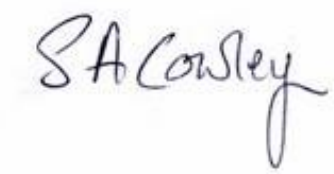
Certificate of analysis

SFC832-03-06LRRK2wt/wtC47

Operator: Rowan Flynn, Olga Perestenko

Date: 29/07/2015

Supervisor: Sally Cowley Date: 28.08.2015

Signature: 

Source of fibroblasts and reprogramming information

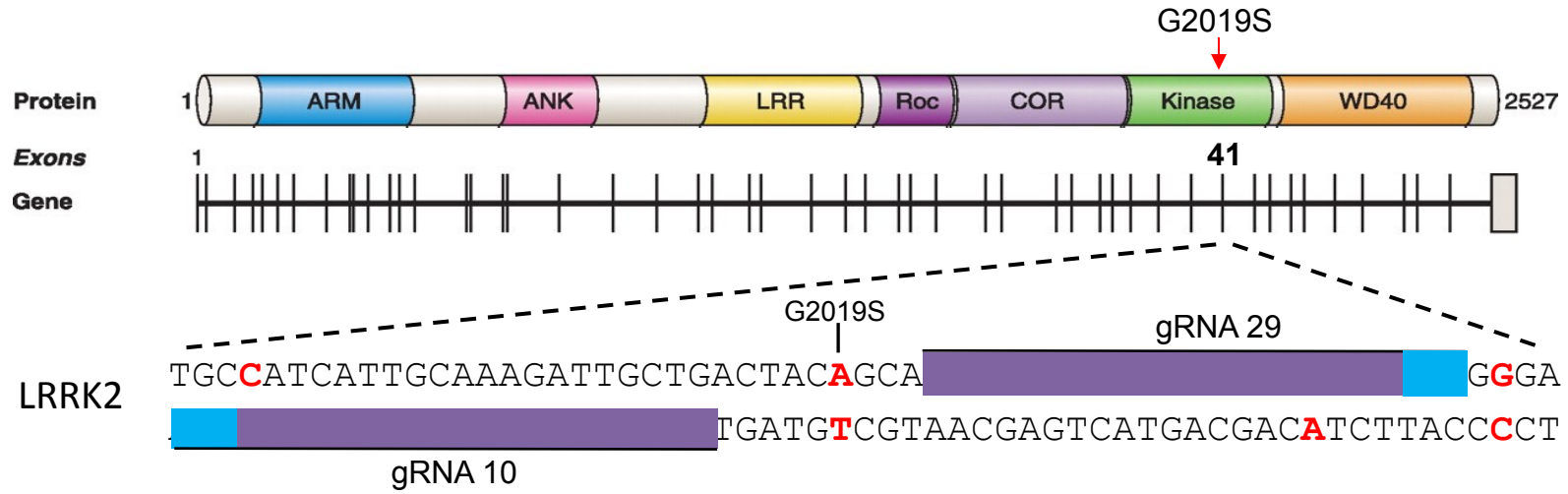
- SF832 from Oxford University Hospitals 03/07/2012
- Reprogrammed at UOXF S
- Reprogrammed on 17/07/2013 at passage 4
- Cytotune v1 WP3 SOP10

Gene editing information

- Strategy: CRISPR/Cas9-mediated double-strand break generated close to G2019S 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 in mutant allele to maintain isogeneic sequence. The G2019S mutation was repaired (G>A), and other silent mutations introduced including mutated protospacer adjacent motifs to prevent recutting of repaired sequence, and a *Pst*I site for identification of repaired clones.
- Transfection of SFC832-03-06 p25 03.10.2014 UOXF [JMSCF] with guide RNA plasmids encoding gRNA10 and gRNA29 and donor template.

RF60	CACCGTCAGCAATCTTTGCAATGA	gRNA10 oligo 1
RF61	AAACTCATTGCAAAGATTGCTGAC	gRNA10 oligo 2
RF239	CACCGTCAGTACTGCTGTAGAATG	gRNA29 oligo 1
RF240	AAACCATTCTACAGCAGTACTGAC	gRNA29 oligo 2

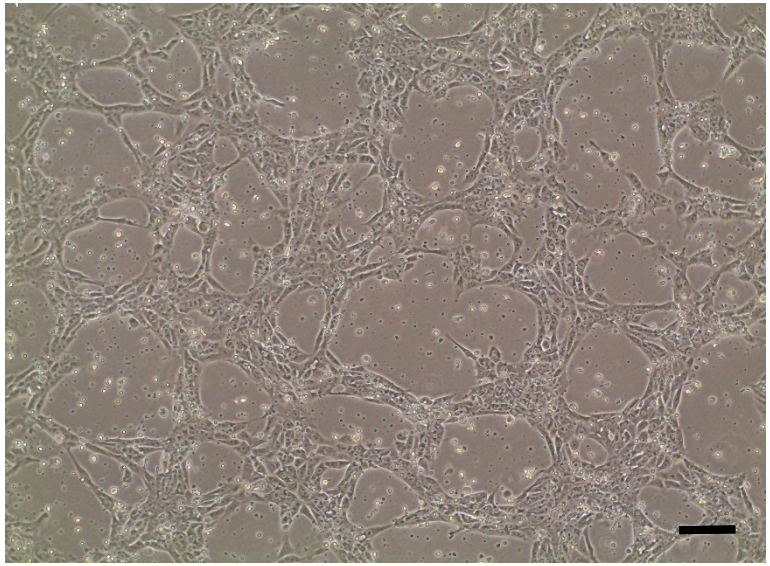
Guide RNA location and donor template design:

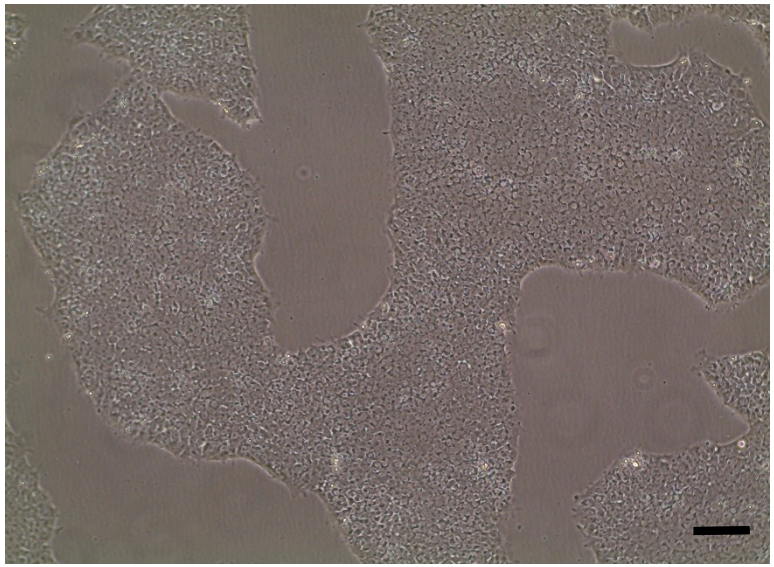


Sequence confirmation of gene editing:

Viability post-thaw and Morphology according to SOP19 passage 40

- Cell count immediately post-thaw 1.48 x 10^6
- Viability immediately post-thaw 74.8%
- Photo at 24h & day 4 post-thaw (scale bar = 100µm):





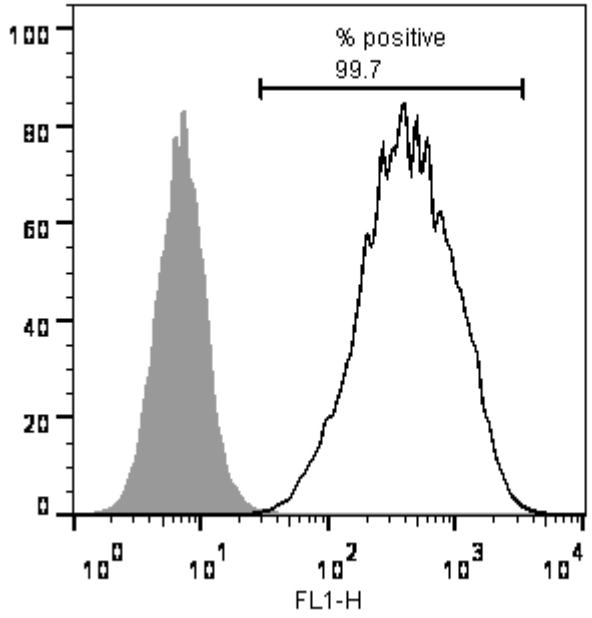
Mycoplasma Test:
According to MycoAlert Lonza LT07-318 undetectable at passage 40

Sample	Clone	Passage number	Initial	Reading 1	Reading 2	Ratio/Status
+ve control				12.68	145	**11.44**
-ve control				7.237	0.318	**0.04**
1	SFC832-03-06LRRK2wt/wt/C47	P40	OP	1.001	0.564	**0.56**

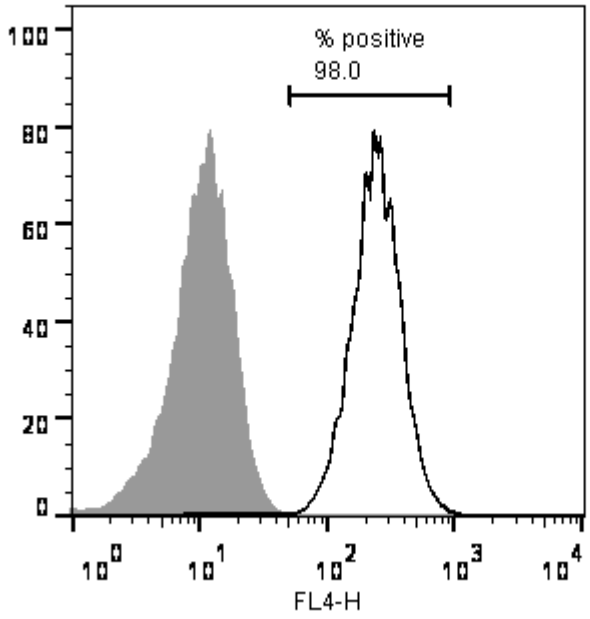
Results mean
Ratio **0 - 0.999** negative for mycoplasma
Ratio **1 – 1.3** Borderline Result (retest required)
Ratio above **1.3** positive for mycoplasma

Flow cytometric analysis according to WP3 SOP 20 and 21 passage p41

Tra-1-60:



NANOG:



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

- Passage 40
- Identity to parent fibroblasts confirmed
- Karyotype abnormalities: none compared to SF832 fibroblasts
- For details and raw data see StemDB