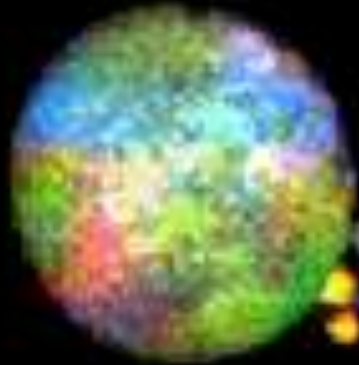


FISH and GISH: molecular cytogenetic tools and their applications



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Disadvantages of conventional cytogenetic technique

- Always study at particular stage of cell division
- Most of aberration are observed during meiosis cell division
- With Q, C, G, N and R bands are restricted to centromere or telomere or heterochromatine regions.

Disadvantages of molecular techniques

- Normal hybridization requires the isolation of DNA or RNA
- PCR
- separating it on a gel
- blotting it into nitrocellulose.
- Ex situ methods

In situ hybridization:

- In situ Hybridization (ISH) is a powerful method **to localize nucleic acid sequences in vivo** i.e. in tissues, cells, organelles, nuclei or chromosomes by using appropriate probes.
- With ISH, nucleic acids are localized in their original or proper place.

Different types localized by ISH

DNA sequence

- Repetitive seq.
- Unique seq
- Whole chromosome or part of chromosome
- Whole genome

RNA seq.

- Helps to study the spatial and temporal pattern of genes expression

Viral sequence

- Forms the basis of diagnosis of several viral diseases.

Principles underlying in situ reactions:

- Most of in situ reactions have a **1° and 2° step**.
- Reaction is carried out on chromosomes in situ involve a
- **1° reaction** and that defines the **specificity of the reaction**,
- **2° reaction** that provides the means **for detecting the product of reaction** . Chemicals used in preparation of chromosomes can determine the specificity of a primary reaction.

FISH and GISH Techniques

- modification of in situ hybridization technique
- **A fluorescent molecule is deposited** at the site of in situ hybridization location of genes or DNA can be visualized on chromosomes:- Fluorescence in situ hybridization (FISH)
- **Total genomic DNA is used as probe in hybridization experiments** :- Genomic in situ hybridization (GISH)

Fluorescence in situ Hybridization (FISH)

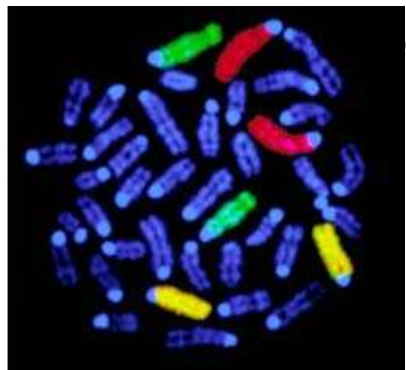
- A process which **distinctly paints** and detects RNA as well as DNA Structures, numbers and location in place in the cell or **in situ**.
- FISH may be used with:
 - Morphologically preserved chromosome preparations (**Metaphase**).
 - Fixed cells or tissue sections (**Interphase**)
- Aids in **gene mapping, toxicological studies, analysis of chromosome structural aberrations, and ploidy determination**

Advantages of Interphase FISH

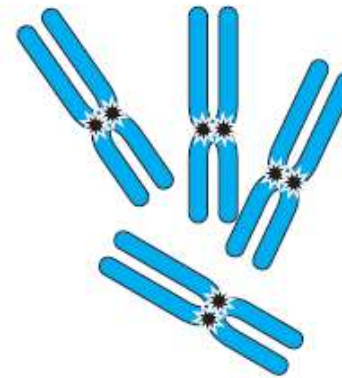
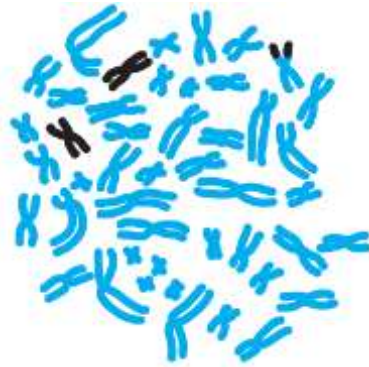
- Interphase cells for FISH **do not require culturing** of the cells and **stimulating division to get metaphase spreads**
 - interphase FISH is **faster** than methods using metaphase cells
 - valuable for analysis of cells that do not divide well in culture, including **fixed cells**.
- 200–500 cells can be analyzed microscopically using FISH
 - the sensitivity of detection is **higher than** that of metaphase procedures, which commonly examine 20 spreads.

Metaphase FISH

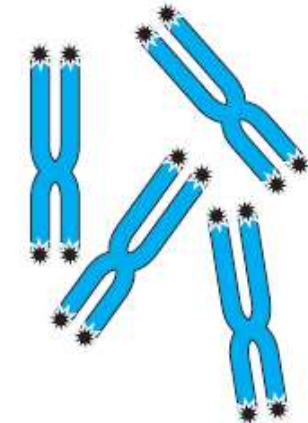
- Uses fluorescent probes that bind to metaphase chromosomal regions or to whole chromosomes.
- **Whole chromosome paints:** Probes that cover the entire chromosome, are valuable for detecting small rearrangements that are not apparent by regular chromosome banding.
- **Telomeric and centromeric probes** are also applied to metaphase chromosomes to de



or



Centromeric probes



Telomeric probes

■ Figure 8-20 Centromeric (left) and telomeric (right) probes on metaphase chromosomes.

Genomic in situ hybridization (GISH)

- Genomic in situ hybridization (GISH) Is a cytogenetic technique that allows the detection and localization of specific nucleic acid sequences on morphologically preserved chromosomes using genomic DNA of donor specie as probe.
- An unlabeled DNA of parental specie is used as competitor DNA .

- **GISH for plants...was developed in 1987 by M.D. Bennett and J.S. Heslophorizon**

Importance of FISH and GISH

- detect **specific nucleotide sequences within cells and tissues**
- **unique link** among the studies of **cell biology**, cytogenetics, and molecular **genetics**.
- it is possible to detect single-copy sequences on chromosome with **probes shorter than 0.8 kb.**

FISH essentially involves seven steps:-

- Probe DNA-Characterization.
- Nick translation labeling of probe DNA.
- Purification of labeled DNA probe.
- Chromosome preparation.
- In situ hybridization.
- Detection of hybridization.
- Microphotography.

GISH essentially involve eight steps: -

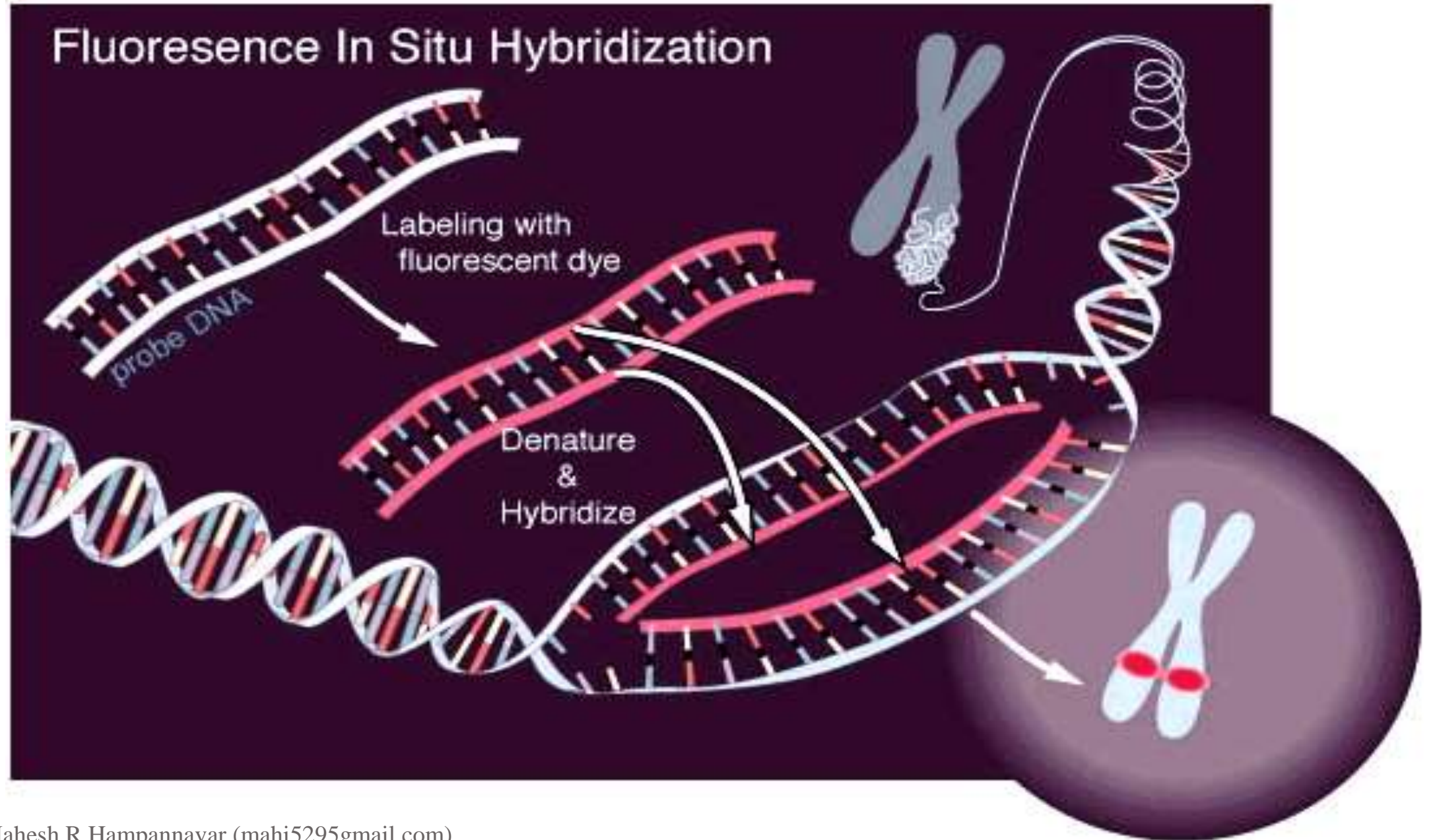
- Probe DNA
- isolation and shearing of probe DNA
- Isolation and sizing the ~~competitor DNA~~
- Nick translation labeling of probe DNA
- Purification of labeled DNA probe
- Chromosome preparation
- In situ hybridization
- Detection of hybridization
- Microphotography.

DNA from a test organism that is denatured and then used in vitro hybridization experiments in which it competes with DNA (homologous) from a reference organism; used to determine the relationship of the test organism to the reference organism.

Steps involved in FISH and GISH

- Denature the chromosomes
- Denature the probe
- Hybridization
- Fluorescence staining
- Examine slides or store in the dark

Fluorescence In Situ Hybridization



ON THE DENATURATION OF CHROMOSOMAL DNA IN SITU

- Denaturation of chromosomal DNA for fluorescence in situ hybridization (FISH) is an essential step
- shorter denaturation time in **70% formamide/2 × SSC at 72°C** provides sufficient denaturation, where the hydrogen bonds are broken between the purines and pyrimidines of the double helix
- denaturation in **alkali (0.07 N NaOH at room temperature) and formamide (90% formamide; 0.1 SSC, pH 7.2) at 65 °C**
- denaturation in HCl (0.24 M) at room temperature and 60% formamide: 2 × 10⁻⁴ M EDTA (pH 8) at 55 °C. The presence of 4% formaldehyde in the denaturation buffer prevents DNA loss.

Ramesh *et al.*, 2009

Barbera *et al.*,

Probes

- complimentary sequences of nucleotide bases
 - 20-40 base pairs or be up to 1000 bp
 - Small probe- penetration
 - RNase resistant
- Types of probe that can be used in performing in situ hybridization
 - ❖ Oligonucleotide probes
 - ❖ Single stranded DNA probes.
 - ❖ Double stranded DNA probes

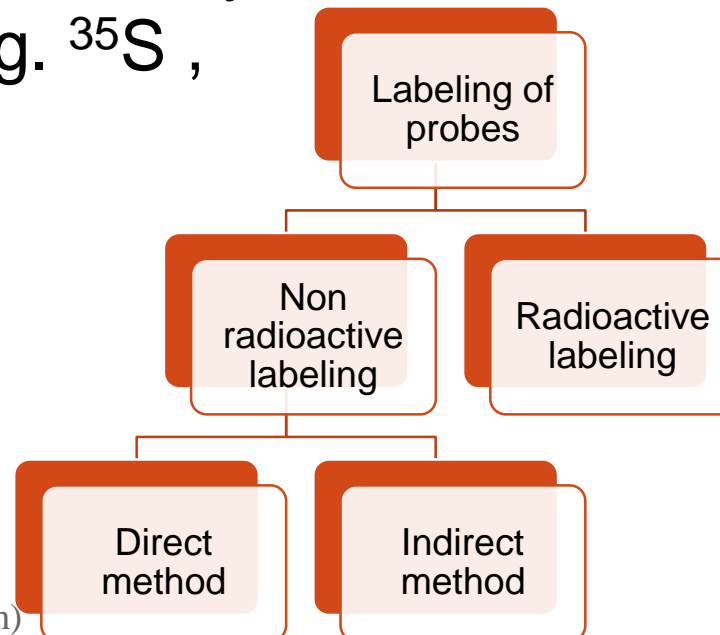
Labels...

Radioactive labels

- Radioactive labels are the isotopes which emit β -particles and are detected by autoradiography . E.g. ^{35}S , ^{32}P , ^3H

Non-radioactive labels

- Non-radioactive labelling procedures are of two types:-
Direct ISH Indirect ISH



Radioactive probe

- Traditionally oligonucleotide probes have been radiolabeled.
- Radiolabeled probes are still the choice for many workers.
- Radiolabeled probes are visualized by exposure of the tissue section or cells against photographic film which is then developed.
- When using radiolabeling, waste disposal and containment measures must be given thought and it must be remembered that the useful **shelf life of labeled probe is inherently dependent on the half life of the radionucleotide.**

Labelling and detection system

³²P

Sensitivity

Down to 10 fg

Application

All high sensitivity applications

Time required

5 min to 3 h

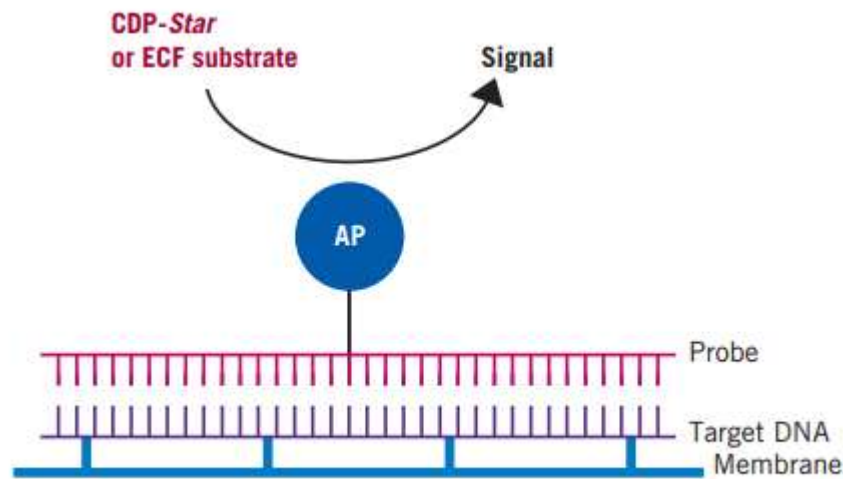
Time from preparation

to detection
On film 1 h to 1 week

Non-radioactive probe

Direct probe

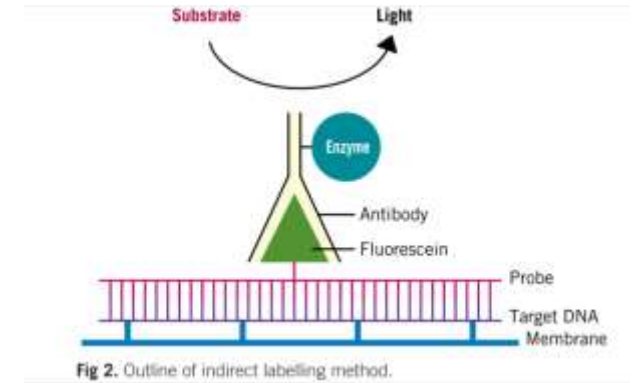
- label is incorporated directly into nucleic acid probe so that hybridization site could be visualized immediately after



Maheetha R. H. (mailto:maheetha@rediffmail.com)

