

CLAIMS

We Claim:

1. A method for improved microbiome sequencing for characterizing a microorganism-related condition for a user associated with a biological sample, the method comprising:
 - generating guide RNA (gRNA) complexes comprising endonucleases and gRNAs for a set of targets corresponding to a set of taxa associated with the microorganism-related condition;
 - processing the biological sample with the gRNA complexes to generate microorganism nucleic acid fragments comprising a set of end regions associated with the set of targets;
 - ligating the set of end regions with a set of adapters sharing an adapter sequence and configured to facilitate next-generation sequencing of the set of targets;
 - amplifying the set of targets based on the ligated set of end regions and a set of primers sharing a primer sequence associated with the adapter sequence;
 - generating a microorganism sequence dataset for the user based on the amplified set of targets; and
 - determining a microbiome-associated characterization for the user for the microorganism-related condition based on the microorganism sequence dataset, wherein the microbiome-associated characterization is configured to facilitate modification of the microbiome of the user in improving a state of the microorganism-related condition.
2. The method of Claim 1, wherein amplifying the set of targets is based on the set of primers consisting of primers sharing the primer sequence to reduce amplification biases.
3. The method of Claim 1, wherein amplifying the set of targets comprises performing, with a bridge amplification substrate of a next generation sequencing platform, multiplex amplification based on the set of primers and the set of adapters, and wherein generating the microorganism sequence dataset comprises generating the microorganism sequence dataset at a computing system operable to communicate with the next generation sequencing platform.

4. The method of Claim 1, wherein generating the gRNA complexes comprises ranking gRNA sequences based upon a set of gRNA design factors for optimizing on-target activity.
5. The method of Claim 4, wherein the gRNA design factors comprises at least one of: a folding energy factor, a hybridization factor, a GC content factor, a nucleotide run factor; a first binding energy factor, a second binding energy factor, and a GC clamp factor.
6. The method of Claim 4, wherein the gRNAs are for a set of targets associated with 16S rRNA regions.
7. The method of Claim 1, wherein the set of taxa corresponding to the set of targets comprises at least one of: *Alistipes* (genus), *Barnesiella* (genus), *Bifidobacterium* (genus), *Campylobacter* (genus), *Peptoclostridium* (genus), *Escherichia-Shigella* (genus), *Fusobacterium* (genus), *Lactobacillus* (genus), *Odoribacter* (genus), *Prevotella* (genus), *Pseudoflavonifractor* (genus), *Roseburia* (genus), *Ruminococcus* (genus), *Salmonella* (genus), *Veillonella* (genus), *Akkermansia muciniphila* (species), *Anaerotruncus colihominis* (species), *Bacteroides fragilis* (species), *Bacteroides vulgatus* (species), *Bifidobacterium longum* (species), *Butyrivibrio crossotus* (species), *Campylobacter jejuni* (species), *Campylobacter coli* (species), *Campylobacter lari* (species), *Peptoclostridium difficile* (species), *Collinsella aerofaciens* (species), *Coproccoccus eutactus* (species), *Desulfovibrio piger* (species), *Dialister invisus* (species), *Escherichia coli* (species), *Escherichia coli O157* (species), *Faecalibacterium prausnitzii* (species), *Methanobrevibacter smithii* (species), *Oxalobacter formigenes* (species), *Ruminococcus albus* (species), *Ruminococcus bromii* (species), *Ruminococcus gnavus* (species), *Salmonella enterica* (species), *Salmonella bongori* (species), *Shigella boydii* (species), *Shigella sonnei* (species), *Shigella flexneri* (species), *Shigella dysenteriae* (species), *Streptococcus sanguinis* (species), *Streptococcus thermophilus* (species), *Vibrio cholerae* (species), *Yersinia enterocolitica* (species), *Alloprevotella* (genus), *Anaerofilum* (genus), *Bacteroides* (genus), *Blautia* (genus), *Butyricimonas* (genus), *Catenibacterium* (genus), *Christensenella* (genus), *Collinsella* (genus),

