

GENOME ANALYSIS

A LABORATORY MANUAL

VOLUME 3

CLONING SYSTEMS

Volume Editors

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Wistar Institute

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University of British Columbia

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Middle: (Left) *S. cerevisiae*; (center) *C. elegans*; (right) *Drosophila*

Bottom: *E. coli*

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VOLUME 4
MAPPING GENOMES

Series Editors

Eric D. Green

National Human Genome Research Institute

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University of British Columbia

Preface

Although biologists have studied the genomes of numerous organisms for decades, the last ten years have brought an enormous increase in the pace of genome research. Problems of a previously unthinkable scope are now routinely solved, and the results of these studies are having a profound impact on biomedical research. Many of these advances are the direct result of the coordinated effort of the Human Genome Project, which was established to produce genomic maps and sequences for a set of well-studied organisms. Recent strategic and technological advances have made it feasible to clone, genetically manipulate, and analyze very large segments of DNA; to identify expressed sequences within large genomic regions; to identify DNA sequence variation associated with phenotypic variation; and to determine the nucleotide sequences of DNA cheaper and more efficiently than before. Indeed, to date, several bacterial genomes and that of baker's yeast have been sequenced in their entirety, and the sequencing of other genomes is well under way.

The purpose of this four-volume manual is to provide newcomers and experienced practitioners alike with theoretical background, laboratory protocols, and resource materials for applying these powerful new techniques of genome analysis to the study of the very large number of genes and genomes yet to be characterized. We feel that there are several compelling reasons for producing such a manual. First, there are few sources that provide detailed information on the application of methods for genome analysis, due to the recent development of these techniques and the rapid rate at which they have evolved. Second, genome research has led to a new way of thinking that allows a completely different scale of question to be addressed. Many of these techniques, while originally developed for the systematic analysis of mammalian genomes, are now applied to specific biological questions in a variety of organisms. We hope to hasten further the increased understanding of basic biological phenomena by making this technology more widely applied. We have therefore provided step-by-step protocols with detailed explanations regarding why key manipulations are performed as described and which of these steps are most critical to success or prone to failure. We also describe how to recognize and avoid common problems and provide guidelines for troubleshooting various aspects of the protocols. Finally, we believe that a manual that covers the vast array of approaches needed at the different stages of a typical genome analysis project will find application in many laboratories.

This manual consists of chapters authored and edited by genome scientists who are experts in, and in many cases, the developers of, the described experimental techniques. The editors wrote some of the chapters themselves and worked closely with the other authors and the staff at Cold Spring Harbor Laboratory Press to develop cohesiveness, a consistent style,

and substantial cross-referencing. The methods are presented with a high level of detail and completeness to allow readers without experience with these methods to evaluate the strategies and successfully implement the protocols. Most of the editors have led laboratory courses at Cold Spring Harbor Laboratory, and thus have an appreciation for the importance of technology transfer and the amount of background explanation and detail that must accompany a protocol to make its use a success, especially in the hands of an inexperienced user. While we have not assumed that the user is experienced in the techniques of genome research, a basic knowledge of molecular biology techniques (i.e., recombinant DNA cloning) is a prerequisite for the successful use of most of the described methods. Appendices containing instructions for preparing reagents, protocols for basic methods used throughout the manual, safety information, and useful reference information are included at the end of each volume.

Volume 1 of this manual contains seven chapters describing basic techniques in genome analysis that are applicable to most of the experimental methods appearing in subsequent volumes. Chapter 1 provides basic protocols for isolating genomic DNA and performing standard manipulations, such as gel transfer for hybridization analysis. Chapter 2 describes protocols for isolating, manipulating, and analyzing high-molecular-weight DNA, many of which are based on technologies developed specifically in response to the need to clone, map, and sequence large genomes. The polymerase chain reaction is now a standard tool in almost all biomedical research laboratories, and the method has many key roles in genome research. Chapter 3 provides an overview of these PCR-based applications in genome analysis. Chapters 4, 5, and 6 describe large-scale DNA sequencing and include general protocols for dideoxy-mediated sequencing, as well as more specific methods for shotgun and directed sequencing strategies. Chapter 7 reviews perhaps the most critical set of all genome methods—those concerned with analyzing and accessing genomic information, particularly sequence data. This chapter is associated with an electronic version available at http://www.cshl.org/books/g_a that contains supplemental information that should be useful to all researchers as well as to those interested in using the vast quantities of mapping and sequencing data generated by the genome project.