Indirect probe

- the label in the probe **cannot be detected** immediately after hybridization .
- second molecule called **reporter is required to detect** the label in probe
- This reporter molecule **is conjugated with signal generating system** which makes the visualization of probe possible .
- Can be: - one step or two step (with 2^o reporter)



Non radioactive probe

Chemical labelling

- Acetylaminofluorine
- Mercury

Enzymatic labelling

- Biotin
- Digoxigenin

Reporter Molecules in non-radioactive probe

- **Biotin** is detected by **avidin or streptavidin**
- **Digoxigenin** is detected by **anti-digoxigenin antibodies**.
- **AAF** is detected by **anti-acetylaminofluorene** antibodies.
- **Mercury** is detected by **ligands having an immunogenic group** which can bind to same antibody

Signal Generating System:

Fluorochromes

- They get excited by **light of one wavelength** and **emit light of another wavelength**
- which is observed as **fluorescence of different colors.**

Enzymes

- Enzymes work by catalyzing the **precipitation of a visible product at hybridization site.**

Metals

- Colloidal gold which is **conjugated to antibodies.**
- Can be visualized with both **light and electron microscope**

Fluorochromes

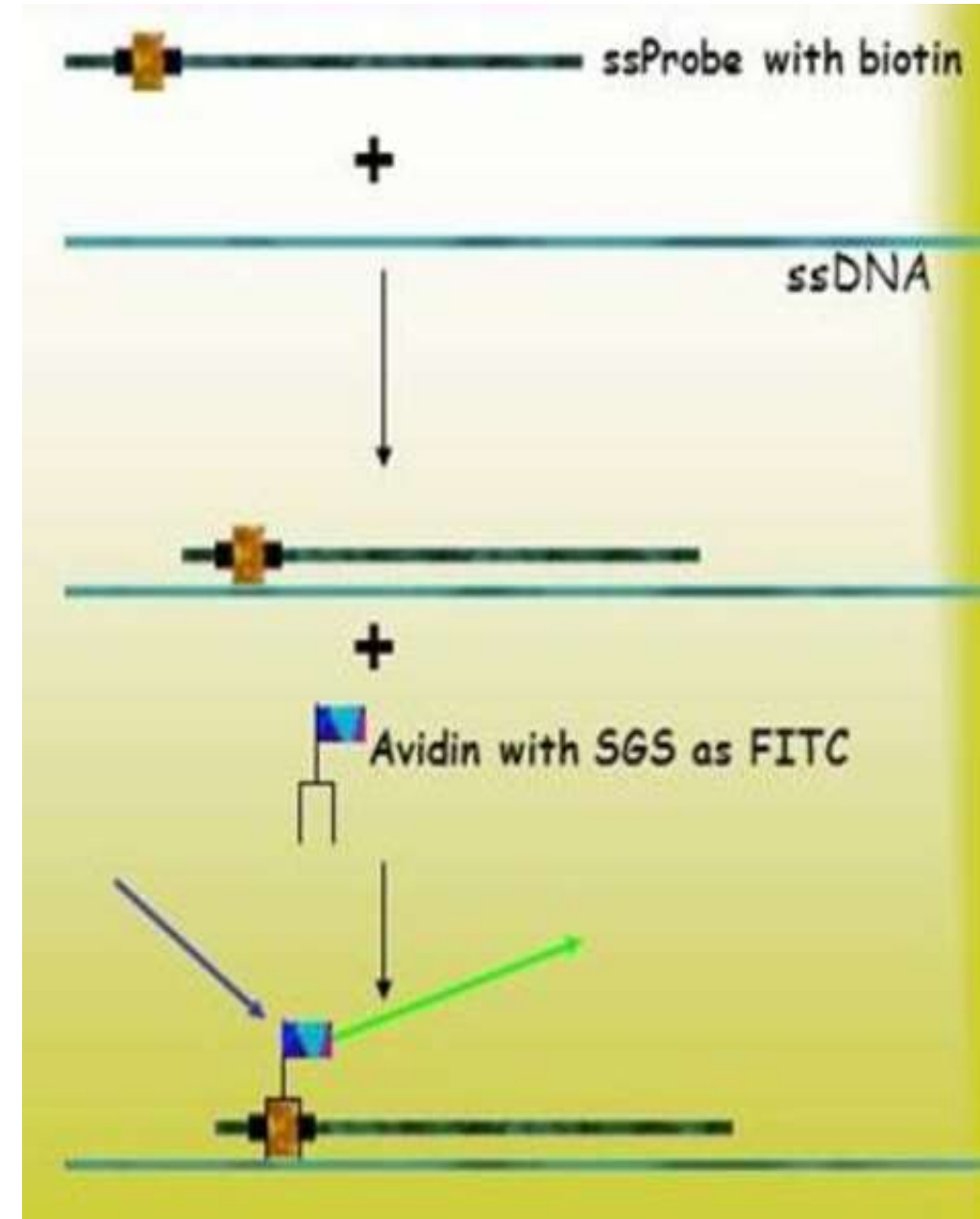
Fluorochrome	Excite	Fluorescence
Fluoresceine isothiocyanate (FITC)	Blue	Green
Tetramethyl rhodamine isothiocyanate (TRITC)	Green	Red
Texas red or sulphorhodamine	Green	Deep red
Amino methyl coumarine acetic acid (AMCA)	UV	Blue

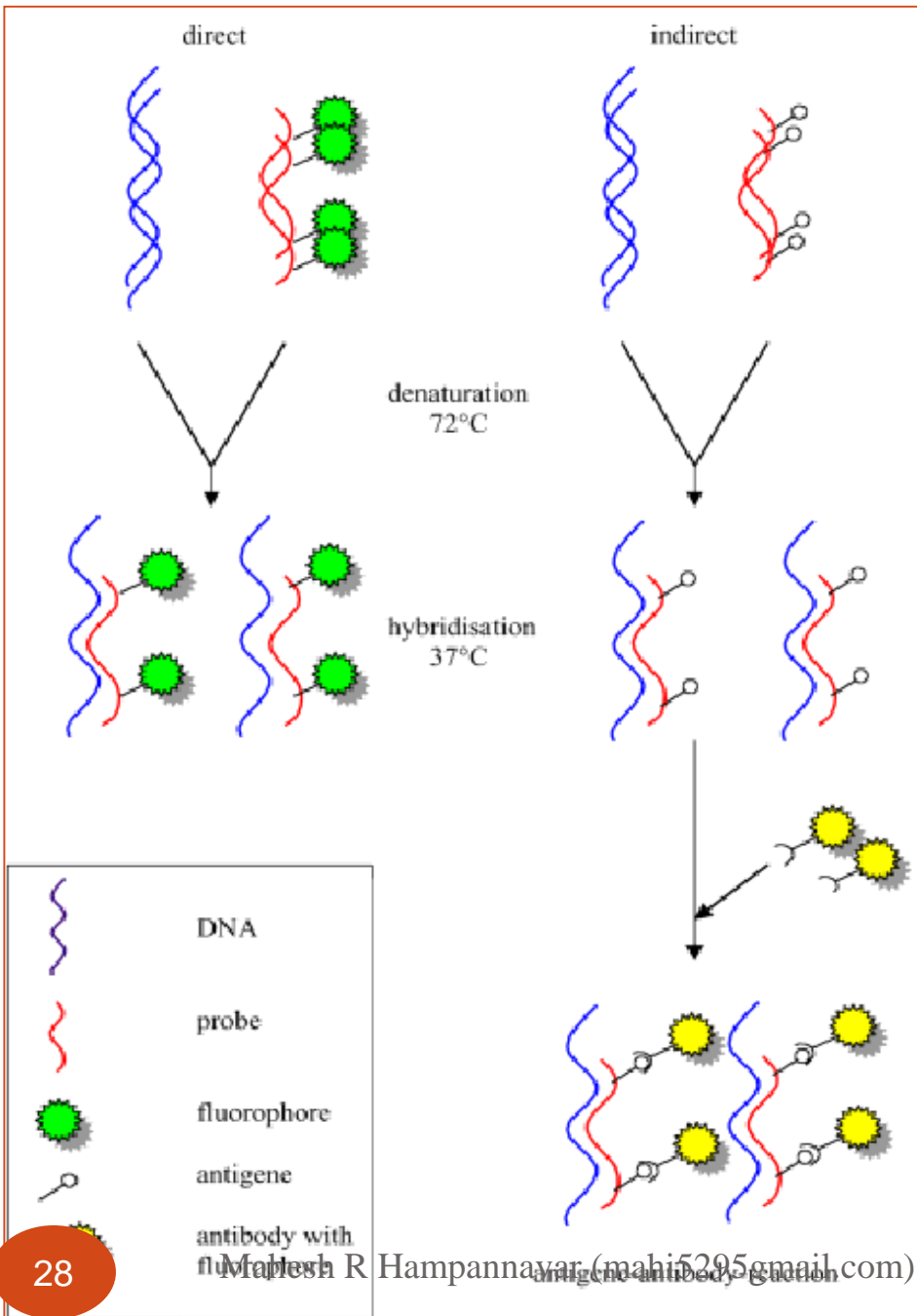
Enzymes.

Enzyme	Substrate	Color of ppt
Horseradish peroxidase	Diamino benzidine(DAB)	Red
Alkaline phosphatase	5-Bromo 4-chloro 3-indolyl phosphate (BCIP)	Blue

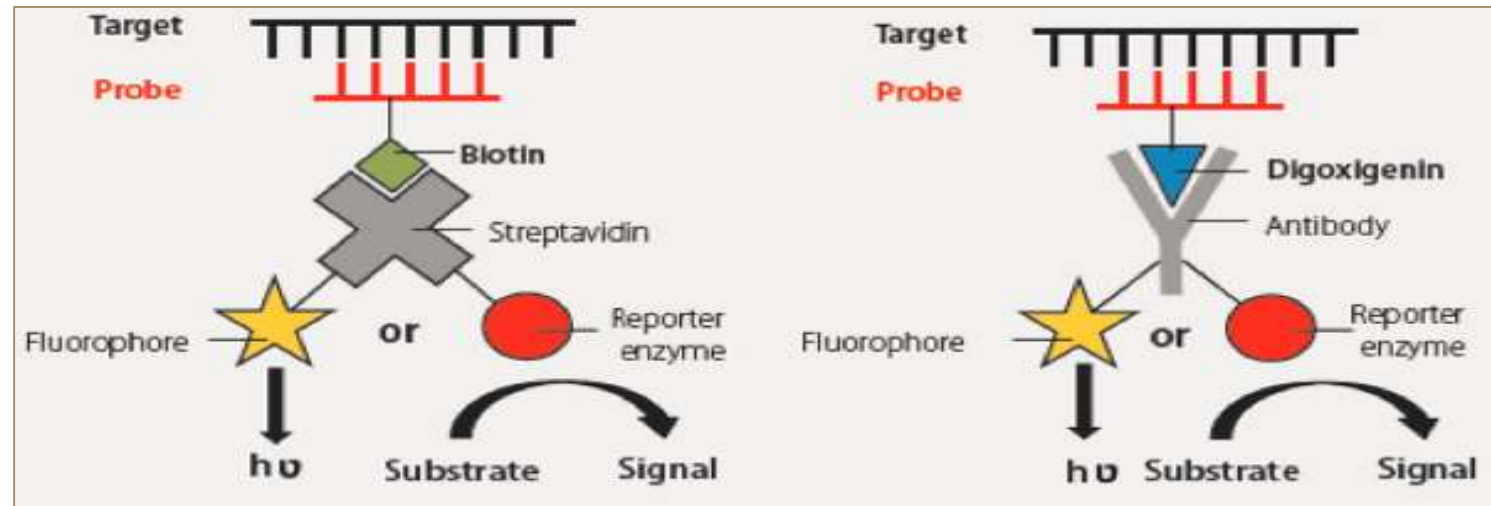
Example- Detection by Biotin

- Biotin, is first introduced enzymatically into NA probe.
- Probe hybridized to target NA.
- Then avidin, conjugated to same signal generating system, (say FITC) is introduced.
- Detected by green colored fluorescence of FITC.
- To enhance the signal strength avidin can further be detected by biotin-antiavidin conjugate.
- Then again avidin, conjugated to some signal generating system is introduced.





- non-radioactive labels used successfully with in situ hybridization include
- **Biotin, digoxin and digoxigenin (DIG), alkaline phosphatase and the fluorescent labels, fluorescein (FITC), Texas Red and rhodamine.**
- Since these nonradioactive labels have no inherent "decay" kinetics, -2°C for later use for as long as 1-2 years with careful storage.



Hybridization issues

- In situ hybridization presents a unique set of problems
 - as the sequence to be detected will be at a **lower concentration**,
 - **be masked** because of associated protein,
 - **protected within a cell** or cellular structure.
- Therefore, in order to probe the tissue or cells of interest one has to increase the **permeability** of the cell and the **visibility of the nucleotide sequence** to the probe without destroying the structural integrity of the cell or tissue.

Permeablization

- Three common reagents used to **permeabilize tissue** are
 - HCl,
 - detergents (Triton or SDS)
 - Proteinase K.

Pretreatment/Prehybridization step(s).

- Pretreatment/Prehybridization is generally carried out to **reduce background staining**
- This can be achieved with peroxidases by treating the tissues with 1% H₂O₂ in methanol for 30 minutes.

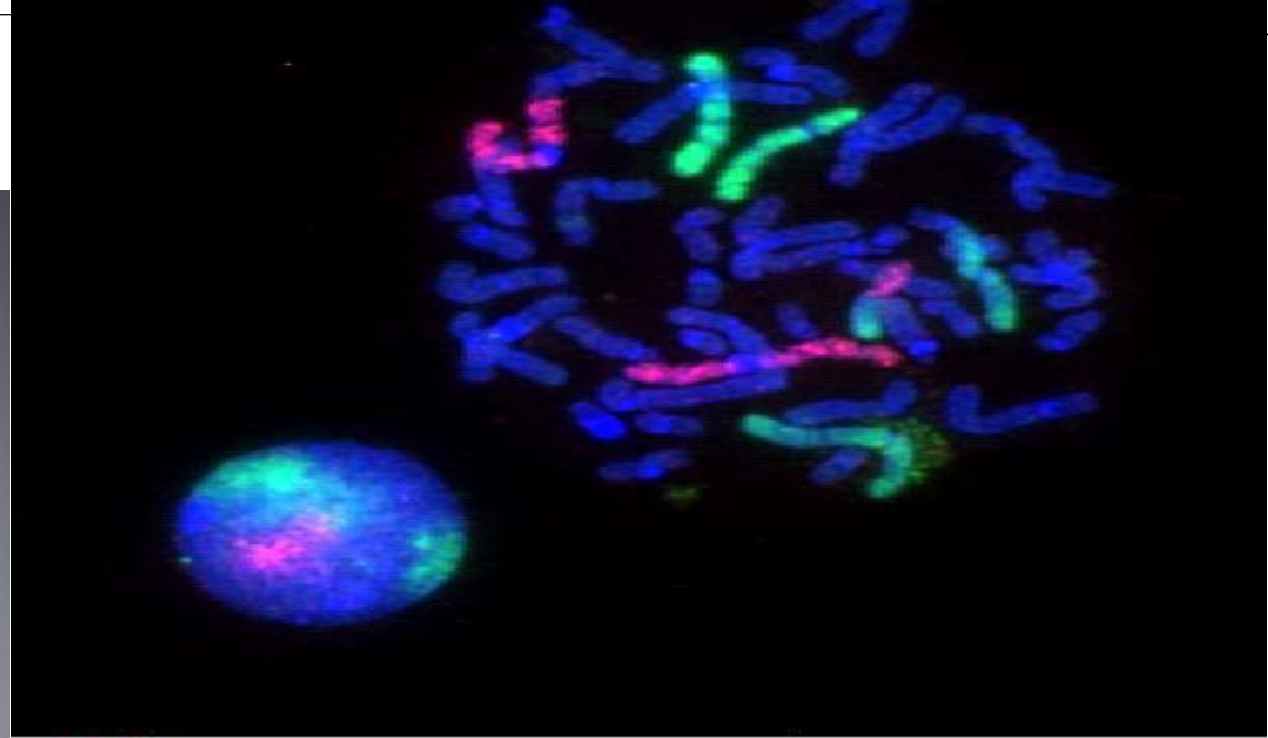
The factors that influence the hybridization of the oligonucleotide probe to the target mRNA are:

- Temperature
- pH
- monovalent cation concentration
- presence of organic solvents

