(19) ORGANISATION AFRICAINE DE LA PROPRIETE INTELLECTUELLE

(51) Inter. Cl.[8] A61K 35/74 (2018.01); A61P 1/00 (2018.01); A61P 37/00 (2018.01)

(11) N° 20538

FASCICULE DE BREVET D'INVENTION

(21) Numéro de dépôt : 1202100153
PCT/EP2019/077332

(22) Date de dépôt : 09/10/2019

(30) Priorité(s) :

EP n° 18199455.9 du 09/10/2018

(24) Délivré le : 10/08/2022

(45) Publié le : 27/10/2022

(73) Titulaire(s) :
4D Pharma Research Limited,
Life Sciences Innovation Building,
Cornhill Road,
ABERDEEN
Aberdeenshire AB25 2ZS (GB)

(72) Inventeur(s) :
MULDER, Imke Elisabeth (GB);
JEFFERY, Ian (IE);
STEVENSON, Alexander (GB)

(74) Mandataire : **SPOOR & FISHER Inc. NGWAFOR & PARTNERS SARL, The House of Gideon, Golf/Bastos Quarters, Opposite The American Embassy, Entrance-Saint John Paul II Boulevard, B.P. 8211, YAOUNDE (CM).**

(54) **Titre : Compositions comprising bacterial strains.**

(57) **Abrégé :**

Provided are compositions comprising a bacterial strain of the genus Bacteroides, for use in a method of increasing the microbiota diversity and/or inducing stability of the microbiota of a subject.

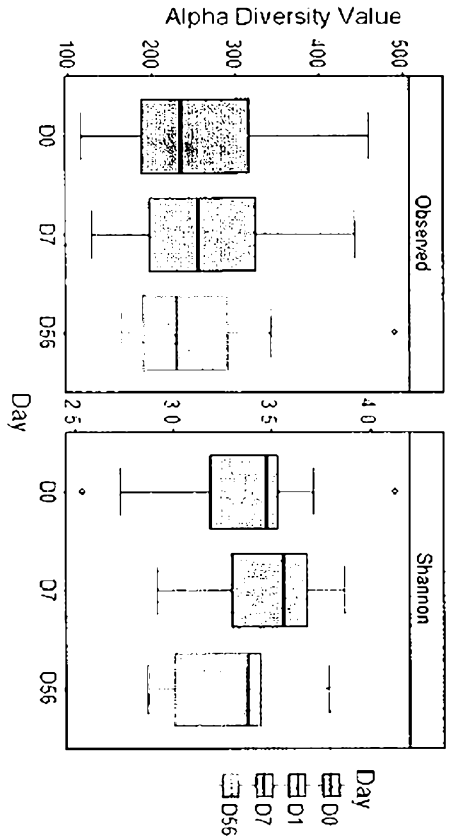


Fig. 1

COMPOSITIONS COMPRISING BACTERIAL STRAINS

TECHNICAL FIELD

This invention is in the field of compositions comprising bacterial strains isolated from the mammalian digestive tract and the use of such compositions in the treatment of disease.

BACKGROUND TO THE INVENTION

The human intestine is thought to be sterile *in utero*, but it is exposed to a large variety of maternal and environmental microbes immediately after birth. Thereafter, a dynamic period of microbial colonization and succession occurs, which is influenced by factors such as delivery mode, environment, diet and host genotype, all of which impact upon the composition of the gut microbiota, particularly during early life. Subsequently, the microbiota stabilizes and becomes adult-like [1]. The human gut microbiota contains more than 1500 different phylotypes dominated in abundance levels by two major bacterial divisions *(phyla)*, the Bacteroidetes and the Firmicutes [2]. The successful symbiotic relationships arising from bacterial colonization of the human gut have yielded a wide variety of metabolic, structural, protective and other beneficial functions. The enhanced metabolic activities of the colonized gut ensure that otherwise indigestible dietary components are degraded with release of by-products providing an important nutrient source for the host and additional health benefits. Similarly, the immunological importance of the gut microbiota is well-recognized and is exemplified in germfree animals which have an impaired immune system that is functionally reconstituted following the introduction of commensal bacteria [3-5].

Dramatic changes in microbiota composition have been documented in gastrointestinal disorders such as inflammatory bowel disease (IBD). For example, the levels of *Clostridium* cluster XIVa bacteria are reduced in IBD subjects whilst numbers of *E. coli* are increased, suggesting a shift in the balance of symbionts and pathobionts within the gut [6-9, 16].

In recognition of the potential positive effect that certain bacterial strains may have on the animal gut, various strains have been proposed for use in the treatment of various diseases (see, for example, [10-13]). A number of strains, including mostly *Lactobacillus* and *Bifidobacterium* strains, have been proposed for use in treating various bowel disorders (see [14] for a review and see [15]).

The relationship between different bacterial strains and different diseases, and the precise effects of particular bacterial strains on the gut and at a systemic level and on any particular types of

diseases, are poorly characterised and results to date are variable and pose more questions than provide answers [16].

While the term 'dysbiosis' has been used in the literature to generically define deleterious fluctuations in the microbiome, there is no universal definition of what does or does not constitute 'dysbiosis'. A more accurate and verifiable metric to assess perturbations in the microbiome is 'microbiota diversity' Loss of diversity is also measured by reductions in the Shannon Diversity Index. As those skilled in the art will be aware, the Shannon Diversity Index accounts for both the abundance (i.e. changes in the the populations of different OTUs present) and evenness (i.e. how numerically similar the populations of different OTUs present in the microbiome are) of species present in the microbiome. A significant variation in either abundance or evenness from the 'healthy' or 'normal' microbiome in a population equates to dysbiosis.

Reduced microbiota diversity is reported in recent studies of obesity, inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), type 2 diabetes and frailer older people [20]. In particular, references [17] and [18] teach that a reduced microbiota diversity is strongly associated with IBD and reference [17] furthers summarises studies concluding that increasing the microbiota diversity has curative effects on IBDs.

Re-establishing the healthy microbiota can be difficult, however, as the bacteria in the gut are resistant to colonisation. This poses a challenge when trying to treat the microbiota of unhealthy subjects by increasing the diversity of the microbiota [19]. The accompanying loss of microbial metabolic function is assumed to be a contributory factor to the symptoms of these pathophysiologies. In contrast to healthy adults in whom the microbiota is stable, the microbiota of unhealthy subjects such as those suffering from IBD, IBS and frail elderly subjects is unstable [16, 20].

There is a requirement for the profile effects of gut bacteria to be positively modified to permit the treatment of diseases or conditions characterised by reduced microbiota diversity and / or evenness.

SUMMARY OF THE INVENTION

The inventors have developed new therapies for treating and preventing diseases and disorders by increasing or maintaining the intestinal microbiota diversity in a subject. In particular, the inventors have unexpectedly identified that bacterial strains from the genus Bacteroides can be

effective in increasing or maintaining the diversity and/or evenness of different types of bacteria in the distal gut of a subject.

As described in the examples, an IBD patient population treated with an organism from the species *Bacteroides thetaiotaomicron* experienced a statistically significant increase in their microbiome diversity and evenness. Additionally, the examples show that treatment with compositions comprising *Bacteroides thetaiotaomicron* increased the stability of the microbiota in IBD subjects throughout the course of the study.

Therefore, in a first embodiment, the invention provides a composition comprising a bacterial strain of the species *Bacteroides thetaiotaomicron*, for use in a method of increasing or maintaining the microbiota diversity. Similarly, there is also provided a method of increasing or maintaining the microbiota diversity in a subject comprising use of a bacterial strain of the species *Bacteroides thetaiotaomicron*. Preferably, the subject has reduced microbiota diversity and/or stability.

The term "increasing or maintaining the microbiota diversity" is used herein to mean increasing or maintaining the number of different types of bacteria and/or the evenness of the different types of bacteria in the microbiota of a subject. In some embodiments, the microbiota diversity is increased. In some embodiments, the number of different genera of bacteria in the microbiota is increased. In some embodiments, the number of different species of bacteria in the microbiota is increased. In some embodiments, the number of different strains of bacteria in the microbiota is increased. In some embodiments, the microbiota diversity is maintained. In some embodiments, the number of different genera of bacteria in the microbiota is maintained. In some embodiments, the number of different species of bacteria in the microbiota is maintained. In some embodiments, the number of different strains of bacteria in the microbiota is maintained. In some embodiments, the number of genera, species and strains in the microbiota is increased or maintained.

The increase in microbiotia diversity may be for non-acetogenic bacteria. It may also be for both acetogenic and non-acetogenic bacteria. Such bacteria are well known in the art. Briefly, acetogenic bacteria produce acetate as an end product of anaerobic respiration or fermentation.

In some embodiments, loss, increase or maintenance of microbiota diversity may be quantified by a measurable reduction, increase or maintenance, respectively, in the number of the sequence-based bacterial classifications or Operational Taxonomic Units (OTUs) in a sample, typically

determined by 16S rRNA amplicon sequencing methods. In some embodiments, loss of diversity may be measured by reductions in the Shannon Diversity Index. Conversely, in some embodiments, an increase of diversity may be measured by an increase in the Shannon Diversity Index. Similarly, in some embodiments, maintenance of diversity may be measured by the same result in the Shannon Diversity Index.

In some embodiments, the evenness of the different types of bacteria is increased. In some embodiments, the relative abundance of the different types of bacteria in the microbiota becomes more even following administration of a composition of the invention.

The inventors have also developed new therapies for treating and preventing diseases and disorders by inducing stability of the intestinal microbiota. In particular, the inventors have identified that bacterial strains from the genus Bacteroides induce stability of the intestinal microbiota. By "induce stability" is meant that the microbiota diversity remains stable and also the relative numbers of the different genera in the microbiota remains stable. Thus, the relative numbers may fluctuate by less than 10%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2% or less than 1%.

Stability of the intestinal microbiota is important as a number of diseases and disorders, including IBS and IBD, are characterised by reduced stability of the microbiota. As described in the examples, oral administration of compositions comprising *Bacteroides thetaiotaomicron* induces stability of the microbiota in stool. Therefore, in a further embodiment, the invention provides a composition comprising a bacterial strain of the species *Bacteroides thetaiotaomicron*, for use in a method of inducing stability of the microbiota in a subject. Similarly, there is also provided a method of inducing stability of the microbiota in a subject comprising use of a bacterial strain of the species *Bacteroides thetaiotaomicron*.

In some embodiments, the relative numbers of the different bacterial species in the microbiota of a subject becomes more stable following treatment or prevention with a composition of the invention, for example in a subject diagnosed with a disease or disorder characterised by a reduction in the diversity of microbiota. In some embodiments, the relative numbers of the different bacterial genera in the microbiota of a subject becomes more stable following treatment or prevention with a composition of the invention, for example in a subject diagnosed with a disease or disorder characterised by a reduction in the diversity of microbiota. The stability of a subject's microbiota can be assessed by comparing the microbiome from the subject at two

different time points. If there is a difference in the microbiome, this can be indicative of disease or of a disorder being present. In some embodiments, the two different time points are at least three days apart (e.g. at least 1 week, 2 weeks, 1 month, 3 months, 6 months, 1 year, 2 years apart). In some embodiments, the two different time points are 3-7 days apart, 1-2 weeks apart, 2-4 weeks apart, 4-8 weeks apart, 8-24 weeks apart, 24-40 weeks apart, 40-52 weeks apart or more than 52 weeks apart. In some embodiments, more than two different time points are used, e.g. three, four, five or more than five time points. Suitable intervals are chosen between the various time points, for example, as set out above.

The bacterial strain may be *Bacteroides thetaiotaomicron* and is preferably the strain deposited under accession number NCIMB 42341. This strain was deposited with the international depositary authority NCIMB, Ltd. (Ferguson Building, Aberdeen, AB21 9YA, Scotland) on 3rd December 2014.

Further *Bacteroides thetaiotaomicron* strains for use in the invention is the type strain ATCC 29148. The 16S rRNA gene sequences for these strains are disclosed as SEQ ID NOs 2. A further preferred *Bacteroides thetaiotaomicron* strain for use in the invention is the strain described in EP1448995. The accession number for the 16S rRNA gene sequence of *Bacteroides thetaiotaomicron* strain WAL 2926 is M58763 (disclosed herein as SEQ ID NO:3). Other suitable *Bacteroides thetaiotaomicron* strains have the 16S rRNA sequences of SEQ ID NOs 4-12.

In some embodiments, the microbiota diversity, evenness and/or the stability of the microbiota refers to the microbiota diversity, evenness and/or the stability in a stool sample from the subject. In some embodiments, the microbiota diversity, evenness and/or the stability of the microbiota refers to the microbiota diversity and/or the stability in the distal gut of the subject. In some embodiments, the microbiota diversity, evenness and/or the stability of the microbiota refers to the microbiota diversity, evenness and/or the stability in the gastrointestinal tract of the subject. In some embodiments, the microbiota diversity, evenness and/or the stability of the microbiota refers to the microbiota diversity, evenness and/or the stability in the caecum. In some embodiments, the microbiota diversity, evenness and/or the stability of the microbiota refers to the microbiota diversity, evenness and/or the stability in the colon.

In some embodiments, the invention provides a composition comprising a bacterial strain of the species *Bacteroides thetaiotaomicron*, for use in a method of treating or preventing a disease or

disorder associated with a level of microbiota diversity that is reduced relative to the microbiota diversity of a healthy subject, or a population of healthy subjects. Such diseases are well known in the art and include, for example, IBS, IBD (such as Crohn's disease and ulcerative colitis) [21], cancer (for example colorectal cancer, or other cancers for example where a reduction in microbiota diversity is observed with concomitant cancer therapy treatment including chemotherapy), obesity [22], autism, allergy, celiac disease, infectious diseases, and graft versus host disease amongst others [23]. The invention is useful for treating these diseases. Preferably, the compositions of the invention are for use in treating IBD, in particular Crohn's disease, or cancer. Whilst these conditions may be associated with reduced microbiota diversity and/or stability this is not an inherent feature of these diseases as patients can suffer from these even if their microbiome diversity/stability is unaffeced. A skilled person can easily ascertain whether a patient suffering from any of these conditions has reduced microbiota diversity and/or stability relative to the levels in a healthy individual, or a population of healthy individuals as explained in further detail below. Thus, in embodiments of the invention, the subject to be treated, who may be diagnosed with one or more of the diseases discussed therein has reduced microbiota diversity and / or stability.

In some embodiments, the treatment or prevention using a composition of the invention results in the microbiota diversity, evenness and / or stability increasing to the levels corresponding to or greater than those present in a healthy individual, or a population of healthy individuals. A healthy individual in this context may be someone who does not suffer from a disease which is associated with reductions in microbiome diversity. A healthy individual may be the subject being treated prior to the onset or diagnosis of their disease; administration of the compositions of the invention may cause the diversity, evenness or stability of their microbiome to revert to their former, pre-disease levels.

In some embodiments, treatment or prevention using a composition of the invention results in the microbiota diversity, evenness and / or stability increasing to levels corresponding to or greater than those present in a population of healthy individuals.

In embodiments of the invention in which changes in microbiome diversity are determined with reference to a healthy individual or a population of healthy individuals, the healthy individual/s is/are resident in the same geographical region (e.g. resides within a 200km radius, within a 100km radius, or within a 50km radius) as the subject, is of a similar/same age to the subject and/or is of a similar/same race to the subject. Similarly, the invention also provides a method of

treatment or prevention of a disease or disorder associated with a level of microbiota diversity that is reduced relative to the microbiota diversity of a healthy individual or population of healthy individuals wherein the method comprises administering a composition comprising a bacterial strain of the genus Bacteroides.

The levels of microbiota diversity in a healthy individual are well known in the art and can be determined by a skilled person using methods known in the art (see, for example, reference [24]).

In some embodiments, the subject is an infant or child with a reduced microbiota diversity compared to a healthy infant or child (or population thereof), respectively. It has been observed that some children who develop a disease associated with a reduced microbiota diversity later in life have a reduced diversity of faecal microbiota as 1 week old infants [25]. Thus, in some embodiments, the infant is less than 1 week old, is less than 2 weeks old, is less than one month old, is less than two months old or is less than four months old. In some embodiments, the subject is an infant who has not been delivered via a vaginal birth. For example, in some embodiments, the subject is an infant who has been delivered by Caesarean section. Reduced microbiota diversity has also been reported in frail elderly subjects. In some embodiments, therefore, the subject is an elderly subject, for example, a frail elderly subject. In some embodiments, the subject is 65 or more years in age (e.g. 70 or more, 75 or more, 80 or more, 85 or more or 90 or more years in age) [20]. The subject may also be an adolescent. For example, the subject may be between 10 and 19 years of age.

It has been estimated that a healthy human individual has approximately 101 different bacterial species and 195 different bacterial strains in its microbiota [26]. Accordingly, in some embodiments, the composition is for use in treating a subject having fewer than 101 different bacterial species (e.g. fewer than 100, 99, 98, 97, 96, 95, 94, 93, 92, 91, 90, 85, 80, 75 or 70 bacterial species) and/or fewer than 195 different strains (e.g. less than 194, 193, 192, 191, 190, 189, 188, 187, 186, 185, 183, 180, 175, 170, 165, 160, 150, 140 bacterial strains) in its microbiota. In some embodiments, the treatment or prevention results in the microbiota diversity increasing to more than 80 bacterial species (e.g. more than 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 bacterial species) or to 101 bacterial species. For example, in some embodiments, the treatment or prevention results in the microbiota diversity increasing to more than 90 bacterial species. For example, in some embodiments, the treatment or prevention results in the microbiota diversity increasing to more than 95 bacterial species. For example, in some

embodiments, the treatment or prevention results in the microbiota diversity increasing to more than 97 bacterial species. For example, in some embodiments, the treatment or prevention results in the microbiota diversity increasing to more than 99 bacterial species. In some embodiments, the treatment or prevention results in the microbiota diversity increasing to more than 160 bacterial strains (e.g. more than 165, 170, 185, 186, 187, 188, 189, 190, 191, 192, 193 or 194 bacterial species) or to 195 bacterial strains. For example, in some embodiments, the treatment or prevention results in the microbiota diversity increasing to more than 175 bacterial strains. For example, in some embodiments, the treatment or prevention results in the microbiota diversity increasing to more than 185 bacterial strains. For example, in some embodiments, the treatment or prevention results in the microbiota diversity increasing to more than 190 bacterial strains.

In some embodiments, the treatment or prevention results in the microbiota diversity increasing by at least one bacterial genus (e.g. by at least two, three, four, five, six, seven, eight, nine or ten bacterial genera). In some embodiments, the treatment or prevention results in the microbiota diversity increasing by at least one bacterial species (e.g. by at least two, three, four, five, six, seven, eight, nine, ten, 12, 15, 17 or 20 bacterial species). In some embodiments, the treatment or prevention results in the microbiota diversity increasing by at least one bacterial strain (e.g. by at least two, three, four, five, six, seven, eight, nine, ten, 12, 15, 17, 20 or 25 bacterial strains).

In some embodiments, the invention provides a composition comprising a bacterial strain of the species *Bacteroides thetaiotaomicron*, for use in a method of treating or preventing a disease or disorder associated with reduced stability of the microbiota compared to the stability of the microbiota in a healthy subject (or compared to a population of healthy subjects). By "reduced stability of the microbiota" is meant that the microbiota diversity does not remain as stable and also the relative numbers of the different genera in the microbiota do not remain as stable as the stability observed in a healthy subject or in a population of healthy subjects. In some embodiments, inducing stability of the microbiota results in the stability being induced to a similar level as is present in a healthy subject, or in a population of healthy subjects. In some embodiments, inducing stability of the microbiota results in the stability being induced to the same level as is present in a healthy subject, or in a population of healthy subjects.

Similarly, the invention provides a method of treating or preventing a disease or disorder associated with reduced stability of the microbiota wherein the method comprises administering a composition comprising a bacterial strain of the species *Bacteroides thetaiotaomicron*. For

example, the pathogenesis of some diseases or disorders is characterised by reduced stability of the microbiota. Examples of such diseases and disorders are IBS, IBD, diabetes (e.g. type 2 diabetes), allergic diseases, autoimmune diseases and metabolic diseases/disorders. Accordingly, in some embodiments, the invention provides a composition comprising a bacterial strain of the species *Bacteroides thetaiotaomicron*, for use in a method of treating or preventing a disease or disorder associated with reduced stability of the microbiota, wherein the treatment or prevention comprises inducing stability of the microbiota. In some embodiments, the disease or disorder is selected from IBS, IBD, diabetes (e.g. type 2 diabetes), allergic diseases, autoimmune diseases and metabolic diseases/disorders. In some embodiments, the disease or disorder is IBS or IBD. In some embodiments, the disease or disorder is Crohn's disease. Accordingly, in some embodiments, the invention provides a composition comprising a bacterial strain of the species *Bacteroides thetaiotaomicron*, for use in a method of treating or preventing IBS or IBD (in particular Crohn's disease), wherein the treatment or prevention comprises inducing stability of the microbiota. In such embodiments, the composition may be administered to a subject having reduced microbiota diversity and / or stability.

In some embodiments, the invention provides a method of treatment or prevention of a disease or disorder associated with a level of microbiota diversity and/or evenness that is reduced relative to the microbiota diversity of a healthy subject or population of healthy subjects wherein the method comprises diagnosing a subject as having a reduced level of microbiota diversity and then if a reduced level of diversity is found to be present, administering a composition comprising a bacterial strain of the species *Bacteroides thetaiotaomicron* to the subject.

In some embodiments, the invention provides a method of treatment or prevention of a disease or disorder associated with reduced stability of microbiota relative to the stability of microbiota in a healthy subject wherein the method comprises diagnosing a subject as having reduced stability of microbiota and then if reduced stability is found to be present, administering a composition comprising a bacterial strain of the species *Bacteroides thetaiotaomicron* to the subject.

