PLANT MOLECULAR CYTOGENETIC ANALYSES

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Molecular cytogenetics involves a combination of molecular biology and cytogenetics. In general, this involves the use of a series of techniques referred to as the fluorescence *in situ* hybridization (FISH), in which DNA probes are labelled with differently coloured fluorescent tags to visualize one or more specific regions of the genome (*Figure 1*). The technology enables the location of DNA sequences such as ribosomal DNA, transgenes and specific ISSR and RFLP markers to identify individual chromosome pairs, and also to distinguish between *Elaeis oleifera* and *E. guineensis* chromosomes in interspecific hybrids.

METHODOLOGY

FISH experiments are performed on oil palm metaphase chromosomes or interphase nuclei in the manner shown in *Figure 2*.

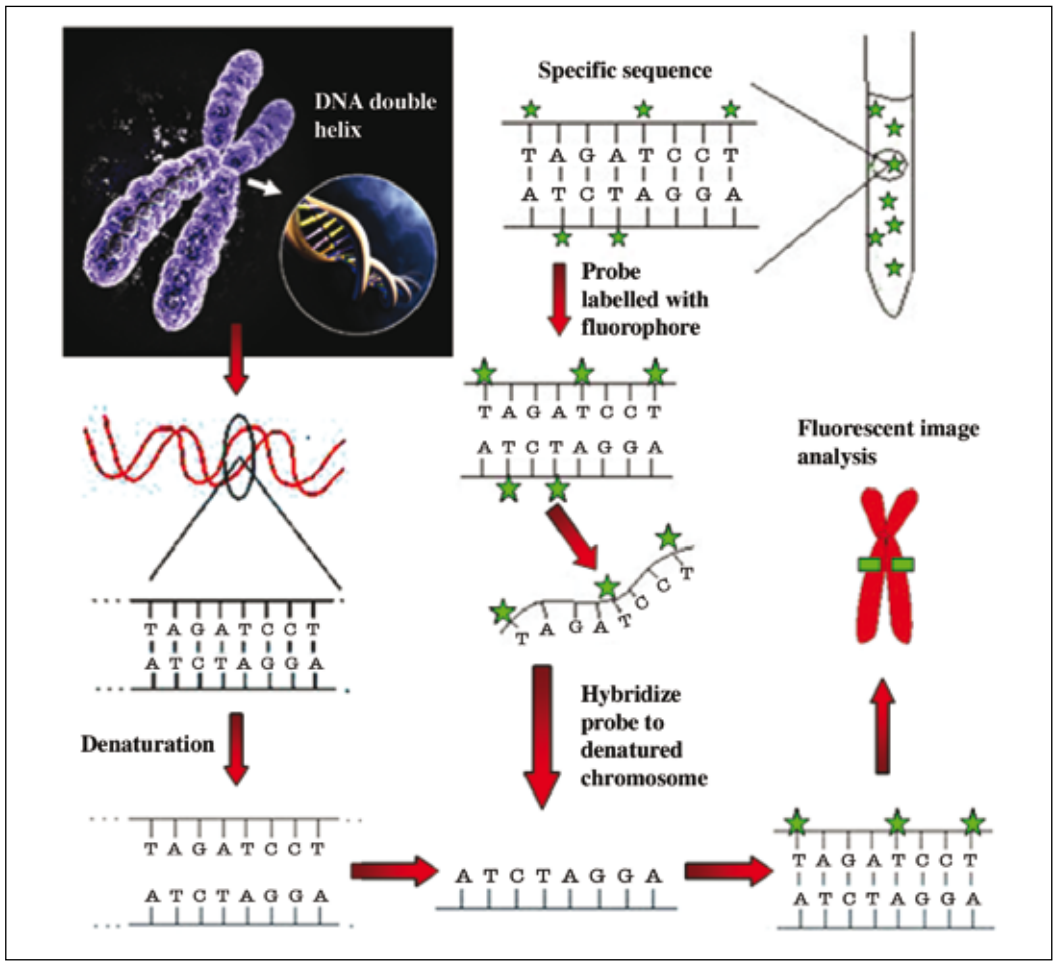

Figure 1. The principles of a FISH experiment to locate selected probes or labelled DNA sequences on chromosomes.

