

DE NOVO SYNTHESIZED GENE LIBRARIES

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 61/862445, filed August 5, 2013 and U.S. Provisional Application No. 61/862457, filed August 5, 2013, which applications are incorporated herein by reference.

BACKGROUND OF THE INVENTION

[0002] Highly efficient chemical gene synthesis with high fidelity and low cost has a central role in biotechnology and medicine, and in basic biomedical research.

[0003] De novo gene synthesis is a powerful tool for basic biological research and biotechnology applications. While various methods are known for the synthesis of relatively short fragments in a small scale, these techniques suffer from scalability, automation, speed, accuracy, and cost. There is a need for devices for simple, reproducible, scalable, less error-prone and cost-effective methods that guarantee successful synthesis of desired genes and are amenable to automation.

SUMMARY OF THE ~~DISCLOSURE~~INVENTION

[0004] As noted above, there exists a pressing need for methods, devices and systems that can quickly synthesize large gene libraries or relatively longer oligonucleotide fragments efficiently with less error. Similarly, there is also a need for methods that can partition and mix liquid reagents in a microfluidic scale for large numbers of individually addressable reactions in parallel. The present ~~disclosure invention~~ addresses these needs and provides related advantages as well.

[0005] In one aspect, the present ~~disclosure invention~~ provides a gene library as described herein. The gene library comprises a collection of genes. In some embodiments, the collection comprises at least 100 different preselected synthetic genes that can be of at least 0.5 kb length with an error rate of less than 1 in 3000 bp compared to predetermined sequences comprising the genes. In another aspect, the present ~~disclosure invention~~ also provides a gene library that comprises a collection of genes. The collection may comprise at least 100 different preselected synthetic genes that can be each of at least 0.5 kb length. At least 90% of the preselected synthetic genes may comprise an error rate of less than 1 in 3000 bp compared to predetermined sequences comprising the genes. Desired predetermined sequences may be supplied by any method, typically by a user, e.g. a user entering data using a computerized system. In various embodiments, synthesized nucleic acids are compared against these predetermined sequences, in some cases by sequencing at least a

portion of the synthesized nucleic acids, e.g. using next-generation sequencing methods. In some embodiments related to any of the gene libraries described herein, at least 90% of the preselected synthetic genes comprise an error rate of less than 1 in 5000 bp compared to predetermined sequences comprising the genes. In some embodiments, at least 0.05% of the preselected synthetic genes are error free. In some embodiments, at least 0.5% of the preselected synthetic genes are error free. In some embodiments, at least 90% of the preselected synthetic genes comprise an error rate of less than 1 in 3000 bp compared to predetermined sequences comprising the genes. In some embodiments, at least 90% of the preselected synthetic genes are error free or substantially error free. In some embodiments, the preselected synthetic genes comprise a deletion rate of less than 1 in 3000 bp compared to predetermined sequences comprising the genes. In some embodiments, the preselected synthetic genes comprise an insertion rate of less than 1 in 3000 bp compared to predetermined sequences comprising the genes. In some embodiments, the preselected synthetic genes comprise a substitution rate of less than 1 in 3000 bp compared to predetermined sequences comprising the genes. In some embodiments, the gene library as described herein further comprises at least 10 copies of each synthetic gene. In some embodiments, the gene library as described herein further comprises at least 100 copies of each synthetic gene. In some embodiments, the gene library as described herein further comprises at least 1000 copies of each synthetic gene. In some embodiments, the gene library as described herein further comprises at least 1000000 copies of each synthetic gene. In some embodiments, the collection of genes as described herein comprises at least 500 genes. In some embodiments, the collection comprises at least 5000 genes. In some embodiments, the collection comprises at least 10000 genes. In some embodiments, the preselected synthetic genes are at least 1kb. In some embodiments, the preselected synthetic genes are at least 2kb. In some embodiments, the preselected synthetic genes are at least 3kb. In some embodiments, the predetermined sequences comprise less than 20 bp in addition compared to the preselected synthetic genes. In some embodiments, the predetermined sequences comprise less than 15 bp in addition compared to the preselected synthetic genes. In some embodiments, at least one of the synthetic genes differs from any other synthetic gene by at least 0.1%. In some embodiments, each of the synthetic genes differs from any other synthetic gene by at least 0.1%. In some embodiments, at least one of the synthetic genes differs from any other synthetic gene by at least 10%. In some embodiments, each of the synthetic genes differs from any other synthetic gene by at least 10%. In some embodiments, at least one of the synthetic genes differs from any other synthetic gene by at least 2 base pairs. In some embodiments, each of the

synthetic genes differs from any other synthetic gene by at least 2 base pairs. In some embodiments, the gene library as described herein further comprises synthetic genes that are of less than 2kb with an error rate of less than 1 in 20000 bp compared to preselected sequences of the genes. In some embodiments, a subset of the deliverable genes is covalently linked together. In some embodiments, a first subset of the collection of genes encodes for components of a first metabolic pathway with one or more metabolic end products. In some embodiments, the gene library as described herein further comprises selecting of the one or more metabolic end products, thereby constructing the collection of genes. In some embodiments, the one or more metabolic end products comprise a biofuel. In some embodiments, a second subset of the collection of genes encodes for components of a second metabolic pathway with one or more metabolic end products. In some embodiments, the gene library is in a space that is less than 100 m³. In some embodiments, the gene library is in a space that is less than 1 m³. In some embodiments, the gene library is in a space that is less than 1 m³.

[0006] In another aspect, the present ~~disclosure invention~~ also provides a method of constructing a gene library. The method comprises the steps of: entering before a first timepoint, in a computer readable non-transient medium at least a first list of genes and a second list of genes, wherein the genes are at least 500 bp and when compiled into a joint list, the joint list comprises at least 100 genes; synthesizing more than 90% of the genes in the joint list before a second timepoint, thereby constructing a gene library with deliverable genes. In some embodiments, the second timepoint is less than a month apart from the first timepoint.

[0007] In practicing any of the methods of constructing a gene library as provided herein, the method as described herein further comprises delivering at least one gene at a second timepoint. In some embodiments, at least one of the genes differs from any other gene by at least 0.1% in the gene library. In some embodiments, each of the genes differs from any other gene by at least 0.1% in the gene library. In some embodiments, at least one of the genes differs from any other gene by at least 10% in the gene library. In some embodiments, each of the genes differs from any other gene by at least 10% in the gene library. In some embodiments, at least one of the genes differs from any other gene by at least 2 base pairs in the gene library. In some embodiments, each of the genes differs from any other gene by at least 2 base pairs in the gene library. In some embodiments, at least 90% of the deliverable genes are error free. In some embodiments, the deliverable genes comprises an error rate of less than 1/3000 resulting in the generation of a sequence that deviates from the sequence of a gene in the joint list of genes. In some embodiments, at least 90% of the deliverable genes comprise an error rate of less than 1 in

3000 bp resulting in the generation of a sequence that deviates from the sequence of a gene in the joint list of genes. In some embodiments, genes in a subset of the deliverable genes are covalently linked together. In some embodiments, a first subset of the joint list of genes encode for components of a first metabolic pathway with one or more metabolic end products. In some embodiments, any of the methods of constructing a gene library as described herein further comprises selecting of the one or more metabolic end products, thereby constructing the first, the second or the joint list of genes. In some embodiments, the one or more metabolic end products comprise a biofuel. In some embodiments, a second subset of the joint list of genes encode for components of a second metabolic pathway with one or more metabolic end products. In some embodiments, the joint list of genes comprises at least 500 genes. In some embodiments, the joint list of genes comprises at least 5000 genes. In some embodiments, the joint list of genes comprises at least 10000 genes. In some embodiments, the genes can be at least 1kb. In some embodiments, the genes are at least 2kb. In some embodiments, the genes are at least 3kb. In some embodiments, the second timepoint is less than 25 days apart from the first timepoint. In some embodiments, the second timepoint is less than 5 days apart from the first timepoint. In some embodiments, the second timepoint is less than 2 days apart from the first timepoint. It is noted that any of the embodiments described herein can be combined with any of the methods, devices or systems provided in the current ~~disclosure~~invention.

[0008] In another aspect, a method of constructing a gene library is provided herein. The method comprises the steps of: entering at a first timepoint, in a computer readable non-transient medium a list of genes; synthesizing more than 90% of the list of genes, thereby constructing a gene library with deliverable genes; and delivering the deliverable genes at a second timepoint. In some embodiments, the list comprises at least 100 genes and the genes can be at least 500 bp. In still yet some embodiments, the second timepoint is less than a month apart from the first timepoint.

[0009] In practicing any of the methods of constructing a gene library as provided herein, in some embodiments, the method as described herein further comprises delivering at least one gene at a second timepoint. In some embodiments, at least one of the genes differs from any other gene by at least 0.1% in the gene library. In some embodiments, each of the genes differs from any other gene by at least 0.1% in the gene library. In some embodiments, at least one of the genes differs from any other gene by at least 10% in the gene library. In some embodiments, each of the genes differs from any other gene by at least 10% in the gene library. In some embodiments, at least one of the genes differs from any other gene by at least 2 base pairs in the

gene library. In some embodiments, each of the genes differs from any other gene by at least 2 base pairs in the gene library. In some embodiments, at least 90% of the deliverable genes are error free. In some embodiments, the deliverable genes comprises an error rate of less than 1/3000 resulting in the generation of a sequence that deviates from the sequence of a gene in the list of genes. In some embodiments, at least 90% of the deliverable genes comprise an error rate of less than 1 in 3000 bp resulting in the generation of a sequence that deviates from the sequence of a gene in the list of genes. In some embodiments, genes in a subset of the deliverable genes are covalently linked together. In some embodiments, a first subset of the list of genes encode for components of a first metabolic pathway with one or more metabolic end products. In some embodiments, the method of constructing a gene library further comprises selecting of the one or more metabolic end products, thereby constructing the list of genes. In some embodiments, the one or more metabolic end products comprise a biofuel. In some embodiments, a second subset of the list of genes encode for components of a second metabolic pathway with one or more metabolic end products. It is noted that any of the embodiments described herein can be combined with any of the methods, devices or systems provided in the current ~~disclosure~~invention.

[0010] In practicing any of the methods of constructing a gene library as provided herein, in some embodiments, the list of genes comprises at least 500 genes. In some embodiments, the list comprises at least 5000 genes. In some embodiments, the list comprises at least 10000 genes. In some embodiments, the genes are at least 1kb. In some embodiments, the genes are at least 2kb. In some embodiments, the genes are at least 3kb. In some embodiments, the second timepoint as described in the methods of constructing a gene library is less than 25 days apart from the first timepoint. In some embodiments, the second timepoint is less than 5 days apart from the first timepoint. In some embodiments, the second timepoint is less than 2 days apart from the first timepoint. It is noted that any of the embodiments described herein can be combined with any of the methods, devices or systems provided in the current ~~disclosure~~invention.

[0011] In another aspect, the present ~~disclosure~~invention also provides a method of synthesizing n-mer oligonucleotides on a substrate. The method comprises a) providing a substrate with resolved loci that are functionalized with a chemical moiety suitable for nucleotide coupling; and b) coupling at least two building blocks to a plurality of growing oligonucleotide chains each residing on one of the resolved loci at a rate of at least 12 nucleotides per hour according to a locus specific predetermined sequence, thereby synthesizing a plurality of oligonucleotides that

are n basepairs long. Various embodiments related to the method of synthesizing n-mer oligonucleotides on a substrate are described herein.

[0012] In any of the methods of synthesizing n-mer oligonucleotides on a substrate as provided herein, in some embodiments, the methods further comprise coupling at least two building blocks to a plurality of growing oligonucleotide chains each residing on one of the resolved loci at a rate of at least 15 nucleotides per hour. In some embodiments, the method further comprises coupling at least two building blocks to a plurality of growing oligonucleotide chains each residing on one of the resolved loci at a rate of at least 20 nucleotides per hour. In some embodiments, the method further comprises coupling at least two building blocks to a plurality of growing oligonucleotide chains each residing on one of the resolved loci at a rate of at least 25 nucleotides per hour. In some embodiments, at least one resolved locus comprises n-mer oligonucleotides deviating from the locus specific predetermined sequence with an error rate of less than 1/500 bp. In some embodiments, at least one resolved locus comprises n-mer oligonucleotides deviating from the locus specific predetermined sequence with an error rate of less than 1/1000 bp. In some embodiments, at least one resolved locus comprises n-mer oligonucleotides deviating from the locus specific predetermined sequence with an error rate of less than 1/2000 bp. In some embodiments, the plurality of oligonucleotides on the substrate deviate from respective locus specific predetermined sequences at an error rate of less than 1/500 bp. In some embodiments, the plurality of oligonucleotides on the substrate deviate from respective locus specific predetermined sequences at an error rate of less than 1/1000 bp. In some embodiments, the plurality of oligonucleotides on the substrate deviate from respective locus specific predetermined sequences at an error rate of less than 1/2000 bp.

[0013] In practicing any of the methods of synthesizing n-mer oligonucleotides on a substrate as provided herein, in some embodiments, the building blocks comprise an adenine, guanine, thymine, cytosine, or uridine group, or a modified nucleotide. In some embodiments, the building blocks comprise a modified nucleotide. In some embodiments, the building blocks comprise dinucleotides or trinucleotides. In some embodiments, the building blocks comprise phosphoramidite. In some embodiments, n of the n-mer oligonucleotides is at least 100. In some embodiments, n is at least 200. In some embodiments, n is at least 300. In some embodiments, n is at least 400. In some embodiments, the surface comprises at least 100,000 resolved loci and at least two of the plurality of growing oligonucleotides can be different from each other.

[0014] In some embodiments, the method of synthesizing n-mer oligonucleotides on a substrate as described herein further comprises vacuum drying the substrate before coupling. In some embodiments, the building blocks comprise a blocking group. In some embodiments, the blocking group comprises an acid-labile DMT. In some embodiments, the acid-labile DMT comprises 4,4'-dimethoxytrityl. In some embodiments, the method of synthesizing n-mer oligonucleotides on a substrate as described herein further comprises oxidation or sulfurization. In some embodiments, the method of synthesizing n-mer oligonucleotides on a substrate as described herein further comprises chemically capping uncoupled oligonucleotide chains. In some embodiments, the method of synthesizing n-mer oligonucleotides on a substrate as described herein further comprises removing the blocking group, thereby deblocking the growing oligonucleotide chain. In some embodiments, the position of the substrate during the coupling step is within 10 cm of the position of the substrate during the vacuum drying step. In some embodiments, the position of the substrate during the coupling step is within 10 cm of the position of the substrate during the oxidation step. In some embodiments, the position of the substrate during the coupling step is within 10 cm of the position of the substrate during the capping step. In some embodiments, the position of the substrate during the coupling step is within 10 cm of the position of the substrate during the deblocking step. In some embodiments, the substrate comprises at least 10,000 vias providing fluid communication between a first surface of the substrate and a second surface of the substrate. In some embodiments, the substrate comprises at least 100,000 vias providing fluid communication between a first surface of the substrate and a second surface of the substrate. In some embodiments, the substrate comprises at least 1,000,000 vias providing fluid communication between a first surface of the substrate and a second surface of the substrate. It is noted that any of the embodiments described herein can be combined with any of the methods, devices or systems provided in the current ~~disclosure~~invention.

