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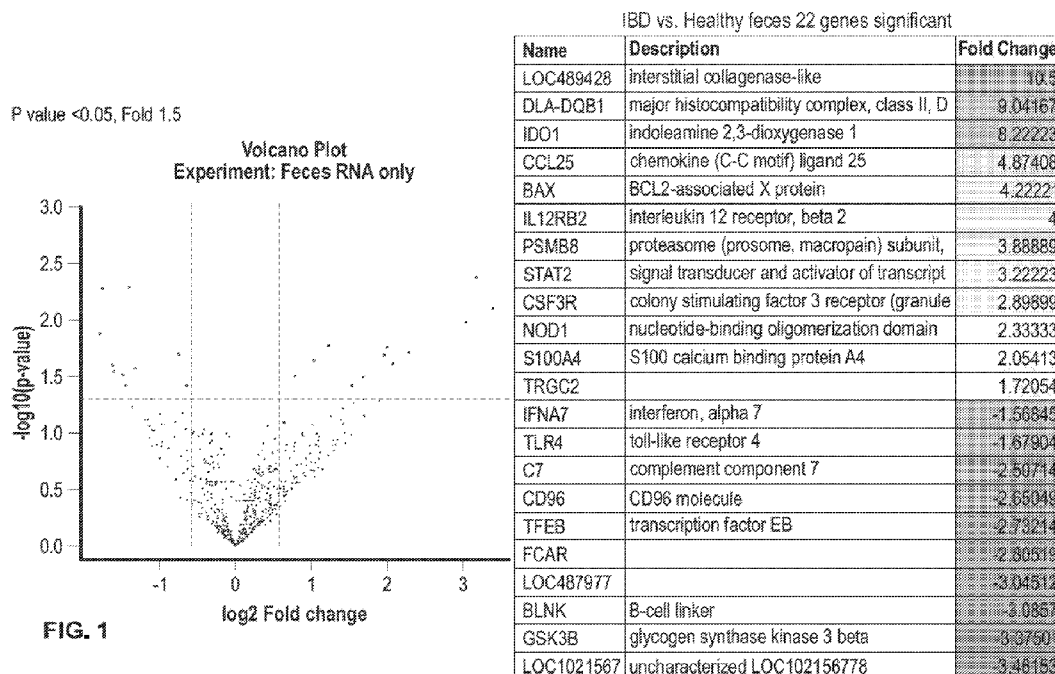
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(54) Title: NON-INVASIVE DIRECT TRANSCRIPTOMIC PROFILING USING STOOL SAMPLES FOR DIAGNOSIS OF GASTROINTESTINAL DISEASES



(57) Abstract: Inflammatory bowel disease (IBD) in humans and dogs and other companion animals is characterized by infiltration of lymphocytes and macrophages into the mucosa and submucosa and clinical signs of gastrointestinal (GI) dysfunction (diarrhea, malabsorption, weight loss). Alteration of the gut environment and development of dysbiosis may allow the overgrowth of pathogenic bacteria and induction of intestinal injury and inflammation in IBD. What is needed are novel methods that allow for rapid diagnosis of IBD (and follow-up monitoring) and treatment of the disease. The present disclosure relates to methods for diagnosing and treating inflammatory bowel disease (IBD) or gastrointestinal lymphoma.

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NON-INVASIVE DIRECT TRANSCRIPTOMIC PROFILING USING STOOL SAMPLES FOR DIAGNOSIS OF GASTROINTESTINAL DISEASES

5 CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 63/274,380, filed November 1, 2021, and U.S. Provisional Application No. 63/290,849, filed December 17, 2021, which are expressly incorporated herein by reference in their entireties.

10 FIELD

The present disclosure relates to methods for diagnosing and treating gastrointestinal disorders.

BACKGROUND

15 Inflammatory bowel disease (IBD) in humans and dogs and other companion animals is characterized by infiltration of lymphocytes and macrophages into the mucosa and submucosa and clinical signs of gastrointestinal (GI) dysfunction (diarrhea, malabsorption, weight loss). Alteration of the gut environment and development of dysbiosis may allow the overgrowth of pathogenic bacteria and induction of intestinal injury and inflammation in IBD. Genetic and
20 environmental factors are also associated with development of IBD in dogs and humans, independent of the role of the intestinal microbiome. Currently the diagnosis of IBD in humans and veterinary species is complicated, time consuming, costly, and often involves the use of endoscopy or colonoscopy procedures, all of which increases cost and risks. What is needed are novel methods that allow for rapid diagnosis of IBD (and follow-up monitoring) and treatment of
25 the disease. Said methods, which would not require invasive GI biopsy procedures, represent an important advance in the diagnosis of IBD in humans and veterinary species (companion animals). The methods disclosed herein address these and other needs.

SUMMARY

30 In some aspects, disclosed herein are method of measuring a level of a coding or non-coding nucleic acid (such as mRNA, microRNA, or DNA) in a stool sample, comprising:

- a) obtaining a stool sample;
- b) extracting the RNA (or DNA) from the stool sample; and
- c) measuring the level of the coding or non-coding RNA or DNA.

5 In some embodiments, the stool sample of step a) is placed or stored in an RNA preservation solution.

In some embodiments, step b) further comprises:

- adding the stool sample in a tube that comprises a lysis buffer, wherein the lysis buffer comprises β -mercaptoethanol;
- mixing the stool sample with the lysis buffer; and
- 10 centrifuging the mixed sample thereby obtaining a first supernatant portion.

In some embodiments, the method of any preceding aspect further comprises adding a solution comprising phenol, chloroform, and isoamyl alcohol into the tube prior to adding the stool sample. In some embodiments, the method of any preceding aspect further comprises adding a solution comprising phenol into the tube prior to adding the stool sample.

15 In some embodiments, the method of any preceding aspect further comprises:

- transferring the first supernatant portion into a tube;
- mixing the first supernatant portion with an inhibitor removal solution in the tube;
- and
- centrifuging the mixed sample thereby obtaining a second supernatant portion.

20 In some embodiments, the method of any preceding aspect further comprises

- transferring the second supernatant portion into a tube;
- mixing the second supernatant portion with a solution comprising binding salts and ethanol; and
- passing the mixture through a binding membrane thereby binding the RNA (or
- 25 DNA) onto the membrane.

In some embodiments, the method of any preceding aspect further comprises adding a DNase to the binding membrane. It should be understood and herein contemplated that the step of adding the DNase is to remove contaminating DNA, while in other applications the DNA would not be removed, and would be the primary analyte instead. In some embodiments, the method of

30 any preceding aspect further comprises washing the binding membrane with a washing buffer

comprising isopropanol or ethanol. In some embodiments, the method of any preceding aspect further comprises adding water to the binding membrane thereby eluting the RNA in the water.

In some embodiments, the method of any preceding aspect comprises measuring the level of the RNA with an RNA sequencing assay, a hybridization-based array, or a polymerase chain reaction (PCR)-based assay (e.g., reverse transcription PCR (RT-PCR) or real-time RT PCR). In some embodiments, the RNA is measured by NanoString analysis, real-time PCR, or Illumina or other high-throughput next generation sequencing analysis.

In some embodiments, the level of the RNA is measured by one or more primers or probes specific for RNAs encoded by genes selected from the group consisting of LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, IFGGC1, HLA-DRB1, MAPK8, STAT2, ITGB1, CSF3R, CEBPB, IDH1, PRDM1, CASP10, NOD1, S100A4, CASP8, TRGC2, LOC484343, CLEC4A, CD22, TNKS, IL21R, CCL17, IFNA7, SLAMF6, IL17F, TLR4, PTGER4, SELL, INPPL1, TANK, SH2D1A, OLIG2, LOC1021559, IRF3, GATA3, MAPK14, LOC481722, C7, CCR2, CD96, TFEB, FCAR, LOC487977, BLNK, GSK3B, and LOC1021567.

In some embodiments, the level of the RNA is measured by one or more primers or probes specific for RNAs encoded by genes selected from the group consisting of LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, STAT2, CSF3R, NOD1, S100A4, and TRGC2.

In some embodiments, the level of the RNA is measured by one or more primers or probes specific for RNAs encoded by genes selected from the group consisting of IFNA7, TLR4, C7, CD96, TFEB, FCAR, LOC487977, BLNK, GSK38, and LOC1021567.

In some embodiments, the level of the RNA is measured by one or more primers or probes specific for RNAs encoded by genes selected from the group consisting of TNFRSF13C, CXCL10, SHMT2, CX3CL1, PPARGC1A, DMBT1, FYN, MAVS, CD163, CCL19, SIGIRR, CD84, TXNIP, TXNIP, PRKCD, IL17F, NRAS, C7, C5, CFD, CSF3, LOC483397, and MAPK1.

In some embodiments, the level of the RNA is measured by one or more primers or probes specific for RNAs encoded by genes selected from the group consisting of MSR1, ARG1, IL11RA, IL2RG, CTSS, RUNX1, CCR1, ADA, TIGIT, MR1, IL27, CRADD, PSMB8, and HLA-DRB3.

In some embodiments, the level of the RNA is measured by one or more primers or probes specific for RNAs encoded by genes selected from the group consisting of S100A8, IDO1, CXCL8, FOS, IL1RN, EGR1, CEBPB, EPCAM, S100A9, PLAUR, S100A12, DUSP1, CD74, NFKBIA, LCP1, C1QBP, TNFAIP3, DLA-DRA, DDX5, LGALS3, CCND2, MAP2K2, MEF2C,

PSMB9, IFNA7, ANXA1, PTEN, MAPK14, MME, C6, LYN, STAT3, CIITA, MAPK8, TRB1, ITGAV, ERBB2, NFKB2, FCER1G, HMGCR, RPS6, HBEGF, NDRG1, STAT2, APP, VIM, MAP2K1, CD28, and GPI.

5 In some embodiments, the level of the RNA is measured by one or more primers or probes specific for RNAs encoded by genes selected from the group consisting of MMP1, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, STAT2, CSF3R, NOD1, S100A4, TRGC2, IFNA7, TLR4, C7, CD96, TFEB, FCAR, LOC487977, BLNK, GSK3B, and LOC102156778.

10 In some embodiments, the level of the RNA is measured by a device. In some embodiments, the device is a PCR machine, a hybridization-based array, or a high-throughput sequencing machine. In some embodiments, the device is a NanoString analysis system.

Also disclosed herein is a system comprising

a collection tube;

a preservation solution for a stool sample; and

15 a set of polynucleotides for measuring levels of target RNAs.

In some embodiments, the system further comprises a device for measuring the levels of target RNAs. In some embodiments, the device is a PCR machine, a hybridization-based array, or a high-throughput sequencing machine. In some embodiments, the system further comprises a NanoString analysis system.

20

Also disclosed herein are methods for diagnosing, treating, and/or monitoring inflammatory bowel disease (IBD) and other GI disorders, including GI cancers, in humans and companion animals. The invention allows diagnosis, treatment, and/or monitoring of inflammatory bowel disease (IBD) and other GI disorders, including GI cancers, to be made by direct analysis
25 of key immune gene expression using a biological sample (e.g., a fecal sample).

In some aspects, the methods, systems, or platforms of any preceding aspect can be used for diagnosing, treating, and/or monitoring a GI disorder. In some aspects, the methods, systems, or platforms of any preceding aspect can be used for noninvasively monitoring responses to a
30 treatment. In some aspects, the methods, systems, or platforms of any preceding aspect can be used for noninvasively monitoring a GI disorder or monitoring responses to a treatment. The original

diagnosis can be made initially through some other approach, such as biopsy. In some embodiments, the GI disorder comprises an enteric infection, GI bacterial overgrowth, GI cancer, inflammatory bowel disease (IBD), or a non-inflammatory GI disease.

In some aspects, disclosed herein is a method for treating inflammatory bowel disease (IBD) in a human or an animal comprising:

obtaining a biological sample from the human or animal;

quantifying an expression level of one or more genes relative to a reference control,

wherein the one or more genes are selected from the group consisting of LOC489428,

DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, IFGGC1, HLA-DRB1,

MAPK8, STAT2, ITGB1, CSF3R, CEBPB, IDH1, PRDM1, CASP10, NOD1,

S100A4, CASP8, TRGC2, LOC484343, CLEC4A, CD22, TNKS, IL21R, CCL17,

IFNA7, SLAMF6, IL17F, TLR4, PTGER4, SELL, INPPL1, TANK, SH2D1A, OLIG2,

LOC1021559, IRF3, GATA3, MAPK14, LOC481722, C7, CCR2, CD96, TFEB,

FCAR, LOC487977, BLNK, GSK3B, and LOC1021567; and

determining that the human or animal is susceptible to or suffering from IBD if the

expression level of one or more of LOC489428, DLA-DQB1, IDO1, CCL25, BAX,

IL12RB2, PSMB8, IFGGC1, HLA-DRB1, MAPK8, STAT2, ITGB1, CSF3R, CEBPB,

IDH1, PRDM1, CASP10, NOD1, S100A4, CASP8, TRGC2, LOC484343, CLEC4A,

and CD22 in the biological sample is higher in comparison to the reference control

and/or if the expression level of one or more of TNKS, IL21R, CCL17, IFNA7,

SLAMF6, IL17F, TLR4, PTGER4, SELL, INPPL1, TANK, SH2D1A, OLIG2,

LOC1021559, IRF3, GATA3, MAPK14, LOC481722, C7, CCR2, CD96, TFEB,

FCAR, LOC487977, BLNK, GSK3B, and LOC1021567 in the biological sample is

lower in comparison to the reference control; and

and if the human or animal is susceptible to or suffering from IBD: i) administering to the human or animal an effective amount of a therapeutic agent for treating the IBD (e.g., treatments for Crohn's disease or ulcerative colitis), ii) changing the diet of the human or animal, and/or iii) performing a fecal transfaunation in the human or animal.

In some embodiments, the one or more genes are selected from the group consisting of LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, STAT2, CSF3R, NOD1,

S100A4, and TRGC2. In some embodiments, the one or more genes are selected from the group consisting of IFNA7, TLR4, C7, CD96, TFEB, FCAR, LOC487977, BLNK, GSK38, and LOC1021567.

5 In some embodiments, the sample is from a human. In some embodiments, the sample is from a companion animal. In other embodiments, the companion animal is a canine. In some embodiments, the companion animal is a dog. In some embodiments, the companion animal is a feline. In some embodiments, the companion animal is a cat. In other embodiments, the companion animal is a horse.

10 In some embodiments, the therapeutic agent for treatment of IBD in humans or the animal is selected from a non-absorbable NSAID, an immunosuppressive drug (e.g., methotrexate, budesonide, or prednisone), a biological agent (e.g., anti-TNF α antibody or anti-integrin antibodies), or a Janus kinase inhibitor (e.g., Xeljanz), a probiotic, or an antibiotic.

In some embodiments, the treatment comprises a non-absorbable NSAID (e.g., 5-aminosalicylates).

15 In some embodiments, the treatment comprises therapeutic monoclonal antibodies (e.g., Humira, Entivyo, or Stelara).

In some embodiments, the treatment comprises Janus kinase inhibitors (e.g., Xeljanz).

In some embodiments, the treatment comprises immunomodulators (e.g., methotrexate, or thiopurines).

20 In some embodiments, the treatment comprises immune suppressive drugs (e.g., budesonide, or prednisone).

In some embodiments, the treatment comprises antibiotics (metronidazole or ampicillin).

25 In some embodiments, the biological sample is a fecal sample, a sputum sample, an oral swab sample, a vaginal swab sample, a urethral swab sample, a nasal swab sample, an ocular swab sample, an aural swab sample, or a skin swab sample. In some embodiments, the biological sample is a fecal sample.

In some aspects, disclosed herein is a method for diagnosing or monitoring inflammatory bowel disease (IBD) in a human or an animal comprising:

30 obtaining a biological sample from the human or animal;

quantifying an expression level of one or more genes relative to a reference control, wherein the one or more genes are selected from the group consisting of LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, IFGGC1, HLA-DRB1, MAPK8, STAT2, ITGB1, CSF3R, CEBPB, IDH1, PRDM1, CASP10, NOD1, S100A4, CASP8, TRGC2, LOC484343, CLEC4A, CD22, TNKS, IL21R, CCL17, IFNA7, SLAMF6, IL17F, TLR4, PTGER4, SELL, INPPL1, TANK, SH2D1A, OLIG2, LOC1021559, IRF3, GATA3, MAPK14, LOC481722, C7, CCR2, CD96, TFEB, FCAR, LOC487977, BLNK, GSK3B, and LOC1021567; and

determining that the human or animal is susceptible to or suffering from IBD if the expression level of one or more of LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, IFGGC1, HLA-DRB1, MAPK8, STAT2, ITGB1, CSF3R, CEBPB, IDH1, PRDM1, CASP10, NOD1, S100A4, CASP8, TRGC2, LOC484343, CLEC4A, and CD22 in the biological sample is higher in comparison to the reference control and/or if the expression level of one or more of TNKS, IL21R, CCL17, IFNA7, SLAMF6, IL17F, TLR4, PTGER4, SELL, INPPL1, TANK, SH2D1A, OLIG2, LOC1021559, IRF3, GATA3, MAPK14, LOC481722, C7, CCR2, CD96, TFEB, FCAR, LOC487977, BLNK, GSK3B, and LOC1021567 in the biological sample is lower in comparison to the reference control.

In some embodiments, the method further comprises administering to the human or animal companion animal an effective amount of a therapeutic agent for treating the IBD, changing the diet of the human or animal, or performing a fecal transfaunation, if the human or animal is susceptible to or suffering from IBD.

In some aspects, disclosed herein is a method for monitoring response to a treatment for inflammatory bowel disease (IBD) in a human or an animal comprising:

obtaining a biological sample from the human or animal, wherein the human or animal has received a treatment for IBD;

quantifying an expression level of one or more genes relative to a reference control, wherein the one or more genes are selected from the group consisting of LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, IFGGC1, HLA-DRB1, MAPK8, STAT2, ITGB1, CSF3R, CEBPB, IDH1, PRDM1, CASP10, NOD1,

S100A4, CASP8, TRGC2, LOC484343, CLEC4A, CD22, TNKS, IL21R, CCL17, IFNA7, SLAMF6, IL17F, TLR4, PTGER4, SELL, INPPL1, TANK, SH2D1A, OLIG2, LOC1021559, IRF3, GATA3, MAPK14, LOC481722, C7, CCR2, CD96, TFEB, FCAR, LOC487977, BLNK, GSK3B, and LOC1021567; and

determining that the human or animal is responsive to the treatment if the expression level of one or more of LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, IFGGC1, HLA-DRB1, MAPK8, STAT2, ITGB1, CSF3R, CEBPB, IDH1, PRDM1, CASP10, NOD1, S100A4, CASP8, TRGC2, LOC484343, CLEC4A, and CD22 in the biological sample is lower in comparison to the reference control and/or if the expression level of one or more of TNKS, IL21R, CCL17, IFNA7, SLAMF6, IL17F, TLR4, PTGER4, SELL, INPPL1, TANK, SH2D1A, OLIG2, LOC1021559, IRF3, GATA3, MAPK14, LOC481722, C7, CCR2, CD96, TFEB, FCAR, LOC487977, BLNK, GSK3B, and LOC1021567 in the biological sample is higher in comparison to the reference control.

Also disclosed herein is a method for treating a gastrointestinal disorder in a human or an animal, comprising:

obtaining a stool sample from the human or animal;

quantifying an expression level of one or more genes relative to a reference control,

wherein the one or more genes are selected from the group consisting of MSR1, ARG1, IL11RA, IL2RG, CTSS, RUNX1, CCR1, ADA, TIGIT, MR1, IL27, CRADD, PSMB8, and HLA-DRB3;

determining that the human or animal is susceptible to or suffering from the gastrointestinal disorder if the expression level of one or more genes selected from MSR1 and ARG1 in the biological sample is higher in comparison to the reference control and/or if the expression level of one or more genes selected from IL11RA, IL2RG, CTSS, RUNX1, CCR1, ADA, TIGIT, MR1, IL27, CRADD, PSMB8, and HLA-DRB3 in the biological sample is lower in comparison to the reference control; and

if the human or animal is susceptible to or suffering from the gastrointestinal disorder:

i) administering to the human or animal an effective amount of a therapeutic agent for treating the gastrointestinal disorder, and/or

- ii) changing the diet of the human or animal.

Also disclosed herein is a method for diagnosing or monitoring a gastrointestinal disorder in a human or animal, comprising:

- 5 obtaining a stool sample from the human or animal;
quantifying an expression level of one or more genes relative to a reference control,
wherein the one or more genes are selected from the group consisting of MSR1, ARG1,
IL11RA, IL2RG, CTSS, RUNX1, CCR1, ADA, TIGIT, MR1, IL27, CRADD, PSMB8,
and HLA-DRB3; and
10 determining that the human or animal is susceptible to or suffering from the gastrointestinal
disorder if the expression level of one or more genes selected from MSR1 and ARG1
in the biological sample is higher in comparison to the reference control and/or if the
expression level of one or more genes selected from IL11RA, IL2RG, CTSS, RUNX1,
CCR1, ADA, TIGIT, MR1, IL27, CRADD, PSMB8, and HLA-DRB3 in the biological
15 sample is lower in comparison to the reference control.

In some embodiments, the gastrointestinal disorder is IBD, GI cancer, a GI infection, or a non-inflammatory GI disease.

- In some aspects, disclosed herein is a method for monitoring response to a treatment for a
20 gastrointestinal disorder in a human or animal, comprising:
obtaining a stool sample from the human or animal, wherein the human or animal has
received a treatment for the gastrointestinal disorder;
quantifying an expression level of one or more genes relative to a reference control,
wherein the one or more genes are selected from the group consisting of MSR1, ARG1,
25 IL11RA, IL2RG, CTSS, RUNX1, CCR1, ADA, TIGIT, MR1, IL27, CRADD, PSMB8,
and HLA-DRB3; and
determining that the human or animal is responsive to the treatment if the expression level
of one or more genes selected from MSR1 and ARG1 in the biological sample is higher
in comparison to the reference control and/or if the expression level of one or more
30 genes selected from IL11RA, IL2RG, CTSS, RUNX1, CCR1, ADA, TIGIT, MR1,

IL27, CRADD, PSMB8, and HLA-DRB3 in the biological sample is lower in comparison to the reference control.

Also disclosed herein is a method for treating a GI cancer (e.g., gastrointestinal lymphoma)

5 in a human or an animal, comprising:

obtaining a stool sample from the human patient or the animal;

quantifying an expression level of one or more genes relative to a reference control,

wherein the one or more genes are selected from the group consisting of S100A8,

IDO1, CXCL8, FOS, IL1RN, EGR1, CEBPB, EPCAM, S100A9, PLAUR, S100A12,

10 DUSP1, CD74, NFKBIA, LCP1, C1QBP, TNFAIP3, DLA-DRA, DDX5, LGALS3,

CCND2, MAP2K2, MEF2C, PSMB9, IFNA7, ANXA1, PTEN, MAPK14, MME, C6,

LYN, STAT3, CIITA, MAPK8, TRB1, ITGAV, ERBB2, NFKB2, FCER1G,

HMGCR, RPS6, HBEGF, NDRG1, STAT2, APP, VIM, MAP2K1, CD28, and GPI;

determining that the human or animal is susceptible to or suffering from the GI cancer (e.g.,

15 gastrointestinal lymphoma) if the expression level of one or more genes selected from

S100A8, IDO1, CXCL8, FOS, IL1RN, EGR1, CEBPB, S100A9, PLAUR, S100A12,

DUSP1, CD74, NFKBIA, LCP1, TNFAIP3, DLA-DRA, DDX5, LGALS3, CCND2,

MAP2K2, MEF2C, PSMB9, ANXA1, PTEN, MAPK14, MME, LYN, STAT3, CIITA,

MAPK8, TRB1, ITGAV, ERBB2, NFKB2, FCER1G, HMGCR, RPS6, HBEGF,

20 NDRG1, STAT2, APP, VIM, MAP2K1, and CD28 in the biological sample is higher

in comparison to the reference control and/or if the expression level of one or more

genes selected from EPCAM, C1QBP, IFNA7, C6, and GPI in the biological sample is

lower in comparison to the reference control; and

administering to the human or animal an effective amount of a therapeutic agent for treating

25 the GI cancer (e.g., gastrointestinal lymphoma) if the human or animal is susceptible

to or suffering from the GI cancer (e.g., gastrointestinal lymphoma).