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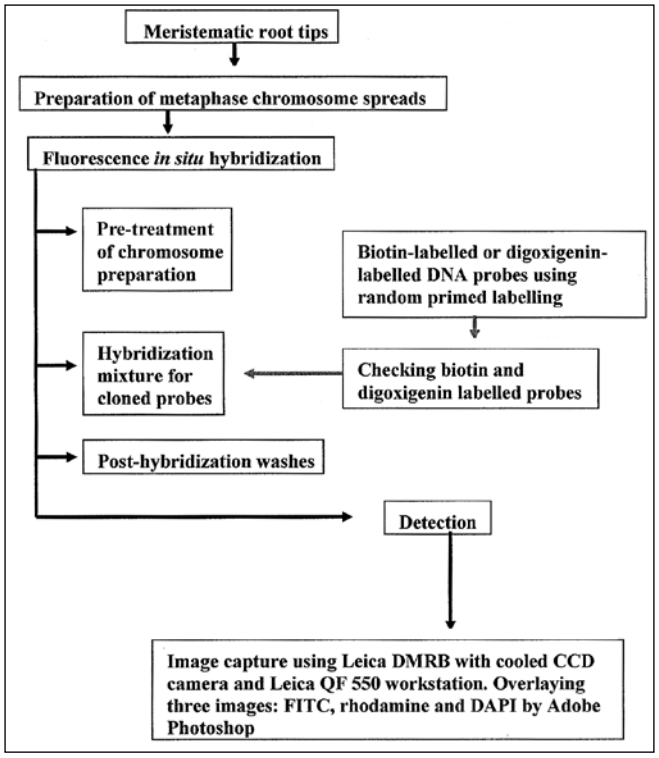

Figure 2. Fluorescence in situ *hybridization (FISH) experimental approaches.*

APPLICATIONS

FISH experiments are performed on oil palm metaphase chromosomes or interphase nuclei in the following applications:

(1) To locate a DNA sequence of interest on the oil palm chromosomes. Several examples follow:
   a) 5S ribosomal DNA (*Figure 3*).
   b) 18S-25S ribosomal DNA (*Figure 4*).
   c) Transgenes or plasmid pME22 carrying *bar, phaC, bktB* and *phaB* genes driven by maize polyubiquitine promoters for synthesizing PHB (*Figure 5*).
   d) Specific ISSR and RFLP markers for oil palm chromosome identification.
   The chromosome number of *E. guineensis* and *E. oleifera* is 2n=32 (Madon *et al., 1998*), and can be divided into three groups on the basis of length. Group I consists of chromosome number 1 (longest), group II consists of chromosome numbers 2-9 (medium long) and group III consists of numbers 10-16 (medium short chromosomes). It is difficult to distinguish between the individual chromosome pairs, hence recently ISSR and RFLP markers that map onto the same linkage group have been used as probes in simultaneous double labeling FISH experiments. *Table 1* shows the corresponding ISSR and RFLP markers used to distinguish between the individual chromosome pairs except for pairs numbers 3, 7, 11, 12 and 14. For chromosome pair number 16, the 18S-25S rDNA probe is used as its specific marker.

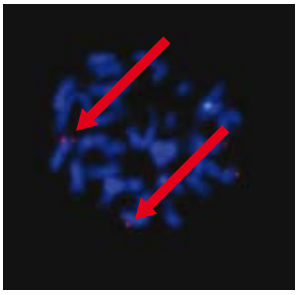

Figure 3. Oil palm metaphase chromosomes showing 5S rDNA sites on the longest chromosome pair No. 1 (red arrows).

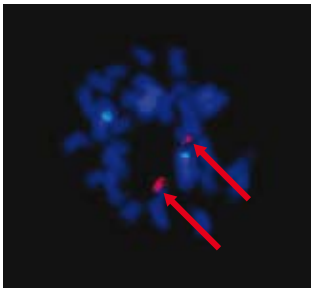

Figure 4. Oil palm metaphase chromosomes show 18S-25S rDNA sites located on the shortest acrocentric chromosome pair No. 16 (red arrows).

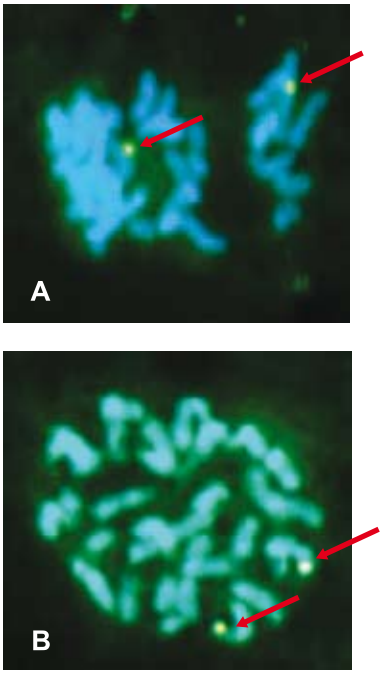

Figure 5. The transgene signals (red arrows) are located on the telomeric chromosomal regions.

