CLAIMS

1. A method of profiling a complex microbiotic composition of a sample comprising the nucleic acid molecules of the complex microbiotic composition in a digital polymerase chain reaction (dPCR) system, the method comprising:

a) partitioning the sample into a sufficient number of partitions such that at least a portion of the partitions comprises no more than one nucleic acid molecule of the complex microbiotic composition;

wherein the method comprises an asymmetric polymerase chain reaction (PCR) that uses a primer set comprising:

a pair of forward and reverse primers for amplifying the nucleic acid molecules or parts thereof to produce double-stranded PCR products; and

a third primer for amplifying one of the two strands of the double-stranded PCR products or a part thereof,

b) contacting the partitions of the sample to a plurality of mismatch-tolerant probes that are capable of binding to a 16s ribosomal nucleic acid (16s rRNA) region of the nucleic acid molecules from different bacterial taxonomic groups or parts thereof under suitable conditions, wherein the plurality of mismatch-tolerant probes is configured to emit multiple channel fluorescence signals and the contacting occurs in an amplification reaction;

c) determining multiple channel fluorescence signals generated by each of the plurality of mismatch-tolerant probes in each partition; and

d) establishing the complex microbiotic composition in the sample based on the multiple channel fluorescence signals comprising computing a melting temperature (Tm) of each of the plurality of mismatch tolerant probes to obtain a Tm signature for each partition based on the multiple channel fluorescence signals generated by each of the plurality of mismatch tolerant probes in each partition,

wherein the plurality of mismatch-tolerant probes is configured to have different Tm signatures for nucleic acid molecules from different bacterial taxonomic groups

.

2. The method according to claim 1, wherein the plurality of mismatch-tolerant probes is configured to collectively generate different signal intensity profiles for different groups of nucleic acid molecules.

3. The method according to claim 2, wherein computing the Tm comprises identifying an inflection point in a signal intensity generated by each of the plurality of mismatch-tolerant probes in each partition.

4. The method according to claim 1, wherein step d) further comprises classifying the Tm signature as belonging to a group of nucleic acid molecules.

5. The method according to claim 1, wherein step d) further comprises counting the number of partitions with the same Tm signature.

6. The method according to claim 5, wherein step d) further comprises determining a proportion of different groups of nucleic acid molecules in the sample based on the count numbers.

7. The method according to claim 1, wherein the forward and reverse primers have a higher annealing temperature than the third primer.

8. The method according to claim 1, wherein the third primer is present at a higher concentration than the forward and reverse primers.

9. The method according to claim 1, wherein the plurality of mismatch-tolerant probes is configured to bind to a 16s ribosomal region of nucleic acid region of the microbiotic composition at temperatures below their melting temperature.

10. The method according to claim 1, wherein the plurality of mismatch-tolerant probes is configured to bind to a V3 region of a 16s ribosomal nucleic acid region of the nucleic acid molecules at temperatures below their melting temperatures.

11. The method according to claim 1, wherein the plurality of mismatch-tolerant probes is configured to have different Tm signatures for nucleic acid molecules from different phyla and/or species of bacteria.

12. The method according to claim 1, wherein the plurality of mismatch-tolerant probes has one or more of the following properties:

is capable of producing a fluorescent signal;

has a greater number of mismatches with the sequences of one group of nucleic acid molecules than another group of nucleic acid molecules;

comprises an oligonucleotide comprising a reporter moiety and a quencher moiety;

comprises a molecular beacon;

each is capable of producing a distinct colorimetric signal, optionally wherein the colorimetric signal is selected from the group consisting of: a green signal, an orange signal and a red signal.

13. The method according to claim 1, wherein the microbiotic composition comprises

one or more bacteria from the genus of Acetobacter, Acinetobacter, Actinomyces, Agrobacterium spp., Azorhizobium, Azotobacter, Anaplasma spp., Bacillus spp., Bacteroides spp., Bartonella spp., Bordetella spp., Borrelia, Brucella spp., Burkholderia spp., Calymmatobacterium, Campylobacter, Chlamydia spp., Chlamydophila spp., Clostridium spp., Corynebacterium spp., Coxiella, Ehrlichia, Enterobacter, Enterococcus spp., Escherichia, Francisella, Fusobacterium, Gardnerella, Haemophilus spp., Helicobacter, Klebsiella, Lactobacillus spp., Lactococcus, Legionella, Listeria, Methanobacterium extroquens, Microbacterium multiforme, Micrococcus luteus, Moraxella catarrhalis, Mycobacterium spp., Mycoplasma spp., Neisseria spp., Pasteurella spp., Peptostreptococcus, Porphyromonas, Pseudomonas, Rhizobium, Rickettsia spp., Rochalimaea spp., Rothia, Salmonella spp., Serratia, Shigella, Staphylococcus spp., Stenotrophomonas, Streptococcus spp., Treponema spp., Vibrio spp., Wolbachia, and Yersinia spp.

