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

The adipose derived mesenchymal cells (ADMCs) at passage 4 were nucleofected with a cocktail of nonintegrating episomal vectors, pCXLE-hOCT3/4-shp53-F (Addgene Plasmid #27077), pCXLE-hSK (Addgene Plasmid #27078), pCXLE-hUL (Addgene Plasmid #27080) and pCXWB-EBNA1 (Addgene Plasmid #37624), using the P2 nucleofection kit and EO-114 program of the 4D-Nucleofector, X Unit System, as per the manufacturer's instructions (Lonza, Basel, Switzerland). The nucleofected 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% CO_2 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 (LVIP02-RB-1) was expanded beyond 10 passages and the excess cells are cryopreserved at every passage for further evaluation and characterization.