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(54) Title: DIAGNOSIS AND TREATMENT OF DISEASES AND CONDITIONS OF THE INTESTINAL TRACT

(57) Abstract: The invention relates to methods of monitoring treatment response, disease resolution, and disease progression in subjects having inflammatory bowel disease (IBD).

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DIAGNOSIS AND TREATMENT OF DISEASES AND CONDITIONS OF THE INTESTINAL TRACT

SEQUENCE LISTING

[0001] The instant application contains a Sequence Listing which has been submitted electronically in XML file format and is hereby incorporated by reference in its entirety. Said XML copy, created on September 12, 2022, is named 51373-019WO1_Sequence_Listing_9_12_22.xml and is 2,275,135 bytes in size.

FIELD OF THE INVENTION

[0002] The invention relates to methods of monitoring treatment response, disease resolution, and disease progression in subjects having inflammatory bowel disease (IBD).

BACKGROUND

[0003] Mammals are colonized by microorganisms in the gastrointestinal (GI) tract, on the skin, and in other epithelial and tissue niches. The gastrointestinal tract of a healthy individual harbors an abundant and diverse microbial community. It is a complex system, providing an environment or niche for a community of many different species or organisms, including diverse strains of bacteria. Hundreds of different species may form a commensal community in the GI tract in a healthy person, and this complement of organisms evolves from the time of birth and is believed to form a functionally mature microbial population by about 3 years of age. Interactions between microbial strains in these populations and between microorganisms and the host, e.g., interactions with the host's immune system, shape the community structure, with availability of and competition for resources affecting the distribution of microorganisms.

[0004] A healthy microbiome may provide a subject with multiple benefits, including colonization resistance to a broad spectrum of pathogens, essential nutrient biosynthesis and absorption, and immune stimulation that plays a role in maintaining a healthy gut epithelium and appropriately controlled systemic immunity. Conversely, an unhealthy (e.g., dysregulated) microbiome may be associated with a disease state.

[0005] There is a need for methods for addressing problems in healthcare through assessment of the microbiome.

SUMMARY OF THE INVENTION

[0006] In one aspect, the disclosure features a method of monitoring the response of a subject to treatment with an anti-inflammatory bowel disease (anti-IBD) therapy, the method comprising (a) determining a level of one or more of SEQ ID NOs: 1-156 in a pre-treatment sample from the subject and (b) determining a level of one or more of SEQ ID NOs: 1-156 in an on-treatment or post-treatment sample from the subject, wherein a level of one or more of SEQ ID NOs: 1-156 that is changed in the on-treatment or post-treatment sample, as compared to the respective reference level in the pre-treatment sample, indicates that the subject is responding to treatment with the anti-IBD therapy.

[0007] In another aspect, the disclosure features a method for determining that a subject having an IBD is responding to treatment with an anti-IBD therapy, comprising the steps of (a) measuring the nucleic acid level of one or more of SEQ ID NOs: 1-156 in a pre-treatment sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156; (b) treating the subject with an anti-IBD therapy; (c) measuring the nucleic acid level of one or more of SEQ ID NOs: 1-156 in an on-treatment or post-treatment sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156; and (d) using the amplification results to determine whether the level of one or more of SEQ ID NOs: 1-156 is changed in the on-treatment or post-treatment sample as compared to the respective reference level in the pre-treatment sample, thereby determining that the subject is responding to treatment with the anti-IBD therapy.

[0008] In some embodiments, a level of one or more of SEQ ID NOs: 1-156 is changed in the on-treatment or post-treatment sample as compared to the respective reference level in the pre-treatment sample, and the method further comprises continuing to administer the anti-IBD therapy to a subject.

[0009] In another aspect, the disclosure features a method of monitoring the response of a subject to treatment with an anti-IBD therapy, the method comprising (a) determining a level of one or more of SEQ ID NOs: 1-156 in a pre-treatment sample from the subject and (b) determining a level of one or more of SEQ ID NOs: 1-156 in an on-treatment or post-treatment sample from the subject, wherein a level of one or more of SEQ ID NOs: 1-156 that not changed in the on-treatment or post-treatment sample, as compared to the respective reference level in the pre-treatment sample, indicates that the subject is not responding to treatment with the anti-IBD therapy.

[00010] In another aspect, the disclosure features a method for determining that a subject having an inflammatory bowel disease (IBD) is not responding to treatment with an anti-IBD therapy, comprising the steps of (a) measuring the nucleic acid level of one or more of SEQ ID NOs: 1-156 in a pre-treatment sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156; (b) treating the subject with an anti-IBD therapy; (c) measuring the nucleic acid level of one or more of SEQ ID NOs: 1-156 in an on-treatment or post-treatment sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156; and (d) using the amplification results to determine whether the level of one or more of SEQ ID NOs: 1-156 is not changed in the on-treatment or post-treatment sample as compared to the respective reference level in the pre-treatment sample, thereby determining that the subject is not responding to treatment with the anti-IBD therapy.

[00011] In some embodiments, a level of one or more of SEQ ID NOs: 1-156 is not changed in the on-treatment or post-treatment sample as compared to the respective reference level in the pre-treatment sample, and the method further comprises discontinuing treatment of the subject with the anti-IBD therapy and/or administering an alternative anti-IBD therapy to the subject.

[00012] In another aspect, the disclosure features a method of identifying a subject who is likely to respond to treatment with an anti-IBD therapy, the method comprising determining a level of one or more of SEQ ID NOs: 1-156 in a sample from the subject, wherein a level of one or more of SEQ ID NOs: 1-156 that is changed relative to a respective reference level for SEQ ID NOs: 1-156 indicates that the subject is likely to benefit from an anti-IBD therapy.

[00013] In another aspect, the disclosure features a method for determining that a subject having an inflammatory bowel disease (IBD) is likely to respond to treatment with an anti-IBD therapy, comprising the steps of (a) measuring the nucleic acid level of one or more of SEQ ID NOs: 1-156 in a sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156; and (b) using the amplification results to determine whether the level of one or more of SEQ ID NOs: 1-156 is changed relative to a respective reference level for SEQ ID NOs: 1-156, thereby determining that the subject is likely to respond to treatment with an anti-IBD therapy.

[00014] In some embodiments, a level of one or more of SEQ ID NOs: 1-156 is changed relative to the respective reference level for SEQ ID NO: 1-156 in the sample from the subject and the method further comprises administering an anti-IBD therapy to a subject.

[00015] In another aspect, the disclosure features a method of determining disease resolution in a subject with an IBD, the method comprising determining a level of one or more of SEQ ID NOs: 1-156 in a sample from the subject, wherein a level of one or more of SEQ ID NOs: 1-156 that is not substantially different from a respective reference level for SEQ ID NOs: 1-156 indicates that the subject has experienced resolution of the IBD.

[00016] In another aspect, the disclosure features a method for determining that disease resolution has occurred in a subject having an IBD, comprising the steps of (a) measuring the nucleic acid level of one or more of SEQ ID NOs: 1-156 in a sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156; and (b) using the amplification results to determine whether the level of one or more of SEQ ID NOs: 1-156 is not substantially different from a respective reference level for SEQ ID NOs: 1-156, thereby determining that disease resolution has occurred in the subject.

[00017] In some embodiments, the subject is being treated with an anti-IBD therapy, a level of one or more of SEQ ID NOs: 1-156 is not substantially different from a respective reference level for SEQ ID NOs: 1-156 in the sample from the subject, and the method further comprises discontinuing treatment of the subject with the anti-IBD therapy.

