VERSION 2

JUN 26, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.81wgbx1q1lpk/v2

Protocol Citation: William J Buchser, Mallory Wright, purva patel, jwaligor, ckremitz 2023. Basic maintenance protocol for human induced pluripotent stem cell (hIPSCs). **protocols.io** https://dx.doi.org/10.17504/protocols.io.81wgbx1q1lpk/v2 Version created by Mallory Wright

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

Created: Jun 23, 2023

Last Modified: Jun 26, 2023

Basic maintenance protocol for human induced pluripotent stem cell (hIPSCs) V.2

William J Buchser[1], Mallory Wright[1], purva patel[1], jwaligor[1], ckremitz[1]

[1]Washington University, Saint Louis

Washington University FIVE @ MGI

Mallory Wright
Washington University, Saint Louis. McDonnell Genome Institu...

ABSTRACT

Human Induced pluripotent stem cell (hIPSCs) maintenance protocol. This includes thawing, passaging, performing a media change, single cell dissociating, and freezing hIPSCs.

PROTOCOL integer ID:
83955

MATERIALS

ROCKi **STEMCELL Technologies Inc. Catalog #72304** -20 °C

Note

- iPSCs like being in clusters. Single cell iPSCs tend to die. ROCKi prevents that from happening
- Need a 10mM stock: dilute 5mg of the powder in 1.56 ml nuclease free water. Aliquot into smaller quantities. Store in -20C.
- RevitaCell is a good alternative, either can be used

ReLeSR **STEMCELL Technologies Inc. Catalog #05872**
Room temperature
TrypLE Select (1X) no phenyl red **Thermo Fisher Scientific Catalog #12563-011**
Room temperature
Matrigel Matrix hESC qualified **Corning Catalog #354277** 20 °C
DMEM/F12 (1:1) **Thermofisher Catalog #11320033** 4 °C
CryoStor CS10-100ml **STEMCELL Technologies Inc. Catalog #07930** 4 °C
DBPS -/- (without calcium / magnesium) **Thermo Fisher Scientific Catalog #14190144**
Room temperature
Antibiotic/Antimycotic (100X) **Thermo Fisher Scientific Catalog #15240062**
-20 °C

Thawing iPSCs

9m

1 Quickly thaw the frozen vial in a 37 °C water bath 00:02:00 2m

Note

Swirl the tube as the ice melts until only a tiny amount of ice is in the vial, and then remove it from the water bath.

2 Using a 10 ml serological pipette, add 9 mL of media into 15 ml conical tube. 2m

3 Add the contents of the cryovial into the 15 ml conical tube with a 5 ml serological pipette

Note

Rinse the cryovial once with 1 ml taken from the 15 ml conical tube.

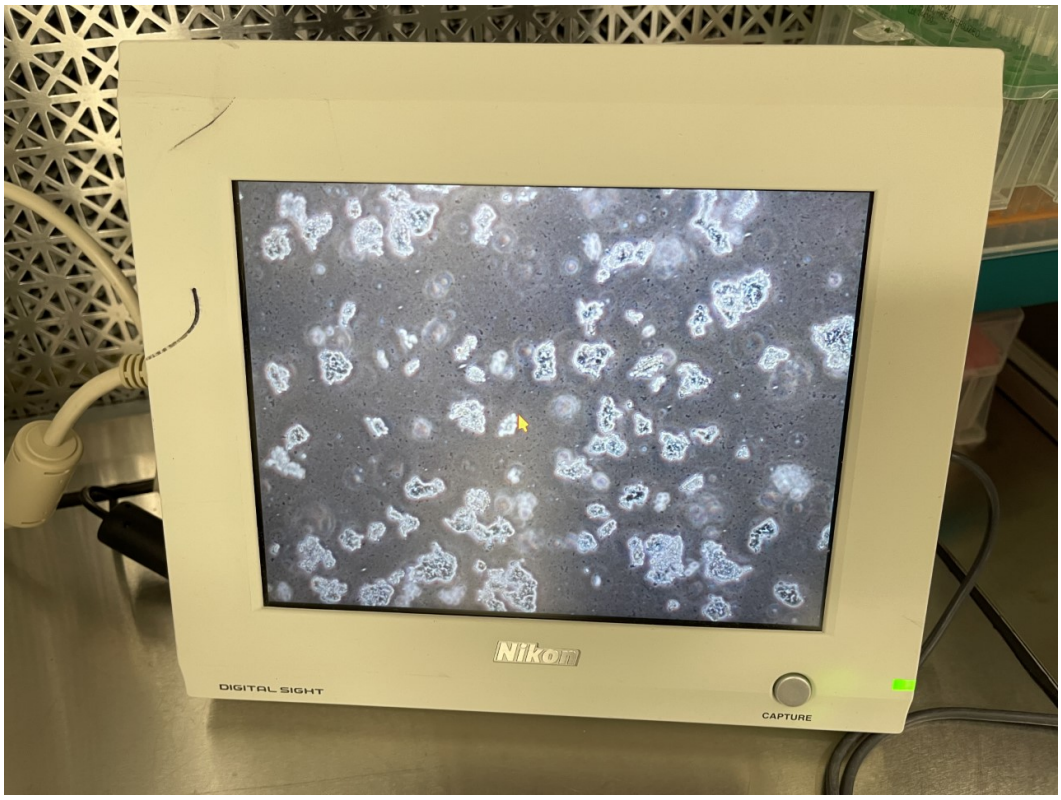
4 Spin down conical tube 1200 rpm, 00:05:00 5m

