Detecting mRNA modifications using single molecule nanopore sequencing

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NANOPORE HUMAN RNA CONSORTIUM

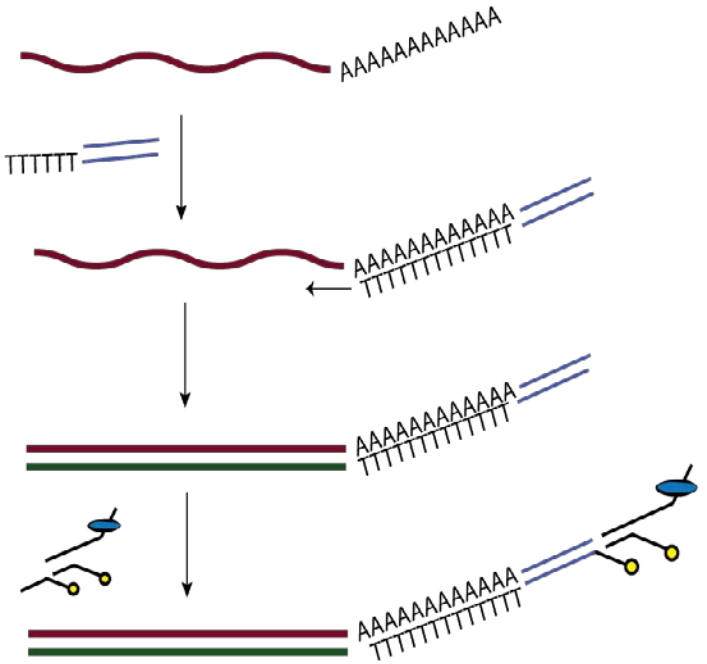
Native RNA sequencing opens new frontiers for long read RNA-seq analysis

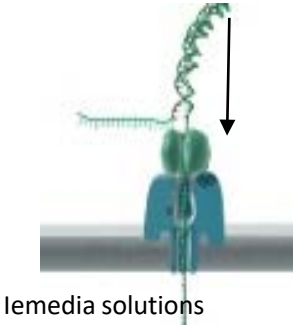
PolyA+ RNA captured

Splint poly-T adapter ligation

Reverse transcription (optional)

