CERTIFICATE OF ANALYSIS
AICS-0075-085:WTC-mEGFP-ACTN2-cl85 (mono-allelic tag)

Product description	Human iPSC clonal line in which ACTN2 has been endogenously tagged with mEGFP using CRISPR/Cas9 technology
Parental cell line	Parental hiPSC line (WTC/AICS-0 at passage 33) derived from fibroblasts reprogrammed using episomal vectors (OCT3/4, shp53, SOX2, KLF4, LMYC, and LIN28). Coriell catalog: GM25256
Publication(s) describing iPSC establishment	Kreitzer et al (2013) Am. J. Stem Cells, 30; 2(2): 119-31
Passage of gene edited iPSC reported at submission	p35[a]
Number of passages at Coriell	0
Media	mTeSR1
Feeder or matrix substrate	Matrigel
Passage method	Accutase
Thaw	500K cells (ea vial) in 10 cm plate - ready for passaging in 4 days
Seeding density	Recommended cell plating of 650K cells/10-cm plate for passage every 4 days, and 1.2M cells/10-cm plate for passage every 3 days for up to 3 passages post-thaw. Reduced seeding densities (500K for 4 days and 1M for 3 days) are recommended once cells recover from thaw (see Figure 2).

Test Description[b]	Method	Specification	Result
Post-Thaw Viable Cell Recovery	hiPSC culture on Matrigel	$>$ 50% confluency 3-4 days post-thaw (10 cm plate)	Pass; crater-like morphology is observed in several recovery passages, but improves with 4 day seeding and continued passaging (see Figure 2)
mEGFP insertion at genomic locus - precise editing	PCR and Sanger sequencing of recombinant and wildtype alleles	C-term insertion of mEGFP in frame with exact predicted recombinant allele junctions. No additional mutations.	Pass
Copy number	ddPCR[c] assay for FP(s) and RPP30 reference gene[d]	FP/RPP30: $\sim$ 0.5 = Mono-allelic $\sim$ 1.0 = Bi-allelic	Mono-allelic (0.48)
Plasmid integration	ddPCR assay to detect plasmid integration into the genome	AmpR/RPP30: $<$ 0.1 = no plasmid integration	Pass (0.00)
Mutational analysis	Whole exome sequencing[f]	Check for acquired mutations (not detected in p8[a] parental line) that: 1) Correspond to off-target sites predicted by Cas-OFFinder[e] 2) Affect genes in Cosmic Cancer Gene Census	Sequencing planned
mEGFP localization	Spinning Disk confocal live cell imaging	Localization to sarcomeres in hiPSC-derived cardiomyocytes	Localizes to striations in myofibrils, consistent with localization to the Z-disk and exclusion from the thin filaments (actin-based, anchored at the Z-disk), thick filaments (myosin-based), and M-line.
Expression of tagged protein	Western blot	Expression of expected size product	Not performed

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Growth rate	ATP quantitation[g]	Comparable to parental line	Pass (measured at p33)[a]
Expression of stem cell markers	Flow cytometry	Transcription factors: OCT4/SOX2/NANOG $\geq$ 85% Surface markers: SSEA3, TRA-1-60 $\geq$ 85%; SSEA1 $\leq$ 15%	Pass
Germ layer differentiation	Trilineage differentiation[h] as assayed by ddPCR gene expression analysis	Expression of endoderm (SOX17), mesoderm (Brachyury), and ectoderm (PAX6) markers upon directed differentiation to all three germ layers	Analysis in progress
Cardiomyocyte differentiation	Modified small molecule differentiation (Lian et al. 2012)[i]	Beating initiated (D7-D14) and Cardiac Troponin T expression (D11-D30) by flow cytometry	Pass
Karyotype	G-banding (30 cell analysis)	Normal karyotype, 46 XY	Pass
Mycoplasma	qPCR (IDEXX)	Negative	Pass
Sterility (bacterial, yeast and fungal testing)	Direct inoculation and incubation for 10 days	No growth after 10 days	Pass
Viral Panel Testing[j]	PCR	Negative when assayed for CMV, EBV, HepB, HepC, HIV1, and HPV	Pass
Identity of unedited parental line[k]	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] All QC assays are performed on stem cells except when noted otherwise.
[c] Droplet digital PCR using Bio-Rad QX200
[d] RPP30 is a reference 2 copy gene used for normalization.
[e] Bae et al (2014) Bioinformatics. 30(10): 1473-1475
[f] Nextera rapid capture exome
[g] Promega CellTiter-Glo Luminescent Cell Viability Assay (Catalog #G7571)
[h] STEMCELL Technologies STEMdiff Trilineage Differentiation Kit (Catalog #05230)
[i] Lian et al (2012) PNAS. 109(27):E1848-E1857
[j] 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.
[k] 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 C-terminus of ACTN2 as shown below.

