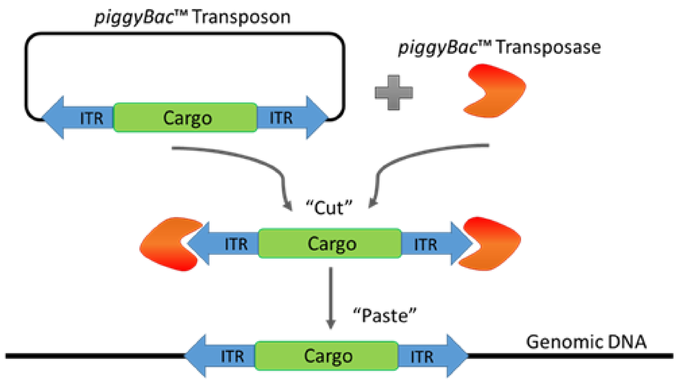
A murine model for chronic liver-specific oligonucleotide efficacy testing

Diane Begemann[1], R. Grace Walton[1], Kyle Rector[1], Marissa O'Callaghan[1], Nicolas Johnston[1], Daniel Machado[1], Chris Brenzel[1], Valeriya Steffey[1], Fallon Noto[1]

[1] Hera BioLabs, Inc. Lexington, KY, USA.

Abstract

Liver-directed oligonucleotide therapies are highly promising, with rapidly emerging chemical modifications requiring careful compound screening for efficacy and side effects. Furthermore, in vitro oligonucleotide activity is not highly predictive of in vivo activity. Thus, numerous candidate molecules must be screened in vivo to identify valid drug candidates. However, transient expression of human genes in mouse liver is not useful for predicting duration of candidate drug efficacy. We have established a model that allows for rapid, high-level, sustained expression of human genes in mouse liver via AAV8-mediated delivery of two piggyBac gene editing system plasmids. The first plasmid contains our liver-specific promoter/enhancer sequence driving the gene of interest (e.g., GFP-Luc). The second plasmid contains an expression vector for the piggyBac transposase (AAV8-sPBase), which mediates genomic integration of the gene of interest. Here we report on a series of experiments aimed at optimizing the system.



Using HepG2 cells, we compared GFP expression driven by our promoter versus several popular liver-specific promoters, including EII-hAAT, pLIVE, and LP1 (with CMV used as a positive control) and found that ours drove higher GFP expression than the others. We then constructed a piggyBac transposon with our promoter driving expression of firefly luciferase packed into AAV8 (AAV8-sPBo-Luc) for delivery to mice. Since mouse strain and sex have been shown to affect AAV8-mediated gene expression, we compared hepatic Luc bioluminescence in Balb/c males, Balb/c females, B6 males, and B6 females following delivery of 1E11vg each of AAV8-sPBo-Luc and AAV8-sPBase, and found that male B6 mice show significantly more bioluminescence than females or Balb/c. In B6 males, we next determined that bioluminescence increases as a function of viral titer (1E12vg > 1E11vg > 1E10vg) and verified liver specificity of Luc expression, with no Luc transcript detected in lung, spleen, or kidney.
Finally, ongoing experiments will determine whether in vivo delivery of anti-luciferase GalNAc-siRNA (with additional 2'OMe, 2'F and pS phosphorothioate modifications) yields consistent knock-down of Luc expression. To our knowledge this is the first high-throughput in vivo platform that allows for testing the chronic effects of liver-directed oligonucleotide therapeutics.

Long Term Stable Expression

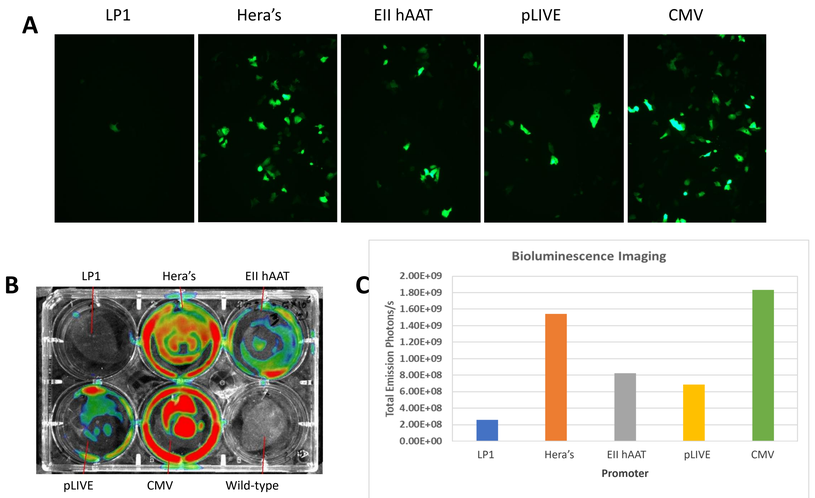


Figure 1: HepG2 cells were transfected with plasmids driving GFP and luciferase expression using various promoter/enhancer constructs. **A:** Representative images showing GFP fluorescence. **B & C:** Luminescence imaging and quantification. Hera's promoter/enhancer construct drives GFP and Luciferase expression at levels that are comparable to CMV.