[0015] In another aspect of the present ~~disclosure~~invention, a system for conducting a set of parallel reactions is provided herein. The system comprises: a first surface with a plurality of resolved loci; a capping element with a plurality of resolved reactor caps. In some embodiments, the system aligns the plurality of resolved reactor caps with the plurality of resolved loci on the first surface forming a temporary seal between the first surface and the capping element, thereby physically dividing the loci on the first surface into groups of at least two loci into a reactor associated with each reactor cap. In some embodiments, each reactor holds a first set of reagents.

[0016] In some embodiments related to any of the systems for conducting a set of parallel reactions as described herein, upon release from the first surface, the reactor caps retain at least a portion of the first set of reagents. In some embodiments, the portion is about 30%. In some embodiments, the portion is about 90%. In some embodiments, the plurality of resolved loci resides on microstructures fabricated into a support surface. In some embodiments, the plurality of resolved loci is at a density of at least 1 per mm². In some embodiments, the plurality of resolved loci is at a density of at least 10 per mm². In some embodiments, the plurality of resolved loci are at a density of at least 100 per mm². In some embodiments, the microstructures comprise at least two channels in fluidic communication with each other. In some embodiments, the at least two channels comprise two channels with different width. In some embodiments, at least two channels comprise two channels with different length. In some embodiments, at least one of the channels is longer than 100 μm. In some embodiments, at least one of the channels is shorter than 1000 μm. In some embodiments, at least one of the channels is wider than 50 μm in diameter. In some embodiments, at least one of the channels is narrower than 100 μm in diameter. In some embodiments, the system further comprises a second surface with a plurality of resolved loci at a density of at least 0.1 per mm². In some embodiments, the system further comprises a second surface with a plurality of resolved loci at a density of at least 1 per mm². In some embodiments, the system further comprises a second surface with a plurality of resolved loci at a density of at least 10 per mm².

[0017] In some embodiments related to any of the systems for conducting a set of parallel reactions as described herein, the resolved loci of the first surface comprise a coating of reagents. In some embodiments, the resolved loci of the second surface comprise a coating of reagents. In some embodiments, the coating of reagents is covalently linked to the first or second surface. In some embodiments, the coating of reagents comprises oligonucleotides. In some embodiments, the coating of reagents has a surface area of at least 1.45 μm² per 1.0 μm² of planar surface area. In some embodiments, the coating of reagents has a surface area of at least 1.25 μm² per 1.0 μm² of planar surface area. In some embodiments, the coating of reagents has a surface area of at least 1 μm² per 1.0 μm² of planar surface area. In some embodiments, the resolved loci in the plurality of resolved loci comprise a nominal arclength of the perimeter at a density of at least 0.001 μm/μm². In some embodiments, the resolved loci in the plurality of resolved loci comprise a nominal arclength of the perimeter at a density of at least 0.01 μm/μm². In some embodiments, the resolved loci in the plurality of resolved loci of the first surface comprise a high energy surface. In some embodiments, the first and second surfaces comprise a different

surface tension with a given liquid. In some embodiments, the high surface energy corresponds to a water contact angle of less than 20 degree. In some embodiments, the plurality of resolved loci are located on a solid substrate comprising a material selected from the group consisting of silicon, polystyrene, agarose, dextran, cellulosic polymers, polyacrylamides, PDMS, and glass. In some embodiments, the capping elements comprise a material selected from the group consisting of silicon, polystyrene, agarose, dextran, cellulosic polymers, polyacrylamides, PDMS, and glass. It is noted that any of the embodiments described herein can be combined with any of the methods, devices or systems provided in the current ~~disclosure~~invention.

[0018] In yet another aspect, the present ~~disclosure~~invention also provides an array of enclosures. The array of enclosures comprise: a plurality of resolved reactors comprising a first substrate and a second substrate comprising reactor caps; at least 2 resolved loci in each reactor. In some cases, the resolved reactors are separated with a releasable seal. In some cases, the reactor caps retain at least a part of the contents of the reactors upon release of the second substrate from the first substrate. In some embodiments, the reactor caps on the second substrate have a density of at least 0.1 per mm². In some embodiments, reactor caps on the second substrate have a density of at least 1 per mm². In some embodiments, reactor caps on the second substrate have a density of at least 10 per mm².

[0019] In some embodiments related to the array of enclosures as provided herein, the reactor caps retain at least 30% of the contents of the reactors. In some embodiments, the reactor caps retain at least 90% of the contents of the reactors. In some embodiments, the resolved loci are at a density of at least 2/mm². In some embodiments, the resolved loci are at a density of at least 100/mm². In some embodiments, the array of enclosures further comprises at least 5 resolved loci in each reactor. In some embodiments, the array of enclosures as described herein further comprises at least 20 resolved loci in each reactor. In some embodiments, the array of enclosures as described herein further comprises at least 50 resolved loci in each reactor. In some embodiments, the array of enclosures as described herein further comprises at least 100 resolved loci in each reactor.

[0020] In some embodiments related to the array of enclosures as described herein, the resolved loci reside on microstructures fabricated into a support surface. In some embodiments, the microstructures comprise at least two channels in fluidic communication with each other. In some embodiments, the at least two channels comprise two channels with different width. In some embodiments, the at least two channels comprise two channels with different length. In some embodiments, at least one of the channels is longer than 100 μm. In some embodiments, at

least one of the channels is shorter than 1000 μm . In some embodiments, at least one of the channels is wider than 50 μm in diameter. In some embodiments, at least one of the channels is narrower than 100 μm in diameter. In some embodiments, the microstructures comprise a nominal arclength of the perimeter of the at least two channels that has a density of at least 0.01 μm / square μm . In some embodiments, the microstructures comprise a nominal arclength of the perimeter of the at least two channels that has a density of at least 0.001 μm / square μm . In some embodiments, the resolved reactors are separated with a releasable seal. In some embodiments, the seal comprises a capillary burst valve.

[0021] In some embodiments related to the array of enclosures as described herein, the plurality of resolved loci of the first substrate comprise a coating of reagents. In some embodiments, the plurality of resolved loci of the second substrate comprises a coating of reagents. In some embodiments, the coating of reagents is covalently linked to the first or second surface. In some embodiments, the coating of reagents comprises oligonucleotides. In some embodiments, the coating of reagents has a surface area of at least 1 μm^2 per 1.0 μm^2 of planar surface area. In some embodiments, the coating of reagents has a surface area of at least 1.25 μm^2 per 1.0 μm^2 of planar surface area. In some embodiments, the coating of reagents has a surface area of at least 1.45 μm^2 per 1.0 μm^2 of planar surface area. In some embodiments, the plurality of resolved loci of the first substrate comprises a high energy surface. In some embodiments, the first and second substrates comprise a different surface tension with a given liquid. In some embodiments, the surface energy corresponds to a water contact angle of less than 20 degree. In some embodiments, the plurality of resolved loci or the reactor caps are located on a solid substrate comprising a material selected from the group consisting of silicon, polystyrene, agarose, dextran, cellulosic polymers, polyacrylamides, PDMS, and glass. It is noted that any of the embodiments described herein can be combined with any of the methods, devices, arrays or systems provided in the current ~~disclosure~~ invention.

[0022] In still yet another aspect, the present ~~disclosure~~ invention also provides a method of conducting a set of parallel reactions. The method comprises: (a) providing a first surface with a plurality of resolved loci; (b) providing a capping element with a plurality of resolved reactor caps; (c) aligning the plurality of resolved reactor caps with the plurality of resolved loci on the first surface and forming a temporary seal between the first surface and the capping element, thereby physically dividing the loci on the first surface into groups of at least two loci; (d) performing a first reaction, thereby forming a first set of reagents; and (e) releasing the capping element from the first surface, wherein each reactor cap retains at least a portion of the first set of reagents in a

first reaction volume. In some embodiments, the portion is about 30%. In some embodiments, the portion is about 90%.

[0023] In some embodiments, the method of conducting a set of parallel reactions as described herein further comprises the steps of: (f) providing a second surface with a plurality of resolved loci; (g) aligning the plurality of resolved reactor caps with the plurality of resolved loci on the second surface and forming a temporary seal between the second surface and the capping element, thereby physically dividing the loci on the second surface; (h) performing a second reaction using the portion of the first set of reagents, thereby forming a second set of reagents; and (i) releasing the capping element from the second surface, wherein each reactor cap can retain at least a portion of the second set of reagents in a second reaction volume. In some embodiments, the portion is about 30%. In some embodiments, the portion is about 90%.

[0024] In practicing any of the methods of conducting a set of parallel reactions as described herein, the plurality of resolved loci can have a density of at least 1 per mm^2 on the first surface. In some embodiments, the plurality of resolved loci have a density of at least 10 per mm^2 on the first surface. In some embodiments, the plurality of resolved loci have a density of at least 100 per mm^2 on the first surface. In some embodiments, the plurality of resolved reactor caps have a density of at least 0.1 per mm^2 on the capping element. In some embodiments, the plurality of resolved reactor caps have a density of at least 1 per mm^2 on the capping element. In some embodiments, the plurality of resolved reactor caps have a density of at least 10 per mm^2 on the capping element. In some embodiments, the plurality of resolved loci have a density of more than 0.1 per mm^2 on the second surface. In some embodiments, the plurality of resolved loci have a density of more than 1 per mm^2 on the second surface. In some embodiments, the plurality of resolved loci have a density of more than 10 per mm^2 on the second surface.

[0025] In practicing any of the methods of conducting a set of parallel reactions as described herein, the releasing of the capping elements from the surface steps such as the releasing steps in (e) and (i) as described herein can be performed at a different velocity. In some embodiments, the resolved loci of the first surface comprise a coating of reagents for the first reaction. In some embodiments, the resolved loci of the second surface comprise a coating of reagents for the second reaction. In some embodiments, the coating of reagents is covalently linked to the first or second surface. In some embodiments, the coating of reagents comprises oligonucleotides. In some embodiments, the coating of reagents has a surface area of at least 1 μm^2 per 1.0 μm^2 of planar surface area. In some embodiments, the coating of reagents has a surface area of at least 1.25 μm^2 per 1.0 μm^2 of planar surface area. In some embodiments, the coating of reagents has a

surface area of at least $1.45 \mu\text{m}^2$ per $1.0 \mu\text{m}^2$ of planar surface area. In some embodiments, the oligonucleotides are at least 25 bp. In some embodiments, the oligonucleotides are at least 200 bp. In some embodiments, the oligonucleotides are at least 300 bp. In some embodiments, the resolved loci of the first surface comprise a high energy surface. In some embodiments, the first and second surfaces comprise a different surface tension with a given liquid. In some embodiments, the surface energy corresponds to a water contact angle of less than 20 degree.

[0026] In some embodiments related to the method of conducting a set of parallel reactions as described herein, the plurality of resolved loci or the resolved reactor caps are located on a solid substrate comprising a material selected from the group consisting of silicon, polystyrene, agarose, dextran, cellulosic polymers, polyacrylamides, PDMS, and glass. In some embodiments, the first and second reaction volumes are different. In some embodiments, the first or second reaction comprises polymerase cycling assembly. In some embodiments, the first or second reaction comprises enzymatic gene synthesis, annealing and ligation reaction, simultaneous synthesis of two genes via a hybrid gene, shotgun ligation and co-ligation, insertion gene synthesis, gene synthesis via one strand of DNA, template-directed ligation, ligase chain reaction, microarray-mediated gene synthesis, solid-phase assembly, Sloning building block technology, or RNA ligation mediated gene synthesis. In some embodiments, the methods of conducting a set of parallel reactions as described herein further comprises cooling the capping element. In some embodiments, the method of conducting a set of parallel reactions as described herein further comprises cooling the first surface. In some embodiments, the method of conducting a set of parallel reactions as described herein further comprises cooling the second surface. It is noted that any of the embodiments described herein can be combined with any of the methods, devices, arrays or systems provided in the current ~~disclosure~~invention.

[0027] In another aspect, the present ~~disclosure~~invention provides a substrate having a functionalized surface. The substrate having a functionalized surface can comprise a solid support having a plurality of resolved loci. In some embodiments, the resolved loci are functionalized with a moiety that increases the surface energy of the solid support. In some embodiments, the resolved loci are localized on microchannels.

[0028] In some embodiments related to the substrate having a functionalized surface as described herein, the moiety is a chemically inert moiety. In some embodiments, the microchannels comprise a volume of less than 1 nl. In some embodiments, the microchannels comprise a density of the nominal arclength of the perimeter of $0.036 \mu\text{m}$ / square μm . In some embodiments, the functionalized surface comprises a nominal surface area of at least $1 \mu\text{m}^2$ per

1.0 μm^2 of planar surface area of the substrate. In some embodiments, the functionalized surface comprises a nominal surface area of at least 1.25 μm^2 per 1.0 μm^2 of planar surface area of the substrate. In some embodiments, the functionalized surface comprises a nominal surface area of at least 1.45 μm^2 per 1.0 μm^2 of planar surface area of the substrate. In some embodiments, the resolved loci in the plurality of resolved loci comprise a coating of reagents. In some embodiments, the coating of reagents is covalently linked to the substrate. In some embodiments, the coating of reagents comprises oligonucleotides. In some embodiments, at least one of the microchannels is longer than 100 μm . In some embodiments, at least one of the microchannels is shorter than 1000 μm . In some embodiments, at least one of the microchannels is wider than 50 μm in diameter. In some embodiments, at least one of the microchannels is narrower than 100 μm in diameter. In some embodiments, the surface energy corresponds to a water contact angle of less than 20 degree. In some embodiments, the solid support comprises a material selected from the group consisting of silicon, polystyrene, agarose, dextran, cellulosic polymers, polyacrylamides, PDMS, and glass. In some embodiments, the plurality of resolved loci is at a density of at least 1/ mm^2 . In some embodiments, the plurality of resolved loci is at a density of at least 100/ mm^2 . It is noted that any of the embodiments described herein can be combined with any of the methods, devices, arrays, substrates or systems provided in the current ~~disclosure~~invention.

[0029] In another aspect, the present ~~disclosure~~invention also provides a method for synthesizing oligonucleotides on a substrate having a functionalized surface. The method comprises: (a) applying through at least one inkjet pump at least one drop of a first reagent to a first locus of a plurality of loci; (b) applying negative pressure to the substrate; and (c) applying through at least one inkjet pump at least one drop of a second reagent to the first locus.

[0030] In practicing any of the methods for synthesizing oligonucleotides on a substrate having a functionalized surface as described herein, the first and second reagents can be different. In some embodiments, the first locus is functionalized with a moiety that increases their surface energy. In some embodiments, the moiety is a chemically inert moiety. In some embodiments, the plurality of loci resides on microstructures fabricated into the substrate surface. In some embodiments, the microstructures comprise at least two channels in fluidic communication with each other. In some embodiments, the at least two channels comprise two channels with different width. In some embodiments, the at least two channels comprise two channels with different length. In some embodiments, at least one of the channels is longer than 100 μm . In some embodiments, at least one of the channels is shorter than 1000 μm . In some

embodiments, at least one of the channels is wider than 50 μm in diameter. In some embodiments, at least one of the channels is narrower than 100 μm in diameter. In some embodiments, the substrate surface comprises a material selected from the group consisting of silicon, polystyrene, agarose, dextran, cellulosic polymers, polyacrylamides, PDMS, and glass.

