Analytical insights in characterization of poly(A) tail by complementary techniques

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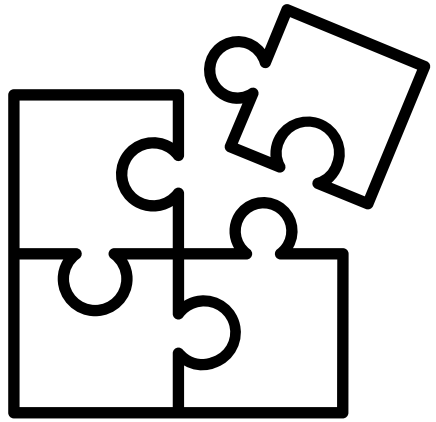
The use of orthogonal methods for mRNA characterization

- **Comprehensive Analysis**: Different methods can provide complementary information, offering a more complete understanding of mRNA characteristics and behavior.
- **Detection of Anomalies**: Orthogonal methods can help identify discrepancies or anomalies that might be missed when using a single method, ensuring more robust and thorough analysis.
- **Validation of Results**: Confirming findings through various techniques strengthens the validity of the conclusions drawn from the data.



mRNA critical quality attributes and analytical methods

Integrity and Purity

- Gel electrophoresis
- Capillary Electrophoresis
- IP-RP-HPLC
- SEC-HPLC
- AX-HPLC

Identity

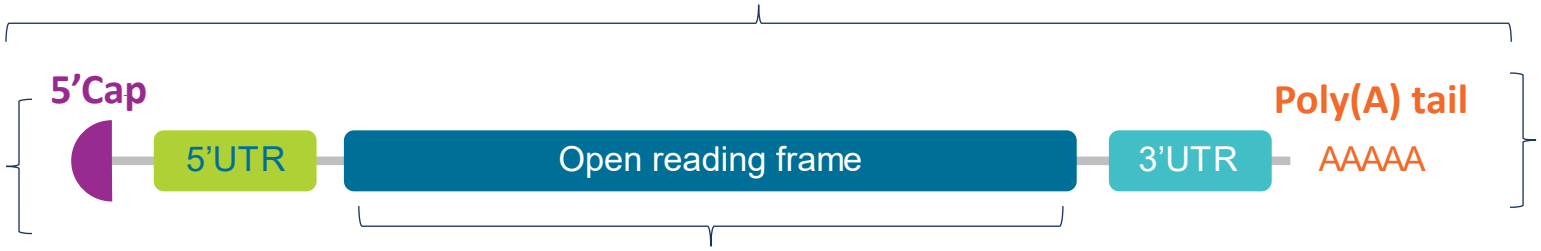
- Sequencing (Sanger, NGS)
- RT-PCRA
- "Fingerprinting" by LC-MS/MS
- Base composition Assay

Content

- UV-Vis Spectroscopy
- RT-qPCR
- RT-dPCR
- IP-RP-HPLC

Capping efficiency

LC-MS
LC-UV



Tail length

LC-MS
LC-UV

Functionality

- In-vitro translation/Western blot (Cell Free assay)
- Cell-based assays (contract out)

Impurities

- **DNA:** qPCR
- **Protein:** Nano Orange; BCA
- **NTPs**: AX-HPLC
- **Solvents:** GC
- **dsRNA:** Immunoblot, ELISA

Others

- **Appearance:** USP <1>, <790>
- **pH:** USP <791>

Safety

- **Endotoxin:** USP <85>
- **Bioburden:** USP <61>, <62>, <1115>

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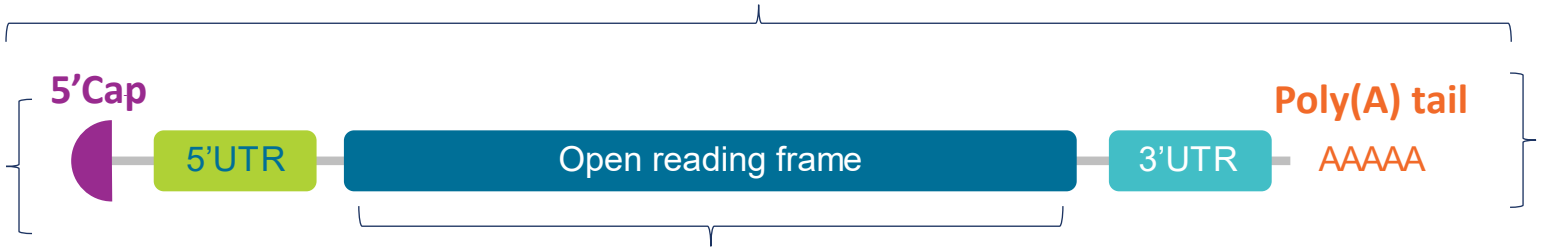