Coprococcus (genus), *Dialister* (genus), *Eggerthella* (genus), *Faecalibacterium* (genus), *Flavonifractor* (genus), *Gelria* (genus), *Haemophilus* (genus), *Holdemania* (genus), *Oscillibacter* (genus), *Oscillospira* (genus), *Parabacteroides* (genus), *Paraprevotella* (genus), *Phascolarctobacterium* (genus), *Streptococcus* (genus), *Turicibacter* (genus), *Tyzzzerella* (genus), *Acetobacter nitrogenifigens* (species), *Acinetobacter baumannii* (species), *Azospirillum brasilense* (species), *Bacillus cereus* (species), *Bacillus coagulans* (species), *Bacillus licheniformis* (species), *Bifidobacterium animalis* (species), *Bifidobacterium bifidum* (species), *Brevibacillus laterosporus* (species), *Christensenella minuta* (species), *Clavibacter michiganensis* (species), *Clostridium butyricum* (species), *Enterococcus italicus* (species), *Fibrobacter succinogenes* (species), *Kocuria rhizophila* (species), *Lactobacillus brevis* (species), *Lactobacillus coryniformis* (species), *Lactobacillus delbrueckii* (species), *Lactobacillus fermentum* (species), *Lactobacillus helveticus* (species), *Lactobacillus kefiranoferiens* (species), *Lactobacillus kunkeei* (species), *Lactobacillus rhamnosus* (species), *Lactobacillus salivarius* (species), *Lactococcus fujiensis* (species), *Lactococcus garvieae* (species), *Lactococcus lactis* (species), *Leptotrichia hofstadii* (species), *Leuconostoc fallax* (species), *Leuconostoc kimchii* (species), *Oenococcus oeni* (species), *Paenibacillus apiarius* (species), *Pediococcus pentosaceus* (species), *Propionibacterium freudenreichii* (species), *Pseudoclavibacter helvolus* (species), *Renibacterium salmoninarum* (species), *Ruminococcus flavefaciens* (species), *Staphylococcus sciuri* (species), *Weissella koreensis* (species), *Clostridium* (genus), and *Clostridium difficile* (species).

8. The method of Claim 7, wherein the microorganism-related condition comprises a gut-related condition comprising at least one of: diarrhea, irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), Crohn's Disease, ulcerative colitis, constipation, abdominal tenderness, bloating, flatulence, obesity, type II diabetes, prediabetes, kidney stones, cardiovascular health, and anxiety.

9. The method of Claim 1, wherein determining the microbiome-associated characterization for the microorganism-related condition comprises determining the microbiome-associated characterization based on at least one of: presence of microbiome features extracted from the microorganism sequence dataset, absence of the microbiome features, relative abundance for taxonomic groups of the set of taxa, diversity of microbiome composition features of the microbiome features, diversity of microbiome functional features of the microbiome features, a ratio between at least two features of the microbiome features associated with the set of taxa, interactions between the set of taxa, and phylogenetic distance between the set of taxa.
10. The method of Claim 1, wherein determining the microbiome-associated characterization for the user comprises determining the microbiome-associated characterization based on at least one of a microbiome composition diversity feature and a microbiome functional diversity feature extracted from the microorganism sequence dataset.
11. The method of Claim 1, wherein the endonucleases of the gRNA complexes are associated with protein type families comprising at least one of: *cas*, *cpf*, *cse*, *csy*, *csn*, *csd*, *cst*, *csf*, *csa*, *csm*, *cmr*, and a CRISPR-associated protein family.
12. A method for improved microbiome sequencing, the method comprising:
 - generating guide RNA (gRNA) complexes comprising proteins and gRNAs for a set of targets corresponding to a set of taxa associated with a microorganism-related condition;
 - processing microorganism nucleic acid material with the gRNA complexes to generate end regions associated with the set of targets;
 - ligating the end regions with a set of adapters sharing an adapter sequence;
 - performing sequencing of the set of targets based on the ligated end regions and a set of primers sharing a primer sequence associated with the adapter sequence; and
 - generating at least one of a microbiome composition diversity dataset and a microbiome functional diversity dataset based on the sequenced set of targets.

