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 (Gibco™, Thermo Fisher Scientific Inc) in a T25 flask at 37°C with 5% CO_2 supply and passaged at a split ratio of 1:3 using TrypLE, for expansion and cryopreservation.

The HDFs at passage 4 were reprogrammed by nucleofection of integration-free episomal plasmids that encode the key reprogramming factors such as *OCT4, SOX2, KLF4, L-MYC, LIN28* and an shRNA against p53. The reprogrammed HDFs were maintained under standard pluripotent stem cell culture conditions. Upon reprogramming, the ESC-like colonies that emerged after 3-4 weeks of nucleofection were manually picked and clonally expanded under xeno-free culture conditions, using the E8 medium™ and vitronectin-coated culture dishes. The clonal cultures were manually passaged till they expand stably and were 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 were cryopreserved at every passage for further evaluation and characterization.