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Haplotype Analysis using Long-read Nanopore Sequencing

Haplotypanalyse mittels Long-Read Nanopore Sequenzierung

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In transfusion medicine, genetic characterization of blood groups is of great importance, particularly for patients requiring regular transfusions. Recent advancements in long-read sequencing accuracy have paved the way for such technologies to become a valuable alternative for genetic analyses in blood group diagnostics. Focusing on the sequencing method by Oxford Nanopore Technologies (ONT), we discuss its particular strength, namely the ability to construct full-gene haplotypes, which is especially challenging when complex structural variation including translocations between paralogous genes is present (as regularly observed in the form of hybrid alleles in the RH and MNS blood group systems). There are currently several enrichment methods available, all having their own advantages and pitfalls. The most cost-efficient and fast method, we present here is based on long-range PCR-based Nanopore sequencing. With this technique, we successfully produced reference haplotypes and resolved single-sample cases of genotype-phenotype discrepancies. Haplotypes for long blood group genes and those prone to forming hybrid alleles are, however, difficult to construct with LR-PCR. Such cases are better addressed using an ONT-specific, amplification-free adaptive sampling approach. As this method does not limit read length, it allows better performance for phasing and structural variant detection and even allows targeting many genetic loci in parallel. We present examples of haplotype construction of all ~50 blood group genes (useful for extended donor-recipient matching) with the inclusion of challenging hybrid alleles. The main hindrance to routinely use this technique is its high cost. The third enrichment technique we present here-with example on hybrid *RHD-CE-D* alleles-is based on long-read capturing by hybridization. This approach allows much higher enrichment (i.e. is much more cost-efficient) while preserving some of the advantages like even read coverage without the risk of allelic drop-outs. We are currently still optimizing the labor-intense pre-sequencing protocols and the moderate read length impairing phasing capabilities.

In summary, we present here different ONT-based sequencing approaches all aiming at enhancing diagnostic antigen-profiling by elucidating the underlying blood group genetics. We outlie their respective benefits and pitfalls along with different examples of blood group gene haplotyping, and provide inside on when to best apply them.