GRAND-SLAM - The Method for quantification of new and old RNA

Reference No: B78083

CHALLENGE

The abundance of all expressed RNAs can be determined by RNA-seq experiments on genome scale. All mRNAs of a sample are fragmented, transcribed into cDNA and millions of these fragments are then analyzed by next-generation sequencing. The number of sequencing „reads“ obtained for a specific mRNA can be used as a measure of its expression strength. If the research question involves studying rapid processes, only a small part of changes in the transcription or degradation rate are reflected in total RNA quantities. RNAs with different turn-over rates (in particular mRNAs with medium and long half-lives) are affected to different degrees. This distorts the results drastically. In order to mitigate this problem, SLAM-seq, TUC-seq or TimeLapse-seq methods were developed and published recently. They describe the combination of metabolic RNA labeling and single nucleotide conversions chemical nucleotide conversion for measuring the RNA newly transcribed by cells in a defined time window. This is based on RNA-seq and exploiting the introduced SNCnucleotide substitutions.

INNOVATION

This technology is based on a computational approach called GRAND-SLAM which provides a powerful tool for the analysis of expression data generated by SLAM-seq, TUC-seq and/or TimeLapse-seq. The unique feature of GRAND-SLAM is to enable fully quantitative analyses of such data to determine a) the new-to-total ratio of RNA (NTR) for each gene representing the percentage of RNA transcribed during the period of labeling b) regulation of mRNAs over time c) RNA half-lives and d) changes based on RNA stability or on altered RNA synthesis rates.

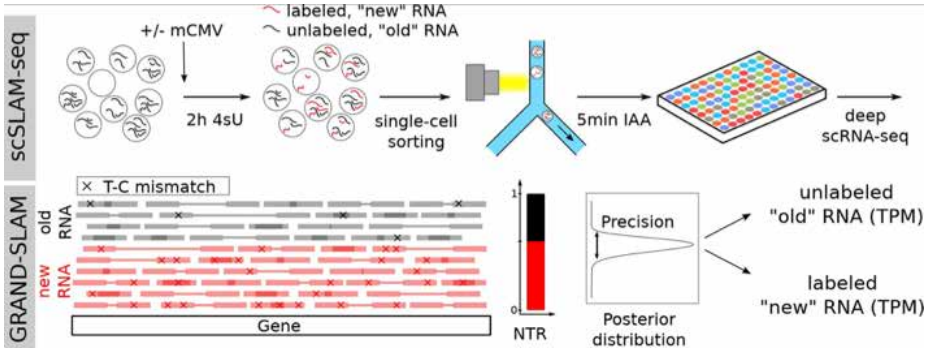

Figure: scSLAM-seq resolves transcriptional activity at the single-cell level. Overview of scSLAM-seq (top) and GRAND-SLAM (bottom). Top, nascent transcripts are labeled concomitantly with cytomegalovirus or mock infection by adding 4sU to the cell culture medium for 2h. After single-cell sorting and RNA isolation, 4sU is converted into a cytosine analogue by IAA and Smart-seq2 libraries are prepared and sequenced. Bottom, GRAND-SLAM identifies thymine-to-cytosine mismatches and estimates both the NTR and the expression of old and new RNA. TPM, transcript per millions.

COMMERCIAL OPPORTUNITIES

- Metabolic labelling using SLAM-seq, TUC-seq or TimeLapse-seq with GRAND-SLAM is applicable to all major model organisms including vertebrates, insects, plants and yeast, *in vitro* as well as *in vivo*.
- SLAM-seq and GRAND-SLAM are applicable to single cell RNA sequencing (scSLAM-seq)[1]
- The additional temporal dimension of such experiments enables to pursue completely new types of research questions.
- SLAM-seq, TUC-seq or TimeLapse-seq with GRAND SLAM combined with CRISPR-based perturbations will greatly improve the sensitivity of the approaches to decipher the molecular mechanisms with major implications for developmental biology, infection and cancer.

DEVELOPMENT STATUS

Proof of concept

REFERENCES:

1 Erhard F et al. scSLAM-seq reveals core features of transcription dynamics in single cells. Nature 2019

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