Biotechnology

Genetic markers

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GENETIC MARKER

- Any character that can be measured in an organism which provides information on its genotype / genetic make up

- Based on their mode of transmission and evolutionary dynamics they are classified into 3

1. ***Morphological markers*** – Identify differences based on phenotypes eg: Albino, Dwarf, Red eye

2. ***Biochemical marker*** – Detect variation at the level of expression of a gene product such as protein or aminoacid eg: Hormone, allozymes

3. ***Molecular marker*** - Is a fragment of DNA that is associated with a certain location on a chromosome within the genome

- It is used to detect variations at the nucleotide level in a DNA sequence generated by deletion, duplication, inversion or insertion Eg:

1. RFLP – Co-dominant marker which works based on southern blotting

   Restriction Fragment Length Polymorphism

2. RAPD – Dominant / recessive marker which works based on PCR

   Randomly Amplified Polymorphic DNA

3. Repetitive DNA based Mini satellite – VNTR and Micro satellite – STR

   Variable Number Tandem Repeats

   Short Tandem Repeats

- A molecular marker should have the following features:

- Must be polymorphic [different structures in different individuals]

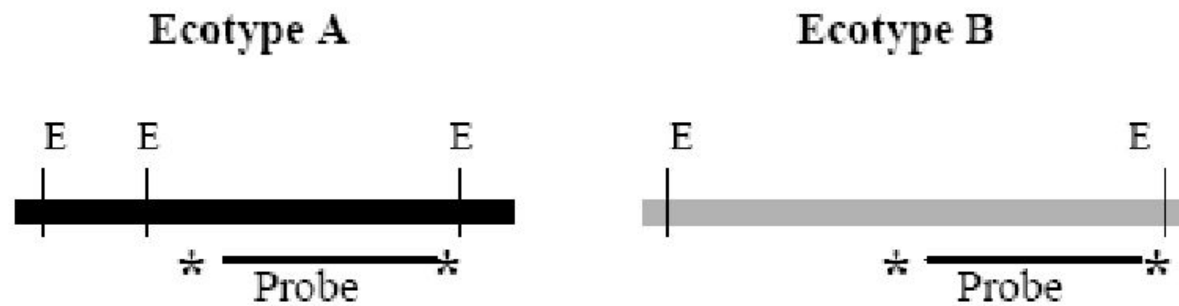
- Should be evenly distributed throughout the genome

- Should distinguish Homozygous and Heterozygous

1. Restriction Fragment Length Polymorphism - RFLP

- Is the dissimilarity in homologous DNA sequence which is identified by differences in length of the DNA fragments generated after digesting of both strands with same restriction enzyme

- Homologous human chromosomes differ in sequences on average at every 1250 bp. These genetic differences create and eliminate new restriction sites



Digestion genomic DNA with restriction enzyme E
Agarose gel electrophoresis
DNA blotting
Hybridisation with probe
Exposure

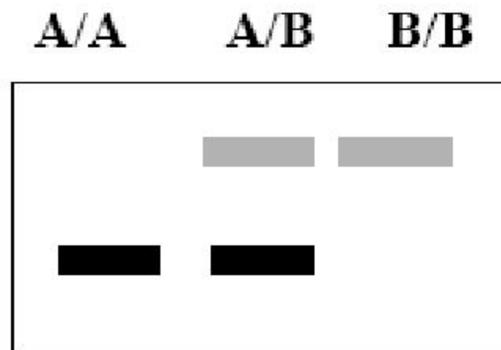


Figure 1. Principle of RFLP markers. This figure illustrates an RFLP marker which utilises a site for the restriction enzyme (E) which is present in line A and not in line B.

PROCESS

1. Genomic DNA is isolated from several strains or related species
2. These DNAs are digested with a selected restriction enzyme
3. The fragments are separated through gel electrophoresis
4. The resulting gel lines are transferred and chemically bound to a nitrocellulose membrane and exposed to a radio labelled probe (Southern blotting)
5. Free probes are removed and those fragments to which probes are hybridized is detected by autoradiography

- An RFLP occurs when the length of a detected fragment varies between individuals

- **APPLICATIONS**

1. Vital tool in genome mapping
2. Genetic disease analysis – Location of a particular disease gene
3. To find out phylogenetic relations
4. Genetic variation with in species (Genetic finger printing)

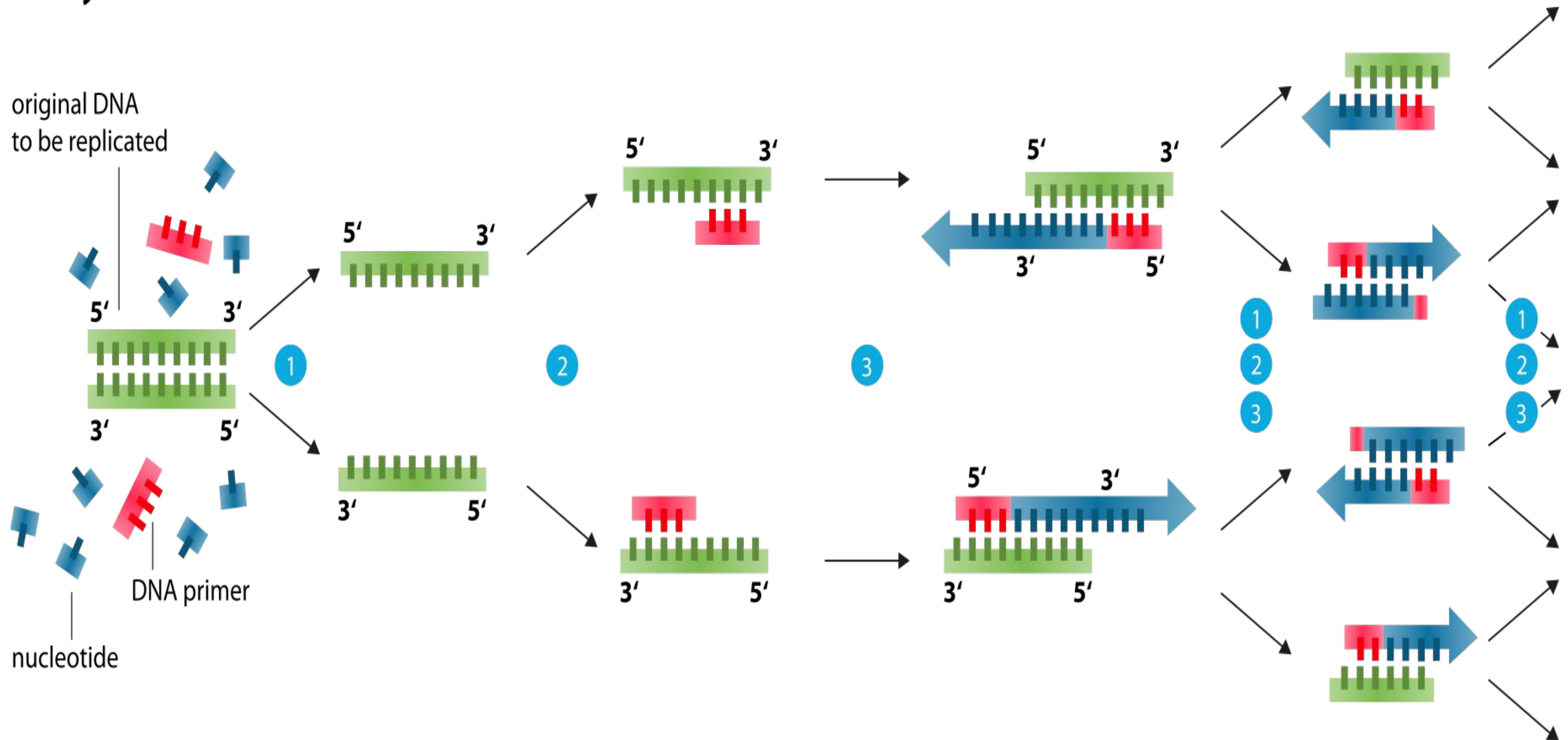
2. Randomly Amplified Polymorphic DNA - RAPD

