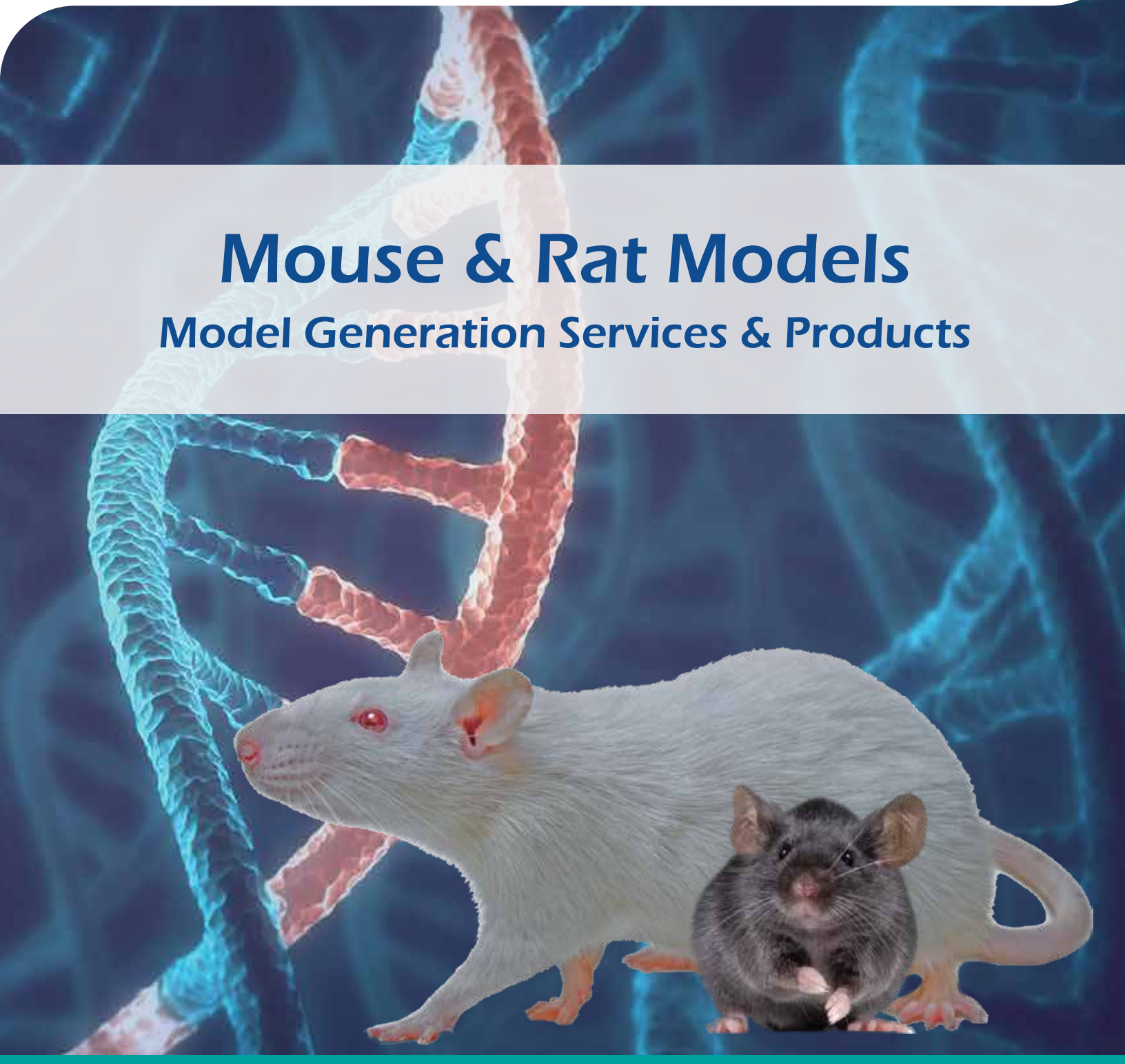
Applied StemCell

Genome editing *in vitro* and *in vivo*

Mouse & Rat Models

Model Generation Services & Products



www.appliedstemcell.com

Applied StemCell

Genome editing *in vitro* and *in vivo*

Genetically Engineered Animal Models for Preclinical Projects

Precision engineering of predictive models of human biology and disease; downstream phenotype analysis, behavior/locomotor activity and drug screening

Why work with Appled StemCell?

- Leading transgenic service company recognized in *Nature Biotechnology**
- Multi-technology genome editing: TARGATT™, CRISPR, and more
- Global service provider for industry & academic researchers
- Animal models made in USA in AAALAC facility
- F1 breeding for germline transmission
- Fast turnaround, dedicated project management

Site-Specific Knock-in Technology
TARGATT™

In vivo gRNA validation for high efficiency, high success rate in CRISPR projects

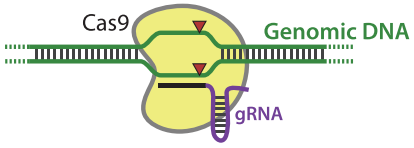
New! Full Service for downstream preclinical assays involving animal models: adoptive transfer, cell replacement therapy models, drug toxicity and screening

Smalley, E. (2016). CRISPR mouse model boom, rat model renaissance. Nature Biotechnology. 34, 893–894 and Baker, M. (2014). Gene editing at CRISPR speed. Nature biotechnology, 32(4), 309-313.

