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STUDY NOTES ON GISH

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GISH (Genomic in-situ Hybridization)

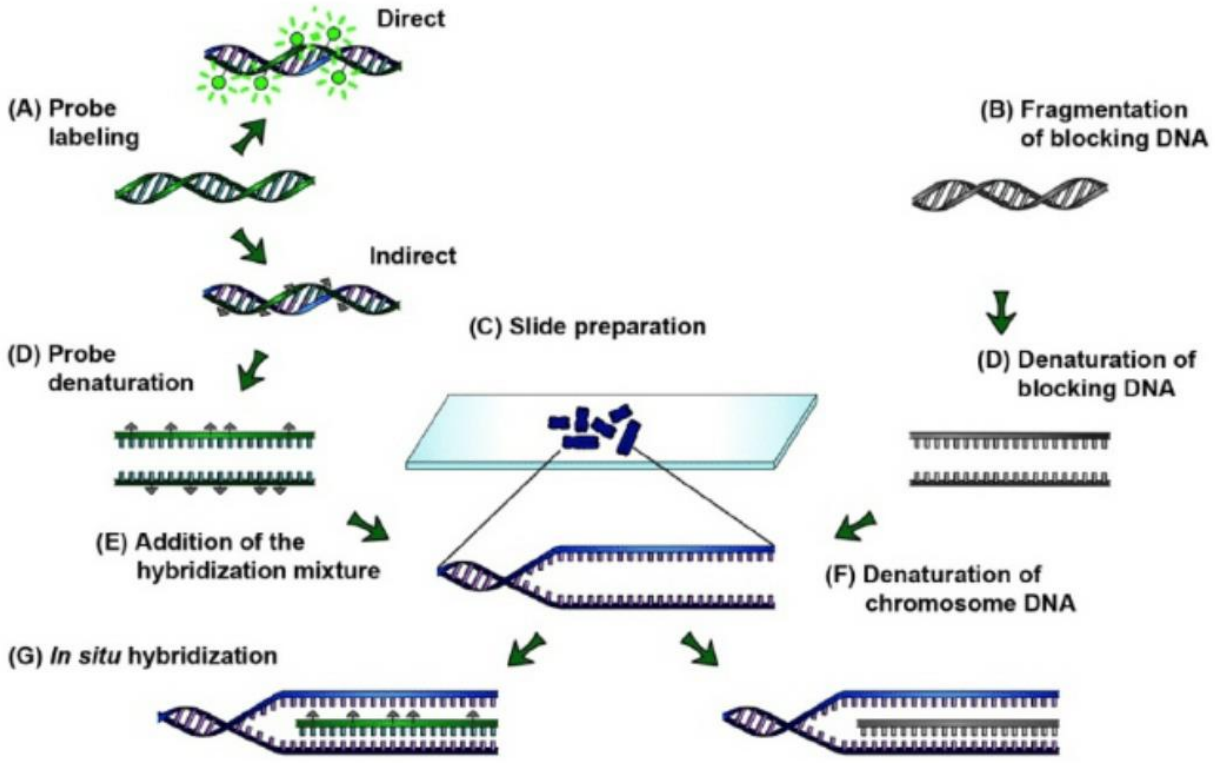
Genomic in situ hybridization (GISH), which is a modification of fluorescent in situ hybridization, has been widely used in the study of plants

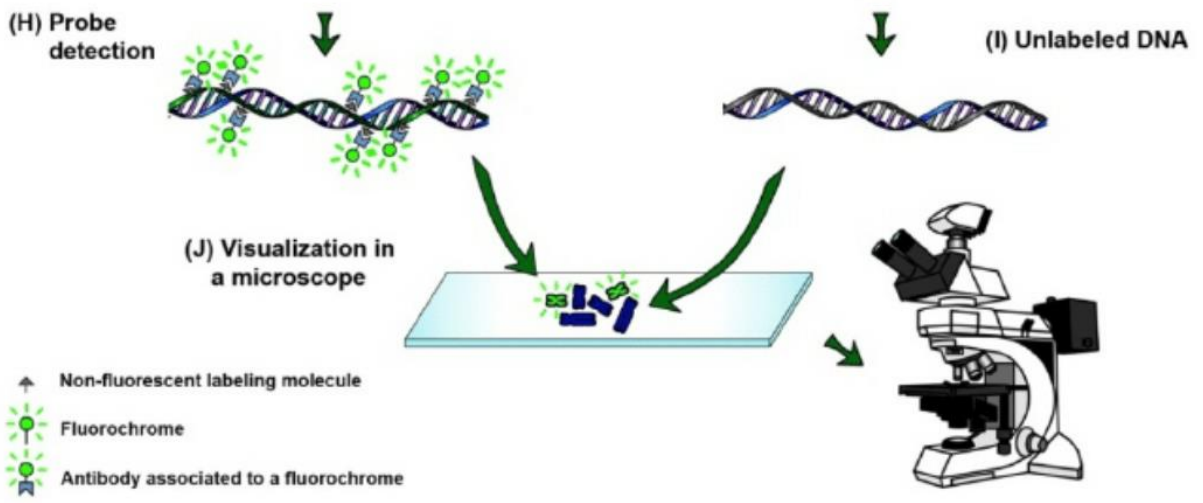
This tool has been applied to the study of genetic improvement programs, and studies of the evolution of polyploids.

In GISH, the probe is easier to obtain, and may be used directly, with no need for amplification, because the amount of DNA is limitless

Probe labeling may be carried out with random primers, PCR, or nick translation; however, labeling using kits for nick translation is more widely used

Usually, genomes that share 80-85% homology can be differentiated using standard GISH conditions





Steps:-

- Direct and indirect probe labeling.
- Fragmentation of the blocking DNA.
- Slide preparation.
- Probe and blocking DNA denaturation in a hybridization mixture.
- Addition of the hybridization mixture with the probe and the blocking DNA.
- Denaturation of the chromosome DNA.
- In situ hybridization of probe and blocking DNA in the target sequence of the chromosome.
- Detection of the probe in the chromosome DNA of one parent, in an indirect labeling.
- Chromosome DNA molecule of the second parent associated to the unlabeled blocking DNA.
- Visualization of hybridization signals associated to a probe (green) in a fluorescence microscope. Unmarked chromosomes are visualized with a counter-staining (blue). When the probe labeling is direct, the detection step of the GISH can be excluded.

Denaturation of the chromosome DNA.

In situ hybridization of probe and blocking DNA in the target sequence of the chromosome. Detection of the probe in the chromosome DNA of one parent, in an indirect labeling. Chromosome DNA molecule of the second parent associated to the unlabeled blocking DNA. Visualization of hybridization signals associated to a probe (green) in a fluorescence microscope. Unmarked chromosomes are visualized with a counter-staining (blue).

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