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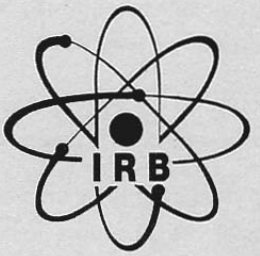


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# VISUALIZATION OF INDUCED VARIATIONS AT DNA AND CHROMOSOME LEVELS

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## Introduction

Variations at DNA and chromosome levels are the basis of mutation breeding because these primary events lead to mutation at the plant and animal levels. Various methods have been developed to detect variations at DNA and chromosome levels. It was difficult to detect and evaluate the variations at DNA level accurately and has been obstacle to improve the efficiency of mutation breeding. On the other hand, chromosomes that are visible genetic materials under a photomicroscope, have been extensively studied for detection of chromosomal mutations and evaluation of mutation efficiency because translocation and deletion are relatively easy to detect by photomicroscopy. Recently detection methods for variation at DNA levels are much developed and they are practically used for the detection of various variation eveu at DNA levels. In this review, various visualization methods for detection of chromosomal variation are outlined and recent advances in visualization methods at DNA level are also summarized.

## 1. Visual detection of variations at chromosome levels

Karyotype analysis and banding methods have been used to detect variations at chromosome levels, such as translocation, deletion, inversion, etc. Recently fluorescence *in situ* hybridization method (FISH, e.g., OHMIDO *et al.* 1998) is frequently used to detect such chromosome aberrations. Figure 1 depicts a result of FISH of rice chromosomes using 45S ribosomal RNA genes as probe and the experimental procedures. Now FISH method enables the detection of DNA sequences as small as 1 kb even in plant chromosome (OHMIDO *et al.* 1998). The key point of FISH is that the method provides positional information on localization of the target genes by fluorescent signal. Thus variability and translocation of even single genes occurred either naturally or artificially can be detected by FISH. Variability in ribosomal DNA loci is detected in the genus *Oryza* (FUKUI *et al.* 1994, SHISHIDO *et al.* 2000), tribe wheat (DUBCOVSKY and DVORAK 1995), *Glycine* (SINGH *et al.* 2001), *Allium* (SCHUBELT *et al.* 1984), etc. by FISH. Comparative genomic hybridization (CGH) enables to detect amplification of certain genes at specific chromosomal

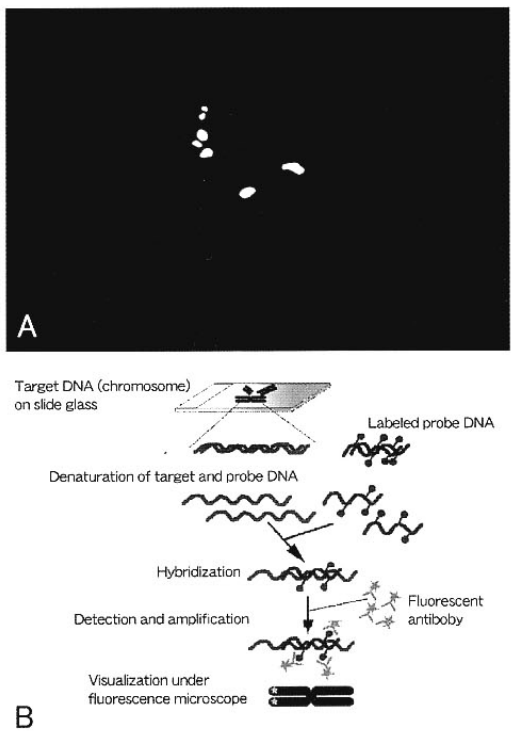


Fig. 1 FISH on rice chromosomes (Indica type) with 45S rDNA probe (A)[4] and schematic representation of FISH method (B).

regions or loss of specific genes. CGH is mainly used in gain and loss of genes in human cancer cells (LARRAMENDY *et al.* 2000).

Genomic *in situ* hybridization (GISH, SCHWARZACHER *et al.* 1989) is also an effective method to detect genomic belongings of chromosomes in allopolyploid species. Figure 2 shows typical example of GISH to paint differently the two different chromosome groups from cultivated tomato (*Lycopersicon esculentum*, 2n = 24) and its wild relative (*Solanum lycopersicoides*, 2n = 24) with disease resistant genes generated by somatic hybridization (ESCALANTE *et al.* 1998). Yellow fluorescent signals inserted in the middle part of the red chromosomes may indicate insertion of wild relative chromosome fragment. Insertion of small chromosome fragment into the other chromosomes after cell fusion is also detected in somatic hybrids of rice (SHISHIDO *et al.* 1998). GISH is thus an effective method to detect intergeneric chromosome aberration both after sexual and somatic hybridization.

