Gene-targeted microfluidic cultivation validated by isolation of a gut bacterium listed in Human Microbiome Project's Most Wanted taxa

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This paper describes a microfluidics-based workflow for genetically targeted isolation and cultivation of microorganisms from complex clinical samples. Data sets from high-throughput sequencing suggest the existence of previously unidentified bacterial taxa and functional genes with high biomedical importance. Obtaining isolates of these targets, preferably in pure cultures, is crucial for advancing understanding of microbial genetics and physiology and enabling physical access to microbes for further applications. However, the majority of microbes have not been cultured, due in part to the difficulties of both identifying proper growth conditions and characterizing and isolating each species. We describe a method that enables genetically targeted cultivation of microorganisms through a combination of microfluidics and on- and off-chip assays. This method involves (*i*) identification of cultivation conditions for microbes using growth substrates available only in small quantities as well as the correction of sampling bias using a "chip wash" technique; and (*ii*) performing on-chip genetic assays while also preserving live bacterial cells for subsequent scale-up cultivation of desired microbes, by applying recently developed technology to create arrays of individually addressable replica microbial cultures. We validated this targeted approach by cultivating a bacterium, here referred to as isolate microfluidicus 1, from a human cecal biopsy. Isolate microfluidicus 1 is, to our knowledge, the first successful example of targeted cultivation of a microorganism from the high-priority group of the Human Microbiome Project's "Most Wanted" list, and, to our knowledge, the first cultured representative of a previously unidentified genus of the *Ruminococcaceae* family.

microscale | anaerobe | aerobe | cultivate | metagenome

This paper describes an integrated microfluidic workflow for genetically targeted cultivation and isolation of microorganisms. Microbes play critical functional roles in diverse environments ranging from soil and oceans to the human gut. The emergence of culture-independent techniques has provided insights into microbial ecology by revealing genetic signatures of uncultured microbial taxa (1–5). It also suggests that certain microbes may impact host phenotypes such as obesity, inflammation, and gastrointestinal integrity (6, 7). This explosion of sequencing data has presented new challenges and opportunities for microbial cultivation, which is critical for allowing direct access to microorganisms to test hypotheses experimentally, and is crucial for proper taxonomic classification, functional annotation of metagenomic sequences, and use of such microbes for environmental remediation, energy applications, and formulation of probiotics. However, a direct approach that cultivates, in a targeted fashion, microbes carrying genes of interest identified in metagenomic data sets remains mostly unexplored. As a result, for example, a list of the "Most Wanted" taxa that are urgently in need of cultivation has been issued by the Human Microbiome Project (HMP) from the National Institutes of Health. These microorganisms are highly prevalent and abundant in the human microbiome but poorly represented in cultured collections (2).

Most microbes do not grow using traditional cultivation methods and hence are referred to as "unculturable" (8–10). Although these microbes could be grown in their natural habitats (9), where effects such as cross-feeding (11) and microbe–host interactions (12, 13) are present, some biological samples, such as clinical biopsies, are often limited in quantity. This makes it challenging to set up cultivation experiments in large scale with these native media, but creates opportunities for miniaturized methods. Further, miniaturized methods that use compartmentalization can eliminate competition among species. Cultivation methods that use miniaturization and compartmentalization, including gel microdroplets (14), miniaturized Petri dishes (15), and microfluidics (16–19), have become increasingly promising as a basis for targeted microbial cultivation and isolation platforms, as they can limit the consumption of precious samples and also control the microenvironment around cells (20). We envisioned implementing targeted cultivation with microfluidics by focusing on two goals. The first goal is to efficiently identify cultivation

Significance

Obtaining cultures of microbes is essential for developing knowledge of bacterial genetics and physiology, but many microbes with potential biomedical significance identified from metagenomic studies have not yet been cultured due to the difficulty of identifying growth conditions, isolation, and characterization. We developed a microfluidics-based, genetically targeted approach to address these challenges. This approach corrects sampling bias from differential bacterial growth kinetics, enables the use of growth stimulants available only in small quantities, and allows targeted isolation and cultivation of a previously uncultured microbe from the human cecum that belongs to the high-priority group of the Human Microbiome Project's "Most Wanted" list. This workflow could be leveraged to isolate novel microbes and focus cultivation efforts on biomedically important targets.

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Conflict of interest statement: R.F.I. has a financial interest in SlipChip Corporation.

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Data deposition: The genome sequences reported in this paper have been deposited in the Joint Genome Institute's Integrated Microbial Genomes database, https://img.jgi.doe.gov/cgi-bin/w/main.cgi (accession no. 2545555870). The 16S rRNA gene sequences of isolate microfluidicus 1 reported in this paper have been deposited in the GenBank database (accession nos. KJ875866 and KJ875867).

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conditions that support growth of target microbes. This can be accomplished by performing a genetic assay with target-specific primers or probes on the pooled microbial culture from a certain cultivation condition before isolation (21); however, designing specific probes based on short reads from high-throughput sequencing can be difficult. Moreover, it can be challenging to detect and cultivate slowly growing strains, as they often fall below the limit of detection, being outcompeted by rapidly growing strains in a complex community. A second goal of targeted cultivation is to focus isolation efforts on microbial targets of interest, thereby minimizing the effort associated with isolating off-target colonies. However, both PCR and fluorescence in situ hybridization (FISH) require access to genetic material, which is often not compatible with the goal of isolating and cultivating live cells. This paper addresses these challenges. In an accompanying paper (22), we describe the design, fabrication, and underlying physics of a microfluidic device to create arrays of individually addressable replica microbial cultures. Here, we integrate this device and additional devices and methods into a workflow for genetically targeted microbial cultivation, and validate this workflow by isolating a bacterium from the Most Wanted taxa.

Results and Discussion

Overview of Workflow for Genetically Targeted Microfluidics-Based Cultivation. We envisioned isolating and cultivating microbial targets identified from metagenomic or 16S ribosomal RNA (16S rRNA) gene high-throughput sequencing studies by combining microfluidics with genetic assays (Fig. 1*A*). To address the goal of streamlining cultivation efforts using genetic assays, we created a general workflow with two major components: identification of cultivation conditions for the target organism (Fig. 1*B*) and isolation of the target (Fig. 1*C*). In both components, single bacterial cells from clinical samples are stochastically confined in nanoliter wells on a microfluidic device to promote the growth of microcolonies. This confinement can be useful for suppression of overgrowth from rapidly growing strains, in favor of slowly growing strains. In the first step, a "chip wash" method is used to monitor bacterial growth on a microfluidic device (Fig. 1*B*) under various conditions; miniaturization allows cultivation experiments that involve limited quantities of natural growth stimulants. In this method, microcolonies grown under each cultivation condition are collected into a single tube by washing the microwells after cultivation, analogously to the plate wash PCR method (21). DNA from the pooled cells is analyzed by sequencing, target-specific primers, or both, to determine whether the cultivation conditions for that chip allowed the growth of the target microorganism. This chip wash method can be repeated sequentially or in parallel until the growth conditions are identified. Then, the target organism is isolated and cultivated (Fig. 1*C*): The sample is cultivated on a separate microfluidic device, described in an accompanying paper (22), under the optimal condition identified during chip wash. After cultivation, this device splits each microcolony into two identical copies. We anticipate that multiple rounds of culture and splitting on the same device could be performed in a similar fashion. PCR is performed on the first copy to identify the compartment containing the target of interest, and then live cells can be retrieved from the corresponding well on the other half of the chip for scale-up cultivation.

To implement this workflow, we relied on the SlipChip platform for three reasons (23). First, it can create thousands of miniaturized reactions without the need for bulky equipment. It can be used in the limited space of an anaerobic chamber, which is widely used to cultivate anaerobes that dominate the human gut microbiota. Second, SlipChip is compatible with PCR (24) and enzymatic assays (25). Third, compartmentalization on SlipChip is reversible and the microcolonies can be spatially indexed as described in an accompanying paper (22), which facilitates the retrieval of reagents and organisms from the device (24, 26).

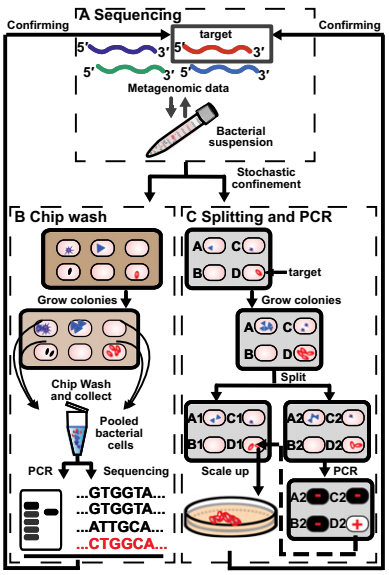

Fig. 1. Illustration representing the workflow for targeted cultivation and isolation of microbial organisms. (*A*) Microbial targets carrying genes of interest are identified by high-throughput sequencing of clinical samples. A representative sequence of the target is shown in red. To cultivate the target, the inoculum is suspended in cultivation medium and loaded onto a microfluidic device, enabling stochastic confinement of single cells and cultivation of individual species (represented by different shapes). (*B*) A chip wash method is used to monitor bacterial growth under different cultivation conditions. Cells are pooled *en masse* into a tube and DNA is extracted for genetic analysis such as sequencing and PCR. (*C*) The target can be isolated by growing the sample under the growth condition identified from the chip wash. The two halves of the device are separated, resulting in two copies of each colony. On one half of the chip, target colonies are identified using PCR. Then, the target colony on the other half of the chip is retrieved for a scale-up culture, after which sequencing is used to validate that the correct target has been isolated.

Chip Wash Device. Fig. 2*A* shows the general workflow of a chip wash experiment. We designed a microfluidic device to perform up to 3,200 microbial cultivation experiments, each on a scale of ~6 nL (Fig. 2*C* and *SI Appendix*). This device enables three capabilities: stochastic confinement of single cells from samples, microbial cultivation, and collection of cultivated cells. To confine single cells, a sample of bacteria suspended in cultivation medium is loaded into the channels and wells (Fig. 2*A*, *ii*). Slipping the bottom plate (dashed layer in Fig. 2*A*) upward enables stochastic confinement of bacterial cells in wells (Fig. 2 *A*, *iii*). To introduce gas into the channel and remove residual sample in the channel, the solution is purged from the channel by vacuum (Fig. 2*A*, *iv*). To cultivate microbes, the device is incubated and some of the single cells grow to microcolonies (Fig. 2*A*, *v*). After cultivation, the microchannel is loaded with buffer solution (Fig. 2*A*, *v*) to avoid the formation of gas bubbles. The presence of gas bubbles in a channel could increase flow resistance (27) and therefore slow down or stop the flow in that channel, resulting in inefficient washing in later steps. To allow collection of the microbial cells, the bottom plate is slipped back to overlay the wells with the channel (Fig. 2*A*, *vi*). A buffer solution is injected to flush the channel (Fig. 2*A*, *vii*) and is collected, from the outlet specifically designed for collection (Fig. 2 *A*, *viii* and *C*), in a pipette tip. The flow of fluid on SlipChip is

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controlled by positive pressure using a pipettor. This process of injection–collection is repeated three times. Immiscible oil is then injected to further displace the remaining aqueous phase. We used a red dye experiment to visualize the device operation described above (Fig. 2*B*), which allowed us to observe that the droplets remained intact during purging when gas was introduced into the channels. In addition, in the chip wash step, the solutions from the channel and the wells were merged and could be visualized by the originally colorless solutions from the channel turning red. The removal of red dye can be observed in Fig. 2*B*, *vii* as the solution in the channel turned back to colorless. To quantify the recovery efficiency of this method, a solution with a fluorescent dye was injected into the device and subsequently collected and quantified using a fluorospectrometer. We determined a recovery rate of 96% when comparing the fluorescence signal from the chip wash solution with the starting stock solution normalized to the same volume. A recovery rate of 83% was observed when *Escherichia coli* cells labeled with red fluorescent protein were used to quantify the recovery efficiency of bacterial cells.

Validating the Chip Wash Method with a Two-Species Model Community. Having validated the device's operation, we next tested the functionality of the chip wash method using a model community from the human gut microbiome (Fig. 3). First, we tested whether chip wash can detect microbial growth on SlipChip. We cultivated a mixture of *Clostridium scindens* and *Enterococcus faecalis* at a 5:1 ratio on the chip or agar plates. The genomic DNA of the starting inoculum and chip wash solution were extracted and quantified by quantitative PCR (qPCR). Cultivation on the chip followed by chip wash resulted in an ~1,000-fold increase of DNA for each strain compared with DNA from the starting inoculum used as a nongrowth control (Fig. 3*E*), showing that chip wash can be used to detect microbial growth.

Second, we hypothesized that chip wash would detect, without bias, the growth of bacteria that grow at different rates but with similar carrying capacity, for the following reason. For the interest of detection, the optimal time for sampling is the late exponential phase or early stationary phase of the target to maximize the yield of biomass. A single cell growing on a plate starts at a density of ~10 cfu mL^{-1} assuming the inoculation density is 300 cfu with 30 mL of medium, whereas a single cell growing in a 6-nL well starts at a density of ~1.7×10^5 cfu mL^{-1}. Typical carrying capacity of the media we used for gut anaerobes is ~10^9 cfu mL^{-1}; therefore, on the device the carrying capacity can be reached more rapidly, and for a larger range of growth

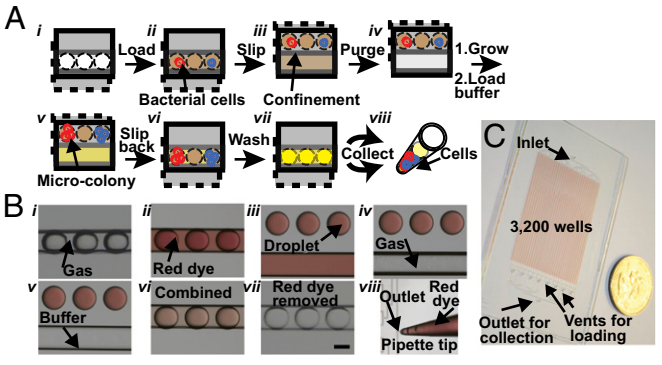

Fig. 2. Design and operation of the chip wash device. (*A*) Schematic drawings of the chip wash method illustrating device design for handling microbial cells. (*B*) Representative photographs showing device operation as visualized with red dye. See text for details. Scale bar in *i–vii*, 200 μm. (*C*) Photograph of 3,200 droplets generated and stored on the chip for chip wash, shown next to a US quarter.

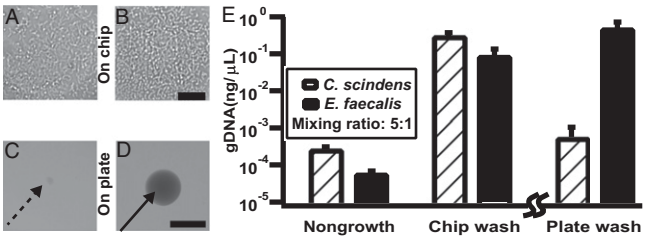

Fig. 3. Validation of the chip wash method with a model community of *C. scindens* and *E. faecalis*. Samples were collected on day 1. (*A* and *B*) Representative optical microscopy of *C. scindens* (*A*) and *E. faecalis* (*B*) grown on SlipChip. (*C* and *D*) Representative photographs of *C. scindens* (*C*) and *E. faecalis* (*D*) grown on an agar plate. (*E*) Graph showing genomic DNA of *C. scindens* and *E. faecalis* recovered from nongrowth negative control, chip wash, and plate wash solutions. The nongrowth control and the chip wash experiments were performed using an identical procedure and can be directly compared. Because the plate wash experiment requires a different protocol, only the relative values can be compared (emphasized by the break in the axis). Error bars indicate SD ($n = 3$). Scale bar, 30 μm for *A* and *B* and 1 mm for *C* and *D*.

rates, than on a plate. To test this hypothesis, we confirmed that under this particular cultivation condition, *E. faecalis* grew faster than *C. scindens* on agar plates, as observed from the difference in colony size on day 1 (Fig. 3 *C* and *D*). The cultivation medium has a similar carrying capacity for the two strains (*SI Appendix*). Consistent with the prediction, the two strains grew on the chip to a comparable density on day 1 (Fig. 3 *A* and *B*). As shown by the quantity of genomic DNA recovered from the two strains, sampling on day 1 by plate wash resulted in an ~1,000-fold bias toward rapidly growing bacteria, whereas the chip wash method effectively corrected this bias, as the genomic DNA was comparable for each strain (Fig. 3*E*). This chip wash method provides an efficient way to detect slowly growing bacteria and is complementary to the plate wash method (21). Because we have shown that SlipChip is compatible with solutions used in membrane protein crystallization (28), we expect that SlipChip would be compatible with testing a wide range of growth media with different viscosities and surface tensions.

Using Splitting to Preserve Cultivar and Perform Genetic Assays. We next tested whether genetic assays could be used to identify and characterize microbes on the chip. We used a replica-SlipChip described in an accompanying paper (22) to split the microcolonies into two halves so that PCR could be performed with one of these halves and live microbes could be preserved on the other. To unambiguously establish the mapping from genotype to phenotype, we used *E. coli* cells expressing DsRed or GFP genes to ensure the genotype could be characterized by PCR, and the phenotype could be monitored by fluorescence microscopy (Fig. 4). We tested if this on-chip PCR approach could reliably distinguish the DsRed-labeled *E. coli* from the GFP-labeled *E. coli*. A mixture of *E. coli* cells labeled with GFP and DsRed proteins was loaded onto the chip, at final densities of 2×10^4 cfu mL^{-1} and 2×10^3 cfu mL^{-1}, respectively. We assume that the cells are distributed in wells randomly and therefore that their distribution is governed by the Poisson statistics. We used a motile strain of *E. coli* to ensure uniform distribution of bacterial cells in both wells within 3 h of incubation. Individual cells were compartmentalized and cultivated, and then the chip was split into two daughter halves, each carrying a copy of the microcolonies (Fig. 4*A*). One chip was mixed with PCR reagents containing primers targeting the plasmid of DsRed and the other was imaged with a fluorescence microscope to check for the presence or absence of fluorescent proteins. We observed 125 wells that contained colonies with GFP *E. coli* and 12 wells

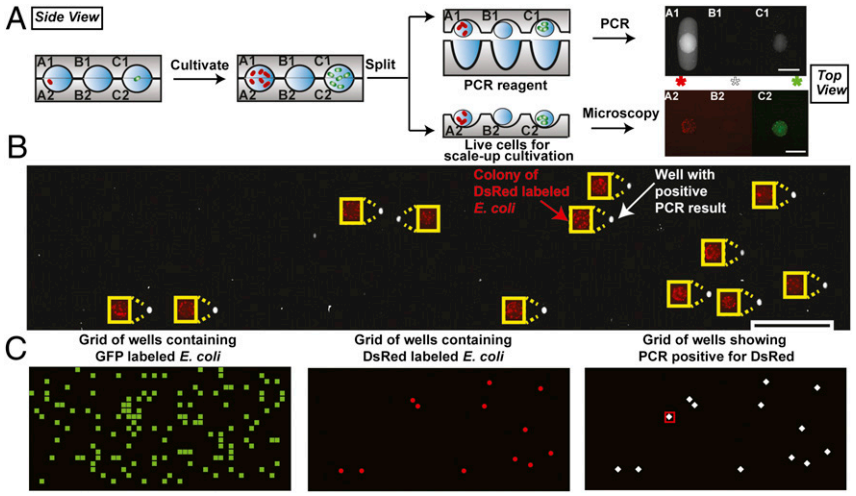

Fig. 4. Cultivating pure microcolonies from a mixture and using PCR to identify specific microcolonies. Schematics show side views, whereas photographs show top views. (*A*) Schematic illustrating the cultivation of single cells from a mixture of *E. coli* expressing GFP and DsRed genes, as well as a method for splitting individual colonies. PCR was used to identify the *E. coli* expressing DsRed gene on one half of the split chip. The PCR reagents wells have an ellipsoidal cross-section from top view. An increase in fluorescence intensity indicated a positive result for PCR, and thus, the presence of the DsRed gene. Fluorescence microscopy identified wells that contained microbes expressing red and green fluorescent proteins, matching corresponding results in PCR. (*B*) To test the accuracy of the PCR assay, results from microscopy imaging (red), indicating *E. coli* colonies expressing DsRed gene, and PCR assay (white) were montaged with an offset to allow visualization without overlap. (*C*) Plot of a 20 × 50-well grid was used to represent the position of each well on the same device. Elements corresponding to wells were colored to highlight the presence of *E. coli* GFP colonies (green squares), *E. coli* DsRed colonies (red dots), and PCR positive results for DsRed (white diamonds). A red square in the third plot denotes a false positive result from PCR. The different shapes of markers used in *C* do not represent the shapes of wells. Scale bar, 200 μm for *A*, 2 mm for *B*. A 200-μm-wide yellow rectangle was used as scale bar for images showing DsRed expressing *E. coli* colonies in *B*. Note: schematics are not to scale; dimensions are provided in *SI Appendix*.

containing DsRed *E. coli*. The wells showing PCR-positive matched the corresponding wells containing DsRed *E. coli* (Fig. 4 *B* and *C*); in contrast, blank wells that contained no bacteria and wells containing GFP-labeled *E. coli* were PCR-negative. We also noticed one well that showed increased fluorescence intensity in the PCR result, but no bacterial colony was detected in the other copy, which indicates that the well may have contained nongrowing cells or cell-free DNA from the solution. Many microbes residing in the human gut are not motile and might also adhere to surfaces. Therefore, we wanted to verify that this method would work with such organisms or whether active mixing inside SlipChip wells (25) would be required.

