Molecular And Computational Biology Methods For Improving Nanopore Sequencing Technology

Tech ID: 32967 / UC Case 2018-406-0

BACKGROUND

Long read sequencing (e.g. nanopore sequencing) involves a tradeoff between the length of the DNA fragment sequenced, which allows for greater ease of data assembly relative to massively parallel sequencing technologies (e.g. Illumina (R) sequencing) and accuracy of individual base calls.

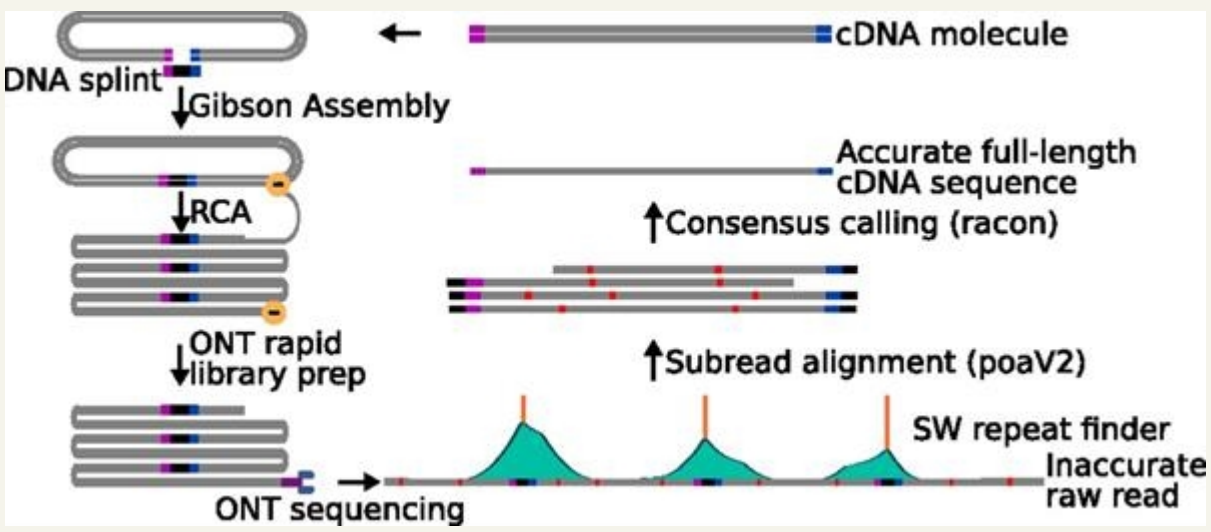
This technology takes advantage of the long read capabilities of nanopore sequencing to *improve* the accuracy of reads of highly variable nucleic acid species, including cDNAs, and which can be highly variable due to alternative RNA splicing.

TECHNOLOGY DESCRIPTION

This technology incorporates both wet lab (concatamer formation by rolling circle amplification) and software (sequence analysis).

The technology involves producing a circularized DNA library via a Gibson Assembly that includes a full-length cDNA and a known heterologous sequence. The Gibson Assembly assures that only full-length cDNA's are included in the library. A rolling circle amplification is performed using the circularized DNA as template. This produces a concatemer or repeating segments of the full-length cDNA and the known heterologous sequence.

A raw sequencing read of the concatemer is obtained using long read sequencing (e.g. nanopore sequencing). The repeating segments in the raw sequencing read can be identified via a Smith-Waterman self-self alignment. From that, a consensus sequence of the full-length cDNA based on the sequences of the repeating segments can be produced.



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OTHER INFORMATION

KEYWORDS

Long Read Sequencing - improved accuracy, Gibson Assembly, Smith-Waterman self-self alignment, Full-length cDNA sequencing, Single Cell Sequencing, Rolling Circle Amplification

CATEGORIZED AS

- **Biotechnology**
  - Bioinformatics
  - Genomics

RELATED CASES

2018-406-0, 2023-911-0

APPLICATIONS

- Long read nucleic acid sequencing
- Single cell cDNA sequencing

ADVANTAGES

- Allows accurate identification of closely related species (e.g. splice variants)
- Allows identification of rare mutants
- Whole cDNA's can be assessed
- Can be used to quantify RNA expression

INTELLECTUAL PROPERTY INFORMATION

Country	Type	Number	Dated	Case
United States Of America	Published Application	20210079461	03/18/2021	2018-406

RELATED MATERIALS

- Improving nanopore read accuracy with the R2C2 method enables the sequencing of highly multiplexed full-length single-cell cDNA - 09/10/2018

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