[00018] In some embodiments, the method comprises determining a level of at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least fifteen, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, or at least 150 of SEQ ID NOs: 1-156 in a sample from the subject.

[00019] In some embodiments, the method comprises determining a level of all 156 of SEQ ID NOs: 1-156 in a sample from the subject.

[00020] In some embodiments, the anti-IBD therapy comprises fecal microbiota transplantation (FMT).

[00021] In some embodiments, the reference level is a pre-assigned level.

[00022] In some embodiments, the reference level is a level in a set of samples from a reference population.

[00023] In some embodiments, the reference population is a population of healthy subjects.

[00024] In some embodiments, the level of each of SEQ ID NOs: 1-156 that is determined in a sample from the subject is a nucleic acid level.

[00025] In some embodiments, the nucleic acid level is a DNA level.

[00026] In some embodiments, the change is a decrease relative to the reference level.

[00027] In some embodiments, the levels of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more than 90% of the sequences for which a level is determined are decreased relative to a respective reference level for the sequence.

[00028] In some embodiments, the change is an increase relative to the reference level.

[00029] In some embodiments, the levels of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more than 90% of the sequences for which a level is determined are increased relative to a respective reference level for the sequence.

[00030] In some embodiments, the sample comprises a sample of the microbiota of the subject.

[00031] In some embodiments, the sample is a fecal sample.

[00032] In another aspect, the disclosure features a kit for monitoring the response of a subject to treatment with an anti-IBD therapy, the kit comprising: (a) polypeptides or polynucleotides capable of determining the level of one or more of SEQ ID NOs: 1-156 in a sample from the subject; and optionally (b) instructions for use of the polypeptides or polynucleotides to determine the level of one or more of SEQ ID NOs: 1-156 in a pre-treatment, on-treatment, and/or post-treatment sample from the subject, wherein a level of one or more of SEQ ID NOs: 1-156 that is changed in the on-treatment or post-treatment sample, as compared to the respective reference level in the pre-treatment sample, indicates that the subject is responding to treatment with the anti-IBD therapy.

[00033] In another aspect, the disclosure features a method of monitoring the response of a subject to treatment with an anti-IBD therapy, the method comprising (a) determining a level of one or more of SEQ ID NOs: 1-156 in a pre-treatment sample from the subject and (b) determining a level of one or more of SEQ ID NOs: 1-156 in an on-treatment or post-treatment sample from the subject, wherein a level of at least 50% of SEQ ID NOs: 1-156 that is changed in the on-treatment or post-treatment sample, as compared to the respective reference level in the pre-treatment sample, indicates that the subject is responding to treatment with the anti-IBD therapy.

[00034] In some embodiments, a level of at least 50% of SEQ ID NOs: 1-156 is increased relative to a respective reference level for SEQ ID NOs: 1-156 in the sample from the subject and the method further comprises continuing to administer the anti-IBD therapy to a subject.

[00035] In some embodiments, a level of at least 60%, 70%, 80%, 90%, or 95% of SEQ ID NOs: 1-318 that is increased in a sample from the subject relative to a respective reference level for SEQ ID NOs: 1-156 and the method further comprises continuing to administer the anti-IBD therapy to a subject.

[00036] In another aspect, the disclosure features a method of treating a subject having an IBD using a therapy appropriate for treating the IBD, wherein the subject was monitored for their response to the therapy by any of the methods provided herein.

[00037] In another aspect, the disclosure features a method of treating a subject having an IBD using a therapy appropriate for treating the IBD, wherein the subject was determined to be responsive to prior treatment with the therapy by any of the methods provided herein.

[00038] In another aspect, the disclosure features a method of treating a subject having an IBD using a therapy appropriate for treating the IBD, wherein the subject was determined to be likely to respond to the treatment with the therapy by any of the methods provided herein.

[00039] In another aspect, the disclosure features a method of treating a subject having an IBD, the method comprising the steps of (a) measuring the nucleic acid level of one or more of SEQ ID NOs: 1-156 in a pre-treatment sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156; (b) administering an anti-IBD therapy to a subject; (c) measuring the nucleic acid level of one or more of SEQ ID NOs: 1-156 in an on-treatment or post-treatment sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156, wherein a level of one or more of SEQ ID NOs: 1-156 that is changed in the on-treatment or post-treatment sample as compared to the respective reference level in the pre-treatment sample indicates the subject is responding to treatment with the anti-IBD therapy; and (d) continuing to administer the anti-IBD therapy to a subject who has been determined to be responding to treatment with the anti-IBD therapy.

[00040] In some embodiments, the nucleic acid level of the one or more of SEQ ID NOs: 1-156 in the sample collected from the subject are measured using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156, and the amplification results are used to determine whether the level of one or more of SEQ ID NOs: 1-156 is changed in the on-treatment or post-treatment sample as compared to the respective reference level in the pre-treatment sample.

[00041] In some embodiments, the method comprises measuring the nucleic acid level of at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least

nine, at least ten, at least fifteen, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, or at least 150 of SEQ ID NOs: 1-156 in the sample from the subject. In some embodiments, the method comprises measuring the nucleic acid level of all 156 of SEQ ID NOs: 1-156 in the sample from the subject.

[00042] In some embodiments, the nucleic acid level is a DNA level.

[00043] In some embodiments, the change is a decrease relative to the reference level. In some embodiments, the levels of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more than 90% of the sequences for which a level is measured are decreased relative to a respective reference level for the sequence.

[00044] In some embodiments, the change is an increase relative to the reference level. In some embodiments, the levels of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more than 90% of the sequences for which a level is measured are increased relative to a respective reference level for the sequence.

[00045] In some embodiments, the sample comprises a sample of the microbiota of the subject. In some embodiments, the sample is a fecal sample.

[00046] In another aspect, the disclosure features a method of treating a subject having an IBD, the method comprising the steps of (a) measuring the nucleic acid level of each of SEQ ID NOs: 1-156 in a pre-treatment sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156; (b) administering an anti-IBD therapy to a subject; (c) measuring the nucleic acid level of each of SEQ ID NOs: 1-156 in an on-treatment or post-treatment sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156, wherein a level of at least 50% of SEQ ID NOs: 1-156 that is changed in the on-treatment or post-treatment sample as compared to the respective reference level in the pre-treatment sample indicates the subject is responding to treatment with the anti-IBD therapy; and (d) continuing to administer the anti-IBD therapy to a subject who has been determined to be responding to treatment with the anti-IBD therapy.

[00047] In some embodiments, the nucleic acid level of each of SEQ ID NOs: 1-156 in the sample collected from the subject are measured using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156, and the amplification results

are used to determine whether the level of each of SEQ ID NOs: 1-156 is changed in the on-treatment or post-treatment sample as compared to the respective reference level in the pre-treatment sample.

