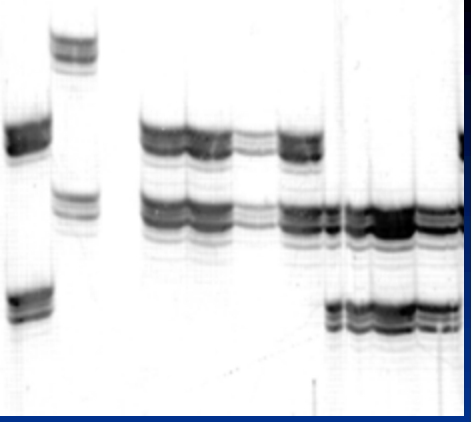
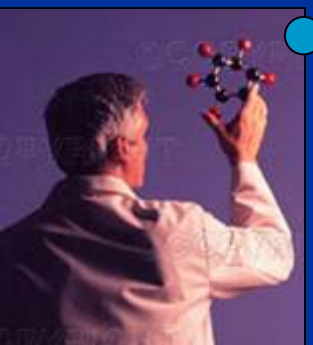
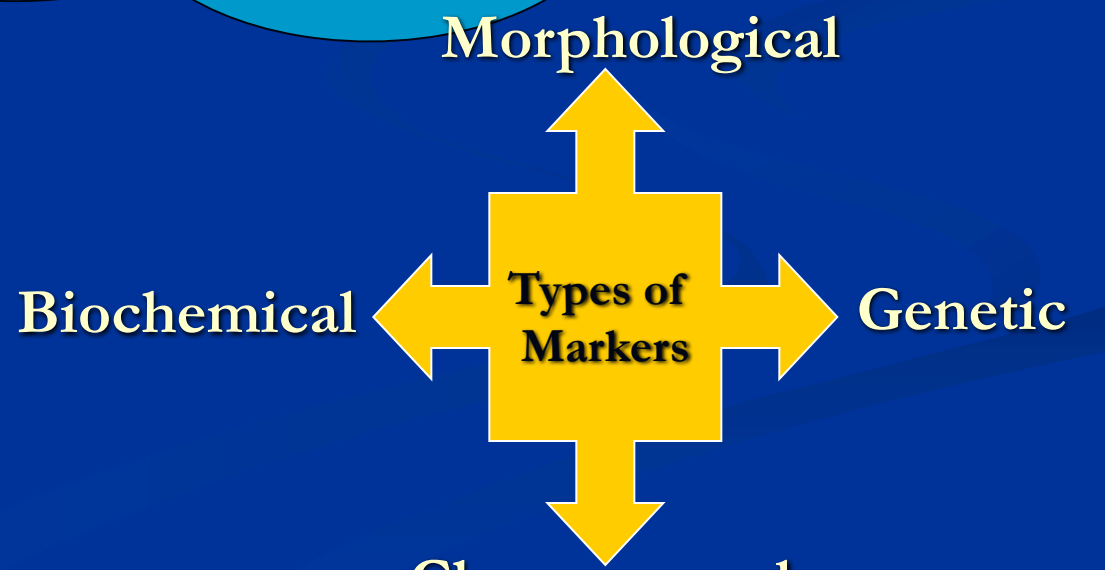


MOLECULAR MARKERS
AND
ITS APPLICATIONS IN LIVESTOCK IMPROVEMENT

What is Marker?

Marker is a piece of DNA molecule that is associated with a certain trait of a organism





Morphological Markers

Animals are selected based on appearance

Eg. PIGMENTATION

Disadvantage: lack of polymorphism

Biochemical Markers

Animals are selected based on biochemical properties

Eg. Hb, AMYLASE, BLOOD GROUPS ETC.

Disadvantage:

- Sex limited
- Age dependent
- Influenced by environment
- It covers less than 10% of genome