5 When done spinning, aspirate supernatant very carefully and resuspend the pellet in 1 ml of mTeSR plus media with Rocki (Y-27632)
ROCKi STEMCELL Technologies Inc. Catalog #72304

Note

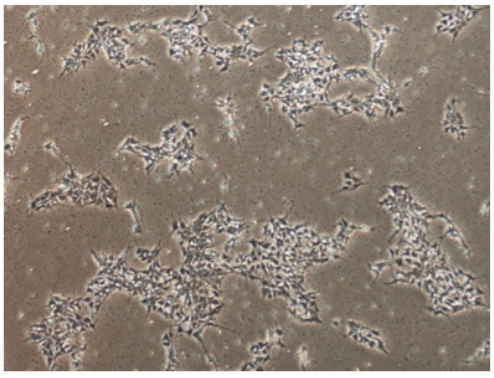
1ul of ROCKi per 1 ml media. For 4 ml media, add 4ul ROCKi.

6 Add the whole cell suspension into one well of a 6-well plate coated with matrigel

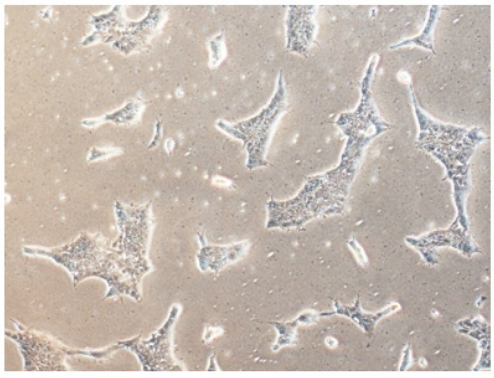


cells right after thawing

- 24 hours post thaw before removing rocki



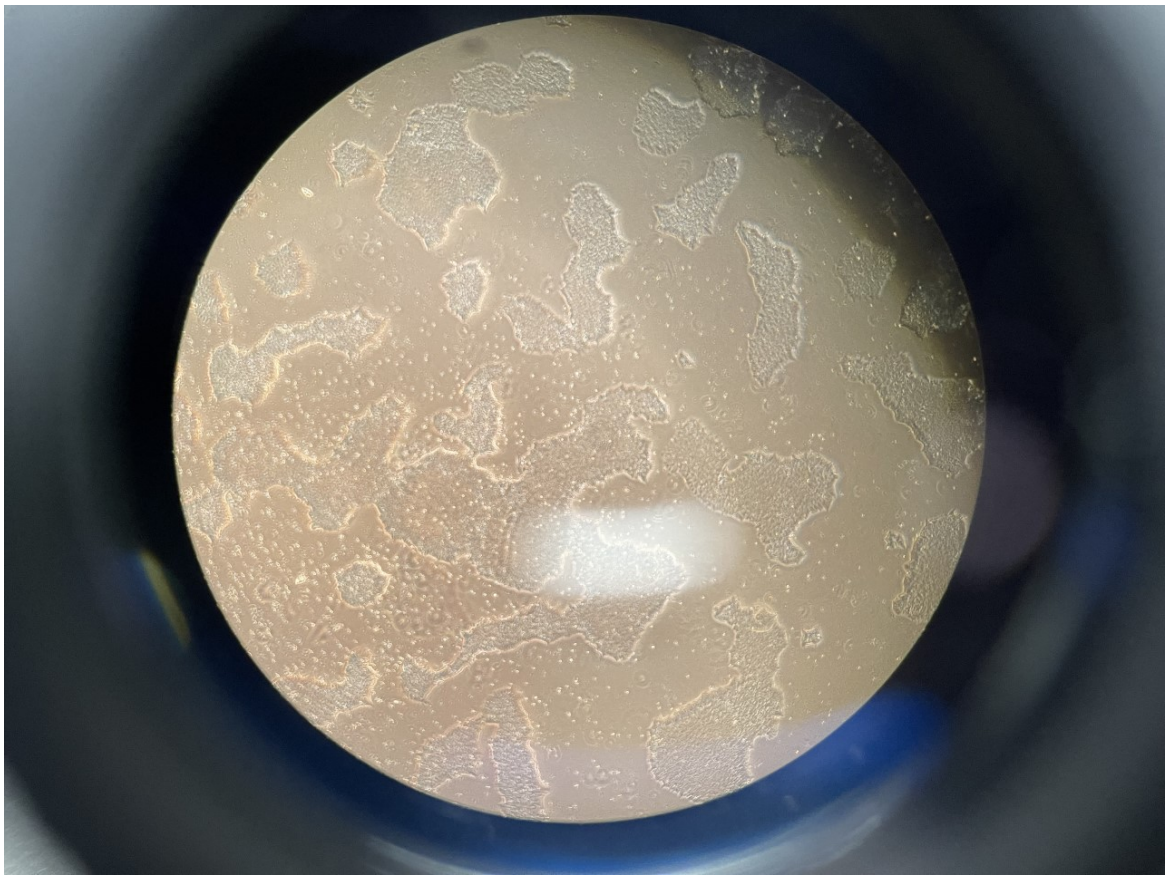
- 26 hours post thaw (2 hours after removing rocki)



