An optimized single tube workflow for robust, low input NGS library preparation

Guillaume Durin1, Elizabeth Weiss1, Jeremy Terry1, Julie Donaldson1, Ulrich Thomann1, Eugenio Daviso1, Jonathan Sampson1, Gianna Garza1, Justin Lenhart2, Sukhinder Sandhu2, Laurie Kurihara2, and Jim Laugharn1
1 Covaris Inc., Woburn, MA, USA and 2Swift Biosciences, Ann Arbor, MI, USA

INTRODUCTION

As Next Generation Sequencing (NGS) moves into the clinical diagnostics space, requiring higher throughput and sensitivity, existing processes need to be improved to ensure accurate data outputs. Mechanical DNA shearing using Covaris® Adaptive Focused Acoustics® (AFA) technology is recognized as the gold-standard for DNA fragmentation in NGS library preparation. AFA hydrodynamic shear force-based DNA fragmentation is strictly mechanical and isothermal, and thus guarantees a highly controlled process while yielding random, unbiased DNA fragment distributions.

We present a new DNA shearing vessel, the Covaris oneTUBE™, that is optimized for low DNA input and allows single tube DNA shearing and NGS library preparation. This new engineered polymer vessel incorporates features that effectively control acoustic cavitation to enable reproducible and precisely-tuned hydrodynamic shear forces, its low microscale surface guarantees NDA recovery as well as ease of nucleic acid handling down to 10 µl and program DNA inputs. Additionally, this vessel is fully compatible with magnetic separation procedures, thermal cylics and liquid handling platforms, sample design is adapted for up to 500 samples, such as 96, 384, and high well densities.

We present data that demonstrates precise and accurate DNA shearing in the Covaris oneTUBE, and validation using a wide range of DNA input quantities and SSCC.

For NGS library construction, DNA shearing in the oneTUBE was paired with Swift Biosciences Accel-NGS 25 K. The 25 K kit preserves complexity from low input samples due to enhanced end repair chemistry for highly efficient adapter ligation in a single tube workflow. This combination enables for the first time DNA shearing, end repair, and adapter ligation in the same vessel, thereby eliminating cumbersome sample transfers. We present sequence data from libraries constructed with this workflow and compare them to libraries constructed according to standard “left transfer” protocol in microTUBE.

WHOLE GENOME SEQUENCING ON HiSeq X TEN

PCR-free WGS Libraries were constructed as described in Figure 3 using 250 ng Covaris miRNA Purified genomic DNA. For each condition, two libraries were constructed (one entirely in the 96 oneTUBE-10 AFA Plate, and one with microTUBE for DNA Shearing and subsequent transfer to PCR plate for library construction (control library). Sequencing was carried out by Novogene on the Illumina HiSeq X Ten platform. Post sequencing, following exclusion filters were applied before analysis (e.g exclusion for oneTUBE and microTUBE Libraries, respectively)

Low-mapping quality reads 4.1% and 4.7%
- Duplicate exclusion 3.0% and 7.9%
- Low base quality 4.5% and 4.2%
- Second observation from an insert with overlapping reads: 2% and 4.1%

CONCLUSIONS

Combination of DNA shearing in the oneTUBE with Swift Biosciences Accel-NGS 25 K enables for the first time DNA shearing, end repair, and adapter ligation in the same vessel, thereby eliminating cumbersome sample transfers and enabling a streamlined workflow. Sequencing performance metrics in oneTUBE are matching or exceeding current benchmark consisting of shearing in microTUBE and then transferring into a PCR plate for library construction.

DNA shearing with the 96 oneTUBE-10 AFA Plate demonstrated robust performance. For both conditions analyzed, 150 and 350 bp, the 48 technical replicates display highly reproducible and tight DNA fragment size distributions. Testing performance with different DNA sizes also highlight that, as expected with mechanical DNA shearing, the protocols are robust across a wide input range.

ARTIFICIAL MICROBIAL COMMUNITY (AMC) SEQUENCING

We constructed an Artificial Microbial Community (AMC) to study how varying species, phyla, and GC bias may impact PCR free WGS libraries.

"BAD PROMOTERS" LIST ANALYSIS FOR WGS DATA

"Characterizing and measuring bias in sequence data” from Ross et al.

Table 3. WGS coverage and alignment tables

Table 2. WGS Coverage Table

Figure 3. Concatenated Read NGS 25 Kit library with 96 oneTUBE-10 AFA Plate. Single reads without transfer step to generate PCR-free from DNA inputs.

Figure 4. Robust and linear DNA shearing

Comparison of DNA fragment size distributions at different DNA inputs, for a range of 1 ng to 1 µg. Human genomic DNA (Promega PN G3041) was sheared on Covaris LE220-plus with the following settings:

- A: Overlay of the 48 technical replicates after amplitude normalization: 150 bp (Blue) or 350 bp (Red).
- Box plot of the 48 replicates for each targeted size (500 bp, 1500 bp, 3500 bp) genomic DNA (Promega PN CI014) was sheared on Covaris LE220 plus with the following settings:

Table 1. Evaluation of transfer yield and base composition bias

<table>
<thead>
<tr>
<th>Input</th>
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<th>DNA Concentration</th>
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<th>Mean Mode Coverage</th>
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<tbody>
<tr>
<td>5 µg</td>
<td>PCR-free</td>
<td>miRNA purified</td>
<td>50 ng</td>
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MATERIALS AND METHODS

Artificial Microbial Community (AMC) sequencing was performed to study how varying species, phyla, and GC bias may impact PCR free WGS libraries.

"BAD PROMOTERS" list analysis for WGS Data

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Figure 8. Coverage of 25 ng PCR-free WGS libraries.

Figure 9. Relative "Bad Promoters" coverage

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