In Volume 2, comprehensive approaches are presented for identifying, isolating, and analyzing genes. Chapter 1 reviews strategies for gene discovery in mammalian systems, including approaches for gene identification, mapping, isolation of transcribed sequences, and assessment of candidate genes. Chapter 2 provides detailed protocols for the construction and screening of normalized cDNA libraries. Methods for gene isolation are presented in Chapters 3 (Direct cDNA Selection), 4 (Exon Trapping), and 5 (Gene Detection by the Identification of CpG Islands). Chapter 6 describes a variety of methods for the detection of DNA sequence variation, including protocols for identifying alterations in electrophoretic mobility and recognizing mismatches.

Volume 3 contains methods for using a variety of genomic cloning systems. Chapter 1 provides general methods for working with bacterial cloning systems, including procedures for construction and storage of bacterial genomic libraries, library screening, contig assembly, and chromosome walking. Chapters 2, 3, and 4 describe three bacterial cloning systems: cosmids, bacteriophage P1, and BACs, respectively. Each of these chapters provides a detailed description of cloning vectors and host strains, as well as methods for library construction, characterization, and handling. Chapter 2 includes protocols for subcloning DNA from YACs into cosmids and for preparing low- and high-density filter arrays of cosmid clones. Chapter 3 includes a general description of the bacteriophage P1 life cycle and methods for preparation of packaging extracts. Chapter 4 includes protocols for recovery and analysis of the large BAC DNA inserts. Finally, Chapter 5 provides a comprehensive treatment of the YAC cloning system, including a primer on yeast genetics and detailed protocols for library construction, screening, and characterization. Methods for working with individual YAC clones include protocols for long-range restriction mapping, isolation of insert ends, and YAC manipulation, such as recombination-based modification, amplification, and transfer of YACs between yeast strains and into mammalian cells by spheroplast fusion.

Volume 4 of this manual includes methods central to the generation and use of genomic maps, including the analysis of DNA polymorphisms, meiotic mapping in humans, meiotic and comparative mapping in the mouse, mapping by fluorescence in situ hybridization, generation of PCR-based markers for genome mapping, and radiation hybrid mapping.

We strongly encourage users of this manual to heed all safety cautions noted in the protocols, in the Appendices, and in the instructions provided by manufacturers. We urge all investigators to be familiar with the safe use of reagents and laboratory equipment, as well as with national, state, local, and institutional regulations regarding the use and disposal of materials described in this manual.

The 1970s and 1980s brought the revolution of molecular biology—initially a field of research but more recently a fundamental set of techniques that have come to have key roles in virtually all studies of biological systems. The “genome revolution” of the 1990s will likely evolve in a similar fashion. Our hope is that the experimental techniques described in this four-volume manual will ultimately be useful for investigators focusing on the study of genomes, as well as for those wishing to manipulate and analyze genomes as a means of gaining insight into basic biological processes.

Eric D. Green
Bruce Birren
Philip Hieter
Sue Klapholz
Richard M. Myers

Dedication

The field of genome research was greatly enriched by the contributions of Dr. Nat Sternberg, a talented scientist, an inspiring teacher, and an admired colleague. A microbiologist by training, Nat's basic research on bacteriophage P1 led to the development of P1 as a cloning system for large DNA. In 1989, Nat initiated a laboratory course at Cold Spring Harbor Laboratory to disseminate the newly emerging techniques for generating and manipulating large-insert clones. He continued to improve upon and share his procedures, preparing Chapter 3 of this manual shortly before his untimely death. Nat is dearly missed by those who were fortunate enough to know him. We have admired and benefited not only from his creativity, his insightfulness, and his accurate scientific instincts, but from his warm and generous spirit as well. We dedicate this book to the memory of our friend and colleague, Nat Sternberg.

