The 454 sequencing-by-synthesis platform, the GS20, is the first of the next generation DNA sequencers to be commercially available. It is capable of producing 300-400,000 ~100bp reads totaling 30-40Mb in a 5 hour run. Although the GS20 produces a large amount of data in a short time, most of the processes upstream of sequencing require a significant investment of labor. At the Broad Institute, we have taken several steps to streamline these processes and significantly reduce the amount of labor needed to conduct a sequencing run.

We focused on optimizing the sizing of DNA fragments, since the 454 technology is most effective when DNA fragments to be sequenced are in a narrow size range. We have developed a new method to shear DNA more efficiently that generates the small fragments required with a higher yield than nebulization. This method uses focused, non-contact acoustics to shear the DNA in an enclosed environment. In addition, we have developed a prototype HPLC-based method that promises to size fractionate the sheared DNA with minimal labor, cheaply and accurately.

Finally, we are automating many steps of the process from library construction to emulsion PCR breakage. These process improvements combined with the automation have allowed us to increase the efficiency and quality of the sample preparation process, and reduce the amount of labor required.

Abstract

The control of the acoustic field obtained with the Covaris instruments enables rapid, isothermal, closed vessel sample preparation. This process, coined Adaptive Focused Acoustics (AFA) by Covaris, sets-up a self-configuring, “mechanical reflux” to maximize the sample retention time in the high intensity focal zone within the sample vessel. In addition, the high frequency of the AFA eliminates many of the constructive and destructive interference patterns intrinsic with lower frequency (e.g., sonicator-type probes) and consequently shearing results are more reproducible. In other applications, the AFA acoustic wave packets may also be down-tuned to gently mix samples containing DNA, such as for washing steps with solid-phase beads, without any shearing.

Platform

454 Process

DNA Shearing: Nebulization

Fragment Purification: Spin Column

Fragment Preparation: Manual

Emulsions Creation: Mixing Mill

Emulsions Breakage: Manual

Broad Process Improvements

DNA Shearing: Covaris*

Fragment Purification: HPLC

Fragment Preparation: Manual

Emulsions Creation: Mixing Mill

Emulsions Breakage: Robotic (filter plate based)

HPLC separation of sheared fragments:

Agilent Chips: 12000 (DNA) and Pico 6000 (sst DNA):

Covaris Shearing

Before (red): 1 µl of Covaris sheared DNA (500ul) template DNA

After (blue): 1 µl of HPCL fractionated sample (concentrated to 15 µl on Qiagen MinElute column)

Single Stranded template DNA

Sequencing Metrics of Covaris Sheared and HPLC Fractionated Library: one lane of 8-lane PicoTiterPlate

<table>
<thead>
<tr>
<th></th>
<th>Total Raw Wells</th>
<th>Total Key Pass Wells</th>
<th>Total Wells Passed Filtering</th>
<th>read length</th>
<th>% reads aligned</th>
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<tbody>
<tr>
<td>Covaris Ultra-sonic Shearing:</td>
<td>17605</td>
<td>17368</td>
<td>7159</td>
<td>95.9</td>
<td>82.2</td>
</tr>
<tr>
<td>HPLC:</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Total bases</td>
<td>565,985</td>
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</tr>
</tbody>
</table>

Discussion

Our improvements to the 454 process will enable us to streamline and automate the platform to significantly reduce the amount of labor needed. Advantages over current process:

Covaris Ultra-sonic Shearing:

- Currently all DNA samples go through the Covaris process
- Non-contact shearing is performed in an enclosed tube
- Minimizes risk of sample cross contamination
- Less input DNA (3 micrograms; working towards 1 microgram or less)
- Decrease shearing volume from 1.6 ml down to 0.5 ml (currently working on 0.1 ml)
- Scalable to 96 well format (E-series instrument)

HPLC:

- Tight size distribution (200-600 bases)
- Automate separation of up to 96 libraries

Robotic Emulsion Breakage:

- Significantly increases walk-away time
- Eliminates learning curve associated with using filter syringes
- More consistent DNA bead recovery
- Reduce possibility of cross-contamination