Strains closely related to the species *Bacteroides thetaiotaomicron* may also be used. Such bacterial strains may have a 16s rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to the 16s rRNA sequence of a bacterial strain of *Bacteroides thetaiotaomicron*. Preferably, the bacterial strain has a 16s rRNA sequence that is at least 95%, 95%, 97%, 98%, 99%, 99.5% or 99.9% identical to any one of SEQ ID NOs:1-12, preferably to SEQ ID NO: 1. Preferably, the bacterial strain has the 16s rRNA sequence of SEQ ID NO:1.

Most preferably, the bacterial strain in the composition is the *Bacteroides thetaiotaomicron* strain deposited under accession number NCIMB 42341.

In certain embodiments, the composition of the invention is for oral administration. Oral administration of the strains of the invention can be effective for increasing the microbiota diversity and/or inducing the stability of the microbiota. Also, oral administration is convenient for subjects and practitioners and allows delivery to and/or partial or total colonisation of the intestine.

In certain embodiments, the composition of the invention comprises one or more pharmaceutically acceptable excipients or carriers.

In certain embodiments, the composition of the invention comprises a bacterial strain that has been lyophilised. Lyophilisation is an effective and convenient technique for preparing stable compositions that allow delivery of bacteria, and is shown to provide effective compositions in the examples.

In certain embodiments, the invention provides a food product comprising the composition as described above.

In certain embodiments, the invention provides a vaccine composition comprising the composition as described above.

Additionally, the invention provides a method of increasing the microbiota diversity and/or inducing the stability of the microbiota and thereby treating or preventing diseases or disorders associated with a reduced microbiota diversity and/or with reduced stability of the microbiota, comprising administering a composition comprising a bacterial strain of the genus Bacteroides.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1: Effect of Thetanix treatment on microbiota diversity using Observed Species and Shannon Diversity Metrics

Figure 2: Effect of Thetanix on microbiota evenness

DISCLOSURE OF THE INVENTION

Bacterial strains

The compositions of the invention comprise a bacterial strain of the genus Bacteroides. The examples demonstrate that bacteria of this genus are useful for increasing the microbiota diversity and/or inducing the stability of the microbiota. The preferred bacterial strains are of the species *Bacteroides thetaiotaomicron*, particularly the bacterium deposited under accession number NCIMB 42341.

Bacteroides is a genus of gram-negative, obligate anaerobic bacteria. Bacteroides species are non endospore-forming bacilli, and may be either motile or nonmotile, depending on the species.

Bacteroides thetaiotaomicron was first described in 1912 under the name *Bacillus thetaiotaomicron* and moved to the genus Bacteroides in 1919. It was originally isolated from adult human feces. *Bacteroides thetaiotaomicron* triggers the nuclear export of the RelA subunit of nuclear kappa-light-chain-enhancer of activated B cells (NK-B), an important nuclear transcription factor, thereby limiting the transcription of downstream pro-inflammatory genes and synthesis of inflammatory factors, including interleukin (IL)-9 and tumor necrosis factor alpha (TNFα).

Bacterial strains closely related to the strain tested in the examples are also expected to be effective for increasing the microbiota diversity and/or inducing the stability of the microbiota. In certain embodiments, the bacterial strain for use in the invention has a 16s rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to the 16s rRNA sequence of a bacterial strain of *Bacteroides thetaiotaomicron*. Preferably, the bacterial strain for use in the invention has a 16s rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to SEQ ID NO:1. Preferably, the bacterial strain for use in the invention has a 16s rRNA sequence that has the sequence of SEQ ID NO:1. Preferably, the bacterial strain for use in the invention belongs to the genus *Bacteroides*.

Bacterial strains that are biotypes of the bacterium deposited under accession number NCIMB 42341 are also expected to be effective for increasing the microbiota diversity and/or inducing the stability of the microbiota. A biotype is a closely related strain that has the same or very similar physiological and biochemical characteristics.

Strains that are biotypes of a bacterium deposited under accession number NCIMB 42341 and that are suitable for use in the invention may be identified by sequencing other nucleotide sequences for a bacterium deposited under accession number NCIMB 42341. For example, substantially the whole genome may be sequenced and a biotype strain for use in the invention may have at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity across at least 80% of its whole genome (e.g. across at least 85%, 90%, 95% or 99%, or across its whole genome). For example, in some embodiments, a biotype strain has at least 98% sequence identity across at least 98% of its genome or at least 99% sequence identity across 99% of its genome. Other suitable sequences for use in identifying biotype strains may include hsp60 or repetitive sequences such as BOX, ERIC, $(GTG)_5$, or REP or [27]. Biotype strains may have sequences with at least 97%, 98%, 99%, 99.5% or 99.9% sequence identity to the corresponding sequence of a bacterium deposited under accession number NCIMB 42341. In some embodiments, a biotype strain has a sequence with at least 97%, 98%, 99%, 99.5% or 99.9% sequence identity to the corresponding sequence of the *Bacteroides thetaiotaomicron* strain deposited under accession number NCIMB 42341 and comprises a 16S rRNA sequence that is at least 99% identical (e.g. at least 99.5% or at least 99.9% identical) to SEQ ID NO:1. In some embodiments, a biotype strain has a sequence with at least 97%, 98%, 99%, 99.5% or 99.9% sequence identity to the corresponding sequence of the *Bacteroides thetaiotaomicron* strain deposited under accession number NCIMB 42341 and has the 16S rRNA sequence of SEQ ID NO:1.

Alternatively, strains that are biotypes of a bacterium deposited under accession number NCIMB 42341 and that are suitable for use in the invention may be identified by using the accession number NCIMB 42341 deposit, and restriction fragment analysis and/or PCR analysis, for example by using fluorescent amplified fragment length polymorphism (FAFLP) and repetitive DNA element (rep)-PCR fingerprinting, or protein profiling, or partial 16S or 23s rDNA sequencing. In preferred embodiments, such techniques may be used to identify other *Bacteroides thetaiotaomicron* strains.

In certain embodiments, strains that are biotypes of a bacterium deposited under accession number NCIMB 42341 and that are suitable for use in the invention are strains that provide the same pattern as a bacterium deposited under accession number NCIMB 42341 when analysed by amplified ribosomal DNA restriction analysis (ARDRA), for example when using Sau3AI restriction enzyme (for exemplary methods and guidance see, for example [28]). Alternatively,

biotype strains are identified as strains that have the same carbohydrate fermentation patterns as a bacterium deposited under accession number NCIMB 42341.

Other *Bacteroides* species that are useful in the compositions and methods of the invention, such as biotypes of a bacterium deposited under accession number NCIMB 42341, may be identified using any appropriate method or strategy. For instance, strains for use in the invention may be identified by culturing bacteria and administering to rats to test in the distension assay. In particular, bacterial strains that have similar growth patterns, metabolic type and/or surface antigens to a bacterium deposited under accession number NCIMB 42341 may be useful in the invention. A useful strain will have comparable microbiota modulatory activity to the NCIMB 42341 strain. In particular, a biotype strain will elicit comparable effects on the microbiota to the effects shown in the Examples.

A particularly preferred strain of the invention is the *Bacteroides thetaiotaomicron* strain deposited under accession number NCIMB 42341. This is the exemplary strain tested in the examples and shown to be effective for increasing the microbiota diversity and/or inducing the stability of the microbiota. Therefore, the invention provides a cell, such as an isolated cell, of the *Bacteroides thetaiotaomicron* strain deposited under accession number NCIMB 42341, or a derivative thereof, for use in therapy, in particular for the diseases and disorders described herein.

A derivative of the strain may be a daughter strain (progeny) or a strain cultured (subcloned) from the original. A derivative of a strain of the invention may be modified, for example at the genetic level, without ablating the biological activity. In particular, a derivative strain of the invention is therapeutically active. A derivative strain will have comparable microbiota modulatory activity to the original strain. In particular, a derivative strain will elicit comparable effects on the microbiota to the effects shown in the Examples, which may be identified by using the culturing and administration protocols described in the Examples. A derivative of the NCIMB 42341 strain will generally be a biotype of the NCIMB 42341 strain.

References to cells of the *Bacteroides thetaiotaomicron* strain deposited under accession number NCIMB 42341 encompass any cells that have the same safety and therapeutic efficacy characteristics as the strains deposited under accession number NCIMB 42341, and such cells are encompassed by the invention.

In preferred embodiments, the bacterial strains in the compositions of the invention are viable and capable of partially or totally colonising the intestine.

Therapeutic uses

In certain embodiments, the compositions of the invention are for use in increasing the microbiota diversity, evenness and/or inducing the stability of the microbiota. Reduced diversity or evenness of the microbiota and/or reduced stability of the microbiota are associated with numerous pathological diseases and disorders, as discussed above, and the examples demonstrate that the compositions of the invention may be effective for increasing the microbiota diversity and evenness and/or inducing the stability of the microbiota. Accordingly, the disease or disorder to be treated or prevented using a composition of the invention is preferably a disease or disorder associated with a level of microbiota diversity and / or evenness that is reduced relative to the microbiota diversity and / or evenness of a healthy subject and/or a disease or disorder that is associated with reduced stability of the microbiota. Thus, in some embodiments, the disease or disorder may be associated with a level of microbiota diversity and / or evenness that is reduced relative to the microbiota diversity of a healthy subject and also be associated with reduced stability of the microbiota.

In certain embodiments, the compositions of the invention are for use in increasing the microbiota diversity, evenness and/or inducing the stability of the microbiota in patients diagnosed with a disease or disorder selected from IBS, IBD (including Crohn's disease), cancer (including colorectal cancer) optionally in patients receiving concomitant anti-cancer therapies such as chemotherapy, obesity, type 2 diabetes, one or more infectious diseases, one or more allergic diseases, one or more autoimmune diseases and one or more metabolic diseases/disorders. Use of the compositions of the invention to increase the microbiota diversity, evenness and/or induce the stability of the microbiota in patients diagnosed with other diseases and disorders is also envisaged. In certain embodiments, the compositions of the invention are for use in treating or preventing IBS or IBD. In certain embodiments, the compositions of the invention are for use in treating or preventing IBS. In certain embodiments, the compositions of the invention are for use in treating or preventing IBD. In certain embodiments, the compositions of the invention are for use in treating or preventing one or more allergic diseases. In certain embodiments, the compositions of the invention are for use in treating or preventing cancer optionally in patients administered concomitant anticancer therapy. In certain embodiments, the compositions of the invention are for use in treating or preventing obesity. In

certain embodiments, the compositions of the invention are for use in treating or preventing one or more infectious diseases. In certain embodiments, the compositions of the invention are for use in treating or preventing one or more autoimmune diseases. In certain embodiments, the compositions of the invention are for use in treating or preventing one or more metabolic diseases/disorders. Preferably, the treatment or prevention comprises increasing the microbiota diversity and/or inducing the stability of the microbiota in the subject. Preferably the disease which is treated is Crohn's disease.

In certain embodiments, the one or more infectious diseases is selected from a viral, bacterial or fungal disease. In certain embodiments, the one or more allergic diseases is asthma. In certain embodiments, the one or more metabolic diseases/disorders is selected from diabetes, e.g. type 2 diabetes, and obesity. In certain embodiments, the one or more autoimmune diseases is selected from multiple sclerosis and rheumatoid arthritis.

In certain embodiments, the compositions of the invention are for use in treating or preventing IBS, IBD (including Crohn's disease), obesity, type 2 diabetes, one of more infectious diseases, one or more allergic diseases, one or more autoimmune diseases or one or more metabolic diseases/disorders by increasing the microbiota diversity in the microbiota. In certain embodiments, the compositions of the invention are for use in treating or preventing IBS or IBD by inducing the stability of the microbiota. In certain embodiments, the compositions of the invention are for use in treating or preventing IBD by inducing the stability of the microbiota

In preferred embodiments, the invention provides a composition comprising a bacterial strain of the species Bacteroides thetaiotaomicron , for use in the treatment or prevention of IBD, IBS, obesity, type 2 diabetes, one or more infectious diseases, one or more allergic diseases, one or more autoimmune diseases or one or more metabolic diseases/disorders, wherein the treatment or prevention comprises increasing the microbiota diversity and/or inducing the stability of the microbiota in the subject.

In some embodiments, the invention provides a composition comprising a bacterial strain of the species *Bacteroides thetaiotaomicron* for use in treating or preventing a disease or disorder selected from IBS, IBD, obesity, type 2 diabetes, one or more infectious diseases, one or more allergic diseases, one or more autoimmune diseases and one or more metabolic diseases/disorders. In some embodiments, the invention provides a method of treating or preventing a disease or disorder selected from IBS, IBD, obesity, type 2 diabetes, one or more

infectious diseases, one or more allergic diseases, one or more autoimmune diseases and one or more metabolic diseases/disorders, comprising administering a composition comprising a bacterial strain of the species *Bacteroides thetaiotaomicron*.

In preferred embodiments, the compositions of the invention comprise the bacterium deposited under accession number NCIMB 42341 and are for use in increasing the microbiota diversity and/or inducing the stability of the microbiota in the subject in the treatment of IBD, IBS, obesity, type 2 diabetes, one or more infectious diseases, one or more allergic diseases, one or more autoimmune diseases or one or more metabolic diseases/disorders. In further preferred embodiments, the compositions of the invention comprise the bacterium deposited under accession number NCIMB 42341 and are for use in treating or preventing IBD, IBS, obesity, type 2 diabetes, one or more infectious diseases, one or more allergic diseases, one or more autoimmune diseases or one or more metabolic diseases/disorders by increasing the microbiota diversity and/or inducing the stability of the microbiota.

In some embodiments, the pathogenesis of the disease or disorder affects the intestine. In some embodiments, the pathogenesis of the disease or disorder does not affect the intestine. In some embodiments, the pathogenesis of the disease or disorder is not localised at the intestine. In some embodiments, the treating or preventing occurs at a site other than at the intestine. In some embodiments, the treating or preventing occurs at the intestine and also at a site other than at the intestine. In certain embodiments, the disease or disorder is systemic.

In certain embodiments, the compositions are for use in subjects that exhibit, or are expected to exhibit, reduced levels of microbiota diversity, for example, when compared to a healthy subject, or a population of healthy subjects. For example, in some embodiments, the composition is for use in treating a subject having less than 101 different bacterial species (e.g. less than 100, 99, 98, 97, 96, 95, 93, 90, 85, 80, 75 or 70 bacterial species) and/or less than 195 different strains (e.g. less than 193, 190, 187, 185, 183, 180, 175, 170, 165, 160, 150, 140 bacterial strains) in its microbiota. For example, in some embodiments, the composition is for use in treating a subject that has at least one bacterial genus (e.g. at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 bacterial genera) fewer in its intestinal microbiota compared to a healthy subject or compared to a population of healthy subjects. In some embodiments, the treatment or prevention comprises a step of diagnosing a subject as having a reduced level of microbiota diversity and then if a reduced level of diversity is found to be present, the subject is then treated with a composition according to the invention.

In certain embodiments, the compositions are for use in subjects that exhibit, or are expected to exhibit, reduced stability of the microbiota. In some embodiments, the compositions are for use in subjects that exhibit, or are expected to exhibit, reduced stability in its microbiota, for example, when compared to a healthy subject, or a population of healthy subjects. In some embodiments, the treatment or prevention comprises a step of diagnosing a subject as having a reduced stability in its microbiota and then if reduced stability is found to be present, the subject is then treated with a composition according to the invention.

In certain embodiments, the subject is an infant. In certain embodiments, the subject is a child. In certain embodiments, the subject is an adult. The subject may be an adolescent, for example a subject with an age between 10 and 19 years.

In certain embodiments, the subject is a healthy subject. For example, in some embodiments in which the composition is used for preventing a disease or disorder, the subject is a healthy subject, optionally one identified as being at risk of developing a disease or disorder characterised by a reduction in microbiota diversity.

In certain embodiments, the subject has previously received, is receiving, or will be receiving anticancer treatment, for example chemotherapy. Accordingly, in some embodiments, the treatment or prevention comprises administering the composition of the invention after, together with, or before anticancer treatment.

In certain embodiments, the subject has previously received, is receiving, or will be receiving antibiotic treatment. Accordingly, in some embodiments, the treatment or prevention comprises administering the composition of the invention after, together with, or before antibiotic treatment. The composition of the invention and the one or more antibiotics may be for separate, simultaneous or sequential administration.

Treatment or prevention may refer to, for example, an alleviation of the severity of symptoms or a reduction in the frequency of exacerbations or the range of triggers that are a problem for the subject.

Bacteria in the microbiota may be detected in faeces from a subject, using standard techniques, such as the qPCR techniques used in the examples.

Modes of administration

Preferably, the compositions of the invention are to be administered to the gastrointestinal tract in order to enable delivery to and / or partial or total colonisation of the intestine with the bacterial strain of the invention. Generally, the compositions of the invention are administered orally, but they may be administered rectally, intranasally, or via buccal or sublingual routes.

In certain embodiments, the compositions of the invention may be administered as a foam, as a spray or a gel.

In certain embodiments, the compositions of the invention may be administered as a suppository, such as a rectal suppository, for example in the form of a theobroma oil (cocoa butter), synthetic hard fat (e.g. suppocire, witepsol), glycero-gelatin, polyethylene glycol, or soap glycerin composition.

In certain embodiments, the composition of the invention is administered to the gastrointestinal tract via a tube, such as a nasogastric tube, orogastric tube, gastric tube, jejunostomy tube (J tube), percutaneous endoscopic gastrostomy (PEG), or a port, such as a chest wall port that provides access to the stomach, jejunum and other suitable access ports.

The compositions of the invention may be administered once, or they may be administered sequentially as part of a treatment regimen. In certain embodiments, the compositions of the invention are to be administered daily. The examples demonstrate that daily administration provides successful delivery and clinical benefits.

In certain embodiments, the compositions of the invention are administered regularly, such as daily, every two days, or weekly, for an extended period of time, such as for at least one week, two weeks, one month, two months, six months, or one year.

In certain embodiments of the invention, treatment according to the invention is accompanied by assessment of the subject's gut microbiota. Treatment may be repeated if delivery of and / or partial or total colonisation with the strain of the invention is not achieved such that efficacy is not observed, or treatment may be ceased if delivery and / or partial or total colonisation is successful and efficacy is observed.

In certain embodiments, the composition of the invention may be administered to a pregnant animal, for example a mammal such as a human in order to prevent reduced levels of diversity in

the microbiota and/or reduced stability of the microbiota developing in her child *in utero* and / or after it is born.

The compositions of the invention may be administered to a subject that has been diagnosed with reduced microbiota diversity relative to a healthy subject and/or reduced stability of the microbiota or a disease or disorder associated with reduced microbiota diversity relative to a healthy subject and/or reduced stability of the microbiota, or that has been identified as being at risk of reduced microbiota diversity relative to a healthy subject and/or reduced stability of the microbiota. The compositions may also be administered as a prophylactic measure to prevent the development of reduced microbiota diversity relative to a healthy subject and/or reduced stability of the microbiota in a healthy subject.

The compositions of the invention may be administered to a subject that has been identified as having an abnormal gut microbiota. For example, the subject may have reduced or absent colonisation by *Bacteroides*, and in particular *Bacteroides thetaiotaomicron.*

The compositions of the invention may be administered as a food product, such as a nutritional supplement.

Generally, the compositions of the invention are for the treatment of humans, although they may be used to treat animals including monogastric mammals such as poultry, pigs, cats, dogs, horses or rabbits. The compositions of the invention may be useful for enhancing the growth and performance of animals. If administered to animals, oral gavage may be used.

Compositions

Generally, the composition of the invention comprises bacteria. In preferred embodiments of the invention, the composition is formulated in freeze-dried form. For example, the composition of the invention may comprise granules or gelatin capsules, for example hard gelatin capsules, comprising a bacterial strain of the invention.

Preferably, the composition of the invention comprises lyophilised bacteria. Lyophilisation of bacteria is a well-established procedure and relevant guidance is available in, for example, references [29-31]. The examples demonstrate that lyophilisate compositions are particularly effective.

Alternatively, the composition of the invention may comprise a live, active bacterial culture.

In some embodiments, the bacterial strain in the composition of the invention has not been inactivated, for example, has not been heat-inactivated. In some embodiments, the bacterial strain in the composition of the invention has not been killed, for example, has not been heat-killed. In some embodiments, the bacterial strain in the composition of the invention has not been attenuated, for example, has not been heat-attenuated. For example, in some embodiments, the bacterial strain in the composition of the invention has not been killed, inactivated and/or attenuated. For example, in some embodiments, the bacterial strain in the composition of the invention is live. For example, in some embodiments, the bacterial strain in the composition of the invention is viable. For example, in some embodiments, the bacterial strain in the composition of the invention is capable of partially or totally colonising the intestine. For example, in some embodiments, the bacterial strain in the composition of the invention is viable and capable of partially or totally colonising the intestine.

In some embodiments, the composition comprises a mixture of live bacterial strains and bacterial strains that have been killed.

In preferred embodiments, the composition of the invention is encapsulated to enable delivery of the bacterial strain to the intestine. Encapsulation protects the composition from degradation until delivery at the target location through, for example, rupturing with chemical or physical stimuli such as pressure, enzymatic activity, or physical disintegration, which may be triggered by changes in pH. Any appropriate encapsulation method may be used. Exemplary encapsulation techniques include entrapment within a porous matrix, attachment or adsorption on solid carrier surfaces, self-aggregation by flocculation or with cross-linking agents, and mechanical containment behind a microporous membrane or a microcapsule. Guidance on encapsulation that may be useful for preparing compositions of the invention is available in, for example, references [32] and [33].

The composition may be administered orally and may be in the form of a tablet, capsule or powder. Encapsulated products are preferred because *Blautia* are anaerobes. Other ingredients (such as vitamin C, for example), may be included as oxygen scavengers and prebiotic substrates to improve the delivery and / or partial or total colonisation and survival *in vivo*. Alternatively, the probiotic composition of the invention may be administered orally as a food or nutritional product, such as milk or whey based fermented dairy product, or as a pharmaceutical product.