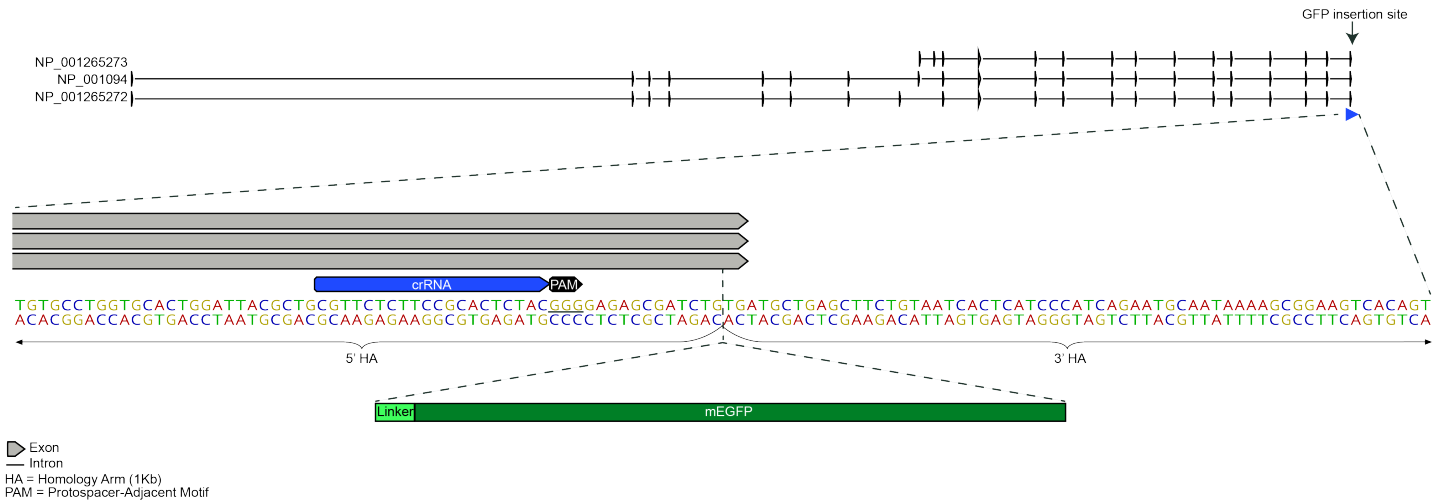

Figure 1: Top: ACTN2 locus showing 3 ACTN2 isoforms; Bottom: Zoom in on mEGFP insertion site at ACTN2 C-terminus; More information on tagging transcriptionally silent genes in hiPSCs will be available soon (Roberts et al. (2019) Stem Cell Reports. In Press (4/4/2019))

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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 using a Leica microscope at 4x and 10x magnification (a-d). Cells were then passaged and seeded into plates for three day and four day growth and imaged at 4x magnification (e, f). This cell line may exhibit transient poor morphology and lower than expected confluence in the first few passages post-thaw (e, f). This suboptimal phenotype resolves with continued passaging.