A modified FISH method which uses chromosome specific DNA probes is now available in detection of specific human chromosomes. Chromosomal rearrangements in human lymphocytes induced by X-rays (0, 0.5, 1.0 and 2.0 Gray) were analyzed using chromosome painting. DNA

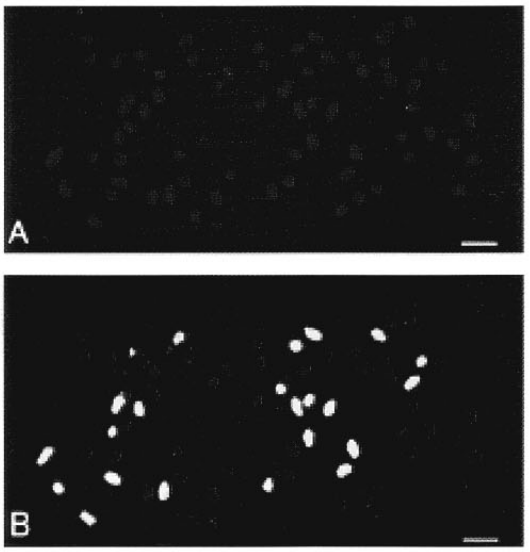


Fig. 2 Chromosomal spread of a somatic hybrid between *Lycopersicon esculentum* and *Solanum lycopersicoides* (A) and signal tagged *S. lycopersicoides* derived chromosomes after GISH[2]. Bar shows 10 $\mu$m.

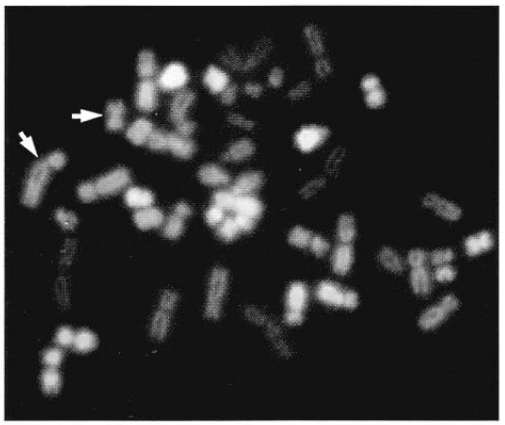

Fig. 3 Detection of reciprocal translocations (solid arrows) differently colored by M-FISH[19].

probes for human chromosomes 1, 3 or 4 alone, and a combination of 1 and 4, were used for analysis (MATSUOKA *et al.* 1994). Combination of all the chromosome specific repetitive sequences allows to paint all human chromosomes in different colors. The method is referred to as M-FISH. Figure 3 demonstrates the M-FISH of human chromosomes in different colors. In this

case combination of five different haptens were used to color 21 autosomes and X and Y chromosomes (HENEGARIU *et al.* 1999). The colors themselves are computer generated false color based on the different wave length from the different fluorescent dyes. Chromosomal aberrations are easily detected not by complicated banding patterns but simply by difference in color. Thus it is concluded that the chromosome painting, represented by GISH and M-FISH methods is a simpler, more objective and more practical method for detecting chromosome rearrangements than conventional banding and karyotype analyses.

## 2. Visual detection of variations at DNA level

Diameter of a DNA molecule is 2 nm and the dimension is far smaller than the wave length of visible light. As a result, it is not possible to see the DNA molecule directly by optical methods. It becomes, however, possible to visualize DNA molecules directly using various methods. Fluorescent staining method is the most frequently used ones among them by the development of efficient fluorescent dyes. Figure 4 shows visualization of single DNA molecules of linear, open circular (OC) or covalently closed circular (CCC) conformation.

