Compact Disc with Both Numeric and Genomic Information as DNA Microarray Platform

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ABSTRACT

The compact disc (CD) is an ideal tool for reading, writing, and storing numeric information. It was used in this work as a support for constructing DNA microarrays suited for genomic analysis.

The CD was divided into two functional areas: the external ring of the CD was used for multiparametric DNA analysis on arrays, and the inner portion was used for storing numeric information. Because polycarbonate and CD resins autofluoresce, a colorimetric method for DNA microarray detection was used that is well adapted for the fast detection necessary when using a CD reader. A double-sided CD reader was developed for the simultaneous analysis of both array and numeric data. The numeric data are engraved as pits in the CD tracks and result in the succession of 0/1, which results from the modulation of the laser reflection when one reads the edges of the pits. Another diffraction-based laser was placed above the CD for the detection of the DNA targets on the microarrays. Both readers fit easily in a PC tower. Both numeric and genomic information data were simultaneously acquired, and each array was reconstituted, analyzed, and processed for quantification by the appropriate software.

INTRODUCTION

Miniaturization has been a main target of the electronic industry and is now invading molecular biology through the technology of microarrays or biochips (3–6). The principle of DNA microarray analysis was known for many years as reverse dot blot analysis, which was first performed on large nitrocellulose or nylon membranes. Efforts in miniaturization were associated with new supports. The electronic chips were first proposed for DNA binding using electric-based targeting (9). The possibility of in silico synthesis of nucleotide sequences combined with photolithography provided surfaces with several thousand different small oligonucleotide capture probes (11,12). Glasses were also activated by introducing aldehyde groups for the covalent binding of aminated DNA capture probes. The miniaturization was obtained for this method through the use of robotic precise automatic spotting (13). In all cases, the hybridized DNA is detected by incorporating fluorescent labels either directly during the copy of target sequences to be analyzed or by a second labeling step with fluorescent streptavidin or antibodies (7,9). The search for electronic-based detection methods (10,11) continues, but the proposed solutions are now becoming of practical use (14).

The search for how to use polymers such as polycarbonate for supporting DNA microarrays was hampered mainly because of their autofluorescence. However, a new colorimetric method has been proposed, based on the deposit of silver precipitation at the DNA location (1,10). This new method allows plastic polymers to be tested as support for the DNA arrays. Although the three-log dynamic range of the colorimetric detection method is lower than the four-log range of the Cy3 fluorescence method, the silver precipitation colorimetric method is as sensitive as the Cy3 fluorescent detection of DNA when it is performed on glass slides (1).

Here we used compact disc (CD) as a support for constructing DNA microarrays. The goal of the project was to use a common platform for storing numeric and genomic data, both being read by one or two reading devices inserted into a PC tower. The first attempt (data not shown) was to take part of the CD technology developed for reading and engraving the numeric information, using the laser reflection process present in a normal CD reader. Indeed, we succeeded in transferring the DNA hybridization site onto the site of numeric information and read it with a normal CD reader, but the solution was impractical because of the constraint of working in a clean atmosphere. We then decided to separate physically on the CD the location of the encoded numeric information from the DNA binding location. This solution gave the best results and is described in this paper.
MATERIALS AND METHODS

Bio-CD

The commercially available CD is a 1.2-mm-thick polycarbonate disc that carries on its upper side one track running from the internal to the external part of the disc. The track is composed of pits that are 1–4 μm long, 0.15 μm deep, and 0.5 μm wide (2). This upper surface is then covered by a reflective aluminium or gold layer that is then protected from oxidation by a varnish layer.

The pits give binary information in a simple way: a laser beam is focused on the surface of the pits and, when the beam is reflected by a flat surface, gives a 0 value. However, when the reflection meets the edge of a pit, it decreases below a threshold value and this deflection is counted as a 1 value. The succession of the 1/0 numeric signals are then converted into data of different kinds like bytes for computers, some of them necessary for the CD reader to recognize the CD, adjust the speed of rotation, and control the position of the head compared to the CD. The reader follows the track as a continuous spiral on the CD with an elaborate servo tracker (part of the CD reader) that corrects for both lateral and vertical variations.