[0031] In some embodiments related to the methods for synthesizing oligonucleotides on a substrate having a functionalized surface as described herein, the volume of the drop of the first and/or the second reagents is at least 2 pl. In some embodiments, the volume of the drop is about 40 pl. In some embodiments, the volume of the drop is at most 100 pl. In some embodiments, the microchannels comprise a density of the nominal arclength of the perimeter of at least 0.01 $\mu\text{m}/\mu\text{m}^2$. In some embodiments, the microchannels comprise a density of the nominal arclength of the perimeter of at least 0.001 $\mu\text{m}/\mu\text{m}^2$. In some embodiments, the functionalized surface comprises a nominal surface area of at least 1 μm^2 per 1.0 μm^2 of planar surface area of the substrate. In some embodiments, the functionalized surface comprises a nominal surface area of at least 1.25 μm^2 per 1.0 μm^2 of planar surface area of the substrate. In some embodiments, the functionalized surface comprises a nominal surface area of at least 1.45 μm^2 per 1.0 μm^2 of planar surface area of the substrate. In some embodiments, the pressure surrounding the substrate is reduced to less than 1 mTorr. It is noted that any of the embodiments described herein can be combined with any of the methods, devices, arrays, substrates or systems provided in the current ~~disclosure~~invention.

[0032] In some embodiments, the method for synthesizing oligonucleotides on a substrate having a functionalized surface as described herein further comprises coupling at least a first building block originating from the first drop to a growing oligonucleotide chain on the first locus. In some embodiments, the building blocks comprise a blocking group. In some embodiments, the blocking group comprises an acid-labile DMT. In some embodiments, the acid-labile DMT comprises 4,4'-dimethoxytrityl. In some embodiments, the method for synthesizing oligonucleotides on a substrate having a functionalized surface as described herein further comprises oxidation or sulfurization. In some embodiments, the method for synthesizing oligonucleotides on a substrate having a functionalized surface as described herein further comprises chemically capping uncoupled oligonucleotide chains. In some embodiments, the method for synthesizing oligonucleotides on a substrate having a functionalized surface as described herein further comprises removing the blocking group, thereby deblocking the growing oligonucleotide chain. In some embodiments, the position of the substrate during the negative pressure application is within 10 cm of the position of the substrate during the coupling

step. In some embodiments, the position of the substrate during the negative pressure application is within 10 cm of the position of the substrate during the oxidation step. In some embodiments, the position of the substrate during the negative pressure application is within 10 cm of the position of the substrate during the capping step. In some embodiments, the position of the substrate during the negative pressure application is within 10 cm of the position of the substrate during the deblocking step. In some embodiments, the first locus resides on a microstructure fabricated into the substrate surface. In some embodiments, at least one reagent for the oxidation step is provided by flooding the microstructure with a solution comprising the at least one reagent. In some embodiments, at least one reagent for the capping step is provided by flooding the microstructure with a solution comprising the at least one reagent. In some embodiments, the first locus resides on a microstructure fabricated into the substrate surface and at least one reagent for the deblocking step can be provided by flooding the microstructure with a solution comprising the at least one reagent. In some embodiments, the method for synthesizing oligonucleotides on a substrate having a functionalized surface as described herein further comprises enclosing the substrate within a sealed chamber. In some embodiments, the sealed chamber allows for purging of liquids from the first locus. In some embodiments, the method for synthesizing oligonucleotides on a substrate having a functionalized surface as described herein further comprises draining a liquid through a drain that is operably linked to the first locus. In some embodiments, after applying the negative pressure to the substrate, the moisture content on the substrate is less than 1 ppm. In some embodiments, the surface energy is increased corresponding to a water contact angle of less than 20 degree. It is noted that any of the embodiments described herein can be combined with any of the methods, devices, arrays, substrates or systems provided in the current ~~disclosure~~invention.

[0033] In yet another aspect, the present ~~disclosure~~invention provides a method of depositing reagents to a plurality of resolved loci. The method comprises applying through an inkjet pump at least one drop of a first reagent to a first locus of the plurality of loci; applying through an inkjet pump at least one drop of a second reagent to a second locus of the plurality of resolved loci. In some embodiments, the second locus is adjacent to the first locus. In still some embodiments, the first and second reagents are different. In still yet some embodiments, the first and second loci reside on microstructures fabricated into a support surface. In yet some embodiments, the microstructures comprise at least one channel that is more than 100 μm deep.

[0034] In practicing any of the methods of depositing reagents to a plurality of resolved loci as described herein, in some embodiments, the microstructures comprise at least two channels in

fluidic communication with each other. In some embodiments, the at least two channels comprise two channels with different width. In some embodiments, the at least two channels comprise two channels with different length. In some embodiments, the first locus receives less than 0.1% of the second reagent and the second locus receives less than 0.1% of the first reagent. In some embodiments, the loci comprise a density of the nominal arclength of the perimeter of at least $0.01 \mu\text{m} / \text{square } \mu\text{m}$. In some embodiments, the loci comprise a density of the nominal arclength of the perimeter of at least $0.001 \mu\text{m} / \text{square } \mu\text{m}$. In some embodiments, the first and second loci comprise a coating of reagents. In some embodiments, the coating of reagents is covalently linked to the substrate. In some embodiments, the coating of reagents comprises oligonucleotides. In some embodiments, at least one of the channels is longer than $100 \mu\text{m}$. In some embodiments, at least one of the channels is shorter than $1000 \mu\text{m}$. In some embodiments, at least one of the channels is wider than $50 \mu\text{m}$ in diameter. In some embodiments, at least one of the channels is narrower than $100 \mu\text{m}$ in diameter. In some embodiments, the support surface comprises a material selected from the group consisting of silicon, polystyrene, agarose, dextran, cellulosic polymers, polyacrylamides, PDMS, and glass. In some embodiments, the plurality of resolved loci is at a density of at least $1/\text{mm}^2$. In some embodiments, the plurality of resolved loci is at a density of at least $100/\text{mm}^2$. In some embodiments, the volume of the drop is at least 2 pl. In some embodiments, the volume of the drop is about 40 pl. In some embodiments, the volume of the drop is at most 100 pl. It is noted that any of the embodiments described herein can be combined with any of the methods, devices, arrays, substrates or systems provided in the current ~~disclosure~~invention.

[0035] In still yet another aspect, the present ~~disclosure~~invention provides a microfluidic system. The microfluidic system comprises a first surface with a plurality of microwells at a density of at least 10 per mm^2 ; and a droplet inside one of the plurality of microwells. In some embodiments, the droplet inside one of the plurality of microwells has a Reynolds number at a range of about 1-1000. In some embodiments, the plurality of microwells is at a density of at least 1 per mm^2 . In some embodiments, plurality of microwells is at a density of at least 10 per mm^2 .

[0036] In some embodiments related to the microfluidic system as provided herein, the microfluidic system further comprises an inkjet pump. In some embodiments, the droplet is deposited by the inkjet pump. In some embodiments, the droplet is moving in the lower half of a first microwell dimension. In some embodiments, the droplet is moving in the middle third of a first microwell dimension. In some embodiments, the plurality of microwells is at a density of at least 100 per mm^2 . In some embodiments, the first microwell dimension is larger than the

droplet. In some embodiments, the microwell is longer than 100 μm . In some embodiments, the microwell is shorter than 1000 μm . In some embodiments, the microwell is wider than 50 μm in diameter. In some embodiments, the microwell is narrower than 100 μm in diameter. In some embodiments, the volume of the droplet is at least 2 pl. In some embodiments, the volume of the droplet is about 40 pl. In some embodiments, the volume of the droplet is at most 100 pl. In some embodiments, each of the plurality of microwells is fluidically connected to at least one microchannel. In some embodiments, the at least one microchannel is coated with a moiety that increases surface energy. In some embodiments, the moiety is a chemically inert moiety. In some embodiments, the surface energy corresponds to a water contact angle of less than 20 degree. In some embodiments, the microwells are formed on a solid support comprising a material selected from the group consisting of silicon, polystyrene, agarose, dextran, cellulosic polymers, polyacrylamides, PDMS, and glass. In some embodiments, the microchannels comprise a density of the nominal arclength of the perimeter of at least 0.01 μm / square μm . In some embodiments, the microchannels comprise a density of the nominal arclength of the perimeter of 0.001 μm / μm^2 . In some embodiments, the surface coated with the moiety comprises a nominal surface area of at least 1 μm^2 per 1.0 μm^2 of planar surface area of the first surface. In some embodiments, the surface coated with the moiety comprises a nominal surface area of at least 1.25 μm^2 per 1.0 μm^2 of planar surface area of the first surface. In some embodiments, the surface coated with the moiety comprises a nominal surface area of at least 1.45 μm^2 per 1.0 μm^2 of planar surface area of the first surface. It is noted that any of the embodiments described herein can be combined with any of the methods, devices, arrays, substrates or systems provided in the current ~~disclosure~~invention. In some embodiments, the droplet comprises a reagent that enables oligonucleotide synthesis. In some embodiments, the reagent is a nucleotide or nucleotide analog.

[0037] In another aspect, the present ~~disclosure~~invention provides a method of depositing droplets to a plurality of microwells. The method comprises applying through an inkjet pump at least one droplet to a first microwell of the plurality of microwells. In some cases, the droplet inside one of the plurality of microwells has a Reynolds number at a range of about 1-1000. In some embodiments, the plurality of microwells has a density of at least 1/ mm^2 . In yet some cases, the plurality of microwells has a density of at least 10/ mm^2 .

[0038] In practicing any of the methods of depositing droplets to a plurality of microwells as provided herein, the plurality of microwells can have a density of at least 100/ mm^2 . In some embodiments, the microwell is longer than 100 μm . In some embodiments, the microwell is

shorter than 1000 μm . In some embodiments, the microwell is wider than 50 μm in diameter. In some embodiments, the microwell is narrower than 100 μm in diameter. In some embodiments, the droplet is applied at a velocity of at least 2 m/sec. In some embodiments, the volume of the droplet is at least 2 pl. In some embodiments, the volume of the droplet is about 40 pl. In some embodiments, the volume of the droplet is at most 100 pl. In some embodiments, each of the plurality of microwells is fluidically connected to at least one microchannel. In some embodiments, the at least one microwell is coated with a moiety that increases surface energy. In some embodiments, the moiety is a chemically inert moiety. In some embodiments, the surface energy corresponds to a water contact angle of less than 20 degree. In some embodiments, the microwells are formed on a solid support comprising a material selected from the group consisting of silicon, polystyrene, agarose, dextran, cellulosic polymers, polyacrylamides, PDMS, and glass. In some embodiments, the microchannels comprise a density of the nominal arclength of the perimeter of at least 0.01 μm / square μm . In some embodiments, the microchannels comprise a density of the nominal arclength of the perimeter of at least 0.001 $\mu\text{m}^2\text{m} / \mu\text{m}^2$. In some embodiments, the surface coated with the moiety comprises a nominal surface area of at least 1 μm^2 per 1.0 μm^2 of planar surface area of the first surface. In some embodiments, the surface coated with the moiety comprises a nominal surface area of at least 1.25 μm^2 per 1.0 μm^2 of planar surface area of the first surface. In some embodiments, the surface coated with the moiety comprises a nominal surface area of at least 1.45 μm^2 per 1.0 μm^2 of planar surface area of the first surface. In some embodiments, a droplet inside a microwell is traveling in the middle third of the microwell. In some embodiments, a droplet inside a microwell is traveling in the bottom half of the microwell. In some embodiments, droplet comprises a reagent that enables oligonucleotide synthesis. In some embodiments, the reagent is a nucleotide or nucleotide analog. It is noted that any of the embodiments described herein can be combined with any of the methods, devices, arrays, substrates or systems provided in the current

disclosureinvention.

[0039] In another aspect, the present ~~disclosure invention~~ also provides a method of partitioning. The method of partitioning comprises contacting a first surface comprising a liquid at a first plurality of resolved loci with a second surface comprising a second plurality of resolved loci; determining a velocity of release such that a desired fraction of the liquid can be transferred from the first plurality of resolved loci to the second plurality of resolved loci; and detaching the second surface from the first surface at said velocity. In some embodiments, the first surface

comprises a first surface tension with the liquid, and the second surface can comprise a second surface tension with the liquid.

[0040] In practicing any of the methods of partitioning as provided herein, a portion of the first surface can be coated with a moiety that increases surface tension. In some embodiments, the moiety is a chemically inert moiety. In some embodiments, the surface tension of the first surface corresponds to a water contact angle of less than 20 degree. In some embodiments, the surface tension of the second surface corresponds to a water contact angle of more than 90 degree. In some embodiments, the first surface comprises a material selected from the group consisting of silicon, polystyrene, agarose, dextran, cellulosic polymers, polyacrylamides, PDMS, and glass. In some embodiments, the plurality of resolved loci comprises a density of the nominal arclength of the perimeter of at least $0.01 \mu\text{m}/\mu\text{m}^2$. In some embodiments, the plurality of resolved loci comprises a density of the nominal arclength of the perimeter of at least $0.001 \mu\text{m}/\mu\text{m}^2$. In some embodiments, the surface coated with the moiety comprises a nominal surface area of at least $1 \mu\text{m}^2$ per $1.0 \mu\text{m}^2$ of planar surface area of the first surface. In some embodiments, the surface coated with the moiety comprises a nominal surface area of at least $1.25 \mu\text{m}^2$ per $1.0 \mu\text{m}^2$ of planar surface area of the first surface. In some embodiments, the surface coated with the moiety comprises a nominal surface area of at least $1.45 \mu\text{m}^2$ per $1.0 \mu\text{m}^2$ of planar surface area of the first surface. In some embodiments, the first plurality of resolved loci is at a density of at least $1/\text{mm}^2$. In some embodiments, the first plurality of resolved loci is at a density of at least $100/\text{mm}^2$. In some embodiments, the first or the second surface comprises microchannels holding at least a portion of the liquid. In some embodiments, the first or the second surface comprises nanoreactors holding at least a portion of the liquid. In some embodiments, the method of partitioning as described herein further comprises contacting a third surface with a third plurality of resolved loci. In some embodiments, the liquid comprises a nucleic acid. In some embodiments, the desired fraction is more than 30%. In some embodiments, the desired fraction is more than 90%. It is noted that any of the embodiments described herein can be combined with any of the methods, devices, arrays, substrates or systems provided in the current ~~disclosure~~invention.

[0041] In yet another aspect, the present ~~disclosure~~invention also provides a method of mixing as described herein. The method comprises: (a) providing a first substrate comprising a plurality of microstructures fabricated thereto; (b) providing a second substrate comprising a plurality of resolved reactor caps; (c) aligning the first and second substrates such that a first reactor cap of the plurality can be configured to receive liquid from n microstructures in the first substrate; and

(d) delivering liquid from the n microstructures into the first reactor cap, thereby mixing liquid from the n microstructures forming a mixture.

