Sep 06, 2022

Electroporation of hPSCs

In 1 collection

Hanqin Li[1], Oriol Busquets[2], Steven Poser[2], Dirk Hockemeyer[1], Frank Soldner[2]

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

1 *Works for me* Share

dx.doi.org/10.17504/protocols.io.b4phqvj6

Devin E Snyder

ABSTRACT

This protocol describes the standard procedure for the delivery of plasmids into human pluripotent stem cells (hPSCs) using electroporation.

Protocol Overview

A. Preparation of MEFs-cultured hPSCs for electroporation
B. Preparing plasmids for electroporation
C. Electroporation

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.b4phqvj6

PROTOCOL CITATION

Hanqin Li, Oriol Busquets, Steven Poser, Dirk Hockemeyer, Frank Soldner 2022. Electroporation of hPSCs. **protocols.io** https://dx.doi.org/10.17504/protocols.io.b4phqvj6

FUNDERS ACKNOWLEDGEMENT

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

COLLECTIONS

Nucleofection (Amaxa) and electroporation (Biorad) of hPSCs

KEYWORDS

ASAPCRN

CREATED

Feb 03, 2022

LAST MODIFIED

Sep 06, 2022

PROTOCOL INTEGER ID

57801

PARENT PROTOCOLS

Part of collection

Nucleofection (Amaxa) and electroporation (Biorad) of hPSCs

MATERIALS TEXT

Citation: Hanqin Li, Oriol Busquets, Steven Poser, Dirk Hockemeyer, Frank Soldner Electroporation of hPSCs https://dx.doi.org/10.17504/protocols.io.b4phqvj6

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
2-Mercaptoethanol	Sigma	M3148
Y-27632	Chemdea	CD0141
Cas9, purified protein, 40μM	Macrolab, QB3 UC Berkeley	
Synthetic pegRNAs	IDT or Synthego	
Synthetic sgRNAs	Synthego	
P3 primary Cell 4D X kit S	Lonza	V4XP-3032
Countess™ Cell Counting Chamber Slides	Thermo Fisher	C10228
pCMV-PE2	Addgene	132775
4D-Nucleofector TM Core + X Unit	Lonza	AAF-1002B, AAF-1002X
5 ml polystyrene round-bottom tube with cell-strainer cap	Corning	352235
Cell-strainer (70 μm)	Fisher	07201431
Gene Pulser Xcell Eukaryotic System	Bio-Rad	1652661
Gene Pulser Electroporation Cuvettes, 0.4 cm gap	Bio-Rad	1652081
Exact N Amp Blood PCR Kit	Sigma	XNAT2-1KT
0.25% Trypsin/EDTA (Trypsin)	Gibco	25200-056

Note: This protocol makes reference to other protocols. Please check for any materials found in those protocols, which might not be listed here

A. Preparation of MEFs-cultured hPSCs for electroporation 2h 18m

1 Incubate the hPSC cultures (on MEF feeders) in hPSC medium containing 10 μM Y-27632 (1:1000 dilution of stock) for at least two hours (overnight works as well) before electroporation. Two to three near confluent 6-well plates should provide sufficient cells for each individual experiment (higher than $10X10^6$ hPSCs/experiment)

Citation: Hanqin Li, Oriol Busquets, Steven Poser, Dirk Hockemeyer, Frank Soldner Electroporation of hPSCs https://dx.doi.org/10.17504/protocols.io.b4phqvj6

For a detailed protocol on hPSC cultures on MEF feeders, refer to the collection "Maintenance and inactivation of mouse embryonic fibroblasts (MEFs) as feeder cells for human pluripotent stem cell culture;" dx.doi.org/10.17504/protocols.io.b4pbqvin

1.1 hPSCs medium

Reagent	Volume
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 (25µg/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)

Citation: Hanqin Li, Oriol Busquets, Steven Poser, Dirk Hockemeyer, Frank Soldner Electroporation of hPSCs
https://dx.doi.org/10.17504/protocols.io.b4phqvj6

