From DBS to WGS: Rapid genomic DNA isolation from Dried Blood Spots and Whole Genome library preparation using sparQ DBS Library Prep Kit

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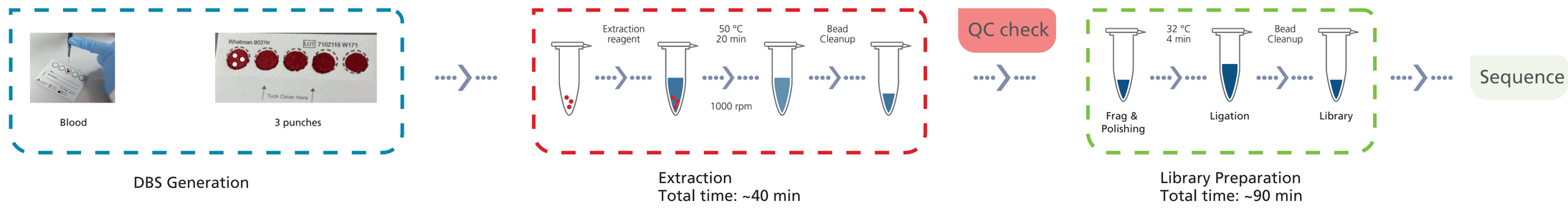
Abstract

Newborn screening is an essential step to monitor clinically significant rare and treatable disorders within the early few days of life. Blood from newborn babies is collected on a specialized paper in the form of dried blood spots (DBS) and used for testing metabolic, endocrine and hemoglobin disorders among other genetic disorders. For whole genome sequencing based newborn screening, we developed a comprehensive methodology (sparQ DBS Library Prep Kit) that includes lysis buffer, magnetic beads, and DNA library prep reagents for isolating gDNA from DBS samples and preparing DNA libraries for whole genome sequencing. The gDNA isolation method required only 40 minutes to achieve ~0.5 µg of high molecular weight gDNA from DBS samples. The library prep reagents provided a streamlined solution for PCR-free library preparation within 90 minutes.

In a pilot study, multiple blood samples were spotted on the W903 filters, which were subjected to whole genome sequencing (WGS) for validation. DBS lysis for DNA isolation generated high molecular weight (>50 kb) genomic DNA (gDNA) with a yield of 50-400 ng from 1-8 punches. PCR-free whole genome libraries were prepared and sequenced on NovaSeq XP with 150 bp pair-end reads to achieve 160 Gb reads from each sample. The sequencing results provide >30X coverage across the genome with a low duplication rate (<8%). The percentage of mismatches, inserts, and deletions was similar to other well-known kits available in the market. The quality and the yield of gDNA make it suitable for NGS and other applications.

Overall, the sparQ DBS Library Prep Kit provides a rapid, cost-effective, and robust solution for whole genome library preparation from DBS samples that will benefit newborn screening across the globe.



Genomic DNA (gDNA) Extraction

- We used 3mm punches from DBS cards to extract genomic DNA.
- The process of gDNA extraction requires three components: sparQ lysis buffer, Proteinase K, and sparQ PureMagBeads.
- Purified gDNA samples were quantified using a Qubit 4 Fluorometer (ThermoFisher Scientific) and analyzed using TapeStation (Agilent).

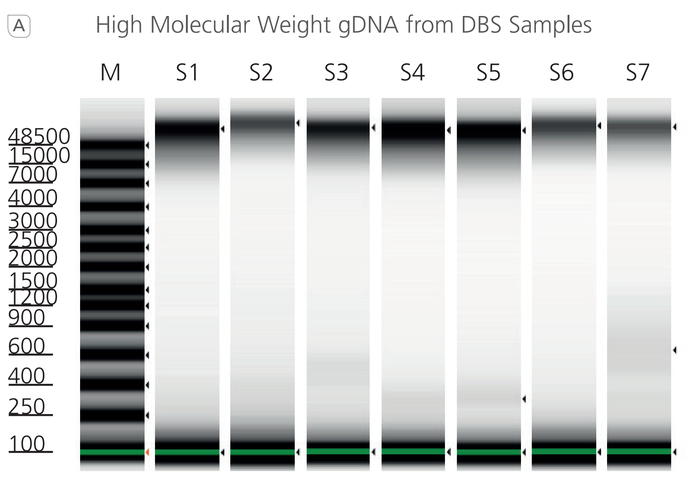


Figure 1A Three punches from each DBS sample were used to extract genomic DNA (S1-S7). The quality of the genomic DNA was verified by Genomic DNA ScreenTape, which demonstrated very high-quality genomic DNA.

