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RESEARCH

# A Comparison of Hybridization Efficiency between Flat Glass and Channel Glass Solid Supports

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**Abstract** Two different solid supports, channel glass and flat glass, were compared for their affect on the sensitivity and efficiency of DNA hybridization reactions. Both solid supports were tested using a set of arrayed, synthetic oligonucleotides that are designed to detect short insertion/deletion polymorphisms (SIDPs). A total of 13 different human SIDPs were chosen for analysis. Capture probes, designed for this test set, were covalently immobilized on substrates. Hybridization efficiency was assessed using fluorescently labeled stacking probes which were preannealed to the target and then hybridized to the support-bound oligonucleotide array; the hybridization pattern was detected by fluorescence imaging. It was found that structural features of nucleic acid capture probes tethered to a solid support and the molecular basis of their interaction with targets in solution have direct implications on the hybridization process. Our results demonstrate that channel glass has a number of practical advantages over flat glass including higher sensitivity and a faster hybridization rate.

**Keywords** Channel glass · Flat glass · Oligonucleotide arrays · Capture probes · Hybridization · Sensitivity

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## Introduction

A wide variety of solid supports have been investigated for DNA analysis, including nylon and nitrocellulose membranes, glass beads, polyacrylamide gels, polystyrene matrices, activated dextrans, avidin-coated polystyrene beads, and glass [1–5]. Proper choice of support material depends on several technical aspects of DNA hybridization and detection. For example, the support material can affect fluorescence-based detection sensitivity by affecting the level of scattering and fluorescence background. Further, the material surface dictates chemical modification procedures, the loading capacity, and the degree of non-specific binding [1, 4–8].

Glass microscope slides (flat glass) are a frequently chosen support due to their low cost, ready availability, and well-characterized chemical and physical properties. Glass is amenable to a variety of chemical modification procedures [3, 5, 8, 9]. However, an inherent limitation of planar glass is the limited surface area available for attachment of DNA probes. This limitation can be overcome by using glass with cylindrical pores (hereafter called channel glass). Channel glass contains thousands of microscopic channels that pass completely through the thin silica substrate. The channels are arranged parallel to each other and allow for flow through of the substrate (see Fig. 1). Oligonucleotides can be immobilized on the inner surfaces of these microchannels, increasing the binding capacity per cross-sectional unit and consequently leading to improved detection sensitivity.

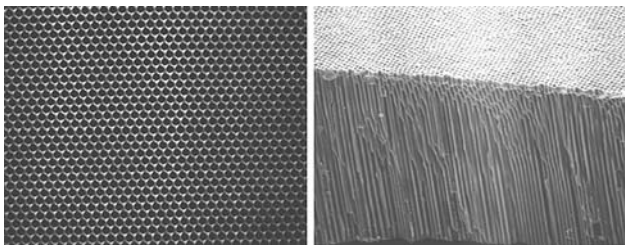

**Fig. 1** Scanning electron micrographs of a portion of channel glass. Viewed from above (left panel) and in cross section (right panel) showing the channels arranged parallel to each other to allow flow through of the sample solution

This structure allows for the interrogation of genetic samples (targets) by flowing through the channels that contain immobilized oligonucleotide probes [10–15].

A notable advantage over flat substrates is avoidance of sample drying during chemical attachment of the oligonucleotide probe. Another advantage is improved accessibility of target molecules to the surface-tethered probes. Targets flowing through the microchannels rapidly encounter the surface-tethered probes, due to the short diffusion distance to the surface of the channel walls. This allows for the analysis of dilute solutions of target molecules [6, 12–14, 16]. Diverse formats of microchannel biosensors have been developed and validated [1, 4, 6, 13, 14, 16–18]. Some have been used for rapid molecular biological testing, offering similar results to that obtained on DNA microarrays prepared on glass slides e.g., for the detection of breast cancer subtypes [17], for the analysis of gene expression profiles [18], and for the detection and differentiation of clinically relevant species of *Staphylococcus aureus* [19]. Likewise, novel strategies involving agitation mechanisms or sample injection have been reported in order to guarantee fast and reproducible hybridization results. For example, surface acoustic waves have been used to agitate small volumes of hybridization solution on glass slide microarrays [15]. This ensured continuous mixing and enhanced the probability for target molecules to bind to specific probes without disturbance by flow effects. Microfluidic channel devices have been aligned and reversibly assembled manually to glass slide microarrays [19, 20]. Other approaches have combined microfluidic hybridization with sample oscillation [4]. In this work, the advantages of using channel glass in carrying out hybridization assays are described, and the performance of this system is compared to planar glass substrates.

