ON-CHIP AMPLIFICATION OF GENOMIC DNA WITH SHORT TANDEM REPEAT AND SINGLE NUCLEOTIDE POLYMORPHISM ANALYSIS

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Introduction

As DNA finger printing proves more useful in fighting crime, new ways of using this technology are being proposed. One area being considered for expansion, point-of-arrest testing, has a need for easy and accurate genotyping methodologies. At Nanogen, we have developed assays on electronic microarrays for determining the genetic identity of individuals, either via short tandem repeat (STR) (1) or single nucleotide polymorphism (SNP) analysis. This technology is amenable to the development of highly automated sample-to-answer test devices.

Using PCR-generated amplicons, concordance studies at three beta sites clearly demonstrated the genotyping accuracy of our microchips. Specifically, the rate of calling SNPs correctly was 100%, while the rate of correctly calling STRs (BODE Technology Group) was > 99.5%. In order to increase the ease of DNA finger printing on our microchips, we developed an on-chip amplification system (2), whereby the generated amplified products could be scored using our already proven identification assays.

In this manuscript, we review the strengths of our electronically-active microarrays (3), as well as briefly explain our discrimination (1) and on-chip amplification assays (2). We then show genotyping results from human DNA purified from blood samples wherein said DNA is amplified and scored directly on our microarray. Finally, we discuss the advantages of this assay.

Discussion

At Nanogen, we develop biological assays for our proprietary electronically-active microchips (an image of various chips is depicted in Figure 1). By utilizing a controlled electronic field, we are able to precisely control the movement of charged molecules, such as DNA or proteins (3). Thus, DNA can be selectively targeted to specific sites on the chip. Moreover, this DNA can be concentrated at these sites, leading to enhanced hybridization. For example, where a passive hybridization might occur in hours, on our chips the same hybridization is complete within minutes.

The electronic microchip is attached within a cartridge (Figure 2). The cartridge, known as the NanoChip™, is used along with the Nanogen Molecular Biology Workstation (Figure 3) to conduct experiments of interest, such as on-chip amplification followed by STR scoring. As the cartridge and research system are fully integrated, minimal hands-on manipulation is required, allowing for more user-friendly assays with less chance of operator error.

While our STR discrimination assay has been described elsewhere [reference (1), a brief overview follows (Figures 4-6). Biotinylated-target DNA, be it generated by PCR or Anchored SDA (described below), is specifically attached to streptavidin in the microchip's permeation layer. Various length stabilizer oligonucleotides, representing the different repeat numbers of the STR being investigated, are then electronically hybridized to the attached amplicons. Fluorescently-labeled reporter is then allowed to passively hybridize to each of the target: stabilizer complexes. Due to enhanced energy imparted by base stacking, thermal discrimination removes the reporter from all complexes except where the target and stabilizer repeat lengths are identical. Thus, the repeat number of the target DNA is discerned. An essentially identical discrimination assay is utilized to determine the genetic identity of an individual's SNPs.

In order to implement a sample-to-answer product, we developed on-chip amplification using the isothermal Strand Displacement Amplification (SDA) assay (2), which bypasses the requirement of preparing amplified products *in vitro* as well as post-purification methodologies. In brief (Figures 7-9), biotinylated SDA primers are electronically attached to specific sites on the microchip. Next, denatured genomic template is hybridized to these primers. Using a nicking enzyme and a polymerase capable of strand displacement, exponential amplification ensues. Once complete, the amplicons are denatured, stabilizer is electronically hybridized, and reporting is done. The advantages of SDA include its isothermal nature, its ability to allow multiplex amplification with minimal optimization of primer pairs, and its ability to allow amplification and genetic determination to occur on the same platform.

Using Anchored SDA and our discrimination protocol, we have been able to accurately determine the genetic makeup of individuals for 2 STRs (TPOX and CSF) and 5 Y-chromosome SNPs (DYS199, DYS271, SRY1532, PN1, and PN2) as demonstrated in Figures 10-13. With our ability to electronically separate red and white blood cells as well as electronically lyse the DNA-containing white blood cells, we hope to have an integrated device whereby blood can be inserted into our platform and an individuals genetic identity will be output.

References

1. Radtkey R, Feng L, Muralhider M, Duhon M, Canter D, DiPierro D, Fallon S, Tu E, McElfresh K, Nerenberg
M, Sosnowski R. Rapid high fidelity analysis of simple sequence repeats on an electronically active DNA microchip.
Nucleic Acids Research, January 2000.

2. Westin L, Xu X., Miller C, Wang L, Edman CF, Nerenberg M. Anchored multiplex amplification on a microelectronic
chip array. Nature Biotechnology 2000;, 18:199-204.

3. Sosnowski R, Tu E, Butler W, O'Connell J, and Heller M. Rapid Determination of Single Base Mismatch Mutations in
DNA Hybrids by Direct Electric Field Control. Proc Natl Acad Sci 1997; 94:1119-1123.

