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Harvesting and irradiation of mouse embryonic fibroblasts (MEFs) for hPSC cultures

In 1 collection

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ABSTRACT

This protocol describes the process of harvesting and irradiating mouse embryonic fibroblasts (MEFs) to use 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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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
0.25% Trypsin/EDTA (Trypsin)	Gibco	25200-056
DPBS w/o Ca & Mg (DPBS)	Corning	MT21031CV
50ml centrifuge tubes	Corning	1495949A
10cm petri dish	Fisher	08757100D
10ml serological pipet	Corning	7200574
15cm tissue culture dish	Corning	0877224

1 Wash the plates once with 10 ml DPBS for each 15-cm plate.

2 Add 3 ml Trypsin and incubate for in 37°C; 5% CO2 for **00:10:00** 10m

3 Add 9 ml MEF medium to neutralize the Trypsin. Collect cell suspension in a 50 ml conical tube.

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

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4 Rinse the plate with 9 ml of new MEF medium to collect the remaining cells.

5 If there are cell clumps that not fully dissociated, let the clumps settle by gravity for 3 min then transfer the single cell suspension to a new conical tube. Cells in clumps are usually not uniformly irradiated and that may lead to proliferating feeders.

6 Parafilm the conical tubes and bring cells to irradiation.

7 Irradiate the cells in conical tubes at 3500 cGy on Precision X-RAD 320 irradiator.

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