To avoid interference between the gene-based signal and the numeric reading that also provides the track and controls the speed, we separated the two signals laterally on the CD and detected them separately with two laser-reading devices. For the lateral separation, the CDs were engraved with a numeric information band of around 1 cm located on the inner part of the CD and covered with an aluminium layer restricted to this location. The outer part was a transparent polycarbonate band coated with a DNA fixation layer. This specially designed CD for microarray technology is called the Bio-CD (Figure 1).

Bio-CD for Staphylococcus Detection

The DNA spotting, PCR amplification, and hybridization were performed as described previously (8). In brief, the femA gene of the various Staphylococci are amplified by a consensus set of primers and then detected on CD microarrays that bear the capture probes specific for the femA of the different Staphylococci species. Array spotting on the CD was performed on an arrayer with a plate that could support 12 CDs (Figure 2).

The spots were 300 μm in diameter, and the DNA bound to the CD through the use of a specific fixation layer coated on the CD (UCB, Drogenbos, Belgium).

Colorimetric Silver Labeling

After hybridization, the CD is washed four times for 1 min with 10 mM maleate buffer containing 15 mM NaCl and 0.1% Tween® 20, pH 7.5. The CD is incubated with a solution of streptavidin-colloidal gold conjugate and then with the Silver Blue solution (AAT, Namur, Belgium) as described earlier (1). The results are digitized and quantified with software that is included in the workstation (AAT).

Bio-CD Reader

A commercially available CD reader (Creative Laboratories, Singapore, Singapore) was used to read the numeric information written onto the CD. A second laser-based reader was attached on the upper part of the CD reader. The reader consists of a laser diode module that illuminates a 50-μm spot on the surface of the CD with a wavelength of 670 nm (Figure 3). The diffracted light is detected by a photodiode, and the data are digitized by an acquisition card (National Instruments, Austin, TX, USA).

The head of the detector moves following a stepping motor-driven radial displacement with a speed of 20 mm/min while the CD is turning. The overall CD surface devoted to the DNA analysis is a 15-mm-wide external band. This surface is scanned in less than 1 min, and the overall digitized data represent 6 MB of information. The data from each array are retreated to recreate the picture of each array present on the CD. Each array is stored in a separate file. Image analysis is then processed by the evaluation of the average gray level of the pixels of each spot, minus the average gray level of pixels surrounding the spot. The means of quadruplicates are then calculated ± SD.

Figure 1. View of a CD used for microarray detection. The center contains numeric information covered by an aluminium layer. The outer part of the CD is covered by 15 hybridization chambers in which a microarray has been spotted.

Figure 2. Axial arrayer developed for the transfer of capture probes present in solution from the multi-well plate onto the surface of the CD. The arm of the robot is a 9-based movement, and the disc is fixed on a rotating platform that has 12 CDs.
RESULTS

The optimization of the conditions for silver precipitation and reading with the double CD reader were first performed on biotinylated probes fixed on the CD. Once the detection was optimized, the CD platform was tested using the DNA hybridization assay.

A normal CD reader was used to read the numeric information on the inner part of the CD. This commercial CD reader also controlled the CD rotation speed during the analysis. The analysis of the outer part of the CD carrying the genomic results was performed by a second laser-based reader (Figure 3), which scans the outer part of the CD and measures the scattered light caused by silver precipitates that result from positive hybridization.

The signal was digitized by a PC acquisition card and stored as files, each one corresponding to one array. The data acquisition was then followed by the use of specific data analysis and data mining software.