Contents of this Brochure:

ASC's Genome Editing Technology Overview

CRISPR/Cas9
Licensed from the Broad Institute



Knockout
(frameshift or targeted deletion)

Point Mutation

Conditional Knockout

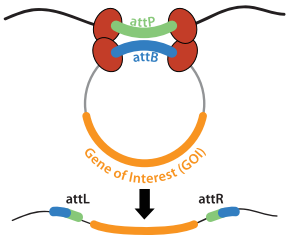
Conditional/ Induced Expression

Gene Overexpression

Gene Tagging

Reporter Gene Knock-in

TARGATT™
Exclusive to ASC



What is TARGATT™ Technology?

Site-specific transgene integration into a pre-selected, safe harbor locus

- Large transgene KI (up to 22kb)
- High integration efficiency (up to 40%)
- High level gene expression
- Works independently of cell division
- Non-immunogenic reagents
- Overcomes problems associated with random insertion

For more options to engineer animal models for your project requirements, we also offer custom mouse/ rat model generation service using our expanded technology portfolio, such as traditional ***homologous recombination, bacterial artificial chromosome (BAC) and random transgenic technologies.***

Comparing Genome Editing Technologies

Project Purpose	CRISPR/Cas9	TARGATT™
Knock-Out (KO)	**Yes**	
Point Mutation	**Yes**	
Conditional KO	**Yes**	
Knock-In <2kb; ssODN	**Yes**	
Knock-In > 2kb; Safe Harbor Loci	**Challenging** (limitations on size)	**Yes** (up to 22 kb)

Mouse Model Generation: *https://www.appliedstemcell.com/research/animal-models/gene-editing-mouse*
Rat Model Generation: *https://www.appliedstemcell.com/research/animal-models/transgenic-rat-models*

Custom Mouse/Rat Model Generation Timelines

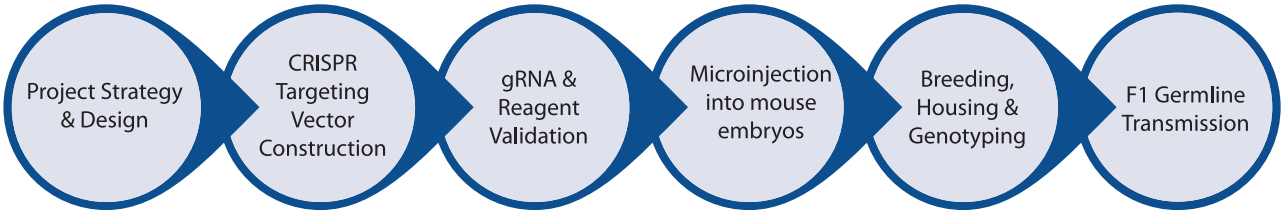
Project Purpose	KO	PM	cKO	KI<2kb	KI>2kb	KI up to 22kb*
Technology	CRISPR	CRISPR	CRISPR	CRISPR	CRISPR	TARGATT™
Timeline	5-8 months	5-8 months	5-8 months	5-8 months	8-10 months	3-5 months

Project Purpose	KO	PM	cKO	KI<2kb	KI>2kb	KI up to 22kb*
Technology	CRISPR	CRISPR	CRISPR	CRISPR	CRISPR	TARGATT™
Timeline	5-8 months	6-10 months	6-10 months	6-10 months	8-10 months	6-8 months

** Turnaround time for F1 breeding; # safe harbor locus KI using TARGATT™; KO: Knockout, PM: Point Mutation; cKO: Conditional Knockout; KI: Knock-in.*

CRISPR/Cas9 Mouse Model Generation Services

ASC is a leading CRISPR service provider, and has engineered several hundred mouse models for researchers worldwide. Our scientists can engineer a variety of mutations in your gene of interest, using proprietary designing strategies, highly optimized protocols. Take advantage of our precise genome editing technologies, fast turnaround, and reasonable cost to research with a mouse model engineered for your project specifications.



Deliverables and Timeline

- Two germline transmitted F1s
- 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)

Knockout
(Region Specific/ Frameshift)

Conditional Gene Knockout

Point Mutation

Transgene Insertion
(Locus Specific/ Safe Harbor Locus)

Reporter Gene Knock-in

Benefits and Applications

- Most up-to-date CRISPR designing strategies
- *In vivo* gRNA validation ensures up to 100% target-site cutting efficiency
- High efficiency, optimized protocols, for >98% project success rate
- Genetically engineer mouse models in many different mouse strains: C57BL/6. FVB, BALB/c, and more

Optional! **Phenotype validation/assessment, custom *in vivo* assay and drug screening services available.** ***Ask for details.***

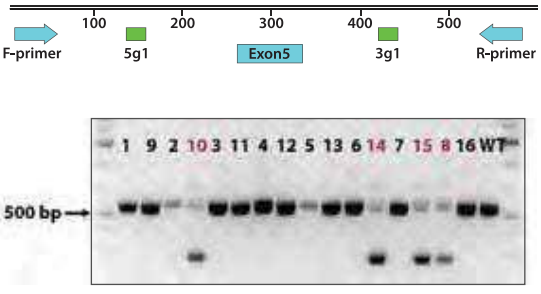
CRISPR/Cas9 Mouse Model Generation: *https://www.appliedstemcell.com/services/crispr-cas9-genome-editing/animal-models*

Case Studies

1. Knockout Mouse Models

Goal: To generate a gene knockout mouse model by removing exon 5 of the gene of interest to generate a frame shift mutation.

