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The Molecular Basis of Feline Xanthinuria

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Abstract

Feline xanthinuria is caused by a mutation in either the *xanthine dehydrogenase* (*XDH*) gene or the *molybdenum cofactor sulfurase* (*MOCOS*) gene. The *XDH* gene was studied to identify the location of the mutation in a hospitalized cat exhibiting the disease. Primers were designed for the 36 exons of the *XDH* gene and each was given a temperature gradient PCR, then used in a PCR to amplify DNA for gel extraction. The resulting DNA was sequenced to compare to the published cat genome. The location of exon 22-23 was identified as a possible site for the mutation, however further sequencing needs to be done to confirm this.

Introduction

Xanthinuria is a rare disease that results in excessive urinary output of xanthine[1], often leading to xanthine crystallization and urinary tract obstruction[2]. Xanthinuria can be acquired by certain medications or, in rare cases, can be due to a genetic error in the *xanthine dehydrogenase* (*XDH*) or *molybdenum cofactor sulfurase* (*MOCOS*) genes[3]. The feline *XDH* gene has 36 exons while the feline *MOCOS* gene has 15 exons. The genetic basis is better understood in humans and dogs[4], while its understanding in cats has been less studied. A cat which was diagnosed with congenital xanthinuria who was treated at Penn Vet is the subject of this study. The purpose of this study is to locate the mutation in the *XDH* gene of the studied cat.

Exon Number	Sequence	Product Length	Optimal Annealing Temperature (°C)
Ex1 Forward	CCCGTAACAATGACAGCGGA	698	60
Ex1 Reverse	TCCCTATGCACCAATGGAGG	698	60
Ex 2 Forward	ATACCTAAGCCCTCCGTGGT	678	60
Ex 2 Reverse	ATCTCCTGAGGAAGGTCCCC	678	60
Ex 3 Forward	ACTTGGGTGCTGTCTGATGG	721	59
Ex 3 Reverse	CCCATATGCAGTTGAGACCA	721	59
Ex 4 Forward	TGGGTGGAGACTTGAGGACA	420	64
Ex 4 Reverse	TCATGCAATGGTCCCAGGAG	420	64
Ex 5-6 Forward	AGAGCCTGAGAGCGTTTGTG	1254	62
Ex 5-6 Reverse	GACCCTGCCAACTGGGATTT	1254	62
Ex 7-8 Forward	CAGCAGCAGGCCTCAATTTC	635	64
Ex 7-8 Reverse	CACAGACTTAGCGGGACCTC	635	64
Ex 9 Forward	GGACCTGGATCCTCGTTCTG	460	58
Ex 9 Reverse	CTGGAGAGCGGTCAGTAAGG	460	58
Ex 10-11 Forward	GGCTGACATTGTGGAAACCG	1080	63
Ex 10-11 Reverse	AAAAGGCCCCATGACCTCAG	1080	63
Ex 12 Forward	AGCTGACCCACATTACCGAC	415	63
Ex 12 Reverse	CATTGAATTGTGGGCCCTGC	415	63
Ex 13 Forward	ACTGTGGGTACCAGAGACCA	452	62
Ex 13 Reverse	TTTACAGACGAGCAAGCCGA	452	62
Ex 14 Forward	CCGGCTTCTGGGAGTGTTAT	570	61
Ex 14 Reverse	TGCTCCCAGGAATCGAGCTA	570	61
Ex 15 Forward	CTGGCAGTTTCTGGAACGAG	442	60
Ex 15 Reverse	AAGGATTCAGTCGGGACCAG	442	60
Ex 16 Forward	GAGATAGATCCAGCCGGCAC	531	61
Ex 16 Reverse	ACTCCTGGCTTTGCACATCA	531	61
Ex 17 Forward	ACTCAGGTTTGCTTTGGCCT	400	62
Ex 17 Reverse	TGAGTCCCTAACCTCTCCCG	400	62
Ex 18 Forward	AGGGCCCAAAAGTGCTGATT	678	61
Ex 18 Reverse	CATCCCTGTACTGATCGCCC	678	61
Ex 19-20 Forward	TTTGGGTCTGCGTCTCTTCA	1125	61
Ex 19-20 Reverse	CGGCCGTACCTGAAACAATG	1125	61
Ex 21 Forward	CCCCAGGCGAGTTGTACATT	583	60
Ex 21 Reverse	AGTTCTGTCGCCTTACCAGC	583	60
Ex 22-23 Forward	TCACCAGTTCTTGGCTGGAT	1121	59
Ex 22-23 Reverse	AGCATCGCCCTTCTATGTGG	1121	59
Ex 24 Forward	AGGTCCTCTCTGCTTCACCT	405	64
Ex 24 Reverse	TAGAGCTTAACCGGGGGTGG	405	64
Ex 25-26 Forward	GGGCATGTCAGGTGAGAGTA	1003	63
Ex 25-26 Forward	AGCACCCTCAAGCACCTTAC	1003	63
Ex 27-28 Forward	CCCTGACTCTGAGCTCCACT	1121	64
Ex 27-28 Reverse	GCTTCTGCTTCTAAGGTGTGC	1121	64
Ex 29-30 Forward	TGAAACGGTCTTGGGGAGAAG	1086	64
Ex 29-30 Forward	CTGTGAAACCCTACGGACCA	1086	64
Ex 31 Forward	TCTTAGTGCTGGGTCAGGGA	443	64
Ex 31 Reverse	GGTCCCACCACGTCGTAAAT	443	64
Ex 32 Forward	AGAAGGCGTCACATAAGGGC	491	61
Ex 32 Reverse	TTCTCCCAGCCACAAAGTGT	491	61
Ex 33-34 Forward	CCTCGGCATTCACGATACCA	1060	63
Ex 33-34 Reverse	ATTGCCCCATCTGCAGTGAA	1060	63
Ex 35 Forward	AGAACGCCATCGAGGTTGAG	504	60
Ex 35 Reverse	CCTCAACGGCACAAGGAAGG	504	60
Ex 36 Forward	GACAGTTTCCCAACCCCCTTC	605	64
Ex 36 Reverse	TTTGTCGTCAGACCCTCACAG	605	64

