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Cre Driver Rats: Spatial and Temporal Gene Expression for Human Diseases

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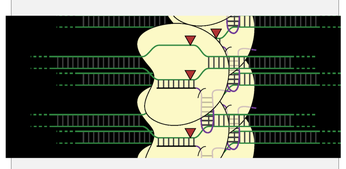
INTRODUCTION

- Transgenic rats are better models for studying certain human diseases compared to mice.
  - Cardiovascular diseases, neurobiology, autoimmunity, cancer models, transplantation biology, inflammation, cancer risk assessment, industrial toxicology, pharmacology, and behavioral and addiction studies
- Recent advances in site-specific genome editing technologies such as CRISPR/Cas9 and TARGATT™ bypasses the need for rat stem cells, thereby successfully generating genetically engineered rat models.
- Based on our successful mouse TARGATT™ models, we have engineered a TARGATT™ rat line in Sprague Dawley rats, with "*attP*" docking sites, at a transcriptionally active, safe genomic locus (rH11), to facilitate single copy integration of large transgenes (up to 22 kb) for basic and applied research..
  - Unidirectional gene integration between non-identical sites: *attP* (on rat genome) and *attB* (on donor vector)
  - High integration efficiency (up to 40%) and high level gene expression
  - Overcomes challenges associated with random gene integration
- We aim to use the complementary CRISPR/Cas9 and TARGATT™ technologies to generate a repository of Cre driver rat lines to address the immediate need for building physiologically predictive animal models.
  - We are generating 21 Cre rat lines, 18 neural lineage-specific Cre lines, 2 cardiovascular specific lines and one Cre-reporter test line to allow temporal or spatially controlled gene expression using the Cre/LoxP system.
- This project will provide an efficient way to create novel and physiologically relevant rat models of human diseases with controlled temporal/ spatial expression, especially suitable for drug target discovery and drug screening.

TARGATT™ For Site-Specific Gene Integration

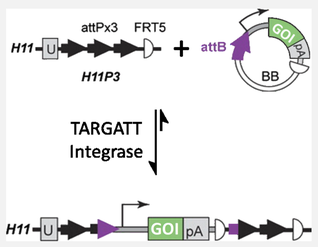
CRISPR/Cas9

Point mutation
Gene knockout
DNA tag insertion
Reporter lines
Conditional KO (CKO)



TARGATT™

Site-specific gene insertion
Works in non-dividing cells
Large fragment KI up to 20kb



Project Purpose	CRISPR/Cas9	TARGATT™
Knock-Out (KO)	Yes	
Point Mutation	Yes	
Conditional KO	Yes	
Knock-In (<2kb ssODN Donor)	Yes	
Knock-In Transgenes in Safe Harbor Loci (>2kb)	Yes (but limitations on size)	Yes (up to 20kb)
Knock-In (Plasmid DNA)	Challenging (but limitations on size)	Yes

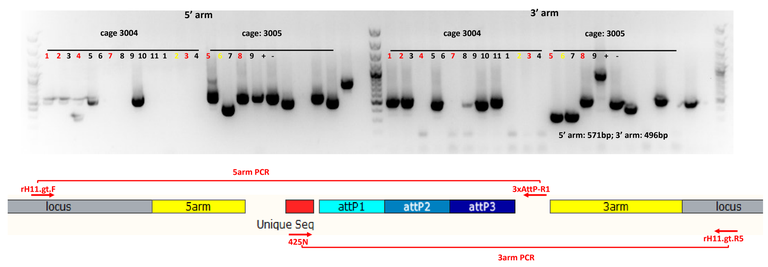
Cre Driver Rat Models to be Generated Using TARGATT™ & CRISPR/Cas9

TARGATT™	
Syn1-Cre	PAG-Cre
Six3-Cre	TH-NFH-Cre
PDGF-Cre	GFAP-Cre
MOR23-Cre	POCx32 Cre
Crh-Cre	SMHC Cre
CAG-L4SL -GFP-lacZ	

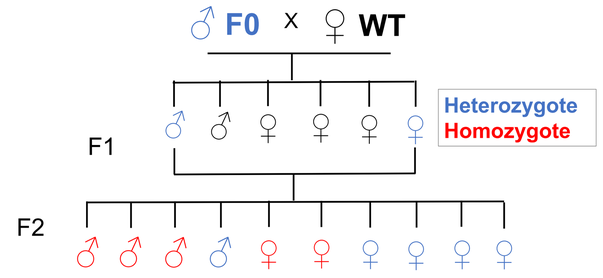
CRISPR/Cas9	
Thy1 -Cre	Tie2-Cre
Pomc-Cre	Drd1a-Cre
Plp1-Cre	Gad67-Cre
Hb9-Cre	Nestin-Cre
Vglut-Cre	Wnt-Cre

