

ABSTRACT

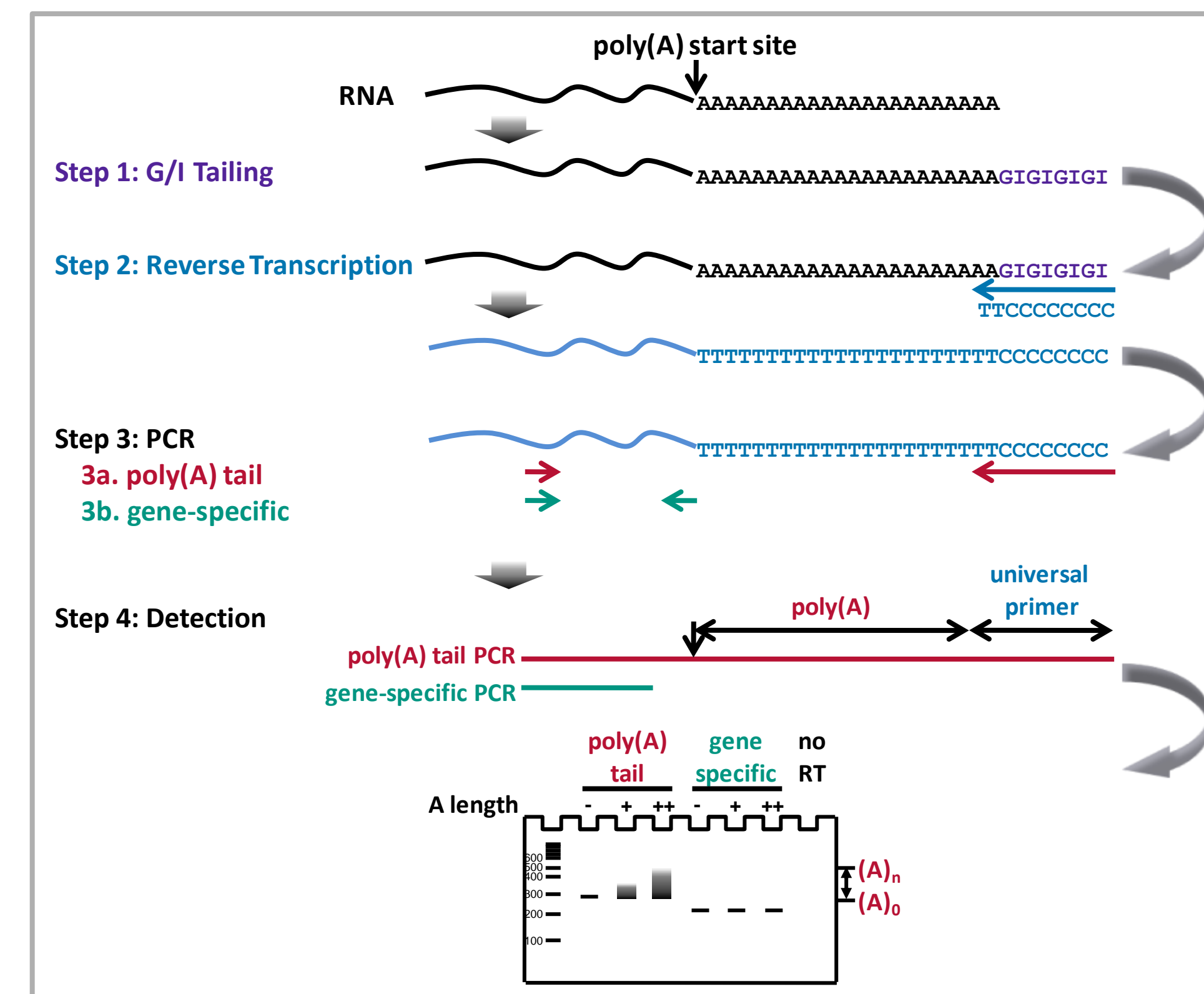
The poly-adenylated(A) tail on nearly all eukaryotic mRNAs plays a number of important roles in mRNA metabolism including enhancing translation, mRNA stability and transport from the nucleus^{1,2}. Recent studies in a variety of model organisms have revealed a pivotal role for regulated deadenylation as being rate limiting for mRNA degradation and deadenylation is now recognized as a mechanism of miRNA mediated gene regulation^{3,4}. Current techniques for analysis of poly(A) tail length include ligation-mediated PCR (LM-PAT) and Northern blotting, both of which are limited to analysis of abundant mRNAs⁵. These method limitations coupled with the heightened interest in poly(A) metabolism encouraged us to develop a convenient and powerful assay to measure poly(A) tail length.

