**Live-Cell Imaging DNA in Cells**

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DMEM (cat# 11995-040 / ThermoFisher)

McCoy’s 5A (cat# 16600-082 / ThermoFisher)

DMEM/F12 1:1 Glutamax (cat# 10565-018 / 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)

Puromycin (cat# P8833 / Sigma)

Doxycycline (cat# D1822 / Sigma)

SiR-DNA (cat# CY-SC007 / Cytoskeleton)

JF646-Hoechst (lot# NF-1-111 / JaneliaFluor)

Protocol:

1. Plate cells on 22 x 22mm acid washed, #1.5 coverglass in a 35mm well [\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_]

* Induce \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ expression with \_\_\_µg/mL doxycycline [\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_]

1. Stain with ⬜ SiR-DNA or ⬜JF646-Hoechst at 125 nM for 40-60 min.
2. Assemble Rose Chamber in L-15 + 10% FBS + imaging treatment [0.1% DMSO or 10 µM spastazoline]
3. Allow chamber to incubate for 10-30 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
4. Image as per Imaging Details

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

(382.52g/mol)

Cells:

Parental:

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

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

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

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

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

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

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

Growth Conditions:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Imaging Details:

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

Objective: 100x 1.45 NA Plan Apo with Wollaston prism

Imaging Mode: Yokogawa W1 spinning disk

Illumination: 640 nm 75mW laser (60%) 100 ms exposure

DIC; LED (70%) 100 ms exposure; Home only

Camera: Prime95B sCMOS

z-step: 1 µm x 2 steps (4 µm volume) around center (PFS on)

Time: 1 min interval, variable duration

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