LC 2019 LONDON CALLING

Signal level RNA modifications detection in eukaryotic ncRNAs

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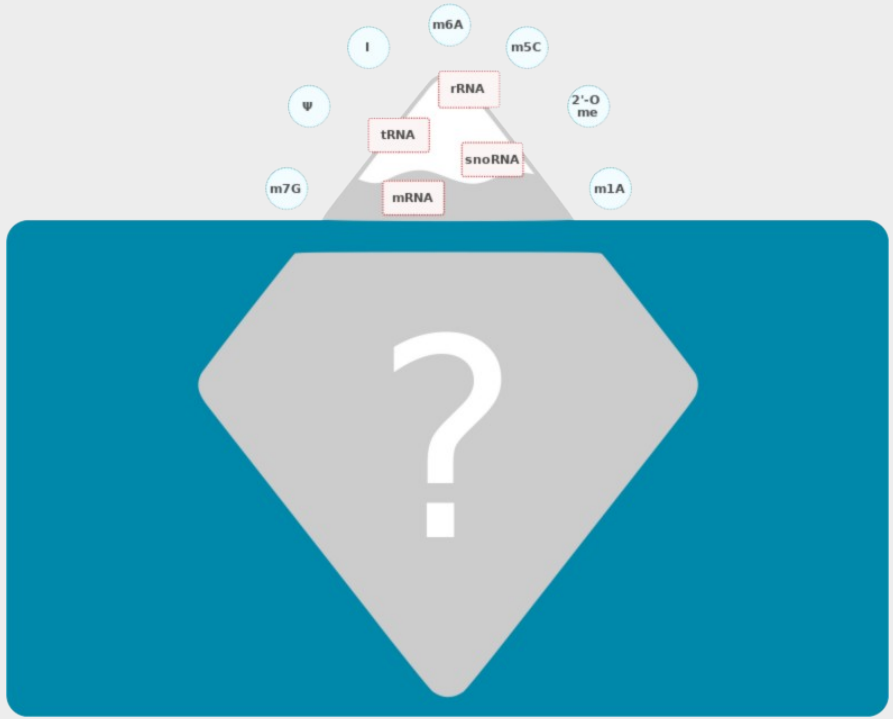
Organised by Oxford NANOPORE Technologies