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Production Editor: Dorothy Brown

Project Coordinator: Mary Cozza
Desktop Editor: Susan Schaefer
Book Design: Nancy Ford, Emily Harste

Contributors

Bruce Birren, *Whitehead Institute/MIT Center for Genome Research, Cambridge*
Kenneth Dewar, *Whitehead Institute/MIT Center for Genome Research, Cambridge*
Ian Dunham, *The Sanger Centre, Cambridge, United Kingdom*
Glen A. Evans, *University of Texas Southwestern Medical Center at Dallas*
Eric D. Green, *National Human Genome Research Institute, Bethesda*
Philip Hieter, *University of British Columbia*
Ung-Jin Kim, *California Institute of Technology, Pasadena*
Valeria Mancino, *California Institute of Technology, Pasadena*
Mark Ross, *The Sanger Centre, Cambridge, United Kingdom*
Hiroaki Shizuya, *California Institute of Technology, Pasadena*
Forrest Spencer, *Johns Hopkins University School of Medicine, Baltimore*
Nat L. Sternberg,* *DuPont Merck Pharmaceuticals, Glenolden, Pennsylvania*

*Deceased

Abbreviations and Acronyms

In addition to standard abbreviations for metric measurements (e.g., ml) and chemical symbols (e.g., HCl), the abbreviations and acronyms below are used throughout this manual.

5FoA	5-fluoroorotic acid
A	adenosine (RNA) or deoxyadenosine (DNA) residue
ACEDB	a <i>C. elegans</i> database
AHC medium	acid-hydrolyzed casein medium
AMCA	7-amino-4-methylcoumarin-3-acetic acid
amp ^r	β -lactamase gene conferring resistance to ampicillin
Amp ^r	ampicillin-resistance phenotype
AMV	avian myeloblastosis virus
AP PCR	arbitrarily primed PCR
ARS	autonomously replicating sequence
AT-2	Artificial Transposon-2
ATCC	American Type Culture Collection
ATP	adenosine triphosphate
ATP γ S	adenosine-5'-O-(3-thiotriphosphate)
AV-FIGE	asymmetric voltage field-inversion gel electrophoresis
BAC	bacterial artificial chromosome
BAP	bacterial alkaline phosphatase
BLAST	basic local alignment search tool
bp	base pair
Bq	Becquerel
BrdU	bromodeoxyuridine
BSA	bovine serum albumin
C	cytidine (RNA) or deoxycytidine (DNA) residue

<i>C. albicans</i>	<i>Candida albicans</i>
<i>cam^r</i>	gene conferring resistance to chloramphenicol
Can	canavanine
C-banding	centromere banding
CCD	charge-coupled device
CCM	chemical cleavage at mismatches
CDGE	constant denaturant gel electrophoresis
cDNA	complementary DNA
<i>C. elegans</i>	<i>Caenorhabditis elegans</i>
CEN	centromere
CEPH	Centre D'Etudes du Polymorphisme Humain
CERN	European Nuclear Research Council
cfu	colony-forming units
CGH	comparative genome hybridization
CHAPS	3-([3-cholamidopropyl]-dimethylammonio)-1-propanesulfonate
CHEF	contour-clamped homogeneous electric field
Ci	Curie
CIP (also known as CIAP)	calf intestinal alkaline phosphatase
cM	centiMorgans
cpm	counts per minute
CTAB	cetyltrimethylammonium bromide
CTP	cytosine triphosphate
Cyh	cycloheximide
DAPI	4',6-diamidino-2-phenylindole
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
DDBJ	DNA Database of Japan
ddGTP	dideoxyguanosine triphosphate
ddNTP	dideoxynucleoside triphosphate
DD PCR	differential display PCR
ddTTP	dideoxythymidine triphosphate
DEPC	diethyl pyrocarbonate
DGGE	denaturing gradient gel electrophoresis
dGTP	deoxyguanosine triphosphate
dITP	deoxyinosine triphosphate
DMD	Duchenne muscular dystrophy
<i>D. melanogaster</i>	<i>Drosophila melanogaster</i>
DMEM	Dulbecco's modified Eagle's medium
DMF	<i>N,N</i> -dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dNMP	deoxynucleoside monophosphate
dNTP	deoxynucleoside triphosphate
DOP PCR	degenerate-oligomer-primed PCR
D-PBS	Dulbecco's phosphate-buffered saline
dpm	disintegrations per minute
DTT	dithiothreitol

