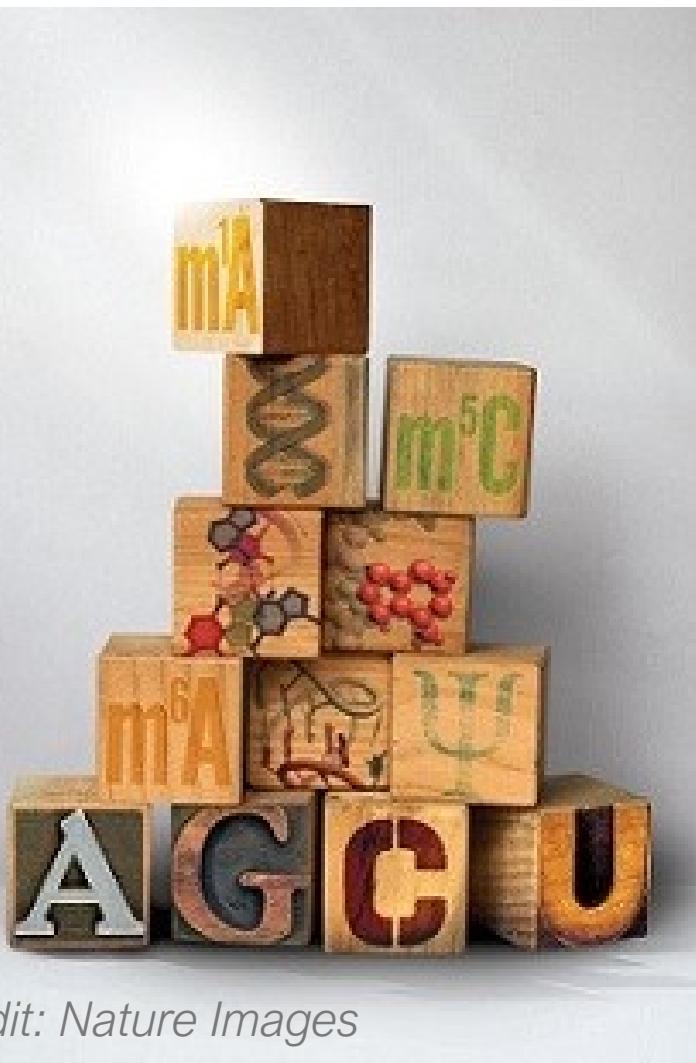


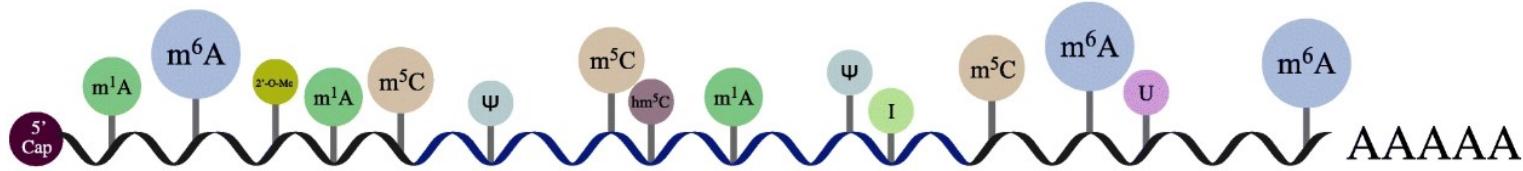
Decoding the epitranscriptome at single molecule resolution



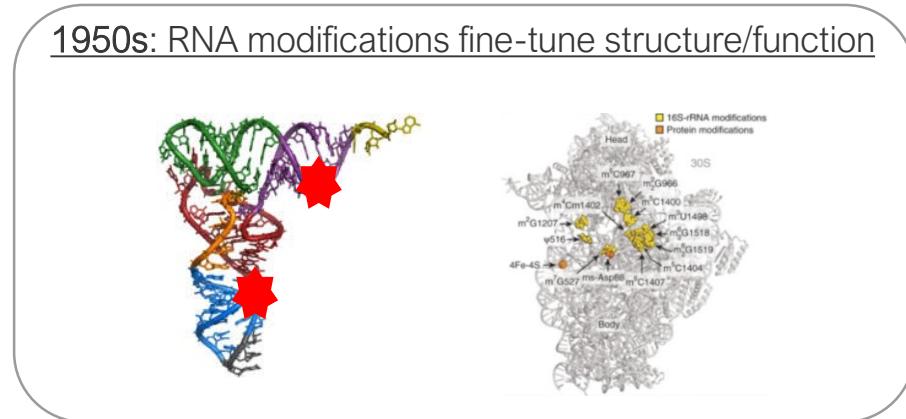
Ivan Milenkovic

Epitranscriptomics & RNA Dynamics
Center for Genomic Regulation (CRG)
Barcelona, Spain

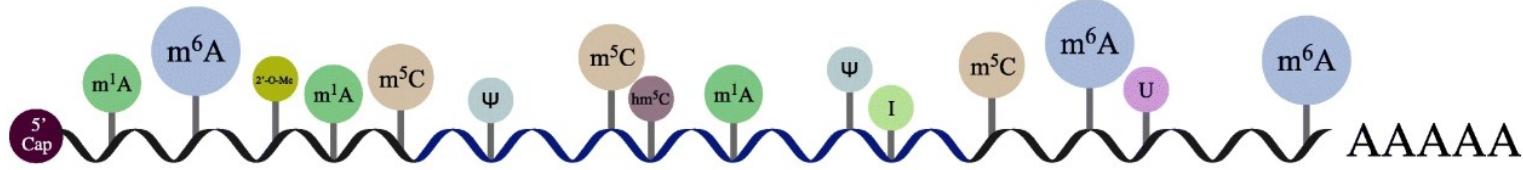
The epitranscriptome



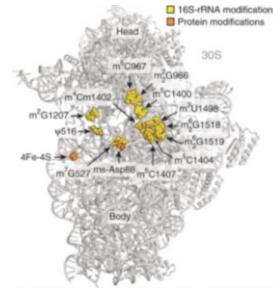
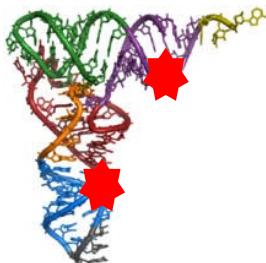
1950s: RNA modifications fine-tune structure/function



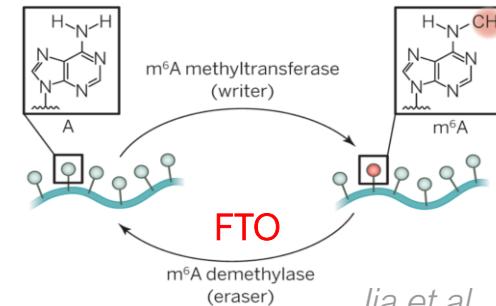
The epitranscriptome



1950s: RNA modifications fine-tune structure/function



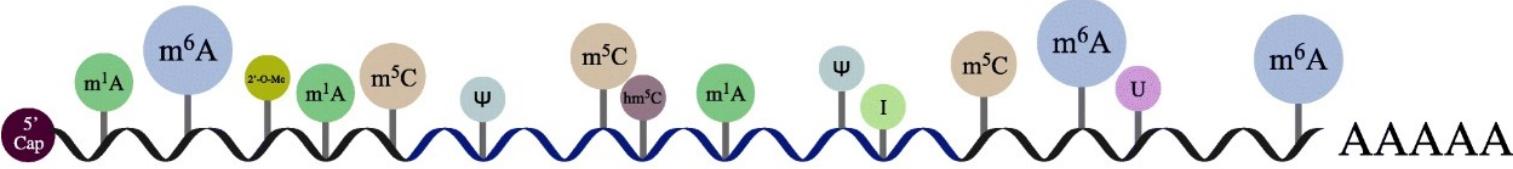
2011: RNA modifications are reversible!



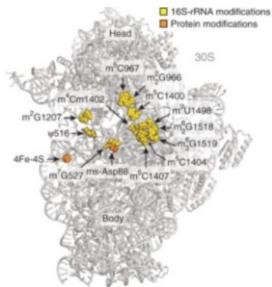
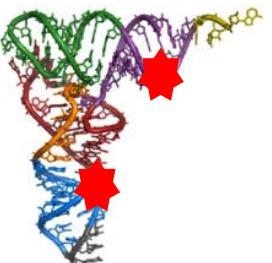
→ Function!

Jia et al., Nat Chem Biol 2011

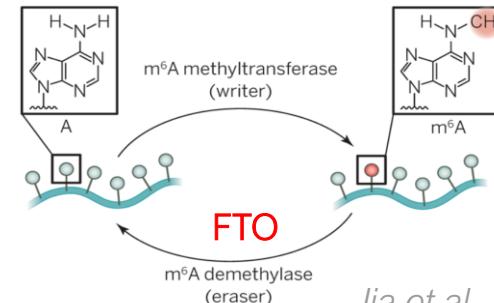
The epitranscriptome



1950s: RNA modifications fine-tune structure/function



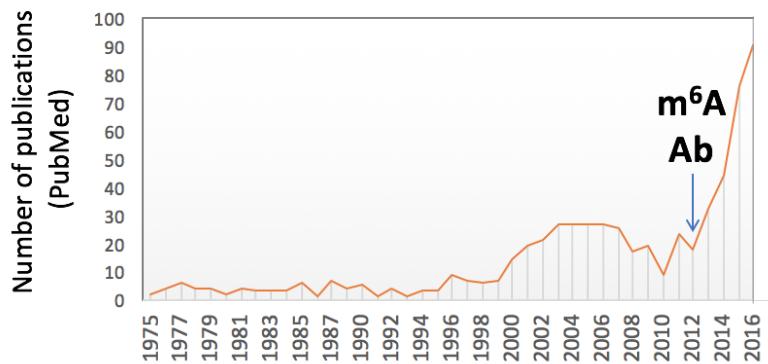
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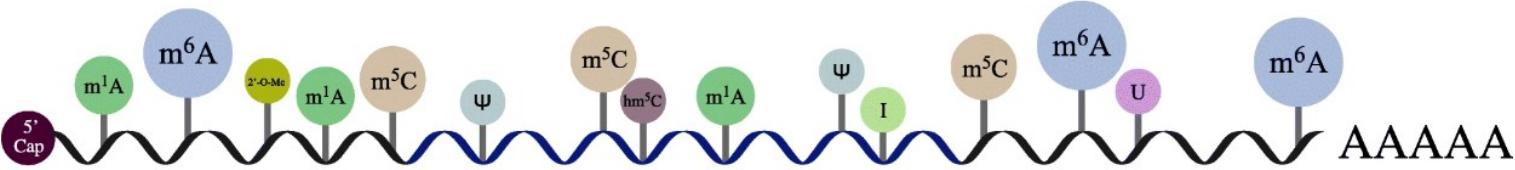
→ Function!

Jia et al., Nat Chem Biol 2011

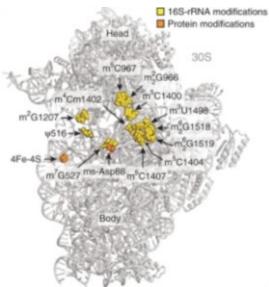
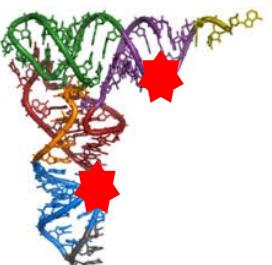
2012: First genome-wide method (m⁶A-Seq)



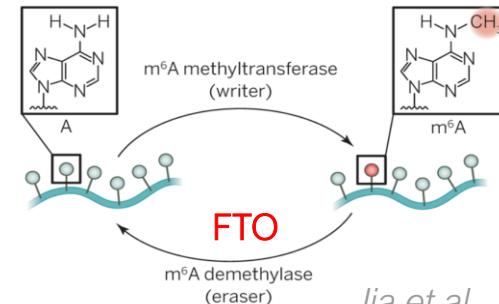
The epitranscriptome



1950s: RNA modifications fine-tune structure/function



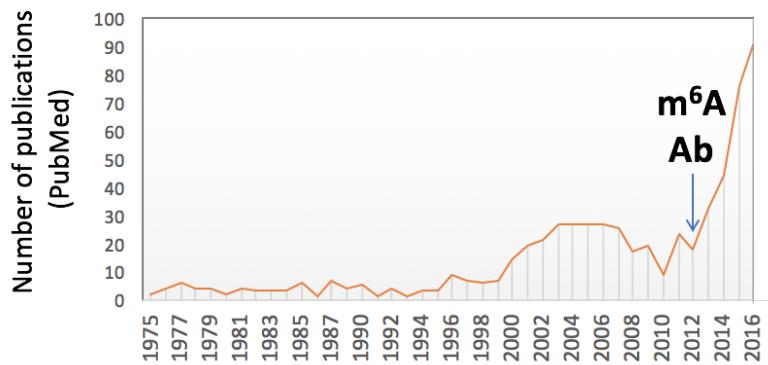
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→ Function!

Jia et al., *Nat Chem Biol* 2011.