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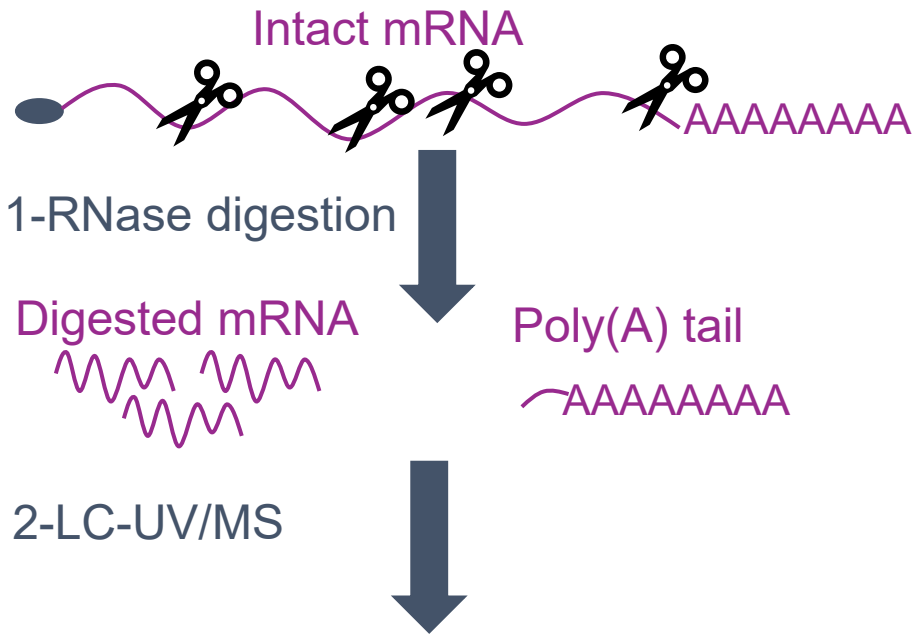
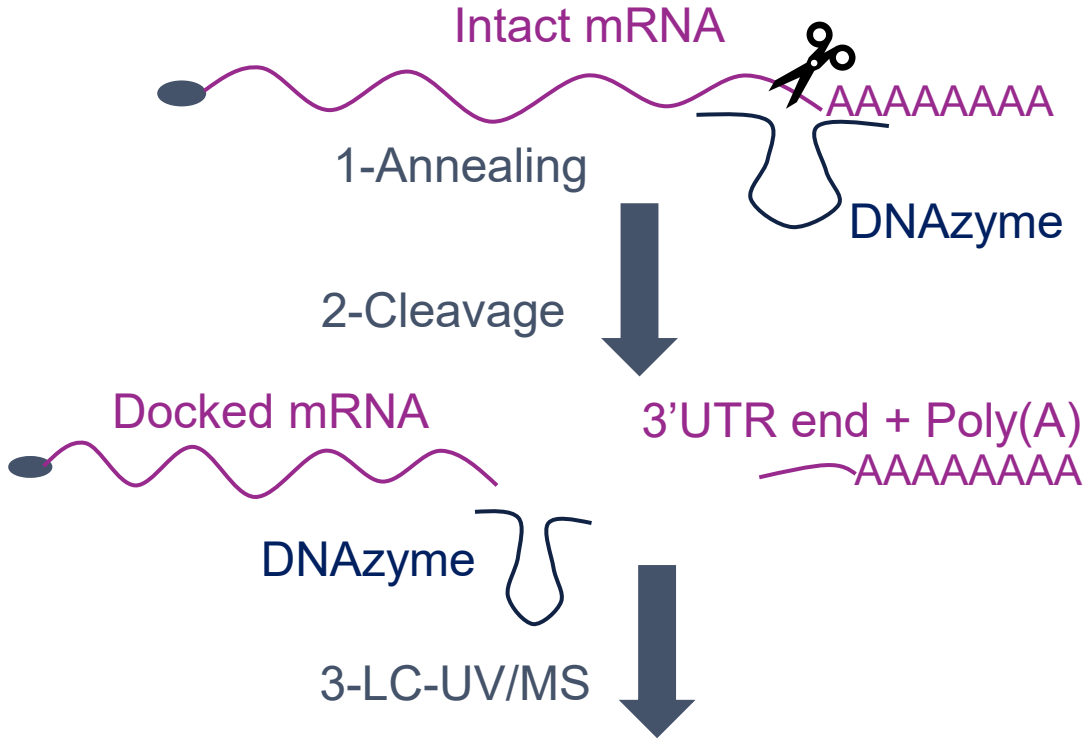
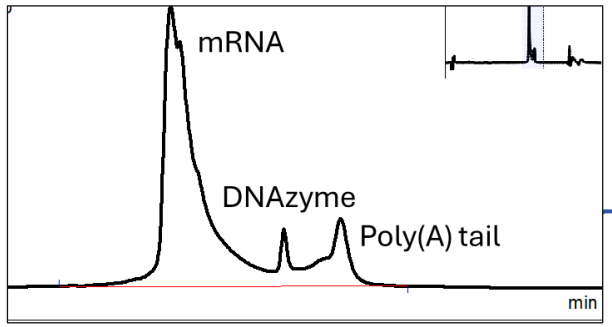
Assays for the analysis of poly(A) tail at TriLink

