

**CERTIFICATE OF ANALYSIS**

**AICS-0102-330:WTC-mTagRFP-T-COXVIII (mitoTag)-Safe harbor locus (AAVS1)-cl330  
(mono-allelic tag)**

<b>Product description</b>	Human iPSC clonal line in which mTagRFP-T has been localized to the mitochondrial matrix via COXVIII (mitoTag) under the control of a CAG promoter at a safe harbor locus (AAVS1) edited 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>	p37 <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, single cell
<b>Thaw</b>	1 million cells (ea vial) in 10 cm plate - ready for passaging in 3-4 days
<b>Seeding density</b>	400K cells/10-cm plate every 4 days or 800K cells/10-cm plate every 3 days (see culture protocol)

<b>Test Description<sup>b</sup></b>	<b>Method</b>	<b>Specification</b>	<b>Result</b>
<b>Post-Thaw Viable Cell Recovery</b>	hiPSC culture on Matrigel	> 50% confluence 3-4 days post-thaw (10 cm plate)	Pass
<b>mTagRFP-T insertion(s) at genomic locus - precise editing</b>	PCR and Sanger sequencing of recombinant and wildtype alleles	Insertion of mTagRFPT at COXVIII (mitoTag) at the AAVS1 locus with exact predicted recombinant allele junctions.	Pass
<b>Copy number</b>	ddPCR <sup>c</sup> assay for FP(s) and RPP30 reference gene <sup>d</sup>	FP/RPP30: ~ 0.5 = Mono-allelic ~ 1.0 = Bi-allelic	mTagRFP-T-COXVIII (mitoTag): Mono-allelic (0.516)
<b>Plasmid integration</b>	ddPCR assay to detect plasmid integration into the genome	AmpR/RPP30 < 0.1: no plasmid integration	Pass (0.00)
<b>To determine the presence of the PPM1D mutation<sup>e</sup> in clonal line</b>	ddPCR assay (PPM1D:PPM1D REF)	PPM1D mutation present in parental line	positive (+)
<b>mTagRFP-T localization</b>	Spinning Disk confocal live cell imaging	Localization of mTagRFP-T to the mitochondrial matrix.	mTagRFP-T-tagged mitochondrial targeting sequence of COXVIII (mitoTag) localizes to the mitochondrial matrix.
<b>Expression of tagged protein</b>	Western blot	Expression of expected size product	Expected size band for mTagRFP-T-tagged mitochondrial targeting sequence of COXVIII (mitoTag).

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<b>Growth rate</b>	ddPCR assay for RPP30 reference gene	Comparable to parental line	Pass (measured at p29) <sup>a</sup>
<b>Expression of stem cell markers</b>	Flow cytometry	Transcription factors: OCT4/SOX2/NANOG ≥ 85% Surface markers: SSEA4, TRA-1-60 ≥ 85%; SSEA1 ≤ 15%	Pass
<b>Germ layer differentiation</b>	Trilineage differentiation <sup>f</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>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>g</sup></b>	PCR	Negative when assayed for CMV, EBV, HepB, HepC, HIV1, and HPV	Pass
<b>Identity of unedited parental line<sup>h</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> All QC assays are performed on stem cells except when noted otherwise.

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

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

<sup>e</sup> Identifier NM\_003620.4(PPM1D):c.1426G>T(p.Glu159X)

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

<sup>g</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>h</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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**Tagging strategy:** CRISPR-Cas9 methodology was used to introduce mTagRFP-T localized to the mitochondrial matrix via COXVIII (mitoTag) at the safe harbor locus (AAVS1) as shown below.

