

Review article

Authentic identification and new drug discovery from natural plant based constituents through DNA bar-coding: A challenging task to the researchers

Kuntal Das*

Krupanidhi College of Pharmacy, Department of Pharmacognosy and Phytochemistry, #12/1, Chikkabellandur, Carmelaram Post, Varthur Hobli, Bengaluru-56003, Karnataka, India

Received September 11, 2019; Revised November 2, 2019; Accepted November 5, 2019; Published online December 30, 2019

Abstract

Education provides thumb impression to signature whereas technology provides signature to thumb impression. Signature is copied in many cases but thumb impression never be copied. Such an important technology is DNA bar coding in plant species which a new biological tool for organismal biologists to increase their understanding of the environment especially authentication of all individual plants and phylogenetic construction. DNA bar code helps to determine the correct identification of a plant sample in a rapid, repeatable, and reliable fashion to conserve world biodiversity. Not only that, it is a powerful tool in systematics, ecology, evolutionary biology, including community assembly, species interaction networks, taxonomic discovery, and assessing priority areas for ecological and environmental protection. Furthermore, plant DNA bar codes are useful in the regulatory areas where endangered species and commercial products (*viz.*, Foods and plant based supplements) are observed by the forensic investigators. Even though, application of genetic markers in the field of biological and commercial products by adopted genomic sequencing technologies are more efficient and cost effective workflow. Therefore, plant based DNA barcode is necessary and essential to preserve in the form of a library (through DNA amplification) which is the major challenges ahead in future, *i.e.*, on building the global plant DNA barcode library to contribute toward the discovery of overlooked plant species around the globe.

Key words: Authentication, amplification, cost effective, DNA barcode, environment, plants

1. Introduction

Technology is a broad term which is the sum of techniques, skills, methods, and processes that used in the new invention from various research developments, technical knowledge and tools independent of product and service initiatives. Today where we are standing is a machine era where machines are working as same as human, known as artificial intelligence. This new technology immensely captured whole health care system as well as in Pharmaceutical segment. Such important innovative technology is DNA bar coding. The DNA bar-coding is mainly aimed with the establishment of a shared community resource of DNA sequences and further utilization for organismal identification and taxonomic clarification. The method is successfully investigated and developed in animals using a portion of the cytochrome oxidase 1 (CO1) mitochondrial gene but establishing a standardized DNA bar-coding system in plant is more challengeable task. Therefore, the successful implementation of the DNA bar-coding helps an authentic plant drug discovery. The DNA bar-coding is a standard short gene fragment (sequences of DNA between 400 and 800 base pairs long) which is easily isolated and

characterized for all plant species, applicable for any botanical species identification in herbal medicines (Hebert *et al.*, 2003; Harris and Bellino, 2013; Hollingsworth *et al.*, 2016) as well as tracks the illegal trade of endangered plant species (Jeanson *et al.*, 2011; Muellner *et al.*, 2011) and so on (Figure 1). This method is rapidly developing in recent years for rapid biological species discrimination, and become a useful tool for biodiversity investigation, their monitoring, molecular phylogeny and various evolutions (Pei *et al.*, 2017). Wherever any new technology is applied, there always risk factors and challenges. Development of new technologies means we are in the last stage of the evaluation where maximum alteration and manipulation occurs with the genes and any misleading in molecular level leads to a total change of the evaluation in the World or may be the end of life. Hence, application of new technologies should be in proper way. Same way, the new technology DNA bar-coding, has many drawbacks and problems that include failure of amplification or sequencing, difficulties in finding universal primers, lack of bar-coding gap, hybridization and introgression in some plants, *etc.* (Collins and Cruickshank, 2013; Zinger and Philippe, 2016) and hence the application of DNA bar coding is really a challenging task to the researchers on building the global plant DNA barcode library and adopting genomic sequencing technologies for a more efficient and cost effective workflow in applying these genetic identification of these biological markers for commercialization. Therefore the review on DNA bar coding in plant species is demonstrated past history, recent progress, major challenges, and possible future directions plant DNA bar-coding.

Author for correspondence: Dr. Kuntal Das

Professor, Department of Pharmacognosy and Phytochemistry, #12/1, Krupanidhi College of Pharmacy, Chikkabellandur, Carmelaram Post, Varthur Hobli, Bangalore-560035, Karnataka, India

E-mail: drkkdsd@gmail.com

Tel.: +91-9632542846

Copyright © 2019 Ukaaz Publications. All rights reserved.

Email: ukaaz@yahoo.com; Website: www.ukaazpublications.com

2. History of DNA bar coding in herbal field

DNA barcode is a new biological tool for organismal biologists to increase their understanding of the herbal natural world. The term DNA bar code was introduced by Hebert *et al.* (2003) with the aim to utilize the information in one or a few gene regions to discriminate among all species of life. The genomics is just inverse of DNA bar-coding, describes in a fewer number of species the function and interactions across many if not all genes. The process of generating and applying plant DNA barcodes for the purpose of identification have two basic steps such as: i. building the DNA barcode library of known species, and ii. matching the DNA barcode sequence of an unknown sample against the DNA barcode library (Figure 2). The first step requires taxonomists to select one to several individuals per species to serve as reference samples in the DNA barcode library. Plant tissue is obtained from specimens already preserved in herbaria or taken directly from live specimens in the field with appropriately pressed, labeled, and mounted voucher specimens. These vouchers serve as a critical permanent record that connects the DNA barcode to a particular species of plant. Once the DNA barcode library is complete for the organisms under study, whether they comprise a geographic region, a taxonomic group, or a target assemblage (*e.g.*, medicinal plants, timber trees, *etc.*), then the DNA barcodes generated for the unidentified samples are compared to the known DNA barcodes using some type of matching set of rules in calculations (Kress, 2017).