Industry Standard: RNase digestion



DNAzyme cleavage





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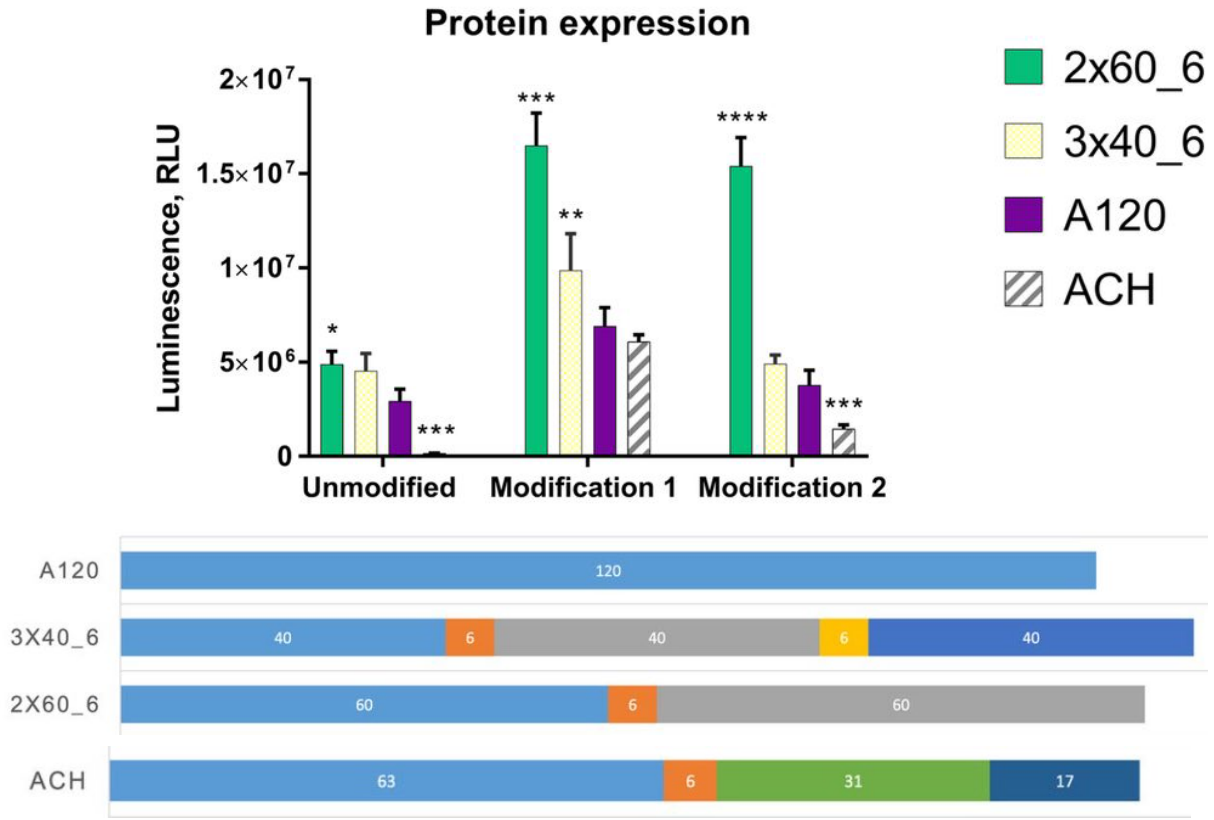
Segmented poly(A) tails may enhance protein expression



"Use of segmented poly(A)2 × 60_6 construct significantly increased protein levels post-transfection in a modification independent manner when compared to poly(A)120 and ACH benchmarks".

Trepotec et al. 2019. Segmented poly(A) tails significantly reduce recombination of plasmid DNA without affecting mRNA translation efficiency or half-life. *RNA*. 4:507-518

Liu et al. 2019. Poly(A) inclusive RNA isoform sequencing (PAIso-seq) reveals wide-spread non-adenosine residues within RNA poly(A) tails. *Nature Communications*. 10:5292

