Evaluation of Next Generation Library Preparation using DNA Extracted from Dried Blood Spots using truXTRAC® DBS DNA kit

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INTRODUCTION

Dried Blood Spots (DBS) provide an easy and inexpensive way to collect and store peripheral blood specimens from infants, children and adults.[1] The use of DBS allows for less invasive procedures for patients and easier shipment while still providing the ability to run molecular or clinical biochemical assays. This convenient method for the long term room-temperature storage of materials also minimizes storage and archival space. The truXTRAC DBS DNA kit is designed for controlled and efficient extraction of next-generation sequencing (NGS)-grade DNA from DBS samples using Adaptive Focused Acoustics® (AFA®). Covaris AFA enables sample rehydration while providing simultaneous cell lysis and controlled mechanical DNA shearing, resulting in high-yield, high-quality and NGS library preparation ready DNA.

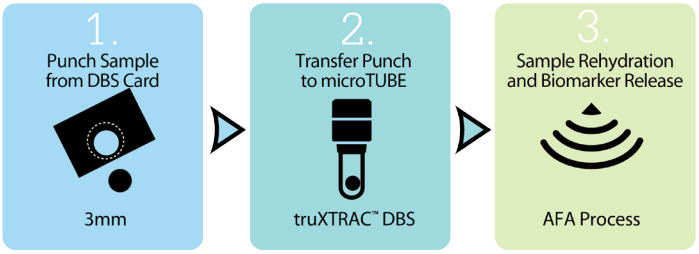
In this study Sanger-sequencing validated known variant patient samples are used for the first time to evaluate the feasibility of using DBS, instead of 1 ml of whole blood, as a viable, equivalent source of sample type for next generation sequencing. [2]

ABOUT GeneDx

GeneDx is a world leader in genomics with acknowledged expertise in rare and ultra-rare genetic disorders, as well as one of the broadest menus of sequencing services available among commercial laboratories. Among other tests, GeneDx focuses on exome sequencing, and has performed clinical exome testing for over forty-seven thousand individuals to date. The GeneDx mission is to make clinical testing affordable and available to people with rare genetic conditions and their families.

Materials and Methods:

FTA cards (GE Healthcare) were spotted with de-identified, venous blood from individuals with known sequence variants in different inherited cancer genes (n=24). Spotted DBS cards were air dried at room temperature overnight before getting punched into 3 mm diameter using semi-automated DBS puncher. DNA from one single 3 mm punch was extracted and sheared using truXTRAC DBS DNA kit (PN 520180). Workflow diagram as shown in FIGURE 1.



Less than 10 mins from a single 3mm punch to NGS-grade DNA

FIGURE 1. Workflow for truXTRAC DBS DNA kit

DNA from 1ml of donor-matched whole blood was extracted using Omega Bio-tek's Mag-Bind® Blood DNA kit on a 96-well robot, and purified DNA was sheared to 300-350 bp range. Extracted DNA was quantified using QUBIT 3.0 Fluorometer, and fragments size distribution was analyzed using the Agilent 2100 Bioanalyzer.

NGS library preparation and sequencing:

DNA libraries were prepared using the KAPA Hyper Prep kit from KAPA Biosystems. The library were captured using custom Agilent baits using hybrid capture technology. The libraries were sequenced on Illumina HiSeq 2500 system using the 2x100bp V2 chemistry. Sequencing data was analyzed and coverage of regions of interest (ROIs) was calculated using a custom-developed and validated pipeline.

Results:

1. DNA Extraction and Library Yield

Peak Incident Power (PIP)	175 W
Duty Factor	10%
Cycles per burst	200
Treatment time (sec)	130
Water Bath Temperature	20° C

TABLE 1: Covaris E220 DBS extraction and shearing parameters

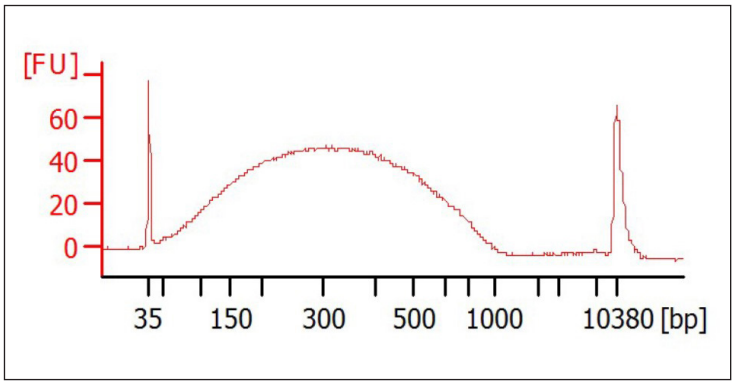


FIGURE 2: DNA profile after following truXTRAC DBS DNA kit protocol. Measured by Agilent 2100 BioAnalyzer HS 12K chip. Average size is 335 bp.

