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CURRENT TOPICS IN PLANT RESEARCH

DNA banking for plant breeding, biotechnology and biodiversity evaluation

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Abstract The manipulation of DNA is routine practice in botanical research and has made a huge impact on plant breeding, biotechnology and biodiversity evaluation. DNA is easy to extract from most plant tissues and can be stored for long periods in DNA banks. Curation methods are well developed for other botanical resources such as herbaria, seed banks and botanic gardens, but procedures for the establishment and maintenance of DNA banks have not been well documented. This paper reviews the curation of DNA banks for the characterisation and utilisation of biodiversity and provides guidelines for DNA bank management. It surveys existing DNA banks and outlines their operation. It includes a review of plant DNA collection, preservation, isolation, storage, database management and exchange procedures. We stress that DNA banks require full integration with existing collections such as botanic gardens, herbaria and seed banks, and information retrieval systems that link such facilities, bioinformatic resources and other DNA banks. They also require efficient and well-regulated sample exchange procedures. Only with appropriate curation will maximum utilisation of DNA collections be achieved.

Keywords Databases · DNA bank · DNA extraction · Exchange · Management · Material transfer agreements

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Introduction

Throughout the world, biodiversity is under serious threat from factors such as intensive agriculture and silviculture, increased habitat fragmentation and exposure to pollution and mass tourism (Wilson 1992; Hodkinson and Parnell 2007a). Countries of the world must honour their commitments to conserve biodiversity under the United Nations Convention on Biological Diversity (OJ L309 1993; http://www.biodiv.org). A number of initiatives is underway to meet these objectives, such as the Global Strategy for Plant Conservation (GSPC 2002) co-ordinated by the Global Partnership for Plant Conservation (http://www.plants2010.org). Furthermore, plant genetic resources of major and minor world crops are also under threat, and activities are ongoing to preserve and characterise them (Ford-Lloyd and Jackson 1986; Maxted et al. 1997; Spooner et al. 2005). For example, the International Plant Genetic Resource Institute (http://www.ipgri.cgiar.org), part of the Consultative Group on International Agricultural Research (http://www.cgiar.org) helps coordinate and promote international plant genetic resource initiatives.

DNA collections have become important resources in worldwide efforts to address the biodiversity crisis, manage the world's genetic resources and maximise their potential. It is evident that plant conservationists are increasingly turning to DNA technologies to answer basic botanical questions that may help formulate appropriate conservation strategies (Karp et al. 1996; Kelleher et al. 2005; Lowe et al. 2004). Such techniques are particularly well suited for measuring and monitoring genetic diversity and resolving complex taxonomic issues (Karp et al. 1996; Soltis and Soltis 2000; Hodkinson et al. 2002a, b; Carolan et al. 2006). The plant taxonomy and systematics community has responded to the biodiversity crisis by defining three major challenges (summarised by Blackmore 2002): (1) completing the inventory of life, (2) discovering evolutionary relationships through phylogenetics, and (3) providing information via the Internet. DNA collections can help with all three of those activities. DNA sequence analysis is useful in the identification and delimitation of species and higher taxa and is also set to become increasingly important via DNA taxonomy and DNA barcoding (Tautz et al. 2002, 2003; Lipscomb et al. 2003; Kristiansen et al. 2005; Chase et al. 2005). It is a powerful resource for molecular phylogenetics and efforts to reconstruct the 'tree of life' (Palmer et al. 2004; http://www.tolweb.org.tree/; Hodkinson and Parnell 2007b). Furthermore, information from DNA collections can easily be made available via the Internet and made globally accessible within existing initiatives such as the Global Biodiversity Information Facility (Bisby 2000; http://www.gbif.net), Species 2000 (http://www.species2000.org) and regional databases such as the Inter-American Biodiversity Network (http://www.iabin.org) and the National Biodiversity Network (http://www.ukbiodiversity.net).

Likewise, plant breeders and biotechnologists rely heavily on DNA collections for genetic resource characterisation and utilisation. These activities include genetic diversity analysis (Karp et al. 1996; Mueller and Wolfenbarger 1999; Hodkinson et al. 2002a; Harbourne et al. 2005), marker-aided selection (MAS) and quantitative trait loci (QTL) analysis (Young 1999; Thomas 2003; Toenniessen et al. 2003) and various aspects of plant biotechnology and genetic engineering. These DNA collections do not generally cover a broad range of taxa but are based on samples from within the immediate gene pool of the target species such as landraces, cultivars or varieties. Some collections, such as the NIAS DNA Bank of Japan (http://www.dna.affrc.go.jp), distribute restricted types of DNA including expressed sequence tags (ESTs), restriction fragment length polymorphism (RFLP) markers, and P1-derived artificial chromosome/bacterial artificial chromosome (PAC/BAC) clones.

DNA as a resource

Collections for plant breeding, biotechnology and biodiversity evaluation have traditionally been based on living plants managed in seed banks, field gene banks, botanic gardens or in situ reserves (Maxted et al. 1997), or based on dried plants managed in herbarium collections (Bridson and Forman 1992). Herbaria, in particular, have played a major role in biodiversity evaluation and allow plant collections to be maintained, catalogued and studied. Estimates of plant species diversity (250,000 angiosperms, 650 gymnosperms, 12,000 ferns and allies, 14,000 bryophytes, 13,500 lichens and 40,000 algae; Prance 1997) were made by taxonomic study of herbarium specimens. Studies of herbarium collections have also produced systems of classification (Cronquist 1981; Dahlgren et al. 1985; Thorne 1992; Takhtajan 1997).

