Yeast Artificial Chromosome Clones of Rice Chromosome 2
Ordered Using DNA Markers

Yosuke UMEHARA, Nori KURATA,* Ikuo ASHIKAWA,* and Takuji SASAKI*

Rice Genome Research Program, National Institute of Agrobiological Resources, 1-2 Kannondai, 2-chome, Tsukuba, Ibaraki 305, Japan/Institute of Society for Techno-Innovation of Agriculture, Forestry and Fisheries, 446-1 Ippaizuka, Kamiyokoba, Tsukuba, Ibaraki 305, Japan

(Received 29 December 1996; revised 13 March 1997)

Abstract
Yeast artificial chromosome (YAC) clones were ordered for the physical mapping of rice chromosome 2, the last of the 12 rice chromosomes to be assigned YACs by the Rice Genome Research Program. A total of 128 restriction fragment length polymorphism markers and 4 sequence-tagged site (STS) markers located on our high-density genetic map were used for YAC clone landing. By colony/Southern hybridization and polymerase chain reaction screening, a total of 239 individual YACs were selected from our YAC library of 6934 clones covering six genome equivalents. The YACs located on the corresponding marker positions in the linkage map formed 43 contigs and islands and were estimated to encompass about 50% of the length of rice chromosome 2.

Key words: yeast artificial chromosome (YAC); physical mapping; rice

1. Introduction
Rice, one of the most important staple crops, supports about half of the world’s human population. Since the growing world population will require increasing production of this crop, it is an urgent matter to understand rice and to improve the productivity of rice through genetic manipulation. Rice is considered to be a model cereal because of its small genome size (4.3 x 10^8 bp) and synteny with other cereals. Much progress has been made recently in rice genome analysis, particularly in the establishment of high-density linkage maps using DNA markers, construction of large fragment genomic clone libraries using yeast artificial chromosomes (YACs) and bacterial artificial chromosomes (BACs), and partial sequencing of cDNA clone resources.

Ordered genomic clones are indispensable for clarification of the rice genome structure and map-based cloning of genes responsible for desired traits. The YAC system is a powerful tool for cloning DNA fragments longer than several hundred kilobases. This capability enables the construction of ordered clone libraries covering entire chromosome lengths with fewer clones than would be required with other DNA fragment cloning systems. Our YAC ordering is done by chromosome landing with a YAC library containing 6934 clones of 350 kb average insert size, which cover about six genome equivalents, and with DNA markers mapped on our high-density linkage map. We have already reported YAC contigs assigned to all the other rice chromosomes — 613, 14, 515, 3 and 16, 4 and 717, 8 and 918, and 10 and 1219 — constructed by the same method of chromosome landing. This report on YAC assignment to chromosome 2 is thus the last in the series.

The genetic distance of this chromosome was previously calculated to be 156.1 cM on our linkage map. From this value, the size of chromosome 2 was estimated by dissecting the rice genome proportionally in centimorgan values to be approximately 43 Mb (156.1 cM x 430 Mb/1575 cM = 43 Mb). The 141 DNA markers mapped on this chromosome are dispersed at an average distance of about 300 kb. Genes responsible for 18 morphological traits on this chromosome, such as the Pyricularia oryzae resistance gene, Pi-b, and the spotted leaf-2 gene, spl-2, have been mapped. An ordered YAC clone library would supply important information for clarification of physical structure and for positional cloning of trait genes on this chromosome.
2. Materials and Methods

2.1. YAC library and DNA markers

The 6934-YAC clone library was constructed from cultured cells of Oryza sativa L. cv. Nipponbare, as described previously.5 DNA markers including restriction fragment length polymorphism (RFLP), sequence-tagged sites (STS), and randomly amplified polymorphic DNA (RAPD) markers had already been located on the rice linkage map of chromosome 2; most of them were at least partially sequenced and deposited at the DNA Data Bank of Japan (DDBJ).5,21 All these DNA markers were used for screening the YAC library to select clones which harbored identical sequences.

2.2. YAC library screening and construction of a YAC contig map

Screening of the 6934 YAC clones was done by colony/Southern hybridization with RFLP markers using the ECL system (Amersham), and YAC clones with STS markers were isolated using the three-step polymerase chain reaction (PCR) as described previously.13 YAC clones carrying a DNA marker sequence were assigned to the marker positions on our molecular linkage map to make an ordered YAC marker sequence. The sizes of YAC clones were scaled by Southern hybridization with a YAC vector probe following contour-clamped homogeneous electric field (CHEF) electrophoresis gel fractionation.

3. Results and Discussion

A total of 128 RFLP markers on chromosome 2 were used to identify YAC clones by colony hybridization. The candidate clones from colony hybridizations were examined by Southern hybridization to confirm the presence of each genomic DNA band which was used for RFLP mapping. In addition to these RFLP markers, four PCR-based markers on chromosome 2 were used with the three-step PCR screening system to identify the corresponding YAC clones.13

A total of 239 YAC clones were selected with 120 RFLP markers and three STS markers and were assigned to chromosome 2 (Fig. 1). YAC contigs, each connecting more than 2 DNA marker positions, were formed in 22 areas (black lines on the genetic map in Fig. 1). Twenty-one other YAC clusters were located independently at separate marker positions. Among these YACs, Y1385 was the most marker-rich clone, containing 13 markers on the distal end of the chromosome and spanning 5.8 cM in region 1 (see Fig. 1). The longest contig, covering 7.4 cM from C1470 to C978 was formed here; the physical length of this contig was estimated to be at least 650 kb (assuming the greatest possible overlap among the three constituent YACs (Y7063, Y1385, and Y3308)) and at most 1250 kb (assuming the least possible overlap among these 3 YAC clones).

