Optimisation of transfection methods using DNA and Protein formats for CRISPR Cas9 mediated gene knock out in Beta-TC-6 cells.

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INTRODUCTION

Beta-tumour cells (βTC) are a group of highly differentiated beta cell lines derived by expression of the SV40 T antigen (Tag) oncoprotein under control of the insulin promoter in transgenic mice.

βTC-6 cells exhibit glucose stimulated insulin secretion which makes them a valuable tool in understanding the mechanisms that regulate insulin secretion.

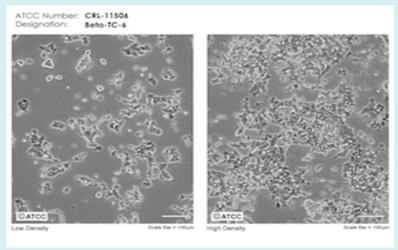

Figure 1

CRISPR/Cas9

Recently emerged as a powerful and highly efficient genome engineering tool.

Provides new approaches for generating *in vitro* disease models presenting an opportunity to study rare genetic diseases [2, 3]

The success of CRISPR genome editing experiments is limited by the intracellular delivery and expression of Cas9 protein and gRNA.

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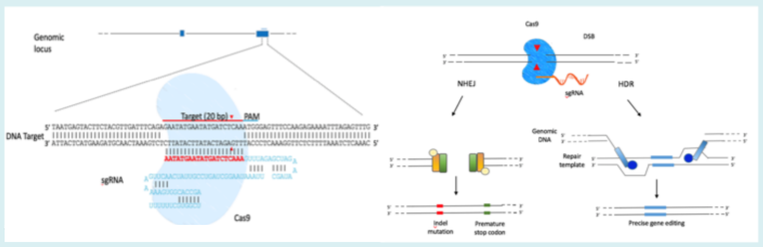


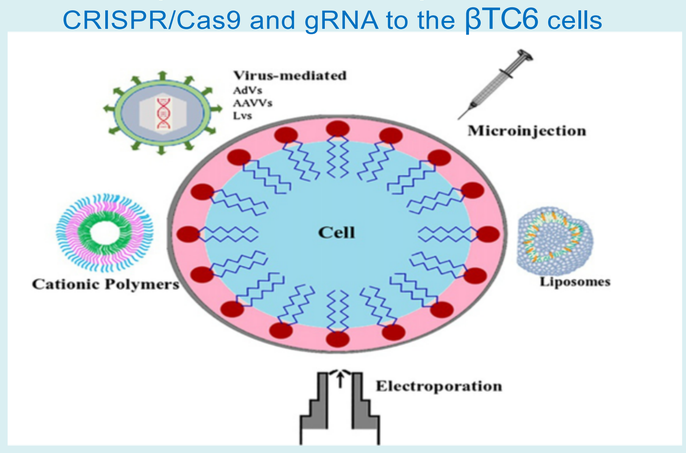
Figure 2

MATERIALS AND METHODS

Gene editing by CRISPR/Cas9

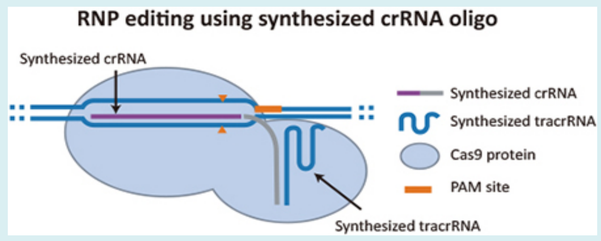
Cloning of gRNA into expression plasmid pX330

Delivery methods for delivery of CRISPR/Cas9 and gRNA to the βTC6 cells



Cas9+gRNA as an RNP complex:

- No transcription of RNA or translation of proteins
- Transient nature → fewer off target effects

