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„The painted gut:
Establishing a comprehensive fluorescence *in situ* hybridization (FISH) probe set to illuminate abundant members of the mouse intestinal microbiota“

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1 ABSTRACT

Inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis are characterized by chronic intestinal inflammation of largely unknown causes. In addition to genetic risk factors that predispose individuals to IBD, the composition of the intestinal microbiota is altered in disease, resulting in the loss of beneficial microbes and increase in opportunistic pathogens. Fluorescence *in situ* hybridization (FISH) is an ideal method for monitoring the abundance of intestinal microorganisms that can serve as health-state biomarkers. This study aimed to illuminate the abundance shifts and possible role of the microbiota in mouse models of intestinal inflammation, but a suitable set of oligonucleotide probes to target abundant microorganisms has been limited. 16S rRNA gene-targeted FISH probes were developed that are specific for 13 bacterial species-level phylotypes in the mouse gut. The probes were systematically evaluated using multiple approaches. As a proof-of-concept, three newly-designed probes targeting phylotypes within an as-yet completely uncultivable group from the order *Bacteroidales* were applied to monitor abundance shifts throughout inflammation induction and resolution. The idea of developing a contemporary multiplex FISH approach using mono-labeled probes resulted in the successful application of two fluorophores that are unusual for FISH. Additionally, a comprehensive list of published FISH probes targeting the intestinal microbiota of humans and animals was created.

Keywords: gut microbiota; *Bacteroidales*; dextran sodium sulfate; colitis; fluorescence *in situ* hybridization; oligonucleotide probes; multiplex FISH

2 INTRODUCTION

The intestinal microbiota plays an essential physiological role in human health, suggesting that the microbiota may be viewed as an additional multifunctional organ (1). Co-evolution of host and microbiota has led to a complex mutualistic relationship, in which the host ensures nutrient-rich and protected ecological niches for growth and propagation (2). The microbiota not only helps to digest and absorb nutrients, but plays essential roles in complementing host metabolism, hinders detrimental colonization by pathogens and stimulates and trains the host immune system (2). The diverse microbial symbionts in the distal intestine form the most densely populated ecosystem of the world, harboring 10^{12} organisms per gram (dry weight) of feces (3). The collective genomic content of the symbiotic microbes, termed the microbiome, exceeds the human genome approximately 150 times (5). All three domains of life and viruses share the complex habitat, though the majority is represented by bacteria with an estimated amount of 160 bacterial species per individual (4).

The intestinal microbiota is shaped by challenging environmental conditions, due to pH, redox potential, peristalsis, rapid turnover of epithelium and mucus, exposure to transient bacteria and food particles as well as to the host immune defense (2). Thus, the microbial composition is highly dynamic and varies between and within individuals, depending on several factors like genotype, age and gender as well as environmental influences such as diet, lifestyle, stress and anatomical site (5, 6, 7). Despite sharing the same living environment, spouses harbor more distinctive microbial compositions than siblings (and particularly monozygotic twins), emphasizing the crucial influence of genetics and immune system on the microbial structure (8).

A healthy gut is characterized by stability of the symbiotic interactions (9). On the part of the microbiota, vital functions do not necessarily depend on single groups, but feature some functional redundancy that is thought to help to increase resistance and resilience to perturbation (10). In many cases dysbiosis, an imbalance of the microbial structure, is reversible, for instance after antibiotic therapy (6, 3). Chronic or relapsing dysbiosis, however, is associated with severe health conditions such as inflammatory bowel disease (IBD).

IBD is a chronic inflammatory disorder of the human gastrointestinal tract that typically emerges in the phenotypes Crohn's disease and ulcerative colitis. IBD is a major human health problem with increasing prevalence, though its causes are unclear. The microbial composition differs significantly not only between healthy individuals and IBD

patients, but also between individuals suffering from Crohn's disease and ulcerative colitis (4, 11). The complex interplay between the three major factors immune system (IS) (innate and adaptive IS), intestinal epithelial barrier, and microbiota that encounter directly in the site of pathology, is imbalanced in disease (12). A contemporary view on the trigger of pathogenesis is that environmental stimuli can lead to elevated immune response in genetically susceptible persons that correlates with dysbiosis in a habitat with altered selective conditions (13).

Several genes have been identified as risk loci involved in predisposing humans to inflammatory diseases. Some susceptibility genes encode elements involved in the signaling pathways of the anti-inflammatory cytokines interleukin-10 (IL-10) and transforming growth factor beta, which regulate intestinal homeostasis (14, 15). Down-regulated IL-10, for instance, has repeatedly been shown to predispose humans and mice to intestinal inflammation, and germfree IL-$10^{-/-}$ mice do not develop colitis but do under specific pathogen free conditions, indicating that the presence of a microbiota is important for disease onset in this model (16, 17, 18).

The onset of intestinal inflammation depends on the presence of bacteria, shown by the amelioration of symptoms in humans and mice after antibiotic treatment (19, 20, 21). The idea that a single pathogenic infection is the cause of IBD remains doubtful (22, 23), since many microbes are indirectly rather than causally linked to disease (24). Though, the disrupted mucosal barrier function enables members of the normal healthy microbiota to contribute to inflammation. Microbes that show this behavior are termed pathobionts (25).

Dysbiosis in IBD patients is characterized by reduced stability of the ecosystem and bacterial species richness and diversity, which is typical for acute murine colitis as well (26, 27, 28, 29). Several studies show the reduction of symbionts with beneficial effects and enrichment of opportunistic pathogens in IBD (reviewed by Berry and Reinisch (13)). Structure or abundance shifts of specific microbes dependent on the host health state and independent of host genotype may be exploited for their use as microbial biomarkers in diagnosis and treatment monitoring (30, 13). The definition of a healthy microbiota is a prerequisite for elucidation of microbial shifts in disease.

Mice are important model organisms for studying intestinal inflammatory disorders, since the intestinal tract of humans and mice shares similar microbial composition (22). Both are dominated by the phyla *Firmicutes* and *Bacteroidetes* and respond to specific genetic and environmental stimuli with structural shifts of the gut microbiota (31). By studying experimental mouse models, where IBD is commonly initiated by bacterial

infection, genetic or immunological susceptibility and/or chemical treatment, intestinal function and homeostasis can be investigated precisely (22). Nevertheless, few studies have comprehensively investigated the structure and dynamics of the murine microbiota in reasonable taxonomic/phylogenetic depth (family-level and higher). Especially the large percentage of uncultured organisms (32) has been vastly neglected. Complicating factors are strong inter-individual differences, the intricate dynamic nature of the ecosystem and different research approaches that lead to discordance of results.

Culture-independent techniques, typically on the basis of analyzing the small subunit ribosomal ribonucleic acid (SSU rRNA or 16S rRNA), as well as high throughput sequencing approaches, are popular tools for describing the gut microbial structure and function. Fluorescence *in situ* hybridization (FISH) is a widely used method to identify gut bacteria. However, the lack of suitable oligonucleotide probes is a serious obstacle leading to misinterpretation. The routinely used probe Bac303, for example, identifying the majority of *Bacteroidetes* was observed to bind only about three quarters of their target organisms (33). An overview of published probes with updated information about the binding behavior could help researchers select probes meaningfully. Considering the huge diversity of the gut microbiota, few probes have been designed to target groups at high taxonomic resolution (genus and species level or higher). Despite that, the limited number of different groups simultaneously detectable in FISH, recently stimulated researchers to find strategies of “multiplexing” (34, 35).

This work is part of the interdisciplinary metagenomics and metatranscriptomics project InflammoBiota (http://gutmicrobiota.univie.ac.at/inflammobiota, 29.5.2013) that aims to identify microorganisms associated with IBD. For that purpose experimental mouse models, are investigated in a colitic and non-inflamed state. IBD is simulated by oral administration of the chemical irritant dextran sodium sulfate (DSS). DSS disrupts the intestinal epithelial barrier, whereupon acute murine colitis is induced that shares similarities with ulcerative colitis (36). Wild type mice and mice deficient in the Janus kinase (JAK) - signal transducer and activator of transcription (STAT) signaling pathway, which plays a central role in immune regulation, are examined. Alterations of the involved key factors potentially affect pathophysiology of intestinal inflammatory disorders. This was shown for STAT in human IBD (37) as well as in acute murine colitis (38) and may further be true for interferon-gamma receptor (IFNGR), which regulates antibacterial immunity via JAK/STAT signaling (39).

The aim of this study was to apply the FISH method for its use in illumination of the human and animal gut microbiota. Potential bacterial health-state indicators were

identified with high taxonomic resolution. In bacterial surveys roughly species-level divisions are often defined by the 16S rRNA encoding gene, in which sequences with similarity of at least 97% are termed “operational taxonomic units” (OTU) or “phylotypes”. FISH probes were designed and optimized to detect the most abundant phylotypes of the healthy murine gut on the one hand and of the inflamed on the other hand, throughout mice with different genetic background (wild type, $IFNGR^{-/-}$ and $STAT1^{-/-}$). Applicability for monitoring bacterial abundance shifts by quantitative FISH was proven in a trial of inflammation induction and recovery of wild type mice.

Furthermore, an idea of a new multicolor FISH approach using mono-labeled probes, SIMPL FISH (single labeled probes in multiplex FISH), was established.

A comprehensive list of published FISH probes for the identification of gut bacteria of humans and animals was created. Information about probe specificity and group coverage of target as well as non-target organisms were updated to provide meaningful selection for further research.

3 MATERIALS AND METHODS

As part of the InflammoBiota project, this study involved already prepared samples of murine intestinal content (colonic, caecal and faecal samples), fixed with 4% paraformaldehyde (PFA) or ethanol according to the protocol of Daims and colleagues (40). Used samples originated from C57BL/6N mouse models of genotypes wild type (wt), STAT1$^{-/-}$ and IFNGR$^{-/-}$. To evoke acute colitis, mice were orally administrated 2% DSS for 7 days, thereupon they received autoclaved drinking water until sacrifice. For detailed information on the animal experiments, see (28).

3.1 Targeted clone library and sequencing

Abundant phylotypes that were found in existing 454 pyrosequencing data throughout trials of wt, IFNGR$^{-/-}$ and STAT1$^{-/-}$ mouse models of intestinal inflammation served as targets, upon which the probe set was established. The phylotypes were chosen by the criterion of ≥ 1% median relative abundance in samples of either non-treated control mice or DSS-treated mice.

Due to the short 454 pyrosequencing read sizes of 300 to 500 nucleotides (nt) (Table SR1), the full-length 16S rRNA sequences of the target bacteria were of interest for the design of specific probes. Nucleic acid samples that showed a relatively high proportion of at least one of the targets in the metagenomics data were picked to recover the target sequences. Near full-length 16S rRNA genes were amplified by polymerase chain reaction (PCR) using the general bacterial primers 8F (5'-AGA GTT TGA TYM TGG CTC-3') and 1492R (5'-GGY TAC CTT GTT ACG ACT T-3') (for details see supplementary materials, Table S7 and Table S8). Using TOPO® TA Cloning® Kit containing pCR^{TM}2.1-TOPO® vector (Invitrogen) according to the manufacturer's instructions, the amplified bacterial 16S rRNA sequences were captured in transformant *Escherichia coli* (chemically competent One Shot® TOP10, Invitrogen) colonies on lysogeny broth (LB) agar plates containing 40 µL 5-bromo-4-chloro-indolyl-β-D-galactopyranoside (X-gal) (40 mg/ml). Parallel to transferring white colonies to a "masterplate", prepared PCR reaction tubes were inoculated to control for the presence of vector inserts. For that purpose polymerase chain reaction (PCR) was performed using M13 forward and reverse primers, M13F (5'-GTA AAA CGA CGG CCA G-3') and M13R (5'-CAG GAA ACA GCT ATG AC-3'), respectively (for details see supplementary materials, Table S7 and Table S9). After purification using QIAquick PCR Purification Kit (Qiagen) following the instructions of the manufacturer, the amplicons were

sequenced by Sanger chain termination synthesis (in house, using established protocols). Only one primer (1492R) was applied at that step in order to match the reads to the short pyrosequencing amplicons that stretch about 300 to 500 nt starting at the 3' prime end of the 16S rRNA genes. Sequences that shared at least 97% sequence similarity with one of the short target amplicons were used for further work. The alignment step was performed with the BLAST function of NCBI (41). Using clones picked from the masterplate, 5 mL liquid LB cultures were grown overnight and plasmids were harvested using QIAprep Spin Miniprep Kit (Qiagen) according to the manufacturer's protocol. Purified plasmids of up to five sequences per target phylotype were sequenced by Sanger chaintermination synthesis (Microsynth) using M13 forward and reverse primers. Reads were assembled and manually inspected in ChromasPro version 1.5 (Technelysium).

Recovered near full-length 16S rRNA gene sequences were aligned to the corresponding target pyrosequencing amplicons using the BLAST function of NCBI. At similarity values below 97%, sequences were excluded from further work. Isolates representing one target phylotype were aligned to each other, to determine their sequence similarities. In case they showed similarities below 97%, they were further analyzed to find out which isolate is abundant in the habitat. Sequencing data generated in other studies, which examined the same habitat (murine intestines), were mined. Reads with ≥ 200 nt length and were recovered from environmental samples, were downloaded from the Sequence Read Archive (SRA) next generation sequencing collection (42), and sorted by environment using BLAST+ (43). Amplicons that originated from murine guts were extracted and aligned to the ambiguous 16S rRNA gene sequence isolates, setting a similarity threshold of ≥ 97% and a coverage limit of 80%. Finally, isolates that matched the most abundant amplicons in this database of mouse intestinal microbiota were chosen as the sequences of interest.

3.2 Classification of the target phylotypes

Assembled sequences were aligned by the use of the SINA online aligner of SILVA (44) and imported into the database of small subunit rRNA (SILVA release 111, SSU Ref). In the ARB program (45) the 16S rRNA gene sequences were introduced into the phylogenetic tree using the "quick add" function.

To illustrate the phylogenic relationship of the target organisms, a neighbor joining tree was calculated using the filter "bacterial position variability" and a Jukes-Cantor DNA correction. The tree was inferred including primarily cultured relatives of the target

isolates. In case target organisms clustered to undefined groups, sequences of uncultured organisms that had high quality values (sequencing, alignment and pintail quality ≥ 85%, respectively), were included for building the tree. These unclassified target organisms were additionally analysed in RDP Classifier using the maximum possible confidence threshold of 95% (RDP Naïve Bayesian rRNA Classifier Version 2.5, May 2012) (46).

3.3 Design of oligonucleotide probes

In the ARB program, functions “probe design” and “probe match” were utilized complemented by manual improvements to create deoxyribonucleic acid (DNA) oligonucleotides of good quality concerning specificity and target group coverage. Attention was paid that the probes specifically target small subgroups to which the target sequences were assigned. Candidate probes were additionally checked using the “probe match” function of ribosomal database project (RDP) II and specificity and coverage of the final probes were analyzed (database release 10, Update 31 (December7, 2012) containing 2 639 157 bacterial and archaeal 16S rRNA sequences) (47). Probe match was restricted to sequences of good quality with a minimum length of 1200 nt. Predicted behavior of the probes was calculated using online tools (see section *in silico* probe evaluation).

Further considered properties of probe candidates were their own structure (guanine plus cytosine (GC) content of about 50%) and the structure of their binding site (secondary structure). For that purpose the *in situ* accessibility of the secondary structure of the *E. coli* 16S rRNA was checked and potential probes that bound largely in less accessible regions (class-5 and class-6) were avoided (48). Probes were placed meaningfully with regard to intensify mismatches to non-target groups (preferably strong mismatches were shifted in the middle region of the probes). Due to the ensured coexistence of the target phylotypes in murine gut samples, particular attention was paid to introduce strong mismatches to the sequences of remaining targets.

3.4 Revelation of chimeric sequences

Chimeric sequences were detected using pintail (version 1.1) (49) and all sequences, including those assumed to be chimeric according to pintail, were checked manually in ARB SILVA. First, quality values (sequencing, alignment and pintail quality) of neighboring sequences in the tree were controlled as well as their own and the target

isolates' branch lengths. In the second step, small trees were constructed using all isolates representing one target organism and few neighboring sequences, preferring cultured strains. After that, the trees were rebuilt three times using different filters, each included one third of the 16S rRNA sequences. If the sequence isolates representing one target phylotype clustered in a similar pattern in all four trees, the sequences were regarded non-chimeric. However, in case isolates switched positions and clustered to different groups in at least one tree, sequences were checked manually to concretize the site the different sequence parts meet and determine the origins of the sequence parts. Third, the sequences were aligned to the NCBI nucleotide database using BLAST, to check whether similar sequences have been recovered in other works, which would increase the probability of natural origin. Ambiguous sequences were excluded from further work.

3.5 *In silico* probe evaluation

The behavior of all potential probes was calculated *in silico* using the online tools mathFISH (http://mathfish.cee.wisc.edu/) (50) and Oligo Analyzer (version 3.1) (http://eu.idtdna.com/analyzer/Applications/OligoAnalyzer/). MathFISH uses mathematical models to predict the thermodynamic nature of interactions between the nucleic acids involved in the FISH process. The change in Gibbs free energy describes the binding probability of the DNA probe to the target rRNA (ΔG^{o}_{1}), as well as the intermolecular structure of the probe (ΔG^{o}_{2}) and the rRNA target site (ΔG^{o}_{3}), predicting the accessibility (51). Finally, from the three individual values the probe affinity ($\Delta G^{o}_{overall}$) and the dissociation profile were calculated (52), as well as the hybridization efficiency (53). All of the described values were calculated in the online tool using the function "general analysis".

In addition the probes' melting temperature (T_m) values and tendencies to build hairpin structures and hetero-dimers were simulated using the functions "analyze", "hairpin", and "self-dimer" in Oligo Analyzer.

3.6 Clone-FISH

In Clone-FISH, the 16S rRNA gene sequence is inserted into an expression vector and cloned into *E. coli* host cells. Overexpression of the rRNA is then induced via a T7 expression system so that the host cells contain extrinsic rRNA structure. Fixed cells can be treated as a pure culture of the target organisms for FISH and newly designed probes can be evaluated.

A valid configuration of the extrinsic rRNA product in the *E. coli* cells is essential to enable binding of the tested probe. Sanger sequencing (in house, using established protocols) using the forward primer M13F, which includes a part of the vector sequence, indicated orientation of the insert ligated into the vector. The insert orientation was concluded by regarding the position of the forward and reverse primers 8F and 1492R of the sequencing reads; in case 8F was found in the beginning of the read, expression of the insert was possible, if 1492R was found, the insert was inversely orientated.

In vivo transcription of the plasmid insert for Clone-FISH was performed according to the standard protocol of Schramm and colleagues (54), using the pCR™2.1-TOPO® vector (Invitrogen). On that account electrocompetent *E. coli* JM109(DE3) cells had been prepared (find the protocol in the Supplementary Materials and Methods section). After transcription cells were harvested by centrifugation (14 000 g, 5 min and 4° C) and fixed by incubation in 2% (PFA) at 4° C overnight. Subsequently, cells were pelleted (14 000 g, 5 min, 4° C), washed two times with 1x phosphate buffer saline (PBS) to remove PFA residues. Finally, the pellet was resuspended in 1 to 2 mL 1x PBS, and the same amount of 99.9% pure ethanol was added for storage at − 20° C.

Clone-FISH was performed with at least one isolate per target phylotype. Sufficient expression of the vector inserts was tested by FISH according to the protocol of Daims and colleagues (40) using the respective newly designed probes in addition to probes EUB338 (S-D-Bact-0338-a-A-18) (5'-GCT GCC TCC CGT AGG AGT-3') (55) and NONEUB (5'-ACT CCT ACG GGA GGC AGC-3' (56) as positive and negative control, respectively. Valid Clone-FISH samples were chosen for probe evaluation by FISH using serially increasing formamide (FA) concentrations of 0, 10, 20, 25, 30, 35, 40, 50, 60 and 70% and correspondingly decreasing NaCl-concentrations, both at constant temperatures, in order to attain dissociation profiles. Thereby, the respective tested probes, conjugated to indocarbocyanine (Cy3), were applied in addition to control probe EUB338 labeled with indodicarbocyanine (Cy5). At least 100 cells per FA concentration within the detectable range were imaged using a confocal laser scanning microscope (CLSM) (LSM 510 META, Zeiss). To achieve comparability of the cell signal intensities the same instrumental settings were kept during analysis of the FA series. Signal intensities were calculated in the software “daime” (57) using the function “evaluate FA series”. Automatically selected objects were corrected manually by excluding those untypical in size and shape and out of focus.

3.7 *In situ* probe evaluation

The probes were tested *in situ* by hybridizing the target organisms directly in environmental samples of murine intestinal content. For that purpose PFA-fixed samples were utilized that were known to have high abundance of target organisms from existing metagenomics data. Each probe was tested in addition to the probe combination “EUB338mix” targeting most bacteria. EUB338mix consists of three bacterial probes EUB338 detecting most bacteria, EUBII (S-*-BactP-0338-a-A-18) (5'-GCA GCC ACC CGT AGG TGT-3') detecting *Planctomycetales* and EUBIII (S-*-BactV-0338-a-A-18) (5'-GCT GCC ACC CGT AGG TGT-3') detecting *Verrucomicrobiales* (58), in equimolar concentrations. To verify the coverage of EUB338mix, some samples were hybridized using the probe mix in addition to staining the cells with 4,6-diamidino-2-phenylindole (DAPI) (59). Subsequently to FISH, the respective microscope slide well was incubated with 15 µL DAPI solution (100 ng/mL) for five minutes, and then the solution was removed. Wells were washed with ultrapure water two times and air-dried. Images were taken using a CLSM (LSM 510 META, Zeiss) and analyzed in daime as described above.

Due to high photostability of the fluorophore and to mind comparability to the Clone-FISH evaluation, newly designed probes were tested conjugated to Cy3. Evaluation was performed in single experiments, each combined with EUB338mix labeled with Cy5 or 5,(6)-carboxfluorescein-N-hydroxysuccimidester (FLUOS). Probes LasB3720_1281 and LasB5944_580, binding non-target *Lachnospiraceae*, were tested linked to Cy5 and evaluated at the same time as the respective target *Lachnospiraceae* phylotype, and without the addition of EUB338mix. As a negative control, samples were tested with probe NONEUB (Cy3-labeled).

In order to obtain reliable comparison to the Clone-FISH evaluation, same FA concentrations were applied to assess the probes *in situ*. Using a CLSM (LSM 510 META, Zeiss), images were taken of at least 100 target cells per FA concentration, to determine the respective signal intensities in daime as described above.

In single cases probes yielded weak signals at relatively low FA concentrations. To clarify whether the fixation type was unsuitable, FISH was additionally performed on ethanol-fixed samples at 10 and 30% FA. Moreover, FISH signal intensities were also tested performing pretreatment with proteinase K. After the ethanol series, according to the FISH protocol, 15 µl of proteinase K solution (15 mg/mL) was applied on microscopy slide wells and incubated for one, two, five, eight and ten minutes to test different treatment intensities. Subsequently, the wells were washed with ultrapure water for one minute and incubated with 15 µl 0.01 M HCl for 15 minutes to inactivate

the enzyme. After two additional washing steps with ultrapure water the slide was dried using an air flow and the FISH protocol was continued as described.

3.7.1 Quantitative FISH

To test specificity of the designed probes, the relative abundance of the target phylotypes was calculated by quantitative FISH and compared to existing metagenomics data. Parallel to the measurement of cell signal intensities at increasing FA concentrations (*in situ* probe evaluations), the images were used to count the targeted cells (in pixels) relative to the whole "bioarea" detected by EUB338mix. In this work, the image area that shows biomass is termed "bioarea". Quantification was performed in daime using the "biovolume fraction" function and automatically selected objects were corrected manually as described.

3.8 Monitoring abundance shifts by quantitative FISH

Abundance shifts of potential bacterial health state indicators throughout a trial of colitis induction and recovery in wild type mice were monitored. The trial was composed of 25 days with a five days period of DSS-treatment followed by a prolonged recovery period of 20 days. DSS-treatment started at day 1 and ended at day 5 and at five time points (day 0, 5, 8, 14, and 25) four mice were sampled, respectively. Three populations of *Bacteroidales* "*cluster S24-7*" were quantified in the PFA-fixed samples of luminal gut content via FISH using probes Bac16065_472, Bac15326_476 and Bac13481_437 (Cy3-conjugates), relative to most bacteria detected by EUB338mix (FLUOS-conjugate), respectively. FISH was performed as described at conditions of 50, 20 and 30% FA for probe Bac16065_472, Bac15326_476 and Bac13481_437, respectively. Images were taken with a CLSM (Leica LSM SP8, Leica Microsystems) and per sample 20 fields of view were analyzed.

3.9 Multicolor FISH

The aim of this experiment was to establish a new multiplex FISH approach using mono-labeled probes. A set of unusual fluorophores was tested for their simultaneous applicability in FISH experiments. New probes designed to detect phylotypes of the order *Bacteroidales* were chosen to test the fluorescent labels. The fluorescent dyes were selected to avoid spectral overlaps (excitation and fluorescence spectra) in order to ease discrimination of FISH signals. Parallel applicability of the newly labeled probes in addition

to EUB338mix was tested by FISH using conditions of 30, 25, 20, and 10% FA. PFA-fixed samples of murine gut content and feces of DSS-treated and non-treated wt mice were used. New probes, which could not be detected, were tested in individual FISH experiments with EUB338mix at conditions of 10 and 30% FA as well. Due to the limitation of hybridization conditions by one probe (Bac15326_476) to a maximum FA concentration of 20%, a serial FISH approach was tested. For serial FISH two hybridization steps were performed in sequence, with decreasing FA concentrations in the hybridization buffer and increasing corresponding NaCl concentrations in the washing buffer. In the first hybridization, probes Bac16065_472, Bac3732_846, Bac11839_649, Bac13481_437, Bac15326_476 Baci731A_87 and Baci731B_87 were applied and hybridized using 30% FA. Subsequently, the dried slide was reused for a second hybridization with probe Bac15326_476 using 20% FA. Different hybridization times were tested for the serial FISH approach; the first probe set was hybridized overnight and for 4 h, and the second steps were hybridized for 5 h and 2 h, respectively.

Images were taken with a CLSM (Leica LSM SP8 CLSM) by applying advised settings according to the datasheets of the fluorophores. Image processing (channel dye separation) was performed using the microscope software (LAS AF version 3.1).

3.10 Creating a comprehensive list of probes for gut bacteria

Published oligonucleotide probes for the identification of bacteria occurring in human and animal intestines were gathered and reevaluated. A search for probes was started in Google.Scholar (http://scholar.google.at) restricted to a set of criteria: "gut" OR "intestinal" AND "oligonucleotide probe" OR "oligonucleotide probes" OR "FISH probe" OR "FISH probes" OR "hybridization probe" OR "hybridization probes" OR "targeted probe" OR "targeted probes". In case a publication found by the search pattern referred to probes designed in another publication, the respective article was obtained. If free access was denied other research sources were browsed, such as PubMed of NCBI (www.ncbi.nlm.nih.gov/pubmed) and Science Direct (www.sciencedirect.com). Probes that bind the small subunit (16S) or large subunit (23S) rRNA were listed amended with important information. For each probe several properties were gathered: the short name (that was created if missing) as well as the systematic probe name after Alm and colleagues (60), the target organisms according to the publication, the reference to the probe's first publication as well as a second reference if it provided additional mentioned information, recommended FA concentration or temperature for specific use, the corresponding *E. coli* binding region according to Brosius and colleagues (61) and the target rRNA (either 16S or 23S).

Each oligonucleotide that binds the 16S rRNA was analyzed performing probe match of RDP II as described above (database release 10, Update 31 (December 7, 2012)), to assess specificity and coverage of the target groups and of perfectly matching non-target groups based on current data. The search was restricted to sequences of good quality with a minimum length of 1200 nt. In case probes did not match any sequences in the database, different sequence orientations (reverse, complement, and reverse complement) were tested and probe sequences were corrected. If no match was yielded nevertheless, a BLAST search of NCBI was performed to check the presence of the target organism in another database. BLAST was also facilitated to check validity of 23S rRNA targeting probes. Furthermore, the newly designed probes of this study were assessed the same way.

4 RESULTS

4.1 13 abundant bacterial phylotypes in the murine gut

Using existing 454 pyrosequencing data, 13 phylotypes were identified that showed median abundance of ≥ 1% in non-treated control mice (seven OTUs) or in DSS-treated colitic mice (eight OTUs), thereby two phylotypes showed ≥ 1% median abundance in both categories (Table 1). Based on the short pyrosequencing reads (Table SR1 of the Supplementary Tables section) taxonomic affiliations could only be assigned to order or family level.

Table 1. Abundance of target phylotypes enriched in either non-colitic control mice or DSS-treated colitic mice, according to metagenomics data. OTU identification (ID) number, taxonomic affiliation and median and maximum relative abundance of the phylotypes throughout mice of different genetic background are given.

OTU ID	Taxonomic affiliation	Median abundance (%)	Maximum abundance (%)
			In non-colitic mice
3720	*Lachnospiraceae*	4.82	25.49
15326	*Bacteroidales*	3.38	9.08
9057	*Lactobacillaceae*	2.28	5.44
13481	*Bacteroidales*	2.10	4.93
16065	*Bacteroidales*	1.18	1.98
2185	*Lactobacillaceae*	1.15	4.04
3732	*Bacteroidales*	0.95	6.48
			In colitic mice
15326	*Bacteroidales*	3.19	8.84
3256	*Erysipelotrichaceae*	2.90	22.41
731	*Bacteroidaceae*	2.20	5.93
14644	*Ruminococcaceae*	1.78	4.32
17903	*Ruminococcaceae*	1.49	7.04
5944	*Lachnospiraceae*	1.21	4.48
13481	*Bacteroidales*	1.01	4.73
11839	*Bacteroidales*	0.99	1.91

Using existing sequence information of the abundant phylotypes, their near full-length 16S rRNA gene sequences (Table SR2) were attained via a targeted clone library. Three to five sequence isolates of each target phylotype were recovered, except for target *Lactobacillus* OTU 2185 and *Lachnospiraceae* OTU 5944 of which only one and two isolates were obtained, respectively. A part of these recovered isolates were left for further work after removal of chimeric sequences and of isolates that showed low abundance in the SRA collection of mouse isolates (Table 2). Taxonomic affiliations of the sequences were inferred using ARB SILVA (see also in Figure 1, details discussed below). In two cases (*Bacteroides* OTU 731 and *Ruminococcaceae* OTU 14644) the respective isolates grouped into two different subclusters, hence were split into two probe target subgroups A and B. The corresponding biomass of the recovered phylotypes in metagenomics data represents the sum of both subgroups A and B. Some phylotypes of the category “abundant in non-colitic mice” were recovered from individuals that were treated with DSS for one day, because inflammation and microbial abundance shifts accommodate gradually. Screening started with samples of non-colitic mice, thus isolates of colitis-associated *Lachnospiraceae* OTU 5944 and of *Bacteroidales* “*cluster S24-7*” OTU 11839 were coincidentally found in samples of non-colitic and one day DSS-treated mice, respectively. All used samples were luminal gut contents consisting of caecal and colonic biomass, except for faecal sample G47.

Table 2. Information about the origin of the recovered 16S rRNA gene sequences of the target phylotypes. The final numbers of recovered isolates per phylotype and the corresponding mouse genotype they originated from are given. The mice treatment (water or DSS) and the duration of DSS treatment in days (d) at the sampling time point are listed. Relative biomass shows the abundance in existing metagenomics data.

Taxonomic affiliation of target phylotypes	Recovered isolates	Genotype	Treatment	Sample ID	Relative biomass
Abundant phylotypes of non-colitic mice					
Lachnospiraceae OTU 3720	4	IFNGR$^{-/-}$	DSS (1d)	C31	24.8%
		wt	Control	G47	3.1%
Bacteroidales "cluster S24-7" OTU 16065	5	IFNGR$^{-/-}$	DSS (1d)	C31	1.0%
		IFNGR$^{-/-}$	DSS (1d)	C32	1.8%
Bacteroidales "cluster S24-7" OTU 3732	1	wt	Control	G33	0.6%
Lactobacillus OTU 2185	1	IFNGR$^{-/-}$	DSS (1d)	C32	4.0%
Lactobacillus OTU 9057	3	IFNGR$^{-/-}$	DSS (1d)	C31	5.4%
		IFNGR$^{-/-}$	DSS (1d)	C32	5.3%
Abundant phylotypes of colitic mice					
Allobaculum OTU 3256	4	IFNGR$^{-/-}$	DSS (7d)	C43	22.4%
Bacteroides OTU 731 A	2	IFNGR$^{-/-}$	DSS (7d)	C43	1.9%*
Bacteroides OTU 731 B	2	STAT1$^{-/-}$	DSS (10d)	A15	1.9%*
		IFNGR$^{-/-}$	DSS (7d)	C43	1.9%*
Ruminococcaceae OTU 14644 A	1	STAT1$^{-/-}$	DSS (10d)	A15	1.2%*
Ruminococcaceae OTU 14644 B	1	STAT1$^{-/-}$	DSS (10d)	A15	1.2%*
Ruminococcaceae OTU 17903	4	STAT1$^{-/-}$	DSS (10d)	A13	4.2%
		STAT1$^{-/-}$	DSS (10d)	A15	5.2%
		IFNGR$^{-/-}$	DSS (7d)	C43	1.9%
Lachnospiraceae OTU 5944	2	IFNGR$^{-/-}$	DSS (7d)	C45	2.1%
		wt	Control	G33	0.6%
Bacteroidales "cluster S24-7" OTU 11839	1	IFNGR$^{-/-}$	DSS (1d)	C32	0.4%
Abundant phylotypes of all mice					
Bacteroidales "cluster S24-7" OTU 15326	2	IFNGR$^{-/-}$	DSS (1d)	C32	2.8%
Bacteroidales "cluster S24-7" OTU 13481	3	IFNGR$^{-/-}$	DSS (1d)	C31	1.6%
		wt	Control	G33	1.6%

*Value represents the sum of subgroups A and B of one phylotype.

4.1.1 Classification of the target phylotypes

The phylogenic relationship of the target organisms, analyzed in ARB SILVA, is represented in a neighbor joining tree (Figure 1). Two *Lactobacillaceae* phylotypes, enriched in non-inflamed murine intestines, could be identified to species-level. *Lactobacillus* OTU 2185 represented *L. reuteri* (100% sequence similarity) and *Lactobacillus* OTU 9057 isolates grouped to *L. taiwanensis, L. johnsonii* and *L. gasseri.* These neighboring species shared ≥ 98% 16S rRNA gene sequence similarity to each other and to *Lactobacillus* OTU 9057. *Bacteroidaceae* OTU 731, found accumulated in inflammation, represents *Bacteroides acidifaciens*. The isolates grouped to two small subgroups within the diverse *Bacteroides acidifaciens* cluster. *Bacteroides* OTU 731 subgroup A and B represent *B. acidifaciens* Group-2 and Group-1, respectively, according to Miyamoto and colleagues (62). 16S rRNA gene sequence similarities showed ≥ 96% between the groups and ≥ 99% within the groups. The isolates of *Erysipelotrichaceae* OTU 3256, abundant members of the colitic murine microbiota, grouped to the genus *Allobaculum*. The isolates exhibited 87% 16S rRNA gene sequence similarity to the only cultured representative of the genus, *Allobaculum stercoricanis* DSM 13633. Analysis in RDP Classifier confirmed the classification to genus *Allobaculum*. *Ruminococcaceae* OTU 14644 and OTU 17903, which increased in inflamed mouse guts, clustered to uncharacterized phylotypes of unaffiliated groups. This was also the case for *Lachnospiraceae* OTU 3720 and 5944, whereby each phylotype accumulated either in non-colitic or in inflamed intestines. *Bacteroidales* phylotypes (OTU 3732, 16065, 11839, 15326 and 13481) were abundant in all mouse categories. The 16S rRNA gene sequence isolates grouped to a family-level *"cluster S24-7"*, which harbors sequences from uncultured organisms exclusively.

The 16S rRNA gene isolates of the undescribed phylotypes were additionally analyzed in RDP Classifier. The *Lachnospiraceae* phylotypes OTU 3720 and OTU 5944 as well as *Ruminococcaceae* OTU 17903 were assigned to unclassified *Lachnospiraceae* and unclassified *Ruminococcaceae*, respectively. *Ruminococcaceae* OTU 14644 was affiliated to the genus *Oscillibacter* and depicts 95% (subgroup A) and 94% (subgroup B) sequence similarity to *Oscillibacter valericigenes.* Most *Bacteroidales* "*cluster S24-7*" isolates were classified to the genus *Barnesiella* (of the family *Porphyromonadaceae*), which is the neighboring cluster harboring the closest cultured relatives according to ARB. OTU 16065 assigned to unclassified *Porphyromonadaceae* in RDP. The closest cultured relatives *Barnesiella intestinalis* and *B. viscerosa* share about 82 to 87% 16S rRNA gene sequence similarity with the *Bacteroidales* "*cluster S24-7*" isolates. Sequence similarity values within the target phylotypes of the large "*cluster S24-7*" were calculated in ARB, using the filter "bacterial position variability" (Table 3).

Table 3. 16S rRNA gene sequence similarities of the *Bacteroidales* "*cluster S24-7*" target phylotypes. Phylotypes are described by OTU identification (ID) number and values represent mean percentages of the respective sequence isolates. Highest and lowest values are highlighted.

OTU ID	16065	3732	11839	15326	13481
16065	**99%**	89%	89%	88%	89%
3732	89%	**100%**	90%	88%	**94%**
11839	89%	90%	**100%**	89%	90%
15326	88%	88%	89%	**100%**	**87.5%**
13481	89%	**94%**	90%	**87.5%**	**99%**

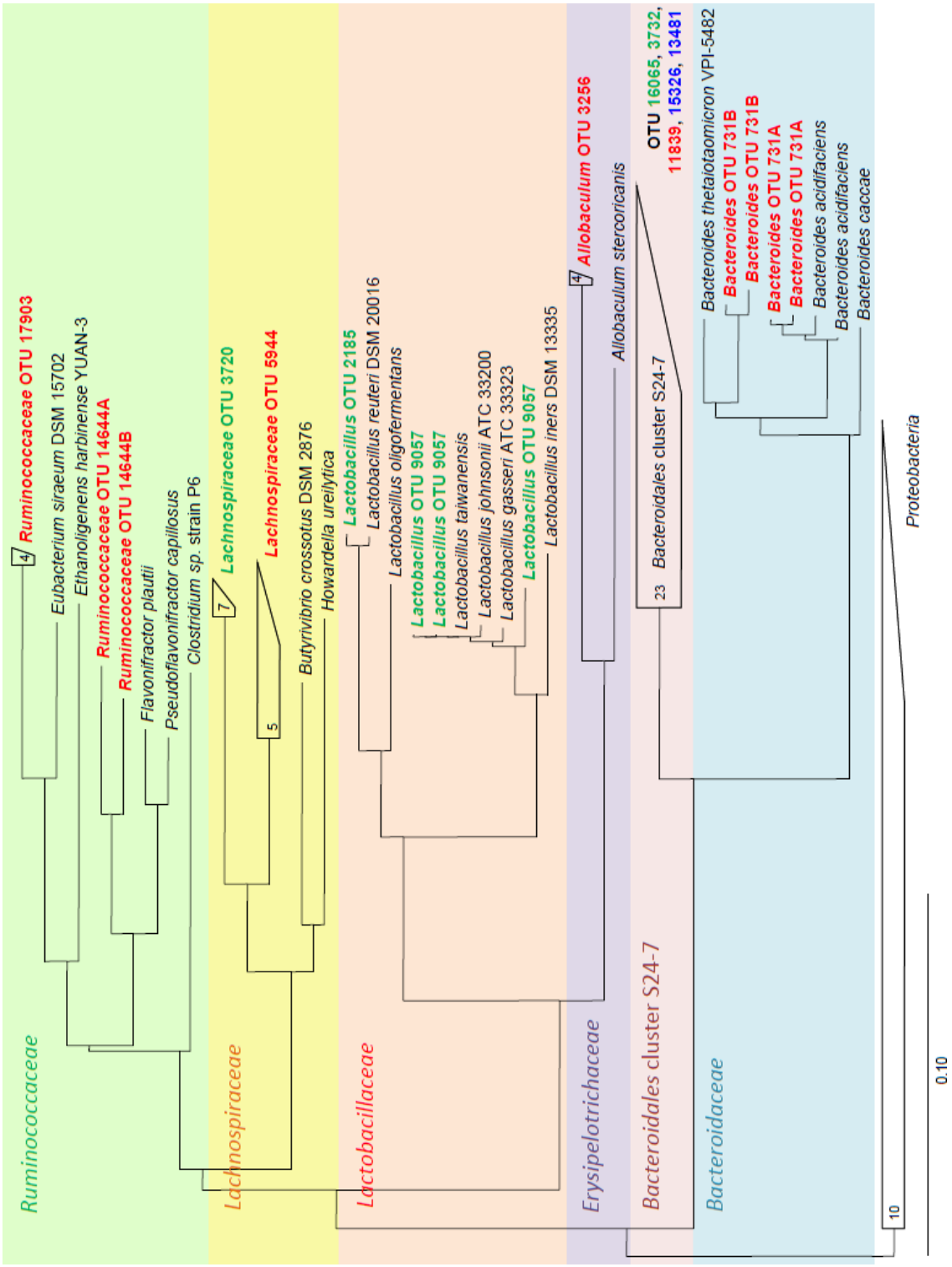

Figure 1. **Neighbor joining tree inferring the phylogeny of the abundant phylotypes in murine intestines.** Background colors illustrate family-levels. Abundant members of the colitic murine microbiota are shown in red, those of the non-treated mice in green and the phylotypes abundant in all mice in blue. The scale bar represents sequence divergence of 10%.

4.2 17 new FISH probes

15 DNA oligonucleotide probes were designed to detect the recovered 16S rRNA sequences and two additional probes (LasB3720_1281 and LasB5944_580) to increase group coverage of *Lachnospiraceae* OTU 3720 and OTU 5944, respectively (Table 4). The highly specific probes bind variable regions V1 to V5 and V8 of the 16S rRNA sequence (63). Target 16S rRNA regions were of relatively good accessibility (class-1 to class-4) in probe evaluations of *E. coli* (48), except for a class-5 (less accessible) region bound by probe Bac11839_649.

Using ARB SILVA, most probes covered the specific groups to a minimum of 77% (Figure 2). The *Lachnospiraceae* targeting probes were complemented by additional probes to increase the coverage to a sum of about 90%. The genus Lactobacillus showed heterogeneous 16S rRNA sequences, which made the design of a specific probe that also covers the majority of the group challenging. Due to the priority of specificity the designed probe (Lab2185_87) covers the subgroup to 47%. Each of the probes targeting *Ruminococcaceae* phylotypes exclusively hit the corresponding recovered isolates. The probe for *Allobaculum* TU 3256 binds one of two large subgroups in the genus.

In the course of the literature search for probes targeting gut bacteria probes specific for *Lactobacillus reuteri* (64) and *Bacteroides acidifaciens* (65) were found that bind target OTU 2185 and OTU 731, respectively, and show similar properties as the probes designed in this study.

Table 4. Basic information of the oligonucleotide probes designed in this work.

