

# Genome-Based Approaches to the Authentication of Medicinal Plants

## Author

Nikolaus J. Sucher, Maria C. Carles

## Affiliation

Centre for Complementary Medicine Research, University of Western Sydney, Penrith South DC, NSW, Australia

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## Correspondence

**Nikolaus J. Sucher**  
 Professor of Herbal  
 Pharmacology  
 The Centre for Complementary  
 Medicine Research  
 University of Western Sydney  
 Locked Bag 1797  
 Penrith South DC  
 NSW 1797  
 Australia  
 Tel.: +61-2-4620-3345  
 Fax: +61-2-4620-3017  
 n.sucher@uws.edu.au

## Abstract

▼ Medicinal plants are the source of a large number of essential drugs in Western medicine and are the basis of herbal medicine, which is not only the primary source of health care for most of the world's population living in developing countries but also enjoys growing popularity in developed countries. The increased demand for botanical products is met by an expanding industry and accompanied by calls for assurance of quality, efficacy and safety. Plants used as drugs, dietary supplements and herbal medicines are identified at the species level. Unequivocal identification is a critical step at the beginning of an extensive process of quality assurance and is of importance for the characterization of the genetic diversity, phylogeny and phylogeography as well as the protection of endangered species. DNA-based methods have been developed for the identification of medicinal plants. Nuclear and chloroplast

DNA is amplified by the polymerase chain reaction and the reaction products are analyzed by gel electrophoresis, sequencing, or hybridization with species-specific probes. Genomic fingerprinting can differentiate between individuals, species and populations and is useful for the detection of the homogeneity of the samples and presence of adulterants. Although sequences from single chloroplast or nuclear genes have been useful for differentiation of species, phylogenetic studies often require consideration of DNA sequence data from more than one gene or genomic region. Phytochemical and genetic data are correlated but only the latter normally allow for differentiation at the species level. The generation of molecular “barcodes” of medicinal plants will be worth the concerted effort of the medicinal plant research community and contribute to the ongoing effort of defining barcodes for every species on earth.

## Introduction

▼ Plants have been used for medicinal purposes not only by humans since prehistoric times [1], [2] but are also used to treat various ailments by our closest relatives, the African great apes [3], [4]. To date, medicinal plants are the source of a large number of chemical compounds used as drugs in Western medicine and serve as the primary therapeutic resource for most of the world's population living in developing countries [5], [6], [7], [8], [9]. At the same time the use of herbal preparations for health care purposes is gaining popularity in developed countries [10], [11]. The increased demand for botanical products is met by an expanding industry and accompanied by calls for assurance of quality, efficacy and safety [12], [13].

The botanical sources of herbal supplements and medicines are identified at the species level by their Latin scientific names and the plant species is the basic unit for the preparation of herbal formulations. National pharmacopoeias such as that of China [14] as well as recent drug monographs (e.g., ref. [15]) prepared for the botanical industry and regulators always start their description of herbal drugs by naming the botanical species used for its preparation. Unequivocal identification and authentication of the plants used for production is therefore an elementary and critical step at the beginning of an extensive quality assurance process. Unfortunately, substitution or adulteration either intentionally, e.g., motivated by the desire to maximize financial gains, or unintentionally, e.g., by clerical errors or lack of knowledge, are not rare occurrences [16] and can have tragic consequences [17]. Authentica-

tion is also of importance for the characterization of the genetic diversity [18], [19], phylogeny and phylogeography [20], [21] as well as the protection and management of endangered species [22].

Identification of plants at the species level is traditionally achieved by careful examination of the specimen's macroscopic and microscopic morphology. This work usually needs to be performed by a specially trained expert. However, morphological identification is often not possible when the original plant material has been processed. Therefore, additional methods of identification at the species level have been sought and genome-based methods have been developed for the identification of medicinal plants starting in the early 1990s [17], [23]. This work followed in the footsteps of the use of DNA for plant systematics in the preceding two decades [24], [25] and was greatly facilitated by the invention of the polymerase chain reaction (PCR) and the introduction of a heat-stable DNA polymerase from the thermophilic bacterium *Thermus aquaticus* [26]. Together, these two achievements have revolutionized the way scientists work with DNA and made molecular cloning and DNA-based analysis accessible to workers in virtually every field concerned with living matter. In fact, molecular taxonomists now envision cataloging all living species on earth using so-called DNA barcodes, the nucleotide sequence of a short DNA fragment [27], [28], [29].

Here, we review the published work using genome-based approaches to the authentication of medicinal plants. Much of this work specifically relates to the authentication of plants used as sources of drugs in Chinese medicine. Chinese herbal medicine is part of a system of medical thought and practice that is distinctly different from that of Western medicine [30] and is the most widely practiced form of herbalism worldwide. In recent years, a number of factors have stimulated interest in Chinese medicine in the West, where an increasing number of patients and medical practitioners use herbal medicines as a supplement to or substitute for prescription drugs. Therefore, interactions between herbal and Western medicines have become an important issue in clinical practice [31], [32]. In China and Japan herbal medicines are listed in the national pharmacopoeias and their use is recognized and promoted by official health care policy on equal footing with Western style (single chemical entity) prescription drugs [33], [34], [35], [36].

### Molecular Biological Techniques used for Genome-Based Authentication

▼ An overview and description of the various techniques that have been used for genome-based authentication of medicinal plants is presented in **Table 1**. These procedures can be broadly divided into two general approaches. In one approach, investigators determine the nucleotide sequence of one or more genetic loci ("genes") in the plants of interest and identify nucleotide sequences that are characteristic (i.e., inherited by all members) of a given species. Examples of techniques that are based on this approach and are described in **Table 2** include allele-specific diagnostic PCR, amplified refractory mutation system (ARMS) and multiplex amplification refractory mutation system (MARMS), DNA microarray, and DNA sequencing. In a second approach, rather than focusing on specific genetic loci, researchers make use of species-specific variations (polymorphisms) of the nucleotide sequence that are spread randomly over the entire genome resulting in characteristic "fingerprints" of genomic

DNA. Examples of techniques that are based on this approach and are described in **Table 2** include amplified fragmented length polymorphism (AFLP), arbitrarily primed PCR (AP-PCR), direct amplification of length polymorphism (DALP), randomly amplified polymorphic DNA (RAPD), restriction length polymorphism (RFLP), inter simple sequence repeat anchored PCR and simple sequence repeat polymorphism (SSR). The PCR and its numerous variations are central to both approaches and virtually all of the published genome-based authentication work employs this technique.

PCR was originally developed for the directed amplification of predetermined regions of genomic DNA using primers with a specific sequence and is used in this way for the cloning and sequencing of specific genetic loci. However, PCR can also be used for the amplification of random stretches of DNA using primer pairs with arbitrary nucleotide sequences [37]. With arbitrary primers, the PCR yields a mixture of amplified products (amplicons) of various sizes that can be analyzed by gel electrophoresis. The amplicon patterns reflect the polymorphisms in different genomic DNA samples and are termed RAPD. This version of the PCR is a more rapid and less laborious replacement for the digestion of genomic DNA by restriction enzymes for the characterization of RFLP [38]. Both RAPD and RFLP result in a mixture of DNA fragments. The fragments are sorted by size using gel electrophoresis. The DNA is visualized either directly in the gel using fluorescent dyes (e.g., ethidium bromide) or indirectly using radioactively labeled probes, which are hybridized to the DNA following its transfer ("blotting") from the gel to a solid membrane (e.g., nitrocellulose or nylon). The latter procedure is referred to as Southern blotting using the name of its inventor as an eponym. The pattern obtained with a specific DNA sample is termed its "fingerprint". Once a "fingerprint" has been established for a control sample, the appearance of additional amplicons in test samples signals the presence of impurities or unexpected genetic variation. RAPD was used by some of the early workers using genome-based methods for the authentication of medicinal plants and their RAPD protocols as well as other modified versions of PCR have been collected in a recently published booklet [39]. As a PCR-based procedure, RAPD requires only nanogram amounts of genomic DNA and rapidly and efficiently generates a large number of genomic markers. Although RAPD is suitable for both the rapid sample authentication as well as the assessment of sample purity, it is often not easy to replicate fingerprint patterns established in one laboratory in another because even slight (instrumentation-dependent) variations during the PCR can result in variant fingerprints even when samples of the same genomic DNA are used. In contrast, sequencing will always yield the same result independent of the particular instrumentation used. DNA sequence data can be deposited as simple text strings (with explanatory meta data) in electronic databases such as GenBank and mined easily using text-based bioinformatics tools in contrast to gel-based fingerprints, which will require more complicated image analysis software. Finally, the advent of automated DNA sequencers and DNA microarrays has resulted in a considerable drop in the costs of using these techniques and should favor their more general and widespread use for genome-based authentication of medicinal plants.

**Table 1** Molecular biological methods used for the authentication of medicinal plants

Name	Acronym	Explanation
Polymerase chain reaction	PCR	PCR provides an <i>in vitro</i> method for the rapid enzymatic amplification of fragments of deoxyribonucleic acid (DNA) [114], [115]. In the PCR procedure, two oligonucleotide primers (often referred to as “upstream” and “downstream” or “forward” and “reverse” primers) that are complementary to the 5’ and 3’ flanking sequences of the DNA to be amplified are used to prime a heat-stable DNA polymerase that performs the copying of each strand of DNA. The denaturation of the DNA double helix, the annealing of the oligonucleotide primers to each complementary strand, and the synthesis of new strands by DNA polymerase are performed at their optimal temperature resulting in a three-step reaction. PCR is conducted in fully programmable thermocyclers that change the reaction temperatures at each step automatically [116].
Allele-specific diagnostic PCR		Primers with allele specific 3’ ends and labeled with different fluorochromes at their 5’ end are used together with a common primer in PCR [117]. The resulting amplicons can be analyzed by gel electrophoresis or capillary electrophoresis using an automated DNA sequencer.
Amplification refractory mutation system	ARMS	This variation of the PCR is based on the fact that the primers only bind to their target sequence when their 3’-ends are complementary. Oligonucleotides with mismatched (“mutated”) 3’ end residues will not bind to the “normal” target sequence and no amplification will take place [118].
Amplified fragmented length polymorphism	AFLP	In this technique, genomic DNA is digested with restriction enzymes. In a ligation reaction specific oligonucleotide adapters are added to the ends of the fragments, which can then be selectively amplified by PCR using primers that are complementary to the adapter and restriction site sequence [119].
Arbitrarily primed PCR	AP-PCR	Similar to RAPD but PCR is performed using sets of two longer primers (>18 nucleotides) of arbitrary sequence.
Direct amplification of length polymorphism	DALP	PCR is conducted with variable forward primers that contain a universal core sequence at their 5’ end and a constant reverse primer resulting in multiple amplicons that can be separated by gel electrophoresis, isolated and directly sequenced [120].
Multiplex PCR		PCR with multiple sets of forward and reverse primers in the same reaction resulting in parallel amplification [116].
PCR-selective restriction	PCR-SR	PCR amplicons obtained with gene specific primers are cut with restriction enzymes and analyzed by gel electrophoresis [121].
Randomly amplified polymorphic DNA	RAPD	Genomic DNA (gDNA) is amplified by PCR using a single, short (10 nucleotides) primer with arbitrary sequence resulting in multiple amplicons of different lengths (“fingerprint” pattern) that are analyzed by gel electrophoresis [37].
Sequence characterized amplified region	SCAR	Distinct amplicons obtained by RAPD are sequenced and amplicon specific primers are designed for use in PCR [122].
Restriction length polymorphism	RFLP	Genomic DNA is cut with sequence specific DNA restriction endonucleases resulting in the generation of a number of small fragments of various lengths, which are separated according to their molecular size by gel electrophoresis. The band pattern obtained with a specific DNA source and a specific restriction enzyme is called a DNA fingerprint of that source.
DNA microarray		A DNA microarrays, also often referred to as gene chip, DNA chip, or gene array, consists of a solid support matrix (e. g. a glass slide, silicon chip or synthetic membrane) to which DNA has been covalently bound in the form of a collection of microscopic spots [123]. Each spot contains DNA of a defined sequence that is referred to as the probe. Fluorescently labeled target DNA is hybridized to the chip, which is washed and then analyzed using a microarray reader.
DNA sequencing		DNA sequencing is now almost exclusively performed using cycle sequencing, which is conducted using a heat stable DNA polymerase and fluorescently labeled dideoxynucleotides in a thermocycler. The resulting polymerase products are separated according to length using capillary electrophoresis, detected by laser-induced fluorescence and automatically analyzed by computer software [124]. Older methods making use of radioactively labeled nucleotides and gel electrophoresis are still in use and may be the only option, when access to automated sequencers is not available.
Inter simple sequence repeat-anchored PCR	ISSR-PCR	In ISSR-PCR, primers anchored at simple sequence repeat (SSR) sequences (e. g., CACACACA; see below) are used to amplify the DNA regions between the flanking SSR [125].
Multiplex amplification refractory mutation system	MARMS	Multiplex PCR using a common primer and multiple mutation specific primers as used in ARMS [126].
Simple sequence repeat polymorphism	SSR	Simple sequence repeats (SSRs) or microsatellites are short sequence motifs consisting of 2 or more nucleotides (e. g., CA and ATG), which repeat in tandem (e. g., CACA-CA and ATGATGATG). The repeats vary in length (e. g., CACACA vs. CACACACACACA) and are ubiquitously and randomly distributed in all eukaryotic genomes. The length-polymorphisms can be easily detected by gel electrophoresis of amplicons generated by PCR using unique pairs of primers flanking the repeat [127].