How: Two *in vivo* validated gRNAs (with activities of 86% and 88%), designed to target introns 4 and 5 of the gene of interest, and the Cas9 protein, were microinjected into C57BL/6 embryos. The new mice born from the microinjection were screened for potential founders (F0), with exon 5 deletion. The FO mice were mated with wild type (WT) mice to generate germline transmitted F1 knockout mice.



Result: Fo # 8, 10, 14, 15) to 6 16 f im e bo n were id nt fied by geo yg P R to conh a 288 bp delet on of h a geted gen .

2. Point Mutation

Goal: To generate a point mutation mouse model in C57BL/6 mice using CRISPR/Cas9.

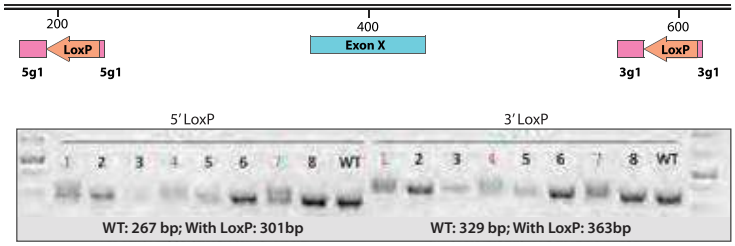
How: A GAC > AAT (D569N) mutation was introduced into the gene of interest using CRISPR/Cas9 by injecting an *in vivo* validated gRNA (88%), a D569N ssODN donor, and Cas9 protein into C57BL/6 embryos. Founder mice (F0) born after injection were identified by sequencing and subsequently bred with WT mice for mutation transmission to F1.

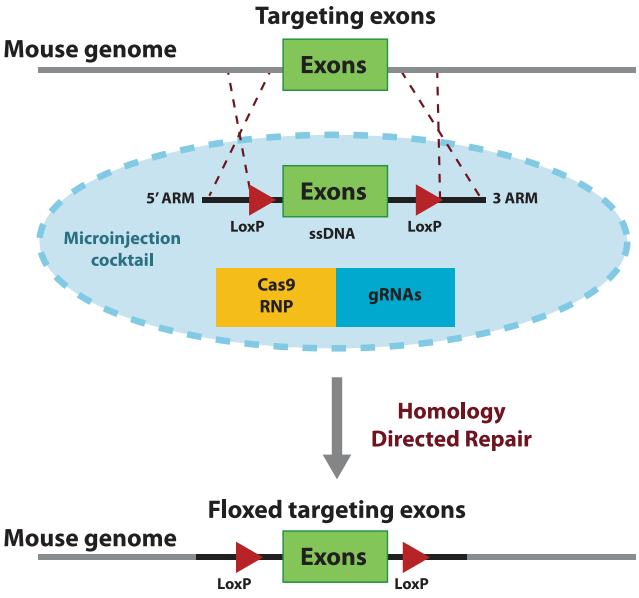
Result: Out of fourteen F1 born, seven mice were identified to be heterozygous mutants.

3. Conditional Knockout Mouse Models

Goal: To generate a conditional knockout (cKO) mouse model through CRISPR/Cas9-mediated cleavage and subsequent homology directed repair (HDR).

How: The cKO mouse model was generated by flanking exonX of the gene of interest with two LoxP sequences. To achieve this, a pair of *in vivo* validated gRNAs, a single-stranded deoxyribonucleic acid (ssDNA) donor, and the Cas9 protein were injected into the mouse embryos. New mice born from injection were screened by genotyping PCR and NGS/ Sanger sequencing to confirm founders (F0). The F0 mice were again mated with WT to ensure transmission of the cKO genotype to F1 generation.



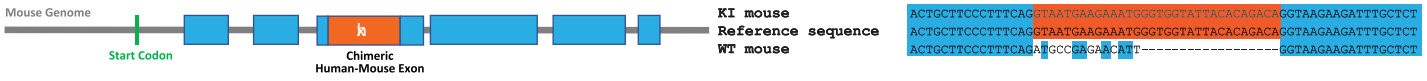


Result: Genotyping PCR (above gel) and NGS/ Sanger sequencing (not shown) confirmed 5' and 3' LoxP insertions in three (#s 1, 4, 7) of out eight F1 pups.

4. Large Fragment Knock-In Mouse Models

Goal: To knock-in a fragment of a human exon of a gene of interest to generate a chimeric mouse model.

How: In this model, part of the mouse exon mX was replaced with a human sequence, hX using HDR. A mixture containing a pair of active gRNA (100% and 80% cutting efficiency), a single-stranded oligo deoxynucleotide (ssODN) donor containing the human sequence, and Cas9 protein was injected into C57BL/6 mouse embryos. The F0 mice confirmed by NGS were mated with WT mouse for germline transmission of chimeric human gene fragment (hX) knock-in in F1 mice.



Result: Genotyping PCR and NGS identified three out of 16 mice born in F1 breeding to have the chimeric transgene containing the human hX replacement fragment.

