Unlocking the Potential of Dried Blood Spot Samples for Clinical Whole Genome Sequencing

Michelle Cipicchio[1], Evan McDaid[1], Dania Zaiter[1], Sophie Low[1], Brendan Blumenstiel[1], Niall Lennon[1]
[1]Broad Clinical Labs, Broad Institute of MIT and Harvard, Cambridge MA, USA

Introduction

Dried blood spots (DBS) are a ubiquitous sample type worldwide that is simple to collect, store, and transport. These samples present unique challenges for applications that require high quality genomic DNA, such as clinical PCR-free whole genome sequencing.

Previous large scale studies from this sample type have required PCR based methods. To avoid the bias and artifacts introduced by PCR, we have developed a method that can reliably produce enough DNA with sufficient quality for PCR-free WGS applications.

Here we present the methodology we have used, and results from validation experiments including

- DNA Extraction quantity and quality from contrived and real world patient samples
- Library quality assessment for a subset of samples using our 30X Clinical Whole Genome product sequenced using Illumina NovaSeq 6000
- Comparison of variant identification for whole blood and dried blood spots matched by donor

Background

Benefits of dried blood spot (DBS) samples:

- Collection - simpler than venous blood draws
- Storage - stable for years at ambient temperature
- Transport - no need for dry ice when shipping
- Low bacterial contamination (compared to saliva)
- Routinely collected and banked for newborn screening for millions of neonates each year in the United States, representing an enormous potential resource for research.

We have optimized a highly scalable fully automated DNA extraction method and found that the majority of DBS samples can yield DNA of adequate quality and quantity for clinical PCR-free whole genome sequencing with at least 30X coverage.

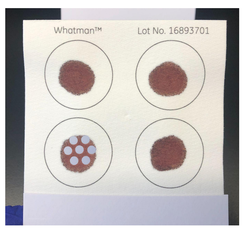

Figure 1. Example DBS card with 3.2 mm punches removed. Our method uses up to 14 punches and incorporates automated sample tracking.

Materials & Methods

Automation
Our lab has a proven system for automated blood card punching and sample tracking that previously processed over 40,000 samples for malaria research. We leveraged this highly scalable system and built liquid handling automation for subsequent DNA purification on a Hamilton Starlet using a high throughput blood extraction kit.

Figure 2. Blood punching automation from BSD Robotics enables up to 14 3.2 mm punches per sample into a single processing well with automated sample tracking by barcode.

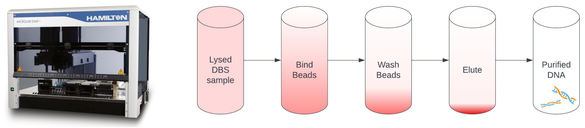

Figure 3. DNA extraction automation. Reagents from the Promega Maxwell® HT 96 gDNA Blood Isolation kit are used with a modified protocol to maximize DNA yield from dried blood spots. All extraction steps are performed on a Hamilton Starlet outfitted with a heated plate shaker and barcode scanner.

Samples

- Venous blood draws from 12 donors were used to prepare contrived blood spots samples for optimization and testing using 70 µL of blood per spot.
- 100% of contrived samples yielded >250 ng DNA using our final process improvements.
- Real world pilot samples were collected in the field using disposable lancets and Perkin Elmer SpotSaver cards and shipped dry at room temperature to our lab.

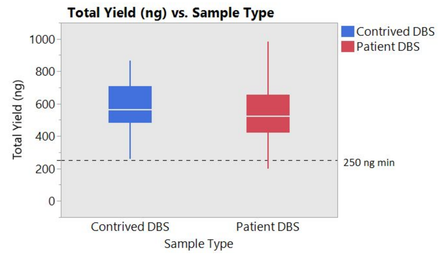

Figure 4. DNA Yield for Pilot samples. 100% of contrived samples and 96% of patient samples yielded the minimum DNA requirement for clinical WGS. N= 56 contrived samples and 52 pilot samples.

Results: Automation Validation

Through a series of optimizations we validated that DNA from our high throughput system met or exceeded the quality and quantity requirements achieved manually.

