

MRNA CRITICAL QUALITY ATTRIBUTE ANALYSIS USING A UPLC-TOF-MS SYSTEM AND CUSTOMIZED SOFTWARE

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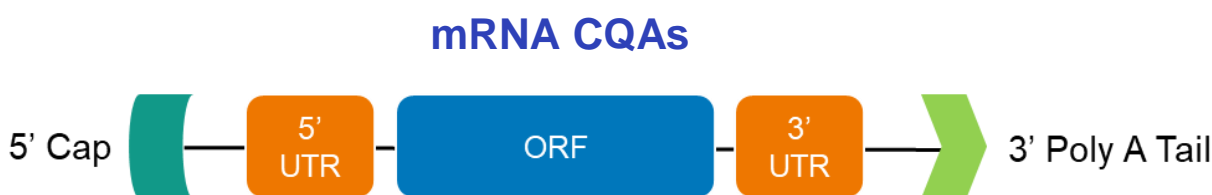
INTRODUCTION

Recent development and approval of the two COVID mRNA-based vaccines has brought RNA therapeutics to the forefront of the biopharma industry. As such, development of analytical methods for monitoring of the CQA of RNA-based therapeutics has become a high priority for ensuring proper control of manufacturing process.

This study illustrated three CQAs (Critical Quality Attributes) analytical workflow for mRNA using a bench top UPLC-TOF MS instruments and customized data processing software.¹⁻³

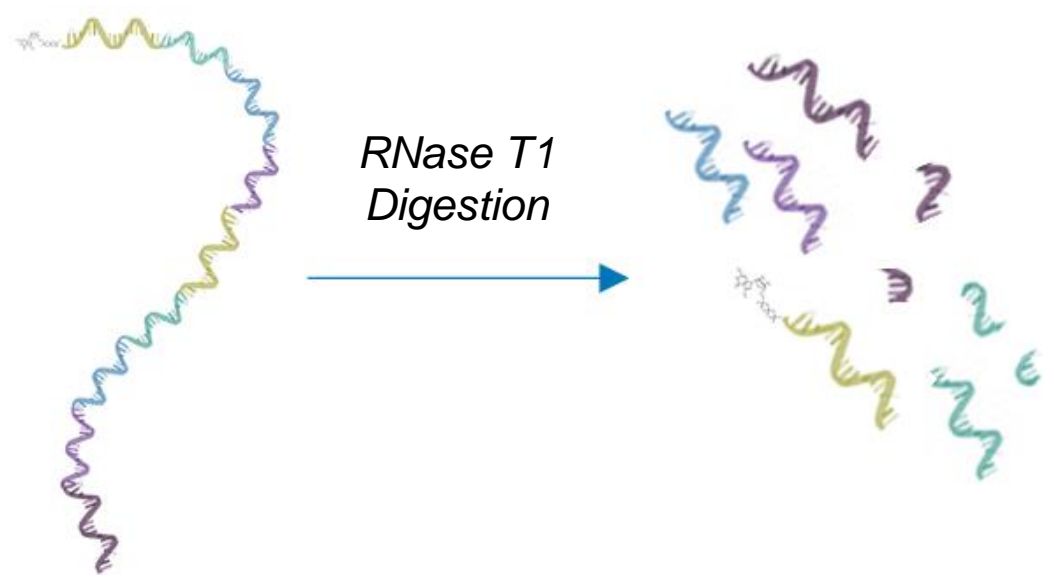
These CQAs are:

- 5' capping
- Poly A tail heterogeneity
- Sequence mapping

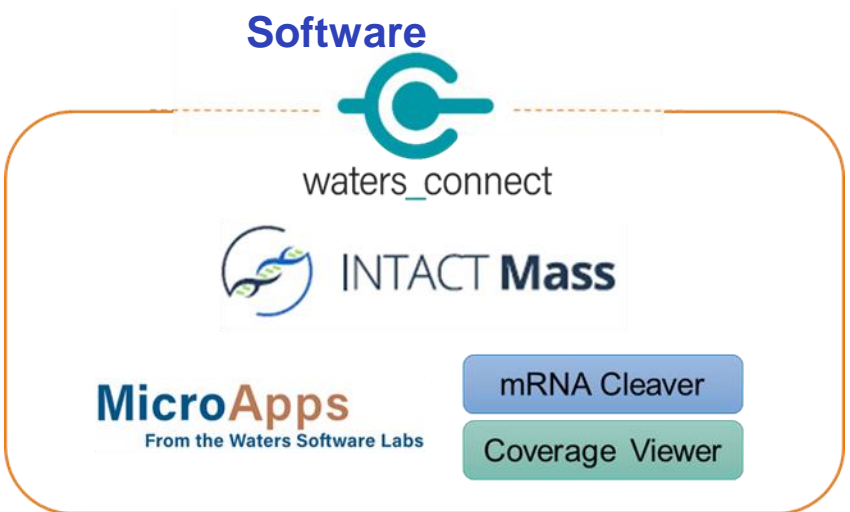


METHODS

Sample Preparation



Software



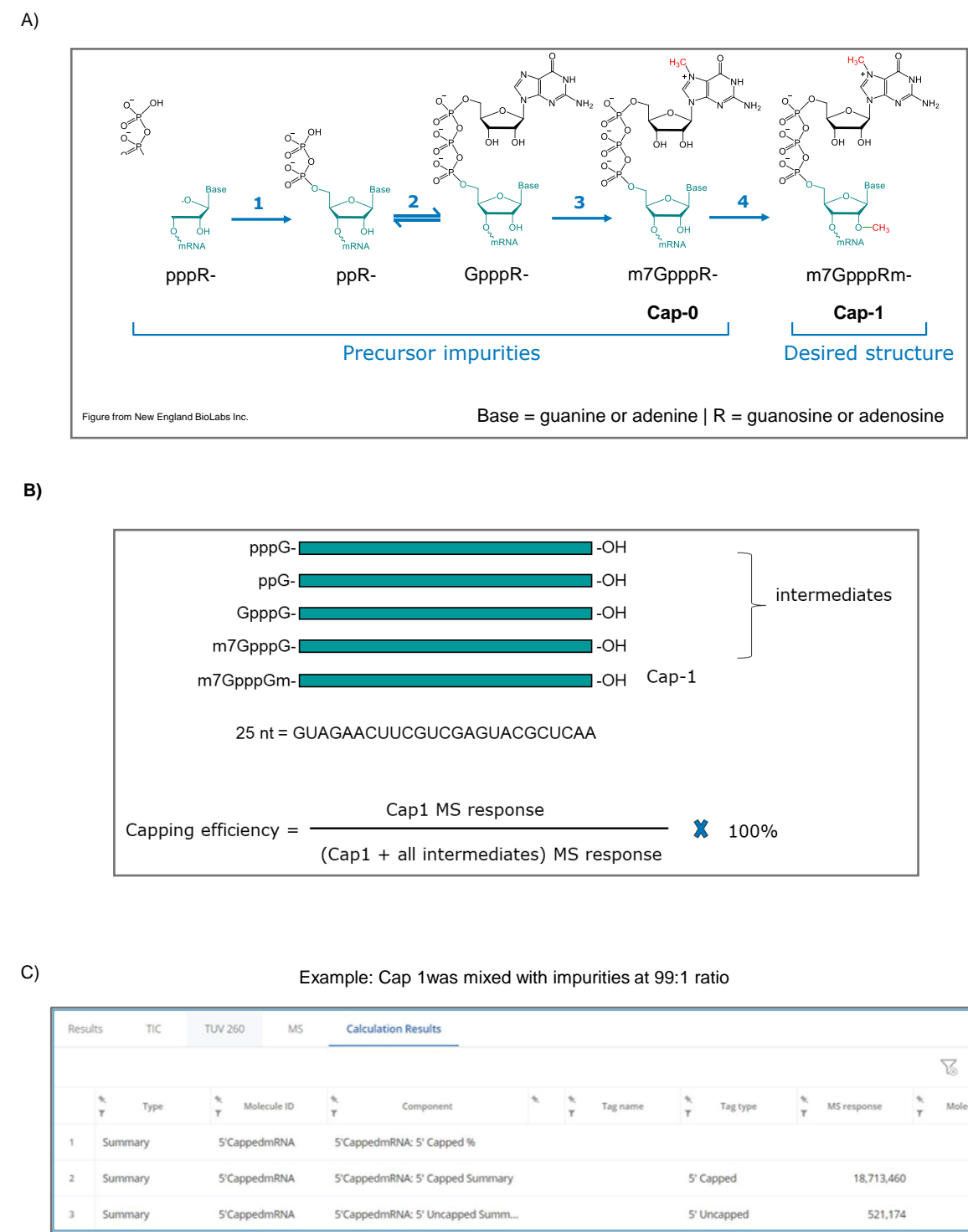
INTACT Mass App (software) was the main software used for 5' capping analysis, sequence mapping and poly A tail heterogeneity profiling.



