CERTIFICATE OF ANALYSIS
AICS-0032-019:WTC-mTagRFPT-CETN2-cl19 (mono-allelic tag)

Product description	Human iPSC clonal line in which CETN2 has been endogenously tagged with mTagRFP-T 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	p22[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	400-800K cells/10-cm plate; every 3-4 days (see culture protocol)

Test Description	Method	Specification	Result
Post-Thaw Viable Cell Recovery	hiPSC culture on Matrigel	> 50% confluency 3-4 days post-thaw (10 cm plate)	Pass
mTagRFP-T insertion at genomic locus - precise editing	PCR and Sanger sequencing of recombinant and wildtype alleles	N-term insertion of mTagRFP-T in frame with exact predicted recombinant allele junctions. No additional mutations in either allele.	Pass
Copy number	ddPCR[b] assay for mTagRFP-T and RPP30 reference gene[c]	mTagRFP-T/RPP30: ~ 0.5 = Mono-allelic ~ 1.0 = Bi-allelic	Mono-allelic (0.56); CETN2 is on chrX
Plasmid integration	ddPCR assay to detect plasmid integration into the genome	AmpR/RPP30: < 0.1 = no plasmid integration	Pass (0.002)
Off-target mutations	1) PCR and Sanger sequencing of 5-10 sites predicted by Cas-OFFinder[d] 2) Whole exome sequencing[e]	No mutations at off-target sites assayed	1) Pass 2) Sequencing in progress
Other mutations	Whole exome sequencing[e]	Check for acquired mutations (not detected in p8[a] parental line) that affect genes in Cosmic Cancer Gene Census	Sequencing in progress
mTagRFP-T localization	Spinning Disk confocal live cell imaging	Localization to centrioles	Localizes to small puncta, often in pairs at the apical tip of cells. These puncta replicate and reorganize during cell division consistent with the centriole duplication cycle.

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Expression of tagged protein	Western blot	Expression of expected size product	Undetectable by Western blot due to low expression levels. Will require immunoprecipitation to assess further.
Growth rate	ATP quantitation[f]	Comparable to parental line	Pass
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 differentation[g]	Expression of endoderm (SOX17), mesoderm (Brachyury), and ectoderm (PAX6) markers upon directed differentiation to all three germ layers	Pass
Cardiomyocyte differentiation	Palpant et al. (2015)[h]	Beating initiated (D7-D14) and Troponin T expression (D20-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[i]	PCR	Negative when assayed for CMV, EBV, HepB, HepC, HIV1, and HPV	Pass
Identity of unedited parental line[j]	STR	29 allelic polymorphisms across 15 STR loci compared to donor fibroblasts	Identity matched

[a] This is the number of passages beyond the orginal parental line (WTC/AICS-0 at passage 33).
[b] Droplet digital PCR using Bio-Rad QX200
[c] RPP30 is a reference 2 copy gene used for normalization.
[d] Bae et al (2014) Bioinformatics. 30(10): 1473-1475
[e] Nextera rapid capture exome
[f] Promega CellTiter-Glo Luminescent Cell Viability Assay (Catalog #G7571)
[g] STEMCELL Technologies STEMdiff Trilineage Differentiation Kit (Catalog #05230)
[h] Palpant et al (2015) Development. 142(18): 3198-3209
[i] 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.
[j] 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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mTagRFP-T tagging strategy: Used CRISPR-Cas9 methodology to introduce mTagRFP-T at N-terminus of CETN2 as shown below.

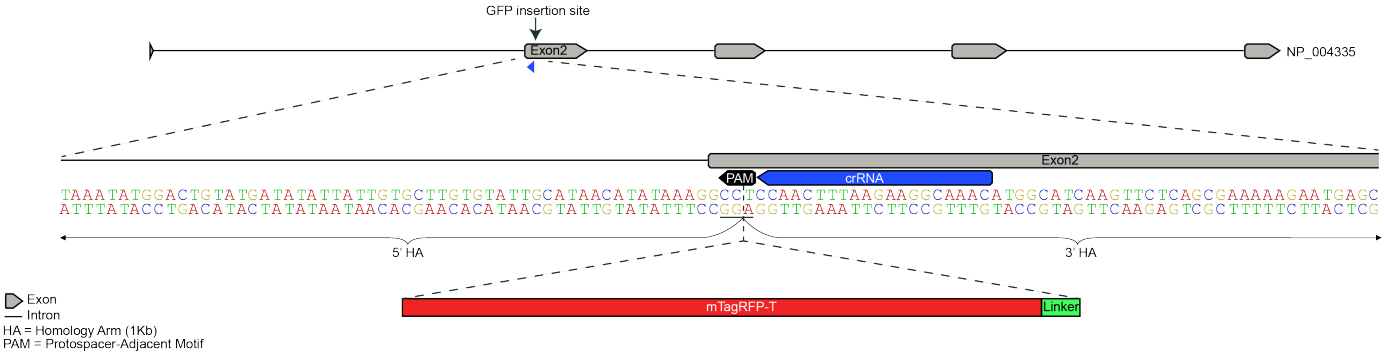

Figure 1: Top: CETN2 locus; Bottom: Zoom in on mTagRFP-T insertion site at CETN2 N-terminus

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.

