

NANOPORE SEQUENCING OF RNA USING REVERSE TRANSCRIPTION

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PATENT STATUS

Country	Type	Number	Dated	Case
Patent Cooperation Treaty	Published Application	WO 2023/196738	10/12/2023	2022-056

BRIEF DESCRIPTION

This invention demonstrates that an engineered cellular reverse transcriptase is a potent motor protein that can processively thread single-stranded RNA (ssRNA) through the MspA biological nanopore in single nucleotide steps while it is synthesizing cDNA. Notably, this represents a first-ever achievement for threading of ssRNA through the engineered *Mycobacterium smegmatis* porin A (MspA) nanopore in discrete steps, and also for ssRNA sequencing with the MspA nanopore.

The inventors constructed the “quadromer map” for ssRNA in the MspA nanopore, which is essentially a table that can convert measured nanopore ion current to RNA sequences, using ssRNAs of known sequences. In addition, the inventors discovered that the single-molecule kinetic rates of the reverse transcriptase are affected by the presence of stable RNA secondary structures. Monitoring this biophysical behavior can be used to determine RNA structures during nanopore sequencing.

Nanopore sequencing is a powerful third generation sequencing technology that offers advantages such as ultra-long read length and direct detection of chemically modified bases. One of the key components of developing a successful nanopore sequencer is identifying potent motor proteins (such as polymerases or helicases) that can thread single-stranded (ss) DNA or ssRNA through the nanopore in discrete steps with high processivity.

SUGGESTED USES

This novel technology can be used:

- » in developing commercial RNA sequencers that can directly sequence RNA extracted from any biological sample
- » to identify expression levels, mutations, secondary structures, and chemical modifications of RNA
- » to sequence full length RNA without the need of fragmentation, which is made possible by the long read nature of nanopore sequencers

ADVANTAGES

The advantages of this nanopore sequencing technology include:

- » ultra-long read length and direct detection of chemically modified bases
- » a potent motor protein that can processively thread ssRNA through the MspA biological nanopore in single nucleotide steps while it is synthesizing cDNA

CONTACT

Laleh Shayesteh
lalehs@berkeley.edu
tel: 510-642-4537.



INVENTORS

- » Marqusee, Susan

OTHER INFORMATION

KEYWORDS

nanopore, expression, RNA, sequencing

CATEGORIZED AS

- » **Biotechnology**
- » Genomics
- » Health
- » **Medical**
- » Research Tools
- » Screening
- » **Nanotechnology**
- » NanoBio

RELATED CASES

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RELATED MATERIALS

ADDITIONAL TECHNOLOGIES BY THESE INVENTORS

- ▶ [Stabilizing an Alternative Conformation of the SARS-CoV-2 Spike Protein](#)



University of California, Berkeley Office of Technology Licensing

2150 Shattuck Avenue, Suite 510, Berkeley, CA 94704

Tel: 510.643.7201 | Fax: 510.642.4566

ipira.berkeley.edu/ | otl-feedback@lists.berkeley.edu

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