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# Electronic microarray for DNA analysis

**Review Article**

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**Abbreviations: AC**, alternate current; DC, direct current; **STRs**, Short Tandem Repeats; **SNPs**, single nucleotide polymorphisms; **CODIS**, Combined DNA Information System.

**Key words**: microarray, electronic hybridization, short tandem repeat, single nucleotide polymorphism, forensics

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## Summary

**Nanogen has developed a microelectronic technology that allows fast and accurate transport and hybridization of DNA on a semiconductor microchip. Based on this technology, we have developed electronic assays for genetic analysis of single nucleotide polymorphisms in several different applications. We have also developed electronic hybridization based short tandem repeats analysis for genetic identification and forensics. Our data show that these assays work in multiple formats under different electronic conditions. A beta instrument is capable of performing these tests on 100-site arrays in a semi-automated fashion. We are also working towards developing an integrated system, which could potentially become a portable device.**

## I. Introduction

Recently there has been tremendous interest in microarray technologies ( for reviews, see Nature Genet. Supplement, Vol 12, 1999). The combination of microfabrication, chemistry and molecular biology have allowed the generation of microarrays that permit rapid and miniaturized multiplex analysis of DNA samples. These microarray technologies are also good platforms for applications in drug discovery and genomics. Most microarray technologies require successful integration of miniaturization, array format, microfabrication and highly sensitive detection system to make genetic analysis on a chip possible. They mostly rely on passive hybridization or diffusion of large biological molecules such as nucleic acid or proteins. Nanogen has developed an electronic microchip technology that allows fast and accurate delivery of electronically charged biological molecules to test sites (electrodes) on microarrays followed by hybridization and detection. The technology (**i**) allows significant acceleration of molecular binding, therefore speeding up reaction time; (**ii**) affords multiplexing and simultaneous analysis of multiple test results from single sample; (**iii**) has an open architecture design, which allows the microarrays to be customized easily and quickly by end users; (**iv**) has high accuracy because it can give end users the ability to precisely manipulate molecular movement electronically and to perform automatic stringency and quality checks; (**v**) has a very wide applicability to charged molecules including DNA; and (**vi**) has broad applicability in biomedical research, medical diagnostics, genomics, and genetic testing and drug discovery.

Nanogen's microchips are made by standard semiconductor processing techniques (Mahajan, 1993). The microchips are 1cm by 1cm in size with 25-site, 100-site or 400-site arrays (Sosnowski et al, 1997). The test sites are clustered in the center of the array in a 5 x 5, 10 x 10 and 20 x 20 fashion (**Fig. 1**). A ring of counter electrodes surrounds them. A platinum wire connects each individual site and their respective counter electrode in the 25 or 100 pad chips. This allows the microinsulation of these sites while in the same time provides equal access to the molecules in an overlaying electrolyte solution. On top of the array is an agarose based permeation layer, which provides the attachment chemistry for anchoring probes as well as an interface between the electrodes and the solution. This interface allows ion flow while retarding penetration of target nucleic acid. It also acts as a buffer zone for DNA from damaging electrochemical reactions on top of the active electrode.

In the electronic DNA assays, probe DNA is loaded to the desired site(s) by electronically activating that electrode(s) with a postive charge and the counter electrodes

with a negative charge (**Fig.2**). The electric field strength generated by this configuration interrogates the solution above the test array and drives the negatively charged DNA molecules to migrate and concentrate over the positively biased sites. Because of the streptavidin attachment chemistry in the agarose permeation layer, we are able to electronically address and anchor our biotinylated capture probes to individual or multiple pads on the array. Subsequently, the target DNA is addressed to these pads electronically in a similar fashion. Our electronic format not only allows fast transport and concentration of target sample on individual test sites, but also affords rapid hybridization of the target DNA to capture probes anchored *in situ*. Conversely, the electronic polarity can be reversed so that the charge on the test site electrodes is negative and counter electrodes positive. This repulses DNA from the test sites (Sosnowski et al, 1997; Gilles et al, 1999). The resulting electronic washing allows the removal of nonbound target DNA, nonspecific DNA and partially hybridized target DNA respectively with increasing electronic field strength.