Target organism	Probe name	OPD code[a]	Probe sequence (5' → 3')	Probe length	Target position[b]	Variable region[c]
Lachnospiraceae OTU 3720	LasA3720_1281	S-*-LspA-1281-a-A-23	GAA CTG AGA CGT TGT TTT TCG GA	23 nt	1281 - 1303	V8
OTU 3720-like *Lachnospiraceae*	LasB3720_1281	S-*-LspB-1281-a-A-23	GAA CTG AGA CGT TAT TTT TCG GA	23 nt	1281 - 1303	V8
Bacteroidales "cluster S24-7" OTU 16065	Bac16065_472	S-*-Bada-0472-a-A-24	CTC CGG GTA CTC TCC AAA CCG CA	24 nt	472 - 494	V3
Bacteroidales "cluster S24-7" OTU 3732	Bac3732_846	S-*-Bada-0846-a-A-24	CGC TTT CGC TGT ATC ACT CAG GGA	24 nt	846 - 869	V5
Lactobacillus OTU 2185	Lab2185_87	S-*-Lbre-0087-a-A-24	TGG TGA TCC ATC GTC AAT CAG GTG	24 nt	87 - 97	V1
Lactobacillus OTU 9057	Lab9057_570	S-S-Lbad-0570-a-A-23	TGA ACC GCC TGC ACT CGC TTT AC	23 nt	570 - 592	V4
Allobaculum OTU 3256	All3256_484	S-*-Allb-0484-a-A-22	CGT GAC TTT CTG GCG GGG CAC C	22 nt	484 - 505	V3
Bacteroides OTU 731 A	Baci731A_87	S-*-Baci-0087-a-A-24	GCG CCG GTC GCC ATC AAA AGT TTG	24 nt	87 - 108	V1
Bacteroides OTU 731 B	Baci731B_87	S-*-Baci-0087-a-A-22	GCC GGT CGC CAT CGG AAG TTT G	22 nt	87 - 106	V1
Ruminococcaceae OTU 14644 A	Rum14644A_184	S-*-Ruco-0184-a-A-23	ACA TCA AAA CCA TGC GGC TTC GA	23 nt	184 - 194	V2
Ruminococcaceae OTU 14644 B	Rum14644B_64	S-*-Ruco-0064-a-A-24	ATC TCT TTC CAA GGC ACT CCG TTC	24 nt	64 - 82	V1
Ruminococcaceae OTU 17903	Rum17903_443	S-*-Ruco-0443-a-A-20	TAC CGT CAT TAT CGC CCC CC	20 nt	443 - 487	V3
Lachnospiraceae OTU 5944	LasA5944_580	S-*-LspA-0580-a-A-24	TTC AGG CTT GCA CAG CCG TCT ACG	24 nt	580 - 603	V4
OTU 5944-like *Lachnospiraceae*	LasB5944_580	S-*-LspB-0580-a-A-24	TTC AGG CTT GCT CAG CCG TCT ACG	24 nt	580 - 603	V4
Bacteroidales "cluster S24-7" OTU 11839	Bac11839_649	S-*-Bada-0649-a-A-23	CAC ACT TCT CGC CCA CTC AAG AC	23 nt	649 - 671	V4
Bacteroidales "cluster S24-7" OTU 15326	Bac15326_476	S-*-Bada-0476-a-A-23	TTT TCT TCG GAT ACA CGC AGT CC	23 nt	476 - 498	V3
Bacteroidales "cluster S24-7" OTU 13481	Bac13481_437	S-*-Bada-0437-a-A-22	CGC CCT TTG CTC CCT GAC AAA A	22 nt	437 - 457	V3

[a]Oligonucleotide probe database (OPD) code (60). [b]Target positions refer to *E. coli* numbering (61). [c]Variable region of 16S rRNA sequence (63).

A

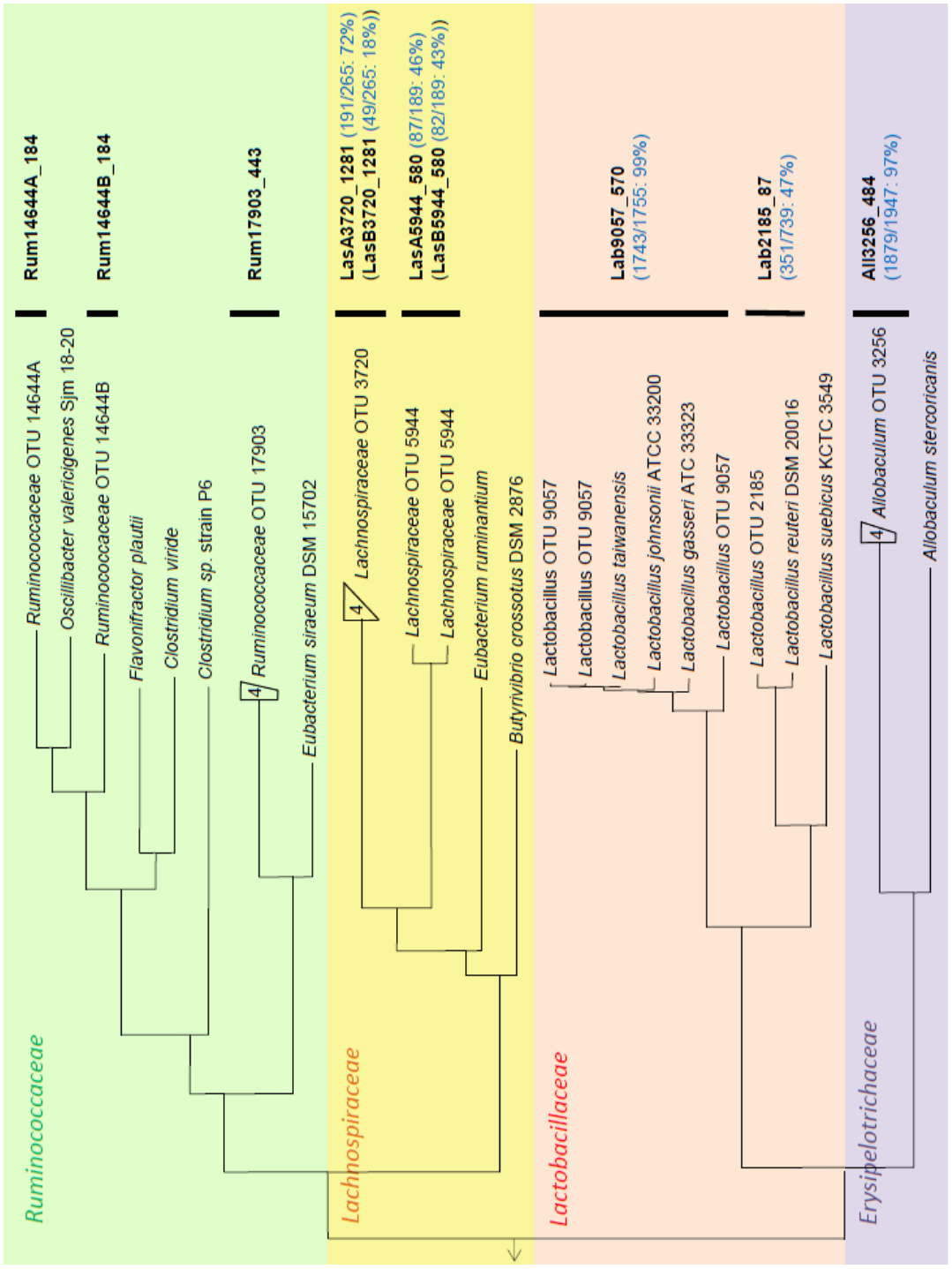

Figure 2. **Specific FISH probes for the target phylotypes of *Firmicutes* (A) *and Bacteroidales* (B).** Two neighbor joining trees inferred based on 16S rRNA phylogeny show the isolates of the target phylotypes and mainly cultured relatives, split by phylum; *Firmicutes* (tree A) and *Bacteroidetes (*tree B*)*. The probes on the right side indicate specificity by bars, and respective group coverage (sequences hit by the probe/total sequences of the subgroup: percent coverage) in blue brackets. The tree showing *Bacteroidetes* includes organisms of two non-target families *Porphyromonadaceae* and *Prevotellaceae* as the closest cultured relatives of the *Bacteroidales "cluster S24-7"* target phylotypes. Scale bars indicate sequence divergence of 10%.

B

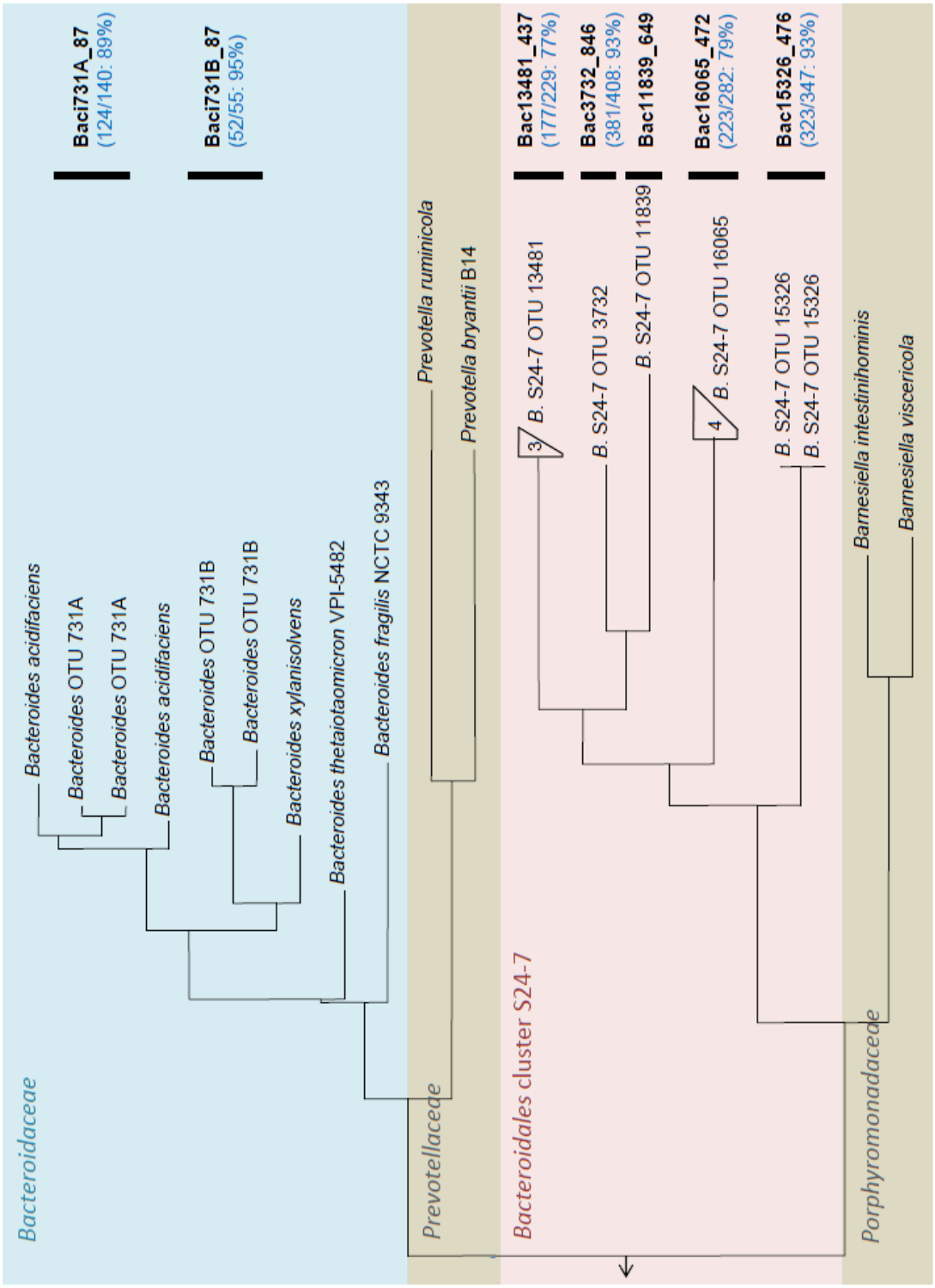

Figure 2. (continued)

4.3 Systematic optimization of FISH probes

4.3.1 In silico probe evaluation

Analyses of the designed oligonucleotides using the online tools OligoAnalyzer and mathFISH are shown in Table 5 and Table 6, respectively. Most probes contain 44 to 58% purine bases, except for probes LasB3720_1281, Bac16065_472 and All3256_484 with GC contents of 39, 61 and 68%, respectively. The probes' tendency to create a homodimer (HD) was limited to four possible base pairs. Each probe showed a simulated hybridization efficiency of one (100%), but probe Rum14644A_184 showed 0.88 (88%). For this probe the lowest melting FA concentration ($[FA]_m$) of 11% was predicted. Melting FA concentration represents an equivalent to melting temperature at 50% dissociated molecules, when FA at constant temperature is adapted to create specific conditions. Suggested melting FA concentration for most probes ranged from 32 to 62%, beside probes LasA3720_1281 and LasB3720_1281, for which 26% and 19% FA is proposed, respectively. The three probes holding lowest melting FA concentration correspondingly yielded lowest melting temperatures (T_m) from 66° to 64° C. Melting temperatures of the remaining probes ranged from 72 to 80 °C.

Table 5. Properties of the designed probes calculated in OligoAnalyzer. GC content (GC), melting temperature (T_m), maximum possible base pairs (bp) to form a homodimer (HD) and corresponding Gibbs free energy values (∆G HD), maximum Gibbs free energy value for building a hairpin structure (∆G hairpin) and corresponding melting temperature (T_m hairpin) are listed.

Probe name	GC	T_m (° C)	HD (bp)	ΔG HD	ΔG hairpin	T_m hairpin (° C)
LasA3720_1281	43.5	66.1	4	-6.3	-5.96	68.1
LasB3720_1281	39.1	63.5	4	-6.3	-5.96	68.1
Bac16065_472	60.9	75.6	4	-9.75	-3.94	57.9
Bac3732_846	54.2	76.4	2	-3.61	-3.2	45.2
Lab2185_87	50.0	72.8	3	-5.02	-4.84	52.2
Lab9057_570	56.5	73.5	4	-7.05	-2.86	54.6
All3256_484	68.2	78.0	2	-3.61	-5.99	56.4
Baci731A_87	58.3	72.5	4	-9.89	-3.38	62.6
Baci731B_87	53.6	75.1	4	-9.75	-5.72	73.1
Rum14644A_184	47.8	66.2	4	-6.76	-3.46	51.7
Rum14644B_64	50.0	77.9	2	-3.61	-1.68	47.9
Rum17903_443	60.0	77.2	2	-3.61	0.17	22.2
LasA5944_580	58.3	77.9	4	-7.81	-7.63	84.9
LasB5944_580	58.3	79.6	4	-7.81	-7.63	84.9
Bac11839_649	56.5	73.2	2	-3.61	-1.13	38.2
Bac15326_476	47.8	72.3	3	-4.64	-3.99	62.1
Bac13481_437	54.5	71.8	4	-5.84	-2.78	41.8

Table 6. Properties of the designed probes calculated in mathFISH. Thermodynamic properties (ΔG values), Hybridization efficiency (HE) and melting FA concentration ($[FA]_m$) of the oligonucleotide probes are given.

Probe name	ΔG°_1 (kcal/mol)	ΔG°_2 (kcal/mol)	ΔG°_3 (kcal/mol)	$\Delta G^{\circ}_{overall}$ (kcal/mol)	HE	$[FA]_m$ (%)
LasA3720_1281	-20.7	0.5	-5.8	-14.7	1	26.0
LasB3720_1281	-19.1	0.5	-5.8	-13.1	1	18.8
Bac16065_472	-28.3	-0.5	-13.1	-14.5	1	44.4
Bac3732_846	-27.3	-0.9	-10.4	-15.8	1	43.6
Lab2185_87	-24.9	0.0	-6.9	-17.5	1	40.4
Lab9057_570	-26.6	0.0	-9.6	-16.6	1	44.3
All3256_484	-28.2	-0.5	-6.4	-21.0	1	61.5
Baci731A_87	-27.5	-1.4	-9.3	-16.7	1	47.3
Baci731B_87	-27.0	-1.5	-11.8	-13.7	1	42.9
Rum14644A_184	-22.3	-0.3	-11.2	-10.5	0.88	11.0
Rum14644B_64	-27.5	-0.3	-6.6	-20.3	1	53.2
Rum17903_443	-27.0	1.1	-8.2	-18.7	1	58.4
LasA5944_580	-28.7	-2.1	-11.2	-15.4	1	50.0
LasB5944_580	-29.3	-2.1	-6.1	-21.1	1	61.6
Bac11839_649	-25.7	0.8	-7.2	-18.3	1	45.4
Bac15326_476	-24.1	-0.4	-9.0	-14.5	1	32.4
Bac13481_437	-25.6	0.1	-11.5	-13.7	1	33.8

4.3.2 Clone-FISH

In order to evaluate the designed probes using Clone-FISH a functionality test was performed resulting in eight samples that were suitable for this approach. Target sequences that were cloned into the expression vector in an orientation that did not match the transcription sense with the T7 polymerase promoter were not applicable. For targets *Lactobacillus* OTU 2185 and *Ruminococcaceae* OTU 14644B only one non-chimeric sequence isolate was recovered, respectively, which were inserted in an inverted orientation. For that reason probes Lab2185_87 and Rum14644B_64 could not be evaluated via this method. At the time of performing *in vivo* transcription experiments, the insert orientation was still unfamiliar. No sample had been prepared for *Bacteroides* OTU 731 subgroup A, hence only probe Baci731B_87 targeting the subgroup B of the phylotype was evaluated. Probably due to insufficient expression of the insert, in some cases Clone-FISH did not show adequate probe signal intensities thus were not considered. Therefore, probes Bac3732_846, Lab9057_570, Rum14644A_184 and Bac15326_476 were not evaluated by Clone-FISH. Besides, probes LasB3720_1281 and LasB5944_580 were not designed for targets of the clone library, hence were not evaluated.

Melting FA concentrations resulting from the method as well as from *in silico* and *in situ* probe evaluations are compared in Table 8 and discussed in section “4.3.4 Different

approaches – different results". Corresponding dissociation profiles depicting signal intensities at increasing FA concentrations are given in Figure S1 of the Supplementary Figures section.

4.3.3 In situ probe evaluation

The newly designed probes were tested on environmental gut samples in combination with EUB338mix. Representative FISH images of the target cells at specific FA concentrations are shown in Figure 3 and resulting FA curves of each probe in Figure S1 (Supplementary Figures). As a control, FISH using the general probe combination EUB338mix was combined with DAPI staining, whereby images of the bright field channel revealed that with the use of EUB338mix as well as DAPI the majority, but not all of the cells were detected in PFA-fixed samples of gut content (Figure S2).

Lactobacillus targeting probes yielded low signals even at low FA concentrations in *in situ* hybridizations using cell samples fixed with PFA, leading to doubtful data. Probe Lab9057_570 tested on an ethanol-fixed sample resulted in bright signals at higher FA concentrations of 30% FA (Figure 4) than on cells fixed with PFA. No signals were detected after proteinase K treatment of samples treated with either fixative. Signal detection by probe Lab2185_87, however, could neither be improved using ethanol-fixed samples (Figure 5), nor by enzymatic treatment. On ethanol-fixed samples EUB338mix signals were drastically reduced compared to signals of the DAPI staining, and in particular to signals using PFA-fixed samples. Additionally, most cells looked battered due to the rough treatment. Probes targeting *Firmicutes* performed well using ethanol-fixed cells, whereas *Bacteroidales* targeting probes yielded weak signals, except for OTU 11839 (Figure S3). Most *Bacteroidales* targets were not stained by EUB338mix (FLUOS, green), whereas low signals were detected using specific probes conjugated to the brighter fluorophore Cy3 (red).

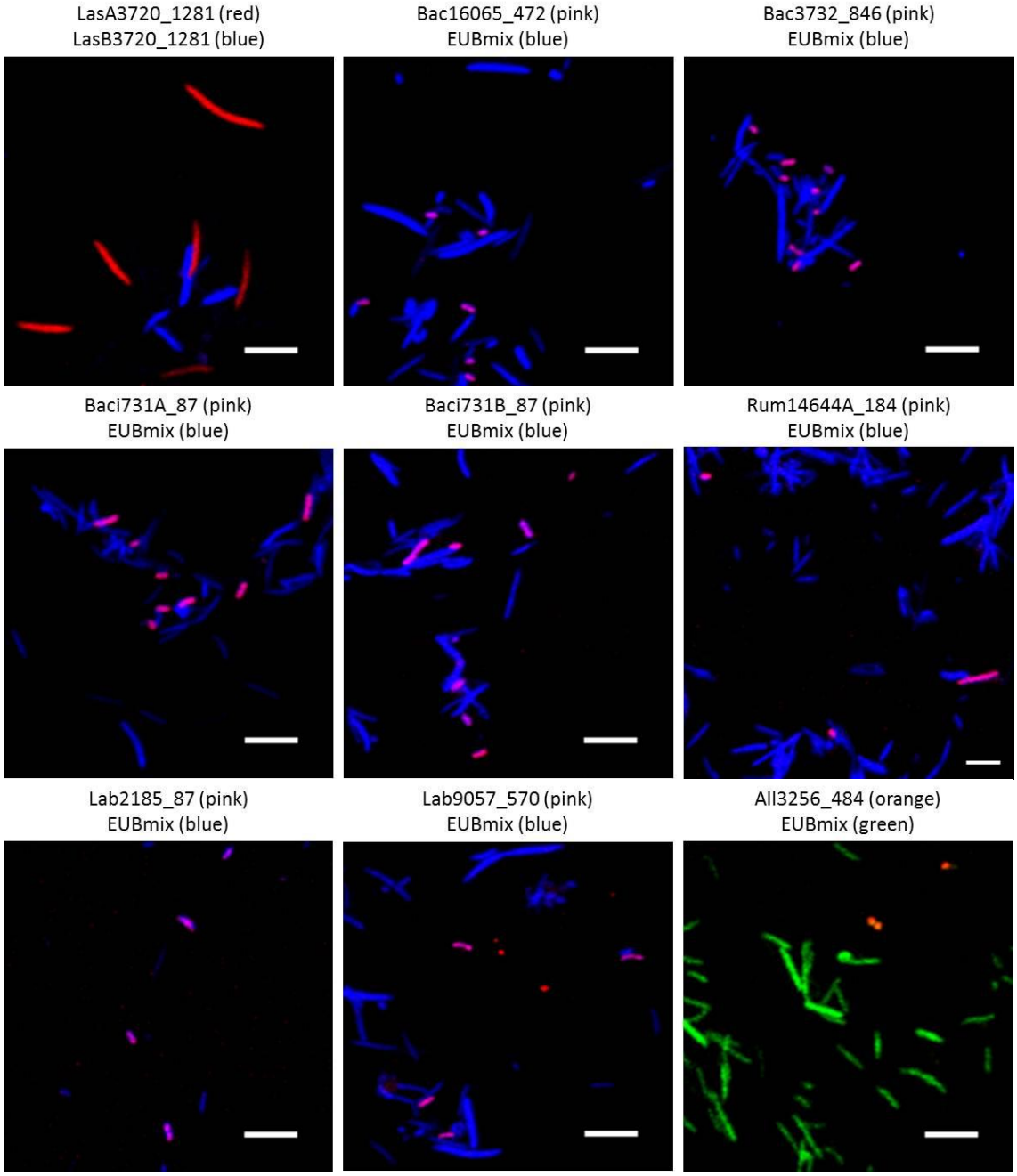

Figure 3. **Representative FISH images of the target phylotypes detected by new probes.** Images were taken for the *in situ* probe evaluation and show the specific probes in warm colors (Cy3) and most other bacteria in blue (Cy5) or green (FLUOS), except for images of *Lachnospiraceae* targeting probes, where the corresponding closely related members of *Lachnospiraceae* are stained blue (Cy5). The scale bars indicate 5 µm.

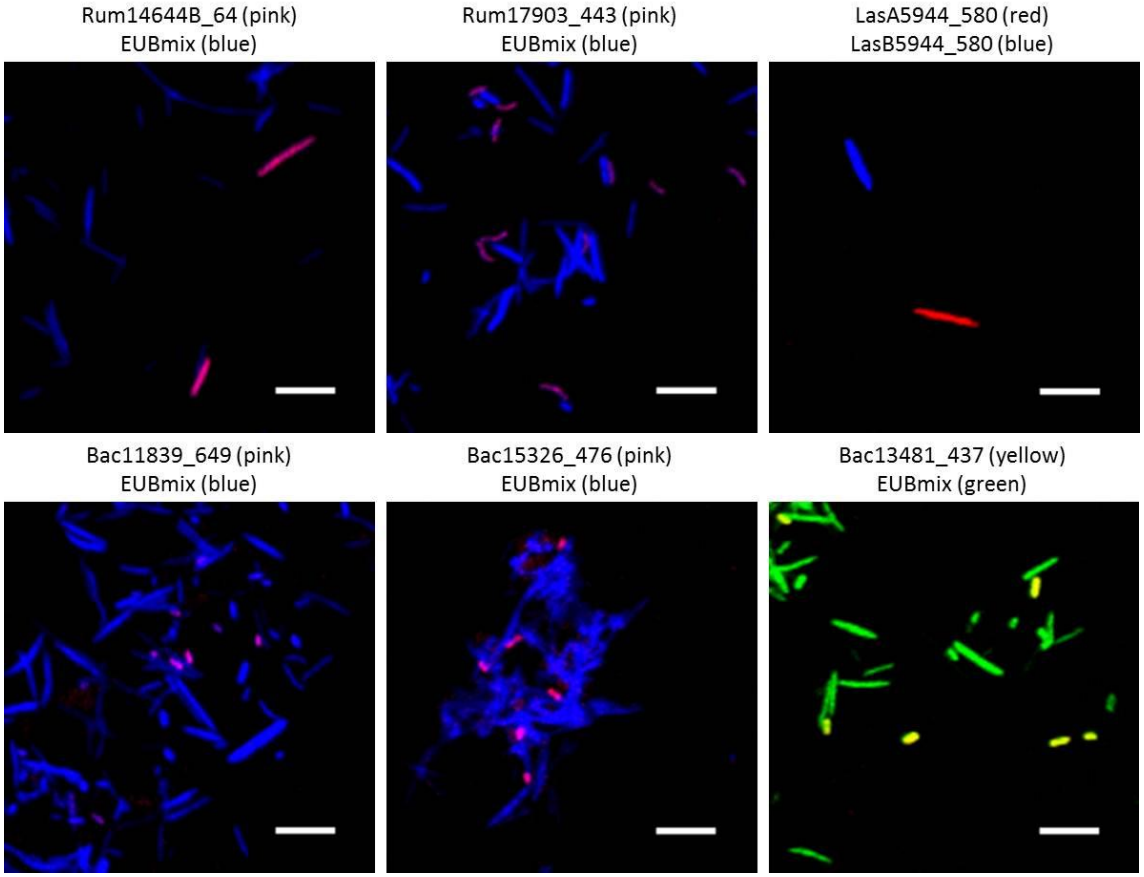

Figure 3. (continued)

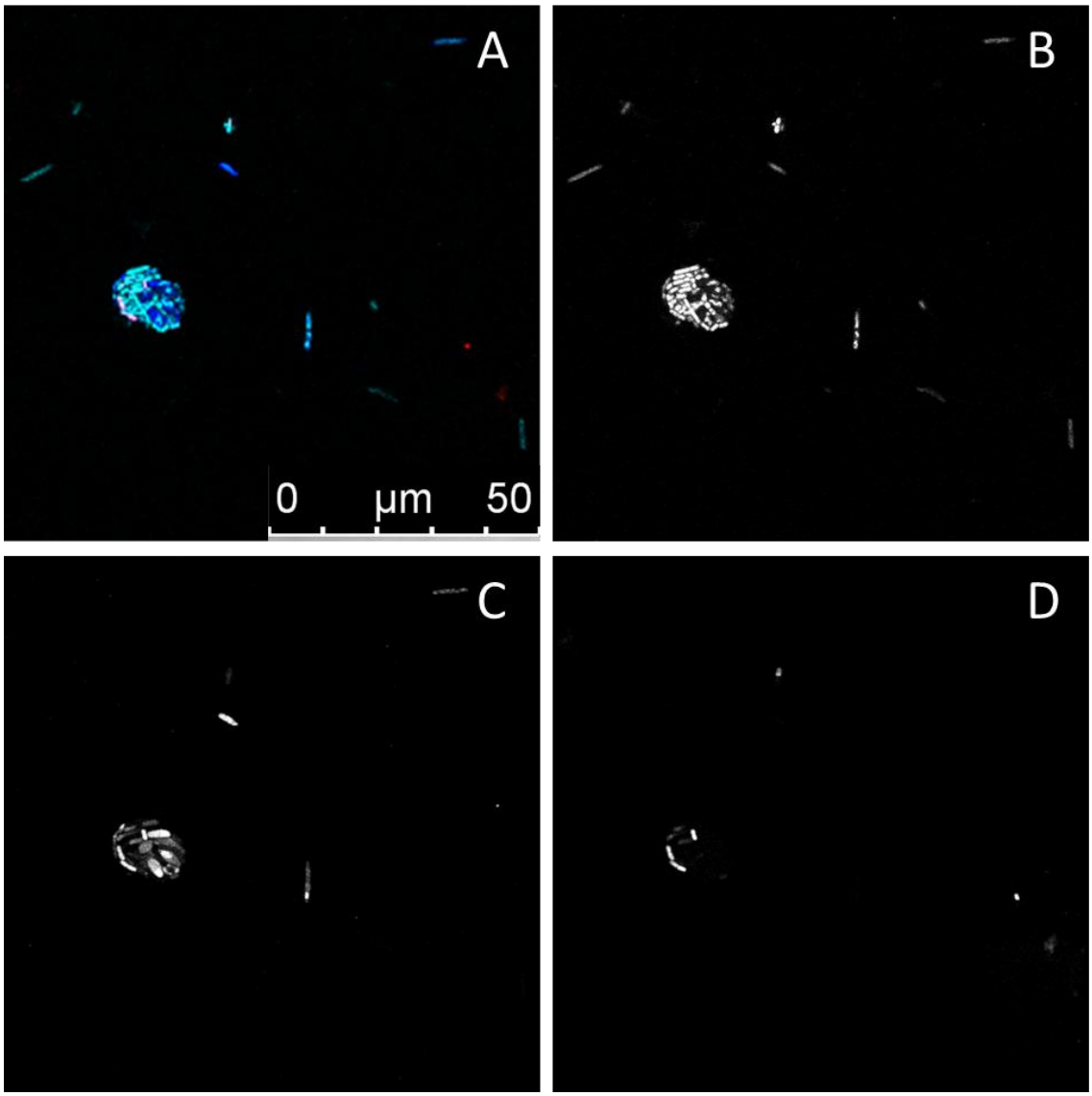

Figure 4. **Probe Lab9057_570 yielded bright signals on ethanol-fixed cells.** Image A shows the overlay of all channels, the single channels are DAPI (cyan) (B), EUB338mix (Cy5, blue) (C), Lab9057_570 (Cy3, light pink) (D).

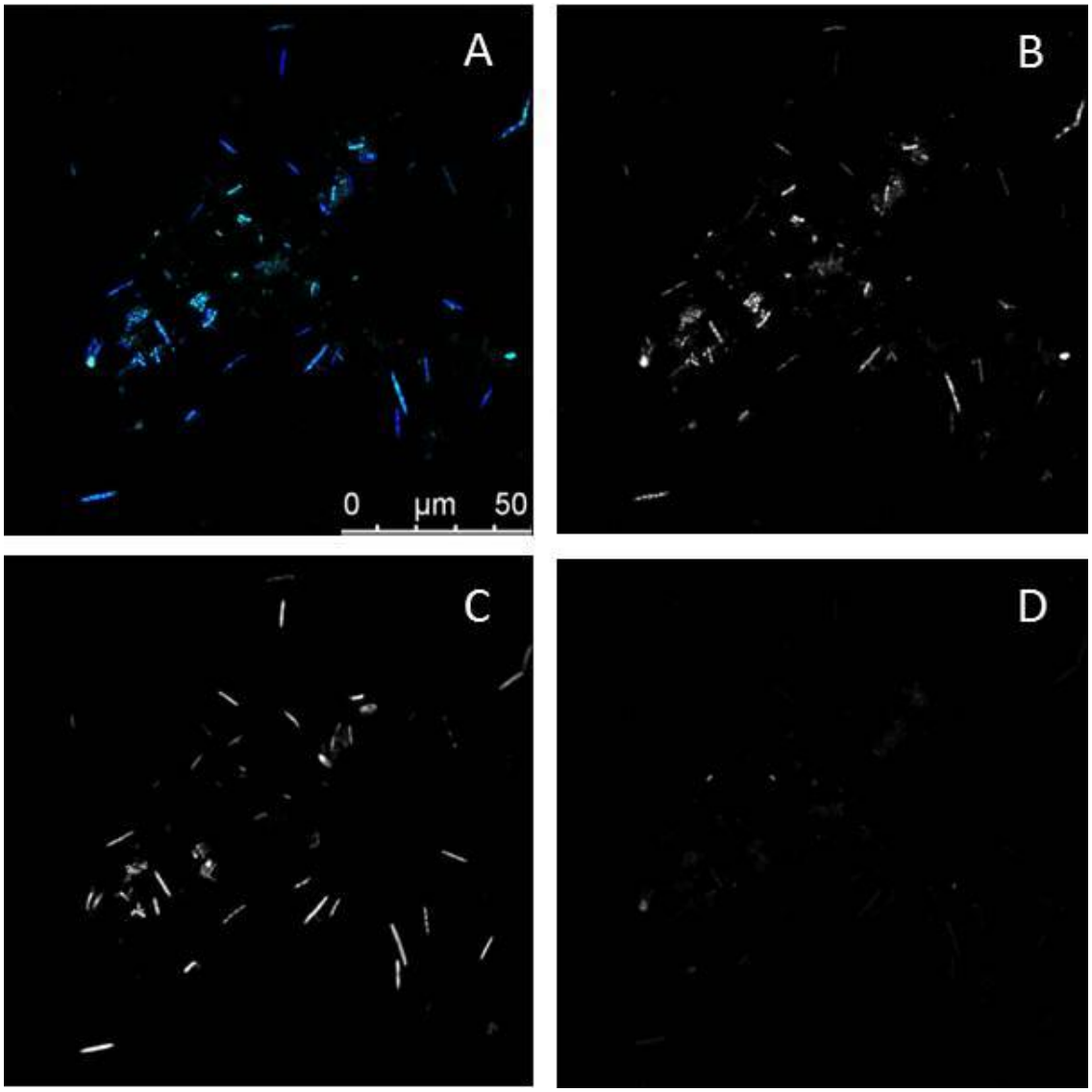

Figure 5. Probe Lab2185_87 yielded poor signals on ethanol-fixed cells. Image (A) shows the overlay of the channels, the single channels are DAPI (cyan) (B), EUB338mix (Cy5, blue) (C), and Lab2185_87 (Cy3, pink) (D).

4.3.3.1 Quantification at increasing stringency

Images of the *in situ* probe evaluation were also used to quantify specific signals of the newly designed probes relative to signals of all bacteria detected by the probe combination EUB338mix (Figure S4 of the Supplementary Figures section). Specificity of the designed probes could be assessed by comparing existing metagenomics data to the abundances at specific FA concentrations (highest FA concentrations the probe signal intensities allowed) (Table 7). The two distinct approaches showed large compliance, whereby in seven of nine cases, sequencing resulted in higher values. In most cases abundances of one approach represented at least 69% of the other

approach and in four cases at least 90%. Larger variation was observed in case of *Allobaculum* OTU 3256 and *Bacteroidales "cluster S24-7"* OTU 15326, yielding 6 and 11% higher relative biomass, respectively, resulting from the sequencing approach. Quantification of *Bacteroides* OTU 731 probes A as well as B by FISH corresponded to the abundance of OTU 731 in sequencing data, suggesting that the single mismatch could not be discriminated when the probes were applied separately. In case of *Ruminococcaceae* OTU 14644, the two probes targeting subclusters A and B, in total acquire 89% of the sequencing biomass.

Due to unreliable signals using PFA-fixed samples, *Lactobacillus* target phylotypes were excluded from the quantitative FISH experiment. *Lachnospiraceae* phylotypes were not quantified, because the respective probes were evaluated without the addition of EUB338mix that is needed for relative quantification.

Table 7. Relative abundance of target phylotypes measured using distinct approaches. Given are numbers of relative biomass resulting from existing pyrosequencing (seq.) data and quantitative (quant.) FISH, respectively. Quantitative FISH is amended with corresponding specific FA concentrations ([FA]). Comparison is specified as relative percentage of the values of the respective other approach. In case phylotypes were split in probe target subgroups A and B, the sequencing data values represent the sum of both groups.

Probe name	Seq. data (%)	Quant. FISH (%)	Specific [FA] (%)	Percent-age (%)
Bac16065_472	2.0	2.8	50	71
Bac3732_846	6.5	4.5	40	69
All3256_484	22.4	1.4	40	6
Baci731A_87	5.3	5.2	40	98
Baci731B_87		4.9	35	93
Rum14644A_184	3.6	0.4	40	11
Rum14644B_64		2.8	35	78
Rum17903_443	7.0	6.4	30	91
Bac11839_649	1.9	1.7	30	90
Bac15326_476	9.1	0.8	20	9
Bac13481_437	2.6	3.3	30	79

4.3.4 Different approaches – different results

The three different probe evaluation methods resulted in poor agreement (Table 8 and Figure 6). Using a tolerance of five percent deviant melting FA concentration, *in situ* evaluations compared to Clone-FISH and *in silico* calculations, presented similar dissociation behavior of five of nine probes, and five of 16 probes, respectively. Two of eight compliant outcomes were observed opposing Clone-FISH to *in silico* evaluations.

Table 8. Comparison of the three probe evaluation methods. The numbers represent the probes' values of melting FA concentration ($[FA]_m$). Due to the use of an unsuitable fixative (PFA) for the detection of *Lactobacillus* OTU 9057 in the *in situ* probe evaluation, the respective $[FA]_m$ value is not considered.

Probe name	*In silico* $[FA]_m$ (%)	Clone-FISH $[FA]_m$ (%)	*In situ* $[FA]_m$ (%)
LasA3720_1281	26.0	41	40
LasB3720_1281	18.8		20
Bac16065_472	44.4	64	60
Bac3732_846	43.6		54
Lab2185_87	40.4		24[a]
Lab9057_570	44.3		-
All3256_484	61.5	56	54
Baci731A_87	47.3		72[b]
Baci731B_87	42.9	48	49
Rum14644A_184	11.0		55
Rum14644B_64	53.2		51
Rum17903_443	58.4	58	42
LasA5944_580	50.0	65	55
LasB5944_580	61.6		61
Bac11839_649	45.4	57	56
Bac15326_476	32.4		30
Bac13481_437	33.8	59	45

[a]Ambiguous value due to low signal intensities even at low FA concentrations.
[b]Ambiguous value, due to unusually flat dissociation profile (Figure S1).

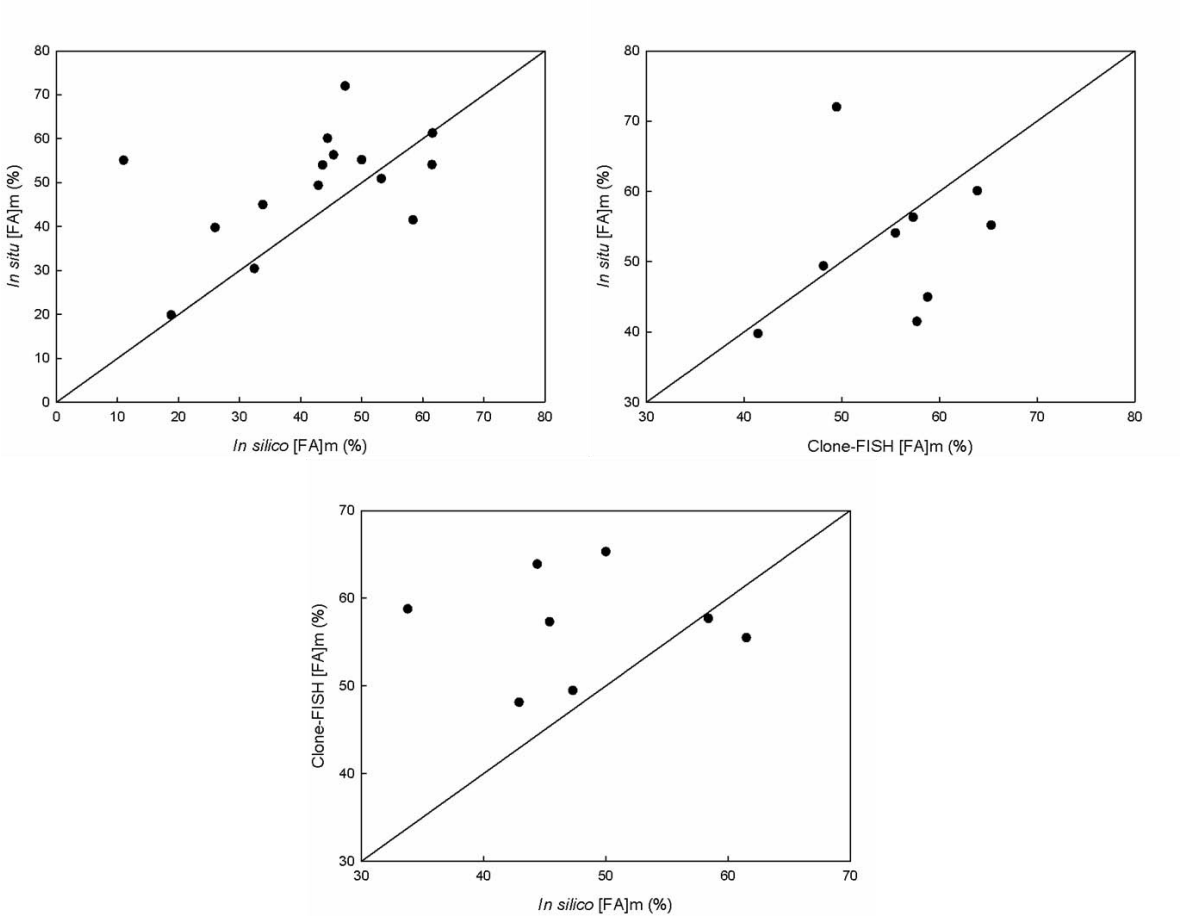

Figure 6. Different evaluation methods result in distinct outcomes. Correlation plots of melting FA concentrations ($[FA]_m$) based on dissociation profiles of the probe evaluations *in silico, in situ*, and via Clone-FISH.

4.4 Colitis-dependent abundance shifts of *Bacteroidales* populations

Newly designed probes were applied to monitor abundance shifts of three *Bacteroidales "cluster S24-7"* phylotypes throughout inflammation induction and resolution in wild type mice (Figure 7). Representative FISH images of each target are shown in Figure 8. The trial was composed of 25 days, whereby DSS-treatment started at day 1 and ended at day 5. Inflammation developed delayed and gained peak severity at day 8. At each of five time points (day 0, 5, 8, 14, and 25) four mice were sampled. The first sampling time point "day 0" depicts the healthy microbial composition of the three *Bacteroidales "cluster S24-7"* populations with OTU 13481 representing 3.5%, OTU 16065 2.1%, and OTU 15326 0.93% of the biomass. OTU 13481 was significantly higher abundant than OTU 15326 (one way analysis of variance (ANOVA) followed by the Holm-Sidak post-hoc test for pairwise comparisons, $p \leq 0.001$). Along with inflammation development, the *Bacteroidales* populations assimilated in numbers, and OTU 16065 as well as OTU 15326 obtained highly similar

concentrations in convalescence (toward day 25) as in healthy mice ("day 0"). Inflammation induction had a dramatic influence on the abundance of OTU 13481 and OTU 16065 that decreased to about 25% of their numbers in healthy mice. OTU 13481 declined significantly between day 0 and each of the sampling days 5, 8, and 14 (one way ANOVA and Holm-Sidak method, $p \leq 0.001$). Relative abundance of OTU 16065 differed significantly between day 0 and 8 ($p < 0.005$) as well as between day 8 and 22 in recovery ($p < 0.005$) (Kruskal-Wallis one way ANOVA on ranks and Tukey test). OTU 15326 in contrast, tended to decrease delayed toward day 8.

OTU 16065 was categorized as "abundant in healthy mice", whereas OTU 13481 and OTU 15326 were found abundant in all mice, however declined toward disease in this trial.