## Materials and Methods

### Polymorphic Sequences Studied

Sequences of human SIDP were provided by the laboratory of Dr. J. L. Weber [21] and were identified through analysis of overlapping genomic sequences or cDNA sequences. http://research.marshfieldclinic.org/genetics/home/index.asp.

### Oligonucleotides

All DNA capture probes, stacking probes, and targets were synthesized by IDT, Inc. (Coralville, IA) by standard phosphoramidite procedure and then desalted. They were spectrophotometrically quantified as previously described [22]. Stacking probes were synthesized with a 5′-Cy3-fluorescent label. Oligonucleotide probes to be immobilized on the channel glass surface were derivatized with a terminal 3′-$NH_2$. A summary of the sequences and their alignment to the target sequence are shown in Fig. 2. For initial studies, a set of capture probes for a marker 8 that varied in length from 6 to 16 nucleotides were prepared for both the insertion and deletion alleles. These probes were evaluated using synthetic target sequences for the insertion and deletion alleles and a common set of stacking probes that varied in length from 10 to 50 nucleotides.

Target sequences, based on the natural alleles, are summarized in Table 1. Two target sequences per allele were prepared and correspond to either the insertion or the deletion allele. These sequences either contain or lack a short (3–14) nucleotide segment. Capture probes to the alleles were also designed. In general, the probes were selected to contain the polymorphism in the central portion of the probe. In this fashion, a common stacking probe can be used to hybridize to either allele of a particular marker. The sequences of the capture and stacking probes are summarized in Table 2. The capture probes varied in length from 6 to 10 nucleotides so as to narrow the range of melting temperatures resulting from duplexes formed from these probes.

### Amplification of Polymorphism 13 from Genomic DNA Sample by PCR

The polymorphic region of the appropriate allele was amplified by PCR from human genomic DNA as described by Weber and May [23]. The amplification was performed in volumes of 100 µl containing 50 mM potassium chloride, 10 mM Tris, pH 8.4, 1.5 mM magnesium chloride, 0.15 µM each primer, 200 µM each deoxyribonucleotide triphosphates (dATP, dCTP, dTTP, and dGTP), 50 ng of genomic DNA, and 0.5 units of *Taq* Polymerase (Perkin Elmer). The cycling reaction was done in a Perkin-Elmer 9600 thermocyler programmed for 1 cycle of 5 min at 95°C, 75 s at 55°C, 1 min at 72°C followed by 29 cycles of 30 s at 95°C, 75 s at 55°C, 60 s at 72°C, followed by a final 5 min hold at 72°C. About 10 µl from each amplification was resolved by electrophoresis in 2% agarose gels and detected by staining with ethidium bromide.


**Fig. 2** Strategy for detection of polymorphisms by stacking hybridization. The DNA target should anneal with a labeled stacking probe (upper case letters, note that the stacking probes are common to both the insertion or the deletion alleles). The capture probes (lower case letters) are designed to hybridize to the target DNA and form a contiguous strand with the stacking probe. Base stacking interactions between the longer stacking probe and the short capture probe allow for increased hybridization stability when binding target

## Hybridization Substrates

Channel glass, obtained from Galileo Electro-Optics Corp. (Sturbridge MA), was prepared for probe attachment by soaking for 15 min in 0.1 N HCl, followed by three washes in deionized water. The glass microscope slides were soaked for 15 min in hexane, followed by three washes in deionized water. Both were then dried in an 80°C oven for several hours. If the attachment reaction was not to be performed immediately, they were kept dry in a desiccator under vacuum at room temperature until the oligonucleotide probes were attached.

