Certificate of analysis

SFC893-07-06

Signature: Theodore Latsis: 11-08-2015

Supervisor signature: Lyle Armstrong

Date: 11-08-2015

Source of fibroblasts and reprogramming information

- SF893 from University of Oxford
- Reprogrammed at UNEW, on 13-02-2015 at passage 5
- Cytotune 2
- This cell line has been difficult to expand mainly due to immediate differentiation when passaged.
- The tendency to differentiate diminished over time for one other clone of the same line however this did not occur for this particular one. Differentiation became clearly evident 2-3 days after passaging resulting in almost complete differentiation of colonies at days 5-6 (see Figure 1). As the image illustrates, ipsc fragments obtained when EDTA (Versene) was used to passage the cells/colonies started to differentiate around the periphery eventually resulting in differentiated colonies (usually towards a neuronal lineage).
- Colonies were cleaned around the periphery to remove differentiated areas, however, this not only did not solve the problem of differentiation but it had an obvious negative impact on the **size of the colonies** (**too small**) rendering them unsuitable to passage. The latter coupled with a **slow proliferation** rate made the expansion of this clone extremely difficult, thus the 3 vials produced.
- The clone has been fully characterised and is positive for ipsc markers Nanog and Tra-1-60 (see Flow cytometric analysis).

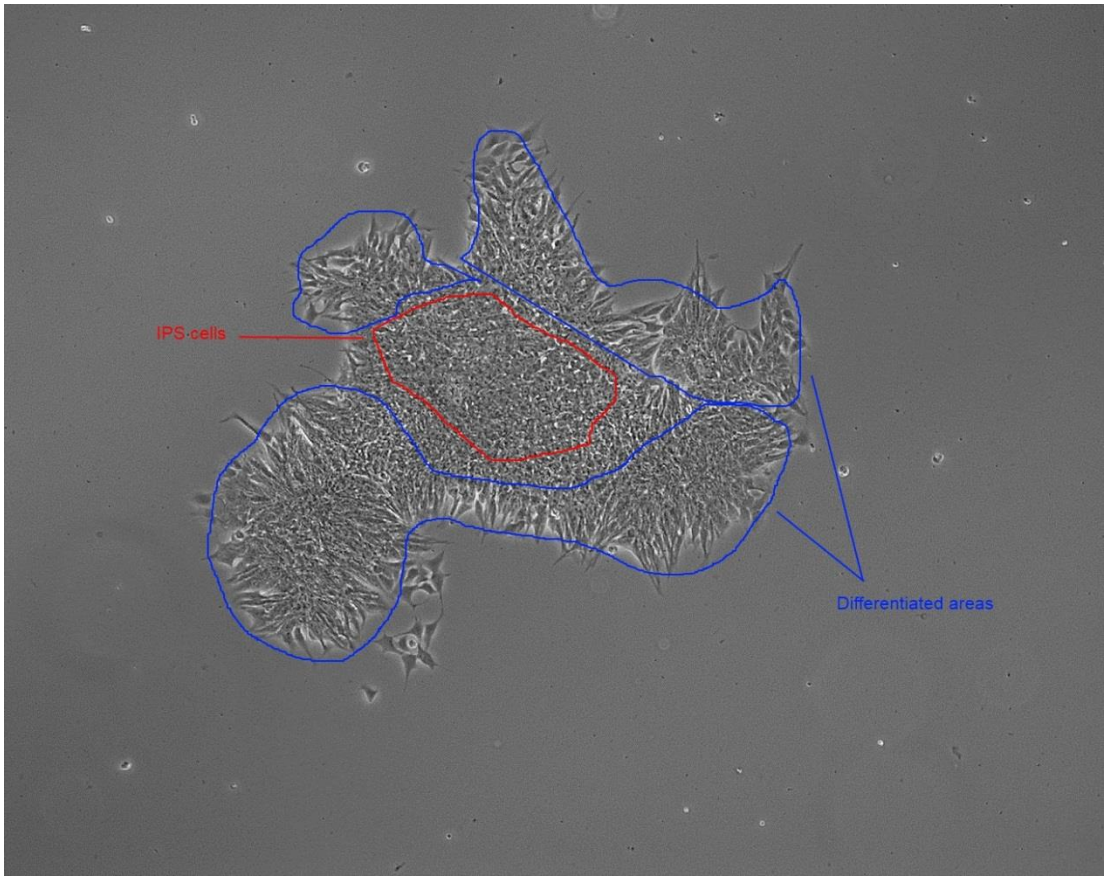
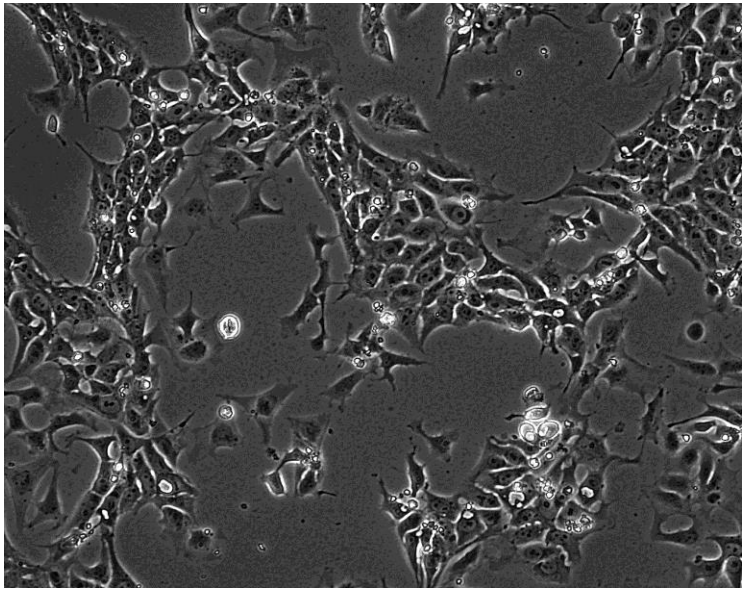


