CERTIFICATE OF ANALYSIS
AICS-0088-083:WTC-mEGFP-PCNA-cl83 (mono-allelic tag)

Product description	Human iPSC clonal line in which PCNA 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	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	375K cells/10-cm plate every 4 days or 800K 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(s) at genomic locus - precise editing	PCR and Sanger sequencing of recombinant and wildtype alleles	N-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 (0.5)
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 of mEGFP to DNA replication foci	The localization of mEGFP-tagged proliferating cell nuclear antigen (PCNA) depends on the cell cycle, including diffuse distribution in the cytoplasm and/or nucleoplasm (G1 and G2) and localization to foci that vary in size and distribution during S-phase, consistent with cell cycle-dependent reorganization of DNA replication foci. During mitosis, mEGFP-tagged PCNA is diffusely cytoplasmic.

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Genome-wide analysis of replication timing[g]	Repli-seq[h]	No significant difference from the parental cell line in the genome-wide replication timing profile by visual inspection and correlation analysis	Pass, no difference from parental line
Expression of tagged protein	Western blot	Expression of expected size product	Expected size band for untagged and mEGFP-tagged proliferating cell nuclear antigen (PCNA). Semi-quantitative results show that 3% of PCNA-encoded protein product is mEGFP labeled.
Growth rate	ATP quantitation[i]	Comparable to parental line	Pass (measured at p26)[a]
Expression of stem cell markers	Flow cytometry	Transcription factors: OCT4/SOX2/NANOG $\geq$ 85% Surface markers: SSEA4, TRA-1-60 $\geq$ 85%; SSEA1 $\leq$ 15%	Pass
Germ layer differentiation	Trilineage differentiation[j] 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	Pass
Cardiomyocyte differentiation	Modified small molecule differentiation (Lian et al. 2012)[k]	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[l]	PCR	Negative when assayed for CMV, EBV, HepB, HepC, HIV1, and HPV	Pass
Identity of unedited parental line[m]	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] We thank David Gilbert and Takayo Sasaki (Florida State University) for the genome-wide analysis of replication timing
[h] Marchal et al (2018) Nature Methods. 13:819-839
[i] Promega CellTiter-Glo Luminescent Cell Viability Assay (Catalog #G7571)
[j] STEMCELL Technologies STEMdiff Trilineage Differentiation Kit (Catalog #05230)
[k] Lian et al (2012) PNAS. 109(27):E1848-E1857
[l] 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.
[m] 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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Important information	**Total proliferating cell nuclear antigen (PCNA) levels are low in this line.** Our semi-quantitative analysis indicates that AICS-0088 clone 83 has 34% of the total levels of PCNA measured in unedited WTC-11 control cells. Our time-lapse imaging and cell growth assays indicate that these cells grow and divide as expected. An additional functional analysis to assess genome-wide replication timing also showed no significant differences in replication timing between AICS-0088 clone 83 and unedited WTC-11 control cells. However, if you plan to use this line for an assay that you believe may be sensitive to low total protein levels of PCNA, you may wish to perform additional functional assays.

