

AUTOMATED SIZE SELECTION OF NUCLEIC ACIDS

Reference to Related Application

[0001] This application claims Paris convention priority from United States application
5 No. 61/497586 filed on 16 June 2011 and entitled Method and Apparatus for Automated
Size Selection of Nucleic Acids which is hereby incorporated herein by reference for all
purposes. For purposes of the United States of America, this application claims the benefit
under 35 U.S.C. §119 of United States application No. 61/497586 filed on 16 June 2011
and entitled Method and Apparatus for Automated Size Selection of Nucleic Acids which
10 is hereby incorporated herein by reference for all purposes.

Technical Field

[0002] This invention relates to the automated size selection of nucleic acids. Aspects of
the invention provide methods and apparatus useful for selecting nucleic acids according
15 to size.

Background

[0003] There are a wide range of applications in which it is desirable to select nucleic
acids, such as DNA or RNA by size. For example, size selection is used in the production
20 of DNA libraries for use in sequencing and other applications.

[0004] Various techniques for DNA size selection exist. Some of these techniques are
undesirably labour intensive. One method for DNA size selection is to perform
electrophoresis of a sample containing DNA in a gel. Since DNA of different sizes have
25 different mobilities in the gel the electrophoresis separates the DNA into different bands
by size. A band containing DNA in the desired size range can be identified and then
manually cut out from the gel. The desired DNA can then be extracted from the gel.

[0005] Some electrophoresis systems comprise wells formed in a gel. DNA can be run
30 into the wells by electrophoresis. Invitrogen E-gels and the Lonza Flash Gel™ provide
such wells.

[0006] Y-channel size selection machines are another technology for DNA size selection.
Examples are the Sage Pippin Prep™ and the Caliper XT™ machines. These machines
35 can extract DNA of a desired size range from a sample by diverting DNA of the desired
size range into a side channel and collecting the diverted DNA against a molecular weight
cut-off filter.

[0007] Solid Phase Reversible Immobilization Beads (SPRI) beads which are available from Beckman Coulter and others may be used to trap DNA of a certain size and then release the DNA after a wash and a change in pH.

- 5 [0008] There remains a need for a DNA size-selection technology that can provide high throughput. There remains a need for a DNA size-selection technology that can provide accurate DNA size selection with reduced labour.

Summary

- 10 [0009] This invention has a number of aspects that may be applied together. Some of the aspects have independent application. One aspect provides apparatus for automated size-selection of nucleic acids. Another aspect provides a computer system for controlling apparatus for automated size-selection of nucleic acids. Another aspect provides methods for automated size-selection of nucleic acids. The nucleic acids may comprise DNA and/or
15 RNA. Another aspect provides cartridges useful *inter alia* for automated size selection of nucleic acids.

- [0010] In one example embodiment, nucleic acids are size selected by loading DNA samples individually into agarose channels, each of which has a loading well at one end of
20 the channel and an extraction well downstream. Electrophoresis is performed on the nucleic acids after loading and the nucleic acids are separated by size as they migrate towards the extraction well. The channel is imaged at regular intervals during this process and a software algorithm uses the images to identify reference bands and predict the time at which the desired nucleic acid fragments will arrive at the extraction well. The channel
25 current is also individually controllable via pulse width modulation of the DC voltage so that if adjacent samples are running at different speeds, the extractions times can be altered so that no two samples need to be extracted at the same moment.

- [0011] Another aspect of the invention provides methods for size-selection of nucleic
30 acids such as DNA, RNA and the like. Such methods comprise moving nucleic acids from a sample along a channel by electrophoresis; automatically monitoring progress of a reference fraction of the nucleic acids along the channel; based on the monitoring, estimating an estimated time of arrival of a target fraction of the nucleic acids at an extraction well in the channel; and extracting fluid containing the target fraction from the
35 extraction well at the estimated time of arrival. The reference fraction may be the same as or different from the target fraction. For example, in some embodiments the reference

fraction may comprise nucleic acids that are abundant in the sample (either originally present in the sample or added to the sample as a size marker) and the target fraction may comprise nucleic acids having sizes different from that of the reference fraction. Progress of the target fraction along the channel may be inferred from progress of the reference

5 fraction. For example, the target fraction may be known to lead or lag behind the reference fraction by a certain percentage. In some embodiments, the target fraction of the nucleic acids comprises an adapter joined to a nucleic acid molecule of interest, and the reference fraction of the nucleic acids comprise the adapter which is not joined to the nucleic acid molecule of interest. In some embodiments, the methods may comprise automatically

10 monitoring progress of a plurality of reference fractions of the nucleic acids along the channel. The plurality of reference fractions may comprise a DNA or RNA ladder of known sizes.

[0012] In some embodiments the monitoring comprises, at spaced apart times, obtaining

15 images of the channel and identifying areas in the images corresponding to the reference fraction. The images may, for example, be acquired by a camera mounted to view the channel. The camera may image a large number of channels simultaneously. Progress of the reference fractions (which are not necessarily the same for different channels) in multiple channels may be monitored using the same set of images. The estimated time of

20 arrival of the target fraction may be estimated in some cases based on an average velocity of the target fraction based on differences between the positions of the reference fraction in two or more of the images. The images may comprise high dynamic range images. For example, the images may be obtained using a high dynamic range sensor or may be assembled from two or more different exposures. In some embodiments the images have a

25 bit-depth of 10-bits or 12-bits or more. In some embodiments obtaining each of the images comprises operating an imaging device to obtain a plurality of different exposures of the channel and combining the plurality of different exposures to yield the image, wherein the image has a greater dynamic range than any of the plurality of different exposures.

30 [0013] Some embodiments comprise specifying a size or size range of the target fraction. For example, the size or size range of the target fraction may be specified in absolute terms or relative to one or more of the reference fractions. For example, the size or size range of the target fraction may be specified as leading or lagging behind the reference fraction by a certain percentage. Some embodiments comprise scheduling a time of arrival for the

35 target fraction at the extraction well; comparing the scheduled time of arrival to the estimated time of arrival and adjusting one or more electrophoresis parameters of an

electrophoresis signal based on any difference between the scheduled time of arrival and the estimated time of arrival. In such embodiments, target fractions in different channels may be caused to arrive at extraction wells at different times (facilitating extraction of the target fractions using a single mechanism such as a robot carrying a pipetter that services
5 each channel at the scheduled time). Also in such embodiments target fractions in different channels may be caused to arrive at extraction wells at the same time (facilitating extraction of the target fractions using a multi-channel mechanism such as a robot carrying a multi-channel pipetter that services several channels simultaneously at the scheduled time).

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[0014] Adjusting the one or more electrophoresis parameters may comprise adjusting a duty cycle of the electrophoresis signal, adjusting potentials of the electrophoresis signal or adjusting other parameters that define the electrophoresis signal.

15 **[0015]** In some embodiments the method determines a location of an extraction well and/or a loading well in one or more channels by image analysis. This facilitates systems in which extraction wells in different channels are at different locations and also facilitates automatic compensation for variations in the positions of extraction wells and/or loading wells.

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[0016] Another aspect of the invention provides apparatus for size-selection of nucleic acids. The apparatus comprises: a channel having first and second ends and an extraction well in the channel; an electrophoresis power supply connected to deliver an electrophoresis signal to the channel to move nucleic acids from a sample along the
25 channel; an imaging device mounted to image the channel; a controller connected to obtain images from the imaging device. the controller is configured to: automatically monitor progress of a reference fraction of the nucleic acids along the channel by analysis of the images; based on the monitoring, estimate an estimated time of arrival of a target fraction of the nucleic acids at the extraction well in the channel; and operate a mechanism to
30 extract fluid containing the target fraction from the extraction well at the estimated time of arrival.

[0017] The imaging device may comprise an electronic camera. The camera may be equipped with a filter that attenuates light outside of an emission band of a dye associated
35 with the nucleic acid.

[0018] In some embodiments the mechanism comprises a robotic system comprising a pipetter operable to transfer a sample into a loading well in the channel and to extract the fluid from the extraction well. In some embodiments the pipetter comprises a multi-channel pipetter capable of simultaneously introducing multiple samples into multiple channels or simultaneously extracting fluids from extraction wells in multiple channels.

[0019] In some embodiments the channel comprises an elongated groove having opposed first and second sides and an electrophoresis medium in the groove and the first and second sides having steps extending longitudinally along the first and second sides, the electrophoresis medium filling the groove up to the steps.

