NANOPORE SEQUENCING TO RESOLVE KIDD BLOOD GROUP DISCREPANCIES

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BACKGROUND

- Constant increase in read accuracy of long-read sequencing → clinical diagnostics
- Nanopore sequencing has great potential for resolving complex phenotype/genotype discrepancies observed in routine blood group antigen typing
- Classical Sanger sequencing not adequate for:
  - detecting structural variants (SVs)
  - phasing novel variants to haplotype background
- Case example: Kidd system (Jk, *SLC14A1*)

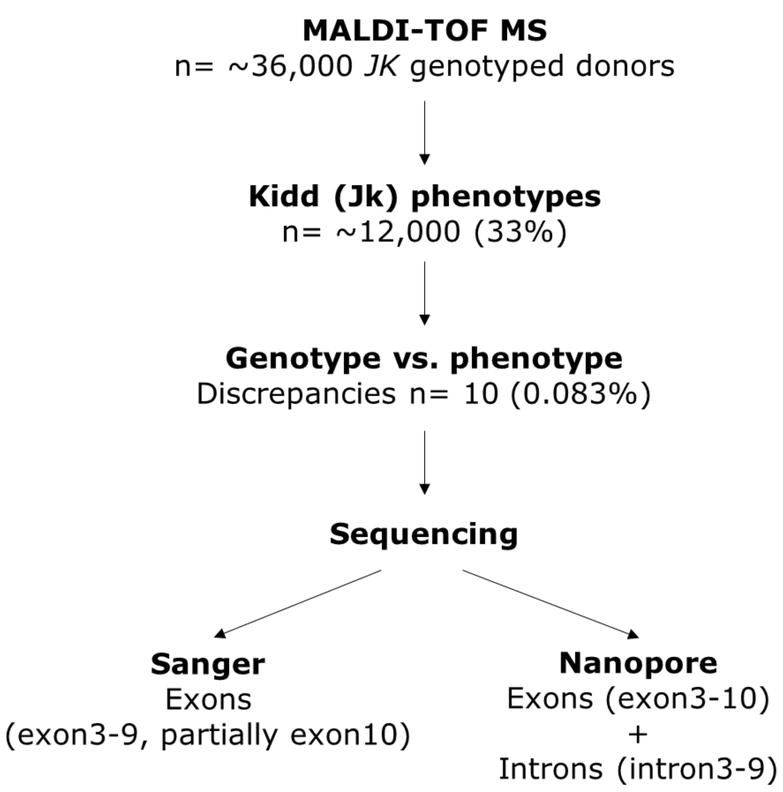

Figure 1. Flowchart of sample analysis.

METHODS

- Routine donor genotyping by MALDI-TOF MS at Blutspende Zurich includes: *JK*A/B* (c.838G>A), *JK*01N.03* (c.582C>G), *JK*02N.01/.02* (c.342-1G>A/C)
- Resolving 10 phenotype/genotype discrepancies (Fig. 1)
  - *SLC14A1* amplified in two overlapping long-range PCRs (Fig. 2)
  - Nanopore sequencing on a MinION (R9.4.1) flow cell
  - Sanger sequencing of exons and 'bridge-PCR' of breakpoints for validation

