APPLICATION NOTE

Ultra-High Capacity Multiplexing With the Twist FlexPrep UHT Workflow Automated on Hamilton NGS STAR

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. 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 Hamilton NGS STAR 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.

METHODOLOGY

The Hamilton NGS STAR automation method was developed, tested, and demonstrated using 100 ng control DNA (Horizon Tru-Q 100% WT Reference) for 96 samples. All thermal cycling was performed off deck with a fragmentation time of 17 minutes and 8 cycles of PCR amplification.

The Twist Bioscience Flexprep UHT Library Preparation workflow consists of:

1. Enzymatic Fragmentation/End Repair/dA-tailing

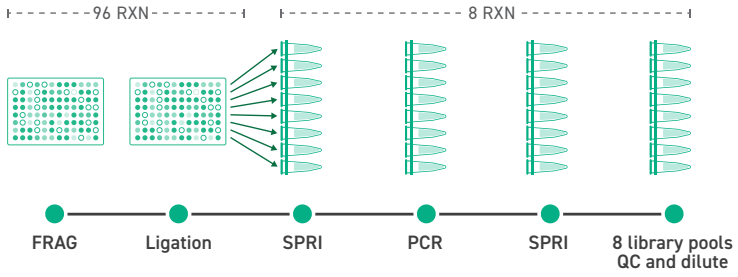
2. Normalization Adapter Ligation

3. Pooling

4. Post-Ligation Bead Cleanup

5. PCR Setup

6. Post-PCR Bead Cleanup



The method deck setup dialog boxes describe how to prepare master mixes and load consumables 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. The user was prompted to remove the plate from the Hamilton plate, seal it, and then place it on the thermal cycler for each of the three programs. A dialog box then prompted the user to spin-down, remove the seal, and return the plate to the deck at the completion of its cycler program. The inclusion of the Hamilton ODTC is also available as an option within the method for thermal cycling. The method was completed in approximately 5 hours.

A total of 96 FlexPrep libraries, reduced to eight 12-plex library pools during ligation, were generated and sequenced. These same library pools were also enriched using the Twist Exome 2.0 and Human RefSeq panels and then sequenced. A NovaSeq S4 flowcell using 200 paired-end cycles was used for sequencing. Capture data was downsampled to 150X.

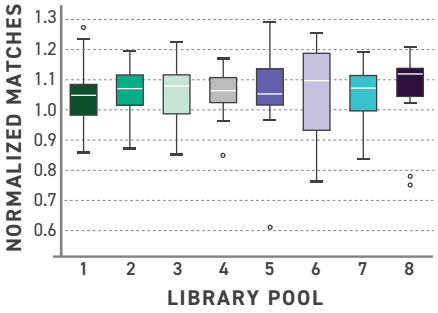


Figure 1. Normalized Matches by Pool for lpWGS.

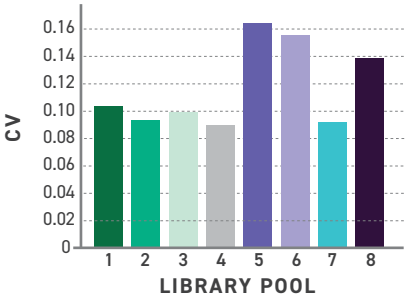


Figure 2. CV by Pool for lpWGS.

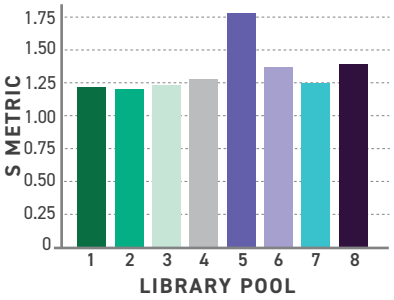


Figure 3. S Metric by Pool for lpWGS.

RESULTS

Low-Pass Whole Genome Sequencing

For lpWGS, 96 samples were processed through library preparation. For all 96 samples, Normalized Matches averaged at 1.06 (**Figure 1**). CV averaged 0.12 (**Figure 2**). Additionally, S metric averaged 1.34 (**Figure 3**). The S metric measures how much 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 1.05 (**Figure 4**). CV averaged 0.14 (**Figure 5**). Furthermore, the S metric averaged at 1.40 (**Figure 6**). These results show that each sample was sequenced very uniformly.

Fold-80 Base Penalty scores averaged 1.38, demonstrating high enrichment uniformity for all captures. Off-Target Rate averaged about 10%, showing capture was efficient. With Mean Target Coverage at 57X, Percent Target Bases 30X at 91%, and Zero Coverage Targets at 1.6%, sequencing depth was shown to be adequate for downstream analysis. HS Library Size averaged 120M, with Duplicate Rates at 10% (**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 Hamilton NGS STAR automation platform for sequencing. The data presented here confirm that the automated workflow produces high-quality lpWGS and enriched libraries suitable for downstream analyses. The method described is also adaptable to the NGS STAR MOA deck layout with use of the Inheco ODTC.

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.

