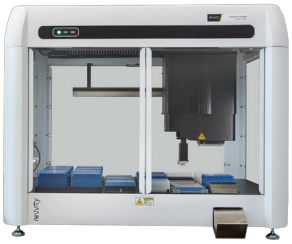
APPLICATION NOTE

Ultra-High Capacity Multiplexing With the Twist FlexPrep UHT Workflow Automated on Revvity Sciclone G3 NGSx Workstation

INTRODUCTION

Next-generation sequencing (NGS) has revolutionized biological research, but current library preparation workflows often present bottlenecks, particularly when scaling to large sample numbers. In addition to being labor-intensive and costly, existing methods can also introduce variability to performance. These characteristics limit the efficiency of high-throughput genomic studies, such as low-pass whole genome sequencing (lpWGS), target capture, and genotyping by sequencing (GBS). Twist Bioscience has developed the FlexPrep UHT Library Preparation Kit to address these challenges. A single kit can prepare enough libraries for up to 1,152 samples to be sequenced in one run. The FlexPrep workflow leverages two key Twist innovations: (1) Normalization-by-Ligation™ (NBL), which eliminates the need for upfront and intermediate sample quantitation, and (2) inline read barcodes, which enable high levels of multiplexing. Furthermore, the FlexPrep UHT Library Preparation Kit uses fill volumes that are automation friendly. This application note details the performance of the FlexPrep workflow on a Sciclone™ G3 NGSx automation platform, presenting data on sequencing metrics. We demonstrate that the FlexPrep workflow, when coupled with automation, delivers significant improvements in throughput and cost-effectiveness while maintaining high-quality sequencing results.



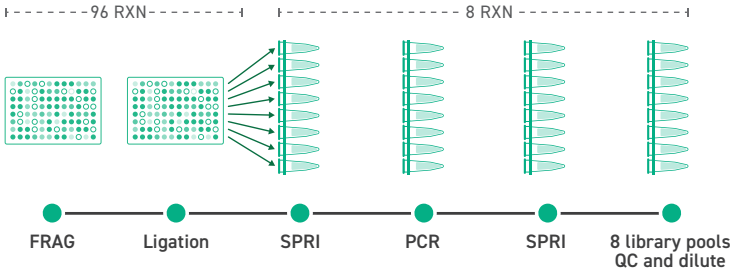
Revvity Sciclone G3 NGSx Workstation.

METHODOLOGY

The Revvity Sciclone G3 NGSx workstation is an automated liquid handler that allows for consistent and reproducible NGS library preparation at scale. The Twist FlexPrep UHT method for the Sciclone G3 NGSx was developed, tested, and demonstrated with 384 samples using 100 ng input of control gDNA (Horizon Tru-Q 100% WT Reference) per sample. All thermal cycling was performed off-deck with a fragmentation time of 22 minutes and 6 cycles of PCR amplification.

The Twist Bioscience Flexprep UHT library workflow consists of:

1. **Enzymatic Fragmentation/End Repair/dA-tailing**
2. **Normalization Adapter Ligation**
3. **Pooling**
4. **Post-Ligation Bead Cleanup**
5. **PCR Setup**
6. **Post-PCR Bead Cleanup**



The Twist Bioscience FlexPrep UHT Workbook describes how to prepare master mixes and reagent plates to begin a run.

For Enzymatic Fragmentation/End Repair/dA-tailing, Normalization Adapter Ligation, and PCR Amplification, an off-deck, full-skirt plate compatible thermal cycler was utilized. For each of the off-deck thermal cycler programs, the user was prompted to remove the plate from the Sciclone deck and place it on the thermal cycler. While the off-deck thermal cycler programs were running, the ligation mix and the PCR mix were broadcasted to streamline subsequent steps. The user was prompted to return the plate to the deck at the completion of its cycler program. The method was completed in approximately 4 hours.

A total of 384 FlexPrep libraries were generated and enriched using manufacturer instructions. Twist Exome 2.0 and Human RefSeq panels were used for target enrichment. For sequencing, 96 libraries were pooled and then sequenced on a NovaSeq S4 flowcell using 200 paired-end cycles. These libraries were selected from quadrant 1 of the 384-well plate and capture data was downsampled to 150X.

