

# CERTIFICATE OF ANALYSIS

AICS-0054-091:WTC-mTagRFPT-CAAX-Safe harbor locus (AAVS1)-cl91  
(mono-allelic tag)

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<b>Product description</b>	Human iPSC clonal line in which CAAX domain of K-RAS tagged with mTagRFP-T under the control of a CAG promoter has been inserted at the safe harbor locus (AAVS1) using CRISPR/Cas9 technology
<b>Parental cell line</b>	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
<b>Publication(s) describing iPSC establishment</b>	Kreitzer et al (2013) Am. J. Stem Cells, 30; 2(2): 119-31
<b>Passage of gene edited iPSC reported at submission</b>	p26 <sup>a</sup>
<b>Number of passages at Coriell</b>	0
<b>Media</b>	mTeSR1
<b>Feeder or matrix substrate</b>	Matrigel
<b>Passage method</b>	Accutase
<b>Thaw</b>	1 million cells (ea vial) in 10 cm plate - ready for passaging in 3-4 days
<b>Seeding density</b>	450-900K cells/10-cm plate; every 3-4 days (see culture protocol)

Test Description	Method	Specification	Result
<b>Post-Thaw Viable Cell Recovery</b>	hiPSC culture on Matrigel	> 50% confluency 3-4 days post-thaw (10 cm plate)	Pass
<b>mTagRFP-T insertion at genomic locus - precise editing</b>	PCR and Sanger sequencing of recombinant and wildtype alleles	Insertion of mTagRFPT-CAAX at the AAVS1 locus	Not sequenced
<b>Copy number</b>	ddPCR <sup>b</sup> assay for mTagRFP-T and RPP30 reference gene <sup>c</sup>	mTagRFP-T/RPP30: ~ 0.5 = Mono-allelic ~ 1.0 = Bi-allelic	Mono-allelic (0.48)
<b>Plasmid integration</b>	ddPCR assay to detect plasmid integration into the genome	AmpR/RPP30: < 0.1 = no plasmid integration	Pass (0.00)
<b>Mutational analysis</b>	Whole exome sequencing <sup>e</sup>	Check for acquired mutations (not detected in p8 <sup>a</sup> parental line) that: 1) Correspond to off-target sites predicted by Cas-OFFinder <sup>d</sup> 2) Affect genes in Cosmic Cancer Gene Census	Sequencing planned

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<b>mTagRFP-T localization</b>	Spinning Disk confocal live cell imaging	Localization to plasma membrane	Surrounds lamellar protrusions at the bottom of the cell, outlines cell boundaries at the middle height of cells, and surrounds microvilli at the very top of cells. This pattern is consistent with localization to the plasma membrane. In addition, bright puncta are present throughout the cell, with a greater number near the top of the cell. These are likely endosomes created by membrane taken into the cell.
<b>Expression of tagged protein</b>	Western blot	Expression of expected size product	Expected band size for mTagRFP-T-tagged CAAX domain of K-Ras protein
<b>Growth rate</b>	ATP quantitation <sup>f</sup>	Comparable to parental line	Pass (measured at p27)
<b>Expression of stem cell markers</b>	Flow cytometry	Transcription factors: OCT4/SOX2/NANOG $\geq$ 85% Surface markers: SSEA3, TRA-1-60 $\geq$ 85%; SSEA1 $\leq$ 15%	Pass
<b>Germ layer differentiation</b>	Trilineage differentiation <sup>g</sup> 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
<b>Cardiomyocyte differentiation</b>	Palpant et al. (2015) <sup>h</sup>	Beating initiated (D7-D14) and Cardiac Troponin T expression (D12-D30) by flow cytometry	Pass
<b>Karyotype</b>	G-banding (30 cell analysis)	Normal karyotype, 46 XY	Pass
<b>Mycoplasma</b>	qPCR (IDEXX)	Negative	Pass
<b>Sterility (bacterial, yeast and fungal testing)</b>	Direct inoculation and incubation for 10 days	No growth after 10 days	Pass
<b>Viral Panel Testing<sup>i</sup></b>	PCR	Negative when assayed for CMV, EBV, HepB, HepC, HIV1, and HPV	Pass
<b>Identity of unedited parental line<sup>j</sup></b>	STR	29 allelic polymorphisms across 15 STR loci compared to donor fibroblasts	Identity matched

<sup>a</sup> This is the number of passages beyond the original parental line (WTC/AICS-0 at passage 33).

