CERTIFICATE OF ANALYSIS
AICS-0059-036:WTC Dual tagged SEC61B-mEGFP/LMNB1-mTagRFPT-cl36 (mono-allelic tags)

Product description	Human iPSC clonal line in which SEC61B and LMNB1 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	p40[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	400K 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 / mTagRFP-T insertion 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. N-term insertion of mTagRFP-T in frame with exact predicted recombinant allele junctions. No additional mutations.	SEC61B-mEGFP: Pass LMNB1-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	SEC61B-mEGFP: Mono-allelic (0.53) LMNB1-mTagRFP-T: Mono-allelic (0.56)
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

CERTIFICATE OF ANALYSIS
AICS-0059-036:WTC Dual tagged SEC61B-mEGFP/LMNB1-mTagRFPT-cl36 (mono-allelic tags)

mEGFP localization and mTagRFP-T localization	Spinning Disk confocal live cell imaging	Localization of mEGFP to the endoplasmic reticulum and localization of mTagRFP-T to the nuclear envelope.	mEGFP localization to ER sheets and ER tubules throughout the cytoplasm and co-localization with mTagRFP-T in the nuclear periphery and co-localization to an extended lamina during cell division.
Expression of tagged protein	Western blot	Expression of expected size product	Expected size bands for untagged and mEGFP-tagged Sec61 beta and for untagged and mTagRFP-T-tagged lamin B1. Semi-quantitative results show that 68% of SEC61B-encoded protein product is mEGFP labeled, and 23% of LMNB1-encoded protein product is mTagRFP-T labeled.
Growth rate	ATP quantitation[g]	Comparable to parental line	Pass (measured at p38)[a]
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 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.

CERTIFICATE OF ANALYSIS
AICS-0059-036:WTC Dual tagged SEC61B-mEGFP/LMNB1-mTagRFPT-cl36 (mono-allelic tags)

Tagging strategy: CRISPR-Cas9 methodology was used to introduce mEGFP at N-terminus of SEC61B as shown below. A SEC61B clone was selected, and mTagRFP-T was introduced at the N-terminus of LMNB1 as shown below to make a dual tag line.

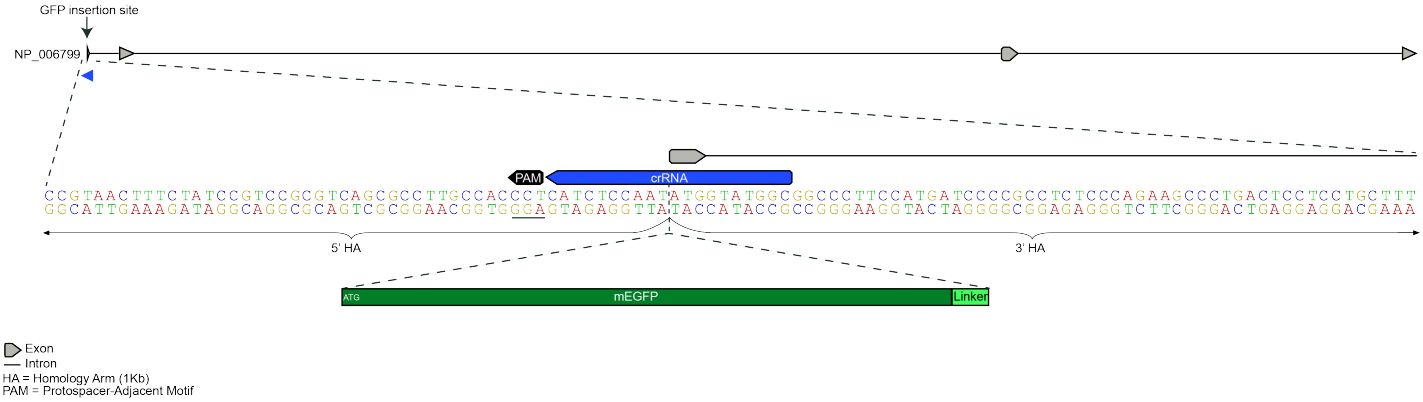

Figure 1: Top: SEC61B locus; Bottom: Zoom in on mEGFP insertion site at SEC61B N-terminal exon