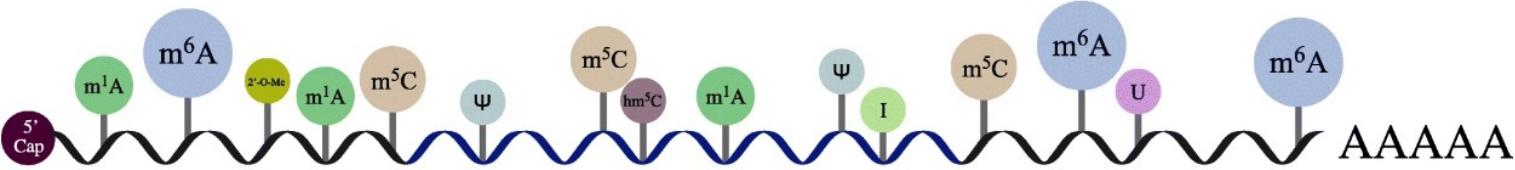
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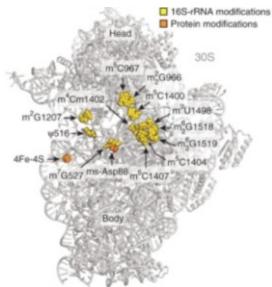
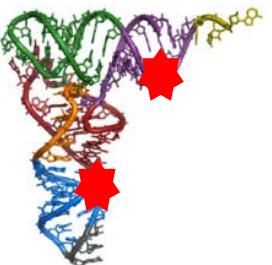
2013-2021: Pivotal roles of m6A in cellular functions

- Cell differentiation (2014)
 - Stress responses (2015)
 - mRNA half lives /RNA stability (2013)
 - Sex determination (2016)
 - Splicing (2018)

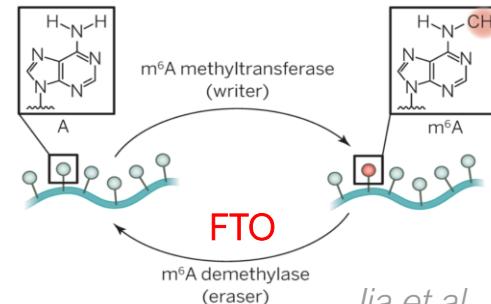
The epitranscriptome



1950s: RNA modifications fine-tune structure/function

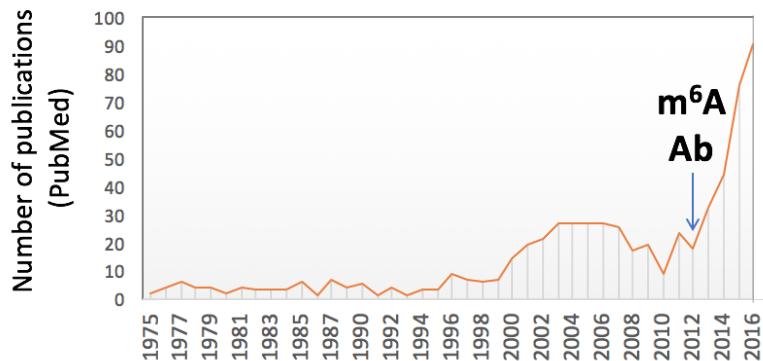


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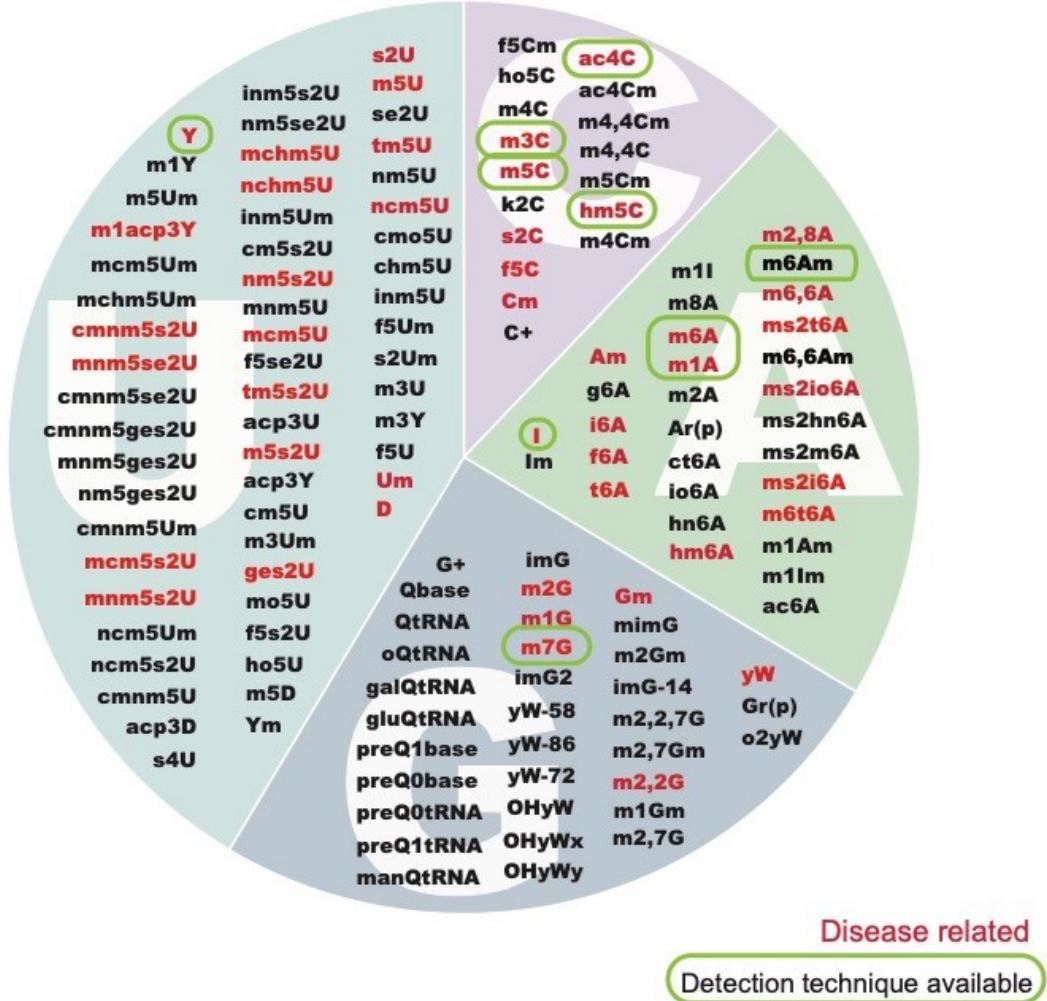


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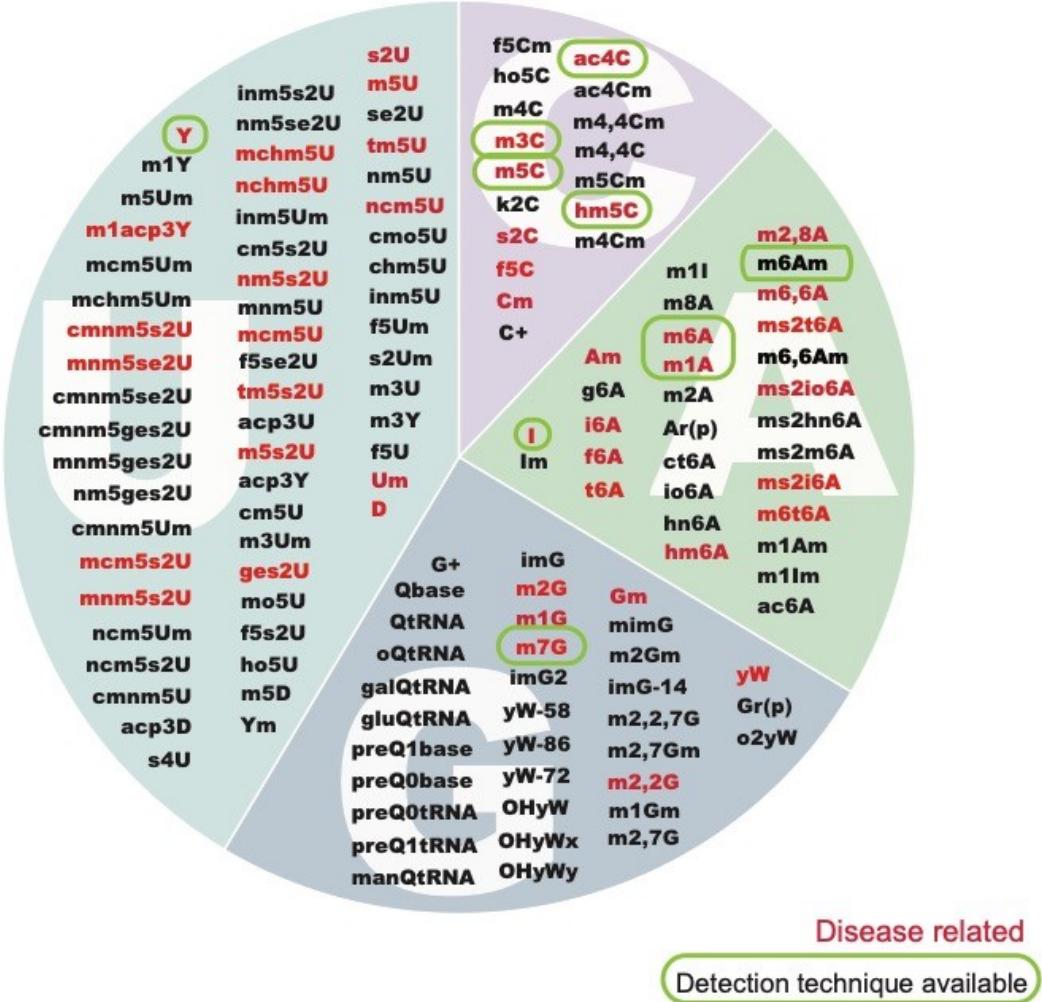
- Cell differentiation (2014)
- Stress responses (2015)
- mRNA half lives /RNA stability (2013)
- Sex determination (2016)
- Splicing (2018)

But... what about other modifications?

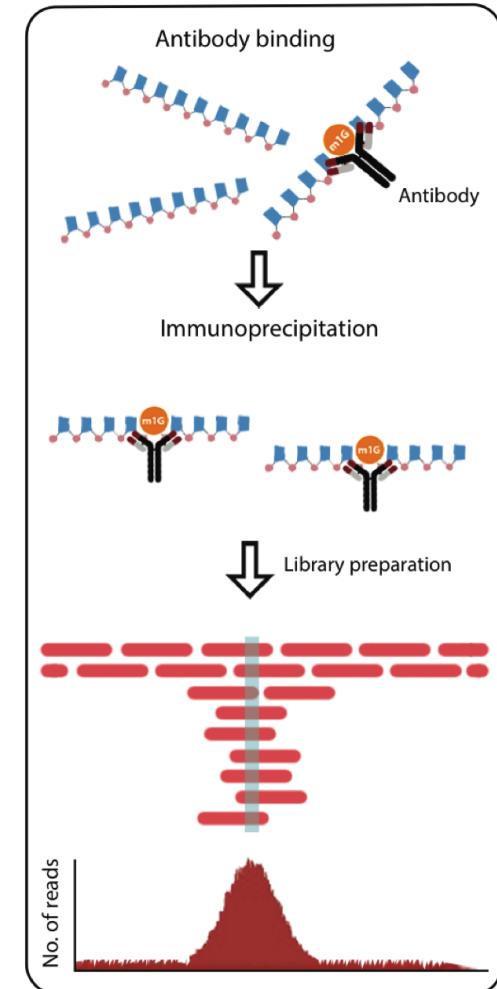
The epitranscriptome comes in more than 170 different flavours



The epitranscriptome comes in more than 170 different flavours

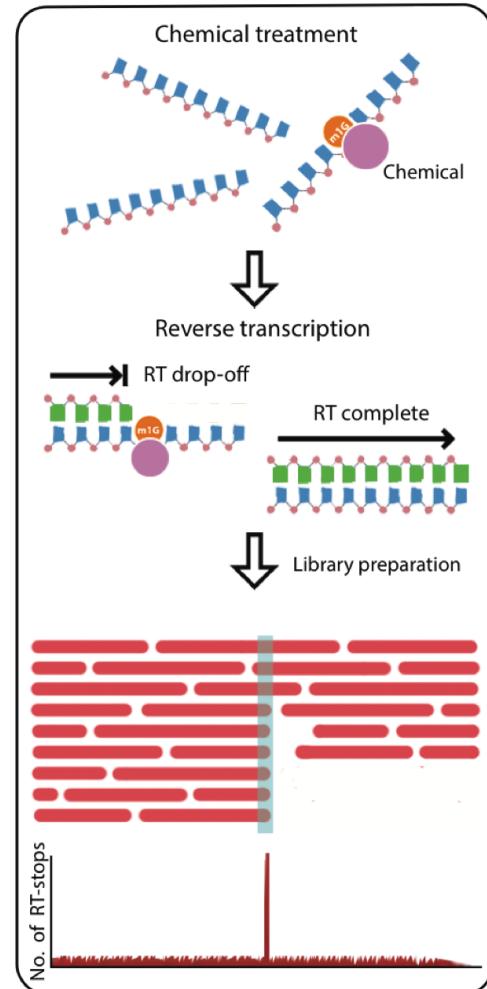


Antibody-based



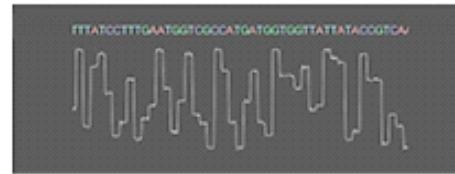
$\text{m}^6\text{A}, \text{m}^1\text{A}$