Initially DNA bar code was designed and applied on animals successfully in the early years but for the plants, the technique was not successfully worked out. After an extensive inventory of gene regions in the mitochondrial, plastid, and nuclear genomes (Kress

and Erickson, 2007; Newmaster *et al.*, 2008), four primary gene regions, *viz.*, *rbcL*, *matK*, *trnH-psbA*, and ITS are identified and used as the standard DNA barcodes for plants authentication. The single DNA bar-coding marker *trnH-psbA* raised relatively high rates of species discrimination, followed by *matK* and *rbcL*. The combination of *rbcL+matK* averagely discriminated globally whereas with the three-locus barcode combination (*rbcL+matK+psbA-trnH*) provided a well-resolved phylogenetic framework. Initially these four DNA bar codes are used to determine the life of the trees and gradually it is a tool for identification that used to diagnose a plant species during all stages of its life history (*i.e.*, fruits, seeds, seedlings, mature plants) as well as in damaged specimens. Biodiversity index evaluation showed more effective and comparable with the aid of standardized method of plant DNA bar-coding where the combination of markers *rbcL+matK+trnH-psbA* are effective for DNA-based studies on forest communities (Pei *et al.*, 2015). Genetic variability of plants is quantified based on the DNA barcode sequence data. DNA bar-coding also helps to flag species (cryptic species) that are potentially new to science. Therefore DNA bar coding is a biodiversity discovery tool (Hebert *et al.*, 2004). DNA bar-coding is also serves as a means to identify regulated species, invasive species, and endangered species, and to test the identity and purity of botanical products, such as commercial herbal medicines and dietary supplements, used to address ecological, evolutionary, and conservation issues, such as the ecological rules controlling the assembly of plant species and determines the most evolutionarily diverse habitats for protection (Shapcott *et al.*, 2017). Over the last decade, the application of plant DNA barcodes is accelerated, especially in the fields of ecology, evolution, and conservation but still under huge challenges for natural herbal plants.

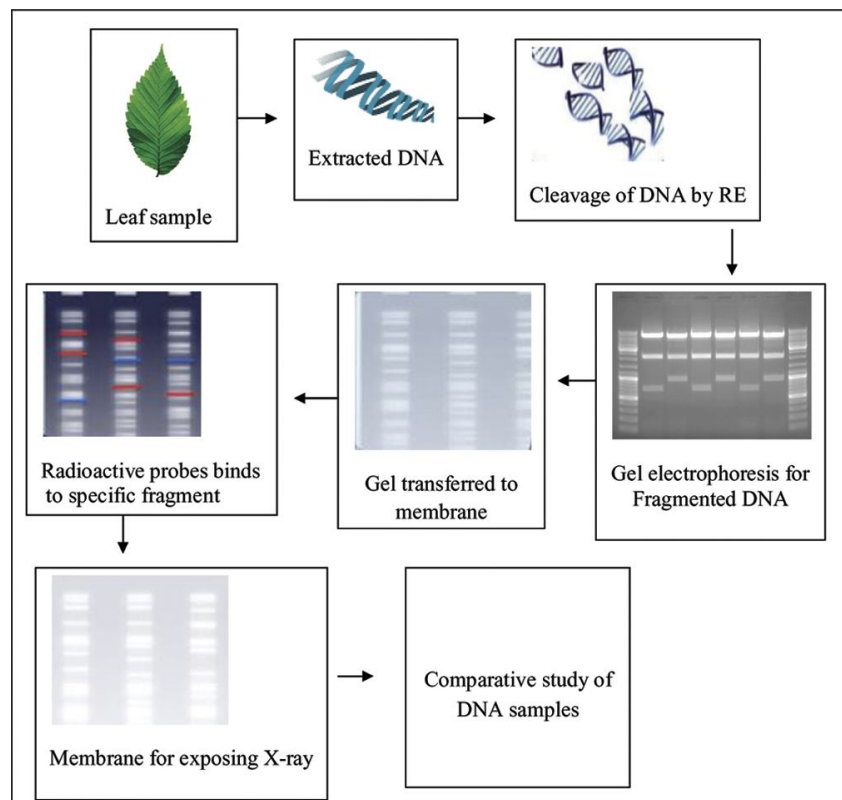


Figure 1: Authentication of medicinal plants by DNA markers.

