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Thawing, Passaging and Freezing of hPSCs on MEFs

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ABSTRACT

This collection contains protocols which describe the standard procedure of culturing human pluripotent stem cells (hPSCs) on inactivated mouse embryonic fibroblasts (MEFs).

Collection overview

Thawing of hPSCs grown on MEFs
Passaging of hPSCs grown on MEFs
Freezing of hPSCs grown on MEFs
A. Freezing of hPSCs as single cell suspension using trypsin
B. Freezing of hPSCs as cell aggregates using collagenase

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. Until otherwise indicated, hPSCs are routinely grown in a humidified cell culture incubator under “low” oxygen conditions. We have successfully maintained hPSCs using either 3% O2 (3% O2, 5% CO2) or 5% O2 (5% O2, 5% CO2) conditions.
3. While freezing hPSCs as single cell solution (using Rock Inhibitor) results in better cell recovery, some laboratories prefer freezing of hPSCs as cell clusters. We have used both approaches and do not observe obvious differences.
4. While bulk/collagenase passaging is used for routine maintenance of hPSC cultures, manual/microdissection passaging is used to enrich for undifferentiated hPSC colonies (“clean-up” of culture based on undifferentiated hPSC colony morphology) or to expand (“pick”) individual colonies (e.g.,for clonal expansion of individual targeted cells in the process of establishing genome edited cell lines). Manual passaging/ microdissection requires (i) the identification and discrimination of undifferentiated and differentiated hPSC colonies and (ii) the capacity to excise the undifferentiated cells and transfer them to a new plate and can be performed using various approaches as established in many hPSC laboratories.

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KEYWORDS

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MATERIALS TEXT

A	B	C
Item	**Vendor**	**Catalog #**
DMEM/F12	Thermo Fisher	11320082
DPBS w/o Calcium and magnesium	Corning	MT21031CV
Fetal Bovine Serum (FBS)	Corning	35-011-CV
Knockout Serum Replacement	Thermo Fisher	10828-028
FB Essence	Avantor	10803-034
Newborn Calf Serum	Sigma	N4762
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
Collagenase type IV	Thermo Fisher	17104019
DMSO	Fisher Scientific	BP231-100
BSA	Sigma	A4503
Y-27632	Chemdea	CD0141
2-Mercaptoethanol	Sigma	M3148
0.25% Trypsin with EDTA	Thermo Fisher	25200114
Styrofoam microtube freezer box	Labnet	R8000
Nalgene® Mr. Frosty® Cryo 1°C Freezing Containers	Thermo Fisher	

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FILES

Thawing of hPSCs grown on MEFs
Version 1
by Devin E Snyder

Passaging of hPSCs grown on MEFs
Version 1
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Freezing of hPSCs grown on MEFs
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