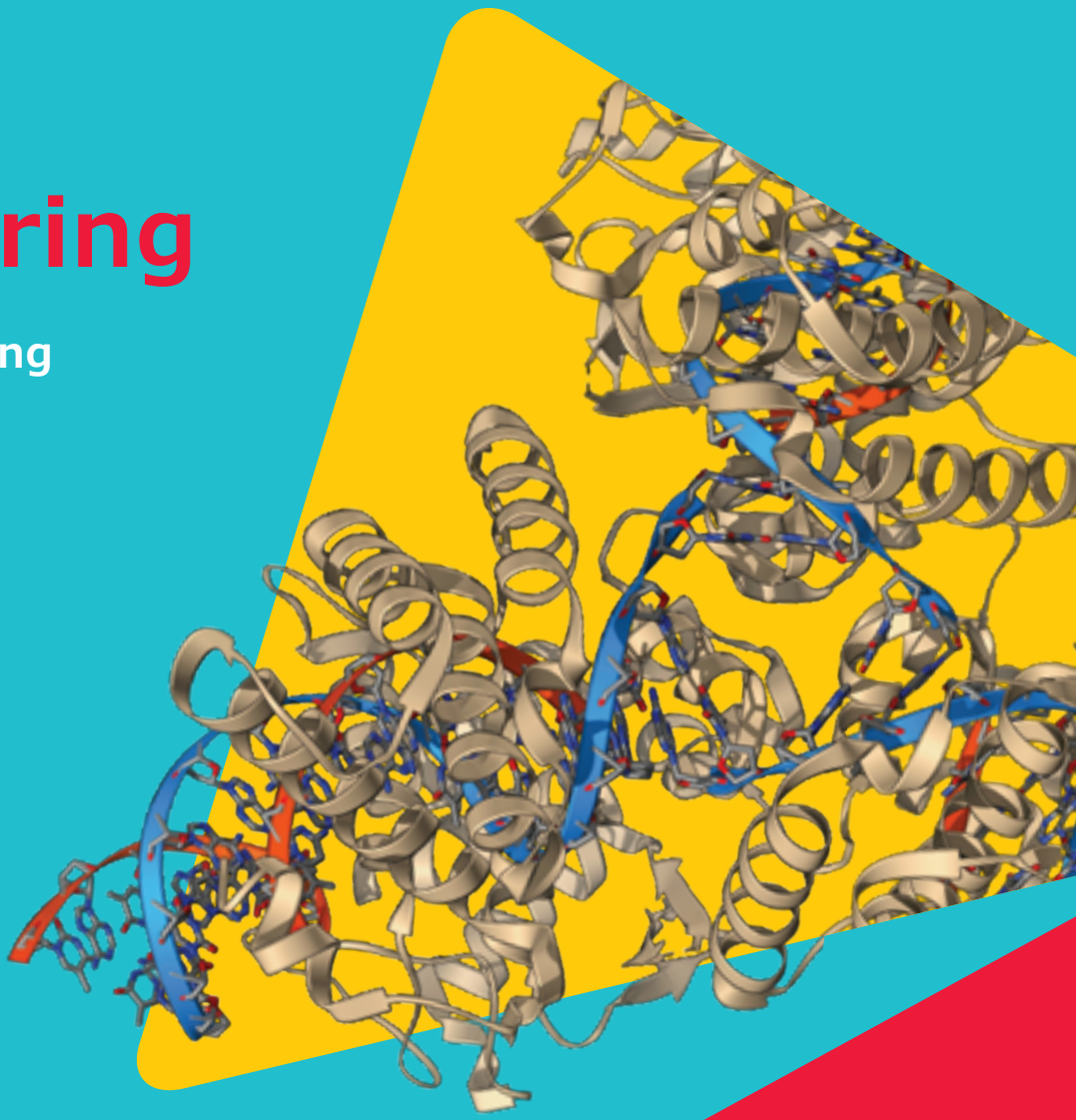
MilliporeSigma

Cell Engineering

Safe Harbor Landing Pad Cell Lines



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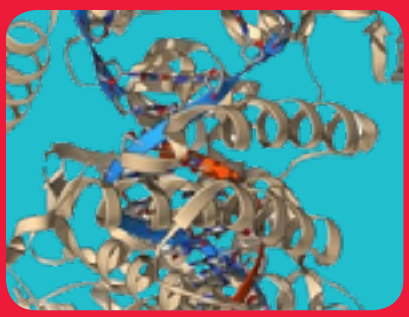

We have applied the gold standard of gene-editing technology to bring you the highest quality engineered cell lines the market has to offer.

