DNA extraction from dried blood spots for Oxford Nanopore sequencing

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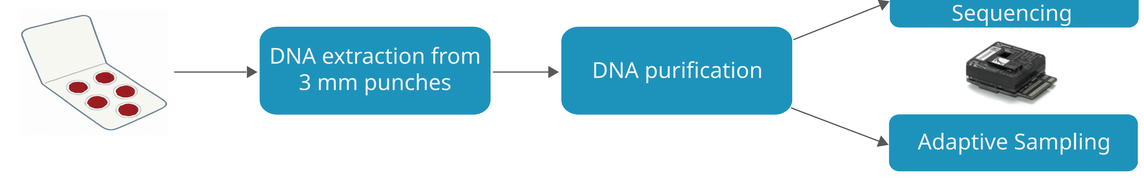
Abstract

Dried blood spot (DBS) cards provide a minimally invasive, stable, and easily transportable method for long-term preservation of blood samples, making them widely used in newborn screening and population-scale genomics. However, extracting high molecular weight (HMW) DNA from DBS remains challenging due to limited input material, variable card chemistries, and potential degradation during storage. Here, we present an optimized workflow for extracting HMW DNA from DBS samples for whole genome sequencing (WGS) and Adaptive Sampling (AS) on Oxford Nanopore Technologies' platforms. Despite using DNA inputs below the recommended amount for library preparation, we obtained whole genome coverage of >20× and read length N50s up to 20 kb from single PromethION flow cells, which is sufficient for variant detection and methylation analysis. AS enrichment demonstrated >80× on-target coverage, allowing for sample multiplexing. Collectively, this work demonstrates that DBS-derived DNA is compatible with Oxford Nanopore sequencing and can generate valuable genomic data from limited inputs. This workflow also opens new opportunities for large-scale, minimally invasive genomic research leveraging the wealth of existing DBS repositories, including precious or archived samples. As extraction and sequencing methods continue to improve, DBS-based Oxford Nanopore sequencing could form the foundation for population-scale genetic and epigenetic screening in both research and translational contexts.

Materials and methods

Varying numbers of 3 mm punches were taken using Unicore Punch Kits (Qiagen) from multiple DBS cards – Whatman™ 903 Protein Saver (Cytiva), QIAcard FTA™ Classic (Qiagen), and Revvity™ 226 Spot Saver Card (Revvity). The punches were incubated with G2 buffer (Qiagen), RNase A, Proteinase K, and PBS. Each sample was then subsequently purified with bead-based recovery and sequencing libraries were prepared with the Ligation Sequencing Kit V14 (SQK-LSK114)[1] as per manufacturer's instructions (Fig. 1a). High-accuracy (HAC) basecalling and alignment to the hg38 reference genome was performed for all sequencing data (dorado 7.6.7). Targeted enrichment using AS (Fig. 1b) was performed enriching for 429 relevant pharmacogenetic (PGx) targets[2] amounting to ~1.7% of the genome. Prior to AS, DNA was sheared to ~10 kb using a Covaris g-TUBE to optimize read length for effective on-target enrichment.

1a)



1b)

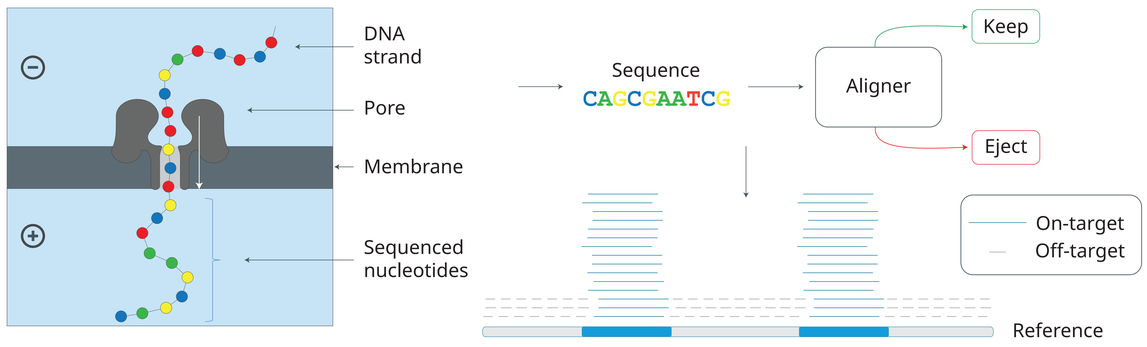


Figure 1. a) Extraction and library preparation workflow for WGS and AS from DBS cards. b) Schematic of AS for the targeted enrichment of DNA.