Sequencing adapter ligation



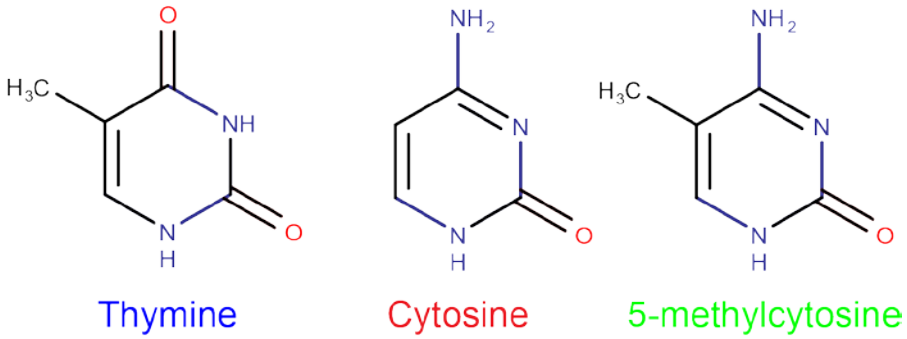


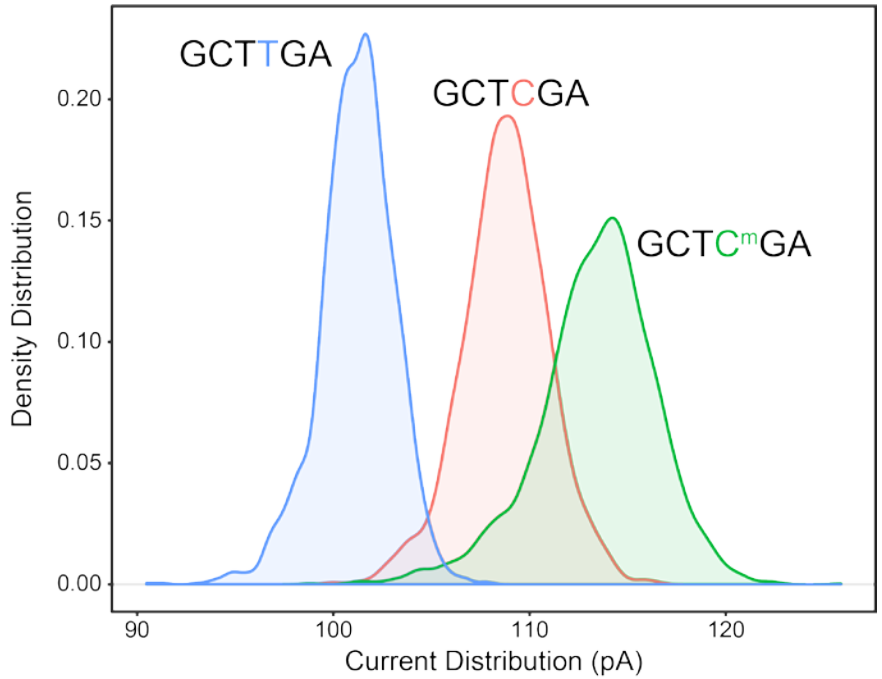
Iemedia solutions

Raw Current Signal

Current Signal K-mers Sequence

Nanopore current is affected by modifications

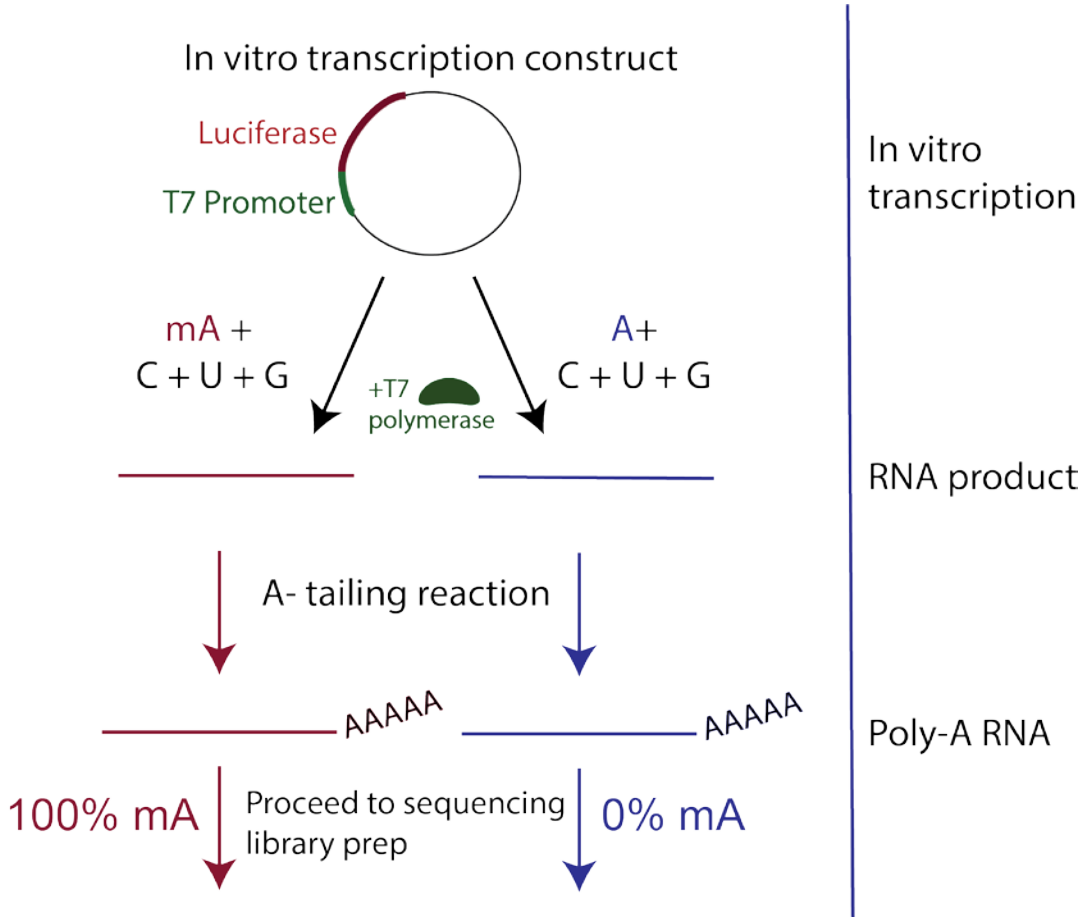




- 5mC detected and modeled in DNA