## II. pH Generation and the zwitterionic buffer system

Our buffer system supports rapid electrode specific transport and concentration of DNA molecules while facilitating accelerated hybridization (Edman et al, 1997). In order to transport nucleic acid efficiently by free solution electrophoresis, low conductivity buffers are preferred. We have utilized zwitterions which have pIs near neutral pH, where they have little net charge. In the case of histidine, conductivity is less than 100uS/cm. This is three magnitudes less than the buffers commonly used in molecular biology electrophoresis (e.g. Tris-Borate). However, at their pI, zwitterions do not permit hybridization under passive conditions because they are unable to support optimal shielding of charges contributed by the nucleic acid phosphodiester backbone. Buffers that support optimal hybridization should have titratable substituents with pKa values at or near pH 7.0. This requirement is particularly important for our electronic system because acid is generated at the test site electrode by electrolysis during DNA transport. In the absence of buffer, a current of 200nA causes a dramatic drop in pH (Edman et al, 1997). We found that some low ionic buffers containing an imidazole ring, such as histidine and imidazole, could maintain a pH above 5 in the region above the anode during electrolysis. The imidazole ring could serve as the primary source of buffering within the pH range. Furthermore, the imidazole ring can be protonated near neutral pH. This provides cations that shield repulsion between the negatively charged phosphodiester backbone of the two DNA strands during hybridization.

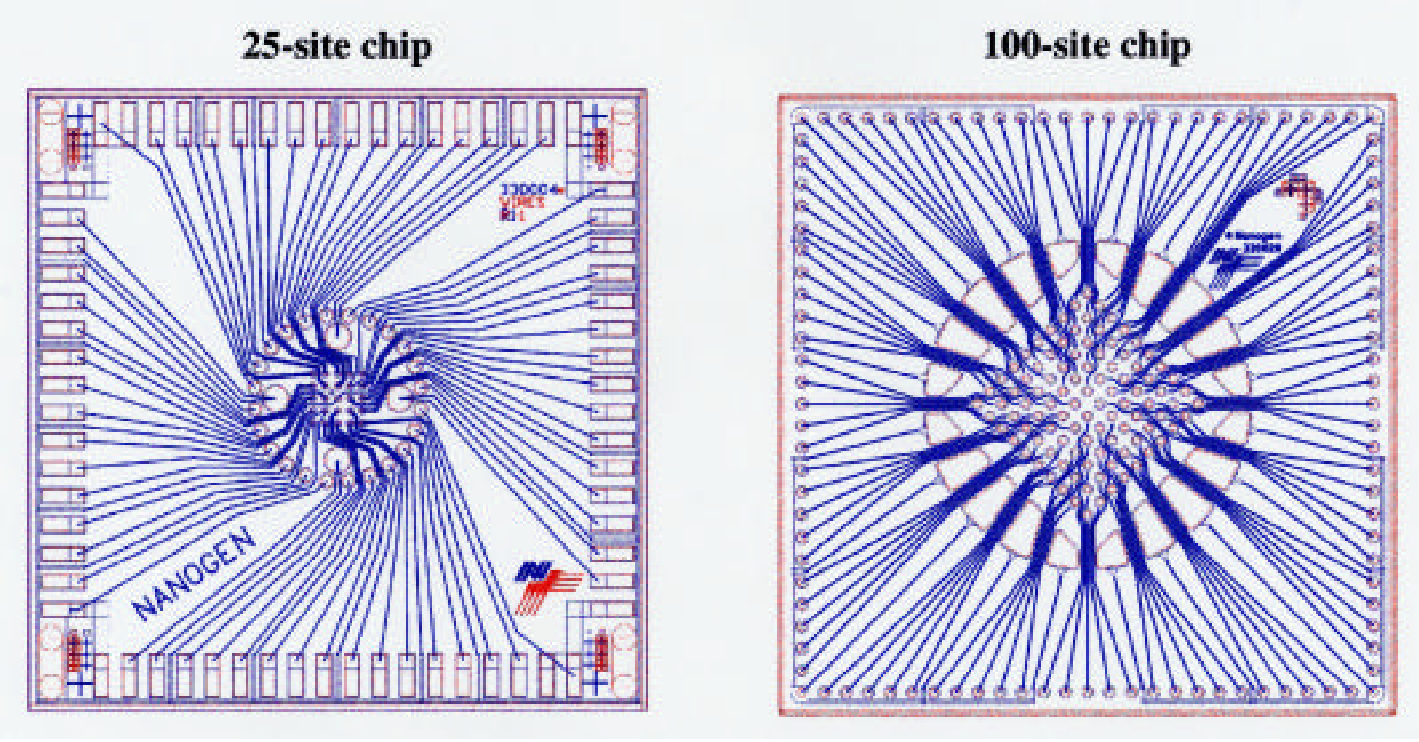


**Figure 1:** Layout for the 25-site and 100-site chips. The array (5 x 5 or 10 x 10) is in the center of the microchip. The ring pads surrounding the array are used as counter electrodes. Contact pads are on the edge of the chip, which are used for contacting with pogo pins that apply electronics to the microchip. For each of the test sites and ring pads, there is a platinum wire connecting it to its respective contact pad. There are two wires for each test site on the 25-site chips.