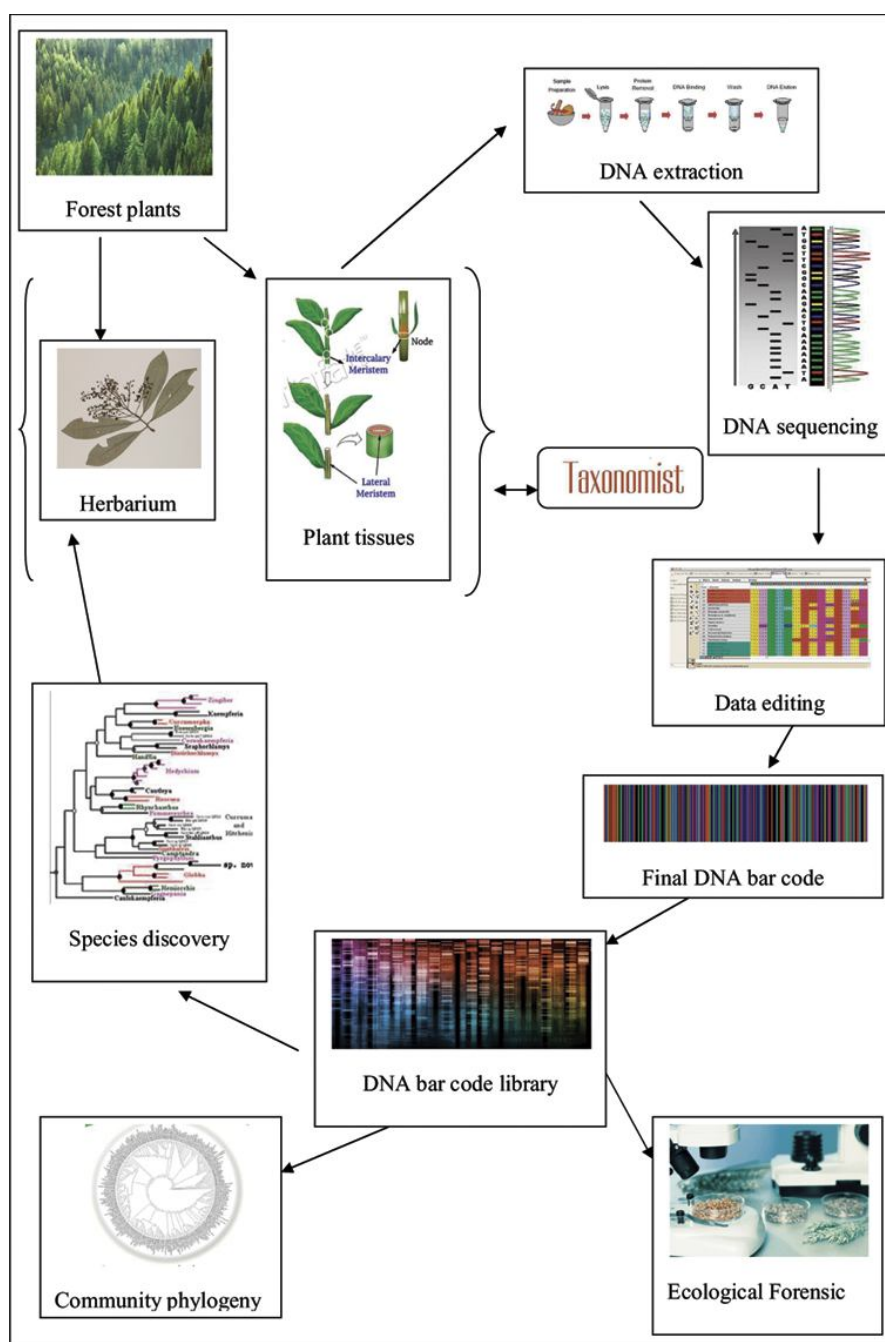


Figure 2: Various steps in plant DNA barcoding.

3. Identification of herbals through DNA bar coding

3.1 DNA extraction methods

DNA bar-coding is performed to the herbal plants when a minimum quantity and quality of DNA is present. A number of extraction methods and commercial kits are available to extract high-quality of DNA from plants (Akkurt, 2012; Sika *et al.*, 2015). After collected herbal plants (in fresh condition), DNA extraction is performed to avoid DNA degradation due to DNA damaging storage conditions and under good laboratory practice to avoid cross-contamination

with other samples. Plant part, storage time, storage conditions, and processing methods affect the quality and quantity of isolated DNA. Thereafter, plant metabolites such as polysaccharides, flavonoids, polyphenols, and terpene lactones are also hinder DNA isolation. The most widely used approaches to extract genomic DNA are the cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1987) and commercial DNA extraction kits (Akkurt, 2012) but the method is not successful in isolation of DNA from plants parts that contain high amounts of secondary metabolites. Roots, rhizomes, and tubers are contain particularly

high levels of polysaccharides and polyphenols which are removed with added high concentrations of CTAB, polyvinylpyrrolidone (PVP), and β -mercaptoethanol (β -Me) during the early stages of DNA extraction (Barnwell *et al.*, 1998; Cavallari *et al.*, 2012). High-quality of DNA is obtained from leaves and flowers because of the low levels of interfering metabolites and fibers. Especially the DNA obtained from fresh and young leaves and flowers are used to prepare a crude extract, and this solution is used to amplify the targeted DNA barcode through direct polymer chain reaction (Han *et al.*, 2016). Some plant leaves like tomato, cotton, and tea contain high concentrations of polyphenols and tannins, which gave barriers in PCR amplification as well as DNA isolation. Therefore, these are unsuitable for direct PCR which is overcome through modified DNA extraction methods for the isolation of DNA from tissues or plants containing high amounts of phenolic compounds and tannins (Peterson *et al.*, 1997). Same way, direct PCR is not possible from dried stems, roots, and fruits due to high content of storage of plant secondary metabolites. In modern phytomedicines, the plant DNA is removed or degraded during the manufacturing processes of herbal products. Hence, the DNA is extracted from capsules, tablets, and liquid extracts as a smear on an analytical agarose gel. But in the herbal product formulations, the DNA extraction is difficult because they contain excipients such as fillers, diluents, binders, glidants, lubricants, pigments, and stabilizers. All the tested excipients, talc, silica, iron oxide, and titanium dioxide, exhibited adsorbent properties that affected the extraction of DNA from the natural herbal products (Costa *et al.*, 2015).

3.2 Selection of loci as DNA barcodes for herbals

The selection of a universal barcode region for identification of herbal plants is quite challengeable and also bar coding plants is more difficult for many reasons. The slow evolutionary rate of the plant mitochondrial genome means that the mitochondrial gene regions, including the COI region, do not sufficiently distinguish plants species. Therefore, relatively fast-evolving plastid and nuclear genomes are as alternative barcodes for plants such common regions are *matK*, *rbcl*, ITS, ITS2, *psbA-trnH*, *atpF-atpH*, *ycf5*, *psbK-1*, *psbM*, *trnD*, *cox1*, *nad1*, *trnL-F*, *rpoB*, *rpoC1*, and *rps16* (Newmaster *et al.*, 2006; Hollingsworth *et al.*, 2011). Among plastid regions, *rbcl* is the best characterized gene. *matK* is one of the most rapidly evolving plastid coding regions and it consistently showed high levels of discrimination among angiosperm species. None of the individual plant DNA barcodes are described to date has both differentiating regions and universal primer regions. Hence, a multilocus plant barcode with combinations of two or three loci is recommended (Kress and Erickson, 2007). The Consortium for the Barcode of Life (CBOL) is suggested that *matK+rbcl* as the preferred plant barcode combination. The method utilizes the easily amplifiable and alignable *rbcl* region as a scaffold on which data from highly variable non-coding regions such as ITS2 or the *trnH-psbA* region are employed for identification of plant species (Table 1). Numerous studies revealed that barcode loci are useful to identify medicinal plants (Techen *et al.*, 2014) such as seven DNA regions, *psbA-trnH*, *matK*, *rbcl*, *rpoC1*, *ycf5*, ITS (consisting of both ITS1 and ITS2), and ITS2 which are evaluated for identification of more than 6600 samples of fresh medicinal plants leaves and their closely related species (Chen *et al.*, 2012) and confirmed as an effective barcode for plants. For example, 24 medicinal plants from the Fabaceae