**Table 2** Nuclear and chloroplast genes used for authentication of medicinal plants

Gene	Genome	Explanation
18S rRNA	Nuclear	The 18S ribosomal ribonucleic acid (rRNA) sequences have been widely used for phylogenetic studies in plants [128].
Internal transcribed spacers (ITS) of 18S, 5.8S and 26S rRNA	Nuclear	In land plants, the 18S, 5.8S and 26S rRNA genes form a linearly arrayed unit (a cistron) in which the individual coding regions separated by 2 internal transcribed spacers (ITS; ITS1 between the 18S and 5.8S genes and ITS2 between 5.8S and 26S genes). The cistron itself is tandemly arrayed separated by external transcribed spacers (ETS) on one or more chromosomes [57], [60], [129]. The ITS region has been used in many phylogenetic studies [58].
Intergenic spacer of the 5S rRNA (5S gene spacer)	Nuclear	In land plants, the genes for the 5S ribosomal RNA (rRNA) are arrayed as tandem repeats separated by intergenic spacers on one or more chromosomes [57]. The 5S rRNA sequence has been used for construction of the phylogenetic tree of major organisms [61].
26S rRNA	Nuclear	The entire coding region of the 26S rRNA gene can be amplified by DNA and was reported to provide ~3 times more phylogenetically informative characters than the 18S rRNA. The 26S rRNA sequence consists of conserved core and highly variable expansion regions [128].
atpA, atpB, atpF, atpH	Chloroplast	Single copy chloroplast genes coding for the ATP synthase subunits $\alpha$ (atpA), $\beta$ (atpB), I (atpF), and $\delta$ (atpH), res [130].
chlB	Chloroplast	A chloroplast gene coding for a subunit of the light-independent protochlorophyllide reductase that catalyzes the reduction of protochlorophyllide to chlorophyllide in photosynthetic bacteria, algae, and gymnosperms but is not present in angiosperms [131].
matK	Chloroplast	The matK gene, which is located within the trnK intron and comprises ~1.6 kbp. It is assumed to be involved in the splicing of group II introns [132].
psbA, psbK, psbI	Chloroplast	The psb genes code for proteins of photosystem II.
rbcl	Chloroplast	Large subunit of the enzyme ribulose-1,5-biphosphate carboxylase (rbcl) is one of the largest (~1.4 kbp) genes in the chloroplast genome. It has been sequenced in a large number of plants beginning in the mid-1980s [55], [56].
rp14, rpl16	Chloroplast	Chloroplast genes coding for the ribosomal proteins L14 and L16, constituents of the large subunit (50S) of the chloroplast ribosome. The chloroplast (70S) and nuclear (80S) ribosomes are of different size [130].
rpoB, rpoC1	Chloroplast	Chloroplast gene coding for DNA-directed RNA polymerase beta and gamma chains, respectively.
rps16	Chloroplast	Chloroplast gene coding for the ribosomal proteins S16, a constituent of the small subunit of the chloroplast ribosome.
trnC, trnD, trnF, trnK, trnL	Chloroplast	Genes coding for the transfer RNA (tRNA) for cystein, aspartate, phenylalanine, lysine, and leucine, respectively. Chloroplast genomes code for 20 to 40 different tRNAs [130]. Regions used in molecular taxonomy include the trnL intron and various tRNA intergenic spacer regions [133].

## Microchip-Based Authentication of Medicinal Plants

▼  
The desire to speed up the often slow and labor-intensive molecular analyses and reduce costs, has driven research and engineering efforts aimed at the automation and miniaturization of molecular biological analytical techniques and the development of miniature chip-based analytical devices with the goal to build a “lab-on-a-chip” [40], [41], [42], [43]. Our own work in this regard has been aimed at the development of microchip-based devices integrating sample preparation, amplification, detection, and analysis for the DNA-based identification of traditional Chinese herbal materials [44], [45], [46], [47], [48]. We chose silicon as primary and glass as secondary substrates for the fabrication of these devices. Silicon, the paramount substrate for the fabrication of electronic microchips, also offers a number of important advantages for the fabrication of lab-on-a-chip devices and we have recently shown that commonly used microfabrication techniques used in the production of electronic circuits can be modified to include biological materials such as DNA and even protein [49]. Using microfabrication methods, we built silicon-

based microchips integrating PCR reactors with built-in electrochemical detection or DNA microarrays and demonstrated their use for the genotyping of Chinese medicinal plants [46], [47]. This work demonstrated that the chips are suitable for the use in the design of automated systems for industrial use and even battery-operated, hand-held devices used as mobile instrumentation in the field.

## Molecular Basis of Genome-Based Authentication

▼  
Plant DNA comprises three independently replicated genomes. In addition to the nuclear genome that is organized in chromosomes, plants contain circular chloroplast and mitochondrial genomes. The nuclear DNA content (C-value) varies approximately 1000-fold across the angiosperms but exact C-values based on genome sequencing have not been obtained for any angiosperm to date [50]. The chloroplast genome in angiosperms ranges in size between 120 and 220 kb [51] and the plant mitochondrial genome varies in size from 200 kb in *Brassica* to over 2.5 Mb in watermelon and is substantially larger than that in animals,

which is only between 15–18 kb [52]. Interestingly, “whole” genome size determined by sequencing is generally smaller than the C-values indicate, as considerable amounts of genomic DNA cannot be cloned and sequenced with currently available techniques [50]. For example, the *Arabidopsis* Genome Initiative estimated the “genome” size of *Arabidopsis thaliana* at ~125 Mb (115.4 Mb in the sequenced regions plus an estimated 10 Mb in unsequenced regions) but recent data indicate that it may be considerably larger at 157 Mb [50].

The use of genome-based methods for the authentication of medicinal plants should be seen in the context of plant phylogenetic studies and a general effort aimed at barcoding of all plants [53], [54], [55], [56], [57]. Genetic loci commonly used for the authentication of medicinal plants have included the internal transcribed spacers (ITS) that separate the coding regions of the nuclear 5.8S, 18S and 26S rRNA genes [58], [59], [60] and the intergenic spacers that separate multiple repeated copies of the nuclear 5S rRNA gene [61]. On the other hand, genetic loci used in phylogenetic studies include several chloroplast-based genes [55], [56] such as *atpF*, *matK*, *rbcL*, *rpoB*, and *rpoC1*, the *trnL* intron and intergenic spacers between the *trnC-trnD*, *trnL-trnF*, *trnH-psbA*, and *psbK-psbKI* genes. It is noteworthy that the ITS and 5S spacers have been found to lack sufficient discriminatory power in some phylogenetic studies. In fact, sequence data from a single gene have proved to be insufficient for barcoding purposes in plants because multiple closely related species have been found to possess identical sequences at some loci. Consequently, the consensus view has developed that the unequivocal identification and barcoding of all plant species will require consideration of sequence data from more than one locus [53], [54], [62]. The generation of molecular “barcodes” of medicinal plants and deposition of sequence data in publicly accessible databases will be worth the concerted effort of the medicinal plant research community and contribute to the ongoing effort of defining barcodes for every (plant) species on earth. Along these lines, future studies aimed at the authentication of medicinal plants using genomic methods should focus on genetic loci that have been found useful for barcoding of plants in general in addition to those previously described in the literature.

### Application of Genome-Based Authentication

An overview of work that has been performed for the genome-based authentication of medicinal plants is presented in **Table 3**, which collates information from 82 published papers. The columns of the Table contain (from left to right): 1) an alphabetical list of the scientific names of the medicinal plant species that have been investigated (Plant) with information on 2) the plant parts (e.g., leaf or root; Part) used for DNA extraction and 3) their condition (e.g., fresh or dry; Condition), an indication of whether 4) a voucher specimen was retained (Voucher), 5) the method (e.g., DNA sequencing; Method), 6) the genetic loci used (Gene) and 7) the number corresponding to the original paper in the list of references (Ref).

Species that have been investigated using genome-based methods for authentication include plants of economical importance such as *Panax* [17], [63], [64], [65], [66], [67], [68], [69], [70], [71], [72], [73], [74], [75], *Fritillaria* [76], [77], [78], [79], [80], and *Ephedra* [81], [82], [83], [84], [85]. Published work furthermore includes species of forensic importance such as *Cannabis* [86], [87], [88], species threatened by extinction such as the

wild orchid *Dendrobium* [89], [90], [91], [92], [93], [94], [95], [96], [97], [98], [99], [100], [101], species of unclear phylogenetic relationship such as *Astragalus* [20], [102], [103], [104], [105], [106], and various toxic species such as *Aconitum*, *Datura* and *Strychnos* [44]. The data show that DNA was generally isolated from fresh leaves, stems or roots but in some cases also from dried material, crude drug, extracts and even finished products such as herbal teas, tablets and capsules [85]. Most of the studies included morphological identification of the plants by experts and deposition of voucher specimens in herbaria and museums. Availability of voucher specimens is useful in case potential discrepancies between past and future studies need to be resolved. A large number of studies have used PCR to establish genetic markers for the authentication of medicinal plants and detection of adulterants. The PCR is one of the most sensitive analytical techniques available and using carefully optimized conditions, it can be used to detect the presence of a single template molecule. In practice, however, pushing the limit of detection is prone to contamination artifacts. Therefore, it is better to use sufficient amounts of good quality template DNA that is free of PCR-inhibiting contaminants than to carry out PCR with a high number of amplification cycles (>35). The best method for the extraction and purification of DNA from a particular plant or drug sample needs to be established empirically. Tehen and colleagues [85] showed that the success of PCR was dependent on both the type of source material (raw plants, herbal teas, tablets, capsules) as well as the specific brand of commercial DNA extraction kit used. Following optimization of extraction and PCR, these workers reported correct identification of *Ephedra* species in complex herbal mixtures containing as little as 1 : 1000 part *Ephedra* tissue [85].

Several investigations examined the correlation of genetic markers with intra- and interspecies geographical and phytochemical variation. For example, workers using the DNA sequence of the 5S rRNA intergenic spacer domain as species identifier found both intra- and interspecies differences in the phytochemical fingerprints established by HPLC [105], [107], [108]. However, only DNA data could resolve species level differences in *Rehmannia* [18]. Not surprisingly, whole-genome RAPD or AP-PCR patterns exhibited more variation at the species level than the sequences of single DNA regions. For example, samples of *Astragalus membranaceus* collected from different geographical regions in China exhibited identical ITS1 sequences but different AP-PCR fingerprints [105]. Similarly, AP-PCR or RAPD fingerprints differentiated samples of *Codonopsis pilosula* from different regions in China [109]. Fruits from *Vitex rotundifolia* obtained from 14 different locations in China could be divided into four closely matching groups based on chemical fingerprinting using HPLC and DNA fingerprinting based on inter simple sequence repeat (ISSR)-anchored PCR [19]. Roots of *Panax notoginseng* collected from a single farm exhibited variation in their AFLP fingerprints which correlated with morphological differences such as variations in leaf color and phytochemical differences such as saponin content [67]. On the other hand, a study of cultivated *Ephedra* plants from different regions in China revealed not only the presence of both *Ephedra sinica* and *Ephedra intermedia* in the same field but also the occurrence of plants with markers for either species and varied morphology [83]. Dong and colleagues determined the DNA sequences of the 5S rRNA spacer, ITS and the 18S rRNA coding region in 10 different taxa of *Astragalus* and used several different bioinformatics tools to construct phylogenetic trees with each genetic region

**Table 3** Quick reference to publications on the application of genome-based methods for the authentication of medicinal plants sorted by species and quick references to experimental methods used (blank = no information provided)

