

# Using CRISPR To Better Understand Cancer

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## Introduction

Rhabdomyosarcoma (RMS) is classified into two major subtypes including the embryonal subtype (ERMS), which is the most common form of the disease and the alveolar subtype (ARMS), which is the more metastatic and aggressive subtype. Embryonal subtype usually affects children in their first 5 years of life, but it can occur at older ages as well. ERMS tends to occur in the head and neck area, bladder, vagina, or in or around the prostate and testicles. Alveolar rhabdomyosarcoma typically affects all age groups equally. It makes up a larger portion of RMS in older children, teens, and adults than in younger children. (“What is Rhabdomyosarcoma?”) *Mus musculus* genetic engineering advancement has enabled scientists to explore genetic mutations at a genomic level.

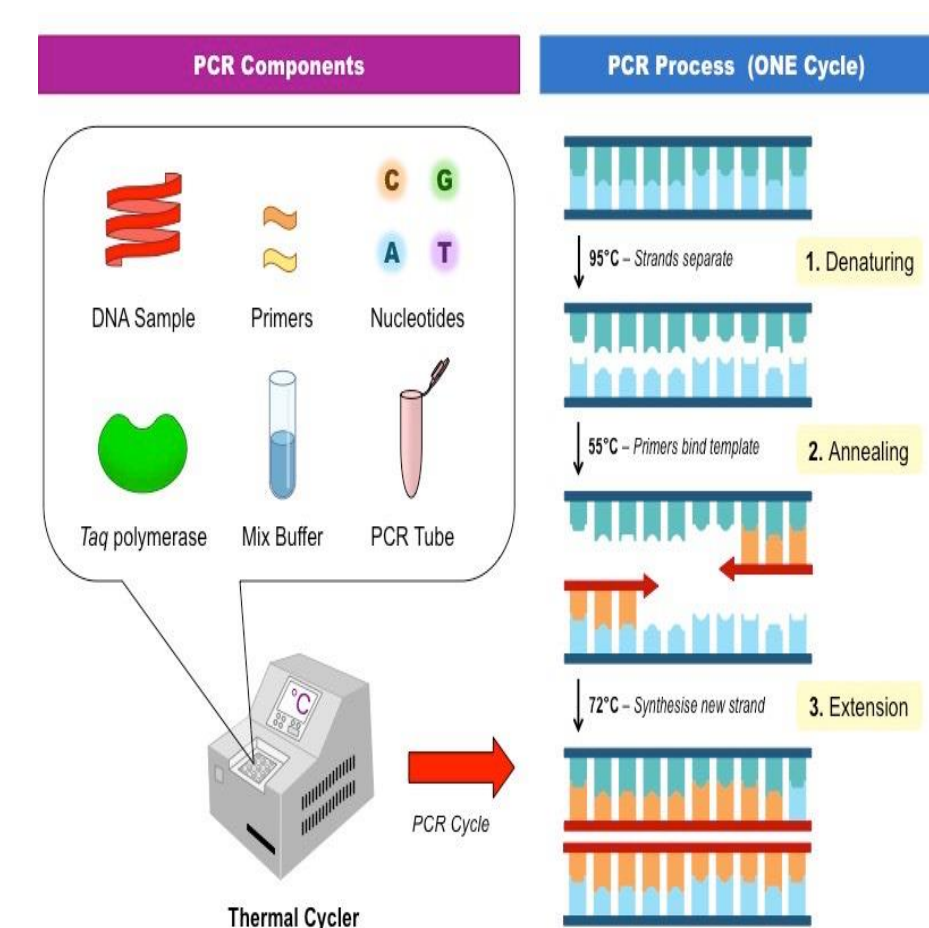
C2C12 cells are myoblast cells from the mouse cell line *Mus musculus*. RH30 cells are found in the bone marrow of patients with alveolar RMS.



