Assignment of YAC Clones Spanning Rice Chromosomes 10 and 12

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Abstract

Yeast artificial chromosome (YAC) clones were assigned on rice (Oryza sativa L. cv. Nipponbare) chromosomes 10 and 12 using DNA markers from our high-density linkage map. Out of 1,383 markers localized in this genetic map, 68 and 74 markers were located on chromosomes 10 and 12, respectively. Screening of the YAC genomic library was conducted by colony hybridization and Southern hybridization using restriction fragment length polymorphism (RFLP) markers or by polymerase chain reaction (PCR) using sequence-tagged site (STS) markers. We have completed the screening of 68 markers on chromosome 10 and 74 markers on chromosome 12. A total of 134 and 103 YACs were assigned to chromosomes 10 and 12, respectively, with an estimated coverage of more than 60% for chromosome 10 and about 47% for chromosome 12. As rice is considered a model plant for genome analysis, the ordered YAC clones on chromosomes 10 and 12 as well as other chromosomes will certainly be helpful for isolation of agronomically and biologically important genes and for understanding the genome structure of these chromosomes.

Key words: rice; DNA marker; genome; yeast artificial chromosome; minimum overlap

1. Introduction

Genome analysis by genetic and physical mapping has become a very important approach towards understanding the large-scale organization of the genome of many organisms.1,2,3 Among cereal crops, rice is considered a model plant for genome analysis because it has the smallest genome size, a well-developed genetic system and known syntenic relationships with other crops.4 The Rice Genome Research Program is undertaking construction of a physical map of the rice genome using a high-resolution genetic map with 1,383 molecular markers.5 At present, several vectors such as cosmids,1,6 bacterial artificial chromosome (BAC),7,8 P1-derived artificial chromosome (PAC)3,9 and yeast artificial chromosome (YAC)2,10 have become primary tools for physical mapping and analysis of complex genomes. In order to clone genomic DNA fragments that range in size up to several hundred kilobase pairs, we used YAC for library construction.11 The resulting YAC rice genomic library covers six equivalents of the rice genome and offers the possibility of covering the whole genome with YAC clones. Using the DNA markers in our high-density linkage map, we are ordering the YAC rice genomic library to construct a physical map of all rice chromosomes.

In this report, we described the construction of YAC contigs by chromosome landing using DNA markers localized on rice chromosomes 10 and 12. In our high-density linkage map, chromosome 10 is the shortest in genetic distance whereas chromosome 12 is the third shortest among the 12 linkage groups of rice. More importantly, agronomically important genes such as fertility restoring gene12 and a rice blast resistance gene13 have been identified and located on these chromosomes.14 Thus, by ordering YAC clones to establish contigs, specific segments of these chromosomes which have been characterized genetically can be manipulated, and biologically important genes can eventually be isolated by map-based cloning.

2. Materials and Methods

2.1. YAC library and DNA markers

The YAC library was prepared as described previously11 and consisted of 6,932 clones with an average insert size of 350 kb. These YAC clones were blotted onto five high-density replica filters. The DNA markers included restriction fragment length polymorphism (RFLP) and sequence-tagged site (STS) markers which...
have already been located on the high-density linkage map of rice. Most of these DNA marker sequences are deposited in the DNA Data Bank of Japan (DDBJ) and clones with their detailed information are available from the MAFF DNA bank at http://bank.dna.affrc.go.jp.

2.2. YAC library screening

The RFLP markers were used for screening of YAC library by colony hybridization using the replica filters. The candidate YAC clones were confirmed by Southern hybridization using the same RFLP marker as described. All PCR-derived markers on chromosomes 10 and 12 have been analyzed as STS and specific primers have been synthesized. The YAC clones were divided into eight super-pools and amplified using established primers (1st screening). The amplified pools were analyzed using three-dimensional DNA pools (2nd screening). Then the individual candidate YACs were amplified to confirm the presence of the mapped DNA fragment (3rd screening).