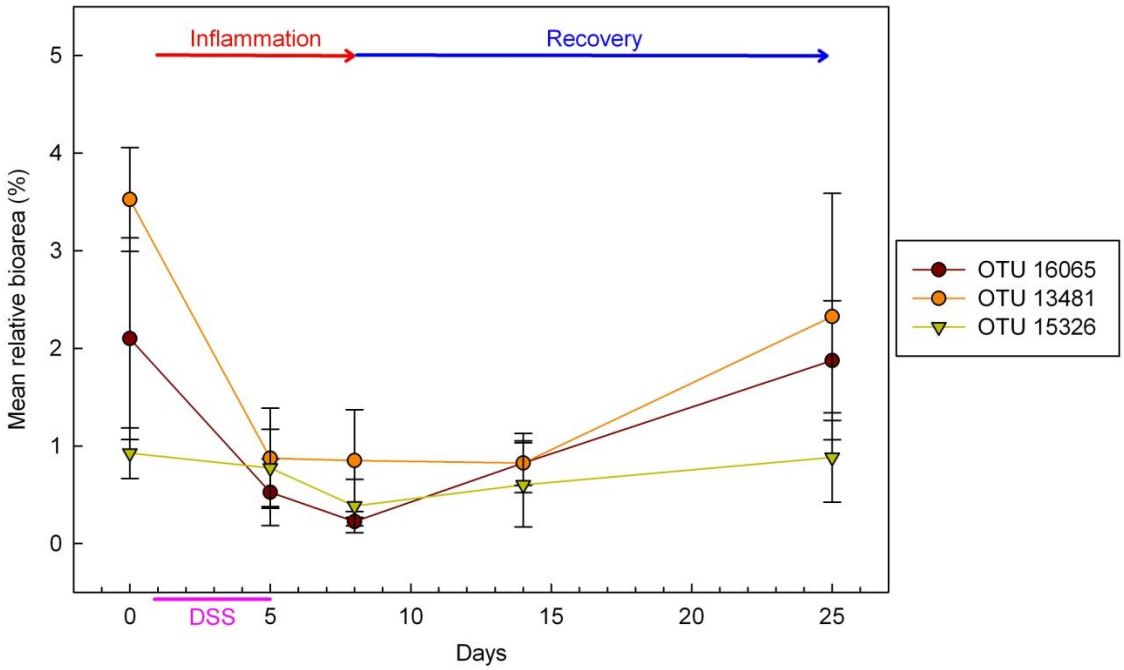

Figure 7. Abundance shifts of phylotypes in response to inflammation induction and resolution in wild type mice. Relative biomass of *Bacteroidales* "*cluster S24-7"* OTU 16065, OTU 13481 and OTU 15326 measured by quantitative FISH. DSS-treatment started at day 1 and ended at day 5 (indicated by the pink bar). The red and the blue arrow depict increase and decrease of the murine physiological symptoms, respectively.

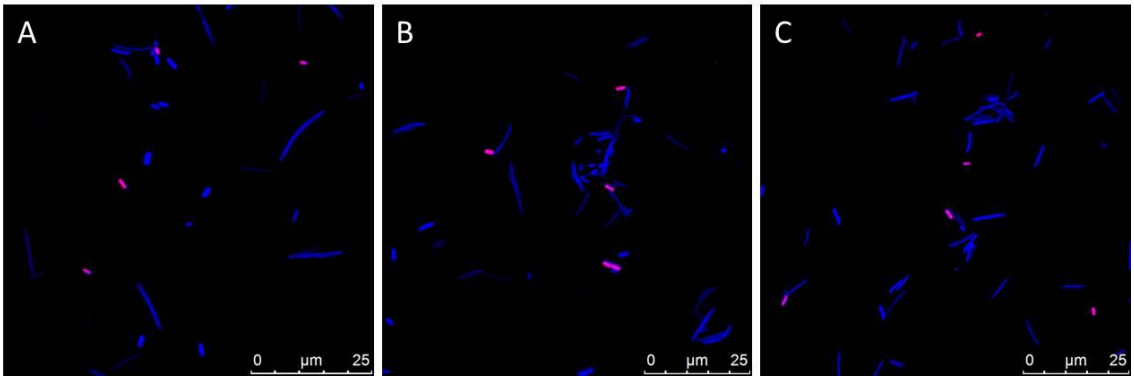

Figure 8. Representative FISH images of the three monitored *Bacteroidales "cluster S24-7"* phylotypes. The target organisms OTU 16065 (A), OTU 13481 (B) and OTU 15326 (C) are visualized in pink (Cy3) and most other bacteria in blue (FLUOS).

4.5 A multicolor FISH approach

Applicability of a multiplex FISH approach using mono-labeled probes with several fluorophores was tested. Target phylotypes of the order *Bacteroidales* were aimed to be detected using the new probes conjugated to distinct fluorophores (Table 9) its simultaneous detection was examined. In the selection of discriminable dyes, the

shortest distance of neighboring excitation and fluorescence spectra obtained 28 and 25 nm, respectively. The undetectable dyes DyLight 405 and ATTO 465 yielded smallest values of molar extinction coefficient (ε_{max}). Quantum yield (QY) of the ATTO dyes was at least four times as high as for Cy3. ATTO 665 and ATTO 465 exhibited smallest and highest decay time (DT) of 2.9 ns and 5 ns, respectively.

Table 9. Properties of fluorophores tested for parallel detection of the *Bacteroidales* targets. Information according to ATTO-TEC product catalogue 2013/2015 (www.atto-tec.com), DyLight Fluors – technology and product guide (www.piercenet.com) and www.glensearch.com.

Target organism	Probe name	Fluorophore	λ_{max} (excitation)	λ_{max} (fluoresc.)	ε_{max} [l/mol x cm]	QY (%)	DT (ns)	MW (g/mol)
Bacteroidales "cluster S24-7" OTU 3732	Bac3732_846	DyLight 405	400	423	3×10^4			793
Bacteroides OTU 731B	Baci731B_87	ATTO 465	453	508	7.5×10^4	75	5.0	396
Bacteroides OTU 731A	Baci731A_87	ATTO 514	511	533	1.15×10^5	85	3.9	868
Bacteroidales "cluster S24-7" OTU 16065	Bac16065_472	Cy3	550	570	1.5×10^5	15		767
Bacteroidales "cluster S24-7" OTU 11839	Bac11839_649	ATTO 594	601	627	1.2×10^5	85	3.9	1137
Bacteroidales "cluster S24-7" OTU 13481	Bac13481_437	ATTO 633	629	657	1.3×10^5	64	3.3	652
Bacteroidales "cluster S24-7" OTU 15326	Bac15326_476	ATTO 665	663	684	1.6×10^5	60	2.9	723

Maximum wavelength of excitation/fluorescence (λ_{max} (excitation/fluoresc.)). Molar extinction coefficient (ε_{max}).
Quantum yield (QY) in water. Decay time (DT). Molecular weight (MW).

The conditions at which all probes showed detectable signals were restricted by probe Bac15326_476, which tolerated a maximum of 20% FA. However, no specific cell signals could be detected by probes Bac15326_476 (ATTO 665), Bac3732_846 (DyLight 405), Baci731A_87 (ATTO 514) and Baci731B_87 (ATTO 465) in any of the trials. The tested variations resulted in the parallel detection of three *Bacteroidales* "*cluster S24-7*" populations by probes Bac16065_472 (Cy3), Bac11839_649 (ATTO 594), Bac13481_437 (ATTO 633) combined with EUB338mix (FLUOS) to stain most bacteria. Figure 9 shows representative images of the serial multiplex FISH approach using 30 and 20% FA conditions, on a faecal sample of a non-treated wt mouse. Signals of the fluorophore ATTO 594 showed poor overlaps with signals of Cy3 and ATTO 633, respectively. By the application of channel dye separation, a feature of the microscope software, discrimination of the channels could be improved (Figure 10). Figure 10 shows images of multiplex FISH on a sample of luminal gut content of a DSS-treated wt mouse, using 20% FA and includes probe Bac15326_476 (ATTO 665) in image series B. The microscope settings used to emit Bac15326_476 (ATTO 665) resulted in the unspecific detection of low fluorescent signals originating from all cells.

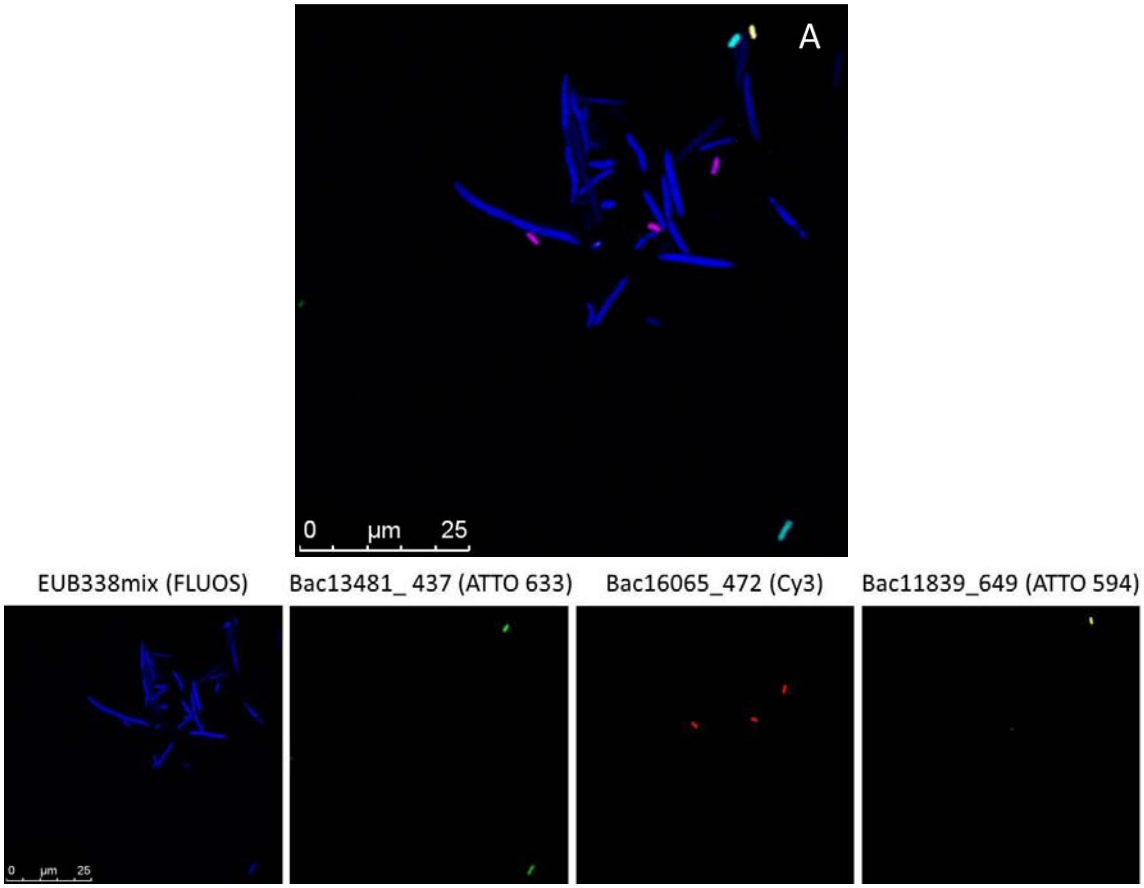

Figure 9. Representative multiplex FISH images of serial FISH showing three *Bacteroidales* "*cluster S24-7*" populations. Corresponding images of the single channels are depicted below the combined images, showing EUB338mix (blue, FLUOS), Bac13481_437 (green, ATTO 633), Bac16065_472 (red, Cy3) and Bac11839_649 (yellow, ATTO 594).

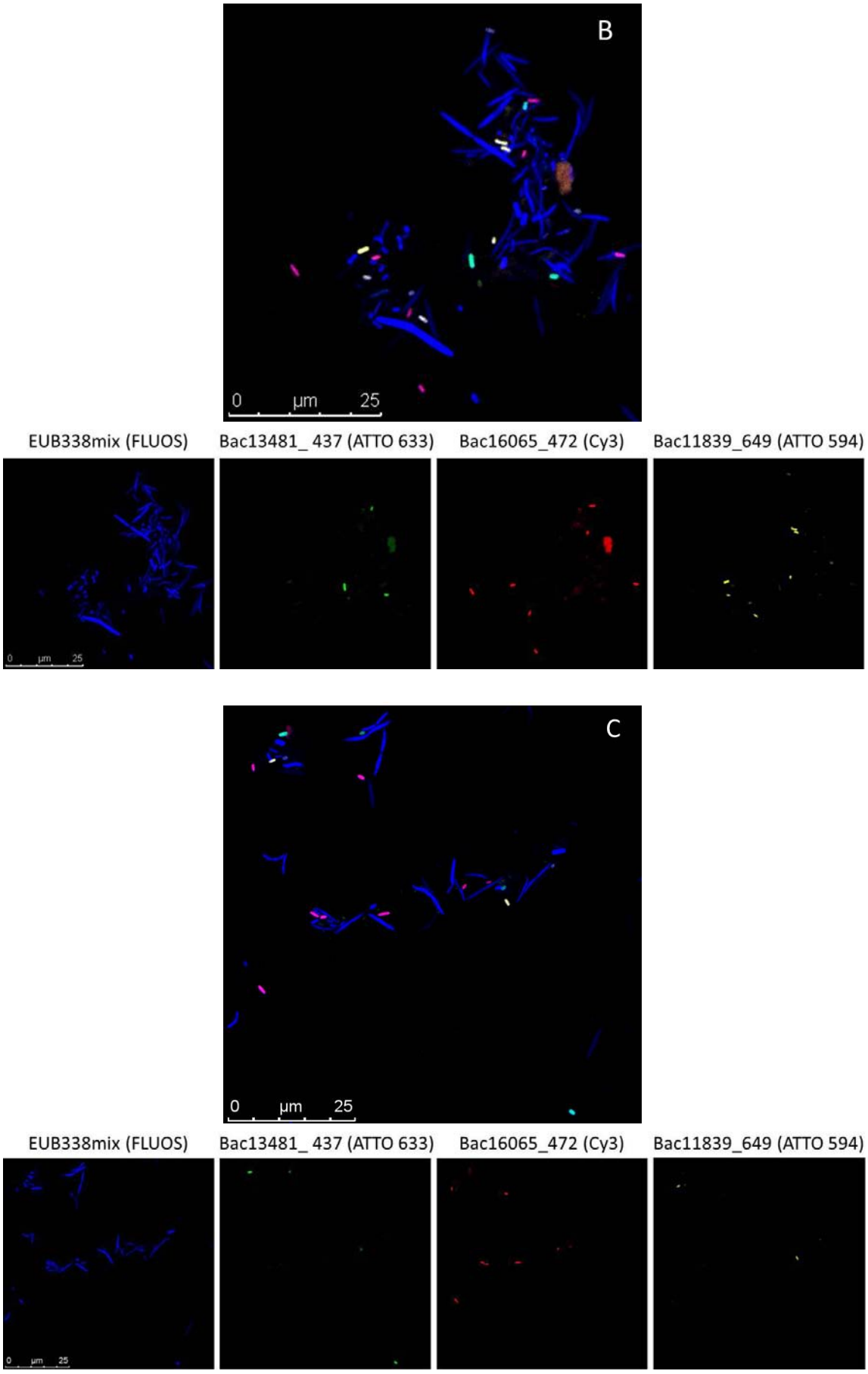

Figure 9. (continued)

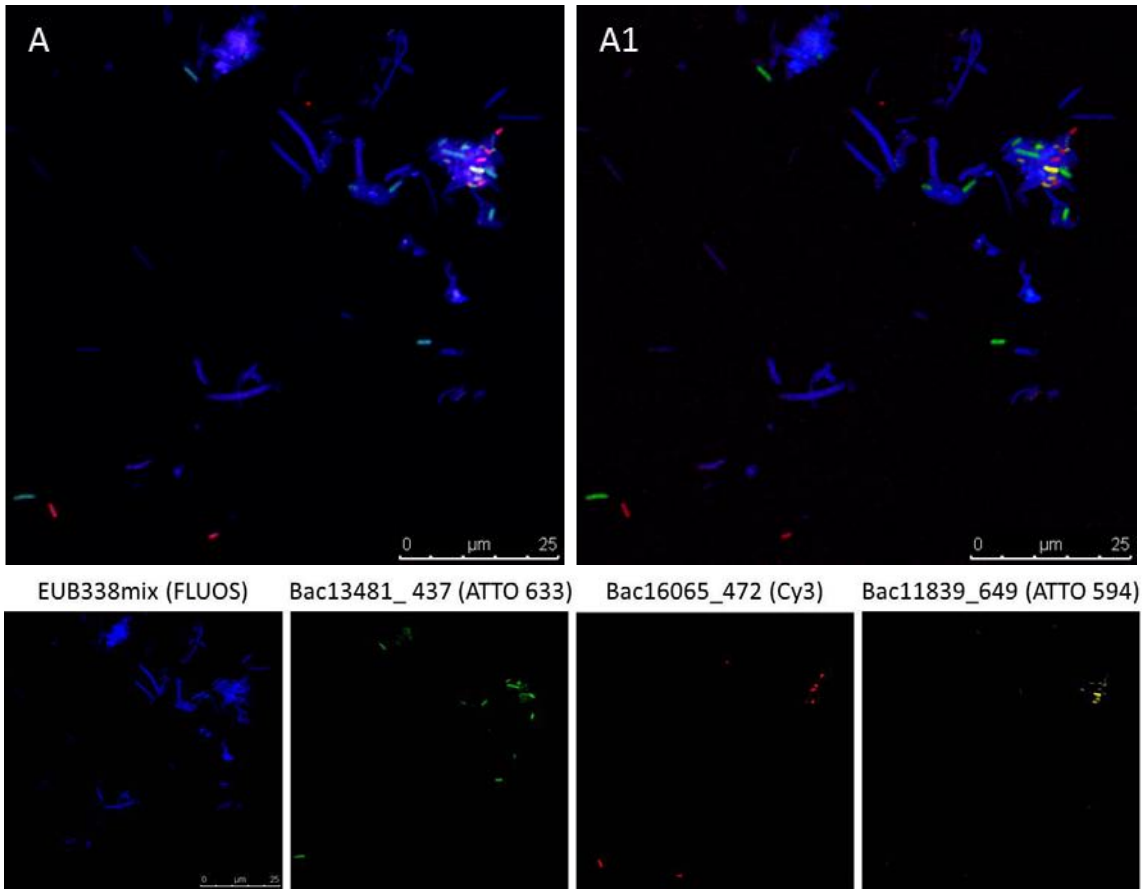

Figure 10. **Representative multiplex FISH images of *Bacteroidales* "*cluster S24-7*" populations before and after image processing.** The large combined images present unprocessed images (A and B) and images processed by channel dye separation (A1 and B1). The small images below depict single channels of probes EUB338mix (blue, FLUOS), Bac13481_ 437 (green, ATTO 633), Bac16065_472 (red, Cy3) and Bac11839_649 (yellow, ATTO 594). Image series B additionally includes the channel of probe Bac15326_476 (magenta, ATTO 665) which was not detected specifically.

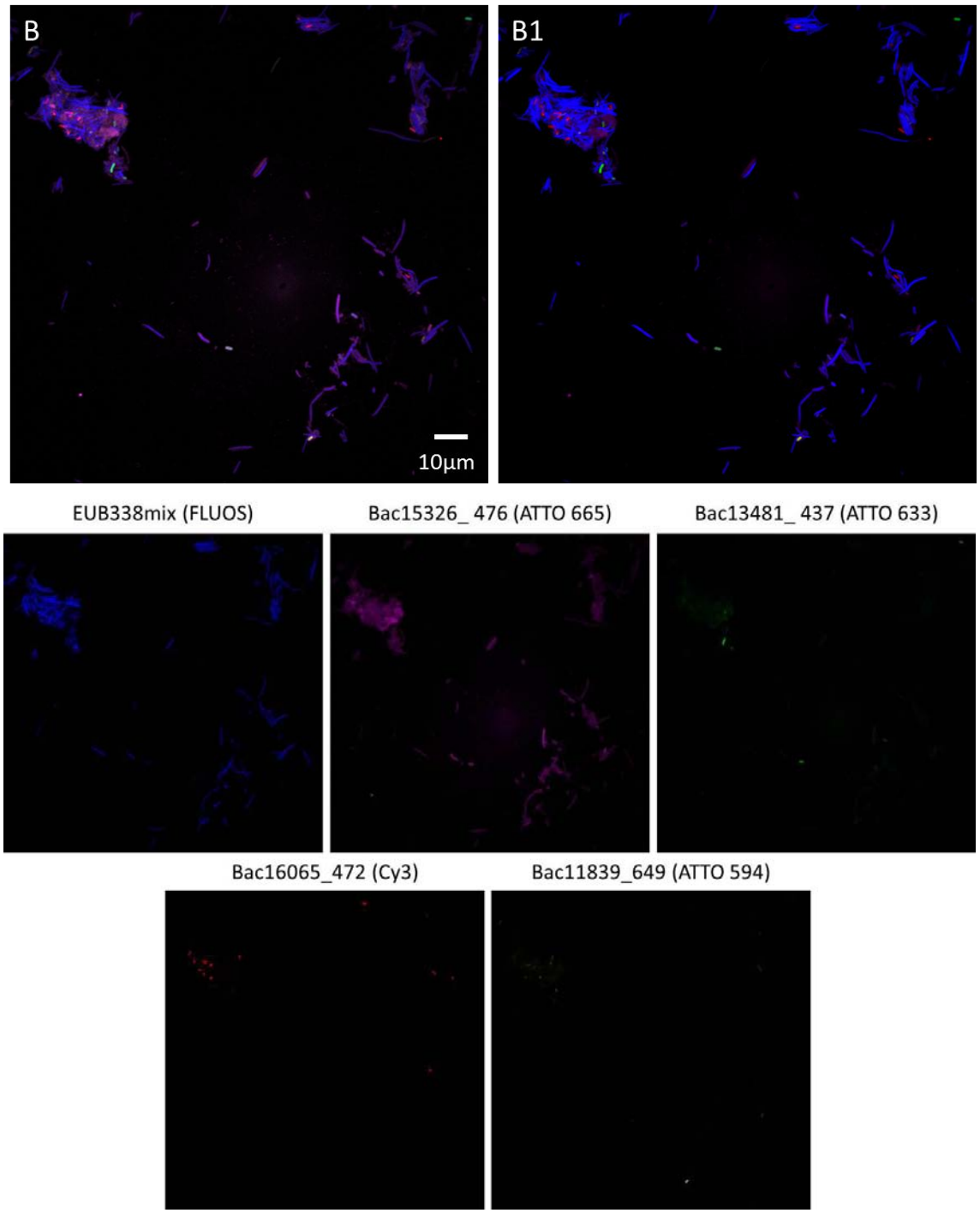

Figure 10. (continued)

4.6 Creation of a comprehensive list of probes detecting gut bacteria

A generous overview of oligonucleotide probes used for the detection of bacteria residing in the human and animal intestines was elaborated and relevant information was updated (Table SR3). The list focuses on probes specific for bacteria at the taxonomic levels of order and higher. Some probes targeting bacteria at phylum-level, which were encountered in several publications were included as well. Corresponding information of the newly designed probes of this study, which has not been included in Table 4 is given in Table 10.

To facilitate easy selection of FISH probes for gut bacteria, the online resource for rRNA-targeted oligonucleotide probes probeBase was amended with the gathered probes as well as the newly designed probes of this study. In probeBase, probes targeting gut microbiota can be found assigned to the categories “human and animal microbiota”, “intestinal microbiota”, “organisms of medical or hygienical relevance” and “organisms at higher taxonomic levels”.

Table 10. Newly designed probes analyzed using RDP II probe match. Total hits represent the sum of sequences in the database that perfectly matched the oligonucleotide. Total non-target (NT) hits show the number of perfectly matching organisms, except for target organisms. Major target and NT taxa list perfectly matching sequences of RDP in percent of the total number of sequences of the respective groups. Important information about the use of probes is given as comment.

Probe name	Total hits	Major target taxa	NT hits	Major NT taxa	FA (%)	Comment	Target organism[a]
LasA3720_1281	186	unclassified_*Lachnospiraceae* (0.7/28237)	/		35	use together with probe LasB3720_1281 (competitors)	"subgroup A" of uncl. *Lachnospiraceae* OTU
LasB3720_1281	49	unclassified_*Lachnospiraceae* (0.2/28237)	2		0	use together with probe LasA3720_1281 (competitors)	"subgroup B" of uncl. *Lachnospiraceae* OTU
Bac16065_472	197	unclassified_*Porphyromonadaceae* (0.6/3167)	178	genus *Barnesiella* (3.1/5792)	50		uncl. *Bacteroidales* "*cluster S24-7*" OTU
Bac3732_846	327	genus *Barnesiella* (5.6/5792)	1		40		uncl. *Bacteroidales* "*cluster S24-7*" OTU
Lab2185_87	1774	genus *Allobaculum* (74.3/2378)	6		40		subgroup of genus *Allobaculum*
Lab9057_570	2482	genus *Lactobacillus* (17.2/14441)	4		n.d.		*Lactobacillus taiwanensis* -*L. johnsonii-L. gasseri* group
All3256_484	365	genus *Lactobacillus* (2.5/14441)	/		n.d.		*Lactobacillus reuteri*
Baci731A_87	131	genus *Bacteroides* (0.4/34620)	1		40	use together with probe Baci731B_87 (competitors)	*Bacteroides acidifaciens* Group-2
Baci731B_87	51	genus *Bacteroides* (0.1/34620)	/		35	use together with probe Baci731A_87 (competitors)	*Bacteroides acidifaciens* Group-1
Rum14644A_184	/				40		uncl. *Oscillibacter* OTU
Rum14644B_64	5	genus *Oscillibacter* (0.2/3063)	/		35		uncl. *Oscillibacter* OTU
Rum17903_443	/				30		uncl. *Ruminococcaceae* OTU
LasA5944_580	246	unclassified_*Lachnospiraceae* (0.9/28237)	/		35	use together with probe LasB5944_580 (competitors)	"subgroup A" of uncl. *Lachnospiraceae* OTU
LasB5944_580	150	unclassified_*Lachnospiraceae* (0.5/28237)	/		35	use together with probe LasA5944_580 (competitors)	"subgroup B" of uncl. *Lachnospiraceae* OTU
Bac11839_649	/				30		uncl. *Bacteroidales* "*cluster S24-7*" OTU
Bac15326_476	374	genus *Barnesiella* (3.3/5792)	184	unclassified_*Porphyro-monadaceae* (5.8/3167)	20		uncl. *Bacteroidales* "*cluster S24-7*" OTU
Bac13481_437	214	genus *Barnesiella* (3.7/5792)	/		30		uncl. *Bacteroidales* "*cluster S24-7*" OTU

[a]Description of the target organisms refers to cluster pattern in ARB SILVA. Non-target (NT). Not determined (n.d.). Unclassified (uncl.).

5 DISCUSSION

5.1 The core gut bacteria of mice in health and colitis

The health of the intestines depends on the balanced interplay between the microbiota, the host immune system and the site they meet, the epithelial barrier (12). Disruption of the co-evolved ecosystem can lead to severe diseases, such as inflammatory bowel disease. Mouse models of DSS-induced intestinal barrier damage are valuable tools to explore the pathology of this as yet incurable disorder (22). The complexity of the habitat, however, including 60 to 80% uncultured microorganisms (32), makes elucidation challenging. Defining the microbiota in health and disease is a first step toward the identification of microbes associated with the host health-state. So-called microbial biomarkers allow a gentle method of diagnosis and treatment monitoring, hence do not have to play a causative role in disease (13). Though, studies illuminating the structure and dynamics of the murine intestinal microbiota in depth are rare. In this work, the core gut microbiota throughout mice of different genetic background was identified in high taxonomic resolution. Specific FISH oligonucleotide probes were designed and systematically optimized to detect abundant members of the non-inflamed ("healthy") or colitic murine intestine. In the healthy mouse gut, five abundant phylotypes of the two families *Lachnospiraceae* and *Lactobacillaceae* were found, as well as of a family-level group termed "*cluster S24-7"* of the order *Bacteroidales* (according to the SILVA database) (Figure 1). One phylotype of the latter group was numerically dominant in colitic mice as well. Furthermore, five phylotypes of four families, *Lachnospiraceae*, *Ruminococcaceae*, *Bacteroidaceae* and *Erysipelotrichaceae,* were enriched in diseased mice. In addition, two phylotypes of the unclassified *Bacteroidales "cluster S24-7"* fitted the criteria for being abundant in both categories with the tendency of higher numbers in healthy mice (Table 1).

The identified abundant members of the murine gut microbiota were assigned to the two phyla *Firmicutes* (Gram positive) and *Bacteroides* (Gram negative), which dominate the gastrointestinal tract of humans, mice and other rodents (31). The frequently observed abundance shifts toward higher numbers of *Enterobacteriaceae* (28, 66, 67, 36) and *Verrucomicrobiae* (28, 29) in murine colitis were not observed in all mice. However, the groups increased largely depending on mouse genotype, e.g. in the STAT1$^{-/-}$ mouse model, for details see the study of Berry and colleagues (28). It is part of the InflammoBiota project and presents the metagenomics survey of the STAT1$^{-/-}$ mouse model, which is partly also used in this study.

5.1.1 Lactobacillus

The two *Lactobacillus* target phylotypes associated with healthy mouse guts were classified to well-described human mutualistic symbionts of strong medical and industrial interest. The loss of numerically dominant health-beneficial species in disease conforms to microbial shifts in human IBD (13).

Lactobacillus OTU 2185 was identified as the heterofermentative *L. reuteri*. The symbiont is highly abundant in the murine gastrointestinal tract, where it is capable of adhering directly to the stratified squamous epithelium cells of the murine forestomach, a cell type which is missing in humans, where *L. reuteri* is autochtonous as well, though in lower numbers (68). The population structure and reduced genome size of *L. reuteri* suggest long term association and host-specific adaptation (68). *L. reuteri* is fitted well to the extreme habitat, e.g. by the ability of taking up 1,2-propanediol by the enzyme diol dehydratase, which is involved in glycerol utilization, the synthesis of the antimicrobial molecule reuterin and of vitamin B12 (68). An additional urease activity provides acid tolerance (69). The probiotic organism was observed to enhance levels of secretory immunoglobulin A (IgA), T helper cells and cytotoxic T cells, stimulate mucosal growth, decrease intestinal permeability and contribute to convalescence of enterocolitic rats (70, 71, 72).

Lactobacillus OTU 9052 was assigned to the homofermentative *Lactobacillus acidophilus* group. The isolates grouped to a cluster involving *L. taiwanensis, L. johnsonii* (formally known as *L. acidophilus* DNA similarity group B2 after Johnson (73)) and *L. gasseri* (*L. acidophilus* DNA similarity group B1) (74). These species are classified to the monophyletic *L. delbrueckii* group (75) of the heterogeneous *L. acidophilus* species group (76). Type strains *L. taiwanensis* BCRC 17755*, L. johnsonii* BCRC 17474, and *L. gasseri* BCRC 14619 share 99.5% similarity of the 16S rRNA gene sequence, but < 90% of the *gyrB* gene and show low tendency of DNA-DNA reassociation of < 45% (77). Recent genome comparisons of *L. johnsonii* and *L. gasseri* strains though, suggested closer phylogenetic relationship, because housekeeping genes shared > 94% sequence similarity (78). Hitherto, *L. johnsonii* and *L. gasseri* were found in digestive tracts of humans and animals exclusively, and lack biosynthesis of amino acids, purine nucleotides and cofactors, indicating strong dependency on the host organism and a potentially obligate symbiotic role (78). Due to beneficial modulation of the host immune system, provision of colonization resistance and epithelial cell attachment (79), the *L. acidophilus* group is ranked among the most important probiotics.

5.1.2 Clostridiales

The families *Lachnospiraceae* (also known as the *Clostridium coccoides-Eubacterium rectale* group) and *Ruminococcaceae* (*Clostridium leptum* group) and the order *Clostridiales* were observed to represent the majority of human and murine intestinal *Firmicutes*, respectively (2, 66). Many beneficial human symbionts are described among the families *Lachnospiraceae* and *Ruminococcaceae*, e.g. butyrate-producers *Roseburia intestinalis* and *Faecalibacterium prausnitzii*, respectively (2), and are observed to be depleted in IBD patients (13). Noteworthy, the short chain fatty acid butyrate is the preferred energy source of colonocytes and is proposed to protect against cancer and ulcerative colitis in consequence (80).

In this study each of the two target phylotypes of the family *Lachnospiraceae* was either abundant in healthy or colitic mice. More superficial investigation on family-level would have hidden the condition-associated shift of the organisms, which is generic for the insufficiency of lower-level analyses to examining gut community dynamics (28). The associated publication of Berry and colleagues (28) presents a metagenomics survey of STAT1$^{-/-}$ mouse model and discusses the results of the same two *Lachnospriraceae* phylotypes. Health-associated OTU 3720 and disease-associated OTU 5944 share 90% 16S rRNA gene sequence similarity with their closest cultured relatives, *Eubacterium ruminantium* and *Blautia producta* (formally known as *Ruminococcus productus*), respectively. The anaerobic butyrate-producing type strain *E. ruminantium* GA 195 was isolated from bovine rumen (81). Strictly anaerobic type strain *B. producta* ATCC 27340, was isolated from human faeces and formerly was believed to represent a dominant member of the human intestinal microbiota (82, 83). The distant relationship of the recovered *Lachnospiraceae* phylotypes to characterized strains emphasizes the high proportion of unknown commensals, which potentially play important roles.

No close relative is described for one of the two *Ruminococcaceae* phylotypes, which were enriched in colitis. The closest cultured relative of *Ruminococcaceae* OTU 17903 is *Eubacterium sireaum*, matching 93% 16S rRNA gene sequence similarity. The type strain *E. siraeum* ATCC 29066 was isolated from human faeces and described as obligate anaerobic (84). OTU 14644 clustered to *Oscillibacter*, sharing 95% (subgroup A) and 94% (subgroup B) sequence similarity to type strain *Oscillibacter valericigenes* Sjm18-20. The anaerobic valeric acid producer was isolated from the alimentary canal of a corbicula clam (85). Interestingly, valeric acid mediates chloride current by modulating the neurotransmitter receptor, γ-aminobutyric acid receptor, which plays a key role in mammalian central nervous system (86). This property is also characteristic

for related *Pseudoflavinofractor plautii* (*Clostridium orbiscindens* strain LBN 208) (87), which shared 91% 16S rRNA gene sequence identity to *O. valericigenes* as well as to *Ruinococcaceae* OTU 14644. Furthermore, morphological variations of *O. valericigenes* were similar to this study, as *Ruminococcus* OTU 14644 subgroup A appeared as short and long rods at same time (below 1 µm and about 10 µm in length) (Figure 3 and Figure S3) (85). The fact that only subgroup A showed this size variation, but subgroup B which shares 99% 16S rRNA gene sequence identity did not, demonstrates potential differences at high taxonomic resolution.

Unclassified *Clostridiales* accumulating in acute colitis were observed in wild type mice (28) and in mice of impaired immunity (67). The same shift of increasing *Ruminococcacaea* toward disease can be seen in the associated study of Berry and colleagues (28). Another study reported significant increase of unclassified *Lachnospiraceae* and OTU richness within that group, as well as a less clear decrease of unclassified *Ruminococcaceae* associated with DSS-induced colitis (29), which is contradictory to results of this work. Inconsistency in observations underlines the need for more studies that examine the intestinal microbiota in-depth including uncultured groups.

5.1.3 Erysipelotrichaceae

An uncultured phylotype of the family *Erysipelotrichaceae*, OTU 3256, was accumulated in diseased mice and was assigned to the genus *Allobaculum*. *Allobaculum* OTU 3256 shared 87% 16S rRNA gene sequence similarity to the only cultured representative of the genus, the butyrate-producing anaerobe *A. stercoricanis* DSM 13633 (type strain), which was isolated from canine faeces (88). The distant relationship of the members of this genus suggests reorganization of the current *Allobaculum* cluster.

Abundance shifts of intestinal *Allobaculum* and *Erysipelotrichaceae* members were observed in studies of different fields. Increasing numbers of an *Allobaculum* representative were observed in mice exposed to carzinogenic 1,2-dimethyl hydrazine in rats (89). Despite that, *Erysipelotrichaceae* and *Allobaculum* were associated with obesity in humans (90, 91) and high cholesterol diet in mice and hamsters, though their role in energy metabolism is still unclear (92, 93). Various conditions which lead to similar outcomes indicate that the group benefits from altered community structure, due to occupation of released niches, e.g. as a consequence of disappeared microbes, which are sensitive to these changes. However, elucidation in deep resolution might

also reveal that different members of the group play distinct roles and individual groups respond to particular environmental conditions.

5.1.4 Bacteroidales

The phylum *Bacteroidetes* of the human and murine microbiota is rather represented by members of the order *Bacteroidales*, and believed to be dominated by the genera *Bacteroides*, *Prevoltellaceae* and *Porphyromonadaceae* (2, 28, 66). Some intestinal *Bacteroidales* species were shown to share a unique ability of synthesizing manifold polysaccharides for phase variation, which multiplies heterogeneity of surface recognition structures within a population, thus might provide a way of escaping attacks (94). This niche indicates long-term adaptation to the challenging habitat and could explain the success of the group.

5.1.4.1 Bacteroides

The only representative of the family *Bacteroidaceae* accumulated in colitis and was identified as *Bacteroides acidifaciens* (OTU 731). Enrichment of *Bacteroides* toward disease is supported by other findings (66, 67, 36) and constitutes a characteristic shift in human IBD as well (13). *Bacteroides* play prominent and diverse roles in the human and mouse intestinal ecosystem, representing a heterogeneous group of obligate anaerobes of which some species elicit non-pathogenic, others pathogenic effects in humans and mice (36). The human symbiont *Bacteroides fragilis*, for instance, was shown to suppress colonization of enterocolitis-inducing *Helicobacter hepaticus* by immune modulation via the synthesis of polysaccharide A in animals (95). Enterotoxigenic *Bacteroides fragilis*, on the other hand, was associated with diarrheal diseases in humans and animals (96). Moreover, pathobiont potential of commensal *Bacteroides* isolates was reported, by induction of colitis in genetically susceptible but not in non-susceptible mice (14).

Recovered 16S rRNA gene sequences of *Bacteroides* OTU 731 assigned to *B. acidifaciens* Group-1 (*Bacteroides* OTU 731 subgroup B) and Group-2 (*Bacteroides* OTU 731 subgroup A) (62). The groups share at least 98% sequence similarity and exhibit 80% DNA-DNA homology, according to Momose and colleagues (65). Type strain *B. acidifaciens* JCM 10556 was isolated from mouse caecum, whereby its pH reductive attribute was eponymous (62). The species exhibits glucose-6-phosphate dehydrogenase and 6-phosphoglucomat dehydrogenase enzyme activities (62) and was shown to stimulate IgA production in the large murine intestine (97).

5.1.4.2 Bacteroidales "cluster S24-7"

In this work, particular attention was paid to *Bacteroidales "cluster S24-7"*, since it comprises sequences of exclusively uncultured phylotypes, mostly recovered from caecal contents and faeces of mice and other mammals. In the manner of "nomen est omen" *"cluster S24-7"* represents the largest target group of this study, involving five of 13 identified abundant murine gut bacteria, independent of category. *Bacteroidales "cluster S24-7"* consists of an astonishing number of sequences, considering that those isolates remain largely unmentioned in the literature. This study's isolates were distributed across the "*cluster S24-7*" and shared about 90% sequence similarity, at that OTU 13481 and OTU 3732 might represent one genus, showing the closest relationship of 94% similarity (Table 3). *Barnesiella species* of the family *Porphyromonadaceae* represent the closest cultured relatives of "*cluster S24-7*" members and most isolates were classified to the genus *Barnesiella* in RDP Classifier. This misclassification probably distorts the current view of microbial community structure in a way that *Porphyromonadaceae* is vastly overrepresented. Small subunit rRNA gene sequences of "*cluster S24-7*" shared about 82 to 87% similarity to the closest cultured relatives *Barnesiella intestinalis* and *Barnesiella viscerosa*. Interestingly, *Barnesiella* was associated with the reduction of vancomycin-resistant *Enterococcus faecium* of the murine microbiota and with resistance to *E. faecium* colonization in hospitalized patients (98).

Some sequences of the *Bacteroidales* "*cluster S24-7"* were recovered in a similar study investigating microbial abundance shifts in response to DSS, in which unclassified members of the *Bacteroidales* group tended to disappear (67). This trend conforms to this study, where more "*cluster S24-7"* populations with higher abundance were observed in healthy mice (Table 1). The pattern was also apparent in the correlated study of Berry and colleagues, who reported DSS-driven increase in *Bacteroidaceae* and decrease in other *Bacteroidales* in wild type and STAT1$^{-/-}$ mice (28). Furthermore, correlation of inflammation with the reduction of *Bacteroidetes* on phylum-level was reported (29).

5.2 17 new FISH probes detecting intestinal bacteria

FISH oligonucleotide probes were designed to identify 13 abundant members of the murine microbiota on phylotype-level and higher. Since the respective isolates of the two target phylotypes *Bacteroides* OTU 731 and *Ruminococcaceae* OTU 14644 clustered to different subgroups, they were split into two probe target subgroups A and

B. In order to increase group coverage of the probes detecting the *Lachnospiraceae* target phylotypes meaningfully, two additional probes were designed to detect closely related organisms of each target OTU. In sum, 17 specific FISH probes were designed and successfully applied to detect the target bacteria at maximum possible taxonomic resolution (Figure 2). Most target groups were covered by the corresponding probes to about 80% and the *Ruminococcaceae* isolates were detected individually. Due to extensive sequence heterogeneity of the *L. reuteri* (OTU 2185) cluster, the designed probe covered about 50% and no single additional probe was found to complement the probe coverage. The published probe for *L. reuteri* exhibits similar group coverage (64). Reasonable selectivity of the *L. reuteri* cluster might necessitate the design of several additional oligonucleotides.

The newly designed probes were systematically evaluated to assess common strategies for optimizing hybridization conditions of probes detecting uncultured bacteria (the three described target organisms were treated equally as the uncultured). Probes were analyzed *in silico*, via Clone-FISH and *in situ* using complex gut samples directly. *In silico* predictions of probe behavior were successfully utilized to preselect for probe candidates. Each designed probe showed appropriate signal intensity in *in situ* hybridizations, except for probes targeting *Lactobacillus*, which was a consequence of cell treatment during the fixation step (discussed below) (Figure 3). Resulting dissociation profiles of the three methods underlined the importance of laboratory experiments. The mathematical *in silico* prediction of the probes' melting FA concentrations matched poorly with the numbers of both laboratory methods (Table 8 and Figure 6). Better but still surprisingly low concordance was observed using Clone-FISH compared to *in situ* evaluations. General trends were revealed of lower FA concentration values resulting from the *in silico* evaluation and higher numbers resulting from Clone-FISH compared to *in situ* evaluations, respectively. The latter phenomenon may be explained by additional unspecific signals that are co-enumerated in hybridizations of environmental samples. Moreover, a far smaller tendency of that kind can be seen in the publication of the development of the Clone-FISH method as well, when dissociation profiles resulting from Clone-FISH were correlated with probe evaluations using pure cultures (54). Discordance may be explained by configurations of the extrinsic rRNA structure in the *E. coli* host cells, which does not conform to the native structure.

In addition to *in situ* probe evaluation, the FISH images were utilized to monitor the relative abundance of cells detected by the specific probes at increasing FA concentration (Figure S4). In theory, the amount of cell-signals declines toward specific

conditions, where they may develop a plateau and signals disappear rapidly at increasing stringency. The resulting relative abundance of each target organism was compared to existing metagenomics data (Table 7). Concordance of quantification via FISH and via 454 pyrosequencing confirms specificity of most newly designed probes. The agreement assorts with previous observations of InflammoBiota, e.g. (28). However, *Allobaculum* OTU 3256 and *Bacteroidales "cluster S24-7"* OTU 15326 were outliers with 1.4 and 0.8% relative abundance in quantitative FISH and 22.4 and 9.1% in sequencing data, respectively. The comparison constitutes below 10% agreement of the methods in these cases. One possible origin for differences is the short length of the 454 pyrosequencing reads (300 to 500 base pairs), which clearly restricts selectivity of the target organisms less than the near full length 16S rRNA gene sequences used for the design of FISH probes. In addition, the low amount of only two recovered isolates for *Bacteroidales "cluster S24-7"* OTU 15326, based on which the probe was designed, might have restricted sequence variability of targets. This applies to *Bacteroidales "cluster S24-7"* OTU 3732 as well its probe attained the third lowest agreement of the quantification approaches of 69% with 4.5% relative abundance resulting from quantitative FISH and 6.5% in metagenomics data.

Quantification experiments emphasize the importance of competitor probes, illustrated by similar cell numbers resulting from quantitative FISH of *Bacteroides* OTU 731 probes A and B applied separately. The probes differ by a single mismatch, hence should be applied simultaneously as they behave competitive. This also applies to probes targeting *Lachnospiraceae* OTU 3720 and OTU 5944 and the respective closely related organisms. Competitor probes compete for the binding position on the rRNA molecule, whereby specific probes exhibit higher affinity than probes with (weak) mismatches. The use of competitor probes is recommended to ensure specificity of probes. If weak mismatches to non-target organisms, which occur in similar habitats, are encountered during the process of probe design, competitor probes should be designed in parallel and might be applied unlabeled. To determine conditions at which unspecific probes are outcompeted, stringency of hybridization and washing steps is adapted either by addition of FA and sodium chloride or by temperature. The new probes detecting *B. acidifaciens* should be tested via dissociation profiling applying both competitive probes in parallel on a pure culture. Both probes detecting unclassified *Lachnospiraceae* were applied simultaneously on environmental samples, which resulted in very distinct specific conditions. LasA3720_1281 yielded bright signals up to 35% FA, whereas probe LasB3720_1281 does not allow addition of formamide (Figure S1).