Figure 1. Colony of 893-07-06 at day 6 showing extensive peripheral differentiation.

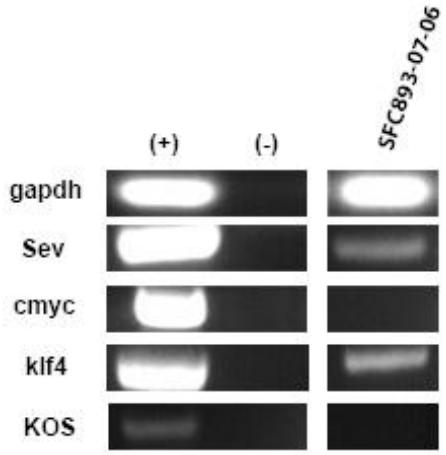
Viability post-thaw and Morphology according to SOP19 passage 16

- Cell count immediately post-thaw: 1,5 X 106
- Viability immediately post-thaw: 85%
- Photo 24h post-thaw

X10 magnification



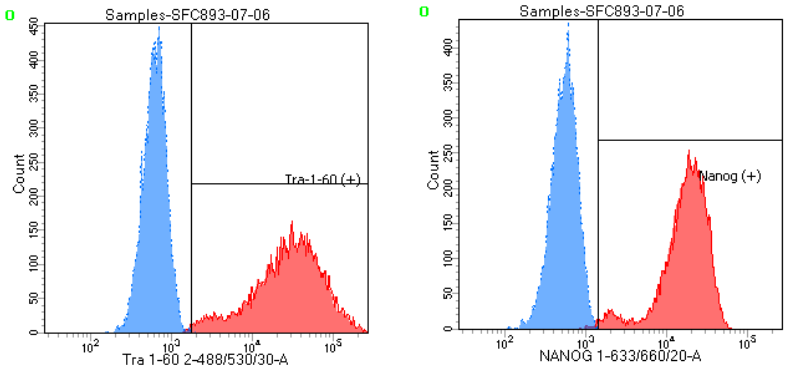
Sendai clearance: according to WP3 SOP15 detectable at passage 16



Mycoplasma test:
According to MycoAlert Lonza LT07-318
Undetectable at passage 16

					Sample
> 1.2		Mycoplasma Contaminated	Positive Control	Negative Control	**SFC893-07-06**
0.9-1.2		Status Unknown - Restest within 24 hours	0.022	0.078	0.014
0-0.9		Mycoplasma Free	1.814	0.005	0.006
			82.081	0.068	0.387

Flow cytometric analysis according to WP3 SOP 20 and 21 passage 16



Tube: SFC893-07-06

Population	#Events	%Parent	%Total
All Events	17,199	####	100.0
Cells	8,856	51.5	51.5
Tra-1-60 (+)	8,779	99.1	51.0
Nanog (+)	8,744	98.7	50.8
Samples/893/All Events	19,359	####	100.0
Samples/893/P1	9,875	51.0	51.0

SNP analysis according to WP3 SOP Preparation of DNA and RNA samples for Illumina arrays

- Passage 16
- Identity to parent fibroblasts confirmed
- Karyotype abnormalities: minor Chr20 abnormality which is not present in parental fibroblasts
- For details and raw data see StemDB