Molecular Cloning and In Vitro Culture of Human Cells

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Introduction

- The Zaret lab's goal is understanding the process of "cell fate control." Cell fate control is the process by which a pluripotent cell becomes specialized into a differentiated cell. DNA is organized in a tightly compacted form called chromatin. Cell fate control is achieved by the deactivation of genes in accessible chromatin known as euchromatin and the repression genes within inaccessible chromatin known as heterochromatin. Understanding how cells determine their fate is important because this can help us with therapeutic applications, such as replacing damaged cells.

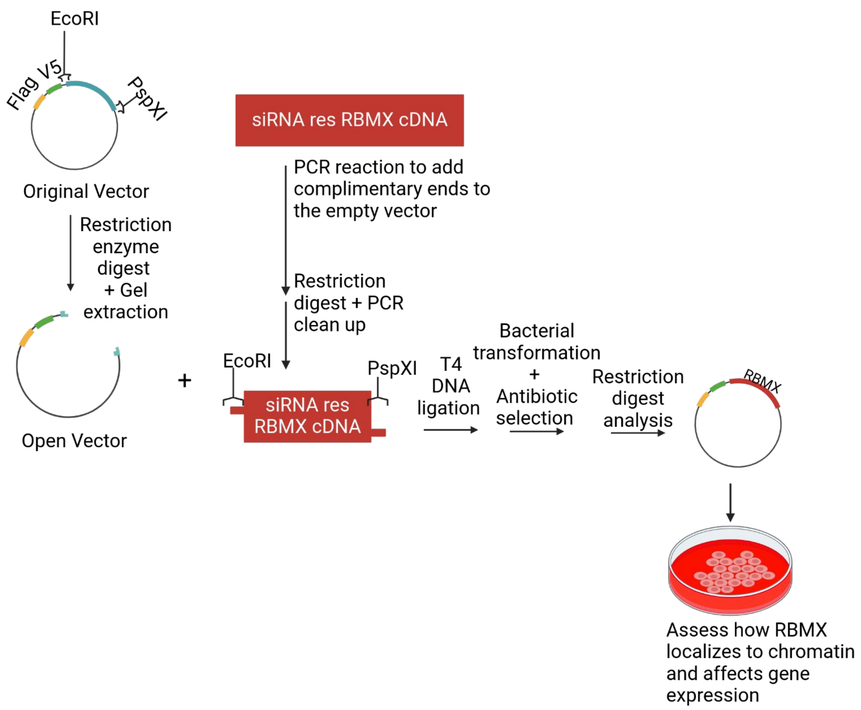
- We were interested in how gene silencing occurs in heterochromatin. The Zaret lab did a previous study to identify proteins present in H3K9me3 heterochromatin. We found that a protein highly present in heterochromatin was RBMX. RBMX is an important protein used to maintain gene expression. We wish to better understand what the heterochromatin enriched proteins are doing to regulate gene expression.

Goal of My Summer Project

- **Question:** How does heterochromatin enriched protein affect gene transcription?

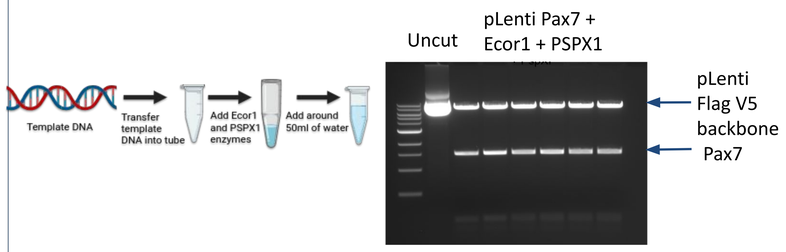
- **Hypothesis:** Heterochromatin enriched proteins are important for the suppression of genes in H3K9me3 heterochromatin.

- **Goal:** To be able to study heterochromatin proteins, we sought to make a plasmid that expresses RBMX at will in human primary cells.

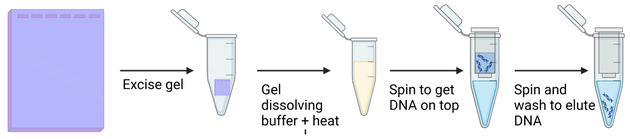


Methodology

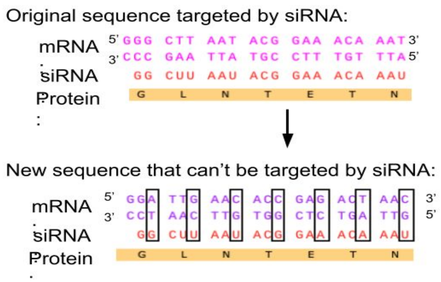
- **Restriction Enzyme Digest:** A restriction enzyme digest is used to open up the plasmid to create overhangs to ligate the RMBX to the plasmid.



