Targeted long-read Nanopore sequencing of the blood group genome by adaptive sampling

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Aim: Determine if Oxford Nanopore adaptive sampling can be used for detecting structural variants (SVs) and calling single-nucleotides variants (SNVs) across the entire blood group genome

What is adaptive sampling:

- Purely computational enrichment method of genomic regions of interest
- During sequencing, reads are mapped in real time (~first 500 nt within 1 sec) against a user-defined reference
- Off-target reads are continuously ejected from the nanopores, leaving pores available for on-target reads
- Typical enrichment: ~5-10x

Methods:

Monarch HMW DNA Extraction Kit for blood

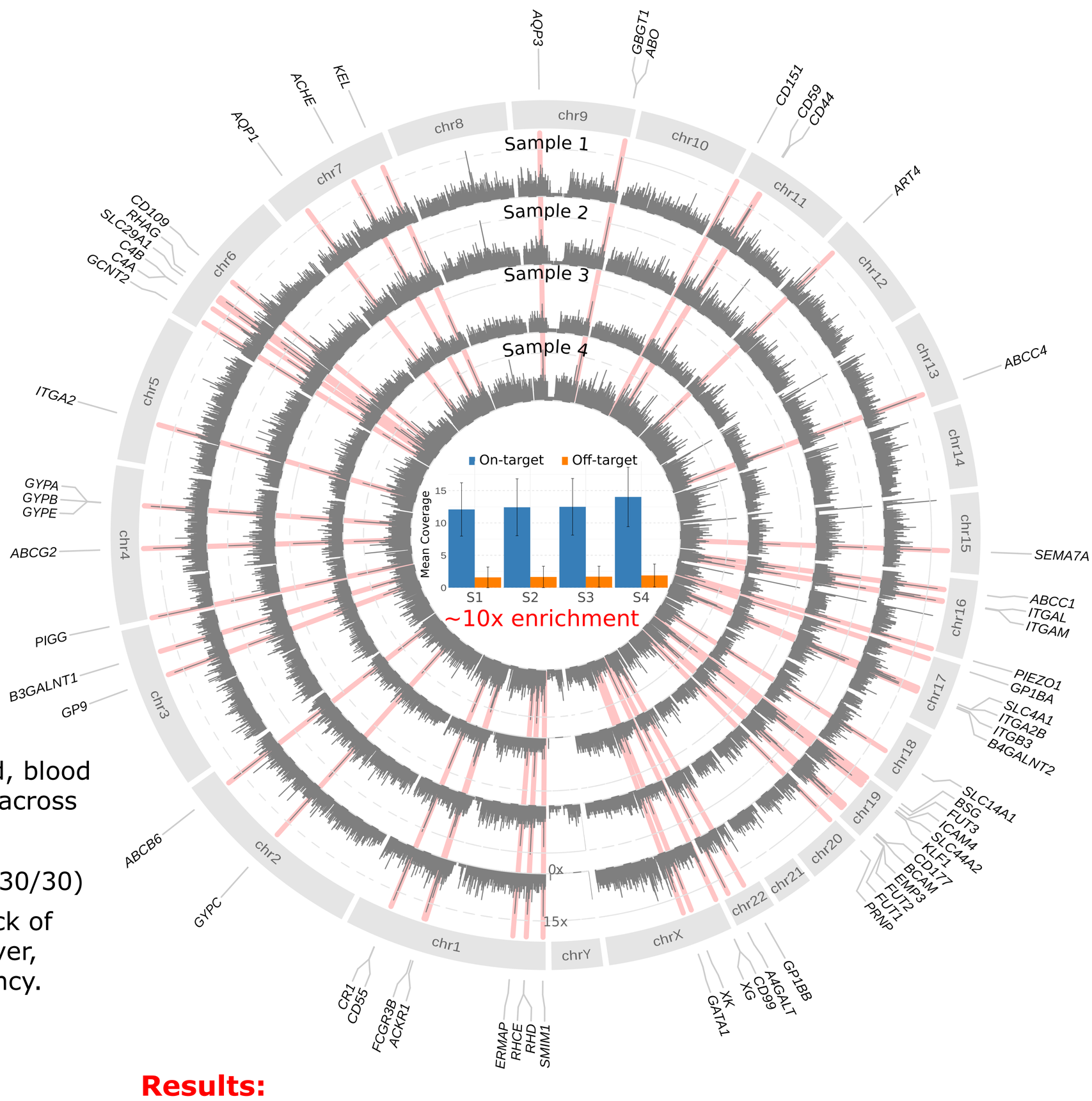
SQK-LSK110 ligation kit with 3-9 µg input DNA

