

# CERTIFICATE OF ANALYSIS

## AICS-0005:WTC-EGFP-PXN-cl50 (mono-allelic tag)

<b>Product description</b>	Human iPSC clonal line in which PXN has been endogenously tagged with EGFP 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>	350-700K 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>EGFP insertion at genomic locus - precise editing</b>	PCR and Sanger sequencing of recombinant and wildtype alleles	C-term insertion of EGFP in frame with exact predicted recombinant allele junctions. No additional mutations in either allele.	Pass
<b>Copy number</b>	ddPCR <sup>b</sup> assay for EGFP and RPP30 reference gene <sup>c</sup>	EGFP/RPP30: ~ 0.5 = Mono-allelic ~ 1.0 = Bi-allelic	Mono-allelic (0.56)
<b>Plasmid integration</b>	ddPCR assay to detect plasmid integration into the genome	AmpR/RPP30: < 0.1 = no plasmid integration	Pass (0.00)
<b>Off-target mutations</b>	1) PCR and Sanger sequencing of 5-10 sites predicted by Cas-OFFinder <sup>d</sup> 2) Whole exome sequencing <sup>e</sup>	No mutations at off-target sites assayed	1) Pass 2) Analysis in progress
<b>Other mutations</b>	Whole exome sequencing <sup>e</sup>	Check for acquired mutations (not detected in p8 <sup>a</sup> parental line) that affect genes in Cosmic Cancer Gene Census	PPM1D G1426T/E476X
<b>EGFP localization</b>	Spinning Disk confocal live cell imaging	Localization to focal adhesions	Localizes to patches at the bottom surface of the cell consistent with a localization to focal adhesions

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<b>Expression of tagged protein</b>	Western blot	Expression of expected size product	Expected size band for untagged and EGFP-tagged Paxillin; semi-quantitative results show ~50% of PXN encoded protein product is EGFP labeled
<b>Growth rate</b>	ATP quantitation <sup>f</sup>	Comparable to parental line	Pass
<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>	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 Troponin T expression (D20-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> Nimblegen V3 capture

<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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**EGFP tagging strategy:** Used CRISPR-Cas9 methodology to introduce EGFP at C-terminus of PXN as shown below.

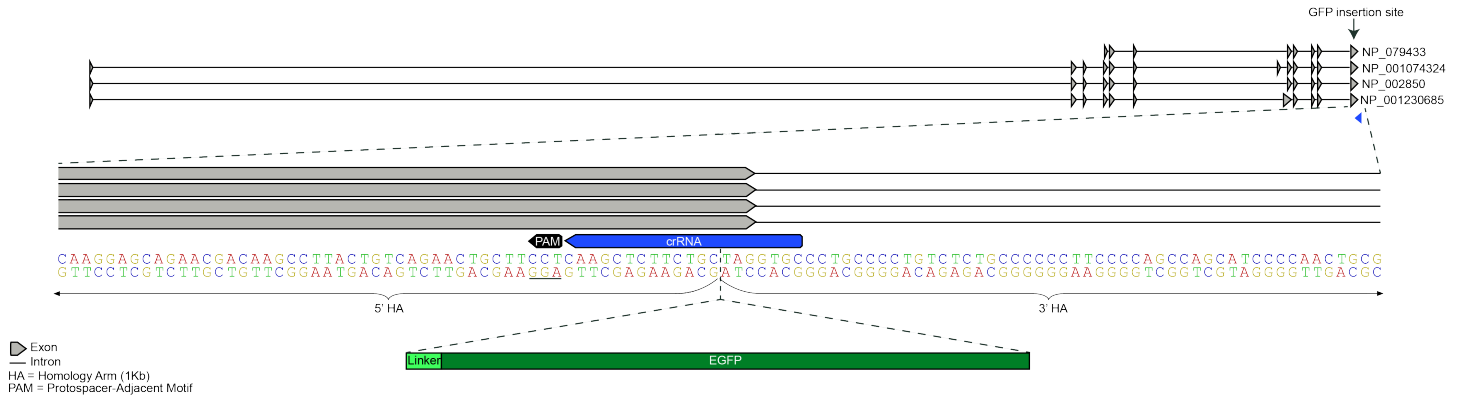


Figure 1: Top: PXN locus showing 4 PXN isoforms; Bottom: Zoom in on EGFP insertion site at PXN C-terminal exon

