CERTIFICATE OF ANALYSIS
AICS-0104: MYH7-H251N in WTC-mEGFP-ACTN2 (mono-allelic tag)

Cell Line description	H251N mutation introduced into the MYH7 gene
Parental cell line	Human iPSC clonal line in which ACTN2 has been endogenously tagged with mEGFP using CRISPR/Cas9. Parental hiPSC line (WTC/AICS-0 passage 33 at acquisition) derived from dermal fibroblasts reprogrammed using episomal vectors (OCT3/4, shp53, SOX2, KLF4, LMYC, and LIN28).
Relevant publications	Kreitzer et al (2013) Am. J. Stem Cells, 30; 2(2): 119-31; Roberts et al. Stem Cell Reports. 2019 May 14; 12(5) 1145 - 1158
Passage of gene edited iPSC reported at submission	p48[a]
Number of passages at Coriell	0
Media	mTeSR1
Feeder or matrix substrate	Matrigel
Passage method	Accutase, single cell
Thaw	1 million cells (ea vial) in 10 cm plate - ready for passaging in 3-4 days
Seeding density	400K cells/10-cm plate every 4 days or 800K cells/10-cm plate every 3 days (see culture protocol)
F primer for PCR/sequencing (5' to 3')	TCTCCTGATTTGAGGCTTGC
R primer for PCR/sequencing (5' to 3')	AAAGACACCTAGCCATGCAG
Protospacer + PAM (5' to 3')	ATTCATTCGAATTCATTTTGGGG

Test Description[b]	**Method**	**Specification**	**Results**			
Clone Number	N/A	N/A	3	85	4	6
Transfection Replicate (A or B)	N/A	Clones were derived from separate replicated transfections. Comparisons between clones of different genotypes recommended from same replicate.	A	B	A	A
Clone PCR/Sanger	PCR and Sanger sequencing of recombinant and wildtype alleles	Determine if predicted mutation occurred with no additional mutations present.	H251N / WT	H251N / WT	WT / WT	WT / WT
ddPCR Assay (allele frequency)	ddPCR assay (MYH7-H251N:RPP30; MYH7-WT:RPP30; MYH7-H251N; MYH7-WT)	Determine if clone has a distribution of expected alleles	pass	pass	pass	pass
Trisomy 12 Test	ddPCR assay (Chr12:RPP30)	pass = trisomy 12 not detected in quantitative ddPCR assay.	pass	pass	pass	pass
Karyotype	G-banding (30 cell analysis)	Normal karyotype, 46 XY	pass	pass	pass	pass

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Cardiac Differentation	Modified small molecule differentiation **(see cardiac differentiation protocol)**	Beating initiated (D7-D14) and Cardiac Troponin T expression (D11- D30) by flow cytometry	pass	pass	pass	pass
Avg % cTnT+	Flow Cytometry	% cTnT+ cells compared to isotype control	81.5%	79.0%	91.5%	49.0%
Sterility	Direct inoculation and incubation for 10 days	No growth after 10 days	pass	pass	pass	pass
Mycoplasma	qPCR (IDEXX)	Negative	pass	pass	pass	pass
Viral Panel Testing of WTC-11 parental line[c]	PCR	Negative when assayed for CMV, EBV, HepB, HepC, HIV1, and HPV	pass			
Identity of Unedited WTC-11 parental line[d]	STR	9 allelic polymorphisms across 15 STR loci compared to donor fibroblasts	Identity matched			

0.1 BLUE = MUTANT CLONES; GREEN = WILDTYPE CLONES

[a] This is the number of passages beyond the original parental line (WTC/AICS-0 at passage 33).
[b] Bacterial, yeast and fungal testing.
[c] Viral panel testing was conducted for the parental WTC line prior to editing. Sterility (bacterial, fungal) and mycoplasma testing were conducted in both the parental and edited lines
[d] STR tests were conducted for the WTC parental line prior to editing. WTC is the only cell line used by AICS. Edited WTC cells were not re-tested because they did not come into contact with any other cell lines.

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Tagging strategy: CRISPR-Cas9 methodology was used to introduce mEGFP at N-terminus of SON as shown below.

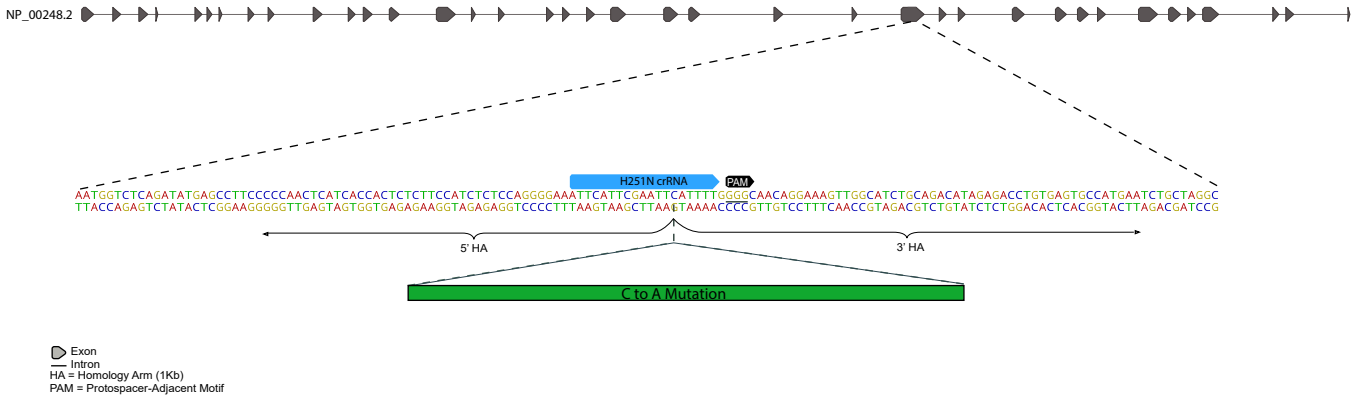

Figure 1: Top: SON locus showing 1 SON isoform; Bottom: Zoom in on mEGFP insertion site at SON N-terminal exon