Chemical-based

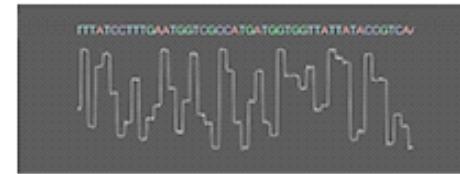


$\Psi, \text{m}^5\text{C}$

A promising solution: *Direct RNA nanopore sequencing*

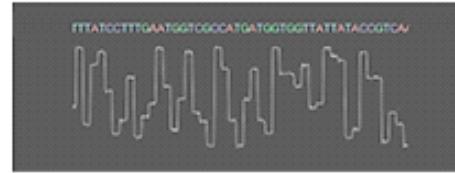


A promising solution: *Direct RNA nanopore sequencing*



- All RNA modifications
- No custom protocols for each modification
- Quantitative (% modified)
- Isoform-specific RNA modifications
- RNA modification co-dependencies
- PolyA tail lengths
- Single molecule resolution
- No PCR bias

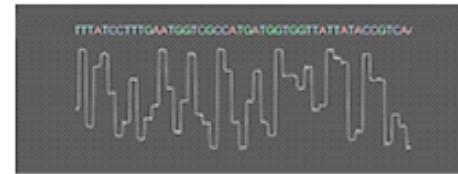
A promising solution: *Direct RNA nanopore sequencing*



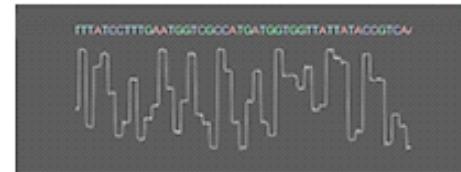
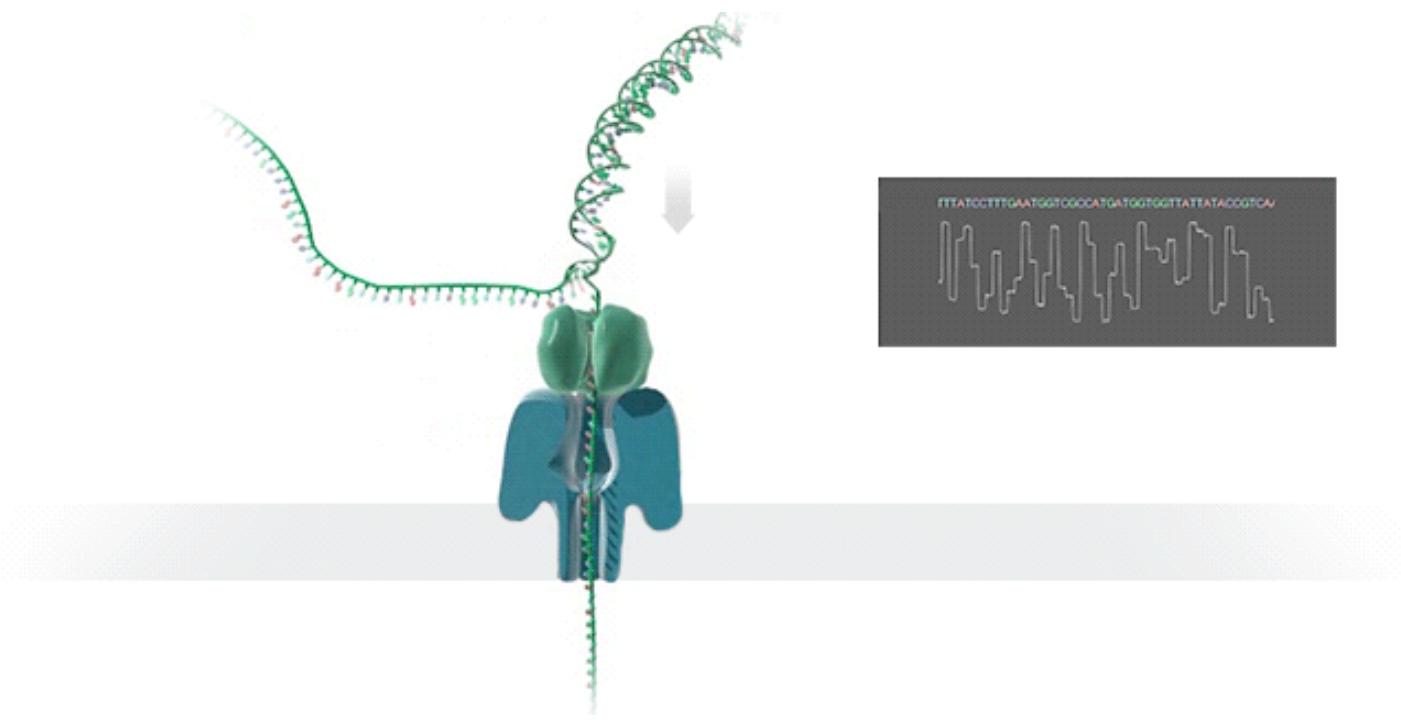
- All RNA modifications
- No custom protocols for each modification
- Quantitative (% modified)
- Isoform-specific RNA modifications
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- Single molecule resolution
- No PCR bias

“Much of what we know about RNA, we actually know about cDNA”

But there are some major challenges



But there are some major challenges



- 1. No basecaller for RNA modifications
- 2. Large input requirements (500ng pA+)
- 3. Limited to polyA+ RNA
- 4. Limited to long RNA (>200nt)

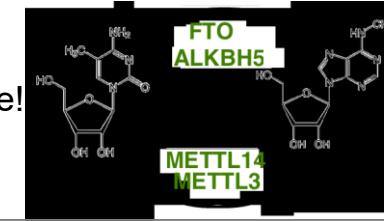
The Epitranscriptomics and RNA Dynamics Lab

WHAT?

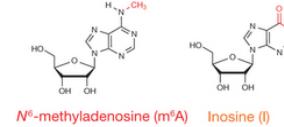
RNA modifications
are regulatory *dynamic* features



Reversible!



Expanding the RNA lexicon



5-methylcytidine (m⁵C)

N⁶-methyladenosine (m⁶A)

Inosine (I)

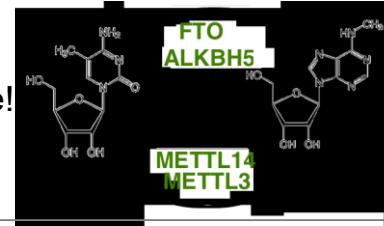
The Epitranscriptomics and RNA Dynamics Lab

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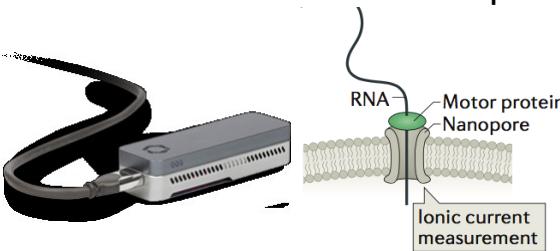


Expanding the RNA lexicon



HOW?

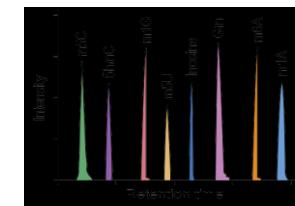
Characterize and map them using novel approaches and technologies



Oxford Nanopore Technologies
(direct RNA sequencing)

CGAGUCGUGAGC
CGAGUCGUGAGC
CGAGUCTUGAGC
CGAGUCGUGAGC
CGAGUCAUGAGC

Illumina sequencing
(non-random misincorporation)



Nucleoside
Mass Spectrometry

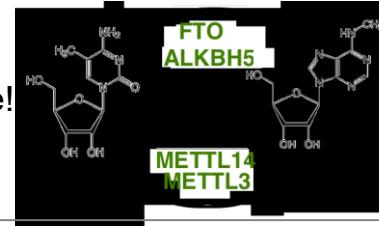
The Epitranscriptomics and RNA Dynamics Lab

WHAT?

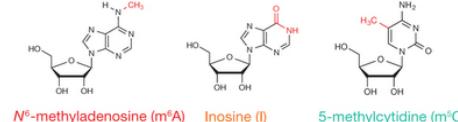
RNA modifications
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Reversible!

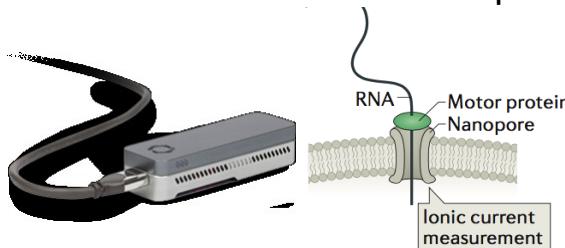


Expanding the RNA lexicon



HOW?

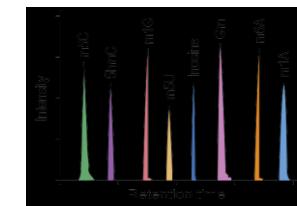
Characterize and map them using novel approaches and technologies



Oxford Nanopore Technologies
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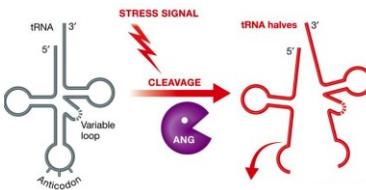
CGAGUCGUGAGC
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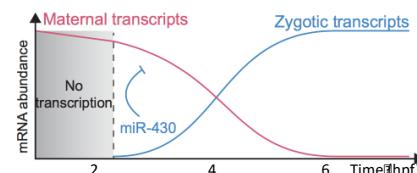
Nucleoside
Mass Spectrometry

WHY?



Role in RNA fate?

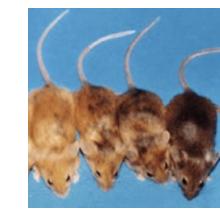
To decipher their role in:



Vertebrate embryogenesis?



Human disease?

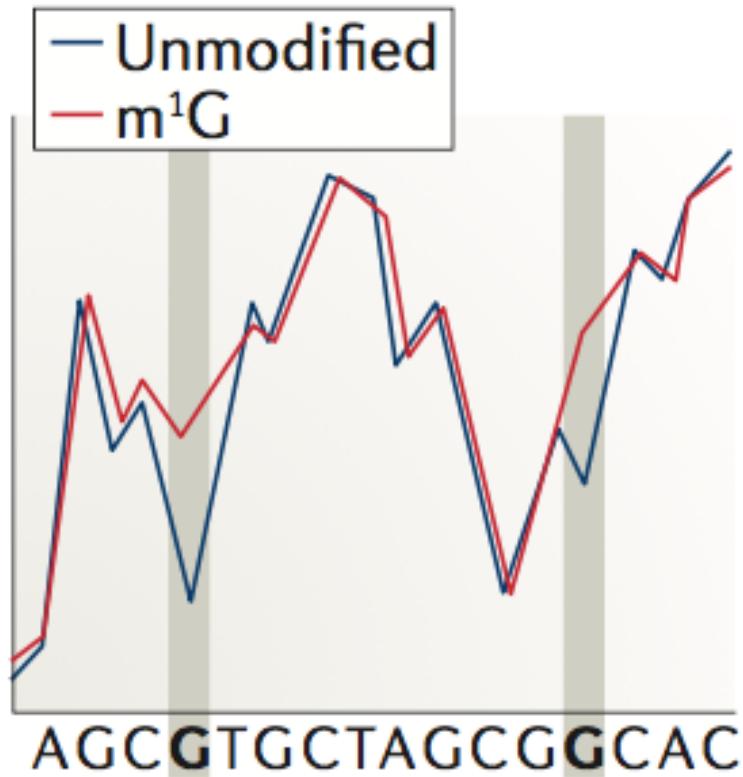


Intergenerational
inheritance?

