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

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ABSTRACT

This collection describes the maintenance and inactivation of mouse embryonic fibroblasts (MEFs) as feeder cells for human pluripotent stem cell (hPSC) culture.

Collection overview

Thawing of mouse embryonic fibroblasts (MEFs) for hPSC cultures
Expansion of mouse embryonic fibroblasts (MEFs) for hPSC cultures
Freezing of mouse embryonic fibroblasts (MEFs) for hPSC cultures
Harvesting and irradiation of mouse embryonic fibroblasts (MEFs) for hPSC cultures
Mitomycin C inactivation of mouse embryonic fibroblasts (MEFs) for hPSC cultures
Preparation of mouse embryonic fibroblast (MEF) feeder plates for hPSC cultures
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 these protocols, 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 or frozen stocks of irradiated or Mitomycin C inactivated MEFs can be used to prepare hPSC feeder cells.
3. The indicated MEF densities are recommended starting densities and might have to be adjusted for each hPSC line and hPSC media formulation (KSR, serum-free versus serum-containing media).
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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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
DMSO	Thermo Fisher	BP231-100
Gelatin powder	Sigma	G2625
0.25% Trypsin/EDTA	Gibco	25200-056
DNase	Roche	4536282001
DPBS w/o Ca & Mg	Corning	MT21031CV
50ml centrifuge tubes	Corning	1495949A
Nunc 1.8 ml cryovials	Thermo Fisher	377267
10cm petri dish	Fisher	08757100D
10ml serological pipet	Corning	7200574
15cm tissue culture dish	Corning	0877224
No5. forceps	Roboz	RS-5010
Microdissecting curved surgical scissors	Roboz	RS-5881
Styrofoam microtube freezer box	Labnet	R8000

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FILES

Thawing of mouse embryonic fibroblasts (MEFs) for hPSC cultures
Version 1
by Devin E Snyder

Expansion of mouse embryonic fibroblasts (MEFs) for hPSC cultures
Version 1
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Freezing of mouse embryonic fibroblasts (MEFs) for hPSC cultures
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Harvesting and irradiation of mouse embryonic fibroblasts (MEFs) for hPSC cultures
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