**Single Cell Ribosome Profiling (scRibo-seq)**

In recent years, single-cell sequencing methods have allowed an in-depth analysis of the diversity of cell types and cell states in a wide range of organisms. These novel tools predominantly focus on sequencing the genomes, epigenomes, and transcriptomes of single cells, however despite recent progress in detecting proteins by mass spectrometry with single-cell resolution, it remains a major challenge to measure translation in individual cells.

Building upon existing ribosome profiling protocols, the van Oudenaarden lab has developed a methodology to sequence ribosome footprints from single cells. Their results demonstrate that scRibo-seq provides a genome-wide approach to measuring translation dynamics in single cells at single-codon resolution and down to the individual transcript level.

**Key advantages**

- This technology quantifies the number and location of translating ribosomes in single cells.
- Integrated with a machine learning approach this technology positions ribosomes with single codon resolution in individual cells.
- This method does not use markers or transgenes, enabling its broad and direct application to varied species, experimental systems and conditions.
- This new technique provides the first steps towards determining the contribution of the translational process to the astonishing diversity between seemingly identical cells.

**Potential impact**

**Single-cell ribosome profiles**

The method was validated by demonstrating that scRibo-seq exhibits the same characteristics that define existing bulk-scale ribosome profiling methods. They also showed that single-cell ribosome profiles are able to accurately define cell states in heterogeneous samples. Next, they demonstrated that limitation for a particular amino acid causes ribosome pausing at a subset of the codons representing this amino acid. Interestingly, this pausing is only observed in a sub-population of cells correlating to its cell-cycle state. The lab further corroborated this phenomenon in non-limiting conditions and detected pronounced GAA pausing during mitosis.
scRibo-seq measures translation in single cells

a. Heatmap of the fold change of the number of ribosome footprints aligning along metagene regions around the start codon (left), in the coding sequence (middle), and around the stop codon (right). The mapping coordinate of the 5' end of each read is reported. b. Length-corrected distributions of footprint mapping frequencies in the 5' UTR, CDS, and 3' UTR. c. Frame and read-length distributions of the 5' end of the footprints and random-forest predicted P-sites averaged across cell types and d. in single cells. e. Number of footprints per cell along a metagene region within coding sequences before (left, reads whose 5' ends align at the given region) and after (right, number of predicted P-sites at each location) the random forest correction.

Data
**Ribosome pausing under amino acid limitation**

a. Codon occupancy in ribosome E, P, and A sites from reads pooled from all single cells from the same condition. b. Heatmap of the fold change in codon occupancy in sites relative to the ribosome active sites. c. UMAP of the single-cell RPF libraries showing derived clusters (outline) and limitation condition (point colour). d. UMAPs showing the mean log2 fold change in codon occupancy for arginine (top) and leucine (bottom) limitation conditions.
Data

e. Average of the P-site occupancy along a section of H3C2 for cells sorted and grouped based on their global arginine pausing.
f. Heatmap showing RPF counts of the top marker genes for each cell cluster.
g. Heatmap showing the single-cell P-site occupancy along H3C2.

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