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Gut Microbiota in Disease Diagnostics

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1. Introduction

A substantial amount of scientific documentation is mounting for the significance of the gastrointestinal microbiota (gut or GI microbiota) on human health. In addition to the obvious connection between the gut microbiota and inflammation in the colon (leading to IBD, IBS and possibly colon cancer), recent articles have highlighted the not-so-obvious connection between the gut microbiota and the brain (Heijtz et al., 2011)) and cardiovascular system (Wang et al., 2011).

Despite several major breakthrough discoveries of the importance of the gut microbiota in human health, this information has not yet been transformed into diagnostic or therapeutic approaches. A major bottleneck in the gut microbiota diagnostics is the reproducibility and throughput of the analytical approaches used.

Since we do not know the growth conditions for most gut bacteria, traditional cultivation based analysis of bacteria cannot be used for a comprehensive overview of the gut microbiota. Currently, quantitative PCR are quite extensively being used to detect the presence of specific pathogens. However, since the gut microbiota is complex and contains hundreds of different microbes, such technology does not hold the promise to be used as a general analysis tool to monitor changes in the gut microbiota that affect health conditions. The current focus in human gut microbiota screenings are based on explorative deep sequencing. It is expected that it will take considerable time and further development before sequencing of the gut microbiota could become a routine diagnostic tool. However, since the human gut microbiota is getting more thoroughly characterized, it is time to look for more targeted approaches rather than the current explorative screenings.

The most widely applied targeted approach to describe microbial diversity are by probes targeting the gene encoding 16S ribosomal RNA. This gene is ancient, universally distributed and comprise highly conserved sequence domains interspersed with more variable regions (Woese, 1987). The conserved regions provide information for classification of higher taxa, while the variable regions can be used for differentiation between closely related species. An average bacterial 16S rRNA molecule has a length of approximately 1 500 nucleotides, making it experimentally manageable by e.g. PCR amplification.

The first step in 16S rRNA gene microbiota analyses is to purify DNA from all bacteria present in a sample without introducing bias due to e.g. differential lysis or recovery. Subsequent to DNA purification, all bacterial 16S rRNA genes in the samples are amplified using primers targeting generally conserved regions in the 16S rRNA gene. The ratio between 16S rRNA gene copies for different bacteria is (in theory) conserved during the PCR amplification process.

Currently the most widely used approach to analyze 16S rRNA gene diversity is through next generation sequencing (Kunin et al 2010). The depth of the information that can be obtained form such sequencing efforts are dependent on the number of reads that are being done.

An alternative approach is the use of high-density microarrays based on 16S rRNA sequence variations. These approaches are explorative, and intended for discovery rather than diagnostics. A major challenge with traditional 16S rRNA gene microarrays is probe specificity, and cross-reactivity between closely related species (Cox et al 2010). For microarrays, this challenge has recently been addressed by tilling probes covering the variable region of the 16S rRNA gene (Rajilic-Stojanovic et al 2009). The principle by tilling is that a large number of overlapping probes cover the region of interest, with the combined probe signals providing a relatively good signal-to-noise ratio. Both for next generation sequencing and microarray analysis, a comprehensive data analysis of the information is required in order to obtain interpretable results.

A more direct approach for characterizing the gut microbiota is the use of highly specific single nucleotide primer extension (SNuPE) probes (Eggesbo et al 2011).The high specificity of the SNuPE assay is obtained by DNA polymerase based incorporation of a labeled dideoxynucleotide (Syvanen et al 1990). The SNuPE probes are constructed so that the probes hybridize adjacent to discriminative gene positions. If the target bacterium is present, a labeled dideoxynucleotide is incorporated by the polymerase.

A technology platform have been developed that can readily be applied to analyze the gut microbiota based on the SNuPE principle. The method – called GA-map™ (Genetic Analysis' Microbiota Analysis Platform) - has already demonstrated its usefulness in applications for assessing various disease states based on analysis of the composition of the gut microbiota.

Here, we discuss the possibility of using a novel comprehensive targeted approach for quantifying the inhabitants of the human gut microbiota in disease diagnostics.

2. Requirements for a gut microbiota test

The general requirements for a gut microbiota test is (i) simple sample collection, (ii) comprehensive, (iii) reproducible, (iv) high-throughput and (v) affordable. In addition, the test must deliver results that can be interpreted and be relevant to disease.

There are still major challenges with all these requirements. Most methods developed for research are actually not well documented, and promises far more than the methods in reality can deliver. A current example of this is the emergence of the next generation sequencing approaches. For instance, it is now being realized that these techniques inflate diversity measures (Kunin et al 2010). New software tools have been developed to reduce

this inflation of diversity, but it is still uncertain if the number of different Operational Taxonomic Units (OTUs) obtained by next generation sequencing techniques gives a correct representation of the actual number of different bacteria present in the sample.

Perhaps the largest challenge in gut microbiota diagnostics is the establishment of the correlation between microbiota patterns and disease. Since the characterization of the gut microbiota is in its infancy, there are not many diseases that have actually been characterized with respect to specific dysbiosis of the gut microbiota. Furthermore, since the analysis represent patterns rather than single bacteria, new diagnostic principles must also be implemented.

Diagnostic approaches related to dysbiosis of the gut microbiota have until now been dominated by culture dependent techniques which only detect a minor portion of the true diversity. Doctors used to such techniques may find it challenging to adapt knowledge about non-cultivable bacteria in their diagnostics because that would include bacteria that they are not familiar with.

We have for instance found that the clostridal family Lachnospiraceae is the most stable and dominant phylogroup in the human gut (Sekelja et al., 2011).This phylogroup has been overlooked by the traditional cultivation dependent techniques, probably because it is strictly anaerobic and that it has special growth requirements that are difficult to simulate on plates.

3. Function vs phylogeny of gut bacteria

A long standing debate is how well functionality of bacteria in the gut correlates to phylogeny. There are recent reports suggesting that the function of gut bacteria is a relatively stable feature, while the microbiota composition is unstable (Turnbaugh et al., 2009).

Although functions cannot directly be deduced from the gut microbiota 16S rRNA gene analyses, correlations between gut microbiota phylogenetic composition and function can be established. We have recently addressed this issue by mapping functions onto a phylogenetic map as illustrated in Figure 1. As seen in this figure, there are good correlations between functions and phylogeny, with different functions being clustered in different phylogroups.

