environments. To survive, microbes have developed amazing sets of biochemical tools, including novel enzymes, unique metabolites, innovative genetic pathways, and strategies for manipulating their environment and their microbial neighbors – powerful solutions that could lead to new insights and products ranging from life-saving antibiotics to fertilizers that improve food production and security.

SUMMARY

The present disclosure describes microbiology systems, apparatus, kits, and methods for streamlining the cultivation workflow, supporting high throughput screening, and/or developing new insights and products in accordance with some examples. Thus, in one aspect, the present disclosure refers to a method of analyzing a sample including a population of biological entities using at least one microfabricated device having a top surface defining an array of microwells, wherein a plurality of the microwells of the array of microwells each comprise a unique tag nucleic acid molecule including: (1) a target-specific nucleotide sequence for annealing to a target nucleic acid fragment of one or more biological entities of interest that may be present in the microwell, and (2) a location-specific nucleotide sequence for identifying the location of the microwell on the microfabricated device, the method comprising: loading a sample onto the microfabricated device such that at least some microwells of the plurality of microwells each include more than one cell of a biological entity as well as an amount of a nutrient; incubating the microfabricated device at predetermined conditions; amplifying, in individual microwells, a selected genetic material of the cells of the biological entities obtained from the incubation in the plurality of microwells, thereby obtaining first amplicons in at least a subset of the plurality of microwells; sequencing an aggregate of the first amplicons collected from the subset the plurality of microwells to obtain sequencing data; based on the sequencing data and the unique tag nucleic molecule included in each of the plurality of microwells, obtaining an identification of the biological entities present in each of the subset of the plurality of microwells of the at least one microfabricated device; and based on the identification of the biological entities present in each of the subset of the plurality of microwells, determining a presence or absence of a relationship regarding co-inhabitance between at least two different types of cells of biological entities in the population of biological entities contained in the sample.

In another aspect, the present disclosure refers to a method of analyzing a sample including a population of biological entities using at least one microfabricated device having a top surface defining an array of microwells, the method comprising: loading into each of a plurality of the microwells of the array of microwells with a unique tag nucleic acid molecule including: (1) a target-specific nucleotide sequence for annealing to a target nucleic acid fragment of one or more biological entities of interest that may be present in the microwell, and (2) a location-specific nucleotide sequence for identifying the location of the microwell on the at least microfabricated device; loading a sample onto the microfabricated device such that at least some microwells of the plurality of microwells each includes more than one cell of a biological entity as well as an amount of a nutrient; incubating the microfabricated device at predetermined conditions; amplifying a selected genetic material of the cells of the biological entities obtained from the incubation in at least a subset of the plurality of microwells of the at least one microfabricated device, thereby obtaining first amplicons in the plurality of microwells; sequencing an aggregate of the first amplicons collected from the subset the plurality of microwells to obtain sequencing data; based on the sequencing data and the unique tag nucleic molecule included in each of the plurality of microwells, obtaining an identification of the biological entities present in each of the subset of the plurality of microwells of the at least one microfabricated device; and based on the identification of the biological entities present in each of the subset of the plurality of microwells, determining a presence or absence of a relationship regarding co-inhabitance between at least two different types of cells of biological entities in the population of biological entities contained in the sample.

In yet another aspect, the present disclosure refers to a method of analyzing a microbiome of microorganisms collected from a specific environment using at least one microfabricated device having a top surface defining an array of microwells, wherein a plurality of the microwells of the array of microwells each comprise a unique tag nucleic acid molecule including: (1) a target-specific nucleotide sequence for annealing to a target nucleic