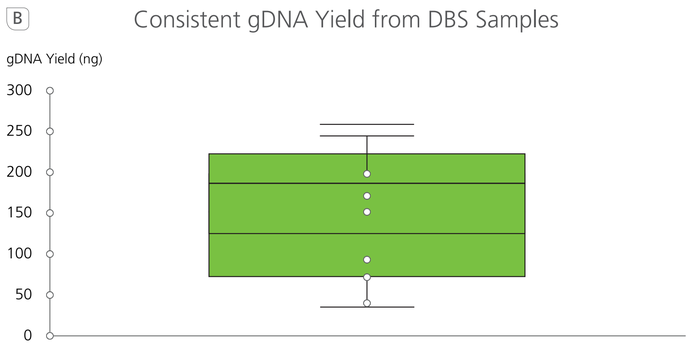


Figure 1B gDNA was quantified using Qubit, which showed consistent and high yield.

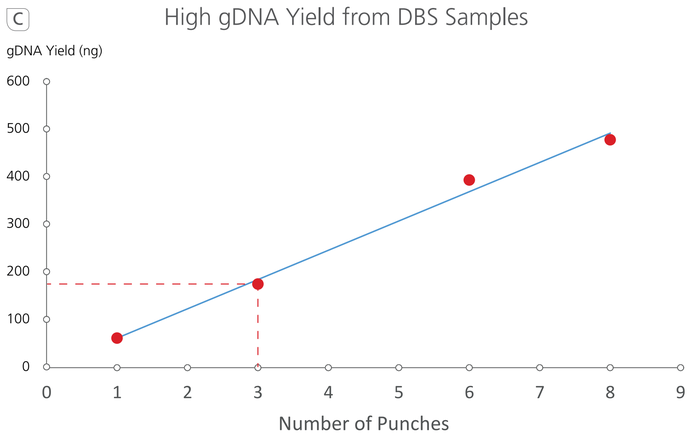


Figure 1C gDNA yield had a strong correlation with the number of punches used. Three punches yielded an average of 175 ng gDNA, sufficient for PCR-free whole genome library preparation.

Whole Genome Library Preparation

- 35 µl gDNA samples were fragmented for 4 min at 32 °C.
- PCR-free libraries were quantified using Qubit (Thermo) and by qPCR using sparQ Universal Library Quant Kit (Quantabio).
- PCR-free libraries were prepared using sparQ DBS Library Prep Kit
- To determine the size of the libraries, 1 µl PCR-free libraries were amplified using HiFi PCR Master Mix and Primer Mix included in the sparQ DBS Library Prep Kit.

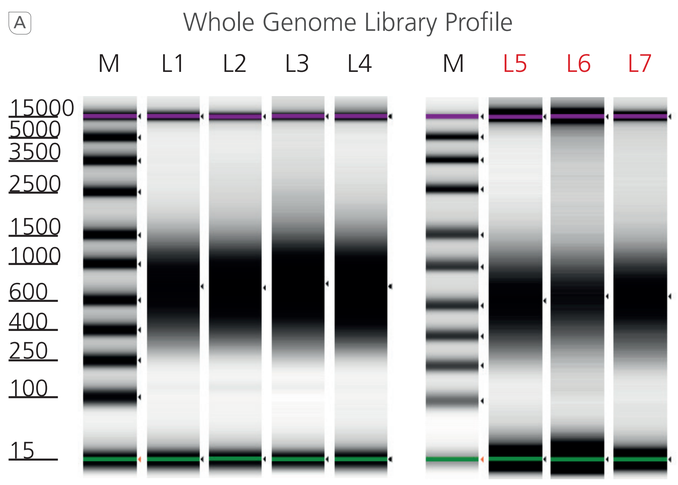


Figure 2A PCR-free libraries with full-length Y-adapters were amplified for three cycles before running TapeStation (D5000). Four minutes of fragmentation generated library size distribution between 700-750 bp. For L1 to L4, 35 µl gDNA extracted from three punches was used directly for library preparation. For L5 to L7, 400 or 100 ng gDNA was used for PCR-free or amplified library preparation.

