Evaluation of *Rbceq2* for High Resolution Genotyping of ABO And Other Relevant Blood Group Genes in Transplantation

Sarah Grasedieck[1], Liam McIntyre[2], Eileen Roulis[2], Krishna Dwarka[1], Paul A Keown[1], William J Lane[3,4], Cathi Murphey[5], Karen R Sherwood[1].

1. Department of Medicine, University of British Columbia, Vancouver, BC, Canada..
2. Australian Red Cross Lifeblood, Kelvin Grove, Queensland, Australia.
3. Department of Pathology, Brigham and Women's Hospital, Boston, MA, USA.
4. Harvard Medical School, Boston, MA, USA.
5. Southwest Immunodiagnostics, Inc., Dallas, Texas, USA.

sgrase@mail.ubc.ca

Background / Aim

Accurate determination of ABO and related blood group genotypes is critical for transplantation but remains challenging in cases with weak subgroups, hybrid alleles, chimerism, or serologic discrepancy. Laboratory methods frequently yield inconclusive results, limiting transplant decision making. This study evaluates the performance and clinical utility of RBCeq2, a comprehensive blood group genotyping tool, applied to long read nanopore sequencing data in transplantation focused use cases.

Methods

Genomic DNA from transplant assessment samples underwent targeted long read sequencing using nanopore adaptive sampling, enriching the *ABO* locus and additional blood group genes relevant to transplantation, including *FUT* genes. Adaptive sampling generated long read sequencing data were analyzed using *RBCeq2* and compared with genotype calls generated by another established blood group genotyping tool (*bloodTyper*). Concordance was assessed in samples with available diagnostic reference typings performed at two independent HLA laboratories.

Results

Across all benchmark samples, *RBCeq2* demonstrated complete concordance with the comparator tool for *ABO* and *FUT* genotypes, with equivalent accuracy for small and structural variants (**Table 1**). *RBCeq2* additionally provided high confidence genotyping across a substantially larger number of blood group genes in a single analysis. In complex ABO cases, RBCeq2 resolved diverse sources of laboratory ambiguity, including weak subgroup phenotypes, rare alleles, and compound variant patterns, enabling definitive genotype assignment where standard testing had remained inconclusive. Phased long read data supported confident allele level interpretation.

