APPLICATION NOTE

Targeted sequencing of DNA from dried blood spot samples.

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Introduction

Dried blood spots (DBS) are frequently used for newborn screening, large population-based surveys, and biobanking. DBS sampling delivers many benefits not realized by other blood sampling protocols including:

- Minimal blood volume required (approximately 30 - 100 µL per spot)
- Simple collection which does not require a phlebotomist
- Easy transportation and space saving storage
- Sample integrity protected during transportation and storage

Because of these benefits, DBS are used in many research, pathogen detection, and biobanking applications. Revvity's optimized DBS workflow solution provides a tested and validated method for targeted sequencing of DNA extracted from DBS.

Improving quality and yield of DNA isolation from DBS

The DBS Puncher™ instrument was used to punch two 4.75 mm dried blood spot samples per extraction into 96-well microtitration plates from 96 blood cards. DNA was isolated from the 96 DBS samples using the chemagic™ DNA CS200 DNA Kit automated on the chemagic™ 360 instrument. Both instruments are capable of high throughput operations, working in a 96 well format.

The chemagic™ 360 instrument is one of several Revvity chemagen™ instruments for nucleic acid extraction using the proprietary chemagen™ separation technology.

Chemagen™ Technology addresses challenges associated with the isolation of high molecular weight (HMW) gDNA from DBS samples by eliminating the alkaline lysis and centrifugation steps traditionally used during isolation which denature and fragment nucleic acids. The chemagen™ Technology's automated magnetic separation procedure (*Figure 1*) features magnetizable rotating rods combining the transfer and suspension of chemagen™ M-PVA magnetic beads to isolate and purify ultra-pure, HMW gDNA from DBS. The M-PVA magnetic beads have a high affinity for nucleic acids and low protein binding, resulting in very pure DNA and RNA elution.

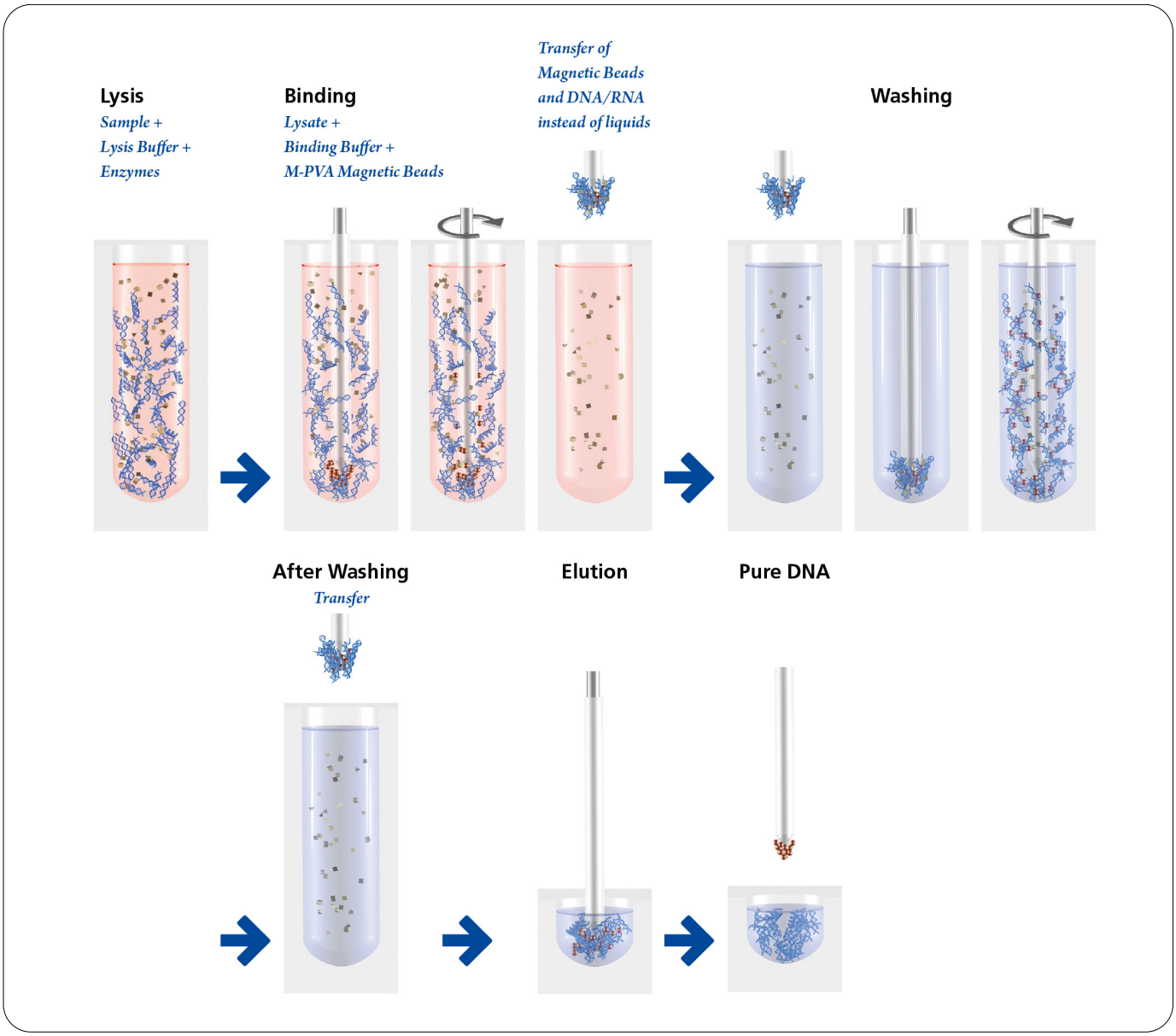

Figure 1

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Nucleic acid automation designed for your lab's needs