Libraries	Total input DNA (ng)	Yield Qubit (nM)	Yield qPCR (nM)
L1	141.4	23.0	28.8
L2	156.1	26.2	33.4
L3	181.3	32.5	41.3
L4	157.85	33.7	34.9
L5	400		48
L6	100		21.1
L7	100 ng with 3 PCR cycles		92.3

Table 1 PCR-free whole genome library yield as determined by Qubit and qPCR.

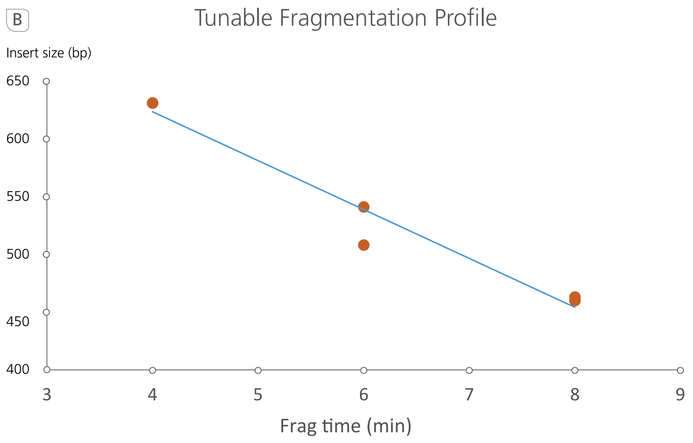


Figure 2B Desired DNA fragment size can be achieved by incubating gDNA with fragmentation master mix at 32°C for recommended fragmentation time.

Whole Genome Sequencing

- Libraries were pooled and sequenced in NovaSeq X using 2 x 150 PE
- Target output was 140 Gb
- FASTQ files were analyzed using CLC Genomics Workbench (Qiagen)

Library	Input DNA (ng)	No. of PCR cycles	Duplication rate (%)	Mapped reads cycles (%)
L5	400 ng	PCR-free	7.4	95.6
L6	100 ng	PCR-free	7.7	95.5
L7	100 ng	3 cycles	8.1	95.5

Table 2 sparQ DBS Library Prep Kit generated high-quality DNA libraries with minimal duplication artifacts and a high percentage of mapped reads.

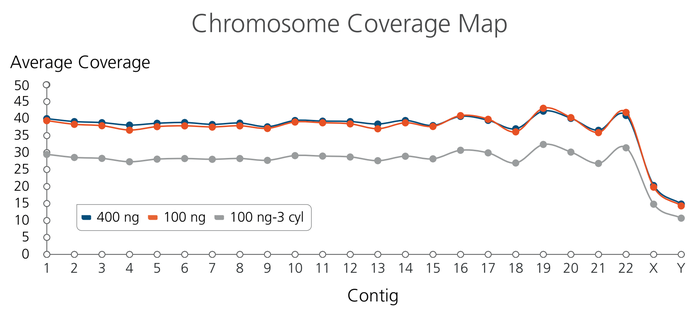


Figure 3 Genome-wide coverage was greater than 30X across all chromosomes. This is essential for determining most germline mutations with high confidence.

	400 ng	100 ng	100 ng-3 cyl
Total variants	7,179,966	7,144,471	7,200,144
Total SNPs	6,014,491	5,987,965	6,098,961
Structural variants	10,978	10,534	8,554
Duplications	160	150	139
Deletions	466	569	483

Table 3 Libraries prepared with the sparQ DBS Library Prep Kit generated low mutational frequency, which indicates less error incorporated during the library preparation stage. Also, mutational frequencies are comparable between different input amounts, and library amplification did not incorporate additional bias.

Summary

- sparQ DBS Library Prep Kit provides a streamlined solution for gDNA extraction to PCR-free whole genome library preparation.
- Rapid gDNA isolation workflow requires 40 minutes, and the PCR-free library prep takes 90 min.
- The extraction method generated high molecular weight gDNA (MW > 50kb) with high yield.
- High-quality libraries generated reliable sequencing data.
- The sparQ DBS Library Prep Kit provides a rapid, cost-effective, and robust solution for whole genome library preparation from DBS samples that will benefit newborn screening across the globe.