

CERTIFICATE OF ANALYSISAICS-0084-018:WTC Dual tagged FBL-mEGFP/NPM1-mTagRFP-T-cl18
(mono-allelic tags)

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|---|--|
| Product description | Human iPSC clonal line in which FBL and NPM1 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 | p41 ^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 | 450K cells/10-cm plate every 4 days or 900K 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 | C-term insertion of mEGFP in frame with exact predicted recombinant allele junctions. C-term insertion of mTagRFP-T in frame with exact predicted recombinant allele junctions. No additional mutations. | FBL-mEGFP: Pass NPM1-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 | FBL-mEGFP: Mono-allelic (0.534) NPM1-mTagRFP-T: Mono-allelic (0.526) |
| Plasmid integration | ddPCR assay to detect plasmid integration into the genome | AmpR/RPP30: < 0.1 = no plasmid integration | Pass (0.042) |
| 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 the dense fibrillar component of the nucleolus and localization of mTagRFP-T to the granular component of the nucleolus | mTagRFP-T-tagged nucleophosmin is visible as textured shells surrounding spheres of mEGFP-tagged fibrillarin within the nucleus consistent with localization to the nucleolar granular component and nucleolar dense fibrillar component, respectively. Localization changes throughout the course of cell division. |
| Expression of tagged protein | Western blot | Expression of expected size product | Expected size bands for untagged and mEGFP-tagged fibrillarin and for untagged and mTagRFP-T-tagged nucleophosmin. Semi-quantitative results show that 28% of FBL-encoded protein product is mEGFP labeled, and 21% of NPM1-encoded protein product is mTagRFP-T labeled. |
| Growth rate | ATP quantitation ^g | Comparable to parental line | Pass (measured at p43) ^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.

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Tagging strategy: CRISPR-Cas9 methodology was used to introduce mEGFP at C-terminus of FBL as shown below. An FBL clone was selected, and mTagRFP-T was introduced at the C-terminus of NPM1 as shown below to make a dual tag line.

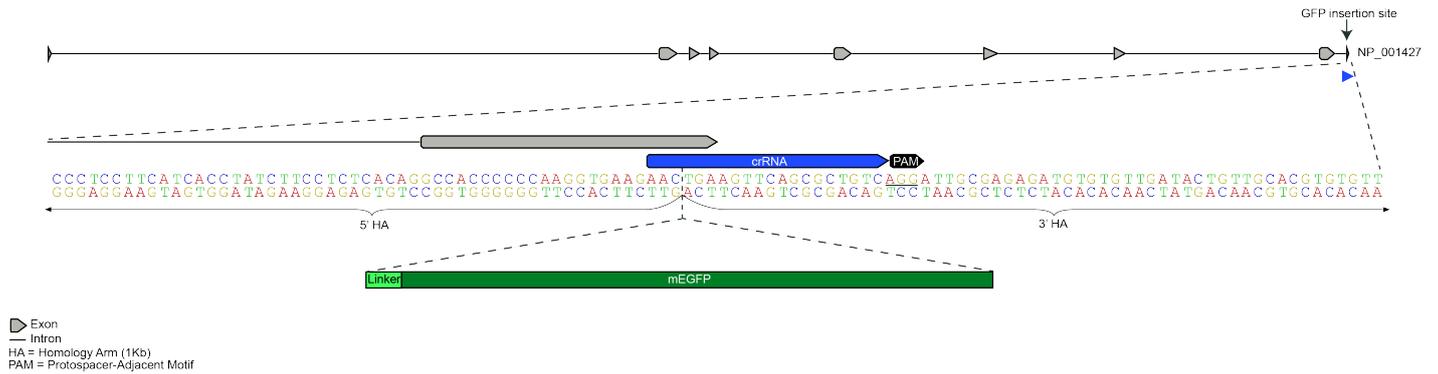


Figure 1: Top: FBL locus; Bottom: Zoom in on mEGFP insertion site at FBL C-terminal exon