Using our CompoZr® Zinc Finger Nucleases, we created an unparalleled range of genetically modified mammalian cell lines for use in basic research, target validation, early drug discovery and drug development.

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As a company offering custom biomolecules globally for genome editing, we are helping drive widespread adoption by researchers to fit your specific gene modification needs.

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Novel Landing Pad Technology

Concept for the Landing Pad

CompoZr® zinc finger nuclease (ZFN) technology is a fast and reliable way to manipulate the genome in a targeted fashion. ZFNs are naturally occurring proteins that can be engineered to bind DNA at a sequence-specific location and create a double strand break (www.sigma.com/zfn). The cell's natural machinery repairs the break in one of two ways: non-homologous end joining or homologous recombination. The non-homologous end joining pathway typically produces small modifications (indels) at the targeted locus that may result in a functional knockout. Single cell clones are then isolated, tested for the desired modification, and expanded to establish stable cell lines [1-3].

The homologous recombination mechanism was used to insert the landing pad construct into the AAVS1 safe harbor locus resulting in expression of mKATE2. A donor construct containing a fluorescent reporter gene (mKATE2) flanked by sequences homologous to the regions on either side of the genomic target site was nucleofected into each cell line along with ZFNs designed to cut near the genomic target site (Figure 1). Integration resulted in endogenous expression of the fluorescent protein mKATE2.

Figure 1:
Illustration of landing pad construct inserted in AAVS1 safe harbor locus. The EF1α promoter and mKATE2 gene are flanked and separated by lox sequences for easy recombination by the Cre protein.

Traditional Cell Line Engineering Workflow

The extensive timelines, cost, and technical expertise required to develop targeted CRISPR/ZFN knock-in cell lines are challenging for many labs. Additionally, project outsourcing is often too expensive for early discovery projects. These researchers require a simpler, cost-effective alternative to custom cell line engineering.

Landing Pad Workflow

Customer workflow for landing pad cells is shown below. Following nucleofection with Cre+ donor, cells can be screened for loss of the mKATE2 signal prior to cloning. Loss of mKATE2 acts as a surrogate for successful payload exchange. Cells can also be enriched for gain of fluorescence if a tagged payload is used. Loss of mKATE2 signal following donor integration takes only one to two weeks from nucleofection. Cells of interest can then be isolated and used downstream. This timeline is significantly faster than the months required for traditional cell line engineering.

Figure 2:
Overview of steps in the Landing Pad cell line engineering process.

References:

1. Carrol, D.
Genome Engineering With Zinc-Finger Nucleases. Genetics 2001; 188: 773-782. doi: 10.1534/genetics.111.131433

2. Chandrasegaran, S.
Recent advances in the use of ZFN-mediated gene editing for human gene therapy. Cell Gene Therapy 2017; 3(1): 33–41. doi:10.18609/cgti.2017.005.

3. Shrivastav M,De Haro1 LP, Nickoloff JA.
2008 Regulation of DNA double-strand break repair pathway choice. Cell Research 2008; 18:134-147.

Proof of Concept

Knock-in cells were sorted into single cells by flow cytometry and then expanded into clonal populations. Testing of these clones was used to select a landing pad clone that contained a single copy of the landing pad construct as a stable cell line. Junction PCR, digital droplet PCR, and fluorescent analysis showed that the landing pad construct is inserted into a single allele in the selected clone.

mKATE2 can easily be exchanged for a payload of the user's choice using Cre recombinase and a targeting vector with appropriate lox sites (Figure 2). Cells can then be sorted via fluorescence-activated cell sorting (FACS) for loss of mKATE2 expression as a surrogate for successful integration of the targeting vector. Approximately 7-10 days are required for loss of the mKATE2 signal in successfully targeted cells.

Figure 3:
Exchange of Landing Pad payload exchange using Cre recombinase and targeting vector with appropriate LoxP elements.

