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Molecular and Cellular Probes 20 (2006) 60–63

Molecular and Cellular Probes

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Optimized DNA microarray assay allows detection and genotyping of single PCR-amplifiable target copies

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Accepted for publication 20 September 2005
Available online 5 December 2005

Abstract

This study was conducted to determine the detection limit of an optimized DNA microarray assay for detection and species identification of chlamydiae. Examination of dilution series of a plasmid standard carrying the target sequence from *Chlamydia trachomatis* and genomic DNA of this organism revealed that a single PCR-amplifiable target copy was sufficient to obtain a specific hybridization pattern. This performance renders the test suitable for routine testing of clinical samples.

Keywords: DNA microarray; Sensitivity; Detection; Chlamydia trachomatis

1. Introduction

While DNA microarray technology has been widely used in gene expression monitoring, genotyping has emerged as another area of application in the last few years. The highly parallel approach, i.e. the possibility to obtain precise sequence information on a variety of genomic loci renders DNA microarrays promising diagnostic tools. Given the complex nature of many bacterial virulence factors, the current PCR-based methods are not capable to fulfill the criteria required for highly informative diagnostic tests in the future. Multi-locus genotyping assays or even genomotyping [1] will supplant the 'one-dimensional' typing methods used nowadays.

Recent applications of DNA microarrays in genotyping include detection of antibiotic resistance genes in gram-positive bacteria [2,3], toxin typing of *Clostridium perfringens* [4], species differentiation among mixed bacterial communities [5], and identification of respiratory pathogens [6]. However, the suitability of DNA microarray assays for routine diagnosis has yet to be demonstrated as most studies have been dealing with bacterial cultures rather than direct examination of clinical specimens. To achieve this goal, any such test should be easy-to-handle and cost efficient, as well as highly sensitive and specific.

The recently developed ArrayTube™ (AT) platform represents an interesting alternative to the widely used, but relatively expensive fluorescence-based glass slide microarray systems. It involves chips of 2.4×2.4 mm size placed on the bottom of 1.5-ml plastic micro-reaction tubes. Hybridization can be conducted on standard laboratory equipment without changing vessels. In a previous paper, we described the development of an AT microarray to differentiate among all nine species of *Chlamydia* (*C.*) and *Chlamydophila* [7]. In the present study, an optimized protocol of this assay was examined to determine detection limits and identify factors limiting sensitivity.

2. Materials and methods

2.1. DNA microarray

The present version of the microarray includes 28 probes for species identification, three genus-specific probes, five probes for the closest relatives, i.e. *Simkania negevensis* and *Waddlia chondrophila*, as well as four positive controls (consensus probes), and one internal staining control (biotin marker). Each probe was spotted fivefold, yielding a total of 289 spots (Print pattern and probe identities, see Supplement 1; Barplot demonstrating specificity and discriminatory power, see Supplement 2).

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doi:10.1016/j.mcp.2005.09.003

2.2. DNA templates

Recombinant plasmid pCR2.1-TOPO + DC38 (map see Supplement 3) served as a model target and was used from a stock solution containing 2.11×10^{10} copies per microlitre. It was prepared by cloning a 1086-bp insert comprising the 3′-domain of the 16S rRNA gene, the intergenic spacer and domain I of the 23S rRNA gene of *C. trachomatis*, into vector pCR2.1-TOPO (invitrogen, Karlsruhe, Germany). The insert also contains the primer binding sites for biotinylation PCR and real-time PCR. Based on a DNA concentration of 0.111 μg/ml as measured in triplicate from UV absorption and a molecular mass of 3.11×10^6 g/mol, the present preparation was calculated to contain 2.11×10^{10} plasmid copies per microlitre.

Chromosomal DNA of purified elementary bodies of *C. trachomatis* strain D was prepared from 100 ml of infected cell culture in BGM cells containing 6.38×10^9 inclusion-forming units (ifu)/ml using standard methodology [8]. Based on a DNA concentration of 600 μg/ml as measured from UV absorption and a molecular mass of 6.42×10^8 g/mol for the *C. trachomatis* genome, the present preparation was calculated to contain 5.64×10^8 genome copies per microlitre. The proportion of residual mammalian DNA from host cells was lower than 0.5% as determined by ß-actin real-time PCR.