[0020] The electrophoresis medium may comprise, for example, a gel such as an agarose gel, an acrylamide gel, a denaturing acrylamide gel, or the like.

[0021] In some embodiments the controller is configured to determine a location of the extraction well in the channel by image analysis of one or more of the images and to move the pipette tip to the determined location of the extraction well.

[0022] In some embodiments the controller is configured to compare the estimated time of arrival of the target fraction at the extraction well to a desired time of arrival of the target fraction at the extraction well and to control the electrophoresis power supply to adjust one or more electrophoresis parameters of the electrophoresis signal based on any difference between the desired time of arrival and the estimated time of arrival.

[0023] In some embodiments the controller is configured to control a rate of movement of the nucleic acids along the channel by proportional feedback control of the one or more electrophoresis parameters based on an error signal comprising a difference between the estimated time of arrival and a desired time of arrival of the target fraction at the extraction well.

[0024] In some embodiments the controller comprises a scheduler configured to generate the desired time of arrival of the target fraction at the extraction well.

[0025] The apparatus may comprise a proportional feedback controller configured to control the electrophoresis power supply to vary an average speed of the target fraction along the channel in response to an error signal representing a difference between the

estimated time of arrival of the target fraction at the extraction well and a desired time of arrival of the target fraction at the extraction well. In some embodiments the controller is configured to reduce a difference between the estimated arrival time and the desired arrival time by temporarily interrupting application of the electrophoresis signal to the channel.

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[0026] Another aspect of the invention provides a cassette for use in size selection of nucleic acids. The cassette comprises a plate having a channel formed in the plate, the channel comprising an elongated groove having opposed first and second sides and an electrophoresis medium in the groove, first and second sides having steps, the

10 electrophoresis medium filling the groove up to the steps. The plate may have one or more holes, grooves or other features for locking the plate into a known location relative to a robot. The channel may include both a loading well and an extraction well at spaced apart locations along the channel. The plate may optionally be transparent at least in its portion below the channel.

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[0027] Further aspects of the invention and features of example embodiments of the invention are illustrated in the accompanying drawings and/or described below.

Brief Description of the Drawings

20 **[0028]** The accompanying drawings depict non-limiting example embodiments of the invention.

[0029] Figure 1 illustrates a method for size-selecting a nucleic acid according to an example embodiment.

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[0030] Figure 1A illustrates an alternative example method.

[0031] Figure 2 illustrates schematically an image of one channel.

30 **[0032]** Figure 2A is a plot illustrating density as a function of position along channel.

[0033] Figure 3 illustrates a method for identifying a peak corresponding to DNA of a predetermined size.

35 **[0034]** Figure 4 illustrates apparatus according to an example embodiment.

[0035] Figure 5 shows an example robot.

[0036] Figure 5A shows an example deck.

5 [0037] Figure 5B shows an example camera assembly.

[0038] Figure 6 is a screen shot of an example graphical display.

[0039] Figure 7 is a plan view of an example channel plate.

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[0040] Figure 7A is a cross section of an individual channel.

[0041] Figure 7B shows an example comb useful for forming loading or extraction wells.

15 [0042] Figure 7C is a plan view of an example channel plate according to another embodiment.

[0043] Figure 7D is a perspective view of a channel plate with combs engaged for forming source and extraction wells.

20

Description

[0044] Throughout the following description specific details are set forth in order to provide a more thorough understanding to persons skilled in the art. However, well known elements may not have been shown or described in detail to avoid unnecessarily
25 obscuring the disclosure. Accordingly, the description and drawings are to be regarded in an illustrative, rather than a restrictive, sense.

[0045] One aspect of the invention provides an automated method for size selection of multiple nucleic acid samples. The method uses imaging in conjunction with predictive
30 algorithms to time extractions and provide size selected nucleic acids of a desired size range. The method can be practiced to advantage in conjunction with automated apparatus comprising one or more electrophoresis channels, a camera which acquires images of the one or more electrophoresis channels and a robot comprising a pipetter for introducing samples into corresponding channels and extracting size-selected nucleic acids from the
35 channels. The channels may be filled, for example, with an agarose gel or an acrylamide

gel. The channels may each have a loading well in the channel and an extraction well spaced apart from the loading well along the channel.

[0046] The following description explains construction and operation of example

5 embodiments being used to size-select DNA. For example, the DNA may comprise cDNA derived from RNA. The DNA to be size-selected may have a size in the range of 10 bp to 10 kbp. However, the invention may be applied to size-selection of other nucleic acids such as RNA. In some embodiments the nucleic acids comprise sheared nucleic acids.

10 [0047] Figure 1 illustrates a method **10** according to an example embodiment of the invention. In block **11** a sample containing DNA is introduced into a loading well in a channel containing a medium through which the DNA can be moved by electrophoresis. The sample containing the DNA may also comprise a dye (e.g., SYBR Green™ dye or ethidium bromide) which is introduced into the loading well together with the DNA. The
15 function of the dye is to facilitate the detection or imaging of the DNA in the medium. In some embodiments, the DNA molecules in the sample may comprise an adapter, which may be useful for downstream applications such as DNA sequencing. The medium may, for example, comprise an agarose or acrylamide gel. In block **12** electrophoresis is commenced. Electrophoresis may be performed by applying an electrical potential
20 difference between electrodes at opposing ends of the channel. The potential difference may comprise a DC electrical potential, a pulsed DC electrical potential or an unbalanced AC electrical potential, for example. The applied electrical potential drives the DNA to migrate from the loading well along the channel toward an extraction well. DNA of different sizes has different mobilities in the channel and so the DNA becomes size
25 segregated.

[0048] Optional block **13** provides a delay to allow the DNA to migrate far enough along the channel that concentrations of DNA of different sizes can be detected. Block **14** comprises determining the location along the channel of target DNA of a desired size
30 range. In some embodiments, block **14** comprises obtaining a sequence of images of the channel with a camera, detecting one or more landmarks in the image(s) corresponding to DNA of one or more known sizes, and determining the position of the target DNA based on the position(s) of the landmark(s). The output of block **14** is a sequence of positions along the channel of the target DNA.

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[0049] Sizing reference(s) (e.g. landmarks) may be specified at or before run time; whether it is a DNA ladder, or an inherent feature expected to be present in the electropherogram. If the size reference used is a DNA ladder, the DNA ladder may be added to the sample prior to loading the sample into the loading wells. In some

5 embodiments, the size reference is inherently present in the sample. For example, to size-select a cDNA sample derived from miRNA, the sample may comprise both cDNA+adapter fragments (e.g., having a size of 109 bp) as well as adapter-adapter fragments (e.g., having a size of 80 bp). The adapter-adapter fragments may be used as a size reference.

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[0050] The size-range of the target DNA may be specified in various ways, for example in absolute terms or relative to given reference(s), allowing for excision of fractions with sizes either dependent or independent of the electropherogram profile of the input sample.

For example, if the input sample is sheared genomic DNA with an expected peak centre at
15 ~250bp, the mobility range of the target fraction may be specified relative to the peak centre (e.g. 110%- 90% of the peak-centre mobility), or the target may be specified as a absolute size range (e.g. 150bp-200bp) independent of the actual mobility of the peak centre.

20 **[0051]** Block **15** determines an average velocity of the target DNA along the channel. Block **15** may, for example, be as simple as dividing a difference of two positions of the target DNA by an elapsed time between the images from which the positions were determined. Block **15** may take into account more than two positions. For example, block **15** may average or find the median of a plurality of velocities.

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[0052] Block **16** estimates a time of arrival of the target DNA at the extraction well. This determination may be based on a known, predetermined location of the extraction well. In some embodiments the location of the extraction well is determined in block **17** by locating the image of the extraction well in the images obtained in block **15** (or else
30 separate images obtained for the purpose of locating the extraction well). This image recognition may be model-based (i.e. it is known in advance what the image of a channel is expected to look like in an image, where each channel is expected to be found in the image, and what the image of an extraction well is expected to look like in the image.

Locating the extraction well may, for example, comprise, finding a location in the image
35 where the correlation with a model image of an extraction well is maximized. In other

simpler embodiments locating the extraction well in the image comprises locating one or more edges corresponding to the extraction well in the image.

5 [0053] For example, the estimated time of arrival may be obtained by adding an estimated travel time to a current time. The estimated travel time may be determined, for example, by dividing a distance between the location of the extraction well and the current location of the target DNA by the current velocity of the target DNA as estimated in block **15**.

