

Arabidopsis

A PRACTICAL APPROACH

Edited by
Z. A. WILSON



The Practical Approach Series
Series Editor: B. D. Hames

<http://www.oup.co.uk/PAS>

Arabidopsis

The Practical Approach Series

SERIES EDITOR

B. D. HAMES

*Department of Biochemistry and Molecular Biology
University of Leeds, Leeds LS2 9JT, UK*

See also the Practical Approach web site at <http://www.oup.co.uk/PAS>

★ indicates new and forthcoming titles

- | | |
|-------------------------------------|---|
| Affinity Chromatography | The Cell Cycle |
| Affinity Separations | ★ Cell Growth, Differentiation and Senescence |
| Anaerobic Microbiology | ★ Cell Separation |
| Animal Cell Culture (2nd edition) | Cellular Calcium |
| Animal Virus Pathogenesis | Cellular Interactions in Development |
| Antibodies I and II | Cellular Neurobiology |
| Antibody Engineering | Chromatin |
| Antisense Technology | ★ Chromosome Structural Analysis |
| ★ Apoptosis | Clinical Immunology |
| Applied Microbial Physiology | Complement |
| ★ Arabidopsis | ★ Crystallization of Nucleic Acids and Proteins (2nd edition) |
| Basic Cell Culture | Cytokines (2nd edition) |
| Behavioural Neuroscience | Cytoskeleton |
| Bioenergetics | Cytoskeleton: signalling and cell regulation |
| Biological Data Analysis | Diagnostic Molecular Pathology I and II |
| Biomechanics—Materials | DNA and Protein Sequence Analysis |
| Biomechanics—Structures and Systems | DNA Cloning 1: Core Techniques (2nd edition) |
| Biosensors | |
| ★ C. Elegans | |
| Carbohydrate Analysis (2nd edition) | |
| Cell-Cell Interactions | |

- DNA Cloning 2: Expression Systems (2nd edition)
- DNA Cloning 3: Complex Genomes (2nd edition)
- DNA Cloning 4: Mammalian Systems (2nd edition)
- ★ DNA Microarrays
 - ★ DNA Viruses
 - Drosophila (2nd edition)
 - Electron Microscopy in Biology
 - Electron Microscopy in Molecular Biology
 - Electrophysiology
 - Enzyme Assays
 - Epithelial Cell Culture
 - Essential Developmental Biology
 - Essential Molecular Biology I and II
 - ★ Eukaryotic DNA Replication
 - Experimental Neuroanatomy
 - Extracellular Matrix
 - Flow Cytometry (2nd edition)
 - Fmoc solid phase peptide synthesis
 - Free Radicals
 - Gel Electrophoresis of Nucleic Acids (2nd edition)
 - ★ Gel Electrophoresis of Proteins (3rd edition)
 - Gene Probes 1 and 2
 - ★ Gene Targeting (2nd edition)
 - Gene Transcription
 - Genome Mapping
 - Glycobiology
 - Growth Factors and Receptors
 - Haemopoiesis
 - ★ High Resolution Chromatography
 - Histocompatibility Testing
 - HIV Volumes 1 and 2
 - HPLC of Macromolecules (2nd edition)
 - Human Cytogenetics I and II (2nd edition)
 - Human Genetic Disease Analysis
 - ★ Image Processing and Analysis
 - ★ Immobilized Biomolecules in Analysis
 - Immunochemistry 1
 - Immunochemistry 2
 - Immunocytochemistry
 - ★ Immunodiagnosics
 - ★ *In Situ* Hybridization (2nd edition)
 - Iodinated Density Gradient Media
 - Ion Channels
 - ★ Light Microscopy (2nd edition)
 - Lipid Modification of Proteins
 - Lipoprotein Analysis
 - Liposomes
 - ★ Lymphocytes (2nd edition)
 - Mammalian Cell Biotechnology
 - Medical Parasitology
 - Medical Virology
 - MHC Volumes 1 and 2
 - Molecular Genetic Analysis of Populations (2nd edition)
 - Molecular Genetics of Yeast

