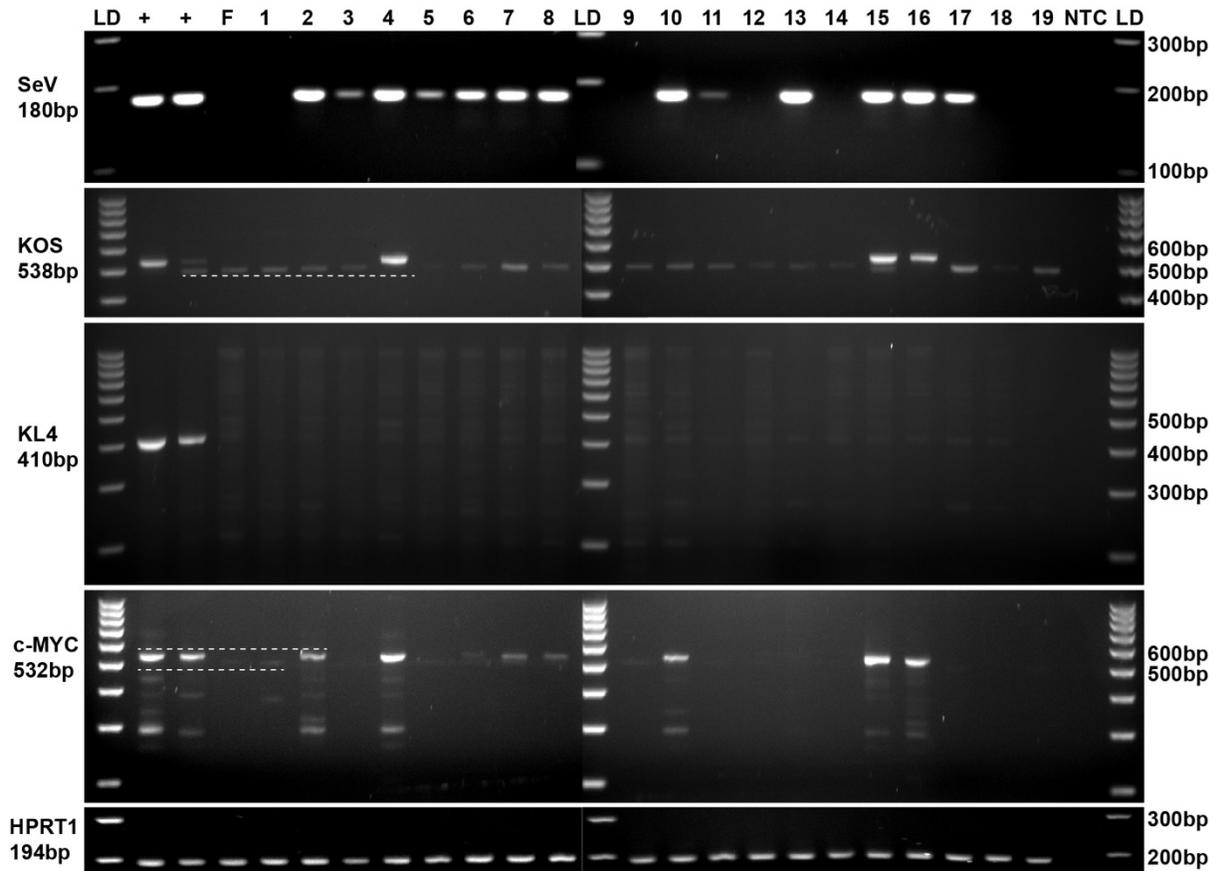
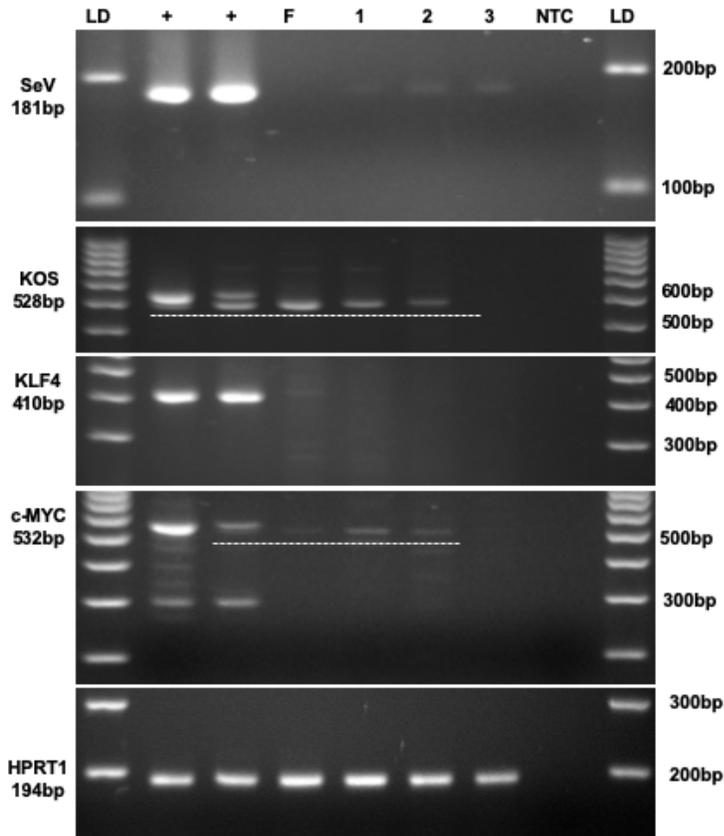


