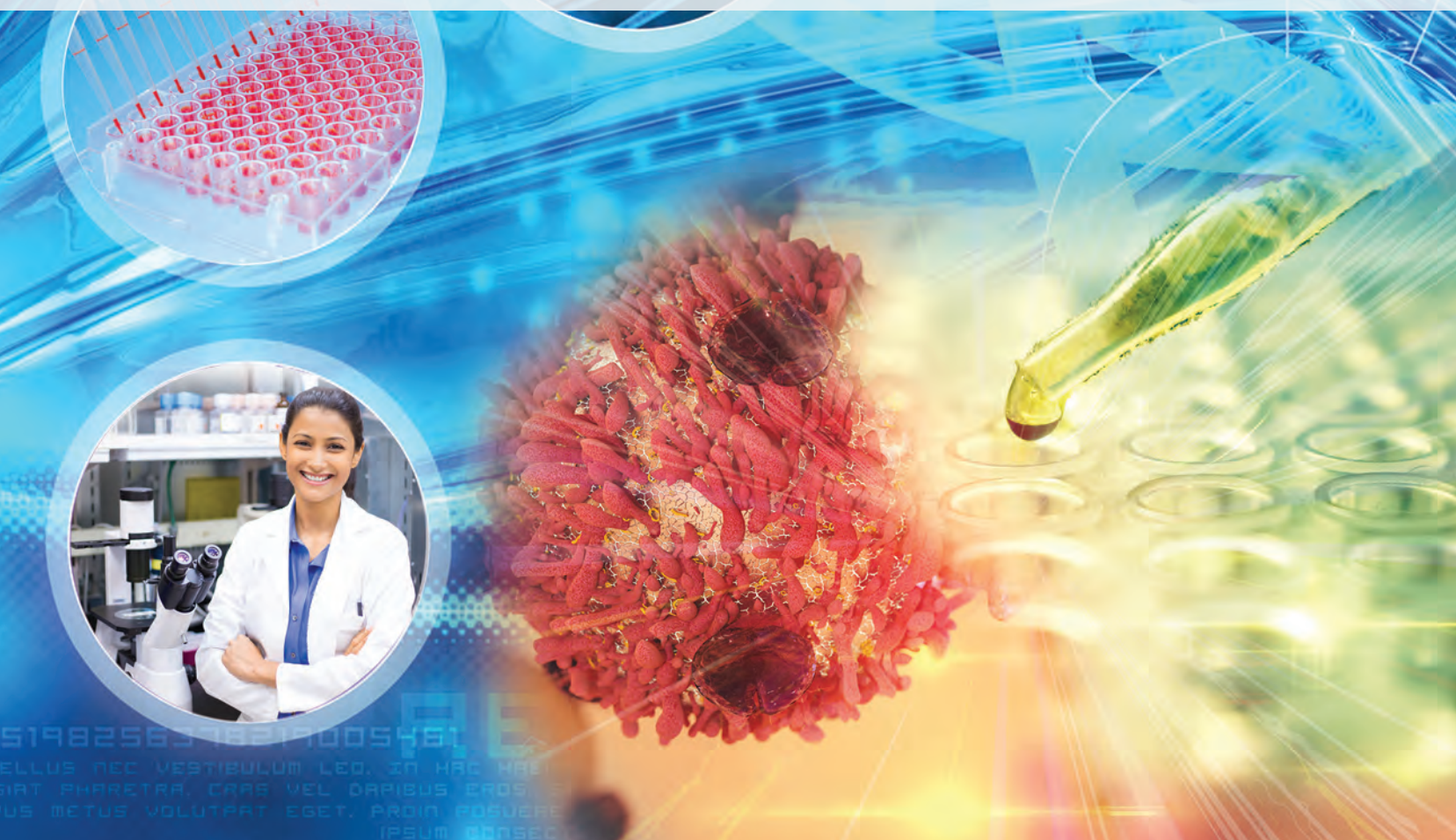
Applied StemCell

Genome editing *in vitro* and *in vivo*

Cell Line Models

Custom Model Generation • Antibody Discovery/Screening

CHO Bioproduction Cell Lines • Preclinical Cell-Based Assays



www.appliedstemcell.com

Knockout/Knock-in Cell Line Model Generation and Cell-Based Assays

Precision engineering of predictive *in vitro* models of human biology and disease; downstream phenotype analysis, and early stage drug screening assays

Why work with ASC?

- Leading service provider for cell line genome editing services and enabling tools for cancer research
- Multi-technology, multi-approach genome editing: TARGATT™, CRISPr/Cas9, Lentivirus, and more
- Successfully delivered > 1300 cell line models in > 200 distinct cell lines
- Downstream custom assay solutions for a comprehensive project package
- FDA and IND compliant documentation
- Seamless workflow from cell lines to animal models

Site-Specific Knock-in Technology TARGATT™

ISO 9001 & 13485 Certified

Custom Knockout cell lines as negative controls for Antibody Validation

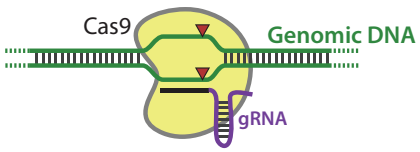
New! UniSelect™ Antibody Discovery and Screening, a fast, unique and efficient way to build your own mammalian cell-based library for Antibody Discovery and Screening...
(more details on page 9)

High-yield CHO Cell lines for Recombinant Protein and Antibody Bioproduction

Contents of this Brochure:

ASC's Genome Editing Technology Overview

CRISPR/Cas9



Knockout
(frameshift or targeted deletion)