Also disclosed herein is a method for diagnosing or monitoring a GI cancer (e.g.,
gastrointestinal lymphoma) in a human or animal, comprising:

30 obtaining a stool sample from the human or animal;

quantifying an expression level of one or more genes relative to a reference control, wherein the one or more genes are selected from the group consisting of S100A8, IDO1, CXCL8, FOS, IL1RN, EGR1, CEBPB, EPCAM, S100A9, PLAUR, S100A12, DUSP1, CD74, NFKBIA, LCP1, C1QBP, TNFAIP3, DLA-DRA, DDX5, LGALS3, CCND2, MAP2K2, MEF2C, PSMB9, IFNA7, ANXA1, PTEN, MAPK14, MME, C6, LYN, STAT3, CIITA, MAPK8, TRB1, ITGAV, ERBB2, NFKB2, FCER1G, HMGCR, RPS6, HBEGF, NDRG1, STAT2, APP, VIM, MAP2K1, CD28, and GPI; and

determining that the human or animal is susceptible to or suffering from the GI cancer (e.g., gastrointestinal lymphoma) if the expression level of one or more genes selected from S100A8, IDO1, CXCL8, FOS, IL1RN, EGR1, CEBPB, S100A9, PLAUR, S100A12, DUSP1, CD74, NFKBIA, LCP1, TNFAIP3, DLA-DRA, DDX5, LGALS3, CCND2, MAP2K2, MEF2C, PSMB9, ANXA1, PTEN, MAPK14, MME, LYN, STAT3, CIITA, MAPK8, TRB1, ITGAV, ERBB2, NFKB2, FCER1G, HMGCR, RPS6, HBEGF, NDRG1, STAT2, APP, VIM, MAP2K1, and CD28 in the biological sample is higher in comparison to the reference control and/or if the expression level of one or more genes selected from EPCAM, C1QBP, IFNA7, C6, and GPI in the biological sample is lower in comparison to the reference control.

Also disclosed herein is a method for monitoring response to a treatment for a GI cancer (e.g., gastrointestinal lymphoma) in a human or animal, comprising:

obtaining a stool sample from the human or animal;

quantifying an expression level of one or more genes relative to a reference control, wherein the one or more genes are selected from the group consisting of S100A8, IDO1, CXCL8, FOS, IL1RN, EGR1, CEBPB, EPCAM, S100A9, PLAUR, S100A12, DUSP1, CD74, NFKBIA, LCP1, C1QBP, TNFAIP3, DLA-DRA, DDX5, LGALS3, CCND2, MAP2K2, MEF2C, PSMB9, IFNA7, ANXA1, PTEN, MAPK14, MME, C6, LYN, STAT3, CIITA, MAPK8, TRB1, ITGAV, ERBB2, NFKB2, FCER1G, HMGCR, RPS6, HBEGF, NDRG1, STAT2, APP, VIM, MAP2K1, CD28, and GPI; and

determining that the human or animal is responsive to the treatment if the expression level of one or more genes selected from S100A8, IDO1, CXCL8, FOS, IL1RN, EGR1, CEBPB, S100A9, PLAUR, S100A12, DUSP1, CD74, NFKBIA, LCP1, TNFAIP3, DLA-DRA, DDX5, LGALS3, CCND2, MAP2K2, MEF2C, PSMB9, ANXA1, PTEN, MAPK14, MME, LYN, STAT3, CIITA, MAPK8, TRB1, ITGAV, ERBB2, NFKB2, FCER1G, HMGCR, RPS6, HBEGF, NDRG1, STAT2, APP, VIM, MAP2K1, and CD28 in the biological sample is lower in comparison to the reference control and/or if the expression level of one or more genes selected from EPCAM, C1QBP, IFNA7, C6, and GPI in the biological sample is higher in comparison to the reference control.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying figures, which are incorporated in and constitute a part of this specification, illustrate several aspects described below.

FIG. 1 shows transcriptomic analysis of immune gene expression in fecal samples from dogs with IBD vs healthy dogs (n = 8 in each group). mRNA abundance was quantitated in fecal samples, using Nanostring analysis, as described in Methods. The overall abundance of significantly ($p < 0.05$) upregulated and downregulated genes is displayed by volcano plot (left), with downregulated genes (red) and upregulated genes (green) displayed above the horizontal 1.5 fold expression axis. A heat map of most upregulated (green) and downregulated (red) genes is displayed on the right.

FIG. 2 shows transcriptomic analysis of immune gene expression in stool (fecal) samples from dogs with inflammatory bowel disease compared to healthy dogs.

FIG. 3 shows diagnostic gene expression signatures for canine inflammatory bowel disease.

FIG. 4 shows transcriptomic analysis of human stool samples revealing immune gene changes following dietary intervention.

FIG. 5 show differentially expressed genes in human stool samples following dietary intervention.

FIG. 6 shows direct stool transcriptome analysis revealing changes in GI immune transcriptomes in dogs with inflammatory bowel (IBD) disease undergoing dietary intervention.

FIG. 7 shows changes in immune gene expression following implementation of a therapeutic diet in dogs with IBD.

FIG. 8 shows stool transcriptome analysis to identify GI lymphoma in cats.

FIG. 9 shows tubes with stool samples.

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DETAILED DESCRIPTION

Disclosed herein are methods for diagnosing and treating inflammatory bowel disease (IBD) in humans and companion animals. To date, there are no commercially available assays for accurately identifying IBD in humans or companion animals using single stool samples. Rather, the diagnosis relies on colonoscopy and/or endoscopy with GI biopsy and histopathological interpretation. However, the accuracy of histopathology in diagnosis IBD in humans and animals is not fully reliable, and there is a great deal of variation from one pathologist to another. As disclosed herein, the inventors have developed a novel, non-invasive and much more reliable method for diagnosing and treating IBD in human and companion animals by determining the expression levels of many different genes provided herein in biological samples (e.g., a fecal sample) obtained from humans or companion animals.

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Reference will now be made in detail to the embodiments of the invention, examples of which are illustrated in the drawings and the examples. This invention may, however, be embodied in many different forms and should not be construed as limited to the embodiments set forth herein.

20

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this disclosure belongs. The term “comprising” and variations thereof as used herein is used synonymously with the term “including” and variations thereof and are open, non-limiting terms. Although the terms “comprising” and “including” have been used herein to describe various embodiments, the terms “consisting essentially of” and “consisting of” can be used in place of “comprising” and “including” to provide for more specific embodiments and are also disclosed. As used in this disclosure and in the appended claims, the singular forms “a”, “an”, “the”, include plural referents unless the context clearly dictates otherwise.

25

The following definitions are provided for the full understanding of terms used in this specification.

30

Terminology

As used herein, the terms “may,” “optionally,” and “may optionally” are used interchangeably and are meant to include cases in which the condition occurs as well as cases in which the condition does not occur. Thus, for example, the statement that a formulation “may include an excipient” is meant to include cases in which the formulation includes an excipient as well as cases in which the formulation does not include an excipient.

Ranges can be expressed herein as from “about” one particular value, and/or to “about” another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent “about,” it will be understood that the particular value forms another embodiment. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint. It is also understood that there are a number of values disclosed herein, and that each value is also herein disclosed as “about” that particular value in addition to the value itself. For example, if the value “10” is disclosed, then “about 10” is also disclosed. It is also understood that when a value is disclosed that “less than or equal to” the value, “greater than or equal to the value” and possible ranges between values are also disclosed, as appropriately understood by the skilled artisan. For example, if the value “10” is disclosed the “less than or equal to 10” as well as “greater than or equal to 10” is also disclosed. It is also understood that the throughout the application, data is provided in a number of different formats, and that this data, represents endpoints and starting points, and ranges for any combination of the data points. For example, if a particular data point “10” and a particular data point 15 are disclosed, it is understood that greater than, greater than or equal to, less than, less than or equal to, and equal to 10 and 15 are considered disclosed as well as between 10 and 15. It is also understood that each unit between two particular units are also disclosed. For example, if 10 and 15 are disclosed, then 11, 12, 13, and 14 are also disclosed.

The term “about” as used herein when referring to a measurable value such as an amount, a percentage, and the like, is meant to encompass variations of $\pm 20\%$, $\pm 10\%$, $\pm 5\%$, or $\pm 1\%$ from the measurable value.

“Administration” to a subject includes any route of introducing or delivering to a subject an agent. Administration can be carried out by any suitable route, including oral, topical, intravenous, subcutaneous, transcutaneous, transdermal, intramuscular, intra-joint, parenteral,

intra-arteriole, intradermal, intraventricular, intracranial, intraperitoneal, intralesional, intranasal, rectal, vaginal, by inhalation, via an implanted reservoir, parenteral (e.g., subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intraperitoneal, intrahepatic, intralesional, and intracranial injections or infusion techniques), and the like. The phrases "concurrent administration", "administration in combination", "simultaneous administration" or "administered simultaneously" as used herein, means that the compounds are administered at the same point in time or immediately following one another.

The term "biological sample" as used herein means a sample of biological tissue or fluid. Such samples include, but are not limited to, tissue isolated from animals. Biological samples can also include sections of tissues such as biopsy and autopsy samples, frozen sections taken for histologic purposes, blood, plasma, serum, sputum, stool, tears, mucus, hair, and skin. Biological samples also include explants and primary and/or transformed cell cultures derived from patient tissues. A biological sample can be provided by removing a sample of cells from an animal, but can also be accomplished by using previously isolated cells (e.g., isolated by another person, at another time, and/or for another purpose), or by performing the methods as disclosed herein in vivo. Archival tissues, such as those having treatment or outcome history can also be used.

As used herein, the term "companion animal" refers to those animals traditionally kept for companionship or enjoyment, such as for example, dogs, cats, horses, birds, reptiles, mice, rabbits, hamsters, and the like.

A "composition" is intended to include a combination of active agent and another compound or composition, inert (for example, a detectable agent or label) or active, such as an adjuvant.

The term "comprising" and variations thereof as used herein is used synonymously with the term "including" and variations thereof and are open, non-limiting terms. Although the terms "comprising" and "including" have been used herein to describe various embodiments, the terms "consisting essentially of" and "consisting of" can be used in place of "comprising" and "including" to provide for more specific embodiments and are also disclosed.

"Encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (i.e., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological

properties resulting therefrom, Thus, a gene encodes a protein if transcription and translation of mRNA.

The “fragments,” whether attached to other sequences or not, can include insertions, deletions, substitutions, or other selected modifications of particular regions or specific amino acids residues, provided the activity of the fragment is not significantly altered or impaired compared to the nonmodified peptide or protein. These modifications can provide for some additional property, such as to remove or add amino acids capable of disulfide bonding, to increase its bio-longevity, to alter its secretory characteristics, etc. In any case, the fragment must possess a bioactive property, such as regulating the transcription of the target gene.

The term “gene” or “gene sequence” refers to the coding sequence or control sequence, or fragments thereof. A gene may include any combination of coding sequence and control sequence, or fragments thereof. Thus, a “gene” as referred to herein may be all or part of a native gene. A polynucleotide sequence as referred to herein may be used interchangeably with the term “gene”, or may include any coding sequence, non-coding sequence or control sequence, fragments thereof, and combinations thereof. The term “gene” or “gene sequence” includes, for example, control sequences upstream of the coding sequence (for example, the ribosome binding site).

The terms “identical” or percent “identity,” in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (i.e., about 60% identity, preferably 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 higher identity over a specified region when compared and aligned for maximum correspondence over a comparison window or designated region) as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters described below, or by manual alignment and visual inspection (see, e.g., NCBI web site or the like). Such sequences are then said to be “substantially identical.” This definition also refers to, or may be applied to, the complement of a test sequence. The definition also includes sequences that have deletions and/or additions, as well as those that have substitutions. As described below, the preferred algorithms can account for gaps and the like. Preferably, identity exists over a region that is at least about 10 amino acids or 20 nucleotides in length, or more preferably over a region that is 10-50 amino acids or 20-50 nucleotides in length. As used herein,

percent (%) nucleotide sequence identity is defined as the percentage of amino acids in a candidate sequence that are identical to the nucleotides in a reference sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, ALIGN-2 or Megalign (DNASTAR) software. Appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared can be determined by known methods.

For sequence comparisons, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Preferably, default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al. (1977) *Nuc. Acids Res.* 25:3389-3402, and Altschul et al. (1990) *J. Mol. Biol.* 215:403-410, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al. (1990) *J. Mol. Biol.* 215:403-410). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score

goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-5787). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01.

The term "isolating" as used herein refers to isolation from a biological sample, i.e., blood, plasma, tissues, exosomes, or cells. As used herein the term "isolated," when used in the context of, e.g., a nucleic acid, refers to a nucleic acid of interest that is at least 60% free, at least 75% free, at least 90% free, at least 95% free, at least 98% free, and even at least 99% free from other components with which the nucleic acid is associated with prior to purification.

The term "nucleic acid" as used herein means a polymer composed of nucleotides, e.g. deoxyribonucleotides (DNA) or ribonucleotides (RNA). The terms "ribonucleic acid" and "RNA" as used herein mean a polymer composed of ribonucleotides. The terms "deoxyribonucleic acid" and "DNA" as used herein mean a polymer composed of deoxyribonucleotides. (Used together with "polynucleotide" and "polypeptide".)

Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. The phrase nucleotide sequence that encodes a protein or an RNA may also include introns to the extent that the nucleotide sequence encoding the protein may in some version contain an intron(s).

As used herein, the term “preventing” a disease, a disorder, or unwanted physiological event in a subject refers to the prevention of a disease, a disorder, or unwanted physiological event or prevention of a symptom of a disease, a disorder, or unwanted physiological event.

5 "Pharmaceutically acceptable" component can refer to a component that is not biologically or otherwise undesirable, i.e., the component may be incorporated into a pharmaceutical formulation of the invention and administered to a subject as described herein without causing significant undesirable biological effects or interacting in a deleterious manner with any of the other components of the formulation in which it is contained.

10 "Pharmaceutically acceptable carrier" (sometimes referred to as a “carrier”) means a carrier or excipient that is useful in preparing a pharmaceutical or therapeutic composition that is generally safe and non-toxic, and includes a carrier that is acceptable for veterinary and/or human pharmaceutical or therapeutic use. The terms "carrier" or "pharmaceutically acceptable carrier" can include, but are not limited to, phosphate buffered saline solution, water, emulsions (such as an oil/water or water/oil emulsion) and/or various types of wetting agents. As used herein, the
15 term "carrier" encompasses, but is not limited to, any excipient, diluent, filler, salt, buffer, stabilizer, solubilizer, lipid, stabilizer, or other material well known in the art for use in pharmaceutical formulations and as described further herein.

As used herein, the terms “treating” or “treatment” of a subject includes the administration of a drug to a subject with the purpose of curing, healing, alleviating, relieving, altering,
20 remedying, ameliorating, improving, stabilizing or affecting a disease or disorder, or a symptom of a disease or disorder. The terms “treating” and “treatment” can also refer to reduction in severity and/or frequency of symptoms, elimination of symptoms and/or underlying cause, and improvement or remediation of damage.

“Effective amount” of an agent refers to a sufficient amount of an agent to provide a desired
25 effect. The amount of agent that is “effective” will vary from subject to subject, depending on many factors such as the age and general condition of the subject, the particular agent or agents, and the like. Thus, it is not always possible to specify a quantified “effective amount.” However, an appropriate “effective amount” in any subject case may be determined by one of ordinary skill in the art using routine experimentation. Also, as used herein, and unless specifically stated
30 otherwise, an “effective amount” of an agent can also refer to an amount covering both therapeutically effective amounts and prophylactically effective amounts. An “effective amount”

of an agent necessary to achieve a therapeutic effect may vary according to factors such as the age, sex, and weight of the subject. Dosage regimens can be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

5 “Therapeutic agent” refers to any composition that has a beneficial biological effect. Beneficial biological effects include both therapeutic effects, e.g., treatment of a disorder or other undesirable physiological condition, and prophylactic effects, e.g., prevention of a disorder or other undesirable physiological condition. The terms also encompass pharmaceutically acceptable, pharmacologically active derivatives of beneficial agents specifically mentioned herein, including,
10 but not limited to, salts, esters, amides, proagents, active metabolites, isomers, fragments, analogs, and the like. When the term “therapeutic agent” is used, or when a particular agent is specifically identified, it is to be understood that the term includes the agent per se as well as pharmaceutically acceptable, pharmacologically active salts, esters, amides, proagents, conjugates, active metabolites, isomers, fragments, analogs, etc.

15 The term “subject” is defined herein to include animals such as mammals, including, but not limited to, primates (e.g., humans), cows, sheep, goats, horses, dogs, cats, rabbits, rats, mice and the like. In some embodiments, the subject is a human.

 As used here, a “substantially pure population” means a population of derived mesenchymal cells that contains at least 99% mesenchymal cells. Cell purification can be
20 accomplished by any means known to one of ordinary skill in the art. For example, a substantially pure population of cells can be achieved by growth of cells or by selection from a less pure population, as described herein.

 The terms “treat,” “treating,” “treatment,” and grammatical variations thereof as used herein, include partially or completely delaying, alleviating, mitigating or reducing the intensity
25 of one or more attendant symptoms of a disorder or condition and/or alleviating, mitigating or impeding one or more causes of a disorder or condition. Treatments according to the invention may be applied preventively, prophylactically, pallatively or remedially. Prophylactic treatments are administered to a subject prior to onset (e.g., before obvious signs of cancer), during early onset (e.g., upon initial signs and symptoms of cancer), or after an established development of cancer.
30 Prophylactic administration can occur for several days to years prior to the manifestation of symptoms of an infection.

“Therapeutically effective amount” or “therapeutically effective dose” of a composition (e.g., a composition comprising an agent) refers to an amount that is effective to achieve a desired therapeutic result. Therapeutically effective amounts of a given therapeutic agent will typically vary with respect to factors such as the type and severity of the disorder or disease being treated and the age, gender, and weight of the subject. The term can also refer to an amount of a therapeutic agent, or a rate of delivery of a therapeutic agent (e.g., amount over time), effective to facilitate a desired therapeutic effect. The precise desired therapeutic effect will vary according to the condition to be treated, the tolerance of the subject, the agent and/or agent formulation to be administered (e.g., the potency of the therapeutic agent, the concentration of agent in the formulation, and the like), and a variety of other factors that are appreciated by those of ordinary skill in the art. In some instances, a desired biological or medical response is achieved following administration of multiple dosages of the composition to the subject over a period of days, weeks, or years.

Systems and Uses thereof for Extracting and Measuring RNAs in Stool Samples

The gold standard for diagnosis of IBD is GI biopsy and histopathology readout. However, there are numerous studies demonstrating the inability of pathologists to agree on interpretation of GI biopsy samples when looking at same samples. In some cases, it is unable to distinguish between biopsies from healthy individuals and individuals with IBD. This is true of both human IBD and veterinary IBD. Thus, there is a large unmet need for a more objective tool to diagnose IBD through measuring many data points (e.g., RNAs or DNAs) simultaneously, which would eliminate the guesswork and biases of pathologists.

However, evaluation of RNAs directly in stool samples remains a challenge, as in such a harsh environment RNAs would be expected to be rapidly and fully degraded. Thus, a skilled artisan would not suggest a rapid diagnostic platform built around the analysis of stool samples that most people would say RNAs cannot be detected at all.

The instant disclosure has demonstrated systems and methods of isolating and measuring nucleic acids (e.g., RNAs) in stool samples from humans and companion animals. The studies disclosed herein used animal models of IBD and tested human from a diet trial to illustrate that the disclosed systems and method can be used for isolation and measurement of nucleic acids (e.g., RNAs) in stool samples. Uses of the systems, platforms, and/or methods disclosed herein enable

the measurement of hundreds or thousands of datapoints in a single small stool sample, providing much greater power and accuracy than measure one or two proteins in stool or in blood.

Accordingly, in some aspects, disclosed herein are methods, systems, platforms, an/or kits relate to using small stool samples and targeted or untargeted transcriptome analysis.

5 In some aspects, disclosed herein is a method of measuring a level of a nucleic acid (such as mRNA, microRNA, or DNA) in a stool sample, comprising:

- a) obtaining a stool sample;
- b) extracting the nucleic acid (such as mRNA, microRNA, or DNA) from the stool sample; and
- 10 c) measuring the level of the nucleic acid (such as mRNA, microRNA, or DNA).

In some embodiments, the stool sample is obtained from a human or a companion animal.

In some embodiments, the stool sample of step a) is placed or stored in a preservation solution (e.g., an RNA preservation solution).

In some embodiments, step b) further comprises:

- 15 adding the stool sample in a tube that comprises a lysis buffer, wherein the lysis buffer comprises β -mercaptoethanol;
- mixing the stool sample with the lysis buffer; and
- centrifuging the mixed sample thereby obtaining a first supernatant portion.

20 In some embodiments, the method of any preceding aspect further comprises adding a solution comprising phenol, chloroform, and isoamyl alcohol into the tube prior to adding the stool sample. In some embodiments, the method of any preceding aspect further comprises adding a solution comprising phenol into the tube prior to adding the stool sample.

In some embodiments, the method of any preceding aspect further comprises:

- transferring the first supernatant portion into a tube;
- 25 mixing the first supernatant portion with an inhibitor removal solution in the tube;
- and
- centrifuging the mixed sample thereby obtaining a second supernatant portion.

In some embodiments, the method of any preceding aspect further comprises

- transferring the second supernatant portion into a tube;
- 30 mixing the second supernatant portion with a solution comprising binding salts and ethanol; and

passing the mixture through a binding membrane thereby binding the nucleic acid (such as mRNA, microRNA, or DNA) onto the membrane.

In some embodiments, the method of any preceding aspect further comprises adding a DNase to the binding membrane. In some embodiments, the method of any preceding aspect further comprises washing the binding membrane with a washing buffer comprising isopropanol or ethanol. In some embodiments, the method of any preceding aspect further comprises adding water to the binding membrane thereby eluting the RNA in the water.

In some embodiments, the method of any preceding aspect comprises measuring the level of the RNA with an RNA sequencing assay, a hybridization-based array, or a polymerase chain reaction (PCR)-based assay (e.g., reverse transcription PCR (RT-PCR), or real-time PCR). In some embodiments, the RNA is measured by NanoString analysis, real-time PCR, other high-throughput next gen sequencing analysis. Uses of a transcriptome analysis technology (e.g., RNA sequencing, or NanoString targeted panels) allows for the direct analysis in a stool sample of the expression of a panel of GI health and disease related genes. NanoString analysis can generate panels of 100 to 800 genes, whose expression can be quantitated using the NanoString cassettes and their readers. For full RNA sequencing, several platforms (e.g., Illumina) can be utilized for measuring mRNAs extracted the fecal samples. For targeted analysis of disease detection (e.g., for inflammatory bowel disease), a custom gene panel can be created to focus in on IBD and subtypes of IBD (e.g., Crohn's vs ulcerative colitis) vs healthy subjects. For the global expression concept with NanoString, the cassettes can use all 800 available spots to create a broader net.

In some embodiments, the nucleic acid is an RNA.

In some embodiments, the level of the RNA is measured by one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) primers or probes specific for RNAs encoded by one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) genes selected from the group consisting of LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, IFGGC1, HLA-DRB1, MAPK8, STAT2, ITGB1, CSF3R, CEBPB, IDH1, PRDM1, CASP10, NOD1, S100A4, CASP8, TRGC2, LOC484343, CLEC4A, CD22, TNKS, IL21R, CCL17, IFNA7, SLAMF6, IL17F, TLR4, PTGER4, SELL, INPPL1, TANK, SH2D1A, OLIG2, LOC1021559, IRF3, GATA3, MAPK14, LOC481722, C7, CCR2, CD96, TFEB, FCAR, LOC487977, BLNK, GSK3B, and LOC1021567.

In some embodiments, the level of the RNA is measured by one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) primers or probes specific for RNAs encoded by one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) genes selected from the group consisting of LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, STAT2, CSF3R, NOD1, S100A4, and TRGC2.

In some embodiments, the level of the RNA is measured by one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) primers or probes specific for RNAs encoded by one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) genes selected from the group consisting of IFNA7, TLR4, C7, CD96, TFEB, FCAR, LOC487977, BLNK, GSK38, and LOC1021567.

In some embodiments, the level of the RNA is measured by one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) primers or probes specific for RNAs encoded by one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) genes selected from the group consisting of TNFRSF13C, CXCL10, SHMT2, CX3CL1, PPARGC1A, DMBT1, FYN, MAVS, CD163, CCL19, SIGIRR, CD84, TXNIP, TXNIP, PRKCD, IL17F, NRAS, C7, C5, CFD, CSF3, LOC483397, and MAPK1. In some embodiments, the accession numbers of the genes are ones in Table 1.

Table 1.

