

Metagenome microarray for screening of fosmid clones containing specific genes

Soo-Je Park¹, Cheol-Hee Kang¹, Jong-Chan Chae² & Sung-Keun Rhee¹

¹Department of Microbiology, Chungbuk National University, Heungduk-gu, Cheongju, Korea; and ²Biotechnology Center for Agriculture and the Environment, School of Environmental and Biological Sciences, Rutgers University, New Brunswick, NJ, USA

Correspondence: Sung-Keun Rhee, Department of Microbiology, Chungbuk National University, 12 Gaeshin-dong, Heungduk-gu, Cheongju 361-763, Korea. Tel.: +82 43 261 2300; fax: +82 43 264 9600; e-mail: rhees@chungbuk.ac.kr

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Abstract

A critical step in the process of metagenome analysis is to screen for clones that contain specific genes among a large number of clones. To form one of the sequence-based screening tools of a metagenome library, we designed a format of microarray [metagenome microarray (MGA)] that is arrayed with fosmid library clone DNA samples on a glass slide. We evaluated the MGA using random prime labeled fluorescent probes prepared from PCR products of the target gene and found that we could obtain specific hybridization signals only for the fosmid clone that contained the target gene. We found that the detection limit of the MGA was $c. 10 \text{ ng } \mu\text{L}^{-1}$ of fosmid clone DNA, and that the MGA-based hybridization was quantitative within a concentration range of 10–200 $\text{ng } \mu\text{L}^{-1}$ of fosmid clone DNA. We used the MGA successfully to identify two fosmid clones that contained 16S rRNA genes from a fosmid library from the sediment of the East Sea, Korea. In conclusion, we have demonstrated that the MGA can be used for screening for fosmid clones containing specific genes in a metagenome library, and that this technology has potential application as a high-throughput metagenome screening tool.

Introduction

Metagenomics is a new and rapidly developing field, and metagenomic techniques are used in an attempt to analyze the complex genomes contained within the microbial community (Kowalchuk *et al.*, 2007; Schmeisser *et al.*, 2007). One general approach of metagenomics begins with the preparation of a library of clones that contain large inserts, which are obtained from microbial communities. Fosmid libraries containing inserts that comprise fragments of environmental genomes that are $c. 35 \text{ kb}$ in size have been constructed for many microbial communities (Quaiser *et al.*, 2002; Grzymanski *et al.*, 2006; Hallam *et al.*, 2006b). Novel biocatalysts and metabolites have been obtained from metagenome libraries (Williamson *et al.*, 2005; Kim *et al.*, 2007; van Hellemond *et al.*, 2007). When clones that contain phylogenetic genes such as the 16S rRNA gene, are retrieved, the DNA sequence information surrounding these genes provides access to the genomes of uncultivated microorganisms and can provide clues to the physiology of such microorganisms (Treusch *et al.*, 2005; Hallam *et al.*, 2006a, b).

A critical step of metagenome analysis is to screen for clones that contain target genes among a large number of clones. There are two different approaches to screening for target gene-containing clones: activity- and sequence-based screening. In the case of activity-based screening, several hundred thousand clones may need to be analyzed in a single screen in order to detect a few functionally active clones (Henne *et al.*, 2000; Majernik *et al.*, 2001; van Hellemond *et al.*, 2007). This is mainly due to a lack of efficient expression of the metagenome-derived genes in the host strains used. Furthermore, in activity-based screening, it is necessary to develop specialized screening systems to detect the activity of the products of the gene of interest. On the other hand, the application of the sequence-based approach involves the design of PCR primers or hybridization probes for the target genes that are derived from conserved regions of known gene families (Kim *et al.*, 2007; Roh *et al.*, 2007). In general, labor-intensive analyses of individual clones or pools of clones within the library are often required for both activity-based and sequence-based screening procedures.

