Simulation of RNA sequencIng with Oxford Nanopore Technologies

Camille Marchet, Leandro Lima

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Camille Marchet[1] and Leandro Lima[2]
[1]Univ Rennes, Inria, CNRS, IRISA, F-35000 Rennes, France
[2]Laboratoire de Biométrie et Biologie Evolutive (LBBE), CNRS : UMR5558, Université Claude Bernard - Lyon I (UCBL), INRIA 69622 Villeurbanne, France

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

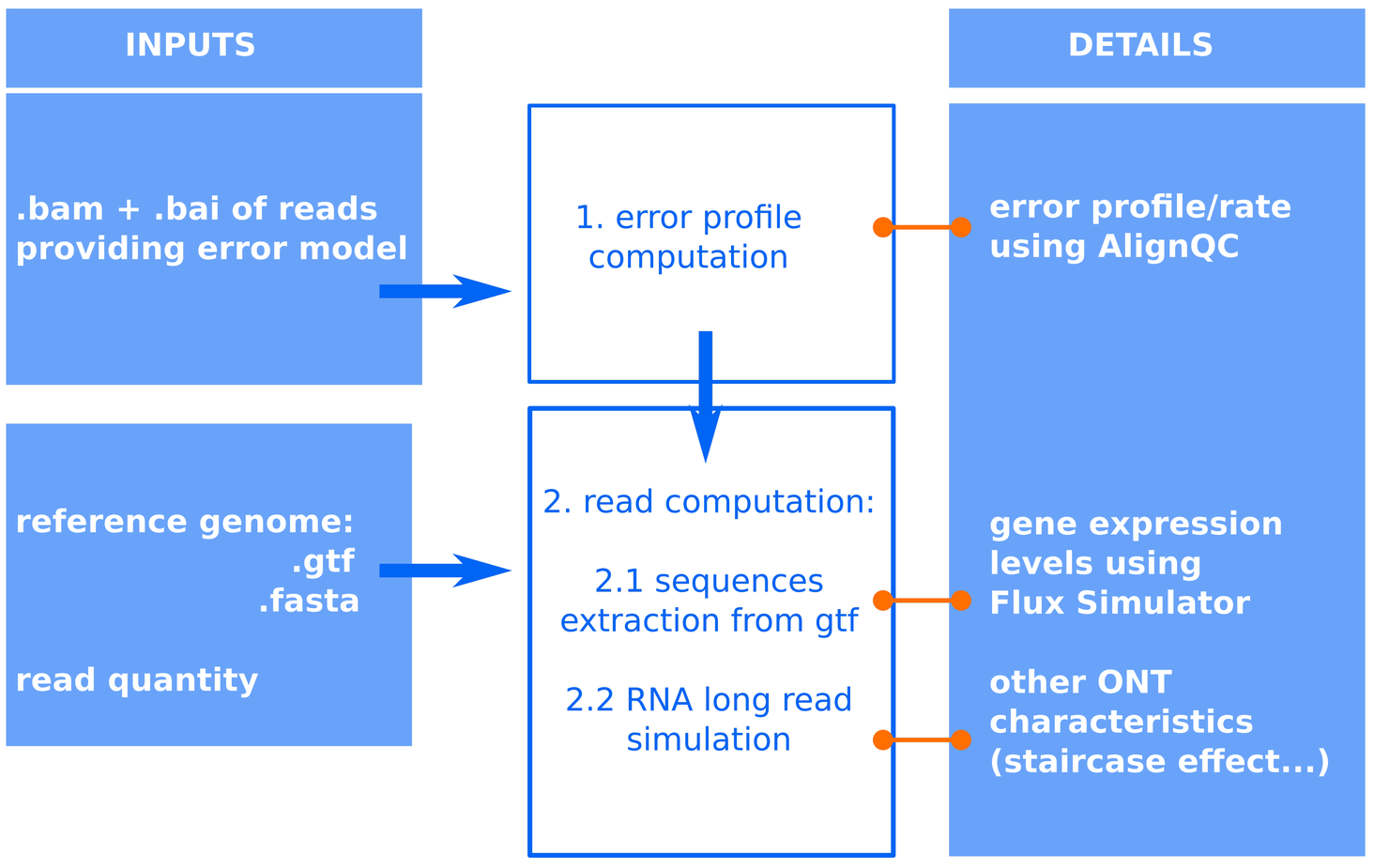
Simulate transcripts

- 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



Experiment

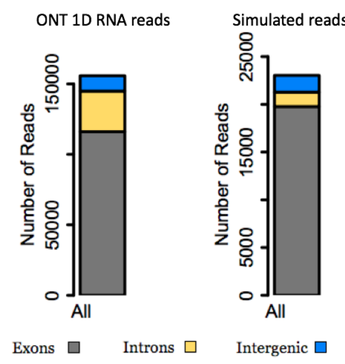
- 300k reads simulation
- Genome reference: *Mus musculus* GRCm38
- Annotation: Ensembl *Mus musculus* GRCm38 release 87
- Template dataset for error training: 740k *Mus musculus* RNA ONT 1D reads
- Comparison of raw reads and simulated reads statistics using AlignQC [2]

Results

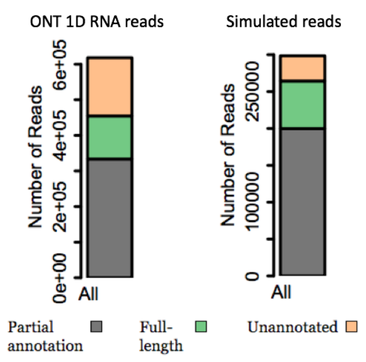
Comparing error rates between ONT 1D RNA reads and the simulated reads shows that the simulator produces reads with realistic error profiles:

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%

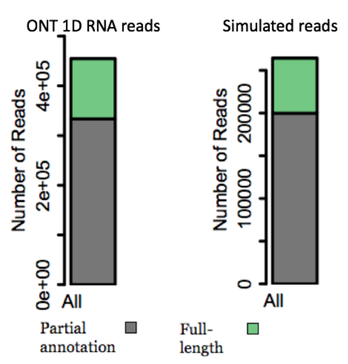
The distribution of reads among genomic features is similar between the real and simulated reads:



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

Acknowledgments

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References

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[3] Thasso Griebel, Benedikt Zacher, Paolo Ribeca, Emanuele Raineri, Vincent Lacroix, Roderic Guigó, and Michael Sammeth. Modelling and simulating generic rna-seq experiments with the flux simulator. *Nucleic acids research*, 40(20):10073–10083, 2012.