Nanopore based native RNA sequencing of human transcriptomes reveals complexity of mRNA modifications and crosstalk between RNA regulatory features

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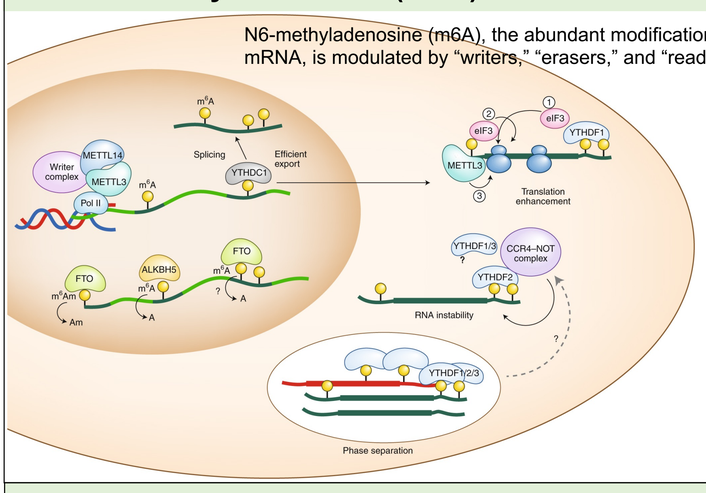
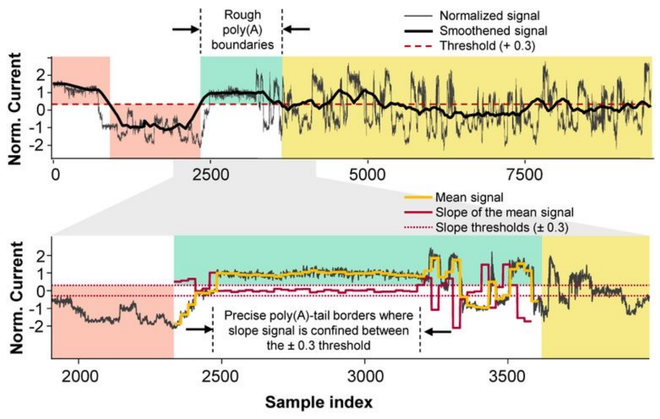
N6-methyladenosine (m6A) RNA modification

N6-methyladenosine (m6A), the abundant modification on mRNA, is modulated by "writers," "erasers," and "readers"



