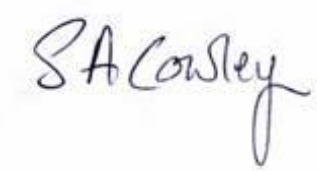
Certificate of analysis

SFC840-03-03 LRRK2wt/R1441C H3

Operator: Rowan Flynn Date: 26/01/2017

Supervisor: Sally Cowley Date: 06/06/2017

Signature: 

Source of fibroblasts and reprogramming information

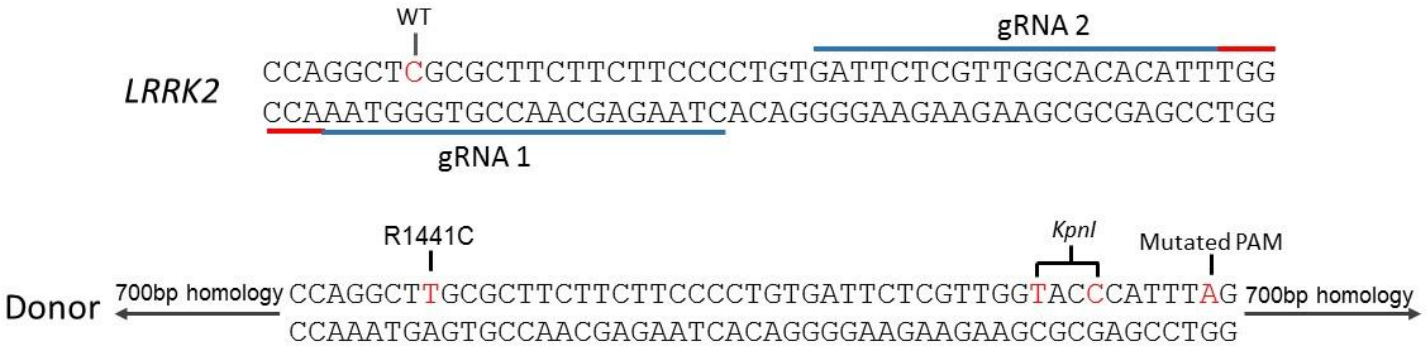
- SF840 from UOXF 04/04/2011
- Reprogrammed at UOXF JMSCF
- Reprogrammed on 13/12/2012 at passage 5
- Cytotune v1 WP3 SOP10

Gene editing information

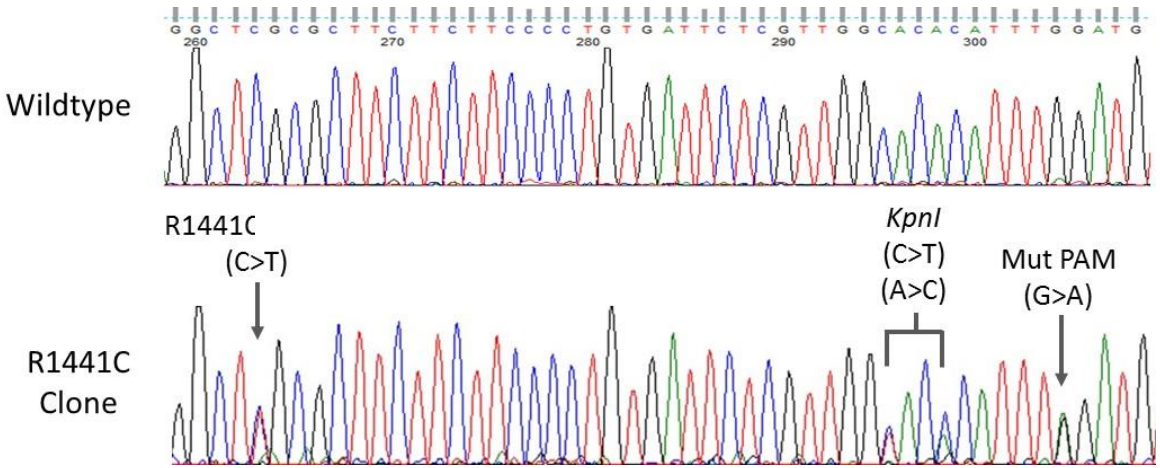
- Strategy: CRISPR/Cas9-mediated double-strand break generated close to R1441C mutation and homology-directed repair with donor sequence.
- Single plasmid CRISPR/Cas9 constructs used (px462 nickase, Zhang lab)
- Donor plasmid: Homology arms generated by amplification of LRRK2 sequence from SFC840-03-03. R1441C and silent mutations to mutate gRNA PAM site and create *KpnI* restriction site for screening were introduced by PCR with long-tailed primers.
- Transfection of SFC840-03-03 p?? 04.04.2011 UOXF [JMSCF] with guide RNA plasmids encoding gRNA57 and gRNA59 and donor template.