Identifying Cultivation Conditions for One of the Most Wanted Microbial Targets. To test this workflow, we focused on isolating microbes from the human gut that belong to the high-priority groups of the Most Wanted list. The genus *Oscillibacter* is frequently observed in the Most Wanted list (2) and other sequencing data sets (29–31), but no human-associated member of this genus has been cultivated yet. To cultivate this genus, we collected samples from the human cecum using a brushing technique to obtain mucosa-associated microbes of high biomedical interest that may directly interact with the host (Fig. 5*A*). To identify microbial targets in the cultivar, we used 16S rRNA gene high-throughput sequencing with the V4 variable region (32) as a first screening. Reads were clustered to operational taxonomic units (OTUs) de novo with mothur software (33). First, the sample was cultured on agar plates in M2GSC medium (see *SI Appendix* for ingredients) and examined by plate wash. No OTU from the cultivar was classified as *Oscillibacter*. Next, miniaturization enabled by microfluidics allowed us to test if we could culture this genus by supplementing the medium with washing fluid from the sampling site in the human cecum. We obtained washing fluid by a lavage technique, autoclaved it, and spiked into the cultivation medium, referred to in this paper as M2LC (see *SI Appendix* for ingredients). The same amount of inoculum as plated on M2GSC agar was cultivated on SlipChip with M2LC medium and then chip wash was performed as described above. High-throughput sequencing of the V4 region of the 16S rRNA gene showed the successful cultivation of *Oscillibacter* on the chip (*SI Appendix*). We performed high-throughput sequencing of the V1V3 region of the 16S rRNA gene to test if the *Oscillibacter* recovered from chip wash belonged to the Most Wanted list. We were able to assign the reads classified as *Oscillibacter* to OTU_158_V1V3 (OTU158 for short, with an estimated ~0.7% relative abundance in stool samples in the HMP dataset) from the list (*SI Appendix*) using a custom script (provided in *SI Appendix*) based on usearch software (34). PCR with OTU158-specific primers (OTU158P) confirmed that the target OTU indeed could be found in the cultivar (*SI Appendix*). OTU158P allowed us to validate results of the 16S survey conclusively by qPCR (Fig. 5*B*). We quantified the genomic DNA of OTU158 using OTU158P and total bacterial genomic DNA using 16S rRNA V4 universal primers. We observed genomic DNA of OTU158 from the chip wash experiment

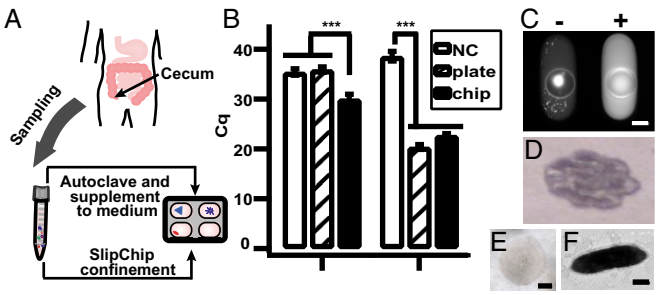

Fig. 5. Targeted isolation of isolate microfluidicus 1 from SlipChip. (*A*) Illustration showing that mucosal biopsies obtained from the human cecum were used for stochastic confinement as well as supplemented into the medium to stimulate growth of microbes. (*B*) Identifying the cultivation condition of the microbial target OTU158 using qPCR. (*Left*) Graph showing that the use of target-specific primers revealed that the target was found in the chip wash solution (M2LC) but not in the blank negative control (NC) or the plate wash solution (M2GSC). (*Right*) Graph showing that the use of universal primers of 16S rRNA gene showed that both chip wash and plate wash solutions contained bacterial genomic DNA. A lower Cq value indicates higher concentration of DNA. Error bars indicate SD ($n = 3$). (*C*) Fluorescence microscopy photograph of on-chip colony PCR after the chip was split, showing a positive well (*Right*) for OTU158. A PCR negative well is shown on the left, as indicated by the low fluorescence intensity of the solution. The bright spot was presumably from cell material stained with SYBR Green. (*D*) Photograph of the first round of scaled-up culture of OTU158. (*E*) Microphotograph of a single colony of isolate microfluidicus 1. (*F*) Transmission electron microscopy image of a single OTU158 cell. Scale bar, 200 μm for *C* and *E*, and 0.5 μm for *F*.

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but not in the blank negative control or the plate wash experiment, whereas both plate wash and chip wash solutions had similar quantities of bacterial DNA that were higher than that of the blank negative control. Chip wash with M2GSC medium did not recover OTU158 (*SI Appendix*). We concluded that the M2LC medium with the washing fluid is an optimal condition to cultivate OTU158.

Isolating OTU158 Using Replica-SlipChip. We further tested isolation and scale-up of microcolonies by cultivating the sample on the replica-SlipChip (22) with the M2LC medium containing the washing fluid from the sampling site. PCR was carried out with primers OTU158P targeting OTU158. We observed two positive wells (one is shown in Fig. 5*C*) from a single device with ~500 microbial colonies (a negative PCR well is shown in Fig. 5*C*, *Left*). We scaled up the cultivar from one of the positive wells on an agar plate using the M2GSC medium. The intact scale-up culture after 3 d of incubation is shown in Fig. 5*D*. The culture contained multiple colonies, as shown in the picture, due to the presence of multiple cfus transferred from the same well of the chip. Although we did not observe the target from plate wash and chip wash experiments in the same medium, the cells could be scaled up on an agar plate with M2GSC medium. It is possible that the target grew in M2GSC medium but was outcompeted by rapidly growing strains in both plate wash and chip wash experiments, or that the target was in a dormant state until it was primed by washing fluid from the sampling site (35). Alternatively, the scaled-up colonies may represent a subpopulation of cells that can be cultivated with M2GSC, and the microcolony grown on the chip offered enough cells to cultivate these rare cells. We expect this observation can be understood as similar isolates are obtained using this method and as improved analytics are developed for quantitatively understanding the uncultivable state of cells from environmental samples (10). Next, we performed colony PCR on this isolate with both species-specific and universal primers in bulk, and confirmed by Sanger sequencing that it was indeed the desired target. Although we observed that this was an almost pure culture (with some minor heterogeneity observed from chromatogram, shown in *SI Appendix*), we streaked the plates five times for purification to obtain single colonies (Fig. 5*D*) of target cells. This isolate, hereafter referred to in this paper as isolate microfluidicus 1, could then be routinely grown in bulk liquid culture to obtain enough biomass to initiate in vivo studies and whole genome sequencing. For example, the draft genome of this isolate was sequenced and assembled into 83 contigs comprising 3.4 Mbp sequences. We observed rod-shaped cells (Fig. 5*F* and *SI Appendix*) and two 16S rRNA gene types of 99.4% sequence identity to one another, each with 99% identical to OTU_158_V1V3 and OTU_896_V1V3 from the Most Wanted list (Table S1). Both OTUs are from the high-priority group classified as *Oscillibacter*, but their relative abundances differ by 20-fold in stool samples surveyed by the HMP (2). Although sequence heterogeneity among multiple 16S rRNA genes on the same genome is not uncommon (36), these two sequence types could either have been derived from a single strain or indicated the presence of two closely related strains. Therefore, we designed two oligonucleotide probes able to distinguish between the two sequence types and used them in FISH experiments (37, 38). All FISH-positive cells bound both sequence type-specific FISH probes (Clost183-I and Clost183-II, Fig. 6*A*), as well as the general probe mix EUB338I-III (*SI Appendix*), which specifically detects most members of the bacteria (39, 40). Together, these results demonstrate the presence of a single *Ruminococcaceae* species in the culture.

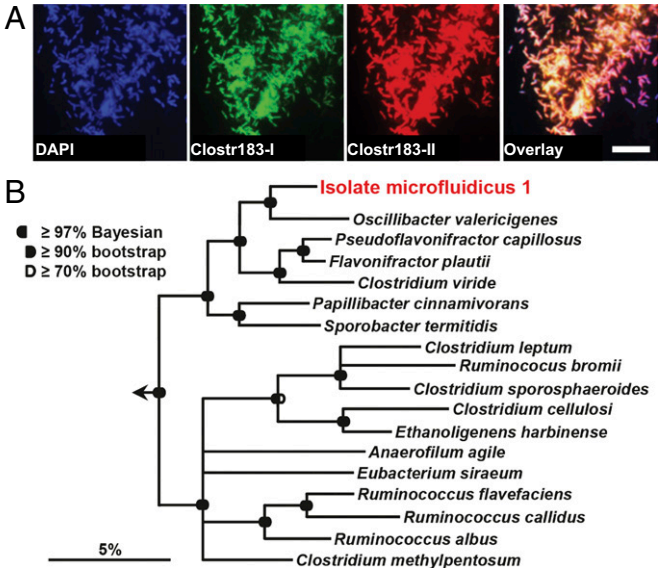

Fig. 6. Phylogenetic affiliation of isolate microfluidicus 1 and validation of the purity of the culture by FISH. (*A*) Fluorescence images showing that both 16S rRNA types obtained from the culture are expressed within the same cells, demonstrating the presence of a single *Ruminococcaceae* species within the culture. Clostr183-I and Clostr183-II indicate FISH probes, each specific to a different sequence type. (Scale bar, 10 μm.) (*B*) 16S rRNA-based consensus tree demonstrating the positioning of isolate microfluidicus 1 within the *Ruminococcaceae* (Clostridia cluster IV). Please see *SI Appendix* for details.

Improved Taxonomic Assignment of the Isolate. Short reads from 16S rRNA high-throughput sequencing may not be sufficient for assignment of taxonomy if the organisms are poorly represented in culture collections. Based on both 16S rRNA V4 and V1V3 high-throughput sequencing, the target was classified as *Oscillibacter* (see *SI Appendix* for Ribosomal Database Project classification). However, the pure culture suggests that isolate microfluidicus 1 is a member of a previously unidentified genus. The closest described relative for which a 16S rRNA sequence is available is *Oscillibacter valericigenes,* isolated from a Japanese clam (*Corbicula*) (41), which exhibits a sequence identity of 93.0% to the isolate of isolate microfluidicus 1. Phylogenetic analyses of the 16S rRNA of isolate microfluidicus 1 confirmed the unique positioning of this microbe within the *Ruminococcaceae* (42, 43) (Fig. 6*B*). These observations suggest that this highly sought (Table S2) bacterium may represent, to our knowledge, the first discovered species of an uncultured genus.

Materials and Methods

Sample Collection. Brush and luminal cecum samples were collected from a healthy volunteer. Samples were transferred into an anaerobic chamber immediately after collection and homogenized in grants buffered saline solution (GBSS) supplemented with 5% DMSO by vortexing for 5 min. Aliquots of the samples were flash frozen with liquid nitrogen and preserved at −80 °C. Work with clinical samples for this project is approved by the Institutional Review Boards at California Institute of Technology and The University of Chicago, and by the Institutional Biosafety Committee.

SlipChip Cultivation. The brush sample was serially diluted in GBSS buffer and then suspended in M2LC medium. This bacterial suspension was then loaded onto SlipChip designed for chip wash and incubated for 3 d.

Chip Wash. The cultivar was collected into an Eppendorf pipette tip by flowing 90 μL PBS buffer three times and then 90 μL tetradecane into the SlipChip. The solution was then transferred into an Eppendorf tube. DNA was extracted using a QiaAmp kit following the manufacturer's protocol and then used to prepare the library for high-throughput sequencing and PCR.

Isolation of Isolate Microfluidicus 1. We used the replica-SlipChip to cultivate and split the microcolonies. One copy was used for colony PCR to identify the wells containing OTU158. The microcolony from the other copy was transferred on an M2GSC agar plate for scale-up culture.

Conclusions

In this paper, we describe an integrated microfluidic workflow for genetically targeted isolation of microbes, and validate it by successful isolation and cultivation of isolate microfluidicus 1 from the HMP's Most Wanted list. To our knowledge, this is the first example of targeted isolation of a high-priority member from the list, and is the first successful targeted cultivation from a complex biological sample of a previously uncultured taxon defined only by short reads from high-throughput sequencing of the 16S rRNA gene. We believe this genetically targeted workflow can become a general method beyond the isolate described in this paper, as in our preliminary experiments, an additional high-priority and three medium-priority organisms on the Most Wanted list have been isolated. We envision that the microfluidics-based workflow described in this paper will be useful for conclusively testing hypotheses generated from culture-independent studies by providing pure cultures of biomedically and environmentally significant microorganisms.

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Gene-targeted microfluidic cultivation validated by isolation of a gut bacterium listed in Human Microbiome Project's Most Wanted taxa

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Supporting Text

All chemicals were purchased from commercial sources and used as received unless otherwise stated.

Homemade Reagents

A protocol for making GBSS buffer can be found on the Schmidt Lab website (1). For H_2Oc or PBSc, 0.2% cysteine was added and the solution was sterilely filtered through 0.22 μm membrane. 0.1 M $Fe(SCN)_3$ solution was sterilely filtered through 0.22 μm membrane (Fisher Scientific) and used as a red dye solution. Tetradecane (Fisher Scientific) was sterilely filtered through 0.22 μm membrane (Fisher Scientific). For assembling SlipChip for depositing PCR reagents, filtered tetradecane was degassed under house vacuum overnight. All plastic consumables and reagents were equilibrated in an anaerobic chamber for more than 24 hours before usage.

Anaerobic Chamber

A Coy lab anaerobic chamber equipped with dehumidifier was used in all anaerobic cultivation experiments. The hydrogen level was maintained at 3–4% and a relative humidity of 30%. The chamber was equipped with recirculating atmosphere filtration system (HePa) to maintain a sterile atmosphere.

Design and Fabrication of SlipChip

SlipChips for chip wash were fabricated as previously reported(2). Photomasks were designed in AutoCAD and ordered from CAD/Art Services, Inc. (Bandon, OR). Soda-lime glass plates with chromium/photoresist and chromium/gold/photoresist coating were purchased from Telic Company (Valencia, CA). The device contained 3,200 microwells for compartmentalization on one plate, and continuous loading channels on the other plate. The depth of wells was 90 μm measured using a profilometer (Dektak 150, Veeco, CA). Through holes were drilled with a 0.035" drill bit (Diamond ball 4F bit, Harvey Tool #74335-C4, Colmar Industrial Supplies, Wheeling, IL) before surface modification. Glass debris from drilling was removed by sonicating the chips in a 1:1 mixture of water and ethanol for more than one hour in a warm water bath. Prior to use, the SlipChips were cleaned with piranha solution, rinsed three times with millipore water followed by 200 proof ethanol, blow dried with nitrogen and silanized with dimethyldichlorosilane using a previously reported protocol (2). An acid piranha solution (caution: this is a corrosive mixture) is used to remove organic contaminants from substrates by mixing 3:1 (v:v) concentrated sulfuric acid (H_2SO_4) with 30% hydrogen peroxide (H_2O_2). Sulfuric Acid (Cat. #A300-212) and 30% Hydrogen peroxide (Cat. #H325-4) can be ordered through Fisher Scientific.

Silanized chips were stored at room temperature in a dessicant box (< 15% humidity, maintained with Drierite). When a glass SlipChip needed to be reused, it was cleaned with Piranha solution first, and then subjected to the same silanization procedure described above.

For the replica-SlipChip, the depth of the features measured by a contact profiler (Dektak 150, Veeco, CA) was 90 μm. The depth was 120 μm for wells, and 60 μm for loading channels of the SlipChip used for depositing PCR reagent wells. The lateral dimensions of cultivation and PCR reagent wells can be measured by photographs from a stereoscope, as shown in the accompanying paper (3).

Performing Chip Wash

The plates of SlipChip were assembled under a layer of tetradecane in a petridish and four binder clips (small binder chips, cat # 429-415, Office Depot) were applied to hold the devices together. The lubricating oil in the loading channel and wells was removed by repeated purging with vacuum for 3 to 5 times at an interval of 1-2 hours between each purging. For anaerobic culture, the SlipChip was placed into the anaerobic chamber for at least 24 hours prior to use. Aqueous solution was loaded onto SlipChip by pipetting, and the device was slipped to form compartments. The solution in the loading channel was removed by purging with a vacuum. In the case of anaerobic cultivation, a gas recirculation pump for atmosphere filtration system was used as a vacuum source. For chip wash experiments with microbial samples, the loading channel can be cleaned by repeated washing with GBSS or PBSc buffer solution to

remove any residual microbial cells and prevent overgrowth of microbes in the loading channel. The continuous loading channels were used as gas exchange channels.

PBS buffer was loaded into the channel to remove the gas phase, and can be used for repeated washing in the case of microbial samples. The SlipChip was designed to collect the chip wash solution with a single outlet (Fig. S1). To collect the solution, 90 µL PBS buffer was injected into the SlipChip using an Eppendorf pipettor and collected into an Eppendorf pipette tip (1-200 µL, cat. No. 02-717-141, Fisher Scientific) and transferred into an Eppendorf tube. This process was repeated for three times. 90 µL tetradecane was loaded into the SlipChip to further remove the diluted aqueous solution. The solution was also transferred from the pipette tip into the Eppendorf tube.

It is important to keep the lubricating oil in the device from drainage during incubation at elevated temperature or for long-term culture. Loss of lubricating oil in the gap between the two glass plates causes cross-contamination among the confined wells, and cannot be used in the chip wash experiment because the washing buffer would flow through the gap and not into the collection outlet. To prevent loss of oil from the device, a piece of Kimwipe was briefly saturated with 1:1 (vol) mixture of water and tetradecane and then placed inside a Petri dish. The SlipChip was then placed into the Petri dish and Parafilm was used to seal the Petri dish. The Petri dish was then incubated at the desired temperature for microbial culture.

E. coli Preparation

The green fluorescent protein (GFP)-labeled *E. coli* RP 437 were obtained as a gift from Guillaume Lambert at Princeton University. *E. coli* RP 437 was purchased from CGSC (catalog #: 12122) and transformed with DsRed plasmid.

E. coli cells labeled with DsRed fluorescent proteins were enriched with 50 µg mL^{-1} of Ampicillin in LB at 37 ^{o}C overnight (12 hours) in a rotary shaking incubator (SI-600 Lab Companion, Jeio Tech) at 200 rpm. Overnight culture was then diluted 100-fold and cultured with 10 µg mL^{-1} of Ampicillin and 40 µmol L^{-1} IPTG in LB media for 3 hrs. Cells from 1 mL culture were then pelleted at 3000 ×g for 5 min and washed 3 times with 1 mL of ice cold 1× PBS buffer before use.

Quantifying the Recovery Efficiency of Chip Wash

To quantitatively test the recovery efficiency of this method, an aqueous solution of 200 nM Alexa Fluor 488 hydrazide (Invitrogen) in PBS buffer was injected into the device and subsequently collected by chip wash method, normalized to a volume of 500 µL, and quantified using a fluorospectrometer (Thermo scientific). 0.45 nM, 0.9 nM, 1.8 nM, 3.6 nM and 7.2 nM of Alexa Fluor 488 hydrazide solutions in PBS buffer were used to obtain a calibration curve. The loading volume of the device was calculated to be 18 µL. Therefore, the concentration of the recovered solution was divided by the concentration of 7.2 nM Alexa Fluor 488 hydrazide to calculate the recovery efficiency.

E. coli cells labeled with DsRed fluorescent proteins were prepared as described above, loaded onto SlipChip and collected immediately using chip wash method. The chip wash solution was normalized to a volume of 500 µL. 18 µL of the same solution was diluted to 500 µL and used as a control. The cells from chip wash solutions as well as the control solution were quantified using INCYTO C-Chip (DHC-N01) under Leica DMI6000 microscope (Leica Microsystems) with a 20 x/0.4NA Leica objective, TX2 filter and a Hamamatsu ORCA-ER camera with 1× coupler with an exposure time of 200 ms. Images were acquired and analyzed by using Metamorph imaging system version 6.3r1 (Universal Imaging). Recovery efficiency was calculated by dividing cell number in the recovered solution by the cell number in the control solution.

Reagents and Equipment for PCR

Primers for PCR were ordered from Integrated DNA Technologies (Coralville, IA). SsoFast EvaGreen Supermix (2X) was purchased from Bio-Rad Laboratories (Hercules, CA). Bovine serum albumin solution (BSA) was purchased from Roche Diagnostics (Indianapolis, IN). PCR Mastercycler and in situ adapter were purchased from Eppendorf (Hamburg, Germany).

Using Chip Wash to Monitor Bacterial Growth with a Single Species Model System

E. coli cells labeled with DsRed fluorescent proteins prepared as described above were serially diluted to a final density of ~10^5 CFU/mL in 10 µg mL^{-1} of Ampicillin and 40 µmol L^{-1} IPTG in LB media or 1× PBS buffer that does not support growth of bacteria as a negative control and loaded onto SlipChip. SlipChip was incubated at 37 ^{o}C overnight. The solution was collected after cultivation using chip wash. Genomic DNA was purified from chip wash solutions using QiaAmp kit (Qiagen). For calibration, genomic DNA was purified from bulk liquid culture of *E. coli* cells labeled with DsRed fluorescent proteins, quantified by Quanti-it DNA high sensitivity quantification kit (Invitrogen), and serially diluted in AE buffer containing 0.01 mg/mL of BSA. The reaction master mixture for qPCR was prepared by mixing 10 µL of 2X SsoFast EvaGreen Supermix, 1 µL of forward and reverse primer (10 µmol L^{-1}), 1 µL of template solution and 8 µL of water (Fisher Scientific, BP 2470-1). qPCR was performed on the Eco real-time PCR machine (Illumina, Inc, San Diego, CA) with 27F(4) (5′-AGAGTTTGATCCTGGCTCAG -3′) and 534R (5) (5′-ATTACCGCGGCTGCTGG-3′) primers. We observed a 10,000-fold increase in DNA concentration (Fig. S1), suggesting that for this particular model system, non-growing cells contribute to 0.01% of the genetic material recovered from chip wash.