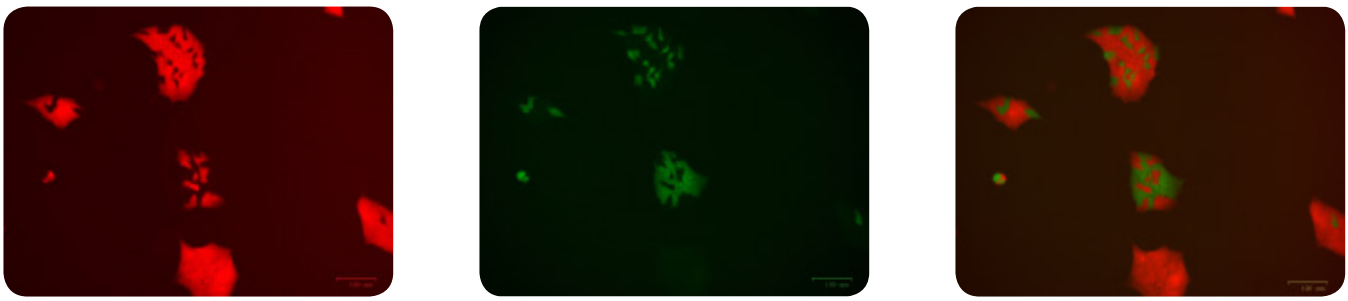


Figure 4:
Landing Pad Payload Exchange- mKATE2 population (red), EGFP population(green), merge.

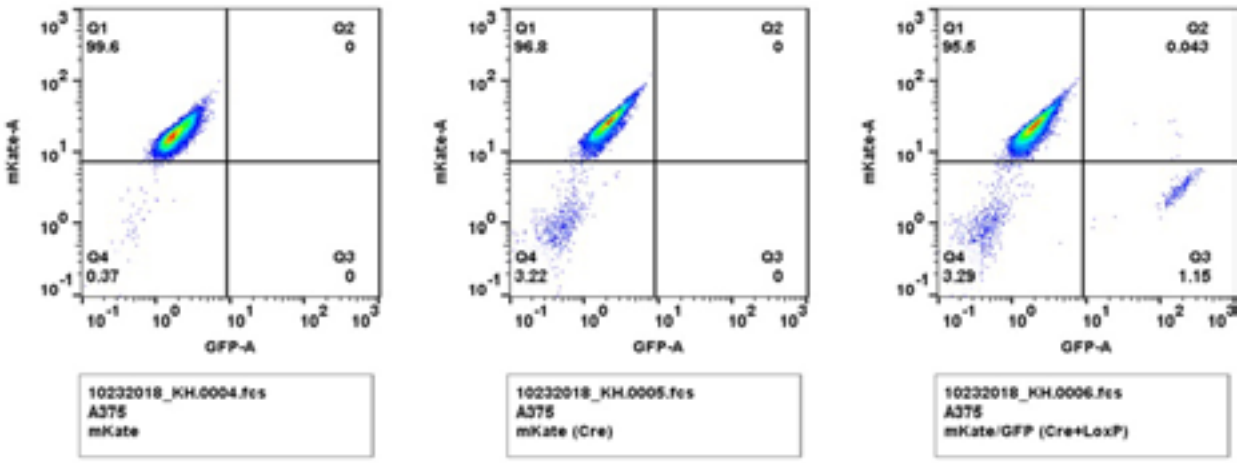


Figure 5:
A375 cell LPCL. Left Panel: Parental unmodified. Middle Panel: nucleofection with Cre only. Right Panel: nucleofection with Cre and GFP exchange plasmid; gate Q3 demonstrates the GFP expressing population 7 days post nucleofection.

