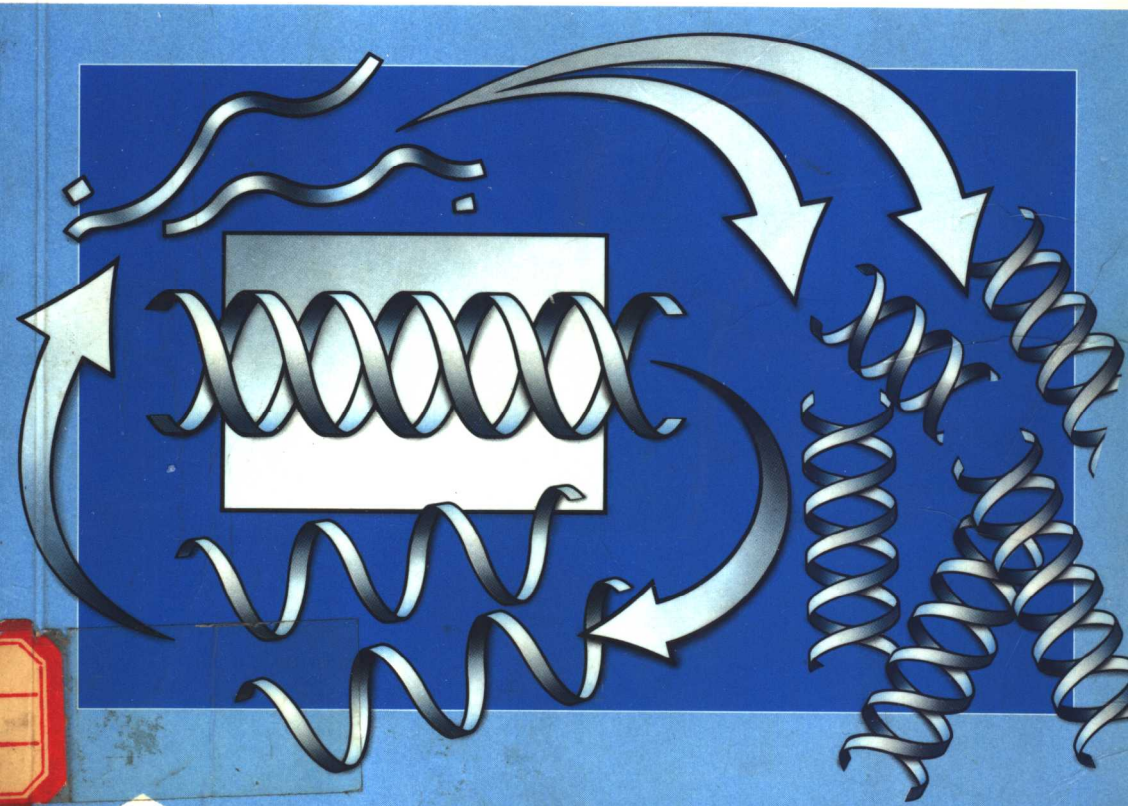
PCR

A Practical Approach

Edited by M. J. McPHERSON,
P. QUIRKE, and G. R. TAYLOR



The Practical Approach Series
SERIES EDITORS: D. RICKWOOD and B. D. HAMES

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Preface

The polymerase chain reaction (PCR) has rapidly become established as one of the most widely used techniques of molecular biology, and with good reason; it is a rapid, inexpensive, and simple means of producing microgram amounts of DNA from minute quantities of source material and is relatively tolerant of poor quality template. Starting materials for gene analysis and manipulation by the PCR may be genomic DNA (in extreme cases from a single cell or a few microdissected chromosome fragments), RNA (perhaps from only a few cells), nucleic acids from archival specimens, cloned DNA, or PCR products themselves.

