Stem Cell Core: iPSC Technology and Therapeutic implications

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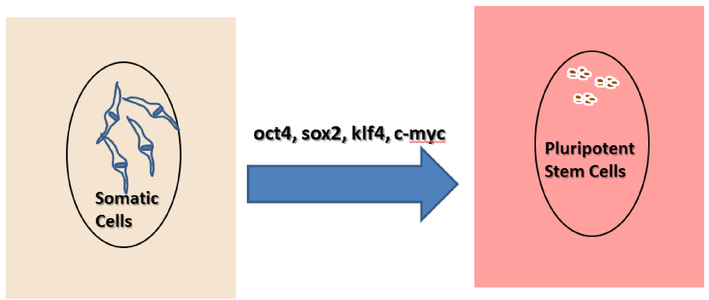
ABSTRACT: The human Stem Cell Core is the latest addition to the UVA School of Medicine Core Facility family that is missioned to provide research grade induced pluripotent stem cells (iPSCs) and their downstream derivatives to investigators to facilitate the generation of clinical quality cells for human cell therapy. Overall, the core will provide three categories of services: Production and Cell Banking (organ-and disease-specific derived iPSCs); Product Development (individualized projects - generation of specific cell types from iPSCs such as neurons, cardiomyocytes, insulin producing β cells); and Education and Training workshops to students and faculties.

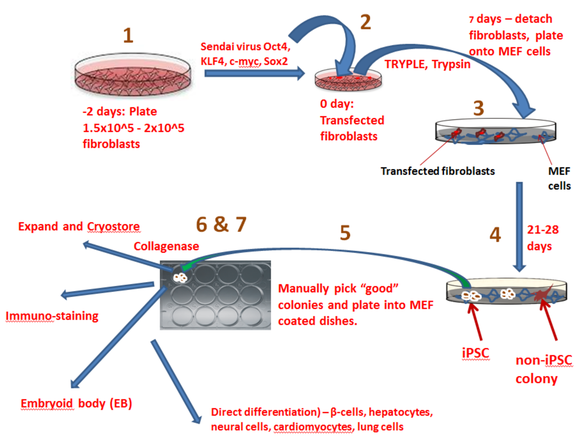
Human pluripotent stem cells including embryonic stem cells (hESC) and induced stem cells (hiPSC) have unlimited ability for self-renewal and can differentiate to all cell types of the three germ layers, which make them well suited for generating autologous and custom-tailored cells for regenerative medicine and to produce *in vitro* disease models to study pathogenesis, treatments, drug screening, and toxicology. While hESCs are derived from inner-cell mass of early embryos, hiPSCs are generated by artificially converting non-pluripotent somatic cells to pluripotency by forced expression of specific key pluripotent stem cell transcription factor genes including Oct-4, Sox2, c-MYC, and KLF4. Here, we show a step-by-step production of iPSC from an established human foreskin fibroblast (ATCC # CRL 2522) using non-integrating Sendai virus reprogramming Kit (LifeTechnology # A13780-01).

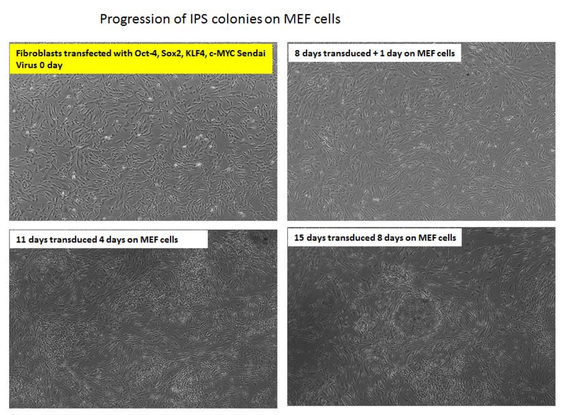
METHODS:

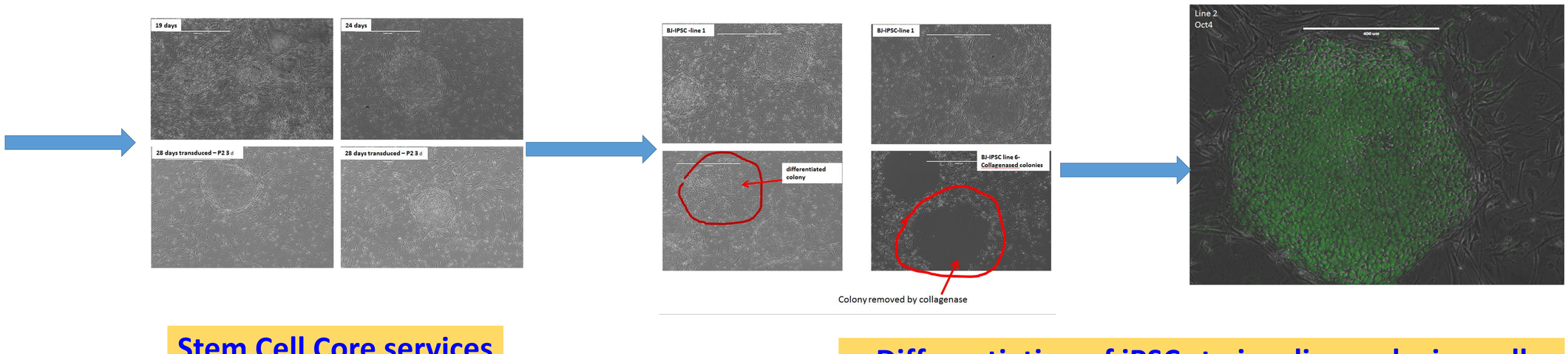
1) Plate 150-200,000 fibroblasts in 2 wells of 6-well plate
2) Transfect with Sendai virus carrying Oct4, Sox2, KLF4, c-MYC
3) After 7 days, harvest transfected cells and plate 200000, 100000, 50000 cells in each of 6 10-cm dishes coated with mouse embryonic fibroblasts (MEF) cells
4) After 3-4 weeks, pick "good" colonies and re-plate in MEF coated 12-well plates (one colony in each well)
5) After few days when the colonies are large, pick "good" colonies from 12-well plates and re-plate into 6-wellplates coated with MEF cells
6) Harvest "good colonies" from each of 6-well plates into 10- cm dishes using collagenase enzyme (25-35 minutes). Collagenase concentration and time of incubation need to be optimized to detach only colonies leaving behind MEF cells.
7) Collect colonies and cryo-store.

