

# CERTIFICATE OF ANALYSIS

AICS-0106: MYH7-S291F mEGFP-ACTN2 (mono-allelic tag)

<b>Cell Collection description</b>	S291F mutation introduced into the MYH7 gene. Additionally, mEGFP added into C terminus of ACTN2.
<b>Parental cell line</b>	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).
<b>Relevant publications</b>	Kreitzer et al (2013) Am. J. Stem Cells, 30; 2(2): 119-31 . PMID: 23862100; PMCID: PMC3708511 Roberts et al (2019) Stem Cell Reports, 12(5): 1145-1158. doi: 10.1016/j.stemcr.2019.03.001 Lee et al (2023) bioRxiv (preprint). doi: 10.1101/2023.06.08.54476
<b>Number of passages at Coriell</b>	0
<b>Media</b>	mTeSR1
<b>Feeder or matrix substrate</b>	Matrigel
<b>Passage method</b>	Accutase, single cell
<b>Thaw</b>	500K cells (per vial) in 10 cm plate - ready for passaging in 4-5 days

Test Description	Method	Specification	Results			
<b>Clone Number</b>	N/A	N/A	104	134	40	88
<b>Transfection Replicate (A or B)</b>	N/A	Clones were derived from separate replicated transfections. Comparisons between clones of different genotypes recommended from same replicate.	A	A	A	B
<b>Clone PCR &amp; Sanger</b>	PCR and Sanger sequencing of MYH7 recombinant and wildtype alleles	Determine if predicted mutation occurred with no additional mutations present.	S291F / WT	S291F / WT	WT / WT	WT / WT
<b>Passage of gene edited iPSC reported at submission</b>	N/A	N/A	p47 <sup>a</sup>	p47 <sup>a</sup>	p47 <sup>a</sup>	p47 <sup>a</sup>
<b>Seeding density</b>	N/A	Recommended seeding densities in 10 cm plate every 4 days or every 3 days, consecutively (see culture protocol)	400K / 800K	400K / 800K	400K / 800K	400K / 800K
<b>Post-Thaw Viable Cell Recovery</b>	hiPSC culture on Matrigel	>50% confluency 4-5 days post-thaw (10cm plate)	Pass	Pass	Pass	Pass
<b>Mono-Clonality Confirmation</b>	ddPCR assay	Verification of genomic copy number of WT and mutant alleles	Pass	Pass	Pass	Pass
<b>Trisomy 12 Test</b>	ddPCR assay (Chr12:RPP30)	pass = trisomy 12 not detected in quantitative ddPCR assay.	Pass	Pass	Pass	Pass
<b>Karyotype</b>	G-banding (30 cell analysis)	Normal karyotype, 46 XY	Pass	Pass	Pass	Pass

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<b>Cardiac Differentiation</b>	Modified small molecule differentiation (see <b>cardiac differentiation protocol</b> )	Beating initiated (D7-D14) and Cardiac Troponin T expression (D11- D30) by flow cytometry	Pass	Pass	Pass	Pass
<b>Avg % cTnT+</b>	Flow Cytometry	% cTnT+ cells compared to isotype control	75.4%	73.0%	73.4%	66.1%
<b>Mycoplasma</b>	qPCR (IDEXX)	Negative	Pass	Pass	Pass	Pass
<b>Sterility (bacterial, yeast and fungal testing)</b>	Direct inoculation and incubation for 10 days	No growth after 10 days	Pass	Pass	Pass	Pass
<b>Viral Panel Testing<sup>b</sup></b>	PCR	Negative when assayed for CMV, EBV, HepB, HepC, HIV1, and HPV	Pass			
<b>Identity of Unedited WTC-11 parental line<sup>c</sup></b>	STR	29 allelic polymorphisms across 15 STR loci compared to donor fibroblasts	Identity matched			

<sup>a</sup> This is the number of passages beyond the original parental line (WTC/AICS-0 at passage 33).

<sup>b</sup> 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

<sup>c</sup> 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.

**BLUE = MUTANT CLONES; GREEN = WILDTYPE CLONES**

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**Tagging strategy:** CRISPR-Cas9 methodology was used to introduce a single base pair mutation to MYH7, and mEGFP at C-terminus of ACTN2 as shown below.

