Oct 24, 2024

live and fixed cell fluorescence imaging of agDD-GFP aggregation and NRF1 activation

Forked from a private protocol

DOI
https://dx.doi.org/10.17504/protocols.io.5jyl823e7l2w/v1

Harper JW[1], Kelsey Hickey[1]

[1]Department of Cell Biology, Harvard Medical School

Kelsey Hickey
Harvard Medical School

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.5jyl823e7l2w/v1

Protocol Citation: Harper JW, Kelsey Hickey 2024. live and fixed cell fluorescence imaging of agDD-GFP aggregation and NRF1 activation. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5jyl823e7l2w/v1

Manuscript citation:
https://www.biorxiv.org/content/10.1101/2022.12.06.519342v1

Protocol status: Working
We use this protocol and it's working

Created: October 18, 2024

Last Modified: October 24, 2024

Protocol Integer ID: 110252

Keywords: ASAPCRN, protein aggregates, cell fluorescence imaging of agdd, nrf1 by fluorescence imaging, fixed cell fluorescence imaging, fluorescence imaging, immunofluorescence, gfp aggregation, gfp aggregate, nrf1 activation, agdd, gfp, nrf1 activation this protocol, nrf1, imaging, fluorescence

Funders Acknowledgements:
ASAP
Grant ID: 000282

Abstract

This protocol describes methods for visualization of agDD-GFP aggregates and NRF1 by fluorescence imaging and immunofluorescence, respectively.

Materials

HeLa-dCas9 cells (Ref 1 and 2) expressing agDD-GFP (Addgene #78289) as described (Ref 3)
PBS; Phosphate buffered saline: ThermoFisher (#14040133)

Dulbecco's MEM (DMEM), high glucose, pyruvate (Gibco / Invitrogen, 11995)
18 or 22 mm-glass coverslips (No. 1.5, 22×22 mm glass diameter, VWR 48366-227)

FluoroBrite DMEM (Thermo Fisher Scientific A, 1896701)
NRF1/NFE2L1 (1:1000; abcam, ab238154)
mounting media (Vectashield H-1000)

AlexaFluor 594 Goat anti-Rabbit IgG (Thermo Fisher Catalog # A-11012)
Hoechst-33342 dye (Thermo Fisher #62249)

Cell Culture

1 HeLa-dCas9 cells (from Ref 1 and 2) were grown in complete DMEM (Dulbecco' Modifies Eagles Medium (DMEM) with 10% fetal bovine serum and optional 1% penicillin-streptomycin), with the addition of shield-1 (S1) ligand at a final concentration of 1 µm (MedChem Express, cat # HY-112210) to keep agDD-GFP in a folded form and maintained in a 5% CO_2 incubator at 37^{o}C. Cells were maintained at <80% confluency throughout the course of experiments.

Imaging of agDD-GFP aggregation in fixed cells

2 Cells in the presence of S1 were plated onto 18 or 22 mm-glass coverslips (No. 1.5, 22×22 mm glass diameter, VWR 48366-227) for imaging. S1 washout was performed as described in Ref 3 using 5 µm FKBP12-F36V in the culture media. The choice of time for the washout will depend on the goals of the experiment but 10 min to 6 h represent the extremes of the time range, typically.

2.1 Cells were then fixed using 4% PFA for 10 min at room temperature.

2.2 Coverslips were then washed 3 times with DPBS + 0.02% tween-20, with the final wash done in the presence of Hoechst33342 dye to mark nuclei, and mounted onto glass slides using mounting media (Vectashield H-1000) and sealed with nail polish.

2.3 The cells were imaged by confocal microscopy using a Yokogawa CSU-W1 spinning disk confocal on a Nikon Ti motorized microscope equipped with a Nikon Plan Apo 100x/1.40 N.A objective lens, and Hamamatsu ORCA-Fusion BT CMOS camera. For the analysis, the equal gamma, brightness, and contrast are applied for each image using FiJi software (FiJi ImageJ V.2.0.0 https://imagej.net/Fiji).

Imaging of NRF1 in fixed cells

3 Cells in the presence of S1 were plated onto 18 or 22 mm-glass coverslips (No. 1.5, 22×22 mm glass diameter, VWR 48366-227) for imaging. S1 washout was performed as described in Ref 3 using 5 µm FKBP12-F36V in the culture media. The choice of time for the washout will depend on the goals of the experiment but 10 min to 6 h represent the extremes of the time range, typically.

3.1 Cells were then fixed using 4% PFA followed by permeabilization with 0.5% Triton-X100.

3.2 Cells were blocked in 3% BSA for 30 minutes, followed by incubation in primary antibodies (e.g. anti-NRF1; 1:200) for 1 hour at room temperature. Cells were washed 3 times with PBS + 0.02% tween-20, followed by incubation in secondary (alexafluor conjugated secondary antibodies such as AlexaFluor 647 Goat anti-Rabbit IgG) for 1 hour at room temperature.

3.3 Coverslips were then washed 3 times with DPBS + 0.02% tween-20 and mounted onto glass slides using mounting media (Vectashield H-1000) and sealed with nail polish.

3.4 The cells were imaged by confocal microscopy. We employ a Yokogawa CSU-W1 spinning disk confocal on a Nikon Ti motorized microscope equipped with a Nikon Plan Apo 100x/1.40 N.A objective lens, and Hamamatsu ORCA-Fusion BT CMOS camera. For the analysis, the equal gamma, brightness, and contrast are applied for each image using FiJi software (FiJi ImageJ V.2.0.0 https://imagej.net/Fiji)

Imaging of agDD-GFP aggregation in live cells

4 Cells in the presence of S1 were plated onto glass glass bottom dishes for imaging. S1 washout was performed as described in Ref 3 using 5 µm FKBP12-F36V in the culture media.

5 Before imaging, S1 washout is started to begin aggregation in PhenolRed free DMEM. Then
cells are imaged using a Yokogawa CSU-W1 spinning disk confocal on a Nikon Ti motorized microscope equipped with a Nikon Plan Apo 100x/1.40 N.A objective lens, and Hamamatsu ORCA-Fusion BT CMOS camera, and a live cell chamber with temperature and carbon dioxide control. For the analysis, the equal gamma, brightness, and contrast were applied for each image using FiJi software (FiJi ImageJ V.2.0.0 https://imagej.net/Fiji).

Protocol references

1. M. Jost *et al.*, Combined CRISPRi/a-Based Chemical Genetic Screens Reveal that Rigosertib Is a Microtubule-Destabilizing Agent. *Mol Cell***68**, 210-223 e216 (2017).
2. L. A. Gilbert *et al.*, CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. *Cell* **154**, 442-451 (2013).
3. Y. Miyazaki *et al.*, A method to rapidly create protein aggregates in living cells. *Nat Commun* **7**, 11689 (2016).