The zoo of RNA post-transcriptional modifications

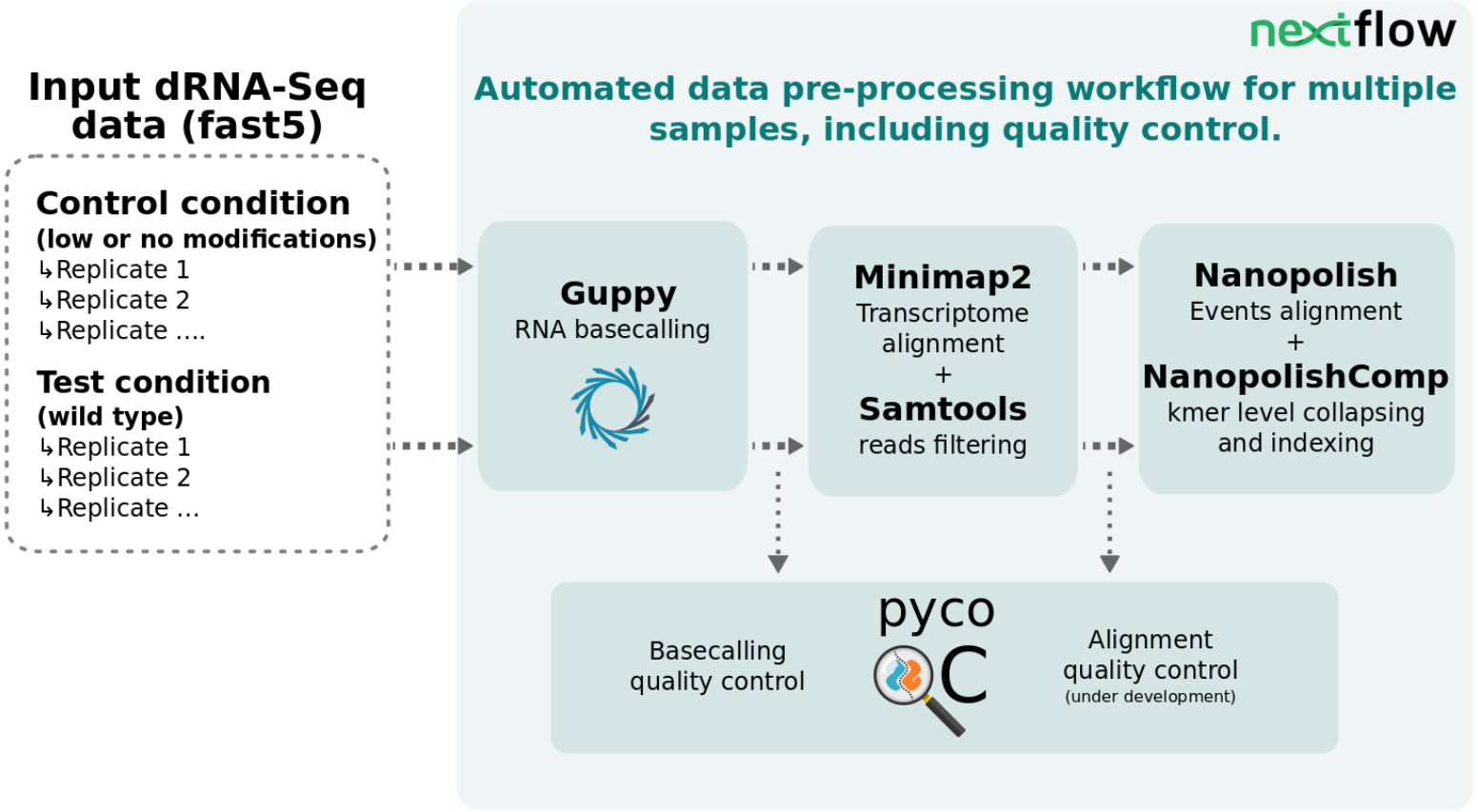
- Highly conserved feature found in archaea, bacteria and eukarya
- Impact on RNA structure and interaction properties
- More than 150 known RNA modifications



Meet Nanocompore

- Identifies signal-level differences between conditions (e.g. WT vs IVT/KD/KO)
- Based on Nanopolish resquiggling
- Robust and flexible statistical framework
- Takes into account biological variability
- Allows for complex statistical designs (e.g. multi-factor designs, batch effects, etc.)

The Nanocompore analysis workflow



The Nanocompore analysis workflow

Compares 2 ONT dRNA sequencing datasets from different experimental conditions expected to have a significant impact on the epitranscriptome. Replicates aware framework with continuous integration testing.

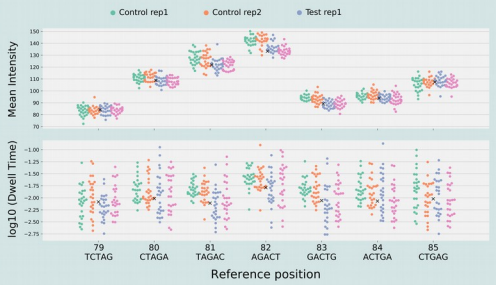
Whitelist

Select reads mapped on transcripts with sufficient coverage

Position-wise data aggregation

Intensity and dwell time values are collected at transcript position level. The replicate structure is conserved

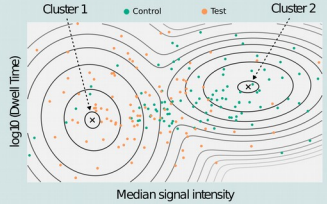
Kmer phased signal intensity and dwell time



Statistical pairwise comparison

- Mann-Whitney, Kolmogorov-Smirnov or T test
- Gaussian mixture model clustering + Anova/logit
- Complex statistical design (multi-factor, batch effects ...)