- Current shifts occur when a non-canonical nucleotide traverses the pore, as we know from DNA studies
- These shifts can be modeled and used to call modifications

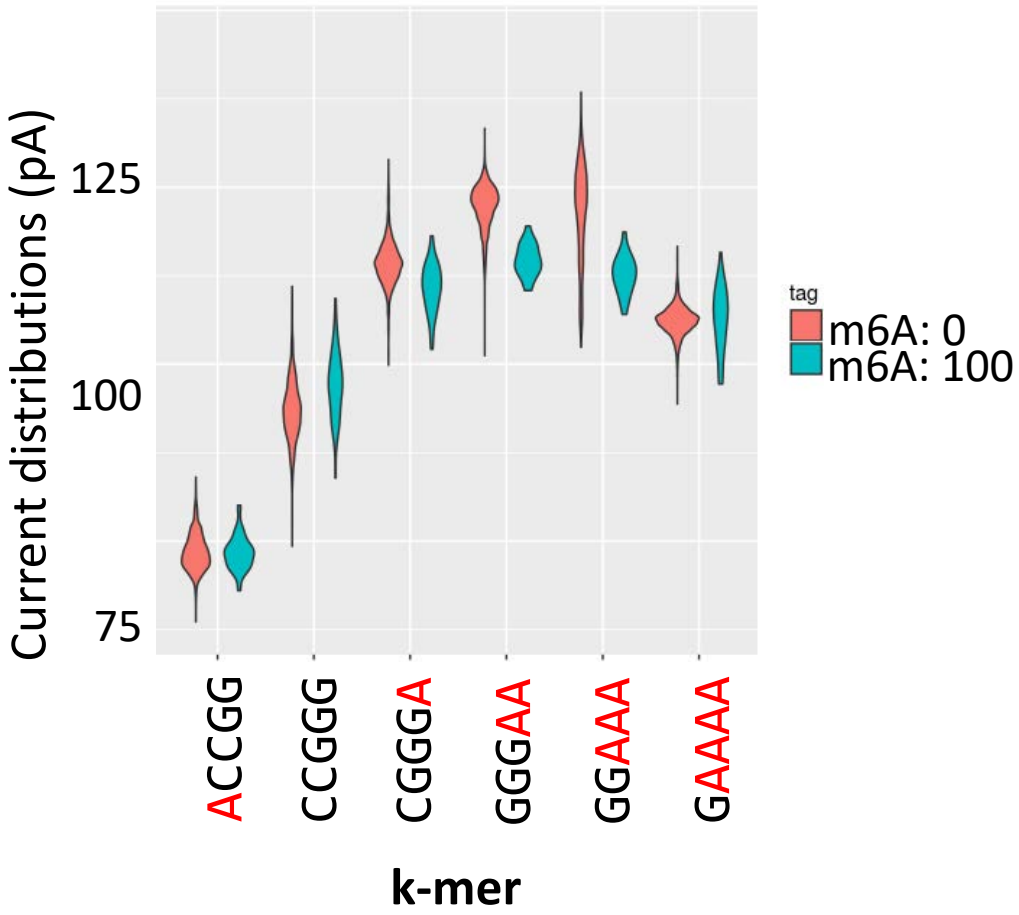
Simpson et. al *Nature* Methods 2017

Training RNA basecaller to recognize modified sites requires truth sets: **modIVT**



