**INTRODUCTION**

- Transgenic rats are better models for studying certain human diseases compared to mice.
- Recent advances in site-specific genome editing technologies such as CRISPR/Cas9 and TARGATT™ bypass the need for rat stem cells, thereby successfully generating genetically engineerd rat models.
  - Transgene integration at prescellected transcriptionally active H11 locus
  - High integration efficiency (up to 40%)
  - Capability of inserting large piece DNA, up to 230kb
- Based on our successful mouse TARGATT™ models, we have generated TARGATT™ “master” rats with an “attP” docking site at the transcriptionally active safe harbor locus, H11.
- 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 100 Cre rat lines, 18 neural specific Cre lines, 2 cardiovascular specific lines and 2 reporter lines to allow transient and spatially controlled gene expression using the TARGATT™ Rat platform.
- This project will provide an efficient way to create novel and relevant rat models of human diseases, especially suitable for drug target discovery and drug screening.

**CRISPR/Cas9 and TARGATT™ For Site-Specific Gene Integration**

- **CRISPR/Cas9**
  - Point mutation
  - Gene knock-out
  - DNA tag insertion
  - Reporter lines
  - Conditional KO (CKO)
- **TARGATT™**
  - Site-specific gene insertion
  - Works in non-dividing cells
  - Large fragment H11 up to 230kb

**TARGATT™ Integrase System For Generating Site-Specific Transgenic Animals**

- **TARGATT™ Rat**
  - Gene expression cassette
- **TARGATT™ Vector**
  - Pronuclear injection of vector containing gene expression cassette + integrase mRNA into TARGATT™ Embryo

**Generation of TARGATT™ “Master” Rats**

- A. Identification of rat H11 locus (H11)
- B. Inserting attP at H11 locus using CRISPR
- C. TARGATT™ rat with attP site
- D. Establishing the TARGATT™ rat colony

**Generation of CAG-GFP Rats Using TARGATT™**

- 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 H11 docking site (locus) in the TARGATT™ attP “master” rat. B. Two founder pups (#1 and #19) were identified to carry the gene of interest by PCR using 4 sets of genotyping primers: 5' Gol, 3' Gol, 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 Wnt gene, using CRISPR/Cas9. Founder rats were identified by genotyping - and bred to F1 generation (data shown). B. Out of 12 pups born in F1 generation, genotyping PCR using 3 sets of primers identified 9 pups (4 #1, 3, 4, 5, 7, 8, 9, 10, 11) with insertion of the gene of interest at the Wnt locus. F1 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 #1R43GM120871-01A1
- 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 Wnt-Cre rat line was generated using CRISPR/Cas9 by insertion of the Cre-mCherry cassette into the endogenous locus of the Wnt gene at the 3’-end.
- Similar strategies is being employed for generating the other Cre rat lines funded by 2R44GM108071-02A1 (NIH SBIR).
- This platform can also be applied to generate customized expression rat models.