14. The method according to claim 1, wherein the microbiotic composition comprises

one or more bacteria from the group consisting of *Acetobacter aurantius, Acinetobacter baumannii, Actinomyces Israelii, Agrobacterium radiobacter, Agrobacterium tumefaciens*, *Azorhizobium caulinodans, Azotobacter vinelandii, Anaplasma phagocytophilum, Anaplasma marginale*, *Bacillus anthracis, Bacillus brevis, Bacillus cereus, Bacillus fusiformis, Bacillus licheniformis, Bacillus megaterium, Bacillus mycoides, Bacillus stearothermophilus, Bacillus subtilis*, *Bacteroides fragilis, Bacteroides gingivalis, Bacteroides melaminogenicus* (*Prevotella melaminogenica*), *Bartonella henselae, Bartonella quintana*, *Bordetella bronchiseptica, Bordetella pertussis*, *Borrelia burgdorferi, Brucella abortus, Brucella melitensis, Brucella suis*, *Burkholderia mallei, Burkholderia pseudomallei, Burkholderia cepacia* complex, *Burkholderia cenocepacia*, *Calymmatobacterium granulomatis, Campylobacter coli, Campylobacter fetus, Campylobacter jejuni, Campylobacter pylori*, *Chlamydia trachomatis*, *Chlamydophila*. (such as *C. pneumoniae*,

Chlamydophila psittaci, *Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Clostridium tetani*), *Corynebacterium diphtheriae, Corynebacterium fusiforme*, *Coxiella bumetii, Ehrlichia chaffeensis, Enterobacter cloacae, Enterococcus avium, Enterococcus durans, Enterococcus faecalis, Enterococcus faecium, Enterococcus galllinarum, Enterobacter gergoviae (now known as Pluralibacter gergoviae), Enterococcus maloratus*, *Escherichia coli, Francisella tularensis, Fusobacterium nucleatum, Gardnerella vaginalis, Haemophilus ducreyi, Haemophilus influenzae, Haemophilus parainfluenzae, Haemophilus pertussis, Haemophilus vaginalis*, *Helicobacter pylori, Klebsiella pneumoniae, Lactobacillus acidophilus, Lactobacillus casei*, *Lactococcus lactis, Legionella pneumophila, Listeria monocytogenes, Methanobacterium extroquens, Microbacterium multiforme, Micrococcus luteus, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium diphtheriae, Mycobacterium intracellulare, Mycobacterium leprae, Mycobacterium lepraemurium, Mycobacterium phlei, Mycobacterium smegmatis, Mycobacterium tuberculosis*, *Mycoplasma fermentans, Mycoplasma genitalium, Mycoplasma hominis, Mycoplasma penetrans, Mycoplasma pneumoniae*, *Neisseria gonorrhoeae, Neisseria meningitidis*, *Pasteurella multocida, Pasteurella tularensis Peptostreptococcus, Porphyromonas gingivalis, Pseudomonas aeruginosa, Rhizobium Radiobacter, Rickettsia prowazekii, Rickettsia psittaci, Rickettsia quintana, Rickettsia rickettsii, Rickettsia trachomae*, *Rochalimaea henselae, Rochalimaea quintana*, *Rothia dentocariosa, Salmonella enteritidis, Salmonella typhi, Salmonella typhimurium*, *Serratia marcescens, Shigella dysenteriae, Staphylococcus aureus, Staphylococcus epidermidis*, *Stenotrophomonas maltophilia, Streptococcus agalactiae, Streptococcus. avium, Streptococcus bovis, Streptococcus cricetus, Streptococcus faceium, Streptococcus faecalis, Streptococcus ferus, Streptococcus gallinarum, Streptococcus lactis, Streptococcus mitior, Streptococcus mitis, Streptococcus mutans, Streptococcus oralis, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus rattus, Streptococcus salivarius, Streptococcus sanguis, Streptococcus sobrinus*, *Treponema pallidum, Treponema denticola*, *Vibrio cholerae, Vibrio comma, Vibrio parahaemolyticus, Vibrio vulnificus*, *Wolbachia*, *Yersinia enterocolitica, Yersinia pestis and Yersinia pseudotuberculosis*.

15. The method according to claim 1, wherein the sample is a medical sample and/or a non-medical sample, optionally

wherein the non-medical sample is a sample of one or more selected from the group consisting of food industries, consumer products, agriculture, and laboratory, or

wherein the medical sample is a biological sample.

16. A complex microbiotic composition profiling kit comprising

a reagent for detecting nucleic acid molecules of a complex microbiotic composition,

wherein the reagent comprises a plurality of mismatch-tolerant probes capable of binding to a 16S ribosomal nucleic acid (16s rRNA) region of nucleic acid molecules from different bacterial taxonomic groups or parts thereof under suitable conditions,

wherein the plurality of mismatch-tolerant probes is configured to emit multiple channel fluorescence signals and

wherein the plurality of mismatch-tolerant probes is configured to have different Tm signatures for different groups of nucleic acid molecules from different bacterial taxonomic groups of the microbiotic composition,

a pair of forward and reverse primers for amplifying the nucleic acid molecules or parts thereof to produce double-stranded PCR products; and

a third primer for amplifying one of the two strands of the double-stranded PCR products or a part thereof.