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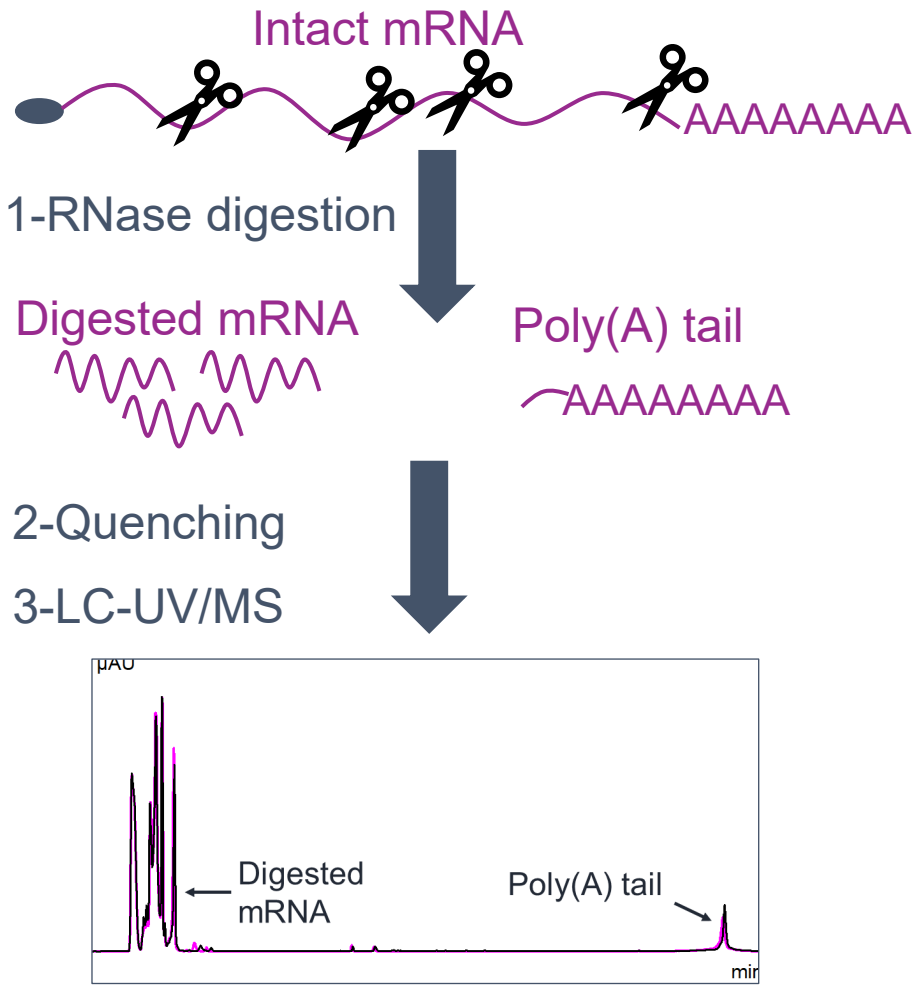
Cutting specificity of the RNases used in the assay

Industry Standard: RNase digestion



RNases typically used in the assay:

- **RNase T1:** digest RNA at G residues.
- **RNase A:** digest RNA at C and U residues.
- **Mixture of RNase T1/RNase A:** digest RNA at G, C and U residues.

