The 12th Symposium on Plant Biotechnology
Vol. 12 : 61~70(1998)

# Multi-dimensional analyses of plant chromosomes and genomes.

Kiichi FUKUI, Nobuko OHMIDO, Toshiyuki WAKO
Laboratory of Rice Genetic Engineering, Hokuriku National Agricultural Experiment Station, Joetsu 943-0193, Japan

## ABSTRACT

Genome and chromosome analyses in plants using fluorescence *in situ* hybridization (FISH) and immuno-staining (IMS) methods are reviewed by presenting the recent results obtained by the Chromosome Link, a group of chromosome and genome researchers. FISH is now effective to detect unique nucleotide sequences with 153 bp on the extended DNA fibers. Genomic *in situ* hybridization (GISH) also allows painting plant chromosomes of different genomes. GISH is quite effective to detect the genomic differentiation in the individual chromosomes within a nucleus. Three dimensional (3D) analyses are now available by confocal microscopy and a deconvolution system. These techniques are invaluable to visualize both the structural and functional dynamics within a nucleus. 3D-FISH revealed the spatial differentiation of different genomes within a nucleus. 3D-FISH also proved structural partition of centromeric and telomeric domains within a barely nucleus. The dynamic acetylation of histone H4 at the specific regions of a genome during a cell cycle is also analyzed using 3D-IMS. It is anticipated that these methods will provide us powerful tools to understand the structural and functional significance of plant chromosomes and genomes.

## INTRODUCTION

Plant chromosomes and genomes have studied for years. Chromosomes in a cell were observed and reported for the first time by Nageli in 1842. The number of rice and oilseed chromosomes were accurately determined as 2n=24 and 2n=38, respectively, in the beginning of the 20th century. The basic genome constituent of wheat was revealed as AABBDD more than a half century ago. Chromosome and genome researches, however, have been long stagnant until recently when several new methods are introduced. The main reasons lie in the fact that the chromosomes and genomes have tremendous amount of information from both the biochemical and informatical points of view. Biochemical and Image analyses of chromosomes were limited due to the very point until recently. In fact, the chromosomes of rice and oilseed rape were objectively identified and their quantitative chromosome maps were developed by imaging methods only recently (Fukui and Iijima, 1991; Kamisugi et al., 1998)

Recent rapid advances in microelectronics and optical engineering have enabled to analyze the structural and functional significance of plant

chromosomes and genomes. Imaging method has advanced rapidly both in the theoretical and applied sides. The recent development of a low noise and high sensitive CCD camera and a new computing system with algorithm to reconstruct 3D images have made both the fluorescence *in. situ* hybridization (FISH) and immuno-staining (IMS) methods the most effective to visually reveal the enigma in the structure and function of plant chromosomes and genomes. Now image analyses of plant chromosomes and genomes do not require any specific image analysis system. An ordinary personal computer is sufficient to carry out image analyses (Kato et al., 1997; Kato and Fukui, 1998). FISH has been employed to physically detect and map nucleotide sequences on plant chromosomes and genomes as an ordinary technique (Ohmido and Fukui, 1997). IMS further reveals the dynamic acetylation of histone H4 at the specific regions of a genome during a cell cycle (Wako et al., 1998). These new analyzing methods for plant chromosomes and gnomes are reviewed presenting representative examples.

## Image analyses of plant chromosomes for the development of objective chromosome maps.

The first generation of chromosome image analyzing system (CHIAS) was developed in 1995 (Fukui, 1986; 1988). Image analyses of plant chromosomes and genomes have rapidly advanced after successful development of the CHIAS. The second generation chromosome image analyzing system, CHIAS II (Fukui and Nakayama, 1996; Nakayama and Fukui, 1997) was then developed improving both the hard- and software (Fukui and Nakayama, 1996). CHIAS had the CP/M operating system and the image file format specific to the CHIAS. CHIAS II was equipped with MS/DOS operating system and with the image files common to most of the other imaging systems.