- Molecular Imaging in Neuroscience
- Molecular Plant Pathology I and II
- Molecular Virology
- Monitoring Neuronal Activity
- ★ Mouse Genetics and Transgenics
- Mutagenicity Testing
- Mutation Detection
- Neural Cell Culture
- Neural Transplantation
- Neurochemistry (2nd edition)
- Neuronal Cell Lines
- NMR of Biological Macromolecules
- Non-isotopic Methods in Molecular Biology
- Nucleic Acid Hybridisation
- ★ Nuclear Receptors
- Oligonucleotides and Analogues
- Oligonucleotide Synthesis
- PCR 1
- PCR 2
- ★ PCR 3: PCR In Situ Hybridization
- Peptide Antigens
- Photosynthesis: Energy Transduction
- Plant Cell Biology
- Plant Cell Culture (2nd edition)
- Plant Molecular Biology
- Plasmids (2nd edition)
- Platelets
- Postimplantation Mammalian Embryos
- ★ Post-translational Processing
- Preparative Centrifugation
- Protein Blotting
- ★ Protein Expression
- Protein Engineering
- Protein Function (2nd edition)
- ★ Protein Localization by Fluorescence Microscopy
- ★ Protein Phosphorylation (2nd edition)
- Protein Purification Applications
- Protein Purification Methods
- Protein Sequencing
- Protein Structure (2nd edition)
- Protein Structure Prediction
- Protein Targeting
- Proteolytic Enzymes
- Pulsed Field Gel Electrophoresis
- RNA Processing I and II
- RNA-Protein Interactions
- Signalling by Inositides
- ★ Signal Transduction (2nd edition)
- Subcellular Fractionation
- Signal Transduction
- ★ Transcription Factors (2nd edition)
- Tumour Immunobiology
- ★ Virus Culture
-

Arabidopsis

A Practical Approach

Edited by

ZOE A. WILSON
*Plant Science Division,
School of Biological Sciences,
University of Nottingham*

OXFORD
UNIVERSITY PRESS

OXFORD

UNIVERSITY PRESS

Great Clarendon Street, Oxford OX2 6DP

Oxford University Press is a department of the University of Oxford
and furthers the University's aim of excellence in research, scholarship,
and education by publishing worldwide in

Oxford New York

Athens Auckland Bangkok Bogotá Buenos Aires Calcutta
Cape Town Chennai Dar es Salaam Delhi Florence Hong Kong Istanbul
Karachi Kuala Lumpur Madrid Melbourne Mexico City Mumbai
Nairobi Paris São Paulo Singapore Taipei Tokyo Toronto Warsaw
and associated companies in Berlin Ibadan

Oxford is a registered trade mark of Oxford University Press

Published in the United States
by Oxford University Press Inc., New York

© Oxford University Press, 2000

All rights reserved. No part of this publication may be reproduced,
stored in a retrieval system, or transmitted, in any form or by any means,
without the prior permission in writing of Oxford University Press.
Within the UK, exceptions are allowed in respect of any fair dealing for the
purpose of research or private study, or criticism or review, as permitted
under the Copyright, Designs and Patents Act, 1988, or in the case
of reprographic reproduction in accordance with the terms of licences
issued by the Copyright Licensing Agency. Enquiries concerning
reproduction outside those terms and in other countries should be
sent to the Rights Department, Oxford University Press,
at the address above.

This book is sold subject to the condition that it shall not, by way
of trade or otherwise, be lent, re-sold, hired out, or otherwise circulated
without the publisher's prior consent in any form of binding or cover
other than that in which it is published and without a similar condition
including this condition being imposed on the subsequent purchaser

Users of books in the Practical Approach Series are advised that prudent
laboratory safety procedures should be followed at all times. Oxford
University Press makes no representation, express or implied, in respect of
the accuracy of the material set forth in books in this series and cannot
accept any legal responsibility or liability for any errors or omissions
that may be made.

A catalogue record for this book is available from the British Library

Library of Congress Cataloging in Publication Data

Arabidopsis : a practical approach / edited by Zoe A. Wilson

(The practical approach series ; PAS/223)

Includes bibliographical references and index.

1. Arabidopsis—Laboratory manuals. 2. Arabidopsis—Molecular aspects—Laboratory
manuals. I. Wilson, Zoe A. II. Practical approach series ; 223.