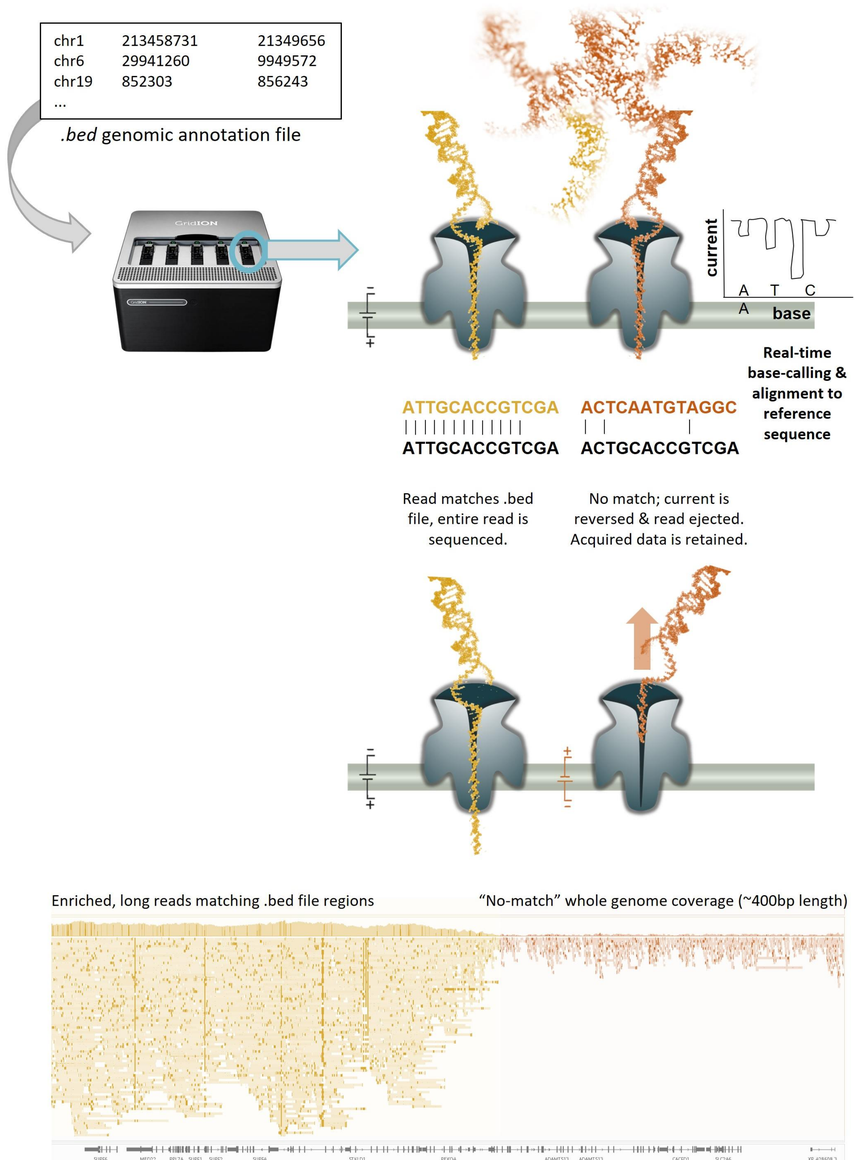


Figure 1. Adaptive Sampling enables real-time selective enrichment of specific genomic regions. A) *An annotation file listing all region of interest coordinates is provided to the ONT sequencer. The ionic current changes measured while each native nucleotide strand passes through a Nanopore is translated in real-time to base information (A,T,C,G, 5mC, 5hmC, 6mA, U, PseudoU, m6A and others) and mapped to the provided reference genome.* **B)** *After sequencing the first ~400 bases, an algorithm checks whether the sequence matches a provided region of interest or not. In case of a match, the complete molecule is sequenced and a long read is produced. In case of a no-match, the current in this particular pore is reversed and the molecule ejected but the ~400bp long read mapping to an off-target region is retained. This process happens in parallel at thousands of pores at a time.* **C)** *The result is an enrichment of genomic target regions that are captured with long reads (left) and a lower whole genome coverage with 400bp long rejected reads (right) that allows copy number and similar analyses.*

Table 1. ***Concordance of adaptive sampling and two different ABO genotyping tools with laboratory typing*.**

Sample	Lab typing	AS+*RBCeq2* Genotype	AS+*BloodTyper* Genotype
8	O+ (R1r)	*ABO*O.01.01/ABO*O.01.09*	*ABO*O.01.01/ABO*O.01.09*
4	B- (rr)	*ABO*B.01/ABO*O.01.09*	*ABO*B.01/ABO*O.01.09*
9	O+ (R1r)	*ABO*O.01.09/ABO*O.01.09*	*ABO*O.01.09/ABO*O.01.09*
10	*ABO*A2.01/ABO*B.01*	*ABO*A2.01/ABO*B.01*	*ABO*A2.01/ABO*B.01*
11	*ABO*O,01.01/ABO*O,01,05* **2nd typing (NGS):** *ABO*O.01.01/ABO*O.01.75*	*ABO*O.01.01/ABO*O.01.75*	*ABO*O.01.01/ABO*O.01.75*
12	O (ambiguous phenotype)	*ABO*O.01.02/ABO*O.01.12 & ABO*O.01.68*	*ABO*O.01.02/ABO*O.01.68(c.595C>T)*

Download and learn more about *RBCeq2* at
https://github.com/limcintyre/RBCeq2
Not for clinical use.

RBCeq2 is an interpretive tool that infers blood-group genotypes and phenotypes from supplied variant calls (*.vcf* files*) using the curated ISBT allele definitions distributed with the package. Deterministic rules select and exclude candidate alleles and pairs; --debug records the evidence and named exclusions behind the result.

***V**ariant **C**all **F**ormat is a standard text file format used in bioinformatics for storing gene sequence or DNA sequence variations:

```
##fileformat=VCFv4.2
##contig=<ID=2,length=51304566>
##INFO=<ID=AC,Number=A,Type=Integer,Description="Allele count in genotypes">
##INFO=<ID=AN,Number=1,Type=Integer,Description="Total number of alleles in called genotypes">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality">
#CHROM  POS ID  REF ALT QUAL FILTER  INFO FORMAT      SAMPLE1      SAMPLE2      SAMPLE3      SAMPLE4      SAMPLE5      SAMPLE6      SAMPLE7
2  81170  .  C   T   .    .       AC=9;AN=7424  GT:DP:GQ  0/0:4:12  0/0:3:9  0/1:1:3  0/1:9:24  1/0:4:12  0/0:5:15  0/0:4:12
2  81171  .  G   A   .    .       AC=6;AN=7446  GT:DP:GQ  0/1:4:12  0/0:3:9  0/0:1:3  0/0:9:24  0/1:4:12  0/1:5:15  0/0:4:12
2  81182  .  A   G   .    .       AC=5;AN=7506  GT:DP:GQ  0/0:5:15  0/0:4:12  0/0:5:15  0/0:9:24  0/0:4:12  0/0:4:12  0/0:4:12
2  81204  .  T   G   .    .       AC=2;AN=7542  GT:DP:GQ  1/0:5:15  0/0:9:27  0/0:10:30  0/0:15:39  0/0:9:27  1/0:13:39  0/1:14:42
```

Currently supported blood group genes:
A4GALT, ABCB6, ABCC1, ABCC4, ABCG2, ABO, AUG, CD59, CO, CROM, CTL2, DI, DO, ER, FUT1/2/3, FY, GATA1, GBGT1, GCNT2, GE, GIL, GLOB, GYPA/B, IN, JK, JMH, KANNO, KEL, KLF1, KN, LU, LW, MAM, OK, PIGG, RAPH, RHAG, SC, SID, VEL, XG, XK, YT

Conclusion

RBCeq2 performs robustly on short and long read sequencing data and offers clear added value for transplantation by enabling comprehensive, high-resolution genotyping of ABO and related blood group genes. Its ability to resolve complex and previously indeterminate cases supports integration of extended blood group genotyping into transplant assessment beyond traditional serologic approaches and makes this tool a strong candidate for future clinical use.

Acknowledgements

GenomeCanada
Genome BritishColumbia

THE UNIVERSITY OF BRITISH COLUMBIA
Precision Medicine in Transplantation
Research Excellence Cluster

GCTC
GENOME CANADA
TRANSPLANT CONSORTIUM

BC HISTOCOMPATIBILITY AND IMMUNOLOGY LABORATORY