Improving FRET real-time translation monitoring technology signal-to-noise in human based on ribo-seq modeling

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Summary

Fluorescence resonance energy transfer (FRET) is a technology that enables probing of molecular interactions between pair of close molecules. Recently it was suggested and demonstrated that this technology can be used for monitoring translation in-vivo via the accurate modification of tRNA molecules. However, the signal produced in this process is very noisy.

In this project we demonstrate an approach for improving the performances of this technology: Based on the analysis of hundreds of ribo-seq experiments, we developed a novel approach that might improve the signal to noise (S/N) ratio, thereby enabling researching the particular gene of interest. To this end, we created a ribo-seq pipeline for detecting ribosomal pausing sites consistent on multiple conditions/experiments. These data is then used for finding tRNAs pairs that will be modified to optimize the FRET signal. Among others, our algorithm suggests tRNA pairs which are related to codon pairs that tend to appear near translation pauses only in the transcript of interest.

Workflow

Background

Ribo-seq Pipeline

A Peak is defined as two STD from mean of RC relative to Codon profile

Reliable peaks appear in multiple experiments

Codon positions categories:
0. No peak
1. Appears in 1 repeat
2. Same lab, other condition
3. More than 1 lab, other condition
4. Appears in >4 labs, other condition
5. Appears in >4 labs, >1 Cell-line, other condition

di-codons measures
RC: sum count of all dicodon pairs for gene vs. background (sum multiple occurrences of the same dicodon in a gene)

Codons demand (RNA): Total ORF’s RNA-seq count of gene, divided by gene length. Multiplied by number of codon pairs (each pair gets the same value)

RC_peaks: how much a codon appears in a peak neighbour-hood (-20/+3)

diComps (sequence-based measure): frequency of dicodon in gene vs. the background.

Results

Optimize transcript signal by proper tRNA