QK495.C9 A687 2000 583'.64—dc21 99-045851

ISBN 0-19-963565-X (Hbk)

0-19-963564-1 (Pbk)

Typeset by Footnote Graphics,
Warminster, Wilts

Printed in Great Britain by Information Press, Ltd,
Eynsham, Oxon.

Preface

Over the past fifteen years *Arabidopsis thaliana* has changed from being an insignificant weed, to one of the most cultivated laboratory plants around the world. This increase in popularity can be put down to its small genome, low levels of repetitive DNA, small size and fast generation time. As such it is an ideal molecular genetic tool for the analysis of development in higher plants. The acknowledgement of the importance of a model plant species was brought about by the desire of researchers around the world to come together in an International effort which will allow the advancement of Plant Sciences across all plant species.

This book provides an introduction to the key techniques required for the use of *Arabidopsis* as an experimental system. It gives a basic introduction to the optimal growth conditions and the genetic resources available for *Arabidopsis*, how this material should be handled, maintained and used. Individual chapters describe strategies for the identification, mapping and characterization of different mutants by microscopy, molecular cytogenetics and gene expression analysis. Different cloning strategies, using transposons, T-DNA and map position are described in detail and provide a means to generate, identify and characterise genes of developmental interest.

I would like to thank all the contributors for their help and patience in completing their chapters and revealing their innermost laboratory secrets. I would also like to thank Dr. Bernard Mulligan who instigated most of the original ground work for this book.

University of Nottingham
September 1999

Z.A.W

This page intentionally left blank

Contents

<i>List of Contributors</i>	xv
<i>Abbreviations</i>	xvii
1. Growth, maintenance, and use of <i>Arabidopsis</i> genetic resources	1
<i>Mary Anderson and Fiona Wilson</i>	
1. What is <i>Arabidopsis</i> ?	1
2. What makes <i>Arabidopsis</i> such an attractive experimental model?	3
3. <i>Arabidopsis</i> genetic resource centres	4
<i>Arabidopsis</i> genetic resources	4
Accessing <i>Arabidopsis</i> resources	5
4. Mutants of <i>Arabidopsis</i>	5
Single gene mutation lines	5
Resources for the identification/investigation of novel genes	8
Mapping tools	10
5. Considerations of available resources for identifying novel genes	11
Forward genetics	12
Reverse genetics	14
6. Growing <i>Arabidopsis</i>	15
How to maintain clean growth conditions	15
Growing <i>Arabidopsis</i> in the glasshouse	16
Chemical control of pests and diseases	19
7. Seed storage	21
8. Growing <i>Arabidopsis</i> with specific growth requirements	23
9. Sterile culture of <i>Arabidopsis</i>	24
References	26
2. Preservation and handling of stock centre clones	29
<i>Randy Scholl, Keith Davis, and Doreen Ware</i>	
1. Introduction	29

Contents

2. Missions of a plant DNA resource centre	29
3. Preservation of stocks	30
Plasmids with small DNA inserts	30
Cosmids	32
Phage and phage libraries	33
Yeast artificial chromosome (YAC) libraries	33
Pools of YAC library cells for PCR screening	37
Distribution of YAC libraries arrayed on nylon filters	38
Other large-insert libraries	38
Yeast expression analysis—‘two-hybrid’ libraries and complementation testing	42
4. Verification of stock identity and purity	42
5. Pooled DNA from T-DNA lines for PCR screening	44
6. Organization of stock information	48
Collecting, maintaining, and disseminating stock data	48
Organizing and distributing patron data	48
7. The future	48
References	49
3. Genetic mapping using recombinant inbred lines	51
<i>Clare Lister, Mary Anderson, and Caroline Dean</i>	
1. Introduction	51
2. Preparation and digestion of <i>A. thaliana</i> genomic DNA	54
Preparation of genomic DNA	56
Identifying an RFLP	60
Southern blotting and hybridization	61
3. Polymorphic markers	62
RFLP markers	62
PCR-based markers	66
Phenotypic and biochemical markers	68
4. Calculating map positions	69
Mapping programs	69
NASC mapping service	70
5. Integration of a mutation into a molecular map	72
References	74