Plant (scientific name)	Part	Condition	Voucher	Method	Gene	Ref
<i>Aconitum carmichaeli</i>	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
<i>Aconitum napellus</i>	Leaves		Yes	AFLP	N/A (not applicable)	[134]
<i>Aconitum pendulum</i>				PCR, sequencing; microarray (silicon)	trnL	[44]
<i>Actaea racemosa</i>	Leaves		Yes	AFLP	N/A	[134]
<i>Actaea cordifolia</i>	Leaves		Yes	AFLP	N/A	[134]
<i>Actaea podocarpa</i>	Leaves		Yes	AFLP	N/A	[134]
<i>Actaea pachypoda</i>	Leaves		Yes	AFLP	N/A	[134]
<i>Adenophora hunanensis</i>		Fresh, crude drug	Yes	PCR, sequencing	5S gene spacer	[135]
<i>Adenophora stricta</i>		Fresh, crude drug	Yes	PCR, sequencing	5S gene spacer	[135]
<i>Adenophora tetraphylla</i>		Fresh, crude drug	Yes	PCR, sequencing	5S gene spacer	[135]
<i>Agastache foeniculum</i>			Yes	PCR, sequencing	18S rRNA; matK	[136]
<i>Agastache rugosa</i>			Yes	PCR, sequencing	18S rRNA; matK	[136]
<i>Alisma canaliculatum</i>	Rhizome	Dried	Yes	PCR, sequencing; RFLP; ARMS	ITS	[137]
<i>Alisma gramineum</i>	Rhizome	Dried	Yes	PCR, sequencing; RFLP; ARMS	ITS	[137]
<i>Alisma lanceolatum</i>	Rhizome	Dried	Yes	PCR, sequencing; RFLP; ARMS	ITS	[137]
<i>Alisma nanum</i>	Rhizome	Dried	Yes	PCR, sequencing; RFLP; ARMS	ITS	[137]
<i>Alisma orientale</i>	Rhizome	Dried	Yes	PCR, sequencing; RFLP; ARMS	ITS	[137]
<i>Alisma plantago-aquatica</i>	Rhizome	Dried	Yes	PCR, sequencing; RFLP; ARMS	ITS	[137]
<i>Alocasia macrorrhiza</i>	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
<i>Angelica acutiloba</i>		Dried	Yes	PCR, sequencing	5S gene spacer	[107]
<i>Angelica acutiloba</i> var. <i>acutiloba</i>	Leaves	Fresh		PCR, sequencing	Spacer between atpF-atpA	[138]
<i>Angelica acutiloba</i> var. <i>iwatensis</i>	Leaves	Fresh		PCR, sequencing	Spacer between atpF-atpA	[138]
<i>Angelica acutiloba</i> var. <i>sugijamae</i>	Leaves	Fresh		PCR, sequencing	Spacer between atpF-atpA	[138]
<i>Angelica acutiloba</i>	Leaves	Fresh		RAPD; RFLP	N/A	[139]
<i>Angelica acutiloba</i> var. <i>Sugiyamae</i>	Leaves	Fresh		RAPD; RFLP	N/A	[139]
<i>Angelica gigas</i>		Dried	Yes	PCR, sequencing	5S gene spacer	[107]
<i>Angelica sinensis</i>		Dried	Yes	PCR, sequencing	5S gene spacer	[107]
<i>Angelica sinensis</i>	Root	Dried		RAPD; RFLP	N/A	[139]
<i>Aralia elata</i>			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
<i>Aralia franchetii</i>			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
<i>Arisaema heterophyllum</i>	Leaves	Fresh	Yes	PCR, sequencing; PCR-SR	Mannose-binding lectin	[121]
<i>Artemisia aponica</i>	Leaves	Fresh		PCR, sequencing, SCAR	N/A	[140]
<i>Artemisia argyi</i>	Leaves	Fresh		PCR, sequencing, SCAR	N/A	[140]
<i>Artemisia capillaries</i>	Leaves	Fresh		PCR, sequencing, SCAR	N/A	[140]
<i>Artemisia iwayomogi</i>	Leaves	Fresh		PCR, sequencing, SCAR	N/A	[140]
<i>Artemisia keiskeana</i>	Leaves	Fresh		PCR, sequencing, SCAR	N/A	[140]
<i>Artemisia princeps</i>	Leaves	Fresh		PCR, sequencing, SCAR	N/A	[140]
<i>Asarum arifolium</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum asaroides</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum asperum</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum blumei</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum canadense</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum caudatum</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum caudigerellum</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum caudigerum</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum caulescens</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum crassum</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum debile</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum dimidiatum</i>			Yes	PCR, sequencing	ITS	[21]
<i>Asarum europaeum</i>			Yes	PCR, sequencing	ITS	[141]

Plant (scientific name)	Part	Condition	Voucher	Method	Gene	Ref
<i>Asarum forbesii</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum fudsinoi</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum gelasinum</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum hartwegii</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum hatsushimae</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum heterotropoides</i> var. <i>heterotropoides</i>			Yes	PCR, sequencing	ITS	[21]
<i>Asarum heterotropoides</i> var. <i>mandshuricum</i>				PCR, sequencing	ITS	[142]
<i>Asarum heterotropoides</i> var. <i>mandshuricum</i>			Yes	PCR, sequencing	ITS	[21]
<i>Asarum heterotropoides</i> var. <i>seoulense</i>			Yes	PCR, sequencing	ITS	[21]
<i>Asarum himalaicum</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum lemonii</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum marmoratum</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum maruyamae</i>			Yes	PCR, sequencing	ITS	[21]
<i>Asarum mikuniense</i>			Yes	PCR, sequencing	ITS	[21]
<i>Asarum minimitanianum</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum minor</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum misandrum</i>			Yes	PCR, sequencing	ITS	[21]
<i>Asarum patens</i>			Yes	PCR, sequencing	ITS	[21]
<i>Asarum pulchellum</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum satsumense</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum savatieri</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum shuttleworthii</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum sieboldii</i>				PCR, sequencing	ITS	[142]
<i>Asarum sieboldii</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum sieboldii</i> f. <i>maculatum</i>			Yes	PCR, sequencing	ITS	[21]
<i>Asarum sieboldii</i> f. <i>seoulense</i>				PCR, sequencing	ITS	[142]
<i>Asarum sieboldii</i> f. <i>siboldii</i>			Yes	PCR, sequencing	ITS	[21]
<i>Asarum sieboldii</i> var. <i>cornutum</i>			Yes	PCR, sequencing	ITS	[21]
<i>Asarum speciosum</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum takaoi</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum tohokuense</i>			Yes	PCR, sequencing	ITS	[21]
<i>Asarum versicolor</i>			Yes	PCR, sequencing	ITS	[21]
<i>Asarum virginicum</i>			Yes	PCR, sequencing	ITS	[141]
<i>Asarum yakusimense</i>			Yes	PCR, sequencing	ITS	[141]
<i>Astragalus aksuensis</i>		Dried	Yes	PCR, sequencing	5S gene spacer, ITS; 18S rRNA	[20]
<i>Astragalus austrosibiricus</i>		Dried	Yes	PCR, sequencing	5S gene spacer; ITS; 18S rRNA	[20]
<i>Astragalus hoantchy</i>		Dried	Yes	PCR, sequencing	5S gene spacer; ITS; 18S rRNA	[20]
<i>Astragalus hoantchy</i> subsp. <i>Dshimensis</i>		Dried	Yes	PCR, sequencing	5S gene spacer; ITA; 18S rRNA	[20]
<i>Astragalus lehmannianus</i>	Leaves, roots	Fresh, crude drug	Yes	PCR, sequencing	5S gene spacer	[104]
<i>Astragalus lehmannianus</i>		Dried	Yes	PCR, sequencing	5S gene spacer; ITS; 18S rRNA	[20]
<i>Astragalus lepsensis</i>		Dried	Yes	PCR, sequencing	5S gene spacer; ITS; 18S rRNA	[20]
<i>Astragalus membranaceus</i>	Leaves, roots	Fresh, crude drug	Yes	PCR, sequencing	5S gene spacer	[104]
		Dried	Yes	PCR, sequencing	5S gene spacer; ITS; 18S rRNA	[20]

Plant (scientific name)	Part	Condition	Voucher	Method	Gene	Ref
	Roots	Fresh		3' untranslated region sequence-based amplified polymorphism (UAP)	3' untranslated regions (3'UTR)	[102]
				RAPD	N/A	[106]
<i>Astragalus membranaceus</i> from 23 locations		Dried		AP-PCR	ITS	[105]
<i>Astragalus membranaceus</i> var. <i>mongholicus</i>	Leaves, roots	Fresh, crude drug	Yes	PCR, sequencing	5S gene spacer	[104]
		Dried	Yes	PCR, sequencing	5S gene spacer; ITS; 18S rRNA	[20]
	Roots	Fresh		3' untranslated region sequence-based amplified polymorphism (UAP)	3' untranslated regions (3'UTR)	[102]
<i>Astragalus membranaceus</i> var. <i>mongholicus</i> from 23 locations		Dried		AP-PCR	ITS	[105]
<i>Astragalus propinquus</i>		Dried	Yes	PCR, sequencing	5S gene spacer; ITS; 18S rRNA	[20]
<i>Astragalus sieversianus</i>		Dried	Yes	PCR, sequencing	5S gene spacer; ITS; 18S rRNA	[20]
<i>Astragalus hoanchy</i>	Leaves, roots	Fresh, crude drug	Yes	PCR, sequencing	5S gene spacer	[104]
<i>Atractylodes chinensis</i>	Crude drug			PCR, sequencing	ITS	[143]
<i>Atractylodes japonica</i>				RAPD	N/A	[144]
<i>Atractylodes japonica</i>	Crude drug			PCR, sequencing	ITS	[143]
<i>Atractylodes lancea</i>				RAPD	N/A	[144]
<i>Atractylodes lancea</i>	Leaves	Fresh		PCR, sequencing, SCAR	N/A	[140]
<i>Atractylodes ovata</i>				RAPD	N/A	[144]
<i>Atractylodes ovata</i>	Crude drug			PCR, sequencing	ITS	[143]
<i>Bacopa monnieri</i>	Leaves	Fresh		RAPD	N/A	[145]
<i>Bupleurum aureum</i>		Fresh		PCR, sequencing	ITS	[146]
<i>Bupleurum chinense</i>		Fresh		PCR, sequencing	ITS	[146]
<i>Bupleurum commelynoideum</i> var. <i>flaviflorum</i>		Fresh		PCR, sequencing	ITS	[146]
<i>Bupleurum krylovianum</i>		Fresh		PCR, sequencing	ITS	[146]
<i>Bupleurum longiradiatum</i>		Fresh		PCR, sequencing	ITS	[146]
<i>Bupleurum marginatum</i> var. <i>stenophyllum</i>		Fresh		PCR, sequencing	ITS	[146]
<i>Bupleurum scorzonerifolium</i>		Fresh		PCR, sequencing	ITS	[146]
<i>Bupleurum sibiricum</i>		Fresh		PCR, sequencing	ITS	[146]
<i>Bupleurum smithii</i>		Fresh		PCR, sequencing	ITS	[146]
<i>Bupleurum tianschanicum</i>		Fresh		PCR, sequencing	ITS	[146]
<i>Bupleurum yinchouwense</i>		Fresh		PCR, sequencing	ITS	[146]
<i>Cannabis sativa</i>	Leaves	Fresh	Yes	ISSR	N/A	[88]
	Leaves, stems, flowering heads	Fresh, dried		RAPD	N/A	[87]
	Leaves, inflorescences	Fresh, dried		AFLP	N/A	[86]
<i>Carthamus tinctorius</i>	Leaf	Fresh, crude drug	Yes	PCR, sequencing	5S gene spacer	[147]
<i>Changium smyrnioides</i>	Leaves	Dried	Yes	RAPD	N/A	[148]
<i>Codonopsis pilulosa</i>	Roots	Dried	Yes	AP-PCR, RAPD	N/A	[109]
<i>Corton tigilium</i>	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
<i>Crocus sativus</i>	Leaf	Fresh, crude drug	Yes	PCR, sequencing	5S gene spacer	[147]



Plant (scientific name)	Part	Condition	Voucher	Method	Gene	Ref
<i>Cultivated Ephedra</i>	Stem	Fresh	Yes	PCR, sequencing	ITS; trnL; trnL-trnF	[83]
<i>Curcuma chuanyujin</i>		Dried, crude drug	Yes	PCR, sequencing	5S gene spacer	[108]
<i>Curcuma kwangsiensis</i>		Dried, crude drug	Yes	PCR, sequencing	5S gene spacer	[108]
<i>Curcuma longa</i>		Dried, crude drug	Yes	PCR, sequencing	5S gene spacer	[108]
<i>Curcuma phaeocalis</i>		Dried, crude drug	Yes	PCR, sequencing	5S gene spacer	[108]
<i>Curcuma wenyujin</i>		Dried, crude drug	Yes	PCR, sequencing	5S gene spacer	[108]
<i>Datura innoxia</i>	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
<i>Datura metel</i>	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
<i>Datura tatula</i>	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
<i>Dendrobium acinaforme</i>	Leaves, stems	Fresh, dried		PCR	ITS	[91]
<i>Dendrobium aduncum</i>	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
<i>Dendrobium aphyllum</i>	Leaves, stems	Fresh, dried		PCR	ITS	[91]
				PCR, sequencing	ITS	[93]
<i>Dendrobium aurantiacum</i>	Leaves, stems	Fresh, dried		PCR	ITS	[91]
<i>Dendrobium aurantiacum</i> var. <i>denneanum</i>	Leaves, stems	Fresh, dried		PCR	ITS	[91]
<i>Dendrobium auriantiacum</i>	Stems			Microarray (nylon)	gDNA	[95]
<i>Dendrobium brymerianum</i>	Leaves, stems	Fresh, dried		PCR	ITS	[91]
<i>Dendrobium candidum</i> (= <i>Dendrobium officinale</i> )	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
<i>Dendrobium cantonensis</i>	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
<i>Dendrobium capillipes</i>	Leaves, stems	Fresh, dried		PCR	ITS	[91]
<i>Dendrobium cariniferum</i>	Leaves, stems	Fresh, dried		PCR	ITS	[91]
<i>Dendrobium chrysanthum</i>	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
				PCR, sequencing	ITS	[93]
<i>Dendrobium chrysotoxum</i>	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
	Stems			Microarray (nylon)	gDNA	[95]
<i>Dendrobium crepidatum</i>	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
				PCR, sequencing	ITS	[93]
<i>Dendrobium crystallinum</i>	Leaves, stems	Fresh, dried		PCR	ITS	[91]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
<i>Dendrobium densiflorum</i>	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
<i>Dendrobium densiflorum</i>		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]

Plant (scientific name)	Part	Condition	Voucher	Method	Gene	Ref
	Leaves, stems	Fresh, dried	Yes		ITS	[91]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
<i>Dendrobium ellipsophyllum</i>	Leaves, stems	Fresh, dried		PCR	ITS	[91]
<i>Dendrobium exile</i>	Leaves, stems	Fresh, dried		PCR	ITS	[91]
<i>Dendrobium falconeri</i>	Leaves, stems	Fresh, dried		PCR	ITS	[91]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
<i>Dendrobium fimbriatum</i>	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
	Stems			Microarray (nylon)	gDNA	[95]
<i>Dendrobium fimbriatum</i> var. <i>occulatum</i>	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
<i>Dendrobium findlayanum</i>	Leaves, stems	Fresh, dried		PCR	ITS	[91]
<i>Dendrobium flexicaule</i>	Leaves, stems	Fresh, dried		PCR	ITS	[91]
<i>Dendrobium funiushanense</i>	Leaves, stems	Fresh, dried		PCR	ITS	[91]
<i>Dendrobium gratiosissimum</i>	Leaves, stems	Fresh, dried	Yes		ITS	[91]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
<i>Dendrobium hancockii</i>	Leaves, stems	Fresh, dried		PCR	ITS	[91]
<i>Dendrobium henanense</i>	Leaves, stems	Fresh, dried		PCR	ITS	[91]
<i>Dendrobium hercoglossum</i>	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
<i>Dendrobium huoshanense</i>	Leaves, stems	Fresh, dried		PCR	ITS	[91]
<i>Dendrobium jenkinsii</i>	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
<i>Dendrobium lindleyi</i>	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
<i>Dendrobium lituiflorum</i>	Leaves, stems	Fresh, dried		PCR	ITS	[91]
				PCR, sequencing	ITS	[93]
<i>Dendrobium loddigesii</i>	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
	Leaves, stems	Fresh, dried	Yes		ITS	[91]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
<i>Dendrobium lohohense</i>	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
<i>Dendrobium miniliforme</i>	Leaves, stems	Fresh, dried		PCR	ITS	[91]
<i>Dendrobium moniliforme</i>	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
<i>Dendrobium moschatum</i>	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]

