

Study of time dependent degradation of mRNA encoding the SPRR3 and S100A9 gene markers in saliva stains

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INTRODUCTION

When a body fluid stain is discovered at a crime scene, it may be beneficial to use the RNA found within to approximate the age of the stain. However, RNA has been shown to be less stable than DNA [1, 2]. Analysis methods for RNA include reverse transcription PCR, also known as RT-PCR [3-5]. Research previously conducted in this laboratory has shown the relationship between the age of a blood and semen stains and their respective amount of RNA degradation [6-8].

OBJECTIVES

Reverse transcription and real time qPCR analysis of specific mRNAs in aged buccal swabs can be used to estimate the age of the sample.

METHODS

Buccal swabs were collected and left to dry in a box in a room temperature environment for up to three weeks. RNA extraction was performed, incorporating inhibition removal to further purify the RNA product. Then the RNA was reverse transcribed to cDNA.

After reverse transcription, the cDNA was diluted to appropriate amounts to prevent oversaturation. For the SPRR3, sites 15, 594, and 768 on the marker were chosen. For S100A9, sites 27, 310, and 453 on the marker were chosen. Real time qPCR was performed on the QuantStudio5 system. Data was analyzed and graphed with Excel.

RESULTS

Figure 1

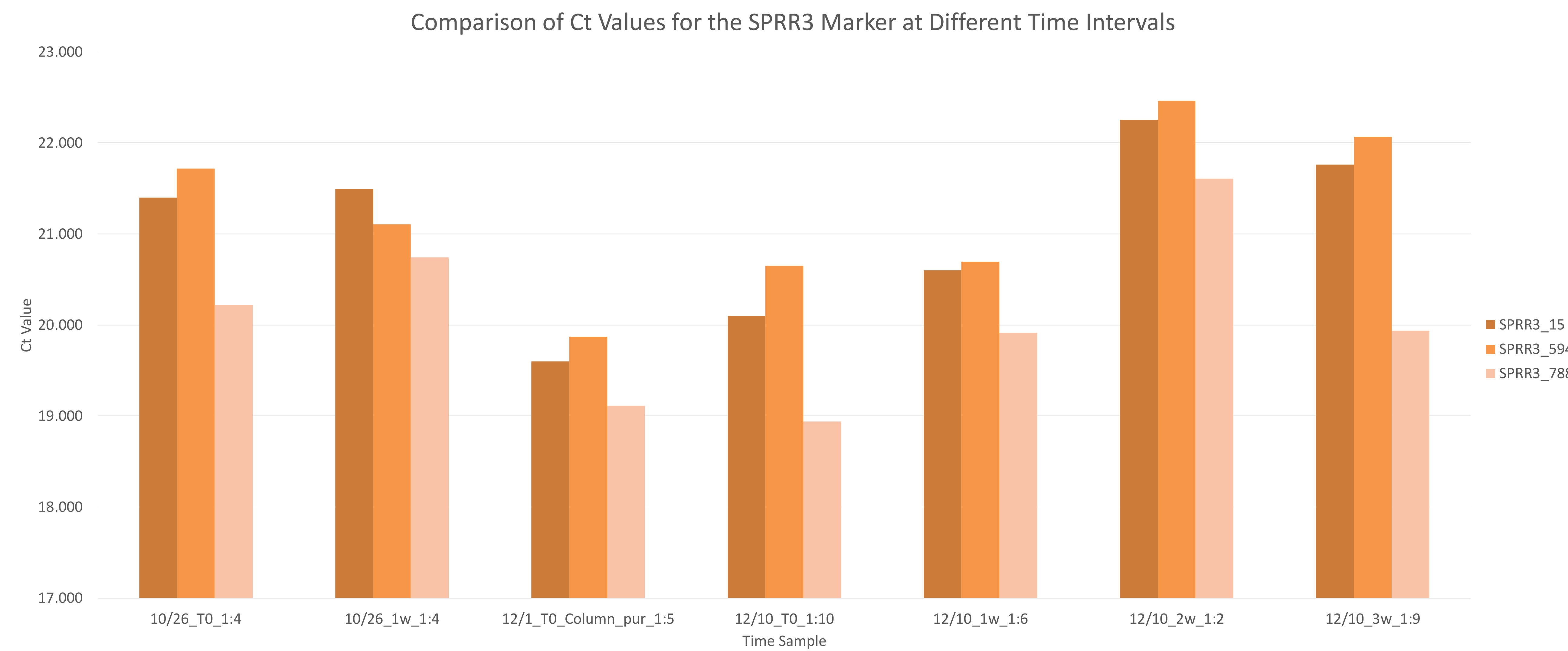
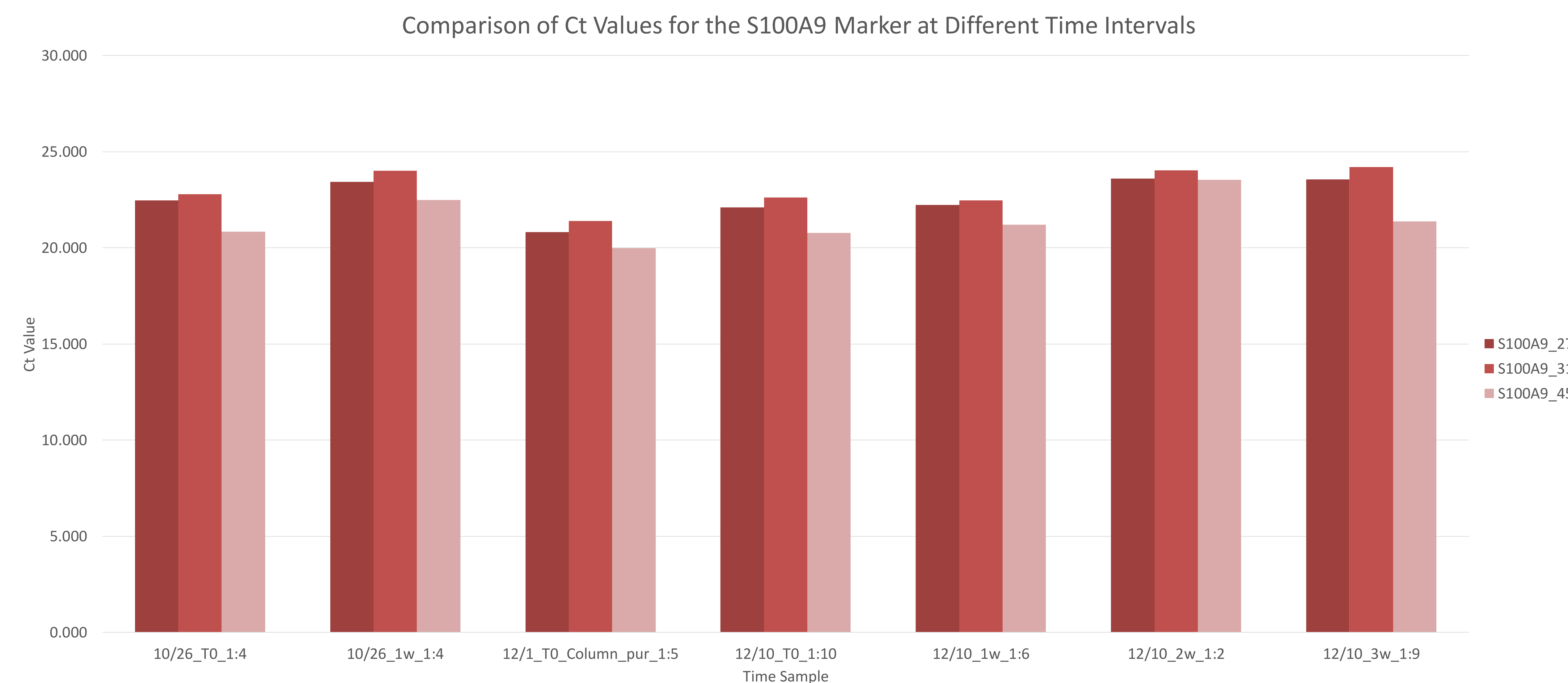


Figure 2



CONCLUSION

Results so far show that as the time between sample acquisition and analysis increases, Ct values for SPRR3 increase, an indication of degradation (Fig. 1). The Ct values of S100A9 stayed relatively constant through time (Fig. 2). ΔCq values, or the difference between the 5' end and 3' end, for both SPRR3 and S100A9 show no relationship (not shown here). Further research will be performed on buccal swabs aged up to four weeks. The housekeeping gene ACTB will also be included in future research.

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