Figures

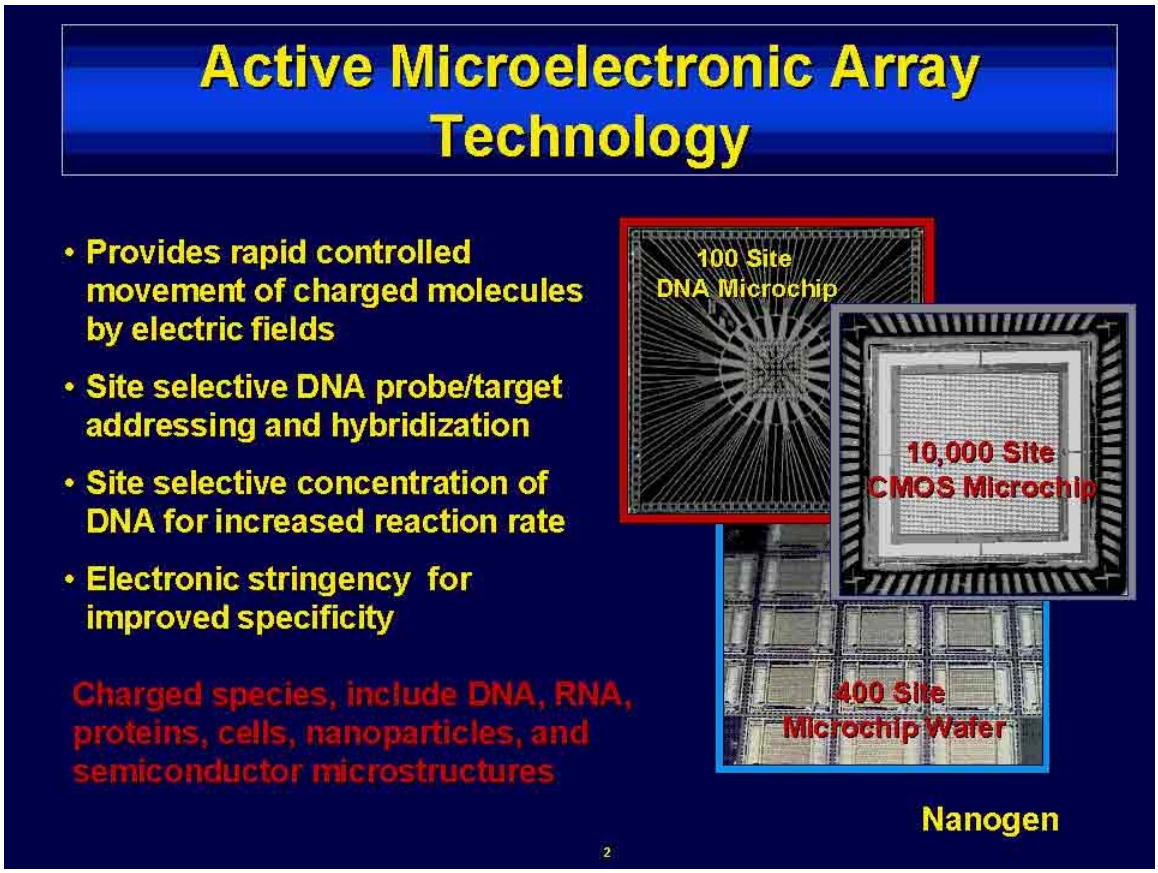

Figure 1. Active microelectronic array technology

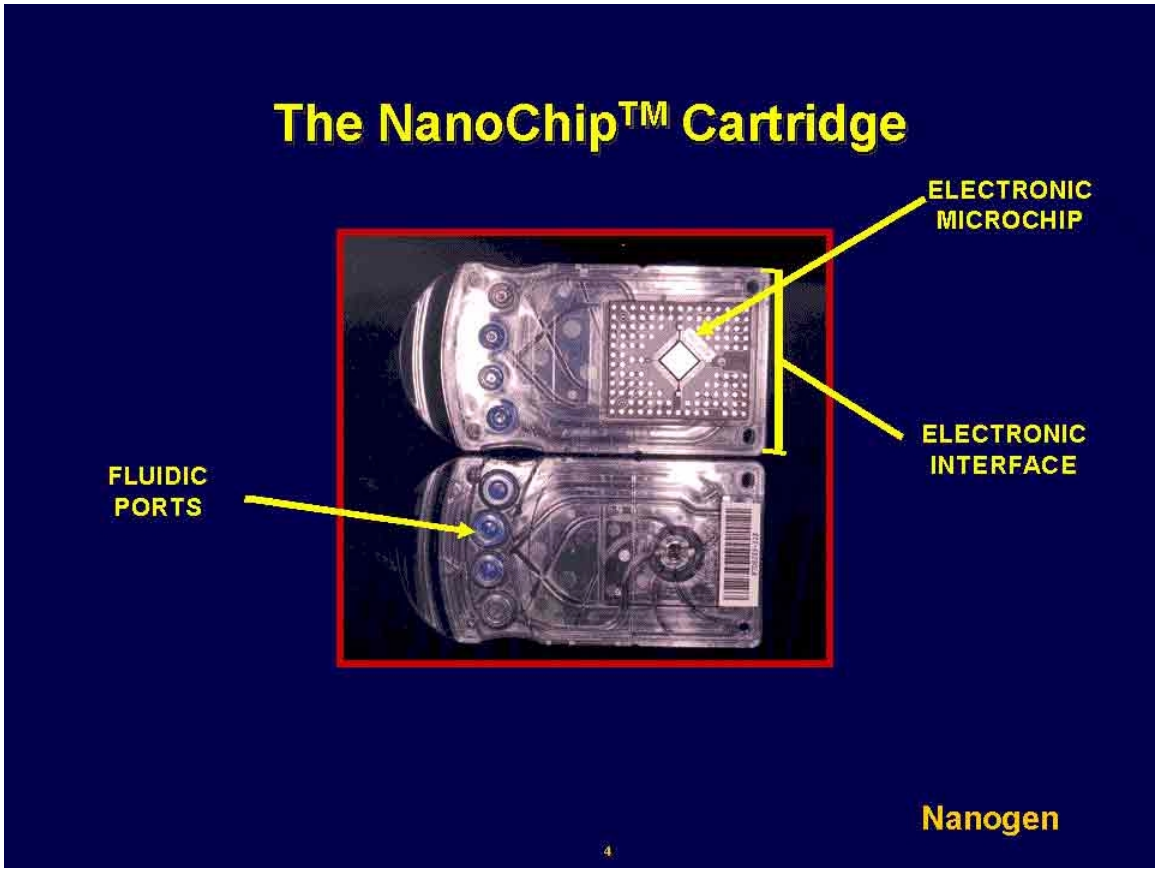

Figure 2. The Nanochip™ cartridge