family and their adulterants were identified using ITS2 (Gao *et al.*, 2010). The major advantage of ITS2 as a barcode for the identification of herbal supplements is its short length (200-230 bp on average). In most herbal products and dietary supplements, the DNA is highly degraded into pieces of less than 500 nucleotides in length due to various processing methods and hence, short-length barcodes that can be easily retrieved from dried, powdered form, or from extracts. Furthermore, the ITS2 locus has some disadvantages and is not suitable for global identification of plants because of (a) the presence of multiple copies of ITS2 within one individual in all plant species. The multiple ITS2 copies, which are not always homogenized by concerted evolution which led to the incorrect identification of species due to their similarity with the copies of the more closely related species and (b) the technical problems in amplification and sequencing that arises due to the presence of DNA from other species (e.g., fungi, which coexist with plants as endophytes and/or mycorrhizal symbionts) (Rodriguez *et al.*, 2009; Ivanova *et al.*, 2016).

Table 1: Primers for amplification of *matK* and *rbcl* genome regions

Primer name	Sequence (5'-3')
<i>matK</i> -390F	5'CGATCTATTCAATATTTTC3'
<i>matK</i> -1326R	5'TCTAGCACACGAAAGTCGAAGT3'
<i>rbcl</i> -BF	5'ATGTCACCACAAACAGAAAC3'
<i>rbcl</i> -724R	5'TCGCATGTACCTGCAGTAGC3'

Few years back, "mini-barcodes" was introduced for short length DNA markers (Figure 3). They are used for the identification of botanical ingredients from processed herbal supplements (Little and Jeanson, 2013). Mini-barcodes are less than 200 bp sequences of DNA from standardized *matK* and *rbcl* barcode regions, which are used to identify and authenticate herbal dietary supplements made from saw palmetto (*Serenoa repens*) fruit (Little and Jeanson, 2013), *Ginkgo biloba* leaf (Little, 2014), and devils claw (*Harpagophytum procumbens* and *Harpagophytum zeyheri*) root and rhizome (Little, 2015) contains herbal dietary supplements. The advantages of mini-barcodes are the easy retrieval of DNA markers from processed dietary materials due to their small amplicon length, and they are genus or species specificity.

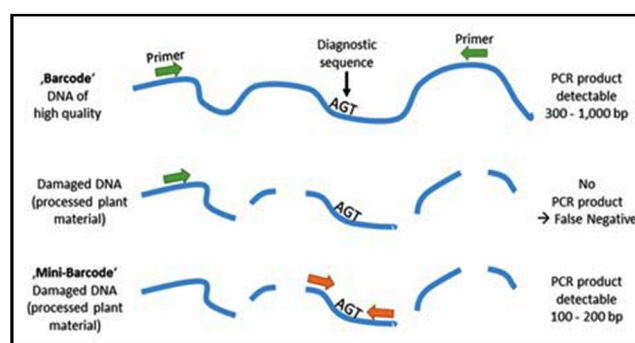


Figure 3: DNA bar code and mini barcode.

3.3 Amplification of PCR

PCR is the molecular method by which a single copy or few copies of DNA piece is amplified into million copies of particular DNA sequence are formed. This method helps in formation of DNA library (Figure 4). This method is carried out with heat stable DNA polymerase, template DNA and DNA oligonucleotides. The general barcoding technique

uses universal primers for rapid identification of plant species (Burgess *et al.*, 2011; Lahaye *et al.*, 2005) which are recommended for barcoding of plant species by amplified DNA from four genomic regions, namely ITS/ITS2 from the nuclear genome, and *matK*, *rbcL*, and *trnH-psbA* from the chloroplast genome. The requirement of number of PCRs depends on the expected frequency of the DNA to be detected. For a sample containing 10% of the low abundant DNA, 2 out of 20 PCRs

resulted in the amplification of low abundant DNA. It is reported that the differences in the melting temperatures of the primers is reduced amplification rate and the affinity of universal primers to template DNA of all known and unknown organisms (Costa *et al.*, 2015). Not only that, the presence of inhibitory secondary metabolites and inactive ingredients in tablets and capsules is also reduced the efficiency of PCR amplification.

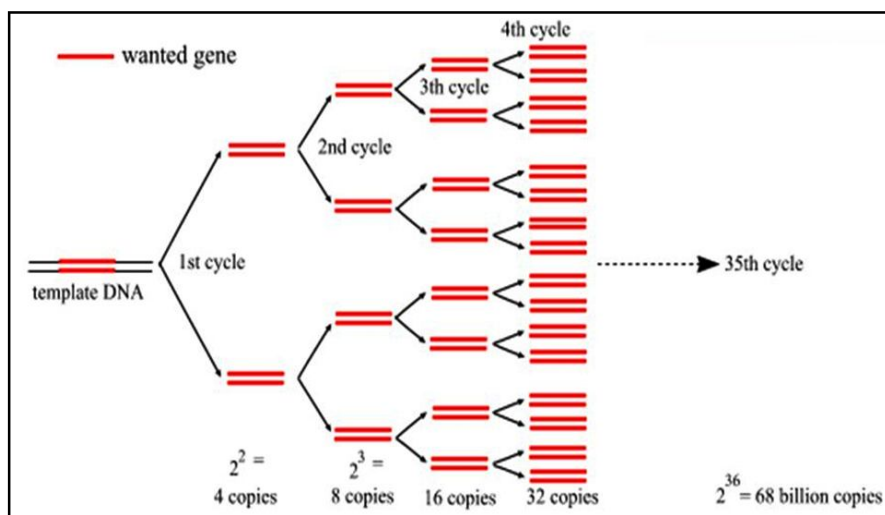


Figure 4: DNA amplification for DNA library.