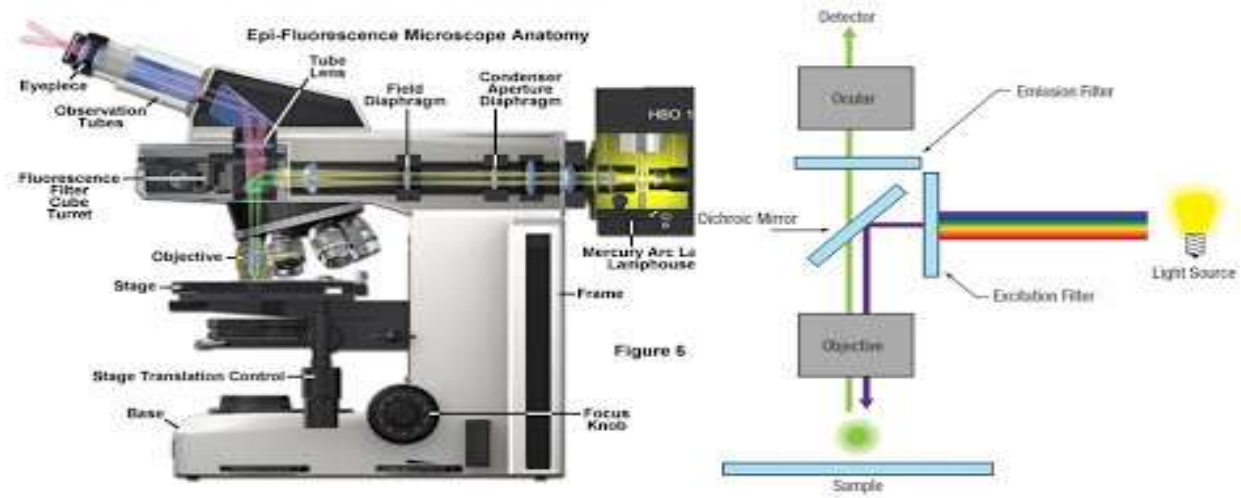
- The following is a typical hybridization solution with a hybridization temperature of around 37°C
- **Dextran sulphate - reduces the amount of hydrating water** for dissolving the nucleotides and therefore effectively **increases the probe concentration in solution** resulting in higher hybridization rates.
- **Formamide and DTT (dithiothreitol) -** These are organic solvents which **reduce the thermal stability of the bonds allowing hybridization to be carried out at a lower temperature.**

- **SSC (NaCl + Sodium citrate)** - Monovalent cations **interact mainly with the phosphate groups** of the nucleic acids decreasing the electrostatic interactions between the two strands.
- **EDTA** - This is a chelator and removes free divalent cations from the hybridization solution, because they strongly **stabilize duplex DNA.**

Detection



Fluorescence Microscopy



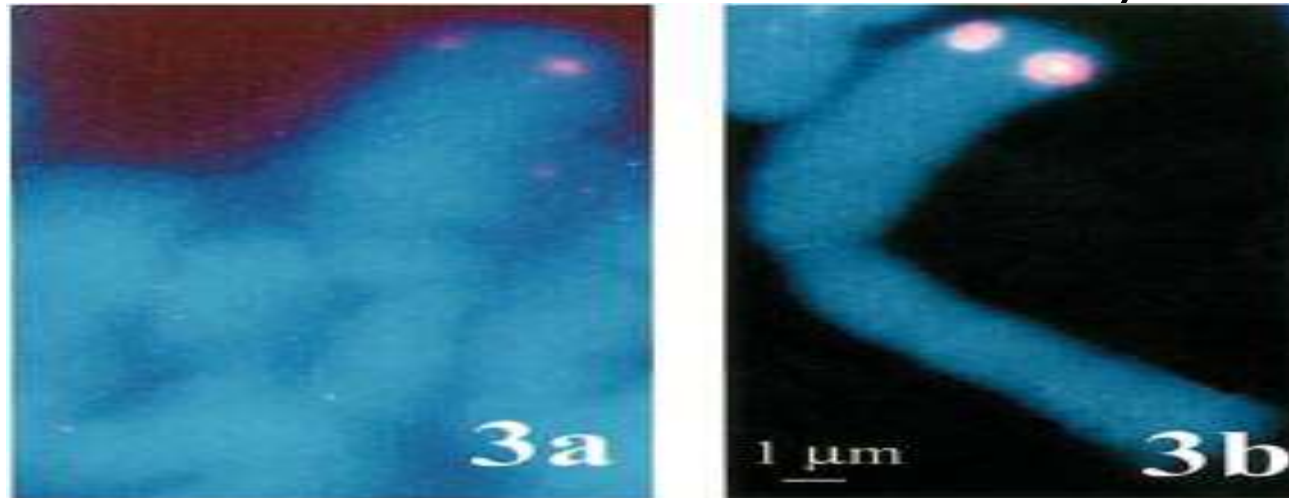
Technical developments

- Although the principle steps of the FISH procedure have stayed the same
- **little technical modification** adopted in plant cytogenetics
 - Tyramid-FISH
 - Three dimensional FISH
 - FISH on super- stretched chromosome
 - FISH on DNA fibers

Tyr FISH

- The **sensitivity of fluorescence in situ hybridization (FISH)** for mapping plant chromosomes of single copy DNA sequences is limited.
- ultra-sensitive FISH **with tyramide signal amplification**
- Increase the detection sensitivity of FISH experiment
- **Use of signal amplification molecule i.e. peroxidase conjugated antibody**
- Use this as first layer of signal detection

- Then to use **fluorochrome- labeled tyramides** as peroxidase substrates to **generate and deposit many fluorochromes** close to the in situ bound peroxidase.
- Sensitivity increased **10-100 times**
- DNA probes smaller than 1 kb were successfully visualized



Chromosome 1 of transgenic shallot line 7-1 L4, accession number 54 probed with 13.3 kb pTOK233 plasmid DNA. (a) **Conventional FISH**; (b) **Tyr-FISH**. DNA was counterstained with DAPI.

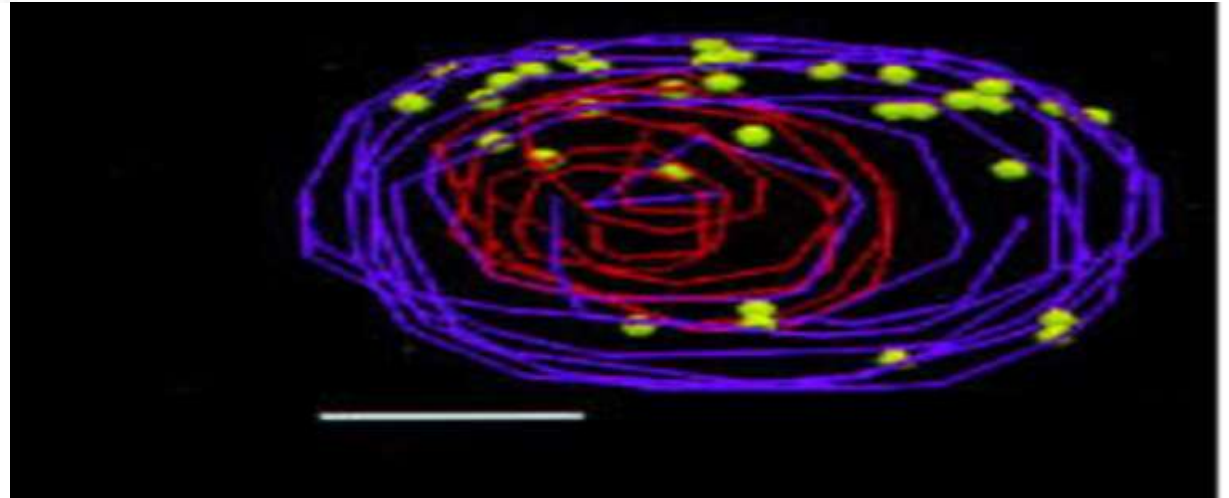
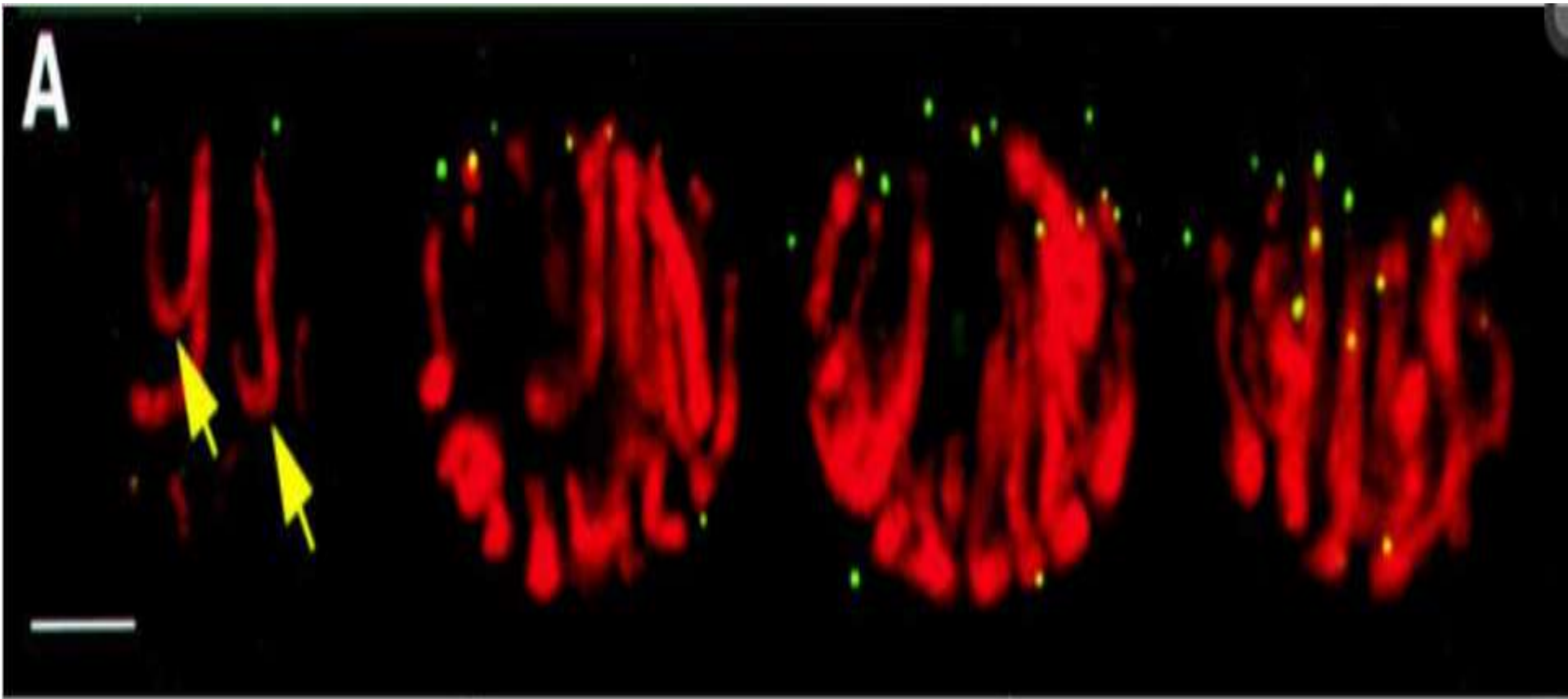
Frequency of single-copy T-DNA detection on metaphase chromosomes of transgenic shallots (*Allium cepa*) using Tyr-FISH and conventional FISH

Probe	Tyramide-FISH		Conventional FISH			
			Conventional microscope		CCD camera	
	No. metaphases observed	% metaphases with signal	No. metaphases observed	% metaphases with signal	No. metaphases observed	% metaphases with signal
pTOK233	542	77.5 [420] ^a	298	0.3 [1]	78	34.6 [27]
<i>uidA</i>	348	36.5 [127]	213	0	72	0

^aAbsolute number of metaphases with a signal.

Three dimensional FISH

- Bass *et al.*, 1997 in maize
- **Precise position of DNA probe on chromosome within the nucleolus.**
- It doesn't help in mapping technique
- It helps to identify the **spatial position** of probe during cell division
- **Stacks the FISH signals** are taken and **composed into 3 dimensional image**

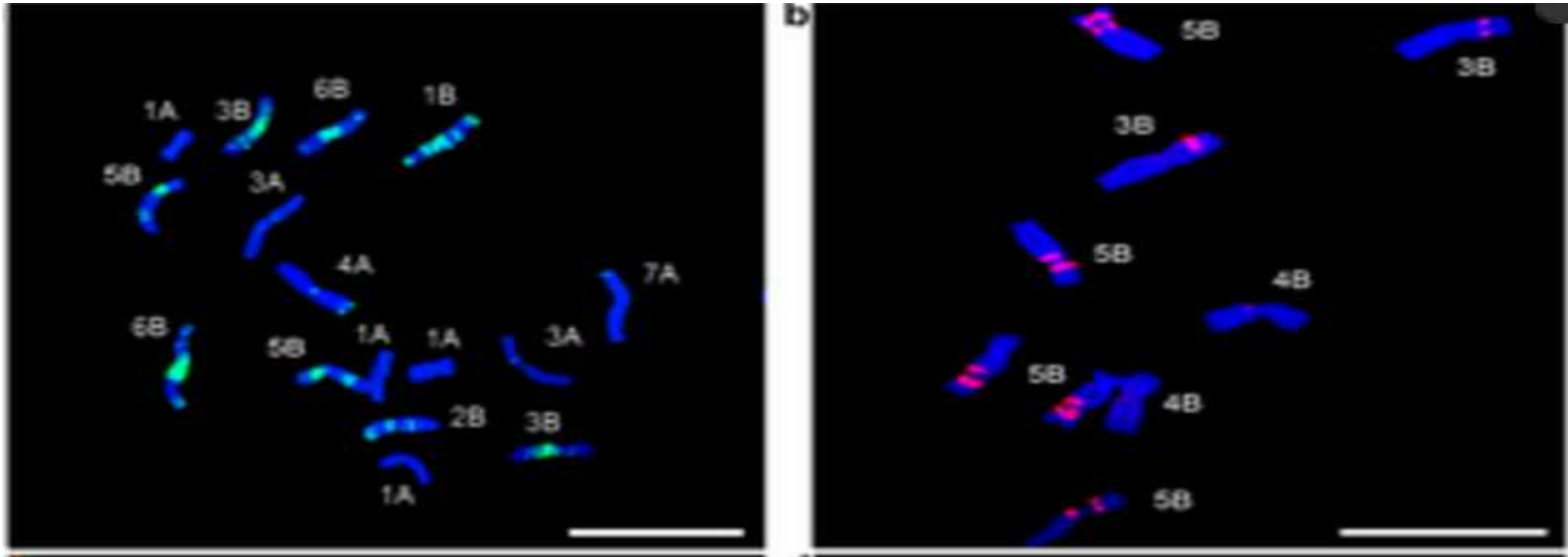


FISH on super- stretched chromosome

- The ability to **isolate single chromosomes**, which represent small units of nuclear genome, is priceless in many areas of plant research including cytogenetics, genomics, and proteomics.
- **Flow cytometry** is the only technique which can provide large quantities of pure chromosome fractions suitable for downstream applications
- **physical mapping, preparation of chromosome-specific BAC libraries, sequencing, and optical mapping.**

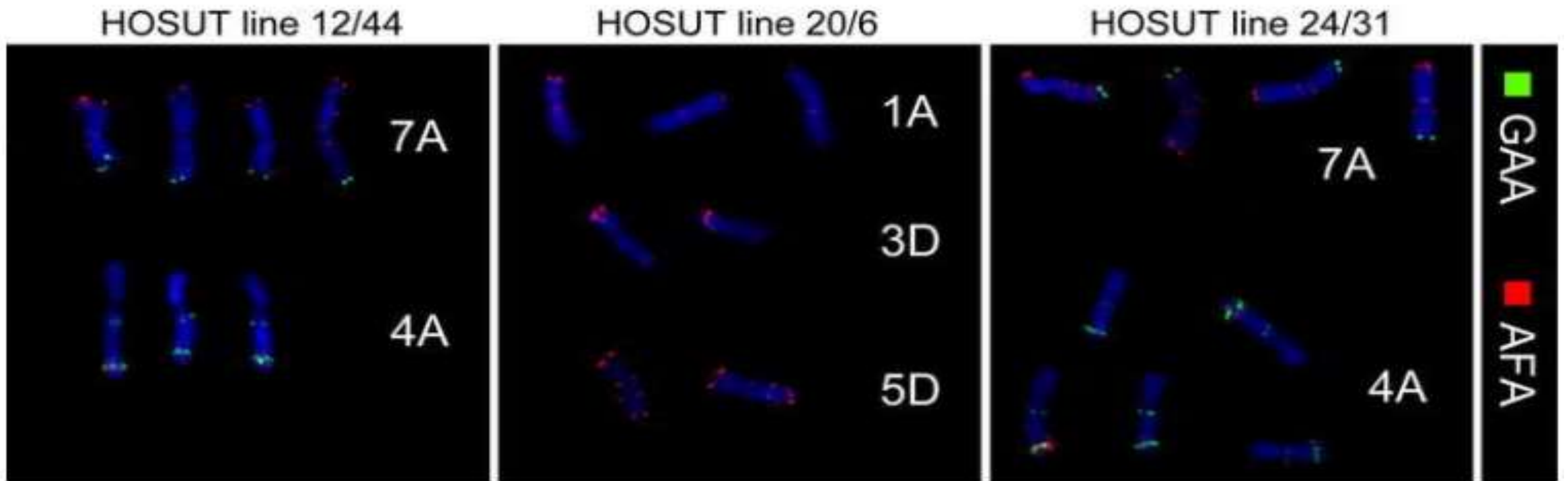
Flow cytometry as an analytical and preparative technique