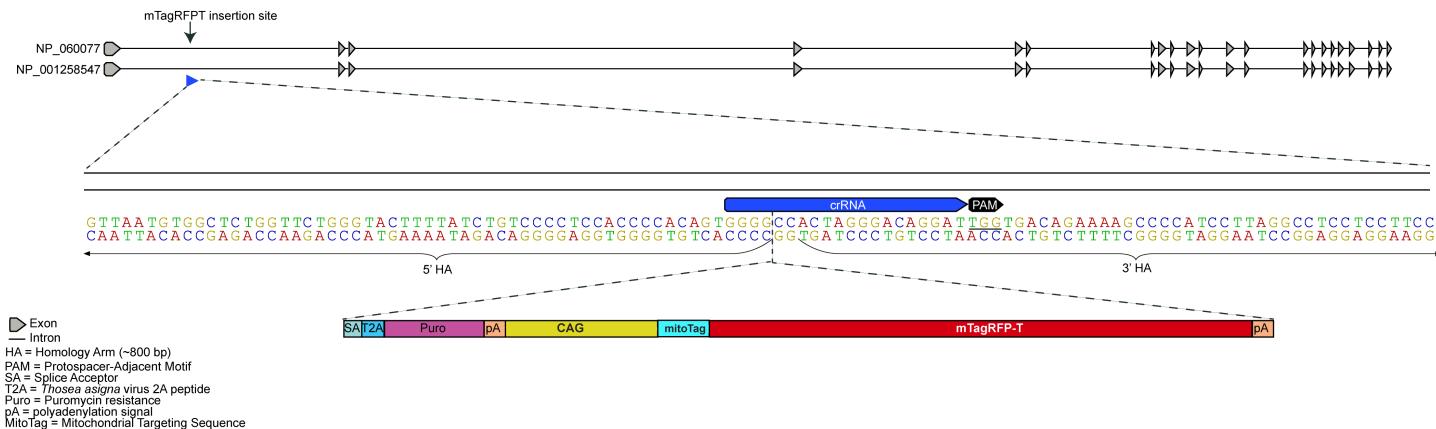


Figure 1: Top: mTagRFP-T insertion site at safe harbor locus (AAVS1) in PPP1R12C intron; Bottom: Zoom in on mTagRFP-T insertion site at safe harbor locus (AAVS1); insertion into AAVS1 is based on Hockmeyer et al (2011) Nat. Biotechnology, 29(8): 731-734

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**Post-thaw imaging:** One vial of distribution lot was thawed (cells were treated with ROCK inhibitor for 24hrs post-thaw). Cultures were observed daily. Colonies were imaged one (a,b), three (c,d) and four (e,f) days post-thaw<sup>1,2</sup> using a Leica microscope.

