Trends in Biochemical Sciences | Technology of the Month

Characterizing Circular RNAs Using Nanopore Sequencing

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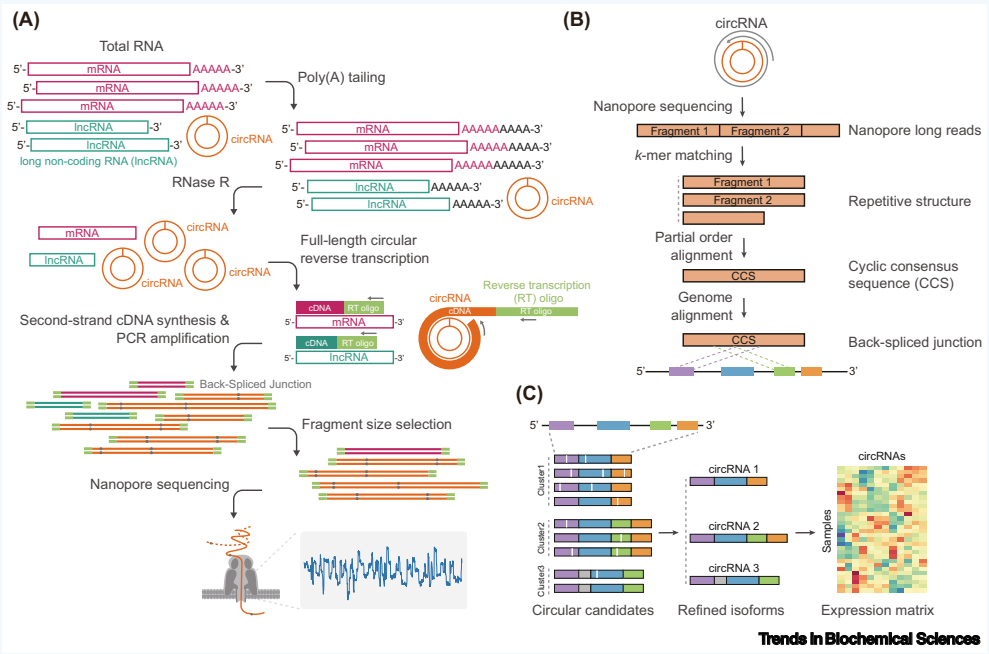
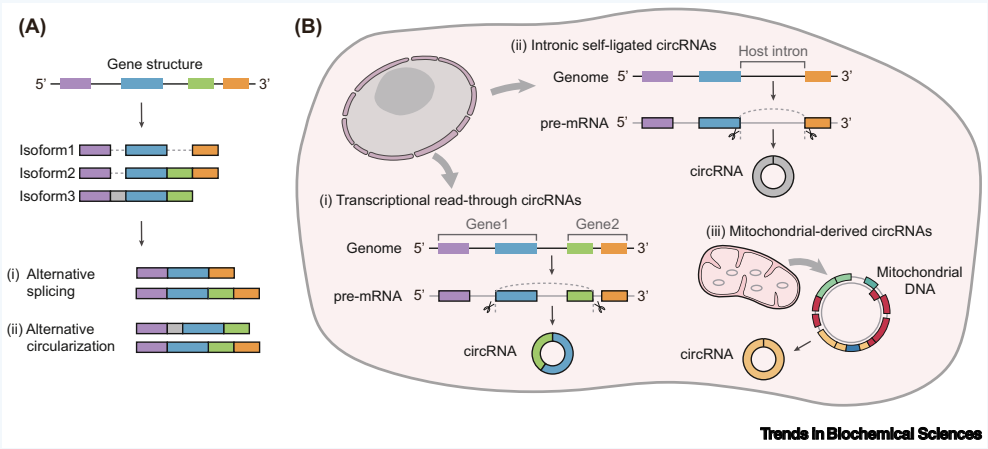


The reconstruction of full-length sequences of circular RNAs (circRNAs) provides important information for circRNA prioritization and function prediction. circRNA identifier using long-read sequencing data (CIRI-long) is a comprehensive experimental and computational approach to determine full-length 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.



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.

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.

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Declaration of Interests

The authors have no interests to declare.

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