Autologous Induced Pluripotent Stem Cell (iPSC)

Banking Certification of Analysis (COA)

PATIENT NAME:	[redacted]
PATIENT NUMBER:	01002530
DOB:	30th March 1960
SERVICE:	Autologous Induced Pluripotent Stem Cell Generation & Banking

iPSC21 Generation Method 1/2

The induced Pluripotent Stem Cell (iPSC) line is derived from the patient's skin fibroblasts via a non-integrative, non-viral reprogramming technology using mRNAs.

The original method for deriving iPSCs from mature cells (e.g. fibroblasts) described by Professor Shinya Yamanaka of Kyoto University remains the basis for iPSC reprogramming today. Recently the field has moved on to non-integrating reprogramming approaches that do not induce genetic changes in reprogrammed cells. This carries the advantage of vastly reducing the risk of unintended genetic changes and also eliminates problems associated with residual viral DNA remaining in the target cell. The cutting edge of these non-integrating approaches that has emerged in the last few years is to utilize synthetic, highly modified mRNAs. These mRNAs only have a transient existence in the patient's skin fibroblasts and allow reprogramming without editing the genome or introducing any lasting foreign genetic material. mRNAs will be used by the cell's own protein construction machinery to synthesize the transcription factor proteins that govern the reprogramming process. After four doses of a cocktail of mRNAs, and once this reprogramming process is under way, the mRNAs will deteriorate entirely meaning the patient's own iPSCs have been produced in a 'scarless' way, leaving no genetic trace of the procedure. This approach creates the ideal starting resource for clinical application in regenerative medicine.

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iPSC21 Generation Method 2/2

When cells are reprogrammed into iPSCs they adopt a 'rejuvenated' state, erasing the 'epigenetic' signatures of ageing and essentially resembling a fetal cell. These cells are called 'pluripotent' because of their ability to form all cell types of adult humans. Because iPSCs have been 'rejuvenated' by the reprogramming process, and given their capacity for almost indefinite self renewal and proliferation, they can be used as an almost limitless source of biologically young therapeutic cells, including Mesenchymal Stem Cells (MSCs) that are at the forefront of clinical stem cell therapy today.

At iPSC21 all iPSC lines are characterized and banked according to the international guidelines outlined by the International Society of Stem Cell Research (ISSCR) – "**Guidelines for Stem Cell Research and Clinical Translation**", 2016. And to the iPSC banking guidelines outlined by Stephen Sullivan et al., as part of the Global Alliance for iPSC Therapies (GAiT) "**Quality control guidelines for clinical-grade human induced pluripotent stem cell lines**", 2018.

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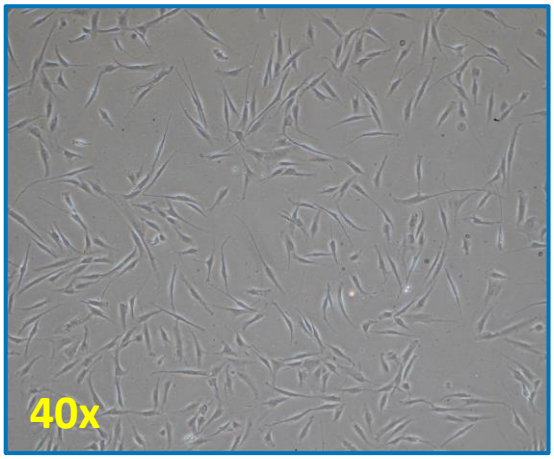
Stage 1: Fibroblast Culture & Banking

Autologous fibroblasts derived from a patient's skin sample are used as the starting cells for iPSC generation. Fibroblasts are extracted form the skin biopsy, expanded to a supply of millions of cells and then a portion is used for iPSC generation and the rest are cryogenically frozen as a stock of the patient's unaltered cells.



HF BANKING RECORD (VIAL#)	Results
HF-AM-01	$1.0x10^6$ Fibroblasts
HF-AM-02	$1.0x10^6$ Fibroblasts
HF-AM-03	$1.0x10^6$ Fibroblasts
HF-AM-04	$1.0x10^6$ Fibroblasts
HF-AM-05	$1.0x10^6$ Fibroblasts

Total Fibroblasts Banked: 5.0 $x10^6$

All vials of the patient's autologous fibroblasts have been frozen according to the SC21 cryogenic freezing protocol which complies with international standards. The fibroblasts are stored long-term in liquid nitrogen suspension at <-180°C.