Performing Chip Wash and Plate Wash Experiment with a Two-Species Model System

Cells of *Clostridium scindens* (ATCC 35704) and *Enterococcus faecalis* (ATCC 49532) were separately enriched in Schaedler Anaerobe Broth (Oxoid) at 37^{o}C in an incubator (model# 10-140E, Quincy lab Inc) overnight (~ 16 hours) in anaerobic chamber (Coylab). The culture was diluted 100 fold and incubated at 37^{o}C for 5 hours (*E. faecalis*) and 8 hours (*C. scindens*). The cells were pelleted at 6000 ×g for 3 minutes and washed with PBSc for 3 times. Cell numbers of the two species were estimated for loading by cell counting using INCYTO C-Chip (DHC-N01) under Leica DMI6000 microscope (Leica Microsystems) with a 20 x/0.4NA Leica objective. The two species were mixed at 5:1 ratio (*C. scindens*: *E. faecalis*, confirmed by separately plating the two species) in Schaedler Anaerobe Broth at a final density of ~10^5 CFU/mL, loaded onto SlipChip and incubated at 37^{o}C for 24 hours for growth. Genomic DNA extracted from 18 μL of the same solution containing mixture of cells using QiaAmp kit (Qiagen) was used as non-growth control. To perform plate wash, 4 μL of the same solution containing mixture of cells was plated on Schaedler Anaerobe medium with 2% (wt/vol) noble agar (Fisher Scientific) to achieve a final density of ~300 colonies (counted on day 3 after cultivation when both of the species reached saturation on an agar plate) to reduce interaction between colonies. The cultivar was collected by chip wash and plate wash. Plate wash was performed following a previously described protocol(6) with minor modifications. Cell scrapers (Fisher Scientific) were used to collect cultivar into 1 mL of GBSS buffer. 50 μL of the combined solution was centrifuged at 6000× g for 10 minutes to pellet the cells. Serial dilutions of genomic DNA from macroscale liquid culture of the two species quantified by Quanti-it DNA high sensitivity quantification kit (Invitrogen) were used to calibrate the qPCR machine. The reaction master mixture for qPCR was prepared by mixing 10 μL of 2X SsoFast EvaGreen Supermix, 1 μL of forward and reverse primer (10 μmol L^{-1}), 1 μL of template solution and 8 μL of water (Fisher Scientific, BP 2470-1). For plate wash, ~1 ng/ μL of genomic DNA was used for qPCR as we are interested in the relative ratio of *C. scindens* and *E. faecalis*. qPCR was performed on the Eco real-time PCR machine (Illumina, Inc, San Diego, CA) with ScinF4 (5′-CGTAACGCGCTCTTTCTTCG-3′) and ScinR4 (5′-CCTTCCTCCAGGTTCTCCCT-3′) for *C. scindens* and E.faecalis_F (5′-CGC TTC TTT CCT CCC GAG T-3′) and E.faecalis_R (5′-GCC ATG CGG CAT AAA CTG-3′). The two pairs of primers are specific to the targeting species, which was confirmed by qPCR.

We cultivated the two strains separately on SlipChip or on agar plates. To monitor bacterial growth on agar plate, cells were plated on Schaedler Anaerobe Agar separately, incubated at 37 ^{o}C and imaged every 24 hours with a Leica MZ 16 stereoscope. The plating experiment was set up with more than three plates for each species. For each time point, one plate was taken out of the anaerobic chamber for imaging and discarded. To image bacterial growth on the SlipChip, bacterial cells from each species were loaded onto a "replica-SlipChip" described in the accompanying paper(3) at a final density of ~10^5 CFU mL^{-1} in Schaedler Anaerobe broth. The device was incubated for 24 hours to allow growth of bacteria. The microcolonies were imaged under Leica DMI6000 microscope (Leica Microsystems) with a 20 x/0.7NA Leica objective and a Hamamatsu ORCA-ER camera with 1× coupler under bright field.

On-chip Cultivation of *E. coli* and Splitting of the Microcolonies

E. coli cells were enriched with 50 μg mL^{-1} of Ampicillin in LB at 34 ^{o}C overnight (12 hours) in a rotary shaking incubator at 200 rpm to reach stationary phase. To synchronize cells, overnight culture of each species was then diluted 100-fold and cultured with 10 μg mL^{-1} of Ampicillin and 40 μmol L^{-1} IPTG in LB media for 3 hrs. 1 mL culture of cells were then pelleted at 3000 ×g for 5 min and washed 5 times with 1 mL of 1× PBS buffer. Cells were finally suspended in 10 μg mL^{-1} of Ampicillin and 40 μmol L^{-1} IPTG in LB media and cell suspension was serially diluted with 10 μg mL^{-1} of Ampicillin and 40 μmol L^{-1} IPTG with 0.5% of ultra-low gelling temperature Type IX-A agarose (Sigma-Aldrich) in LB media and mixed to a final density of 2 × 10^4 and 2 × 10^3 CFU mL^{-1} for *E. coli* strains with GFP and DsRed genes, respectively, and loaded onto replica-SlipChip as described in the accompanying paper(3). SlipChip was incubated at 34 ^{o}C for 3 hours and then split into two halves as described in the accompanying paper(3). The bottom half was kept on the holder for colony PCR and the top was preserved at 10 ^{o}C on Echo therm chilling plate (Torrey Pines Scientific, Carlsbad, CA) under oil in a Petri dish.

Depositing PCR Reagents on SlipChip

The reaction master mixture was prepared by mixing 100 μL of 2X SsoFast EvaGreen Supermix, 1 μL of forward (DSR_F, 5′-GGACGGCTCCTTCATCTACA-3′, 100 μmol L^{-1}) and reverse primer (DSR_R, 5′-GGTGATGTCCAGCTTGGAGT-3′, 100 μmol L^{-1}), 10 μL of 10 μg mL^{-1} BSA solution, and 68 μL of 2.5% (w/v) ultra-low gelling temperature agarose in water. This mixture was then loaded onto the SlipChip for depositing PCR reagents described in the SI of the accompanying paper (3) by replacing tetradecane in loading channels and this SlipChip for depositing PCR reagent was split to obtain 1,000 droplets deposited on one half of the SlipChip.

Combining the Replica-SlipChip with the PCR Chip

The PCR chip preloaded with PCR reagents was taken off the holder and combined with the bottom piece of the replica chip by aligning through-holes with the pins. A binder clip (5/32' inch capacity, 1/2' inch size, officemax, Itasca, IL) was used to clamp the two plates together, allowing the combined SlipChip to be removed from the holder and the oil without misalignment.

Fluorescence Imaging of PCR Results and *E. coli* with GFP and DsRed Fluorescent Proteins

Fluorescence images were acquired with a Leica DMI6000 microscope (Leica Microsystems) with a 10 x/0.4NA Leica objective and a Hamamatsu ORCA-ER camera with 1× coupler. An L5 filter with an exposure time of 500 ms was used to collect images. For quantitative analysis, fluorescent intensity of a fluorescence reference slide for L5 filter was recorded and used for background

correction. Images were acquired and analyzed by using Metamorph imaging system version 6.3r1 (Universal Imaging) and ImageJ by the National Institutes of Health (http://rsb.info.nih.gov/ij/download.html). Processing was applied equally to the entire image.

Preparation of M2GSC Medium

This protocol is adapted from reference (7).

1L M2GSC medium contains the following ingredients:

10 g of casitone, 2.5 g of yeast extract, 4 g of $NaHCO_3$, 2 g of cellobiose, 2 g of soluble starch, 300 mL of rumen fluid, 2 g of glucose, 1 g of cysteine, 0.45 g of K_2HPO_4, 0.45g of KH_2PO_4, 0.9 g of $(NH4)_2SO_4$, 0.9 g of NaCl, 0.09 g of $MgSO_4$ $7H_2O$, 0.09 g of $CaCl_2$, 15 g of Agar Noble and 1 mg of resazurin.

Preparation of M2LC medium

We prepare the basal medium containing: 10 g of casitone, 2.5 g of yeast extract, 4 g of $NaHCO_3$, 2 g of cellobiose, 2 g of soluble starch, 2 g of glucose, 1 g of cysteine, 0.45 g of K_2HPO_4, 0.45g of KH_2PO_4, 0.9 g of $(NH4)_2SO_4$, 0.9 g of NaCl, 0.09 g of $MgSO_4$ $7H_2O$, 0.09 g of $CaCl_2$, 15 g of Agar Noble and 1 mg of resazurin in 700 mL of water.

We mixed 70 µL of the basal medium with 30 µL supernatant of autoclaved luminal sample.

Handling Frozen Stock Solutions of Bacterial Samples in an Anaerobic Chamber

10 µL aliquots of homogenized brush samples and ~50 µL aliquots of homogenized luminal samples were stored in -80 ^oC freezer. For cultivation, the brush sample was transferred from the freezer to an anaerobic chamber on dry ice with GasPak systems.

Microbial Cultivation from the Biopsy with M2GSC Medium and Performing Plate Wash

An aliquot of cecum brush sample was serially diluted in GBSS buffer. 18 µL of the 10^4 dilution was plated onto four M2GSC agar plates (4.5 µL for each plate) yielding ~250 colonies per plate after three days of incubation at 37 ^oC (model# 10-140E, Quincy lab Inc) in an anaerobic chamber (Coylab). Plate wash was performed following a previously described protocol (6) with minor modifications. Cell scrapers were used to collect cultivar into 1 mL of GBSS buffer. The plate wash solutions from four plates were combined into a single tube and mixed by vortexing. 50 µL of the combined solution was centrifuged at 6000× g for 10 minutes to pellet the cells. Genomic DNA was extracted using QiaAmp kit (Qiagen) and quantified by Quanti-it DNA high sensitivity quantification kit (Invitrogen). The experiment was carried out in triplicates. The volume of autoclaved luminal fluid was not enough to prepare plate medium; therefore we did not perform the bulk culture the sample with M2LC medium on agar plates.

Microbial Cultivation from the Biopsy with M2LC and M2GSC Medium and Performing Chip Wash

An aliquot of cecum brush sample was serially diluted in GBSS buffer and then in M2LC. 10^4 dilution of the brush sample was loaded onto a 3,200 well SlipChip (loading volume of 18 µL and is consistent with sample used the plate wash) and incubated (model# 10-140E, Quincy lab Inc) for three days at 37 ^oC in an anaerobic chamber (Coylab). Chip wash was performed and the chip wash solution was centrifuged at 6000× g for 10 minutes to pellet the cells. Genomic DNA was extracted using QiaAmp minikit (Qiagen) and quantified by Quanti-it DNA high sensitivity quantification kit (Invitrogen). The experiment was carried out with triplicate devices.

Designing Primers for 16S V1V3 rRNA Gene for Miseq High Throughput Sequencing

We chose high-throughput sequencing for three reasons: First, high-throughput sequencing can be used to profile the community at great depth, and is cost-effective and less labor-intensive than cloning of universally amplified PCR product and Sanger sequencing; Second, compared with testing with primers targeting different groups of microbes, high-throughput sequencing is intrinsically multiplexed and can be used to detect multiple targets simultaneously. This feature is well suited for the "Most Wanted" list, as 45 high priority targets are defined for human gut microbiome. Third, designing and validating primers or probes for specific targets at the resolution of individual OTUs for a complex community from short reads can be challenging for non-experts, and high-throughput sequencing can be used to retrieve the target of interest before the effort for designing target-specific primers.

The primers for variable region V1V3 of 16S rRNA gene was designed similar to that of V4 region of 16s rRNA gene (8, 9). Primer Prospector (10) was used to design the linker region with reference sequences from Greengenes (11) February 2013 release. Possible interactions between barcodes and new pad and linker regions that may yield secondary structures were also screened with Primer Prospector.

16S rRNA Gene Library Construction for Miseq High Throughput Sequencing

The library was prepared according to published protocol. 20 ng of genomic DNA extracted from chip wash solution with M2LC medium or plate wash solutions with M2GSC medium were used in each 50 µL reaction mix. The mixture contained X µL of template DNA (adjusted to 20 ng), (13.5-X) µL of H_2O (Fisher Scientific, BP 2470), 20 µL of 5 Primer Hot MasterMix (5 prime: cat # 2200410), and 1 µL of Primer mix (10µM of Forward primer and barcoded Reverse primer). The PCR reaction was set up in triplicates and PCR product was purified by Agencourt AmPure XP beads (Beckman Coulter Inc, A63881) followed by Qiaquick PCR

purification kit (Qiagen). The purified PCR product was pooled in equal molar quantified by Kapa library quantification kit (Kapa Biosystems, KK4824) and sent for sequencing at GenoSeq Core of UCLA (Los Angeles, CA).

Data Analysis for High Throughput Sequencing of 16S V4 rRNA Gene

OTUs were chosen *de novo* with mothur (12) to identify candidate targets. The results were summarized as an OTU (Operational taxonomic unit) table. The OTU table from chip wash M2LC medium and plate wash with M2GSC medium was sub-sampled to 12599 reads per sample and summarized at genus level using QIIME (13). The relative abundance of each OTU from the two methods is presented in Fig. S4.

Data Analysis for High Throughput Sequencing of 16S V1V3 rRNA Gene

2×250 bp paired end reads were assembled using PANDASeq (14) and de-replicated with usearch (15). The de-replicated reads were sorted by abundance and clustered at 97% identity. Chimeric reads were detected using UCHIME (16) with both *de novo* and reference-based methods. The filtered reads were then searched against the HMP's "Most Wanted" list for targets within the identity of 97% using usearch v6.0.293. Alignment of the sequence from chip wash with the sequence of OTU158 from the most wanted list is shown in Fig. S5. Detailed scripts are provided in the SI below.

Using qPCR to Quantify Bacterial Genomic DNA from the Total Bacteria and OTU158

Primers for PCR were ordered from Integrated DNA Technologies (Coralville, IA). SsoFast EvaGreen Supermix (2X) was purchased from Bio-Rad Laboratories (Hercules, CA).1.5 ng purified gDNA was used to prepare the reaction mixture of a total volume of 30 µL. Water (Fisher Scientific, BP 2470) was used as negative control. Universal primers for the V4 region of 16S rRNA gene (515F, 5′-GTGCCAGCMGCCGCGGTAA-3′, 806R, 5′-GGACTACHVGGGTWTCTAAT-3′) were used to quantify total bacterial load, and OTU158 specific primers (158F, 5′-AGA ATC TAC TGA AAG AGT TTT CGG A-3′, 158R, 5′-TTC TAG AGG TAC CGT CTT CTG CT-3′) were used to quantify the concentration of OTU158. The mixture was split into 3 aliquots and loaded onto the Eco real-time PCR machine (Illumina, Inc, San Diego, CA). Reactions were incubated for 2 min at 98 °C, followed by 40 cycles of 5 s at 98 °C, 3 s at 60 °C. Data analysis was performed using Eco software.

Using Splitting Technology and the On-chip PCR Method to isolate the bacterium: isolate microfluidicus 1

An aliquot of cecum brush sample was serially diluted in GBSS buffer and then in M2LC medium. 10^4 dilution of the brush sample was loaded onto a 1,000 well replica-SlipChip (3) and incubated (model# 10-140E, Quincy lab Inc) for two days at 37 ^oC in an anaerobic chamber (Coylab). The replica-SlipChip was split as described in the accompanying paper (3), and on-chip PCR was performed and analyzed as described above with the following modification: The PCR reaction master mixture was prepared by mixing 150 µL of KAPA 2G Robust Hot Start Readymix (KAPA BIOSYSTEMS), 1.5 µL of forward and 1.5 µL of reverse primer (100 µmol L^{-1}), 15 µL of 10 µg mL^{-1} BSA solution, and 94.5 µL of 2.5% (w/v) ultra-low gelling temperature agarose in water, and 7.5 µL of 40X SYBR green (Sigma-Aldrich). The staining of cell material with SYBR green was observed and could be used to estimate the number of microcolonies grown on device could be estimated . Microbes from the PCR-positive wells on the plate for sample preservation was retrieved as described in the accompanying paper (3), and plated on M2GSC plate. After two days of incubation, the cluster of colonies was used for both colony PCR and streaking additional plates.

16S rRNA Gene Analysis for isolate microfluidicus 1

Genomic DNA for PCR amplification was isolated using QiaAmp kit (Qiagen) following the manufacturer's protocol with the following modification. We added a bead-beating step using lysing matrix B (MP Biomedicals 6911-500) that was shaken using a Mini-Beadbeater-16 (BioSpec Products, Inc.) for 1 min. The 16S rRNA gene was amplified by PCR using AccuPrimer Pfx DNA polymerase (Invitrogen). Primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) (17) were used for PCR amplification. PCR amplification was performed by Biorad thermocycler with 2 min incubation at 95 ^oC, followed by 34 cycles at 95 ^oC for 15s, 55 ^oC for 30s and 68 ^oC for 90s. Amplified PCR product was cloned into TOPO vector (Invitrogen) and transformed into TOPO10 *E. coli* cells (Invitrogen) on LB/Amp+ medium. The plates were incubated at 37 ^oC overnight and single colonies were picked for liquid culture. Plasmids were purified from cells using Qia miniprep kit (Qiagen). Plasmid DNA was then amplified by PCR with the same protocol as described above using M13F/M13R primers (Invitrogen). PCR products were purified using Qia quick PCR purification kit (Qiagen).

Sequencing PCR Products and Data Analysis

PCR products were sequenced by Laragen, Inc. (Culver City, CA) using T3 and T7 as sequencing primers (Invitrogen). The paired-end reads were assembled in Seqman Pro (DNASTAR) and manually trimmed to remove the adapters and PCR primers in Microsoft Word. 15 assembled sequences were aligned using muscle (18) and usearch (15).

TEM

TEM was performed in the Jensen laboratory electron microscopy facility at the California Institute of Technology with 200 mesh formvar/ carbon grids on TECNAI 120 keV TEM (FEI, Hillsboro, OR) equipped with a Gatan 2k by 2k CCD camera for image acquisition.

Optical Microscopy

Optical microscopy of the isolate was obtained by suspending the cells in PBS buffer and imaged using a 63×/1.2 NA Leica objective with a Leica DMI6000 microscope (Leica Microsystems) and a Hamamatsu ORCAER camera.

Ribosomal Database Project (RDP) classification of 16S rRNA gene of OTU158

Taxonomic assignment of the sequencing results for OTU158 (isolate microfluidicus 1) was generated using the online RDP classifier with 16S rRNA training set 9. The results are summarized below:

Classification on sequence of OTU158 retrieved from high throughput sequencing of V4 region of 16S rRNA gene:
Bacteria(100);Firmicutes(100);Clostridia(100);Clostridiales(100);Ruminococcaceae(100); Oscillibacter(100);

Classification on sequence of OTU158 retrieved from high throughput sequencing of V1V3 region of 16S rRNA gene:
Root(100);Bacteria(100);Firmicutes(100);Clostridia(100);Clostridiales(100);Ruminococcaceae(100);Oscillibacter(99);

Classification on sequence of two types of full length 16S rRNA gene of isolate microfluidicus 1 from Sanger sequencing:
Root(100%) Bacteria(100%) Firmicutes(100%) Clostridia(100%) Clostridiales(100%) Ruminococcaceae(100%) Oscillibacter(75%);
Root(100%) Bacteria(100%) Firmicutes(100%) Clostridia(100%) Clostridiales(100%) Ruminococcaceae(100%) Oscillibacter(84%);

Numbers in parentheses give the classification confidence level; threshold is 80%.

Phylogeny

Phylogenetic analyses of 16S rRNA gene sequences were performed using the software package ARB(19) as well as MrBayes (20). Automatic alignments of sequences obtained from our culture as well as from reference strains were manually refined within ARB. A consensus tree was constructed based on maximum-likelihood (ML) calculation (using the Hasegawa, Kishino and Yano substitution model), and by collapsing all nodes with parsimony bootstrap (5,000 iterations) support ≤50% or Bayesian support below ≤70%. Conditions for Bayesian inference were as follows: 2 parallel runs; 1,000,000 tree generations; sample frequency 100; final split frequency 0.007; potential scale reduction factor 1.006; burnin of 25% of sample. For phylogenetic analyses only sequences of cultured members of the *Clostridia* for which the 16S rRNA sequence was available were considered. Using a manually designed sequence filter we excluded highly variable in-del positions from the analysis, resulting in 1,371 alignment positions for tree calculations.

Fluorescence *in situ* Hybridization

16S rRNA targeted FISH was carried out following established protocols (21). In brief, formaldehyde- and ethanol-fixed samples were hybridized at 46°C with FAM- and Cy3-labeled oligonucleotide probes for 16 hours in a formamide-containing humid chamber. To test whether cell wall digestion leads to an increase in fluorescence detection and/or labeling intensity, before hybridization samples were pre-treated with either (i) 10 mg mL^{-1} lysozyme in TE buffer (1 h at 37°C in a humid chamber); (ii) 15 µg mL^{-1} proteinase K in TE buffer (10 min at room temperature, *i.e.* 23°C) followed by 0.01 M HCl (10 min at 23°C); or (iii) a 1:1 mix of acetone:methanol (15 min at 23°C). Formamide concentrations in the hybridization buffer were as recommended: 20-35% for probe mix EUB338 I-III (22, 23) and control probe NonEUB338 (24); 35% for probe Arch915 (25); 20% for probe EUK516 (22). The two newly designed probes Clostr183-I (AAA GAT CAT GCG ACC TCT) and Clostr183-II (AAG AAT CAT GCG ACC CCT) were hybridized at 15% (at concentrations >20% we did not observe any fluorescence signal). Via competition for the same binding site, these probes are able to distinguish between the two 16S rRNA gene sequence types obtained from our culture. After hybridization, slides were washed for 10 min in pre-warmed washing buffer at 48°C. Then, they were dipped into pre-cooled deionized water (4°C) and dried using pressurized air. Slides were mounted with DAPI/Citifluor and analyzed using an Olympus BX51 epifluorescence microscope. Fluorescence images were analyzed using the software provided by the microscope manufacturer and ImageJ by the National Institutes of Health (http://rsb.info.nih.gov/ij/download.html). No unspecific labeling was observed when control probe NonEUB338 (9) was applied to our samples.

Whole Genome Sequencing

The genome of isolate microfluidicus 1 was sequenced on Illumina Hiseq 2000 and assembled using a combination of velvet (26) and GapCloser (27). We obtained 83 contigs with N50 length of 131 kbp.