The probes designed for both *Lactobacillus* targets could hardly be detected using PFA-fixed samples. Gram positive bacteria often have robust cell walls, which oligonucleotides cannot pass easily, and therefore need special treatment. This applied to *Lactobacillus* OTU 9057, where ethanol fixed samples clearly improved intensity of probe signals (Figure 4). In case of *Lactobacillus* OTU 2185 ethanol fixation was not effective (Figure 5), whereby the treatment could be either too gentle or too stringent. The samples used have already been stored for years which may have amplified the attacking effect of ethanol. The relatively stringent enzymatic pretreatment with proteinase K on PFA fixed samples was insufficient for increasing signals of both *Lactobacillus* groups. In another study *L. reuteri* was detected in formalin fixed biopsy sections via a treatment combination consisting of lysozyme, proteinase K and additional reagents (70). Alternative treatment methods, such as lysozyme pretreatment, should be tested to figure out conditions at that *Lactobacillus* OTU 2185 can be detected.

The applicability of ethanol instead of PFA fixed cells was tested for all probes, resulting in appropriate detection of target groups of Gram positive *Firmicutes*, but largely poor signals of Gram negative *Bacteroidetes* targets (Figure S3). Though, integrity of most cells seemed to suffer dramatically from the rough treatment, resulting in largely weak and patchy signals of the domain level probes detecting most bacteria (EUB338mix).

5.3 Bacterial health state biomarkers

Knowledge about microbial abundance shifts specifically associated with a health-state, e.g. IBD, might be exploited for diagnosis and treatment monitoring (13). The probes designed in this study facilitate detection of characteristic bacterial phylotypes recovered from colitic and healthy murine intestines by the FISH method. To demonstrate monitoring of bacterial shifts in response to conditions, three of the *Bacteroidales* "*cluster S24-7*" target phylotypes were quantified by FISH in a trial of inflammation induction and recovery of wild type mice (Figure 7).

The composition of the three *Bacteroidales* "*cluster S24-7*" populations in health and the distinct patterns in response to colitis induction and resolution suggested occupation of different ecological niches. OTU 13481 was significantly more abundant in health than OTU 15326. Parallel to disease development, the populations assimilated in numbers and diverged again in recovery. OTU 13481 seemed most sensitive to DSS-treatment as it showed fastest significant decrease that lasted over

two weeks and did not regrow to the initial density in the recovery period of three weeks. OTU 16065 was more stable when chemical treatment started, but declined significantly toward the day of most severe inflammation. The phylotype seemed more resistant, since it stabilized back to initial numbers during the time span of convalescence. The numerically smallest population OTU 15326 indicated highest resistance to DSS and the inflammatory environment. It showed delayed and non-significant decrease when colitis was most severe and regrew to similar abundance as in the healthy mice when mice convalesced.

In this trial of wild type mice, *Bacteroidales* "*cluster S24-7*" populations showed a health indicative pattern, decreasing in inflammation and accumulating in convalescence. *Bacteroidales* "*cluster S24-7*" OTU 13481 and OTU 16065 declined significantly during acute colitis. The populations proved their applicability as microbial biomarkers of DSS-induced murine colitis in this trial, thus replicate studies should verify this outcomes. OTU 13481 and OTU 15326 were categorized as "abundant in all mice", since they showed at least one percent median relative abundance in healthy and inflamed mouse guts. The criterion did not account for shifts between categories, though especially OTU 13481 depicted higher numbers in non-colitic mice according to existing metagenomics data (Table 1).

5.4 Multicolor FISH

FISH and its combination with other methods facilitate a large set of applications on single cell basis. The number of differentially detectable organisms is limited though, a constraint that can be overcome owing to developed technique of confocal laser scanning microscopy and image processing. Hitherto, approaches of multiplying the detectable target groups in complex samples were established, such as combinatorial labeling and spectral imaging (CLASI) -FISH (34). In the CLASI-FISH method each target organism is tagged with a unique fluorophore combination by using either two different probes which bind to distinct rRNA regions, or equal probes in two differently labeled versions (34). To design two probes with similar properties and behavior (specificity, efficiency, and hybridization conditions) is highly elaborate and may be impossible on the basis of the short 16S rRNA sequence (34). Targeting the 23S rRNA instead, insufficient data constitutes an obstacle. Since the probe versions compete for the same binding site, the second option suffers from low probe signal intensities and might lead to ambiguous specificity. A derivation of the approach was established to circumvent this limitation by using single probes that are double labeled (DOPE-FISH) with distinct and identical fluorophores (35).

However, the higher the number of applied fluorophores, the higher is the effort of these multiplexing approaches. Due to double labeling of probes, higher costs and image processing are required, including particular algorithms for label assignment in case of CLASI-FISH, which additionally demand the use of standards to define the fluorophore combinations. Elaborate strategies can be outpaced by exploiting contemporary technique of confocal laser scanning microscopy.

In this study, an idea of a multiplex FISH approach was taken up which facilitates combining a set of specific mono-labeled probes in a single experiment of SIMPL FISH (singly labeled probes in multiplex FISH). By exploiting the spectral capacity of the CLSM, a series of fluorophores were tested, most of which are unusual for their application in FISH. Two new fluorescent dyes proved their suitability for FISH experiments. Together with Cy3, ATTO 594 and ATTO 633 were successfully introduced to simultaneously detect three *Bacteroidales* "*cluster S24-7*" populations (OTU 16065, OTU 11839, and OTU 13481) specifically, without the need for special image processing (Figure 9 and Figure 10). In this experiment, FLUOS-labeled FISH probes were used to detect most cells, but DAPI staining may serve the same purpose. With the involvement of additional specific probes labeled with FLUOS and Cy5, it would currently be possible to detect five different bacterial populations specifically via the new SIMPL FISH approach without exploiting labeling by fluorophore combinations. This result almost corresponds to the number of detected fluorophore combinations by the alternative approach of multiplex DOPE-FISH. Several additional fluorophores are offered waiting to be tested to develop SIMPL FISH.

The method is still in its early phases and needs to be processed. Four out of seven simultaneously applied probes could not be detected. Interestingly, each of the ATTO dyes was described as exhibiting much higher values of quantum yield than Cy3 (Table 9). However, regarding the two detected ATTO fluorophores, signal intensities seemed similar to those of Cy3. This might be explained by the reliance of fluorophore properties on conditions of measurement. Quantum yield of Cy3, for instance, was shown to vary depending on the state of bonding to single and double stranded DNA (99). The reasons for difficulties in detection of the fluorophores seem to arise from different origins, since the properties of undetected molecules are similar to the detected dyes or contrary to each other. Two non-detectable fluorophores, ATTO 665 and ATTO 465 for example, display smallest and highest decay time. However, two undetected dyes DyLight 405 and ATTO 465 yielded smallest molar extinction coefficient. Problems might originate from insufficient excitation of some fluorophores, because the detectable range of the microscope was planned to be exploited too

generously. The CLSM in use exhibited a UV diode and a white light laser, and fluorescence was excitable at wavelengths 405 nm and at 470 to 670 nm, respectively. DyLight 405 and ATTO 465 could not be excited at the wavelengths of best absorption (400 nm and 453 nm (420 to 465 nm), respectively), though the use of 405 nm lasers was recommended to excite fluorophore DyLight 405. Admittedly, malfunction of the UV channel at the time of microscopy cannot be excluded. However, signals could neither be detected through the microscope oculars, though emission of DyLight 405 is visible (λ_{max} 420 nm). Excitation and detection of fluorophore ATTO 665 reside in the far red light spectrum, close to the maximum possible excitation wavelength. Interestingly, the molecule seemed to adhere to each cell (Figure 10). Fluorophore ATTO 514 could not be observed for unclear reasons. ATTO 514 is described as suitable for FISH and already proved its functionality in our laboratory. This indicates that the problem originated from combining the fluorophore with other fluorescent molecules, or with the oligonucleotide. Solubility and pH are additional influences to consider. According to the datasheet ATTO 665 is only moderately hydrophilic, but so is ATTO 633, which could be detected. Although ATTO 665 seemed to dissolve well in water, tiny clumps of the dye molecules cannot be excluded. Each fluorophore was described as suitable for DNA and RNA hybridizations and as largely pH tolerant, beside ATTO 465, for which no information on pH dependency was stated. Validation of the problematic probe solutions is needed with regard to proper binding of the fluorophores to the oligonucleotides, solubility and pH.

The three specific probes successfully detected in the SIMPL FISH trial showed some overlapping signals. In the detection range of ATTO 594, signals of both neighboring fluorophores (Cy3 and ATTO 633) were faintly visible. Unspecific binding of the probes can be excluded, since *Bacteroidales* "*cluster S24-7*" OTU 16065 and OTU 13481 share low 16S rRNA gene sequence similarity of 89.2 to 89.8% to OTU 11839 (Table 3) and the probes exhibit several mismatches to the other target organisms' binding positions. Consequently, ATTO 594 signals "bled" into the detection range of the neighboring channels. Much more intense specific signals could be discriminated unambiguously, either by reducing background or with the help of function "channel dye separation" of the microscope software. The function represents relative proportions of distinct channels contributing to a signal of an object, based on that the channels can be readjusted manually. Dealing with gut microbes, which exhibit high ribosomal contents due to permanent feeding on the host and its nutrition, manual correction of the channels was unambiguous. However, investigating oligotrophic environments, spectral "bleeding" of distinct fluorophores as well as unspecific signals

could pose an obstacle that might be circumvented by serial hybridizations using descending FA concentrations or by the design of unlabeled competitor probes.

Some general concerns of multiplex FISH experiments should be noted at that point. First, complex environmental samples mostly contain microbes with different cell wall structures (e.g. Gram negative and Gram positive *Bacteria* and *Archaea*) its cell fixation and permeabilization for FISH necessitates different treatments. Performing a multiplex FISH experiment, one has to either compromise on the set of microorganisms for parallel detection, or essay modified fixation protocols. Besides usual fixation protocols (using PFA for Gram negative and ethanol for Gram positive bacteria) with altered incubation times, strategies have been established, in which PFA is combined with lysozyme using succinct incubations times (100). The second limitation is based on different probe behavior. When performing a perfect multiplex FISH experiment, each introduced oligonucleotide probe should bind its target organism specifically under related hybridization conditions. Otherwise, unspecific and ambiguous signals might appear which cannot be assigned correctly.

Most of the newly designed probes detecting *Bacteroidales* bound specifically at hybridization conditions of 30% FA, except for probes Bac16065_472 and Bac15326_476, which allowed up to 50% FA and 20% FA, respectively. Quantitative FISH of the series of increasing FA concentration proved to be a meaningful tool for determining specificity of probes at particular conditions. Probe Bac16065_472 showed similar probe selectivity at conditions of 25 to 50% FA (Figure S4), thus could be applied at 30% FA in the SIMPL FISH experiment without the risk of unspecific signals. However, at conditions of 20% FA, which was applied to include detection of probe Bac15326_476, specificity of the other probes was probably limited.

By detecting more organisms at the same time, a well-established SIMPL FISH method multiplies the spectrum of applications of the valuable identification method, besides time, labor and cost saving benefits. Application of the FISH method for relative cell quantification proved to be a useful tool. 2D and 3D spatial image analysis provide important structural and functional insights into complex communities, e.g. examining intestinal tissue sections. Functional analyses can be facilitated by combining the identification method with spectroscopy techniques on single cell level, such as Raman spectroscopy and nano secondary ion mass spectrometry (nanoSIMS). FISH-nanoSIMS was successfully introduced recently for tracing back bacterial uptake of host mucosal compounds by measuring stable isotopes (101). By combining these methods complex ecological questions can be raised to shed light on complex environments, such as the additional organ gut microbiota.

5.5 The first comprehensive oligonucleotide probe list for gut bacteria

Another obstacle when planning a FISH experiment is the lack of updated overviews of published oligonucleotide probes dedicated to specific fields of study. For the first time a generous overview of published probes for the detection of intestinal bacteria of humans and higher animals is provided (Table SR3). Relevant information was gathered, such as the updated status of target group specificity and coverage of target groups as well as perfect matches to non-target taxa. All probes can be found at the online resource for rRNA-targeted oligonucleotide probes "probeBase" (www.microbial-ecology.net/probebase) to facilitate quick and easy selection.

6 CONCLUSION

The mammalian gut is recognized as an essential ecosystem its impact stretches beyond conditions concerning the organ itself. Unfortunately, the knowledge about the highly complex ongoing functions, driven by host factors, especially the immune system in interaction with the co-evolved intestinal microbiota, is dramatically limited. As a consequence severe disorders, such as increasing prevalent inflammatory bowel diseases, remain opaque and hence incurable. The main challenge demonstrates the diversity of the microbiota, which is largely uncharacterized in reasonably high resolution (28). Microbes associated with the host health state, so-called microbial biomarkers, have the potential to provide a non-invasive means for diagnosis and treatment monitoring (13). In this study, abundant bacterial members of the murine gut in an inflamed and non-inflamed state, irrespective of the host genotype, are identified at phylotype level (≥ 97% 16S rRNA gene sequence similarity). The 13 newly designed and optimized oligonucleotide probes proved to enable their specific identification and treatment monitoring by (quantitative) FISH. The development of the trusted cultivation-independent method toward a more contemporary approach of multicolor FISH was taken up, aiming to detect several mono-labeled probes in parallel. The vision of SIMPL FISH (singly labeled probes in multiplex FISH) opens the door to a next generation of cultivation-independent studies on single cell basis.

7 ZUSAMMENFASSUNG

Die Ursachen für chronisch entzündliche Darmerkrankungen, wie Morbus Crohn und Colitis ulcerosa, sind weitgehend ungeklärt. Neben genetischen Veranlagungen werden Veränderungen in der Zusammensetzung der Darm-Mikrobiota mit den Erkrankungen in Verbindung gebracht. Dabei kann ein Verlust an potentiell gesundheitsfördernden Mikroorganismen beobachtet werden, während die Anzahl an opportunistischen Pathogenen steigt. Fluoreszenz *in situ* Hybridisierung (FISH) stellt eine ideale Methode dar um die Häufigkeit derjenigen Mikroorganismen zu bestimmen, die als „intestinale mikrobielle Biomarker" Aufschluss über den Gesundheitszustand geben können. Ziel dieser Studie war Veränderungen in der Häufigkeit bestimmter mikrobieller Indikator-Spezies in verschiedenen Maus Entzündungsmodellen zu untersuchen. Hierfür wurden neue 16S rRNA-gerichtete FISH-Sonden für den spezifischen Nachweis von 13 abundanten, zum Teil unkultivierten Bakterienarten der Darm-Mikrobiota von Mäusen entwickelt. Die Sonden wurden unter Verwendung mehrerer Methoden systematisch evaluiert. Anschließend wurden drei dieser neu entwickelten Sonden exemplarisch eingesetzt um Veränderungen in der relativen Häufigkeit unkultivierter Arten der Ordnung *Bacteroidales* über den Verlauf einer Kolitis – von Induktion bis Auflösung der Entzündung – zu bestimmen. Des Weiteren wurden durch den erfolgreichen Einsatz zweier für FISH untypischer Fluorophore erste Erfolge in Richtung der Entwicklung eines neuen multiplex FISH Ansatzes erzielt. Abschließend wurde eine umfassende Liste von bisher publizierten FISH-Sonden für den Nachweis der intestinalen Mikrobiota von Mensch und Tier zusammengestellt und in der Internet-Datenbank probeBase veröffentlicht.

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9 SUPPLEMENTARY INFORMATION

9.1 Supplementary materials and methods

9.1.1 Equipment and software

Table S1. Equipment and software.

Equipment	Software	Company
MILLI-Q Biocel water purification system		EMD Millipore
Confocal laser scanning microscope LSM 510 META	LSM 510 (3.2)	Carl Zeiss AG
Leica LSM SP8	LAS AF (3.1)	Leica Microsystems
NanoDrop ND-1000 (UV-visible spectrophotometer)	ND-1000 (3.2)	Thermo Fisher Scientific Inc.
UST-C30M-8R (UV transilluminator)	Argus X1 (4.1)	Biostep GmbH
Sub-Cell GT (agarose gel electrophoresis system)		Bio-Rad Laboratories
Sub-Cell GT UV-Transparent Gel Tray		Bio-Rad Laboratories
PowerPac Basic Power supply		Bio-Rad Laboratories
Vortex Genie 2		Carl Roth GmbH
M107 High Specification (visible spectrophotometer)		Spectronic Camspec Ltd.
UV Sterilising PCR Workstation		PEQLAB Biotechnolgie GmbH
Shaker Thermo Twister Comfort		QUANTIFOIL
inoLab® pH Level 1 (pH meter)		WTW Wissenschaftlich-Technische Werkstätten GmbH
Microcentrifuge 5804 R		Eppendorf AG
Hybridisation oven UE-500		Memmert GmbH
Eppendorf research pipettes 1 – 1000		Eppendorf AG
iCycler™ (thermal cycler)		Bio-Rad Laboratories
Micropulser™ (electroporation apparatus)		Bio-Rad Laboratories
PowerPac Basic (universal electrophoresis power supply)		Bio-Rad Laboratories
SmartSpec™ 3000 (visible spectrophotometer)		Bio-Rad Laboratories
Sub-Cell GT (agarose gelelectrophoresis system)		Bio-Rad Laboratories
Sub-Cell GT UV-Transparent Gel Tray		Bio-Rad Laboratories
5804 R (microcentrifuge)		Eppendorf AG
Incubation/Inactivation Water Bath 1004		GFL Gesellschaft für Labortechnik mbH
Mikro 20 (microcentrifuge)		Hettich Lab Technology
LaminAir Model 1.2 (laminar flow hood)		JOUAN Nordic A/S
Innova™ 2300 platform shaker		New Brunswick Scientific
C-5050 Zoom (digital camera, used with UV transilluminator)		Olympus Corporation
UV Sterilising PCR Workstation		PEQLAB Biotechnology GmbH
M107 High Specification (visible spectrophotometer)		Camspec

9.1.2 Media, buffers and solutions

Table S2. LB medium. For solid medium (plates) agar was added.

Chemical	Concentration (g/L)
Tryptone	10
Yeast extract	5
Natrium Chloride (NaCl)	5
(Agar)	(5)
Ultrapure water	

Table S3. dYT medium.

Chemical	Concentration (g/L)
Tryptone	16
Yeast extract	10
NaCl	5
Ultrapure water	

Table S4. Phosphate-buffered saline (PBS) stock solution (pH 7.2 – 7.4).

Chemical	Concentration (mmol/L)
NaH_2PO_4	200
Na_2HPO_4	200

Table S5. 1x PBS.

Chemical	Concentration (mmol/L)
PBS stock solution	10
NaCl	130
Ultrapure water	

Table S6. 10x TBE buffer used for agarose gel electrophoresis.

Chemical	Concentration (mmol/L)
Tris	890
Boric acid	890
Ethylenediaminetetraacetic acid (EDTA)	20
Ultrapure water	

9.1.3 Agarose gel electrophoresis and polymerase chain reactions

1.5% agarose gels were made using LE Agarose and 1x TBE buffer. After electrophoresis gels were stained by incubation in an ethidium bromide waterbath (100 µL / L) and images were taken using an UV transilluminator.

Table S7. Reagents and corresponding amounts used for PCR.

Reagent	Amount (µL)
Ultrapure water	33.55
10x *Taq* Buffer (Fermentas)	5
$MgCl_2$ (25mmol / L) (Fermentas)	4
dNTP Mix (2mmol / L) (Fermentas)	4
Forward primer (50pmol / µL) (Fermentas)	1
Reverse primer (50pmol / µL) (Fermentas)	1
Bovine serum albumin (20mg / mL) (Fermentas)	0.25
Taq polymerase (recombinant, 5u / µL) (Fermentas)	0.2
Template DNA / (single E. coli colony)	1
Total reaction amount	50

Table S8. PCR cycle and corresponding temperature, repeats and time used for PCR with general bacterial primers 8F and 1492R.

Cycle	Temperature (° C)	Repeats	Timespan (min)
Prime denaturation	94	1x	03:00
Denaturation	94	25x	01:00
Annealing	60	25x	01:00
Elongation	72	25x	02:00
Final elongation	72	1x	03:00

Table S9. PCR cycles and corresponding temperature, repeats and time used for PCR with M13 primers:

Cycle	Temperature (° C)	Repeats	Timespan (min)
Prime denaturation	94	1x	07:00
Denaturation	94	35x	00:30
Annealing	60	35x	00:30
Elongation	72	35x	01:30
Final elongation	72	1x	10:00

9.1.4 Preperation of electrocompetent E. coli JM109(DE3) cells

(according to an established protocol in our laboratory)

750mL dYT medium was inoculated with an *E. coli* JM109(DE3) overnight LB culture to a final concentration of 1 L and incubated at 37° C on a shaker. At an OD_{600} of 0.5 to 0.7, cells were cooled down at 4° C for 15 min. All following steps were performed at 4° C with cooled solutions and equipment. The culture was filled in 50 mL centrifugation tubes (Greiner) and harvested by centrifugation at 6236 g for 15 min. After washing the cell pellets with ultrapure water, another centrifugation step followed as described above. Cells were washed with 10% glycerol and centrifuged a third time. Finally, cell pellets were resuspended with 1 to 2 mL 10% glycerol and 100 µl aliquots were stored at − 80° C until use.

9.2 Supplementary Results

9.2.1 Supplementary Figures

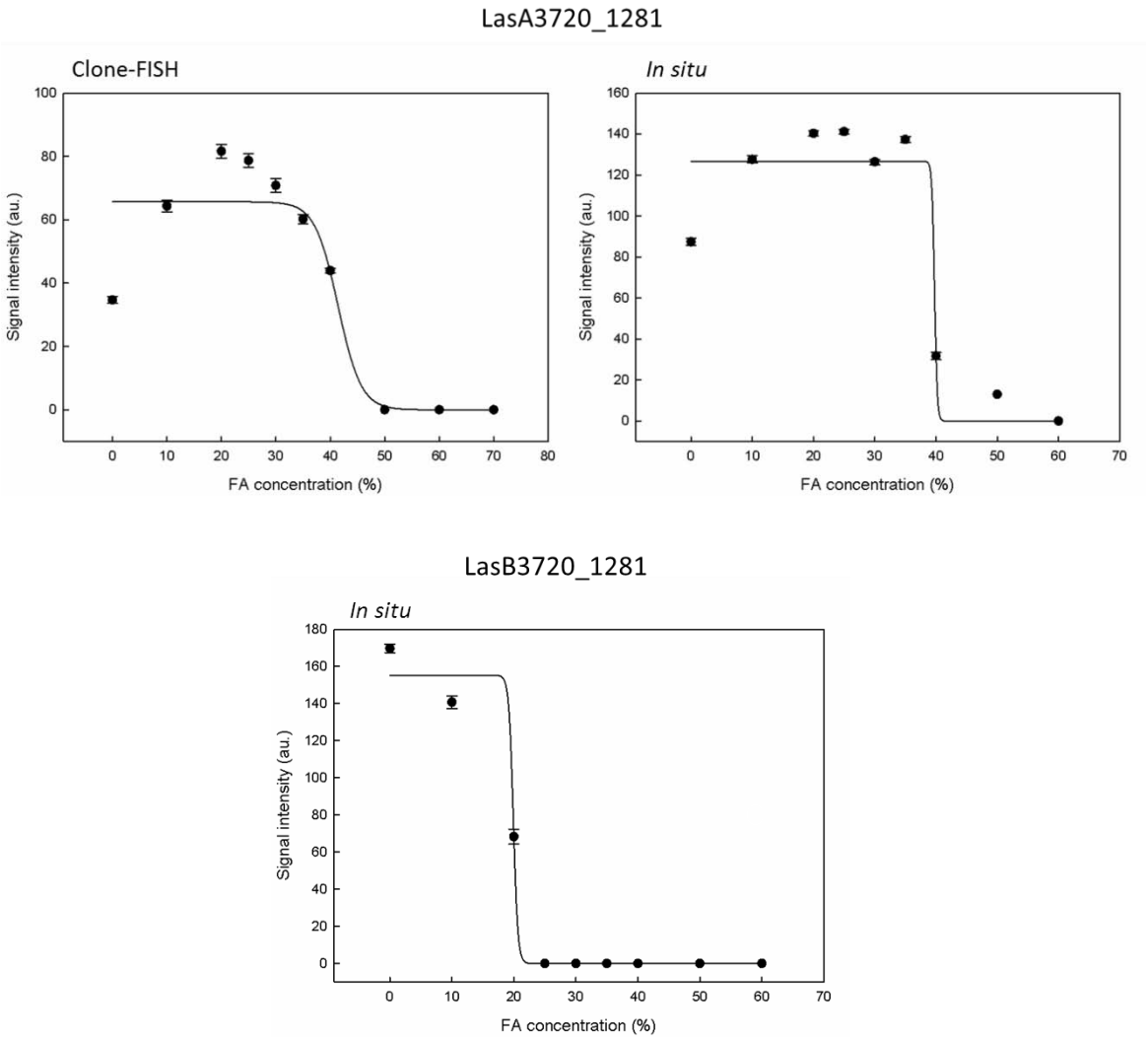


Figure S1. Formamide dissociation profiles of the newly designed probes. Signal intensities developing over increasing FA concentrations are depicted, using Clone-FISH and environmental samples (*in situ* evaluation).

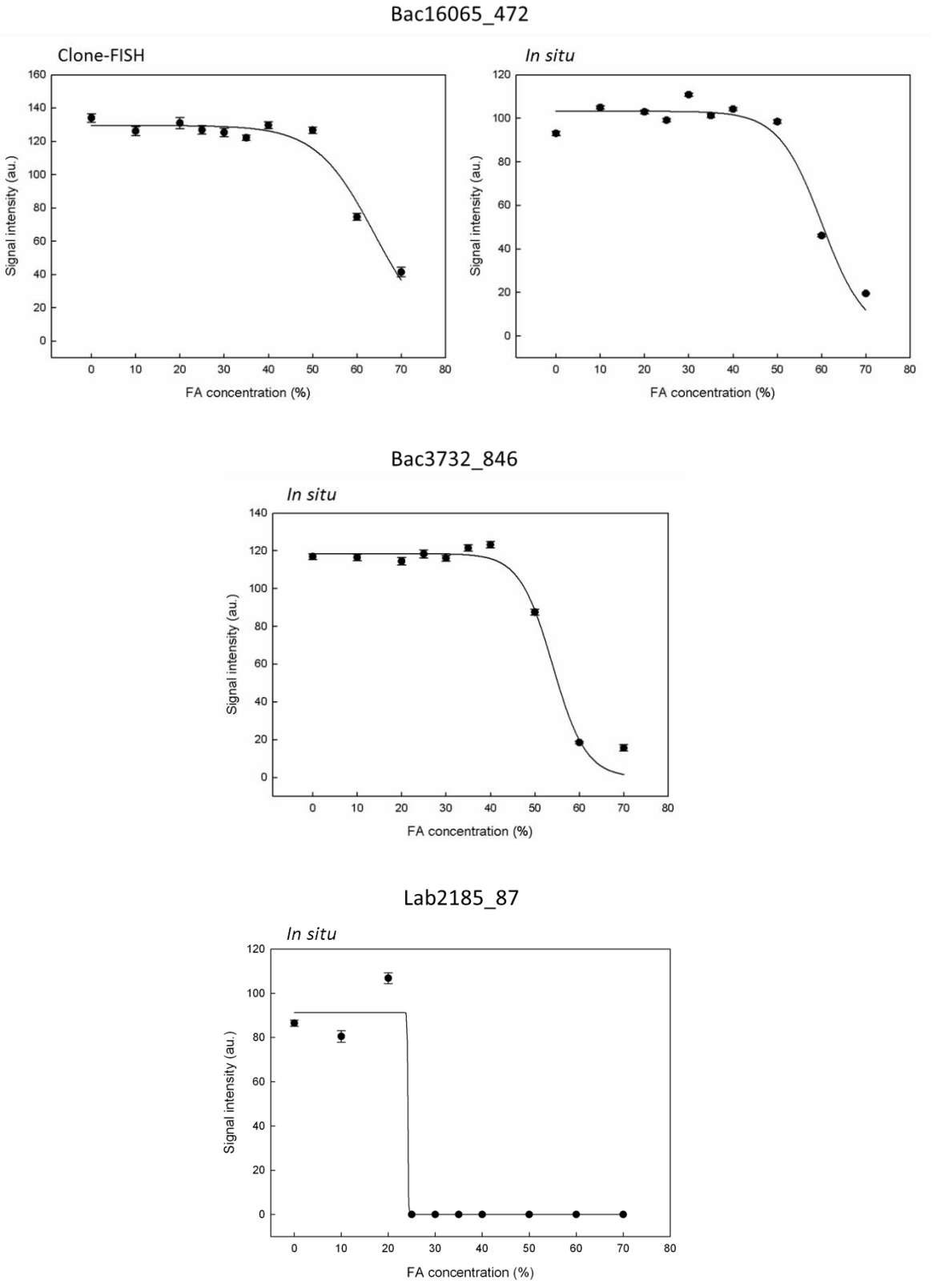
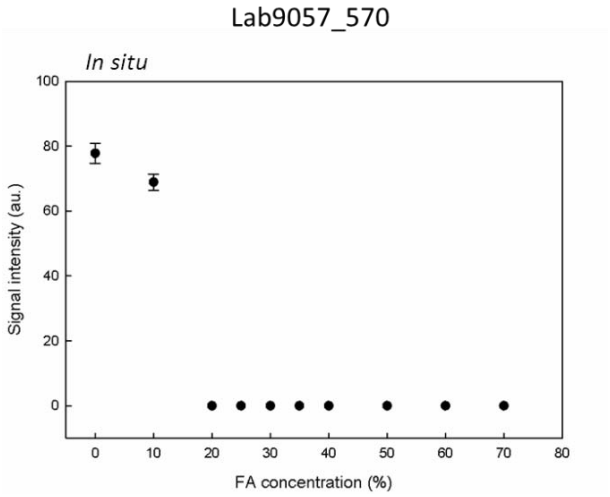
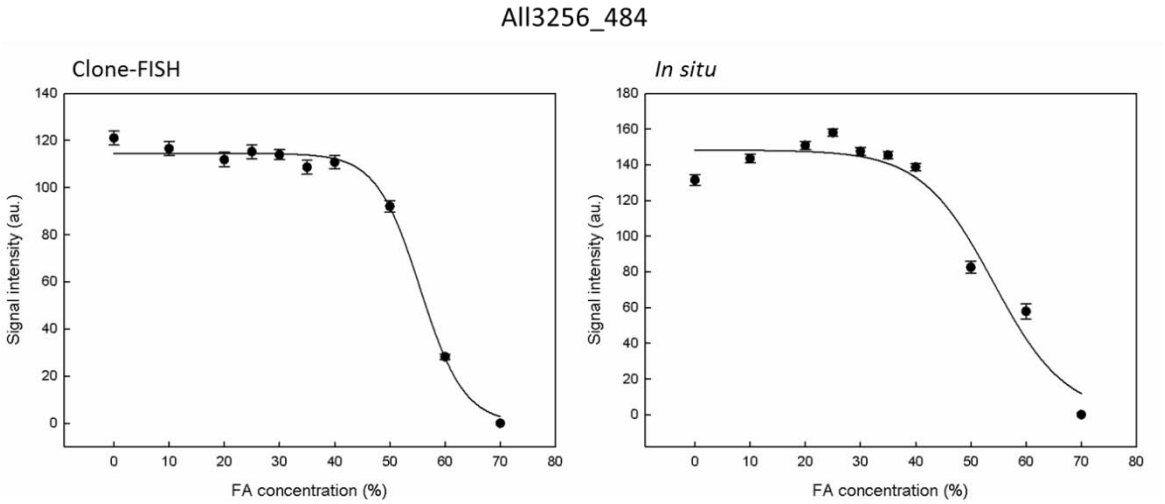


Figure S1. (continued)





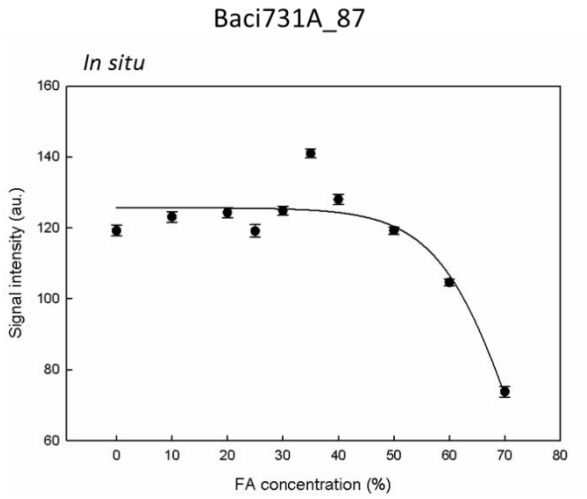


Figure S1. (continued)

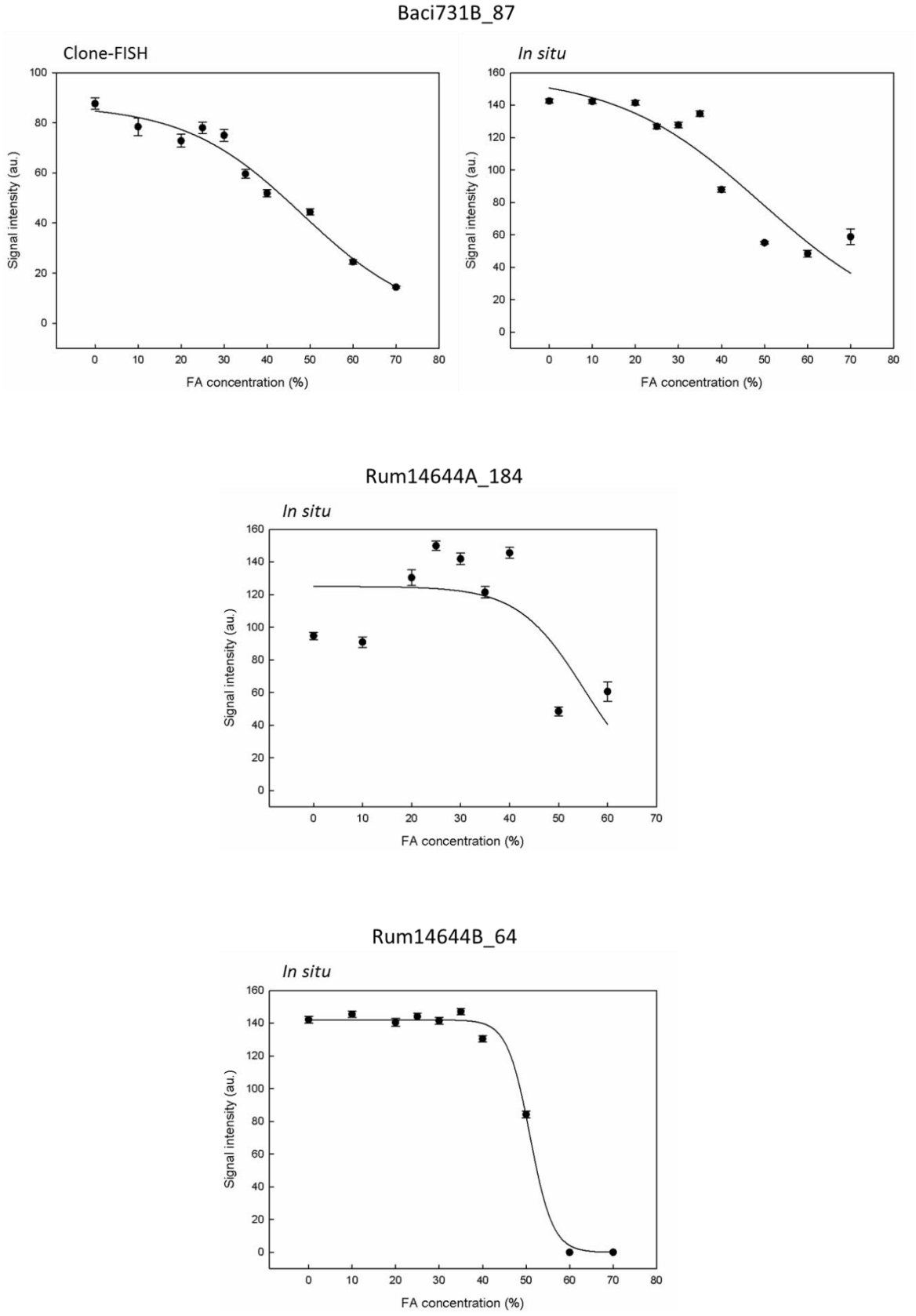


Figure S1. (continued)

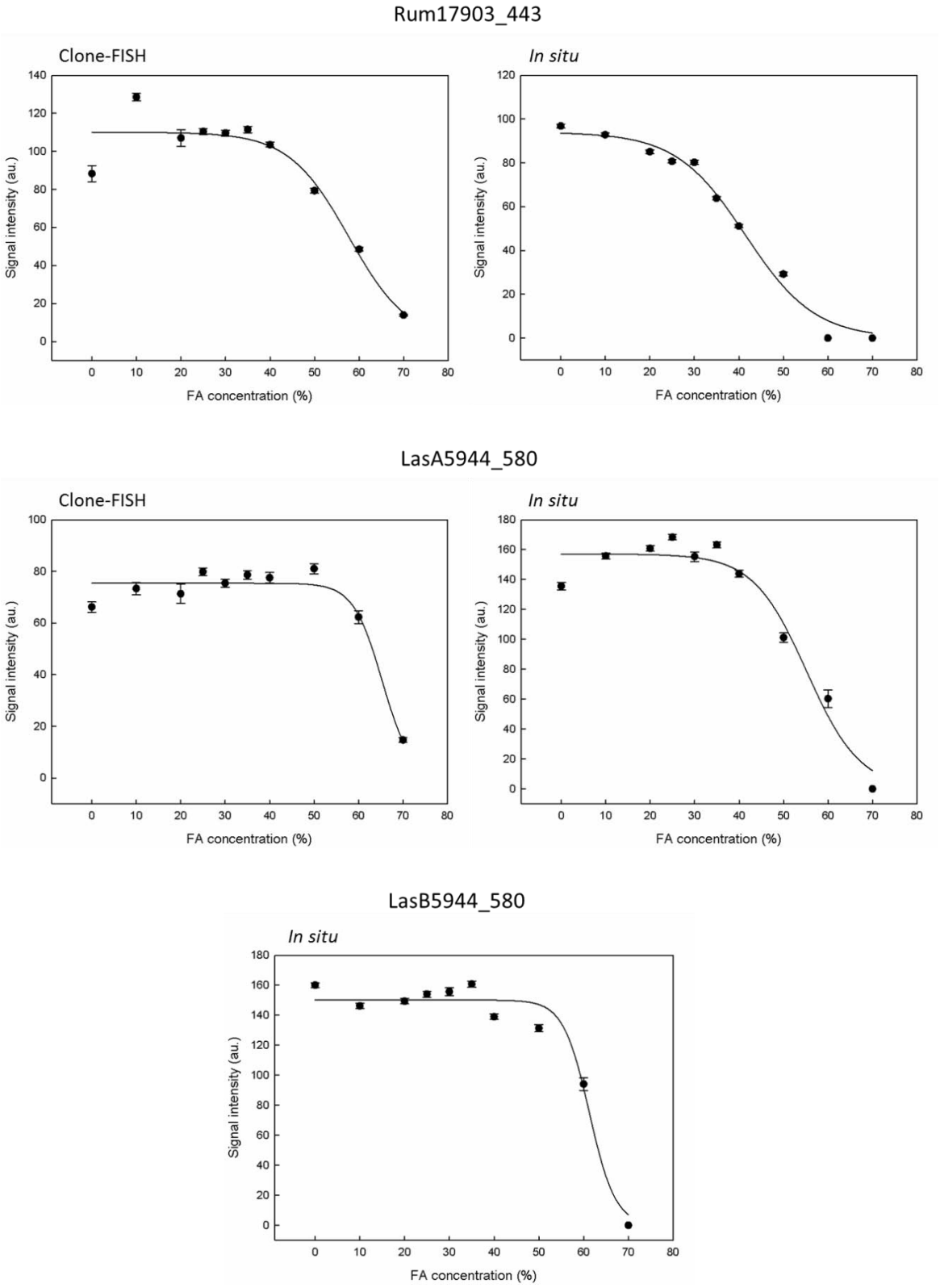


Figure S1. (continued)

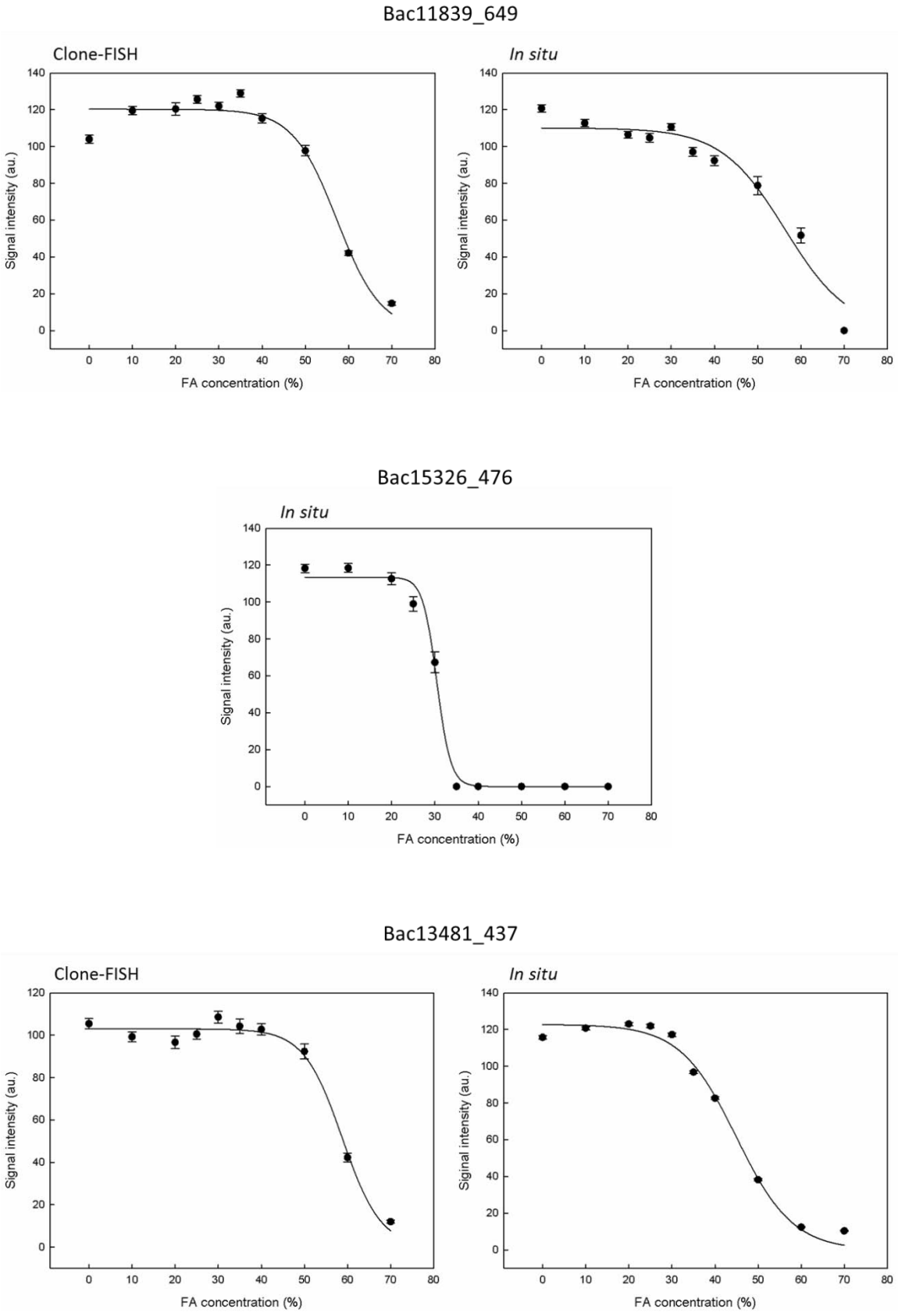


Figure S1. (continued)

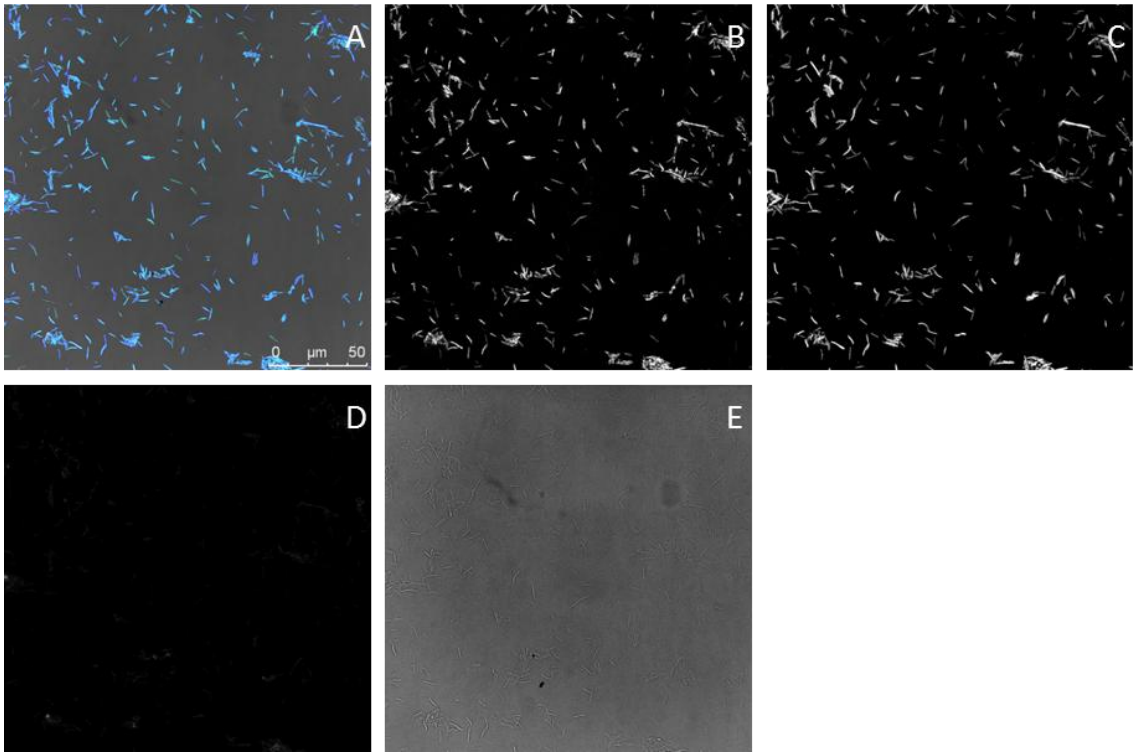


Figure S2. Positive and negative controls of FISH using PFA-fixed mouse gut samples. The first overlay image shows the DAPI stain in blue, FISH with EUB338mix (Cy5) in cyan, NONEUB (Cy3) in red and a bright field image. The monochromatic mages A, B, C and D show the single channels in this order.

