2.5-μL portion of the sample processed on the cartridge system or QIAamp Kit was subjected to PCR to amplify the TH01 locus. Agarose gel electrophoresis results (Fig. 1B) indicated that the relative yield of PCR products obtained from samples prepared using the two methods were similar.

Allelic typing was performed by adding TH01 allelic ladder (Promega) to the PCR products generated from the WBC lysates and subjecting the mixtures to CE analysis (Fig. 1C). The ladder ranged in size from 179 to 203 bp and consisted of the alleles designated 5, 6, 7, 8, 9, 9.3, 10, and 11. The sharp, distinct TH01 allelic peaks observed on the electropherograms indicated that robust PCR amplification and CE separation analysis were achieved on the blood samples processed using the cartridge system. The PCR products exhibited a CE migration time of ~13 min. The total time for processing, amplifying, and analyzing the blood sample was 84 min.

In summary, a cartridge system was used to process a 200-μL sample of whole blood in 9 min for genetic testing. This flow-through sample preparation procedure concentrated, extracted, and lysed WBCs from the blood without requiring vortex-mixing, precipitation, and centrifugation. The quantity and quality of DNA from the WBC lysate automatically prepared on the cartridge were sufficient for PCR and CE analysis of the TH01 STR locus, and the cartridge procedure was at least three times faster than the QIAamp Kit.

References

Fast and Specific Hybridization Using Flow-Through Microarrays on Porous Metal Oxide, Rinie van Beuningen,* Henk van Damme, Piet Boender, Niek Bastiaensen, Alan Chan, and Tim Kievits (PamGene B.V., Burgemeester Loeffplein 70a, 5211RX Den Bosch, The Netherlands; * author for correspondence: fax 31-73-615-8081, e-mail RvBeuningen@PamGene.com)

The advent of sequencing technologies and efforts in sequencing and analysis of polymorphic regions in various viruses, bacteria, and higher organisms, including humans, has led to a wealth of genetic information (1, 2). This information is used to relate genetic information to phenotypic effects, which is used to provide better tools in drug development and a better understanding of the biologic pathways involved in various rare as well as common diseases in humans. A tool in this type of analysis is parallel testing on the basis of microarrays. These arrays, sometimes referred to as “DNA-chips”, usually consist of a flat surface with capture probes at specific positions (spots) directed toward the various targets that may be present in the sample.

The use of microarrays for genomic-based screening and the search for new genes has been well documented by scientific groups using systems (e.g., from Affymetrix and Sequenom) that have integrated many novel, microscale technologic developments (3, 4). The ability to either deposit directly, or synthesize in situ, hundreds or thousands of oligonucleotides on glass surfaces in sub-nanoliter volumes at high density allows for high-throughput simultaneous detection.

These first-generation microarrays, characterized by passive hybridization between targets and probes, are typically performed on planar surfaces. The fundamental problem caused by the concept of working on a basic two-dimensional surface has led many research groups to develop second-generation microarrays that seek to enhance the performance of this platform. The aim is more toward low costs, reproducibility of hybridization signals, and speed. The technologies used by these microarrays have properties beyond simple passive hybridization, such as microfabricated fluidic channels, electronic hybridization, novel posthybridization signaling steps, or flow-through dynamics (5–7).

The rate-limiting step in this planar format is the diffusion of the sample molecules (the target) toward the attached probe during hybridization. Because diffusion is slow, incubation usually takes place overnight. To bypass this limitation, we have used a porous aluminum oxide substrate as solid support. The substrate, with a thickness of 60 μm, has long branched capillaries, which are interconnected inside the substrate (8). The diameter of the
individual pores is ~200 nm. The interstitial volume inside the material is only 30 nL/mm². Compared with a flat two-dimensional surface, the reactive surface in this material is increased 500-fold. In addition, the permeable nature of the microarray facilitates the pressurized movement of fluid, such as the sample solution, through its structure. In contrast to flat, solid-surface arrays, the flow-through microarray substantially reduces hybridization times and increases signal and signal-to-noise ratios.

The flow-through microarrays can be prepared with, for example, an array of oligonucleotides. We used a noncontact inkjet-spotting technique to deposit and covalently link an array of oligonucleotides onto the substrate. The inkjet technology enables accurate spotting of 325 pL in single droplets with a distance of 0.2 mm between the centers of each spot (9).

To show hybridization stringency and speed of this new three-dimensional porous substrate, we prepared an 18-oligonucleotide array that contains several single and multiple mismatches. A fluorescently labeled oligonucleotide was used as a sample and incubated at various temperatures while the solution was pumped, twice per minute, through the substrate; the amount of bound target fluorescent oligonucleotide after each pumping step was detected by a charge-coupled device (CCD)-equipped epifluorescence microscope.