Scripts for Identifying Targets from "Most Wanted" list from paired-end Miseq reads

File name: get_target_all

```
#!/bin/bash
#file created on Dec 2, 2012
#Author= "Liang Ma"
#Email= liangma.chem@gmail.com
# usage example:  get_target_all ~/Downloads/reads/
#set environmental variables
#usearch program
export u=/Users/Liang/scripts/usearch/usearch6.0.293_i86osx32_lm
#reference database for UCHIME
export UCHIME_REFUDB=/Users/Liang/scripts/Database/gold.fa
# fasta file of consensus sequence from most wanted list, high priority group
export MostWanted=/Users/Liang/scripts/Database/MostWanted.fa
export scriptsHome=/Users/Liang/scripts
# $1 is the folder containing fastq files with paired end reads
cd $1
for file in $(ls| grep .*R1.*\.fastq)
do
FN=$(echo $file|grep -o .*R)
FileR="$FN"2_001.fastq
#assemble reads with pandaseq
pandaseq -f $file -r $FileR -o 10 > "$FN.fa"
$u -derep_fulllength $FN.fa -output "derep_$FN.fa".fa –sizeout
readname='results'
mkdir -p $FN$readname
cd $FN$readname
$scriptsHome/get_target1 .."derep_$FN.fa.fa"
cd $1
done
```

File name: get_target1

```
#!/bin/bash
#file created on Dec 2, 2012
#Author= "Liang Ma"
#Email= liangma.chem@gmail.com
# usage example: get_target1 reads.fa
if [ x$1 == x ] ; then
        echo Missing FASTA filename >> /dev/stderr
        exit 1
fi
if [ x$UCHIME_REFUDB == x ] ; then
        echo Must set \$UCHIME_REFUDB >> /dev/stderr
        exit 1
fi
if [ x$MostWanted == x ] ; then
        echo Must set \$MostWanted >> /dev/stderr
        exit 1
fi
# Sort by decreasing size
$u -sortbysize $1 -output bysize.fa
#cluster at 97% identity
$u -cluster_smallmem bysize.fa -id 0.97 -sizein -sizeout -centroids 97.fa
# Chimera filter with UCHIME de novo
$u -uchime_denovo 97.fa -chimeras ch.fa -nonchimeras nonch.fa -uchimeout denovo.uchime
# Chimera filter with UCHIME ref
$u -uchime_ref nonch.fa -db $UCHIME_REFUDB -nonchimeras filtered.fa -strand plus -uchimeout ref.uchime
# find hits with MostWanted list
$u -usearch_global filtered.fa -db $MostWanted -id 0.97 -strand plus -fastapairs mostwanted.fastapairs
```

> Sequence of rrnA of 16S rRNA gene of isolate microfluidicus 1

GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGAGAATCTACTGAAGGAGGATTCGTCCAACGGAAGTAGAGGAAAGTGGCGGACGGGT
GAGTAACGCGTGAGGAACCTGCCTTGAAGAGGGGGACAACAGTTGGAAACGACTGCTAATACCGCATGACGCATAGGGGTCGCATGATTCTTATGCCA
AAGATTTATCGCTTCAAGATGGCCTCGCGTCTGATTAGCTAGTTGGCGGGGTAACGGCCCACCAAGGCGACGATCAGTAGCCGGACTGAGAGGTTGAA
CGGCCACATTGGGACTGAGATACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGGCAATGGGCGCAAGCCTGACCCAGCAACGCCGCG
TGAAGGAAGAAGGCTTTCGGGTTGTAAACTTCTTTTAAGAGGGAAGAGCAGAAGACGGTACCTCTAGAATAAGCCACGGCTAACTACGTGCCAGCAGC
CGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTACTGGGTGTAAAGGGCGTGCAGCCGGGTCTGCAAGTCAGATGTGAAATCCATGGGCTCAAC
CCATGAACTGCATTTGAAACTGTAGATCTTGAGTGTCGGAGGGGCAATCGGAATTCCTAGTGTAGCGGTGAAATGCGTAGATATTAGGAGGAACACCA
GTGGCGAAGGCGGATTGCTGGACGATAACTGACGGTGAGGCGCGAAAGTGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACACTGTAAACG
ATGAATACTAGGTGTGCGGGGACTGACCCCCTGCGTGCCGCAGTAAACACAATAAGTATTCCACCTGGGGAGTACGATCGCAAGGTTGAAACTCAAAG
GAATTGACGGGGGCCCGCACAAGCGGTGGATTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGGTTTGACATCCTGCTAACGAAGTAGA
GATACATTAGGTGCCCTTCGGGGAAAGCAGAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC
AACCCCTATTGTTAGTTGCTACGCAAGAGCACTCTAGCGAGACTGCCGTTGACAAAACGGAGGAAGGTGGGGACGACGTCAAATCATCATGCCCCTTA
TATCCTGGGCTACACACGTAATACAATGGCGGTCAACAGAGGGAAGCAAAGCCGCGAGGCAGAGCAAACCCCCAAAAGCCGTCCCAGTTCGGATTGT
AGGCTGCAACTCGCCTGCATGAAGTCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCA
CACCATGAGAGTCGGGAACACCCGAAGTCCGTAGCCTAACCTGAAAAGGAGGGCGCGGCCGAAGGTGGGTTCGATAATTGGGGTG

> Sequence of rrnB of 16S rRNA gene of isolate microfluidicus 1

GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGAGAATCTACTGAAAGAGTTTTCGGACAATGGAAGTAGAGGAAAGTGGCGGACGGGT
GAGTAACGCGTGAGGAACCTGCCTTGAAGAGGGGGACAACAGTTGGAAACGACTGCTAATACCGCATGACGCATAGAGGTCGCATGATCTTTATGCCA
AAGATTTATCGCTTCAAGATGGCCTCGCGTCTGATTAGCTAGTTGGCGGGGTAACGGCCCACCAAGGCGACGATCAGTAGCCGGACTGAGAGGTTGAA
CGGCCACATTGGGACTGAGATACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGGCAATGGGCGCAAGCCTGACCCAGCAACGCCGCG
TGAAGGAAGAAGGCTTTCGGGTTGTAAACTTCTTTTAAGAGGGAAGAGCAGAAGACGGTACCTCTAGAATAAGCCACGGCTAACTACGTGCCAGCAGC
CGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTACTGGGTGTAAAGGGCGTGCAGCCGGGTCTGCAAGTCAGATGTGAAATCCATGGGCTCAAC
CCATGAACTGCATTTGAAACTGTAGATCTTGAGTGTCGGAGGGGCAATCGGAATTCCTAGTGTAGCGGTGAAATGCGTAGATATTAGGAGGAACACCA
GTGGCGAAGGCGGATTGCTGGACGATAACTGACGGTGAGGCGCGAAAGTGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACACTGTAAACG
ATGAATACTAGGTGTGCGGGGACTGACCCCCTGCGTGCCGCAGTAAACACAATAAGTATTCCACCTGGGGAGTACGATCGCAAGGTTGAAACTCAAAG
GAATTGACGGGGGCCCGCACAAGCGGTGGATTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGGTTTGACATCCTGCTAACGAAGTAGA
GATACATTAGGTGCCCTTCGGGGAAAGCAGAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC
AACCCCTATTGTTAGTTGCTACGCAAGAGCACTCTAGCGAGACTGCCGTTGACAAAACGGAGGAAGGTGGGGACGACGTCAAATCATCATGCCCCTTA
TATCCTGGGCTACACACGTAATACAATGGCGGTCAACAGAGGGAAGCAAAGCCGCGAGGCAGAGCAAACCCCCAAAAGCCGTCCCAGTTCGGATTGT
AGGCTGCAACTCGCCTGCATGAAGTCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCA
CACCATGAGAGTCGGGAACACCCGAAGTCCGTAGCCTAACCTGAAAAGGAGGGCGCGGCCGAAGGTGGGTTCGATAATTGGGGTG

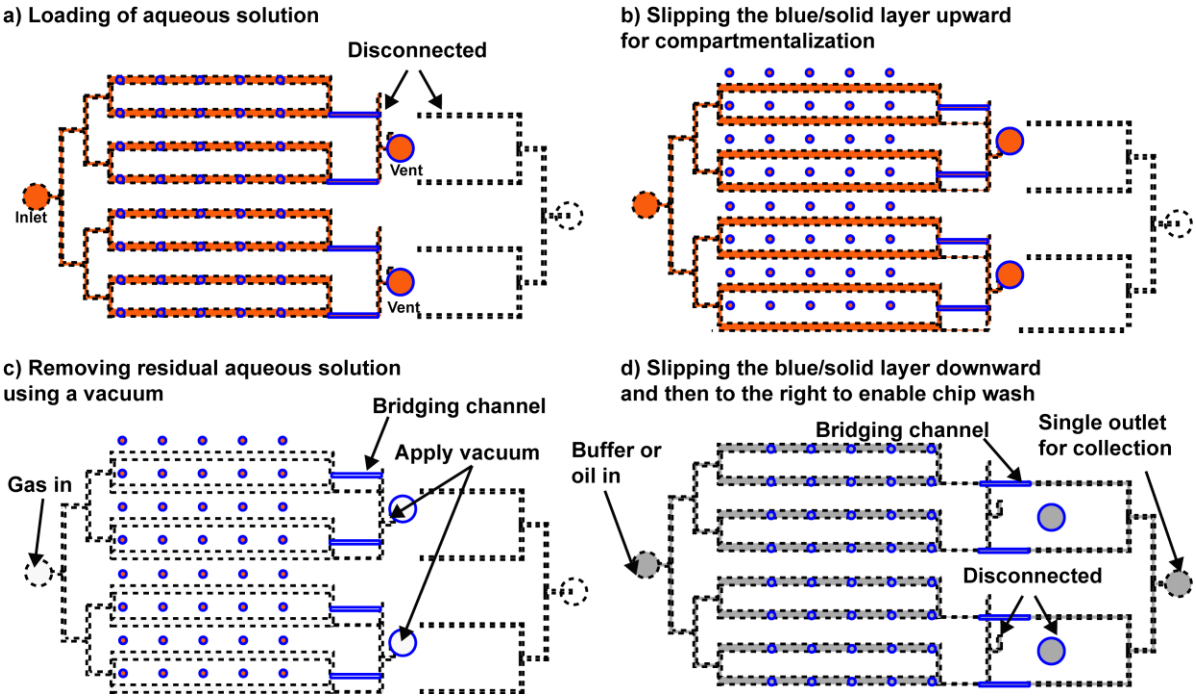

Fig. S1 Illustration of operation of the device showing how the bridging channels, vents and outlet for collection were used during chip wash. This schematic drawing uses a simplified design with fewer wells to illustrate the principle.

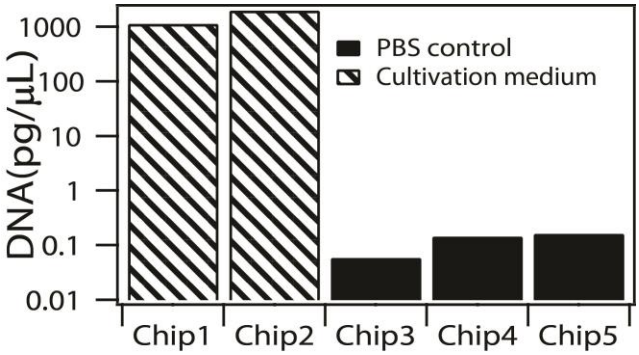

Fig. S2 Validation of the chip wash method with a model microorganism, *E. coli*. *E. coli* cells were loaded onto SlipChip with either medium for cultivation or a PBS buffer that does not support growth of cells. The cultivar was collected by chip wash method and DNA was extracted and quantified using qPCR. A 10,000-fold increase in the amount of DNA was observed between the condition that supports growth of *E. coli* vs. the non-growth control.

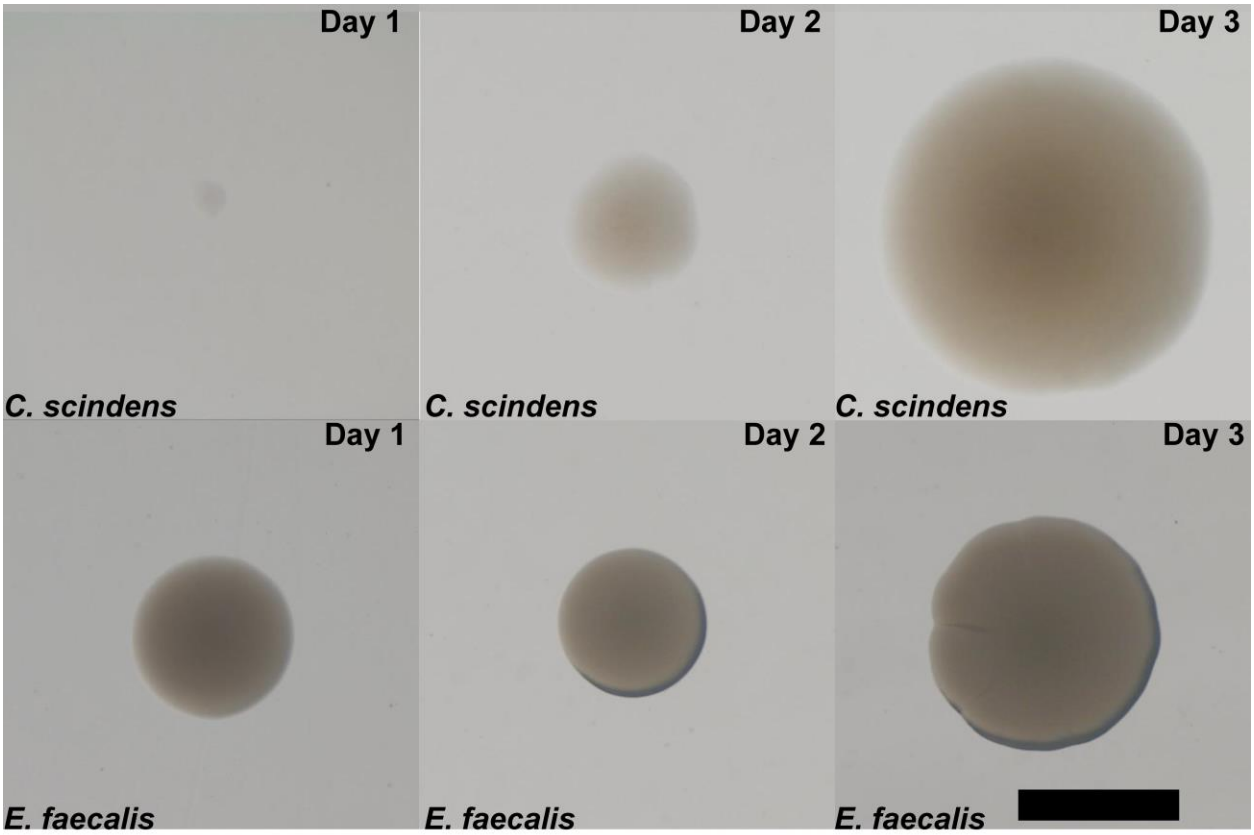

Fig. S3 Photograph showing time series of growth of *Clostridium scindens* and *Enterococcus faecalis* on agar plate. Scale bar is 1 mm.

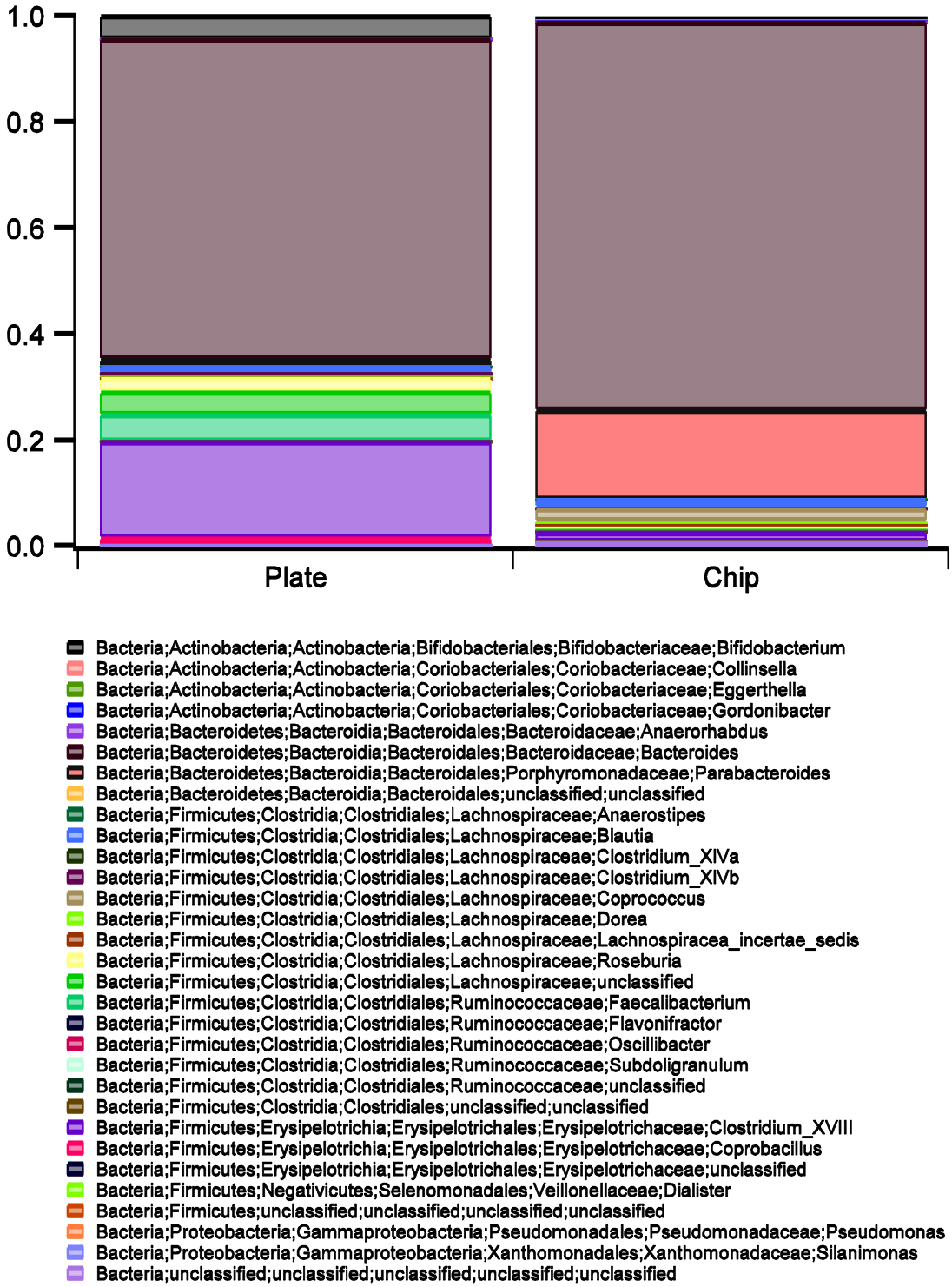

Fig. S4 Bar chart of relative abundance of OTUs grouped at genus level from 16S rDNA V4 high throughput sequencing of chip wash cultivar with M2LC and plate wash cultivar with M2GSC. We observed that more *Clostridium XIII* and *Bifidobacterium* can be observed from the plate wash solution, while some members such as *Gordonibacter*, *Anaerostipes*, *Oscillibacter* and *Silanimonas* can only be observed from chip wash method.

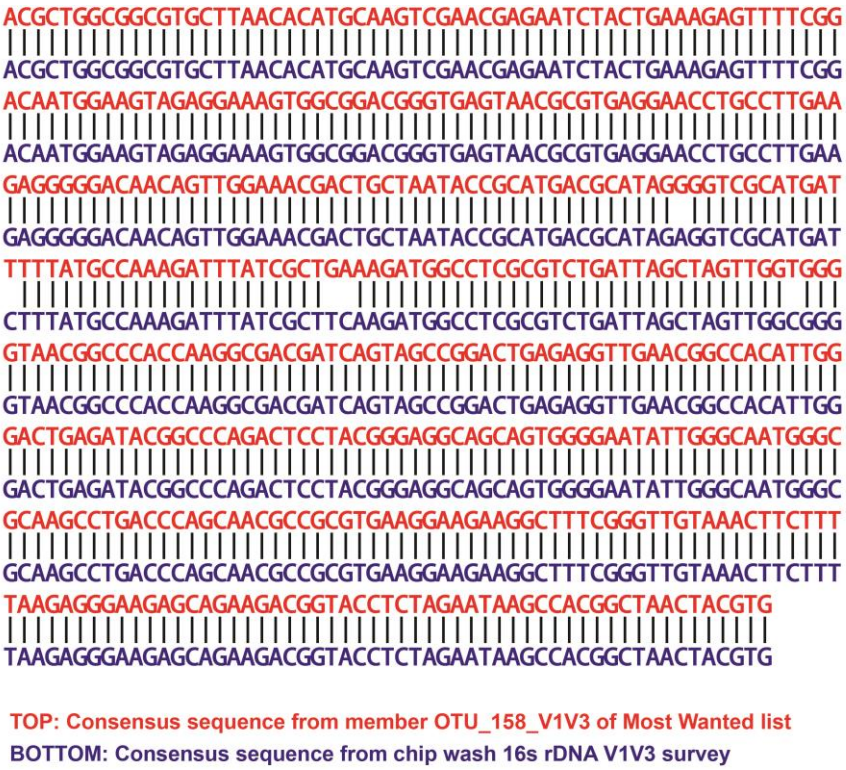

Fig. S5 Identifying OTU158 from high throughput sequencing of V1V3 region of 16S rRNA gene of the chip wash solution. The sequence retrieved from high throughput sequencing aligned well with the consensus sequence of OTU158 from HMP's "Most Wanted" list.