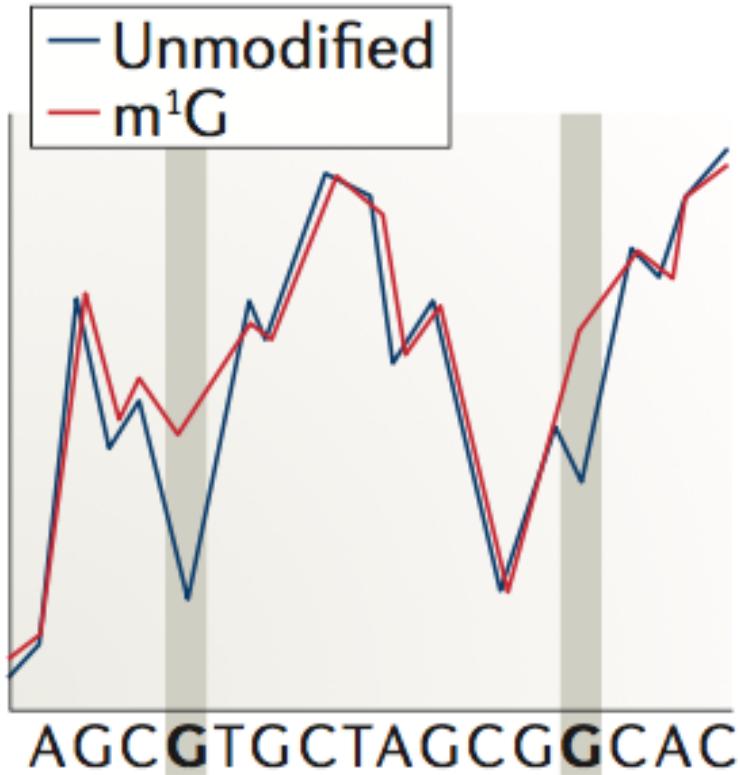
1. RNA MODIFICATIONS

*Can we detect RNA modifications using native
RNA nanopore sequencing?*

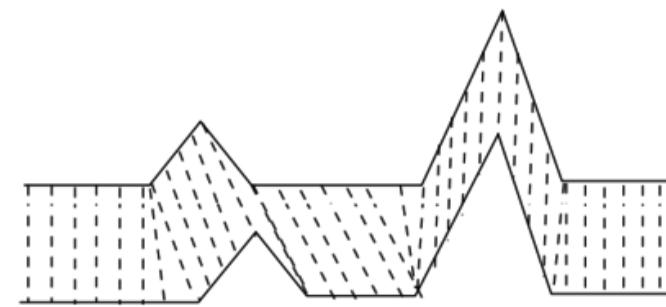
RNA modifications cause changes in current intensity



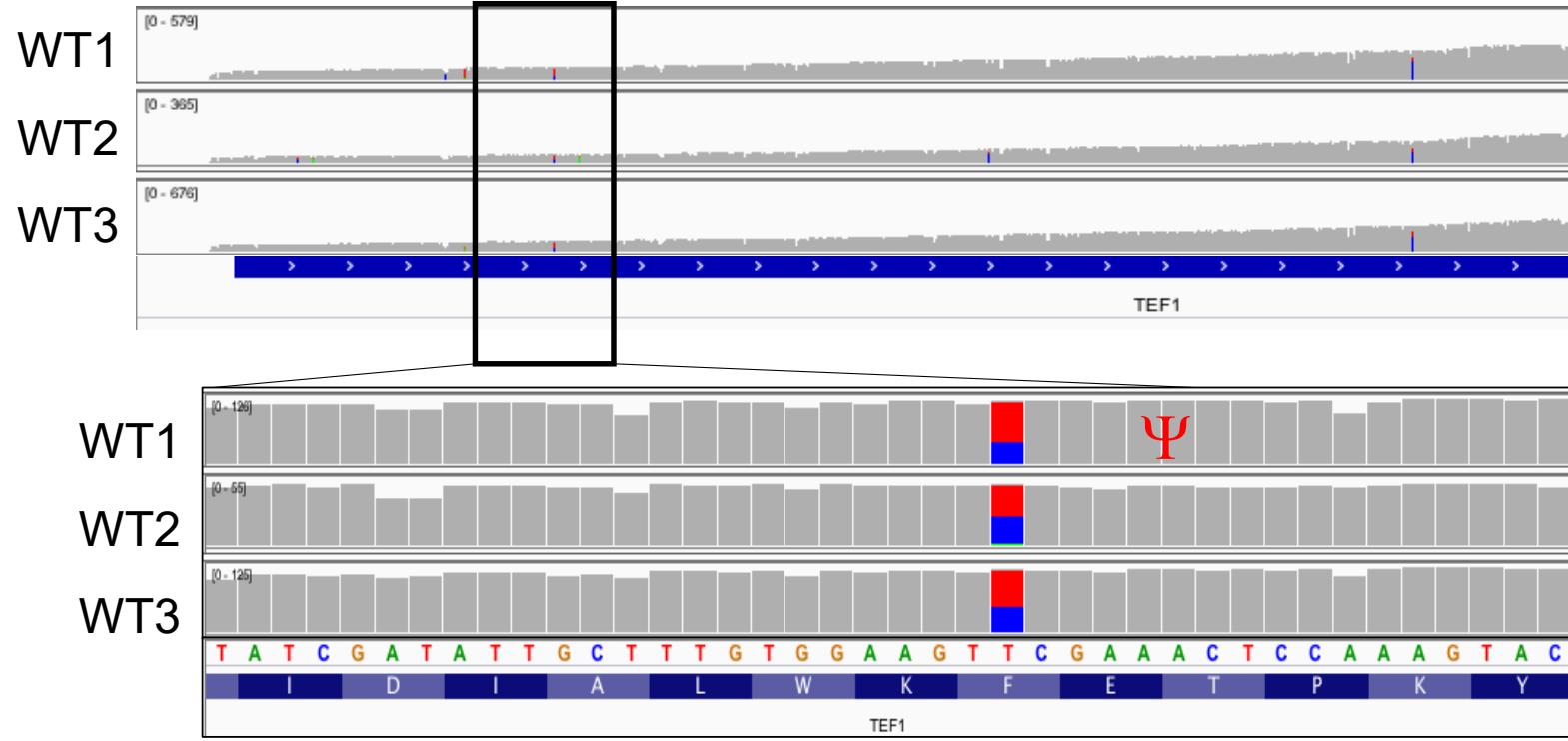
RNA modifications cause changes in current intensity



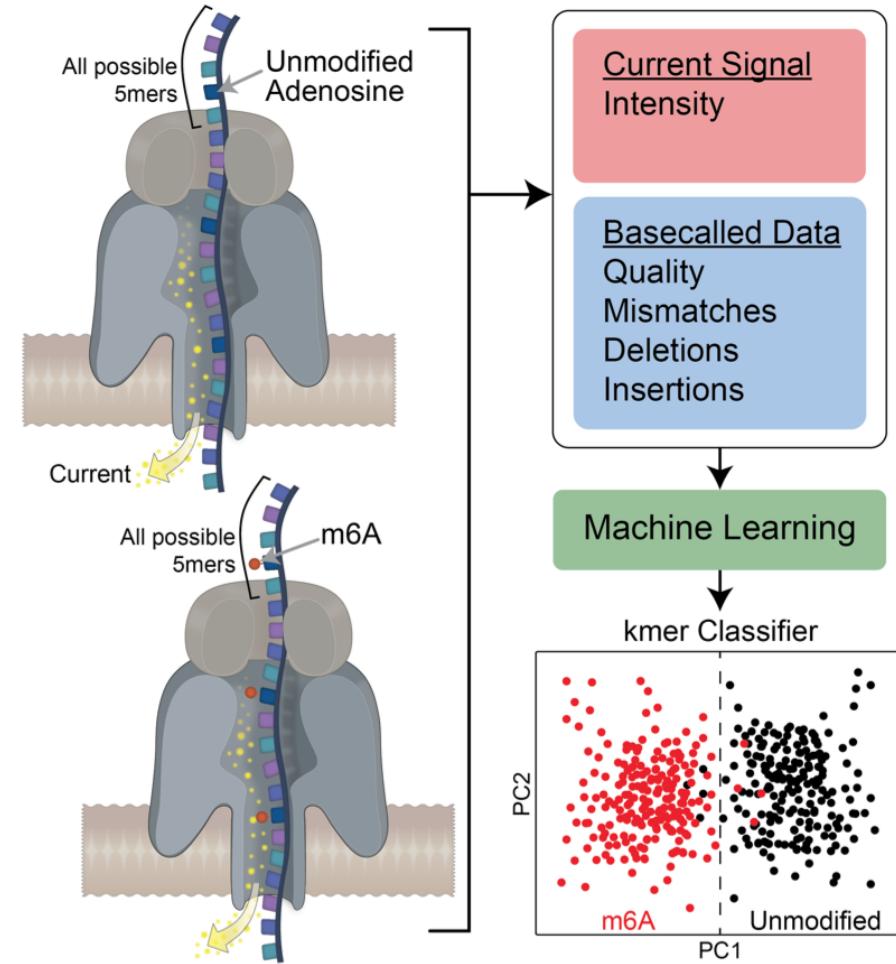
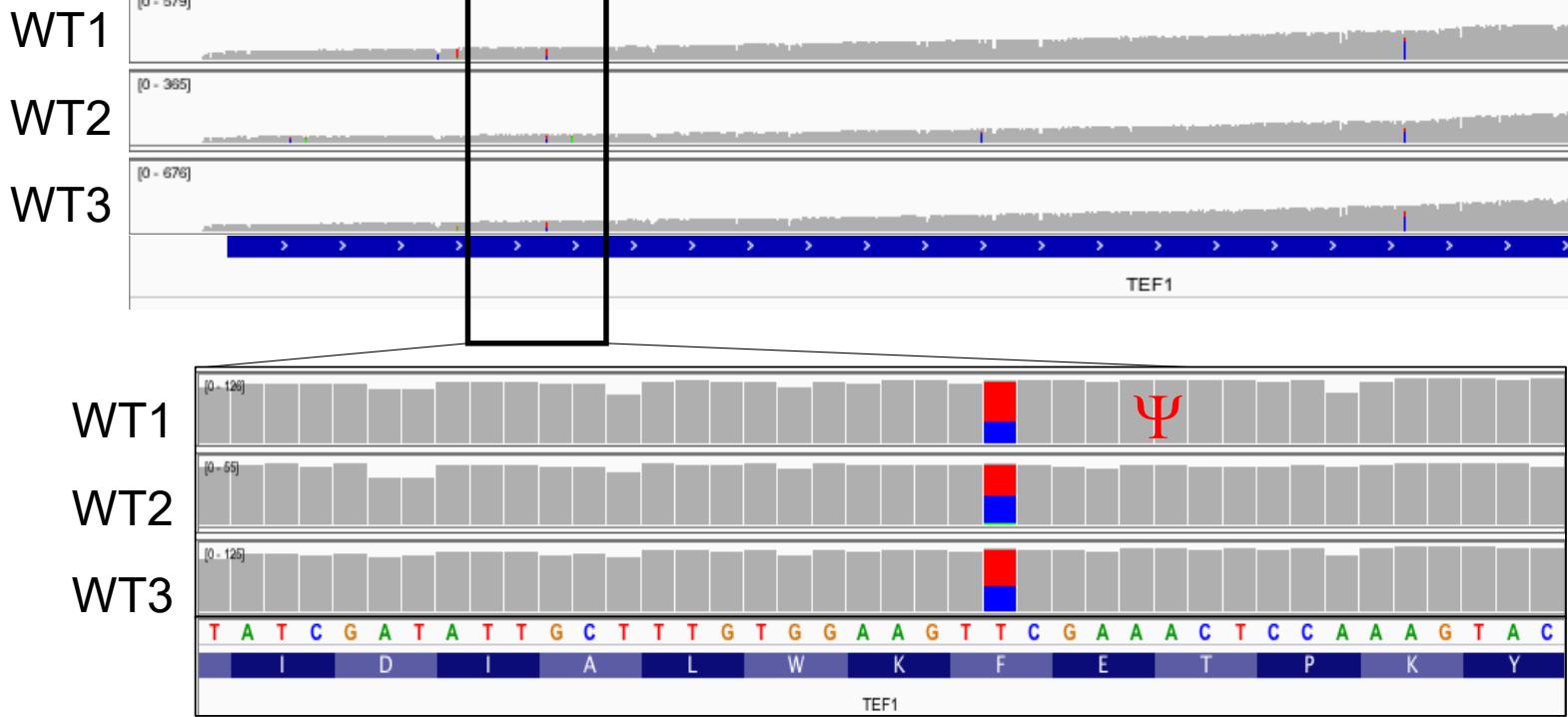
Need to align (or ‘resquiggle’)



