

Oligo Workflow Resource Guide

# End-To-End Workflow Solutions for Short Nucleic Acids and mRNA Analysis

From R&D to QC/production



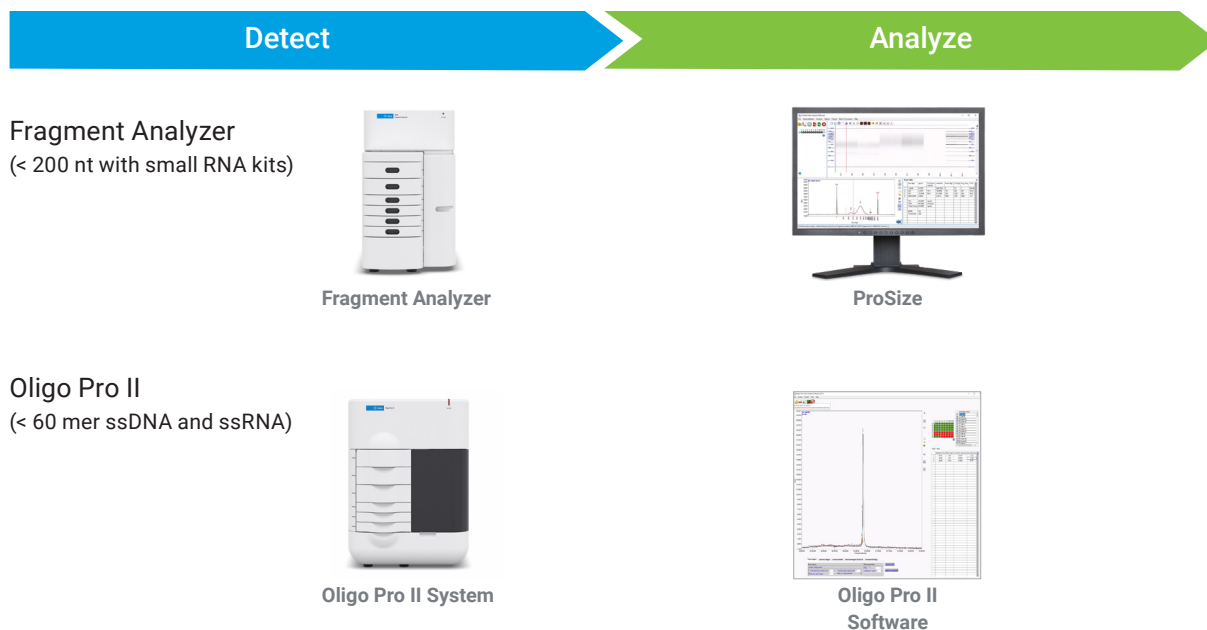
Short synthetic oligonucleotides, such as small interfering RNA, antisense oligonucleotides, aptamers, and CRISPR guides have gained much attention and grown rapidly as a new type of drug in gene therapy. Over the past three years, the success of mRNA vaccines for COVID-19 has generated an explosion of interest in both academia and the pharmaceutical industry. Therefore, there are growing needs and many opportunities for robust analytical QC methods in the development and characterization of these nucleic acids and mRNA vaccine products. Common attributes include size, purity, and melting temperature ( $T_m$ ) of the nucleic acid samples. In addition, the 5'-terminal dinucleotide modification (capping), 3' poly(A) tail of the mRNA, and its sequence are all very critical to the transcription process that should be well-characterized.

### Nucleic Acids (Short) Analysis by Capillary Electrophoresis (CE)

A variety of small (short) RNA types, such as microRNA (miRNA), small-interfering RNA (siRNA) and piwi-interacting RNA (piRNA) are known to be involved in gene silencing, and potentially other mechanisms of gene regulation. Therefore, accurate measurement of the quality of these samples can help ensure the success of downstream applications, and be used to optimize isolation and purification protocols for small RNAs.

The Agilent Fragment Analyzer systems are parallel capillary electrophoresis instruments that are designed for reliable and accurate nucleic acid sample assessment (Figure 1). A broad range of application kits are available. They focus on small RNA and miRNA quality control, concentrating on a narrow range of 200 nucleotides (nt) and below, and allow for high-separation resolution of small RNAs.

The Agilent Oligo Pro II system is an automated parallel CE instrument designed for high-throughput separation and purity analysis of single-stranded DNA (ssDNA) and RNA oligonucleotides. The Oligo Pro II system provides high resolution and direct detection of oligos using a denaturing gel that allows single-stranded nucleic acid samples to migrate through a capillary and separate by size. UV absorption spectroscopy is used for dye-free detection of the sample. The system can accommodate a variety of throughputs with interchangeable arrays, allowing for the simultaneous analysis of 12, 24, or 96 samples. Up to three 96-well plates can be loaded and analyzed without user intervention.