1 2D GMM clustering



2 Compare GMM clusters against condition labels

- ANOVA if replicates
  OR
- Logistic regression

3 Multiple tests correction

- Benjamini/Hochberg

4 Results saving and analysis

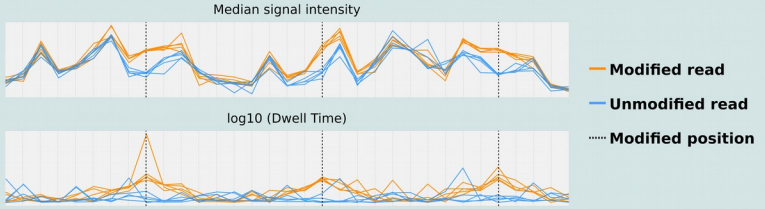
- Save data in lightweight GDBM database
- Extensive plotting API to explore data

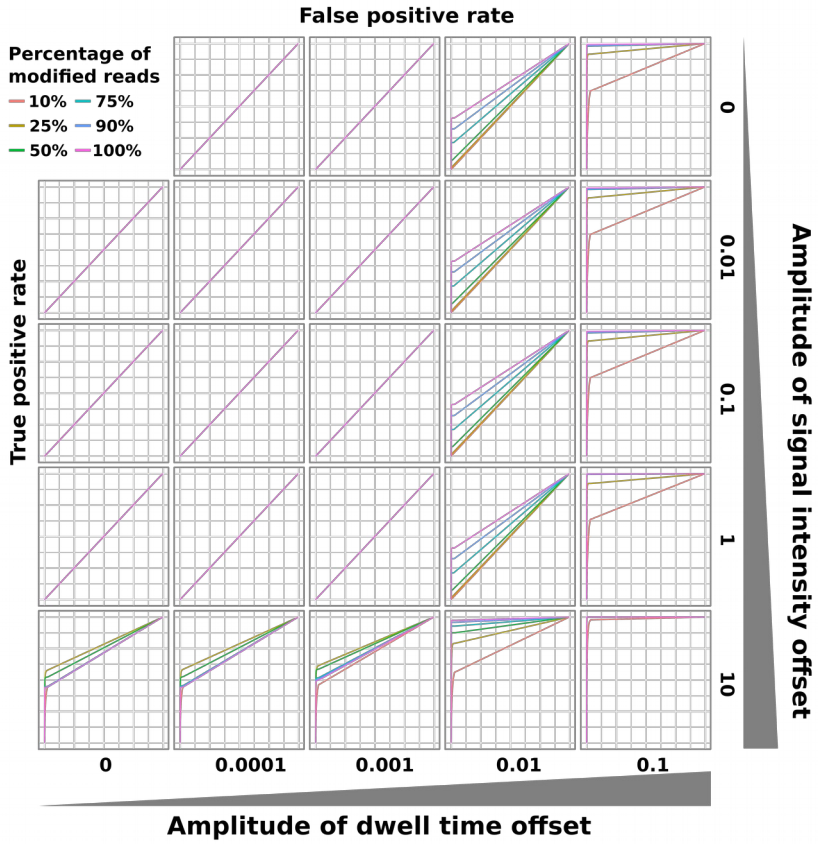
Benchmarks on *in silico* generated data

A **kmers model**
From an in vitro generated non-modified RNA dataset, find the best distribution to fit each kmers for both median intensities (*Logistic*) and dwell time (*Wald*)

B **Artificial reference**
Set of 2000 semi-random references 500 bases long. Covers all 5-mers (median: 970 times per kmer) and 99.67% of the possible 9-mers (median: 4 times per kmer)

C ***In silico* modifications simulation**
100 reads per reference using the probability density functions of the kmer models. Simulation of RNA modification effect on signal by offseting the model density function.

