Australian National University

TECHNICAL SUMMARY

Detection of RNA modifications at a single molecule-level with nanopore sequencing

RNA modifications are known to perform many critical functions in gene expression. Current RNA sequencing technologies produce a stream of signals that can be decoded to uncover the properties of individual molecules. Most of the existing methods are devoted to extracting the sequence of unmodified nucleotides. Yet, there are more than 150 RNA modifications, and knowledge about their individual function in health and disease is limited by the available detection techniques. To combat these limitations, researchers at The Australian National University (ANU) have come up with a computational algorithm and software tool that offers a new way to analyse and interpret signal data to extend the types of nucleotide modifications that can be detected from nanopore sequencing. These methodologies can be used to study the interplay of RNA modifications and their role in RNA processing.

Software features

> Performs deep learning-based signal analysis for the data collected with a nanopore sequencer device from Oxford Nanopore Technologies
> Best-in-class accuracy and depth of detection
> Methylation (CH_3) Estimation Using Ionic current (CHEUI)
> Two models to detect m6A and m5C modifications (**Fig. 1**)
> Can work on any CUDA GPU-equipped computer
> Does not need to be run in real-time. Saved sequence data can be transferred to the neural network when ready for analysis

Discovery benefits

> **Targeted detection:** allows identification of modifications in specific nucleotides within individual RNA molecules
> **Detection sensitivity:** can detect single nucleotide modifications in rare molecules/genes with low expression, often with just one read
> **Improved diagnostics:** potential to use in the identification of disease-specific molecular interactions
> **Better therapeutics:** improved identification of RNA modifications and protein-RNA interactions to aid in mRNA design for therapeutics
> **Scalable and versatile:** capable of simultaneous detection of multiple modifications and re-analysis of the pre-existing data

Potential applications

> Vaccines and drug design
> Disease diagnostics
> Gene therapy
> Biotech design – mRNA design for protein production, bioremediation, agriculture applications, etc.
> Biotechnology and molecular research
> Synthetic biology - construction of molecules for industrial application and validation of molecular reactions

Opportunity

ANU is seeking collaborative R&D partnerships to further develop and expand this technology. Looking to perform extensive biological and modelling exploration using the computational algorithm and software tool, both academically as well as in a consultancy/services type arrangement. Would like to work with others to generate additional modification models and expand the scope of the technology applicability.

IP status

The algorithm, software implementation, and its related IP are owned by the ANU and are the subject of a patent application.

Related publication:
https://www.biorxiv.org/content/10.1101/2022.03.14.484124v2

Key research team

> Eduardo Eyras, Professor and EMBL Australia Group Leader, The John Curtin School of Medical Research
> Pablo Acera Mateos, PhD student, The John Curtin School of Medical Research
> Jiajia Xu, PhD student, The John Curtin School of Medical Research
> Nikolay Shirokikh, PhD and Group Leader, The John Curtin School of Medical Research

Other affiliation: The Shine-Dalgarno Centre for RNA Innovation.

Contact

Dr Lindsay Hogan
Commercial Development Manager
Commercialisation & IP
Office of Research and Innovation Services
The Australian National University
T: +61 2 6125 1492 | E: lindsay.hogan@anu.edu.au

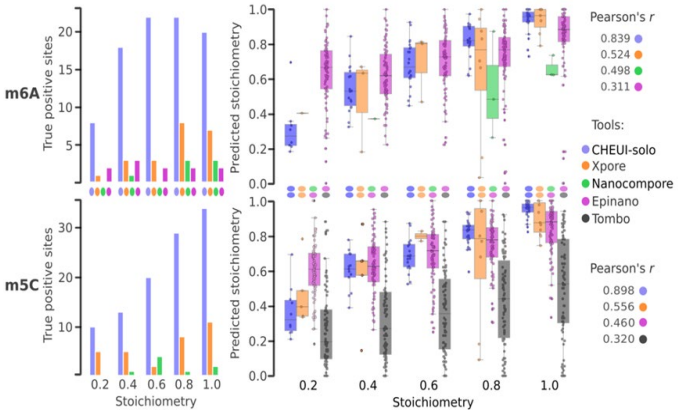

Figure 1: Comparison of CHEUI with other tools for true positives (left panels) and stoichiometry prediction (right panels) at different ground truth stoichiometry values (x axis) using a reference dataset.