HDR Editing Design	
crRNA	ATTCATTCGAATTCATTTTG
PAM	GGG
DNA Donor	CCATCTCTCCAGGGGAAATTCATTCGAATTAAT TTTGGGGCAACAGGAAAGTTGGCATC

Post-thaw imaging: One vial of distribution lot was thawed (cells were treated with ROCK inhibitor for 24hrs post-thaw - refer to culture protocol). Cultures were observed daily. Colonies were imaged one and three days post-thaw[1,2] using a Leica microscope.

1 Representative Image of H251N

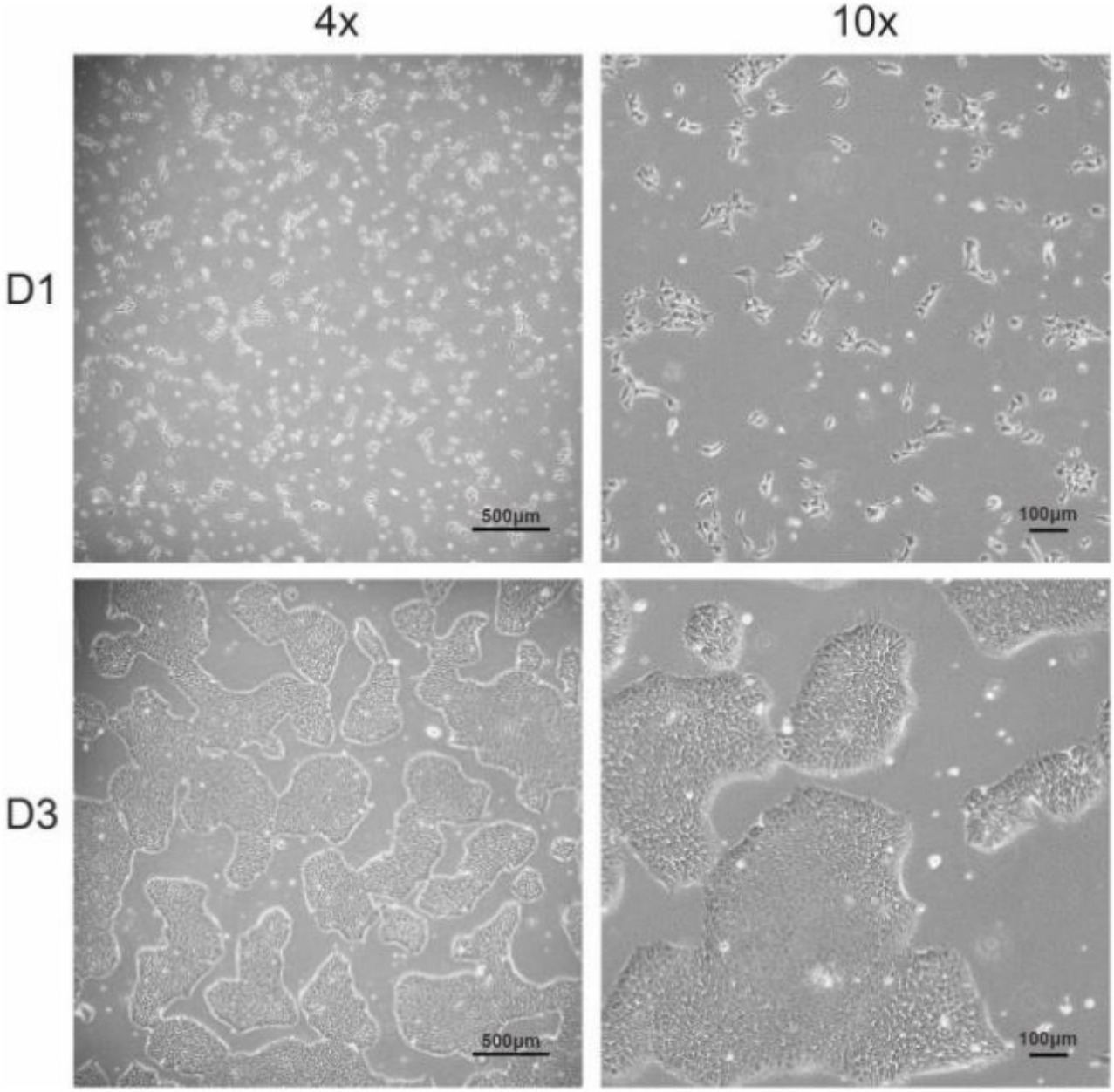

Figure 2: Viability and colony formation one day and three days post-thaw

[1]Cells may take up to 3 passages to recover after thaw
[2]Morphologies observed post-thaw are representative of cell morphologies observed post-passage