Analysis of morphological, chemical and anatomical characteristics of herbarium specimens can be used for assessment of genetic variation within and between species, but none of these can claim to offer the same potential as DNA. After all, genomic DNA samples represent the entire genetic component of the target organism. A relatively small plant genome, such as *Lycopersicum esculatum* L. (tomato), has 1,000 megabase-pairs (Mbp) of DNA, and a large genome, such as *Triticum aestivum* L. (hexaploid wheat), has approximately 16,000 Mbp (Walbot 2000). DNA is easily extracted from most plants and, once purified, can be stored for long periods of time. Techniques to study genetic variation via direct analysis of their DNA are routine for well-equipped molecular laboratories, and, recently, entire plant genomes have been sequenced. The first was *Arabidopsis thaliana* (L.) Heyn. (thale cress), with approximately 15,000 genes and 125 Mbp of DNA (The *Arabidopsis* Genome Initiative 2000), and the second was *Oryza sativa* L. (rice), with approximately 4.2 Mbp of DNA (Adam 2000). The genome of the first model tree *Populus trichocarpa* Torr. & Gray (black cottonwood), has also recently been sequenced and assembled through shotgun DNA sequencing and a fingerprint-based physical map (Tuskan et al. 2006).

The most direct method to study genetic variation is to sequence DNA. One gene, *rbcL*, has been studied more than any other for phylogenetic and taxonomic studies. The *rbcL* gene codes for the large subunit of ribulose, 1,5-bisphosphate carboxylase/oxygenase (RUBISCO; Chase et al. 1993; Soltis et al. 1999;

Sanderson and Driskell 2003), the principal carbon-fixing enzyme in plants, and is possibly the most abundant enzyme on earth. It has been used extensively in plant phylogenetics (Savolainen and Chase 2003; Hodkinson and Parnell 2007b), along with an increasing number of other genes (Soltis et al. 1999). However, other methods of detecting sequence variation, generically known as fingerprinting markers, such as RFLP, amplified fragment length polymorphisms, (AFLPs), and simple sequence repeats (SSRs) (Vos et al. 1995; Karp et al. 1996; Reeves et al. 1998; Wolfe and Liston 1998; Hodkinson et al. 2000; McGrath et al. 2006), are often either more appropriate, or cost-effective, for characterising genetic variation. These methods are particularly appropriate for assessing diversity within species and are being used extensively for QTL and MAS in plant breeding (Young 1999; Thomas 2003; Toenniessen et al. 2003). There has also been an explosion in the information systems and the methods to exploit genetic information that are collectively known as bioinformatics (Boguski 1998; Lewitter 1998; Mount 2004). Furthermore, DNA collections in biological libraries have become increasingly important, such as PAC/BAC clones. For example, EST and genome sequencing projects are working from these resources and can produce molecular markers, for comparative studies and further genome assemblies.

The need for plant DNA banks, appropriate management protocols and networking

Plant DNA banks have emerged as new resources with great potential for characterising and utilising biodiversity. Other botanical collections, genetic resource collections, herbaria, botanic gardens and seed banks are highly valued on the basis that they represent important national and international resources (Waldren et al 1999). Methods of curation for these collections are well developed and documented (Bridson and Forman 1992; Maxted et al. 1997). It is our view that DNA banks should be fully integrated with such facilities and carry equal status. Methods for the curation of DNA banks have, however, not been adequately documented. Savolainen and Reeves (2004) made a 'Plea for DNA banking' specifically for biodiversity evaluation. This paper makes a similar plea but also recognises that procedures for their development and maintenance are still very much in their infancy. We therefore review the curation practices of DNA banks and provide guidelines for their management.

DNA banking and plant DNA banks of the world

A number of attempts has been made to coordinate international networks of DNA banks. For example, DNA Bank-Net (Adams 1988) was created in the late 1980s (Adams 1998) with the aim of establishing an international network of DNA repositories for the storage of genomic DNA on every continent. Members of this network met formally on a number of occasions (Adams and Adams 1992, 1998; Adams et al. 1994) to share experiences of cryo-storage of DNA and DNA-rich materials. Despite the initiation of this network, most DNA banks continue to operate independently, often from within a major research institution. There is a pressing need for more formal co-operation and communication between existing research institutes that house DNA collections, so that experiences and expertise can be shared, material stored safely, and material better exchanged and utilised. One example of a successful partnership is the UK Darwin Initiative-funded collaboration of the Royal Botanic Gardens, Kew, England and the South African National Biodiversity Institute, Kirstenbosch, South Africa, to establish a DNA bank of South African plants (http://www.nbi.ac.za/research/dnabank.htm). There are still relatively few publicly available plant DNA banks. A list of active DNA banks that allow, or promote sharing, usually using Internet links for rapid processing of requests, is provided in Table 1. It is not an exhaustive list but serves as a pointer to some of the major world collections on several continents. The CGIAR (http://www.cgiar.org) is a decentralised structure made up of 16 International Agricultural Research Centres (IARCs) located in 12 developing and three developed countries. Many of these IARCs have DNA collections, such as the International Rice Research Institute (IRRI) (Table 1), but information is generally not readily accessible regarding their holdings via the Internet, nor do they usually have web-based databases or interfaces. Clearly, the compilation of an online exhaustive list, such as exists for the World's herbaria (http://www.sciweb.nybg.org/science2/IndexHerbariorum. asp), would be very useful but requires considerable resource commitment from a major international organisation.

The major operational activities of a generalised DNA bank are summarised in Fig. 1. The main components are collection of plant material, storage of DNA-rich material in a 'tissue bank', DNA extraction, DNA banking, and DNA utilisation (analysis and exchange). These are discussed in detail in the following sections.

