Introduction

The expression of the four human transcription factors (TFs) (Yamanaka Oct4, Klf4, Sox2, and c-Myc) or (Thomson: Oct4, Sox2, Nanog, Lin28) have been shown to reprogram different types of terminally differentiated cells to an embryonic stem (ES) cell-like state known as the induced pluripotent stem (iPS) cells (Yu, et al., 2007; Takahashi, et al., 2007). **abm** has developed the most comprehensive product line for iPSC generation and related stem cell research areas. This includes iPSC purified recombinant proteins, minicircle DNA, iPSC lentiviral and adenoviral particles, EBV-based non-integrated plasmids, and piggyMac vectors. With those tools available, you can generate iPSC from any cell type with choices of both transient (proteins, adenovirus, and plasmids) and stable (lentivirus) iPSC factors. In addition, choices of iPSC factors driven by different promoters in lentivirus (CMV, UBC, PGK, and EF1a) are also available. We have a greater selection of iPSC vectors than any other provider in stem cell research.

Stringent Quality Testing

CBI's application testing ensures the highest quality of reprogramming recombinant proteins, lentiviruses, adenoviruses, and plasmids. Every lot of products have been confirmed to successfully produce iPSCs or to express specific iPSC factors in cell types. With proven quality, you can minimize the need to repeat experiments and advance your project efficiently.

Choices for Different Formats, Concentrations, and Species

CBI's broad iPSC product line enables researchers to select the right product applicable to their research – no need to compromise. Products are provided in the formats of recombinant proteins, viral particles, and plasmids for both human and mouse iPSC factors. Custom development of any specialized iPSC products is also available. All known six reprogramming factors are available as individual viruses and in functional sets.

iPSC Lentivirus Protocol

Recombinant iPSC lentiviral particles (either set or polycistronic particles) from **CBI** are purified by high titer preparation and are ready to use. The following protocol is based on human fibroblasts and will be applicable to most other cell types.

1. Seed HDF 1X10^5 cells in a 6 well plate (~70% confluent) one day before viral infection, including one dish used for GFP control.
2. The following day, dilute 10~50µl of each lentivirus (individual one or polycistronic ones) to 0.5ml complete culture medium at 3:00pm in the afternoon.
3. Aspirate medium from 6-well plate and add diluted virus (0.5ml) to the 6-well plate and add polybrene at a concentration of 2µg/ml. Return the plate to a CO_2 incubator for overnight incubation.

Note: *Polybrene is a polycation that neutralizes charge interactions to increase binding between the pseudoviral capsid and the cellular membrane. The optimal concentration of Polybrene depends on cell type and may need to be empirically determined (usually in the range of 1–8µg/ml). Excessive exposure to Polybrene (>12 hr) can be toxic to some cells.*

4. The following day, aspirate virus-containing medium and replenish with 2 ml iPSC medium.
5. Change medium every 48 hours.
6. Wait for 2-4 weeks for iPSC colonies to form.
7. Once iPSC colonies form, prepare Mytomycin C treated MEF (mouse embryonic fibroblasts) feeder cells in 24-well plate (80% confluent) at a concentration of 10µg/ml for 3 hours in an incubator at 37°C followed by 2X PBS wash.
8. Pick colonies manually into 96-well plate and trypsinize in 96-well plate.
9. Transfer the trypsinized cells from each of the 96 wells into 24-well MEF coated plate.
10. Wait for another 1-2 weeks for iPSC colonies to develop (change medium every 48 hours)
11. When MEF cells become too old (about 2 weeks) or a lot of iPSC colonies have developed in the 24-well plate, prepare MEF feeder layer as described above in 6-well plate to expand.
12. Trypsinize the cells (both MEF and iPSC cells) and spin down at 1000Xg.
13. Seed iPSCs into 6-well MEF coated plate for expansion.
14. Using the same procedures above, iPSCs can be further expanded to bigger culture vessels to meet your lab needs for iPSC analysis or down-stream applications.

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