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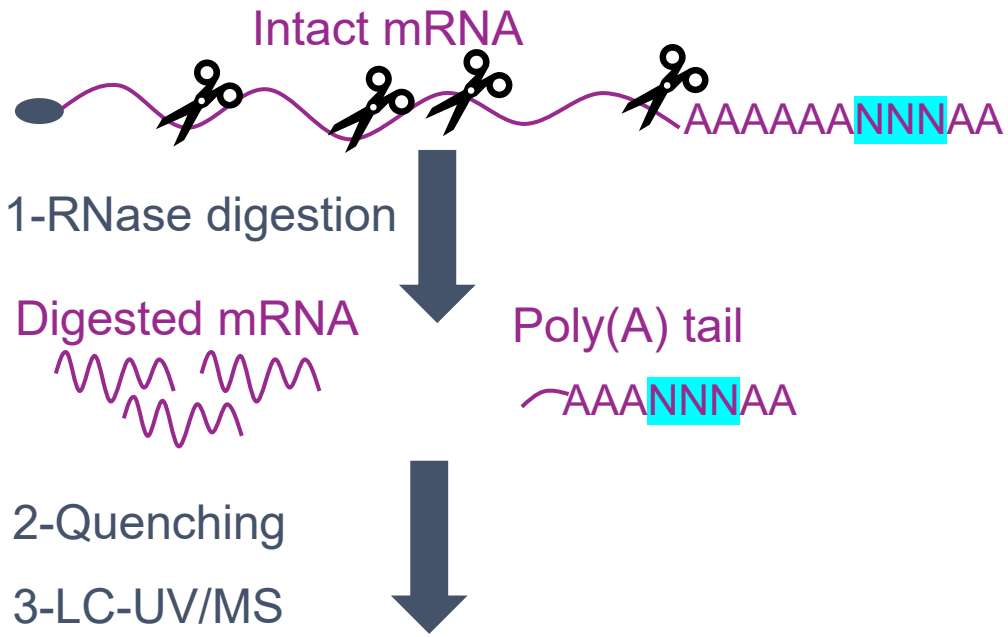
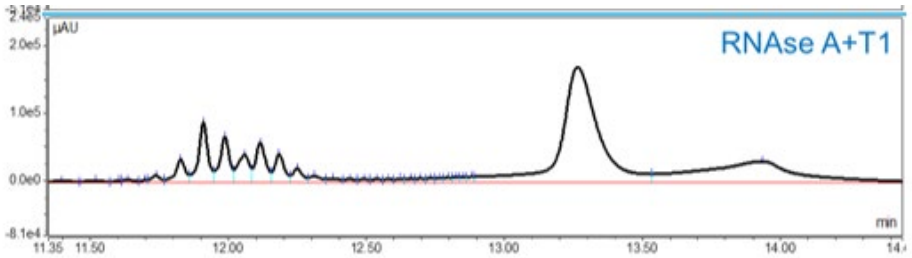
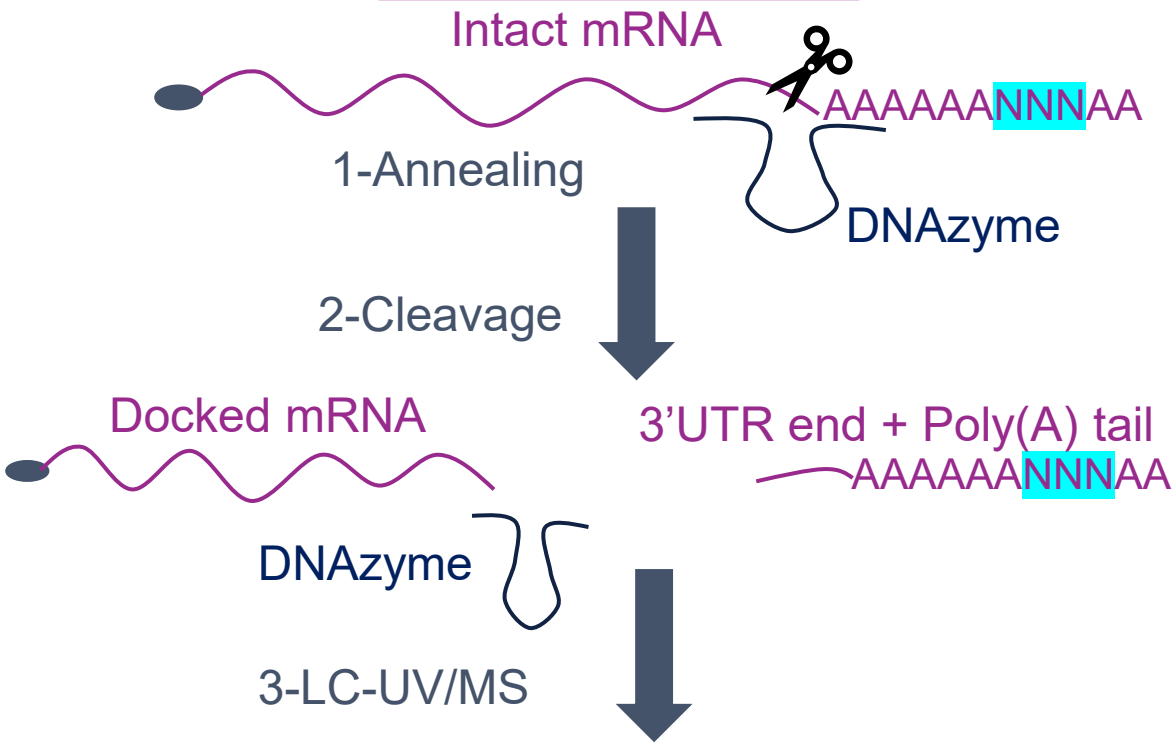
Assays for the analysis of poly(A) tail are complementary