Table 1 Plant DNA banks of the world

DNA bank	Web address
Australian Plant DNA Bank, Centre for Plant Conservation Genetics, Southern Cross University, Lismore, NSW, Australia	http://www.dnabank.com.au/index_files/index.php
BGBM DNA Bank, Botanic Garden and Botanical Museum, Berlin, Germany	http://www.bgbm.org/bgbm/research/dna/
DNA Bank Brazilian Flora Species, Rio de Janeiro Botanic Garden, Brazil	http://www.jbrj.gov.br/pesquisa/div_molecular/bancodna/index.htm
DNA Bank at Kirstenbosch, South African National Biodiversity Institute, Kirstenbosch, South Africa	http://www.nbi.ac.za/research/dnabank.htm
IRRI, DNA Bank, International Rice Research Institute, Philippines	http://www.irri.org/GRC/GRChome/Home.htm
Missouri Botanic Garden DNA Bank, St Louis, MO, USA	http://www.mobot.org/MOBOT/research/diversity/dna_banking.htm
National Herbarium Netherlands DNA Bank, The Netherlands	http://www.nationaalherbarium.nl/taskforcemolecular/dna_bank.htm
NIAS DNA Bank, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan	http://www.dna.affrc.go.jp/
Plant DNA Bank Korea (PDBK), Graduate School of Biotechnology, Korea University, Seoul, Korea	http://www.pdbk.korea.ac.kr/
Royal Botanic Garden Edinburgh DNA Bank, Edinburgh, Scotland	http://www.rbge.org.uk/rbge/web/science/research/
Royal Botanic Garden Kew DNA Bank, Richmond, England	http://www.rbgkew.org.uk/data/dnaBank/
TCD DNA Bank, Department of Botany, School of Natural Sciences, Trinity College, Ireland	http://www.dnabank.bot.tcd.ie
Tropical Plant DNA Bank, Fairchild Tropical Botanic Garden and Florida International University, FL, USA	http://www.ftg.org/research

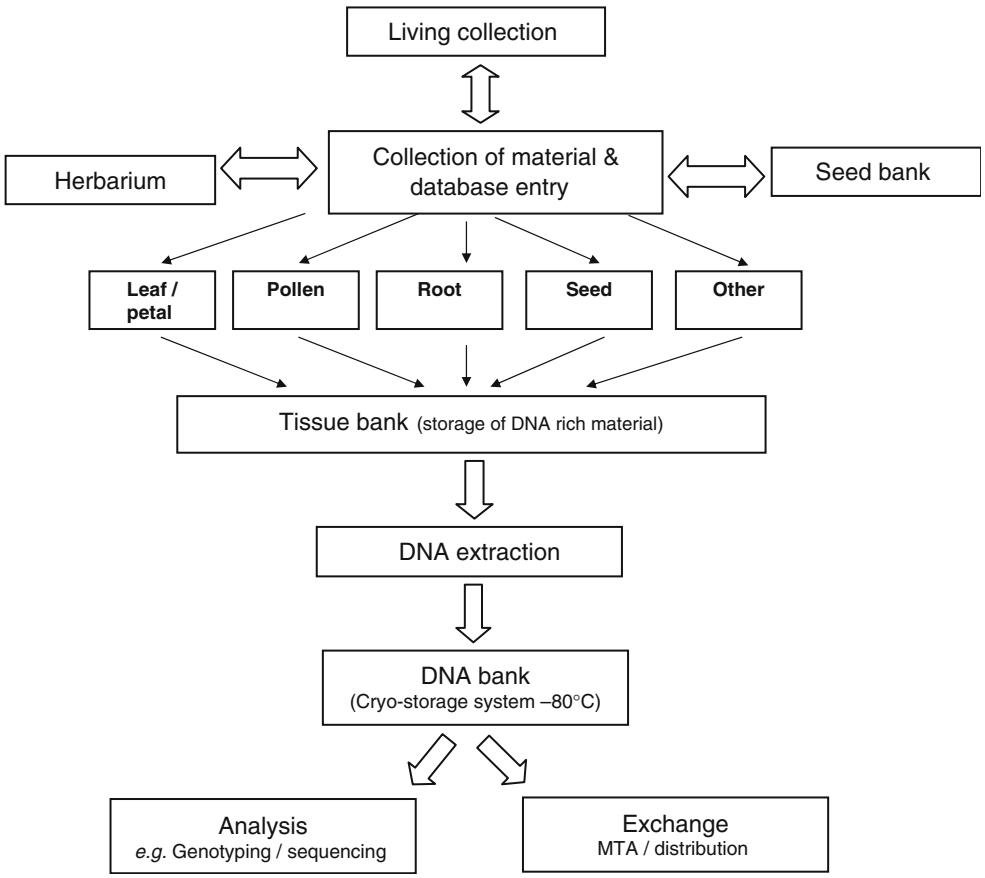

Fig. 1 Summary of main DNA bank operational activities. DNA is often stored in a cryo-storage system (DNA bank), and DNA-rich desiccated plant tissue is stored typically at –20°C (tissue bank)

Collection of plant material

The first part of the DNA banking process involves the collection of high-quality plant material for extraction and the collection of associated information (Fig. 1). Leaves are most generally used for DNA extractions and are best extracted while fresh to optimise DNA yields. Fresh leaves can usually be kept in the refrigerator for 2–3 days in sealed zip-lock bags with a small piece of damp filter paper to keep them moist prior to extraction. Other parts of the plant can also be used for extraction, including petals (Kasajima et al. 2004; Pridgeon et al. 2001), roots (DeVries et al. 2003), buds (Gilmore et al. 1993; Heuertz et al. 2004), and seed (Saini et al. 1999; Robledo and Gil 2005). For many applications, it is important to have clean, or even sterile, source material that is free from contaminating organisms. Contaminating fungi, such as various pathogens or endophytes, may cause problems with subsequent DNA manipulation, and there are documented cases where fungal DNA has been unintentionally amplified instead of the target plant DNA. For example, Zhang et al. (1997) inadvertently amplified fungal ribosomal DNA from leaves of bamboos.