The take home message from this analysis is that the phylogenetic framework can be used to deduce the probability of functions in the gut microbiota, and aid in identifying functions through other targeted approaches. Still, however, our knowledge about the correlations between function and phylogeny is limited. The current major genomic sequencing efforts will aid in these investigations. It will be particularly interesting to determine which functions follow a phylogenetic distribution pattern, and which do not.

For genes located on mobile elements, such as those encoding antibiotic resistance, an important aspect would be to determine the host range of the mobile elements. For instance, given that there are antibiotic resistance genes present on mobile elements in the commensal microbiota with a host range that include pathogens, there will be a severe risk that the antibiotic resistance genes can be transferred to the pathogen.

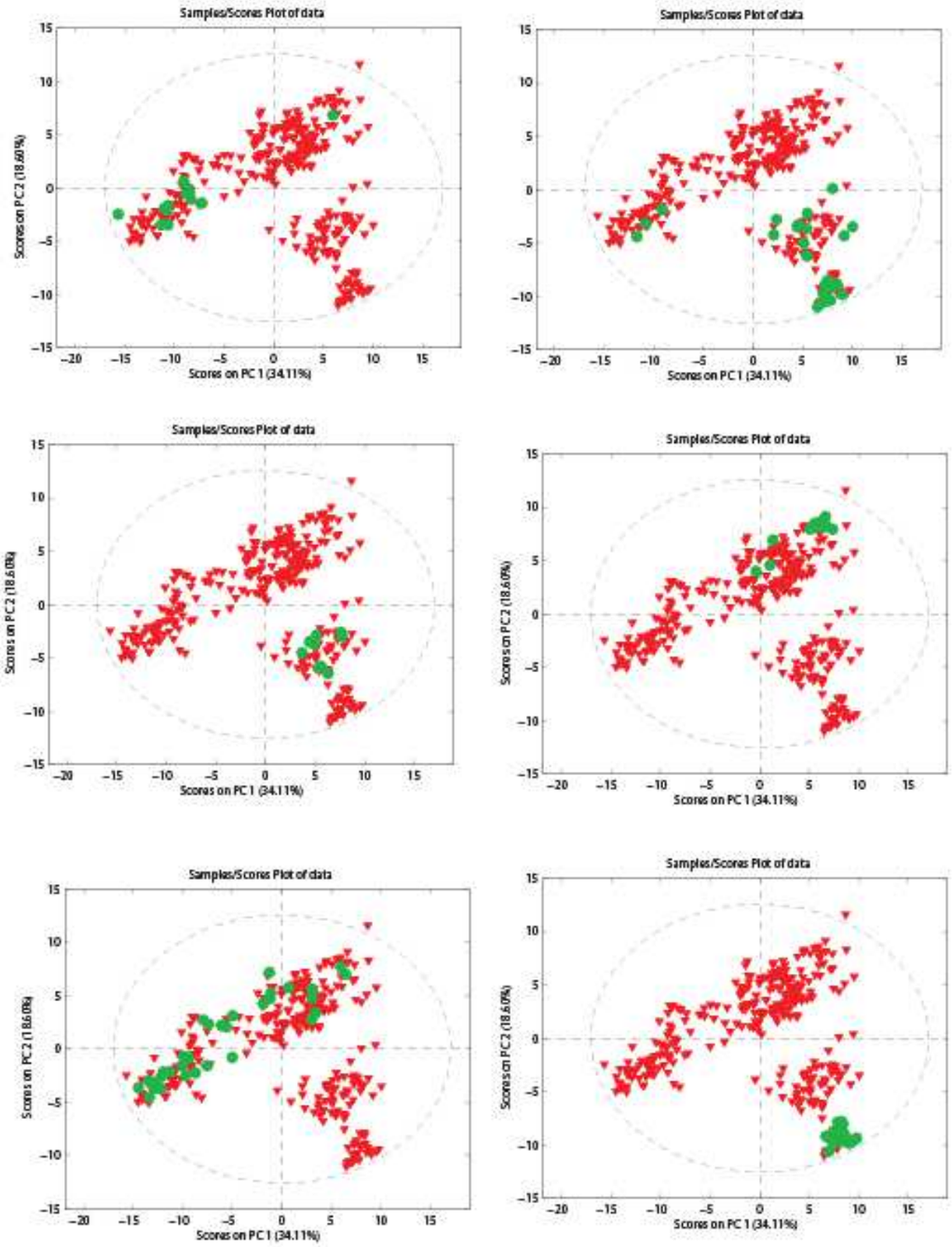

Fig. 1. Properties of gut bacteria in a phylogenetic framework. The PCA biplots represent the 16S rRNA phylogenetic relatedness between the bacteria, while the green circles represent different functions for the different panels. The six panels represent six different functional groups.

4. Diseases with established correlation to the gut microbiota

Necrotizing enterocolitis (NEC) (Wang et al 2009) and inflammatory bowels disease (IBD) (Frank et al 2007, Nell et al 2010) are two diseases that have a recognized established link to the gut microbiota.

NEC is a severe disease affecting preterm infants with a high mortality. About 10% of infants with a birth weight below 1500 g develop NEC, with mortality rate is as high as 30%. It has been suggested that this disease is associated with a reduced diversity of the gut microbiota (Wang et al 2009). Both due to the impact of the test, and because the preterm infant gut microbiota is relatively simple, NEC is an attractive target for developing a human gut microbiota test.

IBD represents a collection of diseases associated with gut inflammation. It has two main sub forms: Crohn's Disease (CD) and Ulcerative colitis (UC). Disturbance of the normally stable GI microbiota are predicted to adversely affect the health of the host (Frank, et al., 2007). Studies of experimental animal models of IBD reveal that germ-free animals show few signs of inflammation; experimental colitis is exhibited only when the animal is exposed to natural microbial communities. Likewise human studies have shown a response of IBD patients to antibiotic and probiotic treatment (Hecht, 2008). In CD patients inflammation most commonly appears in the gut locations where bacterial concentrations are high. Furthermore, diversion of the fecal stream from the lumen is associated with improvement of the inflammation, indicating a role for bacteria in the IBD pathogenesis (Baker, Love, & Ferguson, 2009). Taken together, there are relatively good evidence for a correlation between IBD and the gut microbiota.