Contents

4. <i>Arabidopsis</i> mutant characterization; microscopy, mapping, and gene expression analysis	77
<i>Kriton Kalantidis, L. Greg Briarty, and Zoe A. Wilson</i>	
1. Generation of mutants and their importance for developmental biology	77
2. Mapping and segregation analysis	78
Mapping of mutations	78
Influence of environment on phenotype	82
3. Microscopy	82
Fresh material characterization	82
Fixed material characterization	84
4. Analysis of plant gene expression	89
RNA isolation	89
Northern analysis	91
<i>In situ</i> hybridizations	92
References	103
5. Classical and molecular cytogenetics of <i>Arabidopsis</i>	105
<i>G. H. Jones and J. S. Heslop-Harrison</i>	
1. Introduction	105
2. Mitotic chromosome analysis by light microscopy	108
3. <i>In situ</i> hybridization to mitotic chromosome preparations	112
Photography of <i>in situ</i> hybridizations	117
4. Meiotic chromosome analysis by light microscopy	117
5. Meiotic chromosome analysis by electron microscopy	121
References	123
6. Tissue culture, transformation, and transient gene expression in <i>Arabidopsis</i>	125
<i>Keith Lindsey and Wenbin Wei</i>	
1. Introduction	125
2. Stable transformation by <i>Agrobacterium tumefaciens</i>	128

Contents

3. Transient gene expression in <i>Arabidopsis</i> protoplasts	131
Reporter gene enzyme assays	134
Acknowledgements	139
References	139
7. Transposon and T-DNA mutagenesis	143
<i>Mark G. M. Aarts, Csaba Koncz, and Andy Pereira</i>	
1. Introduction	143
2. Transposon tagging	143
Endogenous transposable elements	143
Transposon tagging systems in <i>Arabidopsis</i>	144
Which system to use?	149
Genetic and molecular analysis of a putatively transposon tagged mutant	151
Further applications of transposon tagging	156
3. T-DNA tagging	158
The use of T-DNA as insertional mutagen	158
Random tagging	158
Available populations of T-DNA transformants	159
Promoter/enhancer trapping	160
Analysis of T-DNA mutants and cloning a tagged gene	161
Further applications of T-DNA tagging	166
References	166
8. Map-based cloning in <i>Arabidopsis</i>	171
<i>Joanna Putterill and George Coupland</i>	
1. Introduction	171
2. Locating the mutation of interest relative to DNA markers	172
Determining an approximate map position	172
Identifying a short genetic interval containing the mutation as a prelude to isolating the gene	175
3. Placing the gene on the physical map	177
Chromosome landing	177
Chromosome walking with YAC clones	178
4. Identification of the gene	189
Location of the gene by molecular complementation	189
Determining the structure of the gene	194
5. Perspectives	194
References	195

Contents

9. Physical mapping: YACs, BACs, cosmids, and nucleotide sequences	199
<i>Ian Bancroft</i>	
1. Introduction	199
2. Genome mapping with YAC clones	199
3. Genome mapping with BAC and P1 clones	202
Communal resources	202
Construction of BAC libraries	208
Genome mapping with BACs	211
4. High resolution mapping with cosmids	216
Approaches to mapping with cosmids	216
Construction of cosmid libraries	216
5. Nucleotide sequences—the ultimate mapping tool	221
The EST sequencing project	221
The genome sequencing project	221
Sequence-based mapping	222
References	223
10. Web-based bioinformatic tools for <i>Arabidopsis</i> researchers	225
<i>Seung Y. Rhee and David J. Flanders</i>	
1. Introduction	225
What is bioinformatics	225
Sources of <i>Arabidopsis</i> bioinformatic data	226
2. Basic tools for the Internet	227
Web basics	227
Getting onto the Web	228
Using your browser	229
Browser tips and errors	232
Privacy issues	235
3. Scenarios of bioinformatic use in <i>Arabidopsis</i> research	235
4. Gene information resources	237
General gene information	237
<i>Arabidopsis</i> gene information	237
Plant gene information	238
5. Maps	239
Genetic maps	239
Physical maps	240
6. Sequencing	242