Point Mutation

Conditional Knockout

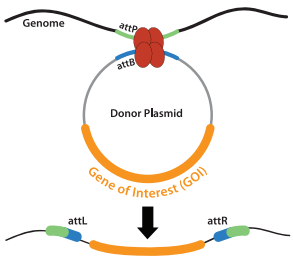
Conditional/ Induced Expression

Gene Overexpression

Gene Tagging

Reporter Gene Knock-in

TARGATT™
Exclusive to ASC



Comparing Genome Editing Technologies

Project Purpose	CRISPR/Cas9	TARGATT™
Knock-Out (KO)	**Yes**	
Point Mutation (PM)	**Yes**	
Conditional KO (CKO)	**Yes**	
Knock-In (KI) < 10kb	**Yes**	
Knock-In > 10kb; Safe Harbor Loci	**Challenging** (limitations on size)	**Yes**

Leverage our extensive expertise in cell line model generation and associated services to advance your research discoveries:

Basic Research

Disease Model Generation

Isogenic Cell Line Models

Drug Efficacy and Toxicity Screening

Protein and Antibody Library Generation

CHO Bioproduction

For hard-to-transfect cell lines, such as primary cells and blood lineage cell lines, we offer an extended technology portfolio using lentivirus technology to generate the cell line model apt for your research needs.

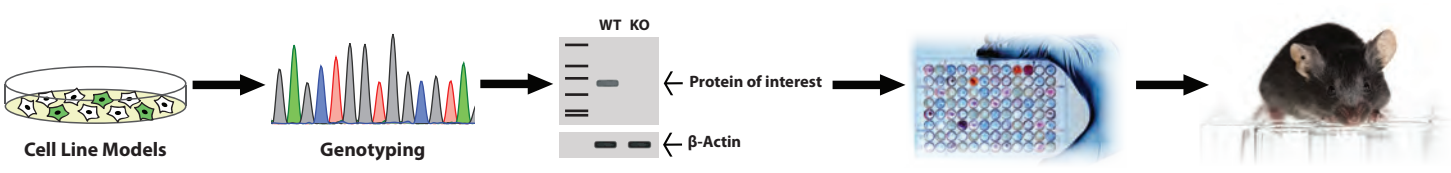
CRISPR/Cas9 Cell Line Model Generation: *https://www.appliedstemcell.com/services/crispr-cas9-genome-editing/cell-line-models*
TARGATT™ Cell Line Model Generation: *https://www.appliedstemcell.com/services/targatttm-genome-editing/gene-knock-in-technology*

Custom Cell Line Model Generation Timelines

Technology:	CRISPR/Cas9	CRISPR/Cas9	CRISPR/Cas9	CRISPR/Cas9	TARGATT™/Cas9
Project Purpose:	KO	PM	cKO	KI	Safe harbor locus KI*
Timeline:	3-5 months	3-5 months	3-5 months	3-5 months	3-5 months

**Knock-in timeline only for TARGATT™ Master Cell Line pre-engineered with an integrase recognizing docking site; does not include TARGATT™ Master Cell Line Generation. Refer to CRISPR/Cas9 KI service for Master Cell Line Generation timeline.*

Add-on Downstream Custom Cell-Assays and/or *In Vivo* Model Generation for a Seamless and Effortless Research and Discoveries



CRISPR Cell Line Model Generation Services

There is no "one-size" fits all CRISPR cell line protocol! With > 10 years genome editing expertise and as a leading CRISPR service provider, ASC understands that each cell line and targeted gene is unique, and therefore uses the most optimized strategies to achieve a >98% success rate in engineering cell line models according to customers' specifications. Our scientists and project managers will work with you at every stage of your project to engineer a mutation of choice in your cell line and gene of interest. Leverage our expertise in engineering biorelevant cell line models to advance cancer research discoveries and antibody validation applications.

Benefits and Applications

- Engineered 1300+ cell line models in >200 distinct mammalian cell lines with a wide variety of mutations
- ISO:9001 certified quality of service
- Custom deliverables such as: choice of homozygous/ heterozygous clones; footprint-free mutations for preclinical gene/ cell therapy applications

Service Includes:

- Targeting vector construction and validation
- Cell culture and optimization
- Transfection, clone screening and confirmation
- Single cell cloning
- Expansion and cryopreservation

Optional! **Phenotype validation/assessment, cell-based assays and drug screening services available.**

Knockout
(Region Specific/ Frameshift)

Point Mutation

Transgene Insertion
(Locus Specific/Safe Harbor Locus)

Reporter Gene Knock-in

Conditional Gene Knockout

Deliverables and Timeline

- Genetically engineered cells with confirmed mutations
- Dedicated project management to provide detailed milestone and final project reports
- Comprehensive report on technical details, genotyping strategy, etc.

Timeline: in as little as 3 months (varies by project type)

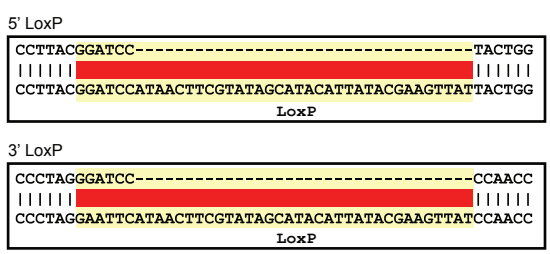
CRISPR/Cas9 Cell Line Service:
https://www.appliedstemcell.com/services/crispr-cas9-genome-editing/cell-line-models/cell-line-modification

Case Studies

1. Conditional Knockout in HCT116 cells

Goal: To create a conditional knockout cell line model for the gene of interest in HCT116 cells.