Figure 3. The Nanogen Molecular Biology Workstation

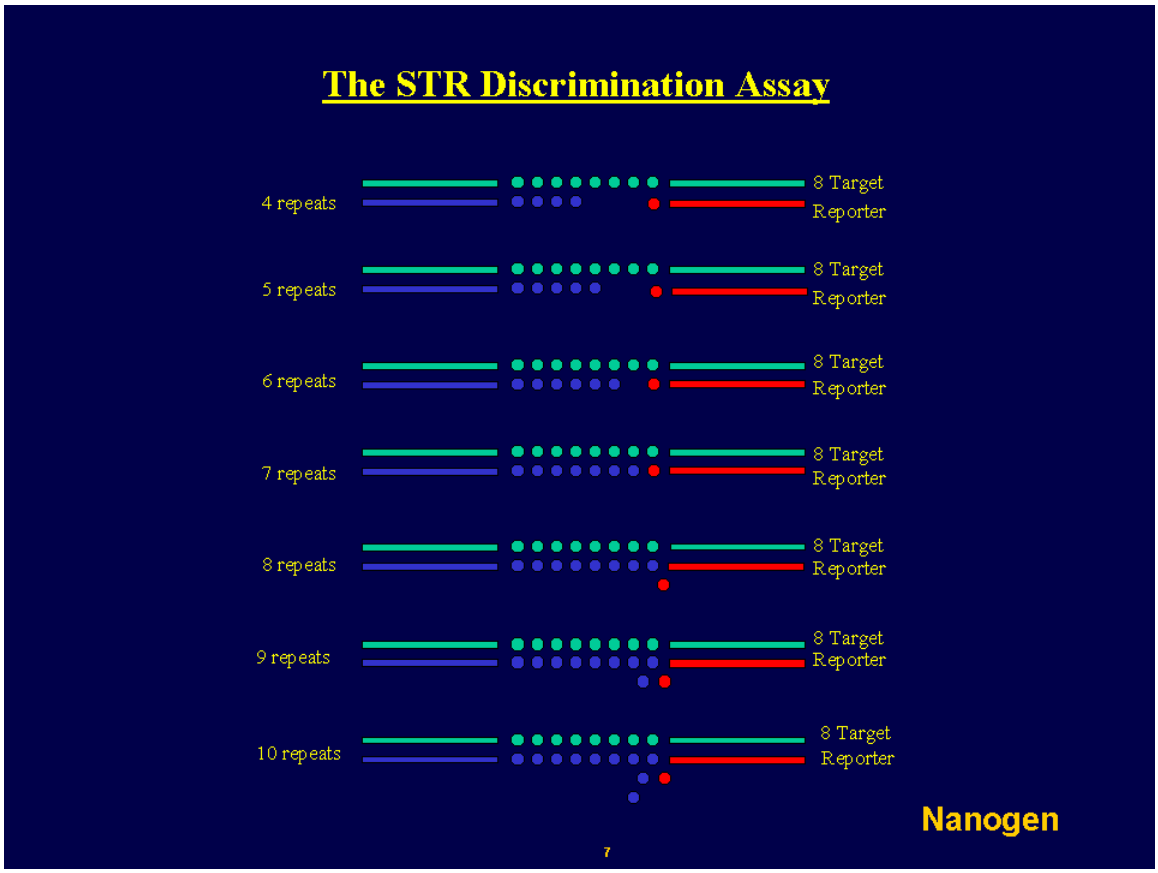

Figure 4. The STR Discrimination Assay – Part. 1

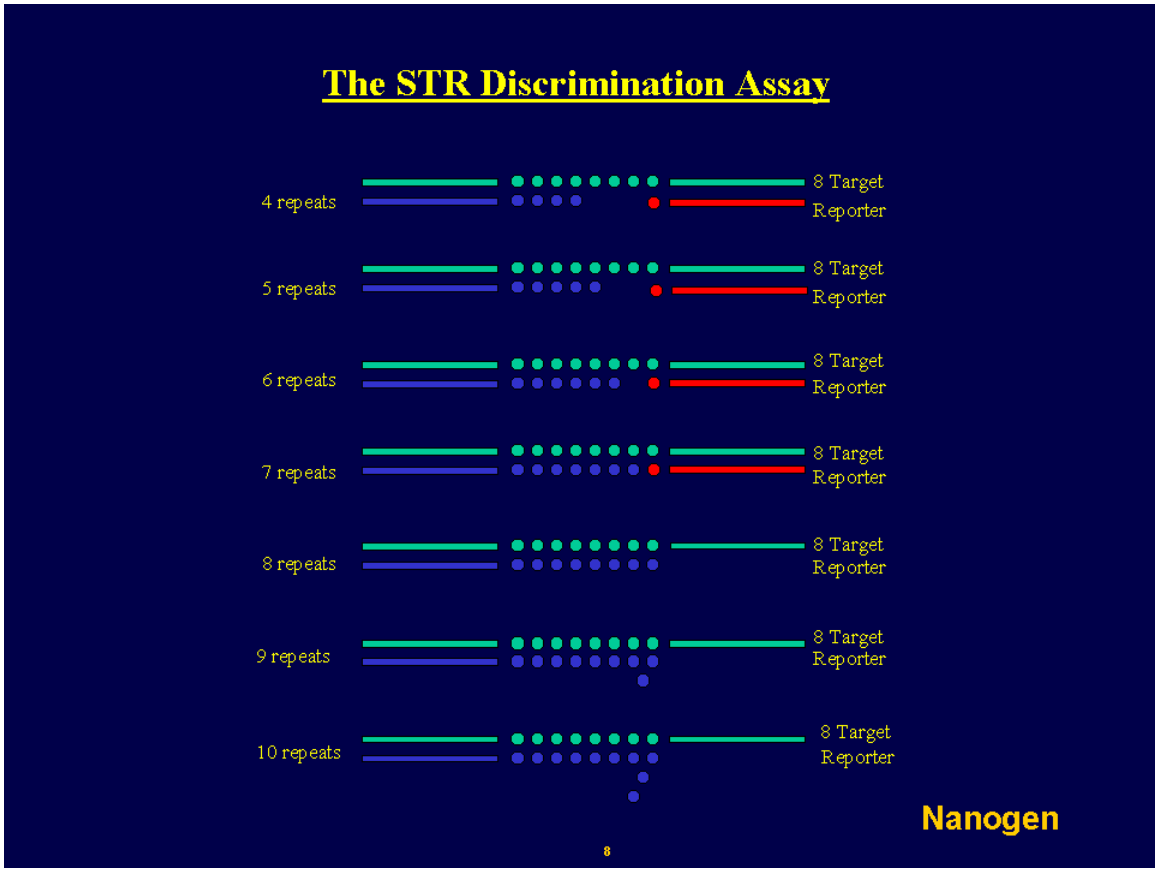

