CERTIFICATE OF ANALYSIS

AICS-0090-391:WTC-CLYBL-dCas9-TagBFP-KRAB-cl391 (mono-allelic tag)

Product description	Human iPSC clonal line in which dCas9-TagBFP-KRAB is expressed from a CAGGS promoter edited into the CLYBL safe harbor (second intron) 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	p33[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	350K cells/10-cm plate every 4 days or 750K 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
TagBFP insertion at genomic locus - precise editing	PCR and Sanger sequencing of recombinant and wildtype alleles (insert is too large for full allele PCR)	Insertion of dCas9-TagBFP-KRAB at the CLYBL locus. No additional mutations.	Pass
Copy number	ddPCR[c] assay for FP(s) and RPP30 reference gene[d]	FP/RPP30: $\sim$ 0.5 = Mono-allelic $\sim$ 1.0 = Bi-allelic	Mono-allelic (0.54)
Plasmid integration	ddPCR assay to detect plasmid integration into the genome	KAN/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-0090-391:WTC-CLYBL-dCas9-TagBFP-KRAB-cl391 (mono-allelic tag)

TagBFP localization	Spinning Disk confocal live cell imaging	Localization of TagBFP to the nucleus	TagBFP localizes to the nucleus where it is dim and heterogeneous in signal intensity between cells, including cells that have little to no fluorescent signal. In addition to the expected nuclear localization, aggregates of TagBFP are observed in the cytoplasm of most cells. Functional testing suggests that the heterogenous expression profile and the presence of aggregates do not impact the functionality of dCas9-KRAB (see below for more details).
dCas9-KRAB functionality in stem cells	Flow based assessment of knockdown of surface marker TFRC	Complete knockdown of TFRC in $\geq$ 90% of cells	Pass
dCas9-KRAB functionality in stem cells	ddPCR based assessment of knockdown of multiple genes	Complete or partial reduction of mRNA abundance	Gene, % reduction mRNA, Result TFRC, 95%, Pass DPH2, 20%, Pass DPY30, 71%, Pass PBRM1, 75-87%, Pass
Expression of tagged protein in stem cells	Western blot	Expression of expected size product	Expected size band for TagBFP-tagged dCas9-KRAB
dCas9-KRAB functionality in cardiomyocytes[g]	Flow based assessment of knockdown of surface marker TFRC in day 12 cardiomyocytes	Complete knockdown of TFRC in $\geq$ 90% of cells	Complete knockdown was observed in 16.5% of cells, partial knockdown was observed in 83.5% of cells
Expression of tagged protein in cardiomyocytes[g]	1. Western blot 2. Flow cytometry	1. Expression of expected product 2. Detection of TagBFP fluorescence	1. No protein detected using an anti-Cas9 antibody (n=2) 2. TagBFP was not detectable above autofluorescence (n=3)
dCas9-KRAB functionality in astrocyte progenitor cells[g,h]	Flow based assessment of knockdown of surface marker TFRC	At least 90% reduction in TFRC staining brightness (median fluorescence intensity)	Pass
Growth rate	ATP quantitation[i]	Comparable to parental line	Pass (measured at p34)[a]
Expression of stem cell markers	Flow cytometry	Transcription factors: OCT4, NANOG $\geq$ 85% Surface markers: SSEA4, TRA-1-60 $\geq$ 85%	Pass
Germ layer differentiation	Trilineage differentiation[j] 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)[k]	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

CERTIFICATE OF ANALYSIS
AICS-0090-391:WTC-CLYBL-dCas9-TagBFP-KRAB-cl391 (mono-allelic tag)

Sterility (bacterial, yeast and fungal testing)	Direct inoculation and incubation for 10 days	No growth after 10 days	Pass
Viral Panel Testing[l]	PCR	Negative when assayed for CMV, EBV, HepB, HepC, HIV1, and HPV	Pass
Identity of unedited parental line[m]	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] For use in differentiated cell types, pilot studies are advised to determine functionality of dCas9-TagBFP-KRAB in the cell lineage of interest.
[h] We thank Martin Kampmann and Kun Leng (UCSF) for dCas9-TagBFP-KRAB functionality experiments in astrocyte progenitors.
[i] Promega CellTiter-Glo Luminescent Cell Viability Assay (Catalog #G7571)
[j] STEMCELL Technologies STEMdiff Trilineage Differentiation Kit (Catalog #05230)
[k] Lian et al (2012) PNAS. 109(27):E1848-E1857
[l] 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.
[m] 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-0090-391:WTC-CLYBL-dCas9-TagBFP-KRAB-cl391 (mono-allelic tag)

Tagging strategy: CRISPR-Cas9 methodology was used to introduce dCas9-KRAB-TagBFP into CLYBL safe harbor locus (located in second intron between exons 2 and 3).