The microarray is a powerful genomic technology that is used widely to study biological processes. Microarray technology has been used successfully to analyze global gene expression in pure cultures of microorganisms (Richmond *et al.*, 1999; Ye *et al.*, 2000; Liu *et al.*, 2003). It has generated tremendous interest among environmental microbiologists and microbial ecologists because of its ability to achieve a high throughput. Therefore, various types of DNA microarray have been applied to study the microbial diversity of various environments. Various types of DNA microarray can be classified according to the nature of the DNA arrayed on the slide, for example, oligonucleotide comprising 20–70 bp (Bodrossy *et al.*, 2003; Ward *et al.*, 2007), cDNA (PCR-amplified DNA fragments) (Wu *et al.*, 2004), and whole-genome DNA (Bae *et al.*, 2005). One of the most promising sequence-based approaches for identifying clones that contain target genes within metagenome libraries is the use of DNA-microarray technology. The use of microarrays to profile metagenome libraries offers an effective approach for characterizing many clones rapidly (Sebat *et al.*, 2003).

In this study, we designed a novel format of microarray in which a fosmid library obtained from marine sediment was arrayed on a glass slide. This format is referred to as a metagenome microarray (MGA). In the MGA format, the 'probe' and 'target' concept is a reversal of those of general cDNA and oligonucleotide microarrays: targets (fosmid clones) were spotted on a slide and a specific gene probe was labeled and used for hybridization (Fig. 1). We evaluated the conditions and performance of the MGA as a tool for the screening of target gene-containing fosmid clones. This format of microarray may offer an effective approach for identifying clones from metagenome libraries rapidly. Because the library is maintained on a slide, it is not necessary to repeat laborious procedures for screening various target genes. We propose that the MGA may be used

as an alternative or a complementary technique to current metagenome-screening technologies.

Materials and methods

Construction of a fosmid library from marine sediment

Marine sediments were collected from a coastal site (128°35'E, 38°20'N, 650 m) of the East Sea, Korea, during July 2005, using a core sampler. Each core sample was placed in a sterile plastic tube using alcohol-sterilized spatulas. The samples were stored at $-80\text{ }^{\circ}\text{C}$ until analysis. Metagenomic DNA was prepared from the marine sediment using a procedure derived from that of Quaiser *et al.* (2002).

The fosmid library was constructed using a **CopyControl™ Fosmid Library Production Kit** (Epicentre, Madison, WI) according to the manufacturer's protocol. Briefly, purified DNA (0.5 μg) was treated enzymatically to end-repair the blunt ends, and was then ligated into the fosmid vector pCC1FOS (Epicentre). After *in vitro* packaging into lambda phages and infection of *Escherichia coli* EPI300-T1^R, the bacterial cells were plated on Luria–Bertani (LB) that contained $12.5\text{ }\mu\text{g mL}^{-1}$ chloramphenicol. The plates were incubated at $37\text{ }^{\circ}\text{C}$ for 24 h before the selection of colonies. Transfected *E. coli* colonies were transferred to 96-well plates that contained 150 μL of chloramphenicol-containing LB medium and were incubated at $37\text{ }^{\circ}\text{C}$ for 24 h.

Extraction of fosmid clone DNA from 96-well plate cultures

Each clone on the library plate was transferred to a deep 96-well plate (Nalgene, Rochester, NY) and incubated in a shaking incubator at 180 r.p.m. and $37\text{ }^{\circ}\text{C}$ in the presence of chloramphenicol and an inducer, which was supplied by the

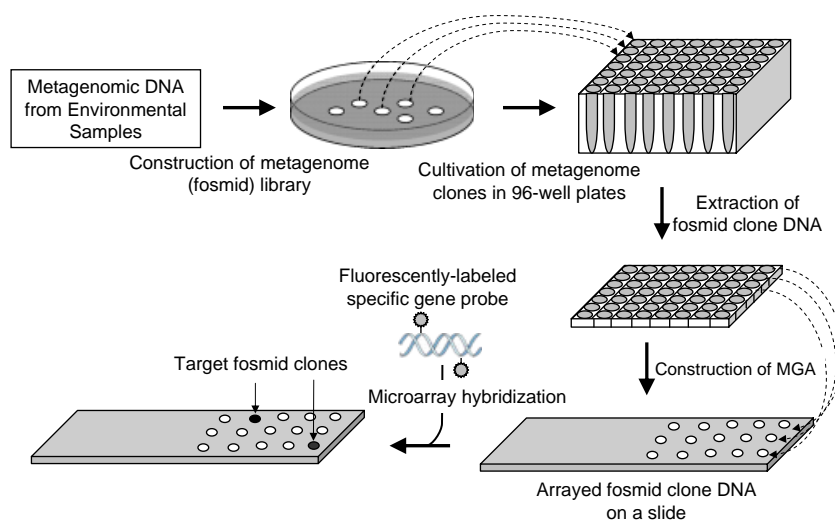


Fig. 1. Schematic diagram of the construction of the MGA and screening of target metagenome clones using the MGA.

manufacturer (Epicentre). Cells were harvested and the fosmid DNA was extracted using a Perfectprep Plasmid 96 Vac DB kit according to the manufacturer's protocol (Eppendorf, Germany). The fosmid DNA samples were resuspended in deionized water to yield a final concentration of 40 ng μL^{-1} .