BRIEF DESCRIPTION OF THE DRAWING

[00048] FIG. 1 is a set of box plots showing the number (count) of metagenomic markers in donors and in patients having inflammatory bowel disease who were responders (R) or non-responders (NR) to treatment for the IBD at the indicated time point (screening, week 4 (Wk4), week 8 (Wk8), or week 16 (Wk16)). Box plots depict median, first and third quartiles and largest/smallest value within 1.5* interquartile range (IQR). The dotted line represents the theoretical maximum number of metagenomic markers (355). Statistical test is a pairwise Wilcoxon test where ns indicates $p > 0.05$, * $p <= 0.05$, and **: $p <= 0.01$.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[00049] The term "changed," as used herein, refers to an observable difference in the level of a marker in a subject (e.g., in a sample from the subject), as determined using techniques and methods known in the art for the measurement of the marker. A marker level that is changed in a subject may result in a difference of at least 1% (e.g., at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%, or at least 2.5-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 15-fold, 20-fold, 25-fold, 50-fold, 75-fold, 100-fold, or more than 100-fold) more or less than a reference level (e.g., a level from a healthy subject or a level prior to treatment) (e.g., up to 100% or up to 100-fold relative to the reference level). In some embodiments, the change is an increase in the level of a marker in a subject. Increasing the marker level in a subject may result in an increase of at least 1% (e.g., at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100%, or at least 2.5-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 15-fold, 20-fold, 25-fold, 50-fold, 75-fold, 100-fold, or more than 100-fold) relative to the reference level (e.g., up to 100% or up to 100-fold relative to the reference level). In other embodiments, the change is a decrease the level of a marker in a subject. Decreasing the marker level in a subject may result in a decrease of at least 1% (e.g., at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%, or at least 2.5-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-

fold, 10-fold, 15-fold, 20-fold, 25-fold, 50-fold, 75-fold, 100-fold, or more than 100-fold) relative to the reference level (e.g., up to 100% or up to 100-fold relative to the reference level).

[00050] In embodiments in which a level is increased or decreased (or reduced) in a subject following a step of administering a therapy described herein, the increase or decrease may take place and/or be detectable within a range of time following the administration (e.g., within six hours, 24 hours, 3 days, a week or longer), and may take place and/or be detectable after one or more administrations (e.g., after 2, 3, 4, 5, 6, 7, 8, 9, 10, or more administrations, e.g., as part of a dosing regimen for the subject).

[00051] In some embodiments, the change in the level of a portion of the markers analyzed is an increase, while the change in the level of another portion of the markers analyzed is a decrease. In some embodiments, the change in at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more (e.g., 100%) of the markers analyzed is an increase relative to a reference level. In some embodiments, the change in at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more (e.g., 100%) of the markers analyzed is a decrease relative to a reference level.

[00052] The term "pharmaceutical composition," as used herein, represents a composition formulated with a pharmaceutically acceptable excipient. For example, a "pharmaceutical composition" can be a composition that is manufactured or sold with the approval of a governmental regulatory agency as part of a therapeutic regimen for the treatment of a disease, disorder, or condition in a mammal, intended for such use, or in development for such use. In some examples, the pharmaceutical composition is a pre-approved composition.

[00053] The term "subject," as used herein, represents a human or non-human animal (e.g., a mammal).

[00054] "Treatment" and "treating," as used herein, refer to the medical management of a subject with the intent to improve, ameliorate, stabilize, prevent, or cure a disease, disorder, or condition. This term includes active treatment (treatment directed to improve the disease, disorder, or condition); causal treatment (treatment directed to the cause of the associated disease, disorder, or condition); palliative treatment (treatment designed for the relief of symptoms of the disease, disorder, or condition); preventative treatment (treatment directed to minimizing or partially or completely inhibiting the development of the associated disease, disorder, or condition); and supportive treatment (treatment employed to supplement another therapy).

[00055] The term "inflammatory bowel disease" or "IBD," as used herein, refers to a condition of the bowel, e.g., the small intestine, large intestine, mouth, esophagus, stomach, rectum, and/or anus, that is characterized by inflammation. Examples of IBD include ulcerative colitis (UC) and Crohn's disease. Other examples include microscopic colitis (e.g., collagenous colitis or lyphocytic colitis), diversion colitis, Behçet's disease, or indeterminate colitis.

I. METHODS OF MONITORING TREATMENT RESPONSE AND DISEASE PROGRESSION IN INFLAMMATORY BOWEL DISEASE

[00040] The invention is based, in part, on the discovery that levels of gut microbiome biomarkers (i.e., markers of bacterial origin), which may be referred to as co-evolved molecules, can be used to monitor treatment response and disease progression in subjects having an inflammatory bowel disease (IBD). Accordingly, the disclosure provides methods of monitoring and treating subjects (e.g., human patients) based on this discovery.

Methods

[00041] In some aspects, the disclosure features methods of monitoring treatment response and disease progression in IBD in a subject (e.g., a human subject), the methods comprising determining a level of one or more of SEQ ID NOs: 1-156 in a sample from the subject, wherein a level of one or more of SEQ ID NOs: 1-156 that is changed relative to a respective reference level for SEQ ID NOs: 1-156 indicates whether the subject is responding to treatment or whether the subject's disease is progressing. SEQ ID NOs: 1-156 are bacterial sequences. In some embodiments, the IBD is, e.g., ulcerative colitis or Crohn's disease. In some aspects, the disclosure features a method for determining that a subject having an IBD is responding to treatment with an anti-IBD therapy, comprising the steps of (a) measuring the nucleic acid level of one or more of SEQ ID NOs: 1-156 in a pre-treatment sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156; (b) treating the subject with an anti-IBD therapy; (c) measuring the nucleic acid level of one or more of SEQ ID NOs: 1-156 in an on-treatment or post-treatment sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156; and (d) using the amplification results to determine whether the level of one or more of SEQ ID NOs: 1-156 is changed in the on-treatment or post-treatment sample as compared to the respective reference level in the pre-treatment sample, thereby determining that the subject is responding to treatment with the anti-IBD therapy.

[00042] In some aspects, the disclosure features a method for determining that a subject having an inflammatory bowel disease (IBD) is not responding to treatment with an anti-IBD therapy, comprising the steps of (a) measuring the nucleic acid level of one or more of SEQ ID NOs: 1-156 in a pre-treatment sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156; (b) treating the subject with an anti-IBD therapy; (c) measuring the nucleic acid level of one or more of SEQ ID NOs: 1-156 in an on-treatment or post-treatment sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156; and (d) using the amplification results to determine whether the level of one or more of SEQ ID NOs: 1-156 is not changed in the on-treatment or post-treatment sample as compared to the respective reference level in the pre-treatment sample, thereby determining that the subject is not responding to treatment with the anti-IBD therapy.

[00043] In some aspects, the disclosure features a method for determining that a subject having an inflammatory bowel disease (IBD) is likely to respond to treatment with an anti-IBD therapy, comprising the steps of (a) measuring the nucleic acid level of one or more of SEQ ID NOs: 1-156 in a sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156; and (b) using the amplification results to determine whether the level of one or more of SEQ ID NOs: 1-156 is changed relative to a respective reference level for SEQ ID NOs: 1-156, thereby determining that the subject is likely to respond to treatment with an anti-IBD therapy.

[00044] In some aspects, the disclosure features a method for determining that disease resolution has occurred in a subject having an IBD, comprising the steps of (a) measuring the nucleic acid level of one or more of SEQ ID NOs: 1-156 in a sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156; and (b) using the amplification results to determine whether the level of one or more of SEQ ID NOs: 1-156 is not substantially different from a respective reference level for SEQ ID NOs: 1-156, thereby determining that disease resolution has occurred in the subject.

[00045] In another aspect, the disclosure features a method of treating a subject having an IBD using a therapy appropriate for treating the IBD, wherein the subject was monitored for their response to the therapy by any of the methods provided herein.