We have taken advantage of the fact that poly(A) polymerase can add a limited number (~25) of guanosine residues to an existing poly(A) tail^{6,7}. We have found that the same polymerase also can add inosine. This is an advantage because inosine forms less self-pairing structure than guanosine. Accordingly, we extend total RNA with a mixture of guanosine and inosine. This tail then provides a priming site for reverse transcription. When coupled with a gene specific internal primer, we can quickly and accurately determine the length distribution of poly(A) tails in any mRNA. This novel method should be of broad utility in the currently very vibrant area of poly(A) tail metabolism.

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ASSAY SCHEMATIC



METHODS

G/I Tailing

adding limited numbers of G and I to 3' ends of poly(A) RNAs

mix 1 μ g of total RNA, Reaction Buffer and Enzyme Mix incubate at 37 °C for 60 min
 Add Stop Solution

Reverse Transcription

convert the tailed RNA to DNA using the newly added G/I tails as the priming sites

mix 5 μ l of tailed RNA, Reaction Buffer and Enzyme Mix incubate at 44 °C for 60 min \rightarrow 92 °C for 10 min

PCR

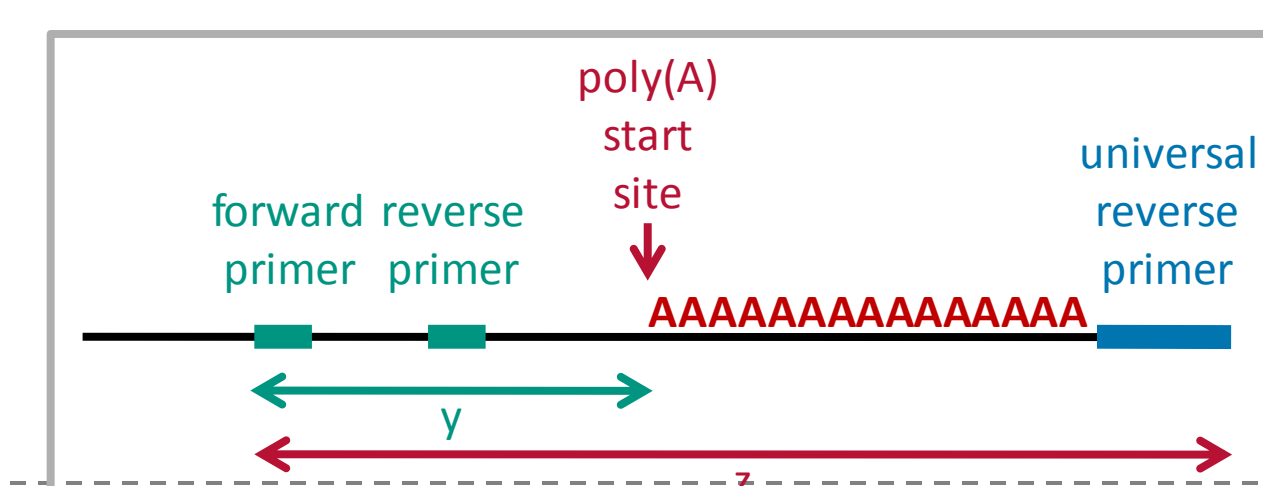
amplification of a control for the gene-of-interest with gene-specific forward and reverse primers designed upstream of the poly(A) start site

amplification of the poly(A) tails with the gene-specific forward primer and the universal reverse primer

mix 1 μ l cDNA, PCR Primers, Reaction Buffer and HotStart-IT Taq
 30 cycles of 94 °C for 10 sec and 60 °C for 30 sec

