

Reverse Transcriptase Fingerprint Method for RNA Caps Detection

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For decades, the N7-methylguanosine cap was believed to be the sole 5′ cap structure in viral and eukaryotic mRNAs, playing a central role in regulating RNA biogenesis, stability, translation, and decay. However, the discovery of non-canonical RNA caps derived from metabolites and cofactors — such as NAD, FAD, CoA, UDP-glucose, UDP−N-acetylglucosamine, and dinucleoside polyphosphates — has expanded the known repertoire of 5′ RNA modifications. Present across all domains of life, these caps, together with canonical 5′ caps, can influence RNA metabolism, including stability, translation initiation, and cellular stress responses, highlighting the need for selective profiling techniques.

Reverse transcription of RNA templates containing modifications generates cDNA that encodes information about these modifications through misincorporation, arrest, or nucleotide skipping events. The cumulative patterns across multiple reverse transcriptases (RTs) produce a distinctive RT fingerprint specific to a given modification. Recent advances have employed machine learning to analyze RT signatures and detect internal RNA modifications, such as m^6G (6-methylguanosine). Building on this concept, we developed an RT fingerprint method for 5′ RNA cap detection, coupled with Nanopore sequencing, to study RNAs with diverse 5′ caps. Using model RNAs with various caps (e.g., m^7Gp_3A, TMG, NAD, Ap_4A) as substrates, we employed a combination of commercially available RTs to prepare cDNA-seq libraries optimized for Oxford Nanopore Technology. A mathematical algorithm was designed to analyze RNA caps in human cell lines based on their characteristic RT patterns.

Analysis of these model systems revealed distinct RT behaviors for different caps, producing unique signatures that can be systematically used to profile RNA caps across diverse human cell lines. Leveraging this framework, we are now studying the distinct capping status of canonical U1 snRNA – a key spliceosomal RNA – and its isoforms in human tumor and non-tumor cell lines.