

SUMMARY

[0006] The present disclosure provides systems, devices and methods for nucleic acid or protein purification and imaging.

[0007] An embodiment of the present disclosure provides a cartridge configured for purifying a hybridized target molecule sample and imaging the hybridized target molecule. The cartridge comprises a sample input area, a first binding chamber, a first elution channel, a second binding chamber, a second elution channel, and a binding area.

[0007a] In one aspect, there is provided a cartridge configured for purifying a hybridized target molecule sample and imaging a hybridized target molecule, comprising:

a sample input area configured to hold a target molecule sample, the sample comprising:

a plurality of hybridized complexes comprising a plurality of target molecules each hybridized with a first probe and a second probe,

a plurality of non-hybridized first probes, and

a plurality of non-hybridized second probes;

a first binding chamber configured to receive and/or contain a first affinity matrix and to receive the sample, wherein:

the first affinity matrix is functionalized with first molecules configured to bind with the non-hybridized first probes and hybridized complexes of the sample during a first period of time;

the first binding chamber is additionally configured to receive a first buffer to remove non-hybridized second probes from the sample after the non-hybridized first probes and hybridized complexes of the sample bind with the first affinity matrix;

a first elution channel configured to receive the first affinity matrix after the first period of time and configured for heating the first affinity matrix to elute a first eluted sample comprising the plurality of hybridized complexes and plurality of non-hybridized first probes;

a second binding chamber configured to receive and/or contain a second affinity matrix and to receive the first eluted sample, wherein:

the second affinity matrix is functionalized with second molecules configured to bind with the hybridized complexes during a second period of time;

the second binding chamber is additionally configured to receive a second buffer to remove at least non-hybridized first probes;

a second elution channel configured to receive the second affinity matrix after the second period of time and configured for heating the second affinity matrix to elute a second eluted sample comprising the plurality of hybridized complexes; and

a binding area having an active binding surface configured to receive the second eluted sample and bind with the hybridized complexes.

[0008] In this aspect, the sample input area may be configured to hold a target molecule sample, e.g., comprising a plurality of hybridized complexes (which include a plurality of target molecules each hybridized with a first probe and/or a second probe), a plurality of non-hybridized first probes, and a plurality of non-hybridized second probes. The first binding chamber may be configured to receive and/or contain a first affinity matrix and/or to receive the sample. The first affinity matrix may be functionalized with first molecules configured to bind with the non-hybridized first probes and/or hybridized complexes of the sample during a first period of time. The first binding chamber may be additionally configured to receive a first buffer to remove non-hybridized second probes from the sample after the non-hybridized first probes and/or hybridized complexes of the sample bind with the first affinity matrix. The first elution channel may be configured to receive the first affinity matrix after the first period of time and/or configured for heating the first affinity matrix to elute a first eluted sample comprising the plurality of hybridized complexes and/or plurality of non-hybridized first probes. The second binding chamber may be configured to receive and/or contain a second affinity matrix and/or to receive the first eluted sample. The second affinity matrix may be functionalized with second molecules configured to bind with the hybridized complexes during a second period of time. The second binding chamber may be additionally configured to receive a second buffer to remove at least non-hybridized first probes. The second elution channel may be configured to receive the second affinity matrix after the second period of time and/or configured for heating the second affinity matrix to elute a second eluted sample comprising the plurality of hybridized complexes. The binding area may have an active binding surface configured to receive the second eluted sample and/or bind with the hybridized complexes.

[0009] In embodiments of this aspect, the target molecule may be a nucleic acid or a protein. In embodiments, the first affinity matrix and/or the second affinity matrix, respectively, correspond to a first set of magnetic beads (e.g., oligonucleotide-coupled magnetic beads, e.g., F magnetic beads) and/or a second set of magnetic beads (e.g., oligonucleotide-coupled magnetic beads, e.g., G magnetic beads). The cartridge may further comprise a plurality of buffer input areas, a plurality of first binding chambers, a plurality of waste output areas, and/or a plurality of bead pads. The bubble vent may be configured to separate the sample input and/or the first binding chamber and/or to eliminate air bubbles. In embodiments, the active binding surface may comprise streptavidin, an avidin (e.g., Neutravidin™), or oligonucleotides. In embodiments, the first probes include capture probes. In embodiments, the second probes include reporter probes. In embodiments, the cartridge may be operatively coupled to a plurality of off-card buffer input valves operatively coupled to a fluidic manifold and/or a plurality of waste valves. In embodiments, the cartridge further comprises a plurality of on-card buffer input valves operatively coupled to a fluidic manifold. The plurality of off-card buffer input valves may be configured to receive the first buffer and/or the second buffer from the fluidic manifold and/or provide the buffer to the cartridge. In embodiments, the flow of the second eluted sample onto the binding area may be done in small steps that may be re-ordered based on pressure profiles. In embodiments, the binding area may be further configured to receive a solution (e.g., comprising includes G-hooks, anti-fade media, and/or fiducials) formulated to immobilize the second eluted sample on the active binding surface after stretching with flow.