Plant (scientific name)	Part	Condition	Voucher	Method	Gene	Ref
<i>Dendrobium nobile</i>	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
<i>Dendrobium officinale</i>	Stems			Microarray (nylon)	gDNA	[95]
	Leaves, stems	Fresh, dried	Yes		ITS	[91]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
	Stems			Microarray (nylon)	gDNA	[95]
	Stems, leaves	Fresh, Dried		PCR, sequencing	ITS	[90]
<i>Dendrobium pendulum</i>	Leaves, stems	Fresh, dried		PCR	ITS	[91]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
<i>Dendrobium primulinum</i>	Leaves, stems	Fresh, dried		PCR	ITS	[91]
		Fresh, medicinal formulation	Yes	PCR, microarray (glass)	ITS	[101]
				PCR, sequencing	ITS	[93]
<i>Dendrobium salaccense</i>	Leaves, stems	Fresh, dried		PCR	ITS	[91]
<i>Dendrobium thyriflorum</i>	Leaves, stems	Fresh, dried		PCR	ITS	[91]
<i>Dendrobium wardianum</i>	Leaves, stems	Fresh, dried		PCR	ITS	[91]
<i>Dendrobium williamsonii</i>	Stem	Fresh	Yes	PCR, sequencing	ITS	[94]
	Leaves, stems	Fresh, dried		PCR	ITS	[91]
<i>Digitalis obscura</i>	Leaves	Fresh		RAPD		[149]
<i>Dioscorea alata</i>				PCR, sequencing	18S rRNA	[150]
<i>Dioscorea japonica</i>				PCR, sequencing	18S rRNA	[150]
<i>Dioscorea persimilis</i>				PCR, sequencing	18S rRNA	[150]
<i>Dioscorea polystachia</i>				PCR, sequencing	18S rRNA	[150]
<i>Dysosma aurantiocaulis</i>	Leaves	Dried	Yes	PCR, RFLP	trnT-trnL; trnD-trnT	[151]
<i>Dysosma difformis</i>	Leaves	Dried	Yes	PCR, RFLP	trnT-trnL; trnD-trnT	[151]
<i>Dysosma majorensis</i>	Leaves	Dried	Yes	PCR, RFLP	trnT-trnL; trnD-trnT	[151]
<i>Dysosma pleiantha</i>				PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
	Leaves	Dried	Yes	PCR, RFLP	trnT-trnL; trnD-trnT	[151]
<i>Dysosma veitchii</i>	Leaves	Dried	Yes	PCR, RFLP	trnT-trnL; trnD-trnT	[151]
<i>Dysosma versipellis</i>		Fresh		PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
	Leaves	Dried	Yes	PCR, RFLP	trnT-trnL; trnD-trnT	[151]
<i>Echinacea angustifolia</i>	Leaves			RAPD	N/A	[152]
				RAPD	N/A	[153]
<i>Echinacea artrorubens</i>				RAPD	N/A	[152]
<i>Echinacea pallida</i>				RAPD	N/A	[152]
				RAPD	N/A	[153]
<i>Echinacea purpurea</i>				RAPD	N/A	[152]
				RAPD	N/A	[153]
<i>Ephedra antisiphilitca</i>	Aerial parts	Dried	Yes	PCR, sequencing	pbsA-trnH	[85]
<i>Ephedra aspera</i>	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
<i>Ephedra californica</i>	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
<i>Ephedra coryi</i>	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
<i>Ephedra distachya</i>	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
<i>Ephedra equisetina</i>		Dried, crude drug	Yes	PCR, sequencing; PCR, RFLP	chlB; ITS	[81]
	Stem	Fresh	Yes	PCR, sequencing	ITS; trnL; trnL-trnF	[84]
	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
<i>Ephedra fasciculata</i>	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
<i>Ephedra fragilis</i>	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
<i>Ephedra fedtschenkkoae</i>	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
<i>Ephedra foeminea</i>	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]

Plant (scientific name)	Part	Condition	Voucher	Method	Gene	Ref
<i>Ephedra frustilata</i>	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
<i>Ephedra gerardiana</i>	Stem	Fresh	Yes	PCR, sequencing	ITS; trnL; trnL-trnF	[84]
<i>Ephedra intermedia</i>		Dried, crude drug	Yes	PCR, sequencing; PCR, RFLP	chlB; ITS	[81]
	Stem	Fresh	Yes	PCR, sequencing	ITS; trnL; trnL-trnF	[84]
<i>Ephedra likiangensis</i>	Stem	Fresh	Yes	PCR, sequencing	ITS; trnL; trnL-trnF	[84]
	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
<i>Ephedra major</i>	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
<i>Ephedra minuta</i>	Stem	Fresh	Yes	PCR, sequencing	ITS; trnL; trnL-trnF	[84]
<i>Ephedra monosperma</i>	Stem	Fresh	Yes	PCR, sequencing	ITS; trnL; trnL-trnF	[84]
<i>Ephedra nevadensis</i>	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
<i>Ephedra ochreatea</i>	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
<i>Ephedra przewalskii</i>		Dried, crude drug	Yes	PCR, sequencing; RFLP	chlB; ITS	[81]
	Stem	Fresh	Yes	PCR, sequencing	ITS; trnL; trnL-trnF	[84]
	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
<i>Ephedra saxatilis</i>	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
<i>Ephedra sinica</i>		Dried, crude drug	Yes	PCR, sequencing; PCR, RFLP	chlB; ITS	[81]
	Stem	Fresh	Yes	PCR, sequencing	ITS; trnL; trnL-trnF	[84]
	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
<i>Ephedra trifurca</i>	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
<i>Ephedra torreyana</i>	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
<i>Ephedra viridis</i>	Aerial parts	Dried	Yes	PCR, sequencing	psbA-trnH	[85]
<i>Epimedium</i>			Yes	PCR, sequencing	5S gene spacer	[154]
<i>Epimedium brevicornu</i>						
<i>Epimedium koreanum</i>			Yes	PCR, sequencing	5S gene spacer	[154]
<i>Epimedium pubescens</i>			Yes	PCR, sequencing	5S gene spacer	[154]
<i>Epimedium sagittatum</i>			Yes	PCR, sequencing	5S gene spacer	[154]
<i>Epimedium wushanense</i>			Yes	PCR, sequencing	5S gene spacer	[154]
<i>Euphorbia discolor</i>			Yes	PCR, sequencing	ITS	[155]
<i>Euphorbia esula</i>			Yes	PCR, sequencing	ITS	[155]
<i>Euphorbia kansui</i>	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
<i>Euphorbia lamprocarpa</i>			Yes	PCR, sequencing	ITS	[155]
<i>Euphorbia lathyris</i>			Yes	PCR, sequencing	ITS	[155]
<i>Euphorbia pekinensis</i>			Yes	PCR, sequencing	ITS	[155]
<i>Euphorbia peplus</i>			Yes	PCR, sequencing	ITS	[155]
<i>Euphorbia turczaninowii</i>			Yes	PCR, sequencing	ITS	[155]
<i>Fritillaria anhuiensis</i>	Leaves, bulbs	Fresh	Yes	PCR, sequencing; restriction digest	5S gene spacer	[77]
<i>Fritillaria cirrhosa</i>	Leaves, bulbs	Fresh	Yes	PCR, sequencing; restriction digest	5S gene spacer	[77]
	Leaves, bulbs	Dried	Yes	PCR, sequencing; PCR, RFLP	ITS	[80]
				PCR, sequencing; PCR, microarray (glass)	26S rRNA	[79]
<i>Fritillaria delavayi</i>	Leaves, bulbs	Dried	Yes	PCR, sequencing; PCR, RFLP	ITS	[80]
				PCR, sequencing; PCR, microarray (glass)	26S rRNA	[64]
<i>Fritillaria hupehensis</i>	Leaves, bulbs	Dried	Yes	PCR, sequencing; PCR, RFLP	ITS	[80]
<i>Fritillaria pallidiflora</i>	Leaves, bulbs	Dried	Yes	PCR, sequencing; PCR, RFLP	ITS	[80]
				PCR, sequencing; PCR, microarray (glass)	26S rRNA	[64]
<i>Fritillaria przewalskii</i>	Leaves, bulbs	Dried	Yes	PCR, sequencing; PCR, RFLP	ITS	[80]
<i>Fritillaria puqiensis</i>	Leaves, bulbs	Fresh	Yes	PCR, sequencing; restriction digest	5S gene spacer	[77]
	Leaves, bulbs	Dried	Yes	PCR, sequencing; PCR, RFLP	ITS	[80]
<i>Fritillaria thunbergii</i>	Leaves, bulbs	Fresh	Yes	PCR, sequencing; restriction digest	5S gene spacer	[77]
	Leaves, bulbs	Dried	Yes	PCR, sequencing; PCR, RFLP	ITS	[80]
<i>Fritillaria thunbergii</i> <i>var. chekiangensis</i>				PCR, sequencing; PCR, microarray (glass)	26S rRNA	[64]
<i>Fritillaria unibracteata</i>	Leaves, bulbs	Dried	Yes	PCR, sequencing; PCR, RFLP	ITS	[80]

Plant (scientific name)	Part	Condition	Voucher	Method	Gene	Ref
				PCR, sequencing; PCR, microarray (glass)	26S rRNA	[64]
<i>Fritillaria ussuriensis</i>	Leaves, bulbs	Dried	Yes	PCR, sequencing; PCR, RFLP	ITS	[80]
				PCR, sequencing; PCR, microarray (glass)	26S rRNA	[64]
<i>Fritillaria walujewii</i>	Leaves, bulbs	Dried	Yes	PCR, sequencing; PCR, RFLP	ITS	[80]
<i>Gentiana straminea</i>			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]
<i>Glehnia littoralis</i>	Leaves	Fresh		RFLP	N/A	[157]
		Fresh, crude drug	Yes	PCR, sequencing	5S gene spacer	[135]
<i>Gnetum gnemon</i>	Aerial parts	Fresh	Yes	PCR, sequencing	psbA-trnH	[85]
<i>Gnetum lepostachyum</i>	Stems	Fresh	Yes	PCR, sequencing	ITS; trnL; trnL-trnF	[84]
<i>Halenia elliptica</i>			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]
<i>Hedysarum polybotris</i>	Leaves, roots	Fresh, crude drug	Yes	PCR, sequencing	5S gene spacer	[104]
				RAPD	N/A	[106]
<i>Hemerocallis citrina</i>	Leaf	Fresh, crude drug	Yes	PCR, sequencing	5S gene spacer	[147]
<i>Hemerocallis fulva</i>	Leaf	Fresh, crude drug	Yes	PCR, sequencing	5S gene spacer	[147]
<i>Humulus hops</i>	Leaves, stems, flowering heads	Fresh, dried		RAPD	N/A	[87]
<i>Hyoscyamus niger</i>	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
<i>Lamium amplexicaule</i>				PCR, sequencing	ITS	[158]
<i>Leonurus chaituroides</i>				PCR, sequencing	ITS	[158]
<i>Leonurus heterophyllus</i>				PCR, sequencing	ITS	[158]
<i>Leonurus pseudomacranthus</i>				PCR, sequencing	ITS	[158]
<i>Leonurus sibiricus</i>				PCR, sequencing	ITS	[158]
<i>Ligularia dentata</i>			Yes	PCR, sequencing	5S gene spacer	[159]
<i>Ligularia knaizensis</i>			Yes	PCR, sequencing	5S gene spacer	[159]
<i>Ligularia lankongensis</i>			Yes	PCR, sequencing	5S gene spacer	[159]
<i>Ligularia lapathifolia</i>			Yes	PCR, sequencing	5S gene spacer	[159]
<i>Ligularia narynensis</i>			Yes	PCR, sequencing	5S gene spacer	[159]
<i>Ligularia nelumbifolia</i>			Yes	PCR, sequencing	5S gene spacer	[159]
<i>Ligularia pleurocaulis</i>			Yes	PCR, sequencing	5S gene spacer	[159]
<i>Ligularia przewalskii</i>			Yes	PCR, sequencing	5S gene spacer	[159]
<i>Ligularia sagitta</i>			Yes	PCR, sequencing	5S gene spacer	[159]
<i>Ligularia subspicata</i>			Yes	PCR, sequencing	5S gene spacer	[159]
<i>Ligularia tongolensis</i>			Yes	PCR, sequencing	5S gene spacer	[159]
<i>Ligularia virgaurea</i>			Yes	PCR, sequencing	5S gene spacer	[159]
<i>Lomatogonium orecharis</i>			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]
<i>Lycium barbarum</i>	Fruit	Dried	Yes	RAPD	N/A	[160]
<i>Lycium barbarum</i> cv. "Tianjinense"	Fruit	Dried	Yes	RAPD	N/A	[160]
<i>Lycium barbarum</i> var. <i>aranticarpum</i>	Fruit	Dried	Yes	RAPD	N/A	[160]
<i>Lycium barbarum</i> var. <i>potaninii</i>	Fruit	Dried	Yes	RAPD	N/A	[160]
<i>Lycium chinense</i>	Fruit	Dried	Yes	RAPD	N/A	[160]
<i>Lycium dasy</i> Stems var. <i>rubricaulium</i>	Fruit	Dried	Yes	RAPD	N/A	[160]
<i>Lycium ruthenicum</i>	Fruit	Dried	Yes	RAPD	N/A	[160]
<i>Lycium truncatum</i>	Fruit	Dried	Yes	RAPD	N/A	[160]
<i>Medicago sativa</i>	Leaves; dried ground material	Fresh, dried		PCR, sequencing; RFLP	ITS	[161]
<i>Mirabilis jalapa</i>	Roots	Fresh, dried	Yes	AP-PCR; RAPD	N/A	[71]
<i>Nandina domestica</i>			Yes	PCR, sequencing	5S gene spacer	[154]
<i>Panax assamicus</i>			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
<i>Panax bipinnatifidus</i> var. <i>angustifolius</i>			Yes	PCR, sequencing	ITS; trnC-trnD	[69]

