments would have a linear amplification whereas the region of interest would have an exponential amplification. Small fragments are removed by using AMPure beads and the remaining DNA is used for sequencing.



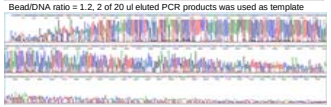
Results:

The bead-to-DNA volume ratios of 0.7 to 2.0 were used to remove small fragments. (The chromatograms generated from sequencing templates of ratios 0.7 and 1.2 are shown here. The beads-to-DNA ratios in this range for cleaning the small fragments do not seem to significantly affect the quality of the sequencing reads. The Sanger approach has been successfully used in gap closure of several prokaryote genomes at JGI.

Bead/DNA ratio = 0.7, 2 of 20 ul eluted PCR products was used as template



Bead/DNA ratio = 1.2, 2 of 20 ul eluted PCR products was used as template



Next Steps:

• Test the bPCR-tag approach on large eukaryote genomes
• Develop a 454 method of closing gaps using the bPCR-tags

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