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PCR – the polymer se ch in re ction

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DNA- s s s l [illegible] (PC) s s s l [illegible] . D l 1985 K B. M ll s, PC l [illegible] (DNA) l . M ll s s ll s s [illegible] ll s f s f s l DNA s. , PC [illegible] s. PC s s l l l s f , l, l f s fi l s. s l B f s s s f PC s f s s. [illegible]

The basic components of PCR include a thermocycler, template DNA, primers, reaction buffer, free nucleotides, polymerase, salt and water (free from nucleases and contaminating DNA). Nearly any DNA region can act as a template for PCR amplification if the base sequences either side of the target region (. ., the particular DNA sequence sought) are known. [illegible] No 35 describes the general structure of DNA and some essential terminology. Within a typical PCR analysis the target region can be copied to almost any extent by repeating a simple three step process (amplification cycle): denaturation, annealing and elongation (Fig. 1). In theory PCR appears simple but, with numerous variables to consider, PCR can be difficult to optimise.

A –

Here the double strands of the DNA helix (dsDNA) are separated by 'melting', a process analogous to pulling apart the opposing teeth of a zipper (Fig. 1A). The denaturation temperature (. .,

90–94 °C) breaks the hydrogen bonds between the base pairs adenine (A) and thymine (T) and between cytosine (C) and guanine (G). The time and temperature required to denature dsDNA is contingent on the DNA sequence. DNA with a large number of GC base pairs requires a higher melting temperature than that with a high AT content. A higher temperature is required because GC base pairs have three hydrogen bonds whereas AT pairs have two. However, denaturation does not break the bonds between the deoxyribose sugars and the nucleoside bases, or between the sugars and phosphate entities. Thus released, the single-stranded DNA molecules (ssDNA), analogous to zipper halves, are left free to act as templates for the amplification cycle.

) B – l

Following denaturation, the temperature is reduced to the range 55–70 °C to allow PCR primers to bind ('anneal') to the ssDNA (Fig. 1B). Primers are short single strands of nucleic acid. Two sets of primers are designed to bind to complementary segments on either side of the target DNA, with their 3

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number of acronyms for PCR variations is also huge and causes much confusion. For example, the acronyms for real-time PCR, reverse transcriptase PCR and real-time reverse transcriptase PCR are all similar and can be perplexing! Moreover, real-time PCR has a variety of alternative names including kinetic PCR (original name), quantitative PCR and quantitative real-time PCR. A standardised nomenclature system has yet to be agreed.

1 DNA – an analytical chemist's view,

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