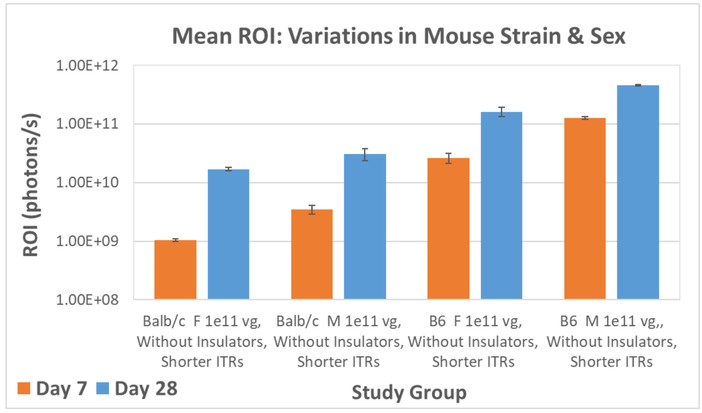


Figure 2: 1e11 vg each of piggyBac™ transposon (AAV8-sPBo-Luc, Hera's promoter) and AAV8-sPBase delivered to female and male Balb/c and C57Bl6 mice (n = 4/grp, IV). Mice were injected with 150 mg/kg luciferin (SubQ) and imaged with AMI HT (Spectral Instruments, Inc). C57Bl/6 males had the highest bioluminesce at 7 and 28-days.

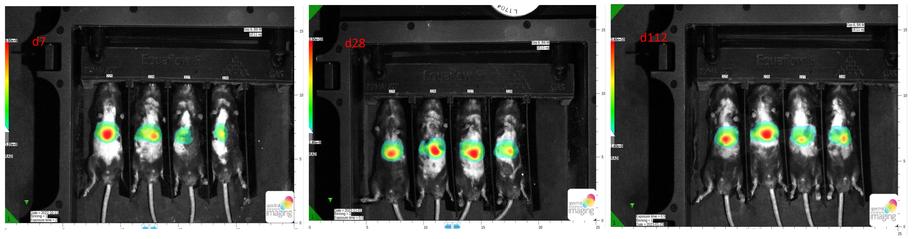


Figure 3: Representative images of C57Bl/6 male mice injected with 1e11 vg at days 7, 28, and 112 post-injection.

Liver Specific Delivery System

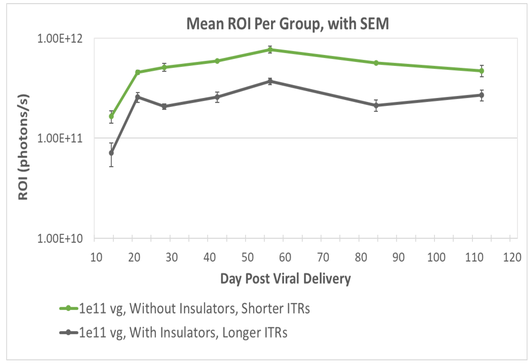


Figure 4: Quantification of bioluminescent imaging over time in mice injected with both 1e11 vg of AAV8-sPBo-Luc with either no insulators and shorter ITRs (Green) or with insulators and longer ITRs (Blue) and AAV8-sPBase.

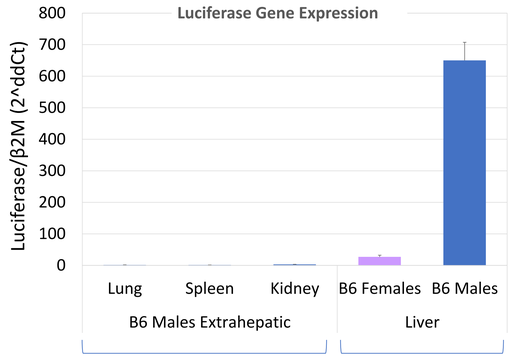


Figure 5: piggyBac™ transposon delivery system (1E11vg each of AAV8-sPBo-Luc and AAV8-sPBase) optimized by Hera BioLabs demonstrates liver specific target gene expression, as indicated by nondetectable extrahepatic luciferase presence.

Conclusions

- Hera BioLabs has demonstrated a stable, customizable, specific viral delivery system that can be utilized to express a variety of unique genes in the mouse liver.
- AAV8-piggyBac hepatic luciferase expression is higher in male B6 mice versus female B6 and Balb/c males or females.
- Inclusion of insulators and long ITRs on the transposon does not enhance luciferase expression stability over 112 days.

Future Research

- Hera BioLabs plans to use the piggyBac transposon rapidly express human genes in mouse liver for siRNA efficacy screening.
- Liver-targeted RNAi therapeutics are commonly GalNac-conjugated, providing months long knockdown of target genes in vivo.
- To silence gene expression in our luciferase model, we will use anti-luciferase GalNAc-siRNA with 2'Ome, 2'F, and pS phosphorothioate modifications to enhance in vivo stability.
- 28 days after viral luciferase induction, we will dose animals with varying concentrations of GalNAc-siRNA, and measure bioluminescence.