For the evaluation experiment, larger amounts of fosmid DNA (200 ng μL^{-1}) were obtained using conical tube (SPL Life Science, Korea) cultures containing 10 mL of LB containing chloramphenicol and an inducer. Each clone was inoculated into a conical tube and incubated in a gyratory shaker at 180 r.p.m. and 37 °C. Cells at the exponential phase were harvested rapidly. The fosmid DNA was extracted using a FosmidMAX™ DNA Purification Kit (Epicentre).

Construction of the MGA using fosmid clone DNA

Ten microliters of each fosmid DNA in the 96-well plate was transferred to a 384-well microplate. In the 384-well microplate, the DNA samples were diluted 1:1 (v/v) in 40% dimethyl sulfoxide (Sigma-Aldrich, St Louis, MO). The fosmid DNA samples were arrayed using a Micro Grid II Compact (Genomic Solutions, Hudson, NH). Each fosmid DNA set was printed in four replicates on a different part of the glass slide. The slides were subjected to posttreatment as described by Bae *et al.* (2005). The PCR product of the 16S rRNA gene amplified from the genomic DNA that was obtained from the marine sediment was printed and used as a positive control as well as a position marker on the slide.

For the evaluation experiment, 200 ng μL^{-1} aliquots of fosmid clone DNA obtained from conical tube (SPL Life Science) cultures were diluted appropriately before printing onto slides. Ten randomly selected fosmid DNAs were printed and used as negative controls.

Fluorescent labeling of probe

In the MGA experiment, we used the terms 'probe' for the fluorescence-labeled DNA used for hybridization, 'library pool' for the fosmid clones arrayed (spotted) on a glass slide, and 'target' for the clone of the library pool that contained the specific gene that corresponded to the probe. For the evaluation experiments, the gene, UDP-N-acetylglucosamine pyrophosphorylase (EU159413), which was obtained by end sequencing of a fosmid clone, was amplified using gene-specific primers (F164: 5'-ACGATCAGTCGGGAGGGAGA-3', R280: 5'-AGGCACTCGCATGAAATCGC-3', R717: 5'-GTATTGCCGTGGACTTGGATC-3') and used for the preparation of the fluorescent probe. PCR products of two different lengths (100 and 550 bp) were amplified using the F164-R280 and F164-R717 primer pairs, respectively. The PCR amplification conditions were as follows: 95 °C for 2 min for one cycle; 94 °C for 1 min, 53 °C for 30 s, and 72 °C

for 30 s for 30 cycles; and then 72 °C for 5 min for one cycle. The PCR product was purified using a QIAquick PCR purification kit (Qiagen, Germany). The labeling of 100 or 500 ng PCR product was performed using the BioPrime DNA Labeling kit (Invitrogen, Carlsbad, CA), as described by Rhee *et al.* (2004). The labeled probe was purified using a QIAquick PCR purification kit (Qiagen), concentrated in a Speedvac for 1 h, and then resuspended in 4.35 μL of deionized water and stored at -20 °C. In the experiment that involved application of the MGA, the 16S rRNA gene was amplified from the genomic DNA of the marine sediment using the 27F and 1492R primer pair, as described by Park *et al.* (2006). The PCR product was labeled as described above and used for screening of 16S rRNA gene-containing fosmid clones using MGA.