The composition may be formulated as a probiotic.

A composition of the invention includes a therapeutically effective amount of a bacterial strain of the invention. A therapeutically effective amount of a bacterial strain is sufficient to exert a beneficial effect upon a subject. A therapeutically effective amount of a bacterial strain may be sufficient to result in delivery to and / or partial or total colonisation of the subject's intestine.

A suitable daily dose of the bacteria, for example for an adult human, may be from about 1 x 10^3 to about 1 x 10^{11} colony forming units (CFU); for example, from about 1 x 10^7 to about 1 x 10^{10} CFU; in another example from about 1 x 10^7 to about 1 x 10^{11} CFU; in another example from about 1 x 10^8 to about 1 x 10^{10} CFU; in another example from about 1 x 10^8 to about 1 x 10^{11} CFU; in another example from about 1 x 10^6 to about 1 x 10^{10} CFU.

In certain embodiments, the dose of the bacteria is at least 10^9 cells per day, such as at least 10^{10}, at least 10^{11}, or at least 10^{12} cells per day.

In certain embodiments, the composition contains the bacterial strain in an amount of from about 1 x 10^6 to about 1 x 10^{11} CFU/g, respect to the weight of the composition; for example, from about 1 x 10^8 to about 1 x 10^{10} CFU/g. The dose may be, for example, 1 g, 3g, 5g, and 10g. In preferred embodiments, the composition contains the bacterial strain in an amount from about 1 x 10^6 to about 1 x $10^{9.5}$.

Typically, a probiotic, such as the composition of the invention, is optionally combined with at least one suitable prebiotic compound. A prebiotic compound is usually a non-digestible carbohydrate such as an oligo- or polysaccharide, or a sugar alcohol, which is not degraded or absorbed in the upper digestive tract. Known prebiotics include commercial products such as inulin and transgalacto-oligosaccharides.

In certain embodiments, the probiotic composition of the present invention includes a prebiotic compound in an amount of from about 1 to about 30% by weight, respect to the total weight composition, (e.g. from 5 to 20% by weight). Carbohydrates may be selected from the group consisting of: fructo- oligosaccharides (or FOS), short-chain fructo-oligosaccharides, inulin, isomalt-oligosaccharides, pectins, xylo-oligosaccharides (or XOS), chitosan-oligosaccharides (or COS), beta-glucans, arable gum modified and resistant starches, polydextrose, D-tagatose, acacia fibers, carob, oats, and citrus fibers. In one aspect, the prebiotics are the short-chain fructo-oligosaccharides (for simplicity shown herein below as FOSs-c.c); said FOSs-c.c. are not digestible carbohydrates, generally obtained by the conversion of the beet sugar and including a saccharose molecule to which three glucose molecules are bonded.

The compositions of the invention may comprise pharmaceutically acceptable excipients or carriers. Examples of such suitable excipients may be found in the reference [34]. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art and are described, for example, in reference [35]. Examples of suitable carriers include lactose, starch, glucose, methyl cellulose, magnesium stearate, mannitol, sorbitol and the like. Examples of suitable diluents include ethanol, glycerol and water. The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as, or in addition to, the carrier, excipient or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s). Examples of suitable binders include starch, gelatin, natural sugars such as glucose, anhydrous lactose, free-flow lactose, beta-lactose, corn sweeteners, natural and synthetic gums, such as acacia, tragacanth or sodium alginate, carboxymethyl cellulose and polyethylene glycol. Examples of suitable lubricants include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. Preservatives, stabilizers, dyes and even flavouring agents may be provided in the pharmaceutical composition. Examples of preservatives include sodium benzoate, sorbic acid, cysteine and esters of p-hydroxybenzoic acid. Antioxidants and suspending agents may be also used. A further example of a suitable carrier is saccharose. A further example of a preservative is cysteine.

The compositions of the invention may be formulated as a food product. For example, a food product may provide nutritional benefit in addition to the therapeutic effect of the invention, such as in a nutritional supplement. Similarly, a food product may be formulated to enhance the taste of the composition of the invention or to make the composition more attractive to consume by being more similar to a common food item, rather than to a pharmaceutical composition. In certain embodiments, the composition of the invention is formulated as a milk-based product. The term "milk-based product" means any liquid or semi-solid milk- or whey- based product having a varying fat content. The milk-based product can be, e.g., cow's milk, goat's milk, sheep's milk, skimmed milk, whole milk, milk recombined from powdered milk and whey without any processing, or a processed product, such as yoghurt, curdled milk, curd, sour milk, sour whole milk, butter milk and other sour milk products. Another important group includes milk beverages, such as whey beverages, fermented milks, condensed milks, infant or baby milks; flavoured milks, ice cream; milk-containing food such as sweets.

In certain embodiments, the compositions of the invention contain a single bacterial strain or species and do not contain any other bacterial strains or species. Such compositions may comprise only *de minimis* or biologically irrelevant amounts of other bacterial strains or species. Such compositions may be a culture or lyophilisate that is substantially free from other species of organism.

In certain embodiments, the compositions of the invention comprise one or more bacterial strains of the genus Bacteroides and do not contain any other bacterial genera, or which comprise only *de minimis* or biologically irrelevant amounts of bacteria from another genus. In certain embodiments, the compositions of the invention comprise a single species of *Bacteroides,* preferably *Bacteroides thetaiotaomicron,* and do not contain any other bacterial species, or which comprise only *de minimis* or biologically irrelevant amounts of bacteria from another species. In certain embodiments, the compositions of the invention comprise a single strain of *Bacteroides,* for example, of *Bacteroides thetaiotaomicron* NCIMB 42341 and do not contain any other bacterial strains or species, or which comprise only *de minimis* or biologically irrelevant amounts of bacteria from another strain or species.

In some embodiments, the compositions of the invention comprise more than one bacterial strain or species. For example, in some embodiments, the compositions of the invention comprise more than one strain from within the same species (e.g. more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40 or 45 strains), and, optionally, do not contain bacteria from any other species. In some embodiments, the compositions of the invention comprise less than 50 strains from within the same species (e.g. less than 45, 40, 35, 30, 25, 20, 15, 12, 10, 9, 8, 7, 6, 5, 4 or 3 strains), and, optionally, do not contain bacteria from any other species. In some embodiments, the compositions of the invention comprise 1-40, 1-30, 1-20, 1-19, 1-18, 1-15, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, 2-50, 2-40, 2-30, 2-20, 2-15, 2-10, 2-5, 6-30, 6-15, 16-25, or 31-50 strains from within the same species and, optionally, do not contain bacteria from any other species. In some embodiments, the compositions of the invention comprise more than one species from within the same genus (e.g. more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, 23, 25, 30, 35 or 40 species), and, optionally, do not contain bacteria from any other genus. In some embodiments, the compositions of the invention comprise less than 50 species from within the same genus (e.g. less than 50, 45, 40, 35, 30, 25, 20, 15, 12, 10, 8, 7, 6, 5, 4 or 3 species), and, optionally, do not contain bacteria from any other genus. In some embodiments, the compositions of the invention comprise 1-50, 1-40, 1-30, 1-20, 1-15, 1-10, 1-9, 1-8, 1-7, 1-6, 1-

5, 1-4, 1-3, 1-2, 2-50, 2-40, 2-30, 2-20, 2-15, 2-10, 2-5, 6-30, 6-15, 16-25, or 31-50 species from within the same genus and, optionally, do not contain bacteria from any other genus. The invention comprises any combination of the foregoing.

In some embodiments, the composition comprises a microbial consortium. For example, in some embodiments, the composition comprises the *Bacteroides* bacterial strain, for example, a *Bacteroides thetaiotaomicron* bacterial strain as part of a microbial consortium. For example, in some embodiments, the *Bacteroides* bacterial strain is present in combination with one or more (e.g. at least 2, 3, 4, 5, 10, 15 or 20) other bacterial strains from other genera with which it can live symbiotically *in vivo* in the intestine. For example, in some embodiments, the composition comprises a bacterial strain of *Bacteroides thetaiotaomicron* in combination with a bacterial strain from a different genus. In some embodiments, the microbial consortium comprises two or more bacterial strains obtained from a faeces sample of a single organism, e.g. a human. In some embodiments, the microbial consortium is not found together in nature. For example, in some embodiments, the microbial consortium comprises bacterial strains obtained from faeces samples of at least two different organisms. In some embodiments, the two different organisms are from the same species, e.g. two different humans. In some embodiments, the two different organisms are an infant human and an adult human. In some embodiments, the two different organisms are a human and a non-human mammal.

In some embodiments, the composition of the invention additionally comprises a bacterial strain that has the same safety and therapeutic efficacy characteristics as the *Bacteroides thetaiotaomicron* strain deposited under accession number NCIMB 42341, but which is not the *Bacteroides thetaiotaomicron* strain deposited under accession number NCIMB 42341, or which is not a *Bacteroides thetaiotaomicron* strain.

In some embodiments in which the composition of the invention comprises more than one bacterial strain, species or genus, the individual bacterial strains, species or genera may be for separate, simultaneous or sequential administration. For example, the composition may comprise all of the more than one bacterial strains, species or genera, or the bacterial strains, species or genera may be stored separately and be administered separately, simultaneously or sequentially. In some embodiments, the more than one bacterial strains, species or genera are stored separately but are mixed together prior to use.

In some embodiments, the bacterial strain for use in the invention is obtained from human adult faeces. In some embodiments in which the composition of the invention comprises more than one bacterial strain, all of the bacterial strains are obtained from human adult faeces or if other bacterial strains are present they are present only in *de minimis* amounts. In some embodiments, the bacteria may have been cultured subsequent to being obtained from the human adult faeces and being used in a composition of the invention.

In some embodiments, the one or more *Bacteroides* bacterial strains (for example the *Bacteroides thetaiotaomicron* strain) is/are the only therapeutically active agent(s) in a composition of the invention. In some embodiments, the bacterial strain(s) in the composition is/are the only therapeutically active agent(s) in a composition of the invention.

The compositions for use in accordance with the invention may or may not require marketing approval.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein said bacterial strain is lyophilised. In certain embodiments, the invention provides the above pharmaceutical composition, wherein said bacterial strain is spray dried. In certain embodiments, the invention provides the above pharmaceutical composition, wherein the bacterial strain is lyophilised or spray dried and wherein it is live. In certain embodiments, the invention provides the above pharmaceutical composition, wherein the bacterial strain is lyophilised or spray dried and wherein it is viable. In certain embodiments, the invention provides the above pharmaceutical composition, wherein the bacterial strain is lyophilised or spray dried and wherein it is capable of partially or totally colonising the intestine. In certain embodiments, the invention provides the above pharmaceutical composition, wherein the bacterial strain is lyophilised or spray dried and wherein it is viable and capable of partially or totally colonising the intestine.

In some cases, the lyophilised or spray dried bacterial strain is reconstituted prior to administration. In some cases, the reconstitution is by use of a diluent described herein.

The compositions of the invention can comprise pharmaceutically acceptable excipients, diluents or carriers.

In certain embodiments, the invention provides a pharmaceutical composition comprising: a bacterial strain as used in the invention; and a pharmaceutically acceptable excipient, carrier or

diluent; wherein the bacterial strain is in an amount sufficient to increase the microbiota diversity in a subject and/or induce stability of the microbiota and/or treat a disorder associated with reduced microbiota diversity and/or reduced stability of the microbiota when administered to a subject in need thereof, the disorder associated with microbiota diversity being selected from, for example, IBS, IBD, cancer, obesity, type 2 diabetes, one or more infectious diseases, one or more allergic diseases, one or more autoimmune diseases or one or more metabolic diseases/disorders.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein the amount of the bacterial strain is from about 1×10^3 to about 1×10^{11} colony forming units per gram with respect to a weight of the composition.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein the composition is administered at a dose of 1 g, 3 g, 5 g or 10 g.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein the composition is administered by a method selected from the group consisting of oral, rectal, subcutaneous, nasal, buccal, and sublingual.

In certain embodiments, the invention provides the above pharmaceutical composition, comprising a carrier selected from the group consisting of lactose, starch, glucose, methyl cellulose, magnesium stearate, mannitol and sorbitol.

In certain embodiments, the invention provides the above pharmaceutical composition, comprising a diluent selected from the group consisting of ethanol, glycerol and water.

In certain embodiments, the invention provides the above pharmaceutical composition, comprising an excipient selected from the group consisting of starch, gelatin, glucose, anhydrous lactose, free-flow lactose, beta-lactose, corn sweetener, acacia, tragacanth, sodium alginate, carboxymethyl cellulose, polyethylene glycol, sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate and sodium chloride.

In certain embodiments, the invention provides the above pharmaceutical composition, further comprising at least one of a preservative, an antioxidant and a stabilizer.

In certain embodiments, the invention provides the above pharmaceutical composition, comprising a preservative selected from the group consisting of sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein said bacterial strain is lyophilised.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein when the composition is stored in a sealed container at about 4°C or about 25°C and the container is placed in an atmosphere having 50% relative humidity, at least 80% of the bacterial strain as measured in colony forming units, remains after a period of at least about: 1 month, 3 months, 6 months, 1 year, 1.5 years, 2 years, 2.5 years or 3 years.

In some embodiments, the composition of the invention is provided in a sealed container comprising a composition as described herein. In some embodiments, the sealed container is a sachet or bottle. In some embodiments, the composition of the invention is provided in a syringe comprising a composition as described herein.

The composition of the present invention may, in some embodiments, be provided as a pharmaceutical formulation. For example, the composition may be provided as a tablet or capsule. In some embodiments, the capsule is a gelatine capsule ("gel-cap").

In some embodiments, the compositions of the invention are administered orally. Oral administration may involve swallowing, so that the compound enters the gastrointestinal tract, and/or buccal, lingual, or sublingual administration by which the compound enters the blood stream directly from the mouth.

Pharmaceutical formulations suitable for oral administration include solid plugs, solid microparticulates, semi-solid and liquid (including multiple phases or dispersed systems) such as tablets; soft or hard capsules containing multi- or nano-particulates, liquids (e.g. aqueous solutions), emulsions or powders; lozenges (including liquid-filled); chews; gels; fast dispersing dosage forms; films; ovules; sprays; and buccal/mucoadhesive patches.

In some embodiments the pharmaceutical formulation is an enteric formulation, i.e. a gastro-resistant formulation (for example, resistant to gastric pH) that is suitable for delivery of the composition of the invention to the intestine by oral administration. Enteric formulations may be particularly useful when the bacteria or another component of the composition is acid-sensitive, e.g. prone to degradation under gastric conditions.

In some embodiments, the enteric formulation comprises an enteric coating. In some embodiments, the formulation is an enteric-coated dosage form. For example, the formulation

may be an enteric-coated tablet or an enteric-coated capsule, or the like. The enteric coating may be a conventional enteric coating, for example, a conventional coating for a tablet, capsule, or the like for oral delivery. The formulation may comprise a film coating, for example, a thin film layer of an enteric polymer, e.g. an acid-insoluble polymer.

In some embodiments, the enteric formulation is intrinsically enteric, for example, gastro-resistant without the need for an enteric coating. Thus, in some embodiments, the formulation is an enteric formulation that does not comprise an enteric coating. In some embodiments, the formulation is a capsule made from a thermogelling material. In some embodiments, the thermogelling material is a cellulosic material, such as methylcellulose, hydroxymethylcellulose or hydroxypropylmethylcellulose (HPMC). In some embodiments, the capsule comprises a shell that does not contain any film forming polymer. In some embodiments, the capsule comprises a shell and the shell comprises hydroxypropylmethylcellulose and does not comprise any film forming polymer (e.g. see [36]). In some embodiments, the formulation is an intrinsically enteric capsule (for example, Vcaps® from Capsugel).

In some embodiments, the formulation is a soft capsule. Soft capsules are capsules which may, owing to additions of softeners, such as, for example, glycerol, sorbitol, maltitol and polyethylene glycols, present in the capsule shell, have a certain elasticity and softness. Soft capsules can be produced, for example, on the basis of gelatine or starch. Gelatine-based soft capsules are commercially available from various suppliers. Depending on the method of administration, such as, for example, orally or rectally, soft capsules can have various shapes, they can be, for example, round, oval, oblong or torpedo-shaped. Soft capsules can be produced by conventional processes, such as, for example, by the Scherer process, the Accogel process or the droplet or blowing process.

Culturing methods

The bacterial strains for use in the present invention can be cultured using standard microbiology techniques as detailed in, for example, references [37-39].

The solid or liquid medium used for culture may be YCFA agar or YCFA medium. YCFA medium may include (per 100ml, approximate values): Casitone (1.0 g), yeast extract (0.25 g), $NaHCO_3$ (0.4 g), cysteine (0.1 g), K_2HPO_4 (0.045 g), KH_2PO_4 (0.045 g), NaCl (0.09 g), $(NH_4)_2SO_4$ (0.09 g), $MgSO_4 \cdot 7H_2O$ (0.009 g), $CaCl_2$ (0.009 g), resazurin (0.1 mg), hemin (1

mg), biotin (1 μg), cobalamin (1 μg), *p*-aminobenzoic acid (3 μg), folic acid (5 μg), and pyridoxamine (15 μg).

Bacterial strains for use in vaccine compositions

The inventors have identified that the bacterial strains of the invention are useful for treating or preventing diseases or disorders associated with a level of microbiota diversity that is reduced relative to the microbiota diversity of a healthy subject (or relative to the microbiota diversity of a population of healthy subjects) and/or diseases or disorders that are associated with reduced stability of the microbiota compared to a healthy subject (or compared to a population of healthy subjects). This is likely to be a result of the effect that the bacterial strains of the invention have on the host immune system. Therefore, the compositions of the invention may also be useful for preventing such diseases or disorders when administered as vaccine compositions. These vaccines comprise a *B. thetaiotaomicron* antigen. In certain such embodiments, the bacterial strains of the invention are viable. In certain such embodiments, the bacterial strains of the invention are capable of partially or totally colonising the intestine. In certain such embodiments, the bacterial strains of the invention are viable and capable of partially or totally colonising the intestine. In other certain such embodiments, the bacterial strains of the invention may be killed, inactivated or attenuated. In certain such embodiments, the compositions may comprise a vaccine adjuvant. In certain embodiments, the compositions are for administration via injection, such as via subcutaneous injection.

General

The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, molecular biology, immunology and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, *e.g.*, references [40] and [41-47], *etc.*

The term "comprising" encompasses "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term "about" in relation to a numerical value *x* is optional and means, for example, $x \pm 10\%$.

The word "substantially" does not exclude "completely" *e.g.* a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

References to a percentage sequence identity between two nucleotide sequences means that, when aligned, that percentage of nucleotides are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of ref. [48]. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in ref. [49].

Unless specifically stated, a process or method comprising numerous steps may comprise additional steps at the beginning or end of the method, or may comprise additional intervening steps. Also, steps may be combined, omitted or performed in an alternative order, if appropriate.

Various embodiments of the invention are described herein. It will be appreciated that the features specified in each embodiment may be combined with other specified features, to provide further embodiments. In particular, embodiments highlighted herein as being suitable, typical or preferred may be combined with each other (except when they are mutually exclusive).

MODES FOR CARRYING OUT THE INVENTION

Example 1 – Effect of Thetanix on Microbiota Diversity

Thetanix is a live biotherapeutic containing the bacterium *Bacteroides thetaiotaomicron* (*B. Theta*) as the active ingredient. It is lyophilised and formulated as gastro-resistant capsules for oral administration. Each capsule contains $10^{7.73\pm1.43}$ colony forming units (CFUs).

Overall study design

The study was a randomised, double-blind, placebo-controlled, multiple dose study in subjects aged 16 to 18 years with Crohn's disease. Subjects suitable for the study were identified from patient lists at appropriate gastroenterology clinics.

The patients received daily dosing over 7.5 days where the first dose was taken on Day 0 (D0) in clinic, the next 13 doses were taken at home and the 15th dose was taken in the clinic. Subject received a dose of *B. Theta* or placebo an hour before food every 12 hours during the 7.5 day dosing period.

Stool samples were collected at D0, D1, D7 and D56. These were analysed by quantitative polymerase chain reaction (PCR) for *B. theta* and other common constituents of the microbiome.

Results

The effect of treatment on microbiota diversity was assessed using the number of Observed species per sample (richness) and the Shannon Diversity Index which represents the number of taxa (richness) and their relative abundances (evenness) within each sample. The effects of Thetanix treatment on microbiota diversity are shown in Figure 1 which shows a significant difference in Shannon Diversity between the study timepoints (D0, D7 and D56). Similarly, microbiota evenness was found to be significant across the study timepoints, as shown in Figure 2.

Conclusions

B. Theta was well tolerated in the study. There were no serious adverse events, deaths or subjects who discontinued from the study after treatment. There were no trends in haematology, clinical chemistry, vital signs, or physical examinations to suggest an adverse effect of *B. Theta* on these parameters.

Although the study was conducted in a small population, Thetanix shows promise as an agent capable of increasing diversity and evenness in the microbiota. Given the association between disease and a loss of microbiota diversity, Thetanix can be expected to treat conditions like Crohn's disease which are associated with reduced microbiome diversity.

Furthermore, a signifant change in the faecal calprotectin levels was observed in several of the patients administered Thetanix over the course of the study indicating the efficacy of Thetanix treatment in Crohn's disease.

The invention has been described above by way of example only and it will be understood that further modifications may be made which fall within the scope of the claims.

