OSU-CHS / Forensic Sciences

Detection of RNA Methylation Patterns of Forensically Relevant Transcripts in Dried Bloodstains

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INTRODUCTION

RNA degradation kinetics can be used to estimate the age of a biological sample found at a crime scene. RNA sequencing of transcripts from various tissue types shows that degradation occurs faster at the 5’ end than the 3’ end. This discovery led to the development of the 5’-3’ assay, which quantifies and compares each end of a transcript in a single reaction to estimate sample age. This assay has been validated on dried bloodstains, however why the 5’ end of the transcript degrades faster than the 3’ end remains unknown.

As this phenomenon is being observed in dried samples, we hypothesize that chemical hydrolysis reactions are responsible for breaking the RNA molecule and thus chemical modifications of the 5’ or 3’ ends of the RNA molecule may affect the degradation rate. A literature exists that suggests that methylation of RNA molecules can alter the kinetics of RNA degradation through affecting transcript stability and also possibly dependent on the RNA binding proteins (RBPs) present.

OBJECTIVES

We aim to interrogate the RNA methylation patterns of two forensically relevant transcripts in dried bloodstains.

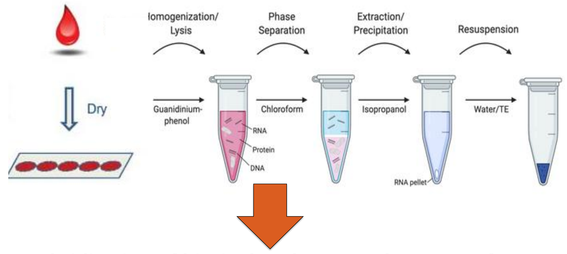
METHODS

We developed a novel RNA enrichment technique that utilizes 120bp DNA probes designed to hybridize to the 5’ or 3’ ends of a transcript for selected target enrichment. The enriched product will then be hydrolyzed into nucleotides for analysis via LC-MS/MS.

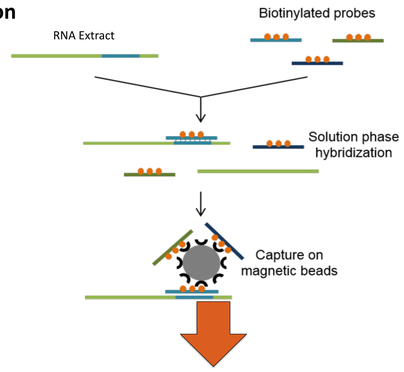
- Two biotinylated, 120 base DNA probes were designed for each transcript in order to enrich the 5’ or 3’ ends.
- Each end of the transcript was enriched in separate reactions

Methods

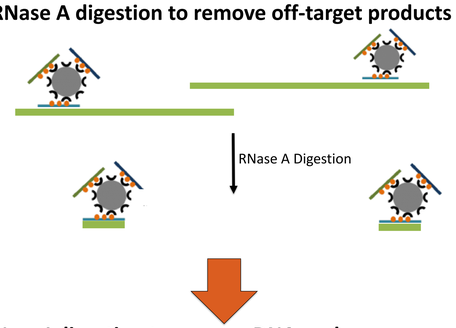
1. RNA Extraction



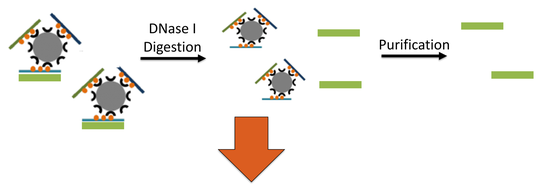
2. Hybridization of biotinylated DNA probe to RNA for target selection



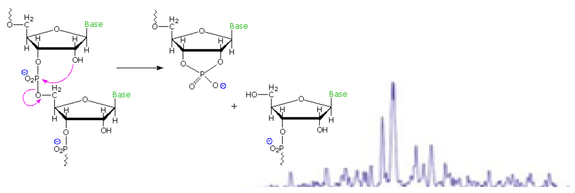
3. RNase A digestion to remove off-target products