Hybridization

The fluorescence-labeled probe was mixed with a hybridization solution. The microarray hybridization solution contained 4.35 μL of labeled DNA, 8.75 μL of formamide (50%, v/v), 3 \times SSC (1 \times SSC contained 150 mM NaCl and 15 mM trisodium citrate), 1.25 μg of unlabeled herring sperm DNA (Promega, Madison, WI), and 0.3% sodium dodecyl sulfate (SDS) in a total volume of 17.5 μL . A reduced volume (7.5 μL) of the hybridization mixture was boiled for 5 min for probe denaturation, and was then deposited directly onto the slides and covered with a coverslip (10 mm \times 15 mm; Sigma-Aldrich). Fifteen microliters of 3 \times SSC was dispensed into the hydration wells on either side of a hybridization chamber (Corning Inc., Corning, NY). The hybridization solution was not kept below the hybridization temperature (50 °C) until it was washed to prevent cross-hybridization. The hybridization chamber was plunged into a 50 °C water bath immediately for overnight hybridization (Rhee *et al.*, 2004). After hybridization, the time that the slide remained at room temperature was minimized in order to prevent cross-hybridization. Each microarray slide was taken out, and the coverslip was immediately removed in wash solution 1 (1 \times SSC and 0.2% SDS). The slides were washed using wash solution 1, wash solution 2 (0.1 \times SSC and 0.2% SDS), and wash solution 3 (0.1 \times SSC) for 5 min each at ambient temperature before being dried. The slides were dried using centrifugation as described by Rhee *et al.* (2004).

Image processing and data analysis

The microarrays were scanned using a ScanArray 4000 Microarray Analysis system (Perkin-Elmer, Wellesley, MA) at a resolution of 10 μm . The laser power and PMT gain were adjusted to avoid saturation of target spots. The scanned images were saved as 16-bit TIFF files. Each spot was quantified using GENEXIP version 6.0 software (Molecular devices Co., Downingtown, PA). The data were analyzed as

described by Rhee *et al.* (2004). The signal-to-noise ratio (SNR) was calculated using the following formula (Verdnick *et al.*, 2002) as a basis: $SNR = (\text{signal intensity} - \text{background}) / \text{SD of background}$. The SNR was used as the hybridization signal intensity. In the formula, the background measurement refers to the local spot background intensity, and the SD of the background was calculated by the GENEPIX version 6.0 software (Molecular devices Co.). The SNRs from four replicate data sets were then averaged to represent the SNR for a particular fosmid clone.

Cloning of the 16S rRNA gene and phylogenetic analysis

The PCR products of the 16S rRNA gene (1.5 kb) obtained from candidate target clones using the 27F and 1492R primer set were ligated into the T&A Cloning Vector (Real Biotech Co., Taiwan) and transformed into *E. coli* DH5 α cells according to the manufacturer's instructions. Plasmid DNAs containing a 1.5-kb insert were prepared using the Plasmid Mini prep kit (Solgent Inc., Korea) according to the manufacturer's instructions and were used as a template in the sequencing reactions, as described by Park *et al.* (2006). Two partial 16S rRNA gene sequences determined in this study were deposited in the GenBank database under the accession numbers EU088099 and EU088100. The DNA sequences obtained were submitted to BLAST (<http://www.ncbi.nlm.nih.gov>) to identify related 16S rRNA gene sequences in GenBank. Multiple alignments, calculation of the evolutionary distances, and construction of a phylogenetic tree were performed as described by Park *et al.* (2006).

Results and discussion

Specificity of MGA hybridization

Specificity is one of the most critical parameters for all assay techniques, including microarray techniques that are used to detect and monitor genetic markers in environmental samples. To study the specificity of MGA hybridization, hybridization probes of two different lengths (100 and 550 bp) were prepared from the gene of a fosmid clone that encodes UDP-*N*-acetylglucosamine pyrophosphorylase (EU159413). We observed that the hybridization signal was obtained only for the fosmid clone that contained the target gene corresponding to the labeled probe. The length and concentration of the probe were proportional to the signal intensity of the target clone (Fig. 2). An increase of probe concentration from 100 to 500 ng caused a 1.6-fold and a 2.0-fold increase in signal intensity for the 100- and 550-bp probes, respectively. An increase of the length of the probe from 100 to 550 bp caused a 2.2-fold and a 2.7-fold increase in signal intensity for probe concentrations of 100 and 500 ng, respectively. A weak hybridization signal, with an