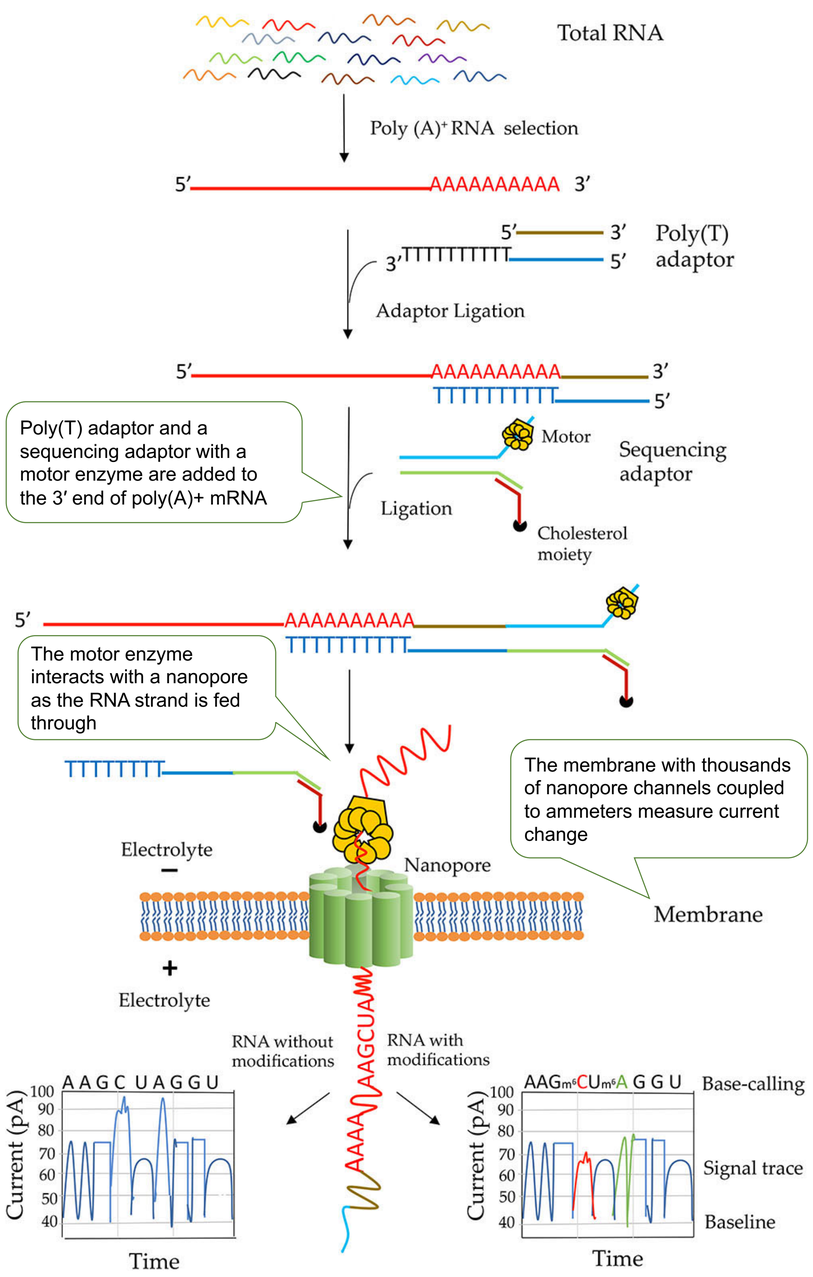
Poly A tail Length detection with Nanopore Direct RNA seq

- The Motor protein (red) is attached to the native RNA molecule (yellow) at the 3'-end by T4 DNA ligation via a double-stranded adapter (light red) with oligo-T overhang
- The motor protein thus feeds the RNA strand to the pore from 3' to 5'



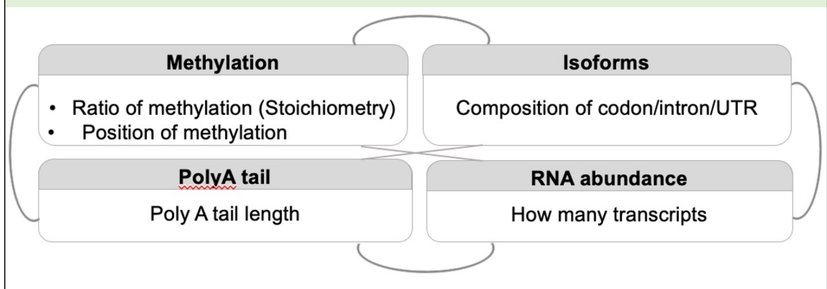
- Representative normalized signal data from eGFP-RNA sequencing
- Red background indicates ONT adapter signal, green background represents rough borders of poly(A) signal, yellow background highlights signal from RNA sequence

Profiling m6A methylation via Nanoporedirect RNA sequencing



- A voltage across the membrane is applied
- RNA moves through the nanopore nucleotide bases cause a characteristic change in current through the pore
- The current output is then used in base-calling

