

deg/yr (see Table 1), more than 35000 times larger than the precession rate of the orbit of the planet Mercury. As this change of the longitude of the periastron gradually allowed us to see the orbit in different orientations, the measurement of a second relativistic parameter γ which combines the time dilation effects due to transverse doppler shift and gravitational redshift, became possible. With these two measurements the orbit could be completely solved for, and the masses of both the components determined. The mass of the pulsar turned out to be 1.442 ± 0.003 solar masses⁵, remarkably close to the Chandrasekhar limit, at which the degenerate core of a massive star is expected to collapse and form a neutron star. This stands as the most accurate measurement of the mass of a neutron star till today. The mass of the companion, too, is very close to this, 1.386 ± 0.003 solar masses, which strongly suggests that even the companion is a neutron star. This companion neutron star is not directly visible to us either because it is not functioning as a pulsar or if it is, its beam of radiation is not directed towards the earth.

The third, and perhaps the most important parameter to be measured for the PSR 1913±16 system was the rate of change of the orbital period, \dot{P}_b . The orbital period is shortening with time, which means that the components are coming closer to each other. The binary is thus losing energy, and the only reason for this could be the emission of gravitational radiation. Thus this was the first, though indirect, proof of the existence of gravitational radiation. In addition, it served as an important verification of the Einstein's theory of gravitation. With the latest measurements (Table 1), the ratio of the observed \dot{P}_b to that predicted by general relativity stands at 1.0023 ± 0.0047 —an impressive agreement²!

These observations have of late also led to an important theoretical activity of parametrizing all existing theories of gravitation in terms of parameters that can be fitted to the timing data on the binary pulsar^{2,6-8}. The predictions from general relativity still fit very well the data on this binary pulsar, and two others that were discovered rather recently. The data already strongly constrain most other the-

ories of gravity. In time, binary pulsar systems will provide even tighter constraints on theories of gravitation, and judging by the present trend, general relativity is well on its way to emerge the final winner.

1. Hulse, R. A. and Taylor, J. H., *Astrophys. J.*, 1975, 195, L51.
2. Taylor, J. H., Wolszczan, A., Damour, T. and Weisberg, J. M., *Nature*, 1992, 355, 132.
3. Smart, L. L. and Blandford, R. D., *Astrophys. J.*, 1976, 207, 574
4. Srinivasan, G. and van den Heuvel, E. P. J., *Astron. Astrophys.*, 1982, 108, 143.
5. Taylor, J. H. and Weisberg, J. M., *Astrophys. J.*, 1989, 345, 434.
6. Damour, T. and Deruelle, N., *Ann. Inst. H. Poincaré (Phys. Théor.)*, 1985, 43, 107.
7. Damour, T. and Deruelle, N., *Ann. Inst. H. Poincaré (Phys. Théor.)*, 1986, 44, 263.
8. Damour, T. and Taylor, J. H., *Phys. Rev.*, 1992, D45, 1840.

Dipankar Bhattacharya, Raman Research Institute, Bangalore.

Nobel Prize for Chemistry

This year's Nobel prize for Chemistry was given to Michael Smith of the University of British Columbia, Vancouver, Canada and Kary B. Mullis of Cetus Corporation, USA (presently a freelance consultant) for developing methods for site-directed mutagenesis and amplification of DNA by polymerase chain reaction (PCR), respectively^{1,2}. These methods have been so revolutionary that they have changed the way molecular biology is done today.

Site-directed mutagenesis allows one to introduce changes in the sequence of a given DNA. It has been instrumental in the study of the structure-function relationship(s) of the nucleic acids and the proteins (by mutating their genes). Further, it has allowed detailed understanding of nucleic acid protein interactions and various aspects of gene expression e.g. transcription, translation and processing of RNA and proteins etc. To perform site-directed mutagenesis one designs an oligodeoxyribonucleotide (oligo), complementary to the DNA to be mutagenized but with mismatches in regions where mutations are sought. Oligos containing deletions or insertions (with respect to the template DNA) can be used

to perform deletion or insertion mutagenesis. DNA is usually cloned in bacteriophage vectors (e.g. M13) to obtain

single-stranded DNA template. Various steps of mutagenesis are depicted in Figure 1. As shown, the procedure results

