accurate instrument reading. This can be problematic if the sample is highly dilute, or if an extraction technique requires the elution of nucleic acids into low volumes. Contamination is also a problem. For example, if measuring DNA, biological molecules such as proteins, RNA, and chaotropic salts from extraction procedures, can falsely elevate nucleic acid concentration estimations. Buffer salts such as Tris, EDTA, and guanidine isothiocyanate absorb strongly at 230 nm and bleed into the 260-nm absorbance range. In addition, free nucleotides present in a sample may influence the UV quantitation method. Changes in sample pH also alter UV readings. An alternative to the spectrometer is adding a fluorescent probe and measuring it in a fluorimeter which requires further handling of the RNA/DNA sample and labeling such molecules with fluorescent molecules, increasing the cost and complexity. All of these approaches have major shortcomings and may require manual intervention and the output of the measurement that is not tied into the microfluidic control of the system.

[0005] Described herein are apparatuses, and in particular, microfluidic apparatuses, that can address these shortcomings.

SUMMARY

[0005a] In one aspect, provided herein is a method for manufacturing a polynucleotide using a microfluidic driver apparatus operating on a cartridge, the method comprising: forming, in a cartridge, a therapeutic polynucleotide, and determining, by the microfluidic driver apparatus, a concentration of the therapeutic polynucleotide by: driving a first blank solution into an ultraviolet (UV) measurement chamber of the cartridge and taking a first absorption measurement through the cartridge, driving a sample solution of the therapeutic polynucleotide into the UV measurement chamber of the cartridge and taking a second absorption measurement through the cartridge, and estimating a concentration of the therapeutic polynucleotide from the first and second absorption measurements.

[0005b] In another aspect, provided herein is a method for manufacturing a polynucleotide using a microfluidic driver apparatus operating on a cartridge, the method comprising: forming, in a cartridge, a therapeutic polynucleotide, wherein the therapeutic polynucleotide comprises a therapeutic mRNA; determining, by the microfluidic driver apparatus, a concentration of the therapeutic mRNA by: driving a first blank solution into an ultraviolet (UV) measurement chamber of the cartridge and taking a first absorption measurement through the cartridge, driving

a sample solution of the therapeutic polynucleotide into the UV measurement chamber of the cartridge and taking a second absorption measurement through the cartridge, driving a second blank solution into the UV measurement chamber of the cartridge and taking a third absorption measurement through the cartridge, and estimating a concentration of the therapeutic polynucleotide from the first, second and third absorption measurements; and adjusting, in a processor of the microfluidic driver apparatus, the operation of the microfluidic driver apparatus based on the estimated concentration of the therapeutic polynucleotide.

[0005c] In another aspect, provided herein is a system for making and/or processing a polynucleotide, the system comprising: a cartridge mount; a plurality of pressure lines; a plurality of fluidic lines, each coupled with or configured to couple with a fluid source, wherein each fluidic line and at least a subset of the pressure lines are configured to be secured to inlet or outlet ports on a cartridge held in the cartridge mount; an ultraviolet (UV) light source; a UV light detector; and a controller configured to control application of pressure through the pressure lines to drive fluid through the cartridge, wherein the controller is further configured to direct the formation of a therapeutic polynucleotide in the cartridge, and to estimate a concentration of the therapeutic polynucleotide by driving a first blank solution through a UV measurement chamber of the cartridge and taking a first absorption measurement using the UV light source and UV light receiver, driving a sample solution including the therapeutic polynucleotide through the UV measurement chamber of the cartridge and taking a second absorption measurement using the UV light source and UV light receiver, further wherein the controller is configured to estimate the concertation of the therapeutic polynucleotide from the first and second absorption measurement.

[0005d] In another aspect, provided herein is a system for making and/or processing a polynucleotide, the system comprising: a cartridge; and a microfluidic driver apparatus comprising: a cartridge mount; a plurality of pressure lines; a plurality of fluidic lines, each coupled with or configured to couple with a fluid source, wherein each fluidic line and at least a subset of the pressure lines are configured to be secured to inlet or outlet ports on a cartridge held in the cartridge mount; an ultraviolet (UV) light source; a UV light detector; and a controller configured to control application of pressure through the pressure lines to drive fluid through the cartridge, wherein the controller is further configured to direct the formation of a therapeutic polynucleotide, further wherein the controller is configured to estimate a

concentration of the therapeutic polynucleotide by driving a first blank solution through a UV measurement chamber of the cartridge and taking a first absorption measurement using the UV light source and UV light receiver, driving a sample solution including the therapeutic polynucleotide through the UV measurement chamber of the cartridge and taking a second absorption measurement using the UV light source and UV light receiver, and driving a second blank solution though the UV measurement chamber of the cartridge and taking a third absorption measurement using the UV light source and UV light receiver; modifying operation of the microfluidic driver apparatus based on the concertation of the therapeutic polynucleotide determined from the first, second and third absorption measurement.

