

CLAIMS

WHAT IS CLAIMED IS:

1. A method for nucleic acid identification, comprising:
 - (a) using a plurality of nucleic acid molecules to generate, in a plurality of chambers, a plurality of double-stranded nucleic acid molecules, wherein: (i) a first subset of said plurality of double-stranded nucleic acid molecules comprises a first double-stranded nucleic acid molecule comprising a first sequence corresponding to a first nucleic acid molecule of said plurality of nucleic acid molecules and an added sequence; and (ii) a second subset of said plurality of double-stranded nucleic acid molecules comprises a second double-stranded nucleic acid molecule comprising a second sequence corresponding to a second nucleic acid molecule of said plurality of nucleic acid molecules and does not comprise said added sequence;
 - (b) denaturing double-stranded nucleic acid molecules of said plurality of double-stranded nucleic acid molecules;
 - (c) detecting signals indicative of said denaturing to generate a plurality of denaturation profiles, wherein:
 - i. a first denaturation profile of said plurality of denaturation profiles is derived from denaturation of said first double-stranded nucleic acid molecule;
 - ii. a second denaturation profile of said plurality of denaturation profiles is derived from denaturation of said second double-stranded nucleic acid molecule; and
 - iii. said first denaturation profile and said second denaturation profile are different; and
 - (d) processing said plurality of denaturation profiles to identify a nucleic acid molecule of said plurality of nucleic acid molecules.
2. The method of claim 1, further comprising, prior to (a), providing said plurality of nucleic acid molecules and a plurality of forward primers to said plurality of chambers.
3. The method of claim 2, wherein said plurality of forward primers comprises (i) a first forward primer comprising a first region complementary to at least a portion of said first nucleic acid molecule and a second region that is not complementary to said first nucleic acid molecule and corresponds to said added sequence and (ii) a second forward primer complementary to at least a portion of said second nucleic acid molecule.

4. The method of claim 3, wherein said plurality of forward primers are not universal primers.
5. The method of claim 3 or claim 4, further comprising, prior to (a), subjecting said plurality of forward primers to primer extension reactions to generate a plurality of first extension products.
6. The method of claim 5, further comprising, prior to (a), contacting said plurality of first extension products with a plurality of reverse primers.
7. The method of claim 6, wherein said plurality of reverse primers are universal primers.
8. The method of claim 6, further comprising, prior to (a), subjecting said plurality of reverse primers to primer extension reactions to generate a plurality of second extension products.
9. The method of claim 8, wherein said plurality of second extension products are said plurality of double-stranded nucleic acid molecules.
10. The method of claim 1, further comprising imaging at least a portion of said plurality of chambers to detect said signals.
11. The method of claim 10, further comprising imaging said plurality of chambers to detect said signals.
12. The method of claim 1, further comprising subjecting said plurality of double-stranded nucleic acid molecules to controlled heating to denature said double-stranded nucleic acid molecules.
13. The method of claim 1, wherein said double-stranded nucleic acid molecules comprise intercalating dyes from which said signals are derived.
14. The method of claim 13, wherein said double-stranded nucleic acid molecules comprise a plurality of different intercalating dyes from which said signals are derived.
15. The method of claim 1, wherein said signals are optical signals.
16. The method of claim 1, wherein a chamber of said plurality of chambers has a volume of less than or equal to about 500 picoliters.

17. The method of claim 16, wherein said volume of said chamber is less than or equal to about 250 picoliters.
18. The method of claim 1, wherein said plurality of chambers comprises greater than or equal to about 1,000 chambers.
19. The method of claim 18, wherein said plurality of chambers comprises greater than or equal to about 10,000 chambers.
20. A system for nucleic acid identification, comprising:
a detection unit configured to collect and process signals for identification of nucleic acid molecules; and
one or more processors operatively coupled to said detection unit, wherein said one or more processors are individually or collectively programmed or otherwise configured to:
- (i) use a plurality of nucleic acid molecules to generate, in a plurality of chambers, a plurality of double-stranded nucleic acid molecules, wherein: (i) a first subset of said plurality of double-stranded nucleic acid molecules comprises a first double-stranded nucleic acid molecule comprising a first sequence corresponding to a first nucleic acid molecule of said plurality of nucleic acid molecules and an added sequence; and (ii) a second subset of said plurality of double-stranded nucleic acid molecules comprises a second double-stranded nucleic acid molecule comprising a second sequence corresponding to a second nucleic acid molecule of said plurality of nucleic acid molecules and does not comprise said added sequence;
 - (ii) denature double-stranded nucleic acid molecules of said plurality of double-stranded nucleic acid molecules;
 - (iii) detect signals indicative of said denaturing to generate a plurality of denaturation profiles, wherein:
 - (A) a first denaturation profile of said plurality of denaturation profiles is derived from denaturation of said first double-stranded nucleic acid molecule;
 - (B) a second denaturation profile of said plurality of denaturation profiles is derived from denaturation of said second double-stranded nucleic acid molecule; and
 - (C) said first denaturation profile and said second denaturation profile are different; and
 - (iv) processing said plurality of denaturation profiles to identify a nucleic acid molecule of said plurality of nucleic acid molecules.

21. The system of claim 20, wherein a chamber of said plurality of chambers has a volume of less than or equal to about 500 picoliters.
22. The system of claim 21, wherein said volume of said chamber is less than or equal to about 250 picoliters.
23. The system of claim 20, wherein said plurality of chambers comprises greater than or equal to about 1,000 chambers.
24. The system of claim 23, wherein said plurality of chambers comprises greater than or equal to about 10,000 chambers.
25. The system of claim 20, wherein said detection unit is configured to image at least a portion of said plurality of chambers.
26. The system of claim 25, wherein said detection unit is configured to image said plurality of chambers.
27. The system of claim 25, wherein said detection unit comprises a camera with a field of view of greater than or equal to about 15 millimeters (mm) by about 15 mm.
28. The system of claim 27, wherein said field of view that is greater than or equal to about 50 mm by about 75 mm.
29. The system of claim 20, wherein said detection unit comprises a camera comprising a complementary metal-oxide-semiconductor (CMOS) sensor.
30. The system of claim 29, wherein said detection unit further comprises a telecentric lens disposed between said camera and said plurality of chambers.
31. The system of claim 20, wherein said detection unit comprises an optical unit configured to collect optical signals.
32. The system of claim 31, wherein said optical unit comprises greater than or equal to four channels, each channel configured to collect a different wavelength of light.
33. The system of claim 20, wherein said system is configured to receive a substrate comprising a plurality of chamber arrays, and wherein a chamber array of said plurality of chamber arrays comprises said plurality of chambers.

34. The system of claim 33, wherein said substrate comprises at least four chamber arrays.
35. The system of claim 33, wherein said chamber array is fluidically isolated from another chamber array.
36. The system of claim 33, wherein said system is configured to receive a plate, and wherein said plate is configured to retain a plurality of substrates comprising said substrate.
37. The system of claim 20, further comprising a thermal unit operatively coupled to said one or more processors, wherein said thermal unit is configured to control a temperature of said plurality of chambers.
38. The system of claim 37, wherein said one or more processors directs said thermal unit to subject said plurality of chambers to controlled heating to denature said double-stranded nucleic acid molecules.
39. The system of claim 37, wherein said thermal unit comprises a thermoelectric temperature control unit.