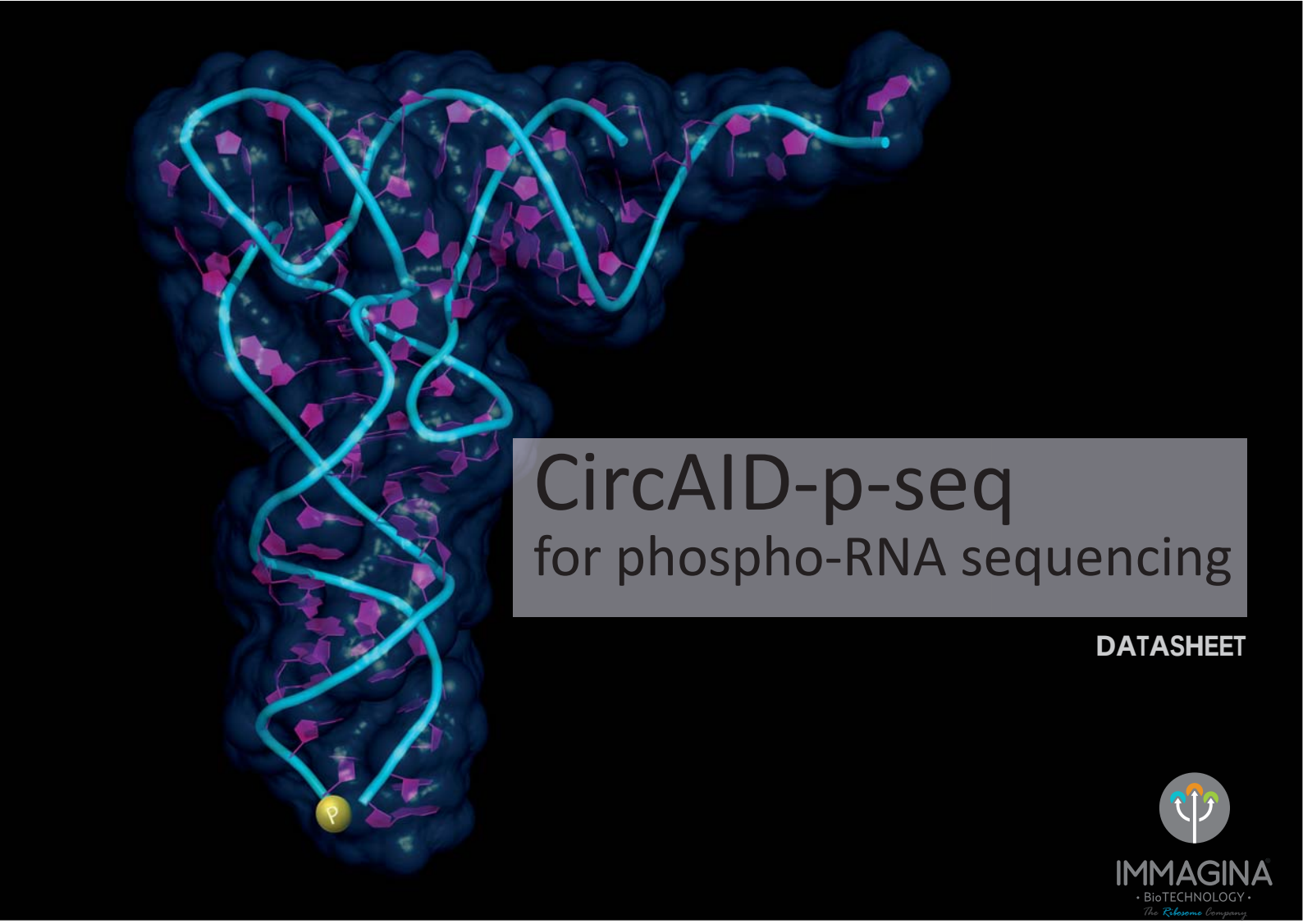

CircAID-p-seq
for phospho-RNA sequencing

DATASHEET

IMMAGINA BioTECHNOLOGY
The Ribosome Company

Introduction

CircAID-p-seq (Circular Amplification and IDentification of short RNA sequences bearing a 3' Phosphate) kit uses our proprietary technology, to generate Oxford Nanopore-compatible sequencing libraries without the need for PCR amplification.

The kit is designed for a quick (1-day) high quality library preparation for short RNAs (20-50 nt) bearing a 3'-phosphate/2',3'-cyclic phosphate (3'-P/2',3'-cP) end.

The final library product is suitable for third-generation sequencing on the Oxford Nanopore platform, thus enabling real-time single-molecule detection of biologically relevant RNA species.

Highlights

Phospho-RNA sequencing

- Selective sequencing of short RNAs bearing a 3'P or 2',3' cP end.

Short and simple workflow

- Quick (1-day) high quality library preparation;
- No PCR amplification.

Wide range of applications

- RNA footprinting;
- Ribosome Profiling;
- Transcriptome analysis.

