CERTIFICATE OF ANALYSIS
AICS-0097: MYH7-G256E mEGFP-ACTN2 (mono-allelic tag)

Cell Collection description	G256E mutation introduced into the MYH7 gene. Additionally, mEGFP added into C terminus of ACTN2.
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 . 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
Number of passages at Coriell	0
Media	mTeSR1
Feeder or matrix substrate	Matrigel
Passage method	Accutase, single cell
Thaw	500K cells (per vial) in 10 cm plate - ready for passaging in 4-5 days

Test Description	**Method**	**Specification**	**Results**				
Clone Number	N/A	N/A	102	141	157	113	174
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	A	B	A	B
Clone PCR & Sanger	PCR and Sanger sequencing of MYH7 recombinant and wildtype alleles	Determine if predicted mutation occurred with no additional mutations present.	G256E / WT	G256E / WT	G256E / WT	WT / WT	WT / WT
Passage of gene edited iPSC reported at submission	N/A	N/A	p50[a]	p50[a]	p50[a]	p50[a]	p50[a]
Seeding density	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	500K / 1M	500K / 1M
Post-Thaw Viable Cell Recovery	hiPSC culture on Matrigel	>50% confluency 4-5 days post-thaw (10cm plate)	Pass	Pass	Pass	Pass	Pass
Mono-Clonality Confirmation	ddPCR assay	Verification of genomic copy number of WT and mutant alleles	Pass	Pass	Pass	Pass	Pass

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Trisomy 12 Test	ddPCR assay (Chr12:RPP30)	pass = trisomy 12 not detected in quantitative ddPCR assay.	Pass	Pass	Pass	Pass	Pass
Karyotype	G-banding (30 cell analysis)	Normal karyotype, 46 XY	Pass	Pass	Pass	Pass	Pass
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	Pass
Avg % cTnT+	Flow Cytometry	% cTnT+ cells compared to isotype control	86%	77%	76%	76%	74%
Mycoplasma	qPCR (IDEXX)	Negative	Pass	Pass	Pass	Pass	Pass
Sterility (bacterial, yeast and fungal testing)	Direct inoculation and incubation for 10 days	No growth after 10 days	Pass	Pass	Pass	Pass	Pass
Viral Panel Testing[b]	PCR	Negative when assayed for CMV, EBV, HepB, HepC, HIV1, and HPV	Pass				
Identity of Unedited WTC-11 parental line[c]	STR	29 allelic polymorphisms across 15 STR loci compared to donor fibroblasts	Identity matched				

[a] This is the number of passages beyond the original parental line (WTC/AICS-0 at passage 33).
[b] 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
[c] 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.

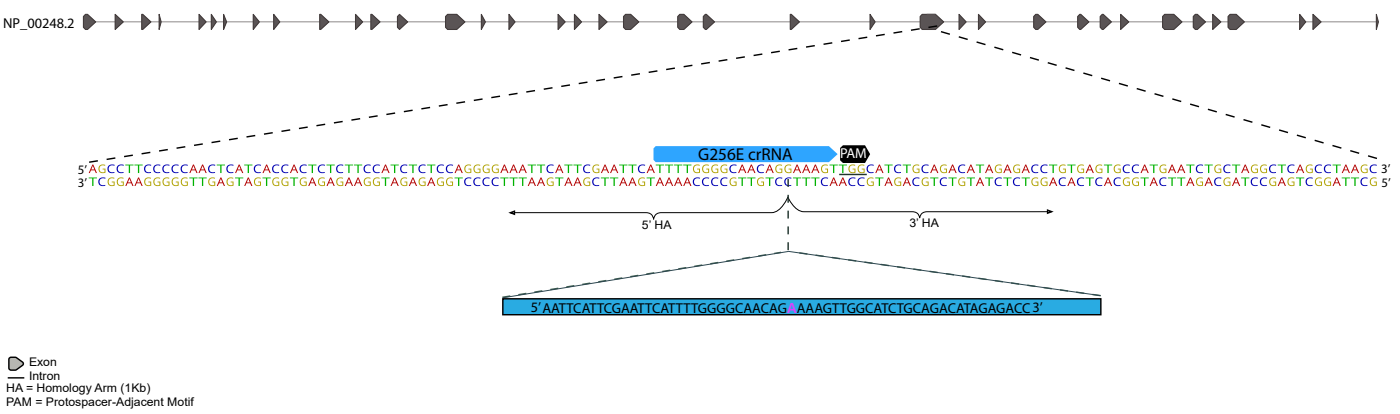

Figure 1: Top: MYH7 locus showing 1 MYH7 isoform; Bottom: Zoom in on mutation site at isoform NM_000257.4(MYH7):c.767G>A(p.Gly256Glu)