5. Diseases with suspected correlation to gut microbiota

There is a very wide range of diseases that have been suggested correlated to the gut microbiota. Among these are diabetes, cardiovascular diseases, rheumatism, metabolic syndrome and obesity (Wang et al. 2011, Wen et al., 2008, Ley et al., 2006). Common for most of these diseases is the correlation to some form of underlying inflammation. Thus, imbalance in the gut microbiota could be a common underlying factor that triggers inflammation. Still, detailed knowledge is lacking about such potential correlations.

Future knowledge building with respect to microbiota composition could open new diagnostic possibilities for these diseases, which all have major impacts on human health. Gut microbiota diagnostics may also help in understanding the etiology of the disease, which potentially could help in developing therapeutic approaches.

The perhaps most surprising correlation to the gut microbiota are cardiovascular disease (Wang et al. 2011). There were relatively strong correlations between microbial metabolites and atherosclerotic disease. Gut microbiota diagnostic in this field could potentially have major impacts on human health.

6. Gut micobiota diagnostic for diffuse conditions

Major diseases with more diffuse symptoms such as irritable bowels disease (IBS) (Salonen et al 2010) and depression (Maes et al 2009) could be targets for development of future

diagnostics. These are diseases representing enormous burden and costs to society. If these diseases can be linked to some kind of gut microbiota disorders, then there may also be potential for intervention and treatment.

Discomfort such as abdominal pain, flatulence and bloating affect our everyday life. It has recently been shown correlation between these discomforts and specific gut bacteria (Jalanka-Tuovinen et al., 2011). Although not life threatening, the quality of life can be severely reduced by these discomforts. Diagnosis of the gut microbiota could potentially help in dietary interventions.

Although the severity of IBS is not pronounced, 10% - 20% of people in the Western world suffer from this (Maxion-Bergemann et al 2001). Therefore, a diagnostics for this condition could have major impact, given the potential for advice that would increase the quality of life through reducing discomforts.

7. Stratification of patients with respect to gut microbiota in clinical trials

It has recently been discovered that the gut microbiota plays a major role in the human metabolome (Nicholson et al 2005), and that the effects of drugs can be dependent on the gut microbiota (Clayton et al 2009). Combined with recent evidence that the human microbiome may consist of only three enterotypes (Arumugam et al 2011), diagnostics of such enterotypes is expected to provide important information with respect clinical trials. Enterotypes represent clusters of bacteria with a high frequency of co-occurrence, suggesting different states of the microbiota with different functionalities. There are several evidences for gut microbiota metabolism of important drugs, such as drugs against Alzheimer (Pieper et al., 2009)

Combined with the enterotype knowledge we believe that stratification will be a highly interesting field for gut microbiota diagnostics. This will be in line with the recent developments of personalized drugs –drugs adapted to individuals. Clearly, a major part of defining an individual would be the composition of gut microbiota. Information about the gut microbiota may therefore help to increase the success rate of clinical trials.

8. Manipulation of gut microbiota with pro- and prebiotics

The diet plays a major role in stratifying the gut microbiota (Muegge, 2011). This gives promise for food related gut microbiota perturbations. Manipulation of the gut microbiota through the diet is an old concept, either through probiotic live bacteria, or through prebiotic oligosaccharides.

Even though there is a range of products on the marked, there have been challenges in documenting the effect of these products. Diagnostic products that will help in substantiating the potential health claims would be of great benefit to the food industry. A main reason for the lack of documentation could actually be the lack of proper tools to describe the microbiota, rather than that the products have no effect.

Given proper documentation, the marked for pro- and prebiotics is very large. In particular, documentation that can be used in marketing would have a high commercial value. New diagnostic approaches could also potentially enable the discovery of new pro- and prebiotic products.

9. GA-map tecnology platform in gut microbiota diagnostics

The GA-map platform has given rise to a pipeline of assays for analysis of disease based on the microbiota composition. This platform includes a DNA purification module, a module for probe design, a patent protected approach for the actual gut microbiota screening, in addition to a diagnostic database. The GA-map assay will help to utilize information in the gut microbiota for diagnostic purposes.

An outline of the GA-map platform for gut microbiota diagnostics is illustrated in Figure 2. The platform can be used to assess health conditions of individuals based on the composition of the gut microbiota. In addition, it can serve both the the pharmaceutical industry and governmental health authorities in epidemiological population screenings and clinical trials. In addition, the technology can be used for early detection of undesirable conditions in the gut that can be corrected before the illnesses are manifested. The core aspects of the technology was developed and patented at the University of Oslo in 1998 (US patent # 6 617 138 Nucleic Acids Detection Methods). The technology has since then been refined at Nofima Mat (Matforsk). Currently the technology is patented worldwide and Genetic Analysis AS has been set up to commercialize the technology.

A high throughput analysis platform based on this technology will make it possible to gain greater understanding of the relationship between the composition of the gut microbiota and health, as well as being used as a diagnostic and prognostic tool in the future.

9.1 GA-map array

Probe labeling is based on the minisequencing principle, where a DNA polymerase extends the probe with a single labeled dideoxy nucleotide (Syvannen et al., 1990). In the GA-map assay several probes are labeled simultaneously, with the detection by reverse hybridization to the complementary strands spotted on to a solid phase (Vebo et al., 2011). This process is illustrated in Figure 3. The probes are constructed so that the probes hybridize adjacent to discriminative gene positions. If the target bacterium is present then a labeled dideoxynucleotide is incorporated by the polymerase. This is illustrated in Figure 3A. The solid phase (i.e. microarray or beads) is used to separate the probes by hybridization to their respective complementary sequences attached to the solid phase, which is illustrated in Figure 3B (exemplified by an array).

9.2 Probe design

There are several steps in the GA-map array process that can lead to wrong patterns if the probes are not properly designed. The probes may bind to the wrong target, and be labeled that way. Furthermore, the probes may be labeled by using itself as a target, or another probe as target. Finally, the probes may bind to the wrong spots on the array. Successful application of the GA-map assay requires therefore the application of a probe design software that takes into account many of the potentially unwanted reactions mentioned above, leading to false results.

The probe design is based on a novel way of bacterial classification based on 16S rRNA gene sequences. Rather than classifying bacteria by traditional phylogenetic tree-based approaches, the bacteria are classified in a coordinate system (Rudi et al., 2006). The benefit of this approach is both that very large numbers of bacteria can be analyzed, and that phylogroups are easily identified for probe design. This is illustrated in Figure 4.