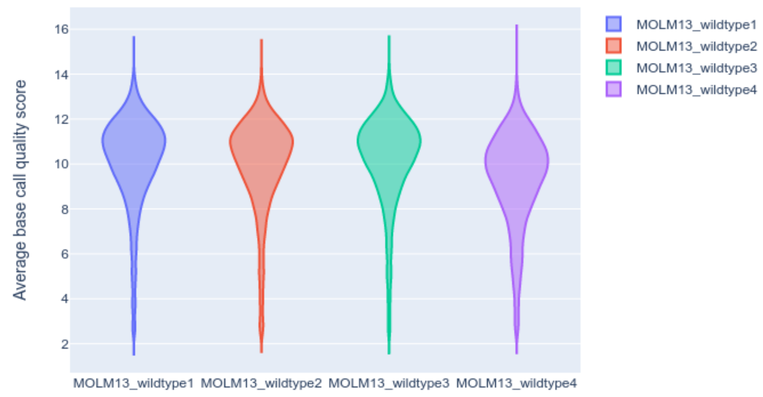
Research Direction



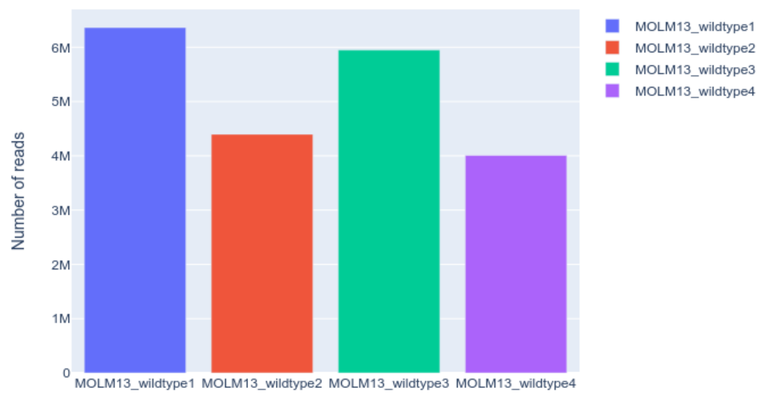
- High resolution landscape of N6-methyladenosine (m6A)
- Crosstalk of N6-methyladenosine (m6A) with other post-transcriptional regulations

Nanopore Direct RNA Seq Quality Control

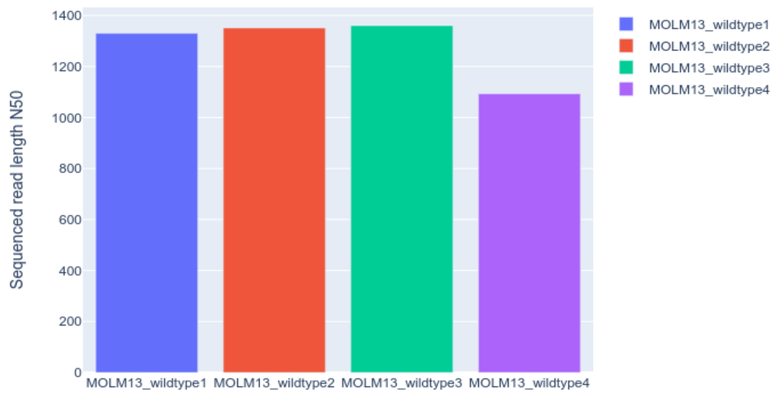
Comparing average base call quality score