## Methodology

WT/ dKO Mice  
Biopsy of Tail or Toes or Fingers  
DNA Isolation

Polymerase Chain Reaction (PCR)  
Gel electrophoresis  
Genotyping  
Sequencing

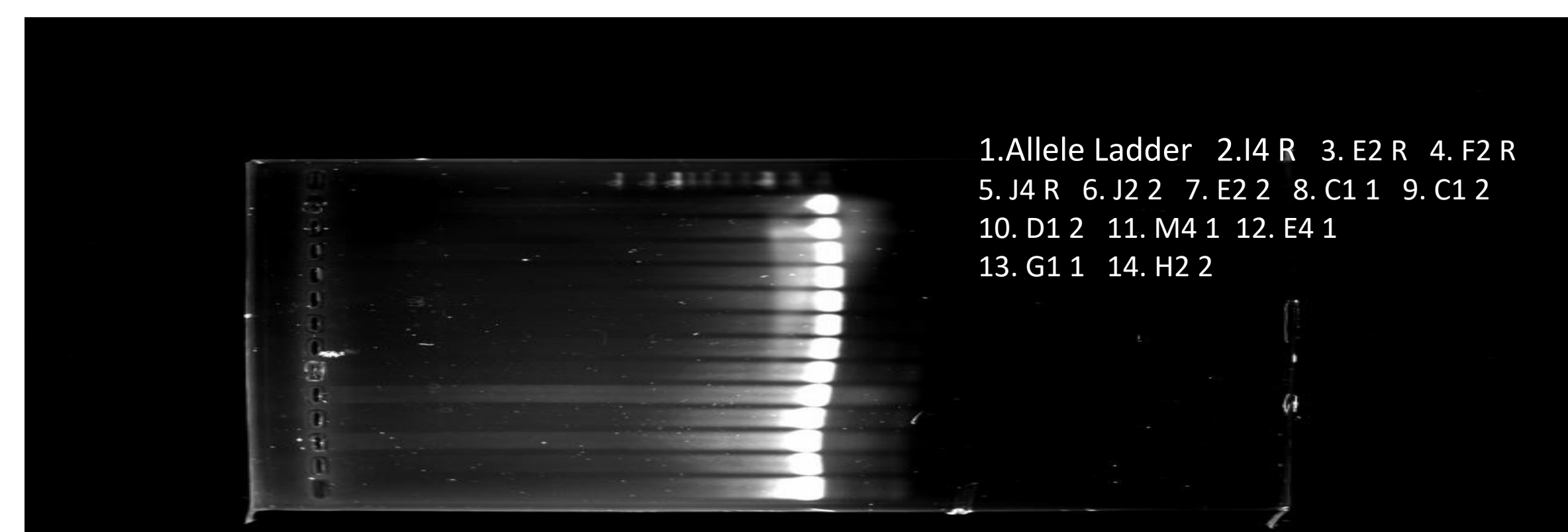


Genotyping using PCR and Electrophoresis Polymerase chain reaction is a technique that takes a small sample of DNA and amplifies it. This is one of the methods used in this research to determine if mouse models contain the wild type allele or have been successfully knocked out also referred to as DKO mice.

