Title: DIR-CLIP, a method for the discovery of RNA-binding protein sites on full length RNAs

Authors:
Mauro Scaravilli[1], Mohamed N.M. Bahrudeen[1], Zileena Zahir[2], Katrina Woodward[3], Aditya Sethi[3], Eduardo Eyras[3], Nikolay E. Shirokikh[3] and Minna-Liisa Änkö[1,2,4]

[1]Faculty of Medicine and Health Technology (MET), Tampere University, Tampere, Finland. [2]Hudson Institute of Medical Research, Melbourne, Australia. [3]The John Curtin School of Medical Research, The Australian National University, Canberra, Australia. [4]Department of Molecular and Translational Science, Monash University, Melbourne, Australia.

Keywords:
Molecular biology, bioengineering and biotechnology.

Abstract
RNA binding proteins dynamically interact with RNA molecules to regulate all aspects of the life of RNA, from biogenesis to degradation. The characterization of protein-bound RNA molecules and the determination of RNA binding protein sites within target RNAs represent fundamental steps in understanding how gene expression is regulated in cells and tissues. Importantly, defects in RNA regulation play a central role in the molecular pathogenesis of many human diseases.
CLIP-seq methods (crosslinking immunoprecipitation followed by sequencing) take advantage of the fact that proteins and RNA located in close proximity can be covalently crosslinked using UV irradiation and enable the mapping of RNA binding protein target sites at high resolution.
However, these methods require the fragmentation of RNA with RNase treatment and the conversion of RNA molecules into cDNA followed by amplification with PCR. Therefore, this approach does not allow the determination of the RNA binding sites within full-length RNA molecules in their native state, and the PCR step introduces an amplification bias.
The aim of this project is to establish a novel CLIP method, named DIR-CLIP (direct-CLIP), to map RNA binding sites in the context of full-length, native RNA molecules using amplification-free long-read sequencing technologies (Oxford Nanopore). DIR-CLIP addresses the major limitations of current CLIP-seq methods by providing a complete map of protein-RNA interactions not limited to a single RNA binding protein or RNA molecule.