If freshly collected material cannot be processed immediately, samples have to be stored to optimise subsequent DNA extraction yields. It is advisable that the material be freeze-dried and then stored in a freezer (Thomson 2002). For field collection, there are two major preservation options; one involves drying and the other involves liquid preservative (pickling). Drying specimens in the field has proven successful for subsequent extraction (tissue bank storage of DNA-rich material; Fig. 1) and can be done by rapid drying of plant material in silica gel (Chase and Hills 1991; Adams et al. 1992; Pyle and Adams 1989). Air-drying (Taylor and Swann 1994), or the use of anhydrous calcium sulphate as a desiccating agent, has also proven successful (Liston et al. 1990). We find that preservation in silica gel is most appropriate as a general drying method; approximately 50 g of silica gel will reliably desiccate 1 g fresh weight of leaf material for most species of higher plants. Desiccated and frozen plant tissues are stable for at least 10 years (T. R. Hodkinson, personal observation) but could potentially provide high-quality DNA extractions for several decades or even centuries.

The alternative to drying is to use a preservative solution. Rogstad (1992) advocated the preservation of material in aqueous NaCl–cetryltrimethyl-ammonium bromide (CTAB, pickling). This method has been adapted by Thomson (2002), who included Na-ascorbate as an antioxidant in Rogstad's NaCl–CTAB preservative and found it to improve DNA extraction, especially with 'hard to extract plant material'. Rogstad's method has also been optimised for DNA extractions by Storchová et al. (2000), who recommend preliminary homogenisation in a sorbitol wash buffer (a rinse) before DNA extraction.

Nickrent (1994) advocated another variant relating to the methods of Rogstad (1992) and Thomson (2002), by homogenising leaves in the field, immediately after collection in buffered NaCl–CTAB containing antioxidants at 95°C, and incubating them at 70–80°C for 30 min prior to short-term storage at ambient temperatures. This denatures the enzymes responsible for oxidative processes and also destroys deoxynucleases. The method preserves the DNA very well but requires the use of heating equipment in the field and may not be a practicable method for many researchers.

We have used both silica gel drying and Thomson's (2002) NaCl–CTAB field pickling method and found both to be highly suitable preservation methods when extractions from fresh material or freeze-dried material are not possible. New preservation and storage techniques may replace these methods in the near future. For example, an alternative and rapid method for preparing genomic DNA from a wide range of organisms has recently been described and involves the use of Whatman FTA filter matrix technology (Tsukaya et al. 2005; Borman et al. 2006). Tsukaya et al. (2005) used FTA cards for the general collection of a large number of DNA samples of wild plants during a botanical expedition in Nepal.

DNA may also be collected from ancient or fossil material, but this can require modified extraction procedures to improve DNA yields from such highly degraded DNA sources (Golenberg 1994; Savolainen et al. 1995; Drábková et al. 2002). Best yields are generally obtained from air-dried herbarium specimens that have not been treated with chemical preservatives, high temperatures or microwaves (Taylor and Swann 1994; Drábková et al. 2002).

Preparation of associated voucher material or maintenance of living collections

A DNA sample is much less useful without detailed collection information and, for many purposes (especially for taxonomy and systematics), an associated herbarium voucher. Herbarium specimens should, therefore, be made for all samples taken for DNA extraction, unless there are strong reasons not to do so (Kristiansen et al. 2005). For example, individuals from a large population genetics study that focuses on one, well-defined, species may sometimes not require

vouchers. Likewise, some genetic resource collections of crop species may not require vouchers. For other plants, the existence of an associated herbarium specimen is critical in order to confirm taxonomic identity (Tautz et al. 2002; Lipscomb et al. 2003; Seberg and Petersen 2007). Herbaria are concentrated sources of biodiversity information (Parnell 2000) and vary enormously in size and management practice. The holdings of most herbaria are not fully catalogued and digitised, even for their type specimens. The National Herbarium of the Netherlands (http://www.nationaal-herbarium.nl) is one of the few to digitise their collection, but other digitising initiatives are underway, such as The African Plants Initiative (Demissew et al. 2005). Digital images of specimens and cataloguing of herbarium holdings will allow other workers to assess the veracity of the DNA record by seeing, on their computer screens, the data source. It will also allow workers to pinpoint likely sources of other material for DNA extraction.

Live plants or propagules may be collected concurrently and maintained in living collections (Fig. 1) such as botanical gardens, field banks, kitchen gardens or seed banks (Ford-Lloyd and Jackson 1986; Maxted et al. 1997). These collections also need to be fully integrated with DNA collections, for maximum utilisation. Seed banks are often associated with botanic gardens and may be integrated to a certain extent, such as the Millennium Seed Bank Project that is closely linked to the Royal Botanic Gardens Kew (http://www.rbgk.org.uk/msbp). For example, many of these facilities document their holdings in databases compatible with the international transfer format (ITF) for botanic gardens (Botanic Gardens Conservation Secretariat 1987). DNA banks linking closely with such resources should consider adopting a compatible system (see also database section below).