## Oligonucleotide Probe Printing and Immobilization

The 3′-$NH_2$ oligonucleotide probes were dissolved to a final concentration of 20 μM in deionized water. An aliquot of 10 nl of each probe was applied in duplicate to the solid supports using a Microlab 2200 workstation (Hamilton Company, Reno, NV) equipped with a precision x–y substage and a solenoid valve-based ink jet using a sapphire dispense tip as described by Hicks et al. [24]. The applied droplets were allowed to air dry, and both slides and channel glass were rinsed three times with deionized water at room temperature to remove unbound oligonucleotides.

## Preannealing

In order to introduce a label into the target, the synthetic target was first annealed to a long (30 bases) stacking probe that carries the label and hybridizes to a position adjacent to the polymorphism. Target DNA was annealed with five times the amount of stacking probe in 100 μl of 5× SSPE buffer (1× SSPE is 0.75 M NaCl, 50 mM $NaH_2PO4$, 5 mM EDTA, pH 7.4, 5% (w/v) polyethylene 8000). The annealing was performed in a thermocycler program of

**Table 1** List of polymorphic sequences studied

| Marker | Synthetic target sequence 5′ → 3′ |
|---|---|
| 1I TCTT | AGTAAGGG*TGAC*TCTT**TATTTAAACTAAAAACATTGGTAATATACA**AATTT |
| 1D | CAGTAGTAA*GGGTGAC***TATTTAAACTAAAAACATTGGTAATATACA**AATTT |
| 2I TTC | GTGCTGA*TAAACA*TTC**TTCTTATGGTTCCAGCCCCTACTTTAGTTA**TTTTC |
| 2D | AACGTGC*TGATAAACA***TTCTTATGGTTCCAGCCCCTACTTTAGTTA**TTTTC |
| 3I GGTGGA | CTAGGGGGA*GGGTGGA*GGTGGA**GGTAGGTTATGGGACAGAGAGGAC**AAGAA |
| 3D | GGCTGGCTAG*GGGGGAG***GGTGGAGGTAGGTTATGGGACAGAGAGGAC**AAGAA |
| 4I AAGAT | TCATATGT*AACA*AAGAT**AAGGACGTGTGCAGGTATAAAACAGAGGCAG**AATCA |
| 4D | GTATTTCA*TATGTAACA***AAGGACGTGTGCAGGTATAAAACAGAGGCA**GAATCA |
| 5I CAAT | TGCACAG*AATAG*CAAT**CAATCAATCAGTCATGTCAATAAAAATAAA**ACAAT |
| 5D | TAAGTGCA*CAGAATAG***CAATCAATCAGTCATGTCAATAAAAATAAA**ACAAT |
| 6I AACA | GCTTCATC*CTCT*AACA**AACAGTGTACACTCCCAGAGCTGATATTCT**GGATT |
| 6D | ATGTGCTT*CATCCTCT***AACAGTGTACACTCCCAGAGCTGATATTCT**GGATT |
| 7I GTT | TATTTAG*GCCA*GTT**GTTGACAGCCACATTATTTTGAGGTGTGGC**TACTT |
| 7D | GTTTATT*TAGGCCA***GTTGACAGCCACATTATTTTGAGGTGTGGC**TACTT |
| 8I TGTTT | CGGGTTGAA*TCC*TGTTT**TGTTTTGTTGTCCCCATCCCTGATGACACA** |
| 8D | CACAACGGG*TTGAATCC***TGTTTTGTTGTCCCCATCCCTGATGACACA** |
| 9I GAA | CAGAACT*GCCTT*GAA**GAAAAGGAATGGACAAGTGGCTTTATTGTA**AAAAT |
| 9D | ACTCAGA*ACTGCCTT***GAAAAGGAATGGACAAGTGGCTTTATTGTA**AAAAT |
| 10I AGA | TGCAACA*GAATTC*AGA**AGAAGAGTCTTAAGGAGGTGATTAGGCCAT**GAGTT |
| 10D | AAATGCA*ACAGAATTC***AGAAGAGTCTTAAGGAGGTGATTAGGCCAT**GAGTT |
| 11I TAT | TACTCT*TAATGTA*TAT**TATTTCATATTTGTTTAACAAAAGCAGCTT**GATGC |
| 11D | ATCTACT*CTTAATGTA***TATTTCATATTTGTTTAACAAAAGCAGCTT**GATGC |
| 12I AGGCATGAACAAAT | GTCACAAAGGGA*AGGCATGAACAAAT***CTTGCCACTCAGTCCCACACAGGGCAGCTG**TTTCA |
| 12D | TCAGGGTTCTTGAAGTCA*CAAAGGGA***CTTGCCACTCAGTCCCACACAGGGCAGCTG**TTTCA |
| 13I CAACAT | AAATTAACAG*GA*CAACAT**CAACATTTGTCCACCTGTGAATAATGGTCA**CTAAT |
| 13D | GCCTATAAAT*TAACAGGA***CAACATTTGTCCACCTGTGAATAATGGTCA**CTAAT |

