Technical Overview

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Quality Control in IVT RNA Workflow using Agilent TapeStation Systems

Introduction

Precise and accurate quality control (QC) analysis is a critical part of the in vitro transcription (IVT) RNA workflow. RNA produced by IVT can be used in a variety of applications including vaccines, gene therapies, cancer treatments, treatments for chronic infections, and therapies for autoimmune disorders. IVT RNA workflows (Figure 1) begin with the initial genetic starting material, which can include linearized DNA plasmids, PCR amplified DNA, or cDNA. After purification of the DNA template, the IVT reaction generates RNA, which is then purified to achieve the final product. QC of the DNA template and final RNA product is critical for IVT workflows.

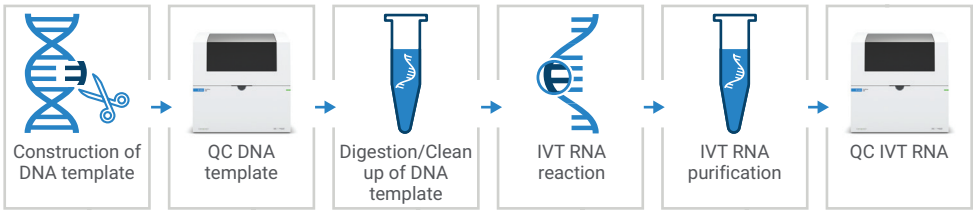

Figure 1. IVT RNA workflow with QC steps where the Agilent TapeStation systems can be used.

Accurate sizing and QC of the DNA assists in verifying amplification of the target DNA, verifies complete linearization of DNA plasmids, and confirms purity. Templates containing DNA from regions outside of the intended transcript interfere with transcription of the target RNA. Additionally, DNA templates with unexpected digestion can produce incomplete RNA products. Ensuring sizing and quality of the final IVT RNA product provides assurance that the IVT RNA is suitable for downstream use. Poor RNA transcription, or contamination and degradation of the final product, impairs the potential therapeutic application of the RNA. It is therefore essential to assess DNA transcripts and final IVT RNA for both size and purity.

The Agilent automated electrophoresis systems, including the TapeStation and Fragment Analyzer systems,[1] can be used for QC and size analysis during the IVT RNA workflow. This technical overview discusses the use of the TapeStation in IVT RNA workflows. The TapeStation system easily switches between DNA and RNA analysis, allowing for quick and reliable quality checks and sizing of DNA and IVT RNA products to help optimize the workflow and ensure a good final product for downstream applications.

Methods

The experiments in this study were performed using the Agilent 4200 TapeStation system (p/n G2991BA), and can be replicated on the Agilent 4150 TapeStation system (p/n G2992AA). DNA templates were prepared from PCR amplification of Lambda DNA with Phusion High-Fidelity DNA polymerase (Thermo Fisher Scientific p/n F530S) and standard protocols. The DNA templates were analyzed on the TapeStation instrument with the Agilent D5000 ScreenTape (p/n 5067-5588) and Agilent D5000 reagents (p/n 5067-5589).

Three IVT RNA samples were generated using Promega T7 RiboMAX Express (Promega p/n P1320). Sample 1, a single IVT control included with the T7 RiboMAX Express kit, yields two fragments with expected sizes of 1,065 nt and 2,346 nt in length. Samples 2 and 3, generated from PCR amplified DNA templates, yield RNA with the expected sizes of 2,055 nt and 4,053 nt, respectively. The IVT RNA was diluted with nuclease-free water, and analyzed on the TapeStation instrument with the Agilent RNA ScreenTape (p/n 5067-5576), Agilent RNA ScreenTape Sample Buffer (p/n 5067-5577), and Agilent RNA ScreenTape Ladder (p/n 5067-5578).

Results and discussion

Assessment of DNA template

DNA templates were assessed for size and purity with the D5000 ScreenTape and reagents. Figure 2 shows the electropherograms of the DNA templates. Sample 1, the control provided in the T7 RiboMAX Express kit, has an estimated size of 4,200 bp. Samples 2 and 3 were generated from PCR amplification of Lambda DNA. Sample 2 has an expected size of 2,073 bp, and sample 3 has an expected size of 4,071 bp. Representative electropherograms from each sample are shown in Figure 2. In all cases, the electropherogram displays a single peak very close to the expected size. The samples were analyzed for both accuracy and precision. Sizing accuracy for each sample was determined by calculating percent error based on the expected size. The precision of each sample was measured by evaluating the percent coefficient of variation (CV). All samples displayed a low percent error (less than 10%) and CV (less than 1%) (Table 1). The single peak and low percent error of each sample indicates minimal off-target amplification. Precise and accurate QC of the DNA templates ensures the samples are ready to move to the next step in the IVT workflow.