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

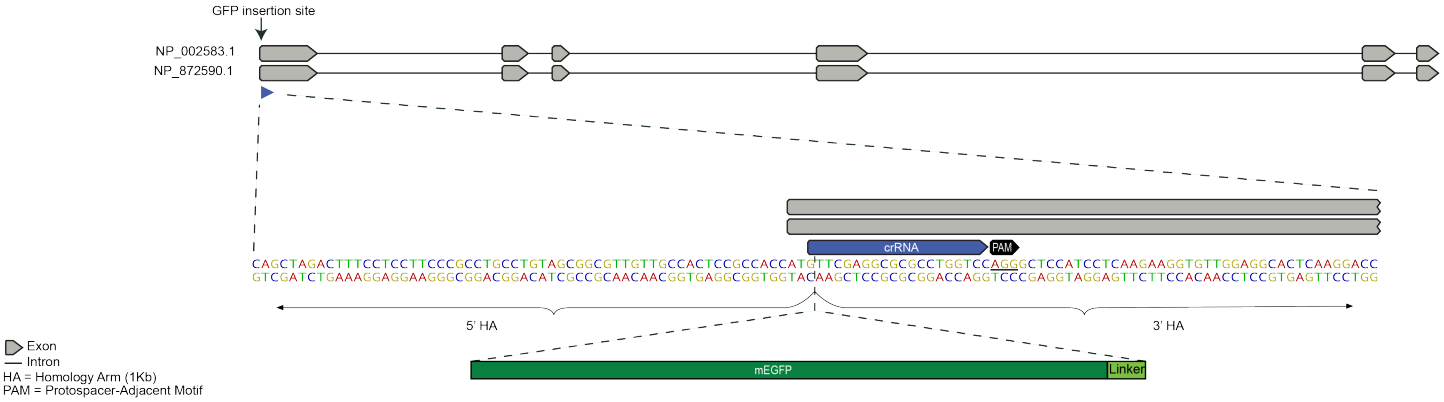

Figure 1: Top: PCNA locus showing 2 PCNA isoforms; Bottom: Zoom in on mEGFP insertion site at PCNA N-terminal exon

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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 three days post-thaw[1,2] using a Leica microscope.

