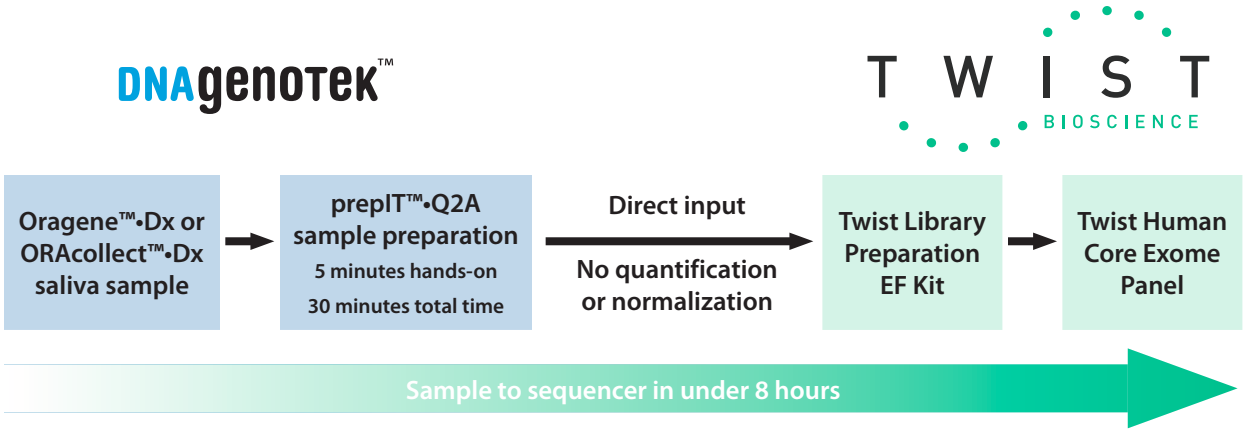
prepIT™•Q2A

prepIT™•Q2A: Streamlined processing of Oragene™•Dx and ORAcollect™•Dx saliva samples for whole exome sequencing

Introduction

The drive towards personalized medicine, pharmacogenomics 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 and ORAcollect™•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 next generation sequencing library preparation and target enrichment solution using a streamlined workflow, from saliva sample to sequencer in under 8 hours.

Technology



DNA Genotek prepIT™•Q2A DNA preparation reagent

The prepIT™•Q2A reagent enables a rapid, liquid-based cleanup of saliva samples collected with Oragene™•Dx and ORAcollect™•Dx devices. The process involves minimal hands-on time and no 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 reagent as an ideal solution for laboratories wanting to scale up their sample throughput — from saliva sample directly to library preparation.

Twist Bioscience target enrichment solution

The Twist Library Preparation EF Kit (Twist Bioscience, Cat. No. 101058) offers a robust and tunable library construction method. When combined with the Twist Universal Adapter System (Twist Bioscience, Cat. No. 101308) with 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 Twist Bioscience target enrichment solutions achieves maximum confidence in variant detection with minimal sequencing.

MK-AN-39 Issue 2/2024-01

Methods

Sample collection and preparation

Oragene™•Dx and ORAcollect™•Dx saliva samples were collected from 27 and 30 healthy donors respectively. These samples were pre-screened for DNA yield using prepIT™•Q2A preparation reagent followed by Quant-iT™ PicoGreen™ (Thermo Fisher Scientific, Cat. No. P7581) fluorescence quantification. Seven samples from each collection type were selected to cover the range of DNA yield observed in a typical population as shown in Figure 1. Since DNA input amounts can influence fragment length, this selection of samples ensured that samples with high DNA content were represented in order to push the limits of the direct-to library capability of this workflow.

DNA was prepared from 100 µL of each sample in duplicate using the prepIT™•Q2A reagent. Purified genomic DNA from cell line NA12878 (Coriell Institute) was normalized in duplicate to 5 ng/µL (50 ng total) and was used as a control.