[00046] In another aspect, the disclosure features a method of treating a subject having an IBD using a therapy appropriate for treating the IBD, wherein the subject was determined to be responsive to prior treatment with the therapy by any of the methods provided herein.

[00047] In another aspect, the disclosure features a method of treating a subject having an IBD using a therapy appropriate for treating the IBD, wherein the subject was determined to be likely to respond to the treatment with the therapy by any of the methods provided herein.

[00048] In another aspect, the disclosure features a method of treating a subject having an IBD, the method comprising the steps of (a) measuring the nucleic acid level of one or more of SEQ ID NOs: 1-156 in a pre-treatment sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156; (b) administering an anti-IBD therapy to a subject; (c) measuring the nucleic acid level of one or more of SEQ ID NOs: 1-156 in an on-treatment or post-treatment sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156, wherein a level of one or more of SEQ ID NOs: 1-156 that is changed in the on-treatment or post-treatment sample as compared to the respective reference level in the pre-treatment sample indicates the subject is responding to treatment with the anti-IBD therapy; and (d) continuing to administer the anti-IBD therapy to a subject who has been determined to be responding to treatment with the anti-IBD therapy.

[00049] In another aspect, the disclosure features a method of treating a subject having an IBD, the method comprising the steps of (a) measuring the nucleic acid level of each of SEQ ID NOs: 1-156 in a pre-treatment sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156; (b) administering an anti-IBD therapy to a subject; (c) measuring the nucleic acid level of each of SEQ ID NOs: 1-156 in an on-treatment or post-treatment sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156, wherein a level of at least 50% of SEQ ID NOs: 1-156 that is changed in the on-treatment or post-treatment sample as compared to the respective reference level in the pre-treatment sample indicates the subject is responding to treatment with the anti-IBD therapy; and (d) continuing to administer the anti-IBD therapy to a subject who has been determined to be responding to treatment with the anti-IBD therapy.

[00050] In some embodiments, the sample comprises a sample of the microbiota (e.g., the gut microbiota) of the subject. In some embodiments, the sample is a fecal sample. In some embodiments, the sample is a colon or rectal biopsy.

[00051] In some embodiments, the method comprises determining or measuring a level of at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at

least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 100, at least 105, at least 110, at least 115, at least 120, at least 125, at least 130, at least 135, at least 140, at least 145, at least 150, or at least of SEQ ID NOs: 1-156 in a sample from the subject, e.g., comprises determining or measuring a level of 1-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 40-45, 45-50, 50-55, 55-60, 60-65, 65-70, 70-75, 75-80, 80-85, 85-90, 90-95, 95-100, 100-105, 105-110, 110-115, 115-120, 120-125, 125-130, 130-135, 135-140, 140-145, 145-150, or 150-156 of SEQ ID NOs: 1-156 in the sample from the subject. In some embodiments, the method comprises determining or measuring a level of all 156 of SEQ ID NOs: 1-156 in a sample from the subject.

[00052] In some embodiments, a change in the level of at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 100, at least 105, at least 110, at least 115, at least 120, at least 125, at least 130, at least 135, at least 140, at least 145, at least 150, or at least 155 of SEQ ID NOs: 1-156 in a sample from the subject, e.g., a change in the level of 1-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 40-45, 45-50, 50-55, 55-60, 60-65, 65-70, 70-75, 75-80, 80-85, 85-90, 90-95, 95-100, 100-105, 105-110, 110-115, 115-120, 120-125, 125-130, 130-135, 135-140, 140-145, 145-150, or 150-156 of SEQ ID NOs: 1-156 in the sample from the subject relative to a respective reference level for SEQ ID NOs: 1-156 indicates that the subject is responding to treatment. In some embodiments, a change in the level of all 156 of SEQ ID NOs: 1-156 relative to a respective reference level for SEQ ID NOs: 1-156 indicates that the subject is responding to treatment.

[00053] In some embodiments, a change in the level of at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 95%, at least 98%, at least 99%, or 100% of the total number of SEQ ID NOs: 1-156 measured in a sample from the subject relative to a respective reference level for SEQ ID NOs: 1-156 indicates that the subject is responding to treatment.

[00054] In some embodiments, a threshold number of one or more of SEQ ID NOs: 1-156 is changed relative to the respective reference level for SEQ ID NO: 1-156 in the sample from the subject (e.g., a number of SEQ ID NOs: 1-156 that has been determined to indicate that the patient is responding to treatment, e.g., a level of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least

30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 100, at least 105, at least 110, at least 115, at least 120, at least 125, at least 130, at least 135, at least 140, at least 145, at least 150, or at least 155 of SEQ ID NOs: 1-156 in a sample from the subject (e.g., a level of 1-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 40-45, 45-50, 50-55, 55-60, 60-65, 65-70, 70-75, 75-80, 80-85, 85-90, 90-95, 95-100, 100-105, 105-110, 110-115, 115-120, 120-125, 125-130, 130-135, 135-140, 140-145, 145-150, or 150-156)) of SEQ ID NOs: 1-156 is changed relative to the respective reference level for SEQ ID NO: 1-156 in the sample from the subject, and the method further comprises administering an anti-IBD therapy to a subject.

[00055] The anti-IBD therapy may be any medicament, treatment, or combination thereof suitable for the treatment of an IBD. In some aspects, the anti-IBD therapy comprises an anti-integrin therapy. In some aspects, the anti-integrin therapy targets integrin $\alpha_4\beta_7$. In some aspects, the anti-integrin therapy is vedolizumab. In some embodiments, the anti-IBD therapy comprises a biologic therapy, an anti-inflammatory agent, an antibiotic, or an immune system suppressor. In some embodiments, the biologic therapy is vedolizumab (ENTYVIO®), infliximab (REMICADE®), adalimumab (HUMIRA®), golimumab (SIMPONI®), certolizumab (CIMZIA®), risankizumab (SKYRIZI®), or ustekinumab (STELARA®); the anti-inflammatory agent is a corticosteroid or an aminosalicylate; the antibiotic is ciprofloxacin (cipro) or metronidazole (Flagyl); or the immune system suppressor is azathioprine (AZASAN®, IMURAN®), mercaptopurine (PURINETHOL®, PURIXAN®), methotrexate (TREXALL®), tofacitinib (XELJANZ®), upadacitinib (RINVOQ®), or ozanimod (ZEPOSIA®). Any one or more of these therapies may optionally be used in any of the methods described herein as employing an anti-IBD therapy. In some aspects, the anti-IBD therapy further comprises treatment by fecal microbiota transplant (FMT).

[00056] In some embodiments, the respective reference level for SEQ ID NOs: 1-156 is a pre-assigned level of one of SEQ ID NOs: 1-156.

[00057] In some embodiments, the respective reference level for SEQ ID NOs: 1-156 is a level in a set of samples from a reference population, e.g., a population of healthy subjects (e.g., a population of subjects not having an IBD and/or a population of subjects having a healthy gut microbiome). In some embodiments, the respective reference level for SEQ ID NOs: 1-156 is a pre-treatment level from the subject or from a set of samples from a reference population (e.g., subjects having IBD).

