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Standard operating procedure for the isolation of genetically engineered hPSCs clones in a high-throughput way

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ABSTRACT

This collection describes a standard procedure for isolating single human pluripotent stem cell (hPSC) clones in a high-throughput way. This collection follows nucleofection of hPSCs as described in detail in the collection "Nucleofection (Amaxa) and electroporation (Biorad) of hPSCs;" dx.doi.org/10.17504/protocols.io.b4qnqvve

Collection overview

Seeding nucleofected hPSCs in 96-well plates using limited dilution
Duplicating 96-well plate-cultured hPSCs clones
Subcloning of genotype-confirmed hPSCs clones

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.

DOI

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COLLECTION CITATION

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A	B	C
Item	**Vendor**	**Catalog #**
DMEM/F12	Thermo Fisher	11320082
DPBS w/o Calcium and magnesium (DPBS)	Corning	MT21031CV
Fetal Bovine Serum (FBS)	Corning	35-011-CV
Knockout Serum Replacement	Thermo Fisher	10828-028
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
Y-27632	Chemdea	CD0141
2-Mercaptoethanol	Sigma	M3148
0.25% Trypsin with EDTA (Trypisin)	Thermo Fisher	25200114
DMSO	Fisher Scientific	BP231-100
Proteinase K	Sigma	P6556

*While we prefer Heat Stable Recombinant Human FGF2, we also have used regular FGF2

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FILES

Seeding nucleofected hPSCs in 96-well plates using limited dilution
Version 1
by Devin E Snyder

Duplicating 96-well plate-cultured hPSCs clones
Version 1
by Devin E Snyder

Subcloning of genotype-confirmed hPSCs clones
Version 1
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