Comprehensive mRNA Characterization by LC-MS/MS using RNase 4

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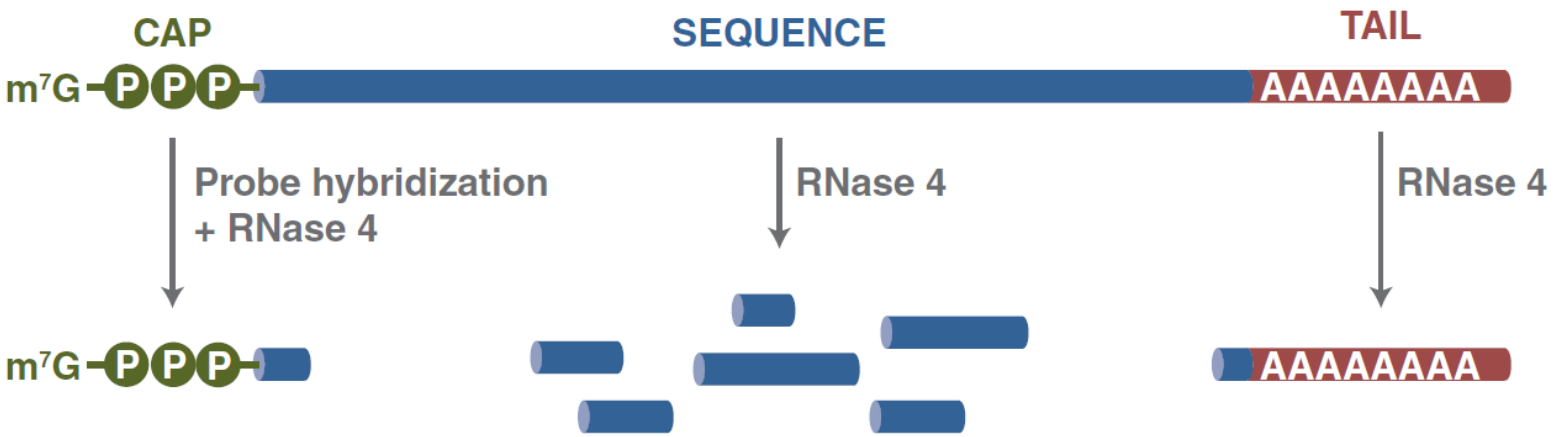
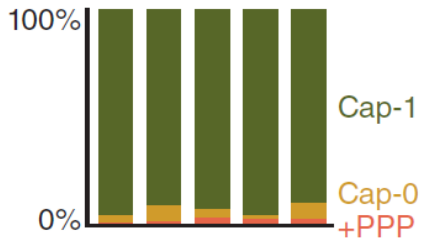
A Uniquely Enabling Nuclease for mRNA Analysis | RNase 4 M1284



LC-MS

Identification & quantitation

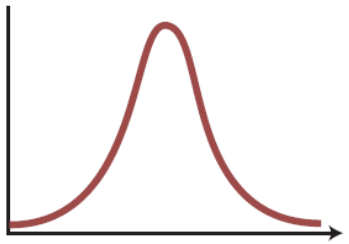


LC-MS

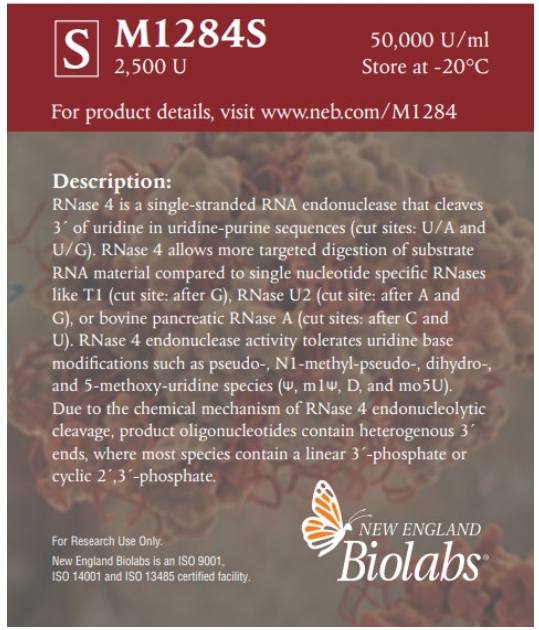
Sequence annotation & mapping

LC-MS

Poly(A) tail length profiling



RNase 4



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Agenda

- General Features of mRNA Analysis by LC-MS/MS
- RNase 4 Workflows for Sequence Annotation and Modification Mapping
- Poly(A)-tail Analysis w/ targeted RNase 4 Digestion
- Determination of 5′ mRNA Cap Structures using a probe directed RNase 4 digestion strategy
- Questions

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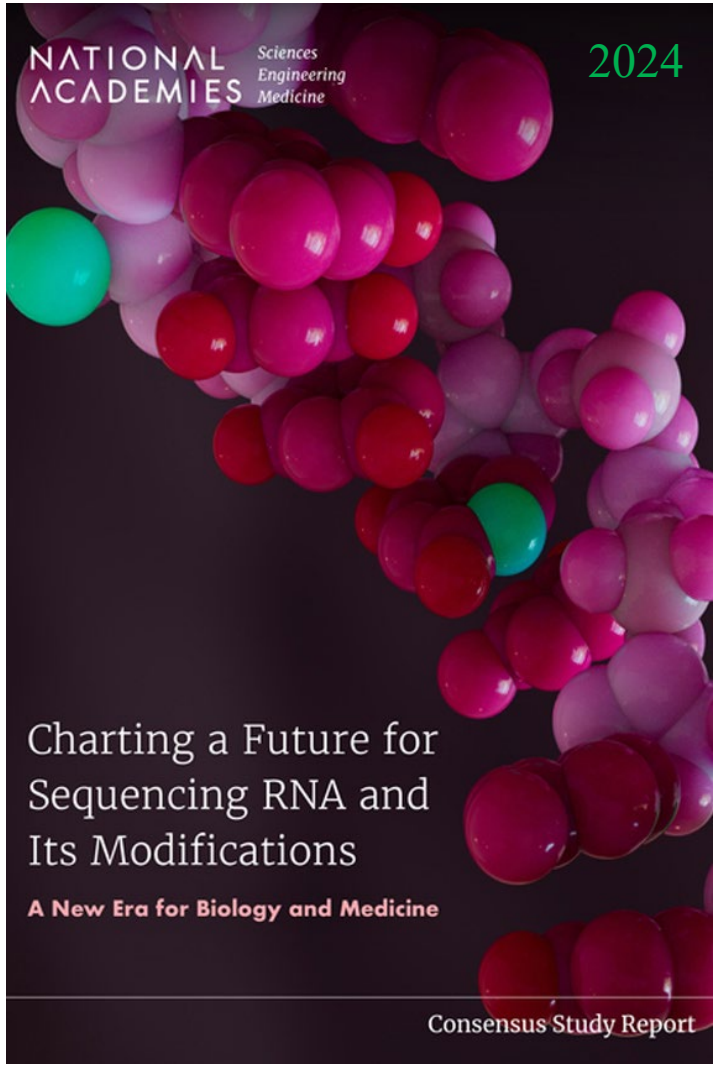
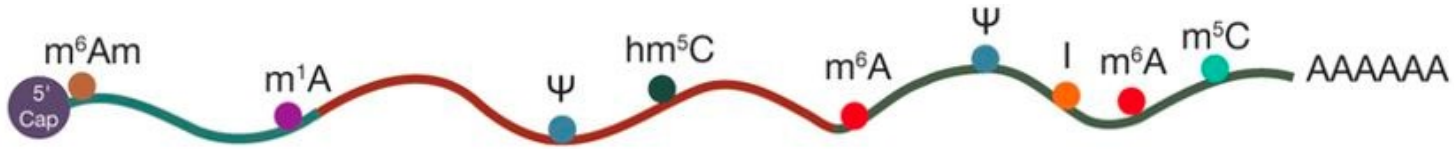
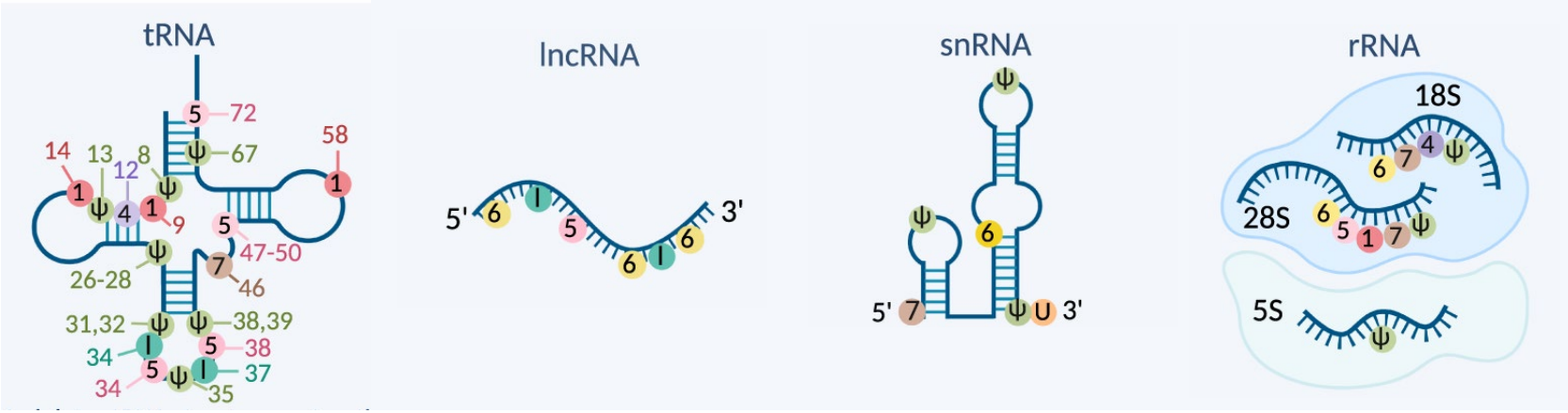
Charting a Future for Sequencing RNA & Its Modifications

