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Variant painting via immunostaining

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Chloe Reno[1]

[1]University of Toronto

IGVF

C Chloe Reno
University of Toronto

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We use this protocol and it's working

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Abstract

High-throughput imaging platform to assay the impact of coding variation on protein localization. Proteins are flag-tagged and immunostained for visualization, alongside additional cellular compartments including the nucleus, mitochondria, and ER.

Day 1: Cell Seeding

1 Seed 4,000-5,000 HeLa Kyoto Cells - 96-well format, 100 µL per well. Media is high glucose DMEM supplemented with 10% FBS, 1% penicillin-streptomycin. Incubate Overnight at 37 °C , 5% CO_2

Day 2: Transfection

2d 0h 15m

2 Prepare Transfection Complex Master Mix in 96-well v-bottom plates

> Note
>
> First check cells to make sure they are at optimal (50-60%) confluency for transfection.

2.1 Per well, mix:

1. 10 uL of diluted DNA (200 ng total)

> Note
>
> Add DNA to wells first. Prepare a master mix of OptiMEM + X-tremeGENE 9. Vortex to mix. Make extra master mix to account for error.

2. 0.3 uL X-tremeGENE 9
3. 30 uL OptiMEM

2.2 Let stand at room temperature for 00:15:00 15m

3 Mix by pipetting up and down. Transfer transfection complex to seeded HeLa cells dropwise.

4 Incubate for 48:00:00 at 37 °C , 5% CO_2 2d

Day 4: Fixation & Staining

3h 55m

5 Stain live cells with Mitotracker:

5.1 Make 1mM stock of tracker dye fresh. Before opening, allow the vial to warm to room temperature and then briefly centrifuge the vial.

> Note
>
> For Mitotracker Red – add 94.1 µL DMSO to vial.

5.2 Dilute the 1 mM stock solution to the final working concentration (100 nM for Mito, 1 µM for Lysotracker) in growth medium (1xDMEM complete).

5.3 Add to cells and incubate for 01:00:00 in the tissue culture incubator. 1h

5.4 Wash cells 3 times with 1xPBS.

6 Fix cells

6.1 Add 30 uL/well of 4% paraformaldehyde diluted in complete DMEM

6.2 Wrap the plate in foil. Incubate for 00:15:00 at room temp 15m

6.3 Wash cells 3 times with 1xPBS.

7 Complete intracellular staining:

7.1 Incubate with 50µL/well of 1xPBS/0.1% Triton X-100 for 00:10:00, room temperature shaking gently for permeabilization. 10m

7.2 Incubate with 50µL/well of 1xPBS/0.1% Triton X-100/1% BSA for 00:30:00, room temperature shaking gently for blocking. 30m

7.3 Incubate with primary antibody of choice diluted in 30µL/well blocking buffer for 01:00:00 , room temperature shaking gently (For anti-FLAG, 1:500). 1h

7.4 Wash three times with 1xPBS.

7.5 Dilute the secondary antibodies (anti-mouse 488, 1:500; Hoechst, 1:5000; Concanavalin A, 1:250) in 30 µL/well blocking buffer. Incubate for 01:00:00 at room temperature shaking gently. 1h

7.6 Wash three times with 1xPBS.

7.7 Leave in 50µL 1xPBS for imaging.