Reprogramming of Somatic Cells to Induced Pluripotent Stem Cells

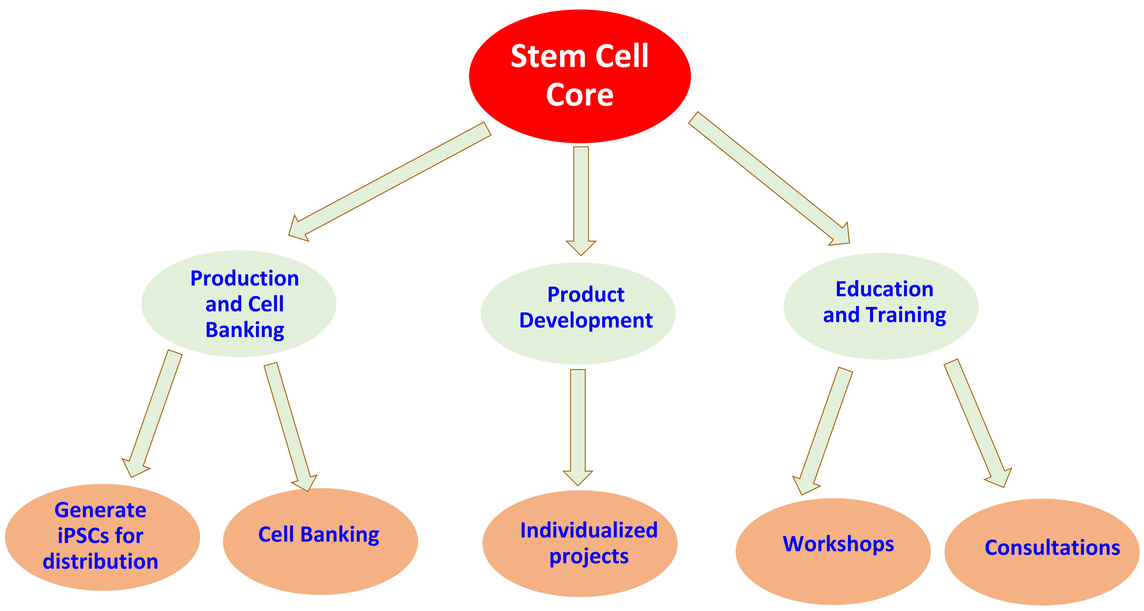




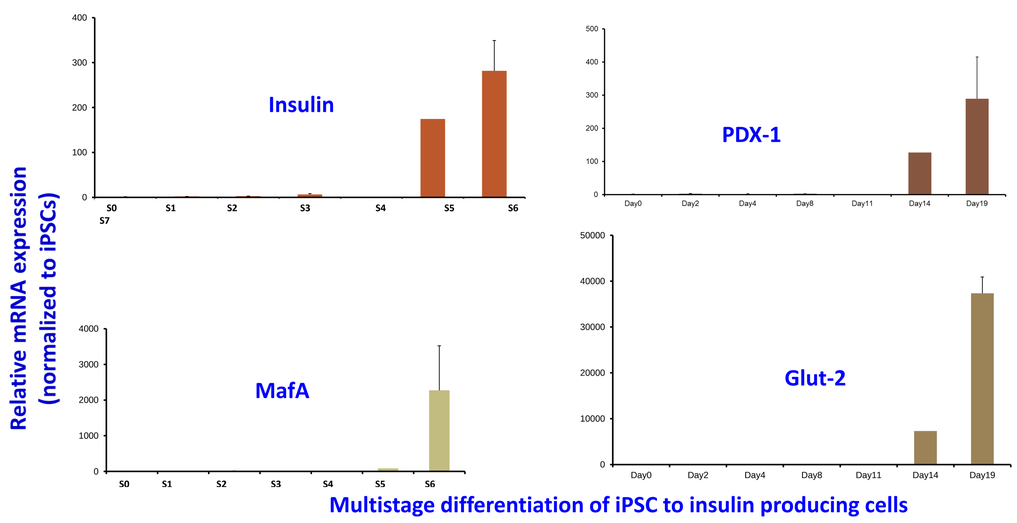




Stem Cell Core services



Differentiation of iPSCs to insulin producing cell



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