Sequences

SEQ ID NO:1 (*Bacteroides thetaiotaomicron* strain NCIMB 42341 16S ribosomal RNA gene)

```
cttttacaat gaagagtttg atcctggctc aggatgaacg ctagctacag
gcttaacaca  60
tgcaagtcga ggggcagcat ttcagtttgc ttgcaaactg gagatggcga
ccggcgcacg  120
ggtgagtaac acgtatccaa cctgccgata actcggggat agcctttcga
aagaaagatt  180
```

aatacccgat ggtataatca gaccgcatgg tttgattatt aaagaatttc ggttatcgat 240

ggggatgcgt tccattaggc agttggtgag gtaacggctc accaaacctt cgatggatag 300

gggttctgag aggaaggtcc cccacattgg aactgagaca cggtccaaac tcctacggga 360

ggcagcagtg aggaatattg gtcaatgggc gcaggcctga accagccaag tagcgtgaag 420

gatgactgcc ctatgggttg taaacttctt ttatatggga ataaagtttt ccacgtgtgg 480

aattttgtat gtaccatatg aataaggatc ggctaactcc gtgccagcag ccgcggtaat 540

acggaggatc cgagcgttat ccggatttat tgggtttaaa gggagcgtag gtggacagtt 600

aagtcagttg tgaaagtttg cggctcaacc gtaaaattgc agttgatact ggctgtcttg 660

agtacagtag aggtgggcgg aattcgtggt gtagcggtga aatgcttaga tatcacgaag 720

aactccgatt gcgaaggcag ctcactggac tgcaactgac actgatgctc gaaagtgtgg 780

gtatcaaaca ggattagata ccctggtagt ccacacagta aacgatgaat actcgctgtt 840

tgcgatatac agtaagcggc caagcgaaag cattaagtat tccacctggg gagtacgccg 900

gcaacggtga aactcaaagg aattgacggg ggcccgcaca agcggaggaa catgtggttt 960

aattcgatga tacgcgagga accttacccg ggcttaaatt gcatttgaat atattggaaa 1020

cagtatagcc gtaaggcaaa tgtgaaggtg ctgcatggtt gtcgtcagct cgtgccgtga 1080

ggtgtcggct taagtgccat aacgagcgca acccttatct ttagttacta acaggtcatg 1140

ctgaggactc tagagagact gccgtcgtaa gatgtgagga aggtggggat gacgtcaaat 1200

cagcacggcc cttacgtccg gggctacaca cgtgttacaa tgggggggtac agaaggcagc 1260

tacctggtga caggatgcta atcccaaaag cctctctcag ttcggatcga agtctgcaac 1320

ccgacttcgt gaagctggat tcgctagtaa tcgcgcatca gccatggcgc ggtgaatacg 1380

ttcccgggcc ttgtacacac cgcccgtcaa gccatgaaag ccgggggtac ctgaagtacg 1440

taaccgcaag gagcgtccta gggtaaaact ggtaattggg gc 1482

SEQ ID NO: 2 (Bacteroides thetaiotaomicron (ATCC 29148) 16S rRNA)

cantgaagag tttgatcctg gctcaggatn aacgctagct acaggcttaa cacatgcaag 60

tcgaggggca gcatttcnnt ttgcttgcaa actnnagatg gcgaccggcg cacgggtgag 120

taacacgtat ccaacctgcc gataactcgg ggatagcctt tcgaaagaaa gattaatacc 180

cgatggcata atcanaccgc atggtcttat tattaaagaa tttcggttat cgatggggat 240

gcgttccatt aggcagttgg tgaggtaacg gctcacnaaa ccttcgatgg ataggggttc 300

tgagaggaag gtcccccaca ttggaactga gacacggtcc naactcctac gggaggcagc 360

agtgaggaat attggtcaat gggcgcaggc ctnaaccagc caagtagcgt gaaggatgac 420

tgccctatgg gttgtaaact nctnttatat gggaataaag tnttccacgt gtggaatttt 480

gtatgtacca tatgaataag gatcggctaa ctccgtgcca gcagccgcgg tnatacggag 540

gatccgagcg ttatccggat ttattgggtt taaagggagc gtaggtggac agttaagtca 600

gttgtgaaag tttgcggctc aaccgtaaaa ttgcagttga tactggctgt cttgagtaca 660

gtagaggtgg gcggaattcg tggtgtagcg gtgaaatgct tagatatcac gaagaactcc 720

gattgcgaag gcagctcact ggactgcaac tgacactgat gctcgaaagt gtgggtatca 780

aacaggatta gataccctgg tagtccacac agtaaacgat gaatactcgc tgtttgcgat 840

atacagtaag cggccaagcg aaagcattaa gtattccacc tggggagtac gccggcaacg 900

gtgaaactca aaggaattga cgggggcccg cacaagcgga ggaacatgtg gtttaattcg 960

atgatacgcg aggaacctta cccgggctta aattgcattt gaataatctg gaaacaggtt 1020

agccgcaagg caaatgtgaa ggtgctgcat ggttgtcgtc agctcgtgcc gtgaggtgtc 1080

ggcttaagtg ccataacgag cgcaaccctt atctttagtt actaacaggt catgctgagg 1140

actctagaga gactgccgtc gtaagatgtg aggaaggtgg ggatgacgtc aaatcagcac 1200

ggcccttacg tccggggcta cacacgtgtt acaatggggg gtacagaagg cagctacctg 1260

gtgacaggat gctnatccca aaagcctctc tcagttcgga tcgaagtctg caacccgact 1320

tcgtgaagct ggattcgcta gtaatcgcgc atcagccatg gcgcggtgaa tacgttcccg 1380

ggccttgtac acaccgcccg tcaanccatg anagccgggg gtacctgaag tacgtaaccg 1440

caaggagcgt cctagggtaa aactggtaat tgggg 1475

SEQ ID NO: 3 (*Bacteroides thetaiotaomicron* strain WAL 2926 (M58763) 16S rRNA)

cttntacaat gaagagtttg atcctggctc aggatnaacg ctagctacag gcttaacaca 60

tgcaagtcna ggggcagcat ttcagtttgc ttgcaaactg gagatggcga ccggcgcacg 120

ggtgagtaac acgtatccaa cctgccgata actcggggat agcctttcga aagaaagatt 180

aatacccnat ggtataatca gaccgcatng tcttrttatt aaagaatttc ggttatcgat 240

ggggatgcgt tccattaggc agttggtgag gtaacggctc acnnaacctt cgatggatag 300

gggttctgag aggaaggtcc cccacattgg aactgagaca cggtccaaac tcctacggga 360

ggcagcagtg aggaatattg gtcaatgggc gcaggcctga accagccaag tagcgtgaag 420

gatgactgcc ctatgggttg taaacttctt ttatatggga ataaagtttt ccacgtgtgg 480

aattttgtat gtaccatatg aataaggatc ggctaactcc gtgccagcag ccncgntnat 540

acggagnatc cgagcgttat ccggatttat tgggtttaaa gggagcgtag gtggacagtt 600

aagtcagttg tgaaagtttg cggctcaacc gtaaaattgc agttgatact ggctgtcttg 660

agtacagtag aggtgggcgg aattcgtggt gtagcggtga aatgcttaga tatcacgaag 720

aactccgatt gcgaaggcag ctcactggac tgcaactgac actgatgctc gaaagtgtgg 780

gtatcaaaca ggattagata ccctggtagt ccacacagta aacgatgaat actcgctgtt 840

tgcgatatac agtaagcggc caagcgaaag cattaagtat tccacctggg gagtacgccg 900

gcaacggtga aactcaaagg aattgacggg ggccngcaca agcggaggaa catgtggttt 960

aattcgatga tacgcgagga accttacccg ggcttaaatt gcatttgaat atattggaaa 1020

cagtatagcc gyaaggcaaa tgtgaaggtg ctgcatggtt gtcgtcagct cgtgccgtga 1080

ggtgtcggct taagtgccat aacgagcgca acccttatct ttagttacta acaggtcatg 1140

ctgaggactc tagagagact gccgtcgtaa gatgtgagga aggtggggat gacgtcaaat 1200

cagcacngcc cntacgtccg gggctacaca cgtgttacaa tgggggggtac agaaggcagc 1260

tacctggtga caggatgcta atcccaaaag cctctctcag ttcggatcga agtctgcaac 1320

ccgacttcgt gaagctggat tcgctagtaa tcgcgcatca gccatggcgc ggtgaatacg 1380

ttcccgggcn ttgtacacac cgcccgtcaa gccatgaaag ccgggggtac ctgaagtacg 1440

taaccgcaag gagcgtccta gggtaaaact ggtaattggg gc 1482

SEQ ID NO:4 (*Bacteroides thetaiotaomicron* gene for 16S rRNA – BT-A)

gttttcccta ggacgctcct tgcggttacg tacttcaggt acccccggct ttcatggctt 60

gacgggcggt gtgtacaagg cccgggaacg tattcaccgc gccatggctg atgcgcgatt 120

actagcgaat ccagcttcac gaagtcgggt tgcagacttc gatccgaact gagagaggct 180

tttgggatta gcatcctgtc accaggtagc tgccttctgt acccccccatt gtaacacgtg 240

tgtagccccg gacgtaaggg ccgtgctgat ttgacgtcat ccccaccttc ctcacatctt 300

acgacggcag tctctctaga gtcctcagca tgacctgtta gtaactaaag ataagggttg 360

cgctcgttat ggcacttaag ccgacacctc acggcacgag ctgacgacaa ccatgcagca 420

ccttcacatt tgccttacgg ctatactgtt tccaatatat tcaaatgcaa tttaagcccg 480

ggtaaggttc ctcgcgtatc atcgaattaa accacatgtt cctccgcttg tgcgggcccc 540

cgtcaattcc tttgagtttc accgttgccg gcgtactccc caggtggaat acttaatgct 600

ttcgcttggc cgcttactgt atatcgcaaa cagcgagtat tcatcgttta ctgtgtggac 660

taccagggta tctaatcctg tttgataccc acactttcga gcatcagtgt cagttgcagt 720

ccagtgagct gccttcgcaa tcggagttct tcgtgatatc taagcatttc accgctacac 780

cacgaattcc gcccacctct actgtactca agacagccag tatcaactgc aattttacgg 840

ttgagccgca aactttcaca actgacttaa ctgtccacct acgctccctt taaacccaat 900

aaatccggat aacgctcgga tcctccgtat taccgcggct gctggcacgg agttagccga 960

tccttattca tatggtacat acaaaattcc acacgtggaa aactttattc ccatataaaa 1020

gaagtttaca acccataggg cagtcatcct tcacgctact tggctggttc aggcctgcgc 1080

ccattgacca atattcctca ctgctgcctc ccgtaggagt ttggaccgtg tctcagttcc 1140

antgtggggg accttcctct cagaacccct atccatcgaa ggtttggtga gccgttacct 1200

caccaactgc ctaatggaac gcatccccat cgataaccga aattctttaa taacaagacc 1260

atgcggtcta attataccat cggatattaa tctttctttc gaaaggctat ccccgagtta 1320

tcggcaggtt ggatacgtgt tactcacccg tgcgccggtc gccatcttca gttgcaagca 1380

aactgaaatg ctgcccctcg acttgcatgg taagcc 1416

SEQ ID NO:5 (*Bacteroides thetaiotaomicron* gene for 16S rRNA – BT-B)

gctccttgcg gttacgtact tcaggtaccc ccggctttca tggcttgacg ggcggtgtgt 60

acaaggcccg ggaacgtatt caccgcgcca tggctgatgc gcgattacta gcgaatccag 120

cttcacgaag tcgggttgca gacttcgatc cgaactgaga gaggcttttg ggattagcat 180

cctgtcacca ggtagctgcc ttctgtaccc cccattgtaa cacgtgtgta gccccggacg 240

taagggccgt gctgatttga cgtcatcccc accttcctca catcttacga cggcagtctc 300

tctagagtcc tcagcataac ctgttagtaa ctaaagataa gggttgcgct cgttatggca 360

cttaagccga cacctcacgg cacgagctga cgacaaccat gcagcacctt cacatttgcc 420

ttgcgactaa cctgtttcca gattattcaa atgcaattta agcccgggta aggttcctcg 480

cgtatcatcg aattaaacca catgttcctc cgcttgtgcg ggcccccgtc aattcctttg 540

agtttcaccg ttgccggcgt actccccagg tggaatactt aatgctttcg cttggccgct 600

tactgtatat cgcaaacagc gagtattcat cgtttactgt gtggactacc agggtatcta 660

atcctgtttg atacccacac tttcgagcat cagtgtcagt tgcagtccag tgagctgcct 720

tcgcaatcgg agttcttcgt gatatctaag catttcaccg ctacaccacg aattccgccc 780

acctctactg tactcaagac agccagtatc aactgcaatt ttacggttga gccgcaaact 840

ttcacaactg acttaactgt ccacctacgc tccctttaaa cccaataaat ccggataacg 900

ctcggatcct ccgtattacc gcggctgctg gcacggagtt agccgatcct tattcatatg 960

gtacatacaa aattccacac gtggaaaact ttattcccat ataaaagaag tttacaaccc 1020

atagggcagt catccttcac gctacttggc tggttcaggc ctgcgcccat tgaccaatat 1080

tcctcactgc tgcctcccgt aggagtttgg accgtgtctc agttccaatg tgggggacct 1140

tcctctcaga acccctatcc atcgaaggtt tggtgagccg ttacctcacc aactgcctaa 1200

tggaacgcat ccccatcgat aaccgaaatt ctttaataac aagaccatgc ggtctaatta 1260

taccatcggg tattaatctt tctttcgaaa ggctatcccc gagttatcgg caggttggat 1320

acgtgttact cacccgtgcg ccggtcgcca tctccagttt gcaagcaaac tgaaatgctg 1380

cccctcgact gca

1393

SEQ ID NO:6 (*Bacteroides thetaiotaomicron* gene for 16S rRNA – BT-C)

gctccttgcg gttacgtact tcaggtaccc ccggctttca tggcttgacg ggcggtgtgt 60

acaaggcccg ggaacgtatt caccgcgcca tggctgatgc gcgattacta gcgaatccag 120

cttcacgaag tcgggttgca gacttcgatc cgaactgaga gaggcttttg ggattagcat 180

cctgtcacca ggtagctgcc ttctgtaccc cccattgtaa cacgtgtgta gccccggacg 240

taagggccgt gctgatttga cgtcatcccc accttcctca catcttacga cggcagtctc 300

tctagagtcc tcagcatgac ctgttagtaa ctaaagataa gggttgcgct cgttatggca 360

cttaagccga cacctcacgg cacgagctga cgacaaccat gcagcacctt cacatttgcc 420

ttacggctat actgtttcca gtatattcaa atgcaattta agcccgggta aggttcctcg 480

cgtatcatcg aattaaacca catgttcctc cgcttgtgcg ggcccccgtc aattcctttg 540

agtttcaccg ttgccggcgt actccccagg tggaatactt aatgctttcg cttggccgct 600

tactgtatat cgcaaacagc gagtattcat cgtttactgt gtggactacc agggtatcta 660

atcctgtttg atacccacac tttcgagcat cagtgtcagt tgcagtccag tgagctgcct 720

tcgcaatcgg agttcttcgt gatatctaag catttcaccg ctacaccacg aattccgccc 780

acctctactg tactcaagac agccagtatc aactgcaatt ttacggttga gccgcaaact 840

ttcacaactg acttaactgt ccacctacgc tccctttaaa cccaataaat ccggataacg 900

ctcggatcct ccgtattacc gcggctgctg gcacggagtt agccgatcct tattcatatg 960

gtacatacaa aattccacac gtggaaaact ttattcccat ataaaagaag tttacaaccc 1020

atagggcagt catccttcac gctacttggc tggttcaggc ctgcgcccat tgaccaatat 1080

tcctcactgc tgcctcccgt aggagtttgg accgtgtctc agttccaatg tgggggacct 1140

tcctctcaga acccctatcc atcgaaggtt tggtgagccg ttacctcacc aactgcctaa 1200

tggaacgcat ccccatcgat aaccgaaatt ctttaataac aagaccatgc
ggtctgatta 1260
taccatcggg tattaatctt tctttcgaaa ggctatcccc gagttatcgg
caggttggat 1320
acgtgttact cacccgtgcg ccggtcgcca tctccagttt gcaagcaaac
tgaaatgctg 1380
cccctcgact gca
1393

SEQ ID NO:7 (*Bacteroides thetaiotaomicron* gene for 16S rRNA – BT-D)

gctccttgcg gttacgtact tcaggtaccc ccggctttca tggcttgacg
ggcggtgtgt 60
acaaggcccg ggaacgtatt caccgcgcca tggctgatgc gcgattacta
gcgaatccag 120
cttcacgaag tcgggttgca gacttcgatc cgaactgaga gaggcttttg
ggattagcat 180
cctgtcacca ggtagctgcc ttctgtaccc cccattgtaa cacgtgtgta
gccccggacg 240
taagggccgt gctgatttga cgtcatcccc accttcctca catcttacga
cggcagtctc 300
tctagagtcc tcagcatgac ctgttagtaa ctaaagataa gggttgcgct
cgttatggca 360
cttaagccga cacctcacgg cacgagctga cgacaaccat gcagcacctt
cacatttgcc 420
ttacggctat actgtttcca gtatattcaa atgcaattta agcccgggta
aggttcctcg 480
cgtatcatcg aattaaacca catgttcctc cgcttgtgcg ggcccccgtc
aattcctttg 540
agtttcaccg ttgccggcgt actccccagg tggaatactt aatgctttcg
cttggccgct 600
tactgtatat cgcaaacagc gagtattcat cgtttactgt gtggactacc
agggtatcta 660

atcctgtttg atacccacac tttcgagcat cagtgtcagt tgcagtccag tgagctgcct 720

tcgcaatcgg agttcttcgt gatatctaag catttcaccg ctacaccacg aattccgccc 780

acctctactg tactcaagac agccagtatc aactgcaatt ttacggttga gccgcaaact 840

ttcacaactg acttaactgt ccacctacgc tccctttaaa cccaataaat ccggataacg 900

ctcggatcct ccgtattacc gcggctgctg gcacggagtt agccgatcct tattcatatg 960

gtacatacaa aattccacac gtggaaaact ttattcccat ataaaagaag tttacaaccc 1020

atagggcagt catccttcac gctacttggc tggttcaggc ctgcgcccat tgaccaatat 1080

tcctcactgc tgcctcccgt aggagtttgg accgtgtctc agttccaatg tgggggacct 1140

tcctctcaga acccctatcc atcgaaggtt tggtgagccg ttacctcacc aactgcctaa 1200

tggaacgcat ccccatcgat aaccgaaatt ctttaataac aagaccatgc ggtctgatta 1260

taccatcggg tattaatctt tctttcgaaa ggctatcccc gagttatcgg caggtggata 1320

cgtgttactc acccgtgcgc cggtcgccat ctccagtttg caagcaaact gaaatgctgc 1380

ccctcgactg catg

1394

SEQ ID NO:8 (*Bacteroides thetaiotaomicron* gene for 16S rRNA – BT-E)

gctccttgcg gttacgtact tcaggtaccc ccggctttca tggcttgacg ggcggtgtgt 60

acaaggcccg ggaacgtatt caccgcgcca tggctgatgc gcgattacta gcgaatccag 120

cttcacgaag tcgggttgca gacttcgatc cgaactgaga gaggcttttg
ggattagcat 180
cctgtcacca ggtagctgcc ttctgtaccc cccattgtaa cacgtgtgta
gccccggacg 240
taagggccgt gctgatttga cgtcatcccc accttcctca catcttacga
cggcagtctc 300
tctagagtcc tcagcatgac ctgttagtaa ctaaagataa gggttgcgct
cgttatggca 360
cttaagccga cacctcacgg cacgagctga cgacaaccat gcagcacctt
cacatttgcc 420
ttacggctat actgtttcca gtatattcaa atgcaattta agcccgggta
aggttcctcg 480
cgtatcatcg aattaaacca catgttcctc cgcttgtgcg ggcccccgtc
aattcctttg 540
agtttcaccg ttgccggcgt actccccagg tggaatactt aatgctttcg
cttggccgct 600
tactgtatat cgcaaacagc gagtattcat cgtttactgt gtggactacc
agggtatcta 660
atcctgtttg atacccacac tttcgagcat cagtgtcagt tgcagtccag
tgagctgcct 720
tcgcaatcgg agttcttcgt gatatctaag catttcaccg ctacaccacg
aattccgccc 780
acctctactg tactcaagac agccagtatc aactgcaatt ttacggttga
gccgcaaact 840
ttcacaactg acttaactgt ccacctacgc tccctttaaa cccaataaat
ccggataacg 900
ctcggatcct ccgtattacc gcggctgctg gcacggagtt agccgatcct
tattcatatg 960
gtacatacaa aattccacac gtggaaaact ttattcccat ataaaagaag
tttacaaccc 1020
atagggcagt catccttcac gctacttggc tggttcaggc ctgcgcccat
tgaccaatat 1080

tcctcactgc tgcctcccgt aggagtttgg accgtgtctc agttccaatg tgggggacct 1140

tcctctcaga acccctatcc atcgaaggtt tggtgagccg ttacctcacc aactgcctaa 1200

tggaacgcat ccccatcgat aaccgaaatt ctttaataac aagaccatgc ggtctgatta 1260

taccatcggg tattaatctt tctttcgaaa ggctatcccc gagttatcgg caggttggat 1320

acgtgttact cacccgtgcg ccggtcgcca tctccagttt gcaagcaaac tgaaatgctg 1380

cccctcgact gcatg

1395

SEQ ID NO:9 (*Bacteroides thetaiotaomicron* gene for 16S rRNA – BT-F)