- Increased lysis reaction volume to enable increased total input material
- Decreased total volumes of binding beads and elution volumes to maximize yield
- Optmized heater shaker conditions to maximize yield without compromising quality

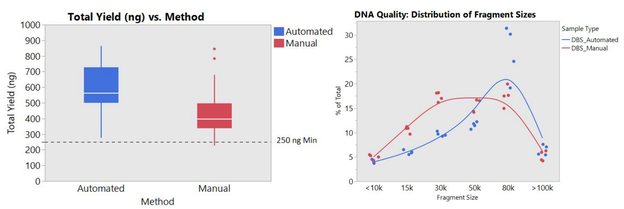

Figure 5. DNA from automated extraction exceeded yield and quality requirements for clinical WGS. Yield (left) and DNA fragment size (right) were both significantly higher using our automated protocol vs a manual protocol using the same reagents. N=36 manual samples N= 48 automated samples.

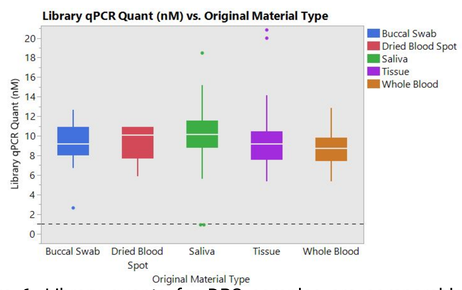

Figure 6. Library quants for DBS samples are comparable to samples from other material types. Libraries were prepared for 8 DBS samples using Covaris shearing and a Kapa HyperPrep kit and compared to over 200 recent libraries from our clinical production WGS process.

Individual	Sample Type	Mean Coverage	Mean Insert Size (bp)	% Chimeras	% Duplication	% Contamination
A	DBS	32.2	420.36	6.46	7.06	0.02
A	WholeBlood	30.8	420.59	3.95	6.40	0.01
B	DBS	33.5	438.05	7.08	6.78	0.02
B	WholeBlood	31.6	419.27	3.69	6.49	0.01
C	DBS	35.4	431.65	6.30	7.37	0.03
C	WholeBlood	36.3	417.79	3.83	7.35	0.01

Figure 7. Picard metrics for PCR free WGS at ~30X coverage for whole blood and DBS matched by donor for 3 individuals. Insert size, duplication rates, and contamination are comparable between sample types. % chimeric reads are higher for DBS.

Discussion

To assess the suitability of DBS samples for clinical NGS applications, we evaluated the performance in three ways:

- Extraction yield and quality - 96% of samples met requirements.
- Library quality and quantity - no significant difference in DBS vs other material types.
- Data quality and accuracy - we examined 30X WGS from matched whole blood and DBS for 3 donors and found excellent concordance in variant calling for both SNPs and Indels.

VARIANT TYPE	INDEL	SNP
VAR_SENSITIVITY	0.96019	0.99090
VAR_SPECIFICITY	0.99999	0.99999
GENOTYPE CONCORDANCE	0.99997	0.99997
NON_REF GENOTYPE CONCORDANCE	0.91997	0.97857

Figure 8. Genotype concordance summary metrics. This analysis compared 30X WGS from DBS to 30X WGS from matched whole blood. The sensitivity for all heterozygous and homozygous variants is TP / (TP + FN) and specificity is TN / (FP + TN)

Conclusion

DBS samples pose undeniable challenges for NGS applications. The quality of the DNA is not sufficient for long reads, and the limited quantity restricts the number of tests that might be performed from a single sample.

However, whole blood or saliva may not always be possible to obtain, due to limited resource availability or patient conditions. This study suggests that high quality clinical WGS can still be achieved when these samples are processed in a way to preserve DNA integrity and maximize yield.

Taken together these results indicate that blood spots represent a powerful source of biological material for a variety of downstream clinical applications including pcr free whole genomes as well as targeted approaches.

Acknowledgements

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Contact:
Michelle Cipicchio
michellec@broadinstiute.org