



Certificate of analysis

SFC840-03-03 LRRK2-/- D10

Operator: Sally Cowley

Date: 07/09/2015

Supervisor: Sally Cowley

Date: 07/09/2015

Signature:

SACowley

Source of parental iPSc line and reprogramming information

- SF840 from UOXF 04/04/2011
- Reprogrammed at UOXF S [JMSCF]
- Reprogrammed on 13/12/2012 at passage 5
- Cytotune v1 WP3 SOP10
- SFC840-03-03 StemBANCC QC passed
22/04/2015

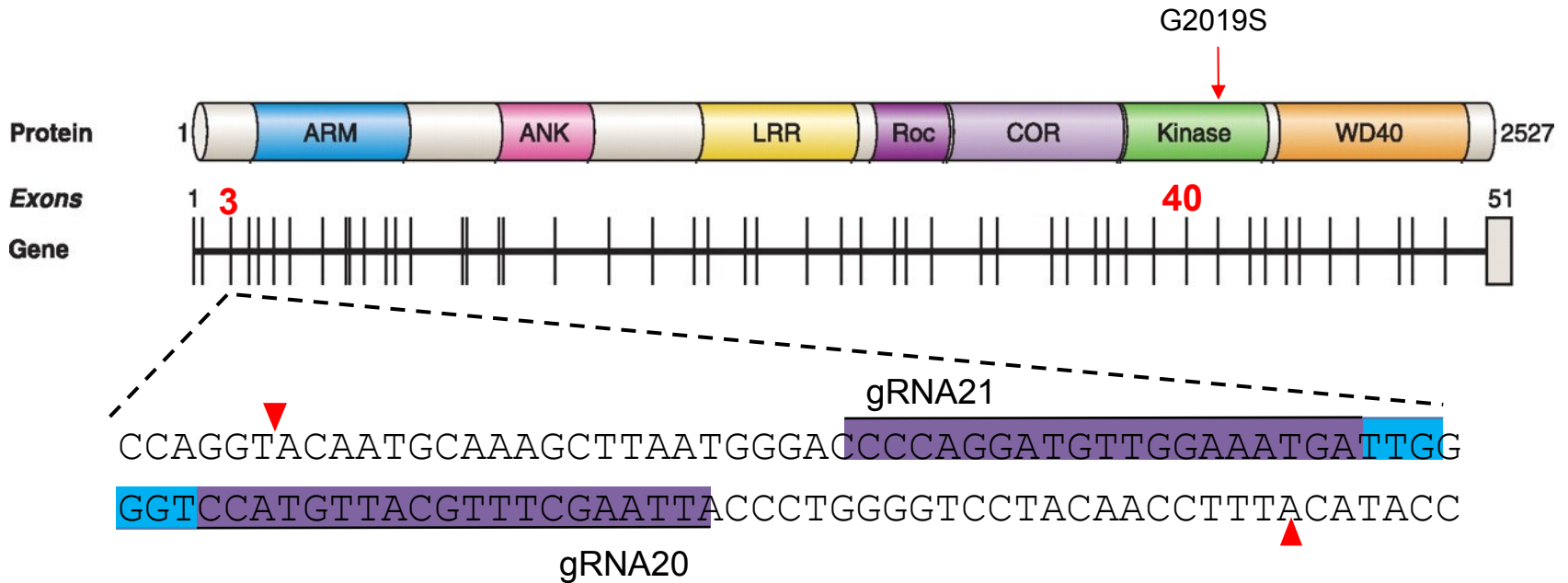
Gene editing information

- Strategy: CRISPR/Cas9-mediated disruption of exon 3 and clone-screening for bi-allelic out-of-frame repair, to create Lrrk2 knockout line
- Neon transfection of SFC840-03-03 p20 20.06.2014 SC UOXF S [JMSCF]
- CRISPR/Cas9 px462 (nickase, Zhang lab) plasmids encoding gRNA20 and gRNA21

