

# General Reprogramming Protocol

1. Use CytoTune-iPS 2.0 Sendai reprogramming kit to transfect fibroblast cells or PMBCs. Approximately 12 days later, pick ES cell-like colonies and expand the cells in mTesR medium.

2. Identify the iPSCs with pluripotency markers Oct 3/4, Sox2, TRA-1-60, Nanog, SEA4 by immunofluorescence in combination with RT-PCR.

3. Analyse the differentiation potential of iPSCs using;

(1) in vitro differentiation: iPSCs are spontaneously differentiated as embryoid bodies and examined with three germ layers markers, smooth muscle (mesoderm), b-tubulin III (ectoderm) and a-fetoprotein (endoderm) by immunofluorescence.

(2) in vivo teratoma formation: iPSCs are injected into SCID mice and are then histologically analyzed by H&E staining to check for the three germ layer tissues.

4. Karyotype the iPSCs for chromosomes analysis by G-banding and manually pairing up chromosomes looking for ploidy and large structural abnormalities.

5. Detection for the absence of the Sendai virus and vectors by RT PCR.