The maximum chromosome coverage by the minimum number of overlapping YACs together with singly arranged YACs, that is the minimum tiling path, was composed of 53 YACs (see clone stretches with markers of filled circles or a square in Fig. 1). On chromosome 2, each marker selected 3.9 YACs on average, and the maximum of 12 clones was identified by R1870A and R1870B. Multiple-copy sequences mapped on other chromosomes assigned 23 YACs to each chromosome,14,15,17,19 Other copies of multiple-copy sequences, which have not yet been mapped genetically on any chromosomes because of lack of polymorphism,13 also identified 110 YACs.

Among the YAC clones selected with distinct markers, 45 YACs (followed by the letter (C) in Fig. 1), were shown to carry additional DNA markers mapped on other chromosomes or at completely different positions within the chromosome separated by other markers. These clones, which consisted of at least 19% of the identified YACs on chromosome 2, are thought to be chimeric.

There are discrepancies between the YAC contigs and linkage map positions of 3 DNA markers, C348 and R2734 in region 1 and P117 in region 3 (see Fig. 1). Revised linkage analysis with more markers around these positions has shown that the orders of these DNA markers in the linkage map are identical to those confirmed in the YAC contigs (data not shown).

Chromosome coverage of rice chromosome 2 with YACs was estimated by the method previously described.14 Two hundred and forty-one distinct YACs on chromosome 2 were identified and distributed in 22 contigs and 22 islands. The 22 YAC contigs covered a genetic distance of 37.1 cM. The 21 YAC islands each covered only one DNA marker position on the linkage map. Among them were seven islands containing single YAC clones and 14 islands containing multiple YACs. Assuming that all the YACs in these islands overlapped by 50% of their length, these contigs and islands covered about 20 Mb (273 kb/cM × 37.1 cM + 7 × 350 kb + 14 × 350 × 1.5 kb) or 46% of the length of chromosome 2. Broadly similar estimates of coverage are obtained with the minimum tiling path; the tentative arrays comprising these 53 YACs would cover at least 20.5 Mb or 48% of chromosome 2 based on the largest overlaps among YAC clones and up to 23.8 Mb or 55%, based on the smallest overlaps.

No YAC clones could be selected with the nine DNA markers indicated on the right side of the linkage map in Fig. 1. The DNA fragments in the regions around these markers would be unclonable in the yeast host AB1380 either, because there were many repeated sequences22 or
Figure 1. YAC contigs on rice chromosome 2. The chromosome is shown in four separate parts, regions 1 to 4. The vertical bars on the right side of each diagram represent our high-density genetic map of this chromosome. Lengths in centimorgans are shown for the end markers of each region. Stretches with black bars show genetic distances of the regions covered by YAC contigs. DNA markers are aligned between the YACs and the linkage map and consist of random genomic clones (G), cDNA clones from callus (C), root (R), and shoot (S), Not I linking clones (L), YAC end clones (Y), DNA clones from visitors (V), wheat DNA clones (W), RAPDs (P), and STSs (T). YACs are shown as short vertical bars on the left side of the figures, with circles representing the DNA markers located on them. YACs including open rectangles were selected by co-existing DNA bands with RFLP markers. YACs carrying black circles or a square indicate the minimum tiling path of YACs. The sizes (in kb) of these YACs are shown on the right side of each YAC. The YACs with the notation (C) are chimeric clones. The gray line in the middle of YAC clone Y3228 indicates a deleted portion where a corresponding DNA marker was absent. The markers shown on the right side of the genetic map were those which failed to identify any YAC or which were not suitable for use in YAC screening (underlined).
Figure 1. Continued.
the DNA was toxic to the yeast. For cloning DNA fragments in these regions, another system, such as BAC, should be investigated.

The YAC high-density replica filters, YAC clones, and DNA markers used in this study are available from the MAFF DNA bank operated by the Ministry of Agriculture, Forestry and Fisheries of Japan. Information about the YAC contig map, genetic map and DNA markers, as well as request forms for markers and clones, are also available on the world wide web at: http://bank.dna.affrc.go.jp/.

Acknowledgments: We thank Hiroshi Tanoue, Zi-Xuan Wang, Shoko Saji, Takanori Shimokawa, Katsuhiro Yoshino, Balataar A. Antonio, Atsuko Idonuma, Makiko Emoto, Jianzhong Wu, Takayuki Momma, Wim Van Houten, Norio Sue, and Yuichi Katayose for valuable advice and much assistance, and Yoshiaki Nagamura, Masahiro Yano, Lisa Monna, and Akio Miyao for providing information on DNA markers and expert advice. We also thank Kaetsu Kobayashi and Koichi Hasegawa for encouragement. This work was supported by the Ministry of Agriculture, Forestry and Fisheries, Japan and by the Japan Racing Association.

References