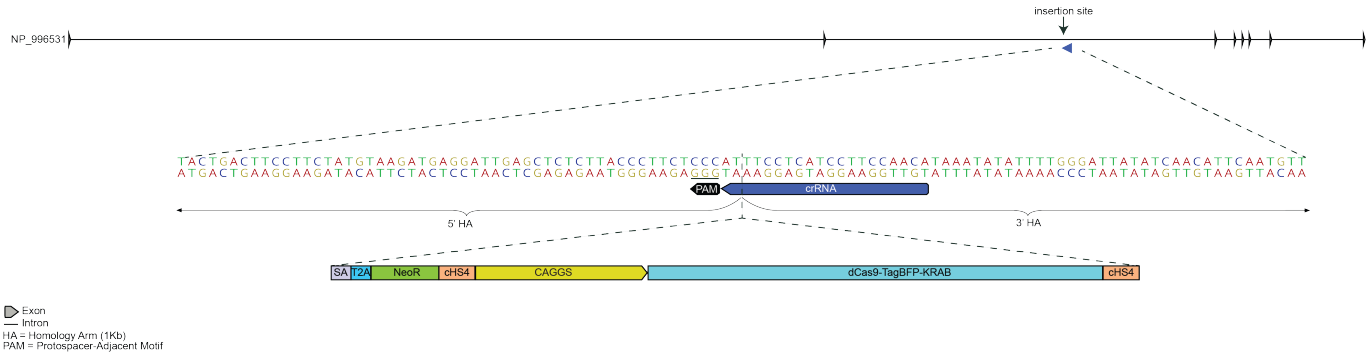

Figure 1: Top: CLYBL locus; Bottom: Zoom in on dCas9-TagBFP-KRAB insertion site at CLYBL safe harbor site between exons 2 and 3

CERTIFICATE OF ANALYSIS
AICS-0090-391:WTC-CLYBL-dCas9-TagBFP-KRAB-cl391 (mono-allelic tag)

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.