[0010] Another embodiment of the present disclosure provides a cartridge configured for purifying a hybridized target molecule sample and imaging the hybridized target molecule. The cartridge comprises a buffer input area, a bubble vent, a first binding chamber, a first elution channel, a second binding chamber, a second elution channel, and a binding area.

[0010a] In one aspect, there is provided a cartridge configured for purifying a hybridized target molecule sample and imaging the hybridized target molecule, comprising:

a buffer input area configured to hold a target molecule sample, the sample comprising:

a plurality of hybridized complexes including a plurality of target molecules, and each of reporter probes and capture probes,

a plurality of non-hybridized reporter probes, and

a plurality of non-hybridized capture probes;

a bubble vent configured to separate the sample input and the first binding chamber and to eliminate air bubbles;

a first binding chamber configured to receive and/or contain F magnetic beads and to receive the sample, wherein:

the F magnetic beads are functionalized with first molecules configured to bind with the non-hybridized reporter probes and hybridized complexes of the sample during a first period of time;

the first binding chamber is additionally configured to receive a first buffer to remove non-hybridized reporter probes from the sample after the non-hybridized reporter probes and hybridized complexes of the sample bind with the F magnetic beads;

a first elution channel configured to receive the F magnetic beads after the first period of time and configured for heating the F magnetic beads to elute a first eluted sample comprising the plurality of hybridized complexes and plurality of non-hybridized reporter probes;

a second binding chamber configured to receive and/or contain G magnetic beads and to receive the first eluted sample, wherein:

the G magnetic beads are functionalized with second molecules configured to bind with the hybridized complexes during a second period of time;

the second binding chamber is additionally configured to receive a second buffer to remove at least non-hybridized capture probes;

a second elution channel configured to receive the G magnetic beads after the second period of time and configured for heating the G magnetic beads to elute a second eluted sample comprising the plurality of hybridized complexes;

a binding area having an active binding surface configured to receive the second eluted sample and bind with the hybridized complexes;

wherein the cartridge is operatively coupled to:

a plurality of off-card buffer input valves operatively coupled to a fluidic manifold, the plurality of off-card buffer input valves being configured to receive the first buffer and the second buffer from the fluidic manifold and provide the first and buffer to the cartridge; and

a plurality of waste valves configured to collect the first and second buffer from the cartridge.

[0011] In this aspect, the buffer input area may be configured to hold a target molecule sample, e.g., comprising a plurality of hybridized complexes (which include a plurality of target molecules, reporter probes, and/or capture probes), a plurality of non-hybridized reporter probes, and/or a plurality of non-hybridized capture probes. The bubble vent may be configured to separate the sample input and the first binding chamber and/or to eliminate air bubbles. The first binding chamber may be configured to receive and/or contain F magnetic beads and/or to receive the sample. The first binding chamber may be additionally configured to receive a first buffer to

remove non-hybridized reporter probes from the sample after the non-hybridized reporter probes and/or hybridized complexes of the sample bind with the F magnetic beads, which may be functionalized with first molecules configured to bind with the non-hybridized reporter probes and/or hybridized complexes of the sample during a first period of time. The first elution channel may be configured to receive the F magnetic beads after the first period of time and/or configured for heating the F magnetic beads to elute a first eluted sample comprising the plurality of hybridized complexes and/or plurality of non-hybridized reporter probes. The second binding chamber may be configured to receive and/or contain G magnetic beads and/or to receive the first eluted sample. The second binding chamber may be additionally configured to receive a second buffer to remove at least non-hybridized capture probes. The G magnetic beads may be functionalized with second molecules configured to bind with the hybridized complexes during a second period of time. The second elution channel may be configured to receive the G magnetic beads after the second period of time and/or configured for heating the G magnetic beads to elute a second eluted sample comprising the plurality of hybridized complexes. The binding area may have an active binding surface configured to receive the second eluted sample and/or bind with the hybridized complexes. The cartridge may be operatively coupled to a plurality of off-card buffer input valves operatively coupled to a fluidic manifold. The plurality of off-card buffer input valves may be configured to receive the first buffer and/or the second buffer from the fluidic manifold and/or provide the first and/or buffer to the cartridge. The plurality of waste valves may be configured to collect the first and/or second buffer from the cartridge.

[0012] In embodiments of this aspect, the target molecule may be a nucleic acid or a protein. In embodiments, the active binding surface may comprise streptavidin, an avidin (e.g., Neutravidin™), or oligonucleotides.

[0013] Yet another embodiment of the present disclosure provides a system for imaging a plurality of hybridized complexes. The system comprises a cartridge of any of the herein described aspects or embodiments, a cartridge tray operatively coupled to the system and configured to hold the cartridge, a first heater operatively coupled to the cartridge, a second heater operatively coupled to the cartridge, a magnet operatively coupled to the imaging device below the cartridge tray, a fluidic manifold operatively coupled to the system above the cartridge tray and configured to hold and/or control the flow of a plurality of buffers, a plurality of off-card buffer input valves

operatively coupled to the fluidic manifold and the cartridge; a plurality of waste valves operatively coupled to the system above the cartridge tray, and an imaging reference surface operatively coupled to the imaging device above the cartridge tray.