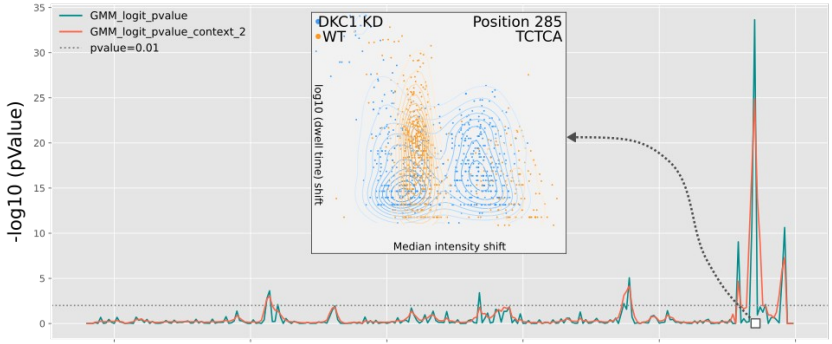




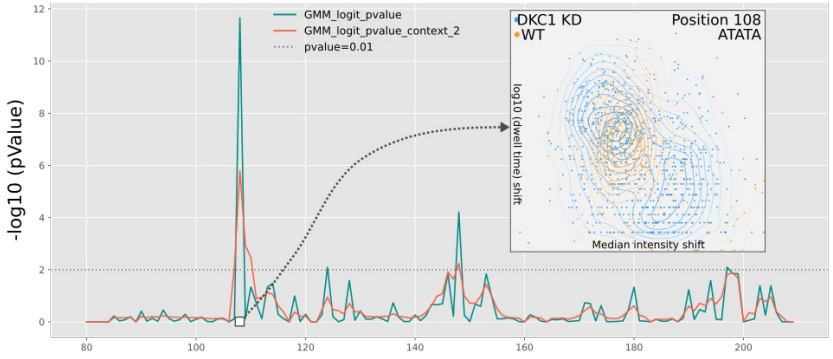
Use-case 1: RNA modifications in targeted human ncRNAs

Pseudouridine

RMRP (ENST00000602361) / MOLM-13 cells WT vs DKC1 KD (ψ)

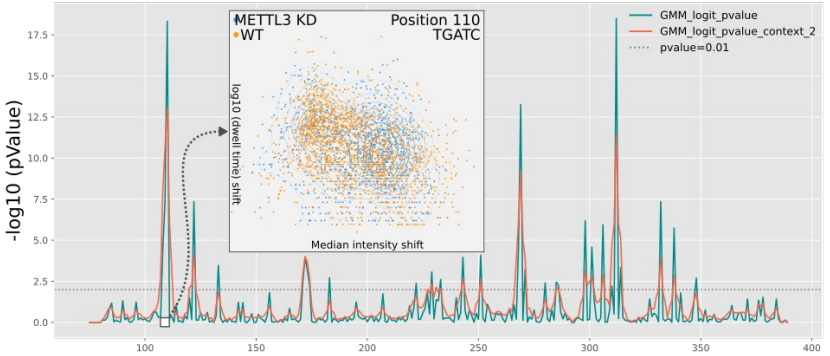


RNU2-1 (ENST00000618664) / MOLM-13 cells WT vs DKC1 KD (ψ)

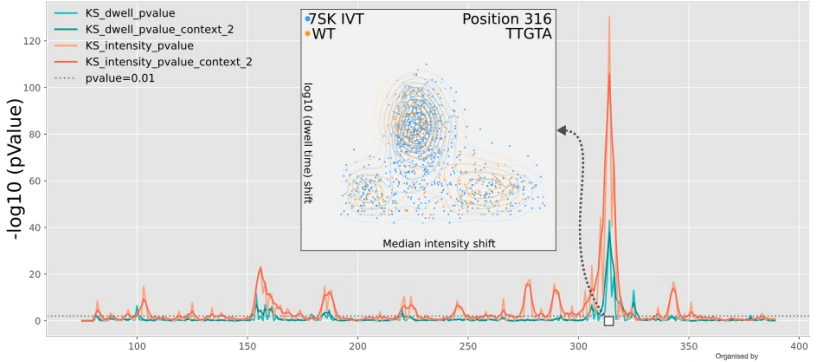


N6-methyl-adenosine

RN7SK (ENST00000636484) / MOLM-13 cells WT vs METTL3 KD (m6A)