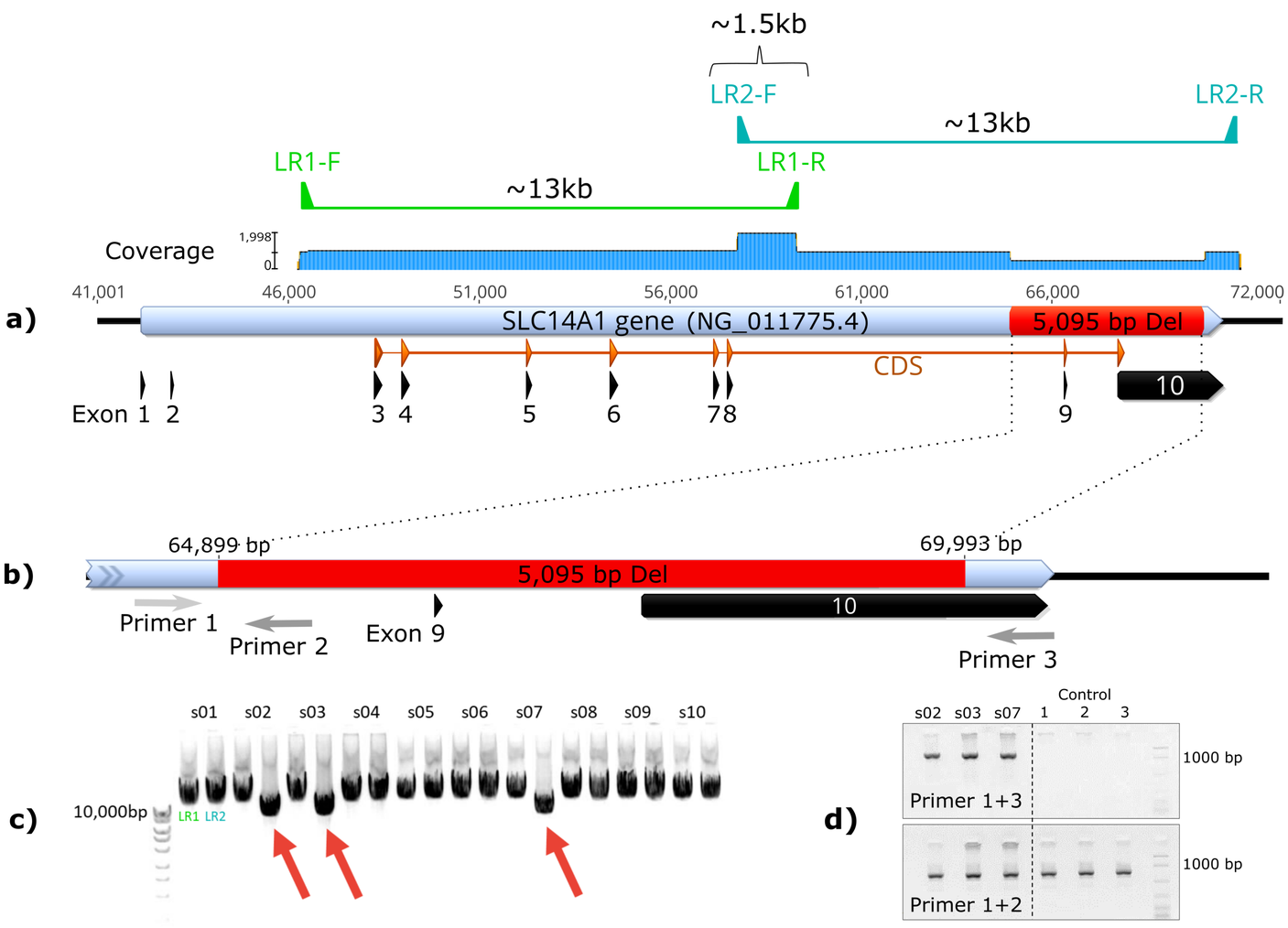

Figure 2. (a) *SLC14A1* gene showing exons, coding DNA sequence (CDS), newly identified ~5 kb deletion (red), coordinates of long-range PCRs (LR1/LR2), and raw reads mapping coverage of sample s02. **(b)** Zoom on the novel ~5 kb deletion with breakpoints and primer positions used for 'bridge-PCR'. **(c)** Long-range PCR products of all samples; red arrows highlight ~5 kb shorter LR2 fragments with novel deletion. **(d)** 'bridge-PCR' products for the three samples with the novel deletion, and control reactions.

RESULTS & CONCLUSIONS

- 10 unexplained Kidd phenotype/genotype discrepancies among ~12,000 donors (Fig. 1)
- All discrepancies resolved using nanopore sequencing (Fig. 2, Table 1):
  - 5/10: rare known weak and null alleles not included in MALDI-TOF MS assay
  - 5/10: novel null alleles
    - SNV-based (n = 2)
    - *JK*A* linked ~5 kb deletion (n = 3)
- Nanopore sequencing particularly useful for challenging diagnostics involving hybrid genes, deletions, and duplications
- Structural variants mostly not detectable by Sanger sequencing → likely more SVs missed so far also in other blood groups
- Established a fast and reliable protocol well adaptable to other blood group systems

Table 1. Summary table of serology, genotyping, and sequencing results for all 10 samples.

Sample	Serology	MALDI-TOF Genotyping	Nanopore Sequencing Haplotype 1	Nanopore Sequencing Haplotype 2
Known weak and null alleles				
s01	Jk(a+^{weak} b-)	Jk(a+b+)	*JK*01W.01*	*JK*02N.08*
s04	Jk(a+b-)	Jk(a+b+)	*JK*01*	*JK*02W.04*
s05	Jk(a-b+)	Jk(a+b+)	*JK*01W.05*	*JK*02*
s06	Jk(a+b-)	Jk(a+b+)	*JK*01*	*JK*02N.09*
s08	Jk(a+^{weak} b-)	Jk(a+b+)	*JK*01W.01*	*JK*02N.06*
Novel null alleles				
s09	Jk(a+b-)	Jk(a+b+)	*JK*01*	*JK*02(G242E)Null*
s10	Jk(a-b+)	Jk(a+b+)	*JK*01(G40D)Null*	*JK*02*
Novel structural variant				
s03	Jk(a-b+)	Jk(a+b+)	*JK*01(Ex9_10del)**	*JK*02*
s04	Jk(a-b+)	Jk(a+b+)	*JK*01(Ex9_10del)**	*JK*02*
s07	Jk(a-b+)	Jk(a+b+)	*JK*01(Ex9_10del)**	*JK*02*

*Only detected by Nanopore sequencing. Confirmed by bridge-PCR (Fig. 2d).

Abstract