Detection

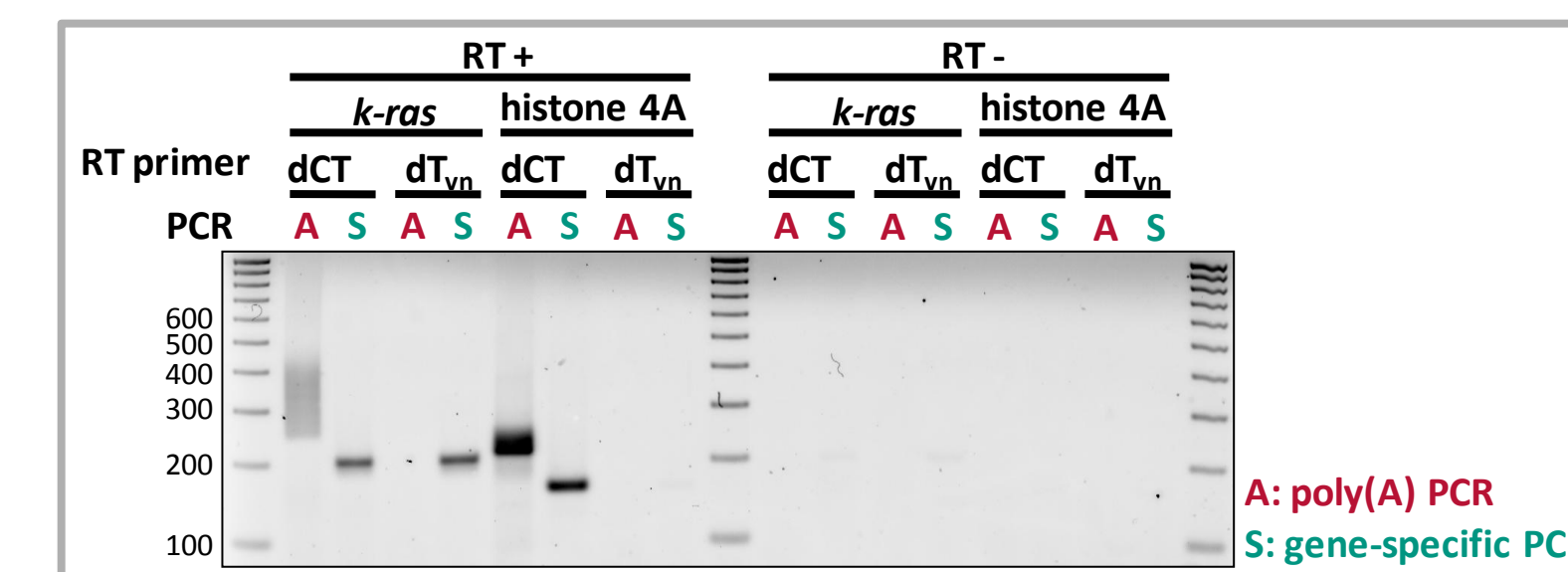
gel electrophoresis of PCR reaction



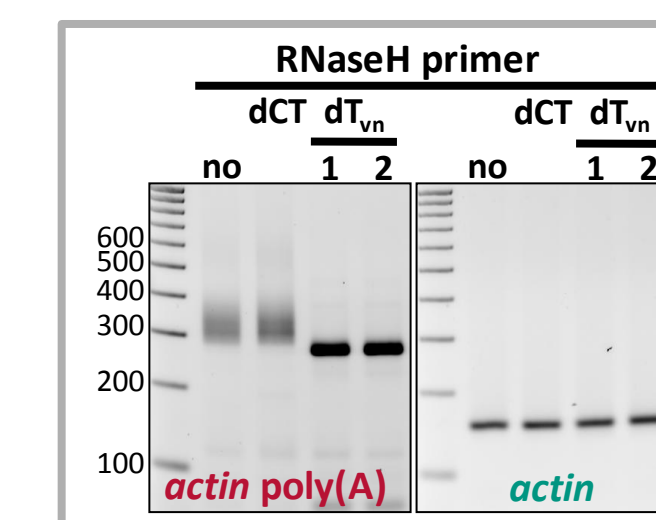
RESULTS

