Supplementary protocol:

Live imaging of mRNA using RNA-stabilized fluorogenic proteins

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% CO_2.
- 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 10^5 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% CO_2 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% CO_2 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% CO_2 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.

Materials

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