Letters shaded in underline represent the polymorphism, letters in italics represent detection region of capture to the target, and the letters in bold represent stacking probe annealing

92°C 5 min, 65°C 30 min followed by a final cooling at 6°C. About 50 µl was immediately hybridized. For flat glass, the annealing solution was concentrated to 10 µl and hybridized to the array. For the channel glass, the annealing solution was prepared in a volume of 250 µl.

## Hybridization on Flat Glass

A 10-µl aliquot of the target solution was applied to the area of the slide containing the array covered with a cover slip and incubated for 12 h at room temperature. The cover slips were removed and then the slides were washed with 1× SSPE solution at 15°C for a minimum of 5 min. Each hybridization experiment was carried out with duplicate probes (per spotted array) and triplicate slides hybridized simultaneously. Thus, each hybridization result represented an average of six parallel determinations.

## Hybridization on Channel Glass

The annealed duplex (target DNA-stacking probe) was placed into the chamber and allowed to flow with forward and backward flow rates of 0.05 ml/min or 0.1 ml/min using a syringe pump for 5 min at different temperatures. After hybridization, the glass was washed for about 30 s with cold 5× SSPE. For this, a chamber for flow-through hybridization was fabricated by Eastern Plastics Inc. (Bristol, CT) using a custom design (Fig. 3). This chamber holds a single 12 × 12 mm channel glass inside a sealed gasket. Small channels (0.020″) are machined and laminated within the gasket to bring the hybridization and washing solutions to and from the chip (see Fig. 3). These fluid passages traverse in three dimensions to bring the fluid entry and exit points to the same face of the holder. This allows for easier assembling and for flush mounting of the chamber to the hybridization detection instruments. An

**Table 2** List of capture and stacking probes sequences

| Marker | Capture probe 5′ → 3′-$NH_2$ | | Stacking probe 5′-Cy3 → 3′ |
|---|---|---|---|
| 1 | d | GTCACCC | TGTATATTACCAATGTTTTTAGTTTAAATA |
| | i | AAGAGTCA | |
| 2 | d | TGTTTATCA | TAACTAAAGTAGGGGCTGGAACCATAAGAA |
| | i | GAATGTTTA | |
| 3 | d | CTCCCC | GTCCTCTCTGTCCCATAACCTACCTCCACC |
| | i | TCCACCC | |
| 4 | d | TGTTACATA | TGCCTCTGTTTTATACCTGCACACGTCCTT |
| | i | ATCTTTGTT | |
| 5 | d | CTATTCTG | TTTATTTTTATTGACATGACTGATTGATTG |
| | i | ATTGCTATT | |
| 6 | d | AGAGGATG | AGAATATCAGCTCTGGGAGTGTACACTGTT |
| | i | TGTTAGAG | |
| 7 | d | TGGCCTA | GCCACACCTCAAAATAATGTGGCTGTCAAC |
| | i | AACTGGC | |
| 8 | d | GGATTCAA | TGTGTCATCAGGGATGGGGACAACAAAACA |
| | i | AAACAGGA | |
| 9 | D | AAGGCAGT | TACAATAAAGCCACTTGTCCATTCCTTTTC |
| | i | TTCAAGGC | |
| 10 | d | GAATTCTGT | ATGGCCTAATCACCTCCTTAAGACTCTTCT |
| | i | TCTGAATTC | |
| 11 | d | TACATTAAG | AAGCTGCTTTTGTTAAACAAATATGAAATA |
| | i | ATATACATTA | |
| 12 | d | TCCCTTTG | CAGCTGCCCTGTGTGGGACTGAGTGGCAAG |
| | i | ATTTGTTCA | |
| 13 | d | TCCTGTTA | TGACCATTATTCACAGGTGGACAAATGTTG |
| | i | ATGTTGTC | |

i = insertion, d = deletion

optically clear acrylic window is laminated to the structure for visualizing the flow of the liquid. This style of hybridization chamber is easily connected to conventional narrow bore tubing.

