MOLECULAR TOOLS FOR SCREENING BIODIVERSITY

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MOLECULAR TOOLS FOR SCREENING BIODIVERSITY

Plants and Animals

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CONTENTS

LIST OF CONTRIBUTORS	xiii
FOREWORD Mark Chase	xix
PREFACE Angela Karp, Peter G. Isaac and David S. Ingram	xxi
INTRODUCTION Etienne Magnien EU DGXII	xxiii
PART ONE DNA EXTRACTION	1
1 DNA Extraction: widely applicable methods	3
1.1 Isolation of animal cellular total DNA <i>De-Xing Zhang and Godfrey M. Hewitt</i>	5
1.2 Plant total DNA extraction <i>Julia Rueda, Rosario Linacero and Ana M. Vázquez</i>	10
1.3 Chloroplast DNA extraction procedure for species high in phenolics and polysaccharides <i>Roselyne Lumaret, Henri Michaud, Jean-Philippe Ripoll and Lamjed Toumi</i>	15
1.4 Quantification of DNA <i>Rosario Linacero, Julia Rueda and Ana M. Vázquez</i>	18
Box 1.1 Miniprep procedures for the isolation of plant DNA <i>Keith J. Edwards</i>	22
2 DNA Extraction: methods for difficult species/tissues	25
2.1 Special DNA extraction methods for some animal species <i>De-Xing Zhang and Godfrey M. Hewitt</i> A: Extraction of total DNA from soft-body invertebrate species (e.g. aphids, woodlice) B: Extraction of total DNA from tissues or organisms with high nuclease activity	27 29
2.2 Methods for difficult plant species/tissues <i>Birgit Ziegenhagen and Florian Scholz</i>	32
Box 2.1 DNA maxipreparations: A comparison of protocols for rapid extraction of good quality DNA from recalcitrant species <i>Birgit Ziegenhagen and Florian Scholz</i>	36

3	Preserved specimens	39
3.1	Isolation of DNA from preserved specimens <i>De-Xing Zhang and Godfrey M. Hewitt</i>	41
	A: Chelex DNA extraction method for PCR-based analysis	42
	B: Extraction of DNA from paraffin-embedded tissues for PCR analysis	44
Box 3.1	Field collection: animals <i>De-Xing Zhang and Godfrey M. Hewitt</i>	46
Box 3.2	Field collection: plants <i>Roger Hyam</i>	49
4	DNA Extraction using anion-exchange chromatography and silica-gel based membranes	51
4.1	Isolation of nucleic acids using anion-exchange chromatography : qiagen-tip based methods <i>Simone Gauch, Ralf Hermann, Petra Feuser, Uwe Oelmüller and Helge Bastian</i>	54
4.2	Isolation of nucleic acids using silica-gel based membranes: methods based on the use of QIAamp spin columns <i>Simone Gauch, Ralf Hermann, Petra Feuser, Uwe Oelmüller and Helge Bastian</i>	59
	A: DNA isolation from animal tissues using QIAamp spin columns	60
	B: Isolation from plant tissue with DNeasy spin columns	61
Box 4.1	Isolation of total RNA from animal tissue using anion-exchange chromatography (qiagen-tips) <i>Simone Gauch, Ralf Hermann, Petra Feuser, Uwe Oelmüller and Helge Bastian</i>	64
Box 4.2	Isolation of total RNA using silica-gel based membranes <i>Simone Gauch, Ralf Hermann, Petra Feuser, Uwe Oelmüller and Helge Bastian</i>	67
PART TWO	BASIC SCREENING METHODS	71
5	Isozymes <i>Gerhard Müller-Starck</i>	73
6	RFLP analysis <i>Reinhold Brettschneider</i>	83
Box 6.1	Nonradioactive probes <i>Peter G. Isaac</i>	96
7	DNA fingerprinting with VNTR sequences <i>Michael W. Bruford and Ilik J. Saccheri</i>	99