Poly(A) Tail Length Assay Specificity by Oligo dCT RT Primer

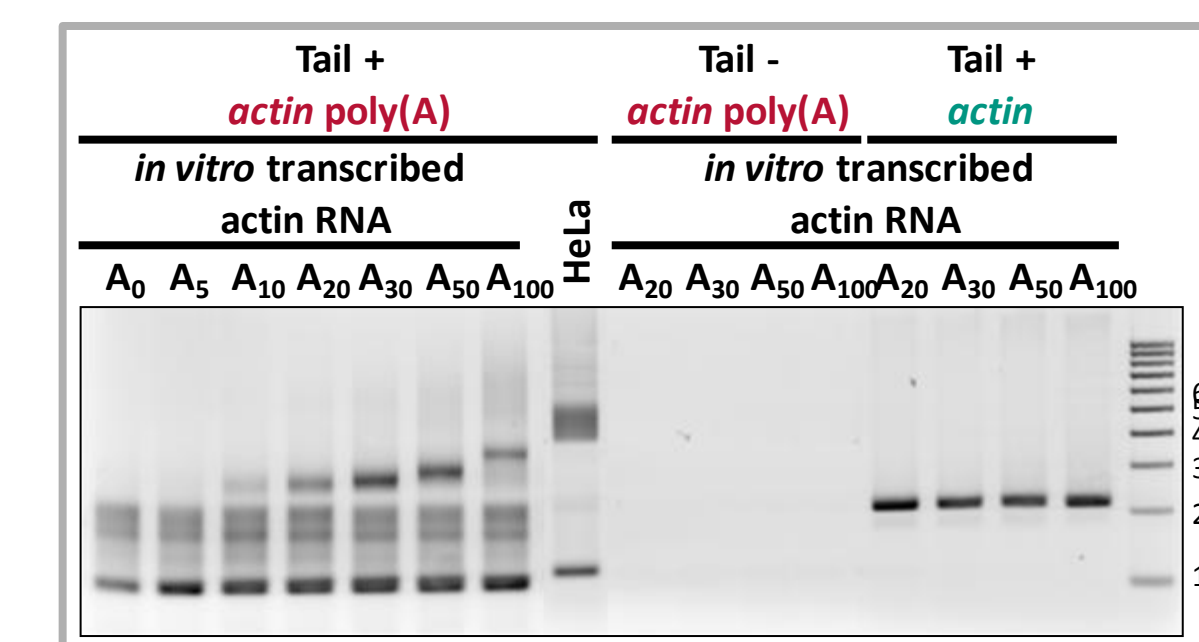
Detection of histone 4A and k-ras Poly(A) Tails