HDR Editing Design for MYH7	
crRNA Target Site	5' TTTTGGGGCAACAGGAAAGTTGG 3'
DNA Donor Sequence	5' AATTCATTCGAATTCATTTTGGGGCAACAG**A**AAAGTTGGCATCT GCAGACATAGAGACC 3'
F primer for PCR/sequencing	5' CCCAACTCATCACCACTCTC 3'
R primer for PCR/sequencing	5' GGAGAGAGAGAGAGGTCAAG 3'

Red = PAM Site; Blue = Mutation

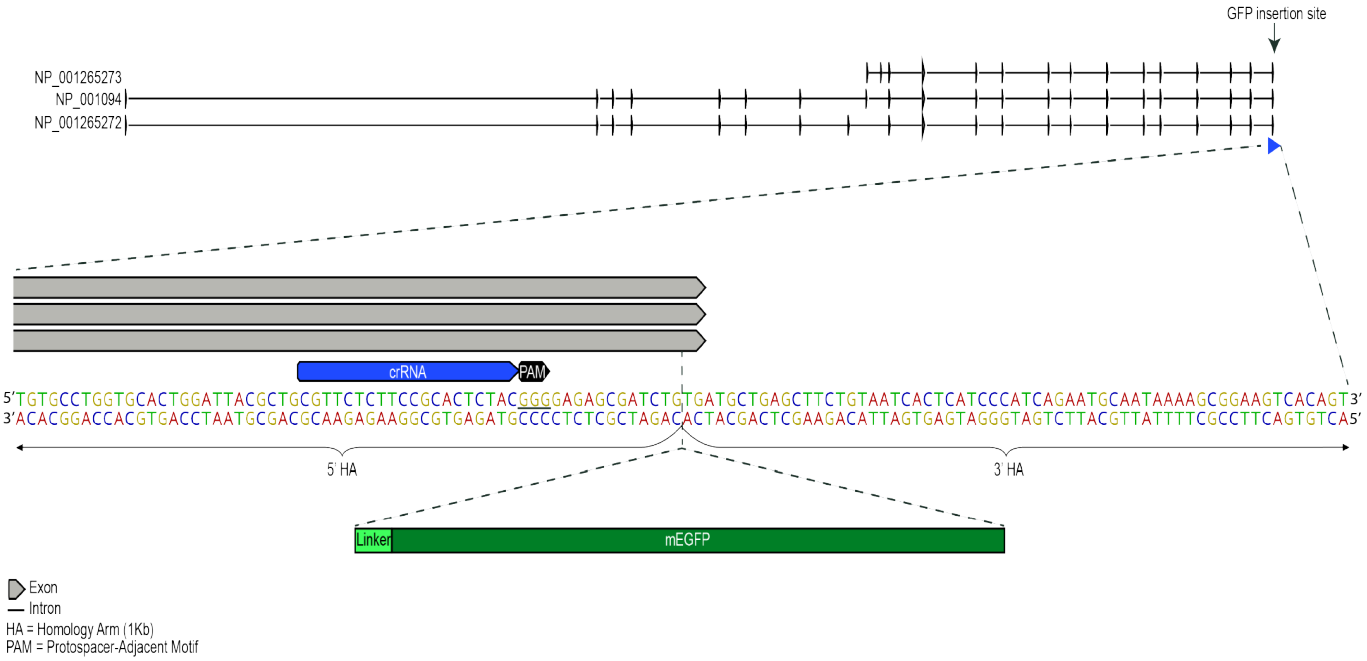

Figure 2: Top: ACTN2 locus showing 3 ACTN2 isoforms; Bottom: Zoom in on mEGFP insertion site at ACTN2 C-terminus

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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[1,2] using a Leica microscope 4x and 10x magnification. 1. clone 141 (G256E/wt) is shown here.