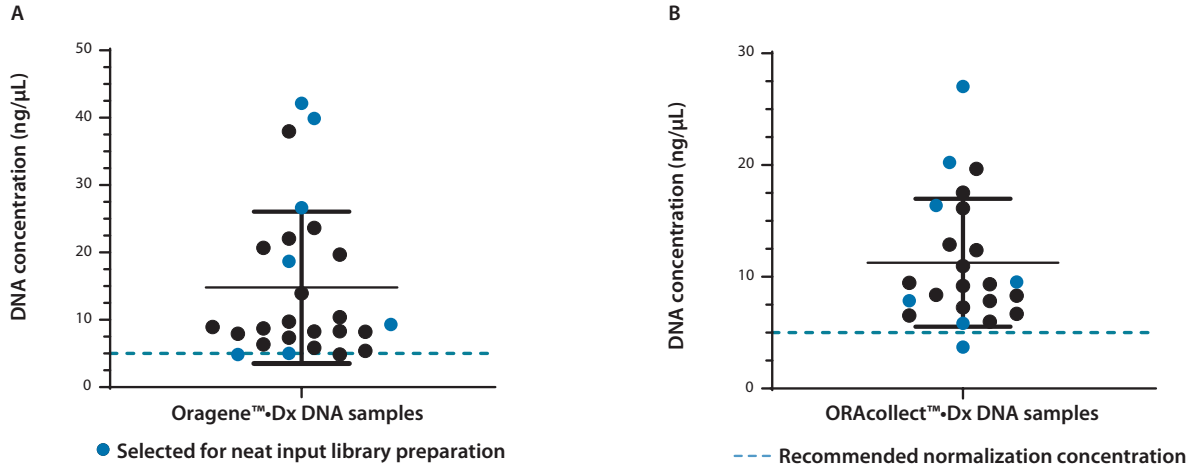


Figure 1. *Distribution of DNA concentration from Oragene™•Dx (A) and ORAcollect™•Dx (B) samples in a healthy population and concentration of selected samples for library preparation.*

Twist library preparation

Samples were prepared according to the Enzymatic Fragmentation and Universal Adapter System protocol (Twist Bioscience) with a few modifications. The standard protocol requires sample normalization 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, reduced fragmentation times of 18 minutes and 12 minutes were tested for Oragene™•Dx samples to ensure that the target fragment size was achieved. The Agilent DNA 7500 kit for the 2100 Bioanalyzer system (Agilent Technologies, Cat. No. 5067-1506) 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. A fragmentation time of 12 minutes was selected for ORAcollect™•Dx sample processing based on the results for the Oragene™•Dx samples.

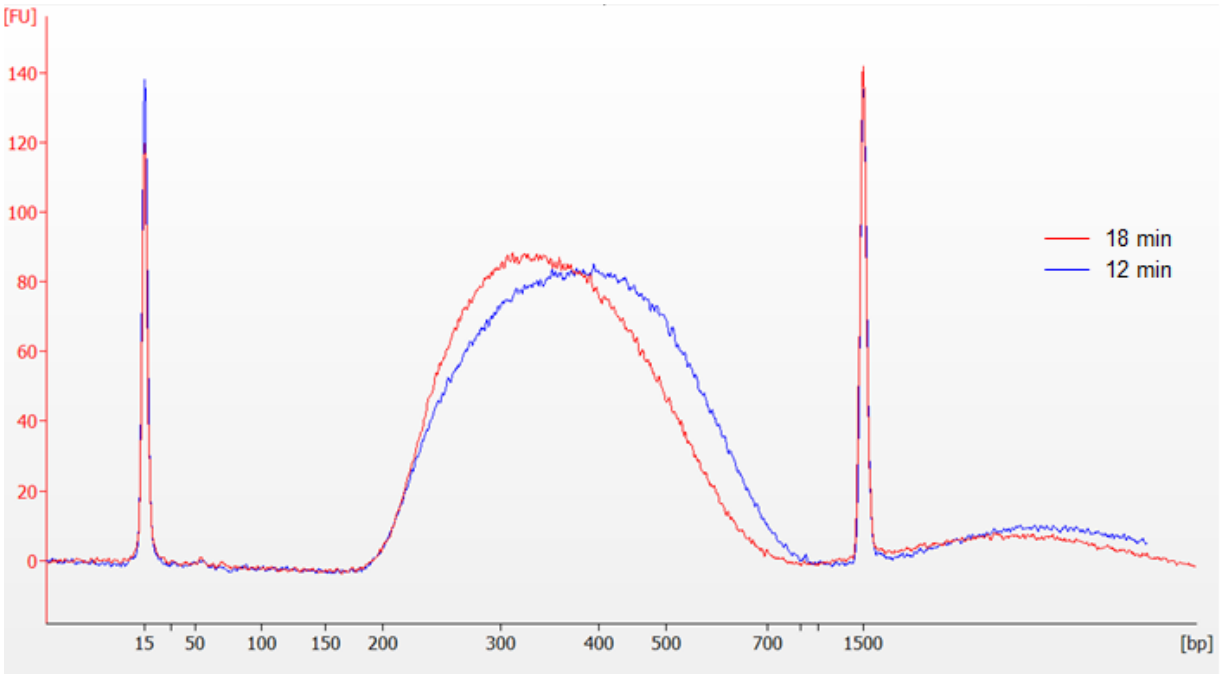


Figure 2. *Tunability using the Enzymatic Fragmentation and Universal Adapter System protocol (Twist Bioscience). At 18 minutes, the average fragment size was 368 base pair (bp). At 12 minutes, the average size was 393 bp. The concentrations were 161 ng/µL and 184 ng/µL respectively. Traces shown here are with a 3 × dilution.*

Target enrichment using the Twist Human Core Exome Panel

Following library preparation, samples (n = 8, including control) were pooled together for a multiplex capture reaction. The Human Core Exome Panel (Twist Bioscience, Cat. No. 102026) was used with the Fast Hybridization protocol (Twist Bioscience) and a hybridization time of two hours. The enriched libraries were sequenced on the NextSeq 500/550 High Output kit v2.5 (Illumina, Cat. No. 20024907) kit to generate 2 × 76 paired-end reads and down-sampled to 150× of targeted bases. Picard HsMetrics tools with a mapping quality of 20 were utilized for sequence performance analysis.

