

# Certificate of analysis

SFC049-03-04

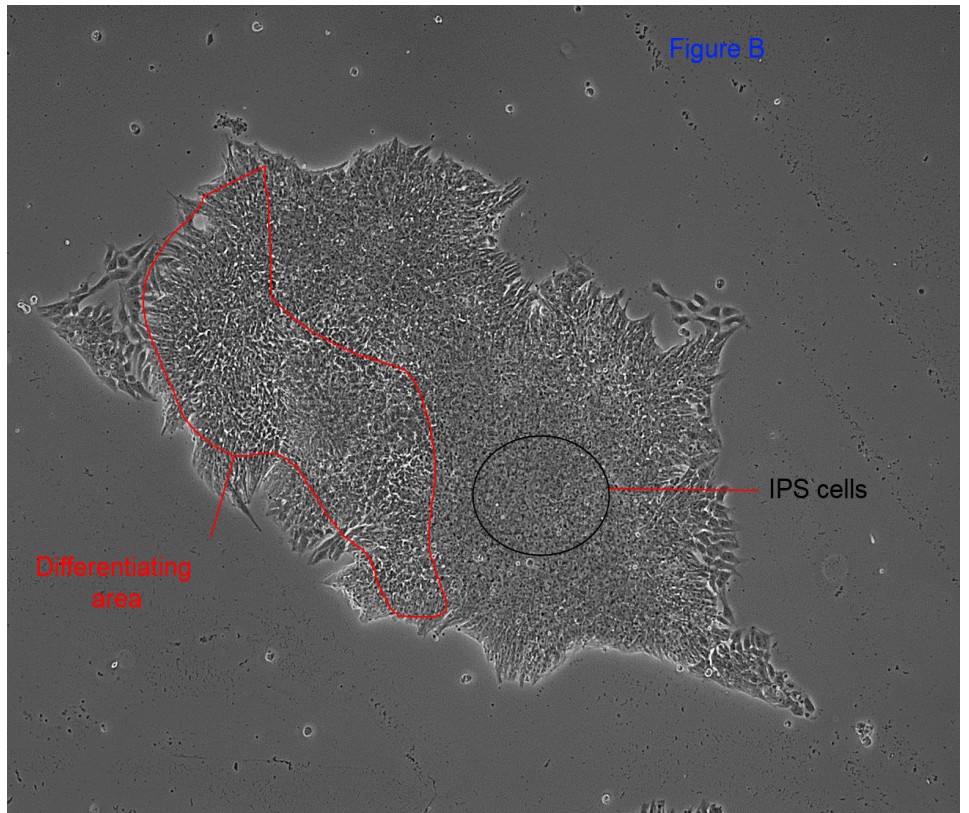
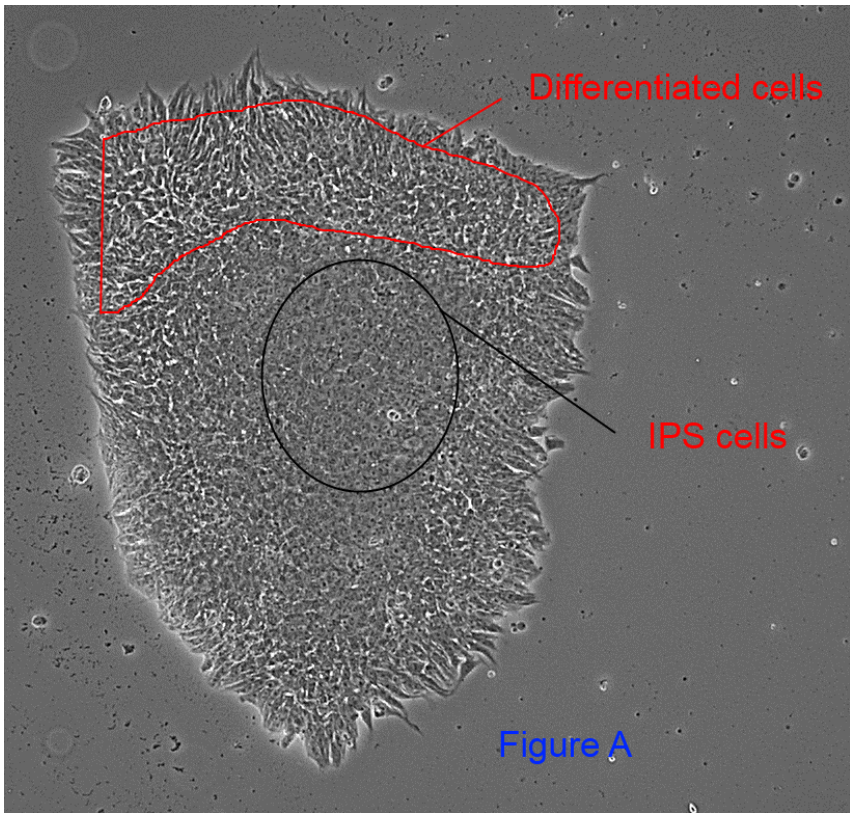
Signature: Theodore Latsis: 11-08-2015

