

Sep 06, 2022

🌐 Passaging of hPSCs grown on MEFs

📁 In 1 collection

DOI

dx.doi.org/10.17504/protocols.io.b4mpqu5n

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Protocol Citation: Hanqin Li, Oriol Busquets, Steven Poser, Dirk Hockemeyer, Frank Soldner 2022. Passaging of hPSCs grown on MEFs. **protocols.io** **<https://dx.doi.org/10.17504/protocols.io.b4mpqu5n>**

Manuscript citation:

Hanqin Li, Oriol Busquets, Yogendra Verma, Khaja Mohieddin Syed, Nitzan Kutnowski, Gabriella R Pangilinan, Luke A Gilbert, Helen S Bateup, Donald C Rio, Dirk Hockemeyer, Frank Soldner (2022) Highly efficient generation of isogenic pluripotent stem cell models using prime editing eLife 11:e79208

<https://doi.org/10.7554/eLife.79208>

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Protocol status: Working

Created: February 03, 2022

Last Modified: May 31, 2024

Protocol Integer ID: 57743

Keywords: ASAPCRN

Funders Acknowledgement:
Aligning Science Across
Parkinson's
Grant ID: ASAP-000486

Abstract

This protocol describes the standard procedure of using collagenase to passage human pluripotent stem cells (hPSCs) on inactivated mouse embryonic fibroblasts (MEFs).

General notes

1. Throughout this protocol, the term hPSC is used to collectively refer to both hiPSCs and hESCs. All described procedures have been tested and work equally well for hiPSCs and hESCs.
2. Until otherwise indicated, hPSCs are routinely grown in a humidified cell culture incubator under “low” oxygen conditions. We have successfully maintained hPSCs using either 3% O₂ (3% O₂, 5% CO₂) or 5% O₂ (5% O₂, 5% CO₂) conditions.
3. While bulk/collagenase passaging is used for routine maintenance of hPSC cultures, manual/microdissection passaging is used to enrich for undifferentiated hPSC colonies (“clean-up” of culture based on undifferentiated hPSC colony morphology) or to expand (“pick”) individual colonies (e.g., for clonal expansion of individual targeted cells in the process of establishing genome edited cell lines). Manual passaging/ microdissection requires (*i*) the identification and discrimination of undifferentiated and differentiated hPSC colonies and (*ii*) the capacity to excise the undifferentiated cells and transfer them to a new plate and can be performed using various approaches as established in many hPSC laboratories.

Materials

A	B	C
Item	Vendor	Catalog #
DMEM/F12	Thermo Fisher	11320082
DPBS w/o Calcium and magnesium (D PBS)	Corning	MT21031CV
Fetal Bovine Serum (FBS)	Corning	35-011-CV
Knockout Serum Replacement	Thermo Fisher	10828-028
Newborn Calf Serum	Sigma	N4762
L-Glutamine	Sigma	G8540
Penicillin & Streptomycin (100X)	Thermo Fisher	15140163
MEM Non-Essential Amino Acids (100X)	Thermo Fisher	11140050
Heat Stable Recombinant Human FGF2	Thermo Fisher	PHG0360
Collagenase type IV	Thermo Fisher	17104019
BSA	Sigma	A4503
2-Mercaptoethanol	Sigma	M3148
0.25% Trypsin with EDTA (Trypsin)	Thermo Fisher	25200114

- 1 Check MEFs plates for suitability to receive passaged hPSCs prior to beginning. Refer to the "Maintenance and inactivation of mouse embryonic fibroblasts (MEFs) as feeder cells for human pluripotent stem cell culture" collection; [dx.doi.org/10.17504/protocols.io.b4pbqvin](https://doi.org/10.17504/protocols.io.b4pbqvin)
- 2 Wash hPSCs with DPBS
- 3 Use 1 ml Collagenase solution/well of a 6-well plate.