Visualization of DNA molecules under a microscope opens various application fields. DNA breakage after irradiation of ionizing radiation, such as $\gamma$-rays, can be easily visualized. The DNA damage is the primary step for all genetic variations leading to stable mutations. It is, however, difficult to quantify the DNA damage accurately after mutagenic treatments. Thus the combing method (MICHALET *et al.* 1997) which is developed to linearly arrange the DNA molecules dissolved in a solution to cover slips, is developed. Figure 5 depicts the DNA molecules extended linearly on the glass chip and some DNA molecules have double strand breakages. Quantification of the break points directly gives the quantitative data for the primary DNA damages after

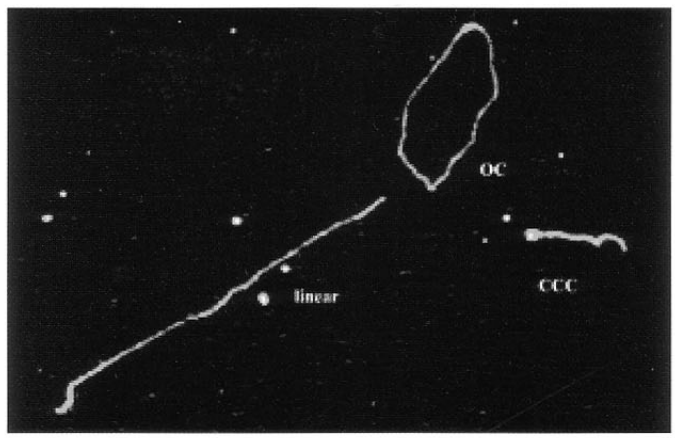


Fig. 4 Visualization of single DNA molecules by fluorescent microscopy.

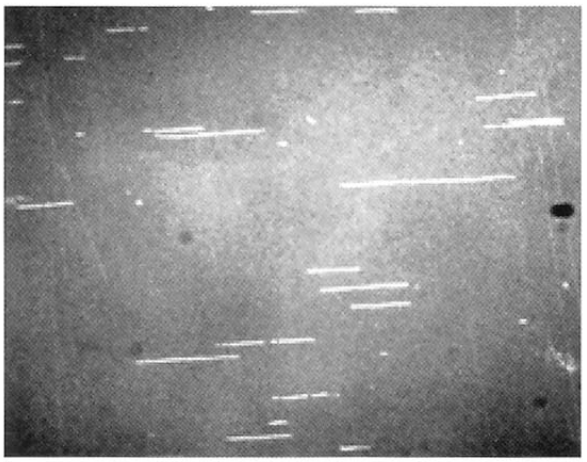

Fig. 5 DNA double strand breakage visualized after $\gamma$-ray irradiation.

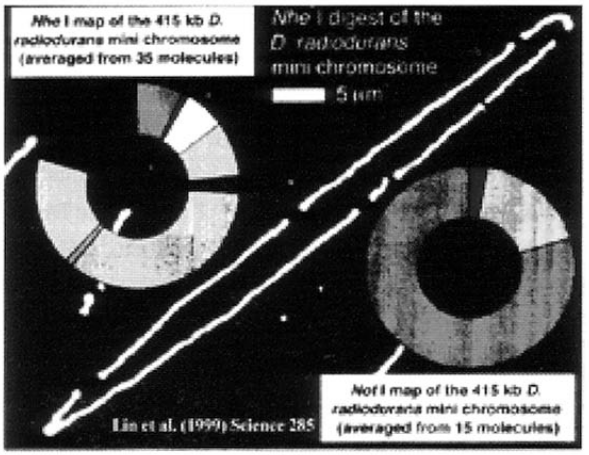


Fig. 6 Optical mapping of restriction sites on *D. radiodurance* chromosome[6].

rradiation of ionizing radiations. This method allows the direct estimation of primary genetic :ffect on the radiation. Now restriction sites can be visually determined in the bacterial DNA (LIN *:t al.* 1999). Figure 6 depicted the restriction sites of *Nhe* I in the circular mini-chromosome of *Dienococcus radiodurance*. The method enables visual detection of the restriction sites and is now :eferred to as optical mapping. As intuitively accepted, the optical mapping method is far more :fficient than the conventional biochemical means to detect restriction sites on DNA providing the :uitable experimental conditions.