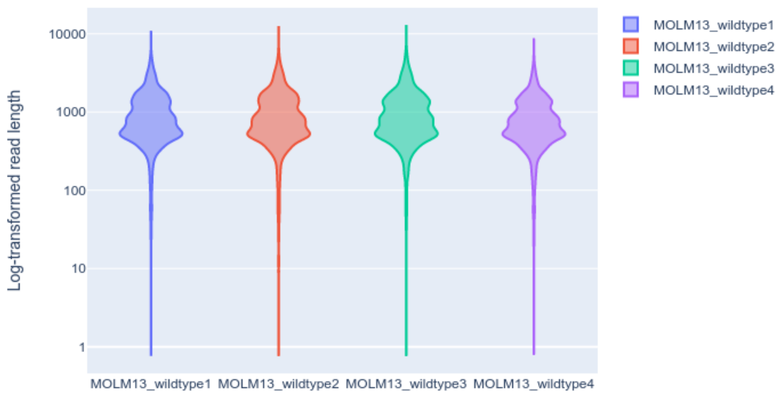
Comparing number of reads



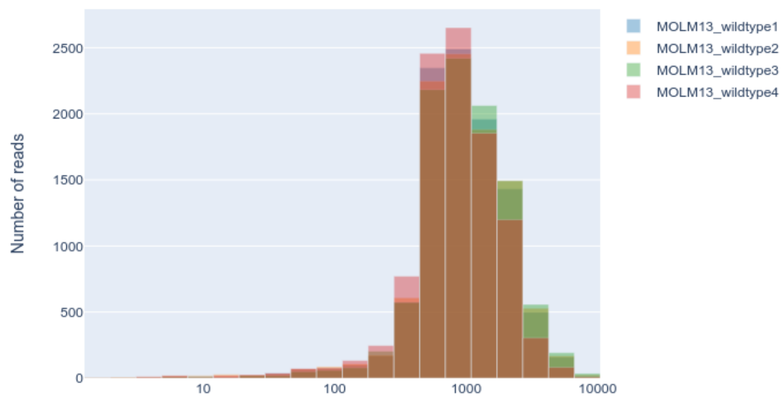
Comparing read length N50



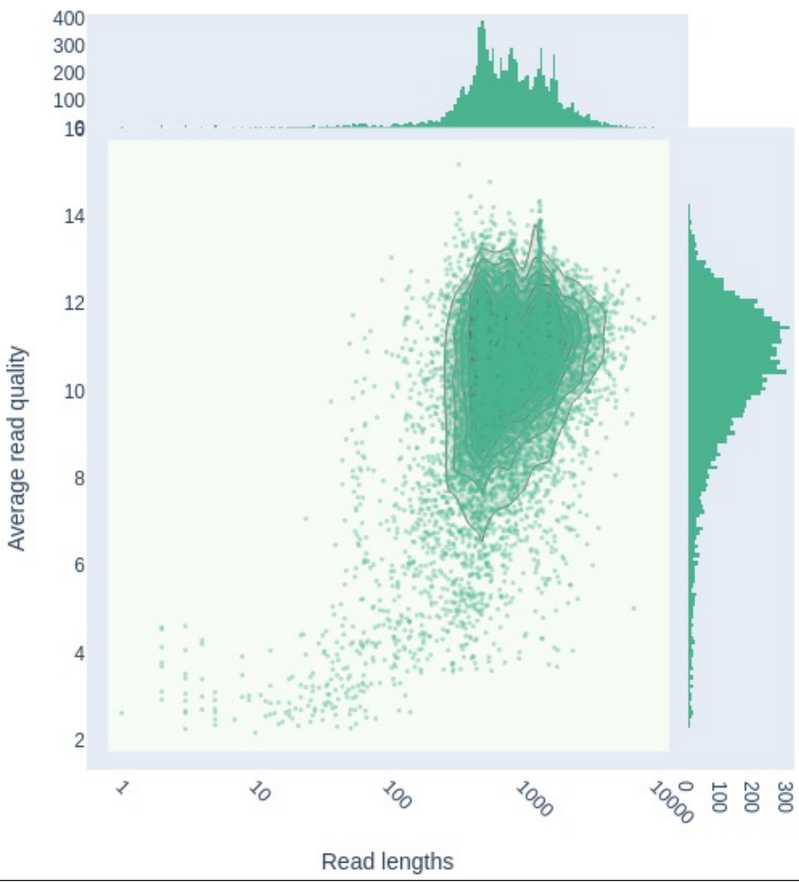
Comparing log transformed read length



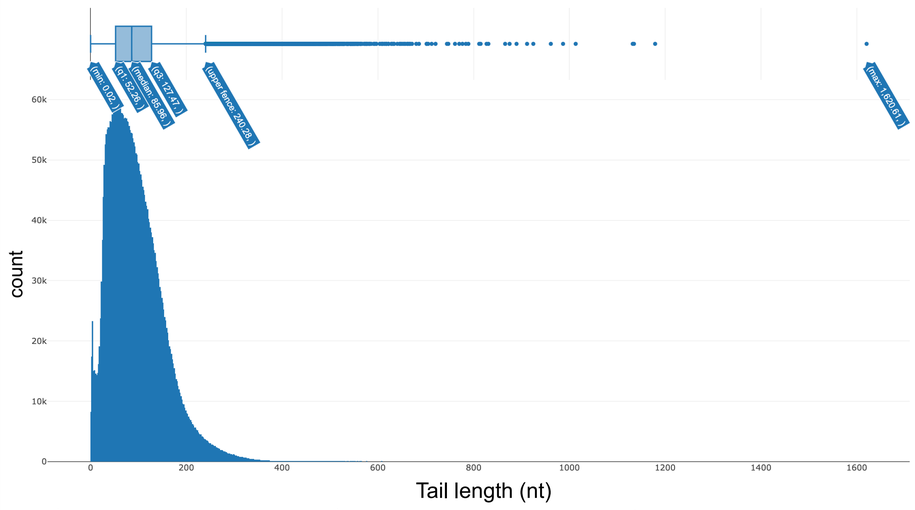
Histogram of log transformed read lengths

