APPLICATION NOTE

Genotyping Bovine Samples Prepared With Twist FlexPrep™ Library Preparation and Target Enrichment Workflows

INTRODUCTION

Next-generation sequencing (NGS) platforms continue to advance by increasing output while decreasing sequencing costs. Researchers interested in exploring population-level genomics for the evaluation of livestock are no longer limited by sequencer output. Instead, library preparation at scale remains an existing bottleneck that is labor-intensive and expensive.

The Twist FlexPrep UHT Library Preparation and Hybridization Kit helps to address these challenges through two technologies. First, Normalization by Ligation™ (NBL) consistently converts genomic DNA (gDNA) samples into libraries independent from mass input. This allows users to skip tedious and costly upfront quantification and normalization steps. Second, inline barcodes tag samples early in the library preparation process allowing for pooling and reducing the number of reactions after ligation by 12-fold. The Twist FlexPrep UHT Library Preparation and Hybridization Kit allows for capture of libraries in a 96-plex format, greatly reducing the number of capture reactions needed. Together these workflow improvements reduce reagent costs and allow more samples to be processed at once. This ultimately allows for more efficient use of high-throughput sequencers.

Here we showcase the Twist FlexPrep UHT Library Preparation and Hybridization Kit using bovine samples from ear punches. We provide guidance for gDNA extraction and a comparison to ground truth PCR-free whole genome sequencing (WGS). Libraries generated with Twist FlexPrep UHT Library Preparation Kit have high concordance to the ground truth datasets, demonstrating similar data quality to microarrays. By combining this kit with custom target enrichment (TE) panels, users are able to easily identify and investigate novel regions of interest that can impact population management and breeding choices.

METHODS

Ear punches were collected from frozen bovine ears and gDNA was extracted using the BioEcho ECHOlution Tissue Extraction kit. Two library preparations were performed with these extracted gDNA samples. First, PCR-free Twist EF 2.0 Library Preparation was performed with 100 ng gDNA. These libraries were sequenced to 30X depth and used to generate ground truth datasets to be used as references for calculating genotype concordance. Second, without prior dilution, a constant volume of gDNA (50-160 ng) was used as input into Twist FlexPrep UHT Library Preparation. Ninety-six gDNA samples were processed at once (sourced from 12 unique bovine samples each repeated eight times). Pools of 12 libraries were then combined, by equal volume and without prior quantification, for a single 96-plex capture reaction using a 9 Mb bovine genotyping panel with blockers designed for the bovine genome. After capture, these samples were sequenced to 150X depth on an Illumina NextSeq2000 and aligned to the bosTau9 genome for genotyping analysis. Germline variants were called with GATK HaplotypeCaller and genotype concordance metrics were collected with VCFs on Picard.

RESULTS

The Twist FlexPrep UHT Library Preparation Kit workflow creates high-quality data for genotyping applications. Coupled with TE, users are able to capture sequencing reads on genomic regions of interest to achieve the required coverage for genotype calls. High genotype concordance is observed between the FlexPrep samples and the PCR-free ground truth WGS (**Figure 1**). The TE panel used is designed to enrich single-nucleotide polymorphism (SNP) regions. Using this panel, the Twist FlexPrep UHT Library Preparation and Hybridization Kit shows 99.3% genotype concordance of SNPs to the PCR-free reference at 100X downsampling. This FlexPrep UHT sample has 97.2% concordance of INDELs at the same downsampling despite the panel not being specifically designed for INDEL detection. If coverage is reduced to 50X the SNP and INDEL genotype concordance remains high at 98.1% and 94.9%, respectively. The Twist FlexPrep UHT Library Preparation and Hybridization Kit workflow provides a simplified process for generating high-quality data that is comparable to microarray genotyping. TE allows for quick iteration in panel design that can be used for identification and genotyping of specific regions of interest.

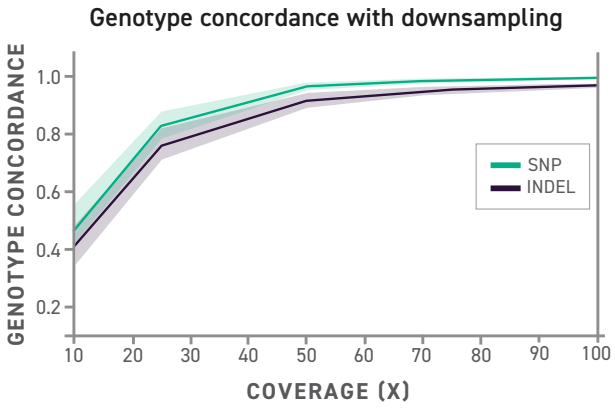


Figure 1. Twist FlexPrep UHT Target Enrichment libraries with various downsampling levels are compared to the PCR-free reference to calculate genotype concordance. Concordance is calculated for SNPs and INDELs independently. Data plotted represent sequencing from three samples, each processed with eight replicates.