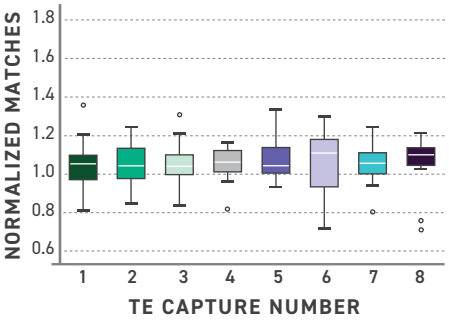


Figure 4. Normalized Matches by Pool for Exome Sequencing.

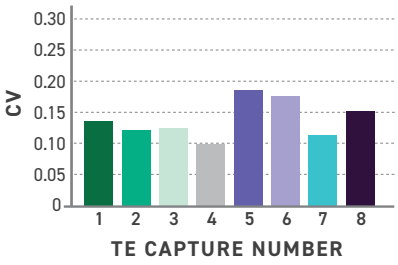


Figure 5. CV by Pool for Exome Sequencing.

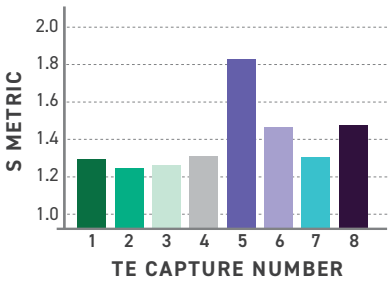


Figure 6. S Metric by Pool for Exome Sequencing.

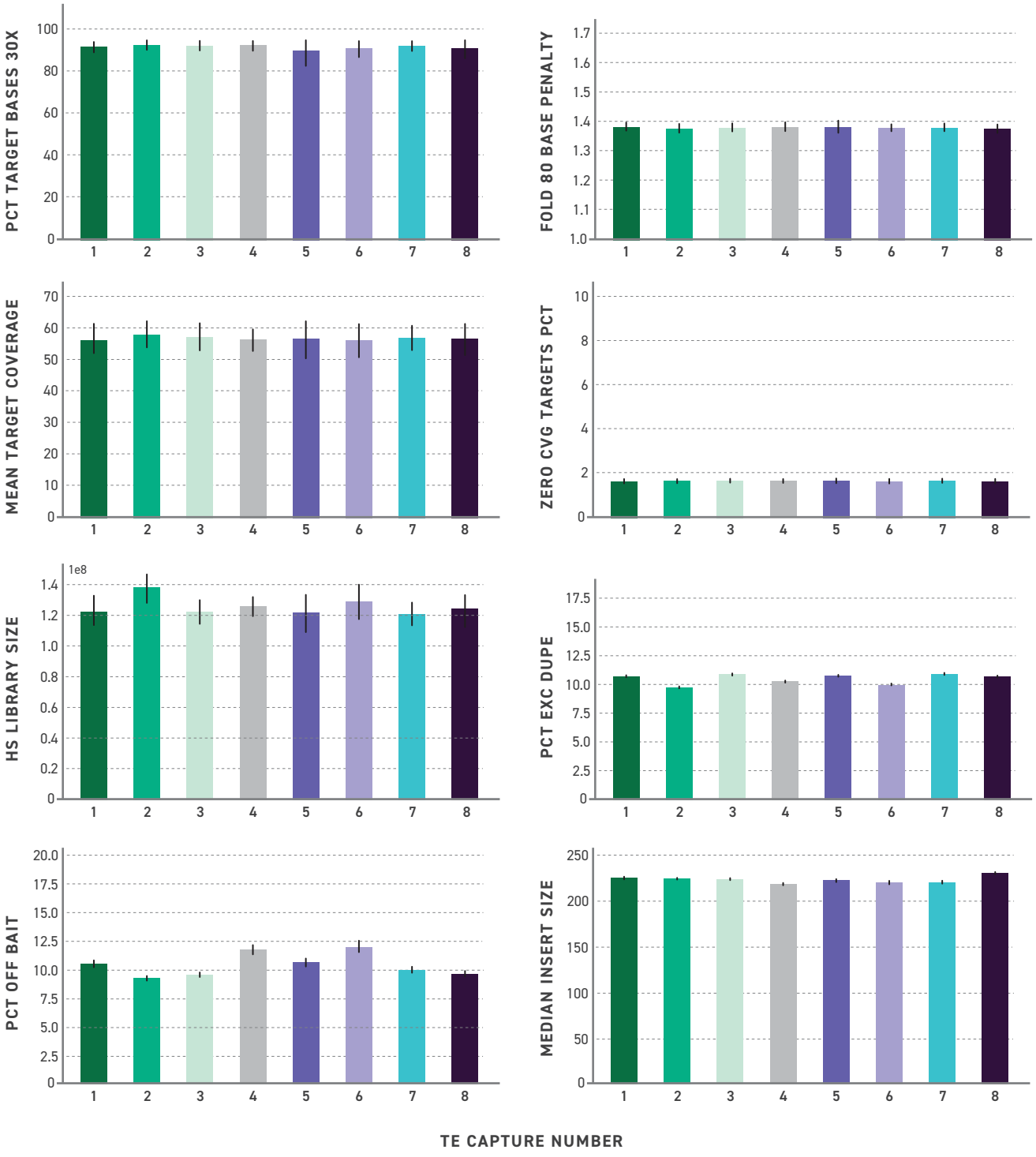


Figure 7. Various Picard Metrics by Pool for Exome Sequencing.