

DNA Bank-Net: an international network of DNA banks and *in vitro* storage institutions

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ABSTRACT

The recent advances in technology for the extraction and immobilization of DNA, coupled with the prospect of the loss of significant plant genetic resources throughout the world, has led to the establishment of DNA Bank-Net (Royal Botanic Gardens, Kew, London, April, 1991). DNA Bank-Net is an association of institutions focused on preserving genomic DNA as well as *in vitro* cryopreservation of plants from tropical endangered and/or economic plants. There are currently over forty institutions, representing 25 nations and every continent, that have expressed interest in DNA Bank-Net. The initial users are likely to be molecular systematists. As the world's horizontal gene pool becomes better characterized, plant breeders will use genes from the banks for transgenic plant production.

RATIONALE FOR THE ESTABLISHMENT OF DNA BANK-NET

Genetic transfers are now being performed in order to attain insect, bacteria, viral and fungal resistance, more nutritionally balanced protein, more efficient photosynthesis, nitrogen fixation, and salt and heavy metal tolerance, to name a few. These kinds of gene transfers from one unrelated organism to another indicate that we must now view the world's genetic resources (genes, DNA) from a horizontal perspective in which gene transfers will cut across species, genera and family boundaries. The world's biota should now be considered a horizontal gene pool.

Previously, we have utilized only vertical gene pools (i.e., breeding with ancestral or derived taxa that are closely related in order to make fertile or semi-fertile crosses and then backcrossing until the gene of interest has been incorporated into the desired cultivar). This vertical gene pool of very closely related taxa severely limits the kinds of genes available for species improvement. The development of pharmaceutical farming, bioreactors and even insect resistance in our field crops will utilize genes from distantly related taxa (i.e., the horizontal gene pool).

However, concurrent with the development of these biotechnologies, we are faced with the most severe loss of genes from the world gene pool since the extinction of the dinosaurs. The kinds of novel insecticides, biocides, medicines, etc., that may exist in nature are scarcely known. Yet, the principal areas of diversity among plants, the lowland tropical forests, will have been cut or severely damaged within the next 20 years (Raven, 1988). The Amazon River system, for example,

contains eight times as many species as the Mississippi River system (Shulman, 1986). Raven (1988) estimated that as many as 1.2 million species would become extinct in the next twenty years. The loss of plant species will mean a loss of potential plant derived pharmaceuticals which are now estimated at \$2 billion/year in the United States alone (US Congress, 1987).

STRUCTURE AND OPERATION OF DNA BANK-NET

DNA Bank-Net is a cooperative network of over forty institutions, representing 25 nations and every continent, that are developing DNA banks or have expressed interest in DNA Bank-Net (Figure 1). At the organizational meeting of DNA Bank-Net (Kew, London, 1991), a task force recommended two kinds of network nodes with the following functions (Adams and Adams, 1992):

Working (DNA dispensing) nodes:

- a. Collection of plant material by taxonomists. This may be the primary function of a particular node or be in association with other organizations such as universities, botanic gardens, etc.
- b. DNA extraction by molecular biologists or trained staff.
- c. Long-term preservation of DNA-rich materials and/or extracted DNA in liquid nitrogen.
- d. DNA analysis/gene replication by molecular biologists or trained staff.
- e. Distribution of DNA (genes, gene segments, oligonucleotides, etc.) by PCR.