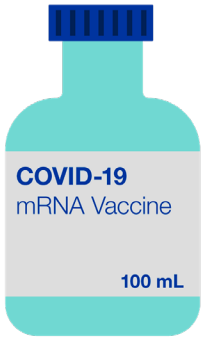


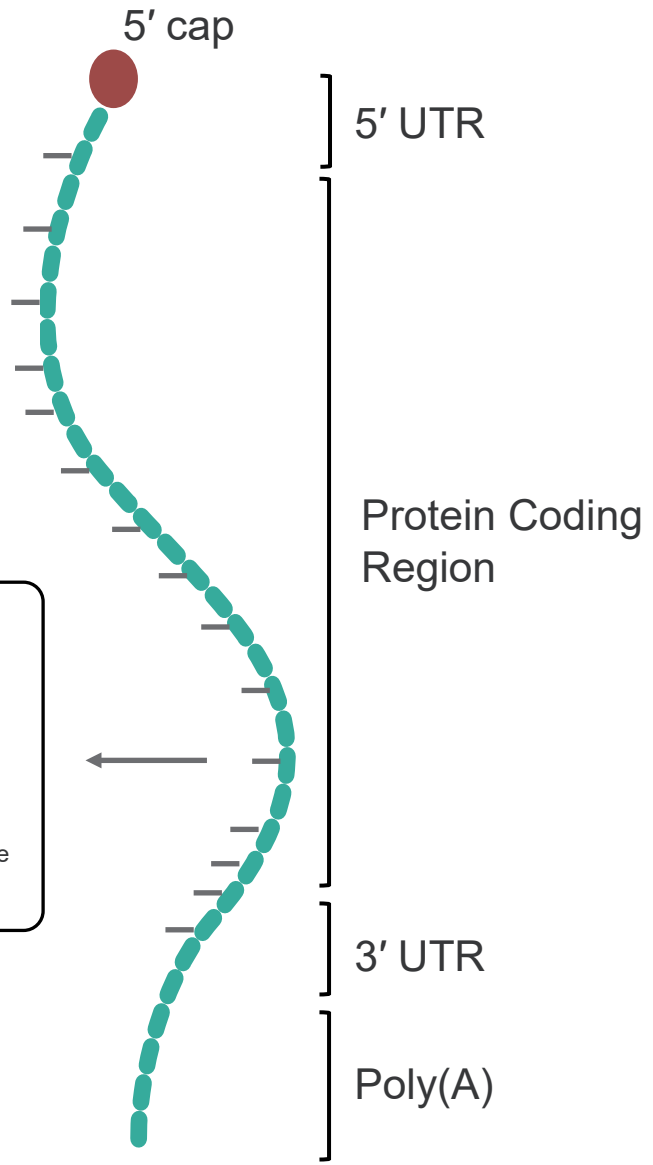
- NASEM Consensus Study Report 2024
  - Importance of RNA modifications in Biology, Disease, Medicine, and Society
  - Tools and technologies for studying RNA modifications
  - Standards and databases for RNA modifications
  - Driving innovation to study RNA modification