10 [0054] Block **18** extracts the target DNA from the extraction well at the estimated arrival time. Block **18** may, for example comprise controlling a robot carrying a pipetter to place the pipetter into the extraction well at or before the estimated arrival time and withdrawing fluid from the extraction well into the pipetter at the estimated arrival time. The robot may then dispense the collected fluid into a reservoir where the fluid may be held or delivered for further processing.

15 [0055] Method **10** has a range of variations. In some embodiments information regarding the velocity and/or position of the target DNA is applied to control the velocity of target DNA. Position information obtained from run-time electropherograms and time information is used in a feedback loop to control electrophoresis speed of the target DNA
20 fraction in addition to its arrival time at the extraction well.

[0056] Feedback control may be applied, for example, to adjust the estimated arrival time of the target DNA at the extraction well. The estimated arrival time may be adjusted, for example, adjusting a duty cycle and/or voltage of an electrophoresis field and/or by
25 pausing application of the electrophoresis field one or more times. Embodiments which adjust velocity of the target DNA by adjusting duty cycle can be advantageous since the relationship between duty cycle and velocity tends to be linear or nearly linear. This simplifies control. Electrophoresis speed and extraction scheduling may be simultaneously controlled for an arbitrary number of samples. In one embodiment, extraction scheduling
30 processes 96 samples running in parallel.

[0057] Figure 1A illustrates an alternative example method 10A which is similar to method **10** except that it includes a block **19** that schedules a scheduled arrival time for the target DNA and a block **20** that compares the estimated arrival time from block **16** to the
35 scheduled arrival time for the target DNA. Block **21** receives a control signal from block **20** and adjusts electrophoresis parameters based on the control signal. Loop **22** may be

repeated periodically at a rate sufficient to control the progress of the target DNA so hat the target DNA arrives at the extraction well at the scheduled time.

[0058] In block 21, the electrophoresis parameters may be adjusted, as appropriate, to
5 retard the progress of the target DNA, to accelerate the progress of the target DNA or to
maintain the current rate of progress of the target DNA. In some embodiments the
adjustment depends merely on the sign of the control signal (i.e. whether the estimated
arrival time is before or after the scheduled arrival time). In other embodiments the
adjustment is based at least in part on a magnitude of the difference between the estimated
10 arrival time and the scheduled arrival time (or equivalently a magnitude of the difference
between an estimated velocity and a velocity that would result in the target DNA arriving
at the extraction well at the scheduled time.

[0059] In typical cases the target DNA does not have a specific size but has a range of
15 sizes. Thus the target DNA will arrive at an extraction well during a time window having a
length determined by the range of sizes in the DNA fraction as well as by the
electrophoresis parameters. In some embodiments a target fraction may be specified
initially and also continuously adjusted until the fraction is extracted.

[0060] Control over the time at which target DNA arrives at an extraction well may be
20 applied to good effect in the case where multiple electrophoresis channels are being
operated at the same time. For example, electrophoresis of DNA in each of a plurality of
channels may be controlled to cause target DNA in each channel to arrive at an extraction
well at a scheduled time such that the scheduled times in different channels are different.
25 The target DNA in different channels may be the same or different. This can facilitate
using a robot to extract target DNA from each of the channels without requiring the same
pipetter of the robot to be extracting fluid from two extraction wells at the same time. The
scheduled times may be assigned to ensure that there is enough time for the robot to make
each of the scheduled extractions.

30 [0061] Control over the electrophoresis velocity may be applied to compensate for
variations between channels in electrophoresis velocity (caused, for example, by
inhomogeneities in the electrophoresis medium or other differences in the electrophoresis
medium used in different channels. The control may also be applied to compensate for
35 differences in the location of the extraction well between channels. The control may also
be applied to compensate for differences in the target DNA for different channels.

[0062] Some embodiments employ a multi-channel robot. For example, such a robot may have a plurality of pipetters arranged so that their tips can be simultaneously inserted into a plurality of extraction wells. For example, the robot may carry 8, 16 or some other number
5 of pipetters. In some such embodiments channels are arranged side-by-side and the robot may be configured to simultaneously introduce fluid into N adjacent loading wells or to simultaneously remove fluid from N adjacent extraction wells.

[0063] In some embodiments which use a multi-channel robot, electrophoresis in a
10 plurality of channels is controlled to cause target DNA in the plurality of channels to reach extraction wells at the same scheduled time. The target DNA may differ among the plurality of channels. The robot may then be controlled to place pipette tips into the extraction wells of the plurality of channels at the scheduled time and to simultaneously extract fluid from the extraction wells. Such embodiments permit different scheduled
15 arrival times to be assigned to groups of channels. Electrophoresis in the individual channels in each group may be separately controlled to cause target DNA in each channel in the group to arrive at the corresponding extraction well at the time scheduled for that group. The scheduled times for different groups of channels may be spaced apart such that the robot has time to make the scheduled extractions. Such embodiments can provide high
20 throughput electrophoresis.

[0064] The principles described above may also be applied in situations where it is desired to extract two or more fractions from the same sample. For such applications, electrophoresis may be performed to bring a first target fraction to an extraction well and
25 to extract the first target fraction. Subsequently, further electrophoresis may be performed to bring a second fraction to the extraction well. The second fraction may then be extracted. If desired, the first and second target fractions may be kept isolated from one another. For example, each of the first and second target fractions may be transferred from the extraction well to a separate destination well. It is also possible to transfer multiple
30 fractions from the same sample to the same destination well if that is desired.

[0065] In some applications, three or more fractions may be extracted from the same sample. Where two or more target fractions are to be extracted from the same sample then extraction of each of the target fractions may be separately scheduled. After a first target
35 fraction has been extracted from a channel at a first scheduled time, electrophoresis

parameters for the channel may be controlled to bring the second target fraction to the extraction well for extraction at a second scheduled time.

[0066] In some embodiments electrophoresis is controlled in a plurality of channels to
5 bring a corresponding plurality of first target fractions to the extraction wells in the
plurality of channels at a first time. A multi-channel pipetter or other multi-channel
extraction mechanism may then be applied to transfer the first target fractions to
corresponding destination wells. Electrophoresis in the plurality of channels may then be
controlled to bring second target fractions to the extraction wells in the channels at the
10 same time. It is not necessary that the spacing between the first target fractions and the
second target fractions be the same between the different channels. Electrophoresis may be
controlled to move the second target fraction toward the extraction well faster in some
channels than in others. In some embodiments electrophoresis is controlled to bring the
second target fractions to extraction wells at the same time in the plurality of channels so
15 that the second target fractions can be simultaneously extracted using the multi-channel
pipetter.

[0067] Figure 2 illustrates schematically an image of one channel **24**. Channel **24**
comprises a strip **25** of a suitable electrophoresis medium with a buffer reservoir **25A**, **25B**
20 at each end. A loading well **26A** is located in medium **25** near buffer reservoir **25A**. An
extraction well is located in medium **25** at a location that is spaced apart from loading well
26A toward buffer reservoir **25B**.

[0068] Also shown in Figure 2 are various bands of DNA that have been carried along
25 medium **25** from loading well **26A** by electrophoresis. Because DNA of different sizes
moves at different rates under electrophoresis, the bands at different locations represent
DNA of different sizes. Different bands may have different densities in the image. The
bands may all represent DNA that is present in a sample. In some embodiments DNA of a
known size or a set of known sizes (e.g. a DNA ladder) may be added to the sample for the
30 purpose of providing a size scale that may be used to determine the location of target
DNA.

[0069] In some embodiments sizing references such as DNA ladders are run in the same
channel **24** as input samples. This ensures sizing accuracy in comparison to embodiments
35 where sizing references and samples are run in separate channels.

[0070] Figure 2A is a plot illustrating density as a function of position along channel **24**. Peaks in curve **27** correspond to the locations of the bands shown in Figure 2. The methods described above may identify a peak in curve **27** corresponding to the target DNA or infer a current position of the target DNA from locations of one or more other peaks
5 corresponding to DNA having a known size relationship(s) to the target DNA.

