Live imaging of mRNA using Pepper RNA-stabilized fluorogenic proteins

Jiahui Wu
Weill Cornell Medicine, Cornell University https://orcid.org/0000-0003-2406-6728
Samie Jaffrey (srj2003@med.cornell.edu)
Weill Cornell Medicine, Cornell University https://orcid.org/0000-0003-3615-6958

Method Article

Keywords: Fluorescent proteins, RNA imaging, Fluorogenic proteins, Fluorescence microscopy

Posted Date: November 20th, 2019

DOI: https://doi.org/10.21203/rs.2.11494/v1

Abstract

mRNA plays critical roles in cell biology. Here, we describe the procedures of imaging mRNA using Pepper RNA-stabilized fluorogenic proteins in living cells.

Introduction

None.

Reagents

DMEM (Thermo Fisher Scientific 11995-065)

Fetal bovine serum (Corning 35-010-CV)

Penicillin-Streptomycin (Thermo Fisher Scientific 15140122)

1xPBS (Thermo Fisher Scientific 10010023)

TrypLE™ Express Enzyme (1x), no phenol red (Thermo Fisher Scientific 12604013)

Opti-MEM (Thermo Fisher Scientific 31985070)

FuGENE HD (Promega 2311)

Phenol red-free DMEM (Thermo Fisher Scientific 31053-028)

GlutaMAX™ (Thermo Fisher Scientific 35050-061)

Sodium Pyruvate (100 mM) (Thermo Fisher Scientific 11360-070)

Immersion oil (N = 1.520) (Applied Precision)

Equipment

T75 cell culture flask (Corning 430641U)

Precision™ shaking water bath (Thermo Fisher Scientific)

15 ml tube (VWR 82050-276)

35 mm imaging dishes (Mattek Corporation P35GC-1.5-14C)

1.7 ml tube (Thomas Scientific C2170)

Epifluorescence inverted microscope (Olympus IX-70)

Evolve® 512 EMCCD OEM camera (Photometrics)

Insight SSI 7 color solid state illumination system (Applied Precision)

Procedure

Cell culture

- Culture U2OS cells in a T75 cell culture flask with culture media (DMEM supplemented with 10% fetal bovine serum, 100 U ml−1 penicillin and 100 µg ml−1 of streptomycin) at 37°C with 5% CO2.

- Prewarm culture media, 1xPBS, and TrypLE Express in a 37°C water bath.

- Aspirate culture media, gently rinse cells with 1xPBS, aspirate 1xPBS, then add 2 ml TrypLE Express to the culture flask, and incubate under 37°C for 5 to 10 minutes to detach cells from the flask.

- Add 8 ml of culture media to the culture flask, and gently pipette up and down to make sure all cells are fully detached from the flask.

- Transfer resuspended cells from the culture flask to a 15 ml tube, then centrifuge resuspended cells in the 15 ml tube at 300 g for 3 minutes.

- Aspirate supernatant, and resuspended cell pellet with fresh culture media, pipette up and down gently, to make sure cells are fully resuspended.

- Count the number of cells using a hemocytometer.

- To each 35 mm imaging dish (poly-D-lysine-coated), seed 2 x 105 cells in 2 ml of culture media. Gently shake the imaging dish to make sure cells are evenly spread. Keep the imaging dish at 37°C with 5% CO2 overnight.

Transfection

- On the next day, transfect U2OS cells. We suggest to determine the optimal amount of plasmid encoding (mNeonGreen)4-tDeg in the transfection. An optimal amount of (mNeonGreen)4-tDeg plasmid will give highest mRNA fluorescent puncta signal-to-noise ratio possible in fluorescence imaging. In our setup, we use 1.4 µg of miniCMV-(mNeonGreen)4-tDeg or 0.28 µg of UbC-(mNeonGreen)4-tDeg with 1.12 µg of pUC19 (as a diluent DNA) as the fluorogenic protein in the transfection. The amount of plasmid encoding (mNeonGreen)4-tDeg should be titrated and optimized case by case.

- For transfection, prepare a 1.7 ml tube for each imaging dish. To this tube, add 125 µl of Opti-MEM, 1.4 µg of mRNA reporter plasmids, an optimal amount of plasmid encoding (mNeonGreen)4-tDeg, and 9.6 µl

of FuGENE® HD reagent. Mix thoroughly by pipetting up and down, then incubate at room temperature for 10 to 15 minutes.

- After incubation, add this mixture drop by drop to the imaging dish with cells. Keep the imaging dish under 37°C with 5% CO2 overnight.

- On the next day, prewarm 1xPBS and culture media.

- Aspirate culture media from the imaging dish, gently rinse cells with 1xPBS, aspirate 1xPBS, add 2 ml fresh culture media to cells. Culture cells under 37°C with 5% CO2 overnight.

