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## INTRODUCTION

Piecing together a crime scene is one of the most significant components of any forensic investigation and estimating the age of biological stains at a scene can be crucial to the case. Several studies have demonstrated a correlation between the degradation kinetics for mRNA transcripts present in dried body fluid stains aged for varying amounts of time. The relationship between the age of a sample and the state of degradation of many transcripts can be exploited to estimate the age of an unknown crime scene stain. In this laboratory, preliminary work has demonstrated that the 5' and 3' ends of many mRNA transcripts degrade at different rates during aging of body fluid stains.

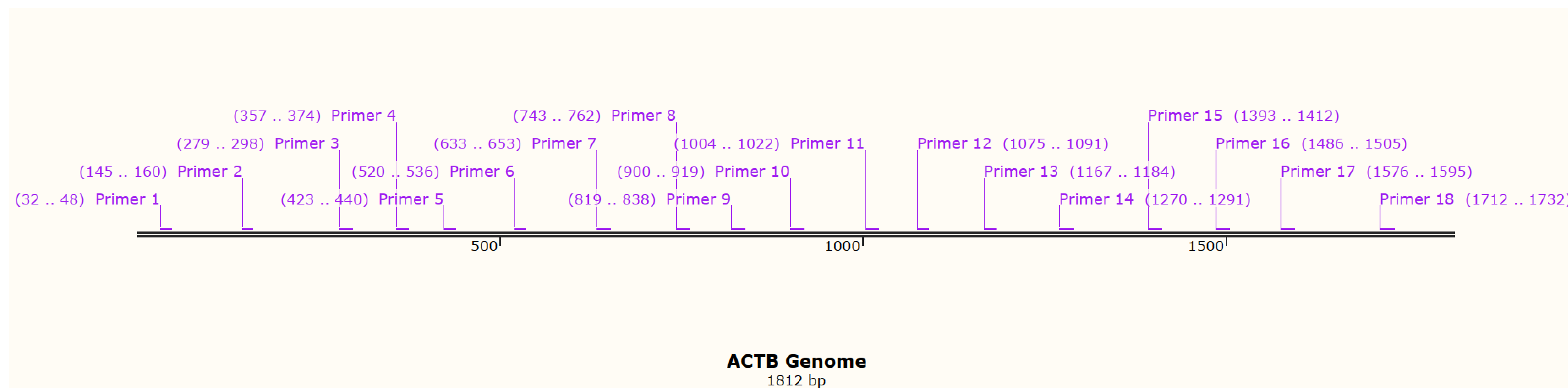
## OBJECTIVES

The purpose of this study is to map the degradation of the entire *ACTB* transcript (~1800 nucleotides long) in dried blood and semen stains and to determine if variances in degradation sensitivity are uniformly distributed along the length of the molecule. We will also evaluate the kinetics of *ACTB* mRNA degradation in dried blood and semen stains to determine whether there are any tissue-specific differences in transcript degradation.