The two image analyzing systems have effectively been used for the image analyses of plant chromosomes and genomes. For example, semi-automatic karyotyping (Fukui, 1986), computer aided idiogram development (Fukui,1988), automatic metaphase finding (Fukui, 1988), simulation of optical and staining chromosomal images (Fukui and Kamisugi, 1991), development of a personal computer-based system (CHIAS-mini, Kamisugi and Fukui, 1990), automatic identification of rice chromosomes (Kamisugi et al., 1993), simulation of human vision to quantify banding patterns (Fukui and Kamisugi, 1995), etc. have been developed.

Quantitative idiograms or chromosome maps were constructed for the first time in *Atriplex rosea* (Fukui and Mukai, 1988), and then, in barley (Fukui and Kakeda, 1990), rice (Fukui and Iijima, 1991), *Paris* spp. (Miyamoto et al., 1991), and *Crepis capillaris* (Fukui and Kamisugi, 1995). Figure 1 shows the quantitative rice chromosome

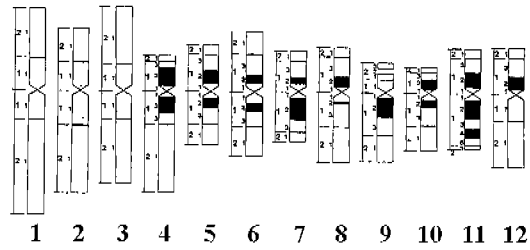


Fig. 1. A quantitative chromosome map of rice based on the condensation pattern (Fukui, 1996).

map. 5S rRNA gene loci detected through *in situ* hybridization have been mapped on the quantitative chromosome maps of barley (Fukui et al., 1994) and rice (Ohmido and Fukui, 1995). Furthermore, *in situ* hybridization signals of the 5S rDNA locus were quantitatively analyzed and localized on the rice chromosome map as shown in Fig. 2. (Kamisugi et al., 1994). Laser microdissection of plant chromosomes has also been carried out based on the quantitative maps

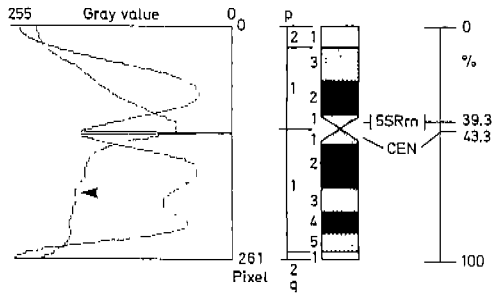


Fig. 2. Image analyses of the signal location of 5S rDNA on rice chromosomes after *in situ* hybridization (Kamisugi et al., 1994).

of barley and rice chromosomes (Fukui et al., 1991; 1992) and of the forest tree chromosomes (Nakamura and Fukui, 1997).

Now a universal imaging system without using specific hard- and software has been constructed. The third generation chromosome image analyzing system, CHIAS III (Kato and Fukui, 1998), which consists of an ordinary personal computer, and does not depend on specific imaging software which runs on limited systems, but uses public domain software available via the Internet. Consistency of the data between those obtained with CHIAS III and the previous systems wa also experimentally confirmed (Kato and Fukui, 1998). The program is obtained via the Internet(http://150.26.36.99/Eudejas/chias3/chias3man/contents.html). A written manual on CHIAS III is also available (Kato et al., 1997). A quantitative chromosome map of sugarcane has been developed by using CHIAS III (Ha et al., submitted).

**Fluorescence *in situ* hybridization to visualize specific nucleotide sequences on plant chromosomes and DNA fibers.**

The FISH method has been applied extensively in human gene mapping for localizing nucleotide sequences directly to chromosomes and genomes. The signals obtained were localized to the defined sites in the fine chromosome map by the sequential application of a banding method after FISH. Identification of the signal-tagged chromosomes or even the signal sites by the banding method greatly contributes to enhance the reliability and effectiveness of human FISH experiments (Trask, 1991).

The application of FISH to plant chromosomes using ribosomal RNA genes began at late 1980s (Jiang and Gill, 1994) and FISH has now become an effective method not only to localize repetitive sequences but also to unique sequences in plant chromosomes as well. Attempts to localize specific nucleotide sequences on identified chromosomes were, however, quite limited in plants. In fact, the systematic FISH, that is a sequential application of chromosome identification and FISH methods was mainly confined to wheat, rye and some other plant species with large chromosomes, where the banding methods allow precise identification of chromosomes and definition of the chromosomal addresses (Jiang and Gill, 1993; Ma et al., 1997).