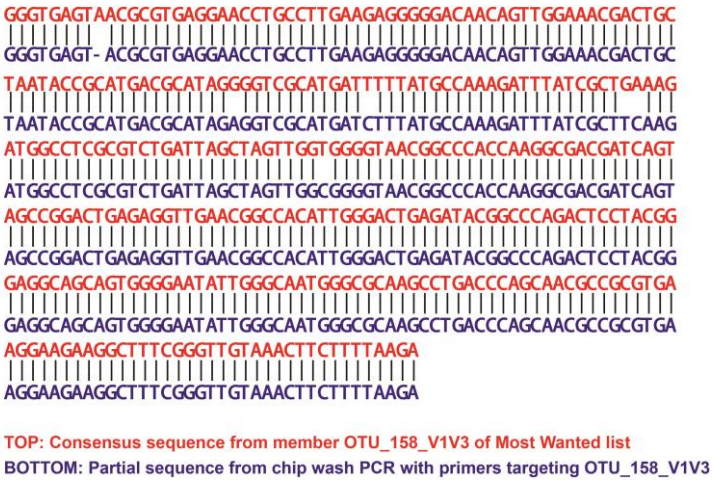

Fig. S6 Validating species specific primers for OTU158 by Sanger sequencing. The sequence retrieved from Sanger sequencing aligned well with the consensus sequence of OTU158 from HMP's "Most Wanted" list.

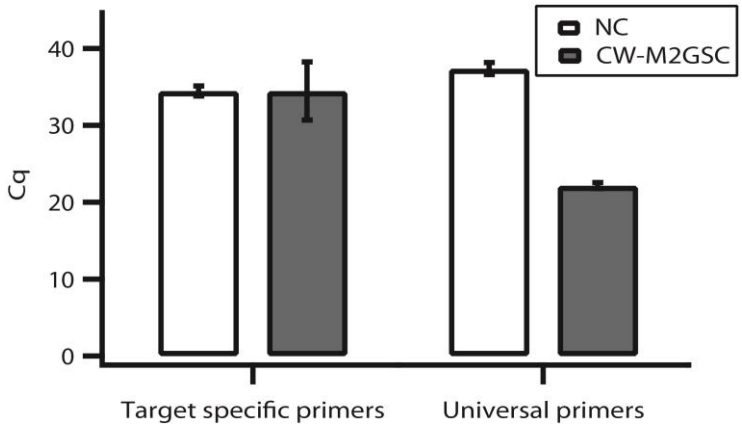

Fig. S7 qPCR with target specific primers (left) showing that the target was not present in chip wash solution of M2GSC nor in the blank negative control; Universal primers of 16s rDNA (right) showed that both chip wash contained bacterial genomic DNA and had a lower Cq value than the blank negative control. NC is negative control, and CW-M2GSC is chip wash with M2GSC medium.

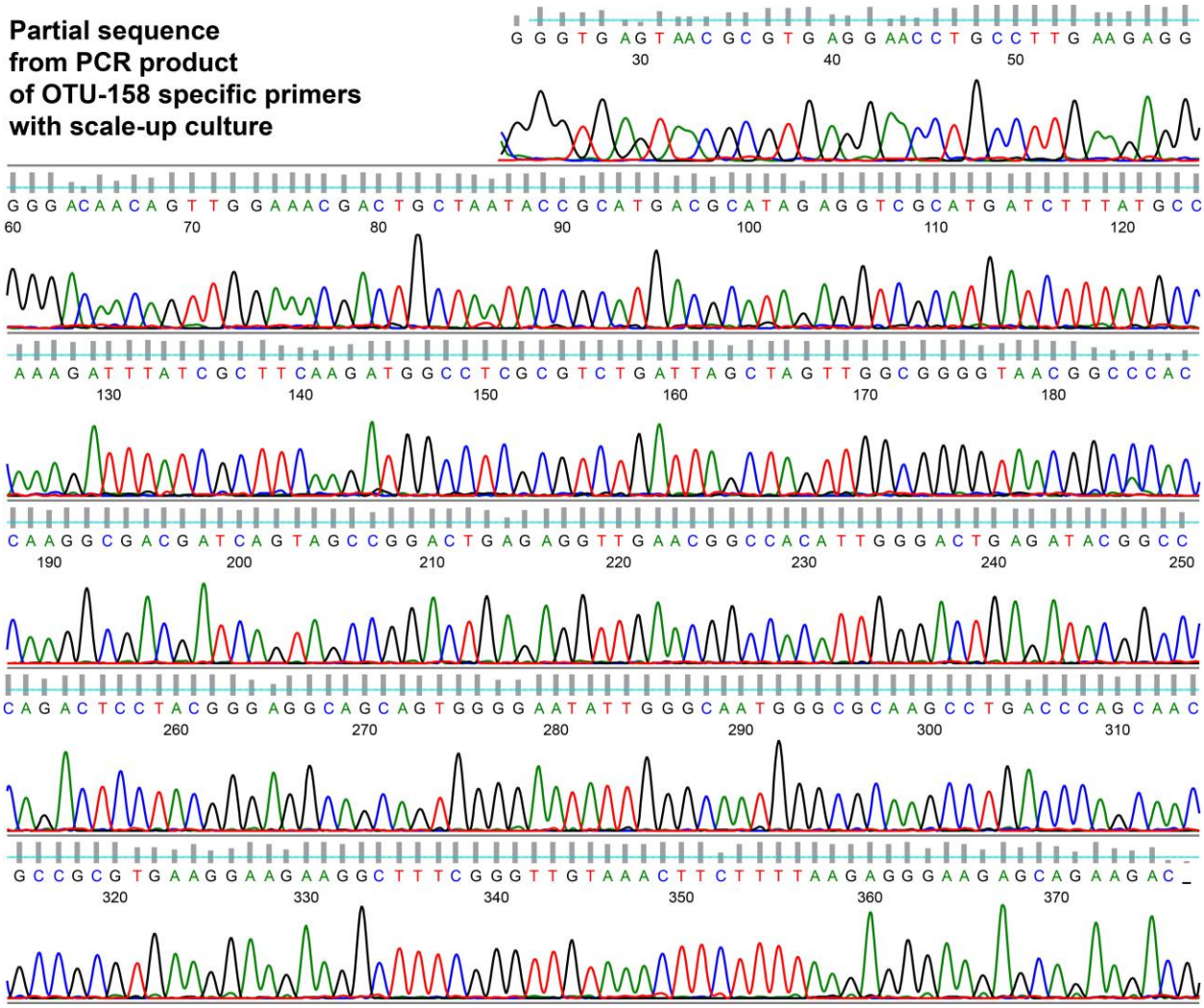

Fig. S8 Chromatogram from Sanger sequencing of positive PCR product of the first scale-up culture with target-specific primers.

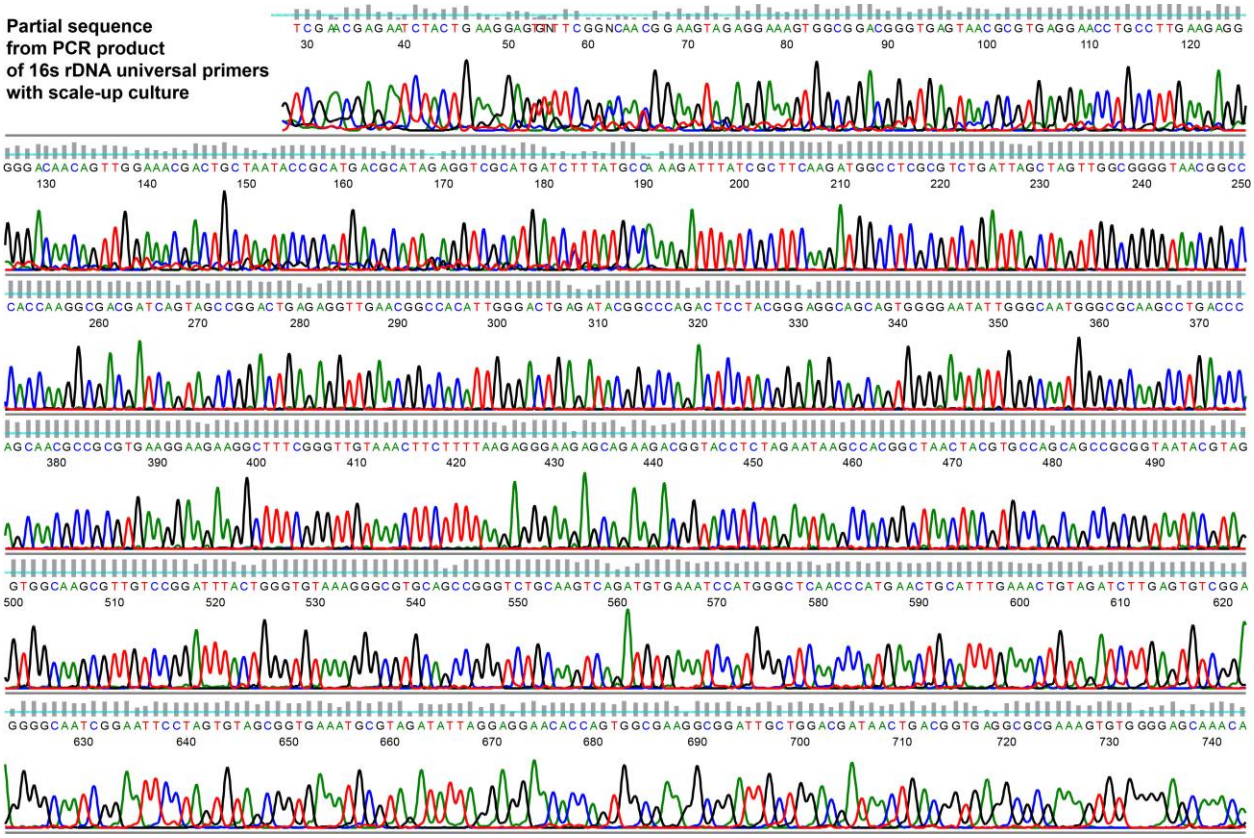

Fig. S9 Validating the scale-up colonies from the splitting SlipChip approach by Sanger sequencing of the positive PCR product of the first scale-up culture using universal primers. No contamination was observed, with minor heterogeneity at the beginning of the chromatogram.

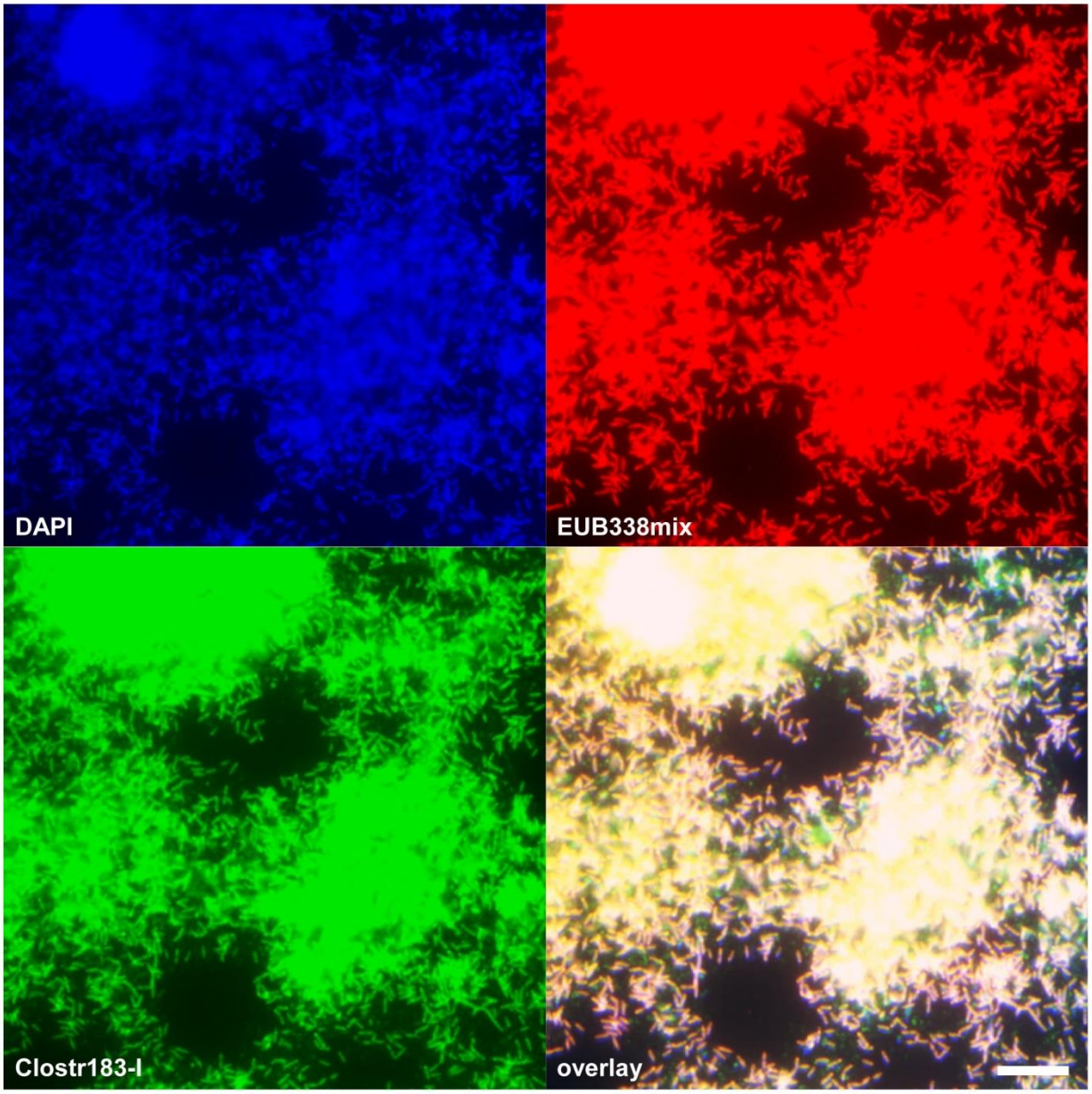

Fig. S10 FISH visualization of bacterial cells within pure culture of isolate microfluidicus 1. All cells detected by the general bacterial probe mix EUB338 I-III are also detected via the newly designed Clostr183-I probe, targeting isolate microfluidicus 1. While some cells (as identified by DAPI) did not bind any of these two probes, no archaea or eukaryotes were detected. Thus, FISH-negative cells are likely sporulating cells with substantially decreased ribosome content as compared to log-phase cells. Scale bar is 10 µm.

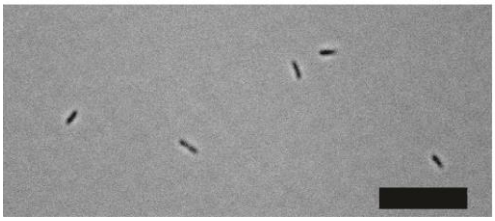

Fig. S11 Optical microscopy of isolate microfluidicus 1. Scale bar is 20 μm.

Detailed Contribution Description

LM and RFI designed the experiment as described in Figure 1. LM designed, performed experiments, and analyzed the data in Figures 2, 4, and 5. LM and JK designed, performed experiments, and analyzed the data in Figure 3. RH designed and performed FISH experiments and phylogenetic analyses for Figure 6, wrote the corresponding Results and Discussion and Supporting Information sections, and helped to edit the paper. NH was involved in clinical specimen acquisition and preparation in Figure 5*A*. IH performed all procedures for clinical specimen acquisition in Figure 5*A*. EC designed the study and clinical operations in Figure 5*A*. MK helped with device infrastructure and performed the experiment in SI Figure 11.

Table S1

#metadata of OTU_158_V1V3 and OTU_896_V1V3, retrieved from http://www.hmpdacc.org/most_wanted/ on Apr 27, 2012

otuID	otu_158_V1V3	otu_896_V1V3
priorityGroup	HIGH PRIORITY/MOST WANTED	HIGH PRIORITY/MOST WANTED
variableRegion	V1V3	V1V3
count454	14888	788
consensusLength	475	463
	ACGCTGGCGGCGTGCTTAACACATGCAAGTCGA	TGCTTAACACATGCAAGTCGAACGAGAATCTGCTG
	ACGAGAATCTACTGAAAGAGTTTTCGGACAATG	AAGGAGGATTCGTCCAACGGAAGTAGAGGAAAGT
	GAAGTAGAGGAAAGTGGCGGACGGGTGAGTA	GGCGGACGGGTGAGTAACGCGTGAGGAACCTGCC
	ACGCGTGAGGAACCTGCCTTGAAGAGGGGGAC	TTGAAGAGGGGGACAACAGTTGGAAACGACTGCT
	AACAGTTGGAAACGACTGCTAATACCGCATGAC	AATACCGCATGACGCATAGGGGTCGCATGATCTTT
	GCATAGGGGTCGCATGATTTTTATGCCAAAGAT	ATGCCAAAGATTTATCGCTTCAAGATGGCCTCGCG
	TTATCGCTGAAAGATGGCCTCGCGTCTGATTAG	TCTGATTAGCTGGTTGGCGGGGTAACGGCCCACCA
	CTAGTTGGTGGGGTAACGGCCCACCAAGGCGA	AGGCGACGATCAGTAGCCGGACTGAGAGGTTGAA
	CGATCAGTAGCCGGACTGAGAGGTTGAACGGC	CGGCCACATTGGGACTGAGATACGGCCCAGACTCC
	CACATTGGGACTGAGATACGGCCCAGACTCCTA	TACGGGAGGCAGCAGTGGGGAATATTGGGCAATG
	CGGGAGGCAGCAGTGGGGAATATTGGGCAATG	GGCGCAAGCCTGACCCAGCAACGCCGCGTGAAGG
	GGCGCAAGCCTGACCCAGCAACGCCGCGTGAA	AAGAAGGCTTTCGGGTTGTAAACTTCTTTTAAGAG
	GGAAGAAGGCTTTCGGGTTGTAAACTTCTTTTA	GGAAGAGCAGAAGACGGTACCTCTAGAATAAGCC
	AGAGGGAAGAGCAGAAGACGGTACCTCTAGAA	ACGGCTAACTACGTG
	TAAGCCACGGCTAACTACGTG	
consensusSequence		
rdpSummary	Root(100);Bacteria(100);Firmicutes(100);Clostridiales(100);Clostridiales(100);Ruminococcaceae(100);Oscillibacter(99)	Root(100);Bacteria(100);Firmicutes(100);Clostridia(100);Clostridiales(100);Ruminococcaceae(100);Oscillibacter(94)
UCHIMERefScore	0.038	0.0612
UCHIMEDenovoScore	0	0.1856
maxUCHIMEScore	0.038	0.1856
UCHIMEVerdict	neither	neither
toGoldGlobalPercentIdentity	90.59	88.44
toGoldGlobalBestHit	249174	32020
toGoldHumanGlobalPercentIdentity	87.29	88.44
toGoldHumanGlobalBestHit	804634	32020
toHMPStrainsGlobalPercentIdentity	87.29	88.44
toHmpStrainsGlobalBestHit	804634	32020
toSilvaGlobalPercentIdentity	98.95	99.78
toSilvaGlobalBestHit	Unl08zxz	Unl022fr
toGreengenesNamedGlobalPercentIdentity	92.45	91.61
toGreengenesNamedGlobalBestHit	607206	607206
toGreengenesUnnamedGlobalPercentIdentity	91.08	91.16
toGreengenesUnnamedGlobalBestHit	244027	812374
maxFraction	0.6979866	0.3489933
maxFractionBodyHabitat	Stool	Stool
454_seqCounts_Stool	13561	709
454_seqCounts_Buccal mucosa	8	3
454_seqCounts_Hard palate	37	1
454_seqCounts_Keratinized gingiva	3	0
454_seqCounts_Palatine Tonsils	6	0
454_seqCounts_Saliva	11	0
454_seqCounts_Subgingival plaque	0	0
454_seqCounts_Supragingival plaque	0	0
454_seqCounts_Throat	19	1
454_seqCounts_Tongue dorsum	0	2
454_seqCounts_Anterior nares	161	6
454_seqCounts_L_Antecubital fossa	364	15
454_seqCounts_L_Retroauricular crease	112	8
454_seqCounts_R_Antecubital fossa	341	37
454_seqCounts_R_Retroauricular crease	204	6
454_seqCounts_Mid vagina	7	0
454_seqCounts_Posterior fornix	10	0
454_seqCounts_Vaginal introitus	44	0
454_seqCounts_positive	0	0
454_seqCounts_water	0	0
454_subjectCounts_Stool	104	52
454_subjectCounts_Buccal mucosa	5	2
454_subjectCounts_Hard palate	9	1

454_subjectCounts_Keratinized gingiva	3	0
454_subjectCounts_Palatine Tonsils	4	0
454_subjectCounts_Saliva	6	0
454_subjectCounts_Subgingival plaque	0	0
454_subjectCounts_Supragingival plaque	0	0
454_subjectCounts_Throat	3	1
454_subjectCounts_Tongue dorsum	0	1
454_subjectCounts_Anterior nares	18	3
454_subjectCounts_L_Antecubital fossa	22	6
454_subjectCounts_L_Retroauricular crease	13	3
454_subjectCounts_R_Antecubital fossa	21	5
454_subjectCounts_R_Retroauricular crease	11	1
454_subjectCounts_Mid vagina	2	0
454_subjectCounts_Posterior fornix	2	0
454_subjectCounts_Vaginal introitus	9	0
454_subjectCounts_positive	0	0
454_subjectCounts_water	0	0
454_subjectfractions_Stool	0.6979866	0.3489933
454_subjectfractions_Buccal mucosa	0.035714287	0.014285714
454_subjectfractions_Hard palate	0.06666667	0.007407407
454_subjectfractions_Keratinized gingiva	0.021276595	0
454_subjectfractions_Palatine Tonsils	0.028368793	0
454_subjectfractions_Saliva	0.045801528	0
454_subjectfractions_Subgingival plaque	0	0
454_subjectfractions_Supragingival plaque	0	0
454_subjectfractions_Throat	0.022222223	0.007407407
454_subjectfractions_Tongue dorsum	0	0.006896552
454_subjectfractions_Anterior nares	0.1294964	0.021582734
454_subjectfractions_L_Antecubital fossa	0.16058394	0.04379562
454_subjectfractions_L_Retroauricular crease	0.084415585	0.019480519
454_subjectfractions_R_Antecubital fossa	0.15	0.035714287
454_subjectfractions_R_Retroauricular crease	0.07096774	0.006451613
454_subjectfractions_Mid vagina	0.02739726	0
454_subjectfractions_Posterior fornix	0.028169014	0
454_subjectfractions_Vaginal introitus	0.13235295	0
454_subjectfractions_positive	0	0
454_subjectfractions_water	0	0
454_RelativeAbundanceStool	0.007333881	3.07E-04
454_RelativeAbundanceBuccal mucosa	0.00000398	9.16E-07
454_RelativeAbundanceHard palate	0.0000995	9.62E-07
454_RelativeAbundanceKeratinized gingiva	0.00000379	0
454_RelativeAbundancePalatine Tonsils	0.00000542	0
454_RelativeAbundanceSaliva	0.0000109	0
454_RelativeAbundanceSubgingival plaque	0	0
454_RelativeAbundanceSupragingival plaque	0	0
454_RelativeAbundanceThroat	0.0000492	7.04E-07
454_RelativeAbundanceTongue dorsum	0	8.48E-07
454_RelativeAbundanceAnterior nares	0.00013	5.07E-06
454_RelativeAbundanceL_Antecubital fossa	0.000338	8.00E-06
454_RelativeAbundanceL_Retroauricular crease	0.000153	4.02E-06
454_RelativeAbundanceR_Antecubital fossa	0.000878	3.11E-05
454_RelativeAbundanceR_Retroauricular crease	0.0001	9.22E-07
454_RelativeAbundanceMid vagina	0.00000316	0
454_RelativeAbundancePosterior fornix	0.00000454	0
454_RelativeAbundanceVaginal introitus	0.0000574	0
454_RelativeAbundancepositive	0	0
454_RelativeAbundancewater	0	0