References

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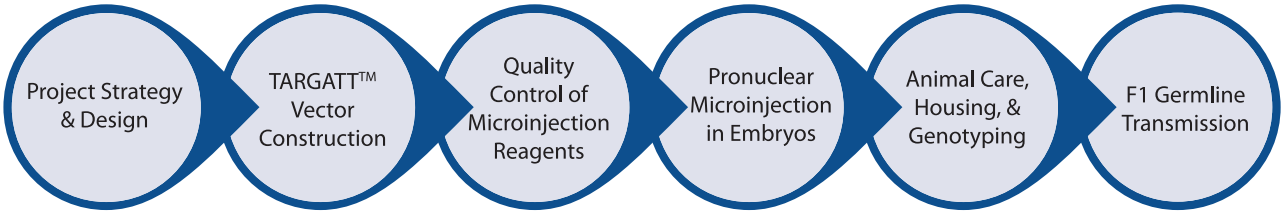
TARGATT™ Fast & Site-Specific Knock-In Mouse Services

With our proprietary, ΦC31 (PhiC31) integrase-mediated transgene integration technology, we will generate your site-specific knock-in mouse in as little as 3 months. Knock-in any gene of interest at a defined, transcriptionally active safe harbor locus (mRosa26 or mH11) engineered with an "attP" docking site, for high level transgene expression without any interruption of gene expression.



Inducible/ Conditional Gene Expression

Tissue-Specific Expression

Gene Overexpression

Gene Tagging

Cre-Driver Lines

Humanized Models

Generate matched mouse lines for precise phenotypic comparison of different transgenes

Deliverables and Timeline

- Two germline transmitted F1s
- Dedicated project management to provide detailed milestone and final project reports
- Comprehensive report on technical details, genotyping strategy, etc.

Timeline: 3-5 months

Benefits and Applications

- Large fragment knock-in (up to 22 kb)
- High integration efficiency (up to 40%)
- Single copy knock-in in an active locus: **avoids gene silencing and genomic instability**
- Fast turnaround

TARGATT™ Mouse Model Generation: *https://www.appliedstemcell.com/services/targatttm-genome-editing/targatt-knock-in-mouse*

Case Studies

1. Site-Specific Knock-In Mice

Goal: To generate site-specific knock-in of a coat color gene in mice.

How: A mixture of donor plasmid (containing the attB sites and transgene) and the ΦC31 mRNA was injected into the pronuclei of H11P3-C57BL/6 mouse embryos. Genomic DNA of founders was analyzed using primer pairs for transgene and H11 locus to verify site-specific insertion.

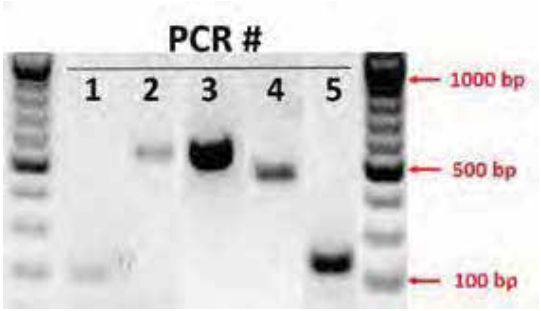
Result: Genotyping PCR after microinjection confirmed F0 founders (center) containing the transgene in the H11 locus. Further breeding with WT mice, generated F1 germline transmitted transgene (right). (Left) H11-C57BL/6 mice used for donor embryos.

2. Large Fragment Knock-In

Goal: To insert a large (8.5 kb) DNA fragment into the H11 safe harbor locus in FVB mice using TARGATT™ integrase based technology.

How: The donor plasmid and integrase mRNA were injected into the pronuclei of H11P3-FVB mouse embryos. Transgene integration at the H11 locus was confirmed by genotyping PCR using primer pairs for transgene and locus primer pairs.

Result: Genotyping PCR identified one founder mouse among 6 pups born. PCR #1 & 5: H11 locus (junction PCR); PCR #2 & 4: site-specific knock-in of transgene; PCR #3: transgene integration .



Do-It-Yourself Products! TARGATT™ Site-Specific Knock-In Mouse

For the transgenic mouse engineering experts! Make your own TARGATT™ knock-in mouse by purchasing all necessary products: the TARGATT™ "attP" Mice, the TARGATT™ Transgenic Kit, TARGATT™ Plasmids, and the TARGATT™ Genotyping Kit directly from Applied StemCell.

Step 1. Purchase plasmids

TARGATT™ Plasmids

AST-3042 TARGATT™ 2 (CAG + Poly A)
AST-3043 TARGATT™ 3 (no promoter + MCS)
AST-3050 TARGATT™ 6.1 (CAG-L4SL-MCS-PolyA)
AST-3047 TARGATT™ 7 (PGK-MCS-PolyA)
AST-3048 TARGATT™ 8 (PCA-MCS-PolyA)
AST-3051 TARGATT™ 9 .1 (PCA-L4SL-MCS-PolyA)

Step 2. Purchase transgenic kits from ASC for injection

TARGATT™ Transgenic Kit

AST-1003 Transgenic Kit (5 rounds of microinjections)
AST-1004 Transgenic Kit (2 rounds of microinjections)

Step 3. Purchase attP mice

TARGATT™ Mouse:

(Purchase from ASC & shipped by Charles River)

MODEL #549 (H11, C57BL/6)

Other strains such as Rosa26-C56BL/6, H11 FVB, Rosa26-FVB, are available upon request; lead time a minimum of 3 months.