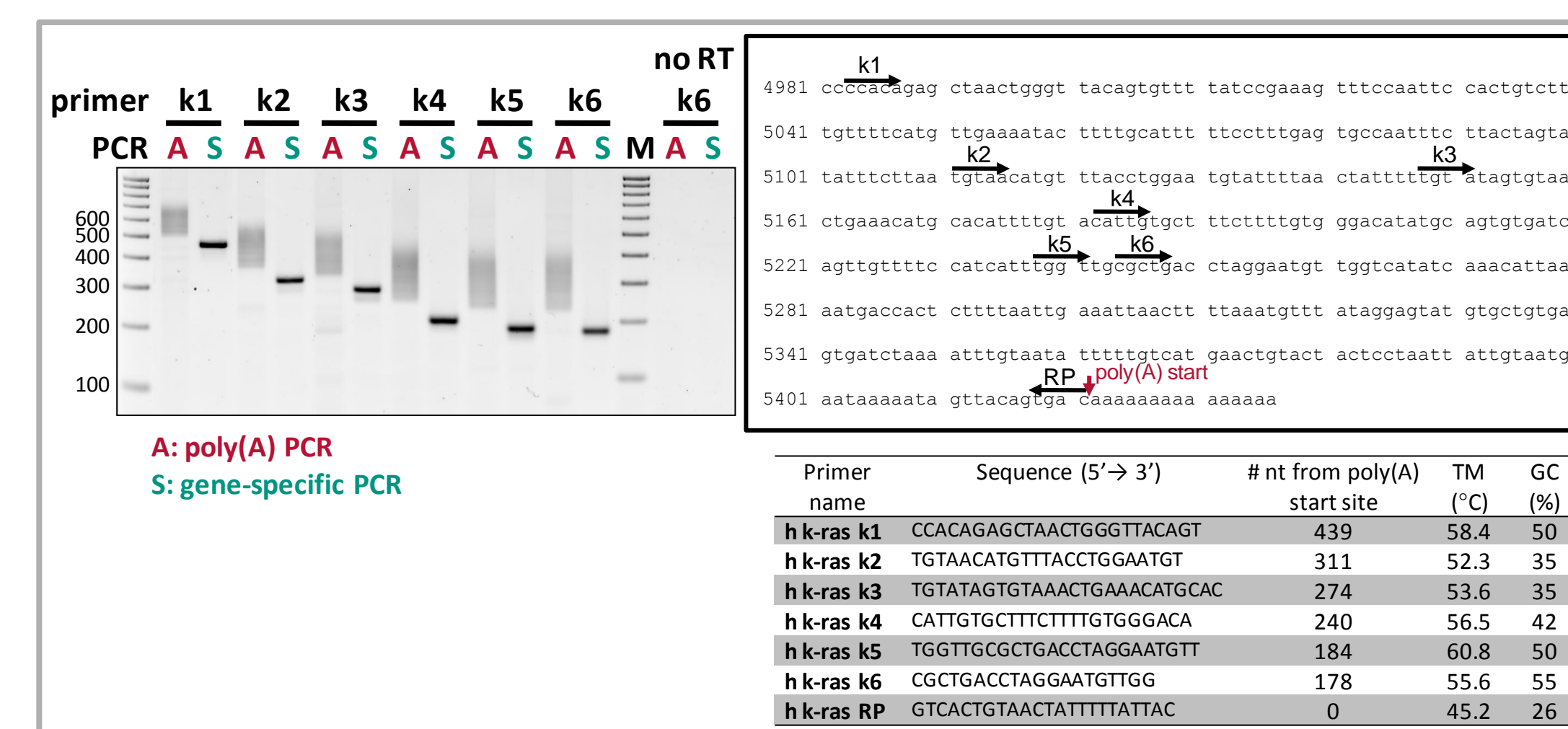
No Poly(A) Tail Length Detection from Oligo dT-RNase H-Cleaved RNA