For small plant chromosomes such as those of rice, *Arabidopsis*, *Brassica*, soybean, etc., measuring 1-2 $\mu$m at mitotic metaphase, it had been difficult to identify their chromosomes objectively by morphological means. No banding methods give crucial cues to soybean and *Brassica* chromosomes or even no band has appeared in *Arabidopsis* and rice chromosomes to date. Therefore sequential FISH incorporating chromosome identification by using banding methods, which ensures the reliability of FISH as in the case of human chromosomes, has not been applicable to these biologically and economically important plant species with small chromosomes. In 1991, Fukui and Iijima (1991) developed an objective identification method for small plant chromosomes, which does not employ any banding method but analyses the condensation pattern i.e., uneven condensation of chromatin fibers occurring at the prometaphase chromosomes.

Figure 3 shows rice chromosomes with typical condensation pattern (Fukui, 1996).

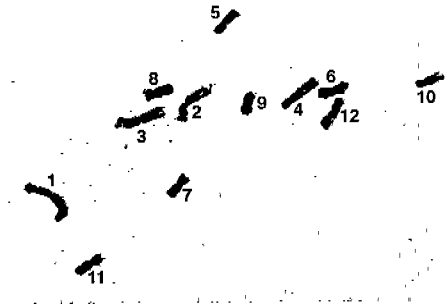


Fig. 3. Rice chromosomes at the prometaphase stage depicting typical uneven condensation of chromatin fibers (Fukui, 1996).

Recently, along with advances in chromosome and genome researches, a great number of DNA clones with a range of sizes have become available. For example, more than two thousands of molecular markers are now genetically mapped on rice chromosomes (Causse et al., 1994; Kurata et al., 1994). Libraries of yeast artificial chromosomes (YACs) and bacterial artificial chromosomes (BACs) have been developed in rice (Nakamura et al., 1997; Zhang et al. 1996). However, a limited number of nucleotide sequences related to traits of agronomic and biological importance has been localized physically on plant chromosomes. Cosmids and RFLP markers, which contain smaller nucleotide sequences than YACs and BACs, have not been widely used for physical mapping of genes (Song et al., 1996). Because physical mapping is effective for localizing genes directly on the chromosome and determining physical length between the markers, information derived by physical mapping would be indispensable for genome research such as map-based cloning of a useful gene.

Ohmido et al. (1998) developed a systematic method in FISH for mapping nucleotide seque nces on specific regions of rice chromosomes effectively and reliably by introducing identification of the target chromosomes before and/or after FISH. Four unique rice nucleotide sequences ranging in size from 1.29 kb to 399 kb were successfully mapped at the defined addresses on the identified rice chromosomes. Figure 4 depict physically mapping RFLP makers on rice chromosome 4 and comparison between the locations of the RFLP clone on the genetic map and the chromosome map was made. The smallest size of the clone that has been detected is 153 bp to date. The extended DNA fiber was used instead of the chromosomes in this case as described below.

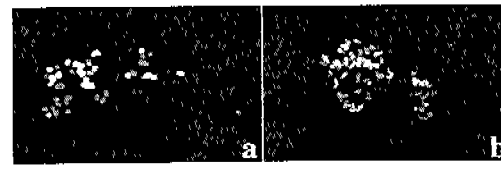


Fig. 4. Physical mapping of an RFLP marker on rice chromosome 4 by FISH (Ohmido et al., 1998).

The higher sensitivity of FISH discussed above is one of the most important technical destinations. There is, however, another aspect to be improved in the FISH technique. Even though the detection sensitivity of FISH is as high as a few hundreds base pairs, there exists another barrier to be overcome in the detection of DNA sequences localized closely. That is a space resolving power of FISH. The space resolving power of FISH on metaphase chromosomes is estimated to be 1 Mb and that on interphase nuclei and pachytene chromosomes, 100 kb (Lawrence et al. 1990). Thus it is reasonably calculated that the FISH signals from two nucleotide sequences sitting closer than 100 kb on the same chromatin fiber will be overlapped on the nucleus and chromosome.