[0071] Some embodiments provide a scheduler. The scheduler may, for example, be implemented in software. The scheduler may schedule: the transfer of samples into source wells **26A** in channels **24**, commencement of electrophoresis in channels **24** and the
10 extraction of target fractions from extraction wells **26B**. In some embodiments the scheduler operates while samples are being run in channels **24** and may re-schedule extraction of target fractions in response to the monitoring of the progress of the target fraction (or a band having a known relationship to the target fraction). The schedule may initially schedule extraction of a target fraction in a time-slot that is separated from a time
15 of commencement of electrophoresis in a channel by a period that is longer than the shortest period in which a target fraction could possibly progress from the source well **26A** to the corresponding extraction well **26B**. The time period used for this initial scheduling may be determined based on a measured distance from the source well **26A** to the extraction well **26B** in some embodiments. The time period may be generated based on an
20 assumed average velocity of the target fraction that is less than a maximum velocity achievable within an available range of electrophoresis parameters. The assumed average velocity may be a function of a size of the target fraction and the characteristics of the medium in which electrophoresis is being performed.

25 [0072] In some embodiments, the length of a period scheduled by the scheduler for extraction of a target fraction is variable and depends on the sizes of nucleic acid included in the target fraction (a target fraction which includes a greater range of sizes will take longer to extract than a target fraction in which the spread of sizes is small). In some embodiments start and stop times for extraction of a target fraction are adjusted on the
30 basis of the estimated times of arrival at the extraction well of leading and trailing edges of the target fraction.

[0073] In some embodiments the scheduler monitors for conflicts between times for extraction of target fractions from different channels **24**. In some such embodiments, in the
35 case of a conflict (i.e. periods assigned to extraction of target fractions from different channels **24** overlap the scheduler may revise the scheduled time for extraction of the

target fraction from one of the channels **24** to remove the conflict. Changing of the scheduled extraction time may automatically result in parameters of the electrophoresis in the rescheduled channel **24** being altered so as to control the progress of the target fraction in the channel **24** so that the target fraction arrives at the extraction well at the rescheduled
5 time.

[0074] In some embodiments the electrophoresis parameters for a channel **24** are controlled such that upon the leading edge of the target fraction arriving at the corresponding extraction well **26B** and extraction commencing, the rate of electrophoresis
10 is increased, thereby reducing the time over which extraction must be continued to extract the entire target fraction.

[0075] Figure 3 illustrates a method **30** for identifying a peak corresponding to DNA of a predetermined size in channel **24**. Block **32** applies electrophoresis to a channel using
15 known electrophoresis parameters for a period of time. Block **33** obtains an image of the channel at the end of block **33**. Block **34** identifies a range of positions along the channel based upon known characteristics for the target DNA. For example, a predetermined calibration curve **35** may be provided which relates position along the channel to DNA size. In some embodiments a plurality of different calibration curves **35** are provided. The
20 different calibration curves may apply to different media that may be used in channels **24**.

[0076] Block **34** estimates a position or range of positions in which target DNA is expected to be found. The estimated position may be a function of the length of time that electrophoresis has been performed, the medium in channel **24**, the electrophoresis
25 parameters and the characteristics (especially size) of the target DNA. An operator may enter a size or size range for the target DNA. Block **34** may use an appropriate calibration curve **35** to identify the expected position of a peak in curve **27** corresponding to the target DNA. Block **36** sets a range **37** (see Figure 2A) and searches curve **27** for a peak within range **37**. If a peak is successfully detected (as determined e.g. by a YES result from block
30 **39** then the peak is identified as the initial location of the target DNA. Once a peak corresponding to target DNA has been identified in one image the peak may be tracked through subsequent images as it propagates along the channel. Prominent features of the electropherogram profile may be identified at run-time and used to help maintain size integrity as faster-moving size references move out of the field of view.

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[0077] Method **30** may be applied to identify one or more peaks corresponding to DNA in a DNA ladder and/or a sample. In some embodiments one or more peaks that are different from the target DNA are identified and tracked as described above. A current location of the target DNA may be identified relative to such peaks. For example, a user may specify
5 the amount by which target DNA is expected to lead or lag one or more such peaks.

[0078] Figure 4 illustrates apparatus **40** according to an example embodiment. Apparatus **40** comprises a robot **42** comprising a pipetter **44** that can be positioned by robot **42** over desired locations in a field **43**. For example, robot **42** may comprise an XYZ stage that
10 supports a single-channel pipette pump, which also supports a buffer loading line and tip ejection mechanism. A source plate **45** comprises a plurality of source wells **45A**. A destination plate **46** comprises a plurality of destination wells **46A**. A plurality of channels **47** is provided within field **43**.

15 [0079] Robot **42** comprises a controller **42A** that can control the position of pipetter **44**. Controller **42A** may, for example, control robot **42** to load a channel by: picking up a pipette tip **48** at a station **48A**, positioning the pipette tip over a selected source well **45A**, lowering the pipette tip into the source well **45A** and drawing fluid into the pipette tip **45A**, raising the pipette tip and repositioning it over a loading well of a selected channel
20 **47**, lowering the pipette tip into the loading well, operating the pipetter to dispense the fluid into the source well, raising the pipette tip and moving to pipette tip to a storage area for used pipette tips and disconnecting the used pipette tip.

[0080] Controller **42A** may, for example, control robot **42** to retrieve target DNA from a
25 channel by: picking up a pipette tip **48** at station **48A**, positioning the pipette tip over the extraction well in the selected channel, just prior to the estimated arrival of the target DNA lowering the pipette tip into the extraction well of the channel, drawing fluid into the pipette tip **45A** over a period of time corresponding to the expected arrival of the target DNA, raising the pipette tip and repositioning it over a destination well **46A**, lowering the
30 pipette tip into the destination well, operating the pipetter to dispense the fluid into the destination well, raising the pipette tip and moving to pipette tip to a storage area for used pipette tips and disconnecting the used pipette tip.

[0081] Apparatus as described herein may be configured to process any sensible number
35 of samples. In some embodiments, apparatus as described herein provides automated size selection for 96 samples concurrently. In other example embodiments apparatus process

multiples of 96 samples concurrently. Other example embodiments are configured to process other numbers of samples.

5 [0082] Robots suitable for use as robot **42** are commercially available. Robots suitable for use as robot **42** may also be made from commercially-available components in ways known to those of skill in the art.

10 [0083] Apparatus **40** comprises an imaging device **50** which may, for example, comprise a camera arranged to obtain images of channels **47**. Imaging device **50** may comprise a high dynamic range imaging device. For example, camera **50** or a controller connected to receive images from camera **50** may be configured to obtain and combine images taken at different exposure times to expand the detectable dynamic range. This allows dim bands to be visible without saturating the brightest bands.

15 [0084] A light source **52** illuminates channels **47** to facilitate imaging of nucleic acids propagating along the channels. Where the DNA is associated with a dye the light source may emit light corresponding to an absorption band of the dye (e.g. a band corresponding to a wavelength that excites a fluorophore of the dye. Light source **52** may comprise a filter that blocks wavelengths outside of this range. For example, SYBR GreenTM dye
20 absorbs light at 488nm. The light source may emit blue light. For example, the light source may comprise an array of blue LEDs. Alternatively or additionally, the light source may emit UV light. Camera **50** may include a filter that preferentially admits fluorescence of the dye. for example, SYBR GreenTM dye emits light at 520nm. The camera may have a bandpass or notch filter that passes light at 520 nm but attenuates light at other
25 wavelengths.

[0085] For example, the camera may be fixed to a component of robot **42** such that the camera is at a fixed distance from channels **47**. In a prototype embodiment camera **50** and LED illuminator **52** are fixed to a Y-axis arm of robot **42**.
30

[0086] A multi-channel electrophoresis power supply **54** is configured to provide electrophoresis potentials across channels **47**. Power supply **54** may comprise a single unit or a plurality of separate units. A controller **55** is connected to receive images from camera **50** to control power supply **54** and to coordinate actions of robot **42**. A user interface **56**
35 allows users to provide control inputs and information to guide operation of apparatus **40**.

[0087] In operation of an example system, source and destination plates **45**, **46** are loaded along with two tip boxes containing pipette tips. Plates comprising channels **47** are set on the deck and electrode arrays are placed so that their electrodes are in electrical contact with channels **47** in the ends of the channel plate. In some embodiments the electrodes are
5 mounted to a structure which permits them to be introduced into buffer wells at each end of each channel. For example, the apparatus may comprise a hinged frame carrying first and second electrodes corresponding to each channel. The first and second electrodes may be mounted on the hinged frame and the hinged frame may be movable between a first position wherein the first and second electrodes project into first and second buffer
10 reservoirs of a channel and a second position wherein the first and second electrodes are removed from the first and second buffer reservoirs.