How? The CRISPR/Cas9 system was used to insert two LoxP sites, flanking exons 4-6 of the gene of interest. A set of two gRNAs was used for this approach.

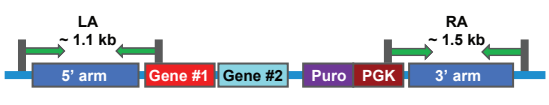


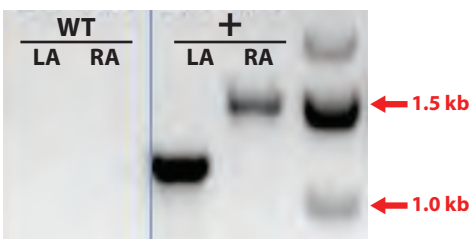
Result: Two homozygous conditional knockout clones containing the floxed allele were identified and confirmed by PCR and sequencing.

2. Large "Fusion" Transgene Knock-in in A549 Cell Line

Goal: To insert an 8.5 kb fusion transgene downstream of a specific locus in A549 cancer cell line.

How? A donor plasmid was constructed with Gene #2 sequences downstream of specific Gene #1 sequence. After transfection with validated gRNA, donor plasmid and Cas9, transfected cells were selected by puromycin selection. Transgene knock-in and expression was confirmed by PCR gel electrophoresis and qPCR (not shown).





Result: PCR gel electrophoresis using primers for 5' arm (LA) and 3' arm (RA) showed a ~1.1 kb and a ~1.5 kb fragment, respectively, confirming site-specific insertion of transgene.

Selected Cell Lines Successfully Modified Using CRISPR/Cas9

Cell	Species	Tissue	Cell Type	Disease
Blood Lineage Cells:				
BCWM-1*	Human	Bone marrow	Lymphoplasmacytic	Waldenstrom macroglobulinemia
Jurkat	Human	Peripheral blood	T lymphocyte	Acute T cell leukemia
JVM2	Human	Peripheral blood	Lymphoblast	Mantle Cell Lymphoma
K562	Human	Bone Marrow	Lymphoblast	Chronic myelogenous leukemia (CML)
KG-1	Human	Bone	Lymphoblast	Acute myelogenou leukemia
KHYG-1*	Human	Peripheral blood	T lymphocyte	Natural killer cell leukemia
MOLM-13	Human	Peripheral blood	Monocyte-like	Acute myeloid leukemia
MWCL-1	Human	Bone marrow	Lymphoplasmacytic	Waldenstrom macroglobulinemia
T2	Human	Blood lineage	Lymphocyte	
TF-1	Human	Bone marrow	Erythroblast	Erythroleukemia
U937	Human	Lymphocyte	Monocyte	Histiocytic lymphoma
Cancer Cell Lines:				
786-0	Human	Kidney	Epithelial	Renal cell adenocarcinoma
A375	Human	Skin	Epithelial	Malignant melanoma
A549	Human	Lung	Epithelial	Carcinoma
DLD-1	Human	Colon	Epithelial	Dukes' type C, colorectal adenocarcinoma
HCT116	Human	Colon	Epithelial	Colorectal carcinoma
HEK293; HEK293T	Human	Embryonic kidney	Epithelial	
Hela	Human	Cervix	Epithelial	Cervical cancer
HepG2	Human	Liver	Epithelial	Hepatocellular carcinoma
HT1080	Human	Connective Tissue	Epithelial	Fibrosarcoma
Huh7	Human	Liver	Epithelial	Hepatocellular carcinoma
KYSE-270	Human	Esophagus	Epitheloid	Esophageal squamous cell carcinoma
LNCaP	Human	Prostrate	Epithelial	Prostrate adenocarcinoma
MALME-3M	Human	Lung (metastatic)	Fibroblast	Malignant melanoma
MCF7	Human	Mammary gland	Epithelial	Adenocarcinoma
PANC1	Human	Pancreas/duct	Epithelial	Epithelioid carcinoma
RKO	Human	Colon	Epithelial	Carcinoma
SH-SY5Y	Human	Bone Marrow	Epithelial	Neuroblastoma
T47D	Human	Mammary gland	Epithelial	Ductal carcinoma
U-2 OS	Human	Bone	Epithelial	Osteosarcoma
Other Cell Lines:				
ARPE-19	Human	Eye	Retinal (pigmented epithelium)	Normal
BEAS-2B	Human	Lung	Epithelial	Normal
Fibroblast (primary)	Human		Primary fibroblast	Normal
HaCaT	Human	Skin	Keratinocyte	Normal
hTERT RPE	Human	Retina (pigmented epithelium)	Epithelial	
MCF10	Human	Mammary gland	Epithelial	Fibrocystic disease
Other Species:				
AGMK GL37	African Green Monkey	Kidney	Epithelial	Normal
CHO-S	Hamster	Ovary	Epithelial-like	
3T3-Swiss albino	Mouse	Embryo	Fibroblast	Normal
B16-F10	Mouse	Skin	Spindle/Epithelial-like	Melanoma
CT-26	Mouse	Colon	Fibroblast	Carcinoma
Mc-38	Mouse	Colon	Epithelial	Colon adenocarcinoma
mEERL	Mouse	Lung	Epithelia	Orpharyngeal squamous cell carcinoma
Neuro-2a	Mouse	Brain	Neuroblast	Neuroblastoma
NIH/3T3	Mouse	Embryo	Fibroblast	Normal
PCCL3	Rat	Thyroid	Epithelium	
RAW 264.7	Mouse	Ascites	Macrophage	Abelson murine leukemia virus-induced tumor
Renca	Mouse	Kidney	Epithelial	Renal adenocarcinoma
RCS	Rat	n/a	Chondrocytes	Chondrosarcoma
SW10	Mouse	Neuronal Schwann cell	Neuronal	
Stem Cells: Induced Pluripotent Stem Cells (iPSCs) and Embryonic Stem Cells (ESCs)				
iPSC	Human	PBMC/ Skin/ Cord blood	PBMC/Fibroblasts	Normal/ Disease
ESC	Human	inner cell mass	Embryonic stem cell	Normal/ Disease
iPSC	Mouse, Primate, Others	Skin	Fibroblast	Normal
ESC	Mouse, Rat, Macaque	inner cell mass	Embryonic stem cell	Normal