Storage of DNA-rich material in a tissue bank

Preserved plant material should be catalogued as part of the DNA bank so that a tissue bank of DNA-rich material is maintained (Fig. 1). A DNA sample is a finite resource, but, if sufficient source tissue is stored, then this can be re-utilised to provide another DNA sample. Alternatively, a living or culture collection (of, for example, microalgae or fungi) can be re-used. It is neither desirable nor feasible for tissue samples (of DNA-rich material) to be collected for many purposes, or during all botanical collecting trips, but field workers should at least consider the option of collecting a small amount of plant tissue solely for the purpose of future DNA analysis. Herbarium specimens should not be unnecessarily sacrificed for DNA extraction purposes. It takes very little time to preserve some plant tissue by freeze drying or by using one of the preservation methods discussed above. Desiccated material (freeze dried or air dried by desiccating agent) is best kept in a freezer at, for example, –20°C, but hermetically sealed packets containing this material and some silica gel could also be attached to the herbarium specimen at room temperature to provide secure long-term storage. Liquid-preserved samples are generally less suitable for long-term storage but can be kept in refrigerators or at room temperature for at least a few months (Thomson 2002). A DNA bank, therefore, has two components, namely a core DNA bank and a tissue bank of DNA-rich material for subsequent extraction (Fig. 1).

DNA extraction

Extracted DNA often needs to be of sufficient purity and quantity to allow use in many applications, such as gene library construction, amplification by polymerase chain reaction (PCR) and digestion with restriction enzymes. Sometimes, specific plastid or mitochondrial DNA is required at high concentrations for subsequent manipulation (Dowling et al. 1996). Plants vary in primary and secondary compounds, and numerous isolation buffers have been proposed that are optimal for different taxa, but there are few published reviews available for these. Some are more generally used than others, but these universally used methods are not necessarily the best for the plant group in question. Problems in obtaining good-quality DNA extractions are often encountered when one is working with plant material rich in polyphenolics/tannins, polysaccharides mucilage/gums and resins (Thomson 2002). The quinone oxidation products of polyphenolics and polysaccharide-like contaminants can be particularly troublesome (Katterman and Shattuck 1983; Couch and Fritz (1990); Rowland and Nguyen 1993; Lichtenstein and Draper 1986; Gilmore et al. 1993).

Various components of DNA isolation buffers may need optimisation, such as their pHs, the concentrations of protective agents, and the type of detergents used. The pH is usually optimised to avoid optima for degradative enzymes (Milligan 1992). Lipolytic enyzmes and lipoxygenases generally have optima between pH 5 and 6, and nuclear DNases tend to have optima around pH 7 (Dunham and Bryant 1983), so most DNA extraction buffers are pH 8–9. Reducing or sulphydryl agents such as 2-mercaptoethanol, cysteine, dithiothreitol (DTT), glutathione or other thiols are

often used to protect against disulphides, peroxidases, polyphenoloxidases and quinones (Herrmann et al. 1980). Polyvinylpyrrolidone (PVP) is also often added, to decrease the effect of polyphenols, quinones and tannins (Porebski et al. 1997; Karakousis and Langridge 2003).

Other components of isolation buffers can also protect DNA from degradation by native enzymes or secondary compounds released upon disruption of the cells. Ethylene-diamine-tetra-acetate (EDTA) inhibits metal-dependent enzymes by chelating divalent cations such as Ca^{2+} or Mg^{2+} (Milligan 1992). However, one EDTA-stimulated DNase is known from wheat (Jones and Boffey 1984; Milligan 1992). Bovine serum albumin (BSA) is often used to promote surface denaturation of degradative enzymes (Price 1983). DNA may co-isolate with contaminants such as polysaccharides that can prevent re-suspension of DNA after precipitation or can prevent enzyme manipulation. Ion exchange chromatography is often used to overcome this problem (Do and Adams 1991).

DNA extraction buffers 'Hot CTAB', 'SDS' and 'miniprep' methods

Commonly used methods to extract total DNA from plants vary in the buffers they use. More specifically, they vary in the detergents used to solubilise the cellular membranes and the means of separating the DNA from proteins and other cellular components. Chloroplast DNA or mitochondrial DNA isolations are sometimes required (reviewed in Milligan 1992; Dowling et al. 1996). Furthermore, clone banks or libraries can be constructed (these are often cloned DNA restriction endonuclease fragments) and stored for future use and distribution (Sugiura et al. 1986).

Hot CTAB, a cationic detergent, is commonly used as an extraction solution, as it can solubilise plant membranes and form complexes with DNA. The 'hot CTAB method' (Murray and Thompson 1980; Doyle and Doyle 1987) is a general-purpose technique (see Appendix 1 for a method adapted from Doyle and Doyle 1987 and frequently used by ourselves, and researchers at the Royal Botanic Gardens Kew, England, for a wide range of plant material). It involves grinding (sometimes with a preliminary liquid nitrogen step) the tissue in hot (60–65°C) CTAB extraction buffer with high salt concentrations, extracting with chloroform/isoamyl alcohol, and then precipitating the DNA with alcohol. This is then followed by ethanol washing, and often further purification (see Appendix 2). Sodium dodecyl sulphate (SDS) is often used as a substitute detergent for CTAB (Edwards 1998).

Many of the general techniques for DNA extraction and purification can be scaled down as 'minipreps'. This may be desirable if only small amounts of DNA are required, if only small quantities of plant material are available for extraction, or if higher extraction throughput is required. For example, the generalised hot CTAB method (Appendix 1) can be scaled down for extractions in microcentrifuge tubes (see also Hillis et al. 1996). Miniprep methods are reviewed by Edwards (1998) and Rueda et al. (1998). An efficient and effective miniprep method was described by Edwards et al. (1991) that involved DNA isolation from a small disk of leaf in a microcentrifuge tube, then mixing with an SDS-based isolation buffer, centrifugation, decanting of supernatant and isopropanol precipitation of its DNA, followed by ethanol rinsing and re-suspension in Tris-EDTA (TE) buffer (see Milligan 1992 for a modification of this method).