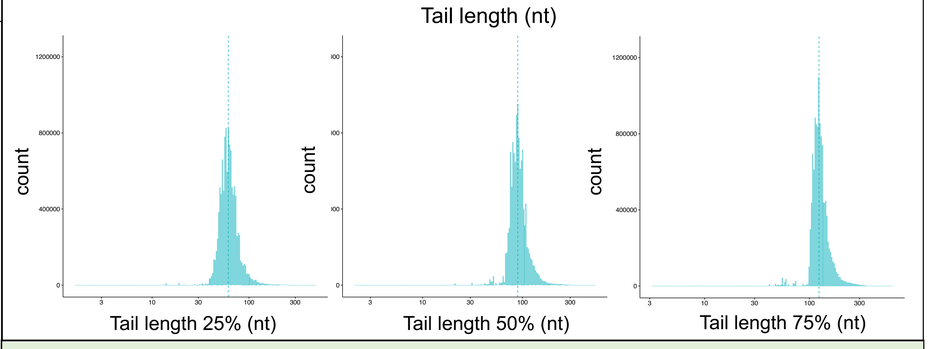


Read lengths vs Average read quality KDE plot

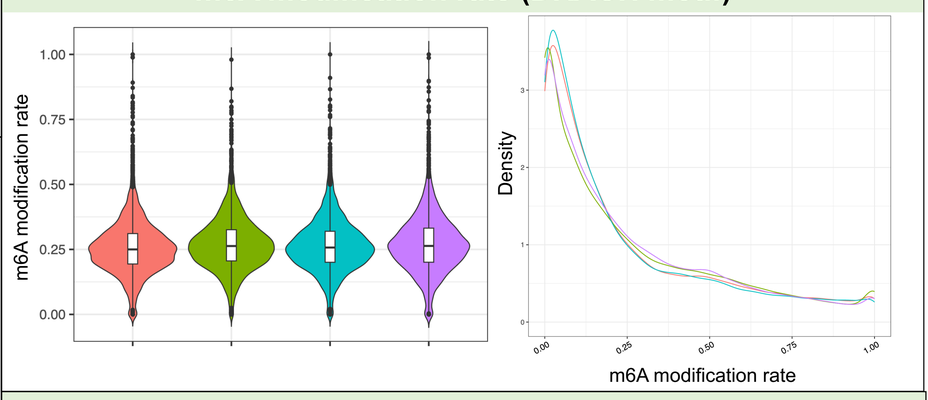


Poly A tail length Distribution

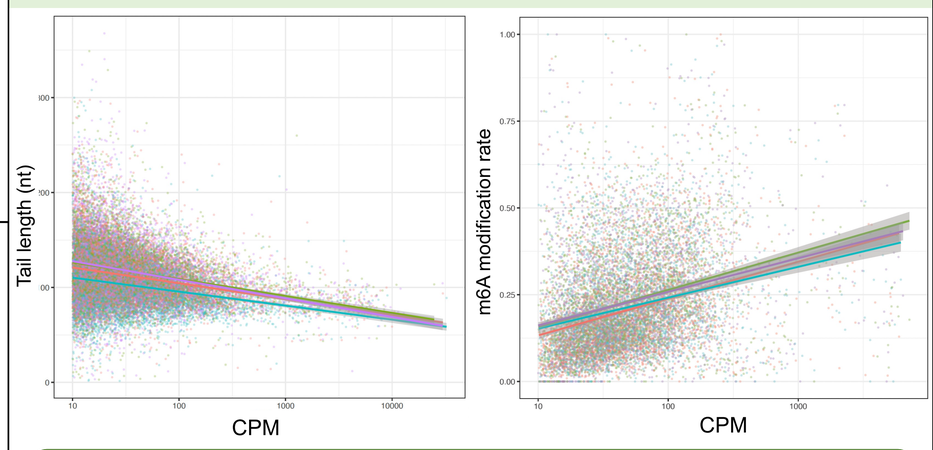




m6A modification rate (DRACH motif)

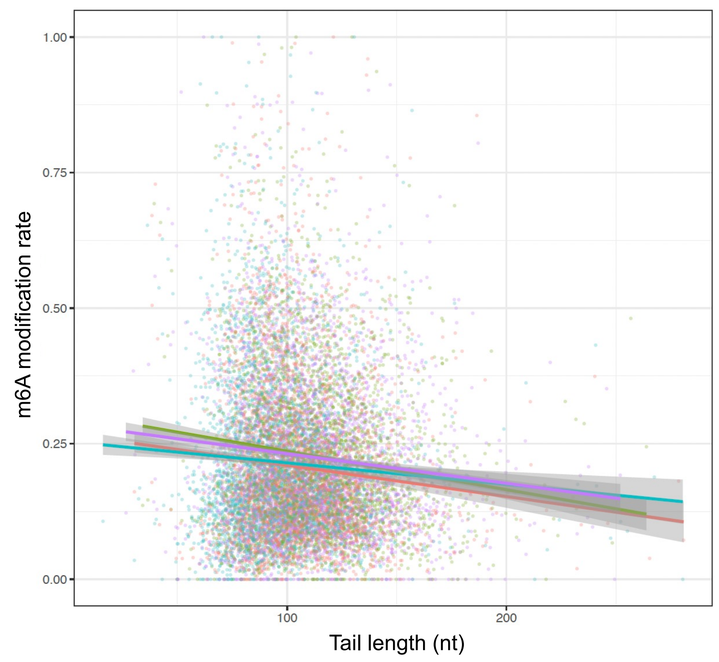


Crosstalk among *m6A modification rate*, *Transcript expression* and *Poly A tail length*



- When transcript expression level is high, poly A tail lengths tend to be shorter
- When transcript expression level is low, poly A tail lengths tend to be longer
- On global level, correlation between transcript expression and m6A modification rate is weak

Crosstalk between *m6A modification rate* and *Poly A tail length*



- Wide range of m6A modification rate is observed around tail length 100 (nt)
- Around tail length 100 (nt) m6A modification rate tend to be less than 0.5
- Tail lengths either very long or short tend to have low m6A modification rate

Future Direction

- Oxford Nanopore Technology's direct RNA sequencing offers a promising way to uncover important insights into post-transcriptional regulation biology.
- Despite recent advances, we still lack a comprehensive understanding of how RNA methylation dynamics intersect with other gene expression regulatory mechanisms.
- We plan to analyze different post-transcriptional regulations in detail, as no clear relationship has been found between m6A modification rates and poly A tail lengths on a global scale.
- By examining variations in tail length (long, medium, and short) and their correlation with other regulatory mechanisms, we aim to uncover more nuanced insights into post-transcriptional regulation.
- Investigating the reciprocal relationship between m6A modification rates and poly A tail lengths promises to shed new light on the post-transcriptional modification landscape.
- To fill a gap in our understanding, we aim to study how the location of m6A methylation affects other post-transcriptional regulations.