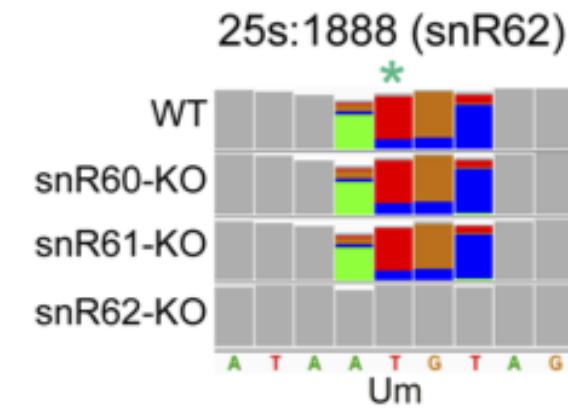
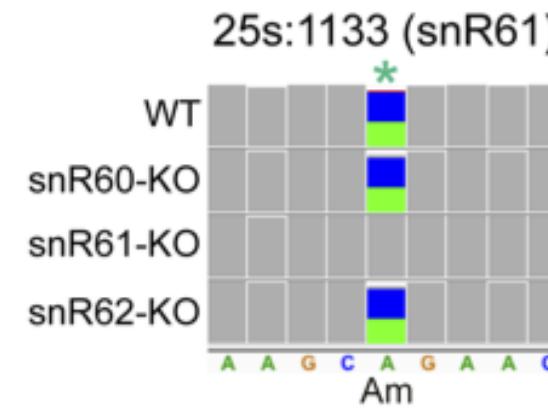
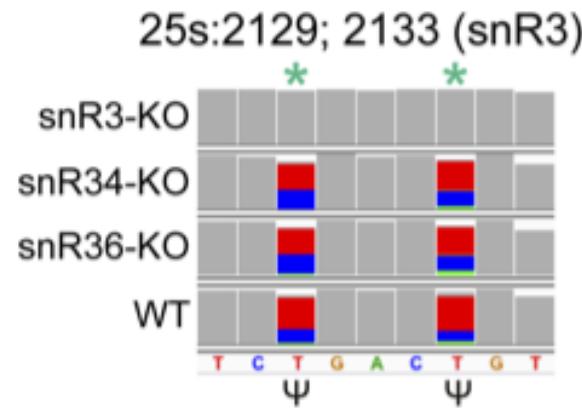
RNA modifications cause systematic basecalling 'errors'



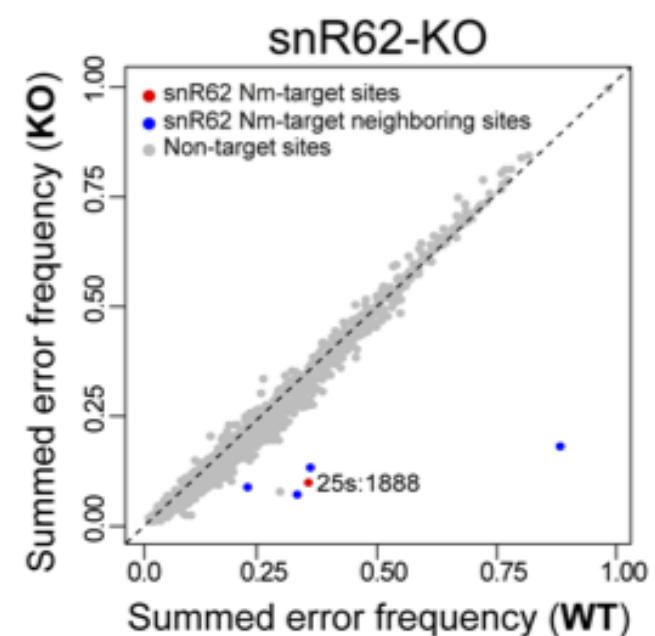
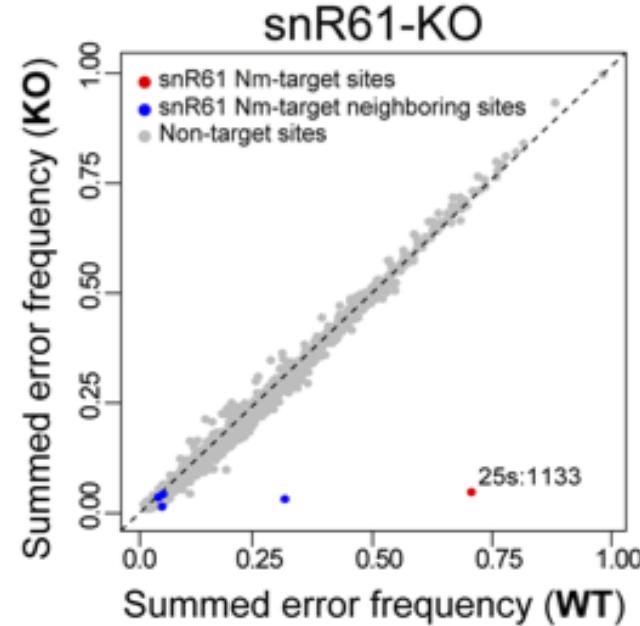
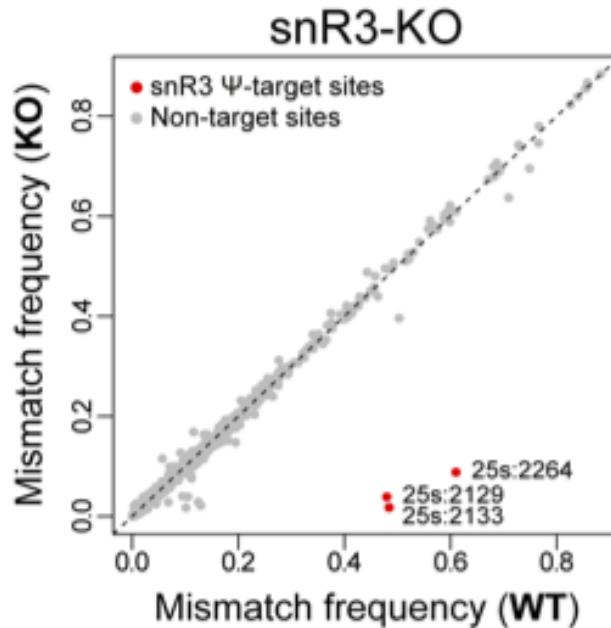
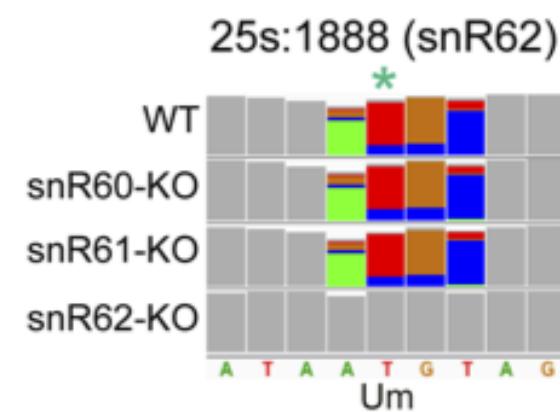
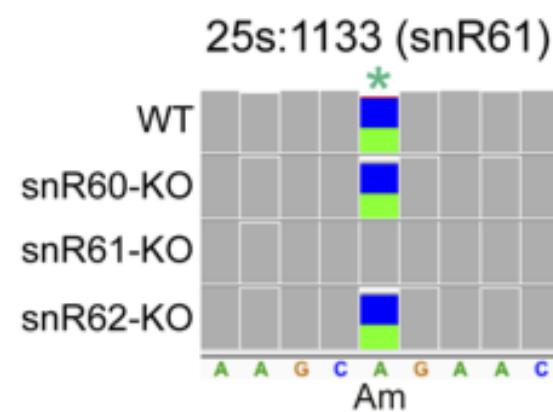
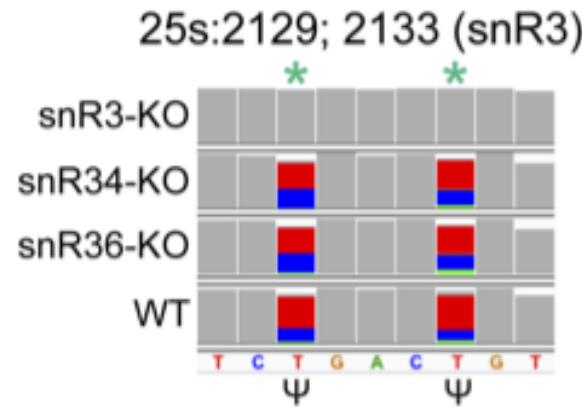
RNA modifications cause systematic basecalling 'errors'



Not just m6A... many different RNA modification types can be seen with nanopore

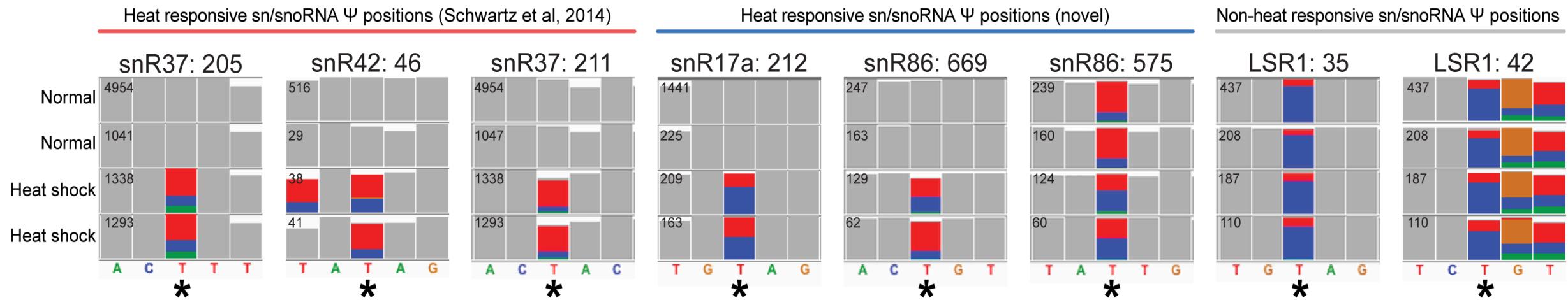


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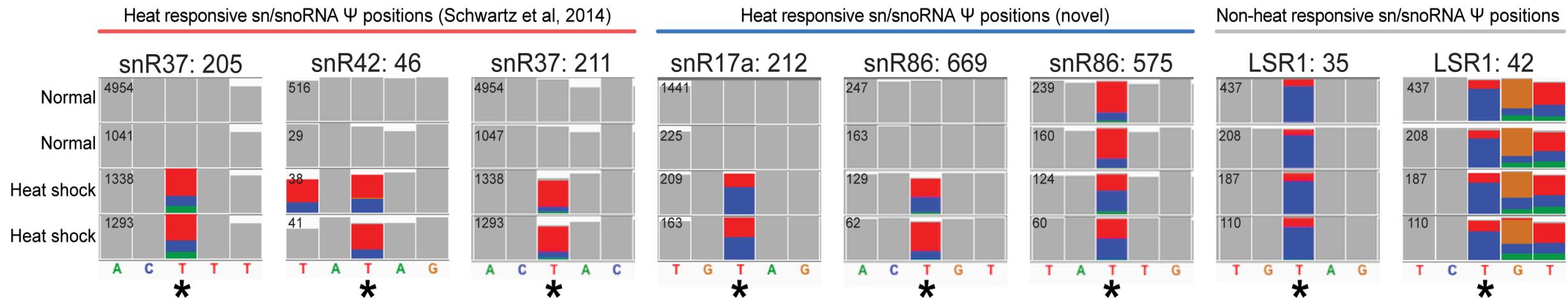


Can we apply it to study RNA modification dynamics?

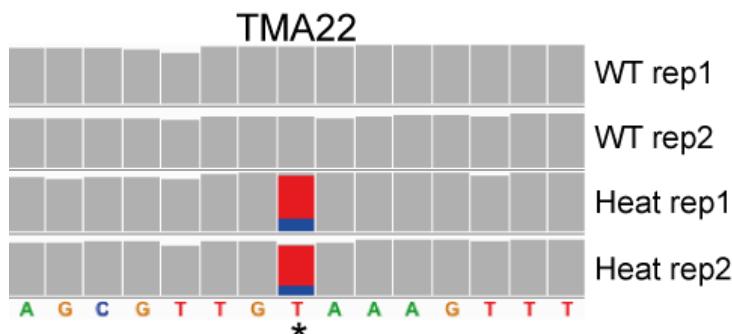
Sn/snoRNA Y modifications are dynamically regulated upon heat stress