## Imaging

Fluorescent signals were captured by a GeneTAC 1000 analyzer system with GT Imaging & Review software (Genomic Solutions, Ann Arbor, MI). The GeneTAC imaging system has a dynamic range of 2–3 logs and displays detection linearity when the average pixel intensity is between 5,000 and 50,000. Each support was imaged for a period which yielded average pixel intensity for the brightest spots of slightly below 50,000 (typically 30,000). For each hybridization result the average pixel intensities for the six determinations were averaged, and after subtraction of background signal (average pixel intensity typically <100) the hybridization result was assigned a qualitative intensity value of 'strong' (average pixel intensity 10,000–50,000), 'weak' (average pixel intensity <10,000 but typically >5,000), or 'absent/undetected' (average pixel intensity <1,000). To statistically compare the hybridization patterns obtained with the three spotted slides in each hybridization, SigmaStat version 2.03 was used for Friedman repeated measures analysis of variance on ranks. This non-parametric test consistently yielded

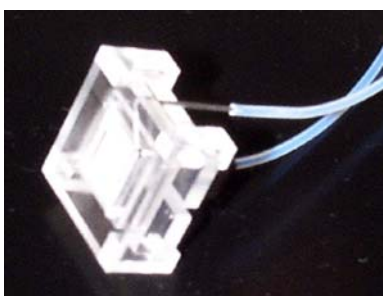

**Fig. 3** Photograph of chamber used for flow-through hybridization

*P*-values of 1.000 for replicate hybridization intensities, thus the differences between experimental replicates were not statistically significant, supporting the conclusion that these hybridization experiments were highly reproducible.

## Results and Discussion

Channel glass is a high-surface area substrate that offers substantial advantages in hybridization assays [6, 12–18]. To quantify these advantages, a model DNA diagnostic system based on stacking hybridization and diallelic markers was used. In the stacking hybridization approach, a short "capture" probe is immobilized onto the surface of the sensor. These immobilized probes capture target DNA sequence that has been previously annealed to a "stacking" probe. The stacking probe binds to the region adjacent to the capture probe, allowing for contiguous base stacking between the capture and stacking probes. Previous work has demonstrated the advantages of stacking probes for hybridization-based analyses [25–27]. A key benefit is an increase in hybridization specificity and stability. Compared to direct hybridization with short (<20 nucleotides) oligonucleotide probes, the thermodynamic stability of a perfectly matched complement compared to a mismatched complement is improved [28–30].

A set of 13 diallelic containing genetic sequences was selected and used to evaluate the advantages offered by the combination of stacking hybridization and channel glass in genosensor-based diagnostic screens. Target sequences, based on natural alleles, were synthesized; two target sequences per allele correspond to either an insertion or deletion allele. These sequences either contain or lack a short (3–14) nucleotide segment. Capture probes to the alleles were also prepared. These capture probes were designed such that their 5′-end is diagnostic of the insertion or deletion. In this fashion, a common stacking probe can be used to hybridize to either allele of a particular marker.

Initial DNA hybridization studies were performed on channel glass to examine the influence of the length of the capture and stacking probes on hybridization efficiency. Using marker 8, a set of capture probes that varied in length from 6 to 16 nucleotides were prepared for both the insertion and deletion alleles. These probes were evaluated using synthetic target sequences for the insertion and deletion alleles and a common set of stacking probes that varied in length from 10 to 50 nucleotides. Using a standard set of hybridization conditions, the hybridization efficiency varied depending on the length of the capture probe. Capture probe lengths of less than nine nucleotides worked poorly when compared to longer capture probes as seen in Fig. 4. However, probe lengths of greater than 15 nucleotides led to non-specific hybridization. These longer probes show cross hybridization to the other allele. These results were independent of the length of the stacking probe. However, the shorter stacking probes (10 and 15 nucleotides) were less efficient in capturing the target than those between 20 and 40 nucleotides (Fig. 5). The longest stacking probe evaluated, 50 nucleotides, also showed a lower ability to capture the target sequence.