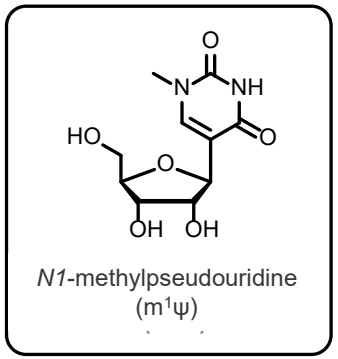


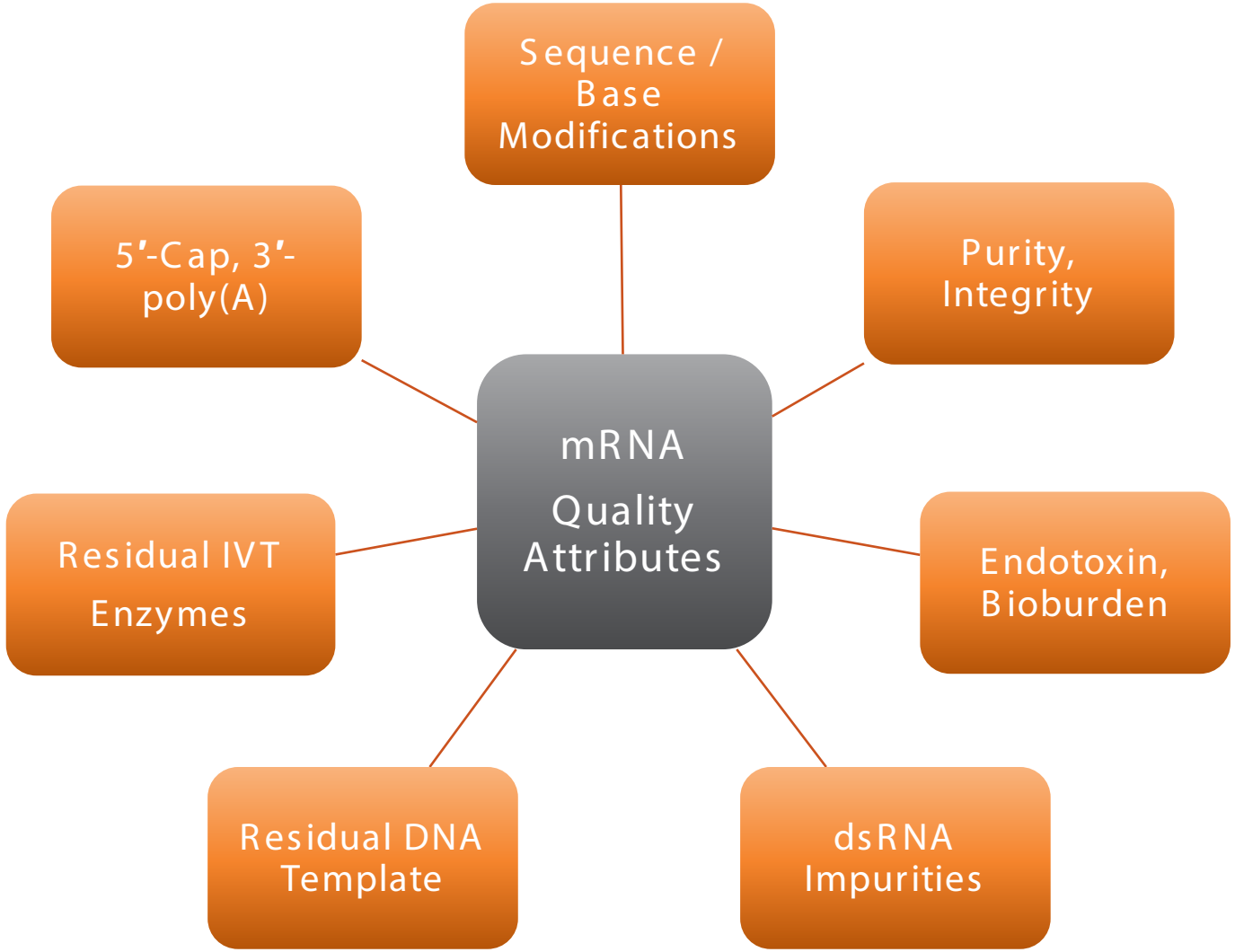


Key features of mRNA | **USP Critical Quality Attributes**

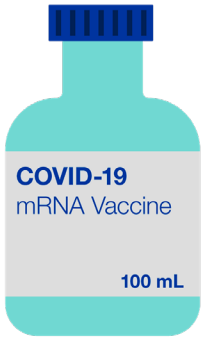


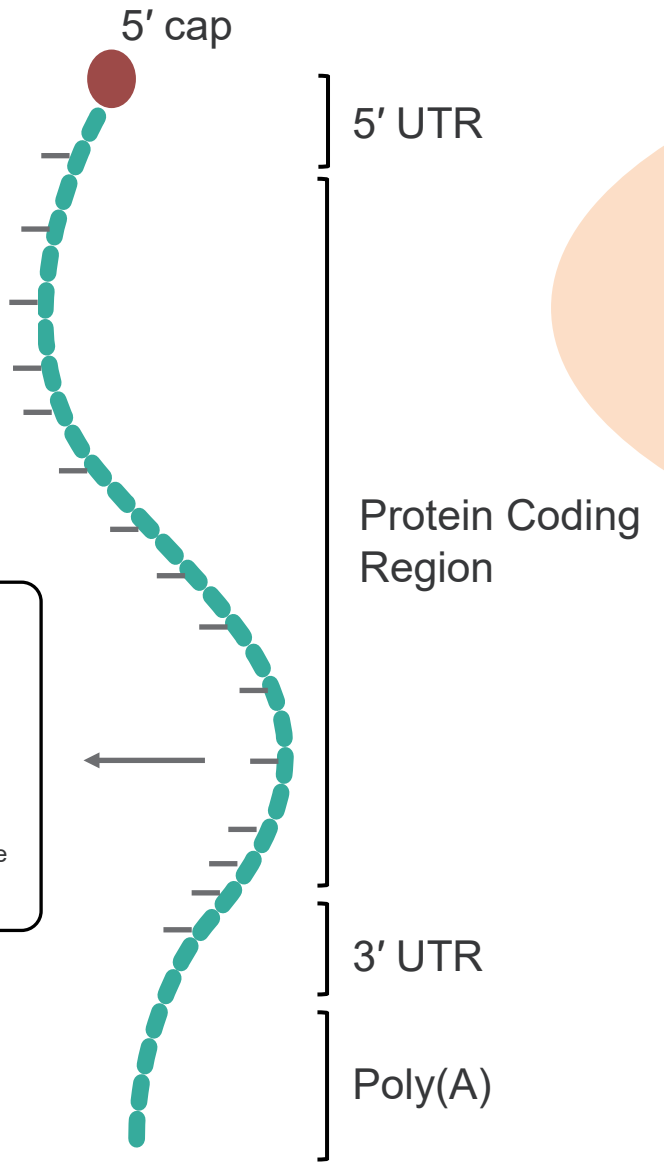


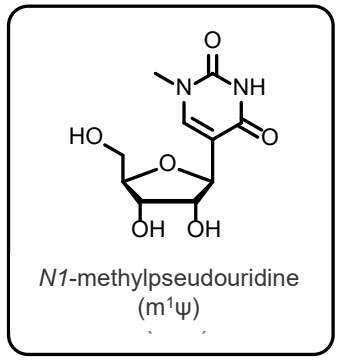


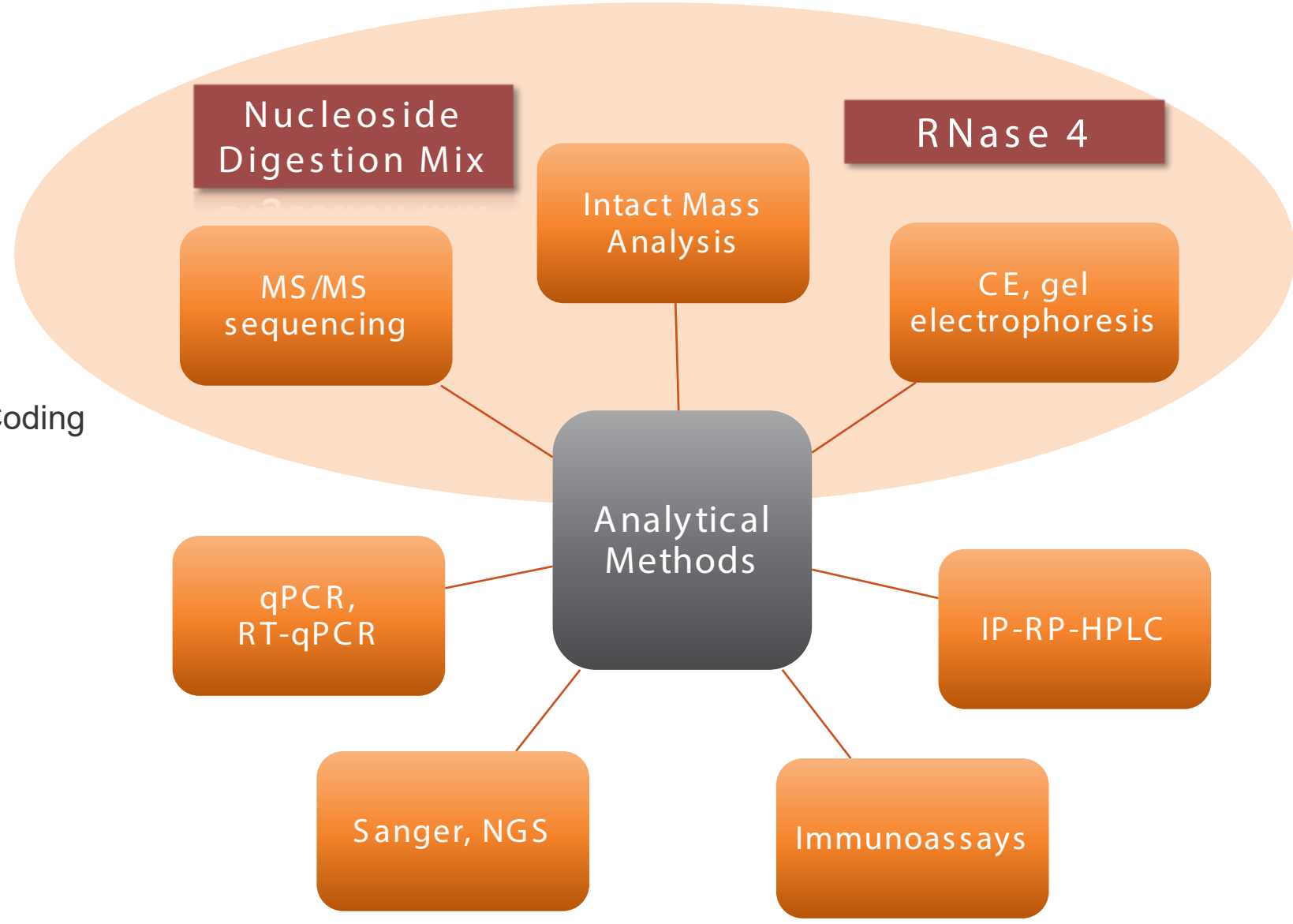


Key features of mRNA | **Methods of Assessment**





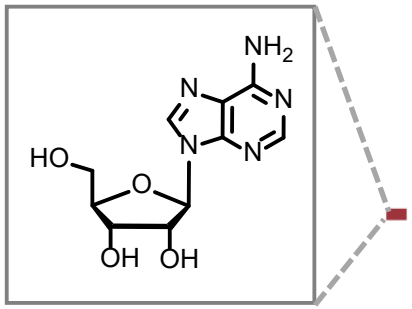




NEB Products for Bottom-Up RNA Analysis by LC-MS/MS

Nucleoside Analysis

Nucleoside Digestion Mix (#M0649)



nucleosides

- ✓ nucleoside composition
- ✓ identify and quantify RNA modifications

Oligonucleotide Analysis

RNase 4 (#M1284)

RNase 4 Digestion & 3′ End Repair Mix (#M1288)

oligonucleotides

- ✓ map the position of RNA modifications
- ✓ verify RNA sequence and identity

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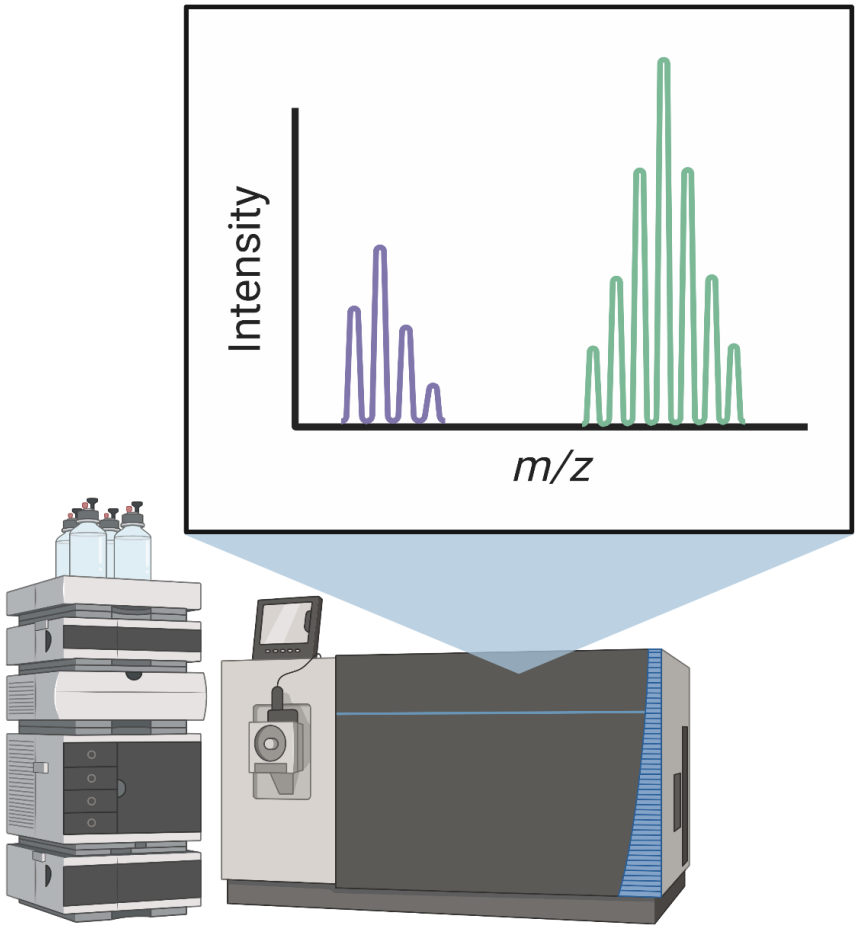
RNA Analysis by Mass Spectrometry (MS)



Key differentiators:

- ✓ direct analysis — eliminates RT and amplification biases
- ✓ detects nucleotide modifications alongside sequence
- ✓ untargeted — applicable to all modifications
- ✓ high accuracy
- ✓ orthogonal to RNA-seq

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Liquid Chromatography (LC)-MS/MS Instrumentation for RNA Analysis at NEB

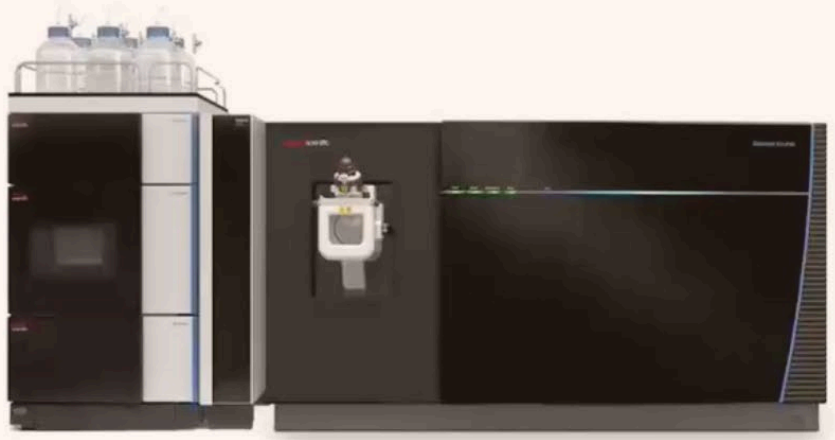
Agilent 1290 Infinity II UHPLC – 6135 XT MS

