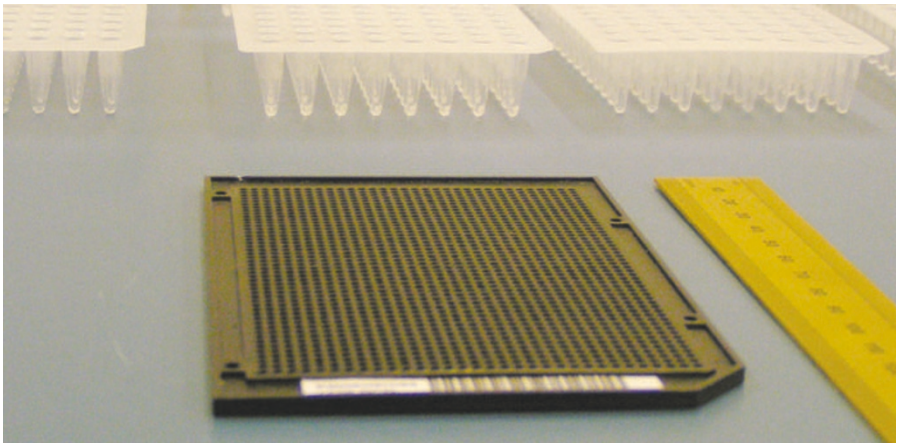
Replicating success

PCR often gets taken for granted, but there are ways of making it faster, more accurate and easier to perform. Pete Moore investigates.

As a means of rapidly copying a selected template sequence from a DNA mixture *in vitro*, PCR by itself and in combination with other techniques has found a vast range of applications. These range from sequence detection and isolation for research, forensics and species identification to detecting mutations and polymorphisms and amplifying RNA-derived cDNAs for microarray analysis of gene expression. As well as standard PCR, the technique now comes in the form of real-time quantitative PCR (real-time PCR or qPCR). This uses fluorescent probes to monitor the amount of product at the end of each cycle, and real-time PCR machines look for the cycle at which they can first detect fluorescence. This relates to the number of copies of original template — the greater the number of starting copies, the fewer cycles are needed to reach fluorescence detection.

PCR can also be used to monitor RNA by adding a reverse transcriptase enzyme at the beginning to generate a DNA template. This reverse transcription PCR (RT-PCR) can then be taken a step further by adding the quantification protocols, resulting in real time RT-PCR.



KBIOSCIENCES

More reactions in the same space with 1,536 wells.

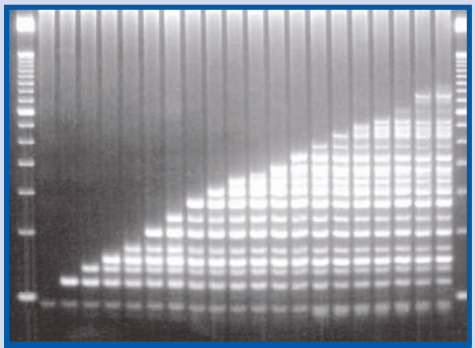
There can be few life-science laboratories without a PCR thermal cycler tucked in a corner, happily churning out short DNA sequences to order with tried and tested protocols. But newer applications for PCR, such as single-nucleotide polymorphism (SNP) detection and screening, need faster throughput, and this is now achievable.

One approach is to abandon the traditional 96-well plate in favour of 384 wells or more. The new high-throughput 7900HT fast real-time PCR system from Applied Biosystems in Foster City, California, takes 96- and 384-well plates and runs a full set of amplification cycles in about 35 minutes. "Our new high-speed system can alleviate some of the burden of instrument sharing by reducing cycling time," says Peter Dansky, senior director and general manager of core PCR at Applied Biosystems. If you need

HOT FROM THE VENT

Isolated from the heat-loving bacterium *Thermus aquaticus*, Taq DNA polymerase launched PCR. It was great, and versions of the original recombinant Taq are still widely used — but its error rate of between 1 in 10,000 and 1 in 50,000 base pairs (bp) is too high for some applications, such as the detection of single-nucleotide mutations. You can now take your pick from a plethora of Taq-based polymerases engineered to have higher fidelity and to go faster. And if damaged DNA is the problem, Restorase from Sigma-Aldrich of St Louis, Missouri, is a mixture of Sigma's AccuTaq blend and a repair enzyme, and works with fragment lengths from 200 to 20,000 bp.

Developers have also gone back to the planet's hot springs and hydrothermal vents to find a new generation of thermostable polymerases with the 3'–5' exonuclease proofreading capacity that makes for higher accuracy. The archaeal genus *Pyrococcus* has been mined for high-fidelity DNA polymerases with accuracies some 40 to 50 times greater than Taq. *P. abyssi* is the source of the Isis proofreading DNA polymerase from Qbiogene of Irvine, California, with an error rate of one mismatched base per 1.5 million bases per duplication. Other hot offerings come from Stratagene, of La Jolla, California, which claims an error rate of 1 in around 660,000 for its *Pfu* DNA polymerase from *P. furiosus*, while Bio-Rad of Hercules, California, has the highly processive iProof High-Fidelity DNA polymerase, a *Pyrococcus*-type polymerase fused to a double-stranded DNA-binding protein to give additional grip, which the company claims is 50 times more accurate than Taq. As well as a DNA polymerase isolated from *P. woesi*, Roche Applied Science of Indianapolis, Indiana, offers a thermostable reverse transcriptase from *Carboxydothermus hydrogenoformans* for RT-PCR, and Toyobo Company of Osaka, Japan, supplies a very fast DNA polymerase from *P. kodakaraensis* (now renamed *Thermococcus*).



INVITROGEN

Clean multiplexing with AccuPrime.

Proofreading enzymes are generally more finicky than Taq or enzyme blends. To address this problem, Invitrogen's AccuPrime *Pfx* DNA polymerase is a *P. kodakaraensis* polymerase in a mix containing the company's proprietary AccuPrime accessory proteins (also available as a mix with the company's Taq polymerase), which improve specificity by ensuring that primers only bind to their complementary sequence. The QuantiTect Multiplex PCR kits from Qiagen, of Hilden, Germany, also aim to provide trouble-free multiplex PCR by including a novel reaction chemistry in the buffer that helps avoid competition between PCR products and ensures efficient primer hybridization. **P.M.**