Passaging IPSCs

6m

7 Ideal confluency for passaging IPSCs is about ~60%. This would be about 1 million cells in a 6-well plate.



Example image of ideal confluency for passaging

8 Aspirate the media off the wells from the plate

9 Rinse each well or flask with DPBS -/- (1ml per well of a 6 well, 3 ml per T-25)

10 Aspirate the DPBS -/-. You can keep this in the hood at room temperature for storage.

11 Add 1 ml ReLeSR* per well of 6-well plates or 3 ml ReLeSR for a T-25. Start your timer and leave it on the cells for 00:01:00 at Room temperature 1m

Note

*ReLeSR is an enzyme-free reagent for the dissociation of cells as aggregates **without** manual selection of differentiated areas or scraping to remove cell aggregates*

12 Fully aspirate ReLeSR and leave the plate in the incubator for 00:05:00 5m

Note

The plate will have a little residual moisture only. If cells were recently treated in ROCKi, leave cells for 5-10 more minutes). After the incubation period, you may observe gap formations in the colonies.

13 Remove the plate/flask from the incubator, and use a 5ml serological to add 1 ml of mTeSR plus media in each well of the 6-well, or 3ml of that media in a T-25 (to inactivate the ReLeSR)

14 Use the same serological pipette to rinse the wells 3-4 times to capture all the cells.

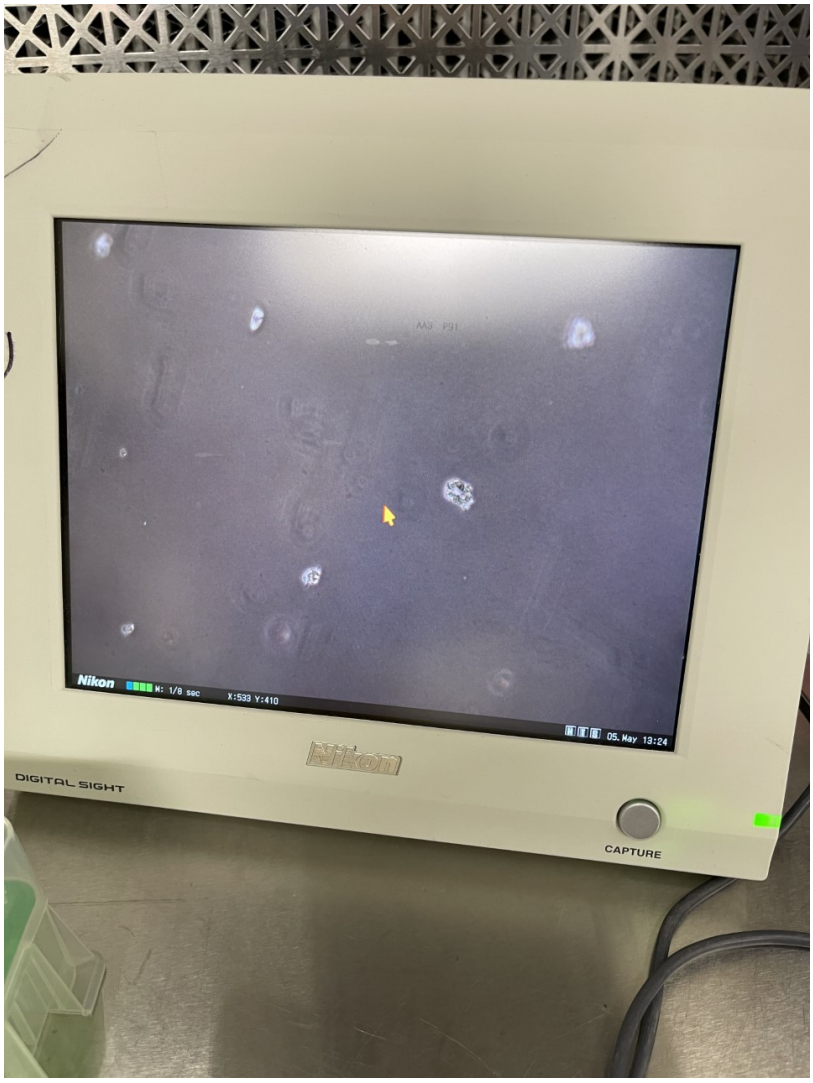
15 Use the same pipette to transfer cells to a 15 ml conical tube. Triturate gently without bubbles once or twice with a P-1000.