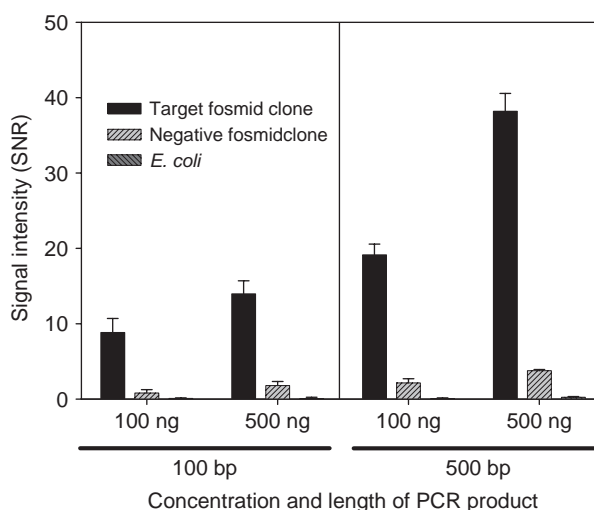


Fig. 2. Result of analysis of the hybridization specificity of the MGA. Combinations of two different lengths and concentrations of PCR product were used for the preparation of fluorescent hybridization probes. Fosmid DNA and *Escherichia coli* genomic DNA of $50 \text{ ng } \mu\text{L}^{-1}$ were arrayed on a glass slide.

SNR of < 3.0 , was observed with all negative-control spots from the fosmid clones. The signal intensity obtained from spots of genomic DNA from *E. coli* DH5 α was negligible (an SNR below 0.1).

Sensitivity and quantification of the MGA hybridization

Detection sensitivity is an important factor in the use of this technique because the preparation of a large amount of fosmid DNA for fabrication of the MGA from cultures in 96-deep-well plates is not feasible. The detection sensitivity of MGA hybridization was analyzed by probing fosmid DNAs containing target genes that had been serially diluted in distilled-deionized water ($200\text{--}5 \text{ ng } \mu\text{L}^{-1}$) and arrayed on the slide. When the probe prepared from 500 ng of the 100- or 550-bp PCR product was used, the hybridization signal was measurable ($SNR > 3$) with more than $10 \text{ ng } \mu\text{L}^{-1}$ of target fosmid DNA, but the signal intensity was barely detectable at $5 \text{ ng } \mu\text{L}^{-1}$ (Fig. 3). It is considered that the effect of fosmid clone size on hybridization is ignorable because the size range of fosmid clones ($36 \pm 3 \text{ kb}$) is quite narrow.

The commonly accepted criterion for the minimum signal (threshold) intensity that can be accurately quantified is an SNR of 3 (Verdnick *et al.*, 2002). Therefore, the detection limit of target fosmid DNA in the MGA must be at least $10 \text{ ng } \mu\text{L}^{-1}$ using randomly labeled fluorescent probes prepared from PCR products. Because we can extract $c. 40\text{--}50 \text{ ng } \mu\text{L}^{-1}$ of fosmid DNA from each well of

a 96-deep-well plate culture, we assume that the MGA could be used successfully to screen target gene-containing fosmid clones under our experimental conditions. Reliable linear relationships were also observed for signal intensity and concentrations of target fosmid clone DNA that ranged from 5 to 200 ng μL^{-1} ($R^2 = 0.98$ and 0.96 for probes prepared from 100- and 550-bp PCR products, respectively) (Fig. 3). These results indicate that the MGA-based hybridization is quantitative for the detection of specific gene-containing

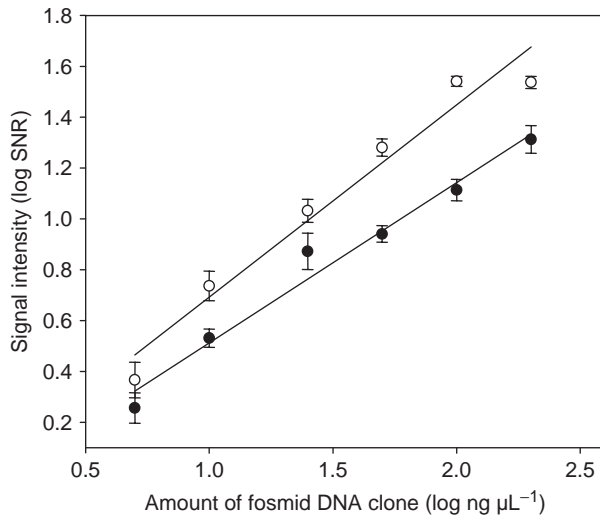


Fig. 3. Relationship of the hybridization signal intensity with the concentration of fosmid DNA arrayed on the slide. A PCR product of (●) 500 ng of 100 bp or (○) 500 ng of 550 bp was used for preparation of a fluorescence-labeled probe.

fosmid clones within a wide range of fosmid DNA concentrations.