Agilent 1290 Infinity II UHPLC – 6495C QQQ MS

Agilent 1290 Infinity II Prep HPLC

Waters H-Class UPLC

Thermo Vanquish Horizon UHPLC – Q-Exactive Plus MS



Thermo Vanquish Horizon UHPLC – Orbitrap Eclipse Tribrid MS

Thermo Vanquish Neo UHPLC

mRNA Sequence Identity & Modification Mapping Using RNase 4 prior to LC-MS/MS

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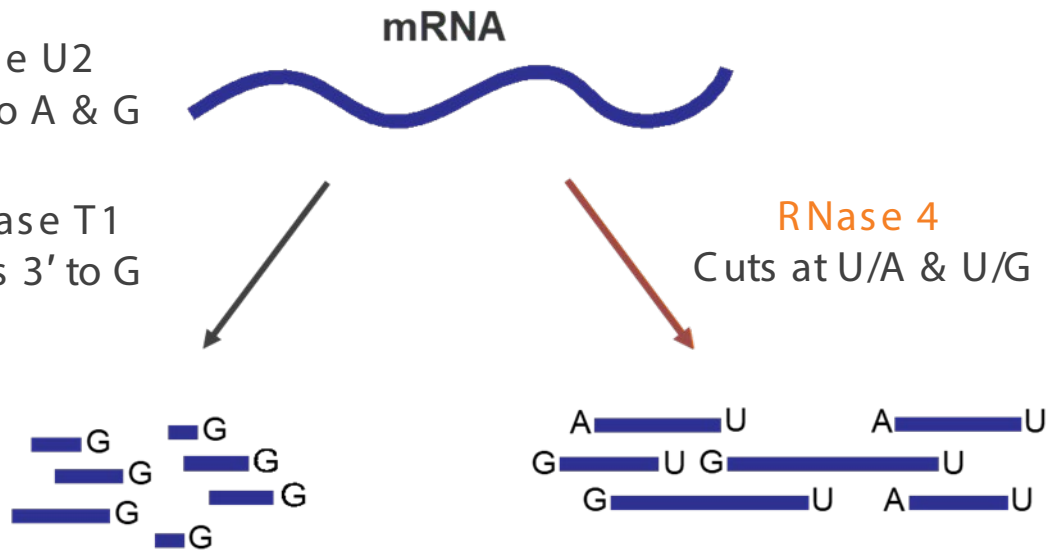
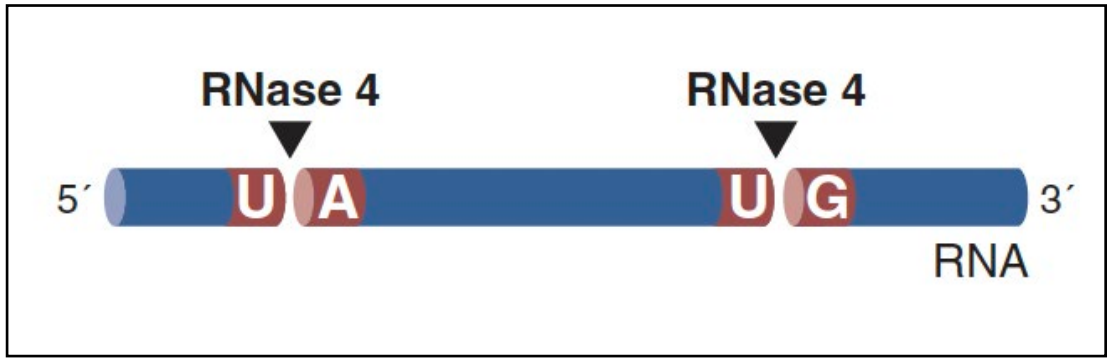
RNase 4 has 'Goldilocks' Dinucleotide Cut-Site Specificity

RNase A
Cuts 3′ to U & C

RNase U2
Cuts 3′ to A & G

RNase T1
Cuts 3′ to G



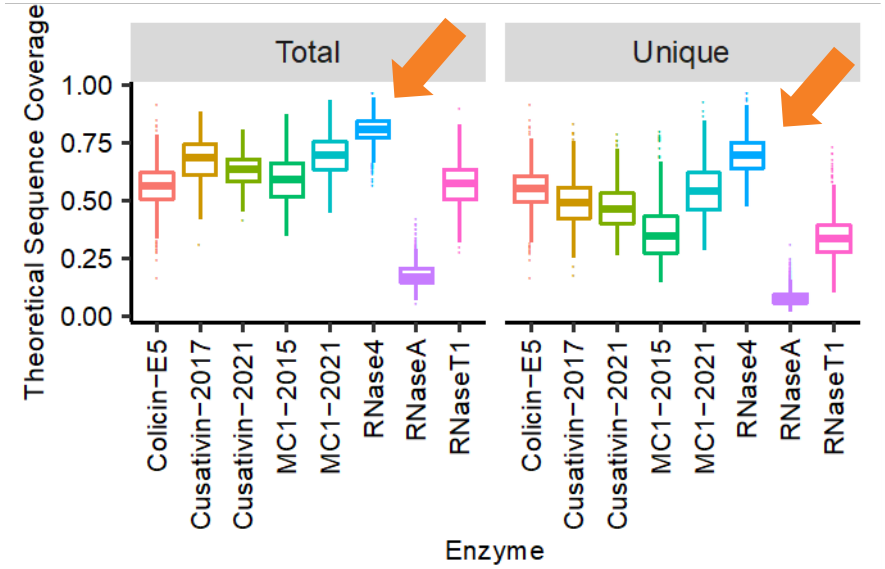


Sequence Mapping by LC-MS/MS

More Complete Coverage

RNase 4 Theoretical & Experimental Sequencing Coverage

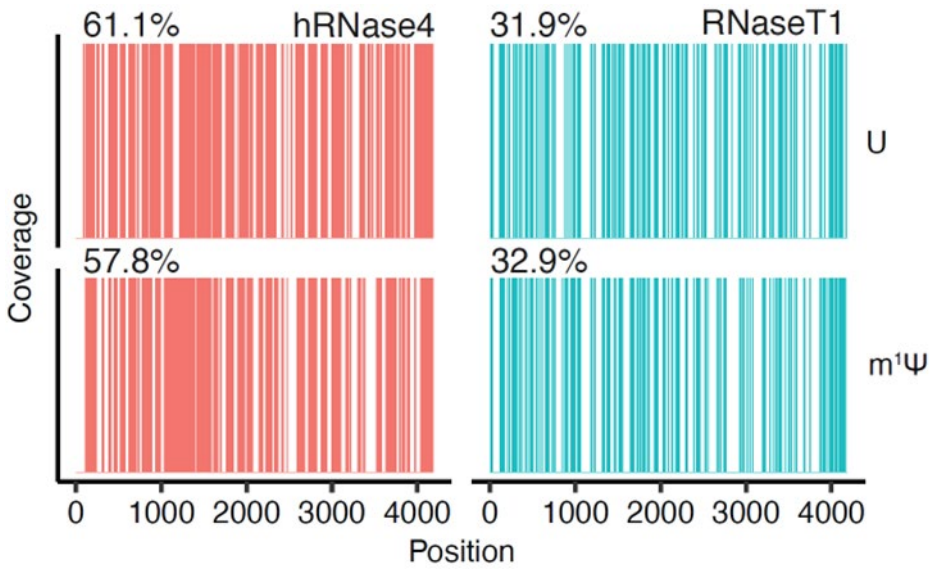
RNase 4 shows highest predicted mRNA sequencing coverage



