1 – Abstract

DNA library preparation is the entry point of the 454 Sequencing™ sample preparation process for many applications. The current protocol is amenable for parallel processing of a large number of samples by one individual. We have adopted the GS FLX Standard shotgun DNA library protocol to allow the parallel processing of up to 96 libraries using a commercially available liquid handling automation.

Nebulization has been replaced by fragmentation on a Covaris® E210 instrument. This allows for the unattended fragmentation of up to 96 samples in 3.5 hours. Because there is no DNA loss during fragmentation with the Covaris® instrument we have been able to reduce the DNA input requirement from 5 to 3 μg of double stranded DNA.

All post-fragmentation steps are carried out in a 96-well plate format on a Hamilton MICROLAB® STAR liquid handler. All Qiagen® MiniElute® clean ups have been eliminated and replaced by purification using a combination of Agencourt AMPure® SPRI® beads and Qiagen® QIAquick® 96 well plate. The processing time from using SPRI® to single stranded library takes approximately 5 hours.

The automated processing appears robust and reproducible. We have also validated the method with Multiplexing Identifiers (MiDps). We find library quality to be equivalent to the manual method. The method is currently in production at the 454 Sequencing Center.

2 – Introduction

Project Context:
• Large BAC sequencing project
• 81,000 BACs
• 5,000 BAC pools to be converted into FLX Standard libraries
• Each BAC pool needs MID-adaptors
• Multiple MID-libraries to be pooled for sequencing on a single PGM

Challenges to Overcome:
• Fragmentation by nebulization too cumbersome
• Need automated fragmentation solution
• BAC library provider could not guarantee minimum DNA requirement of 5μg
• Need to reduce DNA input requirement
• Manual library preparation protocol has limited throughput (≤100 ltl/FT/Day)
• Need higher throughput method
• Must be cost effective

Platform Choices:
• Fragmentation on Covaris® E210
• Able to do unattended processing with 96-well rack
• Self-contained sample processing reduces sample loss
• Reproducible fragmentation results
• Hamilton MICROLAB® STAR liquid handler for remaining steps
• Uses only off-the-shelf Hamilton equipment on robot desk
• System is flexible with two pipetting heads and 2 plate handling devices

3 – Automation Solution

Covaris® E210

Covaris heater

Covaris Meter

96-well tube holder and lid

Hamilton MICROLAB® STAR

96-tip Pipetting Head

Vacuum Manifold

Heater Block

Magnet Plate

8-tip Pipetting Tool

Plate Shaker

Vacuum Pump

4 – Workflow Modifications

DNA fragmentation by nebulisation (5 μg)

DNA input reduced to 3 μg

Nebulization replaced by Covaris®

Removed Master Mix Clean up Step Post-Fragmentation

Fragment End Polishing

Removal BSA / Added Town 10

Removed Master Mix Clean up by AMPure® SPRI®

Adapter Ligation

Removed Post-Ligation Clean up

MID-Library Pooling Step (Optional)

Library transformation

Fill-in Reaction

single stranded DNA Library isolation

Replaced Master Mix Clean up by Agarose® 96

5 – Comparison Between Manual and Automated Methods


- The current Covaris® protocol uses the following settings: 99% duty cycle, 3 intensity, 209 cycles per burst, 0.6 seconds per sample.
- Fragmenting 96 samples on Covaris® using these settings takes 3.5 hours, including manual sample transfer in and out of the tubes.
- The automated Covaris® protocol on the Covaris® E210 takes approximately 9 hours starting at SPRI® clean up (post-fragmentation) and ending with eluted ssLibrary.

- We have successfully automated the 454 Sequencing™ FLX Standard library preparation protocol.
- We have replaced labor intensive manual nebulization by fragmentation on a Covaris® E210 instrument.
- We have adopted the FLX Standard library protocol to make it compatible with automated liquid handling.
- OTS components used: No custom equipment.

- Processing of up to 96 samples can be done in parallel over a 2-day period.
- Fragmenting of 96 samples takes 3.5 hours.
- Completing the rest of library prep takes 9 hours.

- For one researcher creating 96 FLX Standard Libraries:
  - 8.5 hours using the automated protocol.
  - 68 hours using the manual protocol (≤100 ltl/FT/Day).

- Automated protocol is being adopted to GS FLX Titanium shotgun library preparation

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