

Editorial

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## Challenges in Transcriptome Applications Using Oxford Nanopore Sequencing Technology Kan Liu<sup>1,2</sup>

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## Editorial

Technological breakthroughs in sequencing enabled huge impact in biological advancements. Recently a nanopore-based portable device for long-read sequencer (MinION) was developed for single molecule sequencing (third-generation sequencing) by measuring characteristic current changes of the bases. Nanopore RNA sequencing aims to sequence complete RNA strands directly and in real-time, which means it sequences the full-length of transcripts either with or without PCR amplification [1]. The ability of MinION to generate very long read (1-100 kb) at a low capital cost presents the opportunity for many important applications in genomics and transcriptomics [2]. While next-generation sequencing data usually have lower error rates and have become the mainstream target of sequence analysis, short reads cannot offer sufficient information for long continuous assemblies of genome or full-length transcripts identification. MinION RNA-seq data are necessary to investigate isoforms usage, novel exon detection and allelic-specific expression. However, the applications of reads generated with the instrument are far from satisfaction due to its current limits and disadvantages.

MinION, like its peer PacBio [3], has the advantage of long read lengths yet higher sequencing error rates and lower throughput compared with the next-generation sequencing platform such as Illumina [4]. Usually, many MinION reads fail to be mappable due to the high sequencing error of MinION. Due to such limits, high quality short reads or thoroughly annotated reference genome sequences are always indispensable to improve the error correction of MinION data [5]. Currently, some researches using MinION data for the transcriptomic sequencing were reported due to its late publish. In the work of Goodwin et al. [6], the nanopore reads had a high proportion of error reads that should be preprocessed before the read alignment. A thorough comparison of the performance between RNA-seq data of MinION and other sequencers present the result including isoform identification, quantification and discovery of complex transcriptome events [7]. Based on the error pattern of MinION reads, SNP and small insertion/deletion calling might be erroneous with a large proportion of false positives. A significant number of chimeric reads were also identified, which may be generated by either library preparation artifacts or failure of removing adapters. Therefore, besides improving the sequencing quality and accuracy of the instrument itself, sophisticated data analysis and bioinformatics methods are also urgently needed for MinION read processing such as error correction and read alignment algorithm for low quality long-reads.

Despite such obstacles, the long length of MinION is yet very informative for transcriptome analysis, especially for discovery of novel genes and isoforms. Suzuki et al. described a new approach to phase alternative splicing events detection designed for long-read RNA-seq data [8]. Bolisetty et al. discovered a high isoform discovery of Drosophila samples, which illustrated the utility of MinION reads in characterizing complex transcriptomic regulations [9]. Although many methods have been developed to predict or identify differential alternative splicing events or isoforms for short-read RNA-seq data, software tools for long-read RNA-seq analysis for the alternative splicing are not intensively developed. Robust evaluation tools and analytical software for the rapidly-evolving technology should be on the horizon to achieve a better application of MinION data.

Although MinION data showed high error rate and context-specific mismatches, the advantages are also obvious, such as long read length, high throughput, and low cost, which make MinION promising for a broader area of applications. The combination of MinION data and other short-read data for the transcriptomic analysis is an acceptable protocol to offset the disadvantages discussed above.

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