DISTRIBUTION AND USES OF LEGUME DNA CLONE RESOURCES

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Abstract

Since 1990, my lab has developed and distributed various DNA clone resources for the legumes. In the first several years, the focus was on members of the tropical genus, *Vigna*, including the widely cultivated species, mungbean (*V. radiata*) and cowpea (*V. unguiculata*). Both of these grain legumes play key roles in agriculture in developing countries of Asia (mungbean) and Africa (cowpea). Moreover, because there is substantial genome conservation among legumes [1], these genetic resources have also been utilized by a wide range of researchers in other crop species. In 1997, my lab began to focus on the development and distribution of a new generation of DNA clone resources; Bacterial Artificial Chromosomes (BAC). A library of these clones was constructed in soybean (*Glycine max*) the most important legume species worldwide in terms of economic value. Again, the library has become a valuable resource for the legume research community and has been widely used in studies of legume genomics.

1. INTRODUCTION

1.1. Genome studies in *Vigna*

The first DNA marker maps for mungbean and cowpea were published in 1993 [2, 3]. The mungbean map consisted of 171 restriction fragment length polymorphism (RFLP) markers, while the cowpea map consisted of 83 RFLPs. Since that time, the number of RFLPs placed on the mungbean has increased to more 260. Many of these markers, especially those derived from mungbean or cowpea, were developed in my lab at the University of Minnesota. The RFLP clones found on these maps and developed in my lab are available to researchers worldwide who are interested in *Vigna* or other legume crops.

1.2.Genome studies in soybean based on the BAC library

BAC libraries have several applications in the growing field of genomics. Because they have very large inserts, BACs can be used for chromosome walking and positional cloning. This is one of the most powerful methods for isolating genes known only by map position today. BAC libraries form the basis of physical mapping. In the next decade, complete physical maps for many important crop species, including soybean and perhaps other legumes, will probably be completed using BAC libraries. Finally, BAC libraries are also well suited for comparative genomics, especially for questions of microsynteny. In these studies, the question is one of comparing genomes of different organisms at the micro-level. There is a growing body of evidence that gene order is often maintained, even at the kilobase level of resolution.

2. MATERIALS AND METHODS

2.1. Construction of a *Vigna* RFLP library

A DNA library suitable for RFLP analysis was constructed from mungbean and cowpea genomic DNA. To prepare these libraries, DNA was digested with *PstI* (a methylation-sensitive enzyme) and separated according to size by sucrose gradient centrifugation. The fraction between 500 and 3000 base pairs was collected and ligated into pUC19 by standard methods [4].

2.2. Construction of a soybean BAC library

Ten day-old soybean plants, cultivar Faribault, were used for preparation of high molecular weight DNA in agarose plugs. These DNA samples were partially digested with *EcoR*I and separated on a 1% low melting point agarose gel using a clamped homogeneous electric field device. The isolated DNA was ligated into vector, pECSBAC4 (Dr. Richard Michelmore, University of California, U.S.A.) and transformed into *E. coli* by electroporation. White recombinant BAC clones were picked manually and transferred to 384-well plates. For long-term storage, the library was replicated three times. A total of 78 000 BAC colonies were isolated with an average insert size of 120 kilobase pairs [5].

3. RESULTS AND DISCUSSION

3.1. Clone distribution

3.1.1. Vigna Clone Distribution

Since 1992, when the first set of *Vigna* clones were distributed, we have responded to a total of 35 requests for biological materials. Approximately 2000 clones have been sent out to fill these requests, which came from researchers in eleven different countries including, United States, Republic of China, Australia, Nigeria, Germany, India, Italy, United Kingdom, Japan, Korea, and Israel. Twenty-two of the requests were filled before 1995, while 13 have been filled since.

3.1.2. BAC Library distribution

In 1998, the entire BAC library for soybean has been on deposit at the Clemson Genomics Center under the direction of Dr. Rod Wing. This facilities provides libraries, high density filters, and support for numerous plant BAC libraries. Requests to use the library are still routed through my lab at the University of Minnesota, but the distribution of physical resources comes from Clemson University. The BAC library consists of approximately 78 000 clones each with an insert of soybean DNA approximately 120 000 base pairs in length. Thus, the library provides approximately seven-fold coverage of the soybean genome.

There have already been 11 requests for the BAC library, high density filters derived from the library, or pools of BAC clones suitable for PCR screening. These requests have come primarily from scientists in the United States, along with one request from China.