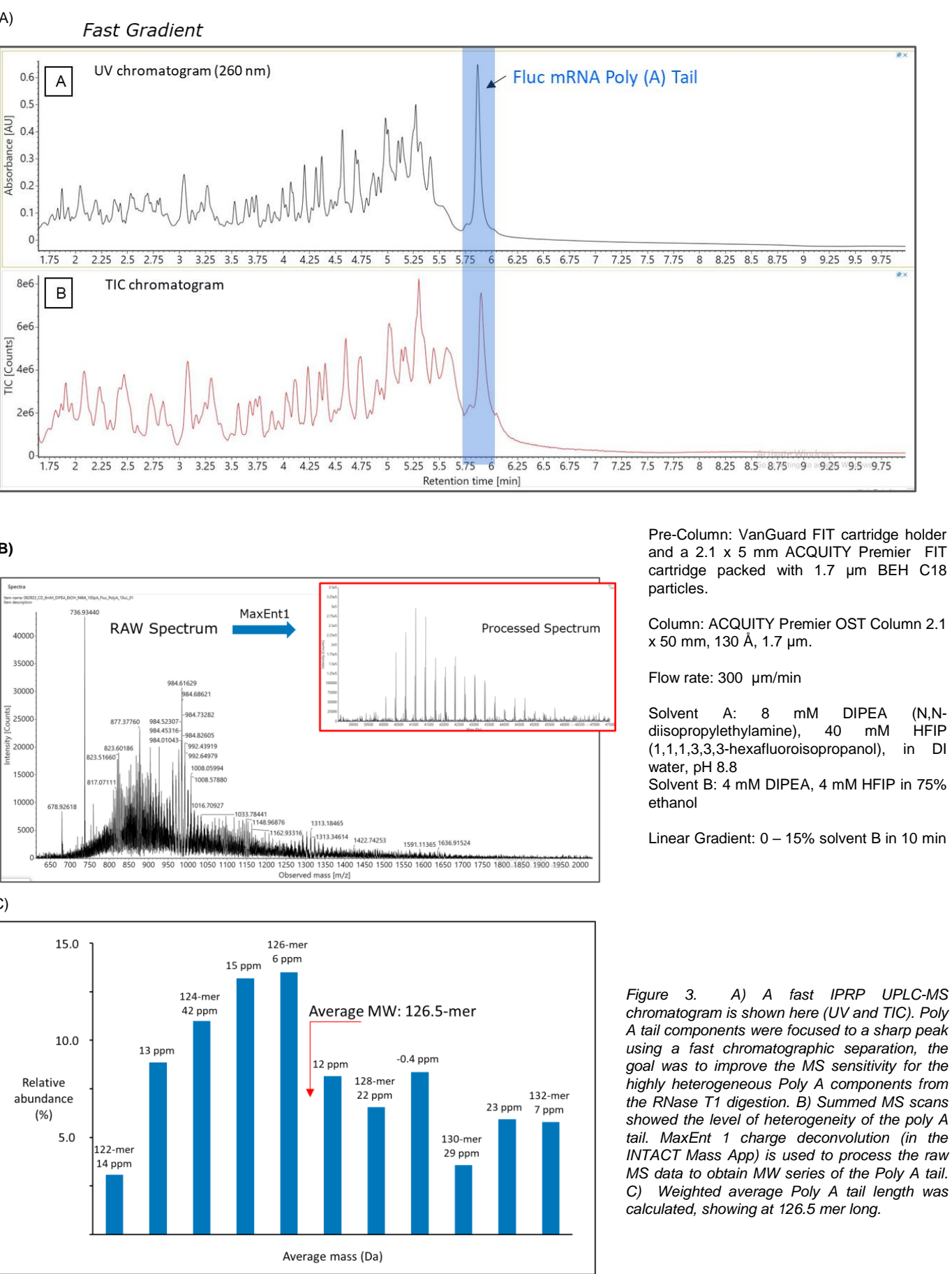
Figure 1: BioAccord™ UPLC-TOF MS System with Max Peak™ High Performance Surfaces.
Column used: ACQUITY™ Premier OST Column 1.7 μm, 130 Å, 2.1 x 150 mm
More method information can be found in the three application notes listed under the reference.