Step 4. Confirm sequence with genotyping kit

AST-2005 H11 Mouse Genotyping Kit
AST-2006 Rosa26 Mouse Genotyping Kit

FAQs

- **What is background strain for the TARGATT™ "attP" mice**
  The TARGATT™ "attP" mice have been generated in C57BL/6J background.

- **Where can I order TARGATT™ "attP" mice?**
  The TARGATT™ "attP" C57BL/6-H11 mice (Model #549) are available for order directly from Applied StemCell (ASC): (1) From the ASC website; (2) By email: info@appliedstemcell.com; (3) By phone: +1 (866) 497-4180. Charles River Laboratories ship the TARGATT™ attP mice directly to you.

- **Besides H11 and Rosa26, can gene be inserted at other loci?**
  Yes. This would be a customized service. We need to first insert the docking attP site into a desired locus using CRISPR/Cas9 and then insert the gene of interest into the attP site using TARGATT™.

- **What is the efficiency of TARGATT™ Mouse Model Generation technology?**
  TARGATT™ has up to a 40% Knock-in efficiency for transgenes <1kb. The efficiency decreases with increasing fragment size.

- **What is the difference in terminologies for TARGATT™ "attP" mice and TARGATT™ transgenic mice?**
  The term, TARGATT™ "attP" mice, refers to the "docking site-ready" mouse models where the attP sequence has been inserted into either the mRosa26 or mHipp11 safe harbor loci. TARGATT™ transgenic mice, refers to the transgenic mice generated using TARGATT™ technology and containing your gene of interest integrated into the safe harbor locus (i.e. a knock-in mouse model).

Please visit our website for more FAQs and details regarding our unique TARGATT™ site-specific knock-in technology.

Publications

Description of the technology

• Tasic, B., Hippenmeyer, S., Wang, C., Gamboa, M., Zong, H., Chen-Tsai, Y., & Luo, L. (2011). Site-specific integrase-mediated transgenesis in mice via pronuclear injection. Proceedings of the National Academy of Sciences of the United States of America, 108(19), 7902–7907. http://doi.org/10.1073/pnas.1019507108.

Applications for mice generated by TARGATT™

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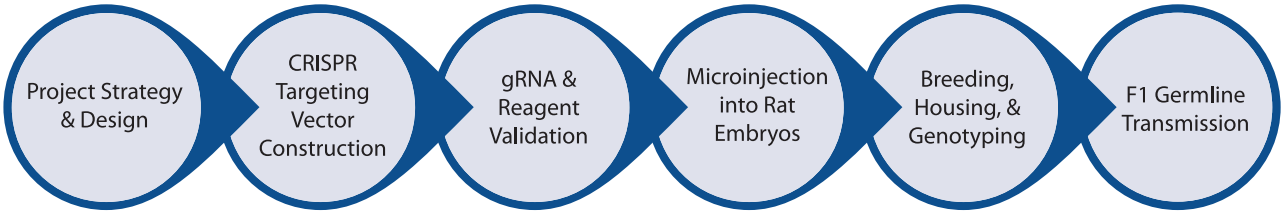
• Li, K., Wang, F., Cao, W. B., Lv, X. X., Hua, F., Cui, B., & Yu, J. M. (2017). TRIB3 Promotes APL Progression through Stabilization of the Oncoprotein PML-RARα and Inhibition of p53-Mediated Senescence. Cancer Cell, 31(5), 697-710.

For more citaitons, please visit our website: https://www.appliedstemcell.com/services/targatttm-genome-editing/publications

CRISPR/Cas9 Rat Model Generation Services

The successful isolation of rat embryonic stem cells (rES) and complementary advances in site-directed mutagenesis using techniques such as CRISPR/Cas9 have made the generation of genetically engineered rat models possible. Leverage ASC's expertise in animal model engineering and CRISPR/Cas9 technology to generate physiologically relevant rat models to meet your specifications and budget.



Benefits and Applications

- ASC is a premier CRISPR/Cas9 service provider for animal model engineering
- Highly optimized and efficient protocols for precision genome editing
- *In vivo* gRNA validation ensures up to 100% target-site cutting efficiency
- Rat models are generated in the USA in a AAALAC accredited animal facility

Knockout
(Region Specific/ Frameshift)

Conditional Gene Knockout

Point Mutation

Transgene Insertion
(Locus Specific/ Safe Harbor Locus)

Reporter Gene Knock-in

Deliverables and Timeline

- Two germline transmitted F1s
- Dedicated project management to provide detailed milestone and final project reports
- Comprehensive report on technical details, genotyping strategy, etc.

Timeline: in as little as 5 months (varies by project type)

Pair your conditional knockout rat models with our neuronal tissue-specific Cre-rat lines or a Cre line of your choice. Inquire!

