

Technology Offer

Unique multifunctional oligonucleotide enabling ultrafast RNA-protein interactions in cells and other uses thereof

Ref.-No.: MI 0214-5029-MG-ZE

Unique multifunctional oligonucleotide for the ultrafast transcriptome-wide identification of RNA-binding protein (RBP) targets.

Background

RNA-binding proteins (RBP) play a central role in RNA-metabolism and therefore regulate gene expression by posttranscriptional modifications. The identification of *in vivo* targets of RBPs is crucial for the understanding of complex gene regulatory networks and RBP-related diseases such as neurological, sensory and muscular disorders or cancer.

Commonly used state of the art methods for the identification of RBP-RNA interaction sites are often time consuming and cost-intensive due to multiple gel electrophoresis steps and the use of radioactivity. Hence there is an urgent need to develop a faster, scalable, more efficient and cost-saving alternative to the established protocols that provides sequencing results of the same quality.

Technology

Scientists at the Max-Planck-Institute of Immunobiology and Epigenetics developed a RNA-cloning technique comprising a unique multifunctional oligonucleotide and an optimized protocol for the transcriptome-wide identification of targets of RBPs to generating a sequencing library in 1.5 days (1,2). In a first sample preparation step RBPs are cross-linked *in vivo* to their targets and the resulting complexes are subsequently enriched by affinity purification. In a second step the multifunctional oligonucleotide is applied which harbors the following properties:

- Reversibly blocked to suppress undesired product formation
- Self-primed for reverse transcription
- sequence for multiplexing and error correction
- binding-sites for library oligonucleotides

This unique oligonucleotide design enables the rapid conversion of RBP-bound RNA into circularized DNA without the addition of any additional linkers or oligonucleotides. The resulting DNA is eventually the template for the generation of the final sequencing library (1,2).

Benefits & applications

- transcriptome-wide identification of targets of RBPs is matter of hours and not of days anymore
- cost-saving due to abandonment of the use of radioactivity and electrophoresis steps
- high quality sequencing data
- improved efficiency by multiplexing (at least 12 samples processed in parallel) or even high-throughput approaches

This method is compatible to various next generation sequencing platforms such as Illumina™ or Ion Torrent™.

Further applications of the oligonucleotide

- development of Multiplexed Affinity Purification of Capped RNA (MAPCap) that allows ultra-fast and high-resolution detection of transcription start sites (3)
- pull-down of modified RNA such as m6A

Patent Information

In July 2015 an EP priority application was filed. The patent application was regionalized in EP and US.

References

1. Ibrahim Avsar Ilik, Tugce Aktas, Daniel Maticzka, Rolf Backofen, Asifa Akhtar, FLASH: ultra-fast protocol to identify RNA–protein interactions in cells, *Nucleic Acids Research*, Volume 48, Issue 3, 20 February 2020, Page e15, <https://doi.org/10.1093/nar/gkz1141>
2. Aktaş, T., Avşar Ilik, İ., Maticzka, D. et al. DHX9 suppresses RNA processing defects originating from the Alu invasion of the human genome. *Nature* 544, 115–119 (2017). <https://doi.org/10.1038/nature21715>
3. Bhardwaj, V., Semplicio, G., Erdogdu, N.U. *et al.* MAPCap allows high-resolution detection and differential expression analysis of transcription start sites. *Nat Commun* 10, 3219 (2019). <https://doi.org/10.1038/s41467-019-11115-x>

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