**Generation of an Alpha-1 Antitrypsin Knockout Mouse Model Using CRISPR/Cas9 System**

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### Background

Alpha-1 antitrypsin (AAT) deficiency is a common autosomal co-dominant genetic disorder. This condition affects 1:2500 individuals of European ancestry, leading to the development of lung and liver disease. Within North American and Northern European populations, an estimated 4% of individuals are carriers of deficient genes for AAT. AAT deficiency presents with an emphysema-like phenotype in the lungs of older subjects. AAT deficient subjects also suffer from liver disease of varying severity; however, lung disease is the principle cause of death. Belonging to the serpin family, AAT is a protease inhibitor predominantly synthesized in the liver. Upon secretion into the blood stream, AAT is directed towards the lungs where it inactivates excess neutrophil elastase, thereby preventing damage to the alveoli. Mutations of the SerpinA gene can lead to reduced serum levels of AAT and decreased protein functionality, allowing for unrestricted elastin breakdown, pulmonary inflammation and eventual emphysema. Currently, an animal model simulating the lung condition does not exist, which severely limits the development of innovative therapeutics.

### Experimental Design

1. Design gRNA's that target coding regions within each SerpinA gene (5 copies per chromosome).
2. Test efficiency of gRNA constructs using a Single Strand Annealing Assay
3. Design primers to screen for AAT mutations (In/Del) using PCR assay
4. In vitro: Screen embryonic stem cells co-transfected with gRNA and CRISPR/Cas9 system
   - **In vivo**: Screen microinjected transgenic zygotes (gRNA: 20ng/μL, Cas9 mRNA: 50ng/μL)
5. Perform mouse specific AAT ELISA to validate knockout candidates
6. Sequence bands from PCR products of suspected knockout embryonic stem (ES) cells and transgenic mice

### Results

**5 Copies of the SerpinA Gene**

![Diagram of 5 copies of the SerpinA gene](image)

- **gRNAs Cas9**

**Amplicons Showing Deletion Patterns**

![Amplicons showing deletion patterns](image)

**Identifying AAT Knockouts**

![Identifying AAT Knockouts](image)

**Cutting Patterns Exhibited by AAT KO Mouse #7**

![Cutting patterns exhibited by AAT KO Mouse #7](image)

**Conclusions**

- From screening results, AAT knockout mice were created using CRISPR/Cas9 genome editing technology (mice #7, 24, 31).
- Additionally, several other mice exhibit considerable AAT knockdown (mice: 10, 15, 33).

**Future Plans**

We will exacerbate the lung condition and characterize this model by performing necessary pathological and histological analysis of affected tissues. With significant clinical relevance, this developmental model presents the opportunity to create novel therapeutics that will help to treat future patients affected by AAT deficiency.

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