8	Polymerase chain reaction	109
8.1	Polymerase chain reaction <i>Rita Aert, Marleen Voet, Steven Van Campenhout, Jacqueline Vander Stappen and Guido Volckaert</i>	111
8.2	PCR sequencing <i>Guido Volckaert, Rita Aert, Marleen Voet, Steven Van Campenhout and Peter Verhasselt</i>	119
Box 8.1	Routes to DNA sequencing <i>Guido Volckaert</i>	125
8.3	Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLPs) <i>Keith J. Edwards</i>	131
8.4	TGGE and DGGE <i>Michael Etscheid and Detlev Riesner</i>	135
	A: Temperature gradient gel electrophoresis	138
	B: Denaturing gradient gel electrophoresis	141
Box 8.2	Theoretical aspects of thermal denaturation of nucleic acids <i>Michael Etscheid and Detlev Riesner</i>	144
Box 8.3	Modifications for the improvement of TGGE and DGGE <i>Michael Etscheid and Detlev Riesner</i>	147
Box 8.4	Temporal TGGE <i>Michael Etscheid and Detlev Riesner</i>	149
Box 8.5	TGGE and SSCP <i>Michael Etscheid and Detlev Riesner</i>	150
8.5	Single-strand conformation polymorphism (SSCP) analysis <i>William.C. Jordan, Katherine Foley and Michael Bruford</i>	152
8.6	A rapid screening procedure for detecting mtDNA haplotypes in humpback whales (<i>Megaptera novaeangliae</i>) <i>Anja H. Larsen, Pernille Feddersen and Per J. Palsbøll</i>	157
Box 8.6	Modifications to SSCP analysis conditions <i>William C. Jordan, Katherine Foley and Michael Bruford</i>	164
Box 8.7	Automation and the polymerase chain reaction <i>Richard G. Keightley</i>	165
9	RAPDs	169
9.1	Randomly amplified polymorphic DNAs (RAPDs) <i>Keith J. Edwards</i>	171

Box 9.1	Reproducibility testing of RAPDs by a network of European laboratories <i>C.J. Jones, K.J. Edwards, S. Castiglione, M.O. Winfield, F. Sala, C. Van der Wiel, B.L. Vosman, M. Matthes, A. Daly, R. Brettschneider, P. Bettini, M. Buiatti, E. Maestri, N. Marmioli, R.L. Aert, G. Volckaert, J. Rueda, A. Vazquez and A. Karp</i>	176
10 AFLP		181
10.1	Amplified fragment length polymorphism (AFLP) <i>Michaela C. Matthes, Allan Daly and Keith J. Edwards</i>	183
Box 10.1	Reproducibility testing of AFLPs by a network of European laboratories <i>C.J. Jones, K.J. Edwards, S. Castiglione, M.O. Winfield, F. Sala, C. Van der Wiel, B.L. Vosman, M. Matthes, A. Daly, R. Brettschneider, E. Maestri, N. Marmioli, R.L. Aert, G. Volckaert and A. Karp</i>	191
11 SSRs		193
11.1	Genotyping with microsatellite markers <i>Claudio Ciofi, Stephan M. Funk, Trevor Coote, David J. Cheesman, Robert L. Hammond, Ilik J. Saccheri and Michael W. Bruford</i>	195
Box 11.1	Characteristics of microsatellites <i>Michael W. Bruford, Claudio Ciofi, Stephan M. Funk</i>	202
11.2	PCR analysis of SSR polymorphisms in plants using agarose gels <i>Michele Morgante, Antonella Pfeiffer, Irena Jurman, Gianpaolo Paglia and Angelo M. Oliveiri</i>	206
Box 11.2	Fluorescent labelling of SSRs and automated detection <i>Michele Morgante, Antonella Pfeiffer, Irena Jurman, Gianpaolo Paglia and Angelo M. Oliveiri</i>	208
Box 11.3	Reproducibility testing of SSRs by a network of European laboratories <i>C.J. Jones, K.J. Edwards, C. Van der Wiel, B.L. Vosman, M. Matthes, A. Daly, P. Bettini, M. Buiatti, E. Maestri, N. Marmioli, J. Rueda, A. Vazquez and A. Karp</i>	209
PART THREE SOURCES OF PROBES AND PRIMERS		213
12 Probes		215
12.1	Cloning of random amplified polymorphic DNA (RAPD) to generate codominant genetic markers <i>Christoph Sperisen and Urs B��chler</i>	217
12.2	cpDNA <i>Kornel Burg, Silvia Fluch and Giovanni G. Vendramin</i>	223