Figure 5 shows representative demonstration of multi-color FISH (McFISH) on a chromosome and nucleus using TrsA and telomere sequences.

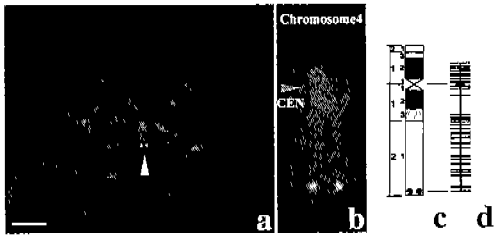


Fig. 5. McFISH using the rice A genome specific tandem repeat sequence A and telomere sequences on rice chromosomes (a) and nucleus (b) (Ohmido and Fukui, 1997).

TrsA is tandem repeated sequences specifically amplified in rice A genome species (Ohtsubo et al., 1991). TrsA has been localized at the terminal region of long arms by ordinary FISH and it is overlapped with telomere sequences in the nucleus suggesting that the TrsA and telomere sequences are located less than 100 kb (Ohmido and Fukui, 1997). Thus FISH on extended DNA fibers (EDF-FISH) has been applied to resolve the accurate positional relationship between the telomere sequences and TrsA. EDF-FISH was first developed to resolve the inner structure of 45S rDNA and contig of cosmids in tomato and *Arabidopisis* by Fransz et al. (1996). Instead of chromosome samples, they used isolated and extended DNA fibers from nuclei. They also calculated that 1m of signal track on DNA fibers corresponds to 3.7 kb of actual nucleotide length (Fransz et al., 1996) indicating that EDF-FISH gives much higher space resolving power than any other methods developed to date. It is also reasonably anticipated to quantify copy number and/or length of target nucleotide sequences by using EDF-FISH.

**Genomic *in situ* hybridization effective to analyze the genome constituent in plants.**

Polyploidy is prominent among angiosperm plants and 30 % to 35 % of the known species are estimated to polyploid. Furthermore, almost 75 % of the species in Gramineae are polyploid. Genomic *in situ* hybridization (GISH) is effective to differently paint the chromosomes originated from different genomes. Two rice amphidiploids of BBCC (*O. minuta, O. officinalis*) and CCDD (*O. latifolia, O. grandglumis, O. glumaepatura*) are known among wild rice species. BBCC species distribute mainly in Southeast Asia and CCDD species in Central and South Americas, however a diploid rice species with the D genome has not been found to date. The rice D genome exists only in the combination form with the C genome as an amphidiploid, CCDD. The D genome is estimated to be valuable genetic source for vigorous growth and brown plant hopper resistance (D. S. Brar personal communication). Thus it is worthwhile to characterize the D genome not only from the phylogenetic and evolutional aspects but also from the point of practical breeding view.

Thus genomic *in situ* hybridization (GISH) method was applied to determine the chromosomes belonging to the D genome. Fukui et al. (1997) used two wild rice species *O. minuta* (BBCC) and *O. latifolia* (CCDD) and a donor of the C genome DNA, *O. officinalis* (a diploid species with the C genome) were chosen to the GISH experiment. Total genomic DNA was isolated from *O. officinalis* and biotin labeled. Then the labeled C genome DNA was *in situ* hybridized both to the chromosomes of *O. minuta* and *O. latifolia*. No blocking DNA was included in a hybridization mixture as in the case of ordinary GISH but thresholding the signal intensity was carefully determined in order to discriminate target chromosomes using CHIAS II, instead. Figure 6 shows the results of GISH with *O. minuta* and *O. latifolia*. Twenty four chromosomes of the C genome were clearly painted with the labeled genomic DNA isolated from the C genome in both cases. Detailed comparison on both the hybridization patterns

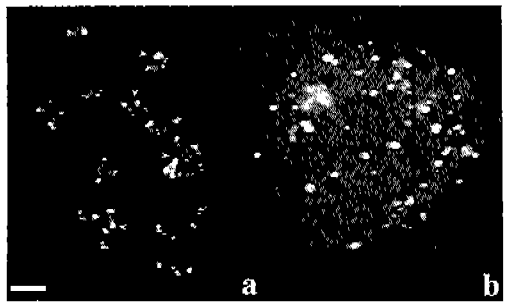


Fig. 6. Rice chromosomes originated from the C genome in the tetraploid species were painted brightly by GISH using the genomic DNA isolated from the C genome species (Fukui et al., 1997).