acid fragment of one or more biological entities of interest that may be present in the microwell, and (2) a location-specific nucleotide sequence for identifying the location of the microwell on the microfabricated device, the method comprising: loading a sample prepared from the microbiome onto the at least one microfabricated device such that the plurality of microwells each include, on average, 2 to 20 cells of any microorganism of the microbiome as well as an amount of a nutrient; incubating the microfabricated device at predetermined conditions; amplifying a selected genetic material of the microorganisms obtained from the incubation in at least a subset of the plurality of microwells in the at least one microfabricated device, thereby obtaining first amplicons in the plurality of microwells; sequencing an aggregate of first amplicons collected from the subset of the plurality of microwells to obtain sequencing data; and based on the sequencing data and the unique tag nucleic molecule included in each of the plurality of microwells, obtaining an identification of microorganisms present in each of the subset of the plurality of microwells of the at least one microfabricated device; and based on the identification of the microorganisms present in the plurality of microwells, determining a presence or absence of a relationship regarding co-inhabitance between at least two different types of microorganisms in the microbiome. In a further example, the present disclosure refers to a method of analyzing a relationship among microorganisms of a microbiome collected from a specific environment, comprising: partitioning microorganisms of the microbiome into a plurality of portions each including, on average, 2 to 20 cells of any microorganism of the microbiome; incubating each portion of the plurality of portions of microorganisms in a separate compartment at predetermined conditions in the presence of a nutrient; identifying the microorganisms present in each of at least a subset of the isolated compartments after incubation; and determining a presence or absence of a relationship between at least two different types of the microorganisms in the microbiome based on a comparison across the subset of compartments which types of microorganisms as identified are present or not present in the same microwells.

In some examples, a method of analyzing a sample including a population of biological entities is described. The methods employs at least one microfabricated device having a top surface defining (or comprising) an array of microwells, wherein a plurality of the microwells of the array of microwells each comprise a unique tag nucleic acid molecule including: (1) a target-specific nucleotide sequence for annealing to a target nucleic acid fragment of one or more biological entities of interest that may be present in the microwell, and (2) a location-specific nucleotide sequence for identifying the location of the microwell on the microfabricated device. The method comprises: loading a sample onto the microfabricated device such that at least some microwells of the plurality of microwells each include more than one cell of a biological entity as well as an amount of a nutrient; incubating the microfabricated device at predetermined conditions; amplifying, in individual microwells, a selected genetic material of the cells (e.g., genomic DNA of the cells) of the biological entities obtained from the incubation in the plurality of microwells, thereby obtaining first amplicons in at least a subset of the plurality of microwells; sequencing an aggregate of the first amplicons collected from the subset the plurality of microwells to obtain sequencing data; and based on the sequencing data and the unique tag nucleic molecule included in each of the plurality of microwells, obtaining an identification of the biological entities present in each of the subset of the plurality of microwells of the at least one microfabricated device.

In some examples of the method, based on the identification of the biological entities present in each of the subset of the plurality of microwells, a presence or absence of a relationship between at least two different types of biological entities in the population of biological entities contained in the sample is

determined. The relationship can be one of a dependent relationship, a symbiotic relationship, or a destructive relationship. In some examples, the presence or absence of a relationship can be determined by comparing across the plurality of microwells which types of biological entities are present or not present in the same microwells after incubation.

In some examples of the method, loading the sample onto the microfabricated device can be performed such that each microwell of the plurality of microwells includes, on average, N cells of any biological entity (the N cells can be same type or different types), where N is a number and equal to 2 or greater. For example, N can be a number between 2 and 100, between 2 and 50, between 2 and 20, between 2 and 10, etc.

In some examples of the method, the biological entities in the sample comprise bacteria. The bacteria can include bacteria of different strains, different species, or different genera. The population of the of the population of the biological entities in the sample can include a collection of microorganisms naturally occurring in a specific environment. For example, the biological entities can be a collection of microorganisms obtained from human stool, human gut, human skin, human nasal cavity, vagina, soil, rhizosphere, water, etc. In other examples, the biological entities in the sample can include viruses, fungi, or eukaryotic cells.

In some examples of the method, prior to incubation, a membrane can be applied on the top surface of the microfabricated device to retain the biological entities loaded in the plurality of microwells. The nutrient can be pre-loaded in the microwells and retained by the membrane. The nutrient can include a plurality of different nutrients loaded across the microwells of the at least one microfabricated device. In such a case, determining the determining the presence or absence of a relationship between at least two types of biological entities can include determining such a relationship dependent on the different nutrients. In some examples, the nutrient can be included in a reservoir provided on the membrane and external to the microwells. In such a case, the membrane can be permeable to the nutrient and allows the nutrient to migrate from the reservoir into the microwells through the membrane.

In some examples, the method further comprises transferring at least some of the cells of the biological entities obtained from the incubation to a target location. In such a case, the amplifying and sequencing can be performed on either the cells not transferred to the target location or the cells transferred to the target location.