Supplementary Figure 3: a) Confirmation of vector-free NWR iPSCs from the first reprogramming experiment. A 3% Metaphor gel (Lonza) of RT-PCR for the Sendai vectors, showing the complete clearance of the virus by passage 11 in NWR 9947-c501. The two positive controls are pooled fibroblast samples collected during reprogramming, at time of re-plating ~ day six post-transduction (+). Fibroblasts from 9947 collected prior to transduction (F), iPSCs from passage 6 (P6), passage 11 (P11), passage 16 (P16), and passage 21 (P21) with a no template control (NTC) and 100 base pair ladder (LD). The fragments represent the Sendai genome (SeV) and vector-specific primers for the cassettes KOS, (KLF4, POU5F1(OCT4), and SOX2), KLF4, and MYC. The observed non-specific bands in KOS and MYC amplifications were presumably caused by non-specific amplification since one primer from each of the KOS and MYC sets was a 100% match with the available SWR genome sequence, and most likely matched the NWR also. Non-specific bands observed in untransduced fibroblasts confirms the non-specific amplification. The lack of the SeV backbone band present in the P11 sample confirms the bands present in the MYC samples are also non-specific even though the band in the fibroblasts is almost non-detectable. Housekeeping gene HPRT1 is included as a positive control for all samples.



Supplementary Figure 3: b) A 3% Metaphor gel (Lonza) indicating which clones are vector free. iPSCs derived from the 2nd – 4th reprogramming experiments. The two Sendai-positive controls are pooled fibroblast samples collected during reprogramming, at time of re-plating ~ day six post-transduction (+). Fibroblasts from 9947 (F), 5764-c503 P12 (1), 5765-c506 P22 (2), 5765-c507 P20 (3), 5765-c508 P25 (4), 5765-c509 P20 (5), 6571-c501 P18 (P6), 6571-c502 P21 (7), 6571-c505 P13 (8), 8173-c505 P15 (9), 8174-c501 P23 (10), 8175-c501 P10 (11), 9939-c5101 P11 (12), 9947-c5101 P13 (13), 17626-c501 P12 (14), 17626-c5101 P20 (15), 17626-c5103 P14 (16), 21328-c560 P14 (17), 21409-c550 P12 (18), 21409-c5156 P8 (19), no template control (NTC), and 100bp ladder (LD). Samples 4, 15 and 16 are still positive for the reprogramming cassettes KOS and cMYC. The majority of the samples are still positive for the SeV backbone without showing expression of any reprogramming cassettes. Housekeeping gene HPRT1 is included as a positive control for all samples.



Supplementary Figure 3c: A 3% Metaphor gel (Lonza) of samples post five days of culture at 38.5 C and selective sub-cloning of the three clones still positive for the reprogramming cassettes. Faint expression of the SeV backbone is still present in the samples, however all of the clones are clear of the reprogramming cassettes. The two Sendai positive controls are pooled fibroblast samples collected during reprogramming, at time of re-plating ~ day six post-transduction (+). Fibroblasts from 9947 (F), Clone 5765-c508 (1), Clone 17626-c5101 (2), and clone 17626-c5103 (3), no template control (NTC), and 100bp ladder (LD). Housekeeping gene HPRT1 is included as a positive control for all samples.