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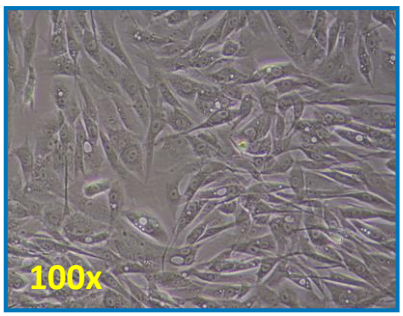
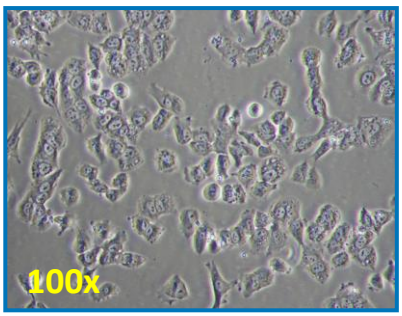
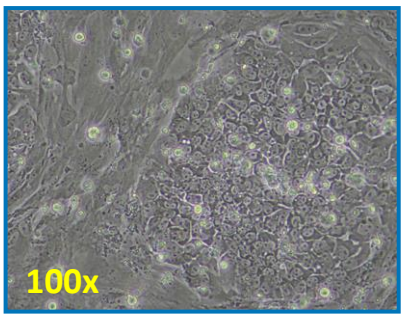
Stage 2: Induced Pluripotent Stem Cell (iPSC) Generation

The patient's fibroblasts are incubated with a reagent that forms a complex with the mRNAs that will reprogram the cells. This complex attaches to the cell membranes forming buds that eventually break off, entering the cell's interior. Once inside the mRNAs will be used as the code to make proteins by the cell's protein construction machinery. The key proteins constructed are 'transcription factors' that activate cell systems reprogramming the cell into an induced pluripotent stem cell.

Around 10 days after the reprogramming process is started iPSC colonies start to emerge among the fibroblasts. Fast-growing colonies of compact cells are used to visually identify iPSCs which are then manually isolated and expanded as a genetically pure reprogrammed line ahead of stabilization and characterization.

Day 1	Day 6	Day 12
		

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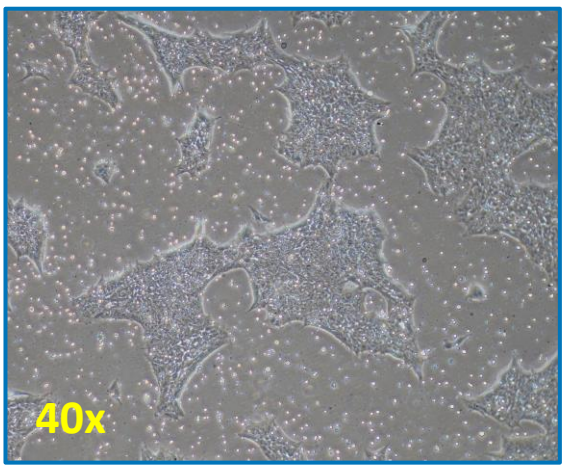
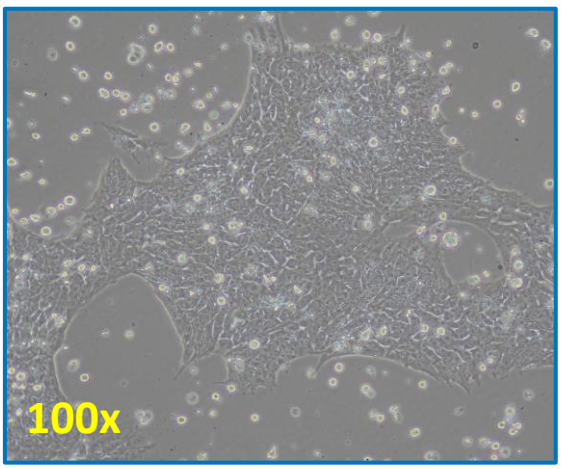
Stage 3: iPSC clonal expansion

Six to twelve iPSC colonies are manually isolated, expanded and monitored. After 5-10 days the best colonies are chosen for further expansion and characterization.

One of the key characterization tests is flow cytometry to assess the percentage of reprogrammed cells expressing both the early reprogramming cell surface marker SSEA4, and the mature iPSC nuclear marker OCT4. These two genes are also confirmed visually by immunocytochemistry (ICC).

