CERTIFICATE OF ANALYSIS
AICS-0036-028:WTC-mEGFP-Safe harbor locus (AAVS1)-cl28 (mono-allelic tag)

Product description	Human IPSC clonal line in which mEGFP under the control of a CAGGS promoter has been inserted at the safe harbor locus (AAVS1) 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	p28[a]
Number of passages at Coriell	0
Media	mTeSR1
Feeder or matrix substrate	Matrigel
Passage method	Accutase
Thaw	1 million cells (ea vial) in 10 cm plate - ready for passaging in 3-4 days
Seeding density	450K cells/10-cm plate every 4 days or 900K cells/10-cm plate every 3 days (see culture protocol)

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
mEGFP insertion at genomic locus - precise editing	PCR and Sanger sequencing of recombinant and wildtype alleles	Internal insertion; No additional mutations.	Not sequenced
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 (0.003)
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	Exome not sequenced; RNA-Seq shows presence of PPM1D G1426T/E476X
mEGFP localization	Spinning Disk confocal live cell imaging	Localization to cell cytoplasm	Localizes to the cytoplasm and nucleus. Spaces between cells appear dark. Weaker intensity is observed in the nucleolus within the nucleus. Some variation in intensity visible between cells.
Expression of tagged protein	Western blot	Expression of expected size product	Expected size band for mEGFP

Growth rate	ATP quantitation[g]	Comparable to parental line	Pass (measured at p27)[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	Palpant et al. (2015)[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] Palpant et al (2015) Development. 142(18): 3198-3209

[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 safe harbor locus (AAVS1) of PPP1R12C gene as shown below.

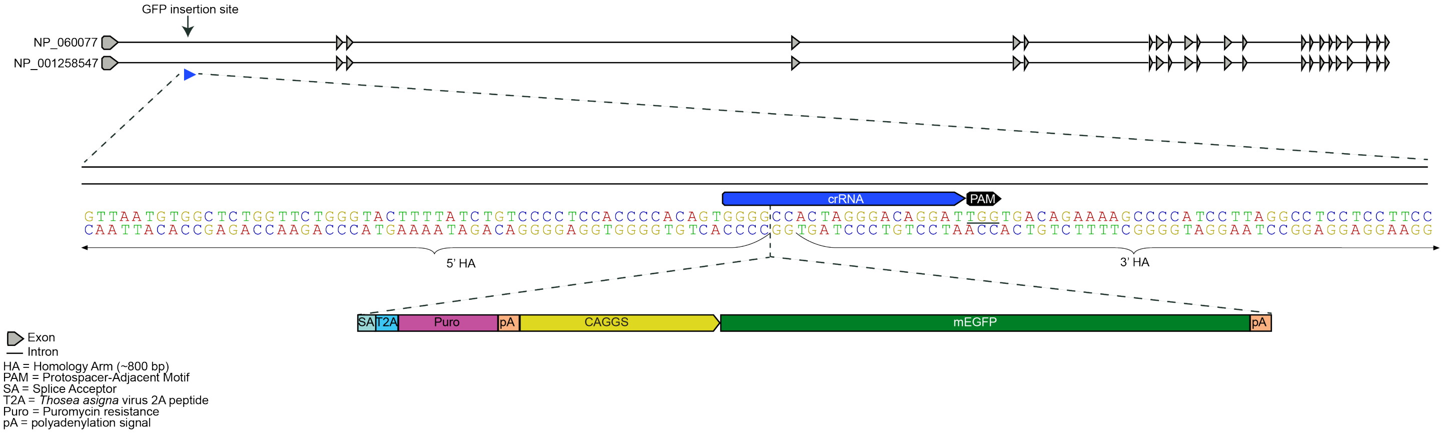

Figure 1: Top: mEGFP insertion site at safe harbor locus (AAVS1) in PPP1R12C intron; Bottom: Zoom in on mEGFP insertion site at safe harbor locus (AAVS1)

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 photographed one and three days post-thaw[1,2] using a Nikon microscope at 4x and 10x magnification.