- Amplification of sequences using random primers without any previous knowledge of the DNA sequence of the targeted organism
- The primers will bind somewhere in the, sequence but not certain exactly where
- Consequently, random sample of DNA markers are obtained

PROCESS

1. PCR amplification of random locations in the genome
2. Many random oligo nucleotide primers of 10bp length are designed using different combination of nucleotides
3. The DNA amplification product is generated from a region flanked by a pair of 10primer sequences
4. Genomic DNA of two individuals produce different RAPDs and hence used to identify individuals

Polymerase chain reaction - PCR



1 **Denaturation** at 94-96°C

2 **Annealing** at ~68°C

3 **Elongation** at ca. 72 °C

APPLICATIONS

1. Used as genetic marker to construct genetic maps
2. Used to characterise and trace phylogeny of different plant and animal species
3. Used to tag genes of high economic importance in segregating populations eg: resistant traits against pathogens
4. Genetic finger printing

- Advantages over RFLP

- No species specific probes are required for different species
- Data can be collected quickly
- Crude DNA preparation may be used for analysis of whole genome
- Only small amount of DNA is required
- Not require blotting or hybridization

• Limitations of RAPD

- Being dominant markers it is not possible to distinguish whether the DNA segment is amplified from a locus that is heterozygous (1 copy) or homozygous (2 copy)
- PCR is an enzymatic reaction and therefore the laboratory conditions may influence the process
- Since the probes are random preparations mis-matches may result causing total absence or decreased amount of production

3. Variable Number Tandem Repeats - VNTR

- Is a location in a genome where a short nucleotide sequence that range in length from 10-100bp are arranged as tandem repeats (clustered and oriented toward same direction)

  Eg: A-T-T-C-G-A-T-T-C-G-A-T-T-C-G

- The repeated sequences may same from person to person (usually GC rich) but the length and number of times they are repeated tends to vary among individuals

- Individuals repeats can be removed from (or added to) VNTR via recombination or replication errors

- The flanking region adjacent to tandem repeats are segments of non-coding regions having restriction enzyme recognition sites
- Hence VNTRs can be extracted using restriction enzymes and is an important source of RFLP
- The VNTR alleles follows the rules of inheritance. In matching an individual with parent , the person must have an allele that matches one from each parent
- If the relation is more distant, such as sibling or grand parent, then matches should be consistent with degree of relatedness

•Applications

- DNA finger printing – Paternity & Maternity testing, linking DNA samples of relatives with a missing person
- Forensic studies – Link a suspect with a sample of blood, semen or hair taken from a crime
- Diagnosis and identification of human diseases
- Population studies
- Conservation biology

4. Short Tandem Repeats - STR

- Or Simple Sequence Repeats (SSR) are sequences of 2-6 base pairs that can be repeated 3 to 100 times in a loci

Di nucleotide repeat : A-C-A-C-A-C-A-C

Tri nucleotide repeat: C-A-G-C-A-G-C-A-G

- STR polymorphism occurs when homologous STR loci differ in the number of repeats between individuals
- The variability of microsatellites are due to higher rate of mutation

Applications

- Changes in genotypes are observed when mutations occur in STR sequences
- Act as marker for some genetic diseases.
- In humans 90% of microsatellites are found in non-coding region but when found in coding regions they occur as tri-nucleotide repeats. This leads to frame shift mutation and associated disorders
- Microsatellites provide a necessary source of genetic variation