3.4 Sequencing method

Sanger's di-deoxy method of sequencing is a conventional method for generating DNA sequence data to obtain a barcode from PCR amplicons (Sanger *et al.*, 1977). Sanger's sequencing technology is capable of generating sequencing reads of up to 1000 bases and has been the approach used for DNA sequencing in most of the DNA barcode analyses. The Sanger sequencing method is suitable for herbal materials that contain only a single medicinal plant because Sanger-based DNA sequencing has low throughput and, hence require

in high concentrations of DNA (100-500 ng) to avoid errors. If the herbal dietary preparation contains multiple plant species or excipients, co-amplification of barcode sequences from other material than the intended one can occur due to the nature of the universal primers during the PCR amplification step. This leads to the production of multiple/overlapping sequencing peaks and, consequently, a failure of sequencing because the correct DNA sequence of the barcode cannot be determined (Figure 5). Moreover, multiple sequences may also create confusion in the identification of the "true" barcode and other sequences.

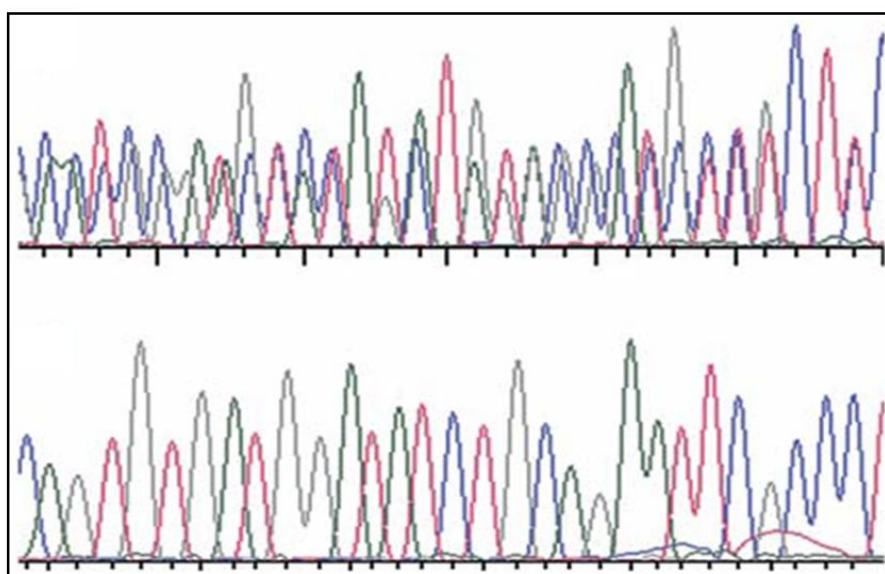


Figure 5: Sequencing signals obtained with Sanger sequencing through electropherograms.

To overcome the limitations of Sanger-based sequencing for DNA bar coding of processed or mixed samples, a high-throughput sequencing method called next-generation sequencing (NGS) is used (Kircher and Kelso, 2010). The NGS technology allows parallel sequencing of multiple DNA fragments from various DNA templates in a single reaction. For example, the whole chloroplast sequence of *Ceratophyllum demersum* was obtained by Moore *et al.* (2007) using the 454 Life Sciences sequencing platform and complete plastomes of 37 *Pinus* species were assembled by Parks *et al.* (2009) on a multiplex Illumina sequencing platform. Furthermore, the NGS “meta-barcoding” method combines with DNA barcoding and high-throughput DNA sequencing for mass analyze DNA barcodes from sediments or environmental, ancient/historical, or processed samples.

4. Authentication of herbals with DNA bar coding

Plant DNA bar coding is launched in 2009 when the core of two DNA regions from the chloroplast, RuBisCO large subunit (*rbcL*), and group II intron maturase (*matK*) genes are accepted by CBOL Plant Working Group for land plants. Since the adoption of the loci to be used in plant DNA barcode. DNA bar coding studies for the botanicals is essential for the identification of a universal marker or

suitable barcode locus/loci for herbal raw material authentication. It was reported that six different species of *Phyllanthus* were available on the market based on morphological studies. Seventy-six percent of the market samples contained *Phyllanthus amarus* as the predominant species and the remaining included five different species, namely *P. debilis*, *P. fraternus*, *P. urinaria*, *P. maderaspatensis*, and *P. kozhikodanus*. Species-specific DNA barcode signatures are developed for the tested *Phyllanthus* species using the chloroplast DNA region *psbA-trnH* (Srirama *et al.*, 2010). The DNA barcoding analysis using *matK*, *rbcL*, and ITS2 regions confirmed species belonging to the correct genus of the samples. Commercial tea samples (*Camellia sinensis*) is analyzed with 90% success identification rates using *rbcL* and *matK* barcode loci and reported 33% adulterations in herbal teas (Stoeckle *et al.*, 2011). Black cohosh (*Actaea racemosa*) samples were analyzed with a mini-barcode approach using the *matK* locus (Baker *et al.*, 2012). DNA from other plant species is accidentally introduced at any stage of processing, for example, at the time of collection of herbal plant material, during storage, drying, grinding, at various stages of the product manufacturing process, or during the analysis in the quality control laboratory (Figure 6). An overview of DNA bar coding technique is given in Figure 7.