Figure 5. The STR Discrimination Assay – Part 2

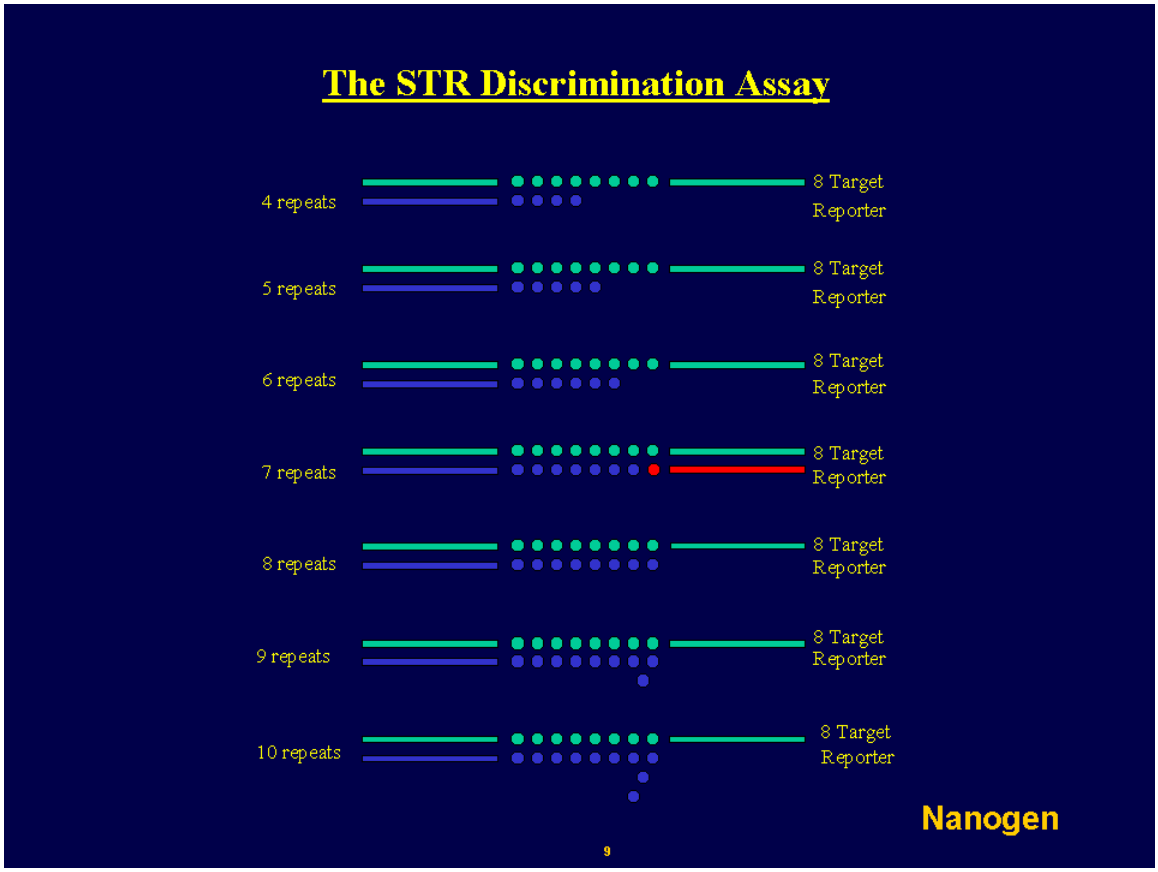

Figure 6. The STR Discrimination Assay – Part 3