Contents

The <i>Arabidopsis</i> genome initiative (AGI)	243
Annotation of sequences by AGI	243
Caveats in annotation	244
Sequence contigs from AtDB	245
7. Sequence analysis tools	245
<i>BLAST</i>	246
<i>FASTA</i>	250
EST databases	252
Gene identification programs	253
Gene family analyses	255
Motif analyses	255
Protein structures	258
Comprehensive sequence analysis tools	259
8. Current issues and future directions in bioinformatics	260
Some important bioinformatic issues	260
Bioinformatic tools currently under development for <i>Arabidopsis</i> research	
9. Conclusion	261
Acknowledgements	261
References	261
Appendix 1	263
Glossary	264
<i>Appendix</i>	267
<i>Index</i>	273

Contributors

MARK G. M. AARTS

CPRO-DLO, Postbus 16, 6700 AA Wageningen, The Netherlands.

MARY ANDERSON

John Innes Centre, Colney Lane, Norwich NR4 7UH, UK.

IAN BANCROFT

John Innes Centre, Colney Lane, Norwich NR4 7UH, UK.

L. GREG BRIARTY

Plant Science Division, School of Biological Sciences, The University of Nottingham, University Park, Nottingham NG7 2RD, UK.

GEORGE COUPLAND

John Innes Centre, Colney Lane, Norwich NR4 7UH, UK.

KEITH DAVIS

Arabidopsis Biological Resource Center, The Ohio State University, Columbus, OH, USA.

CAROLINE DEAN

John Innes Centre, Colney Lane, Norwich NR4 7UH, UK.

DAVID J. FLANDERS

PBI Cambridge, Maris Lane, Trumpington, Cambridge CB2 2LQ, UK.

J. S. HESLOP-HARRISON

Karyobiology Group, John Innes Centre, Colney Lane, Norwich NR4 7UH, UK.

G. H. JONES

School of Biological Sciences, University of Birmingham, Birmingham B15 2TT, UK.

KRITON KALANTIDIS

Plant Molecular Biology Unit, Institute of Molecular Biology and Biotechnology, The Forth Institute, Crete.

CSABA KONCZ

MPI für Züchtungsforschung, Carl-von-Linné-Weg 10, D-50829, Köln, Germany.

KEITH LINDSEY

Department of Biological Sciences, University of Durham, South Road, Durham DH1 3LE, UK.

Contributors

CLARE LISTER

John Innes Centre, Colney Lane, Norwich NR4 7UH, UK.

ANDY PEREIRA

CPRO-DLO, Postbus 16, 6700 AA Wageningen, The Netherlands.

JOANNA PUTTERILL

School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand.

SEUNG Y. RHEE

Department of Genetics, School of Medicine, Stanford University, Stanford, CA 94305-5120, USA.

RANDY SCHOLL

Arabidopsis Biological Resource Center, The Ohio State University, 1735 Neil Avenue, 309 Botany and Zoology Building, Columbus, OH, USA.

DOREEN WARE

Arabidopsis Biological Resource Center, The Ohio State University, 1735 Neil Avenue, 309 Botany and Zoology Building, Columbus, OH, USA.

WENBIN WEI

Department of Biological Sciences, University of Durham, South Road, Durham DH1 3LE, UK.

FIONA WILSON

Nottingham Arabidopsis Stock Centre, Plant Science Division, School of Biological Sciences, The University of Nottingham, University Park, Nottingham NG7 2RD, UK.

ZOE A. WILSON

Plant Science Division, School of Biological Sciences, The University of Nottingham, University Park, Nottingham NG7 2RD, UK.