Hybridization results for the entire set of 13 diallelic markers are shown in Fig. 6. Also displayed is a comparison of hybridization performance on channel glass and conventional glass substrates. In each comparison, an equivalent quantity of each synthetic target was used and applied to either the channel glass or the microscope slide surface. However, the volume of the hybridization solution was 25-fold greater for the channel glass, reducing the target concentration an equivalent amount. For the flow-through experiments, the target solution was passed and returned through the channel glass one time over the course of 20 min. For the microscope slides, the target solution was simply incubated under a cover slip for an equivalent period. Despite the significantly lower concentration, the channel glass shows much higher detection sensitivity. Detectable hybridization is observed using a 10-fold lower amount of target molecules (25 fmol of a 50-pM solution). Longer incubation times improved the hybridization sensitivity on both substrates (data not shown). For example, 25 fmol sensitivity is observed on the microscope slide after a 24-h hybridization time. By extending the hybridization time to 1 h on channel glass, detectable hybridization is improved to 5 fmol.

As seen in the Fig. 6, the different markers do not hybridize with similar efficiency even when using synthetic target sequences at known concentrations. General correlations between melting temperature and probe intensity are not evident nor do changes in the hybridization conditions yield significantly different results. Using channel glass, the optimal hybridization temperature was evaluated for markers: 2, 5, 8, 9 and 13; using PCR to amplify the target alleles. Figure 7 displays the results when using a quantity of 0.5 pmol of each PCR product as the target material. Of the five temperatures evaluated, the maximum signal intensity was observed when hybridization occurred at 15°C. The signal intensity drops off significantly when hybridizing at higher temperatures.

The lack of discernable signal for the other markers can be due to several factors that require additional optimization. Presumably, the re-hybridization of the PCR products prevents hybridization to the immobilized probe [31, 32]. Cross hybridization of an allele to probes for other markers was also problematic. The sequence design of the probes is an important issue Computer programs have emerged and focus on minimizing the probability of non-specific (mismatched) hybridization between the probe and nucleic acid

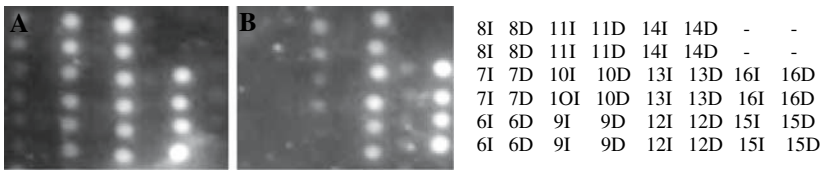


**Fig. 4** Hybridization pattern for marker 8. Each probe was spotted in duplicate onto the channel glass. The patterns of fluorescence are shown on the left and an alphanumeric representation of the orientation of each probe on the channel glass is shown on the right. Hybridization signals correspond to either the insertion (**A**) or the deletion (**B**) allele

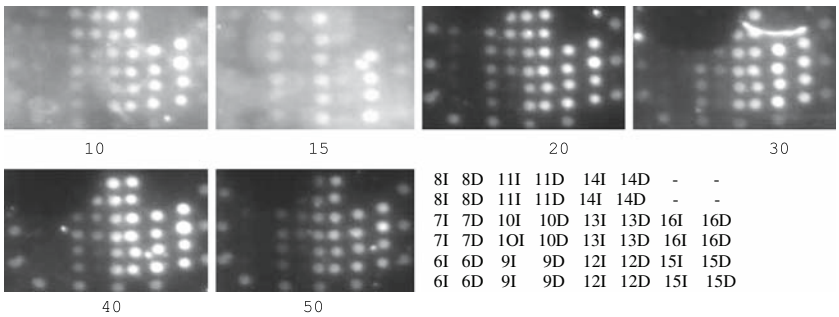


**Fig. 5** Effect of the length of the capture probes and the stacking probes on hybridization. Each probe was spotted in duplicate onto the channel glass. Capture probe lengths of less than 9 nucleotides showed weak experimental hybridization signals. However, the shorter stacking probes (10 and 15 nucleotides) were less efficient in capturing the target than those between 20 and 40 nucleotides. Also, the longest stacking probe (50 nucleotides) showed a lower ability to capture the target sequence. The numbers under each image correspond to the length of stacking probe. The alphanumeric representation shows the orientation each probe on the channel glass

