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G542X & F508del Mutations of Cystic Fibrosis

Cystic Fibrosis (CF) is a recessive human genetic disease that is caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene, responsible for transport of Cl- and HCO3- anions in epithelial cells

Although there are ~2000 mutations responsible for CF, G542X is found in 84% of those impacted, and F508del is found in 4.6%

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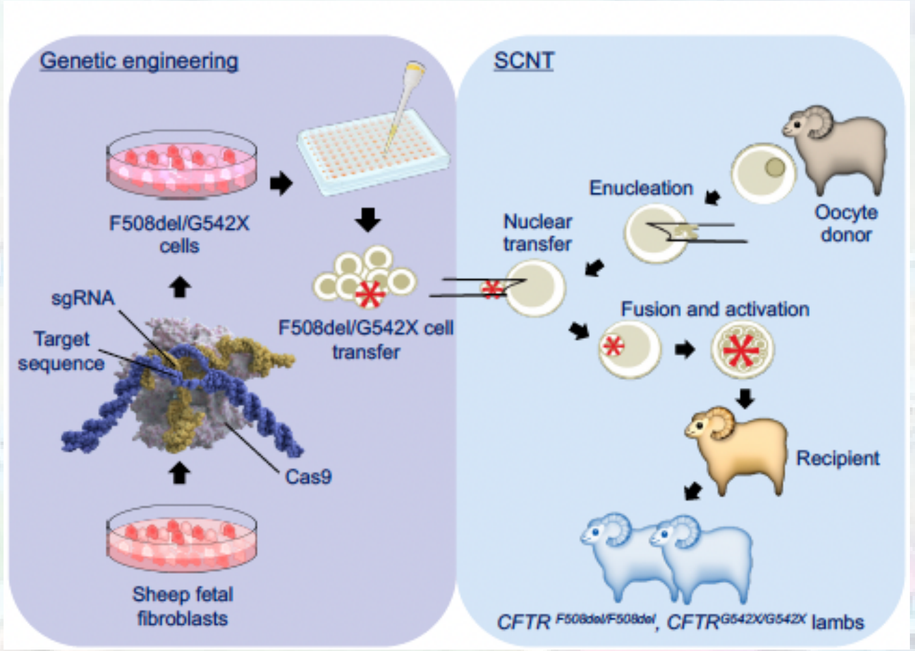
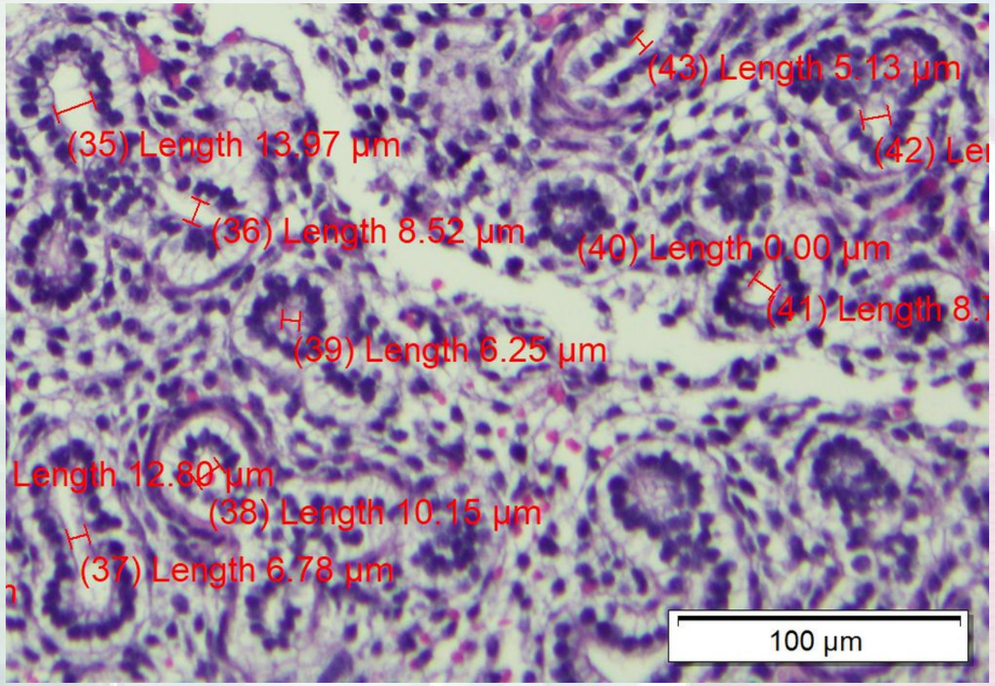
Animal Models

