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Short communication

Quantitative oligonucleotide microarray data analysis with an artificial standard probe strategy

Byeong Hee Hwang, Hyung Joon Cha *

Department of Chemical Engineering, Pohang University of Science and Technology, Pohang 790-784, Republic of Korea

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Abstract

Quantitative data analysis is an important element in several applications of DNA microarray, including mRNA expression profiling and estimation of infectious doses for pathogens. Here, we introduce an artificial standard probe strategy for quantitative pathogen detection using an oligonucleotide chip as a model system. The standard capture probe sequence was artificially designed to prevent non-specific hybridization with bacterial targets. Based on the fluorescence intensities of artificial standard spots, the raw fluorescence intensity data for specific spots could be corrected to generate linear correlations with target concentrations. Therefore, our novel artificial standard probe may be effectively applied for the correction of chip-to-chip variations and quantitative data analysis of a one-color labeled DNA microarray system.

Keywords: DNA chip; Oligonucleotide microarray; Quantitative data analysis; Artificial standard probe

1. Introduction

DNA microarray is a powerful tool in medical science and biotechnology (Heller, 2002). With this technology, a single short and simple experiment is capable of generating vast quantities of useful data, equivalent to information obtained from several thousand Southern blots. In particular, oligonucleotide microarrays can successfully detect up to a single nucleotide polymorphism. These attractive characteristics allow its use in several applications, including mRNA expression profiling and disease diagnosis (Guschin et al., 1997; Heller et al., 1997; Yershov et al., 1996).

Quantification of DNA microarray is currently an important issue, because several applications require quantitative, rather than qualitative analysis. One typical procedure that involves quantitative analysis is mRNA expression profiling, since the amount of transcribed mRNA is used as a comparison standard. To date, several strategies to facilitate accurate and reproducible quantitative data analysis of the DNA microarray system have been investigated. Two- and three-color fluorescence hybridizations were applied for relative quantification between control and experimental data (Hessner et al., 2003; Schena et al., 1995). Quantitative real-time polymerase chain reaction (RT-PCR) was performed separately to validate DNA microarray results (Qin et al., 2006; Rajeevan et al., 2001). Image analysis with statistics, such as normalization, is yet another approach aimed at improving the reliability of quantification (Olshen and Jain, 2002; Wang et al., 2001). For mRNA expression profiling, reference DNA molecules, such as lambda phage, and internal standard probes for correlation between two-color intensities were additionally introduced for accurate quantitative data analysis (Behr et al., 1999; Bredel et al., 2005; Cho and Tiedje, 2002).

In the present study, we utilized a novel artificial standard capture/target probe strategy. The artificial standard capture probe was designed to incorporate <85% similarity to prokaryotic (and even eukaryotic) sequences to avoid non-specific hybridization which previously reported methods might have. The artificial standard target probe was chemically synthesized and attached to a fluorescent tag to prevent participation in further labeling steps (no reaction bias). These characteristics might enable to be the artificial capture/target probe as a more precise standard that can establish a standard curve for quantification of data with a few experiments in the fluorescence-based DNA microarray system which has relatively high intensity variation due to easy fluorescence destruction by light exposure. Therefore, our

* Corresponding author. Tel.: +82 54 279 2280; fax: +82 54 279 2699.
E-mail address: hjcha@postech.ac.kr (H.J. Cha).

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artificial standard probe strategy might be used more precisely and generally for prokaryotic and eukaryotic DNA chip systems than previously reported standard probes. Even though this strategy needs additional artificial capture/target probes, format, and correction analysis, it enables to perform more efficient and cost-effective experiment by one-color labeling and one-step scanning than other reported quantitative methods. To examine the efficacy of our novel quantitative strategy, we used a pathogen detection oligonucleotide microarray as the model system based on 16S rDNA sequence information as the detection principle (Eom et al., 2007). In pathogen detection, quantification of the target pathogen is a desired outcome, since the infectious dose of pathogen that causes disease is a critical factor for pathogenesis.