**Workflow 1:** Nucleic acids (short) analysis by capillary electrophoresis.

## mRNA purity analysis by capillary electrophoresis (Agilent Fragment Analyzer and Agilent TapeStation) and UV-Vis (Agilent Cary 3500 UV-Vis)

mRNA produced by in vitro transcription (IVT) can be used in a variety of applications, including vaccines, gene therapies, cancer treatments, treatments for chronic infections, and therapies for autoimmune disorders. Precise and accurate quality control (QC) analysis is a critical part of the IVT mRNA workflow (Figure 2).

The Agilent Fragment Analyzer systems can be used for several QC steps at different checkpoints in mRNA vaccine development, providing accurate and precise sizing and purity analysis. These QC steps are to determine the quality and size of the linearized plasmid, the size and purity of the IVT mRNA, length of the poly(A) tail, and purity of the final mRNA vaccine product.

Similarly, the Agilent TapeStation systems can size PCR-amplified DNA templates and intermediate IVT mRNA products. QC of the DNA template ensures appropriate templates are used in the IVT reaction, saving time and money. The Fragment Analyzer and TapeStation systems can easily switch between DNA and RNA analysis, allowing

for quick and reliable quality checks, and sizing of DNA and IVT mRNA products to help optimize the workflow and ensure a good final product for downstream applications.

UV-Vis spectrophotometers are widely used for nucleic acid quantification and QC using the fact that nucleic acids have a maximum absorbance at 260 nm. As well as determining the concentration of the mRNA sample, a UV-Vis measurement can provide information on whether there are any contaminants in the sample. Because UV-Vis spectrophotometers can do this simply and robustly, they are widely used in QC. The Agilent Cary 3500 Multicell UV-Vis spectrophotometer is ideal for measuring small-volume nucleic acid samples very rapidly and with very high accuracy, as the instrument has a highly focused beam of less than 1.5 mm width and can measure up to seven samples with four independent Peltier blocks for accurate temperature control if needed. Agilent Cary UV Workstation software enables the concentration of the sample to be reported.

## High-throughput mRNA quantification and purity assessments using Agilent BioTek microplate readers

The Agilent BioTek Synergy H1 multimode reader is a modular multimode microplate reader with a variety of detection modes available, including UV/Vis absorbance, fluorescence, and luminescence. This system can measure UV/Vis absorbance from 230 – 999 nm with tunable monochromator-based optics. Measurements for nucleic acid quantification and purity can be taken in UV transparent microplates, as well as the Agilent BioTek Take3 microvolume plate. The Take3 plate enables detection and purity assessment of nucleic acids in

very small volumes (2  $\mu$ L) and cuvettes. Fluorometric measurements of nucleic acids can also be obtained with the Synergy H1 with intercalating nucleic acid dyes. The benefit of a microplate reader format includes measurements at higher throughput (up to 384-well microplates). Microplate-based formats also enable automation of nucleic acid quantification workflows using standard automated microplate loading platforms.

Detect

Analyze

Fragment Analyzer  
(< 9000 nt)



Fragment Analyzer

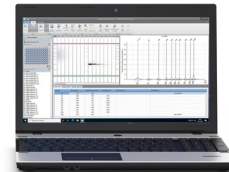


ProSize

TapeStation  
(< 6000 nt)



4200 TapeStation



TapeStation Analysis Software

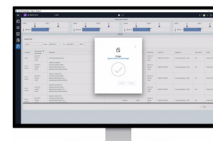
Cary 3500  
(Cuvette based UV)  
or BioTek  
(Microplate-based  
UV and fluorescence)



Cary 3500



Synergy H1  
Multimode Reader



Cary UV  
Workstation



BioTek Gen6  
Software

Workflow 2: mRNA purity analysis by capillary electrophoresis or UV-Vis.

### mRNA 5' capping efficiency and 3' Poly-A sequence workflows

mRNA 3' poly(A) tail and 5'-capping are important for the translation process. The percentage of successfully capped material, the type of capping structure, and the length of a poly(A) tail have all been shown to affect

translation efficiency and the rate of mRNA degradation. Therefore, the 5' capping and 3' poly(A) sequences are critical quality attributes (CQAs) that should be thoroughly characterized and monitored (Figure 3).



Figure 3. Structure of a typical human protein coding mRNA.

