

Preface

For thousands of years, Chinese medicinal materials have been used to cure and prevent diseases. In recent years, scientific research has confirmed the pharmacological values of many of these materials. Unfortunately, the lack of proper quality control compromises the reputation of Chinese medicine and deters its wider application. Both in the marketplace and in literature, it is not uncommon to find that a given material has been called several different names and that several different herbs share a name. The challenge of correct identification is compounded by substitutions and unscrupulous adulterations, in some instances leading to herbal poisoning. In order to establish a healthy system for the application of traditional Chinese medicine, it is essential to properly authenticate raw materials and finished products. To this end, many countries have begun implementing standards for quality assurance of Chinese medicines, particularly for those that are imported. Since the great majority of Chinese medicinal materials originate from either plant or animal sources, DNA analysis becomes an important tool to complement organoleptic, morphological, anatomical and chemical parameters. As compared to phenotypic markers, molecular markers are more consistent and are definitive of a specific taxon. The wide array of sequences and patterns in the genomic, chloroplast and mitochondrial DNA offers numerous physical markers for characterising a species.

This book is the first international publication on molecular authentication of Chinese medicinal materials. We have gathered a number of the pioneers in this field to reveal their experiences in the methodology, application and evaluation of molecular approaches for authenticating plant and animal medicinal materials. In order for those researchers who are unfamiliar with molecular techniques to appreciate the technology and carry out similar experiments in their laboratories, the principles and procedures

of each DNA technique are provided in detail. We hope that this book will stimulate wider application of molecular techniques in the authentication and facilitate the advancement of this promising field.

The first chapter (P.-C. Shaw *et al.*) reviews the current status of the authentication of Chinese medicinal materials and introduces a wide range of the applicable DNA techniques. The second chapter (F.-N. Ngan and F.C.-F. Yau) lists the experimental procedures, equipment and reagents commonly used. For some procedures, precautions to be taken are also noted.

Chapters 3 to 6 describe techniques that apply polymerase chain reaction (PCR) at various levels of relaxed stringency to reveal the differences in genotype among organisms. These techniques do not require any prior knowledge of an organism's DNA sequence. Chapter 3 (H. Cao *et al.*) demonstrates two similar techniques: Random Amplified Polymorphic DNA (RAPD) and Arbitrarily Primed PCR (AP-PCR) used in differentiating *Elephantopus* and *Panax* species from their substitutes. Chapter 4 (K.-T. Cheng) focuses on the use of RAPD to distinguish herb samples of *Astragalus membranaceus*, *Atractylodes macrocephala* and *Ledebouriella seseloides* and the application of this technique to identify components from a mixed prescription. In Chapter 5, J. Wang *et al.* circumvent the low specificity of random-primed PCR by generating Sequence Characterised Amplification Region (SCAR) from the RAPD fingerprint. This method is demonstrated by differentiating *Panax ginseng* and *P. quinquefolius* from their adulterants. Chapter 6 (W.-Y. Ha *et al.*) elucidates the method of Direct Amplification Length Polymorphisms (DALP). Its uniqueness is found in the design of the primer which contains the 5' core sequence of the M13 sequencing primer. Hence, specific bands in the DALP fingerprint can be sequenced immediately for primer design.

Chapter 7 (W.-Y. Ha *et al.*) demonstrates another powerful fingerprinting technique, Amplified Fragment Length Polymorphism (AFLP), which combines both PCR and Restriction Fragment Length Polymorphism (RFLP). The AFLP primers contain a variable nucleotide at the 3' end to selectively amplify different sets of restriction fragments. *P. ginseng* and *P. quinquefolius* can be differentiated by this highly reproducible method. The application of Direct Amplification of a Minisatellite-region DNA (DAMD) polymorphism is also demonstrated.

Chapters 8 to 11 deal with techniques employing specific primers to generate DNA fragments for analysis and sequencing. In Chapter 8, (Y.Q. Wang and K.Y. Zhou) Cyt *b* and 12s rRNA sequences from the mitochondrial genome are used to authenticate animal medicinal materials including snakes, tortoise and turtle shells, and seahorses. In Chapter 9, D.T.-W. Lau *et al.* employ the ITS II region of rDNA to authenticate *Dendrobium* species and its adulterants. The complications in authenticating dried material are also outlined. Chapter 10 (H. Mizukami) demonstrates the use of 5s rRNA and *trnK* to authenticate *Angelica* and *Atractylodes species*, respectively. The author also suggests the use of PCR-RFLP and PCR-SSCP for differentiating crude drugs originating from *Atractylodes species*. In Chapter 11, K. Komatsu uses 18s RNA and *matK* genes as authentication markers for Chuanxionsgs and ginsengs. The author also applies PCR-RFLP and Amplification Refractory Mutation System (ARMS) for further analysis.

Finally, in Chapter 12, P.-C. Shaw *et al.* evaluate the advantages and limitations of molecular technologies and suggest areas for further development and other possible applications of molecular authentication.

We would like to thank all the contributors for providing their first-hand experience in molecular authentication. We are grateful to Prof. Ping-Chung Leung of the Chinese University for encouragement and Ms. Sook-Cheng Lim of the World Scientific Publishing Pte Ltd. for co-ordinating the project. We also gratefully acknowledge the expert editorial assistance from Ms. Carolyn Whitehead of WordCraft Communications. Our research work at various stages has been generously supported by the Chinese University of Hong Kong, the Research Grants Council of the Government of Hong Kong S.A.R., Health Food Enterprises Ltd., the Environment and Conservation Fund, and the National Research Institute of Chinese Medicine, Taiwan. Last but not least, we thank the Innovation and Technology Fund (AF/181/97 and AF/154/98) of the Government of Hong Kong S.A.R. and Chung Chi College, Chinese University of Hong Kong for providing financial support for the publication of this book.

P.-C. Shaw
J. Wang
P.P.-H. But