

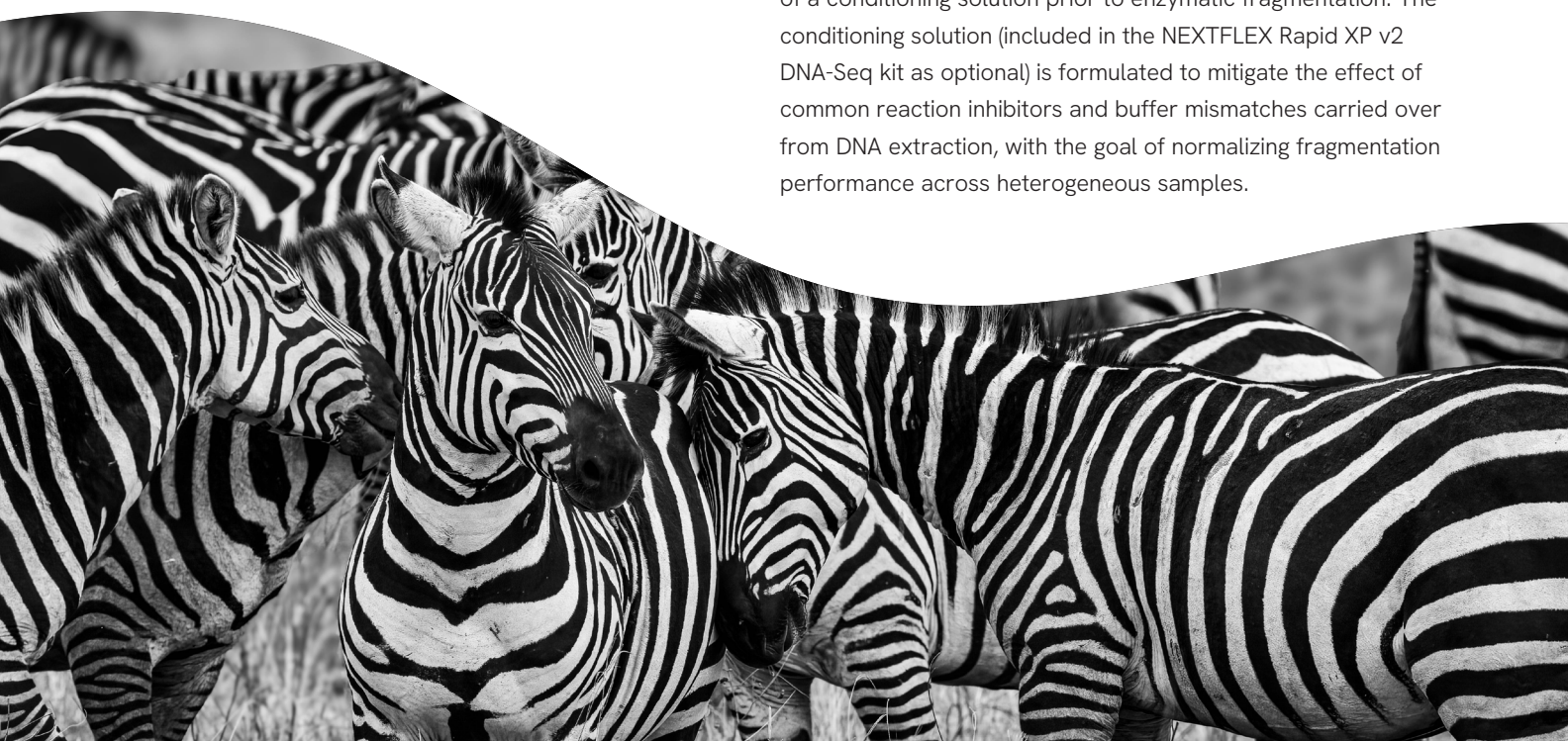
Reducing fragmentation variability in WGS library prep.

Introduction

Whole-genome sequencing (WGS) enables comprehensive detection of genomic variation across the entire genome. As sequencing costs continue to decline and run formats to support higher multiplexing, WGS is increasingly adopted in research settings for a broad range of applications.

For laboratories with limited NGS experience, the process of WGS library construction can appear challenging. Automation on robotic systems, such as the Fontus™ NGS Workstation (Revvity), substantially reduces hands-on time and improves reproducibility. Nevertheless, certain steps can remain sources of variability even under automation. Chief among these is enzymatic fragmentation of the input DNA, whose efficiency is affected by attributes such as molecular weight distribution, residual solvent content, carryover salts and chelators, polysaccharides or phenolic compounds from extraction, and overall integrity. In practice, these factors lead to sample-to-sample variability in insert sizes, cluster density, duplication rates, and coverage uniformity, all undesirable outcomes in high-throughput WGS studies.