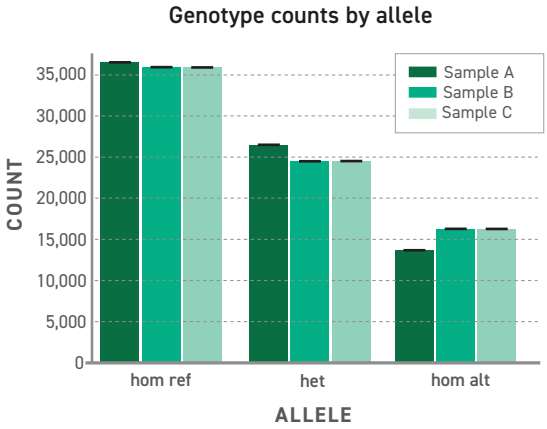
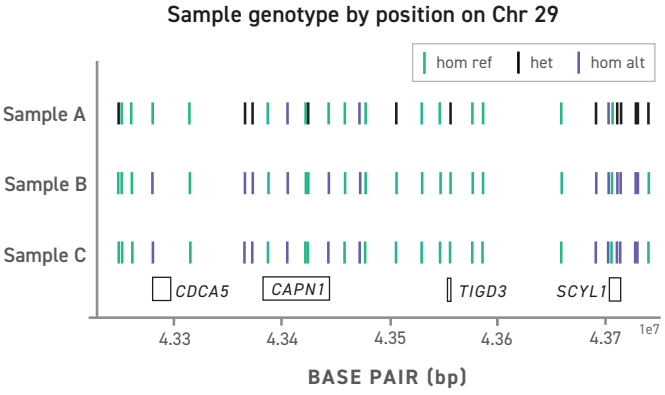


Figure 2. Genotype counts by allele for three representative samples after Twist FlexPrep UHT Library Preparation and Target Enrichment with 150X downsampling. Each sample was processed with eight replicates. Alleles are categorized as homozygous reference (hom ref), heterozygous (het), or homozygous alternate (hom alt).

The Twist FlexPrep UHT workflow is useful for genotype identification at target regions. For three example samples, genotypes were called for each SNP enriched by the panel (**Figure 2**). When using this 9 Mb TE panel, more than 75k SNPs were identified for each sample. Consistently, sample A was found to have more heterozygous alleles and fewer homozygous alternate alleles compared to samples B and C. In an example region of chromosome 29, the higher heterozygous count in sample A is displayed at the *TIGD3* and *SCYL1* genes (**Figure 3**). Samples B and C have identical genotype calls at this region suggesting that these two individuals may be related and could have similar phenotypic traits. In addition, at the *CDCA5* gene, sample A is homozygous for the reference allele and samples B and C are homozygous for the alternate allele. This analysis demonstrates the ease of genotyping bovine samples when using the Twist FlexPrep UHT Library Preparation and Hybridization Kit.



CONCLUSION

The Twist FlexPrep UHT Library Preparation and Hybridization Kit provides a useful workflow for generating libraries from bovine ear punch samples or from samples of other livestock. These libraries are suitable for downstream genotype calls critical to breeding and population management decisions. Utilizing the streamlined workflow that relies on normalization adapters and inline barcoding, along with a highly multiplexed target enrichment workflow, many samples can be processed at once with reduced reagent costs.

Figure 3. Allele distribution for three samples on an example region of Bovine chromosome 29. Subset of annotated transcripts from NCBI RefSeq is displayed at the bottom. The color of the bar indicates the genotype of each sample. Alleles are categorized as homozygous reference (hom ref), heterozygous (het), or homozygous alternate (hom alt).

Get in touch with us to explore the future of Agrigenomics.

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ORDERING INFORMATION

FlexPrep UHT Library Preparation Kit
109220: Twist FlexPrep UHT Library Preparation Kit, 192 Samples
109223: Twist FlexPrep UHT LP and Hybridization Kit, 192 Samples
109224: Twist FlexPrep UHT Library Preparation Kit, 1152 Samples
109226: Twist FlexPrep UHT LP and Hybridization Kit, 1152 Samples