

CERTIFICATE OF ANALYSIS

AICS-0078-079:WTC Dual tagged

TOMM20-mEGFP/mTagRFP-T-TUBA1B-cl79 (mono-allelic tags)

Product description	Human iPSC clonal line in which TOMM20 and TUBA1B have been endogenously tagged with mEGFP and mTagRFP-T, respectively, 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	p45 ^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	500K cells/10-cm plate every 4 days or 1 million 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 / mTagRFP-T insertion(s) at genomic locus - precise editing	PCR and Sanger sequencing of recombinant and wildtype alleles	C-term insertion of mEGFP in frame with exact predicted recombinant allele junctions. N-term insertion of mTagRFP-T in frame with exact predicted recombinant allele junctions. No additional mutations.	TOMM20-mEGFP: Pass TUBA1B-mTagRFP-T: Pass
Copy number	ddPCR ^c assay for FP(s) and RPP30 reference gene ^d	FP/RPP30: ~ 0.5 = Mono-allelic ~ 1.0 = Bi-allelic	TOMM20-mEGFP: Mono-allelic (0.52) TUBA1B-mTagRFP-T: Mono-allelic (0.58)
Plasmid integration	ddPCR assay to detect plasmid integration into the genome	KanR/RPP30 < 0.1 AmpR/RPP30 < 0.1 = no plasmid integration	Pass KanR/RPP30: 0.021 AmpR/RPP30: 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

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mEGFP localization and mTagRFP-T localization	Spinning Disk confocal live cell imaging	Localization of mEGFP to mitochondria and mTagRFP-T to microtubules	mEGFP-tagged Tom20 localizes to the mitochondrial outer membrane. mTagRFP-T-tagged alpha tubulin localizes to microtubules, mitotic spindles, primary cilia, and midbodies; some diffuse signal throughout the cytosol consistent with depolymerized tubulin.
Expression of tagged protein	Western blot	Expression of expected size product	Expected size bands for untagged and mEGFP-tagged Tom20 and for untagged and mTagRFP-T-tagged alpha tubulin. Semi-quantitative results show that 29% of TOMM20-encoded protein product is mEGFP labeled and 20% of TUBA1B-encoded protein product is mTagRFP-T labeled.
Growth rate	ATP quantitation ^g	Comparable to parental line	Pass (measured at p42) ^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 ^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	Pass
Cardiomyocyte differentiation	Modified small molecule differentiation (Lian et al. 2012) 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 Lian et al (2012) *PNAS*. 109(27):E1848-E1857

^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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Important information	<p>Total alpha-tubulin and total Tom20 protein levels are low in this line. Our semi-quantitative analysis indicates that AICS-0078 clone 79 has 37% and 26% of the total levels of alpha-tubulin and Tom20 protein, respectively, in unedited WTC-11 control cells. Our time-lapse imaging and cell growth assays indicate that these cells grow and divide as expected. Further, we cannot detect any abnormalities in microtubule organization or mitochondrial morphology. However, if you plan to use this line for an assay that you believe may be sensitive to low total protein levels of Tom20 and/or alpha-tubulin, you may wish to perform additional functional assays.</p>
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Tagging strategy: CRISPR-Cas9 methodology was used to introduce mTagRFP-T at the N-terminus of TUBA1B as shown below. A TUB1AB clone was selected, and mEGFP was introduced at the C-terminus of TOMM20 as shown below to make a dual tag line.

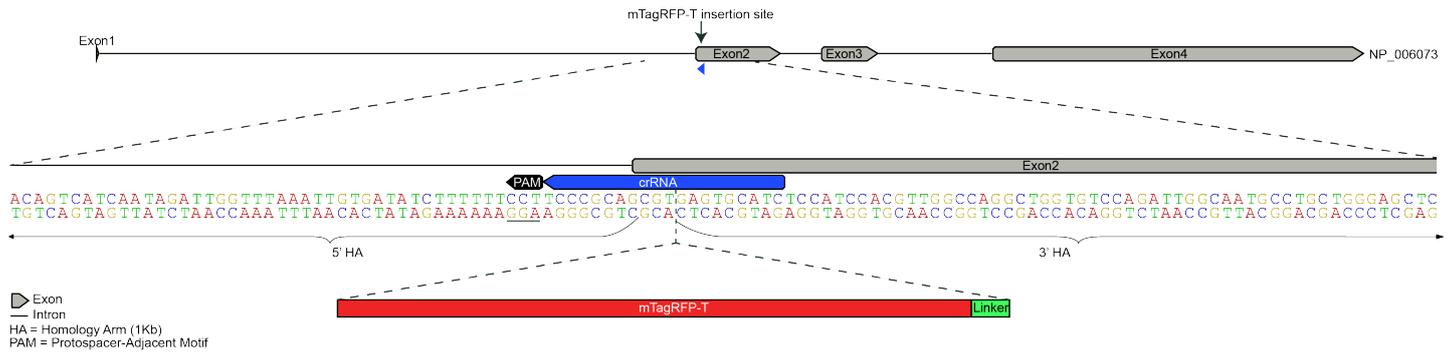


Figure 1: Top: TUBA1B locus; Bottom: Zoom in on mTagRFP-T insertion site at TUBA1B N-terminus.