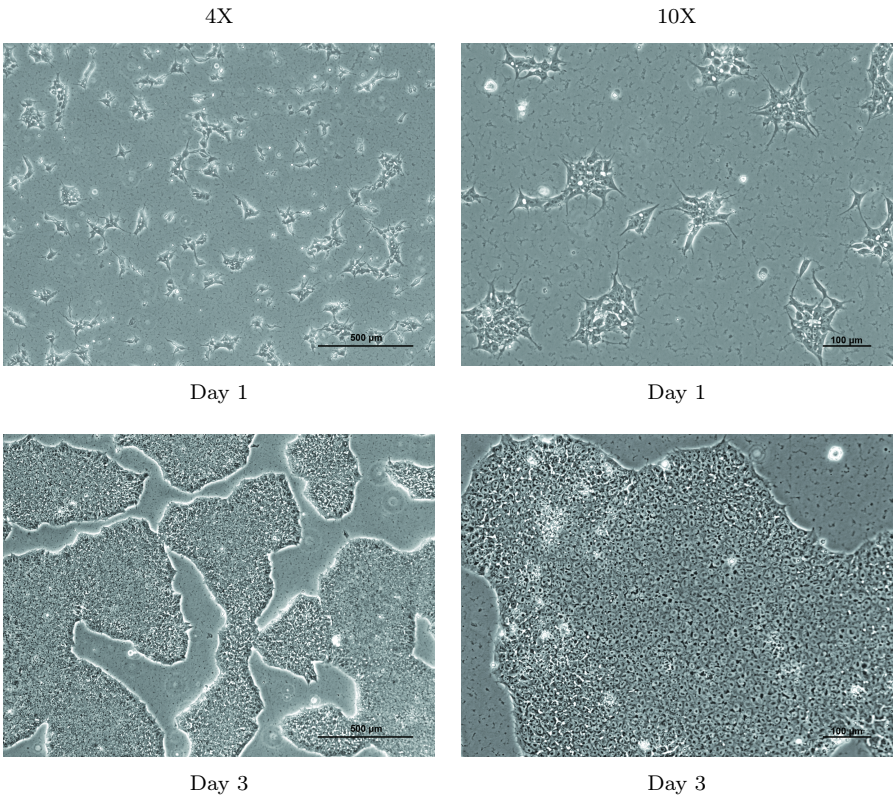

Figure 2: Viability and colony formation one day and three days post-thaw

[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-free mTeSR media (STEMCELL Technologies). Our most common microscope configuration are a Zeiss spinning disk fluorescence microscope with a Yokogawa CSUX1 head, Hamamatsu CMOS camera, and a 561 laser (mTagRFP-T). 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.

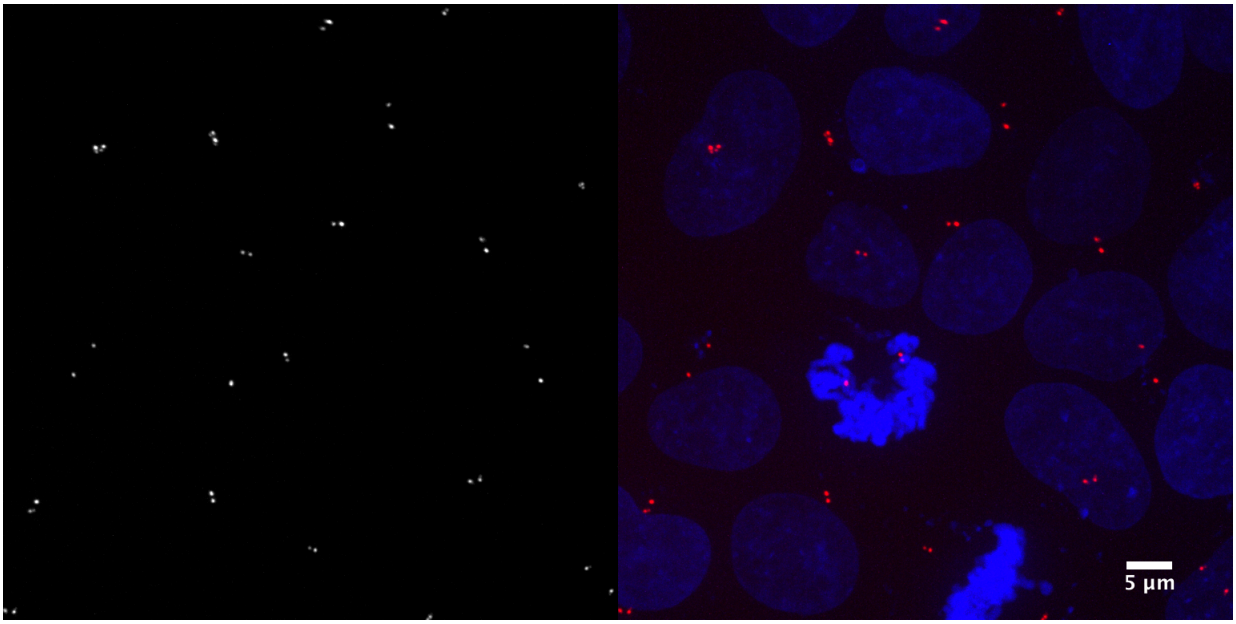

Figure 3: mTagRFPT-tagged CETN2 localization in an hiPSC colony. Image is a maximum intensity projection of a 3D spinning disk confocal z-stack of a live hiPSC colony. Right image shows centrin (red) overlaid with DNA (Hoechst, blue).