[0042] In practicing any of the methods of mixing as described herein, the plurality of resolved reactor caps can be at a density of at least $0.1/\text{mm}^2$. In some embodiments, the plurality of resolved reactor caps are at a density of at least $1/\text{mm}^2$. In some embodiments, plurality of resolved reactor caps are at a density of at least $10/\text{mm}^2$. In some embodiments, each of the plurality of microstructures can comprise at least two channels of different width. In some embodiments, the at least one of the channels is longer than $100\text{ }\mu\text{m}$. In some embodiments, the at least one of the channels is shorter than $1000\text{ }\mu\text{m}$. In some embodiments, the at least one of the channels is wider than $50\text{ }\mu\text{m}$ in diameter. In some embodiments, the at least one of the channels is narrower than $100\text{ }\mu\text{m}$ in diameter. In some embodiments, the at least one of the channels is coated with a moiety that increases surface energy. In some embodiments, the moiety is a chemically inert moiety. In some embodiments, the microstructures are formed on a solid support comprising a material selected from the group consisting of silicon, polystyrene, agarose, dextran, cellulosic polymers, polyacrylamides, PDMS, and glass. In some embodiments, the microchannels comprise a density of the nominal arclength of the perimeter of at least $0.01\text{ }\mu\text{m} / \text{square }\mu\text{m}$. In some embodiments, the microchannels comprise a density of the nominal arclength of the perimeter of at least $0.001\text{ }\mu\text{m} / \mu\text{m}^2$. In some embodiments, the surface coated with the moiety comprises a nominal surface area of at least $1\text{ }\mu\text{m}^2$ per $1.0\text{ }\mu\text{m}^2$ of planar surface area of the first surface. In some embodiments, the surface coated with the moiety comprises a nominal surface area of at least $1.25\text{ }\mu\text{m}^2$ per $1.0\text{ }\mu\text{m}^2$ of planar surface area of the first surface. In some embodiments, the surface coated with the moiety comprises a nominal surface area of at least $1.45\text{ }\mu\text{m}^2$ per $1.0\text{ }\mu\text{m}^2$ of planar surface area of the first surface. In some embodiments, the plurality of microstructures comprises a coating of reagents. In some embodiments, the coating of reagents is covalently linked to the first surface. In some embodiments, the coating of reagents comprises oligonucleotides. In some embodiments, the microstructures are at a density of at least $1/\text{mm}^2$. In some embodiments, the microstructures are at a density of at least $100/\text{mm}^2$.

[0043] In some embodiments related to the methods of mixing as described herein, after step (c), which is aligning the first and second substrates such that a first reactor cap of the plurality can be configured to receive liquid from n microstructures in the first substrate, there is a gap of less than $100\text{ }\mu\text{m}$ between the first and the second substrates. In some embodiments, after step (c), there is a gap of less than $50\text{ }\mu\text{m}$ between the first and the second substrates. In some embodiments, after step (c), there is a gap of less than $20\text{ }\mu\text{m}$ between the first and the second substrates. In some

embodiments, after step (c), there is a gap of less than 10 μm between the first and the second substrates. In some embodiments, the mixture partially spreads into the gap. In some embodiments, the method of mixing further comprises sealing the gap by bringing the first and the second substrate closer together. In some embodiments, one of the two channels is coated with a moiety that increases surface energy corresponding to a water contact angle of less than 20 degree. In some embodiments, the moiety is a chemically inert moiety. In some embodiments, the delivering is performed by pressure. In some embodiments, the volume of the mixture is greater than the volume of the reactor cap. In some embodiments, the liquid comprises a nucleic acid. In some embodiments, n is at least 10. In some embodiments, n is at least 25. In some embodiments, n, the number of microstructures from which the liquid is mixed forming a mixture, can be at least 50. In some embodiments, n is at least 75. In some embodiments, n is at least 100. It is noted that any of the embodiments described herein can be combined with any of the methods, devices, arrays, substrates or systems provided in the current ~~disclosure~~invention.

[0044] In yet another aspect, the present ~~disclosure~~invention also provides a method of synthesizing n-mer oligonucleotides on a substrate as described herein. The method comprises: providing a substrate with resolved loci that are functionalized with a chemical moiety suitable for nucleotide coupling; and coupling at least two building blocks to a plurality of growing oligonucleotide chains each residing on one of the resolved loci according to a locus specific predetermined sequence without transporting the substrate between the couplings of the at least two building blocks, thereby synthesizing a plurality of oligonucleotides that are n basepairs long.

[0045] In practicing any of the methods of synthesizing n-mer oligonucleotides on a substrate as described herein, the method can further comprise coupling at least two building blocks to a plurality of growing oligonucleotide chains each residing on one of the resolved loci at a rate of at least 12 nucleotides per hour. In some embodiments, the method further comprises coupling at least two building blocks to a plurality of growing oligonucleotide chains each residing on one of the resolved loci at a rate of at least 15 nucleotides per hour. In some embodiments, the method further comprises coupling at least two building blocks to a plurality of growing oligonucleotide chains each residing on one of the resolved loci at a rate of at least 20 nucleotides per hour. In some embodiments, the method further comprises coupling at least two building blocks to a plurality of growing oligonucleotide chains each residing on one of the resolved loci at a rate of at least 25 nucleotides per hour. In some embodiments, at least one

resolved locus comprises n-mer oligonucleotides deviating from the locus specific predetermined sequence with an error rate of less than 1/500 bp. In some embodiments, at least one resolved locus comprises n-mer oligonucleotides deviating from the locus specific predetermined sequence with an error rate of less than 1/1000 bp. In some embodiments, at least one resolved locus comprises n-mer oligonucleotides deviating from the locus specific predetermined sequence with an error rate of less than 1/2000 bp. In some embodiments, the plurality of oligonucleotides on the substrate deviate from respective locus specific predetermined sequences at an error rate of less than 1/500 bp. In some embodiments, the plurality of oligonucleotides on the substrate deviate from respective locus specific predetermined sequences at an error rate of less than 1/1000 bp. In some embodiments, the plurality of oligonucleotides on the substrate deviate from respective locus specific predetermined sequences at an error rate of less than 1/2000 bp.

[0046] In some embodiments related to the method of synthesizing n-mer oligonucleotides on a substrate as described herein, the building blocks comprise an adenine, guanine, thymine, cytosine, or uridine group, or a modified nucleotide. In some embodiments, the building blocks comprise a modified nucleotide. In some embodiments, the building blocks comprise dinucleotides. In some embodiments, the building blocks comprise phosphoramidite. In some embodiments, n is at least 100. In some embodiments, wherein n is at least 200. In some embodiments, n is at least 300. In some embodiments, n is at least 400. In some embodiments, the substrate comprises at least 100,000 resolved loci and at least two of the plurality of growing oligonucleotides are different from each other. In some embodiments, the method further comprise vacuum drying the substrate before coupling. In some embodiments, the building blocks comprise a blocking group. In some embodiments, the blocking group comprises an acid-labile DMT. In some embodiments, the acid-labile DMT comprises 4,4'-dimethoxytrityl. In some embodiments, the method further comprise oxidation or sulfurization. In some embodiments, the method further comprise chemically capping uncoupled oligonucleotide chains. In some embodiments, the method further comprise removing the blocking group, thereby deblocking the growing oligonucleotide chain. In some embodiments, the substrate comprises at least 10,000 vias providing fluid communication between a first surface of the substrate and a second surface of the substrate. In some embodiments, the substrate comprises at least 100,000 vias providing fluid communication between a first surface of the substrate and a second surface of the substrate. In some embodiments, the substrate comprises at least 1,000,000 vias providing fluid communication between a first surface of the substrate and a

second surface of the substrate. It is noted that any of the embodiments described herein can be combined with any of the methods, devices, arrays, substrates or systems provided in the current ~~disclosure~~invention.

[0047] In yet another aspect, the present ~~disclosure~~invention also provides a method of constructing a gene library as described herein. The method comprises: entering at a first timepoint, in a computer readable non-transient medium a list of genes, wherein the list comprises at least 100 genes and wherein the genes are at least 500 bp; synthesizing more than 90% of the list of genes, thereby constructing a gene library with deliverable genes; preparing a sequencing library that represents the gene library; obtaining sequence information; selecting at least a subset of the deliverable genes based on the sequence information; and delivering the selected deliverable genes at a second timepoint, wherein the second timepoint is less than a month apart from the first timepoint.

[0048] In practicing any of the methods of constructing a gene library as described herein, the sequence information can be obtained via next-generation sequencing. The sequence information can be obtained by Sanger sequencing. In some embodiments, the method further comprises delivering at least one gene at a second timepoint. In some embodiments, at least one of the genes differ from any other gene by at least 0.1% in the gene library. In some embodiments, each of the genes differ from any other gene by at least 0.1% in the gene library. In some embodiments, at least one of the genes differ from any other gene by at least 10% in the gene library. In some embodiments, each of the genes differ from any other gene by at least 10% in the gene library. In some embodiments, at least one of the genes differ from any other gene by at least 2 base pairs in the gene library. In some embodiments, each of the genes differ from any other gene by at least 2 base pairs in the gene library. In some embodiments, at least 90% of the deliverable genes are error free. In some embodiments, the deliverable genes comprise an error rate of less than 1/3000 resulting in the generation of a sequence that deviates from the sequence of a gene in the list of genes. In some embodiments, at least 90% of the deliverable genes comprise an error rate of less than 1 in 3000 bp resulting in the generation of a sequence that deviates from the sequence of a gene in the list of genes. In some embodiments, a subset of the deliverable genes are covalently linked together. In some embodiments, a first subset of the list of genes encode for components of a first metabolic pathway with one or more metabolic end products. In some embodiments, the method further comprises selecting of the one or more metabolic end products, thereby constructing the list of genes. In some embodiments, the one or more metabolic end products comprise a biofuel. In some

embodiments, a second subset of the list of genes encode for components of a second metabolic pathway with one or more metabolic end products. In some embodiments, the list comprises at least 500, genes. In some embodiments, the list comprises at least 5000 genes. In some embodiments, the list comprises at least 10000 genes. In some embodiments, the genes are at least 1kb. In some embodiments, the genes are at least 2kb. In some embodiments, the genes are at least 3kb. In some embodiments, the second timepoint is less than 25 days apart from the first timepoint. In some embodiments, the second timepoint is less than 5 days apart from the first timepoint. In some embodiments, the second timepoint is less than 2 days apart from the first timepoint. It is noted that any of the embodiments described herein can be combined with any of the methods, devices, arrays, substrates or systems provided in the current ~~disclosure~~invention.

[0049] Provided herein, in some embodiments, is a microfluidic device for nucleic acid synthesis, comprising a substantially planar substrate portion comprising n groupings of m microfluidic connections between opposite surfaces, wherein each one of the $n*m$ microfluidic connections comprises a first channel and a second channel, and wherein the first channel within each of the n groupings is common to all m microfluidic connections, wherein the plurality of microfluidic connections span the substantially planar substrate portion along the smallest dimension of the substrate, and wherein n and m are at least 2. In some embodiments, the second channel is functionalized with a coating that is capable of facilitating the attachment of an oligonucleotide to the device. In some embodiments, the device further comprises a first oligonucleotide that is attached to the second channels in k of the n groupings. In some embodiments, k is 1. In some embodiments, the device further comprises a second oligonucleotide that is attached to l of the n groupings. In some embodiments, l is 1. In some embodiments, the none of the groupings in the l groupings are in the k groupings.

[0050] In some embodiments, the oligonucleotide is at least 10 nucleotides, 25 nucleotides, 50 nucleotides, 75 nucleotides, 100 nucleotides, 125 nucleotides, 150 nucleotides, or 200 nucleotides long.

[0051] In some embodiments, the first and the second oligonucleotides differ by at least 2 nucleotides, 3 nucleotides, 4 nucleotides, 5 nucleotides, or 10 nucleotides.

[0052] In some embodiments, the $n*m$ microfluidic connections are at most 5 mm, 1.5 mm, 1.0 mm, or 0.5 mm long. In some embodiments, the first channel within each of the n groupings is at most 5 mm, 1.5 mm, 1.0 mm, or 0.5 mm long. In some embodiments, the first channel within each of the n groupings is at least 0.05 mm, 0.75 mm, 0.1 mm, 0.2 mm, 0.3 mm, or 0.4 mm long. In some embodiments, the second channel in each of the $n*m$ microfluidic

connections is at most 0.2 mm, 0.1 mm, 0.05 mm, 0.04 mm, or 0.03 mm long. In some embodiments, the second channel in each of the $n*m$ microfluidic connections is at least 0.001 mm, 0.005 mm, 0.01 mm, 0.02 mm, or 0.03 mm long. In some embodiments, the cross section of the first channel within each of the n groupings is at least 0.01 mm, 0.025 mm, 0.05 mm, or 0.075 mm. In some embodiments, the cross section of the first channel within each of the n groupings is at most 1 mm, 0.5 mm, 0.25 mm, 0.1 mm, or 0.075 mm. In some embodiments, the cross section of the second channel in each of the $n*m$ microfluidic connections is at least 0.001 mm, 0.05 mm, 0.01 mm, 0.015 mm, or 0.02 mm. In some embodiments, the cross section of the second channel in each of the $n*m$ microfluidic connections is at most 0.25 mm, 0.125 mm, 0.050 mm, 0.025 mm, 0.02 mm. In some embodiments, the standard deviation in the cross section of the second channels in each of the $n*m$ microfluidic connections is less than 25%, 20%, 15%, 10%, 5%, or 1% of the mean of the cross section. In some embodiments, the variation in the cross section within at least 90% of the second channels of the $n*m$ microfluidic connections is at most 25%, 20%, 15%, 10%, 5%, or 1%.

[0053] In some embodiments, n is at least 10, 25, 50, 100, 1000, or 10000. In some embodiments, m is at least 3, 4, or 5.

[0054] In some embodiments, the substrate comprises at least 5 %, 10%, 25%, 50%, 80%, 90%, 95%, or 99% silicon.

[0055] In some embodiments, at least 90% of the second channels of the $n*m$ microfluidic connections is functionalized with a moiety that increases surface energy. In some embodiments, the surface energy is increased to a level corresponding to a water contact angle of less than 75, 50, 30, or 20 degrees.

[0056] In some embodiments, the aspect ratio for at least 90% of the second channels of the $n*m$ microfluidic connections is less than 1, 0.5, or 0.3. In some embodiments, the aspect ratio for at least 90% of the first channels in the n groupings is less than 0.5, 0.3, or 0.2.

[0057] In some embodiments, the total length of at least 10%, 25%, 50%, 75%, 90%, or 95% of the $n*m$ fluidic connections are within 10%, 20%, 30%, 40%, 50%, 100%, 200%, 500%, or 1000% of the smallest dimension of the substantially planar substrate.

[0058] In some embodiments, the substantially planar portion of the device is fabricated from a SOI wafer.