CRISPR Blood Lineage Cell Line Gene Editing Services

Leverage our CRISPR expertise for hard-to-modify cell lines! ASC offers full service to genetically modify "hard-to-transfect" blood lineage cell line models (such as Jurkat, TF-1, Lymphocytes) using CRISPR/Cas9 technology. Our custom gene modification services include knockout, knock-in, and point mutation to name a few.

Benefits and Applications

- High success rate in genome editing hematopoietic lineage cell lines
- ISO:9001 certified quality of service
- Adherent/ suspension cell lines; slow growing and hard-to-transfect
- Multi-approach protocols using DNA-based, protein, or viral-based CRISPR reagents
- Choice of custom deliverables such a homozygous or heterozygous clones
- Custom Cas9 expressing cell line(s) and isogenic cell line generation

Deliverables and Timeline

- Genetically engineered cells with confirmed mutations
- Dedicated project management to provide detailed milestone and final project reports
- Comprehensive report on technical details, genotyping strategy, etc.

Timeline: in as little as 3 months (varies by project type)

Service Includes:

- Targeting vector construction and validation
- Cell culture and optimization
- Single cell cloning
- Transfection, clone screening and confirmation
- Expansion and cryopreservation

CRISPR/Cas9 Cell Line Service:
https://www.appliedstemcell.com/services/crispr-cas9-genome-editing/cell-line-models/crispr-blood-cells

Lentivirus Stable Cell Line Generation:
https://www.appliedstemcell.com/research/services/stable-cell-line-generation-service

Case Studies

1. Targeted Large Fragment Deletion in Jurkat Cells

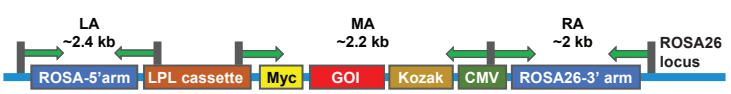
Goal: To delete a targeted (~5 kb) region in the enhancer region of the gene of interest in Jurkat cells.

How? Validated gRNAs were co-transfected with Cas9-expressing plasmid. After puromycin selection and single cell cloning, PCR gel electrophoresis followed by Sanger sequencing was used to identify heterozygous clones with ~5 kb deletion.

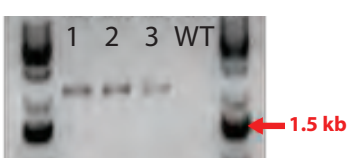
Result: Sequence alignment of target region between positive heterozygous clone (#2) and wild type (WT) confirmed deletion of required sequence.

2. Large Transgene Knock-in in T2 Cell Line

Goal: To insert a 5 kb gene fragment into the hROSA26 locus in T2 lymphoblastic cell line.



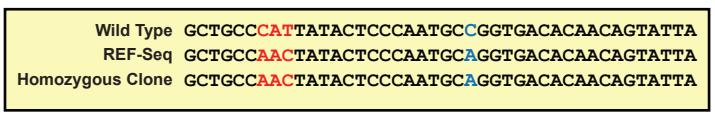
How? T2 cells were transfected with donor plasmid and a biscistronic vector cloned with a validated gRNA targeting hROSA26 locus, and Cas9. Single cell clones were screened by PCR/ gel electrophoresis with primer sets to confirm site-specific insertion of the gene of interest (GOI) at the right locus, followed by Sanger Sequencing (not shown): LA: Left arm primers; RA, right arm primers; MA: transgene primers.



Result: Three clones (1-3) containing the GOI at the hROSA26 locus were confirmed using PCR gel electrophoresis with primers for transgene (MA) and locus (LA, RA; not shown).

3. Homozygous Point Mutation in K562 Cell Line

Goal: To obtain a homozygous point mutation (CAT > AAC) in the gene of interest in K562 cells.



How? K562 cells were transfected with Cas9 protein, validated gRNA, donor plasmid containing point mutation and a silent mutation in the gRNA cut site, and a DNA-dependent protein kinase to improve HDR efficiency.

Result: Representative sequence alignment of a homozygous clone with reference sequence (Ref-seq) and wildtype sequence confirmed required mutation in targeted locus.

Gene Fusions Cell Lines for Cancer Research

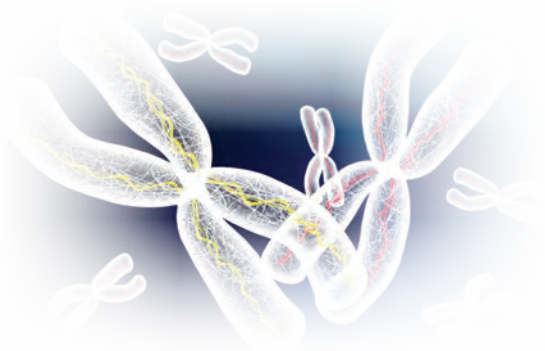
Genome Level Engineered Gene Fusions! Leverage ASC's expertise in CRISPR/Cas9 cell line genome engineering to advance your immuno-oncology research and discoveries with clinically relevant engineered gene fusion cell lines. We can custom engineer cell lines suited for your cancer research niche with your preferred chromosomal re-arrangement between your genes of interest in cell of your choice.

