mRNA vaccine production: ensure quality control with the NanoDrop Spectrophotometers

Introduction

In the height of the SARS-CoV-2 pandemic, messenger RNA (mRNA) vaccines became increasingly popular in the fight to end the pandemic due to their safety, efficacy, and rapid production capabilities.[1] mRNA vaccines function by training cells to produce a foreign protein that is sufficient to yield an immune response. The subsequent immune response triggers the development of antibodies specific to the target protein, killing pathogenic viruses as they enter the body in the future.[2] In this note, the use of the Thermo Scientific™ NanoDrop™ One/One^{C} Microvolume UV-Vis Spectrophotometer or the NanoDrop Eight Spectrophotometer in the mRNA vaccine manufacturing protocols as a quality control checkpoint was investigated.

Sequencing

The early production of mRNA vaccines begins with an extraction of nucleic acids and preparation for sequencing. To successfully sequence nucleic acids, it is crucial that the starting material is a certain quality and quantity.[3] The Genomics Core Facility at the Pennsylvania State University requests total RNA for sequencing that is free of contaminants as any foreign material can cause "variance…in the transcript profile." With the large number of nucleic acid extraction kits and protocols available, the concentration yield and purity can vary with each kit or protocol. Implementing the NanoDrop One/One^{C} Spectrophotometer or the NanoDrop Eight Spectrophotometer after the nucleic acid extraction step can serve as a quality control checkpoint prior to sequencing.

The Thermo Scientific™ Acclaro™ Sample Intelligence Technology, which is built into the NanoDrop One/One^{C} instrument and the NanoDrop Eight instrument software, serves as an important factor in quality control. Historically, nucleic acid purity ratios, A260/A280 and A260/A230, served as the method for quantifying purity. However, for traditional UV-Vis spectroscopic measurements, any material that absorbs at the analysis wavelength will contribute to the overall absorbance and concentration calculations. For example, contaminating dsDNA in an RNA preparation will co-absorb as both nucleic acids absorb at 260 nm, thus overestimating the concentration. To solve this drawback, the Acclaro technology utilizes chemometric algorithms to distinguish RNA and dsDNA as well as common extraction reagent materials such as phenol and chaotropic agents. The Acclaro technology provides an advantage to users in the sequencing workflow because contaminated RNA will interfere with the library production.[3] Once the genome has been sequenced successfully, a vaccine target is determined and plasmid production proceeds.