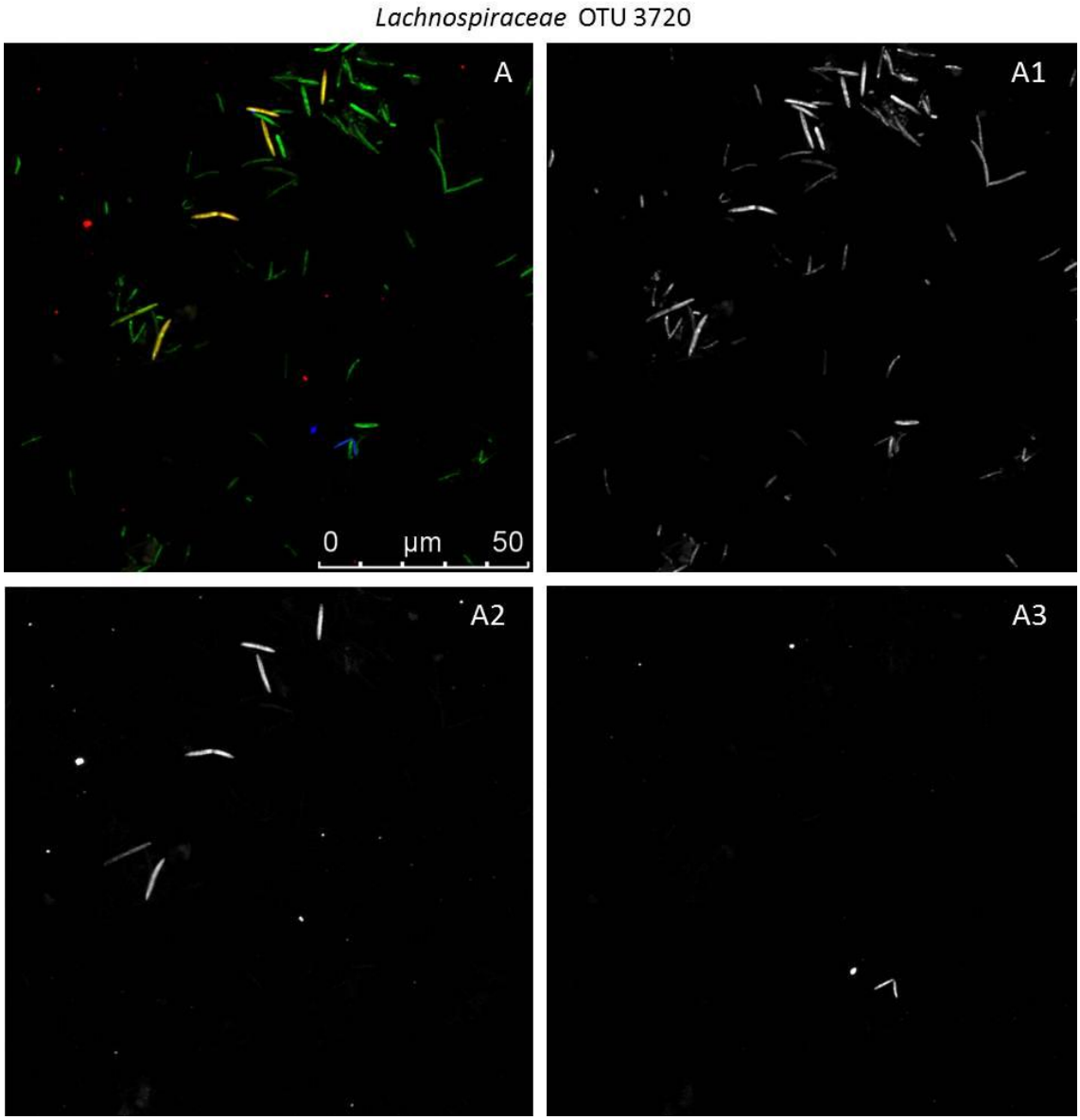


Figure S3. Applicability of the designed probes on ethanol-fixed mouse gut samples. Images show specific target bacteria in warm colors (Cy3, red, orange or yellow) with the single channel shown in the corresponding images "2" and all other bacteria in green (FLUOS) with the single channel shown in the corresponding images "1". In images of *Lachnospiraceae* OTUs (A and B), additionally the corresponding closely related *Lachnospiraceae* are shown in blue (Cy5), with the single channels given in corresponding images 3. No signals were detected for *Bacteroidales "cluster S24-7"* OTU 3732 and OTU 15326 (not shown) and the *Lactobacillus* phylotypes are given in Figure 4 and Figure 5.

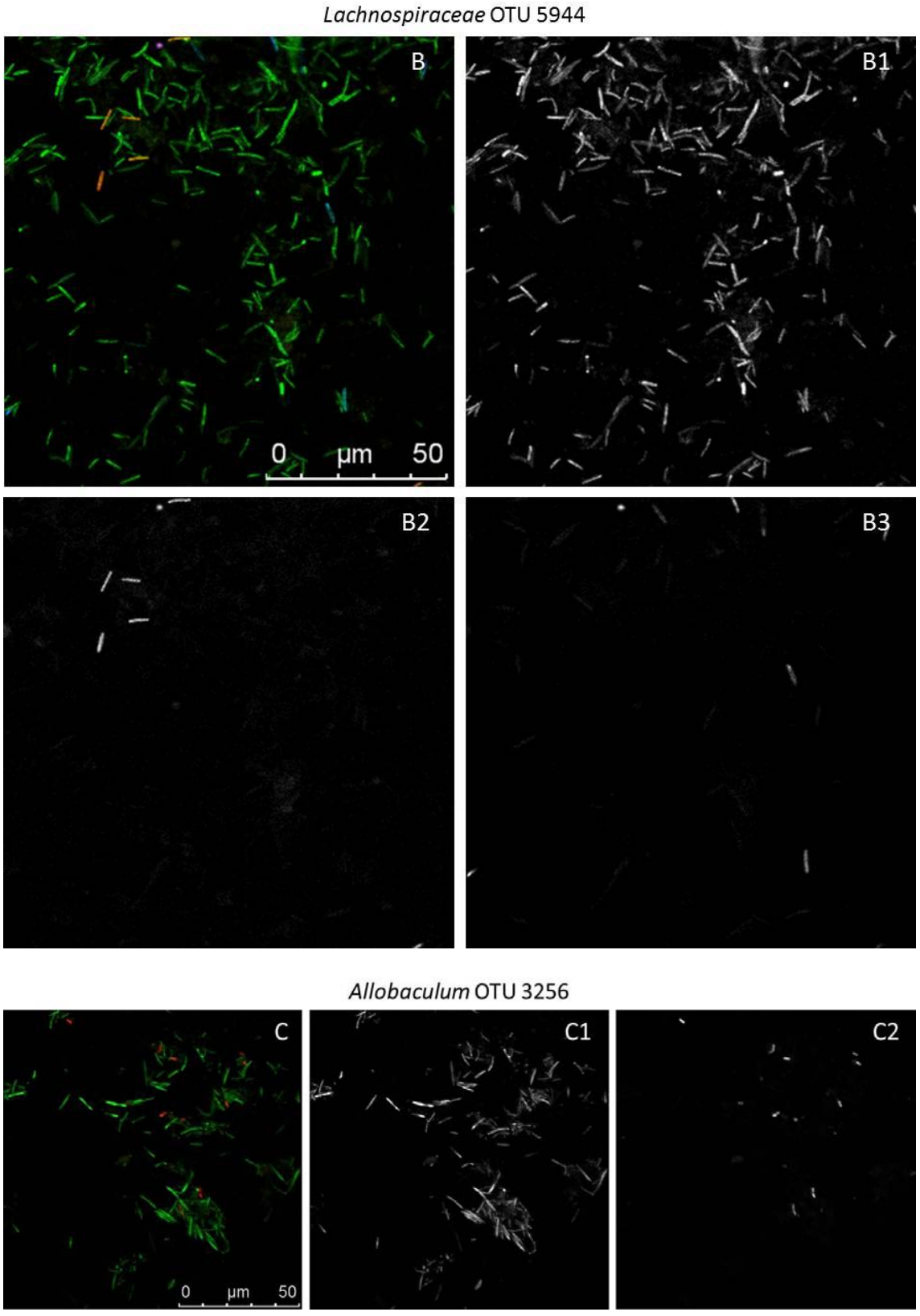


Figure S3. (continued)

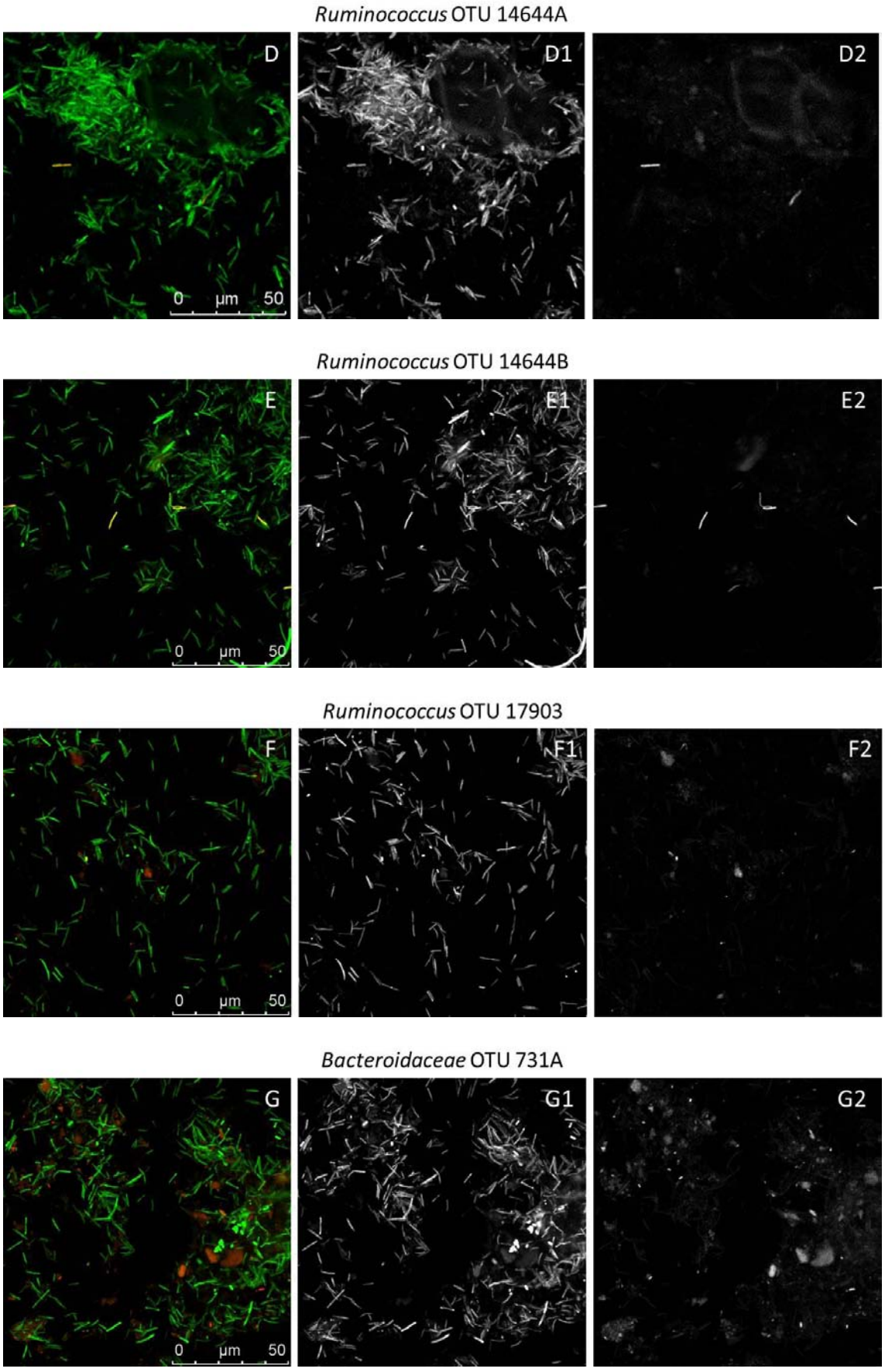


Figure S3. (continued)

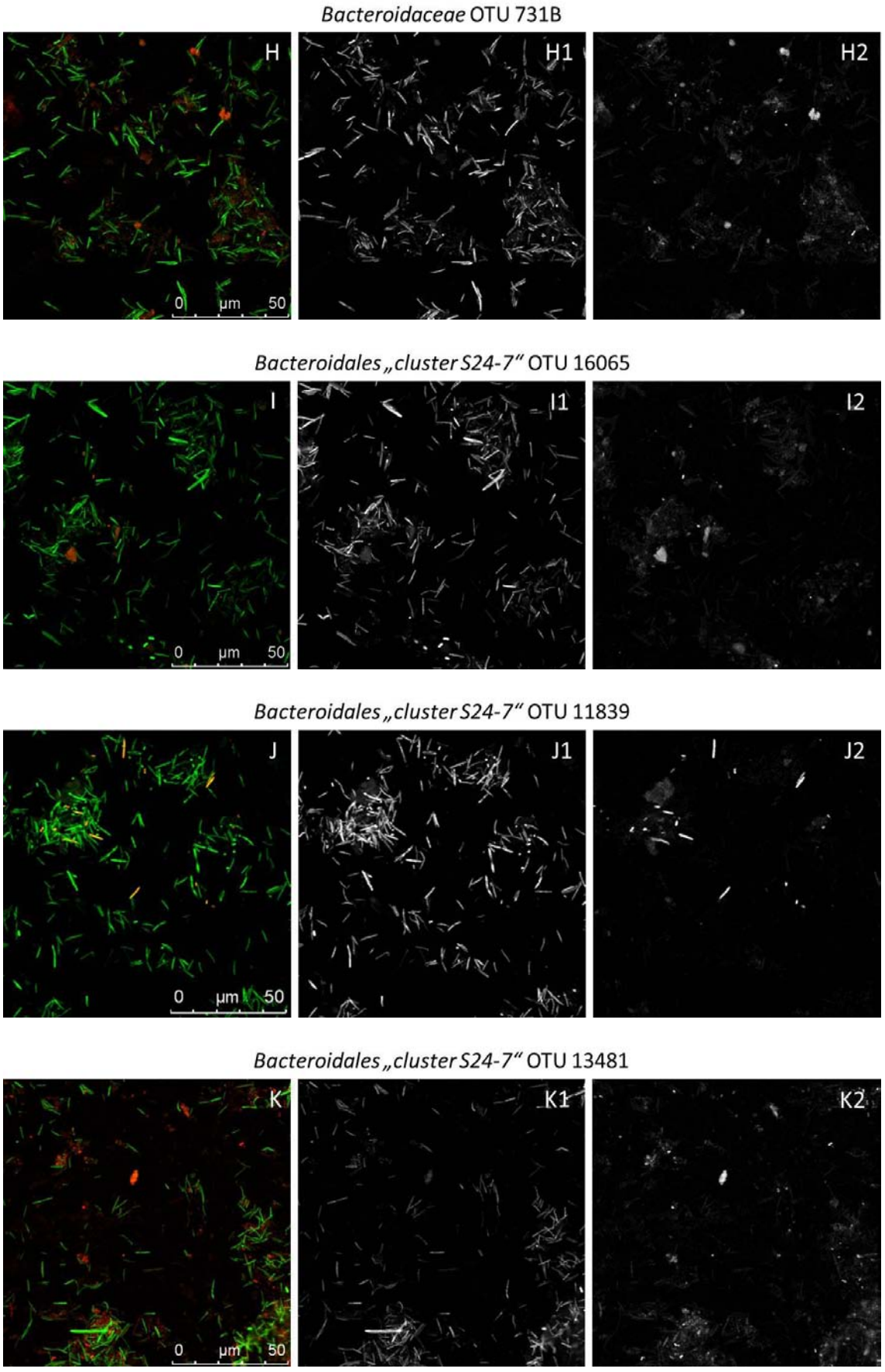


Figure S3. (continued)

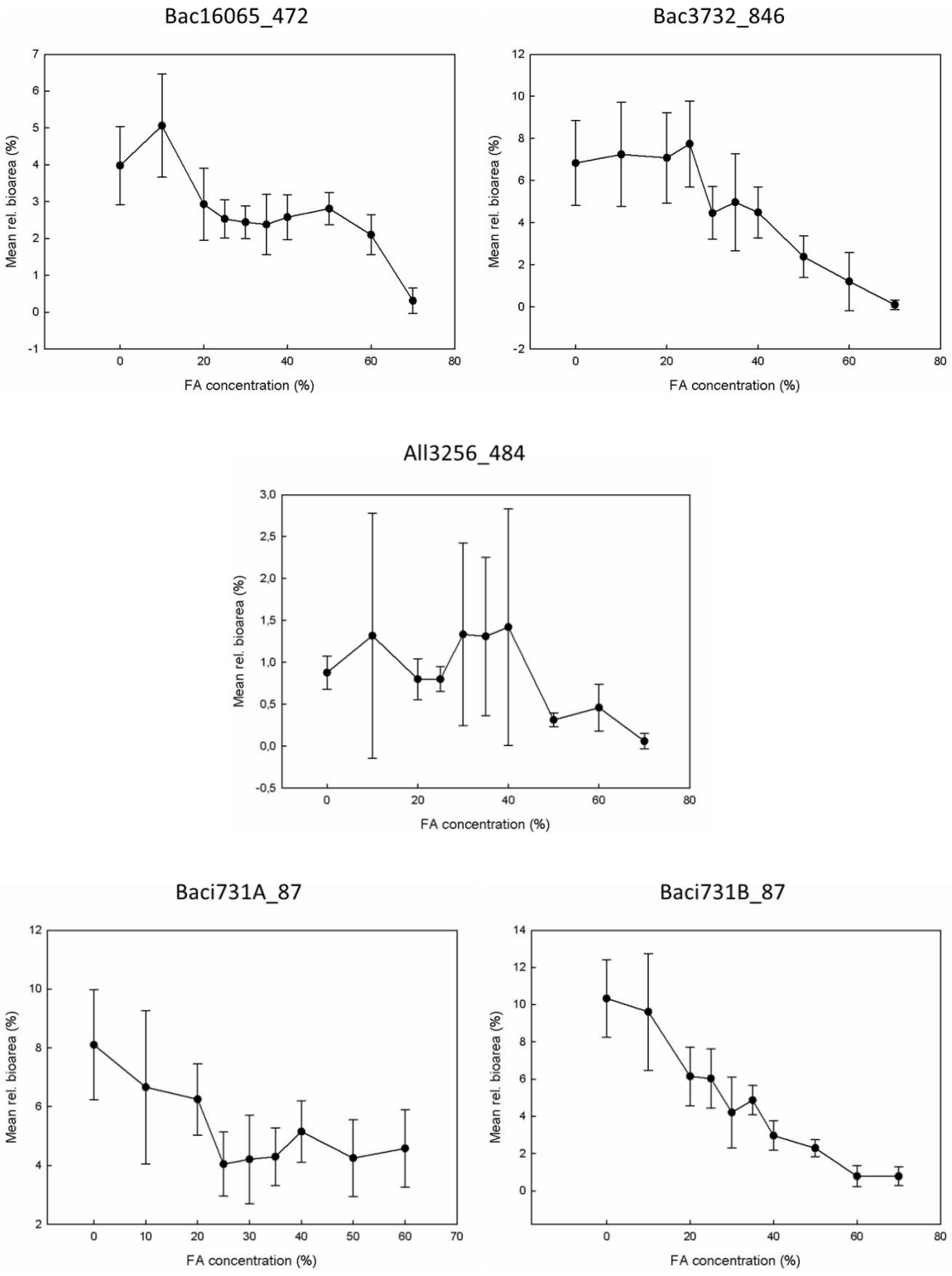


Figure S4. Quantitative FISH of the target phylotypes at increasing FA concentration. The graphs show the development of the specific probe signals relative to signals of EUB338mix at increasing FA concentration. In some cases EUB338mix signals showed stronger decrease than specific probes at high FA concentrations, hence relative bioarea increased (data points are sometimes skipped).

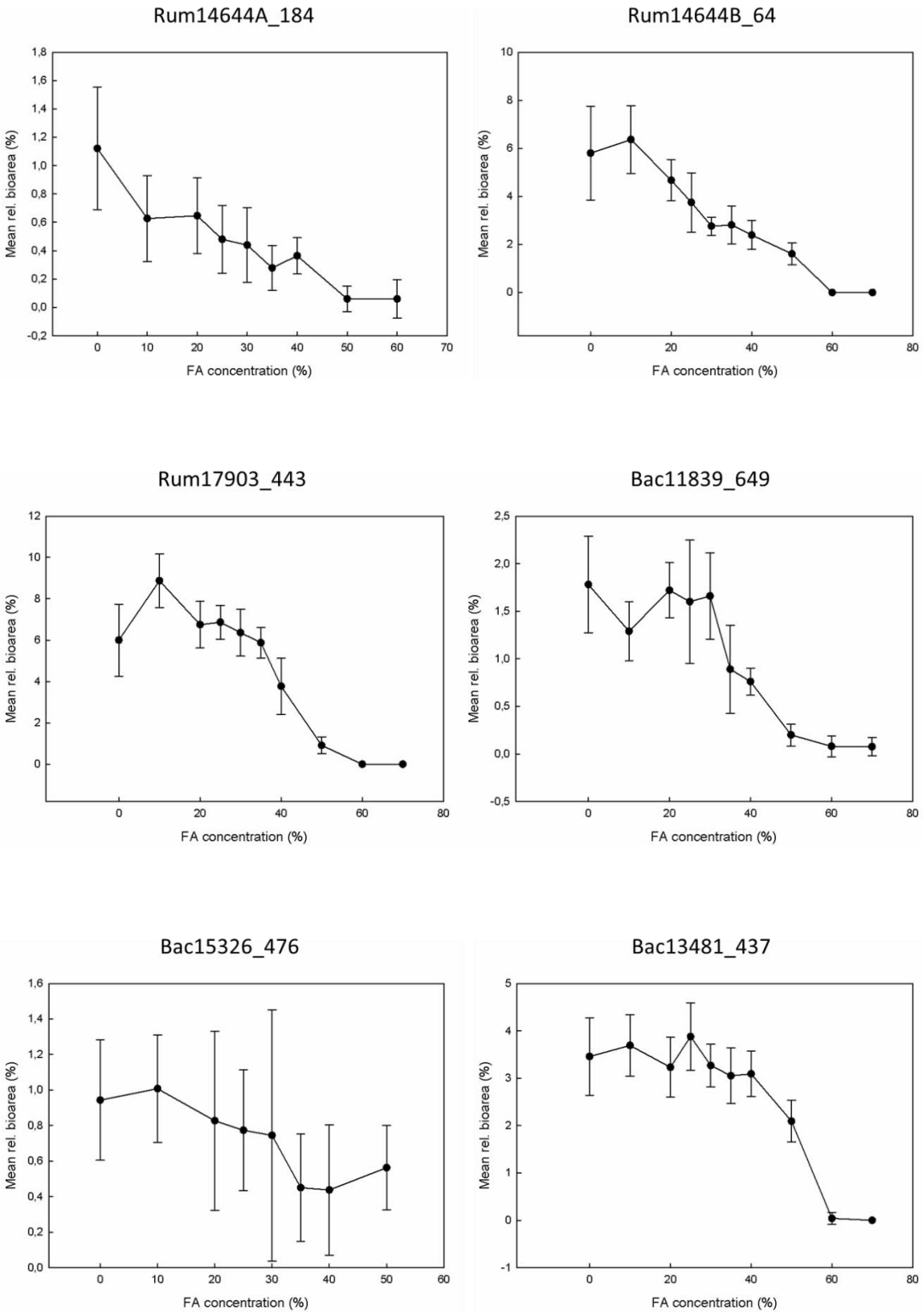


Figure S4. (continued)

9.2.2 Supplementary Tables

Table SR1. List of the 454 pyrosequencing reads of the target phylotypes. Using primer 1492R nucleotides 1000 to 1450 of the 16S rRNA gene sequence were identified (numbers refer to *E. coli* numbering according to Brosius and colleagues (61)).

> OTU_3720
TCTTGACATCCCGATGACGAGTGAGCAAAGTCACTTTCCCTTCGGGGCATTGGAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTC
GTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTATTTCCAGTAGCCAGCAAGAAAGATGGGAACTCTGGAGAGACTGCCCG
GGATAACTGGGAGGAAGGCGGGGATGACGTCAAATCATCATGCCCCTTATGATCAGGGCTACACACGTGCTACAATGGCGTAAACAAA
GGGAAGCGAAGTGGTGACACGAAGCAAATCCGAAAAACAACGTCTCAGTTCGGATTGTAGTCTGCAACTCGACTACATGAAGCTGGAA
TCGCTAGTAATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGTCGGAAATG
CCCGAAGTCAGTGACCCAACCGAGAGGAGGGAGCTGCCGAAGGTGGAGCCGGTAACTGGGGTGA

> OTU_16065
CGAGGAACCTTACCCGGGCTCAAACGCAGGAGGGATGTTTTTGAAAGGAGGCAGCCAGCAATGGTCTCCTGCGAGGTGCTGCATGGTT
GTCGTCAGCTCGTGCCGTGAGGTGTCGGCTTAAGTGCCATAACGAGCGCAACCCCTACCTACAGTTGCCATCGCGTGATGGCGGGCAC
TCTGTGGGGACTGCCTGCGCAAGCAGTGAGGAAGGCGGGGATGACGTCAAATCAGCACGGCCCTTACGTCCGGGGCGACACACGTGTT
ACAATGGCAGGTACAGCGGGAAGCCACCCGGCGACGGGGCGCGGAACCCGAAAACCTGTCTCAGTTCGGATTGGAGTCTGCAACCCGA
CTCCATGAAGCTGGATTCGCTAGTAATCGCGCATCAGCCACGGCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGC
CATGGAAGCCGGGGGTGCCTGAAGTGCGTGACCGCAAGGAGCGCCCTAGGGTAAAACCGGTGACTGGGGCTA

>OTU_3732
CAGGAATACTTTTGAAAGGAGGTAGCTCTACGGAGCCTGTGTCGAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGC
TTAAGTGCCATAACGAGCGCAACCCCCATCGCCAGTTACCAGCAAGTCAAGTTGGGGACTCTGGCGAGACTGCCGGCGCAAGCTGTGA
GGAAGGCGGGGATGACGTCAAATCAGCACGGCCCTTACGTCCGGGGCGACACACGTGTTACAATGGCAGGTACAGAGAGAAGCAATGC
GGCGACGCAAAGCGGAACTTGAAAGCCTGTCTCAGTTCGGATTGGAGTCTGCAACTCGACTCCATGAAGCTGGATTCGCTAGTAATCG
CGCATCAGCCATGGCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGGAAGCCGGGAGTGCCTGAAGTGTGC
AACCGCAAGGAGCGCCCTAAGGTAAAACTGGTGACTGGGGCTA

> OTU_2185
GGTCTTGACATCTTGCGCTAACCTTAGAGATAAGGCGTTCCCTTCGGGGACGCAATGACAGGTGGTGCATGGTCGTCGTCAGCTCGTG
TCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTTACTAGTTGCCAGCATTAAGTTGGGCACTCTAGTGAGACTGCCG
GTGACAAACCGGAGGAAGGTGGGGACGACGTCAGATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAA
CGAGTCGCAAGCTCGCGAGAGTAAGCTAATCTCTTAAAGCCGTTCTCAGTTCGGACTGTAGGCTGCAACTCGCCTACACGAAGTCGGA
ATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTTGTAAC
GCCCAAAGTCGGTGGCCTAACCTTTATGGAGGGAGCCGCCTAAGGCGGGACAGATGACTGGGGTGA

> OTU_9057
TGCAAACCTAAGAGATTAGGTGTTCCCTTCGGGGACGCTGAGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGG
TTAAGTCCCGCAACGAGCGCAACCCTTGTCATTAGTTGCCATCATTAAGTTGGGCACTCTAATGAGACTGCCGGTGACAAACCGGAGG
AAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAACGAGAAGCGAACCTG
CGAAGGCAAGCGGATCTCTTAAAGCCGTTCTCAGTTCGGACTGTAGGCTGCAACTCGCCTACACGAAGCTGGAATCGCTAGTAATCGC
GGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTCTGTAACACCCAAAGCCGGTGG
GATAACCTTTATAGGAGTCAGCCGTCTAAGGTAGGACAGATGATTAGGGTGA

> OTU_3256
CATTGGAGGTCAACATCCACACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTCAAGTCCCGCAACGAGCGCAA
CCCTTGTGGCATGTTGCTAACAGGAAAAGCTGAGGACTCATGCCAGACTGCCGGTGACAAACCGGAGGAAGGCGGGGATGACGTCAAA
TCATCATGCCCCTTATGGCCTGGGCTACACACGTACTACAATGGCGGCTACAAAGAGCAGCGAGACAGGGATGTCGAGCGAATCTCAT
AAAAGCCGTCCCAGTTCGGATTGGAGGCTGCAACCCGCCTCCATGAAGTTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGA
ATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAACCATGGGAGTCGGTAATGCCCGAAGCCGGTGGCATGACCTCATAAGAGGAGT
GAGCCGTCGAAGGCAGGATCGATGACTGGGGTTA

Table SR1. (continued)

> OTU_731
TACGCGAGGAACCTTACCCGGGCTTAAATTGCACCTGAATAATGTGGAAACATGTTAGCCGTAAGGCAGGTGTGAAGGTGCTGCATGG
TTGTCGTCAGCTCGTGCCGTGAGGTGTCGGCTTAAGTGCCATAACGAGCGCAACCCTTATCTTCAGTTACTAACAGGTCATGCTGAGG
ACTCTGGAGAGACTGCCGTCGTAAGATGTGAGGAAGGTGGGGATGACGTCAAATCAGCACGGCCCTTACGTCCGGGGCTACACACGTG
TTACAATGGGAGGTACAGAAGGCCGCTACCTGGCGACAGGATGCCAATCCCCAAAACCTCTCTCAGTTCGGATCGAAGTCTGCAACCC
GACTTCGTGAAGCTGGATTCGCTAGTAATCGCGCATCAGCCATGGCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAA
GCCATGAAAGCCGGGGGTACCTGAAGTACGTAACCGCAAGGATCGTCCTAGGGTAAAACTGGTAATTGGGGCTA

> OTU_14644
AACCCCTATTGTTAGTTGCTACGCAAGAGCACTCTAGCGAGACTGCCGTTGACAAAACGGAGGAAGGCGGGGACGACGTCAAATCATC
ATGCCCCTTATGTCCTGGGCTACACACGTAATACAATGGCGGTTAACAAAGGGATGCAAAGCCGCGAGGCAGAGCGAACCCCAAAAAG
CCGTCCCAGTTCGGATCGCAGGCTGCAACCCGCCTGCGTGAAGTCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACG
TTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTCGGGAACACCCGAAGTCCGTAGCCTAACCGCAAGGAGGGCGCGGCCG
AAGGTGGGTTCGATAATTGGGGTGA

> OTU_17903
GGGTCTTGACATCGAGTGAAAGGTCAAGAGATTGATCCCTCTCTTCGGAGACACGAAGACAGGTGGTGCATGGTTGTCGTCAGCTCGT
GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCATTAGTTGCTACGCAAGAGCACTCTAATGAGACTGCCGTTGA
CAAAACGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTTATGACCCGGGCTACACACGTACTACAATGGTGTTTAACAAAGA
GAAGCGAAGCCGCGAGGCGGAGCAAATCTCGAAAAAACATCTCAGTTCAGATTGCAGGCTGCAACTCGCCTGCATGAAGTCGGAATTG
CTAGTAATCGTAGGTCAGCATACTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAACCATGAGAGTTGGCAACACCC
GAAGTCGGTAGTCTAACCGCAAGGAGGACGCCGCCGAAGGTGGGGTTGATGATTAGGGTTA

> OTU_5944
AACCCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGGCCGGGGCTACACACGTGCTACAATGGCGTAAACAAAGGGA
AGCGAGAGAGTGATCTTAAGCGAAACTCAAAAATAACGTCCCAGTTCGGATTGCAGTCTGCAACTCGACTGCATGAAGCTGGAATCGC
TAGTAATCGCGGATCAGAATGCCGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGCCGGGAATGCCCG
AAGTCGGTGACCTAACCTGAAAAGGGAGGAGCCGCCGAAGGCAGGTCTGGTAACTGGGGTGA

> OTU_11839
TAATGACGACAGTTGAAAGATTGTTTCCCTTCGGGGCATTATGCGAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGG
CTTAAGTGCCATAACGAGCGCAACCCCTATCGACAGTTGCTAACGAGTCGAGTCGAGGACTCTGTCGAGACTGCCGGCGCAAGCTGTG
AGGAAGGCGGGGATGACGTCAAATCAGCACGGCCCTTACGTCCGGGGCGACACACGTGTTACAATGGCAGGGACAGCGGGAAGCCACC
AAGCGATTGGGCGCGGAACCCGAAACCCTGTCTCAGTTCGGATTGGAGTCTGCAACTCGACTCCATGAAGCTGGATTCGCTAGTAATC
GCGCATCAGCCATGGCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGGGAGTCGGGAGTGCCTGAAGTTCG
TGACCGTATAGGAGCGACCTAGGGCAAGACTGATGACTGGGGCTA

> OTU_15326
CGAGGAACCTTACCCGGGCTCAAACGCAACCGGAATGTATCCGAAAGGGTACAGCTCTACGGAGTCTGTTGCGAGGTGCTGCATGGTT
GTCGTCAGCTCGTGCCGTGAGGTGTCGGCTTAAGTGCCATAACGAGCGCAACCCCTATCGACAGTTGCTAACGAGTTAAGTCGAGGAC
TCTGTCGAGACTGCCGGCGCAAGCTGTGAGGAAGGCGGGGATGACGTCAAATCAGCACGGCCCTTACGTCCGGGGCGACACACGTGTT
ACAATGGCGACTACAGCGGGAAGCCACTTGGCGACAAGGAGCGGAACCCGAAAAGTCGTCTCAGTTCGGATTGGAGTCTGCAACTCGA
CTCCATGAAGCTGGATTCGCTAGTAATCGCGCATCAGCCATGGCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGC
CATGGAAGCCGGGGGTGCCTGAAGTGCGTGACCGCAAGGAGCGCCCTAAGGTAAAACCGGTGACTGGGGCTA

> OTU_13481
GGGCTCAAACGACGGATGGATGTTTCTGAAAGGAGGCAGTCCTACGGGACATCCGTCGAGGTGCTGCATGGTTGTCGTCAGCTCGTGC
CGTGAGGTGTCGGCTTAAGTGCCATAACGAGCGCAACCCCCATCGCCAGTTACCAGCAAGTAAAGTTGGGGACTCTGGCGAGACTGCC
GGCGCAAGCTGTGAGGAAGGCGGGGATGACGTCAAATCAGCACGGCCCTTACGTCCGGGGCGACACACGTGTTACAATGGCAGGTACA
GAGAGAAGCGATGCGGCGACGCAGAGCGGAACTTCAAAGCCTGTCTCAGTTCGGATTGGAGTCTGCAACCCGACTCCATGAAGCTGGA
TTCGCTAGTAATCGCGCATCAGCCACGGCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGGAAGCCGGGAG
TGCCTGAAGTGTGCAACCGCAAGGAGCGCCCTAAGGTAAAACCGGTGACTGGGGCTA

Table SR2. Near full-length 16S rRNA gene sequences of the target phylotypes. Sequences were attained by Sanger sequencing using primer pair 8F and 1492R. Sequences are named by the organisms' taxonomic affiliations inferred by ARB SILVA and OTU number according to 454 pyrosequencing data (≥ 97% similarity).