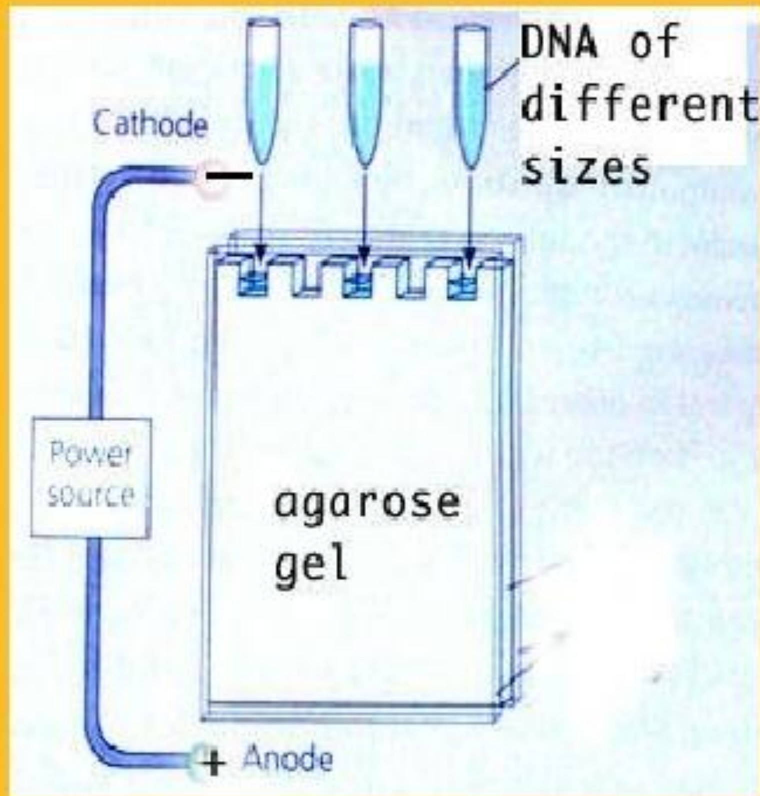
DNA Finger Printing

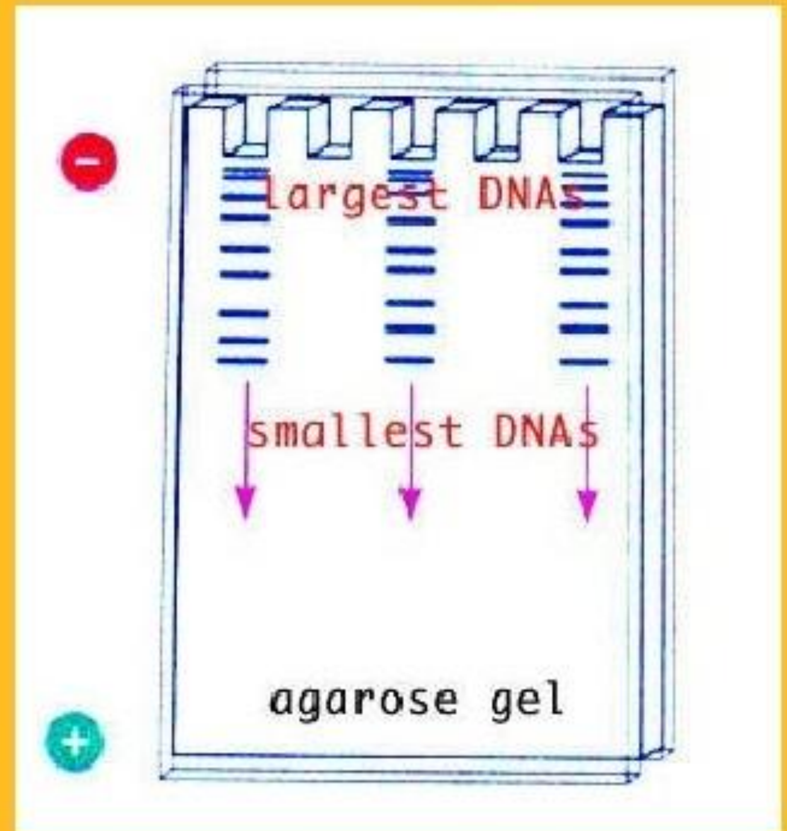
- Is the method of isolating and distinguishing individuals on the basis of their DNA analysis
- Developed by Alec Jeffreys in 1984
- The method relies on the restrictions made on mini satellites VNTR using restriction enzymes and subsequent formation of RFLPs
- Each organism except identical twins has a unique pattern of this minisatellites
- As they follow Mendelian pattern of inheritance it is applicable to find out DNA relationships among humans

1. Obtain the sample cells containing DNA (skin, blood or hair)
2. Extract and purify DNA
3. Cut at specific points along the strand using restriction enzymes which produce fragments of varying length
4. Sort them placing on a gel and subject to electrophoresis
5. Sorted double stranded fragments are subjected to Southern blotting
6. ds fragments are denatured to ss by soaking them in 0.5M NaOH

7. Then they are transferred on to a nitrocellulose filter membrane using a blotting assembly
8. The nirocellulose membrane with ss DNA blotted on it are then baked at 80°C for 2-3hrs to fix DNA permanently on it
9. Subjected to hybridisation with a radioactively labelled probe
10. Probes with bound digested DNA are visualised by auto radiography and compared

* Unrelated individuals generally have mini satellite that differ in length but children inherit one set of minisatellite DNA sequence from each parent


DNA of
different
sizes
Cathode
Power
source
agarose
gel
Anode


largest DNAs
smallest DNAs
agarose gel

MOM DAD D1 D2 S1 S2

•Applications

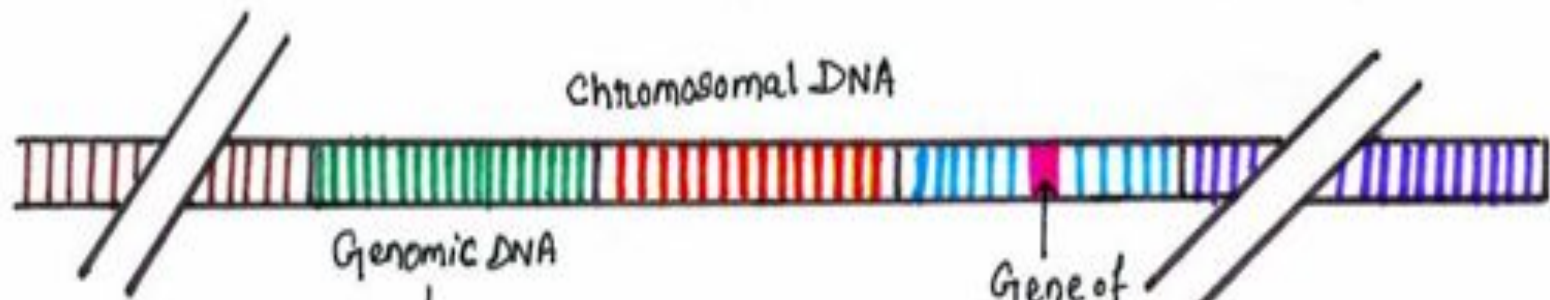
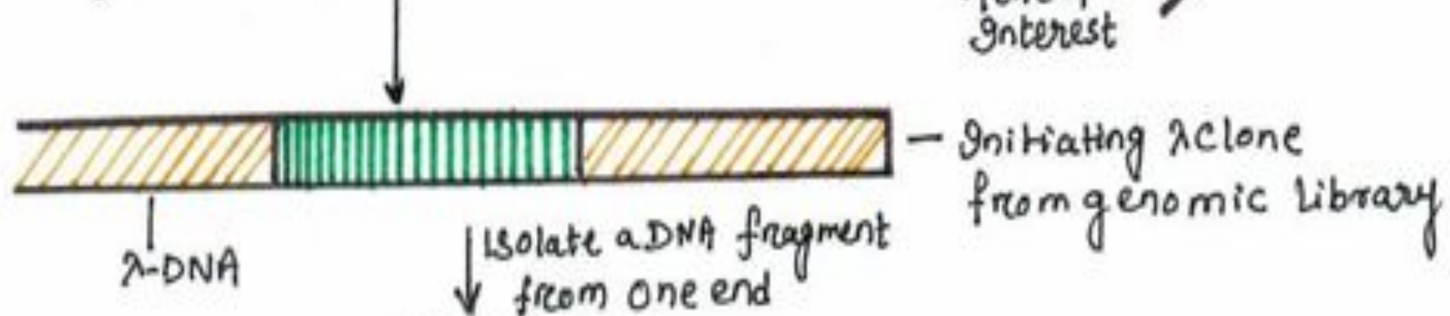
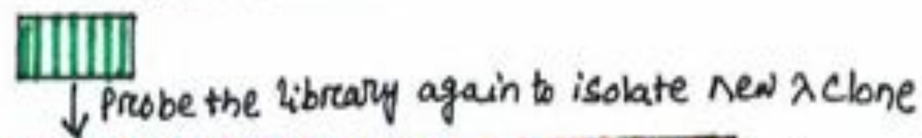
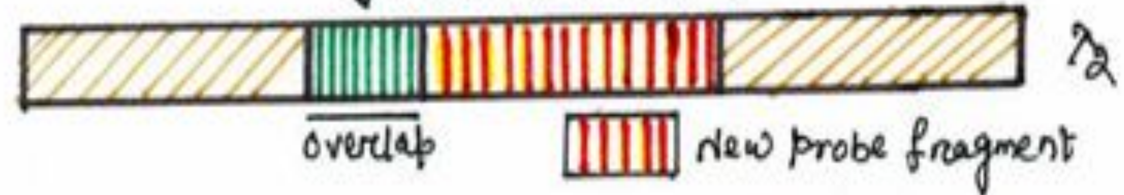
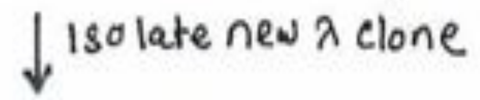
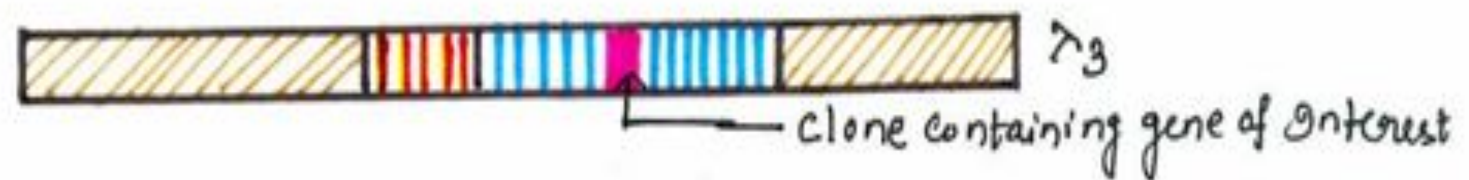
- Paternity & Maternity testing, linking DNA samples of relatives with a missing person
- Forensic studies – Link a suspect with a sample of blood, semen or hair taken from a crime
- Identify of burnt unidentifiable dead body
- Cases of child stealing, mix up between babies in hospitals
- Problem of immigration

