Generation of patient specific iPSC line by reprogramming of HDFs to iPSCs

The full thickness skin biopsy was aseptically harvested from the retro-auricular region of the patient with their informed consent. The tissue was digested using collagenase (1mg/mL) for 3 h at 37°C and the digested tissue was cultured in Medium 106 and LSGS supplement (GibcoTM, Thermo Fisher Scientific Inc) in a T25 flask at 37°C with 5% CO2 supply and passaged at a split ratio of 1:3 using TrypLE, for expansion and cryopreservation.

The human dermal fibroblasts (HDFs) at passage 4 were HDFs at passage 4 was used for reprogramming, using the integration-free, cell therapy solutions grade, CTS™ CytoTune™-iPS 2.1 Sendai Reprogramming Kit, which encodes for the human stemness genes such as, *OCT4, SOX2, KLF4* and *L-MYC.* The transduced cells were plated on Matrigel™ (Corning) coated plates (1:100 dilution) and cultured using Essential 8™ Medium (Thermo Fisher Scientific) and incubated at 37°C with 5% CO2 with media changes on alternate days for up to 30 days. Well reprogramed hiPSC colonies with distinct margins that emerged at D20-D25 are manually cut into 5-10 cell clusters using a flame-pulled glass pasture pipette with a hooked tip and are further clonally expanded on Matrigel™ coated plates. The clonal cultures are manually passaged till they expand stably and are further passaged using the cell dissociation solution (CDS) containing 0.5 mM EDTA and 30 mM NaCl in 1X DPBS. The stably expanded clonal line was passaged at 70-80% confluency on every 4-5 days at 1:6 split ratio. The freshly passaged cells were cultured in E8 medium, with ROCK inhibitor (10 μM Y-27632) supplementation only during the initial 16-18 h of passaging. The clonal line was expanded beyond 10 passages and the excess cells are cryopreserved at every passage for further evaluation and characterization.