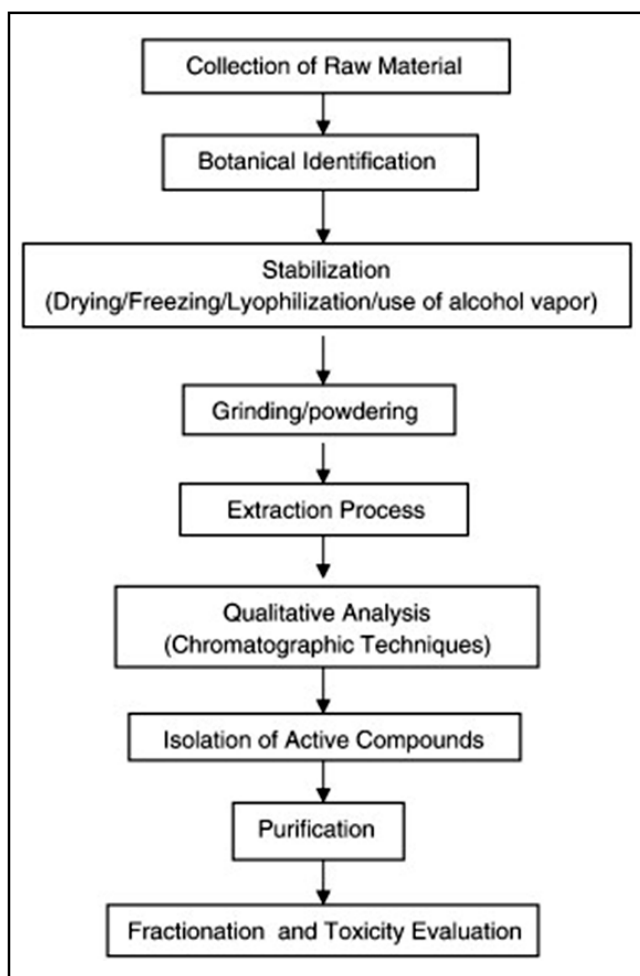


Figure 6: Various steps in the processing of plant material during which exogenous DNA is introduced into a sample.

5. Challenges

In last few years DNA bar coding in herbals plays significant role for authentication and development of biomarkers and still the technique is necessary to identify a vast number plant species which are not explored. Therefore the bar coding technique has few challenges that may be tough but remain promising. Such as: i. Building the impetus to generate DNA barcodes using multiple plant DNA markers for all woody species at forest community levels, which requires investment as well as significant research resources; ii. Analyzing massive DNA bar coding sequence data, which needs powerful computational systems and critical infrastructures to perform multidisciplinary research projects in mass scale, and iii. Promoting theoretical innovation, which calls for raising novel scientific hypotheses and publishing valuable research papers in top academic journals as well as patenting of research works.

6. Future directions

Plant DNA bar-coding is essential for comparative analyses of community phylogenies from forest dynamics plots or natural reserves and when the technology is combined with conservative plant traits (*e.g.*, flowering phenology), effects of individuals on assembly patterns within communities, dissimilarity of diverse communities along an environmental gradient, and non-random processes therein are more thoroughly explored. Therefore, some possible directions are: i. Proposing a “purpose-driven barcode” (*e.g.*, metabarcoding and mini-barcode) fit for multi-level applications such as identifying living organisms, reconstructing community phylogenies, detecting environmental biodiversity information, and exploring ecological network structure, ii. Developing new integrative sequencing strategies (*e.g.*, genome skimming) to generate mega-phylogenies in face of the post-genomic era, iii. Constructing national-level DNA barcode sequence libraries

of economically valuable tree species for commercial authentication and endangered plant taxa against illegal international trade, and iv. Establishing intelligent identification systems for land plants integrating genetic, morphological and environmental information, which will make DNA-based plant identification more precise and convenient. This DNA bar coding technology is valuable to explore large-scale biodiversity patterns, the origin and evolution of life, and will also facilitate preservation and utilization of biological resources. Furthermore, the application of other sequencing technologies, such as the utilization of microfluidic PCR-based target offers a faster and less expensive option for large-scale multi-locus plant DNA bar coding.

7. Conclusion

DNA bar coding uses specific short regions of DNA in order to identify plant species in herbal products and dietary supplements. It is a highly reliable and promising tool under specific conditions, such as the correct stage of analysis when the DNA could be detected, primer affinity for successful PCR amplification, and absence of contaminating DNA. The detection of adulteration of botanical ingredients are improved if DNA barcoding is routinely and appropriately used for authentication of herbal materials. At last, intense research is focused on their effectiveness, integration with High Throughput Sequencing (HTS) methods, and application to a wide range of basic and applied research.