Table 1. Analysis of DNA templates using the Agilent D5000 ScreenTape assay. The expected size, measured size, percent error, standard deviation, and percent CV are shown. n=3 for each sample.

Sample ID	DNA Template Expected Size (bp)	Measured Size (bp)	% Error	Standard Deviation	%CV
1	4,200	4,275	1.79%	7.35	0.17%
2	2,073	2,133	2.91%	4.71	0.22%
3	4,071	4,423	8.63%	3.50	0.08%

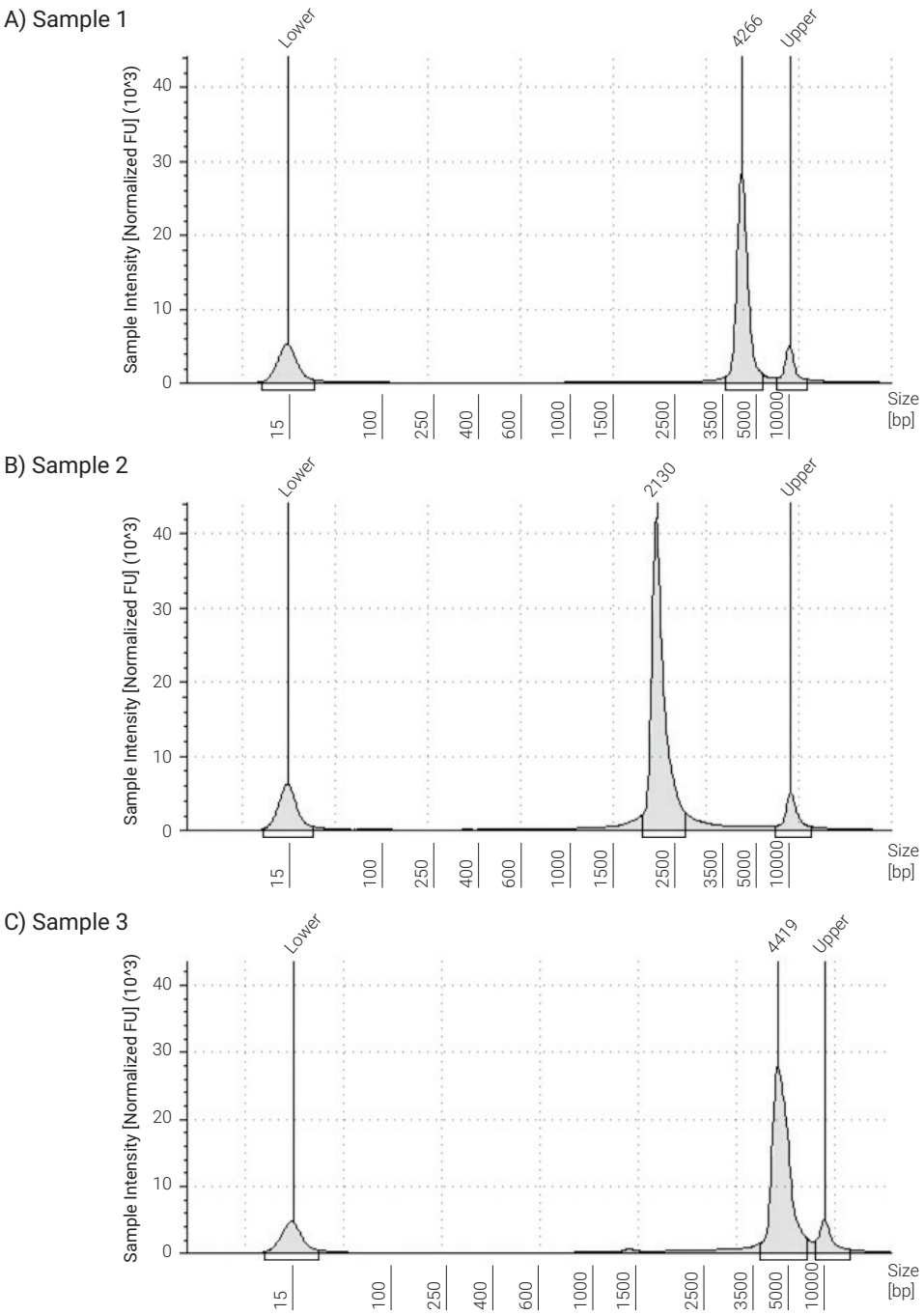

Figure 2. DNA templates of (A) sample 1, (B) sample 2, and (C) sample 3 were analyzed on the Agilent 4200 TapeStation system with the Agilent D5000 assay. Example electropherograms of the DNA templates analyzed at a concentration of approximately 40 ng/µL are shown.

