PCR and the 3 Rs

The story goes like this: on a night in late spring in 1984, a young scientist was driving the windy roads of Northern California with his girlfriend, headed to a cabin for a weekend getaway, when he had an epiphany—one that would usher in the genetic revolution and thereby change the world. Kary Mullis, a native of South Carolina with an undergraduate degree in chemistry and a PhD in biochemistry, was working at the Emeryville, CA-based biotech firm Cetus. His daily work involved cloning genes into bacteria to amplify them—a time-consuming and error-prone process, but one that no one had yet been able to improve.

That night, when Mullis pulled the car over to the side of the road, he scribbled down a series of chemical reactions that would become known as the polymerase chain reaction, or PCR. This simple yet elegant technique would be used to make millions of exact copies of genes in a matter of hours. The brilliance of PCR lies in its simplicity: short DNA 'primers' are used in combination with purified DNA polymerase enzyme to rapidly amplify a target sequence through a repeated series of denaturing, annealing, and extension reactions.

Mullis' revolutionary concept earned him the 1993 Nobel Prize for Chemistry and a $10,000 bonus from Cetus—which would eventually sell that patent to Hoffman-La Roche for $300 million. Mullis has developed a reputation as a womanizing surfer who frequently uses LSD and is known to hold a number of unusual and even deeply controversial views: among them, that HIV does not cause AIDS. Despite this negative reputation, Mullis and his work have undoubtedly changed the way biological science is done. The completion of the Human Genome Project would not have been possible without PCR. Cancer researchers use PCR to study the genes responsible for tumor development. PCR-based tests are now routinely used by forensic scientists to solve crimes and have been used to free individuals wrongly convicted of crimes such as murder and rape.

PCR is also becoming an increasingly useful tool to those working in laboratory animal science. PCR-amplified genes can be injected into developing embryos to create transgenic mice. The rat and the mouse are just two of the common laboratory animals that have had their genomes sequenced with the assistance of PCR. Sentinel animals can be checked for viral infections by using PCR.

Now, PCR can be considered a replacement technique as well. In this issue, authors Blank *et al.* (p. 26) describe the use of PCR to replace mouse antibody production (MAP) testing. Prior to use in *in vivo* studies, biological materials must be tested for contamination with infectious agents that might interfere with the experiment. This has traditionally been done by injecting mice (or rats or hamsters in the cases of RAP and HAP tests) with the material in question, waiting up to four weeks, and then testing the mice to see if they had produced antibodies to any of a number of potential contaminating microbes. PCR can now be used to directly test for these agents, eliminating the use of animals, and demonstrating both the versatility of this technique, and how innovations in a surprising variety of research fields can directly contribute to the advancement of animal welfare.