Screening of Sunflower BAC Library for the Identification of Specific BAC Clones

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Abstract

To facilitate molecular cytogenetic research and the integration of cytogenetic and genetic linkage groups of sunflower, one BAC and one BIBAC library from HA 89 with BamHI and HindIII, respectively, were developed. The average insert sizes were estimated to be 140 kb and 137 kb in the BamHI and HindIII libraries, respectively. The combined libraries consist of 192,000 clones and represent approximately 8.9 haploid genomes of sunflower. The frequencies of clones that carry chloroplast or mitochondrial DNA were about 2.35% and 0.04%, respectively; and the highly repetitive DNA sequences were roughly 41%. A subset of the library screened by 36 overgo probes, derived from RFLP markers, identified 195 positive clones. These chromosome-specific BAC or BIBAC clones provide crucial tools for association of genetic linkage groups with their corresponding chromosomes using FISH analysis.

Introduction

Sunflower (Helianthus annuus L.) is an important oil crop in the world. The genomic resources for sunflower have been developed, including genetic maps, molecular markers (Berry et al. 1994; Jan et al. 1998; Gedil et al. 2001; Tang et al. 2002), and several BAC libraries (Gentzbittel et al. 2002; Özdemir et al. 2004). However, the genetic linkage groups have not been associated with corresponding chromosomes, partly due to unreliability of molecular cytogenetic technology in sunflower. However, recent studies have demonstrated that FISH (fluorescence in situ hybridization) technology combined with large insert BAC clones provide an efficient and reliable technique for chromosome identification (Dong et al. 2000; Howell et al. 2002; Kim et al. 2002, 2005). Therefore, the identification of chromosome-specific BAC clones for FISH analysis would allow cross-referencing the genetic linkage groups with particular chromosomes.

To develop molecular cytogenetic tools, we constructed and characterized one BAC and one BIBAC library. The objective of this project was to identify a set of sunflower linkage group-specific BAC or BIBAC clones from a subset of the libraries by overgo hybridization. These specific clones will be employed to assign each linkage group to its respective sunflower chromosome.
Materials and Methods

An inbred line, HA 89, released by the USDA-ARS and the Texas Agricultural Experiment Station in 1971 as a maintainer line was used for BAC and BIBAC library construction. Leaves were collected from 2-week-old seedlings and stored at -80°C for DNA extraction.

Library Construction

The libraries were constructed according to the protocol developed by Zhang (2000) (also see Wu et al. 2004). High-molecular-weight genomic DNA was extracted from the intact nuclei of HA 89 and partially digested by BamHI and HindIII, followed by size selection. The size-selected DNA fragments were ligated into the cloning vectors pECBAC1 and pCLD04541, respectively. The ligations that produced clones with an average insert size of 130 kb or larger were selected for large-scale transformation, and the resulting clones were used in assembling the libraries.

Library screening

The BAC and BIBAC clones of the libraries were robotically gridded on 22.5 × 22.5-cm Hybond N+ membranes (Amersham, USA) in a 4 × 4 format using the GeneTAC™ G3 Robotic Workstation (Genomic Solutions, Inc., USA). One to three RFLP markers were selected from each linkage group (Jan et al. 1998) for designing overgo primers. All overgos were individually labeled with [α-32P]dATP and [α-32P]dCTP, and combined into pools following the method developed by J. D. McPherson (http://www.tree.caltech.edu). The libraries were screened in two steps. In the first step, all overgos were used to hybridize all high-density clone filters of the subset of libraries in a single hybridization. The positive clones were re-arrayed in 384-well microtiter plates, and used to produce secondary filters. In the second step, all overgos were divided into different row pools and column pools, and hybridized to the secondary filters.

Library evaluation

Three barley chloroplast DNA probes, ndhA, rbcL, and psbA, and one wheat mitochondrial DNA probe, coxII, were labeled by using the Rediprime II Random prime labeling system (Amersham Biosciences, RPN1633), and used for screening the BAC and BIBAC libraries to identify the genome origin of the clones.