[00058] In some embodiments, the level of each of SEQ ID NOs: 1-156 that is determined or measured in a sample from the subject is a nucleic acid level, e.g., a DNA level or an RNA level. In some embodiments, the nucleic acid level is a DNA level, which may be detected, e.g., using a PCR-based method. In other embodiments, detection of a level of one or more of SEQ ID NOs: 1-156 can optionally comprise, for example, detection of RNA levels, which can be achieved by, e.g., RT-PCR, RNA-Seq, and/or methods including the use of microarrays, as are known in the art. In other embodiments, the methods can focus on the detection of protein levels, which can be carried out using standard approaches (e.g., immunoassay-based approaches).

[00059] In some embodiments, the change in a level of one or more of SEQ ID NOs: 1-156 in the sample from the subject is a decrease relative to the respective reference level for SEQ ID NOs: 1-156 (e.g., a decrease of at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, e.g., a decrease of, e.g., at least 2.5-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 15-fold, 20-fold, 25-fold, 50-fold, 75-fold, 100-fold, or more than 100-fold) relative to the respective reference level for SEQ ID NOs: 1-156; or a decrease of 1-5%, 5-10%, 10-15%, 15-20%, 20-25%, 25-30%, 30-35%, 35-40%, 40-45%, 45-50%, 50-55%, 55-60%, 60-65%, 65-70%, 70-75%, 75-80%, 0-85%, 85-90%, 90-95%, or 95-100% relative to the respective reference level for SEQ ID NOs: 1-156).

[00060] In some aspects, the levels of at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or more than 99% of the sequences for which a level is determined or measured in the sample from the subject (i.e., one or more of SEQ ID NOs: 1-156) are decreased relative to a respective reference level for the sequence, e.g., 5-10%, 10-15%, 15-20%, 20-25%, 25-30%, 30-35%, 35-40%, 40-45%, 45-50%, 50-55%, 55-60%, 60-65%, 65-70%, 70-75%, 75-80%, 0-85%, 85-90%, 90-95%, or 95-100% of the sequences for which a level is determined or measured are decreased relative to a respective reference level for the sequence.

[00061] In some embodiments, the change in a level of one or more of SEQ ID NOs: 1-156 in the sample from the subject is an increase relative to the respective reference level for SEQ ID NOs: 1-156 (e.g., an increase of at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or more than 100%,e.g., an increase of, e.g., 2.5-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 15-fold, 20-fold, 25-fold, 50-fold, 75-fold, 100-fold, or more than 100-fold, relative to the respective level for SEQ ID NOs: 1-156; or an increase of 1-5%, 5-10%, 10-15%, 15-20%, 20-25%, 25-30%, 30-35%, 35-40%, 40-45%, 45-50%, 50-55%, 55-60%, 60-65%, 65-70%, 70-75%, 75-80%, 0-85%, 85-90%, 90-95%, 95-100%, or more than 100%, relative to the respective reference level for SEQ ID NOs: 1-156).

[00062] In some aspects, the levels of at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or more than 99% of the sequences for which a level is determined or measured in the sample from the subject (i.e., one or more of SEQ ID NOs: 1-156) are increased relative to a respective reference level for the sequence, e.g., 5-10%, 10-15%, 15-20%, 20-25%, 25-30%, 30-35%, 35-40%, 40-45%, 45-50%, 50-55%, 55-60%, 60-65%, 65-70%, 70-75%, 75-80%, 0-85%, 85-90%, 90-95%, or 95-100% of the sequences for which a level is determined or measured are increased relative to a respective reference level for the sequence.

[00063] In some aspects, a level of at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of SEQ ID NOs: 1-156 that is increased in a sample from the subject relative to a respective reference level for SEQ ID NOs: 1-156 indicates that the subject is responding to treatment.

[00064] Determination of whether a difference detected is significant can be carried out using standard methods, as well as statistical analysis. In some embodiments, a difference detected is a change of at least 5%, 10%, 20%, 30%, 40%, 50%, 75%, 100%, or more, e.g., at least 2.5-fold,

3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 15-fold, 20-fold, 25-fold, 50-fold, 75-fold, 100-fold, or more than 100-fold, relative to a reference level.

II. ARTICLES OF MANUFACTURE AND KITS

[00065] In another aspect of the invention, an article of manufacture or kit containing materials useful for the diagnosis, prognostic assessment, and/or treatment of individuals is provided.

[00066] In one aspect, the disclosure features a kit or article of manufacture for diagnosing inflammatory bowel disease (IBD) in a subject, the kit comprising (a) reagents for determining a level of one or more of SEQ ID NOs: 1-156 in a sample from the subject (e.g., polynucleotides or polypeptides capable of use in determining the level of one or more of SEQ ID NOs: 1-156 in a sample from the subject); and optionally (b) instructions for use of the polynucleotides or polypeptides to determine the level of one or more of SEQ ID NOs: 1-156 in the sample from the subject, wherein a change in the level of one or more of SEQ ID NOs: 1-156 relative to a respective reference level for SEQ ID NOs: 1-156, as described herein, indicates whether the subject is responding to treatment or whether their disease is progressing. In some embodiments, reagents are included for determining a level of at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or more than 99% of the sequences of SEQ ID NOs: 1-156.

[00067] In some aspects, the reagents for determining a level of one or more of SEQ ID NOs: 1-156 in a sample from the subject comprise one or more polynucleotides (e.g., PCR primers) that hybridize to a complement of a locus of one or more of SEQ ID NOs: 1-156 under stringent conditions and may be used to amplify all or a portion of any one or more of SEQ ID NOs: 1-156, as described herein. In some aspects, the instructions indicate that the one or more oligonucleotides (e.g., PCR primers) may be used to evaluate the presence and/or level of one or more of SEQ ID NOs: 1-156 in a sample from the subject and provide instructions for using the polynucleotide(s) for evaluating the presence and/or level of one or more of SEQ ID NOs: 1-156 in the sample.

[00068] For polynucleotide-based articles of manufacture or kits, the article of manufacture or kit may include, for example: (1) an oligonucleotide, e.g., a detectably labeled oligonucleotide, which hybridizes to a nucleic acid sequence encoding a protein, (2) a pair of primers useful for amplifying a nucleic acid molecule, or (3) a microarray comprising multiple

oligonucleotide probes. For protein-based articles of manufacture or kits, the article of manufacture or kit may include, for example, one or more antibody-based reagents. The article of manufacture or kit can also include, e.g., a buffering agent, a preservative, or a protein-stabilizing agent. The article of manufacture or kit can further include components necessary for detecting the detectable label (e.g., an enzyme or a substrate). The article of manufacture or kit can further include components necessary for analyzing the sequence of a sample (e.g., a restriction enzyme or a buffer). The article of manufacture or kit can also contain a control sample or a series of control samples that can be assayed and compared to the test sample (e.g., a reference sample, as described herein). Each component of the article of manufacture or kit can be enclosed within an individual container and all of the various containers can be within a single package, along with instructions for interpreting the results of the assays performed using the kit.

[00069] The following examples are meant to illustrate the invention. They are not meant to limit the invention in any way.

III. EXAMPLES

Example 1. Identification of markers

A. Assembly and annotation of metagenomic markers

[00070] Paired end reads from healthy Human Microbiome Project 1 (HMP1; Human Microbiome Project Consortium, *Nature*, 486(7402): 207-214, 2012) individuals were downloaded from the National Center for Bioinformatics (NCBI) Short Read Archive (SRA) and assembled using metaSPAdes (cab.spbu.ru/software/spades/). Metagenomic markers were annotated using antiSMASH4.0 (docs.antismash.secondarymetabolites.org/) with the following non-default parameters: -c 3 --smcogs --disable-embl. Annotated metagenomic markers were clustered using all vs. all diamond (https://github.com/bbuchfink/diamond) blastx. Blastx results were filtered using a python script requiring (a) E-value < 1×10^{-5}, (b) 90% coverage of length of coding sequence, and (c) > 50% of coding sequences in a metagenomic marker present. Metagenomic markers were then grouped using markov clustering, resulting in a dereplicated library of 8,211 representative metagenomic markers identified in healthy human gut metagenomes.