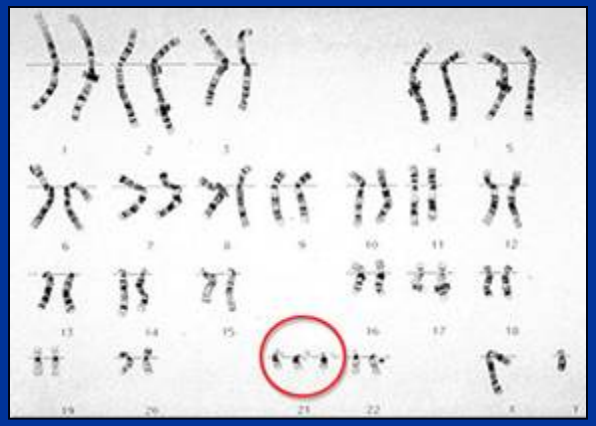
Chromosomal Markers

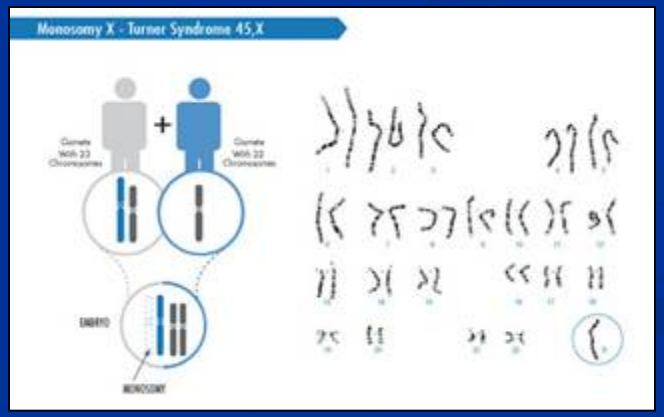
Animals are selected based on structural & numerical variations

Eg. Structural and Numerical Variations

Structural- Deletions, Insertions etc.

