the DNA polymerase (Thermo Sequenase). For this purpose, a special four-colour instrument, FD-003, was developed at Asper Ltd. The latter uses a total internal reflection fluorescence (TIRF)-based excitation mechanism combined with four lasers, filter wheel and scientific grade CCD camera. Image acquisition is fast (0.5–2 min for 4-colour) and automatic, followed by software analysis to convert the fluorescence information into DNA sequence data. Signal-to-noise ratio is at least 30:1, allowing comfortable analysis of heterozygous positions in DNA. This system is used for mutation detection in β -globin, BRCA1 and BRCA2 genes, resequencing of p53 gene fragments, SNP testing and gene expression using mtComplex 1 genes.

Mills, Jr, Allen

Interconversion of electrical and DNA data for molecular computation

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To make practical use of the large-scale computing possibilities associated with a molecular computer based on DNA hybridization¹ it will be necessary to quickly convert data back and forth from electrical to molecular form. In a DNA computer configured as an analogue neural network² we will let a subset of all single-stranded DNA q -mers E_i be in 1:1 correspondence with the basis vectors e_i , $i = 1, 2, \dots, m$ in the abstract m -dimensional vector space used to represent the network. The concentration $[E_i]$ of strands with index i in a DNA sample representing a vector $V = \sum V_i e_i$ is proportional to the amplitude V_i of the i -th component of the vector.

Suppose that input data are presented in the form of images of $n \times n = m$ pixels. The images may be flashed on a DNA microplate³ whose pixels match the image pixels and each of which is covered with unique DNA strands E_i attached by hybridization to complementary anchored strands. The image flash causes local heating⁴ that melts some of the double-stranded DNA. The liberated single stranded DNA is subsequently collected to yield a solution containing a concentration of E_i molecules proportional to the image intensity at the i -th pixel for $i = 1, 2, \dots, m$. The DNA image solution may be used for DNA computation and may be reconverted to electrical form at any time, that is "read out", by allowing a portion of the solution to rehybridize with a single-stranded microarray. The amount of duplex DNA at each pixel may be determined by fluorescence analysis in the usual fashion to reconstitute the original image.

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Mir, Kalim U.

What length of probe is optimal for microarrays?

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DNA microarrays, comprising spotted PCR products of 0.6Kb or above, have become a major platform for functional genomics. It is considered that array performance could be improved by turning to oligonucleotides instead of cDNAs and by controlling the site of attachment. Furthermore, to bring the

approach within the reach of methods for combinatorial synthesis, the length of the probe needs to be minimised.

However, under the low stringency conditions required for short nucleic acid probes to form stable interaction, the longer single-stranded targets are able to engage in secondary structure. This can mask the desired interaction between target and probe.

We find that the extent of interaction of target with short complementary probes varies sharply even at adjacent positions. The factors that affect hybridisation have been studied using combinatorial arrays and the well-characterised yeast tRNA^{phe} as a model. We find that heteroduplexes giving high hybridisation yield incorporate target single- and double-stranded portions and that specific structural features may increase or decrease yield.

We have conducted studies to find the optimal length of probe for achieving efficient hybridisation across all target sites in nucleic acids. A combinatorial "scanning" array was synthesised *in situ* comprising a set of probes which ranged in length from 1 to 76 nucleotides—a length that is within the constraints for efficient oligonucleotide synthesis.

We find that the effects of secondary structure can be overcome with probes beyond a specific length when hybridisation is performed within a specific temperature range and in an appropriate buffer.

DNA has a negatively charged backbone that requires cations to form a duplex. We have also studied an alternative to DNA, the Peptide Nucleic Acids (PNA) which have a neutral backbone. An array of PNA probes complementary to tRNA^{phe} was used to study the hybridisation behaviour of PNA in comparison to DNA and to see if hybridisation could be enhanced (in collaboration with J. Hoheisel, S. Wuerz, S. Matysiak).

Our work has demonstrated that the molecular basis of target-probe interactions can be exquisitely studied using combinatorial arrays and that this is an important resource for developing and optimising applications of array technologies.

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Very large-scale arrays of biomolecules

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ArrayChips are semiconductor devices that enable rapid and flexible fabrication of very large-scale arrays of biomolecules. Array fabrication is controlled by electronic hardware on the chip and is software configurable. This feature enables very rapid turnaround times of new array designs for testing and optimization. Biomolecules in the array are synthesized *in situ* or immobilized at selected addressable locations. Synthesis occurs directly within the interstices of a hydrated and highly porous membrane that overlays the ArrayChip. Synthesis is accomplished *in situ* using reagents that are generated electrochemically at individually addressable microelectrodes on ArrayChips. We have developed technologies for *in situ* synthesis of arrays of oligonucleotides, peptides and small molecules. ArrayChips obviate any need for microfluidics or other unconventional semiconductor hardware. First-generation ArrayChips have microelectrode array densities of over 1,000 individually addressable sites per cm². Second-generation hardware designs have over 500,000 individually addressable sites per cm².