- IVT based RNA synthesis allows incorporation of labeled nucleotides
- All or none reaction right now, T7 has a strong preference for the unmodified nucleotides, making mixtures hard

Training RNA basecaller to recognize modified sites requires truth sets: **modIVT**

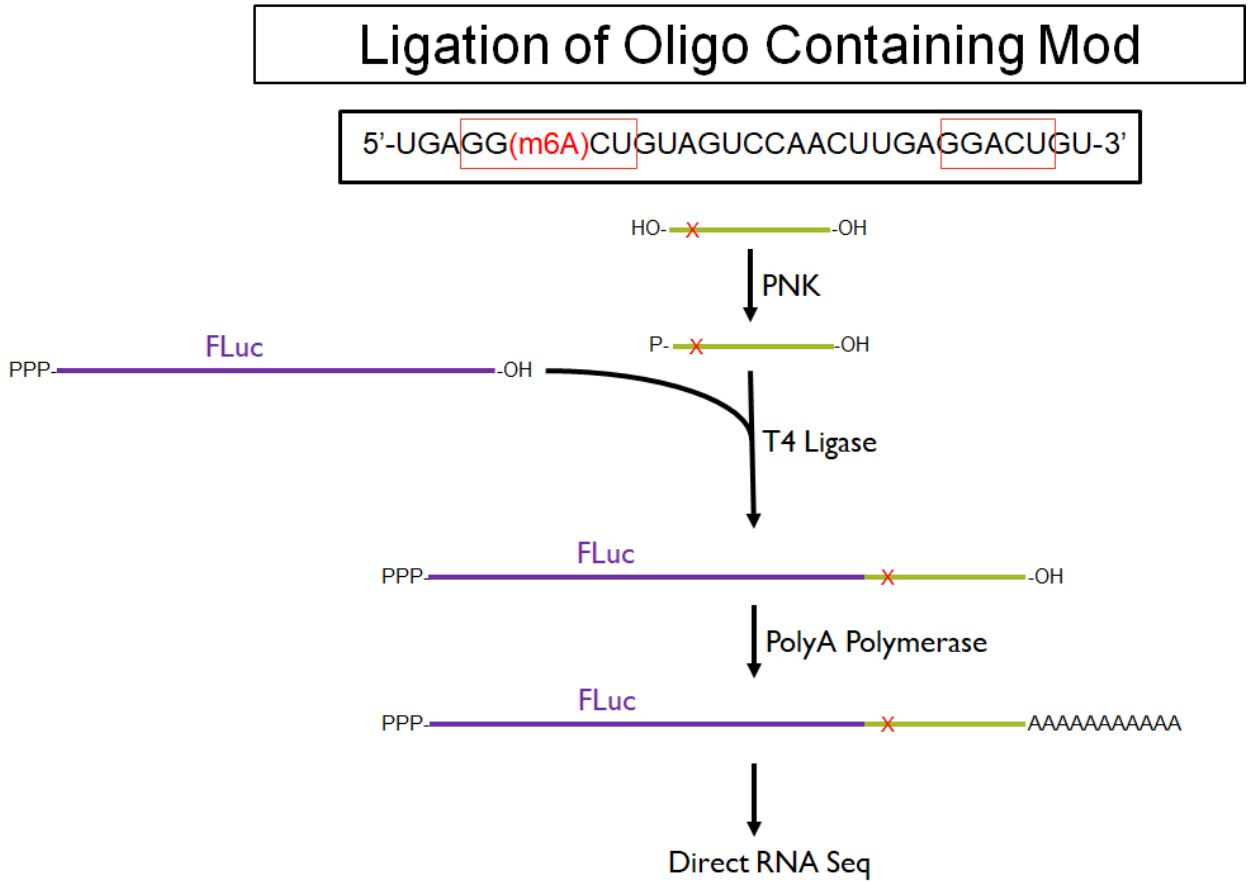


- From luciferase we can already see strong signal depending on context
- Using nanopolish eventalign, we can extract the distribution of current values along the RNA strand