1000 Random Human Transcripts (<5 kB)

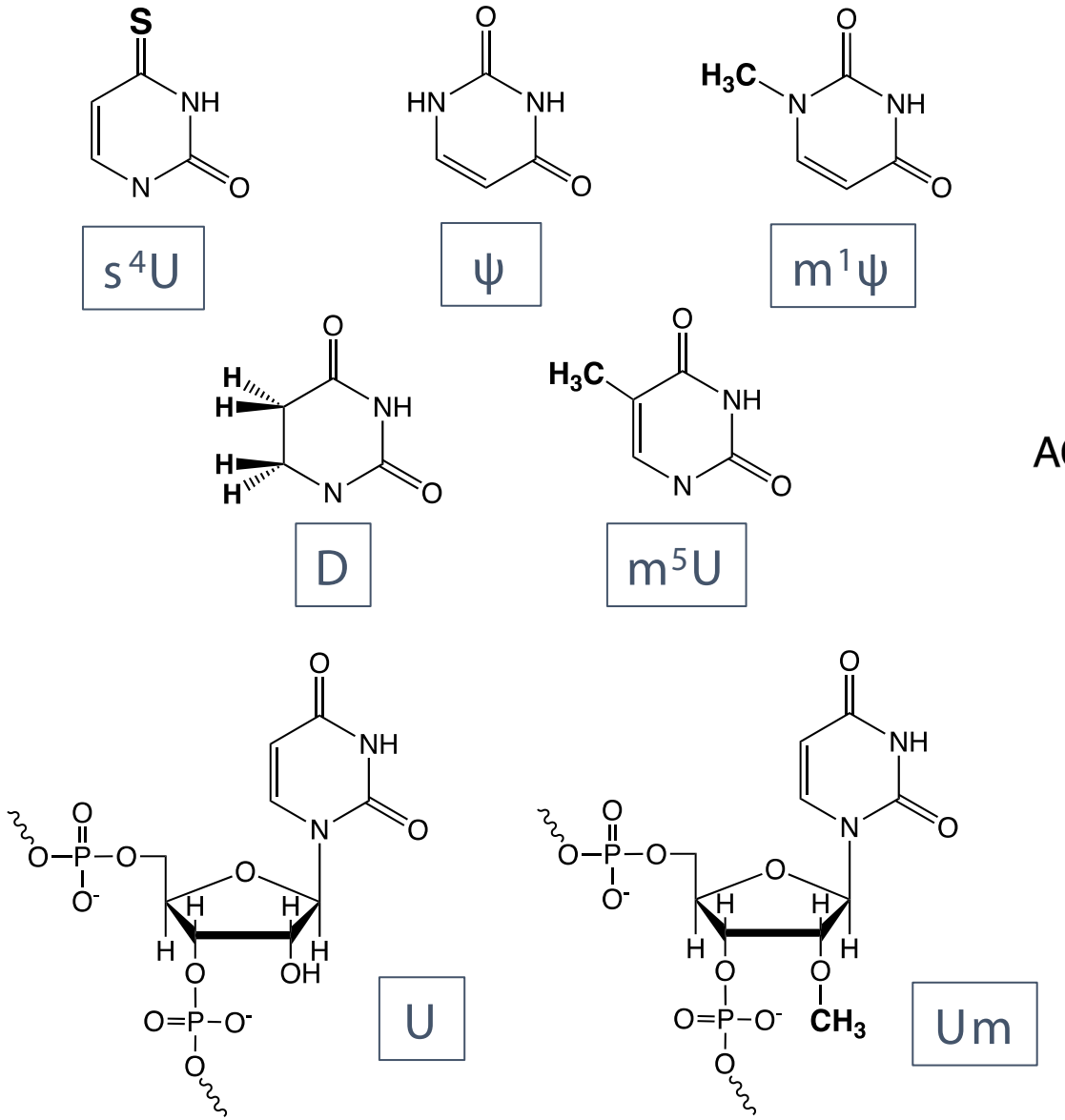
4nt < Oligo length < 40nt

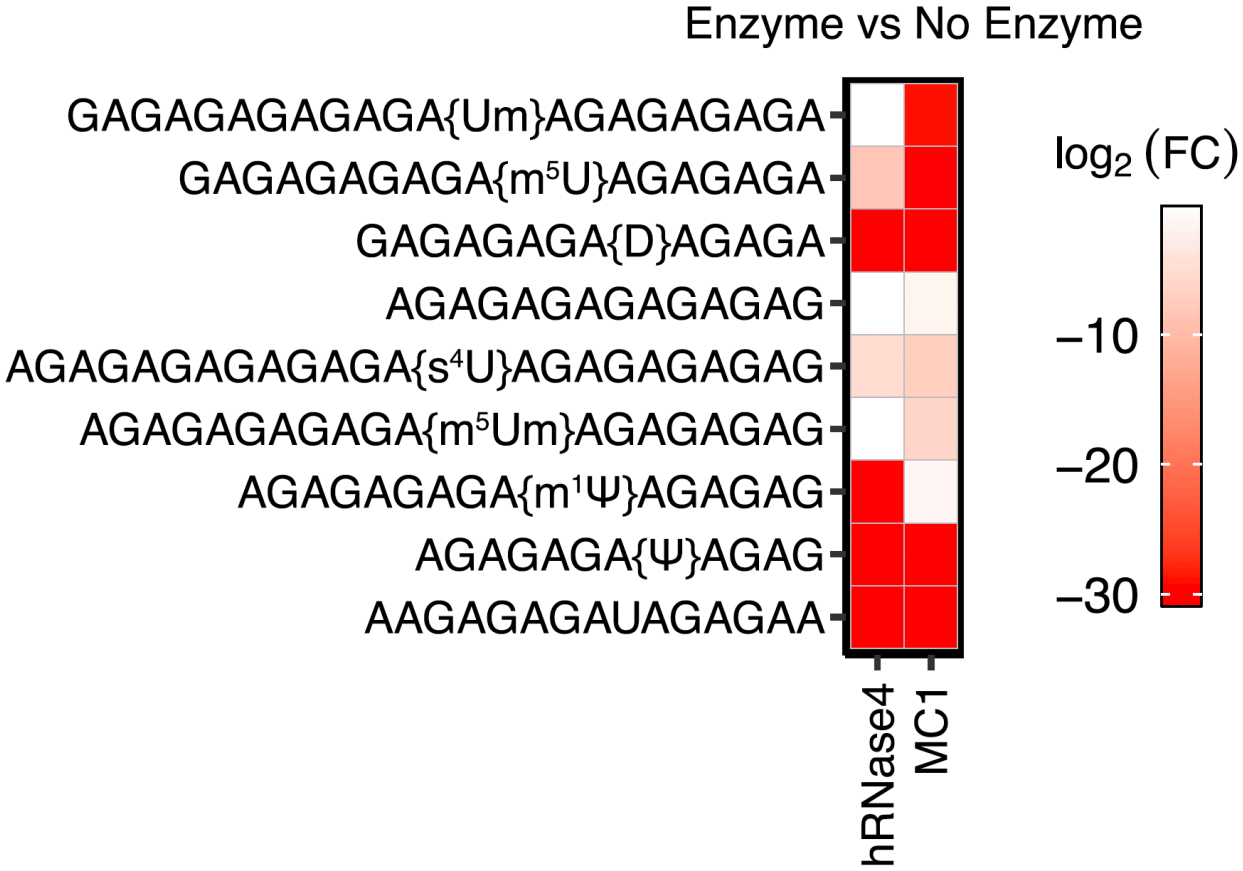
Mapping coverage of 4,187nt mRNA encoding BNT162b2 SARS-CoV-2 vaccine



Wolf EJ et al. ***Nucleic Acids Res.*** *2022, 50, e106*

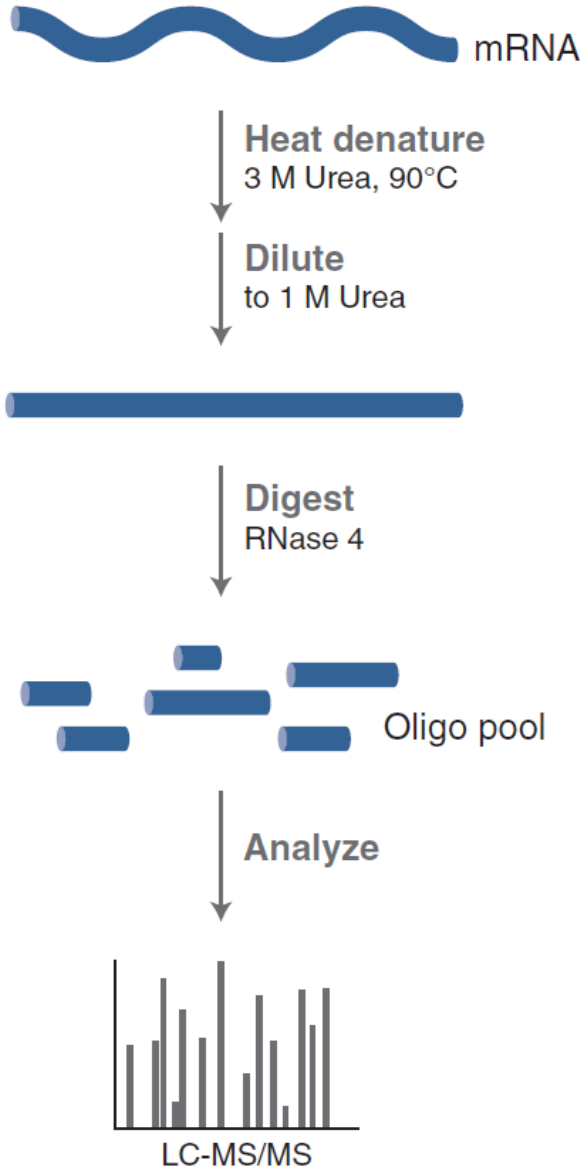
RNase 4 Endonuclease Activity Tolerant of Uridine Chemical Modifications



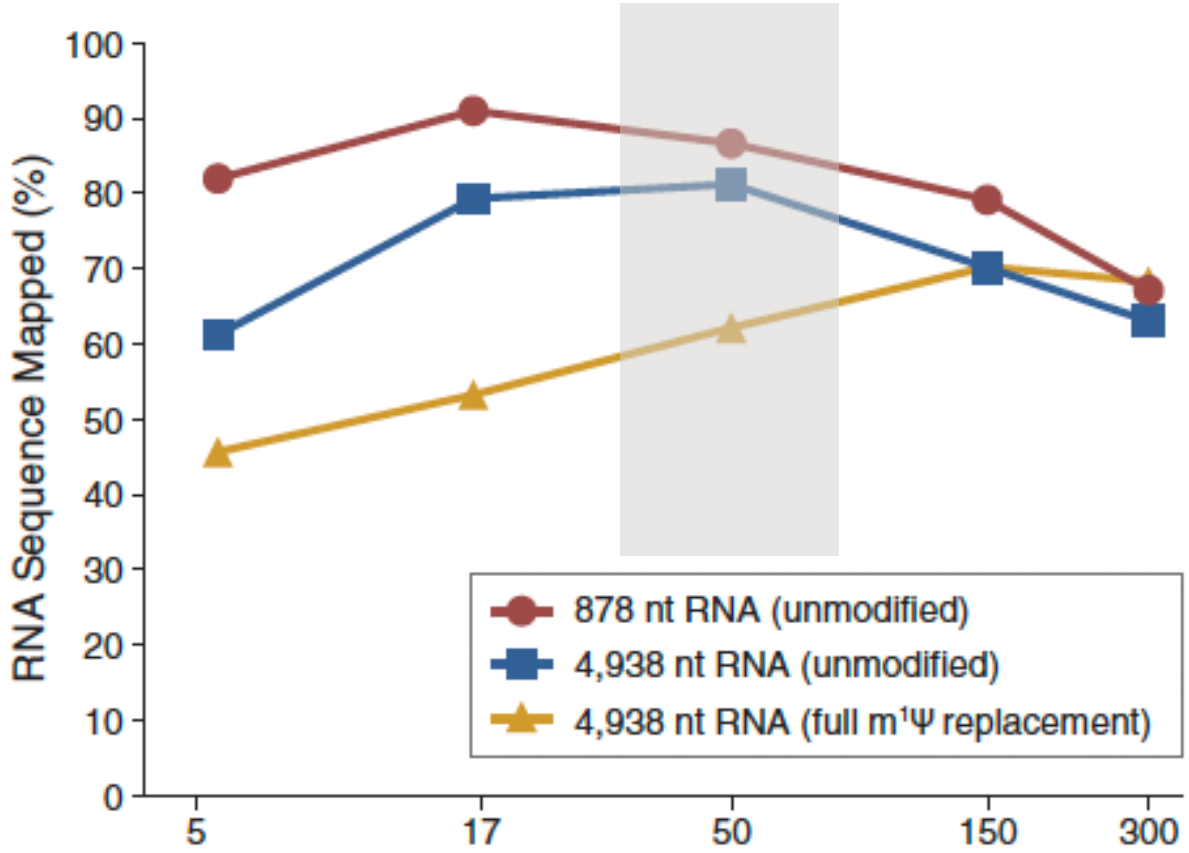


Wolf EJ et al. ***Nucleic Acids Res.*** *2022, 50, e106*

Denatured mRNA Digestion Protocol
RNase 4 is Single Strand-Specific



Sequencing Coverage by mRNA Length & Modification



Units of RNase 4

#M1284 (50 U/µl)