- Flow cytometry, **accompanied by sorting, permits isolation of individual chromosomes** for further study, and generates highly pure and chromosome-specific DNA preparations.
- FCM involves the passage of chromosome suspensions through the focus of intense light sources, typically lasers, using one or two DNA-specific fluorochromes to provide fluorescent signals related to the DNA content and base-pair composition of the individual chromosomes.
- The end products are “flow karyotypes” providing **one- or two-dimensional representations** of the distributions of the different chromosomes (histograms or



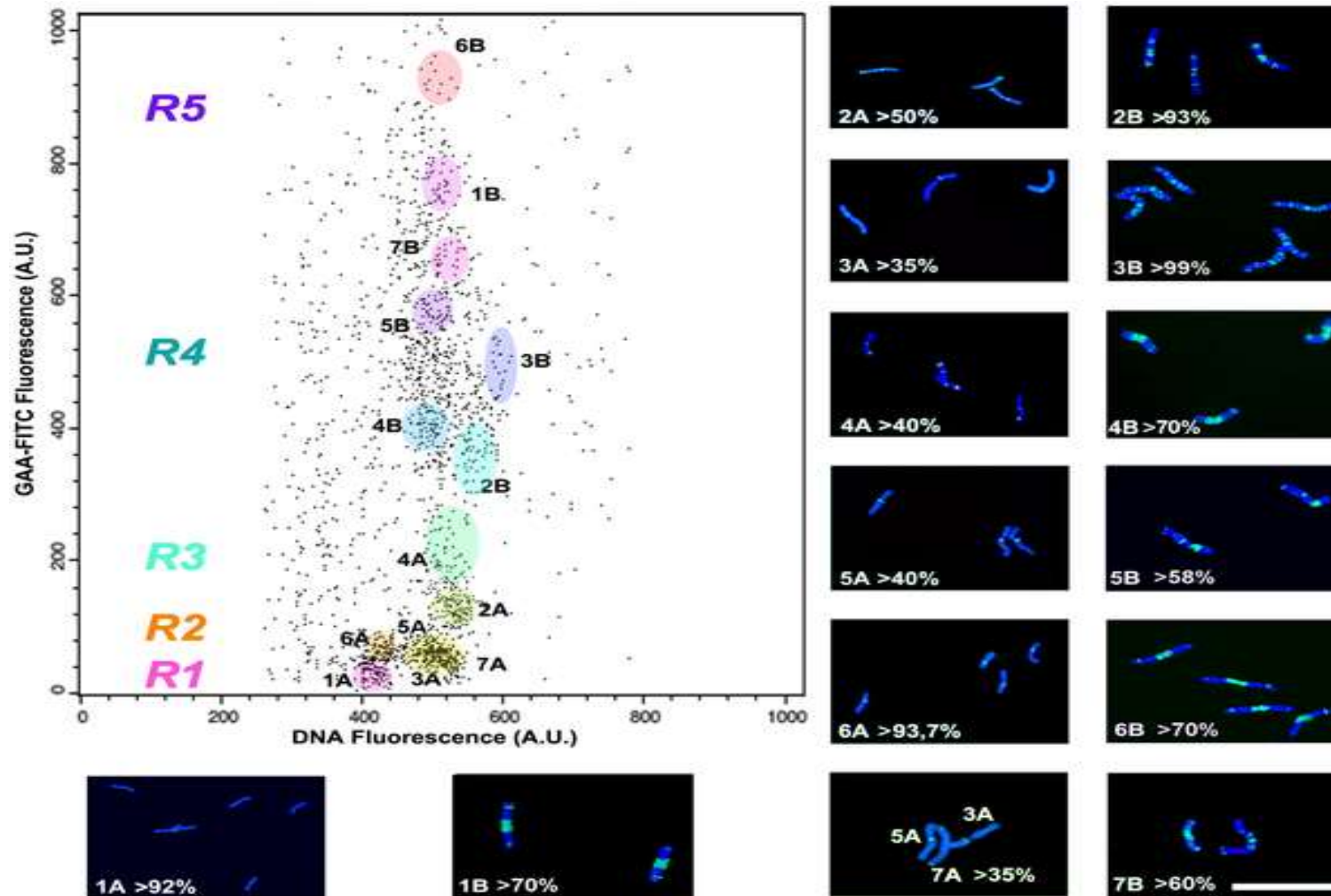
FISHIS of pasta wheat cv Creso chromosome suspensions.

- a) Chromosome suspensions hybridized with $(GAA)_7$ -FITC;
- b) flow-sorted chromosomes 3B, 4B and 5B following hybridization with $(AG)_{12}$ -Cy3



Representative images of chromosomes flow-sorted from three HOSUT (Transgenic lines of hexaploid wheat carrying barley (*Hordeum vulgare*) sucrose transporter HvSUT1 (SUT) gene) lines

Figure 2. Biparametric dot plot analysis of pasta wheat cv Creso chromosomes.



Giorgi D, Farina A, Grosso V, Gennaro A, Ceoloni C, et al. (2013) FISHIS: Fluorescence In Situ Hybridization in Suspension and Chromosome Flow Sorting Made Easy. PLOS ONE 8(2): e57994. <https://doi.org/10.1371/journal.pone.0057994>
<http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0057994>

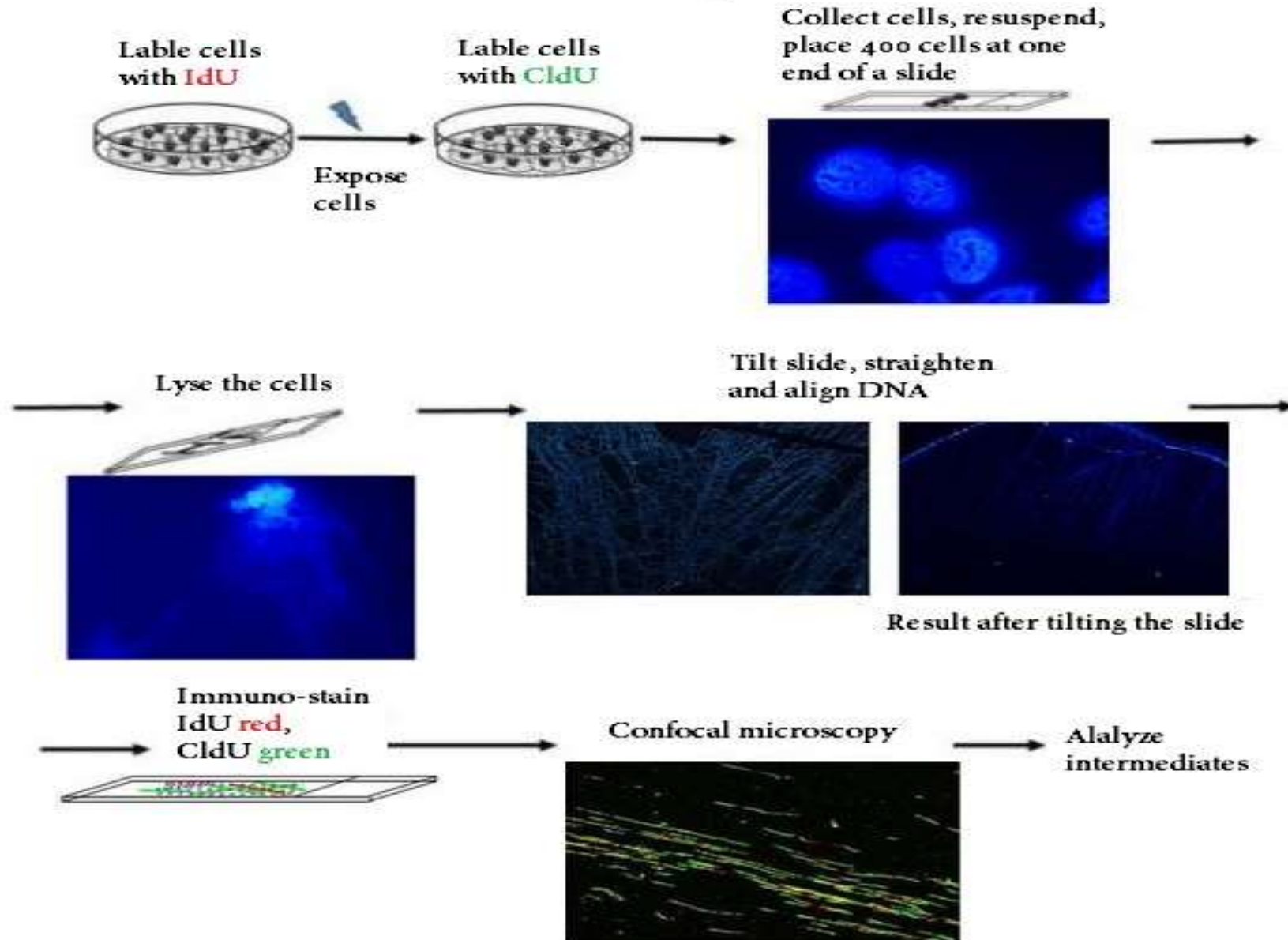
Mahesh R Hampannavar (mahi5295gmail.com)

- Flow sorted plant chromosome can be stretched to more than 100 times their original size
- It facilitate the brighter signal in FISH
- Mapping resolution of up to 70kb

FISH on DNA fiber

- Due to higher molecular weight or large insert size of DNA clones (2.5-3.5kb/um)
- Fiber DNA method provide **fine mapping resolution of up to a few kilobases.**
- So this method used in various mapping
- This method used to measure the **size of physical gaps in mapping**

Method for Generating DNA Fibers



Limits of Fiber FISH

- Not applicable to large genome size crops like Wheat, because extensive amount of repetitive DNA
- Problem to distinguish short fiber FISH (<1kb) from background stain

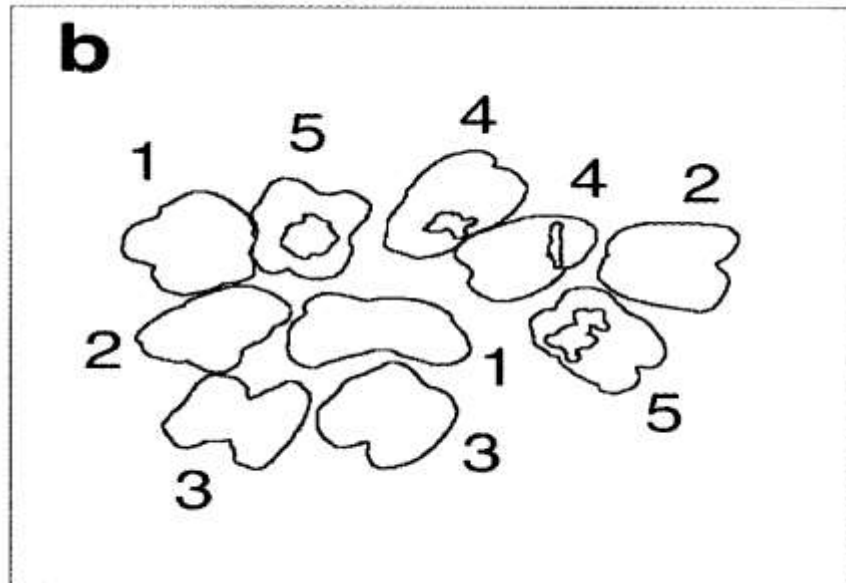
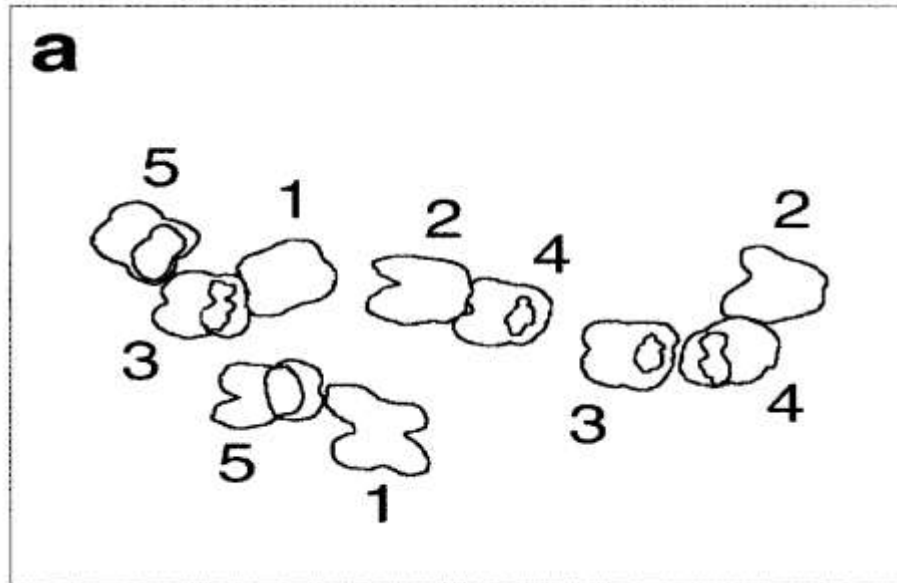
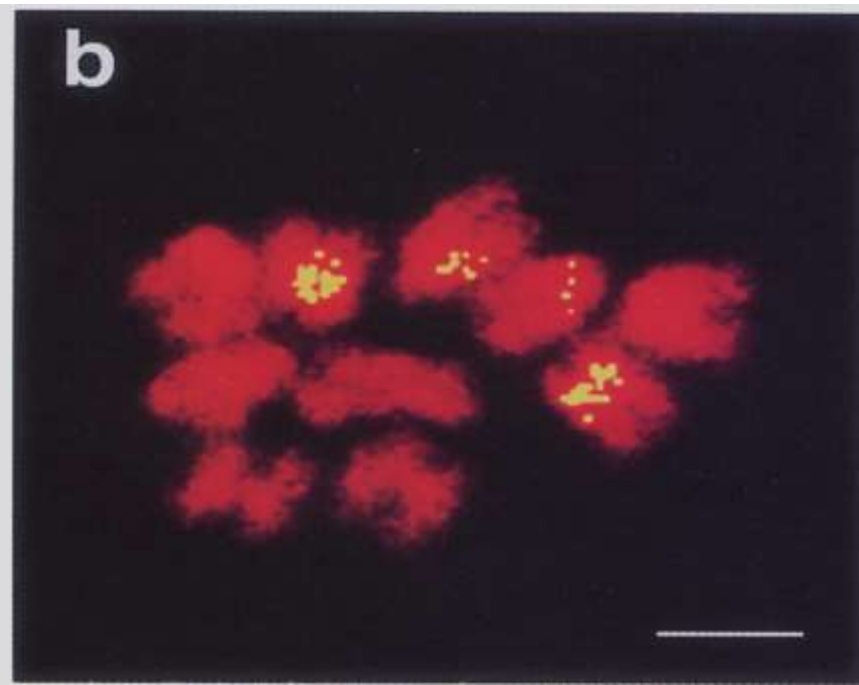
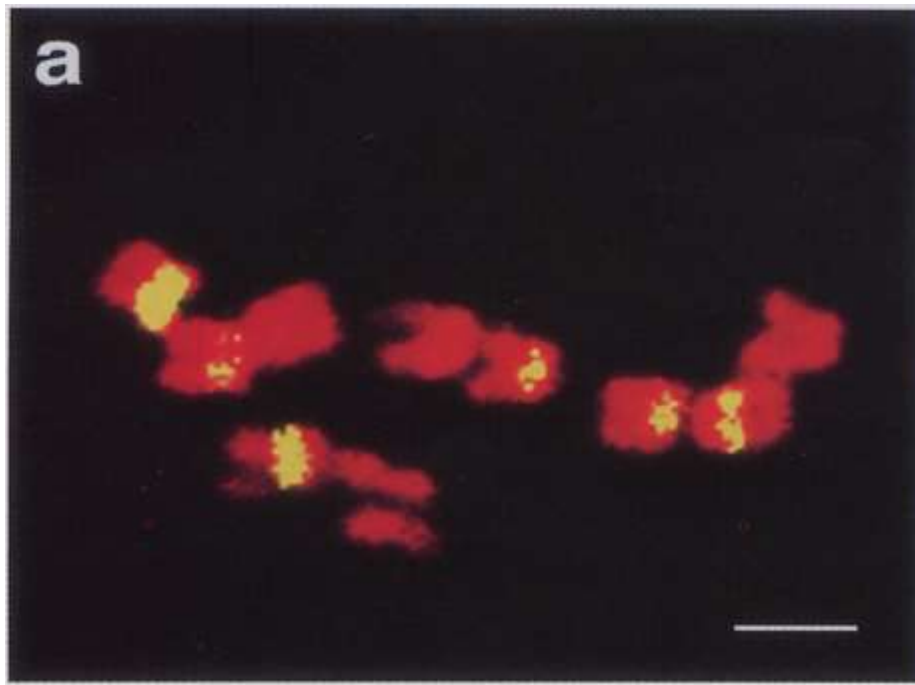
• Applications