[0059] In another aspect, the ~~disclosure invention~~ relates to a method of nucleic acid amplification, comprising: (a) providing a sample comprising n circularized single stranded nucleic acids, each comprising a different target sequence; (b) providing a first adaptor that is hybridizable to at least

one adaptor hybridization sequence on m of the n circularized single stranded nucleic acids; (c) providing conditions suitable for extending the first adaptor using the m circularized single stranded nucleic acids as a template, thereby generating m single stranded amplicon nucleic acids, wherein each of the m single stranded amplicon nucleic acids comprises a plurality of replicas of the target sequence from its template; (d) providing a first auxiliary oligonucleotide that is hybridizable to the first adaptor; and (e) providing a first agent under conditions suitable for the first agent to cut the m single stranded amplicon nucleic acids at a plurality of cutting sites, thereby generating a plurality of single stranded replicas of the target sequences in the m circularized single stranded nucleic acids. In some embodiments, n or m is at least 2. In some embodiments, n or m is at least 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 75, 100, 150, 200, 300, 400, or 500. In some embodiments, m is less than n . In some embodiments, the sample comprising the n circularized single stranded nucleic acid is formed by providing at least n linear single stranded nucleic acids, each comprising one of the different target sequences and circularizing the n linear single stranded nucleic acids, thereby generating the n circularized single stranded nucleic acids. In some embodiments, the first adaptor is hybridizable to both ends of the n linear single stranded nucleic acids concurrently. In some embodiments, the different target sequences in the n linear single stranded nucleic acids are flanked by a first and a second adaptor hybridization sequence. In some embodiments, the at least n linear single stranded nucleic acids are generated by de novo oligonucleotide synthesis. In some embodiments, the first adaptor hybridization sequence in each of the n linear single stranded nucleic acids differ by no more than two nucleotide bases. In some embodiments, the first or the second adaptor hybridization sequence is at least 5 nucleotides long. In some embodiments, the first or the second adaptor hybridization sequence is at most 75, 50, 45, 40, 35, 30, or 25 nucleotides long. In some embodiments, the ends of the n linear single stranded nucleic acids pair with adjacent bases on the first adaptor when the first adaptor is hybridized to the both ends of the linear single stranded nucleic acid concurrently. In some embodiments, the locations of the plurality of cutting sites are such that the adaptor hybridization sequence is severed from at least 5% of a remainder sequence portion of the m circularized single stranded nucleic acid replicas. In some embodiments, at least 5% of the sequence of the m circularized single stranded nucleic acid replicas other than the at least one adaptor hybridization sequence remains uncut. In some embodiments, the locations of the plurality of cutting sites are outside the at least one adaptor hybridization sequence. In some embodiments, the locations of the plurality of cutting sites are independent of the target sequences. In some embodiments, the locations of the plurality of cutting sites are determined

by at least one sequence element within the sequence of the first adaptor or the first auxiliary oligonucleotide. In some embodiments, the sequence element comprises a recognition site for a restriction endonuclease. In some embodiments, the first auxiliary oligonucleotide or the first adaptor oligonucleotide comprises a recognition site for a Type IIS restriction endonuclease. In some embodiments, the recognition sites are at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides away from the cutting sites. In some embodiments, the plurality of cutting sites are at junctures of single and double stranded nucleic acids. In some embodiments, the double stranded nucleic acids comprise the first adaptor and the first auxiliary oligonucleotide. In some embodiments, the single stranded nucleic acids consists essentially of the m different target sequences. In some embodiments, the m different target sequences have at most 95% pairwise similarity. In some embodiments, the m different target sequences have at most 90% pairwise similarity. In some embodiments, the m different target sequences have at most 80% pairwise similarity. In some embodiments, the m different target sequences have at most 50% pairwise similarity. In some embodiments, generating the m single stranded amplicon nucleic acid comprises strand displacement amplification. In some embodiments, the first auxiliary oligonucleotide comprises an affinity tag. In some embodiments, the affinity tag comprises biotin or biotin derivative. In some embodiments, the method further comprises isolating double stranded nucleic acids from the sample. In some embodiments, the isolating comprises affinity purification, chromatography, or gel purification. In some embodiments, the first agent comprises a restriction endonuclease. In some embodiments, the first agent comprises at least two restriction endonucleases. In some embodiments, the first agent comprises a Type IIS restriction endonuclease. In some embodiments, the first agent comprises a nicking endonuclease. In some embodiments, the first agent comprises at least two nicking endonucleases. In some embodiments, the first agent comprises at least one enzyme selected from the group consisting of MlyI, SchI, AlwI, BccI, BceAI, BsmAI, BsmFI, FokI, HgaI, PleI, SfaNI, BfuAI, BsaI, BspMI, BtgZI, EarI, BspQI, SapI, SgeI, BceFI, BslFI, BsoMAI, Bst7II, FagI, AceIII, BbvII, BveI, LguI, BfuCI, DpnII, FatI, MboI, MluCI, Sau3AI, Tsp509I, BssKI, PspGI, StyD4I, Tsp45I, AoxI, BscFI, Bsp143I, BssMI, BseENII, BstMBI, Kzo9I, NedII, Sse9I, TasI, TspEI, AjaI, BstSCI, EcoRII, MaeIII, NmuCI, Psp6I, MnlI, BspCNI, BsrI, BtsCI, HphI, HpyAV, MboII, AcuI, BciVI, BmrI, BpmI, BpuEI, BseRI, BsgI, BsmI, BsrDI, BtsI, EciI, MmeI, NmeAIII, Hin4II, TscAI, Bce83I, BmuI, BsbI, BscCI, NlaIII, Hpy99I, TspRI, FaeI, Hin1II, Hsp92II, SetI, TaiI, TscI, TscAI, TseFI, Nb.BsrDI, Nb.BtsI, AspCNI, BscGI, BspNCI, EcoHI, FinI, TsuI, UbaF11I, UnbI, Vpak11AI, BspGI, DrdII, Pfl1108I, UbaPI, Nt.AlwI, Nt.BsmAI, Nt.BstNBI, and Nt.BspQI, and variants thereof. In some

embodiments, the first agent comprises essentially the same function, recognizes the same or essentially the same recognition sequence, or cuts at the same or essentially same cutting site, as any of the listed first agents and variants.. In some embodiments, the at least two restriction enzymes comprise MlyI and BciVI or BfuCI and MlyI. In some embodiments, the method further comprises (a) partitioning the sample into a plurality of fractions; (b) providing at least one fraction with a second adaptor that is hybridizable to at least one adaptor hybridization sequence on k of the n different circularized single stranded nucleic acids; (c) providing conditions suitable for extending the second adaptor using the k circularized single stranded nucleic acids as a template, thereby generating k single stranded amplicon nucleic acids, wherein the second single stranded amplicon nucleic acid comprises a plurality of replicas of the target sequence from its template; (d) providing a second auxiliary oligonucleotide that is hybridizable to the second adaptor; and (e) providing a second agent under conditions suitable for the agent to cut the k single stranded amplicon nucleic acids at a second plurality of cutting sites, thereby generating a plurality of single stranded replicas of the target sequences in the k circularized single stranded nucleic acids. In some embodiments, the first and the second adaptors are the same. In some embodiments, the first and the second auxiliary oligonucleotides are the same. In some embodiments, the first and the second agents are the same. In some embodiments, $k + m$ is less than n . In some embodiments, k is at least 2. In some embodiments, the sample comprising the n circularized single stranded nucleic acid is formed by single stranded nucleic acid amplification. In some embodiments, the single stranded nucleic acid amplification comprises: (a) providing a sample comprising at least m circularized single stranded precursor nucleic acids; (b) providing a first precursor adaptor that is hybridizable to the m circularized single stranded precursor nucleic acids; (c) providing conditions suitable for extending the first precursor adaptor using the m circularized single stranded precursor nucleic acids as a template, thereby generating m single stranded precursor amplicon nucleic acids, wherein the single stranded amplicon nucleic acid comprises a plurality of replicas of the m circularized single stranded precursor nucleic acid; (d) providing a first precursor auxiliary oligonucleotide that is hybridizable to the first precursor adaptor; and (e) providing a first precursor agent under conditions suitable for the first precursor agent to cut the first single stranded precursor amplicon nucleic acid at a plurality of cutting sites, thereby generating the m linear precursor nucleic acids. In some embodiments, the method further comprises circularizing the m linear precursor nucleic acids, thereby forming replicas of the m circularized single stranded precursor nucleic acids. In some embodiments, the m circularized single stranded precursor nucleic acid is amplified by at

least 10, 100, 250, 500, 750, 1000, 1500, 2000, 3000, 4000, 5000, 10000-fold, or more in single stranded replicas. In some embodiments, at least one of the m circularized single stranded nucleic acids is at a concentration of about or at most about 100 nM, 10 nM, 1 nM, 50 pM, 1 pM, 100 fM, 10 fM, 1 fM, or less. In some embodiments, circularizing comprises ligation. In some embodiments, ligation comprises the use of a ligase selected from the group consisting of T4 DNA ligase, T3 DNA ligase, T7 DNA ligase, e.coli DNA ligase, Taq DNA ligase, and 9N DNA ligase.

[0060] In yet a further aspect, the ~~disclosure~~^{invention}, in various embodiments relates to a kit comprising: (a) a first adaptor; (b) a first auxiliary oligonucleotide that is hybridizable to the adaptor; (c) a ligase; and (d) a first cleaving agent, comprising at least one enzyme selected from the group consisting of MlyI, SchI, AlwI, BccI, BceAI, BsmAI, BsmFI, FokI, HgaI, PleI, SfaNI, BfuAI, BsaI, BspMI, BtgZI, EarI, BspQI, SapI, SgeI, BceFI, BslFI, BsoMAI, Bst71I, FagI, AceIII, BbvII, BveI, LguI, BfuCI, DpnII, FatI, MboI, MluCI, Sau3AI, Tsp509I, BssKI, PspGI, StyD4I, Tsp45I, AoxI, BscFI, Bsp143I, BssMI, BseENII, BstMBI, Kzo9I, NedII, Sse9I, TasI, TspEI, AjnI, BstSCI, EcoRII, MaeIII, NmuCI, Psp6I, MnlI, BspCNI, BsrI, BtsCI, HphI, HpyAV, MboII, AclI, BciVI, BmrI, BpmI, BpuEI, BseRI, BsgI, BsmI, BsrDI, BtsI, EciI, MmeI, NmeAIII, Hin4II, TscAI, Bce83I, BmuI, BsbI, BscCI, NlaIII, Hpy99I, TspRI, FaeI, Hin1II, Hsp92II, SetI, TaiI, TscI, TscAI, TseFI, Nb.BsrDI, Nb.BtsI, AspCNI, BscGI, BspNCI, EcoHI, FinI, TsuI, UbaF11I, UnbI, Vpak11AI, BspGI, DrdII, Pfl1108I, UbaPI, Nt.AlwI, Nt.BsmAI, Nt.BstNBI, and Nt.BspQI, and variants thereof. In some embodiments, the first agent comprises essentially the same function, recognizes the same or essentially the same recognition sequence, or cuts at the same or essentially same cutting site as any of the listed first agents and variants.. In some embodiments, the kit further comprises a second cleaving agent. In some embodiments, the second cleaving agent comprises and enzyme selected from the group consisting of MlyI, SchI, AlwI, BccI, BceAI, BsmAI, BsmFI, FokI, HgaI, PleI, SfaNI, BfuAI, BsaI, BspMI, BtgZI, EarI, BspQI, SapI, SgeI, BceFI, BslFI, BsoMAI, Bst71I, FagI, AceIII, BbvII, BveI, LguI, BfuCI, DpnII, FatI, MboI, MluCI, Sau3AI, Tsp509I, BssKI, PspGI, StyD4I, Tsp45I, AoxI, BscFI, Bsp143I, BssMI, BseENII, BstMBI, Kzo9I, NedII, Sse9I, TasI, TspEI, AjnI, BstSCI, EcoRII, MaeIII, NmuCI, Psp6I, MnlI, BspCNI, BsrI, BtsCI, HphI, HpyAV, MboII, AclI, BciVI, BmrI, BpmI, BpuEI, BseRI, BsgI, BsmI, BsrDI, BtsI, EciI, MmeI, NmeAIII, Hin4II, TscAI, Bce83I, BmuI, BsbI, BscCI, NlaIII, Hpy99I, TspRI, FaeI, Hin1II, Hsp92II, SetI, TaiI, TscI, TscAI, TseFI, Nb.BsrDI, Nb.BtsI, AspCNI, BscGI, BspNCI, EcoHI, FinI, TsuI, UbaF11I, UnbI, Vpak11AI, BspGI, DrdII, Pfl1108I, UbaPI, Nt.AlwI, Nt.BsmAI, Nt.BstNBI, and Nt.BspQI, and

variants thereof. In some embodiments, the second agent comprises essentially the same function, recognizes the same or essentially the same recognition sequence, or cuts at the same or essentially same cutting site as any of the listed second agents and variants. In some embodiments, the first cleaving agent comprises MlyI. In some embodiments, the second cleaving agent comprises BciVI or BfuCI.

[0061] In yet another aspect, the ~~disclosure invention~~ relates to a method of nucleic acid amplification, comprising: (a) providing a sample comprising n circularized single stranded nucleic acids, each comprising a different target sequence; (b) providing a first adaptor that is hybridizable to at least one adaptor hybridization sequence on m of the n circularized single stranded nucleic acids; (c) providing conditions suitable for extending the first adaptor using the m circularized single stranded nucleic acids as a template, thereby generating m single stranded amplicon nucleic acids, wherein each of the m single stranded amplicon nucleic acids comprises a plurality of replicas of the target sequence from its template; (d) generating double stranded recognition sites for a first agent on the m single stranded amplicon nucleic acids; and (e) providing the first agent under conditions suitable for the first agent to cut the m single stranded amplicon nucleic acids at a plurality of cutting sites, thereby generating a plurality of single stranded replicas of the target sequences in the m circularized single stranded nucleic acids. In some embodiments, the double stranded recognition sites comprise a first portion of the first adaptor on a first strand of the double stranded recognition sites and a second strand of the first adaptor on the second strand of the double stranded recognition sites. In some embodiments, the adaptor comprises a palindromic sequence. In some embodiments, the double stranded recognition sites are generated by hybridizing the first and second portions of the first adaptor to each other. In some embodiments, the m single stranded amplicon nucleic acids comprise a plurality of double stranded self-hybridized regions.

[0062] In a yet further aspect, the ~~disclosure invention~~ relates to a method for generating a long nucleic acid molecule, the method comprising the steps of: (a) providing a plurality of nucleic acids immobilized on a surface, wherein said plurality of nucleic acids comprises nucleic acids having overlapping complementary sequences; (b) releasing said plurality of nucleic acids into solution; and (c) providing conditions promoting: i) hybridization of said overlapping complementary sequences to form a plurality of hybridized nucleic acids; and ii) extension or ligation of said hybridized nucleic acids to synthesize the long nucleic acid molecule.

[0063] In another aspect, the ~~disclosure invention~~ relates to an automated system capable of processing one or more substrates, comprising: an inkjet print head for spraying a microdroplet comprising a

chemical species on a substrate; a scanning transport for scanning the substrate adjacent to the print head to selectively deposit the microdroplet at specified sites; a flow cell for treating the substrate on which the microdroplet is deposited by exposing the substrate to one or more selected fluids; an alignment unit for aligning the substrate correctly relative to the print head each time when the substrate is positioned adjacent to the print head for deposition; and not comprising a treating transport for moving the substrate between the print head and the flow cell for treatment in the flow cell, wherein said treating transport and said scanning transport are different elements.

[0064] In yet another aspect, the ~~disclosure invention~~ relates to an automated system for synthesizing oligonucleotides on a substrate, said automated system capable of processing one or more substrates, comprising: an inkjet print head for spraying a solution comprising a nucleoside or activated nucleoside on a substrate; a scanning transport for scanning the substrate adjacent to the print head to selectively deposit the nucleoside at specified sites; a flow cell for treating the substrate on which the monomer is deposited by exposing the substrate to one or more selected fluids; an alignment unit for aligning the substrate correctly relative to the print head each time when the substrate is positioned adjacent to the print head for deposition; and not comprising a treating transport for moving the substrate between the print head and the flow cell for treatment in the flow cell, wherein said treating transport and said scanning transport are different elements.

[0065] In yet a further aspect, the ~~disclosure invention~~ relates to an automated system comprising: an inkjet print head for spraying a microdroplet comprising a chemical species on a substrate; a scanning transport for scanning the substrate adjacent to the print head to selectively deposit the microdroplet at specified sites; a flow cell for treating the substrate on which the microdroplet is deposited by exposing the substrate to one or more selected fluids; and an alignment unit for aligning the substrate correctly relative to the print head each time when the substrate is positioned adjacent to the print head for deposition; and wherein the system does NOT comprise a treating transport for moving the substrate between the print head and the flow cell for treatment in the flow cell.