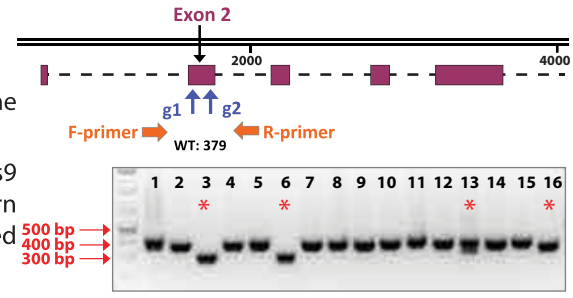
CRISPR Rat Model Generation: *https://www.appliedstemcell.com/services/crispr-cas9-genome-editing/rat-models*

Case Studies

1. Knockout Rat Models

Goal: To generate a gene knockout rat model by nucleotide deletion within the gene of interest in Sprague-Dawley (SD) rat strain.

How: Two active gRNAs designed to target the gene of interest, and the Cas9 protein, were microinjected into the embryos of SD rats. The new pups born from the microinjection were screened for potential founders (F0), and mated with wild type (WT) mice to generate germline transmitted F1 knockout rats.

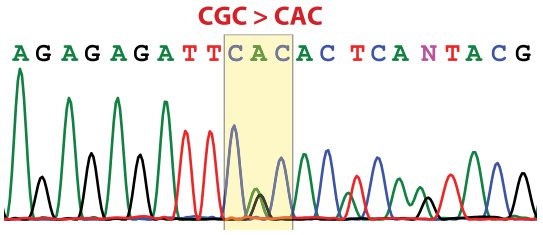


Result: Four pups (*) were identified by genotyping PCR and Sanger sequencing (not shown) to contain deletion mutation.

2. Point Mutation

Goal: To generate a point mutation rat model in SD rats using CRISPR/Cas9.

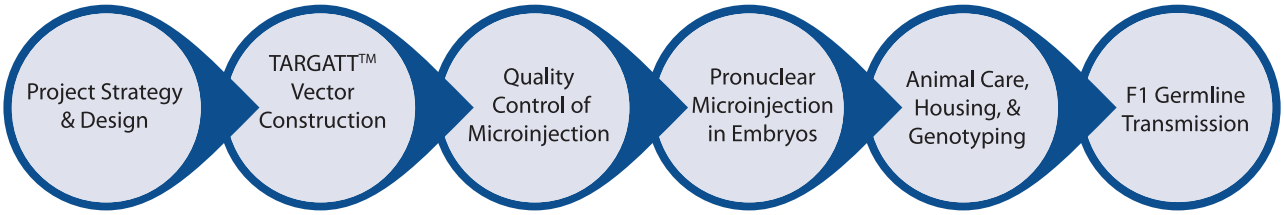
How: A CGC > CAC (R237H) mutation was introduced into the gene of interest using CRISPR/Cas9 by injecting an *in vivo* validated gRNA, a ssODN donor, and the Cas9 protein into rat embryos. Founder rats (F0) born after injection were identified by genotyping PCR and sequencing, and subsequently bred with wild type SD rats for germline transmission of mutation to F1.



Result: Eight out of seventeen rats were confirmed to have the desired point mutations.

TARGATT™ Fast & Site-Specific Knock-In Rat Service

Applied StemCell's proprietary TARGATT™ technology enables generation of physiologically relevant transgenic rat models suitable for a variety of applications including reporter gene expression, gene knock-down, conditional gene expression and disease modeling. This technology uses the Phic31 integrase to mediate an irreversible integration of large transgene(s) into a preselected, safe harbor locus for high level gene expression.



Deliverables and Timeline

- Two germline transmitted F1s
- Dedicated project management to provide detailed milestone and final project reports
- Comprehensive report on technical details, genotyping strategy, etc.

Timeline: in as little as 6 months

Benefits and Applications

- Transgenic knock-in rat model service platform is built from our very successful TARGATT™ mouse model services
- Single copy knock-in in an active locus: **avoids gene silencing and genomic instability**
- Direct microinjection into rat zygotes without the need for rat ES cells
- Ideal for tissue-specific/ inducible expression rat models, reporter gene knock-in, gene overexpression, and humanized/ chimeric rat models

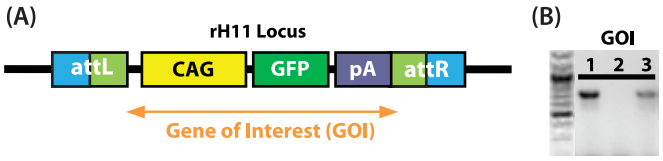


Figure. TARGATT™ GFP Rat Generation by knock-in of a CAG-GFP transgene into the rH11 locus of the TARGATT™ attP Sprague-Dawley rats. (A) Schematic representation of the knock-in strategy; (B) PCR gel electrophoresis showing results from 2 representative F0 rats identified by genotyping PCR and confirmed by Sanger sequencing #1 and #3 were (not shown).