gctccttgcg gttacgtact tcaggtaccc ccggctttca tggcttgacg ggcggtgtgt 60

acaaggcccg ggaacgtatt caccgcgcca tggctgatgc gcgattacta gcgaatccag 120

cttcacgaag tcgggttgca gacttcgatc cgaactgaga gaggcttttg ggattagcat 180

cctgtcacca ggtagctgcc ttctgtaccc cccattgtaa cacgtgtgta gccccggacg 240

taagggccgt gctgatttga cgtcatcccc accttcctca catcttacga cggcagtctc 300

tctagagtcc tcagcatgac ctgttagtaa ctaaagataa gggttgcgct cgttatggca 360

cttaagccga cacctcacgg cacgagctga cgacaaccat gcagcacctt cacatttgcc 420

ttacggctat actgtttcca gtatattcaa atgcaattta agcccgggta aggttcctcg 480

cgtatcatcg aattaaacca catgttcctc cgcttgtgcg ggcccccgtc aattcctttg 540

agtttcaccg ttgccggcgt actccccagg tggaatactt aatgctttcg cttggccgct 600

tactgtatat cgcaaacagc gagtattcat cgtttactgt gtggactacc agggtatcta 660

atcctgtttg atacccacac tttcgagcat cagtgtcagt tgcagtccag tgagctgcct 720

tcgcaatcgg agttcttcgt gatatctaag catttcaccg ctacaccacg aattccgccc 780

acctctactg tactcaagac agccagtatc aactgcaatt ttacggttga gccgcaaact 840

ttcacaactg acttaactgt ccacctacgc tccctttaaa cccaataaat ccggataacg 900

ctcggatcct ccgtattacc gcggctgctg gcacggagtt agccgatcct tattcatatg 960

gtacatacaa aattccacac gtggaaaact ttattcccat ataaaagaag tttacaaccc 1020

atagggcagt catccttcac gctacttggc tggttcaggc ctgcgcccat tgaccaatat 1080

tcctcactgc tgcctcccgt aggagtttgg accgtgtctc agttccaatg tgggggacct 1140

tcctctcaga acccctatcc atcgaaggtt tggtgagccg ttacctcacc aactgcctaa 1200

tggaacgcat ccccatcgat aaccgaaatt ctttaataac aagaccatgc ggtctgatta 1260

taccatcggg tattaatctt tctttcgaaa ggctatcccc gagttatcgg caggtaggat 1320

acgtgttact cacccgtgcg ccggtcgcca tctccagttt gcaagcaaac tgaaatgctg 1380

cccctcgact gcatg

1395

SEQ ID NO:10 (*Bacteroides thetaiotaomicron* gene for 16S rRNA – BT-G)

tttactagga cgctcttgcg gttacgtact tcaggtaccc ccggctttca tggcttgacg 60

ggcggtgtgt acaaggcccg ggaacgtatt caccgcgcca tggctgatgc gcgattacta 120

gcgaatccag cttcacgaag tcgggttgca gacttcgatc cgaactgaga gaggcttttg 180

ggattagcat cctgtcacca ggtagctgcc ttctgtaccc cccattgtaa cacgtgtgta 240

gccccggacg taagggccgt gctgatttga cgtcatcccc accttcctca catcttacga 300

cggcagtctc tctagagtcc tcagcatgac ctgttagtaa ctaaagataa gggttgcgct 360

cgttatggca cttaagccga cacctcacgg cacgagctga cgacaaccat gcagcacctt 420

cacatttgcc ttacggctat actgtttcca gtatattcaa atgcaattta agcccgggta 480

aggttcctcg cgtatcatcg aattaaacca catgttcctc cgcttgtgcg ggcccccgtc 540

aattcctttg agtttcaccg ttgccggcgt actccccagg tggaatactt aatgctttcg 600

cttggccgct tactgtatat cgcaaacagc gagtattcat cgtttactgt gtggactacc 660

agggtatcta atcctgtttg atacccacac tttcgagcat cagtgtcagt tgcagtccag 720

tgagctgcct tcgcaatcgg agttcttcgt gatatctaag catttcaccg ctacaccacg 780

aattccgccc acctctactg tactcaagac agccagtatc aactgcaatt ttacggttga 840

gccgcaaact ttcacaactg acttaactgt ccacctacgc tccctttaaa cccaataaat 900

ccggataacg ctcggatcct ccgtattacc gcggctgctg gcacggagtt agccgatcct 960

tattcatatg gtacatacaa aattccacac gtggaaaact ttattcccat
ataaaagaag 1020
tttacaaccc atagggcagt catccttcac gctacttggc tggttcaggc
ctgcgcccat 1080
tgaccaatat tcctcactgc tgcctcccgt aggagtttgg accgtgtctc
agttccaatg 1140
tgggggacct tcctctcaga acccctatcc atcgaaggtt tggtgagccg
ttacctcacc 1200
aactgcctaa tggaacgcat ccccatcgat aaccgaaatt ctttaataac
aagaccatgc 1260
ggtctgatta taccatcggg tattaatctt tctttcgaaa ggctatcccc
gagttatcgg 1320
caggttggat acgtgttact cacccgtgcg ccggtcgcca tctccagttg
caagcaaact 1380
gaaatgctgc ccctcgactg catgtgtagc cg
1412

SEQ ID NO:11 (*Bacteroides thetaiotaomicron* gene for 16S rRNA – BT-H)

ggacgctcct tgcggttacg tacttcaggt acccccggct ttcatggctt
gacgggcggt 60
gtgtacaagg cccgggaacg tattcaccgc gccatggctg atgcgcgatt
actagcgaat 120
ccagcttcac gaagtcgggt tgcagacttc gatccgaact gagagaggct
tttgggatta 180
gcatcctgtc accaggtagc tgccttctgt acccccccatt gtaacacgtg
tgtagccccg 240
gacgtaaggg ccgtgctgat ttgacgtcat ccccaccttc ctcacatctt
acgacggcag 300
tctctctaga gtcctcagca tgacctgtta gtaactaaag ataagggttg
cgctcgttat 360
ggcacttaag ccgacacctc acggcacgag ctgacgacaa ccatgcagca
ccttcacatt 420

tgccttacgg ctatactgtt tccagtatat tcaaatgcaa tttaagcccg
ggtaaggttc 480
ctcgcgtatc atcgaattaa accacatgtt cctccgcttg tgcgggcccc
cgtcaattcc 540
tttgagtttc accgttgccg gcgtactccc caggtggaat acttaatgct
ttcgcttggc 600
cgcttactgt atatcgcaaa cagcgagtat tcatcgttta ctgtgtggac
taccagggta 660
tctaatcctg tttgataccc acactttcga gcatcagtgt cagttgcagt
ccagtgagct 720
gccttcgcaa tcggagttct tcgtgatatc taagcatttc accgctacac
cacgaattcc 780
gcccacctct actgtactca agacagccag tatcaactgc aattttacgg
ttgagccgca 840
aactttcaca actgacttaa ctgtccacct acgctccctt taaacccaat
aaatccggat 900
aacgctcgga tcctccgtat taccgcggct gctggncacg gagttagccg
atccttattc 960
atatggtaca tacaaaattc cacacgtgga aaactttatt cccatataaa
agaagtttac 1020
aacccatagg gcagtcatcc ttcacgctac ttggctggtt caggcctgcg
cccattgacc 1080
aatattcctc actgctgcct cccgtaggag tttggaccgt gtctcagttc
caatgtgggg 1140
gaccttcctc tcagaacccc tatccatcga aggtttggtg agccgttacc
tcaccaactg 1200
cctaatggaa cgcatcccca tcgataaccg aaattcttta ataacaagac
catgcggtct 1260
gattatacca tcgggtatta atctttcttt cgaaaggcta tccccgagtt
atcggcaggt 1320
tggatacgtg ttactcaccc gtgcgccggt cgccatctcc agtttgcaag
caaactgaaa 1380

tgctgcccct cgactgca
1398

SEQ ID NO:12 (*Bacteroides thetaiotaomicron* gene for 16S rRNA – BT-I)

gctccttgcg gttacgtact tcaggtaccc ccggctttca tggcttgacg
ggcggtgtgt 60
acaaggcccg ggaacgtatt caccgcgcca tggctgatgc gcgattacta
gcgaatccag 120
cttcacgaag tcgggttgca gacttcgatc cgaactgaga gaggcttttg
ggattagcat 180
cctgtcacca ggtagctgcc ttctgtaccc cccattgtaa cacgtgtgta
gccccggacg 240
taagggccgt gctgatttga cgtcatcccc accttcctca catcttacga
cggcagtctc 300
tctagagtcc tcagcatgac ctgttagtaa ctaaagataa gggttgcgct
cgttatggca 360
cttaagccga cacctcacgg cacgagctga cgacaaccat gcagcacctt
cacatttgcc 420
ttgcggctaa cctgtttcca gawtattcaa atgcaattta agcccgggta
aggttcctcg 480
cgtatcatcg aattaaacca catgttcctc cgcttgtgcg ggcccccgtc
aattcctttg 540
agtttcaccg ttgccggcgt actccccagg tggaatactt aatgctttcg
cttggccgct 600
tactgtatat cgcaaacagc gagtattcat cgtttactgt gtggactacc
agggtatcta 660
atcctgtttg atacccacac tttcgagcat cagtgtcagt tgcagtccag
tgagctgcct 720
tcgcaatcgg agttcttcgt gatatctaag catttcaccg ctacaccacg
aattccgccc 780
acctctactg tactcaagac agccagtatc aactgcaatt ttacggttga
gccgcaaact 840

ttcacaactg acttaactgt ccacctacgc tccctttaaa cccaataaat ccggataacg 900

ctcggatcct ccgtattacc gcggctgctg gcacggagtt agccgatcct tattcatatg 960

gtacatacaa aattccacac gtggaaaact ttattcccat ataaaagaag tttacaaccc 1020

atagggcagt catccttcac gctacttggc tggttcaggc tttcgtccat tgaccaatat 1080

tcctcactgc tgcctcccgt aggagtttgg accgtgtctc agttccaatg tgggggacct 1140

tcctctcaga acccctatcc atcgaaggtt tggtgagccg ttacctcacc aactgcctaa 1200

tggaacgcat ccccatcgat aaccgaaatt ctttaataac aagaccatgc ggtctaatta 1260

taccatcggg tattaatctt tctttcgaaa ggctatcccc gagttatcgg caggttggat 1320

acgtgttact cacccgtgcg ccggtcgcca tctccagttt gcaagcaaac tgaaatgctg 1380

cccctcgact gca 1393

REFERENCES

[1] Spor *et al.* (2011) *Nat Rev Microbiol.* 9(**4**):279-90.
[2] Tap *et al.* (2009), *Environ Microbiol,* 11(10):2574-84
[3] Macpherson *et al.* (2001) *Microbes Infect.* 3(12):1021-35
[4] Macpherson *et al.* (2002) *Cell Mol Life Sci.* 59(12):2088-96.
[5] Mazmanian *et al.* (2005) *Cell* 15;122(1):107-18.
[6] Frank *et al.* (2007) *PNAS* 104(34):13780-5.
[7] Scanlan *et al.* (2006) *J Clin Microbiol.* 44(11):3980-8.
[8] Kang *et al.* (2010) *Inflamm Bowel Dis.* 16(12):2034-42.
[9] Machiels *et al.* (2013) *Gut.* 63(8):1275-83.
[10] WO 2013/050792
[11] WO 03/046580
[12] WO 2013/008039
[13] WO 2014/167338
[14] Lee and Lee (2014) *World J Gastroenterol.* 20(27): 8886-8897.
[15] Xie et al. (2016) Journal Dairy Sci. 99:6913–6921
[16] YQ et al. (2016), *J. Dig. Dis., "Therapeutic Modulation of the Gut Microbiota in IBD - More Questions to Be Answered"*, Oct 15, 1751-2980, 12422, Epub ahead of print.
[17] Gong et al. Gastroenterol Res Pract. 2016;2016:6951091

[18] Ott and Schreiber Gut. 2006 Aug; 55(8): 1207.
[19] Lozupone (2012). Nature. 2012 September 13; 489 (7415): 220–230
[20] Claesson, et al. (2012) Nature, 488, 178-184.
[21] Hansen, et al., (2010), Curr. Opin. Gastroenterol., 26(6): 564-571.
[22] Turnbaugh et al. Nature, 457(7228): 480-484.
[23] Mosca et al. (2016) Front.Microbiol.7:455
[24] Faith *et al.* (2013), Science, 341(6141): 1237439
[25] Wang et al. (2009) ISME J. 3(8): 944-954.
[26] Faith *et al.* (2013), Science, 341(6141): 1237439
[27] Masco *et al.* (2003) *Systematic and Applied Microbiology,* 26:557-563.
[28] Srůtková *et al.* (2011) *J. Microbiol. Methods*, 87(1):10-6.
[29] Miyamoto-Shinohara *et al.* (2008) *J. Gen. Appl. Microbiol.*, 54, 9–24.
[30] Cryopreservation and Freeze-Drying Protocols, ed. by Day and McLellan, Humana Press.
[31] Leslie *et al.* (1995) *Appl. Environ. Microbiol.* 61, 3592–3597.
[32] Mitropoulou *et al.* (2013) *J Nutr Metab.* (2013) 716861.
[33] Kailasapathy *et al.* (2002) *Curr Issues Intest Microbiol.* 3(2):39-48.
[34] Handbook of Pharmaceutical Excipients, 2nd Edition, (1994), Edited by A Wade and PJ Weller
[35] Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985)
[36] US 2016/0067188
[37] *Handbook of Microbiological Media, Fourth Edition (*2010) Ronald Atlas, CRC Press.
[38] *Maintaining Cultures for Biotechnology and Industry* (1996) Jennie C. Hunter-Cevera, Academic Press
[39] Strobel (2009) *Methods Mol Biol.* 581:247-61.
[40] Gennaro (2000) *Remington: The Science and Practice of Pharmacy.* 20th edition, ISBN: 0683306472.
[41] *Molecular Biology Techniques: An Intensive Laboratory Course*, (Ream *et al.*, eds., 1998, Academic Press).
[42] *Methods In Enzymology* (S. Colowick and N. Kaplan, eds., Academic Press, Inc.)
[43] *Handbook of Experimental Immunology*, Vols. 1-IV (D.M. Weir and C.C. Blackwell, eds, 1986, Blackwell Scientific Publications)
[44] Sambrook *et al.* (2001) *Molecular Cloning: A Laboratory Manual*, 3rd edition (Cold Spring Harbor Laboratory Press).
[45] *Handbook of Surface and Colloidal Chemistry* (Birdi, K.S. ed., CRC Press, 1997)
[46] Ausubel *et al.* (eds) (2002) *Short protocols in molecular biology*, 5th edition (Current Protocols).
[47] *PCR (Introduction to Biotechniques Series)*, 2nd ed. (Newton & Graham eds., 1997, Springer Verlag)
[48] *Current Protocols in Molecular Biology* (F.M. Ausubel *et al.*, eds., 1987) Supplement 30
[49] Smith & Waterman (1981) *Adv. Appl. Math.* 2: 482-489.

CLAIMS

1. A composition comprising a bacterial strain of the species *Bacteroides thetaiotaomicron*, for use in a method of increasing the microbiota diversity and/or inducing stability of the microbiota of a subject.
2. A composition comprising a bacterial strain having a 16s rRNA sequence that is at least 95%, 97%, 98%, 99%, 99.5% or 99.9% identical to the 16s rRNA sequence of a bacterial strain of *Bacteroides thetaiotaomicron* deposited under accession number NCIMB 42341, for use in a method of increasing the microbiota diversity and/or inducing stability of the microbiota of a subject.
3. The composition for use according to claim 1 or claim 2, wherein the composition is for use in a method of treating or preventing a disease or disorder in a subject, wherein the disease or disorder is associated with a level of microbiota diversity that is reduced relative to the microbiota diversity of a healthy subject, and wherein the treatment or prevention comprises increasing the microbiota diversity in the subject.
4. The composition for use according to any one of claims 1 to 3, wherein the subject has:
    (a) a reduced microbiota diversity relative to a healthy subject;
    (b) fewer than 99 different bacterial species; and/or
    (c) less than 190 different bacterial strains in its microbiota.
5. The composition for use according to claim 1 or claim 2, wherein the composition is for use in a method of treating or preventing a disease or disorder in a subject, wherein the disease or disorder is associated with reduced stability of the microbiota compared to a healthy subject, and wherein the treatment or prevention comprises inducing stability of the microbiota in the subject.
6. The composition for use according to any one of claims 1 to 5, wherein the subject has reduced stability of its microbiota compared to a healthy subject.
7. The composition for use of any one of claims 1 to 6 wherein the composition is for use in a method of treatment or prevention of IBS, IBD, obesity, type 2 diabetes, one or more infectious diseases, cancer, one or more allergic diseases, one or more autoimmune diseases or one or more metabolic diseases/disorders.
8. The composition for use of claim 7, wherein:
    (a) the disease is Crohn's disease;
    (b) the disease is asthma;

(c) the autoimmune disease is rheumatoid arthritis or multiple sclerosis; or

(d) the disease is cancer.

9. The composition for use of claim 8(d), wherein the subject is concomitantly treated with chemotherapy.

10. The composition for use of any preceding claim, wherein the increase in microbiota diversity and/or induction of stability of microbiota is for:

(a) non-acetogenic bacteria; or

(b) both acetogenic and non-acetogenic bacteria.

11. The composition for use of any one of the preceding claims, wherein the subject is:

(a) an infant who has been delivered by Caesarean section;

(b) a frail elderly subject; or

(c) between 10 and 19 years of age.

12. The composition for use of any one of the preceding claims, wherein the microbiota diversity is increased and/or stability of the microbiota is induced in:

(a) the intestine of the subject; and/or

(b) the distal gut of the subject.

13. The composition for use of claim 1 or claim 2, wherein the composition comprises a bacterial strain of the species *Bacteroides thetaiotaomicron*, for use in a method of increasing the microbiota diversity and/or inducing the stability of the microbiota in a subject diagnosed with IBD.

14. The composition for use of claim 13, wherein the IBD is Crohn's disease.

15. The composition for use of any preceding claim, wherein the bacterial strain has a 16s rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to the sequence of:

(a) any one of SEQ ID NOs: 1-12; or

(b) SEQ ID NO: 1.

16. The composition for use of claim 15(b), wherein the bacterial strain is the *Bacteroides thetaiotaomicron* bacterium deposited under accession number NCIMB 42341.

17. The composition for use of any preceding claim, wherein the composition is for oral administration, optionally wherein the composition comprises one or more pharmaceutically acceptable excipients or carriers.

18. The composition for use of any preceding claim, wherein the bacterial strain is:

(a) lyophilised;

(b) viable; and/or

(c) capable of partially or totally colonising the intestine.

19. The composition for use of any preceding claim, wherein the composition comprises:

(a) a single strain from the genus *Bacteroides*;

(b) a bacterial strain from the genus *Bacteroides* and does not comprise bacteria from any other genera or comprises such other bacteria only in *de minimis* amounts; and/or

(c) *Bacteroides thetaiotaomicron* and does not comprise bacteria from any other species or comprises such other bacteria only in *de minimis* amounts.

20. The composition for use of any one of claims 1-19, wherein the composition comprises *Bacteroides thetaiotaomicron* as part of a microbial consortium.

21. A food product comprising the composition as defined in any preceding claim, for the use of any preceding claim.

22. A vaccine composition comprising the composition as defined in any one of claims 1-20, for the use of any one of claims 1-20.

23. A composition comprising a bacterial strain having a 16s rRNA sequence that is at least 95%, 97%, 98%, 99%, 99.5% or 99.9% identical to the 16s rRNA sequence of a bacterial strain of *Bacteroides thetaiotaomicron* deposited under accession number NCIMB 42341, for use in a method of increasing or maintaining the intestinal microbiota diversity in a subject and/or inducing stability of the intestinal microbiota in a subject.

24. The composition for use according to claim 23, wherein the bacterial strain is of the species *Bacteroides thetaiotaomicron.*

Abstract

Provided are compositions comprising a bacterial strain of the genus *Bacteroides*, for use in a method of increasing the microbiota diversity and/or inducing stability of the microbiota of a subject.

