

Supporting Information Table S1. List of primers used in this study.

| Gene | Forward Primer | Reverse Primer |
|---------------------------|----------------------------|------------------------------|
| For PCR | | |
| <i>neo</i> | ATGATGGATACTTTCTCGGCAGGA | TGCCACGTTGTGAGTTGGATAGTT |
| <i>tk</i> | AATCCAGGATAAAAGACGTGCATGG | GACAATCGCGAACATCTACACCAC |
| <i>C-Myc-SOX2</i> | GAAGTTCTCCTCCTCGTCGCAGTA | CCTGCAGTACAACCTCCATGACCAG |
| <i>SOX2-KLF4</i> | TTCTCCGTCTCCGACAAAAGTTTC | AAGAGTTCCCATCTCAAGGCACAC |
| <i>KLF4-OCT4</i> | GATCGTTGAACTCCTCGGTCTCTC | ATGTGGTCCGAGTGTGGTTCTGTA |
| <i>OriP</i> | ATGGCTATGGGCAACACATAATCC | CTCTCAGCGACCTCGTGAATATGA |
| <i>EBNA-1</i> | CCTCATCTCCATCACCTCCTTCAT | TCCAACCCGAAATTTGAGAACATT |
| <i>AMPpUC</i> | TCCCGGCAACAATTAATAGACTGG | TGAAGCCAGTTACCTTCGGAAAAA |
| <i>NANOG-LIN28</i> | AACCCTTCCATGTGCAGCTTACTC | CTGCTGGGGAAGGCCTTAATGTA |
| <i>miR302/367 cluster</i> | ACTTATTTCTCTCCTTCACCCCGATG | GGAAATCATGATCATCCCTTCTCCT |
| <i>OCT4</i> | CAGGCTCTGAGGTGTGG | GACTCCTGCTTCGCCCTCA |
| <i>NR5A2</i> | TGTCAATTTGGCAGTTCTGGTTTT | AGGGGTTTTATGCGATGGAGTTTC |
| <i>GAPDH</i> | GCAATGCCTCCTGTACCACC | CCTGGAAGATGGTGTATGG |
| For RT-qPCR | | |
| <i>hOCT4</i> | ATGGGAGCCCTCACTTCACT | CCGCCAACTTGAGAAGGTC |
| <i>hSOX2</i> | CCCCCAGCAGACTTCACAT | GCCAGCGAGTTCAACAAAG |
| <i>hKLF4</i> | GACCAGGCACTACCGTAAACA | GGGTTCTCCTCCACGTCG |
| <i>hC-MYC</i> | GCTGGCGATGTTGAAAGTAAC | TGCTGCTGCTGGTAGAAGTTC |
| <i>hNANOG</i> | TTCTCCTGCCAGTGACTTGG | CCGCCAACTTGAGAAGGTC |
| <i>hLIN28</i> | GTGGCGTGAAACAGACTTTGA | CGCACGTTGAACCACTTACA |
| <i>pOCT4</i> | CAAAGTGAAGGTGCCTGCCCTTC | ATTGAACTTCACCTTCCCTCCAACC |
| <i>pSOX2</i> | GTTCCATGGGCTCAGTGGTCAAG | AAGCGTACCGGGTTTTTCTCCATAC |
| <i>pKLF4</i> | CCCTTCAACCTGGCAGACAT | CACAAACTTGCCCATCAGCC |
| <i>pC-MYC</i> | ATCCAAGACCACCACCACTG | GTTACAGCAACATTCAGGTAGA |
| <i>pNANOG</i> | AGCCCCAGCTCCAGTTTCAGC | AATGATCGTCACATATCTTCAGGCTGTA |
| <i>pLIN28</i> | TGCCGGCATCTGTAAATGGT | ACTCTGGTGACAAAAGACGT |

