Gene-Editing Myostatin to Achieve Muscle Hypertrophy

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ACKNOWLEDGEMENTS

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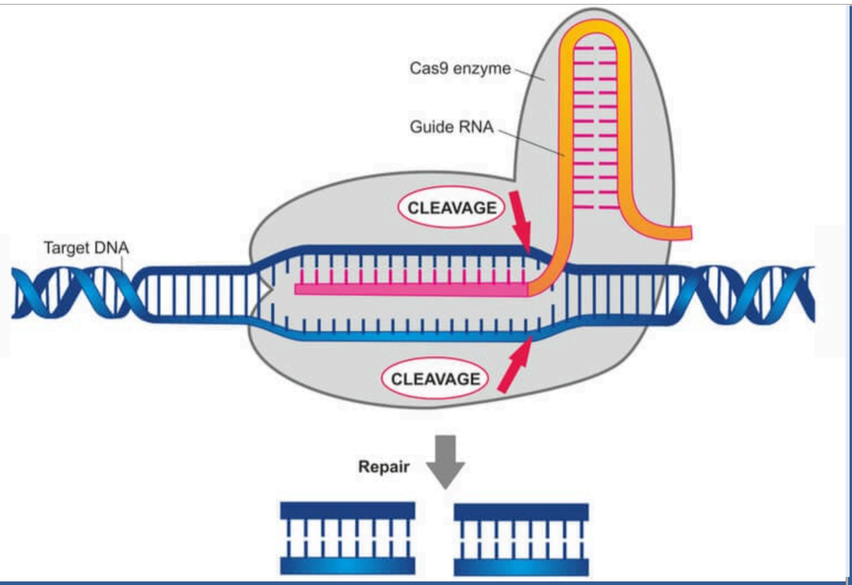


1. Introduction/Background

Found on chromosome 2, myostatin, also known as Growth Differentiation Factor 8 (GDF-8), is a crucial negative regulator of muscle growth. Mutations in the myostatin gene can lead to muscle hypertrophy. Its role in potentially treating muscle degenerative conditions like Duchenne's Muscular Dystrophy and muscle loss in athletes highlights its therapeutic value. Innovations in CRISPR/Cas-9 technology allow precise targeting and editing of specific genes, enhancing the potential to manipulate the myostatin gene for medical benefits.

2. Hypothesis

Using CRISPR/Cas-9 technology to selectively delete segments of the myostatin gene in human and mouse cell lines will result in the inactivation of the myostatin protein, leading to an increase in muscle mass.

Related Work

Moro, L. N., Viale, D. L., Bastón, J. I., Arnold, V., Suvá, M., Wiedenmann, E., Olguín, M., Miriuka, S., & Vichera, G. (2020). Generation of myostatin edited horse embryos using CRISPR/Cas9 technology and somatic cell nuclear transfer. Scientific Reports, 10, Article number: 15587. https://doi.org/10.1038/s41598-020-72657-7.

3. Methodology

Custom sgRNAs were designed to target exon 1 of the myostatin gene. CRISPR technology was used to transfect the guide RNAs into mouse and human cell lines to achieve gene knockout. Confirmation of successful gene editing was conducted via PCR amplification and gel electrophoresis using custom primers designed around the CRISPR cut sites.

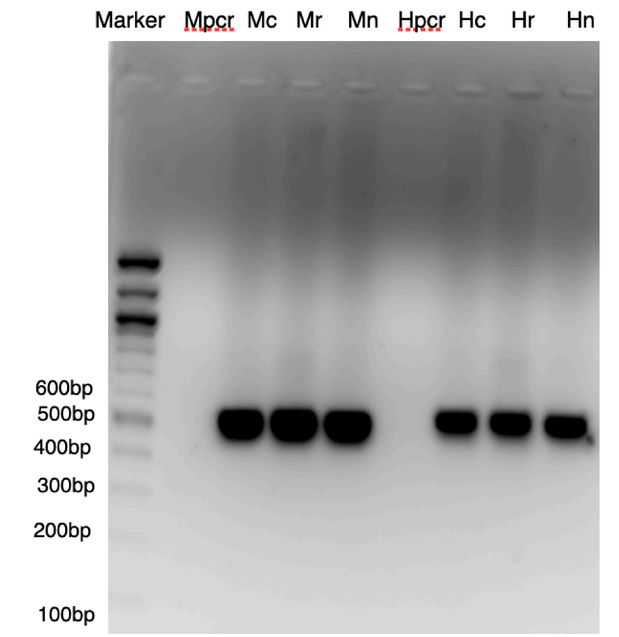
- **Primer/sgRNA design**
- **Cell culture**
- **DNA/RNA extraction**
- **PCR amplification**
- **Gel electrophoresis**

4. Results

The results indicated that the expected deletion in the myostatin gene was not observed in the gel. It is possible that the deletion was too small to detect via in the gel, or the alignment of the deleted base pairs prevented an effective frameshift mutation.

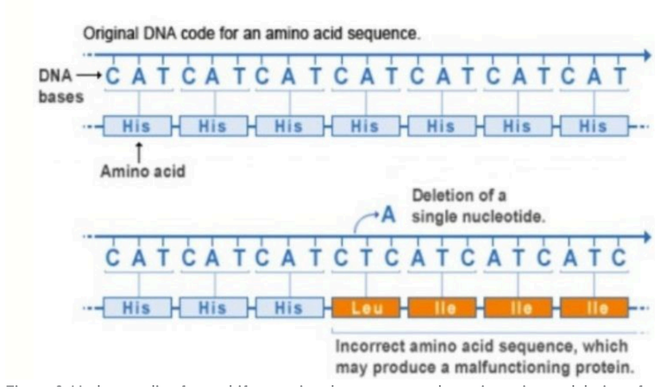
MARKER= NEB 100 BP LADDER

Mpcr = mouse PCR control
Mc = transfection control
Mr = reaction
Mn = untreated normal cells
Hpcr = human PCR control
Hc = transfection control
Hr = reaction
Hn = untreated normal cells



5. Analysis

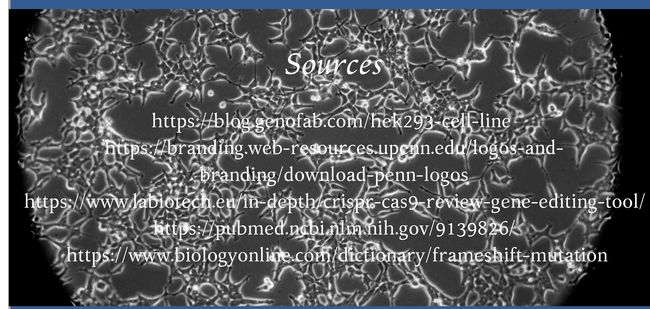
The sgRNA was designed to cause a 77-base pair deletion, and the PCR primers spanned 500 base pairs. Because this deletion is small compared to the amplified DNA length, the deletion is difficult to detect through gel. Further sequencing could identify whether the deletion caused a frameshift that impaired the functionality of the gene.



Example frameshift caused by deletion

6. Future Work

In the next phase of this project, sgRNAs and primers will continue to be designed and optimized to facilitate precise deletions. This strategy will be focused on achieving a frameshift mutation, ensuring the effective knockout of the myostatin gene, and confirming its subsequent loss of function.



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