13. The method of Claim 12, wherein processing the microorganism nucleic acid material comprises processing biological samples from a population of subjects with the gRNA complexes, wherein the method further comprises:
 - receiving a supplementary dataset, associated with at least a subset of the population of subjects, wherein the supplementary dataset is informative of the microorganism-related condition; and
 - transforming the supplementary dataset and features extracted from the at least one of the microbiome composition diversity dataset and the microbiome functional diversity dataset into a characterization model for the microorganism-related condition.
14. The method of Claim 13, further comprising:
 - collecting a user biological sample from a user, wherein the user biological sample comprises a set of user targets sharing target sequences with the set of targets;
 - sequencing the set of user targets based on user-associated gRNA complexes sharing sequences with the proteins and the gRNAs, user-associated adapters sharing the adapter sequence, and user-associated primers sharing the primer sequence;
 - generating a user microbiome dataset based on the sequenced set of user targets; and
 - determining a microbiome-associated characterization for the user for the microorganism-related condition based on the user microbiome dataset and the characterization model.
15. The method of Claim 14, further comprising promoting a therapy to the user with the microorganism-related condition based on the microbiome-associated characterization and a therapy model, wherein the therapy modulates user microbiome composition to improve a state of the microorganism-related condition.
16. The method of Claim 12, wherein generating the gRNA complexes comprises selecting gRNA sequences based upon at least one of: a folding energy factor, a hybridization factor, a GC content factor, a nucleotide run factor; a first binding energy factor, a second binding energy factor, and a GC clamp factor.

17. The method of Claim 16, wherein the proteins of the gRNA complexes comprise endonucleases for generating microorganism nucleic acid fragments comprising a set of blunt end regions associated with the set of targets, and wherein ligating the end regions comprises ligating the set of blunt end regions with the set of adapters sharing the adapter sequence.
18. The method of Claim 12, wherein the set of targets corresponding to the set of taxa comprises a plurality of targets associated with at least one of: *Alistipes* (genus), *Barnesiella* (genus), *Bifidobacterium* (genus), *Campylobacter* (genus), *Peptoclostridium* (genus), *Escherichia-Shigella* (genus), *Fusobacterium* (genus), *Lactobacillus* (genus), *Odoribacter* (genus), *Prevotella* (genus), *Pseudoflavonifractor* (genus), *Roseburia* (genus), *Ruminococcus* (genus), *Salmonella* (genus), *Veillonella* (genus), *Akkermansia muciniphila* (species), *Anaerotruncus colihominis* (species), *Bacteroides fragilis* (species), *Bacteroides vulgatus* (species), *Bifidobacterium longum* (species), *Butyrivibrio crossotus* (species), *Campylobacter jejuni* (species), *Campylobacter coli* (species), *Campylobacter lari* (species), *Peptoclostridium difficile* (species), *Collinsella aerofaciens* (species), *Coprococcus eutactus* (species), *Desulfovibrio piger* (species), *Dialister invisus* (species), *Escherichia coli* (species), *Escherichia coli O157* (species), *Faecalibacterium prausnitzii* (species), *Methanobrevibacter smithii* (species), *Oxalobacter formigenes* (species), *Ruminococcus albus* (species), *Ruminococcus bromii* (species), *Ruminococcus gnavus* (species), *Salmonella enterica* (species), *Salmonella bongori* (species), *Shigella boydii* (species), *Shigella sonnei* (species), *Shigella flexneri* (species), *Shigella dysenteriae* (species), *Streptococcus sanguinis* (species), *Streptococcus thermophilus* (species), *Vibrio cholerae* (species), and *Yersinia enterocolitica* (species).
19. The method of Claim 18, wherein the set of adapters sharing the adapter sequence and the set of primers sharing the primer sequence facilitate substantially simultaneous sequencing of the plurality of targets.

