[Protocol 4.00] Reprogramming using Sendai virus_CytoTune2.0

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Protocol 4.00 Reprogramming using Sendai virus_CytoTune2.0
Version: 1.0 (21.11.2023)

Media and Reagents:
Fibroblast medium (see appendix)
Essential 8 (E8) Medium (see protocol 1.07)
DMEM/high Glucose (Thermo Fischer, 41965039)
FBS (Biochrom, S0115)
KO DMEM/F-12 (Thermo Fischer, 12660-012)
CytoTune-iPS 2.0 Reprogramming Kit (Thermo Fischer, A16517)
Polybrene Transfection Reagent (Millipore, TR-1003-G)
0,1% Gelatin solution (Millipore, ES-006-B)
TrypLE Select (Thermo Fischer, 12563029)
Geltrex (Thermo Fischer, A1413302)
PBS w/o Ca2+, Mg2+ (Biochrom, L1825)

Materials and Equipment:
Geltrex coated culture vessels (refer to protocol 1.05)
Aspiration Pump
5ml/10ml pipettes
15/50 ml falcon
Cell counting equipment
Cell culture dishes or IVF-dish (Falcon, 353653)
Stereo microscope build in safety bench

1. Introduction and Purpose
This protocol describes the reprogramming of fibroblasts using Sendai virus vectors (CytoTune-iPS 2.0 Reprogramming Kit) to generate iPS cell lines.

Note: Sendai virus vectors are potentially infectious! Handling of CytoTune-iPS 2.0 Reprogramming Kit is only allowed in class 2 safety cabinets which are located in S2 laboratories. It is strictly forbidden to work with Sendai virus vectors in S1 laboratories!

2. Cell seeding
1. Two days before transduction, seed different densities of fibroblasts into a Geltrex coated 24-well plate. Use 1 ml/well of preferred fibroblast medium (or see appendix).
2. Perform complete medium change (1 ml fibroblast medium/well) the next day.

3. Transduction

Note: All steps of transduction, seeding, picking and cultivation of new iPS cells have to be performed in a S2 laboratory until PCR test confirms absence of Sendai virus in newly generated iPSC cultures!

1. Perform cell count from one well for each of the different densities to determine the appropriate seeding density for transduction. The optimal cell density for transduction is about 5 x 10^4 cells in one 24-well. Use two wells for transduction!
2. Transfer two vials á 8 µl of pre-mixed Sendai virus vectors from -80°C freezer to S2 laboratory.
3. Perform spray disinfection of the virus vials and thaw inside the S2 safety cabinet.
4. Aspirate fibroblast media from the cells. Pipet 250 µl new fibroblast media supplemented with polybrene (5 ug to 10 ug per ml) to the cells and add 8 µl of Sendai virus mix to each well.
5. Use closable buckets to perform spin infection of the 24 well plate and centrifuge plate 20 minutes at 800g and RT. Change direction of the plate and spin one more time for 10 minutes at 800g and RT.
6. Incubate the cells overnight in a 37 °C incubator with a humidified atmosphere of 5% CO2 (hypoxia incubator with 5% O2 and 5% CO2 is possible as well).
7. 24 hours after transduction, replace the virus mix with 1 ml fresh fibroblast medium.
Note: During the first time (24 -48 h) after transduction it is possible to see some cytotoxicity, which can affect >50% of the cells. This is an indication of high virus uptake.
8. Culture the cells for 6 more days, changing the spent medium with 1 ml fresh fibroblast medium every other day. Take microscopy pictures to document changes in cell morphology.

4. Seeding of transduced cells

1. At day 7 after transduction prepare Geltrex coating of 3 wells of a 6-well plate.
2. Wash cells once with PBS w/o Ca2+, Mg2+.
3. To remove the cells from the 24-well plate, use 250 µl of TrypLE Select reagent or 0.05% Trypsin/EDTA per well and incubate at room temperature for 2 - 3 minutes.

4. When the cells have rounded up, add 1 ml of fibroblast medium into each well, and collect the cells in a 15 ml conical tube.
Note: Because the cells can be very sensitive to trypsin at this point, minimize trypsin exposure time and incubate the cells at room temperature!
5. Centrifuge cells for 5 minutes at 200g.
6. Aspirate supernatant and resuspend cells in 1 ml fibroblast medium.
7. Perform cell count.
8. Seed increasing cell densities into the three wells of the Geltrex coated 6-well plate using 2 ml fibroblast medium (for example seed 1.3 x 10^4 cells/well, 1.0 x 10^4 cells /well and 0.5 x 10^4 cells/well).
Important: Remaining cells should be centrifuged again for 5 minutes at 200 x g. Harvest cell pellet for RNA isolation! Store sample at -80°C in RLT buffer until RNA isolation. This will be a positive control for later Sendai virus test PCR.
9. 24 hours later, change the medium to 2 ml Essential 8 Medium (E8), and replace the spent medium every day thereafter.
Note: On weekends, it is possible to perform a double feed on a Friday, with the next medium change on Sunday. However, it is not recommended to either go longer than 1 day without a medium change, or to feed the cultures every other day continuously.
10. Observe wells every day under a microscope for the appearance of cell clumps indicative of reprogrammed cells. Around day 12 after transduction the first colonies should appear.
11. Three to four weeks after transduction, colonies should have grown to an appropriate size for transfer/picking.

Appendix

Fibroblast medium

Ingredients	500 mL	250 mL
DMEM	500 mL	250 mL
10% FBS	50 mL	25 mL
1% L-Glutamine	5 mL	2,5 mL
1% Pen/ Strep	5 mL	2,5 mL