and threshold levels between the two wild species allowed to conclude that the overall similarity of nucleotide sequences between the C and D genomes is higher than those between the B and C genomes. Figure 7 depicts a schematic representation of the phylogenetic relationship among the three rice genomes examined putting the C genome as a pivotal genome. The phylogenetic relationship among the B, C and D genomes deduced by the GISH experiment is

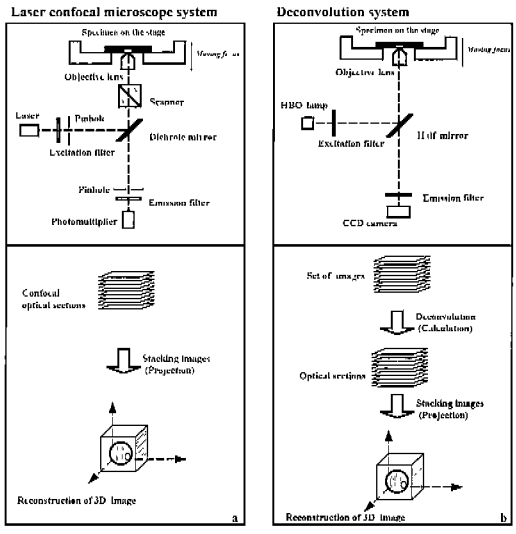


Fig. 7. Phylogenetic representation among the rice B, C and D genomes (Fukui et al., 1997).

consistent with that estimated by the nucleotide sequence homology among the chloroplast DNAs (Dally and Second, 1990).

Sequential application of GISH and a laser-dissection method presents further possibility to recover the DNA sequences from the missing D genome. Micro-laser irradiation through an objective has already been proved to be effective to dissect out certain regions of chromosomes as shown in Figure 8 (Fukui et al., 1992, Kamisugi et al. 1993). 45S rRNA genes from chromosomes of a woody plant, *Sequoiadendron giganteum* were also successfully recovered and used in FISH (Nakamura and Fukui, 1997). Thus elimination and recovery of *in situ* labeled 12 C genome chromosomes from the complement is technically feasible. DOP-PCR and linker aided PCR would also provide a method to amplify the D genome specific nucleotide sequences.

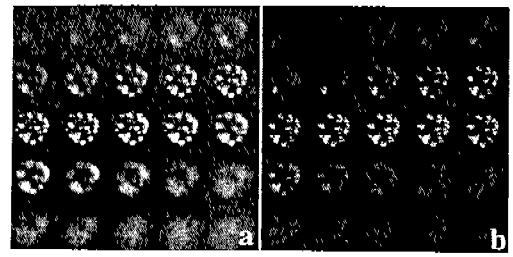


Fig. 8. Laser dissection of barley chromosomes under a microscope (Fukui et al. 1992).

Somatic cell fusion and remote hybridization through embryo rescue allow even artificial polyploids in plants with various genome combinations. Back crossing and back fusion techniques are both effective to introduce useful traits in wild plant species to cultivated species. Recurrent back crossing and selection are both important to derive a cultivate species with the target trait of wild species. One back crossing with cultivated species reduces a half of the wild genome theoretically. GISH also provides an effective technique to reveal presence of different

genomes and to monitor the processes of reduction of the wild genome after the hybridization between cultivated and wild species.

### Three dimensional analyses of plant chromosomes and genomes

It is not possible to observe a nucleus and chromosomes three-dimensionally using a conventional optical microscope because out-of-focus information obscures detail in the focal plane, and degrades the image by reduction of contrast. For three-dimensional (3D) analysis, out-of-focus signals can be removed by two methods, confocal microscopy and computational deconvolution. The confocal microscope produces an image without out-of-focus information by mechanical means setting a pin hole just before a photomultiplier, while deconvolution removes out-of-focus information from the captured image by computation (Figure 9). After removing the out-of-focus signals, both methods reconstruct 3D images from a series of two-dimensional (2D) sectioned images, which are collected by focusing the microscope at different focal planes through the specimen (Wako et al. 1998).