CHROMOSOME WALKING

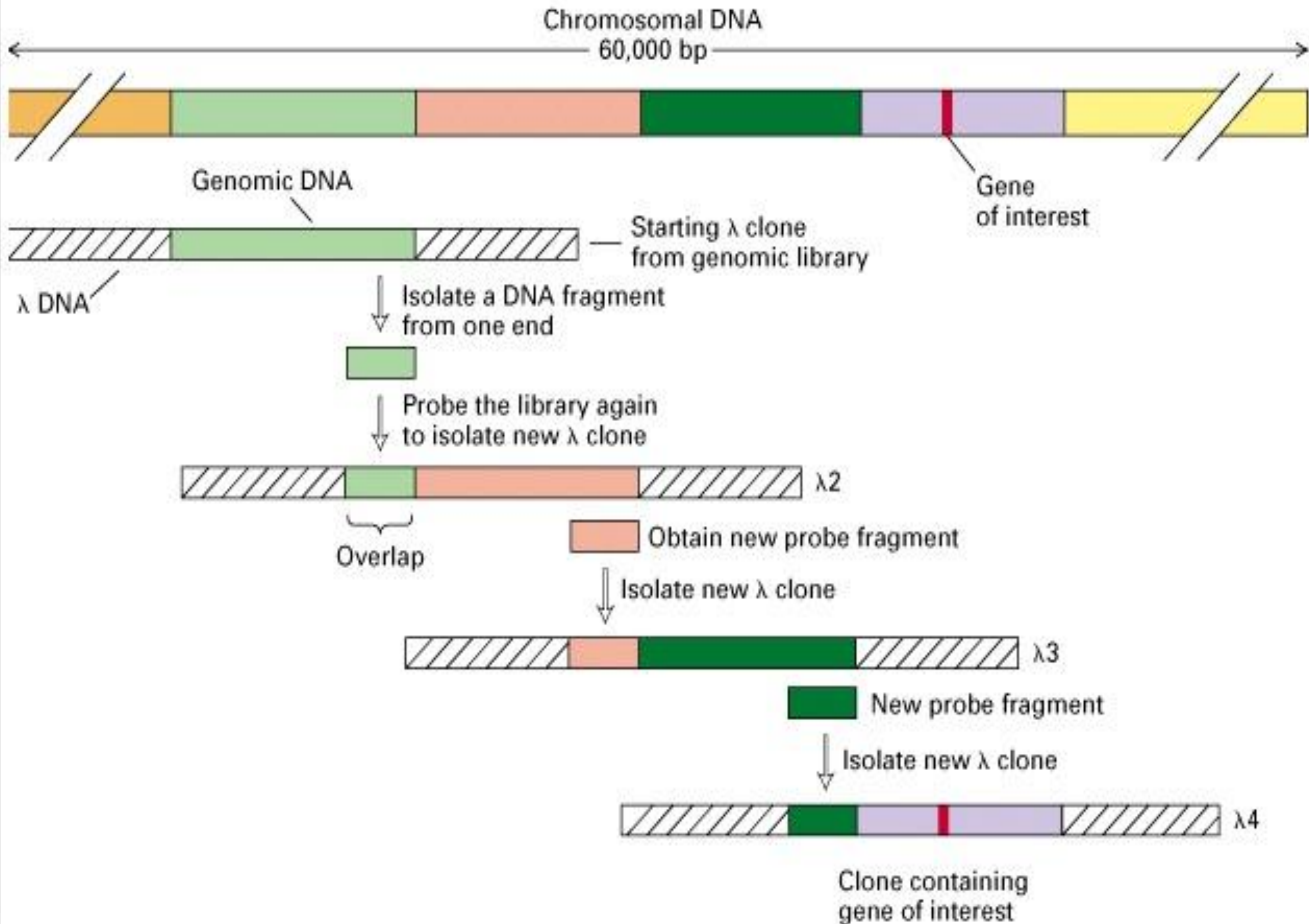
- Move systematically along a chromosome from a known location and to clone overlapping genomic clones that represent progressively longer parts of a particular chromosome

- Used as a means of finding adjacent genes – Positional cloning

1. From genomic library select a clone of interest
2. Sub clone a small fragment from one end of the clone
3. The sub cloned fragment of the selected gene may hybridize with other clone in the library due to overlapping sequences
4. The end of the second clone is then sub cloned and used for hybridisation with other clone
5. From this a third clone is identified and so on….