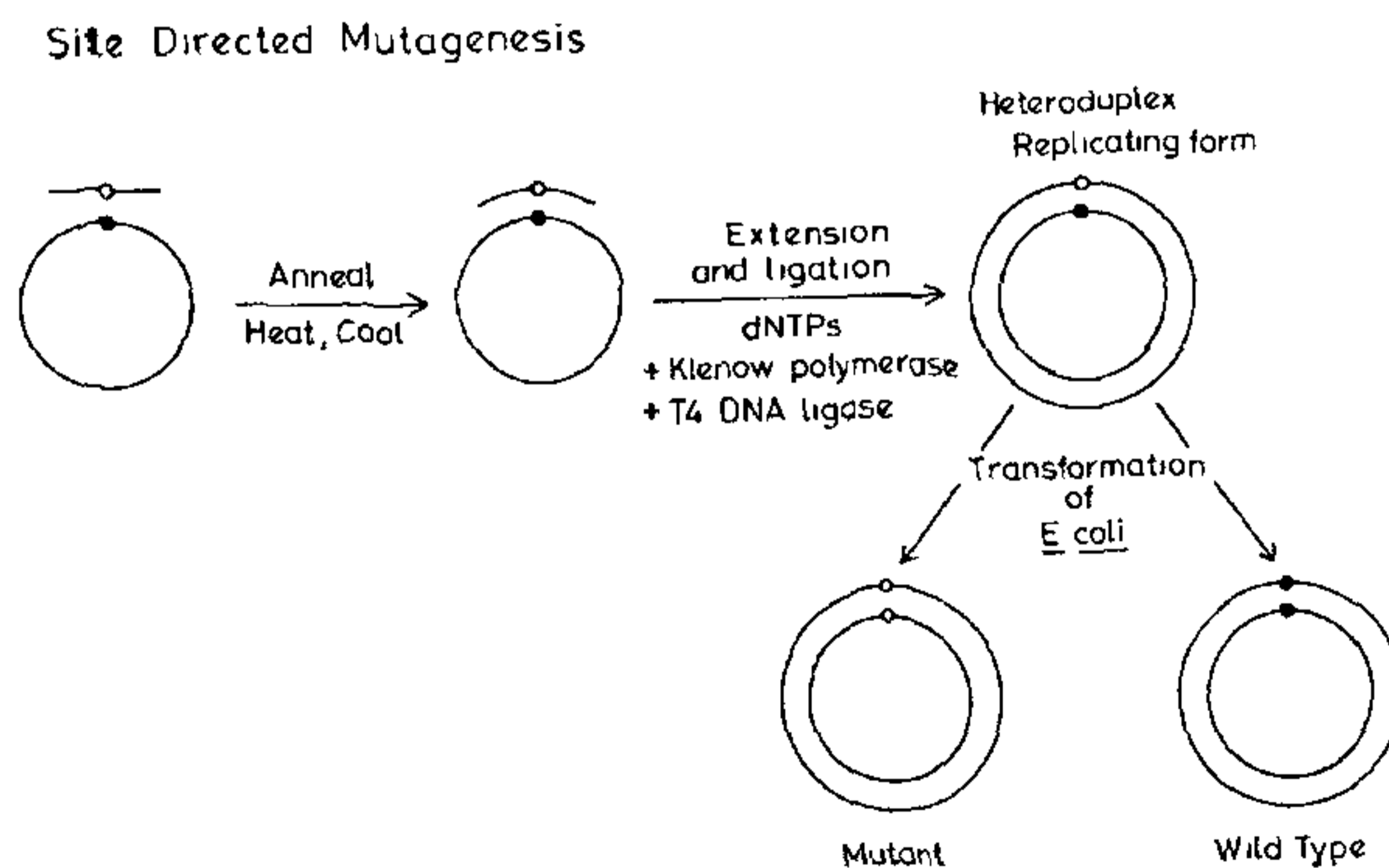


Figure 1. Site-directed mutagenesis. Single-stranded DNA from M13 recombinants is used as template. A complementary mutagenic oligo containing a mutation is used as primer. Second strand synthesis is carried out in the presence of dNTPs, Klenow polymerase and T4 DNA ligase. The heteroduplex replicating forms (RF) are used to transform *E. coli*. Hollow circles (o) and filled circles (•) represent mutated and wild type DNA sequence, respectively.