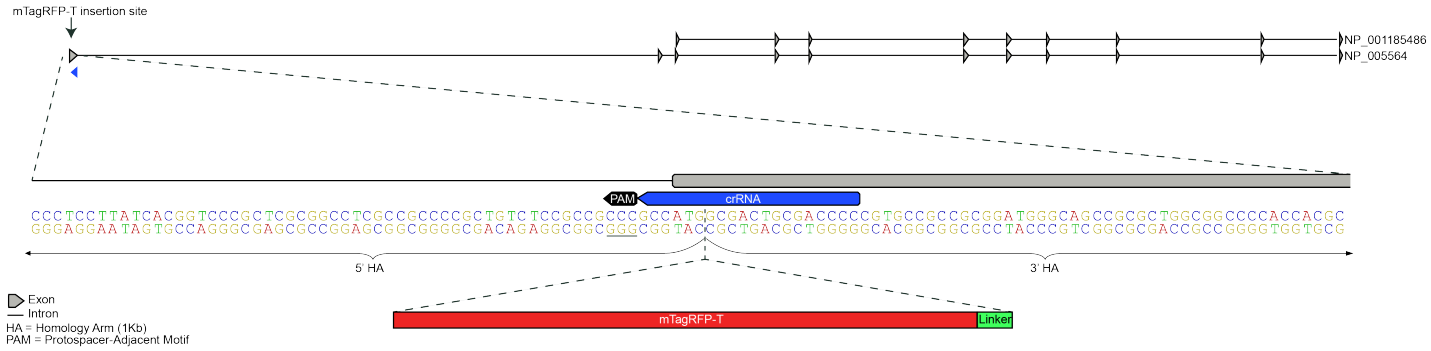

Figure 2: Top: LMNB1 locus showing 2 LMNB1 isoforms; Bottom: Zoom in on mTagRFP-T insertion site at LMNB1 N-terminal exon

CERTIFICATE OF ANALYSIS
AICS-0059-036:WTC Dual tagged SEC61B-mEGFP/LMNB1-mTagRFPT-cl36 (mono-allelic tags)

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 Leica microscope at 4x and 10x magnification. Cells were not ready to be passaged at day 3 (Figure 3c, d) and were grown for an extra day (Figure 3e, f) before passaging. Another distribution vial was thawed, and the cells were again underconfluent at day 3. If your cultures also appear underconfluent 3 days post thaw, we recommend growing for one additional day before passaging.