Application of the MGA for screening of 16S rRNA gene-containing clones in a metagenome library obtained from marine sediment

The sensitivity and specificity experiments indicated that the format of the MGA described herein has the potential to be used as a screening tool for specific gene-containing clones. We prepared a small-scale MGA using the fosmid library from sediment obtained from the East Sea of Korea, and tested the potential of the MGA in this application. We extracted each fosmid DNA using four 96-deep-well plate cultures, which corresponds to about 13 Mb of metagenome (36 kb \times 380 clones), and arrayed them on a microarray slide. In this test, we attempted to screen for fosmid clones containing the 16S rRNA gene. The PCR product of the 16S rRNA gene from the metagenomic DNA of the East Sea sediment was labeled and used as a hybridization probe. After hybridization, we observed two putative positive spots (2F8, 4D5) (Fig. 4a) with an average signal intensity for all four replicates on the microarray of 23.2 ± 2.5 and 20.4 ± 1.8 , respectively (Fig. 4b). All other, negative, spots had SNR values of less than 3.

Comparative 16S rRNA gene sequence study showed that the 16S rRNA genes of the fosmid clones clearly belonged to the *Roseobacter* clade of *Alphaproteobacteria*, which comprise a large marine group inhabiting various oligotrophic marine environments (Allgaier *et al.*, 2003). The 16S rRNA gene sequences between two clones, 2F8 and 4D5, exhibited 97.9% similarity and both genes were most closely related to

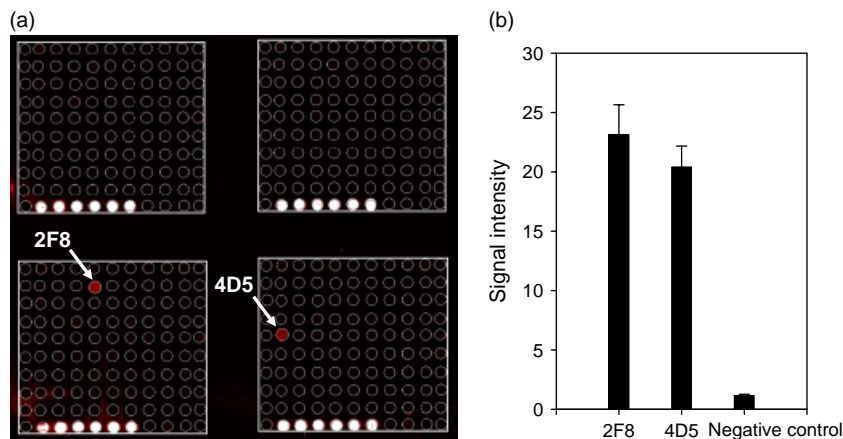
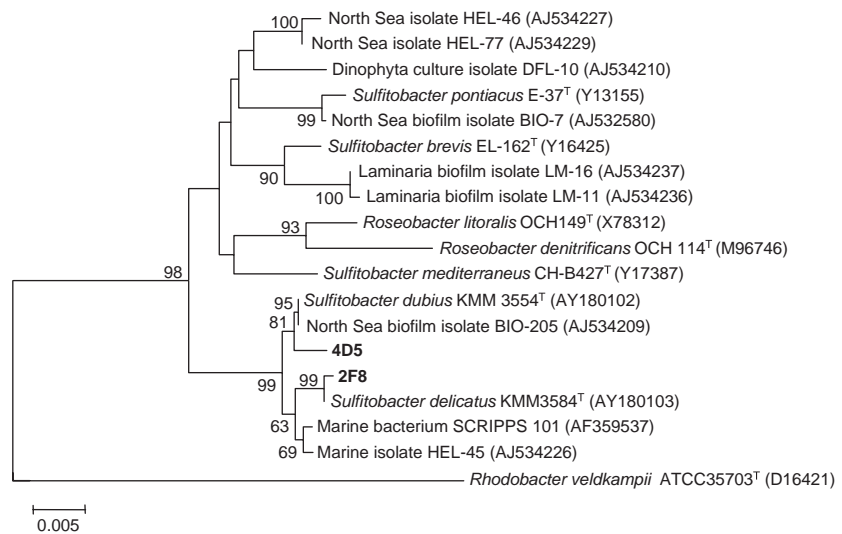