Sn/snoRNA Y modifications are dynamically regulated upon heat stress



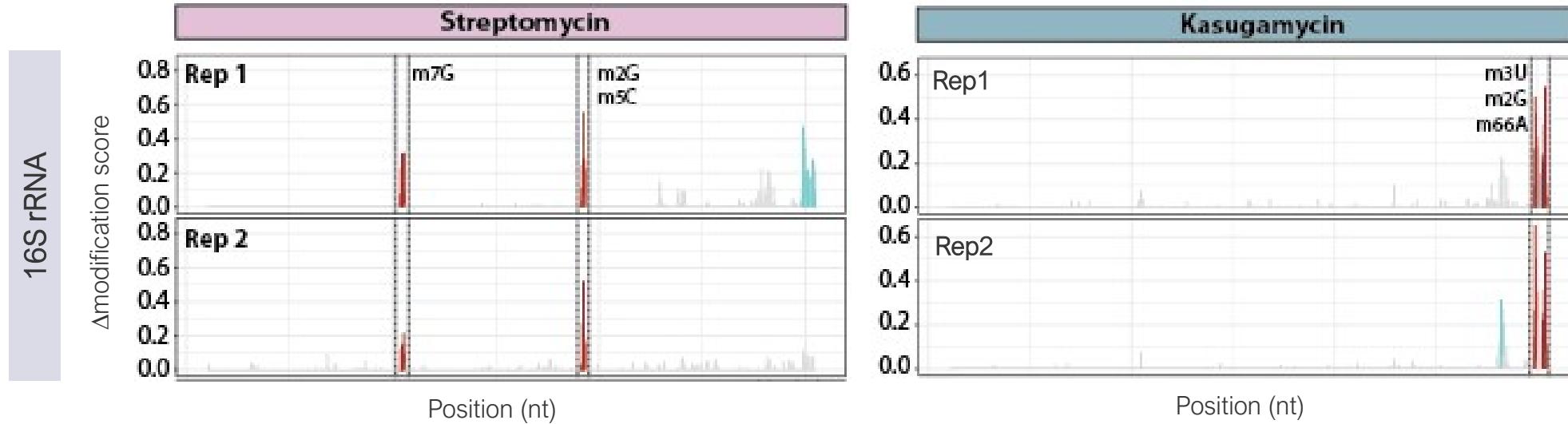
And mRNAs



rRNA modifications are dynamically regulated upon antibiotic exposures



Anna Delgado



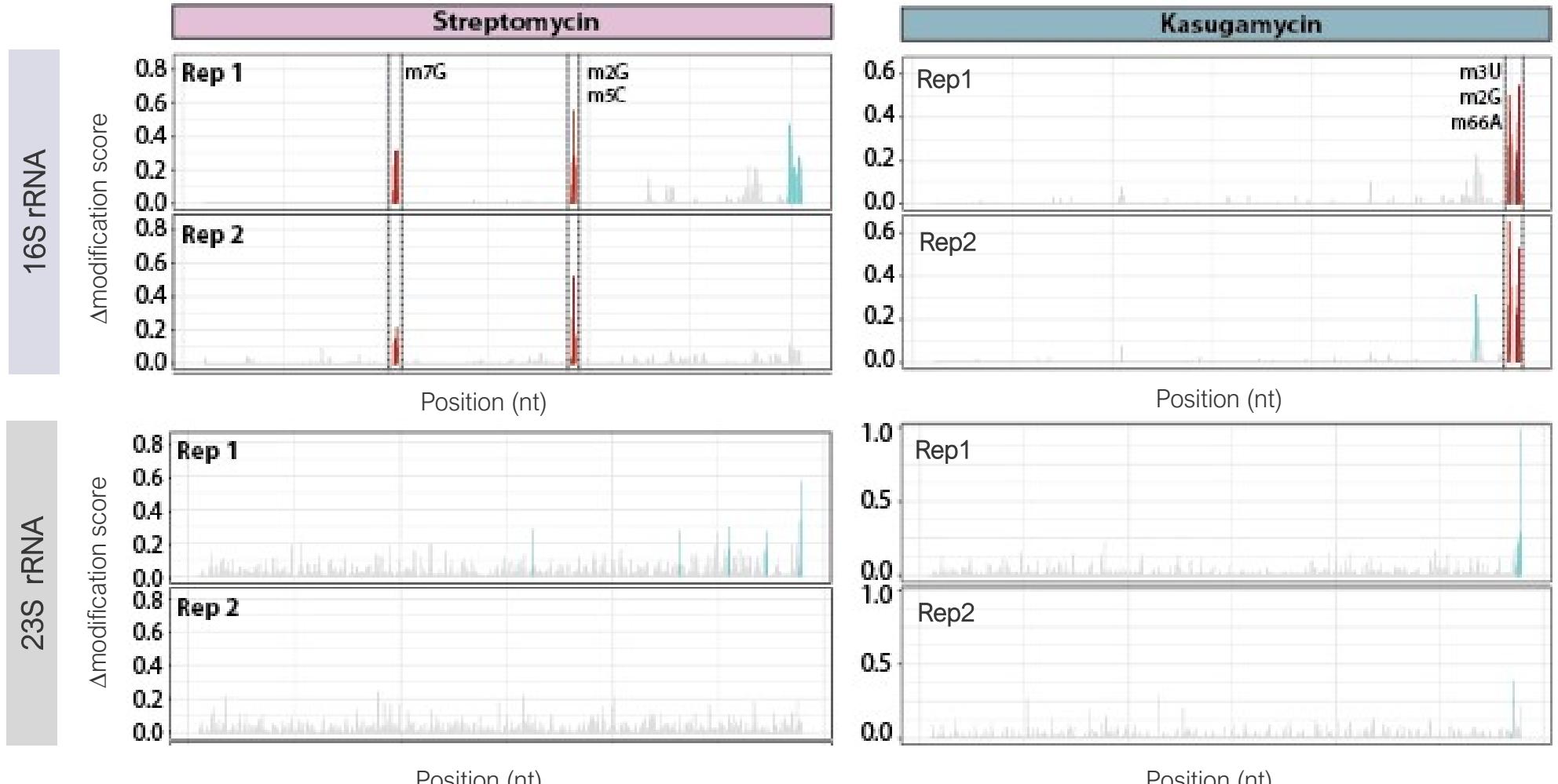
□ Not significant ■ Significant ■ Significant and replicable

Delgado-Tejedor et al, *bioRxiv* 2023

rRNA modifications are dynamically regulated upon antibiotic exposures



Anna Delgado



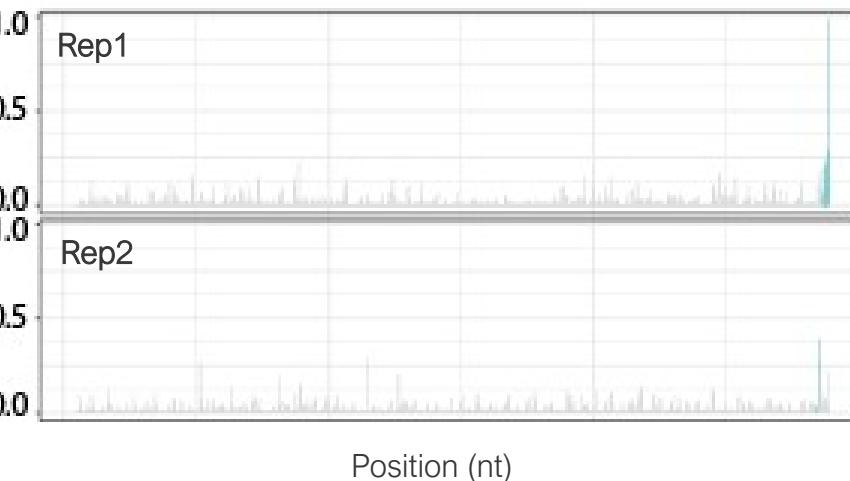
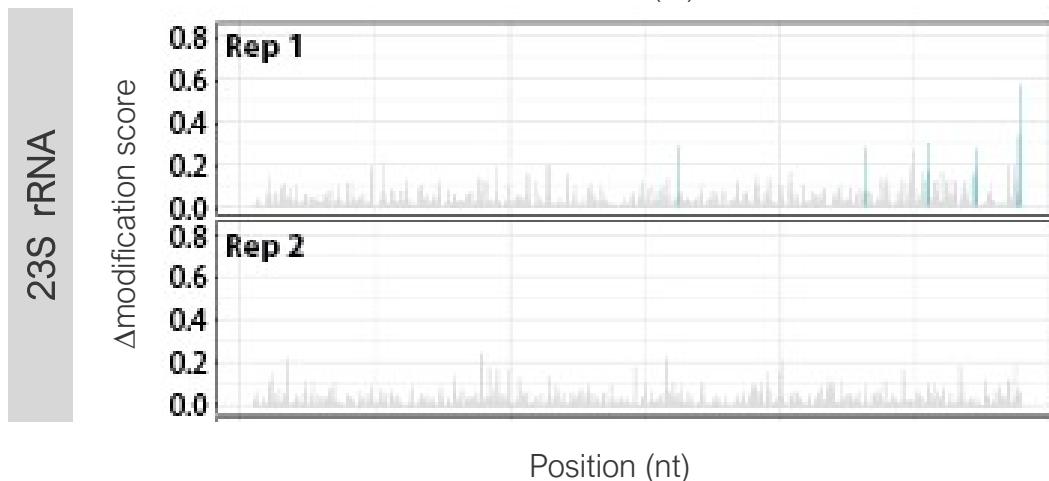
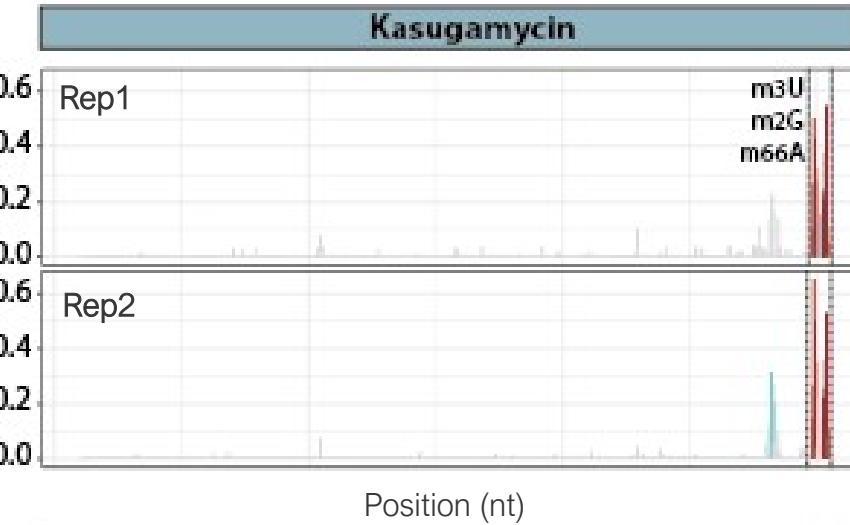
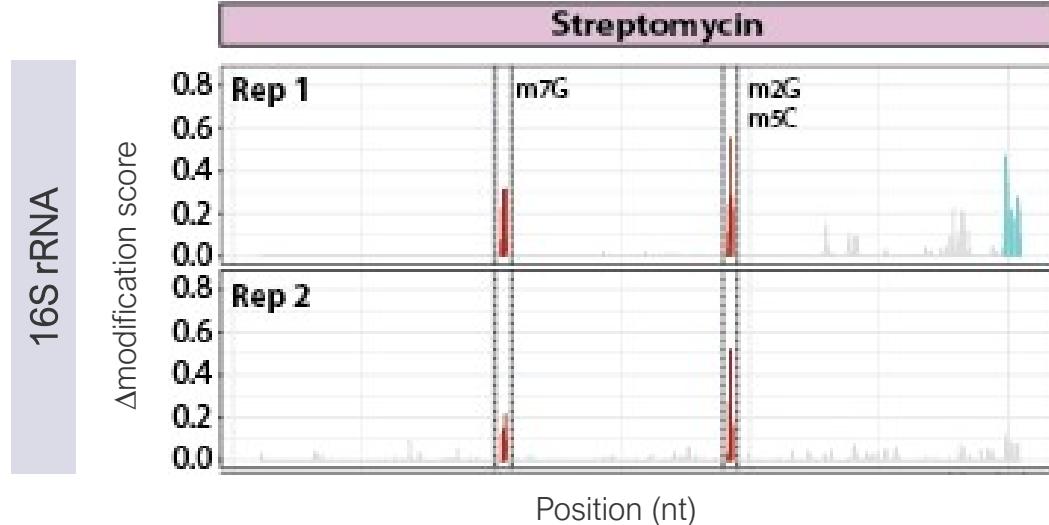
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Delgado-Tejedor et al, *bioRxiv* 2023

rRNA modifications are dynamically regulated upon antibiotic exposures



Anna Delgado



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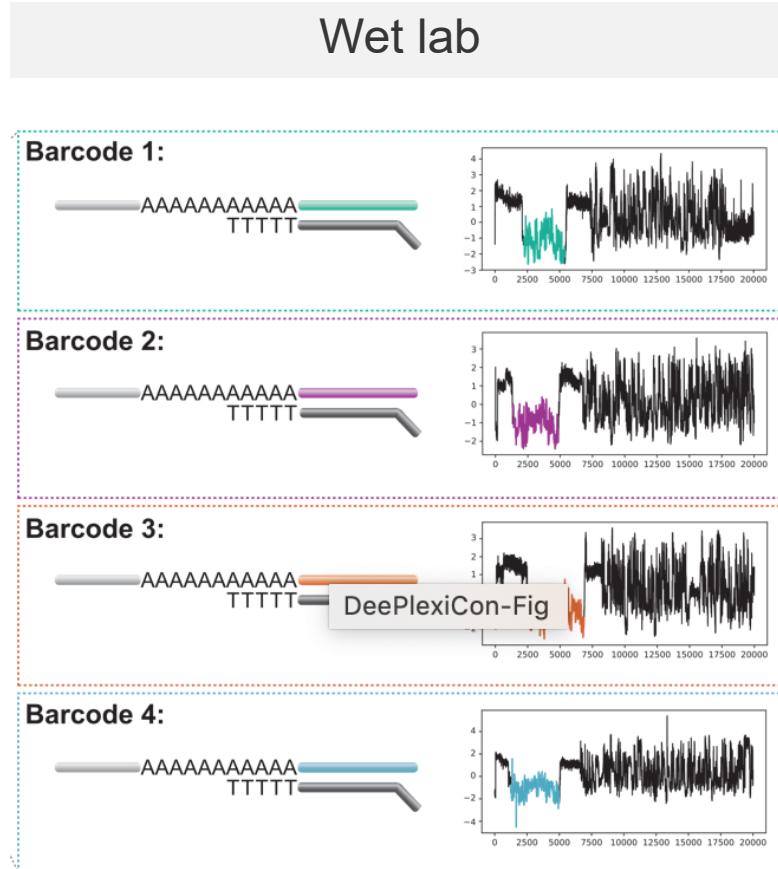
Delgado-Tejedor et al, *bioRxiv* 2023

2. MULTIPLEXING

Can we start with less material?