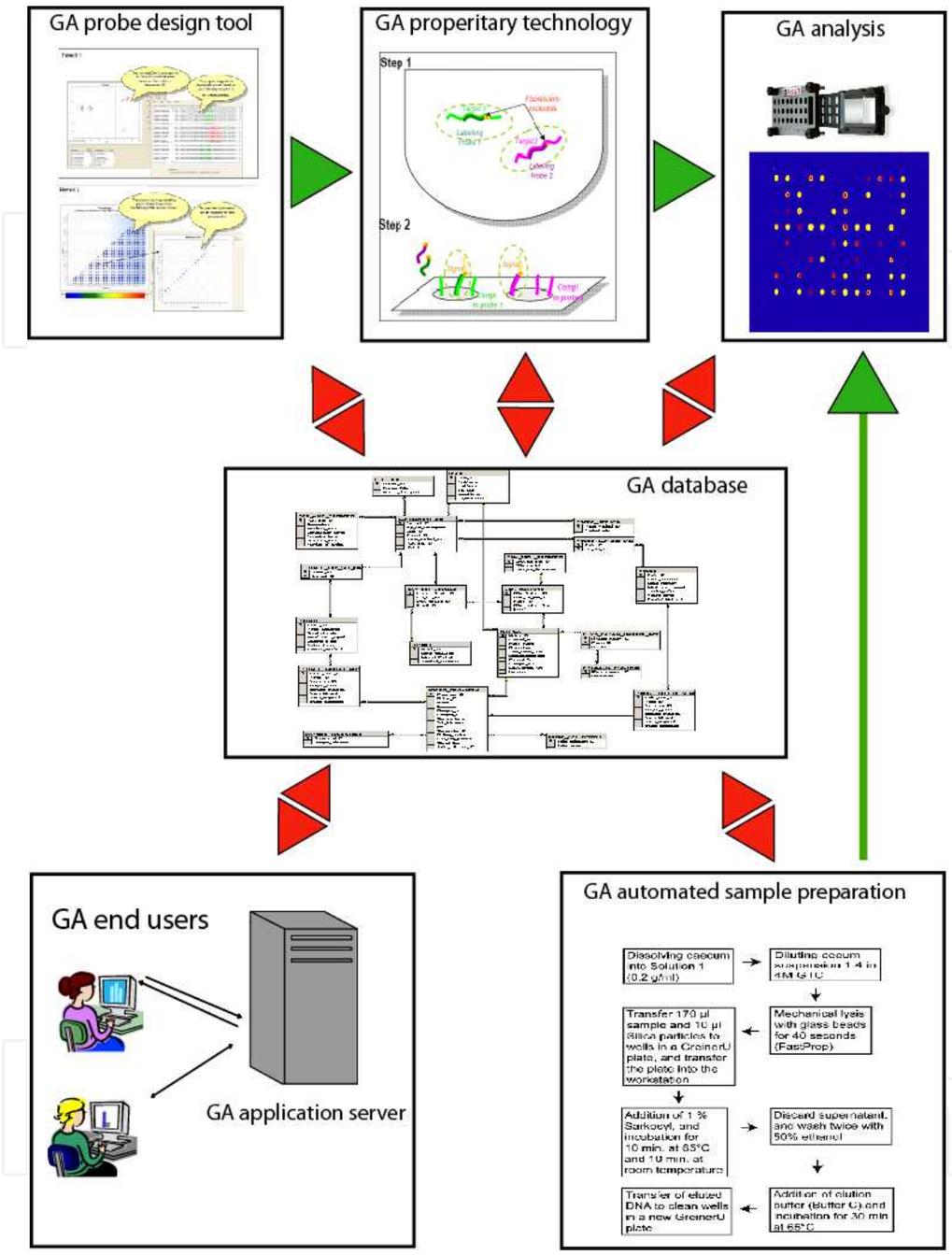

Fig. 2. GA pipeline. This figure illustrates the GA core technology and pipeline. Based on signatures in the 16S rRNA gene sequences GA probes are designed and evaluated *in silico* using the GA probe design tool. The GA analysis involves automated sample preparation combined with array hybridization . The whole information flow is stored in the GA database including the information about sample preparation and storage. The results to the end user are in the form of a direct description of the microbiota with respect to consequences for health and disease. Using this proprietary GA technology, a GA array is obtained, giving a specific "fingerprint" of each persons gut microbiota.

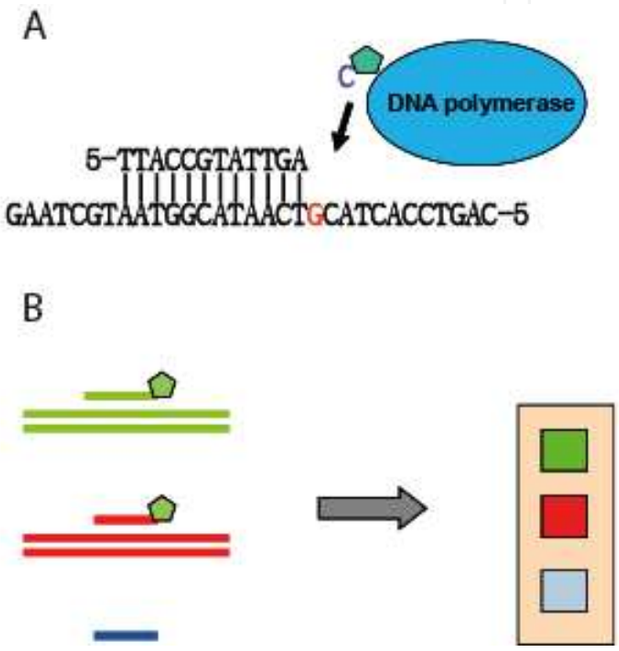

Fig. 3. Schematic outline of the GA map assay(A) Illustration of the SNuPE principle. An unlabelled probe hybridizes adjacent to a discriminative guanine on the complementary DNA strand. A DNA polymerase single-base extends the probe with a labeled cytosine dideoxy nucleotide. (B) Illustration of array hybridization of SNuPE labeled probes. Three probes are illustrated by green - , red – and blue bars. The green and the red probe are labeled. The probes are hybridized to their complementary sequences on an array as illustrated with the squares. The green and red probes will give a signal on the array due to the label, while the blue probe will not give a signal since it is not labeled.