12.3	RFLP markers <i>Ahmed Jahoor</i>	229
13	Primers	237
13.1	Nuclear DNA primers <i>Joanne Russell, Gordon Machray and Robbie Waugh</i>	239
13.2	Primers for animal mitochondrial DNA: the importance of species-specific primers <i>Per J. Pasbøll and Peter Arctander</i>	249
13.3	cpDNA and mtDNA primers in plants <i>Rémy J. Petit, Brigitte Demesure and Sylvie Dumolin</i>	256
Box 13.1	Variations on a theme <i>Ben Vosman</i>	262
14	Ribosomal DNA probes and primers <i>Christian Schlötterer</i>	265
15	Probes and primers for simple repeats	277
15.1	Isolation of microsatellite markers in animals <i>Robert L. Hammond, Ilik J. Saccheri, Claudio Ciofi, Trevor Coote, Stephan M. Funk, W. Owen McMillan, Michele K. Bayes, Emma Taylor and Michael W. Bruford</i>	279
Box 15.1	VNTR probes <i>Michael W. Bruford</i>	286
Box 15.2	Designing SSR primer pairs using MacVector Software <i>Michael W. Bruford, Claudio Ciofi, Stephan M. Funk, and Robert L. Hammond</i>	287
15.2	Isolation of microsatellite markers in plants <i>Michele Morgante, Anotella Pfeiffer, Irena Jurman, Gianpaolo Paglia and Angela M. Olivieri</i>	288
PART FOUR	DATA ANALYSIS	297
16	Measures of polymorphism within and among populations	299
16.1	Definitions <i>Antoine Kremer, Rémy J. Petit and Odile Pons</i>	301
16.2	Diversity and differentiation at the allelic level <i>Antoine Kremer, Rémy J. Petit and Odile Pons</i>	303
16.3	Diversity and differentiation at the nucleotide level <i>Antoine Kremer, Rémy J. Petit and Odile Pons</i>	306

17	Distance data	313
17.1	Measuring genetic distance <i>Mark A. Beaumont, Kamal M. Ibrahim, Pierre Boursot and Michael W. Bruford</i>	315
18	Discrete data	327
18.1	Binary data analysis <i>Michael W. Bruford and Mark A. Beaumont</i>	329
18.2	Reading DNA sequences <i>Gitte Petersen</i>	332
18.3	Multiple alignment <i>Jotun Hein</i>	334
Box 18.1	Selected software packages for personal computers <i>Ole Seberg and Gitte Petersen</i>	341
18.4	Constructing phylogenies from discrete data – parsimony methods <i>Ole Seberg and Gitte Petersen</i>	344
18.5	Constructing phylogenies from discrete data – maximum likelihood <i>Ole Seberg and Gitte Petersen</i>	353
19	Molecules vs morphology <i>Gitte Petersen and Ole Seberg</i>	357
Box 19.1	Gene trees vs species trees <i>Gitte Petersen and Ole Seberg</i>	365
PART FIVE	CASE STUDIES	367
20	Accessions and collections	369
20.1	Comparison of molecular marker assays in inbreeding (barley) and outbreeding (potato) species <i>Dan Milbourne, Joanne Russell and Robbie Waugh</i>	371
20.2	The use of molecular markers for the identification of tomato cultivars <i>Ben Vosman</i>	382
20.3	Molecular analysis of variation in <i>Lactuca</i> <i>Clemens van de Wiel and Ben Vosman</i>	388
20.4	Evaluation of the AFLP and RAPD molecular marker technologies with regard to the genetic diversity of commercial wheat cultivars <i>Allan Daly</i>	394
Box 20.1	Screening germplasm collections <i>Gitte Petersen and Ole Seberg</i>	398