Cot-1 DNA was prepared as described by Zwick et al (1997) with minor modifications. Briefly, total sunflower genomic DNA was isolated and sheared by autoclaving for 2 min at 100°C, and repeated for one more cycle. The sheared DNA fragments ranging from 300 to 800 bp were denatured, reannealed for the appropriate Cot-1 time and subjected to S1 nuclease digestion and phenol-chloroform purification. All the positive clones identified were spotted on the filters manually. The resulting Cot-1 DNA was labeled with [α-32P]dCTP by using the same random priming method, and then hybridized to the positive clone filters. Those clones containing repetitive DNA sequences were expected to give the strongest hybridization signals.
Results and Discussion

1. Construction of BAC and BIBAC libraries

The resulting sunflower BAC library contains 107,136 clones, with an average insert size of 140 kb. The BIBAC library contains 84,864 clones, with an average insert size of 137 kb. The two libraries combined contain 192,000 clones and are equivalent to approximately 8.9 haploid genomes of sunflower (3,000 Mb/1C), and provide a greater than 99% probability of obtaining a clone of interest.

2. Library screening with RFLP markers

To isolate BACs and/or BIBACs specific for each chromosome of sunflower (x = 17), overgo primers designed from the sequences of previously mapped RFLP markers (Jan et al. 1998) were used to screen the libraries. A total of 36 overgos distributed across 19 linkage groups were employed to screen a subset library representing a 5.1× genome coverage.

We screened the subset library in two steps. In the first step, all 36 overgos were labeled and used as a single pool to screen the subset library. A total of 206 positive clones were identified and subsequently double-spotted on a new nylon filter. In the second step, the 36 overgos were aligned into 6 row pools and 6 column pools and hybridized to the new filter. In the second round screening, 195 positive clones were confirmed and the overgo-clone relationships determined (Table 1). These 195 BACs or BIBACs were distributed on the 19 linkage groups, with a range of 3-23 positive clones for each linkage group. This result also indicated that the sunflower genome is well covered by these libraries.

3. Estimation of the proportion of organellar DNA and repetitive DNA

To evaluate the quality of the libraries and estimate the proportion of clones that originated from organellar DNA, we screened a fraction of the library with a mixture of three chloroplast genome-specific genes (ndhA, rbcL, and psbA) and one mitochondrial gene (coxII) as probes, respectively. The results demonstrated that 2.35% and 0.04% of the clones contained chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA), respectively, in the combined libraries.

In general, large insert BAC DNA allows the generation of observable FISH signals; however, repetitive DNA in a majority of the BAC clones would cause dispersed FISH signals on each chromosome. In practice, Cot-1 DNA or total genomic DNA has been used to block repetitive DNA sequences in FISH analysis. Since Cot-1 DNA is highly enriched for repetitive DNAs, it can compete with the labeled probe for repetitive sequences. To estimate the repetitive DNA content in the libraries, Cot-1 DNA from sunflower HA 89 was labeled and hybridized to filters that contained 195 positive clones. Of these clones, 79 clones hybridized strongly, 75 moderately, and 41 gave weak signals with the Cot-1 DNA. This suggested that roughly 41% of the library clone contain highly repetitive DNA sequences. These clones should be avoided for FISH analysis because they cause dispersed FISH signals along every chromosome.

In conclusion, we constructed one BAC library and one BIBAC library from sunflower, and
screened the libraries with overgos derived from RFLP markers. This library provides an excellent resource for sunflower chromosome identification and molecular cytogenetics research. FISH mapping of RFLP marker-targeted BAC clones would allow construction of a sunflower physical map. This physical map could be directly related to the genetic linkage maps of sunflower.

Table 1. Positive clones identified with 36 overgos.

<table>
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<tr>
<th>Linkage group</th>
<th>Overgos (RFLP markers)</th>
<th>No of positive clones</th>
<th>Linkage group</th>
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References


fragment length polymorphism linkage map for cultivated sunflower. Genome 44:213-221.


Acknowledgements

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