Gene ID	Gene Name	Accession Number
TNFRSF13C	TNF receptor superfamily member 13C;TNFRSF13C;ortholog	Q96RJ3
CXCL10	C-X-C motif chemokine 10;CXCL10;ortholog	P02778
SHMT2	Serine hydroxymethyltransferase;SHMT2;ortholog	P34897
CX3CL1	C-X3-C motif chemokine ligand 1;CX3CL1;ortholog	P78423
PPARGC1A	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha;PPARGC1A;ortholog	Q9UBK2
DMBT1	Deleted in malignant brain tumors 1 protein;DMBT1;ortholog	Q9UGM3
FYN	Tyrosine-protein kinase;FYN;ortholog	P06241
MAVS	Mitochondrial antiviral signaling protein;MAVS;ortholog	Q7Z434
CD163	Scavenger receptor cysteine-rich type 1 protein M130;CD163;ortholog	Q86VB7

CCL19	C-C motif chemokine;CCL19;ortholog	Q99731
SIGIRR	Single Ig and TIR domain containing;SIGIRR;ortholog	Q6IA17
CD84	CD84 molecule;CD84;ortholog	Q9UIB8
TXNIP	Thioredoxin interacting protein;TXNIP;ortholog	Q9H3M7
TXNIP	Thioredoxin interacting protein;TXNIP;ortholog	Q9H3M7
PRKCD	Protein kinase C delta type;PRKCD;ortholog	Q05655
IL17F	Interleukin 17F;IL17F;ortholog	Q96PD4
NRAS	NRAS proto-oncogene, GTPase;NRAS;ortholog	P01111
C7	Complement C7;C7;ortholog	P10643
C5	Complement C5;C5;ortholog	P01031
CFD	Complement Factor D	P00746
CSF3	Granulocyte colony-stimulating factor;CSF3;ortholog	P09919
LOC483397	Uncharacterized protein;LOC483397;ortholog	A0A8P0PLR9
MAPK1	Mitogen-activated protein kinase;MAPK1;ortholog	P28482

In some embodiments, the level of the RNA is measured by one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) primers or probes specific for RNAs encoded by one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) genes selected from the group consisting of MSR1, ARG1, IL11RA, IL2RG, CTSS, RUNX1, CCR1, ADA, TIGIT, MR1, IL27, CRADD, PSMB8, and HLA-DRB3. In some embodiments, the accession numbers of the genes are the ones in Table 2.

Table 2.

Gene ID	Gene Name	Accession Number
MSR1	Macrophage scavenger receptor types I and II	P21757
ARG1	Arginase-1	P05089
IL11RA	Interleukin-11 receptor subunit alpha	Q14626
IL2RG	Cytokine receptor common subunit gamma	P31785
CTSS	Cathepsin S	P25774
RUNX1	Runt-related transcription factor 1	Q01196
CCR1	C-C chemokine receptor type 1	P32246
ADA	Adenosine deaminase	P00813
TIGIT	T-cell immunoreceptor with Ig and ITIM domains	Q495A1
MR1	Probable hydrolase PNKD	Q8N490
IL27	Interleukin-27 subunit alpha	Q8NEV9
CRADD	Death domain-containing protein CRADD	P78560

PSMB8	Proteasome subunit beta type-8	P28062
HLA-DRB3	HLA class II histocompatibility antigen, DR beta 3 chain	P79483

In some embodiments, the level of the RNA is measured by one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) primers or probes specific for RNAs encoded by one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) genes selected from the group consisting of S100A8, IDO1, CXCL8, FOS, IL1RN, EGR1, CEBPB, EPCAM, S100A9, PLAUR, S100A12, DUSP1, CD74, NFKBIA, LCP1, C1QBP, TNFAIP3, DLA-DRA, DDX5, LGALS3, CCND2, MAP2K2, MEF2C, PSMB9, IFNA7, ANXA1, PTEN, MAPK14, MME, C6, LYN, STAT3, CIITA, MAPK8, TRB1, ITGAV, ERBB2, NFKB2, FCER1G, HMGCR, RPS6, HBEGF, NDRG1, STAT2, APP, VIM, MAP2K1, CD28, and GPI. In some embodiments, the accession numbers of the genes are the ones in Table 3.

Table 3.

Gene ID	Gene Name	Accession Number
S100A8	Protein S100-A8	P05109
IDO1	Indoleamine 2,3-dioxygenase 1	P14902
CXCL8	Interleukin-8	P10145
FOS	Proto-oncogene c-Fos	P01100
IL1RN	Interleukin-1 receptor antagonist protein	P18510
EGR1	Early growth response protein 1	P18146
CEBPB	CCAAT/enhancer-binding protein beta	P17676
EPCAM	Epithelial cell adhesion molecule	P16422
S100A9	Protein S100-A9	P06702
PLAUR	Urokinase plasminogen activator surface receptor	Q03405
S100A12	Protein S100-A12	P80511
DUSP1	Dual specificity protein phosphatase 1	P28562
CD74	HLA class II histocompatibility antigen gamma chain	P04233
NFKBIA	KF-kappa-B inhibitor alpha	P25963
LCP1	Plastin-2	P13796
C1QBP	Complement component 1 Q subcomponent binding protein, mitochondria	Q07021
TNFAIP3	Tumor necrosis factor alpha-induced protein 3	Q96KP6
DLA-DRA	MHC class II DR alpha chain	A0A8C0RKW1

DDX5	Probable ATP-dependent RNA helicase DD5	P17844
LGALS3	Galectin-3	P17931
CCND2	G1/S-specific cyclin-D2	P30279
MAP2K 2	Dual specificity mitogen-activated protein kinase kinase 2	P36507
MEF2C	Myocyte-specific enhancer factor 2C	Q06413
PSMB9	Proteasome subunit beta type-9	P28065
IFNA7	Interferon alpha-7	P01567
ANXA1	Annexin A1	P04083
PTEN	Phosphatidylinositol 3,4,5-triphosphate 3-phosphatase and dual specific	P60484
MAPK1 4	Mitogen-activated protein kinase 14	P49137
MME	Macrophage metalloelastase	P39900
C6	Complement component C6	P13671
LYN	Tyrosine-protein kinase Lyn	P07948
STAT3	Signal transducer and activator of transcription 3	P40763
CIITA	MHC class II transactivator	P33076
MAPK8	Mitogen-activated protein kinase 8	P45983
TRB1	Tribbles Homolog 1	Q96RU8
ITGAV	Integrin alpha-V	P06756
ERBB2	Receptor tyrosine-protein kinase erbB-2	P04626
NFKB2	Nuclear factor NK-kappa-B p100 subunit	Q00653
FCER1G	High affinity immunoglobulin epsilon receptor subunit gamma	P30273
HMGCR	3-hydroxy-3-methylglutaryl-coenzyme A reductase	P04035
RPS6	40S ribosomal protein S6	P62753
HBEGF	Proheparin-binding EGF-like growth factor	Q99075
NDRG1	Protein NDRG1	Q92597
STAT2	Signal transducer and activator of transcription 2	P52630
APP	Amyloid-beta precursor protein	P05067
VIM	Vimentin	P08670
MAP2K 1	Dual specificity mitogen-activated protein kinase kinase 1	Q02750
CD28	T-cell-specific surface glycoprotein CD28	P10747
GPI	Glucose-6-phosphate isomerase	P06744

In some embodiments, the level of the RNA is measured by one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) primers or probes specific for RNAs encoded by one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or

5

ten or more) genes selected from the group consisting of MMP1, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, STAT2, CSF3R, NOD1, S100A4, TRGC2, IFNA7, TLR4, C7, CD96, TFEB, FCAR, LOC487977, BLNK, GSK3B, and LOC102156778.

In some embodiments, the level of the RNA is measured by one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, twenty or more, thirty or more, forty or more, fifty or more, sixty or more, seventy or more, eighty or more, ninety or more, a hundred or more, three hundreds or more, three hundreds or more, four hundreds or more, five hundreds or more, six hundreds or more, or seven hundreds or more) primers or probes specific for RNAs encoded by one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, twenty or more, thirty or more, forty or more, fifty or more, sixty or more, seventy or more, eighty or more, ninety or more, a hundred or more, three hundreds or more, three hundreds or more, four hundreds or more, five hundreds or more, six hundreds or more, or seven hundreds or more) genes selected from Table 4.

Also disclosed herein is a system comprising

a collection tube;

a preservation solution for a stool sample; and

a set of polynucleotides for measuring levels of target nucleic acids (such as mRNA, microRNA, or DNA).

In some embodiments, the primers or probes are specific for RNAs encoded by one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) genes selected from the group consisting of LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, IFGGC1, HLA-DRB1, MAPK8, STAT2, ITGB1, CSF3R, CEBPB, IDH1, PRDM1, CASP10, NOD1, S100A4, CASP8, TRGC2, LOC484343, CLEC4A, CD22, TNKS, IL21R, CCL17, IFNA7, SLAMF6, IL17F, TLR4, PTGER4, SELL, INPPL1, TANK, SH2D1A, OLIG2, LOC1021559, IRF3, GATA3, MAPK14, LOC481722, C7, CCR2, CD96, TFEB, FCAR, LOC487977, BLNK, GSK3B, and LOC1021567.

In some embodiments, the primers or probes are specific for RNAs encoded by one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more,

nine or more, or ten or more) genes selected from the group consisting of LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, STAT2, CSF3R, NOD1, S100A4, and TRGC2.

In some embodiments, the primers or probes are specific for RNAs encoded by one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, 5 nine or more, or ten or more) genes selected from the group consisting of IFNA7, TLR4, C7, CD96, TFEB, FCAR, LOC487977, BLNK, GSK38, and LOC1021567.

In some embodiments, the primers or probes are specific for RNAs encoded by one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, 10 nine or more, or ten or more) genes selected from the group consisting of TNFRSF13C, CXCL10, SHMT2, CX3CL1, PPARGC1A, DMBT1, FYN, MAVS, CD163, CCL19, SIGIRR, CD84, TXNIP, TXNIP, PRKCD, IL17F, NRAS, C7, C5, CFD, CSF3, LOC483397, and MAPK1.

In some embodiments, the primers or probes are specific for RNAs encoded by one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, 15 nine or more, or ten or more) genes selected from the group consisting of MSR1, ARG1, IL11RA, IL2RG, CTSS, RUNX1, CCR1, ADA, TIGIT, MR1, IL27, CRADD, PSMB8, and HLA-DRB3.

In some embodiments, the primers or probes are specific for RNAs encoded by one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, 20 nine or more, or ten or more) genes selected from the group consisting of S100A8, IDO1, CXCL8, FOS, IL1RN, EGR1, CEBPB, EPCAM, S100A9, PLAUR, S100A12, DUSP1, CD74, NFKBIA, LCP1, C1QBP, TNFAIP3, DLA-DRA, DDX5, LGALS3, CCND2, MAP2K2, MEF2C, PSMB9, IFNA7, ANXA1, PTEN, MAPK14, MME, C6, LYN, STAT3, CIITA, MAPK8, TRB1, ITGAV, ERBB2, NFKB2, FCER1G, HMGCR, RPS6, HBEGF, NDRG1, STAT2, APP, VIM, MAP2K1, CD28, and GP.

In some embodiments, the primers or probes are specific for RNAs encoded by one or more 25 (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) genes selected from the group consisting of MMP1, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, STAT2, CSF3R, NOD1, S100A4, TRGC2, IFNA7, TLR4, C7, CD96, TFEB, FCAR, LOC487977, BLNK, GSK3B, and LOC102156778.

In some embodiments, the level of the RNA is measured by one or more (two or more, 30 three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, twenty or more, thirty or more, forty or more, fifty or more, sixty or more, seventy or

more, eighty or more, ninety or more, a hundred or more, three hundreds or more, three hundreds or more, four hundreds or more, five hundreds or more, six hundreds or more, or seven hundreds or more) primers or probes specific for RNAs encoded by one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, twenty or more, thirty or more, forty or more, fifty or more, sixty or more, seventy or more, eighty or more, ninety or more, a hundred or more, three hundreds or more, three hundreds or more, four hundreds or more, five hundreds or more, six hundreds or more, or seven hundreds or more) genes selected from Table 4.

In some aspects, disclosed herein uses of the systems or methods of any preceding aspect for treatment, diagnosis, and/or detection of IBD, GI cancers, GI infections, inflammatory diseases, or non-inflammatory GI diseases. In some embodiments, the systems or methods comprise broad screening diagnostic panels and/or more tailored panels specific to a disease. In some embodiments, the methods or systems comprise uses of the primers or probes disclosed herein for measurement of the nucleic acids. In some embodiments, the methods or systems further comprise using NanoString for measurement of the nucleic acids.

Methods for Treatment and Diagnosis

As noted above, currently the diagnosis of IBD in humans and veterinary species is complicated, time consuming, costly, and often involves the use of endoscopy or colonoscopy procedures, all of which increases cost and risks. Thus, there is a large unmet need for a more objective tool to diagnose IBD through measuring many data points (e.g., RNAs or DNAs) simultaneously, which would eliminate the guesswork and biases of pathologists.

In some aspects, disclosed herein is a method for treating inflammatory bowel disease (IBD) in a human or companion animal comprising:

- obtaining a biological sample from the human or companion animal;
- quantifying an expression level of one or more genes relative to a reference control, wherein the one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) genes are selected from the group consisting of LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, IFGGC1, HLA-DRB1, MAPK8, STAT2, ITGB1, CSF3R, CEBPB, IDH1, PRDM1, CASP10, NOD1, S100A4, CASP8, TRGC2, LOC484343, CLEC4A,

CD22, TNKS, IL21R, CCL17, IFNA7, SLAMF6, IL17F, TLR4, PTGER4, SELL, INPPL1, TANK, SH2D1A, OLIG2, LOC1021559, IRF3, GATA3, MAPK14, LOC481722, C7, CCR2, CD96, TFEB, FCAR, LOC487977, BLNK, GSK3B, and LOC1021567;

determining that the human or companion animal is susceptible to or suffering from IBD if the expression level of one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) of LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, IFGGC1, HLA-DRB1, MAPK8, STAT2, ITGB1, CSF3R, CEBPB, IDH1, PRDM1, CASP10, NOD1, S100A4, CASP8, TRGC2, LOC484343, CLEC4A, and CD22 in the biological sample is higher in comparison to the reference control and/or if the expression level of one or more of TNKS, IL21R, CCL17, IFNA7, SLAMF6, IL17F, TLR4, PTGER4, SELL, INPPL1, TANK, SH2D1A, OLIG2, LOC1021559, IRF3, GATA3, MAPK14, LOC481722, C7, CCR2, CD96, TFEB, FCAR, LOC487977, BLNK, GSK3B, and LOC1021567 in the biological sample is lower in comparison to the reference control; and

if the human or companion animal is susceptible to or suffering from IBD:

- i) administering to the human or companion animal an effective amount of a therapeutic agent for treating the IBD,
- ii) changing the diet of the human or companion animal, or
- iii) performing a fecal transfaunation.

The term “reference control” refers to a level in detected in a subject in general or a study population (e.g., healthy control).

In some embodiments, the therapeutic agent for treatment of IBD in humans or the companion animal is selected from an non-absorbable NSAID, an immunosuppressive drug (e.g., methotrexate, budesonide, or prednisone), a biological agent (e.g., anti-TNF α antibody or anti-integrin antibodies), or a Janus kinase inhibitor (e.g., Xeljanz), a probiotic, or an antibiotic.

In some embodiments, the treatment comprises a non-absorbable NSAID (e.g., 5-aminosalicylates).

In some embodiments, the treatment comprises therapeutic monoclonal antibodies (e.g., Humira, Entivyo, or Stelara).

In some embodiments, the treatment comprises Janus kinase inhibitors (e.g., Xeljanz).

In some embodiments, the treatment comprises immunomodulators (e.g., methotrexate, or thiopurines).

In some embodiments, the treatment comprises immune suppressive drugs (e.g., budesonide, or prednisone).

In some embodiments, the quantifying is carried out to detect RNA expression levels. In some embodiments, the quantifying is carried out by one or a combination of Polymerase Chain Reaction, Real Time-Polymerase Chain Reaction, Real Time Reverse Transcriptase-Polymerase Chain Reaction, Real-time quantitative RT-PCR, Northern blot analysis, in situ hybridization, and probe array. In some embodiments, the RNA is measured by NanoString analysis, real-time PCR, other high-throughput next gen sequencing analysis. Uses of a transcriptome analysis technology (e.g., RNA sequencing, or NanoString targeted panels) allows for the direct analysis in a stool sample of the expression of a panel of GI health and disease related genes. NanoString analysis can generate panels of 100 to 800 genes, whose expression can be quantitated using the NanoString cassettes and their readers. For full RNA sequencing, several platforms (e.g., Illumina) can be utilized for measuring mRNAs extracted the fecal samples. For targeted analysis of disease detection (e.g., for inflammatory bowel disease), a custom gene panel can be created to focus in on IBD and subtypes of IBD (e.g., Crohn's vs ulcerative colitis) vs healthy subjects. For the global expression concept with NanoString, the cassettes can use all 800 available spots to create a broader net.

In some embodiments, the quantifying is carried out to detect protein expression levels. In some embodiments, the quantifying is carried out by one or a combination of Western blot, ELISA, flow cytometry, immunohistochemistry, and other methods of detection using antibodies (for example, antibodies to recognize the proteins or polypeptides encoded by the genes provided herein).

In some embodiments, the expression level of the one or more of the genes described herein is about at least 10% higher (for example, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about a 100% higher or any increase between 10-100% as compared to a reference

level, or at least about a 2-fold, or at least about a 3-fold, or at least about a 4-fold, or at least about a 5-fold, or at least about a 10-fold higher, or any increase between 2-fold and 10-fold or greater as compared to a reference level so long as the increase is statistically significant, wherein the genes are selected from the group consisting of LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, IFGGC1, HLA-DRB1, MAPK8, STAT2, ITGB1, CSF3R, CEBPB, IDH1, PRDM1, CASP10, NOD1, S100A4, CASP8, TRGC2, LOC484343, CLEC4A, and CD22.

In some embodiments, the expression level of the one or more of the genes described herein is about at least 10% lower (for example, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about a 100% lower or any decrease between 10-100% as compared to a reference level, or at least about a 2-fold, at least about a 3-fold, at least about a 4-fold, at least about a 5-fold, or at least about a 10-fold lower, or any decrease between 2-fold and 10-fold or more as compared to a reference level so long as the decrease is statistically significant, wherein the genes are selected from the group consisting of TNKS, IL21R, CCL17, IFNA7, SLAMF6, IL17F, TLR4, PTGER4, SELL, INPPL1, TANK, SH2D1A, OLIG2, LOC1021559, IRF3, GATA3, MAPK14, LOC481722, C7, CCR2, CD96, TFEB, FCAR, LOC487977, BLNK, GSK3B, and LOC1021567.

In some aspects, disclosed herein is a method for treating inflammatory bowel disease (IBD) in a human or a companion animal comprising:

obtaining a biological sample from the human or the companion animal;

quantifying an expression level of one or more genes relative to a reference control, wherein the one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) genes are selected from the group consisting of MMP1, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, STAT2, CSF3R, NOD1, S100A4, TRGC2, IFNA7, TLR4, C7, CD96, TFEB, FCAR, LOC487977, BLNK, GSK3B, and LOC102156778;

determining that the human or companion animal is susceptible to or suffering from IBD if the expression level of one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) of MMP1, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, STAT2, CSF3R, NOD1, S100A4, and TRGC2 in the biological sample is higher in comparison to the

reference control and/or if the expression level of one or more of IFNA7, TLR4, C7, CD96, TFEB, FCAR, LOC487977, BLNK, GSK3B, and LOC102156778 in the biological sample is lower in comparison to the reference control; and
if the human or companion animal is susceptible to or suffering from IBD:

- i) administering to the human or companion animal an effective amount of a therapeutic agent for treating the IBD,
- ii) changing the diet of the human or companion animal, or
- iii) performing a fecal transfaunation.

In some aspects, disclosed herein is a method for treating inflammatory bowel disease (IBD) in a companion animal comprising:

obtaining a biological sample from the companion animal;

quantifying an expression level of one or more genes relative to a reference control, wherein the one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) genes are selected from the group consisting of TNFRSF13C, CXCL10, SHMT2, CX3CL1, PPARGC1A, DMBT1, FYN, MAVS, CD163, CCL19, SIGIRR, CD84, TXNIP, TXNIP, PRKCD, IL17F, NRAS, C7, C5, CFD, CSF3, LOC483397, and MAPK1;

determining that the animal is susceptible to or suffering from IBD if the expression level of one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) of TNFRSF13C, CXCL10, SHMT2, CX3CL1, PPARGC1A, DMBT1, FYN, MAVS, CD163, CCL19, SIGIRR, CD84, TXNIP, TXNIP, PRKCD, IL17F, NRAS, C7, C5, CFD, CSF3, LOC483397, and MAPK1 in the biological sample changes in comparison to the reference control; and

if the animal is susceptible to or suffering from IBD:

- i) administering to the companion animal an effective amount of a therapeutic agent for treating the IBD,
- ii) changing the diet of the companion animal, or
- iii) performing a fecal transfaunation.

In some embodiments, the method of treatment, if the animal is susceptible to or suffering from IBD, comprises administering to the human or companion animal an effective amount of a therapeutic agent for treating IBD. In some embodiments, the method of treatment, if the animal is susceptible to or suffering from IBD, comprises changing the diet of the human or companion animal. In some embodiments, the method of treatment, if the animal is susceptible to or suffering from IBD, comprises performing a fecal transfaunation.

In some embodiments, the one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) genes are selected from the group consisting of LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, STAT2, CSF3R, NOD1, S100A4, and TRGC2. In some embodiments, the one or more genes (two or more, three or more, four or more, five or more, or six or more) are selected from the group consisting of LOC489428, DLA-DQB1, IDO1, CCL25, BAX, and IL12RB2.

In some embodiments, the one or more genes (two or more, three or more, four or more, five or more, or six or more) are selected from the group consisting of IFNA7, TLR4, C7, CD96, TFEB, FCAR, LOC487977, BLNK, GSK38, and LOC1021567. In some embodiments, the one or more genes (two or more, three or more, or four or more) are selected from the group consisting of TFEB, FCAR, LOC487977, GSK38, and LOC1021567.

In some embodiments, the companion animal is a canine. In some embodiments, the companion animal is a dog. In some embodiments, the companion animal is a feline. In some embodiments, the companion animal is a cat. In some embodiments, the companion animal is a horse.

In some embodiments, instead of a companion animal, the subject may be a human.

In some embodiments, the biological sample is a fecal sample, a sputum sample, an oral swab sample, a vaginal swab sample, a urethral swab sample, a nasal swab sample, an ocular swab sample, an aural swab sample, or a skin swab sample. In some embodiments, the biological sample is a fecal sample.

In some embodiments, the therapeutic agent is selected from an antibiotic, an immunosuppressive agent, or a probiotic. In some embodiments, the therapeutic agent is an antibiotic. In some embodiments, the therapeutic agent is an immunosuppressive agent. In some embodiments, the therapeutic agent is a probiotic.

In some embodiments, the antibiotic comprises metronidazole, tylosin, or ampicillin. In some embodiments, the antibiotic is metronidazole. In some embodiments, the antibiotic is tylosin. In some embodiments, the antibiotic is ampicillin.

5 In some embodiments, the immunosuppressive agent comprises prednisone, prednisolone, budesonide, cyclosporine, mycophenolate, or chlorambucil. In some embodiments, the immunosuppressive agent is prednisone. In some embodiments, the immunosuppressive agent is cyclosporine. In some embodiments, the immunosuppressive agent is mycophenolate. In some embodiments, the immunosuppressive agent is chlorambucil.

10 In some embodiments, the method further comprises administering to the human or companion animal an effective amount of a therapeutic agent for treating the IBD or changing the diet of the human or companion animal, if the animal is susceptible to or suffering from IBD.

In some embodiments, the fecal sample is a fresh sample. In some embodiments, the fecal sample can be a frozen sample.

15 In some embodiments, the method further comprises a step for processing the fecal sample to produce a fecal bacterial suspension. In some embodiments, the method further comprises a step wherein the fecal bacterial suspension is centrifuged to obtain a bacterial pellet. In some embodiments, the bacterial pellet is resuspended and incubated with a detecting antibody.

20 In some aspects, disclosed herein is a method for diagnosing inflammatory bowel disease (IBD) in a human or companion animal comprising:

obtaining a biological sample from the human or companion animal;

quantifying an expression level of one or more (two or more, three or more, four or more,

five or more, six or more, seven or more, eight or more, nine or more, or ten or more)

genes relative to a reference control, wherein the one or more genes are selected from

25 the group consisting of LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, IFGGC1, HLA-DRB1, MAPK8, STAT2, ITGB1, CSF3R, CEBPB, IDH1,

PRDM1, CASP10, NOD1, S100A4, CASP8, TRGC2, LOC484343, CLEC4A, CD22,

TNKS, IL21R, CCL17, IFNA7, SLAMF6, IL17F, TLR4, PTGER4, SELL, INPPL1,

TANK, SH2D1A, OLIG2, LOC1021559, IRF3, GATA3, MAPK14, LOC481722, C7,

30 CCR2, CD96, TFEB, FCAR, LOC487977, BLNK, GSK3B, and LOC1021567; and

determining that the animal is susceptible to or suffering from IBD if the expression level of one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) of LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, IFGGC1, HLA-DRB1, MAPK8, STAT2, ITGB1, CSF3R, CEBPB, IDH1, PRDM1, CASP10, NOD1, S100A4, CASP8, TRGC2, LOC484343, CLEC4A, and CD22 in the biological sample is higher in comparison to the reference control and/or if the expression level of one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) of TNKS, IL21R, CCL17, IFNA7, SLAMF6, IL17F, TLR4, PTGER4, SELL, INPPL1, TANK, SH2D1A, OLIG2, LOC1021559, IRF3, GATA3, MAPK14, LOC481722, C7, CCR2, CD96, TFEB, FCAR, LOC487977, BLNK, GSK3B, and LOC1021567 in the biological sample is lower in comparison to the reference control.