21 Populations	401
21.1 PCR-RFLP analysis of chloroplast DNA of various gymnosperms: a rapid tool for assessing genetic variation at the above-species level <i>Birgit Ziegenhagen and Matthias Fladung</i>	403
21.2 Analysis of hypervariable chloroplast microsatellites in <i>Pinus halepensis</i> reveals a dramatic genetic bottleneck <i>Michele Morgante, Nicoletta Felice and Giovanni G. Vendramin</i>	407
21.3 Genetic variation of mitochondrial DNA reveals subdivision of Norway spruce (<i>Picea abies</i> (L.) Karst.) <i>Christoph Sperisen, Urs Büchler and Gábor Mátyás</i>	413
21.4 Diversity in insect species using DNA sequences <i>Godfrey Hewitt</i>	418
21.5 The usefulness of parallel analysis of uni- and bi-parental markers: the North Atlantic humpback whale <i>Per J. Palsbøll, Phil J. Clapham, Hanne Jørgensen, Finn Larsen, David Mattila, Richard Sears, Johann Sigurjonsson and Oswaldo Vasquez</i>	426
21.6 Partitioning of genetic diversity in the house mouse <i>Pierre Boursot</i>	431
22 Classification and phylogeny	435
22.1 Molecular studies on the phylogeny of the genus barley (<i>Hordeum</i> ; Poaceae) <i>Gitte Petersen and Ole Seberg</i>	437
22.2 The genus <i>Rhododendron</i> : a case study to test the value of various molecular techniques as measures of biodiversity <i>David Chamberlain and Roger Hyam</i>	441
22.3 RAPD fingerprinting as a tool for taxonomic studies in the genus <i>Populus</i> <i>Francesco Sala, Gejiao Wang, Stefano Castiglione and Stefano Bisoffi</i>	449
23 Screening for 'useful variation'	457
23.1 Screening for 'useful variation' using RAPD markers <i>Robbie Waugh and Joanne Russell</i>	459
23.2 Application of stress responsive genes RFLP analysis to the evaluation of genetic diversity in plants <i>Nelson Marmioli, Aliosha Malcevski and Elena Maestri</i>	464
23.3 Exploiting genome plasticity for the detection of hypervariable sequences <i>Marcello Buiatti and Patrizia Bogani</i>	471
Appendix Safety in the molecular laboratory <i>Peter G. Isaac and Keith J. Edwards</i>	485
Index	491

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FOREWORD

Mark Chase

There are many literature resources available to molecular biologists wishing to assess genetic variation, but the myriad of techniques and approaches potentially available to the plant breeder and the evolutionary biologist is truly bewildering, and most have never been evaluated side-by-side on the same sets of samples.

Additionally, it is often not recognized that tools that are useful for breeders can often be adapted for use in evolutionary studies and vice versa, but this is generally the case. The borderline between population genetics and phylogenetics is vague and difficult to assess, and a combination of both types of tools is best when it is not clear with which area one is dealing. Furthermore, it is not now appropriate to use just one type of marker in any kind of study; most markers have the potential to misinform under certain conditions, so it is always wise to incorporate at least two different types of assessments into any project. This volume is designed to facilitate this sort of multiple approach and provides comparative data on most currently available methods so that researchers can more intelligently select those appropriate to their area of interest, regardless of whether it is in the realm of breeding or evolutionary biology.

Assessment of biodiversity has become such a commonly used term that what it actually means is not clear to many people. In some circles it has become synonymous with assessing the degree of genetic divergence among cultivars or strains, whereas to others it means evaluating the potential of naturally occurring variants for breeding programmes. To evolutionary biologists, it means assessing not only the within and between species variation but also phylogenetic relationships of species and other higher-level taxa, such as genera and families. Some workers would doubt the applicability of a generalized suite of techniques to all these types of studies, but I am convinced that this is indeed possible. Up to this point, there has been a lack of communication between people interested in these different areas but this volume presents techniques and approaches that are generally applicable. There are topics of importance in this volume for everyone, regardless of their research emphasis. The commercial breeder, the research geneticist and the evolutionary biologist will all find several chapters of relevance. The coverage of phylogenetic data and implications for population genetics of natural species are given the least attention, but there are good treatments of these areas by other volumes.

No other recent book has devoted so much time to problems with extracting DNA. The major crops and breeds are relatively easy to handle, but when dealing with a wider array of strains and species, one quickly encounters problem taxa. By accident, one of the selected systems to test the tools was *Rhododendron* (Ericaceae), and from personal experience a better target genus could not have been found.