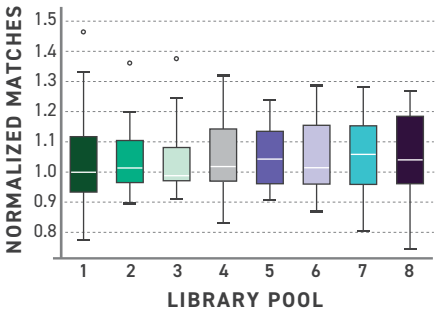


Figure 1. Normalized Matches by Pool for lpWGS.

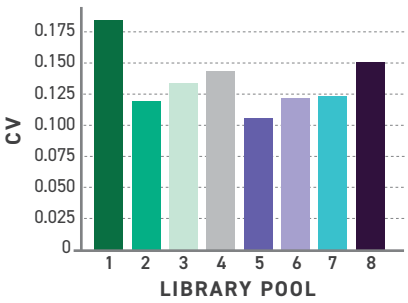


Figure 2. CV by Pool for lpWGS.

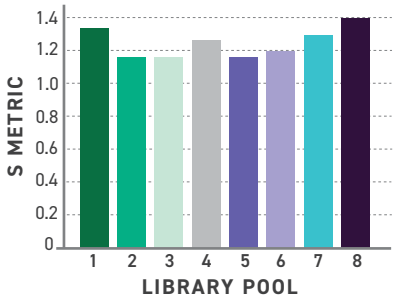


Figure 3. S Metric by Pool for lpWGS.

RESULTS

Low-Pass Whole Genome Sequencing

For lpWGS, 96 samples went through library preparation. For all 96 samples, Normalized Matches averaged 1.05 (**Figure 1**). Additionally, CV averaged 0.14 (**Figure 2**). The S metric had an average of 1.25 (**Figure 3**). The S metric measures how much more relative sequencing is required for the least sequenced sample in a pool to equal the mean sequencing depth of the pool. A lower value indicates less variability among the coverage of the individual samples. A hypothetical case of perfect uniformity would have an S metric of 1. These results show that sequencing across each sample was very uniform.

Exome Sequencing

In total, 96 samples underwent library preparation and target enrichment. Picard metrics show that the workflow produces high-quality enriched libraries.

Across all 96 samples, Normalized Matches averaged 1.06 (**Figure 4**). CV averaged 0.22 (**Figure 5**). Furthermore, the S metric averaged 1.39 (**Figure 6**). In general, this shows that each sample was sequenced very uniformly.

Fold-80 Base Penalty score averaged 1.44, demonstrating high enrichment uniformity for all captures. Off-Target Rates averaged 7.9%, showing capture was efficient. Average Mean Target Coverage was 55X, Percent Target Bases 30X was 88%, and Zero Coverage Targets was 1.7%. This shows that sequencing depth was adequate for downstream analysis. HS Library Size was 99M. Duplicate Rate averaged 14% (**Figure 7**). These results show that all libraries had sufficient complexity for downstream analysis.

The performance of this automated workflow shown here is concordant with manual workflows. For details on manual workflow performance, please see the Twist FlexPrep UHT Library Preparation Kit Datasheet and the Twist FlexPrep UHT Library Preparation Kit product sheet. The workflow steps are detailed in the library preparation and target enrichment protocols.

CONCLUSION

These results demonstrate the successful implementation of the Twist FlexPrep UHT Library Preparation Kit on the Sciclone G3 NGSx automation platform for target capture sequencing. The data presented here confirm that the automated workflow produces high-quality lpWGS and enriched libraries suitable for downstream analyses. By integrating the high-throughput capabilities of the FlexPrep workflow with automation, this approach offers substantial advantages in terms of improved reproducibility, reduced processing time, and decreased labor costs, all without compromising data quality.

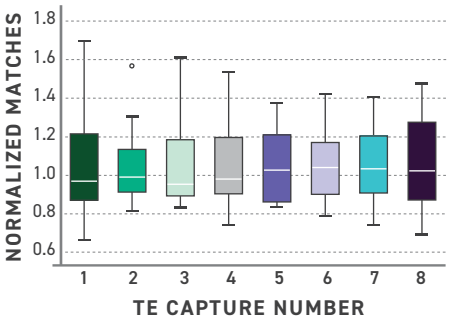


Figure 4. Normalized Matches by Pool for Exome Sequencing.

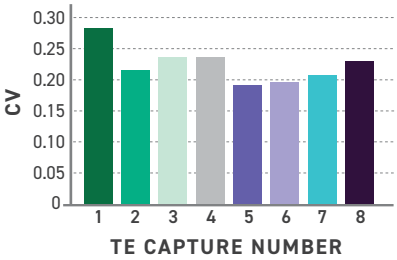


Figure 5. CV by Pool for Exome Sequencing.