We previously generated CFTRF508del/F508del and CFTRG542X/G542X lambs using CRISPR/Cas9 and Somatic Cell Nuclear Transfer (SCNT) techniques.

Although successful, these lambs had several lethal phenotypic issues such as Meconium Ileus, which although present in affected humans, often does not result in death. However the most surprising discovery what the impact on development!





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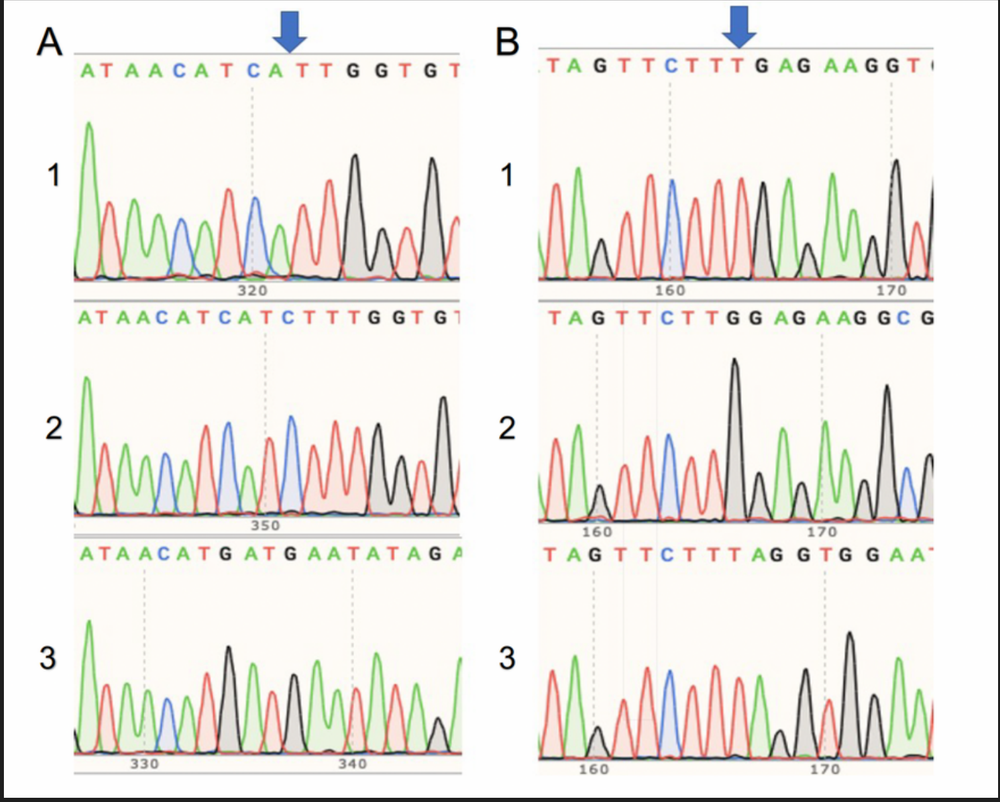
We constructed single guide RNAs (sgRNA) and transfected cells harvested from our animal models with CFTR mutations F508del and G542X.



Sequencing results from single-bacterial cloning containing the correction of (A) F508del and (B) G542X. Rows 1, Non-corrected mutation; row 2, Corrected to WT; row 3, indel mutations randomly introduced.

