Sep 06, 2022

Thawing of mouse embryonic fibroblasts (MEFs) for hPSC cultures

In 1 collection

Hanqin Li[1], Oriol Busquets[2], Steven Poser[2], Dirk Hockemeyer[1], Frank Soldner[2]

[1]University of California, Berkeley; [2]Albert Einstein College of Medicine

dx.doi.org/10.17504/protocols.io.b4n4qvgw

Devin E Snyder

ABSTRACT

This protocol describes the thawing of mouse embryonic fibroblasts (MEFs) as feeder cells for human pluripotent stem cell (hPSC) culture.

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. MEFs were obtained as described in Manipulating the Mouse Embryo: A Laboratory Manual, Third Edition (ISBN: 0879695919)

Andras Nagy, Marina Gertsenstein, Kristina Vintersten, & Richard Behringer. Manipulating the Mouse Embryo: A Laboratory Manual, 3rd ed.. Cold Spring Harbor Laboratory Press.

DOI

dx.doi.org/10.17504/protocols.io.b4n4qvgw

PROTOCOL CITATION

Hanqin Li, Oriol Busquets, Steven Poser, Dirk Hockemeyer, Frank Soldner 2022. Thawing of mouse embryonic fibroblasts (MEFs) for hPSC cultures. **protocols.io** https://dx.doi.org/10.17504/protocols.io.b4n4qvgw

FUNDERS ACKNOWLEDGEMENT

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

COLLECTIONS

Maintenance and inactivation of mouse embryonic fibroblasts (MEFs) as feeder cells for human pluripotent stem cell culture

KEYWORDS

ASAPCRN

CREATED

Feb 03, 2022

LAST MODIFIED

Sep 06, 2022

PROTOCOL INTEGER ID

57788

PARENT PROTOCOLS

Part of collection
Maintenance and inactivation of mouse embryonic fibroblasts (MEFs) as feeder cells for human pluripotent stem cell culture

MATERIALS TEXT

Item	Vendor	Catalog #
DMEM	Corning	10-013-CV
FB Essence	Avantor	10803-034
FBS	Gibco	10437028
200mM L-Glutamine	Sigma	G8540
Penicillin & Streptomycin	Gibco	15140-122
MEM Non-Essential Amino Acids	Gibco	11140-050
Gelatin powder	Sigma	G2625

Citation: Hanqin Li, Oriol Busquets, Steven Poser, Dirk Hockemeyer, Frank Soldner Thawing of mouse embryonic fibroblasts (MEFs) for hPSC cultures https://dx.doi.org/10.17504/protocols.io.b4n4qvgw

BEFORE STARTING

All cell culture plates which are used as feeders to maintain hPSCs are coated for at least 1 hour with autoclaved 0.2% gelatin solution at room temperature. Remove gelatin solution immediately before plating MEF cells.

0.2% Gelatin Solution

A	B
Sterile H2O	1L
Gelatin powder	2g

After preparation, the gelatin solution should be autoclaved. Final volume: 1L

1 To recover frozen stocks for MEF expansion, P0 MEF tubes will be thawed in a water bath at **37 °C** by gently shaking

2 Thawed cells are transferred into a 15 ml conical tube containing 9 ml pre-warmed MEF medium. Centrifuge the tube at **250 x g, 00:05:00** 5m

2.1 MEF medium

A	B
DMEM	435 ml
FB Essence/FBS*	75 ml
200mM L-Glutamine	5 ml
Penicillin & Streptomycin (100x)	5 ml
MEM Non-Essential Amino Acids	5 ml

*We have successfully used either FB Essence or FBS and have not observed an obvious difference. Final volume: 500ml

3 Resuspended MEFs (10x10^6 cells/10 cm plate) are plated in fresh MEF medium and maintained in a humidified incubator (37°C; 5% CO2)

4 3-4 days after thawing, the cells should be ready for passaging (90-95% confluent)

Citation: Hanqin Li, Oriol Busquets, Steven Poser, Dirk Hockemeyer, Frank Soldner Thawing of mouse embryonic fibroblasts (MEFs) for hPSC cultures https://dx.doi.org/10.17504/protocols.io.b4n4qvgw