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# Simulation of RNA sequencing with Oxford Nanopore Technologies



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## Introduction

Until recently, transcriptomics applications with long reads were realized with Pacific Biosciences' Iso-seq protocol. Pioneer works start dealing with characterization of isoforms or gene expression quantification using Oxford Nanopore Technologies (ONT) reads.

Despite the existence of long reads simulators for genomic data sets, a current lack is the possibility to adequately simulate long reads from **ONT RNA protocols**, which would help with the developments of new tools to handle this kind of data. The simulation of transcriptomics sequencing is a more complex task than in genomics because the gene expression and transcript variability have to be modeled.

# Methods

#### Error rates and profiles

- learn error rates and profiles by training using real read data sets such as Nanosim [1]
- compute error rates and percentages of deletion, insertion and substitution, as well as homopolymer errors using AlignQC [2]
- pre-computed error profiles can also be input

#### Simulate transcripts

- extract template transcripts from the GTF file of the desired reference using gffreads (http: //ccb.jhu.edu/software/stringtie/gff.shtml)
- extract realistic expression levels from Flux Simulator [3] results

#### Integrate errors and transcripts in synthetic reads

- novel implementation in C++ that generates the final reads
- adds the errors to the sequences extracted from the GTF
- deals with regular versus homopolymer errors
- adds supplementary characteristics such as the staircase effect

# Pipeline **INPUTS DETAILS** error profile/rate .bam + .bai of reads 1. error profile using AlignQC providing error model computation 2. read computation: reference genome: gene expression .gtf levels using 2.1 sequences .fasta Flux Simulator extraction from gtf read quantity other ONT 2.2 RNA long read characteristics simulation (staircase effect...)

# Experiment

- 300k reads simulation
- reference: musculus• Genome GRCm38
- Ensembl Mus musculus• Annotation: GRCm38 release 87
- Template dataset for error training: 740k Mus musculus RNA ONT 1D reads
- Comparison of raw reads and simulated reads statistics using AlignQC [2]

# Results

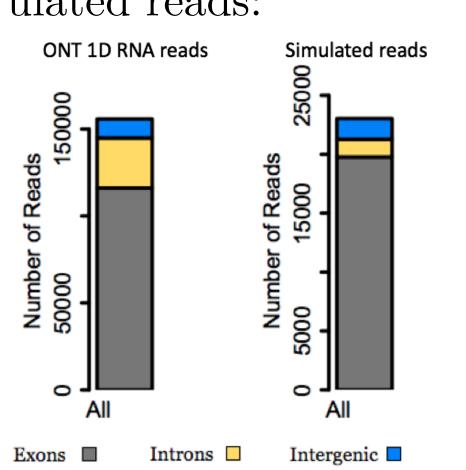
Comparing error rates between ONT 1D RNA reads and the simulated reads shows that the simulator produces reads with realistic error profiles:

Metric	ONT 1D RNA reads	Simulated reads
ERROR RATE	13.697%	11.592%
MISMATCHES	5.089%	5.848%
DELETION	7.348%	4.85%
INSERTION	1.26%	0.894%
NON HOMOPOLYMER DELETION	4.397%	3.069%
HOMOPOLYMER DELETION	2.951%	1.782%
NON HOMOPOLYMER INSERTION	0.874%	0.558%
HOMOPOLYMER INSERTION	0.387%	0.337%

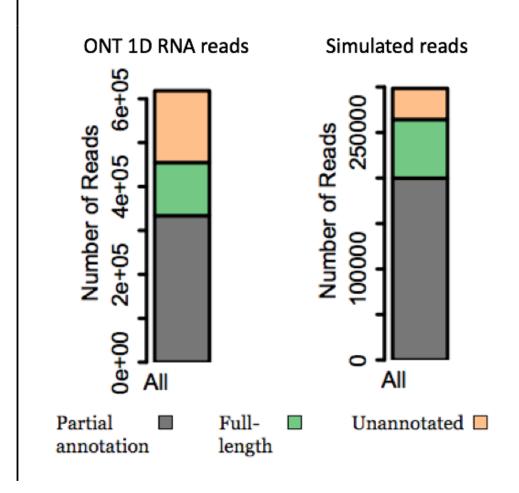
# References

- [1] Chen Yang, Justin Chu, René L Warren, and Inanç Birol. Nanosim: nanopore sequence read simulator based on statistical characterization. GigaScience, 6(4):1-6, 2017.
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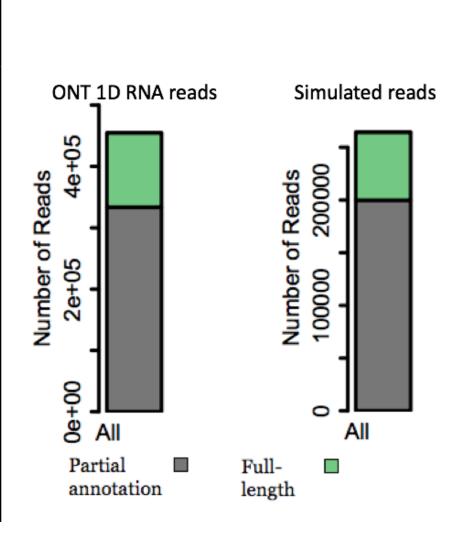
distribution of reads among genomic features is similar between the real and simulated reads:



As well as the distribution of of annotated reads:



And the distribution of identified reference transcripts:



# Conclusion

### Main messages

- Work in progress, **first tool** to simulate transcriptome sequencing with ONT
- Availability: github.com/kamimrcht/RNA-long-reads-Simulator

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