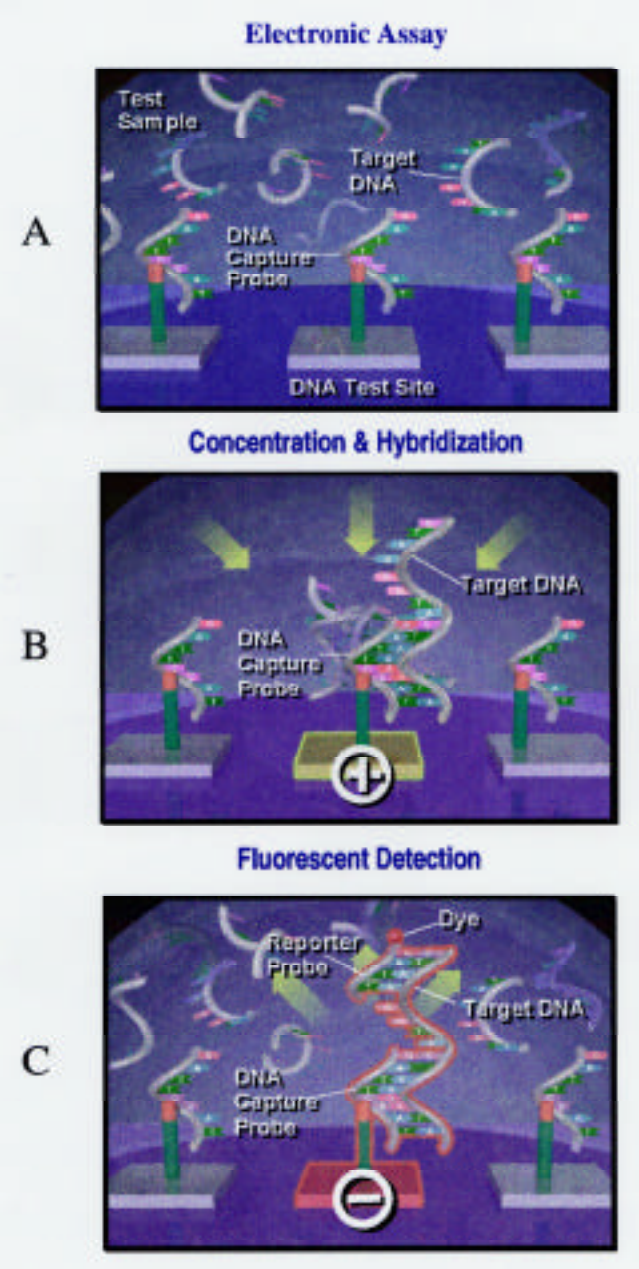


**Figure 2:** (**A**) Capture oligos are biotinylated at the 5' end. When a test site is positively charged, it will allow negatively charged oligos to move to the site which will become bound in the permeation layer.

(**B**) Target DNA is electronically hybridized to the capture oligo on a desired pad by applying positive charge to that pad.

(**C**) A fluorophore dye labeled reporter oligo is passively hybridized to the target DNA in order to detect the presence of the target. By reversing the polarity of that pad, nonhybridized target DNA will be removed from the vicinity of the test site.

Most importantly, the ability of histidine buffer to support DNA hybridization is dependent on electronic activation at the test sites. Since buffer molecules have very little net charge under neutral pH without electroactivation, they serve to keep complementary single stranded target DNA, such as PCR product from reannealing in solution. In summary, our microelectronic array has the advantage of a programmed pH gradient that allows discrete activation of hybridization zones.

## III. AC vs. DC current

One drawback of electronic addressing is the generation of damaging agents by electrolysis in solution above the permeation layer. Purines in DNA are particularly susceptible to attack by compounds such as radicals. One way to avoid this is to use thicker permeation layer which allows a greater distance over which the buffer may neutralize these radicals. Another approach is to use a higher

concentration of buffer to serve as a scavenger. A third approach is to use alternate current (AC) rather than direct current (DC). The polarity on the DNA test sites is alternated between positive and negative, with the duration under positive current longer than that of negative current. This asymmetric biasing ensures the directed movement and concentration of DNA samples on microarray pads while allowing adequate time for inactivation of damaging radicals. In addition, AC current is better than DC at controlling pH gradient generated by electrolysis at the electrode as it facilitates diffusion and maintains the pH as close to neutral as possible for optimal hybridization. However, in exchange for operating closer to equilibrium, transport and hybridization are slowed down.

