Duplicating 96-well plate-cultured hPSCs clones

Sep 06, 2022

In 1 collection

Hanqin Li[1], Yogendra Verma[1], Dirk Hockemeyer[1], Frank Soldner[2]

[1]University of California, Berkeley; [2]Albert Einstein College of Medicine

dx.doi.org/10.17504/protocols.io.b4mjqu4n

Devin E Snyder

ABSTRACT

This protocol describes a standard procedure for duplicating 96-well plate-cultured human pluripotent stem cells (hPSCs).

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

DOI

dx.doi.org/10.17504/protocols.io.b4mjqu4n

PROTOCOL CITATION

Hanqin Li, Yogendra Verma, Dirk Hockemeyer, Frank Soldner 2022. Duplicating 96-well plate-cultured hPSCs clones. **protocols.io** https://dx.doi.org/10.17504/protocols.io.b4mjqu4n

FUNDERS ACKNOWLEDGEMENT

Aligning Science Across Parkinson's
Grant ID: ASAP-000486

COLLECTIONS

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

KEYWORDS

ASAPCRN

CREATED

Feb 03, 2022

LAST MODIFIED

Sep 06, 2022

PROTOCOL INTEGER ID

57739

PARENT PROTOCOLS

Part of collection

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

Citation: Hanqin Li, Yogendra Verma, Dirk Hockemeyer, Frank Soldner Duplicating 96-well plate-cultured hPSCs clones https://dx.doi.org/10.17504/protocols.io.b4mjqu4n

MATERIALS TEXT

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

1 Change medium to hPSCs medium + Rock inhibitor one day ahead of plate duplication.

1.1 hPSC medium

Citation: Hanqin Li, Yogendra Verma, Dirk Hockemeyer, Frank Soldner Duplicating 96-well plate-cultured hPSCs clones https://dx.doi.org/10.17504/protocols.io.b4mjqu4n

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

Citation: Hanqin Li, Yogendra Verma, Dirk Hockemeyer, Frank Soldner Duplicating 96-well plate-cultured hPSCs clones https://dx.doi.org/10.17504/protocols.io.b4mjqu4n

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

Final volume: 500ml

2 Prepare 11 ml DPBS, 3 ml Trypsin, 15 ml hPSCs medium + Rock inhibitor and 3 ml 2x Crude lysis buffer (with proteinase K) into separate reservoirs.

2.1 Crude lysis buffer (2x)

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

pH: 8

3 Aspirate the medium from plates, wash by dispensing 100 µl DPBS per well using a Multichannel pipette. Gently swirl plates to wash the content of the wells.

4 Aspirate DPBS from the plates using multichannel aspirator.

5 Add 25 µl Trypsin to each well using a Multichannel pipette.

6 Incubate at **37 °C** **00:05:00** 5m

7 Take a 96-well PCR plate and dispense 25 µl of 2X Crude lysis buffer with Proteinase K per well onto the plates. Label the plates according to your tissue culture plates.

Citation: Hanqin Li, Yogendra Verma, Dirk Hockemeyer, Frank Soldner Duplicating 96-well plate-cultured hPSCs clones https://dx.doi.org/10.17504/protocols.io.b4mjqu4n

8 Take a new 96-well MEFs plate. Aspirate MEF medium and add 75 µl of hPSCs medium + Rock inhibitor to the wells.

9 Take the Trypsin-dissociated cells out from 37°C . Dispense 25 µl hPSCs medium + Rock inhibitor onto each well using a Multichannel pipette to inactivate trypsin.

NOTES: Try not to touch the base of each well, this way you keep reusing the same pipette tips for multiple wells without crossing or mixing cells between wells. Or else, use fresh pipette tips for each well.

10 Use a fresh 200 µl filter tip box for this step:

Set the Multichannel at 25 µl, load it with pipette tips, break down the trypsinized wells by pipette at least 7-8 times, focusing mostly on the center of the well, also pipette 2-3 times near the circumference of the wells.

11 Using the same tips, take out 25 µl of the dissociated cells, and dispense them over the new MEFs plate from step 8.

12 Take the same tips to pipette out the remaining approx. 25 µl cell suspension to the PCR plate from step 7. This becomes the gDNA plate. The location for all wells on tissue culture plate should correspond with their particular gDNA collection plate.

Throughout steps 10-12, make sure to use fresh tips for each well. Eject out the used tips before proceeding to other wells. To make it easier to follow, mimic the location of the tips in the tip box with the location of the wells on tissue culture and gDNA plates. Also make sure to prioritize adding the first 25 µl of cells to the new tissue culture plate , and then go back to collect the remaining 25 µl to be added to gDNA plate.

13 After adding all samples to tissue culture and gDNA plates, transfer the tissue culture plates to **37 °C** . Do not shake. Medium change every other day.

Citation: Hanqin Li, Yogendra Verma, Dirk Hockemeyer, Frank Soldner Duplicating 96-well plate-cultured hPSCs clones https://dx.doi.org/10.17504/protocols.io.b4mjqu4n

14 Seal the gDNA plate using an adhesive plate seal. Ensure they are tightly covered to prevent evaporation.

15 Place the gDNA plate at **50 °C** **Overnight**

16 Heat inactivate at **95 °C** **00:05:00** in a thermocycler 5m

17 Chill **On ice**

18 Proceed to PCR, NGS and analysis to identify properly edited clones.

19 Genotyping needs to be done in a week before the duplicated cells overgrow.

Citation: Hanqin Li, Yogendra Verma, Dirk Hockemeyer, Frank Soldner Duplicating 96-well plate-cultured hPSCs clones https://dx.doi.org/10.17504/protocols.io.b4mjqu4n