2.3. Amplification, labeling and quantitation of DNA templates used for the microarray test

Target DNA was amplified and biotin labeled for the AT microarray assay in 40 cycles of 94 °C/30 s, 55 °C/30 s, and 72 °C/30 s, using primers U23F-19 (5′-ATTGAMAGGC-GAWGAAGGA-3′) and 23R-22 (5′-biotin-GCYTACTAA-GATGTTTCAGTTC-3′). Hybridization was conducted as described previously [7]. Hybridizing spots were visualized using 3,3′5,5′-tetramethyl benzidine (TMB) as substrate for streptavidine-conjugated horseradish peroxidase. Hybridization signals were processed using the Iconoclust version 2.3 software (Clondiag, Jena, Germany).

Real-time PCR was conducted on a Mx 3000 (Stratagene, La Jolla, CA) using a modified version of the procedure of Everett et al. [9], which included primers Ch23S-F (5′-CTGAAACCAGTAGCTTATAAGCGGT-3′), Ch23S-R (5′-ACCTCGCCGTTTAACTTAACTCC-3′), and probe Ch23S-p (FAM-CTCATCATGCAAAAGGCACGCCG-TAMRA). Each dilution series was examined in triplicate by each test.

3. Results and discussion

To evaluate the sensitivity of the microarray assay, we examined decimal dilution series of recombinant plasmid pCR2.1-TOPO + DC38. Fig. 1 illustrates that a single copy was sufficient to obtain a species-specific hybridization pattern on the microarray after PCR amplification. When chromosomal DNA of *C. trachomatis* was tested in an analogous trial, the detection limit was near 0.05 fg of DNA, which is equivalent to 56 genomic copies or 1.87 ifu (see Supplement 4). This prompted us to examine three different templates by quantitative real-time PCR (Fig. 2). The fact that chromosomal DNA was detected with lower sensitivity than

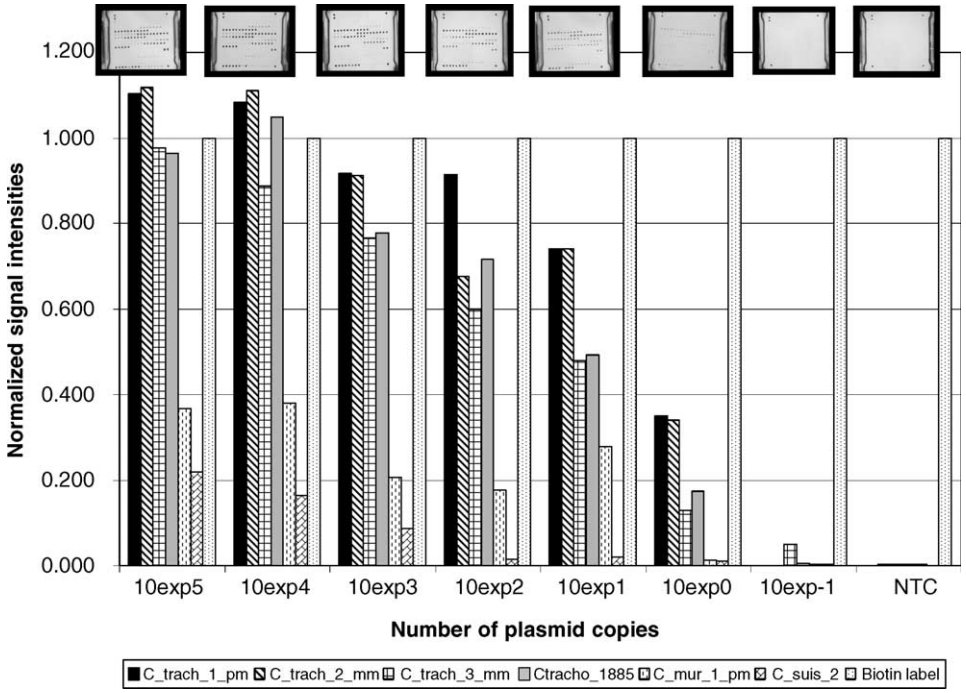

Fig. 1. Examination of a dilution series of recombinant plasmid pCR2.1-TOPO + DC38 using the AT microarray assay. Upper line: images of microarray hybridization patterns obtained with plasmid copy numbers indicated on the abscissa. Diagram shows normalized signal intensities of *C. trachomatis* probes (pm perfect match; mm single mismatch) and its closest relatives, *C. suis* and *C. muridarum*, for comparison. Each array included an arbitrary biotinylated 26-mer oligonucleotide probe as internal staining control and four consensus probes representing genomic sequences conserved in all chlamydial species (hybridization controls).