Chromosomal DNA
Genomic DNA
Gene of Interest

Initiating λ clone from genomic library
λ-DNA
Isolate a DNA fragment from one end

Probe the library again to isolate new λ clone

λ2
overlap

New probe fragment
Isolate new λ clone

λ3
Clone containing gene of Interest

	01	04	12	06	13	14	02	14	07	19	11	15	05	08	16	03	10	09	20	18
A							1	1	1	1										
B																1	1	1	1	
C				1	1	1	1	1												
D								1	1	1	1	1								
E														1	1	1	1			
F		1	1	1	1															
G											1	1	1	1						



Chromosomal DNA
60,000 bp
Genomic DNA
Gene
of interest
Starting λ clone
from genomic library
λ DNA
Isolate a DNA fragment
from one end
Probe the library again
to isolate new λ clone
λ2
Overlap
Obtain new probe fragment
Isolate new λ clone
λ3
New probe fragment
Isolate new λ clone
λ4
Clone containing
gene of interest

FISH – Fluorescent In Situ Hybridisation

- Use of fluorescently labelled nucleic acid sequences or probes for visualisation of specific DNA or RNA sequences on mitotic chromosome preparations or in interphase cells

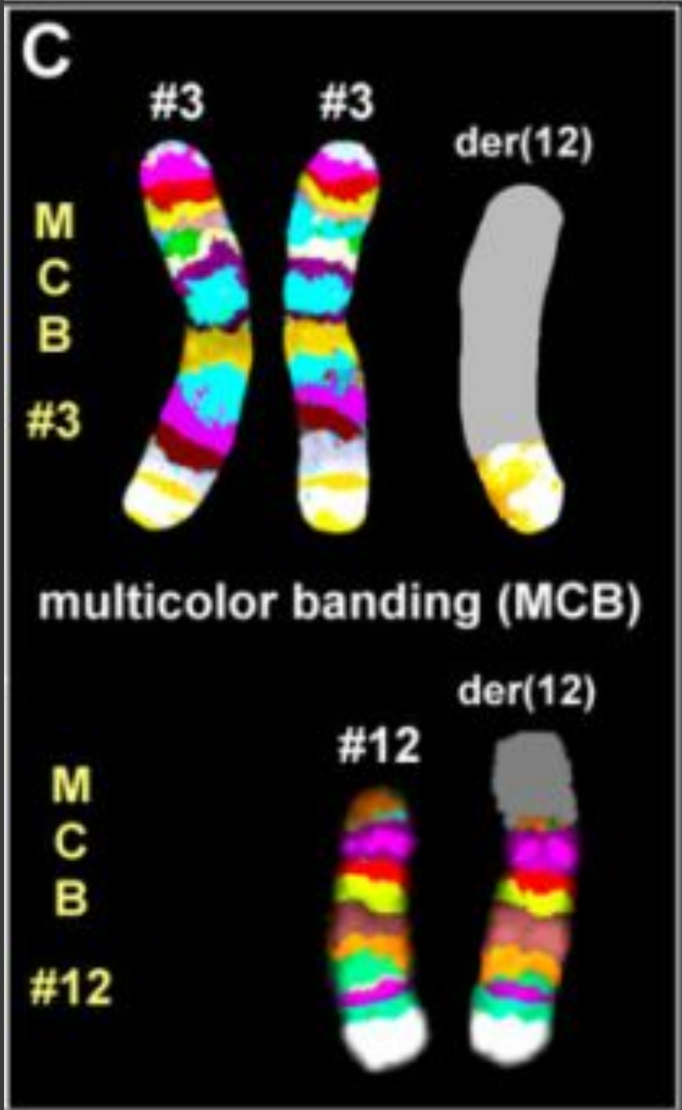
- The method also known as *Chromosome painting* since different gene probes that are labelled with different fluoro chrome vividly paint different genes or chromosomes in different colours

