Empowering Newborn Sequencing with Streamlined High Molecular Weight Genomic DNA Extraction from DBS and Whole Genome Library Preparation using sparQ DBS-seq Workflow

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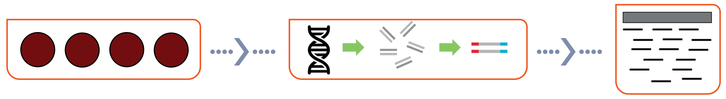
Newborn Screening

- NBS
  - Blood spot screening
  - Pulse oximetry screening
  - Hearing screening

Uses a few drops of blood from the baby's heel to generate Dried Blood Spots (**DBS**) and uses technologies like mass spectrometry, qPCR, and targeted sequencing.

Whole Genome Sequencing for Newborn Testing



Whole genome sequencing (WGS) provides comprehensive, reliable, and fast genetic information regarding genetic disorders present in newborns.

Challenge: Current extraction methods do not support a quick and cost-effective extraction of high-quality genomic DNA (gDNA) from DBS samples

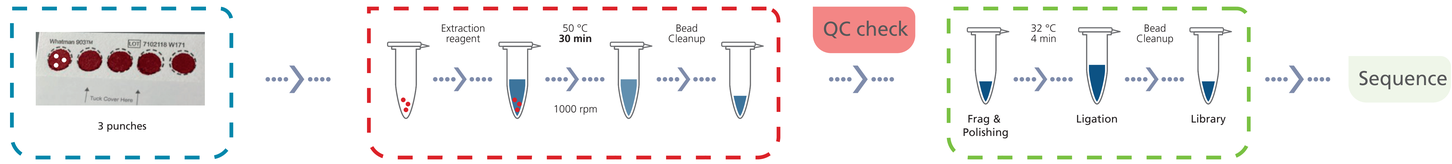
sparQ DBS-seq Workflow: Complete Solution for Whole Genome Sequencing

sparQ Lysis Kit:
gDNA Extraction from Dried Blood Spots (DBS) And other samples

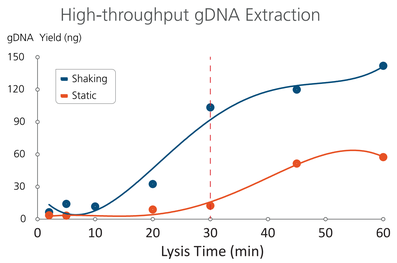
+

sparQ DNA Frag & Library Prep Kit:
DNA Library Preparation with Enzymatic Fragmentation

sparQ DBS-seq Workflow

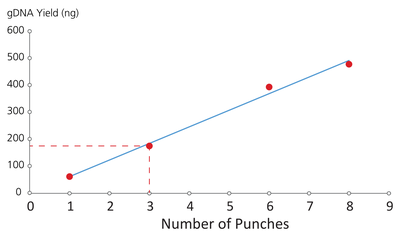


High-throughput gDNA Extraction



To achieve a high yield of genomic DNA (gDNA), it is essential to incubate at 50 °C while shaking at 1000 rpm. The lysis reaction should be carried out for a minimum of 30 minutes to ensure consistent gDNA yield. For the lysis step, using an Eppendorf ThermoMixer® is recommended.

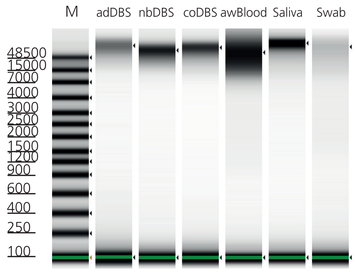
High gDNA Yield Fulfilling Different Applications

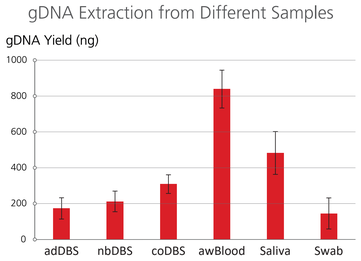


gDNA yield had a strong correlation with the number of punches used. Three punches yielded an average of 175 ng gDNA, sufficient for PCR-free whole genome library preparation.

If a higher gDNA yield is needed for other applications, consider increasing the number of DBS punches to achieve this goal.

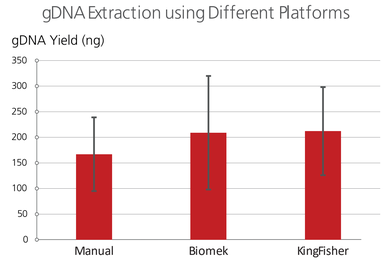
gDNA Extraction from Different Samples





adDBS: Three 3.2 mm punches from dried adult blood spots
coDBS: Three 3.2 mm punches from dried cord blood spots
Saliva: 400 µl stabilized saliva
nbDBS: Three 3.2 mm punches from dried newborn blood spots
awBlood: 200 µl whole adult blood
Swab: 400 µl stabilized cheek swab

gDNA Extraction using Different Platforms



gDNA was extracted from three 3.2 mm DBS punches, either manually or using the Biomek i7 Automated Workstation (Beckman) or KingFisher (ThermoFisher Scientific) automated platforms.

Both manual and automated workflows provided consistent and high yields of gDNA.

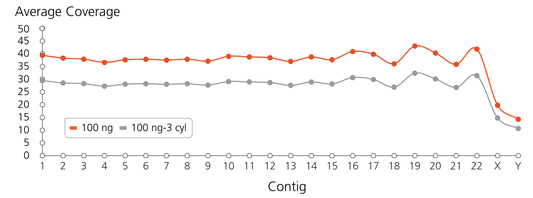
Whole Genome Sequencing

Libraries were pooled and sequenced in NovaSeq X (Illumina) using 2x150 PE. Target output was 140 Gb. FASTQ files were analyzed using CLC Genomics Workbench (Qiagen).

Input DNA (ng)	No. of PCR cycles	Library yield (nM)	Duplication rate (%)	Mapped reads cycles (%)
100 ng	PCR-free	21	7.7	95.5
100 ng	3 cycles	92	8.1	95.5

sparQ DBS-seq workflow generated high-quality DNA libraries with minimal duplication artifacts and a high percentage of mapped reads.

Chromosome Coverage Map



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

Input DNA (ng)	Total variants	Total SNPs	Structural variants	Duplications	Deletions
100 ng	7,144,471	5,987,965	10,534	150	569
100 ng-3 cyl	7,200,144	6,098,961	8,554	139	483

Libraries prepared with the sparQ DBS-seq workflow generated low mutational frequency, which means less error was 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-seq workflow provides a streamlined solution for gDNA extraction to whole genome library preparation.
- Rapid gDNA isolation workflow requires 50 minutes, and the PCR-free library prep takes 90 min. Both gDNA extraction and library preparation are amendable to high-throughput and automation.
- The extraction method generates high molecular weight gDNA (MW > 50kb) with high yield.
- Using this workflow, gDNA can be extracted from DBS, whole blood, saliva and other sample types.
- High-quality libraries generated reliable sequencing data.
- sparQ DBS-seq workflow provides a rapid, cost-effective, and robust solution for whole genome library preparation from various samples that will benefit newborn sequencing and other applications across the globe.