in the production of phage progeny containing both the wild type and the mutated forms of the DNA insert. The mutants can be identified by screening. As the T_m values of hybrids of small oligos with wild type and mutated DNA differ significantly, plaque hybridizations using radiolabelled oligo probes are generally used to identify the mutants. Although the theoretical yield of obtaining mutant plaques is 50% (Figure 1), in practice it is much lower. However, many modifications to this general procedure have been described which increase the efficiency of the mutant strand selection³⁻⁵.

PCR is used for amplification of DNA of interest up to several million-fold for rapid and convenient analysis. The technique is so powerful that DNA can be amplified even from single cells mixed with excess of unrelated DNA. The procedure, as shown in Figure 2, involves three basic steps, (i) heat denaturation of double-stranded templates, (ii) annealing of oligomeric primers (forward- and reverse-primers) to the templates and, (iii) extension of the primers with DNA polymerase. These steps are repeated 30 to 40 times (hence the name polymerase chain reaction) which lead to enormous amplification of DNA region between the two primers. Depending on the conditions, the procedure usually takes only a few hours. When the PCR was first reported in 1985, Klenow polymerase from *E. coli* was used for primer extensions. But because of its thermolability, addition of a fresh aliquot at each extension step was required. However, in 1988, Mullis and his coworkers introduced use of thermostable DNA polymerase from *Thermus aquaticus* which eliminated the need to supplement the reactions after heat denaturation steps⁶. Several modifications of this technique have since been described for various applications, e.g. one can perform single strand-specific PCR for direct sequencing from single-stranded DNA. Another related technique of inverse PCR allows one to perform DNA crawling by amplification and cloning of regions flanking a known DNA sequence⁷. Because of the simplicity and its amenability to a wide variety of modifications, the PCR technology has gained immense usefulness in forensic science, detection of HIV in early stages of infection (where immunological tests may be insensitive) and diagnosis of several genetic diseases e.g. thalassemias, cystic fibrosis, Duchenne muscular dystrophy etc. DNA from appro-