Jurkat T Lymphocytes

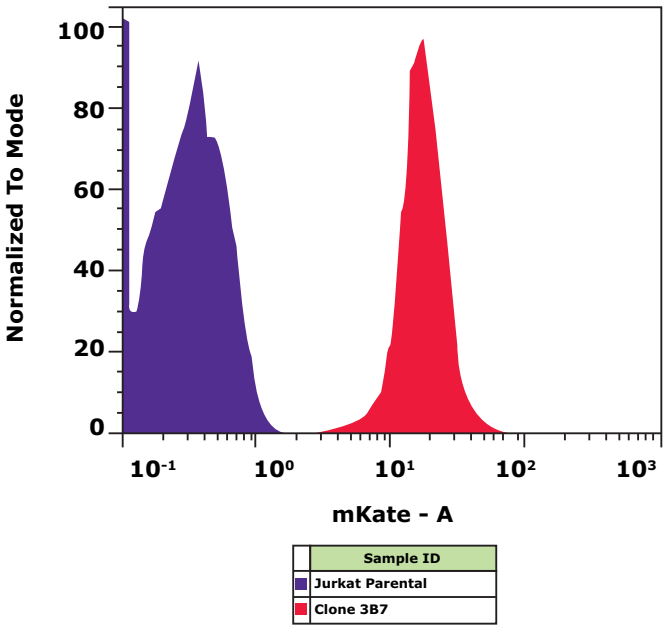
Jurkat T lymphocytes are a human, acute T cell lymphoma cell line isolated in the late 1970s from the peripheral blood of a young male patient suffering from T cell leukemia. The cells possess a pseudodiploid karyotype and have been characterized as expressing CD3 and, upon stimulation, interleukin-2. This line has also been used to determine the cytotoxicity of the cytolethal distending toxin (CDT) holotoxin and its components and the study of expression of bisphenol A exposed estrogen receptor-β (ERβ) and estrogen-related-receptor-α (ERRα) *in vitro.*

Cell Line Description

- Organism: *homo sapiens* (human)
- Tissue: Lymphocyte; peripheral blood
- Age: 14 years
- Gender: Male
- Morphology: Lymphoblast
- Growth properties: Suspension

Benefits

- Single copy integration into the AAVS1 Safe Harbor locus; confirmed by junction PCR (jPCR) and CNV analyses.
- Continuous stable expression of the far-red fluorophore mKATE2.
- Rapid and robust Cre mediated exchange with matching lox2272 and loxP plasmid or PCR amplicon with your gene of interest (GOI).
- Select for mKATE2 negative population by FACS sorting.
- Have an enriched mKATE2 negative population within 2-4 weeks.



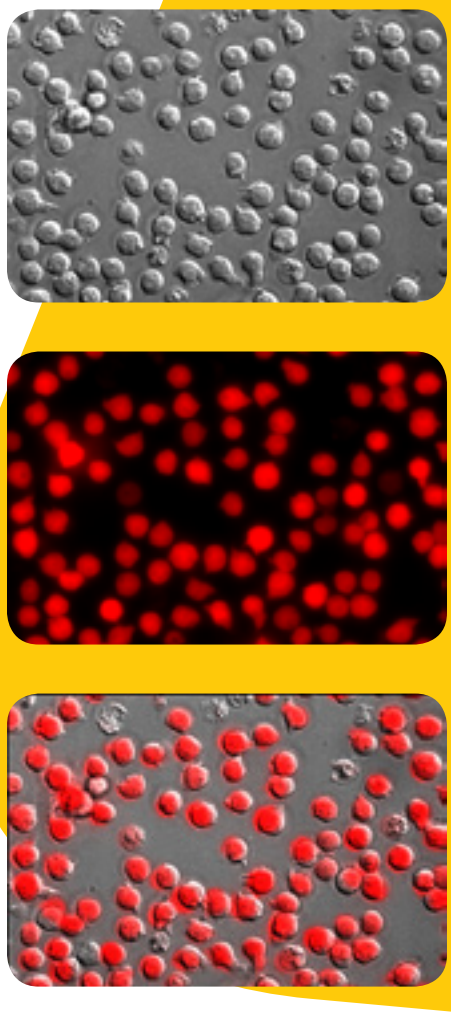


Figure 6.
Imaging of the Jurkat landing pad cell line at 40X magnification. Top image demonstrates the homogenous expression of mKATE2 in the cell line. Middle, a bright field image of the same culture showing 90% confluent Jurkat culture. Bottom, merged image showing all the cells in the culture are stably expressing mKATE2.