>*Lachnospiraceae* OTU 3720 clone 1

AGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGGCTCATATTGAAACCTAGTGATTTATGAGTTAGTGGCGGACGGG
TGAGTAACGCGTGGAAAACCTGCCGTATACTGGGGGATAACACTTAGAAATAGGTGCTAATACCGCATAAGCGCACAGCTTCGCATGA
AGTGGTGTGAAAAACTCTGGTGGTATACGATGGTTCCGCGTCTGATTAGCTTGTTGGTGGGGTAATGGCTCACCAAGGCGACGATCAG
TAGCCGGCCTGAGAGGGTGAACGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAA
TGGGGGAAACCCTGATGCAGCGACGCCGCGTGAGTGAAGAAGTATTTCGGTATGTAAAGCTCTATCAGCAGGGAAGAAAATGACGGTA
CCTGATTAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTATCCGGATTTACTGGGTGTAAAG
GGAGCGTAGACGGCAGCGCAAGTCTGAAGTGAAATGCCGGGGCTTAACCCCGGAACTGCTTTGGAAACTGTGCAGCTAGAGTGCAGGA
GAGGTAAGTGGAATTCCTAGTGTAGCGGTGAAATGCGTAGATATTAGGAGGAACACCAGTGGCGAAGGCGGCTTACTGGACTGTAACT
GACGTTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGATTACTAGGTGTTGGGGG
ACCAAGGTCCTTCGGTGCCGTCGCAAACGCATTAAGTAATCCACCTGGGGAGTACGTTCGCAAGAATGAAACTCAAAGGAATTGACGG
GGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCTGTCTTGACATCCCGATGACGAGTGAGCA
AAGTCACTTTCCCTTCGGGGCATTGGAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC
GAGCGCAACCCCTATTTCCAGTAGCCAGCAAGAAAGATGGGAACTCTGGAGAGACTGCCCGGGATAACTGGGAGGAAGGCGGGGATGA
CGTCAAATCATCATGCCCCTTATGATCAGGGCTACACACGTGCTACAATGGCGTAAACAAAGGGAAGCGAAGTGGTGACACGAAGCAA
ATCCGAAAAACAACGTCTCAGTTCGGATTGTAGTCTGCAACTCGACTACATGAAGCTGGAATCGCTAGTAATCGCGAATCAGAATGTC
GCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGTCGGAAATGCCCGAAGTCAGTGACCCAACCGAGAGG
AGGGAGCTGCCGAAGGTGGAGCCGGTAACTGGGGTG

>*Lachnospiraceae* OTU 3720 clone 2

AGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGGCTCATATTGAAACCTAGTGATTTATGAGTTAGTGGCGGACGGG
TGAGTAACGCGTGGAAAACCTGCCGTATACTGGGGGATAACACTTAGAAATAGGTGCTAATACCGCATAAGCGCACAGCTTCGCATGA
AGTGGTGTGAAAAACTCCGGTGGTATACGATGGTTCCGCGTCTGATTAGCTTGTTGGTGGGGTAATGGCTCACCAAGGCGACGATCAG
TAGCCGGCCTGAGAGGGTGAACGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAA
TGGGGGAAACCCTGATGCAGCGACGCCGCGTGAGTGAAGAAGTATTTCGGTATGTAAAGCTCTATCAGCAGGGAAGAAATAGCGACCT
TTGGGTCGCCAGACGGTACCTGATTAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTATCCG
GATTTACTGGGTGTAAAGGGAGCGTAGACGGCAGCGCAAGTCTGAAGTGAAATGCCGGGGCTTAACCCCGGAACTGCTTTGGAAACTG
TGCAGCTAGAGTGCAGGAGAGGTAAGTGGAATTCCTAGTGTAGCGGTGAAATGCGTAGATATTAGGAGGAACACCAGTGGCGAAGGCG
GCTTACTGGACTGTAACTGACGTTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATG
ATTACTAGGTGTTGGGGGACCAAGGTCCTTCGGTGCCGTCGCAAACGCATTAAGTAATCCACCTGGGGAGTACGTTCGCAAGAATGAA
ACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCTGGTCTTGACA
TCCCGATGACGAGTGAGCAAAGTCACTTTCCCTTCGGGGCATTGGAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATG
TTGGGTTAAGTCCCGCAACGAGCGCAACCCCTATTTCCAGTAGCCAGCAAGAAAGATGGGAACTCTGGAGAGACTGCCCGGGATAACT
GGGAGGAAGGCGGGGATGACGTCAAATCATCATGCCCCTTATGATCAGGGCTACACACGTGCTACAATGGCGTAAACAAAGGGAAGCG
AAGTGGTGACACGAAGCAAATCCGAAAAACAACGTCTCAGTTCGGATTGTAGTCTGCAACTCGACTACATGAAGCTGGAATCGCTAGT
AATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGTCGGAAATGCCCGAAGT
CAGTGACCCAACCGAGAGGAGGGAGCTGCCGAAGGTGGAGCCGGTAACTGGGGTG

>*Lachnospiraceae* OTU 3720 clone 3

AGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGACTCATATTGAAACCTAGTGATTTATGAGTTAGTGGCGGACGGG
TGAGTAACGCGTGGAAAACCTGCCGTATACTGGGGGATAACACTTAGAAATAGGTGCTAATACCGCATAAGCGCACAGCTTCGCATGA
AGTGGTGTGAAAAACTCTGGTGGTATACGATGGTTCCGCGTCTGATTAGCTTGTTGGCGGGGTAATGGCTCACCAAGGCGACGATCAG
TAGCCGGCCTGAGAGGGTGAACGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAA
TGGGGGAAACCCTGATGCAGCGACGCCGCGTGAGTGAAGAAGTATTTCGGTATGTAAAGCTCTATCAGCAGGGAAGAAATAGCGACCT
TTGGGTCGCCAGACGGTACCTGATTAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTATCCG
GATTTACTGGGTGTAAAGGGAGCGTAGACGGCAGCACAAGTCTGAAGTGAAATGCCGGGGCTTAACCCCGGAACTGCTTTGGAAACTG
TGCAGCTAGAGTGCAGGAGAGGTAAGTGGAATTCCTAGTGTAGCGGTGAAATGCGTAGATATTAGGAGGAACACCAGTGGCGAAGGCG
GCTTGCTGGACTGTAACTGACGTTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATG
ATTACTAGGTGTTGGGGGACCAAGGTCCTTCGGTGCCGTCGCAAACGCATTAAGTAATCCACCTGGGGAGTACGTTCGCAAGAATGAA
ACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCTGGTCTTGACA
TCCCGATGACGAGTGAGCAAAGTCACTTTCCCTTCGGGGCATTGGAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATG
TTGGGTTAAGTCCCGCAACGAGCGCAACCCCTATTTCCAGTAGCCAGCAAGAAAGATGGGAACTCTGGAGAGACTGCCCGGGATAACT
GGGAGGAAGGCGGGGATGACGTCAAATCATCATGCCCCTTATGATCAGGGCTACACACGTGCTACAATGGCGTAAACAAAGGGAAGCG
AAGTGGTGACACGAAGCAAATCCGAAAAACAACGTCTCAGTTCGGATTGTAGTCTGCAACTCGACTACATGAAGCTGGAATCGCTAGT
AATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGTCGGAAATGCCCGAAGT
CAGTGACCCAACCGAGAGGAGGGAGCTGCCGAAGGTGGAGCCAGTAACTGGGGTG

Table SR2. (continued)

>*Lachnospiraceae* OTU 3720 clone 4

AGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGGCTCATATTGAAACCTAGTGATTTATGAGTTAGTGGCGGACGGG
TGAGTAACGCGTGGAAAACCTGCCGTATACTGGGGGATAACACTTAGAAATAGGTGCTAATACCGCATAAGCGCACAGCTTCGCATGA
AGTGGTGTGAAAAACTCTGGTGGTATACGATGGTTCCGCGTCTGATTAGCTTGTTGGTGGGGTAATGGCTCACCAAGGCGACGATCAG
TAGCCGGCCTGAGAGGGTGAACGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAA
TGGGGGAAACCCTGATGCAGCGACGCCGCGTGAGTGAAGAAGTATATCAGCAGGGAAGAAATAGCCACCAGGGAAGAAAAAGACGGTA
CCTGATTAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTATCCGGATTTACTGGGTGTAAAG
GGAGCGTAGACGGCAGCGCAAGTCTGAAGTGAAATGCCGGGGCTTAACCCCGGAACTGCTTTGGAAACTGTGCAGCTAGAGTGCAGGA
GAGGTAAGTGGAATTCCTAGTGTAGCGGTGAAATGCGTAGATATTAGGAGGAACACCAGTGGCGAAGGCGGCTTACTGGACTGTAACT
GACGTTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGATTACTAGGTGTTGGGGG
ACCAAGGTCCTTCGGTGCCGTCGCAAACGCATTAAGTAATCCACCTGGGGAGTACGTTCGCAAGAATGAAACTCAAAGGAATTGACGG
GGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCTGTCTTGACATCCCGATGACGAGTGAGCA
AAGTCACTTTCCCTTCGGGGCATTGGAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC
GAGCGCAACCCCTATTTCCAGTAGCCAGCAAGAAAGATGGGAACTCTGGAGAGACTGCCCGGGATAACTGGGAGGAAGGCGGGGATGA
CGTCAAATCATCATGCCCCTTATGATCAGGGCTACACACGTGCTACAATGGCGTAAACAAAGGGAAGCGAAGTGGTGACACGAAGCAA
ATCCGAAAAACAACGTCTCAGTTCGGATTGTAGTCTGCAACTCGACTACATGAAGCTGGAATCGCTAGTAATCGCGAATCAGAATGTC
GCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGTCGGAAATGCCCGAAGTCAGTGACCCAACCGAGAGG
AGGGAGCTGCCGAAGGTGGAGCCGGTAACTGGGGTG

>*Bacteroidales* "*cluster S24-7*" OTU 16065 clone 1

AGGATGAACGCTAGCGACAGGCTTAACACATGCAAGTCGAGGGGCAGCGGGGGTGATGGCTTGCCATCACCTGCCGGCGACCGGCGCA
CGGGTGAGTAACACGTATGCAACCTGCCTGCCACAGCCGGATAATCGGGAGAAATTCCGTCTAATACGGCATAATGCATCGACGGGAC
ATCCTGTTGGTGCCAAAGGTTAATTCCGGTGGCGGATGGGCATGCGTCGCATTAGTTAGTTGGCGGTGTAACGGACCACCAAGACGAC
GATGCGTAGGGGTTCTGAGAGGAAGGTCCCCCACACTGGTACTGAGACACGGACCAGACTCCTACGGGAGGCAGCAGTGAGGAATATT
GGTCAATGGGCGGGAGCCTGAACCAGCCAAGTCGCGTGAGGGATGACGGCCCTATGGGTTGTAAACCTCTTTTGCCGGGGAGCAAAGT
GCCGCACGTGTGCGGTTTGGAGAGTACCCGGAGAAAAAGCATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATGCGAGC
GTTATCCGGATTTATTGGGTTTAAAGGGTGCGTAGGCGGACGCTTAAGTCAGCGGTAAAATTGCGGGGCTCAACCTCGTCGAGCCGTT
GAAACTGGGTGCCTTGAGTGGGCGAGAAGTACGCGGAATGCGTGGTGTAGCGGTGAAATGCATAGATATCACGCAGAACTCCGATTGC
GAAGGCAGCGTACCGGCGCCCAACTGACGCTGAAGCACGAAGGCGTGGGTATCGAACAGGATTAGATACCCTGGTAGTCCACGCGGTA
AACGATGAATGCTAGTTGTCCGGGGCGATTGAGTTCTGGGTGACACAGCGAAAGCGTTAAGCATTCCACCTGGGGAGTACGCCGGCAA
CGGTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGAGGAACATGTGGTTTAATTCGATGATACGCGAGGAACCTTACCCGGG
CTCAAACGCAGGAGGGATGTTTTTGAAAGGAGGCAGCCAGCAATGGTCTCCTGCGAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGT
GAGGTGTCGGCTTAAGTGCCATAACGAGCGCAACCCCTACCTACAGTTGCCATCGCGTGATGGCGGGCACTCTGTGGGGACTGCCTGC
GCAAGCAGTGAGGAAGGCGGGGATGACGTCAAATCAGCACGGCCCTTACGTCCGGGGCGACACACGTGTTACAATGGCAGGTACAGCG
GGAAGCCACCCGGCGACGGGGCGCGGAACCCGAAAACCTGTCTCAGTTCGGATTGGAGTCTGCAACCCGACTCCATGAAGCTGGATTC
GCTAGTAATCGCGCATCAGCCACGGCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGGAAGCCGGGGGTGC
CTGAAGTGCGTGACCGCAAGGAGCGCCCTAGGGTAAAACCGGTGACTGGGGCT

>*Bacteroidales* "*cluster S24-7*" OTU 16065 clone 2

AGGATGAACGCTAGCGACAGGCTTAACACATGCAAGTCGAGGGGCAGCGGTGGTGAAGGCTTGCCTTCACCAGCCGGCGACCGGCGCA
CGGGTGAGTAACACGTATGCAACCTGCCTGCCACAGCCGGATAATCGGGAGAAATTCCGTCTAATACGGCATAATGCATCGACGGGAC
ATCCTGTTGGTGCCAAAGGTTAATTCCGGTGGCGGATGGGCATGCGTCGCATTAGTTAGTTGGCGGTGTAACGGACCACCAAGACGAC
GATGCGTAGGGGTTCTGAGAGGAAGGTCCCCCACACTGGTACTGAGACACGGACCAGACTCCTACGGGAGGCAGCAGTGAGGAATATT
GGTCAATGGGCGGGAGCCTGAACCAGCCAAGTCGCGTGAGGGATGACGGCCCTATGGGTTGTAAACCTCTTTTGCCGGGGAGCAAAGT
GCCGCACGTGTGCGGTTTGGAGAGTACCCGGAGAAAAAGCATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATGCGAGC
GTTATCCGGATTTATTGGGTTTAAAGGGTGCGTAGGCGGACGCTTAAGTCAGCGGTAAAATTGCGGGGCTCAACCTCGTCGAGCCGTT
GAAACTGGGTGCCTTGAGTGGGCGAGAAGTACGCGGAATGCGTGGTGTAGCGGTGAAATGCATAGATATCACGCAGAACTCCGATTGC
GAAGGCAGCGTACCGGCGCCCAACTGACGCTGAAGCACGAAGGCGTGGGTATCGAACAGGATTAGATACCCTGGTAGTCCACGCAGTA
AACGATGAATGCTAGTTGTCCGGGGCGATTGAGTTCTGGGTGACACAGCGAAAGCGTTAAGCATTCCACCTGGGGAGTACGCCGGCAA
CGGTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGAGGAACATGTGGTTTAATTCGATGATACGCGAGGAACCTTACCCGGG
CTCAAACGCAGGAGGGATGTTTTTGAAAGGAGGCAGCCAGCAATGGTCTCCTGCGAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGT
GAGGTGTCGGCTTAAGTGCCATAACGAGCGCAACCCCTACCTACAGTTGCCATCGCGTGATGGCGGGCACTCTGTGGGGACTGCCTGC
GCAAGCAGTGAGGAAGGCGGGGATGACGTCAAATCAGCACGGCCCTTACGTCCGGGGCGACACACGTGTTACAATGGCAGGTACAGCG
GGAAGCCACCCGGCGACGGGGCGCGGAACCCGAAAACCTGTCTCAGTTCGGATTGGAGTCTGCAACCCGACTCCATGAAGCTGGATTC
GCTAGTAATCGCGCATCAGCCACGGCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGGAAGCCGGGGGTGC
CTGAAGTGCGTGACCGCAAGGAGCGCCCTAGGGTAAAACCGGTGACTGGGGCT

Table SR2. (continued)

>Bacteroidales "cluster S24-7" OTU 16065 clone 3

AGGATGAACGCTAGCGACAGGCTTAACACATGCAAGTCGAGGGGCAGCGGTGGTGAAGGCTTGCCTTTACCAGCCGGCGACCGGCGCA
CGGGTGAGTAACACGTATGCAACCTGCCTGCCACAGCCGGATAATCGGGAGAAATCCCGTCTAATACGGCATAATGCATCGACGGGAC
ATCCTGTTGGTGCCAAAGGTTGATTCCGGTGGCGGATGGGCATGCGTCGCATTAGTTAGTTGGCGGTGTAACGGACCACCAAGACGAC
GATGCGTAGGGGTTCTGAGAGGAAGGTCCCCCACACTGGTACTGAGACACGGACCAGACTCCTACGGGAGGCAGCAGTGAGGAATATT
GGTCAATGGGCGGGAGCCTGAACCAGCCAAGTCGCGTGAGGGATGACGGCCCTATGGGTTGTAAACCTCTTTTGCCGGGGAGCAAAGT
GCCGCACGTGTGCGGTTTGGAGAGTACCCGGAGAAAAAGCATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATGCGAGC
GTTATCCGGATTTATTGGGTTTAAAGGGTGCGTAGGCGGACGCTTAAGTCAGCGGTAAAATTGCGGGGCTCAACCTCGTCGAGCCGTT
GAAACTGGGTGCCTTGAGTGGGCGAGAAGTACGCGGAATGCGTGGTGTAGCGGTGAAATGCATAGATATCACGCAGAACTCCGATTGC
GAAGGCAGCGTACCGGCGCCCAACTGACGCTGAAGCACGAAGGCGTGGGTATCGAACAGGATTAGATACCCTGGTAGTCCACGCAGTA
AACGATGAATGCTAGTTGTCCGGGGCGATTGAGTTCTGGGTGACACAGCGAAAGCGTTAAGCATTCCACCTGGGGAGTACGCCGGCAA
CGGTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGAGGAACATGTGGTTTAATTCGATGATACGCGAGGAACCTTACCCGGG
CTCAAACGCAGGAGGGATGTTTTTGAAAGGAGGCAGCCAGCAATGGTCTCCTGCGAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGT
GAGGCGTCGGCTTAAGTGCCATAACGAGCGCAACCCCTACCTACAGTTACCATCGCGTGATGGCGGGCACTCTGTGGGGACTGCCTGC
GCAAGCAGTGAGGAAGGCGGGGATGACGTCAAATCAGCACGGCCCTTACGTCCGGGGCGACACACGTGTTACAATGGCAGGTACAGCG
GGAAGCCACCCGGCGACGGGGCGCGGAACCCGAAAACCTGTCTCAGTTCGGATTGGAGTCTGCGACCCGACTCCATGAAGCTGGATTC
GCTAGTAATCGCGCATCAGCCACGGCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGGAAGCCGGGGGTGC
CTGAAGTGCGTGACCGCAAGGGGCGCCCTAGGGTAAAACCGGTGACTGGGGCT

>Bacteroidales "cluster S24-7" OTU 16065 clone 4

AGGATGAACGCTAGCGACAGGCTTAACACATGCAAGTCGAGGGGCAGCGGTGGTGAAGGCTTGCCTTTACCAGCCGGCGACCGGCGCA
CGGGTGAGTAACACGTATGCAACCTGCCTGCCACAGCCGGATAATCGGGAGAAATCCCGTCTAATACGGCATAATGCATCGACGGGAC
ATCCTGTTGGTGCCAAAGGTTGATTCCGGTGGCGGATGGGCATGCGTCGCATTAGTTAGTTGGCGGTGTAACGGACCACCAAGACGAC
GATGCGTAGGGGTTCTGAGAGGAAGGTCCCCCACACTGGTACTGAGACACGGACCAGACTCCTACGGGAGGCAGCAGTGAGGAATATT
GGTCAATGGGCGGGAGCCTGAACCAGCCAAGTCGCGTGAGGGATGACGGCCCTATGGGTTGTAAACCTCTTTTGCCGGGGAGCAAAGT
GCCGCACGTGTGCGGTTTGGAGAGTACCCGGAGAAAAAGCATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATGCGAGC
GTTATCCGGATTTATTGGGTTTAAAGGGTGCGTAGGCGGACGCTTAAGTCAGCGGTAAAATTGCGGGGCTCAACCTCGTCGAGCCGTT
GAAACTGGGTGCCTTGAGTGGGCGAGAAGTACGCGGAATGCGTGGTGTAGCGGTGAAATGCATAGATATCACGCAGAACTCCGATTGC
GAAGGCAGCGTACCGGCGCCCAACTGACGCTGAAGCACGAAGGCGTGGGTATCGAACAGGATTAGATACCCTGGTAGTCCACGCAGTA
AACGATGAATGCTAGTTGTCCGGGGCGATTGAGTTCTGGGTGACACAGCGAAAGCGTTAAGCATTCCACCTGGGGAGTACGCCGGCAA
CGGTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGAGGAACATGTGGTTTAATTCGATGATACGCGAGGAACCTTACCCGGG
CTCAAACGCAGGAGGGATGTTTTTGAAAGGAGGCAGCCAGCAATGGTCTCCTGCGAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGT
GAGGCGTCGGCTTAAGTGCCATAACGAGCGCAACCCCTACCTACAGTTACCATCGCGTGATGGCGGGCACTCTGTGGGGACTGCCTGC
GCAAGCAGTGAGGAAGGCGGGGATGACGTCAAATCAGCACGGCCCTTACGTCCGGGGCGACACACGTGTTACAATGGCAGGTACAGCG
GGAAGCCACCCGGCGACGGGGCGCGGAACCCGAAAACCTGTCTCAGTTCGGATTGGAGTCTGCGACCCGACTCCATGAAGCTGGATTC
GCTAGTAATCGCGCATCAGCCACGGCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGGAAGCCGGGGGTGC
CTGAAGTGCGTGACCGCAAGGGGCGCCCTAGGGTAAAACCGGTGACTGGGGCT

>Bacteroidales "cluster S24-7" OTU 16065 clone 5

AGGATGAACGCTAGCGACAGGCTTAACACATGCAAGTCGAGGGGCAGCGGTGGTGAAGGCTTGCCTTTACCAGCCGGCGACCGGCGCA
CGGGTGAGTAACACGTATGCAACCTGCCTGCCACAGCCGGATAATCGGGAGAAATCCCGTCTAATACAGCATAATGCATCGACGGGAC
ATCCTGTTGGTGCCAAAGGTTAATTCCGGTGGCGGATGGGCATGCGTCGCATTAGTTAGTTGGCGGTGTAACGGACCACCAAGACGAC
GATGCGTAGGGGTTCTGAGAGGAAGGTCCCCCACACTGGTACTGAGACACGGACCAGACTCCTACGGGAGGCAGCAGTGAGGAATATT
GGTCAATGGGCGGGAGCCTGAACCAGCCAAGTCGCGTGAGGGATGACGGCCCTATGGGTTGTAAACCTCTTTTGCCGGGGAGCAAAGT
GCCGCACGTGTGCGGTTTGGAGAGTACCCGGAGAAAAAGCATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCGAGC
GTTATCCGGATTTATTGGGTTTAAAGGGTGCGTAGGCGGACGCTTAAGTCAGCGGTAAAATTGCGGGGCTCAACCTCGTCGAGCCGTT
GAAACTGGGTGCCTTGAGTGGGCGAGAAGTACGCGGAATGCGTGGCGTAGCGGTGAAATGCATAGATATCACGCAGAACTCCGATTGC
GAAGGCAGCGTACCGGCGCCCAACTGACGCTGAAGCACGAAGGCGTGGGTATCGAACAGGATTAGATACCCTGGTAGTCCACGCAGTA
AACGATGAATGCTAGTTGTCCGGGGCGATTGAGTTCTGGGTGACACAGCGAAAGCGTTAAGCATTCCACCTGGGGAGTACGCCGGCAA
CGGTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGAGGAACATGTGGTTTAATTCGATGATACGCGAGGAACCTTACCCGGG
CTCAAACGCAGGAGGGATGTTTTTGAAAGGAGGCAGCCAGCAATGGTCTCCTGCGAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGT
GAGGTGTCGGCTTAAGTGCCATAACGAGCGCAACCCCTACCTACAGTTGCCATCGCGTGATGGCGGGCACTCTGTGGGGACTGCCTGC
GCAAGCAGTGAGGAAGGCGGGGATGACGTCAAATCAGCACGGCCCTTACGTCCGGGGCGACACACGTGTTACAATGGCAGGTACAGCG
GGAAGCCACCCGGCGACGGGGCGCGGAACCCGAAAACCTGTCTCAGTTCGGATTGGGGTCTGCAACCCGACTCCATGAAGCTGGATTC
GCTAGTAATCGCGCATCAGCCACGGCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGGAAGCCGGGGGTGC
CTGAAGTGCGTGACCGCAAGGAGCGCCCTAGGGTAAAACCGGTGACTGGGGCT

Table SR2. (continued)

>*Bacteroidales* "*cluster S24-7*" OTU 3732 clone 1

AGCCCCAGTCACCAGTTTTACCTTAGGGCGCTCCTTGCGGTTGCACACTTCAGGCACTCCCGGCTTCCATGGCTTGACGGGCGGTGTG
TACAAGGCCCGGGAACGTATTCACCGCGCCATGGCTGATGCGCGATTACTAGCGAATCCAGCTTCATGGAGTCGAGTTGCAGACTCCA
ATTCGAACTGAGACAGGCTTTCAAGTTCCGCTTTGCGTCGCCGCATTGCTTCTCTCTGTACCTGCCATTGTAACACGTGTGTCGCCCC
GGACGTAAGGGCCGTGCTGATTTGACGTCATCCCCGCCTTCCTCACAGCTTGCGCCGGCAGTCTCGCCAGAGTCCCCAACTTGACTTG
CTGGTAACTGGCGATGGGGGTTGCGCTCGTTATGGCACTTAAGCCGACACCTCACGGCACGAGCTGACGACAACCATGCAGCACCTCG
ACACAGGCTCCGAAGAGCTACCTCCTTTCAAAAGTATTCCTGTGTCGTTTGAGCCCGGGTAAGGTTCCTCGCGTATCATCGAATTAAA
CCACATGTTCCTCCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCACCGTTGCCGGCGTACTCCCCAGGTGGAATACTTAACGC
TTTCGCTGTATCACTCAGGGATCATTCTCCCCGAACAATTAGTATTCATCGTTTACTGTGTGGACTACCAGGGTATCTAATCCTGTTC
GATACCCACACTTTCGTGCATGAGCGTCAGTTGAGCGCCGGTATGCTGCCTTCGCAATCGAAGTTCTGCGTGATATCTATGCATTTCA
CCGCTACACCACGCATTCCGCATACTTCTCGCTCACTCAAGAAAACCAGTTTCAACGGCTCGAAGAGGTTGAGCCTCTCAATTTTACC
GCTGACTTGATCTTCCGCCTGCGCACCCTTTAAACCCAATAAATCCGGATAACGCTCGCATCCTCCGTATTACCGCGGCTGCTGGCAC
GGAGTTAGCCGATGCTTTTTCTTCGGGTACTCTCGGAACGCCACGCGTGGCGTCTTTTGCTCCCCGACAAAAGAGGTTTACAATCCAT
AGGACCGTCTTCCCTCACGCGACTTGGCTGGTTCAGGCTCGCGCCCATTGACCAATATTCCTCACTGCTGCCTCCCGTAGGAGTCTGG
TCCGTGTCTCAGTACCAGTGTGGGGGACCTTCCTCTCAGAACCCCTACGCATCGTCGCCTTGGTGGGCCGTTACCCCGCCAACTAGCT
AATGCGCCGCATGGCCATCCGTAGCCGGTGTTACCCTTTAAACTCCATGAGATGCCTCTCGGAGTTATTACGCGGTATTAGACGGAAT
TTCTTCCGCTTATCCCCCTGCTACGGGCAGGTTCCATACGTGTTACTCACCCGTGCGCCGGTCGCCGGCGGAAGTATTGCTACCTCCC
CGCTGCCCCTCGACTTGCATGTGTTAAGCCTGTCGCTAGCGTTCATCCT

>*Lactobacillus* OTU 2185 clone 1

AGGATGAACGCTGGCGGTGTGCCTAATACATGCAAGTCGTACGCACTGGCCCAACTGATTGATGGTGTTTGCACCTGATTGACGATGG
ATCACCAGTGAGTGGCGGACGGGTGAGTAACACGTAGGTAACCTGCCCCGGAGCGGGGGATAACATTTGGAAACAGATGCTAATACCG
CATAACAACAAAAGCCACATGGCTTTTGTTTGAAAGATGGCTTTGGCTATCACTCTGGGATGGACCTGCGGTGCATTAGCTAGTTGGT
AAGGTAACGGCTTACCAAGGCGATGATGCATAGCCGAGTTGAGAGACTGATCGGCCACAATGGAACTGAGACACGGTCCATACTCCTA
CGGGAGGCAGCAGTAGGGAATCTTCCACAATGGGCGCAAGCCTGATGGAGCAACACCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAA
GCTCTGTTGTTGGAGAAGAACGTGCGTGAGAGTAACTGTTCACGCAGTGACGGTATCCAACCAGAAAGTCACGGCTAACTACGTGCCA
GCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTGCTTAGGTCTGATGTGAA
AGCCTTCGGCTTAACCGAAGAAGTGCATCGGAAACCGGGCGACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGGAA
TGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACA
GGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGAGTGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGGAGCTAACGCATT
AAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAA
TTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATCTTGCGCTAACCTTAGAGATAAGGCGTTCCCTTCGGGGACGCAATGACAG
GTGGTGCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGTAACCCTTGTTACTAGTTGCCAGCATT
AAGTTGGGCACTCTAGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGACGACGTCAGATCATCATGCCCCTTATGACCTGGGCT
ACACACGTGCTACAATGGACGGTACAACGAGTCGCAAGCTCGCGAGAGTAAGCTAATCTCTTAAAGCCGTTCTCAGTTCGGACTGTAG
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GCCCGTCACACCATGGGAGTTTGTAACGCCCAAAGTCGGTGGCCTAACCTTTATGGAGGGAGCCGCCTAAGGCGGGACAGATGACTGG
GGTG

>*Lactobacillus* OTU 9057 clone 1

AGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAGCTTGCCTAGATGATTTTAGTGCTTGCACTAAATGAAACTAGAT
ACAAGCGAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCAAGAGACTGGGATAACACCTGGAAACAGATGCTAATACCGGAT
AACAACACTAGACGCATGTCTAGAGTTTAAAAGATGGTTCTGCTATCACTCTTGGATGGACCTGCGGTGCATTAGCTAGTTGGTAAGG
TAACGGCTTACCAAGGCAATGATGCATAGCCGAGTTGAGAGACTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGG
AGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAGCTC
TGTTGGTAGTGAAGAAAGATAGAGGTAGTAACTGGCCTTTATTTGACGGTAATTACCTAGAAAGTCACGGCTAACTACGTGCCAGCAG
CCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGTGCAGGCGGTTCAATAAGTCTGATGTGAAAGCC
CTCGGCTCAACCGGAGAATTGCATCAGAAACTGTTGAACTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTGGAATGCG
TAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGAT
TAGATACCCTGGTAGTCCATGCCGTAAACGATGAGTGCTAAGTGTTGGGAGGTTTCCGCCTCTCAGTGCTGCAGCTAACGCATTAAGC
ACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCG
AAGCAACGCGAAGAACCTTACCAAGGCTTGACATACCTTTGACAGATGTAGAGATACATTTTTCCTTCGGGACAAGGGATACAGGTGG
TGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCATTAGTTGCCATCATTAAGT
TGGGCACTCTAATGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGACCTGGGCTACAC
ACGTGCTACAATGGACGGTACAACGAGAAGCGAACCTGCGAAGGCAAGCGGATCTCTTAAAGCCGTTCTCAGTTCGGACTGTAGGCTG
CAACTCGCCTACACGAAGCTGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC
GTCACACCATGAGAGTCTGTAACACCCAAAGCCGGTGGGATAACCTTTATAGGAGTCAGCCGTCTAAGGTAGGACAGATGATTAGGGT
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Table SR2. (continued)

>*Lactobacillus* OTU 9057 clone 2

AGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAGCTTGCCTAGATGATTTTAGTGCTTGCACTAAATGAAACTAGAT
ACAAGCGAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCAAGAGACTGGGATAACACCTGGAAACAGATGCTAATACCGGAT
AACAACACTAGACGCATGTCTAGAGTTTAAAAGATGGTTCTGCTATCACTCTTGGATGGACCTGCGGTGCATTAGCTAGTTGGTAAGG
TAACGGCTTACCAAGGCAATGATGCATAGCCGAGTTGAGAGACTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGG
AGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAGCTC
TGTTGGTAGTGAAGAAAGATAGAGGTAGTAACTGGCCTTTATTTGACGGTAATTACCTAGAAAGTCACGGCTAACTACGTGCCAGCAG
CCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGTGCAGGCGGTTCAATAAGTCTGATGTGAAAGCC
TTCGGCTCAACCGGAGAATTGCATCAGAAACTGTTGAACTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTGGAATGCG
TAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGAT
TAGATACCCTGGTAGTCCATGCCGTAAACGATGAGTGCTAAGTGTTGGGAGGTTTCCGCCTCTCAGTGCTGCAGCTAACGCATTAAGC
ACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCG
AAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCAGTGCAAACCTAAGAGATTAGGTGTTCCCTTCGGGGACGCTGAGACAGGTGG
TGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACTCTTGTCATTAGTTGCCATCATTAAGT
TGGGCACTCTAATGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGACCTGGGCTACAC
ACGTGCTACAATGGACGGTACAACGAGAAGCGAACCTGCGAAGGCAAGCGGATCTCTTAAAGCCGTTCTCAGTTCGGACTGTAGGCTG
CAACTCGCCTACACGAAGCTGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC
GTCACACCATGAGAGTCTGTAACACCCAAAGCCGGTGGGATAACCTTTATAGGAGTCAGCCGTCTAAGGTAGGACAGATGATTAGGGT
G

>*Lactobacillus* OTU 9057 clone 3

AGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAGCTTGCCTAGATGATTTTAGTGCTTGCACTAAATGAAACTAGAT
ACAAGCGAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCAAGAGACTGGGATAACACCTGGAAACAGATGCTAATACCGGAT
AACAACACTAGACGCATGTCTAGAGTTTAAAAGATGGTTCTGCTATCACTCTTGGATGGACCTGCGGTGCATTAGCTAGTTGGTAAGG
TAACGGCTTACCAAGGCAATGATGCATAGCCGAGCTGAGAGACTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGG
AGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAGCTC
TGTTGGTAGTGAAGAAAGATAGAGGTAGTAACTGGCCTTTATTTGACGGTAATTACCTAGAAAGTCACGGCTAACTACGTGCCAGCAG
CCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGTGCAGGCGGTTCAATAAGTCTGATGTGAAAGCC
TTCGG
CTCAACCGGAGAATTGCATCAGAAACTGTTGAACTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTGGAATGCGTAGAT
ATATGGAAGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGAT
ACCCTGGTAGTCCATGCCGTAAACGATGAGTGCTAAGTGTTGGGAGGTTTCCGCCTCTCAGTGCTGCAGCTAACGCATTAAGCACTCC
GCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCA
ACGCGAAGAACCTTACCAGGTCTTGACATCCAGTGCAAACCTAAGAGATTAGGTGTTCCCTTCGGGGACGCTGAGACAGGTGGTGCAT
GGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCATTAGTTGCCATCATTAAGTTGGGC
ACTCTAATGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGACCTGGGCTACACACGTG
CTACAATGGACGGTACAACGAGAAGCGAACCTGCGAAGGCAAGCGGATCTCTTAAAGCCGTTCTCAGTTCGGACTGTAGGCTGCAACT
CGCCTACACGAAGCTGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAC
ACCATGAGAGTCTGTAACACCCAAAGCCGGTGGGATAACCTTTATAGGAGTCAGCCGTCTAAGGTAGGACAGATGATTAGGGTG

>*Allobaculum* OTU 3256 clone 1

AGGATGAACGCTGGCGGCATGCCTAATACATGCAAGTCGAACGAGAGACCTTCGGGTCTCTAGTGGCGAACGGGTGAGTAACACGTAG
GGAACCTGCCCGCGCACCGGGAATACGCTCTGGAAACGGAGAACAAATCCCGATGTACAGGAAGGAGGCATCTTCTTTCTGTGAAACA
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CCTGAGAGGGCGGACGGCCACATTGGGACTGAGACACGGCCCAGACTCCTGCGGGAGGCAGCAGTAGGGAATTTTCGTCAATGGGCGC
AAGCCTGAACGAGCGATGCCGCGTGAGTGAAGAAGGTCTTCGGGTCGTAAAGCTCTGTTGCGGGGGAAAAAAGGCAGCATCAGGAAAT
GGGTGCTGACTGATGGTGCCCCGCCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCGAGCGTTATCCG
GAATGATTGGGCGTAAAGGGTGCGCAGGCGGTCCTGCAAGTCTGGAGTGAAACGCATGAGCTCAACTCATGCGTGGCTTTGGAAACTG
GAGGACTGGAGAGCAGGAGAGGGCGGTGGAACTCCATGTGTAGCGGTAAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCG
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AGGCGGGGATGACGTCAAATCATCATGCCCCTTATGGCCTGGGCTACACACGTACTACAATGGCGGCTACAAAGAGCAGCGAGACAGG
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GATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAACCATGGGAGTCGGTAATGCCCGAAGCCGGTGGC
ATGACCTCATAAGAGGAGTGAGCCGTCGAAGGCAGGATCGATGACTGGGGTT

Table SR2. (continued)

>*Allobaculum* OTU 3256 clone 2

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TCCTTTAGGGGATGGGGCGCGGACGGACCTGCGGTGCATTAGTTGGTTGGCGGGGTAAAGGCCCACCAAGACGATGATGCATAGCCGG
CCTGAGAGGGCGGACGGCCACATTGGGACTGAGACACGGCCCAGACTCCTGCGGGAGGCAGCAGTAGGGAATTTTCGTCAATGGGCGC
AAGCCTGAACGAGCGATGCCGCGTGAGTGAAGAAGGCCTTCGGGTCGTAAAGCTCTGTTGCGGGGGAAAAAAGGCAGCATCAGGAAAT
GGGTGCTGACTGATGGTGCCCCGCCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCGAGCGTTATCCG
GAATGATTGGGCGTAAAGGGTGCGCAGGCGGTCCTGCAAGTCTGGAGTGAAACGCATGAGCTCAACTCATGCATGGCTTTGGAAACTG
GAGGACTGGAGAGCAGGAGAGGGCGGTGGAACTCCATGTGTAGCGGTAAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCG
GCCGCCTGGCCTGTTGCTGACGCTGAGGCACGAAAGCGTGGGGAGCAAATAGGATTAGATACCCTAGTAGTCCACGCCGTAAACGATG
AGGACCAAGTGTTGGGGGTGAAACCTCAGTGCTGAAGTTAACGCAGTGAGTCCTCCGCCTGGGGAGTATGCACGCAAGTGTGAAACTC
AAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATGGG
ATGCGAAGATGCAGAGATGCATTGGAGGTCAACATCCACACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTCA
AGTCCCGCAACGAGCGCAACCCTTGTGGCATGTTGCTAACAGGAAAAGCTGAGGACTCATGCCAGACTGCCGGTGACAAACCGGAGGA
AGGCGGGGATGACGTCAAATCATCATGCCCCTTATGGCCTGGGCTACACACGTACTACAATGGCGGCTACAAAGAGCAGCGAGACAGG
GATGTCGAGCGAATCTCATAAAAGCCGTCCCAGTTCGGATTGGAGGCTGCAACCCGCCTCCATGAAGTTGGAATCGCTAGTAATCGCG
GATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAACCATGGGAGTCGGTAATGCCCGAAGCCGGTGGC
ATGACCTCATAAGAGGAGTGAGCCGTCGAAGGCAGGATCGATGACTGGGGTT

>*Allobaculum* OTU 3256 clone 3

AGGATGAACGCTGGCGGCATGCCTAATACATGCAAGTCGAACGAGAGACCTTCGGGTCTCTAGTGGCGAACGGGTGAGTAACACGTAG
GGAACCTGCCCGCGCACCGGGAATACGCTCTGGAAACGGAGAACAAATCCCGATGTACAGGAAGGAGGCATCTTCTTCCTGTGAAACA
TCCTTTAGGGGATGGGGCGCGGATGGACCTGCGGTGCATTAGTTGGTTGGCGGGGTAAAGGCCCACCAAGACGATGATGCATAGCCGG
CCTGAGAGGGCGGACGGCCACATTGGGACTGAGACACGGCCCAGACTCCTGCGGGAGGCAGCAGTAGGGAATTTTCGTCAATGGGCGC
AAGCCTGAACGAGCGATGCCGCGTGAGTGAAGAAGGCCTTCGGGTCGTAAAGCTCTGTTGCGGGGGAAAAAAGGCAGCATCAGGAAAT
GGGTGCTGACTGATGGTGCCCCGCCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCGAGCGTTATCCG
GAATGATTGGGCGTAAAGGGTGCGCAGGCGGTCCTGCAAGTCTGGAGTGAGACGCATGAGCTCAACTCATGCATGGCTTTGGAAACTG
GAGGACTGGAGAGCAGGAGAGGGCGGTGGAACTCCATGTGTAGCGGTAAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCG
GCCGCCTGGCCTGTTGCTGACGCTGAGGCACGAAAGCGTGGGGAGCAAATAGGATTAGATACCCTAGTAGTCCACGCCTTAAACGATG
AGGACCAAGTGTTGGGGGTGAAACCTCAGTGCTGAAGTTAACGCAGTGAGTCCTCCGCCTGGGGAGTATGCACGCAAGTGTGAAACTC
AAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATGGG
ATGCGAAGATGCAGAGATGCATTGGAGGTCAACATCCACACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTCA
AGTCCCGCAACGAGCGCAACCCTTGTGGCATGTTGCTAACAGGAAAAGCTGAGGACTCATGCCAGACTGCCGGTGACAAACCGGAGGA
AGGCGGGGATGACGTCAAATCATCATGCCCCTTATGGCCTGGGCTACACACGTACTACAATGGCGGCTACAAAGAGCAGCGAGACAGG
GATGTCGAGCGAATCTCATAAAAGCCGTCCCAGTTCGGATTGGAGGCTGCAACCCGCCTCCATGAAGTTGGAATCGCTAGTAATCGCG
GATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAACCATGGGAGTCGGTAATGCCCGAAGCCGGTGGC
ATGACCTCATAAGAGGAGTGAGCCGTCGAAGGCAGGATCGATGACTGGGGTT

>*Allobaculum* OTU 3256 clone 4

AGGATGAACGCTGGCGGCATGCCTAATACATGCAAGTCGAACGAGAGACCTTCGGGTCTCTAGTGGCGAACGGGTGAGTAACACGTAG
GGAACCTGCCCGCGCACCGGGAATACGCTCTGGAAACGGAGAACAAATCCCGATGTACAGGAAGGAGGCATCTTCTTTCTGTGAAACA
TCCTTTAGGGGATGGGGCGCGGATGGACCTGCGGTGCATTAGTTTGTTGGCGGGGTAAAGGCCCACCAAGGCGATGATGCATAGCCGG
CCTGAGAGGGCGGACGGCCACATTGGGACTGAGACACGGCCCAGACTCCTGCGGGAGGCAACAGTAGGGAATTTTCGTCAATGGGCGC
AAGCCTGAACGAGCGATGCCGCGTGAGTGAAGAAGGCCTTCGGGTCGTAAAGCTCTGTTGCGGGGGAAAAAAGGCAGCATCAGGAAAT
GGGTGCTGACTGATGGTGCCCCGCCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCGAGCGTTATCCG
GAATGATTGGGCGTAAAGGGTGCGCAGGCGGTCCTGCAAGTCTGGAGTGAAACGCATGAGCTCAACTCATGCATGGCTTTGGAAACTG
GAGGACTGGAGAGCAGGAGAGGGCGGTGGAACTCCATGTGTAGCGGTAAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCG
GCCGCCTGGCCTGTTGCTGACGCTGAGGCACGAAAGCGTGGGGAGCAAATAGGATTAGATACCCTAGTAGTCCACGCCGTAAACGATG
AGGACCAAGTGTTGGGGGTGAAACCTCAGTGCTGAAGTTAACGCAGTGAGTCCTCCGCCTGGGGAGTATGCACGCAAGTGTGAAACTC
AAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATGGG
ATGCGAAGATGCAGAGATGCATTGGAGGTCAACATCCACACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTCA
AGTCCCGCAACGAGCGCAACCCTTGTGGCATGTTGCTAACAGGAAAAGCTGAGGACTCATGCCAGACTGCCGGTGACAAACCGGAGGA
AGGCGGGGATGACGTCAAATCATCATGCCCCTTATGGCCTGGGCTACACACGTACTACAATGGCGGCTACAAAGAGCAGCGAGACAGG
GATGTCGAGCGAATCTCATAAAAGCCGTCCCAGTTCGGATTGGAGGCTGCAACCCGCCTCCATGAAGTTGGAATCGCTAGTAATCGCG
GATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAACCATGGGAGTCGGTAATGCCCGAAGCCGGTGGC
ATGACCTCATAAGAGGAGTGAGCCGTCGAAGGCAGGATCGATGACTGGGGTT

Table SR2. (continued)

>*Bacteroides* OTU 731A clone 1

AGGATGAACGCTAGCTACAGGCTTAACACATGCAAGTCGAGGGGCAGCATGAAAGTTTGCTTGCAAACTTTTGATGGCGACCGGCGCA
CGGGTGAGTAACACGTATCCAACCTGCCTCATACTCGGGGATAGCCTTTCGAAAGAAAGATTAATACCCGATGTTATAGTCCTACCGC
ATGATGGGATTATTAAAGAATTTCGGTATGGGATGGGGATGCGTTCCATTAGTTAGTTGGCGGGGTAACGGCCCACCAAGACAACGAT
GGATAGGGGTTCTGAGAGGAAGGTCCCCCACATTGGAACTGAGACACGGTCCAAACTCCTACGGGAGGCAGCAGTGAGGAATATTGGT
CAATGGACGAGAGTCTGAACCAGCCAAGTAGCGTGAAGGATGACTGCCCTATGGGTTGTAAACTTCTTTTATATGGGAATAAAACGTT
CCACGTGTGGGATTTTGTATGTACCATATGAATAAGGATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATCCGAGCGTT
ATCCGGATTTATTGGGTTTAAAGGGAGCGTAGGTGGATTGTTAAGTCAGTTGTGAAAGTTTGCGGCTCAACCGTAAAATTGCAGTTGA
AACTGGCAGTCTTGAGTACAGTAGAGGTGGGCGGAATTCGTGATGTAGCGGTGAAATGCTTAGATATCACGAAGAACTCCGATTGCGA
AGGCAGTTCACTGGACTGCAACTGACACTGAGGCTCGAAAGTGTGGGTATCAAACAGGATTAGATACCCTGGTAGTCCACACAGTAAA
CGATGAATACTCGCTGTTTGCGATATACAGCAAGCGGCCAAGCGAAAGCATTAAGTATTCCACCTGGGGAGTACGCCGGCAACGGTGA
AACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGAGGAACATGTGGTTTAATTCGATGATACGCGAGGAACCTTACCCGGGCTTAAA
TTGCACCTGAATAATGTGGAAACATGTTAGCCGTAAGGCAGGTGTGAAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTC
GGCTTAAGTGCCATAACGAGCGCAACCCTTATCTTCAGTTACTAACAGGTCATGCTGAGGACTCTGGAGAGACTGCCGTCGTAAGATG
TGAGGAAGGTGGGGATGACGTCAAATCAGCACGGCCCTTACGTCCGGGGCTACACACGTGTTACAATGGGAGGTACAGAAGGCCGCTA
CCTGGCGACAGGATGCCAATCCCCAAAACCTCTCTCAGTTCGGATCGAAGTCTGCAACCCGACTTCGTGAAGCTGGATTCGCTAGTAA
TCGCGCATCAGCCATGGCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGAAAGCCGGGGGTACCTGAAGTA
CGTAACCGCAAGGATCGTCCTAGGGTAAAACTGGTAATTGGGGCT

>*Bacteroides* OTU 731A clone 2

AGGATGAACGCTAGCTACAGGCTTAACACATGCAAGTCGAGGGGCAGCATGAAAGTTTGCTTGCAAACTTTTGATGGCGACCGGCGCA
CGGGTGAGTAACACGTATCCAACCTGCCTCATACTCGGGGATAGCCTTTCGAAAGAAAGATTAATACCCGATGTCATAGTCCTACCGC
ATGATGGGATTATTAAAGAATTTCGGTATGGGATGGGGATGCGTTCCATTAGTTAGTTGGCGGGGTAACGGCCCACCAAGACAACGAT
GGATAGGGGTTCTGAGAGGAAGGTCCCCCACATTGGAACTGAGACACGGTCCAAACTCCTACGGGAGGCAGCAGTGAGGAATATTGGT
CAATGGACGAGAGTCTGAACCAGCCAAGTAGCGTGAAGGATGACTGCCCTATGGGTTGTAAACTTCTTTTATATGGGAATAAAACGTT
CCACGTGTGGAATTTTGTATGTACCATATGAATAAGGATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATCCGAGCGTC
ATCCGGATTTATTGGGTTTAAAGGGAGCGTAGGTGGATTGTTAAGTCAGTTGTGAAAGTTTGCGGCTCAACCGTAAAATTGCAGTTGA
AACTGGCAGTCTTGAGTACAGTAGAGGTGGGCGGAATTCGTGGTGTAGCGGTGAAATGCTTAGATATCACGAAGAACTCCGATTGCGA
GGGCAGTTCACTGGACTGCAACTGACACTGAGGCTCGAAAGTGTGGGTATCAAACAGGATTAGATACCCTGGTAGTCCACACAGTAAA
CGATGAATACTCGCTGTTTGCGATATACAGCAAGCGGCCAAGCGAAAGCATTAAGTATTCCACCTGGGGAGTACGCCGGCAGCGGTGA
AACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGAGGAACATGTGGTTTAATTCGATGATACGCGAGGAACCTTACCCGGGCTTAAA
TTGCACCTGAATAATGTGGAAACATGTTAGCCGTAAGGCAGGTGTGAAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTC
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TGAGGAAGGTGGGGATGACGTCAAATCAGCACGGCCCTTACGTCCGGGGCTACACACGTGTTACAATGGGAGGTACAGAAGGCCGCTA
CCTGGCGACAGGATGCCAATCCCCAAAACCTCTCTCAGTTCGGATCGAAGTCTGCAACCCGACTTCGTGAAGCTGGATTCGCTAGTAA
TCGCGCATCAGCCATGGCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGAAAGCCGGGGGTACCTGAAGTA
CGTAACCGCAAGGATCGTCCTAGGGTAAAACTGGTAATTGGGGCT