2. Materials and methods

2.1. Design and synthesis of capture and model target probes

We used previously designed specific capture probes (Eom et al., 2007) based on the first variable region (positions 67–108 corresponding to *Escherichia coli* K12 numbering) of the 16S rDNA sequence from three selected target pathogens, *Salmonella typhimurium* (12529; Institute for Fermentation, Osaka, Japan), *Vibrio parahaemolyticus* (17802; American Type Culture Collection, Manassas, VA, USA), and *Vibrio vulnificus* (27562; ATCC), and a positive control capture probe (Eom et al., 2007) based on the conserved region (positions 15–34 corresponding to *E. coli* K12 numbering) of 16S rDNA (Eom et al., 2007) (Table 1). A standard capture probe (20 bp) was designed as an artificial sequence absent in bacterial databases to avoid non-specific hybridization with bacterial sequences (Table 1). The thermodynamic properties of these capture probes were calculated using Primer Premier 5 (Premier Biosoft International, Palo Alto, CA, USA). Capture probes were chemically synthesized with a 5′ amino linker modification to contain a 6-atom spacer composed of ethylene glycol units between the oligonucleotide and amine (MWG-Biotech, Ebersberg, Germany).

Three model targets for each pathogen, also designed previously using the first variable and nearby conserved regions (Eom et al., 2007) were chemically synthesized with the 5′ Cy5 fluorescent dye modification (MWG-Biotech) (Table 1). These targets hybridize with both the positive control and specific capture probes. An artificial standard target with a complementary sequence (Table 1) to the artificial standard capture probe was chemically synthesized with the 3′ Alexa Fluor 647 (Cy5 substitute) modification (Integrated DNA Technologies, Coralville, IA, USA).

2.2. Design of DNA microarray format

We designed a capture probe arrangement containing the repeated elements specified in Fig. 1A. Black, gray, and white spots represent the artificial standard, positive control, and specific capture probes, respectively. Four repeat spots of each specific capture probe were surrounded by 5 replicate spots of the artificial standard capture probe in a rectangular shape. Consequently, each oligonucleotide microarray contained 5 × 5 spots of the artificial standard capture probe, 1 × 4 spots of the positive control capture probe on the first line, and 1 × 4 spots of the each specific capture probe. The interval between spots within an array set was 400 μm, sufficient to minimize the cross-talk effect. The total dimension of the spotted area was 3.2 mm × 3.2 mm, and the whole array set was duplicated at intervals of 28.3 mm to permit two experiments on a single chip.

2.3. Preparation of DNA microarray

Each NH_2-modified oligonucleotide (20 μM) was dissolved in 3× SSC spotting buffer solution (450 mM NaCl, 3 mM tri-

Table 1
Oligonucleotide sequences employed as capture and target probes

No.	Capture probe	(5′-3′, 3′-Amine-spacer, spacer: C6)	Length (bp)	T_m[a] (°C)	Probe quality on rating[b]	16S rRNA accession no.
1	Artificial standard	CCCAAGGGAACCCAAGGGAAA	21	66.8	85	
2	Positive control	GCCGCCAGCGTTCAATCTGA	20	66.5	91	
3	*Salmonella typhimurium*	ACTCGTCAGCAAAGCAGCAAGC	22	64.5	100	Z49264, U90316
4	*Vibrio parahaemolyticus*	CGTTATCGTTCCCCGAAGTTCAGAT	25	66.9	93	X56580
5	*Vibrio vulnificus*	AAACAAGTTTCTCTGTGCTGCCGC	24	66.9	91	X76333

No.	Target probe	Sequence (5′ → 3′)	Length (bp)
6	Artificial standard target	TTTCCCTTGGGTTCCCTTGGG-Alexa fluor 647	21
7	*S. typhimurium*	Cy 5-TGGCTCAGATTGAACGCTGGCGGCAGGCCTAACA CATGCAAGTCGAACG-GTAACAGGAAGCAGCTTGCTGCTTCGCTGACGAGTGGCGGACGGGTGAGTAA	101
8	*V. parahaemolyticus*	Cy 5-TGGCTCAGATTGAACGCTGGCGGCAGGCCTAACA CATGCAAGTCGAGCGGAAAC-GAGTTATCTGAACTTCGGGGAACGATAACGGCGTCGAGCGGCGGACGGGTGAGTAA	110
9	*V. vulnificus*	Cy 5-TGGCTCAGATTGAACGCTGGCGGCAGGCCTAACA CATGCAAGTCGAGCGGCAGCACA-GAGAAACTTGTTTCTCGGGTGGCGAGCGGCGGACGGGTGAGTAA	101

[a] Calculated by Primer Premier 5.
[b] High rating of probe quality means low stability of hairpin, dimmer, false priming, and cross-dimmer calculated by Primer Premier 5.