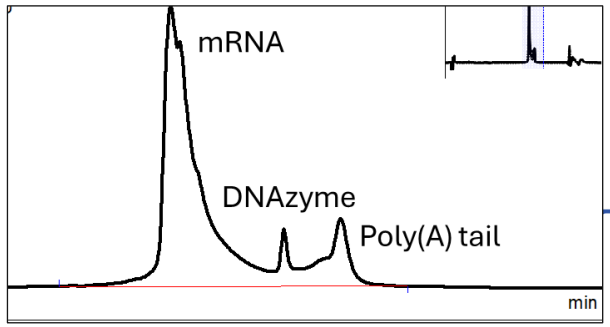
RNase digestion





DNAzyme cleavage





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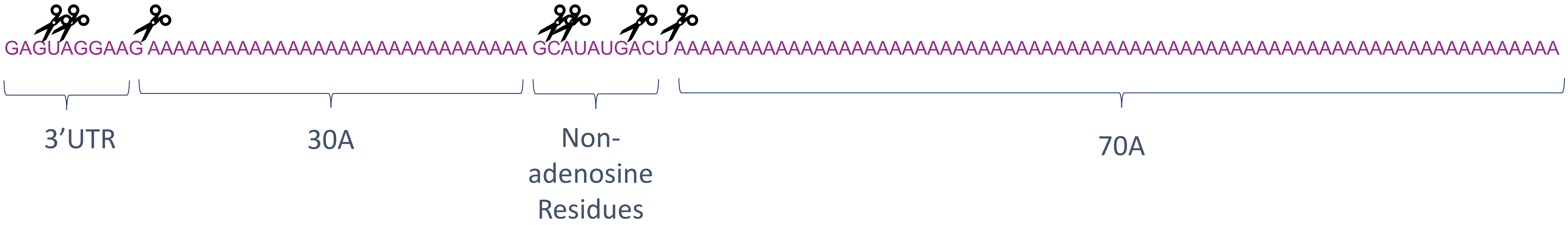
Case study with a segmented poly(A) tail