DNA banking: quantification and storage

Quantity and purity of DNA can be assessed by UV spectrophotometry or by fluorescence assay (Sambrook et al. 1989; Ausubel et al. 2002). If the sample is relatively pure (without significant amounts of contaminants such as agarose, phenol, proteins and RNA), then the measurement of the amount of ultraviolet light absorbed by the DNA, using a UV spectrophotometer, is an accurate and simple method to quantify DNA (Sambrook et al. 1989; Ausubel et al. 2002). Sometimes, an assay quantifying fluorescence of DNA intercalated with a fluorochrome such as ethidium bromide is preferred (Sambrook et al. 1989; Ausubel et al. 2002).

With absorption spectrophotometry, absorbance is measured with an UV spectrophotometer at 260 nm and usually also at 280 nm. The optical density (OD) reading at 260 nm allows calculation of the concentration of nucleic acid in the sample, as an OD of 1 equates to approximately 50 μg ml^{-1} for double-stranded DNA. An estimate of the purity of the nucleic acid can also be obtained by calculating the ratio of the readings at 260 nm and 280 nm (OD_{260}/OD_{280}). Pure preparations of DNA and RNA have OD_{260}/OD_{280} values of 1.8 and 2.0, respectively (Sambrook et al. 1989).

If the samples are impure or are of low concentrations of DNA (<250 ng ml^{-1}), fluorescence methods are more reliable than absorption spectrophotometry (Sambrook et al. 1989; Ausubel et al. 2002). One of the simplest methods involves the use of ultraviolet-induced fluorescence emitted by ethidium bromide

molecules intercalated into the DNA. Small drops (spots) of DNA sample can be stained with ethidium bromide and visualised under UV light and compared with a series of stained standards (Sambrook et al. 1989). Alternatively, and probably the most frequently used method, is to use agarose electrophoresis mini-gels. In this method a small volume of the sample is run on an agarose gel, stained with ethidium bromide, visualised under UV light and compared with standards loaded on the same gel (the DNA sample may need to be diluted if it contains high concentrations of DNA). The intensity of fluorescence of sample and standards is compared by eye. There are numbers of commercially available gel digital documentation systems that can also automate this process of measuring fluorescence. The agarose gel method can be particularly appropriate if the samples contain significant proportions of RNA. It also allows visual inspection of the physical state of the sample at the same time. An alternative to ethidium bromide fluorescence is to use the DNA-binding fluorochrome Hoechst 33258 (Ausubel 2002).

DNA, once sufficiently pure, can be stored in many ways. It is often stored in TE buffer (10 mM Tris–HCl, 1.0 M EDTA) at –80°C in ultra-cold freezers. Commercially available cryoware tubes and storage racks are useful for storage purposes. TE is a suitable solution for post-extraction analysis and prevents DNA degradation by nucleases and bacterial contamination. DNA may be lyophilised (dried) for storage, but we prefer an aqueous sample that can be worked on immediately following thawing without the need for re-suspension. Cycles of freezing and thawing should be minimised to maintain DNA quality. Samples can also be kept in liquid nitrogen, but this is more demanding on resources than ultra-cold freezers.

Ultra-cold freezers often have an alarm in the event of a failure, and their contents can be easily transferred to another freezer. Back-up freezers should be an integral part of any DNA bank; a back-up power supply is also desirable. Some DNA bankers recommend collections be duplicated in other locations to safeguard their content (Adams and Adams 1992). This idea has been slow to catch on but is advisable; it is routinely used in seed banks, for example. Alternatively, material from the tissue bank can be housed elsewhere, so that it can be re-extracted following a loss (or following exhaustion of the extracted DNA).

Efficient utilisation: databases and distribution

A DNA collection is pointless without utilisation, and different DNA banks have different goals. A DNA bank at the Royal Botanic Garden Kew, England (Table 1), contains over 20,000 samples, and the collection reflects research projects conducted in the molecular laboratory that are mainly of phylogenetic or taxonomic nature. For example, the collection contains approximately 2,000 orchid DNA samples. Another collection at the Missouri Botanical Garden (Table 1) supports taxonomic or phylogenetic studies but does not include large numbers of samples from a single species. Other DNA banks, including the Trinity College Dublin (TCD) DNA bank, also cater for genetic studies within an individual species, so that infraspecific variation can be assessed. IARCs may also house DNA collections for crop genetic resources and focus on a few target species.

DNA bank databases

Databases are critical to the efficient utilisation of DNA banks. The purpose of a DNA bank database is to allow the quick and easy retrieval of a maximum amount of associated information. The system should be made available via the world wide web for easy access. We have developed a web-based system for the TCD DNA bank. The HTML pages, MySQL tables and Perl script developed by us to run the database are freely available upon request. MySQL is a relational database in which the collection of data is organised into tables that are then further divided into columns, representing the different attributes of the data, and rows, that gather the data itself. Instead of interacting directly with the database management system, the user connects to the database by an easy to use web page. Each request is performed using an HTML form that allows both simple and complex searches to be made, depending on the needs of the researcher. For more information concerning the database, its fields and operation, readers can refer to the web page for help (http://www.tcd.ie/botany/dna).

DNA bank databases generally have numerous fields corresponding to different items of information, such as plant family, genus, author of extraction, and type of DNA product or marker generated from the DNA. A DDBJ/EMBL/GenBank DNA sequence accession number may be incorporated where appropriate). Important passport data, such as collection locality, rarity and habitat type, can also be accommodated.

