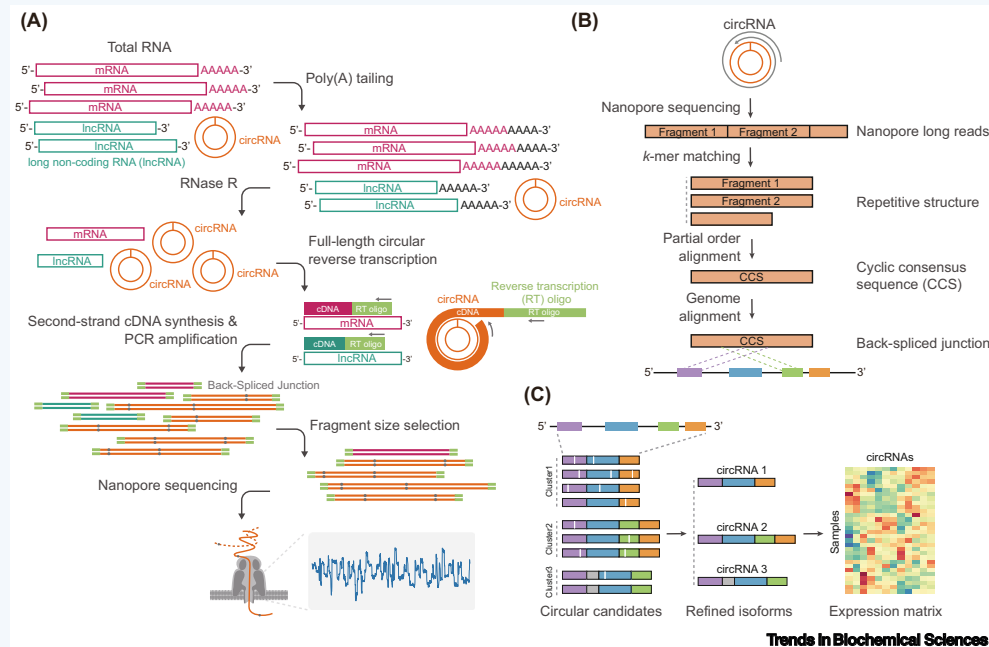


Characterizing Circular RNAs Using Nanopore Sequencing

Jinyang Zhang ^{1,2} and Fangqing Zhao ^{1,2,*}

¹Beijing Institutes of Life Science, Chinese Academy of Sciences, Beijing 100101, China

²University of Chinese Academy of Sciences, Beijing 100049, China



ADVANTAGES:

Rolling circle reverse transcription produces long cDNA molecules containing multiple copies of full-length circRNA sequences, providing direct evidence of the internal structure and presence of the circular templates.

CIRI-long uses poly(A) tailing and fragment size selection for the enrichment of circRNA-derived cDNAs, which allows effective detection of circRNAs with variable lengths using the optimized protocol.

The CIRI-long algorithm has been optimized for accurate detection of full-length circRNAs using error-prone nanopore reads.

CIRI-long determines the widespread alternative circularization and alternative splicing events with better sensitivity than previous methods using Illumina short-read sequencing strategies.

The reconstruction of full-length sequences of circular RNAs (circRNAs) provides important information for circRNA prioritization and function prediction. circRNA identifier using long-read sequencing data (CIRI-long) is a comprehensive experimental and computational approach to determine full-length circRNA isoforms using nanopore sequencing. Using rolling circular reverse transcription, CIRI-long constructs long cDNA libraries containing multiple full-length template sequences and characterizes circRNA structures using a *k*-mer based strategy.

CIRI-long implements partial order alignment to generate error-corrected circRNA sequences and uses dynamic programming for the aggregation of results from multiple samples. CIRI-long can thereby effectively reconstruct the full-length sequences of refined circRNA isoforms.

The CIRI-long method reveals the complex diversity of circRNAs generated from alternative splicing and alternative circularization events. This new method also provides strong evidence for the existence of identified circRNAs.

CIRI-long provides insights into the diversity of circRNAs, including the mitochondria-derived circRNAs, transcriptional read-through circRNAs, and a novel type of intronic self-ligated circRNAs.

