How to use RiboFitter.

Introduction

Ribofitter was developed to determine the number of ribosomes translating an mRNA and the exact time at which individual ribosomes initiate translation. The model reconstituted a raw intensity trace by positioning one or more single ribosome translation events along the trace, using the theoretical intensity profile of a single translating ribosome [1]. The sum intensity for each time point of all theoretical single ribosome intensity traces is calculated and compared to the raw intensity trace. By optimizing the number and the position of ribosomes in an iterative fashion the model generates the optimal fit to the data. See for a detailed description of the fitting approach Boersma and Khuperkar et al.[1]. We here describe how to use the Ribofitter Script, written in the R-coding language.

Instructions

Ribofitter requires the following information to perform fitting of the translation initiation events:

- Raw (bleach corrected) intensity traces quantified by a spot tracking tool, like TransTrack[1].
- The acquisition interval.
- The experimentally determined build-up time, i.e. the time it takes to translate the epitope array used to visualize translating ribosomes that translate in frame one.
- The elongation time, i.e. the time it takes to translate the remaining part of the coding sequence of the reporter.
- The single ribosome intensity of a ribosome translating in frame one.
- The experimentally determined build-up and elongation time and the single ribosome intensity of a translating ribosome translating in the second translation frame (not required when using the script for just one translation frame).

The following steps need to be performed in order to run the Ribofitter script:

1. Create a .csv file (make sure that the file is saved as a comma delimited .csv file!) of the measured intensity traces for both translation frames, as given below. Each column should contain one intensity trace. The first column is used to indicate the frame. The translation initiation times of one translation frame can also be determined, in that case only one .csv file is required (see below).

Frame	mRNA1	mRNA2	mRNA3	mRNA4	mRNA5	mRNA6
1	161.9490741	-77.51080247	-37.12191358	-24.60846561	5.892416226	53.42592593
2	206.1534392	-64.8904321	-18.80246914	4.374485597	39.51388889	116.9300412
3	206.8765432	66.22839506	-20.50970018	8.209876543	8.770723104	37.02645503
4	213.7314815	83.22530864	-25.23280423	-32.81069959	47.73765432	3.549382716
5	339.9104938	23.4691358	-18.77983539	-17.73662551	18.60339506	3.021604938
6	216.1657848	13.06481481	-42.32098765	-80.32304527	-17.58201058	5.39329806
7	265.2932099	-1.660493827	-55.98919753	-70.72839506	20.62786596	-8.012345679
8	208.5329218	-14.9329806	-137.6496914	42.79365079	22.5308642	20.47839506
9	194.970679	-6.476851852	4.154320988	10.41481481	-65.21164021	-0.027777778
“”	“”	“”	“”	“”	“”	“”

2. Before running the script make sure that the build-up and elongation time as well as the single ribosome intensity and the acquisition interval (in seconds!) are set correctly, at, respectively, lines 5 to 9 in the

script for the first translation frame and lines 15 to 17 in the script for the second translation frame. In lines 23 to 28 the computational fitting parameters can be adjusted. Please note that these fitting parameters were determined experimentally, and that it is not recommended to change these values!

3. If the correct parameters are set, the entire script can be selected and executed.
4. Dialog boxes will appear, allowing you to select the .csv-file containing the intensity traces for the first translation frame, followed by the file containing the intensity traces second translation frame. When only looking at one translation frame, press "CANCEL" once the second dialog box has appeared.
5. Ribofitter will now start to determine the most probable translation initiation site. During the calculations, the process is monitored and will be displayed in the Console window of the R interface as:

```
[1] "mRNA 1; iteration 0/1000"
[1] "mRNA 1; iteration 100/1000"
[1] "mRNA 1; iteration 200/1000"
[1] "mRNA 1; iteration 300/1000"
```

6. When the script has finished the following message will appear in the Console window of the R interface

```
[1] "Finished"
```

A dialog box will now appear allowing you to select a folder to save the files.

The script will provide the following files:

- A Plots.pdf file containing plots displaying the intensity traces, fits to these traces and the time points at which ribosomes initiated translation along these traces. Ribosomes initiating translation in the first translation frame are represented by green triangles, the ribosomes initiating translation in the second translation frame by triangles in blue. The number of initiating ribosomes as well as the RMSE is displayed on the right side of the plot, where green and blue numbers represent values determined for the first and second translation frame, respectively.
- An All_results.csv file containing the following information based on the best fit:
    - Column 1: mRNA ID
    - Column 2: Number of ribosomes translating in the first frame
    - Column 3: Initiation times of ribosomes translating in the first frame (in seconds)
    - Column 4: The error of the fit to the data corresponding to the first translation frame.
    - Column 5: Number of ribosomes translating in the second frame
    - Column 6: Initiation times of ribosomes initiating translation in the second frame (in seconds)
    - Column 7: The error of the fit to the data corresponding to the second translation frame
    - Column 8: The time corresponding to the intensity trace
    - Column 9: The number of ribosomes translating the first frame at a given frame*
    - Column 10: The number of ribosomes translating the second frame at a given frame*
    - Column 11: The time difference between two consecutive initiation events in the data corresponding to the first translation frame (in seconds)
    - Column 12: The time difference between two consecutive initiation events in the data corresponding to the second frame (in seconds)

* Note that the identified initiation times are rounded off to the nearest frame.