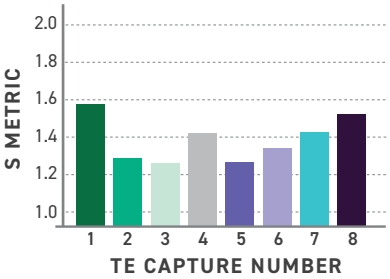


Figure 6. S Metric by Pool for Exome Sequencing.

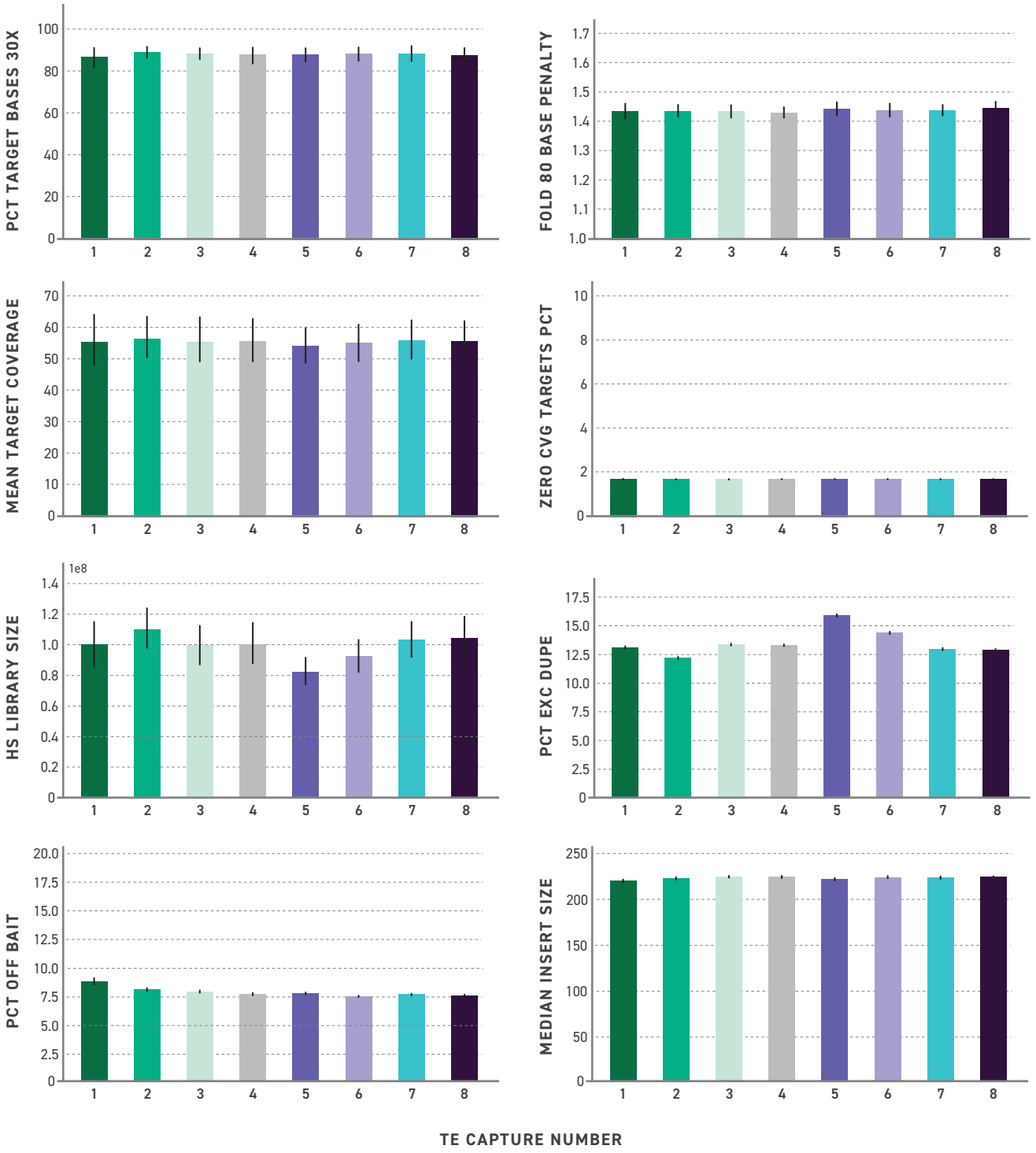


Figure 7. Various Picard Metrics by Pool for Exome Sequencing.