In some examples, the unique tag nucleic acid molecule in each of the plurality of microwells constitutes a portion of a PCR primer used in the amplification.

In some examples, the surface density of the arrays of microwells of the at least one microfabricated device can be at least 150 microwells per cm^2, at least 250 microwells per cm^2, at least 400 microwells per cm^2, at least 500 microwells per cm^2, at least 750 microwells per cm^2, at least 1,000 microwells per cm^2, at least 2,500 microwells per cm^2, at least 5,000 microwells per cm^2, at least 7,500 microwells per cm^2, at least 10,000 microwells per cm^2, at least 50,000 microwells per cm^2, at least 100,000 microwells per cm^2, or at least 160,000 per cm^2. In some examples, each microwell of the arrays of microwells of the at least one microfabricated device can have a diameter of from about 5 μm to about 500 μm, from about 10 μm to about 300 μm, or from about 20 μm to about 200 μm.

In some examples, a method of analyzing a sample including a population of biological entities using at least one microfabricated device having a top surface defining an array of microwells is described. The method comprises: loading into each of a plurality of the microwells of the array of microwells with a unique tag nucleic acid molecule including: (1) a target-specific nucleotide sequence for annealing to a target nucleic acid fragment of one or more biological entities of interest that may be

CLAIMS

1. A method of analyzing a sample including a population of biological entities using at least one microfabricated device having a top surface defining an array of microwells, wherein a plurality of the microwells of the array of microwells each comprise a unique tag nucleic acid molecule including: (1) a target-specific nucleotide sequence for annealing to a target nucleic acid fragment of one or more biological entities of interest that may be present in the microwell, and (2) a location-specific nucleotide sequence for identifying the location of the microwell on the microfabricated device, the method comprising:

loading a sample onto the microfabricated device such that at least some microwells of the plurality of microwells each include more than one cell of a biological entity as well as an amount of a nutrient;

incubating the microfabricated device at predetermined conditions;

amplifying, in individual microwells, a selected genetic material of the cells of the biological entities obtained from the incubation in the plurality of microwells, thereby obtaining first amplicons in at least a subset of the plurality of microwells;

sequencing an aggregate of the first amplicons collected from the subset the plurality of microwells to obtain sequencing data;

based on the sequencing data and the unique tag nucleic molecule included in each of the plurality of microwells, obtaining an identification of the biological entities present in each of the subset of the plurality of microwells of the at least one microfabricated device; and

based on the identification of the biological entities present in each of the subset of the plurality of microwells, determining a presence or absence of a relationship regarding co-inhabitance between at least two different types of cells of biological entities in the population of biological entities contained in the sample.

2. The method of claim 1, wherein the relationship comprises a dependent relationship, a symbiotic relationship, and a destructive relationship.

3. The method of claim 1, where the presence or absence of a relationship is determined by comparing across the plurality of microwells which types of cells of biological entities are

present or not present in the same microwells after incubation.

4. The method of claim 1, wherein loading the sample onto the microfabricated device is performed such that each microwell of the plurality of microwells includes, on average, N cells of any of the biological entities, where N is a number and equal to 2 or greater.

5. The method of claim 4, wherein N is smaller or equal to 20.

6. The method of claim 1, wherein the biological entities comprise bacteria.

7. The method of claim 6, wherein the two different types of biological entities comprise two different strains, two different species, or two different genera of bacteria.

8. The method of claim 1, wherein the population of the biological entities in the sample comprises a collection of microorganisms naturally occurring in a specific environment.

9. The method of claim 1, wherein the biological entities comprise eukaryotic cells.

10. The method of claim 1, further comprising:

prior to incubation, applying a membrane on the top surface of the microfabricated device to retain the biological entities loaded in the plurality of microwells.

11. The method of claim 10, wherein the nutrient is pre-loaded in the microwells and retained by the membrane.

12. The method of claim 1, wherein loading the sample comprises loading a plurality of different nutrients across the microwells of the at least one microfabricated device.

13. The method of claim 1, wherein loading the sample comprises loading a plurality of different nutrients across the microwells of the at least one microfabricated device, and wherein determining the presence or absence of a relationship between at least two types of biological entities comprises determining such a relationship dependent on the different nutrients.