DNA databases would have greater applicability for biodiversity evaluation and utilisation if they were made compatible with other major databases. For example, DNA banks specialising in collections for taxonomy and systematics may look for compatibility

with the ITF for botanic gardens and databases of other initiatives, such as the Global Biodiversity Information Facility (http://www.gbif.net), Species 2000 (http://www.species2000.org), and regional databases, such as the Inter-American Biodiversity Network (http://www.iabin.org) and the National Biodiversity Network (http://www.ukbiodiversity.net). A major challenge for DNA banks will be to integrate with DDBJ/EMBL/GenBank and other bioinformatic databases.

DNA exchange, access, material transfer agreements and benefit sharing

A number of international conventions, agreements and guidelines govern the use of biodiversity, genetic resources and related issues of biotechnology and intellectual property rights (IPRs) (Dutfield 2000; System-wide genetic resources programme [SGRP]) 2003). It is a highly complex system, but biodiversity collections, including DNA banks, should consider the importance of protecting and conserving biodiversity/plant genetic resources for future generations and should particularly adhere to the Convention on Biological Diversity and the International Undertaking on Plant Genetic Resources adopted by the Food and Agriculture Organization of the United Nations (FAO). For crop genetic resources, collections should consider the Commission on Genetic Resources for Food and Agriculture (CGRFA) (2004). It includes 35 crops and crop complexes and 29 forage species in a multilateral system for access and benefit sharing.

In practice, a material transfer agreement (MTA) is often required before plant DNA (material) is exchanged. In this, the recipient generally accepts that the material is held in trust under the terms of this agreement and that the recipient has no rights to obtain IPRs on the material or related information (SGRP 2003). The recipient may utilise and conserve the material for breeding, research and training but cannot claim ownership over the material, or exchange it with others, without a legally binding written agreement. In the MTA, the DNA bank that exchanges the DNA does not take any warranties regarding the quality or purity of the material sent. The DNA bank agrees to exchange associated data (e.g. passport data), and the recipient agrees to furnish the DNA donor bank with related data and information collected during evaluation and utilisation. A small handling fee is sometimes levied. At present, few DNA banks can verify purity of samples, but we anticipate that procedures will be developed to provide the recipient with documentation of sample quality. A mechanism for exchange of living plant material among botanic gardens and other institutions, the International Plant Exchange Network (IPEN; http://www.bcgi.org.uk/abs/ipen), has recently been developed. It provides a register of cultivated plant material that can be traced back to original MTAs relevant to source collection, it and greatly facilitates exchanges among botanic gardens. Development of a similar protocol among DNA banks would be beneficial.

Once the terms of the MTA have been accepted, the DNA samples are exchanged. There are a number of difficulties with the physical exchange of DNA. Firstly, the DNA needs to be kept stable, so that it remains sufficiently intact for use. Secondly, the material has to be dispensed efficiently from the DNA bank without exhausting its supply and by minimising the handling stages. Usually, a small sample (in terms of the proportion of remaining DNA in the storage collection of the DNA bank but sufficient for the recipient) is dispensed in solution (or lyophilised) and kept cold in cool bags or dry ice during transit (often by courier or express mail). If material is of sufficient purity, it can sometimes be exchanged via the post without cooling. Material from clone banks or libraries can also be exchanged (Sugiura et al. 1986). Associated data are also exchanged, either in printed or electronic format.

A new approach to the distribution and storage of genetic resources was outlined by Hayashizaki and Kawai (2004). They recognise the difficulty in exchanging samples of DNA and describe ways in which DNA may be more efficiently exchanged in the future. Among recent innovative technology for exchange are ‘DNA books’ and ‘DNA printing technology’ (Hayashizaki and Kawai 2004). The first application of this technology is the RIKEN Mouse Genome Encyclopaedia DNA Book (The RIKEN genome exploration research group phase II team and the FANTOM consortium 2001), a compendium, in the form of a book, of mouse cDNA clones. The construction of genomic libraries of DNA accessions may be a possibility worth considering for DNA banks to help with exchange, especially of high demand/value material, but it is not currently a viable option for many DNA banks operating in the research fields of biodiversity and plant genetic resource evaluation.

Conclusions

The importance of DNA collections to biodiversity and plant genetic resource evaluation and utilisation cannot be overstated, but these DNA banks should be integrated with other facilities, such as herbaria,

botanic gardens, genebanks, seed banks and bioinformatic resources, to fully maximise their utility. They should be compatible with other databases from biological collections if they wish to be globally available. They may accommodate a tissue bank of DNA-rich material for subsequent extraction, and they should have policies and procedures in place for material transfer if they plan to share resources [under the guidelines of the Convention on Biodiversity (CBD) and the Undertaking on Plant Genetic Resources]. We have provided a review of DNA banking practice that we hope will be of use for others wishing to set up or manage such a facility. Our database system and protocol for DNA extraction (Appendix 1) are available on request.

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Appendix 1

Protocol for DNA extraction using hot CTAB

The following protocol,(adapted from Doyle and Doyle 1987), will work well for a wide range of plant material and is particularly well suited to low sample throughput (typically 8–32 samples per day). If a large number of samples is to be extracted, then several improved grinding methods have been developed to speed up extractions. For example, Karakousis and Langridge (2003) used liquid nitrogen and ball bearings in a high throughput plant DNA extraction process. Other methods have used glass beads in microtitre plates (Steiner et al. 1995). Several commercially available kits allow partial or full automation and higher throughput. For example, McGrath et al. (2006) successfully developed a high throughput extraction protocol that used plant material ground with a mill (Retsch bead mill) or pestle and mortar and the magnetic bead-based method (Qiagen MagAttract Plant DNA core kit) that had been modified for use on a Hamilton MicrolabStar robotic system.