## IV. Assay formats

Although several different formats are possible, the simplest one for our microelectronic assays is the capture down sandwich format (**Fig.2**). Capture probes are designed to encompass matched and mismatched regions of the alleles of the target and are complementary to the target sequence. First they are electronically addressed to individual or multiple pads on the microarray (**Fig. 2A**). The 5' end of these capture probes is biotinylated so that they will be anchored to the permeation layer via biotin/streptavidin interaction. Subsequently, the target DNA, in the form of amplified product such as PCR product or genomic DNA, is electronically concentrated and hybridized to the pads containing appropriate capture probes (**Fig. 2B**). Because electronic conditions can be readily fine-tuned on our microchip, we can achieve hybridization under stringent conditions within seconds. Furthermore, we can multiplex readily since different electronic conditions can be administered on different pads based on the optimal hybridization conditions for individual genes. After target hybridization, a fluorescence-labeled reporter oligo is then annealed to the single stranded target DNA outside the capture region to detect the presence of the target. The reporter oligo is long enough so that it will not be pulled apart from the target during subsequent stringency steps. Electronic stringency can be applied to these sites to quickly remove the nonspecifically bound target (**Fig. 2C**). Importantly, a higher stringency current can then be applied to achieve discrimination between matched and mismatched alleles. Such stringency may be applied serially to obtain optimal discrimination for each test site. Alternatively, the stringency can be achieved thermally. This allows more rapid parallel processing when multiple sites have similar characteristics. A fluorescence scan is then conducted to measure the ratio between the match and the mismatch in order to make a call. Alternatively, fluorescence scans can be integrated into the stringency steps to monitor the discrimination process. Using a reporter oligo has certain advantages over labeling the target DNA directly. It decreases the background without sacrificing sensitivity. So far, we have developed assays for a wide range of SNPs (Gilles et al, 1999; Canter et al, Submitted) and STRs (Short Tandem Repeats) genotyping for forensics and human identification (Radtkey et al, Submitted).

## V. Comparison of array formats: multiple SNPs per chip vs. multiple patients per chip

Most of the microarray technologies currently available focus on large arrays (Lipshutz et al, 1999; Chee et al, 1996; Brown et al, 1999). They provide useful formats for monitoring gene expression, gene screening and drug discovery. But they are less optimal for clinical applications such as medical diagnostics, where relevant target genes are more limited in number. The best examples would be most of the SNPs tests for screening for genetic diseases and drug resistance of microorganisms and cancer cells (Evans 1998; Koza et al, 1996; Wang et al, 1998, 1999). With the electronic platform, screening can be performed in two formats: (**i**) Multiple SNPs from one patient may be analyzed on one chip. (**ii**) One SNP from multiple patients may be determined on one chip. The former is a useful format for screening an individual for polymorphisms conferring drug resistance, such as the large numbers of polymorphism in HIV (Vahey et al, 1999). Though passive hybridization may be used, electronic hybridization should provide speed and flexibility and permit different hybridization conditions to be used simultaneously on multiple genes. There is little concern of cross-contamination between patients since only one patient is applied per chip. The second format is better used for higher throughput screening for the same genetic defect (Wang et al, 1999). An example would be a survey for hemochromatosis mutations in a population (Barton et al, 1997). In this case, it is almost impossible to use passive hybridization schemes in an open format since multiple patient samples must be applied to different test sites on the same array. Our microelectronic technology, using an amplicon down format, will perform this with ease because we are able to target DNA sample to specific pads without significant cross-contamination to adjacent sites. The design of cartridges allows easy washes between multiple sample applications (**Fig. 4**). Therefore, our microchip platform could accommodate both scenarios and perform both assays equally well.

## VI. Comparison between capture down and amplicon down formats

Although the capture down format for SNP and STR assays is convenient for electronic hybridization, it has its limitations (**Fig.3**). The two strands of target DNA are kept apart by our histidine buffer in the absence of electronic activation. However, during electronic activation, histidine is protonated and generates a hybridization zone above the pads. This allows not only hybridization between capture probe and complementing target strand, but also the reannealing between the two target strands. This could result in a decrease in capture probe hybridization efficiency.

Secondly, anchored capture probes are much shorter than target DNA. This may create a steric hindrance problem when short captures near the surface are forced to anneal with a longer DNA that is present in very small quantity. Further, in comparison with transport or hybridization alone, this step is more technically demanding because transport of very small amounts of long DNA strands is coupled simultaneously with hybridization to capture probes,. To get around this problem, we have developed an amplicon down scheme (Canter et al, Submitted).