In some aspects, disclosed herein is a method for diagnosing inflammatory bowel disease (IBD) in a human or companion animal comprising:

obtaining a biological sample from the human or companion animal;

quantifying an expression level of one or more genes relative to a reference control, wherein the one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) genes are selected from the group consisting of MMP1, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, STAT2, CSF3R, NOD1, S100A4, TRGC2, IFNA7, TLR4, C7, CD96, TFEB, FCAR, LOC487977, BLNK, GSK3B, and LOC102156778; and

determining that the animal is susceptible to or suffering from IBD if the expression level of one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) of MMP1, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, STAT2, CSF3R, NOD1, S100A4, and TRGC2 in the biological sample is higher in comparison to the reference control and/or if the expression level of one or more of IFNA7, TLR4, C7, CD96, TFEB, FCAR, LOC487977, BLNK, GSK3B, and LOC102156778 in the biological sample is lower in comparison to the reference control.

In some aspects, disclosed herein is a method for diagnosing inflammatory bowel disease (IBD) in a human or companion animal comprising:

obtaining a biological sample from the human or companion animal;

5 quantifying an expression level of one or more genes relative to a reference control, wherein the one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) genes are selected from the group consisting of TNFRSF13C, CXCL10, SHMT2, CX3CL1, PPARGC1A, DMBT1, FYN, MAVS, CD163, CCL19, SIGIRR, CD84, TXNIP, TXNIP, PRKCD, 10 IL17F, NRAS, C7, C5, CFD, CSF3, LOC483397, and MAPK1;

determining that the animal is susceptible to or suffering from IBD if the expression level of one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) of TNFRSF13C, CXCL10, SHMT2, CX3CL1, PPARGC1A, DMBT1, FYN, MAVS, CD163, CCL19, SIGIRR, 15 CD84, TXNIP, TXNIP, PRKCD, IL17F, NRAS, C7, C5, CFD, CSF3, LOC483397, and MAPK1 in the biological sample changes in comparison to the reference control.

In some embodiments, the method further comprises administering to the human or companion animal an effective amount of a therapeutic agent for treating the IBD, changing the 20 diet of the human or companion animal, or performing a fecal transfaunation, if the animal is susceptible to or suffering from IBD.

Also disclosed herein is a method for treating a gastrointestinal disorder in a human or a companion animal, comprising:

25 obtaining a stool sample from the human or companion animal;

quantifying an expression level of one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) genes relative to a reference control, wherein the one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or 30 more, or ten or more) genes are selected from the group consisting of MSR1, ARG1,

IL11RA, IL2RG, CTSS, RUNX1, CCR1, ADA, TIGIT, MR1, IL27, CRADD, PSMB8, and HLA-DRB3;

determining that the human or companion animal is susceptible to or suffering from the gastrointestinal disorder if the expression level of one or more genes selected from MSR1 and ARG1 in the biological sample is higher in comparison to the reference control and/or if the expression level of one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) genes selected from IL11RA, IL2RG, CTSS, RUNX1, CCR1, ADA, TIGIT, MR1, IL27, CRADD, PSMB8, and HLA-DRB3 in the biological sample is lower in comparison to the reference control; and

if the human is susceptible to or suffering from the gastrointestinal disorder:

- i) administering to the human or companion animal an effective amount of a therapeutic agent for treating the gastrointestinal disorder, and/or
- ii) changing the diet of the human or companion animal.

In some embodiments, the human or companion animal is susceptible to or suffering from IBD, GI cancers, GI infections, or non-inflammatory GI diseases. In some embodiments, the human is susceptible to or suffering from Crohn's disease, ulcerative colitis, or microscopic colitis. In some embodiments, the human susceptible to or suffering from Crohn's disease is treated with an anti-inflammatory medicine (e.g., sulfasalazine, mesalamine, balsalazide, corticosteroids, or a nonsteroidal anti-inflammatory drug), an antibiotic, an antidiarrheal medication, or probiotics.

Also disclosed herein is a method for diagnosing a gastrointestinal disorder in a human or companion animal, comprising:

obtaining a stool sample from the human or companion animal;

quantifying an expression level of one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) genes relative to a reference control, wherein the one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) genes are selected from the group consisting of MSR1, ARG1, IL11RA, IL2RG, CTSS, RUNX1, CCR1, ADA, TIGIT, MR1, IL27, CRADD, PSMB8, and HLA-DRB3; and

determining that the human or companion animal is susceptible to or suffering from the gastrointestinal disorder if the expression level of one or more genes selected from MSR1 and ARG1 in the biological sample is higher in comparison to the reference control and/or if the expression level of one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) genes selected from IL11RA, IL2RG, CTSS, RUNX1, CCR1, ADA, TIGIT, MR1, IL27, CRADD, PSMB8, and HLA-DRB3 in the biological sample is lower in comparison to the reference control.

Also disclosed herein is a method for treating gastrointestinal lymphoma in a human or companion animal, comprising:

obtaining a stool sample from the human or companion animal;

quantifying an expression level of one or more genes relative to a reference control,

wherein the one or more (two or more, three or more, four or more, five or more, six

or more, seven or more, eight or more, nine or more, or ten or more) genes are selected

from the group consisting of S100A8, IDO1, CXCL8, FOS, IL1RN, EGR1, CEBPB,

EPCAM, S100A9, PLAUR, S100A12, DUSP1, CD74, NFKBIA, LCP1, C1QBP,

TNFAIP3, DLA-DRA, DDX5, LGALS3, CCND2, MAP2K2, MEF2C, PSMB9,

IFNA7, ANXA1, PTEN, MAPK14, MME, C6, LYN, STAT3, CIITA, MAPK8, TRB1,

ITGAV, ERBB2, NFKB2, FCER1G, HMGCR, RPS6, HBEGF, NDRG1, STAT2,

APP, VIM, MAP2K1, CD28, and GPI;

determining that the human or companion animal is susceptible to or suffering from

gastrointestinal lymphoma if the expression level of one or more (two or more, three

or more, four or more, five or more, six or more, seven or more, eight or more, nine or

more, or ten or more) genes selected from S100A8, IDO1, CXCL8, FOS, IL1RN,

EGR1, CEBPB, S100A9, PLAUR, S100A12, DUSP1, CD74, NFKBIA, LCP1,

TNFAIP3, DLA-DRA, DDX5, LGALS3, CCND2, MAP2K2, MEF2C, PSMB9,

ANXA1, PTEN, MAPK14, MME, LYN, STAT3, CIITA, MAPK8, TRB1, ITGAV,

ERBB2, NFKB2, FCER1G, HMGCR, RPS6, HBEGF, NDRG1, STAT2, APP, VIM,

MAP2K1, and CD28 in the biological sample is higher in comparison to the reference

control and/or if the expression level of one or more (two or more, three or more, four

or more, or five or more) genes selected from EPCAM, C1QBP, IFNA7, C6, and GPI in the biological sample is lower in comparison to the reference control; and administering to the human or companion animal an effective amount of a therapeutic agent for treating the gastrointestinal lymphoma if the human or companion animal is susceptible to or suffering from gastrointestinal lymphoma.

Also disclosed herein is a method for diagnosing gastrointestinal lymphoma in a human or companion animal, comprising:

obtaining a stool sample from the human or companion animal;

quantifying an expression level of one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) genes relative to a reference control, wherein the one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) genes are selected from the group consisting of S100A8, IDO1, CXCL8, FOS, IL1RN, EGR1, CEBPB, EPCAM, S100A9, PLAUR, S100A12, DUSP1, CD74, NFKBIA, LCP1, C1QBP, TNFAIP3, DLA-DRA, DDX5, LGALS3, CCND2, MAP2K2, MEF2C, PSMB9, IFNA7, ANXA1, PTEN, MAPK14, MME, C6, LYN, STAT3, CIITA, MAPK8, TRB1, ITGAV, ERBB2, NFKB2, FCER1G, HMGCR, RPS6, HBEGF, NDRG1, STAT2, APP, VIM, MAP2K1, CD28, and GPI; and

determining that the human or companion animal is susceptible to or suffering from gastrointestinal lymphoma if the expression level of one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) genes selected from S100A8, IDO1, CXCL8, FOS, IL1RN, EGR1, CEBPB, S100A9, PLAUR, S100A12, DUSP1, CD74, NFKBIA, LCP1, TNFAIP3, DLA-DRA, DDX5, LGALS3, CCND2, MAP2K2, MEF2C, PSMB9, ANXA1, PTEN, MAPK14, MME, LYN, STAT3, CIITA, MAPK8, TRB1, ITGAV, ERBB2, NFKB2, FCER1G, HMGCR, RPS6, HBEGF, NDRG1, STAT2, APP, VIM, MAP2K1, and CD28 in the biological sample is higher in comparison to the reference control and/or if the expression level of one or more (two or more, three or more, four

or more, or five or more) genes selected from EPCAM, C1QBP, IFNA7, C6, and GPI in the biological sample is lower in comparison to the reference control.

In some aspects, disclosed herein are uses of the system or platform of any preceding aspect for treatment and/or diagnosis of IBD comprising

obtaining a stool sample from the human or companion animal;

quantifying an expression level of one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, twenty or more, thirty or more, forty or more, fifty or more, sixty or more, seventy or more, eighty or more, ninety or more, a hundred or more, three hundreds or more, three hundreds or more, four hundreds or more, five hundreds or more, six hundreds or more, or seven hundreds or more) genes selected from Table 4; and

determining that the human or companion animal is susceptible to or suffering from IBD if the expression level of one or more (two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, twenty or more, thirty or more, forty or more, fifty or more, sixty or more, seventy or more, eighty or more, ninety or more, a hundred or more, three hundreds or more, three hundreds or more, four hundreds or more, five hundreds or more, six hundreds or more, or seven hundreds or more) genes selected from Table 4 in the biological sample is lower in comparison to the reference control.

In some embodiments, the accession numbers of the genes provided herein are those listed in Table 4.

Table 4.

Symbol	accession number	log2FoldChange (IBD vs Healthy)
ABCB1	NM_001003215.1	0.489584
ABL1	XM_005625232.1	0
ACOD1	XM_542615.6	-0.36257
ACVR1	XM_022414922.1	0.045088
ADA	XM_005635097.2	0.799701
ADAMTS2	XM_843844.4	-0.05648
ADGRE5	NM_001048110.1	0.370478

ADORA2A	NM_001003278.1	0.598956
ADORA3	NM_001003178.1	-0.06936
AICDA	NM_001003380.1	0.164445
AIRE	XM_022413324.1	0.553598
AKT1	XM_548000.4	-0.16128
AKT3	XM_022421141.1	0.807355
ALCAM	NM_001313804.1	-0.24553
AMBP	XM_538807.6	-0.05881
ANKRD22	XM_005636647.3	0.5517
ANXA1	XM_533524.2	0.241501
APC	XM_014111995.2	0.104843
APOE	XM_022425020.1	0.1668
APP	NM_001293279.1	0.152003
AR	NM_001003053.1	0
ARG1	XM_532053.2	0
ARG2	XM_022421753.1	-0.40696
ATF1	XR_295821.1	-0.09077
ATF2	XM_005640334.3	0
ATG10	XM_005618157.3	0.10806
ATG12	XM_022425133.1	-0.1835
ATG16L1	XM_845571.5	0.304855
ATG5	XM_014118180.2	0
ATG7	XM_022406360.1	0.859822
ATM	NM_001130828.1	0
ATRX	XM_014111550.2	-0.05663
AXL	XM_022405907.1	0
B2M	NM_001284479.1	0.437495
BATF	XM_849670.1	1
BAX	NM_001003011.1	2.078003
BCL10	XM_547304.2	0.654503
BCL2	NM_001002949.1	-0.04389
BCL2L1	NM_001003072.1	-0.10673
BCL6	NM_001195404.1	0.011229
BCR	XM_014108205.2	0.360423
BHLHE40	XM_541795.6	0.444785
BID	NM_001251938.1	-0.00893
BIRC5	NM_001003348.1	0.347923
BLK	XM_543206.4	-0.26298
BLNK	XM_005637573.3	-1.6256
BMI1	NM_001287063.1	0

BRAF	XM_022403736.1	-0.15446
BRCA1	NM_001013416.1	-0.1835
BRCA2	NM_001006653.4	0
BST1	XM_545938.6	0.136663
BST2	XM_860510.5	-0.11134
BTK	XM_005641566.2	0.192019
BTLA	XM_545097.2	0.078003
C1QA	XM_535367.6	0.200366
C1QB	XM_544507.5	-0.12553
C1QBP	XM_546568.6	-0.15147
C1R	XM_534901.6	-0.14917
C1S	XM_005637210.3	0.035189
C2	XM_014118342.2	0.168295
C3	XM_022407130.1	0.402965
C3AR1	XM_005637196.3	-0.53945
C4BPA	XM_003434950.4	0.361795
C5	XM_022425033.1	-0.05242
C6	XM_022417885.1	0.212885
C7	XM_022417892.1	-1.32604
C8A	XM_003639022.3	-0.18569
C8B	XM_536694.6	-0.05242
C8G	XM_014116920.2	-0.31603
C9	XM_005619370.3	0
CALHM6	XM_850260.5	0
CAMP	NM_001003359.1	0.384664
CARD11	XM_005621150.3	0
CARD9	XM_844178.1	-0.17243
CASP10	XM_005640530.3	1.254101
CASP3	NM_001003042.1	0.520916
CASP8	NM_001048029.1	1.015942
CCL1	NM_001005252.1	-0.06301
CCL13	NM_001003966.1	0.63743
CCL14	XM_537723.6	0.351126
CCL16	XM_537724.6	0.985353
CCL17	NM_001003051.1	-0.54507
CCL19	NM_001005256.1	0
CCL2	NM_001003297.1	-0.29155
CCL20	NM_001005254.1	-0.05242
CCL21	NM_001005258.1	0
CCL22	XM_003433778.2	0.117173

CCL23	XM_537722.6	0
CCL24	NM_001003967.1	1.289507
CCL25	NM_001005259.1	2.285128
CCL26	NM_001005253.1	0
CCL27	NM_001003968.1	0.2358
CCL28	NM_001005257.1	-0.05716
CCL3	NM_001005251.1	0
CCL4	NM_001005250.1	-0.04064
CCL5	NM_001003010.2	-0.28526
CCL7	NM_001010960.1	0.071577
CCL8	NM_001005255.1	0.117173
CCND1	NM_001005757.1	-0.05242
CCND2	XM_849493.1	0
CCND3	XM_859764.3	-0.04786
CCR1	NM_001038606.1	0
CCR10	XM_844228.3	0.157961
CCR2	XM_541906.1	-1.36257
CCR3	NM_001005261.1	0
CCR4	NM_001003020.1	0.127178
CCR5	NM_001012342.2	0
CCR6	XM_846017.4	-0.03634
CCR7	XM_548131.2	0
CCR9	NM_001284476.1	-0.29844
CCRL2	XM_541904.5	0.086737
CD14	XM_843653.5	0.117418
CD160	XM_005630717.1	0
CD163	NM_001048020.1	0
CD164	XM_532256.6	0.098334
CD180	XM_544362.6	0.444785
CD19	XM_005621381.3	0
CD1A6	NM_001128837.1	0
CD1B	NM_001130830.1	0.064752
CD1C	NM_001128836.1	0
CD1D	XM_005640956.2	0
CD1E	XM_005640914.2	-0.71348
CD2	XM_844126.1	0.830075
CD200	XM_005639479.2	0
CD207	XM_850409.2	0
CD209	NM_001130832.1	-0.00893
CD22	XM_862798.3	0.424748

CD244	XM_005640944.1	-0.26298
CD247	XM_005622970.1	0
CD27	XM_849371.1	0.146991
CD274	XM_541302.2	-0.05242
CD276	XM_849111.1	-1.1281
CD28	NM_001003087.1	0
CD34	NM_001003341.1	0
CD36	NM_001177734.2	0.136663
CD37	XM_541497.4	0
CD38	NM_001003143.1	0.053265
CD3D	XM_536556.2	0
CD3E	NM_001003379.1	0.215954
CD3EAP	XM_022405335.1	0.374396
CD3G	XM_005619711.3	0.279842
CD4	NM_001003252.1	-0.16424
CD40	NM_001002982.1	0
CD40LG	NM_001002981.1	0
CD44	NM_001197022.1	-0.26298
CD47	NM_001080721.1	0.147487
CD48	XM_545759.5	0
CD5	XM_005631690.3	-0.0857
CD53	XM_003639132.2	0.117173
CD55	XM_022420853.1	0.048409
CD58	XM_014120568.2	0.116899
CD59	XM_022405336.1	0.13493
CD6	XM_014121151.1	0
CD63	XM_005625462.1	-0.01207
CD68	XM_022418524.1	0.033165
CD7	XM_844435.4	-0.15612
CD70	XM_542136.4	-0.00893
CD74	XM_005619298.3	1.282765
CD79A	NM_001313834.1	0.124689
CD79B	XM_005624254.3	0
CD80	NM_001003147.1	0
CD81	XM_014120935.1	0.104843
CD83	XM_847554.5	0.672195
CD84	XM_005640884.3	0
CD86	NM_001003146.1	0
CD8A	XM_850452.1	-0.06622
CD8B	XM_859954.4	-0.29992

CD9	XM_005637440.2	0
CD96	XM_545094.2	-1.40626
CD99	XM_014111551.1	0.454566
CDH1	NM_001287125.1	0.523562
CDH5	XM_546894.6	0
CDK1	XM_014112825.1	0.315502
CDK4	XM_022424104.1	0
CDKN1A	XM_532125.2	0.644094
CDKN2A	XM_003431598.2	-0.42866
CDKN2B	NM_001146269.1	0.096862
CDKN2C	XM_005629013.2	-0.05242
CEACAM1	NM_001097557.1	0.742512
CEBPA	XM_005616767.1	-0.05098
CEBPB	XM_005635170.3	1.421701
CFB	XM_003431676.3	0.152003
CFD	XM_542213.6	0.006165
CFI	XM_014109975.2	-0.2475
CFP	XM_548978.6	-0.21818
CH25H	NM_001313827.1	-0.21503
CHEK2	XM_022410496.1	-0.34346
CHUK	XM_005637611.3	0.546603
CIITA	XM_547128.2	-0.035
CISH	XM_541873.6	0.035979
CKLF	NM_001252340.1	-0.45003
CLDN1	XM_845155.5	0.119446
CLEC4A	XM_014108320.2	0.633967
CLEC5A	XM_014119959.2	-0.36257
CLEC7A	XM_022411028.1	0.485427
CLU	NM_001003370.1	0.300395
CMA1	NM_001013424.1	-0.33997
CMKLR1	XM_022410212.1	0.559427
CNP	XM_005624476.3	0.113178
COL1A1	NM_001003090.1	0.460552
COL3A1	XM_845916.1	0
COLEC12	XM_022421537.1	-0.12278
CPA3	XM_542828.6	0
CR1L	XM_005622317.2	0.0199
CR2	XM_005622319.1	-0.52696
CREB1	XM_014110992.2	0.343699
CREB5	XM_022427523.1	-0.23397

CREBBP	XM_003434864.4	0
CRP	XM_545746.2	0
CSF1	XM_005621807.2	0
CSF1R	XM_546306.6	-0.63941
CSF2	NM_001003245.1	0
CSF2RB	XM_538397.6	-0.08955
CSF3	XM_022423955.1	0.31797
CSF3R	XM_005628934.3	1.53555
CTLA4	NM_001003106.1	-0.31818
CTSG	XM_014115810.2	-0.31223
CTSH	XM_536212.3	-0.05772
CTSS	NM_001002938.1	0.736966
CTSW	XM_540846.5	0
CX3CL1	NM_001284456.1	0
CX3CR1	NM_001284491.1	0.011229
CXCL10	NM_001010949.1	0.917538
CXCL11	XM_003640114.2	0.011825
CXCL12	NM_001128097.1	-0.63176
CXCL13	XM_845089.3	-0.38565
CXCL14	XM_005626560.1	-0.05242
CXCL16	XM_844211.3	0.155278
CXCL8	NM_001003200.1	0.747854
CXCR1	XM_005640640.2	0.495411
CXCR2	NM_001003151.2	-0.47335
CXCR3	NM_001011887.1	0
CXCR4	NM_001048026.1	0
CXCR5	XM_546496.3	0.090289
CXCR6	XM_846798.4	-0.89308
CYBB	NM_001100291.1	1.088091
CYFIP2	XM_005619254.3	0.024453
CYLD	XM_005617569.3	0
DDR1	XM_022425821.1	0.260892
DDX5	NM_001286941.1	0.651785
DDX58	XM_003639385.2	0
DEFB1	NM_001113713.1	-0.14101
DLA-12	NM_001014379.1	0.590124
DLA-64	NM_001014378.1	0.02057
DLA-79	NM_001020810.1	0.084889
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DLA-DMB	NM_001271070.1	0.301867
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DNMT3A	XM_022404353.1	-0.36257
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DOK1	XM_540216.6	0.350534
DOK2	XM_543259.6	0.024453
DUSP1	XM_014112785.1	-0.05445
DUSP6	XM_860257.1	0.029294
EBI3	XM_005633060.3	-0.26298
EGFR	XM_533073.2	0
EGR1	XM_846145.3	0.230006
ELANE	NM_001003378.2	0
ENTPD1	XM_005637555.3	-0.79553
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EP300	XM_022424377.1	0
EPCAM	XM_005626238.1	0.513102
EPHA5	XM_005628243.2	-0.26838
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ERBB3	XM_538226.6	-0.15612
ERBB4	XM_003434277.4	0
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ESR2	XM_861041.4	0
ETS1	XM_546405.5	-0.03717
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FAS	XM_005636650.1	0.269698
FASLG	XM_848916.1	1
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FBXW11	XM_861445.4	0
FCAR	XM_014116440.1	-1.4881
FCER1A	NM_001110766.2	0.416789
FCER1G	NM_001003171.2	0.241862
FCER2	XM_022407108.1	0.253573
FCGR1A	XM_014120558.2	-0.13486

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FGFR3	XM_005618534.2	0
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FN1	XM_536059.2	0.576029
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FOXA1	XM_847261.4	0
FOXP3	NM_001168461.1	0.453005
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GATA3	XM_844060.1	-1.14018
GBP5	XM_547291.4	0
GFAP	XM_005624376.3	0.308481
GH1	NM_001003168.1	-0.32013
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GSK3B	XM_535751.2	-1.75489
GZMA	XM_544335.2	-0.00412
GZMB	XM_547752.2	-0.10001
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IDO2	XM_022404003.1	0.005742
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IL12A	NM_001003293.2	0
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IL12RB1	XM_005632710.1	0.118557
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IL13	NM_001003384.1	0
IL13RA1	XM_538150.6	-0.27915
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IL17F	XM_538959.1	-0.69441
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IL19	XM_547384.3	0.060123
IL1A	NM_001003157.2	0.238046
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IL1R1	XM_005625999.3	-0.2475
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IL1RN	NM_001003096.1	0.169839
IL2	NM_001003305.1	0
IL21	NM_001003347.1	-0.16008
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IL22	XM_538274.1	0
IL22RA1	XM_850020.4	0.263071
IL22RA2	XM_014112310.2	0
IL23A	XM_538231.2	0
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IL24	XM_846427.3	0.321402
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IL26	XM_846075.2	-0.05888
IL27	XM_844736.5	0.262033
IL29L	NM_001114853.1	0.101711
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IL2RB	XM_847855.1	-0.1343
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IL31RA	XM_014108431.2	0
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IL34	XM_022419223.1	-0.00198
IL3RA	XM_014111816.1	0
IL4	NM_001003159.1	-0.59704
IL4R	XM_547077.2	0.30102
IL5	NM_001006950.1	-0.182
IL5RA	XM_022407196.1	0
IL6	NM_001003301.1	-0.05242
IL6R	XM_850012.1	0.563429