Numerical- Trisomy, Monosomy, Nullysomy

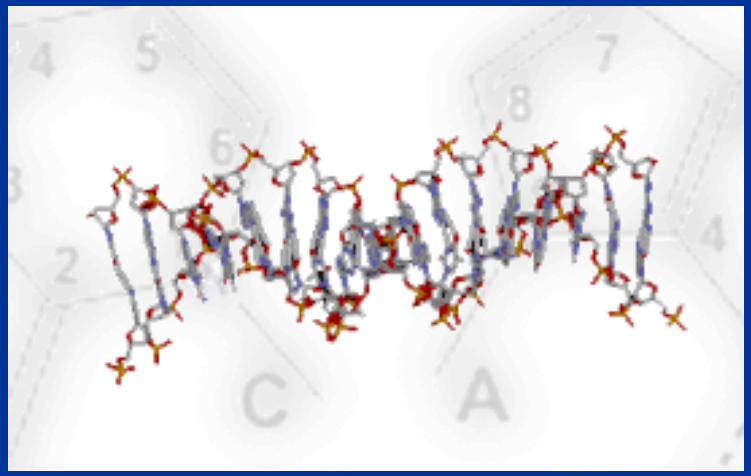




Disadvantage: low polymorphism

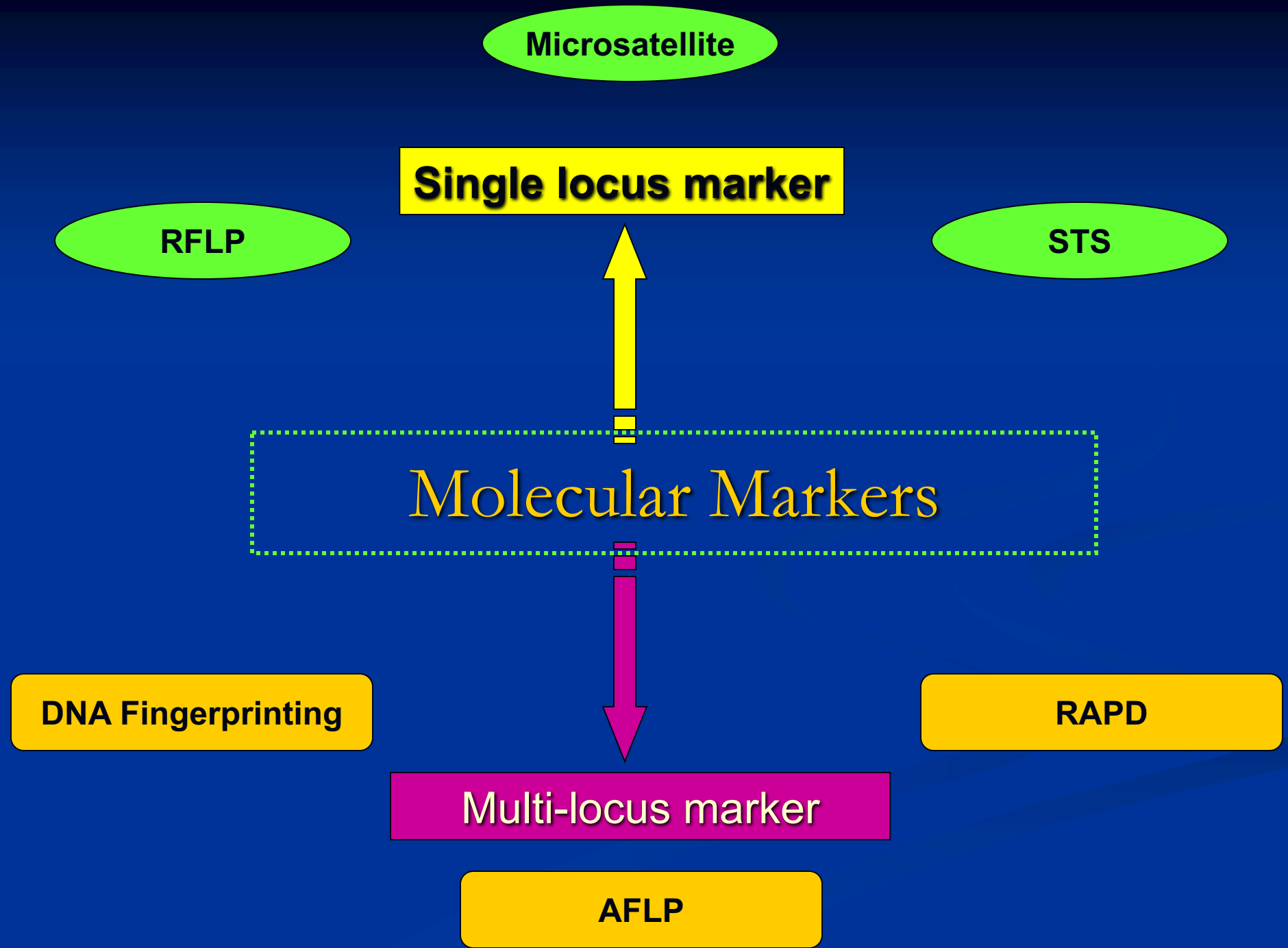
Molecular Marker

- Revealing variation at a DNA level
- Characteristics:
  - Co-dominant expression
  - Nondestructive assay
  - Complete penetrance
  - Early onset of phenotypic expression
  - High polymorphism
  - Random distribution throughout the genome
  - Assay can be automated



Methodological Advantages

- DNA isolated from any tissue eg. Blood, hair etc.
- DNA isolated at any stage even during foetal life
- DNA has longer shelf-life readily exchangeable b/w labs
- Analysis of DNA carried out at early age/ even at the embryonic
- Stage irrespective of sex.


Microsatellite
Single locus marker
RFLP
STS
Molecular Markers
DNA Fingerprinting
RAPD
Multi-locus marker
AFLP

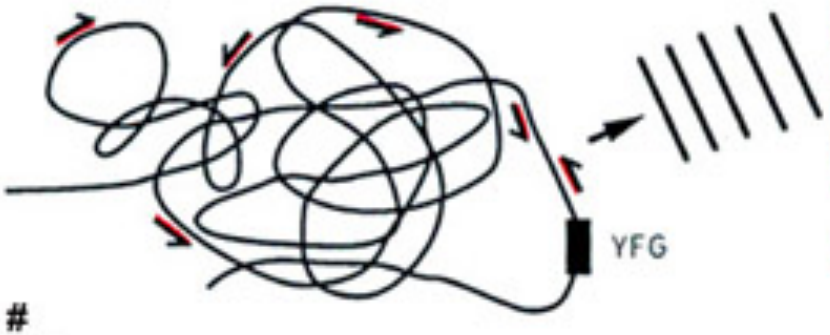
Randomly Amplified Polymorphic DNA (RAPD)

- PCR based marker with 10-12 base pairs
- Random amplification of several fragments
- Amplified fragments run in agarose gel detected by EtBr
- Unstable amplification leads to poor repeatability

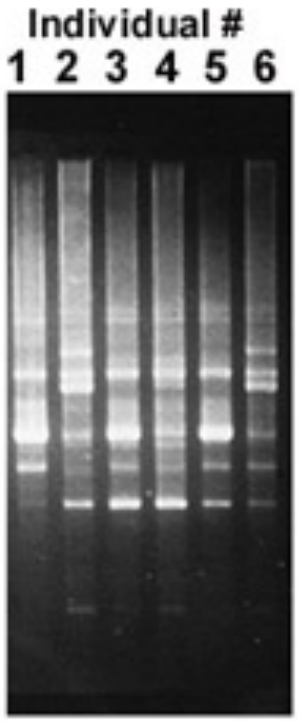
RAPD (Randomly Amplified Polymorphic DNA) marker

Basic technique

Half arrows: 10-nucleotide primer that will find an identical matching site at many different locations in the whole genome (black blob). Only primers that point towards eachother AND are in close enough proximity will result in a product during PCR-amplification reactions.