[0088] The control software is configured with the location of the samples (a whole 96 well plate or less or more), type of samples and positions of the channel plate(s).
15

[0089] When a run commences, buffer wells in the channel plates are filled. The samples are loaded sequentially (e.g. by the robot into the loading wells in channels **47**) and electrophoresis commences. In one embodiment, the on board camera **50** is used to locate the extraction well in each channel **47** avoiding the requirement to manually configure the
20 location of the extraction wells. This facilitates the possibility of providing extraction wells at different locations within their channels **47**.

[0090] Some embodiments comprise a mechanism for measuring and/or setting the Y position of the pipette tip. Knowing the exact position of the pipette tip facilitates precise
25 loading and retrieval of nucleic acid samples in small wells. Such a mechanism is useful because the ends of different pipette tips can be at somewhat different locations relative to the robot when mounted to the pipetter. In an example embodiment the mechanism comprises a switch (which can be for example a microswitch, proximity switch or the like) that changes state when a pipette tip is in a predetermined location relative to the switch.
30 The switch may be at a convenient location in the field of the robot.

[0091] In some embodiments, the switch is located near a supply of fresh pipette tips such that the Y position of each new pipette tip may be set by moving the robot to bring the pipette tip against the switch. In such embodiments the pipette tip may be positioned near
35 to the switch and then moved toward the switch in the Y direction until the switch changes state. This mechanism may be used to individually measure the location of the end of each

pipette tip after a tip is loaded. The measured location may be used to compensate for slight misalignments in different pipette tips. The illustrated system **40** comprises a switch **49** arranged to switch when a pipette tip presses against the switch in a Y direction (a direction parallel to channels **47**).

5

[0092] Figure 5 shows an example robot. Figure 5 shows a lower deck which accommodates controllers and power supplies, and an upper deck which accommodates channel plates and electrodes. Above that is the pipetting head with pump, buffer delivery system and camera and lights for imaging the channels.

10

[0093] Figure 5A shows an example deck. Figure 5A shows deck locator plates that hold the deck in position. Tip boxes, source places, destination plates and channel plates are all mounted to the deck. At least the source places, destination plates and channel plates are removable from the deck. Spring pins are provided to hold the plates against locator pins so that source wells, destination wells and channels will be in known positions when the plates are installed on the deck.

15

[0094] Figure 5B shows an example camera system comprising a camera **50** and LED arrays **52**. LED arrays **52** comprise blue light emitting light sources such as blue LEDs of LEDs covered by blue filters in some embodiments.

20

[0095] In an example embodiment, controller **55** comprises a processor configured to execute instructions provided in software. The software creates a run protocol (which sample runs in which channels, in which order, and what destination wells the respective extractions will end up in) based on data input by the user. This is communicated to the user graphically.

25

[0096] Figure 6 is a screen shot of an example graphical display. Figure 6 shows the display mid-run. Samples have been loaded sequentially starting in the lower left and the first two and half plates have completed runs. The remaining samples to the right are running and each channel's status is shown graphically based on the most recent image. The plot at top is an electropherogram for a channel selected by the user, showing a size reference peak **59A** and target region **59B**.

30

[0097] Another aspect of the invention that may be used together with a robot as described above but also may have other applications provides channel **plates** for use in separation

35

of nucleic acids. In some embodiments one or more channels is provided on a plate. The plate may be removably placed within the field of a robot as described above, for example. Providing DNA separation media in channels as opposed to slabs (e.g. slab gels) has the advantage that the possibility of cross-contamination from one sample to another is
5 reduced.

[0098] Figure 7 is a plan view of an example channel plate **80**. Plate **80** comprises location features **81** such as holes (see Figure 7A) or notches (see Figure 7C) for receiving locating pins or other locating features that permit plate **80** to be repeatably positioned in the field
10 of a robot or other apparatus. A plurality of channels **24** extend along plate **80**. Each channel **24** comprises a strip **25** of a suitable electrophoresis medium. A buffer reservoir **25A**, **25B** is provided at each end of strip **25**. A loading well **26A** is located in strip **25** near buffer reservoir **25A**. An extraction well **26B** is located in strip **25** at a location that is spaced apart from loading well **26A** toward buffer reservoir **25B**. In some applications
15 extraction wells **26B** of different channels are aligned with one another but this is not mandatory. In some applications it may be convenient to provide extraction wells **26B** that are at different locations along strip **25** in different channels **24**.

[0099] Figure 7A is a cross section of an individual channel **24**. Channel **24** optionally has
20 a small step edge on either side of strip **25**. In the illustrated embodiment steps **83** are shown. Steps **83** provide corners **84**. Corners **84** run length-wise along strip **25** parallel to one another. In the illustrated embodiment, corners **84** are parallel to flat top and bottom surfaces **84A** and **84B** of plate **80**. Medium **86** (for example an agarose gel, an acrylamide gel or the like) fills strip **25** up to the level of corners **84**. Steps **83** help to make the top
25 surface of material **86** in strip **85** flat along the length of strip **25**. The presence of corners **84** as material **86** is introduced into strip **25** helps to reduce the tendency of surface tension of material **86** to form a meniscus at the surface of material **86**. Optional features such as small divots or dimples **89** (See Figure 7C) may be formed in walls of strip **25** near the ends of strip **25** in order to mechanically lock material **86** in place.

30
[0100] Dimensions of channel **24** may be varied. In an example embodiment, strip **25** has a depth in the range of about 6 to 12 mm, preferably 8 to 10 mm. In an example embodiment, strip **25** has a width of 3 mm to 11mm, preferably 4 mm to 7 mm. The principles described herein may be applied, however, to channels of other dimensions.

35

[0101] Plate **80** may be made of a suitable plastic or other electrically-insulating material. In some embodiments plate **80** is injection molded however, plate **80** may also be fabricated by machining or in any other suitable manner.

5 [0102] A plate **80** may be prepared by temporarily damming or filling buffer reservoirs **25A** and **25B** and pouring a suitable amount of a settable material **86** into strips **25**. Preferably the entire volume of each buffer reservoir is filled while material **86** is cast so that material **86** is unable to flow into the buffer reservoirs. For example, an agarose gel may be poured into strips **25** while the gel is in a liquid form and then allowed to set in
10 strips **25**. The amount of material **86** introduced into each strip may be just enough that a surface of the material is at the level of corners **84**.

[0103] Loading and extraction wells may be formed in material **86** while the material is being cast into strips **25**. In other embodiments the loading and/or extraction wells may be
15 formed after material **86** has set. In some embodiments loading and/or extraction wells are formed by placing loading and/or extraction combs at appropriate locations along strips **25**. Figure 7B shows an example comb **87**. Each comb **87** comprises a row of pins **87A**. A comb **87** may be placed on plate **80** transversely to strips **25** such that pins **87A** are arranged to project into strips **25** of the channels **24** crossed by the comb **87**.

20 [0104] Plate **80** may comprise locating features **88** to place combs **87** in desired alignment for forming loading wells and/or extraction wells. Multiple sets of locating features **88** may be provided to facilitate forming extraction wells at different locations along strips **25**. As noted above, it can be desirable to provide extraction wells at a location that is
25 tailored to the separation to be performed. The best length of separation channel between loading well **26A** and extraction well **26B** depends on the length of DNA or other target nucleic acid and the desired degree of separation.

[0105] A comb **87** for forming extraction wells may have pins **87A** that are somewhat
30 wider than the pins **87A** used to form loading wells. Providing loading wells **26A** that do not extend the full width of strips **25** helps to avoid loss of sample at the sides of a loading well. Extraction wells **26B** may extend the full width of strips **25** or nearly the full width of strips **25**.

35 [0106] A range of embodiments provide channels in which loading wells are wider than extraction wells. In one particular example embodiment, the loading well has a dimension

of 1.2 x 3.5 x 9 mm, and the extraction well has a dimension of 1.2 x 5.5 x 9 mm (i.e., 2 mm wider than the loading well). A loading well having a dimension of 1.2 x 3.5 x 9 mm allows a sample having a volume of up to 37.8 µl to be loaded. An extraction well having a dimension of 1.2 x 5.5 x 9 mm allows a volume of up to 59.4 µl to be withdrawn.

5

[0107] Combs **87** may be designed so that pins **87A** that form the wells can ‘float’ slightly (e.g. about 0.25mm) in their mounting frames. This facilitates removing combs **87** after material **86** has set.