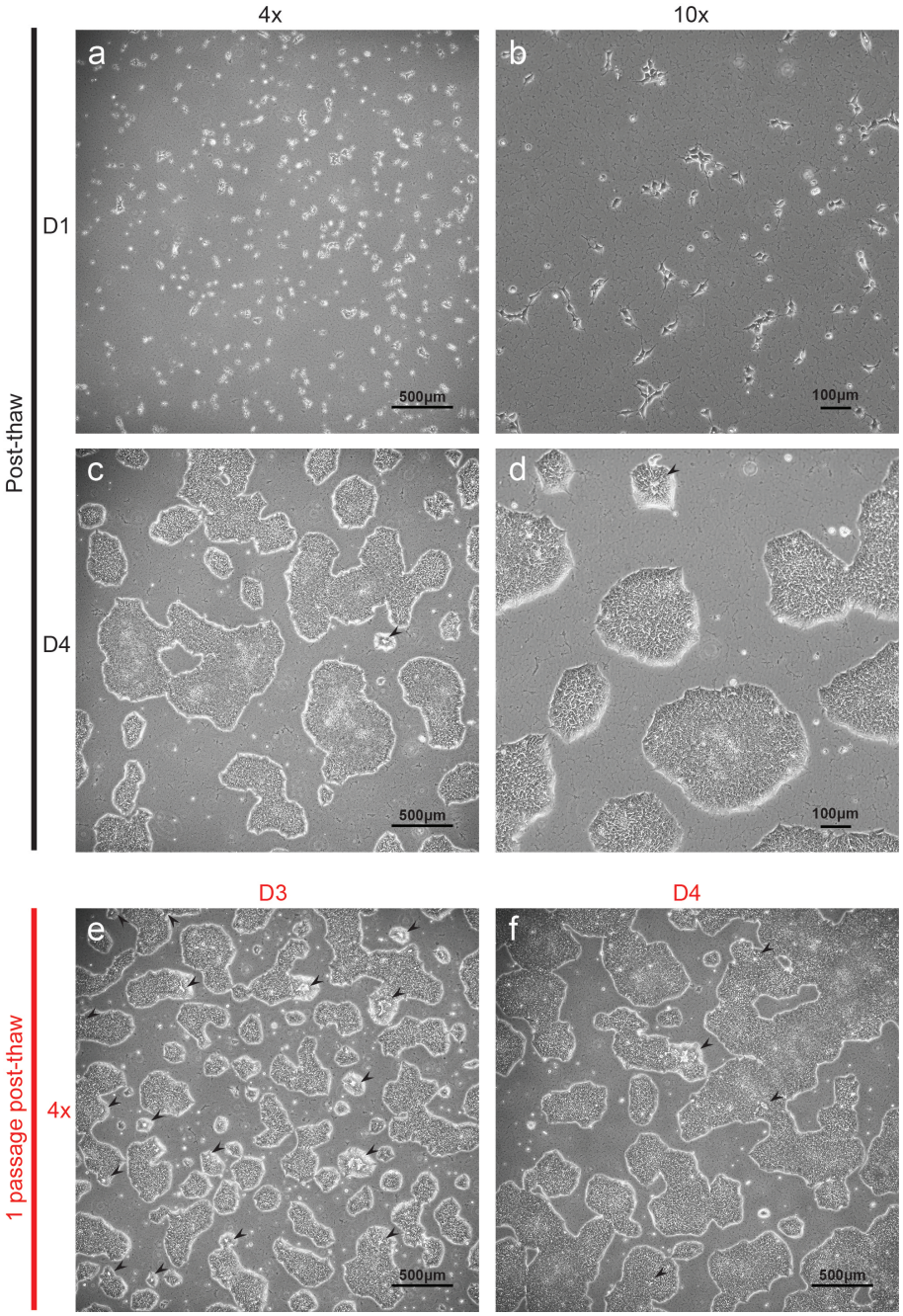

Figure 2: Viability and colony formation post-thaw. a-d: Crater-like morphology is rare post-thaw (∼1% of population). e-f: Morphology worsens the first few passages post-thaw when seeding at 1.2M/10cm dish for three days and 650K cells/10cm dish for four days. e: Craters can be seen in >10% of colonies, and optimal confluence is not reached in three days of growth. f: Lower density seeding for four days of growth improves morphology (fewer craters, better packing) and confluence. Colony morphology and craters resolve with continued passaging and become comparable to those observed post-thaw as shown in c and d. Scale bars are as shown.

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Imaging labeled structures in endogenously tagged hiPSC derived cardiomyocytes: The tagged proteins are expressed endogenously and therefore may not appear as bright as they would in an overexpressed system. For imaging, we plate cells onto high-quality glass bottom 24-well plates (Cellvis) coated with 0.1% w/vol polyethylenimine (PEI) and 25μg/ml laminin. Cells are imaged in phenol red-free RPMI 1640 media (Gibco) supplemented with B-27 containing insulin (Gibco). Our most common microscope configuration is a Zeiss spinning disk fluorescence microscope with a Yokogawa CSUX1 head, Hamamatsu CMOS camera, and a 488 laser (mEGFP). Cells are imaged with a 40x 1.2 NA water immersion objective at 37°C and 5% CO_2 in a temperature-controlled chamber. The approximate laser power measured at the sample for our standard 100x images is ~2.5 mW.