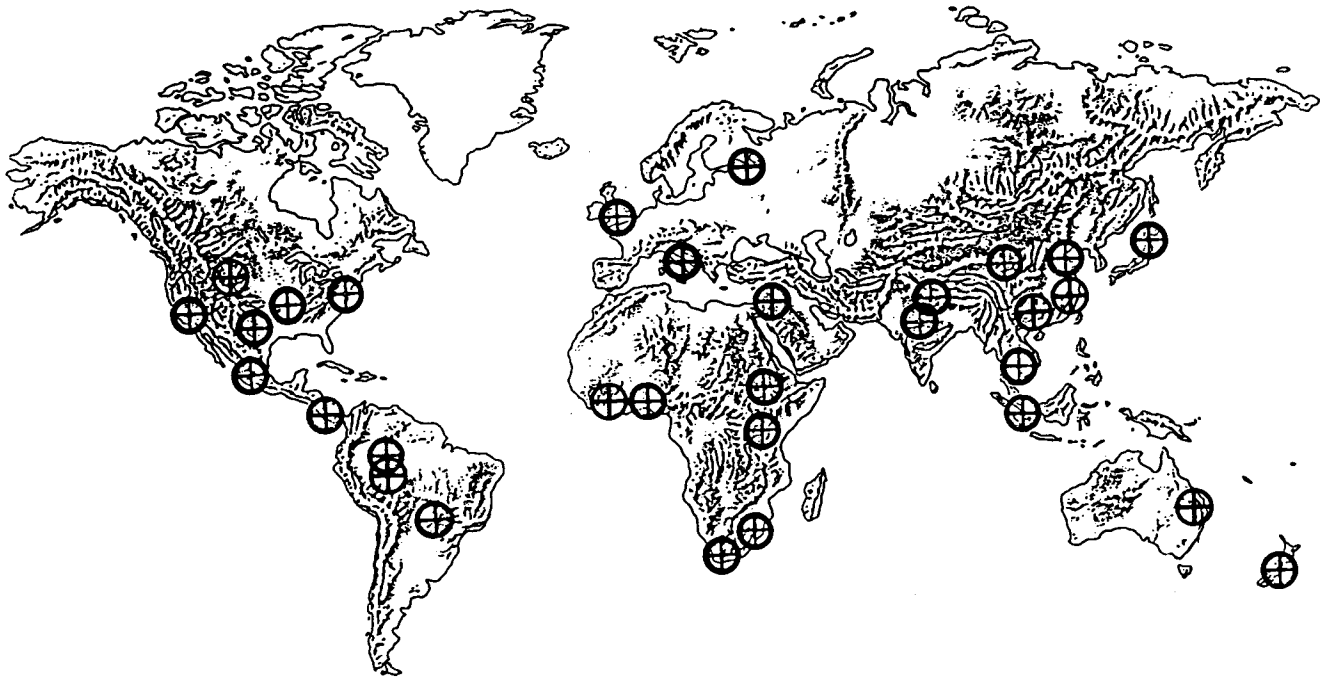


Figure 1. Map of individuals/institutions currently interested in DNA Bank-Net.

Reserve (base) nodes:

- Long-term DNA preservation in liquid nitrogen and monitoring of potential DNA degradation.
- Act as genetic reserve buffer for working nodes.
- Replenishment of DNA if a working node experiences the catastrophic loss of storage parameters and DNA.

Figure 2 depicts the relationship between working and reserve nodes. Note the projected flow of plant materials and DNA through the working (DNA dispensing) node. It is likely that some of the working nodes would be actively acquiring and/or dispensing DNA from some

geographic area (e.g., Africa), yet maintain separate cryovats, functioning as a reserve (base) node for another area (e.g., South America).

From recent experience in China, it also appears that a third kind of node will be defined that will function for plant material acquisition and storage of desiccated materials in liquid nitrogen, without having significant expertise in molecular biology on site. These nodes may be called Regional Working Nodes. They will fill a gap between the centralized molecular laboratories and the strictly reserve nodes. The regional working nodes may in fact be the primary groups that intensively collect floristic elements in a geographic region. For example, Northwest Normal University in Lanzhou already has responsibility for training teachers for northwestern China. It is probably that they will be given the responsibility to collect materials from endangered plants of that region. DNA rich materials would likely be cryopreserved in Lanzhou and replicates sent to Beijing for further disbursement, utilization and storage.

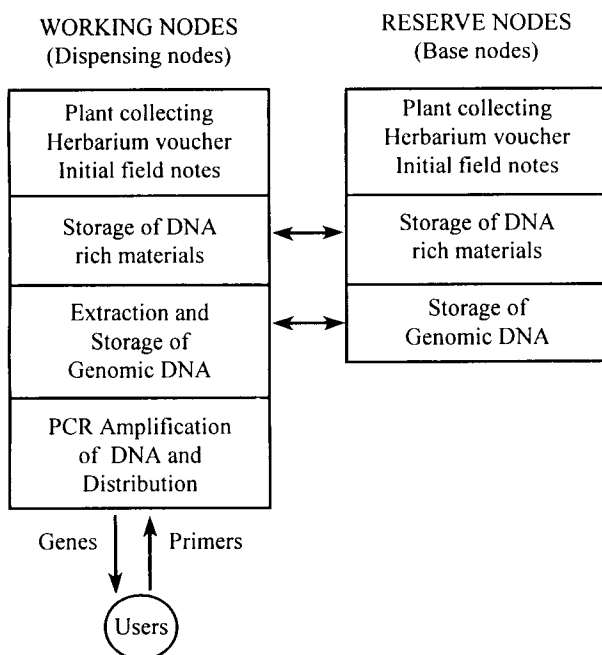


Figure 2. Schematic representation of the flow of materials and the relationship between working (DNA dispensing), reserve (base) nodes and users.

