Sep 06, 2022

Freezing of mouse embryonic fibroblasts (MEFs) for hPSC cultures

In 1 collection

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ABSTRACT

This protocol describes the freezing of mouse embryonic fibroblasts (MEFs), which can later be used as feeder cells for human pluripotent stem cell (hPSC) culture.

General notes

1. This protocol can be used to freeze MEFs before or after inactivation.
2. 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.
3. 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

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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
0.25% Trypsin/EDTA (Trypsin)	Gibco	25200-056
DPBS w/o Ca & Mg (DPBS)	Corning	MT21031CV
50ml centrifuge tubes	Corning	1495949A
Nunc 1.8 ml cryovials	Thermo Fisher	377267
10ml serological pipet	Corning	7200574
Styrofoam microtube freezer box	Labnet	R8000

1 Wash the plates twice with DPBS

2 Add Trypsin (5 ml for 15-cm plate) and incubate for **00:05:00** (37°C; 5% CO2)

5m

3 Add 10 ml MEF medium to neutralize the Trypsin and collect the solution into a 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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10m

4 Centrifuge the cell suspension at **250 x g, Room temperature, 00:10:00**

5 Discard supernatant, and re-suspend the cells in MEF Freezing Medium I at a concentration 2X the desired final freezing concentration (Freezing concentration will vary with MEF usage.)

5.1 MEF Freezing Medium I

A	B
FB Essence/FBS*	5 ml
MEF media	5 ml

*We have successfully used either FB Essence or FBS and have not observed an obvious difference. Final volume: 10ml

6 While swirling, slowly and drop-wise, add an equal volume of MEF Freezing Medium II to the cell suspension.

6.1 MEF Freezing Medium II

A	B
FB Essence/FBS*	8 ml
DMSO	2 ml

*We have successfully used either FB Essence or FBS and have not observed an obvious difference. Final volume: 10ml

7 Dispense cell mixture into pre-labeled cryovials ($10x10^6$) including type of cells, passage number, date frozen, and who handled cells.

8 Place cryovials into Styrofoam microtube freezer box or NALGENE™ Cryo 1°C Freezing Container pre-filled with 250 ml room temperature isopropanol.(This ensures that a -1°C/min rate of cooling is achieved, which is critical to cell viability.)

9 Freeze at **Overnight** **-80 °C**

10 For long term storage, store cryovials in liquid nitrogen (-196ºC).

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