Barcoding and demultiplexing direct RNA nanopore sequencing runs

Multiplexing decreases costs and permits application to low input samples



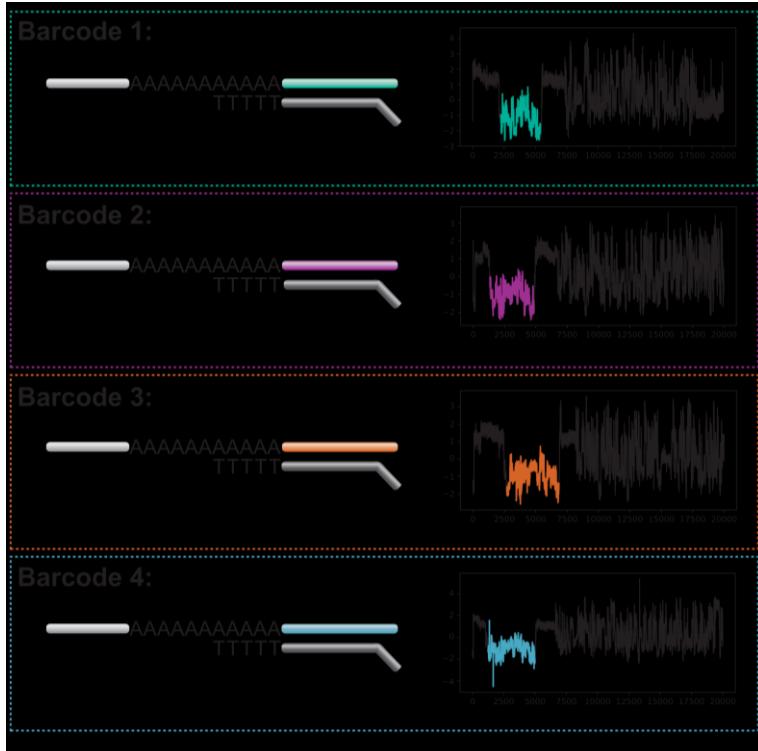
Barcoding and demultiplexing direct RNA nanopore sequencing runs

Multiplexing decreases costs and permits application to low input samples



Martin Smith

Wet lab



Dry lab

DeepMultiplexion

97% accuracy
93% recovery

500ng input / sample
to
125ng input/sample
(4bc)

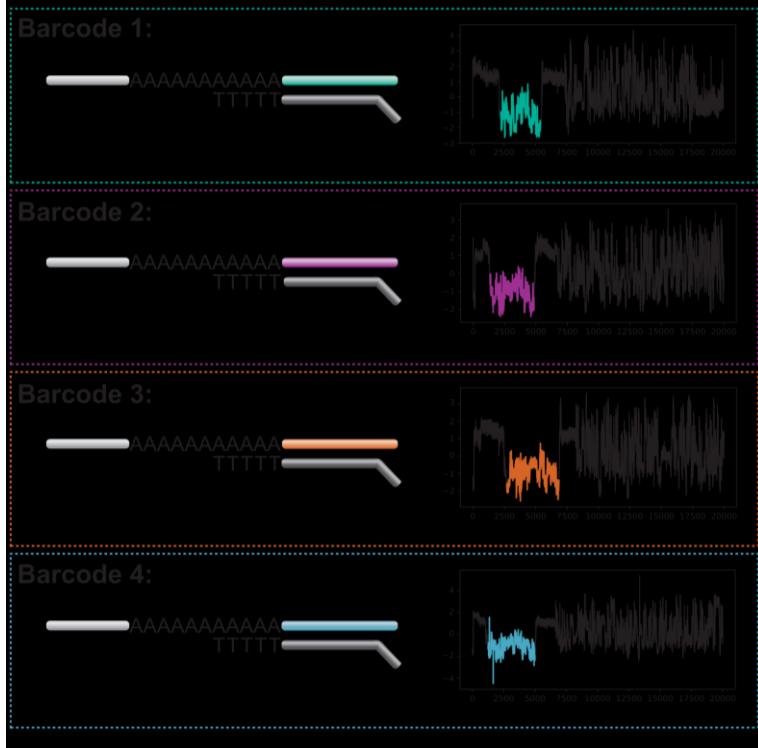
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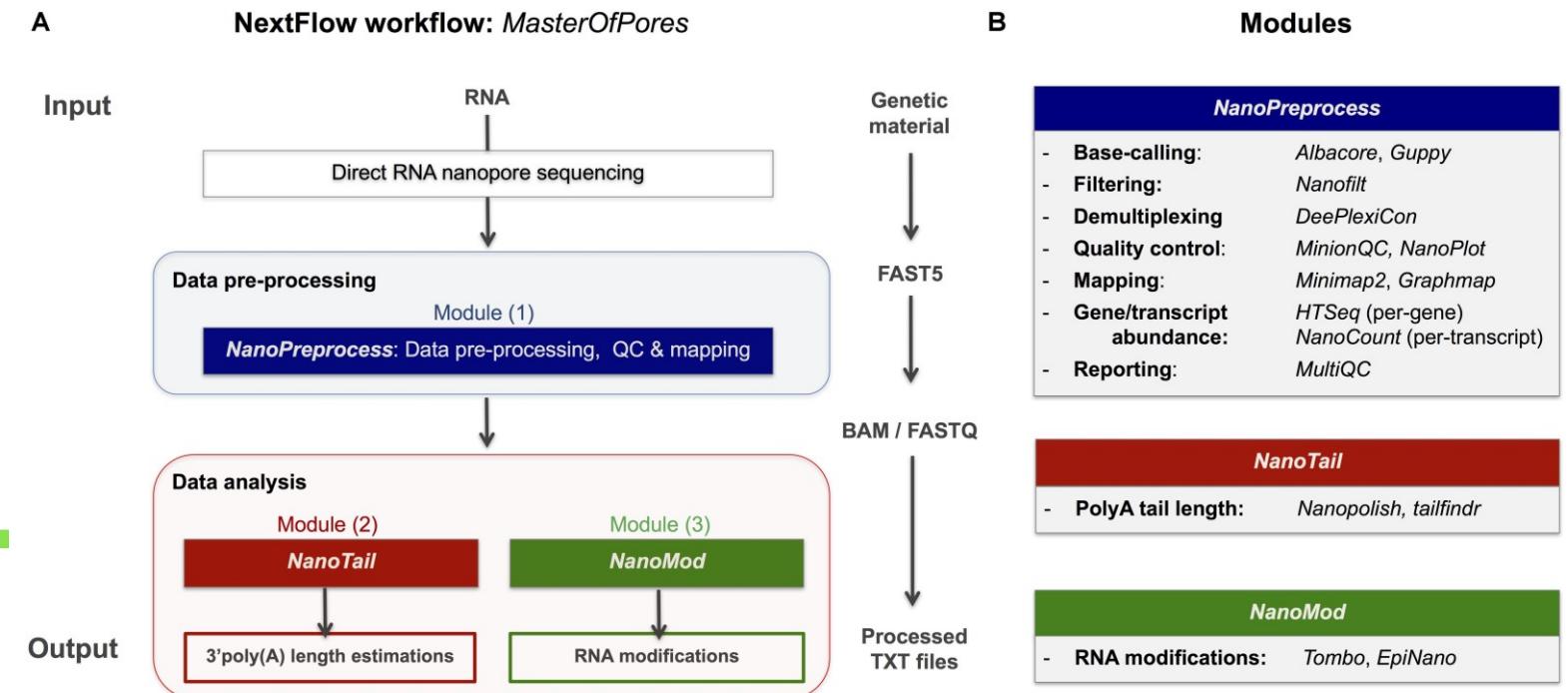
- NOW: 96 barcodes

github.com/biocorecrg/MOP2

biocorecrg/ master_of_pores

Nextflow pipeline for analysis of direct RNA
Nanopore reads

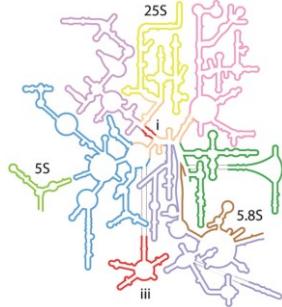
4 Contributors 0 Issues 78 Stars 15 Forks



3. SMALL RNA SEQUENCING

Let's sequence some tRNAs!

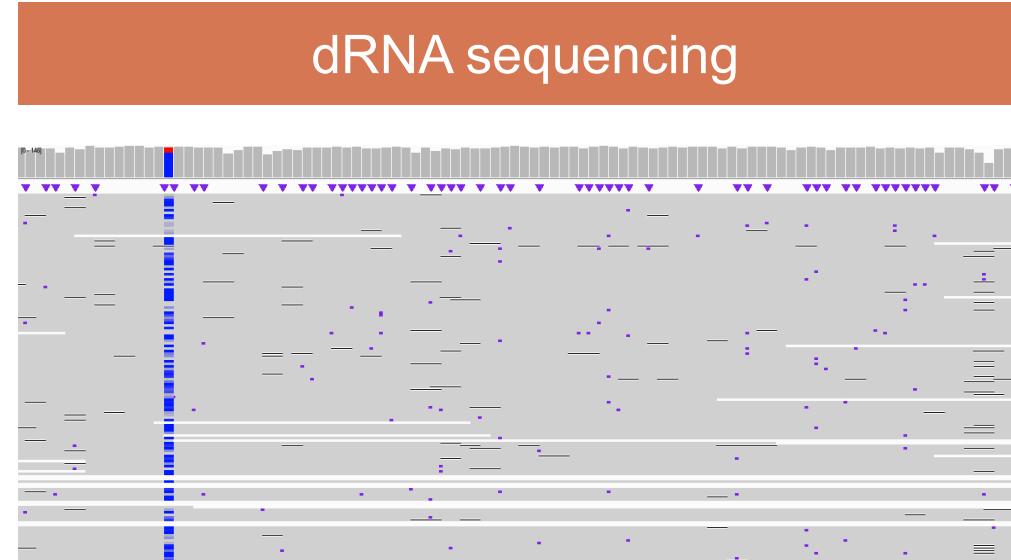
Sequencing small RNAs with nanopore is not as simple as it sounds....



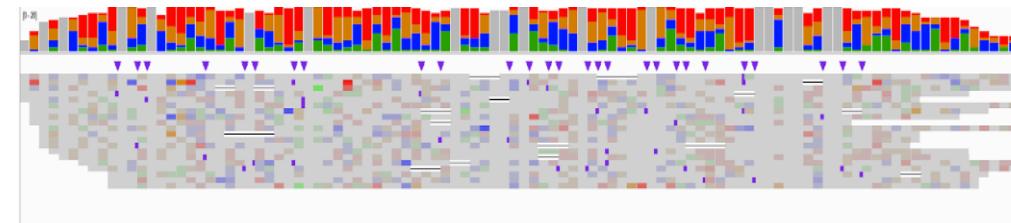
rRNA
mRNA



tRNA



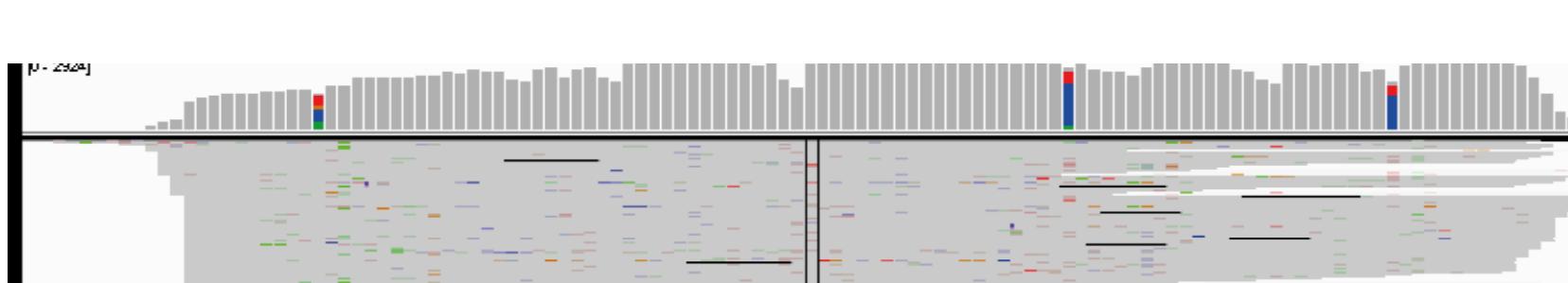
1-2 million reads



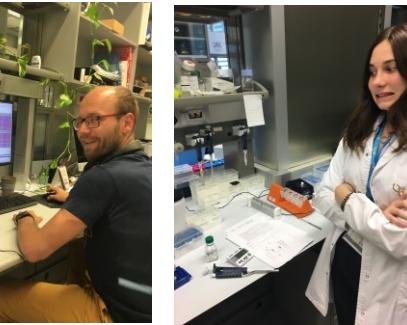
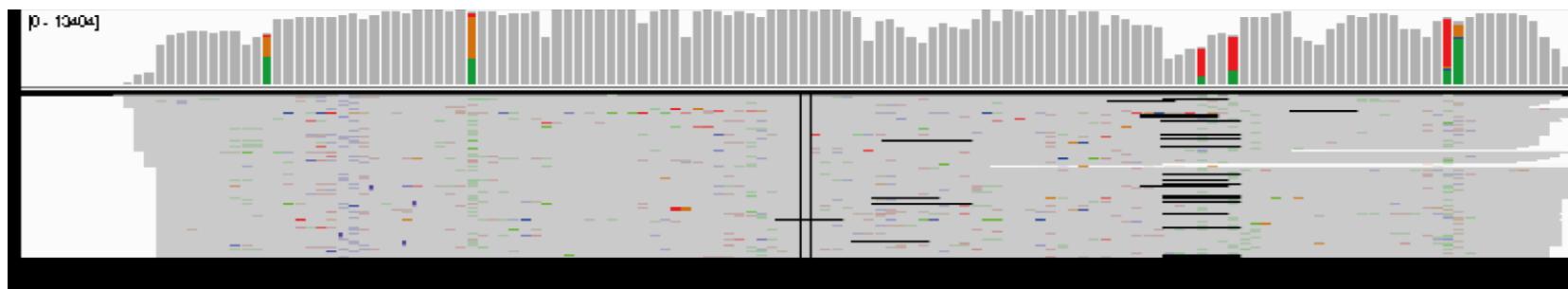
~50,000 reads