Plant (scientific name)	Part	Condition	Voucher	Method	Gene	Ref
<i>Panax bipinnatifidus</i> var. <i>bipinnatifidus</i>			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
<i>Panax elegantior</i>			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
<i>Panax ginseng</i>	Roots			AP-PCR	N/A	[17]
	Roots	Fresh, dried	Yes	AP-PCR; RAPD	N/A	[71]
	Roots	Fresh, dried	Yes	RAPD, sequencing; SCAR	N/A	[162]
	Roots	Fresh, dried	Yes	RAPD, DALP, sequencing	N/A	[66]
	Leaves, roots	Fresh, crude drug	Yes	MARMS	trnK, 18S rRNA	[74]
				PCR	SSR	[163]
		Crude drug		RAPD	N/A	[72]
			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
<i>Panax japonicus</i>	Leaves, roots	Fresh, crude drug	Yes	MARMS	trnK, 18S rRNA	[74]
		Crude drug		RAPD	N/A	[72]
			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
<i>Panax major</i>			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
<i>Panax notoginseng</i>	Roots	Fresh, dried	Yes	AP-PCR; RAPD	N/A	[71]
	Leaves, roots	Fresh, crude drug	Yes	MARMS	trnK, 18S rRNA	[74]
		Crude drug		RAPD	N/A	[72]
			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
	Roots	Fresh		AFLP; PCR, sequencing	ITS 2	[67]
<i>Panax omeiensis</i>			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
<i>Panax pseudoginseng</i>			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
<i>Panax quinquefolium</i>	Roots			AP-PCR	N/A	[17]
	Roots	Fresh, dried	Yes	AP-PCR; RAPD	N/A	[71]
	Roots	Fresh, dried	Yes	RAPD, sequencing; SCAR	N/A	[162]
	Roots	Fresh, dried	Yes	RAPD, DALP, sequencing	N/A	[66]
	Leaves, roots	Fresh, crude drug	Yes	MARMS	trnK, 18S rRNA	[74]
				PCR	Microsatellite marker	[163]
<i>Panax quinquefolium</i>		Crude drug		RAPD	N/A	[72]
			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
<i>Panax shangianus</i>			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
<i>Panax sinensis</i>			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
<i>Panax stipulenatus</i>			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
<i>Panax trifolius</i>			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
<i>Panax variabilis</i>			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
<i>Panax vietnamensis</i>	Leaves, roots	Fresh, crude drug	Yes	MARMS	trnK, 18S rRNA	[74]
			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
<i>Panax wangianus</i>			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
<i>Panax zingiberensis</i>			Yes	PCR, sequencing	ITS; trnC-trnD	[69]
<i>Perilla frutescens</i>				PCR, sequencing	ITS	[164]
<i>Perilla frutescens</i> var. <i>arguta</i>				PCR, sequencing	ITS	[164]
<i>Perilla frutescens</i> var. <i>auriculato-dentata</i>				PCR, sequencing	ITS	[164]
<i>Perilla frutescens</i> var. <i>crispa</i>				PCR, sequencing	ITS	[164]
<i>Pholidota cantonensis</i>	Stems	Fresh	Yes	PCR, sequencing	ITS	[94]
<i>Phyllanthus amarus</i>			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
<i>Phyllanthus arenarius</i>			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
<i>Phyllanthus calcynus</i>			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
<i>Phyllanthus clakei</i>			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
<i>Phyllanthus cochinchinensis</i>			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
<i>Phyllanthus distichus</i>	Leaves	Fresh		RAPD, sequencing SCAR	N/A	[166]
<i>Phyllanthus emblica</i> (= <i>Emblica officinalis</i> )	Leaves	Fresh and dried		RAPD, sequencing; SCAR	N/A	[166]
<i>Phyllanthus emblica</i> (= <i>Emblica officinalis</i> )			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
<i>Phyllanthus flexuosus</i>			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]

Plant (scientific name)	Part	Condition	Voucher	Method	Gene	Ref
<i>Phyllanthus glaucus</i>			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
<i>Phyllanthus guangdongensis</i>			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
<i>Phyllanthus hainanensis</i>			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
<i>Phyllanthus indofischeri</i>	Leaves	Fresh		RAPD, sequencing; SCAR	N/A	[166]
<i>Phyllanthus lokohensis</i>			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
<i>Phyllanthus myrtifolius</i>			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
<i>Phyllanthus niruri</i>	Leaves	Fresh		RAPD, sequencing; SCAR,	N/A	[166]
			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
<i>Phyllanthus nummulariifolius</i>			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
<i>Phyllanthus parvifolius</i>			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
<i>Phyllanthus reticulatus</i>	Leaves	Fresh		RAPD, sequencing; SCAR	N/A	[166]
<i>Phyllanthus reticulatus</i>			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
<i>Phyllanthus ruber</i>			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
<i>Phyllanthus simplex</i>	Leaves	Fresh		RAPD, sequencing; SCAR	N/A	[166]
<i>Phyllanthus taxodiifolius</i>			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
<i>Phyllanthus urinaria</i>	Leaves	Fresh		RAPD, sequencing; SCAR	N/A	[166]
			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
<i>Phyllanthus ussuriensis</i>			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
<i>Phyllanthus virgatus</i>			Yes	PCR, sequencing; multiplex PCR	ITS; atpB; rbcL	[165]
<i>Phytolacca acinosa</i>	Roots	Fresh, dried	Yes	AP-PCR; RAPD	N/A	[71]
<i>Pinellia cordata</i>	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
<i>Pinellia pedatisecta</i>	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
	Leaves	Fresh	Yes	PCR, sequencing; PCR-SR	Mannose-binding lectin	[121]
<i>Pinellia pedatisecta</i>				PCR, sequencing	18S rRNA	[167]
				PCR, sequencing	18S rRNA	[153]
<i>Pinellia ternata</i>	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
	Leaves	Fresh	Yes	PCR, sequencing; PCR-SR	Mannose-binding lectin	[121]
				RAPD	N/A	[168]
				PCR, sequencing	18S rRNA	[167]
				PCR, sequencing	18S rRNA	[153]
<i>Plantago ovata</i>	Seedlings	Fresh		RAPD	N/A	[169]
<i>Platicodon grandiflorum</i>	Roots	Fresh, dried	Yes	AP-PCR; RAPD	N/A	[71]
<i>Plectranthus barbatus</i>	Leaves		Yes	AFLP	N/A	[170]
<i>Plectranthus grandis</i>	Leaves		Yes	AFLP	N/A	[170]
<i>Plectranthus ornatus</i>	Leaves		Yes	AFLP	N/A	[170]
<i>Pogostemon cablin</i>			Yes	PCR, sequencing	18S rRNA; matK	[136]
<i>Pueraria lobata</i>			Yes	PCR, sequencing	ITS; 5S gene spacer	[171]
<i>Pueraria montana</i>			Yes	PCR, sequencing	ITS; 5S gene spacer	[171]
<i>Pueraria thomsonii</i>			Yes	PCR, sequencing	ITS; 5S gene spacer	[171]
<i>Pulsatilla vulgaris</i>	Leaves		Yes	AFLP	N/A	[134]
<i>Rehmannia chingii</i>	Leaves	Dried	Yes	PCR, sequencing	ITS, trnL-trnF, rps16	[18]
<i>Rehmannia elata</i>	Leaves	Dried	Yes	PCR, sequencing	ITS, trnL-trnF, rps16	[18]
<i>Rehmannia glutinosa</i>	Leaves	Dried	Yes	PCR, sequencing	ITS, trnL-trnF, rps16	[18]

Plant (scientific name)	Part	Condition	Voucher	Method	Gene	Ref
<i>Rehmannia henryi</i>	Leaves	Dried	Yes	PCR, sequencing	ITS, trnL-trnF, rps16	[18]
<i>Rehmannia piasezkii</i>	Leaves	Dried	Yes	PCR, sequencing	ITS, trnL-trnF, rps16	[18]
<i>Rehmannia solanifolia</i>	Leaves	Dried	Yes	PCR, sequencing	ITS, trnL-trnF, rps16	[18]
<i>Rheum compactum</i>	Leaves and roots	Dried	Yes	PCR, sequencing	trnL-trnF	[172]
<i>Rheum hoatoense</i>	Leaves and roots	Dried	Yes	PCR, sequencing	trnL-trnF	[172]
<i>Rheum likiangense</i>	Leaves and roots	Dried	Yes	PCR, sequencing	trnL-trnF	[172]
<i>Rheum nanum</i>	Leaves and roots	Dried	Yes	PCR, sequencing	trnL-trnF	[172]
<i>Rheum officinale</i>	Leaves and roots	Dried	Yes	PCR, sequencing	trnL-trnF	[172]
<i>Rheum palmatum</i>	Leaves and roots	Dried	Yes	PCR, sequencing	trnL-trnF	[172]
<i>Rheum przewalskyi</i>	Leaves and roots	Dried	Yes	PCR, sequencing	trnL-trnF	[172]
<i>Rheum pumilum</i>	Leaves and roots	Dried	Yes	PCR, sequencing	trnL-trnF	[172]
<i>Rheum reticulatum</i>	Leaves and roots	Dried	Yes	PCR, sequencing	trnL-trnF	[172]
<i>Rheum sublanceolatum</i>	Leaves and roots	Dried	Yes	PCR, sequencing	trnL-trnF	[172]
<i>Rheum tanguticum</i>	Leaves and roots	Dried	Yes	PCR, sequencing	trnL-trnF	[172]
<i>Rheum undulatum</i>	Leaves and roots	Dried	Yes	PCR, sequencing	trnL-trnF	[172]
<i>Rheum wittrockii</i>	Leaves and roots	Dried	Yes	PCR, sequencing	trnL-trnF	[172]
<i>Rhodiola chrysanthemifolia</i>	Leaves	Fresh		ISSR-PCR	N/A	[173]
<i>Rhododendrom molle</i>				PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
<i>Salvia bowleyana</i>				PCR, sequencing	ITS	[174]
<i>Salvia chinensis</i>				PCR, sequencing	ITS	[174]
<i>Salvia miltiorrhiza</i>				PCR, sequencing	ITS	[174]
<i>Salvia miltiorrhiza f. alba</i>				PCR, sequencing	ITS	[174]
<i>Salvia plebeia</i>				PCR, sequencing	ITS	[174]
<i>Salvia przewalskii</i>				PCR, sequencing	ITS	[174]
<i>Salvia substansifara</i>				PCR, sequencing	ITS	[174]
<i>Salvia trijuga</i>				PCR, sequencing	ITS	[174]
<i>Salvia yunnanensis</i>				PCR, sequencing	ITS	[174]
<i>Scutellaria altissima</i>	Leaves	Fresh	Yes	PCR, sequencing	rpl16; rpl16-rpl14	[175]
<i>Scutellaria baicalensis</i>	Leaves	Fresh	Yes	PCR, sequencing	rpl16; rpl16-rpl14	[175]
				RAPD	N/A	[176]
	Leaves		Yes	RAPD	N/A	[177]
<i>Scutellaria gelericulata</i>	Leaves	Fresh	Yes	PCR, sequencing	rpl16; rpl16-rpl14	[175]
	Leaves		Yes	RAPD	N/A	[177]
<i>Scutellaria incana</i>	Leaves	Fresh	Yes	PCR, sequencing	rpl16; rpl16-rpl14	[175]
<i>Scutellaria indica</i>	Leaves	Fresh	Yes	PCR, sequencing	rpl16; rpl16-rpl14	[175]
<i>Scutellaria lateriflora</i>	Leaves	Fresh	Yes	PCR, sequencing	rpl16; rpl16-rpl14	[175]
	Leaves		Yes	RAPD	N/A	[177]
<i>Sinopodophyllum hexandrum</i>	Leaves	Dried	Yes	PCR, RFLP	trnT-trnL; trnD-trnT	[151]
<i>Stellera chamaejasme</i>				PCR, sequencing; microarray (silicon)	trnL	[44]
<i>Strychnos nux-vomica</i>	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
<i>Swertia angustifolia</i>			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]
<i>Swertia chirayita</i>			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]



Plant (scientific name)	Part	Condition	Voucher	Method	Gene	Ref
<i>Swertia dichotoma</i>			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]
<i>Swertia erythrosticta</i>			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]
<i>Swertia luquanensis</i>			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]
<i>Swertia macrosperma</i>			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]
<i>Swertia mileensis</i>			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]
<i>Swertia mussoitii</i>			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]
<i>Swertia prsewalskii</i>			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]
<i>Swertia punicea</i>			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]
<i>Swertia tetraptera</i>			Yes	PCR, sequencing; allele-specific diagnostic PCR	rpl16; ITS	[156]
<i>Talinum paniculatum</i>	Roots	Fresh, dried	Yes	AP-PCR; RAPD	N/A	[71]
<i>Trifolium pratense</i>	Leaves; dried ground material	Fresh, dried		PCR, sequencing; RFLP	ITS	[161]
<i>Thymus vulgaris</i>	Leaves			RAPD	N/A	[178]
<i>Typhonium divaricatum</i>	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
<i>Typhonium flagelliforme</i>	Leaves	Fresh		RAPD	N/A	[179]
<i>Typhonium giganteum</i>	Leaves	Fresh	Yes	PCR, sequencing; microarray (silicon)	5S gene spacer	[44]
<i>Typhonium roxburghii</i>	Leaves	Fresh		RAPD	N/A	[179]
<i>Typhonium trilobatum</i>	Leaves	Fresh		RAPD	N/A	[179]
<i>Vitex rotundifolia</i>	Fruits and leaves	Fresh	Yes	ISSR-PCR	N/A	[19]
<i>Welwitschia mirabilis</i>	Aerial parts	Fresh	Yes	PCR, sequencing	psbA-trnH	[85]

as input [20]. Although the overall results were similar, these authors found that the 5S rRNA spacer exhibited more sequence variation than either the ITS or 18S coding sequences and therefore proved best suited for the phylogenetic analysis of the *Astragalus* taxa examined [20]. Although the levels of isoflavonoids and astragalosides in each of 10 *Astragalus* taxa collected from 28 different regions exhibited variation, the phytochemical profiles did not allow for species level differentiation [110].

