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Transient Transfection of Cultured Mammalian Cells for Live 3D Fluorescence Microscopy

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We use this protocol and it's working

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Abstract

This is a protocol for transient transfection of plasmids encoding tagged mitochondrial proteins into cultured mammalian cells. The result is a sample of live cells in which various features of mitochondrial morphometrics can be observed by fluorescence microscopy.

Guidelines

Timeline

- **D0:** Seed U2OS cells (typically at ~ 20-30% confluency, so that they are at ~ 80% confluency on the day of the experiment).
- **D1:** Perform transfection – Replace with fresh media 5 hours post-transfection.
- **D2:** Perform experiment 24 hours post-transfection.

Materials

Reagents:

- Complete DMEM media

1. 500mL DMEM high glucose no glutamine **Thermo Fisher Scientific Catalog #**11960044
2. 55mL
   Fetal Bovine Serum, heat inactivated, qualified, One Shot™, United States **Thermo Fisher Catalog #**A3840101
3. 5.5 mL L-Glutamine (200 mM) **Thermo Fisher Catalog #**25030081
4. 5.5mL Penicillin-Streptomycin **Gibco - Thermo Fisher Scientific Catalog #**15140122

- Opti-MEM™ I Reduced Serum Medium **Thermo Fisher Scientific Catalog #**31985070
- Lipofectamine™ 2000 Transfection Reagent **Thermo Fisher Scientific Catalog #**11668030
- Plasmids

1. mCherry-TOMM20-N-10 **addgene Catalog #**55146
2. ATP5F1B (NM_001686) Human Tagged ORF Clone **OriGene Catalog #**RG201638

- 35-nm glass bottom dishes (Mattek, P35GC-1.5-14-C)
- Pipettes
- Pipette tips
- 1.5mL or 2mL microtubes

Equipment:

- Incubator
- Biosafety cabinet
- Fluorescence microscope

Procedure

1d 5h 15m

1 Seed U2OS cells in DMEM complete medium onto 35-nm glass bottom imaging dishes.

2 Incubate the cells at 37 °C, 5% CO_2 for ~ 24:00:00. 1d

3 Thaw plasmid(s) and measure your desired concentration for transfection (500 ng for mCherry-TOMM20-N-10 and 250 ng for ATP5F1B-tGFP).

4 Take an aliquot of Opti-MEM and let it warm at Room temperature in the dark.

5 Take a microfuge tube: add 100 µL of Opti-MEM and 4 µL of lipofectamine 2000 per transfection (example: if you are doing two transfections, you will need 200 µL Opti-MEM and 8 µL lipofectamine 2000). Mix gently.

6 Take microfuge tubes. In each tube, add 100 µL of Opti-MEM and your desired concentration of plasmid (example: 500 ng for mCherry-TOMM20-N-10 / 250 ng for ATP5F1B-GFP). Mix gently.

7 Add 104 µL of the Opti-MEM/Lipofectamine 2000 mix in each of the tubes prepared in step 6. Mix gently.

8 Let the tubes incubate at Room temperature for 00:15:00. 15m

9 Take the cells out of the incubator and add the transfection mixture dropwise to cells.

10 Incubate the cells at 37 °C, 5% CO_2 for 05:00:00. 5h

11 Replace media with fresh DMEM complete media.

12 Perform experiment 24 hours post-transfection.

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