1 REPRESENTATIVE IMAGE FOR ALL CLONES

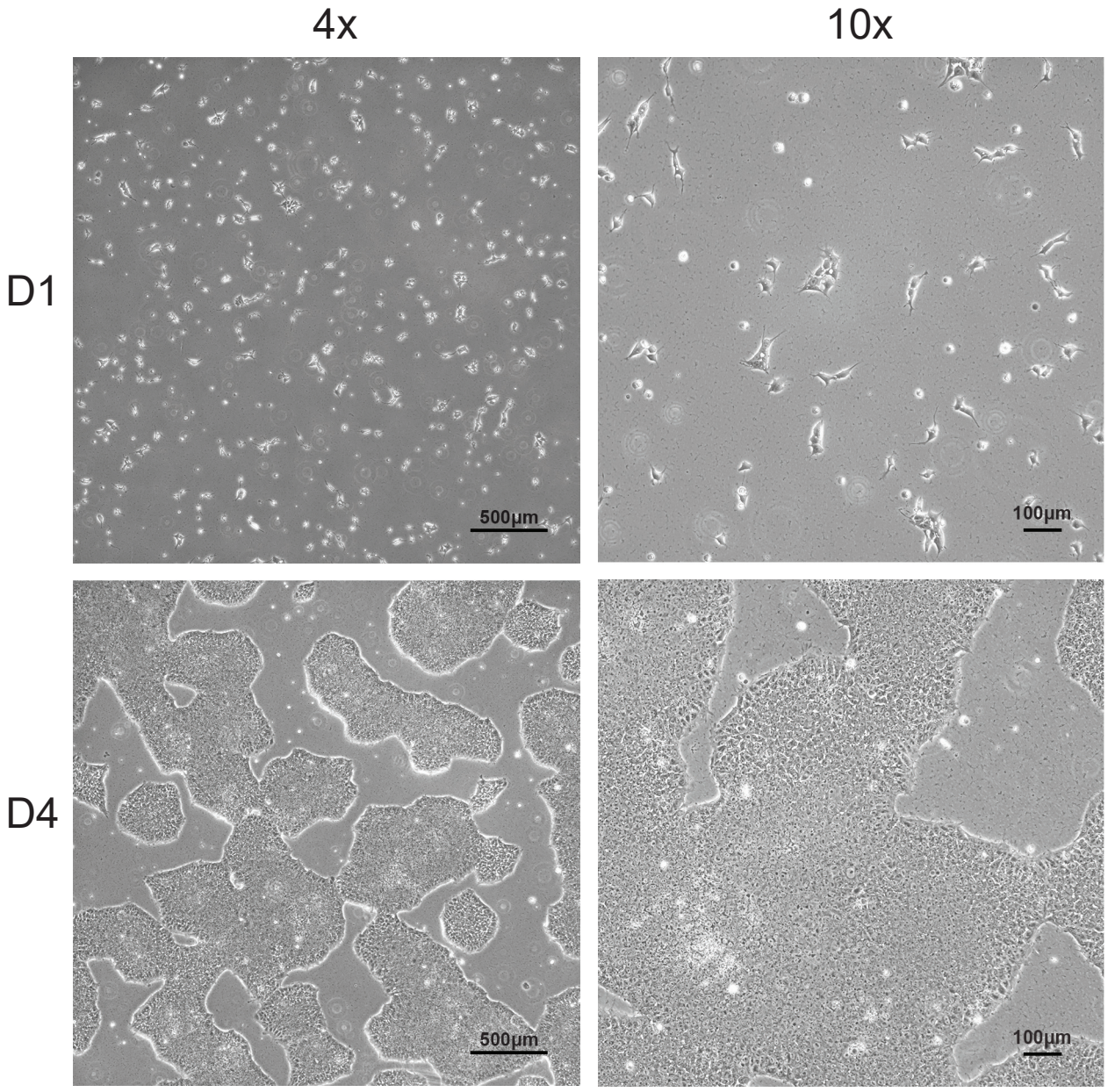

Figure 3: Four panel image of clone 141. Viability and colony formation one day and four days post-thaw. Scale bars are shown.