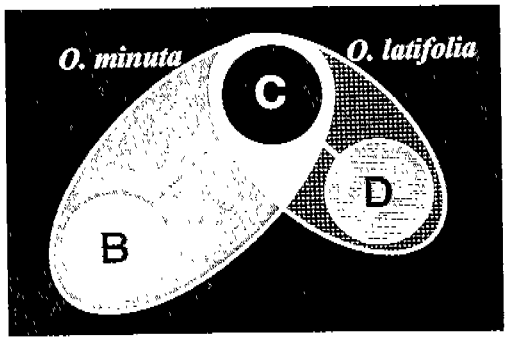


Fig. 9. Schematic representation of the two 3D analysis systems of confocal microscope (a) and deconvolution system (b) (Wako et al., 1988).

3D topographical analysis of the spatial differentiation of distribution of the different genomes within a nucleus was unequivocally proved by the 3D analysis using confocal microscopy. The rice E genome specific retrotransposon (RIRE1, Nakajima et al.,1996) was *in situ* hybridized to hybrid cells between *O. sativa* (AA) and *O. australiensis* (EE). As shown in Figure 10, RIRE distributes abundant in *O. australiensis* chromosomes (Uozu et al., 1997) and the chromosomes derived from *O. australiensis* clustered within a nucleus occupying the different nuclear domain from the chromosomes derived from *O. sativa*. Three dimensional multi-color FISH (McFISH) using centromeric and telomeric probes simultaneously revealed that the centromeres and telomeres occupies opposite domains within a barley nucleus.

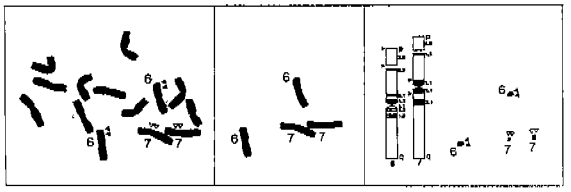

Fig. 10. 3D-FISH using the retrotransposon, RIRE1 analyzed through the deconvolution system (Ohmido, in preparation).

## CONCHUSION

We present several examples of one-dimensional, two-dimensional and even three dimensional analyses in plant chromosomes and genome using mainly FISH and IMS methods. It is important to understand the function and morphology of plant chromosomes and genomes without loosing their live structures. Rapid development of optical and computational methods makes the research based on human visual perception reliable and indispensable for chromosome and genome researches in plants. It is anticipated that these methods will be further developed until the functional and structural

dynamics of the chromosomes and genomes could be visualized in living cells in the near future.