Table S2

searching for 16S rRNA gene of Caecococcus microfluidicus isolated in this paper in nr database by BLAST
Database: nr
Fields:subject ids, % identity, alignment length, mismatches, gap opens, q. start, q. end, s. start, s. end, evalue, bit score
#apply filter: percent identity greater or equal to 97%, and alignment length greater or equal to 250

gi|319500066|gb|HQ792927.1|	99.86	1453	2	0	1	1453	1	1453	0	2673
gi|261261403|gb|GQ897250.1|	99.72	1453	4	0	1	1453	19	1471	0	2662
gi|261261244|gb|GQ897091.1|	99.72	1453	4	0	1	1453	19	1471	0	2662
gi|126112143|gb|EF401832.1|	99.66	1453	5	0	1	1453	21	1473	0	2656
gi|126111973|gb|EF401662.1|	99.66	1453	5	0	1	1453	21	1473	0	2656
gi|126111971|gb|EF401660.1|	99.66	1453	5	0	1	1453	21	1473	0	2656
gi|126111482|gb|EF401171.1|	99.66	1453	5	0	1	1453	21	1473	0	2656
gi|126111445|gb|EF401134.1|	99.66	1453	5	0	1	1453	21	1473	0	2656
gi|126111306|gb|EF400995.1|	99.66	1453	5	0	1	1453	21	1473	0	2656
gi|126111276|gb|EF400965.1|	99.66	1453	5	0	1	1453	21	1473	0	2656
gi|126111154|gb|EF400843.1|	99.66	1453	5	0	1	1453	21	1473	0	2656
gi|261261148|gb|GQ896995.1|	99.59	1453	6	0	1	1453	19	1471	0	2651
gi|126112165|gb|EF401854.1|	99.59	1453	6	0	1	1453	21	1473	0	2651
gi|126111704|gb|EF401393.1|	99.59	1453	6	0	1	1453	21	1473	0	2651
gi|126111580|gb|EF401269.1|	99.59	1453	6	0	1	1453	21	1473	0	2651
gi|126111559|gb|EF401248.1|	99.59	1453	6	0	1	1453	21	1473	0	2651
gi|126111536|gb|EF401225.1|	99.59	1453	6	0	1	1453	21	1473	0	2651
gi|126111075|gb|EF400764.1|	99.59	1453	6	0	1	1453	21	1473	0	2651
gi|319493440|gb|HQ786301.1|	99.52	1453	7	0	1	1453	1	1453	0	2645
gi|261261697|gb|GQ897544.1|	99.52	1453	7	0	1	1453	19	1471	0	2645
gi|261261684|gb|GQ897531.1|	99.52	1453	7	0	1	1453	19	1471	0	2645
gi|261261500|gb|GQ897347.1|	99.52	1453	7	0	1	1453	19	1471	0	2645
gi|261261113|gb|GQ896960.1|	99.52	1453	7	0	1	1453	19	1471	0	2645
gi|126115125|gb|EF404805.1|	99.52	1453	7	0	1	1453	22	1474	0	2645
gi|126111760|gb|EF401449.1|	99.52	1453	7	0	1	1453	21	1473	0	2645
gi|126111328|gb|EF401017.1|	99.52	1453	7	0	1	1453	21	1473	0	2645
gi|126111235|gb|EF400924.1|	99.52	1453	7	0	1	1453	21	1473	0	2645
gi|126111138|gb|EF400827.1|	99.52	1453	7	0	1	1453	21	1473	0	2645
gi|319495953|gb|HQ788814.1|	99.93	1434	1	0	20	1453	1	1434	0	2643
gi|126111646|gb|EF401335.1|	99.52	1453	6	1	1	1453	21	1472	0	2643
gi|126111403|gb|EF401092.1|	99.52	1453	6	1	1	1453	21	1472	0	2643
gi|126111287|gb|EF400976.1|	99.52	1453	6	1	1	1453	21	1472	0	2643
gi|261262538|gb|GQ898389.1|	99.45	1453	8	0	1	1453	20	1472	0	2639
gi|261262369|gb|GQ898220.1|	99.45	1453	8	0	1	1453	20	1472	0	2639
gi|126115648|gb|EF405325.1|	99.45	1453	8	0	1	1453	22	1474	0	2639
gi|126115366|gb|EF405043.1|	99.45	1453	8	0	1	1453	22	1474	0	2639
gi|126115362|gb|EF405039.1|	99.45	1453	8	0	1	1453	22	1474	0	2639
gi|126111838|gb|EF401527.1|	99.45	1453	8	0	1	1453	21	1473	0	2639
gi|6456061|gb|AF132255.1|	99.52	1452	3	4	4	1453	24	1473	0	2639
gi|319482775|gb|HQ775636.1|	99.52	1449	7	0	5	1453	1	1449	0	2638
gi|319499423|gb|HQ792284.1|	99.45	1450	8	0	2	1451	1	1450	0	2634
gi|319488021|gb|HQ780882.1|	99.65	1441	5	0	4	1444	3	1443	0	2634
gi|261262337|gb|GQ898188.1|	99.38	1453	9	0	1	1453	20	1472	0	2634
gi|261261861|gb|GQ897712.1|	99.38	1453	9	0	1	1453	19	1471	0	2634
gi|126115512|gb|EF405189.1|	99.38	1453	9	0	1	1453	22	1474	0	2634
gi|126115207|gb|EF404884.1|	99.38	1453	9	0	1	1453	22	1474	0	2634
gi|126112029|gb|EF401718.1|	99.38	1453	9	0	1	1453	21	1473	0	2634
gi|126111899|gb|EF401588.1|	99.38	1453	9	0	1	1453	21	1473	0	2634
gi|126111893|gb|EF401582.1|	99.38	1453	9	0	1	1453	21	1473	0	2634
gi|126111713|gb|EF401402.1|	99.38	1453	9	0	1	1453	21	1473	0	2634
gi|126111061|gb|EF400750.1|	99.38	1453	9	0	1	1453	21	1473	0	2634
gi|319487719|gb|HQ780580.1|	99.38	1450	9	0	4	1453	1	1450	0	2628
gi|261262554|gb|GQ898405.1|	99.31	1453	10	0	1	1453	20	1472	0	2628
gi|126112132|gb|EF401821.1|	99.31	1453	10	0	1	1453	21	1473	0	2628
gi|126112111|gb|EF401800.1|	99.31	1453	10	0	1	1453	21	1473	0	2628
gi|126112073|gb|EF401762.1|	99.31	1453	10	0	1	1453	21	1473	0	2628
gi|126112033|gb|EF401722.1|	99.31	1453	10	0	1	1453	21	1473	0	2628

gi|126112002|gb|EF401691.1|	99.31	1453	10	0	1	1453	21	1473	0	2628
gi|126111979|gb|EF401668.1|	99.31	1453	10	0	1	1453	21	1473	0	2628
gi|126111925|gb|EF401614.1|	99.31	1453	10	0	1	1453	21	1473	0	2628
gi|126111813|gb|EF401502.1|	99.31	1453	10	0	1	1453	21	1473	0	2628
gi|126111660|gb|EF401349.1|	99.31	1453	10	0	1	1453	21	1473	0	2628
gi|126111649|gb|EF401338.1|	99.31	1453	10	0	1	1453	21	1473	0	2628
gi|126111596|gb|EF401285.1|	99.31	1453	10	0	1	1453	21	1473	0	2628
gi|126111542|gb|EF401231.1|	99.31	1453	10	0	1	1453	21	1473	0	2628
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gi|126111465|gb|EF401154.1|	99.31	1453	10	0	1	1453	21	1473	0	2628
gi|126111399|gb|EF401088.1|	99.31	1453	10	0	1	1453	21	1473	0	2628
gi|126111378|gb|EF401067.1|	99.31	1453	10	0	1	1453	21	1473	0	2628
gi|126111376|gb|EF401065.1|	99.31	1453	10	0	1	1453	21	1473	0	2628
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gi|126111353|gb|EF401042.1|	99.31	1453	10	0	1	1453	21	1473	0	2628
gi|126111323|gb|EF401012.1|	99.31	1453	10	0	1	1453	21	1473	0	2628
gi|126111201|gb|EF400890.1|	99.31	1453	10	0	1	1453	21	1473	0	2628
gi|126111135|gb|EF400824.1|	99.31	1453	10	0	1	1453	21	1473	0	2628
gi|126111085|gb|EF400774.1|	99.31	1453	10	0	1	1453	21	1473	0	2628
gi|319490773|gb|HQ783634.1|	99.31	1450	10	0	4	1453	1	1450	0	2623
gi|261261792|gb|GQ897639.1|	99.24	1453	11	0	1	1453	19	1471	0	2623
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gi|126114933|gb|EF404613.1|	99.24	1453	11	0	1	1453	22	1474	0	2623
gi|126112161|gb|EF401850.1|	99.24	1453	11	0	1	1453	21	1473	0	2623
gi|126112160|gb|EF401849.1|	99.24	1453	11	0	1	1453	21	1473	0	2623
gi|126112061|gb|EF401750.1|	99.24	1453	11	0	1	1453	21	1473	0	2623
gi|126111968|gb|EF401657.1|	99.24	1453	11	0	1	1453	21	1473	0	2623
gi|126111930|gb|EF401619.1|	99.24	1453	11	0	1	1453	21	1473	0	2623
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gi|126111879|gb|EF401568.1|	99.24	1453	11	0	1	1453	21	1473	0	2623
gi|126111753|gb|EF401442.1|	99.24	1453	11	0	1	1453	21	1473	0	2623
gi|126111737|gb|EF401426.1|	99.24	1453	11	0	1	1453	21	1473	0	2623
gi|126111613|gb|EF401302.1|	99.24	1453	11	0	1	1453	21	1473	0	2623
gi|126111405|gb|EF401094.1|	99.24	1453	11	0	1	1453	21	1473	0	2623

gi|126111362|gb|EF401051.1|	99.24	1453	11	0	1	1453	21	1473	0	2623
gi|319488801|gb|HQ781662.1|	99.24	1452	10	1	2	1452	1	1452	0	2619
gi|319499925|gb|HQ792786.1|	99.44	1441	8	0	13	1453	1	1441	0	2617
gi|319492434|gb|HQ785295.1|	99.17	1454	11	1	1	1453	2	1455	0	2617
gi|319488912|gb|HQ781773.1|	99.24	1450	11	0	4	1453	1	1450	0	2617
gi|261262501|gb|GQ898352.1|	99.17	1451	12	0	3	1453	20	1470	0	2617
gi|261260989|gb|GQ896836.1|	99.17	1453	12	0	1	1453	19	1471	0	2617
gi|126111846|gb|EF401535.1|	99.17	1453	12	0	1	1453	21	1473	0	2617
gi|126111498|gb|EF401187.1|	99.17	1453	12	0	1	1453	21	1473	0	2617
gi|126111494|gb|EF401183.1|	99.17	1453	12	0	1	1453	21	1473	0	2617
gi|126111063|gb|EF400752.1|	99.17	1453	12	0	1	1453	21	1473	0	2617
gi|126115692|gb|EF405369.1|	99.17	1453	11	1	1	1453	22	1473	0	2615
gi|319484767|gb|HQ777628.1|	99.38	1442	9	0	12	1453	1	1442	0	2614
gi|319494580|gb|HQ787441.1|	99.17	1450	12	0	4	1453	1	1450	0	2612
gi|261261902|gb|GQ897753.1|	99.11	1453	13	0	1	1453	19	1471	0	2612
gi|261261832|gb|GQ897683.1|	99.11	1453	13	0	1	1453	18	1470	0	2612
gi|126111936|gb|EF401625.1|	99.11	1453	13	0	1	1453	21	1473	0	2612
gi|126111726|gb|EF401415.1|	99.11	1453	13	0	1	1453	21	1473	0	2612
gi|126111600|gb|EF401289.1|	99.11	1453	13	0	1	1453	21	1473	0	2612
gi|126111231|gb|EF400920.1|	99.11	1453	13	0	1	1453	21	1473	0	2612
gi|18644507|gb|AF371798.1|	99.58	1431	4	2	1	1430	21	1450	0	2612
gi|319494186|gb|HQ787047.1|	99.17	1448	12	0	4	1451	1	1448	0	2608
gi|319493693|gb|HQ786554.1|	99.1	1451	13	0	3	1453	1	1451	0	2608
gi|240005432|gb|GQ158590.1|	99.17	1448	12	0	6	1453	1	1448	0	2608
gi|319499385|gb|HQ792246.1|	99.37	1439	8	1	9	1446	1	1439	0	2606
gi|319494484|gb|HQ787345.1|	99.24	1444	11	0	1	1444	5	1448	0	2606
gi|319499757|gb|HQ792618.1|	99.24	1443	11	0	5	1447	1	1443	0	2604
gi|319466460|gb|HQ759321.1|	99.24	1440	11	0	14	1453	1	1440	0	2601
gi|319500228|gb|HQ793089.1|	99.17	1444	12	0	7	1450	1	1444	0	2601
gi|319500039|gb|HQ792900.1|	99.37	1435	9	0	1	1435	3	1437	0	2601
gi|319494363|gb|HQ787224.1|	99.03	1450	14	0	4	1453	1	1450	0	2601
gi|126115689|gb|EF405366.1|	98.97	1453	15	0	1	1453	22	1474	0	2601
gi|126115469|gb|EF405146.1|	98.97	1453	15	0	1	1453	22	1474	0	2601
gi|126115408|gb|EF405085.1|	98.97	1453	15	0	1	1453	22	1474	0	2601

gi|319517965|gb|HQ810826.1|	99.03	1449	14	0	3	1451	1	1449	0	2599
gi|319494413|gb|HQ787274.1|	99.17	1442	12	0	12	1453	1	1442	0	2597
gi|319489028|gb|HQ781889.1|	99.1	1444	13	0	10	1453	1	1444	0	2595
gi|240006081|gb|GQ159239.1|	98.9	1453	16	0	1	1453	1	1453	0	2595
gi|126115476|gb|EF405153.1|	98.9	1453	16	0	1	1453	22	1474	0	2595
gi|126115363|gb|EF405040.1|	98.9	1453	16	0	1	1453	22	1474	0	2595
gi|319495888|gb|HQ788749.1|	99.24	1438	10	1	17	1453	1	1438	0	2593
gi|319494567|gb|HQ787428.1|	99.1	1443	13	0	11	1453	1	1443	0	2593
gi|319494832|gb|HQ787693.1|	99.17	1439	12	0	1	1439	8	1446	0	2591
gi|319466750|gb|HQ759611.1|	99.37	1430	9	0	19	1448	1	1430	0	2591
gi|319487696|gb|HQ780557.1|	99.03	1444	14	0	1	1444	7	1450	0	2590
gi|240006082|gb|GQ159240.1|	98.83	1453	17	0	1	1453	1	1453	0	2590
gi|240006079|gb|GQ159237.1|	98.83	1454	15	2	1	1453	1	1453	0	2590
gi|319493543|gb|HQ786404.1|	99.1	1440	13	0	14	1453	1	1440	0	2588
gi|319488000|gb|HQ780861.1|	99.03	1443	14	0	1	1443	10	1452	0	2588
gi|240006083|gb|GQ159241.1|	98.83	1453	16	1	1	1453	1	1452	0	2588
gi|170522519|gb|EU531967.1|	99.17	1438	10	2	1	1438	1	1436	0	2588
gi|319496000|gb|HQ788861.1|	99.16	1436	12	0	18	1453	1	1436	0	2586
gi|319494044|gb|HQ786905.1|	99.03	1441	14	0	1	1441	4	1444	0	2584
gi|319487727|gb|HQ780588.1|	98.89	1447	16	0	2	1448	1	1447	0	2584
gi|319485110|gb|HQ777971.1|	98.96	1444	15	0	10	1453	1	1444	0	2584
gi|319449343|gb|HQ742204.1|	99.1	1438	13	0	1	1438	1	1438	0	2584
gi|324959751|gb|HQ716104.1|	98.76	1453	18	0	1	1453	19	1471	0	2584
gi|240006080|gb|GQ159238.1|	98.76	1454	15	3	1	1453	1	1452	0	2582
gi|319517807|gb|HQ810668.1|	98.96	1437	15	0	17	1453	1	1437	0	2571
gi|319494034|gb|HQ786895.1|	98.96	1436	15	0	18	1453	1	1436	0	2569
gi|319499806|gb|HQ792667.1|	98.75	1443	18	0	11	1453	1	1443	0	2566
gi|319495841|gb|HQ788702.1|	98.88	1434	16	0	18	1451	1	1434	0	2560
gi|319466752|gb|HQ759613.1|	98.54	1441	20	1	4	1443	1	1441	0	2543
gi|319466850|gb|HQ759711.1|	98.33	1440	24	0	12	1451	1	1440	0	2527
gi|319487726|gb|HQ780587.1|	98.33	1436	22	2	1	1436	3	1436	0	2518
gi|319466599|gb|HQ759460.1|	98.52	1423	19	2	12	1434	2	1422	0	2510
gi|319500123|gb|HQ792984.1|	97.99	1445	28	1	1	1445	6	1449	0	2507
gi|110446538|gb|DQ806753.1|	99.56	1368	6	0	1	1368	32	1399	0	2494

gi|219533792|gb|FJ510597.1|	99.63	1364	5	0	2	1365	13	1376	0	2492
gi|319499960|gb|HQ792821.1|	97.65	1448	34	0	4	1451	1	1448	0	2486
gi|110446177|gb|DQ806392.1|	99.42	1370	6	2	1	1368	33	1402	0	2484
gi|388933029|gb|JQ188146.1|	99.85	1350	2	0	1	1350	1	1350	0	2483
gi|388933200|gb|JQ188317.1|	99.85	1350	2	0	1	1350	1	1350	0	2483
gi|388932914|gb|JQ188031.1|	99.85	1350	2	0	1	1350	1	1350	0	2483
gi|110441168|gb|DQ800768.1|	99.42	1368	8	0	1	1368	33	1400	0	2483
gi|319466572|gb|HQ759433.1|	98.71	1397	18	0	5	1401	1	1397	0	2481
gi|219534086|gb|FJ510891.1|	99.49	1363	7	0	1	1363	10	1372	0	2479
gi|388928035|gb|JQ183152.1|	99.78	1350	3	0	1	1350	1	1350	0	2477
gi|388935405|gb|JQ190522.1|	99.78	1350	3	0	1	1350	1	1350	0	2477
gi|388933350|gb|JQ188467.1|	99.78	1350	3	0	1	1350	1	1350	0	2477
gi|388933228|gb|JQ188345.1|	99.78	1350	3	0	1	1350	1	1350	0	2477
gi|110449771|gb|DQ809986.1|	99.34	1368	9	0	1	1368	32	1399	0	2477
gi|110449667|gb|DQ809882.1|	99.34	1368	9	0	1	1368	33	1400	0	2477
gi|110448825|gb|DQ809040.1|	99.34	1368	9	0	1	1368	21	1388	0	2477
gi|110436401|gb|DQ796001.1|	99.34	1368	9	0	1	1368	21	1388	0	2477
gi|388933272|gb|JQ188389.1|	99.7	1352	2	2	1	1350	1	1352	0	2473
gi|219531115|gb|FJ507920.1|	99.63	1354	5	0	12	1365	1	1354	0	2473
gi|388931523|gb|JQ186640.1|	99.7	1350	4	0	1	1350	1	1350	0	2471
gi|388935595|gb|JQ190712.1|	99.7	1350	4	0	1	1350	1	1350	0	2471
gi|388935367|gb|JQ190484.1|	99.7	1350	4	0	1	1350	1	1350	0	2471
gi|388935044|gb|JQ190161.1|	99.7	1350	4	0	1	1350	1	1350	0	2471
gi|219536114|gb|FJ512919.1|	99.34	1365	9	0	1	1365	11	1375	0	2471
gi|219531116|gb|FJ507921.1|	99.34	1365	9	0	1	1365	11	1375	0	2471
gi|192988166|gb|EU778163.1|	99.27	1368	10	0	1	1368	33	1400	0	2471
gi|192981066|gb|EU775089.1|	99.27	1368	10	0	1	1368	33	1400	0	2471
gi|192976430|gb|EU772453.1|	99.27	1368	10	0	1	1368	33	1400	0	2471
gi|169278176|gb|EU462701.1|	99.27	1368	10	0	1	1368	21	1388	0	2471
gi|169278092|gb|EU462617.1|	99.27	1368	10	0	1	1368	21	1388	0	2471
gi|110441305|gb|DQ800905.1|	99.27	1368	10	0	1	1368	33	1400	0	2471
gi|110436243|gb|DQ795843.1|	99.27	1368	10	0	1	1368	33	1400	0	2471
gi|388933965|gb|JQ189082.1|	99.7	1350	3	1	1	1350	1	1349	0	2470
gi|219531114|gb|FJ507919.1|	99.34	1365	8	1	1	1365	11	1374	0	2470