The CD detection method was first tested for the detection of Staphylococci strains as already developed on glass (8). The principle of the method is to amplify a part of the femA gene that is present in all Staphylococcus species using consensus primers and to detect them by hybridization on capture probes specific for each of the species. This array detects the five most common Staphylococci species. The array also contained a consensus capture probe for the genus Staphylococcus identification and a capture probe for the mecA gene. The mecA gene is associated with the methicillin resistance of the Staphylococcus. The image of each array is reconstituted, and the spot analysis was processed as followed. The program first identifies the spots and corrects for the deformation of the rectangular shape of the array given the circular reading on the CD. The average intensity of each spot inside its boundary is then calculated. The mean value of the background around each spot is subtracted from the spot values. The means of the replicates are then calculated with two standard deviations, and values are assigned to the sequence of a specific bacteria.

A first comparative assay for the detection of the femA and mecA sequence from methicillin-resistant S. epidermidis on glass slide and on CD is presented in Figure 4. The positive signals are in dark on the glass, resulting from light absorption of the illuminated glass, while they appear as bright signals on the CD because of the laser diffraction detected by the photodiode. Besides this difference, both patterns of hybridization were similar with the S. epidermidis, the consensus, and the mecA spots being positive on both arrays.

The experiment was then extended with a single CD spotted with 10 arrays. Hybridization chambers were stuck around each array. FemA and mecA sequences from nine Staphylococci species were then amplified by a duplex PCR using the consensus primers and products hybridized on the arrays. A negative PCR control was also added. After silver precipitation, the arrays were analyzed in the double CD reader, which was inserted into a computer (Figure 5). These reconstituted data were processed, and the quantification of these 10 arrays obtained after image analysis is shown (Figure 6).

All the detections were specific for their respective products. The consensus femA capture probe and the mecA were positive for all the samples, while the specific capture probes only detect their respective products, such as S. aureus, S. epidermidis, S. haemolyticus, S. hominis, and S. saprophyticus. The quantitative data confirmed such conclusions because the signal-to-noise ratio was above 50 for all positive signals.

DISCUSSION

The CD is probably one of the most commonly used data storage support in our daily lives. Here we demonstrated that a CD platform could be adapted for performing DNA analysis while keeping its powerful storage of numeric information. Coupling both genomic and numeric information was achieved through the use of the same physical CD support.
The inner part of the CD contains all the registered information needed for performing the CD reading and controlling the CD reader. This information also provides the location and identification of the various arrays and the DNA capture probes present on the CD. In addition, it can include the quantification program necessary for the data management. Recordable area is yet another possible alternative for writing and storing the biological results of the microarrays on the CD.

One of the main advantages of the CD is its very large surface, which can afford many different arrays or a few very large ones. Figure 1 shows a CD that has a working surface of 74.5 cm² for biological analysis. Using spots of 0.2 mm every 0.4 mm, it would be possible to detect 45,000 sequences on one CD. Both the CD and the CD reader inserted in the computer constitute low-cost technologies.

The fast scanning of the overall CD is another advantage of the system because it can be done within 1 min and data processing is performed automatically by the software, thus making the reading step easy for the user.

In combination with electronic and glass supports, we propose the CD as an alternative platform for making and detecting arrays that are well suited for the routine and multi-sample analysis required in diagnostic DNA and research applications.

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Figure 5. Photograph of the double-sided CD detector. It is composed of a normal laser CD reader to read the numeric information of the CD and a second laser head to detect the presence of positive DNA hybridization onto the microarray of the Bio-CD.

Figure 6. Results of the hybridization of nine duplex PCR products made on nine plasmids containing fem A from nine different Staphylococcus species in the presence of a second plasmid containing the mec A gene. The assay also contains one negative PCR control. The hybridizations were made on 10 microarrays present on the same disc platform. Detection is performed with silver precipitation technology. The nine species are S. aureus, S. epidermidis, S. gallineri, S. hominis, S. saprophyticus, S. schleiferi, S. sciuri, S. simulans, and S. xylosus. Five microliters of each PCR product were first hybridized and washed, incubated with a solution of streptavidin-gold for 45 min, then washed again and incubated with Silver Blue solution for 10 min (1). The results show the specificity of the detection even between homologous sequences and the simultaneous detection of several samples in one incubation and reading run. The values are the x ± so of gray level for four spots minus the gray level of the background.