20. The method of Claim 12, wherein the set of adapters sharing the adapter sequence facilitates next generation sequencing with a next generation sequencing platform, and wherein performing sequencing of the set of targets comprises performing next generation sequencing with the next generation sequencing platform based on the set of primers and the set of adapters.
21. The method of Claim 12, wherein performing sequencing of the set of targets comprises performing amplification of the set of targets based on the set of primers sharing the primer sequence associated with the adapter sequence.
22. The method of Claim 12, wherein the proteins of the gRNA complexes are associated with protein type families comprising at least one of: *cas*, *cpf*, *cas*, *cse*, *csy*, *csn*, *csd*, *cst*, *csh*, *csa*, *csm*, and *cmr*.
23. The method of Claim 22, wherein the proteins of the gRNA complexes are associated with protein types comprising at least one of: Cas9 and Cpf1.
24. The method of Claim 12, wherein processing the microorganism nucleic acid material with the gRNA complexes to generate the end regions comprises:
- processing the microorganism nucleic acid material with the gRNA complexes to generate sticky end regions associated with the set of targets; and
 - processing the sticky end regions to generate blunt end regions associated with the set of targets, wherein ligating the end regions with the set of adapters sharing the adapter sequence comprises ligating the blunt end regions with the set of adapters.
25. The method of Claim 12, wherein the set of taxa corresponding to the set of targets comprises at least one of: *Actinomyces* (genus), *Aerococcus* (genus), *Alloiococcus* (genus), *Anaerococcus* (genus), *Anaeroglobus* (genus), *Anaerostipes* (genus), *Anaerotruncus* (genus), *Arcanobacterium* (genus), *Arthrospira* (genus), *Atopobium* (genus), *Bacteroides* (genus), *Bulleidia* (genus), *Campylobacter* (genus), *Catenibacterium* (genus), *Coriobacteriaceae* (family), *Corynebacterium* (genus), *Dialister* (genus), *Eggerthella* (genus), *Enterococcus* (genus), *Escherichia* (genus), *Finegoldia* (genus), *Fusobacterium* (genus), *Gardnerella* (genus), *Gemella* (genus), *Lactobacillaceae* (family), *Lactobacillales* (order), *Lactobacillus* (genus), *Leptotrichia* (genus), *Megasphaera* (genus), *Mobiluncus*

(genus), *Moryella* (genus), *Mycoplasma* (genus), *Papillibacter* (genus), *Parvimonas* (genus), *Peptococcus* (genus), *Peptoniphilus* (genus), *Peptostreptococcus* (genus), *Porphyromonadaceae* (family), *Porphyromonas* (genus), *Prevotella* (genus), *Prevotellaceae* (family), *Pseudomonas* (genus), *Ruminococcus* (genus), *Segniliparus* (genus), *Shigella* (genus), *Sneathia* (genus), *Staphylococcus* (genus), *Streptococcus* (genus), *Treponema* (genus), *Ureaplasma* (genus), *Veillonella* (genus), *Veillonellaceae* (family), *Aerococcus christensenii* (species), *Aerococcus* spp. (genus), *Algoriphagus aquatilis* (species), *Anaerococcus* spp. (genus), *Anaerococcus tetradius* (species), *Anaerococcus vaginalis* (species), *Anoxybacillus pushchinoensis* (species), *Atopobium* spp. (genus), *Atopobium vaginae* (species), *Bacteroides fragilis* (species), *Bacteroides* spp. (genus), *Bifidobacterium animalis* subsp. *lactis* (species), *Bifidobacterium dentium* (species), *Bifidobacterium lactis* (species), *Bifidobacterium longum* subsp. *suis* (species), *Bulleidia extructa* (species), *Burkholderia fungorum* (species), *Burkholderia phenoliruptrix* (species), *Caldicellulosiruptor saccharolyticus* (species), *Campylobacter* spp. (genus), *Campylobacter ureolyticus* (species), *Candida albicans* (species), *Candida glabrata* (species), *Candida krusei* (species), *Candida lusitanae* (species), *Candidatus Mycoplasma girerdii* (species), *Catenibacterium* spp. (genus), *Chlamydia trachomatis* (species), *Chondromyces robustus* (species), *Clostridiales BVAB2* (species), *Clostridiales BVAB3* (species), *Clostridium cavendishii* (species), *Clostridium viride* (species), *Cryobacterium psychrophilum* (species), *Dialister micraerophilus* (species), *Dickeya chrysanthemi* (species), *Eggerthia catenaformis* (species), *Erwinia chrysanthemi* (species), *Escherichia coli* (species), *Escherichia fergusonii* (species), *Exiguobacterium acetylicum* (species), *Fusobacterium nucleatum* (species), *Fusobacterium* spp. (genus), *Gardnerella* spp. (genus), *Gardnerella vaginalis* (species), *Gemella* sp. (genus), *Haemophilus ducreyi* (species), *Klebsiella granulomatis* (species), *Lachnospiraceae BVAB1* (species), *Lactobacillus acidophilus* (species), *Lactobacillus brevis* (species), *Lactobacillus casei* (species), *Lactobacillus casei Shirota* (species), *Lactobacillus crispatus* (species), *Lactobacillus delbrueckii* (species), *Lactobacillus fermentum* (species), *Lactobacillus gasseri* (species), *Lactobacillus iners* (species), *Lactobacillus jensenii* (species),