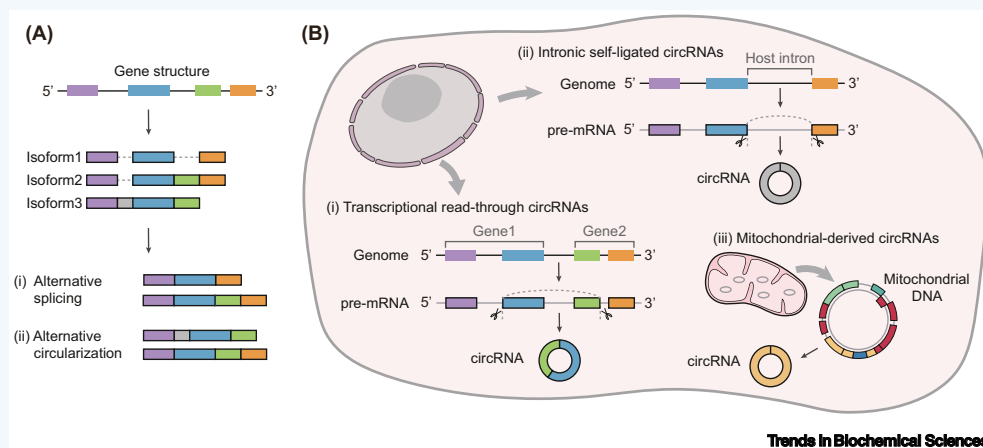
CHALLENGES:

The relatively high error rate of nanopore sequencing affects the accuracy of circRNA detection, which has been improved with the update of the latest R10.3 nanopore chemistry.

CIRI-long requires fragment size selection, which may result in the preference for detecting longer circRNAs.

CIRI-long requires a high sequencing depth of nanopore reads to achieve the saturated detection of circRNAs, which makes it costly compared with other Illumina-based techniques.

*Correspondence:
zhfq@biols.ac.cn (F. Zhao).
Twitter: @Fangqing_Zhao.



Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (32025009, 31722031, 91640117, 91940306).

Declaration of Interests

The authors have no interests to declare.

Literature

1. Zhang, J. *et al.* (2021) Comprehensive profiling of circular RNAs with nanopore sequencing and CIRI-long. *Nat. Biotechnol.* 39, 836–845
2. Xin, R. *et al.* (2021) isoCirc catalogs full-length circular RNA isoforms in human transcriptomes. *Nat. Commun.* 12, 266
3. Zhao, Q. *et al.* (2020) Targeting mitochondria-located circRNA SCAR alleviates NASH via reducing mROS output. *Cell* 183, 76–93.e22
4. Chen, L.-L. (2020) The expanding regulatory mechanisms and cellular functions of circular RNAs. *Nat. Rev. Mol. Cell Biol.* 21, 475–490
5. Zheng, Y. *et al.* (2019) Reconstruction of full-length circular RNAs enables isoform-level quantification. *Genome Med.* 11, 2
6. Gao, Y. *et al.* (2016) Comprehensive identification of internal structure and alternative splicing events in circular RNAs. *Nat. Commun.* 7, 12060
7. Zhang, J. *et al.* (2020) Accurate quantification of circular RNAs identifies extensive circular isoform switching events. *Nat. Commun.* 11, 90
8. Gao, Y. *et al.* (2018) Computational strategies for exploring circular RNAs. *Trends Genet.* 34, 389–400
9. Ji, P. *et al.* (2019) Expanded expression landscape and prioritization of circular RNAs in mammals. *Cell Rep.* 26, 3444–3460.e5
10. Wu, W. *et al.* (2020) CircAtlas: an integrated resource of one million highly accurate circular RNAs from 1070 vertebrate transcriptomes. *Genome Biol.* 21, 101
11. Gao, Y. *et al.* (2018) Circular RNA identification based on multiple seed matching. *Brief. Bioinform.* 19, 803–810
12. Gao, Y. *et al.* (2015) CIRI: an efficient and unbiased algorithm for *de novo* circular RNA identification. *Genome Biol.* 16, 4