Fig. 1, Primers and Temperature Gradient Results.

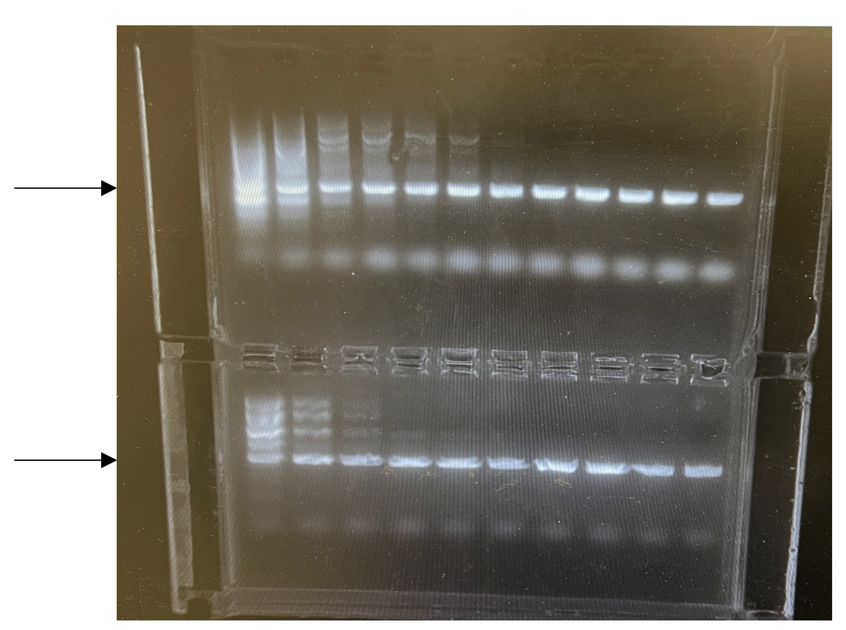

Fig. 2, Temperature Gradient, Exons 13 & 14.