>*Bacteroides* OTU 731B clone 1

AGGATGAACGCTAGCTACAGGCTTAACACATGCAAGTCGAGGGGCAGCATGGAAGTTTGCTTGCAAACTTCCGATGGCGACCGGCGCA
CGGGTGAGTAACACGTATCCAACCTGCCGATAACTCGGGGATAGCCTTTCGAAAGAAAGATTAATATCCGATAGCATATCAAGGTCGC
ATGATCCTGATATTAAAGAATTTCGGTTATCGATGGGGATGCGTTCCATTAGTTAGTTGGCGGGGTAACGGCCCACCAAGACGACGAT
GGATAGGGGTTCTGAGAGGAAGGTCCCCCACATTGGAACTGAGACACGGTCCAAACTCCTACGGGAGGCAGCAGTGAGGAATATTGGT
CAATGGACGAGAGTCTGAACCAGCCAAGTAGCGTGAAGGATGACTGCCCTATGGGTTGTAAACTTCTTTTATATGGGAATAAAACAGG
GTATGCATACCCTCTTGTATGTACCATATGAATAAGGATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATCCGAGCGTT
ATCCGGATTTATTGGGTTTAAAGGGAGCGTAGGTGGATTGTTAAGTCAGTTGTGAAAGTTTGCGGCTCAACCGTAAAATTGCAGTTGA
AACTGGCAGTCTTGAGTACAGTAGAGGTGGGCGGAATTCGTGGTGTAGCGGTGAAATGCTTAGATATCACGAAGAACTCCGATTGCGA
AGGCAGCTCACTAGACTGCAACTGACACTGATGCTCGAAAGTGTGGGTATCAAACAGGATTAGATACCCCGGTAGTCCACACAGTAAA
CGATGAATACTCGCTGTTTGCGATATACAGTAAGCGGCCAAGCGAAAGCATTAAGTATTCCACCTGGGGAGTACGCCGGCAACGGTGA
AACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGAGGAACATGTGGTTTAATTCGATGATACGCGAGGAACCTTACCCGGGCTTAAA
TTGCACCTGAATAACGTGGAAACATGTTAGCCGCAAGGCAGGTGTGAAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTC
GGCTTAAGTGCCATAACGAGCGCAACCCTTATCTTTAGTTACTAACAGGTCATGCTGAGGACTCTAGAGAGACTGCCGTCGTAAGATG
TGAGGAAGGTGGGGATGACGTCAAATCAGCACGGCCCTTACGTCCGGGGCTACACACGTGTTACAATGGGGGGTACAGAAGGCCGCTA
CCTGGCGACAGGATGCTAATCCCAAAAACCTCTCTCAGTTCGGATCGAAGTCTGCAACCCGACTTCGTGAAGCTGGATTCGCTAGTAA
TCGCGCATCAGCCATGGCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGAAAGCCGGGGGTACCTGAAGTA
CGTAACCGCAAGGAGCGTCCTAGGGTAAAACTGGTAATTGGGGCT

Table SR2. (continued)

>*Bacteroides* OTU 731B clone 2

AGGATGAACGCTAGCTACAGGCTTAACACATGCAAGTCGAGGGGCAGCATGGGAGTTTGCTTGCAAACTTCCGATGGCGACCGGCGCA
CGGGTGAGTAACACGTATCCAACCTGCCGATAACTCGGGGATAGCCTTTCGAAAGAAAGATTAATATCCGATAGGATATCAAGGTCGC
ATGATCTTGATATTAAAGAATTTCGGTTATCGATGGGGATGCGTTCCATTAGTTAGTTGGCGGGGTAACGGTCCACCAAGACGACGAT
GGATAGGGGTTCTGAGAGGAAGGTCCCCCACATTGGAACTGAGACACGGTCCAAACTCCTACGGGAGGCAGCAGTGAGGAATATTGGT
CAATGGACGAGAGTCTGAACCAGCCAAGTAGCGTGAAGGATGACTGCCCTATGGGTTGTAAACTTCTTTTATATGGGAATAAAACAGG
GTATGCATACCCTCTTGTATGTACCATATGAATAAGGATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATCCGAGCGTT
ATCCGGATTTATTGGGTTTAAAGGGAGCGTAGGTGGATTGTTAAGTCAGTTGTGAAAGTTTGCGGCTCAACCGTAAAATTGCAGTTGA
AACTGGCAGTCTTGAGTACAGTAGAGGTGGGCGGAATTCGTGGTGTAGCGGTGAAATGCTTAGATATCACGAAGAACTCCGATTGCGA
AGGCAGCTCACTGGACTGCAACTGACACTGATGCTCGAAAGTGTGGGTATCAAACAGGATTAGATACCCTGGTAGTCCACACAGTAAA
CGATGAATACTCGCTGTTTGCGATATACGGTAAGCGGCCAAGCGAAAGCATTAAGTATTCCACCTGGGGAGTACGCCGGCAACGGTGA
AACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGAGGAACATGTGGTTTAATTCGATGATACGCGAGGAACCTTACCCGGGCTTAAA
TTGCACCTGAATAACGTGGAGACATGTTAGCCGCAAGGCAGGTGTGAAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTC
GGCTTAAGTGCCATAACGAGCGCAACCCTTATCTTTAGTTACTAACAGGTTCTGCTGAGGACTCTAGAGAGACTGCCGTCGTAAGATG
TGAGGAAGGTGGGGATGACGTCAAATCAGCACGGCCCTTACGTCCGGGGCTACACACGTGTTACAATGGGGGGTACAGAAGGCAGCTA
CCTGGCGACAGGATGCTAATCCCCAAAACCTCTCTCAGTTCGGATCGAAGTCTGCAACCCGACTTCGTGAAGCTGGATTCGCTAGTAA
TCGCGCATCAGCCATGGCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGAAAGCCGGGGGTACCTGAAGTA
CGTAACCGCGAGGAGCGTCCTAGGGTAAAACTGGTAATTGGGGCT

>*Ruminococcaceae* OTU 14644A clone 1

AGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGGGCACCCCTGAAGGAGTTTTCGGACAACGGATGGGAATGCTTAG
TGGCGAACTGGTGAGTAACGCGTGAGGAACCTGCCTTTCGGAGGGGGACAACAGTTGGAAACGACTGCTAATACCGCATGATACATCG
AAGCCGCATGGTTTTGATGTCAAAGATTTATCGCCGAAAGATGGCCTCGCGTCTGATTAGCTTGTTGGTGAGGTAACGGCTCACCAAG
GCGACGATCAGTAGCCGGACTGAGAGGTTGGCCGGCCACATTGGGACTGAGATACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGA
ATATTGGGCAATGGGCGCAAGCCTGACCCAGCAACGCCGCGTGAAGGAAGAAGGCTTTCGGGTTGTAAACTTCTTTTAAGGGGGAAGA
GCAGAAGACGGTACCCCTTGAATAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTT
ACTGGGTGTAAAGGGCGTGCAGCCGGAGAGGCAAGTCAGATGTGAAATCCGCGGGCTCAACCCGCGAACTGCATTTGAAACTGCTTCC
CTTGAGTATCGGAGAGGTAACCGGAATTCCTAGTGTAGCGGTGAAATGCGTAGATATTAGGAAGAACACCAGTGGCGAAGGCGGGTTA
CTGGACGACAACTGACGGTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATCGATAC
TAGGTGTGCGGGGACTGACCCCCTGCGTGCCGGAGTTAACACAATAAGTATCGCACCTGGGGAGTACGATCGCAAGGTTGAAACTCAA
AGGAATTGACGGGGGCCCGCACAAGCGGTGGATTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGGCTTGACATCCTGC
TAACGAAGTAGAGATACATTAGGTGCCCTTCGGGGAAAGCAGAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTG
GGTTAAGTCCCGCAACGAGCGCAACCCCTATTGTTAGTTGCTACGCAAGAGCACTCTAGCGAGACTGCCGTTGACAAAACGGAGGAAG
GCGGGGACGACGTCAAATCATCATGCCCCTTATGTCCTGGGCTACACACGTAATACAATGGCGGTAAACAAAGGGATGCGAATCCGCG
AGGAGGAGCGAACCCCCAAAAGCCGTCCCAGTTCGGATCGCAGGCTGCAACCCGCCTGCGTGAAGTCGGAATCGCTAGTAATCGCGGA
TCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTCGGGAACACCCGAAGTCCGTAGCCT
AACCGCAAGGAGGGCGCGGCCGAAGGTGGGTTCGATAATTGGGGTG

>*Ruminococcaceae* OTU 14644B clone 1

AGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGAGTGCCTTGGAAAGAGATTTCGGTCAATGGAAGAGGTTACTTAG
TGGCGGACGGGTGAGTAACGCGTGAGGAACCTGCCTTTCAGAGGGGGACAACAGTTGGAAACGACTGCTAATACCGCATAATATATAT
TTGTCGCATGGCAGATATATCAAAGATTTATCGCTGAGAGATGGCCTCGCGTCTGATTAGCTAGTTGGTAGGGTAACGGCCTACCAAG
GCGACGATCAGTAGCCGGACTGAGAGGTTGGCCGGCCACATTGGGACTGAGATACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGA
ATATTGGGCAATGGGCGCAAGCCTGACCCAGCAACGCCGCGTGAAGGAAGAAGGCTTTCGGGTTGTAAACTTCTTTTAAGAGGGAAGA
GCAGAAGACGGTACCTCTTGAATAAGCTCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGAGCGAGCGTTGTCCGGATTT
ACTGGGTGTAAAGGGCGTGCAGCCGGGAGTACAAGTCAGATGTGAAATCCCGCGGCTCAACCGCGGAACTGCATTTGAAACTGTATTT
CTTGAGTACTGGAGAGGCAGACGGAATTCCTAGTGTAGCGGTGAAATGCGTAGATATTAGGAGGAACACCAGTGGCGAAGGCGGTCTG
CTGGACAGCAACTGACGGTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGGATAC
TAGGTGTGCGGGGACTGACCCCCTGCGTGCCGCAGTTAACACAATAAGTATCCCACCTGGGGAGTACGATCGCAAGGTTGAAACTCAA
AGGAATTGACGGGGGCCCGCACAAGCGGTGGATTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCAGGGCTTGACATCCTGC
TAACGAGGTAGAGATACGTCAGGTGCCCTTCGGGGAAAGCAGAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTG
GGTTAAGTCCCGCAACGAGCGCAACCCTTATTGTTAGTTGCTACGCAAGAGCACTCTAGCGAGACTGCCGTTGACAAAACGGAGGAAG
GTGGGGACGACGTCAAATCATCATGCCCCTTATGTCCTGGGCTACACACGTAATACAATGGCGGTCAACAGAGGGATGCAAAACCGTG
AGGTGGAGCGAACCCCTAAAAGCCGTCCCAGTTCAGATCGCAGTCTGCAACCCGACTGCGTGAAGTCGGAATCGCTAGTAATCGCGGA
TCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTCGGGAACACCCGAAGTCCGTAGCCT
AACCGCAAGGAGGGCGCGGCCGAAGGTGGGTTCGATAATTGGGGTG

Table SR2. (continued)

>*Ruminococcaceae* OTU 17903 clone 1

AGGACGAACGCTGGCGGCACGCCTAACACATGCAAGTCGAACGGTGAAGGGGGAGCTTGCTCCCCCGGAACAGTGGCGGACGGGTGAG
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AATACCAAAGGAGCAATCCGCTGAAAGATGGGCTCGCGTCTGATTAGATAGTTGGTGAGGTAACGGCTCACCAAGTCGACGATCAGTA
GCCGGACTGAGAGGTTGAACGGCCACATTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGGATATTGGACAATG
GGGGAAACCCTGATCCAGCGATGCCGCGTGAGGGAAGAAGGTTTTCGGATTGTAAACCTCTGTGGAGGGGGGCGATAATGACGGTACC
CCCTTAGGAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCGAGCGTTGTCCGGAATTACTGGGTGTAAAGGG
AGTGTAGGCGGGAAAGCAAGTCAGAAGTGAAAACTATGGGCTTAACCCATAGCCTGCTTTTGAAACTGTTTTTCTTGAGTGAAGTAGA
GGCAAGCGGAATTCCAAGTGTAGCGGTGAAATGCGTAGATATTAGGAGGAACACCAGTGGCGAAGGCGGCTTGCTGGGCTTTTACTGA
CGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATCACTAGGTGTGGGGAGCG
AACCTTTCCGTGCCGGAGTAAACACAATAAGTGATCCACCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCC
CGCACAAGCAGTGGAGTATGTGGTCTAATTCGAAGCAACGCGAAGAACCTTACCGGGTCTTGACATCGAGTGAAAGGTCAAGAGATTG
ATCCCTCTCTTCGGAGACACGAAGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC
GCAACCCTTATCATTAGTTGCTACGCGAGAGCACTCTAATGAGACTGCCGTTGACAAAACGGAGGAAGGTGGGGATGACGTCAAATCA
TCATGCCCTTTATGACCCGGGCTACACACGTACTACAATGGTGTTTAACAAAGAGAAGCGAAGCCGCGAGGCGGAGCAAATCTCGAAA
AAACATCTCAGTTCAGATTGCAGGCTGCAACTCGCCTGCATGAAGTCGGAATTGCTAGTAATCGTAGGTCAGCATACTACGGTGAATA
CGTTCCCGGGCCTTGTACACACCGCCCGTCAAACCATGAGAGTTGGCAACACCCGAAGTCGGTAGTCTAACCGCAAGGAGGACGCCGC
CGAAGGTGGGGTTGATGATTAGGGTT

>*Ruminococcaceae* OTU 17903 clone 2

AGGACGAACGCTGGCGGCACGCCTAACACATGCAAGTCGAACGGTGAAGGGGGAGCTTGCTCCTCCGGAACAGTGGCGGACGGGTGAG
TAACACGTGAGCAACCTGCCTTTCAGAGGGGGACAACAGTTGGAAACGACTGCTAATACCGCATAATGTATTTTGGCGGCATCGCCGG
AATACCAAAGGAGCAATCCGCTGAAAGATGGGCTCGCGTCTGATTAGATAGTTGGTGAGGTAACGGCTCACCAAGTCGACGATCAGTA
GCCGGACTGAGAGGTTGAACGGCCACATTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGGATATTGGACAATG
GGGGAAACCCTGATCCAGCGATGCCGCGTGAGGGAAGAAGGTTTTCGGATTGTAAACCTCTGTGGAGGGGGGGCGATAATGACGGTAC
CCCTTTAGGAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCGAGCGTTGTCCGGAATTACTGGGTGTAAAGG
GAGTGTAGGCGGGAAAGCAAGTCAGAAGTGAAAACTATGGGCTTAACCCATAGCCTGCTTTTGAAACTGTTTTTCTTGAGTGAAGTAG
AGGCAAGCGGAATTCCTAGTGTAGCGGTGAAATGCGTAGATATTAGGAGGAACACCAGTGGCGAAGGCGGCTTGCTGGGCTTTTACTG
ACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATCACTAGGTGTGGGGAGC
GAACCTTTCCGTGCCGGAGTAAACACAATAAGTGATCCACCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAACTGACGGGGGC
CCGCACAAGCAGTGGAGTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCGGGTCTTGACATCGAGTGAAAGGTCAAGAGATT
GATCCCTCTCTTCGGAGACACGAAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG
CGCAACCCTTATCATTAGTTGCTACGCAAGAGCACTCTAATGAGACTGCCGTTGACAAAACGGAGGAAGGTGGGGATGACGTCAAATC
ATCATGCCCTTTATGACCCGGGCTACACACGTACTACAATGGTGTTTAACAAAGAGAAGCGAAGCCGCGAGGCGGAGCAAATCTCGAA
AAAACATCTCAGTTCAGATTGCAGGCTGCAACTCGCCTGCATGAAGTCGGAATTGCTAGTAATCGTAGGTCAGCATACTACGGTGAAT
ACGTTCCCGGGCCTTGTACACACCGCCCGTCAAACCATGAGAGTTGGCAACACCCGAAGTCGGTAGTCTAACCGCAAGGAGGACGCCG
CCGAAGGTGGGGTTGATGATTAGGGTT

>*Ruminococcaceae* OTU 17903 clone 3

AGGATGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACGGTGAAGGGGGAGCTTGCTCCCCCGGAACAGTGGCGGACGGGTGAG
TAACACGTGAGCAACCTGCCTTTCAGAGGGGGACAACAGTTGGAAACGACTGCTAATACCGCATAATGTATTTTGGCGGCATCGCCGG
AATACCAAAGGAGCAATCCGCTGAAAGATGGGCTCGCGTCTGATTAGATAGTTGGTGAGGTAACGGCTCACCAAGTCGACGATCAGTA
GCCGGACTGAGAGGTTGAACGGCCACATTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGGATATTGGACAATG
GGGGAAACCCTGATCCAGCGATGCCGCGTGAGGGAAGAAGGTTTTCGGATTGTAAACCTCTGTGGAGGGGGGCGATAATGACGGTACC
CCTTTAGGAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCGAGCGTTGTCCGGAATTACTGGGTGTAAAGGG
AGTGTAGGCGGGAAAGCAAGTCAGAAGTGAAAACTATGGGCTTAACCCATAGCCTGCTTTTGAAACTGTTTTTCTTGAGTGAAGTAGA
GGCAAGCGGAATTCCTAGTGTAGCGGTGAAATGCGTAGATATTAGGAGGAACACCAGTGGCGAAGGCGGCTTGCTGGGCTTTTACTGA
CGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATCACTAGGTGTGGGGAGCG
AACCTTTCCGTGCCGGAGTAAACACAATAAGTGATCCACCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCC
CGCACAAGCAGTGGAGTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCGGGTCTTGACATCGAGTGAAAGGTCAAGAGATTG
ATCCCTCTCTTCGGAGACACGAAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC
GCAACCCTTATCATTAGTTGCTACGCGAGAGCACTCTAATGAGACTGCCGTTGACAAAACGGAGGAAGGTGGGGATGACGTCAAATCA
TCATGCCCTTTATGACCCGGGCTACACACGTACTACAATGGTGTTTAACAAAGAGAAGCGAAGCCGCGGGGCGGAGCAAATCTCGAAA
AAACATCTCAGTTCAGATTGCAGGCTGCAACTCGCCTGCATGAAGTCGGAATTGCTAGTAATCGTAGGTCAGCATACTACGGTGAATA
CGTTCCCGGGCCTTGTACACACCGCCCGTCAAACCATGAGAGTTGGCAACACCCGAAGTCGGTAGTCTAACCGCAAGGAGGACGCCGC
CGAAGGTGGGGTTGATGATTAGGGTT

Table SR2. (continued)

>*Ruminococcaceae* OTU 17903 clone 4

AGGACGAACGCTGGCGGCACGCCTAACACATGCAAGTCGAACGGTGAAGGGGGAGCTTGCTCCCCCGGAACAGTGGCGGACGGGTGAG
TAACACGTGAGCAACCTGCCTTTCAGAGGGGGACAACAGTTGGAAACGACTGCTAATACCGCATAATGTATTTTGGCGGCATCGCCGG
AATACCAAAGGAGCAATCCGCTGAAAGATGGGCTCGCGTCTGATTAGATAGTTGGTGAGGTAACGGCTCACCAAGTCGACGATCAGTA
GCCGGACTGAGAGGTTGAACGGCCACATTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGGATATTGGACAATG
GGGGAAACCCTGATCCAGCGATGCCGCGTGAGGGAAGAAGGTTTTCGGATTGTAAACCTCTGTGGAGGGGGGCGATAATGACGGTACC
CCTTTAGGAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCGAGCGTTGTCCGGAATTACTGGGTGTAAAGGG
AGTGTAGGCGGGAAAGCAAGTCAGAAGTGAAAACTATGGGCTTAACCCATAGCCTGCTTTTGAAACTGTTTTTCTTGAGTGAAGTAGA
GGCAAGCGGAATTCCTAGTGTAGCGGTGAAATGCGTAGATATTAGGAGGAACACCAGTGGCGAAGGCGGCTTGCTGGGCTTTTACTGA
CGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATCACTAGGTGTGGGGAGCG
AACCTTTCCGTGCCGGAGTAAACACAATAAGTGATCCACCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCC
CGCACAAGCAGTGGAGTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCGGGTCTTGACATCGAGTGAAAGGTCAAGAGATTG
ATCCCTCTCTTCGGAGACACGAAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC
GCAACCCTTATCATTAGTTGCTACGCAAGAGCACTCTAATGAGACTGCCGTTGACAAAACGGAGGAAGGTGGGGATGACGTCAAATCA
TCATGCCCTTTATGACCCGGGCTACACACGTACTACAATGGTGTTTAACAAAGAGAAGCGAAGCCGCGAGGCGGAGCAAATCTCGAAA
AAACATCTCAGTTCAGATTGCAGGCTGCAACTCGCCTGCGTGAAGTCGGAATTGCTAGTAATCGTAGGTCAGCATACTACGGTGAATA
CGTTCCTGGGCCTTGTACACACCGCCCGTCAAACCATGAGAGTTGGCAACACCCGAAGTCGGTAGTCTAACCGCAAGGAGGACGCCGC
CGAAGGTGGGGTTGATGATTAGGGTT

>*Lachnospiraceae* OTU 5944 clone 1

AGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGGGATGCATCAAGAAGGAGTGCTTGCATTCCGGAAGGATGTATCT
TAGTGGCGGACGGGTGAGTAACGCGTGGGTAACCTGCCCCATACCGGGGGATACCACCTGGAAACAGGTGCTAATACCGCATAAGCGC
ACGGGAGCGCATGCTCCTGTGTGAAAAACTCCGGTGGTATGGGATGGACCCGCGTCTGATTAGCCAGTTGGCAGGGTAACGGCCTACC
AAAGCGACGATCAGTAGCCGGCCTGAGAGGGTGGACGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGG
GGAATATTGCACAATGGGGGAAACCCTGATGCAGCGACGCCGCGTGAGTGAAGAAGTATTTCGGTATGTAAAGCTCTATCAGCAGGAA
CGAGACAAGACGGTACCTGACTAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTATCCGGAA
TCACTGGGTGTAAAGGGAGCGTAGACGGCTGTGCAAGCCTGAAGTGAAAGGCGGGGGCCCAACCCCCGGACTGCTTTGGGAACTGTAC
GGCTGGAGTGCAGGAGAGGTAAGTGGAATTCCTAGTGTAGCGGTGAAATGCGTAGATATTAGGAGGAACACCAGTGGCGAAGGCGGCT
TACTGGACTGTAACTGACGTTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATT
ACTAGGTGTCGGGGGGCAGAGGCCCTTCGGTGCCGCAGCAAACGCAATAAGTAATCCACCTGGGGAGTACGTCCGCAAGGATGAAACT
CAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCCGGCCTTGACATCC
CCCTGACAGTGTATGTAATGTACATTCCCTTCGGGGCAGGGGAGACAGGTGGTGCATGGTTGTCGTCAGCTCCGTGTCGTGAGATGTT
GGGTTAAGTCCCGCAACGAGCGCAACCCTTACCCACAGTAGCCAGCACAAAAGGTGGGCACTCTGTGGGGACTGCCGGGGACAACCCG
GAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGGCCGGGGCTACACACGTGCTACAATGGCGTAAACAAAGGGAAGCGAG
AGAGTGATCTTAAGCGAAACTCAAAAATAACGTCCCAGTTCGGATTGCAGTCTGCAACTCGACTGCATGAAGCTGGAATCGCTAGTAA
TCGCGGATCAGAATGCCGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGCCGGGAATGCCCGAAGTCG
GTGACCTAACCTGAAAAGGGAGGAGCCGCCGAAGGCAGGTCTGGTAACTGGGGTG

>*Lachnospiraceae* OTU 5944 clone 2

AGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGGGATGCATTAAGAAGGAGTGCTTGCATTCCGGAAGGATGTATCT
TAGTGGCGGACGGGTGAGTAACGCGTGGGTAACCTGCCCCATACCGGGGGATACCACCTGGAAACAGGTGCTAATACCGCATAAGCGC
ACGGGAGCGCATGCTCCTGTGTGAAAAACTCCGGTGGTATGGGATGGACCCGCGTCTGATTAGCCAGTTGGCAGGGTAACGGCCTACC
AAAGCGACGATCAGTAGCCGGCCTGAGAGGGTGGACGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGG
GGAATATTGCACAATGGGGGAAACCCTGATGCAGCGACGCCGCGTGAGTGAAGAAGTATTTCGGTATGTAAAGCTCTATCAGCAGGAA
CGAGACAAGACGGTACCTGACTAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTATCCGGAA
TCACTGGGTGTAAAGGGAGCGTAGACGGCTGTGCAAGCCTGAAGTGAAAGGCGGGGGCCCAACCCCTGGACTGCTTTGGGAACTGTAC
GGCTGGAGTGCAGGAGAGGTAAGTGGAATTCCTAGTGTAGCGGTGAAATGCGTAGATATTAGGAGGAACACCAGTGGCGAAGGCGGCT
TACTGGACTGTAACTGACGTTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATT
ACTAGGTGTCGGGGGGCAGAGGCCCTTCGGTGCCGCAGCAAACGCAATAAGTAATCCACCTGGGGAGTACGTCCGCAAGGATGAAACT
CAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCCGGCCTTGACATCC
CCCTGACAGCATATGTAATGTATGTTCCCTTCGGGGCAGGGGAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTG
GGTTGAGTCCCGCAACGAGCGCAACCCTTACCCACAGTAGCCAGCACAAAAGGTGGGCACTCTGTGGGGACTGCCGGGGACAACCCGG
AGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGGCCGGGGCTACACACGTGCTACAATGGCGTAAACAAAGGGAAGCGAGA
GAGTGATCTTAAGCGAAACTCAAAAATAACGTCCCAGTTCGGATTGCAGTCTGCAACTCGACTGCATGAAGCTGGAATCGCTAGTAAT
CGCGGATCAGAATGCCGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGCCGGGAATGCCCGAAGTCGG
TGACCTAACCCAAAAACGAACAAGTTCGTCTGGGGAGGAGCCGCCGAAGGCAGGTCTGGTAACTGGGGTG

Table SR2. (continued)

>*Bacteroidales* "*cluster S24-7*" OTU 11839 clone 1

AGGATGAACGCTAGCGACAGGCCTAACACATGCAAGTCGAGGGGCAGCGGGGGAGTAGCAATACTCCTGCCGGCGACCGGCGCACGGG
TGAGTAACACGTATGGAACCTGCCCGCCACAGGGGGATAAGCGGAAGAAATTCCGTCTAATACCGCATAACGTCCTGCCGGGGCATCC
CGGCGGGACCAAAGGGTAACACCGGTGACGGATGGCCATGCGCCGCATTAGCTGGTTGGCGGGGTAACGGCCCACCAAGGCGACGATG
CGTAGGGGTTCTGAGAGGAAGGTCCCCCACACTGGTACTGAGACACGGACCAGACTCCTACGGGAGGCAGCAGTGAGGAATATTGGTC
AATGGGCGAGAGCCTGAACCAGCCAAGTCGCGTGAGGGAAGACGGCCCTACGGGTTGTAAACCTCTTTTGTCGGGGAGCAAAGAGCGG
GACGCGTCCCGCGTCGAGAGTACCCGAAGAAAAAGCATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATGCGAGCGTTA
TCCGGATTTATTGGGTTTAAAGGGTGCGTAGGCGGGTTGTTAAGTCAGCGGTAAAATTTGGAGGCTCAACCTCCACGAGCCGTTGAAA
CTGGCGGTCTTGAGTGGGCGAGAAGTGTGCGGAATGCGTGGTGTAGCGGTGAAATGCATAGATATCACGCAGAACCCCGATTGCGAAG
GCAGCATACCGGTGCACAACTGACGCTCAGGCACGAAAGCGTGGGTATCGAACAGGATTAGATACCCTGGTAGTCCACGCAGTAAACG
ATGAATGCTACCTGTTTGGCCAGATTGAAGGCTGAGCGGGACAGCGAAAGCGTTAAGCATTCCACCTGGGGAGTACGCCGGCAACGGT
GAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGAGGAACATGTGGTTTAATTCGATGATACGCGAGGAACCTTACCCGGGCTCA
AACGCATAATGACGACAGTTGAAAGATTGTTTCCCTTCGGGGCATTATGCGAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGTGAGG
TGTCGGCTTAAGTGCCATAACGAGCGCAACCCCTATCGACAGTTGCTAACGAGTCGAGTCGAGGACTCTGTCGAGACTGCCGGCGCAA
GCTGTGAGGAAGGCGGGGATGACGTCAAATCAGCACGGCCCTTACGTCCAGGGCGACACACGTGTTACAATGGCAGGGACAGCGGGAA
GCCACCAAGCGATTGGGCGCGGAACCCGAAACCCTGTCTCAGTTCGGATTGGAGTCTGCAACTCGACTCCATGAAGCTGGATTCGCTA
GTAATCGCGCATCAGCCATGGCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGGGAGTCGGGAGTGCCTGA
AGTTCGTGACCGTATAGGAGCGACCTAGGGCAAGACTGATGACTGGGGCT

>*Bacteroidales* "*cluster S24-7*" OTU 15326 clone 1

AGGATGAACGCTAGCGGCAGGCTTAACACATGCAAGTCGAGGGGCATCGGGAGGAAAGCTTGCTTTCCTTGCCGGCGACCGGCGCACG
GGTGAGTAACACGTATGCAACCTGCCCTCTTCAGGGGGACAACCTTCCGAAAGGGAGGCTAATCCCGCGTATATCGGTTTCGGGCATC
CGAGATCGAGGAAAGATTCATCGGAAGAGGATGGGCATGCGGCGCATTAGCTAGACGGCGGGGTAACGGCCCACCGTGGCGACGATGC
GTAGGGGTTCTGAGAGGAAGGTCCCCCACACTGGTACTGAGACACGGACCAGACTCCTACGGGAGGCAGCAGTGAGGAATATTGGTCA
ATGGGAGAGATCCTGAACCAGCCAAGCCGCGTGAGGGAAGACGGCCCTATGGGTTGTAAACCTCTTTTGTCGGAGAACAAAACCCGGG
ACGAGTCCCGGACTGCGTGTATCCGAAGAAAAAGCATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATGCGAGCGTTAT
CCGGATTTATTGGGTTTAAAGGGTGCGTAGGCGGATTTTTAAGTCAGCGGTAAAATGTCCGGGCTCAACCCGGGCCGGCCGTTGAAAC
TGGGGATCTTGAGTGGGCGAGAAGTATGCGGAATGCGTGGTGTAGCGGTGAAATGCATAGATATCACGCAGAACTCCGATTGCGAAGG
CAGCATACCGGCGCCCTACTGACGCTGAGGCACGAAAGCGTGGGTATCGAACAGGATTAGATACCCTGGTAGTCCACGCAGTAAACGA
TGAATGCTAGCTGTCCGGTTCTACGGGACTGGGTGGCACAGCGAAAGCGTTAAGCATTCCACCTGGGGAGTACGCCGGCAACGGTGAA
ACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGAGGAACATGTGGTTTAATTCGATGATACGCGAGGAACCTTACCCGGGCTCAAAC
GCAACCGGAATGTATCCGAAAGGGTACAGCTCTACGGAGTCTGTTGCGAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGTGAGGTGT
CGGCTTAAGTGCCATAACGAGCGCAACCCCTATCGACAGTTGCTAACGAGTTAAGTCGAGGACTCTGTCGAGACTGCCGGCGCAAGCT
GTGAGGAAGGCGGGGATGACGTCAAATCAGCACGGCCCTTACGTCCGGGGCGACACACGTGTTACAATGGCGACTACAGCGGGAAGCC
ACTTGGCGACAAGGAGCGGAACCCGAAAAGTCGTCTCAGTTCGGATTGGAGTCTGCAACTCGACTCCATGAAGCTGGATTCGCTAGTA
ATCGCGCATCAGCCATGGCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGGAAGCCGGGGGTGCCTGAAGT
GCGTGACCGCAAGGAGCGCCCTAAGGTAAAACCGGTGACTGGGGCT

>*Bacteroidales* "*cluster S24-7*" OTU 15326 clone 2

AGGATGAACGCTAGCGGCAGGCTTAACACATGCAAGTCGAGGGGCATCGGGAGGAAAGCTTGCTTTCCTTGCCGGCGACCGGCGCACG
GGTGAGTAACACGTATGCAACCTGCCCTCTTCAGGGGGACAACCTTCCGAAAGGGAGGCTAATCCCGCGTATATCGGTTTCGGGCATC
CGAGATCGAGGAAAGATTCATCGGAAGAGGATGGGCATGCGGCGCATTAGCTAGACGGCGGGGTAACGGCCCACCGTGGCGACGATGC
GTAGGGGTTCTGAGAGGAAGGTCCCCCACACTGGTACTGAGACACGGACCAGACTCCTACGGGAGGCAGCAGTGAGGAATATTGGTCA
ATGGGAGAGATCCTGAACCAGCCAAGCCGCGTGAGGGAAGACGGCCCTATGGGTTGTAAACCTCTTTTGTCGGAGAACAAAACCCGGG
ACGAGTCCCGGACTGCGTGTATCCGAAGAAAAAGCATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATGCGAGCGTTAT
CCGGATTTATTGGGTTTAAAGGGTGCGTAGGCGGATTTTTAAGTCAGCGGTAAAATGTCCGGGCTCAACCCGGGCCGGCCGTTGAAAC
TGGGGATCTTGAGTGGGCGAGAAGTATGCGGAATGCGTGGTGTAGCGGTGAAATGCATAGATATCACGCAGAACTCCGATTGCGAAGG
CAGCATACCGGCGCCCTACTGACGCTGAGGCACGAAAGCGTGGGTATCGAACAGGATTAGATACCCTGGTAGTCCACGCAGTAAACGA
TGAATGCTAGCTGTCCGGTTCTACGGGACTGGGTGGCACAGCGAAAGCGTTAAGCATTCCACCTGGGGAGTACGCCGGCAACGGTGAA
ACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGAGGAACATGTGGTTTAATTCGATGATACGCGAGGAACCTTACCCGGGCTCAAAC
GCAACCGGAATGTATCCGAAAGGGTACAGCTCTACGGAGTCTGTTGCGAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGTGAGGTGT
CGGCTTAAGTGCCATAACGAGCGCAACCCCTATCGACAGTTGCTAACGAGTTAAGTCGAGGACTCTGTCGAGACTGCCGGCGCAAGCT
GTGAGGAAGGCGGGGATGACGTCAAATCAGCACGGCCCTTACGTCCGGGGCGACACACGTGTTACAATGGCGACTACAGCGGGAAGCC
ACTTGGCGACAAGGAGCGGAACCCGAAAAGTCGTCTCAGTTCGGATTGGAGTCTGCAACTCGACTCCATGAAGCTGGATTCGCTAGTA
ATCGCGCATCAGCCATGGCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGGAAGCCGGGGGTGCCTGAAGT
GCGTGACCGCAAGGAGCGCCCTAAGGTAAAACCGGTGACTGGGGCT

Table SR2. (continued)

>*Bacteroidales* "*cluster S24-7*" OTU 13481 clone 1

AGGATGAACGCTAGCGACAGGCTTAACACATGCAAGTCGAGGGGCAGCGGGGGAGTAGCAATACTCCCGCCGGCGACCGGCGCACGGG
TGAGTAACACGTATGAAACCTGCCCGTAGCAGGGGGATAAGCGGAAGAAATTCCGTCTAATACCGCGTGACAACCCATGGAGGCATCT
CCGTGGGTTTAAAGGAAGCGATTCCGGCTACGGATGGTCATGCGTCGCATTAGCTTGTTGGCGGGGTAACGGCCCACCAAGGCGACGA
TGCGTAGGGGTTCTGAGAGGAAGGTCCCCCACACTGGTACTGAGACACGGACCAGACTCCTACGGGAGGCAGCAGTGAGGAATATTGG
TCAATGGCCGCAGGGCTGAACCAGCCAAGTCGCGTGAGGGATGACGGTCCTATGGATTGTAAACCTCTTTTGTCAGGGAGCAAAGGGC
GCCACGTGTGGCGTTTTGCGAGTACCTGAAGAAAAAGCATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATGCGAGCGT
TATCCGGGTTTATTGGGTTTAAAGGGTGCGCAGGCGGAATGTCAAGTCAGCGGTAAAATTTCGGGGCTCAACCCCGTCGTGCCGTTGA
AACTGGCGTTCTTGAGTGAGCGAGAAGTATGCGGAATGCGTGGTGTAGCGGTGAAATGCATAGATATCACGCAGAACTCCGATTGCGA
AGGCAGCATACCGGCGCTCAACTGACGCTCATGCACGAAAGCGTGGGTATCGAACAGGATTAGATACCCTGGTAGTCCACGCAGTAAA
CGATGAATACTAACTGTCCGGGCAGAATGATGCCTGGGTGGTACAGCGAAAGCGTTAAGTATTCCACCTGGGGAGTACGCCGGCAACG
GTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGAGGAACATGTGGTTTAATTCGATGATACGCGAGGAACCTTACCCGGGCT
CAAACGACGGATGGATGTTTCTGAAAGGAGGCAGTCCTACGGGACATCCGTCGAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGTGA
GGTGTCGGCTTAAGTGCCATAACGAGCGCAACCCCCATCGCCAGTTACCAGCAAGTAAAGTTGGGGACTCTGGCGAGACTGCCGGCGC
AAGCTGTGAGGAAGGCGGGGATGACGTCAAATCAGCACGGCCCTTACGTCCGGGGCGACACACGTGTTACAATGGCAGGTACAGAGAG
AAGCGATGCGGCGACGCAGAGCGGAACTTCAAAGCCTGTCTCAGTTCGGATTGGAGTCTGCAACCCGACTCCATGAAGCTGGATTCGC
TAGTAATCGCGCATCAGCCACGGCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGGAAGCCGGGAGTGCCT
GAAGTGTGCAACCGCAAGGAGCGCCCTAAGGTAAAACCGGTGACTGGGGCT

>*Bacteroidales* "*cluster S24-7*" OTU 13481 clone 2

AGGATGAACGCTAGCGACAGGCTTAACACATGCAAGTCGAGGGGCAGCGGGGGAGTAGCAATACTCCCGCCGGCGACCGGCGCACGGG
TGAGTAACACGTATGAAACCTGCCCGTAGCAGGGGGATAAGCGGAAGAAATTCCGTCTAATACCGCGTAACAACCCATGGAGGCATCT
CCGTGGGTTTAAAGGAAGCGATTCCGGCTACGGATGGTCATGCGTCGCATTAGCTTGTTGGCGGGGTAACGGCCCACCAAGGCGACGA
TGCGTAGGGGTTCTGAGAGGAAGGTCCCCCACACTGGTACTGAGACACGGACCAGACTCCTACGGGAGGCAGCAGTGAGGAATATTGG
TCAATGGCCGCAGGGCTGAACCAGCCAAGTCGCGTGAGGGATGACGGTCCTATGGATTGTAAACCTCTTTTGTCAGGGAGCAAAGGGC
GCCACGTGTGGCGTTTTGCGAGTACCTGAAGAAAAAGCATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATGCGAGCGT
TATCCGGATTTATTGGGTTTAAAGGGTGCGCAGGCGGAATGTCAAGTCAGCGGTAAAATTTCGGGGCTCAACCCCGTCGTGCCGTTGA
AACTGGCGTTCTTGAGTGAGCGAGAAGTATGCGGAATGCGTGGTGTAGCGGTGAAATGCATAGATATCACGCAGAACTCCGATTGCGA
AGGCAGCATACCGGCGCTCAACTGACGCTCATGCACGAAAGCGTGGGTATCGAACAGGATTAGATACCCTGGTAGTCCACGCAGTAAA
CGATGAATACTAACTGTCCGGGCAGAATGATGCCTGGGTGGTACAGCGAAAGCGTTAAGTATTCCACCTGGGGAGTACGCCGGCAACG
GTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGAGGAACATGTGGTTTAATTCGATGATACGCGAGGAACCTTACCCGGGCT
CAAACGACGGATGGATGTTTCTGAAAGGAGGCAGTCCTACGGGACATCCGTCGAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGTGA
GGTGTCGGCTTAAGTGCCATAACGAGCGCAACCCCCATCGCCAGTTACCAGCAAGTAAAGTTGGGGACTCTGGCGAGTCTGCCGGCGC
AAGCTGTGAGGAAGGCGGGGATGACGTCAAATCAGCACGGCCCTTACGTCCGGGGCGACACACGTGTTACAATGGCAGGTACAGAGAG
AAGCGATGCGGCGACGCAGAGCGGAACTTCAAAGCCTGTCTCAGTTCGGATTGGAGTCTGCAACCCGACTCCATGAAGCTGGATTCGC
TAGTAATCGCGCATCAGCCACGGCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGGAAGCCGGGAGTGCCT
GAAGTGTGCAACCGCAAGGAGCGCCCTAAGGTAAAACCGGTGACTGGGGCT

>*Bacteroidales* "*cluster S24-7*" OTU 13481 clone 3

AGGATGAACGCTAGCGACAGGCTTAACACATGCAAGTCGAGGGGCAGCGGGGGAGTAGCAATACTCCCGCCGGCGACCGGCGCACGGG
TGAGTAACACGTATGAAACCTGCCCGTAGCAGGGGGGTAAGCGGAAGAAATTCCGTCTAATACCGCGTAACAACCCATGGAGGCATCT
CCTCGGGTTTAAAGGAAGCGATTCCGGCTACGGATGGTCATACGTCGCATTAGCTTGTTGGCGGGGTAACGGCCCACCAAGGCGACGA
TGCGTAGGGGTTCTGAGAGGAAGGTCCCCCACACTGGTACTGAGACACGGACCAGACTCCTACGGGAGGCAGCAGTGAGGAATATTGG
TCAATGGCCGCAGGGCTGAACCAGCCAAGTCGCGTGAGGGATGACGGTCCTATGGATTGTAAACCTCTTTTGTCAGGGAGCAAAGGGC
GTCACGTGTGGCGCTTTGCGAGTACCTGAAGAAAAAGCATCCGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATGCGAGCGT
TATCCGGATTTATTGGGTTTAAAGGGTGCGCAGGCGGAATGTCGAGTCAGCGGTAAAATTTCGGGGCTCAACCCCGTCGTGCCGTTGA
AACTGGCGTTCTTGAGTGAGCGAGAAGTATGCGGAATGCGTGGTGTAGCGGTGAAATGCATAGATATCACGCAGAACTCCGATTGCGA
AGGCAGCATACCGGCGCTCAACTGACGCTCATGCACGAAAGCGTGGGTATCGAACAGGATTAGATACCCTGGTAGTCCACGCAGTAAA
CGATGAATACTAACTGTCCGGGCAGAATGATGCCTGGGTGGTACAGCGAAAGCGTTAAGTATTCCACCTGGGGAGTACGCCGGCAACG
GTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGAGGAACATGTGGTTTAATTCGATGATACGCGAGGAACCTTACCCGGGCT
CAAACGACGGATGGATGTTTCTGAAAGGAGGCAGTCCTACGGGACATCCGTCGAGGTGCTGCATGGTTGTCGTCAGCTCGTGCCGTGA
GGTGTCGGCTTAAGTGCCATAACGAGCGCAACCCCCATCGCCAGTTACCAGCAAGTAAAGTTGGGGACTCTGGCGAGACTGCCGGCGC
AAGCTGTGAGGAAGGCGGGGATGACGTCAAATCAGCACGGCCCTTACGTCCGGGGCGACACACGTGTTACAATGGCAGGTACAGAGAG
ATGCGATGCGGCGACGCAGAGCGGAACTTCAAAGCCTGTCTCAGTTCGGATTGGAGTCTGCAACCCGACTCCATGAAGCTGGATTCGC
TAGTAATCGCGCATCAGCCACGGCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGCCATGGAAGCCGGGAGTGCCT
GAAGTGTGCAACCGCAAGGAGCGCCCTAAGGTAAAACCGGTGACTGGGGCT

Table SR3. List of published oligonucleotide probes to detect members of the intestinal microbiota of humans and higher animals. Probes for 16S rRNA were analyzed by RDP II probe match. Total (Tot.) hits represent the sum of sequences in the database that perfectly matched the oligonucleotide. Total non-target (NT) hits show the number of perfectly matching organisms, which are not included in the target groups according to the respective publication. Group coverage values (major target taxa and major NT taxa) are given as percentages of fully matching sequences in the database, relative to the total number of sequences in the respective groups. Major NT taxa are mentioned (at least 100 hits per group, or over 10% in case of very small groups). Probes that target 23S rRNA were analyzed in NCBI BLAST exclusively. Information obtained from an additional source is cited under secondary reference (Ref.). Probe position (pos.) refers to *E. coli* numbering according to Brosius and colleagues (61). Additional important information, such as references to competitor probes and suggested hybridization conditions under formamide-free conditions, is given in column "comments". Corrected probe sequences as well as newly created short names are highlighted and commented. The target organisms according to the publication are amended with currently valid names according to NCBI taxonomy. Oligonucleotide probe database (OPD) code (60). Hybridization temperature (Th). Washing temperature (Tw).