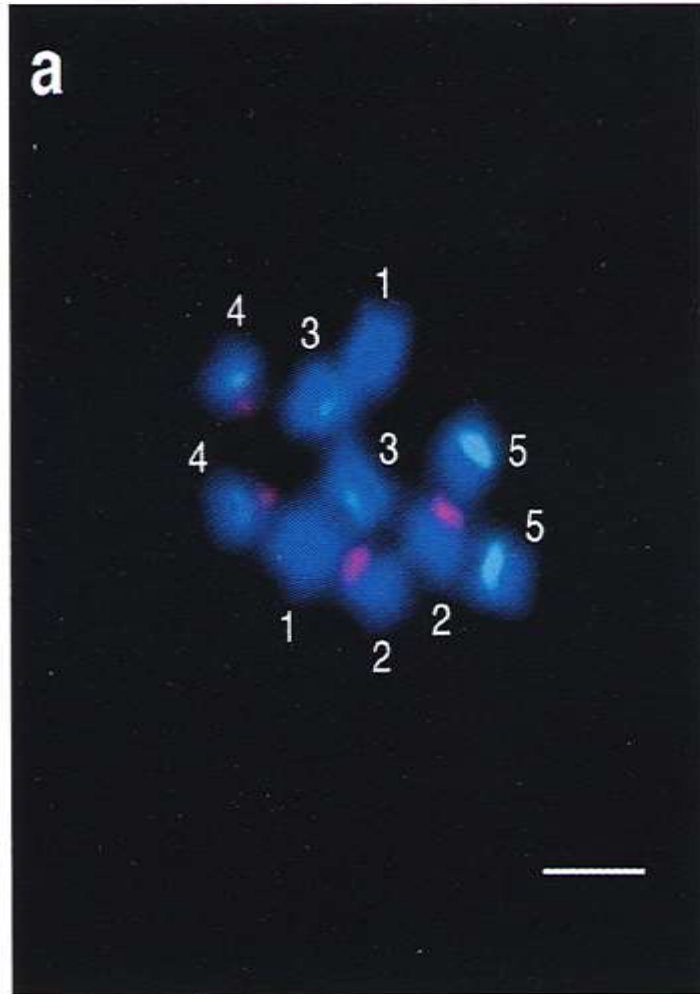
Chromosome mapping

- Species specific, repetitive sequence, ribosomal sequence and unique sequence.
- Ribosomal genes have the great value in karyotyping and comparative genomics
- 5S and 18S-5.8S-26S rDNA multi gene family

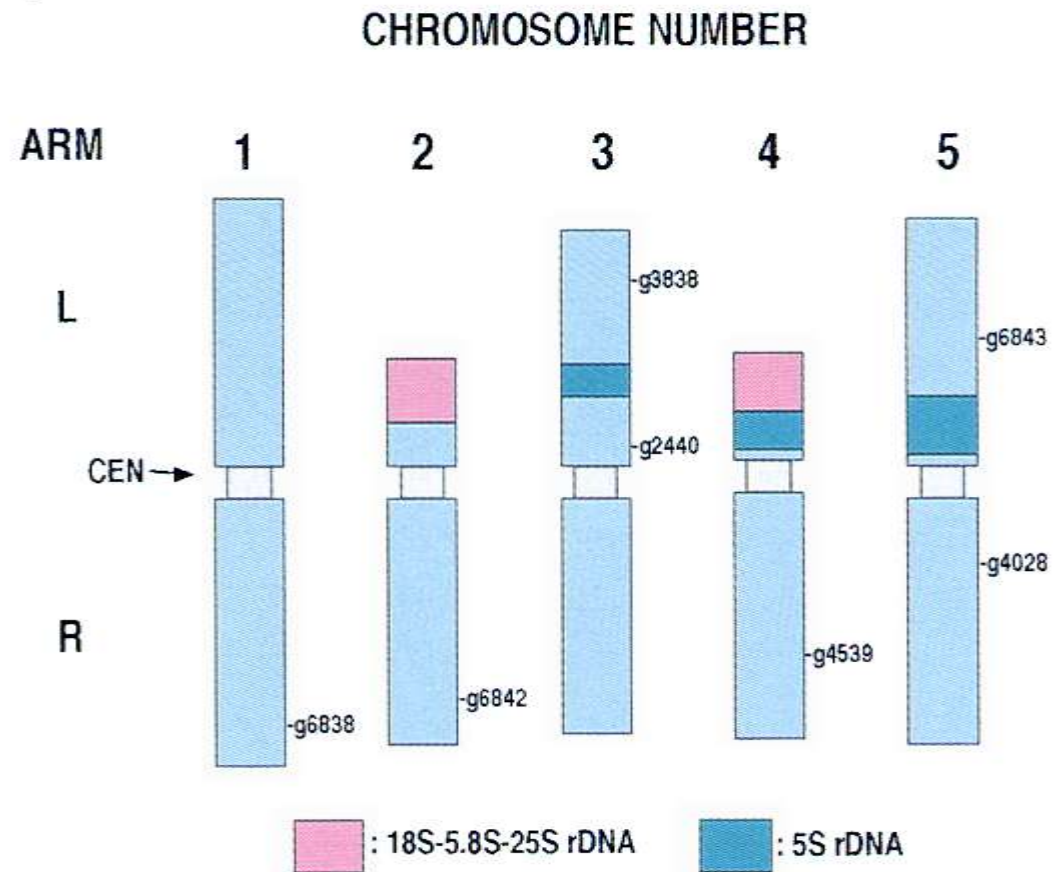
Physical mapping of the 5S ribosomal RNA genes in *Arabidopsis thaliana* by multi-color fluorescence *in situ* hybridization with cosmid clones

- **The 5S ribosomal RNA genes were mapped to mitotic chromosomes of *Arabidopsis thaliana* by fluorescence *in situ* hybridization (FISH).**
- **ecotypes, Columbia and Wassilewskija**





(b)



Genome analyze

- Hybrid plants, allopolyploidy and recombinant inbred line
- mFISH and whole total genomic DNA probe is promising approach in amphidiploid
- Helps to know the progenitors of allopolyploidy

Simultaneous discrimination of the three genomes in hexaploid wheat by multicolor fluorescence *in situ* hybridization using total genomic and highly repeated DNA probes

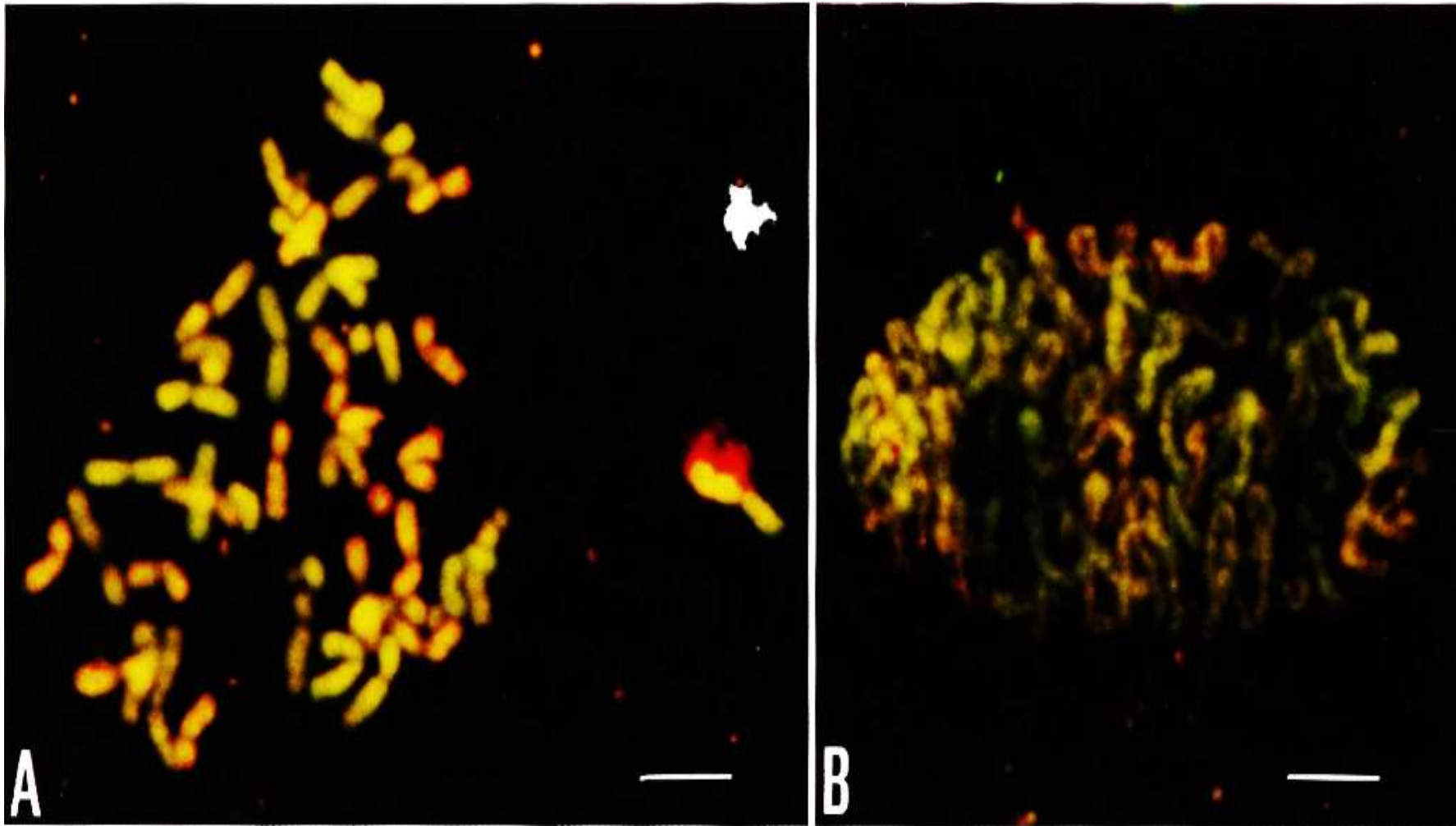
YASUHIKO MUKAI¹ AND YUMIKO NAKAHARA

*Laboratory of Plant Molecular Genetics, Division of Natural Science, Osaka Kyoiku University,
4-698-1 Asahigaoka, Kashiwara, Osaka 582, Japan*

AND

MAKI YAMAMOTO

- Common wheat, *Triticum aestivum*, is an allohexaploid species consisting of three different genomes (A, B, and D).
- Double ditelo **1A** line of cv. **Chinese Spring** wheat
- **Biotinylated** total genomic DNA of the diploid **A genome progenitor *Triticum urartu***, **digoxigenin-labeled** total genomic DNA of the diploid **D genome progenitor *Aegilops squarrosa***, and **nonlabeled** total genomic DNA of one of the possible **B genome progenitors *Ae. speltoides*** were hybridized *in situ* to metaphase chromosome spreads of *Triticum aestivum* cv. Chinese Spring.