5

Figure 1

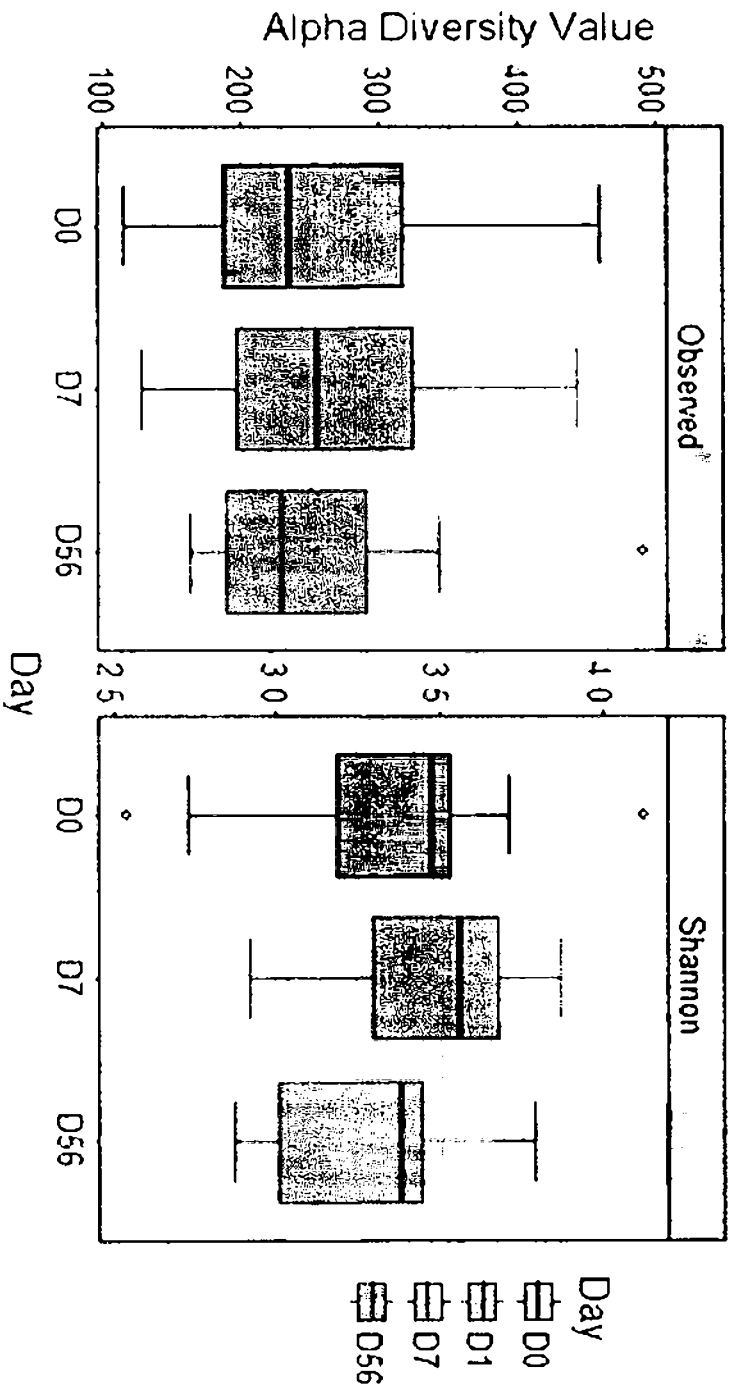
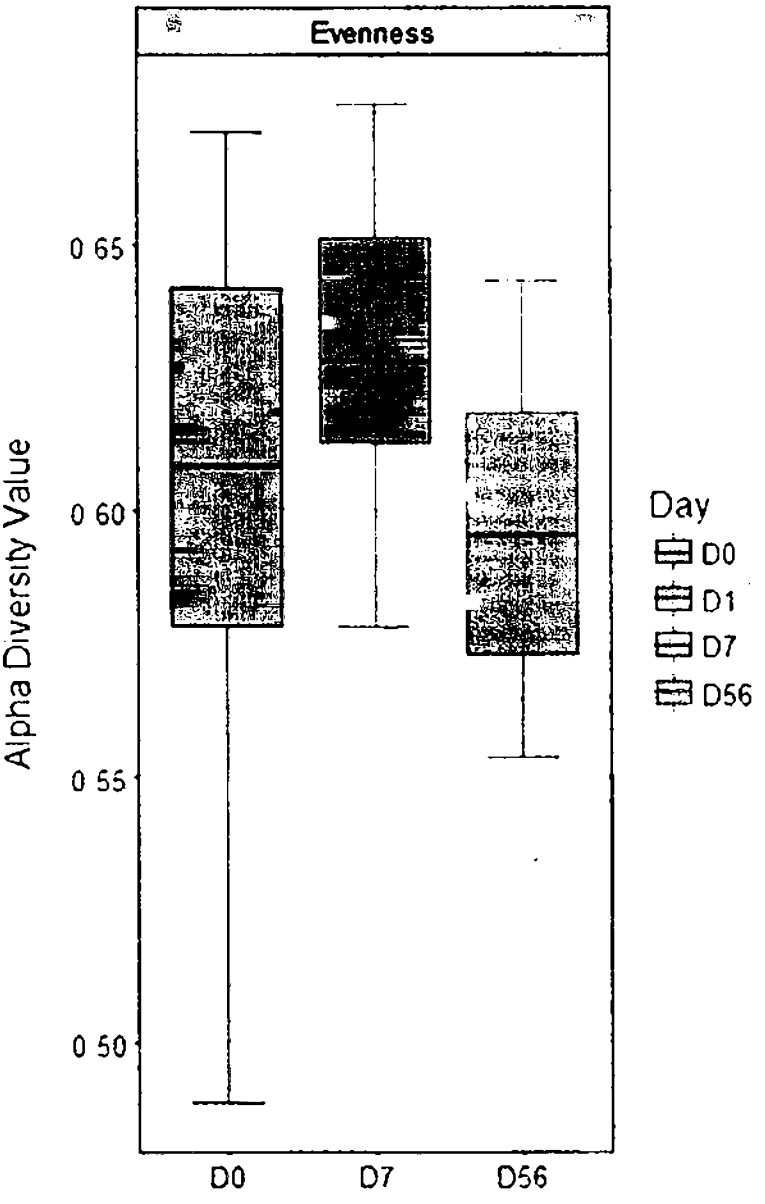

Alpha Diversity Value
100
200
300
400
500
D0
D7
D56
Observed
Day
2.5
3.0
3.5
4.0
D0
D7
D56
Shannon
Day
D0
D1
D7
D56

Figure 2


Evenness
Alpha Diversity Value
0 65
0 60
0 55
0 50
D0
D7
D56
Day
D0
D1
D7
D56

SEQUENCE LISTING

<110> 4D PHARMA RESEARCH LIMITED

<120> COMPOSITIONS COMPRISING BACTERIAL STRAINS

<130> P074041WO

<140>
<140> 2019-10-09

<150> EP18199455.9
<151> 2018-10-09

<160> 13

<170> SeqWin2010, version 1.0

<210> 13
<211> 33038
<212> DNA
<213> Bacteroides thetaiotaomicron VPI-5482

<400> 13
atgaacaaac cgaagattat acagattatt gatgttgttt ccaatgcgat agacggaaac 60
cgtatcgacg aggattttat aaagagttgt atttacggca aagtgaatgc ggaactgtat 120
gcgcatcttt taggcaaata caggggtat gacggggatt tctttcagtt ttatttgggg 180
acggacgatc ggataaaccg ggctttgctg gagaacctgg ggattaaggt cgaacccgac 240
aaatatccgg actatgatag ccggattgtt gcgcaggtcg tccagggaaa gaaaaggttt 300
gatatttatc ctttcgagtt ggaagccttt aacagatatg ccatgtttgg caataataat 360
gcgctttcat gcctgaaagg gatttccccg acagccgggc agaccgtccg ggaaaacggt 420
attaacgagt atggaaacgc cttgaattgg tcgctgttct ggataaaggc aaatccggag 480
gacaaagccc tgttagtgga tcacgttttg aatatccccg aacgatgata gcggaatatt 540
tcatttaccg gagaaagggg gataaagagc cgtttatctc cctgggggaa atgccgcaat 600
acgggttaag gccgaaacag aagtttaccg ggaaaaaact taaaatagag gtgatccgca 660
ggctttccgg tgtggaaatc gagcagacgg ccaccactcc gcagataaac gcctatatcg 720
aggcgaatat ctatgatacc gaacgttggc cggagtaccg gaagttatac cgacaggtgg 780
ccggagaggt ggaaaccgtg gccgacatct ttacgctcca gtatatttta gtggccgaac 840
tggaggatca gaccaggacg ggaaaggact gccagccgca gccgacagat ccgaaagacg 900
agaggctgat acacctaatc cggtgcgagc ttatgggtga acccctggaa atgtacaaga 960
cgatgataaa cccgatcatc gccttaaaga aacgtttcgt ttgatactac atctactaaa 1020
aatagtagat actacttta gtattttagt tcgtacgctc aatcctggga gaaaaactaa 1080
tcgggatatt cattgagttc ccggtatgcc ttagctaact cttcttgggt gtgacgtttg 1140
aacttttac ggcttatgat gaaaggcaac ctctgattca tttcattctc gtactcgtcc 1200
aggagatgtt tacccttcc ggtaaggacg acatgggtac ggtcgtcgga aaagcggatc 1260
tcttttaaat cttccattgt atctaatata cggaggatga ttttctcgta aatgggggta 1320
cgcagtaacg ggtgtgcgag catgggggaa ttgtatttta tatcgaaatt ctcgatgatc 1380
tccgtctgca cttcctctat atgcttggga agtgcttttc ccagcgtgta gtaaacagct 1440
atctgaaagt ctttgtaatt tggaaacatg ggcgtatatg gtttattggg aacgtggata 1500
tttttaatat ttccgttccg ggattcttcg gccgaaagat aaaaaaatct ctcgaataat 1560
ctttgggata gatgcgcttt ttttagtaaa tactaaaaat agtatttttg taatgcgcat 1620
tacagtagtg tattttgtaa acgtaacgtg tacttattca agtatttttag taaaatgggt 1680
atcttttata tccggttata ctaatattag tattttagta tttttataat gtacattaca 1740
aaaatatccg tatctttgca ttcagattga aaaacaaaag gcttatgata acgacgatcc 1800
agtttaagac gttggacgac ttgtatcagt tttacaacga cgcaattttt aacggtgagc 1860
tttcggaatg tatcgtgaat atgtcccgtc acggtggggc gttcggcttt ttcgccgcca 1920
accgttggag aggggacggg caggaaaaaa aggtcgttca cgagatcagc atcaaccccg 1980
attttatgaa ccgggaagat cgggactggc attctaccct ggtacatgaa atgtgccatt 2040
tgtggcagga ggatttcggc aggccgagcc ggggagggta tcataattcg caatgggctg 2100
acaagatgat ccaggtcgga ctgatgccgt ccgacaccgg ggaggccgga ggcaaaagga 2160
caggccagag cattacgcat tatatcattc cgggagggaa gttcgagcag gttttcaata 2220
cgctaagccg ggaggatctg caaaacttgc ggcttaggta caagccgact ttggcggccg 2280
tacctacccg gccgatccgc atcgccggaa gcgacgggga cgaaacggaa gaaccggaag 2340
atccggacga gggggagagc aaaagcggaa agcggaagaa atacacctgt gggtgcggct 2400
gtaacgtgtg gggaaaatcc gggctggtgt taaggtgcgg actgtgcgac actgatttta 2460
cggaacaata ggaaacagtg caaaggtcag aagcccccag taaaaacccc gccggaggca 2520
gatttcaagc atactcgttt tactaaaata ctaagataag tctatactaa tcttagtatt 2580
ttagtattat tgtaatgtgc attacaatat aggggtatat gaaaaagtgg ggatgtcgta 2640

atacgtcaat tcgccccact tttaaggctt gacgtgcaat agccgtcagc ccccagacgg 2700
acgggaacca ccccggccgc acacaaatat agaaaatctt ccggatttaa ccaaacaggt 2760
cgggatcttt ctttttcccc ctggccttga taccgaaatc ctcacgcagc atctttgcca 2820
gtgaattttg tgcgtgggct gtactcattt caccgctgca aaccttatcg tcgtaatatg 2880
cacaacggtc gtacacgtct ttcagccgtc cgctttccag gatcttgtcg agtgccctta 2940
ccggggtgtt gtcgctcgga tagcttaaag caccgtaaac ccaacggtaa acgaacgagt 3000
attgttgttg ctggtcgcct cgcagttctt ggggatttct cgcatggatg aacaggataa 3060
tcgtgtcggc tttcggtttt tgccccttct taggcgtttt gcagatgaat ttgtaatcga 3120
accatacatc ggcctcgctt tccttgattc gtgtctgtgc aggccttaaa acctttttggt 3180
cgatctgtgc attcgagtag gaggccggga tctccaggtc tttgcagaat tggtcgatcg 3240
agtaataata ctcccgtctg tcccgttggc tgcaaatgat cttgtagagc cgcttggtgt 3300
aattgccccg gagggttaag gcgatgttct tcccgtacct tgtaccgccg acacccgtac 3360
cgtagtagag caggaaaggg atcgcccagg ggttgagcgt cagggctacc cggtttgtgc 3420
ctttgatgtt gtgtaccgtc gttatcacca ccccgaacgt ttccaccgtc tggtggatat 3480
tgggatgcac ccagcgaaag gaaaagtttt tccgcatcat ggcgatagcc tctttcagaa 3540
ctttgctctt gttgttcttc cccccggctt cgtcgcattt gatctcgacg acaggctggt 3600
taaagaggtc tttaggcagt tgtttttccc tggtcatgtg ttgctggatc tggtcgctta 3660
tgagcgtcag gaggttttct tgccactcgg tcacagagta ctggccgaat gttacgttat 3720
tcggctggat taacattact tcttcctccg taggaggaat atagagggtg atgtcgttgg 3780
tcgccatagg ctttttttt tgagttcggg acaaagttaa agaaataaaa gataccgcca 3840
aaaaatacca agtacttttt attgtaacct ttagtaaaat gggtatttat tggaggggt 3900
aattttagta aaataagtat tttccccctt cggattttag taaaatgagt attttaggtt 3960
gtgtcttttc acctataacg atttgtattt cagttaatta taaatgaaaa aacgctttct 4020
tacaaagaac ctctacaaag actattctca caaagaagga aaatcttagg gaatcacagg 4080
cttcaaaaac ggctggaaaa tgtgcatttt gaaaacggaa aatgagaaac ggagaaaaaa 4140
tgagcgaagc gtttctttag tgcgatttt ccgcgcgtta aaatgtgtaa aattttgttt 4200
ttgtgttgta aaatataagg tgatcggaac gattgccgcc gttttacaaa tgtacacttt 4260
tttgggggc ggtgtacatt tgtaatgtgc attttggggg ctgggtgtct gatgtattgg 4320
agcgaagccg tgcggagcgt tccaggagcg acgagcgaag caaggagaga gagaacgacg 4380
gtttaccaaa gtgagcaagc agtcattgga cttagtccaa tgctgatttt ccaaatcact 4440
gatatataac tgtttacttg tttatatgta tgcgaattgc atacatattg catacattat 4500
tttgataatt cattgaatta tagtatattg tagccgatat ttatgtatgc aaaccctat 4560
acgtgaaatt atggcaacaa caagtaaaga tacgtctatc cgtataaagg agagtacaag 4620
gtttaggctt gatatgctga aaggaaacaa atcgcacgat gcgttcgtgg ccgaaatgtt 4680
actctatttt gaaacaacgg ggatcacccc gcagtcgaat gtaatgccgc cgaacattgc 4740
ggccaaagag caggcaagcc gggtgatcga agttgtccgg ggcattgaga aatccactaa 4800
cgtaaggctg aaaaacatcg aacaactcct tttgtcgctt gtcggggaag tgaaaacgcc 4860
gggggacaat ccggacgaat atatgcacat ctcacaggta caggaacttt tggagcgttc 4920
caagcaactg gaacaggaag ccagggaaaa ccgggagaag gccgggaaac tgcaaacgga 4980
tctcgaaata gcccggcagg agaaaggaac tccggccgtc ggatgcaata cgcacaagat 5040
cctggagatc gtggagcgta tcgacgaggt gaagaagata ccgacattca acgatacggt 5100
ttatgagata gaccgtaata cgctggatat gtgggtgaaa aggttaaagg acgaactaaa 5160
gagataagcc tatgtttgcc aaagtacacc cggccgagga cgtgaaaagc ggaaatacgg 5220
gaagctgtca cgacctggtg cgttatctgg aaaaggaaac gggggaggga cagcgttttt 5280
tctcccatac ggagcaggat atttcacccg aacgggtaat tatggatata gacgggaaca 5340
aaaaggcgtt aggggctaac gacgctaaat tcttcatgct ttcgctcaat ccgtcgcagt 5400
cggaacaaat gcaccttatc ggccggaaag tggacgattt taaggaactt acaccacagg 5460
agaaaaaaga ggttttccaa aagctggagg cgttcacccg ttcggctatg gatgaatacg 5520
ccttgaactt cggacgggac aatattaggg gaggccagga tctcatgtat tacgcacgtg 5580
tggaaacgga acgatcctat catccggaag atgaagaggt gaaacagggg atcgccagga 5640
tcggagagcc taaacccggt ctgaacttac acgttcacgt aatcgtttca cgcaagagcc 5700
tggacgggaa agtgaaactt tctccggggg ctaaatcggc cgggaacacc tgggaactgg 5760
agggcagggg gacggtgaaa cggggttttt cacatgaggg ctggaaagtc agggtacagg 5820
aatgttttaa ccggaaattc gattaccagg ccaaagaggg ggaaacttat gtacgtccgc 5880
aggtatcggc cgagatcggg aagatcacga acccggagct taaaaggata ttgcaggacg 5940
agcagtttac ggcggcaaac cagatcgtcg cagccatgag ggaacaaggt tacacgcacc 6000
aggtaaggaa gggggtgcat tccttttcac gggagggtga agtgttccag gttgaacaca 6060
ggctcttgaa agcgttcgaa caacctctgt ctgacgagca gctaaaaagc ataacggaac 6120
gtttcgactt gacaaagtac gaggcgaacc cggccgggta cagggagaac ggtttgcagg 6180
taaaggatat aagtttctcg acatatgtaa aagatgaagc acagaagggg gaaaaggcct 6240
tgaaagaggt cgcttataaa gtggtttatg atgaacaaaa ccatacgacg gtatcgttcg 6300
ccaccgtccg gcagttcgca tacgagcatc aaataaacct ggtgaaaagc gaaccgacgg 6360
ccgaagccgt cttgaagaag atcaagaacc cggagcttaa aaacctgttg gagaactacc 6420
gttttacgtc cgctaaccag attgtcgtag ccatgaaaga acaaggctac acgcataagg 6480
tacggaaagg ggtacattcc ttttcacggg agggtgaacg ggtgagcata cggcacaagg 6540
atctgaaaaa attcgccgat ccgaaactgg agagccgcca catggagggc attatcgaac 6600
ggtttaacct gtacaagtac aagcaggagg gcgtggccta ccgggaaaac ggcctggaag 6660
ccaagaatat ttctttcctg acataccaga aagtgccgat cgagccggaa gaggataaaa 6720
gcgtgaccgg aaagccggaa acaaaggagg aagcgcagca gccacagcag gaaacgccgg 6780

