Preparation of mouse embryonic fibroblast (MEF) feeder plates for hPSC cultures

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In 1 collection

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ABSTRACT

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

Protocol overview

A. Starting with frozen irradiated or Mitomycin C inactivated MEFs (optional)
B. Starting with fresh irradiated or Mitomycin C inactivated 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. Either fresh (start at step 4) or frozen stocks of irradiated or Mitomycin C inactivated MEFs can be used to prepare hPSC feeder cells.
3. The indicated MEF density are recommended starting densities and might have to be adjusted for each hPSC line and hPSC medium formulation (KSR, serum-free versus serum-containing medium).
4. 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.

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COLLECTIONS

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

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PARENT PROTOCOLS

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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
50ml centrifuge tubes	Corning	1495949A
10ml serological pipet	Corning	7200574
15cm tissue culture dish	Corning	0877224
DMEM/F12	Thermo Fisher	11320082
Fetal Bovine Serum (FBS)	Corning	35-011-CV
Knockout Serum Replacement	Thermo Fisher	10828-028
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
2-Mercaptoethanol	Sigma	M3148
BSA	Sigma	A4503

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

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A. Starting with frozen irradiated or Mitomycin C inactivated MEFs (optional)

1 To recover frozen stocks of irradiated or Mitomycin C inactivated MEFs (up to passage P4), thaw MEF tubes in in a water bath at **37 °C** by gently shaking.

2 Thawed cells are transferred into a 15 ml conical tube containing pre-warmed MEF medium and centrifuged 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 Re-suspend MEFs in fresh MEF medium

B. Starting with fresh irradiated or Mitomycin C inactivated

4 Take two sets of 10 µl of inactivated MEFs suspension (either irradiated or Mitomycin C inactivated). Mix each set with 10 µl trypan blue dye, which comes with the Countess™ Cell Counting Chamber Slides.

For a protocol on irradiation of MEFs or Mitomycin C inactivation of MEFs, refer to the collection "Maintenance and inactivation of mouse embryonic fibroblasts (MEFs) as feeder cells for human pluripotent stem cell culture." A link to this collection can be found in the title section of this protocol, located above.

5 Count cells with Countess automated cell counter or hemocytometer, average the counts from the two sets.

6 Dilute MEFs needed to $1.67x10^5$cells/ml in MEF medium

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7 Add 2.5 ml diluted MEFs to each well of 6-well plates. This gives ~4x10^4 feeders/cm2.

The indicated MEF seeding density is a recommended starting density for growing hPSCs in serum-containing medium, and might have to be adjusted for each hPSC line and hPSC media formulation (KSR, serum-free versus serum-containing media).

7.1 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 (25µg/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 H2O	500 ml

2-Mercaptoethanol (10,000X)

2-Mercaptoethanol	0.78 ml
MilliQ H2O	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

8 Shake the plates to distribute cells evenly. Maintain plates in a humidified incubator (37°C; 5% CO2). Feeders shall be used within 2 weeks after plating.

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9 For the irradiated MEFs leftover, freeze at $10x10^6$cells/cryovial for future use. Each vial usually can be thawed into three 6-well plates directly, if cells recover well.

For a protocol on freezing and thawing MEFs, refer to the collection "Maintenance and inactivation of mouse embryonic fibroblasts (MEFs) as feeder cells for human pluripotent stem cell culture." A link to this collection can be found in the title section of this protocol, located above.

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