[0065a] In yet a further aspect, the present disclosure provides a method of method of synthesizing nucleic acids, comprising:

- a) providing predetermined sequences for at least 150 preselected nucleic acids;
- b) providing a structure comprising a surface, wherein the surface comprises a plurality of loci, wherein each locus comprises a first plurality of molecules and is adjacent to a region that comprises a second plurality of molecules, wherein each molecule of the first plurality of molecules binds to the surface and comprises a reactive group capable of binding to a nucleoside phosphoramidite, and wherein each molecule of the second plurality of molecules binds to the surface and lacks the reactive group capable of binding to the nucleoside phosphoramidite;

c) synthesizing at least 15,000 non-identical polynucleotides extending from to the surface, wherein the at least 15,000 non-identical polynucleotides are each at least 50 bases long; and assembling the at least 150 preselected nucleic acids, wherein the at least 150 preselected nucleic acids comprise sequences encoded by the at least 15,000 non-identical polynucleotides, wherein the assembled at least 150 preselected nucleic acids encode sequences with an aggregate error rate of less than 1 in 1500 bases compared to the predetermined sequences without correcting errors in the at least 150 assembled preselected nucleic acids.

[0065b] In yet a further aspect, the present disclosure provides a method of synthesizing nucleic acids, comprising:

a) providing predetermined sequences for at least 6,000 preselected nucleic acids;

b) providing a structure comprising a patterned surface, wherein the patterned surface comprises predetermined regions coated with a first molecule, wherein the first molecule comprises a silane, siloxane, or N-(3-triethoxysilylpropyl)-4-hydroxybutyramide, and wherein each predetermined region coated with the first molecule is surrounded by a region coated with a second molecule, wherein the second molecule binds to the patterned surface and lacks an available reactive group that binds to a nucleoside phosphoramidite;

c) synthesizing at least 800,000 of non-identical polynucleotides attached to the patterned surface, wherein the at least 800,000 non-identical polynucleotides are each at least 125 bases long and extend from the patterned surface at the predetermined regions coated with the first molecule;

d) releasing the at least 800,000 non-identical polynucleotides from the patterned surface;

e) suspending the at least 800,000 non-identical polynucleotides in a solution; and

f) subjecting the solution comprising the at least 800,000 non-identical polynucleotides to a polymerase chain assembly reaction to assemble the at least 6,000 preselected nucleic acids, wherein the assembled at least 6,000 preselected nucleic acids encode sequences with an aggregate error rate of less than 1 in 1500 bases compared to the predetermined sequences without correcting errors in the assembled at least 6,000 preselected nucleic acids, and wherein the patterned surface provides for the aggregate error rate of less than 1 in 1500 bases.

[0065c] In yet a further aspect, the present disclosure provides a method for synthesizing a library of oligonucleic acids encoding for predetermined sequences, the method comprising:

a) providing predetermined sequences for a library of at least 100,000 non-identical oligonucleic acids;

b) providing a structure having a patterned surface comprising a plurality of loci for oligonucleic acid extension, wherein each locus comprises a first plurality of molecules and is adjacent to a region that comprises a second plurality of molecules, wherein each molecule of the first plurality of molecules binds to the patterned surface and comprises a reactive group capable of

c) binding to a nucleoside, and wherein each molecule of the second plurality of molecules binds to the patterned surface and lacks the reactive group capable of binding to the nucleoside;

d) adding a droplet of fluid comprising an extension reaction reagent specific to a locus, wherein the extension reaction reagent comprises a nucleoside phosphoramidite;

e) allowing sufficient time for an extension reaction step to occur; and
repeating steps (c) and (d) to synthesize the at least 100,000 non-identical oligonucleic acids, wherein each non-identical oligonucleic acid is at least 50 bases in length and is attached to the patterned surface at a predetermined locus, and wherein the synthesized at least 100,000 non-identical oligonucleic acids encode sequences with an aggregate error rate of less than 1 in 1000 bases compared to the predetermined sequences, and wherein the patterned surface provides for the aggregate error rate of less than 1 in 1000 bases.

[0065d] In yet a further aspect, the present disclosure provides a device for synthesizing oligonucleic acids, the device comprising:

a silicon plate having formed therein:

a plurality of channels, wherein each channel extends vertically to a depth into of the silicon plate from an opening on a top side of the silicon plate, and wherein each channel has a width of 0.5 mm to 1.5 mm; and

for each channel, 50 to 500 microchannels in fluid communication with said channel, wherein each microchannel extends vertically to a height from an opening on a bottom side of the silicon plate to the channel, wherein each microchannel has a width of 1 μ m to 100 μ m, and wherein a ratio of width to height of each microchannel is from 1 to 0.1 and provides for rapid exchange of chemical exposure during de novo synthesis of oligonucleic acids.

[0065e] In yet a further aspect, the present disclosure provides a device for nucleic acid assembly, comprising:

a) a first plate, the first plate comprising:

a base layer of the first plate; and

one or more wells, wherein each well of the one or more wells comprises:

a side wall that extends vertically from the base layer;

an interior region of the well; and

an exterior region of the well, wherein the exterior region of the well has a lower surface energy than the interior region of the well; and

b) a second plate, wherein the second plate comprises a plurality of clusters, wherein each cluster comprises a plurality of loci, wherein each locus comprises a physically distinct region

comprising molecules capable of binding to a nucleoside phosphoramidite, and wherein each cluster is vertically aligned to a single well of the one or more wells.

[0065f] In yet a further aspect, the present disclosure provides an oligonucleic acid library, comprising at least 100,000 non-identical oligonucleic acids that collectively encode sequences for at least 750 preselected genes, each preselected gene having a predetermined sequence, wherein each of the at least 100,000 non-identical oligonucleic acids is synthesized and comprises at least 30 bases in length, and wherein the at least 100,000 non-identical oligonucleic acids encode sequences with an aggregate error rate of less than 1 in 1000 bases compared to the predetermined sequences for the at least 750 preselected genes.

[0065g] In yet a further aspect, the present disclosure provides A gene library, comprising a collection of at least 750 preselected genes, each gene having a predetermined sequence, wherein each of the at least 750 preselected genes is at least 0.5 kb length in length, wherein each preselected gene is synthesized without correcting errors, and wherein the at least 750 preselected genes have an aggregate error rate of less than 1 in 1000 bases compared to the predetermined sequences for the at least 750 preselected genes.

INCORPORATION BY REFERENCE

[0066] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

WHAT IS CLAIMED IS:

1. A method of synthesizing nucleic acids, comprising:
 - a) providing predetermined sequences for at least 150 preselected nucleic acids;
 - b) providing a structure comprising a surface, wherein the surface comprises a plurality of loci, wherein each locus comprises a first plurality of molecules and is adjacent to a region that comprises a second plurality of molecules, wherein each molecule of the first plurality of molecules binds to the surface and comprises a reactive group capable of binding to a nucleoside phosphoramidite, and wherein each molecule of the second plurality of molecules binds to the surface and lacks the reactive group capable of binding to the nucleoside phosphoramidite;
 - c) synthesizing at least 15,000 non-identical polynucleotides extending from the surface, wherein the at least 15,000 non-identical polynucleotides are each at least 50 bases long; and
 - d) assembling the at least 150 preselected nucleic acids, wherein the at least 150 preselected nucleic acids comprise sequences encoded by the at least 15,000 non-identical polynucleotides, wherein the assembled at least 150 preselected nucleic acids encode sequences with an aggregate error rate of less than 1 in 1500 bases compared to the predetermined sequences without correcting errors in the at least 150 assembled preselected nucleic acids.
2. The method of claim 1, wherein the first plurality of molecules comprises a silane, siloxane, or N-(3-triethoxysilylpropyl)-4-hydroxybutyramide.
3. The method of claim 1, wherein the first plurality of molecules comprises 11-acetoxyundecyltriethoxysilane, n-decyltriethoxysilane, (3-aminopropyl)trimethoxysilane, or (3-aminopropyl)triethoxysilane.
4. The method of claim 1, wherein the second plurality of molecules comprises a fluorosilane.
5. The method of claim 4, wherein the fluorosilane is (tridecafluorotetrahydrooctyl)-triethoxysilane.

6. The method of claim 1, wherein the at least 15,000 non-identical polynucleotides attached to the surface are clustered, wherein each cluster is separated from another cluster by a structural barrier on the surface, and wherein each cluster includes a subset of the at least 15,000 non-identical polynucleotides that collectively encodes for a single nucleic acid.

7. The method of claim 6, wherein each cluster comprises 50 to 500 loci.

8. The method of claim 7, wherein each locus is separated from another locus by a structural barrier on the surface.

9. The method of claim 6, wherein each subset of the at least 15,000 non-identical polynucleotides collectively encodes for a single nucleic acid and is located within a channel 1.0 to 1.5 mm in diameter.

10. The method of claim 1, comprising releasing the at least 15,000 non-identical polynucleotides release from the surface after synthesis, wherein the at least 15,000 non-identical polynucleotides are released and clustered, wherein each cluster includes a subset of the at least 15,000 non-identical polynucleotides, and wherein the subset of the at least 15,000 non-identical nucleotides collectively encodes for a single nucleic acid.

11. The method of claim 1, comprising releasing the at least 15,000 non-identical polynucleotides from the surface after synthesis, wherein the release is by gas cleavage.

12. The method of claim 1, wherein steps a) to d) are completed in less than one week.

13. The method of claim 1, wherein steps a) to d) are completed in less than 48 hours.

14. The method of claim 1, wherein the assembled at least 150 preselected nucleic acids are at least 1 kb long on average.

15. The method of claim 1, further comprising treating the assembled at least 150 preselected nucleic acids with an error correction enzyme.

16. A method of synthesizing nucleic acids, comprising:

a) providing predetermined sequences for at least 6,000 preselected nucleic acids;

b) providing a structure comprising a patterned surface, wherein the patterned surface comprises predetermined regions coated with a first molecule, wherein the first molecule comprises a silane, siloxane, or N-(3-triethoxysilylpropyl)-4-hydroxybutyramide, and wherein each predetermined region coated with the first molecule is surrounded by a region coated with a second molecule, wherein the second

molecule binds to the patterned surface and lacks an available reactive group that binds to a nucleoside phosphoramidite;

c) synthesizing at least 800,000 of non-identical polynucleotides attached to the patterned surface, wherein the at least 800,000 non-identical polynucleotides are each at least 125 bases long and extend from the patterned surface at the predetermined regions coated with the first molecule;

d) releasing the at least 800,000 non-identical polynucleotides from the patterned surface;

e) suspending the at least 800,000 non-identical polynucleotides in a solution; and

f) subjecting the solution comprising the at least 800,000 non-identical polynucleotides to a polymerase chain assembly reaction to assemble the at least 6,000 preselected nucleic acids, wherein the assembled at least 6,000 preselected nucleic acids encode sequences with an aggregate error rate of less than 1 in 1500 bases compared to the predetermined sequences without correcting errors in the assembled at least 6,000 preselected nucleic acids, and wherein the patterned surface provides for the aggregate error rate of less than 1 in 1500 bases.

17. A method for synthesizing a library of oligonucleic acids encoding for predetermined sequences, the method comprising:

a) providing predetermined sequences for a library of at least 100,000 non-identical oligonucleic acids;

b) providing a structure having a patterned surface comprising a plurality of loci for oligonucleic acid extension, wherein each locus comprises a first plurality of molecules and is adjacent to a region that comprises a second plurality of molecules, wherein each molecule of the first plurality of molecules binds to the patterned surface and comprises a reactive group capable of binding to a nucleoside, and wherein each molecule of the second plurality of molecules binds to the patterned surface and lacks the reactive group capable of binding to the nucleoside;

c) adding a droplet of fluid comprising an extension reaction reagent specific to a locus, wherein the extension reaction reagent comprises a nucleoside phosphoramidite;

- d) allowing sufficient time for an extension reaction step to occur; and
- e) repeating steps (c) and (d) to synthesize the at least 100,000 non-identical oligonucleic acids, wherein each non-identical oligonucleic acid is at least 50 bases in length and is attached to the patterned surface at a predetermined locus, and wherein the synthesized at least 100,000 non-identical oligonucleic acids encode sequences with an aggregate error rate of less than 1 in 1000 bases compared to the predetermined sequences, and wherein the patterned surface provides for the aggregate error rate of less than 1 in 1000 bases.

18. The method of claim 17, wherein the first plurality of molecules comprises a silane, siloxane, or N-(3-triethoxysilylpropyl)-4-hydroxybutyramide.

19. The method of claim 17, wherein the first plurality of molecules comprises 11-acetoxyundecyltriethoxysilane, n-decyltriethoxysilane, or (3-aminopropyl)trimethoxysilane.

20. The method of claim 17, wherein the second plurality of molecules comprises a fluorosilane.

21. The method of claim 20, wherein the fluorosilane is (tridecafluorotetrahydrooctyl)-triethoxysilane.

22. The method of claim 17, wherein each locus of the plurality of loci is separated from another locus by a structural barrier of the patterned surface.

23. The method of claim 17, wherein steps (a) to (e) are completed in less than 1 month, 14 days, 7 days, or 2 days.

24. The method of claim 17, wherein each of the at least 100,000 non-identical oligonucleic acids comprises a region which is complementary to another of the at least 100,000 non-identical oligonucleic acids.

25. The method of claim 17, wherein more than 90% of the synthesized at least 100,000 non-identical oligonucleic acids encode sequences with an aggregate error rate of less than 1 in 1000 bases compared to the predetermined sequences.

26. The method of claim 17, wherein each non-identical oligonucleic acid is at least 100 bases in length.

27. A device for synthesizing oligonucleic acids, the device comprising:
a silicon plate having formed therein:

a plurality of channels, wherein each channel extends vertically to a depth into of the silicon plate from an opening on a top side of the silicon plate, and wherein each channel has a width of 0.5 mm to 1.5 mm; and

for each channel, 50 to 500 microchannels in fluid communication with said channel, wherein each microchannel extends vertically to a height from an opening on a bottom side of the silicon plate to the channel, wherein each microchannel has a width of 1 μ m to 100 μ m, and wherein a ratio of width to height of each microchannel is from 1 to 0.1 and provides for rapid exchange of chemical exposure during de novo synthesis of oligonucleic acids.

28. The device of claim 27, comprising a first molecule, wherein the first molecule is bound to an interior surface of each of the 50 to 500 microchannels, and wherein the first molecule comprises a reactive group capable of binding a nucleoside phosphoramidite.

29. The device of claim 28, wherein the first molecule is 11-acetoxyundecyltriethoxysilane, n-decyltriethoxysilane, (3-aminopropyl)trimethoxysilane, (3-aminopropyl)triethoxysilane, or N-(3-triethoxysilylpropyl)-4-hydroxybutyramide.

30. The device of claim 28, wherein the first molecule is a silane.

31. The device of claim 28, comprising a second molecule, wherein the second molecule is bound to an interior surface of each channel of the plurality of channels and lacks a reactive group capable of binding a nucleoside phosphoramidite.

32. The device of claim 31, wherein the second molecule is a fluorosilane.

33. The device of claim 32, wherein the fluorosilane is (tridecafluorotetrahydrooctyl)-triethoxysilane.

34. The device of claim 27, wherein the plurality of channels comprises 2 to 250 channels.

35. The device of claim 27, wherein a total number of the microchannels in the silicon plate is at least 30,000 microchannels.