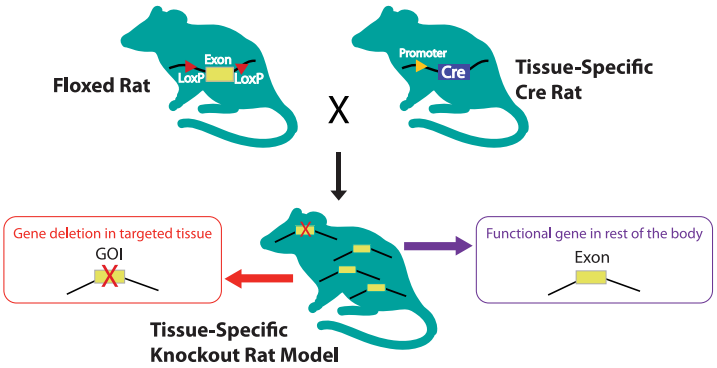
TARGATT™ Knock-in Rat Service: *https://www.appliedstemcell.com/services/targatttm-genome-editing/targatt-knock-in-rat*

Cre-Expressing Rat Lines

Applied StemCell is excited to provide a Cre-driver rat repository for paired breeding with "floxed" conditional rat models. These Cre rat lines will express Cre under tissue-specific promoters (see table) for generation of tissue-specific, conditional/ inducible rat models. The Cre rat lines will provide a much needed resource for generating physiologically relevant rat models.

Key Features

- **21 Cre-driver rat models:** 18 neuronal lineages, 2 cardiovascular lineages, and 1 Cre reporter line
- Generated using complementary TARGATT™ and CRISPR/Cas9 genome editing technologies
- Sprague Dawley Rats
- Optional! We can generate your "floxed" conditional rat models and/or breed your tissue-specific gene expression/ knockout rat models for you



Syn1-Cre	PAG-Cre
Thy -Cre	Tie2-Cre
Pomc-Cre	Drd1a-Cre
Plp1-Cre	Gad67-Cre
Hb9-Cre	Nestin-Cre

Six3-Cre	TH-NFH-Cre
PDGF-Cre	GFAP-Cre
MOR23-Cre	Wnt-Cre
Crh-Cre	Vglut-Cre

POCx32 Cre
SMHC Cre
CAG-L4SL -GFP-lacZ

Phenotype Evaluation, Drug Discovery & Drug Screening Services

As a long-standing leader in genome editing and animal model engineering technologies, Applied StemCell also offers fully customizable downstream animal service solutions that goes far beyond our standard genome engineering service offerings. We can meet the unique needs of our clients by customizing projects piecemeal to fit any requirement/stage of your research pipeline.

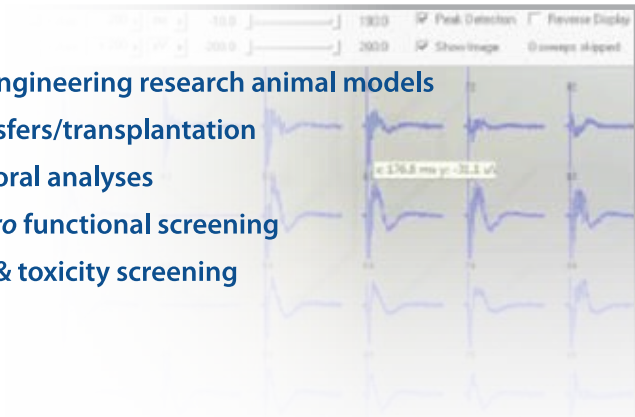
1. Disease Model Generation
- Genetically modified mouse and rat models
- Adoptive cell transfer, teratomas
- Surgically/drug induced models

2. *In Vivo* Assays
- Behavioral assessments: cognition & locomotor activity
- Automated *in vivo* measurements: ECG, EEG, EMG
- *In vivo* pharmacokinetics

3. *In Vitro*/Postmortem Assays
- Electrophysiology: neurological & cardiac assays; patch-clamp, MEA
- Tissue collection and end-of-study analyses: western blots, immunohistochemistry, RT-PCR

Key Features

- AAALAC accreditation and NIH OLAW assurance
- DEA licenses: Schedule I & Schedule II-V
- State-of-the-art vivarium with automated behavioral assessment cages & devices
- Multidisciplinary team of experts to design a comprehensive project plan
- Stress-free projects with dedicated project managers



Designing & engineering research animal models

Adoptive transfers/transplantation

***In vivo* behavioral analyses**

***In vivo* & *in vitro* functional screening**

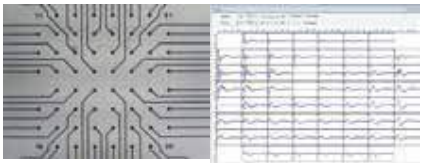
Drug efficacy & toxicity screening

Benefits and Applications

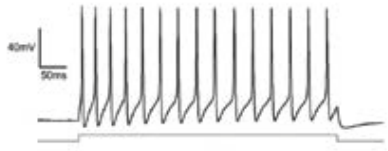
- Advance your preclinical pipeline by leveraging our animal engineering and stem cell technology expertise
- Dedicated project managers and detailed project workflow from initiation to completion
- Projects executed under GLP principles
- FDA compliant documentation for clearance and approval of preclinical studies

Electrophysiology Services For Disease Modeling, Drug Discovery & Drug Screening

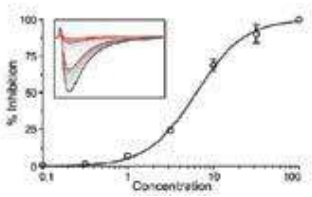
MEA Recording



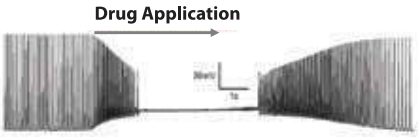
Current Clamp



IC50 Determination



Cell Excitability



Selected Applications Enabled by ASC's *In Vivo*-Based Assays

1. Drug Efficacy Testing in Mouse Model of Epilepsy for Drug Discovery and Drug Screening

A Pentylenetetrazol (PTZ)-induced mouse model of epilepsy (40 mg/kg, i.p.) was used to test the anti-epileptic properties of drug candidate, X. Compound X and conventional anti-epileptics were administered by intraperitoneal injection at 30 min post-PTZ injection. The latency between PTZ-induced seizures and death was used as an indication of drug efficacy.