Supervisor signature: Lyle Armstrong

Date: 11-08-2015

# Source of fibroblasts and reprogramming information

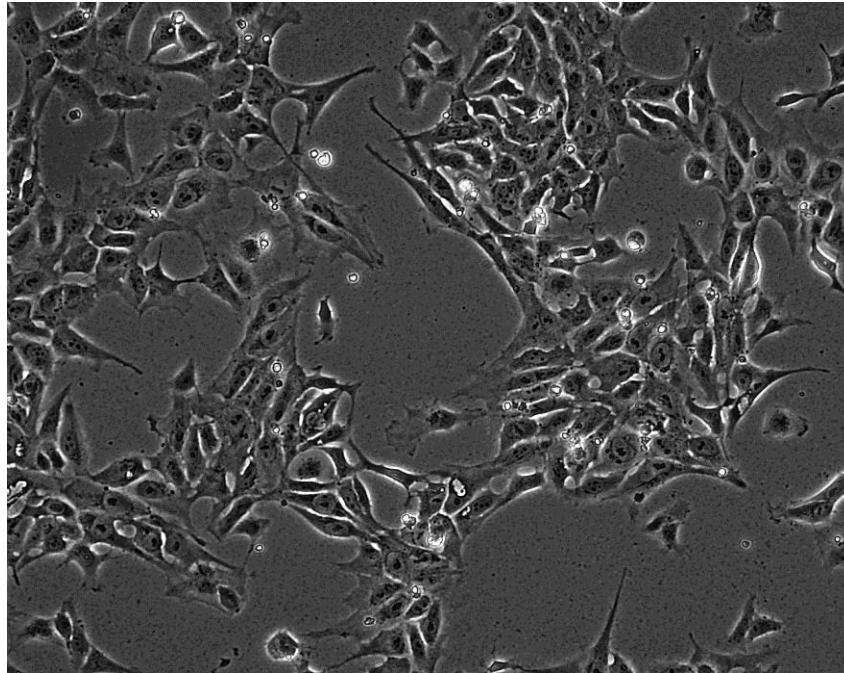
- SF049 from University of Oxford
- Reprogrammed at UNEW, on 13-01-2015 at passage 5
- Cytotune 2
- This cell line has been difficult to expand mainly due to immediate differentiation when passaged.
- The tendency to differentiate diminished over time for (2) other clones of the same line however this did not occur for this particular one. Differentiation is clearly evident from day 3 onwards after passaging (see image below). As the image illustrates, ipsc fragments, obtained when EDTA (Versene) is used to passage the cells/colonies, start to differentiate around the periphery eventually resulting in a completely differentiated colony (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 rendering them unsuitable (too small) to passage. As a result, the expansion of this particular clone has been extremely difficult thus the 6 vials produced.
- The clone has been fully characterised and is positive for ipsc markers Nanog and Tra-1-60 (see Flow cytometric analysis).