36. The device of claim 27, wherein the ratio of width to height is 0.5, 0.2, or 0.1.

37. The device of claim 27, wherein each channel of the plurality of channels has a width of 1 mm to 1.5 mm.

38. The device of claim 27, wherein each of the 50 to 500 microchannels has a height of 10 μ m to 500 μ m.

39. A device for nucleic acid assembly, comprising:
- a) a first plate, the first plate comprising:
 - a base layer of the first plate; and
 - one or more wells, wherein each well of the one or more wells comprises:
 - a side wall that extends vertically from the base layer;
 - an interior region of the well; and
 - an exterior region of the well, wherein the exterior region of the well has a lower surface energy than the interior region of the well;
 - and
 - b) a second plate, wherein the second plate comprises a plurality of clusters, wherein each cluster comprises a plurality of loci, wherein each locus comprises a physically distinct region comprising molecules capable of binding to a nucleoside phosphoramidite, and wherein each cluster is vertically aligned to a single well of the one or more wells.
40. The device of claim 39, wherein the second plate is a silicon plate.
41. The device of claim 39, wherein the first plate is a silicon or plastic plate.
42. The device of claim 41, wherein the first plate further comprises an insulator layer on top of the base layer, wherein the insulator layer comprises silicon dioxide.
43. The device of claim 39, wherein the side wall has a length from 0.1 to 0.6 mm, and wherein the interior region of the well has a width from 0.8 to 2.0 mm.
44. The device of claim 39, wherein each well of the one or more wells comprises an aqueous liquid, wherein the aqueous liquid has a surface tension from 12 to 80 millinewtons per meter.
45. The device of claim 39, wherein the width of the interior region of the well is 1.15 mm, and wherein the length of the side wall is 0.45 mm.
46. The device of claim 39, wherein the device further comprises an enclosure, wherein the first plate and second plate are both within the enclosure.
47. The device of claim 39, wherein each well of the one or more wells comprises a sealing means located at a distal end of the side wall of said well.
48. The device of claim 39, wherein the interior region of each well of the one or more wells comprises a silane.

49. The device of claim 48, wherein the silane is methoxyethoxyundecyltrichlorosilane, methacryloxypropyltrimethoxysilane, ethyltrimethoxysilane, propyltrimethoxysilane, trifluoropropyltrimethoxysilane, 3-(2-aminoethyl)aminopropyltrimethoxysilane, p-tolyltrimethoxysilane, cyanoethyltrimethoxysilane, aminopropyltriethoxysilane, acetoxypentyltrimethoxysilane, phenyltrimethoxysilane, chloropropyltrimethoxysilane, mercaptopropyltrimethoxysilane, trichloro-octodecyl-silane, methyltrimethoxysilane, butyl-aldehyde-trimethoxysilane, heptadecafluorodecyltrimethoxysilane, or glycidoxypentyltrimethoxysilane.

50. The device of claim 39, wherein the exterior region of each well of the one or more wells comprises a fluorosilane.

51. The device of claim 50, wherein the fluorosilane is (tridecafluorotetrahydrooctyl)-triethoxysilane.

52. The device of claim 39, wherein each cluster of the plurality of clusters of the second silicon plate has a lower surface energy than the interior region of each well the plurality of wells of the first silicon plate.

53. The device of claim 39, where each locus of the plurality of loci comprises a microchannel.

54. An oligonucleic acid library, comprising at least 100,000 non-identical oligonucleic acids that collectively encode sequences for at least 750 preselected genes, each preselected gene having a predetermined sequence, wherein each of the at least 100,000 non-identical oligonucleic acids is synthesized and comprises at least 30 bases in length, and wherein the at least 100,000 non-identical oligonucleic acids encode sequences with an aggregate error rate of less than 1 in 1000 bases compared to the predetermined sequences for the at least 750 preselected genes.

55. A gene library, comprising a collection of at least 750 preselected genes, each gene having a predetermined sequence, wherein each of the at least 750 preselected genes is at least 0.5 kb length in length, wherein each preselected gene is synthesized without correcting errors, and wherein the at least 750 preselected genes have an aggregate error rate of less than 1 in 1000 bases compared to the predetermined sequences for the at least 750 preselected genes.

CLAIMS

WHAT IS CLAIMED IS:

1. A gene library, comprising a collection of genes, wherein the collection comprises at least 50 different preselected synthetic genes that are each of at least 0.5 kb length with an error rate of less than 1 in 3000 bp compared to predetermined sequences comprising the genes.
2. A gene library, comprising a collection of genes, wherein the collection comprises at least 50 different preselected synthetic genes that are each of at least 0.5 kb length, wherein at least 90% of the preselected synthetic genes comprise an error rate of less than 1 in 3000 bp compared to predetermined sequences comprising the genes.
3. The gene library according to claims 1 or 2, wherein at least 90% of the preselected synthetic genes comprise an error rate of less than 1 in 5000 bp compared to predetermined sequences comprising the genes.
4. The gene library according to claims 1 or 2, wherein at least 0.05% of the preselected synthetic genes are error free.
5. The gene library according to claims 1 or 2, wherein the collection comprises at least 75 different preselected synthetic genes.
6. The gene library according to claims 1 or 2, wherein the collection comprises at least 100 different preselected synthetic genes.
7. The gene library according to claims 1 or 2, wherein the preselected synthetic genes comprise a deletion rate of less than 1 in 3000 bp compared to predetermined sequences comprising the genes.
8. The gene library according to claims 1 or 2, further comprising at least 1000000 copies of each synthetic gene.
9. The gene library according to claims 1 or 2, wherein the collection comprises at least 500 genes.
10. The gene library according to claims 1 or 2, wherein the preselected synthetic genes are at least 1kb.
11. The gene library according to claims 1 or 2, wherein each of the synthetic genes differ from any other synthetic gene by at least 1%.
12. The gene library according to claims 1 or 2, wherein the gene library is in a space that is less than 10 cm³.
13. A method of constructing a gene library, comprising:

(a) entering before a first timepoint, in a computer readable non-transient medium at least a first list of genes and a second list of genes, wherein the genes are at least 500 bp and wherein, when compiled into a joint list, the joint list comprises at least 100 genes;

(b) synthesizing more than 90% of the genes in the joint list before a second timepoint, thereby constructing a gene library with deliverable genes;

wherein the second timepoint is less than a month apart from the first timepoint.

14. The method of claim 13, further comprising delivering at least one gene at a second timepoint.

15. The method of claim 13, wherein each of the genes differ from any other gene by at least 1% in the gene library.

16. The method of claim 13, wherein at least 90% of the deliverable genes comprise an error rate of less than 1 in 3000 bp resulting in the generation of a sequence that deviates from the sequence of a gene in the joint list of genes.

17. The method of claim 13, wherein the joint list comprises at least 500 genes.

18. The method of claim 13, wherein the genes are at least 1kb.

19. The method of claim 13, wherein the second timepoint is less than 5 days apart from the first timepoint.

20. The method of claim 13, wherein the second timepoint is less than 2 days apart from the first timepoint.

21. A method of constructing a gene library, comprising:

(a) entering at a first timepoint, in a computer readable non-transient medium a list of genes, wherein the list comprises at least 100 genes and wherein the genes are at least 500 bp;

(b) synthesizing more than 90% of the list of genes, thereby constructing a gene library with deliverable genes; and

(c) delivering the deliverable genes at a second timepoint, wherein the second timepoint is less than a month apart from the first timepoint.

22. A method of synthesizing n-mer oligonucleotides on a substrate, comprising:

(a) providing a substrate with resolved loci that are functionalized with a chemical moiety suitable for nucleotide coupling; and

(b) coupling at least two building blocks to a plurality of growing oligonucleotide chains each residing on one of the resolved loci at a rate of at least 10

nucleotides per hour according to a locus specific predetermined sequence, thereby synthesizing a plurality of oligonucleotides that are n basepairs long.

23. The method of claim 22, further comprising coupling at least two building blocks to a plurality of growing oligonucleotide chains each residing on one of the resolved loci at a rate of at least 12 nucleotides per hour.

24. The method of claim 22, further comprising coupling at least two building blocks to a plurality of growing oligonucleotide chains each residing on one of the resolved loci at a rate of at least 15 nucleotides per hour.

25. The method of claim 22, wherein at least one resolved locus comprises n-mer oligonucleotides deviating from the locus specific predetermined sequence with an error rate of less than 1/500 bp.

26. The method of claim 22, wherein the plurality of oligonucleotides on the substrate deviate from respective locus specific predetermined sequences at an error rate of less than 1/500 bp.

27. The method of claim 22, wherein the building blocks comprise an adenine, guanine, thymine, cytosine, or uridine group, or a modified nucleotide.

28. The method of claim 22, wherein n is at least 100.

29. The method of claim 22, wherein the substrate comprises at least 100 resolved loci and wherein at least two of the plurality of growing oligonucleotides are different from each other.

30. The method of claim 22, further comprising vacuum drying the substrate before coupling.

31. The method of claim 22, wherein the building blocks comprise a blocking group.

32. The method of claim 31, wherein the blocking group comprises an acid-labile DMT.

33. The method of claim 32, wherein the acid-labile DMT comprises 4,4'-dimethoxytrityl.

34. The method of claim 29, wherein the substrate comprises at least 1000 vias providing fluid communication between a first surface of the substrate and a second surface of the substrate.

35. The method of claim 29, wherein the substrate comprises at least 10,000 vias providing fluid communication between a first surface of the substrate and a second surface of the substrate.

36. A method for synthesizing oligonucleotides on a substrate having a functionalized surface, comprising:

- (a) applying through at least one inkjet pump at least one drop of a first reagent to a first locus of a plurality of loci;
- (b) applying negative pressure to the substrate; and
- (c) applying through at least one inkjet pump at least one drop of a second reagent to the first locus.

37. The method of claim 36, wherein the pressure surrounding the substrate can be reduced to less than 1 mTorr.

38. A method of constructing a gene library, comprising:

- (a) entering at a first timepoint, in a computer readable non-transient medium a list of genes, wherein the list comprises at least 100 genes and wherein the genes are at least 500 bp;
- (b) synthesizing more than 90% of the list of genes, thereby constructing a gene library with deliverable genes;
- (c) preparing a sequencing library that represents the gene library;
- (d) obtaining sequence information;
- (e) selecting at least a subset of the deliverable genes based on the sequence information; and
- (f) delivering the selected deliverable genes at a second timepoint, wherein the second timepoint is less than a month apart from the first timepoint.

39. The method of claim 38, wherein the second timepoint is less than 5 days apart from the first timepoint.

40. The method of claim 38, wherein the second timepoint is less than 2 days apart from the first timepoint.

41. A method of nucleic acid amplification, comprising:

- (a) providing a sample comprising n circularized single stranded nucleic acids, each comprising a different target sequence;
- (b) providing a first adaptor that is hybridizable to at least one adaptor hybridization sequence on m of the n circularized single stranded nucleic acids;
- (c) providing conditions suitable for extending the first adaptor using the m circularized single stranded nucleic acids as a template, thereby generating m single stranded

amplicon nucleic acids, wherein each of the m single stranded amplicon nucleic acids comprises a plurality of replicas of the target sequence from its template;

(d) providing a first auxiliary oligonucleotide that is hybridizable to the first adaptor; and

(e) providing a first agent under conditions suitable for the first agent to cut the m single stranded amplicon nucleic acids at a plurality of cutting sites, thereby generating a plurality of single stranded replicas of the target sequences in the m circularized single stranded nucleic acids.

42. The method of claim 41, wherein n or m is at least 2.

43. The method of claim 41, wherein n or m is at least 25.

44. The method of claim 41, wherein m is less than n .

45. The method of claim 41, wherein the sample comprising the n circularized single stranded nucleic acid is formed by providing at least n linear single stranded nucleic acids, each comprising one of the different target sequences and circularizing the n linear single stranded nucleic acids, thereby generating the n circularized single stranded nucleic acids.

46. The method of claim 45, wherein the first adaptor is hybridizable to both ends of the n linear single stranded nucleic acids concurrently.

47. The method of claim 45, wherein the different target sequences in the n linear single stranded nucleic acids are flanked by a first and a second adaptor hybridization sequence.

48. The method of claim 45, wherein the at least n linear single stranded nucleic acids are generated by de novo oligonucleotide synthesis.

49. The method of claim 47, wherein the first adaptor hybridization sequence in each of the n linear single stranded nucleic acids differ by no more than two nucleotide bases.

50. The method of claim 47, wherein the first or the second adaptor hybridization sequence is at least 5 nucleotides long.

51. The method of claim 47, wherein the first or the second adaptor hybridization sequence is at most 75 nucleotides long.

52. The method of claim 46, wherein the ends of the n linear single stranded nucleic acids pair with adjacent bases on the first adaptor when the first adaptor is hybridized to the both ends of the linear single stranded nucleic acid concurrently.

53. The method of claim 41, wherein the locations of the plurality of cutting sites are such that the adaptor hybridization sequence is severed from at least 5% of a remainder sequence portion of the m circularized single stranded nucleic acid replicas.

54. The method of claim 41, wherein at least 5% of the sequence of the m circularized single stranded nucleic acid replicas other than the at least one adaptor hybridization sequence remains uncut.

55. The method of claim 41, wherein the locations of the plurality of cutting sites are outside the at least one adaptor hybridization sequence.

56. The method of claim 41, wherein the locations of the plurality of cutting sites are independent of the target sequences.

57. The method of claim 41, wherein the locations of the plurality of cutting sites are determined by at least one sequence element within the sequence of the first adaptor or the first auxiliary oligonucleotide.

58. The method of claim 57, wherein the sequence element comprises a recognition site for a restriction endonuclease.

59. The method of claim 41, wherein the first auxiliary oligonucleotide or the first adaptor oligonucleotide comprises a recognition site for a Type IIS restriction endonuclease.

60. The method of claim 41, wherein the recognition sites are at least 3 nucleotides away from the cutting sites.

61. The method of claim 41, wherein the plurality of cutting sites are at junctures of single and double stranded nucleic acids.

62. The method of claim 61, wherein the double stranded nucleic acids comprise the first adaptor and the first auxiliary oligonucleotide.

63. The method of claim 61, wherein the single stranded nucleic acids consist essentially of the m different target sequences.

64. The method of claim 63, wherein the m different target sequences have at most 95% pairwise similarity.

65. The method of claim 63, wherein the m different target sequences have at most 90% pairwise similarity.

66. The method of claim 63, wherein the m different target sequences have at most 80% pairwise similarity.

67. The method of claim 63, wherein the m different target sequences have at most 50% pairwise similarity.

68. The method of claim 41, wherein generating the m single stranded amplicon nucleic acid comprises strand displacement amplification.

