Nanopore Based Full-length mRNA Sequencing

BMKGENE

RNA sequencing has been an invaluable tool for comprehensive transcriptome analysis. Doubtlessly, traditional short-read sequencing achieved numerous important development in here. Nevertheless, it often encounters limitations in full-length isoform identifications, quantification, PCR bias

Nanopore sequencing distinguishes itself from other sequencing platforms, in that the nucleotides are read directly without DNA synthesis and generates long read at tens of kilobases. This empowers direct read-out crossing full-length transcripts and tackling the challenges in isoform-level studies

Service Specifications

Library	Platform	Data recommended	Data QC	Sample requirements
cDNA-PCR (ployA)	PromethION P48	6 Gb	Average quality score: Q10 Full-length ratio≥70%	Conc. ≥ 100 ng/μl Total amount ＞ 1.2 μg RIN ≥ 7-7.5 Limited or no DNA and protein contamination

Bioinformatics Analysis Content

1. Raw data QC
2. Full-length transcripts identification
3. Reference genome alignment
4. Isoform-level expression

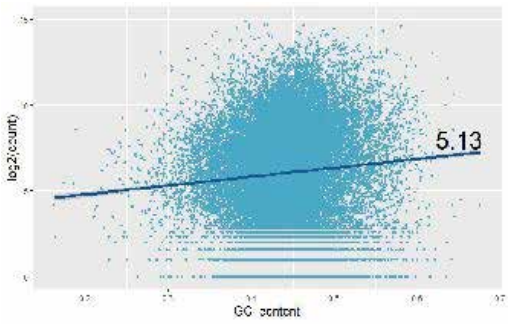
• Functional Interpretation on DEGs and DETs •

- √ Expression in isoform level
- √ Differentially expressed genes (DEGs)
- √ Differentially expressed transcripts(DETs)
- √ Function annotation and enrichment
- √ Fusion genes
- √ Alternative splicing analysis and differential alternative splicing events
- √ Alternative poly-adenylation (APA)
- √ Gene structure optimization
- √ Novel transcripts prediction

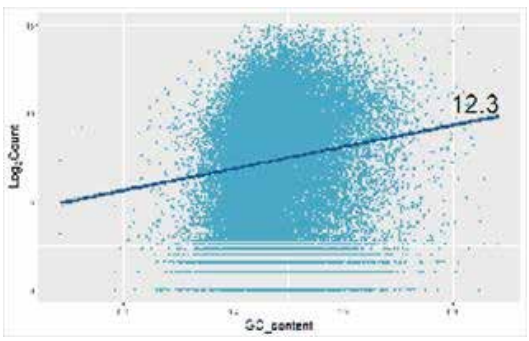
Service Higlights

• Low sequence-specific bias

GC-bias, a constant issue in traditional methods, leads to under-representation of sequences with low or high-level GC contents. Nanopore overcome the challenges by introducing much less-biased read with limited PCR reactions.



