

# UNIVERSITY OF CALGARY

### INTRODUCTION

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology is commonly used to make efficient, reliable and defined targeted changes in the genome of living cells. Commonly, CRISPR technology has been used to make precise edits allowing existing genes to be removed and/or new ones added. In this project, we utilize the technology of CRISPR to activate genes through the use of a modified Cas9 activation nuclease.

Facioscapulohumeral muscular dystrophy (FSHD) is a disease characterized by myasthenia and atrophy and has an estimated prevalence of 1 in 20,000 people. This disease is caused by genetic changes involving the D4Z4 region on chromosome 4. This region contains the DUX4 gene, which is silenced in normal function. In patients diagnosed with FSHD, hypomethylation of the D4Z4 region prevents the DUX4 gene from being silenced.

With the use of CRISPR technology, we will be able to effectively generate expression of the DUXBL gene (homologous to the DUX4 gene in humans) in mice to model FSHD and illustrate the effectiveness of CRISPR activation technology.



### MATERIALS / METHODS



# USING CRISPR/CAS9 TECHNOLOGY TO MODEL FACIOSCUPULOHUMERAL **MUSCULAR DYSTROPHY (FSHD)**

# Sanchit Chopra, Derek Toms, Mark Ungrin

## Department of Compartive Biology & Experimental Medicine, Faculty of Veterinary Medicine









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Amplify ~800bp of Duxbl fragment from Assembled Plasmid

Amplfication of both the backbone vector plasmid (pmaxGFP) as well as desired genomic DNA fragment (Duxbl gene) was possible through the designed primers and PCR protocol. Assembly of both these fragments was achieved through the ease of the Gibson Assembly protocol, which promises the use of this method in the future. Our desired CRISPR activation reporter was consturcted successfully, next step is to test the consturct into an easy-to-transfect cell type by deliver CRISPR (Cas9 VP64 and designed gRNA's).

### Genome Wide Editing Screening Point mutations, deltions/insertion (genes/genomic fragments Knockout libraries, loss of - function screens, **Transcriptional** gain - of - function Regulation screens CRISPR Activation/Repression, /Cas9 **Epigenomic Modulation**

Using CRISPR technology, researchers can insert synthesized gene drive systems into host organism's genome with a high level of precision and reliability. Specifically, CRISPR activation and repression technology can be used to target practically any promoter region in living organisms to model or even treat genetic illnesses.

- Anti-viral and Cancer Therapies
- Correction of genetic
- abnormalities Engineered Cas9 to alter
- methylation (and regulation) Basic science
- applications
- > Therapeutic use in human cells

# cells

- organisms





## email@sanchitchopra.com 403 918 5633

### CONCLUSION / APPLICATIONS



### FUTURE

Transfect promoter plasmid in embryonic stem

Delivery of CRISPR activator (Cas9 VP64) Fuse gene activating Cas9 proteins to different domains for optimized gene activation potential Delivery mechanisms of CRISPR into



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