**Live-Cell Imaging Flp-In Cells**

Materials

DMEM (cat# 11995-040 / ThermoFisher)

Leibovitz’s L-15, no phenol red (cat# 21083027 / ThermoFisher)

Fetal Bovine Serum (FBS) (cat# 7678 / Sigma)

Tet-Free FBS (cat# 631107 / Takara Bio)

L-Glutamine (Q) (cat# 25030-081 / ThermoFisher)

coverslips (cat# 12-545-81 / Fisher)

DMSO (cat# D2650 / Sigma)

Blasticidin (cat# ANT-BL-1 / InvivoGen)

Hygromycin B (cat#10687010 / Invitrogen)

Doxycycline (cat# D1822 / Sigma)

Protocol:

1. Prior to experiment, grow cells in DMEM + 10% Tet-Free FBS + 1% Q + 250 µg/mL hygromycin B + 20 µg/mL blasticidin
2. 20-48 hrs before imaging, plate cells on 22 x 22mm acid washed, #1.5 coverglass in a 35mm well [ \_\_\_\_\_\_\_ x 106 cells] in media with regular FBS
3. 20-30 hrs before imaging, induce \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ expression with 1µg/mL doxycycline
4. Assemble Rose Chamber in L-15 + 10% FBS + imaging treatment:  
   \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
5. Allow chamber to incubate for 20-40 min at 35-37ºC while screening for cells on the Nikon Ti2-E microscope:
   1. Screen for metaphase cells using brightfield and add to XY multipoint list in Elements (v.5.02.00)
   2. Check cells from list for appropriate fluorescence signal using snapshots with laser light
6. Image as per Imaging Details

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spastazoline (\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_­­­­­­­­­­\_\_\_\_\_\_\_\_\_\_\_)

(382.52g/mol)

Cells:

Parental: HeLa TREx Flp-In (R71470 / ThermoFisher)

Designation:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Relevant Plasmid(s):\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Generation:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Thawed:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Mycoplasma Free: ⬜ page no.\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Sequencing: ⬜ page no.\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Cell Type Verified: ⬜ page no.\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Imaging Details:

Filename: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Objective: 100x 1.45 NA Plan Apo ⬜ Wollaston prism

Imaging Mode: Yokogawa W1 spinning disk

Illumination: 488 nm 100mW laser (40%) 100 ms exposure

DIC; LED (60%) 50 ms exposure

Camera: Prime95B sCMOS

z-step: 1 µm x 5 steps (4 µm volume) around center (PFS on)

Time: 3 min interval, variable duration

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Sequencing: ⬜ page no.\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Cell Type Verified: ⬜ page no.\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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