A	B
Y-27632	5 mg
DMSO	1.56 ml

hPSCs medium + Rock inhibitor

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

Final volume: 500ml

2 Wash the hPSC plates twice with DPBS

3 Add 1 ml Trypsin/well of a 6-well plate and incubate for **00:05:00** at **37 °C** until colonies start detaching from MEF feeders. 5m

4 Add 2 ml hPSC medium to inactivate the trypsin.

5 Collect single cell or small clump solution by trituration with P1000 pipette tips into 50 ml conical tube.

6 Gravity precipitate cell solution for **00:05:00** min and remove large clumps of feeders with 5 ml pipette from the bottom of the conical tube. 5m

7 Centrifuge at **225 x g, 00:10:00** 10m

8 Remove the supernatant and re-suspend the cells at high concentration (higher than 10X10^6cells/700 µl) in ice-cold DPBS.

B. Preparing plasmids for electroporation

Citation: Hanqin Li, Oriol Busquets, Steven Poser, Dirk Hockemeyer, Frank Soldner Electroporation of hPSCs https://dx.doi.org/10.17504/protocols.io.b4phqvj6

9 Thaw plasmids **On ice**

10 Dependent on the specific experiment (ZNFs-, TALEN- or CRISPR/Cas9-based genome editing) we recommend keeping the overall amount of plasmid DNA for each experiment below 50 µg.

11 For **CRISPR/Cas9 genome editing**, use:
16 µg pX330-GFP gRNA (alternative: pX330-mcherry/BFP/YFP)
34 µg ssODN (single strand oligonucleotide containing modification, ~100 bp)

12 For **TALEN genome editing**, use
7.5 µg for each (left and right) TALEN-nuclease plasmid
26 µg ssODN (single strand oligonucleotide containing modification, ~100 bp)
10 µg pEGFP-N1 (or comparable fluorescent protein expression vector)

13 For **ZFN genome editing**, use
7.5 µg for each (left and right) ZFN-nuclease plasmid
26 µg ssODN (single strand oligonucleotide containing modification, ~100 bp)
10 µg pEGFP-N1 (or comparable fluorescent protein expression vector)

14 For prime editing **PE2 strategy,** use
33 µg pCMV-PE2-GFP
12 µg pU6-pegRNA

15 For prime editing **PE3 strategy**, use
33 µg pCMV-PE2-GFP
12 µg pU6-pegRNA
5 µg pBPK1520-ngRNA

16 Pipet the proper amount of each plasmid into a micro centrifuge tube and keep **On ice** (4°C) until electroporation.

C. Electroporation 10m

17 Mix cell suspension (higher than 10X10^{6}cells/700 µl DPBS) with pre-made transfection solution (plasmid DNA), transfer in Biorad electroporation cuvette (4 mm) and incubate on ice for **00:10:00** 10m

18 Resuspend cell suspension cuvettes (either by careful vortexing or by hand) and pulse

Citation: Hanqin Li, Oriol Busquets, Steven Poser, Dirk Hockemeyer, Frank Soldner Electroporation of hPSCs
https://dx.doi.org/10.17504/protocols.io.b4phqvj6

cuvettes using Biorad electroporator (Gene Pulser Xcell System, Bio-Rad: 250 V, 500 µF, 4 mm cuvettes).

19 Carefully collect the cells and transfer to pre-warmed hPSC medium containing 10 µM Y-27632 (1:1000 dilution of stock) and distribute electroporated cell solution on an entire 6-well MEF feeder plate (DR4-MEFs for selection-based experiments and ICR-MEFs for subsequent FACS sorting)

20 After plating shake the plate/s gently in the incubator and let it sit **Overnight** (37°C; 5% CO_2; 5% O_2).

21 Refresh hPSC medium daily until FACS or drug selection (usually after 48 – 72 hours).

Citation: Hanqin Li, Oriol Busquets, Steven Poser, Dirk Hockemeyer, Frank Soldner Electroporation of hPSCs
https://dx.doi.org/10.17504/protocols.io.b4phqvj6