Seeding nucleofected hPSCs in 96-well plates using limited dilution

In 1 collection

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dx.doi.org/10.17504/protocols.io.b4miqu4e

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ABSTRACT

This protocol describes a standard procedure for seeding nucleofected human pluripotent stem cells in 96-well plates using limited dilution. This protocol 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

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.

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PROTOCOL CITATION

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COLLECTIONS

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

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

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MATERIALS TEXT

A	B	C
Item	**Vendor**	**Catalog #**
DMEM/F12	Thermo Fisher	11320082
Fetal Bovine Serum (FBS)	Corning	35-011-CV
Knockout Serum Replacement (KSR)	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
Proteinase K	Sigma	P6556
DMSO	Fisher Scientific	BP231-100

1 For an editing with 10% efficiency, prepare two 96-well MEFs plates with a density at 2 million cells/plate at least 1 day ahead. Do not shake the plate after seeding MEFs. It generates swirls and causes cells to accumulate at the center. If editing efficiency is expected to be low, prepare more 96-well plates.

2 Calculate the number of cells needed. Cells should be seeded at a density that gives 20-30 colonies/plate. The density depends on cell survival post nucleofection, and varies between cell lines.

For WIBR3 cells, we seed 1,000 cells per plate.

3 For every seeding density, make and label falcon tubes with 11 ml of hPSC medium + Rock inhibitor. Add the appropriate amount of cell solution to reach your desired cell concentration.

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3.1 hPSC medium

A	B
DMEM/F12	385 ml
Fetal Bovine Serum (FBS)	75 ml
Knockout Serum Replacement	25 ml
L-Glutamine (100X)	5 ml
Penicillin & Streptomycin (100X)	5 ml
MEM Non-Essential Amino Acids (100X)	5 ml
2-Mercaptoethanol (10,000X)	50 µl
Heat Stable Recombinant Human FGF2 (25ug/ml)*	80 µl

*While we prefer Heat Stable Recombinant Human FGF2, we also have used regular FGF2. Final volume: 500ml

L-Glutamine (100X)

L-Glutamine, powder	14.6 g
MilliQ H2O	500 ml

2-Mercaptoethanol (10,000X)

2-Mercaptoethanol	0.78 ml
MilliQ H2O	9.22 ml

Heat Stable Recombinant Human FGF2 (25µg/ml)

A	B
Heat Stable Recombinant Human FGF2	500 µg
0.1% BSA	20 ml

Final volume: 20ml

Y-27632 (1,000X)

Y-27632	5 mg
DMSO	1.56 ml

hPSC medium + Rock inhibitor

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A	B
hPSCs medium	500 ml
Y-27632 (1,000X)	500 µl

Final volume: 500ml

4 Take out 96-well MEF plates, carefully label them with their cell seeding number.

5 Aspirate feeder medium from the plates, then add 100 µl/well using a multichannel pipette and 200 µl filter tips. Use a sterile reagent reservoir for medium.

6 Additionally, prepare one well of a 6-well plate for each seeding density on your 96-well format. Seed the same number of cells that were seeded to the 96-well plate to this well, e.g. 1,000 cells. These will be analyzed in parallel to track the approximate number of expected colonies/clones from the 96-well plates.

7 Seed the rest of cells to another well of a 6-well plate. This will become the bulk sample for estimating the editing efficiency. Change medium daily starting from day 3 for this well and prepare crude cell lysis once it reaches a good confluency.

7.1 Crude lysis buffer (2x)

A	B
KCl	100mM
MgCl2	4mM
NP-40	0.9%
Tween-20	0.9%
Tris	20mM
Proteinase K (add before use)	500µg/ml

pH: 8

8 Change medium for your 96-well plates by using a multi-channel pipet and aspirator every three days. Reduce it to once every two days once the colonies in your plates grow bigger, or during the second week post-nucleofection.

9 Keep cells in culture for 10-14 days. Proceed with plate duplication if the well in the 6-well plate (originally seeded with 1,000 cells) shows 20-50 colonies.

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