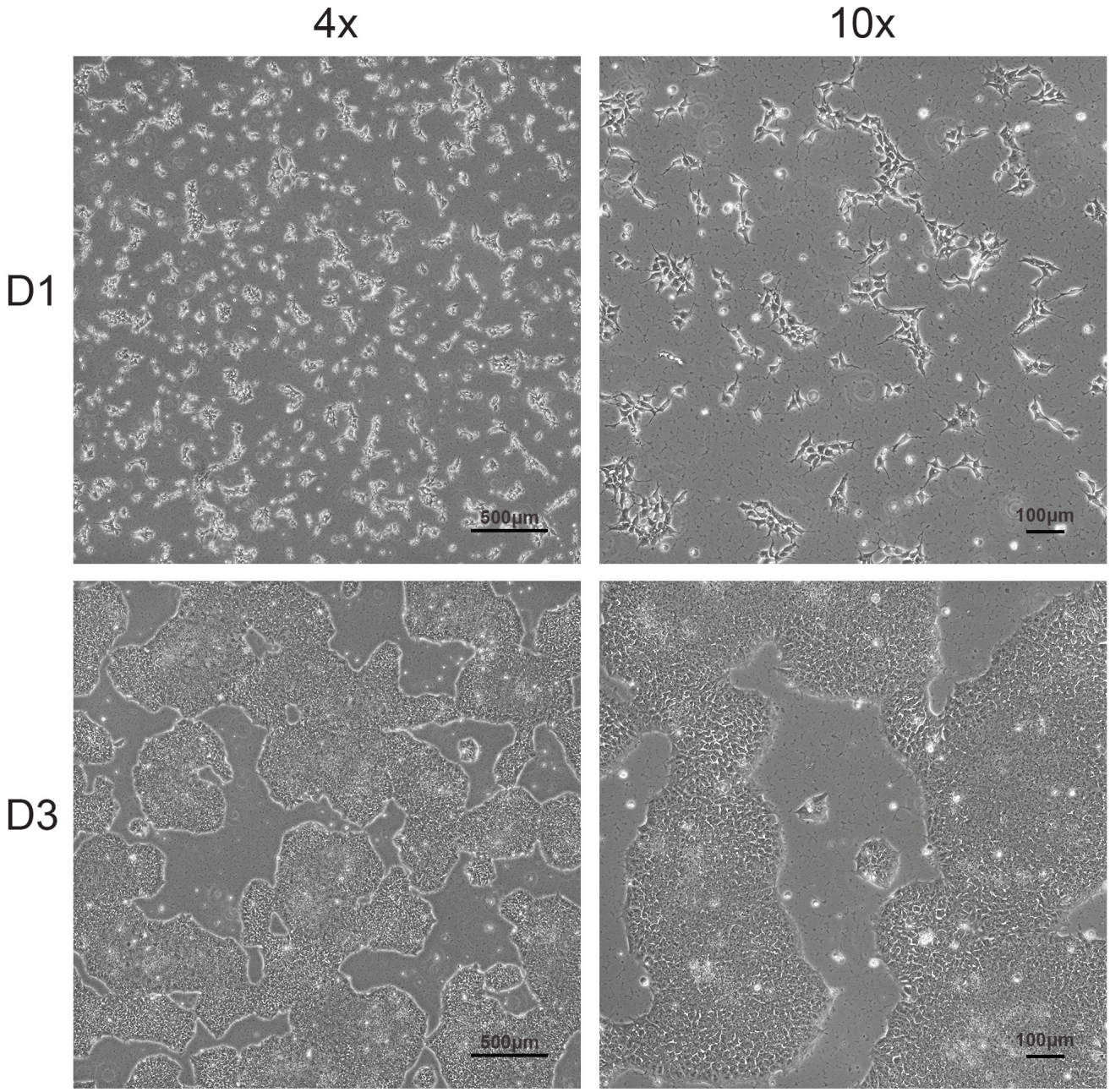

Figure 2: Viability and colony formation one day and three days post-thaw

[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

CERTIFICATE OF ANALYSIS

AICS-0090-391:WTC-CLYBL-dCas9-TagBFP-KRAB-cl391 (mono-allelic tag)

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 405 laser (TagBFP). 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.

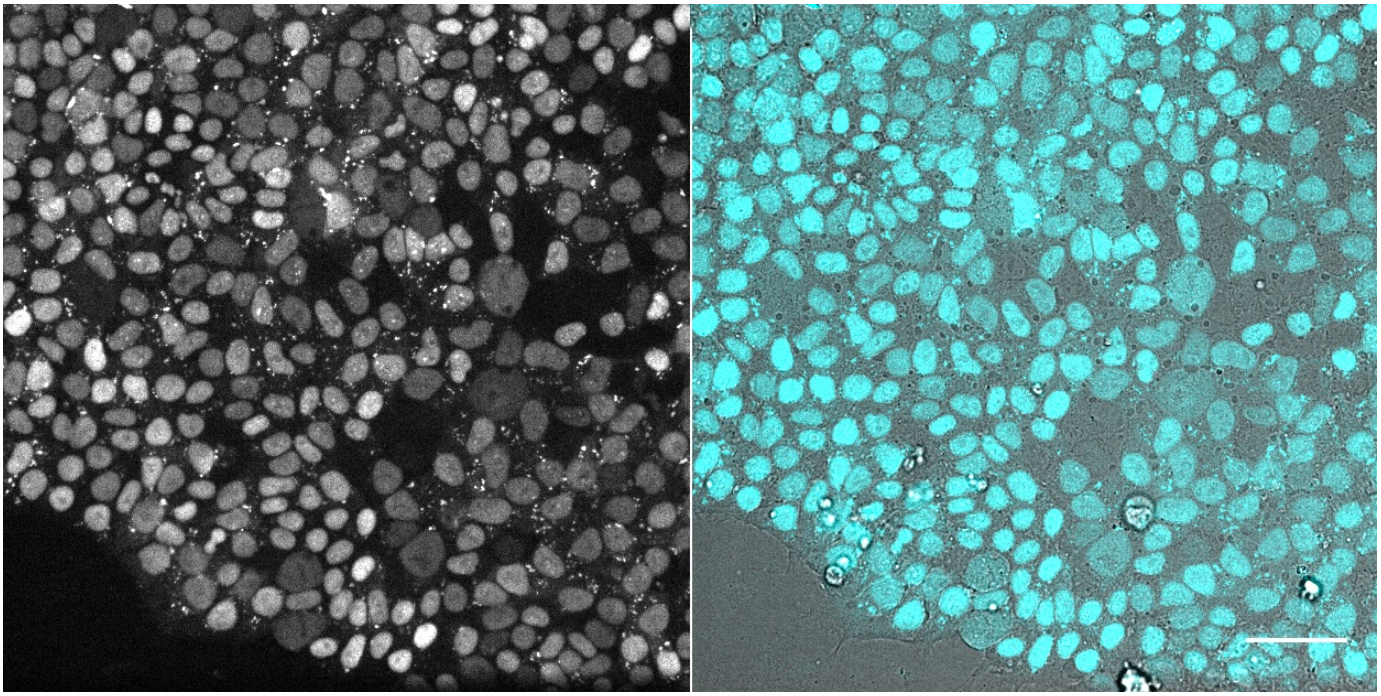

Figure 3: Single, mid-level plane of cells in a live hiPS cell colony expressing TagBFP-tagged dCas9-KRAB. TagBFP-tagged dCas9-KRAB (left) and overlay onto transmitted light image (right) are shown. Cells were imaged in 3D on a spinning-disk confocal microscope. Scale bar, 50 μm.