RNase digestion

RNase A — $AGGAAGA_{30}GC$ — A_{70}

RNase T1 — $A_{30}G$ — $ACUA_{70}$

RNase A & T1 — $A_{30}G$ — A_{70}

DNAzyme cleavage

$UAGGAAG\ A_{30}\ GCAUAUGACU\ A_{70}$

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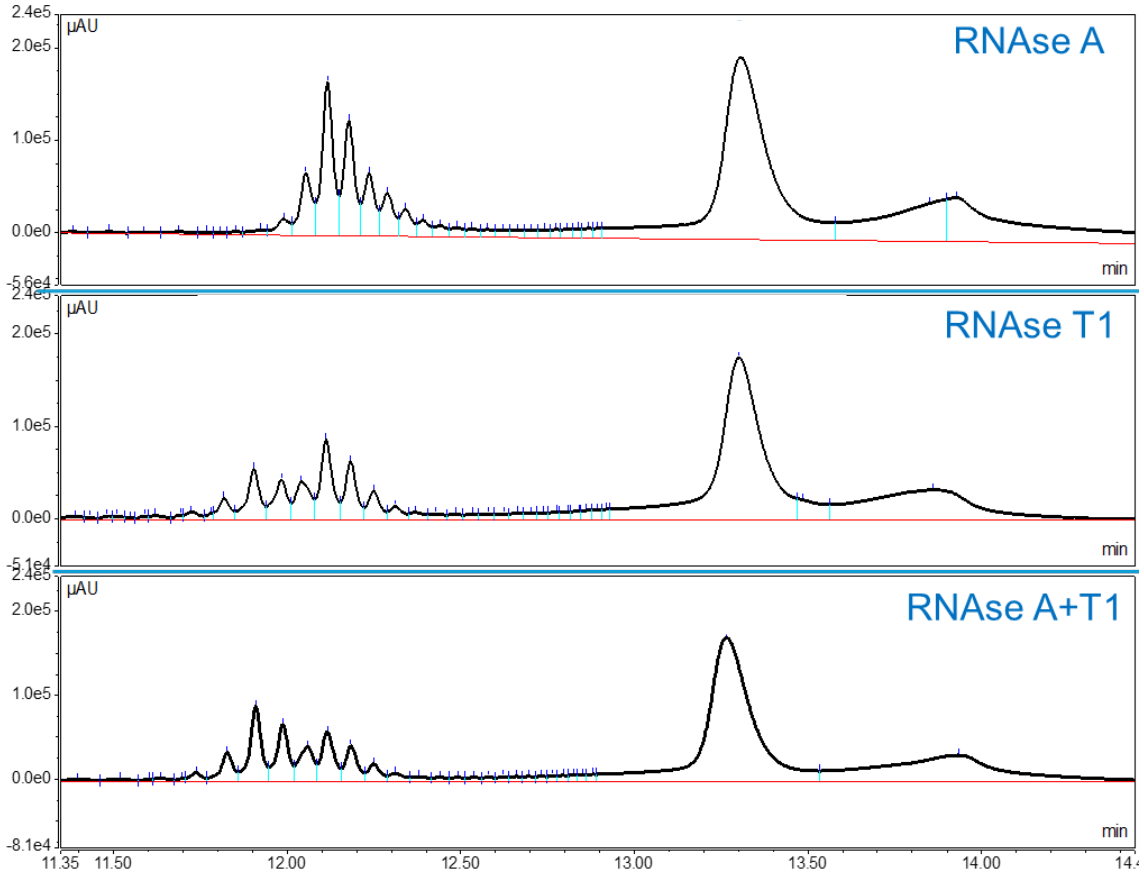
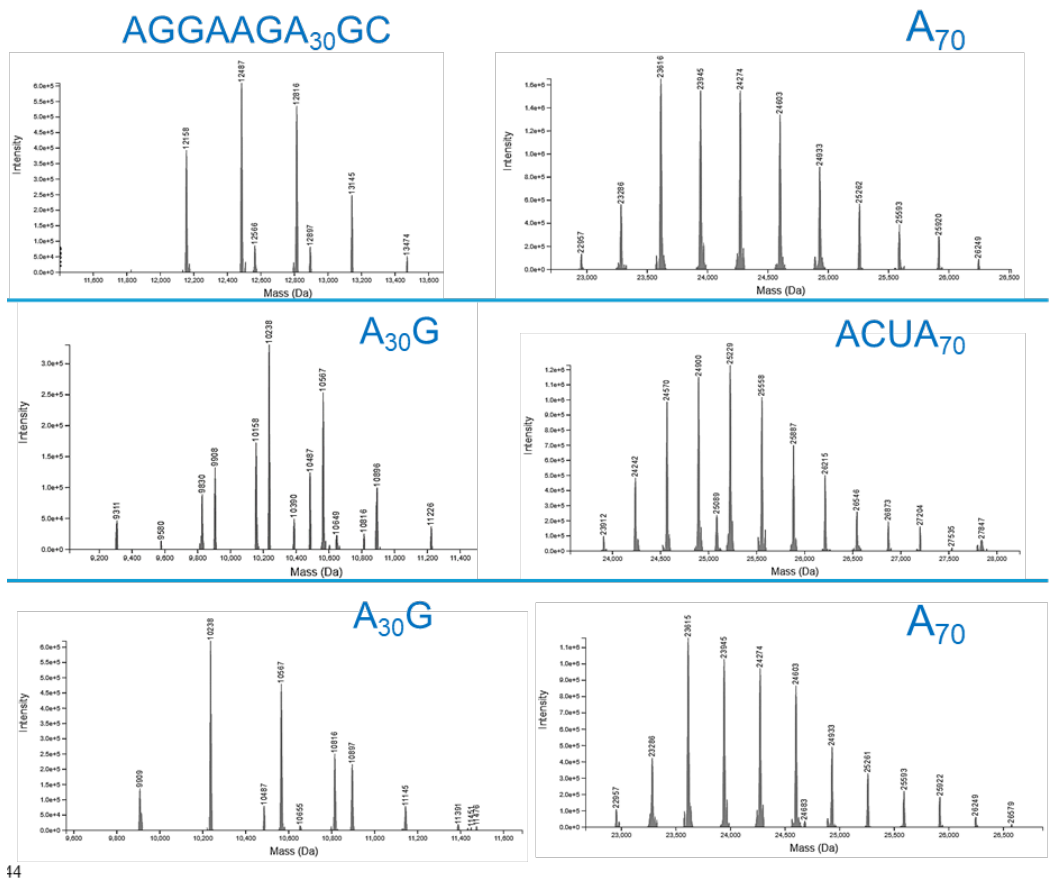
Case Study: DNAzyme cleaved mRNA