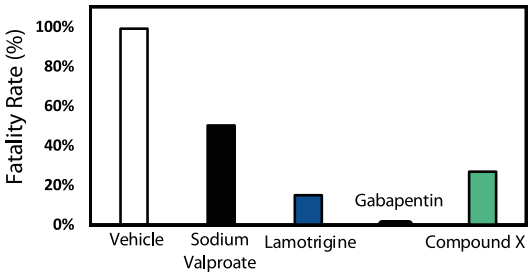
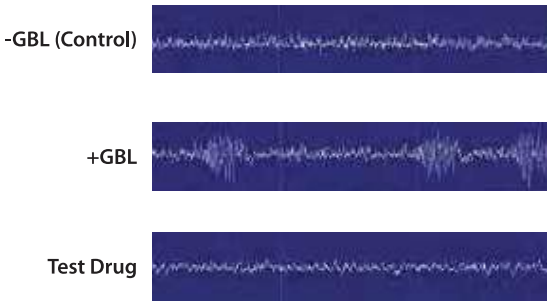


Figure 1. Compound X showed a decrease in the number of PTZ-induced deaths similar to the decrease observed after administration of conventional anti-epileptics in a drug-induced mouse model of epilepsy.

2. *In Vivo* Functional Drug Screening in Rat Models Using EEG/ EMG Recordings

Absence seizures (spike-wave discharges; SWD) were induced in rats using γ-butyrolactone (GBL, 100 mg/kg, i.p.). SWD was measured using an automated 16-channel EEG/EMG recording system. Test drugs were administered prior to GBL treatment.

Figure 2. Pretreatment of the test compound prior to injection of GBL blocked the abnormal wave pattern observed with GBL-induced SWD.



3. Cardiac Ion Channel Safety Screening for Potential Cardiotoxicity Using Manual Patch Recording

Utilizing our expertise in patch-clamp electrophysiology, drugs can be screened against an array of ion channels including recombinant human ether-a-go-go deleted gene (hERG), Nav1.5, Cav1.2, and using human iPSC-derived cardiomyocytes.

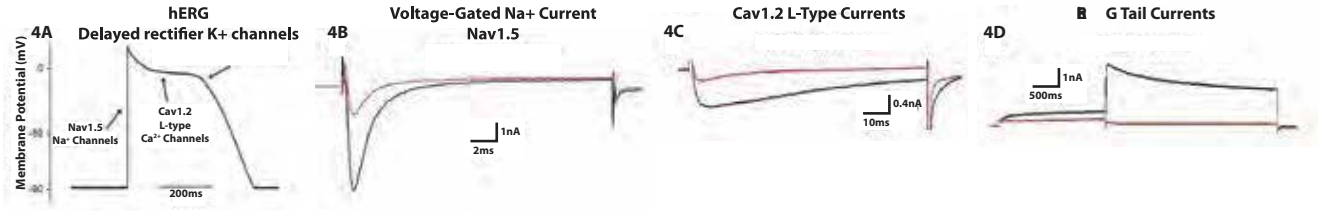


Figure 3. Several ion channels involved in the cardiac action potential are implicated in QT interval prolongation. (B-D) Example recordings show drug inhibition of human Nav1.5, Cav1.2 and hERG currents. Black traces represent control currents and red traces show currents in the presence of representative inhibitory drugs.

Preclinical *In Vivo*-Based Assays: *https://www.appliedstemcell.com/research/animal-models/custom-animal-services*

Autobioluminescent Cell Lines & Vectors for CDX/PDX Model

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.

Benefits and Applications

- Decreased costs
- Increased imaging flexibility; image samples repeatedly without cellular destruction.
- Reduced hands-on time
- Non-invasive *in vivo* tumor tracking in small animal models - imaging possible directly through tissue
- Easy integration into automated systems

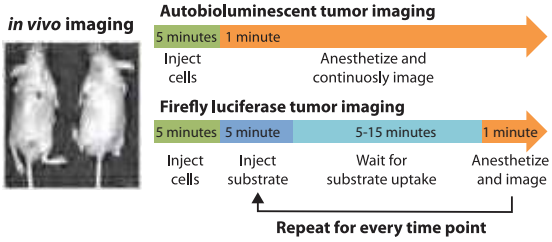


Figure. Comparison between substrate-dependent tumor imaging and substrate-independent tumor imaging techniques highlights the advantages of using ASC's autobioluminescent cell lines for tumor imaging and drug screening.

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

www.appliedstemcell.com

Contact us at:

Email: info@appliedstemcell.com
Phone: 1-866-497-4180
Fax: 1-650-800-7179

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