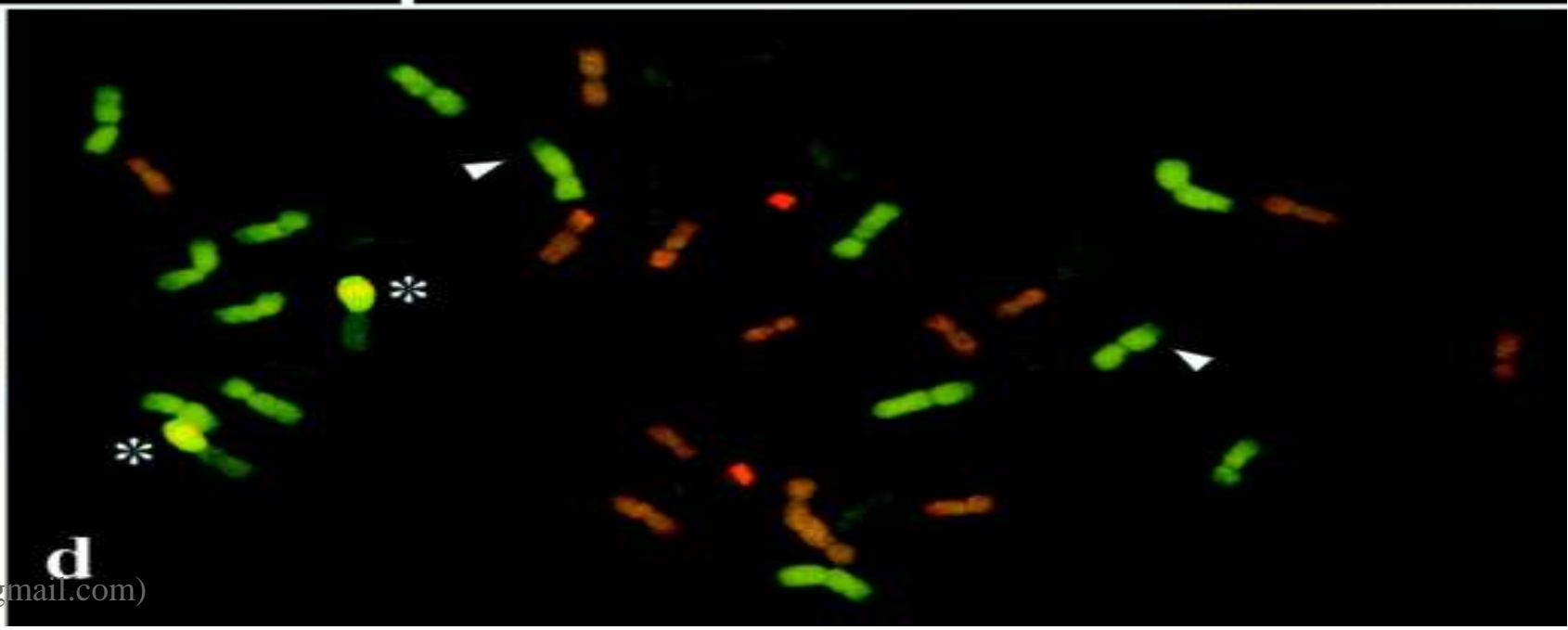
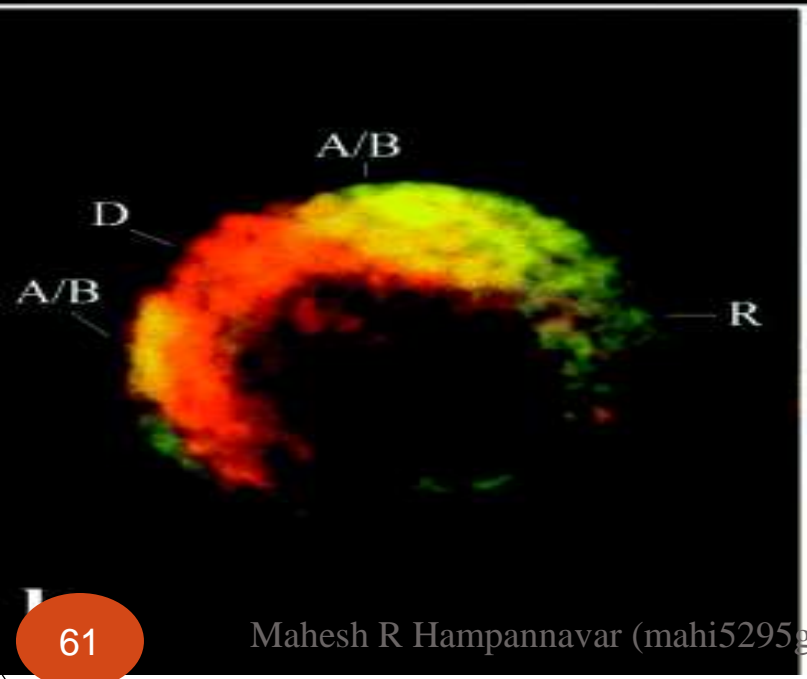
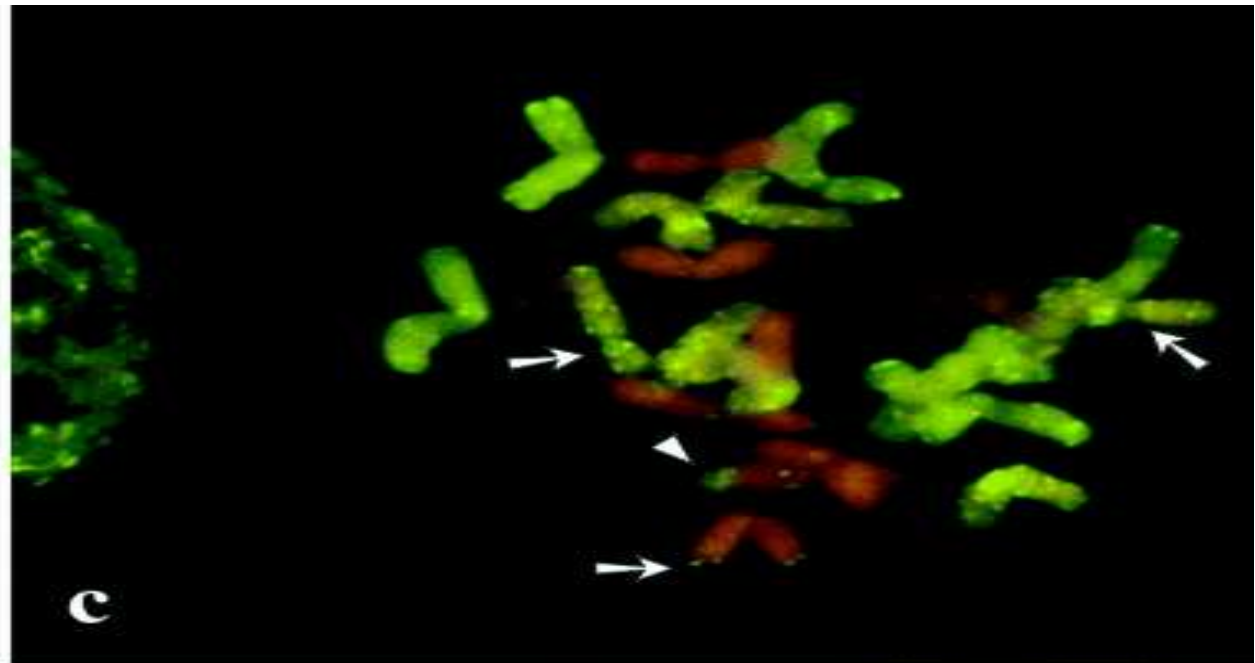
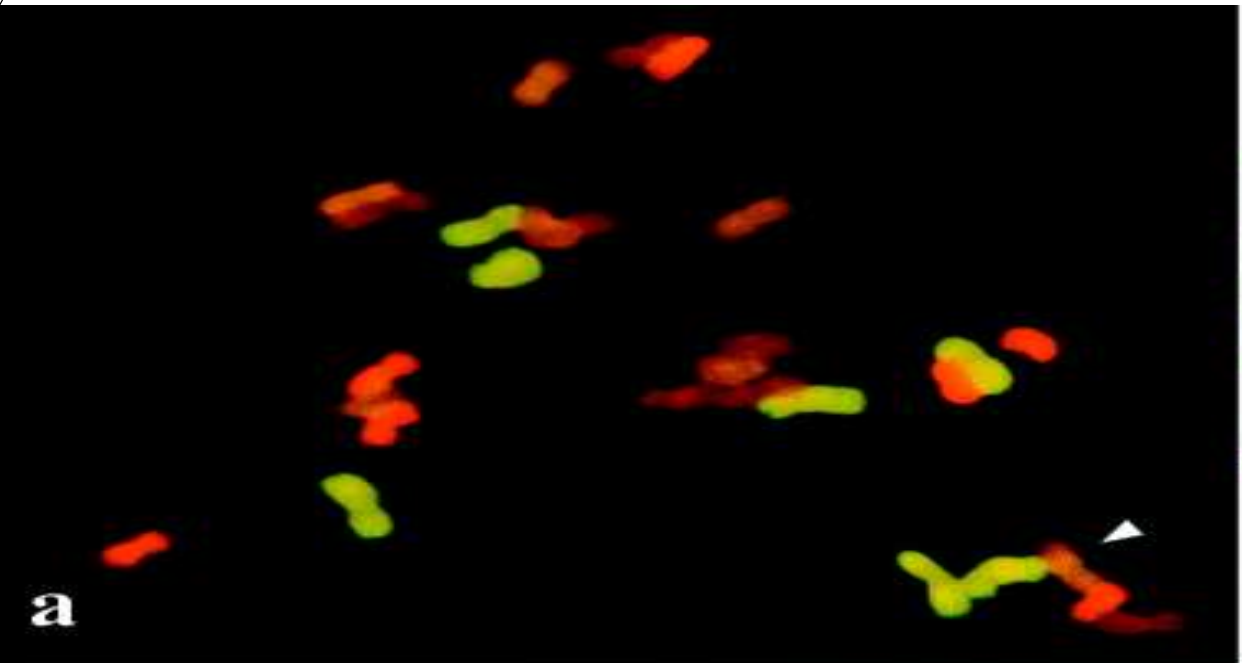
The hybridization sites of the **A genome** probe were detected by **yellow fluorescence**, while those of the **D genome** probe were detected by **orange fluorescence**. The **B genome** chromosomes were **neither labeled yellow nor orange but appeared faint brown as a result of cross-hybridization of the A and B genome probes**

Simultaneous identification of A, B, D and R genomes by genomic *in situ* hybridization in wheat–rye derivatives

E. SÁNCHEZ-MORÁN, E. BENAVENTE & J. ORELLANA*

*Unidad de Genética, Departamento de Biotecnología, ETSI Agrónomos, Universidad Politécnica de Madrid,
28040-Madrid, Spain*

- Multicolour genomic *in situ* hybridization was carried out in wheat*rye hybrids and in a wheat*rye translocation line.
- Different hybridization conditions and mixture compositions were used, and A, B and D genomes of hexaploid wheat as well as the R genome of rye were distinguished simultaneously in somatic cells.



Phylogenetic relationship

- **GISH offers new** opportunities in phylogenetic and taxonomic study
- The 5S rRNA genes of higher plants are organized into clusters of tandem repeats with thousands of copies at one or more position in the genome.
- **Each repeat consist of a highly conserved in a species**
- The variation in sizes and sequences of the NTS of the 5S rRNA gene was found to be useful for phylogenetic reconstruction of spices.

Chromosomal localization of 5S rRNA gene loci and the implications for relationships within the *Allium* complex

S. H. Lee, G. S. Do & B. B. Seo*

Department of Biology, Kyungpook National University, Taegu 702-701, Korea; Tel: +82-53-950-5350; Fax: +82-53-953-3066; email address: bbseo@kyungpook.ac.kr







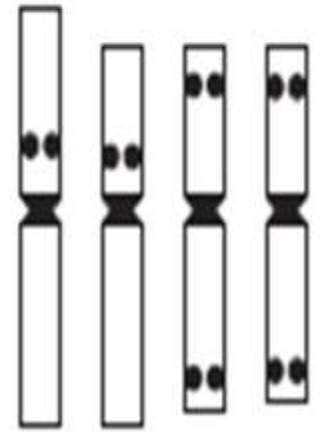
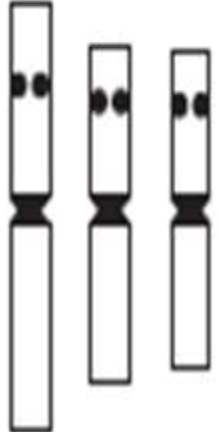
* Correspondence

Received 14 September 1998; received in revised form and accepted for publication by G. Sutherland, 12 November 1998

5S rRNA gene probe was obtained using total genomic DNA of *Allium stulosum*.

Table 1. Somatic chromosome numbers and collection sites or cultivars of the *Allium* species studied.

Species	Chromosome number (2n)	Collection site or cultivar
Diploid		
<i>A. anisopodium</i>	16	Chungpook Sokri Mt.
<i>A. cepa</i>	16	Cultivar
<i>A. cyaneum</i>	16	Jeonpook Jiri Mt.
<i>A. cyaneum</i> var. <i>deltoides</i>	16	Kyungnam Kaya Mt.
<i>A. deltoide-fistulosum</i>	16	Jeonnam Segeol Mt.
<i>A. fistulosum</i>	16	Cultivar
<i>A. sativum</i>	16	Cultivar
<i>A. senescens</i> var. <i>minor</i>	16	Kangwoen Inje-gun
<i>A. splendens</i>	16	Kyungpook Bisl Mt.
<i>A. thunbergii</i>	16	Jeonnam Hongdo Is.
<i>A. victorialis</i> var. <i>platyphyllum</i>	16	Kyungpook Ulreung Is.
Allopolyploid		
<i>A. wakegi</i>	16	Cultivar
<i>A. deltoide-fistulosum</i>	32	Jeonpook Gogseong
<i>A. senescens</i>	32	Kyungpook Ulreung Is.

PLOIDY	DIPLOID					POLYPLOID			
TYPE	A	B	C	D	E	F	G	H	
IDIOPHASE									
	5 7	7	7	7	7	9 15	10 13 14 15	8 14 15	
SPECIES	A, Y, Y ^{del} , D ¹⁶ , P, T	E ^{min} , F	C	S	V	W	D ³²	E	

Analysis of Somaclonal variations:

- In tissue culture
- Chromosomal breakage and DNA transposition leads to change in karyotype.
- Examination of the chromosomal distribution of 5S and 18S-26S rRNA is useful to identifying the types of genomic changes



Analysis of somaclonal variation through tissue culture and chromosomal localization of rDNA sites by fluorescent *in situ* hybridization in wild *Allium tuberosum* and a regenerated variant

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The specific aims of the present investigation were:
(1) to generate plants from callus derived from flower bud explants of *A. tuberosum*
(2) to analyze the karyotypic change of At30 in comparison with the wild type by using 5S and 18S-5.8S-26S rDNA as probes.

Table 2. Effects of growth regulators on regeneration of plantlets and somatic variants formation from flower bud explants on MS medium. Shoot was induced from 2 g of callus cluster per treatment

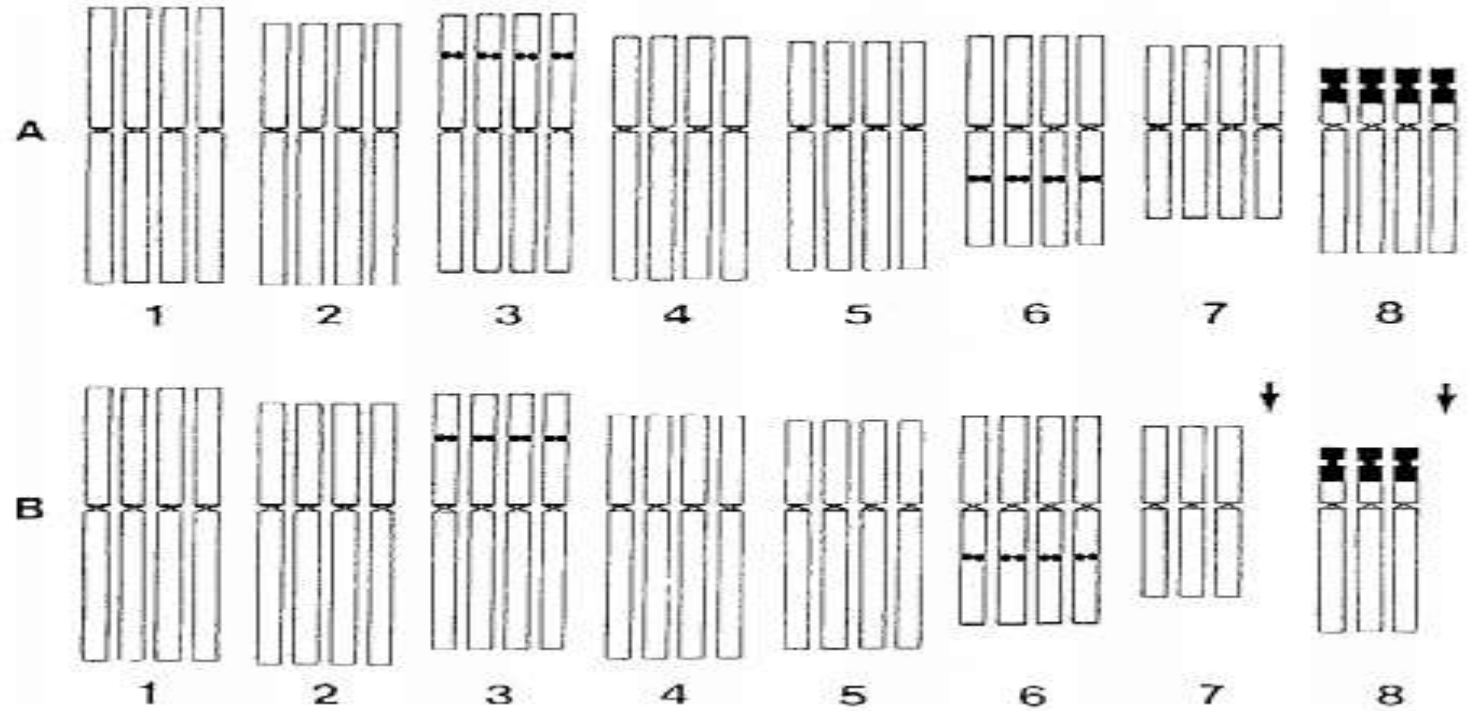
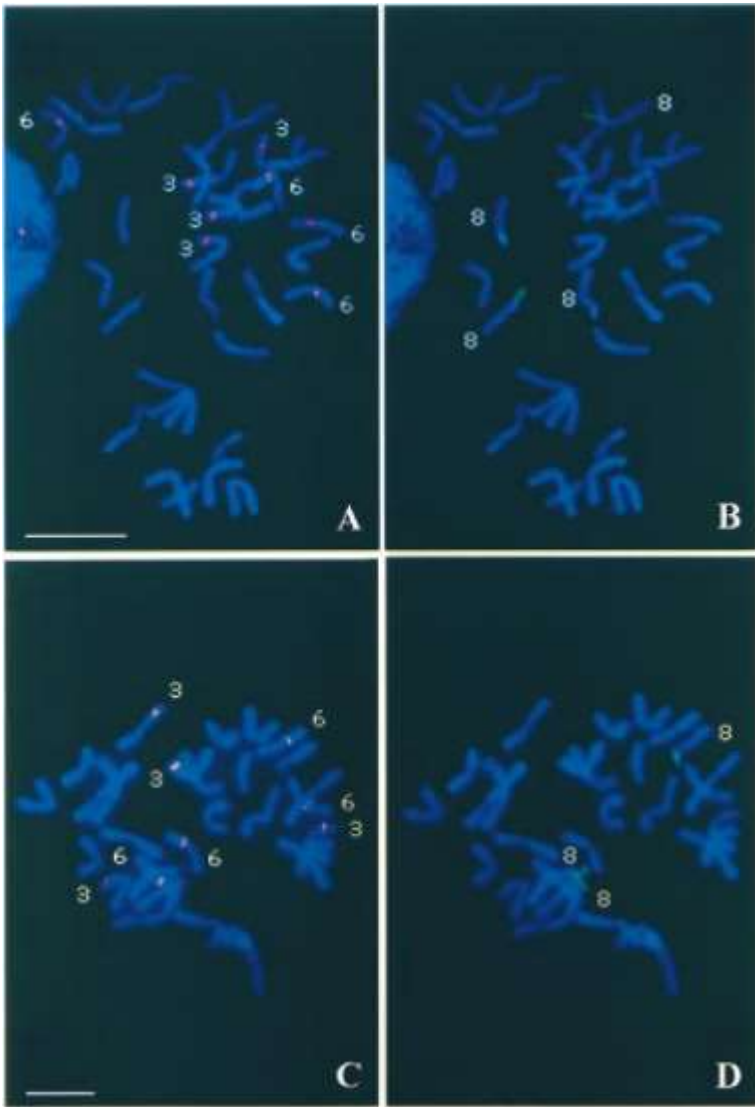
Treatment (mg l ⁻¹)	No. of shoots (<i>in vitro</i>)	No of regenerants in soil	No of hypotetraploids (%) ^c							
			2n = 28	2n = 29	2n = 30	2n = 31	2n = 32	2n = 33		
0.2 2,4-D	2 2ip	13 (5/6/2) ^a	6 (2/4/0) ^b			6				
	2 BAP	2 (1/1)	1 (1/0)			1				
	2 KIN	38 (8/14/12/3/1)	23 (7/10/4/1/1)			2	1	19	1	
0.2 NAA	2 2ip	40 (9/18/10/2/1)	26 (7/10/6/2/1)			26				
	2 BAP	94 (21/51/13/8/1)	75 (18/43/8/5/1)			3	34	11	27	
	2 KIN	14 (3/6/3/1/1)	7 (1/4/1/1/0)			2	1	2	2	
No auxin	2 2ip	21 (5/7/6/2/1)	15 (3/5/4/2/1)			2			12	1
	2 BAP	12 (4/7/1)	6 (2/3/1)			3			3	
	2 KIN	10 (3/6/1)	4 (2/2/0)			4			4	
Total	244	163	5 (3.1)	1 (0.6)	43 (26.4)	12 (7.4)	100 (61.3)	2 (1.2)		

^a Number of shoots regenerated / subculture.