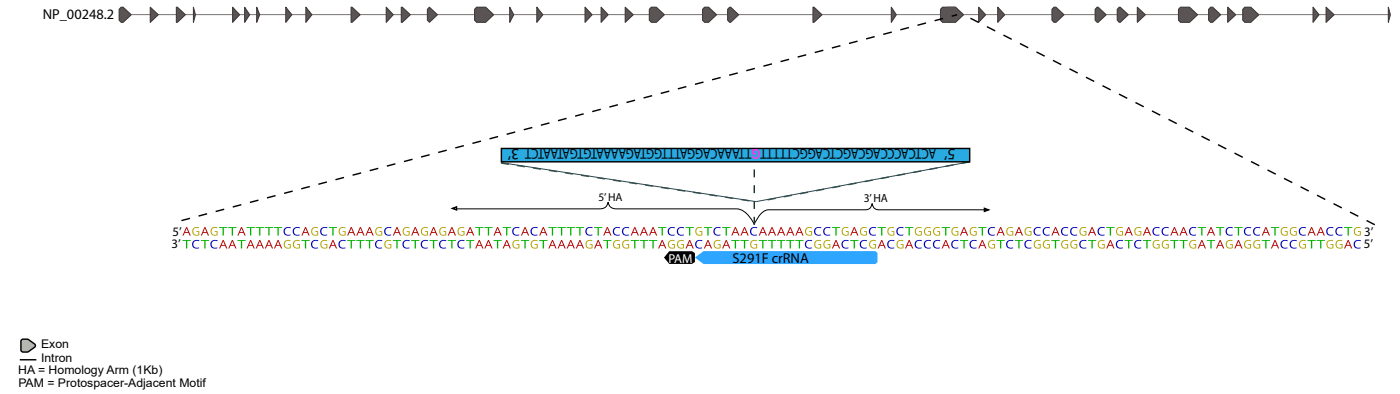


Figure 1: Top: MYH7 locus showing 1 MYH7 isoform; Bottom: Zoom in on mutation site at isoform NM\_000257.4(MYH7):c.872C>T(p.Ser291Phe)

HDR Editing Design for MYH7	
crRNA Target Site	5' GCTCAGGCTTTTTGTTAGACAGG 3'
DNA Donor Sequence	5' ACTCACCAGCAGCTCAGGCTTTTGTAAACAGGATTTGGTAGAAAATGTGATAATCT 3'
F primer for PCR/sequencing	5' GCTAGGTGTCTTTCTCTGGG 3'
R primer for PCR/sequencing	5' GATCAGCAGCATGTCTAGGG 3'

Red = PAM Site; Blue = Mutation

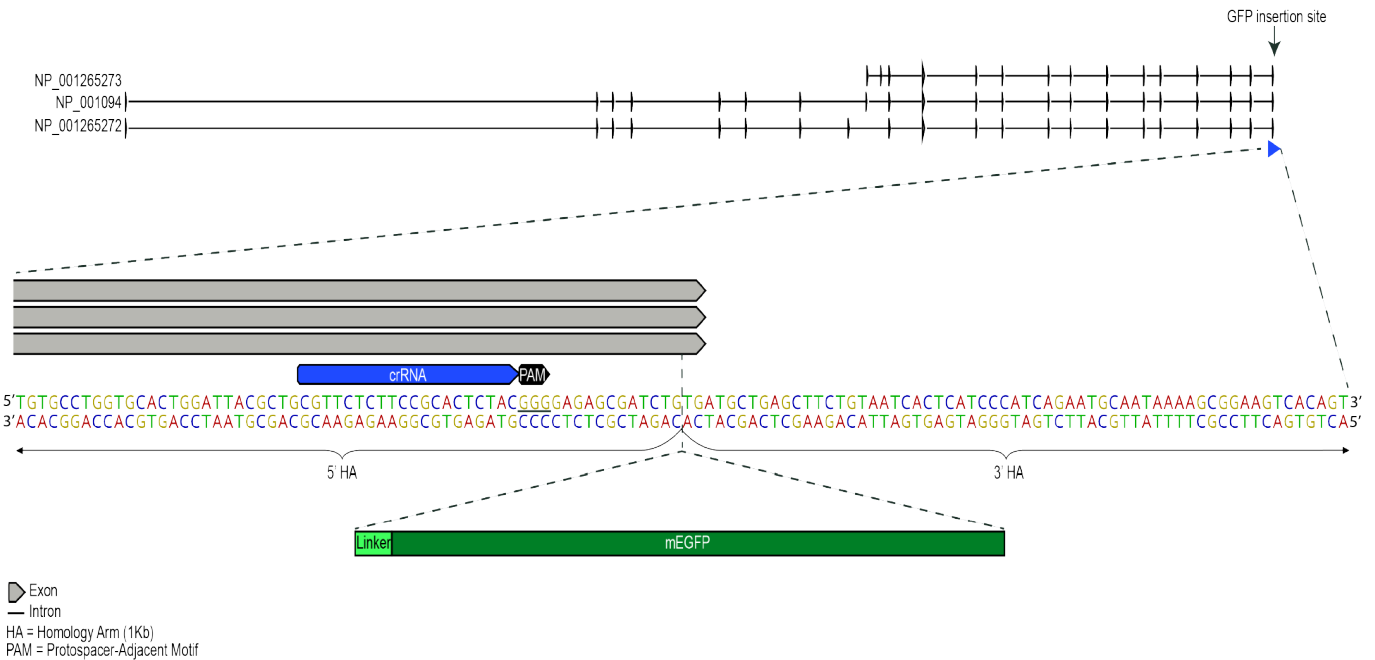


Figure 2: Top: ACTN2 locus showing 3 ACTN2 isoforms; Bottom: Zoom in on mEGFP insertion site at ACTN2 C-terminus.