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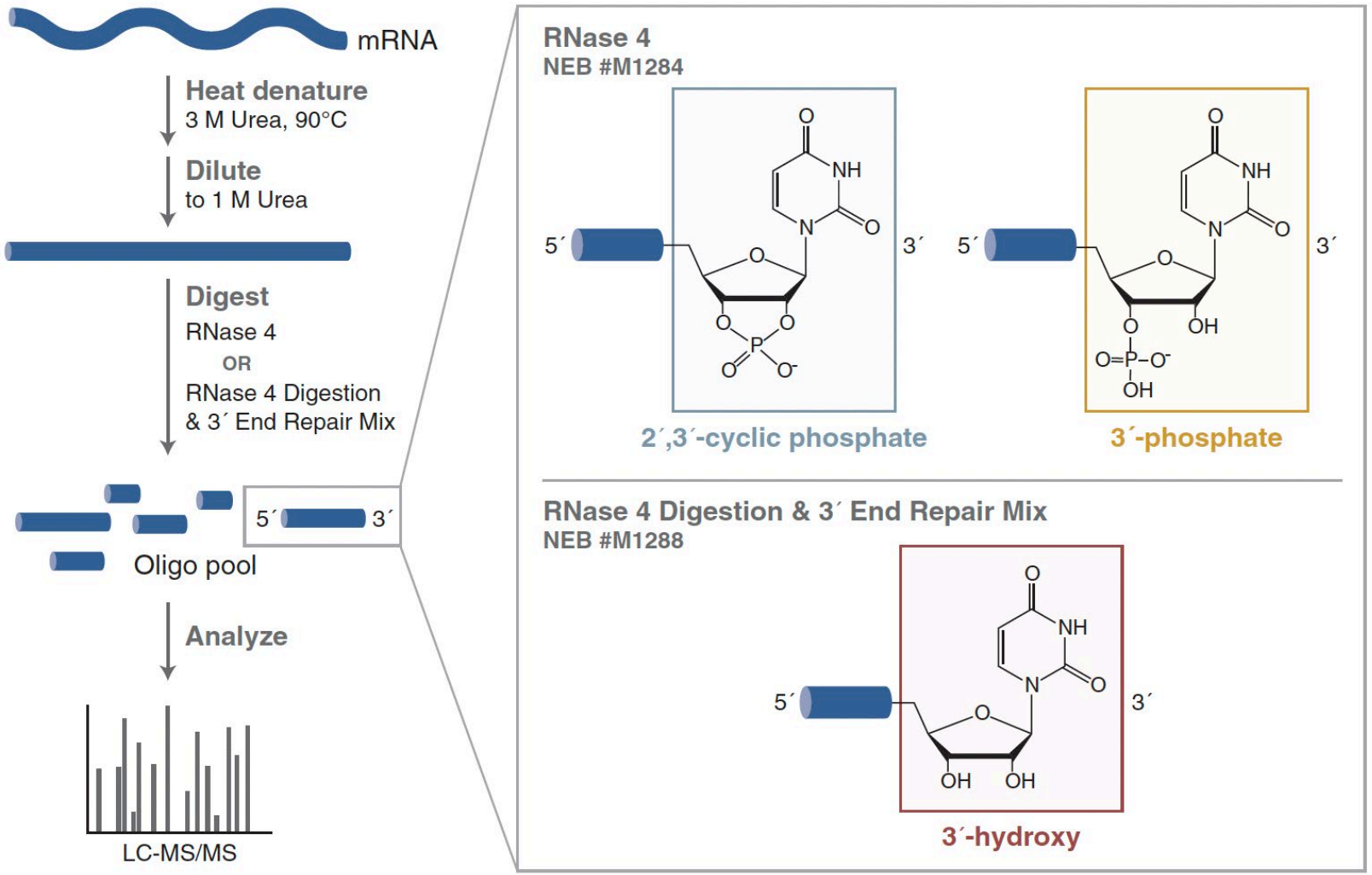
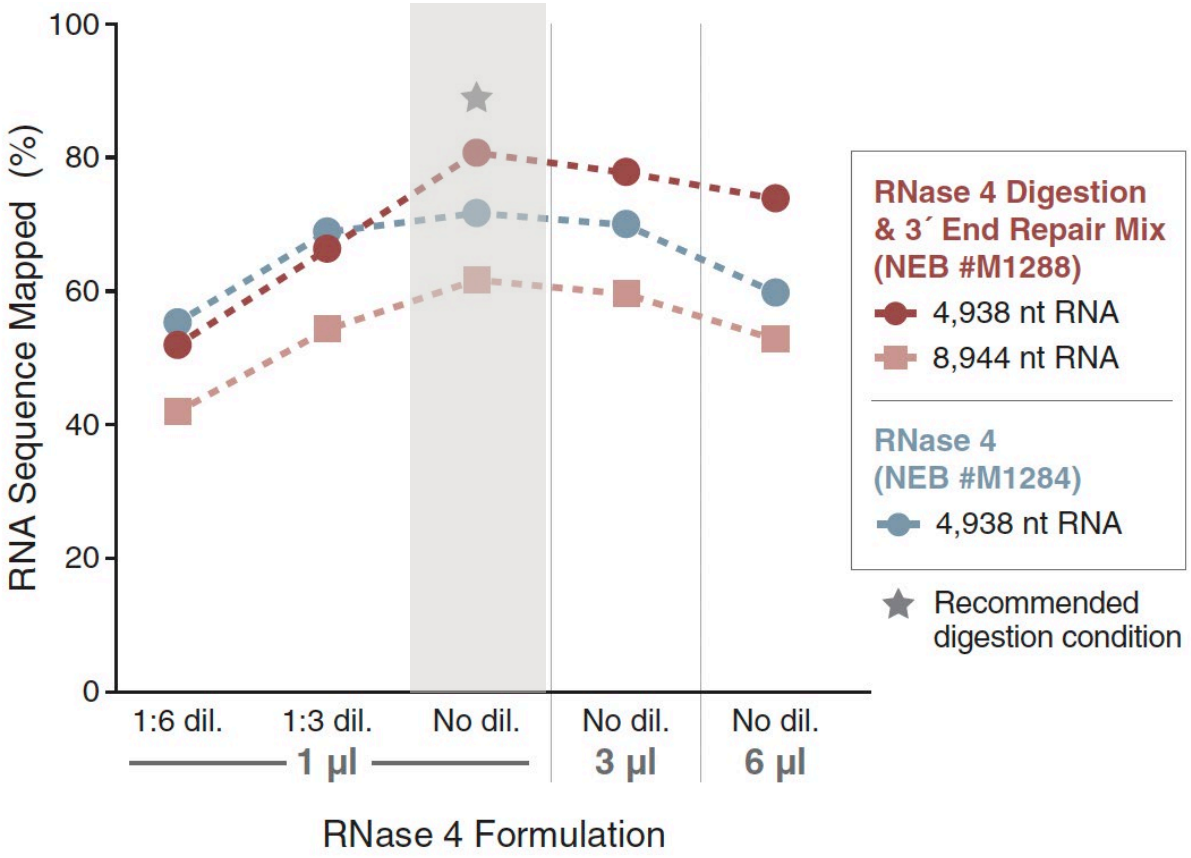
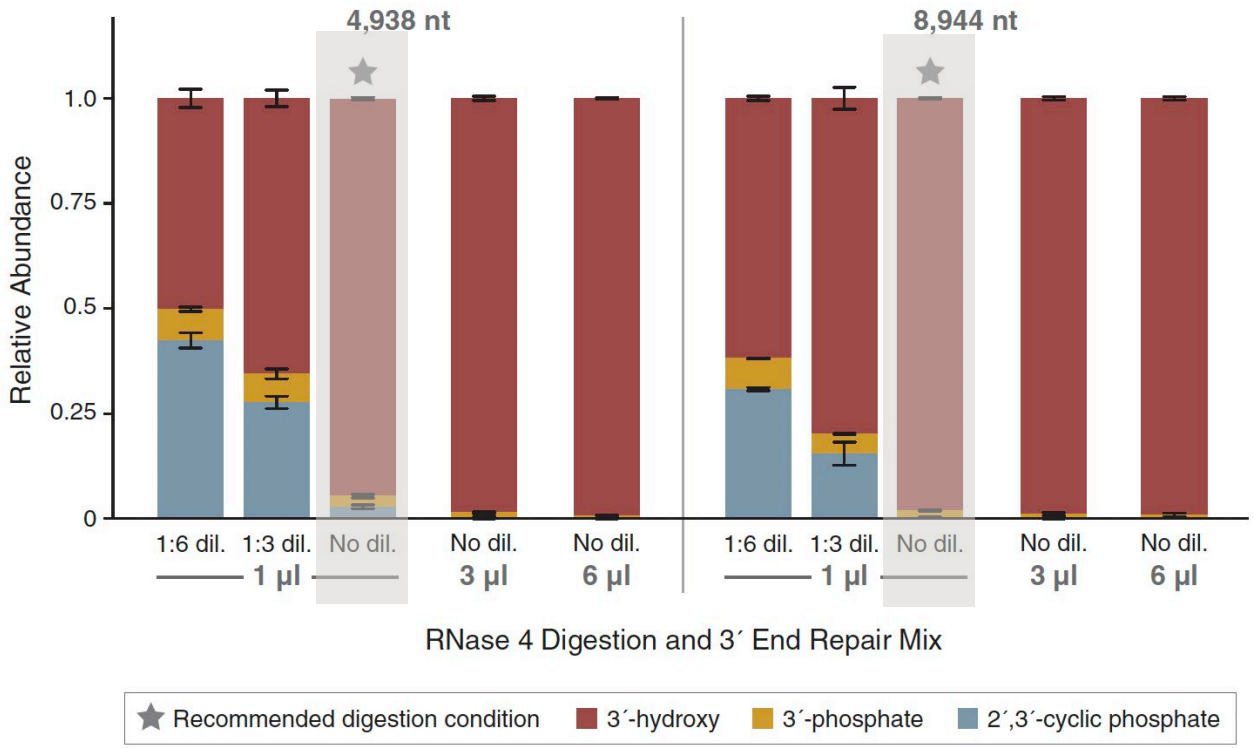
M1288 RNase 4 Digestion & 3′ End Repair Mix Reduces 3′-Heterogeneity of Digestion Products