^b Number of plants transferred to soil / month.

^c Number of plants / total number of regenerants in soil.

- Among these variants, **At30 revealed the highest percentage of aneuploid cells (26.4%).**
- One of the most interesting findings was the fact that **At30 showed better viability and growth than wild-type plant and other aneuploid plants during the culture period for two generations.**
- At30 was **phenotypically normal**, developed extensive roots and grew into healthy plants **with normal flowering**



FISH patterns using the 5S and 18S-5.8S-26S rDNA probes of wild *A. tuberosum* metaphase chromosome (A and B) and At30 (C and D) (bar=10 μ m). Digoxigenin-labelled 5S rDNA probe detected with antidigoxigenin-rhodamine conjugate (red) (A and C) and biotin-labelled 18S-5.8S-26S rDNA probe detected with avidin-FITC conjugate (green) (B and D). Numbers indicate corresponding chromosomes showing rDNA hybridization sites.

to analyze the karyotypic change of At30 in comparison with the wild type by using 5S and 18S- 5.8S-26S rDNA as probes.

Interspecific and intergenetic crosses

- Alien chromosome, chromosome segment and gene can be identify and characterization by FISH and GISH.

Detection of chromosomal aberration

- Almost aberration observed during meiosis
- Than plant its more important to human being
- mFISH for all 24 chromosome by using the human genomics library

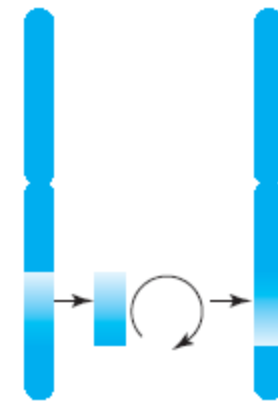
FISH Identifies chromosomal abnormalities



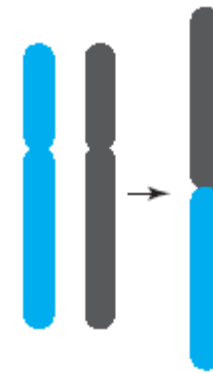
Translocation



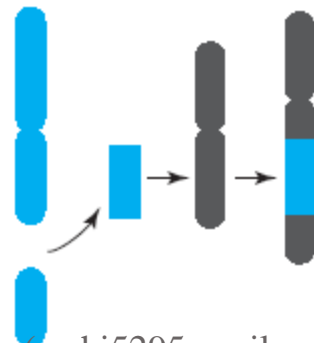
Deletion



Inversion



Isochromosome



Insertion



Ring chromosome

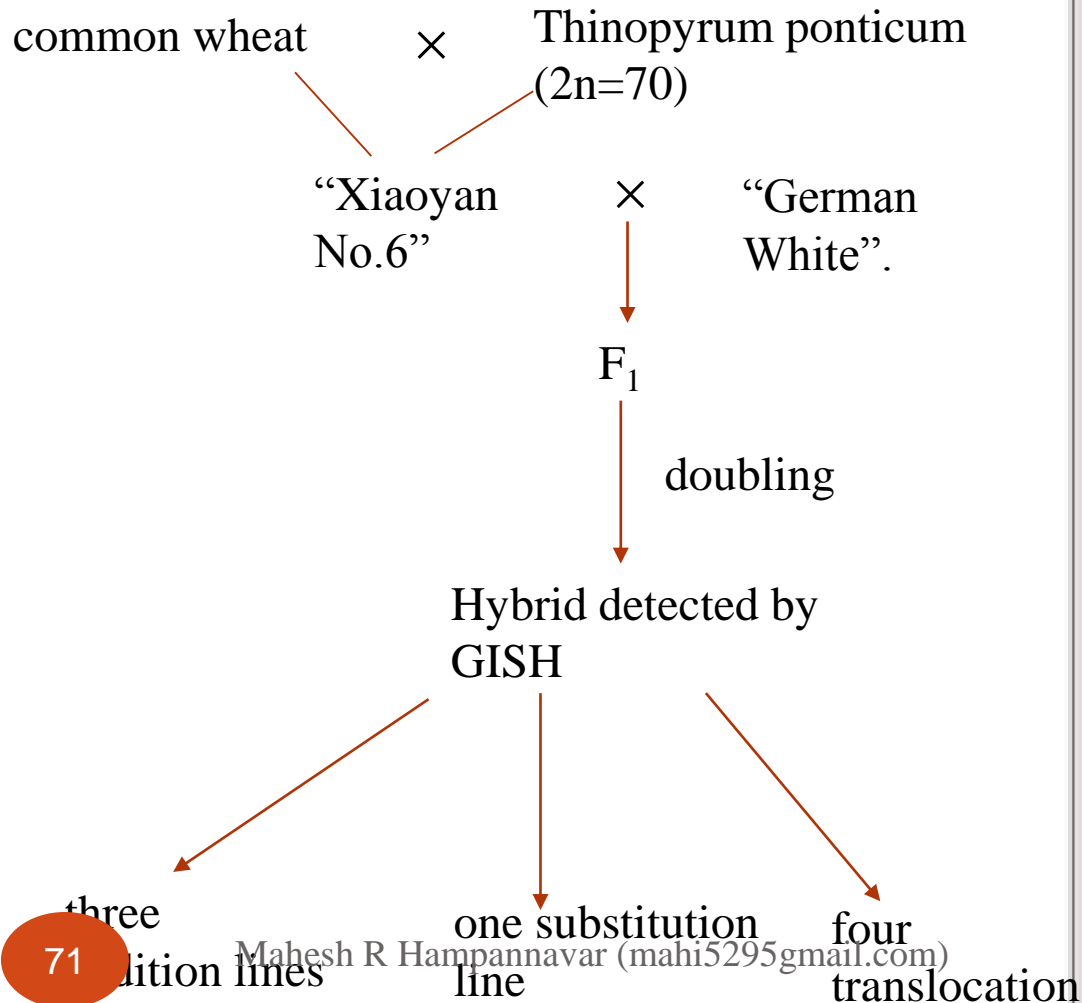


Derivative chromosome

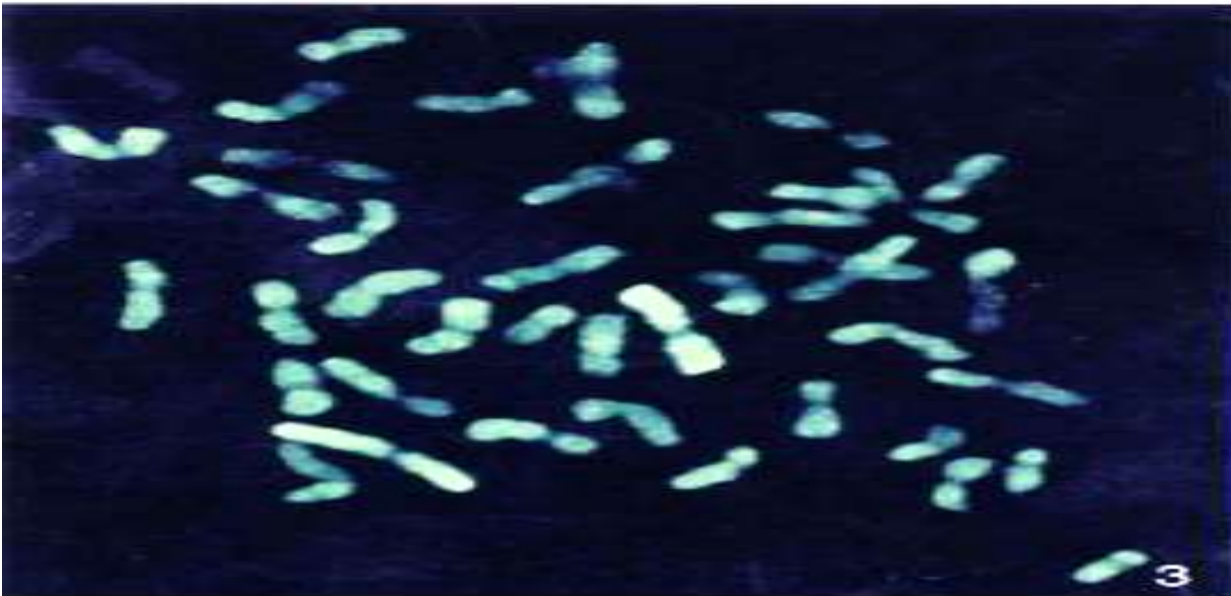
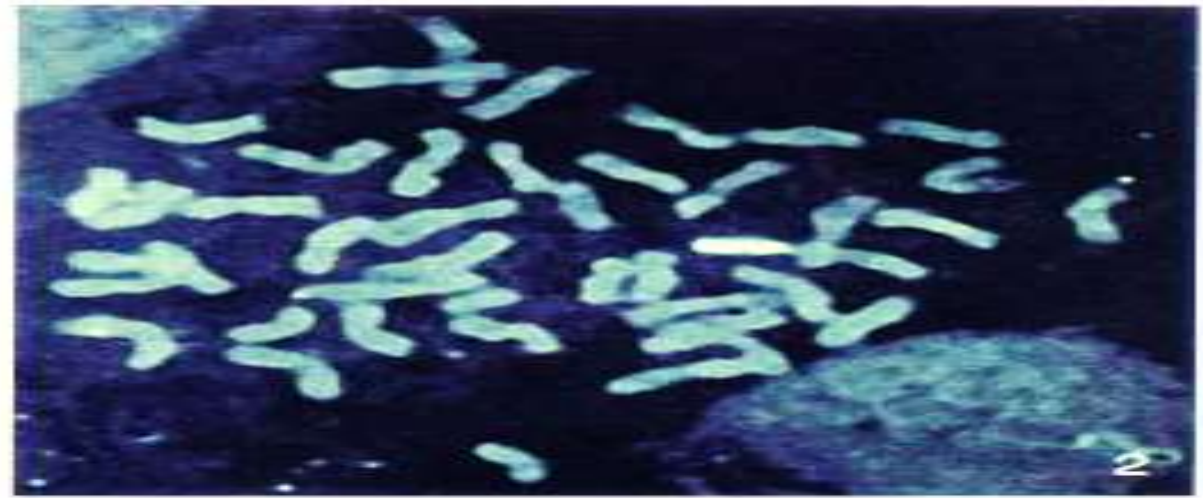
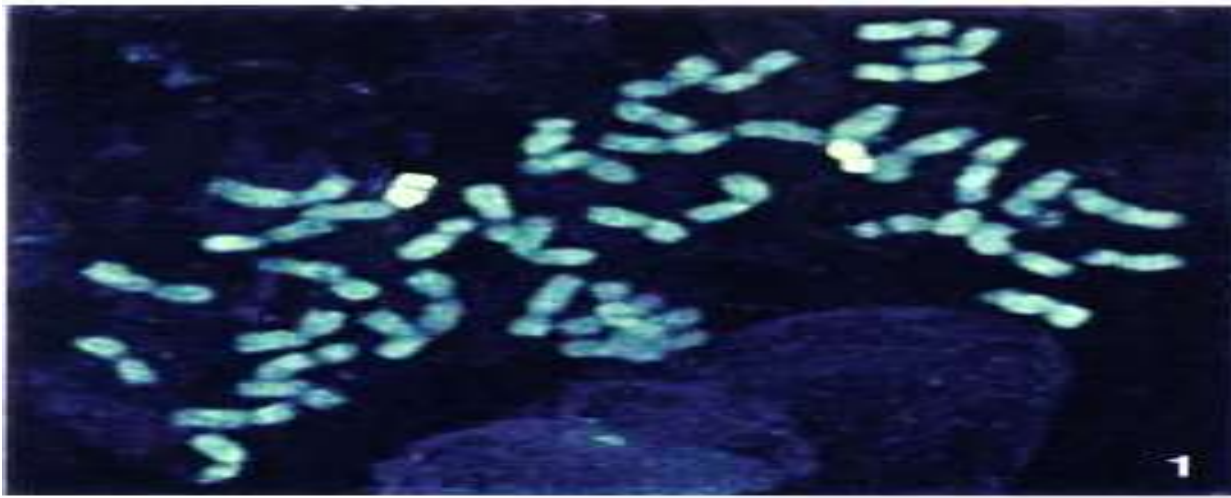
Fluorescent *In Situ* Hybridization Analysis of Rye Chromatin in the Background of “Xiaoyan No.6”

WANG Zhi-Guo¹, AN Tiao-Guo¹, LI Jun-Ming^{1*}, Molnar-Lang MARTA²

JI Jun¹, ZHONG Guan-Chang¹, MU Su-Mei¹



- Wheat-rye hybrids were produced by crossing winter wheat (*Triticum aestivum* L.) cultivar “Xiaoyan No.6” and winter rye (*Secale cereale* L.) cultivar “German White”.
- “Xiaoyan No.6 × rye” were detected by GISH. Eight lines were found carrying rye chromosomes or segments
- Two highly repeated DNA sequences **pSc119.2** and **pAs1** were labeled with **Fluorogreen** and **Fluororedvia** nick translation respectively.
- Two-color fluorescent in situ hybridization (FISH) was used to identify the genome composition and individual chromosome of the hybrids using pSc119.2 labeled with fluorogreen



Figs. 1–4. Detection of rye chromosomes or segments in the background of “Xiaoyan No.6” by GISH. Using genomic DNA of rye (*Secale cereale*) as a probe, and 35-fold genomic DNA of wheat as blocking DNA. The bright-green hybridization signals indicated the rye component. **1.** Translocation line BC116-1. **2.** Translocation line BC152-1. **3.** Substitution line BC97-2. **4.** Addition line BC122-3.