RF128 CACCGATTAAGCTTTGCATTGTACC
RF129 AAACGGTACAATGCAAAGCTTAATC
RF130 CACCGCCCAGGATGTTGGAAATGAT
RF131 AAACATCATTTC AACATCCTGGGC

gRNA20 oligo 1
gRNA20 oligo 2
gRNA21 oligo 1
gRNA21 oligo 2

Sequence confirmation of gene editing: out of frame deletion on both alleles



WT TCTGT**CCA**GGTACAATGCAAAGCTTAATGGGACCCCAGGATGTTGGAAATGAT**TGG**GAAGT

Clone D10

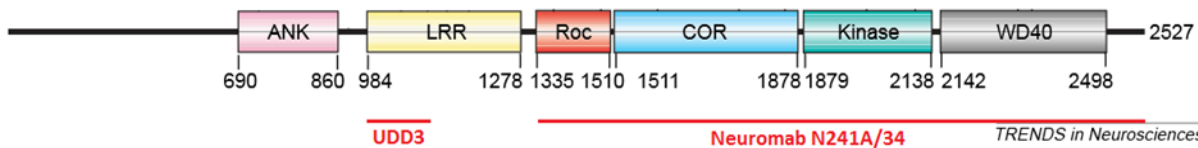
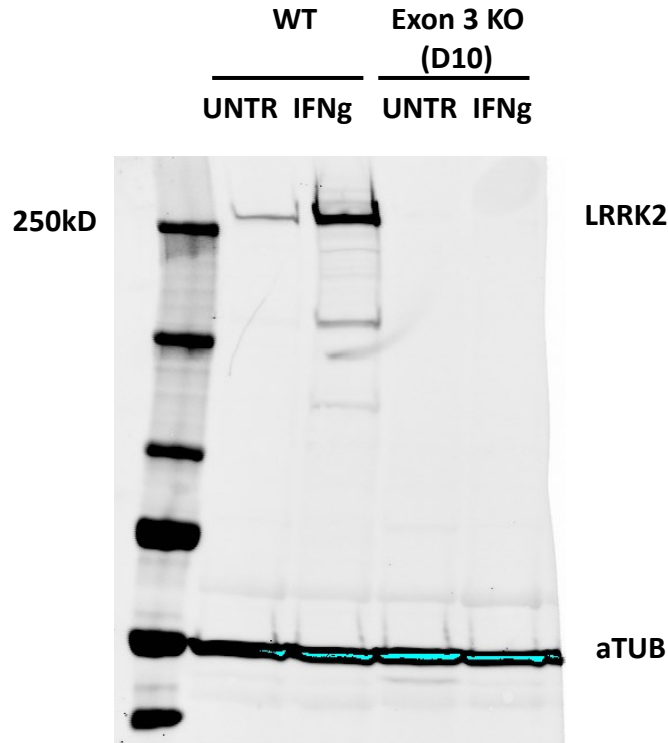
Δ16bp TCTGT**CCA**GGTACAATGCAAAG-----GATGTTGGAAATGAT**TGG**GAAGT

+37bp TCTGT**CCA**GGTACAATGCAAAGCTTAATGGGACCCCAGGATGTTGGAAATGAT**TGG**GAAGT

ACAATGCAAAGCTTAATGGGACCCCAGGATGTTGGAA

Protein expression confirmation of gene editing:

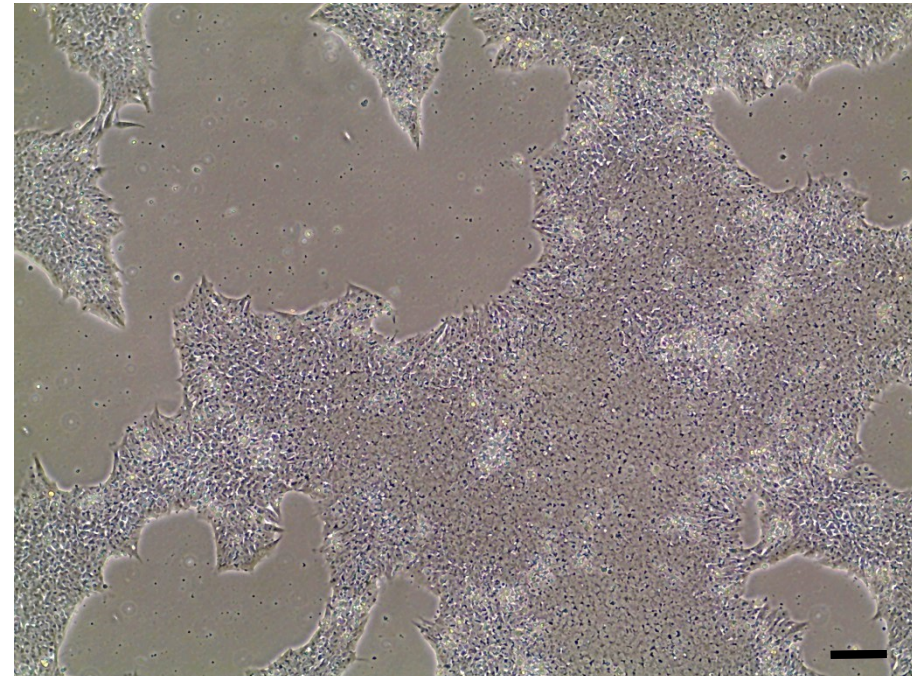
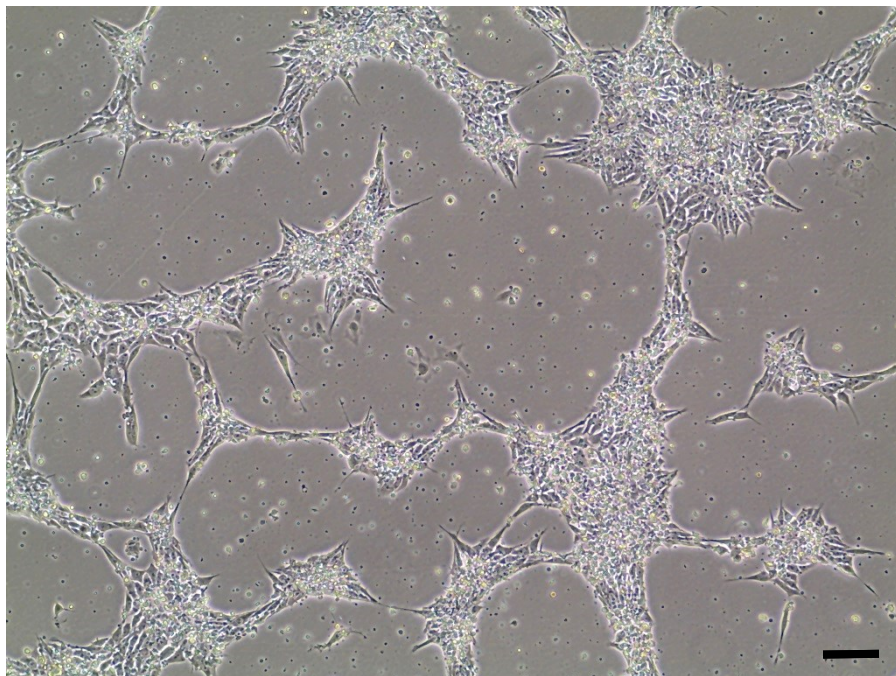
Differentiation to macrophages for Lrrk2 expression confirms KO by Western Blot [neuromab antibody] vs wt cells + maximal activation of expression by IFNg [Lrrk2 286kD]



Viability post-thaw and Morphology according to SOP19 passage 31

NB recommend thawing to 12 well format due to lower than optimal cell count and viability

- Cell count immediately post-thaw 0.91×10^6
- Viability immediately post-thaw **75.8%**
- Photo at 48h and 4d post-thaw (scale bar = 100 μ m):



Mycoplasma test:

According to MycoAlert Lonza LT07-318

Undetectable at passage 33

Sample	Clone	Passage number	Initial	Reading 1	Reading 2	Ratio/Status
+ve control				9.447	176.8	18.71
-ve control				11.15	0.958	0.09
6	SFC840-03-03 Lrrk2-/- D10	p33	SC	4.028	1.365	0.34

SNP analysis

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

- Passage 31
- Identity to parent fibroblasts confirmed
- Karyotype abnormalities: none detected
- For details and raw data see StemDB