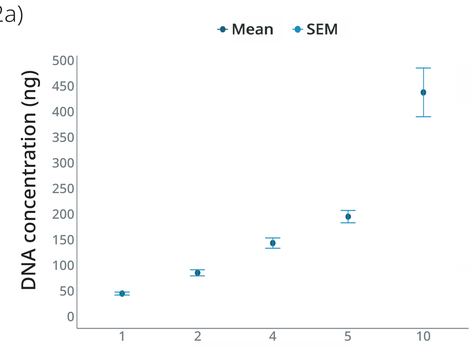
References

[1]https://nanoporetech.com/document/genomic-dna-by-ligation-sqk-lsk114?device=PromethION
[2]https://nanoporetech.com/document/pgx-sequencing-workflow-with-adaptive-sampling-blood-cells-saliva-
[3]https://github.com/epi2me-labs/wf-human-variation
[4]https://github.com/brentp/mosdepth

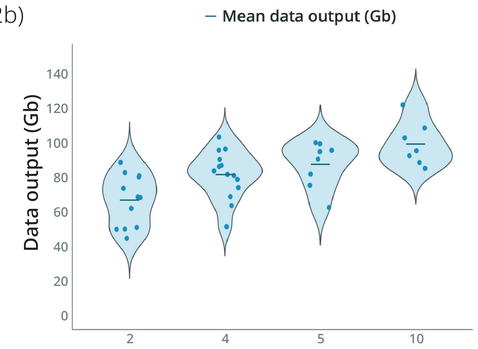
Performance of DBS DNA extractions and sequencing

Using this workflow, we demonstrated a clear relationship between punch number and DNA yield from 3 mm punches on QIAcard™ FTA Non-Indicating cards (Qiagen), with average yields exceeding 400 ng from 10 punches (Fig. 2a). Despite using DNA inputs below the recommended amount for SQK-LSK114 library preparation, we obtained high WGS yields from just 2x 3 mm punches, averaging 67 Gb of pass-filter data (Q-score > 9) per run, equivalent to ~22× whole genome coverage (Fig. 2b). Notably, we also observed long read lengths from all three DBS card types, with N50s consistently exceeding 15 kb across most extractions (Fig. 2c), while preserving methylation. This demonstrates that HMW DNA can be reliably recovered from DBS cards to support long-read applications such as structural variant detection and phasing using Oxford Nanopore's EPI2ME™ wf-human-variation pipeline[3].

2a)



2b)



2c)

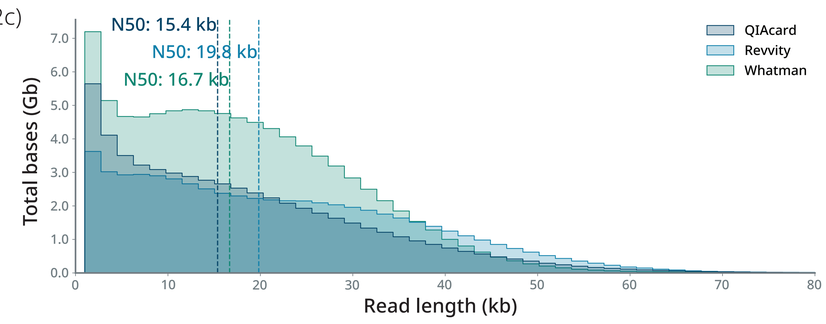


Figure 2. a) DNA concentrations (ng) obtained from 1, 2, 4, 5, and 10 × 3 mm punches extracted from DBS cards. b) Pass-filter (Q-score > 9) basecalled data outputs from WGS of DNA extracted using 2, 4, 5, and 10 x 3 mm punch inputs. c) Read length distributions from a representative sample of each of the three DBS card input types using WGS and 4x 3 mm punches as input.

Targeted enrichment using Adaptive Sampling

Using AS, we achieved average on-target coverages of ~80× and ~106× from 2 and 4 input punches, respectively, across 429 PGx targets. Coverage metrics calculated using mosdepth[4] highlight the specificity of AS in enriching regions of interest, as illustrated by the increased depth over the *CYP2C19* gene, a key pharmacogenomic locus, when compared to WGS results (Fig. 3). Notably, >10× average coverage was also retained across non-targeted regions, supporting broader low-pass or imputation alongside targeted analysis. These results demonstrate that AS can significantly enhance coverage of regions of interest, even from low-input DBS samples, allowing for improved variant detection.

3)

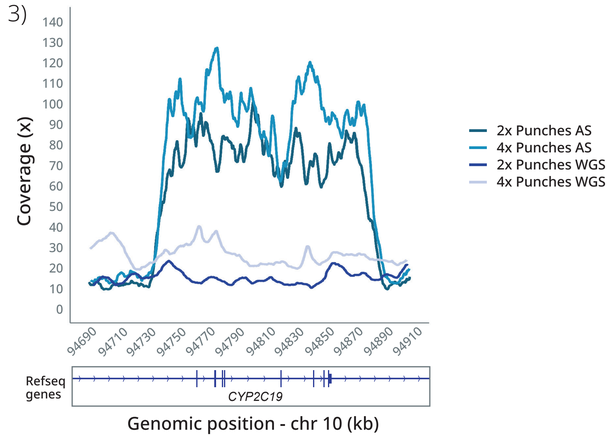


Figure 3. Coverage across the *CYP2C19* locus (chr10: 94,690,000-94,910,000) from AS and WGS sequencing results.

Conclusion

We present a workflow for DNA extraction from DBS cards that is compatible with multiple card types and supports downstream sequencing even with reduced DNA input. High-quality data were generated from as few as two 3 mm punches, yielding >20× WGS coverage and >80× on-target coverage using AS. Consistently long read lengths (N50s >15 kb) show that the workflow preserves DNA integrity, enabling robust variant detection and suitability for long-read applications. This approach enables comprehensive genomic analysis from low-input material, making it well-suited for large-scale research with limited samples, such as newborn screening and clinical genomics.