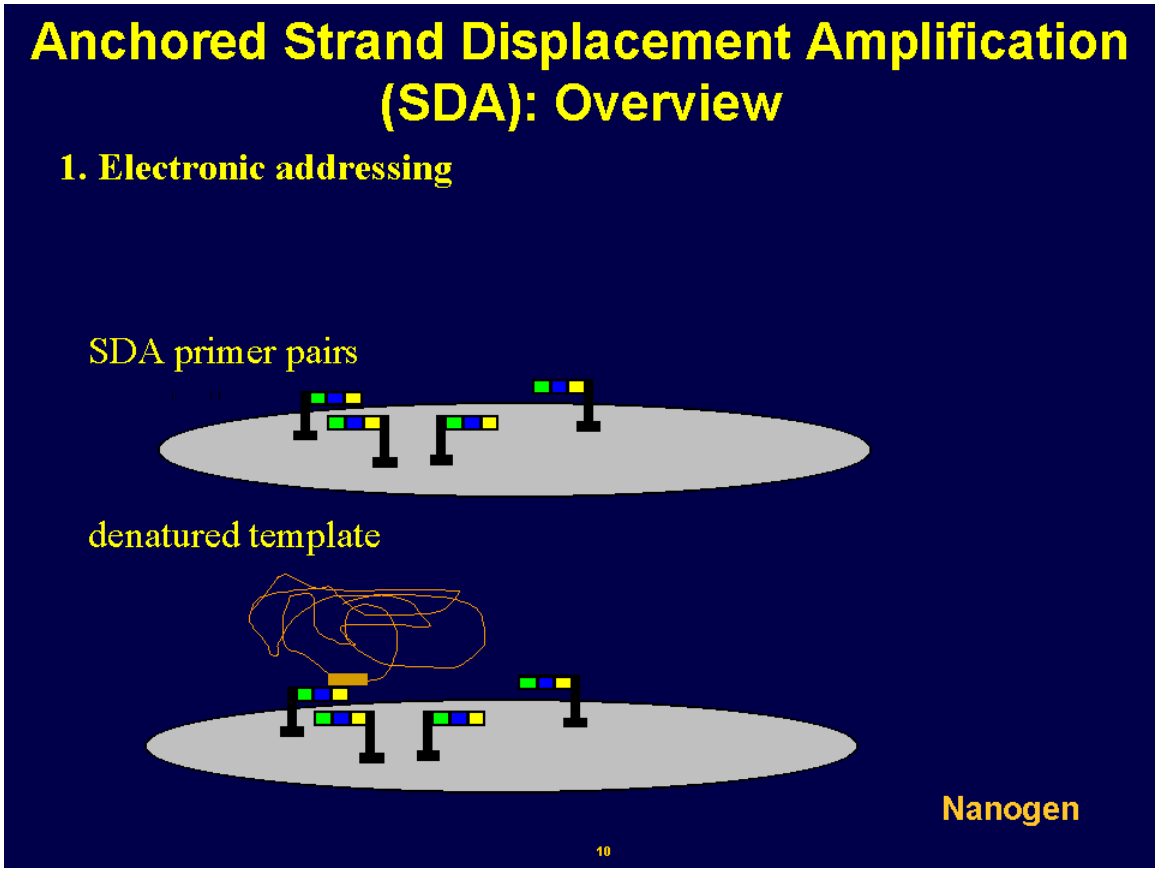

Figure 7. Anchored SDA: Overview – Part 1

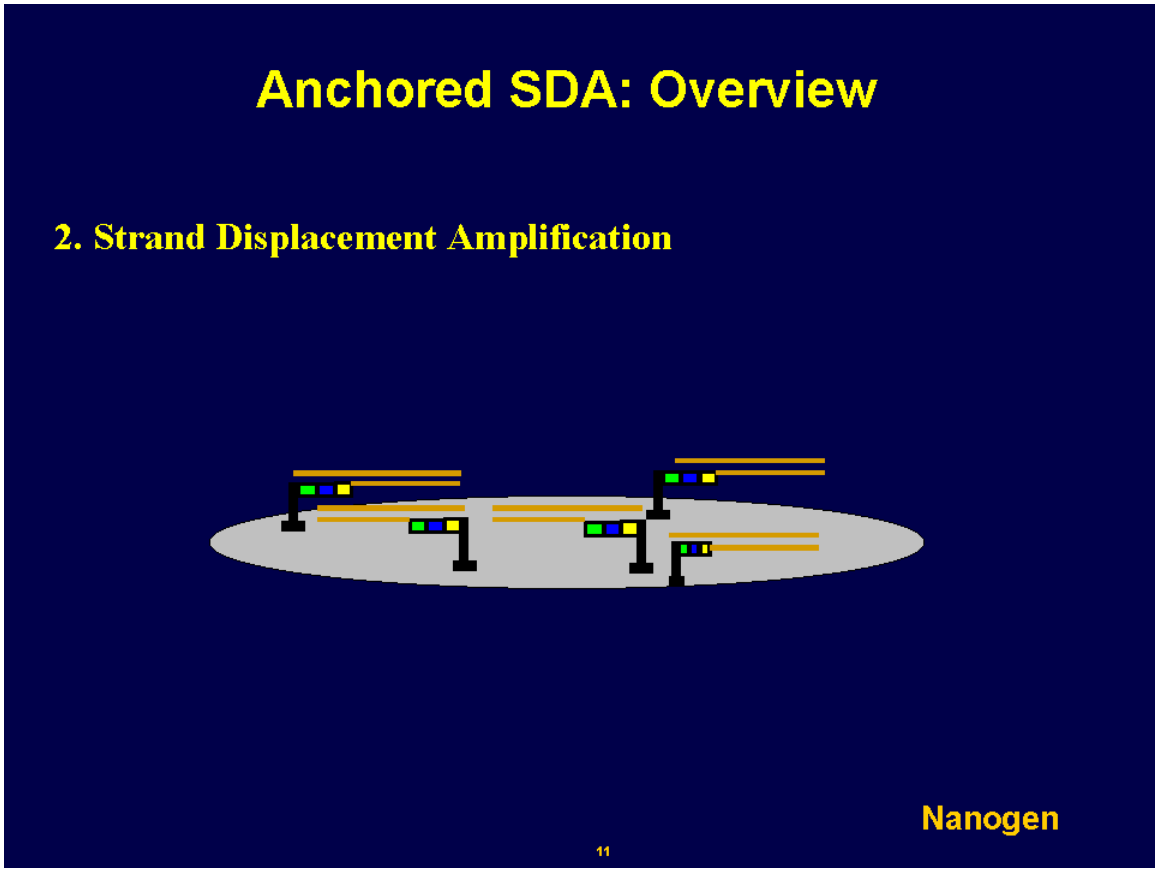

Figure 8. Anchored SDA: Overview – Part 2

