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Efficient workflow for demultiplexing and mapping of direct tRNA sequencing

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Accurate classification and analysis of transfer RNAs (tRNAs) using Nanopore sequencing remain challenging due to the high similarity among tRNAs and basecalling errors induced by RNA modifications. We have created a robust workflow for direct tRNA Nanopore sequencing that addresses these challenges through optimized barcoding, demultiplexing, and read mapping strategies. We used barcoding sequences within RNA adapters for precise classification of reads post-basecalling with the Dorado demultiplexing tool We optimized the demultiplexing parameters to ensure high accuracy while minimizing read loss during the demultiplexing process. Additionally, mapping strategies were refined to minimize multimapping events arising from tRNA sequence similarity and modification-related errors, ensuring accurate downstream analysis. This workflow enables the accurate classification of tRNA Nanopore reads across six barcodes, offering a reliable and efficient approach for tRNA sequencing applications. By mitigating common sources of error and optimizing critical processing steps, our work provides a significant advancement in the field of tRNA Nanopore sequencing and its application in understanding tRNA biology.

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