GENERAL REQUIREMENTS FOR NODES IN THE DNA BANK-NET

Several general recommendations came from the task groups (Adams and Adams, 1992):

a. DNA should be extracted from cryo-preserved DNA-rich materials only when the DNA is needed. Delaying the extraction has the advantage of letting technology catch up, so advanced techniques can be used as they become available.

b. Working nodes should generally be an existing organization with adequate biochemical expertise and have an associated herbarium. Having an herbarium on site would not be required but a very close, local (in the city) association with a recognized herbarium is required.

c. For the working as well as reserve nodes, it is necessary to have a strong institutional commitment, not just a personal commitment, in order that the collection be maintained in perpetuity not just for the lifetime of one person who has committed himself to the idea.

d. Consideration should be made concerning the availability of dependable electricity and liquid nitrogen in determining the feasibility of establishing a node.

e. Considerable interest was shown in the concept of storing composite DNA samples (e.g., a composite of DNA from all the legumes in a region) to be used for screening or retrieval of unusual genes.

f. Each DNA collection should be split initially into at least 2 or 3 portions. One sample (DNA-rich material or extracted DNA) should be stored at a working (DNA dispensing) node and another portion(s) be stored in at least one, but desirably two back-up reserve (base) nodes. The reserve nodes should be in different countries and if possible on different continents to safeguard the DNA samples against various natural and man-made catastrophes.

PLANT SPECIMEN COLLECTORS: AN UNDERUTILIZED RESOURCE

Collections of plant specimens have been utilized for the formulation of our understanding of morphological variation among taxa. Indeed, without the great herbaria and botanic gardens of the world, our knowledge of plant evolution would be fragmented at the very least. During the 1960s, taxonomists began utilizing chemical data for systematic and evolutionary studies, but methods of preserving plant materials for future (chemical) work were largely ignored. Taxonomists were usually content to file a voucher specimen to document the chemical studies. With the previous level of support for plant collections, very few of the world's plant species have been preserved by freezing in order that scientists might have access to the study of secondary compounds, enzymes, or DNA/RNA in the coming centuries.

The cheapest and most practical way to preserve the largest percentage of plant genes would be to utilize the current (and additional) floristic collectors (such as those of the Missouri Botanical Garden, Royal Botanic Gardens, etc.), who are already in the field and familiar with the vegetation in the region. The collections of DNA-rich material (leaves) can be done with little additional effort when specimens are collected.

The plant specimen collectors are professional botanists who are constantly at work throughout the world, collecting, pressing and identifying plants daily for shipment to the major herbaria of the world. With just a few additional steps, these botanists can also field preserve materials for DNA use. The conserved DNA will have numerous uses: molecular phylogenetics and systematics of extant and extinct taxa; production of previously characterized secondary compounds in transgenic cell cultures; production of transgenic plants using genes from gene families; *in vitro* expression and study of enzyme structure and function; and genomic probes for research laboratories.

In addition to random (or pseudo-random) collections by field taxonomists, priority will be given to indigenous plant species that are tended and/or otherwise used by local people. These species will not include the major crop plants of commercial usage that are widely cultivated (e.g., maize, rice, wheat, etc.).

When possible, fossil materials will be included in DNA Bank-Net. There are now several reports of the extraction and utilization of DNA from plant fossils (Giannasi, 1992). With improved DNA extraction and amplification methods, fossil, museum and herbarium materials are likely to yield useable DNA and these materials need to be included in DNA banks to allow access to genes by investigators.

INTERIM FIELD PRESERVATION OF SPECIMENS

Botanists conducting floristic research will collect many of the specimens from tropical rare and endangered species. They often collect specimens from scores of different species in a single day. The generalist collector, working in tropical areas cannot be expected to preserve hundreds or thousands of collections for months under tropical conditions and then arrange transport through customs, all the while keeping the individual specimens frozen.

Fortunately, at least as far as DNA preservation is concerned, interim preservation in silica gel or drierite is an effective way to keep plant materials in the field and/or in transit for several months at ambient temperatures (Liston *et al.*, 1990; Adams *et al.*, 1992).

FUTURE UTILIZATION OF DNA BANK-NET

The primary method for gene retrieval (at present) appears to be by PCR technology. It is expected that primers would be sent to DNA banks and the desired gene obtained by DNA amplification. Research is in progress on immobilization of the original genomic DNA, such that only copies would be disbursed. In a world of vanishing genes, DNA Bank-Net will function as a 'genetic insurance policy' to save genes from our most threatened species.

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