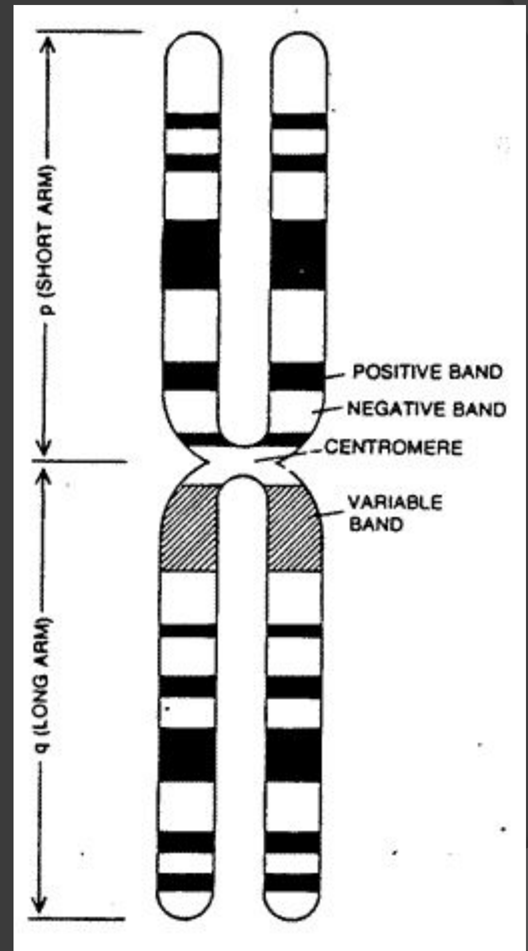
- Invented by Christoph Lengauer

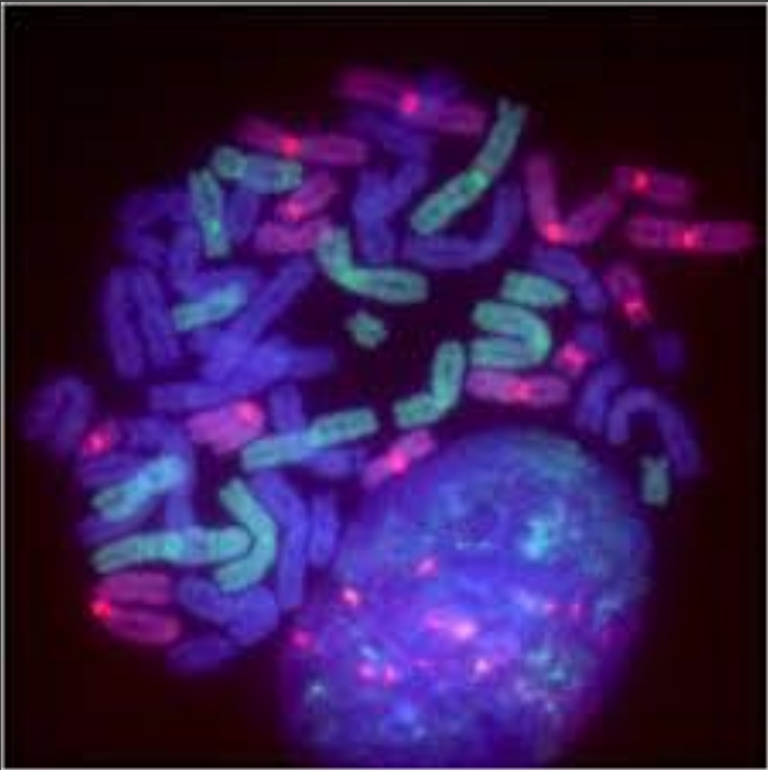
Types of FISH probes:

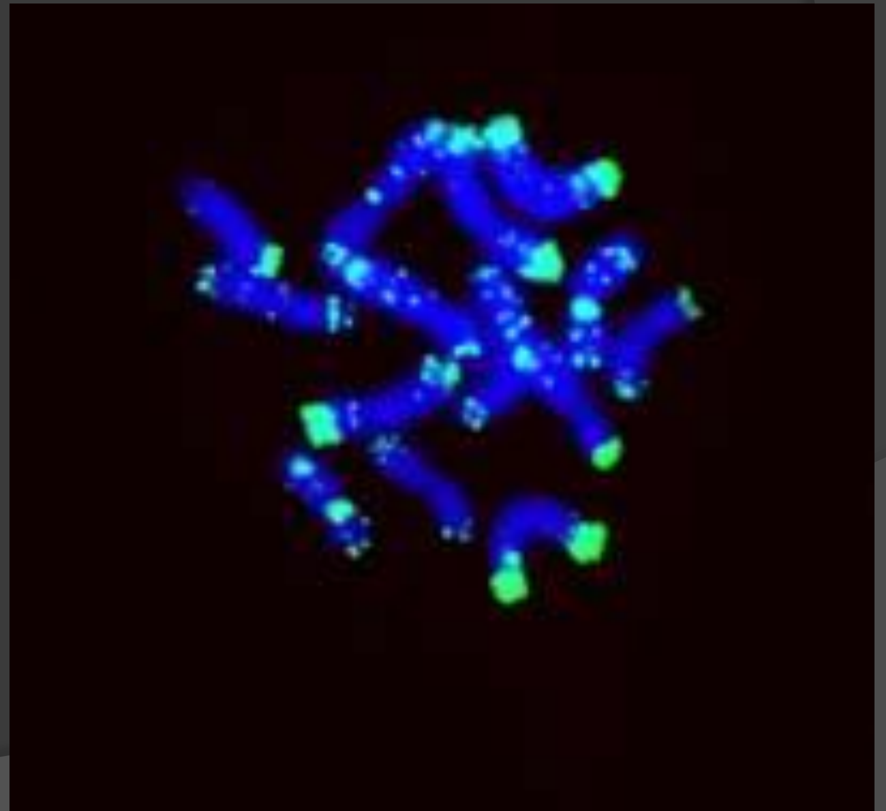
- *Locus specific probe* – hybridise to a particular region on chromosome and helpful to identify the chromosome on which a particular gene is located
- *Centromeric repeat probe* – are generated from repetitive sequences present on a chromosome. Since chromosomes are painted in different colours easy to count and identify genetic abnormalities (Extra copies)
- *Whole chromosome Probe* – Collections of smaller probes each of which hybridise to a different sequence along the length of the same chromosome

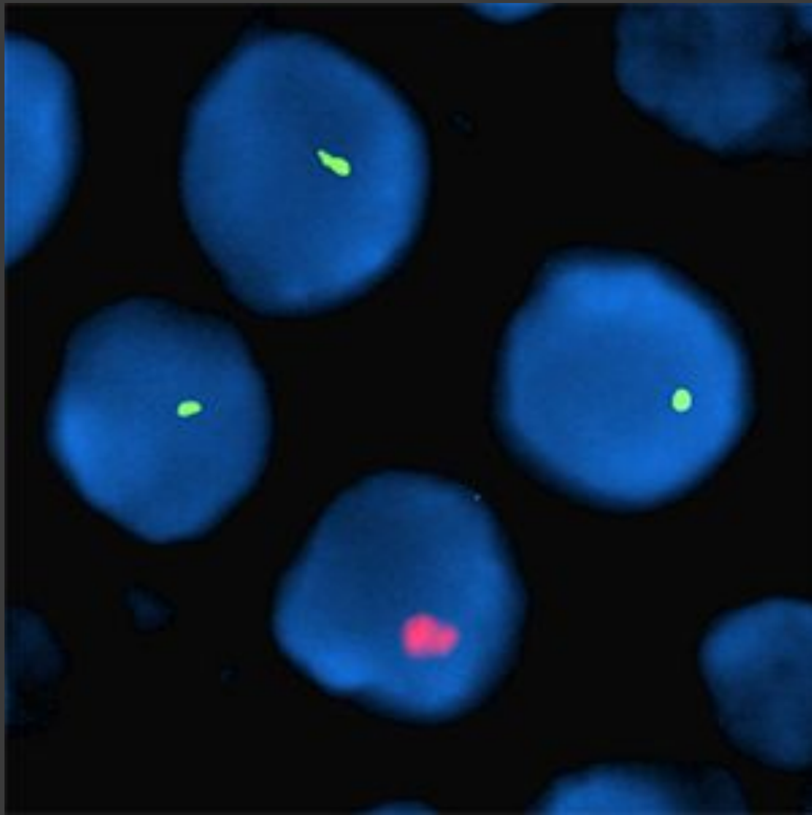
- Here, chromosomes can be identified base on colours rather than by old dark and white banding pattern
- **Applications**
- Used to study telomere shortening
- Mapping of genes on human chromosomes
- Useful technique in Human genome project

