

## **Abstract**

### **Cellular reprogramming as a tool for harvesting patient specific stem cells**

In the year 2006, Dr. Yamanaka surprised the entire field of medicine, by reporting a technique of inducing pluripotency in somatic cells. In his article, he had displayed that fibroblasts could be reprogrammed to pluripotent stem cell state, by ectopic expression of four transcription factors namely OCT4, SOX2, c-MYC and KLF4. His discovery made a paradigm shift in the field of reprogramming because previous methods of reprogramming were dependent on use of human oocytes and this raised ethical concerns. Moreover, his technique of cellular reprogramming broadened the spectrum of application of somatic cells in regenerative medicine.

Objectives of my research were focused on; development of an optimised protocol for detection of mycoplasma that commonly infects animal tissue culture; detailed characterization of reprogrammed clones; targeted differentiation of iPSC towards myogenic lineage, and construction of an expression vector, optimised for miRNA expression.

For detecting mycoplasma infection, we adapted the protocol of Uphoff et al. (2002). By skipping the DNA extraction step (reported in the original protocol) and instead directly using cell culture supernatant and a robust polymerase enzyme for performing PCR, we made the modified protocol more sensitive, robust and rapid.

DPSC isolated from natal tooth produced heterogenous population of cells that expressed haematopoietic stem cell makers CD34 and CD45 in addition to normal DPSC markers.

DPSC derived from male donors displayed higher cell death on transduction of Sendai virus reprogramming vectors compared to DPSC derived from female donors. Furthermore, DPSC derived from female donors expressed higher reprogramming efficiency than two human fibroblasts cell lines.

nDPSC derived iPSC clone closely resembled hESC in terms of morphology, expression of pluripotency markers and gene expression profile. Furthermore, the iPSC clone spontaneously differentiated into derivatives of three embryonic germ layers when cultured as embryonic bodies. In conclusion, the selected iPSC clone passed all the necessary steps included in characterization, thus confirming true induction of pluripotency.

iPSC clones derived from DPSC and WI38, successfully committed to myogenic fate upon treatment with CHIR 99021. Furthermore, among the two iPSC clones, myogenic progenitor specific marker PAX7, was more upregulated in DPSC derived iPSC clone.

Cloning of intronic sequence within DsRed2 reporter gene increased percentage of cells expression the vector by 4 % as compared to native DsRed2 gene.