With flexibility in throughput, capacity, and sample type, high quality manufacturing standards, and outstanding customer support, Revvity's chemagic™ nucleic isolation automation delivers solutions designed to meet your specific need.

chemagic™ 360 Instrument
With the ability to isolate DNA from 96 to 1,000 DBS samples per day and the flexibility to isolate DNA or RNA from any other human sample type, the chemagic™ 360 Instrument is designed to meet labs' high-throughput nucleic acid isolation needs.

chemagic™ Prepito® Instrument
The chemagic™ Prepito® instrument is ideal for lower-throughput labs, isolating DNA from less than 100 samples per day.

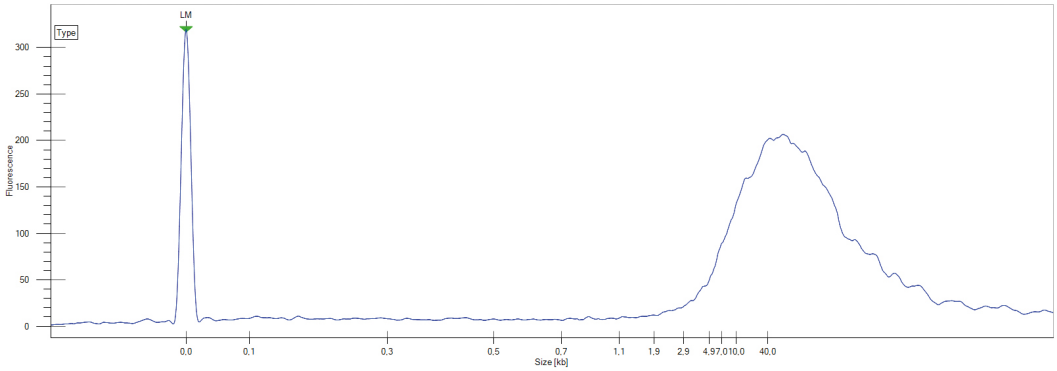

Figure 2: Representative electropherogram made with the LabChip® GX Touch™ nucleic acid analyzer using the Genomic DNA chip of DNA extracted from DBS with the chemagic™ 360 instrument.

The DNA extracted from each sample was eluted into 60 µL of elution buffer. With an average yield of ~600 ng of genomic DNA at an average concentration of 10 ng/µL, enough DNA was extracted from every sample for downstream NGS analysis.

To ensure the quality the extracted DNA was analyzed using the LabChip® GX Touch™ nucleic acid analyzer with the Genomic DNA chip and reagents kit (*Figure 2*).

DNA extracted using the chemagen™ Technology from DBS can be taken for a variety of downstream applications. Data below illustrates its application with targeted sequencing which relies on pure, high quality DNA as input material.

Targeted sequencing from DNA extracted from DBS samples

Five of the afore mentioned DBS gDNA samples served as input for targeted sequencing. The DNA was sheared to a target peak size of approximately 150 - 200 bp using the Covaris® E220 focused ultrasonicator. Libraries were constructed from 200 ng of DNA with the Agilent® SureSelect® XT low input reagent kits for library preparation and the Agilent® SureSelect® XT DMD kit for hybrid-capture-based target enrichment. Both the library preparation and target capture protocols were automated on the Sciclone® G3 NGSx workstation. The samples were sequenced on an Illumina® NovaSeq® sequencer.

Table 1: Sequencing data generated from libraries constructed using the Agilent® SureSelect® XT low input reagent kit for library preparation and the Agilent® SureSelect® XT DMD kit for hybrid-capture-based target enrichment from DNA isolated from 5 DBS samples using the chemagic™ 360 instrument.

DBS sample	Total reads	% Mapped reads	% Duplicate reads	Avg. Align coverage (BED)	% Target			
					10 - 20x	20 - 30x	30 - 40x	>40x
1	10470234	99%	10%	162.49	0.88	1.1	1.37	96.24
2	21593676	99%	10%	183.68	0.63	0.8	0.96	97.41
3	19451036	99%	17%	138.55	0.76	0.94	1.1	96.8
4	18497402	99%	18%	134.26	0.51	0.74	0.87	88.12
5	10512000	100%	14%	180.96	0.58	0.79	0.85	97.6

Conclusion

Genomic DNA extracted from DBS is an attractive sample type because of the many handling and storage benefits it offers. Using the chemagen™ Technology, DBS can now be used for NGS analysis, which has been challenging in the past, because other technologies could not deliver such pure and high quality DNA required for NGS analysis. The described DBS workflow consisting of sampling, transportation, gDNA extraction, DNA and library QC, and library preparation automation facilitates targeted NGS sequencing of DNA extracted from DBS.

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