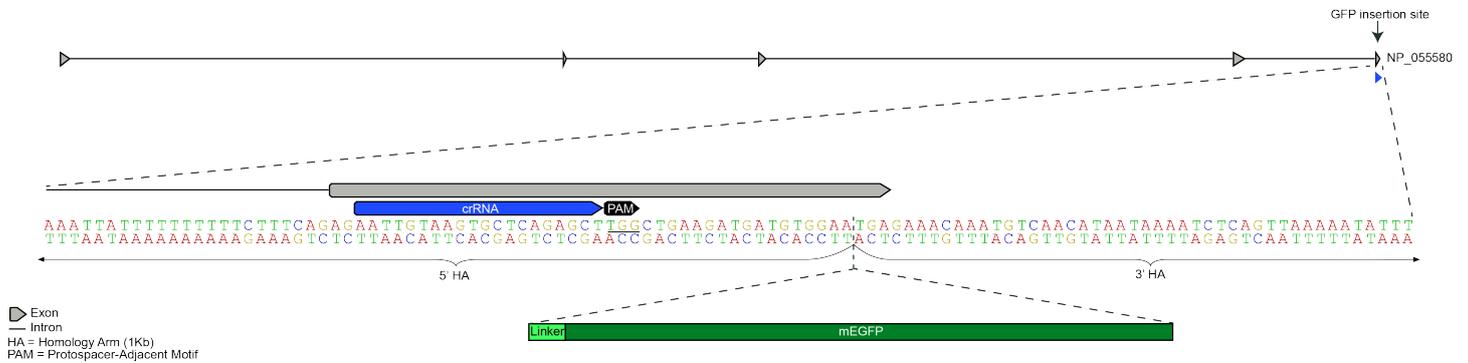


Figure 2: Top: TOMM20 locus; Bottom: Zoom in on mEGFP insertion site at TOMM20 C-terminal exon.

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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.