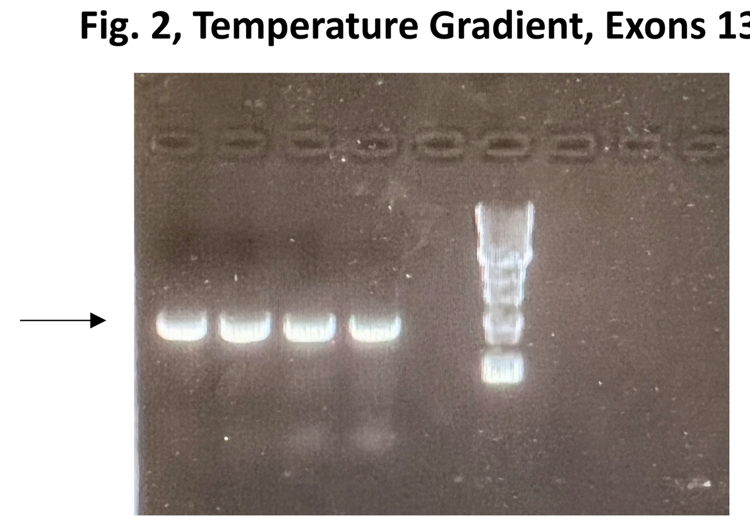

Fig. 3, Exon 33, Pre-Extraction.

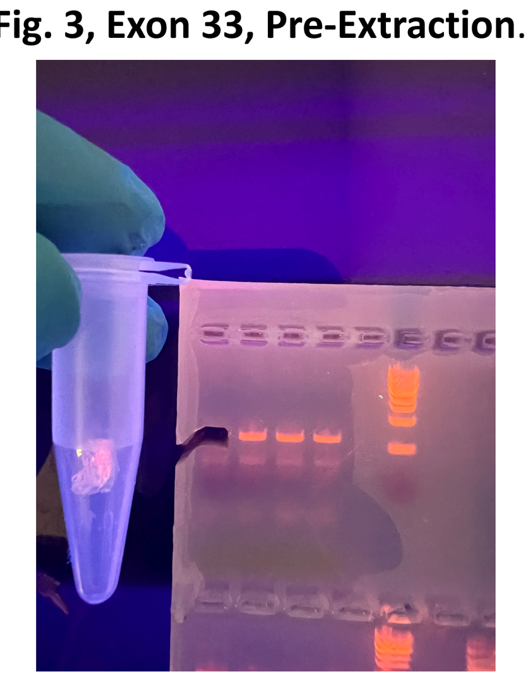

Fig. 4, Gel Extraction, Exon 7.

Methods and Materials

The primary outline of this project is as follows:

- Primer design of the 36 exons of the *XDH* gene via the NCBI database (Fig. 1)
  - Total of 27 primers (some were made to fit two close exons)
- DNA extraction from the blood of affected cat
- Temperature Gradient PCR of each primer to ensure an optimal annealing temperature (Fig. 2)
- PCR using each primer and the DNA from the patient
- Gel extraction of each exon (Fig. 4)
- DNA sequencing of each exon
- Data Analysis of the Sequences (Fig. 7)
  - Comparing found sequences with known sequences

Results

Exons 1-4,7-15,17-20,25, and 31-36 were successfully sequenced. The remaining exons either produced inconclusive sequences that need to be redone or show signs of mutations that need to be further analyzed. In the exons that have been successfully sequenced, a few polymorphisms were found and further analysis will prove if these polymorphisms are disease causing variants or not.

A candidate exon for the mutation was identified when attempting to extract DNA while using the exon 22-23 primers. As compared to the other exons not successfully sequenced, this location was never found to produce bands when shown on a gel, indicating this could be the mutation. When using these primers in a PCR reaction to prepare for gel extraction, no bands appeared at the optimal annealing temperate as well as various other surrounding temperatures. To ensure that the primers were operating effectively, a temperature gradient was run (Fig. 6) using the forward and reverse 22-23 primers with the affected DNA and healthy DNA as a control. The result showed a standard temperature with the control DNA and no discernable bands with the affected DNA suggesting that a mutation is in the proximity of exon 22-23. With this region of the *XDH* gene in focus, two more PCR reactions were run. One with the 21 forward primer and 22-23 reverse primer and the other with the 22-23 forward primer and the 24 reverse primer (Fig. 5). The former PCR showed no discernable bands while the latter PCR produced bands. This suggests that a mutation may be present around the annealing site of the 22-23 reverse primer. This hypothesis will be confirmed or denied pending sequencing information.