[0005e] In yet another aspect, provided herein is a method for manufacturing a polynucleotide using a microfluidic driver apparatus operating on a cartridge, the method comprising: forming, in a cartridge, a therapeutic polynucleotide; and determining, by the microfluidic driver apparatus, a concentration of the therapeutic polynucleotide by: driving a first blank solution into a concentration measurement chamber of the cartridge and taking a first measurement through the cartridge, driving a sample solution of the therapeutic polynucleotide into the concentration measurement chamber of the cartridge and taking a second measurement through the cartridge, and estimating a concentration of the therapeutic polynucleotide from the first and second measurements; and comparing, by a processor of the microfluidic driver apparatus, the estimated concentration of the therapeutic polynucleotide to a concentration range, and based on this comparison, directing the therapeutic polynucleotide for one of: pooling with one or more additional batches of therapeutic polynucleotide formed in the cartridge, waste, or further analysis.

[0005f] In yet another aspect, provided herein is a system for making and/or processing a polynucleotide, the system comprising: a cartridge mount; a plurality of pressure lines; a plurality of fluidic lines, each coupled with or configured to couple with a fluid source, wherein each fluidic line and at least a subset of the pressure lines are configured to be secured to inlet or outlet ports on a cartridge held in the cartridge mount; and a controller configured to control application of pressure through the pressure lines to drive fluid through the cartridge, wherein the controller is further configured to direct the formation of a therapeutic polynucleotide in the cartridge, and to estimate a concentration of the therapeutic polynucleotide by driving a first blank solution through a measurement chamber of the cartridge and taking a first measurement

of the first blank sample in the measurement chamber, driving a sample solution including the therapeutic polynucleotide through the measurement chamber of the cartridge and taking a second measurement of the sample solution in the measurement chamber, further wherein the controller is configured to estimate the concertation of the therapeutic polynucleotide from the first and second measurement.

[0005g] In yet another aspect, provided herein is a method comprising: emitting ultraviolet (UV) light through a UV measurement chamber of a cartridge, the UV measurement chamber being configured to receive fluid; receiving the UV light at a UV light detector, the received UV light having passed through the UV measurement chamber of the cartridge; measuring a maximum voltage of the UV light detector, measuring a standard deviation of a signal from UV the light detector; determining an upper limit of quantitation (LoQ) based on the measured maximum voltage and based on the measured standard deviation; determining a lower LoQ based on the measured maximum voltage and based on the measured standard deviation; determining whether the upper LoQ and the lower LoQ are acceptable; and either: allowing the cartridge to be used to generate a therapeutic polynucleotide if the upper LoQ and the lower LoQ are acceptable, or providing an alert if one or both of the upper LoQ or the lower LoQ is not acceptable.

[0005h] In yet another aspect, provided herein is a method comprising: emitting ultraviolet (UV) light through a UV measurement chamber of a cartridge, the UV measurement chamber containing fluid; receiving the UV light at a UV light detector, the received UV light having passed through the UV measurement chamber of the cartridge; obtaining a first plurality of concentration measurements, each concentration measurement of the first plurality of concentration measurements indicating a respective concentration of fluid in the UV measurement chamber; calculating a mean of the first plurality of concentration measurements; comparing each concentration measurement of the first plurality of concentration measurements to the mean of the first plurality of concentration measurements; identifying an outlier from among the first plurality of concentration measurements relative to the mean of the first plurality of concentration measurements; establishing a second plurality of concentration measurements, the second plurality of concentration measurements comprising the first plurality of concentration measurements with the identified outlier removed; calculating a mean of the second plurality of concentration measurements; and reporting the mean of the second plurality

of concentration measurements.

[0006] Described herein are microfluidic apparatuses (e.g., systems, devices, etc.) and methods for microfluidic detection of polynucleotide concentration. These apparatuses may include removable, single-use or reusable microfluidic members that may include one or more channels, chambers and/or substrates for performing microfluidic maneuvers. For example, the microfluidic member may be a microfluidic cartridge, microfluidic chips ("biochips") or the like and may be referred to herein as "cartridges". Any of these cartridges may be generally flat, planar structures that may be inserted, held and/or seated in a microfluidic driver apparatus that may oversee and control operations within one or more cartridges. The microfluidic driver apparatus may be referred to herein as a microfluidic path device and may include actuators, sensors, controllers (including control circuitry, processors, etc.). As will be described in detail below, the cartridges may be adapted to include one or more polynucleotide quantification regions that are adapted for optical sensing and quantification of polynucleotides, e.g., DNA, RNA (e.g., mRNA), etc. The polynucleotide quantification regions are configured for spectrophotometric readings by an emitter and detector which may be part of the microfluidic driver apparatus. The microfluidic driver apparatus may be further adapted to control activity of