Specimen Type	Average DNA Extraction Yield	DNA Input into Library Preparation	Adapter Ligated Library Yield
Blood (N = 24)	104.12 ng/µL	500 ng	3763 ± 940 ng
DBS (N = 24)	2.28 ng/µL	25 – 100 ng	1808 ± 252 ng

TABLE 2. DNA extraction yields, library input and library yields for blood and DBS samples

As part of truXTRAC DBS DNA kit workflow (TABLE 1), DNA is extracted and sheared to desired fragments distribution in a one-step process without transfer steps. Under the conditions selected for this experiment the average size of DNA recovered was 335 bp (FIGURE 2) and 0.55-5.30 ng/µl of DNA (TABLE 2) were extracted from a single 3 mm punch DBS, which is equivalent to about 25-100 ng total DNA. As 5-20 times less DNA was extracted from DBS than whole blood, two times more PCR cycles were used for DBS DNA than for whole blood-derived DNA (10 in DBS versus 5 in whole blood). Library yield from DBS DNA sample was 1.8 µg on average, indicating high amplifiability.

2. Equivalency in Sequence Coverage & Variant Calls

1) Equivalency in coverage depth: Despite the lower DNA input from DBS than blood, all DBS samples met minimum coverage threshold (64X) defined from historical whole blood-derived samples for all 490 Regions of Interest (ROI) (FIGURE 3).

2) Equivalency in 490 ROI Coverage: There is a >90% correlation between ROI coverage data for DBS and whole blood samples, (FIGURE 4) shown with an R-squared value.

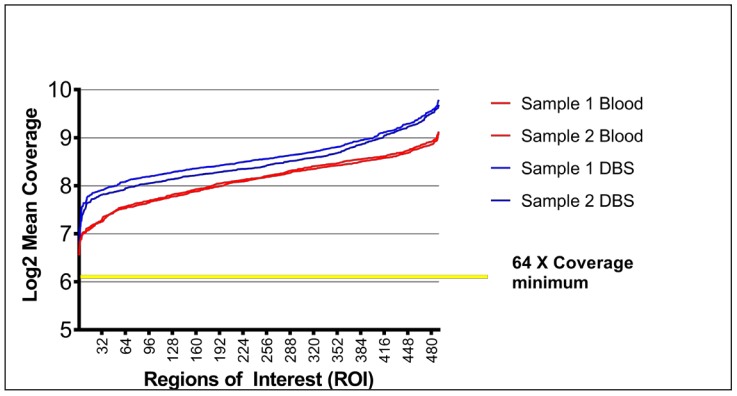


FIGURE 3. Comparison of depth of coverage between blood and DBS samples. Shown as two representative sample results.

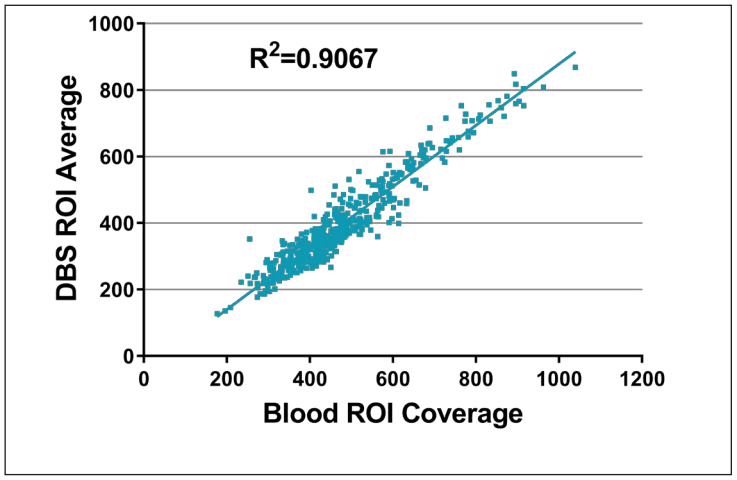


FIGURE 4. Correlation between ROI coverage data for DBS and Blood samples.