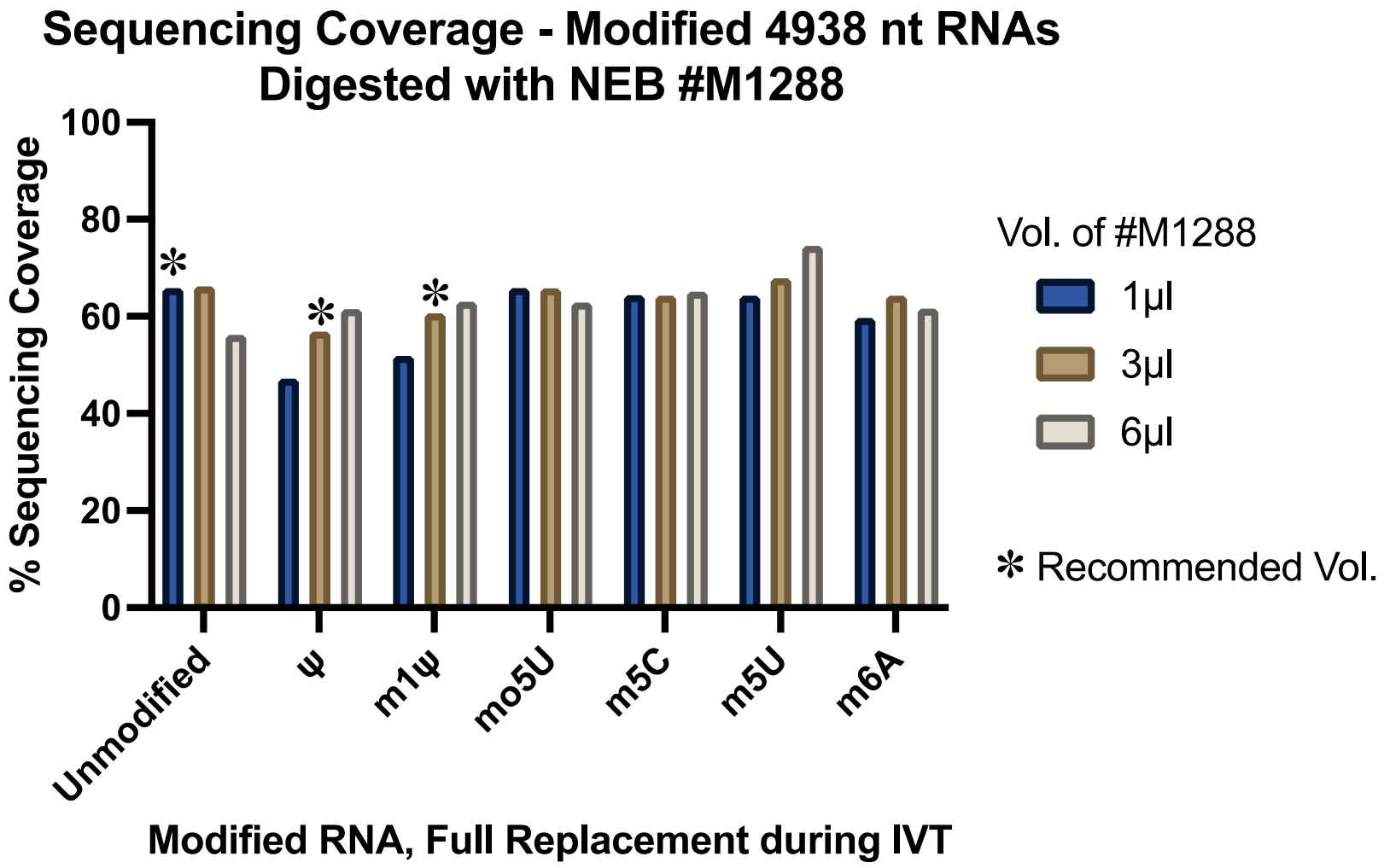


M1288 RNase 4 Digestion & 3′ End Repair Mix Simplifies Spectral Analysis





M1288 RNase 4 Digestion & 3′ End Repair Mix Tolerates Common RNA Modifications



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Targeted RNase 4 Digestion for Poly(A)-Tail Analysis

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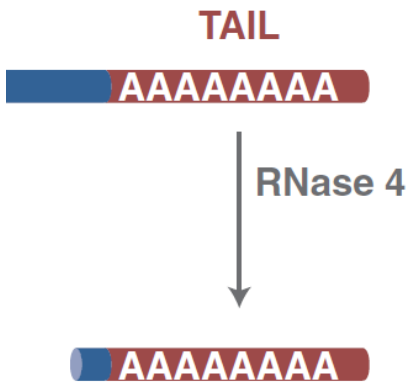
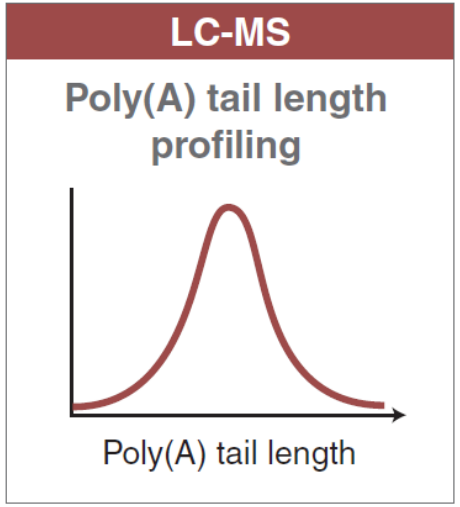
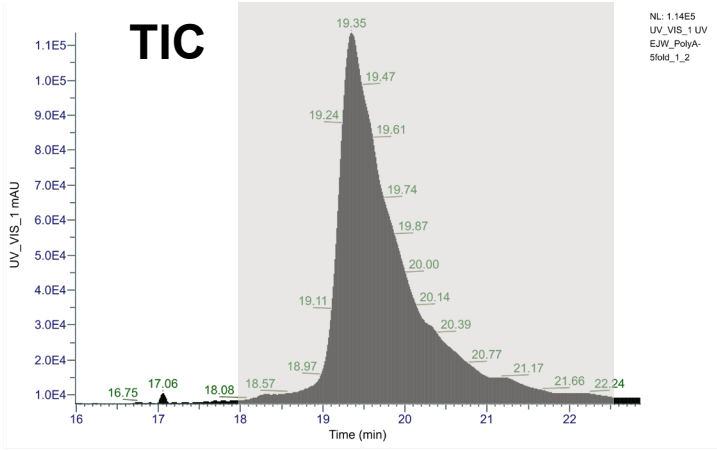
Targeted RNase 4 Digestion for Estimating mRNA Poly(A)-Tail Length