In the amplicon down format, the target DNA (usually amplicon) is anchored to the permeation layer. This is achieved by using a biotinylated PCR primer to synthesize one target strand. After heat denaturation, the amplicons are then electronically addressed in histidine buffer to the desired pads. Since there is no hybridization involved, we utilize a condition that affords maximum transport. At this point, most of the bound DNA should be biotinylated single stranded target DNA. A brief alkaline denaturation step is then performed to remove any residual annealed strand. The two oligo components which comprise the sandwich in the amplicon down format do not contain biotin groups (**Fig. 3**). They hybridize to the anchored target and form a base-stacked complex (Canter et al, Submitted; Radtkey et al, Submitted). One contains the fluorophore and is the reporter oligo. The advantages of the amplicon down format are multifold. First, it is easier to transport very small amounts of amplicon DNA to test sites without having to accommodate hybridization. Second, the additional alkaline denaturation step after putting down target DNA on the permeation layer ensures that the bound DNA is single stranded, thereby enhancing the assay efficiency. Third, there is less potential steric hindrance during hybridization. Since the complementary region to the capture is in the middle of the target molecule, and the anchor is at the 5' end, it gives the capture oligo plenty of room to interact with the target. Most importantly, the capture oligos, which are shorter and in excess, are the free diffusing component in this hybridization reaction. This is a kinetically favored reaction simply due to mass action.