69. The method of claim 41, wherein the first auxiliary oligonucleotide comprises an affinity tag.

70. The method of claim 69, wherein the affinity tag comprises biotin or biotin derivative.

71. The method of claim 41, further comprising isolating double stranded nucleic acids from the sample.

72. The method of claim 71, wherein the isolating comprises affinity purification, chromatography, or gel purification.

73. The method of claim 41, wherein the first agent comprises a restriction endonuclease.

74. The method of claim 73, wherein the first agent comprises at least two restriction endonucleases.

75. The method of claim 73 or 74, wherein the first agent comprises a Type IIS restriction endonuclease.

76. The method of claim 41, wherein the first agent comprises a nicking endonuclease.

77. The method of claim 76, wherein the first agent comprises at least two nicking endonucleases.

78. The method of claim 41, wherein the first agent comprises at least one enzyme selected from the group consisting of MlyI, SchI, AlwI, BccI, BceAI, BsmAI, BsmFI, FokI, HgaI, PstI, SfaNI, BfuAI, BsaI, BspMI, BtgZI, EarI, BspQI, SapI, SgeI, BceFI, BslFI, BsoMAI, Bst7II, FagI, AceIII, BbvII, BveI, LguI, BfuCI, DpnII, FatI, MboI, MluCI, Sau3AI, Tsp509I, BssKI, PspGI, StyD4I, Tsp45I, AoxI, BscFI, Bsp143I, BssMI, BseENII, BstMBI, Kzo9I, NedII, Sse9I, TasI, TspEI, AjnI, BstSCI, EcoRII, MaeIII, NmuCI, Psp6I, MnlI, BspCNI, BsrI, BtsCI, HphI, HpyAV, MboII, AclI, BciVI, BmrI, BpmI, BpuEI, BseRI, BsgI, BsmI, BsrDI, BtsI, EciI, MmeI, NmeAIII, Hin4II, TscAI, Bce83I, BmuI, BsbI, BscCI, NlaIII, Hpy99I, TspRI, FaeI, Hin1II, Hsp92II, SetI, TaiI, TscI, TscAI, TseFI, Nb.BsrDI, Nb.BtsI, AspCNI, BscGI, BspNCI, EcoHI, FinI, TsuI, UbaF11I, UnbI, Vpak11AI, BspGI, DrdII, Pfl1108I, UbaPI, Nt.AlwI, Nt.BsmAI, Nt.BstNBI, and Nt.BspQI.

79. The method of claim 74, wherein the at least two restriction enzymes comprise MlyI and BciVI or BfuCI and MlyI.

80. The method of claim 41, further comprising:

- (a) partitioning the sample into a plurality of fractions;

(b) providing at least one fraction with a second adaptor that is hybridizable to at least one adaptor hybridization sequence on k of the n different circularized single stranded nucleic acids;

(c) providing conditions suitable for extending the second adaptor using the k circularized single stranded nucleic acids as a template, thereby generating k single stranded amplicon nucleic acids, wherein the second single stranded amplicon nucleic acid comprises a plurality of replicas of the target sequence from its template;

(d) providing a second auxiliary oligonucleotide that is hybridizable to the second adaptor; and

(e) providing a second agent under conditions suitable for the agent to cut the k single stranded amplicon nucleic acids at a second plurality of cutting sites, thereby generating a plurality of single stranded replicas of the target sequences in the k circularized single stranded nucleic acids.

81. The method of claim 80, wherein the first and the second adaptors are the same.

82. The method of claim 80, wherein the first and the second auxiliary oligonucleotides are the same.

83. The method of claim 80, wherein the first and the second agents are the same.

84. The method of claim 80, wherein $k + m$ is less than n.

85. The method of claim 80, wherein k is at least 2.

86. The method of claim 41, wherein the sample comprising the n circularized single stranded nucleic acid is formed by single stranded nucleic acid amplification.

87. The method of claim 86, wherein the single stranded nucleic acid amplification comprises:

(a) providing a sample comprising at least m circularized single stranded precursor nucleic acids;

(b) providing a first precursor adaptor that is hybridizable to the m circularized single stranded precursor nucleic acids;

(c) providing conditions suitable for extending the first precursor adaptor using the m circularized single stranded precursor nucleic acids as a template, thereby generating m single stranded precursor amplicon nucleic acids, wherein the single stranded amplicon nucleic acid comprises a plurality of replicas of the m circularized single stranded precursor nucleic acid;

(d) providing a first precursor auxiliary oligonucleotide that is hybridizable to the first precursor adaptor; and

(e) providing a first precursor agent under conditions suitable for the first precursor agent to cut the first single stranded precursor amplicon nucleic acid at a plurality of cutting sites, thereby generating the m linear precursor nucleic acids.

88. The method of claim 87, further comprising circularizing the m linear precursor nucleic acids, thereby forming replicas of the m circularized single stranded precursor nucleic acids.

89. The method of claim 87, wherein the m circularized single stranded precursor nucleic acid is amplified by at least 10-fold in single stranded replicas.

90. The method of claim 41, wherein at least one of the m circularized single stranded nucleic acids is at a concentration of 1 nM or less.

91. The method of claims 45 or 88, wherein circularizing comprises ligation.

92. The method of claim 90, wherein ligation comprises the use of a ligase selected from the group consisting of T4 DNA ligase, T3 DNA ligase, T7 DNA ligase, *e.coli* DNA ligase, Taq DNA ligase, and 9N DNA ligase.

93. A microfluidic device for nucleic acid synthesis, comprising a substantially planar substrate portion comprising n groupings of m microfluidic connections between opposite surfaces,

wherein each one of the $n \times m$ microfluidic connections comprises a first channel and a second channel,

wherein the first channel within each of the n groupings is common to all m microfluidic connections,

wherein the plurality of microfluidic connections span the substantially planar substrate portion along the smallest dimension of the substrate, and

wherein n and m are at least 2.

94. A system for conducting a set of parallel reactions, comprising:

(a) a first surface with a plurality of resolved loci;

(b) a capping element with a plurality of resolved reactor caps;

wherein the system aligns the plurality of resolved reactor caps with the plurality of resolved loci on the first surface forming a temporary seal between the first surface and the capping element, thereby physically dividing the loci on the first

surface into groups of at least two loci into a reactor associated with each reactor cap, and wherein each reactor holds a first set of reagents.

95. The system of claim 94, wherein the plurality of resolved loci is at a density of at least 1 per mm^2 .

96. The system of claim 94, wherein upon release from the first surface, the reactor caps retain at least a portion of the first set of reagents.

97. The system of claim 96, wherein the at least a portion is at least 30%.

98. The system of claim 94, wherein the plurality of resolved loci reside on microstructures fabricated into a support surface.

99. The system of claim 98, wherein the microstructures comprise at least two channels in fluidic communication with each other.

100. The system of claim 99, wherein the at least two channels comprise two channels with different width.

101. The system of claim 99, wherein the at least two channels comprise two channels with different length.

102. The system of claim 99, wherein at least one of the channels is longer than 100 μm .

103. The system of claim 99, wherein at least one of the channels is narrower than 100 μm in diameter.

104. The system of claim 94, further comprising a second surface with a plurality of resolved loci at a density of at least 0.1 per mm^2 .

105. The system of claim 94, wherein the resolved loci of the first surface comprise a coating of reagents.

106. The system of claim 104, wherein the resolved loci of the second surface comprise a coating of reagents.

107. The system of claim 105 or 106, wherein the coating of reagents comprises oligonucleotides.

108. The system of claim 107, wherein the coating of reagents has a surface area of at least 1 μm^2 per 1.0 μm^2 of planar surface area.

109. The system of claim 94, wherein the plurality of resolved loci comprise a nominal arclength of the perimeter of a density of at least 0.001 μm / square μm .

110. The system of claim 94, wherein the plurality of resolved loci of the first surface comprise a high energy surface corresponding to a water contact angle of less than 20 degree.

111. An array of enclosures, comprising:
- (a) a plurality of resolved reactors comprising a first substrate and a second substrate comprising reactor caps; and
 - (b) at least 2 resolved loci in each reactor;
- wherein the resolved reactors are separated with a releasable seal and wherein the reactor caps retain at least a part of the contents of the reactors upon release of the second substrate from the first substrate.
112. A method of conducting a set of parallel reactions, comprising:
- (a) providing a first surface with a plurality of resolved loci;
 - (b) providing a capping element with a plurality of resolved reactor caps;
 - (c) aligning the plurality of resolved reactor caps with the plurality of resolved loci on the first surface and forming a temporary seal between the first surface and the capping element, thereby physically dividing the loci on the first surface into groups of at least two loci;
 - (d) performing a first reaction, thereby forming a first set of reagents; and
 - (e) releasing the capping element from the first surface, wherein each reactor cap retains at least a portion of the first set of reagents in a first reaction volume.
113. The method of claim 112, further comprising:
- (a) providing a second surface with a plurality of resolved loci;
 - (b) aligning the plurality of resolved reactor caps with the plurality of resolved loci on the second surface and forming a temporary seal between the second surface and the capping element, thereby physically dividing the loci on the second surface;
 - (c) performing a second reaction using the portion of the first set of reagents, thereby forming a second set of reagents; and
 - (d) releasing the capping element from the second surface, wherein each reactor cap retains at least a portion of the second set of reagents in a second reaction volume.
114. The system of claim 112, wherein the plurality of resolved loci have a density of at least 1 per mm^2 on the first surface.
115. The method of claim 112, wherein the plurality of resolved reactor caps have a density of at least 0.1 per mm^2 on the capping element.
116. The method of claim 113, wherein the releasing in steps e) and i) are performed at a different velocity.

117. The method of claim 112, wherein the resolved loci of the first surface comprise a coating of reagents for the first reaction.

118. The method of claim 112, wherein the resolved loci of the second surface comprise a coating of reagents for the second reaction.

119. The method of claim 112, wherein the coating of reagents comprises oligonucleotides.

120. The method of claim 119, wherein the coatings of reagents has a surface area of at least $1\ \mu\text{m}^2$ per $1.0\ \mu\text{m}^2$ of planar surface area.

121. The method of claim 112, wherein the oligonucleotides are at least 25 bp.

122. The method of claim 112, wherein the resolved loci of the first surface comprise a high energy surface corresponding to a water contact angle of less than 20 degree..

123. A substrate having a functionalized surface, comprising:

(a) a solid support having a plurality of resolved loci, wherein the resolved loci are functionalized with a moiety that increases the surface energy of the solid support, wherein the resolved loci are localized on microchannels.

124. The substrate of claim 123, wherein the moiety is a chemically inert moiety.

125. The substrate of claim 123, wherein the microchannels comprise a volume of less than 1 nl.

126. The substrate of claim 123, wherein the microchannels comprise a density of the nominal arclength of the perimeter of at least $0.01\ \mu\text{m}$ / square μm .

127. The substrate of claim 123, wherein the microchannels comprise a density of the nominal arclength of the perimeter of at least $0.001\ \mu\text{m}$ / square μm .

128. The substrate of claim 123, wherein the functionalized surface comprises a nominal surface area of at least $1\ \mu\text{m}^2$ per $1.0\ \mu\text{m}^2$ of planar surface area of the substrate.

129. The substrate of claim 123, wherein at least one of the microchannels is longer than $100\ \mu\text{m}$.

130. A method of depositing reagents to a plurality of resolved loci, comprising

(a) applying through an inkjet pump at least one drop of a first reagent to a first locus of the plurality of loci;

(b) applying through an inkjet pump at least one drop of a second reagent to a second locus of the plurality of resolved loci, wherein the second locus is adjacent to the first locus,

wherein the first and second reagents are different, wherein the first and second loci reside on microstructures fabricated into a support surface and wherein the microstructures comprise at least one channel that is more than 100 μm deep.

131. The method of claim 130, wherein the microstructures comprise at least two channels in fluidic communication with each other.

132. The method of claim 131, wherein the at least two channels comprise two channels with different width.

133. The method of claim 131, wherein the at least two channels comprise two channels with different length.

134. The method of claim 130, wherein the first locus receives less than 0.1% of the second reagent and wherein the second locus receives less than 0.1% of the first reagent.

135. The method of claim 130, wherein the plurality of resolved loci are at a density of at least $1/\text{mm}^2$.

136. The method of claim 130, wherein the plurality of resolved loci are at a density of at least $100/\text{mm}^2$.

137. The method of claim 130, wherein the volume of the drop is at least 2 μl .

138. The method of claim 130, wherein the volume of the drop is about 40 μl .

139. The method of claim 130, wherein the volume of the drop is at most 100 μl .

140. A microfluidic system, comprising:

(a) a first surface with a plurality of microwells; and

(b) a droplet inside one of the plurality of microwells;

wherein the droplet inside one of the plurality of microwells has a Reynolds number at a range of about 1-1000.

141. The microfluidic system of claim 140, wherein the microwell is longer than 100 μm .

142. The microfluidic system of claim 140, wherein the volume of the droplet is at least 2 μl .

143. The microfluidic system of claim 140, wherein the droplet comprises a reagent that enables oligonucleotide synthesis.

144. The microfluidic system of claim 143, wherein the reagent is a nucleotide or nucleotide analog.

145. A method of depositing droplets to a plurality of microwells, comprising

applying through an inkjet pump at least one droplet to a first microwell of the plurality of microwells;

wherein the droplet inside one of the plurality of microwells has a Reynolds number at a range of about 1-1000.

146. A method of partitioning, comprising

(a) contacting a first surface comprising a liquid at a first plurality of resolved loci with a second surface comprising a second plurality of resolved loci, wherein the first surface comprises a first surface tension with the liquid, the second surface comprises a second surface tension with the liquid;

(b) determining a velocity of release such that a desired fraction of the liquid is transferred from the first plurality of resolved loci to the second plurality of resolved loci; and

(c) detaching the second surface from the first surface at said velocity.

147. The method of claim 146, wherein the surface tension of the first surface corresponds to a water contact angle of less than 20 degree.

148. The method of claim 146, wherein the surface tension of the second surface corresponds to a water contact angle of more than 90 degree.

149. A method of mixing, comprising:

(a) providing a first substrate comprising a plurality of microstructures fabricated thereto;

(b) providing a second substrate comprising a plurality of resolved reactor caps;

(c) aligning the first and second substrates such that a first reactor cap of the plurality is configured to receive liquid from n microstructures in the first substrate; and

(d) delivering liquid from the n microstructures into the first reactor cap, thereby mixing liquid from the n microstructures forming a mixture.

150. The method of claim 149, wherein n is at least 10.

151. A method of nucleic acid amplification, comprising:

(a) providing a sample comprising n circularized single stranded nucleic acids, each comprising a different target sequence;

(b) providing a first adaptor that is hybridizable to at least one adaptor hybridization sequence on m of the n circularized single stranded nucleic acids;

(c) providing conditions suitable for extending the first adaptor using the m circularized single stranded nucleic acids as a template, thereby generating m single stranded

amplicon nucleic acids, wherein each of the m single stranded amplicon nucleic acids comprises a plurality of replicas of the target sequence from its template;

(d) generating double stranded recognition sites for a first agent on the m single stranded amplicon nucleic acids; and

(e) providing the first agent under conditions suitable for the first agent to cut the m single stranded amplicon nucleic acids at a plurality of cutting sites, thereby generating a plurality of single stranded replicas of the target sequences in the m circularized single stranded nucleic acids.

152. The method of claim 151, wherein the double stranded recognition sites comprise a first portion of the first adaptor on a first strand of the double stranded recognition sites and a second strand of the first adaptor on the second strand of the double stranded recognition sites.

153. The method of claim 152, wherein the adaptor comprises a palindromic sequence.

154. The method of claim 152, wherein the double stranded recognition sites are generated by hybridizing the first and second portions of the first adaptor to each other.

155. The method of claim 151, wherein the m single stranded amplicon nucleic acids comprise a plurality of double stranded self-hybridized regions.

156. A method for generating a long nucleic acid molecule, the method comprising the steps of:

(a) providing a plurality of nucleic acids immobilized on a surface, wherein said plurality of nucleic acids comprises nucleic acids having overlapping complementary sequences;

(b) releasing said plurality of nucleic acids into solution; and

(c) providing conditions promoting:

(i) hybridization of said overlapping complementary sequences to form a plurality of hybridized nucleic acids; and

(ii) extension or ligation of said hybridized nucleic acids to synthesize the long nucleic acid molecule.