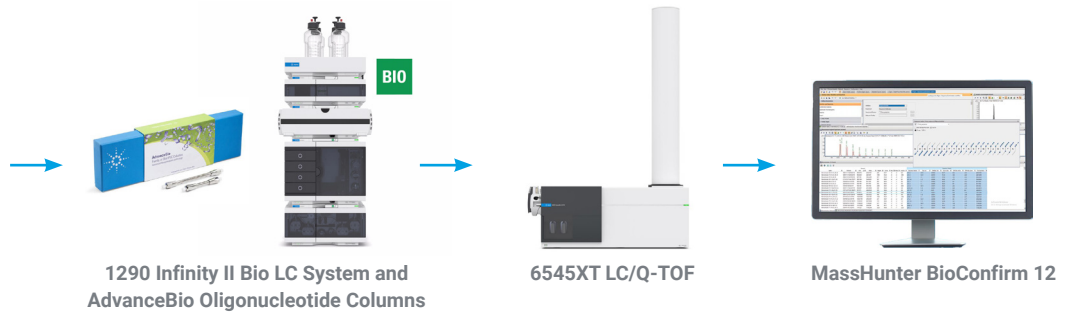


5' Capped  
Oligonucleotides  
LC/MS

- Site-directed RNase-H cleavage
- Sample cleanup

3' Poly-A sequences  
LC/MS

- RNase T1 digestion
- Poly-A sample enrichment by oligo-dT magnetic beads



Fragment Analyzer

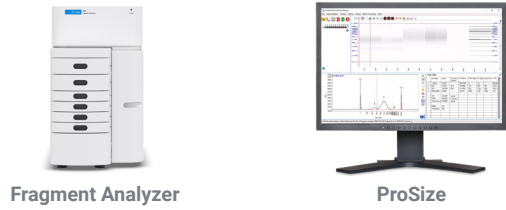


Figure 4. Workflow 3: mRNA 5' capping efficiency and 3' Poly-A sequence workflows.



## References

### Short Nucleic Acids Analysis by CE

- 1) Comparison of Small RNA Analysis using the Agilent Bioanalyzer and Agilent Fragment Analyzer Systems. Agilent Technologies technical overview, publication number [5994-4860EN](#), 2022.
- 2) Performance Characteristics of the Agilent Oligo Pro II System. Agilent Technologies technical overview, publication number [5994-3667EN](#), 2021.
- 3) Nucleic Acid Analysis for Sample Quality Assessment Using the Agilent Fragment Analyzer Systems. Agilent Technologies application compendium, publication number [5994-2813EN](#), 2022.
- 4) Single-Guide RNA Quality Assessment with the Agilent 5200 Fragment Analyzer System. Agilent Technologies application note, publication number [5994-0523EN](#), 2019.
- 5) Accurate Assessment of Oligonucleotide Purity. Agilent Technologies brochure, publication number [5994-0421EN](#), 2021.

### mRNA Purity Analysis




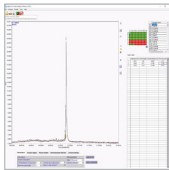
- 1) Best Practices for Analysis of IVT mRNA Using the Agilent Fragment Analyzer Systems. Agilent Technologies technical overview, publication number [5994-6568EN](#), 2023.
- 2) Best Practices for Analysis of In Vitro Transcribed (IVT) mRNA Using the Agilent Fragment Analyzer Systems. Agilent Technologies technical overview, publication number [5994-5927EN](#), 2023.
- 3) Quality Control in IVT RNA Workflow using Agilent TapeStation Systems. Agilent Technologies technical overview, publication number [5994-4882EN](#), 2022.
- 4) Best Practice for Nucleic Acid Thermal Stability Measurements Using the Cary 3500 UV-Vis Spectrophotometer. Agilent Technologies white paper, publication number [5994-4028EN](#), 2022.
- 5) Assessment of Long IVT mRNA Fragments with the Agilent 5200 Fragment Analyzer system. Agilent Technologies application note, publication number [5994-0878EN](#), 2019.
- 6) Benefits of Quality Control in the IVT RNA Workflow Using the Agilent 5200 Fragment Analyzer System. Agilent Technologies application note, publication number [5994-0512EN](#), 2023.
- 7) TekTalk Newsletter: [Nucleic Acid Quantification](#)

### mRNA 5' Capping Efficiency and 3' Poly-A Sequence workflow








- 1) Best Practices for Analysis of In Vitro Transcribed (IVT) mRNA Using the Agilent Fragment Analyzer Systems. Agilent Technologies technical overview, publication number [5994-5927EN](#), 2023.
- 2) Analyzing Poly(A) Tails of In Vitro Transcribed RNA with the Agilent Fragment Analyzer System. Agilent Technologies application note, publication number [5994-5325EN](#), 2022.
- 3) Rapid Analysis of mRNA 5' Capping with High Resolution LC/MS. Agilent Technologies application note, publication number [5991-3984EN](#), 2021.
- 4) Analysis of mRNA Poly-A Sequence Variants by High-Resolution LC/MS. Agilent Technologies application note, publication number [5994-3005EN](#), 2021.