ggagcgaggc acatccggaa gaaacggaaa acgaagcgga aacgcccatg cctgaacccg 6840
atccggtgaa atacaggaaa gagcttaaag aggtgtctta tgatgtactg tttgataggg 6900
aaacgaaaac ctatgtgccg atttcggcga tccggaaata cgcatacgag aatgagatca 6960
atctgataga ccgatacaag catgggtacg cagtcaggaa cgaggatctt agggagtgcc 7020
tggcaaatcc cgaataccgg acggtaaggc agataaacaa ggccatgagg gagaggggat 7080
atacgatcga aagggacgag gccggaaact atacctacat caaggggaa agttctttct 7140
ttatggaacg ccgggatttg cttgccttta ccgggtacgc caaagatacc ggaggccggg 7200
aaaggggaac gcaccgaagc gcagacaaga cggtcgggtt tatcggtggt aaggccaaac 7260
aaaagctgat aaacgagatc ctgggggata gcttccggac ggaaaggatg cttgtaggca 7320
acgtgaaaaa ggccgtcagc cttatacaga acccggctaa tataaaaatg atgctgataa 7380
aacagatcgg cagttttcta aacccgttta aagagttatg atatgatgat gtatattatt 7440
atagggatcg cctgcctggt ggggatcatc gttgttgtgc tgttgcttcc aaattccggg 7500
aaacagcagc aaaaaggaca gaaataccgt tttgaactgt cggccggggg agggaggaaa 7560
ataacctttg ccgatccgtt cgataatttc ctggtctatg gtggggcgaa ctccggaaag 7620
accaaaagca taggaaagcc cttgttaagc cagtatatac aggccgggtt tgccggattt 7680
gtctataatt acaaggattt cgatctggca aggacagccg tacacctggt aaaaaaacat 7740
aattaccctt atgggtgctt ccagatcagt ttcacggata tggaacggac gcaccggaca 7800
aatccgatac gtccttccgt ggtgaagaat gaaaccttgt tcttgcagct aatggatgat 7860
atgctgaccg cctaccaggg aaaggacggc aaacgggacg agtggtttaa cggggcgttg 7920
gggatattaa ggggcgtttc gatccggttt tataacgatt atccgcagtt ttgcacgata 7980
ccccatatcg ttaatttcat ttgttcggcc ggaactgtcc ggataacgtc gttcctggag 8040
ggcaagcacc agagccgggt actggccggg gcattcctgg acgcaaagga cagccccaag 8100
acgcaaagca gctatctaag cagccttacc aacagtctaa gcacgctcgc caacgaaaaa 8160
aaggtgtgtt atgtcctttc cggaaatgat ttcgacttta acctgataga tccggaatgt 8220
ccgaaattgg ttgtcgtatc gaacgcctat cagatcgaaa acctgatttc cccggttata 8280
tcgctcatgc tttccatttc gtcccggcgt ttcacgctgg cgaacaaagt gccgttcttc 8340
tactttctgg atgaagcgac cacgttccgg atcgccgatt tcgagaaact gccgtccgtt 8400
ctaagggaat acctttgcag tttcgttttc ctcacgcagt cggcggcgaa gatcgagaag 8460
atatacggga aatatgaccg cagttcgata gaatccaatt tcgggaacca gttctttggc 8520
cggacaaagg acatagaggc cttgaaaagc tacccgcttg tttttggaaa agaggagagg 8580
cagagggttt caaagaccac cggaagcagc cggggagggg agaaccgcag ccgtacagtt 8640
tccacgcaga aagaggaaat atacgacacg aatttcttta ccagcctgaa atcgggcgag 8700
ttcgtgggga gtgcggcaca ttccaacatg aggaactttc atttgaggtt tgaaatgtac 8760
gaggacaagg aagatccgct tccgatcgtg catcccgtac tggcctcgga tatagaggaa 8820
aattaccagc agatcataag ggatatacaa ggcattgaat aacaagggaa gtgtggcgac 8880
attctacaaa gtgtgggaca actccacact tttttgtgga atataaaaaa ggggtaaaat 8940
tataaaagtt tgataattag tgtatttatt aaaacagtag tgtattatgt caaattggaa 9000
tggattttgt acaatataat tgtataaata cactccagtt tgaaactcca gtgcaatagc 9060
aaaggttgtt tttggattgg ccggataaga atttatttac ttaaatactt aaaaataaac 9120
tccttatgaa aacgcctttg tttatcttgc tccaggctac tggaggtatc agaaatgaag 9180
taaacacatt ccttagtgat tatgccgttc cggtgatcgc catgctcctg atcgtgggag 9240
tggggatcgg tgtggtgatg aattatgata agattataga ccgggacgga cagggaacaa 9300
gaaaagaggg tattgtaaac ttactctggg tagttggtta catcatcatc gggcttgcga 9360
tcattgcagc cgttatcgca cttatcaata gcaaacttaa aatgtccctc taatggactt 9420
ccaggttaga aaggggctgg aaacacccct gaaagtgcat ggaatgagaa cgaagttttt 9480
ttgcatctat tgtattatcc tggctgtgat tgttcttttt gtcgtgggct tcctgacaag 9540
tgccatgagt ggggagggat ctttttttggc ctttatcgtt tcgctggtgg ccggggcgtt 9600
cgcctctgtg gtcttgcgga tcgtctttat caacctttcc atgcagcgca agttcggcaa 9660
gttcaaaaga caggtatttg tcatatcgaa taaggatctg ttaaacagcc tgtaaacact 9720
ctttttatga aaatatcggc agcgcaagcg tactgtatct tggatacggt aggatcttcc 9780
atacttacca aatccggcga aatatccgtt tcctacctgt tggaattgcc ggaggcctat 9840
tcgctggatc gctcggatct ggaggaaagg cataacgagt tattccgggc tttccagtat 9900
gtccgaaccg ggtttataca taagcaggac gtgtttttac gcaggaaatt caagccggaa 9960
tatgtgatcg agggggaggg ctatatacag acggccgaaa ggaagtattt cgacggacgg 10020
gaatacctgc atcacttctg catattgtcc tttacgcttt cgggactgca aagccttgaa 10080
aaagcgtacc aggccaaccc gttggcgtat agcgagaaac tggcgaaggc agaccgggac 10140
aggctggccg agtttctgga atcggtggag agtgcagttt ccattatccg gaatatccgt 10200
ggcacgcaga tccggccgtt gaacgtggcc gaggtgaaac agcacctttt ccggtacgtg 10260
aacggtttcc atgacgacga gggactaagg gatatacagt gttccgatat gataaggatc 10320
gggcagaaga aagggatatt ctttgccgtt tgtgatgaaa ggtatctgcc cgacaggatg 10380
aaagtatatg ttacggacag cactttgcag gaggcaaaca gccagcttta tatgtcgatg 10440
ctggaaagga tcggcgttca tgtgccctgt tcccatgtgg taaaccagat atggaaattc 10500
gccgggggaa gctaccggga ggaactggcc gagagggtga agctgttcgg ccgttaccgg 10560
gagtttgaca aggccataaa aagccgttat gacggcctgg cctcgtatga gcaggagatc 10620
atcaacgagg aaaacgtgct ttgcaaaacg cacctgaacg tgttgctgtt ggaggatgac 10680
gaaacgatcc tgaaccgaca gacggaacag gtaaaaatga ttttcacgaa cgccgggttt 10740
aaatattaca ttccaagtta cgaggggctt tataatatct ttataggctc gatcatcggc 10800
cgggaaaaca acctgaatcc ggactacctg tttctttcgg atctgcattc gtcgctctgc 10860
atggggatca attactctac gttccgcagc gacaaggagg ggatcttgtt taacgaacgg 10920

```
ctgtatcaaa caccgattcg aaaggacgtt tgggacgcag ataagaaacg cattccggca  10980
cgtaacatga ttatcgtcgc atcgacgggt gggggaaagt cggttacgtc gttgaacatc  11040
atccagcaga acatcgagca gaattataaa cagatcgtcg tcgagttcgg aaagagcttt  11100
taccagcttt cgcagttgta cccggacagg tcgctgcata ttgattatga cggcagcagc  11160
ccgttgggga tcaatccgtt ctatacggcc gggaagcagc cggacaacga gaagataaag  11220
acgcttgtaa acctggtgct taaattctgg cggtctaagg cgatcatgga ggacacgaaa  11280
caagttgttt cgctgacaaa gatcatttgc cgttattacg aggatattcc cgacggccat  11340
tgtttcccgg acttttacag gtacgtccaa agagagggga aaaaactgta tgagcgtttg  11400
aatatcctgc cggaatactt cgatatagac agcttcctgc acgtgtgttc ggaatttatg  11460
ccgggggat tttatgagaa cgtgtgcaag ccgtccccac tggagaacga tatgcagaac  11520
cgggatttta tcgtctttga gctgacgaag ataaagaaag atccgttcct gatttccgtc  11580
attatgacaa tccttttcga tacgatagag aacaagatcc tttcggatcg ttccgtcagg  11640
gggatgctga tctttgacga gtacgcagaa agccagtcta taaaggacac cttttcaggt  11700
gcggacatac attcgacggt ggcgttctgt taccagaaac tccgcaagga aaacggggcg  11760
gtgggtacga tcgtccagtc attggcgcag cttcccgata acgagtacac gaaaggcatt  11820
atcgccaata cgcaactctt atacgtgctt ccggccaacg agatcgttta cgaccagacg  11880
gtagaggcgt tccatatcaa gaaccggagc cacgtaaacc tgatgaagtc gatccgtaac  11940
gacttttcag gggtacggcc gtattcggag atattcatcc ggtttatgga tagttatgca  12000
acggtggtac gcctggagct ttcaccggaa aaattgcttg cattccagac cgacggcgag  12060
aaatggaaca aattgcagga gatttacaag gaggccggaa gcatggaaac ggccatagag  12120
aagtataaac aattaaaaca gaaaagttat gaaacggaaa tgtctatgta gtttgcttgt  12180
gatgatgtgc ttttactgta cggcgttcgg ccagtgggtg gtgtccgatc cggccttgac  12240
gcagctatcg cagataacct gggcgaaaga acttaaacag gcatacgaac agtttcaagt  12300
gctgggggaa tccagggaca tactgactaa gagcctggat ctgtaccggc aggtgaacgg  12360
ggttatcaaa aactctaaaa tggtgttgca ggtgctttcc atgcaggggg aaatgttgga  12420
actggcggcg aaagagtgca cccggtcgga cgtgtttacc ggacaggagg catacgggga  12480
atatacgaac gtgctgaaca agatcatgga ggaaagcgtg ctttcctttg acctgatccg  12540
gacgatcata tcgccctcgg tgagcatgac cgacggggag aggataaaaa ttatcgtgga  12600
tctggataac aagctgaaag agaaccggga caagatgctc gacgagagag cgaggtttaa  12660
tacggtcaat gacgctataa aacggatcgc agccctgaaa tcaacagcca agaagtgagc  12720
ctatggaagt gatcgacaat gtgattagcg catacgaggc cgtcaagatg aacggcgtca  12780
gccaggggat catcgccctt tgttccttta tcgccgttat catcgtgata caaaagttcg  12840
tgacggcctg gaaagaggcc tttagcgagg gggacaagcc cgtcgatatg aaacagttct  12900
tcaaactgtt ctatatctat atttatgtgt tcgccattat catggtcgca ccgttcgcct  12960
ttacgatcgt ggaaacggca cttggaaatc ttcagaacga attgataagc cattatcagg  13020
aggacgtgga tctaagcatt gacgaggcga tcgtaacctt tacaaaggac tatatcgagg  13080
acgtgcaaag gcggcataac tgggtagggc agcagattca ggaggttatc atgctgccgt  13140
tcaatattgc ggcttatacg atactcctgt acgcaacgaa atatatattt ttcttctttg  13200
cgtcagcccg gtatctgtat cttatactcc tggagatcgt cacgccgata gccgtaatac  13260
tctacatgga cgagaaaacg aggcattata cgcacgcata cctgaaaaac ctgtttgtct  13320
gttacatgac gatcccggcg ttcctgatag ccaacgccct ggggtctatc attgcggaga  13380
atatcatgca catgtgcgga cagaacaaat ataccatgct gggacttctt ttcgctttcg  13440
tgttcaaact tttcctgttt gccaaaagcg tgaaattttg tcgtgaactg ttttaaatcc  13500
tgatcttatg aataaagacg atatactgaa agattttgat acactggccg aggccaagcg  13560
caaaggagcg gtaaacttaa aattgtgcct gggctttgcc ctggtggtgg ttgtcctggt  13620
gctggcctgg ggactggtgg tgaattttac ggcactggat aaggtggtcg tggtggagcg  13680
atccggcgag tacctgaaaa cgcacgccga ggatagcgag gctctgtttc ttgccctggt  13740
gaaaaagacg tgcgccgagg cgaccgggta cgctaacagc ttcgatcgcc tgaacctgaa  13800
aaaaaatcag gcgcacgccg ctttttatgt caataagaac gatctgaatg ccgtgttctc  13860
aaagtattat aatgataagg cctattttga tgcggtgcag aacggggcgg tctataaatg  13920
cgagatagac agcatccaga cgatcgccgg ggacaatgag ccgtacaagg tggctttcac  13980
ttccaccctg acgatttacg gcgtttcggg gcagaagcta aggtttctta tcaggacgaa  14040
aggggagatc gtgagaacca cgccgcaatt tccggagaac gtgacgggat ttttctttaa  14100
ccagtttata cagcagattg tccggataga acaagaacct gaaaaaaagt aacactatgg  14160
ataaggccac gaaaacaaaa ataatcctgt tcgcatccct ggggatcttc gtgctggtgg  14220
tgctggccgt tacgctggca ctgaccggga aaggggacaa ccgggaagag gacgggagcg  14280
tgaacacttc gcttagggca cagcagcaaa gcgattttac cctggacgat atgatgaata  14340
cggacggacg gccggagtac gagaatttta ccgatgaagt gtaccgggaa cgttcgggag  14400
ctatgtaccg ggaagatccg gaagtgatag ccttgcagga acagttaagg cagaaccaga  14460
gagccgacag tctgaaagcg gccgcagcca tgaaacggag aacggcgaaa gcccggccga  14520
aaaaggaaac gccgaagaag gaagagcctg aaaggacagt aggacgtttc ttttcggggg  14580
aagaaccgga gaacaaggga aacacgatag aggccattgt gtcgggcgat cagaagatca  14640
cgaacgggag cgtgataaaa cttgtcacgc tccaagaaat gcaactgccc ggcggcatgg  14700
tgattaacaa ggggacggcc gttttcgggg tggtgaagct cgcacaggac aggatcaata  14760
taacggttga atccgtccgg atcgggaaca gcatctatga gatgcaaaag accgtgtacg  14820
acagggacgg actgcccggt atatatgtgc ccttgaacat caaggcggaa gcgacaaagg  14880
aggcggccga cgaggtggtt agcgatatga acgttacctc ttacgggacg gacgttctaa  14940
gtacgggggt taatgcggtg agcaatgcgg caaagagcgt gttccggaag aagaacaacc  15000
aggtcgttgt caccgtgaag tcgaactata aactatatct gaaatgaaag ctatttgttc  15060
```

ttttatcctg gcgttggccg tttcctcggc cggggcgcag atccgcccgg tggaaagcct 15120
gcctatcgca gtgaattatt ccaagacgat acatcttgta ttcccctcgg ccgtgaagta 15180
taaccaggcc gttacggact tcgtggccgt cgataacccg gagagcgtgc ctaatatctt 15240
gcgcataaag gccaaccgga aaagtttcag caagcagacg accgtcagcg tggcgaccga 15300
gggaggtttt ttctattcgt ttaacgtgac ctatgccgac agcctggagc atacgaacta 15360
tttcctgccc gatatgtcga gcatacggcc ggacactatc tatctgaatg aggtgtcgca 15420
aacgcacctg atcgcaccgg aaaaggtgat ttatatagat tacggcgata cctgcataca 15480
ggtaagcaag gccgagaata ccgagaatat cgtgcgaatg atcgcagcca cgggaaaggt 15540
ggaggaattt cccaggcaga caaacgtgtc gctggcgacc gaaggggga agttctacac 15600
gttcaacgtg gattatcggc agcagccgga ggcgttcgtc tatgagatag gggagaaacg 15660
gccggagaaa aaggcgaacg tgatcctgac cgacaacatt atcccggccg gggaaaggga 15720
tcaggtcatg agccgggtat ataacgcaaa aaggggata ttcaacaagg ggatcgttcg 15780
caacaagatc gttttctccg tgaacaacct gcatatatat gataacttgc tcctgtttac 15840
ctttgagatc gagaacaaaa gcaaactgcc ttatgatata gattatatcc ggtattacat 15900
catcgacaag aaaacggcca agctgaccgc atcccaggaa gtggatcagc aggctttgtt 15960
ctcggagaat tattcgccca ggatagaggg gaacggccgg atgaaatacg tgatcgcttt 16020
cgataagttc accattccgg acgagaaagt tttccgtatc gagatcaacg agaagaacgg 16080
aggccgtcac gttctttttg acctggagaa cagcgacatc gtgaatgtgg aggatattta 16140
gccttgcgat ccttgcgctg atccctgtcg gccgggtggg ggcgcaggac aaacgttcgc 16200
tgatcgtgga ggtgttgcag accgcagggg attacgggga catcgccccg gtgtgggga 16260
tcgtccaaaa ggacggggat atttaccgtt tcgtccctc tgtcgcaccc ctgaaagaga 16320
aataccggat ctccgaccgt tacggctacc ggacgcatcc gataagcggt gaaaggcagt 16380
ttcacgcagg cctggatatg gcggcggtgt atgcggctac cgtacacgcc gcagccagtg 16440
ggacggtgac gttcgcaggt gaaacgcccg gatatgggaa aacggtagtc gttacgcacc 16500
gtttcggatt tcagaccaga tacgcacatc tgacactcat ttacacccgg aaaggtgcga 16560
aggtggaaaa gggggacgtg atcgggttcg tgggaagcac cgggataagt accgggaatc 16620
atttgcatta tgaggtgata aaaaaccaaa agagaataaa ccctttaaat ttcatttatg 16680
gaactaagta atgaattgaa agtagaaagg atcaggctaa gcctgacggc gaaaagcgtt 16740
gccgaggaaa tggggatcag ccgccagcag ctttgcaaca tcgagcagtc ggaaaccgcc 16800
cccgtggtgg tgaaatacat cgcttttcta aggagcaaag gggtggatct gaacgctctg 16860
tttgaccgta ttatcgtgaa caaataaaat aaatcgtatg agaaaattag ttaccgcctt 16920
gtgcgctttg gtctttttgt cgtcttgcga cgacgaactg aaacttgtca gccgggattt 16980
ttccgtgacg ctgaaaagta tagacggcaa tacggccgta gtggggaagc ccattaattg 17040
caccttacg atctcggatc tcgatccgga caacggggat cagatattga cacgctttga 17100
ggtcagggac ggggacggcg ttatcctggt ggataacaac gaatacagcc ccggagaaac 17160
gtttgaatat gatttcaagg caaacaatcg gctggatttc gattttatcc cggcaaccga 17220
gggagaggcg tatatcgtca tgggggtagc cagcgaactg gtgacacgat ccgacagcat 17280
caaactgaaa gtttccagcc cggagataaa catacgtttc cagaacgtgc cggatcttat 17340
gctggtatcg gaggaagcgg aattttattt gcagcttgat acggaacttt acggggtgaa 17400
agcgtcggcc aggttcgtaa agggaagcgg ccgggtgtat atttccgggt atgatgcgac 17460
aaggggcgag ggcgtggcac tggaaaaaaa caacctggtg acgttccggc cggatgcgac 17520
cgggcaggcg gtgatcgagt ttaccgtttc aagccgttac ggtctgccag tgaaagagaa 17580
tgtaacgata caagtaaatc aatgaggtta tgagaaaaat tgccgtatta tcgctctttt 17640
tgtgcctggt ggccgtgtgc agggcacaat atatcggaag ccagtatatg aacgtgaagc 17700
tgggctatat cgtggatgaa agtcaggtgc aggggacgtt cggttttgga aaggtgtaca 17760
aggctttcaa ggtgggggca aaccttaatt accggaatct aaaccgggat ctggtaaagg 17820
caaacaccgt gacggtcggc ccggagctgg cctattacgc cctgaaagga cggaaattct 17880
cgctcctggg gatcgcagcc gggacgatcg gctaccagaa agccaaggcg aaaagcgatc 17940
ttgtgtacct ggagaaaagc aaggctttcg tgtatgggta cgaggtcggg ataaggccgg 18000
aaatgttgct aagcccgaaa gtggctcttt ttgccgaata caggtttgaa atgttgttta 18060
actccattct ccggaataac aaccatgtcg gtctggggtg cgtgatctat ttataaactt 18120
aaataattga aggtatgaga aagtatttgt atttgtcagc cgtatgcgtg tgcatggcat 18180
tgtgtttcgt cggttgcagc aaggacgacg acgaaccggg aggaaaaggg gcgatgtatg 18240
aggtaacgat cgagcagtcg ggagatttcc gcagcttcat taagtcggtc gtggtagtcg 18300
ccaacggtac gcagttgaaa gacggggcga cgggggaaag cctcgcaagc ccggtgattc 18360
taagcgatga agaactggcc gtcgaaaagg tgacgttaag cacgacggga aaggcgatcg 18420
agtttgccgt ttccggtggt gtcgtggacg gagaggacgg cgttgtgaac gagccgatgc 18480
agtgggtagt gaccgtttac aaaaacggaa aggagatcga gaaaaagagc cttgttttcc 18540
gggacgggaa ggagataagt acggatgatc ttaatctgta ttataattga cgtgacggcc 18600
tgaatgtccc ccggatgaat gttcggggga ttctttttct tttgggcgtt cccgtgggac 18660
ggtcgggctt tacgctccaa gtcctcgcct aaaggctgtg ggctttactc tgcaatccct 18720
aacgcaattt tgggtgtccg ctttgtcatt ctgttaccgg gatttgtctg catggtggga 18780
gaaccgcacc gatcagccag gcacgcaata tcattttgtc attgccagca ggctgttttt 18840
atggtgtaac gctccgatat tgctttcttt ttgatagata tgcaaatgca agaagccccc 18900
cggcgaaaac cccgccggag gcaattatag aaagtctgtg tatcatttcc acaaaatgtg 18960
gaaatgatac acagataaca gttcttcaat aaatatatgt gttttttgta tgcttttgat 19020
tatcaggtat atatgacttg tttatttgtt tggcacaatg tttgttcttt gtctttcaga 19080
aaccatatgt taaatattta aatagattgg attatgaaaa aggtattatt tgcagtagca 19140
cttgttatgg gattgggtag ttcagtggca ttcgccggca acatggtatc ggatgttgag 19200