3) Equivalency in mean coverage by 24 known gene variants: All 24 sequence variants previously identified in liquid blood samples were also detected in the DBS samples, indicating 100% concordance in variant calling using DBS samples. (FIGURE 5) For those 24 known gene variants, DBS sample (blue) had 75-93% coverages of blood (red). A 7% increase in average duplication rate due to higher PCR cycle requirements of DBS samples was responsible for at least some of this reduction. (Data not shown)

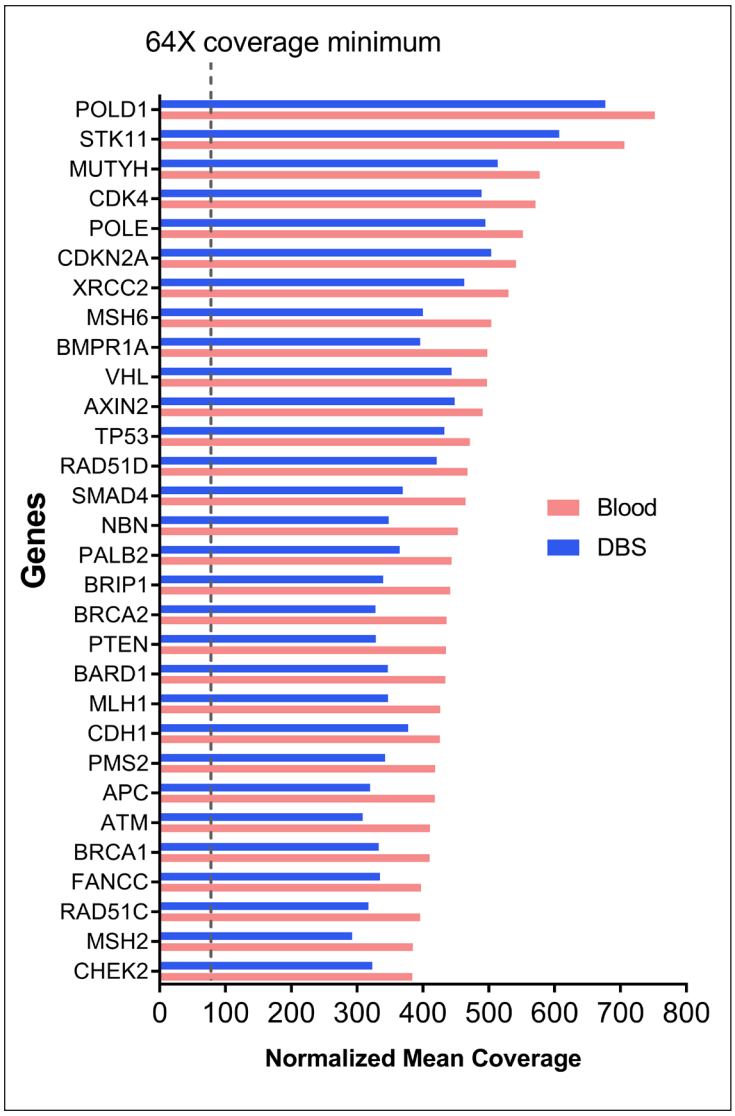


FIGURE 5. Normalized mean coverage by gene.

CONCLUSIONS:

This study demonstrates that the Covaris truXTRAC DBS DNA kit enabled the preparation of high quality NGS libraries from a single 3 mm punch of a standard DBS card. These findings position DBS as an alternative to conventional blood samples for sophisticated genomics applications. Performance was evaluated by multiple performance metrics including (1) DNA yield, (2) depth of coverage of all ROIs; (3) average and minimum gene level coverage; (4) concordance to the gold standard in variant calling.

The truXTRAC DBS DNA kit offers the advantage of extraction and controlled shearing of DNA in a single step workflow, thus making it perfectly suited for routine and highthroughut NGS library preparation and sequencing.

REFERENCES:

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2. Poulsen JB et. al., "High Quality Exome sequencing of whole-genome amplified neonatal dried blood spot DNA" PLoS One 2016; 11(4) PMC4835089

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