Feb 11, 2026

# VarChamp Cell Painting

DOI
https://dx.doi.org/10.17504/protocols.io.36wgqnwkxgk5/v1

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**DOI:** https://dx.doi.org/10.17504/protocols.io.36wgqnwkxgk5/v1

**Protocol Citation:** Tanisha Teelucksingh, Florent Laval 2026. VarChamp Cell Painting . **protocols.io** https://dx.doi.org/10.17504/protocols.io.36wgqnwkxgk5/v1



**Protocol status:** In development
**We are still developing and optimizing this protocol**

**Created:** October 07, 2024

**Last Modified:** February 11, 2026

**Protocol Integer ID:** 109318

**Keywords:** varchamp cell painting, missense variants on protein, subcellular localization, missense variant

**Funders Acknowledgements:**
**National Human Genome Research Institute (NHGRI)**
Grant ID: UM1HG011989

## Abstract

Assessment of the impact of missense variants on protein subcellular localization

## Virus Production - Seeding transfection plates (96-well format)

1 Seed HEK293T Cells - 96-well format, 100 µL per well. Media used: DMEM 4h 30m

> Note
>
> All media contains 10% FBS and 5% penicillin streptomycin

1. if transfecting packaging plasmids on the same day, seed $2.4\times10^4$ cells/well. Allow at least 04:00:00 for cells to adhere, incubating at 37 °C, 5% $CO_2$
2. if transfecting packaging plasmids the next day, seed $1.2\times10^4$ cells/well. Incubate Overnight at 37 °C, 5% $CO_2$

2 Remember to harvest mycoplasma sample for testing downstream

## Virus Production - Preparing packaging/transfection mix (96-well format)

3 Prepare packaging mix - 10 µL /well

1. 50ng/well psPAX2
2. 5ng/well pVSV-G
3. Top up to 10 µL /well with OptiMEM

4 Prepare transfection mix - 10 µL /well

1. 9.4 µL OptiMEM/well
2. 0.6 µL TransIT/well

> Note
>
> Make sure to add the TransIT drop wise and mix well

5 Prepare 96-well plate - 33.33 uL total volume per well 30m

1. 10uL packaging mix
2. 10uL transfection mix
3. 10uL sterile water
4. 3.33uL lentiviral expression plasmid (normalized to 15ng/uL)

5. Incubate at room temperature for 00:30:00

Note

Mix well before incubation

6 Transfer entire mix (33.33uL) to HEK293T cells
1. Incubate for 48:00:00 at 37ºC, 5% $CO_2$

2d

## Transduction of U2OS cells

7 Seed U2OS Cells - 384-well format, 40uL per well. Media used: McCoy's 5A with 10% FBS and 1% penicillin streptomycin
1. Seed 1000 cells/well → include 10ug/mL polybrene
2. Allow cells to settle for ~ 1 hour at room temperature before incubating at 37ºC, 5% $CO_2$ for an additional 3 hours

8 Remember to harvest mycoplasma sample for testing downstream

9 Spinfection - 384-well format
1. Add 6uL of virus per well
2. Spin plates at 1200 x g, 37°C for 30 minutes
3. Incubate for 48 hours at 37ºC, 5% $CO_2$

## Puromycin Selection of Transduced U2OS cells

10 Dilute puromycin in media (McCoy's 5A with 10% FBS and 1% penicillin streptomycin) to a final concentration of 1.5ug/mL
1. Flick media from 384-well plates
2. Add 30uL of diluted puromycin to each well
3. Incubate for 48:00:00 at 37ºC, 5% $CO_2$
4. Make sure to monitor untransduced control for complete selection

2d

## Mycoplasma Testing

11 Before proceeding with staining and fixation of cells, samples taken on steps 2 and 8 should be tested for mycoplasma as this will impact downstream applications

## Mitotracker Staining

12 Dilute MitoTracker Deep Red in McCoy's 5A ( with 10% FBS and 1% penicillin streptomycin) to a final concentration of 500nM
1. Flick media from 384-well plates
2. Add 20uL of diluted MitoTracker Deep Red to each well
3. Incubate for 1 hour at 37ºC, 5% $CO_2$

## Cell Fixation

13 Dilute 32% paraformaldahye to a final concentration of 4% in McCoy's 5A ( with 10% FBS and 1% penicillin streptomycin)
1. Flick media from 384-well plates
2. Add 20uL of diluted PFA to each well
3. Cover with tin foil - PFA is light sensitive
4. Incubate for 15-30 minutes at room temperature
5. Wash each plate 4 times with 1x HBSS
6. If not staining right away, store plates with 50uL 1x HBSS per well, covered in tin foil at 4ºC

## Cell Staining

14 Prepare Staining and Permeabilization Buffer in 1x HBSS:
1. 0.1% Triton (vol/vol)
2. 1% BSA (wt/vol)

15 Dilute dyes to the following concentrations:
1. Phalloidin 568 - 0.125uL/mL (stock 6.6uM)
2. Hoechst 33342 - 1ug/mL (stock 10mg/mL)
3. WGA 555 - 1.5ug/mL (stock 1mg/mL)

> Note
>
> All dyes are spun down before use according to Cell Painting Protocol V3

16 Flick out 1x HBSS from cells after fixation
1. Add 20uL of staining solution per well
2. Centrifuge at 500 x g for 1 min to remove air bubbles
3. Incubate, covered in tin foil for 30 minutes
4. Wash each plate 4 times with 1x HBSS
5. Leave 50uL 1x HBSS per well

## Imaging

17 All confocal images are captured on a Perkin Elmer Opera Phenix Microscope with the following conditions:
1. 20X water objective
2. Single plane (-7um)
3. 384 wells, 9 fields
4. Separate all channels:

| | Channel | Excitation | Emission | Exposure | Power |
|---|---|---|---|---|---|
| | DAPI | 405 | 435-480 | 100 ms | 60% |
| | Alexa488 | 488 | 500-550 | 60 ms | 30% |
| | Alexa568 | 561 | 571-596 | 60 ms | 30% |
| | Mitotracker Deep Red | 640 | 650-760 | 20 ms | 20% |
| | Brightfield High | Transmissio n | | 100 ms | 50% |
| | Brightfield | Transmissio n | | 100 ms | 50% |
| | Brightfield Low | Transmissio n | | 100 ms | 50% |

Note

Current run time is 2hr 12min for 1 plate