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**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 four days post-thaw<sup>1,2</sup> using a Leica microscope at 4x and 10x magnification. 1. clone 88 (S291F/wt) is shown here.

## 1 REPRESENTATIVE IMAGE FOR ALL CLONES

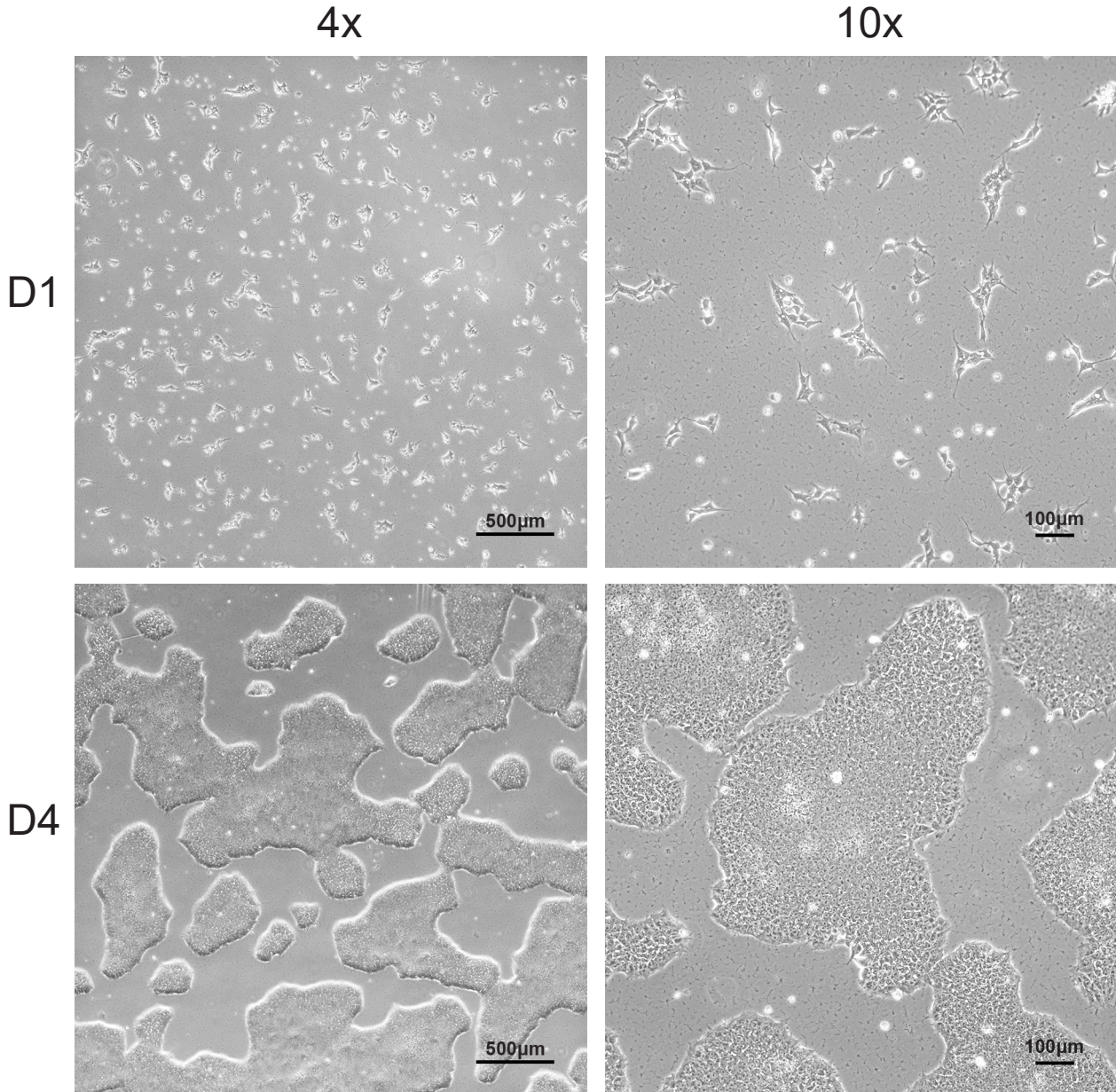


Figure 3: Four panel image of clone 88. Viability and colony formation one day and four days post-thaw. Scale bars are shown.

<sup>1</sup>Cells may take up to 3 passages to recover after thaw

<sup>2</sup>Morphologies observed post-thaw are representative of cell morphologies observed post-passage