Fig. 4. Fluorescence images showing hybridization of the MGA constructed using a fosmid library from marine sediment. (a) A circular grid was overlapped onto the fluorescent image using GENEPiX version 6.0 software to aid identification of arrayed spots. Arrows indicate the position of spots of candidate 16S rRNA gene-containing clones. The PCR product of the 16S rRNA gene (50 ng μL^{-1}) amplified from the genomic DNA that was obtained from the marine sediment was arrayed on the last row of each panel of the slide (six spots) and used as a positive control, which was saturated in this hybridization and scanning condition and shown by a white color. (b) The signal intensities of the candidate fosmid clone spots and negative spots were calculated and compared.

Fig. 5. Phylogenetic tree of 16S rRNA genes obtained from the fosmid clones screened using the MGA. Representative sequences were chosen from *Sulfitobacter* of the *Roseobacter* clade to show the closest relatives to the 16S rRNA gene sequences of the fosmid clones. The scale bar indicates 0.005 substitutions per nucleotide. Bootstrap values of more than 50% from 1000 replicates are given at branch points.



those of *Sulfitobacter* isolates from diverse marine habitats (Fig. 5). Screening of fosmid clones that contain the bacterial 16S rRNA genes using general PCR amplification of the 16S rRNA gene might lead to a false-positive identification because of the biased PCR amplification of 16S rRNA genes from trace amounts of contaminating host genomic DNA, as shown by Rondon *et al.* (2000). The quantitative nature of microarray could provide another advantage of hybridization-based screening using MGA over PCR-based screening.

In conclusion, we have evaluated the specificity, sensitivity, and quantitation of the MGA and demonstrated that this type of microarray can be used successfully to screen for fosmid clones containing specific genes in a metagenome library. Our results suggest that MGA has potential application as a sequence (hybridization)-based high-throughput metagenome screening tool.

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References

- Allgaier M, Uphoff H, Felske A & Wagner-Dobler I (2003) Aerobic anoxygenic photosynthesis in *Roseobacter* clade bacteria from diverse marine habitats. *Appl Environ Microbiol* **69**: 5051–5059.
- Bae JW, Rhee SK, Park JR, Chung WH, Nam YD, Lee I, Kim H & Park YH (2005) Development and evaluation of genome-probing microarrays for monitoring lactic acid bacteria. *Appl Environ Microbiol* **71**: 8825–8835.
- Bodrossy L, Stralis-Pavese N, Murrell JC, Radajewski S, Weilharther A & Sessitsch A (2003) Development and validation of a diagnostic microbial microarray for methanotrophs. *Environ Microbiol* **5**: 566–582.
- Grzymalski JJ, Carter BJ, DeLong EF, Feldman RA, Ghadiri A & Murray AE (2006) Comparative genomics of DNA fragments from six Antarctic marine planktonic bacteria. *Appl Environ Microbiol* **72**: 1532–1541.
- Hallam SJ, Konstantinidis KT, Putnam N *et al.* (2006a) Genomic analysis of the uncultivated marine crenarchaeote *Cenarchaeum symbiosum*. *Proc Natl Acad Sci USA* **103**: 18296–18301.
- Hallam SJ, Mincer TJ, Schleper C, Preston CM, Roberts K, Richardson PM & DeLong EF (2006b) Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine Crenarchaeota. *PLoS Biol* **4**: e95.
- Henne A, Schmitz RA, Bomeke M, Gottschalk G & Daniel R (2000) Screening of environmental DNA libraries for the presence of genes conferring lipolytic activity on *Escherichia coli*. *Appl Environ Microbiol* **66**: 3113–3116.
- Kim BS, Kim SY, Park J, Park W, Hwang KY, Yoon YJ, Oh WK, Kim BY & Ahn JS (2007) Sequence-based screening for self-sufficient P450 monooxygenase from a metagenome library. *J Appl Microbiol* **102**: 1392–1400.
- Kowalchuk GA, Speksnijder AG, Zhang K, Goodman RM & van Veen JA (2007) Finding the needles in the metagenome haystack. *Microb Ecol* **53**: 475–485.

