



Standard Operating Procedure for **REPROGRAMMING FIBROBLASTS INTO iPSC**

1.0 PURPOSE

This procedure describes how to generate induced pluripotent stem cells (iPSC) from human fibroblasts.

2.0 SCOPE (Should include which Cores this SOP applies to)

This procedure applies to all personnel who wish to generate human iPSC.

3.0 PROCEDURE

3.1 Day -4

Thaw the cells in 37 °C water, seed 2×10^5 cells on a 6-cm dish and culture them overnight with DMEM medium (DMEM, 10% FBS; 6 mM L-glutamin; 0.1 mM MEM nonessential amino acids; 50 units/ml of penicillin and 50 µg/ml of streptomycin).

Day -3

1. Preparation of Virus

Take virus stock from -70 °C. Thaw virus in 37 °C water, then put them on ice.

2. Infection

- Using DMEM medium, prepare 5 ml virus mixture (MOI=22.5) containing 6 µg/ml polybrene.
- Wash the Fibroblast with DMEM without FBS 2-3 times. Add the virus mixture onto the cells and incubate overnight in a cell incubator.

Day -2

- Remove the infection medium from Fibroblasts, wash the cells with DMEM without FBS 2-3 times and add 10 ml DMEM medium.
- Prepare 4 gelatin-coated, 10-cm dishes for each cell line. Seed feeder cells to dishes with a density of 2×10^4 cell/ cm² and incubate overnight in a cell incubator.

Day -1

- Treat the infected fibroblasts with 0.05% trypsin, stop the action of trypsin with DMEM medium and wash the cells twice with medium.
- Wash feeder dishes with DMEM without FBS 2-3 times. Put the infected cells into four gelatin-coated, 10 cm feeder dish. Culture the cells in DMEM medium overnight.

Day 0

- Change the medium with hES medium containing 2% FBS, 1 µg/ml Dox, 0.1 mM Vit C.

Day 1

- Change the medium with hES medium containing 2% FBS, 1 µg/ml Dox, 0.1 mM Vit C and 0.5 mM sodium butyrate.

Day 2

2. Change the medium with hES medium containing 1% FBS, 1 $\mu\text{g/ml}$ Dox, 0.1 mM Vit C and 0.5 mM sodium butyrate.

Day 3

3. Change the medium with hES medium containing 1% FBS, 1 $\mu\text{g/ml}$ Dox, 0.1 mM Vit C and 0 and 0.5 mM sodium butyrate.

Day 4-10

1. Change the medium with hES medium containing 1 $\mu\text{g/ml}$ Dox, 0.2 mM Vit C and 0.5 mM sodium butyrate.

Day 11-25

1. Change the medium with hES medium containing 1 $\mu\text{g/ml}$ Dox and 0.2 mM Vit C. When real iPS colonies appear, the usage of Dox can be stopped.

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