dTTP	deoxythymidine triphosphate
dUTP	deoxyuridine triphosphate
EBI	European Bioinformatics Institute
EBV	Epstein-Barr virus
EC number	Enzyme Commission number
<i>E. coli</i>	<i>Escherichia coli</i>
EDTA	ethylenediaminetetraacetic acid
EEO	electroendosmosis
EGTA	ethylene glycol-bis(β -amino-ethyl ether) <i>N,N,N',N'</i> -tetraacetic acid
ELISA	enzyme-linked immunosorbent assay
E-mail	electronic mail
EMBL	European Molecular Biology Laboratory
EPPS	<i>N</i> -(2-hydroxyethyl)piperazine- <i>N'</i> -(3-propanesulfonic acid)
ES	embryonic stem
EST	expressed sequence tag
EUCIB	European Backcross Collaborative Group
FBS	fetal bovine serum
FCS	fetal calf serum
FIGE	field-inversion gel electrophoresis
FISH	fluorescence in situ hybridization
FITC	fluorescein isothiocyanate
FPLC	fast-performance liquid chromatography
FRT	FLP (Flp recombinase) recombination target
ftp	file transfer protocol
F.W.	formula weight
G	guanosine (RNA) or deoxyguanosine (DNA) residue
G-banding	Giemsa banding
gDGGE	genomic denaturing gradient gel electrophoresis
GSS division	GenBank division for genome survey sequences
GTP	guanosine triphosphate
HAP	hydroxyapatite
HBSS	Hanks' balanced salt solution
HEPES	<i>N</i> -(2-hydroxyethyl)piperazine- <i>N'</i> -(2-ethanesulfonic acid)
HGMP	Human Genome Mapping Program
HIV	human immunodeficiency virus
HMBD	histidine-tagged methyl-CpG-binding domain
HMW DNA	high-molecular-weight DNA
HPLC	high-performance liquid chromatography
HSV-TK	herpes simplex virus thymidine kinase
HTG division	GenBank division for data from high-throughput genome sequencing centers
HTML	hypertext markup language
http	hypertext transfer protocol
<i>H. wingei</i>	<i>Hansenula wingei</i>
IgG	immunoglobulin G

IPTG	isopropylthio- β -D-galactoside
IRS PCR	interspersed-repetitive-sequence-based PCR
<i>kan^r</i>	gene conferring resistance to kanamycin
Kan ^r	kanamycin-resistance phenotype
kb	kilobase pair
kD	kilodalton
KGB	potassium glutamate buffer
lb	pound(s)
LB medium/plate	Luria-Bertani medium/plate
LIDS	lithium dodecyl sulfate
LINE	long interspersed nuclear element
LTR	long terminal repeat
M	molar
M13 RF	M13 replicative form
MACAW	multiple alignment construct and analysis workbench
Mb	megabase pair
MBD	methyl-CpG-binding domain
Mbytes	megabytes
α -MEM	α -minimum essential medium
MCD	multiple complete digest
MES	2-(<i>N</i> -morpholino)ethanesulfonic acid
MMLV	Moloney murine leukemia virus
m.o.i.	multiplicity of infection
MOPAC	mixed oligonucleotide-primed amplification of cDNA
MOPS	3-(<i>N</i> -morpholino)propanesulfonic acid
mRNA	messenger RNA
m.w.	molecular weight
N	normal
β -NAD	β -nicotinamide adenine dinucleotide
NCBI	National Center for Biotechnology Information
NCHGR-DOE	National Center for Human Genome Research/Department of Energy
NGM	nematode growth medium
NIGMS	National Institute for General Medical Sciences
NIH	National Institutes of Health
NMR	nuclear magnetic resonance
NOR staining	nuclear organizing region staining
NP-40	Nonidet P-40
OFAGE	orthogonal field alternation gel electrophoresis
ORF	open reading frame
ori	origin of replication
PAC	P1-derived artificial chromosome
PACE	programmable autonomously controlled electrodes
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate-buffered saline
PCR	polymerase chain reaction

