Embryoid body formation assay

The growing hiPSC colonies (@ passage 10) at 80-90% confluence was harvested using the Cell Dissociation Solution. The cell density was estimated and a suspension of 0.5-1X10^6 cells in 10 mL was prepared in 2% hESC qualified Matrigel™ containing Essential 8 medium. About 100 µL of the cell suspension was then plated in each well of a round bottomed, non-adherent, 96-well plate (BD Falcon) at a final cell density of 0.5-1X10^4 cells per well and incubated at 37°C with 5% CO_2. The proliferating cells formed EBs after 18-24 h of culture and day 3 EBs were resuspended in differentiation medium *(*DMEM/F-12 supplemented with 4% KOSR, 1X Non-Essential Amino Acids, 1X GlutaMAX™ and 1X Penicillin-Streptomycin), plated on Matrigel coated 60 mm dishes and cultured in adherence for up to three weeks to enable spontaneous differentiation into all three lineages. At day 20, the EBs are harvested for RNA isolation using the TRIZOL reagent. The total RNA was converted to cDNA using a reverse transcriptase (Superscript III; Invitrogen) and amplified by PCR using gene specific primers to analyze the expression of different stemness markers and three lineage markers.