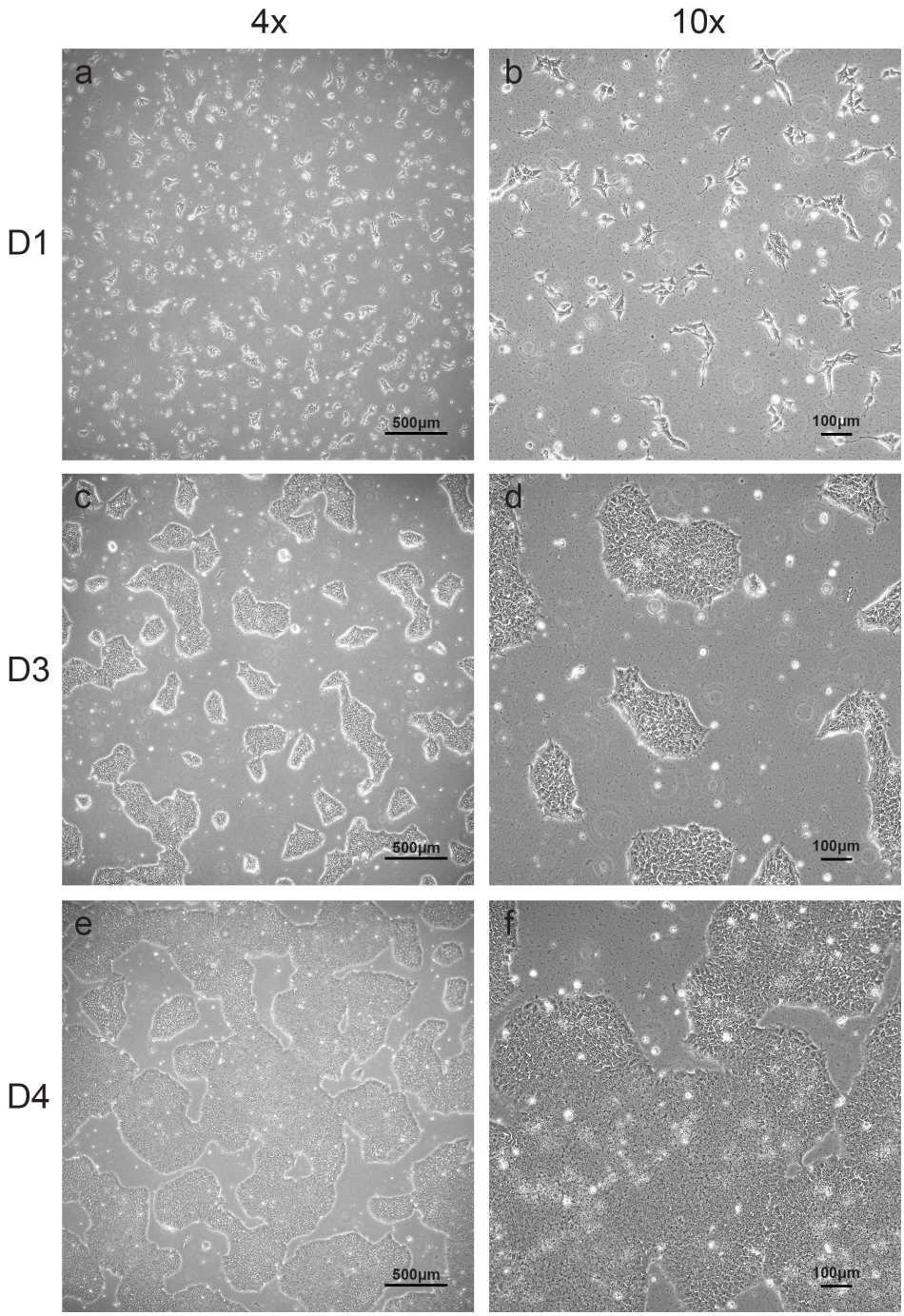

Figure 3: Viability and colony formation one day (a, b) and three days (c, d) post-thaw; e, f: colony morphology after one additional day of growth.

[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

CERTIFICATE OF ANALYSIS
AICS-0059-036:WTC Dual tagged SEC61B-mEGFP/LMNB1-mTagRFPT-cl36 (mono-allelic tags)

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_2 in a temperature-controlled chamber. The approximate laser power measured at the sample for our standard 100x images is ∼2.5 mW.

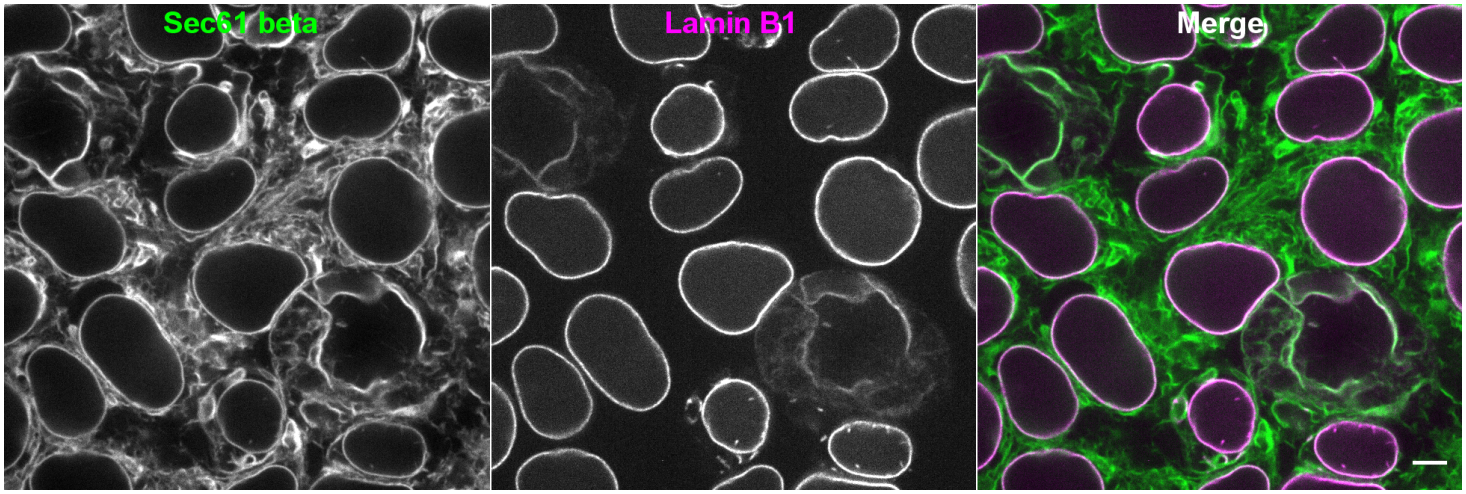

Figure 4: Single, mid-level plane of cells in a live hiPS cell colony expressing mEGFP-tagged Sec61 beta and mTagRFP-T-tagged lamin B1. Panels show individual channels for Sec61 beta (left) and lamin B1 (middle) and the overlay of the two (right). Imaged region captures cells at different stages of the cell cycle to highlight cell cycle-dependent variation in co-localization of Sec61 beta and Lamin B1. Cells were imaged in 3D on a spinning-disk confocal microscope. Scale bar, 5 μm.