## Conclusions

A large number of molecular techniques have been used to authenticate medicinal plants based on species-specific variations in the sequences of various chloroplast and nuclear DNA regions. Using PCR-based methods, species identification has been achieved using DNA that was isolated from fresh and dried plant parts, plant extracts, processed herbal drugs, as well as finished products such as herbal teas, tablets and capsules. Genomic fingerprinting can differentiate between individuals, species and populations and has proven useful for the characterization of sample homogeneity and detection of adulterants. DNA-based authentication of medicinal plants is a work in progress that offers powerful new tools and entry points for measures aimed at quality control and quality assurance in medical plant research as well as the production, clinical use, and foren-

sic examination of herbal medicines. For example, genome-based methods can be useful in quickly and efficiently pinpointing adulterated or misidentified raw materials, which can then be discarded without further need for time- and resource-consuming morphological, physical and phytochemical examinations. However, DNA-based species identification alone will rarely be sufficient for quality control and assurance because, as living organisms, plants are the product of both the genome and the environment. Although both qualitative and quantitative properties of plant metabolic pathways are largely predetermined genetically, overall metabolic activity is strongly influenced by the environment. Moreover, metabolites are often distributed unequally in different parts of the plant such as roots, stems or leaves, for example. Considering the important role that the chemical metabolites are thought to play in mediating the pharmacologic effects of herbal medicines [111], [112], the importance of extensive and standardized phytochemical characterization of medicinal plants by chromatographic and spectroscopic methods will continue to grow [113].

## References

- 1 Hart BL. The evolution of herbal medicine: behavioral perspectives. *Anim Behav* 2005; 70: 975–89
- 2 Halberstein RA. Medicinal plants: historical and cross-cultural usage patterns. *AEP* 2005; 15: 686–99

- 3 Huffman MA. Self-medicative behavior in the African great apes: an evolutionary perspective into the origins of human traditional medicine. *Bioscience* 2001; 51: 651–61
- 4 Krief S, Hladik CM, Haxaire C. Ethnomedicinal and bioactive properties of plants ingested by wild chimpanzees in Uganda. *J Ethnopharmacol* 2005; 101: 1–15
- 5 Jones WP, Chin Y-W, Kinghorn AD. The role of pharmacognosy in modern medicine and pharmacy. *Current Drug Targets* 2006; 7: 247–64
- 6 Koehn FE, Carter GT. The evolving role of natural products in drug discovery. *Nat Rev Drug Discov* 2005; 4: 206–20
- 7 Newman DJ, Cragg GM, Snader KM. The influence of natural products upon drug discovery. *Nat Prod Rep* 2000; 17: 215–34
- 8 Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981–2002. *J Nat Prod* 2003; 66: 1022–37
- 9 WHO traditional medicine strategy 2002–2005. Geneva: World Health Organization Geneva; 2002
- 10 Ernst E. The efficacy of herbal medicine – an overview. *Fundam Clin Pharmacol* 2005; 19: 405–9
- 11 Tindle HA, Davis RB, Phillips RS, Eisenberg DM. Trends in use of complementary and alternative medicine by US adults: 1997–2002. *Altern Ther Health Med* 2005; 11: 42–9
- 12 Cardellina JHI. Challenges and opportunities confronting the botanical dietary supplement industry. *J Nat Prod* 2002; 65: 1073–84
- 13 Ernst E. Herbal medicines – they are popular, but are they also safe? *Eur J Clin Pharmacol* 2006; 62: 1–2
- 14 Zhonghua Renmin Gongheguo wei sheng bu yao dian wei yuan hui. Pharmacopoeia of the People's Republic of China. English ed. Beijing: Chemical Industry Press; 2000
- 15 Chinese Drug Monographs and Analysis. Kötzing: Verlag für Ganzheitliche Medizin Dr. Erich Wühr GmbH; 2004
- 16 Zhao Z, Hu Y, Liang Z, Yuen JP, Jiang Z, Leung KS. Authentication is fundamental for standardization of Chinese medicines. *Planta Med* 2006; 72: 865–74
- 17 Cheung KS, Kwan HS, But PP, Shaw PC. Pharmacognostical identification of American and Oriental ginseng roots by genomic fingerprinting using arbitrarily primed polymerase chain reaction (AP-PCR). *J Ethnopharmacol* 1994; 42: 67–9
- 18 Albach DC, Li HQ, Zhao N, Jensen SR. Molecular systematics and phytochemistry of *Rehmannia* (Scrophulariaceae). *Biochem Syst Ecol* 2007; 35: 293–300
- 19 Hu Y, Zhang Q, Xin H, Qin LP, Lu BR, Rahman K et al. Association between chemical and genetic variation of *Vitex rotundifolia* populations from different locations in China: its implication for quality control of medicinal plants. *Biomed Chromatogr* 2007; 21: 967–75
- 20 Dong TT, Ma XQ, Clarke C, Song ZH, Ji ZN, Lo CK et al. Phylogeny of *Astragalus* in China: molecular evidence from the DNA sequences of 5S rRNA spacer, ITS, and 18S rRNA. *J Agric Food Chem* 2003; 51: 6709–14
- 21 Yamaji H, Fukuda T, Yokoyama J, Pak J-H, Zhou C, Yang C et al. Reticulate evolution and phylogeography in *Asarum* sect. *asiarum* (Aristolochiaceae) documented in internal transcribed spacer sequences (ITS) of nuclear ribosomal DNA. *Mol Phylogenet Evol* 2007; 44: 863–84
- 22 Soltis PS, Gitzendanner MA. Molecular systematics and the conservation of rare species. *Conserv Biol* 1999; 13: 471–83
- 23 Mizukami H, Ohbayashi K, Kitamura Y, Ikenaga T. Restriction fragment length polymorphisms (RFLPs) of medicinal plants and crude drugs. I. RFLP probes allow clear identification of *Duboisia* interspecific hybrid genotypes in both fresh and dried tissues. *Biol Pharm Bull* 1993; 16: 388–90
- 24 Crawford DJ. Plant macromolecular systematics in the past 50 years: one view. *Taxon* 2000; 49: 479–501
- 25 Hillis DM. Molecular versus morphological approaches to systematics. *Annu Rev Ecol Syst* 1987; 18: 23–42
- 26 Bartlett J, Stirling D. A short history of the polymerase chain reaction. In: Bartlett J, Stirling D, editors. *Methods in molecular biology: PCR protocols*, 2nd edition Totowa, NJ: Humana Press Inc.; 2003: 1–6
- 27 Hebert PDN, Cywinska A, Ball SL, deWaard JR. Biological identification through DNA barcodes. *Proc R Soc Lond B Biol Sci* 2003; 270: 313–21
- 28 Savolainen V, Cowan RS, Vogler AP, Roderick GK, Lane R. Towards writing the encyclopedia of life: an introduction to DNA barcoding. *Philos Trans R Soc Lond B Biol Sci* 2005; 360: 1805–11
- 29 Ratnasingham S, Hebert PDN. BOLD: the barcode of life data system ([www.barcodinglife.org](http://www.barcodinglife.org)). *Mol Ecol Notes* 2007; 7: 355–64
- 30 Porkert M. The theoretical foundations of Chinese medicine: systems of correspondence. Cambridge: MIT Press; 1973: 368
- 31 Hu Z, Yang X, Ho PC, Chan SY, Heng PW, Chan E et al. Herb-drug interactions: a literature review. *Drugs* 2005; 65: 1239–82
- 32 Miller LG. Herbal medicinals: selected clinical considerations focusing on known or potential drug-herb interactions. *Arch Intern Med* 1998; 158: 2200–11
- 33 Chen CF, Shum YC, Yang SP. The modernization of traditional Chinese medicine in Taiwan—past, present and future. *Adv Exp Med Biol* 2004; 546: 35–42
- 34 Zhu YP, Woerdenbag HJ. Traditional Chinese herbal medicine. *Pharm World Sci* 1995; 17: 103–12
- 35 Yu F, Takashi T, Morya J, Kawaura K, Yamakawa J, Kusaka K et al. Traditional Chinese medicine and Kampo: a review from the distant past for the future. *J Int Med* 2006; 34: 231–9
- 36 Saito H. Regulation of herbal medicines in Japan. *Pharmacol Res* 2000; 41: 515–9
- 37 Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res* 1990; 18: 6531–5
- 38 Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am J Hum Genet* 1980; 32: 314–31
- 39 Shaw PC, Wang J, But PP-H. Authentication of Chinese medicinal materials by DNA technology. Singapore: World Scientific Publishing Co. Pte. Ltd.; 2002
- 40 Dittrich PS, Tachikawa K, Manz A. Micro total analysis systems. Latest advancements and trends. *Anal Chem* 2006; 78: 3887–908
- 41 Reyes DR, Iossifidis D, Auroux PA, Manz A. Micro total analysis systems. 1. Introduction, theory, and technology. *Anal Chem* 2002; 74: 2623–36
- 42 Auroux PA, Iossifidis D, Reyes DR, Manz A. Micro total analysis systems. 2. Analytical standard operations and applications. *Anal Chem* 2002; 74: 2637–52
- 43 Auroux PA, Koc Y, deMello A, Manz A, Day PJ. Miniaturised nucleic acid analysis. *Lab Chip* 2004; 4: 534–46
- 44 Carles M, Cheung MK, Moganti S, Dong TT, Tsim KW, Ip NY et al. A DNA microarray for the authentication of toxic traditional Chinese medicinal plants. *Planta Med* 2005; 71: 580–4
- 45 Carles M, Lee T, Moganti S, Lenigk R, Tsim KW, Ip NY et al. Chips and Qi: microcomponent-based analysis in traditional Chinese medicine. *Fredericus J Anal Chem* 2001; 371: 190–4
- 46 Trau D, Lee TM, Lao AI, Lenigk R, Hsing IM, Ip NY et al. Genotyping on a complementary metal oxide semiconductor silicon polymerase chain reaction chip with integrated DNA microarray. *Anal Chem* 2002; 74: 3168–73
- 47 Lee TM, Carles MC, Hsing IM. Microfabricated PCR-electrochemical device for simultaneous DNA amplification and detection. *Lab Chip* 2003; 3: 100–5
- 48 Lee TM, Hsing IM, Lao AI, Carles MC. A miniaturized DNA amplifier: its application in traditional Chinese medicine. *Anal Chem* 2000; 72: 4242–7
- 49 Trau D, Jiang J, Sucher NJ. Preservation of the biofunctionality of DNA and protein during microfabrication. *Langmuir* 2006; 22: 877–81
- 50 Bennett MD, Leitch IJ. Nuclear DNA amounts in angiosperms: progress, problems and prospects. *Ann Bot* 2005; 95: 45–90
- 51 Lilly JW, Havey MJ, Jackson SA, Jiang J. Cytogenomic analyses reveal the structural plasticity of the chloroplast genome in higher plants. *Plant Cell* 2001; 13: 245–54
- 52 Levings CS, Brown GG. Molecular biology of plant mitochondria. *Cell* 1989; 56: 171–9
- 53 Kress WJ, Wurdack KJ, Zimmer EA, Weigt LA, Janzen DH. Use of DNA barcodes to identify flowering plants. *Proc Natl Acad Sci U S A* 2005; 102: 8369–74
- 54 Newmaster SG, Fazekas AJ, Ragupathy S. DNA barcoding in land plants: evaluation of rbcL in a multigene tiered approach. *Can J Bot/Rev Can Bot* 2006; 84: 335–41
- 55 Olmstead RG, Palmer JD. Chloroplast DNA systematics: a review of methods and data analysis. *Am J Bot* 1994; 81: 1205–24
- 56 Palmer JD, Jansen RK, Micals HJ, Chase MW, Manhart JR. Chloroplast DNA variation and plant phylogeny. *Ann Mo Bot Gard* 1988; 75: 1180–206
- 57 Small RL, Cronn RC, Wendel JF. Use of nuclear genes for phylogeny reconstruction in plants. *Aust Syst Bot* 2004; 17: 145–70

