ProMab Biotechnologies, Inc.

Protocol

Human iPS Cell Reprogramming Retrovirus Kit

OVERVIEW

Human induced pluripotent stem cells (iPSCs) can be derived from somatic cells through a reprogramming process driven by ectopic expression of a defined set of reprogramming factors: Oct4, Sox2, Klf4 and c-Myc. These hiPSCs share the properties of self-renewal and pluripotency with human ES cells, and can therefore be used as a renewable source for all differentiated cell types of the body. Human iPSCs can be generated from patients of virtually any genetic background.
Retroviruses are efficient tools for delivering heritable genes into the genome of dividing cells. The VSV-G pseudotyped retrovirus has a wide range of targets including both mammalian and non-mammalian cells, and is usually silenced in ES cells. The retrovirus is commonly used in generating iPSCs because of its high reprogramming efficiency. The Human iPS Cell Reprogramming Retrovirus Kit offers such opportunity to generate iPSCs from various tissues and cell types.

Materials you will need:
Human ES medium

Description	Vendor	Cat. #	Concentration	Volume
DMEM/F12	Invitrogen	10565---042		
Pen/strep	Invitrogen	15140---122	1X	1 mL
L---Glutamax	Invitrogen	35050-061	2 mM	2 mL
nonessential amino acids	Invitrogen	11140---050	0.1 mM	2 mL
2---mercaptoethanol	Sigma	M7522	0.1 mM (1000x)	0.2 mL
knockout serum replacement	Invitrogen	10828---028	20%	40 mL
bFGF	StemRD	bFGF-050	10 ng/mL	0.2 mL
				Total 200 mL

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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.

Images on next page

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Progress of reprogramming human fibroblasts