Assessment of IVT RNA

The DNA templates were used to generate three IVT RNA samples. Sample 1, the positive control, produces two fragments with expected sizes of 1,065 nt and 2,346 nt. Samples 2 and 3 were generated from PCR-amplified DNA templates, and have fragments with expected sizes of 2,055 nt and 4,053 nt, respectively. Each sample was analyzed for size and purity on the TapeStation system with the RNA ScreenTape assay. A representative electropherogram for each sample is shown in Figure 3. All samples contain clearly defined peaks, which correspond to the expected sizes of IVT RNA.

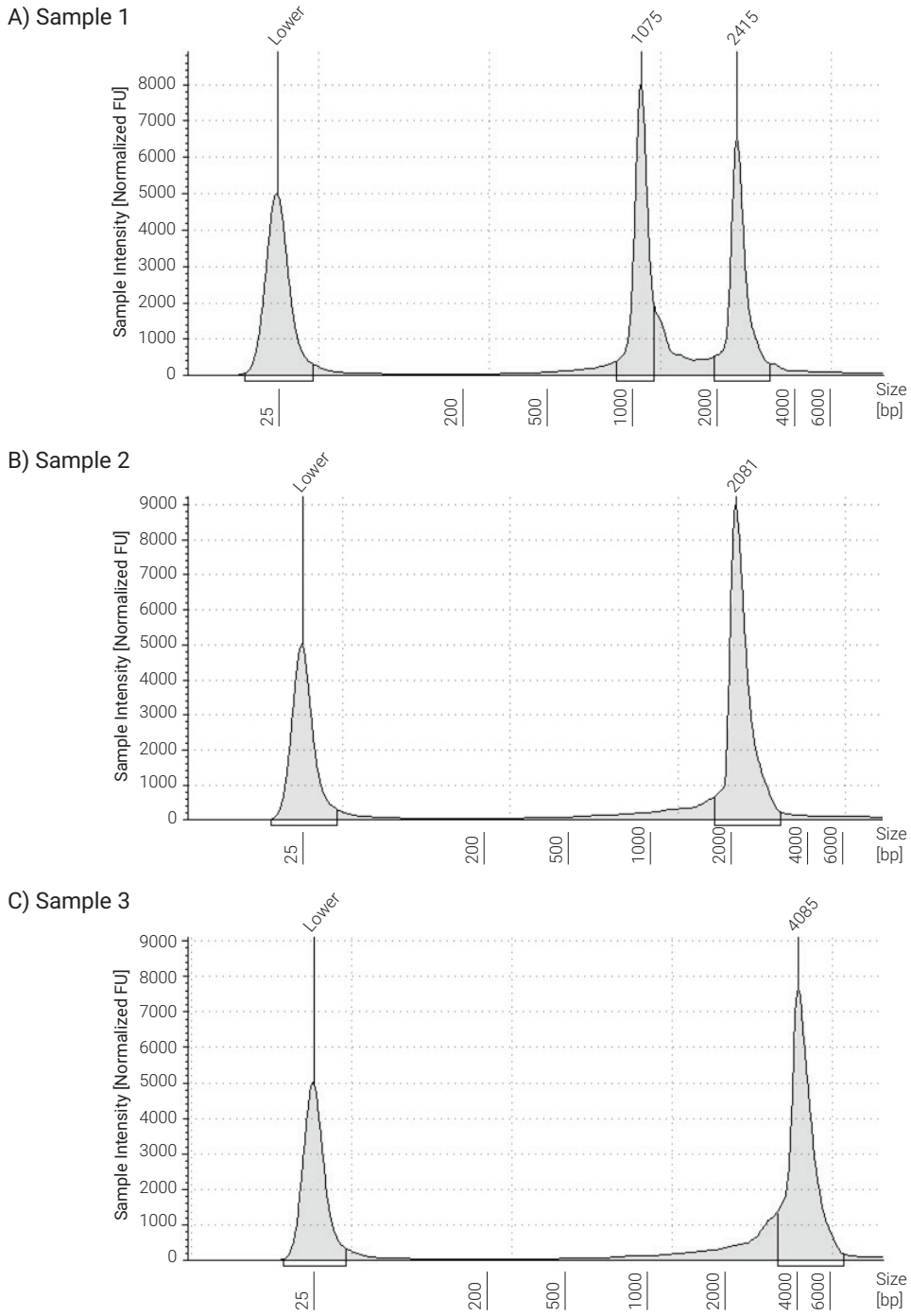

Figure 3. IVT RNA samples were analyzed on the Agilent 4200 TapeStation system with the Agilent RNA ScreenTape assay. Approximately 100 ng/µL of RNA for each sample was analyzed. The electropherogram from (A) sample 1 displays two peaks, as expected. The electropherograms from (B) sample 2 and (C) sample 3 each display the expected single peak.