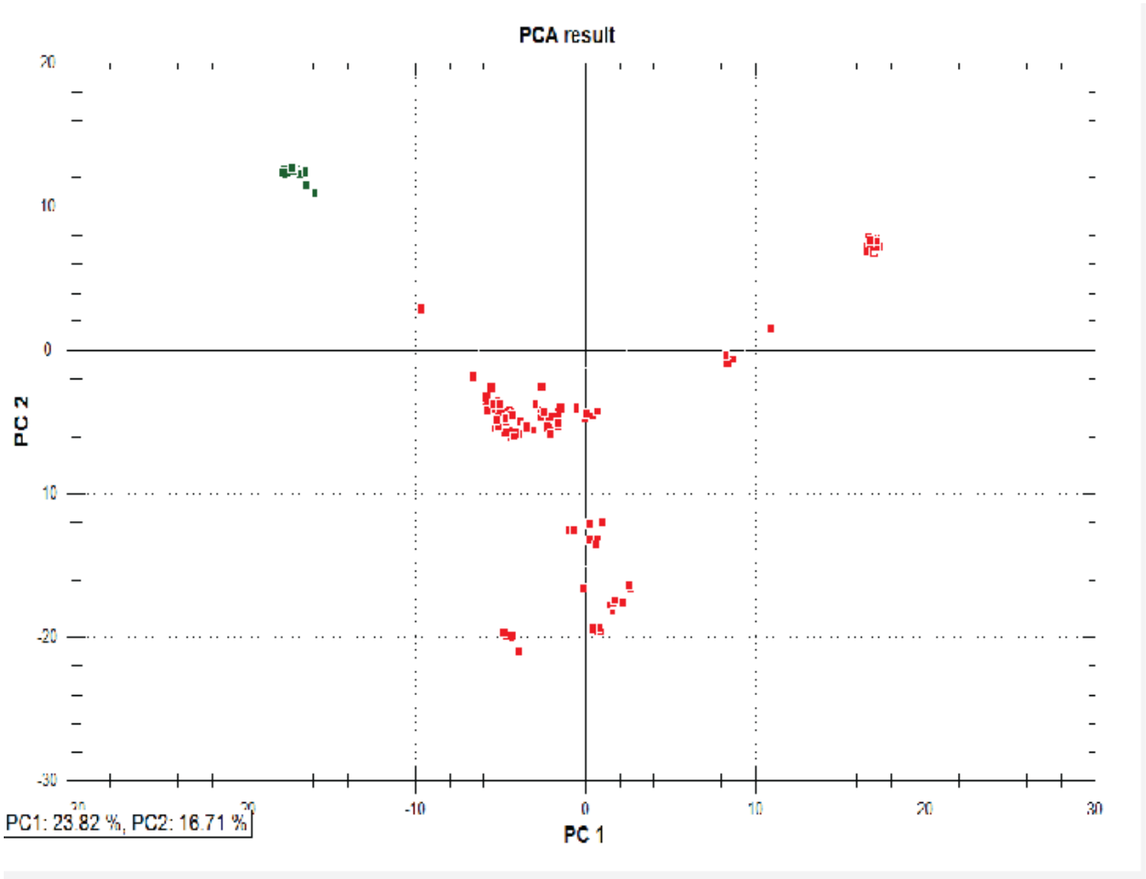

Fig. 4. Illustration of coordinate based classification of bacteria related to IBD. Each point in the plot represent a 16S rRNA gene sequence from a single bacterium. The distances between the points reflect the relatedness between the bacteria. For illustration, the points labeled green are target organisms for probe design, while those labeled red are non-target organisms.

After a set of probes have been constructed, the probes are evaluated with respect to if they will self-label (Figure 5A), whether they can cross-label (Figure 5B), or whether they will bind to a wrong spot on the array (Figure 6C). This bioinformatics evaluation is crucial for the successful construction of functional probe-set based on the GA-map technology (Vebo et al., 2011).

Validation of probes constructed with the probe design software have shown a high success rate (Vebo et al., 2011). Prior to the development of the software, probes were identified manually from multiple sequence alignments. The conclusion from the manual constructions, however, was that these probes did not perform satisfactory, and that there were too many considerations when performing this probe construction to make it possible to do manually.