**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.

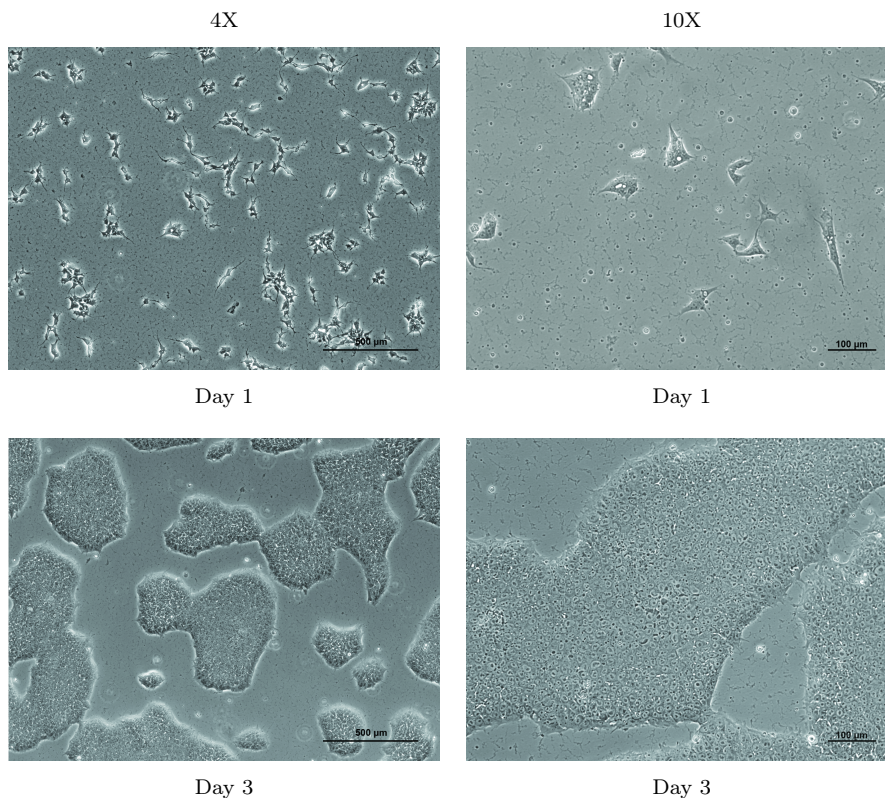


Figure 2: Viability and colony formation one day and three days post-thaw

<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-free mTeSR media (STEMCELL Technologies). Our most common microscope configuration are a Zeiss spinning disk fluorescence microscope with a Yokogawa CSUX1 head, Hamamatsu CMOS camera, and a 488 laser (GFP). 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.

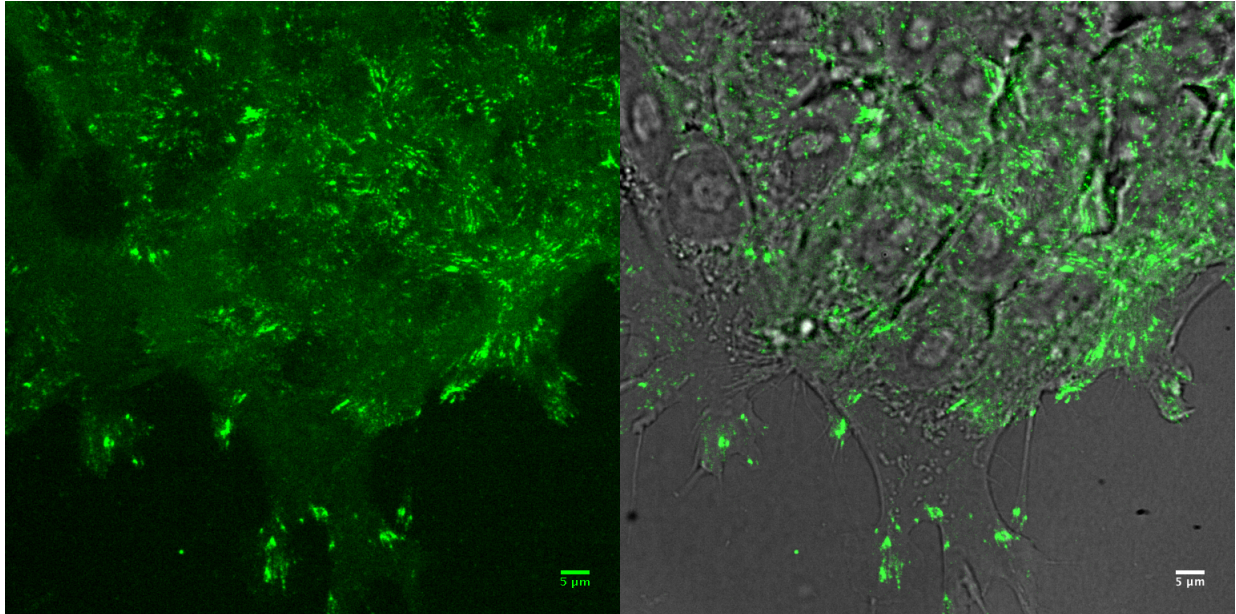


Figure 3: EGFP-tagged Paxillin localization in hiPSC colony. Left panel: Single slice of a spinning disk confocal z-stack of a live hiPSC colony. Right panel: Image on the left is overlaid onto one slice of the same z-stack in the transmitted light channel.