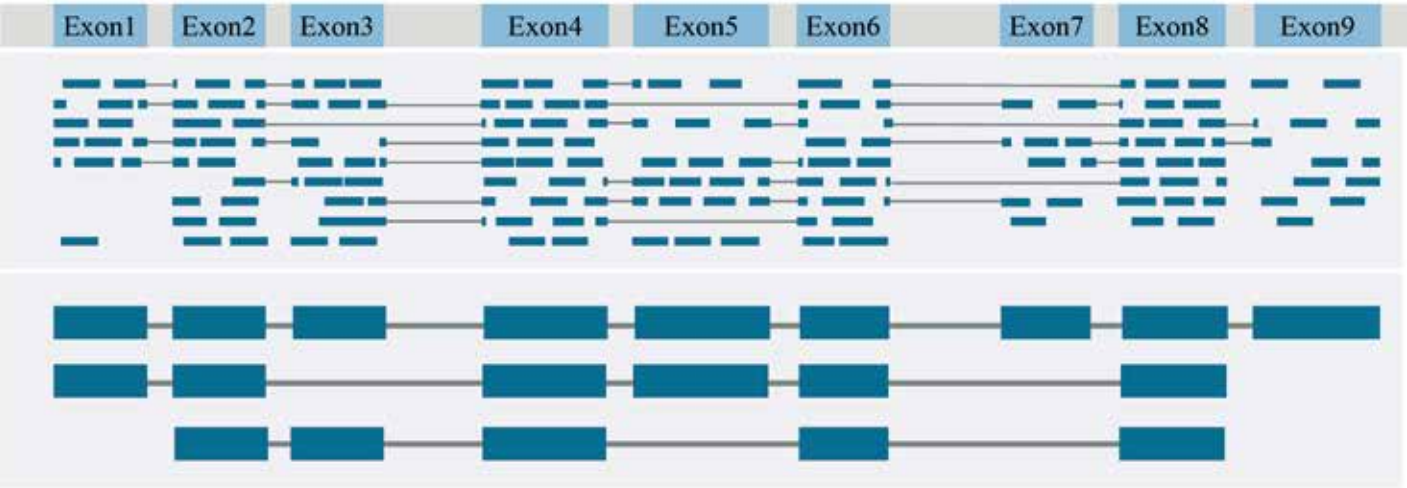
Nanopore



Next Generation Sequencing

• Identification of multiple isoforms per gene

Assembly-based alternative splicing events identification is always problematic due to in identifying all constituent exons. enables generating of complete transcript isoform sequences, which achieves isoform-level resolution. This makes complex transcriptome structural studies possible.

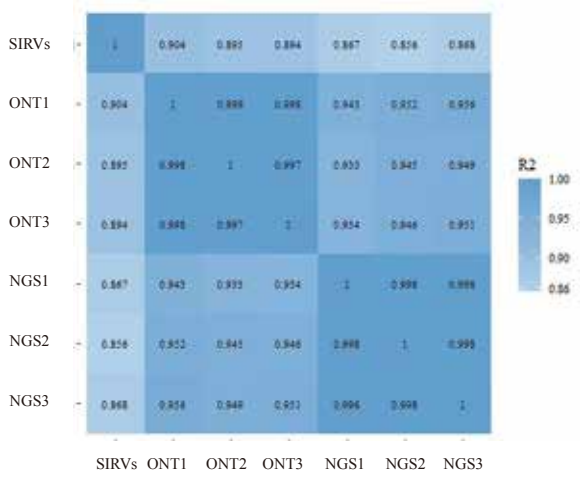


• Less data required to cover same number of transcripts

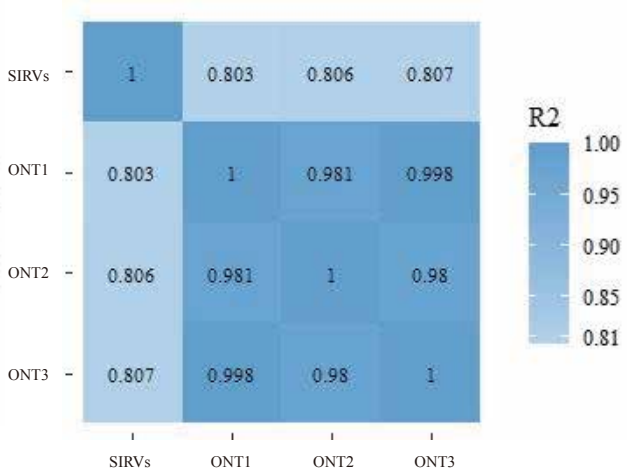
Full-length read out of cDNA molecule could largely reduced multiple-locus alignments, which increased data usage. Compared to short-read technologies, nanopore requires 7-folder fewer data to cover the same number of transcripts.

• Expression quantification in isoform level

Differential expression analysis in gene level is very likely to mask the changes in isoform level. With reliable isoform identification capacity, nanopore empowers both more accurate gene expression quantification and that in transcripts. In-house data of BMK demonstrated that by introducing SIRVs as known standards, the accuracy of Nanopore-based accuracy achieved over 90%, which is higher than that of NGS-based expression quantification. The performance in isoform level expression was also shown to be impressive , which achieved 80%.



Correlatio among standards (SIRVs), Nanopore (ONT) and NGS in gene expression



Correlation between standards and Nanopore in Transcript quantification

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