[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

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LIVE IMAGING OF CARDIOMYOCYTES

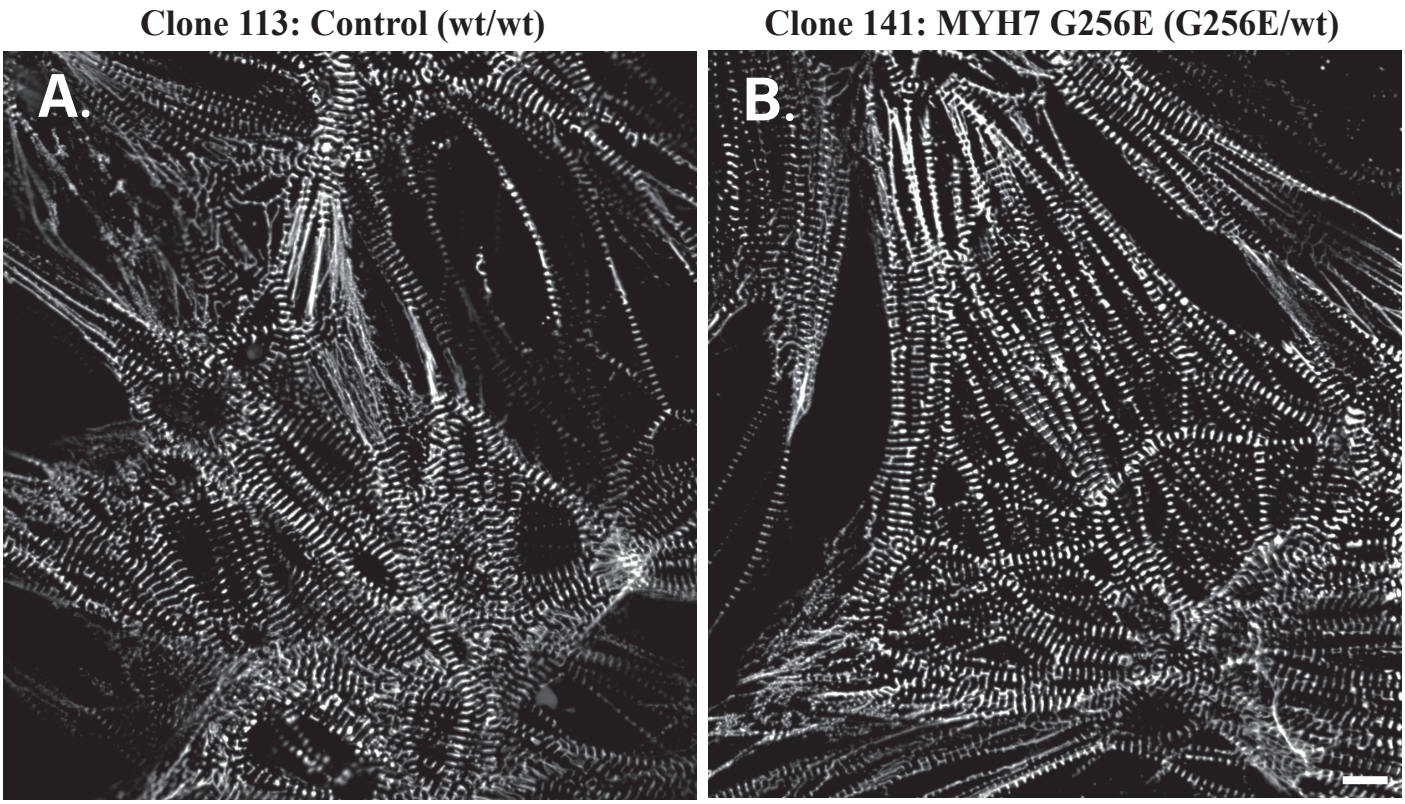

Figure 4: Live-cell imaging of cardiomyocytes with the G256E point mutation in the MYH7 locus introduced into the AICS-0075 cell line (which expresses mEGFP-tagged alpha-actinin-2). Twelve days after the onset of differentiation, cells were plated on PEI and laminin coated glass and imaged in 3D on a spinning disk confocal microscope 18 days later (30 days total after the onset of differentiation). A. clone 113 (wt/wt) and B. clone 141 (G256E/wt). Images are maximum intensity projections of 3 Z slices. Scale bar 10μm.

Image system details: 3i (Denver, CO) spinning disk microscope with a Zeiss (Thornwood, NY) 63x/1.2 NA alpha-plan APOCHROMAT water objective, a CSU-W1 Yokogawa (Sugar Land, TX) spinning disk head, and Hammamatsu (Hammamatsu City, Japan) Orca Flash 4.0 camera.