- 58 Baldwin BG, Sanderson MJ, Wojciechowski MF, Campbell CS, Donoghue MJ. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Ann Mo Bot Gard* 1995; 82: 247–77
- 59 Álvarez I, Wendel JF. Ribosomal ITS sequences and plant phylogenetic inference. *Mol Phylogenet Evol* 2003; 29: 417–34
- 60 Hillis DM, Dixon MT. Ribosomal DNA: molecular evolution and phylogenetic inference. *Q Rev Biol* 1991; 66: 411–53
- 61 Hori H, Osawa S. Origin and evolution of organisms as deduced from 5S ribosomal RNA sequences. *Mol Biol Evol* 1987; 4: 445–72
- 62 Pennisi E. TAXONOMY: Wanted: A Barcode for Plants. *Science* 2007; 318: 190–1
- 63 Cui GH, Tang XJ, Huang LQ. [Application of multiplex allele-specific PCR for authentication of *Panax ginseng* and *P. quinquefolius*]. *Zhongguo Zhong Yao Za Zhi* 2006; 31: 1940–3
- 64 Cui XM, Lo CK, Yip KL, Dong TT, Tsim KW. Authentication of *Panax notoginseng* by 5S-rRNA spacer domain and random amplified polymorphic DNA (RAPD) analysis. *Planta Med* 2003; 69: 584–6
- 65 Ha WY, Shaw PC, Liu J, Yau FC, Wang J. Authentication of *Panax ginseng* and *Panax quinquefolius* using amplified fragment length polymorphism (AFLP) and directed amplification of minisatellite region DNA (DAMD). *J Agric Food Chem* 2002; 50: 1871–5
- 66 Ha WY, Yau FC, But PP, Wang J, Shaw PC. Direct amplification of length polymorphism analysis differentiates *Panax ginseng* from *P. quinquefolius*. *Planta Med* 2001; 67: 587–9
- 67 Hong DY, Lau AJ, Yeo CL, Liu XK, Yang CR, Koh HL et al. Genetic diversity and variation of saponin contents in *Panax notoginseng* roots from a single farm. *J Agric Food Chem* 2005; 53: 8460–7
- 68 Komatsu K, Zhu S, Fushimi H, Qui TK, Cai S, Kadota S. Phylogenetic analysis based on 18S rRNA gene and matK gene sequences of *Panax vietnamensis* and five related species. *Planta Med* 2001; 67: 461–5
- 69 Lee C, Wen J. Phylogeny of *Panax* using chloroplast trnC-trnD intergenic region and the utility of trnC-trnD in interspecific studies of plants. *Mol Phylogenet Evol* 2004; 31: 894–903
- 70 Mihalov JJ, Marderosian AD, Pierce JC. DNA identification of commercial ginseng samples. *J Agric Food Chem* 2000; 48: 3744–52
- 71 Shaw PC, But PP. Authentication of *Panax* species and their adulterants by random-primed polymerase chain reaction. *Planta Med* 1995; 61: 466–9
- 72 Tanaka H, Fukuda N, Shoyama Y. Identification and differentiation of *Panax* species using ELISA, RAPD and eastern blotting. *Phytochem Anal* 2006; 17: 46–55
- 73 Um JY, Chung HS, Kim MS, Na HJ, Kwon HJ, Kim JJ et al. Molecular authentication of *Panax ginseng* species by RAPD analysis and PCR-RFLP. *Biol Pharm Bull* 2001; 24: 872–5
- 74 Zhu S, Fushimi H, Cai S, Komatsu K. Phylogenetic relationship in the genus *Panax*: inferred from chloroplast trnK gene and nuclear 18S rRNA gene sequences. *Planta Med* 2003; 69: 647–53
- 75 Zhu S, Fushimi H, Cai S, Komatsu K. Species identification from Ginseng drugs by multiplex amplification refractory mutation system (MARMS). *Planta Med* 2004; 70: 189–92
- 76 Bian Y, Li P, Gao Z, Wang Y, Zhou K, Tsim KW et al. [Application of RAPD in the taxonomy of the genus *Fritillaria*]. *Zhong Yao Cai* 2000; 23: 13–6
- 77 Cai ZH, Li P, Dong TT, Tsim KW. Molecular diversity of 5S-rRNA spacer domain in *Fritillaria* species revealed by PCR analysis. *Planta Med* 1999; 65: 360–4
- 78 Li YF, Li YX, Lin J, Xu Y, Yan F, Tang L et al. Identification of bulb from *Fritillaria cirrhosa* by PCR with specific primers. *Planta Med* 2003; 69: 186–8
- 79 Tsoi PY, Woo HS, Wong MS, Chen SL, Fong WF, Xiao PG et al. Genotyping and species identification of *Fritillaria* by DNA chips. *Yao Xue Xue Bao* 2003; 38: 185–90
- 80 Wang CZ, Li P, Ding JY, Jin GQ, Yuan CS. Identification of *Fritillaria pallidiflora* using diagnostic PCR and PCR-RFLP based on nuclear ribosomal DNA internal transcribed spacer sequences. *Planta Med* 2005; 71: 384–6
- 81 Guo Y, Tsuruga A, Yamaguchi S, Oba K, Iwai K, Sekita S et al. Sequence analysis of chloroplast chlB gene of medicinal *Ephedra* species and its application to authentication of *Ephedra* herb. *Biol Pharm Bull* 2006; 29: 1207–11
- 82 Joshi VC, Khan I. Macroscopic and microscopic authentication of Chinese and North American species of *Ephedra*. *J AOAC Int* 2005; 88: 707–13
- 83 Kakiuchi N, Nakajima I, Kurita Y, Long C, Cai S, Mikage M. Studies on cultivated *Ephedra* plants in inner Mongolia autonomous region and Ningxia autonomous region. *Biol Pharm Bull* 2006; 29: 746–9
- 84 Long C, Kakiuchi N, Takahashi A, Komatsu K, Cai S, Mikage M. Phylogenetic analysis of the DNA sequence of the non-coding region of nuclear ribosomal DNA and chloroplast of *Ephedra* plants in China. *Planta Med* 2004; 70: 1080–4
- 85 Techen N, Khan IA, Pan Z, Scheffler BE. The use of polymerase chain reaction (PCR) for the identification of ephedra DNA in dietary supplements. *Planta Med* 2006; 72: 241–7
- 86 Datwyler SL, Weiblen GD. Genetic variation in hem and marijuana (*Cannabis sativa* L.) according to amplified fragment length polymorphisms. *J Forensic Sci* 2006; 51: 371–5
- 87 Jagadish V, Robertson J, Gibbs A. RAPD analysis distinguishes *Cannabis sativa* samples from different sources. *Forensic Sci Int* 1996; 79: 113–21
- 88 Kojoma M, Iida O, Makino Y, Sekita S, Satake M. DNA fingerprinting of *Cannabis sativa* using inter-simple sequence repeat (ISSR) amplification. *Planta Med* 2002; 68: 60–3
- 89 Ding G, Ding XY, Shen J, Tang F, Liu DY, He J et al. [Genetic diversity and molecular authentication of wild populations of *Dendrobium officinale* by RAPD]. *Yao Xue Xue Bao* 2005; 40: 1028–32
- 90 Ding X, Wang Z, Zhou K, Xu L, Xu H, Wang Y. Allele-specific primers for diagnostic PCR authentication of *Dendrobium officinale*. *Planta Med* 2003; 69: 587–8
- 91 Ding X, Xu L, Wang Z, Zhou K, Xu H, Wang Y. Authentication of stems of *Dendrobium officinale* by rDNA ITS region sequences. *Planta Med* 2002; 68: 191–2
- 92 Ding XY, Wang ZT, Xu H, Xu LS, Zhou KY. [Database establishment of the whole rDNA ITS region of *Dendrobium* species of “fengdou” and authentication by analysis of their sequences]. *Yao Xue Xue Bao* 2002; 37: 567–73
- 93 Ding XY, Xu LS, Wang ZT, Xu H, Zhou KY. [Molecular authentication of *Dendrobium chrysanthum* from its allied species of *Dendrobium*]. *Zhongguo Zhong Yao Za Zhi* 2002; 27: 407–11
- 94 Lau DT, Shaw PC, Wang J, But PP. Authentication of medicinal *Dendrobium* species by the internal transcribed spacer of ribosomal DNA. *Planta Med* 2001; 67: 456–60
- 95 Li T, Wang J, Lu Z. Accurate identification of closely related *Dendrobium* species with multiple species-specific gDNA probes. *J Biochem Biophys Methods* 2005; 62: 111–23
- 96 Shen J, Ding XY, Ding G, Liu DY, Tang F, He J. [Studies on population difference of *Dendrobium officinale* II establishment and optimization of the method of ISSR fingerprinting marker]. *Zhongguo Zhong Yao Za Zhi* 2006; 31: 291–4
- 97 Xu H, Li XB, Wang ZT, Ding XY, Xu LS, Zhou KY. [rDNA its sequencing of *Herba Dendrobii* (Huangcao)]. *Yao Xue Xue Bao* 2001; 36: 777–83
- 98 Ying Y, Xu H, Wang ZT. [Allele-specific diagnostic PCR authentication of *Dendrobium thyrsiflorum*]. *Yao Xue Xue Bao* 2007; 42: 98–103
- 99 Zhang M, Huang HR, Liao SM, Gao JY. [Cluster analysis of *Dendrobium* by RAPD and design of specific primer for *Dendrobium candidum*]. *Zhongguo Zhong Yao Za Zhi* 2001; 26: 442–7
- 100 Zhang T, Xu LS, Wang ZT, Zhou KY, Zhang N, Shi YF. [Molecular identification of medicinal plants: *Dendrobium chrysanthum*, *Dendrobium fimbriatum* and their morphologically allied species by PCR-RFLP analyses]. *Yao Xue Xue Bao* 2005; 40: 728–33
- 101 Zhang YB, Wang J, Wang ZT, But PP, Shaw PC. DNA microarray for identification of the herb of *Dendrobium* species from Chinese medicinal formulations. *Planta Med* 2003; 69: 1172–4
- 102 Chen G, Wang XL, Wong WS, Liu XD, Xia B, Li N. Application of 3′ untranslated region (UTR) sequence-based amplified polymorphism analysis in the rapid authentication of *Radix astragalii*. *J Agric Food Chem* 2005; 53: 8551–6
- 103 Cheng KT, Su B, Chen CT, Lin CC. RAPD analysis of *Astragalus* medicines marketed in Taiwan. *Am J Chin Med* 2000; 28: 273–8
- 104 Ma XQ, Duan JA, Zhu DY, Dong TT, Tsim KW. Species identification of *Radix Astragalii* (Huangqi) by DNA sequence of its 5S-rRNA spacer domain. *Phytochemistry* 2000; 54: 363–8
- 105 Yip PY, Kwan HS. Molecular identification of *Astragalus membranaceus* at the species and locality levels. *J Ethnopharmacol* 2006; 106: 222–9
- 106 Zhang X, Xu Q, Xiao H, Liang X, Huang L, Liu J. Study on authentication of *Astragalus membranaceus* by DNA fingerprints. *World Science And Technology/Modernization of Traditional Chinese Medicine and Materia Medica* 2006; 8: 33–6