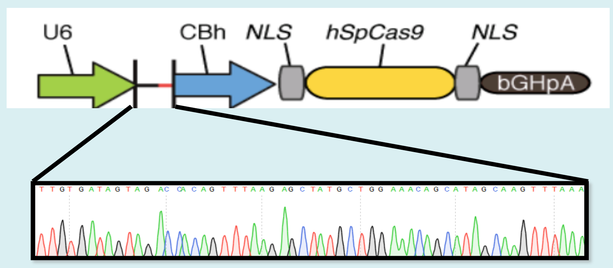


RESULTS

Design of gRNAs to disrupt the ABCC8 gene

gRNA1	CGCTCACCGATGAAGAGGATGGG
gRNA2	GCATGTACAAATGGAGGTGGCGG
gRNA3	CCTCCAGATGGCCCGGGCGCCCT

Cloning of each gRNA in pX330



The cloning of the gRNA into the plasmid was confirmed by Sanger sequencing

Optimisation of Transfection using GFP plasmid

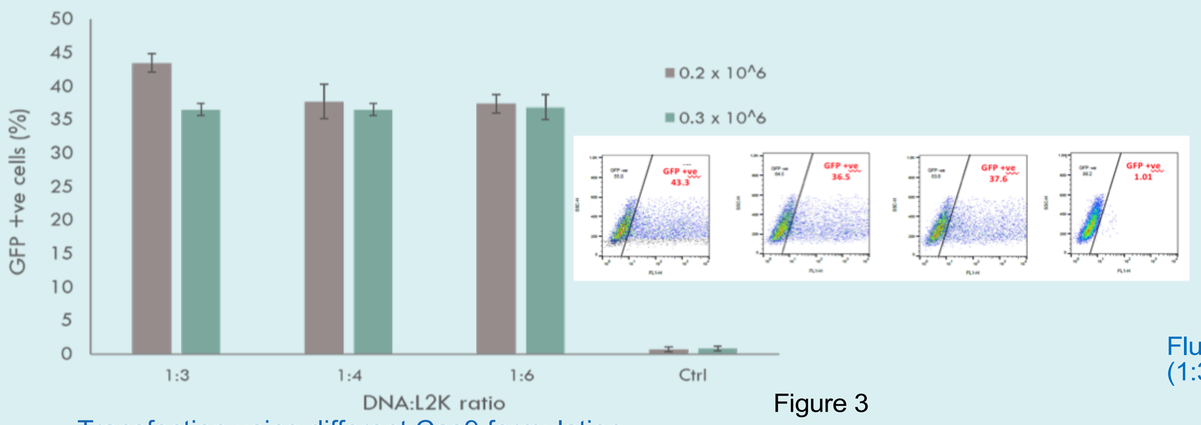
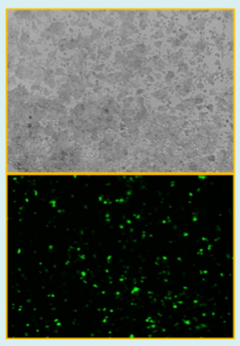


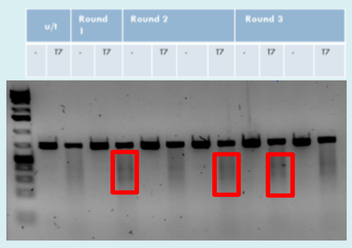
Figure 3

Fluorescent microscopy (1:3) GFPpl: L2K

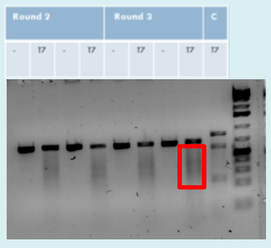


Transfection using different Cas9 formulation

IDT Cas9



True Cut Cas9



Target	PCR product Size (bp)	T7 Endo digest product (bp)	
		Fragment 1	Fragment 2
ABCC8 Exon3	701	460	241

Figure 4

Inference of CRISPR edits (ICE) analysis

Figure 5

CONCLUSIONS AND FUTURE WORK

Conclusions:

- Design of gRNAs to target areas of *Abcc8*
- Cloning of gRNAs into Cas9 plasmid
- Transfections of βTC 6 with the Cas9 + gRNA plasmid & RNP complex

Future Work:

- Optimising the delivery of Cas9/sgRNA system using electroporation
  - gRNA-RNP complex
  - gRNA plasmid
- Molecular validation of the KO *Abcc8* model
  - T7 ENDO assay
  - Sanger sequencing

ACKNOWLEDGEMENT

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References

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