Imaging

- Prior to live-cell imaging, prewarm 1xPBS, and imaging media (phenol red-free DMEM supplemented with 10% fetal bovine serum, 100 U ml−1 penicillin and 100 µg ml−1 of streptomycin, 1x GlutaMAX™, and 1 mM sodium pyruvate).

- Aspirate culture media from the imaging dish, gently rinse cells with 1xPBS, aspirate 1xPBS, add 2 ml of imaging media to the imaging dish.

- Move the imaging dish to the microscope in the prewarmed environmental chamber.

- Add sufficient immersion oil (N = 1.520) on a 100×/1.4-NA oil objective, and use this objective under phase illumination to focus on adhered cells.

- Switch from phase to fluorescence acquisition with FITC filter sets (with excitation filter 475 ± 14 nm, dichroic mirror with a reflection band of 481-502 nm, and a transmission band of 506-543 nm, and emission filter 525 ± 25 nm).

- Determine the proper exposure time by acquiring images with an exposure time between 50 ms to 200 ms. A proper exposure time will allow for highest possible signal without any pixel saturation.

- Set up software to acquire fluorescence images.

Troubleshooting

None.

Time Taken

~ four days

Anticipated Results

Pepper-tagged mRNAs were observed as fluorescent puncta, as shown in Figure 1.

References

None.

Acknowledgements

We thank the Bio-Imaging Resource Center at Rockefeller University for technical support.

Figures

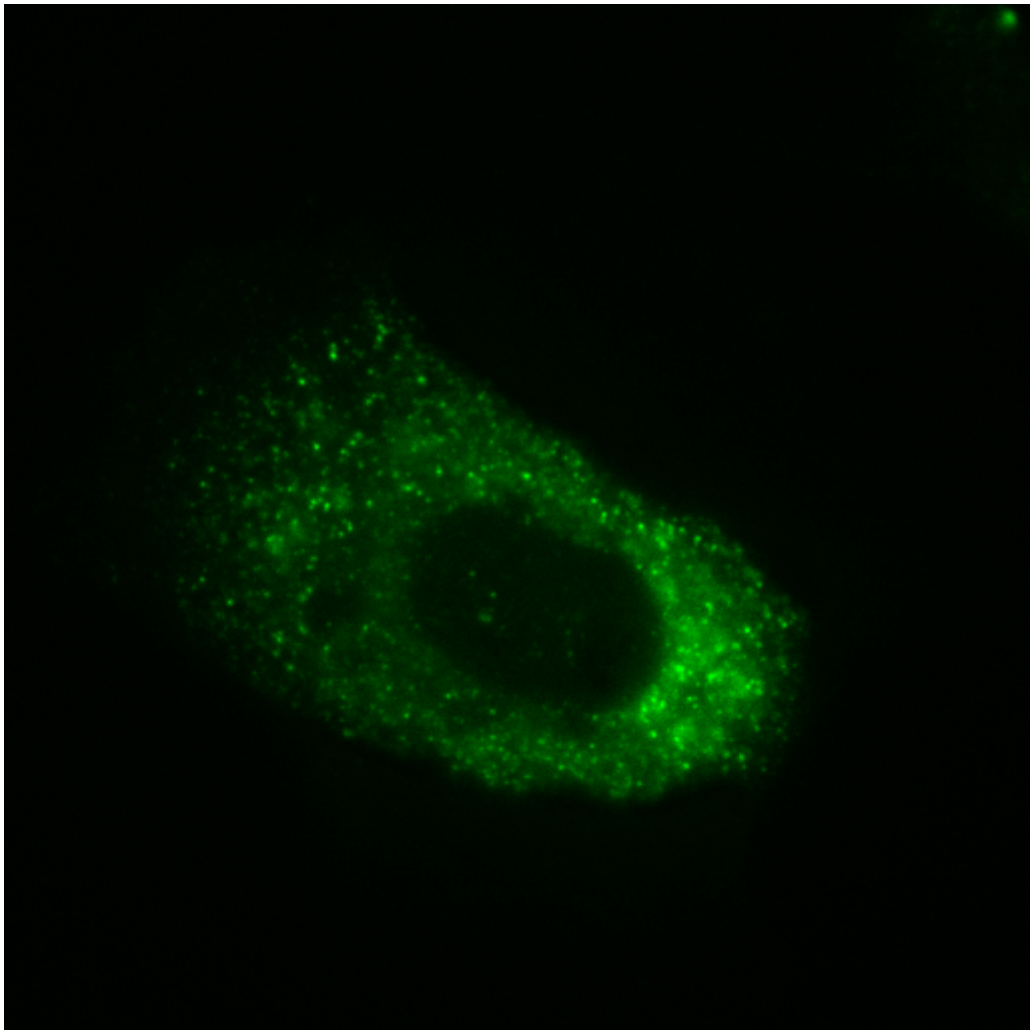

Figure 1

mCherry reporter mRNA tagged with (F30-2xPepper)10 was imaged in U2OS cells coexpressing fluorogenic protein, (mNeonGreen)4-tDeg.