Translocation

Inversion

Deletion

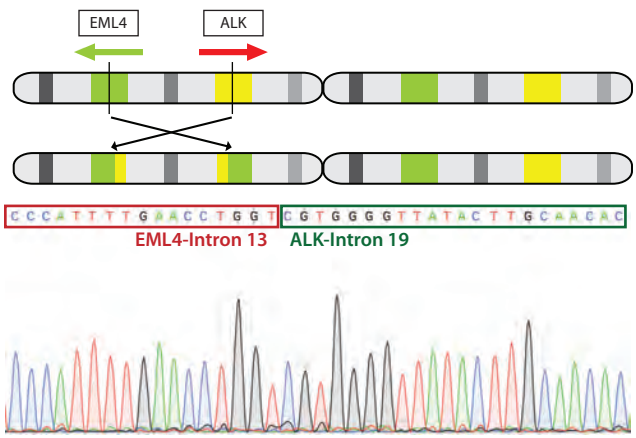
Insertion



Key Features:

- Two types: Genome level and cDNA KI
- One cell line: multiple analysis (DNA, RNA, Protein)
- Most representative of true clinical samples
- Footprint-free gene editing
- Paired isogenic cell lines

Detect with Reverse Transcription PCR, RNA-Seq, IHC, FISH and Western Blot



Application Note

Genome Level Gene Inversion in HCT116 cells

Result: Representative sequence chromatogram of a clone containing the EML4[13]-ALK[20] inversion mutation in HCT116 cell line. Gene inversion between EML4 and ALK genes in chromosome 2 of HCT116 was achieved by co-transfection of single guide RNAs (sgRNAs) and Cas9 to bind and cut the targeted intronic regions of the ALK and EML4. Single cell clones were confirmed to have mutation by Sanger sequencing, and fusion gene expression was confirmed by mRNA sequencing and Western blot (not shown).

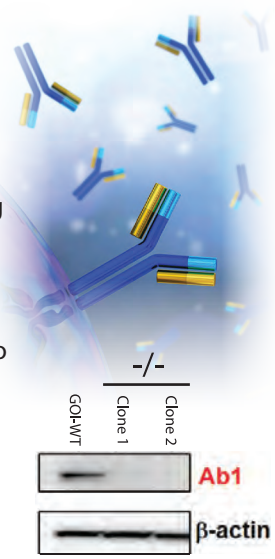
Gene Knockout Cell Lines for Antibody Validation

The Ultimate Negative Control for Genetic Validation of Your Antibodies! Use our CRISPR/Cas9 gene knockout cell lines for evaluating epitope-specificity and non-specific binding of your antibodies. ASC can generate frameshift mutations or precisely deleted targeted region(s) of the gene in any cell line of interest, to serve as the ideal negative controls for your antibody validation assays.

Key Features:

- Custom gene knockout in your cell lines
- Preliminary expression level confirmation by RT-PCR or Western Blot
- Gene targeting and knockout cell line engineering using CRISPR/Cas9
- Optional knockout validation by locus-specific sequencing, RT-PCR, or Western
- Downstream integration of knockout cell lines into FFPE Blocks

Suitable for applications involving: western blot, immunohistochemistry, immunocytochemistry, flow cytometry, ELISA, immunoprecipitation



Other Validation Strategies

Inducible/ Conditional Knockout
for tissue-specific/ essential gene deletion

Affinity Tag Knock-in
compare target antibody to tag-specific antibody

Target Protein Overexpression
positive control by knock-in of target gene into a cell line that doesn't express it normally

Figure: Antibody (Ab1) against a gene of interest (GOI) was validated in GOI-knockout cell lines using Western blot analysis. No Ab1 binding in knockout clones (-/-; 1-2). β-actin: positive control; WT: wild type.

Genetic Validation of Antibodies: *https://www.appliedstemcell.com/research/cell-line-models/antibodyvalidation-knockout-celllines*

Custom FFPE Services

Generate custom FFPE blocks, slides, or scrolls of your own lab's cell lines.

Applications: Controls for IHC, ISH, qPCR, sequencing • Reference materials for *in vitro* research & development • Perfect for pairing drug discovery with companion diagnostics development.

Homogenous Consistent

Site-specific Knock-in Cell Lines Using TARGATT™ & Other Technologies

	Random Insertion	ZFN, TALEN, CRISPR/Cas9	TARGATT™
Site - Specific Gene Insertion	No	Yes	Yes
Reproducible	No	Yes	Yes
Copy # of Inserted Gene	Multiple copies	Single copy	Single copy
Protein Yield	Varies	High/ varies	High
Stability of Integration	Varies	Yes/ varies	Yes
Selectable Marker	Less	Yes	No
Gene Insertion Efficiency	Low	Low	High
Bacterial Backbone	Yes	Yes	No
Turnaround Time	Long	Long	Short

TARGATT™ Cell Line Model Generation Service

Single Copy, Single Site, Consistent Transgene Expression! Applied StemCell's proprietary site-specific TARGATT™ technology can be used to generate stable, knock-in of large transgenes in cell lines, including stem cells, very efficiently and quickly. Knock-in is mediated by PhiC31 integrase at a pre-engineered "docking site" in an intergenic, transcriptionally active genomic locus (safe harbor locus) for guaranteed gene expression without disruption of internal genes. This technology allows only a single-copy integration with very high efficiency with or without clonal selection.