16 Transfer cells to a 15 ml conical tube. Triturate gently without bubbles once or twice with a P-1000. Transfer the desired amount of cells (typically a 1:10 split) into a new matrigel coated well (make sure you remove matrigel media from the new well and leave the well in 2 ml mTeSR media before you add cells).

Note

Use the distributed technique: aspirate 100uL with a P1000, go to a corner of the plate, and put the tip just under the surface. Watch the liquid level and dispense about ⅕ of the pipette tip, count to 3, then keep the tip submerged, go to the other corners, and finally, the center of the plate, repeating the process. Lid the plate and immediately gently tap on two sides in a Left/Right motion and a Front/Back motion to distribute cells (a circular motion leads to poor distribution).

17 Place wells back into incubator



Transferred cells immediately after plating (this may be a little low density, see first image for a higher density version)

Performing a media change

18 Change the media daily for the iPSCs. Aspirate media off, rinse with DPBS-/-, and then add 2 ml mTeSR plus per well of a 6-well plate and 6ml of media per T-25 flask

Note

If you cannot come in over the weekend, add 4 ml media per well in the 6-well plate, and 8 ml media in a T-25 on Friday evening and then perform a media change on Monday morning.

Single-cell dissociating iPSC

19 Aspirate media and Rinse with DPBS-/-. Add 1X TrypLE, 1ml per 6-well, 2ml per T-25, 3ml per T-75 (can use as low as 0.75X TrypLE according to GESC, dilute in 0.5mM EDTA pH 8).

20 Add 1X TrypLE, 1ml per 6-well, 2ml per T-25, 3ml per T-75 (can use as low as 0.75X TrypLE according to GESC, dilute in 0.5mM EDTA pH 8).

21 Put in the incubator until you see cells detaching (2 minutes to 5 minutes MAX), move the flask around to aid in that process.

22 After cells detach, add 2x the original volume minimum of fresh media in the vessel (to stop enzymatic reactions)

23 Take cells and put them in a 15ml conical tube. Spin down at 200 rpm, 00:05:00 (200g/RCF). 5m

24 Take off the supernatant, and resuspend the cell pellet in the media. Media per well in a 6-well is 2ml, 12-well is 1ml, T-25 is 5ml, T-75 is 20 mL.

Note

IMPORTANT: Add ROCKi once you replate cells. 1ul ROCKi per 1 ml of media in the vessel. Remove ROCKi via media change after 24 hrs. (ROCKi is not in the NComm paper, but is suggested by YiHsien and Vijay since it is commonly used to promote survival after single-cell dissociation and is used in the protocol. We have tested with and without and found significantly better differentiation to MNs with ROCKi). Note: RevitaCell is another ROCK inhibitor (may be better than Y-27632).

Freezing iPSCs

7m

25 Aspirate the media and rinse with 1 ml DPBS-/- per well. Add 1mL of ReLeSR to each well. Aspirate ReLeSR after 1-minute incubation

26 Add 1mL of ReLeSR to each well. Aspirate ReLeSR after 1-minute incubation

27 Allow the vessel to sit for 00:07:00 5-7min in the incubator. Checking every minute to ensure gaps are forming in the clusters. 7m

28 Aspirate 1mL of Cryostor CS10 (keep Cryostor on ice) for each well using a 5mL serological pipette.

29 Dispense 1mL of Cryostor CS10 into each well. Each well is rinsed 2-3 times, then the resuspension is transferred to a vial (keep Cryostor on ice) using the 5mL serological bore to scrape/press on the surface of each well