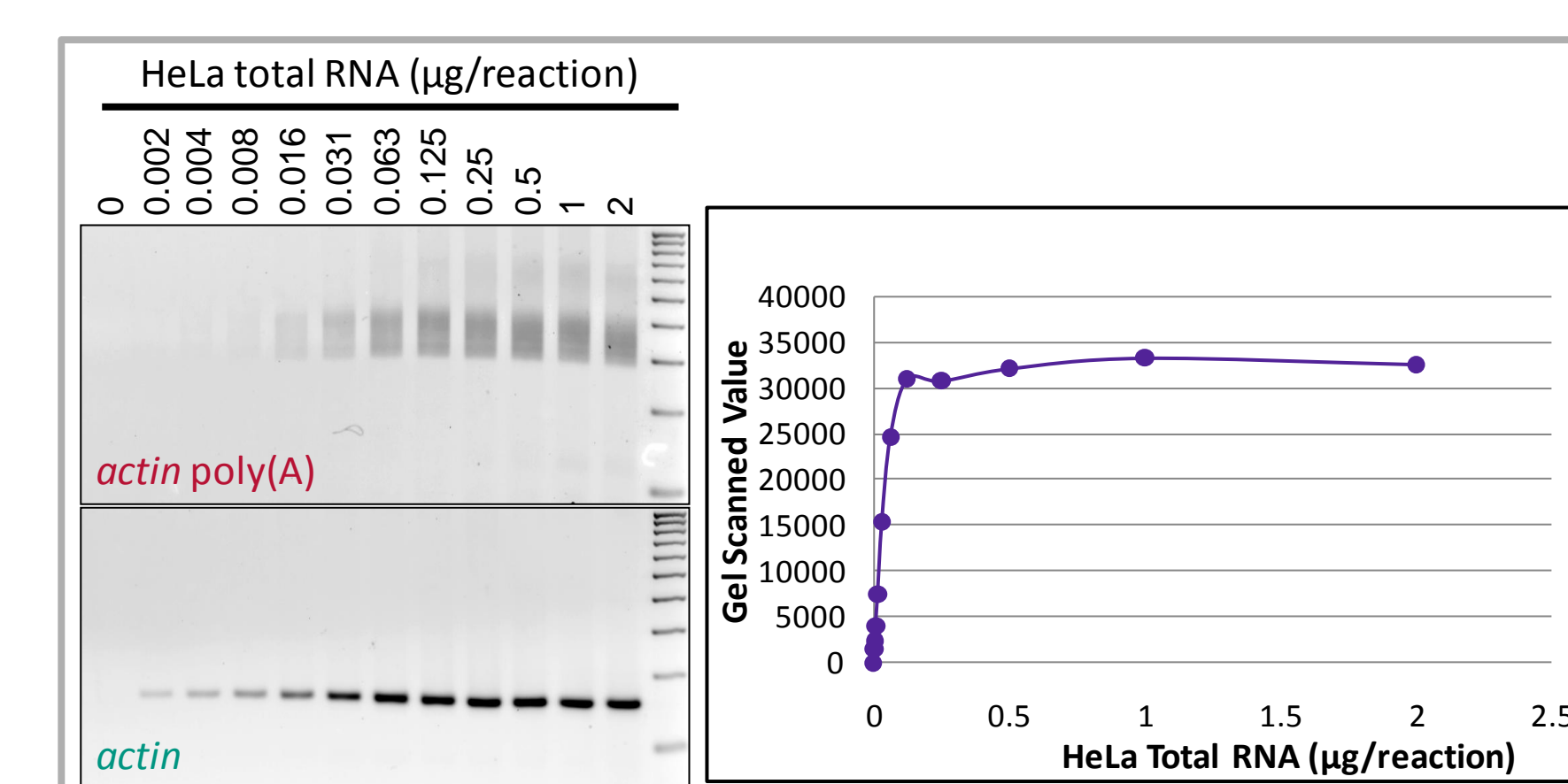
Detection of RNA with Defined Poly(A) Lengths



