Human iPS Cell Reprogramming Retrovirus Kit

PROCEDURE

Retroviral transduction of human dermal fibroblasts

1. When human Fibroblasts reach 80% confluence, aspirate medium, wash twice with PBS, cover cells with 0.05%trypsin, and incubate for 5 min at 37°C.

2. Inactivate trypsin with fresh culture medium, and collect cells into a 15 ml conical tube.
3. Centrifuge cells at 200x g at room temperature for 5 min and discard the supernatant.
4. Resuspend the cells in 1 ml fresh culture medium and count the cell number using a hemacytometer.
5. Plate 1 x 105 cells in each well of 6-well plate, and incubate cells at 37°C, 5% CO_2, for 6 hours.
6. Aspirate medium to remove dead cells, and add 2 ml of fresh culture medium.
7. Add retroviruses carrying hOCT4, hSOX2, hKLF4 and hc-MYC, respectively. Infect one well with retroviruses at MOI 10 and one well with retrovirus carrying GFP and one with empty vector as control.
8. Add 4 µl of 500x TransPlus (cat# V020, ProMab Biotechnologies) solution into each well, and mix gently by swirling the plate.
9. Repeat steps 7 and 8 next day.
10. One day after final infection, remove the viral supernatant, wash three times with PBS, and add 3 ml of fresh culture medium.
11. Four days after infection, plate 2 x 10^6 mitomycin C treated MEF cells in a 100-mm dish or two 60-mm dishes (precoated with 0.1% gelatin, cat. no. M500, ProMab Biotechnologies). Incubate until the next day.

12. On day 5 after first infection, trypsinize the infected cells and plate them in a 100-mm dish at different cell densities between 5 x 10^4 to 2 x 10^5 cells or in a 60-mm dish at densities between 2 x 10^4 to 1 x 10^5 cells.
13. Two days later, aspirate medium and replace with hES medium.
14. Change medium everyday with hES medium.
15. After about 3-4 weeks, check the colony formation and pick the with ES-like morphology manually for expansion in hES media.

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Progress of reprogramming human fibroblasts