attgtggtaa tggtgaacga ttataagcca atcgaggtaa aggatctgcc gcaggcggtt 19260
caggacacga tcaagaagga tttcgcagac ctgacaatca aggaagccgc agttgaggaa 19320
agcgaggaag gcacaaagac ctacaaggtg acatttaccg atgcagaagg cacggacagc 19380
gaagtgttct tcaacgagaa gggcgaagtc ctgaaataaa gcaaatgaac agcaaaaaga 19440
gaattcaatt aaactgtgtc agcaaaatta atattttatt gctttgctga cacagtttgt 19500
tttacagccc ctccggttcc ggtgacttgt aaggcttgaa agcaacccga acctccttat 19560
gctcatcgat gacgaaagtc tgctccggct ccacagaacg aataatgacg ccattgactt 19620
tccaatacat gattgtccat gatttggaat gggtagcacg aaaatgcacc gttgtgcctt 19680
tcgctatttc agccggagag atcaattctc ctatcttttc cgcagccgta ttttccaact 19740
ccggtttagc cgaaagctga ccttcaccct gatccacacc aaatgccagc actacttttt 19800
ccactgcggg aggaagagga ggatcttcat cattgtcaca agaaaccgca aatagcgtaa 19860
gcccatacaa caaaacaacg acaaaagaga gataaccgat ctgtctcatt aagccgattt 19920
ctttttttte catagcagtt ttcataagct tatttttgt ttacgaccgc ataaagataa 19980
caatttgttg gctaaaaggg tttattaaat atagtgttta tcacttaatg atttaaggtt 20040
aatatcctgt aggttgcagg gagaacaaac gggaacagcc gttcccgccc ctttcggatt 20100
gtatgccagc acccctaaaa aatgccttgt caaagacaag tagtaacacg cctgtgttac 20160
gttttcgact tttcagccgt ccgaccgcca ccccgtccga tttgacttgc ctttgaggtt 20220
atatatgcaa atgcaggaag cccccagcga aaaccccgcc gggggcaata tttttaaaca 20280
ggctttaaaa atacacgttc agcataaggt ataaaaagac gttcttatcc ttagtgctgt 20340
ggtgttcaag ttcgaattgg attttgtcct cacgtgcgag ccaacctatt gcgctggcag 20400
cttccaggtc attgaaccct gttgcatcct tgacttcttg taaagtccat tttcggtgta 20460
cgtctgtgct taataagcgc cagagtttcc ctgcgttctc tccgatgatg tgcttatcca 20520
tactttgtta tttgttataa attgataaat ggtttcaaat ggataatgaa aagcactttt 20580
acatcaatag tgttacgcac tgcaaacatt ccagcgttta tcgtttatcc ttttgatacg 20640
ttgtatcact ctttcctgat atacagcaaa ccgtttcgcc gttcgatgta tatcttattc 20700
tcacgtgcga gccacccggc ggcgagtgcg gtttcatcag ccgcaaaatt tagccttctc 20760
gcaagctcgg caatggttat aagtccggtt tctcctaatt tttcaaagat gatcccggct 20820
ttaaatccta tatttgcttt ttccatgata aagtttatat tttaatggtt caaaaatatg 20880
tatatatttt attattcaaa gcgtgtagaa atacaaatta gaatttaccc cgttaataaa 20940
ttttaacaat atagatatta cgttctattc atttcatttt gcggatgaaa agaacttttac 21000
aactctatct tatagggggt taatttagta ataccttact gaaaaacaac gctctgaaac 21060
attccctgtc accgttaaac aggcacaaat atctcccctt tacatcctca aagaagatat 21120
aatacaggca ggaattaatg ccgaatctat ttgttccgat gatctttgca tggcttaatt 21180
tttggcgtac ttgtttttcc gtcagccctt cgatgtatat caaccctttc atgtttccca 21240
tttgtccggg ggtgatctta agcgaactaa gtagttcttt gaggtcaaac agcttaggag 21300
tgtttgacaa gcagtttagg gactgttttc gctgtgtctt ttccattgct ataaggattt 21360
cgcttgtgcc ctacaccaag ccaataaaca taaaaaaagc ggaagggcat tcgcctatcc 21420
gcaagcgagg gagtccaagc cctatacaca caaacaggag atacccccg catatcacgg 21480
ggagcattta tctgtttctc tgtgtataat gcaatttttg gacttttcgc ttgcacagaa 21540
atcagctaaa cgctttttt ctcaaaatgt aaaaacacgg tcggtttacg accgcacaaa 21600
gataacaaat tgttggctaa aagggtttac gaacctttta ttttttgcc ctattatctt 21660
aaattcagtg tttatgaaat accaagttct ttttgaacat ccttgtttgc agatgaaaag 21720
atacttttgc agtaccttaa tgttgtccgc atggtgggag aaccgtaccg atcagccggg 21780
tgcgcaatat cattttataa taaccgatag gttgttttta tggtgtaacg ccccgatatc 21840
gctttctttt tttgtatctt tatgtagaaa atattgcgta ttaactgtta atttcgaaaa 21900
atgatgtatc tttgcggctt tcaaaattgt aagtatagaa tttataatta aaatattagg 21960
tttcaaaagt ataaaggttt ttggttagtt gctctttctg gggtagcaga tattttatag 22020
taagttatat gcaaaaaaaa cagaaaaaag ttggaaatat tgatttttt tataaacaca 22080
cacacacaca cacacacaca cacacacgag tgtgtgtaga aacctatatt taagactttt 22140
caaggtaatt ctccttttctt ttttatcgtg ttccgttctt ttcgtttccg ctcaagagac 22200
acgggattcc gtgaaaatat atttccacaa gggtagaacg gaaatagatc tctctttacg 22260
ggataacagg cggtcgttag agaagatcag ccgccggata ttggaataca ggccggacac 22320
ggctttctct atctgcaaga ttatggtggt cggtgcggcg tcccccgagg ggagcatcga 22380
actgaacagg cgtttgtctg aactaagggc gaaacgtttg ttcgatcacg tgtgcggtta 22440
ctcgccgcta cccgacacgc tgaaaacgtc ggtattcgtg gggcgtgact ggaggggatt 22500
gctgcgtttg gtggagaatg atcccggaat gcccggtaaa tcggaaacgg tggagcttct 22560
gcaaggtatt gtttccgagg tggaagcaag tggaaacggg gagaacaaga taaatgaatt 22620
gaaacgcttg cggtacggca tcccatatcg gtacatgtat cggaatttgt ttccggatct 22680
tcgtgcatct tgtttgtata tttggtatga atcgaaacgg aagtcgttcc ctgccatgca 22740
ggttatctcc cacctgcatg atacattgta cttaccttgt aagctttctc cgttcggtgg 22800
agaaacgggc ggtttccagt ctggggaaaa acatgggcct ttttacatgg ccgtgaaaac 22860
gaacatgctt tatgatgcgt tgcttgtgcc gaatataggg gtggagtttt acgtcggaaa 22920
gaactggtcc ttggccggga attggatgta tgcctggtgg aaaagcgacc ggcaccataa 22980
ctactggcgg ctctatggcg gggatctgga agtgcgccgc tggtttggca ggaaagcgtc 23040
ggaaaaaccg cttgcagggc atcacatcgg tctctacggt cggatattca cctacgactt 23100
cgagaccggg gggacgggct acatgggcgg caagcctggc ggtacgcttt gggataagat 23160
gaactattcg gtaggtctgg agtatggtta ttcgctgccc gtcgcccgtc gtctgaatct 23220
ggatttcgtc atcggtgtgg gctattgggg tggagaatac cataaatatg cccctatcga 23280
cggccattat gtctggaagg agacccgacg gcgccactgg tttggtctca cggaggcgga 23340

gatttcgctc gtatggctct tgggccgtgg caactacaac gagaagaaag gaggcaggca 23400
gtgatactga tacgctttag tctggtgttc atatttttat ggatgggact gacggcttgt 23460
gagcataagg atttatgtta cgatcatccg cactttgcca cggtgcgggt agtattcgac 23520
tggaccaaga tttccaatca tgacaagcct gaaggcatga gggtcgtatt ttatccgacc 23580
gatgatgaaa gcaacacgtg gatatttgat ttccccggag gagaggatgg ggaagtggag 23640
ctccccgaga atgattaccg ggtaatttgc ttcaactacg ataccgacgg gatggtttgg 23700
aaagaaaacg gcagttacac gctttttact gccgatacgc gtgatgttcg gtcgccggat 23760
aaccagacaa tggccgtcac cccaccctgg ctgtgtggcg accatataga cagagttatc 23820
ctcaaggaca ttccggaagg aagcacgaag ataatacggc taactcccgt aaacatggta 23880
tgtcactaca cgtatgaggt gaacggaatt cggggactgg acagggtggc ggatttgcgg 23940
gccgctcttt ccggcatgtc cggctcgctt aacatgtccg gcgacagttt gcccgccgat 24000
ctttcggaga gcctgctctt tgatggaatg gtttcacgga atcagattat aggcggattc 24060
tatacgtttg gacattccgc acttgaagga gagcccaatg ttttccggct gtatctcaag 24120
aaccgttcag gcagcatgtc tgtattggaa caggacgtga gcgaccaagt gcatgatgtc 24180
ccggtagcgg ggcatatcgg tgatgtgcat ctggtgttga attttgatta tgaggtacca 24240
tccgagccgg gaagtggcgg tccgggattt gatgtggacg ttgatgattg ggatgatgtg 24300
aatgtggata tagtgttgta atcgagtttt attaattata tttattaaca attttattca 24360
ttttatgaaa aaaagtacag taatgctttg ggcaatcttt ggagtactac tgatgagttg 24420
ctcagaagag gagattgcga atgtggaaac ctcctcacga aatgcgattg gttttaacgt 24480
gttaagtaat gcggcggaaa cgagggctac ccctactacc aacaccaact tgaagaacac 24540
cgatttcgac gtgttcgctt ttacggcgga tggtactgcc ttcatgggaa agaacgacac 24600
ggaatttgaa catgacggag tgaagatcgt gtacaaaaat ggtaagtggg attatgacaa 24660
tgcgagcgac cttcgttatt ggcctaccga ggctttggac ttttacgcgt tcaatcccgg 24720
aacggttagt gaagacatga tggcgtttta tagttgggaa gccaccaagg atgtccaaaa 24780
gataagttat acctgcatgg atgagtatgg aagcggtact actcatgcaa actacgacgt 24840
gatgtatgcc atggccaaag gccagacaaa agacatgaac aacggaatag tgaaatttaa 24900
tttcaaacat attctgtcac aggtcgtatt caaagcaaag acccaatacg ataacatgca 24960
ggtggacatc gacgtgatca agattcataa tttcaaattt gccggcgcct tcacattgcc 25020
tgcggcagcc gatggaacgg gaagttggag ttcgtctgat ttagcatttc cacacgcatt 25080
taccgtagtg aaaaacgcaa acatcacggt caatagcaat accgaagcta ccgatattac 25140
cacaaatact cctatgctga atataccaca ggagctgact gcttggaaag tttccgaaac 25200
agccaccaaa agcaagttgg aggccgataa tgcgaagcaa tgttatctgg agatcgcctg 25260
caaaatccgg cagtccggag cttatctgtt gggttcagca agcgaataca aaacaattta 25320
tgttccgttc ggagatacat gggaacaggg caagcgtcat atctacacgt tgattttcgg 25380
tggtggttat gatgatcagg gagaggctgt gttgaatccg atccagtttg atgcagagac 25440
aacaggctgg gttgatgcag ataaagacgt aaacgtccaa ccttaatcca aacgtttatt 25500
aatatcgtac ccgttggcag gttaaacata cgcaagatta accctgccaa cgggtttttt 25560
atagggaatc gcattttcaa tatacttttt gatgtgtagg ggtgcttttt gcgtaccttt 25620
ttcctggaga atttcttttg tggagtgttt gccctgtata ttcccgatac gctgttatag 25680
cagggaactg tttttatcca agtagtcaga tatagcctct tgcaataccg attttagcga 25740
atcgcctttg cggatcgcga tcagctttaa gttctggtga taggtttcgt ccatgacaaa 25800
attacaatgc acggttttcg ctttcttcgg cttttgagcc tctttttgcc cggccgtgga 25860
ttggatcagg ctgtctaatc ccccggtcag cccggccgac atactgtttt ttaaatcgtt 25920
cttctttgcc atatggttac tttatttctg ttagcagttc gttacataca ccctcgtagt 25980
cctctgcccc ggcagatttg ggggcatagt gaaaaatatc ctgcccttgt gtcggggctt 26040
cggccagcgc aatggagttg cgtacatggg tgctgaacac tttgccctgg aatgtttcct 26100
gtaccagttc cgaaacgctc ttgttcaggt tctttcttcc gtcgtactgg gttatcagaa 26160
ccccggcgat cgaaagatcc gagttcaaac gttgctgcac cttgtggact acctgcataa 26220
gtttggccat tccacgcatt gccaggaatt gagcctgaac cgggataatc aaccgatccg 26280
aggccgtcag tgcgttaagc gtgagcagcg acagcgaggg aggacagtcg atcaggatat 26340
aatcgaattt ctccttttgg cctttgataa ggtgggctaa gataagctcc cgtccggcct 26400
cgttgattaa ctccgtttca acggccgaga gatccaggca ggagggaacg acgctaaggc 26460
cgtccttatg ctcgtagatc ggcaggtcgt attcgccttt catcgcaccg tagatcgttt 26520
ggggaagctc cgcagagaag cccagcgatt cggtcaggtt ggcctgcccg tcaaggtcga 26580
tcaaaagaac cttgtaccct ttttgacgta acgcaccgcc caggttgatc gtggttgtgg 26640
tctttcctac tccccccttg tgattcaata cggagattac ttttgctttg ctcataaatt 26700
cagtttttta aatggttgtc atgtccgcaa atatagttaa aatactaata tactaaaata 26760
ctaaaagtag tatttttcgt ttttgcgtg ttttttgtgc cgatcgggga agcgtaccga 26820
aataaatcct gtttccgtct ttcggtacgt tttgtttctg taccggaatt aatcaaagat 26880
tagaataacc ataaatgttt ttatacgtta atcatacgta tgtatatatg tatatatttg 26940
tttttgagta tctttgtgtg ctataaataa aaaataaaag acatgagcac ttattcgtat 27000
aaaaatccga agtttataaa ttcccctaaa ggggtggttg aagttgtaga agtgatttat 27060
gacggcaaag atgatccggc ctattcactg gctattatta aatgggaaaa tacctataaa 27120
ttaggtattc gttggaacat cgcttatagt gaatgggacg attaccgcaa acagaacggg 27180
caagatgaat gtataggtaa tccacagtcg agaggtattc ccacctggtt tgttttgccc 27240
gatgatatga tgtttggtga gaagttcagc ggtgcgatgc agaggcttga tgaattaaga 27300
aaaggtaagt aagcaatgga acagggagaa atcatattat accagccgga tgaagcggtc 27360
aagttggaag tgaggttgga agatgaaacc gtttggctga cacaagaaca aatagccgac 27420
ttgttcggga ctaaaaggcc agctattacc aaacatttga acaacattta caaaagcggc 27480

gaacttgata tagatagcac atgttccatt ttggaacata tgggtaatga cggcaaacag 27540
agatatacta ctaaatatta taatcttgat gcgattctgt caataggata tcgggttaat 27600
agcaagaacg caaccctttt taggaaatgg gcgaatagtg tattaaagga ttatctttta 27660
aaggggtatt ccattaataa acgtttgtca gaacttgaaa ggactgtcgc tcaacatacc 27720
gagaagatag atttttttcgt gcgtactgcc ttgccccggg tcgaaggcat tttttataac 27780
ggccagattt tcgatgcgta caagttcgcc accgatctgg taaagtcggc caggcgttcg 27840
atcgtgctta tcgacaacta tgtggacgaa acggtcttgc tcatgttgag taaacgcagc 27900
gtcggtgtct cggccaccat ttacacacag cgtatcacgc agcaactcca acttgatctt 27960
gacaggcata acagccagta cccacctatc gacatcagga cgtaccggga cagtcacgac 28020
cgtttcctga tcgttgatga aacggacgtg taccatatcg gagcctcgtt aaaggatctg 28080
gggaaaaaga tgttcgcttt ctcaaagctg gatattccgg cggctgtgat aactgacctg 28140
ttataatatc ccttgtctta tgaatgtggt tatctattca cgtgtaagct cgcagtcggc 28200
caggcaatcg accgaacggc aggttgtcga tctggagagg ttcgcagccg gacggggtta 28260
cgaggtgacg gcggtctttg aggaaaagat aagcggacga aaggccaata tcgaacgccc 28320
ggttttaagc cgttgcctgg aatactgcac agatccgcag aaccgggtgg atatgctgtt 28380
gctgaccgag atctcacgcc tggggcgttc caccctggag atactaaagg cactcgatac 28440
gctgcacacg cataaaatat gcgtgtatat ccaaaacttg aacctggaaa cattacggcc 28500
ggacaagacg gtaaacccct tgtcctctct gattactacc ctattggggg aactggccgc 28560
aatcgaacgc caggggatca ttgaccgcct taatagcgga cgggagttgt acatccagaa 28620
agggggcagg ctgggacgga agccgggaag ccggaaaacg gccgaacaga ggaaagagga 28680
ataccgggag gcgatcgcct tgttgaagaa aggctactcg atccggaacg tggcgaagct 28740
cacgggaaag gccgtttcga cgatacaaca agtaaaaaaa gacttcatta atagctgatt 28800
atcggaacaa atattgtatt tttgcaacaa atacgagtaa atgcacgtaa ttaaaaacga 28860
gtatttgcac gaaaataatc agaatctaaa taaaaacggt tatggcaaca atggcggaga 28920
aaatggcggc ttcactggag gaattaagaa agctccagga gaaagaccgc tgtgtcgtcc 28980
tgcaagggac ggccgagata gggaggacgc atttaacccg gctgttggat aacggctggt 29040
tgcaggaagt aatgaaaggc tggtatattg cggccaggcc gggaacagag ggggacacga 29100
cggtctggta cacctcattc tggtatttta ttgcgaaata cgcggccgtc cggctggggg 29160
aacaatggtg cttgacggcc gaccagtcgc tggatctgta ttcggggaaa acgaccgtgc 29220
ccgtacaggt ggttatcaag tcgccgaaag gccataacaa cacgcaaaag ctgatgtatg 29280
atacctcgct tttggtgttt caaagcgaga ttccggatca ggtatataaa gagccggaat 29340
acggccttaa tctctatccg cttgccgaag ccctggtata taccacgccg agatatttcc 29400
aggtggagaa gatcgcagcc cgcacctgcc tggccatgat acgggacgct gccgatatat 29460
taaaggtact gacgaaaaac ggggcttcgc ttcgtgccgg aagaatagcc ggggcgttcc 29520
ggaacatcgg gaacagcgaa atcgccgaca gcatcgtttc cacgatgcgg gggttcgggt 29580
atgacgtgag agaagaagat ccgttcgagg atcagccacg aacgcccctt gtttatgagg 29640
tgtcgcctta cgtcacacgc ttgcgcctga tgtgggagaa catgagggat aaggttgtag 29700
aactgtttcc cgaagccccc ggaaagattg atgacgtgga ggggtatttg agatccgtcg 29760
atgaaaagta ttcggaggac gcttatcatt ctctctcgat cgagggatat agggtttccc 29820
cggaactgat cgagaaagtg cgtgtcggga actggaagcc ggaggaagaa gataaggagc 29880
ataaaaacgc actggtggca cgtgggtatt accaggcgtt ccaggctgtc agggggacga 29940
tcgccgatat actgaaagga aagaacgcag gggaagcggt aagggcggat catccggtct 30000
ggtatatgca gatgtggatg ccgttcgtta cggtggggat cttgcaaagg gaggatcttg 30060
tgggttatcg taccgggcag gtctatatac ggggatcgca acatatcccg ttgaacccga 30120
aagcggtcag ggacgctatg cccgtccttt tcgatctctt gaagaatgag ccgcacccgg 30180
cggtaagggc tgtcttggga cacttctttt tcgtgtatat tcaccctat atggacggga 30240
acgggagaat gggacggttc gtcttgaacg ccatgcttgc gtctggaggc tataactgga 30300
cggttgttcc ggtggagcgt agaaaagaat atatgaaagc gttggaaaag gccagcgtgg 30360
agggggatat ttcggagttt acgaaagtaa tcgcatcatt ggtcaagtaa tgatatttat 30420
aataatgaca atatgaaatc aacggaatat atcgaatggg ataaactgga gcagatcccg 30480
ttctgccttt gccggatcgc ggaggatgaa gaaaaccagg aaatagatgt ttattatctg 30540
gataaacggg tttgccatga ttacgatcat gtggggcatt attttcgtac tgcgataatc 30600
atgtttagaa ggatcaggaa cataacggcc gattgggtga acctcaaaaa cctttggttg 30660
ctaagggact gtatcaggga gaattttaat catggcctgg aggtggacga tctgatcttc 30720
ggggaaacat tcgacggtga ggatccggaa actataaagc cgctaaccaa agaacggctg 30780
ttcaagatta aaaaggtgat tcaggaaaaa gatccatacg ctaccgtata ggggggggagt 30840
atataaaaac aaaaccctgg agtttgtctt gcgacaggga caacagggga ttactacgca 30900
aatataggga gtatatctcg tttatacaag gattttatgg cttttctgga ttgattttaa 30960
aaagaataga ggcttgttcc ctggaatgta gtatttacta cttttagtat tttagtatat 31020
tagtacgctt ttcttttgga ggatttgggc gttcccgtaa acggtcgggc tttccgcctc 31080
caagtcctcg cctaaaggct gtgggctttc cactgcaatc cctaacgcat gaatacaccc 31140
actcacccga ttaaggtagg ctatttccgg atagagccgt tcggtgaagc cagccggata 31200
aaagccgcct gacttttcac tgatcggcac aatccgggta ctccggtagc cgattgtcga 31260
acccggtttt aaacccccct tcaggggaga gaggtatttt agtttttccg gcgtggccgc 31320
cttgcgttta ccttgaccgg ggtttttgtc tgcaaaggtc aaacgttttt ttccatatcc 31380
caagttgagc cgcaagcggc ttttagaaaa aatctccagc ctggctaaag ccaggtagta 31440
tttttcctaa aaaacttgtg ctatggaaaa aaacgtttgg ggtgttctca tacaaaaacc 31500
cctcggaact caaggtaaac gcaagatgac cacaccgaca gccccgaaaa accaaaatga 31560
ggttgtcttg atacaaaaac atcggaatct taggcatccg caaggttttt tgggggggag 31620

agggtgcgag gtggggagtt tactatcgga aaaaatcagg tagtaaatac taaatttagt 31680
atatactaaa atactacttt ttcatataat tatctgataa atagttagtt aaatgttgtt 31740
taaatacttg catatctcaa ataaaagtgc tatattagag gtatagaaaa agagataaca 31800
acctttagaa ctggctcacc caccaggata aagaacgggg gacgtagtta tgaaaactga 31860
ttatttcgac aaggcagcgc aacagtttgc aaatttgatg atcgagaaga tccagcaggt 31920
agaggacaat tggcaaaagc cctggatcac cattgcggcg aatacacgca atttctttcc 31980
gcagaacctc acaggacgca ggtacaccgg aggcaacgcc tttttactcc ttttcctttg 32040
cgagaaattc cagtaccaga cacccgtatt tatgacattc aaccaggcga aagaggctgg 32100
gatctccgtg ctgaaaggat cgaaatcgtt tccggtctat tacttccttt tctacgtgta 32160
ccacaaggaa acacgcaaga aaataacctt tgaggaatac aaggcgttaa gccgggagca 32220
gcaacaggaa tacaacgtta taccgaccta taaatattac tcggtcttta acctggatca 32280
gaccaatttt tcggacgtga ggccggaaga atgggaagcg ttaagggaga agttcagagg 32340
gggacaggca gagcagcccg acgggacaac cggagaggcg caccccgaaa tagatgccat 32400
gctggaggcg aaaagctggt tttgcccgat acgggagcag caaggcgaca gggctttcta 32460
ttctccttta gccgattata tcgtagtgcc gcttcgctcg cagttcgtcg atatgcagag 32520
cttttacgaa acgcttctgc atgaaatggg acattccacg ggacacccca cccgattgaa 32580
ccgggatctt gcgcacccgt tcggatcaga ggaatacggc aaggaggaat taacggccga 32640
gttcgccgca gccctggcag gaatgttttt cgggatagcc gaacatatac gcactgaaaa 32700
cgccgcttat ctgaaaagct ggataaaggc gatccgggaa gaacccaagt tcattttaaa 32760
cgtactggcc gacgctatga aaattgtcaa gatgatagcc gggaaactgg atatctccgt 32820
agaaagcgag gaaacggccg cagatgaagc ggaacaggtg gcatgataca aggaagatcc 32880
cccgaaaggg ggacagcctt tcggggtgag ttctttttgg cactcgcccc aaaaagaacc 32940
aaaaaacgct ttgccgctac actccgtttc gctttcatag ctctgataat gcgtttttg 33000
attggataga tatatatttg tagataaaat aatgtaat 33038