Chromosome specific painting

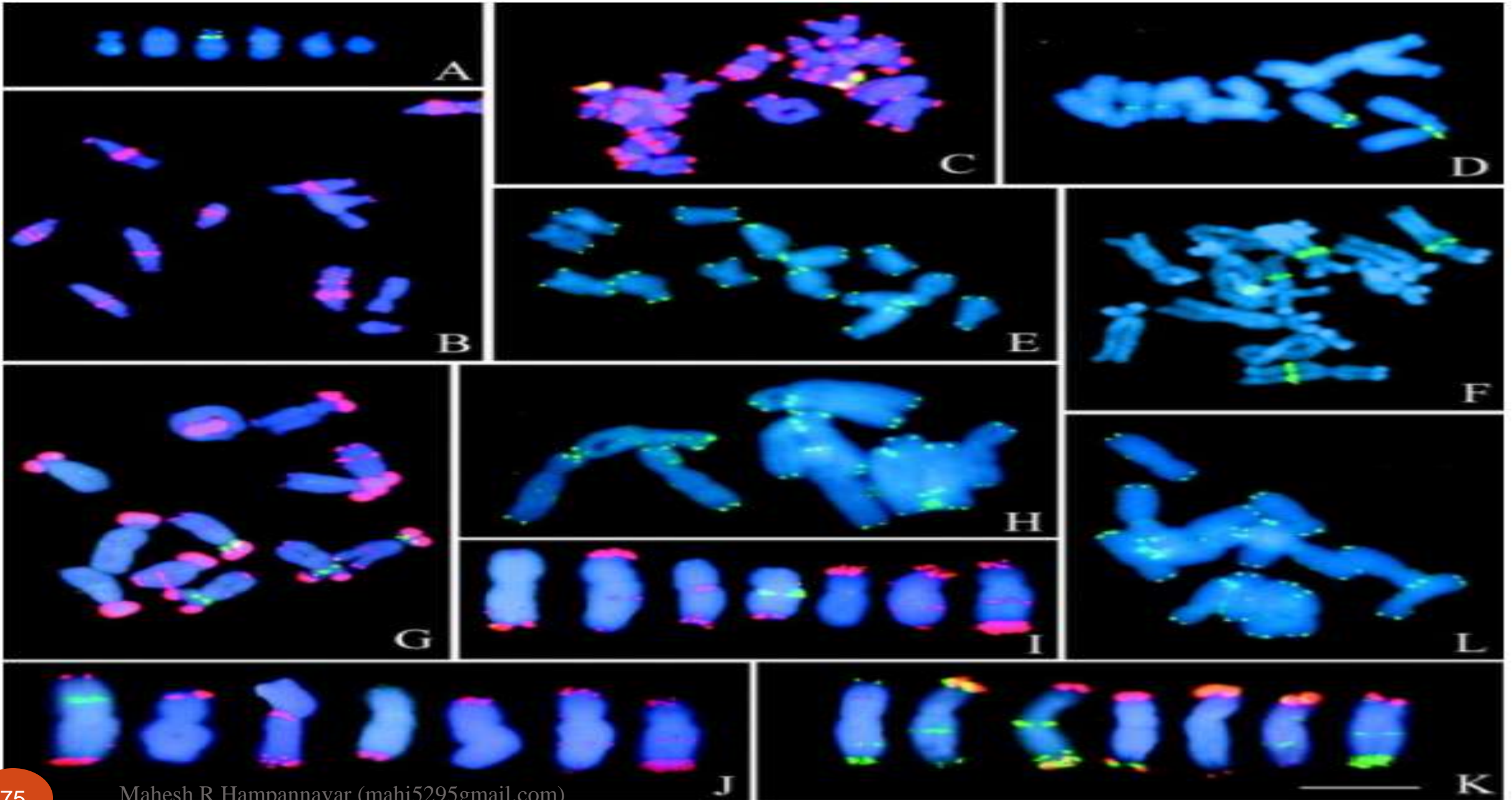
- Determination karyotype based on chromosome size, centric index and banding pattern has its own limitation.
- Discrimination of similar chromosomes.
- Helps to discriminate the same size chromosome within its own genome

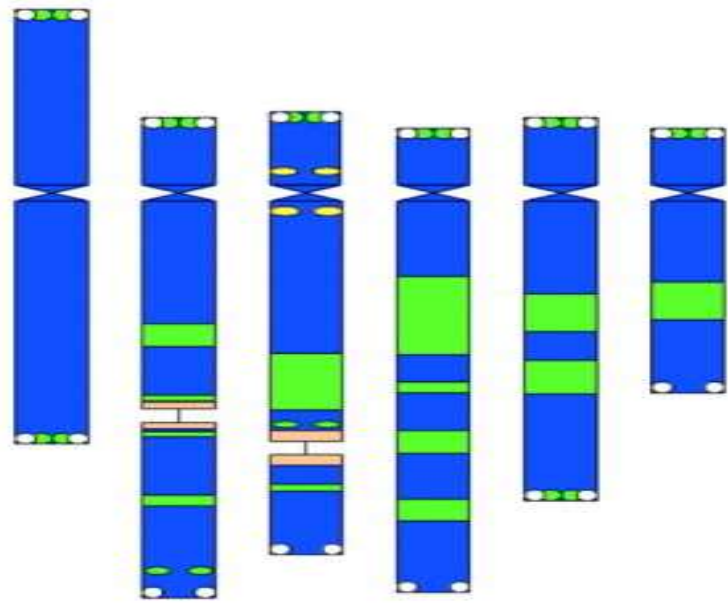
Karyotype Analysis of Four *Vicia* Species using *In Situ* Hybridization with Repetitive Sequences

[ALICE NAVRÁTILOVÁ](#),¹ [PAVEL NEUMANN](#),¹ and [JIŘÍ MACAS](#)¹

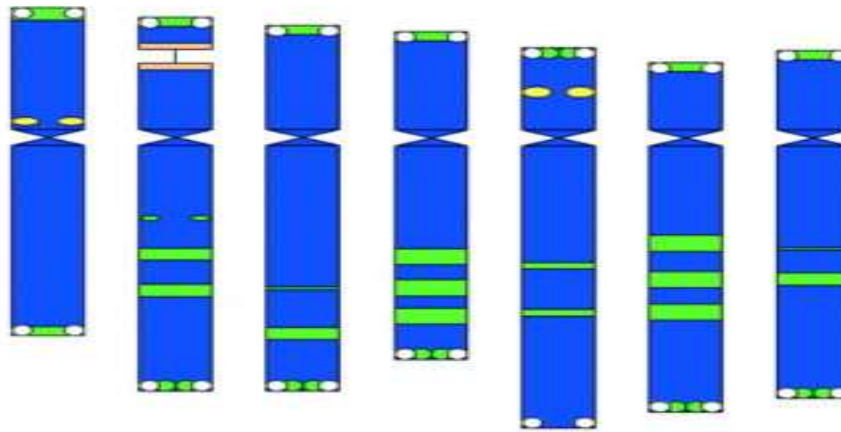
- Four *Vicia* species (*V. sativa*, *V. grandiflora*, *V. pannonica* and *V. narbonensis*).
- DNA sequences (18S–25S and 5S rDNA, telomeres) and genus-specific satellite repeats (**VicTR-A and VicTR-B**)
- the VicTR-A and -B sequences, in particular, produced highly

Species	Chromosome number (2n)	DNA content (pg)	Abundance of VicTR repeats	
			VicTR-A	VicTR-B
<i>V. sativa</i>	12	2-3	–	10^6 – 5×10^6
<i>V. grandiflora</i>	14	3-4	100–1000	10^6 – 5×10^6
<i>V. pannonica</i>	12	6-8	10^5 – 10^6	~100
<i>V. narbonensis</i>	14	7-3	10^5 – 10^6	10^5 – 10^6

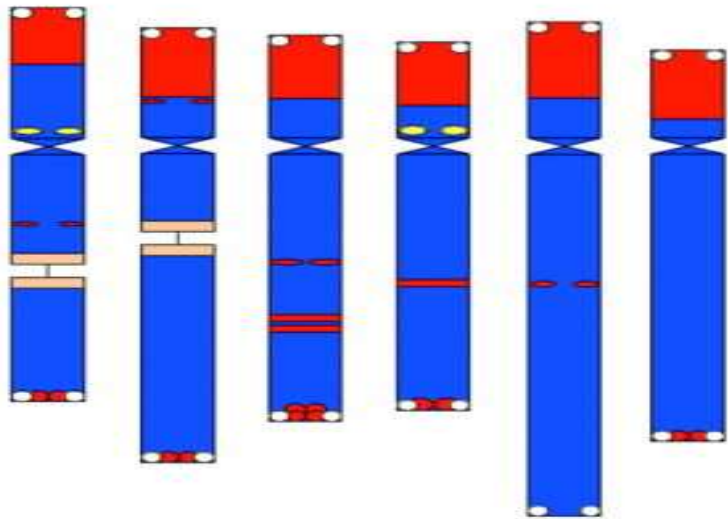




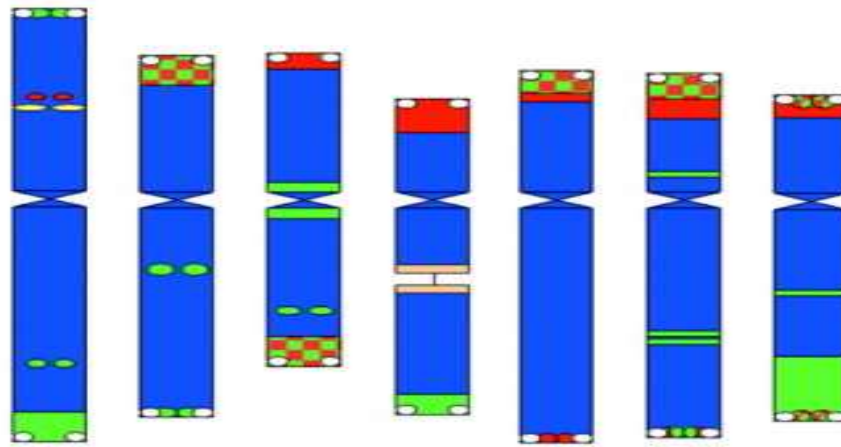
A *Vicia sativa*



B *Vicia grandiflora*

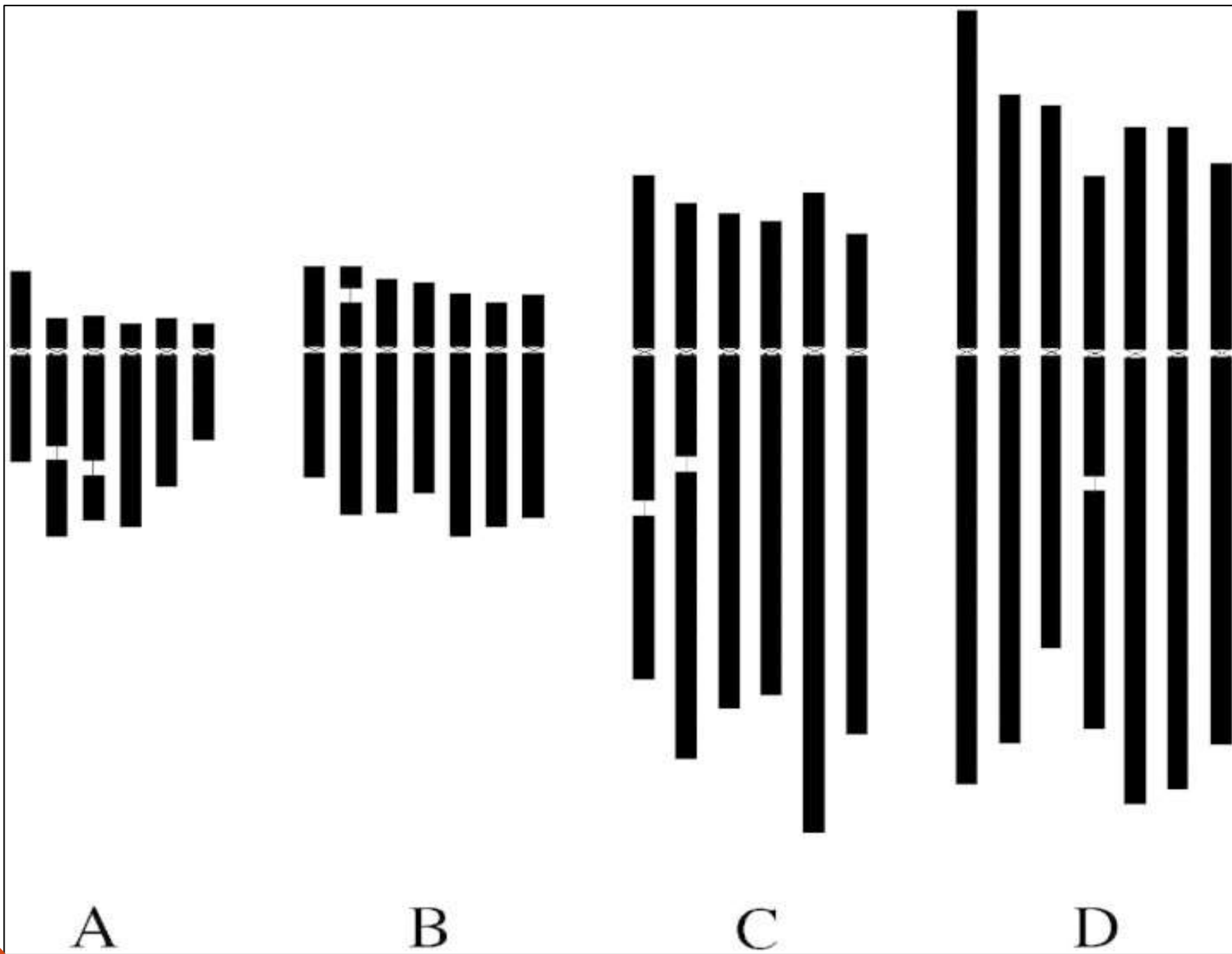


C *Vicia pannonica*



D *Vicia narbonensis*

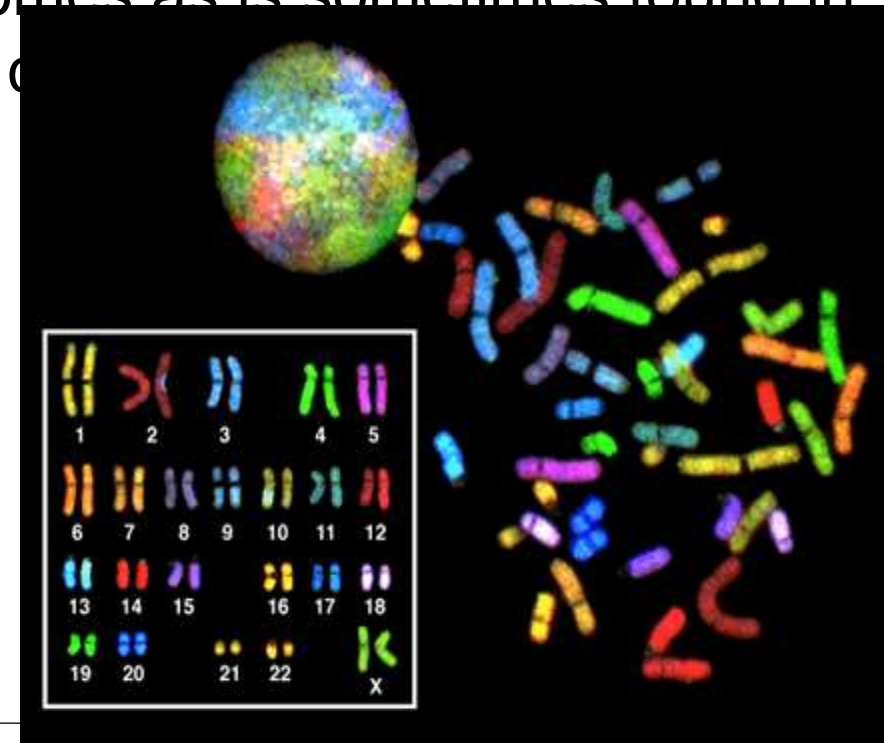
Idiograms of *Vicia sativa* (A), *V. grandiflora* (B), *V. pannonica* (C) and *V. narbonensis* (D). Presence and distribution of 18S–25S rRNA genes (orange), 5S rRNA genes (yellow), VicTR-A (red), VicTR-B (green) and telomeres (white) is indicated.



Schematic comparison of chromosome sizes among *Vicia sativa* (A), *V. grandiflora* (B), *V. pannonica* (C) and *V. narbonensis* (D). Relative chromosome lengths were derived from the data in Table 2 and scaled according to the nuclear DNA content of the species (Table 1).

Spectral karyotyping (SKY) and multiple fluorescent hybridization (M-FISH)

- By mixing combinations of five fluorors and using special imaging software, can distinguish all 23 chromosomes by chromosome specific colors.
- This type of analysis can be used to detect abnormalities that affect multiple chromosomes as is sometimes found in cancer cells or immortalized cells.



limitations of FISH

- The inability to identify chromosomal **changes other than those at the specific binding region of the probe.**
- Preparation of the sample **is critical in interphase FISH analysis**
 - to permeabilize the cells for optimal probe target interaction
 - to maintain cell morphology.
- Cannot detect small mutations.
- Probes are not yet commercially available for all chromosomal regions

- Relatively expensive
- Multi color FISH can only be used successfully on polyploid with **at least one known progenitor species**
- **Closely related genomes** in certain allopolyploids cannot be discriminate using GISH technique
- mFISH is less sensitive and **shows a lower degree of detection resolution than single color FISH** due to multiple exposure photographs

• Conclusions

- Thank you