The flow-through microarray was made of activated 1 × 4 cm aluminum oxide substrate (Whatman). This substrate was placed between two sheets of pressure-sensitive adhesive foil (2.5 × 7.6 cm), each containing four aligned holes 4 mm in diameter on a pitch distance of 9 mm. These openings, called arrays, enable access to the aluminum oxide substrate. A set of 21mer oligonucleotides (Isogen) was synthesized (Table 1) and spotted in 325-pL droplets, containing 0.1 mmol/L of the oligonucleotide mixture, on the arrays with a Packard BioChip Arrayer (Meriden). The foil containing the oligonucleotide arrays was used in a reusable anodized aluminum holder (PamGene) that had four wells that matched the arrays on the foil. A water bath was coupled to an inlet and an outlet on the holder to control the reaction temperature. O-rings were used in the aluminum holder to eliminate crossover between each of the arrays. A syringe pump (Hamilton) was attached to the wells of the aluminum holder. This allowed increased or decreased pressure to be applied to the arrays to pump the sample solution twice per minute across the substrate at a flow rate of 25 μL/15 s.

A 25-μL sample containing 10 nmol/L 5'-fluorescein-labeled oligonucleotide (Isogen) in phosphate-buffered saline (NPBI) was added to a well of the aluminum holder and pumped twice per minute across the substrate.

After each pumping step, the fluorescence was recorded by an epifluorescence microscope (Olympus) and digitized with a CCD camera (Sony). The image information was converted into spot intensity values by a MatLab software image analysis package (PamGene).

The limit of detection of the epifluorescence CCD microscope system was ~5 × 10⁶ fluorescent molecules per spot with a two-log dynamic range when an 8-bit CCD camera was used.

The fluorescent oligonucleotide target was rapidly bound inside the interconnected pores of the substrate by the applied pressure difference of 10 000 Pa, which allows the sample to pass through the substrate twice per minute (Fig. 1A).

The hybridization results after 0, 1, 2, 9, 16, 31, 38, 44, 50, and 55 min are shown in Fig. 1A. The signal increased from 0 to 31 min. The specificity of the binding was determined by changing the hybridization stringency at Table 1. Sequences of the 21mer oligonucleotides.a

| Probe | T | T | G | T | A | C | A | G | A | A | A | T | G | G | A | A | A | G | A | Mismatches |
| A     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 0 |
| B     |   |   |   |   | G |   |   |   |   |   |   | G |   |   |   |   |   |   |   |   |   | 2 |
| C     |   |   |   |   | A |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 2 |
| D, neg. control |   |   |   | C |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 1 |
| E     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 1 |
| F, Fl. control |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 1 |
| G     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 1 |
| H     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 1 |
| I     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 1 |
| J     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 1 |
| K     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 1 |
| L     |   | C | A | T | G |   | C |   | G | A |   | A |   |   |   |   |   |   |   |   |   | 11 |
| M     |   | A | T | A |   |   | G | A | C | T |   | C |   | G | A | C | T |   |   |   |   | 13 |
| N     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 3 |
| O     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 1 |
| P     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 1 |
| Q     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 2 |
| R     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 1 |

a Both D, the negative control, and F, the fluorescence-positive control, are sequences unrelated to the target. The positive control is used to focus the fluorescence microscope.
38, 44, 50, and 55 min. This was achieved by adjusting the temperatures from 25 °C, thus increasing the hybridization stringency to 48, 54, 60, and 63 °C, respectively. The end result was a specific hybridization with a single specific spot on the array (Fig. 1A).

A comparison of the signal intensity values of the perfectly matched spot A compared with spots B–R is shown in Fig. 1B. Increasing the temperature to 48 °C and higher increased the specificity and therefore the signal difference between the perfect match and single or multiple mismatches. At 63 °C, the signal of the perfect match was discriminated from all other single mismatches by a factor of four.

The flow-through aluminum oxide matrix with its interconnecting pores offers many features that are not found on existing silica or membranous microarrays. This three-dimensional structure enables real-time hybridization kinetics, which until recently were associated only with platforms such as spectrophotometry or surface plasmon resonance (Biacore). This technology is scalable and enables parallel analysis of many hundreds of samples on many hundreds of different nucleic acid sequences while using a temperature profile to ensure the highest specificity. This second-generation microarray format will address part of the challenge to provide tools for clinical diagnostics and life science research.

### References