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IL9	XM_003431593.3	0.991067
ILF3	XM_005632891.3	-0.25364
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IRAK2	XM_005632168.3	0
IRAK4	XM_005636956.2	0
IRF1	XM_003639368.4	0.808087
IRF2	XM_005629996.3	0.137264
IRF3	XM_005616307.2	-1.11464
IRF4	XM_005640152.1	-0.12013
IRF5	XM_014119060.2	0
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ISG15	XM_003639053.4	1.146157
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ITGA4	XM_545551.2	0
ITGA5	XM_022411219.1	0.117173
ITGA6	XM_005640324.2	1.451017
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ITGAV	XM_014110784.1	0.344648
ITGAX	XM_547049.4	0.124689
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JAK2	XM_541301.2	0.436423
JAK3	XM_022406886.1	0
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KLRF1	XM_849098.1	0.113868
KLRG1	XM_849133.1	0
KLRK1	XM_005637160.1	-0.14018
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LAIR1	XM_541424.2	0.732587
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LAMP2	XM_005641765.2	-0.24361
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LBP	XM_542993.6	0
LCK	XM_005617639.1	-0.05539
LCN2	XM_022423769.1	-0.18728
LCP1	XM_005633885.2	0
LFNG	XM_547009.5	-0.16914
LGALS1	NM_001201488.1	0.113868
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LOC478984	XM_022415348.1	-0.19002
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LTA	XM_843793.4	0
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LTBR	XM_005637256.3	-0.14756
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LY9	XM_005640882.3	0
LY96	XM_005638051.3	-0.11641
LYN	XM_535078.6	-0.05242
MAF	XM_014113996.1	-0.3846
MAP2	XM_022415214.1	0
MAP2K1	NM_001048094.1	0.138624
MAP2K2	NM_001048136.1	0.158033
MAP2K4	XM_022418586.1	0.441078
MAP3K1	XM_005617402.2	0.302484
MAP3K5	XM_533420.7	-0.13053
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MAP4K2	XM_005631510.2	-0.26946
MAPK1	NM_001110800.1	0.052467
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MAPK14	NM_001003206.1	-1.15612
MAPK3	NM_001252035.1	0.208179
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MARCO	XM_005631917.3	-0.26298
MASP1	XM_005639816.3	-0.63387
MASP2	XM_544572.7	-0.14018
MAVS	NM_001122609.1	-0.05242
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MCAM	XM_022418207.1	0.289507
MDM4	XM_022415421.1	0.250407
MEF2C	XM_014112231.2	0
MEFV	XM_005621638.2	-0.26298
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MGMT	NM_001003376.1	-0.14581
MIF	ENSCAFT00030041867.1	-0.8834
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MRC1	XM_005617091.3	-0.36257
MS4A1	NM_001048028.1	0
MS4A2	NM_001003172.2	-0.05242
MSR1	XM_843168.1	-0.15107
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MYC	NM_001003246.2	0.072268
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PTGDR2	NM_001048107.1	0
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PVR	XM_005616467.3	0.066844
PYCARD	XM_014114362.2	0.63743
RAD51	NM_001003043.1	0
RAF1	XM_005632141.1	0
RAG2	XM_005631105.1	-0.17681
RB1	XM_534118.2	0
REL	XM_005626131.1	0
RELA	XM_005631473.2	0.204471
RELB	XM_005616459.2	0
RET	NM_001197099.1	0.124689
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RORA	XM_535503.7	0.072008
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RUNX1	XM_844282.4	0.104843
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SETD2	XM_014121990.2	0.117173
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SLAMF1	NM_001003084.1	0
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SLAMF7	XM_847365.1	0
SLC11A1	NM_001013851.1	-0.1835
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SMARCA4	XM_014122046.2	0
SOCS1	XM_005622079.1	0.464593
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SPIB	XM_003432720.4	-0.1835
SPP1	XM_003434023.4	0.228624
SPRY2	XM_003433059.4	0
SRC	XM_005635039.3	0
ST6GAL1	XM_005639812.2	0
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STAT2	XM_014117155.1	1.688056
STAT3	XM_548090.2	0
STAT4	XM_005640471.1	-0.26298

STAT5A	XM_548091.6	0.242292
STAT5B	XM_548092.2	-0.02554
STAT6	XM_005625532.1	-0.24709
STK11	XM_005633151.3	0.218008
SYK	XM_005615953.2	0
TAL1	XM_022427965.1	0
TANK	XM_005640244.3	-0.99644
TAP1	NM_001284496.2	0.63743
TAPBP	NM_001048101.2	-0.1835
TBK1	XM_022424157.1	0
TBX21	XM_548164.2	-0.05164
TCF7	XM_003639372.2	0
TERT	NM_001031630.1	0.037968
TFE3	XM_014111858.2	-0.22827
TFEB	XM_014118431.2	-1.45003
TGFB1	NM_001003309.1	0
TGFB2	XM_545713.6	-0.3202
THBD	NM_001006953.2	-0.56743
THBS1	XM_544610.2	0.29758
THY1	NM_001287129.1	-0.05445
TICAM1	XM_005633023.3	0.315502
TICAM2	NM_001204337.1	1.096862
TIGIT	XM_545108.4	0
TIRAP	XM_014113300.2	0.117586
TLR1	XM_014112326.2	0.63743
TLR10	NM_001173127.1	-0.21818
TLR2	NM_001005264.2	4.91E-16
TLR3	XM_005629968.3	-0.03328
TLR4	NM_001002950.1	-0.74764
TLR5	XM_005640757.2	0
TLR6	XM_022417244.1	-0.3202
TLR7	XM_005641003.3	0
TLR8	XM_005641119.3	-0.26298
TLR9	NM_001002998.1	-0.16326
TNF	NM_001003244.4	0.034089
TNFAIP3	XM_014112321.1	1.052467
TNFRSF11A	XM_005615669.3	-0.31835
TNFRSF11B	XM_003639448.4	-0.11464
TNFRSF12A	NM_001193299.2	1.038528
TNFRSF13B	XM_005620179.3	-0.56902

TNFRSF13C	XM_843968.4	0
TNFRSF14	XM_549666.2	0.152003
TNFRSF17	XM_005621530.2	0.429322
TNFRSF18	NM_001190744.1	-0.13124
TNFRSF1A	XM_005637258.1	-0.02272
TNFRSF1B	XM_005617982.1	-0.44922
TNFRSF4	XM_546720.2	-0.97728
TNFRSF8	XM_014111450.1	0
TNFRSF9	XM_845243.1	0
TNFSF10	NM_001130836.1	0.169839
TNFSF11	XM_846672.1	0.258918
TNFSF12	NM_001205168.1	0.013578
TNFSF13	NM_001205169.1	-0.26298
TNFSF13B	NM_001161710.2	0.544321
TNFSF14	XM_849235.1	-0.2292
TNFSF15	XM_848586.3	0.222392
TNFSF18	XM_005622966.1	0
TNFSF4	XM_003639167.2	1.096862
TNFSF8	XM_850342.4	0.762961
TNKS	XM_844295.5	-0.40901
TNKS2	XM_022412163.1	0.277888
TOLLIP	XM_540778.5	-0.64203
TOX	XM_005638006.1	-0.13124
TP53	NM_001003210.1	0.243151
TP63	XM_022414176.1	0
TRAC	Canis_TRAC.1	0.659031
TRAF1	XM_850435.1	0.141705
TRAF2	XM_005625072.2	-0.15107
TRAF3	XM_022422397.1	1.321928
TRAF6	XM_003432322.4	0.530515
TRAT1	XM_535733.5	-0.31521
TRBC	Canis_TRBC.1	-0.23103
TREM1	NM_001284492.1	0.576029
TREM2	XM_005627313.3	0.309855
TRGC2	Canis_TRGC2.1	0.78286
TRGC3	Canis_TRGC3.1	-0.13486
TRGC8	Canis_TRGC8.1	0
TRIB1	NM_001313808.1	0.542273
TSC2	XM_859874.1	0.439491
TUBB3	XM_005620536.3	0.170553

TXK	XM_022426965.1	0
TXNIP	XM_533037.5	0.353976
TYK2	XM_022407058.1	0.16759
TYMS	NM_001252174.1	0
TYROBP	NM_001197117.1	0.213931
VCAM1	XM_005621302.3	0.222392
VEGFA	NM_001003175.2	-0.20623
VEGFC	XM_540047.6	0
VIM	NM_001287023.1	1.073529
VSIR	XM_014112877.2	1.117423
XCR1	XM_005632632.3	-0.23329
ZAP70	XM_005626046.2	0.488328
ZEB1	XM_003638879.4	0

EXAMPLES

The following examples are set forth below to illustrate the compositions, devices, methods, and results according to the disclosed subject matter. These examples are not intended to be inclusive of all aspects of the subject matter disclosed herein, but rather to illustrate representative methods and results. These examples are not intended to exclude equivalents and variations of the present invention which are apparent to one skilled in the art.

Example 1. Non-Invasive GI Transcriptome Analysis and Diagnostic Platform Technology

These studies used Nanostring transcriptome quantification technology to assess the relative abundance of mRNA encoding immune-related genes ($n = 750$ genes) directly in fecal samples from dogs ($n = 8$) with clinically confirmed inflammatory bowel disease (IBD) and $n = 8$ healthy, age-matched control dogs. This unbiased analysis was able to identify a panel of $n = 25$ most highly upregulated and $n = 25$ most highly downregulated genes in dogs with IBD compared to healthy dogs. Similar analysis can also identify significantly upregulated and downregulated genes in fecal samples from humans with IBD, as well as other companion animal species (eg, cats).

This work is important because it demonstrates the unexpected finding that mRNA is present in feces in concentrations sufficient for direct analysis by Nanostring technology. The studies also demonstrate the application of the invention to the elucidation of immune pathways in IBD, using a “whole gut analysis” approach, which allows sampling of the entire gut

transcriptome throughout the GI tract, without requiring invasive GI biopsies, or amplification of mRNA by conventional RT-PCR approaches. This work also demonstrates that a similar approach can be applied to analysis of the immune or other transcriptomes in other body fluids or mucosal samples, including sputum, oral swabs, vaginal or urethral swabs, nasal swabs, ocular swabs, aural
5 swabs, and skin swabs.

Technology overview and applications: The invention described here consists of the application of specific mammalian gene expression signatures, typically 100-200 genes, to identify and characterize specific GI diseases, in humans and veterinary species, using stool samples collective non-invasively. The test is done by using transcriptomic analysis of shed gastrointestinal
10 cells in small stool samples to identify unique patterns of gene expression that can be used to diagnose specific GI diseases, including inflammatory bowel diseases (e.g., Crohn's disease and ulcerative colitis), functional GI diseases (e.g., irritable bowel syndrome) chronic GI infections and parasitism (e.g., Clostridium difficile, hookworms), and GI cancers (e.g., colon and gastric cancers).

The key aspects supporting this technology include the non-invasive use of stool samples, the application of next generation sequencing approaches to analyze small numbers of shed cells (both GI cells and immune and cancer cells) in stool samples, and in the identification of unique gene signatures associated with each of these diseases. This technology therefore allows specific, rapid, and non-invasive diagnosis of many different GI diseases, and also allows repeated analysis
15 of stool samples during treatment to help guide treatment decisions.

The new technology overcomes current challenges in diagnosing complex GI diseases by directly examining the transcriptomes of GI cells in stool samples, thereby eliminating the need for expensive GI biopsy procedures such as colonoscopy and endoscopy. Because the test examines all the GI cells shed in a stool sample, it provides a much more complete picture of GI
25 health and disease. Moreover, because diagnosis is more accurate, the new testing technology also eliminates the need for other less specific blood tests (e.g., C-reactive protein) and stool tests (e.g., calprotectin).

Several different technology platforms can be utilized to perform the GI transcriptomic analysis, using RNA extracted from small volumes (1-2 gm) of stool sample. The stool sample, collected by the patient (humans) or pet owner (canine, feline patients) is shipped to the laboratory
30 in a suitable RNA preservative solution (e.g., Norgen Stool Sample Collection Device). In the

laboratory, the RNA is extracted using an optimized extraction protocol (Figure 9). Next, the RNA is subjected to either to full RNA sequencing, or to targeted analysis using a custom gene panel and a robust RNA analysis technology such as Nanostring. The decision on which approach to choose is based on whether an unknown disease discovery test was intended, in which case full RNA seq would be preferred, or whether the patient was being evaluated for a more specific disease condition (e.g., IBD) in which case an IBD specific diagnostic panel can be employed (e.g., Nanostring custom panel). A number of different GI specific Nanostring panels are developed, including specific diagnostic panels for IBD, GI infection, GI maldigestion/malabsorption, or GI cancer.

Finally, this GI transcriptome analysis technology also allows direct comparisons of the GI cellular transcriptome with the results of GI microbiome analysis. This comparison can allow the user (e.g., researcher, physician, veterinarian) to identify correlations between the microbiome and specific GI abnormalities, as reflected in the GI transcriptome analysis. For example, the analysis can include comparison of microbiome populations with specific GI immune cell populations, to determine directly how the two populations interact. The net result of GI transcriptome analysis can be a much richer picture of overall GI health, and a better understanding of how the microbiome impacts the GI health profile. Such a test could eventually be marketed to the general public to complement the current microbiome analysis technologies being used now being used.

Figure 2 shows transcriptomic analysis of immune gene expression in stool (fecal) samples from dogs with inflammatory bowel disease compared to healthy dogs. RNA was extracted from stool samples of $n = 8$ healthy dogs and $n = 8$ dogs with IBD using an optimized protocol (see Figure 8), and the mRNA abundance of specific immune related genes was then quantitated, using Nanostring analysis. This study used the canine Nanostring IO panel, which consists of a canine specific immune panel of 800 immune related genes. The analysis was done using a Nanostring nCounter analysis instrument. The overall abundance of significantly upregulated and downregulated genes ($p < 0.1$, fold change > 1.25), as determined by ANOVA with adjustment for multiple comparisons) is displayed by volcano plot (left). In the heat map (right), differential gene expression analysis (DEG) was done to identify the most significantly downregulated genes (red) and upregulated genes (green). This analysis revealed that there were 51 genes whose expression was significantly different in dogs with inflammatory bowel disease compared to healthy dogs. This DEG panel can then be used to identify a unique expression signature that can be used as a

specific diagnostic test for IBD in dogs. The same approach can be applied to generating a specific gene expression panel for diagnosis of IBD in humans, using only stool samples, and not requiring GI biopsies.

The data presented in Figure 2 illustrate the ability of the non-invasive stool transcriptome analysis technology to measure eukaryotic gene expression using small stool samples, and importantly to then identify unique gene signatures associated in this case with inflammatory bowel disease. The same approach can be used to identify unique gene expression signatures associated with other GI diseases in humans and veterinary patients (dogs, cats, horses, cattle) in addition to IBD, including chronic infections, GI malabsorption or maldigestion, and GI cancers.

Using the stool transcriptomic analysis approach detailed in Figure 2, a diagnostic expression signature for IBD in dogs was created (Figure 3), consisting of 51 differentially expressed (up or down-regulated) genes. A similar unique signature, generated by analysis of stool samples from known IBD disease patients, can be used to create a diagnostic panel for screening stool specimens from human patients to identify those with a particular type of IBD, for example whether Crohn's or ulcerative colitis.

Healthy human volunteers ($n = 6$) were placed on a dietary supplement, and changes in their GI immune gene expression were assessed using mRNA extracted from stool samples and subjected to Nanostring immune gene analysis (Figure 4). The baseline gene expression (blue) is compared to gene expression after 8 weeks of dietary supplementation (red). Using this technology, significant changes in expression of 15 genes were identified, thereby illustrating the potential for the technology to measure changes in the GI immune transcriptome directly, bypassing the need for biopsy procedures or indirect, insensitive blood tests.

The heat map in Figure 5 displays the 15 significantly ($p < 0.05$) upregulated (red) or downregulated (blue) genes following a dietary trial in healthy human volunteers, as described in Figure 4. This gene signature provides an example of the sensitivity of the non-invasive GI transcriptome technology to identify immune gene changes directly in stool samples, in a setting where conventional blood immune testing does not identify changes.

Dogs ($n = 12$) with previously diagnosed IBD were placed on a new therapeutic diet, and stool samples collected before the diet change (magenta) and 2 weeks after the diet change (teal) (Figure 6). RNA was extracted from the stool samples and analyzed by Nanostring analysis, using a canine 800 gene IO panel, to assess changes in the GI immune transcriptome. This analysis

revealed changes in overall gene expression patterns, as assessed by principle component analysis (PCA plots).

Dogs with IBD (n = 12) were placed on a new elemental diet, and immune gene expression patterns were assessed using stool samples obtained before and 2-weeks after the dietary change, as described in Figure 6. This analysis revealed 11 genes whose expression was significantly up or downregulated following the dietary change, including the 3 genes as depicted (Figure 7). This analysis demonstrated the ability of the new stool transcriptome test to identify rapid changes in the GI immune transcriptome, using non-invasive testing with stool samples. Many of the genes identified in the dogs and cats can also be picked up in human IBD samples.

Stool samples were collected from a health cat and a cat with biopsy confirmed GI lymphoma, and immune transcriptomes were analyzed using a canine 800 gene Nanostring IO immune panel. This analysis identified 50 genes that were differentially expressed (upregulated) in the stool specimen from the cat with GI lymphoma, compared to the healthy cat (Figure 8). These findings highlight the utility of the GI transcriptome analysis technology for use in the non-invasive diagnosis of GI cancers, including lymphoma and other types of GI neoplasia. The technology also allows the discrimination of GI inflammatory disease from cancer, with greater sensitivity than current GI biopsy procedures and analysis.

Study Materials and Methods

Fecal samples and RNA extraction. Fecal samples (0.25 to 0.5 gms, frozen or refrigerated samples) were used for RNA analysis. Total RNA was extracted using an RNAeasy PowerSoil Total RNA kit, according to manufacturer recommendations. RNA amounts were quantitated to assure that at least 400 ng RNA was available for analysis.

Transcriptome analysis. mRNA relative abundance was determined using a Nanostring Canine IO gene panel, containing primers for 750 canine immune genes. Similar Nanostring panels exist currently for human immune genes, and custom panels can be readily created once broad screens identify specific genes of interest. For example, custom GI panels can use approximately 100-150 genes of interest for diagnostic evaluation of fecal samples (human or veterinary) as an initial screen for the presence of IBD or other GI disorders (eg, enteric infections, GI bacterial overgrowth, GI cancer). Once a diagnosis of IBD is made, more specific panels can be used to subclassify the disease into Crohn's or ulcerative colitis categories (human IBD).

The analysis of the most highly upregulated and downregulated genes in fecal samples was done using software (nCounter and Rosalind) provided by Nanostring. This analysis identified genes upregulated by at least 1.5 log₂ analysis, with a p-value set at 0.1. With additional samples (both healthy and IBD), the analysis parameters can be tightened to, for example, p = 0.05 for additional statistical predictive power.

Study Findings, Applications of the Technology

Immune gene signatures of IBD. The analysis revealed key upregulated genes useful for diagnosis of IBD in dogs (and by extension, human IBD). The key top 5 upregulated genes include MMP-1, MHC class II, IDO, CCL25, BAX, and IL12RB2. For most downregulated, the top 5 would include LOC1021567, glycogen kinase synthase 3, LOC487977, FACR, and TFEB.

Additional applications of direct transcriptome analysis using feces and other mucosal samples. The immune transcriptomic profile created using Nanostring analysis of the fecal microbiome could also be combined with microbiome sequencing to help better understand the relationship between the immunome and the microbiome in the GI tract and other sites colonized by bacteria (e.g., nasal and oral microbiomes, female reproductive tract, urinary tract, skin). This combined “immuno-microbiome” signature could be a powerful tool for disease characterization, discovery, and diagnosis in various body sites.

Example 2. Optimized Protocol for RNA Extraction from Stool Samples for Transcriptome analysis.

1. Sample collection and handling.

Stool samples (1 to 2 gm) should be collected as quickly as possible following passage, using a stool sample collection device and then placed in a commercial RNA preservative solution (eg, Norgen Biotek, Stool Nucleic Acid Collection and Preservation Tubes) prior to shipment to the laboratory for processing. Once in RNA preservative solution, stool samples are shipped to the laboratory on a cold pack for processing.

2. Material required for RNA extraction from stool specimens.

a. RNA extraction kit. RNA extractions are performed using the Qiagen PowerMicrobiome kit, with specific additional equipment.

b. Equipment needed for extraction, not provided in kit

- Microcentrifuge (13,000 x g)

- Pipettor (1.5–1000 µl)
- Vortex-Genie® 2 Vortex
- Vortex Adapter for 24 (1.5–2.0 ml) tubes (cat. no. 13000-V1-24)
- 70% ethanol
- 5 ▪ β-mercaptoethanol (β-ME) – 99.9% pure, 14 M
- +/- Phenol–chloroform–isoamyl alcohol (25:24:1) pH 6.7–8.0 (optional)

c. Technical notes

- 10 ▪ Prepare Solution PM1 (from PowerMicrobiome kit) by adding 10 µl β-mercaptoethanol (β-ME) for every 990 µl Solution PM1 (a total of 1 ml for each prep though you only add 650 mL per sample).
- Heat up water bath. Solution PM1 must be warmed at 55°C for 5–10 min prior to use.
- Shake to mix Solution PM5 before use.
- 15 ▪ Prepare DNase I stock solution by adding 550 µl RNase-Free Water to the DNase I (RNase-free) lyophilized powder and mixing gently. Aliquot the DNase I stock enzyme in 50 µl portions and store at -30°C to -15°C for long term storage (but do not freeze–thaw more than 3 times). To prepare DNase I solution, thaw and combine 5 µl DNase I stock enzyme with 45 µl DNase Digestion solution per prep.

20 **3. Detailed RNA extraction procedure**

Step 1. Prepare **PM1-b-ME** solution (1 part b-ME to 99 parts PM1, see above), warm at 55°C for 10 minutes before starting.

Step 2. Aliquot 0.2 to 0.25 gm stool sample into a PowerBead Tube, Glass 0.1 mm (see below).

25 Note: If phenol-based lysis is desired, add 100 µl phenol–chloroform–isoamyl alcohol (pH 6.5–8.0) to the PowerBead Tube before adding the sample. For the dog fecal sample, I have been adding in phenol.

Step 3. Add 650 µl Solution **PM1-β-ME** to the PowerBead Tube. Alternatively, you may add 650 µl PM1 and 6.5 µl β-ME to the PowerBead Tube.

30 Step 4. Secure the PowerBead Tube horizontally to a Vortex Adapter (cat. no. 13000-V1-24). Orient tube caps to point toward the center of the Vortex Adapter.

Step 5. Vortex at maximum speed for 10 min. Centrifuge at 13,000 x g for 1 min at room temperature (15–30°C). Transfer the supernatant (circled in photo below) to a clean 2 ml Collection Tube (provided) with a P1000 pipette.

Note: If you add phenol–chloroform–isoamyl alcohol, remove the upper aqueous layer and transfer to a clean 2 ml Collection Tube (provided).

Note: The sample is homogenized using mechanical bead beating and a lysis buffer that protects the RNA released into the supernatant. As the sample spins, proteins and cellular debris are pelleted with the beads and the supernatant contains RNA and DNA from both mammalian and bacterial cells.

Step 6. Add 150 µl Solution **IRS** and vortex briefly to mix. Incubate in cold room (2–8°C) for 5 min.

Note: Solution IRS is the Inhibitor Removal Solution which completes the IRT process and removes the contaminants from the sample that would cause problems with PCR and other downstream applications.

Step 7. Centrifuge at 13,000 x g for 1 min.

Step 8. Avoiding the pellet (typically big and white), transfer 650 µl of the supernatant to a clean 2 ml Collection Tube (provided).

Note: Do not transfer more than 650 µl at this step.

Step 9. Add 650 µl of Solution **PM3** and 650 µl of **70% ethanol** (if you want to co-purify small RNAs, then use Solution **PM4** instead of ethanol). Vortex briefly to mix, the proceed to step 9.

Note: Solution PM3 contains the binding salts for total nucleic acid purification and Solution PM4 is 100% ethanol. These solutions set up the conditions for RNA and DNA binding to the Spin Filter.

Note: To prevent small RNAs (5s RNAs, tRNAs and degraded RNAs) from co-purifying with mRNA and rRNA, use 650 µl 70% ethanol instead of Solution PM4.

To purify small RNAs, such as microRNAs and siRNAs, transfer the lysate to a larger tube to accommodate a higher volume (2.6 ml) and add an additional 650 µl 100% ethanol (user supplied) to the lysate. *

Step 10. Load 650 µl of the mixture from step 8 into an MB RNA Spin Column and centrifuge the tubes at 13,000 x g for 1 min. Discard the flow-through and repeat until all the supernatant has been processed through the Spin Column (typically takes 3x).

Note: Total nucleic acids are bound to the Spin Column by passing through the membrane using centrifugation.