A549 Cancer Cells

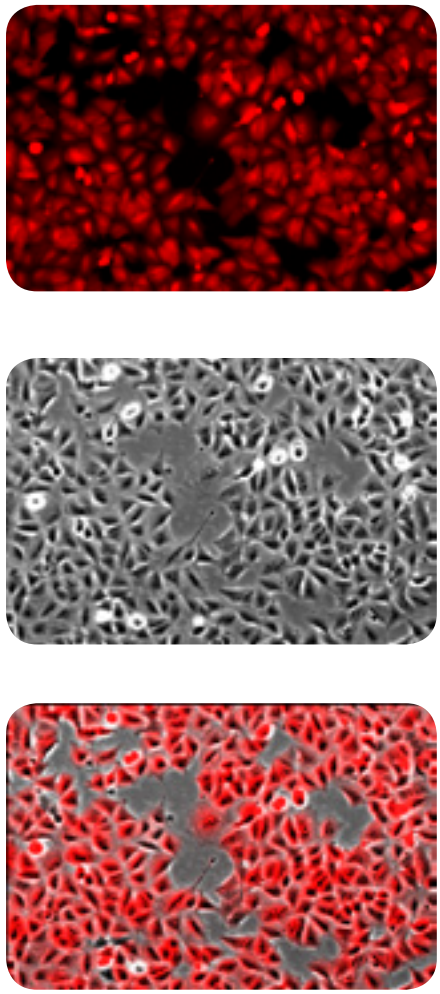
A549 is a human lung carcinoma cell line isolated in 1972 from a lung tumor of a male patient suffering from carcinoma. The cells possess a hypotriploid karyotype, express EGFR and mutant KRAS. A549 cells have been used to study proliferation induced by IGF1 as well as growth inhibition by BChTT. In addition, these cells have been used to model alveolar type II pneumocyte differentiation.

Cell Line Description

- Organism: *homo sapiens* (human)
- Tissue: Carcinoma; lung
- Age: 58 years
- Gender: Male
- Ethnicity: Caucasian
- Morphology: Epithelial
- Growth properties: Adherent

Benefits

- One copy of the cassette integrated into the AAVS1 Safe Harbor locus; confirmed by junction PCR (jPCR) and CNV analyses.
- Continuous expression of the far-red fluorophore, mKATE2.
- Rapid and robust Cre mediated exchange with matching lox2272 and loxP plasmid or PCR amplicon containing your gene of interest (GOI).
- Select for mKATE2 negative population by FACS sorting or other fluorescence assisted instrument.



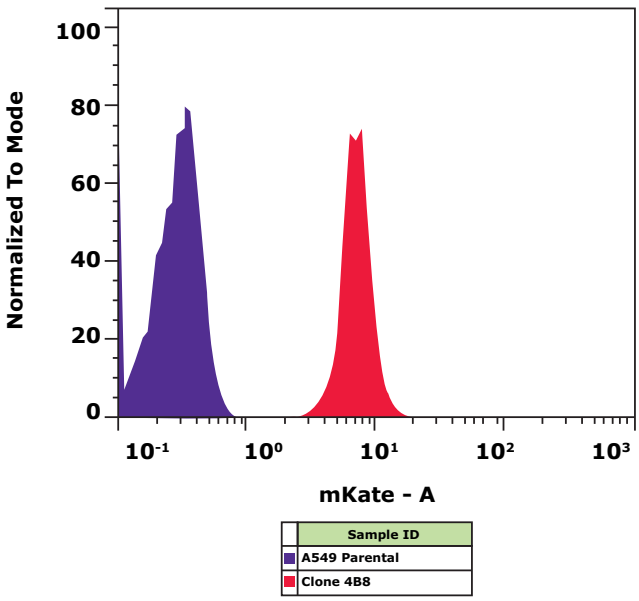


Figure 7.
Imaging of A549 cells landing pad cell line at 10X magnification. Top image demonstrates the homogenous expression of mKATE2 in the cell line. Middle, a bright field image of the same culture showing 90% confluent A549 culture. Bottom, merged image showing all the cells in the culture are stably expressing mKATE2.

A375 Cancer Cells

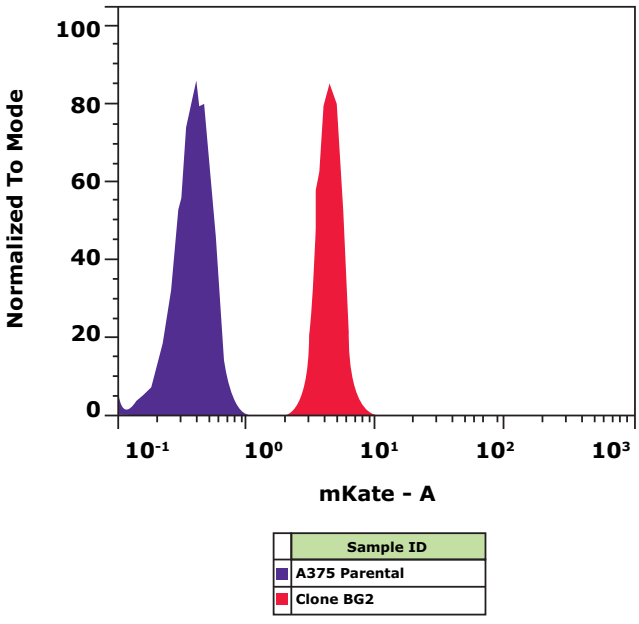
A375 cancer cells are a human malignant melanoma cell line isolated from a 54-year old female. The cells possess a hypotriploid karyotype. The cell line produces rapidly growing amelanotic melanomas in anti-thymocyte serum treated NIH Swiss mice. A375 cells have been used to obtain paracrine factors for prolonged culture of mesenchymal stromal cells (MSCs). It has also been used to study oncolytic activity of the peptide LTX-315.