Benefits and Applications

- Site-specific transgene integration into pre-selected safe harbor locus
- Large transgene knock-in
- High integration efficiency
- Consistent and uniform transgene expression
- Ideal for building mammalian cell libraries

Gene Overexpression

Inducible Gene Expression

Isogenic Cell Line Generation

Reporter Gene Integration

Mammalian Cell Library Generation

One-cell; One-site, One-insert Only

Service Features:

1. TARGATT™ Master Cell Line Generation: (Timeline: 3-5 months)	Insertion of the "attP" landing pad into the desired locus of your cell line of interest
2. TARGATT™ Knock-In Cell Line Generation: (Timeline: 3-5 months)	Integration of your gene of interest into a TARGATT™ Master Cell Line which has a pre-engineered "attP" landing pad.

TARGATT™ Cell Line Service: *https://www.appliedstemcell.com/services/targatttm-genome-editing/gene-knock-in-technology*

TARGATT™ Knock-in Master Cell Line & Transgenic Kits

Do-it-yourself Option! Use our TARGATT™ Master Cell Lines (HEK293T or CHO) and transgenic kits to generate site-specific knock-in cell lines efficiently (even large transgene,) in your own laboratories. The TARGATT™ Master Cell Lines are ideal for large isogenic cell library construction and high-yield bioproduction research.

Higher knock-in efficiency, with or without ± clonal selection

Fast and simple gene knock-in protocol

Single copy integration

No integration of bacterial backbone

Uniform, high-level gene expression

AST-1200	TARGATT™ CHO-S (Master Cell Line) Knock-in Kit
AST-1300	TARGATT™ HEK293T Master Cell Line & Knock-in Kit

TARGATT™ Master Cell Line & Kits: *https://www.appliedstemcell.com/products/targatt-genome-editing/targatt-locus-knockin-kit*

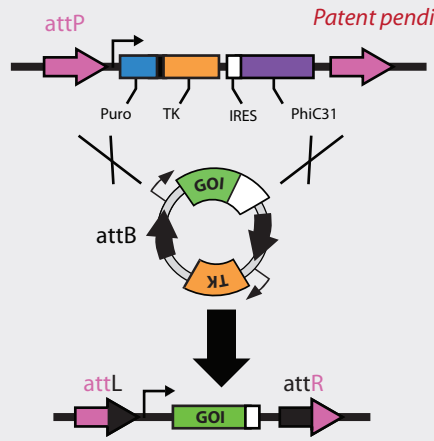
UNISELECT™ Antibody Discovery & Screening

Better than Lentivirus Mammalian Cell Libraries! Applied StemCell provides custom TARGATT™ master cell generation in your cell lines, and ready-to-use TARGATT™-HEK293, and TARGATT™- CHO Master Cell Lines to build protein libraries using site-specific transgene integration at a preselected safe harbor locus. This is a fast, unique and efficient platform for biopanning, including bispecific monoclonal antibodies (mAbs), membrane proteins, CAR-T cell screening, and for bioprocessing.

Benefits and Applications

- Single copy integration
- Homogenous expression of protein variants
- Preselected safe harbor locus
- Stable protein expression
- No bacterial backbone insertion
- Inducible expression compatible for membrane proteins (optional)

UNISELECT™ Cassette Exchange System



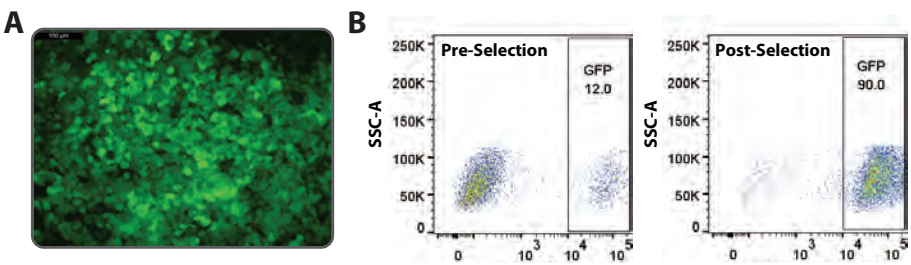


Figure: Uniform transgene expression in TARGATT™ HEK293T Master Cell Line. (A) GFP expression after fast knock-in by fluorescent microscopy; (B) High integration of GFP transfection in TARGATT™ HEK293T master cell line: pre-selection, 12% and post-selection (GCV selection), 90%.

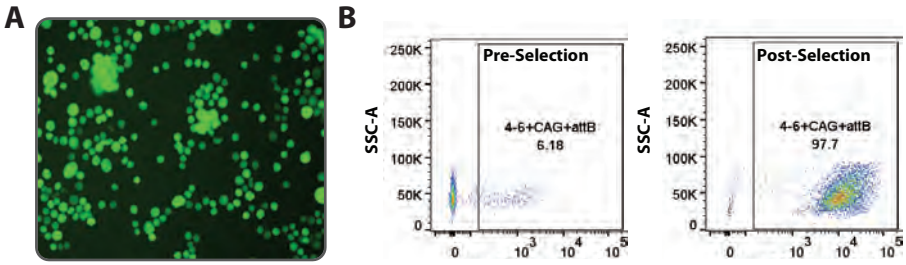
UNISELECT™ Antibody Screening: *https://www.appliedstemcell.com/research/cell-line-models/antibody-discovery-screening-hek293*

High-yield CHO Cell Lines for Bioproduction

TARGATT™ for Bioproduction CHO cells. We provide custom service to genetically engineer and optimize transgene expression levels in your CHO cell lines using our site-specific TARGATT™ technology, for bioproduction applications. This technology enables **site-specific, single-copy insertion** of large transgenes into a preselected safe harbor locus in the CHO cell genome, for guaranteed gene expression without disruption of internal genes. We also have ready-to-use TARGATT™ CHO Master Cell Lines already optimized for bioproduction research and scalability (for licensing).