Rhododendrons are amongst the most difficult plants with which to work, and thus they represent an almost perfect case study. If DNA can be extracted easily and reliably from these plants, then these methods are generally reliable for application across the diversity of plant species that exists in nature.

No single book can expect to cover all areas in great depth, and this one is no exception. From my perspective, the single most important contribution of this volume is the extent to which it provides a measure of the reliability, reproducibility, and degree of ease of application that is found with the various techniques available. For example, everyone has suspected that RAPDs are subject to a greater than desirable degree of lack of reproducibility, but Chapter 9, Box 9.1 and Chapter 23.1 discuss this issue in depth. The same is true for the other various techniques: in addition to a description of how to carry out the necessary protocols there is a discussion of the practical matters that are as important as how to collect the data. There is nothing currently available that covers all these topics in such a critical and comparative manner. It is simply not a matter of following instructions; there are a multitude of other issues that need to be dealt with in practical and simple terms, and these topics are a major feature of this volume. Attention is paid as to whether a technique can provide assessments of heterozygosity and if the data are suitable for phylogenetic analysis as well as assessments of levels of variations within and between populations.

The last two issues that are important and that set this volume apart from others are its inclusion of non-radioactive and automated methods. The first is an obvious and desirable goal, but there has been a call for 'low-tech' methods by many workers so that costs of expensive automated machines could be avoided. There are indeed many 'low-tech' options discussed here, but it has also been recognized that if a technique cannot be automated it will limit the amount of information that can be collected and the number of samples that can be added to the data set. This ultimately is the benefit of automation: the ability to produce massive data sets and to use more than one approach. It has become increasingly clear to me over the last two years that automation is essential if we are to do our jobs and serve the interests of those who fund our research, and techniques that cannot be automated will not serve us well. 'Low-tech', regardless of lower costs, cannot compare with 'high-tech'.

PREFACE

The conservation of natural genetic variation is important not only for ethical and aesthetic reasons but also to ensure that the earth's living resources may be used even more efficiently and sustainably in agriculture, forestry, food production and other industries. More needs to be known about the variation that already exists and how it can be conserved and accessed effectively. In the past 10 years there have been many advances in molecular biology that have provided a suite of powerful tools with enormous potential for the screening and evaluation of biodiversity. This volume was compiled in order to provide a comprehensive description and assessment of these tools. It includes a laboratory manual, in which detailed protocols for the techniques and analysis of the data they yield are described. In addition, a discussion is included of how the techniques can best be employed to tackle different diversity problems, using case studies of animals and plants as examples.

The book is aimed at investigators of all disciplines, with interests in the conservation, population biology, taxonomy, evolution, management, breeding and industrial application of plant and animal diversity. It is divided into five parts. In Part 1 there are four main chapters on DNA extraction, including: widely applicable methods; procedures for difficult species/tissues; methods for preserved specimens; and DNA extraction using commercial kits. Each chapter is divided into separate sub-chapters covering plants and animals. In Part 2 protocols are given for the many different screening methods available, ranging from isozymes and hybridization-based procedures to a whole range of PCR-based methods. In both Parts 1 and 2, all the contributors have introduced their subject with a review of the other methods available, and have presented not only the steps required for the procedures to work effectively, but also comments and tips that in their experience help to ensure success. The protocol chapters are supplemented by small boxes containing either additional information, or even additional methods, to ensure that as many features as possible are covered.

Part 3 of the book comprises chapters on sources of probes and primers and is a necessary complement to Part 2. The chapters vary in style depending on the subject matter. For example, detailed procedures are given for the isolation of microsatellite markers in plants and animals from enriched libraries (similar to Parts 1–2), whereas sources of nuclear, chloroplast and mitochondrial DNA are dealt with more descriptively. Part 4, which deals with data analysis, is largely composed of descriptive chapters which explain the different terminologies used and the methods of analysis that are essential for the effective and appropriate use of molecular data. The chapters are supplemented by boxes providing information of additional interest.