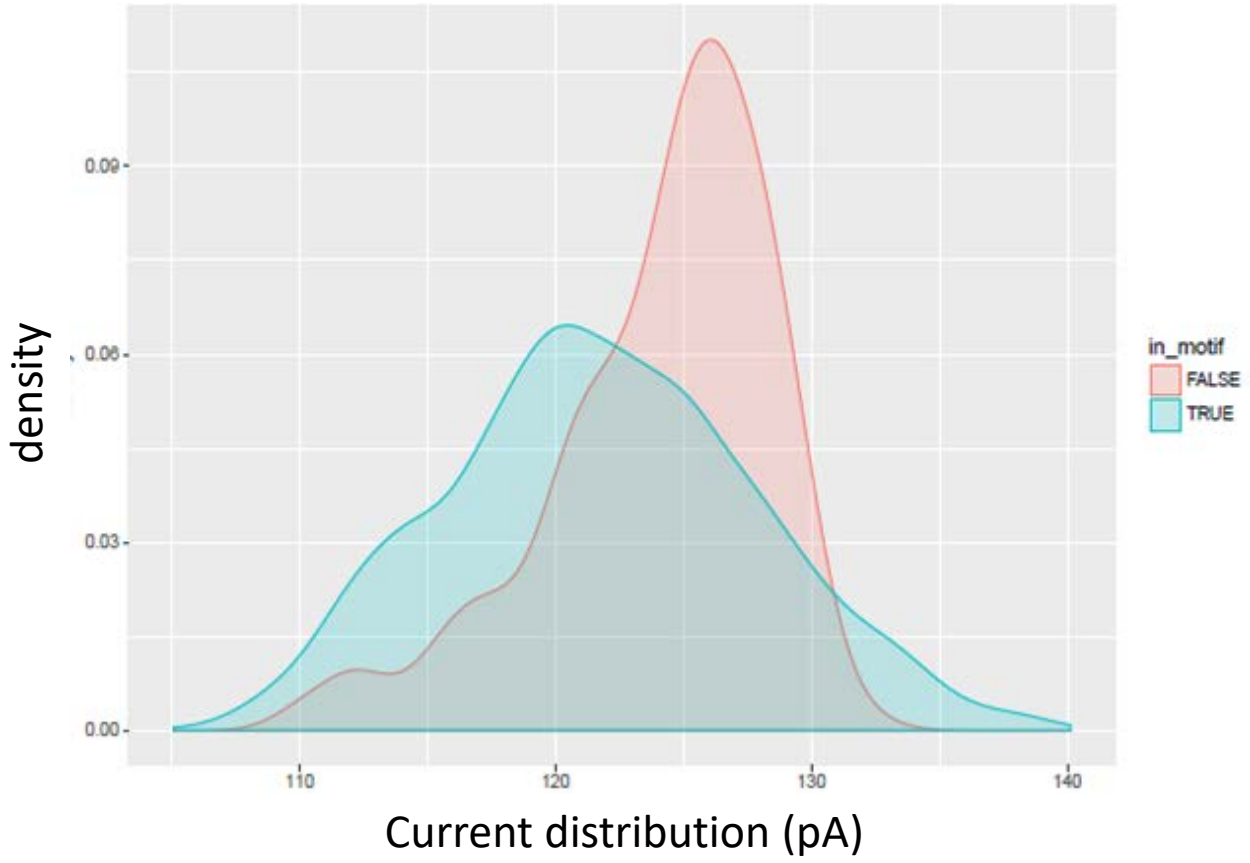
Nanopolish eventalign: https://github.com/jts/nanopolish

