# Seed and Molecular Resources for Arabidopsis

**Randy L. Scholl*, Sean T. May, and Doreen H. Ware**

Department of Plant Biology, Ohio State University, 1735 Neil Avenue, Columbus, Ohio 43210 (R.L.S., D.H.W.); and School of Biosciences, Plant Science Division, Life and Environmental Sciences Building University Park, Nottingham NG7 2RD Nottingham, United Kingdom (S.T.M.)

Arabidopsis, because of its diminutive size, small genome, and rapid life cycle, has become the premiere research organism in plant biology. The power of Arabidopsis as a model system will become even greater now that all five of its chromosomes have been sequenced.

Before 1990, extensive collections of Arabidopsis mutants were maintained by individual scientists, most notably Drs. A. R. Kranz, G. P. Rèdei, and Maarten Koornneef. It is fortunate that it was recognized early on that the establishment of seed stock centers would greatly expedite the dissemination of Arabidopsis research material to the international research community, and thus three major facilities were established. These include the National Science Foundation-supported Arabidopsis Biological Resource Center (ABRC) in the United States, the Biotechnology and Biological Sciences Research Council-supported Nottingham Arabidopsis Stock Centre (NASC) in the UK, and Sendai Arabidopsis Seed Stock Center (SASSC) in Japan.

The role of a seed stock center is to collect, propagate, preserve, and distribute seed lines which are of use to researchers. Mutant lines are the primary core of a seed collection. Arabidopsis researchers are fortunate to have access, through the stock centers, to approximately 1,000 mutants affecting several hundred separate gene loci. As we enter the post-genomic era this number will be augmented greatly. The mutant lines currently available possess a wide range of interesting characteristics useful to modern biological research. The genes affected by many of these mutations have been cloned and characterized, and there is potential benefit to be derived from the use of appropriate mutants in all areas of plant research.

To date, over 4,000 scientists of diverse backgrounds have utilized the stock centers. The numbers of stocks distributed from and users accessing the stock centers have increased steadily since their inception. ABRC currently sends out 50,000 seed samples and 20,000 DNA stocks annually, and NASC sends 25,000 stocks per year. Distribution statistics for the previous five years are shown in Figure 1. Among seed stocks 5,000+ samples of mutant lines are sent annually and 25,000 samples of T-DNA lines are distributed by ABRC.

* Corresponding author; e-mail scholl.1@osu.edu; fax 614–292–0603.

## SEED STOCKS

Diverse resources are available to Arabidopsis researchers from public and private sources. Various molecular resources are also provided: ABRC maintains and distributes a comprehensive collection of DNA stocks and NASC distributes plant DNA for reverse genetic applications.

Various single and group stocks, which aid in genetic analyses associated with basic investigations and gene cloning, have been developed. Mapping resources include mapped mutants, multiple marker stocks, four populations of recombinant inbred lines, trisomic lines, and a population organized by tetrads. Transposon and T-DNA populations with random insertions throughout the genome have been produced which enable a line to be identified by phenotype and utilized to identify and characterize the associated insertion point. Sets of randomly generated T-DNA lines have been donated so that the current holdings of T-DNA stocks exceed 100,000. Large numbers of characterized lines, having transpositions to random locations, are available from the stock centers. A population with transposed Ds elements is maintained by Dr. R. Martienssen, Cold Spring Harbor Laboratory (Cold Spring Harbor, NY) from which lines of interest can be obtained. In addition to the above, lines transformed with specific transgenes and molecular tags, transposon parental stocks for generation of new transpositions by researchers, and several related species are carried by ABRC and NASC. Populations of seeds mutagenized by chemicals or radiation and bulk quantities of several ecotypes can be purchased from Lehle Seeds (Round Rock, TX).

Natural variants ("ecotypes") collected in the wild from around the world have proven to be useful tools for genetic analyses of Arabidopsis. The availability of these from ABRC, NASC, and SASSC has enabled researchers to characterize variation associated with diverse environmental factors and to clone novel genes.

## MOLECULAR RESOURCES

### DNA Stock Centers

The DNA resources available from ABRC are distributed in different forms including single characterized plasmids, expressed sequence tags (ESTs),

libraries, and isolated plant DNA. The characterized clones include known genes, expression marker constructions, and binary vectors used for plant transformation. A large collection of EST cDNAs derived from several different EST projects is maintained. Analysis of the clones suggest that the ESTs currently represent approximately one-third of the genes of Arabidopsis, and the representation is expected to increase in the near future as new collections are added. A minimized EST collection is available as a set on microtiter plates or as colony blots of clones onto nylon hybridization filters. ESTs from similar collections are also available from Incyte Corporation (Palo Alto, CA).

