Process Optimization in Production
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Missions, Interactions, and Projects

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.

Libraries

Goal of Project:
Enhance 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.

Next Steps:
• Saving of ~$800,000 annually (3 Big Dye, no more EdgeBio kits)
• Develop automated platform in 96-well format to replace the manual pipetting steps.

Finishing: Bubble PCR

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

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.

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

454: Barcode and High Throughput Library Construction

BARCODE SEQUENCE DESIGN REQUIREMENTS
- Design unique "barcodes"
- Develop a workflow for parallel library construction and successfully constructed 12 libraries

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.

5 µg Shear 5 µg Shear
0.1 µg Shear
End Repair Beads
Beads
Library
Library
Library

Beads
End Repair
Shear
Gel
Library
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.

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

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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.

Next Steps:
• Further optimization of downstream procedures involved in the construction of Sanger libraries
• Test software capabilities to ensure successful separation of libraries by "barcode" sequences

Next Steps:
• Develop automated platform in 96-well format to replace the manual pipetting steps

454: Quantitative PCR

Goal of Project:
Develop a robust method to quantify libraries of the NextGen 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 pCRY-TOPo vector. A series of diluted standards ranging from 10 to 10^6 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

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