In the final Part case studies are of: (i) accessions and collections; (ii) populations; (iii) classification and phylogeny and; (iv) 'useful' variation. We did not restrict authors to a specific format. Some of the chapters, therefore, are short overviews (e.g. Chapter 21.4, Diversity in insect species using DNA sequences), whereas others describe individual studies (e.g. Chapter 21.5, The usefulness of parallel analysis of uni- and bi-parental markers: the North Atlantic humpback whale). All, however, have been chosen to reveal the usefulness of different markers.

The editors and contributors are all collaborators in an EU-funded project on 'Molecular Genetic Screening Tools', funded by the DGXII Biotechnology Unit. Exceptions are Jotun Hein, who kindly helped by writing the chapter on multiple alignment (18.3), and Mark Chase, who generously wrote the Foreword. We hope that our collective experience of these techniques, and their use in biodiversity studies, will be passed on through the book to all researchers who share an interest in plant and animal biodiversity.

Angela Karp
Peter G. Isaac
David S. Ingram

INTRODUCTION

Dr Etienne Magnien

Modern biotechnology, that is the use of molecular and cellular methods to gain knowledge of and to master more precisely biological processes, dates back only two decades. With the number of applications steadily increasing, a purely technological change gradually becomes a societal change. Humankind can never again look at living things with the same eyes. The manufacturing processes of cells, tissues and organisms lend themselves to programmed design, also referred to as bioprocess engineering. The EU research programme refers to it as the cell factory concept. With an increasing role devolved to technicians in managing and exploiting the living world, many new questions on issues of safety for human health, sustainability, biomedical and environmental ethics, preservation of human dignity and animal welfare come to the fore. Society is increasingly interested in knowing what dictates whether a biological process, once in human hands, may go right or wrong, and how predictable the fate of this new relationship of man with living resources may turn out to be.

A widely shared concern seems to be the risk that biotechnology might contribute further to the uniformity of plant and animal populations because of the exclusive use of a few high performing varieties or animal races. Beside the fact that this is not a claim specific to biotechnology, but applicable to any straightforward intensification in agricultural production, the evidence today points to the contrary. Biotechnology now allows genes to circulate from species to species, making the total gene pool available for breeding and selection much wider than ever before. New genes have been introduced into crop plants to provide novel ways to resist pests and pathogens. Plants can even be engineered to produce new products such as haemoglobin, for human health care applications. Biotechnological techniques have also led to increased use of genetic resources through the provision of new genetic markers which give greater ability to locate genes more precisely, and to follow their inheritance during breeding cycles. The trend towards uniformity or diversity is not imposed by the technology, it results from the value of rare products amenable to a breeding scheme and the relative abundance and diversity of such products in the pool. With biotechnology, numerous products of remote origins will become accessible and may end up in valuable new genetic combinations.

The difference is made by the attractiveness of the genetic attributes which could be picked up more widely, and not by the technology to bring about such attributes.

This is a reassuring principle, because the putatively endangered biodiversity, challenged by mechanization, deforestation, urbanization, and so on, will find an ally in technology whose side effect is to increase access to the global gene pool and which makes genetic innovation more directly dependent upon the range of diversity that can be made available.

Hence, the timeliness of the EU programme supporting research on molecular tools to characterize genetic diversity, part of Biotechnology R&D (1994–1998). Even before biotechnology starts bearing fruits, this molecular screening will make it possible to describe the extent of diversity in a species from any given habitat, population or inside collections. Traits will be traced or predicted. Gene frequencies will be monitored. Kinship will be measured. In

other words, genetic fingerprinting and other tools derived from it will be developed for a wide range of applications until genetic monitoring is a reality for the conservation, management and utilization of genetic resources.

It is to the credit of the members of the EU Molecular Screening Tools project, coordinated overall by A. Karp, that they shared this belief and made rapid progress to deliver screening methods usable across many different species in various operational conditions. The utility of the techniques was confirmed by the setting-up of a platform of users in 1996: the Biotechnology for Biodiversity Platform. New jobs are created to satisfy the demand in standardized analytical kits for large-scale applications. An ultimate though vital step is now being made in the compilation of the methods developed so far for the benefit of breeders, curators, conservationists, taxonomists and others. In this way, scientists under contract with the EU are disseminating their technological advances for the widest possible social benefit.