B. EMP selection

[00071] A subset of metagenomic markers prevalent in healthy cohorts, referred to as essential microbial products (EMPs), were identified by clustering metagenomic markers presence/absence across 592 healthy patients from various geographic, genetic, and lifestyle backgrounds. Clusters with a mean prevalence > 0.7 and z-score > 10 within cohort were selected. To take sample imbalance into account, a proportion test was performed to assess stability across cohorts. This resulted in 1321 EMPs. For diagnostic analyses, metagenomic markers were subsetted from the full dataset, resulting in 1171 EMPs. Metagenomic markers were further subsetted, resulting in 590 EMPs.

C. Metagenomic data

[00072] Raw metagenomic reads from Paramsothy et al., *Gastroenterology,* 156(5): 1440-1454.E2, 2019 were downloaded from the European Nucleotide Archive (ENA). A total of 284 metagenomes were available. The Paramsothy et al. study presents the results of an analysis of fecal matter samples in a double-blind trial of 81 patients having active ulcerative colitis (UC) who were treated with an initial colonoscopic infusion and then intensive multidonor fecal microbiota transplantation (FMT) or placebo enemas, 5 days per week for 8 weeks.

D. Modeling

[00073] EMP abundance data were binarized and filtered to include only 355 EMPs associated with inflammatory bowel disease (IBD) from an orthogonal analysis.

E. Visualization

[00074] Boxplots (Fig. 1) depict median, first and third quartiles, and largest/smallest value within 1.5* interquartile range (IQR). The dotted line represents the theoretical maximum number of metagenomic markers. Statistical test is a pairwise Wilcoxon test where ns indicates p > 0.05, * p <= 0.05, and **: p <= 0.01

F. Conclusions

[00075] A set of 156 metagenomic markers (SEQ ID NOs: 1-156) were found to be significant predictors in a model discriminating between IBD patients who were responders vs. non-responders to anti-IBD therapy (Fig. 1).

OTHER EMBODIMENTS

[00076] Various modifications and variations of the described invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are obvious to those skilled in the art are intended to be within the scope of the invention.

[00077] Other embodiments are in the claims.

CLAIMS

What is claimed is:

1. A method of monitoring the response of a subject to treatment with an anti-inflammatory bowel disease (anti-IBD) therapy, the method comprising (a) determining a level of one or more of SEQ ID NOs: 1-156 in a pre-treatment sample from the subject and (b) determining a level of one or more of SEQ ID NOs: 1-156 in an on-treatment or post-treatment sample from the subject, wherein a level of one or more of SEQ ID NOs: 1-156 that is changed in the on-treatment or post-treatment sample, as compared to the respective reference level in the pre-treatment sample, indicates that the subject is responding to treatment with the anti-IBD therapy.

2. A method for determining that a subject having an IBD is responding to treatment with an anti-IBD therapy, comprising the steps of:

(a) measuring the nucleic acid level of one or more of SEQ ID NOs: 1-156 in a pre-treatment sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156;

(b) treating the subject with an anti-IBD therapy;

(c) measuring the nucleic acid level of one or more of SEQ ID NOs: 1-156 in an on-treatment or post-treatment sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156; and

(d) using the amplification results to determine whether the level of one or more of SEQ ID NOs: 1-156 is changed in the on-treatment or post-treatment sample as compared to the respective reference level in the pre-treatment sample, thereby determining that the subject is responding to treatment with the anti-IBD therapy.

3. The method of claim 1 or 2, wherein a level of one or more of SEQ ID NOs: 1-156 is changed in the on-treatment or post-treatment sample as compared to the respective reference level in the pre-treatment sample, and the method further comprises continuing to administer the anti-IBD therapy to a subject.

4. A method of monitoring the response of a subject to treatment with an anti-IBD therapy, the method comprising (a) determining a level of one or more of SEQ ID NOs: 1-156 in a pre-treatment sample from the subject and (b) determining a level of one or more of SEQ ID NOs: 1-156 in an on-treatment or post-treatment sample from the subject, wherein a level of one or more

of SEQ ID NOs: 1-156 that not changed in the on-treatment or post-treatment sample, as compared to the respective reference level in the pre-treatment sample, indicates that the subject is not responding to treatment with the anti-IBD therapy.

5. A method for determining that a subject having an inflammatory bowel disease (IBD) is not responding to treatment with an anti-IBD therapy, comprising the steps of:

(a) measuring the nucleic acid level of one or more of SEQ ID NOs: 1-156 in a pre-treatment sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156;

(b) treating the subject with an anti-IBD therapy;

(c) measuring the nucleic acid level of one or more of SEQ ID NOs: 1-156 in an on-treatment or post-treatment sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156; and

(d) using the amplification results to determine whether the level of one or more of SEQ ID NOs: 1-156 is not changed in the on-treatment or post-treatment sample as compared to the respective reference level in the pre-treatment sample, thereby determining that the subject is not responding to treatment with the anti-IBD therapy.

6. The method of claim 4 or 5, wherein a level of one or more of SEQ ID NOs: 1-156 is not changed in the on-treatment or post-treatment sample as compared to the respective reference level in the pre-treatment sample, and the method further comprises discontinuing treatment of the subject with the anti-IBD therapy and/or administering an alternative anti-IBD therapy to the subject.

7. A method of identifying a subject who is likely to respond to treatment with an anti-IBD therapy, the method comprising determining a level of one or more of SEQ ID NOs: 1-156 in a sample from the subject, wherein a level of one or more of SEQ ID NOs: 1-156 that is changed relative to a respective reference level for SEQ ID NOs: 1-156 indicates that the subject is likely to benefit from an anti-IBD therapy.

8. A method for determining that a subject having an inflammatory bowel disease (IBD) is likely to respond to treatment with an anti-IBD therapy, comprising the steps of:

(a) measuring the nucleic acid level of one or more of SEQ ID NOs: 1-156 in a sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156; and

(b) using the amplification results to determine whether the level of one or more of SEQ ID NOs: 1-156 is changed relative to a respective reference level for SEQ ID NOs: 1-156, thereby determining that the subject is likely to respond to treatment with an anti-IBD therapy.

9. The method of claim 7 or 8, wherein a level of one or more of SEQ ID NOs: 1-156 is changed relative to the respective reference level for SEQ ID NO: 1-156 in the sample from the subject and the method further comprises administering an anti-IBD therapy to a subject.

10. A method of determining disease resolution in a subject with an IBD, the method comprising determining a level of one or more of SEQ ID NOs: 1-156 in a sample from the subject, wherein a level of one or more of SEQ ID NOs: 1-156 that is not substantially different from a respective reference level for SEQ ID NOs: 1-156 indicates that the subject has experienced resolution of the IBD.