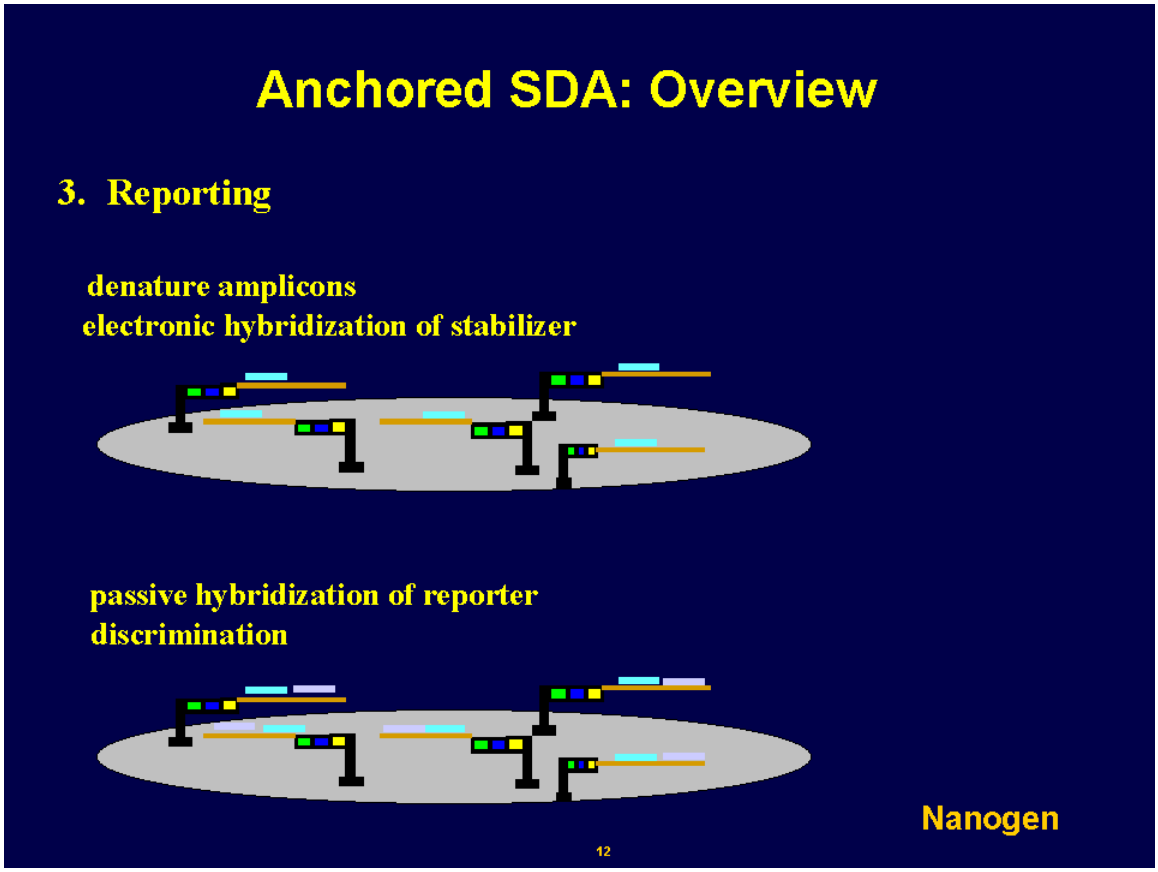

Figure 9. Anchored SDA: Overview – Part 3

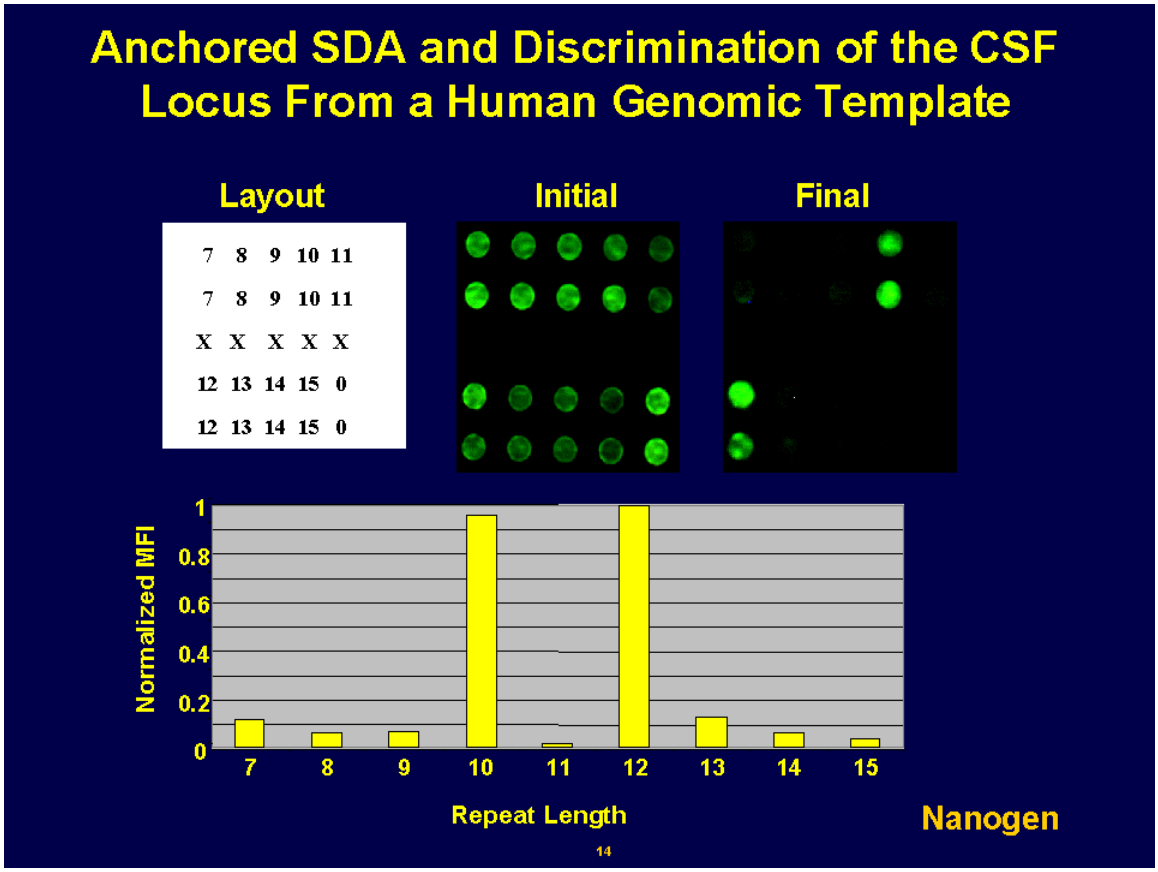

Figure 10. Anchored SDA and discrimination of the CSF locus from a human genomic template

