1. Induced pluripotent stem cell generation protocol:

Generation of retroviruses

Two protocols have been successfully used in our lab to generate retroviruses.

- Protocol 1:
  - Phoenix-A packaging cells were transfected with each pMXs-cDNA vector (listed below) separately using Fugene HD.
  - Collect the viral supernatant 2 days post-transfection, combine and filter (designated VCM1). Add fresh transfection media to cells
  - Next day, collect the second viral supernatant, combine and filter using 0.45 um syringe filters (VCM2).

- Protocol 2:
  - 293-GPG packaging cells were transfected with each pMXs-cDNA vector (listed below) separately using Fugene HD. Next day, add fresh media.
  - Collect the viral supernatant 4 days post-transfection, combine and filter through 0.45 um syringe filters (VCM0). Add fresh transfection media to cells.
  - Next day, collect the viral supernatant, combine and filter through 0.45 um syringe filters (VCM1). Add fresh transfection media to cells.
  - Next day, collect the viral supernatant, combine and filter through 0.45 um syringe filters. (VCM2)

Note: VCM0 is kept at +4˚C to be combined with VCM1 for infection.

pMXs-cDNA vectors were generated by cloning human cDNAs of *Oct4*, *Sox2*, *Nanog*, *Klf4* and *Lin28*, amplified by direct PCR from human ES cell cDNA, into pMXs retroviral vector (available from Addgene). The generated construct are:

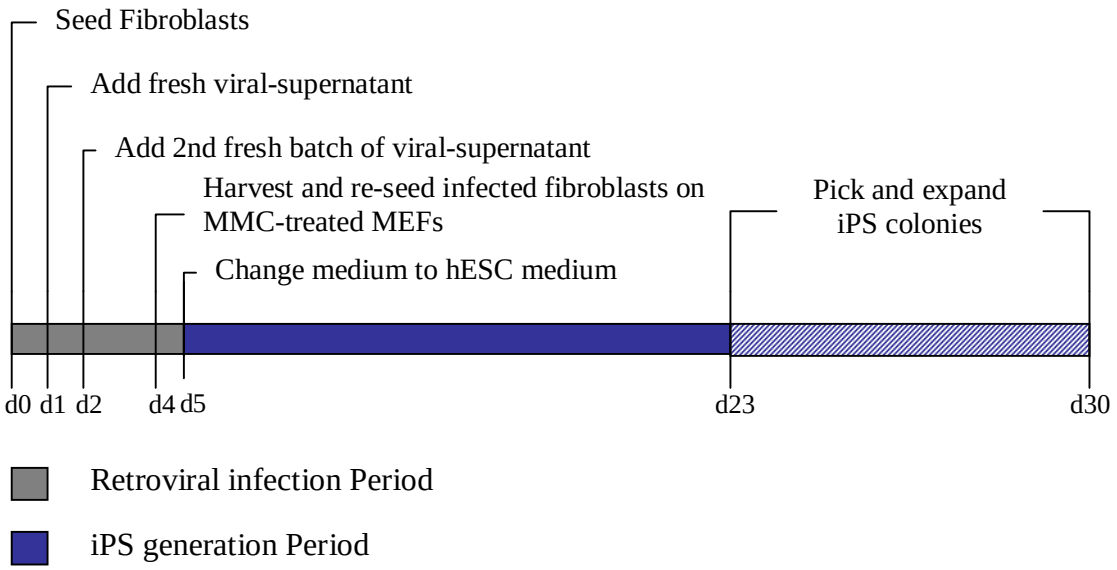
- pMXs-OCT4
- pMXs-SOX2
- pMXs-NANOG
- pMXs-LIN28
- pMXs-KLF4

Infection of fibroblasts

- Day 0: Setup fibroblasts at 0.8-1.2 x 10^6 cells / 10cm culure dish.
- Day 1: Infect cells as follows:
  - Protocol 1:
    - Add Polybrene at 8ug/mL to VCM1, then add all of VCM 1 (approx. 8mLs) to infect fibroblasts.
    - Incubate for 4hrs, centrifuge plates at 2500rpm twice for 15min. each, while rotating the plate after each centrifugation, and then incubate overnight.

  - Protocol 2:
    - Add Polybrene at 8ug/mL to combined mixture of VCM0 + VCM1, then add max. 4 mls to infect fibroblasts.
    - Incubate for 4-5hrs.
    - Add 6 mLs of regular media and incubate overnight.
- Day 2: Infect cells a second time:
  - Protocol 1: Same as above but with VCM2.
  - Protocol 2: Same as above, but this time combine VCM2 with what is left over from VCM0/VCM1 mix to infect cells
- Day 3:
  - Add fresh fibroblast media to infected cells.
  - Treat mouse embryonic fibroblasts (MEFs) with mitomycin C (MMC) for 2-3 hrs.
  - Prepare MMC-treated MEFs at 0.5-1.0 x 10^6 cells / 10cm gelatin-treated culure dish.
- Day 4:
  - Transfer infected fibroblasts onto MMC-treated MEF plates.
    - 1 x 10 cm dish of infected fibroblasts onto 2 x 10cm dish of MMC-treated MEFs
- Day 5:
    - Change media to human ES cell media

Change Media every 2 days. Cells may be kept over the weekend without changing the media. iPS Colonies can be picked between days 23 and 30. Normally, iPS clusters begin to appear around day 16 with clear morphology by day 20. For adult fibroblasts, iPS clusters appear around day 26. The schematic below illustrates the iPS induction protocol:



The following cell lines have generated iPS clones successfully:

- IMR90: Human foetal lung fibroblast
- MRC5: Human foetal lung fibroblast
- HFF: Human foreskin fibroblast
- ASF1: Adult human dermal fibroblast (not available yet)
- ASF2d: Adult human dermal fibroblast (not available yet)

Below are the combinations of factors successfully used in our lab:

- OCT4 / SOX2 / KLF4 (only for HFF cell line / not available yet)
- OCT4 / SOX2 / NANOG / LIN28
- OCT4 / SOX2 / NANOG / LIN28 / KLF4

Please refer to link below for media preparations and cell culture protocols:
http://research.med.helsinki.fi/bscc/protocols.htm

2. An attached table lists the established iPS clones and the respective iPS criteria that was tested for each clone.

Note: Retroviral transfection and fibroblast media are identical to MEF media on our website. More detailed retrovirus preparation protocols, retroviral constructs and iPS clones are available upon request.