gRNA 57	CCAGGCTCGCGCTTCTTCTTCCC	gRNA 59	GATTCTCGTTGGCACACATTTGG
RF403	**CACC**GGGAAGAAGAAGCGCGAGCC	RF407	**CACC**GATTCTCGTTGGCACACATT
RF404	**AAAC**GGCTCGCGCTTCTTCTTCCC	RF408	**AAAC**AATGTGTGCCAACGAGAATC

Guide RNA location and donor template design:



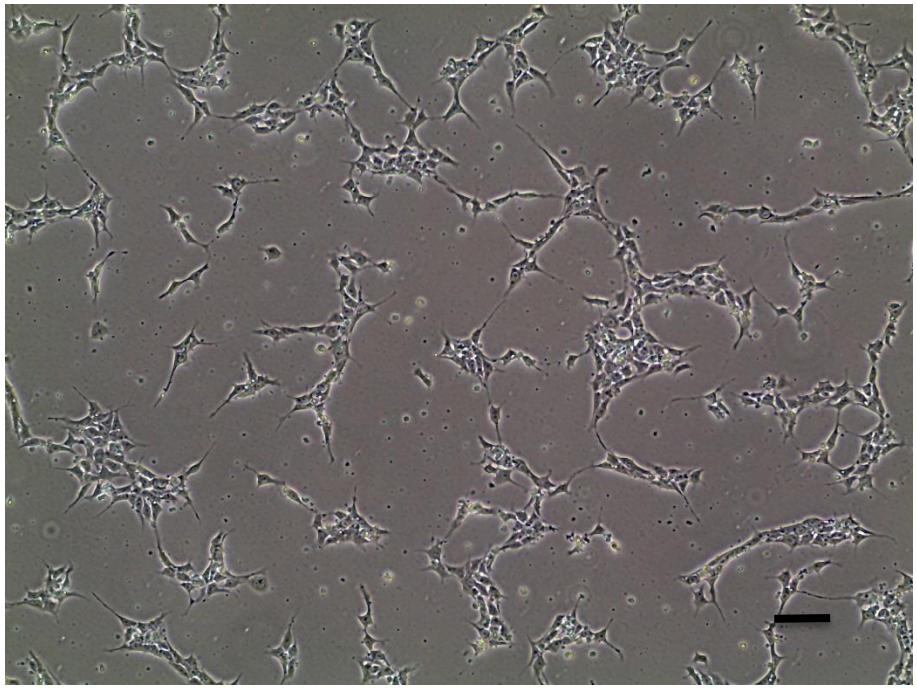
Sequence confirmation of gene editing:



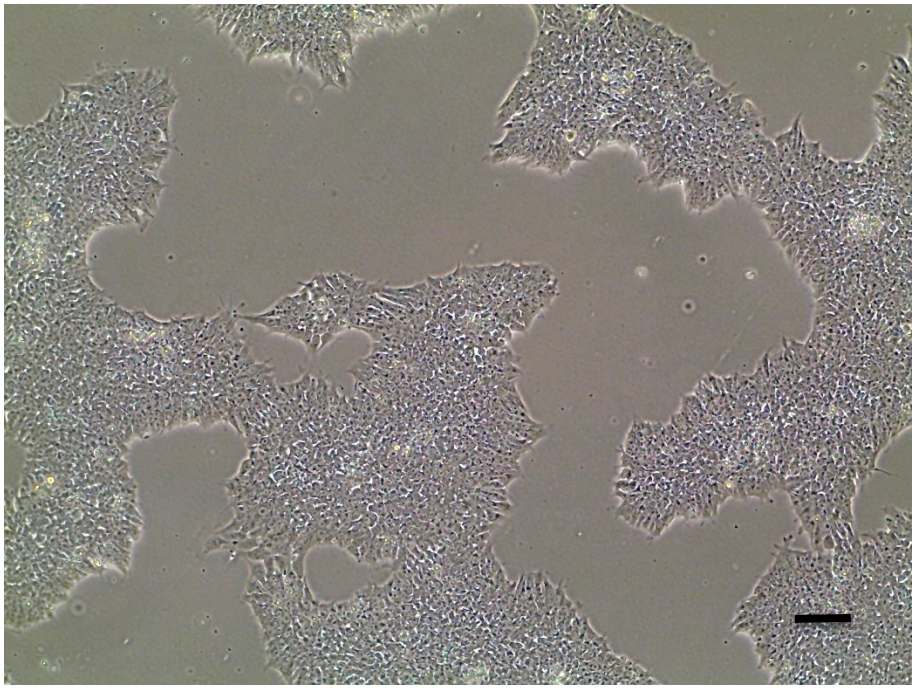
Viability post-thaw and Morphology according to SOP19 passage 31

- Cell count immediately post-thaw 3.0 x 10^6
- Viability immediately post-thaw 83%
- Photo at 24h and day 3 post-thaw (scale bar = 100µm):