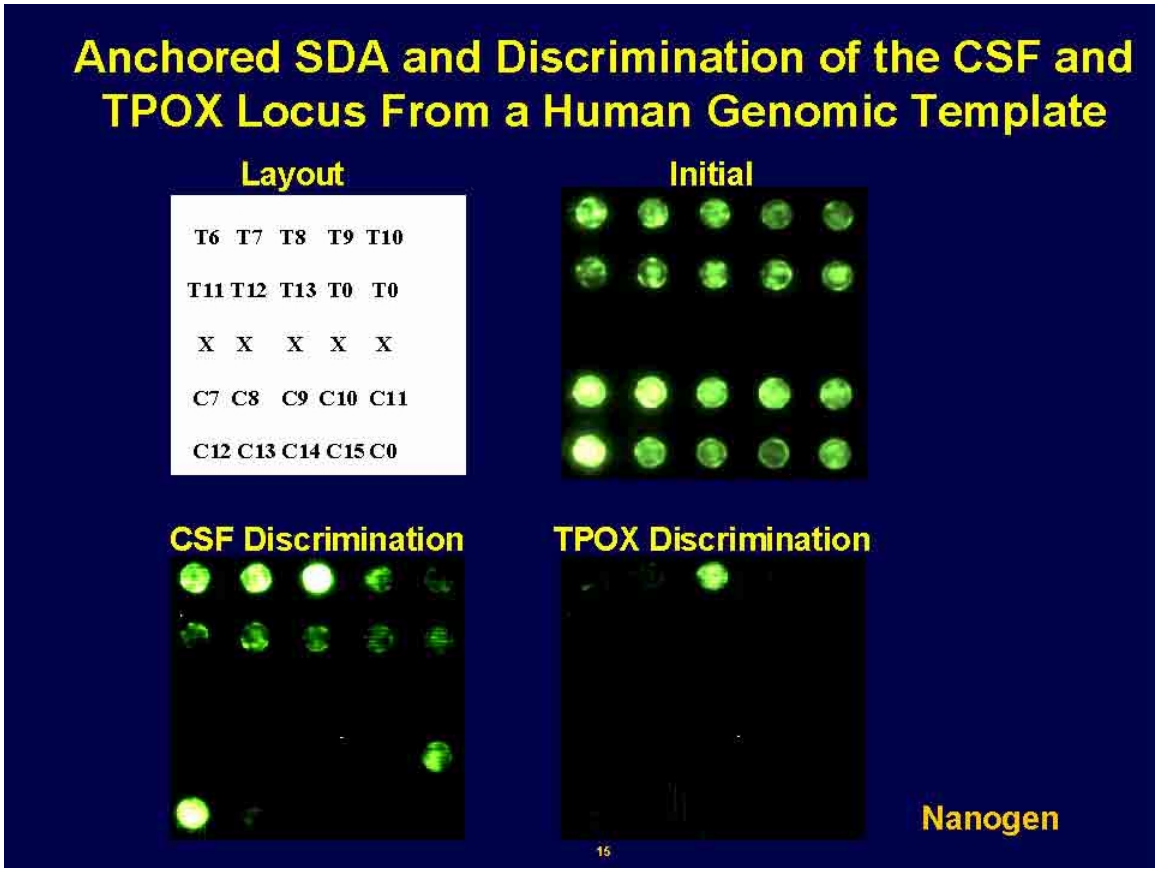

Figure 11. Anchored SDA and discrimination of the CSF and TPOX locus from a human genomic template – Part 1

