VERSION 2
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Nucleofection (Amaxa) and electroporation (Biorad) of hPSCs V.2

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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.

MATERIALS

	Item	Vendor	Catalog #
	DMEM/F12	Thermo Fisher	11320082
	DPBS w/o calcium and magnesium	Corning	MT21031CV

Protocol status: Working
We use this collection and it's working

Created: Jan 12, 2023

Last Modified: Jan 12, 2023

COLLECTION integer ID: 75230

Keywords: ASAPCRN

	Item	Vendor	Catalog #
	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

	Item	Vendor	Catalog #
	Exact N Amp Blood PCR Kit	Sigma	XNAB2-1TK

FILES

Protocol

NAME
Preparing MEF-cultured hPSCs for nucleofection

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Protocol

NAME
Preparing feeder-free hPSCs for nucleofection

VERSION 1

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NAME
Preparing plasmids for nucleofection of hPSCs

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NAME
Preparing mRNA for nucleofection of hPSCs

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In vitro assembling of RNP for nucleofection of hPSCs

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Nucleofection of hPSCs

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Electroporation of hPSCs

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Protocol

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FACS-based enrichment of transfected hPSCs

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