4. DNase I digestion to remove DNA probe



5. Hydrolysis and LC-MS/MS Analysis

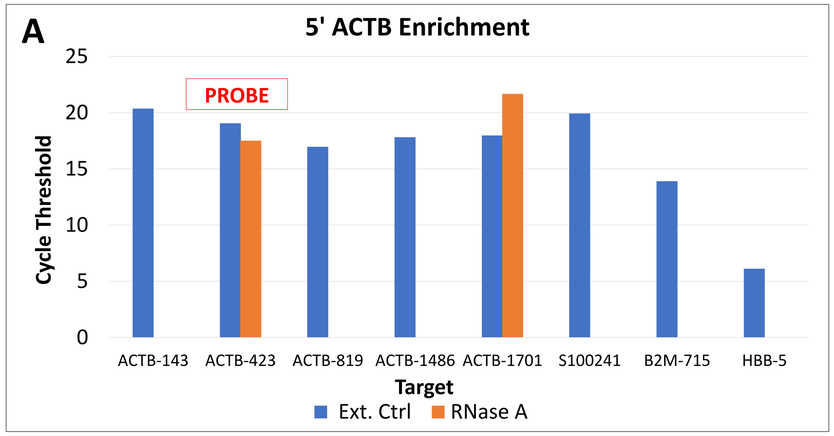


Preliminary Results

Table 1. RNA enrichment data for the 5’ (A) and 3’ (B) ends of ACTB. Enriched products were not digested with RNase A in order to quantify the whole transcript. Delta Ct represents enriched product Ct – control Ct. Bolded data represents where the DNA probe hybridizes.

A Target	Control Ct	Enriched Ct	ΔCt
ACTB-143	20.356	29.462	9.106
ACTB-423	**19.053**	**17.240**	**-1.814**
ACTB-819	16.960	29.328	12.368
ACTB-1486	17.816	31.766	13.949
ACTB-1701	17.970	25.534	7.564
S100241 (Off target)	19.928	36.269	16.341
B2M-715 (Off target)	13.903	32.517	18.614
HBB- 5’ (Off target)	6.118	24.029	17.911

B Target	Control Ct	Enriched Ct	ΔCt
ACTB-143	20.356	33.560	13.204
ACTB-423	19.053	19.939	0.886
ACTB-819	16.960	34.709	17.748
ACTB-1486	17.816	38.457	20.641
ACTB-1701	**17.970**	**18.419**	**0.449**
S100241 (Off target)	19.928	33.877	13.949
B2M-715 (Off target)	13.903		
HBB- 5’ (Off target)	6.118	32.546	26.428



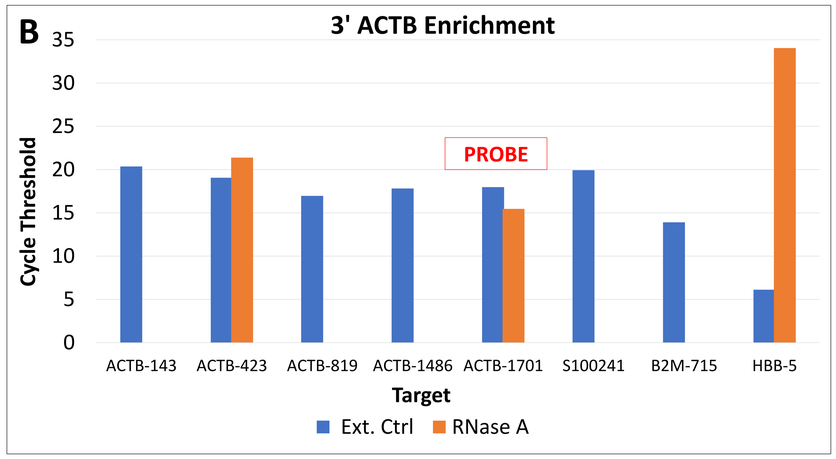


Figure 1. Ct values after enrichment of the 5’ (A) and 3’ (B) ends of the ACTB transcript, following RNase A digestion. The location of the probe is shown on each graph.

ON GOING WORK

- Optimize protocol to rid all off-target RNA sequences.
- Validate with 5’ and 3’ DNA probes on a second transcript
- Validate hydrolysis protocol

CONCLUSION

Preliminary results show that this approach can enrich for our selected target over 150,000-fold. We anticipate observing differences in RNA methylation patterns between the 5’ and 3’ ends of our selected transcripts, potentially explaining the differential degradation rates of the 5’ and 3’ ends of RNA molecules.

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