<sup>b</sup> Droplet digital PCR using Bio-Rad QX200

<sup>c</sup> RPP30 is a reference 2 copy gene used for normalization.

<sup>d</sup> Bae et al (2014) *Bioinformatics*. 30(10): 1473-1475

<sup>e</sup> Nextera rapid capture exome

<sup>f</sup> Promega CellTiter-Glo Luminescent Cell Viability Assay (Catalog #G7571)

<sup>g</sup> STEMCELL Technologies STEMdiff Trilineage Differentiation Kit (Catalog #05230)

<sup>h</sup> Palpant et al (2015) *Development*. 142(18): 3198-3209

<sup>i</sup> 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.

<sup>j</sup> 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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## AICS-0054-091:WTC-mTagRFPT-CAAX-Safe harbor locus (AAVS1)-cl91 (mono-allelic tag)

**mTagRFPT-T tagging strategy:** Used CRISPR-Cas9 methodology to introduce mTagRFPT at safe harbor locus (AAVS1) of PPP1R12C gene as shown below.

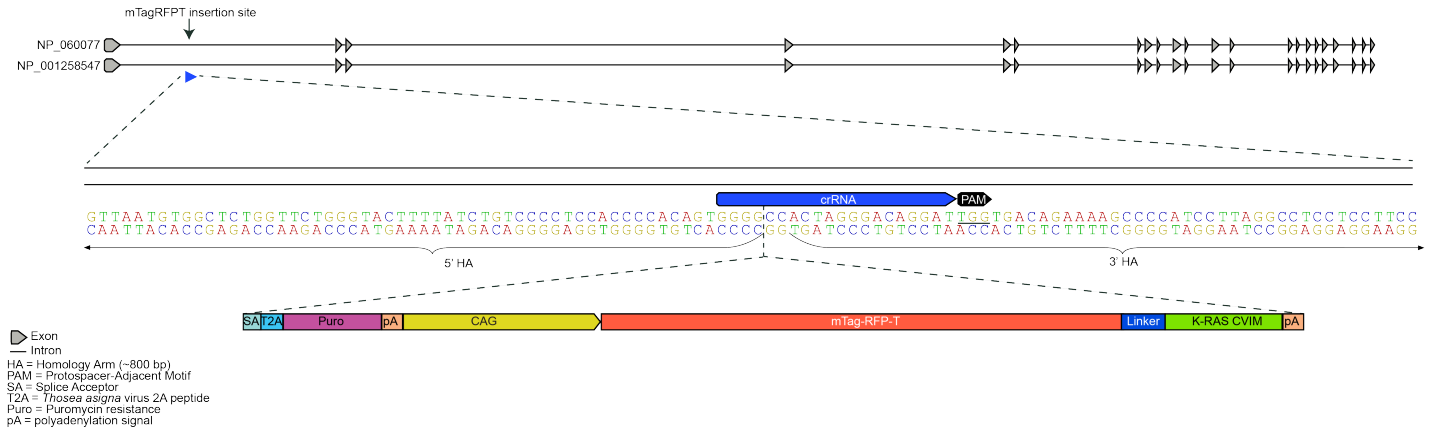


Figure 1: Top: mTagRFPT insertion site at safe harbor locus (AAVS1) in PPP1R12C intron; Bottom: Zoom in on mTagRFPT insertion site at safe harbor locus (AAVS1); insertion into AAVS1 is based on Hockmeyer et al (2011) Nat. Biotechnology, 29(8): 731-734

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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 photographed one and three days post-thaw<sup>1,2</sup> using a Nikon microscope at 4X and 10x magnification.