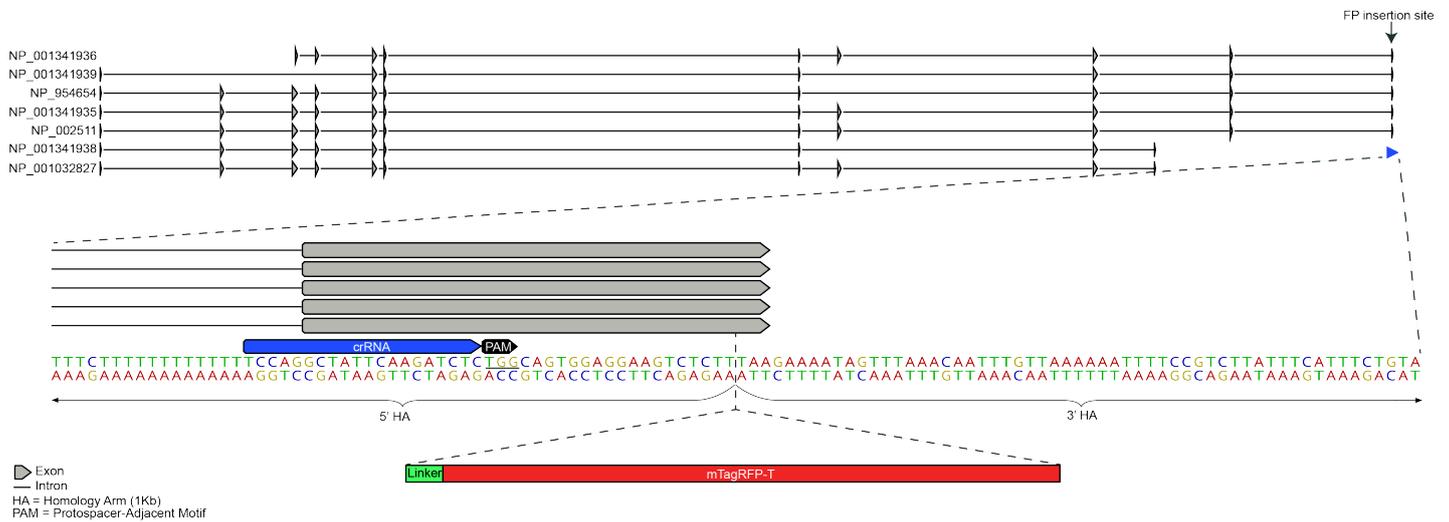


Figure 2: Top: NPM1 locus showing 7 NPM1 isoforms; Bottom: Zoom in on mTagRFP-T insertion site at NPM1 C-terminal exon

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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.

