Sep 06, 2022

Nucleofection of hPSCs

In 1 collection

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dx.doi.org/10.17504/protocols.io.b4pcqviw

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ABSTRACT

This protocol describes the standard procedure for the delivery of plasmids, mRNA or ribonucleoprotein (RNP) into human pluripotent stem cells (hPSCs) using nucleofection.

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.b4pcqviw

PROTOCOL CITATION

Hanqin Li, Oriol Busquets, Steven Poser, Dirk Hockemeyer, Frank Soldner 2022. Nucleofection of hPSCs. **protocols.io** https://dx.doi.org/10.17504/protocols.io.b4pcqviw

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

57796

PARENT PROTOCOLS

Part of collection

Nucleofection (Amaxa) and electroporation (Biorad) of hPSCs

MATERIALS TEXT

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
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
2-Mercaptoethanol	Sigma	M3148
mTeSR-plus	STEMCELL Technologies	100-0276
StemFlex	Thermo Fisher	A3349401
Vitronectin (VTN-N) Recombinant Human Protein, Truncated	Thermo Fisher	A14700

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Accutase	Thermo Fisher	SCR005
Dispase	STEMCELL Technologies	NC9995391
Y-27632	Chemdea	CD0141
Cas9, purified protein, 40uM	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	XNAB2-1TK

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

1 For each nucleofection, prepare 20 µl nucleofection solution by mixing 16.4 µl solution I and 3.6 µl supplement.

2 Prepare plasmids, RNA, or RNP as described in their associated protocols in the collection "Nucleofection (Amaxa) and electroporation (Biorad) of hPSCs." A link to this collection can be found in the title section of this protocol, located above. The specific protocol titles for each preparation type are listed below.

Plasmids: Preparing plasmids for nucleofection
RNA: Preparing mRNA for nucleofection
RNP: In vitro assembling of RNP for nucleofection

3 Prepare cells for nucleofection as described in protocols “Preparing MEF-cultured hPSCs for nucleofection” or “Preparing feeder-free hPSCs for nucleofection," which are both part of the collection "Nucleofection (Amaxa) and electroporation (Biorad) of hPSCs." A link to this collection can be found in the title section of this protocol, located above

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4 Re-suspend cell pellets in nucleofection solution. Mix using P1000 or P200 tips to ensure single cell suspension.

5 Transfer 20 µl cell suspension to the microcentrifuge tube containing the materials to be nucleofected. Mix well.

6 Transfer the entire volume to one cuvette in the 16-cuvette strip. Mark the cuvette used on the lid.

7 Gently stamp the strip on bench to help the small amount of cell suspension enter the narrow space between the metal paddles properly.

8 Nucleofect with program, P3 primary cell, CA137

> The Soldner lab has successfully used previous generation Amaxa nucleofection (Nucleofector 2b device, Amaxa Human Stem Cell Nucleofector Kit 2, program B016) for genome editing of hPSCs.

9 After nucleofection, immediately add 150 µl hPSCs medium or feeder-free medium + Rock Inhibitor to the cuvette according to the culturing system that will be used.

For a detailed description of hPSCs medium, refer to the protocol "Preparing MEF-cultured hPSCs for nucleofection" in the collection "Nucleofection (Amaxa) and electoporation (Biorad) of hPSCs. A link to this collection can be found in the title section of this protocol, located above.

For a detailed description of Feeder-free medium + Rock Inhibitor, refer to the collection "Feeder-free culturing of hPSCs;" dx.doi.org/10.17504/protocols.io.b4mcqu2w

10 Pipet thoroughly using a P200 tip

11 Transfer all cells to a new microcentrifuge tube

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12 Transfer proper amount of cells to MEFs plate or VTN/Matrigel/Geltrex-coated plate

For a detailed protocol on plating hPSCs on MEFS 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

For a detailed protocol on coating plates with VTN/Matrigel/Geltrex, refer to the collection "Feeder-free culturing of hPSCs;" dx.doi.org/10.17504/protocols.io.b4mcqu2w

13 Place the plate in the low oxygen incubator, 3% O2, 5% CO2.

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