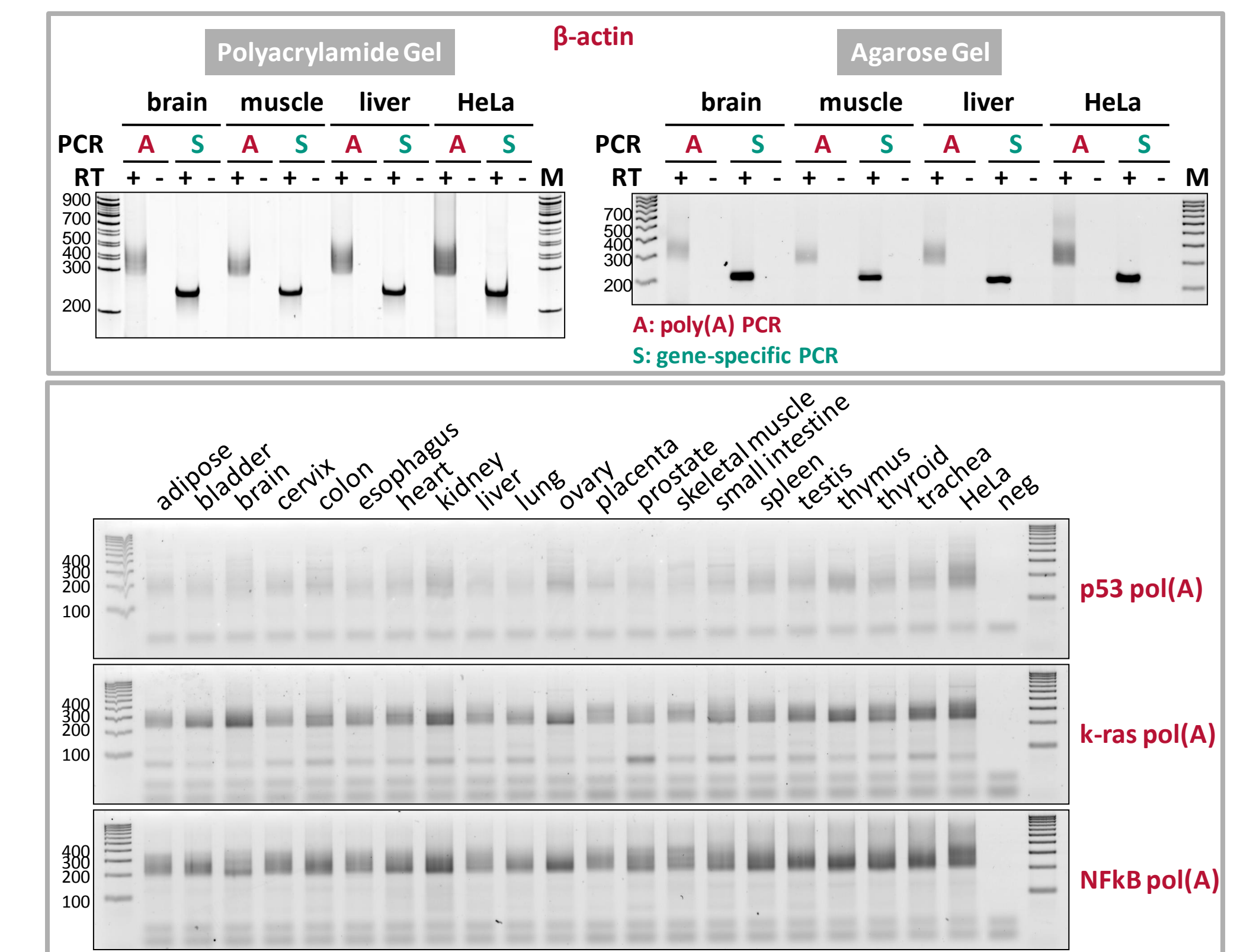
Gene-Specific Primer Selection



Detection Sensitivity



Poly(A) Tail Length Expression Profiling



CONCLUSIONS

We have demonstrated that poly(A) tail length determination can be performed by adding a limited number of guanosine and inosine with poly(A) polymerase and PCR amplification.

Major advantages of this approach are;

- improved efficiency using poly(A) polymerase,
- improved sensitivity and specificity by PCR, and
- improved precision using a priming site located immediately downstream of the poly(A) tail.

REFERENCES

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