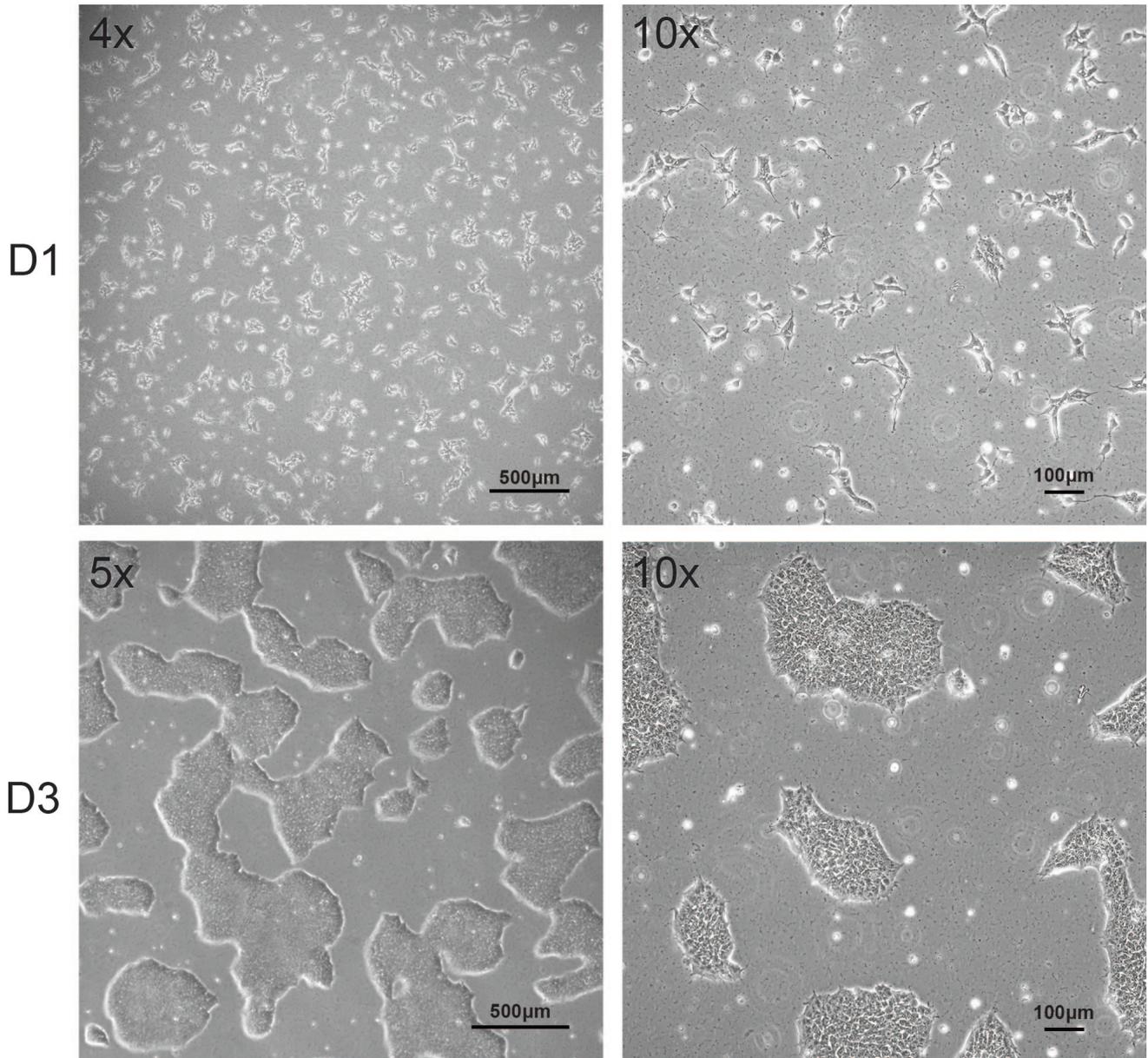


Figure 3: Viability and colony formation one day and three days post-thaw

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 (mTagRFPT-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.

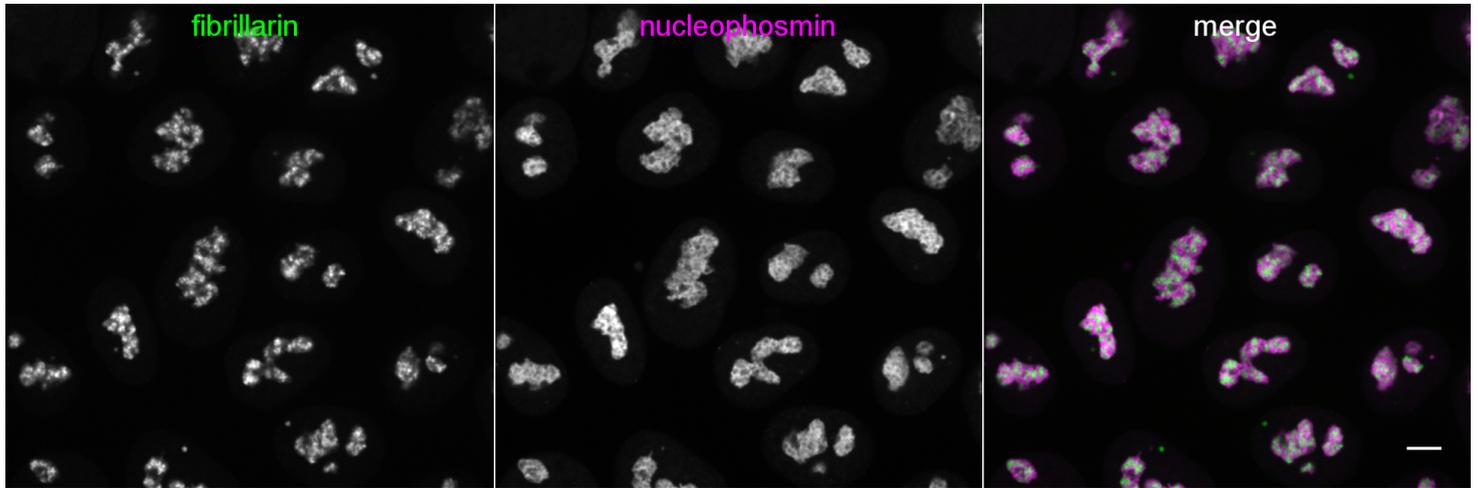


Figure 4: Single, mid-level plane of cells in a live hiPS cell colony expressing mEGFP-tagged fibrillar and mTagRFPT-T-tagged nucleophosmin. Panels show individual channels for fibrillar (left) and nucleophosmin (middle) and the overlay of the two (right). Cells were imaged in 3D on a spinning-disk confocal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