3.2. Clone distribution packages

3.2.1. Vigna clone distribution package

The current package of *Vigna* clones that is sent upon request includes the following:

- Seventy-three single-copy DNA clones from mungbean and cowpea.
- Two moderately repetitive, highly polymorphic mungbean clones.
- Genomic DNA from mungbean, cowpea, soybean, common bean, and pigeon pea.
- Spreadsheet datafile with: locus name, cloning vector, insert size, map position, mapping enzyme, chromosomal locations in common bean and soybean, if known.
- Current RFLP linkage maps for mungbean and cowpea.
- DNA transformation, plasmid miniprep, and PCR amplification protocols.

3.2.1 Soybean BAC library distribution package

- Five high density filters containing all 78 000 soybean BAC clones spotted in duplicate on nylon filters. These filters are suitable for hybridization analysis with radiolabeled DNA probes.
- Pools of BAC clones that make it possible to screen the entire library using Polymerase Chain Reaction (PCR) technology with only 100 total PCR reactions.
- Upon request, specific clones from the library are prepared and distributed, either in the form of bacterial suspensions or purified clone.

3.3. Typical applications of clone resources

3.3.1 Typical Applications of Vigna Clones

The research on *Vigna* clones has focused in areas such as: 1) comparative genome mapping with related legume crop species, 2) mapping and characterization of quantitative trait loci (QTLs), and 3) mapping and tagging genes involved in disease and pest resistance. Table 1 briefly describes the scientists who have worked with the clones and the types of research projects involved.

3.3.2. Typical Applications of Soybean BAC Clones

Research with the soybean BAC clones has focused on two areas: 1) positional cloning of important disease resistance genes and 2) comparative genomic analysis aimed at understand microsyntenic relationships among legume genera. Table 2 briefly describes the scientists who have worked with the clones and the types of research projects involved.

TABLE 1. RESEARCH GROUPS THAT HAVE RECEIVED *VIGNA* RFLP CLONES AND BRIEF DESCRIPTION OF THEIR RESEARCH ACTIVITIES (1992–1998)

Principle investigator	Location	Research Application
E. Vallejos	U. Florida	Comparative mapping
G. Kuo	Taiwan	Disease resistance
J. Manners	CSIRO	Mungbean mapping
R. Shoemaker	Iowa State	Comparative mapping
D. Pignone	Bari, Italy	*In situ* hybridization in
P. Heslop-Harr.	Norwich, UK	cowpea and relatives
J. McCallum	New Zealand	Disease resistance in pea
N.-S. Kim	Korea	Mapping in Korean cultivars
L. Kumar	India	Disease resistance
S. N. Raina	India	Taxonomic relations in *Vicia*
S.R. Rangasamy	Tamil Nadu	Disease resistance
G. Kochert	Univ. Georgia	Peanut mapping
S. Abbo	Rehovot, Israel	QTLs in chickpea
A. Karasawa	Aoba-ku, Japan	CMV resistance in cowpea
C. Lambrides	CSIRO	Mungbean mapping and breeding
R. K. Sahu	India	Bruchid resistance
C. Mendenez	U. Cal., Davis	Seed weight QTL in cowpea
M. Ishimoto	Japan	Disease resistance
C. Liu	CSIRO	*Lablab* mapping
A. Paterson	Texas A&M	Peanut mapping
C.-S. Chen	Taiwan	Disease resistance
S. Chao	Taiwan	Disease resistance
S. Lee	U. Georgia	Comparative genomics
B. Sharma	India	*Vigna* mapping
J. Specht	U. Nebraska	Soybean mapping
V. Sant	India	Disease resistance
S. Lakhanpaul	India	*Vigna* mapping
M. Timko	U. Virginia	*Striga* resistance in cowpea

TABLE 2. RESEARCH GROUPS THAT HAVE RECEIVED SOYBEAN BAC CLONES AND BRIEF DESCRIPTION OF THEIR RESEARCH ACTIVITIES (1998-PRESENT)

Principle investigator	Location	Research Application
E. Vallejos	Florida	Comparative mapping
R. Bolla	Missouri	Disease resistance
P. Cregan	Maryland	Sequence polymorphism search
R. Innes	Indiana	Disease resistance
P. Keim	Arizona	Disease resistance
H. Knap	S. Carolina	Disease resistance
D. Lightfoot	Illinois	Disease resistance
F. Liu	P.R. China	Genome organization
R. Shoemaker	Iowa	Genome organization
L. Vodkin	Illinois	Genome organization
R. Wing	S. Carolina	BAC library characterization

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