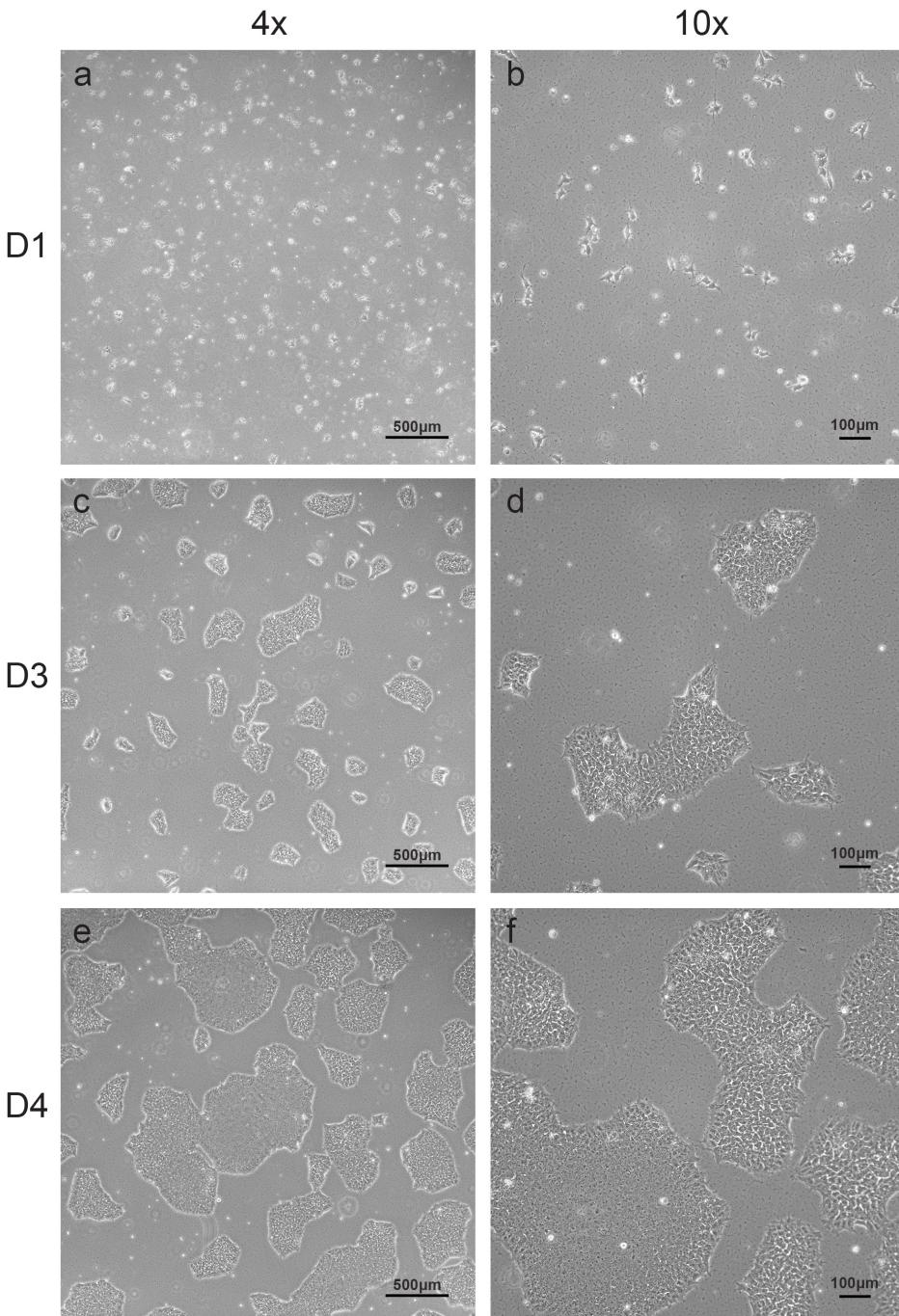


Figure 2: Viability and colony formation one day, three and four days post-thaw.

<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 488 laser (mEGFP) and 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.

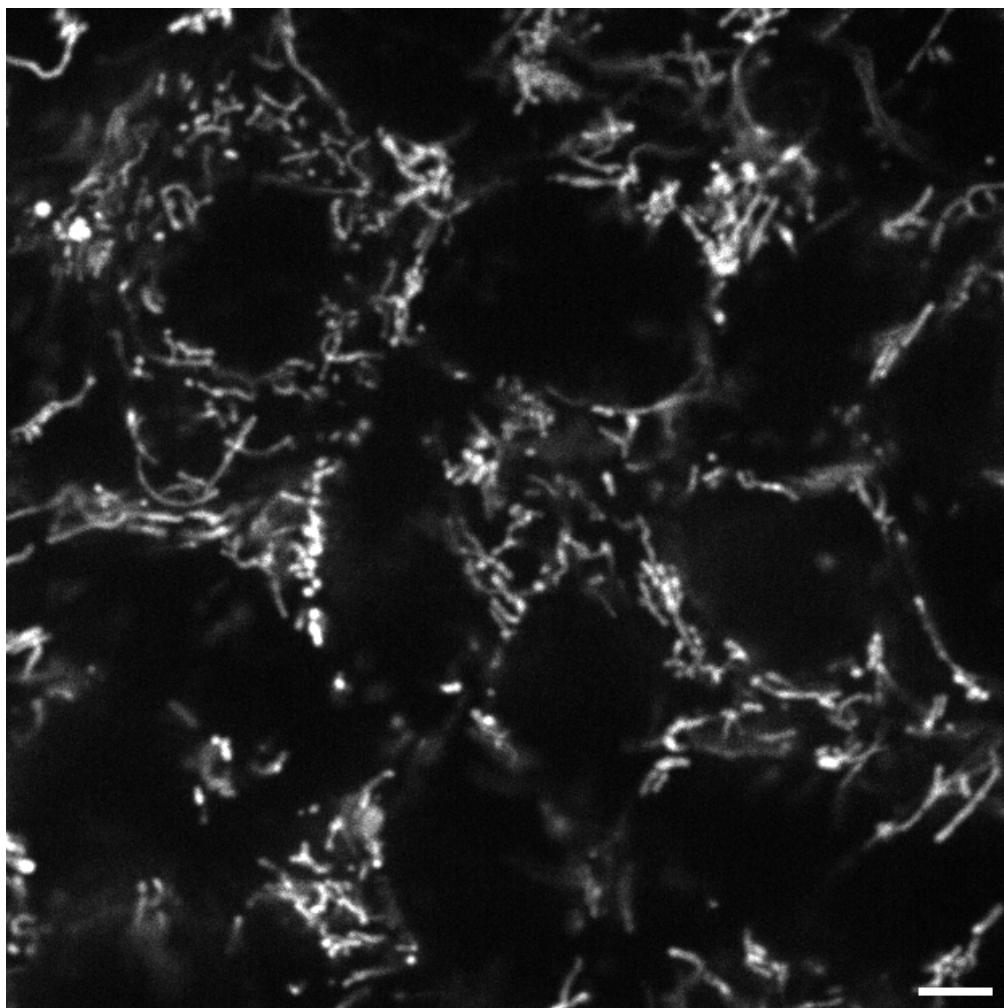


Figure 3: hiPS cells expressing mTagRFP-T-tagged mitochondrial targeting sequence of COXVIII (mitoTag). Image is a single slice near the bottom of the cell (scalebar, 5 μm). Cells were imaged live in 3D on a spinning-disk confocal microscope.