It is now also practical to apply FISH method to the DNA molecules extended on glass slides

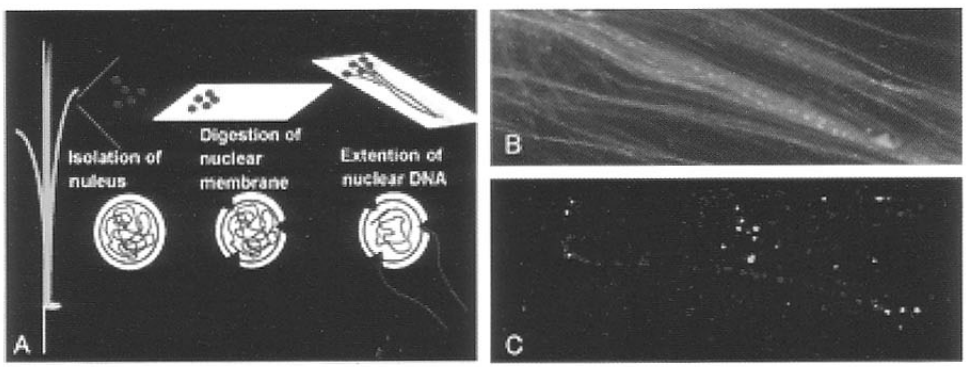


Fig. 7 Preparation of extended DNA fibers (A), DAPI stained rice DNA fibers (B) and signal tracks of TrsA (red) and telomere sequence (green) on a rice extended DNA fiber (C)[10].

(FRANSZ *et al.* 1996, ZHONG *et al.* 1998). The method referred to as extended DNA fibers FISH (EDF-FISH) requires the extension of DNA fibers on glass slides first and then usual FISH method follows. Figure 7 shows the outline of this method, extended rice DNA fibers on a glass slide, and result of the multi-color FISH using telomere and subtelomeric (TrsA) sequences. Rice nuclei are isolated from rice leaves and placed at one end of glass slide and dried once. After application of a nuclear membrane digestion buffer, the glass slide is tilted at suitable angle and allows the extraction and extension of DNA fibers from rice nuclei. The genomic DNAs extended on the glass slide are stained with diaminido-phenylindole (DAPI). Although bundles of DNA fibers are visualized after staining with DAPI, a fluorescent dye of YOYO-1 enables to visualize single DNA fibers (Fig. 7B). Simultaneous detection of telomere sequences with green fluorescent dots and tandem repeat sequence A (TrsA) with red fluorescent signal tracks revealed the relative potions of telomere and TrsA sequences (Fig. 7C). Furthermore the copy number of telomere and TrsA can be estimated just measuring the length of each signal track providing each unit length. As a result, telomeric structure of rice chromosome at DNA level is visually revealed. Variation between rice chromosome 6 and 12, which have TrsA repeats at the end of long arms, is also detected visually (OHMIDO *et al.* 2001).

DNA molecules is visualized with scanning prove microscopy (SPM) as well. It is practical to visualize single DNA molecules by SPM and there are many trials to visualize even the nucleotide sequences of DNA molecules by atomic force microscopy, which is a type of SPM. Using the complex of RecA proteins and complementary DNA sequences, it is possible to produce complex of RecA proteins and complementary DNA sequence at the target DNA site, *in vitro*. The knob-like complex produced on the DNA fiber is a good morphological marker indicating that the place is the target region. The knob-like structure is visible by atomic force microscopy (SEONG *et al.* 2000).

## Conclusion

Visualization methods for genetic materials are developing very rapidly as mentioned above. Now methodological development enables it practical from painting each chromosome in different color by M-FISH to detection of single nucleotide change by rolling circle amplification (ZHONG *et al.* 2001). Not only can we visualize single DNA fibers, but also detect specific nucleotide sequences and double strand breakage visually. As a result, it is concluded that the visualization methods are now quite effective for mutation research. Second, it is anticipated to understand all the mutational processes by visual approaches. Third, it is also anticipated that the mutation genetics and breeding are to be accelerated by the aid of visualization methods because all these technologies provide us quite flexible approaches to the studies on genetic materials of chromosomes and DNAs.

## Acknowledgments

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## Summary

Current status of visualization methods for DNA and chromosomes is reviewed in relation to mutation genetics and breeding. Now it is quite practical to paint each chromosome in different color by different constitution of repetitive sequences in each chromosome using M-FISH method. It allows easy, reliable and objective detection of chromosome aberration. It is also feasible to detect even single nucleotide changes and double strand breakage visually. All these technological developments provide us more efficient and powerful tools for mutation research.

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