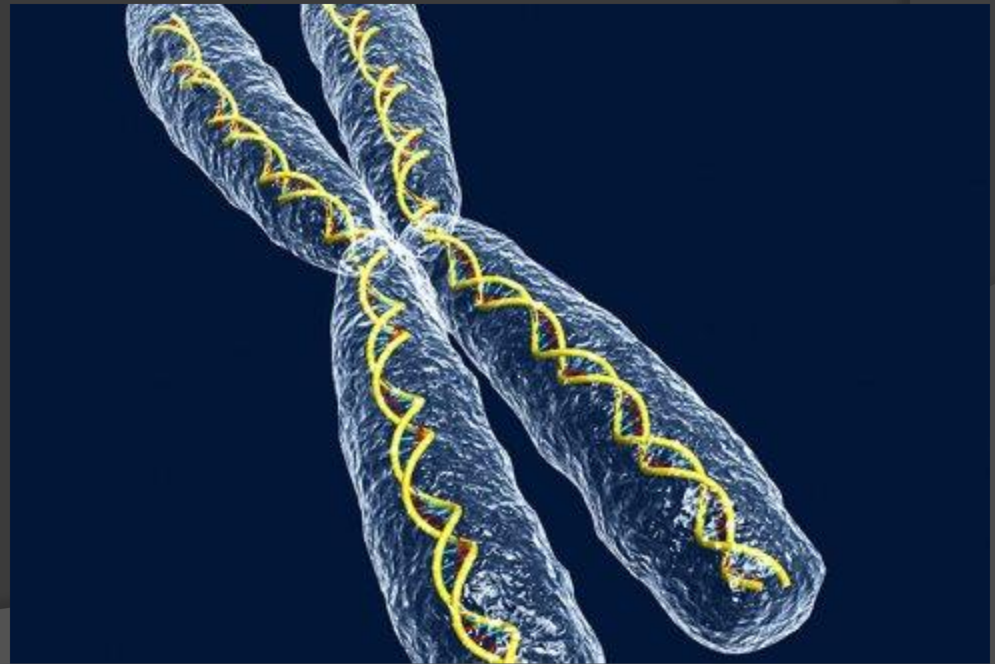

C
#3
#3
der(12)
M
C
B
#3
multicolor banding (MCB)
der(12)
#12
M
C
B
#12


p (SHORT ARM)
q (LONG ARM)
POSITIVE BAND
NEGATIVE BAND
CENTROMERE
VARIABLE BAND







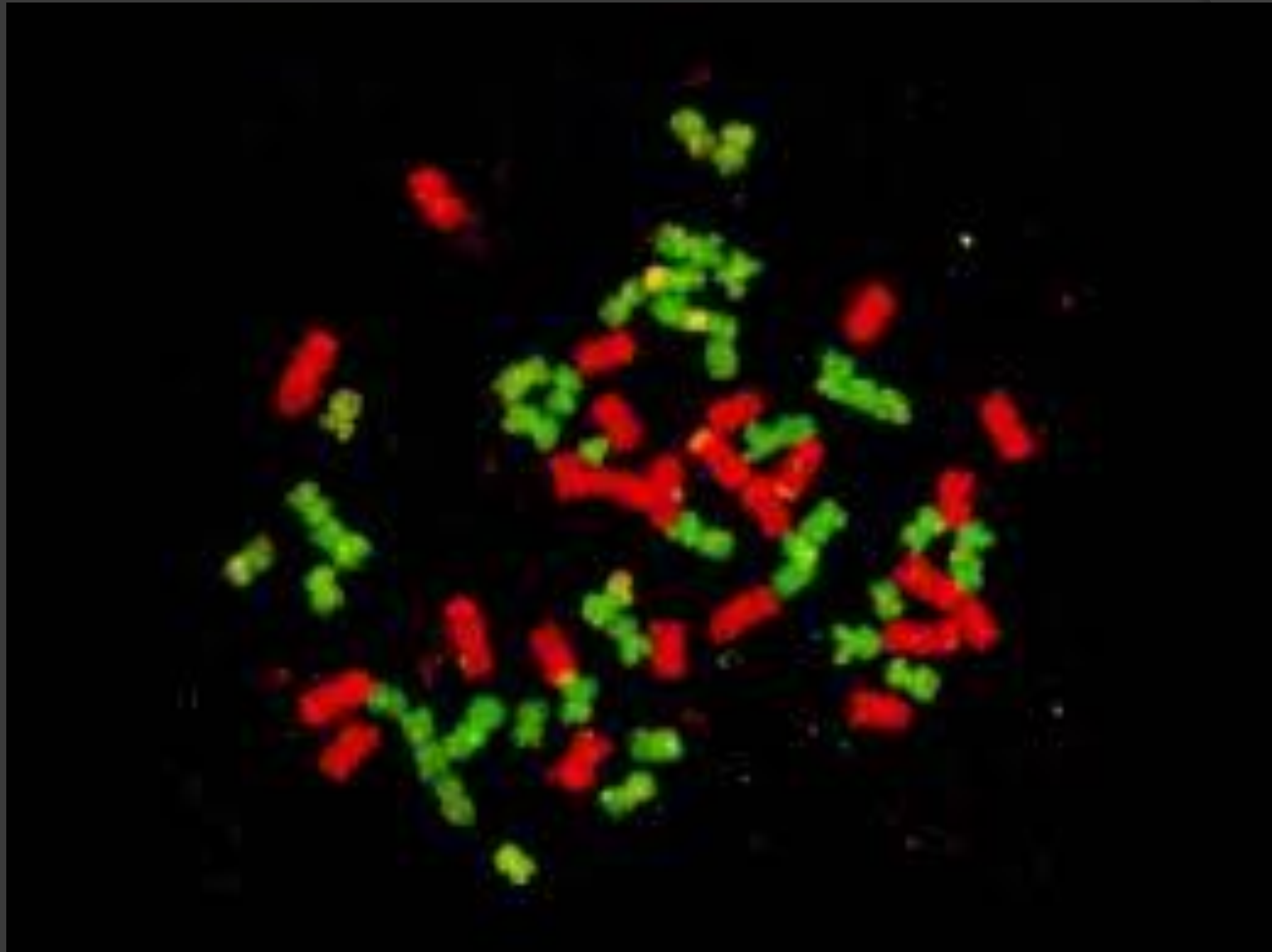


GISH – Genomic In Situ Hybridisation

- Is a molecular cytogenetic technique which allow to distinguish the entire nuclear DNA of a species by means of differential hybridisation of entire genomic probes
- Analyse Polyploidy in plants
- Determine genome origin of paired and unpaired chromosomes in metaphase I configuration (Paternal or Maternal)
- Examine inter sepeics distribution and organisation

PROCESS

- Extract genomic DNA from one species of interest for use as probes
- Allow to hybridise with chromosome preparations from the species being studies using southern blotting or insitu hybridisation
- Many of the DNA sequences within the two or more genome under investigations being sufficiently different, the genomic probe discriminates them



Genomic library

- Also clone bank or gene bank, is a collection of DNA from a single organism, ideally though not necessarily containing its entire genomic DNA sequence.
- The DNA from the source organism of interest is divided into multiple fragments and packaged within cloning vectors such that each carries a portion of it.
- The vector DNA can then be inserted into host organisms - commonly a population of bacteria - for amplification and retrieval.