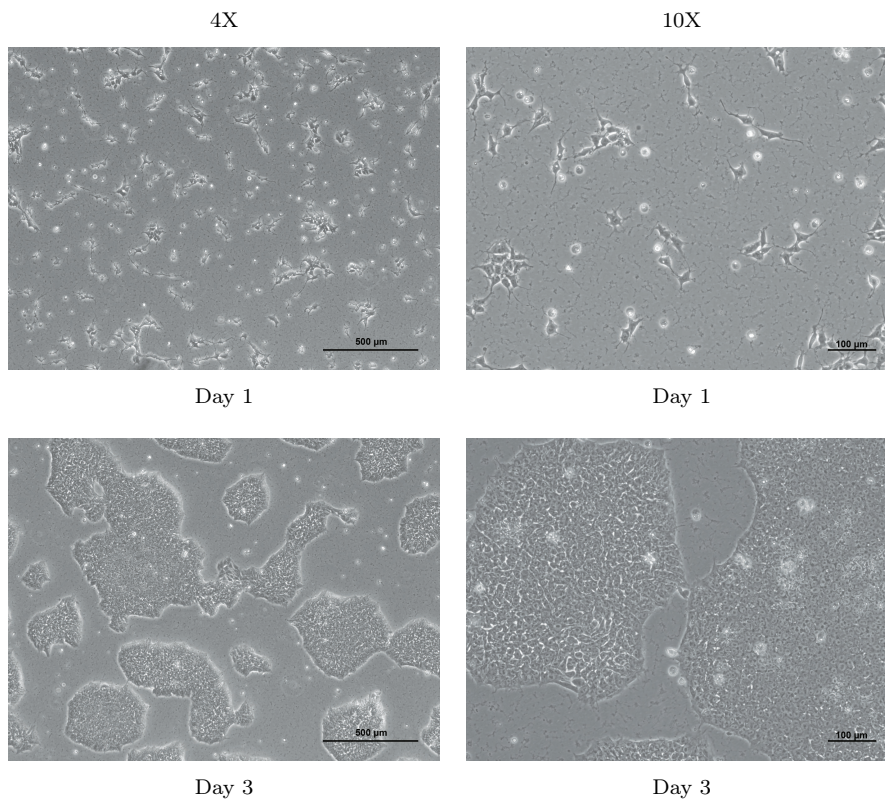


Figure 2: Viability and colony formation one day and three days post-thaw

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<sup>1</sup>Cells may take up to 3 passages to recover after thaw

<sup>2</sup>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 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<sub>2</sub> in a temperature-controlled chamber. The approximate laser power measured at the sample for our standard 100x images is ~2.5 mW.

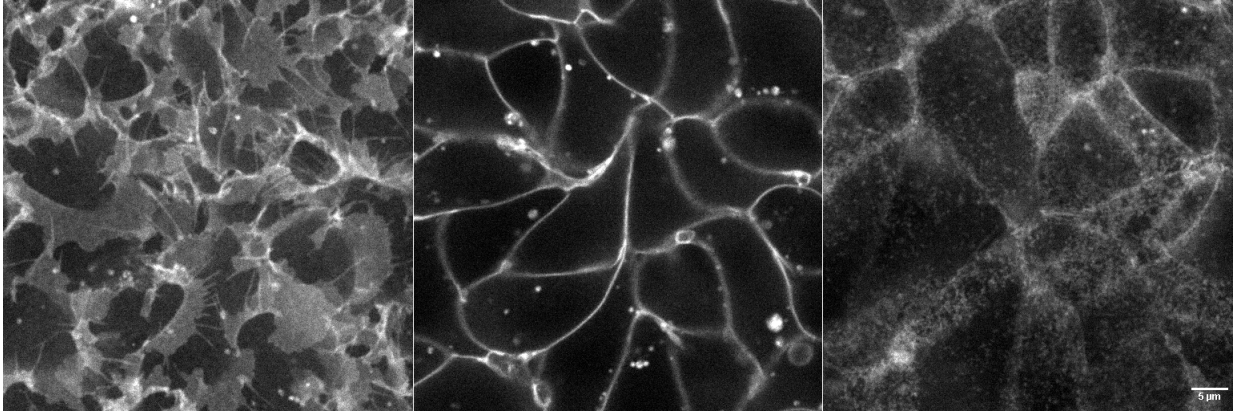


Figure 3: Single-planes of live hiPS cell colony expressing the CAAX domain of K-Ras tagged with mTagRFP-T expressed from a safe harbor locus. Z-planes shown are at the bottom (left), middle (middle) and top (right) of cells. Cells were imaged in 3D on a spinning-disk confocal microscope. Scale bar, 5  $\mu m$ .