TECHNICAL NOTE

AUTOMATED DNA SHEARING AND LIBRARY PREPARATION FOR PACBIO® LONG-READ WHOLE GENOME SEQUENCING WITH THE DREAMPREP® NGS COMPACT AND DREAMPREP NGS.

INTRODUCTION.

DNA shearing is often a resource- and time-consuming process in genomic workflows. For this reason, automated DNA shearing methods have been developed on DreamPrep NGS platforms. These methods generate DNA fragments approximately 15- to 20-kb in length that are suitable for library preparation and subsequent PacBio sequencing, thereby enabling and simplifying assay scalability.

MATERIALS AND METHODS.

The DNA shearing methods were developed using Tecan tips for both the 8-channel Flexible Channel Arm™ (FCA) and Multiple Channel Arm™ 96 (MCA96). FCA is present in both DreamPrep NGS configurations (DreamPrep NGS and DreamPrep NGS Compact) and MCA in DreamPrep NGS. Full sample number flexibility (1 to 96 samples) and walk-away time are supported in both methods. Regarding labware, Tecan conductive filtered 200 µl tips (Tecan Part Number 30057815) and specific deep well plates are required for both methods. The protocols for these methods are designed to be intuitive and accessible through integrated touchscreens with TouchTools™, ensuring ease of use. The subsequent library prep workflow PacBio SMRTbell® prep kit 3.0 has already been fully qualified on DreamPrep NGS Compact to process up to 48 samples, and a respective example script is also available for the larger DreamPrep NGS, allowing for the simultaneous processing of up to 96 samples.

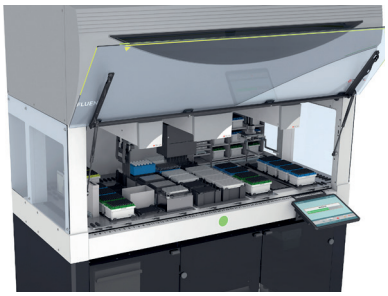


Figure 1: DreamPrep NGS with three independent arms (FCA, MCA, and Robotic Gripper Arm™ [RGA]). On-deck thermocycler (ODTC) and reader are also included.

Studies were performed with 8 samples of Promega Human Male DNA (Catalog Number G1471), in which more than 90% of fragments are longer than 50-kb, and the concentration was 15 ng/µl in a total volume of 200 µl. Mixing cycles are performed in high pipetting speeds to maximize shearing. The FCA method uses dispense speeds of 900 µl/s for 400 cycles and the MCA96 method uses 750 µl/s for 200 cycles. After shearing, fragment sizes were assessed with Femto Pulse system (Agilent Technologies). The sheared DNA with the FCA method was sent to PacBio for manual library preparation using PacBio SMRTbell prep kit 3.0 and size-selected with an 8-kb cut-off using PippinHT (Sage Science). Sequencing of pooled samples was performed on the Revio™ system using a single SMRT Cell. Running time for the MCA96 shearing method is 6 min, which allows 96 samples to be processed in parallel. Whereas for the FCA shearing method, 9 minutes is required per column.

Figure 2: DreamPrep NGS Compact with two independent arms (FCA and RGA). ODTC and reader are also included.

RESULTS AND DISCUSSION.

Although different arms and methods were used, individual optimized parameters for each method were found to generate similar and reproducible DNA fragment distributions, which are all within specifications from PacBio for downstream library prep and subsequent sequencing (Figures 3A, 3B and 4). Sequencing results (Figure 4) further confirmed that DNA sheared using FCA produces high quality data and sequencing yields greater than 100 Gb with mean read lengths of around 16-kb.

TECAN.

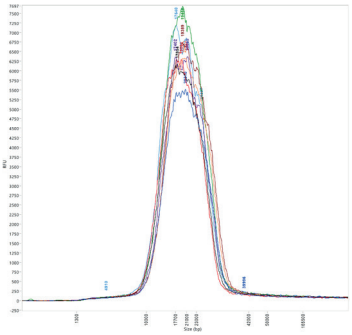


Figure 3A: Femto traces of DNA fragments generated by automated shearing using FCA. Average fragment size was 19326 bp.

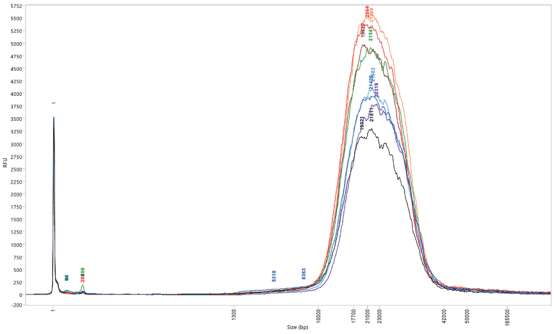
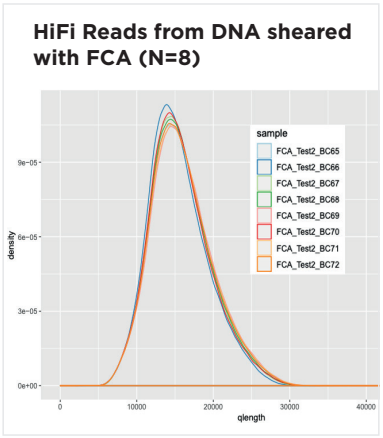


Figure 3B: Femto traces of DNA fragments generated by automated shearing using MCA96. Average fragment size was 21251 bp.

The MCA96 shearing method is quite efficient as it enables desired DNA shearing at lower speeds and less mixing cycles (for a total of 6 minutes), thus matching high throughput requirements and holds potential for generating stronger shearing forces, if required. Future testing will be performed to generate further evidence on how DreamPrep NGS with MCA96 can cover a full workflow from tip shearing down to library prep with short read eliminator (SRE) in walk-away mode. Both FCA and MCA DNA shearing methods are cost-effective as only standard automation-related labware is needed (i.e., conductive tips and plates).

CONCLUSIONS AND OUTLOOK.

Similarly to the previous results from the technical note of the PacBio SMRTbell prep kit 3.0 with DreamPrep NGS Compact (402700 V1.0, 2023-08), DNA shearing with either FCA or MCA96 has also been shown to be a reliable solution that considerably optimizes lab resource efficiency and minimizes user interaction. Therefore, automating and combining upstream DNA shearing with library preparation protocols in DreamPrep NGS solutions will enable users to upscale their genomic projects faster.



Sequencing Metrics	Values
HiFi reads	6.6 M
HiFi base yield	103.9 Gb
HiFi read length (mean)	15.9-kb
Q30+ bases	91%

Figure 4: Sequencing results of DNA fragments generated by automated shearing using FCA. Density plot shows the read length distribution for each of the channels of the FCA, while the table shows the HiFi sequencing metrics.

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