Stabilized iPSCs – week 2





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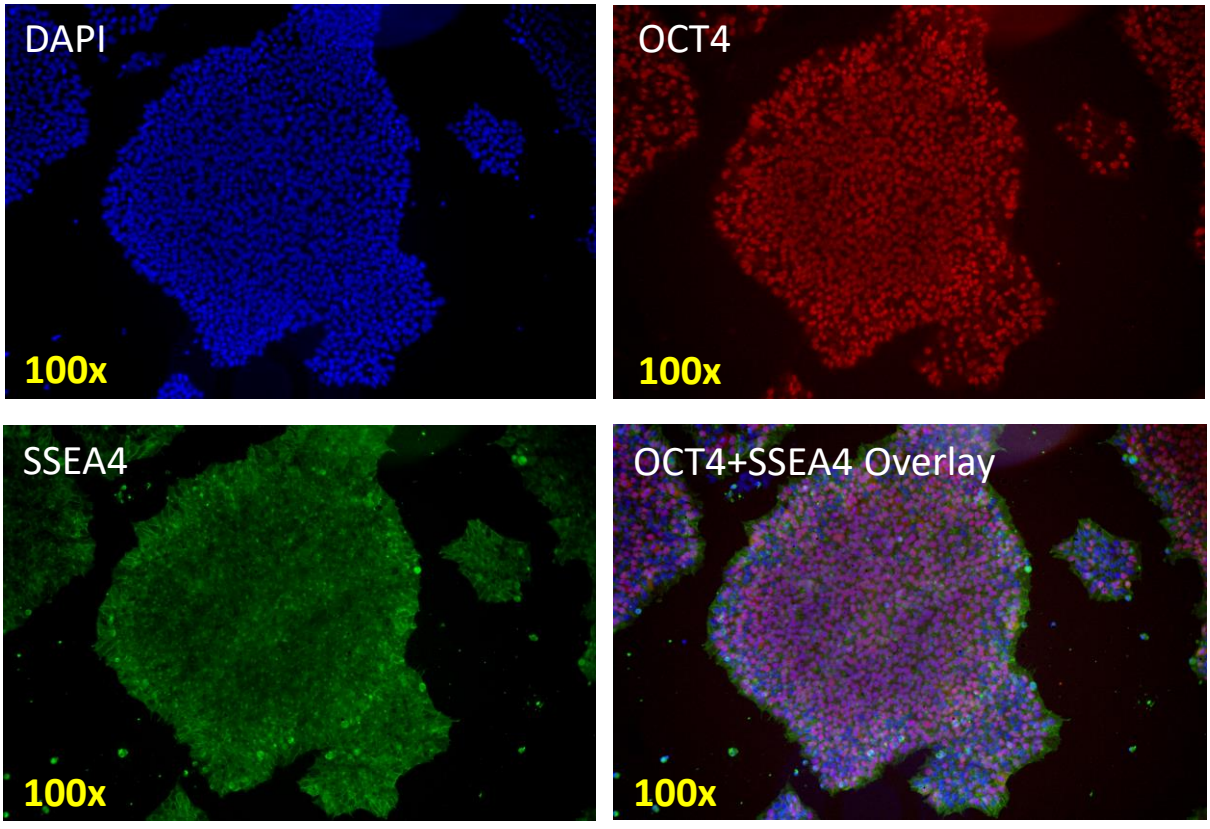
Stage 4(a): iPSC Validation: Staining and Immunofluorescence imaging 1/2

Using immunocytochemistry (ICC) the key iPSC markers SSEA4 and OCT4 are labelled with fluorescent proteins which allows them to be observed under a fluorescent microscope to confirm iPSC identity.



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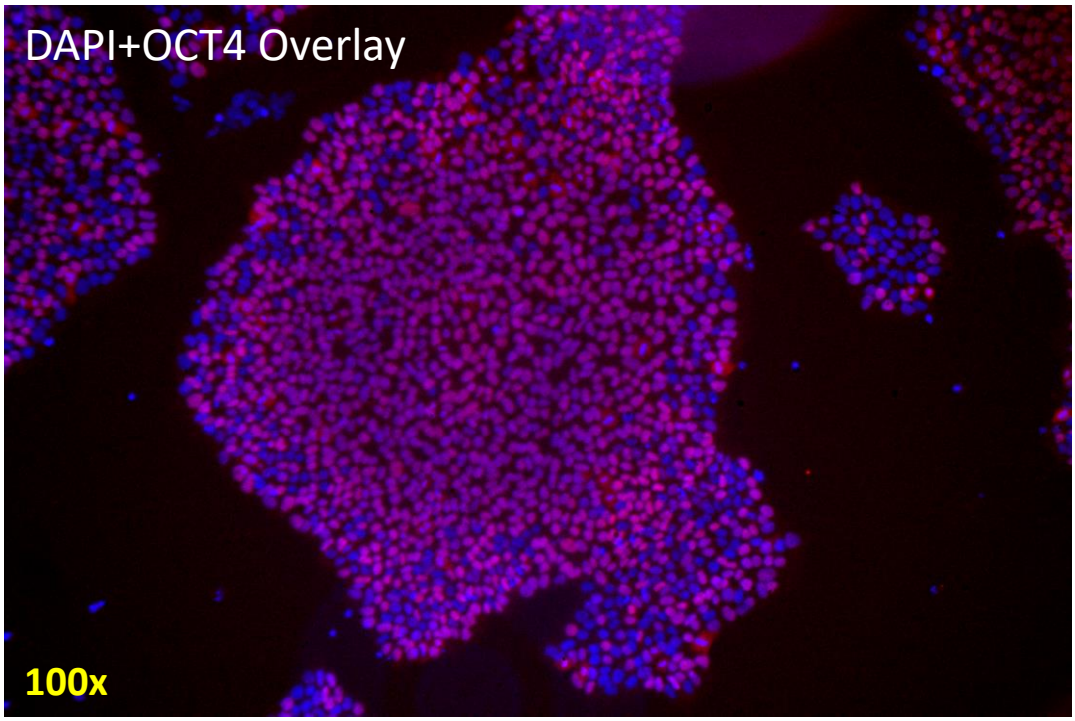
Stage 4(a): iPSC Validation: Staining and Immunofluorescence imaging 2/2



