Optimizing Cre Recombination to Develop *in vitro* Models for ABCA3 Deficiency

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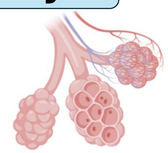
ABCA3 Protein Deficiency

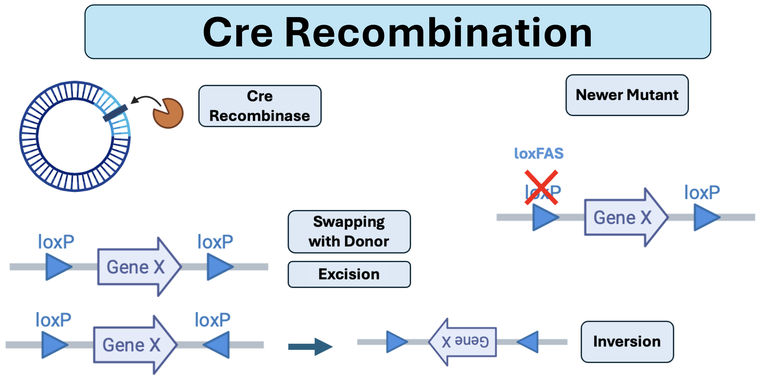


- The ABCA3 glycoprotein is made by lung AT2 cells and maintains healthy surface tension in the lungs
- ABCA3 deficiency is a fatal genetic condition seen soon after birth
- The primary treatment method is lung transplantation, which is particularly difficult to access for infants

Project Goal

Creating lung AT2 cell lines that can model ABCA3 deficiency can provide an *in vitro* tool for use in testing genetic therapy for ABCA3 deficiency.

Cre Recombination



The Cre Recombinase enzyme can be used as a tool to add the Wild Type ABCA3 gene or its mutants to A549 lung AT2 cells. The gene of interest can be provided by a donor plasmid and swapped with a "landing pad" sequence integrated into the cell genome.

Hypotheses

- The CMV promoter needs to be removed in order to create compatible donor plasmids
- The loxFAS/loxp configuration will increase the ratio of swapping to excision
- Delaying addition of Cre enzyme will increase ratio of swapping to excision

Cell Line Generation

VSVg pseudotyped Lentiviral transduction was done with reporter integrant plasmid, followed by puromycin selection.

Integrant plasmid Sequence

Lox site	Ds red	Stop codon	Loxp site	EGFP	IRES	Puromycin resistance

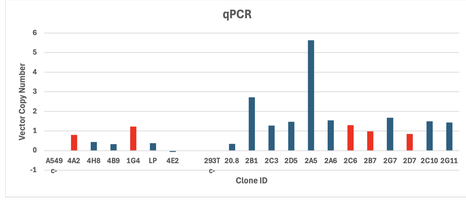
Flow Cytometry

Figure 1 : Fluorescent Protein Expression

Next step: A second round of puromycin selection was done

qPCR

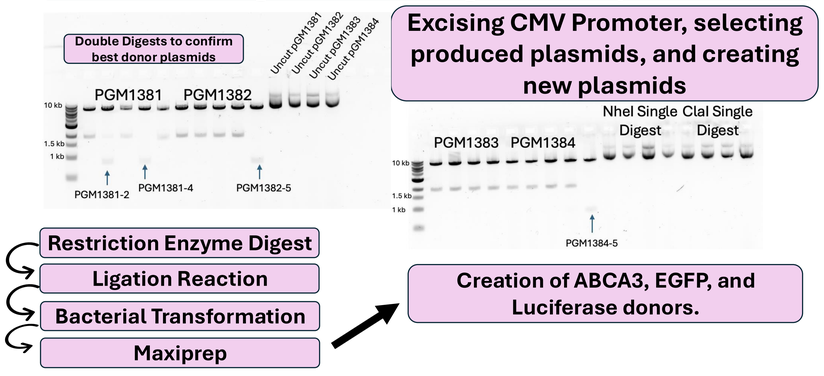
Figure 2 : Assessment of Vector Copy Number



Five cell lines were identified with Vector Copy Number close to 1

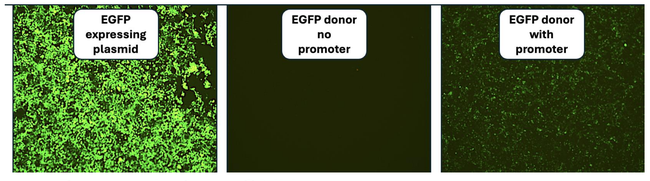
CMV Promoter Removal

Figure 3: Cloning Summary



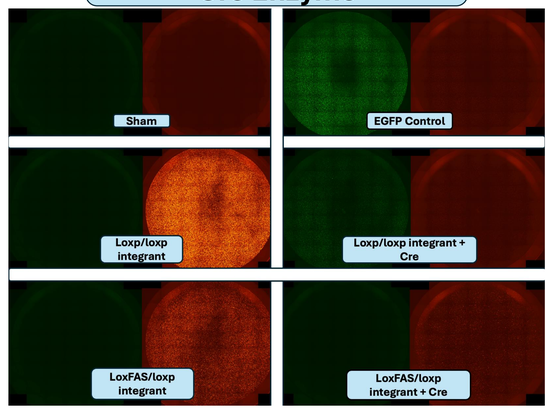
Transfection

Figure 4: Transfection after Removal of Promoter



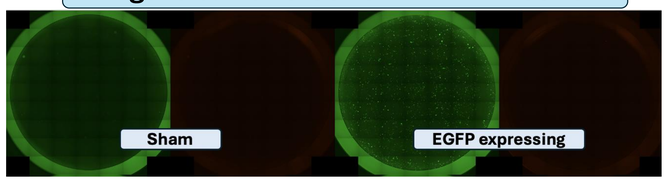
Removal of the CMV promoter was necessary

Figure 5: Transfection +/- Cre Enzyme



The above results suggest that inversion is occurring with the loxFAS/loxp configuration when Cre enzyme is present

Figure 6: Transfection with AT2 cells



Transfection efficiency of A549 cells is significantly lower than that of HEK293T cells.

Conclusions and Future Directions

- After reversing the orientation of the loxFAS sequence, Cre recombination can be re-evaluated in model cell lines
- Transfection of A549 cells should be optimized before further studies are conducted
- After successful ABCA3 swapping, functional assays such as ATPase or lamellar body monitoring can offer insight on effects of ABCA3 mutations
- A549 lines 1G4 and 2B9 were saved for future experimentation due to qualities that indicate vector copy number close to 1

Acknowledgments

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