- 10 [0108] A plate **80** comprising one or more channels **24** may be provided in the form of a pre-prepared cassette provided in sterile packaging. The packaging may, for example, comprise a sterile cover that can be peeled off to reveal channels **24**. In some embodiments the cassette may be supplied with combs inserted into the loading and/or extraction wells. A user may remove the combs prior to use.

15

[0109] Figure 7C is a plan view of an example channel plate according to another embodiment.

- [0110] Figure 7D is a perspective view of a channel plate with combs **87-1** and **87-2**
20 inserted in preparation for casting an electrophoresis medium into channels **24**. Comb **87-1** may have narrower pins than comb **87-2** in some embodiments.

- [0111] although a camera provides a convenient tool for imaging a plurality of channels and simultaneously tracking progress of one or more reference fractions in each of the
25 channels, other tools may be used in place of a camera. For example, a 1-D line scanner could be provided to measure a concentration of a nucleic acid as a function of position along a channel. Further, it is not mandatory that the camera view the channels from above. In some embodiments trays carrying the channels are transparent, at least in their parts underlying the channels and the camera views the channels from below through the
30 plates.

Interpretation of Terms

[0112] Unless the context clearly requires otherwise, throughout the description and the claims:

- "comprise," "comprising," and the like are to be construed in an inclusive sense, as opposed to an exclusive or exhaustive sense; that is to say, in the sense of "including, but not limited to" .
- "connected," "coupled," or any variant thereof, means any connection or coupling, either direct or indirect, between two or more elements; the coupling or connection between the elements can be physical, logical, or a combination thereof.
- "herein," "above," "below," and words of similar import, when used to describe this specification shall refer to this specification as a whole and not to any particular portions of this specification.
- "or," in reference to a list of two or more items, covers all of the following interpretations of the word: any of the items in the list, all of the items in the list, and any combination of the items in the list.
- the singular forms "a", "an" and "the" also include the meaning of any appropriate plural forms.

15

[0113] Words that indicate directions such as "vertical", "transverse", "horizontal", "upward", "downward", "forward", "backward", "inward", "outward", "vertical", "transverse", "left", "right", "front", "back", "top", "bottom", "below", "above", "under", and the like, used in this description and any accompanying claims (where present) depend on the specific orientation of the apparatus described and illustrated. The subject matter described herein may assume various alternative orientations. Accordingly, these directional terms are not strictly defined and should not be interpreted narrowly.

[0114] Embodiments of the invention may be implemented using specifically designed hardware, configurable hardware, programmable data processors configured by the provision of software (which may optionally comprise 'firmware') capable of executing on the data processors, special purpose computers or data processors that are specifically programmed, configured, or constructed to perform one or more steps in a method as explained in detail herein and/or combinations of two or more of these. Examples of specifically designed hardware are: logic circuits, application-specific integrated circuits ("ASICs"), large scale integrated circuits ("LSIs"), very large scale integrated circuits ("VLSIs") and the like. Examples of configurable hardware are: one or more programmable logic devices such as programmable array logic ("PALs"), programmable logic arrays ("PLAs") and field programmable gate arrays ("FPGAs"). Examples of programmable data processors are: microprocessors, digital signal processors ("DSPs"), embedded processors, graphics processors, math co-processors, general purpose

computers, server computers, cloud computers, mainframe computers, computer workstations, and the like. For example, one or more data processors in a control circuit for a device may implement methods as described herein by executing software instructions in a program memory accessible to the processors.

5

[0115] Processing may be centralized or distributed. Where processing is distributed, information including software and/or data may be kept centrally or distributed. Such information may be exchanged between different functional units by way of a communications network, such as a Local Area Network (LAN), Wide Area Network
10 (WAN), or the Internet, wired or wireless data links, electromagnetic signals, or other data communication channel.

[0116] For example, while processes or blocks are presented in a given order, alternative examples may perform routines having steps, or employ systems having blocks, in a
15 different order, and some processes or blocks may be deleted, moved, added, subdivided, combined, and/or modified to provide alternative or subcombinations. Each of these processes or blocks may be implemented in a variety of different ways. Also, while processes or blocks are at times shown as being performed in series, these processes or blocks may instead be performed in parallel, or may be performed at different times.
20 In addition, while elements are at times shown as being performed sequentially, they may instead be performed simultaneously or in different sequences.

[0117] Software and other modules may reside on servers, workstations, personal computers, embedded processors, process controllers, tablet computers, and other devices
25 suitable for the purposes described herein.

[0118] The invention may also be provided in the form of a program product. The program product may comprise any non-transitory medium which carries a set of computer-readable instructions which, when executed by a data processor, cause the data
30 processor to execute a method of the invention. For example, the computer readable instructions may program a computer to control a robotic nucleic acid sizing system as described herein and/or to schedule operations in a nucleic acid sizing system as described herein. Program products according to the invention may be in any of a wide variety of forms. The program product may comprise, for example, non-transitory media such as
35 magnetic data storage media including floppy diskettes, hard disk drives, optical data storage media including CD ROMs, DVDs, electronic data storage media including

ROMs, flash RAM, EPROMs, hardwired or preprogrammed chips (e.g., EEPROM semiconductor chips), nanotechnology memory, or the like. The computer-readable signals on the program product may optionally be compressed or encrypted.

5 **[0119]** In some embodiments, the invention may be implemented in software. For greater clarity, “software” includes any instructions executed on a processor, and may include (but is not limited to) firmware, resident software, microcode, and the like. Both processing hardware and software may be centralized or distributed (or a combination thereof), in whole or in part, as known to those skilled in the art. For example, software and other
10 modules may be accessible via local memory, via a network, via a browser or other application in a distributed computing context, or via other means suitable for the purposes described above.

[0120] Where a component (e.g. a software module, processor, assembly, device, circuit,
15 etc.) is referred to above, unless otherwise indicated, reference to that component (including a reference to a “means”) should be interpreted as including as equivalents of that component any component which performs the function of the described component (i.e., that is functionally equivalent), including components which are not structurally equivalent to the disclosed structure which performs the function in the illustrated
20 exemplary embodiments of the invention.

[0121] Specific examples of systems, methods and apparatus have been described herein for purposes of illustration. These are only examples. The technology provided herein can be applied to systems other than the example systems described above. Many alterations,
25 modifications, additions, omissions and permutations are possible within the practice of this invention. This invention includes variations on described embodiments that would be apparent to the skilled addressee, including variations obtained by: replacing features, elements and/or acts with equivalent features, elements and/or acts; mixing and matching of features, elements and/or acts from different embodiments; combining features,
30 elements and/or acts from embodiments as described herein with features, elements and/or acts of other technology; and/or omitting combining features, elements and/or acts from described embodiments.

[0122] It is therefore intended that the following appended claims and claims hereafter
35 introduced are interpreted to include all such modifications, permutations, additions, omissions and sub-combinations as may reasonably be inferred. The scope of the claims

should not be limited by the preferred embodiments set forth in the examples, but should be given the broadest interpretation consistent with the description as a whole.

[0123] While a number of exemplary aspects and embodiments have been discussed
5 above, those of skill in the art will recognize certain modifications, permutations, additions
and sub-combinations thereof. It is therefore intended that the following appended claims
and claims hereafter introduced are interpreted to include all such modifications,
permutations, additions and sub-combinations.

WHAT IS CLAIMED IS:

1. A method for size-selection of nucleic acids, the method comprising:
moving nucleic acids from a sample along a channel by electrophoresis;
5 automatically monitoring progress of a reference fraction of the nucleic
acids along the channel;
based on the monitoring, estimating an estimated time of arrival of a target
fraction of the nucleic acids at an extraction well in the channel; and
extracting fluid containing the target fraction from the extraction well at the
10 estimated time of arrival.
2. A method according to claim 1 wherein the reference fraction is the same as the
target fraction.
- 15 3. A method according to claim 1 wherein the reference fraction is different from the
target fraction and estimating the estimated time of arrival of the target fraction
comprises estimating a difference between an estimated arrival time of the
reference fraction at the extraction well and the estimated arrival time of the target
fraction at the extraction well.
- 20 4. A method according to any one of claims 1 to 3 wherein the monitoring comprises,
at spaced apart times, obtaining images of the channel and identifying areas in the
images corresponding to the reference fraction.
- 25 5. A method according to claim 4 wherein estimating the estimated time of arrival of
the target fraction comprises determining an average velocity of the target fraction
based on differences between the positions of the reference fraction in two or more
of the images.
- 30 6. A method according to claim 4 or 5 comprising, prior to moving the nucleic acids
along the channel combining the nucleic acids with a dye.
7. A method according to claim 6 wherein the dye comprises a fluorophore or a
chromophore.