Results

The quality of the capture reaction was evaluated using Picard metrics. prepIT™•Q2A samples prepared from both Oragene™•Dx and ORAcollect™•Dx saliva samples had consistent performance with the control NA12878 cell line DNA as demonstrated in Figure 3, with similarly high on-target rates (> 89 %), 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 input of prepIT™•Q2A-prepared samples into library preparation without negative impact on downstream performance.

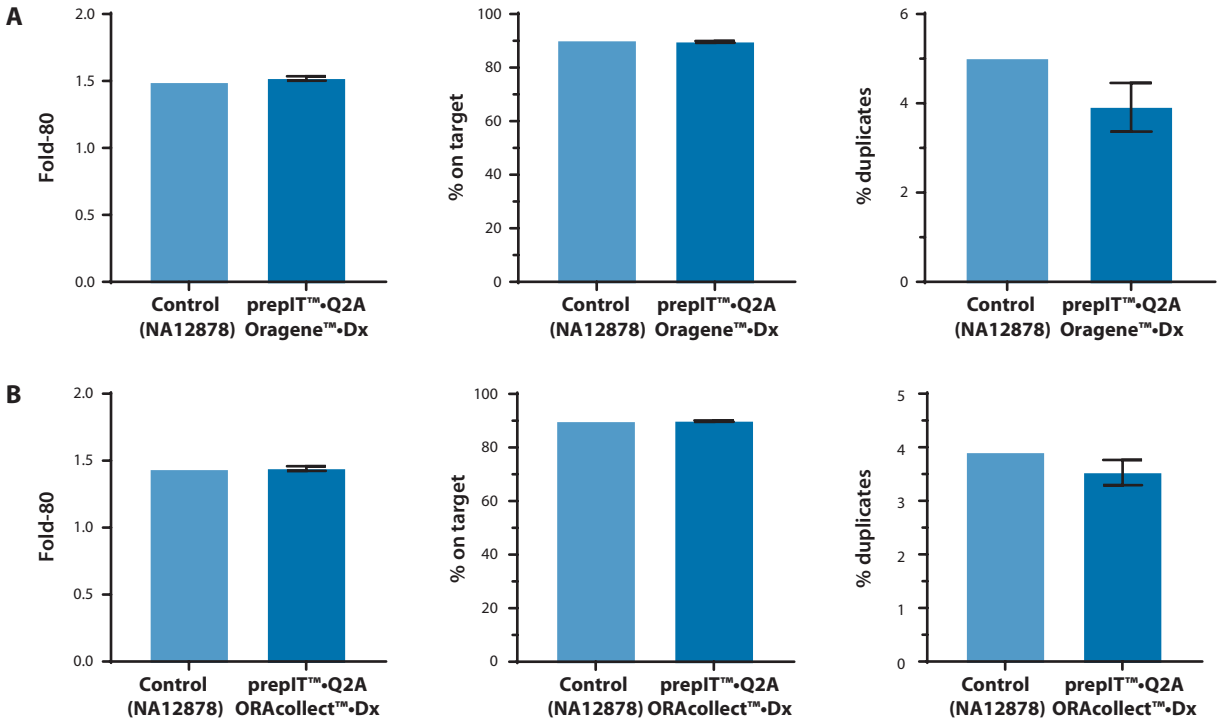


Figure 3. *Selected Picard metrics for comparison between control and A) Oragene™•Dx samples prepared by prepIT™•Q2A reagent and B) ORAcollect™•Dx samples prepared by prepIT™•Q2A reagent.*

Conclusions

Oragene™•Dx and ORAcollect™•Dx devices are the saliva DNA collection kits preferred by researchers and direct-to-consumer and pharmacogenomics companies. The non-invasive means of collection and convenience of ambient temperature shipping and storage of kits are key features that overcome sample-access barriers typical of blood collections, enabling the growth of personalized medicine, health and lifestyle industries. The rise in saliva sample collections has created sample-throughput challenges, highlighting the need for a streamlined workflow.

A workflow comprised of sample collection with Oragene™•Dx or ORAcollect™•Dx devices, sample preparation using prepIT™•Q2A reagent and downstream processing with Twist NGS library preparation and target enrichment solutions was presented. The workflow demonstrated the compatibility of samples prepared with the prepIT™•Q2A reagent used directly in Twist NGS library preparation and targeted exome sequencing assay, with no need to quantify or normalize the DNA samples. The results illustrate the robust performance with high on-target rates, low duplication rates and high uniformity across samples and address throughput challenges with a streamlined workflow, enabling sample to sequencer capability within a standard 8-hour workday.

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

Oragene•Dx is FDA cleared for in vitro diagnostic use [k110701, k152556].
ORAcollect•Dx is FDA cleared for in vitro diagnostic use, for prescription use [k152464] and over-the-counter (direct-to-consumer) use [k212745].
Some DNA Genotek products may not be available in all geographic regions.
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