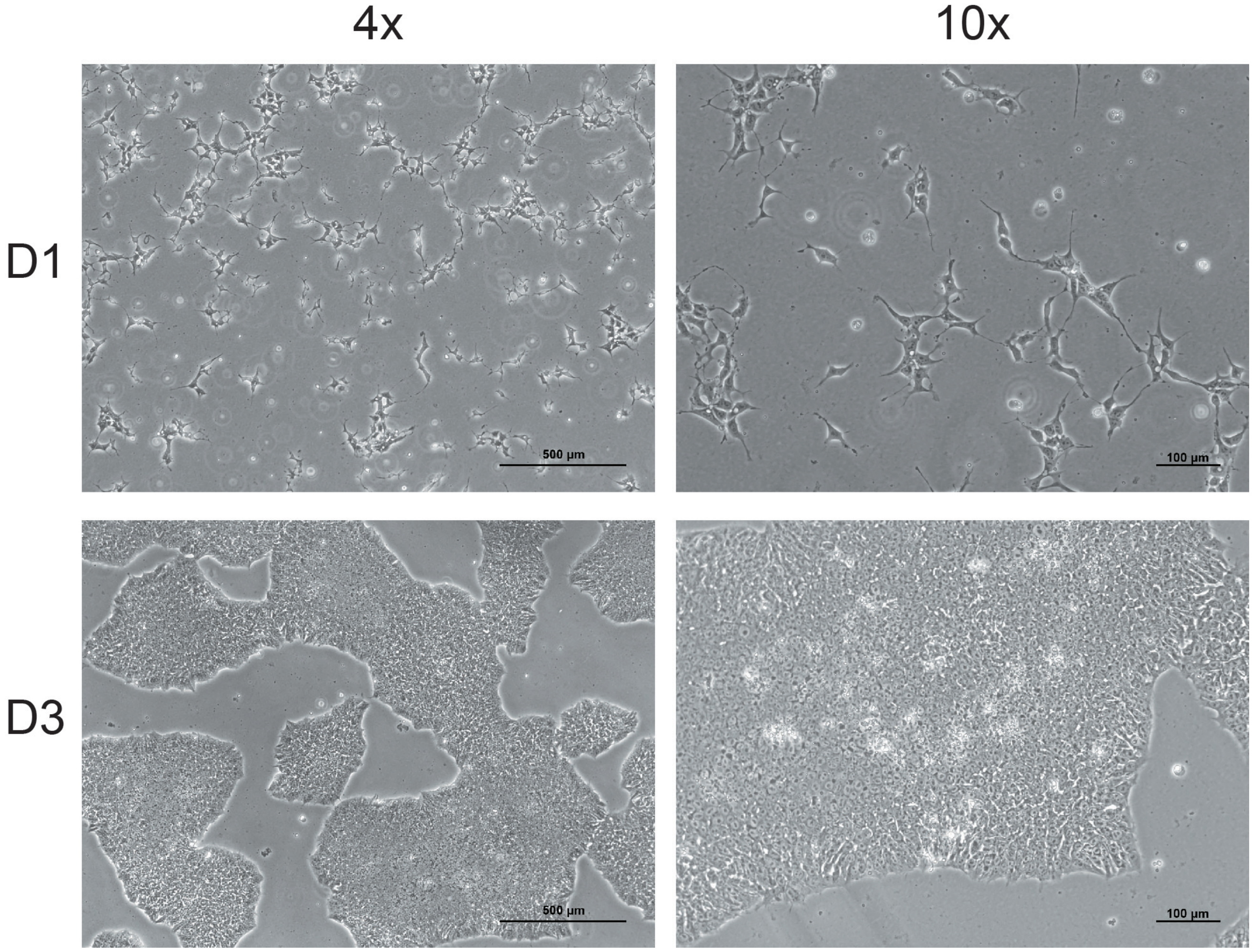

Figure 2: Viability and colony formation one day and three days post-thaw

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[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

Imaging labeled structures in endogenously tagged cells: 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 matrigel-coated high-quality glass bottom coverslips (Cellvis) and image cells in phenol red-free mTeSR media (STEMCELL Technologies). 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 either with a 20x 0.8NA objective for lower magnification or 100x 1.25NA water immersion objective for higher magnification, 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.

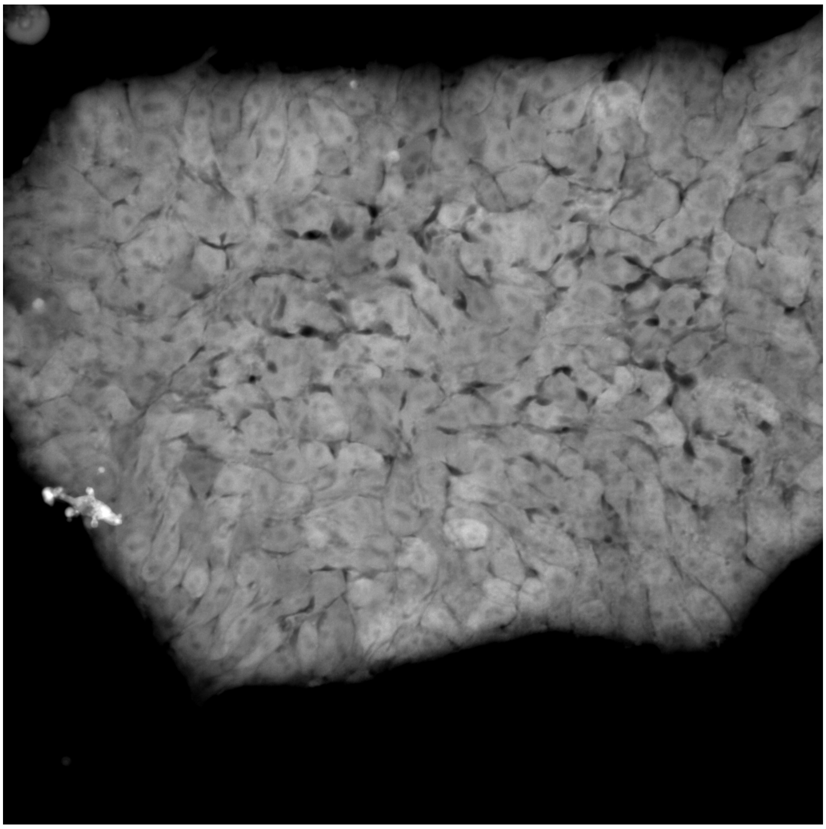

Figure 3: Localization of mEGFP expressed from a safe harbor locus in hiPSC colony. The bright spots are dead cells on top of the colony. Some variation in intensity levels is seen between cells. Image is a maximum intensity projection of a 3D spinning-disk confocal z-stack of a live hiPSC colony.