	Non-Corrected	Corrected	Indels	Total
F508del	6 (17%)	7 (19%)	23 (64%)	36
G542X	21 (53%)	14 (35%)	5 (13%)	40

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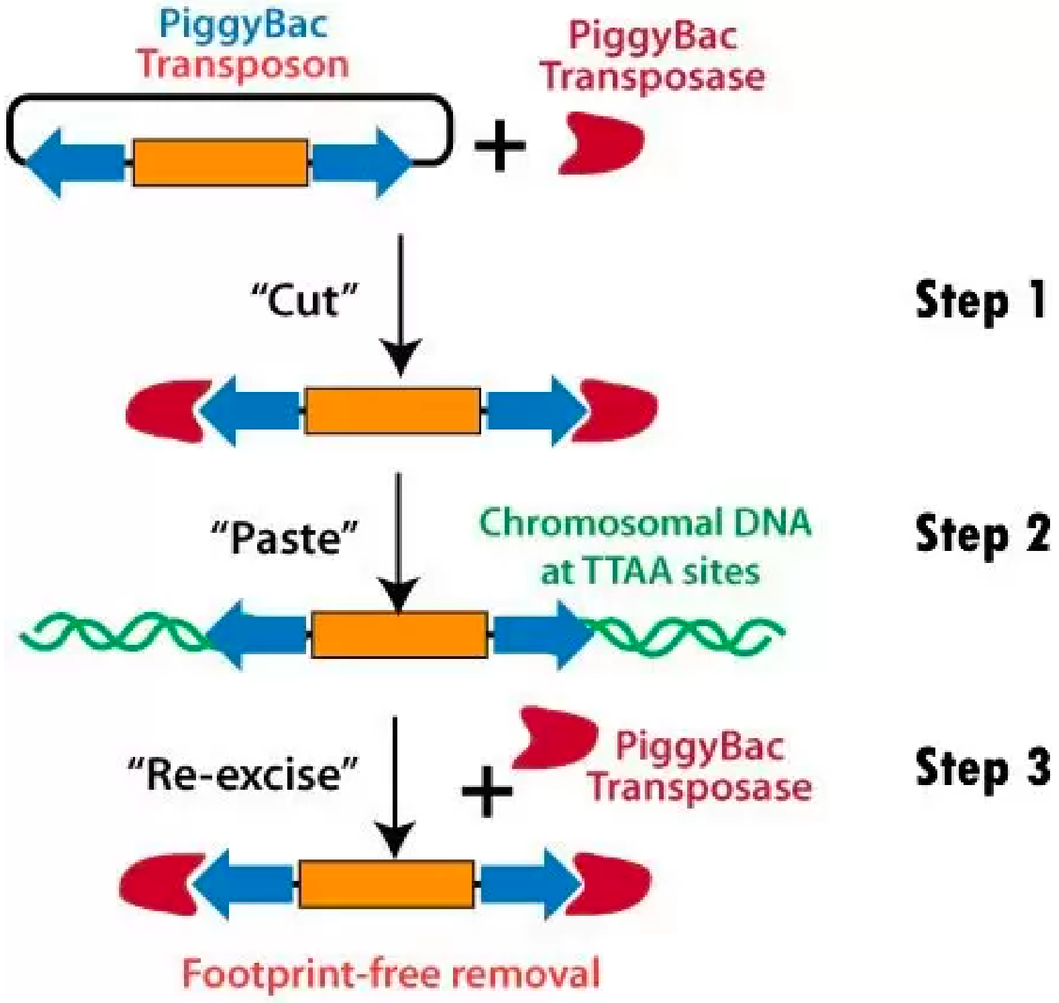
piggyBac Transposon

A Class II DNA transposon, the integration of a DNA transposon in new site results in a duplication of a target sequence on either side of the Transposon. Additionally, the ability of piggyBac to excise itself without traces results in no indels.

We hypothesize that using a piggyBac Transposon will reduce the rate of indels resulting in increased efficiency in correcting the G542X and F508del mutations.

In this ongoing study, we will construct and evaluate the potential of the piggyBac Transposon as a means of gene therapy in Cystic Fibrosis.