8. A method according to claim 6 or 7 wherein obtaining each of the images comprises operating an imaging device to obtain a plurality of different exposures of the channel and combining the plurality of different exposures to yield the image, wherein the image has a greater dynamic range than any of the plurality of different exposures.
5
9. A method according to any one of claims 6 to 8 comprising illuminating the channel with visible light or UV light while obtaining the images.
- 10 10. A method according to claim 9 wherein the visible light or the UV light comprises a wavelength that corresponds to an absorption band of the dye.
11. A method according to any one of claims 1 to 10 comprising scheduling a time of arrival for the target fraction at the extraction well; comparing the scheduled time of arrival to the estimated time of arrival and adjusting one or more electrophoresis parameters of an electrophoresis signal based on any difference between the scheduled time of arrival and the estimated time of arrival.
15
12. A method according to claim 11 wherein adjusting the one or more electrophoresis parameters comprises adjusting a duty cycle of the electrophoresis signal.
20
13. A method according to claim 11 or 12 wherein adjusting the one or more electrophoresis parameters comprises adjusting a potential of the electrophoresis signal.
25
14. A method according to any one of claims 11 to 13 comprising controlling a rate of movement of the nucleic acids along the channel by proportional feedback control of the one or more electrophoresis parameters based on an error signal comprising a difference between the scheduled time of arrival and the estimated time of arrival.
30
15. A method according to any one of claims 11 to 14 comprising reducing a difference between the estimated arrival time and the scheduled arrival time by temporarily interrupting application of an electrophoresis signal to the channel.
35

16. A method according to any one of claims 1 to 15 comprising determining a location of the extraction well in the channel by image analysis.
- 5 17. A method according to claim 16 wherein estimating the estimated time of arrival of the target fraction of the nucleic acids at the extraction well is based in part on the location of the extraction well as determined by the image analysis.
- 10 18. A method according to any one of claims 1 to 17 wherein extracting fluid containing the target fraction from the extraction well is performed over a period determined automatically from a range of sizes of nucleic acid included in the target fraction.
- 15 19. A method according to claim 18 comprising estimating estimated times of arrival at the extraction well of leading and trailing edges of the target fraction and adjusting start and stop times for extraction of the target fraction on the basis of the estimated times of arrival at the extraction well of the leading and trailing edges of the target fraction.
- 20 20. A method according to claim 19 comprising automatically controlling the electrophoresis for the channel such that upon the leading edge of the target fraction arriving at the extraction well, the rate of electrophoresis is increased.
- 25 21. A method according to any one of claims 1 to 20 wherein the channel is one of a plurality of channels and the method is performed in parallel to process a plurality of samples in the plurality of channels.
- 30 22. A method according to claim 21 wherein the electrophoresis parameters of each of the plurality of channels are independently controlled.
- 35 23. A method according to claim 21 or 22 further comprising operating a robotic system comprising a pipetter to transfer the plurality of samples into the plurality of channels.
24. A method according to claim 23 wherein extracting the fluid containing the target fraction from the extraction well comprises operating the robotic system to place a pipette tip into the extraction well, operating the pipetter to withdraw the fluid

from the extraction well and operating the robotic system to transfer the removed fluid to a destination well.

25. A method according to claim 24 comprising automatically controlling the moving
5 of the nucleic acids in first and second ones of the channels such that the estimated time of arrival of the target fraction for the first channel is different from the estimated time of arrival of the target fraction for the second channel.
26. A method according to claim 23 wherein the pipetter comprises a multi-channel
10 pipetter and extracting the fluid containing the target fraction from the extraction well is performed simultaneously for a plurality of the channels by operating the robotic system to place pipette tips for the channels of the multi-channel pipetter into the extraction wells of a corresponding plurality of the channels, operating the
15 pipetter to withdraw the fluid from the extraction wells and operating the robotic system to transfer the removed fluid to a corresponding plurality of destination wells.
27. A method according to claim 26 comprising automatically controlling the moving
20 of the nucleic acids in the plurality of the channels such that the estimated time of arrival of the target fractions for each of the plurality of the channels is the same.
28. A method according to claim 27 wherein the target fractions for the plurality of channels comprise a plurality of different target fractions.
- 25 29. A method according to any one of claims 1 to 28 wherein the target fraction of the nucleic acid comprises an adapter joined to a nucleic acid molecule of interest, and the reference fraction of the nucleic acids comprise the adapter which is not joined to the nucleic acid molecule of interest.
- 30 30. A method according to any one of claims 1 to 29 wherein the monitoring
comprises automatically monitoring progress of a plurality of reference fractions of the nucleic acids along the channel wherein the reference fraction is one of the plurality of reference fractions of the nucleic acids.
- 35 31. A method according to claim 30 wherein said plurality of reference fractions of the nucleic acids comprise a DNA or RNA ladder of known sizes.

32. A method according to any one of claims 1 to 31 comprising specifying a size or size range of the target fraction of the nucleic acids.
- 5 33. A method according to any one of claims 1 to 32 wherein the nucleic acids comprise at least a first target fraction and a second target fraction, and the estimating and extracting steps comprise:
- estimating an estimated time of arrival of the first target fraction at the extraction well in the channel;
- 10 extracting fluid containing the first target fraction from the extraction well at the estimated time of arrival of the first target fraction;
- continuing electrophoresis in the channel;
- estimating an estimated time of arrival of the second target fraction at the extraction well in the channel; and
- 15 extracting fluid containing the second target fraction from the extraction well at the estimated time of arrival of the second target fraction.
34. A method according to claim 33 comprising transferring the fluid containing the first target fraction and the fluid containing the second target fraction to separate destination wells.
- 20
35. A method according to claim 33 comprising transferring the fluid containing the first target fraction and the fluid containing the second target fraction to the same destination well.
- 25
36. A method according to any one of claims 33 to 35 comprising scheduling a first scheduled time for extracting the first target fraction, and scheduling a second scheduled time for extracting the second target fraction.
- 30 37. A method according to claim 36 comprising controlling one or more electrophoresis parameters for the channel to bring the first target fraction to the extraction well at the first scheduled time.
38. A method according to claim 36 comprising controlling one or more
- 35 electrophoresis parameters for the channel to bring the second target fraction to the

extraction well at the second scheduled time after the first target fraction has been extracted.

39. A method according to any one of claims 33 to 38 wherein the nucleic acids
5 comprise a plurality of target fractions and the plurality of target fractions are
extracted from the extraction well of the channel at a plurality of different times.
40. A method according to any one of claims 21 to 28 wherein the nucleic acids in
10 each one of the plurality of channels comprise a plurality of target fractions and the
plurality of target fractions in each one of the channels are extracted from the
extraction well at a plurality of times.
41. A method according to claim 40 comprising controlling one or more
15 electrophoresis parameters for the channels to bring first target fractions of the
plurality of target fractions of the plurality of channels to the extraction wells at a
common first scheduled time.
42. A method according to claim 41 comprising controlling one or more
20 electrophoresis parameters for the channels to bring first target fractions of the
plurality of target fractions of the plurality of channels to the extraction wells at
different times.
43. A method according to claim 41 or 42 comprising controlling one or more
25 electrophoresis parameters for the channels to bring second target fractions of the
plurality of target fractions of the plurality of channels to the extraction wells at a
common second scheduled time.
44. A method according to claim 41 or 42 comprising controlling one or more
30 electrophoresis parameters for the channels to bring second target fractions of the
plurality of target fractions of the plurality of channels to the extraction wells at
different times.
45. A method for size selection of nucleic acids comprising:
35 loading samples containing nucleic acids into a plurality of channels, each
channel comprising an extraction well;
moving the nucleic acids toward the extraction wells by electrophoresis;

scheduling scheduled times for extraction of target fractions of the nucleic acids from the extraction wells;

monitoring progress of the target fractions toward the corresponding extraction wells; and

5 independently controlling electrophoresis in the channels based on the monitoring to cause the target fractions to reach the extraction wells at the scheduled times.