Vaccine production workflow

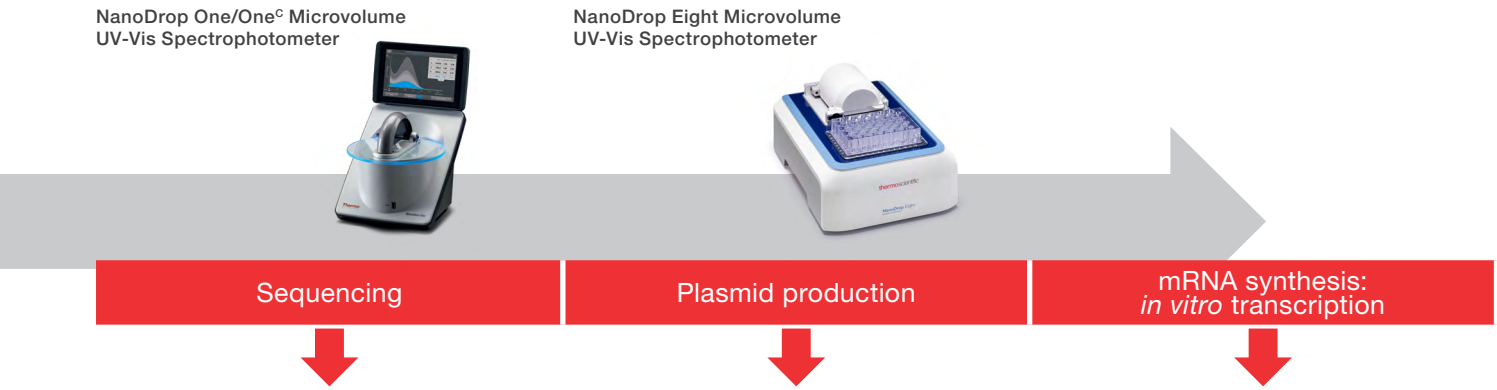

Figure 1: mRNA vaccine workflow displaying the integration of the NanoDrop One/One^{C} Spectrophotometer or the NanoDrop Eight Spectrophotometer as quality control checkpoints.

Plasmid production

After completion of the sequencing process, the recombinant plasmid DNA is constructed by inserting the target gene in the plasmid via restriction endonucleases and a DNA ligase. The recombinant plasmid is transformed into *Escherichia coli* (*E. coli*), where the bacterial cells are then picked based on antibiotic resistance selection or colony color screening.[4] After the plasmid-containing colonies are selected and grown in a medium, the OD600 of the culture is measured on a NanoDrop Spectrophotometer to determine whether the density is appropriate for plasmid purification, typically 2.0–4.0 OD at 600 nm depending on the purification kit.

The plasmid is next purified from the cell culture with a kit to remove genomic DNA or other contaminants. Contaminating salts or proteins will affect downstream success, causing experiments to fail and forcing the user to troubleshoot, which is time consuming and costly. To mitigate downstream troubleshooting of failed experiments, the purified plasmid is measured on the NanoDrop One/One^{C} Spectrophotometer or the NanoDrop Eight Spectrophotometer, where the Acclaro technology algorithms can identify residual contaminants post-purification. The additional quality control measures for plasmid production can be easily implemented with the NanoDrop Spectrophotometers to provide full spectral data in fifteen seconds or less, depending on the instrument model.

mRNA synthesis: *In vitro* transcription

When the recombinant plasmid is determined to be pure from measuring on a NanoDrop Spectrophotometer, the plasmid is linearized and the polymerase begins transcription ahead of the gene of interest. The polymerase synthesizes mRNA from the DNA template and the mRNA is capped to ensure efficient translation to protein.[5] When the mRNA synthesis is complete, the DNA is degraded with DNase, and the mRNA undergoes a final purification to remove contaminating transcription materials.[1] As a final quality control checkpoint with the NanoDrop Spectrophotometers, the purified mRNA is checked for purity before the mRNA solution is filled into the vaccine vials.

The Acclaro technology available on the NanoDrop One/One^{C} Spectrophotometer and the NanoDrop Eight Spectrophotometer provides an enhanced quality control checkpoint post-synthesis. It is crucial to determine purity of the final mRNA as contamination can reduce the vaccine potency via protein translation inhibition.[1] The contaminant identification and helpful onboard troubleshooting features of the Acclaro technology eliminate the guesswork of determining purity from the A260/A280 and A260/A230 ratios and ensures a pure final mRNA product.

Conclusion

The NanoDrop One/One^{C} Spectrophotometer and the NanoDrop Eight Spectrophotometer enhance the quality control mechanisms of mRNA vaccine production from start to finish. The automated pathlength feature of all NanoDrop Spectrophotometers allows the user to measure an increased concentration range in 1–2 µL without the need for diluting samples. Along with the microvolume measurements, the NanoDrop instruments provide full spectral data in 15 seconds or less, depending on the instrument model.

With software available for the NanoDrop One/One^{C} Spectrophotometer and the NanoDrop Eight Spectrophotometer that allows users to comply with 21 CFR Part 11, the instruments are ready to be implemented into the mRNA vaccine workflow at a GMP laboratory. The addition of the Acclaro technology built into the software allows the user to identify contaminants and provides recommended troubleshooting steps, saving overall cost and time from repeating failed experiments. With the increased interest in mRNA vaccines, the NanoDrop Spectrophotometers provide a quick and simple method for improving quality control.

References

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