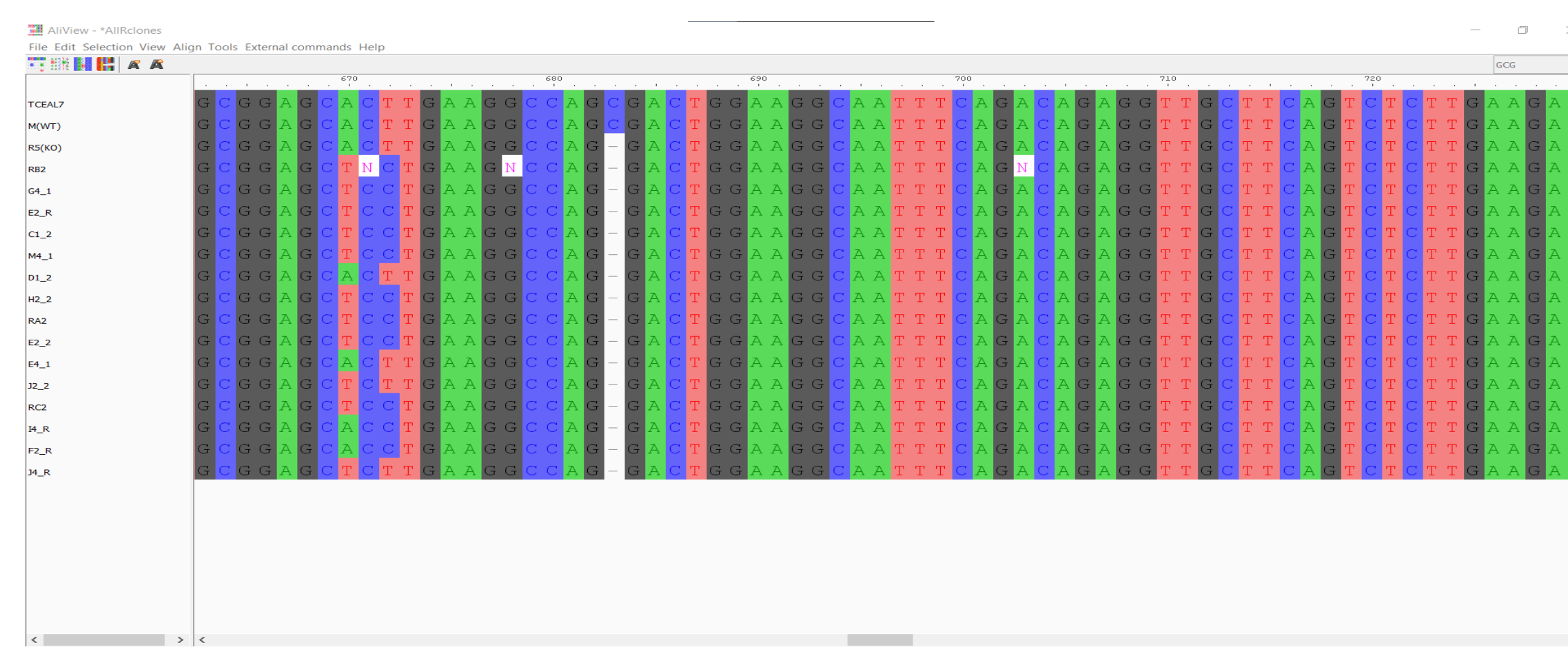
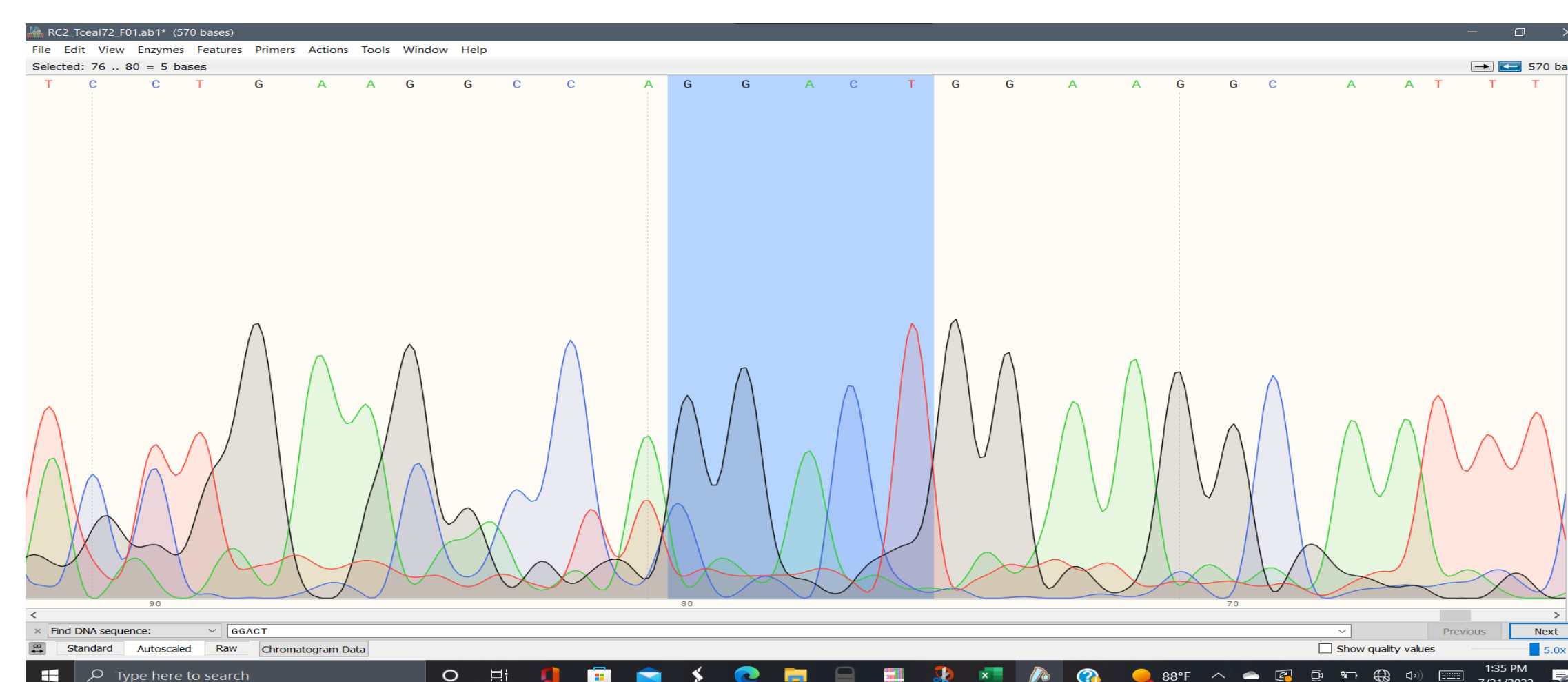
Sequencing, as shown in Figures 1 & 2, is a process that shows the whole genomic sequence displayed as one of the four nucleic acids. Mutations may occur when a change in the DNA sequence occurs during cell division, exposure to a mutagen, along with other known causes.