Training RNA basecaller to recognize modified sites requires truth sets: **oligo ligation**

- Short oligo (easier/cheaper to synthesize) ligated to handle (to give needed length for sequencing)
- Oligo containing modified and unmodified METTL3 motif



Training RNA basecaller to recognize modified sites requires truth sets: **oligo ligation**

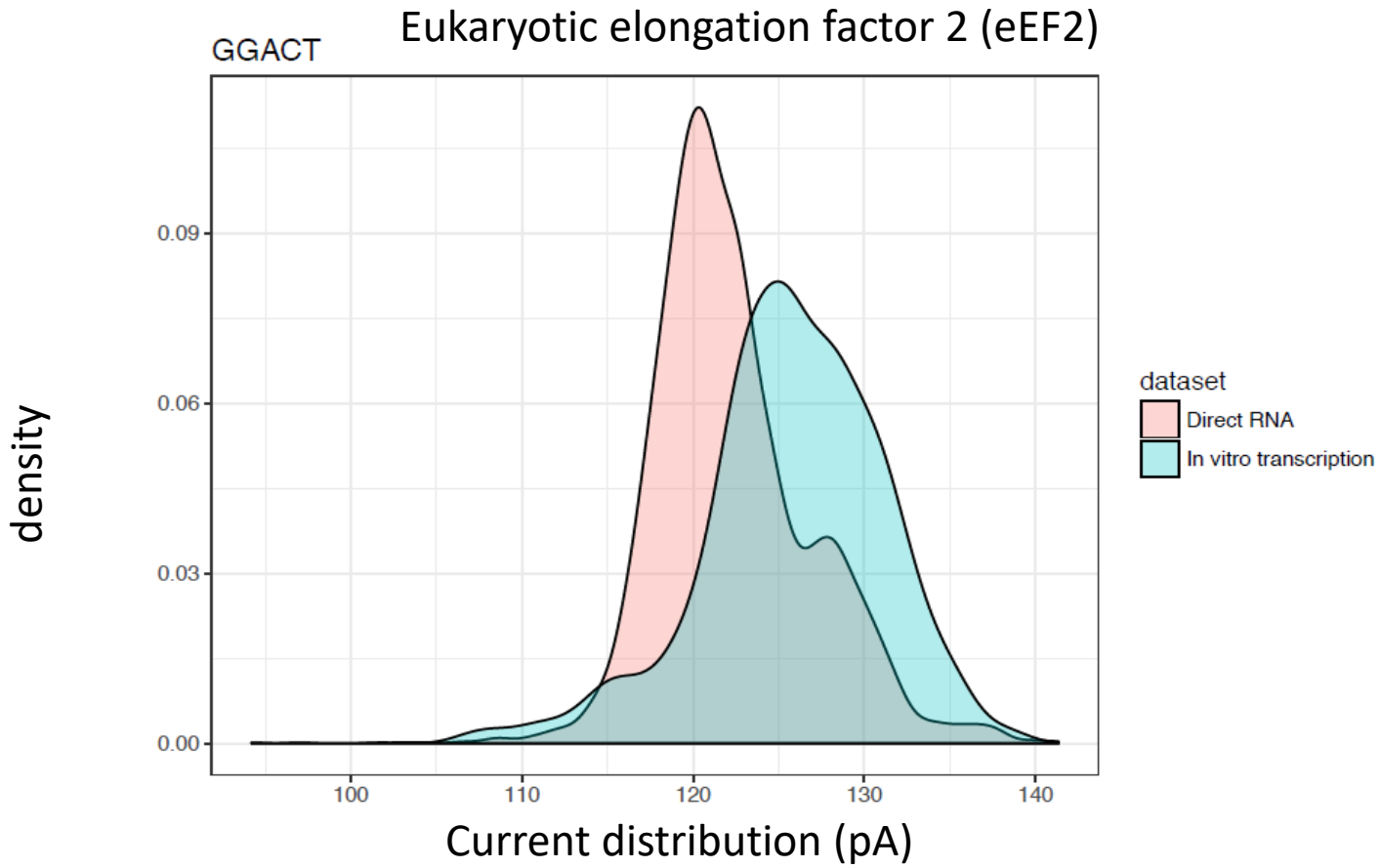


- Similar current distribution shift observed in modification–containing kmers

Nanopolish eventalign: https://github.com/jts/nanopolish

Independently identified m6A peaks in GM12878 reveal hints of mod signature