Benefits and Applications

- High integration efficiency ± clonal selection
- Stable knock-in cell lines
- Uniform and high level transgene expression
- No integration of bacterial backbone
- Reproducibility of clones
- Overcomes challenges posed by traditional random insertion methods



Patent pending

TARGATT™ Bioproduction Services: *https://www.appliedstemcell.com/research/cell-line-models/high-expression-cho-cells*

Figure. High integration efficiency using TARGATT™ CHO Master Cell Lines. (A) GFP expression; (B) GFP Transfection in TARGATT™ CHO master cell line: pre-selection, >6%; and post-selection (GCV selection.), >97%.

TARGATT™ EvolvOne™ Protein Evolution & Screening

The TARGATT™-based EvolvOne™ platform enables generation of **stable, isogenic cell line libraries** for mammalian display-mediated antibody engineering, protein evolution screening, mammalian two-hybrid (M2H) screens and more. It allows only a **1:1 variant-to-cell ratio**, and guarantees uniform and consistent expression of the gene/protein for efficient screening.

EvolvOne™ Protein Evolution & Screening: *https://www.appliedstemcell.com/research/services/targatttm-genome-editing/protein-evolution-services*

Patent pending

Virus Packaging Service

Applied StemCell provides lentiviral and retroviral packaging services to help advance your research without the struggle of designing and preparing your own viruses. We also provide custom virus packaging for efficient delivery of CRISPR components into *in vivo* and *in vitro* models. Our service includes broad tropism viruses, high titer amplification, qRT-PCR verfieid titers, and a fast turnaround (~10 days).

Applications of recombinant lenti and retroviruses:

- Transgene expression in both dividing and non-dividing cells
- Useful for enabling long-term transgene expression in infected cells
- Suitable for hard-to-transfect cell lines
- Enables direct local injection to targeted tissue in animals

Features:

- Ready-to-transduce viral particles
- High quality and high titer viral particles in as little as 10 days
- VSV-G pseudotyped viruses that exhibit broad tropism across a range of cell types
- High titer amplification of viruses (up to 10^9 IFU/mL)
- qRT-PCR quantified viral titers

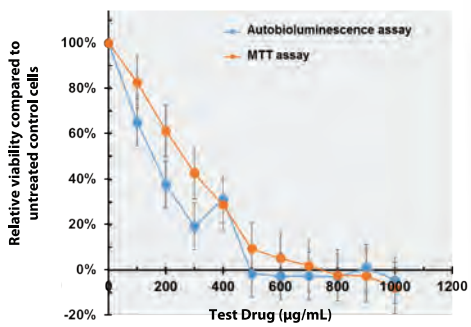
Autobioluminescent Cell Lines and Vectors

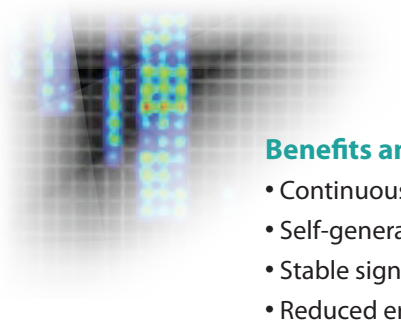
Accelerate the pace of cancer research, and preclinical metabolic and toxicity screening with reduced cost and effort using Applied StemCell's substrate-free autobioluminescence vectors for stable, human expression-optimized synthetic luciferase reporter gene cassette in a cell line of choice for stress-free in vivo imaging experiments.

Directly test your test drug in the autobioluminescent cell lines

Use as CDX model to track tumor cells

Ready-to-use or custom autobioluminescent cell lines





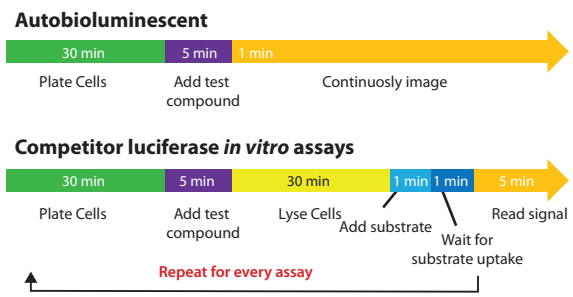


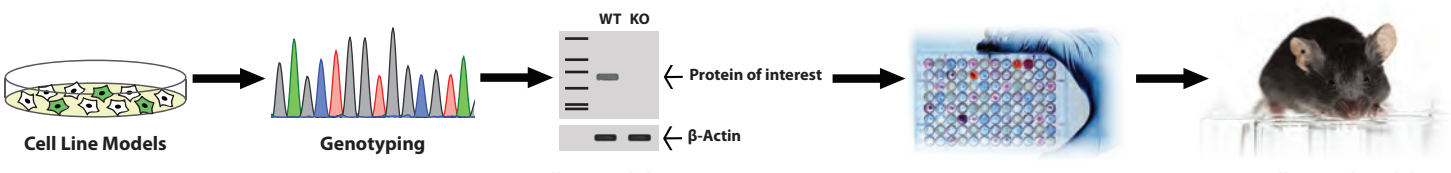
Figure. MTT Assay using autobioluminescent HEK293 cells for drug toxicity screening (at 96h post-treatment).