Cell Line Description

- Organism: *homo sapiens* (human)
- Tissue: Carcinoma; lung
- Age: 54 years
- Gender: Female
- Ethnicity: Caucasian
- Morphology: Epithelial
- Growth properties: Adherent

Benefits

- One copy of the cassette integrated into the AAVS1 Safe Harbor locus; confirmed by junction PCR (jPCR) and CNV analyses.
- Continuous expression of the far-red fluorophore, mKATE2.
- Rapid and robust Cre mediated exchange with matching lox2272 and loxP plasmid or PCR amplicon containing your gene of interest (GOI).
- Select for mKATE2 negative population by FACS sorting or other fluorescence assisted instrument.
- A375 landing pad cell lines can be used to accelerate the study of transporters and other ADME/tox related genes.



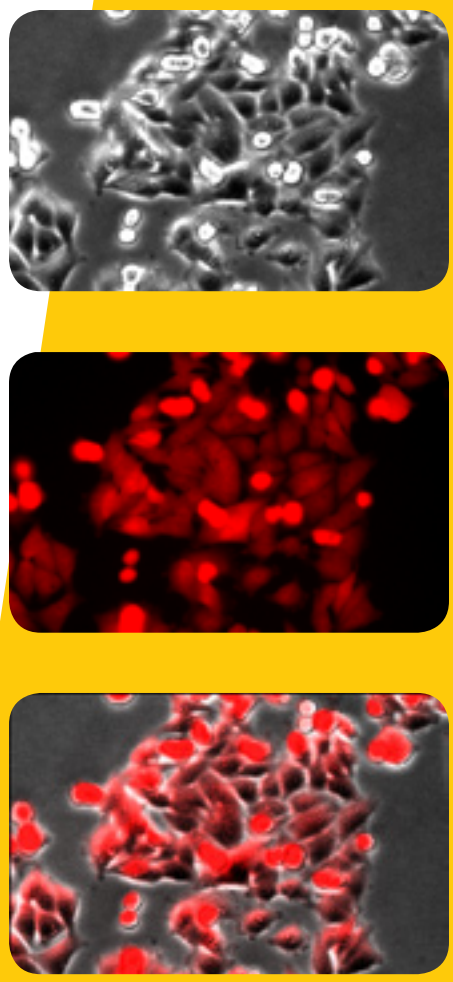


Figure 8.
Imaging of A375 cells landing pad cell line at 20X magnification. Top image demonstrates the homogenous expression of mKATE2 in the cell line. Middle, a bright field image of the same culture showing 90% confluent A375 culture. Bottom, merged image showing all the cells in the culture are stably expressing mKATE2.

HCT-116 Cancer Cells

HCT-116 cancer cells are a human colorectal carcinoma cell line isolated from the colon of an adult male. The cells possess a near diploid karyotype and express carcinoembryonic antigen.

Cells are tumorigenic in nude mice and form colonies on agarose. HCT-116 cells have been used to study the importance of cyclin D1 for the activity of lithocholic acid hydroxyamide (LCAHA).