46. Apparatus for size-selection of nucleic acids, the apparatus comprising:
10 a channel having first and second ends and an extraction well in the channel;
an electrophoresis power supply connected to deliver an electrophoresis signal to the channel to move nucleic acids from a sample along the channel;
an imaging device mounted to image the channel;
15 a controller connected to obtain images from the imaging device and configured to:
automatically monitor progress of a reference fraction of the nucleic acids along the channel by analysis of the images;
based on the monitoring, estimate an estimated time of arrival of a target
20 fraction of the nucleic acids at the extraction well in the channel; and
operate a mechanism to extract fluid containing the target fraction from the extraction well at the estimated time of arrival.

47. Apparatus according to claim 46 wherein the imaging device comprises a camera.
25

48. Apparatus according to claim 47 wherein the imaging device comprises an illuminator configured to illuminate the channel with visible light or UV light.

49. Apparatus according to any one of claims 46 to 48 wherein the mechanism
30 comprises a robotic system comprising a pipetter operable to transfer a sample into a loading well in the channel and to extract the fluid from the extraction well.

50. Apparatus according to any one of claims 46 to 49 wherein the channel comprises
35 an elongated groove having opposed first and second sides and an electrophoresis medium in the groove, the first and second sides having steps extending

longitudinally along the first and second sides, the electrophoresis medium filling the groove up to the steps.

- 5 51. Apparatus according to claim 50 wherein the electrophoresis medium comprises a gel.
52. Apparatus according to claim 51 wherein the gel comprises an agarose gel.
- 10 53. Apparatus according to claim 51 wherein the gel comprises an acrylamide gel.
54. Apparatus according to claim 50 comprising first and second buffer reservoirs respectively located at first and second ends of the groove.
- 15 55. Apparatus according to claim 54 comprising first and second electrodes respectively in the first and second buffer reservoirs wherein the first and second electrodes are connected to the electrophoresis power supply.
- 20 56. Apparatus according to claim 55 comprising a hinged frame wherein the first and second electrodes are mounted on the hinged frame and the hinged frame is movable between a first position wherein the first and second electrodes are in the first and second buffer reservoirs and a second position wherein the first and second electrodes are removed from the first and second buffer reservoirs.
- 25 57. Apparatus according to any of claims 50 to 56 wherein the groove has a width in the range of 3 mm to 11 mm.
58. Apparatus according to any of claims 50 to 56 wherein the groove has a depth in the range of 6 mm to 12 mm.
- 30 59. Apparatus according to any one of claims 50 to 58 comprising a loading well in the medium wherein a layer of the medium lies between the loading well and each of the first and second sides of the groove.
- 35 60. Apparatus according to any one of claims 46 to 59 wherein the mechanism comprises a robot carrying a pipetter and the controller is configured to move a

pipette tip on the pipetter to a position over the extraction well, lower the pipette tip into the extraction well, and draw the fluid into the pipette tip.

- 5 61. Apparatus according to claim 60 wherein the controller is configured to determine a location of the extraction well in the channel by image analysis of one or more of the images and to move the pipette tip to the determined location of the extraction well.
- 10 62. Apparatus according to any one of claims 46 to 61 wherein the controller is configured to compare the estimated time of arrival of the target fraction at the extraction well to a desired time of arrival of the target fraction at the extraction well and to control the electrophoresis power supply to adjust one or more electrophoresis parameters of the electrophoresis signal based on any difference between the desired time of arrival and the estimated time of arrival.
- 15 63. Apparatus according to claim 62 wherein the one or more electrophoresis parameters comprise a duty cycle of the electrophoresis signal.
- 20 64. Apparatus according to claim 62 or 63 wherein the one or more electrophoresis parameters comprises a potential of the electrophoresis signal.
- 25 65. Apparatus according to any of claims 46 to 61 wherein the controller is configured to control a rate of movement of the nucleic acids along the channel by proportional feedback control of the one or more electrophoresis parameters based on an error signal comprising a difference between the estimated time of arrival and a desired time of arrival of the target fraction at the extraction well.
- 30 66. Apparatus according to claim 65 wherein the controller comprises a scheduler configured to generate the desired time of arrival of the target fraction at the extraction well.
- 35 67. Apparatus according to any one of claims 46 to 61 comprising a proportional feedback controller configured to control the electrophoresis power supply to vary an average speed of the target fraction along the channel in response to an error signal representing a difference between the estimated time of arrival of the target

fraction at the extraction well and a desired time of arrival of the target fraction at the extraction well.

68. Apparatus according to any of claims 62 to 67 wherein the controller is configured to reduce a difference between the estimated arrival time and the desired arrival time by temporarily interrupting application of the electrophoresis signal to the channel.
69. Apparatus according to any one of claims 46 to 68 comprising a plurality of channels wherein the channel is one of the plurality of channels.
70. Apparatus according to claim 65 comprising a plurality of channels wherein the channel is one of the plurality of channels and the controller is configured to control the electrophoresis signal for each of the plurality of channels such that the estimated time of arrival of the target fractions for each of the plurality of the channels is the same.
71. Apparatus according to claim 70 wherein the mechanism comprises a multi-channel pipetter configured to support a plurality of pipette tips and the pipette tips are spaced apart for simultaneous insertion into the extraction wells of the plurality of channels.
72. Apparatus according to claim 70 or 71 wherein the target fractions for the plurality of channels comprise a plurality of different target fractions.
73. Apparatus according to claim 65 comprising a plurality of channels wherein the channel is one of the plurality of channels and the controller is configured to control the electrophoresis signal for each of the plurality of channels such that the estimated time of arrival of the target fractions for each of the plurality of the channels is different.
74. Apparatus for size selection of nucleic acids comprising:
a plurality of channels each containing an electrophoresis medium with an extraction well formed therein;
a multi-channel electrophoresis power supply connectable to apply an electrophoresis potential between ends of each channel, the power supply

configured to independently control the electrophoresis potential in each of the plurality of channels;

a camera located to image the plurality of channels;

5 a controller connected to receive images from the camera and to control the power supply, the controller configured to process the images, monitor progress of target fractions of nucleic acid along the channels based on the images, and control the power supply to alter the electrophoresis potentials in the channels based on the monitored progress of the corresponding target fractions.

10 75. Apparatus according to claim 74 wherein the controller comprises a schedule for extracting the target fractions from the extraction wells and the controller is configured to control the electrophoresis potentials to cause the target fractions to arrive at the extraction wells at times according to the schedule.

15 76. Apparatus according to claim 75 comprising a robotic pipetting device wherein the controller is configured to control the robotic pipetting device to extract the target fractions from the extraction wells at the times according to the schedule.

20 77. A cassette for use in electrophoresis of nucleic acids, the cassette comprising a plate having a channel formed in the plate, the channel comprising an elongated groove having opposed first and second sides and an electrophoresis medium in the groove, first and second sides having steps, the electrophoresis medium filling the groove up to the steps.

25 78. A cassette according to claim 77 comprising a loading well and an extraction well at spaced apart locations along the channel.

79. A cassette according to claim 78 wherein the plate is transparent at least in its portion below the channel.

30 80. A cassette according to any one of claims 77 to 79 comprising first and second buffer reservoirs respectively located at first and second ends of the groove.

35 81. A cassette according to any one of claims 77 to 80 comprising a plurality or parallel channels arranged on the plate.

82. A cassette according to claim 81 comprising a first comb having pins projecting into the loading wells.
- 5 83. A cassette according to claim 82 comprising a first set of features aligned with the loading wells of the plurality of channels, wherein the first comb is engaged with the first set of features.
84. A cassette according to claim 83 wherein the pins of the first comb are narrower than the grooves.
- 10 85. A cassette according to any one of claims 82 to 84 comprising a second comb having pins projecting into the extraction wells.
- 15 86. A cassette according to claim 85 wherein the pins of the second comb are wider than the pins of the first comb.
87. A cassette according to claim 85 or 86 comprising a second set of features aligned with the extraction wells of the plurality of channels, wherein the second comb is engaged with the second set of features.
- 20 88. A cassette according to any one of claims 77 to 87 wherein the first and second walls of the channels comprise one or more projections or indentations for retaining the medium at locations near each of the first and second buffer reservoirs.
- 25 89. A cassette according to any one of claims 77 to 88 wherein the groove has a width in the range of 3 mm to 11 mm.
- 30 90. A cassette according to any one of claims 77 to 88 wherein the groove has a depth in the range of 6 mm to 12 mm.
91. A cassette according to any one of claims 77 to 90 comprising a sterile peel-off cover adhered over the channel.