gi|388928800|gb|JQ183917.1|	99.63	1350	5	0	1	1350	1	1350	0	2466
gi|388935165|gb|JQ190282.1|	99.63	1350	5	0	1	1350	1	1350	0	2466
gi|388934187|gb|JQ189304.1|	99.63	1350	5	0	1	1350	1	1350	0	2466
gi|219536113|gb|FJ512918.1|	99.27	1365	10	0	1	1365	11	1375	0	2466
gi|192981058|gb|EU775081.1|	99.2	1368	11	0	1	1368	33	1400	0	2466
gi|110446130|gb|DQ806345.1|	99.2	1368	11	0	1	1368	4	1371	0	2466
gi|62764679|gb|AY985189.1|	99.63	1350	5	0	1	1350	1	1350	0	2466
gi|219533341|gb|FJ510146.1|	99.27	1364	10	0	1	1364	11	1374	0	2464
gi|110449111|gb|DQ809326.1|	99.2	1368	10	1	1	1368	33	1399	0	2464
gi|388931569|gb|JQ186686.1|	99.56	1352	4	2	1	1350	1	1352	0	2462
gi|388931221|gb|JQ186338.1|	99.56	1350	6	0	1	1350	1	1350	0	2460
gi|388930259|gb|JQ185376.1|	99.56	1350	6	0	1	1350	1	1350	0	2460
gi|388928405|gb|JQ183522.1|	99.56	1350	6	0	1	1350	1	1350	0	2460
gi|388934145|gb|JQ189262.1|	99.56	1350	6	0	1	1350	1	1350	0	2460
gi|388933765|gb|JQ188882.1|	99.56	1350	6	0	1	1350	1	1350	0	2460
gi|219536112|gb|FJ512917.1|	99.19	1365	11	0	1	1365	11	1375	0	2460
gi|219536107|gb|FJ512912.1|	99.19	1365	11	0	1	1365	11	1375	0	2460
gi|219536108|gb|FJ512913.1|	99.19	1365	11	0	1	1365	11	1375	0	2460
gi|219533791|gb|FJ510596.1|	99.19	1365	11	0	1	1365	11	1375	0	2460
gi|192976454|gb|EU772477.1|	99.12	1368	12	0	1	1368	33	1400	0	2460
gi|169278049|gb|EU462574.1|	99.12	1368	12	0	1	1368	21	1388	0	2460
gi|110449321|gb|DQ809536.1|	99.12	1368	12	0	1	1368	33	1400	0	2460
gi|219533340|gb|FJ510145.1|	99.19	1364	11	0	1	1364	11	1374	0	2459
gi|214017356|gb|FJ362969.1|	99.41	1355	8	0	1	1355	1	1355	0	2459
gi|219533343|gb|FJ510148.1|	99.26	1360	10	0	5	1364	15	1374	0	2457
gi|169276654|gb|EU461179.1|	99.05	1370	11	2	1	1368	21	1390	0	2457
gi|388929811|gb|JQ184928.1|	99.48	1351	6	1	1	1350	1	1351	0	2455
gi|388928407|gb|JQ183524.1|	99.48	1350	7	0	1	1350	1	1350	0	2455
gi|388935013|gb|JQ190130.1|	99.48	1350	7	0	1	1350	1	1350	0	2455
gi|388935324|gb|JQ190441.1|	99.48	1350	7	0	1	1350	1	1350	0	2455
gi|388932549|gb|JQ187666.1|	99.48	1351	6	1	1	1350	1	1351	0	2455
gi|219536106|gb|FJ512911.1|	99.12	1365	12	0	1	1365	11	1375	0	2455
gi|192981007|gb|EU775030.1|	99.05	1368	13	0	1	1368	25	1392	0	2455
gi|110448398|gb|DQ808613.1|	99.05	1368	13	0	1	1368	21	1388	0	2455

gi|62760514|gb|AY981024.1|	99.48	1350	7	0	1	1350	1	1350	0	2455
gi|388935424|gb|JQ190541.1|	99.41	1354	4	4	1	1350	1	1354	0	2453
gi|219536110|gb|FJ512915.1|	99.12	1364	12	0	2	1365	13	1376	0	2453
gi|219535544|gb|FJ512349.1|	99.12	1364	12	0	2	1365	11	1374	0	2453
gi|219533017|gb|FJ509822.1|	99.12	1364	12	0	1	1364	11	1374	0	2453
gi|219533015|gb|FJ509820.1|	99.19	1361	11	0	4	1364	14	1374	0	2453
gi|388930479|gb|JQ185596.1|	99.41	1350	8	0	1	1350	1	1350	0	2449
gi|388930251|gb|JQ185368.1|	99.41	1350	8	0	1	1350	1	1350	0	2449
gi|388928126|gb|JQ183243.1|	99.41	1350	8	0	1	1350	1	1350	0	2449
gi|388934631|gb|JQ189748.1|	99.41	1350	8	0	1	1350	1	1350	0	2449
gi|388934877|gb|JQ189994.1|	99.41	1350	8	0	1	1350	1	1350	0	2449
gi|388934922|gb|JQ190039.1|	99.41	1350	8	0	1	1350	1	1350	0	2449
gi|388934445|gb|JQ189562.1|	99.41	1350	8	0	1	1350	1	1350	0	2449
gi|388933151|gb|JQ188268.1|	99.41	1350	8	0	1	1350	1	1350	0	2449
gi|322160786|gb|JF175380.1|	99.41	1350	8	0	1	1350	1	1350	0	2449
gi|219535545|gb|FJ512350.1|	99.12	1363	11	1	3	1365	12	1373	0	2449
gi|219533789|gb|FJ510594.1|	99.05	1366	11	2	1	1365	11	1375	0	2449
gi|219533342|gb|FJ510147.1|	99.12	1362	12	0	3	1364	13	1374	0	2449
gi|214018748|gb|FJ364361.1|	99.26	1356	10	0	1	1356	21	1376	0	2449
gi|169291316|gb|EU475841.1|	98.98	1368	14	0	1	1368	21	1388	0	2449
gi|110436585|gb|DQ796185.1|	98.98	1368	14	0	1	1368	21	1388	0	2449
gi|62764715|gb|AY985225.1|	99.41	1350	8	0	1	1350	1	1350	0	2449
gi|62763312|gb|AY983822.1|	99.41	1350	8	0	1	1350	1	1350	0	2449
gi|388933707|gb|JQ188824.1|	99.41	1350	6	2	1	1348	1	1350	0	2447
gi|219533339|gb|FJ510144.1|	99.19	1358	11	0	7	1364	6	1363	0	2447
gi|219533338|gb|FJ510143.1|	99.05	1364	13	0	1	1364	11	1374	0	2447
gi|110436110|gb|DQ795710.1|	99.05	1364	13	0	1	1364	33	1396	0	2447
gi|388934590|gb|JQ189707.1|	99.33	1352	7	2	1	1350	1	1352	0	2446
gi|219531117|gb|FJ507922.1|	99.26	1354	10	0	12	1365	1	1354	0	2446
gi|388934888|gb|JQ190005.1|	99.26	1356	4	6	1	1350	1	1356	0	2444
gi|388934140|gb|JQ189257.1|	99.33	1351	8	1	1	1350	1	1351	0	2444
gi|388933499|gb|JQ188616.1|	99.33	1350	9	0	1	1350	1	1350	0	2444
gi|322160776|gb|JF175370.1|	99.33	1350	9	0	1	1350	1	1350	0	2444
gi|219535543|gb|FJ512348.1|	98.97	1365	14	0	1	1365	11	1375	0	2444

gi|219531288|gb|FJ508093.1|	99.33	1350	9	0	1	1350	16	1365	0	2444
gi|219531287|gb|FJ508092.1|	99.33	1350	9	0	1	1350	16	1365	0	2444
gi|219531286|gb|FJ508091.1|	99.33	1350	9	0	1	1350	16	1365	0	2444
gi|219531284|gb|FJ508089.1|	99.33	1350	9	0	1	1350	16	1365	0	2444
gi|219531285|gb|FJ508090.1|	99.33	1350	9	0	1	1350	16	1365	0	2444
gi|214022757|gb|FJ368373.1|	99.19	1356	11	0	1	1356	1	1356	0	2444
gi|214018691|gb|FJ364304.1|	99.19	1356	11	0	1	1356	1	1356	0	2444
gi|192968905|gb|EU764690.1|	99.33	1350	9	0	1	1350	1	1350	0	2444
gi|62765741|gb|AY986251.1|	99.33	1350	9	0	1	1350	1	1350	0	2444
gi|62764956|gb|AY985466.1|	99.33	1350	9	0	1	1350	1	1350	0	2444
gi|219536109|gb|FJ512914.1|	98.97	1364	14	0	2	1365	13	1376	0	2442
gi|219536105|gb|FJ512910.1|	98.97	1365	13	1	1	1365	11	1374	0	2442
gi|219531289|gb|FJ508094.1|	99.41	1346	8	0	1	1346	16	1361	0	2442
gi|219531118|gb|FJ507923.1|	99.19	1355	11	0	11	1365	1	1355	0	2442
gi|388928236|gb|JQ183353.1|	99.26	1352	8	2	1	1350	1	1352	0	2440
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gi|388931911|gb|JQ187028.1|	99.26	1350	10	0	1	1350	1	1350	0	2438
gi|388931775|gb|JQ186892.1|	99.26	1350	10	0	1	1350	1	1350	0	2438
gi|388931727|gb|JQ186844.1|	99.26	1350	10	0	1	1350	1	1350	0	2438
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gi|388928164|gb|JQ183281.1|	99.26	1350	10	0	1	1350	1	1350	0	2438
gi|388928479|gb|JQ183596.1|	99.26	1351	9	1	1	1350	1	1351	0	2438
gi|388928666|gb|JQ183783.1|	99.26	1350	10	0	1	1350	1	1350	0	2438
gi|388935278|gb|JQ190395.1|	99.26	1350	10	0	1	1350	1	1350	0	2438
gi|388934715|gb|JQ189832.1|	99.26	1350	10	0	1	1350	1	1350	0	2438
gi|388934682|gb|JQ189799.1|	99.26	1350	10	0	1	1350	1	1350	0	2438
gi|388933162|gb|JQ188279.1|	99.33	1347	9	0	4	1350	4	1350	0	2438
gi|388933068|gb|JQ188185.1|	99.26	1350	10	0	1	1350	1	1350	0	2438
gi|388930723|gb|JQ185840.1|;gi|388	99.26	1350	10	0	1	1350	1	1350	0	2438
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gi|219533788|gb|FJ510593.1|	98.9	1365	15	0	1	1365	11	1375	0	2438
gi|219531276|gb|FJ508081.1|	99.26	1350	10	0	1	1350	16	1365	0	2438

gi|219531277|gb|FJ508082.1|;gi|219	99.26	1350	10	0	1	1350	16	1365	0	2438
gi|214017889|gb|FJ363502.1|	99.12	1356	12	0	1	1356	1	1356	0	2438
gi|214017490|gb|FJ363103.1|	99.12	1356	12	0	1	1356	1	1356	0	2438
gi|192970039|gb|EU765824.1|	99.26	1350	10	0	1	1350	1	1350	0	2438
gi|192968817|gb|EU764602.1|	99.26	1350	10	0	1	1350	1	1350	0	2438
gi|192968594|gb|EU764379.1|	99.26	1350	10	0	1	1350	1	1350	0	2438
gi|192981510|gb|EU775533.1|	98.83	1368	16	0	1	1368	33	1400	0	2438
gi|169285159|gb|EU469684.1|	98.83	1368	16	0	1	1368	32	1399	0	2438
gi|62759235|gb|AY979745.1|;gi|3889	99.26	1350	10	0	1	1350	1	1350	0	2438
gi|62765196|gb|AY985706.1|	99.26	1350	10	0	1	1350	1	1350	0	2438
gi|62765128|gb|AY985638.1|	99.26	1350	10	0	1	1350	1	1350	0	2438
gi|62765091|gb|AY985601.1|	99.26	1351	8	2	1	1350	1	1350	0	2438
gi|62764908|gb|AY985418.1|	99.26	1350	10	0	1	1350	1	1350	0	2438
gi|62764829|gb|AY985339.1|	99.26	1350	10	0	1	1350	1	1350	0	2438
gi|62763469|gb|AY983979.1|	99.26	1350	10	0	1	1350	1	1350	0	2438
gi|62763277|gb|AY983787.1|	99.26	1350	10	0	1	1350	1	1350	0	2438
gi|62757651|gb|AY978161.1|	99.26	1350	10	0	1	1350	1	1350	0	2438
gi|62757223|gb|AY977733.1|	99.26	1351	8	2	1	1350	1	1350	0	2438
gi|322160944|gb|JF175538.1|	99.26	1350	9	1	1	1350	1	1349	0	2436
gi|322153252|gb|JF167846.1|	99.26	1350	9	1	1	1350	1	1349	0	2436
gi|219533790|gb|FJ510595.1|	98.9	1365	14	1	1	1365	11	1374	0	2436
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gi|214018920|gb|FJ364534.1|	98.83	1367	16	0	1	1367	1	1367	0	2436
gi|388931650|gb|JQ186767.1|	99.19	1352	9	2	1	1350	1	1352	0	2435
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gi|219532157|gb|FJ508962.1|	98.9	1363	14	1	1	1363	11	1372	0	2435
gi|62765608|gb|AY986118.1|	99.19	1353	8	3	1	1350	1	1353	0	2435
gi|62765449|gb|AY985959.1|	99.19	1352	9	2	1	1350	1	1352	0	2435
gi|388931501|gb|JQ186618.1|	99.19	1350	11	0	1	1350	1	1350	0	2433
gi|388928698|gb|JQ183815.1|	99.19	1350	11	0	1	1350	1	1350	0	2433
gi|388928240|gb|JQ183357.1|	99.19	1350	11	0	1	1350	1	1350	0	2433
gi|388935772|gb|JQ190889.1|	99.19	1350	11	0	1	1350	1	1350	0	2433
gi|388934425|gb|JQ189542.1|	99.19	1350	11	0	1	1350	1	1350	0	2433

gi|322146999|gb|JF161593.1|	99.19	1350	11	0	1	1350	1	1350	0	2433
gi|219531283|gb|FJ508088.1|	99.19	1350	11	0	1	1350	16	1365	0	2433
gi|219531282|gb|FJ508087.1|	99.19	1350	11	0	1	1350	16	1365	0	2433
gi|214021126|gb|FJ366742.1|	99.26	1348	9	1	10	1356	35	1382	0	2433
gi|214020057|gb|FJ365672.1|	99.26	1347	10	0	10	1356	2	1348	0	2433
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gi|62765226|gb|AY985736.1|;gi|1929	99.19	1350	11	0	1	1350	1	1350	0	2433
gi|62765100|gb|AY985610.1|	99.19	1350	11	0	1	1350	1	1350	0	2433
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gi|62764776|gb|AY985286.1|	99.19	1350	11	0	1	1350	1	1350	0	2433
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gi|62759806|gb|AY980316.1|;gi|3889	99.19	1350	11	0	1	1350	1	1350	0	2433
gi|62757414|gb|AY977924.1|	99.19	1350	11	0	1	1350	1	1350	0	2433
gi|219531272|gb|FJ508077.1|	99.26	1346	10	0	1	1346	16	1361	0	2431
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gi|219531270|gb|FJ508075.1|	99.26	1346	10	0	1	1346	16	1361	0	2431
gi|214026068|gb|FJ371687.1|	99.04	1355	13	0	1	1355	1	1355	0	2431
gi|214018797|gb|FJ364410.1|	98.83	1364	16	0	1	1364	1	1364	0	2431
gi|62763276|gb|AY983786.1|	99.19	1350	10	1	1	1350	1	1349	0	2431
gi|388928008|gb|JQ183125.1|	99.11	1353	9	3	1	1350	1	1353	0	2429
gi|219531290|gb|FJ508095.1|	99.26	1345	10	0	1	1345	16	1360	0	2429
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gi|219531268|gb|FJ508073.1|	99.26	1345	10	0	1	1345	16	1360	0	2429
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gi|388928632|gb|JQ183749.1|	99.11	1351	10	2	1	1350	1	1350	0	2427
gi|388928637|gb|JQ183754.1|	99.11	1350	12	0	1	1350	1	1350	0	2427
gi|388930665|gb|JQ185782.1|	99.11	1351	10	2	1	1350	1	1350	0	2427
gi|388928400|gb|JQ183517.1|	99.11	1350	12	0	1	1350	1	1350	0	2427
gi|388935714|gb|JQ190831.1|	99.11	1354	5	6	2	1350	3	1354	0	2427
gi|388934827|gb|JQ189944.1|	99.11	1350	12	0	1	1350	1	1350	0	2427
gi|388933351|gb|JQ188468.1|	99.11	1350	12	0	1	1350	1	1350	0	2427
gi|319484570|gb|HQ777431.1|	97.6	1417	32	2	19	1435	1	1415	0	2427

gi|322160977|gb|JF175571.1|	99.11	1350	12	0	1	1350	1	1350	0	2427
gi|219535541|gb|FJ512346.1|	98.75	1365	17	0	1	1365	11	1375	0	2427
gi|214026245|gb|FJ371864.1|	98.97	1357	13	1	1	1356	1	1357	0	2427
gi|192970466|gb|EU766251.1|	99.11	1350	12	0	1	1350	1	1350	0	2427
gi|192970348|gb|EU766133.1|	99.11	1350	12	0	1	1350	1	1350	0	2427
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gi|192966307|gb|EU762092.1|	99.11	1350	12	0	1	1350	1	1350	0	2427
gi|192966162|gb|EU761947.1|	99.11	1350	12	0	1	1350	1	1350	0	2427
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gi|110433710|gb|DQ793310.1|	98.68	1368	18	0	1	1368	21	1388	0	2427
gi|62759181|gb|AY979691.1|	99.11	1350	12	0	1	1350	1	1350	0	2427
gi|62765793|gb|AY986303.1|	99.11	1350	12	0	1	1350	1	1350	0	2427
gi|62764702|gb|AY985212.1|	99.11	1350	12	0	1	1350	1	1350	0	2427
gi|388934664|gb|JQ189781.1|	99.04	1354	9	4	1	1350	1	1354	0	2425
gi|322147187|gb|JF161781.1|	99.11	1350	11	1	1	1350	1	1349	0	2425
gi|219531281|gb|FJ508086.1|	99.11	1350	11	1	1	1350	16	1364	0	2425
gi|219531274|gb|FJ508079.1|	99.18	1346	11	0	1	1346	16	1361	0	2425
gi|219531271|gb|FJ508076.1|	99.18	1346	11	0	1	1346	16	1361	0	2425
gi|219531269|gb|FJ508074.1|	99.18	1346	11	0	1	1346	16	1361	0	2425
gi|219531279|gb|FJ508084.1|	99.11	1349	10	2	1	1348	16	1363	0	2423
gi|62764920|gb|AY985430.1|	99.04	1353	10	3	1	1350	1	1353	0	2423
gi|388930615|gb|JQ185732.1|	99.04	1350	13	0	1	1350	1	1350	0	2422
gi|388930442|gb|JQ185559.1|	99.04	1350	13	0	1	1350	1	1350	0	2422
gi|388929030|gb|JQ184147.1|	99.04	1350	13	0	1	1350	1	1350	0	2422
gi|388928543|gb|JQ183660.1|	99.04	1351	11	2	1	1350	1	1350	0	2422
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gi|214026073|gb|FJ371692.1|	98.89	1356	15	0	1	1356	1	1356	0	2422
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gi|192970356|gb|EU766141.1|	99.04	1351	12	1	1	1350	1	1351	0	2422
gi|192969098|gb|EU764883.1|	99.04	1350	13	0	1	1350	1	1350	0	2422
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gi|169287503|gb|EU472028.1|	98.61	1368	19	0	1	1368	33	1400	0	2422
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gi|62765215|gb|AY985725.1|	99.04	1350	13	0	1	1350	1	1350	0	2422
gi|322147075|gb|JF161669.1|	99.04	1350	12	1	1	1350	1	1349	0	2420
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gi|388928362|gb|JQ183479.1|	98.96	1350	14	0	1	1350	1	1350	0	2416
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gi|319498064|gb|HQ790925.1|	98.4	1375	20	2	23	1396	1	1374	0	2416
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gi|192970493|gb|EU766278.1|	98.96	1351	13	1	1	1350	1	1351	0	2416
gi|192966008|gb|EU761793.1|	98.96	1352	11	3	1	1350	1	1351	0	2416
gi|169285267|gb|EU469792.1|	98.54	1368	20	0	1	1368	33	1400	0	2416
gi|169278106|gb|EU462631.1|	98.54	1369	18	2	1	1368	21	1388	0	2416
gi|169278096|gb|EU462621.1|	98.54	1368	20	0	1	1368	21	1388	0	2416
gi|169276721|gb|EU461246.1|	98.54	1368	20	0	1	1368	33	1400	0	2416
gi|110445418|gb|DQ805633.1|	98.54	1369	19	1	1	1368	21	1389	0	2416
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gi|388935011|gb|JQ190128.1|	98.89	1350	15	0	1	1350	1	1350	0	2410
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gi|214017608|gb|FJ363221.1|	98.67	1356	18	0	1	1356	1	1356	0	2405
gi|214017504|gb|FJ363117.1|	98.67	1356	18	0	1	1356	1	1356	0	2405
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gi|214026456|gb|FJ372075.1|	99.03	1340	13	0	17	1356	3	1342	0	2403
gi|192970718|gb|EU766503.1|	98.74	1354	13	4	1	1350	1	1354	0	2403
gi|192980909|gb|EU774932.1|	98.32	1368	23	0	1	1368	21	1388	0	2399
gi|192980191|gb|EU774214.1|	99.1	1335	12	0	1	1335	21	1355	0	2399
gi|388930741|gb|JQ185858.1|	98.67	1354	14	4	1	1350	1	1354	0	2398
gi|388930096|gb|JQ185213.1|	98.67	1355	13	4	1	1350	1	1355	0	2398
gi|388928799|gb|JQ183916.1|	98.67	1355	13	5	1	1350	1	1355	0	2398
gi|192970812|gb|EU766597.1|	98.67	1355	13	5	1	1350	1	1355	0	2398
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gi|192980961|gb|EU774984.1|	98.25	1368	24	0	1	1368	33	1400	0	2394
gi|219531267|gb|FJ508072.1|	98.88	1340	14	1	1	1340	16	1354	0	2390
gi|322145055|gb|JF159649.1|	98.59	1351	18	1	1	1350	1	1351	0	2388
gi|214018819|gb|FJ364432.1|	98.45	1356	21	0	1	1356	1	1356	0	2388
gi|192968638|gb|EU764423.1|	98.53	1356	14	6	1	1350	1	1356	0	2388
gi|388930393|gb|JQ185510.1|	98.38	1362	10	12	1	1350	1	1362	0	2383
gi|192981465|gb|EU775488.1|	98.1	1368	26	0	1	1368	33	1400	0	2383
gi|110437046|gb|DQ796646.1|	98.1	1370	23	3	1	1368	33	1401	0	2383
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gi|169278110|gb|EU462635.1|	98.03	1368	27	0	1	1368	21	1388	0	2377
gi|62765348|gb|AY985858.1|	98.31	1363	10	13	1	1350	1	1363	0	2377
gi|214026121|gb|FJ371740.1|	98.23	1356	24	0	1	1356	21	1376	0	2372
gi|169285018|gb|EU469543.1|	98.66	1339	17	1	1	1339	1	1338	0	2372
gi|214026227|gb|FJ371846.1|	98.51	1345	15	5	17	1356	1	1345	0	2368
gi|192970133|gb|EU765918.1|	98.3	1353	19	4	1	1350	1	1352	0	2368
gi|322145018|gb|JF159612.1|	98.3	1350	22	1	1	1350	1	1349	0	2364