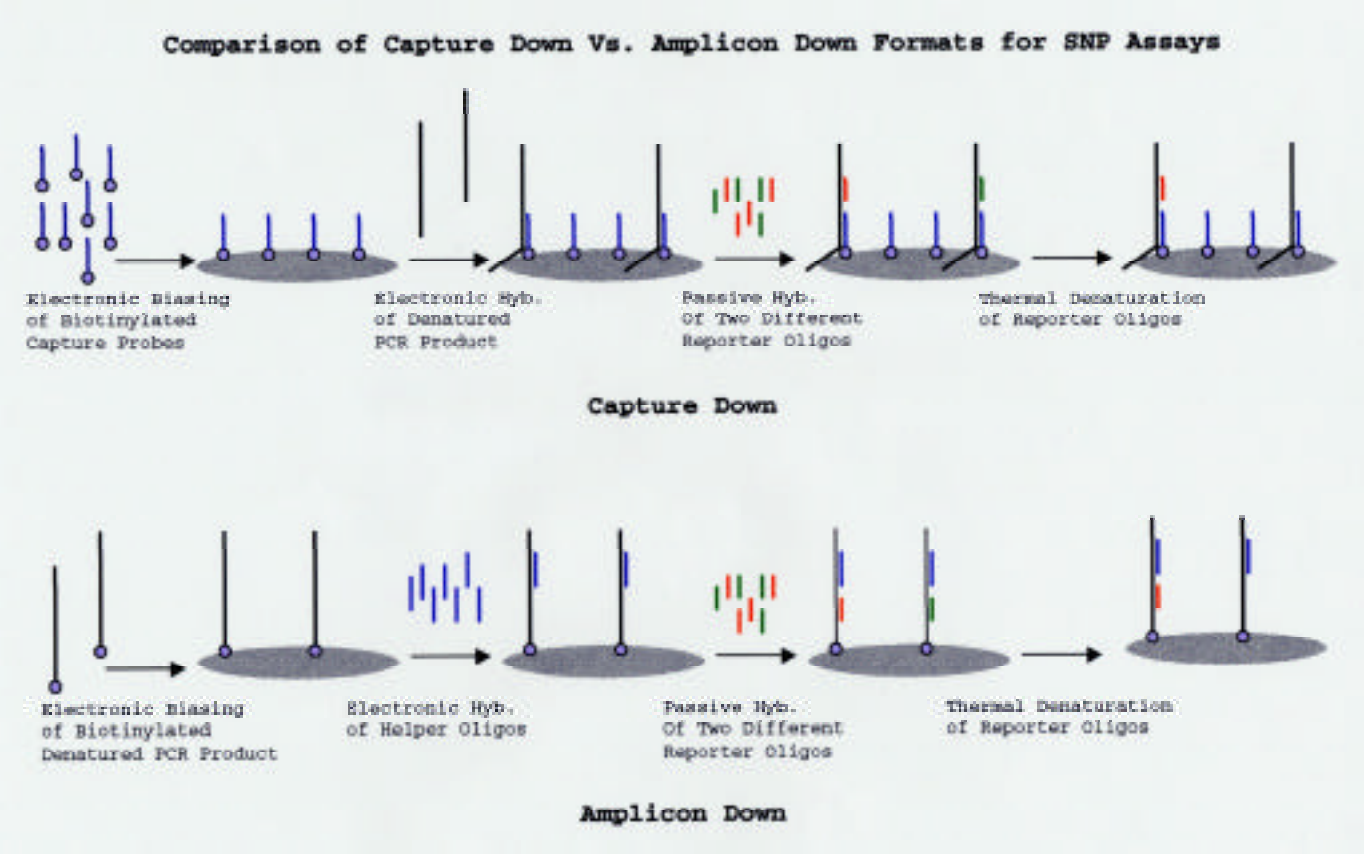


**Figure 3:** Comparison of two different formats for SNP assays. In the capture down format, capture probes are loaded on the pads first. Then the denatured amplicon molecules are electronically hybridized to the captured probes on site. A mixture of reporter oligos that encompass the polymorphic region is passively hybridized to the target. The two different reporter oligos are labeled with different fluorophore dyes and represent either wild type or SNP alleles of the gene. Finally, thermal denaturation is applied to the array. As a result, the reporter which contains the mismatched allele in the polymorphic region is removed and that of the matched allele stays on. The signal ratio between two colors allows us to make a call. The amplicon down format is similar to the capture down format with several exceptions. First, biotinylated amplicon DNA is initially addressed to the site. Second, oligo probes are hybridized to the anchored amplicon.

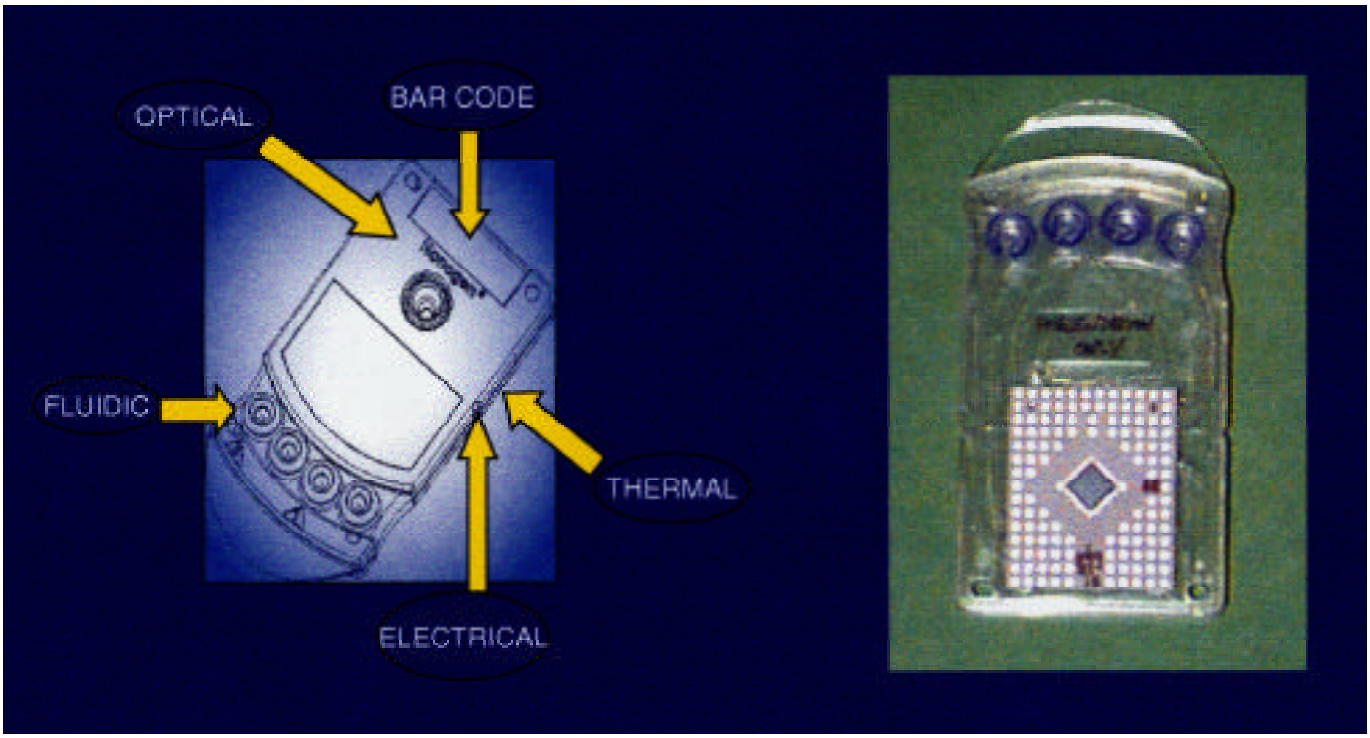


**Figure 4:** The Nanogen microchip cartridge. The cartridge contains a 100-site microchip mounted on a substrate that has contact areas for electrical pogo pins (panel on the right). A flow cell sits on top of the array and serves as a chamber for electronics, washing and optical window (panel on the left). The cartridge also contains multiple valves and interconnected channels leading to the flow cell for sample application and washing.

