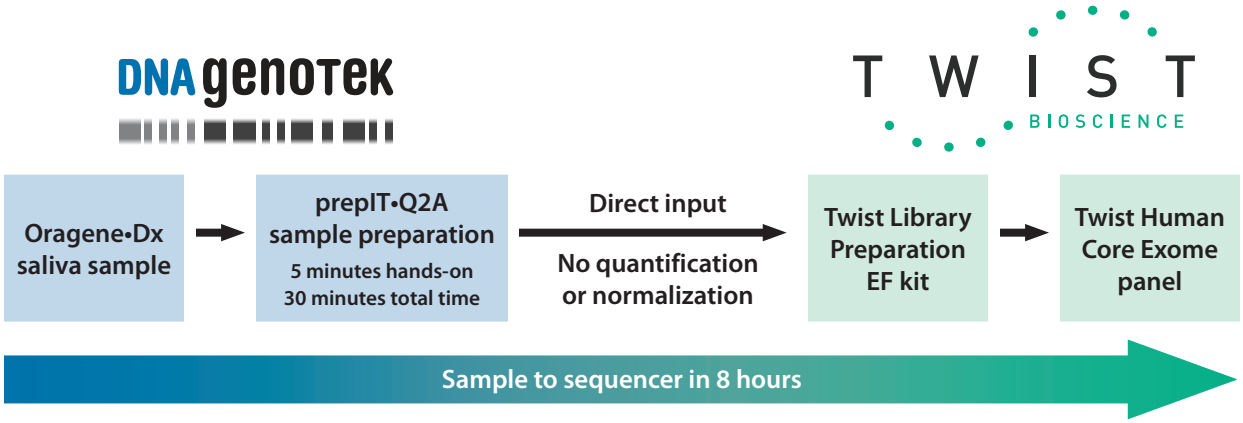
prepIT®•Q2A

prepIT®•Q2A: Streamlined processing of Oragene®•Dx saliva samples for whole exome sequencing

Introduction

The drive towards personalized medicine, pharmacogenomic testing and growth in the direct-to-consumer market has increased the number of genomic samples collected and analyzed year over year. The non-invasive, easy self-collection of DNA from saliva, using Oragene®•Dx collection kits, increases access to donors and contributes to this growth. As such, genomic laboratories are challenged to process more samples, more efficiently. Routine handling, such as DNA extraction, quantification and normalization, adds considerable time and cost to laboratory workflows, often limiting the number of samples that can be processed in a single day. In collaboration, DNA Genotek and Twist Bioscience demonstrate a high quality target enrichment solution using a streamlined workflow, going from saliva sample to sequencer in under 8 hours.

Technology



DNA Genotek prepIT®•Q2A DNA Preparation Reagent

prepIT•Q2A reagent enables a rapid, liquid-based removal of inhibitors found in Oragene•Dx saliva samples. The process involves minimal hands-on steps without the need for centrifugation, making it compatible with automated liquid handlers. It also eliminates the need to perform DNA extraction, quantification and normalization prior to library preparation, positioning prepIT•Q2A as an ideal solution for laboratories wanting to scale up their sample throughput—from saliva sample, direct-to-library preparation.

Twist Bioscience Target Enrichment Solution

The Twist Library Preparation Kit with Enzymatic Fragmentation offers a robust and tunable library construction method. When used with the Twist Universal Adapter System (384 UDI primers), a maximum yield of libraries is generated, forming the foundation for high-throughput sequencing. Coupling this high-quality library with the efficiency of the Twist Target Enrichment Solution, maximum confidence in variant detection with minimal sequencing is achieved.

MK-AN-00039 Issue 1/2019-10

Methods

Sample collection and preparation

Oragene•Dx saliva samples were collected from 27 healthy donors. These samples were pre-screened for DNA yield by using prepIT•Q2A preparation reagent followed by PicoGreen™ fluorescence quantification. Seven of these samples were selected to cover the range of DNA yield observed in a typical population, as well as emphasize donors with high DNA content to push the limits of the direct-to library capability of this workflow (Figure 1). DNA was prepared from the selected samples in duplicate using prepIT•Q2A. Purified genomic DNA from cell line NA12878 (Coriell Institute) was normalized to 5ng/µL (50 ng total) and was used as a control in duplicate.

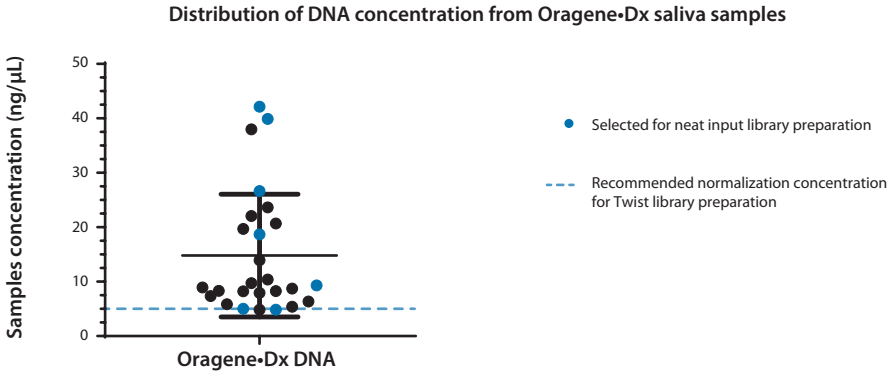


***Figure 1**: Distribution of DNA concentration from Oragene•Dx saliva in a healthy population and concentration of selected samples for library preparation.*

Twist library preparation

Samples were prepared according to Enzymatic Fragmentation and Universal Adapter System protocol with the following modifications. The standard protocol requires samples normalized to 5 ng/µL with a 10 µL input volume, while in this study, 10 µL neat was used directly in the workflow without normalization. In addition, fragmentation time was reduced to 18 and 12 minutes to ensure that the correct target fragment size was achieved. The Agilent Bioanalyzer DNA 7500 assay was used to confirm fragment length, as shown in Figure 2, and all samples achieved the target fragment size despite the sample input amount used. Hybridization steps were performed as written in the protocol.

