Reprogramming efficiency of human embryonic fibroblasts using polycistronic lentiviral expression of reprogramming factors OSKM. Testing of factors that enhance efficiency

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Method Article

Keywords: human reprogramming iPS enhancement

Posted Date: March 19th, 2013

DOI: <https://doi.org/10.1038/protex.2013.032>

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Abstract

This protocol describes the testing of gene products for enhancement of reprogramming to iPS cells from human embryonic fibroblasts. OSKM factors together with the gene of interest are expressed using lentiviruses.

Introduction

see the associated publication

Reagents

see the associated publication

Equipment

see the associated publication

Procedure

This protocol is associated with the following reference: W.W.M. Pim Pijnappel, Daniel Esch, Marijke P.A. Baltissen, Guangming Wu, Nikolai Mischerikow, Atze J. Bergsma, Erik van der Wal, Dong Wook Han, Hermann vom Bruch, Sören Moritz, Phillip Lijnzaad, A.F. Maarten Altelaar, Katrin Sameith, Holm Zaehres, Albert J. R. Heck, Frank C.P. Holstege, Hans R. Schöler and H.Th. Marc Timmers (2013). A central role for TFIID in the pluripotent transcription circuitry. Nature, in press.

Day 1 • Plate 293T cells into 10 cm dishes (4 dishes per virus) in a final volume of 9 ml MEF medium per dish. Plate enough cells to obtain 70% confluence on the next day

Day 2 • Transfect the 293T cells from day 1 (cells should look 70% confluent, nicely spread, evenly distributed, without clumps) with Fugene 6 (Promega) as follows: Perform for each virus a separate transfection. Use for 4 dishes (this will yield 1000 µl virus after concentration)

Tube 1: 328 µl Optimem with 72 µl Fugene 6

Tube 2: In a total volume of 400 µl Optimem: 12 µg LV-OSKM or LV-gene of interest 8 µg psPAX2 4 µg pCMV-VSVG

Incubate both tubes for 5 minutes at room temperature. Add tube 2 to tube 1, mix gently by flicking and incubate 15 minutes at room temperature. Add 200 µl of the mixture per 10cm dish, shake and incubate for 72 hours.

Day 4 • Plate F134 human embryonic fibroblasts at 100,000 cells per well in a 6 wells plate in MEF medium (the dish does not need to be gelatinized).

Day 5 • Collect supernatant of dishes with transfected 293T cells and filter through 0.45 µm PVDF filters. Spin filtered supernatant by ultra-centrifugation at 20 krpm in an SW32Ti rotor (Beckman) for 2 hr at 4 °C. Remove supernatant by decanting and dissolve the pellet into 1000 µl DMEM low glucose. Use the virus directly to infect cells or store virus for less than 1 week at 4 °C or for longer periods in aliquots at -80 °C. Note: When virus production was efficient a white/brown pellet will appear after ultra centrifugation

• Infect the plated F134 cells from Day 4 with 50 µl of concentrated OSKM virus and 50 µl of the concentrated virus expressing the gene of interest. Add to each well 6 µg/ml protamine

sulfate directly into the medium and mix by swirling. Note: use titration of the amount of OSKM virus to determine the optimal amount of virus that gives a moderate amount of iPS colonies to allow for testing enhancers of reprogramming efficiency. Day 6 • Replace medium of the infected F134 cells by washing three times with PBS followed by MEF medium Note: it is also possible to perform this step one day later. Optimal: Check after washing fluorescent signal of tomato red. 48 hours after infection, a light tomato red signal should appear in at least 40 % of the cells. After a couple of days this signal will increase. Day 10 • Plate CF-1 MEF feeder cells with $1,7 \times 10^4$ cells per cm^2 into a gelatinized 6 wells plate. Note: these feeders will be used for 13 days. It is therefore important not to plate the feeders earlier than day 10. Day 11 • Trypsinize infected F134 cells and plate 10,000 cells per well onto the feeders plated at day 11. Change medium to iPS medium. Day 12 and onwards • Daily refresh the medium with iPS medium. Day 29: count the amount of colonies with iPS-like morphology, and/or process for Tra1-60 colony staining or single cell FACS analysis. Medium composition: MEF medium -445ml DMEM low glucose \(\text{PAA, E15-005}\) -50ml Fetal Bovine Serum \(\text{FBS, Invitrogen \# 16000-044}\) -5ml Pen/Strep/Glu 100x \(\text{PAA, P11-013}\) iPS medium -390ml DMEM/F12 \(\text{Invitrogen, \# 21331046}\) -100ml KO serum replacement \(\text{Invitrogen \# 10828}\) -5mL Non-essential amino acids \(\text{PAA, M11-003}\) -5mL Pen/Strep/Glu 100x \(\text{PAA, P11-013}\) -1ml β -Mercaptoethanol \(\text{Invitrogen \# 31350010}\) -1ml basic fibroblast growth factor \(\text{bFGF, 5 } \mu\text{g/ml stock solution in 0.1\% BSA/PBS, Peprotech 100-18b}\) Sterile filter through 0.2 μm filter Store at 4°C for up to 2 weeks

Timing

29 days

Troubleshooting

see details in the protocol

Anticipated Results

When using low lentiviral titers that result in a few colonies after OSKM infection, co-infection with TAF4 virus should lead to a strong increase in the amount of colonies and Tra1-60 positive cells after FACS analysis.

References

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Acknowledgements

We thank Martina Radstaak and Boris Burr for technical assistance