- 107 Zhao KJ, Dong TT, Tu PF, Song ZH, Lo CK, Tsim KW. Molecular genetic and chemical assessment of *Radix Angelica* (Danggui) in China. *J Agric Food Chem* 2003; 51: 2576–83
- 108 Xia Q, Zhao KJ, Huang ZG, Zhang P, Dong TT, Li SP et al. Molecular genetic and chemical assessment of *Rhizoma Curcumae* in China. *J Agric Food Chem* 2005; 53: 6019–26
- 109 Zhang YB, Ngan FN, Wang ZT, Ng TB, But PP, Shaw PC et al. Random primed polymerase chain reaction differentiates *Codonopsis pilosula* from different localities. *Planta Med* 1999; 65: 157–60
- 110 Ma XQ, Shi Q, Duan JA, Dong TT, Tsim KW. Chemical analysis of *Radix Astragali* (Huangqi) in China: a comparison with its adulterants and seasonal variations. *J Agric Food Chem* 2002; 50: 4861–6
- 111 Hamburger M, Hostettmann K. 7. Bioactivity in plants: the link between phytochemistry and medicine. *Phytochemistry* 1991; 30: 3864–74
- 112 Briskin DP. Medicinal plants and phytomedicines. Linking plant biochemistry and physiology to human health. *Plant Physiol* 2000; 124: 507–14
- 113 Holmes E, Tang H, Wang Y, Seger C. The assessment of plant metabolite profiles by NMR-based methodologies. *Planta Med* 2006; 72: 771–85
- 114 Saiki RK, Chang CA, Levenson CH, Warren TC, Boehm CD, Kazazian HH Jr et al. Diagnosis of sickle cell anemia and beta-thalassemia with enzymatically amplified DNA and nonradioactive allele-specific oligonucleotide probes. *N Engl J Med* 1988; 319: 537–41
- 115 Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA et al. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 1985; 230: 1350–4
- 116 Dieffenbach C, Dveksler G. PCR primer: A laboratory manual, 2nd edition. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 2003: 520
- 117 Gupta PK, Roy JK, Prasad M. Single nucleotide polymorphisms: A new paradigm for molecular marker technology and DNA polymorphism detection with emphasis on their use in plants. *Curr Sci* 2001; 80: 524–35
- 118 Newton CR, Graham A, Heptinstall LE, Powell SJ, Summers C, Kalsheker N et al. Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). *Nucleic Acids Res* 1989; 17: 2503–16
- 119 Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M et al. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res* 1995; 23: 4407–14
- 120 Desmarais E, Lanneluc I, Lagnel J. Direct amplification of length polymorphisms (DALP), or how to get and characterize new genetic markers in many species. *Nucleic Acids Res* 1998; 26: 1458–65
- 121 Lin J, Zhou X, Gao S, Wu W, Liu X, Sun X et al. Authentication of *Pinellia ternata* and its adulterants based on PCR with specific primers. *Planta Med* 2006; 72: 844–7
- 122 Paran I, Michelmore RW. Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. *Theor Appl Genet* 1993; 85: 985–93
- 123 Schena M, Heller RA, Thériault TP, Konrad K, Lachenmeier E, Davis RW. Microarrays: biotechnology's discovery platform for functional genomics. *Trends Biotechnol* 1998; 16: 301–6
- 124 Kretz K, Callen W, Hedden V. Cycle sequencing. *PCR Methods Appl* 1994; 3: S107–12
- 125 Zietkiewicz E, Rafalski A, Labuda D. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* 1994; 20: 176–83
- 126 Fortina P, Dotti G, Conant R, Monokian G, Parrella T, Hitchcock W et al. Detection of the most common mutations causing beta-thalassemia in Mediterraneans using a multiplex amplification refractory mutation system (MARMS). *PCR Methods Appl* 1992; 2: 163–6
- 127 Weber JL, May PE. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am J Hum Genet* 1989; 44: 388–96
- 128 Kuzoff RK, Sweere JA, Soltis DE, Soltis PS, Zimmer EA. The phylogenetic potential of entire 26S rDNA sequences in plants. *Mol Biol Evol* 1998; 15: 251–63
- 129 Wendel JF, Álvarez I. Ribosomal ITS sequences and plant phylogenetic inference. *Mol Phylogenet Evol* 2003; 29: 417–34
- 130 Sugiura M. The chloroplast genome. *Plant Mol Biol* 1992; 19: 149–68
- 131 Liu X, Xu H, Huang C. Chloroplast chlB gene is required for light-independent chlorophyll accumulation in *Chlamydomonas reinhardtii*. *Plant Mol Biol* 1993; 23: 297–308
- 132 Heubl G, Bringman G, Meimberg H. Molecular phylogeny and character evolution of carnivorous plant families in Caryophyllales – revisited. *Plant Biol* 2006; 8: 821–30
- 133 Chase MW, Salamin N, Wilkinson M, Dunwell JM, Kesanakurthi RP, Haidar N et al. Land plants and DNA barcodes: short-term and long-term goals. *Philos Trans R Soc Lond Ser B Biol Sci* 2005; 360: 1889–95
- 134 Zerega NJC, Mori S, Lindqvist C, Zheng Q, Motley TJ. Using amplified fragment length polymorphisms (AFLP) to identify black cohosh (*Actaea racemosa*). *Econ Bot* 2002; 56: 154–64
- 135 Zhao KJ, Dong TT, Cui XM, Tu PF, Tsim KW. Genetic distinction of *radix adenophorae* from its adulterants by the DNA sequence of 5S-rRNA spacer domains. *Am J Chin Med* 2003; 31: 919–26
- 136 Luo JP, Cao H, Liu YP. [DNA sequencing and molecular identification of Patchouli and its substitute wrinkled giant hyssop]. *Yao Xue Xue Bao* 2002; 37: 739–42
- 137 Li X, Ding X, Chu B, Ding G, Gu S, Qian L et al. Molecular authentication of *Alisma orientale* by PCR-RFLP and ARMS. *Planta Med* 2007; 73: 67–70
- 138 Hosokawa K, Hishida A, Nakamura I, Shibata T. The sequences of the spacer region between the atpF and atpA genes in the plastid genome allows discrimination among three varieties of medicinal *Angelica*. *Planta Med* 2006; 72: 570–1
- 139 Watanabe A, Araki S, Kobari S, Sudo H, Tsuchida T, Uno T et al. In vitro proagation, restriction fragment length polymorphism, and random amplified polymorphic DNA analyses of *Angelica* plants. *Plant Cell Rep* 1998; 18: 187–92
- 140 Lee MY, Doh EJ, Park CH, Kim YH, Kim ES, Ko BS et al. Development of SCAR marker for discrimination of *Artemisia princeps* and *A. argyi* from other *Artemisia* herbs. *Biol Pharm Bull* 2006; 29: 629–33
- 141 Kelly LM. Phylogenetic relationships in *Asarum* (Aristolochiaceae) based on morphology and ITS sequences. *Am J Bot* 1998; 85: 1454–67
- 142 Liu CS, Bai GB, Yan YN. [Studies on the botanical sources and DNA molecular identification of Herba Asari based on ITS sequence]. *Zhongguo Zhong Yao Za Zhi* 2005; 30: 329–32
- 143 Guo Y, Kondo K, Terabayashi S, Yamamoto Y, Shimada H, Fujita M et al. DNA authentication of So-jutsu (*Atractylodes lancea* rhizome) and Byaku-jutsu (*Atractylodes* rhizome) obtained in the market based on the nucleotide sequence of the 18S-5.8S rDNA internal transcribed spacer region. *J Nat Med* 2006; 60: 149–56
- 144 Chen K-T, Su Y-C, Lin J-G, SHsin L-H, Su Y-P, Su C-H et al. Identification of *Atractylodes* plants in Chinese herbs and formulations by random amplified polymorphic DNA. *Acta Pharmacol Sin* 2001; 22: 493–7
- 145 Darokar MP, Khanuja SPS, Shasany AK, Kumar S. Low levels of genetic diversity detected by RAPD analysis in geographically distinct accessions of *Bacopa monnieri*. *Genet Resour Crop Evol* 2001; 48: 555–8
- 146 Yang ZY, Chao Z, Huo KK, Xie H, Tian ZP, Pan SL. ITS sequence analysis used for molecular identification of the *Bupleurum* species from northwestern China. *Phytomedicine* 2007; 14: 416–23
- 147 Ma XQ, Zhu DY, Li SP, Dong TT, Tsim KW. Authentic identification of stigma Croci (stigma of *Crocus sativus*) from its adulterants by molecular genetic analysis. *Planta Med* 2001; 67: 183–6
- 148 Fu C, Qiu Y, Kong H. RAPD analysis for genetic diversity in *Changium smyrnioides* (Apiaceae), an endangered plant. *Bot Bull Acad Sin* 2003; 44: 13–8
- 149 Nebauer SG, Castillo-Agudo Ld, Segura J. RAPD variation within and among natural populations of outcrossing willow-leave foxglove (*Digitalis obscura* L.). *Theor Appl Genet* 1999; 98: 985–94
- 150 Liu Y-p, He B-z, Cao H. Application of gene technology in quality control of Chinese drugs (II) – Identification of Chinese yam (*Dioscorea polystachia* rhizome) using DNA sequencing. *Chin J Tradit Herb Drugs* 2001; 32: 113–7
- 151 Gong W, Fu C-X, Luo Y-P, Qiu Y-X. Molecular identification of *Sinopodophyllum hexandrum* and *Dysosma* species using cpDNA sequences and PCR-RFLP markers. *Planta Med* 2006; 72: 650–2
- 152 Kapteyn J, Goldsbrough PB, Simon JE. Genetic relationships and diversity of commercially relevant *Echinacea* species. *Theor Appl Genet* 2002; 105: 369–76
- 153 Zhang Y, Liu W, Ai T. DNA molecular identification of the three *Echinacea* species. *Chin J Information on Tradit Chin Med* 2002; 9: 11–2

- 154 Sun Y, Fung KP, Leung PC, Shi D, Shaw PC. Characterization of medicinal *Epimedium* species by 5S rRNA gene spacer sequencing. *Planta Med* 2004; 70: 287–8
- 155 Xue HG, Zhou SD, He XJ, Yu Y. Molecular authentication of the traditional Chinese medicinal plant *Euphorbia pekinensis*. *Planta Med* 2007; 73: 91–3
- 156 Xue CY, Li DZ, Lu JM, Yang JB, Liu JQ. Molecular authentication of the traditional Tibetan medicinal plant *Swertia mussotii*. *Planta Med* 2006; 72: 1223–6
- 157 Mizukami H, Ohbayashi K, Umetsu K, Hiraoka N. Restriction fragment length polymorphism of medicinal plants and crude drugs. II. Analysis of *Glehnia littoralis* of different geographical origin. *Biol Pharm Bull* 1993; 16: 611–2
- 158 Yang ZY, Chao Z, Huo KK, Wu BY, Pan SL. [Nuclear ribosomal DNA internal transcribed spacer 1 sequences of 4 *Leonurus* species]. *Nan Fang Yi Ke Da Xue Xue Bao* 2006; 26: 1593–5
- 159 Zhang M, Zhang DZ, Xu XH, Zhang T, Wang ZT. 5S rRNA gene spacer sequences from *Ligularia* medicinal plants and the identification of HPAs-containing species. *Chin J Nat Med* 2005; 3: 38–40
- 160 Zhang KY, Leung HW, Yeung HW, Wong RN. Differentiation of *Lycium barbarum* from its related *Lycium* species using random amplified polymorphic DNA. *Planta Med* 2001; 67: 379–81
- 161 Lum MR, Potter E, Dang T, Heber D, Hardy M, Hirsch AM. Identification of botanicals and potential contaminants through RFLP and sequencing. *Planta Med* 2005; 71: 841–6
- 162 Wang J, Ha WY, Ngan FN, But PP, Shaw PC. Application of sequence characterized amplified region (SCAR) analysis to authenticate *Panax* species and their adulterants. *Planta Med* 2001; 67: 781–3
- 163 Hon CC, Chow YC, Zeng FY, Leung FC. Genetic authentication of ginseng and other traditional Chinese medicine. *Acta Pharmacol Sin* 2003; 24: 841–6
- 164 Luo YM, Zhang WM, Ding XY, Shen J, Bao SL, Chu BH et al. SNP marker and allele-specific diagnostic PCR for authenticating herbs of *Perilla*. *Acta Pharm Sin* 2006; 41: 840–5
- 165 Lee SK, Li PT, Lau DT, Yung PP, Kong RY, Fong WF. Phylogeny of medicinal *Phyllanthus* species in China based on nuclear ITS and chloroplast atpB-rbcL sequences and multiplex PCR detection assay analysis. *Planta Med* 2006; 72: 721–6
- 166 Dnyaneshwar W, Preeti C, Kalpana J, Bhushan P. Development and application of RAPD-SCAR marker for identification of *Phyllanthus emblica* LINN. *Biol Pharm Bull* 2006; 29: 2313–6
- 167 Liu Y-p, Cao H, Wang X-t. Application of gene technology in quality control of Chinese drugs(I) – identification of *Pinellia ternata* species from Yuncheng, Shandong using DNA sequencing. *Chin J Pharm Anal* 2001; 21: 423–6
- 168 Yang J, Zhu X, Luo C. RAPD analysis on the germplasm resources of *Pinellia ternata*. *Chin J Information on Tradit Chin Med* 2007; 14: 42–5
- 169 Das M, Raychaudhuri SS. Estimation of genetic variability in *Plantago ovata* cultivars. *Biol Plant* 2003/4; 47: 459–62
- 170 Passinho-Soares H, Felix D, Kaplan MA, Margis-Pinheiro M, Margis R. Authentication of medicinal plant botanical identity by amplified fragmented length polymorphism dominant DNA marker: inferences from the *Plectranthus* genus. *Planta Med* 2006; 72: 929–31
- 171 Sun Y, Shaw PC, Fung KP. Molecular authentication of Radix Puerariae Lobatae and Radix Puerariae Thomsonii by ITS and 5S rRNA spacer sequencing. *Biol Pharm Bull* 2007; 30: 173–5
- 172 Yang M, Zhang D, Liu J, Zheng J. A molecular marker that is specific to medicinal rhubarb based on chloroplast trnL/trnF sequences. *Planta Med* 2001; 67: 784–6
- 173 Xia T, Chen S, Chen S, Zhang D, Zhang D, Gao Q et al. ISSR analysis of genetic diversity of the Qinghai-Tibet plateau endemic *Rhodiola chrysanthemifolia* (Crassulaceae). *Biochem Syst Ecol* 2007; 35: 209–14
- 174 Wang H, Wang Q. Analysis of rDNA ITS sequences of Radix et Rhizoma *Salviae miltiorrhizae* and plants of *Salvia* L. *Chin Tradit Herb Drugs* 2005; 36: 1381–5
- 175 Hosokawa K, Minami M, Nakamura I, Hishida A, Shibata T. The sequences of the plastid gene rpl16 and their rpl16-rpl14 spacer region allow discrimination among six species of *Scutellaria*. *J Ethnopharmacol* 2005; 99: 105–8
- 176 Shao A-J, Li X, Huang L-Q, Lin S-F, Chen J. RAPD analysis of *Scutellaria baicalensis* from different germplasms. *China J Chin Mater Med* 2006; 31: 452–5
- 177 Hosokawa K, Minami M, Kawahara K, Nakamura I, Shibata T. Discrimination among three species of medicinal *Scutellaria* plants using RAPD markers. *Planta Med* 2000; 66: 270–2
- 178 Echeverrigaray S, Agostini G, Atti-Serfini L, Paroul N, Pauletti GF, dos Santos AC. Correlation between the chemical and genetic relationships among commercial thyme cultivars. *J Agric Food Chem* 2001; 49: 4220–3
- 179 Rout GR. Identification of *Tinospora cordifolia* (Willd.) Miers ex Hook F & Thomas using RAPD markers. *Z Naturforsch [C]* 2006; 61: 118–22