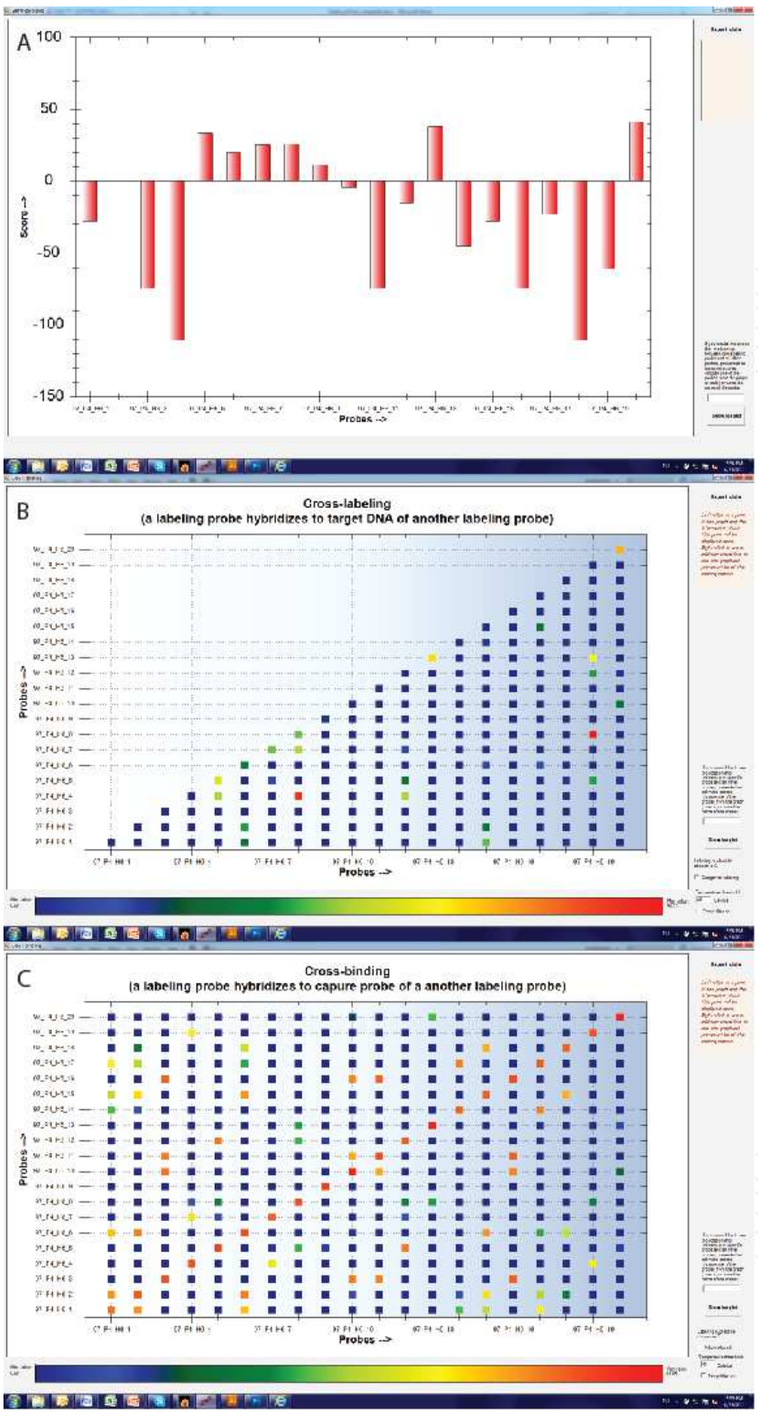

Fig. 5. Illustration of steps in probe evaluation. (A) The first step is to evaluate whether the probe will self label. A high value here indicates a high risk of self labeling. (B) The next step is to evaluate the potential of cross-labeling. A color code is used to illustrate the risk of cross-labeling. High values indicate high risk. (C) The final evaluation is whether the probe will bind to the right spot on the array. The red diagonal line indicates correct hybridization, while red squares outside the diagonal would indicate wrong hybridization.

9.3 Application of the GA-map array to describe the temporal development of the gut microbiota in infants

We have evaluated the recently developed GA-map infant microarray as a high throughput assay for screening of the gut microbiota. We analyzed 216 faecal samples collected from a cohort of 47 infants from 1 day until 2 years of age. To test the predictive ability of the assay we asked the question whether we could predict the age of the infants based on the microarray data.

The Prevention of Allergy Among Children in Trondheim (PACT) study is a large population based intervention study in Norway focused on childhood allergy (Oyen et al., 2006). The samples included here is a subset from the PACT study. Mechanical lysis was used for cell disruption, and an automated magnetic bead-based method was used for DNA purification. The approach is previously described by Skånseng et al. (2006)

We experienced that the primer pairs commonly used for amplification of the full-length 16S rRNA gene showed poor amplification of bifidobacteria. To circumvent this problem we developed a novel primer pair to obtain a near full-length 16S rRNA universal amplicon. The amplicon was evaluated both theoretically based on sequences in the RDP II database, and experimentally for bacterial species expected in the infant gut. We found that all the currently known infant gut bacteria were amplified with this new, optimized primer pair. A primer pair that is able to representably amplify the 16S rRNA gene from all the bacteria present in the sample is critical for proper analysis of the sample.

9.3.1 Temporal development of the infant gut microbiota

Based on signals from the GA-map array we determined the temporal development of the gut microbiota in the infants. These results are pressented in Figure 6.

The prevalence data showed that proteobacteria and lactobacilli reached a maximum between one month and one year, while the bacteroides subgroup containing B. fragilis reached a maximum after one to two years. Surprisingly, we found a high prevalence of bifidobacteria for infants older than one month. This is in contrast to another recent microarray screening of the infant gut microbiota, where they found that bifidobacteria were underrepressented (Palmer et al., 2007). This demsontrates the importance of using an optimal primer pair in the amplification of the 16S rRNA gene.

9.3.2 Modelling age as a function of the microbiota composition

We used the temporal information in the gut microbiota to determine if it is possible to describe age as a function of the composition. This was done using generalized additive models (GAM).

The following function was derived:

$$\begin{aligned} age = \quad & s(E.coli) + s(Clostridium) + s(Staphylococcus) \\ & + s(Bif.breve) + s(Bacteroides.dorei.vulg.theta.frag.) \end{aligned}$$

The functions s to the data are shown in Figure 7.

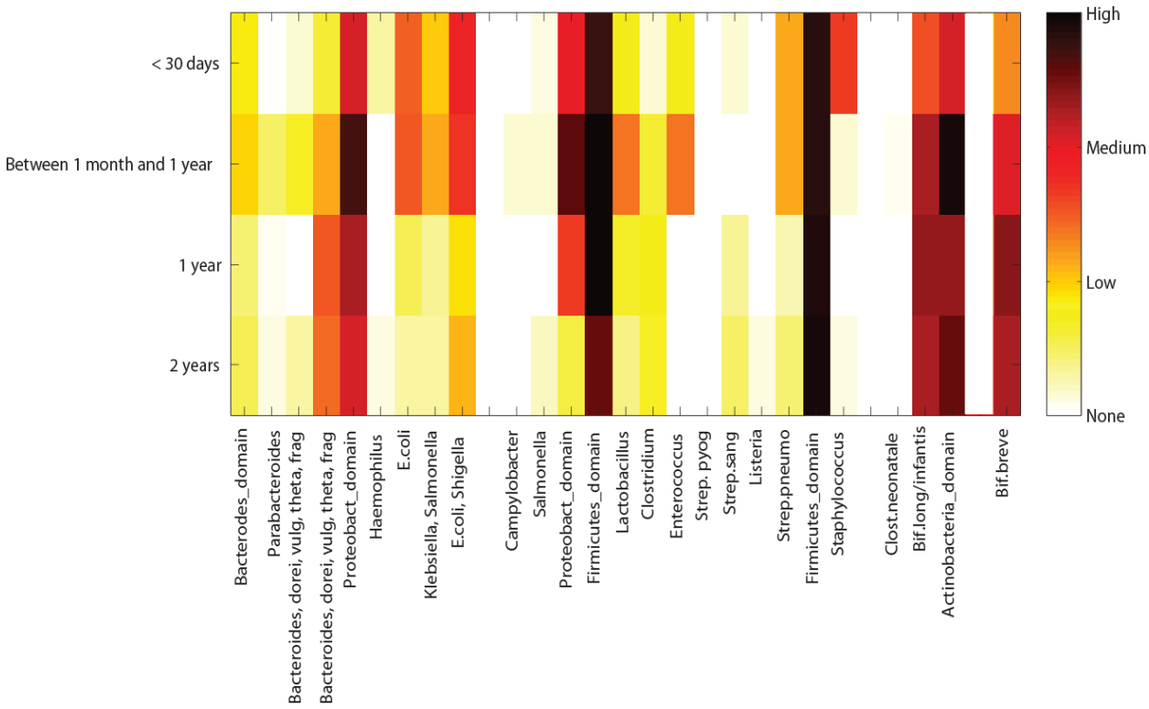

Fig. 6. The prevalence of the G-map bacteria was determined within age groups. The color code indicates the prevalence from absent (white) to present in all samples (black).

