

Induced pluripotent stem cells (iPS)

From: Duke/UNC/UTA/EBI ENCODE group

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Source of Cells:

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Room/incubator conditions

The following protocols take place in a sterile mammalian tissue culture room. Growth is performed in 5% CO₂ at 37°C.

Production of retrovirus for generation of human iPS cells

293 cells expressing stably expressing the viral gag and pol genes (gp293) were cultured in DMEM supplemented with 10% FBS, 2mM l-glutamine, BME, and NEAA. gp293 cells were plated at 75% confluency in a T-75 flask and allowed to attach overnight. The following day cells were transfected with plasmid DNAs using Lipofectamine. To produce virus, each T-75 was transfected with a plasmid containing the viral envelope gene VSV-g (concentration) and one of the following pMX-based retroviral vectors (provided by Shinya Yamanaka, Kyoto, Japan): OCT4, SOX2, KLF4, and MYC. The medium was changed after 24 hours (10ml per T-75). At 48 hours, the medium was collected from each flask and replaced with fresh medium (10ml per T-75). Collected medium was spun at 1,000rpm for 10 minutes to remove debris. The supernatant containing virus was collected and used immediately or stored at 4°C for up to 10 hours. The process was repeated at 72 hours.

Human iPS cell derivation

Human fibroblast cell cultures were obtained from the Coriell Institute for Medical Research (Campden, NJ). Human fibroblasts were cultured in DMEM supplemented with 10% FBS, l-glut, NEAA, BME, and Pen/Strep. Cells were plated at 1.33×10^3 /cm² in a T-75 flask the night before infection. For infection, fibroblast culture medium was removed and 2ml of medium from each of the four viral productions (OCT4, SOX2, KLF4, MYC) was supplemented with 8ug/ml polybrene (Sigma) and added to each T-75 of fibroblasts (for a total of 8ml). Control plates were also infected with each virus individually in order to monitor the proportion of cells infected by each virus by quantitative RT-PCR or immunocytochemistry. Fibroblasts were infected four times (time 0, +6 hours, +24 hours, and +30 hours). At 48 hours, virus containing medium was removed and replaced with fresh culture medium. On day 5, cells were trypsinized and plated at a density of 868 cells/cm² into 6-well plates containing feeders. Feeders were prepared the previous day as described for human ES cells. Infected human fibroblasts were allowed to attach overnight in fibroblast culture medium containing serum. The following morning, cells were fed with human ES cell medium and then fed every 48 hours until day 10. After day 10, cells were fed everyday with MEF-conditioned medium to account for the progressive loss of feeders during the extended culture period. Individual human ES-like colonies were picked on days 24-42 and expanded.

iPS Cell Culture

Human ES/iPS cell medium consists of DMEM-F12 (Invitrogen), 20% KSR, 20ng/ml FGF2 (R&D Systems), 2mM L-glutamine, 0.1mM 2-mercaptoethanol, and 1x non-essential amino acids. Human iPS/ES cells were passaged every 4-6 days with enzymes (1mg/ml collagenase (type IV, Invitrogen) and 1mg/ml dispase (Invitrogen)) and trituration into small clumps of ~10-100 cells. MEFs were irradiated with 30-60 gray and were seeded as feeders at a density of 1.88×10^4 cells/cm² for human ES cell and iPS cell cultures. MEFs were maintained with DMEM supplemented with 10% FBS, 2mM L-glutamine, and 0.1mM 2-mercaptoethanol. All cells were grown on Nunclon Δ -treated dishes or multiwell plates (Fisher Scientific) coated for 2 hours at 37°C with 0.1% (w/v) gelatin (Sigma).