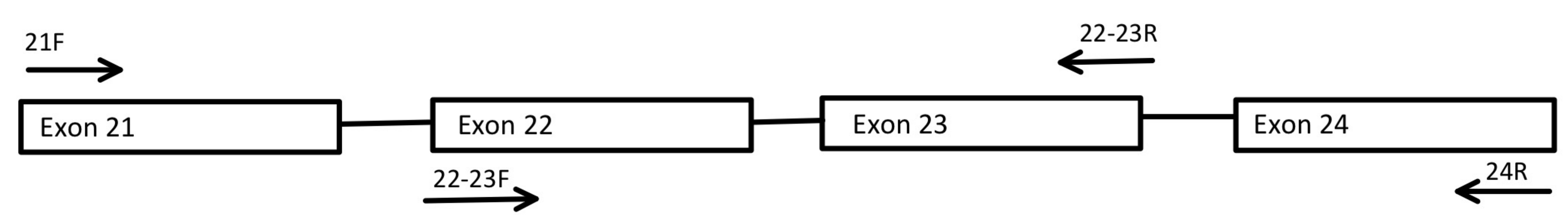

Fig. 5, Exons 21-24 and Primer Placement, Not Drawn to Scale.

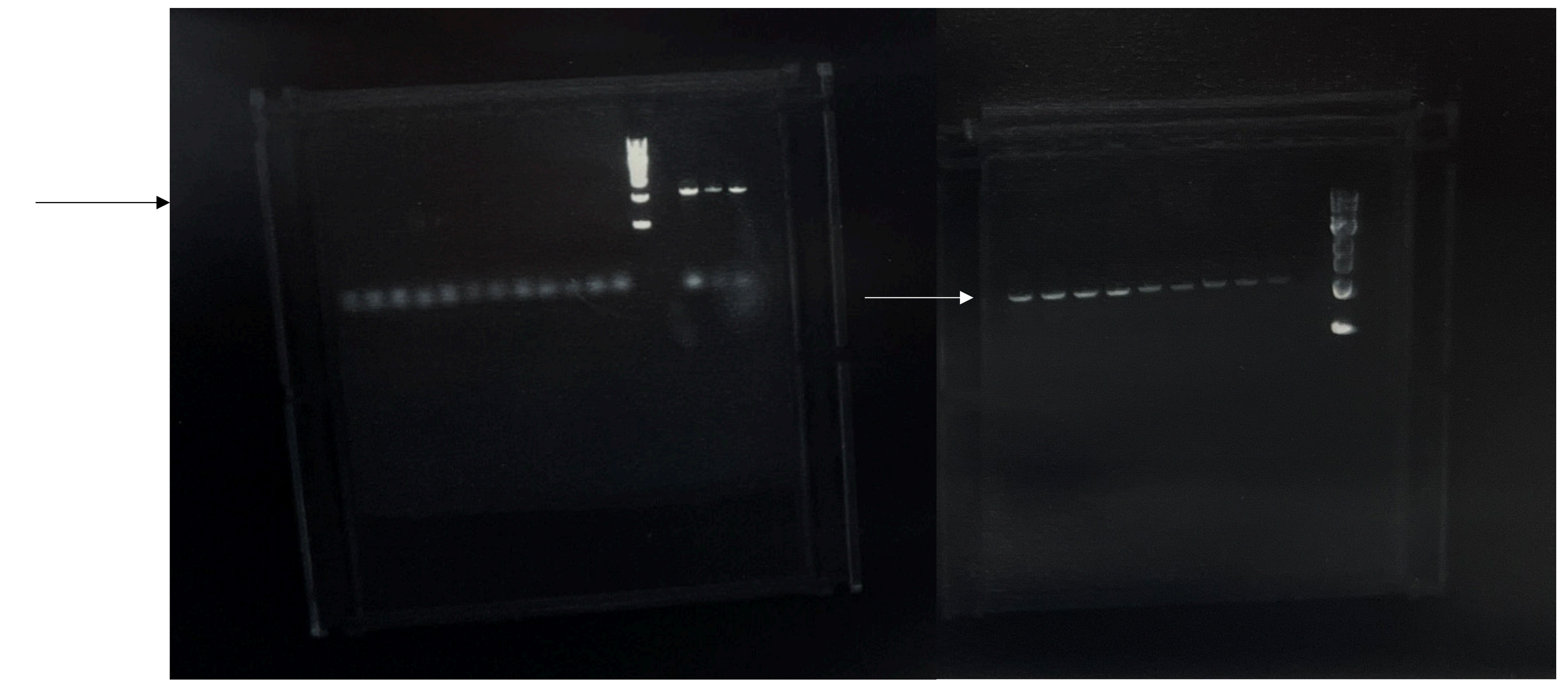

Fig. 6, Temperature Gradient of Exon 22-23 Affected DNA (left), Unaffected DNA (left).