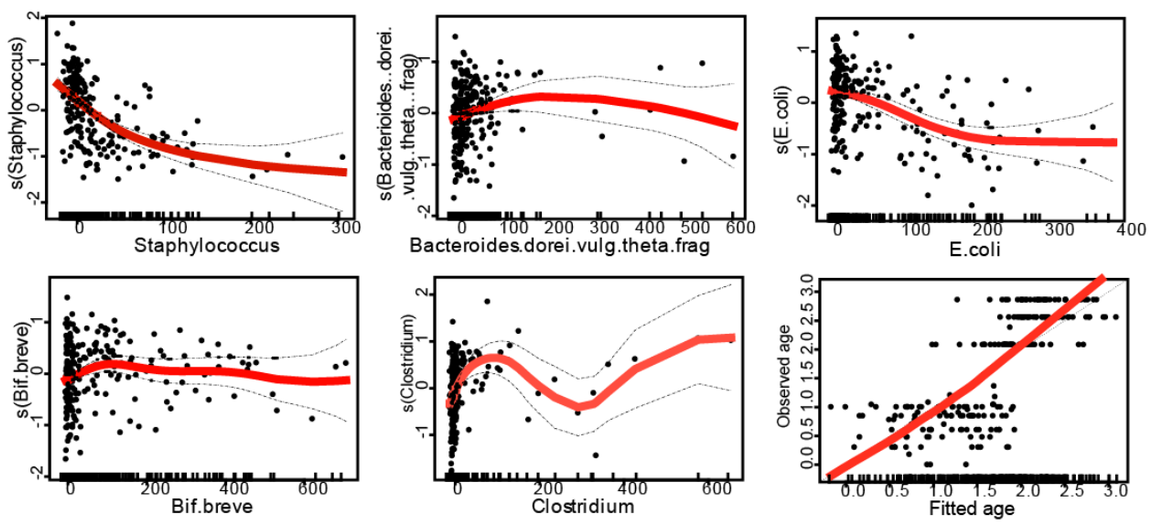

Fig. 7. Bacteria which are important for age prediction. For each bacterium the age contribution is a function of probe signal. Adding all the age contributions gives the predicted age (see function above). The final panel shows a regression between the observed and predicted ages for all the samples in our data.

High E. coli and staphylococci abundance predict an early age, while for the bacteroidetes and B. breve a medium abundance predicts a high age (Fig. 7 B and D). However, very high abundance predicts an early age. The Clostridium probe, on the other hand, shows a complex association with age.

Figure 7 shows that the age can be modelled with high accuracy from the microbiota composition.We found that the observed and modelled age gave a squared regression coefficient of 0.6. These results show that the development of the human gut microbiota is very structured with age, and that it can be predicted. The consequence is that it could potentially be possible to determine the normal development of the microbiota in infants, and use deviations from the normal pattern in identifying disorders. For instance, from our results we can conclude that high levels of staphylococcus for older children would clearly indicate some kind of deviation form the normal development, and indicate some kind of diseased state.

9.4 Application and development of the GA-map assay for diagnostic purposes

We are currently adapting the GA-map assay for detection of the human adult core microbiota, as defined by (Arumugam, et al., 2011). This will enable an assay for the normal gut microbiota, and in identifying the gut microbiota enterotypes. We foresee that, in addition to disease-specific diagnosis, such an assay could potentially be valuable for the pharmacutical industry in population stratification in clinical trials. This tool could also be used by the food industry to determine whether their pro- prebiotic products have an impact on the normal gut microbiota.

9.4.1 IBD

Diagnostic of IBD is an important target for the GA-map assay since there are established correlations between gut microbiota composition and disease. The intention with the assay would be early detection of IBD, and potentially in the following up of IBD treatments. Alternatively, it can be used to rule out IBD from IBS patients. In the future, given a causal relation between gut bacteria and IBD, knowledge about the microbial composition could be used in disease treatment. Preliminary data using a very limited probe-set illustrates that it should be possible to develop IBD diagnostics using the GA-map technology (Frøyland, 2010). The assay is currently being refined by the addition of more probes, and through validations on clinical material. Since we are using feces and not mucosal samples in our analysis we were surprised to obtain the relatively high specificity for CD.

9.4.2 NEC

The GA-map assay is also currently being evaluated for the analysis of NEC. NEC is a very severe disease where rapid and precise diagnostics are crucial. As for IDB there are already established correlations between gut microbiota and the disease. Furthermore, for this disease there are currently no good diagnostic approaches. Preliminary data show promise for separation NEC samples from that of controls. The aim is to further improve the assay and model in order to develop a prognostic assay for NEC development. Such a test would be of great help for pediatricians and neonatologists working with pre-term infants as a guide in treatment regimes.

	CD patients	Control patients
Diagnosed as CD	35	28
Diagnosed as non-CD	25	77
Total number of patients	60	105
	Sensitivity	Specificity
	58,3%	73,3%

Table 1. Single probe GA-map diagnostics of CD (Frøyland, 2010)

9.4.3 Test for assessing health condition of individuals

Many medical doctors find that an analysis of a patients gut microbiota is helpful in obtaining a more complete picture of the condition of the patient, and thus determining the disease state and best treatment. Several such tests exists in the marketplace. The GA-map technology is positioned to become a very powerful test for this purpose, with its complex probe selection and high through-put capabilities.

The general process for the application of the GA-map assay for diagnostic purposes is illustrated in Figure 8.

After implementation of the pilot diagnostic assays, new approaches and diseases will be implemented under the GA-map umbrella. There will also be a transformation over time towards decentralized analyses.