## VII. Amplification on the test site

Although the amplicon down format is not as easily multiplexed as the capture down format, its biggest advantage is to provide a platform for a natural extension of our technology. Chip based nucleic acid tests, especially ones for medical diagnostics, have multiple requirements. These include sample preparation, target amplification, hybridization and detection. In addition to speed and miniaturization, the microelectronics provides a platform for integration of some of these elements. The cartridge used in our semi-automated instruments contains a microelectronic chip mounted on a substrate and a flow cell on top of the array (**Fig**.**4** ). The flow cell can serve as a suitable chamber for aspects of sample prep, for example, providing fluidics steps such as washing the array in between steps. It can also serve as an compartment for on site amplification of the target DNA (Westin et al, Submitted). This could include linear or exponential amplification processes. On site amplification could provide badly needed simplicity and streamlined integration of technologies currently available separately on the market. The on site amplification on our microchip could also potentially alleviate the difficulties of multiplexing in existing amplification schemes.

## VIII. STR typing in forensics and the CODIS database

Short Tandem Repeat sequences are dispersed widely throughout the human genome. They are highly polymorphic markers and the number of copies of the repeat sequence differentiates alleles of these loci (Edwards et al, 1991). STR DNA typing is well established in forensics, paternity testing, cancer diagnostics and plant breading. In the area of forensics, a large DNA database, the Combined DNA Information System (CODIS) has been implemented by Department of Justice to collect DNA genotypes of convicted felons (Budowle et al, 1998; Budowle et al, In Press). Small amounts of biological material collected at a crime scene can be typed, and results run against the CODIS database to determine if a match is found.

STRs are not easily analyzed by conventional hybridization technology. The identical nature of repeats in STR loci makes accurate discrimination fastidious due to slippage during hybridization. Thus, current typing technologies are mainly based on sizing of amplicons which allow for estimation of the numbers of repeats (Budowle et al, 1998). Such analysis is quite labor intensive and does not meet the needs of high throughput and multiplex

analysis. Furthermore, none of these technologies is streamlined enough to facilitate a portable device used at a crime scene. Nanogen's microarray technology allows fast and accurate analysis due to its electronic hybridization, miniaturization of otherwise cumbersome techniques, and potential for highly multiplexed testing (Radtkey et al, Submitted).

## IX. Utility of SNPs in forensics

Recently, utilization of single nucleotide polymorphism in forensic genotyping has become more and more attractive as a complement to STRs. This is partly due to the limitations of the STR system such as microvariants, stutter bands and difficulties dealing with mixed samples (Budowle et al, 1998). Even if SNP analysis does not replace the STR system, it could paly a complementary role. A perfect example is the Y chromosome polymorphism. In recent years these Y chromosome SNPs have surfaced in a number of human population genetic studies (Jobling et al, 1997). With the large number of rape and sexual assault cases in this country, they can be extremely useful in determining the male identity in sexual assault specimens. Nanogen's semi-automated microchip device provides an attractive platform for this potential application, particularly in an integrated system where amplification can be performed on the chip followed by genetic assay.