U|AGC-Poly(A)$_{60}$





RT 16-23



Probe-Directed RNase 4 Digestion & Enrichment of mRNA 5′- Ends

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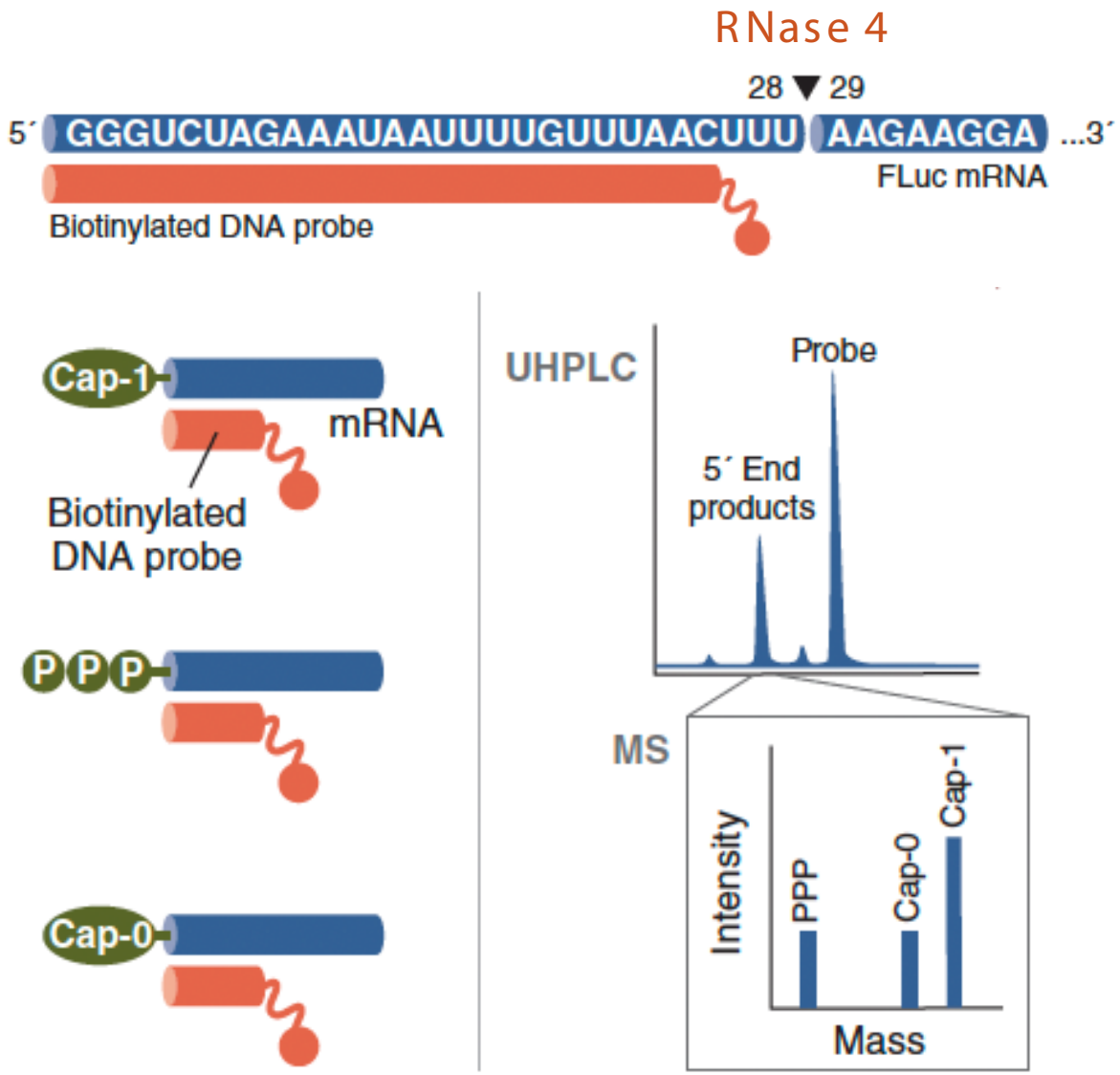
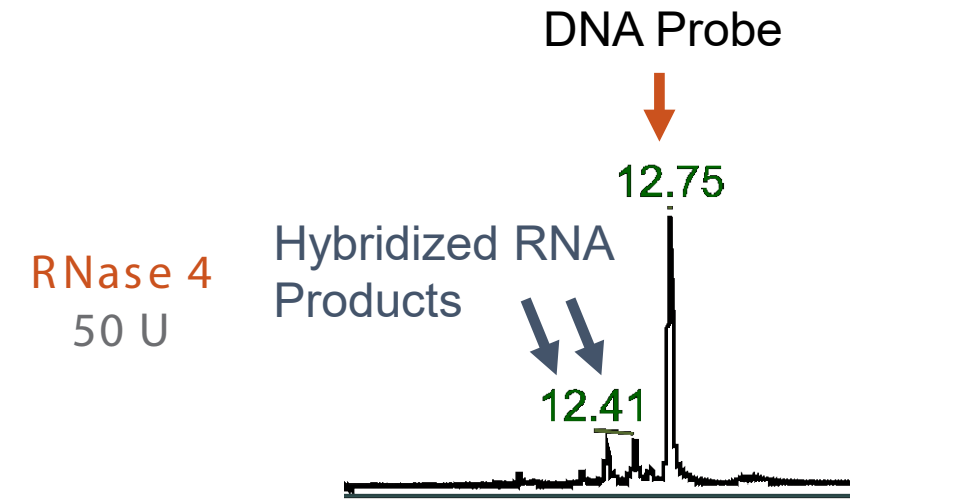
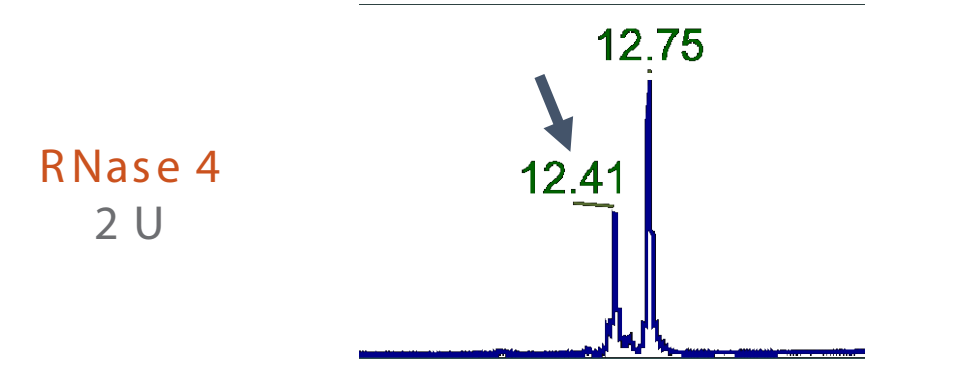
Simplified 5′ Cap Analysis Workflow
Probe-Directed RNase 4 Cleavage & Enrichment







Coming Soon:
RNase 4 Cap Analysis Application Note

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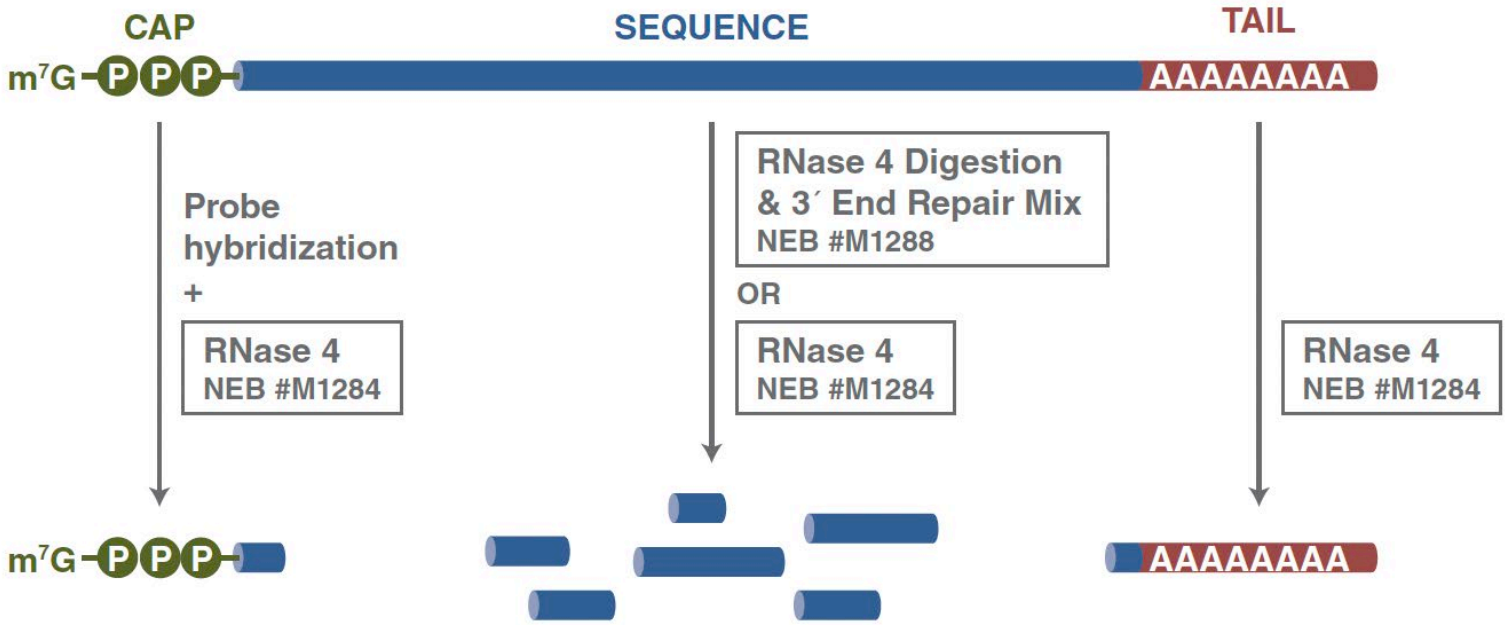
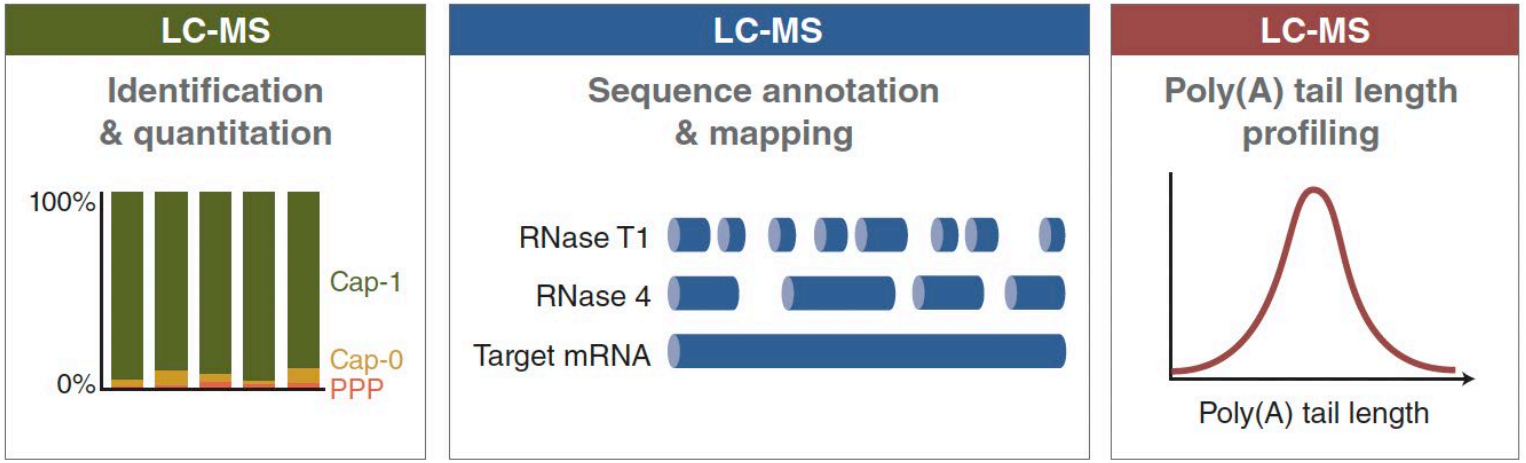
- Glycobiology & Protein Analysis

87 Results

Summary





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Thank You USP Virtual Summit Attendees!

Research
Eric Wolf
Nan Dai
Ivan Correa
Sebastian Gruenberg
Erbay Yigit
Siuhong Chan

Director RNA AppDev
Joe Whipple

Marketing
Tasha Jose
Breton Hornblower

NEB R&D Leadership
Sal Russello
Tom Evans
Nicole Nichols

RNase 4 Product Testimonials

"We tested RNase 4 digestion on a FLuc mRNA from TriLink BioTechnologies® for LCMS/MS fingerprinting applications. Along with the enzyme, a protocol, and a publication on the use of RNAse 4 in this type of application was provided. The protocol was easy to follow and yielded great results. Activity was observed at UA and UG as described in the companion study."
Camila Ortega Ramirez, Ph.D.
Trilink Biotechnologies

"The use of hRNase 4 simplified the sample preparation for subsequent LC-MSMS sequencing of long oligonucleotides (>100 nts). It eased protocols and improved data quality compared to commonly used RNases."
Michael Rühl, Ph.D.
BIOSPRING