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Licensing Opportunity

TTO – Technology Transfer Office

Construction of Sequencing libraries from RNA using tailing and ligation of cDNA (TLC)

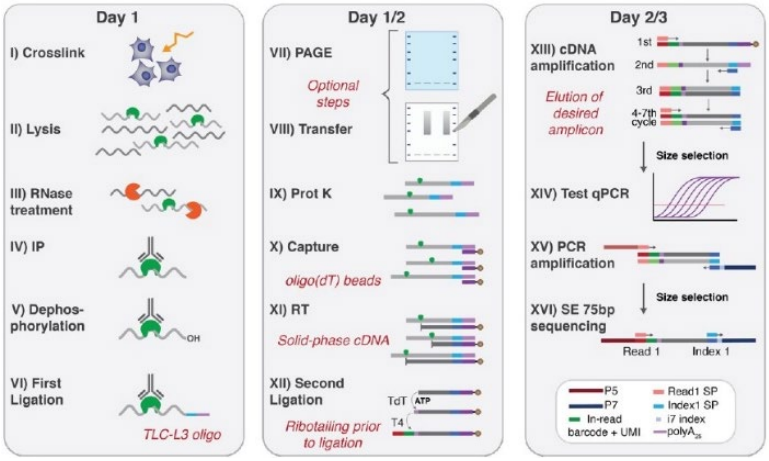

Figure 1: Example of TLC Library Preparation applied to the crosslinking and immunoprecipitation (CLIP) workflow to profile RNA-protein interactions.

Ref. Nr

6.2306

Keywords

RNA sequencing, NGS, library preparation, CLIP

Intellectual Property

EP Application No.22166453.5
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Publication

"Efficient and sensitive profiling of RNA-protein interactions using TLC-CLIP", bioRxiv (2022) 488168

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Description

The generation of double-stranded cDNA is a crucial step during the preparation of high-throughput sequencing libraries starting from RNA input material. In many cases the preservation of, and priming from the original 3' end of the first-strand cDNA molecule is desirable to extract additional information.

Tailing and ligation of cDNA (TLC) provides an alternative strategy to generate full-length cDNA, preserving the original 5' end of the template RNA molecule.

TLC relies on the incorporation of a short stretch of non-template ribonucleotides to the 3' end of first-strand cDNA molecules, which effectively mimics the 3' end of an RNA molecule to increase the efficiency of subsequent ligation reactions. TLC, unlike frequently used template switch oligos (TSOs), uncouples the tailing reaction from reverse transcription, giving higher flexibility in RT conditions, such as higher reaction temperatures that are beneficial for long and/or structured molecules. Unlike TSO-based approaches, TLC is not prone to concatemerization, thus reducing the amount of non-specific background.

TLC allows the generation of sequencing libraries form low amounts of RNA and offers a fully bead-based library preparation strategy that can be implemented on liquid-handling platforms.

We have demonstrated the feasibility of TLC in combination with cross-linking and immune-precipitation (CLIP) to profile RNA-protein interactions at nucleotide resolution starting from as few as 500 cells.

Advantages

- Alternative strategy to template switch oligos (TSOs)
- Higher flexibility in reverse transcription conditions improves transcript coverage of long or structured RNAs
- No adapter concatemerization
- Fully bead-based library preparation strategy amenable to automation

Applications

Library Preparation for high-throughput sequencing from various RNA input sources:

- Cross-linking and immunoprecipitation (CLIP) Single-cell RNA sequencing
- Full-length cDNA sequencing on long-read sequencing platforms
- SmallRNA sequencing
- Ribosome footprinting
- Profiling of 5' ends of RNA molecules
- Profiling of RNA modifications