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Comprehensive single-molecule resolution discovery of RNA modification sites in the human transcriptome

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RNA modifications (RMs) are critical for diverse biological processes, but the lack of accurate, quantitative detection methods has limited their study. A large-scale and high-quality training dataset is an essential component for accurate deep learning, but such dataset has been absent for RM detection, resulting in low accuracies. We developed DeepRM (Deep learning for RNA Modification), a sophisticated deep learning framework powered by Nanopore sequencing. DeepRM dataset is a massive-scale, three orders of magnitude larger than the comparable previous ones, and unprecedentedly high-quality dataset that closely mirrors endogenous transcript environments. Accordingly, DeepRM detects RM sites and measures their modification stoichiometries with a near-perfect accuracy. Using DeepRM, we constructed a comprehensive, human m^6A atlas at a single-molecule resolution that reveals a large number of previously underappreciated non-canonical m^6A sites and differentially modified transcripts, highlighting the complexity and dynamic nature of the human epitranscriptome. DeepRM is freely available, providing a unique, powerful opportunity for understanding the biological functions of RMs. DeepRM can also be expanded to various other RMs and organisms, potentially becoming a future standard for investigating the epitranscriptome.