PEG	polyethylene glycol
PERT	phenol emulsion reassociation technique
PFG	pulsed-field gel
PFGE	pulsed-field gel electrophoresis
pfu	plaque-forming unit
PIPES	piperazine- <i>N,N'</i> -bis(2-ethanesulfonic acid)
PL	prehead lysate
PMSF	phenylmethylsulfonyl fluoride
poly(A) ⁺	polyadenosine residues
PPL	packaging protein lysate
PRINS labeling	primed in situ labeling
PTT	protein truncation test
Q-banding	quinacrine banding
QFD-banding	Q-banding by fluorescence using DAPI
r_{avg}	average radius
RACE	rapid amplification of cDNA ends
RAPD	random amplified DNA polymorphism
RARE cleavage	RecA-assisted restriction enzyme cleavage
R-banding	replication or reverse banding
RC	recombinant congenic strains
RCRE	rare-cutting restriction enzyme
RDA	representational difference analysis
RF	replicative form
RFLP (also known as RFLV)	restriction fragment length polymorphism (variant)
RGE	rotating gel electrophoresis
RI strain	recombinant inbred strain
RNA	ribonucleic acid
RNase	ribonuclease
rpm	revolutions per minute
rRNA	ribosomal RNA
RT-PCR	reverse transcription followed by PCR
RZPD	German Human Genome Project RZPD (Ressourcen Zentrum Primar Datenbank)
<i>S. cerevisiae</i>	<i>Saccharomyces cerevisiae</i>
SC medium	synthetic complete medium
SD	synthetic dextrose
SDS	sodium dodecyl sulfate
SINEs	short interspersed repeated DNA sequences
<i>S. pombe</i>	<i>Schizosaccharomyces pombe</i>
sq. in.	square inch(es)
SSC	standard saline citrate
SSCP	single-strand conformational polymorphism
SSLP	simple-sequence length polymorphism
ssp.	subspecies
STS	sequence-tagged site
<i>S. typhimurium</i>	<i>Salmonella typhimurium</i>
SV40	simian virus 40
T	thymidine (DNA) residue

TAE	Tris-acetate/EDTA
TAFE	transverse alternating field electrophoresis
TAIL-PCR	thermal asymmetric interlaced polymerase chain reaction
TAK buffer	Tris-acetate/potassium acetate buffer
<i>Taq</i> DNA polymerase	DNA polymerase from <i>Thermus aquaticus</i>
TAR	transformation-associated recombination
<i>T. aquaticus</i>	<i>Thermus aquaticus</i>
TBE	Tris-borate/EDTA
TB medium	Terrific Broth medium
TCA	trichloroacetic acid
TE	Tris/EDTA
TEL	telomere
TEMED	<i>N,N,N',N'</i> -tetramethylethylenediamine
<i>tet^r</i>	gene conferring resistance to tetracycline
Tet ^r	tetracycline-resistance phenotype
TFA	trifluoroacetic acid
TGE	Tris/glucose/EDTA
TK	thymidine kinase
T_m	melting temperature
TMGD	Tris/magnesium chloride/gelatin/DNase
TNE	Tris/sodium chloride/EDTA
tRNA	transfer RNA
TTP	deoxythymidine triphosphate
U	uridine (RNA) residue
UDG	uracil DNA glycosylase
UNG	uracil- <i>N</i> -glycosylase
URL	uniform resource locator
UTP	uridine triphosphate
UV	ultraviolet
V	volt
VLP	virus-like particle
VNTR	variable number of tandem repeats
v/v	volume/volume
w/v	weight/volume
X-gal	5-bromo-4-chloro-3-indolyl- β -D-galactoside
YAC	yeast artificial chromosome
YNB	yeast nitrogen base
YPD	yeast extract/peptone/dextrose
ZIFE	zero integrated field electrophoresis