2.3. Ordering of YAC clones

All positive YAC clones for the RFLP markers and STS markers were ordered following the high-density RFLP linkage map. A database of all information derived as a result of ordering YAC clones in the 12 chromosomes of rice was constructed and YACs assigned to chromosomes 10 and 12 were analyzed using this database to determine double assignment of the same YACs at other chromosome positions. The insert size of YACs which consisted of 39 YAC clones. A total genetic distance of 2.4 cM was covered by YACs which identified two or more markers located at separate loci. As with chromosome 10, most of the assigned YACs were single-position YACs which could identify only one or more markers located at one locus position.

Ordered YAC clones spanning chromosome 12 are shown in Fig. 2 with a minimal overlapping YAC array which consisted of 39 YAC clones. A total genetic distance of 2.4 cM was covered by YACs which identified two or more markers located at separate loci. As with chromosome 10, most of the assigned YACs were single-position YACs which corresponded to marker positions covered by 1 YAC per marker as well as marker positions covered by two or more YACs per marker. Positions with two or more markers covered by single or overlapping YAC clones were mostly tightly linked at 0 cM.

The chromosome coverage of ordered YAC clones in chromosomes 10 and 12 are summarized in Table 1. Our group used a unified method of calculation for all chromosomes. Estimation of coverage length was carried out as described. The sum of the physical distance estimated from the total genetic distance of 2 or more marker positions covered by 1 or more YACs and the physical length of single-position YACs was divided with the estimated physical distance of the entire chromosome based on the genome size and genetic map distance of rice. The physical length of marker positions covered by 1 YAC/marker was calculated using the 350 kb average insert size of the YAC clones in our library. However, in the case of

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Figure 1. Ordered YAC clones on rice chromosome 10. The genetic map used for ordering YACs is represented on the right side by a long line marked with DNA markers. Markers in the middle between the linkage map and YAC alignments are those which could select YACs in our library. Markers on the right could not select YACs and Kasalath-dominant type markers (underlined). YAC clones are represented by bars with black and white circles. YACs with black circles constitute a minimal overlapping YAC array. Squares represent YACs selected by DNA bands which co-exist with the mapped RFLP band of the marker. Black squares on the YAC clones represent end of YAC clone. The YAC clones numbered with letter (C) are chimera. The gray lines encompassed by black lines in YAC clones represent deletions where no markers on those gray lines are detected.
Chromosome 12

Figure 2. Ordered YAC clones on rice chromosome 12. All symbols are same as that described in Fig. 1.
Table 1. Coverage of chromosomes 10 and 12 by ordered YAC clones.

|                        | Chr. 10 | Chr. 12 |
|------------------------|---------|---------|
| Genetic distance (cM)  | 85.6    | 112.1   |
| Estimated physical distance (Mb)* | 23.4    | 30.6    |
| Total distance of regions with 2 or more markers covered by 1 or more YACs (cM) | 6.9     | 2.4     |
| Number of marker positions and total length covered by 1 YAC/marker | 10 (3.5 Mb) | 13 (4.6 Mb) |
| Number of marker positions and total length covered by 2 or more YACs/marker** | 19 (10 Mb) | 16 (8.4 Mb) |
| Total length covered with YACs | 15.4 Mb | 13.6 Mb |

*1 cM was estimated to be 273 kb, since the genome size of rice is 430 Mb and total genetic map distance is 1575 cM.
**Based on the assumption that average insert size of YAC is 350 kb and overlap among plural YACs is 50% (525 kb/single marker position).
***Chromosome coverage:

\[(b) \times 273 \text{ kb/cM} + (c) \times 350 \text{ kb} + (d) \times 525 \text{ kb}\]

mark positions covered by 2 or more YACs/marker, it was assumed that there was approximately 50% overlap between assigned YACs.\(^7\) The physical length of markers tightly linked at 0 cM was calculated as single-position YACs covered by either 1 YAC/marker or 2 or more YACs/marker. Using this method of calculation, about 66% and 44% chromosome coverage of positioned YACs were obtained for chromosomes 10 and 12, respectively. In chromosome 10, the number of marker positions covered by 2 or more YACs/marker with an overlap of 50% between YACs totalled 19. This could be translated into 10 Mb which is almost 40% of the total physical distance. As a result, although the number of contigs was minimal, the chromosome coverage of ordered YACs for chromosome 10 was calculated as about 66%. In the case of chromosome 12, the chromosome coverage is relatively low because many markers could not identify any YAC in our library or could not be used for selection. In addition, most markers are distantly separated so that the total genetic distance of YAC contigs as well as regions with 2 or more markers covered by YACs is relatively short.