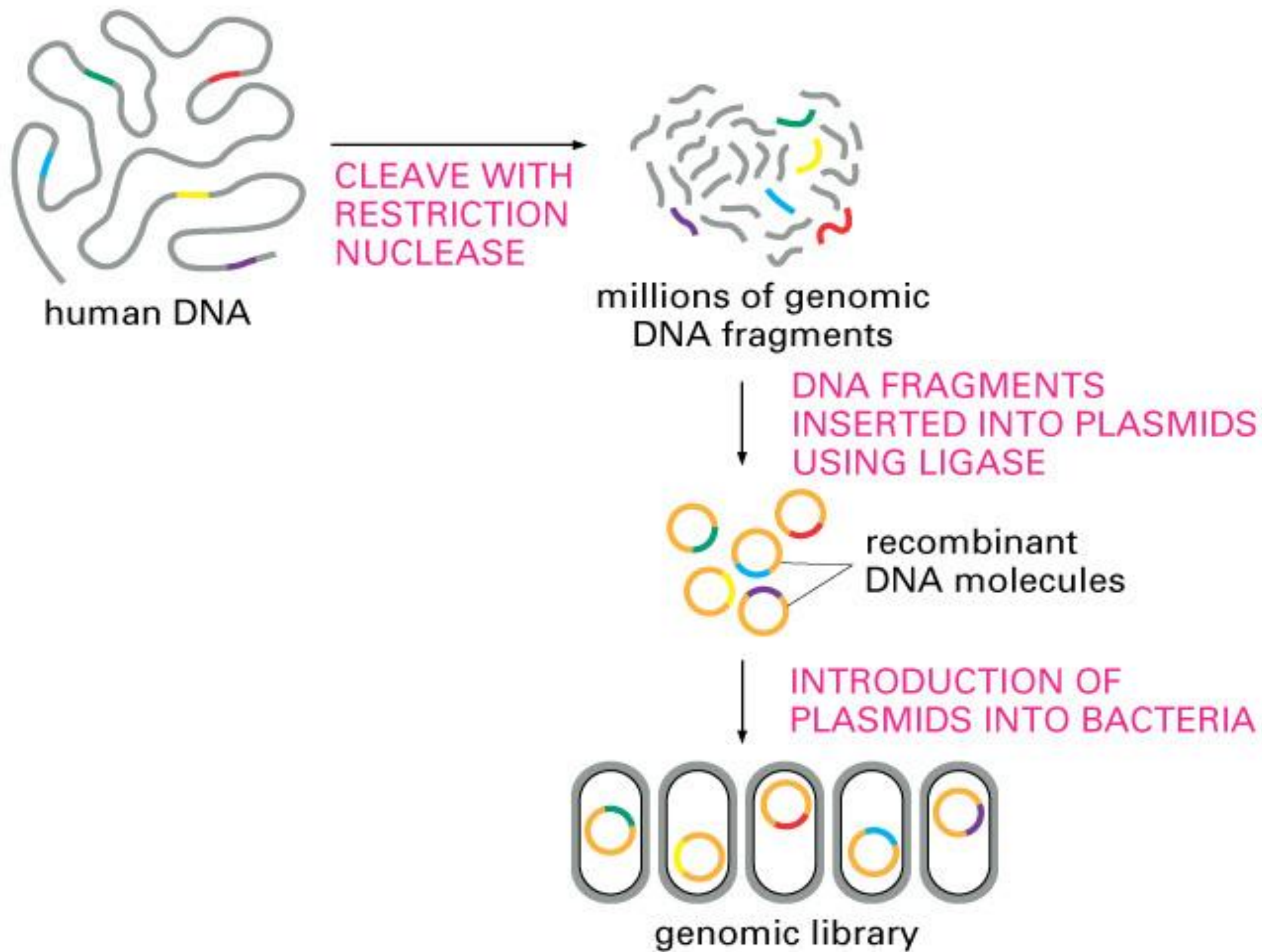
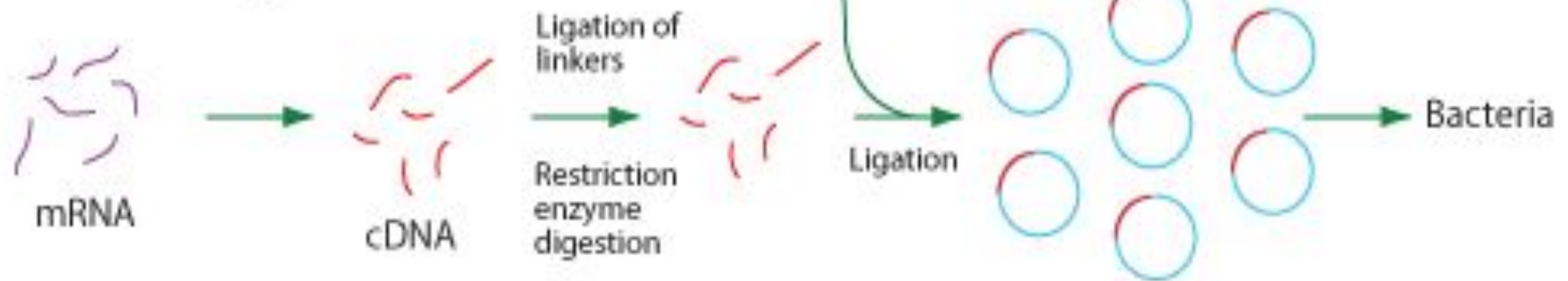


Figure 10-23 **Essential Cell Biology, 2/e. (© 2004 Garland Science)**

cDNA library

- Is a combination of cloned cDNA (complementary DNA) fragments inserted into a collection of host cells, which together constitute some portion of the transcriptome of the organism
- cDNA is produced from fully transcribed mRNA found in the nucleus and therefore contains only the expressed genes of an organism
- To make cDNA, mRNA is isolated from a tissue or whole organism, and DNA is copied from the mRNA template using an enzyme called **reverse transcriptase**.

Genomic Library
Cell
DNA
Restriction enzyme digestion
Ligation
Bacteria
Restriction enzyme digestion
Vector


Restriction enzyme digestion
cDNA Library
mRNA
cDNA
Ligation of linkers
Restriction enzyme digestion
Ligation
Bacteria