Abbreviations

2,4-D	2,4-dichlorophenoxyacetic acid
2-ip	2-isopentenyladenine
A ₂₆₀	absorbance (260 nm)
A ₂₈₀	absorbance (280 nm)
ABRC	<i>Arabidopsis</i> Biological Research Centre
Ac	Activator
AFLP	amplified fragment length polymorphism
AGI	<i>Arabidopsis</i> genome initiative
AGR	<i>Arabidopsis</i> Genome Resources
AIMS	<i>Arabidopsis</i> Information Management System
ARMS	<i>Arabidopsis</i> RFLP mapping set
AtDB	<i>Arabidopsis thaliana</i> database
ATGC	<i>Arabidopsis thaliana</i> Genome Centre
ATP	adenosine triphosphate
BAC	bacterial artificial chromosome
BLAST	basic local alignment search tool
bp	base pair
BSA	bovine serum albumin
CAPS	cleaved amplified polymorphic DNAs
cDNA	complementary DNA
CIAP	calf intestinal alkaline phosphatase
CIM	callus inducing medium
cM	centimorgan
Col	Columbia
CTAB	cetyltrimethylammonium bromide
Cvi	Cape Verde Islands
DAPI	4',6-diamidino-2-phenylindole
dCTP	deoxycytidine triphosphate
ddW	double distilled water
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dNTP	deoxynucleoside triphosphate
Ds	Dissociation
dSpm	defective Suppressor-mutator
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
EM	electron microscopy
EMS	ethylmethane sulfonate
En	Enhancer

Abbreviations

EST	expressed sequence tag
FISH	fluorescence <i>in situ</i> hybridization
fm	femtomole
FTP	file transfer protocol
FW	fresh weight
GDE	Genetic Data Environment
GFP	green fluorescent protein
GMI	germination medium I
GUS	β -glucuronidase
HPT	hygromycin phosphotransferase
HSP	high scoring segment pair
hyg ^r	hygromycin resistant
<i>I</i>	Inhibitor element
IAA	indole-3-acetic acid
IP	Internet protocol
IPCR	inverse polymerase chain reaction
IPTG	isopropyl- β -D-thiogalactopyranoside
kb	kilobase pair
LA-IPCR	long-range IPCR
<i>Ler</i>	Landsberg <i>erecta</i>
LLR	log likelihood ratio
LM	light microscopy
LMP	low melting point
LUC	luciferase
MAFF	Ministry of Farming and Fisheries
Mb	megabase pair
MES	2-(<i>N</i> -morpholino)ethanesulfonic acid
MOPS	morpholinopropanesulfonic acid
mRNA	messenger RNA
MSU	Michigan State University
MU	4-methylumbelliferone
MUG	4-methylumbelliferyl glucuronide
NASC	Nottingham <i>Arabidopsis</i> Stock Centre
NBT	nitro blue tetrazolium salt
NCBI	National Centre for Biotechnology Information
NM	nitrosomethyl biuret
NMU	nitrosomethyl urea
NOR	nucleolar organizer region
NPG	<i>NetPlantGene</i>
ORF	open reading frame
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PEG	polyethylene glycol
PFGE	pulsed-field gel electrophoresis

Abbreviations

PIR	International Protein Sequence Database
PMSF	phenylmethylsulfonyl fluoride
RAPD	random amplified polymorphic DNA
r.c.f.	relative centrifugal force
RFLP	restriction fragment length polymorphism
RI	recombinant inbred
RIL	recombinant inbred lines
RNase	ribonuclease
RTF	rich text format
SAM	S-adenosylmethionine
SC	synaptonemal complexes
SCOP	Structural Classification of Proteins
SDS	sodium dodecyl sulfate
SEM	shoot elongation medium
SIM	shoot inducing medium
SOM	shoot overlay medium
<i>Spm</i>	Suppressor-mutator
SRS	Sequence Retrieval System
SSC	standard saline citrate
SSLP	simple sequence length polymorphism
SSPE	standard saline/phosphate/EDTA
strep ^r	streptomycin resistant
TAC	transformable artificial chromosome
TBE	Tris/borate/EDTA buffer
TC	tentative consensus
T-DNA	transfer DNA
TE	Tris/EDTA buffer
TIGR	The Institute for Genomic Research
TIR	terminal inverted repeat
tRNA	transfer RNA
U.Minn	University of Minnesota
UV	ultraviolet
VAST	Vector Alignment Search Tool
WS	Wassilewskija
WT	wild-type (normal)
X-gluc	5-bromo-4-chloro-3-indoyl glucuronide
X-phosphate	5-bromo-4-chloro-3-indoyl phosphate
YAC	yeast artificial chromosome