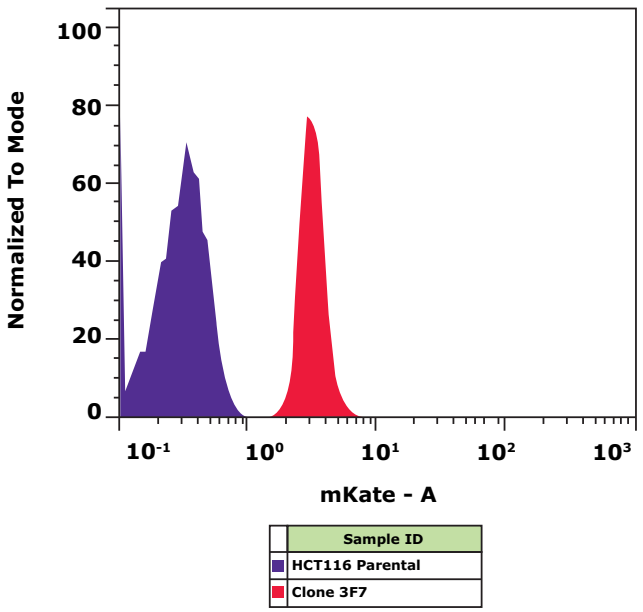
In addition the cells have been used in studies pertaining to iron uptake.

Cell Line Description

- Organism: *homo sapiens* (human)
- Tissue: Carcinoma; colon
- Gender: Male
- Morphology: Epithelial
- Growth properties: Adherent

Benefits

- One copy of the cassette integrated into the AAVS1 Safe Harbor locus; confirmed by junction PCR (jPCR) and CNV analyses.
- Continuous expression of the far-red fluorophore, mKATE2.
- Rapid and robust Cre mediated exchange with matching lox2272 and loxP plasmid or PCR amplicon containing your gene of interest (GOI).
- Select for mKATE2 negative population by FACS sorting or other fluorescence assisted instrument.
- HCT-116 landing pad cell lines can be used to accelerate the study of transporters and other ADME/tox related genes.



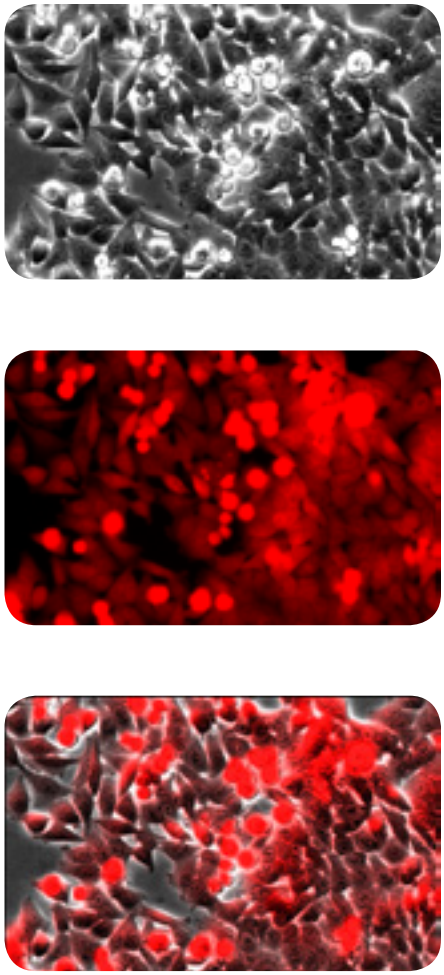


Figure 9.
Imaging of HCT-116 cells landing pad cell line at 20X magnification. Top image demonstrates the homogenous expression of mKATE2 in the cell line. Middle, a bright field image of the same culture showing 90% confluent HCT-116 culture. Bottom, merged image showing all the cells in the culture are stably expressing mKATE2.

THP-1 Cells*

Derived from the peripheral blood of a 1-year old male with acute monocytic leukemia. THP-1 cells have Fc and C3b receptors and lack surface and cytoplasmic immunoglobulins. These cells also stain positive for α-napthhyl butyrate esterase, produce lysozymes and are phagocytic (both latex beads and sensitized erythrocytes). THP-1 cells can also restore the response of purified T lymphocytes to Concanavalin A, show increased CO_2 production on phagocytosis, and can be differentiated into macrophage-like cells using for example DMSO.

Cell Line Description

- Organism: *homo sapiens* (human)
- Tissue: Monocyte; peripheral blood
- Age: 1 year
- Gender: Male
- Morphology: Monocyte
- Growth properties: Suspension

Benefits

- THP-1 cells are highly sensitive to exogenous DNA making gene editing challenging.
- Cre mediated DNA exchange allows for more efficient integration of genes of interest using mKATE negative selection.
- Expands capabilities of THP-1 cells for genetic engineering.

