chemically functionalized fluorescent dyes. EPICENTRE Biotechnologies in Madison, Wisconsin, has incorporated this approach into its TargetAmp kits for RNA amplification. The company says these kits can deliver up to 5-million-fold amplification, and even allow the study of single cells. "We can get down to 10 picograms of starting RNA," says Shervin Kamkar, a technical-sales specialist at the firm, "so it's really useful for people doing stem-cell work or laser capture."

Designer labelling

Genisphere of Hatfield, Pennsylvania, uses a unique labelling approach for its 3DNA kits that is based on fluorescently tagged DNA-based dendrimers. These contain sequences complementary to 'capture' sequences added to cDNA during sample amplification. Different dendrimers are available with varying quantities of linked fluorophore molecules that determine the limits of detection, down to less than a microgram of starting material. Detection can be further augmented with the company's SenseAmp kits, which use one or two rounds of a non-Eberwine amplification strategy to produce sense-strand RNA. "We've gone as low as 0.1 nanograms of total RNA," says Bob Getts, Genisphere's director of research and development, "which is typically from ten cells."

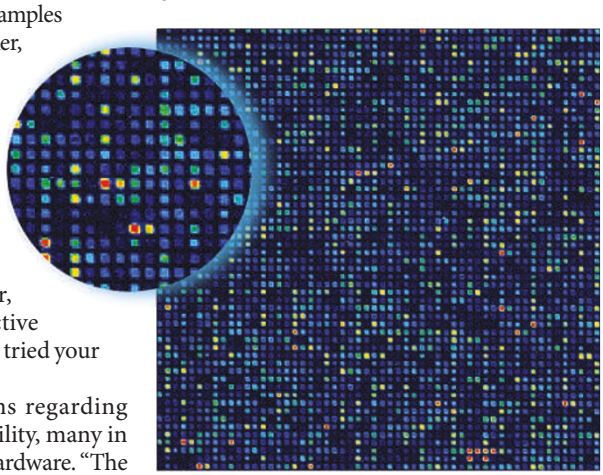
Another problem is posed by the increasing use of microarrays for analysis of clinically prepared formalin-fixed paraffin-embedded (FFPE) samples, such as tumour biopsies. These may have been in storage for anything from months to decades, and the resulting RNA degradation is a serious challenge for reagent designers.

Several companies, including EPICENTRE and Genisphere, are working on this. According to Getts, SenseAmp is well-suited to FFPE work. "We routinely use samples that have been degraded to between 50 and 250 bases long," he says. Array manufacturer Illumina of San Diego, California, offers an alternative with the DNA-mediated annealing, selection, extension and ligation (DASL) assay. This is an adaptation of its GoldenGate genotyping technology that takes advantage of a universal array consisting of large numbers of specific tag sequences in order to quantify PCR-amplified primer-extension products. "It's been shown to work on samples as old as 20 years," says Shawn Baker, Illumina's scientific product manager for gene expression.

Still further optimization will be needed as researchers continue to target smaller and more biologically relevant specimens. "There's just so much variation in the material that you're given," says Kamkar, "and it's hard to know how effective a technology is until people have tried your approach on their own samples."

When asked about concerns regarding microarray experimental reliability, many in the field are quick to defend the hardware. "The microarray instrument itself, if used correctly, is precise and accurate," says Rafael Irizarry, a biostatistician at the Johns Hopkins University in Baltimore, Maryland. "The quality of commercial arrays is improving, whereas their price is dropping, so commercial arrays are supplanting the 'home-brew' approach more and more," says Quackenbush. Different manufacturers have opted for various strategies to improve experimental quality and to minimize opportunities for human error (see 'Hands off!').

John Blume, vice-president of assay and application product development at Affymetrix, cites ever-increasing probe density and genome coverage as a secret of Affymetrix's success. Their Human Genome U133 Plus 2.0 GeneChip microarrays feature 11 different oligonucleotide probes for each transcript, which



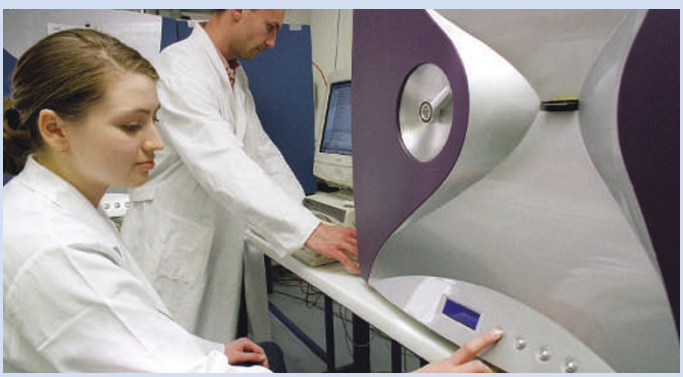
Super-sensitive: high-density arrays of long probes are offered by NimbleGen Systems.

HANDS OFF!

To err is human. Scientists are keenly aware of this, and the multistage nature of microarray experiments provides ample opportunity for the human aspect of experimental error. Robotics could offer a solution, as well as potential for greater experimental efficiency.

"It used to be that the only people who thought about automation were the drug screeners, the diagnostic folks, people who had to do things hundreds of thousands of times," says John Blume, vice-president of assay and application product development at Affymetrix of Santa Clara, California. "But a lot of early-stage discovery-type work now happens at the level of hundreds of things at a time."

Affymetrix uses a modular approach, developing units that automate individual experimental stages. Several other companies have taken a similar path after finding that many customers had already taken matters into their own hands. "A lot of them have already adopted automation infrastructure," says Kevin Meldrum, director of genomics marketing at Agilent Technologies, Santa Clara, California. "They don't want to have to go out and buy a completely new system."



febit's GENIOM automates many steps of microarray experiments.

San Diego-based array manufacturer Illumina offers an 'arrays of arrays' format inherently designed for higher-throughput, and so further automation is a lower priority, although the company offers an AutoLoader that can process the scanning of up to 32 BeadChips — containing up to 256 arrays in total — in 24 hours.

Chip-builder and service provider NimbleGen Systems, based in Madison, Wisconsin, has taken an opposing approach. "Our mission is to make everything automated," says Emile Nuwaysir, vice-president of business development. "So that analysis is monitored and quality controlled by humans, but not run by humans." He projects near-complete automation of the full process by the end of the year.

febit Biotech in Heidelberg, Germany, brings this philosophy to the benchtop with the GENIOM, a system that fully automates most processes, including array synthesis, hybridization and analysis. Current GENIOM arrays are limited to 6,000 features but will soon expand to 15,000, and Peer Stähler, vice-president of marketing and sales at febit, believes that the instrument's speed and flexibility offer benefits for rapid experimental probe design and optimization. "You can easily import and synthesize the results of other people's bioinformatics," he says, "and you can export any capture probe that you have evaluated." M.E.