EXPERIMENTAL PROCEDURES

Cell Culture

HDF from facial dermis of 36-year-old Caucasian female and HFLS from synovial tissue of 69-year-old Caucasian male were purchased from Cell Applications, Inc. When received, the population doubling was less than 16 in HDF and 5 in HFLS. We used these cells for the induction of iPS cells within six and four passages after the receipt. BJ fibroblasts from neonatal foreskin and NTERA-2 clone D1 human embryonic carcinoma cells were obtained from American Type Culture Collection. Human fibroblasts, NTERA-2, PLAT-E, and PLAT-A cells were maintained in Dulbecco's modified eagle medium (DMEM, Nacalai Tesque, Japan) containing 10% fetal bovine serum (FBS, Japan Serum) and 0.5% penicillin and streptomycin (Invitrogen). 293FT cells were maintained in DMEM containing 10% FBS, 2 mM L-glutamine (Invitrogen), 1 3 10_4 M nonessential amino acids (Invitrogen), 1 mM sodium pyruvate (Sigma) and 0.5% penicillin and streptomycin. PA6 stroma cells (RIKEN Bioresource Center, Japan) were maintained in a-MEM containing 10% FBS and 0.5% penicillin and streptomycin. iPS cells were generated and maintained in Primate ES medium (ReproCELL, Japan) supplemented with 4 ng/ml recombinant human basic fibroblast growth factor (bFGF, WAKO, Japan). For passaging, human iPS cells were washed once with PBS and then incubated with DMEM/F12 containing 1 mg/ml collagenase IV (Invitrogen) at 37_C. When colonies at the edge of the dish started dissociating from the bottom, DMEF/F12/collangenase was removed and washed with Primate ES cell medium. Cells were scraped and collected into 15 ml conical tube. An appropriate volume of the medium was added, and the contents were transferred to a new dish on SNL feeder cells. The split ratio was routinely 1:3. For feeder-free culture of iPS cells, the plate was coated with 0.3 mg/ml Matrigel (growth-factor reduced, BD Biosciences) at 4_C overnight. The plate was warmed to room temperature before use. Unbound Matrigel was aspirated off and washed out with DMEM/F12. iPS cells were seeded on Matrigel-coated plate in MEF-conditioned or nonconditioned primate ES cell medium, both supplemented with 4 ng/ml bFGF. The medium was changed daily. For preparation of MEF-conditioned medium, MEFs derived from embryonic day 13.5 embryo pool of ICR mice were plated at 1 3 106 cells per 100 mm dish and incubated overnight. Next day, the cells were washed once with PBS and cultured in 10 ml of primate ES cell medium. After 24 hr incubation, the supernatant of MEF culture was collected, filtered through a 0.22 mm pore-size filter, and stored at -20°C until use.