RESULTS

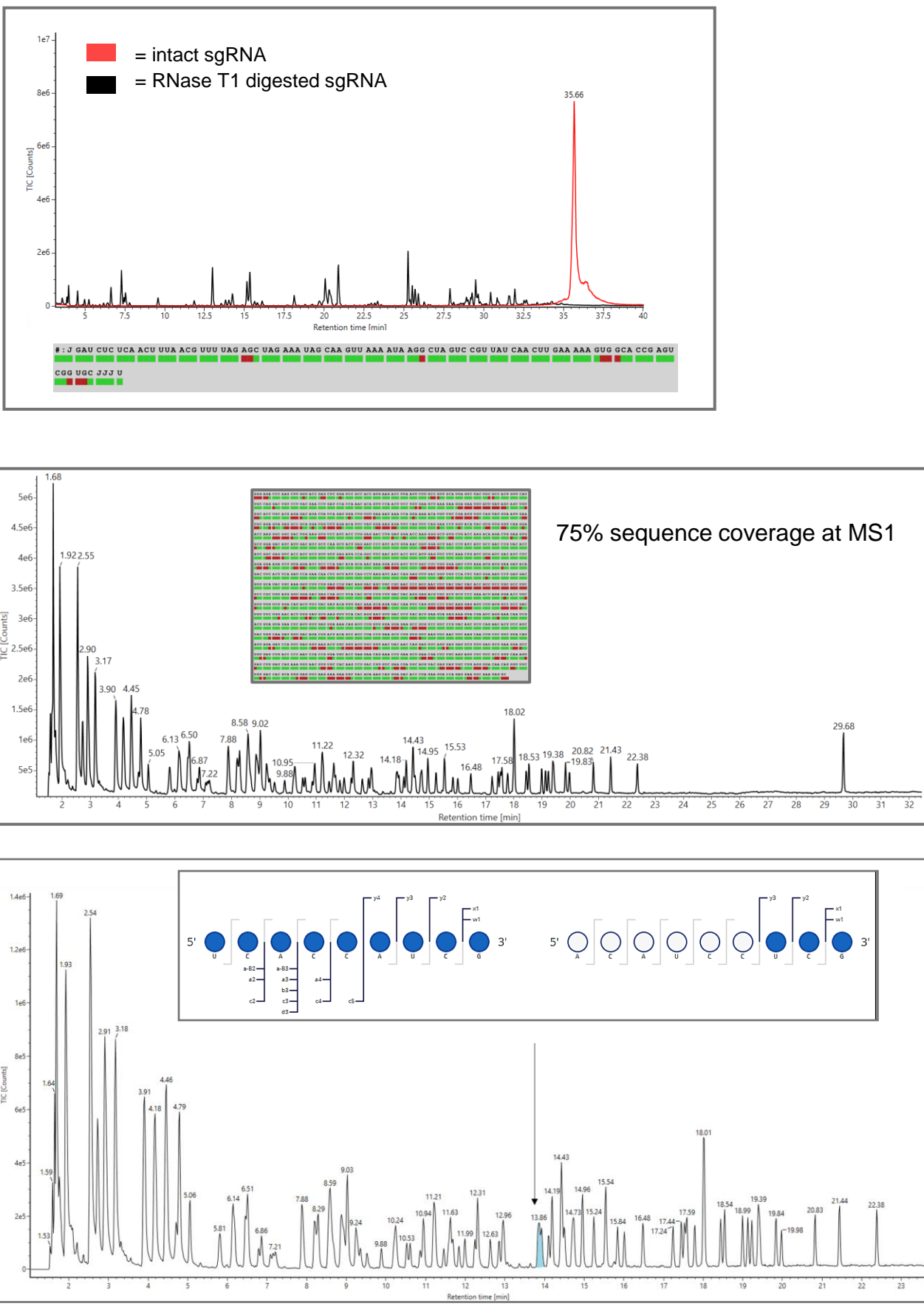
5'capping



Poly A Tail



Sequence Confirmation



CONCLUSION

- THREE ANALYTICAL WORKFLOW ARE DEVELOPED ON THE BIOACCORD LC-MS SYSTEM AND WATERS_CONNECT INFORMATICS PLATFORM FOR THREE MRNA CQA ANALYSIS. THESE CQAS ARE 5' CAPPING EFFICIENCY, POLY A TAIL HETEROGENEITY AND SEQUENCE MAPPING ANALYSIS.
- SOFTWARE TOOL PLAYS CRITICAL ROLE IN REDUCING THE TIME USED FOR DATA INTERPRETATION. THE SOFTWARE USED IN THIS STUDY IS INTACT MASS APP WITH NEWLY ADDED CAPABILITIES SUCH AS ENZYME CLEAVAGE TOOL AND SEQUENCE MAPPING VIEWER.
- FUTURE WORK WILL BE FOCUSED ON IMPROVING THE SEQUENCE COVERAGE BY 1) COMBINING MS1 AND MS2 RESULTS; 2) AUTOMATED THE ENTIRE DATA PROCESSING WITH LESS MANUAL INTERVENTION TO REDUCE THE LEVEL OF AMBIGUOUS ASSIGNMENTS.

References

- WATERS APPLICATION NOTE: 720008130 "RNA CQA ANALYSIS USING THE BIOACCORD LC-MS SYSTEM AND INTACT MASS WATERS_CONNECT APPLICATION".
- WATERS APPLICATION NOTE: 720007329 "RAPID ANALYSIS OF SYNTHETIC MRNA CAP STRUCTURE USING ION-PAIRING RPLC WITH THE BIOACCORD LC-MS SYSTEM".
- WATERS APPLICATION NOTE: 720007925 "ION PAIRING REVERSED PHASE LC-MS ANALYSIS OF POLY (A) TAIL HETEROGENEITY USING THE BIOACCORD LC-MS SYSTEM".