11. A method for determining that disease resolution has occurred in a subject having an IBD, comprising the steps of:

(a) measuring the nucleic acid level of one or more of SEQ ID NOs: 1-156 in a sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156; and

(b) using the amplification results to determine whether the level of one or more of SEQ ID NOs: 1-156 is not substantially different from a respective reference level for SEQ ID NOs: 1-156, thereby determining that disease resolution has occurred in the subject.

12. The method of claim 10 or 11, wherein the subject is being treated with an anti-IBD therapy, a level of one or more of SEQ ID NOs: 1-156 is not substantially different from a respective reference level for SEQ ID NOs: 1-156 in the sample from the subject, and the method further comprises discontinuing treatment of the subject with the anti-IBD therapy.

13. The method of any one of claims 1-8, wherein the method comprises determining or measuring a level of at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least fifteen, at least 20, at least 30, at least 40,

at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, or at least 150 of SEQ ID NOs: 1-156 in the sample from the subject.

14. The method of claim 13, wherein the method comprises determining or measuring a level of all 156 of SEQ ID NOs: 1-156 in the sample from the subject.

15. The method of any one of claims 1-9 and 12-14, wherein the anti-IBD therapy comprises fecal microbiota transplantation (FMT).

16. The method of any one of claims 7-15, wherein the reference level is a pre-assigned level.

17. The method of any one of claims 7-16, wherein the reference level is a level in a set of samples from a reference population.

18. The method of claim 17, wherein the reference population is a population of healthy subjects.

19. The method of any one of claims 1-18, wherein the level of each of SEQ ID NOs: 1-156 that is determined or measured in the sample from the subject is a nucleic acid level.

20. The method of claim 19, wherein the nucleic acid level is a DNA level.

21. The method of any one of claims 1-20, wherein the change is a decrease relative to the reference level.

22. The method of claim 21, wherein the levels of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more than 90% of the sequences for which a level is determined or measured are decreased relative to a respective reference level for the sequence.

23. The method of any one of claims 1-20, wherein the change is an increase relative to the reference level.

24. The method of claim 23, wherein the levels of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more than 90% of the sequences for which a level is determined or measured are increased relative to a respective reference level for the sequence.

25. The method of any one of claims 1-24, wherein the sample comprises a sample of the microbiota of the subject.

26. The method of any one of claims 1-25, wherein the sample is a fecal sample.

27. A kit for monitoring the response of a subject to treatment with an anti-IBD therapy, the kit comprising:

(a) polypeptides or polynucleotides capable of determining the level of one or more of SEQ ID NOs: 1-156 in a sample from the subject; and optionally

(b) instructions for use of the polypeptides or polynucleotides to determine the level of one or more of SEQ ID NOs: 1-156 in a pre-treatment, on-treatment, and/or post-treatment sample from the subject, wherein a level of one or more of SEQ ID NOs: 1-156 that is changed in the on-treatment or post-treatment sample, as compared to the respective reference level in the pre-treatment sample, indicates that the subject is responding to treatment with the anti-IBD therapy.

28. A method of monitoring the response of a subject to treatment with an anti-IBD therapy, the method comprising (a) determining a level of one or more of SEQ ID NOs: 1-156 in a pre-treatment sample from the subject and (b) determining a level of one or more of SEQ ID NOs: 1-156 in an on-treatment or post-treatment sample from the subject, wherein a level of at least 50% of SEQ ID NOs: 1-156 that is changed in the on-treatment or post-treatment sample, as compared to the respective reference level in the pre-treatment sample, indicates that the subject is responding to treatment with the anti-IBD therapy.

29. The method of claim 28, wherein a level of at least 50% of SEQ ID NOs: 1-156 is changed relative to a respective reference level for SEQ ID NOs: 1-156 in the sample from the subject and the method further comprises continuing to administer the anti-IBD therapy to a subject.

30. The method of claim 28 or 29, wherein a level of at least 60%, 70%, 80%, 90%, or 95% of SEQ ID NOs: 1-318 that is changed in a sample from the subject relative to a respective reference level for SEQ ID NOs: 1-156 and the method further comprises continuing to administer the anti-IBD therapy to a subject.

31. A method of treating a subject having an IBD using a therapy appropriate for treating the IBD, wherein the subject was monitored for their response to the therapy by a method of any one of claims 1, 3, 4, 6, 13-26, and 28-30.

32. A method of treating a subject having an IBD using a therapy appropriate for treating the IBD, wherein the subject was determined to be responsive to prior treatment with the therapy by a method of any one of claims 2, 3, and 13-26.

33. A method of treating a subject having an IBD using a therapy appropriate for treating the IBD, wherein the subject was determined to be likely to respond to the treatment with the therapy by a method of any one of claims 8, 9, and 13-26.

34. A method of treating a subject having an IBD, the method comprising the steps of:

(a) measuring the nucleic acid level of one or more of SEQ ID NOs: 1-156 in a pre-treatment sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156;

(b) administering an anti-IBD therapy to a subject;

(c) measuring the nucleic acid level of one or more of SEQ ID NOs: 1-156 in an on-treatment or post-treatment sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156, wherein a level of one or more of SEQ ID NOs: 1-156 that is changed in the on-treatment or post-treatment sample as compared to the respective reference level in the pre-treatment sample indicates the subject is responding to treatment with the anti-IBD therapy; and

(d) continuing to administer the anti-IBD therapy to a subject who has been determined to be responding to treatment with the anti-IBD therapy.

35. The method of claim 34, wherein the nucleic acid level of the one or more of SEQ ID NOs: 1-156 in the sample collected from the subject are measured using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156, and the

amplification results are used to determine whether the level of one or more of SEQ ID NOs: 1-156 is changed in the on-treatment or post-treatment sample as compared to the respective reference level in the pre-treatment sample.

36. The method of claim 34 or 35, wherein the method comprises measuring the nucleic acid level of at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least fifteen, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, or at least 150 of SEQ ID NOs: 1-156 in the sample from the subject.

37. The method of claim 36, wherein the method comprises measuring the nucleic acid level of all 156 of SEQ ID NOs: 1-156 in the sample from the subject.

38. The method of any one of claims 34-37, wherein the nucleic acid level is a DNA level.

39. The method of any one of claims 34-38, wherein the change is a decrease relative to the reference level.

40. The method of claim 39, wherein the levels of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more than 90% of the sequences for which a level is measured are decreased relative to a respective reference level for the sequence.

41. The method of any one of claims 34-38, wherein the change is an increase relative to the reference level.

42. The method of claim 41, wherein the levels of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more than 90% of the sequences for which a level is measured are increased relative to a respective reference level for the sequence.

43. The method of any one of claims 34-42, wherein the sample comprises a sample of the microbiota of the subject.

44. The method of any one of claims 34-43, wherein the sample is a fecal sample.

45. A method of treating a subject having an IBD, the method comprising the steps of:

(a) measuring the nucleic acid level of each of SEQ ID NOs: 1-156 in a pre-treatment sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156;

(b) administering an anti-IBD therapy to a subject;

(c) measuring the nucleic acid level of each of SEQ ID NOs: 1-156 in an on-treatment or post-treatment sample collected from the subject using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156, wherein a level of at least 50% of SEQ ID NOs: 1-156 that is changed in the on-treatment or post-treatment sample as compared to the respective reference level in the pre-treatment sample indicates the subject is responding to treatment with the anti-IBD therapy; and

(d) continuing to administer the anti-IBD therapy to a subject who has been determined to be responding to treatment with the anti-IBD therapy.