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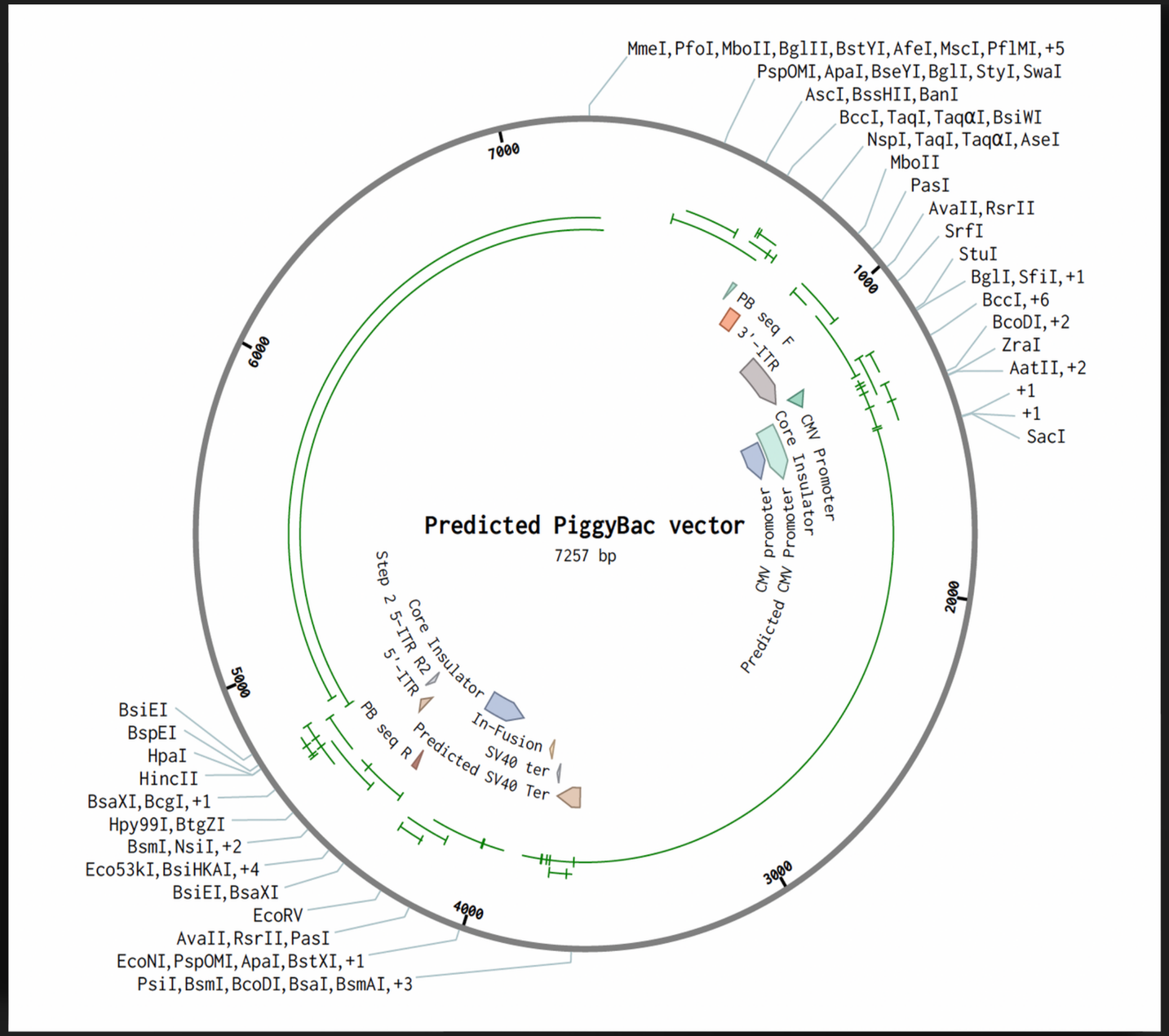
PiggyBac Construction



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What's next? What are we doing now?

Summary

We demonstrated that CRISPR/Cas9 RNP can be effectively used to correct both F508del and G542X mutations in vitro; thus, showing its potential to be an effective tool for correcting genetic diseases.

Current work

The piggyBac Transposon and Prime editing a "search-and-replace" genome editing technology in molecular biology both show potential to be more accurate than CRISPR/Cas-9

Future

Our work shows promise for those impacted by genetic diseases and its potential to be an effective tool for correcting genetic diseases.