AG08C5 iPSC pluripotency analysis by RT-qPCR

RT-qPCR analysis of pluripotency markers: Total RNAs were extracted using RNeasy Plus Mini Kit (Qiagen, cat. No. 74134) according to the manufacturer's recommendations. RNA were reverse transcribed with random hexamers primers by RevertAid™ Reverse Transcriptase (Thermo Fischer Scientific, cat, No. EP0451). Quantitative PCR (qPCR) analysis was then performed using SYBR Green Mastermix (Qiagen) in the CFX-connect system (Bio-Rad). RT-qPCR assays were carried out in triplicate following the manufacturer's protocols. Relative expression levels were normalized to GAPDH house-keeping gene expression using the ΔΔCt method. Sets of gene-specific primers used were as follows :
GAPDH : GTGGACCTGACCTGCCGTCT / GGAGGAGTGGGTGTCGCTGT,
NANOG: TGAACCTCAGCTACAAACAG / TGGTGGTAGGAAGAGTAAAG,
OCT4: CCTCACTTCACTGCACTGTA / CAGGTTTTCTTTCCCTAGCT
SOX2: CCCAGCAGACTTCACATGT / CCTCCCATTTCCCTCGTTTT.

Result: We validated the activation of endogenous pluripotency genes, namely NANOG, OCT4 and SOX2 in AG08C5 iPS compared to fibroblasts.