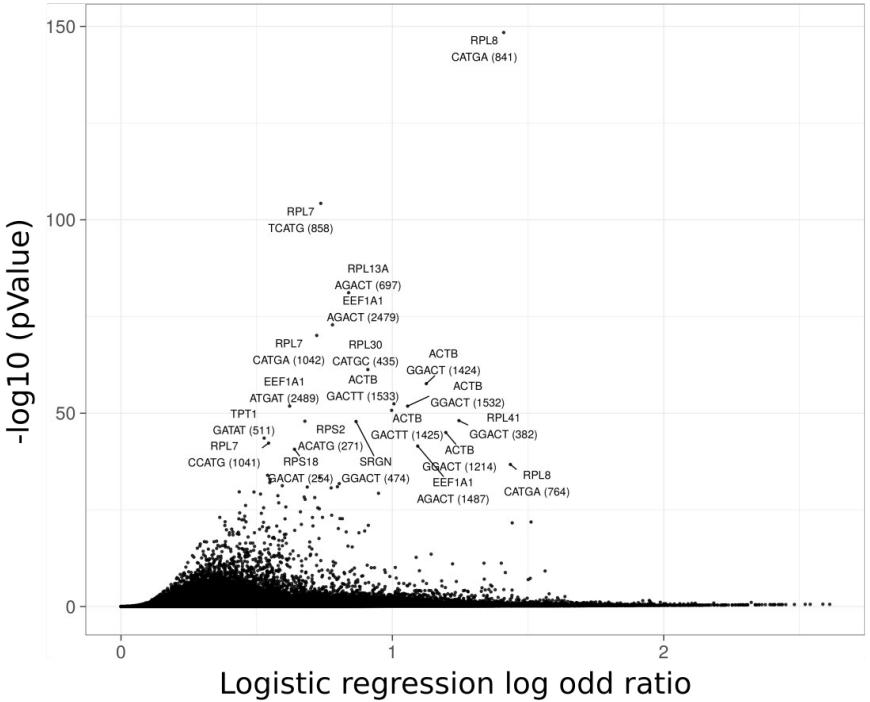


RN7SK (ENST00000636484) / MOLM-13 cells WT vs IVT 7SK (all mods)



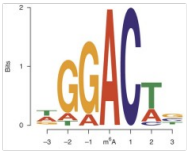
Use-case 2: m6A in a human polyA transcriptome

Top candidate kmer sites found by Nanocompore



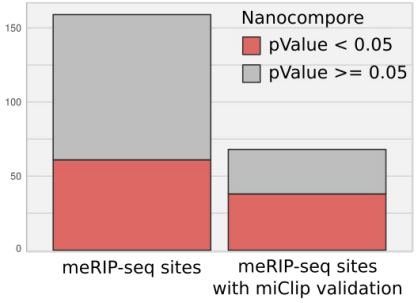
De novo motif enrichment analysis in top 100 hits

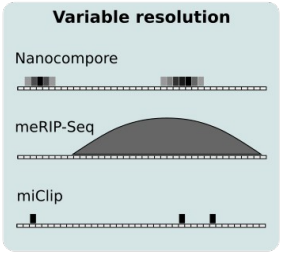
Rank	Motif	P-value	log P-pvalue	% of Targets
1		1e-2	-5.971e+00	22.45%
2		1e0	-1.172e-01	6.12%



Dominissini et al.
Nat Prot vol 8, (2013)

Orthogonal METTL3-dependent m6A mapping methods





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