Impact of pcr (polymerase chain reaction) on biology

PCR- How has this technique revolutionised molecular biology in the last 30 years?

PCR (Polymerase Chain Reaction) has been in existence for several decades now and in that time has become one of the most commonly used of all lab techniques in biology. In its simplest form it is a way of enzymatically replicating a chosen section of DNA *in vitro* , without the need for any living cells. Using minute quantities of starting material PCR can generate large amounts of replicated DNA in an exponential manner and it is now possible to replicate sections of DNA up to 30 or 40 kb in length. From its invention as a somewhat labour-intensive technology, PCR has become more streamlined and user-friendly and now has a vast number of potential applications, not just within biological research but also within the fields of medicine, forensics and archaeology.

Perhaps one of the most important applications for PCR in a lab setting has been its use in molecular cloning strategies. For example, an experiment may require the design of a plasmid which incorporates a reporter gene such as GFP controlled by a mammalian promoter. Prior to the advent of PCR the construction of such a vector would have entailed the almost unfeasible process of trying to isolate enough of the promoter sequence from genomic DNA sources to insert into the vector. Using PCR, it is a relatively simple matter to design primers to amplify the desired sequence and clone it into the vector (Figure 1.). It is even possible to use PCR to give the fragment specific ends which can be cut with restriction enzymes to allow for easier cloning (Voet, D. *et al.* 2005, p71; Alberts, B. *et al.* 2002, p510F). Further refinements to this process include the generation of targeted mutations

within a PCR fragment, used for example when the function of certain regions of promoter or enhancer sequence are to be analysed (Alberts, B. 2002, p540). The ease with which such vectors can be created and modified using PCR has allowed for the generation of transgenic organisms in a way which would have been almost impossible without PCR amplification techniques.

The subsequent generation of transgenic organisms means that there are often large numbers of such organisms which must then be screened to ensure that they have been successfully transformed with the transgene construct. Here too PCR is able to turn the formidable chore of screening large numbers of potential transgenics into a more manageable task. Without PCR it might be necessary to screen such organisms by extracting genomic DNA from each one and subjecting it to the time-consuming and sometimes unreliable technique of Southern blotting (Sambrook, J. *et al.* 2000, 6. 39). Although Southern blotting can show which organisms are hemizygous or homozygous for a particular transgene, it takes several days to complete and is laborious. PCR allows for much faster screening: genomic DNA from each organism to be screened can simply be included in a PCR reaction with primers designed to amplify a part of the transgene, such as the antibiotic resistance gene commonly included in such constructs. This allows genotyping of transgenics in several hours instead of several days and permits non-transformed organisms to be identified and removed much more quickly.

A more complex application of PCR which is now in use in a great many molecular biology labs is that of real-time PCR. This methodology is

employed when levels of gene expression are being investigated as it gives a quantitative measurement of the amounts of RNA being transcribed within a given cell or tissue and thus reveals how active a particular gene is (Provenanzo, M. *et al.* 2007). Although Northern blotting can also be used to quantify gene expression levels it is time consuming and requires a large amount of cellular starting material from which to harvest the RNA (Sambrook, J. *et al.* 2000, 7. 35). In contrast, real-time PCR can be used to examine gene expression levels in much smaller samples and has even been used to analyse gene expression in single cells, something which until recently would have been thought impossible (Brandt, S. *et al.* 1999).

The revolution in molecular biology brought about by PCR has not been confined to the lab. The power and sensitivity of PCR means that it has become a very useful technique in forensic medicine, where it is employed in “ genetic fingerprinting”. The tiny amounts of DNA, often in the order of picograms, which can be recovered from blood, saliva or semen stains can be subjected to PCR analysis to determine whether the bodily fluids came from a particular individual. PCR can be used to amplify short tandem repeat (STR) sections of an individual’s genomic DNA producing a “ genetic fingerprint” which can then be used to eliminate suspects from an inquiry due to the differences in length and frequency of STR regions between individuals (Figure 2.) (Rudin, N. *et al.* 2001, p. 17, 21; Alberts, B. *et al.* 2002 p. 509, 511F). Similarly, the sensitivity of PCR has also found a use in the relatively new area of ancient DNA recovery which involves the recovery and analysis of DNA from fossilised plant or animal tissue. Combining the sensitivity of the PCR technique with the relative stability of the DNA

molecule has allowed DNA to be successfully recovered from a number of ancient sources including fossilized bone material and even ancient bacteria (Pruvost, M. *et al.* 2007; Coolen, M. J. *et al.* 2007). Although both genetic fingerprinting and ancient DNA recovery require less DNA than previously used methods, it is important to note that they are also more sensitive to contamination from other sources of DNA which can obscure results.

Since its inception, PCR has been put to a multitude of uses both within and outside of the lab and it is by no means a static technique. New refinements are being constantly developed such as the more recent TAIL-PCR which allows for easier cloning of DNA regions flanking known sequences (Liu, Y. G. *et al.* 1995). As further applications are developed, it seems likely that this revolutionary technique will remain a crucial part of molecular biotechnology for some time to come.

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