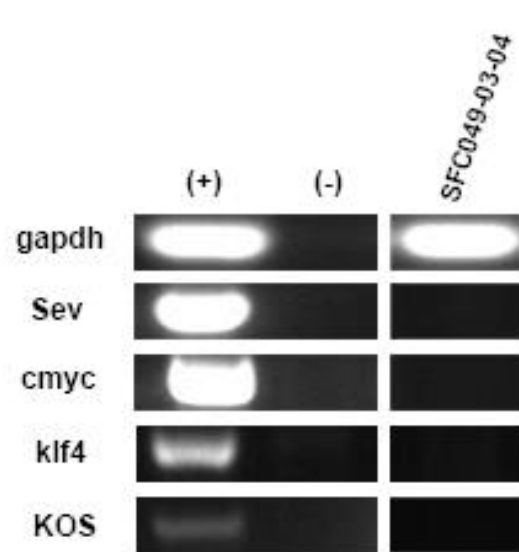
Figures A&B :Differentiating Colonies at day 3 after passaging

# Viability post-thaw and Morphology according to SOP19 passage 20

- Cell count immediately post-thaw:  $2 \times 10^6$
- Viability immediately post-thaw: 86%
- Photo 24h post-thaw






Sendai clearance:  
according to WP3 SOP15  
undetectable at passage 20



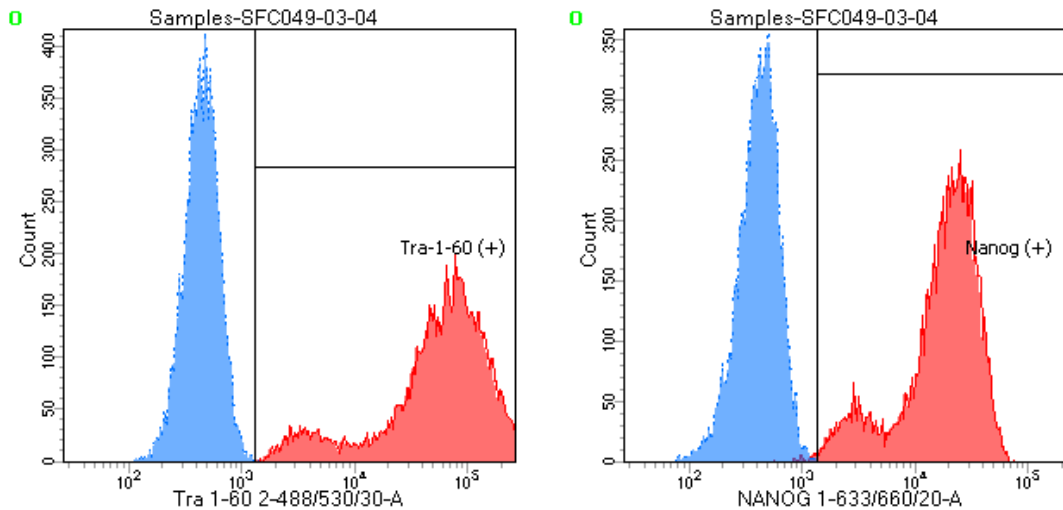
# Mycoplasma test:

## According to MycoAlert Lonza LT07-318

### Undetectable at passage 20

> 1.2		Mycoplasma Contaminated	Positive Control	Negative Control	Cell name	SFC-049-03-04
0.9-1.2		Status Unknown - Restest within 24 hours	0.053	0.062	A	0.013
0-0.9		Mycoplasma Free	0.963	0.010	B	0.007
			18.068	0.166	B/A	0.493

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



Tube: SFC049-03-04

Population	#Events	%Parent	%Total
■ All Events	20,000	####	100.0
■ Cells	9,603	48.0	48.0
☒ Tra-1-60 (+)	9,599	100.0	48.0
☒ Nanog (+)	9,522	99.2	47.6
■ Samples/49/All Events	20,000	####	100.0
■ Samples/49/P1	9,231	46.2	46.2

# SNP analysis

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

- Passage 20
- Identity to parent fibroblasts confirmed
- Karyotype abnormalities: none detected
- For details and raw data see StemDB