## Optimized workflows: configuration and ordering information






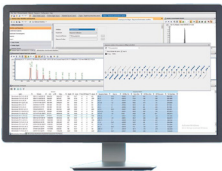

### Workflow 1: Short nucleic acids analysis by capillary electrophoresis

Bundle	Fragment Analyzer		Oligo Pro II	
Instrument		<p>5400 Fragment Analyzer (M5312AA)</p> <p>5300 Fragment Analyzer (M5311AA)</p> <p>5200 Fragment Analyzer (M5310AA)</p>		<p>Oligo Pro II (M5340AA)</p> <p>Oligo Pro II 12-Capillary Array Long (A2500-1275-5580)</p> <p>Oligo Pro II 24-Capillary Array Long (A2500-2475-5580)</p> <p>Oligo Pro II 96-Capillary Array Long (A2500-9675-5580)</p>
Software		ProSize		Oligo Pro II Software
Reagents	Small RNA Kit	DNF-470-0275		<p>Capillary Conditioning Solution (DN-475-1000)</p> <p>Oligo ssDNA Gel (DN-415-0250)</p> <p>ssDNA Oligel Buffer (DN-465-1000)</p>

### Workflow 2: mRNA purity analysis

Bundle	Fragment Analyzer	TapeStation	Cary 3500 (UV-Vis) (Cuvette based UV)	BioTek (Microplate-based UV and Fluorescence)
Instrument	 <p>5400 Fragment Analyzer (M5312AA)</p> <p>5300 Fragment Analyzer (M5311AA)</p> <p>5200 Fragment Analyzer (M5310AA)</p>	 <p>4200 TapeStation (G2991BA)</p>	 <p>Cary 3500 Multicell UV-Vis Spectrophotometer (G9874A #001)</p> <p>Option: G9874A #002: Purge kit</p>	<p>BioTek Take3 Plate (TAKE3-SN)</p> <p>BioTek Synergy H1 Multimode Reader (UV-Vis and Fluorescence) (SH11M-SN)</p> <p>Option: BioTek Epoch 2 Microplate Spectrophotometer (UV-Vis) (EPOCH2NS-SN)</p>
Software	 <p>ProSize</p>	 <p>TapeStation Analysis Software</p>	 <p>Cary UV Workstation Software</p> <p>G5194AA: Cary UV Workstation Plus w/o PC</p> <p>G5195AA: Cary UV Workstation Plus with PC</p> <p>G6894AA: Cary UV Networked Workstation software w/o PC</p> <p>G6896AA: Cary UV Networked Workstation software with PC</p>	 <p>BioTek Gen6 Software (GEN6)</p>
Reagents	RNA Kit (15 nt)	<p>RNA ScreenTape 5067-5576</p> <p>RNA ScreenTape Sample Buffer 5067-5577</p> <p>RNA ScreenTape Ladder 5067-5578</p>		

### Workflow 3: mRNA 5' capping efficiency and 3' Poly-A sequence workflows

Bundle	LC/Q-TOF (5' Capping)	LC/Q-TOF (3' Poly-A)	Fragment Analyzer (3' Poly-A)
Column	 <p><b>AdvanceBio Oligonucleotide</b> (659750-702)</p>	 <p><b>InfinityLab Poroshell 120 HPH-C18</b> (699675-702) <b>Agilent PLRP-S</b> (PL1912-1502)</p>	
LC	 <p><b>1290 Infinity II BioLC</b> Required: G7131A or G7123A, G7137A, and G7116B Optional: G7114B (VWD) or G7117B (DAD)</p>		
MS	 <p><b>6545XT AdvanceBio LC/Q-TOF</b> (G6549AA)</p>		<p><b>5400 Fragment Analyzer</b> (M5312AA) <b>5300 Fragment Analyzer</b> (M5311AA) <b>5200 Fragment Analyzer</b> (M5310AA)</p>
Software	 <p><b>BioConfirm 12</b> (M6025AA) Single workstation: M6026AA or M6027AA Network workstation: M6025AA or M6026AA or M6027AA</p>		 <p><b>ProSize</b></p>



Learn more:

[www.agilent.com/oligonucleotides](http://www.agilent.com/oligonucleotides)

Buy online:

[www.agilent.com/chem/store](http://www.agilent.com/chem/store)

Get answers to your technical questions and access resources in the Agilent Community:

[community.agilent.com](http://community.agilent.com)

U.S. and Canada

**1-800-227-9770**

[agilent\\_inquiries@agilent.com](mailto:agilent_inquiries@agilent.com)

Europe

[info\\_agilent@agilent.com](mailto:info_agilent@agilent.com)

Asia Pacific

[inquiry\\_lsca@agilent.com](mailto:inquiry_lsca@agilent.com)

DE40332016

This information is subject to change without notice.

© Agilent Technologies, Inc. 2023, 2024  
Published in the USA, January 23, 2024  
5994-6933EN