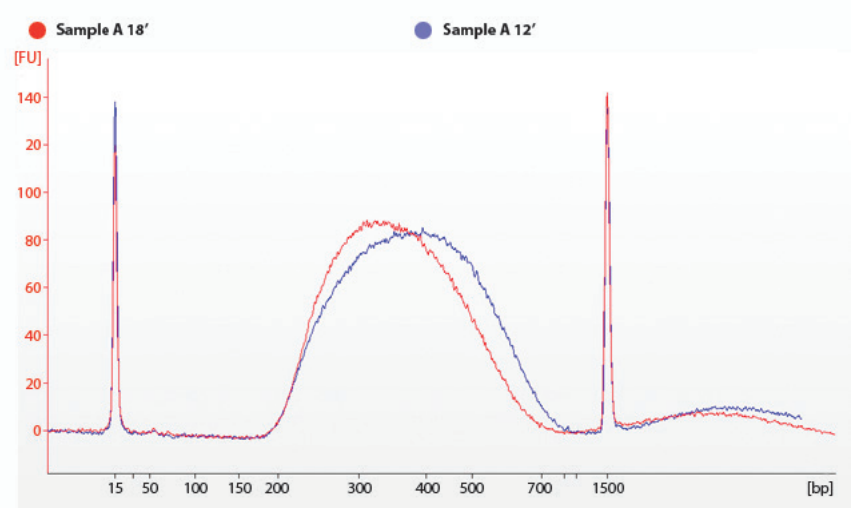


***Figure 2**: Tunability using enzymatic fragmentation. At 18 minutes the average fragment size was 368 bp. At 12 minutes the average size was 393 bp. The concentrations were 161 ng/µL and 184 ng/µL respectively. Shown here with a 3x dilution.*

Twist target enrichment using the Human Core Exome Panel

Following library preparation, samples (N=8 including control) were pooled together for a multiplex capture reaction. The Twist Human Core Exome Panel was used with Fast Hybridization Protocol with a hybridization time of two hours. The enriched libraries were sequenced on the NextSeq 500/550 High Output v2 kit (Illumina®) to generate 2x76 paired-end reads and down-sampled to 150x of targeted bases. Picard HS_metrics tools with a mapping quality of 20 were utilized for sequence analysis.

Results

The quality of the capture reaction was evaluated using Picard metrics[1]. prepIT•Q2A samples had consistent performance with the control NA12878 as demonstrated in Figure 3 by similarly high on target rates (> 90 %), low duplication rates (< 5 %), and high uniformity (fold 80 < 1.5). In addition, there was no relationship between sample input amount and quality metrics, indicating that this workflow is robust and compatible with direct prepIT•Q2A sample input into library preparation without impact on downstream performance.

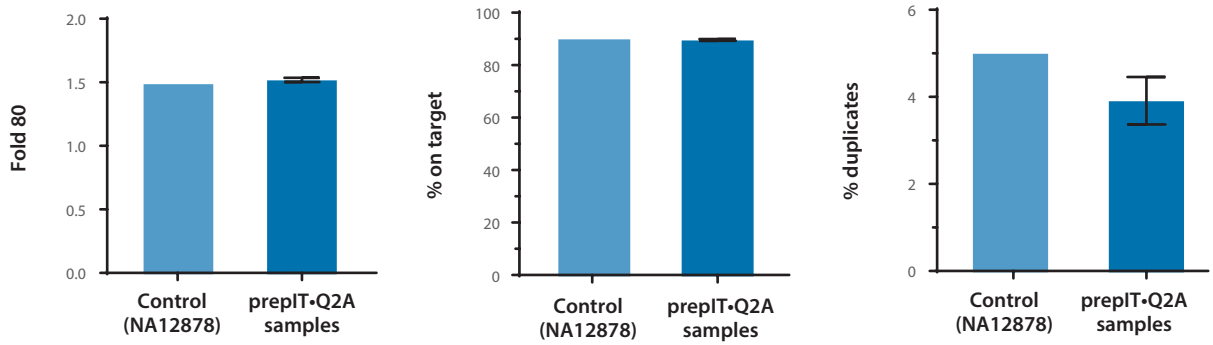


***Figure 3**: Selected Picard metrics for comparison between control and prepIT•Q2A samples.*

Conclusion

Oragene•Dx is the preferred DNA from saliva collection kit by researchers, pharmacogenomic and direct-to-consumer companies. Oragene•Dx's non-invasive means of collection overcomes sample-access barriers typical of blood collections and has enabled the growth of the personalized medicine and health and lifestyle industries. Consequently, genomic laboratories are now experiencing sample throughput challenges.

A workflow for Oragene•Dx using prepIT•Q2A with Twist NGS library preparation and target enrichment components was presented. The workflow demonstrated the compatibility of saliva prepared with prepIT•Q2A direct into Twist NGS library preparation and exome sequencing, with no need to quantify or normalize DNA samples. The results illustrate the robust performance with high on-target rates, low duplication rates, and high uniformity across samples. Further to this, the workflow is streamlined and enables sample to sequencer within a standard 8-hour work day.

Technical support is available Monday to Friday (9h00 to 17h00 ET):

- Toll-free (North America): 1.866.813.6354, option 6
- All other countries: +1.613.723.5757, option 6
- Email: support@dnagenotek.com