The NEXTFLEX™ Rapid XP v2 DNA-Seq Kit (Revvity) was designed to streamline WGS library construction while maintaining performance across a range of inputs and sample qualities. A fully automated implementation of this workflow on the Fontus NGS Workstation is available. Here we describe the impact of a simple intervention intended to reduce variability: the addition of a conditioning solution prior to enzymatic fragmentation. The conditioning solution (included in the NEXTFLEX Rapid XP v2 DNA-Seq kit as optional) is formulated to mitigate the effect of common reaction inhibitors and buffer mismatches carried over from DNA extraction, with the goal of normalizing fragmentation performance across heterogeneous samples.



Methods

Coriell DNA (NA12878) was used as input material. DNA was provided in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0, commonly referred to as low-EDTA Tris buffer. The presence of 1 mM EDTA chelates divalent cations such as Mg^{2+} to inhibit nuclease activity but can interfere with enzymatic DNA fragmentation.

A total of 192 libraries were prepared on the Fontus NGS Workstation with the NEXTFLEX Rapid XP v2 kit using 20 min of fragmentation time. 96 libraries (80 ng input) were processed without conditioning solution and 96 (67 ng input) with conditioning solution. In both cases protocol downstream of fragmentation was identical.

Final libraries were then quantified using the Qubit™ dsDNA High Sensitivity Assay kit (Thermo Fisher Scientific) to check for recovery and examined using the LabChip™ GX Touch™ nucleic acid analyzer with the NGS 3K Assay Kit (Revvity) for proper sizing.

Results

When using the Fontus NGS workstation, library preparation reagents are maintained at 4°C, while samples in the 96-well PCR plate remain chilled at 4°C on the Inheco Thermoshake device. If the conditioning solution is used, the Fontus Varispan™ pipetting arm first dispenses it into the DNA samples before the fragmentation buffer is added. Subsequently, the instrument mixes each column three times to ensure homogeneity.

The fragmentation enzyme is not a hot-start enzyme and therefore is active upon contact with DNA. The enzyme is dispensed into a clean 96-well PCR plate on the On-Deck Thermal Cycler (ODTC), which is set to 4°C prior to incubation. Sample and buffer are aspirated and mixed with the enzyme on the ODTC, and the reaction is sealed by the gripper with an ODTC lid to proceed with the uniform fragmentation incubation program.

This synchronized setup ensures that all wells begin enzymatic fragmentation simultaneously, minimizing variability in exposure time and resulting fragment sizes. This timing can be very difficult to achieve manually. To evaluate fragmentation efficiency, we plotted library yield (proportional to input amount) versus peak fragment size for samples prepared with (+) and without (-) the conditioning solution (Figure 1).

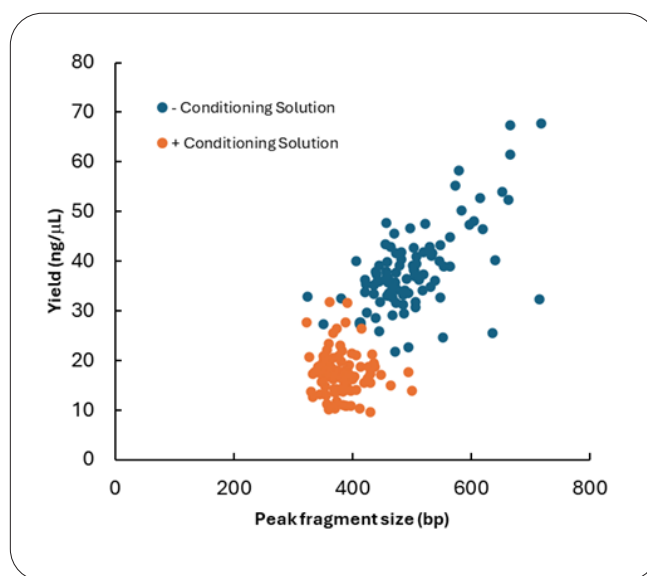


Figure 1: Yield vs peak fragment size for libraries without (-) and with (+) conditioning solution.

Libraries generated without conditioning solution displayed a higher variability in fragment size (CV = 14.8%), whereas those treated with conditioning solution showed improved uniformity (CV = 8.6%). These results demonstrate that even for high-quality reference DNA, the conditioning solution enhances fragmentation consistency across samples.

Conclusions

Fragmentation efficiency depends on both the inclusion of the conditioning solution and the characteristics of the DNA input (e.g., concentration, purity, and integrity). The use of the conditioning solution prior to enzymatic fragmentation on the Fontus NGS workstation improves library size uniformity and minimizes variability across samples, supporting higher reproducibility in automated WGS workflows.

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