Benefits and Applications

- Continuous autobioluminescent output
- Self-generated and self-directed signal by the cell
- Stable signal output for multiple passages
- Reduced error, variability and human interaction
- Increased data acquisition through continuous monitoring
- Easy integration into automated and high throughput systems
- No need to change detection equipment or assays

Autobioluminecence Cell Lines and Vectors:
https://www.appliedstemcell.com/research/products/xenograft-model-research-tools/substrate-free-autobioluminescent-cell-lines

CRO Services for Preclinical Research & Discoveries



ASC provides fully customizable solutions for preclinical development of your drug discovery and early-stage drug screening pipeline. Starting from *in vitro* model generation, phenotype assessments, and screening test compounds, we can also smoothly transition to *in vivo* model generation and assays. We will work with you at every step of your project to meet your requirements with the best possible strategies. We are committed to provide you service with ISO:9001 quality, as well as procedures consistent with the principles of GLP.

Versatile, diverse scientific and technology expertise

Full suite of Biosafety Level 2 Laboratories

QA Review of Protocols

Experienced in IND-enabling studies

Project management from project initiation to completion

AAALAC-accredited vivarium for subsequent animal studies

Cell-Based Assays

Have custom cell line models? Avail ASC's downstream cell validation and phenotype characterization services to evaluate your cell function and behavior. We have extensive experience in culturing and handling a wide variety of cell lines as well as **custom cell-based assay development**. We will work with you at every stage of your project to fit it to the exact needs of your research.

Custom assay development	**Cell viability**	**Cell biomarker analysis**
2D proliferation assay	**Cytotoxicity**	**Cell imaging**
Cellular morphology	**Cell motility**	**Electrophysiology**

Cancer cell lines

Immortalized cell lines

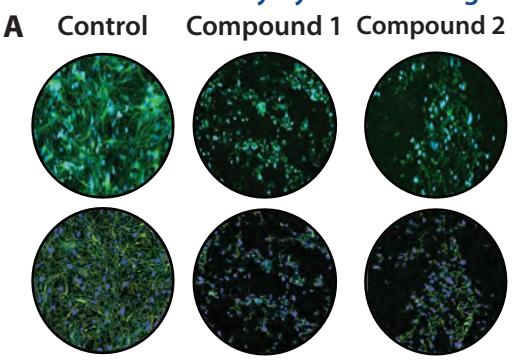
Stem Cell (iPSC/ ESC)

Differentiated cell lines

If you need a biorelevant cell line disease model for better translation of your research findings, add-on our **custom cell line model generation** using CRISPR/Cas9 for a full-suite of custom service tailored specifically for your project specifications. See details on page 4.

Application Note

In Vitro Neurotoxicity by Neurite Outgrowth



Result: Dose response assay to evaluate the impact of different concentration of two test compounds on neurite outgrowth in iPSC-derived neurons. High content imaging and automated phenotype analysis showed compound 1 to have a lower toxicity threshold as compared to compound 1.

Cell-based assays: *https://www.appliedstemcell.com/research/cell-line-models/cell-based-assay*

Cell-Based Drug Toxicity & Efficacy Screening

Make Informed Go-No-Go Decisions Early in Your Drug Development! **No Process!** Applied StemCell (ASC), an ISO-certified service provider offers a flexible assay platform with a wide-range of functional endpoints for early-stage *in vitro* screening of preclinical drug candidates. We use a comprehensive cell-based test battery from which you can choose assays for efficacy, safety or target discovery that suit your therapeutic pipeline.

Screen your compounds in a wide-variety of cell lines	• Healthy/ disease/ genetically modified • Cancer cell lines (solid tumor and blood cancer) • Stem cells (iPSC, ESCs, multipotent cells) • Primary cells
Flexible/customizable assays to suit your screening needs *Don't see an assay you are looking for, please inquire.*	• Cytotoxicity & Cell Viability Assays • Mitochondrial Toxicity Testing • Functional Assays • Quantitative Gene Expression • Morphology • Custom Assays

CNS

Cardiovascular

Metabolic

Cancer

Immunotherapy

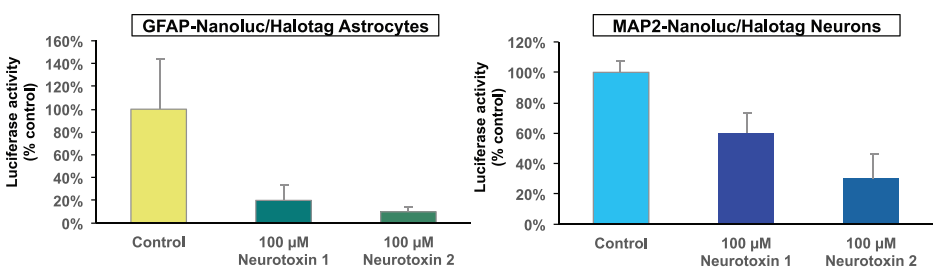


Figure: Luciferase-based cell viability was significantly reduced by up to 90% in astrocytes and neurons derived from lineage-specific reporter iPSC lines, when exposed to two neurotoxins. Luciferase activity was measured as % of control (DMSO-treated cells).

www.appliedstemcell.com

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