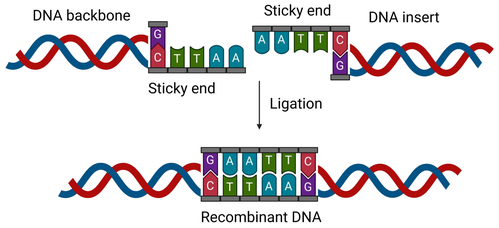
- **Gel Extraction:** The gel extraction isolates our desired DNA fragment.



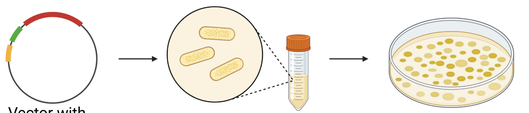
- **siRNA knockdown:** We are changing the nucleotide sequence to make the mRNA resistant to siRNA without changing the resulting protein.

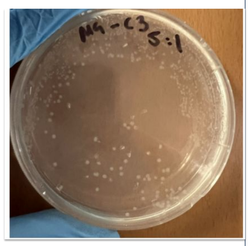


- **Ligation:** The ligation process allows us to put our RMBX construct into our isolated backbone.



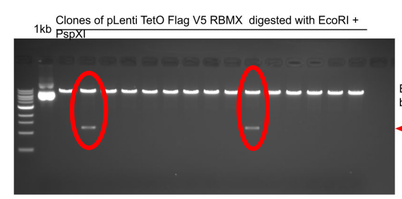
- **Bacterial transformation:** We introduce our completed DNA structure into bacteria to amplify the construct.

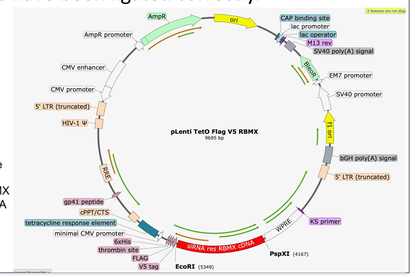




Results

- We ran an agarose gel to determine which bacterial colony was ligated correctly, i.e. has our desired DNA. The circled constructs have been ligated correctly.

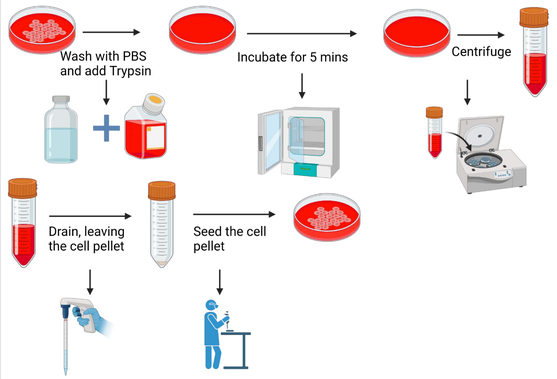


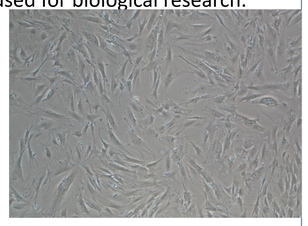


Protocol for Culturing Human Cells

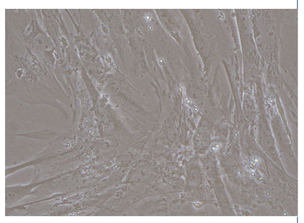
- While testing our hypothesis for heterochromatin during the program, I've also been working on cell culturing. The cells I cultured were called BJ Fibroblast cells (BJ FB).

- **BJ FB:**BJ Fibroblast cells are versatile human skin cells used for biological research. We used them to study the effects of protein absence.





BJ FB cells 10x



BJ FB cells 20x

Conclusion/Future Steps

- We were able to make 2 constructs that can be used for future testing. This means that we were able to insert the RBMX into the backbone and can test for RBMX in human fibroblasts. I also successfully cultured cells for the entirety of my research experience.

- For the future of the experiment, Diego plans to insert the constructs into the BJ FB cells that will be able to express RBMX and further his experiment.

- My time here has been an overall very good experience and I'm glad to be able to work in a lab at this age, which is an opportunity not many people get.

Acknowledgements

I would like to thank Ken Zaret for hosting me in his lab, my mentor Diego helping me through this project and being an incredible teacher, and I would like to thank Tarence and Dr. Shuda for allowing me the opportunity of working in a lab through OER.