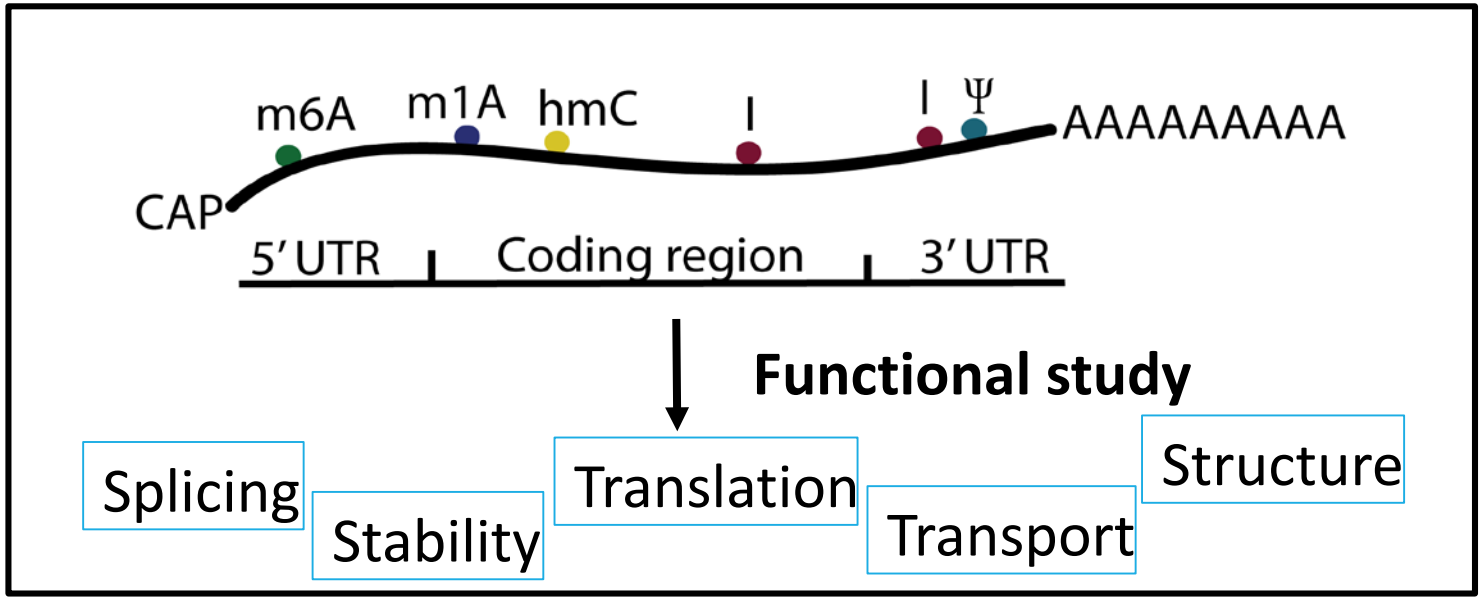
- Direct RNA data shown is publically available! https://github.com/nanopore-wgs-consortium/NA12878/blob/master/RNA.md
- Shift in current distribution at peak site in Eukaryotic elongation factor 2 (eEF2)



Target gene found using m6A immunoprecipitation peak datasets from: Molinie et al 2016. Nat Methods. Roost et al 2015. JACS.

Future directions for RNA modification detection

- Global m6A calling
- Simultaneous detection of multiple mods
- Investigation of relationship between modifications, splice variation and poly-A tail lengths



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