## REFERENCES


1. Causse, M. A., Fulton, T. M., Cho, Y. G., Ahn, S. N., Chunwongse, J., Wu, K., Xiao, J., Yu, Z., Ronald, P. C., Harrington, S. E., Second, G., McCouch, S. R., and Tanksley, S. D. (1994) Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics 138:1251-1274.
2. Dally, a. M., and Second, G. (1990) Chloroplanst DNA diversity in wild and cultivated species of rice (Genus *Oryza*, section Oryza). Cladistic-mutation and genetic-distance analysis. Theor. Appl. Genet. 80: 209-222.
3. Fransz, P. F., Alonso-Blanco, C., Liharska, T. B., Peeters, A. J. M., Zabel, P., and de Jong, J. H. (1996) High resolution physical mapping in *Arabidopsis thaliana* and tomato by fluorescence *in situ* hybridization to extended DNA fibers. Plant J 9:421-430.
4. Fukui, K. (1986) Standardization of karyotyping plant chromosomes by a newly developed chromosome image analyzing system (CHIAS). Theor. Appl. Genet. 72:27-32.
5. Fukui, K. (1988) Analysis and utility of chromosome information by using the chromosome image analyzing system, CHIAS. Bull. Natl. Inst. Agrobiol. Resour. 4:153-176.
6. Fukui, K. (1996a) Advances in rice chromosome research, 1990-1995. In: G. S. Khush (ed.). Rice Genetics III. Proceedings of the Third International Rice Genetics Symposium, pp.117-130. International Rice Research Institute, Manila
7. Fukui, K., and Iijima, K. (1991) Somatic chromosome map of rice by imaging methods. Theor. Appl. Genet. 81:589-596.
8. Fukui, K., and Kakeda, K. (1990) Quantitative karyotyping of barley chromosomes by image analysis methods. Genome 33:450-458.
9. Fukui, K., and Kamisugi, Y. (1991) Simulation of microscopic images by a desktop imaging system. In: M. Doyama et al. (eds.), Computer Aided Innovation of New Materials, pp. 101-104. Elsevier Science Publishers, Amsterdam.
10. Fukui, K., and Kamisugi, Y. (1995) Mapping of C-banded Crepis chromosomes by imaging methods. Chromosome Res. 3:79-86.
11. Fukui, K., Kamisugi, Y., and Sakai, F. (1994) physical mapping of 5SrDNA loci by direct-coned biotinylated probes in barley chromosomes. Genome 37:105-111.
12. Fukui, K., Minezawa, M., Kamisugi, Y., Yanagisawa, T., Fujishita, M., and Sakai, F. (1991) Microdissection of barley chromosome by the cell workstation. Barley Genet. 6:272-276.
13. Fukui, K., Minezawa, M., Kamisugi, Y., Ohmido, N., Ishikawa, T., Yanagisawa, T., Fujishita, M., and Sakai, F. (1992) Microdissection of plant chromosomes by argon ion laser beam. Theor. Appl. Genet. 84:787-791.
14. Fukui, K., and Mukai, Y. (1988) Condensation pattern as a new image parameter for identification of small chromosomes in plants. Jpn. J. Genet. 63:359-366.
15. Fukui, K., and Nakayama, S. (1996) Analysis of chromosome information. In: K. Fukui and S. Nakayama (eds.), Plant Chromosomes: Laboratory Methods, pp. 247-261. CRC Press Inc, Boca Raton.
16. Fukui, K., Shishido, R., and Kinoshita, K. (1997) Identification of the rice D genome chromosomes by genomic in situ hybridization. Theor. Appl. Genet. 95:1239-1245..
17. Jiang, J., and Gill, B. S. (1993) Sequential chromosome banding and *in situ* hybridization analysis. Genome 36: 792-795.
18. Jiang, J., and Gill, B. S. (1994) Nonisotopic *in*

