Protocols for HPSCreg Files

RT/qRT-PCR:

RNA was collected using the RNeasy MiniKit (QUIAGEN). cDNA for RT-PCR and qRT-PCR was generated from 1µg of DNaseI treated RNA using GoScript™ Reverse Transcription System (Promega) with random primers. cDNA was diluted 1:10 before doing RT and qRT-PCR (BioRad S1000™ / BioRad C1000™, respectively). cDNA was generated using the following PCR parameters: [1] 95ºC for 10 min, [2] 94ºC for 15 s, [3] 65ºC for 1 min, [4] repeat 2-3 for 35x, [5] 65ºC for 5 mins. For qRT-PCR, Cq-values were normalized to *GAPDH*, analyzed via $2^{-\Delta\Delta Ct}$ method, and calculated SEM.

Immunofluorescence Staining:

Passage 17 cells were fixed and stained directly in the well. Lines were evaluated for 5 pluripotent markers (OCT4, SOX2, NANOG, TRA-1-81, SSEA4) according to the StemLight™ Pluripotency Antibody Kit instructions.

Teratoma Assay:

Passage 16 cells were digested into single cells with Accutase® (Innovative Cell Technologies). Cells were resuspended in basal iPSC media (DMEM/F12+GLUTAMAX, 20% knockout serum replacement,1xMEM-NEAA, 55nM β-Me) to generate ≈ $1.5x10^8$ cells/mL. Cells were then injected into the interstitial space of 6-week-old NOD/SCID mouse testes (8µL). Tumors were collected 2-3 months post-transplant, fixed in 10% formalin, and histologically processed and stained with hematoxylin/eosin for teratoma analysis.