However, as far as chromosome 10 is concerned, about 66% coverage was obtained although the genetic distance covered by YAC clones was short so that it is necessary to confirm the chromosome coverage using another method of calculation. Thus the chromosome coverage was also determined using actual insert size of all assigned YACs which comprised the minimum overlap YAC array. An overlap of 50% was also assumed for YACs that form contigs. Based on this method of calculation, the simple sum of the insert size for single-position YACs was about 12.9 Mb and the total length of YAC contigs was 1.2 Mb which correspond to a total of 14.1 Mb and about 60% coverage for chromosome 10. Thus a coverage of about 60% was also obtained using this method of calculation. In the case of chromosome 12, the simple sum of the insert size for single-position YACs was about 14 Mb and 1.4 Mb for YAC contigs. A total of 15.4 Mb or about 50% coverage was obtained for chromosome 12. The higher coverage than the estimated value using our unified method of calculation for chromosome 12 is due to the large insert size which is over 350 kb of most ordered YACs. In region 1 of chromosome 10, the upper part with G89, R2309, C709B and R1933 stretching 4.5 cM could not identify any YAC clone in our library. Also, in region 1 of chromosome 12, the upper part with R1552, R1684 and R2501B covering 0.8 cM could not identify even a single clone in our YAC library. Additionally, several markers which are separated from neighboring markers such as C121 and C16 in chromosome 10 as well as R1709, C185 and P4 in chromosome 12 could also not identify any YAC clone. These results suggest that the specific regions of the chromosomes containing those DNA markers are not represented in our YAC library. It is possible that these particular regions represent DNA fragments which are difficult to clone as long inserts in this yeast artificial chromosome cloning system.

Three YACs in region 2 of chromosome 10 (Y1450, Y5563 and Y7065) were selected for G37 and G2155 separated by 12.5 cM while three markers in between such as R1877, C1361 and G4003 were not located in those YACs. Moreover, Y4570 was assigned to G37 and C188 separated by 12.5 cM while four markers (R1877, C1361, G4003 and G2155) in between were not identified. These YACs may be deletion or chimeric clones representing two unlinked regions of the genome which have been re-arranged or ligated in these YAC clones. However, it is also possible that some characteristic features such as
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those relating to chromatin folding of this region of the chromosome would be revealed when a more precise analysis is conducted. There was a case when the genetic position of some markers changed as a result of ordering YAC clones. As an example, in the upper part of region 1 of chromosome 12, L405 and W326 were separated by 5.2 cM with three markers in between. These two markers form a contig in Y2252. Re-mapping of L405 on a more refined genetic map showed that these two markers were tightly linked (data not shown).

An 11.8 cM fragment in the distal region of chromosome 12 (region 2) was reported to have a conserved duplicated segment in 10 cM distal region of chromosome 11.\textsuperscript{18} We were able to select 10 YACs covering this region of chromosome 12. These results will be useful in analysis of the genomic structure of the conserved region of both chromosomes and details will be published elsewhere soon by Wu et al. (in preparation).

The assigned YAC clones described here represent the first stage of physical map construction for chromosomes 10 and 12. In the next stage, we will use additional markers mapped on our RFLP linkage map with more than 2,200 DNA markers. The remaining gaps will be analyzed by chromosome walking and other methods in order to construct a more precise physical map. We will also try the fingerprinting method to clarify overlaps among contigs.

The physical map of chromosomes 10 and 12 will certainly be useful in the overall analysis of these chromosomes as well as the entire rice genome. This will also provide valuable information for the isolation of genes for agronomically and biologically important traits in rice. The images of aligned YAC clones with the high-density maps of chromosomes 10 and 12 have been released and are accessible at the website http://bank.dna.affrc.go.jp.

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