

HEATED NANOWELLS FOR POLYNUCLEOTIDE SYNTHESIS

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. provisional patent application number 62/575,287 filed on October 20, 2017, which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on October 16, 2018, is named 44854-744_601_SL.txt and is 685 bytes in size.

BACKGROUND

[0003] De novo gene synthesis is a powerful tool for basic biological research and biotechnology applications. While various methods are known for the design and synthesis of relatively short fragments in a small scale, these techniques often suffer from predictability, scalability, automation, speed, accuracy, and cost.

BRIEF SUMMARY

[0003a] In one aspect, provided herein is a method for polynucleotide synthesis, the method comprising: a. providing: a. a substrate comprising a surface; a plurality of structures for polynucleotide extension located on the surface, wherein each structure has a width of about 10 nm to about 1000 nm, wherein each structure is in contact with a heating unit, wherein the heating unit comprise an addressable electrode, a solvent distributed across the surface, predetermined sequences for a library of polynucleotides; b. synthesizing the library of polynucleotides at each structure, and c. heating of the solvent at each structure by applying an electrical current through the addressable electrode, causing the solvent to be in the gas phase which prevents deblocking of at least one polynucleotide extending from the at least one region of the surface.

[0004] Provided herein are devices for polynucleotide synthesis comprising: a solid support comprising a surface; a plurality of structures for polynucleotide extension located on the the surface, wherein each structure has a width of about 10 nm to about 1000 nm, wherein each structure is in contact with a heating unit, and wherein the heating unit comprises at least one electrode; and a solvent distributed across the surface, wherein the solvent is a polar solvent. Further provided herein are devices wherein the surface comprises at least 30,000 loci for nucleic acid synthesis. Further provided herein are devices wherein the surface comprises at least 50,000

loci for nucleic acid synthesis. Further provided herein are devices wherein the surface comprises at least 100,000 loci for nucleic acid synthesis. Further provided herein are devices wherein the surface comprises at least 200,000 loci for nucleic acid synthesis. Further provided herein are devices wherein the surface comprises at least 1,000,000 loci for nucleic acid synthesis. Further provided herein are devices wherein the one or more electrodes are addressible electrodes that heats one or more individual loci. Further provided herein are devices wherein the solid support further comprises a cooling unit. Further provided herein are devices wherein the distance between the centers of any two structures is about 10 nm to about 1000 nm. Further provided herein are devices wherein the distance between the centers of any two structures is about 100 nm to about 500 nm. Further provided herein are devices wherein the distance between the

CLAIMS

1. A method for polynucleotide synthesis, the method comprising:
 - a. providing:
 - a substrate comprising a surface;
 - a plurality of structures for polynucleotide extension located on the surface, wherein each structure has a width of about 10 nm to about 1000 nm, wherein each structure is in contact with a heating unit, wherein the heating unit comprise an addressable electrode,
 - a solvent distributed across the surface,
 - predetermined sequences for a library of polynucleotides;
 - b. synthesizing the library of polynucleotides at each structure, and
 - c. heating of the solvent at each structure by applying an electrical current through the addressable electrode, causing the solvent to be in the gas phase which prevents deblocking of at least one polynucleotide extending from the at least one region of the surface.
2. The method of claim 1, wherein synthesizing the library of polynucleotides extending from the surface comprises contacting the surface with a first nucleotide phosphoramidite.
3. The method of claim 2, wherein synthesizing the library of polynucleotides extending from the surface further comprises contacting the surface with a second nucleotide phosphoramidite, wherein the solvent is not removed between contact with the first nucleotide phosphoramidite and the second nucleotide phosphoramidite.
4. The method of claim 1, wherein synthesizing the library of polynucleotides extending from the surface further comprises condensing the solvent present at the at least one region of the surface, and deblocking at least one extended polynucleotide extending from the surface in the at least one region.
5. The method of claim 1, wherein the solvent has a boiling temperature no more than about 75 degrees C.
6. The method of claim 5, wherein the solvent has a boiling temperature no more than about 65 degrees C.

7. The method of claim 1, wherein the solvent has a boiling temperature of between 30 degrees C to 82 degrees C.
8. The method of claim 7, wherein the solvent has a boiling temperature of between 55 degrees C to 82 degrees C.
9. The method of claim 1, wherein the surface comprises at least 30,000 loci for nucleic acid synthesis.
10. The method of claim 9, wherein the surface comprises at least 50,000 loci for nucleic acid synthesis.
11. The method of claim 10, wherein the surface comprises at least 100,000 loci for nucleic acid synthesis.
12. The method of claim 11, wherein the surface comprises at least 200,000 loci for nucleic acid synthesis.
13. The method of claims 12, wherein the surface comprises at least 1,000,000 loci for nucleic acid synthesis.
14. The method of claim 1, wherein the solvent has a boiling temperature no more than about 82 degrees C.
15. The method of claim 1, wherein the solvent is a polar solvent.
16. The method of claim 15, wherein the solvent is acetonitrile.
17. The method of claim 1, wherein the solvent is a non-polar solvent.
18. The method of claim 1, wherein the solvent when in a liquid phase allows deblocking of at least one polynucleotide extending from the at least one region of the surface.
19. The method of claim 1, wherein the method further comprises condensing the solvent after deblocking of at least one polynucleotide extending from the at least one region of the surface.