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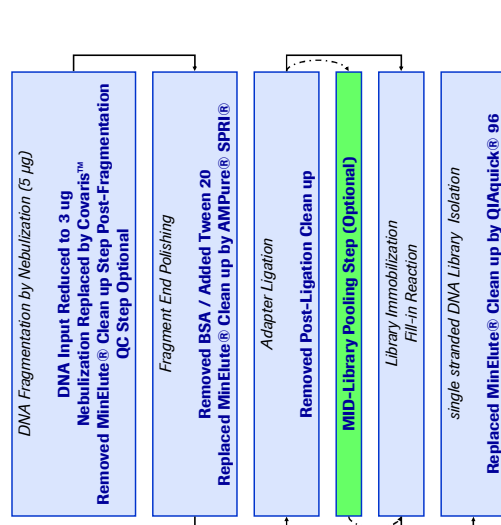
1 – Abstract

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 adapted the GS FLX 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Nebulization has been replaced by fragmentation on a Covaris™ E210 instrument.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 6.5 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 ug 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® MinElute® 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 sizing 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 (MIDs). We find library quality to be equivalent to the manual method. The method is currently in production at the 454 Sequencing Center.

4 – Workflow Modifications



Legend: Italic black font corresponds to the standard manual shotgun protocol. Bold blue font corresponds to changes made to the protocol. The green box shows the optional library pooling step inserted after adapter ligation.

2 – Introduction

Project Context:

Large BAC sequencing project:

- 80,000 BACs
- 5,000 BAC pools to be converted into FLX libraries
- Each BAC pool needs MID-adapters
- Multiple MID-libraries to be pooled for sequencing on a single PTP™

Challenges to Overcome:

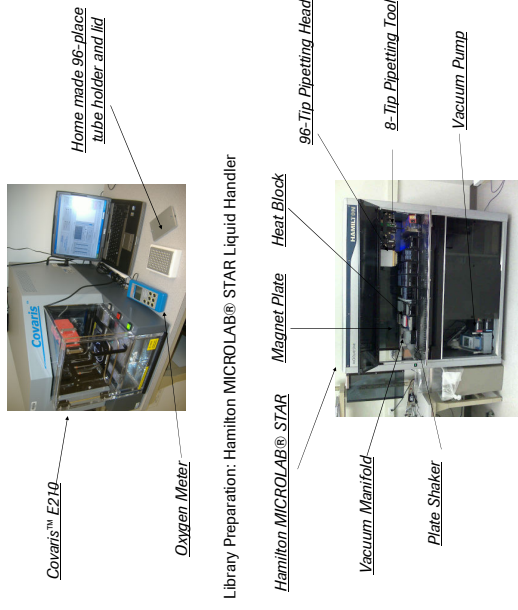
- Fragmentation by nebulization too cumbersome
- Need automated fragmentation solution
- BAC library provider could not guarantee minimum DNA requirement of 5ug
- Need to reduce DNA input requirement
- Manual library preparation protocol cumbersome (8-12 ssLib/FTE/day)
- Need higher throughput method
- Must be cost effective

Platform Choices:

- Fragmentation on Covaris™ E210
- Amenable to unattended processing with custom rack
- Self-contained sample processing reduces sample loss
- Reproducible fragmentation results
- Hamilton MICROLAB® STAR liquid handler for post-fragmentation steps
- Uses only off-the-shelf Hamilton equipment on robot deck
- System is flexible with two pipetting heads and 2 plate handling devices

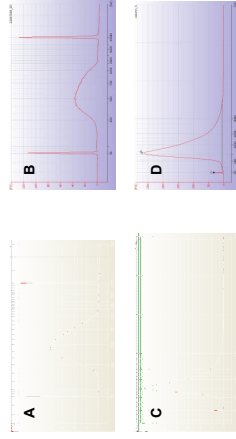
3 – Automation Solution

DNA Fragmentation: Adaptive Focused Acoustics technology™ (AFA)



Library Preparation: Hamilton MICROLAB® STAR Liquid Handler

5 – Comparison Between Manual and Automated Methods



Legend: Agilent Bioanalyzer electropherogram examples. **A)** Standard manual protocol, post-nebulization & post-SPRI® clean up. **B)** Automated protocol, post-Covaris™ fragmentation & post-SPRI® clean up. **C)** Single stranded library completed by standard manual protocol. **D)** Single stranded library completed by automated protocol (including Covaris™ fragmentation). This sample is comprised of multiple MID-libraries pooled post ligation.

- The current Covaris™ protocol uses the following settings: 9% duty cycle, 5 intensity, 200 cycles per burst, 240 seconds per sample.
- Fragmenting 96 samples on Covaris™ using these settings takes 6.5 hours, excluding manual sample transfer in and out of the tubes.
- The subsequent automated protocol on the Hamilton MICROLAB® STAR takes approximately 5 hours starting at SPRI® clean up (post-fragmentation) and ending with eluted sslibrary.

6 – Conclusions

- We have successfully automated the 454 Sequencing™ FLX library preparation protocol.
- We have replaced labor intensive manual nebulization by fragmentation on a Covaris™ E210 instrument.
- We have adapted the FLX library protocol to make it compatible with standard liquid handling automation.
- OTS components used: No custom equipment or instrumentation.
- Processing of up to 96 samples can be done in parallel over a 2-day period
- Fragmentation of 96 samples takes 6.5 hours
- Processing samples through the rest of library prep takes 5 hours
- Improvements on the way:
 - Switch to new Covaris™ 100 µl tube w/ AFA fiber will allow decreasing fragmentation time from 240s to 90s per sample.
 - New tube is also wider and more shallow, more amenable to automation liquid handling.
 - Automated protocol being adapted to Titanium shotgun library preparation protocol.

Acknowledgment:

We would like to thank the Broad Institute of MIT and Harvard for sharing with us their Covaris™ experiences at the beginning of this project.