FACS-based enrichment of transfected hPSCs

In 1 collection

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ABSTRACT

This protocol describes the procedure FACS-based enrichment of transfected human pluripotent stem cells (hPSCs).

Protocol overview

A. Preparation of samples for FACS sorting
B. After FACS

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.
2. Dependent on the genome editing strategy, we use either drug selection or FACS sorting to enrich transfected cell populations based on the presence of a fluorescent protein (either as part of the transfected plasmids or through co-transfection of a fluorescent protein expressing plasmid).
3. Either drug selection or FACS sorting will be usually performed 48 – 72 hours after electroporation/nucleofection.
4. Carefully plan the timeline of FACS experiments and schedule required FACS sorting time at core facility (requires approximately 45 min sorting time per sample)
5. Every FACS sorting experiment requires an additional non-electroporated well of hPSCs (parental cell line) as a negative control to determine the appropriate FACS gates.

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PROTOCOL CITATION

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COLLECTIONS

Nucleofection (Amaxa) and electroporation (Biorad) of hPSCs

KEYWORDS

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LICENSE

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57802

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 (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
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

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

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A. Preparation of samples for FACS sorting 2h 15m

1 Incubate the hPSC cultures (either feeder-free in mTeSR media or on MEF feeders in hPSC medium) supplemented with 10 µM Y-27632 (1:1000 dilution of stock) for at least . 2h
02:00:00

For a detailed protocol on growth of hPSCs in feeder-free media refer to the collection "Feeder-free culturing of hPSCs;" dx.doi.org/10.17504/protocols.io.b4mcqu2w

For a detailed protocol on growth of hPSCs on 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

2 Wash the plates twice with DPBS

3 Add 1 ml of 0.05% Trypsin and incubate for **00:05:00** **37 °C** 5m

4 Add 2 ml of hPSC medium or trypsin inhibitor solution per well of a 6-well plate to inhibit trypsin

5 Collect single cell solution by careful trituration with P1000.

6 Filter cell solution through cell strainer (70 µm) into 50 ml conical tube.

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

8 Remove the supernatant and re-suspend the cells in 0.5 ml hPSC medium or feeder-free medium with 10 µM Y-27632 (1:1000 dilution of stock).

9 Transfer and filter re-suspended single cell solution in 5 ml FACS tubes with cell strainer snap Cap (40 µm)

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10 Substitute filter caps for completely closed ones (maintain sterile conditions) and place the tubes on ice.

11 Provide FACS core facility with cells, negative controls, collection tubes (containing 1 ml of hPSC medium or feeder-free medium with 10 µM Y-27632 (1:1000 dilution of stock)) and detailed experimental information to enable the setup of appropriate FACS parameters.

B. After FACS

12 **Clonal expansion:**
The FACS-sorted (transfected) cells can be plated at low density on MEF feeders (approx. 2000-3000 cells/well of a 6-well plate) in hPSC medium containing 10 µM Y-27632 (1:1000 dilution of stock) (only for the first 24 hours after plating). This will allow for identification individual single cell derived colonies 10-14 days after plating.

For a detailed protocol on maintenance of hPSCs 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

13 **Bulk genotyping:**
The FACS-sorted (transfected) cells can be directly subjected to DNA extraction and next-generation sequencing (NGS). Depending on the cell number (small cell number) for DNA-extraction, we usually use Exact N Amp Blood PCR Kit (Sigma) according to the manufacturers' instructions.

For a detailed protocol on NGS genotyping refer to the collection "Genotyping by next generation sequencing;" dx.doi.org/10.17504/protocols.io.b4n3qvgn

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