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WarpDemuX - METHOD FOR DEMULTIPLEXING OF RNA-SEQUENCING DATA

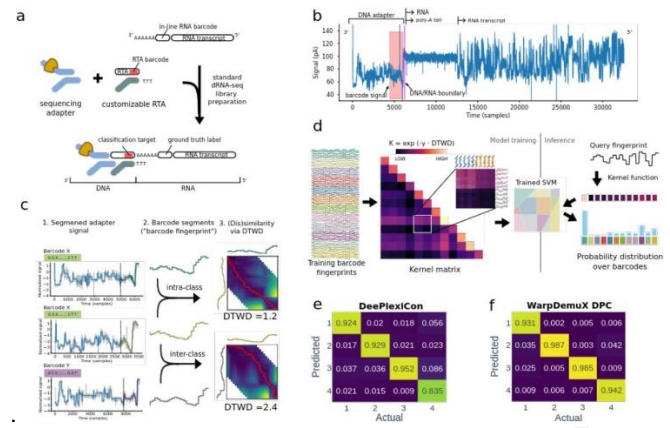
Keywords: WarpDemuX, nanopore sequencing, RNA, multiplexing, barcode, single-cell sequencing, dRNA-seq, demultiplexing, dynamic time warping

INVENTION NOVELTY

WarpDemuX is a method that improves the accuracy and efficiency of molecular analysis by using barcode adaptors attached to the molecule of interest, where the barcode molecule is an instance of a set of optimized sequences, and by applying advanced signal processing techniques. It reduces misclassification rates and simplifies the process of barcode identification, providing a more reliable and efficient solution for molecular diagnostics and research, particularly for nanopore direct RNA sequencing (dRNA-seq).

VALUE PROPOSITION

Nanopore direct RNA sequencing (dRNA-seq) enables unique insights into (epi-)transcriptomics. However, applications are currently limited by the lack of accurate and cost-effective sample multiplexing. Sample multiplexing, or multiplex sequencing, is a common sequencing practice that uses barcodes to allow multiple samples to be pooled and sequenced simultaneously in a single run. While (de)multiplexing protocols have been developed for DNA-based nanopore sequencing, no protocols have been released for multiplexing dRNA-seq samples.



WarpDemuX model: (a) Generation of training data, (b) direct RNA sequencing, electric current measurement (c) Segmentation of adapter signal (d) Model training and classification of barcode fingerprints
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TECHNOLOGY DESCRIPTION

The method involves attaching a barcode adaptor to the molecule of interest. During analysis, an electrical signal derived from the barcode molecule is compared against known barcode signals. This process includes temporarily aligning the electrical signal related to the unknown barcode molecule and predicting the instance of the barcode molecule from the similarity between the aligned electrical signal and the known barcode signals stored in the data memory. The method uses dynamic time warping (DTW) to handle the temporal alignment of electrical signals, improving the accuracy of molecule identification. A signal processing unit and a computer program arranged for executing the above-mentioned methods is provided. This approach is particularly useful for applications in dRNA-seq, where it preserves native RNA modifications by avoiding the conversion to cDNA. However, the method can be adapted to sequence analysis of other molecules, like DNA or polypeptides.

COMMERCIAL OPPORTUNITY

The instance of the barcode molecule can be used for multiplexed sequencing and quantification of the amount of molecule attached to a specific barcode, for use in diagnostics or to control sequencing instruments for multiplexed sequencing. WarpDemuX is a promising solution in the dRNA-seq demultiplexing landscape. The technology is available for co-development and/or licensing.

DEVELOPMENT STATUS

The method has been validated in laboratory settings, demonstrating its efficacy in accurately identifying and quantifying molecules of interest. Optimizing the process for large-scale applications and integrating it with existing sequencing platforms is ongoing.

PATENT SITUATION

A PCT priority application was filed in April 2024 (PCT/EP2024/061629). A second PCT application was filed in April 2025 (PCT/EP2025/061358)

FURTHER READING

van der Toorn W et al., Demultiplexing and barcode-specific adaptive sampling for nanopore direct RNA sequencing. Nat Commun. 2025 Apr 21;16(1):3742. doi: 10.1038/s41467-025-59102-9. van der Toorn et al. *WarpDemuX-tRNA: barcode multiplexing for nanopore tRNA sequencing.* Nucleic Acids Res. 2025;53(17):gkaf873. doi: 10.1093/nar/gkaf873.

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