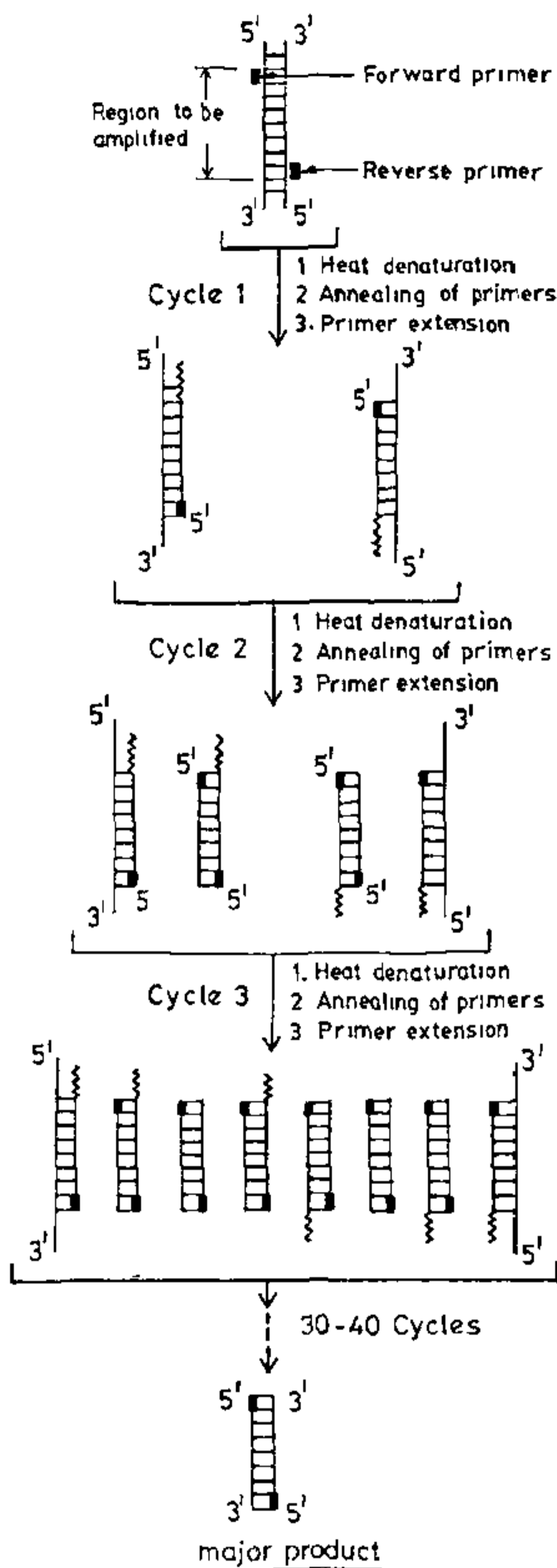


Figure 2. Polymerase chain reaction. Schematic representation of only 3 cycles is given. As the number of shorter DNA fragments increases exponentially, a major product of the reaction is the DNA bounded by the forward- and the reverse-primers.

priate cells such as hair follicles, blood cells, sperms or amniocentesis samples, is used for amplifications and fingerprint analysis. Direct analysis of microbial contamination is another area where PCR has become popular. It eliminates the need for culturing different micro-organisms in their specialized media which is a time-consuming and labour-intensive process. In addition, several forms of microbial contaminants can be tested in a single reaction in a short time. For most of

these applications one does not need to purify the DNA and the procedure works even in presence of detergents such as NP-40, Triton X-100 etc.

The PCR technique has also been an important tool in the analysis of partially degraded prehistoric DNA samples. However, an ingenious use of PCR was shown by Tuerk and Gold⁸ in a process that they termed SELEX (systematic evolution of ligands by exponential enrichment). From a random RNA population they selected two molecules that had a binding site for T4 DNA polymerase. Sequence of one of these was the same as found in the T4 bacteriophage mRNA whereas the other contained variations at 4 positions. Both of these sequences bind to T4 DNA polymerase with similar affinities; therefore, the second molecule represents a sequence that could have evolved in nature but did not. Approaches similar to the SELEX have since been used to obtain ('evolve') other DNA/RNA molecules from random pools. Some of the efficient ribozymes could be obtained by this method. Certainly the PCR technology has provided molecular biologists with an incredible power in their hands and it has become an indispensable research tool even to perform routine tasks of cloning, sequencing, and mutagenesis etc⁷.

1. Hutchinson III, C. A., Phillips, S., Edgell, M. H., Gillam, S., Jahnke, P. and Smith, M., *J. Biol. Chem.*, 1978, 253, 6551-6560.
2. Saiki, R. K., Scharf, S., Faloona, F. A., Mullis, K. B., Horn, G. T., Erlich, H. A. and Arnheim, N., *Science*, 1985, 230, 1350-1354
3. Nakamaye, K. and Eckstein, F., *Nucl. Acids Res.*, 1986, 14, 9679-9698.
4. Kunkel, T. A., *Proc. Natl. Acad. Sci. USA*, 1985, 82, 488-492.
5. Kramer, W., Drutsa, V., Jansen, H-W., Kramer, B., Pflugfelder, M and Fritz, H-J., *Nucl. Acids Res.*, 1984, 12, 9441-9456.
6. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B and Erlich, H. A., *Science*, 1988, 239, 487-491
7. Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J. (eds), *PCR Protocols—A Guide to Methods and Applications*, Academic Press, Calif, 1990
8. Tuerk, C. and Gold, L., *Science*, 1990, 249, 505-510.

Umesh Varshney, Centre for Genetic Engineering, Indian Institute of Science, Bangalore