



PCR – the polymerase chain reaction

Analytical Methods Committee, AMCTB No 59

DNA- s s s l }
) l l l) l s) (PC)
 s s s l) .D l 1985 K B.
 M ll s, PC l)) l (DNA)
 l . M ll s s) ll s s)
 ll s f s f s l) DNA) s , PC
)) s. PC s s l l l) s)
 f ,) l l f s f l s. s l B f
 s) s s f PC s f) s.



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 (i.e., the particular DNA sequence sought) are known. 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.

1.1 Denaturation

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 (i.e.,

Published on 10 December 2013. Downloaded on 12/12/2013 12:31:12.

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

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.

F }

1 DNA – an analytical chemist's view, ,

original the

455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000