11-17-2021

Testing the Role of Wfs1 in Calcium Regulation in Drosophila Cells

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Testing the Role of Wfs1 in Calcium Regulation in *Drosophila* Cells

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Introduction

- Wfs1 is a transmembrane protein in the endoplasmic reticulum (ER)
- This gene and its functions can be studied using *Drosophila*
- Wfs1 is the gene that codes for/makes the protein Wolframin, a resident component found in the ER (1)
- Wfs1 thought to regulate calcium levels with transport of calcium in and out of the ER (2)
- Calcium is vital for many physiological functions (3)
- Research findings indicated that Wolframin protein assists in the maintenance of calcium (4)

Goal

- To knock out Wfs1 gene in *Drosophila* (fruit fly) cells
- To see if gene is involved in regulating intracellular calcium levels

Methods

Cell Culture

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Transfection and selection for 3 days

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Genomic DNA Extraction

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PCR for Wfs1

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DNA purification

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Sequencing

Wfs1 Gene

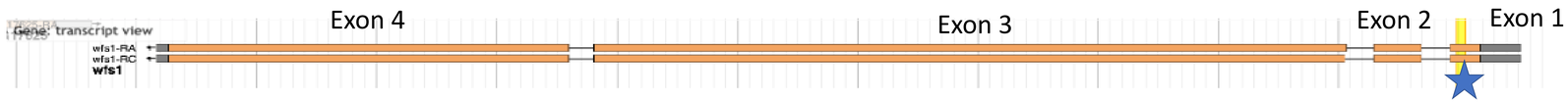


Figure 1: Wfs1 Gene structure with its respective exons. Blue star = target sequence (where it will be cut)

Results and Data

Figure 2: Gel Electrophoresis Image from cells; 10.22.21

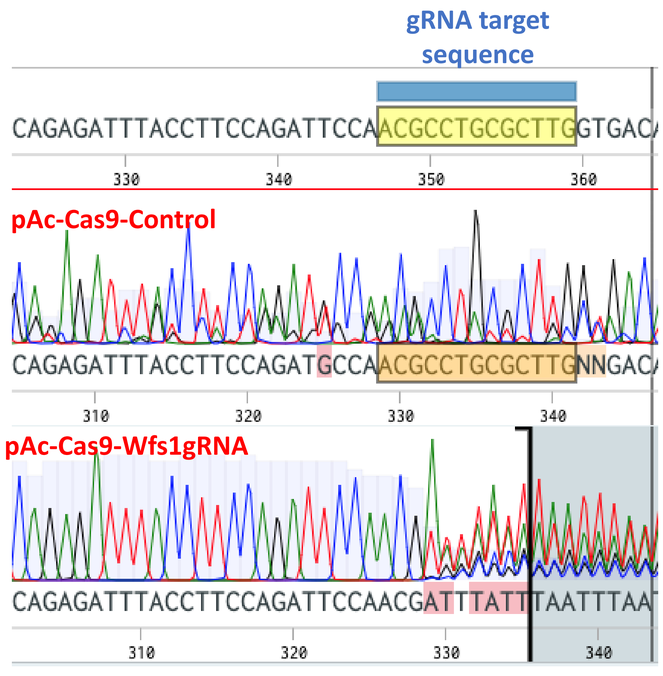


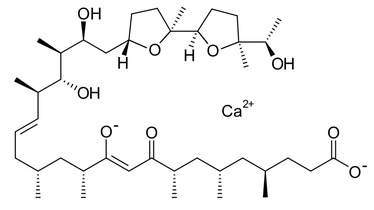
Figure 3: Sequencing Results

Conclusions

- CRISPR-Cas9 molecular tool works/worked
- Gene was successfully knocked out using CRISPR-Cas9 molecular tools
- There were mutations (gene was mutated)

Future Directions

- Treat cells with ionomycin to increase intracellular calcium levels and measure cell death
- Results for Wfs1 knockout cells will be compared to wild type cells with normal Wfs1
- We hypothesize that cells lacking Wfs1 will be more sensitive to ionomycin, as it binds to calcium ions
- Ionomycin sensitivity and gene knockout will prevent cells from properly transporting calcium from the ER = cell death



References

- Ionomycin calcium salt | >99% (HPLC) | CAS 56092-82-1 | Alomone Labs
- VBCF | Vienna BioCenter Core Facilities
- 1.) Rigoli et al., 2011; 2.) Sakakibara et al., 2018; 3.) Martin et al., 2000; 4.) Takei et al., 2006