

Book review

DNA Amplification: Current Technologies and Applications

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This book is full of new and clever techniques for DNA amplification and demonstrates how they are pushing the science to new limits. To anyone involved or interested in the field, the book is 'a must read'. For those in healthcare planning, the book would be a useful introduction to the scope these technologies cover and where they will undoubtedly influence future clinical practice, particularly in the field of human genetics.

The book is spread over four sections covering: (1) enzymes used in DNA amplification; (2) thermocycling methods of DNA amplification; (3) isothermal methods of DNA amplification; and (4) DNA amplification in the detection of non-DNA analytes. These sections contain, respectively, three, six, six and two chapters, each section covering a diversity of topics under the umbrella of the section theme. Each chapter also contains a methodology component, which provides detail to complement the theory described.

Topics covered in section 1 include: (a) chimeric DNA polymerases; (b) ϕ 29 DNA polymerase; and (c) high fidelity ligases. The enhanced DNA adherence of the chimeric polymerases resulting from the fusion of DNA binding domains adjacent to the catalytic site are extolled. A major benefit of this molecular add-on is the increased ability of the polymerase to function in a high salt environment, bringing the holy grail of DNA amplification without extraction into view if not quite into sharp relief. The sheer processivity of the ϕ 29 polymerase—70 000 base extensions at one sitting—and the pin-point focus of the ligase enzymes for SNP analysis are well written.

Section 2 covers six topics in all. The chapter on real-time quantification holds no great surprises but I understand the nuances of the technique better now. Two chapters focus on solutions to the conundrum that is multiplexing, each offering a different way through the maze. The chapters addressing long-distance single molecule PCR and digital PCR are reminiscent of the approach taken for selecting single hybridomas when making monoclonal antibodies. The trick is to dilute beyond the estimated end-point to ensure that where amplification takes place (antibody production in the case of monoclonal antibodies) it does so from a single molecule, with attendant advantages. The final chapter, describing PCR on a chip, is very exciting for those of us in the diagnostic world. It presents a version of nested PCR, with the outer primers free in solution and the internal pair tethered through an amino-link to the slide. Amplification in the presence of a fluorophore-labelled nucleotide results in the capture of labelled positive and negative strands

onto the chip surface. Exit the very tricky hybridization step; and hands-on time reduced to 5 min.

Section 3, covering again six topics, introduces us to the very powerful world of isothermal amplification, championed by the strand-displacement principle. Polymerase enzymes that not only chain-elongate but also displace any downstream primers blocking their path (usually of the random hexamer variety) can just keep on going. If their target is cloned into a circular plasmid then the process continues until it exhausts the supply of nucleotides. The technical term of rolling circle amplification (RCA) sums up one variety of this method. Loop-mediated isothermal amplification is a further ingenious solution to the production of almost limitless amplified product, using a single enzyme (large fragment of *Bst* DNA polymerase) and very clever primer design. Our old friend from section one— ϕ 29 DNA polymerase—returns and shows its pedigree for equimolar genome amplification and for generating quality sequence template. No word of NASBA (nucleic acid sequence based amplification) or TMA (transcription mediated amplification) however, although these would be regarded as primarily techniques for amplification of RNA templates.

Finally, section 4 deals with the less common but growing technique of immuno-PCR (IPCR). The basis of IPCR is the replacement of the normal enzyme conjugated anti-species antibody with one conjugated to nucleic acid. In the first example in this section, the antibody is conjugated to a stretch of dsDNA, which then becomes the target for either real-time detection or an enzyme-linked oligonucleotide sorbent assay (ELOSAs). The latter is more akin to a standard ELISA than the real-time protocol, but overall not the approach favoured by the authors. The second form of IPCR uses an RCA approach to generate signal. The conjugate consists of an oligonucleotide antibody complex, with the oligonucleotide priming a circular piece of ssDNA. After the immune complex is formed, a strand-displacement polymerase and nucleotide triphosphates are added to begin the RCA reaction. This process generates the amplification of an elongating ssDNA molecule, which continues to grow until the reaction is stopped. The amplified product remains tethered to the detector antibody and can be detected by incorporation of labelled nucleotides or oligonucleotide probes. This tethering allows microbead and microarray applications to be undertaken.

If one were being churlish, then the book could be held to account for a little individuality of style between chapters. However, I think this corrugation celebrates the individuality of the contributors and infuses the reader with a sense of forward progression through each section. The book is highly readable from start to finish and any churlishness will rapidly evaporate.

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