Nano-tRNAseq: native tRNA nanopore sequencing

Synthetic



Biological



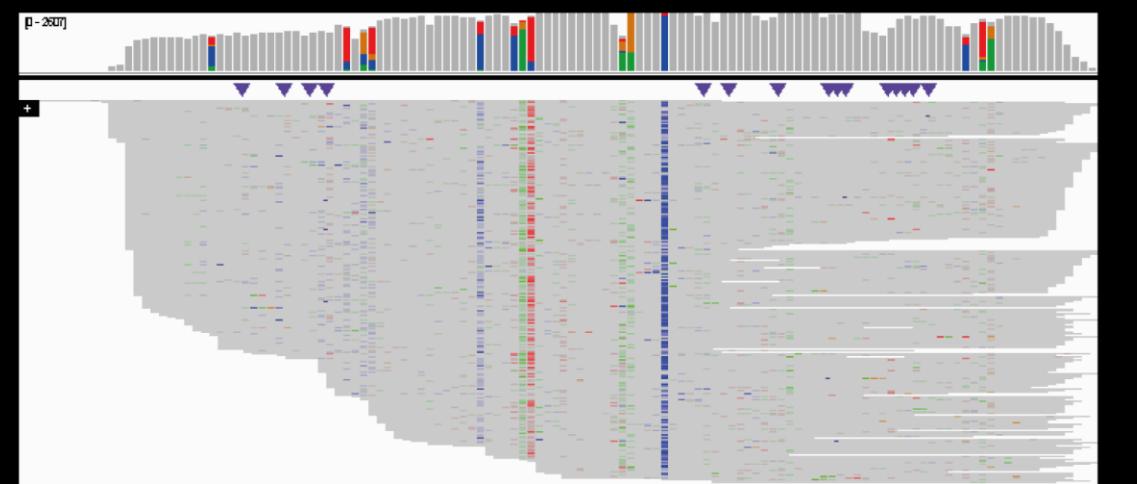
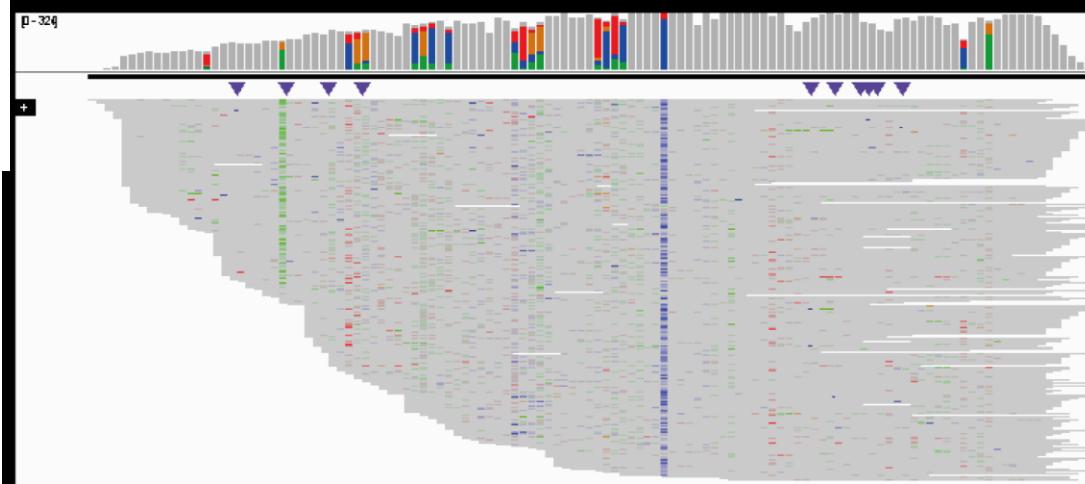
Morgan Lucas
Leszek Pryszzcz

- A mismatch
- C mismatch
- G mismatch
- T mismatch
- No mismatch

Nano-tRNAseq: native tRNA nanopore sequencing

1

WT

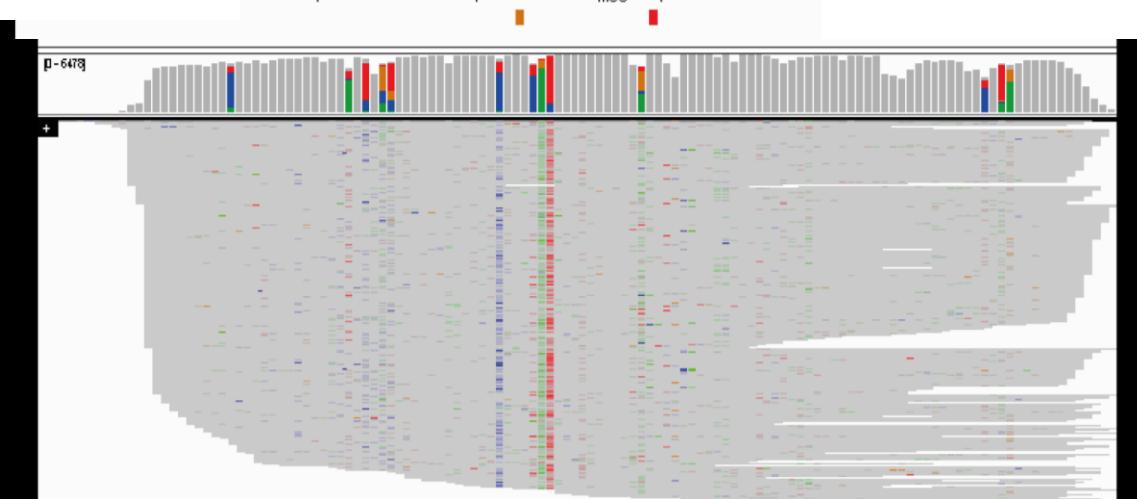
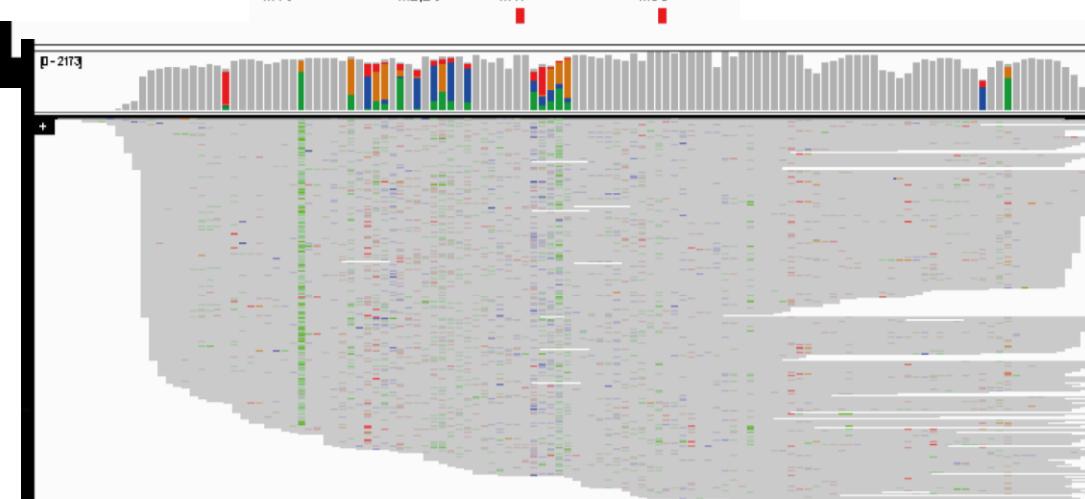


RNA mods

m1G m2,2G m1I m5U

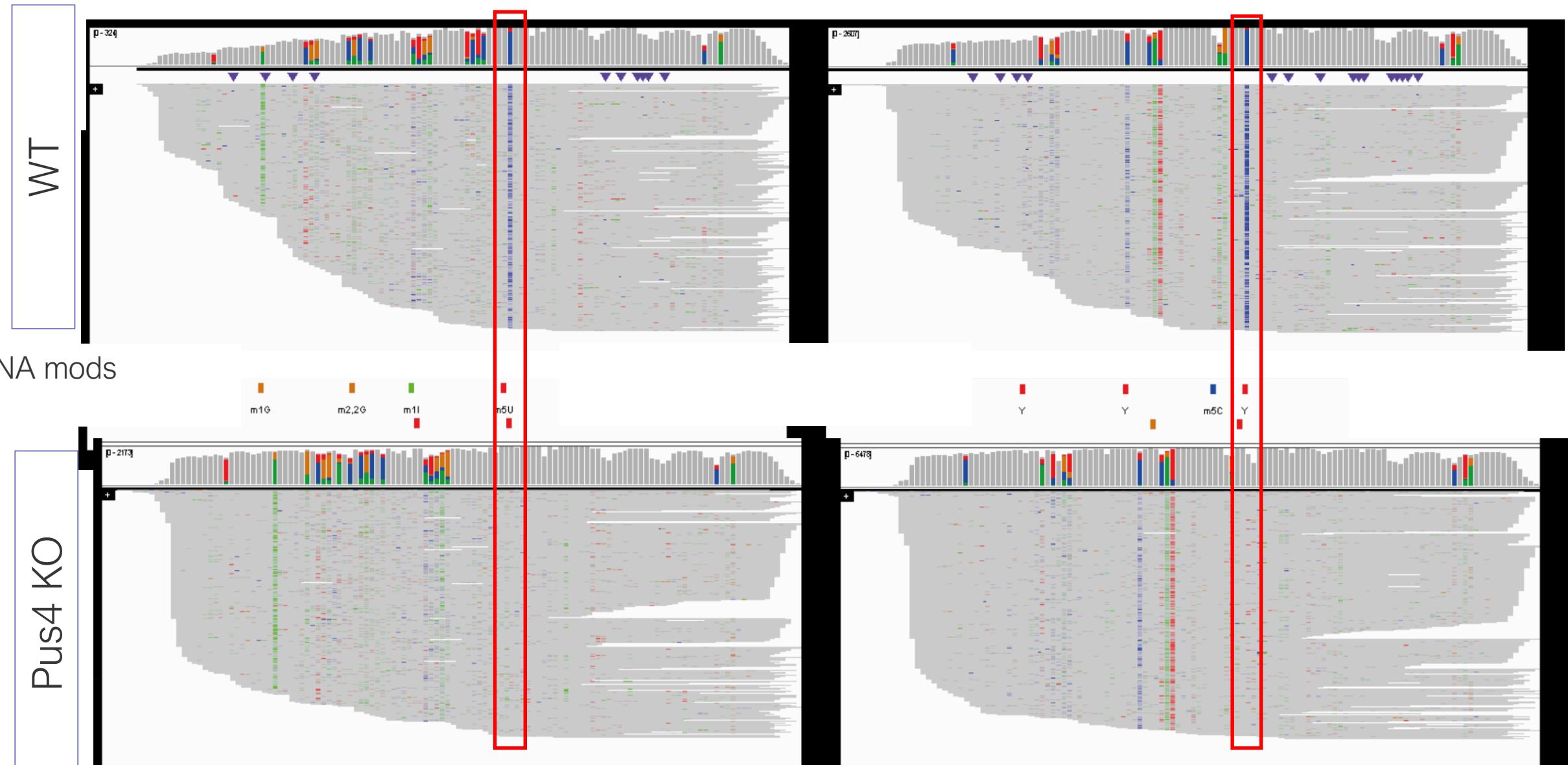
Y Y m5C Y

PUS4 KO



Nano-tRNAseq: native tRNA nanopore sequencing

1



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Conflict of Interest: SAB for Immagina Biotech

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Thank you

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