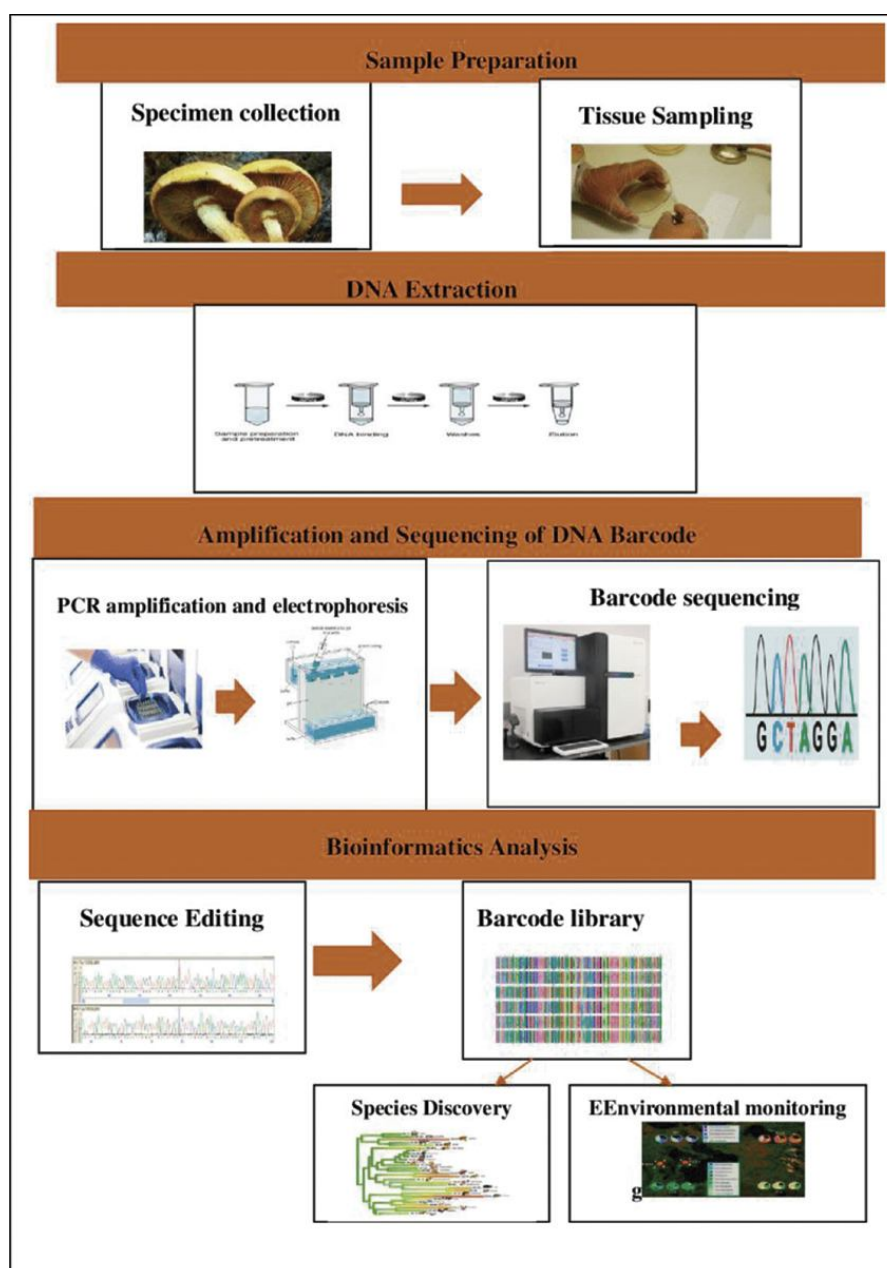


Figure 7: An overview of DNA bar coding technique.

Conflict of interest

The author declared that there is no conflicts of interest in the course of conducting the research. The author has final decision regarding the manuscript and decision to submit the findings for publication.

References

- Akkurt, M. (2012). Comparison between modified DNA extraction protocols and commercial isolation kits in grapevine (*Vitis vinifera* L.). *Genet. Mol. Res.*, **11**:2343-2351.
- Baker, D.A.; Stevenson, D.W. and Little, D.P. (2012). DNA barcode identification of black cohosh herbal dietary supplements. *J. AOAC Int.*, **95**:1023-1034.
- Barnwell, P.; Blanchard, A.N.; Bryant, J.A.; Smirnov, N. and Weir, A.F. (1998). Isolation of DNA from the highly mucilaginous succulent plant *Sedum telephium*. *Plant Mol. Biol. Rep.*, **16**:133-138.
- Burgess, K.S.; Fazekas, A.J.; Kesanakurti, P.R.; Graham, S.W.; Husband, B.C.; Newmaster, S.G.; Percy, D.M. and Hajibabaei, M. (2011). Barrett SCH. Discriminating plant species in a local temperate flora using the *rbcL+matK* DNA barcode. *Methods Ecol. Evol.*, **2**:333-340.
- Cavallari, M.M.; Siqueria, M.V.B.M.; Val, T.M.; Pavanelli, J.C.; Monteiro, M.; Grando, C.; Pinheiro, J.B.; Zucchi, M.I. and Gimenes, M.A. (2012). A modified acidic approach for DNA extraction from plant species containing high levels of secondary metabolites. *Gen. Mol. Res.*, **13**:6497-6502.
- Chen, C.H.; Dickman, K.G.; Moriya, M.; Zavadil, J.; Sidorenko, V.S.; Edwards, K.L.; Gnatenko, D.V.; Wu, L.; Turesky, R.J.; Wu, X.R.; Pu, Y.S. and Grollman, A.P. (2012). Aristolochic acid-associated urothelial cancer in Taiwan. *Proc. Natl. Acad. Sci.*, **109**:8241-8246.
- Collins, R. A. and Cruickshank, R. H. (2013). The seven deadly sins of DNA barcoding. *Mol. Ecol. Resour.*, **13**:969-975. doi: 10.1111/1755-0998.12046.
- Costa, J.; Amaral, J.S.; Fernandes, T.J.R.; Batista, A.; Oliveira, M.B. and Mafra, I. (2015). DNA extraction from plant food supplements: Influence of different pharmaceutical excipients. *Mol. Cell Probes.*, **29**:473-478.
- Doyle, J.J. and Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.*, **19**:11-15.
- Gao, T.; Yao, H.; Song, J.; Liu, C.; Zhu, Y.; Ma, X.; Pand, X.; Xu, H. and Chen, S. (2010). Identification of medicinal plants in the family Fabaceae using a potential DNA barcode ITS2. *J. Ethnopharmacol.*, **130**:116-121.
- Han, J.; Pang, X.; Liao, B.; Yao, H.; Song, J. and Chen, S. (2016). An authenticity survey of herbal medicines from markets in China using DNA barcoding. *Sci. Rep.*, **6**:18723.
- Harris, S. E. and Bellino, M. (2013). DNA barcoding from NYC to Belize. *Science*, **342**:1462-1463. doi: 10.1126/science.1230006.
- Hebert, P.D.N.; Cywinska, A.; Ball, S.L. and deWaard, J.R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, **270**:313-321.
- Hebert, P.D.N.; Penton, E.H.; Burns, J.M.; Janzen, D.H. and Hallwachs, W. (2004). Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences, USA*, **101**:14812-14817.
- Hollingsworth, P.M.; Graham, S.W. and Little, D.P. (2011). Choosing and using a plant DNA barcode. *PLoS One*, **6**:e19254.
- Hollingsworth, P. M.; Li, D. Z.; van der Bank, M. and Twyford, A. D. (2016). Telling plant species apart with DNA from barcodes to genomes. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **371**:20150338. doi: 10.1098/rstb.2015.0338.
- Ivanova, N.V.; Kuzmina, M.L.; Braukmann, T.W.A.; Borisenko, A.V. and Zakharov, E.V. (2016). Authentication of herbal supplements using next-generation sequencing. *PLoS One*, **11**:e0156426.
- Jeanson, M.L.; Labat, J.N. and Little, D.P. (2011). DNA barcoding: A new tool for palm taxonomists? *Ann Bot.*, **108**:1445-1451.
- Kircher, M. and Kelso, J. (2010). High-throughput DNA sequencing-concepts and limitations. *Bioessays.*, **32**:524-536.
- Kress, W.J. and Erickson, D.L. (2007). A two-locus global DNA barcode for land plants: The coding *rbcL* gene complements the non-coding *trnH-psbA* spacer region. *PLoS ONE*, **2**:e508.
- Kress, W.J. (2017). Plant DNA bar codes: Applications today and in the future. *Journal of Systematics and Evaluation*, **55**(4):291-307.
- Lahaye, R.; Van der Bank, M.; Bogarin, D.; Warner, J.; Pupulin, F.; Gigot, G.; Maurin, O.; Duthoit, S.; Barraclough, T.G. and Savolainen, V. (2005). DNA barcoding the floras of biodiversity hotspots. *Proc. Natl. Acad. Sci.*, **105**:2923-2928.
- Little, D.P. and Jeanson, M.L. (2013). DNA barcode authentication of saw palmetto herbal dietary supplements. *Sci. Rep.*, **3**:3518.
- Little, D.P. (2014). Authentication of *Ginkgo biloba* herbal dietary supplements using DNA barcoding. *Genome.*, **57**:513-516.
- Little, D.P. (2015). Confirming species identity of herbal dietary supplements, an example from devil's claw. *Adulteration and Fraud in Food Ingredients and Dietary Supplements Workshop*, Rockville, MD.
- Moore, M.J.; Bell, C.D.; Soltis, P.S. and Soltis, D.E. (2007). Using plastid genome scale data to resolve enigmatic relationships among basal angiosperms. *Proc. Natl. Acad. Sci.*, **104**:19363-19368.
- Muellner, A.N.; Schaefer, H. and Lahaye, R. (2011). Evaluation of candidate DNA barcoding loci for economically important timber species of the mahogany family (Meliaceae). *Mol. Ecol. Resour.*, **11**:450-460.
- Newmaster, S.G.; Fazekas, A.J. and Ragupathy, S. (2006). DNA barcoding in land plants: Evaluation of *rbcL* in a multigene tiered approach. *Botany*, **84**:335-341.
- Newmaster, S.G.; Fazekas, A.J.; Steeves, R.A.D. and Janovec, J. (2008). Testing candidate plant barcode regions in the Myristicaceae. *Molecular Ecology Resources*, **8**:480-490.
- Parks, M.; Cronn, R. and Liston, A. (2009). Increasing phylogenetic resolution at low taxonomic levels using massively parallel sequencing of chloroplast genomes. *BMC Biol.*, **7**:84.
- Pei, N.; Erickson, D.L.; Chen, B. F.; Ge, X. J.; Mi, X. C.; Swenson, N. G.; Zhang, J.L.; Jones, F.A.; Huang, C.L.; Ye, W.; Hao, Z.; Hsieh, C.F.; Lum, S.; Bourg, N.A.; Parker, J.D.; Zimmerman, J.K.; McShea, W.J.; Lopez, L.C.; Sun, L.F.; Davies, S.J.; Ma, K. and Kress, W.J. (2015). Closely-related taxa influence woody species discrimination via DNA bar coding: Evidence from global forest dynamics plots. *Sci. Rep.*, **5**:15127. doi: 10.1038/srep15127.
- Pei, N.; Chen, B. and Kress, W. J. (2017). Advances of community-level plant DNA barcoding in China. *Front Plant Sci.*, **8**:225. doi: 10.3389/fpls.2017.00225.