## METHODS

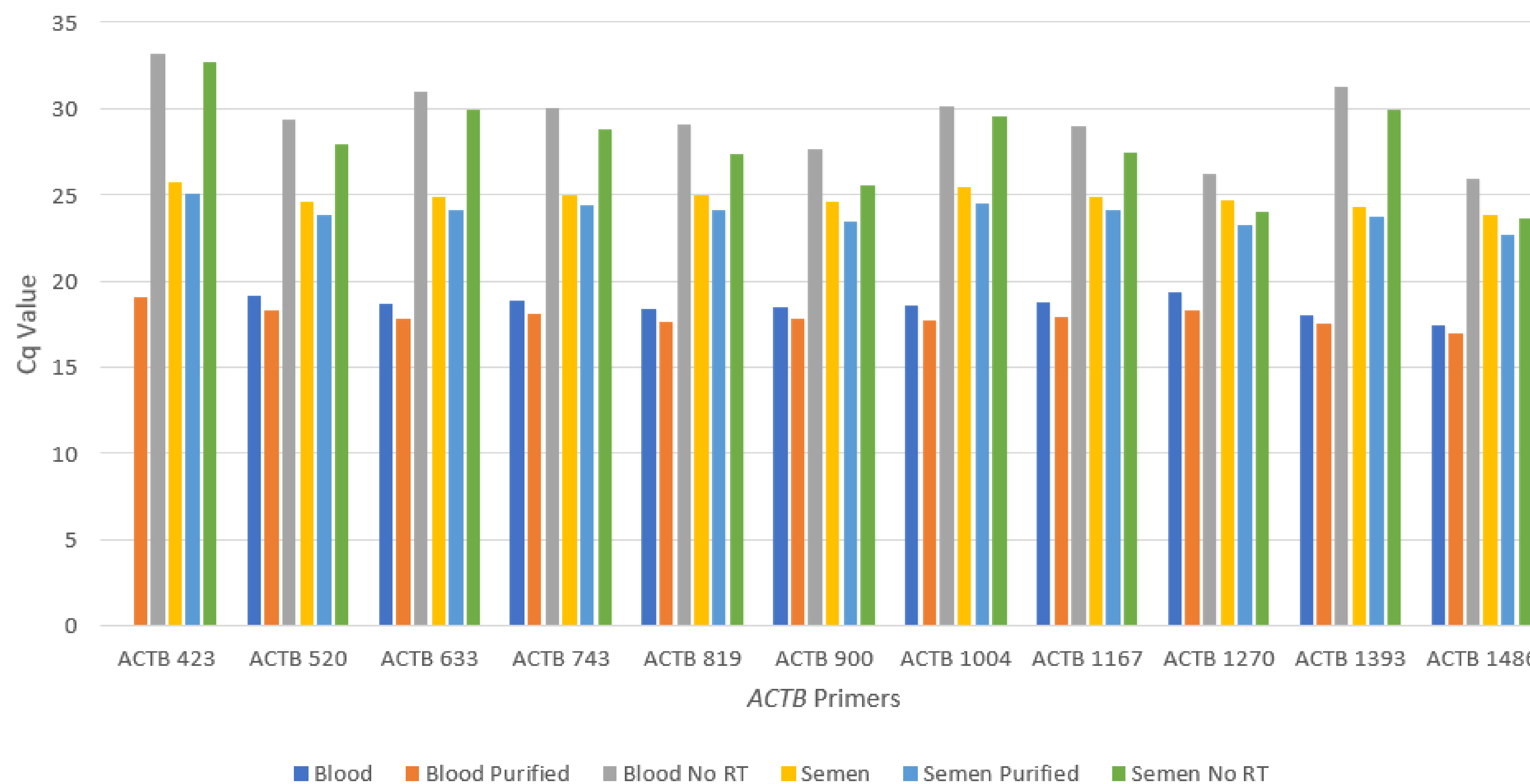
We have designed qPCR primers from National Center for Biotechnology Information (NCBI) to span the entire length of the *ACTB* transcript to direct the amplification of ~100 base pair qPCR amplicons. Blood and semen RNA was extracted from collection cards. For each primer site, we separated the blood and semen stains into three categories as noted in **FIGURE 2** followed by real time qPCR using QuantStudio5. We have identified the amplification efficiencies for our collection of primers through qPCR.

## RESULTS



**FIGURE 1:** All 18 of our designed primers (pictured: forward primers) spanning along the 1812 nucleotide length of the *ACTB* genome.

## ACTB Primers - Preliminary



**FIGURE 2:** Preliminary testing of our first set of primers.

## CONCLUSION

Now that we have our qPCR primers and have determined their amplification efficiencies, we can begin the study of *ACTB* degradation in our blood and semen stains stored for increasing periods of time up to eight weeks. In order to achieve the best results, we will continue to use reverse transcription and the addition of the inhibitor removal for further purification. The findings of this study will contribute to the knowledge that will be needed to apply this technology to estimate the age of biological evidence recovered from a crime scene.

## REFERENCES

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