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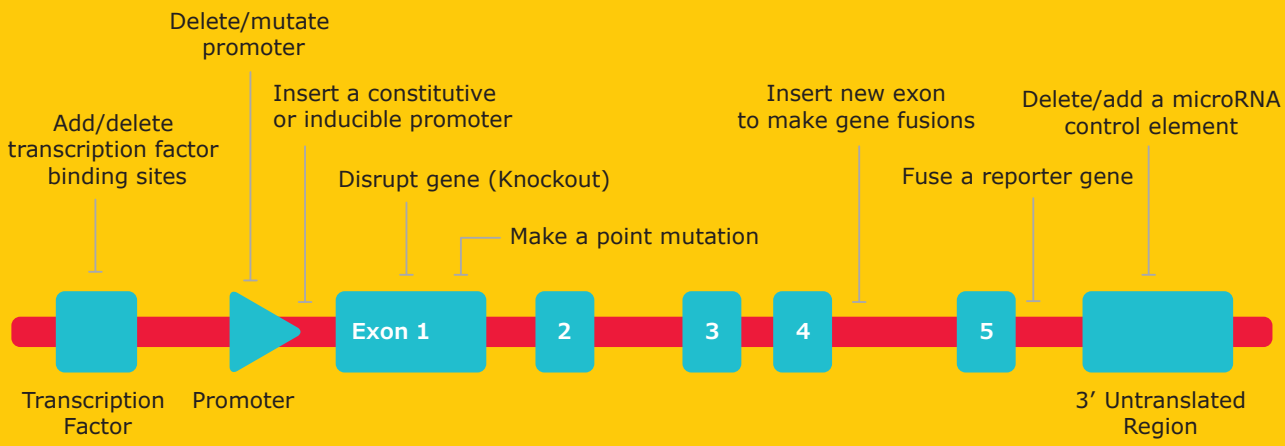

Evaluation → Nuclease Design and Assembly → Transfection and Activity Confirmation → Single Cell Isolation and Expansion → Genotype Confirmation → Safety and Sterility Testing → Delivery

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- Cellular Reporters
- Drug Screening
- Target ID/Validation
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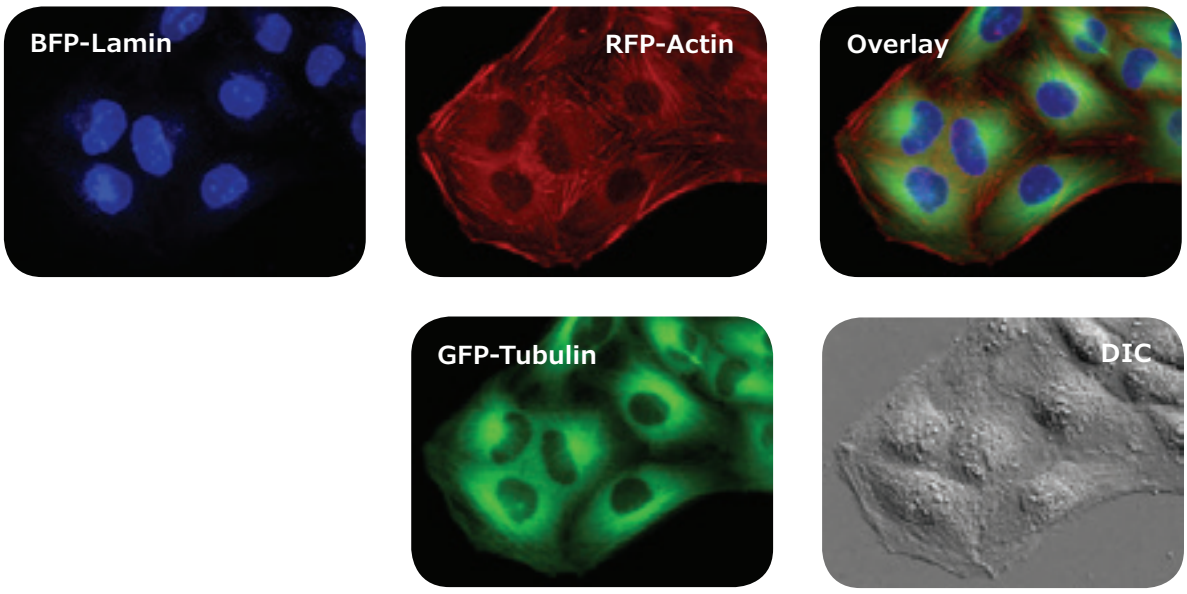

Triple-tagged reporter cell line used for high content imaging and small molecule screening

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