Segmented poly(A) tail: UAGGAAG **30A** GCAUAUGACU **70A**



UV chromatograms



Deconvoluted Spectra

The poly(A) tail is digested into two main poly(A) populations based on the RNase specificity confirming the presence of the non-adenosine residues.

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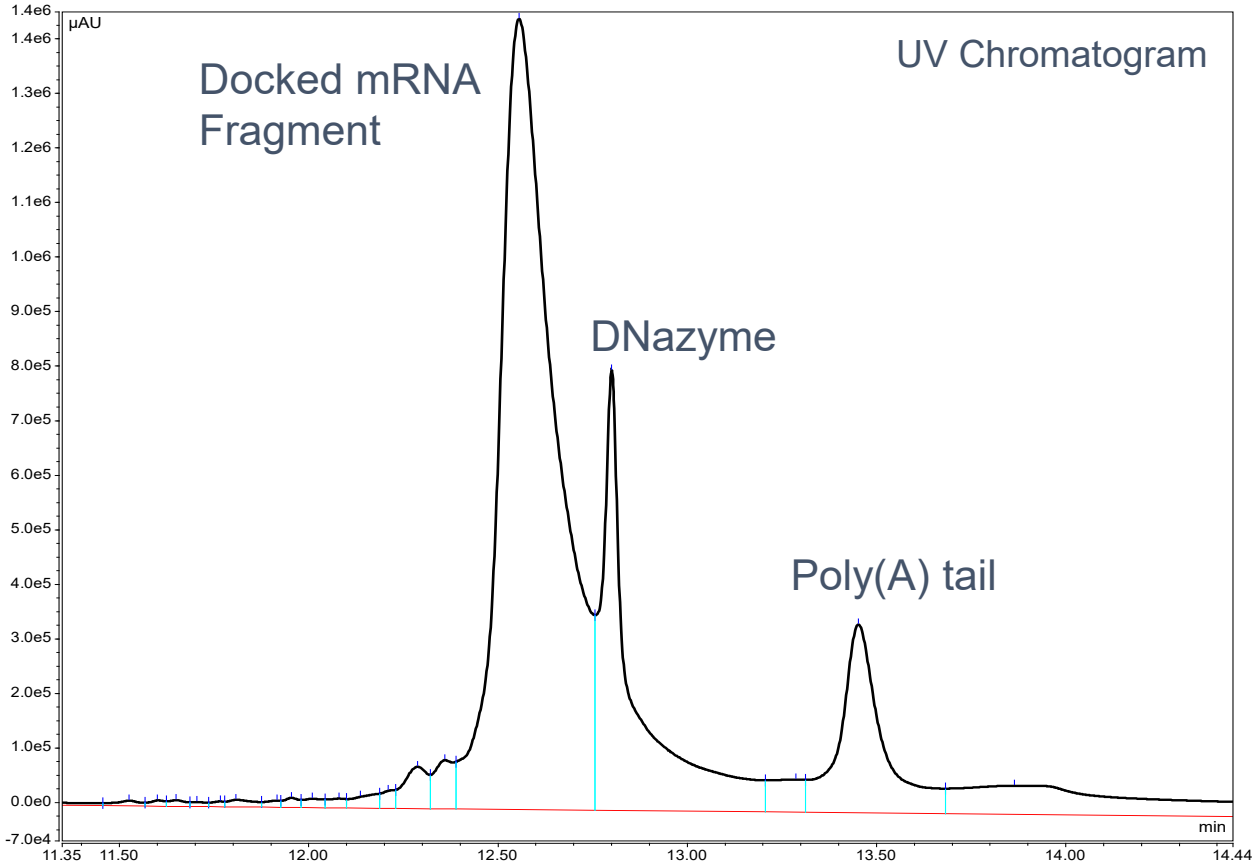
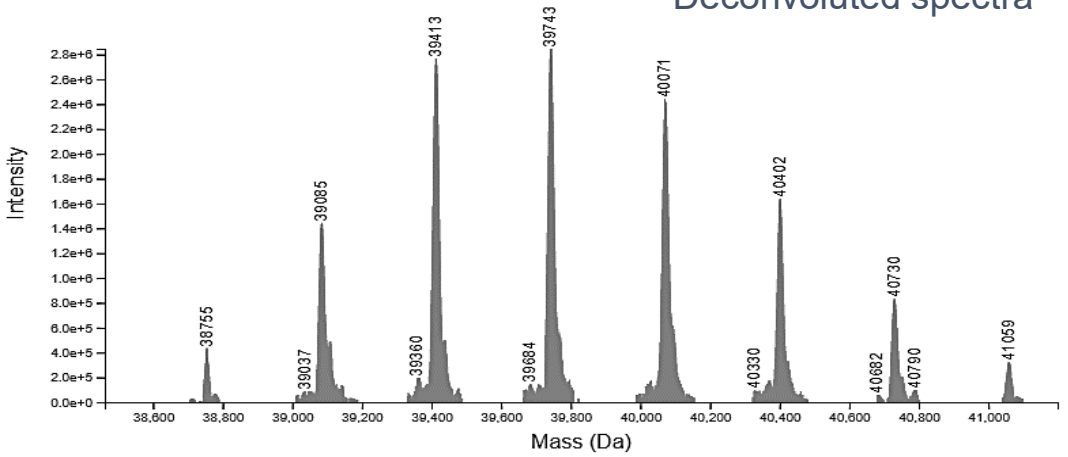
Case Study: DNAzyme cleaved mRNA

Segmented poly(A) tail: UAGGAAG **30A** GCAUAUGACU **70A**

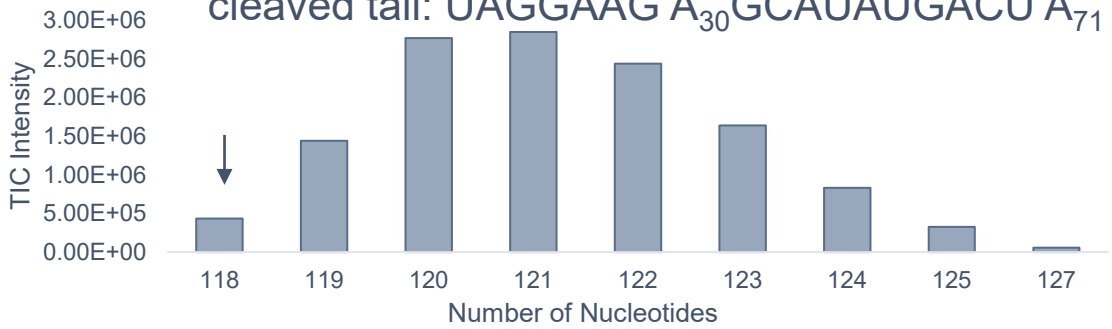


Deconvoluted spectra



Poly(A) tail distribution

cleaved tail: UAGGAAG A_{30}GCAUAUGACU A_{71}



DNAzyme cleavage results in one poly(A) tail distribution that matches the theoretical sequence and confirms the full poly(A) tail length.

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Key takeaways

RNase digestion

- Two poly(A) tail distributions were observed in the range of A_{30} and A_{70}, confirming the presence of non-adenosine residues between the homopolymeric tail distribution.
- This assay did not confirm the full length of the poly(A) tail.

DNAzyme cleavage

- DNAzyme cleavage confirmed the full length of the poly(A) tail and its distribution, including the non-adenosine residues.

The methods provided complementary information enabling full characterization of the complex segmented tail.

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