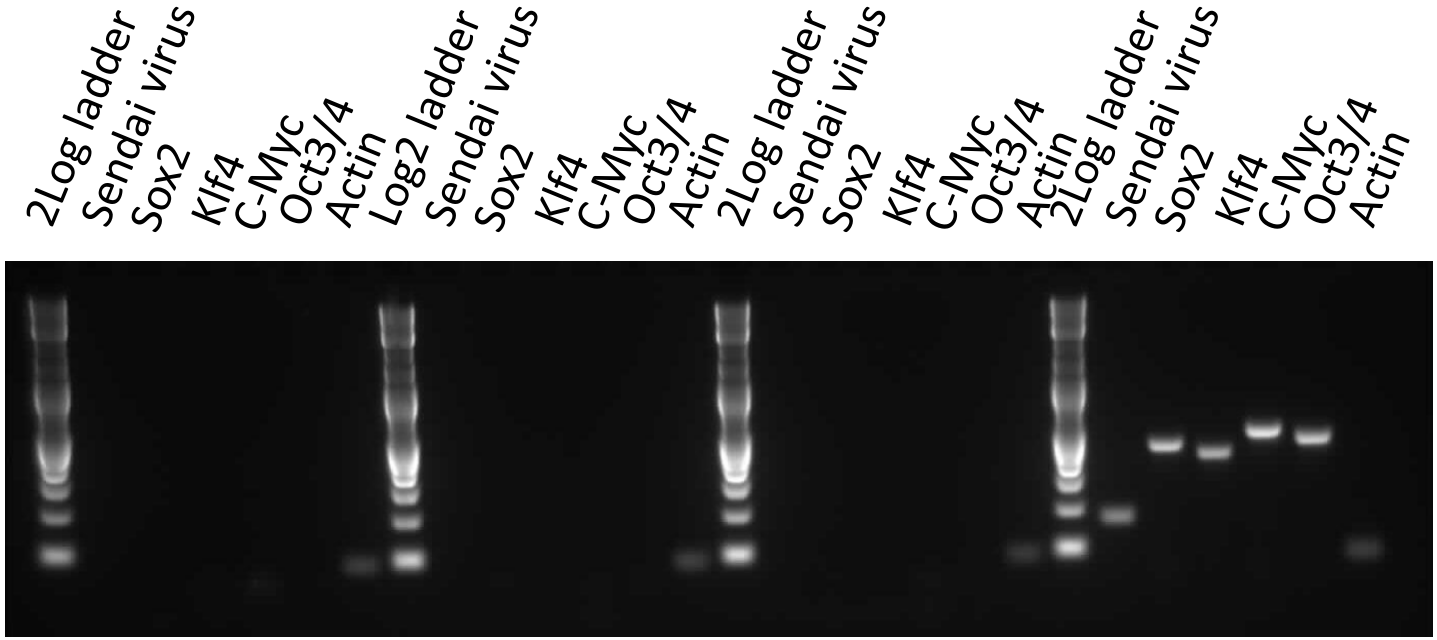
24h post-thaw 80% plated



Day 3 post-thaw 80% plated



Sendai clearance: according to WP3 SOP15 undetectable at p19



SFC821-03-02 **SFC840-03-03** SFC828-03-04 + control

Product sizes: SeV 181bp; SeV-Sox 451bp; SeV-Klf 410bp; SeV-Myc 532bp; SeV-Oct 483bp; Actin 92bp

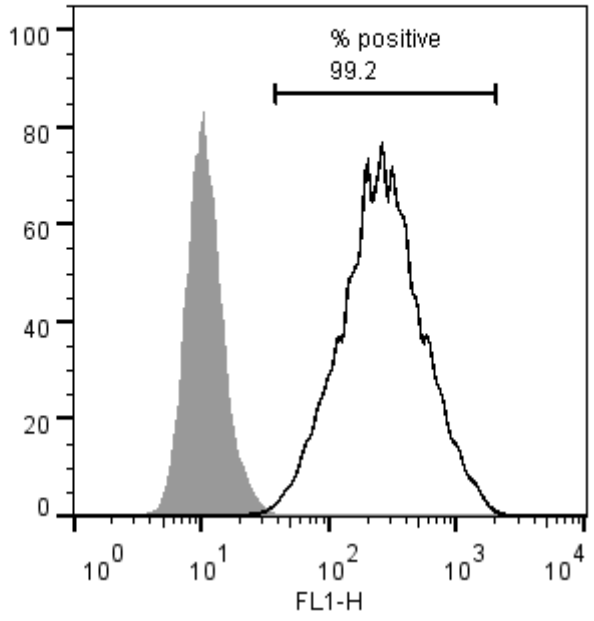
Mycoplasma test:
According to MycoAlert Lonza LT07-318
Undetectable at passage 31

Sample	Passage number	Initial	Reading 1	Reading 2	Ratio/Status
+ve control			5.477	116.6	**21.29**
-ve control			6.458	1.612	**0.25**
SFC840-03-03 R1441C KI H3	p14+17	JV	1.828	0.424	**0.23**

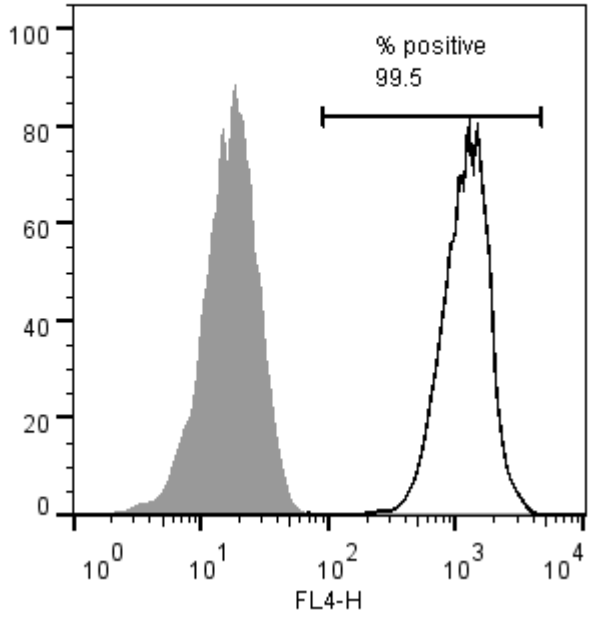
Results mean
Ratio **0 - 0.999** negative for mycoplasma
Ratio **1 – 1.3** Borderline Result (retest required)
Ratio above **1.3** positive for mycoplasma

Flow cytometric analysis according to WP3 SOP 20 and 21 passage p14+17

Tra-1-60:



NANOG:



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

- Passage p31
- No gross karyotypic abnormalities observed, 6 small indels as in parent line
- For details and raw data see StemDB