14. The method of claim 10, wherein the nutrient is included in a reservoir provided on the

membrane and external to the microwells, wherein the membrane is permeable to the nutrient, allowing the nutrient to migrate from the reservoir into the microwells through the membrane.

15. The method of claim 1, wherein the selected genetic material of the cells is genomic DNA of the cells.

16. The method of claim 1, further comprising:

transferring at least some of the cells of the biological entities obtained from the incubation to a target location, wherein the amplifying and sequencing is performed on either the cells not transferred to the target location or the cells transferred to the target location

17. The method of claim 1, wherein the unique tag nucleic acid molecule in each of the plurality of microwells constitutes a portion of a PCR primer used in the amplification.

18. The method of claim 1, wherein the surface density of the arrays of microwells of the at least one microfabricated device is at least 150 microwells per cm^2, at least 250 microwells per cm^2, at least 400 microwells per cm^2, at least 500 microwells per cm^2, at least 750 microwells per cm^2, at least 1,000 microwells per cm^2, at least 2,500 microwells per cm^2, at least 5,000 microwells per cm^2, at least 7,500 microwells per cm^2, at least 10,000 microwells per cm^2, at least 50,000 microwells per cm^2, at least 100,000 microwells per cm^2, or at least 160,000 per cm^2.

19. The method of claim 1, wherein each microwell of the arrays of microwells of the at least one microfabricated device has a diameter of from 5 μm to 500 μm, from 10 μm to 300 μm, or from 20 μm to 200 μm.

20. A method of analyzing a sample including a population of biological entities using at least one microfabricated device having a top surface defining an array of microwells, the method comprising:

loading into each of a plurality of the microwells of the array of microwells with a unique tag nucleic acid molecule including: (1) a target-specific nucleotide sequence for annealing to a target nucleic acid fragment of one or more biological entities of interest that may be present in

the microwell, and (2) a location-specific nucleotide sequence for identifying the location of the microwell on the at least microfabricated device;

loading a sample onto the microfabricated device such that at least some microwells of the plurality of microwells each includes more than one cell of a biological entity as well as an amount of a nutrient;

incubating the microfabricated device at predetermined conditions;

amplifying a selected genetic material of the cells of the biological entities obtained from the incubation in at least a subset of the plurality of microwells of the at least one microfabricated device, thereby obtaining first amplicons in the plurality of microwells;

sequencing an aggregate of the first amplicons collected from the subset the plurality of microwells to obtain sequencing data;

based on the sequencing data and the unique tag nucleic molecule included in each of the plurality of microwells, obtaining an identification of the biological entities present in each of the subset of the plurality of microwells of the at least one microfabricated device; and

based on the identification of the biological entities present in each of the subset of the plurality of microwells, determining a presence or absence of a relationship regarding co-inhabitance between at least two different types of cells of biological entities in the population of biological entities contained in the sample.

21. A method of analyzing a microbiome of microorganisms collected from a specific environment using at least one microfabricated device having a top surface defining an array of microwells, wherein a plurality of the microwells of the array of microwells each comprise a unique tag nucleic acid molecule including: (1) a target-specific nucleotide sequence for annealing to a target nucleic acid fragment of one or more biological entities of interest that may be present in the microwell, and (2) a location-specific nucleotide sequence for identifying the location of the microwell on the microfabricated device, the method comprising:

loading a sample prepared from the microbiome onto the at least one microfabricated device such that the plurality of microwells each include, on average, 2 to 20 cells of any microorganism of the microbiome as well as an amount of a nutrient;

incubating the microfabricated device at predetermined conditions;

amplifying a selected genetic material of the microorganisms obtained from the incubation in at least a subset of the plurality of microwells in the at least one microfabricated

device, thereby obtaining first amplicons in the plurality of microwells;

sequencing an aggregate of first amplicons collected from the subset of the plurality of microwells to obtain sequencing data; and

based on the sequencing data and the unique tag nucleic molecule included in each of the plurality of microwells, obtaining an identification of microorganisms present in each of the subset of the plurality of microwells of the at least one microfabricated device; and

based on the identification of the microorganisms present in the plurality of microwells, determining a presence or absence of a relationship regarding co-inhabitance between at least two different types of microorganisms in the microbiome.