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Expansion of mouse embryonic fibroblasts (MEFs) for hPSC cultures

In 1 collection

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ABSTRACT

This protocol describes the expansion 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.

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PROTOCOL CITATION

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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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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
0.25% Trypsin/EDTA (Trypsin)	Gibco	25200-056
DPBS w/o Ca & Mg (DPBS)	Corning	MT21031CV
10cm petri dish	Fisher	08757100D
10ml serological pipet	Corning	7200574
15cm tissue culture dish	Corning	0877224

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 Wash the plates twice with DPBS

2 Add Trypsin and incubate for **00:05:00** (37°C; 5% CO2) 5m

3 Add MEF medium to neutralize the Trypsin and collect the solution into a conical tube.

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3.1 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

4 Centrifuge at **250 x g, 00:05:00** 5m

5 Remove the supernatant. Re-suspend the cell pellet in fresh MEF medium to plate on gelatin-coated plates (Dilution ratio 1:4) and maintain in a humidified incubator (37°C; 5% CO2).

6 MEFs need to be passaged once confluent (every 3-4 days) and can be expanded up to passage 4 (P4) and frozen before or after inactivation using irradiation or Mitomycin C treatment.

For more information on freezing MEFs, as well as irradiation and Mitomycin C treatment 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

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