Materials

2× CTAB buffer, (100 mM Tris–HCl pH 8.0 (use Tris base and set pH using HCl); 1.4 M NaCl; 20 mM EDTA (Na ethylene-diamine-tetra-acetate); 2% CTAB (hexadecyl-trimethyl-ammonium bromide, w/v)
CI, 24:1 chloroform:isoamyl alcohol

Method

Caution: gloves should be worn at all times.

1. Preheat 5 ml of 2× CTAB extraction buffer and 20 µl mercapto-ethanol (in a 12 ml chloroform-resistant capped centrifuge tube) and a pestle and mortar at 65°C in a water bath.
2. Weigh out approximately 0.3–0.5 g of fresh leaf or 0.05–0.1 g dry leaf (or other plant tissue).
3. Cut leaf into small pieces using scissors or razor blade.
4. Grind leaf material in the pre-heated mortar using a small amount of extraction buffer (sterile sand may be added to aid grinding or liquid nitrogen used prior to grinding).
5. Add the remaining buffer, grind further, and swirl to suspend the slurry.
6. Pour the slurry back into the 12 ml centrifuge tube. Screw on the lid and then incubate the mixture at 65°C for at least 10 min with occasional mixing.
7. Add 5 ml of CI. Replace the lid and mix. Release the lid briefly to release gas and then tighten the lid.
8. Place on the shaker in a horizontal position for approximately 30 min.
9. Centrifuge the tube at 4,000 relative centrifugal force (rcf) for 10 min.
10. Gently remove the tube from the centrifuge, taking care not to disturb the separation. Remove the aqueous (upper) phase containing the DNA, using a transfer pipette, into a 50 ml conical-base tube. Ideally, the upper phase will be clear and colourless, but this is often not the case and does not interfere with the latter stages of the protocol.
11. Add an equal volume of isopropanol and invert the tube gently to precipitate the DNA. You may see the DNA precipitate at this stage.
12. Place the sample into the –20°C freezer to further precipitate the DNA (sometimes it is necessary to leave the sample overnight or longer).
13. Centrifuge the sample at 2,000 rcf for 5 min to pelletise the DNA.
14. Pour off the supernatant and add 1.5 ml of the wash buffer. Mix gently.
15. Centrifuge the sample at 2,000 rcf for 3 min to pelletise the DNA once more.
16. Pour off the supernatant and then gently place the tube upside down for 5 min on a paper towel to let the excess wash buffer drain away.
17. Turn the tube the right way up and let the pellet dry further for about 20 min (it is important to remove all traces of ethanol).

18. Dissolve the pellet in 0.5 ml of TE buffer (considerable mixing with a transfer pipette may be necessary to dissolve the pellet).
19. Transfer the DNA solution from the 50 ml tube into the labelled 1.5 ml centrifuge tube and store DNA in a freezer until required (preferably at –80°C).

The DNA is often of sufficient purity for many applications, but samples can be purified further if necessary using caesium chloride gradient centrifugation, genomic DNA purification kits, or selective binding of DNA to a silica matrix in the presence of a chaotrope (see Appendix 2).

Appendix 2

DNA purification

Crude purification can often be achieved by precipitation/washing (e.g. the ethanol washing steps of Appendix 1) and may yield DNA of sufficient purity for the required application. However, such DNA may need to be further purified. One of the best methods to purify DNA from previous extractions, or crude homogenates, is to use equilibrium gradient centrifugation using caesium chloride (Sambrook et al. 1989; Dowling et al. 1996; Ausubel et al. 2002). However, this procedure requires long ultracentrifugation and large amounts of toxic ethidium bromide. However, many other laboratories prefer faster, cheaper and less toxic methods. These sometimes compromise purity or stability but are generally adequate for most applications.

A standard method to remove protein from DNA extracts is to extract first with phenol:chloroform and then with chloroform (to remove any traces of phenol; Sambrook et al. 1989) or proteolytic enzymes such as pronase or proteinase K before extracting with organic solvents (Sambrook et al. 1989). Powell and Gannon (2002) describe a method where DNA extracts are purified with phenol extraction and ethanol precipitation. Dialysis can also be applied to remove salt, detergents (such as SDS and CTAB) and even some enzyme inhibitors (see Sambrook et al. 1989 and Ausubel et al. 2002 for dialysis procedures; or Powell and Gannon 2002 for a simple drop dialysis method). Another method of removing cellular proteins and polysaccharides is to precipitate them prior to precipitating the DNA. Some of these methods have the advantage of not requiring organic solvent extractions. These methods are often used in rapid miniprep methods (Milligan 1992; Edwards 1998).

Other methods of DNA purification have been developed that are based on selective binding of DNA to a silica matrix in the presence of a chaotrope (Vogelstein and Gillespie 1979; Marko et al. 1982; Gilmore et al. 1993). In these methods DNA is selectively bound to a silica matrix in a solution of chaotrope, washed with chaotrope to remove unbound contaminants, including RNA, carbohydrates and proteins, washed with ethanol to remove the chaotrope, and then eluted from the matrix. Modifications of these methods are used in various commonly used commercial genomic DNA purification kits. Even PCR purification spin columns can be used for genomic DNA purification if the investigator is not concerned about losing some high molecular weight DNA. The method of Gilmore et al. (1993) is an efficient purification technique that involves the selective binding of DNA to diatomite (diatomaceous earth) in the presence of a chaotrope.

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