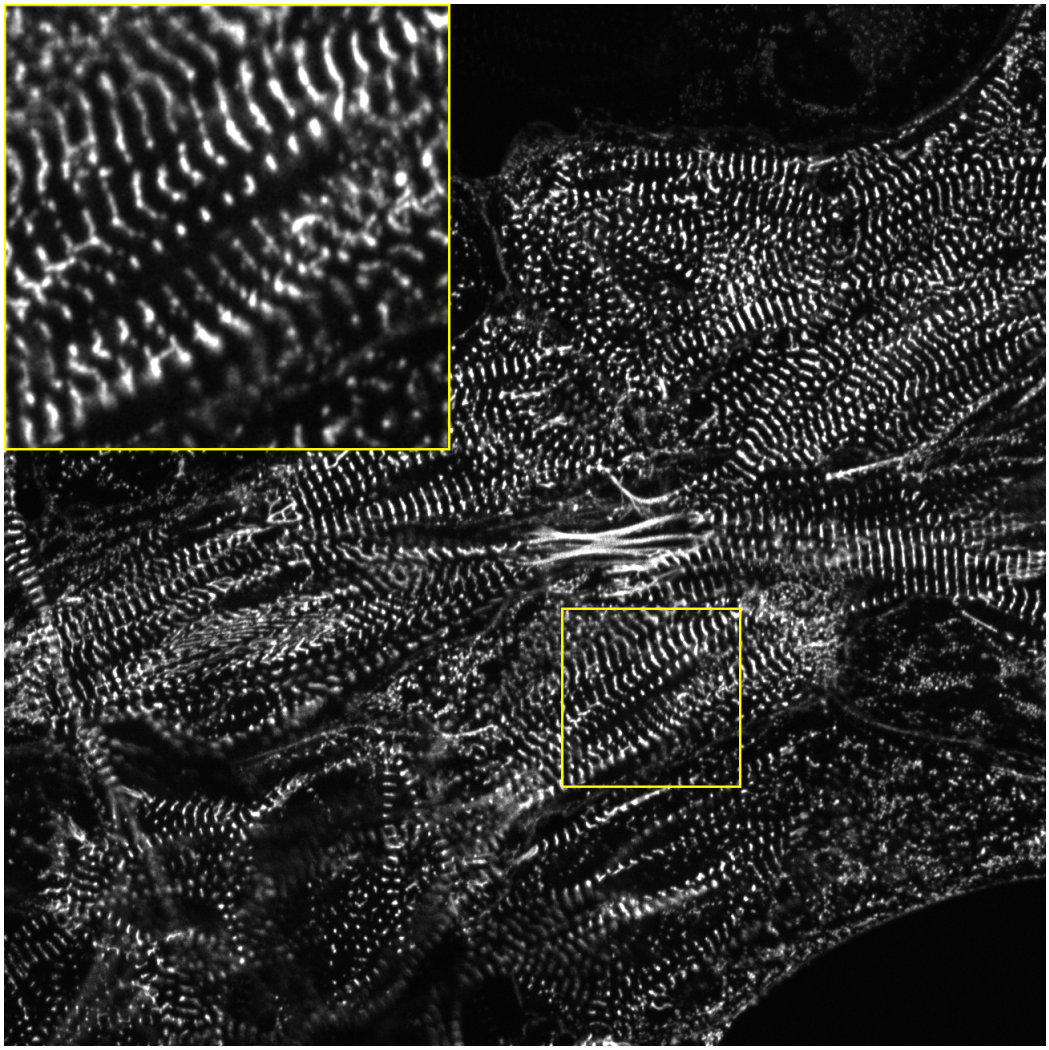

Figure 3: Single, mid-level plane of live hiPSC-derived cardiomyocytes expressing mEGFP-tagged alpha-actinin-2 protein. Inset shows 2.5x enlargement of boxed region to show myofilament detail. Twelve days after the onset of differentiation, cells were plated on PEI and laminin coated glass and imaged in 3D on a spinning-disk confocal microscope 7 days later (19 days total after the onset of differentiation). Scale bar, 20 μm.