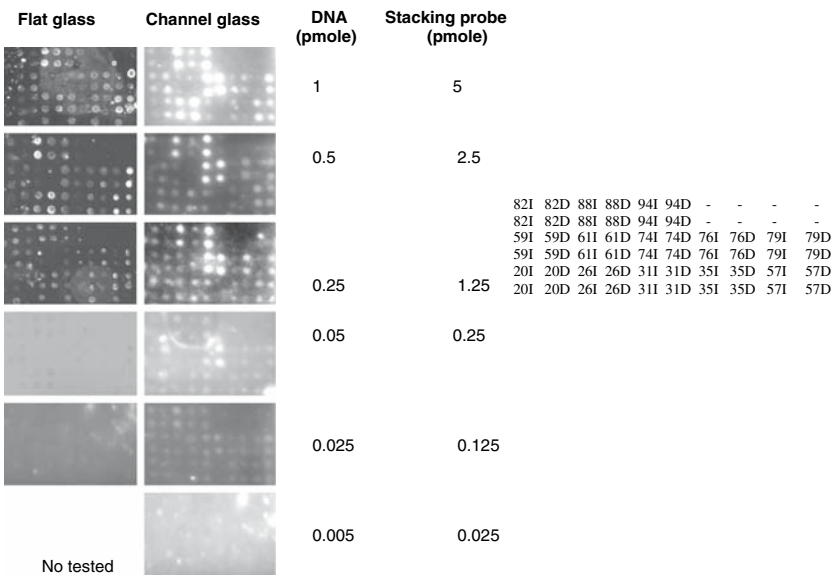


**Fig. 6** Comparison between flat glass and channel glass for hybridization of a synthetic DNA target to an array of probes. A detection limit of 125 fmol was obtained using channel glass and flow-through hybridization. Channel glass also allowed for a substantial reduction in the reagent and sample volumes while reducing the hybridization time to 5 min. By contrast, 12 h was required for detecting barely 250 fmol in the flat glass system. The alphanumeric representation of the orientation each probe on both substrates is shown on the right

sequence other than the intended target site [33]. These programs which can be used for selecting the most suitable probes for microarrays based upon several factors that could affect the hybridization process. These factors include thermal stability, secondary structure, and alternative binding sites within the nucleic acid analyte. A complete understanding of the thermal stability of hybrids formed between probes and nucleic acid molecules requires information about the energetic contributions for all the possible interactions that can take part in the hybridization process [32, 34, 35]. However, it is possible to find optimal hybridization conditions for specific binding of any given probe with its target molecule, but when the hybridization reaction is carried out with numerous probes and target molecules (as with microarrays), a loss in specificity can occur [36–38].

A comparison of the detection sensitivity observed for one of the markers (13) is shown in Fig. 8. As seen, the