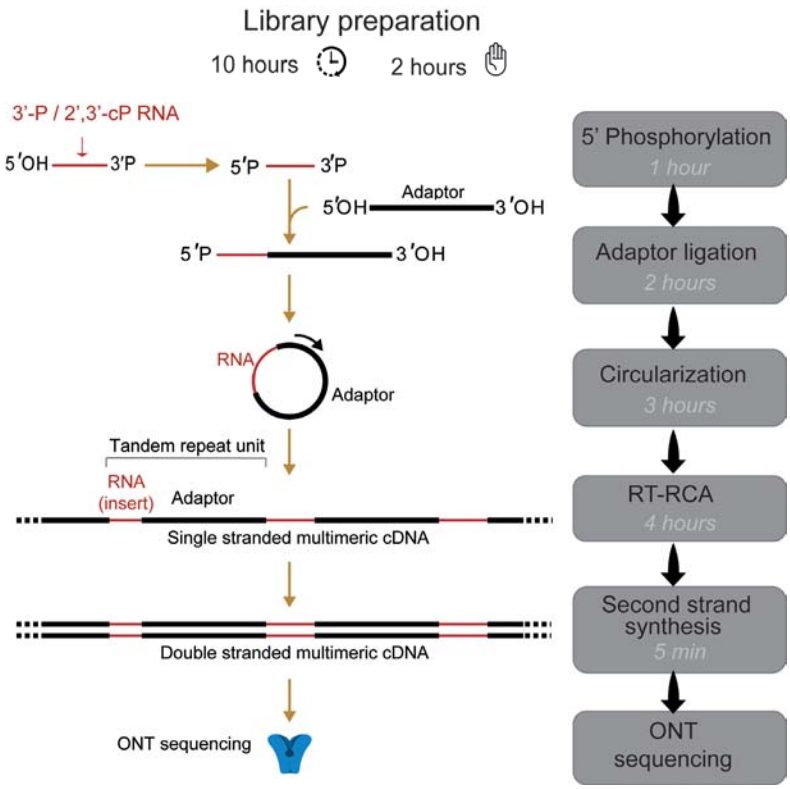

Fig. 1: Wokflow for circAID-p-seq library preparation.

circAID-p-seq workflow

The complete workflow comprises five steps (Fig. 1): (1) phosphorylation, (2) ligation, (3) intramolecular circularization, (4) RT-RCA-multimeric cDNA synthesis, (5) second strand cDNA synthesis. The phosphorylation of the 5' terminus is required to block self-ligation. The circular RNA-adaptor is then subjected to RT-RCA to synthesise the first cDNA strand and generate long single stranded cDNA molecules carrying multiple copies of the RNA fragment. The final cDNA library, after second strand synthesis, is then suitable for ONT library preparation kit (SQK-DCS109 and SQK-LSK109) and can be sequenced on all Oxford Nanopore device according to manufacturer's recommendations.

circAidMe: a dedicated bioinformatic pipeline

The sequencing output is analysed with an in-house computational pipeline (Fig. 2) called circAidMe (available on demand) to accurately extract the sequence of the RNA fragments from the multimeric cDNA molecules. CircAidMe generates a highly accurate consensus sequence of each RNA fragments.

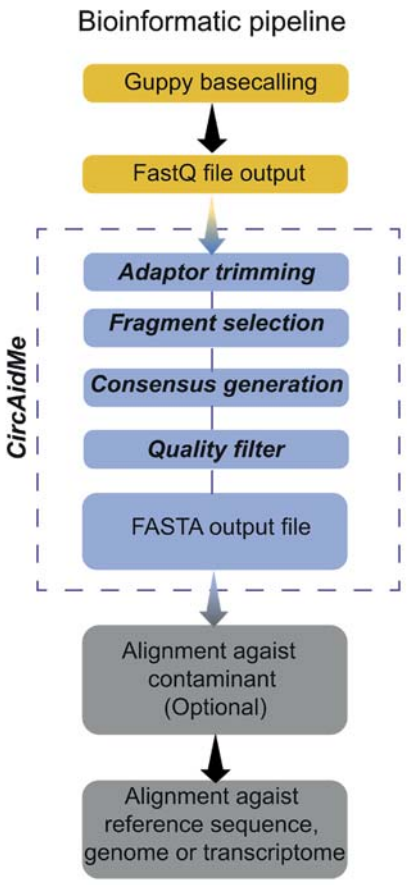

Fig. 2: Workflow of the circAidMe pipeline.

Biological relevance

RNA molecules bearing a 3'-P/2'-3'cP are generated by ribonucleases, ribozymes or toxins. The RNA fragments produced by these endonuclease activities have been involved in several biological processes, such as RNA metabolism, rRNA and tRNA biogenesis[1], mRNA splicing, unfolding protein response and stress granules production. In addition, the of 3'-P/2'-3'cP RNA fragments produced inside the cell and/or released in biological fluids were found to be deregulated in several diseases, such as cancer, viral infection and Amyotrophic Lateral Sclerosis[2]. Therefore, RNA bearing a 3' end phosphate are likely potential markers of desease, nonetheless currently available library preparation and RNA-seq analysis are biased against 3'-P/2'-3'cP ends resulting in a potential loss of useful information[3,4].

(1) Ivanov P et al., Mol Cell. (2011).
(2) Thiyagarajan N et al., Nat Commun. (2012).
(3) Honda S et al., Proc Natl Acad Sci (2015).
(4) Giraldez MD et al., EMBO J. (2019).

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Ordering information

Product name	Catalog no.	No. of reactions
CircAID-p-seq for phospho-RNA-seq	#CA001	6

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