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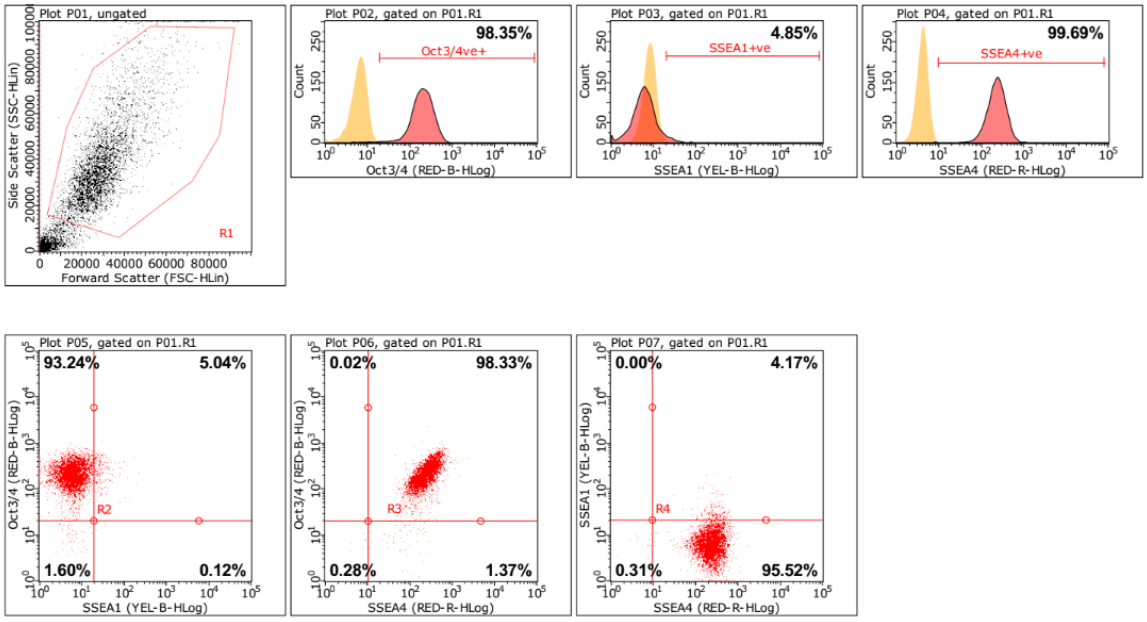
Stage 4(b): Validation by flow cytometry

Flow cytometry allows for population characteristics to be assessed. Looking at the same key markers as for ICC (OCT4 and SSEA4) flow cytometry provides single-cell resolution allowing the purity of the line to be determined, as well as viability, cell count and the size and shape of the cells. All data was compared to isotype controls.



Total overall OCT4+SSEA4 double positive cells: 98.3%

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Stage 5: iPSC cryogenic Banking

After successful generation, stabilization, characterization and expansion the validated iPSCs are cryogenically frozen for long-term storage. All autologous iPSCs are frozen according to the SC21 cryogenic freezing protocol that complies with international iPSC banking standards (Global Alliance for iPSC Therapies (GAiT) "Quality control guidelines for clinical-grade human induced pluripotent stem cell lines", 2018). Vials of 1m iPSCs are stored at < -180°C until use.

IPSC BANKING RECORD (VIAL#)	Results
IPSC-AM-01	10^6 iPSCs
IPSC-AM-02	10^6 iPSCs
IPSC-AM-03	10^6 iPSCs
IPSC-AM-04	10^6 iPSCs
IPSC-AM-05	10^6 iPSCs
IPSC-AM-06	10^6 iPSCs
IPSC-AM-07	10^6 iPSCs
IPSC-AM-08	10^6 iPSCs
IPSC-AM-09	10^6 iPSCs
IPSC-AM-010	10^6 iPSCs

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Overseen by:

Dr Nicholas Boyd-Gibbins (MEng, PhD)

Recorded by:

Julie Ann D. Mendoza (MSc)