Supplementary Table-1 (ST1)

Comparison CE2-CIRI2

CIRCexplorer2	CIRI2	Recommendations/Limitations
mapping (outside) > detection of circRNAs > annotation (and validation)	mapping > characterization of circRNAs	
Alignment to reference genome		
CE2 is compatible with many aligners. However, our experience is limited to CE2 analysis of the mapping performed by STAR (mode paired-end).	CIRI2 is able to use only mapping performed by BWA-MEM.	Statistics are never identical when you used CE2 and CIRI2. Statistics about the number of reads considered and statistics of the number of reads supporting a circular junction.
Main method/filter to avoid false positive		
The use of an annotation file to validate only annotated circRNAs	Identification of a backsplicing signature outside the interval defined by the coordinates of the circRNA.	
For CE2, the signature of backsplicing is the presence of the end of an exon next to the start of an upstream exon in reads supporting the circular junction.	CIRI2 search of canonical splicing sites (SS) as backsplicing signatures.	
Output List		
More concise output because the output list contains only annotated circRNAs.	Generally, produces a more extensive list.	Unfortunately, novice users often select a tool with a reputation for producing a long list of circRNAs. This choice can lead to a high risk of false positives and unknowns.
Do not use the CE2 pre-output file (before annotation) under the pretext that the genome-annotation file is poor.		
Threshold on the number of reads supporting the circular junction		Applying a threshold on the number of reads supporting the circular junction improves the relevance of detected circRNAs. But It is preferable to apply this threshold to the final output list.
This option is not proposed by CE2	We recommend applying a threshold, but we advise against using this option to do so	
Other thresholds		
CE2 can report a circRNA if its genomic size is < 999,999 pb.	CIRI2 can report a circRNA only when 135 pb < genomic size < 199,999 pb.	Filter out exonic circRNAs shorter than 200 bp to improve data quality.
We observed that CE2 is able to report exonic circRNA with a genomic size of 30 pb.		
We observed that CE2 is able to report circRNAs from mitochondrial genome.	CIRI2 does not report circRNAs from mitochondrial genome.	Do not retain circRNAs from mitochondrial genome
False Positives		
Infrequent	Often generates a list with more false positives, complicating data refinement.	Be particularly wary of a circRNA tool that provides output lists of variable length within the same group of samples (biological replicates).

CIRCexplorer2	CIRI2	Recommendations/Limitations
Use of a genome-annotation file		
Essential for CE2 to produce the output list containing only circRNAs validated by CE2.	Optional (no effect on the length of the output file).	Consider that CIRI2 infers information rather than providing true annotations.
circRNA annotation		
The use of a genome-annotation file is required for the second step.	Requiring the annotation to be performed by CIRI2 is not a full satisfying option.	Do not confuse identification of the gene present in the region with identification of the parent gene.
Contents of the circRNA output list		
CE2 recognizes backspliced circRNAs and identifies the parental gene. The exons involved in backsplicing are clearly identified.	CIRI2 infers information (from reference genome and annotation file) to suggest a parental gene but does not recognize specific exons involved.	Personal observation: Most CIRI2 circRNAs "from exons" result from backsplicing of known exons, especially with a threshold of 4–5 supporting reads. Personal observation: Only 80 % of exonic circRNAs identified by CE2 are also identified by CIRI2.
CE2 also identifies circRNAs susceptible to derive from introns likely to be ciRNAs or intronic circles. All are improperly labeled as "ciRNAs."	Rarely includes ciRNAs or intronic circles (due to the specificity of CIRI2 in search of canonical SS as a backsplicing signature).	Use a tool optimized for single-end mapping for accurate identification of ciRNAs and intronic circles.
The identification of ciRNA and intron circle should be completed by the user.	The circRNAs identified by CIRI2 in intronic regions are based on hypotheses about potential splice sites used in backsplicing.	Carefully review and validate results to minimize false positive.
Completely impossible to find circRNAs in unannotated regions	Can identify circRNAs in unannotated regions but relies on hypotheses about potential splice sites used in backsplicing.	Be cautious about false positives generated by this approach.
Completely impossible detection of subexonic circRNA from single-exon genes	Highly unlikely detection of subexonic circRNA from single-exon genes	
Evaluation of the expression of linear counterparts		
Not possible by using only CE2.	Not possible by using only CIRI2.	CirComPara2 is a tool which aggregates circRNA lists from seven methods, retaining only those detected by at least two approaches
CE2 is compatible with CLEAR module (CE3)	CIRI2 is compatible with CIRIquant	
This calculation is supported by the number of split reads that are just outside of the exonic circRNA. (CLEAR module uses the same STAR-PE mapping).	CIRIquant performs a new alignment against pseudo circRNA reference sequences and reference genome using HISAT2	CirComPara2 includes a procedure to calculate the linear expression related to the circRNAs.

For references

CE2 Zhang et al. 2016 [25] and Ma et al. 2021 [24]

CE3 see Ma et al. 2019 [44]

CIRI2 see Gao et al. 2018 [26] Gao et al. 2015 [27]

CIRIquant see Zhang et al. 2020 [40]

CirComPara2 see Gaffo et al. [41]