Cell culture and iPSC reprogramming

Induced pluripotent stem cell (iPSC) lines were generated from peripheral blood mononuclear cells (PBMCs) of the patient. PBMCs were isolated by centrifugation using Vacutainer® CPT™ (BD Biosciences, CA, USA) from peripheral blood of the patient. After 3-4 days of culturation, the PBMCs were subsequently transduced with CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific, MA, USA) for 48 h. At day 3 post transduction, sendai virus were removed and cells were replated onto 96-well culture plate. Monoclones with dense cell growth could be formed after continuous culture for about 30 d. The ones with good state were selected and replated onto 24-well coated with Matrigel (Corning, NY, USA) and cultured in mTeSR1 medium (STEMCELL Technologies, BC, Canada). The mTeSR1 medium was changed for culture every day. The cell clones proliferated and spreaded around. When the cell density covered the surface of the culture vessel by 70-80%, ACCUTASE (Life Technologies, MD, USA) was used for normal digestion and passage at the density of 1:4-1:8. During subculture, Rock inhibitor of y-27632 dihydrometer (Tocris, Bristol, UK) was added into the medium to promote iPSC adherent-wall production and the single-cell layer was formed to cover the surface of the culture vessel. Cells were maintained at 37°C and 5% CO_2 in a humidified atmosphere. Pluripotency was assessed by immunofluorescence for OCT4 and by Flow cytometry for SSEA-4, TRA-1-60, OCT4.