Embryoid body formation assay

The growing hiPSC colonies were passaged at 80-90% confluence and cell density was estimated. For a 96-well format, nearly 9000-10000 cells/well are required. Therefore, approximately $1x10^6$ cells were dispensed in 10 mL Essential 8 medium containing 2% hESC-qualified Matrigel™. Approximately 100 µL cell suspension was dispensed in each well of a round-bottom, non-adherent 96-well plate and incubated at 37°C with 5%CO_2. The formed EBs can be visualized under a microscope. On day 2, 100 µL EB-differentiation medium (DMEM/F-12 supplemented with 4% KOSR, 1X Non-Essential Amino Acids, 1X GlutaMAX™, and 1X Penicillin-Streptomycin) was added to each well. On day3, EBs were collected and resuspended in the differentiation medium and plated on Matrigel coated 60-mm dish and cultured for 3 weeks to enable random differentiation into all three lineages. At day 20, EBs were harvested and subjected to RNA isolation using the TRIZol method. The RNA was converted to cDNA using reverse transcriptase enzyme (Superscript III, Invitrogen), and differentiation potentiality was assessed by analysing the expression of lineage-specific transcripts using semi-quantitative RT-PCR.