For each sample, two concentrations of approximately 100 ng/µL and 50 ng/µL were analyzed for accuracy (percent error) and precision (%CV). In all cases, the samples displayed low percent error (less than 20%) and %CV (less than 5%), indicating the TapeStation instrument and RNA ScreenTape measurements for IVT RNA are accurate and precise (Table 2). The concentrations tested do not impact the sizing, as shown in the overlay of electropherograms for sample 1 (Figure 4).

Table 2. Accuracy and precision of IVT RNA samples. Approximately 100 ng/µL and 50 ng/µL of samples 1-3 were analyzed on the Agilent TapeStation system with the RNA ScreenTape assay. For all samples, n=3.

		100 ng/µL			50 ng/µL		
Sample	Theoretical Size (nt)	Average Measured Size (nt)	% Error	%CV	Average Measured Size (nt)	% Error	%CV
1	1,065	1,092	2.54%	2.09%	1,104	3.66%	2.12%
	2,346	2,466	5.12%	1.54%	2,502	6.65%	2.20%
2	2,055	2,123	3.31%	2.21%	2,154	4.82%	2.94%
3	4,053	4,085	0.79%	4.28%	4,392	8.36%	1.73%

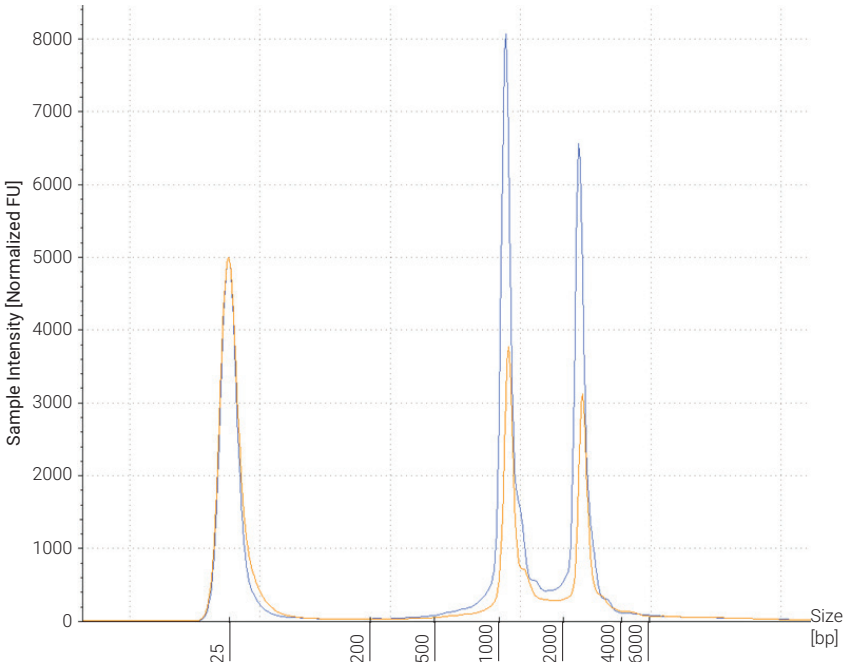

Figure 4. Overlay of the IVT RNA fragments analyzed on the Agilent 4200 TapeStation system with the Agilent RNA ScreenTape assay. IVT RNA fragments from sample 1, with expected sizes of 1,065 and 2,346 nt, were analyzed from dilutions of approximately 100 ng/µL (blue) and 50 ng/µL (yellow).

Summary

The Agilent 4200 TapeStation system with the Agilent D5000 ScreenTape and Agilent RNA ScreenTape assays provides accurate and precise sizing for PCR-amplified DNA templates and final IVT RNA products. The TapeStation software automatically provides peak size, which assists the user in ensuring the accuracy of both the DNA templates and final IVT RNA products. QC of the DNA template ensures appropriate templates are used in the IVT reaction, saving time and money. The TapeStation system and ScreenTape assays assist researchers in verifying that the final IVT RNA is suitable for the intended downstream applications.

References

1. Benefits of Quality Control in the IVT RNA workflow using the Agilent 5200 Fragment Analyzer System. *Agilent Technologies application note*, publication number 5994-0512EN, **2019**.

www.agilent.com/genomics/tapestation

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