- Peterson, D.G.; Boehm, K.S. and Stack, S.M. (1997). Isolation of milligram quantities of nuclear DNA from tomato (*Lycopersicon esculentum*), a plant containing high levels of polyphenolic compounds. *Plant Mol. Biol. Rep.*, **15**:148-153.
- Rodriguez, R.; White, J. Jr.; Arnold, A. and Redman, R. (2009). Fungal endophytes: Diversity and functional roles. *New Phyto.*, **182**:314-330.
- Sanger, F.; Nicklen, S. and Coulson, A.R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci.*, **74**:5463-5467.
- Shapcott, A.; Liu, Y.; Howard, M.; Forster, P.L.; Kress, W.J.; Erickson, D.L.; Faith, D.P.; Shimizu, Y. and McDonald, W.J.F. (2017). Comparing floristic diversity and conservation priorities across south east Queensland regional rain forest ecosystems using phylodiversity indexes. *International Journal of Plant Sciences*, **178**:211-229.
- Sika, K.C.; Kefela, T.; Adoukonou-Sagbadja, H.; Ahoton, L.; Saidou, A.; Baba-Moussa, L.; Baptiste, L.J.; Korconi, S.O. and Gachomo, E.W. (2015). A simple and efficient genomic DNA extraction protocol for large scale genetic analyses of plant biological systems. *Plant Gene*, **2**:43-45.
- Srirama, R.; Senthilkumar, U.; Sreejayan, N.; Ravikanth, G.; Gurumurthy, B.R.; Shivanna, M.B.; Sanjappa, M.; Ganeshiah, K.N. and Uma Shaanker, R. (2010). Assessing species admixtures in raw drug trade of *Phyllanthus*, a hepato-protective plant using molecular tools. *J. Ethnopharmacol.*, **130**:208-215.
- Stoeckle, M.Y.; Gamble, C.C.; Kirpekar, R.; Young, G.; Ahmed, S. and Little, D.P. (2011). Commercial teas highlight plant DNA barcode identification successes and obstacles. *Sci. Rep.*, **1**:1-7.
- Techen, N.; Parveen, I.; Pan, Z. and Khan, I.A. (2014). DNA barcoding of medicinal plant material for identification. *Curr. Opin. Biotechnol.*, **25**:103-110.
- Zinger, L. and Philippe, H. (2016). Coalescing molecular evolution and DNA barcoding. *Mol. Ecol.*, **25**:1908-1910. doi: 10.1111/mec.13639.

Citation: Kuntal Das (2019). Authentic identification and new drug discovery from natural plant based constituents through DNA bar-coding: A challenging task to the researchers. *Ann. Phytomed.*, **8(2):19-27.**