## Results

While growing these cells a cost efficient and a high yield gDNA extraction method was found to be used in growing cells later. A protocol that required the harvesting of high salt gDNA from 13 cell lines was much more cost efficient than other protocols.



**Figure 1.** The PCR results show that the TCEAL7 primers are working and that amplification was successful. In any normal situation the gene TCEAL7 would be present in these cells, but since the mutation has occurred TCEAL7 isn't present.



**Figure 2 & 3.** Results of sequencing showing that the mutation occurred at the 683 position of the following C2C12 cell culture. The two programs used are AliView and Snap Gene Viewer. For all of the high salt samples a knock out gene is present at the 683 gene indicating where the mutation has occurred.

## Conclusion

Rhabdomyosarcoma's (RMS) are rare forms of cancers that are known to affect the major muscle groups of the body. Researchers use *Mus musculus* as a model to conduct experiments dealing with the mechanisms and genes that affect Rhabdomyosarcoma. Genetic engineering of mice allows us to generate “knockout mice” which entails that a gene has either been deleted or inactivated. Amplification using polymerase chain reaction (PCR) allows DNA to be multiplied and viewed through the use of gel electrophoresis. This technique, as shown in **Figure 1.**, brightens samples, substantially, to view and determine if samples are displaying a gene or if amplification has worked properly. Sequencing results provided by **Figures 2 + 3** show where a mutation has occurred. Further work should be shown to determine if genetically altering the mutated position will cure the disease of occurring again.

## Future Directions

- Alignment of the cancer lines will take place to determine if the mutation has occurred.
- Test proliferation to determine how these cells grow.
- Other characteristics will be looked at to determine what else may be affected by the knock out.
- Testing how this may affect other cancer lines could also take place.

## References

1. “What Is Rhabdomyosarcoma?” *American Cancer Society*, <https://www.cancer.org/cancer/rhabdomyosarcoma/about/what-is-rhabdomyosarcoma.html>.

## Acknowledgements

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