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, described 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, described 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, described 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 concentration of the therapeutic polynucleotide from the first and second absorption measurement.

[0005d] In another aspect, described 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 for holding the cartridge; 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 the 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 concentration of the therapeutic polynucleotide determined from the first, second and third absorption measurement.

[0005e] In yet another aspect, described 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) concentration measurement chamber of the cartridge and taking a first measurement through the cartridge, driving a sample solution of the therapeutic polynucleotide into the UV 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, described 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 an ultraviolet (UV) measurement chamber of the cartridge and taking a first measurement of the first blank sample in the UV measurement chamber, driving a sample solution including the therapeutic polynucleotide through the UV measurement chamber of the cartridge and taking a second measurement of the sample solution in the UV measurement chamber, further wherein the controller is configured to estimate the concentration of the therapeutic polynucleotide from the first and second measurement.

[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

CLAIMS

What is claimed is:

1. 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.

2. The method of claim 1, further comprising 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.

3. The method of claim 1 or 2, further comprising 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.

4. 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.

5. 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 concentration of the therapeutic polynucleotide from the first and second absorption measurement.

6. The system of claim 5, wherein the controller is further configured to modify operation of the system based on the estimated concentration of the therapeutic polynucleotide.

7. The system of claim 5 or 6, wherein the controller is further configured to compare the estimated concentration of the therapeutic polynucleotide to a concentration range and, based on this comparison, to direct the therapeutic polynucleotide for one of: pooling with one or more additional batches of therapeutic polynucleotide formed in the cartridge, waste, or further analysis.

8. A system for making and/or processing a polynucleotide, the system comprising: a cartridge; and

a microfluidic driver apparatus comprising:

a cartridge mount for holding the cartridge;

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 the 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 concentration of the therapeutic polynucleotide determined from the first, second and third absorption measurement.

9. 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) concentration measurement chamber of the cartridge and taking a first measurement through the cartridge,

driving a sample solution of the therapeutic polynucleotide into the UV 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.

10. The method of claim 9, wherein the first measurement comprises a first absorption measurement, and the second measurement comprises a second absorption measurement.

11. The method of claim 10, wherein estimating the concentration of the therapeutic polynucleotide further comprises changing one or more of a sensitivity of a UV detector and an intensity of a UV emitter of the microfluidic driver apparatus in response to the second absorption measurement through the cartridge.

12. The method of claim 10 or 11, further comprising pretreating the UV measurement chamber with UV light prior to taking the first absorption measurement through the cartridge.

13. 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 an ultraviolet (UV) measurement chamber of the cartridge and taking a first measurement of the first blank sample in the UV measurement

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

14. The system of claim 13, further comprising:

an ultraviolet (UV) light source; and

a UV light detector;

wherein the controller is configured to take the first measurement in the form of a first absorption measurement using the UV light source and the UV light receiver;

wherein the controller is configured to take the second measurement in the form of a second absorption measurement using the UV light source and the UV light receiver;

wherein the controller is configured to estimate the concentration of the therapeutic polynucleotide from the first and second absorption measurement.

15. The system of claim 14, wherein the controller is further configured to change one or more of a sensitivity of the UV detector and an intensity of the UV light source in response to the second absorption measurement through the cartridge.

16. The system of claim 14 or 15, wherein the controller is further configured to pretreat the UV measurement chamber with UV light prior to taking the first absorption measurement through the cartridge.