Discussion

Due to the time limitations of this project with the PURM program being 10 weeks, this is still ongoing research. Therefore, the evidence presented at this time is preliminary and will be continued on in the future to provide a more definite conclusion. In addition, this project consisted of the sequencing of solely the *XDH* gene. Even if a mutation is found in the *XDH* gene, it cannot be ruled out that there is a mutation in the *MOCOS* gene, leading to xanthinuria.

Xanthinuria is a very rare life-threating disease in cats, as well as other species such as humans and dogs. Only a handful of occurrences in cats have been documented. Understanding the genetic basis of the disease has implications for future treatment.

Conclusions

The primary objective of this study was to identify the genetic cause of xanthinuria in the cat being studied. A candidate location for the mutation was identified around the area of the 22nd and 23rd exon. Ongoing study will be necessary to clarify the results.

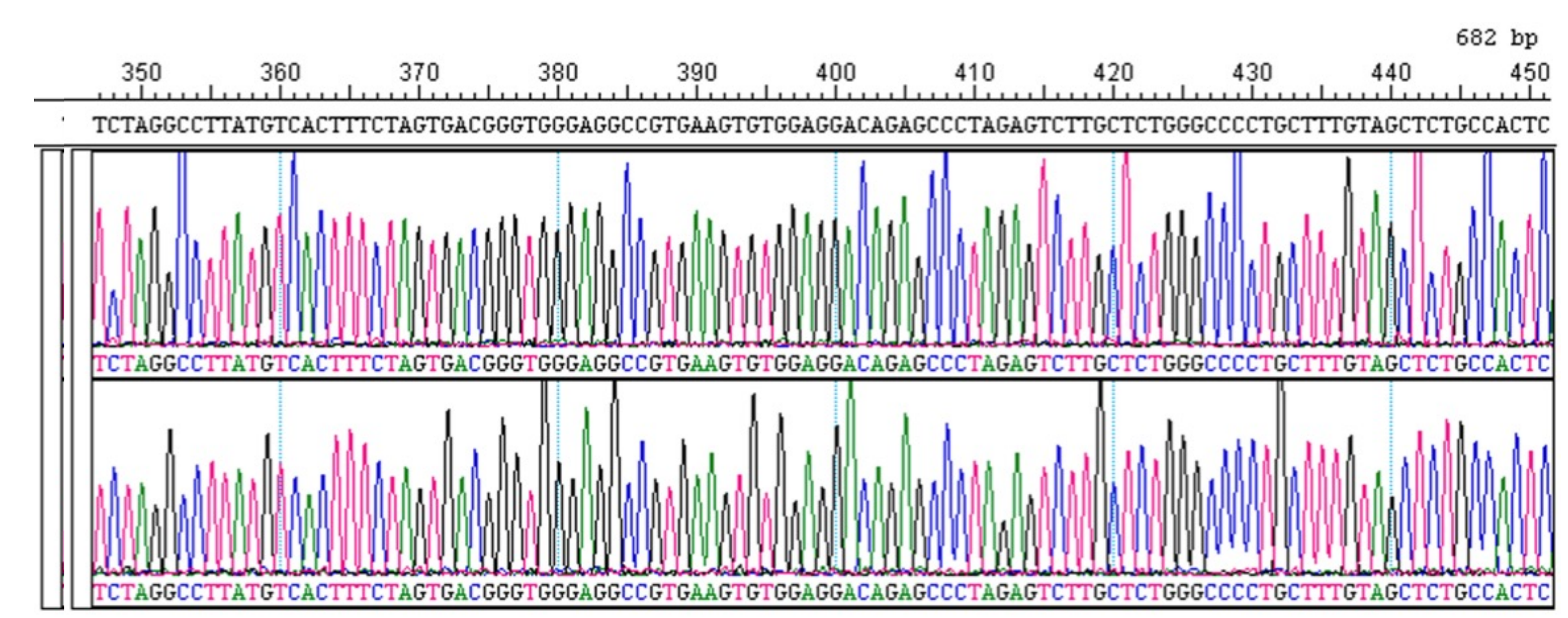

Fig. 7, Exon 2 DNA Trace.

Acknowledgments

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3. Erika Lutz, Undergraduate Researcher

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