Various cDNA and genomic libraries can be obtained from ABRC. The cDNA libraries have been isolated from different sources including whole plants, seedlings, and flowers. Some libraries have been size-selected to facilitate isolation of larger clones. Depending on the vector, these libraries may be used for DNA hybridizations, antibody screening for expressed peptides, and protein-protein interactions in the case of two hybrid libraries. Genomic libraries available from the ABRC include phage, cosmid, yeast artificial chromosomes, bacterial artificial chromosomes (BACs), and plant-transformable BACs (transformable BACs and binary BACs). Available BACs include those utilized by the Arabidopsis Genome Initiative public genome sequencing projects. The BACs are useful for positional cloning. The BAC libraries are spotted onto nylon so that the filters can be ordered, hybridized to a probe of interest, and the positive clone(s) obtained from the Center. The individual transformable BACs can be used for complementation testing. The available BACs and ESTs, in conjunction with the mapping resources of the stock centers, provide a powerful set of resources for positional gene cloning, complementation, expression, and protein characterization.

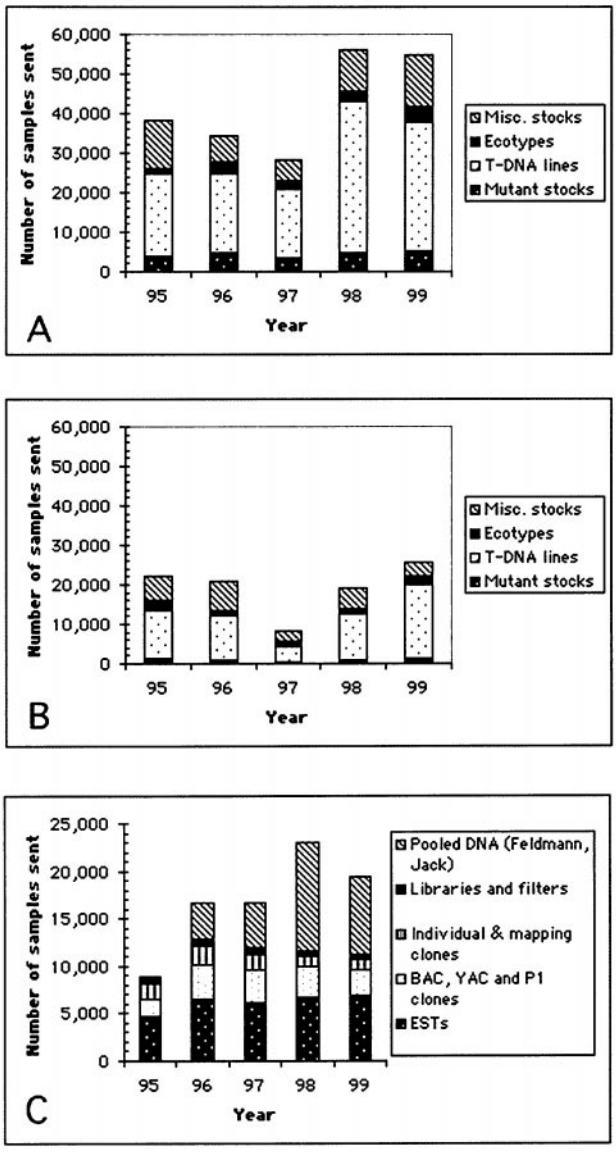


**Figure 1.** Number of stocks sent annually, by stock type, for ABRC seed stocks (A), NASC seed stocks (B), and ABRC DNA stocks (C).

In conjunction with the seeds available for forward genetics, DNA pools are offered by the stock centers for reverse genetics and can be assayed in the user's laboratory for insertion in their specific gene(s). These DNA samples complement the "knockout" services of the Arabidopsis Knockout Facility (AKF), which conducts PCR screens using primers provided by participating laboratories. DNA isolated from 12,000 T-DNA lines is currently available from ABRC and will be expanded to a total of 40,000+. NASC has DNA for 20,000 transposon lines, which will be expanded to 40,000+, including some lines identified by sequences flanking the insertions.

### Service Centers

NASC is currently developing a microarray hybridization service similar to the type offered by the Arabidopsis Functional Genomics Consortium (AFGC). This service will have similar organizational details and is described in the accompanying article on microarray analysis. ESTs used in the generation of AFGC microarrays are available from ABRC.

The AKF, which is part of AFGC, conducts PCR screens using primers provided by participating laboratories. This, in conjunction with the DNA samples distributed by the stock centers, provides substantial populations for reverse genetics. The follow-up seeds for the AKF service are distributed from ABRC.

An antibody service available from Rose Biotechnology (Winchendon, MA), a private company, is currently being established. This is in collaboration with Secant Chemicals (Winchendon, MA) and Rose Scientific (Winchendon, MA). A few antibodies are currently available, including ones raised against vesicular proteins, essential components of the secre-

tion pathway in plants. An additional 15 antibodies will shortly be made available as markers for a range of cellular processes and organelle-specific antibodies. An Antibody Distribution Center is planned that if supported by donations from the community will provide an infrastructure for redistribution of antibodies developed in academic laboratories.

