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LIPID-MEDIATED TRANSFECTION OF iPSCs (Basic Protocol 2)

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We use this protocol and it's working

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Abstract

This protocol describes the lipid-mediated transfection of iPSCs maintained in E8 medium on Matrigel. While several lipid-based transfection reagents are commercially available, Lipofectamine Stem is used here because it is specifically optimized for delivery of DNA plasmids into hiPSCs. If preferred, similar results may also be achieved by electroporation or nucleofection, and other lipid reagents are available for *in vitro*-transcribed RNA or *in vitro*-translated ribonucleoproteins (RNPs). Furthermore, while this protocol provides the steps for a general transfection, specific details are provided below regarding insertion of the transgene cassettes relevant for neural differentiation (See **Basic Protocols 5** and **7**).

Attachments

PDF

fernandopulle2018.pd...

1.7MB

Guidelines

Passaging with Accutase immediately before transfection improves efficiency by generating a single-cell suspension that increases exposure to the Lipofectamine reagent; however, if Accutase passaging for certain iPSC lines results in low viability, transfection may also be performed on EDTA-passaged cells or on adherent cells at low confluency
(20 % to 30 %). Transfection efficiency may be monitored by including a fluorescent protein reporter under a promoter that is active in human stem cells (e.g., CAG, PGK, EF-1α containing introns; not CMV) and viewing the cells 1 day after transfection. This reporter does not need to be integrated, as transient expression should persist for 3 to 4 days after transfection. Finally, increased cell death is typical for 1 to 2 days after transfection, and can result in the accumulation of debris, so the culture medium should be changed daily, and cells may also be washed with PBS after aspiration of spent medium to further reduce debris carryover. The transfected iPSCs should be passaged for expansion, enrichment, and/or clonal selection (**Basic Protocol 3** or **4**) after the cells have reached approximately 80 % confluency, which commonly occurs 2 to 4 days after transfection.

Materials

- Lipofectamine Stem (Invitrogen, cat. no. STEM00001) or other lipid-based transfection reagent

Lipofectamine™ Stem Transfection Reagent **Invitrogen - Thermo Fisher Catalog #**STEM00001

- Opti-MEM I Reduced Serum Medium (Gibco, cat. no. 31985062)

Opti-MEM™ I Reduced Serum Medium **Gibco - Thermo Fisher Scientific Catalog #**31985062

- DNA plasmid(s) (e.g., CRISPR-Cas9 and guide RNA, TALENs, and/or DNA insert with appropriate homology arms; DNA obtained from an endotoxin free maxi-prep kit)

- Additional reagents and equipment for general iPSC culture (**Basic Protocol 1**) and counting cells (Phelan & May, 2015)

Troubleshooting

Safety warnings

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

1 Grow a sufficient number of iPSCs for transfection and prepare cells as for an Accutase split (see **Basic Protocol 1**).

Note

One or two wells of a 6-well dish at **80 % confluency** should provide more than enough cells for one transfection.

2 Count the cells (Phelan & May, 2015), transfer **1.5 × 10^6** cells to a 15-ml conical tube, and centrifuge 00:05:00 at 300 x g , Room temperature .

3 Aspirate the supernatant and resuspend in 2 mL of E8 medium supplemented with [M] 10 micromolar (µM) Y-27632 ROCK inhibitor.

Note

If iPSCs are normally maintained in a Flex medium, it is best to transition to regular E8 medium on the day of transfection to improve efficiency.

4 Pipet the medium and cells into 1 well of a 6-well dish **pre-coated with Matrigel** and return plate to the incubator. Gently shake the plate front-to-back and side-to-side.

5 Allow the cells to adhere in the incubator for 01:00:00 to 02:00:00 before adding the transfection solution.

6 For each transfection, add 100 µL of Opti-MEM and 3 µg of total DNA to one 1.5-ml microcentrifuge tube and vortex for 00:00:02 to 00:00:03 . In a second tube, combine 100 µL of Opti-MEM and 10 µL of Lipofectamine Stem reagent, and vortex for 00:00:02 to 00:00:03 .

Note

For TALEN-mediated insertion to the AAVS1 or CLYBL locus, such as for the hNGN2 (Addgene, **cat. no. 105840**) and hNIL (Addgene. **cat. no. 105841**) differentiation cassettes, use a 2:1:1 ratio of 1.5 µg donor construct with 0.75 µg of each of the site-specific TALENs.

For AAVS1: 0.75 µg of pTALdNC-AAVS1_T2 (Addgene, **cat. no. 80496**) and 0.75 µg of pTALdNC-AAVS1_T1 (Addgene, **cat. no. 80495**) per transfection.

For CLYBL: 0.75 µg of pZT-C13-R1 (Addgene, **cat. no. 62197**) and 0.75 µg of pZT-C13-L1 (Addgene, **cat. no. 62196**) per transfection.

7 Combine the contents of the two tubes and vortex again for 00:00:02 to 00:00:03 . Incubate this mixture for 00:10:00 at Room temperature .

8 Retrieve the cells plated in steps 3 to 4, and, using a P200 pipet tip and repeat pipettor, add 200 µL of the complete transfection solution from step 6 **dropwise**, evenly across the surface of the well. Return the cells to the incubator overnight.

9 24:00:00 after transfection, aspirate transfection medium and replace with fresh E8.
If applicable, evaluate transfection efficiency by fluorescence microscopy.

Note

All cells transfected with the hNGN2 (Addgene, **cat. no. 105840** or **110492**) and hNIL constructs (Addgene, **cat. no. 105841** or **105842**) will transiently express mCherry for **3 to 4 days**, while only those cells with transgene insertion will maintain **stable expression** of mCherry for longer periods of time.
See **Basic Protocols 3** and **4** for options for enrichment and clonal isolation.

10 Change medium daily with normal culture medium, and wash with PBS if necessary to remove debris. Once the cells have reached **80 % confluency**, they may be passaged to a new dish for expansion or used for enrichment or clonal selection.