TABLE 1. THE CORRESPONDING ISSR AND RFLP MARKERS ARE USED TO DISTINGUISH THE FOLLOWING INDIVIDUAL CHROMOSOME PAIRS EXCEPT FOR CHROMOSOME PAIR NUMBER 16

Chromosome pair number	Specific RFLP and ISSR markers
1	RFLP markers: pOP-MET4, pOP-SFB56 and pOP-SFB82, and ISSR marker: UBC881-1500.
2	RFLP marker: pOP-G246 and ISSR marker: UBC835/818-550/500.
4	(a) RFLP marker: pOP-MT135 and ISSR marker: UBC836-780 (b) RFLP marker: pOP-MT194 and ISSR marker: UBC845-750.
5	RFLP markers: M1C and M6B, and ISSR marker: UBC853-1050.
6	RFLP markers: pOP-SFB154 and pOP-SFB147, and ISSR marker: UBC834-530.
8	(a) RFLP marker: pOP SN1 and ISSR marker: UBC 825-750. (b) RFLP marker: pOP G16 and ISSR marker: UBC 890-725.
9	RFLP markers: pOP-ME6 and pOP-ME51, and ISSR marker: UBC 823-715.
10	(a) RFLP marker: pOP-SFB34 and ISSR marker: UBC 880/860-250. (b) RFLP markers: pOP-SFB34 and pOP G18, and ISSR marker: UBC 834-330.
13	(a) RFLP marker: pOP-G39 and ISSR marker: UBC 880-980/900. (b) RFLP marker: pOP-MT30 and ISSR marker: UBC 830/834-300.
15	RFLP markers: pOP-G39 and pOP-MT 40, and ISSR marker: UBC 808/834-700.
16	pTA 71 (18S-25S rDNA).

- **Linkage group number 4 associated with chromosome pair number 4.**
  With the following specific markers (*Figure 6*):
  RFLP marker: pOP-MT194
  ISSR marker: UBC845-750

- **Linkage group number 5 associated with chromosome pair number 5.**
  With the following specific markers (*Figure 7*):
  RFLP markers: M1C and M6B
  ISSR markers: UBC853-1050

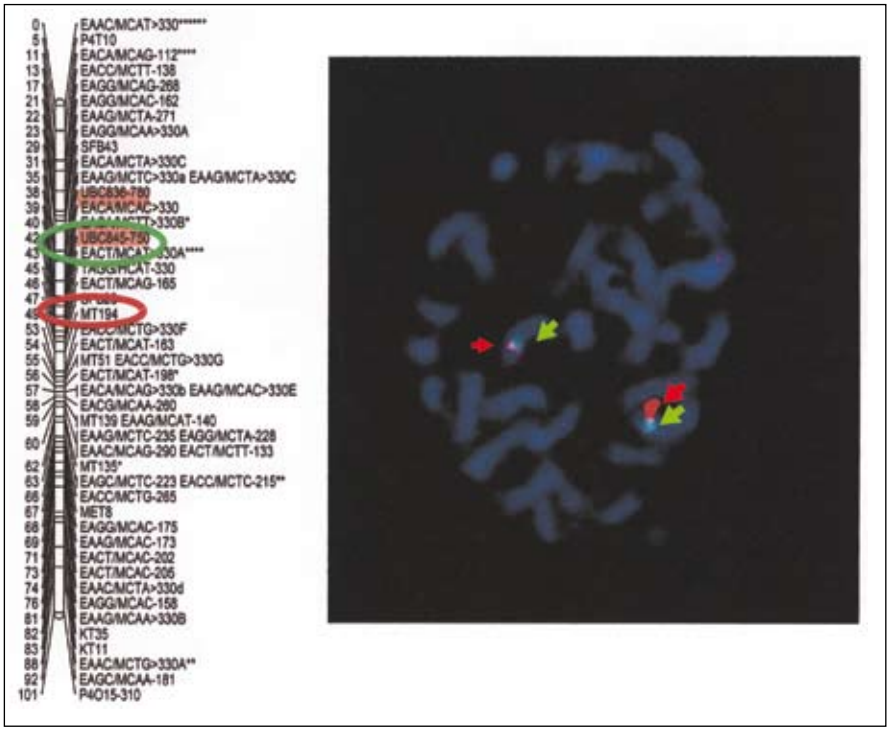

Figure 6. Linkage group 4 (left) shows the locations of pOP-MT194 (RFLP marker, red oval) and UBC 845-750 (ISSR marker, green oval). Chromosome spreads (right) show hybridization of pOP-MT194 (red) and UBC 845-750 (green) on the same medium length chromosome pair.