*situ* hybridization and plant genome mapping: the first 10 years. Genome 37: 717-725.
19. Kamisugi, Y., and Fukui, K. (1990) Automatic karyotyping of barley chromosomes by imaging techniques. BioTechniques 8:290-295.
20. Kamisugi, Y., Furuya, N., Iijima, K., and Fukui, K. (1993) Computer-aided automatic identification f rice chrom0somes by image parameters. Chromosome Res. 1:189-196.
21. Kamisugi, Y., Nakayama, S., Nakajima, R., Ohtsubo, H., Ohtsubo, E., and Fukui, K. (1994) Physical mapping of the 5S ribosomal RNA genes on rice chromosome 11. Mol. Gen. Genet. 245:133-138.
22. Kamisugi, Y., Sakai, F., Minezawa, M. Fujishita, M., and Fukui, K. (1992) Recovery of the dissected C-banded regions in *Crepis* chromosomes. Theor. Appl. Genet. 85:825-828.
23. Kato, S., and Fukui, K. (1998) Condensation pattern (CP) analysis of plant chromosomes by an improved chromosome image analyzing system, CHIAS III. Chromosome Res. (in press).
24. Kato, S., Hirose, T., Akiyama, Y., ONeill, C. M., and Fukui, K. (1997) Manual on the chromosome image analyzing system III, CHIAS III. Res. Rep. Agr. Devel. Hokuriku Area 36:1-76.
25. Kurata, N., Nagamura, Y., Yamamoto, K., Harushima, Y., Sue, N., Wu, J., Antonio, B. A., Shomura, A., Shimizu, T., Lin, S. Y., Inoue, T., Fukuda, A., Shimano, T., Kuboki, Y., Toyama, T., Miyamoto, Y., Kirihara, T., Hayasaka, K., Miyao, A., Monna, L., Zhong, H. S., Tamura, Y., Wang, Z. X., Momma, T., Umehara, Y., Yano, M., Sasaki, T., and Minobe, Y. (1994) A 300 kilobase interval genetic map of rice including 883 expressed sequences. Nature Genet 8: 365-372.
26. Lawrence, J. B., Singer, R. H., and McNeil, J. A. (1990) Interphase and metaphase resolution of different distance within the human dystrophia gene. Science 249: 928-932.
27. Ma, Y., Tomita, M., Nakata, N., Yasumuro, Y., Ohmido, N., and Fukui, K. (1997) Analysis of the chromosomes composition of common wheat-*Agropyron intermedium* partial amphiploid, Yuan Zhong 2 by *in situ* hybridization. Acta Genet. Sinica 24:344-349.
28. Miyamoto, J., Kurita, S., and Fukui, K. (1991) Image analysis of C-banded patterns in two herbs: *Paris tetraphylla* A. Gray and *Paris verticillata* M. Bieb, (Liliaceae). Jpn. J. Genet. 66:335-345.
29. Nakajima, R., Noma, K., Ohtsubo, H., and Ohtsubo, E. (1996) Identification and characterization of two tandem repeat sequences (TrsA and TrsC) and retrotransposon (RIRE1) as genome-general sequences in rice. Genes Genet. Syst.71:373-382.
30. Nakamura, S., Asakawa, S., Ohmido, N., Fukui, K., Shimizu, N., and Kawasaki, S. (1997) Construction of an 800-kb contig in the near-centromeric region of the rice blast resistance gene *Pi-ta2* using a highly repetitive rice BAC library. Mol. Gen. Genet. 254: 611-620.
31. Nakamura, M., and Fukui, K. (1997) A chromosome-oriented approach to genome analysis in a woody plant - *Sequoiadendron giganteum* (Lindl.) Buchholz. In: Z. Borzan and S. E. Schlarbaum (eds.), Cytogenetic studies of forest trees and shrub species. Univ. Zagreb, Zagreb, Croatia.
32. Nakayama, S., and Fukui, K. (1997) Quantitative chromosome mapping of small plant chromosomes by improved imaging on CHIAS II. Genes Genet. Syst. 72:35-40.
33. Ohmido, N., and Fukui, K. (1995) Cytological studies of African cultivated rice *Oryza glaberrima* Steud. Theor. Appl. Genet. 91:212-217.
34. Ohmido, N., and Fukui, K. (1997) Visual verification of close disposition between a rice A genome-specific DNA sequence (TrsA) and the telomere sequence. Plant Mol. Biol. 35:

963-968.

35. Ohmido, N., Akiyama,Y., and Fukui, K. (1998) Systematic mapping of unique nucleotide sequences on identified rice chromosomes. Plant Mol. Biol. (in press)
36. Ohtsubo, H., Umeda, M., and Ohtsubo, E. (1991) Organization of DNA sequences highly repeated in tandem in rice genomes. Jpn J. Genet. 66:241-254.
37. Song, W. Y., Wang, G. L., Chen, L. L., Kim, H. S., Pi, L. Y., Holstein, T., Gardner, J., Wang, B., Zhai, W. X., Zhu, L. H., Fauguet, C., and Ronald, P. (1996) A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa-21*. Science 270: 1804-1806.
38. Trask, B. J. (1991) Fluorescence *in situ* hybridization: application in cytogenetics and gene mapping. Trends Genet. 7: 149-154.
39. Uozu, S., Ikehashi, H., Ohmido, N. Ohtsubo, H., Ohtsubo, E., and Fukui, K. (1997) Repetitive sequences: cause for variation in genome size and chromosome morphology in the genus *Oryza*. Plant Mol. Biol. 35:963-968.
40. Wako, T., Fukuda, M., Furushima-Shimogawara, R., Belayed, N. D., Turner, B. M., and Fukui, K. (1998) Comparative analysis of the topographical distribution of acetylated histone H4 using confocal microscopy and deconvolution system. Anal. Chim. Acta (in press).
41. Zhang, H. B., Choi, S., Woo, S. S., Li, Z., and Wing, R. A. (1996) Construction and characterization of two rice bacterial artificial chromosome libraries from the parents of a permanent recombinant inbred mapping population. Mol. Breed. 2:11-24.