Step 11. Shake to mix Solution **PM5**. Add 650 µl Solution **PM5** to the MB RNA Spin Column and centrifuge at 13,000 x g for 1 min.

Note: Solution PM5 is a wash buffer that contains isopropanol to remove salts from the membrane for optimal performance of the on-column DNase step.

Step 12. Discard flow-through and centrifuge at 13,000 x g for 1 min to remove residual wash.

Step 13. Place the MB RNA spin column into a clean 2 ml Collection Tube (provided). To the center of the Spin Column, add 50 µl **DNase I Solution** (prepared by mixing 45 µl DNase Digestion Solution and 5 µl DNase I stock enzyme; see “Notes before starting”).

Step 14. Incubate at room temperature for 15 min. Add 400 µl Solution **PM7** and centrifuge at 13,000 x g for 1 min.

Note: DNase Digestion Solution is a DNase digestion buffer. The DNase in DNase Digestion Solution soaks into the membrane and digests the genomic DNA in the column. Solution PM7 inactivates the DNase enzyme and removes it from the column membrane along with digested DNA.

Step 15. Discard flow-through. Add 650 µl Solution **PM5**. Centrifuge at 13,000 x g for 1 min.

Step 16. Discard flow-through. Add 650 µl Solution **PM4**. Centrifuge at 13,000 x g for 1 min.

Note: Solution PM5 and PM4 are isopropanol- and ethanol-containing wash buffers, respectively, and are used to desalt the column before the elution step.

Step 17. Discard flow-through. Centrifuge at 13,000 x g for 2 min.

Note: The final dry spin ensures all ethanol is cleared from the membrane so that the elution will be efficient.

Step 18. Place the MB RNA Spin Column into a clean 2 ml Collection Tube (provided).

Step 19. Add 100 µl **RNase-Free Water** (provided) to the center of the white filter membrane. Incubate at room temperature for at least 1 min.

Note: Eluting with 100 µl RNase-Free Water will maximize RNA yield. For more concentrated RNA, as little as 50 µl RNase-Free Water can be used.

5 Step 20. Centrifuge at 13,000 x g for 1 min. Discard the MB Spin Column. The RNA is now ready for downstream applications.

Note: RNA is solubilized from the Spin Filter membrane into RNase-Free Water and is ready for use.

10 Step 21. Keep samples on ice and Nanodrop each sample to determine RNA concentration, then aliquot RNA samples into smaller tubes and freeze, store at -20 °C.

Table 5. Correlation of immune transcriptome in GI tract with the selected microbiome. A correlation (statistically significant, as defined by FDR < 0.05) between certain upregulated inflammatory immune genes and certain families of bacteria is shown.

Feature ID	FDR step up (k__Bacteria;p__Fusobacteria;c__Fusobacteriia;o__Fusobacteriales;f__Fusobacteriaceae)	Pearson correlation (k__Bacteria;p__Fusobacteria;c__Fusobacteriia;o__Fusobacteriales;f__Fusobacteriaceae)
TNFRSF13C	0.0192	0.8554
CXCL10	0.0192	0.8601
SHMT2	0.0192	0.8573
CX3CL1	0.0192	0.8669
PPARGC1A	0.0249	0.8280
DMBT1	0.0249	0.8403
FYN	0.0249	0.8259
MAVS	0.0249	0.8251
CD163	0.0249	0.8352
CCL19	0.0251	0.8216
SIGIRR	0.0293	0.8105
CD84	0.0293	0.8105
Feature ID	FDR step up (k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae)	Pearson correlation (k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae)
TXNIP	0.0042	0.9127

Feature ID	FDR step up (k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae)	Pearson correlation (k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae)
TXNIP	0.0090	0.9006
PRKCD	0.0270	0.8641
Feature ID	FDR step up (k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae)	Pearson correlation (k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae)
IL17F	0.0789	0.8547
Feature ID	FDR step up (k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae)	Pearson correlation (k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae)
NRAS	0.0449	0.8684
Feature ID	FDR step up (k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae)	Pearson correlation (k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae)
C7	0.0006	0.9381
C5	0.0468	0.8483
CFD	0.0468	0.8306
CSF3	0.0468	0.8228
LOC483397	0.0468	0.8199
MAPK1	0.0468	0.8179

Table. 6

Symbol	accession number	Alias	Description	log2FoldChange (IBD vs Healthy)
MMP1	XM_546546.2	MMP1 LOC489428	interstitial collagenase; matrix metalloproteinase 1 (interstitial collagenase)	3.392317423
DLA-DQB1	NM_001014381.1	DLA-DQB	major histocompatibility complex class II_DQ beta 1	3.176588732
IDO1	XM_532793.2	INDO	indoleamine 2,3-dioxygenase 1	3.039528364

CCL25	NM_001005259.1	-	chemokine (C-C motif) ligand 25	2.285128177
BAX	NM_001003011.1	-	BCL2-associated X protein	2.078002512
IL12RB2	NM_001025399.1	-	interleukin 12 receptor_ beta 2	2
PSMB8	NM_001048085.1	ATP6V0D2	proteasome (prosome_ macropain) subunit_ beta type_ 8 (large multifunctional peptidase 7)	1.959358016
STAT2	XM_014117155.1	-	signal transducer and activator of transcription 2_ 113kDa	1.688055994
CSF3R	XM_005628934.3	-	colony stimulating factor 3 receptor (granulocyte)	1.535550307
NOD1	XM_005628676.3	-	nucleotide-binding oligomerization domain containing 1	1.222392421
S100A4	NM_001003161.1	MTS1	S100 calcium binding protein A4	1.038528229
TRGC2	Canis_TRGC2.1	NA	T Cell Receptor Gamma Constant 2	0.78286036
IFNA7	NM_001006654.1	CalFN-alpha 7	interferon_ alpha 7	-0.649341729
TLR4	NM_001002950.1	-	toll-like receptor 4	-0.747635312
C7	XM_022417892.1	-	complement component 7	-1.326044203
CD96	XM_545094.2	-	CD96 molecule	-1.406255606
TFEB	XM_014118431.2	-	transcription factor EB	-1.450032921
FCAR	XM_014116440.1	NA	immunoglobulin alpha Fc receptor	-1.488100961
LOC487977	XM_005639485.2	NA	CD200 receptor 1; cell surface glycoprotein CD200 receptor 1	-1.606495662
BLNK	XM_005637573.3	-	B-cell linker	-1.625604485
GSK3B	XM_535751.2	-	glycogen synthase kinase 3 beta	-1.754887502
LOC102156778	XR_002615925.1	-	uncharacterized LOC102156778, Predicted model non-protein-coding transcript	-1.791413378

Example 3. Gastrointestinal Health Diagnostic.

Described herein is gastrointestinal transcriptome analysis using stool samples for detection and classification of immune, neoplastic, and infectious diseases of the GI tract.

The Enteric-Immune Assay, or EIA, is a diagnostic test for assessing the overall immune health status of the GI tract, using only small specimens of stool for testing. The EIA assesses expression of a panel of immune genes (up to at least 750) by direct measurement of mRNA levels in feces, using NanoString methodology. The test comprises a stool collection vial with RNA preservative, 250-500 ng RNA extracted from 1-2 g of feces, a custom designed NanoString cassette capable of quantitating up to at least 750 immune genes of interest, and proprietary algorithms designed for classification of gene signatures associated with particular types of inflammatory bowel disease (e.g., ulcerative colitis, celiac disease, Crohn's disease).

By measuring a large panel of genes, the EIA diagnostic test allows much greater precision in detecting GI immune abnormalities associated with clinical or subclinical inflammatory bowel diseases. The inclusion of large numbers of immune genes in the EIA panel also provides greater insights into GI disease immune mechanism, which in turn allows for disease subclassification and more personalized medical diagnoses and treatment planning. Use of NanoString technology for mRNA quantitation is robust in that RNA quality is not a major factor in assay performance. The new EIA test developed herein allows the detection and classification of GI inflammatory diseases without the need for GI biopsy or other invasive procedures.

The EIA test is innovative in that it directly measures mRNA abundance in stool specimens, without the need for technically complicated amplification steps typically required in other tests for measurement of mRNA concentrations (e.g., RT-PCR tests). It had been assumed previously that mRNA shed from immune cells and epithelial cells in the gut would be too rapidly degraded for effective detection by conventional RT-PCR or by Nanostring approaches. However, we have discovered that in fact sufficient mRNA from host immune cells (i.e., mammalian mRNA) is present in feces to allow direct detection and quantification by NanoString analysis is possible, using simple fecal RNA extraction from small amounts of feces (e.g., 1-2 gm), without further target amplification or other laborious preparation of larger fecal samples. In addition, sufficient mRNA is preserved in frozen or refrigerated fecal samples to be readily detected.

Proof of concept data has been obtained using canine fecal samples from healthy dogs and dogs with inflammatory bowel disease (IBD), which demonstrated that the test can generate immune gene signatures that discriminated healthy from IBD animals. Importantly, many GI

immune responses in human and dog IBD patients are shared between dogs and humans, thus the same EIA technology can discriminate healthy vs IBD human patients. Moreover, the fact that mammalian mRNA can be quantitated in dogs strongly indicates that the EIA test can detect host mRNA from mammalian species, as there is no reason based on numerous prior studies (e.g., histopathology, immuno-histochemistry, flow cytometry, RNAseq) to believe that the dog GI tract and immune cell populations are substantially different in number or function from other major species. Moreover, this study has found that the EIA gene signatures associated with IBD in dogs overlap considerably with human IBD-associated genes.

This test has several applications for diagnosis and management of GI disorders (inflammatory diseases, cancer, chronic infections, among others) of humans, companion animals (e.g., dogs, cats, horses) and livestock (e.g., cattle, sheep, swine, poultry). While the EIA test was developed for detection and classification of IBD in humans, numerous other possible applications exist. For example, a cancer screening test can be designed for use with stool samples for early detection of GI cancers, including colorectal cancer, gastric cancer, or esophageal cancer. A custom probe set can be readily designed for detection of transcripts associated with specifically mutated genes in CRC, including mutated KRAS and b-actin and hemoglobin genes (fecal blood). Multiple probe sets spanning common KRAS mutations can be included, as well as mutated genes associated with gastric cancer (e.g., mutant TP53, erbB-2), and built into a single GI cancer panel.

Another application of the technology is a custom stool sample test for diagnosis of chronic infections of the GI tract, including the viral pathogens CMV, herpesvirus infection, HPV infection, and the bacterial pathogens *Yersinia*, *Mycobacterium* (both *M. tuberculosis* and *M. avium*), *Actinomyces*, and *Treponema*. A diagnostic test for enteric pathogens would include probe sets specific for individual pathogens and could be designed as a narrow or broad screening test for chronic GI infections.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of skill in the art to which the disclosed invention belongs. Publications cited herein and the materials for which they are cited are specifically incorporated by reference.

Those skilled in the art will appreciate that numerous changes and modifications can be made to the preferred embodiments of the invention and that such changes and modifications can

be made without departing from the spirit of the invention. It is, therefore, intended that the appended claims cover all such equivalent variations as fall within the true spirit and scope of the invention.

CLAIMS

We claim:

1. A method of measuring a level of an RNA in a stool sample, comprising:
 - a) obtaining a stool sample;
 - b) extracting the RNA from the stool sample; and
 - c) measuring the level of the RNA.
2. The method of claim 1, wherein the stool sample of step a) is placed or stored in an RNA preservation solution.
3. The method of claim 1 or 2, wherein step b) further comprises:
adding the stool sample in a tube that comprises a lysis buffer, wherein the lysis buffer comprises β -mercaptoethanol;
mixing the stool sample with the lysis buffer; and
centrifuging the mixed sample thereby obtaining a first supernatant portion.
4. The method of claim 3, further comprising adding a solution comprising phenol, chloroform, and isoamyl alcohol into the tube prior to adding the stool sample.
5. The method of claim 3, further comprising adding a solution comprising phenol into the tube prior to adding the stool sample.
6. The method of any one of claims 3-5, further comprising:
transferring the first supernatant portion into a tube;
mixing the first supernatant portion with an inhibitor removal solution in the tube;
and
centrifuging the mixed sample thereby obtaining a second supernatant portion.
7. The method of claim 6, further comprising:
transferring the second supernatant portion into a tube;

mixing the second supernatant portion with a solution comprising binding salts and ethanol; and
passing the mixture through a binding membrane thereby binding the RNA onto the membrane.

8. The method of claim 7, further comprising adding a DNase to the binding membrane.
9. The method of claim 7 or 8, further comprising washing the binding membrane with a washing buffer comprising isopropanol or ethanol.
10. The method of any one of claims 7-9, further comprising adding water to the binding membrane thereby eluting the RNA in the water.
11. The method of any one of claims 1-10, wherein the level of the RNA is measured by an RNA sequencing assay, a hybridization-based array, or a polymerase chain reaction (PCR)-based assay.
12. The method of any one of claims 1-11, wherein the level of the RNA is measured by one of more primers or probes specific for RNAs encoded by genes selected from the group consisting of LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, IFGGC1, HLA-DRB1, MAPK8, STAT2, ITGB1, CSF3R, CEBPB, IDH1, PRDM1, CASP10, NOD1, S100A4, CASP8, TRGC2, LOC484343, CLEC4A, CD22, TNKS, IL21R, CCL17, IFNA7, SLAMF6, IL17F, TLR4, PTGER4, SELL, INPPL1, TANK, SH2D1A, OLIG2, LOC1021559, IRF3, GATA3, MAPK14, LOC481722, C7, CCR2, CD96, TFEB, FCAR, LOC487977, BLNK, GSK3B, and LOC1021567.
13. The method of any one of claims 1-12, wherein the level of the RNA is measured by one of more primers or probes specific for RNAs encoded by genes selected from the group consisting of LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, STAT2, CSF3R, NOD1, S100A4, and TRGC2.

14. The method of any one of claims 1-12, wherein the level of the RNA is measured by one of more primers or probes specific for RNAs encoded by genes selected from the group consisting of IFNA7, TLR4, C7, CD96, TFEB, FCAR, LOC487977, BLNK, GSK38, and LOC1021567.
15. The method of any one of claims 1-12, wherein the level of the RNA is measured by one of more primers or probes specific for RNAs encoded by genes selected from the group consisting of TNFRSF13C, CXCL10, SHMT2, CX3CL1, PPARGC1A, DMBT1, FYN, MAVS, CD163, CCL19, SIGIRR, CD84, TXNIP, TXNIP, PRKCD, IL17F, NRAS, C7, C5, CFD, CSF3, LOC483397, and MAPK1.
16. The method of any one of claims 1-12, wherein the level of the RNA is measured by one of more primers or probes specific for RNAs encoded by genes selected from the group consisting of MSR1, ARG1, IL11RA, IL2RG, CTSS, RUNX1, CCR1, ADA, TIGIT, MR1, IL27, CRADD, PSMB8, and HLA-DRB3.
17. The method of any one of claims 1-12, wherein the level of the RNA is measured by one of more primers or probes specific for RNAs encoded by genes selected from the group consisting of S100A8, IDO1, CXCL8, FOS, IL1RN, EGR1, CEBPB, EPCAM, S100A9, PLAUR, S100A12, DUSP1, CD74, NFKBIA, LCP1, C1QBP, TNFAIP3, DLA-DRA, DDX5, LGALS3, CCND2, MAP2K2, MEF2C, PSMB9, IFNA7, ANXA1, PTEN, MAPK14, MME, C6, LYN, STAT3, CIITA, MAPK8, TRB1, ITGAV, ERBB2, NFKB2, FCER1G, HMGCR, RPS6, HBEGF, NDRG1, STAT2, APP, VIM, MAP2K1, CD28, and GPI.
18. A system comprising
 - a collection tube;
 - a preservation solution for a stool sample; and
 - a set of polynucleotides for measuring levels of target RNAs.
19. The system of claim 18, wherein polynucleotides are primers or probes.

20. The system of claim 19, wherein the primers or probes are specific for RNAs encoded by genes selected from the group consisting of LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, IFGGC1, HLA-DRB1, MAPK8, STAT2, ITGB1, CSF3R, CEBPB, IDH1, PRDM1, CASP10, NOD1, S100A4, CASP8, TRGC2, LOC484343, CLEC4A, CD22, TNKS, IL21R, CCL17, IFNA7, SLAMF6, IL17F, TLR4, PTGER4, SELL, INPPL1, TANK, SH2D1A, OLIG2, LOC1021559, IRF3, GATA3, MAPK14, LOC481722, C7, CCR2, CD96, TFEB, FCAR, LOC487977, BLNK, GSK3B, and LOC1021567.
21. The system of claim 19, wherein the primers or probes are specific for RNAs encoded by genes selected from the group consisting of LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, STAT2, CSF3R, NOD1, S100A4, and TRGC2.
22. The system of claim 19, wherein the primers or probes are specific for RNAs encoded by genes selected from the group consisting of IFNA7, TLR4, C7, CD96, TFEB, FCAR, LOC487977, BLNK, GSK38, and LOC1021567.
23. The system of claim 19, wherein the primers or probes are specific for RNAs encoded by genes selected from the group consisting of TNFRSF13C, CXCL10, SHMT2, CX3CL1, PPARGC1A, DMBT1, FYN, MAVS, CD163, CCL19, SIGIRR, CD84, TXNIP, TXNIP, PRKCD, IL17F, NRAS, C7, C5, CFD, CSF3, LOC483397, and MAPK1.
24. The system of claim 19, wherein the primers or probes are specific for RNAs encoded by genes selected from the group consisting of MSR1, ARG1, IL11RA, IL2RG, CTSS, RUNX1, CCR1, ADA, TIGIT, MR1, IL27, CRADD, PSMB8, and HLA-DRB3.
25. The system of claim 19, wherein the primers or probes are specific for RNAs encoded by genes selected from the group consisting of S100A8, IDO1, CXCL8, FOS, IL1RN, EGR1, CEBPB, EPCAM, S100A9, PLAUR, S100A12, DUSP1, CD74, NFKBIA, LCP1, C1QBP, TNFAIP3, DLA-DRA, DDX5, LGALS3, CCND2, MAP2K2, MEF2C, PSMB9, IFNA7, ANXA1, PTEN, MAPK14, MME, C6, LYN, STAT3, CIITA, MAPK8, TRB1, ITGAV,

ERBB2, NFKB2, FCER1G, HMGCR, RPS6, HBEGF, NDRG1, STAT2, APP, VIM, MAP2K1, CD28, and GPI.

26. The system of any one of claims 18-26, further comprising a device for detection of the target RNAs.
27. The system of claim 26, wherein the device is a polymerase chain reaction (PCR) machine, a hybridization-based array, or a high-throughput sequencing machine.
28. The system of claim 26 or 27, wherein the device is a NanoString analysis system.
29. A method of treating a gastrointestinal disorder in a subject comprising using the system of any one of claims 18-28.
30. A method of diagnosing or monitoring a gastrointestinal disorder in a subject comprising using the system of any one of claims 18-28.
31. A method of monitoring a treatment of a gastrointestinal disorder in a subject comprising using the system of any one of claims 18-28.
32. The method of any one of claims 29-31, wherein the subject is a human, a companion animal, or a livestock.
33. The method of any one of claims 29-32, wherein the gastrointestinal disorder comprises a gastrointestinal infection, a gastrointestinal cancer, inflammatory bowel disease (IBD), or a non-inflammatory gastrointestinal disease.
34. A method for treating inflammatory bowel disease (IBD) in a human or a companion animal, comprising:
 - obtaining a biological sample from the human or companion animal;
 - quantifying an expression level of one or more genes relative to a reference control, wherein the one or more genes are selected from the group consisting of LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, IFGGC1, HLA-DRB1,

MAPK8, STAT2, ITGB1, CSF3R, CEBPB, IDH1, PRDM1, CASP10, NOD1, S100A4, CASP8, TRGC2, LOC484343, CLEC4A, CD22, TNKS, IL21R, CCL17, IFNA7, SLAMF6, IL17F, TLR4, PTGER4, SELL, INPPL1, TANK, SH2D1A, OLIG2, LOC1021559, IRF3, GATA3, MAPK14, LOC481722, C7, CCR2, CD96, TFEB, FCAR, LOC487977, BLNK, GSK3B, and LOC1021567;

determining that the animal is susceptible to or suffering from IBD if the expression level of one or more genes selected from LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, IFGGC1, HLA-DRB1, MAPK8, STAT2, ITGB1, CSF3R, CEBPB, IDH1, PRDM1, CASP10, NOD1, S100A4, CASP8, TRGC2, LOC484343, CLEC4A, and CD22 in the biological sample is higher in comparison to the reference control and/or if the expression level of one or more genes selected from TNKS, IL21R, CCL17, IFNA7, SLAMF6, IL17F, TLR4, PTGER4, SELL, INPPL1, TANK, SH2D1A, OLIG2, LOC1021559, IRF3, GATA3, MAPK14, LOC481722, C7, CCR2, CD96, TFEB, FCAR, LOC487977, BLNK, GSK3B, and LOC1021567 in the biological sample is lower in comparison to the reference control; and

if the animal is susceptible to or suffering from IBD:

- i) administering to the companion animal an effective amount of a therapeutic agent for treating the IBD,
- ii) changing the diet of the human or companion animal, or
- iii) performing a fecal transfaunation.

35. The method of claim 34, wherein the one or more genes are selected from the group consisting of LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, STAT2, CSF3R, NOD1, S100A4, and TRGC2.

36. The method of claim 34 or 35, wherein the one or more genes are selected from the group consisting of IFNA7, TLR4, C7, CD96, TFEB, FCAR, LOC487977, BLNK, GSK3B, and LOC1021567.

37. The method of any one of claims 34-36, wherein the companion animal is a canine.

38. The method of claim 37, wherein the companion animal is a dog.
39. The method of any one of claims 34-38, wherein the companion animal is a feline.
40. The method of claim 39, wherein the companion animal is a cat.
41. The method of any one of claims 34-40, wherein the therapeutic agent is selected from an antibiotic, an immunosuppressive agent, or a probiotic.
42. The method of claim 41, wherein the antibiotic comprises metronidazole, tylosin, or ampicillin.
43. The method of claim 41, wherein the immunosuppressive agent comprises prednisone, prednisolone, budesonide, cyclosporine, mycophenolate, or chlorambucil.
44. The method of any one of claims 34-43, wherein the biological sample is a fecal sample, a sputum sample, an oral swab sample, a vaginal swab sample, a urethral swab sample, a nasal swab sample, an ocular swab sample, an aural swab sample, or a skin swab sample.
45. A method for diagnosing inflammatory bowel disease (IBD) in a human or companion animal, comprising:
obtaining a biological sample from the human or companion animal;
quantifying an expression level of one or more genes relative to a reference control,
wherein the one or more genes are selected from the group consisting of LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, IFGGC1, HLA-DRB1, MAPK8, STAT2, ITGB1, CSF3R, CEBPB, IDH1, PRDM1, CASP10, NOD1, S100A4, CASP8, TRGC2, LOC484343, CLEC4A, CD22, TNKS, IL21R, CCL17, IFNA7, SLAMF6, IL17F, TLR4, PTGER4, SELL, INPPL1, TANK, SH2D1A, OLIG2, LOC1021559, IRF3, GATA3, MAPK14, LOC481722, C7, CCR2, CD96, TFEB, FCAR, LOC487977, BLNK, GSK3B, and LOC1021567; and

determining that the animal is susceptible to or suffering from IBD if the expression level of one or more genes selected from LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, IFGGC1, HLA-DRB1, MAPK8, STAT2, ITGB1, CSF3R, CEBPB, IDH1, PRDM1, CASP10, NOD1, S100A4, CASP8, TRGC2, LOC484343, CLEC4A, and CD22 in the biological sample is higher in comparison to the reference control and/or if the expression level of one or more genes selected from TNKS, IL21R, CCL17, IFNA7, SLAMF6, IL17F, TLR4, PTGER4, SELL, INPPL1, TANK, SH2D1A, OLIG2, LOC1021559, IRF3, GATA3, MAPK14, LOC481722, C7, CCR2, CD96, TFEB, FCAR, LOC487977, BLNK, GSK3B, and LOC1021567 in the biological sample is lower in comparison to the reference control.

46. The method of claim 45, wherein the method further comprises administering to the human or companion animal an effective amount of a therapeutic agent for treating the IBD, changing the diet of the human or companion animal, or performing a fecal transfaunation, if the animal is susceptible to or suffering from IBD.
47. The method of claim 45 or 46, wherein the one or more genes are selected from the group consisting of LOC489428, DLA-DQB1, IDO1, CCL25, BAX, IL12RB2, PSMB8, STAT2, CSF3R, NOD1, S100A4, and TRGC2.
48. The method of any one of claims 45-47, wherein the one or more genes are selected from the group consisting of IFNA7, TLR4, C7, CD96, TFEB, FCAR, LOC487977, BLNK, GSK38, and LOC1021567.
49. The method of any one of claims 45-48, wherein the companion animal is a canine.
50. The method of claim 49, wherein the companion animal is a dog.
51. The method of any one of claims 45-50, wherein the companion animal is a feline.
52. The method of claim 51, wherein the companion animal is a cat.