Lactobacillus johnsonii (species), *Lactobacillus kefiranofaciens* (species), *Lactobacillus paracasei* FJ861111.1 (species), *Lactobacillus pentosus* strain S-PT84 (species), *Lactobacillus plantarum* (species), *Lactobacillus reuteri* (species), *Lactobacillus reuteri* RC-14 (species), *Lactobacillus rhamnosus* (species), *Lactobacillus rhamnosus* (strain BMX 54) (species), *Lactobacillus rhamnosus* BMX 54 (species), *Lactobacillus rhamnosus* GR-1 (species), *Lactobacillus salivarius* (species), *Lactobacillus vaginalis* (species), *Leptotrichia* spp. (genus), *Maribacter orientalis* (species), *Megasphaera genomosp* (species), *Megasphaera micronuciformis* (species), *Megasphaera* spp. (genus), *Microbacterium halophilum* (species), *Mobiluncus curtisii* (species), *Mobiluncus mulieris* (species), *Moorella glycerini* (species), *Mycoplasma genitalium* (species), *Mycoplasma hominis* (species), *Mycoplasma muris* (species), *Neisseria gonorrhoeae* (species), *Paeniclostridium sordellii* (species), *Papillibacter* spp. (genus), *Parastreptomyces abscessus* (species), *Parvimonas micra* (species), *Parvimonas* spp. (genus), *Pasteurella multocida* (species), *Pediococcus ethanolidurans* (species), *Peptoniphilus harei* (species), *Peptoniphilus indolicus* (species), *Peptoniphilus* spp. (genus), *Peptostreptococcus anaerobius* (species), *Peptostreptococcus massiliae* (species), *Peptostreptococcus* spp. (genus), *Porphyromonas gingivalis* (species), *Porphyromonas levii* (species), *Porphyromonas* sp. (genus), *Porphyromonas uenonis* (species), *Prevotella amnii* (species), *Prevotella bivia* (species), *Prevotella disiens* (species), *Prevotella intermedia* (species), *Prevotella oralis* (species), *Prevotella oris* (species), *Prevotella timonensis* (species), *Pseudomonas* spp. (genus), *Ralstonia pickettii* (species), *Ruminococcus* spp. (genus), *Sanguibacter keddieii* (species), *Sneathia amnii* (species), *Sneathia sanguinegens* (species), *Sneathia* spp. (genus), *Staphylococcus aureus* (species), *Staphylococcus mulans* (species), *Staphylococcus pasteurii* (species), *Staphylococcus simiae* (species), *Staphylococcus simulans* (species), *Staphylococcus* spp. (genus), *Staphylococcus warneri* (species), *Streptococcus agalactiae* (species), *Streptococcus anginosus* (species), *Streptococcus intermedius* (species), *Streptococcus pyogenes* (species), *Streptococcus viridans* (species), *Thermosipho atlanticus* (species), *Thermovirga lienii* (species), *Treponema pallidum* (species), *Trichomonas vaginalis* (species), *Trueperella bernardiae* (species), *Ureaplasma parvum* (species),

Ureaplasma urealyticum (species), *Veillonella montpellierensis* (species), *Veillonella parvula* (species), *Virgibacillus proomii* (species), *Zobellia laminariae* (species), HPV 3 (virus variant), HPV 6 (virus variant), HPV 16 (virus variant), HPV 18 (virus variant), HPV 31 (virus variant), HPV 33 (virus variant), HPV 35 (virus variant), HPV 39 (virus variant), HPV 43 (virus variant), HPV 45 (virus variant), HPV 51 (virus variant), HPV 52 (virus variant), HPV 53 (virus variant), HPV 54 (virus variant), HPV 56 (virus variant), HPV 58 (virus variant), HPV 59 (virus variant), HPV 66 (virus variant), HPV 68 (virus variant), HPV (virus), and HPV (multiple type) (virus).