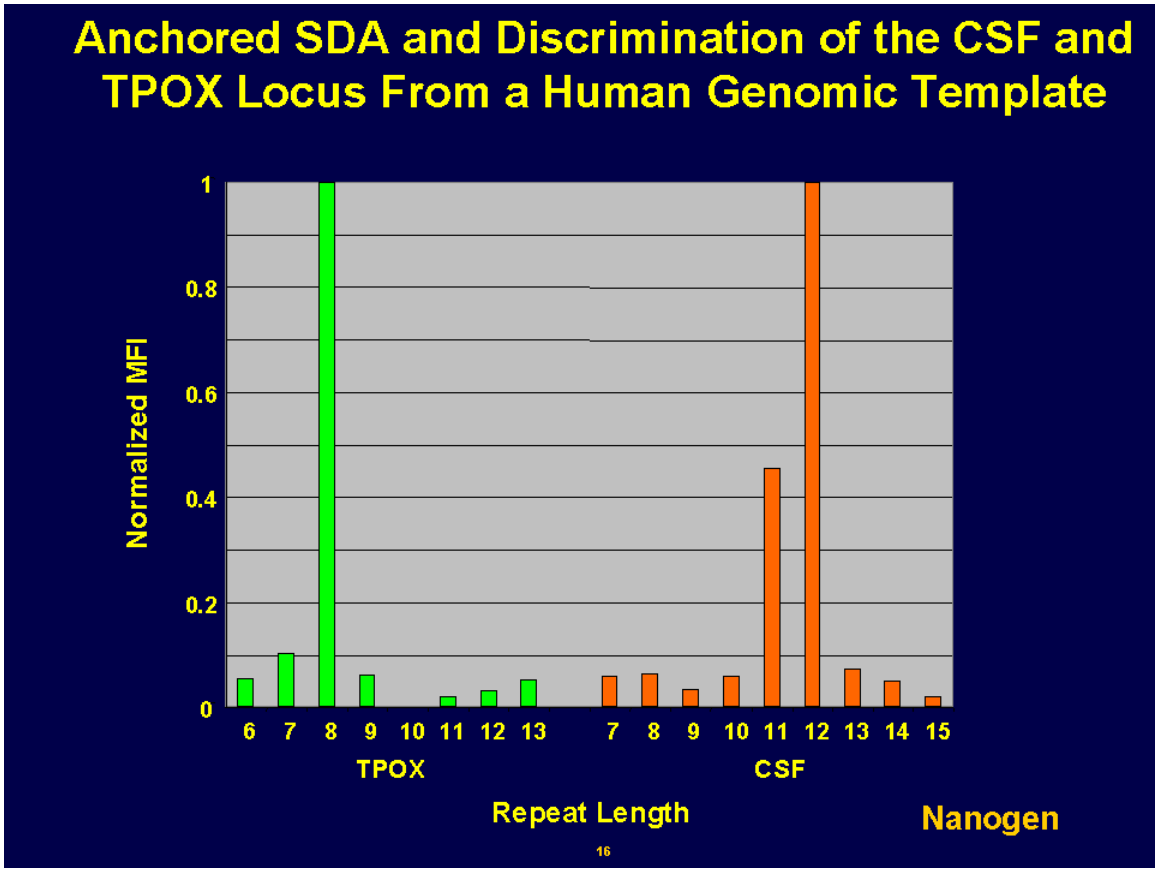

Figure 12. Anchored SDA and discrimination of the CSF and TPOX locus from a human genomic template – Part 2

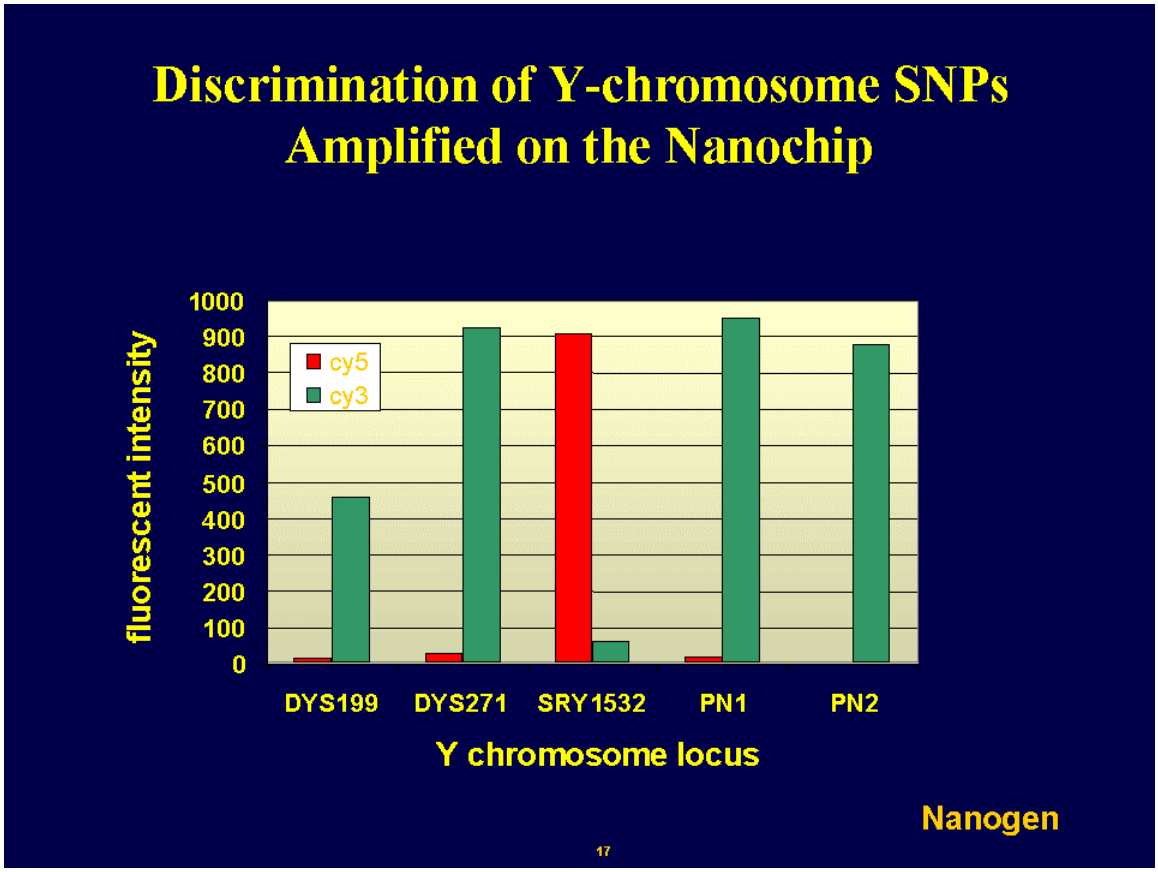

Figure 13. Discrimination of Y-chromosome SNPs Amplified from a human genomic template on the NanoChip™