10. Future directions of gut microbiota diagnostics

There is currently a major focus on exploring the gut microbiota. This is mainly done through the application of explorative techniques such as next generation sequencing. The next phase will be the validation where the discoveries are validated using targeted techniques such as microarray analyses or quantitative PCR. The final phase will be to identify correlations between microbiota and disease that will give some added value

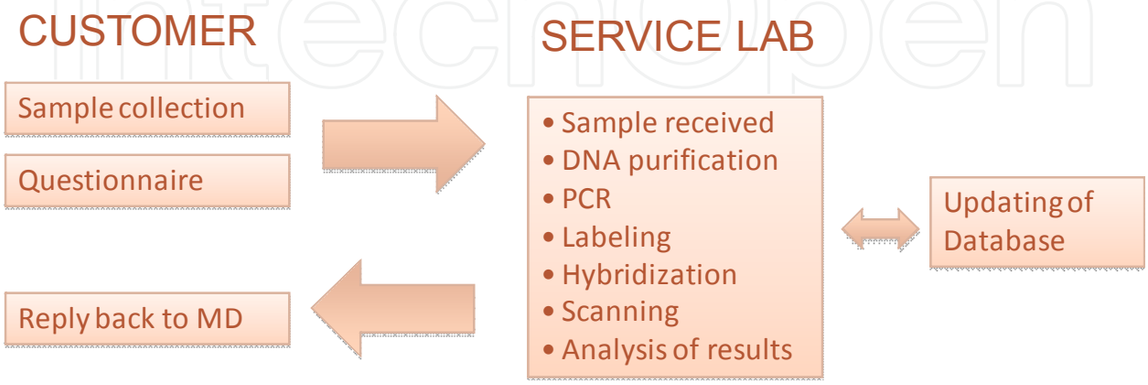

Fig. 8. Illustration of the GA-map diagnostic procedure. The GA-map assay will initially be offered through centralized service labs. A database covering relationships between profiles and disease will constantly be updated based on accumulated information, and a web-based interface with the customers will be available.

through diagnostics. This would open up for various applications of tests for profiling the gut microbiota in disease management.

As the importance of the gut microbiota on health becomes more established and recognized, profiling the composition of the gut microbiota will become an important diagnostic and prognostic tool in the future. This will give an additional window for health professionals to assess the conditions of the patient.

As technology advances and miniaturization and sophisticated Point-of-Care devises are being developed, such comprehensive tests will become easier to perform and more affordable. We see at least three areas where such a test would be applied in health care in the future:

1. **Disease specific diagnostics**

As discussed in this paper, certain health conditions are related to imbalance in the gut microbiota. A test that can correlate imbalances in the gut microbiota to specific health conditions will have a value as a diagnostic and prognostic tool. Furthermore, such tests

could also act as a general help for physicians in determining the best treatment for patients.

2. **Personalized medicine**

Understanding how different bacteria in the gut influences the metabolism of drugs and foodstuff, leads to a more personalized, tailor-made drug treatment regime. Since it is generally recognized that the composition of the gut microbiota has a profound effect on health as well as how foodstuff and drugs are metabolized, a profiling of the gut microbiota would constitute an important aspect of the personalized medicine approach. It is expected that personal medicine would lead to more effective treatments, as well as cost-saving on healthcare budget due to optimizing the drug treatment for each person.

3. **Deviations from each individuals normal flora**

The search for a common core gut microbiota has revealed that each individual has a unique and fairly stable microbiota, at least in adults. Therefore, each individual's own microbiota may be the best control for that person. This calls for routine checks where deviations from its own "normal" core microbiota will be evaluated. Such a test would also include other genetic and physical markers, which combined would give a status of the individual's health condition.

With future technical development we believe the next generation gut microbiota diagnostic will integrate phylogeny and functionality of the gut microbiota. A functionality that is particularly relevant is antibiotic resistance. There is currently a major fear for the development of multi-resistant pathogens, and that the current treatment regimes with broad-spectrum antibiotics will eradicate beneficial bacteria. In this regard targeted narrow spectrum antibiotics could be the solution. Narrow-spectrum antibiotics, however, would require better diagnostics of the actual disease causing organisms. Therefore, a profiling of the gut microbiota may be essential prior to administration of such narrow antibiotics to ensure optimal treatment.

Another aim in treatment of gut microbiota disorders is to restore the diseased gut microbiota towards the healthy state. This could be done either through tailor-made administration of probiotics, prebiotics and antibiotics, or a combination of these substances. Another approach would be to transplant the gut microbiota from close relatives or samples from the same individual collected before disease occurs. Such an approach would resemble what is currently done for treatment of *Clostridium difficile* infections where the gut microbiota is transplanted from relatives.

11. Conclusions

As more data are being generated, the importance of the gut microbiota on health will become more recognized. The advancement in characterizing the composition of the gut microbiota in humans paves the way for new and better health treatments. The techniques used for exploring the gut microbiota and characterizing the functions of the microbiota, may not necessarily be the same techniques used to diagnose and monitor treatment of gut health related diseases. The GA-map assay is a promising tool for the development of gut microbiota diagnostics in routine applications. Such assays has the potential to be set up as a high through-put service, and can also be incorporated into smaller Point-of-Care devices,

opening up new, exciting applications to health management. A comprehensive and rapid characterization of the gut microbiota will be part of the tool-box physicians will use in the future to improve diagnosis and treatments of patients.

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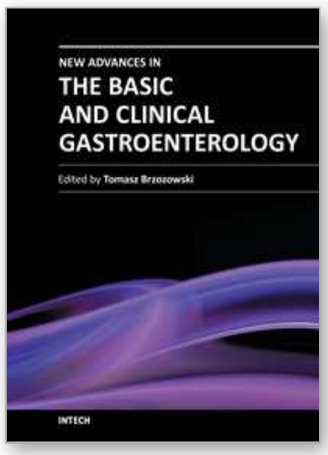

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The purpose of this book was to present the integrative, basic and clinical approaches based on recent developments in the field of gastroenterology. The most important advances in the pathophysiology and treatment of gastrointestinal disorders are discussed including; gastroesophageal reflux disease (GERD), peptic ulcer disease, irritable bowel disease (IBD), NSAIDs-induced gastroenteropathy and pancreatitis. Special focus was addressed to microbial aspects in the gut including recent achievements in the understanding of function of probiotic bacteria, their interaction with gastrointestinal epithelium and usefulness in the treatment of human disorders. We hope that this book will provide relevant new information useful to clinicians and basic scientists as well as to medical students, all looking for new advancements in the field of gastroenterology.

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