[0013a] In one aspect, there is provided a system for imaging a plurality of hybridized complexes comprising:

- a cartridge as described herein;
- a cartridge tray operatively coupled to the system and configured to hold the cartridge;
- a first heater operatively coupled to the cartridge;
- a second heater operatively coupled to the cartridge;
- a magnet operatively coupled to the imaging device below the cartridge tray;
- a fluidic manifold operatively coupled to the system above the cartridge tray and configured to hold and control the flow of a plurality of buffers;
- a plurality of off-card buffer input valves operatively coupled to the fluidic manifold and the cartridge;
- a plurality of waste valves operatively coupled to the system above the cartridge tray; and
- an imaging reference surface operatively coupled to the imaging device above the cartridge tray.

[0014] In embodiments of this aspect, the first heater may be configured to heat the first elution channel. In embodiments, the second heater may be configured to heat the second elution channel. In embodiments, the magnet may be configured to move the first magnetic beads and/or the second magnetic beads within the first and/or second binding chambers and/or the first and/or second elution channels. In embodiments, the magnet may be configured to move parallel to the cartridge tray. In embodiments, the plurality of off-card buffer input valves may be configured to receive the plurality of buffers from the fluidic manifold and/or provide the plurality of buffers to the cartridge. In embodiments, the plurality of waste valves may be configured to collect the plurality of buffers from the cartridge. In embodiments, the system further comprises a cam contact pad operatively coupled to the imaging device and configured to allow preloading against at least one contact pad, at least one adjustable contact between a moving clamp and a base of the imaging device, the at least one adjustable contact configured to allow for datum A adjustment, and a clamp motor operatively coupled to the imaging device and configured to move the moving clamp. In embodiments, at least one of the plurality of off-card buffer input valves and the plurality of waste valves operatively coupled to the system above the cartridge tray may be pneumatically controlled.

[0015] Another aspect provides a method for purifying a hybridized target molecule sample and imaging the hybridized target molecule, comprising:

- (a) receiving a hybridized sample, the sample comprising a plurality of hybridized, complexes comprising target molecules hybridized with first probes and second probes, a plurality of non-hybridized first probes, and a plurality of non-hybridized second probes,
- (b) binding the non-hybridized first probes and hybridized complexes of the sample to a first affinity matrix during a first period of time to produce a first mixture,
- (c) flowing a first buffer through the first mixture to remove non-hybridized second probes from the first mixture after the non-hybridized first probes and hybridized complexes of the sample bind with the first affinity matrix,

- (d) heating the first mixture to free the non-hybridized first probes and hybridized complexes from the first affinity matrix and elute a first eluted sample comprising the plurality of hybridized complexes and plurality of non-hybridized first probes,
- (e) binding the hybridized complexes of the first eluted sample to a second affinity matrix during a second period of time to produce a second mixture,
- (f) flowing a second buffer through the second mixture to remove the non-hybridized first probes from the first eluted sample after the hybridized complexes bind with the second affinity matrix,
- (g) heating the second mixture to free the hybridized complexes from the second affinity matrix to elute a second eluted sample comprising the plurality of hybridized complexes, and
- (h) binding the hybridized complexes to an active binding surface for imaging thereof.

[0016] In embodiments of this aspect, the target molecule may be a nucleic acid or a protein. In embodiments, the first affinity matrix and the second affinity matrix, respectively, correspond to a first set of magnetic beads and a second set of magnetic beads, respectively. In embodiments, the active binding surface may surface may comprise streptavidin, an avidin (e.g., Neutravidin™), or oligonucleotides.

[0017] A further aspect provides a method for purifying a hybridized target molecule sample and imaging the hybridized target molecule, comprising:

- (a) providing the cartridge of any of the herein described aspects or embodiments,
- (b) receiving a hybridized sample, the sample comprising a plurality of hybridized complexes comprising target molecules hybridized with first probes and second probes, a plurality of non-hybridized first probes, and a plurality of non-hybridized second probes,
- (c) binding the non-hybridized first probes and hybridized complexes of the sample to a first affinity matrix in a first binding chamber during a first period of time,
- (d) flowing a first buffer into the first binding chamber to remove non-hybridized second probes from the sample after the non-hybridized first probes and hybridized complexes of the sample bind with the first affinity matrix,
- (e) directing the first affinity matrix into a first elution channel,
- (f) heating the first affinity matrix to elute a first eluted sample comprising the plurality

[0019] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. In the Specification, the singular forms also include the plural unless the context clearly dictates otherwise; as examples, the terms “a,” “an,” and “the” are understood to be singular or plural and the term “or” is understood to be inclusive. By way of example, “an element” means one or more element. Throughout the specification the word “comprising,” or variations such as “comprises” or “comprising,” will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from the context, all numerical values provided herein are modified by the term “about.”

[0020] Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. The references cited herein are not admitted to be prior art to the claimed disclosure. In the case of conflict, the present Specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting. Other features and advantages of the disclosure will be apparent from the following detailed description and claim.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

[0022] The accompanying appendices and/or drawings illustrate various non-limiting, example, innovative aspects in accordance with the present descriptions:

[0023] FIGURE 1A shows block diagrams of the major processes according to some embodiments.