the not-for-profit SNP Consortium.

Of the more than 4 million SNPs mapped so far and deposited in public and private databases, only 3% are within genes. "People are not interested in all the polymorphisms; they want to look at those that are functionally relevant," says Dennis Gilbert, vice-president of genomics applications at Applied Biosystems in Foster City, California. This generally means those SNPs within genes and their regulatory regions. The company's Assays-by-Design service offers a route to this goal — it delivers pre-tested, ready-to-use assays for a customer's chosen SNPs.

Customers send in their target DNA sequence, with the SNP locations indicated. Applied Biosystems then develops optimal primers and 'TaqMan' probes to detect both alleles of the selected SNP sequences. Customers receive the probes for each SNP in a single tube with a two-dimensional bar code for sample tracking.

The assay is designed to run on Applied Biosystems' Sequence Detection Systems, real-time quantitative instruments for the polymerase chain reaction (PCR) that use the company's TaqMan probe technology. The use of a non-fluorescent quencher dye increases assay performance to the point at which it is possible to score 1,000 samples for 250 SNPs — that is, 250,000 genotypes — in a day, the company says.

Applied Biosystems also sells complementary off-the-shelf assays and currently has more than 120,000 validated assays for SNPs in and around genes for linkage disequilibrium mapping studies. "You can go to our public website and type in a gene or region of the genome, and get a whole list of SNP assays for your association studies," says Gilbert.

The more the merrier

For those intending to genotype on a massive scale, Illumina of San Diego, California, has recently launched its hybridization-based BeadArray system. Each high-density array of probe-coated microbeads fits into a well of a standard 96-well microtitre plate and can simultaneously detect up to 1,152 different SNPs in a single DNA sample. The fully automated system can process more than a million assays a day. And reduced reagent volumes mean considerable savings in running costs, says Jay Flatley, Illumina's president and chief executive. "The strategy is to bring down the costs of high-throughput and highly accurate genotyping," he says.

The first system was sold to Génome Québec, a member of the International HapMap Project which is mapping SNP haplotypes in the human genome (see 'Haplotypes or pools?', page 921). The UK arm of HapMap, at the Wellcome Trust Sanger Institute in Hinxton near Cambridge, will also use Illumina's technology, and last autumn the US

APPLERA

Dennis Gilbert: online shopping for SNP assays.

National Institutes of Health awarded the company $9 million to genotype 15% of the HapMap itself.

Illumina's million-dollar system is beyond the means of most academic researchers, but using the company's custom services, a screen for 2,000 SNPs, for example, would cost some 35 cents for each SNP assayed. "As the studies get larger the price drops considerably," says Flatley. "People who run our system can genotype at less than a nickel a data point."

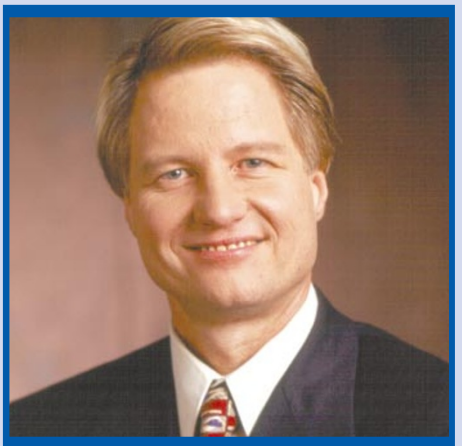
The company has also developed a fine-mapping product — an extensive set of validated SNP assays — from a large genotyping study in collaboration with

RISK ASSESSMENT

THIRD WAVE TECHNOLOGIES

Even small numbers of single-nucleotide polymorphisms (SNPs) can work as predictive tools to assist clinical decisions, and this is the area that interests Third Wave Technologies. Based in Madison, Wisconsin, the company has set low-volume SNP-genotyping tests for individual diagnostics as its priority.

"We started out in the cardiovascular arena and helped to create the field for 'economy-class syndrome' mutation detection," explains chief executive Lance Fors. Third Wave developed a reasonably priced test for mutations in the blood-clotting factors V and II that can trigger venous blood clots in the brain and legs. Women who carry the factor V mutation (V Leiden) and are also taking contraceptive pills are at 100 times greater risk of deep-vein thrombosis and stroke, and today an increasing number of women taking contraceptives are tested for these variants. "It's moving towards mainstream medicine," says Fors. "An individual test for a specific SNP may turn into a $10- or $30-million market."



Lance Fors: a future for predictive testing.

Low false negatives and low false positives are the winning solution in diagnostics, Fors adds. Third Wave's platform uses the firm's Invader RNA Assay. Two probes are designed to overlap or 'invade' each other to make a particular three-dimensional structure. If the mutation is present, there is an invasion and the structure is formed. A nuclease then recognizes this overlapping complex and cuts it. With 'normal' DNA, the probes cannot invade and the DNA remains intact, Fors explains. Structure-specific cleavage is far more precise than conventional methods based on the polymerase chain reaction (PCR), so mistakes are very rare, he points out.

The Invader technology is a one-step assay that simply requires the sample to be added. "Some of our clinical customers could never adopt PCR, because they couldn't afford the equipment or the infrastructure required to minimize contamination," Fors says.

Invader technology can scale up to detecting hundreds of thousands of SNPs, as its fluorescence readout is readily adaptable for high-throughput. It was used to screen 768 Japanese cardiovascular patients for the presence of 92,000 SNPs located in genes that are thought to confer a risk of cardiovascular disease, and to identify a candidate gene associated with susceptibility to heart attack on chromosome 6p21 (K. Ozaki *et al. Nature Genet.* **32,** 650–654; 2002). **L.M.**