53. The method of any one of claims 45-52, wherein the therapeutic agent is selected from an antibiotic, an immunosuppressive agent, or a probiotic.
54. The method of claim 53, wherein the antibiotic comprises metronidazole, tylosin, or ampicillin.
55. The method of claim 53, wherein the immunosuppressive agent comprises prednisone, prednisolone, budesonide, cyclosporine, mycophenolate, or chlorambucil.
56. The method of any one of claims 45-55, wherein the biological sample is a fecal sample, a sputum sample, an oral swab sample, a vaginal swab sample, a urethral swab sample, a nasal swab sample, an ocular swab sample, an aural swab sample, or a skin swab sample.
57. A method for treating a gastrointestinal disorder in a human or companion animal, comprising:
obtaining a stool sample from the human or companion animal;
quantifying an expression level of one or more genes relative to a reference control,
wherein the one or more genes are selected from the group consisting of MSR1, ARG1, IL11RA, IL2RG, CTSS, RUNX1, CCR1, ADA, TIGIT, MR1, IL27, CRADD, PSMB8, and HLA-DRB3;
determining that the human or companion animal is susceptible to or suffering from the gastrointestinal disorder if the expression level of one or more genes selected from MSR1 and ARG1 in the biological sample is higher in comparison to the reference control and/or if the expression level of one or more genes selected from IL11RA, IL2RG, CTSS, RUNX1, CCR1, ADA, TIGIT, MR1, IL27, CRADD, PSMB8, and HLA-DRB3 in the biological sample is lower in comparison to the reference control;
and
if the human or companion animal is susceptible to or suffering from the gastrointestinal disorder:
i) administering to the human or companion animal an effective amount of a therapeutic agent for treating the gastrointestinal disorder, and/or

- ii) changing the diet of the human or companion animal.

58. A method for diagnosing a gastrointestinal disorder in a human or companion animal, comprising:
obtaining a stool sample from the human or companion animal;
quantifying an expression level of one or more genes relative to a reference control,
wherein the one or more genes are selected from the group consisting of MSR1, ARG1, IL11RA, IL2RG, CTSS, RUNX1, CCR1, ADA, TIGIT, MR1, IL27, CRADD, PSMB8, and HLA-DRB3; and
determining that the human or companion animal is susceptible to or suffering from the gastrointestinal disorder if the expression level of one or more genes selected from MSR1 and ARG1 in the biological sample is higher in comparison to the reference control and/or if the expression level of one or more genes selected from IL11RA, IL2RG, CTSS, RUNX1, CCR1, ADA, TIGIT, MR1, IL27, CRADD, PSMB8, and HLA-DRB3 in the biological sample is lower in comparison to the reference control.
59. The method of claim 57 or 58, wherein the gastrointestinal disorder is IBD, a gastrointestinal cancer, a gastrointestinal infection, or a non-inflammatory gastrointestinal disease.
60. A method for treating gastrointestinal cancer in a human or companion animal, comprising:
obtaining a stool sample from the human or companion animal;
quantifying an expression level of one or more genes relative to a reference control,
wherein the one or more genes are selected from the group consisting of S100A8, IDO1, CXCL8, FOS, IL1RN, EGR1, CEBPB, EPCAM, S100A9, PLAUR, S100A12, DUSP1, CD74, NFKBIA, LCP1, C1QBP, TNFAIP3, DLA-DRA, DDX5, LGALS3, CCND2, MAP2K2, MEF2C, PSMB9, IFNA7, ANXA1, PTEN, MAPK14, MME, C6, LYN, STAT3, CIITA, MAPK8, TRB1, ITGAV, ERBB2, NFKB2, FCER1G, HMGCR, RPS6, HBEGF, NDRG1, STAT2, APP, VIM, MAP2K1, CD28, and GPI;
determining that the human or companion animal is susceptible to or suffering from gastrointestinal cancer if the expression level of one or more genes selected from

S100A8, IDO1, CXCL8, FOS, IL1RN, EGR1, CEBPB, S100A9, PLAUR, S100A12, DUSP1, CD74, NFKBIA, LCP1, TNFAIP3, DLA-DRA, DDX5, LGALS3, CCND2, MAP2K2, MEF2C, PSMB9, ANXA1, PTEN, MAPK14, MME, LYN, STAT3, CIITA, MAPK8, TRB1, ITGAV, ERBB2, NFKB2, FCER1G, HMGCR, RPS6, HBEGF, NDRG1, STAT2, APP, VIM, MAP2K1, and CD28 in the biological sample is higher in comparison to the reference control and/or if the expression level of one or more genes selected from EPCAM, C1QBP, IFNA7, C6, and GPI in the biological sample is lower in comparison to the reference control; and

administering to the human or companion animal an effective amount of a therapeutic agent for treating the gastrointestinal cancer if the human or companion animal is susceptible to or suffering from gastrointestinal lymphoma.

61. A method for diagnosing gastrointestinal cancer in a human or companion animal, comprising:

obtaining a stool sample from the human or companion animal;

quantifying an expression level of one or more genes relative to a reference control, wherein the one or more genes are selected from the group consisting of S100A8, IDO1, CXCL8, FOS, IL1RN, EGR1, CEBPB, EPCAM, S100A9, PLAUR, S100A12, DUSP1, CD74, NFKBIA, LCP1, C1QBP, TNFAIP3, DLA-DRA, DDX5, LGALS3, CCND2, MAP2K2, MEF2C, PSMB9, IFNA7, ANXA1, PTEN, MAPK14, MME, C6, LYN, STAT3, CIITA, MAPK8, TRB1, ITGAV, ERBB2, NFKB2, FCER1G, HMGCR, RPS6, HBEGF, NDRG1, STAT2, APP, VIM, MAP2K1, CD28, and GPI; and

determining that the human or companion animal is susceptible to or suffering from gastrointestinal cancer if the expression level of one or more genes selected from S100A8, IDO1, CXCL8, FOS, IL1RN, EGR1, CEBPB, S100A9, PLAUR, S100A12, DUSP1, CD74, NFKBIA, LCP1, TNFAIP3, DLA-DRA, DDX5, LGALS3, CCND2, MAP2K2, MEF2C, PSMB9, ANXA1, PTEN, MAPK14, MME, LYN, STAT3, CIITA, MAPK8, TRB1, ITGAV, ERBB2, NFKB2, FCER1G, HMGCR, RPS6, HBEGF, NDRG1, STAT2, APP, VIM, MAP2K1, and CD28 in the biological sample is higher in comparison to the reference control and/or if the expression level of one or more

genes selected from EPCAM, C1QBP, IFNA7, C6, and GPI in the biological sample is lower in comparison to the reference control.

62. The method of claim 60 or 61, wherein the gastrointestinal cancer is colorectal cancer, gastric cancer, or esophageal cancer.

IBD vs. Healthy feces 22 genes significant

Name	Description	Fold Change
LOC489428	interstitial collagenase-like	10.5
DLA-DQB1	major histocompatibility complex, class II, D	9.04167
IDO1	indoleamine 2,3-dioxygenase 1	8.22223
CCL25	chemokine (C-C motif) ligand 25	4.87408
BAX	BCL2-associated X protein	4.22221
IL12RB2	interleukin 12 receptor, beta 2	4
PSMB8	proteasome (prosome, macropain) subunit,	3.88889
STAT2	signal transducer and activator of transcript	3.22223
CSF3R	colony stimulating factor 3 receptor (granule	2.89899
NOD1	nucleotide-binding oligomerization domain	2.33333
S100A4	S100 calcium binding protein A4	2.05413
TRGC2		1.72054
IFNA7	interferon, alpha 7	-1.56845
TLR4	toll-like receptor 4	-1.67904
C7	complement component 7	-2.50714
CD96	CD96 molecule	-2.65049
TFEB	transcription factor EB	-2.73214
FCAR		-2.80519
LOC487977		-3.04512
BLNK	B-cell linker	-3.0857
GSK3B	glycogen synthase kinase 3 beta	-3.37501
LOC1021567	uncharacterized LOC102156778	-3.46153

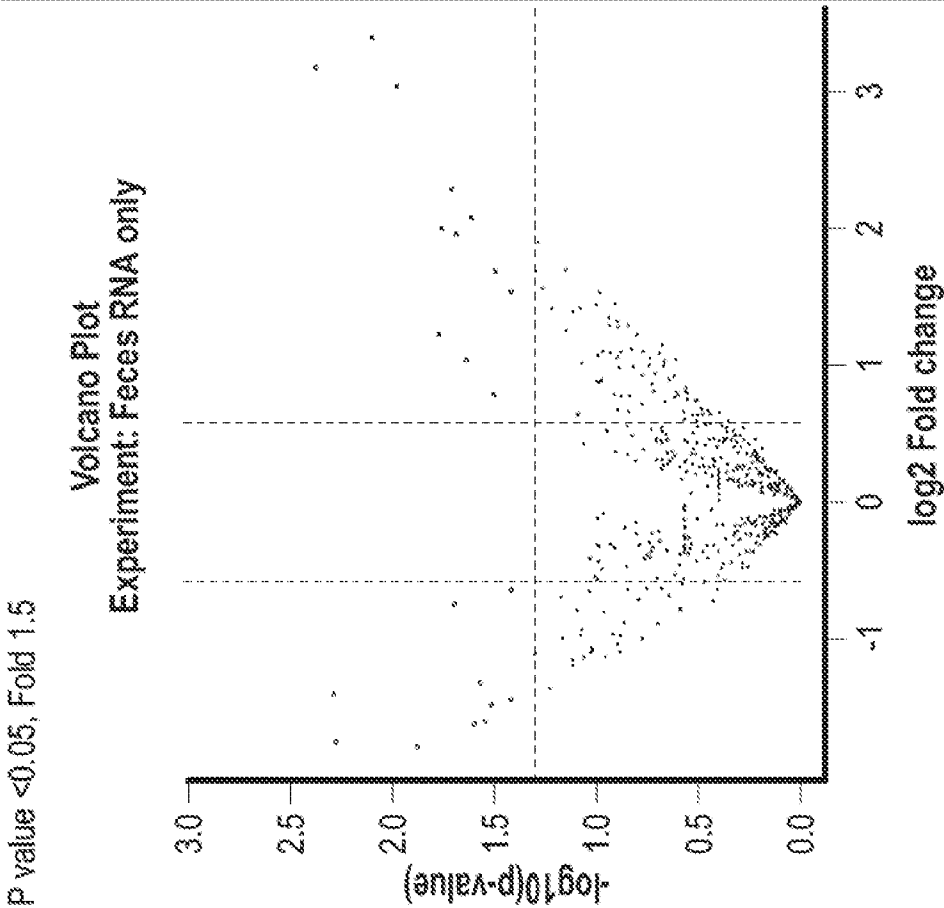
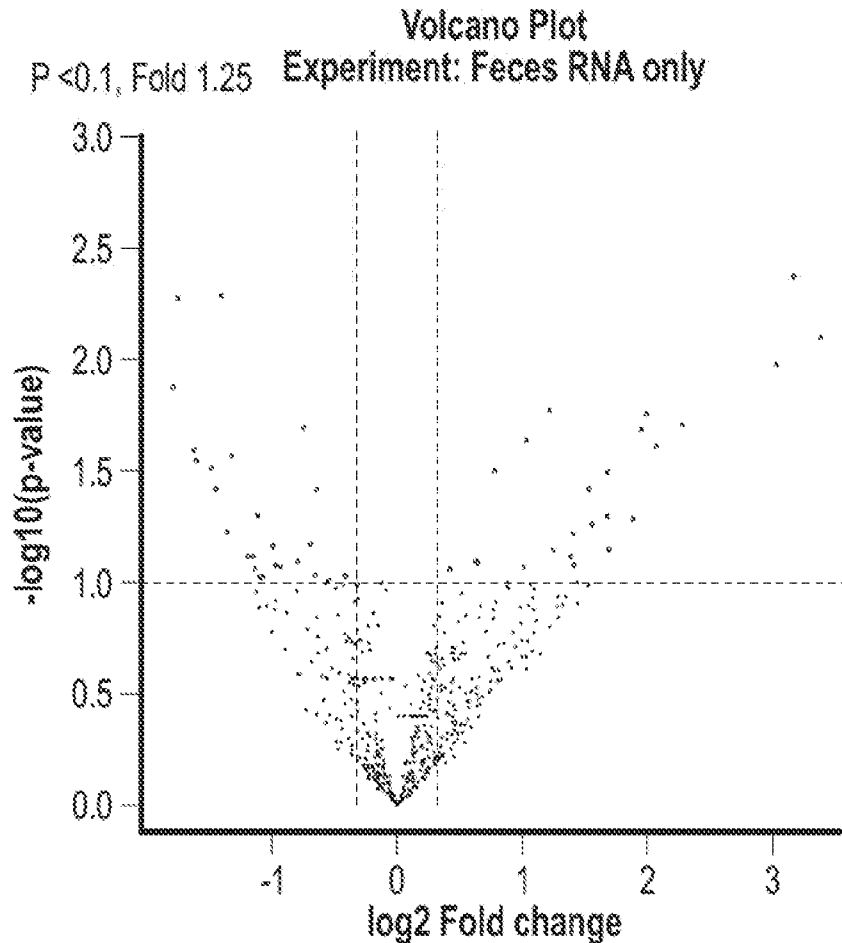


FIG. 1



IBD vs Healthy 51 genes

Name	Description	Fold Change	p-Value
LOC489428	interstitial collagenase-like	10.5	0.007933
DLA-DQB1	major histocompatibility comp	9.04167	0.004216
IDO1	indoleamine 2,3-dioxygenase 1	8.22223	0.010497
CCL25	chemokine (C-C motif) ligand 2	4.87408	0.01956
BAX	BCL2-associated X protein	4.22221	0.02445
IL12RB2	interleukin 12 receptor, beta 2	4	0.017504
PSMB8	proteasome (prosome, macrop	3.88889	0.020574
IFGCG1	interferon-inducible GTPase 1-	3.70941	0.05181
HLA-DRB1	MHC class II DLA DRB1 beta ch	3.24836	0.071214
MAPK8	mitogen-activated protein kina	3.22223	0.050271
STAT2	signal transducer and activator	3.22223	0.032047
ITGB1	integrin, beta 1 (fibronectin rec	2.95556	0.054795
CSF3R	colony stimulating factor 3 rece	2.89899	0.038159
CEBPB	CCAAT/enhancer binding prote	2.67901	0.083625
IDH1	isocitrate dehydrogenase 1 (NA	2.66667	0.060376

FIG. 2

PRDM1	PR domain containing 1, with 2	2.625	0.076674
CASP10	caspase 10, apoptosis-related	2.38518	0.071567
NOD1	nucleotide-binding oligomeriza	2.33333	0.016861
S100A4	S100 calcium binding protein A	2.05413	0.022969
CASP8	caspase 8, apoptosis-related cy	2.02222	0.085316
TRGC2		1.72054	0.031506
LOC484343		1.5681	0.081763
CLEC4A	C-type lectin domain family 4,	1.55183	0.080178
CD22	CD22 molecule	1.34234	0.086894
TNKS	tankyrase, TRF1-interacting an	-1.32777	0.093936
IL21R	interleukin 21 receptor	-1.33548	0.092883
CCL17	chemokine (C-C motif) ligand 1	-1.45909	0.097925
IFNA7	interferon, alpha 7	-1.56845	0.038219
SLAMF6	SLAM family member 6	-1.57482	0.092391
IL17F	interleukin 17F	-1.61823	0.067458
TLR4	toll-like receptor 4	-1.67904	0.020202
PTGER4	prostaglandin E receptor 4 (sub	-1.734	0.080261
SELL	selectin L	-1.90909	0.085147
INPPL1	inositol polyphosphate phospho	-1.96241	0.083692
TANK	TRAF family member-associate	-1.99507	0.068678
SH2D1A	SH2 domain containing 1A	-2.10389	0.094534
OLIG2	oligodendrocyte lineage transc	-2.12143	0.095189
LOC1021559	uncharacterized LOC102155900	-2.14286	0.094404
IRF3	interferon regulatory factor 3	-2.16541	0.050195
GATA3	GATA binding protein 3	-2.20409	0.086719
MAPK14	mitogen-activated protein kina	-2.22857	0.076264
LOC481722		-2.28572	0.076481
C7	complement component 7	-2.50714	0.026888
CCR2	chemokine (C-C motif) recepto	-2.57143	0.059408
CD96	CD96 molecule	-2.65049	0.005173
TFEB	transcription factor EB	-2.73214	0.037979
FCAR		-2.80519	0.030636
LOC487977		-3.04512	0.028287
BLNK	B-cell linker	-3.0857	0.025414
GSK3B	glycogen synthase kinase 3 bet	-3.37501	0.005306
LOC1021567	uncharacterized LOC102156778	-3.46153	0.013246

FIG. 2 (Cont.)

Diagnostic gene expression signatures for canine inflammatory bowel disease

(Upregulated genes)

Name	Description	Fold Change	p-Value
LOC489428	interstitial collagenase-like	10.5	0.007933
DLA-DOB1	major histocompatibility comp	9.04167	0.004216
IDO1	indoleamine 2,3-dioxygenase 1	8.22223	0.010497
CCL25	chemokine (C-C motif) ligand 2	4.87408	0.01956
BAX	BCL2-associated X protein	4.22221	0.02445
IL12RB2	interleukin 12 receptor, beta 2	4	0.017504
PSMB8	proteasome (prosome, macrop	3.88889	0.020574
IFGCG1	interferon-inducible GTPase 1-	3.70941	0.05181
HLA-DRB1	MHC class II DLA DRB1 beta ch	3.24836	0.071214
MAPK8	mitogen-activated protein kina	3.22223	0.050271
STAT2	signal transducer and activator	3.22223	0.032047
ITGB1	integrin, beta 1 (fibronectin rec	2.95556	0.054795
CSF3R	colony stimulating factor 3 rec	2.89899	0.038159
CEBPB	CCAAT/enhancer binding prote	2.67901	0.083625
IDH1	isocitrate dehydrogenase 1 (NA	2.66667	0.060376
PRDM1	PR domain containing 1, with Z	2.625	0.076674
CASP10	caspase 10, apoptosis-related	2.38518	0.071567
NOD1	nucleotide-binding oligomeriza	2.33333	0.016861
S100A4	S100 calcium binding protein A	2.05413	0.022969
CASP8	caspase 8, apoptosis-related cy	2.02222	0.085316
TRGC2		1.72054	0.031506
LOC484343		1.5681	0.081763

(Downregulated genes)

Gene	Description	Fold Change	p-Value
TNKS	tankyrase, TRF1-interacting an	-1.32777	0.093936
IL21R	interleukin 21 receptor	-1.33548	0.092883
CCL17	chemokine (C-C motif) ligand 1	-1.45909	0.097925
IFNA7	interferon, alpha 7	-1.56845	0.038219
SLAMF6	SLAM family member 6	-1.57482	0.092391
IL17F	interleukin 17F	-1.61823	0.067458
TLR4	tol-like receptor 4	-1.67904	0.020202
PTGER4	prostaglandin E receptor 4 (sub	-1.734	0.080261
SELL	selectin L	-1.90909	0.085147
INPPL1	inositol polyphosphate phospho	-1.96241	0.083692
TANK	TRAF family member-associate	-1.99507	0.068678
SH2D1A	SH2 domain containing 1A	-2.10389	0.094534
OLIG2	oligodendrocyte lineage transc	-2.12143	0.095189
LOC1021559	uncharacterized LOC102155900	-2.14286	0.094404
IRF3	interferon regulatory factor 3	-2.16541	0.050195
GATA3	GATA binding protein 3	-2.20409	0.086719
MAPK14	mitogen-activated protein kina	-2.22857	0.076264
LOC481722		-2.28572	0.076481
C7	complement component 7	-2.50714	0.026888
CCR2	chemokine (C-C motif) recepto	-2.57143	0.059408
CD96	CD96 molecule	-2.65049	0.005173
TFEB	transcription factor EB	-2.73214	0.037979
FCAR		-2.80519	0.030636
LOC487977		-3.04512	0.028287
BLNK	B-cell linker	-3.0857	0.025414
GSK3B	glycogen synthase kinase 3 bet	-3.37501	0.005306
LOC1021567	uncharacterized LOC102156778	-3.46153	0.013246

FIG. 3

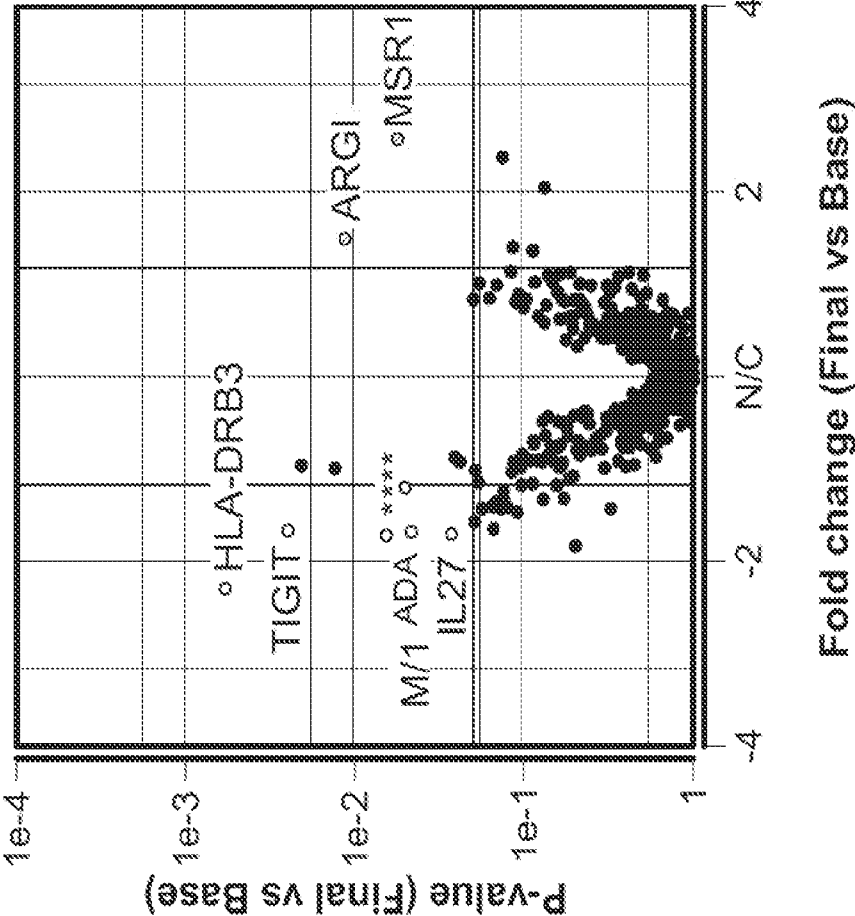
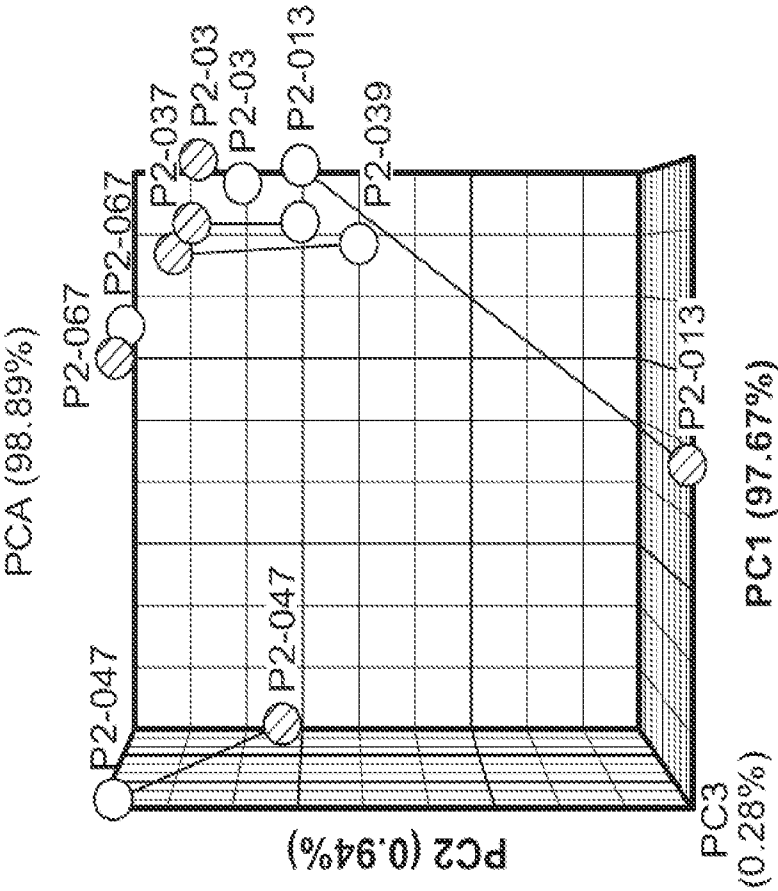
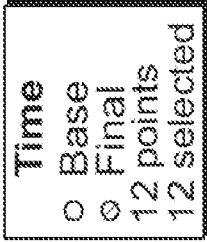


