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Creating a Knockout Cell Line: Using CRISPR/Cas9 as a Tool for Huntington's Disease Research

Emma Jerome

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Honors Capstone

By Emma Jerome

Advised by Dr. Jeff Cantle

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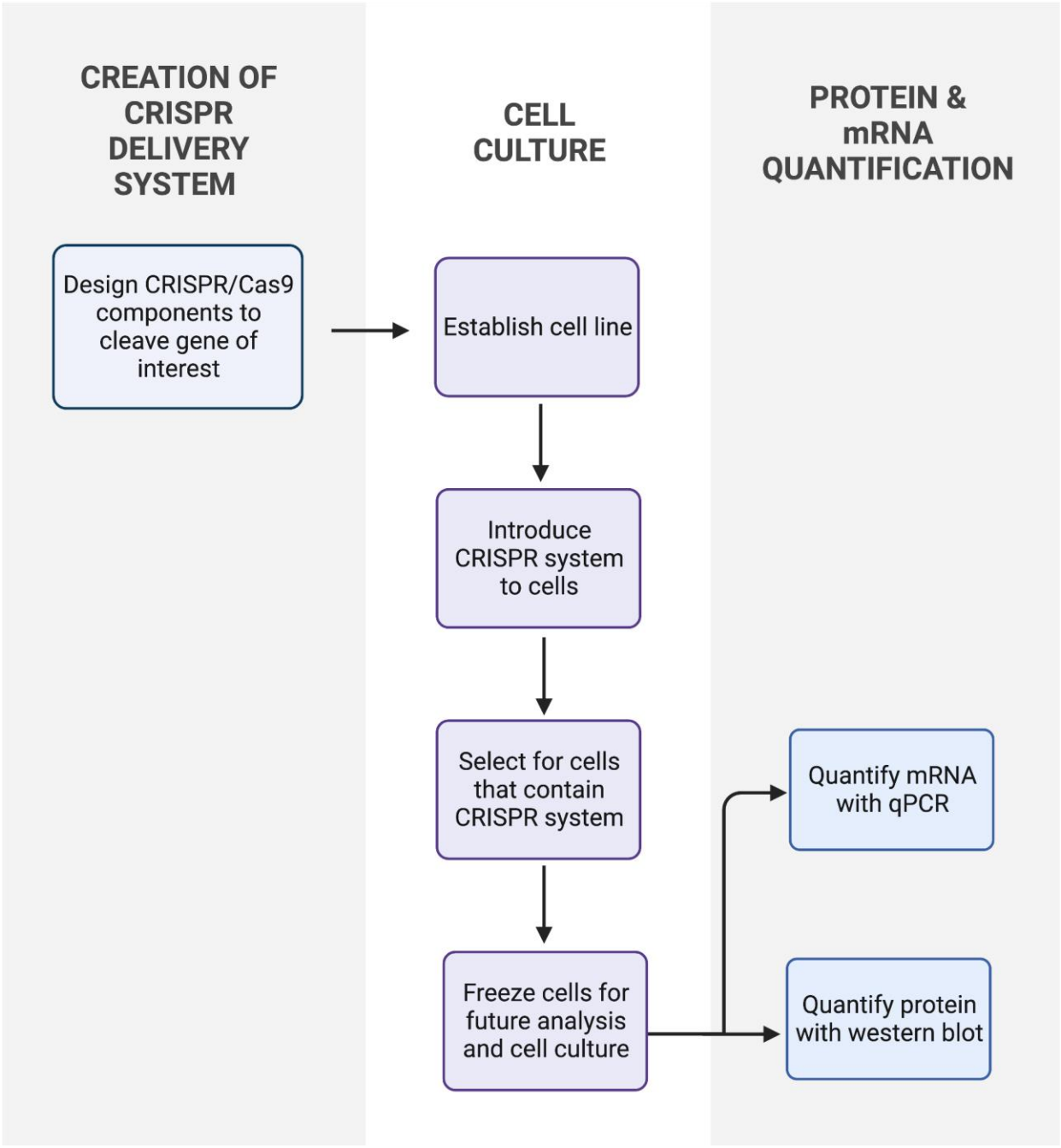
Abstract

This flowchart outlines the experimental methodology used to create and validate a gene knockout in an immortalized cell line. CRISPR/Cas9 gene editing technology was employed to inactivate the gene that encodes a protein of interest previously found to interact with huntingtin, the protein implicated in Huntington's disease. CRISPR/Cas9 was modified to inactivate the gene to prevent the production of the protein it encodes. This CRISPR system was then introduced to a cell line that served as the host for this experiment. Following a several month cell culture, samples were collected to compare the protein content in CRISPR cells versus wildtype controls. Western blot was employed to quantify protein content and quantitative polymerase chain reaction (qPCR) was employed to measure RNA transcripts of the protein of interest. The resulting cell line can be used for future protein-protein interaction research to better understand the function of huntingtin. This work is crucial for understanding the loss-of-function phenotypes thought to contribute to Huntington's disease pathology. The identity of the protein of interest is proprietary information and is withheld from this outline.


CREATION OF CRISPR DELIVERY SYSTEM
CELL CULTURE
PROTEIN & mRNA QUANTIFICATION
Design CRISPR/Cas9 components to cleave gene of interest
Establish cell line
Introduce CRISPR system to cells
Select for cells that contain CRISPR system
Freeze cells for future analysis and cell culture
Quantify mRNA with qPCR
Quantify protein with western blot