



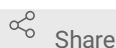
Sep 06, 2022

Nucleofection (Amaxa) and electroporation (Biorad) of hPSCs

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1 Works for me



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Devin E Snyder

ABSTRACT

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

Collection Overview

Preparing MEF-cultured hPSCs for nucleofection

Preparing Feeder-free hPSCs for nucleofection

Preparing plasmids for nucleofection

Preparing mRNA for nucleofection

In vitro assembling of RNP for nucleofection

Nucleofection of hPSCs

Electroporation of hPSCs

A. Preparation of MEFs-cultured hPSCs for electroporation

B. Preparing plasmids for electroporation

C. Electroporation

FACS-based enrichment of transfected cells

A. Preparation of samples for FACS sorting

B. After FACS

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.

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COLLECTION CITATION

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KEYWORDS

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Feb 04, 2022

LAST MODIFIED

Sep 06, 2022

COLLECTION INTEGER ID

57838

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
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

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FILES

-   **Preparing MEF-cultured hPSCs for nucleofection**
Version 1
by Devin E Snyder
-   **Preparing feeder-free hPSCs for nucleofection**
Version 1
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-   **Preparing plasmids for nucleofection of hPSCs**
Version 1
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-   **Preparing mRNA for nucleofection of hPSCs**
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