3.1 Collagenase solution



Collagenase type IV	10 mg
KSR medium	10 ml

Final volume: 10ml

KSR medium

DMEM/F12	385 ml
Knockout Serum Replacement	100 ml
L-Glutamine (200 mM)	5 ml
Penicillin & Streptomycin (100X)	5 ml
MEM Non-Essential Amino Acids (100X)	5 ml

Final volume: 500ml

- 4 Incubate  00:45:00  37 °C . Watch for edge curling of the colonies as this indicates that collagenase incubation is complete.
- 5 Add 2 ml wash medium to each well.

45m

5.1 Wash medium

DMEM/F12	470 ml
Newborn Calf Serum	25 ml
Penicillin & Streptomycin (100X)	5 ml

Final volume: 500ml

- 6 Pipette repeatedly with 5 ml pipette to lift colonies, careful not to carry over too many MEFs.
- 7 Collect into 15 ml conical tube.
- 8 Add 4-5 ml Wash Medium.
- 9 Gravity precipitate cells 5- 10 min.
- 10 Aspirate Wash Medium, leave 0.5 ml. Don't disturb the colonies that are loosely pelleted.
- 11 Repeat steps 8-10 once
- 12 Add 5 ml hPSCs medium

12.1 hPSCs Medium

A	B
DMEM/F12	385 ml
Fetal Bovine Serum (FBS)	75 ml
Knockout Serum Replacement	25 ml
L-Glutamine (100X)	5 ml
Penicillin & Streptomycin (100X)	5 ml
MEM Non-Essential Amino Acids (100X)	5 ml
2-Mercaptoethanol (10,000X)	50 µl
Heat Stable Recombinant Human FGF2 (25ug/ml)*	80 µl

*While we prefer Heat Stable Recombinant Human FGF2, we also have used regular FGF2.
Final volume: 500ml

**L-Glutamine (100X)**

L-Glutamine, powder	14.6 g
MilliQ H ₂ O	500 ml

2-Mercaptoethanol (10,000X)

2-Mercaptoethanol	0.78 ml
MilliQ H ₂ O	9.22 ml

Heat Stable Recombinant Human FGF2 (25µg/ml)

A	B
Heat Stable Recombinant Human FGF2	500 µg
0.1% BSA	20 ml

Final volume: 20ml

- 13 Repeat steps 9-10 once
- 14 Add 3 ml hPSCs medium
- 15 Use a 10 ml strip pipette and triturate the colonies 5-10 times against the bottom of the tube to break up cell clusters

Note

- a. The objective is to reduce cluster size, not to completely dissociate to single cells.
- b. Avoid introducing air bubbles.

- 16 In preparation for the hPSCs, plate 1 ml hPSCs medium onto each MEFs well.
- 17 Add more hPSCs medium to the triturated colonies suspension to a proper dilution so that each MEFs wells will receive 2 ml. Splitting ratio varies between different cell lines and different operators. It's usually within a range of 1:6 to 1:20.



18 Mix well, distribute 2 ml to each MEFs wells.

Note

- a. Use of 5 ml pipette to mix cell suspension is preferred as it has the widest bore size, but 10 ml can be used if the volume of cells to plate is large.
- b. When mixing, expel and take up the entire volume of medium and cells 2 - 3 times, then take 4 ml of cell suspension to plate two wells at a time.
- c. Repeat until all cells are plated.

19 Place the plate in the low oxygen incubator

Note

- a. Do not stack the plates more than two high, preferably leave them as single level.
- b. Spread the cells by moving the plate in left-right, then backward-forward motion.
- c. Repeat cycle 2 - 3 times.
- d. Give the plate a final jerking motion in the backward-forward direction as the last spreading motion.
- e. Close the door of the incubator gently as to not disturb the position of the cell clusters.

20 From day 3, change 2-3 ml pre-warmed hPSCs medium for each well daily.

21 When hPSC density reaches 50-70% confluency, passage again. We usually adjust the splitting ratio to passage every 7 days.