Generation of TARGATT™ "Master" Rats

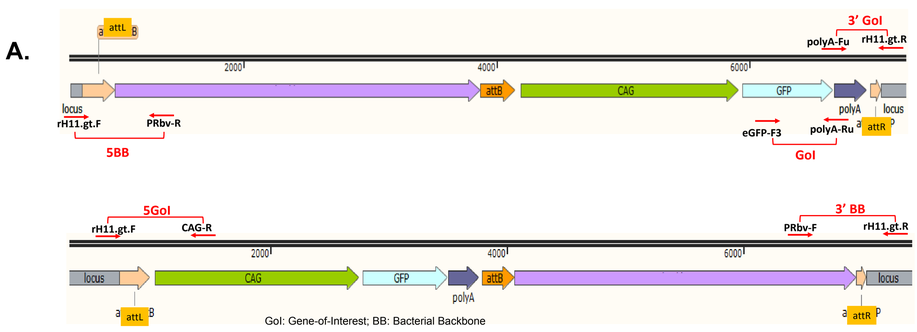
A. Generation of TARGATT™-H11 rat line

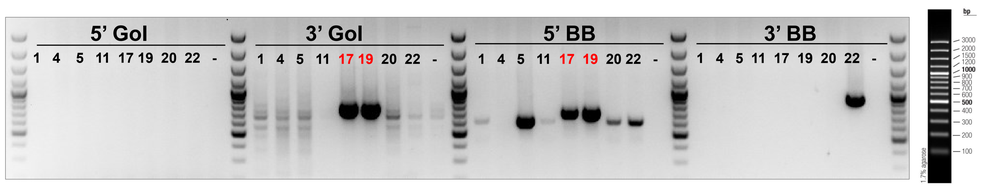


B. Establishment of TARGATT™ rat colony



Generation of TARGATT™-GFP Rats

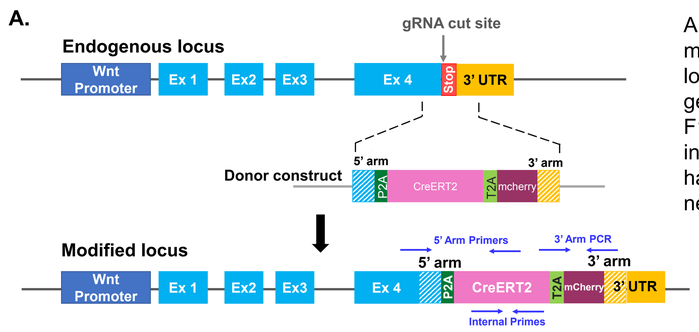


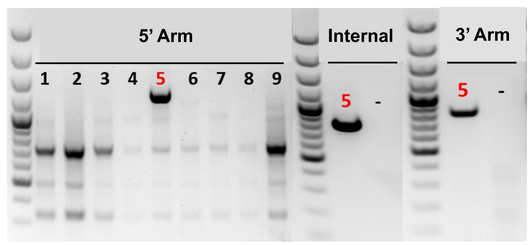


5' GoI: 412bp
3' GoI: 750bp
5' BB: 708bp
3' BB: 919bp

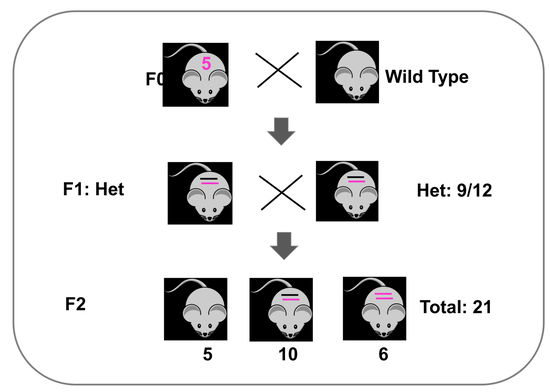
A. Schematic representation of the design and construct of CAG-GFP rat using TARGATT™. A. CAG-GFP transgene was inserted by integration of the gene cassette using PhiC31 integrase at the rH11 docking site (locus) in the TARGATT™ attP "Master" rat. B. Two founder pups (#17 and #19) were identified to carry the gene of interest by PCR using 4 sets of genotyping primers: 5' GoI, 3' GoI, 5' BB, and 3' BB. Note: -: negative control; GeneRuler™ 100 bp plus DNA ladder.

Generation of Wnt-Cre Rats Using CRISPR/Cas9





A. Schematic representation of the design and construct of Wnt-Cre rat. A CreERT2 - mCherry expression cassette was knock-in downstream of exon 4 at the endogenous locus of the rWnt gene, using CRISPR/cas9; B. Founder rats were identified by genotyping, and bred to F1 and F2 generations; C. Breeding scheme for rWnt rats for F1 and F2 generations. Nine out of 12 pups born in F1 generation, and 3 out of 21 rats in the F2 generation, were identified by genotyping PCR using 3 sets of primers to have the insertion of the gene of interest at the rWnt locus. F0: Founder; Note: -: negative control; GeneRuler™ 100 bp plus DNA ladder.



CONCLUSIONS

- Phase I: Successfully generated a TARGATT "Master" rat line, funded by the NIH SBIR grant #1R43GM1z08071-01A1
- We have demonstrated that TARGATT™ technology to be an efficient platform to generate knock-in rat models in trial runs using a GFP reporter cassette.
- Phase II: To generate the twenty-one Cre-driver rat lines funded by 2R44GM108071-02A1 (NIH SBIR)
- The goal is to establish and provide a centralized rat model resource to the scientific community to study human diseases, identify novel drug targets as well as for preliminary screening of drug candidates in a physiological relevant animal model.
- We have successfully used two complementary genome editing technologies, CRISPR/Cas9 and TARGATT™ to knock-in lineage-specific Cre transgenes.
- The rWnt-Cre, rGad67-Cre, rPomc-Cre, and rTie2-Cre rat lines were generated using CRISPR/Cas9 by insertion of the Cre-mCherry cassette into the endogenous loci of the respective gene, at the 3'-end.
- These rats are currently being characterized for their phenotypes.
- This platform can also be applied to generate customized expression rat models.

If you would like to collaborate regarding these Cre- rat lines, please contact us.

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