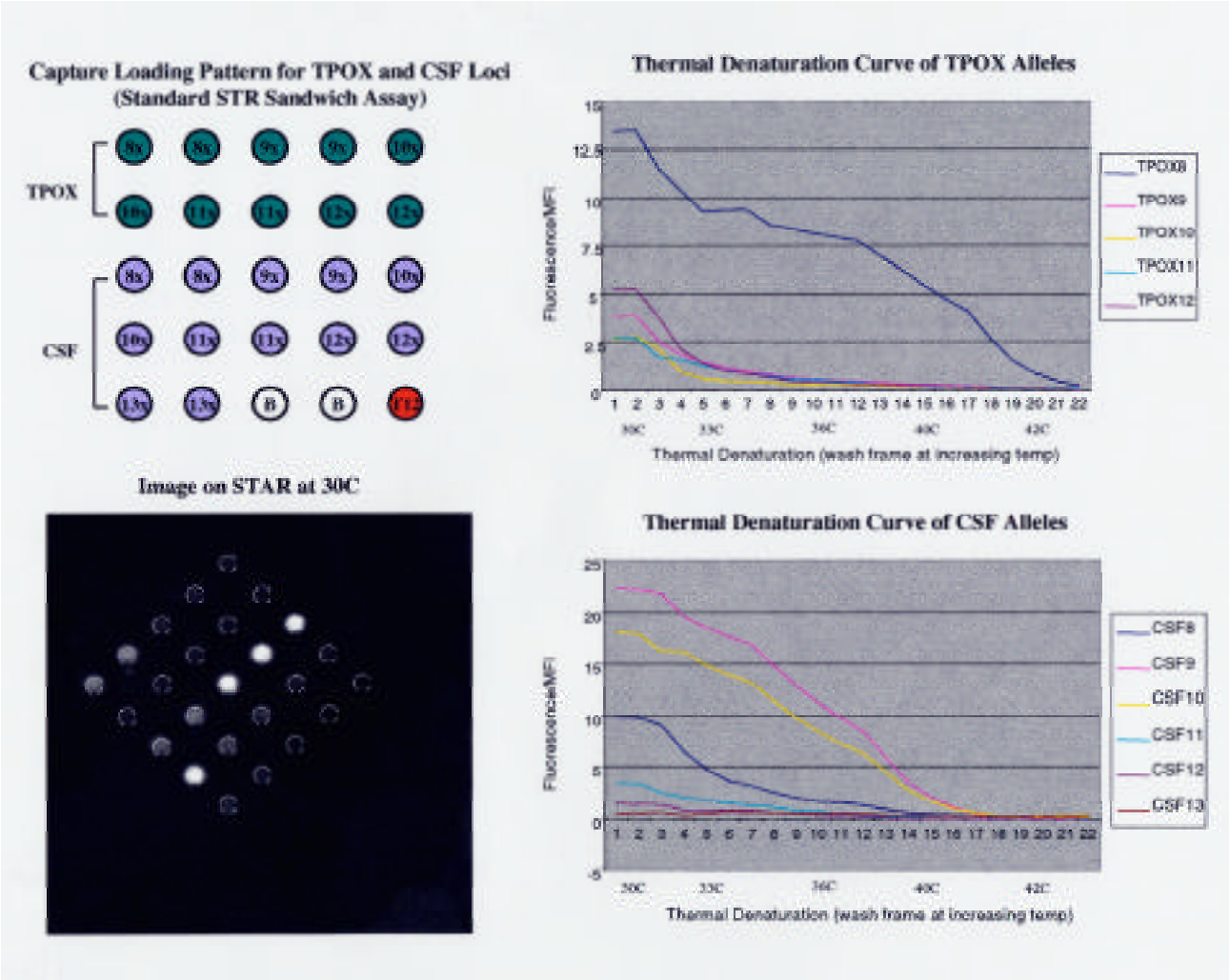


**Figure 5:** Example of a capture down STR assay on a 25-site chip. Capture probes representing various alleles of TPOX and CSF genes are loaded to the array according to the map on the upper left panel of the figure. A labeled T12 oligo is used for control of capture attachment in the permeation layer. It contains a biotin at the 5' end and a Bodipy Texas Red (BTR) dye at the 3' end. The CTT (CSF, THO1 and TPOX loci) triplex PCR product from a patient is then hybridized to the array. During thermal denaturation, multiple images are taken at each elevated temperature. The lower right panel represents one of those images. The fluorescence signal of each pad is measured within each frame and plotted against increased temperatures. A genotype is determined based on the fact that the reporter for matched alleles is more stable than the mismatched alleles under thermal denaturation. In this case, the patient is 8-repeat homozygote for TPOX locus, and 9 and 10-repeat heterozygote for CSF locus.

## X. Prospect of a portable device

In addition to the CODIS national database, several states, including Virginia and Florida have set up similar databases to help them solve crimes within state. This kind of initiative not only requires high-throughput technologies for databasing, it also needs testing at the crime scene to minimize potential contamination and to include or exclude suspects at the scene. The National Institute of Justice has supported DNA chip technology development with an emphasis on portable devices that will accommodate samples such as blood, semen and skin cells at the crime scene (Schmalzing et al, 1997). We are developing a small integrated device containing the cartridges in which the microchips are packaged. The small flow cell will serve as a reaction chamber for amplification. Afterwards, the cartridges can be inserted into a portable reader for assay and detection. Future development which may facilitate miniaturization of instruments may include a direct (nonfluorescent) detection systems, perhaps sensing changes in resistance of magnetism. Since no colormetric detection would be required, we could eliminate the fluorescence scanner that composes a large part of the instrument.

## XI. Conclusions

In conclusion, the electronic microchip technology could have wide applications in biomedical research, medical diagnostics, genomics, genetic testing and drug discovery. We have developed assays for multiple SNP applications and STR analysis for forensic genotyping. These, in conjunction with the development of integrated systems and portable devices, provide an alternative to the current array technologies available.

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