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gi|214018742|gb|FJ364355.1|	98.5	1332	20	0	1	1332	1	1332	0	2350
gi|388928549|gb|JQ183666.1|	97.87	1361	17	12	1	1350	1	1360	0	2342
gi|388934849|gb|JQ189966.1|	97.67	1375	7	25	1	1350	1	1375	0	2338
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gi|110438803|gb|DQ798403.1|	99.38	1288	6	2	67	1352	15	1302	0	2333
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gi|110449954|gb|DQ810169.1|	97.37	1368	33	3	1	1368	25	1389	0	2324
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gi|110441294|gb|DQ800894.1|	97.01	1370	37	3	1	1368	33	1400	0	2300
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gi|110436797|gb|DQ796397.1|	99.29	1261	8	1	109	1368	1	1261	0	2278
gi|169282930|gb|EU467455.1|	97.3	1335	35	1	1	1335	34	1367	0	2265
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gi|169287428|gb|EU471953.1|	99.05	1261	9	3	111	1368	1	1261	0	2259
gi|169282916|gb|EU467441.1|	97.22	1331	36	1	1	1331	35	1364	0	2252
gi|169287473|gb|EU471998.1|	97.13	1288	32	3	84	1368	88	1373	0	2169
gi|169285037|gb|EU469562.1|	98.94	1130	12	0	222	1351	136	1265	0	2021
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gi|298391468|gb|HM478330.1|	100	1001	0	0	345	1345	1	1001	0	1849
gi|298391173|gb|HM478035.1|	100	1001	0	0	345	1345	1	1001	0	1849
gi|298390963|gb|HM477825.1|	100	1001	0	0	345	1345	1	1001	0	1849
gi|298390562|gb|HM477424.1|	100	1001	0	0	344	1344	1	1001	0	1849
gi|298390543|gb|HM477405.1|	100	1001	0	0	345	1345	1	1001	0	1849
gi|298389976|gb|HM476838.1|	100	1001	0	0	345	1345	1	1001	0	1849
gi|298389938|gb|HM476800.1|	100	1001	0	0	345	1345	1	1001	0	1849

gi|298389589|gb|HM476451.1|	100	1001	0	0	344	1344	1	1001	0	1849
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gi|298389379|gb|HM476241.1|	100	1001	0	0	344	1344	1	1001	0	1849
gi|298389231|gb|HM476093.1|	100	1001	0	0	345	1345	1	1001	0	1849
gi|298388837|gb|HM475699.1|	100	1001	0	0	345	1345	1	1001	0	1849
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gi|258688642|emb|FP083302.1|	100	1001	0	0	345	1345	1	1001	0	1849
gi|258688592|emb|FP083252.1|	100	1001	0	0	345	1345	1	1001	0	1849
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gi|258685243|emb|FP075647.1|	100	1001	0	0	345	1345	1	1001	0	1849
gi|258684705|emb|FP075308.1|	100	1001	0	0	345	1345	1	1001	0	1849
gi|258684256|emb|FP075075.1|	100	1001	0	0	345	1345	1	1001	0	1849
gi|258684063|emb|FP081284.1|	100	1001	0	0	345	1345	1	1001	0	1849
gi|258683838|emb|FP081059.1|	100	1001	0	0	344	1344	1	1001	0	1849
gi|258681398|emb|FP084863.1|	100	1001	0	0	345	1345	1	1001	0	1849
gi|258681389|emb|FP084854.1|	100	1001	0	0	345	1345	1	1001	0	1849
gi|258680819|emb|FP084484.1|	100	1001	0	0	345	1345	1	1001	0	1849
gi|258680338|emb|FP078738.1|	100	1001	0	0	344	1344	1	1001	0	1849
gi|258680294|emb|FP078694.1|	100	1001	0	0	344	1344	1	1001	0	1849
gi|298392754|gb|HM479616.1|	99.9	1001	1	0	345	1345	1	1001	0	1844
gi|298391479|gb|HM478341.1|	99.9	1001	1	0	344	1344	1	1001	0	1844
gi|298391366|gb|HM478228.1|	99.9	1001	1	0	344	1344	1	1001	0	1844
gi|298390465|gb|HM477327.1|	99.9	1001	1	0	345	1345	1	1001	0	1844
gi|298390259|gb|HM477121.1|	99.9	1001	1	0	345	1345	1	1001	0	1844
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gi|298389217|gb|HM476079.1|	99.9	1001	1	0	345	1345	1	1001	0	1844
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gi|258680303|emb|FP078703.1|	99.7	1001	3	0	344	1344	1	1001	0	1832
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gi|258689133|emb|FP077748.1|	98.8	1001	12	0	344	1344	1	1001	0	1783
gi|258679670|emb|FP078270.1|	98.8	1003	8	2	345	1345	1	1001	0	1783
gi|62753249|gb|AY920132.1|	97.69	1039	21	2	325	1362	1	1037	0	1783
gi|258689128|emb|FP077743.1|	98.6	1001	14	0	344	1344	1	1001	0	1772
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gi|258679990|emb|FP078589.1|	98.5	1001	15	0	344	1344	1	1001	0	1766
gi|258679817|emb|FP078417.1|	98.3	1001	17	0	344	1344	1	1001	0	1755
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gi|310840978|gb|GU105506.1|	99.07	968	9	0	27	994	1	968	0	1738
gi|258680809|emb|FP084474.1|	97.9	1002	20	1	344	1344	1	1002	0	1733
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gi|258688530|emb|FP083190.1|	97.7	1001	23	0	344	1344	1	1001	0	1722
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gi|258687267|emb|FP082522.1|	97.7	1001	23	0	345	1345	1	1001	0	1722
gi|258680259|emb|FP078659.1|	97.7	1002	21	2	345	1345	1	1001	0	1722
gi|258679892|emb|FP078491.1|	97.7	1001	23	0	345	1345	1	1001	0	1722
gi|258679706|emb|FP078306.1|	97.6	1002	22	2	344	1344	1	1001	0	1716
gi|258687656|emb|FP082713.1|	97.5	1001	25	0	344	1344	1	1001	0	1711
gi|310841110|gb|GU105638.1|	98.75	961	12	0	27	987	1	961	0	1709
gi|298392813|gb|HM479675.1|	97.4	1001	24	2	344	1344	1	999	0	1703
gi|258684733|emb|FP075336.1|	97.4	1001	24	2	344	1344	1	999	0	1703
gi|258680334|emb|FP078734.1|	97.3	1001	27	0	344	1344	1	1001	0	1700
gi|258680314|emb|FP078714.1|	97.3	1001	27	0	345	1345	1	1001	0	1700

gi|310840826|gb|GU105354.1|	98.95	949	10	0	27	975	1	949	0	1698
gi|258680175|emb|FP084224.1|	97.3	1001	25	2	344	1344	1	999	0	1698
gi|310840972|gb|GU105500.1|	99.36	935	6	0	27	961	1	935	0	1694
gi|310841115|gb|GU105643.1|	98.94	944	10	0	32	975	2	945	0	1688
gi|73427009|gb|DQ144095.1|	99.57	925	4	0	511	1435	1	925	0	1687
gi|258681714|emb|FP079519.1|	97	1001	30	0	345	1345	1	1001	0	1683
gi|258689064|emb|FP077679.1|	97.01	1004	23	7	345	1345	1	1000	0	1681
gi|310840973|gb|GU105501.1|	99.04	936	9	0	27	962	1	936	0	1679
gi|310840928|gb|GU105456.1|	98.83	942	11	0	27	968	1	942	0	1679
gi|310840975|gb|GU105503.1|	98.93	936	10	0	27	962	1	936	0	1674
gi|310840927|gb|GU105455.1|	99.03	932	9	0	27	958	1	932	0	1672
gi|310840976|gb|GU105504.1|	98.82	936	11	0	27	962	1	936	0	1668
gi|310841112|gb|GU105640.1|	99.03	928	9	0	27	954	1	928	0	1664
gi|310841093|gb|GU105621.1|	97.72	967	20	2	27	992	1	966	0	1663
gi|310841111|gb|GU105639.1|	99.24	918	7	0	24	941	1	918	0	1657
gi|310841173|gb|GU105701.1|	99.34	912	4	2	28	938	1	911	0	1650
gi|310841037|gb|GU105565.1|	99.12	913	8	0	29	941	1	913	0	1642
gi|383385719|gb|JQ799156.1|	97.89	948	18	2	507	1453	1	947	0	1639
gi|310840926|gb|GU105454.1|	99.12	910	8	0	27	936	1	910	0	1637
gi|310841163|gb|GU105691.1|	98.91	915	10	0	27	941	1	915	0	1635
gi|310840866|gb|GU105394.1|	99.01	912	9	0	27	938	1	912	0	1635
gi|310836892|gb|GU101420.1|	99.55	897	4	0	29	925	1	897	0	1635
gi|310840980|gb|GU105508.1|	99.33	901	6	0	27	927	1	901	0	1631
gi|310842043|gb|GU106571.1|	98.8	915	11	0	24	938	1	915	0	1629
gi|310841348|gb|GU105876.1|	99.12	905	8	0	24	928	1	905	0	1628
gi|310840979|gb|GU105507.1|	99.44	894	5	0	27	920	1	894	0	1624
gi|310842898|gb|GU107426.1|	98.9	908	10	0	31	938	1	908	0	1622
gi|310840856|gb|GU105384.1|	99.77	881	2	0	28	908	1	881	0	1616
gi|310840807|gb|GU105335.1|	99.77	880	2	0	26	905	1	880	0	1615
gi|239620487|gb|FJ651880.1|	99.44	889	5	0	1	889	27	915	0	1615
gi|310841256|gb|GU105784.1|	98.89	898	10	0	28	925	1	898	0	1604
gi|239620493|gb|FJ651886.1|	99.21	889	7	0	1	889	27	915	0	1604
gi|310842899|gb|GU107427.1|	98.36	912	15	0	27	938	1	912	0	1602
gi|310842632|gb|GU107160.1|	98.78	900	11	0	27	926	1	900	0	1602

gi|310838465|gb|GU102993.1|	98.89	897	10	0	24	920	1	897	0	1602
gi|310842230|gb|GU106758.1|	98.88	896	10	0	24	919	1	896	0	1600
gi|310843290|gb|GU107818.1|	99.77	868	2	0	24	891	1	868	0	1592
gi|310842840|gb|GU107368.1|	98.45	905	13	1	28	931	1	905	0	1592
gi|310841113|gb|GU105641.1|	98.55	898	13	0	29	926	1	898	0	1587
gi|291331103|gb|GU957635.1|	98.88	889	10	0	1	889	27	915	0	1587
gi|310842044|gb|GU106572.1|	98.66	894	12	0	24	917	1	894	0	1585
gi|310841152|gb|GU105680.1|	98.77	892	10	1	27	918	1	891	0	1585
gi|310840857|gb|GU105385.1|	98.55	897	13	0	28	924	1	897	0	1585
gi|291328328|gb|GU954860.1|	99.43	873	5	0	1	873	29	901	0	1585
gi|310841879|gb|GU106407.1|	99.54	868	4	0	33	900	2	869	0	1581
gi|295651187|gb|HM013421.1|	98.76	889	11	0	1	889	28	916	0	1581
gi|239620005|gb|FJ651398.1|	98.76	889	11	0	1	889	16	904	0	1581
gi|291328488|gb|GU955020.1|	98.76	889	10	1	2	889	3	891	0	1580
gi|310841909|gb|GU106437.1|	99.54	866	4	0	27	892	1	866	0	1578
gi|239619923|gb|FJ651316.1|	99.42	869	5	0	21	889	1	869	0	1578
gi|169279765|gb|EU464290.1|	98.02	908	18	0	461	1368	18	925	0	1578
gi|291331104|gb|GU957636.1|	98.65	889	12	0	1	889	27	915	0	1576
gi|239620484|gb|FJ651877.1|	98.65	889	12	0	1	889	27	915	0	1576
gi|239620008|gb|FJ651401.1|	98.65	889	12	0	1	889	17	905	0	1576
gi|310839154|gb|GU103682.1|	98.98	879	9	0	27	905	1	879	0	1574
gi|310841445|gb|GU105973.1|	99.77	857	2	0	21	877	1	857	0	1572
gi|291328286|gb|GU954818.1|	98.54	889	13	0	1	889	28	916	0	1570
gi|239620488|gb|FJ651881.1|	98.54	889	13	0	1	889	27	915	0	1570
gi|239619382|gb|FJ650775.1|	98.54	889	13	0	1	889	27	915	0	1570
gi|310840977|gb|GU105505.1|	99.42	864	5	0	27	890	1	864	0	1568
gi|291328529|gb|GU955061.1|	98.43	889	14	0	1	889	28	916	0	1565
gi|310842046|gb|GU106574.1|	98.53	885	13	0	24	908	1	885	0	1563
gi|310841342|gb|GU105870.1|	99.77	852	2	0	24	875	1	852	0	1563
gi|310838205|gb|GU102733.1|	98.32	892	13	2	25	916	1	890	0	1563
gi|310837149|gb|GU101677.1|	99.42	860	5	0	33	892	3	862	0	1561
gi|310835970|gb|GU100498.1|	99.3	863	6	0	29	891	1	863	0	1561
gi|291331110|gb|GU957642.1|	98.31	889	15	0	1	889	27	915	0	1559
gi|239619213|gb|FJ650606.1|	98.31	888	15	0	1	888	27	914	0	1557

gi|310842067|gb|GU106595.1|	99.53	854	4	0	24	877	1	854	0	1555
gi|310841114|gb|GU105642.1|	99.3	860	6	0	29	888	1	860	0	1555
gi|239619393|gb|FJ650786.1|	98.63	877	12	0	1	877	27	903	0	1554
gi|529080432|gb|KF229853.1|	98.96	866	9	0	24	889	1	866	0	1550
gi|310840808|gb|GU105336.1|	98.74	872	11	0	27	898	1	872	0	1550
gi|239619345|gb|FJ650738.1|	98.63	874	12	0	1	874	27	900	0	1548
gi|310841380|gb|GU105908.1|	99.53	849	4	0	29	877	1	849	0	1546
gi|291331651|gb|GU958183.1|	97.98	890	16	2	1	889	28	916	0	1543
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gi|310839536|gb|GU104064.1|	98.62	870	10	2	24	892	1	869	0	1539
gi|291331102|gb|GU957634.1|	99.41	848	5	0	42	889	1	848	0	1539
gi|239620288|gb|FJ651681.1|	98.73	866	11	0	1	866	11	876	0	1539
gi|310838146|gb|GU102674.1|	98.73	866	10	1	25	890	1	865	0	1537
gi|529083001|gb|KF232422.1|	98.62	867	12	0	1	867	23	889	0	1535
gi|529080529|gb|KF229950.1|	98.84	861	10	0	2	862	1	861	0	1535
gi|291329410|gb|GU955942.1|	98.73	864	11	0	1	864	14	877	0	1535
gi|529080654|gb|KF230075.1|	99.06	854	8	0	1	854	21	874	0	1533
gi|310841035|gb|GU105563.1|	98.5	868	13	0	28	895	1	868	0	1531
gi|169287659|gb|EU472184.1|	97.44	898	23	0	471	1368	1	898	0	1531
gi|310838633|gb|GU103161.1|	99.64	837	3	0	33	869	3	839	0	1530
gi|310842151|gb|GU106679.1|	99.4	840	5	0	27	866	1	840	0	1524
gi|310842099|gb|GU106627.1|	99.4	840	5	0	27	866	1	840	0	1524
gi|310841955|gb|GU106483.1|	99.4	840	5	0	24	863	1	840	0	1524
gi|310841878|gb|GU106406.1|	99.4	840	5	0	24	863	1	840	0	1524
gi|310838817|gb|GU103345.1|	99.64	834	3	0	29	862	1	834	0	1524
gi|310838576|gb|GU103104.1|	99.52	837	3	1	27	863	1	836	0	1522
gi|310841036|gb|GU105564.1|	98.38	865	14	0	28	892	1	865	0	1520
gi|164454293|dbj|AB265290.1|	97.53	889	21	1	478	1366	1	888	0	1519
gi|310841954|gb|GU106482.1|	99.05	845	8	0	33	877	1	845	0	1517
gi|310841907|gb|GU106435.1|	99.4	835	5	0	29	863	1	835	0	1515
gi|310838717|gb|GU103245.1|	99.52	833	3	1	33	865	3	834	0	1515
gi|310833518|gb|GU098046.1|	99.52	832	4	0	33	864	1	832	0	1515
gi|310841908|gb|GU106436.1|	99.28	837	6	0	27	863	1	837	0	1513
gi|118136060|gb|EF071471.1|	98.82	849	10	0	1	849	20	868	0	1513

gi|310841536|gb|GU106064.1|	99.16	838	7	0	29	866	1	838	0	1509
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gi|310838778|gb|GU103306.1|	99.16	838	7	0	28	865	1	838	0	1509
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gi|310842517|gb|GU107045.1|	98.15	863	16	0	27	889	1	863	0	1506
gi|310833629|gb|GU098157.1|	98.82	845	10	0	22	866	1	845	0	1506
gi|310840786|gb|GU105314.1|	98.93	840	9	0	27	866	1	840	0	1502
gi|310839537|gb|GU104065.1|	98.81	843	10	0	24	866	1	843	0	1502
gi|310841875|gb|GU106403.1|	98.47	851	13	0	27	877	1	851	0	1500
gi|310840855|gb|GU105383.1|	98.7	845	11	0	27	871	1	845	0	1500
gi|310837024|gb|GU101552.1|	98.36	856	11	3	27	879	1	856	0	1500
gi|310838419|gb|GU102947.1|	99.88	814	1	0	50	863	4	817	0	1498
gi|110440903|gb|DQ800503.1|	99.04	835	8	0	1	835	33	867	0	1498
gi|310842583|gb|GU107111.1|	98.36	852	14	0	25	876	1	852	0	1496
gi|310840877|gb|GU105405.1|	98.81	840	10	0	27	866	1	840	0	1496
gi|310837892|gb|GU102420.1|	98.81	840	10	0	24	863	1	840	0	1496
gi|310833517|gb|GU098045.1|	99.16	831	7	0	33	863	1	831	0	1496
gi|310842068|gb|GU106596.1|	98.24	854	15	0	24	877	1	854	0	1495
gi|399141153|gb|JQ265125.1|	98.69	842	10	1	38	878	1	842	0	1493
gi|310842262|gb|GU106790.1|	99.39	823	5	0	21	843	1	823	0	1493
gi|310833423|gb|GU097951.1|	99.16	829	7	0	36	864	4	832	0	1493
gi|310842231|gb|GU106759.1|	98.58	843	12	0	24	866	1	843	0	1491
gi|310842154|gb|GU106682.1|	98.69	840	11	0	24	863	1	840	0	1491
gi|310842135|gb|GU106663.1|	98.69	840	11	0	27	866	1	840	0	1491
gi|310842045|gb|GU106573.1|	98.69	840	11	0	24	863	1	840	0	1491
gi|310841956|gb|GU106484.1|	98.69	840	11	0	24	863	1	840	0	1491
gi|310842138|gb|GU106666.1|	98.57	840	12	0	27	866	1	840	0	1485
gi|310838495|gb|GU103023.1|	99.03	828	8	0	24	851	1	828	0	1485
gi|239620227|gb|FJ651620.1|	98.34	844	14	0	35	878	2	845	0	1482
gi|399141562|gb|JQ265534.1|	98.68	835	9	2	1	833	65	899	0	1480
gi|310842150|gb|GU106678.1|	98.57	837	12	0	27	863	1	837	0	1480
gi|310842101|gb|GU106629.1|	98.45	840	13	0	24	863	1	840	0	1480
gi|310842097|gb|GU106625.1|	98.45	840	13	0	27	866	1	840	0	1480
gi|310841108|gb|GU105636.1|	97.79	861	14	5	39	895	2	861	0	1480
gi|310837285|gb|GU101813.1|	98.91	828	9	0	24	851	1	828	0	1480

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