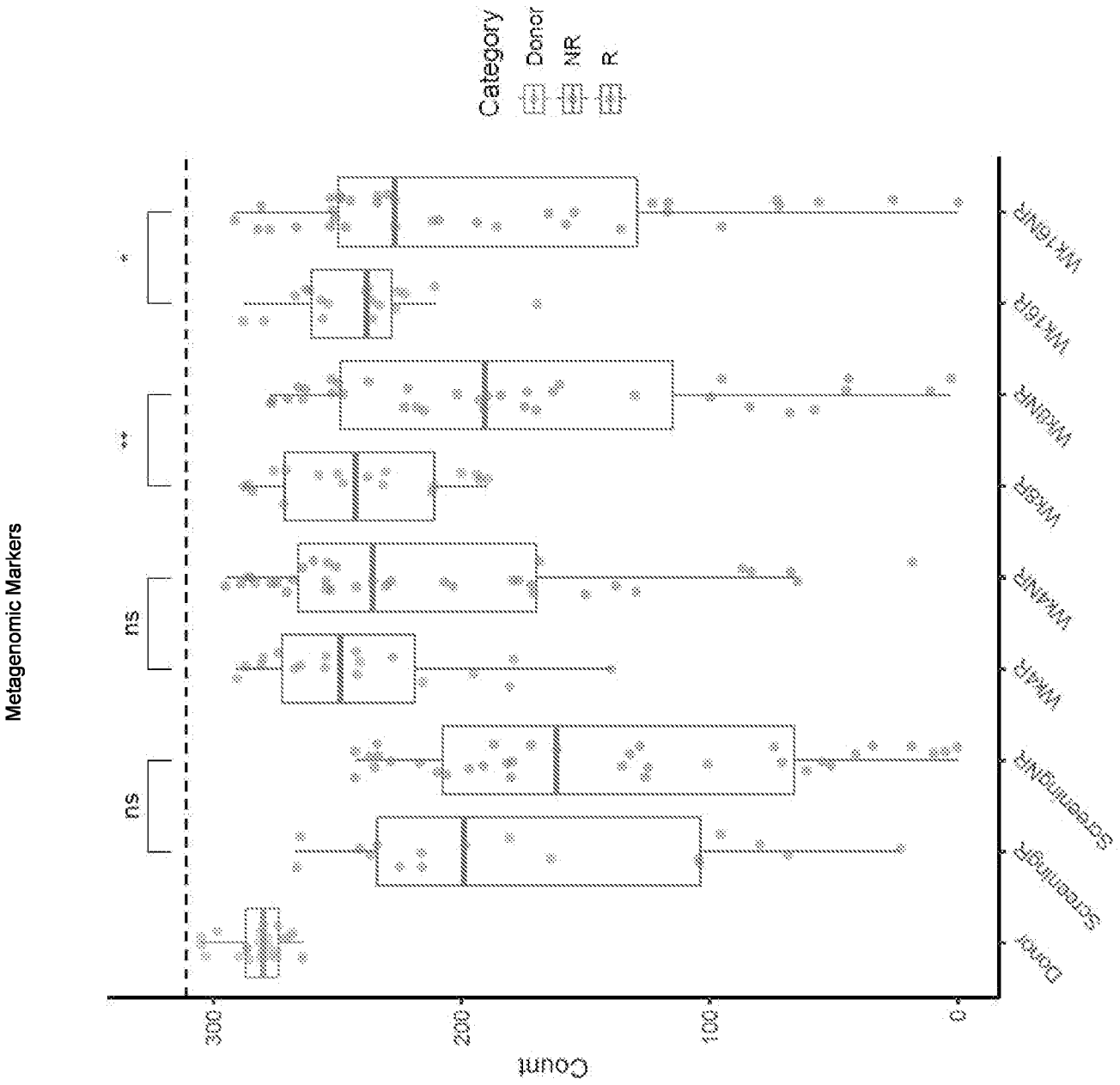
46. The method of claim 45, wherein the nucleic acid level of each of SEQ ID NOs: 1-156 in the sample collected from the subject are measured using amplification and one or more pairs of primers specific for each of the one or more of SEQ ID NOs: 1-156, and the amplification results are used to determine whether the level of each of SEQ ID NOs: 1-156 is changed in the on-treatment or post-treatment sample as compared to the respective reference level in the pre-treatment sample.

47. The method of any one of claims 1 to 26 and 28 to 46, wherein the anti-IBD therapy to which a response is being determined or monitored, or that is administered after the determining or monitoring, comprises a biologic therapy, an anti-inflammatory agent, an antibiotic, an immune system suppressor, or an anti-integrin therapy that optionally targets integrin $\alpha_4\beta_7$.

48. The method of claim 47, wherein the biologic therapy is vedolizumab (ENTYVIO®), infliximab (REMICADE®), adalimumab (HUMIRA®), golimumab (SIMPONI®), certolizumab (CIMZIA®), risankizumab (SKYRIZI®), or ustekinumab (STELARA®); the anti-inflammatory agent is a corticosteroid or an aminosalicylate; the antibiotic is ciprofloxacin (cipro) or

metronidazole (Flagyl); or the immune system suppressor is azathioprine (AZASAN®, IMURAN®), mercaptopurine (PURINETHOL®, PURIXAN®), methotrexate (TREXALL®), tofacitinib (XELJANZ®), upadacitinib (RINVOQ®), or ozanimod (ZEPOSIA®).

49. The method of 47 or 48, wherein the anti-IBD therapy further comprises treatment by fecal microbiota transplant (FMT).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MetaPhlAnNR
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Metagenomic Markers

FIG. 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2022/076928

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/6883
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2013/045874 A1 (EHRLICH STANISLAV [FR]) 21 February 2013 (2013-02-21) tables 1-2 abstract the whole document -----	1-49
X	KELLY A. SHAW ET AL: "Dysbiosis, inflammation, and response to treatment: a longitudinal study of pediatric subjects with newly diagnosed inflammatory bowel disease", GENOME MEDICINE, vol. 8, no. 1, 13 July 2016 (2016-07-13), XP055434103, DOI: 10.1186/s13073-016-0331-y figures 3-4 abstract the whole document -----	1-49

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[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 January 2023	07/03/2023

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Helliot, Bertrand

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2022/076928

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GAVINM DOUGLAS ET AL: "Multi-omics differentially classify disease state and treatment outcome in pediatric Crohn's disease", MICROBIOME, BIOMED CENTRAL LTD, LONDON, UK, vol. 6, no. 1, 15 January 2018 (2018-01-15), pages 1-12, XP021252578, DOI: 10.1186/S40168-018-0398-3 figure 3 abstract the whole document	1-49
X	WO 2021/119358 A1 (UNIV WASHINGTON [US]) 17 June 2021 (2021-06-17) claim 17 abstract the whole document	1-49
X	US 2018/148770 A1 (ELINAV ERAN [IL] ET AL) 31 May 2018 (2018-05-31) claim 2 paragraphs [0009] - [0012] abstract the whole document	1-49

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2022/076928

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2022/076928

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims;; it is covered by claims Nos.:

1-49(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-49(partially)

The subject-matter of claims 1-49, wherein the microbial marker is SEQ ID N° 1.

2-156. claims: 1-49(partially)

The subject-matter of claims 1-49, wherein the microbial marker is selected among SEQ ID N° 2-156.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2022/076928

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		CN 102939391 A	20-02-2013
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