FIG. 4



Feature ID	Gene Description	P-value (Final vs. Base)	FDR step up (Final vs. Base)	Log2(Ratio) (Final vs. Base)	Fold change (Final vs. Base)
MSR1	Macrophage scavenger receptor types I and II	1.80E-02	9.76E-01	1.28	2.43
ARG1	Arginase-1	8.81E-03	9.76E-01	0.74	1.67
IL11RA	Interleukin-11 receptor subunit alpha	3.93E-02	9.76E-01	-0.43	-1.35
IL2RG	Cytokine receptor common subunit gamma	4.18E-02	9.76E-01	-0.47	-1.38
CTSS	Cathepsin S	4.86E-03	9.37E-01	-0.48	-1.39
RUNX1	Runt-related transcription factor 1	1.50E-02	9.76E-01	-0.49	-1.41
CCR1	C-C chemokine receptor type 1	7.73E-03	9.76E-01	-0.50	-1.41
ADA	Adenosine deaminase	2.00E-02	9.76E-01	-0.60	-1.52
TIGIT	T-cell immunoreceptor with Ig and ITIM domains	4.06E-03	9.37E-01	-0.83	-1.78
MR1	Probable hydrolase PNKD	2.16E-02	9.76E-01	-0.84	-1.79
IL27	Interleukin-27 subunit alpha	3.78E-02	9.76E-01	-0.85	-1.80
CRADD	Death domain-containing protein CRADD	1.55E-02	9.76E-01	-0.86	-1.81
PSMB8	Proteasome subunit beta type-8	1.56E-02	9.76E-01	-0.86	-1.82
HLA-DRB3	HLA class II histocompatibility antigen, DR beta 3 chain	1.72E-03	9.37E-01	-1.15	-2.21

FIG. 5

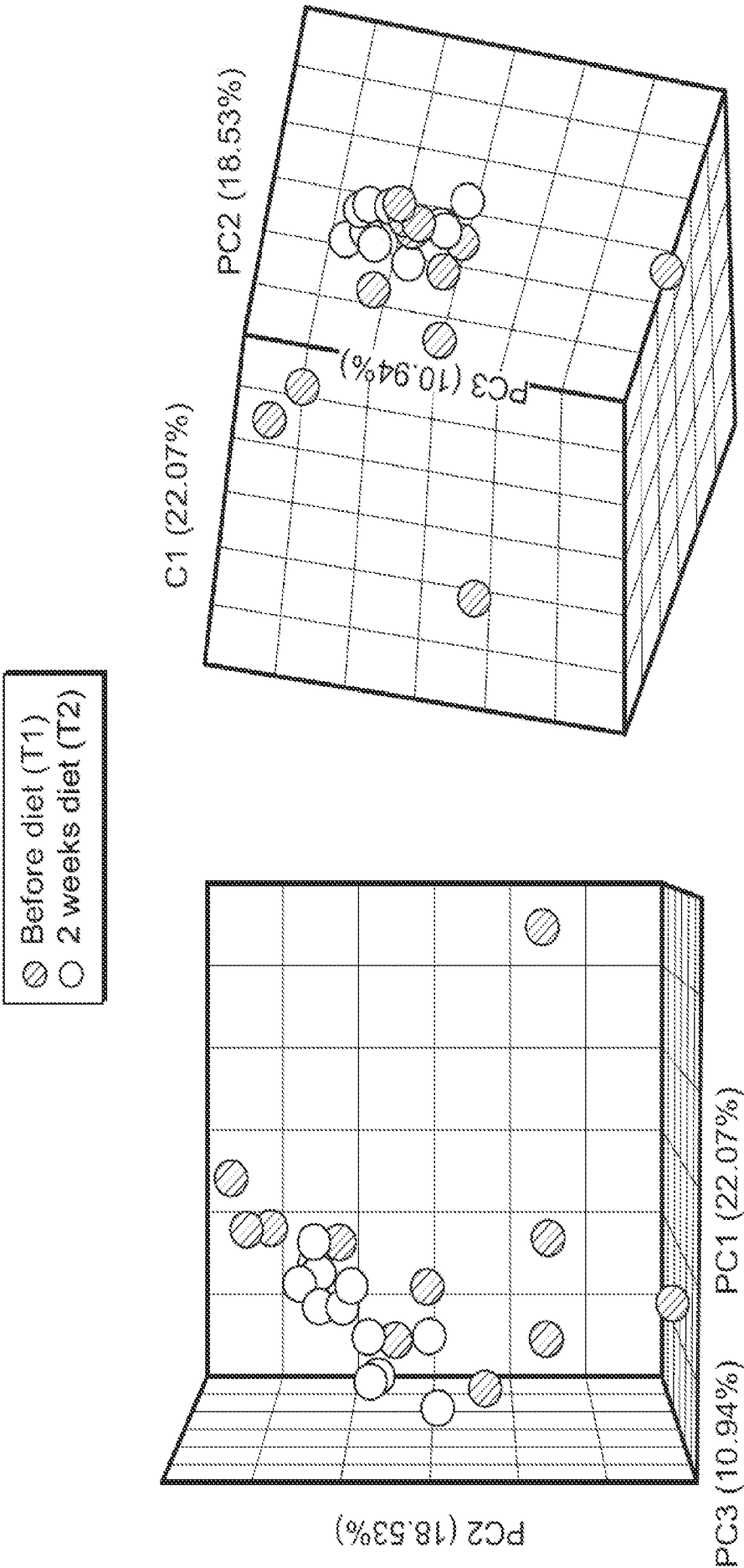
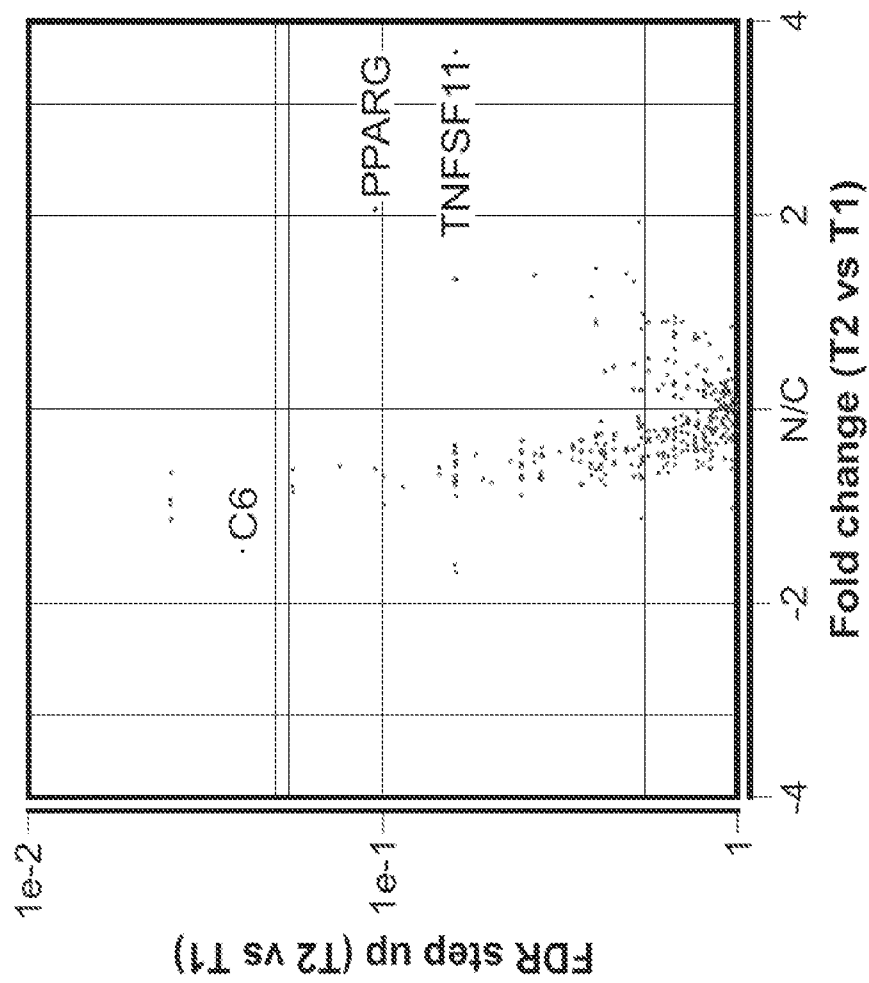


FIG. 6



2 wks diet vs. before diet

gene	fold change	P-value	FDR step up
C6	-1.66	6.59×10^{-4}	.04
PPARG	2.03	3.89×10^{-3}	.10
TNFSF11	3.58	.02	.16

FIG. 7

Probe Name	Description	Lymphoma-Cat	Healthy- Cat
S100A8	Protein S100-A8	2804.25	16.83
IDO1	Indoleamine 2,3-dioxygenase 1	2533.51	72.49
CXCL8	Interleukin-8	1926.38	23.3
FOS	Proto-oncogene c-Fos	1561.29	737.83
IL1RN	Interleukin-1 receptor antagonist protein	964.01	22.01
EGR1	Early growth response protein 1	813.87	76.37
CEBPB	CCAAT/enhancer-binding protein beta	637.48	16.83
EPCAM	Epithelial cell adhesion molecule	581.69	634.28
S100A9	Protein S100-A9	575.13	10.36
PLAUR	Urokinase plasminogen activator surface receptor	568.56	33.66
S100A12	Protein S100-A12	509.49	19.42
DUSP1	Dual specificity protein phosphatase 1	454.52	188.99
CD74	HLA class II histocompatibility antigen gamma chain	379.04	32.36
NFKBIA	NF-kappa-B inhibitor alpha	306.02	73.78
LCP1	Plastin-2	269.92	5.18
C1QBP	Complement component 1 Q subcomponent-binding protein, mitochondrial	263.36	371.51
TNFAIP3	Tumor necrosis factor alpha-induced protein 3	256.8	20.71
DLA-DRA	MHC class II DR alpha chain	201.01	75.08
DDX5	Probable ATP-dependent RNA helicase DDX5	179.68	69.9
LGALS3	Galectin-3	176.39	124.27
CCND2	G1/S-specific cyclin-D2	159.16	19.42
MAP2K2	Dual specificity mitogen-activated protein kinase kinase 2	146.04	76.37
MEF2C	Myocyte-specific enhancer factor 2C	142.76	60.84
PSMB9	Proteasome subunit beta type-9	128.81	37.54
IFNA7	Interferon alpha-7	125.53	173.46
ANXA1	Annexin A1	124.71	71.19
PTEN	Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN	124.71	27.18
MAPK14	Mitogen-activated protein kinase 14	120.6	32.36
MME	Macrophage metalloelastase	109.12	41.42
C6	Complement component C6	108.3	143.68
LYN	Tyrosine-protein kinase Lyn	100.91	18.12

FIG. 8

STAT3	Signal transducer and activator of transcription 3	98.45	23.3
CIITA	MHC class II transactivator	95.99	24.59
MAPK8	Mitogen-activated protein kinase 8	91.89	81.55
TRIB1	Tribbles homolog 1	89.43	27.18
ITGAV	Integrin alpha-V	88.61	75.08
ERBB2	Receptor tyrosine-protein kinase erbB-2	85.33	69.9
NFKB2	Nuclear factor NF-kappa-B p100 subunit	85.33	11.65
FCER1G	High affinity immunoglobulin epsilon receptor subunit gamma	84.5	12.94
HMGCR	3-hydroxy-3-methylglutaryl-coenzyme A reductase	82.86	53.07
RPS6	40S ribosomal protein S6	82.86	31.07
HBEGF	Proheparin-binding EGF-like growth factor	81.22	42.72
NDRG1	Protein NDRG1	81.22	32.36
STAT2	Signal transducer and activator of transcription 2	80.4	25.89
APP	Amyloid-beta precursor protein	78.76	54.37
VIM	Vimentin	77.12	15.53
MAP2K1	Dual specificity mitogen-activated protein kinase kinase 1	75.48	29.77
CD28	T-cell-specific surface glycoprotein CD28	74.66	3.88
GPI	Glucose-6-phosphate isomerase	74.66	76.37
PLAU	Urokinase-type plasminogen activator	74.66	18.12

FIG. 8 (Cont.)

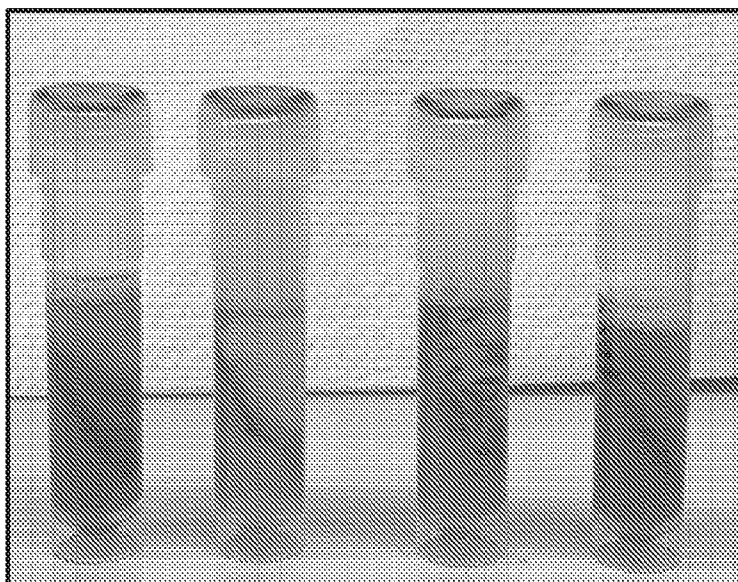


FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US22/48614

A. CLASSIFICATION OF SUBJECT MATTER		
IPC - INV. C12Q 1/6883; A61P 1/00; C12Q 1/6806; C12Q 1/6813; G01N 33/50 (2023.01)		
ADD.		
CPC - INV. C12Q 1/6883; A61P 1/00; C12Q 1/6806; C12Q 1/6813; G01N 33/5091		
ADD. C12Q 2527/119; C12Q 2527/137; G01N 33/53		
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) See Search History document		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched See Search History document		
Electronic database consulted during the international search (name of database and, where practicable, search terms used) See Search History document		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	(QIAGEN) RNeasy PowerMicrobiome Kit Handbook. Kit handbook (online). Qiagen. 27 February 2020 [2 March 2023]. Retrieved from the Internet: [URL: https://www.qiagen.com/au/resources/download.aspx?id=84c1f2e7-8db6-4957-a504-92bf9f82dd84&lang=en]; page 6, 1st paragraph; page 10, 5th, 6th, 7th and 8th paragraphs; page 17, 1st paragraph; page 18, 4th paragraph.	1-5, 18-19, 26/18-19, 27/26/18-19 — 20-21, 26/20-21, 27/26/20-21
X — Y	(WILKE, VL et al.) Gene expression in intestinal mucosal biopsy specimens obtained from dogs with chronic enteropathy. American Journal of Veterinary Research. August 2012, Vol. 73, No. 8; pages 1219-1229; abstract; page 1219, 1st column, 1st paragraph and 2nd column, 1st paragraph; page 1223, 2nd column, 1st paragraph; page 1226, 1st column, 3rd paragraph; DOI: 10.2460/ajvr.73.8.1219	34-35, 45-47 — 20-21, 26/20-21, 27/26/20-21
A	(WANG, YD et al.) Expression of matrix metalloproteinase-1 and tissue inhibitor of metalloproteinase-1 in ulcerative colitis. World Journal of Gastroenterology. 7 October 2006, Vol. 12, No. 37; pages 6050-6053; entire document; DOI: 10.3748/wjg.v12.i37.6050	1-5, 18-21, 26-27, 34-35, 45-47
☐ Further documents are listed in the continuation of Box C. ☐ 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 "D" document cited by the applicant in the international application "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 03 March 2023 (03.03.2023)		Date of mailing of the international search report MAR 27 2023
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300		Authorized officer Shane Thomas Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US22/48614

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.: 6-17, 28-33, 37-44, 48-56
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:
-***-Please See Supplemental Page-***-

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 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.:
Groups I+, Claims 1-5, 18-27, 34-36, 45-47, 57-62, and LOC489428 (gene for which RNA expression is measured), IBD (gastrointestinal disorder).

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.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US22/48614

-Continued From Box No. III: Observations where unity of invention is lacking-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-5, 18-27, 34-36, 45-47, 57-62, and LOC489428 (gene for which RNA expression is measured), IBD (gastrointestinal disorder) are directed towards gene RNA levels in samples and methods and systems associated therewith.

The gene RNA levels in samples and methods and systems of Claims 1-5, 18-19, 20-21 (each in-part), 26-27 (each in-part), 34-35 (each in-part), 45-47 (each in-part) are believed to encompass the first named invention of Groups I+ and are the claims that will be searched without fee to the extent that they encompass LOC489428 (first exemplary gene for which RNA expression is measured), IBD (first exemplary gastrointestinal disorder). This first named invention of Group I+ has been selected to encompass the first species of each of the genus found in claims 20-21, 34-35, 45, and 47 based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines.

Applicant is invited to elect additional gene(s), gastrointestinal disorder(s) to be searched. Additional gene(s), gastrointestinal disorder(s) will be searched upon the payment of additional fees. Applicants must specify the searchable claims that encompass any additionally elected gene(s), gastrointestinal disorder(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. Exemplary elections would be S100A8 (gene for which RNA expression is measured), gastrointestinal cancer (gastrointestinal disorder).

Groups I+ were considered to share the technical features including: a method of measuring a level of an RNA in a stool sample, comprising: a. obtaining a stool sample; b. extracting the RNA from the stool sample; and c. measuring the level of the RNA; a system comprising a collection tube; a preservation solution for a stool sample; and a set of polynucleotides for measuring levels of target RNAs; a method for treating inflammatory bowel disease or IBD in a human or a companion animal, comprising: obtaining a biological sample from the human or companion animal; quantifying an expression level of one or more genes relative to a reference control; determining that the animal is susceptible to or suffering from IBD if the expression level of one or more genes in the biological sample is higher in comparison to the reference control; and if the animal is susceptible to or suffering from IBD: i. administering to the companion animal an effective amount of a therapeutic agent for treating the IBD, ii. changing the diet of the human or companion animal, or iii. performing a fecal transfaunation; a method for diagnosing inflammatory bowel disease or IBD in a human or companion animal, comprising: obtaining a biological sample from the human or companion animal; quantifying an expression level of one or more genes relative to a reference control; and determining that the animal is susceptible to or suffering from IBD if the expression level of one or more genes in the biological sample is higher or lower in comparison to the reference control; a method for treating a gastrointestinal disorder in a human or companion animal, comprising: obtaining a stool sample from the human or companion animal; quantifying an expression level of one or more genes relative to a reference control; determining that the human or companion animal is susceptible to or suffering from the gastrointestinal disorder if the expression level of one or more genes is higher or lower in comparison to the reference control; and if the human or companion animal is susceptible to or suffering from the gastrointestinal disorder: i. administering to the human or companion animal an effective amount of a therapeutic agent for treating the gastrointestinal disorder, and/or ii. changing the diet of the human or companion animal; a method for diagnosing a gastrointestinal disorder in a human or companion animal, comprising: obtaining a stool sample from the human or companion animal; quantifying an expression level of one or more genes relative to a reference control; and determining that the human or companion animal is susceptible to or suffering from the gastrointestinal disorder if the expression level of one or more genes in the biological sample is higher or lower in comparison to the reference control; these shared technical features are previously disclosed by WO 2019/116234 A1 to Nestec S.A. (hereinafter "Nestec").

Nestec discloses a method of measuring a level of an RNA in a stool sample, comprising: a. obtaining a stool sample; b. extracting the RNA from the stool sample; and c. measuring the level of the RNA (method for detecting human and microbiome ribosomal RNA levels in a stool sample, comprising extracting RNA from the stool sample and performing an assay to determine the abundance of an RNA level; paragraphs [0027], [0034], [0104]); a system comprising a collection tube; a preservation solution for a stool sample; and a set of polynucleotides for measuring levels of target RNAs (the stool sample is placed in a container (collection tube) and shipped in the presence of transport media including reagents that stabilize nucleic acid or microbial communities in the stool sample (preservation solution for a stool sample), wherein the transport media contains a DNA or RNA oligonucleotide that is used as an internal control when assessing the contents of the stool sample (a set of polynucleotides for measuring levels of target RNAs); paragraph [0143]); a method for treating inflammatory bowel disease or IBD in a human or a companion animal (a method for determining the efficacy of a clinical treatment, such as a drug or antibody, in a human subject having IBD; paragraphs [0004], [0024], [0058], [0060]), comprising: obtaining a biological sample from the human or companion animal; quantifying an expression level of one or more genes relative to a reference control; determining that the animal is susceptible to or suffering from IBD if the expression level of one or more genes in the biological sample is higher or lower in comparison to the reference control (obtaining a stool sample (biological sample) from the human, quantifying an expression level of one or more rRNA genes relative to a reference control, determining that the human is suffering from IBD if the expression level of the rRNA genes in the stool sample is higher in comparison to the reference control; paragraphs [0058], [0059], [0060], [0073]); and if the animal is susceptible to or suffering from IBD: i. administering to the companion animal an effective amount of a therapeutic agent for treating the IBD (determining the efficacy of a clinical treatment, such as a drug or antibody (therapeutic agent), in a human subject having IBD, thereby indicating that an adjusted dose or a different drug would be administered if the clinical treatment tested is not found to be efficacious; paragraphs [0004], [0024], [0058], [0060]), ii. changing the diet of the human or companion animal, or iii. performing a fecal transfaunation; a method for diagnosing inflammatory bowel disease or IBD in a human or companion animal, comprising: obtaining a biological sample from the human or companion animal; quantifying an expression level of one or more genes relative to a reference control; and determining that the animal is susceptible to or suffering from IBD if the expression level of one or more genes in the biological sample is higher or lower in comparison to the reference control (method for identifying a gastrointestinal-related condition, such as IBD, in a human subject, comprising obtaining a stool (biological) sample from the human, quantifying an expression level of one or more rRNA genes relative to a reference control, and determining that the human is suffering from IBD if the expression level of the rRNA genes in the stool sample is higher in comparison to the reference control; paragraphs [0004], [0024], [0034], [0058], [0061], [0073]); a method for treating a gastrointestinal disorder in a human or companion animal (a method for determining the efficacy of a clinical treatment, such as a drug or antibody, in a human subject having IBD; paragraphs [0004], [0024],

-Continued Within the Next Supplemental Box-

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US22/48614

-***-Continued from previous Supplemental Box-***-

[0058], [0060]), comprising: obtaining a stool sample from the human or companion animal; quantifying an expression level of one or more genes relative to a reference control; determining that the human or companion animal is susceptible to or suffering from the gastrointestinal disorder if the expression level of one or more genes is higher or lower in comparison to the reference control (obtaining a stool sample (biological sample) from the human, quantifying an expression level of one or more rRNA genes relative to a reference control, determining that the human is suffering from IBD if the expression level of the rRNA genes in the stool sample is higher in comparison to the reference control; paragraphs [0058], [0059], [0060], [0073]); and if the human or companion animal is susceptible to or suffering from the gastrointestinal disorder: i. administering to the human or companion animal an effective amount of a therapeutic agent for treating the gastrointestinal disorder (determining the efficacy of a clinical treatment, such as a drug or antibody (therapeutic agent), in a human subject having IBD, thereby indicating that an adjusted dose or a different drug would be administered if the clinical treatment tested is not found to be efficacious; paragraphs [0004], [0024], [0058], [0060]), and/or ii. changing the diet of the human or companion animal; a method for diagnosing a gastrointestinal disorder in a human or companion animal, comprising: obtaining a stool sample from the human or companion animal; quantifying an expression level of one or more genes relative to a reference control; and determining that the human or companion animal is susceptible to or suffering from the gastrointestinal disorder if the expression level of one or more genes in the biological sample is higher or lower in comparison to the reference control (method for identifying a gastrointestinal-related condition, such as IBD, in a human subject, comprising obtaining a stool (biological) sample from the human, quantifying an expression level of one or more rRNA genes relative to a reference control, and determining that the human is suffering from IBD if the expression level of the rRNA genes in the stool sample is higher in comparison to the reference control; paragraphs [0004], [0024], [0034], [0058], [0061], [0073]).

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Nestec reference, unity of invention is lacking.