Probe name	Probe sequence (5' → 3')	Tot. hits	Major target taxa (perfect match(%)/tot. group number)	NT hits	Major NT taxa (perfect match(%)/tot. group number)	Ref.	2nd ref.	OPD code	Probe pos.	FA (%)	Comments	Target organisms (according to publication)	rRNA
phylum *Bacteroidetes*													
CFB935	CCA CAT GTT CCT CCG CTT GT	83705	phylum *Bacteroidetes* (61.8/135261)	61	order *Clostridiales* (0.01/132883); *unclassified_Bacteria* (0.1/34924)	(1)			935 - 954		Tw: 62° C	*Bacteroides-Porphyromonas-Prevotella* subgroup	16S
CFB719	AGC TGC CTT CGC AAT CGG	46140	phylum *Bacteroidetes* (33.9/135261)	295	class *Alphaproteobacteria* (0.4/65801)	(2)			719 - 736	30		phylum Cytophaga-Flavobacterium-Bacteroides	16S
CFB286	TCC TCT CAG AAC CCC TAC	27829	phylum "*Bacteroidetes*" (20.6/135261): mainly class *Bacteroidia* (33.0/78282)			(2)			286 - 304	50		phylum *Cytophaga-Flavobacterium-Bacteroides*	16S
CFB563	GGA CCC TTT AAA CCC AAT	21881	phylum *Bacteroidetes* (16.1/135261): mainly class *Flavobacteria* (87.9/24429); class *Bacteroidetes_incertae_sedis* (20.8/1364)	85	class *Methanomicrobia* (2.5/3353) of domain *Archaea*	(2)			563 - 580	20		phylum Cytophaga-Flavobacterium-Bacteroides	16S
CF319a	TGG TCC GTG TCT CAG TAC	56020	phylum *Bacteroidetes* (40.7/135261): class *Bacteroidia* (22.4/78282); class *Flavobacteria* (85.2/24429); class *Sphingobacteria* (17.5/15889)	952	phylum *Proteobacteria*(0.3/322871); class B*acteroidetes_incertae_sedis* (59.8/1364); un*classified_Bacteroidetes* (85.8/15297)	(3)			319 - 336	35	target most *Bacteroidetes* together with CF319b	most members of F*lavobacteria-Cytophaga* group (most *Flavobacteria,* some *Bacteroidetes,* some *Sphingobacteria)*	16S
CF319b	TGG TCC GTA TCT CAG TAC	2237	phylum *Bacteroidetes* (1.7/135261): class *Bacteroidia* (0.1/78282); family *Porphyromonadaceae* (0.2/17890); class *Flavobacteria*(5.5/24429); family *Flavobacteriaceae* (6.0/21665); class *Sphingobacteria* (4.7/15889)	5	*unclassified_Bacteroidetes* (0.6/15297)	(3)			319 - 336	35	target most *Bacteroidetes* together with CF319a	some members of F*lavobacteria-Cytophaga* group and genus *Porphyromonas*	16S

Table SR3. (continued)

Probe name	Probe sequence (5' → 3')	Tot. hits	Major target taxa (perfect match(%)/tot. group number)	NT hits	Major NT taxa (perfect match(%)/tot. group number)	Ref.	2nd ref.	OPD code	Probe pos.	FA (%)	Comments	Target organisms (according to publication)	rRNA
CFB972	CCT TGG TAA GGT TCC TCG	6406	phylum *Bacteroidetes* (4.5/135261) (mainly order *Flavobacteriales* (24.3/24429))	43	order *Actinomycetales* (0.03/ 166237) of phylum Actinobacteria	(2)			972 - 989	20		phylum Cytophaga-Flavobacterium-Bacteroides	16S
CFB1082	TGG CAC TTA AGC CGA CAC	71199	order *Bacteroidales* (90.2/ 78282): family *Bacteroidaceae* (97.4/34683); family *Porphyro-monadaceae* (91.6/17890); family *Prevotellaceae* (94.9/18900)	612	*unclassified_Bacteroidetes* (3.7/15297); family *Rikenellaceae* (3.9/2128)	(2)			1082 - 1100			mainly human-associated CFB (*Prevotella, Bacteroides, Porphyromonas*)	16S
Bacto1080	GCA CTT AAG CCG ACA CCT	71226	order *Bacteroidales* (89.9/ 78282)	862	class *Clostridia* (0.2/135053); *unclassified_Bacteroidetes* (3.7/15297)	(4)	(5)	S-*-Bacto-1080-a-A-18	1080 - 1097	20	Tw: 50° C	*Bacteroides, Prevotella,* and *Porphyromonas*	16S
Bac303	CCA ATG TGG GGG ACC TT	53636	order *Bacteroidales* (68.5/78282): family *Bacteroidaceae* (96.8/ 34683); family *Porphyromonada-ceae* (22.0/17890); family *Prevotellaceae* (80.5/18900)	28	family *Rikenellaceae* (1.9/2128); *unclassified_Bacteroidetes* (0.1/15297)	(3)			303 - 319	0		most *Bacteroidaceae* and *Prevotellaceae,* some *Porphyromonadaceae*	16S
Bacid1	AAC ATG TYT CCA CGT TAT TCA GG	54	genus *Bacteroides* (0.2/34620)			(6)			1001 - 1023		Th: 50° C, Tw: 50° C	*Bacteroides acidifaciens* Group-1	16S
Bacid2	AAC ATG TTT CCA CAT TAT TCA GG	139	genus *Bacteroides* (0.4/34620)			(6)			1001 - 1023		Th: 50° C, Tw: 50° C	*Bacteroides acidifaciens* Group-2	16S
Bacid3	GGC ATG TCT CCA CGC CGT TCA TT	105	genus *Bacteroides* (0.3/34620)	1		(6)			1001 - 1023		Th: 50° C, Tw: 50° C	*Bacteroides acidifaciens* Group-3	16S
Bot	GCC TTA CGG CTA TAC TGT T	2654	genus *Bacteroides* (7.7/34620)			(6)			1016 - 1037		Th: 50° C, Tw: 50° C	*Bacteroides ovatus* and *Bacteroides thetaiotaomicron*	16S
Bova	CAA CAG CCT TAC GGC TA	1322	genus *Bacteroides* (3.4/34620)	138		(6)			1023 - 1042		Th: 50° C, Tw: 50° C	*Bacteroides ovatus*	16S
Bthe	CAT TTG CCT TGC GGC TA	1373	genus *Bacteroides* (4.0/34620)			(6)			1024 - 1042		Th: 50° C, Tw: 50° C	*Bacteroides thetaiotaomicron*	16S
Buni	GAC ATG TCT CCA CAT CAT TCA GT	2165	genus *Bacteroides* (6.3/ 34620)			(6)			1001 - 1023		Th: 50° C, Tw: 50° C	*Bacteroides uniformis*	16S
Bcac	TAA AAC CCA TGC GGG AAA TAT ATG C	1795	genus *Bacteroides* (5.2/34620)			(6)			182 - 206		Th: 50° C, Tw: 50° C	*Bacteroides caccae*	16S
Bvulg 1017	AGA TGC CTT GCG GCT TAC GGC	11647	genus *Bacteroides* (33.6/34620)	1		(7)		S-S-Bvulg-1017-a-A-21	1017 - 1037	30		*Bacteroides vulgatus*	16S
Bfrag 998	GTT TCC ACA TCA TTC CAC TG	3766	genus *Bacteroides* (10.9/34620)	1		(7)		S-S-Bfrag-998-a-A-21	998 - 1017	30		*Bacteroides fragilis*	16S

Table SR3. (continued)

Probe name	Probe sequence (5' → 3')	Tot. hits	Major target taxa (perfect match(%)/tot. group number)	NT hits	Major NT taxa (perfect match(%)/tot. group number)	Ref.	2nd ref.	OPD code	Probe pos.	FA (%)	Comments	Target organisms (according to publication)	rRNA
Bovat 1028	CCT TCA CAA CAG CCT TAC G	1180	genus *Bacteroides* (3.4/34620)			(7)		S-S-Bovat-1028-a-A-19	1028 - 1046	30		*Bacteroides ovatus*	16S
Bdist 1025	CGC AAA CGG CTA TTG CTA G	1207	genus *Parabacteroides* (45.1/2670)	2		(7)		S-S-Bdist-1025-a-A-19	1025 - 1043	30		*Bacteroides distasonis* (*Parabacteroides distasonis*)	16S
Bputre 698	GTT CTG TAT GAT CTC TAA GC	1512	genus *Alistipes* (80.1, 1886)	2		(7)		S-S-Bputre-698-a-A-20	698 - 717	30		*Bacteroides putredinis* (*Alistipes putredenis*)	16S
FFE8b	CAG CCG CAC ACC CGT CTT	11	genus *Terrimonas* (2.9/381)			(3)			225 - 242	20		*Flavobacterium ferrugineum*	16S
phylum *Firmicutes*													
LGC354A	TGG AAG ATT CCC TAC TGC	25954	phylum *Firmicutes* (6.3/407377): class *Bacilli* (10.3/248081) (mainly order *Bacillales* (2.9/158306) and order *Lactobacillales*(23.5/89758))	146	phylum *TM7* (1.4/2060); *unclassified_Bacteria* (0.3/34924)	(8)	(9)		354–371	35	detect most Firmicutes together with LGC354B and LGC354C	*Firmicutes* (low G+C Gram+); mainly members of *Lactobacillales* and *Bacillales*	16S
LGC354B	CGG AAG ATT CCC TAC TGC	156624	phylum *Firmicutes*(38.4/407377): class *Bacilli* (63.0/248081) (mainly order *Bacillales* (93.2/158306) and order *Lactobacillales* (9.9/89758))	174	*unclassified_Bacteria* (0.5/34924)	(8)	(9)		354 - 371	35	detect most Firmicutes together with LGC354A and LGC354C	mainly members of *Bacillales* and *Lactobacillales*	16S
LGC354C	CCG AAG ATT CCC TAC TGC	59041	phylum *Firmicutes* (14.5/407377): class *Bacilli* (23.5/248081) (mainly order *Lactobacillales*(64.2/89758)) and class E*rysipelotrichia* (7.9/7038)	47	phylum *Tenericutes* (0.8/3026)	(8)	(9)		354 - 371	35	detect most Firmicutes together with LGC354A and LGC354B	Mainly members of *Lactobacillales*	16S
LGC	TCA CGC GGC GTT GCT C	246346	phylum Firmicutes (59.1/407377)	5604	class Mollicutes (44.9/3026) of phylum Tenericutes; class Cyanobacteria (21.8/14545) of phylum Cyanobacteria/Chloroplast; unclassified_Bacteria (1.4/34924)	(10)			399 - 414			*Firmicutes* (low G+C Gram+)	16S
LGC0355	GGA AGA TTC CCT ACT GCT G	181189	phylum Firmicutes (44.4/407377): mainly class Bacilli (72.8/248081); class Clostridia (0.1/135053)	320	genus TM7_genera_incertae _sedis (1.4/2060) of phylum TM7; unclassified_Bacteria (0.8/34924)	(11)			355 - 373	20		*Firmicutes* (low G+C Gram+)	16S
LGC353b	GCG GAA GAT TCC CTA CTG C	156481	class *Bacilli* (98.7/248081)	252	unclassified_Bacteria (0.5/34924)	(12)			353 - 371	20		*Bacillus*	16S
Prop853	ATT GCG TTA ACT CCG GCA C	13904	order *Selenomonadales* (92.3/13598)	1356	family *Ruminococcaceae* (743/32086); genus *Clostridiales _Incertae Sedis XVIII* (2.3/381)	(13)			853 - 871	0	Th: 50° C	*Clostridial* cluster IX	16S

Table SR3. (continued)

Probe name	Probe sequence (5' → 3')	Tot. hits	Major target taxa (perfect match(%)/tot. group number)	NT hits	Major NT taxa (perfect match(%)/tot. group number)	Ref.	2nd ref.	OPD code	Probe pos.	FA (%)	Comments	Target organisms (according to publication)	rRNA
UCA1124	GAG TTC CTA ACT WAA TAT TGG CAA C	429	*unclassified_Clostridiales* (4.4/7522)	95	genus *Gp4* (2.1/1822) of phylum *Acidobacter* and genus *GpXIII* (2.8/1090) of phylum *Cyanobacteria/Chloroplast*	(1)			1124 - 1149		Tw: 52° C	*Firmicutes* (low G+C Gram+) subgroup	16S
UCB807	CCT AGC AYT CAT CGT TTA CAG TGT	1657	*unclassified_Clostridiales* (17.7/7522)	326	family *Ruminococcaceae*(0.3/32086); *unclassified_Firmicutes* (2.2/3598); *unclassified_Bacteria* (0.4/34924)	(1)			830 - 807		Tw: 60° C	*Firmicutes* (low G+C Gram+) subgroup	16S
Lab158	GGT ATT AGC AYC TGT TTC CA	22714	family *Lactobacillaceae* (92.9/ 14944); family *Enterococcaceae* (96.7/5386)	3617	order *Actinomycetales* (0.1/166237); order *Clostridiales* (1.4/132883); family *Leuconostocaceae* 52.7/2639)	(14)		S-G-Lab-0158-a-A20	157 - 176	25		*Lactobacilli* and *Enterococci*	16S
Strc493*	GTT AGC CGT CCC TTT CTG G	52052	family *Streptococcaceae* (96.9/53688)	34		(15)			493 - 511				16S
Strc493c	TTT AGC CGT CCC TTT CTG G					(16)			493 - 511		competitor probe for Strc493		16S
Clit135	GTT ATC CGT GTG TAC AGG G	3234	family *Peptostreptococcaceae* (66.8/4819)	14		(15)		S-*-Clit-0135-a-A-19	135 - 153			some of *Clostridium lituseburense* group (*Clostridium* cluster XI)	16S
Erec482	GCT TCT TAG TCA RGT ACC G	52337	family *Lachnospiraceae* (79.2/64988)	885	class *Betaproteobacteria* (0.2/ 76088); *unclassified_Clostridiales* (7.8/7522); family *Eubacteriaceae* (12.1/766)	(15)		S-*-Erec-0482-a-A-19	482 - 501	0		most of *Clostridium coccoides-Eubacterium rectale* group (*Clostridium* cluster XIVa and XIVb)	16S
LAC435	TCT TCC CTG CTG ATA GA	53835	family *Lachnospiraceae* (81.7/64988)	708	family *Clostridiaceae* 1 (0.2/8896); family *Eubacteriaceae* (11.7/766); family *Ruminococcaceae* 0.1/32086); *unclassified_Clostridiales* (7.3/7522)	(17)		S-S-Lac-0435-a-16	435 - 451	35		*Lachnospiraceae* clones	16S
Chis150	TTA TGC GGT ATT AAT CTY CCT TT	8082	family *Clostridiaceae 1* (86.5/8896)	387	phylum *Bacteroidetes* (0.3/135261) (mainly genus *Arcicella* (59.8/246))	(15)		S-*-Chis-0150-a-A-23	150 - 172			most of the *Clostridium histolyticum* group (*Clostridium* cluster I and II)	16S
Clep866*	GGT GGA TWA CTT ATT G	20690	family *Ruminococcaceae* (48.8/32086)	5022	genus *Gemmiger* (93.7/287) of class *Alphaproteobacterium;* phylum *Bacteroidetes* (0.1/135261); family *Eubacteriaceae* (4.8/766); family *Lachnospiraceae* (4.1/64988); *unclassified_Clostridiales* (2.6/7522)	(16)		S-*-Clep-0866-a-A-18	866 - 883			*Clostridium leptum*	16S
Clep866c1	GGT GGA AWA CTT ATT GTG					(16)			866 - 883		competitor probe1 for Clep866		16S
Clep866c2	GGT GGA TWA CTT ATT GCG					(16)			866 - 883		competitor probe2 for Clep866		16S
III1421	CTA CGG ACT TCG GGT GTT CCC	2394	family *Ruminococcaceae* (7.4/32086)	31		(1)			1421 - 1442		Tw: 65° C	*Clostridiaceae* cluster III	16S

Table SR3. (continued)

Probe name	Probe sequence (5' → 3')	Tot. hits	Major target taxa (perfect match(%)/tot. group number)	NT hits	Major NT taxa (perfect match(%)/tot. group number)	Ref.	2nd ref.	OPD code	Probe pos.	FA (%)	Comments	Target organisms (according to publication)	rRNA
IV815	CCC ACA CCT AGT AAT CAT CGT T	6175	family *Ruminococcaceae* (18.5/32086)	253	family *Lachnospiraceae* (0.1/64988); *unclassified_Bacteria* (0.5/34924)	(1)			815 - 836		Tw: 58° C	*Clostridiaceae* cluster IV	16S
IX854	CTT ATT GCG TTA ACT CCG GCA C	2394	family *Ruminococcaceae* (7.4/32086)	31		(1)			854 - 875		Tw: 60° C	*Clostridiaceae* cluster IX	16S
RUM831	GGT CAG TCC CCC CAC A	4235	family *Ruminococcaceae* (13.2/32086)	8		(17)		S-S-Rum-0831-a-16	831 - 846	35		Rumen clones in family *Ruminococcaceae*	16S
RFL155	TAC CAT CCG TTT CCA GA	667	family *Ruminococcaceae* (2.1/32086)	2		(17)		S-S-Rum-0155-a-15	155 - 171	45		*Ruminococcus flavefaciens* related clones	16S
Clept1240	GTT TTR TCA ACG GCA GTC	28501	family *Ruminococcaceae* (76.9/32086)	3835	phylum *Acidobacteria* (9.7/13454); phylum *Proteobaceria* (0.1/322871); phylum *Crenarchaeota* (0.4/6985)	(18)		S-G-Clept-1240-a-A-18	1240 - 1257		only dot blot hybridization ; Tw: 48.5° C	*Clostridium leptum* subgroup	16S
UCC926	ACT GCT TGT GCG GGC TCC	714	family *Ruminococcaceae* (2.1/32086)	30	*unclassified_Clostridiales* (0.2/7522)	(1)			943 - 926		Tw: 68° C	*Firmicutes* (low G+C Gram+) subgroup	16S
RAL1436	CGG TTA GAA CAC AGG	25	genus *Ruminococcus* (1.1/2317)			(17)		S-S-Rum-1436-15	1436 - 1450	20		*Ruminococcus albus* related clones	16S
Fprau645	CCT CTG CAC TAC TCA AGA AAA AC	9480	genus *Faecalibacterium* (88.6/9825)	777	*Gemmiger* (10.1/287) of class *Alphaproteobacterium*	(19)		S-*-Fprau-0645-a-A-23	645 - 667	15		*Fusobacterium (Faecalibacterium) prausnitzii*	16S
Fpra655	CGC CTA CCT CTG CAC TAC	9388	genus *Faecalibacterium* (94.8/9825)	74	genus *Subdoligranulum* (28.3/46); genus *Anaerofilum* (24.3/37)	(20)		S-*-Fpra-0655-a-A-18	655 - 673		newly created short name; Th: 58° C	*Faecalibacterium prausnitzii*	16S
E.con1122	***TCA TCG GTC GCC AAA TCC GG***	1	reverse sequence: genus *Clostridium XlVa* (0.0/4039) (only *Eubacterium contortum* L34615)			(21)		S-S-E.con-1122-a-A-20	1122		probe sequence corrected; Th: 55° C	*Eubacterium contortum*	16S
Erec207	GGT GGT GTA CAA GAC CCG	19	genus *Clostridium XlVa* (0.1/4039)	13	class *Betaproteobacteria* (0.01/76088)	(20)		S-S-Erec-0207-a-A-18	207		newly created short name; Th: 52° C	*Eubacterium rectale sp. strain A1-86*	16S
E.mon84	***GCC CTT TAC CTA ATC GCC***	328	reverse sequence: genus *Clostridium sensu stricto* (4.3/7640)	2		(21)		S-S-E.mon-0084-a-A-18	84		probe sequence corrected; Th: 51° C	*Eubacterium moniliforme*	16S
Esab1467	AGT TAT CCT CCC TGC CTT	42	genus *Eubacterium* (2.2/447)	32	*unclassified_Clostridiales* (0.2/7522); *unclasssified Lachospiraceae* (0.05/28237)	(21)		S-S-Esab-1467-a-A-18	1467			*Eubacterium saburreum*	16S
E.bar1237	CCT TTG TCC CAA CCC ATT	8	genus *Eubacterium* (1.8/447)			(21)		S-S-E.bar-1237-a-A-18	1237		Th: 37° C, Tw: 51° C	*Eubacterium barkeri*	16S
E.lim1433	GCG GTT CTC TCA CAG GCT	83	genus *Eubacterium* (18.6/447)			(21)		S-S-E.lim-1433-a-A-18	1433		Th: 51° C	*Eubacterium limosum*	16S

Table SR3. (continued)

Probe name	Probe sequence (5' → 3')	Tot. hits	Major target taxa (perfect match(%)/tot. group number)	NT hits	Major NT taxa (perfect match(%)/tot. group number)	Ref.	2nd ref.	OPD code	Probe pos.	FA (%)	Comments	Target organisms (according to publication)	rRNA
LPLANG	TAT CAT TGC CAT GGT GA	1290	genus *Lactobacillus* (8.9/14441)	5		(22)	(23)		267 - 284	25		*Lactobacillus plantarum*	16S
LSAL	GAA TGC AAG CAT TCG GTG TA	165	genus *Lactobacillus* (1.1/14441)			(24)	(23)		75 - 91	25		*Lactobacillus salvirius*	16S
Lacto722	YCA CCG CTA CAC ATG RAG TTC CAC T	16785	genus *Lactobacillus* (91.9/14441)	3514	family *Staphylococcaceae* (0.2/ 109523); order *Lactobacillales* (18.4/ 89758) (mainly family *Leuconostocaceae* (96.5/2639)); genus *Pediococcus* (97.8/370)	(25)		S-G-Lacb-0722-a-A-25	669 - 694		only dot blot hybrisization ; Tw: 54° C	*Lactobacillus* group	16S
Laci66	GAA TCT GTT GGT TCA GCT CGC	189	genus *Lactobacillus* (1.3/14441)			(26)	(27)		66 - 86		newly created short name; only dot blot hybrisization.	*Lactobacillus acidophilus*	16S
Lfer66	CAA TCA ATT GGG CCA ACG CGT	415	genus *Lactobacillus* (2.9/14441)			(26)	(27)		66 - 86		newly created short name; only dot blot hybrisization.	*Lactobacillus fermentum*	16S
Lpla67	CCA ATC AAT ACC AGA GTT CG	1075	genus *Lactobacillus* (7.4/14441)			(26)	(27)		67 - 86		newly created short name; only dot blot hybrisization.	*Lactobacillus plantarum*	16S
Lrum67	TTC GGT GAA AGA AAG CTT CG	79	genus Lactobacillus (0.5/14441)			(26)	(27)		67 - 86		newly created short name; only dot blot hybrisization.	*Lactobacillus ruminis*	16S
Lreu92	GAT CCA TCG TCA ATC AGG	368	genus Lactobacillus (2.5/14441)			(28)			92 - 109		newly created short name; only dot blot hybrisization; Th: 40° C, Tw: 50° C	*Lactobacillus reuteri*	16S
Lbre90	TGT TGA AAT CAG TGC AAG	223	genus *Lactobacillus* (1.5/14441)			(28)			90 - 107		newly created short name; only dot blot hybrisization; Th: 40° C, Tw: 48° C	*Lactobacillus brevis*	16S
Lcarh	GCA GGC AAT ACA CTG ATG					(29)					newly created short name; only dot blot hybrisization	*Lactobacillus casei* and *L. rhamnosus*	23S
Lpcar	CTG ATG TGT ACT GGG TTC					(29)					newly created short name; only dot blot hybrisization	*Lactobacillus casei, L. paracasei* and *L. rhamnosus*	23S

Table SR3. (continued)

Probe name	Probe sequence (5' → 3')	Tot. hits	Major target taxa (perfect match(%)/tot. group number)	NT hits	Major NT taxa (perfect match(%)/tot. group number)	Ref.	2nd ref.	OPD code	Probe pos.	FA (%)	Comments	Target organisms (according to publication)	rRNA
Ldel	AAG GAT AGC ATG TCT GCA					(29)					newly created short name; only dot blot hybrisization	*Lactobacillus delbrueckii*	23S
Lpar	CAC TGA CAA GCA ATA CAC					(29)					newly created short name; only dot blot hybrisization	*Lactobacillus paracasei*	23S
Lsak	TTA ATG ATA ATA CTC GAT T					(30)					newly created short name; only dot blot hybrisization	*Lactobacillus sakei*	23S
Lba	TCT TTC GAT GCA TCC ACA					(31)			1159-1180		Th: 40° C, Tw: 49° C	*Lactobacillus acidophilus* (cluster AI)	23S
Lbj	ATA ATA TAT GCA TCC ACA G					(31)			1158-1179		Th: 40° C, Tw: 49° C	*Lactobacillus johnsonii* (cluster B2)	23S
Lbg	TCC TTT GAT ATG CAT CCA					(31)			1160-1 178		Th: 40° C, Tw: 50° C	*Lactobacillus gasseri* (cluster B1)	23S
Acac194	CTA TAC TGC CAG GGC TTT	41	genus *Anaerostipes* (2.6/1563)			(20)		S-S-Acac-0194-a-A-18	194		newly created short name; Th: 46° C	*Anaerostipes caccae*	16S
E.had579	***GCA TCC ACC ATA CCG TTC AG***	1865	reverse sequence: genus *Anaerostipes* (88.1/1563)	488	genus *Lachnospiracea_incertae_sedis* (1.2/13942); *unclassified_Lachnospiraceae* (1.1/28237)	(21)		S-S-E.had-0579-a-A-20	579		probe sequence corrected; Th: 54° C	*Eubacterium hadrum*	16S
E.len194	***TTT CGG GTC TGC CGT TCC***	65	reverse sequence: genus *Eggerthella* (50.7/73)		*unclassified_Coriobacteriaceae* (8.0/352)	(21)		S-S-E.len-0194-a-A-18	194		probe sequence corrected; Th: 51° C	*Eubacterium lentum* (*Eggerthella lenta*)	16S
E.dol183	***GCT CCG TAG AGC CTC TGT***	13	reverse sequence: genus *Erysipelo-trichaceae_incertae_sedis* (1.1/1238)			(21)		S-S-E.dol-0183-a-A-18	183		probe sequence corrected; Th: 51° C	*Eubacterium dolichum*	16S
Etor727	AGA CCA GGC AAC CGC CTT	40	genus *Erysipelotrichaceae_incertae_sedis* (2.7/1238)	7	genus *Ignavibacterium* (0.8/655)	(21)		S-S-Etor-0727-a-A-18	727			*Eubacterium tortuosum*	16S
Etor129	CCA GTT ACA TGG GTA GGT	1	genus *Erysipelotrichaceae_incertae_sedis* (0.1/1238)			(21)		S-S-Etor-0129-b-A-18	129			*Eubacterium tortuosum*	16S
E.bif462	CAC TCA CTC ATC ATT CCC	705	genus *Erysipelotrichaceae_incertae_sedis* (56.9/1238)			(21)		S-S-E.bif-0462-a-A-18	462		Th: 51° C	*Eubacterium biforme*	16S
E.cyl461	ACC CAC GGA TCA TTC CCT	11	genus *Erysipelotrichaceae_incertae_sedis* (0.9/1238)			(21)		S-St-E.cyl-0461-a-A-18	461		Th: 51° C	Eubacterium cylindroides	16S

Table SR3. (continued)

Probe name	Probe sequence (5' → 3')	Tot. hits	Major target taxa (perfect match(%)/tot. group number)	NT hits	Major NT taxa (perfect match(%)/tot. group number)	Ref.	2nd ref.	OPD code	Probe pos.	FA (%)	Comments	Target organisms (according to publication)	rRNA
E.cyl466	CCG TCA CCC ACA TAG CAT	108	genus *Erysipelotrichaceae _incertae_sedis* (1.3/1238)			(21)		S-St-E.cyl-0466-a-A-18	466		Th: 51° C	*Eubacterium cylindroides*	16S
E.ven66	***GCT TCG TGG AAC CTG TCT***	119	reverse sequence: genus *Lachno-spiracea_incertae_sedis* (0.8/13942)	5		(21)		S-S-E.ven-0066-a-A-18	66		probe sequence corrected; Th: 55° C	*Eubacterium ventriosum*	16S
Ehal578	TTG CAC TGC CAC CTA CGC	824	genus *Lachnospiracea _incertae_sedis* (5.9/13942)	2		(20)		S-*-Ehal-0578-a-A-18	578		newly created short name; Th: 58° C	*Eubacterium hallii*	16S
Emul183	GTT CCT TCA TGC GAA GGT	2911	genus *Lachnospiracea _incertae_sedis* (20.3/13942)	82	unclassified *Lachnospiraceae* (0.2/28237)	(21)		S-S-Emul-0183-a-A-18	183			*Eubacterium multiforme*	16S
Erec834	CGA GAA GCA AUG CUU CCC	2849	genus *Lachnospiracea _incertae_sedis* (19.8/13942)	92	unclassified *Lachnospiraceae* (0.3/28237)	(21)		S-S-Erec-0834-a-A-18	834			*Eubacterium rectale*	16S
Erec838	CGG CAC CGA GAA GCA ATG CT	2911	genus *Lachnospiracea _incertae_sedis* (20.3/13942)	82	unclassified *Lachnospiraceae* (0.2/28237)	(21)		S-S-Erec-0838-b-A-20	838			*Eubacterium rectale*	16S
LSPotu11021-1127	TTC CCA TCT TTC TTG CTG GC	238	unclassified_*Lachnospiraceae* (0.8/28237)	6		(32)		S-*-Lsp-1127-a-A-20	1127	30	specific use together with LSPotu11021-1448	*uncl. Lachnospiraceae* phylotype 11021	16S
LSPotu11021-1448	GCA GCT CCC TCC TCT CGG	194	unclassified_*Lachnospiraceae* (0.6/28237)	14		(32)		S-*-Lsp-1448-a-A-18	1448	30	specific use together with LSPotu11021-1127	*uncl. Lachnospiraceae* phylotype 11021	16S
LSPotu9468-999	CTT TGC CCA TAC GGC GTC CG	78	unclassified_*Lachnospiraceae* (0.3/28237)	1		(32)		S-*-Lsp-999-a-A-20	999	10	specific use together with LSPotu9468-1259	*uncl. Lachnospiraceae* phylotype 9468	16S
LSPotu9468-1259	TGC TCA ACG TCA CCG TCT CG	197	unclassified_*Lachnospiraceae* (0.7/28237)	8		(32)		S-*-Lsp-1259-a-A-20	1259	10	specific use together with LSPotu9468-999	*uncl. Lachnospiraceae* phylotype 9468	16S
BFI826	ATG GCA CCC AAC ACC TAG	67	genus *Butyrivibrio* (11.0/572)	4		(17)		S-S-But-0826-a-18	826 - 843	35		*Butyrivibrio fibrisolvens* related clones	16S
Rrec584	TCA GAC TTG CCG YAC CGC	5668	genus *Roseburia* (63.5/3545)	3416	genus *Lachnospiracea_ incertae_sedis* (21.8/13942)	(13)			584 - 602	0	Th: 50° C	*Roseburia*	16S
Rint623	TTC CAA TGC AGT ACC GGG	6428	genus *Roseburia* (87.2/3545)	3338	genus *Lachnospiracea_ incertae_sedis* (21.9/13942)	(20)		S-*-Rint-0623-a-A-18	623 - 641		Th: 54° C	*Roseburia intestinalis* subcluster	16S
Rint623 helper	GTT GAG CCC CGG GCT TT					(33)			607 - 623				16S

Table SR3. (continued)

Probe name	Probe sequence (5' → 3')	Tot. hits	Major target taxa (perfect match(%)/tot. group number)	NT hits	Major NT taxa (perfect match(%)/tot. group number)	Ref.	2nd ref.	OPD code	Probe pos.	FA (%)	Comments	Target organisms (according to publication)	rRNA
Rint1102	GCT TAC CCG CTG GCT ACT	681	genus *Roseburia* (18.7/3545)	18		(20)		S-S-Rint-1102-a-A-18	1124 - 1140		newly created short name; Th: 46° C	*Roseburia intestinalis*	16S
Eubac576	AGC CTT CCG CCT GCG CTC	1027	genus *Roseburia* (28.4/3545)	20		(20)		S-St-xxxx-0576-a-A-18	576 - 594		newly created short name; Th: 58° C	*Eubacterium sp. strain L1-83*	16S
Phasco741	TCA GCG TCA GAC ACA GTC	1441	genus *Acidaminococcus*(91.0/177); genus *Succiniclasticum*(83.2/279); genus *Phascolarctobacterium* (97.5/943)	1280	family *Veillonellaceae* (0.7/12114); *unclassified _Acidaminococcaceae* (69.4/49)	(34)			741 - 759	0		*Phascolarctobacterium faecium, Acidaminococcus fermentans, Succiniclasticum ruminis*	16S
Copr60	CAC CGA TCT TCT CTC GTT	14	genus *Coprococcus* (1.2/1059)	1		(20)		S-St-xxxx-0060-a-A-18	65 - 82		newly created short name; Th: 54° C	*Coprococcus sp. strain L2-50*	16S
Ceut705	GTC AGT AGC AGT CCA GTA AGT	228	genus *Coprococcus* (21.5/1059)			(20)		S-*-Ceut-0705-a-A-21	734 - 754		newly created short name; Th: 54° C	*Coprococcus eutactus*	16S
SER1410	GTC ATT CCA TCG AAA CAT A					(35)		L-G-Serp-1410-a-A-19	1410 - 1428		Th and Tw: 37° C	*Brachyspira*	23S
Bx183	CTA CAC TTATGT GTC AAG	13	genus *Brachyspira* (3.3/395)			(36)		S-S-B.x-0183-a-A-18	183 - 194		Th and Tw: 37° C	*Brachyspira aalborgi* clusters 2 and 3	16S
Aalborgi 183	CTA CGC TTT AGC GTC AAG	33	genus *Brachyspira* (8.4/395)			(37)		S-S-B.aalborgi-0183-a-A	183 - 200		Th and Tw: 37° C	*Brachyspira aalborgi* cluster 1	16S
Pilosi209	GCT CAT CGT GAA GCG AAA	81	genus *Brachyspira* (20.5/395)			(38)		S-S-S.pilo-0209-a-A-18	209 - 226		Th and Tw: 37° C	*Brachyspira (Serpulina) pilosicoli*	16S
Hyo1210	CTC ACG ATG AAC CTT CGA C					(38)		L-S-S.hyo-1210-a-A-19	1210 - 1228		Th and Tw: 37° C	*Brachyspira hyodysenteriae*	23S
Pilosi1405	TTC CAT CGA AAC ATA CTA TA					(38)		L-S-S.pilo-1405-a-A-20	1405 - 1424		Th and Tw: 37° C	*Brachyspira (Serpulina) pilosicoli*	23S
phylum *Proteobacteria*													
Probe D	TGC TCT CGC GAG GTC GCT TCT CTT	36457	order *Enterobacteriales* (53.0/68741)			(39)	(9)		1251 - 1274	25		*Enterobacteriaceae*	16S

Table SR3. (continued)

Probe name	Probe sequence (5' → 3')	Tot. hits	Major target taxa (perfect match(%)/tot. group number)	NT hits	Major NT taxa (perfect match(%)/tot. group number)	Ref.	2nd ref.	OPD code	Probe pos.	FA (%)	Comments	Target organisms (according to publication)	rRNA
Enter1418	CTT TTG CAA CCC ACT	23153	order *Enterobacteriales* (62.9/31695)	3212	many others of class *Gamma-proteobacteria* (15.8/145725)	(18)	(40)	S-G-Enter-1418-a-A-15	1418 - 1432	40	Tw: 43° C	*Enterobacteria*	16S
687 (DSV687)	TAC GGA TTT CAC TCC T	6143	order *Desulfovibrionales* (93.9/3531); order *Desulfuromonadales* (61.1/1330)	2015	*unclassified_Deltaproteobacteria* (1.1/5936); genus *TM7_genera_incertae_sedis* (89.8/2060) of phylum TM7; genus *Halorubrum* (0.3/380) of *Euryarchaeota*	(41)	(42)	S-F-Dsv-0687-a-A-16	687 - 702	15		most *Desulfovibrionales* (excluding *Lawsonia*), many *Desulfuromonales*	16S
Ent1	CCG CTT GCT CTC GCG AG	28928	family *Enterobacteriaceae* (90.5/31695)	247	others in class *Gamma-proteobacteria* (19.8/145725)	(43)			1273 - 1289			*Enterobacteriaceae*	16S
Law1	AAC CGG AGC AGT CTC TCT AG	7	genus *Lawsonia* (1.2/606)			(35)			1148 - 1168		Th: 45° C, Tw: 45° C	*Lawsonia intracellularis*	16S
Sal3	AAT CAC TTC ACC TAC GTG					(44)		L-S-Sal-1713-a-A-18	1713 - 1730		Th: 45°C	*Salmonella enterica*	23S
DB6	CAC ACA ATC GTA ACA TCC TA					(45)			140 - 158		Th: 42° C, Tw: 47° C	*Enterococcus faecium*	23S
DB8	TAG GTG TTG TTA GCA TTT CG					(45)			342 - 361		Th: 42° C, Tw: 47° C	*Enterococcus faecalis*	23S
DB4	CAC TCA TGA ATA ACT GCT AG					(45)					Th: 42° C, Tw: 47° C	*Lactobacillus lactis*	23S
DB9	TAG GTG CCA GTC AAA TTT TG					(46)			342 - 361		Th: 42° C, Tw: 47° C	*Enterococcus avium, E. malodoratus, E. pseudoavium, E. raffinosus*	23S
Eduhi9b	CAC GCA AAC GTA ACA TCC					(46)			148 - 165	30	Th: 37° C	*Enterococcus durans, E. hirae*	23S
Ega9b	CAC AAC TGT GTA ACA TCC					(46)			148 - 165	10	Th: 37° C	*Enterococcus gallinarum*	23S
Ecafl9b	CAC GCA GAC GTA ACA TCC					(46)			148 - 165	30	Th: 37° C	*Enterococcus casseliflavus, E. flaveszenz,*	23S
Enc38a	CTC TAC CTC CAT CAT TCT					(46)			1214 - 1232	20	Th: 42° C	*Enterococci*	23S
phylum *Actinobacteria*													
Bif164	CAT CCG GCA TTA CCA CCC	949	genus *Bifidobacterium* (85.2/1103)	9		(47)		S-G-Bif-0164-a-A-18	164 - 181			*Bifidobacterium spp.*	16S
Bif1278	CCG GTT TTC AGG GAT CC	726	genus *Bifidobacterium* (65.5/1103)	3		(47)		S-G-Bif-1278-a-A-17	1278 - 1294		Td: 56.6° C	*Bifidobacterium spp.*	16S
Bif228	GAT AGG ACG CGA CCC CAT	1153	genus Bifidobacterium (96.6/1103)	87	many others of family *Bifidobacteriaceae* (94.6/1218)	(48)		S-G-Bif-228-a-A-18	228 - 245	40		Bifidobacterium	16S

Table SR3. (continued)

Probe name	Probe sequence (5' → 3')	Tot. hits	Major target taxa (perfect match(%)/tot. group number)	NT hits	Major NT taxa (perfect match(%)/tot. group number)	Ref.	2nd ref.	OPD code	Probe pos.	FA (%)	Comments	Target organisms (according to publication)	rRNA
PAD	GCT CCC AGT CAA AAG CG	188	genus *Bifidobacterium* (17.0/1103)			(49)			434 - 450		only dot blot hybridization; Th: 50° C, Tw: 60°C	*Bifidobacterium adolescentis*	16S
PBI	GCA GGC TCC GAT CCG A	382	genus *Bifidobacterium* (14.7/1103)	220	phylum *Bacteroidetes* (0.1/ 135261); many others in family *Bifidobacteriaceae* (16.0/1218)	(49)			1302 - 1317		only dot blot hybridization; Th: 55° C, Tw: 65°C	*Bifidobacterium bifidum*	16S
PBR	AAG GTA CAC TCA ACA CA	105	genus *Bifidobacterium* (9.4/1103)	1	genus *Gardnerella* (2.2/46)	(49)			475 - 491		only dot blot hybridization; Th: 40° C, Tw: 50° C	*Bifidobacterium breve*	16S
PIN	TCA CGC TTG CTC CCC GAT A	59	genus *Bifidobacterium* (5.3/1103)			(49)			440 - 458		only dot blot hybridization; Th: 55° C, Tw: 70° C	*Bifidobacterium infantis*	16S
PLO	TCT CGC TTG CTC CCC GAT A	178	genus *Bifidobacterium* (16.1/1103)			(49)			440 - 458		only dot blot hybridization; Th: 55° C, Tw: 70° C	*Bifidobacterium longum*	16S
lm3	CGG GTG CTN CCC ACT TTC ATG	790	genus *Bifidobacterium* (65.5/1103)	67	other *Bifidobacteriaceae:* mainly genus *Gardnerella* (91.3/46) and *Scardovia* (50.0/28)	(50)			1412 - 1432		only colony hybridization	*Bifidobacterium*	16S
ATO291	GGTCGGTCTCTCAA CCC	1878	genus *Atopobium* (96.3/434)	1460	many others of family *Coriobacteriaceae* (78.8/2354)	(51)		S-*-Ato-0291-a-A-17	291 - 308		Th: 50° C	*Atopobium* cluster	16S
Cor653	CCCTCCCMTACCGG ACCC	999	genus *Collinsella* (95.6/1036), genus *Coriobacterium* (90.0/10)			(51)		S-*-Cor-0653-a-A-18	653 - 671		Th: 50° C	*Coriobacterium* and *Collinsella* ("*Coriobacterium* group")	16S
phylum *Deferribacteres*													
Mcs487	GCC GGG GCT GCT TAT ACA GGT	189	genus *Mucispirillum* (96.4/196)			(32)		S-S-Mcs-0487-a-A-21	487 - 507	30		*Mucispirillum spp.*	16S
Mcs547	CAG TCA CTC CGA ACA ACG CT	218	genus *Mucispirillum* (96.9/196)	28	genus *Nitrospira* (1.4/1192)	(32)		S-S-Mcs-0547-a-A-20	547 - 566	30		*Mucispirillum spp.*	16S
phylum *Verrucomicrobia*													
Akk1437	CCT TGC GGT TGG CTT CAG AT	285	genus *Akkermansia* (10.7/2645)	1		(52)	(32)		1437 - 1455	30		*Akkermansia spp.*	16S
phylum *Fibrobacteres*													
Fibr225	AAT CGG ACG CAA GCT CAT CCC	187	genus *Fibrobacter* (60.6/279)	18		(53)	(17)	S-G-Fibr-0225-a-A-21	225 - 245	20	Tw: 56° C	*Fibrobacter succinogenes* related clones	16S

Table SR3. (continued)

Probe name	Probe sequence (5' → 3')	Tot. hits	Major target taxa (perfect match(%)/tot. group number)	NT hits	Major NT taxa (perfect match(%)/tot. group number)	Ref.	2nd ref.	OPD code	Probe pos.	FA (%)	Comments	Target organisms (according to publication)	rRNA
phylum *Spirochaetes*													
SPIRO1400	CTC GGA TGG TGT GAC GGG CG	1771	family *Spirochaetaceae* (18.2/8209)	274	phylum *Synergistetes* (6.8/1110); *unclassified_Bacteria* (0.4/34924); phylum *Euryarchaeota* (0.1/11855) of domain *Archaea*	(1)	(5)		1400 - 1419	20	Tw: 57° C	subgroup of the *Spirochaetaceae*	16S

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