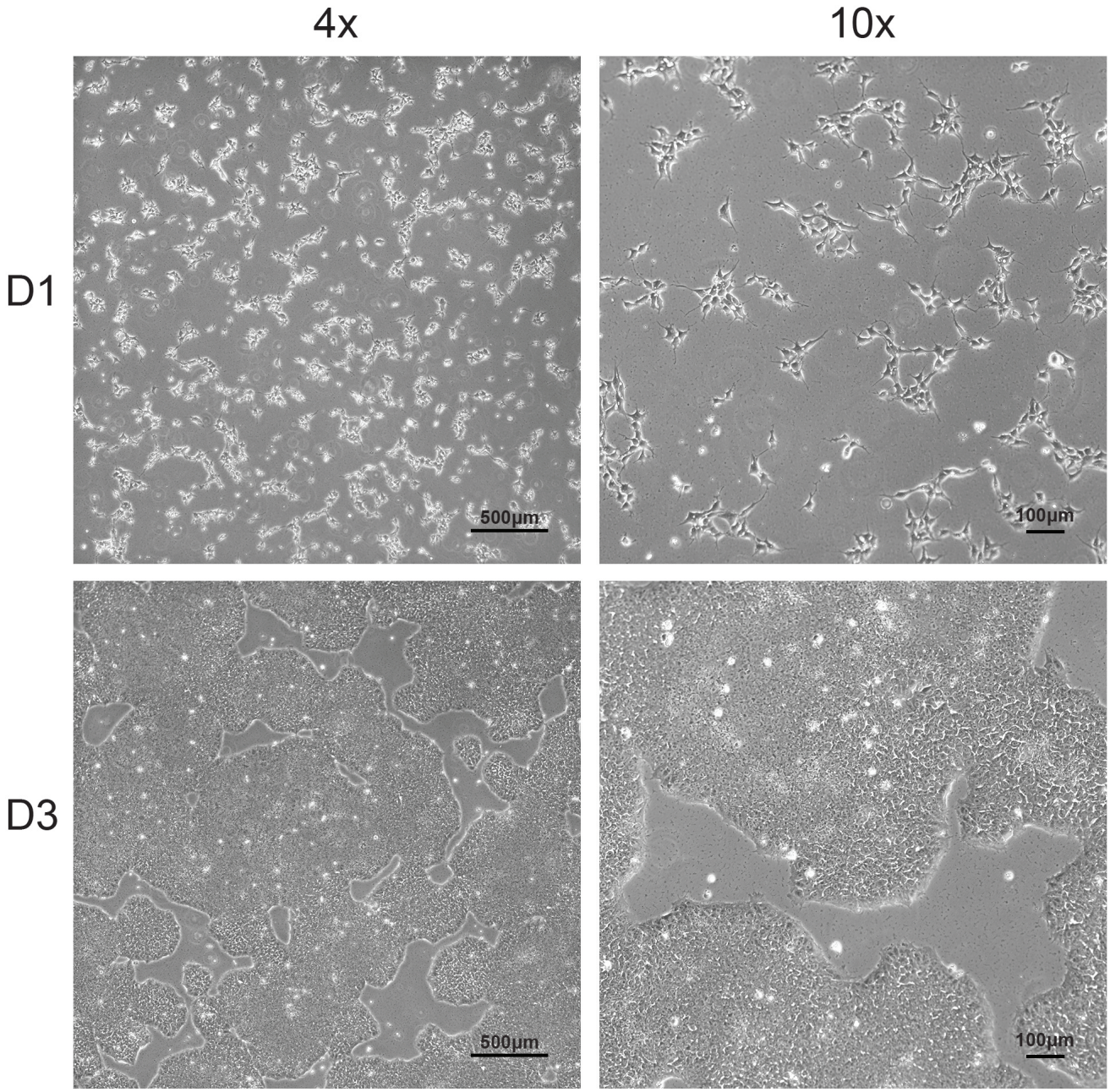

Figure 2: Viability and colony formation one day and three days post-thaw.

[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

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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.

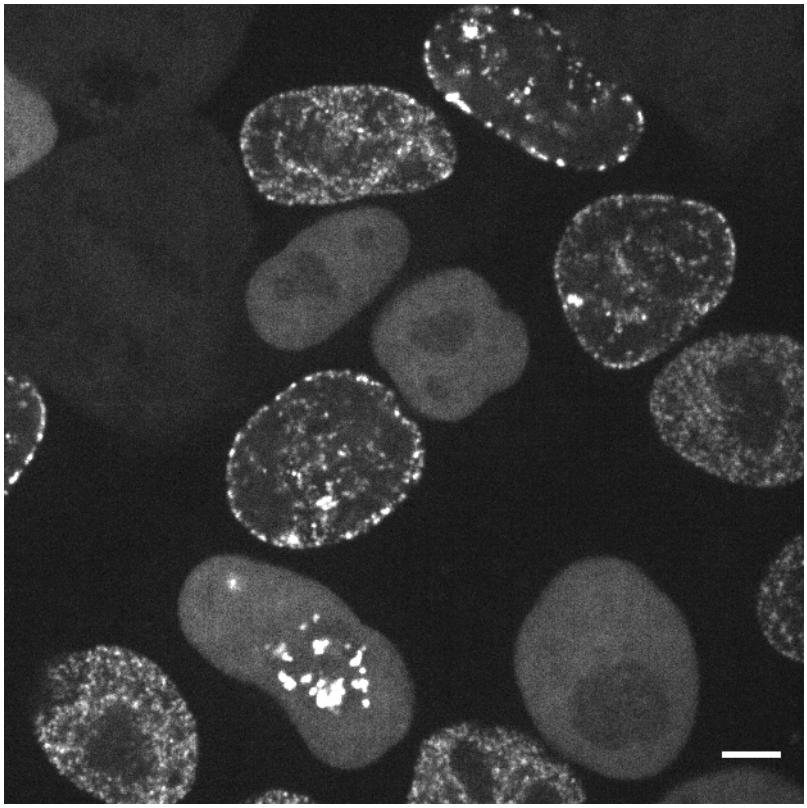

Figure 3: Single, mid-level plane of cells in a live hiPS cell colony expressing mEGFP-tagged proliferating cell nuclear antigen (PCNA). Cells were imaged in 3D on a spinning-disk confocal microscope. Scale bar, 5 μm.