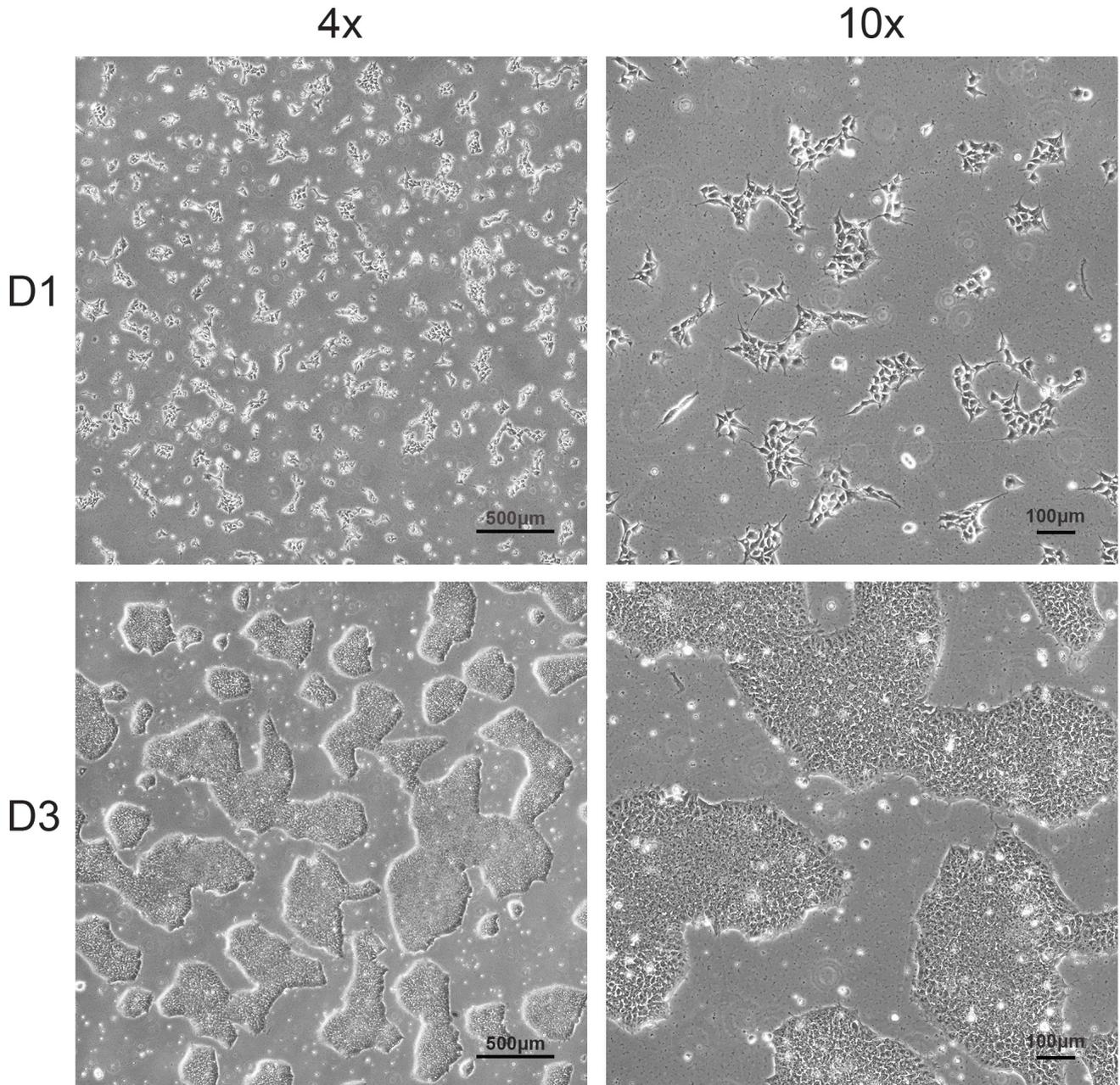


Figure 3: Viability and colony formation one day and three days post-thaw.

¹Cells may take up to 3 passages to recover after thaw

²Morphologies 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% CO₂ in a temperature-controlled chamber. The approximate laser power measured at the sample for our standard 100x images is ~2.5 mW.

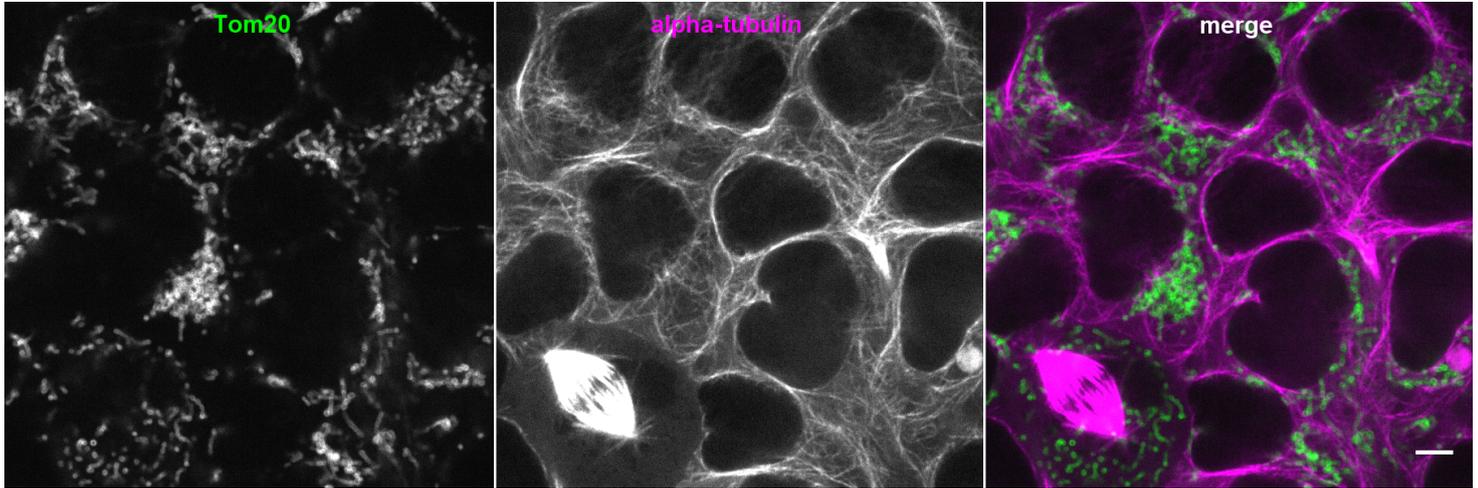


Figure 4: Single, mid-level plane of cells in a live hiPS cell colony expressing mEGFP-tagged Tom20 and mTagRFP-T-tagged alpha-tubulin. Panels show individual channels for Tom20 (left), alpha-tubulin (middle), and the overlay of the two (right). Cells were imaged in 3D on a spinning-disk confocal microscope. Scale bar, 5 μ m.