Certificate of analysis

SFC047-03-03

Signature: Katja Gassner Date: 18.05.2015

Supervisor signature: Linda Lako

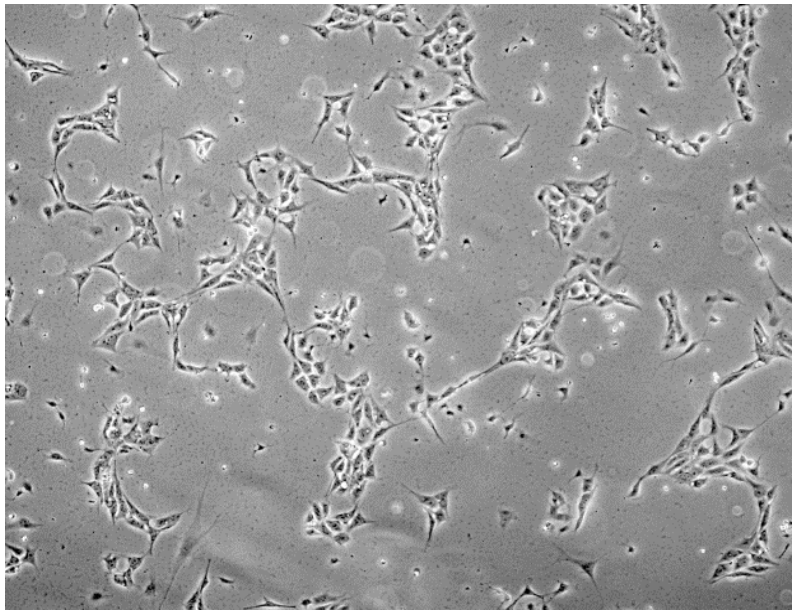
Date: 19.05.2015

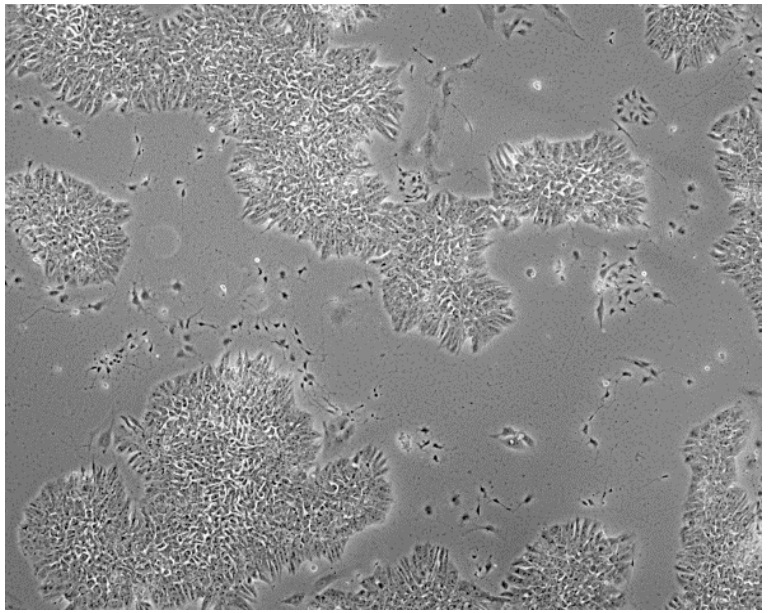
Source of fibroblasts and reprogramming information

- SF047 from UOXF
- Reprogrammed at UNEW
- Reprogrammed on 16/01/2015 at passage 8
- Cytotune 2

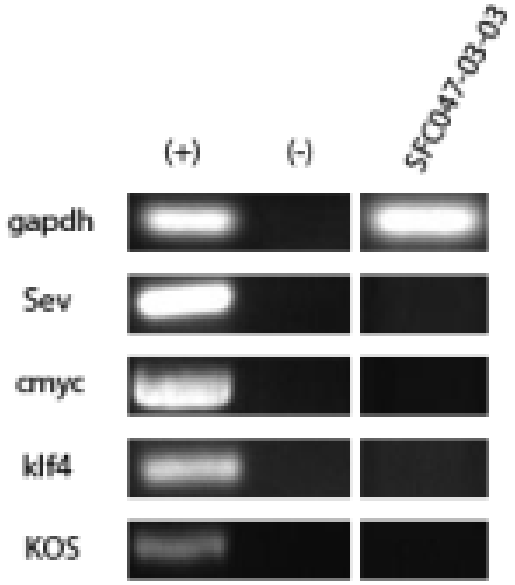
Viability post-thaw and Morphology according to SOP19 passage 7

