Utilization of the Covaris® 96 oneTUBE-10 AFA Plate Streamlines NGS Sample Prep

Justin Abreu[1], Laurie Holmes[1], Cole Walsh[1], Elizabeth Weiss[2], Gianna Garza[2], Jeremy Terry[2], Eugenio Daviso[2], Uli Thomann[2], Tim De Smet[1] Stacey Gabriel[1]

[1]Genomics Platform, Broad Institute, 320 Charles Street, Cambridge, MA 02141 [2]Covaris Inc. 14 Gill Street, Woburn, MA 01801

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The most commonly used approach for fragmenting genomic material utilizes mechanical shearing. This method is advantageous in that it introduces little to no bias as a result of processing. However, mechanical shearing methods necessitate the use of specialized equipment and consumables and, currently, require several manual transfer steps for processing. The workflow presented here eliminates the need for additional transfer steps, reducing the possibility for sample loss and decreased process yield. Additionally, this workflow addresses many of the inconveniences currently associated with gDNA fragmentation on Covaris Focused-ultrasonicators, such as:

- Monitoring water levels
- Time associated with water conditioning steps, such as:
    - degassing
    - reducing water temperature to 4 °C
- Adaptive Focused Acoustics® (AFA®) fiber interferes with tips used by liquid handlers
- AFA tubes and plates do not fit in typical labware used for downstream NGS library processing

Technology Improvements

Current Method: Constant monitoring and adjusting of water level
Improvement: Automated water level detection and z-height adjustment

Current Method: Acoustic shearing performed at 4 °C necessitating additional time to chill the water bath
Improvement: Shearing performed at room temperature. Chiller system used to maintain water temperature

Current Method: Degassing of the water bath must be performed and engaged by the user, potentially causing delays in processing
Improvement: Scheduling software provides the ability to initialize the system including water degas with no user intervention

Current Method: AFA shearing vessels were not compatible with most thermocyclers, heat blocks, and magnets

- Necessitated the transfer of material in and out of the AFA vessel
- Prevented further processing of sheared material within the AFA vessel

Improvement: 96 oneTUBE-10 AFA Plate now fits most thermocyclers, heat blocks and magnet plates

- Reduction of the number of transfer steps
- 96 oneTUBE-10 AFA Plate can be used for further downstream processing as it pertains to NGS library construction

Current Method: AFA fiber in shearing vessels can interfere with tips of automated liquid handlers presenting issues with liquid aspiration
Improvement: 96 oneTUBE-10 AFA Plate is specially designed polymer vessel integrated with cavitation sites for use with AFA

(a) 

(b) 

Figure 1 a-b: Image of the LE220-plus Focused-ultrasonicator and 96 oneTUBE-10 AFA Plate.

NGS Workflow Improvements

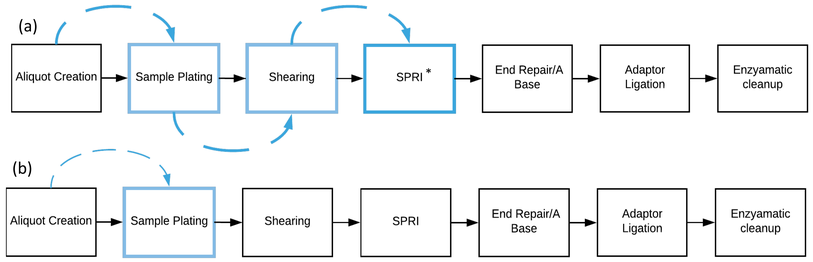

Figure 2a and b: Comparison of a (a) traditional NGS Library construction workflow and an NGS library construction workflow using the 96 oneTUBE-10 AFA Plate (b) Transfer steps highlighted in blue.

The LE220-plus paired with the 96 oneTUBE-10 AFA Plate resulted in:

- Reduction in the number of transfer steps of genomic material
    - Traditional NGS workflow: 3 transfers
    - NGS workflow using 96 oneTUBE-10 AFA Plate: 1 transfer

- Reduction in overall sample treatment time
    - When compared with the standard WGS and WEX production workflows within the Genomics Platform at the Broad
        - Exome: 39% reduction
        - WGS: 44% reduction

Protocol	Instrument	Plate Type	Time (seconds)	Mean Fragment Size (bp)
WGS	LE220-plus	96 oneTUBE-10 AFA Plate	30	411
			45	312
	LE220	96 microTUBE	80	335
Exome	LE220-plus	96 oneTUBE-10 AFA Plate	220	154
	LE220	96 microTUBE	360	150

Table 1 : Comparison of a (a) traditional NGS library construction workflow and an NGS library construction workflow using the 96 oneTUBE-10 AFA Plate (b) Transfer steps highlighted.