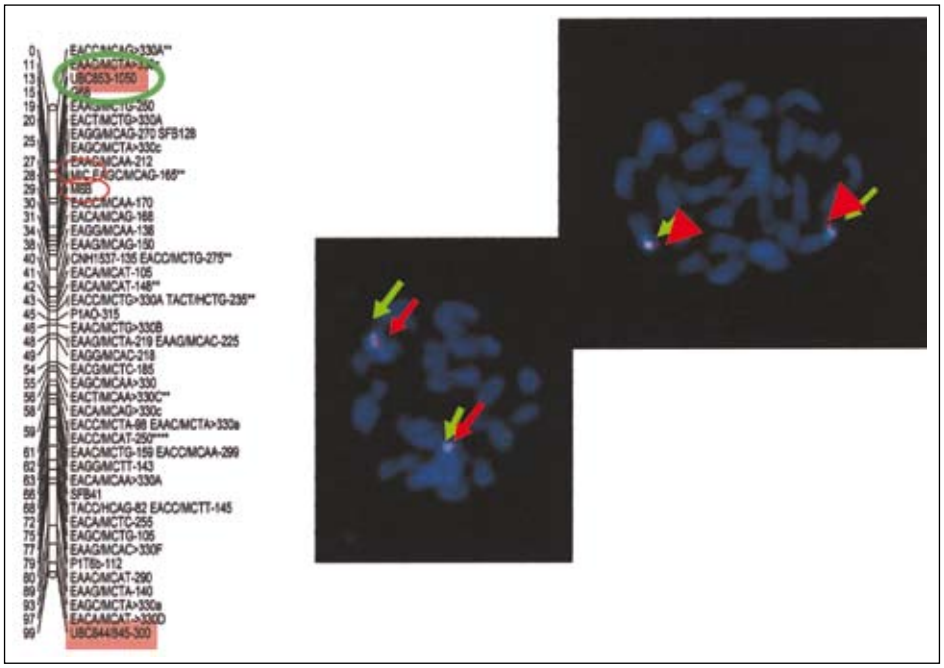

Figure 7. Linkage group 5 (left) shows the locations of M1C and M6B (RFLP markers, red oval) and UBC 853-1050 (ISSR marker, green oval). Chromosome spreads (right) show hybridization signals of M1C and M6B (red) and UBC 853-1050 (green) on the same medium length chromosome pair.

(2) To distinguish between *E. oleifera* and *E. guineensis* chromosomes in interspecific hybrids. This technique called genomic *in situ* hybridization (GISH) assists breeders involved in interspecific breeding programmes.

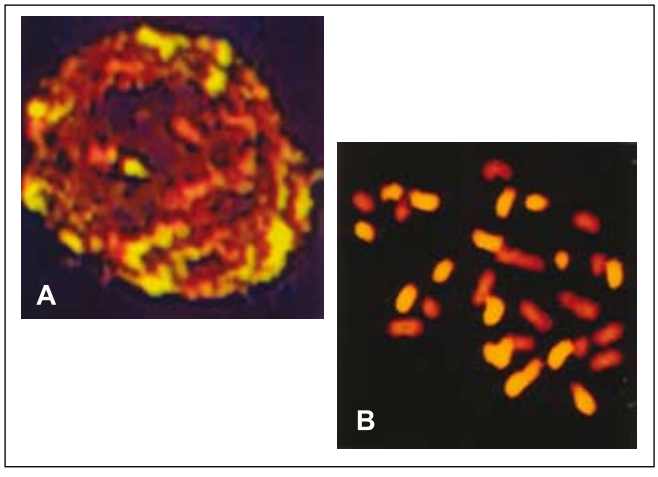

Figure 8. (A) The interphase nuclei of the OxG hybrids had groups of chromosomes from both parental genomes in discrete, non-intermixed domains indicating non-random organization of the nucleus. (B) F1 hybrids showed clear differentiation between the 16 E. oleifera *(yellow) and 16* E. guineensis *(red) chromosomes.*

CONCLUSION

Molecular cytogenetics, which allows the linking of molecular biology with cytogenetics, has revolutionized the investigation of structure, function, organization and evolution of genes and genomes by FISH techniques. As illustrated by the limited examples given above, these tools in turn provide platforms for holistic basic studies on the genome and cytogenetic mapping for crop improvement.

These services are available at a service fee varying between RM 5000 and RM 10 000 for a maximum of 15 FISH analysis, consultancy and data interpretation. However, prior to engaging the service, detailed discussions are needed with the principal investigator on the scope of analyses required.

REFERENCE

MADON, M; CLYDE, M M and CHEAH, S C (1998). Cytological analysis of *Elaeis guineensis* and *Elaeis oleifera* chromosomes. *J. Oil Palm Research Vol. 10 No. 1*: 68-91.

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