- Liu Y, Zhou J, Omelchenko MV *et al.* (2003) Transcriptome dynamics of *Deinococcus radiodurans* recovering from ionizing radiation. *Proc Natl Acad Sci USA* **100**: 4191–4196.
- Majernik A, Gottschalk G & Daniel R (2001) Screening of environmental DNA libraries for the presence of genes conferring Na(+)(Li(+))/H(+) antiporter activity on *Escherichia coli*: characterization of the recovered genes and the corresponding gene products. *J Bacteriol* **183**: 6645–6653.
- Park SJ, Kang CH & Rhee SK (2006) Characterization of the microbial diversity in a Korean solar saltern by 16S rRNA gene analysis. *J Microbiol Biotechnol* **16**: 1640–1645.
- Quaiser A, Ochsenreiter T, Klenk HP, Kletzin A, Treusch AH, Meurer G, Eck J, Sensen CW & Schleper C (2002) First insight into the genome of an uncultivated crenarchaeote from soil. *Environ Microbiol* **4**: 603–611.
- Rhee SK, Liu X, Wu L, Chong SC, Wan X & Zhou J (2004) Detection of genes involved in biodegradation and biotransformation in microbial communities by using 50-mer oligonucleotide microarrays. *Appl Environ Microbiol* **70**: 4303–4317.
- Richmond CS, Glasner JD, Mau R, Jin H & Blattner FR (1999) Genome-wide expression profiling in *Escherichia coli* K-12. *Nucleic Acids Res* **27**: 3821–3835.
- Roh C, Villatte F, Kim BG & Schmid RD (2007) Screening and purification for novel cytochrome b5 from uncultured environmental micro-organisms. *Lett Appl Microbiol* **44**: 475–480.
- Rondon MR, August PR, Bettermann AD *et al.* (2000) Cloning the soil metagenome: a strategy for accessing the genetic and functional diversity of uncultured microorganisms. *Appl Environ Microbiol* **66**: 2541–2547.
- Schmeisser C, Steele H & Streit WR (2007) Metagenomics, biotechnology with non-culturable microbes. *Appl Microbiol Biotechnol* **75**: 955–962.
- Sebat JL, Colwell FS & Crawford RL (2003) Metagenomic profiling: microarray analysis of an environmental genomic library. *Appl Environ Microbiol* **69**: 4927–4934.
- Treusch AH, Leininger S, Kletzin A, Schuster SC, Klenk HP & Schleper C (2005) Novel genes for nitrite reductase and Amo-related proteins indicate a role of uncultivated mesophilic crenarchaeota in nitrogen cycling. *Environ Microbiol* **7**: 1985–1995.
- van Hellemond EW, Janssen DB & Fraaije MW (2007) Discovery of a novel styrene monooxygenase originating from the metagenome. *Appl Environ Microbiol* **73**: 5832–5839.
- Verdnick D, Handran S & Pickett S (2002) *Key Considerations for Accurate Microarray Scanning and Image Analysis*. DNA Press, Salem, MA.
- Ward BB, Eveillard D, Kirshtein JD, Nelson JD, Voytek MA & Jackson GA (2007) Ammonia-oxidizing bacterial community composition in estuarine and oceanic environments assessed using a functional gene microarray. *Environ Microbiol* **9**: 2522–2538.
- Williamson LL, Borlee BR, Schloss PD, Guan C, Allen HK & Handelsman J (2005) Intracellular screen to identify metagenomic clones that induce or inhibit a quorum-sensing biosensor. *Appl Environ Microbiol* **71**: 6335–6344.
- Wu L, Thompson DK, Liu X, Fields MW, Bagwell CE, Tiedje JM & Zhou J (2004) Development and evaluation of microarray-based whole-genome hybridization for detection of microorganisms within the context of environmental applications. *Environ Sci Technol* **38**: 6775–6782.
- Ye RW, Tao W, Bedzyk L, Young T, Chen M & Li L (2000) Global gene expression profiles of *Bacillus subtilis* grown under anaerobic conditions. *J Bacteriol* **182**: 4458–4465.