| | | | | | | | | | | | |
|------------------------------|---|---|---|---|---|---|---|---|---|---|---|
| pM3-5-5 | Y | N | | | | | | | | | |
| pM3-6-1 | N | N | Y | Y | Y | N | Y | Y | N | Y | N |
| pM3-6-2 | Y | Y | | | | | | | | | |
| pM3-6-3 | Y | Y | | | | | | | | | |
| pM3-6-4 | N | N | Y | Y | Y | N | Y | N | N | Y | N |
| pM3-6-5 | N | Y | | | | | | | | | |
| Clones from pMaster12 | | | | | | | | | | | |
| pM12-1-1 | N | N | Y | N | Y | N | N | N | N | Y | N |
| pM12-1-2 | N | Y | | | | | | | | | |
| pM12-1-3 | Y | N | | | | | | | | | |
| pM12-1-4 | Y | N | | | | | | | | | |
| pM12-1-5 | N | N | Y | Y | Y | N | N | N | N | Y | N |
| pM12-1-6 | Y | Y | | | | | | | | | |
| pM12-1-7 | N | Y | | | | | | | | | |
| pM12-2-1 | N | N | Y | Y | Y | N | N | N | N | Y | N |
| pM12-2-2 | N | Y | | | | | | | | | |
| pM12-2-3 | N | N | N | Y | Y | N | N | N | N | Y | N |
| pM12-2-4 | N | N | N | Y | Y | N | N | N | N | Y | N |
| pM12-2-5 | N | N | Y | Y | Y | N | N | N | N | Y | N |
| pM12-3-1 | N | Y | | | | | | | | | |
| pM12-3-2 | N | Y | | | | | | | | | |
| pM12-3-3 | N | N | Y | N | Y | N | N | N | N | Y | N |
| pM12-3-4 | N | N | Y | N | Y | N | N | N | N | Y | N |
| pM12-3-5 | N | N | Y | Y | Y | N | N | N | N | Y | N |
| pM12-3-6 | N | N | N | Y | Y | N | N | N | N | Y | N |
| pM12-3-7 | N | Y | | | | | | | | | |
| pM12-3-8 | Y | N | | | | | | | | | |
| pM12-3-9 | N | Y | | | | | | | | | |
| pM12-3-10 | N | Y | | | | | | | | | |
| pM12-4-1 | N | N | Y | Y | Y | N | N | N | N | Y | N |
| pM12-4-2 | N | N | Y | Y | Y | N | N | N | N | Y | N |
| pM12-4-3 | Y | Y | | | | | | | | | |
| pM12-4-4 | N | N | Y | Y | Y | N | N | N | N | Y | N |
| pM12-4-5 | N | N | Y | Y | Y | N | N | N | N | Y | N |
| pM12-4-6 | N | Y | | | | | | | | | |
| pM12-5-1 | N | N | Y | Y | Y | N | N | N | N | Y | N |
| pM12-5-2 | N | N | Y | Y | Y | N | N | N | N | Y | N |
| pM12-5-3 | Y | N | | | | | | | | | |
| pM12-5-4 | Y | Y | | | | | | | | | |
| pM12-5-5 | N | N | Y | Y | Y | N | N | N | N | Y | N |
| pM12-5-6 | N | N | Y | Y | Y | N | N | N | N | Y | N |
| pM12-5-7 | N | Y | | | | | | | | | |
| pM12-6-1 | N | N | Y | Y | Y | N | N | N | N | Y | N |
| pM12-6-2 | N | N | N | Y | Y | N | N | N | N | Y | N |

| | | | | | | | | | | | |
|-----------|---|---|---|---|---|---|---|---|---|---|---|
| pM12-6-3 | N | N | N | Y | Y | N | N | N | N | Y | N |
| pM12-6-4 | N | N | Y | N | Y | N | N | N | N | Y | N |
| pM12-6-5 | N | N | Y | Y | Y | N | N | N | N | Y | N |
| pM12-6-6 | N | N | Y | Y | Y | N | N | N | N | Y | N |
| pM12-6-7 | N | N | Y | Y | Y | N | N | N | N | Y | N |
| pM12-6-8 | N | N | Y | N | Y | N | N | N | N | Y | N |
| pM12-6-9 | N | N | Y | Y | Y | N | N | N | N | Y | N |
| pM12-6-10 | N | N | N | Y | Y | N | N | N | N | Y | N |
| pM12-6-11 | N | N | Y | Y | Y | N | N | N | N | Y | N |
| pM12-7-1 | Y | Y | | | | | | | | | |
| pM12-7-2 | Y | Y | | | | | | | | | |
| pM12-7-3 | Y | Y | | | | | | | | | |
| pM12-7-4 | Y | Y | | | | | | | | | |
| pM12-7-5 | Y | Y | | | | | | | | | |
| pM12-8-1 | N | N | Y | Y | Y | N | N | N | N | Y | N |
| pM12-8-2 | N | N | Y | Y | Y | N | N | N | N | Y | N |
| pM12-8-3 | N | N | Y | Y | Y | N | N | N | N | Y | N |
| pM12-8-4 | N | N | Y | Y | Y | N | N | N | N | Y | N |
| pM12-8-5 | N | N | Y | Y | Y | N | N | N | N | Y | N |

Y, transgene intact; N, transgene removed.

Note: Even after FIAU selection, some clones still contained the *HSVtk* gene. This is likely due to gene silencing, or partial loss of the *HSVtk* gene expression component. The persistence of negative selection marker genes including *HSVtk* and *DTA* is also commonly observed in gene targeting experiments in ES cells.