Many variations on the basic procedure have now been described and applied to a range of disciplines. In medicine, for example, the PCR has had a major impact on the diagnosis and screening of genetic diseases and cancer, the rapid detection of fastidious or slow growing microorganisms and viruses, such as mycobacteria and HIV, the detection of minimal residual disease in leukaemia, and in HLA typing. The amplification of archival and forensic material has applications in forensic pathology and evolutionary biology. PCR has established a central role in the human genome project, particularly through the concepts of sequence tagged sites, microsatellites, and interspersed repetitive sequence PCR. In most molecular biology laboratories, the PCR has found routine use in processes such as probe preparation, clone screening, mapping and subcloning, and preparation of sequencing templates, as well as for more advanced applications such as cloning very low abundance transcripts, cloning gene families, directed mutagenesis, and sophisticated gene recombination.

This volume is intended to provide a general introduction to the PCR for those new to this area, and then to cover a range of more specialized topics and applications including template preparation, gene analysis and mapping, gene cloning and manipulation, and the fidelity of DNA polymerases in PCRs. Throughout the volume there is an emphasis on practical aspects with detailed protocols forming a central feature. Occasional overlap between chapters reflects the inclusion of alternative protocols to tackle a similar problem. In a single volume of this size it is not possible to cover the complete range of applications of the PCR technology, particularly since new developments are appearing at an unprecedented rate. Nevertheless, the editors hope this volume will provide something of interest to every reader; that it will serve as a starting point for those new to the PCR yet eager to start a voyage of discovery and that it will provide a useful reference to those already well down the PCR trail.

Finally we wish to thank the authors for their valuable contributions and the staff at OUP for the speed of production of this volume.

February 1991

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Abbreviations

AMD	amplification and mismatch detection
AMV	avian myeloblastosis virus
ARMS	amplification refractory mutation system
ASO	allele-specific oligonucleotide
ATP	adenosine triphosphate
BSA	bovine serum albumin
βME	β-mercaptoethanol (2-mercaptoethanol)
cDNA	complementary DNA
CEPH	Centre Etude Polymorphisme Humain
CFTR	cystic fibrosis transmembrane regulator
CIP	calf intestinal phosphatase
COP	competitive oligonucleotide priming
CTAB	cetyl trimethyl ammonium bromide
ddH_2O	double distilled water
dNTP	deoxyribonucleotide triphosphate
DEPC	diethylpyrocarbonate
DGGE	denaturing gradient gel electrophoresis
DMSO	dimethylsulphoxide
DTT	dithiothreitol
EDTA	ethylene diamine tetraacetic acid
EtBr	ethidium bromide
GAWTS	genome amplification with transcript sequencing
HPRNI	human placental ribonuclease inhibitor
HPRT	hypoxanthine guanine phosphoribosyltransferase
IL-2	interleukin-2
IPCR	inverse polymerase chain reaction
IPTG	isopropyl-β-D-thiogalactoside
IRS	interspersed repetitive sequences
LMP	low melting point
LTR	long terminal repeat
MES	4-morpholine-ethanol-sulphonic acid
MLV	murine leukaemia virus
mRNA	messenger RNA
nPCR	nested polymerase chain reaction
nRT	nested reverse transcriptase
OTC	orthinine transcarbamylase
PAGE	polyacrylamide gel electrophoresis
PBL	peripheral blood lymphocytes
PBS	phosphate buffered saline

PCR	polymerase chain reaction
PEG	polyethylene glycol
p.f.u.	plaque forming unit
PIC	polymorphism information content
Pipes	1,4-piperazinebis (ethane-sulphonic acid)
r.t.	room temperature
RT	reverse transcriptase
RFLP	restriction fragment length polymorphism
RNase	ribonuclease
SDS	sodium dodecyl sulphate
SSC	standard saline citrate
SOE	splicing by overlap extension
SSPE	standard saline phosphate-EDTA
SSPEn	subacute sclerosing panencephalitis
SSPR	single-strand-producing reaction
TAE	Tris-acetate-EDTA
TBE	Tris-borate-EDTA
Taq	*Thermus aquaticus*
TE	Tris-EDTA
UV	ultraviolet
VNTR	variable number of tandem repeat
VRC	vanadyl ribonucleoside complex
YAC	yeast artificial chromosome

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