- Improved reproducibility of fragment distribution

Time (seconds)	Mean Fragment Size (bp)	Standard Deviation	% CV
15	704	54.31	7.72
30	411	20.74	5.04
45	312	11.34	3.63
90	223	5.43	2.43
220	154	5.88	3.81
240	151	2.28	1.51

Table 2 : Outline of the fragment distribution and reproducibility of the 96 oneTUBE-10 AFA Plate shearing plate on the LE220-plus for multiple fragment sizes. Data provided by Covaris.

Summary

The newly released LE220-plus Focused-ultrasonicator combined with the 96 oneTUBE-10 AFA Plate improves turnaround time and decreases the overall cost of library prep. Pairing these technologies enables an NGS sample prep workflow in which sample normalization, mechanical shearing and library construction is performed within a single vessel. Streamlining the library preparation workflow has been found to provide libraries of equivalent quality as those generated with a more traditional method while also providing a more standardized solution.

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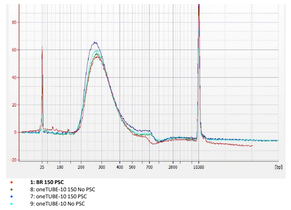
Evaluating Library Quality

- Use of the 96 oneTUBE-10 AFA Plate, in combination with the LE220-plus, resulted in libraries of equivalent quality as compared to libraries created using the 96 microTUBE Plate and LE220 Focused-ultrasonicator.

(a) 

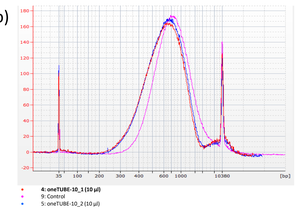
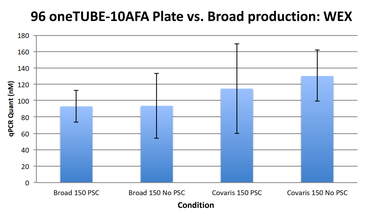
(b) 

Figure 3: Electropherograms demonstrating the consistency in fragment distributions for libraries created using the 96 oneTUBE-10 AFA Plate for (a) Exome and (b) WGS libraries.

- Fragment distributions and yields (nM) for PCR-free WGS libraries were consistent with that observed when compared with production-grade Broad PCR-free WGS libraries.



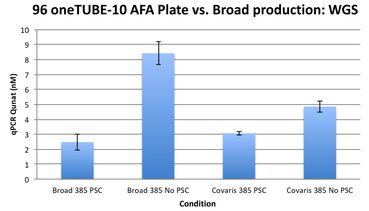


Figure 4 (top): (a) qPCR results of process yields for both WEX and PCR-free WGS (b) libraries created using the 96 oneTUBE-10 AFA Plate workflow as well as the Genomics Platform production workflow.

Figure 5 (right): Comparison of normalized coverage across the GC spectrum for PCR-free WGS libraries created using a conventional NGS workflow (red) and those created using the 96 oneTUBE-10 AFA Plate workflow (green and purple).

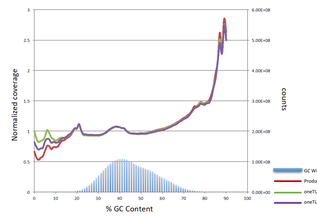


Table 3: Comparison of several Picard pipeline metrics for PCR-free WGS libraries generated using a modified workflow featuring the Covaris 96 oneTUBE-10 AFA Plate along with the LE220-plus Focused-ultrasonicator and PCR-free WGS libraries created using the current Broad production workflow.

Condition	Estimated Library Size	Mean Coverage	Gb/1X	Q score (Cref)
Broad Production	2,699,122,933	4.43	3.72	47
Covaris 96 oneTUBE-10 AFA Plate LE220-plus	2,892,129,705	5.09	3.66	46

Estimated Library Size: Estimation of the number of unique molecules observed within a sample. Used a means to assess library molecular diversity.
Q score (Cref): Numerical score used to assess the extent of oxidative damage associated with sample shearing. Generally scores greater than 40 are desired.
Gb/1X: Amount of passing filter gigabases necessary to generate 1X coverage.

Acknowledgments

Data used in this poster were generated at the Broad. For more information please visit: http://genomics.broadinstitute.org/

Data were created in collaboration with Covaris.