R9.4.1 flow cell washed and reloaded every ~20h

Reference file contained 63 regions (~0,26% of the human genome)

- 50 HEA genes
- 7 HPA genes
- 4 HNA genes
- 2 TFs

Coverage Blood Group Genome

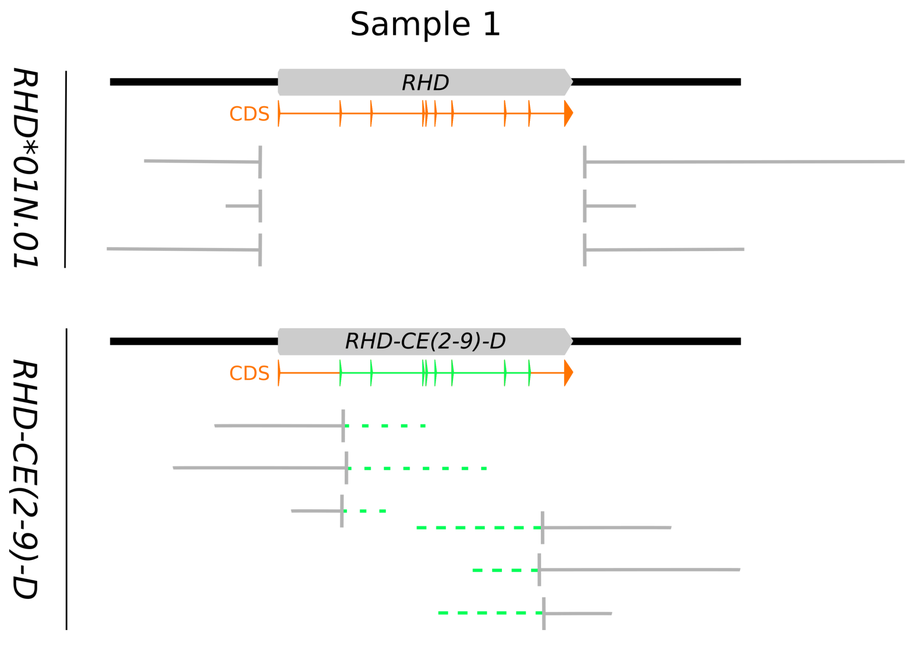


SNV calling

Comparison of genotypes at pre-typed, blood group relevant SNVs (n = 39) spread across 18 systems for Sample 3:

- 100% congruency for called SNVs (30/30)
- 9 positions not called because of lack of coverage. Manual inspection, however, showed strong support for congruency.

Resolving SVs



Results:

- From extracted gDNA to first sequences in ~2h
- 7-8 Gb output per run (MinION flow cell)
- ~10x enrichment; mean coverage of ~12-14x across 63 targeted regions
- High congruency with genotypes at pre-typed SNV positions
- Facilitated identification of SVs in *RH* locus

Conclusions:

- Promising tool for cost-effective and straightforward long-read sequencing of all blood group genes
- Highly versatile: targeted genes can be adapted by adjusting genomic coordinates in a FASTA/BED file
- Sequencing output and coverage still relatively low for MinION

Abstract

33rd Regional Congress of the ISBT, 2023, Gothenburg, Sweden