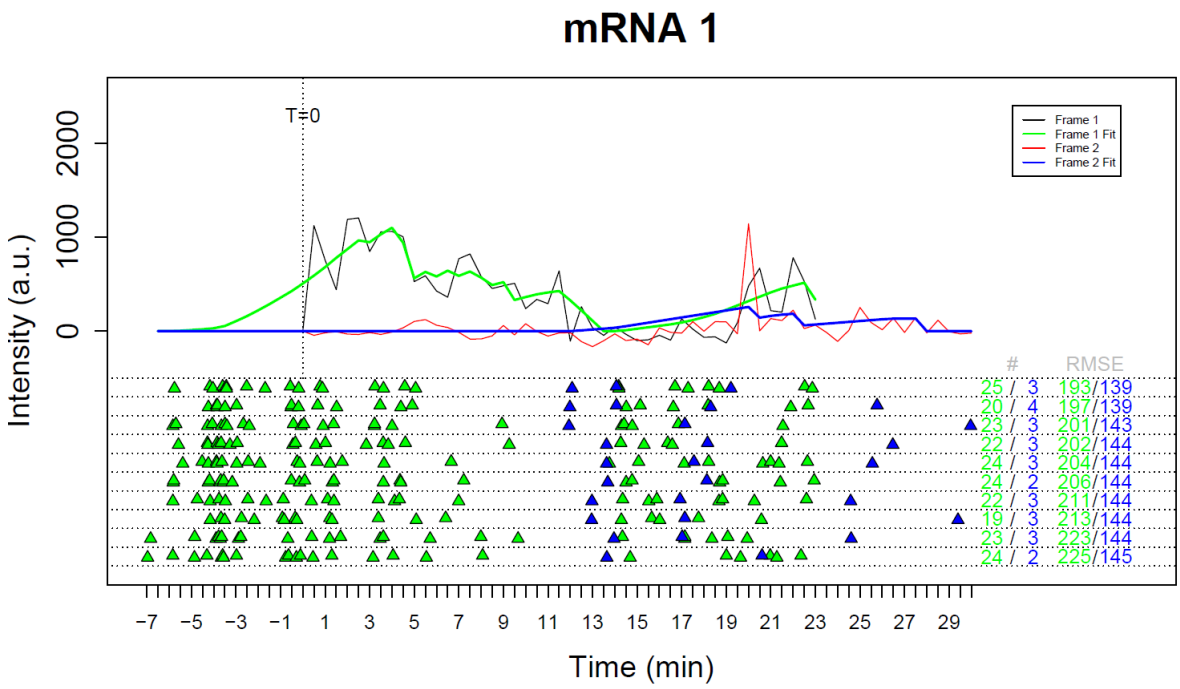

Figure 1. An example dual-color intensity trace of a single MashTag mRNA with frame 1 being the SunTag translation frame in green and frame 2 being the MoonTag translation frame in blue. The black and red line indicate experimentally observed intensities for the SunTag and MoonTag frame, respectively. The green and blue line display the optimal fit to respecitvely the Sun- and MoonTag intensity traces. Colored triangles represent translation initiation events. Each line (underneath the graph) represents a single parallel run. The number of initiating ribosomes as well as the RMSE is displayed on the right side of the plot, where green and blue numbers represent values determined for the SunTag and MoonTag frame, respectively.

mRNA	Frame1_ number_of_ ribosomes	Frame1_ initiation_ times	Frame1_ error	Frame2_ number_of_ ribosomes	Frame2_ initiation_ times	Frame2_ error	time	Frame1_ number_of_ ribosomes_per_	Frame2_ number_of_ ribosomes_	Frame1_Inititiation_ Timing_Difference	Frame2_Inititiation_ Timing_Difference
1	25	74.40512276	192.816	3	1145	138.903	-7	0	0	95.51196408	119.139222
1		169.9170868			1264.139222		-6.5	1	0	7.990177029	308.4581706
1		177.9072639			1572.597393		-6	1	0	26.14542983	
1		204.0526937					-5.5	1	0	6.59731479	
1		210.6500085					-5	1	0	3.355434159	
1		214.0054426					-4.5	3	0	0.34683321	
1		214.3522759					-4	7	0	54.46081061	
1		268.8130865					-3.5	7	0	49.75087223	
1		318.5639587					-3	8	0	67.91256584	
1		386.4765245					-2.5	8	0	22.4255943	
1		408.9021188					-2	9	0	57.47515477	
1		466.3772736					-1.5	9	0	6.441333925	
1		472.8186075					-1	10	0	141.4001661	
1		614.2187736					-0.5	11	0	22.93791841	
1		637.156692					0	11	0	57.34442848	
1		694.5011205					0.5	13	0	29.22448945	
1		723.7256099					1	13	0	546.8773796	
1		1270.60299					1.5	13	0	0.395314683	
1		1270.998304					2	13	0	151.0016958	
1		1422					2.5	13	0	35.33428336	

Figure 2. An example of the All_results.csv output.

References:

[1] *Multi-color single-molecule imaging uncovers extensive heterogeneity in mRNA decoding*, Boersma and Khuperkar et al, Cell, June 2019