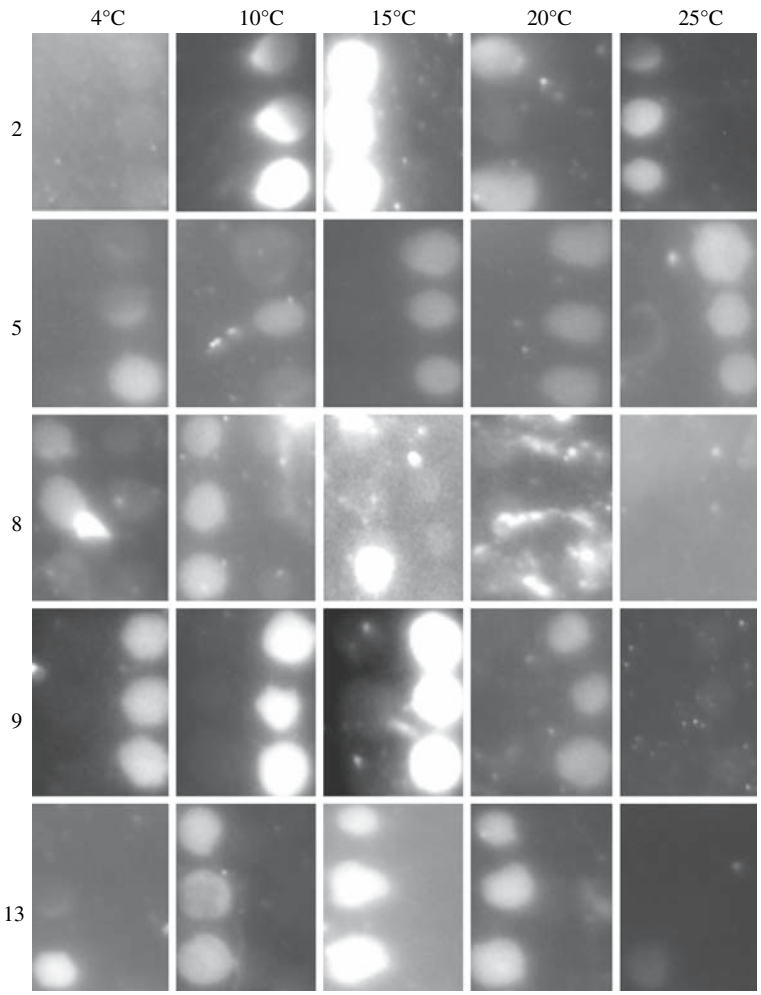


**Fig. 7** Effect of the hybridization temperature on detection of SIDPs. In all cases, channel glass was used as well as tandem hybridization

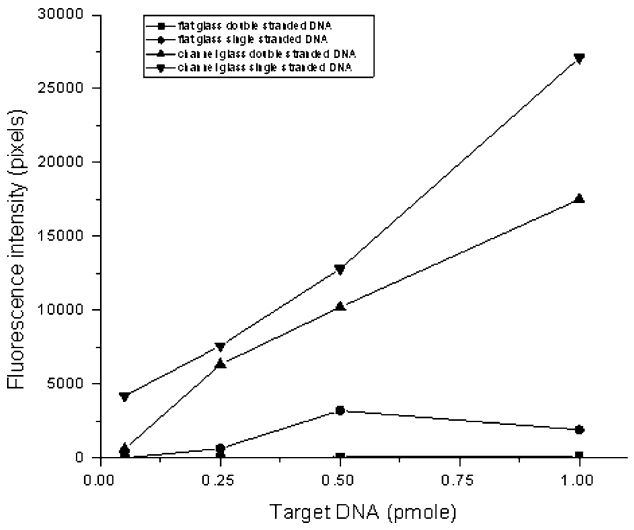


**Fig. 8** Comparison of the detection sensitivity for marker 13. As seen from the graph, channel glass was more efficient compared to flat glass; likewise, the signal intensity is lower when double stranded DNA is hybridized

signal intensity increases with increased concentration of target sequence for both substrates. However, the normalized signal intensity is several fold higher when using the flow-through substrate. Also shown is the concentration dependent signal when using a double stranded target. For these experiments, a 161-bp amplicon of the target allele was prepared from genomic DNA. The signal intensity is generally lower when compared to the synthetic single stranded target. The hybridization is barely detectable using a quantity of 1 pmol and a microscope slide as a substrate. With the channel glass substrate, DNA hybridization is easily detected and concentration dependent.

## Conclusion

The channel glass structures used here allow for a greatly increased surface area and for sample flow to occur through the substrate. These features enhance the speed and sensitivity of DNA hybridization reactions when compared to planar glass substrates. Additionally, stacking hybridization

was employed to increase the stability and specificity of complexes formed from short capture probes. Capture probes of 12–14 nucleotides worked best while the optimal length of the stacking probe was found to be ~40 nucleotides. Further design of effective hybridization probes is needed. Nevertheless, channel glass substrates can play an important role in fast and effective genotyping strategies.

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