- Cell count immediately post-thaw 1.5 x 10^6
- Viability immediately post-thaw 95%
- Photo at 24h and 4 days post-thaw:





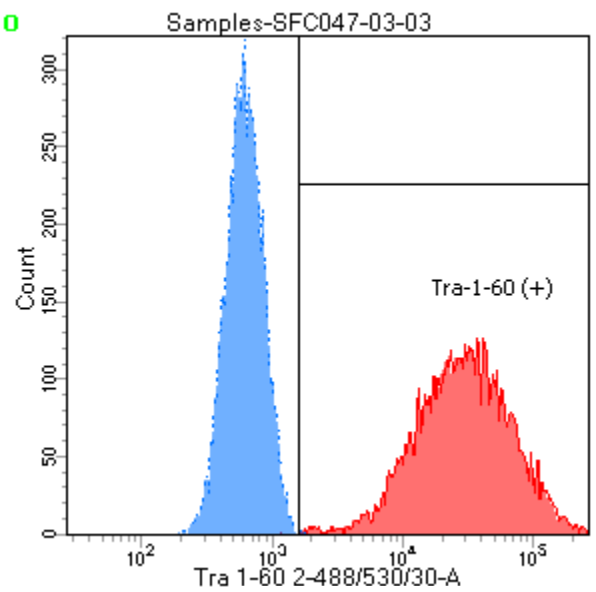
Sendai clearance: according to WP3 SOP15 undetectable at passage 7

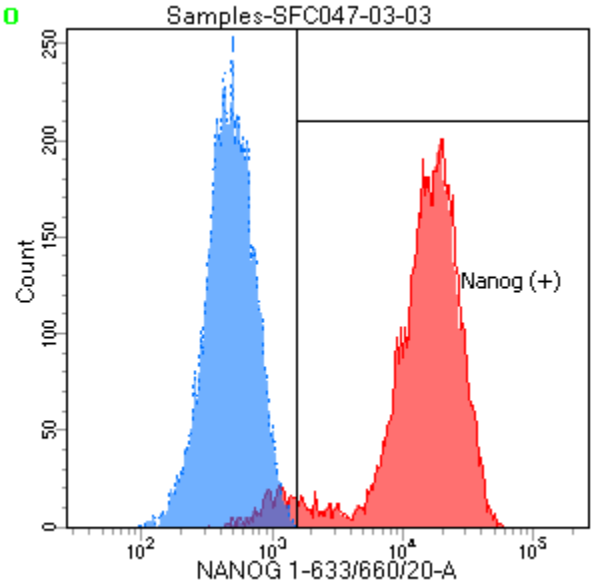


Mycoplasma test:
According to MycoAlert Lonza LT07-318
Undetectable at passage 7

Owner	KG					
Date	08/05/2015					
Cell name	sfc047-03-03					
A	0.0265					
B	0.0146					
B/A	0.550943396					
> 1.2		Mycoplasma Contaminated			Positive Control	Negative Control
0.9-1.2		Status Unknown - Restest within 24 hours			0.0404	0.0906
0-0.9		Mycoplasma Free			2.339	0.0118
					57.8960396	0.130242826

Flow cytometric analysis according to WP3 SOP 20 and 21 passage 7





Tube: SFC047-03-03

Population	#Events	%Parent	%Total
All Events	20,000	####	100.0
Cells	6,931	34.7	34.7
Tra-1-60 (+)	6,918	99.8	34.6
Nanog (+)	6,510	93.9	32.6
Samples/47UN/All Events	20,000	####	100.0
Samples/47UN/P1	6,650	33.2	33.2

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

- Passage 7
- Identity to parent fibroblasts confirmed
- Karyotype abnormalities: none detected
- For details and raw data see StemDB

Comments

- Freezing with accutase and ROCK might promote differentiation, ReLESR passaging recommended
  - Add 1ml ReLESR, incubate for 1 min at 37˚C
  - Remove ReLESR, incubate for 4 min at RT
  - Add medium **dropwise** to collect undifferentiated cells