## ORDERING OF STOCKS AND BIOINFORMATICS

The bioinformatics components of the stock centers are focused on providing detailed stock information and ordering facilities. The ABRC site, AIMS (http://aims.cse.msu.edu/aims/), is maintained by Dr. Sakti Pramanik and associates of the Computer Science and Engineering Department at Michigan State University. AIMS is a relational database which is utilized for processing stock orders, checking progress of orders, and examining the order histories of individual stocks. The NASC web site (http://Arabidopsis.org.uk/) has a similar ordering facility, stock information, and an insert sequence blast facility. In addition, a public mapping service derived from the Lister and Dean RI lines resides at the NASC site as part of a synteny-driven database of genetic information for Arabidopsis and crop plants—UK Cropnet (http://ukcrop.net/).

All stocks of ABRC and NASC can be ordered through the web sites (Table I). The ordering pages can be found as links to the home pages and used to enter patron information and request stocks. AIMS requires a login ID and password which can be obtained by request through the web site. Users may track the progress of their orders in AIMS. For NASC, an order form linked to the home page can be filled out and is automatically entered in the database.

In addition to placement of orders, scientists can use the stock center databases to locate stocks, obtain detailed stock information, and communicate with center staff. In AIMS there are separate search pages for seed and DNA stocks. The simplest search mechanism is the "keyword" search. This window, linked to the main seed and DNA search pages, queries all stock categories relative to any single-word term. Thus "apetala" could be used to find all stocks of this gene class, or "apetala1" could be used to locate all stocks relevant to the *ap1* locus. A phenotypic term such as "glabrous" could be entered, as could the name of a potential stock donor such as "Sundaresan." Once a list of stocks is found, any combination of items can be selected for ordering. The search window for stocks in the NASC database operates in much the same way. The NASC server incorporates a "shopping cart" mechanism for collecting found stocks for ordering purposes. Another search mechanism of the AIMS and NASC databases is the "text-based" search. This search page allows more advanced searches to be executed. A simple example would be the entry of "apetala" in the "gene name" window—this query identifies stocks that carry this gene name and excludes cases where the term may be mentioned in the general description of the stock. Entry of terms in each of two windows of the page for a search utilizes these jointly, connected by a logical "and" in AIMS and a logical "or" in NASC.

## FEES ASSOCIATED WITH STOCKS

The ABRC and NASC provide stocks to Arabidopsis users for fees that are less than the production/distribution costs. Prior to 1997 the stock centers' only outside source of support were the granting agencies, but in recent years partial recovery of operating costs has been achieved through a user fee system. Two types of fees are levied: annual laboratory user fees, and individual fees for aggregate stocks. The annual academic usage fee is $250 (ABRC) or £168 (NASC). Bulk seed and DNA stocks such as sets of T-DNA lines, recombinant inbred populations, and libraries have associated individual fees, which are levied in addition to the annual laboratory user fees. Details of other usage levels and individual stocks are described on the web sites (Table I).

## FUTURE PLANS

As plant research moves to a "post-genomic" era, the stock centers face the challenge of adapting to the accelerating pace of science, including the incorporation of increasing numbers of novel resources. In some cases choices may have to be made and re-

**Table I.** *Web sites of Arabidopsis stock centers, companies, and laboratories distributing Arabidopsis resources*

| Resource | Websites |
|---|---|
| ABRC | http://aims.cse.msu.edu/aims/ |
| NASC | http://arabidopsis.org.uk/ |
| SASSC | http://shigen.lab.nig.ac.jp/arabidopsis/ |
| Rose Biotechnology, Inc. Secant Chemicals, Inc. (distributor) | http://www.rosebiotech.com |
| Dr. Robert Martienssen | http://clio1.cshl.org:80/gradschool/martien_.html |
| Lehle Seeds | http://arabidopsis.com |
| Incyte Genomics, Inc. | http://www.incyte.com/reagents/index.shtml |

sources organized to maximize their usefulness and allow the greatest number of items to be distributed. However, over time, an organized set of loss-of-function mutants corresponding to each Arabidopsis gene will effectively replace the random insertion populations. Novel mutational approaches are also being applied and the resulting strains and/or populations will be sought. New collections of natural variants, related species, and mapping populations will also be added. For the DNA collection it is expected that a complete set of ESTs will be received, with most if not all of these being in the form of full-length clones. The collection of BACs will be expanded to encompass greater numbers of transformable BAC clones. The stock centers will continue to improve the information resources associated with the stocks, specifically by achieving higher degrees of integration with The Arabidopsis Information Resource and other public databases. Information regarding donation and ordering of stocks can be obtained from the web sites listed in Table I. Donations of seed, clone, and antibody stocks are welcomed from all researchers.

Received September 12, 2000; accepted September 24, 2000.