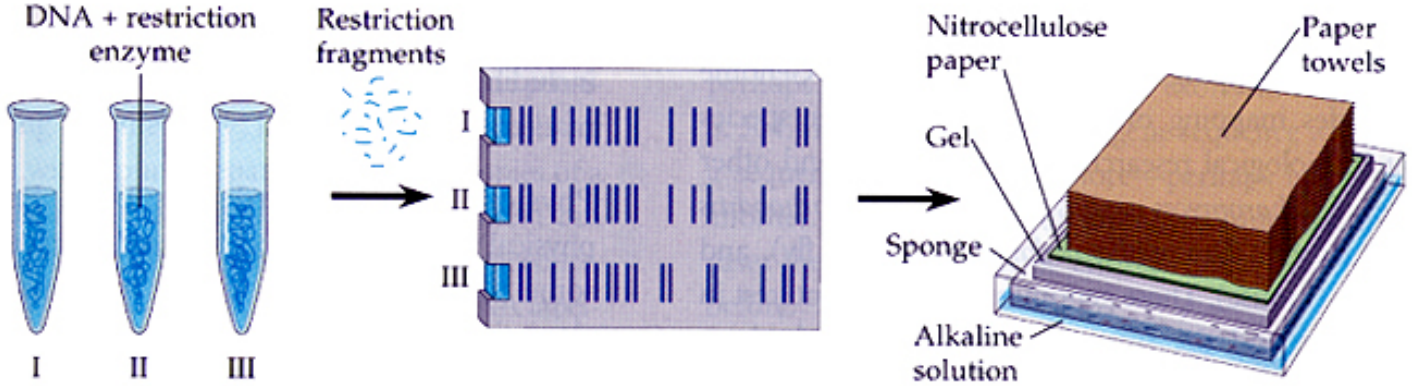
PCR amplified fragment (RAPD marker linked to YFG, Your Favorite Gene)



Example of a RAPD agarose gel. A mixture of many different PCR-amplified fragments has been separated in size by electrophoresis.

Restriction Fragment Length Polymorphism (RFLP)

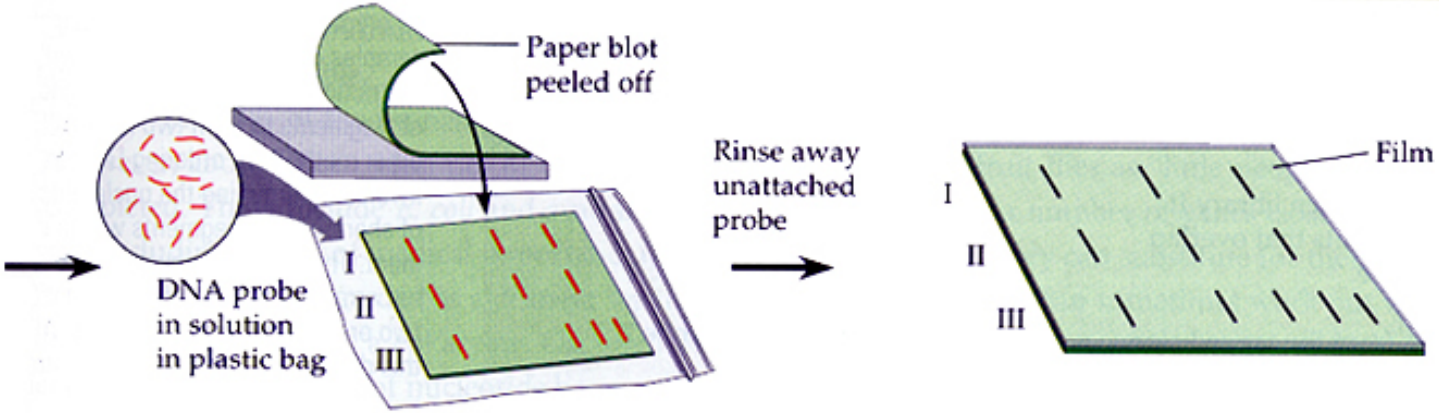
- Genomic DNA digested with Restriction Enzymes
- DNA fragments separated via electrophoresis and transfer to nylon membrane
- Membranes exposed to probes labelled with P^{32} via southern hybridization
- Film exposed to X-Ray



1 Restriction fragment prepartion. DNA samples to be tested (in this case identified as samples I, II, and III) are prepared from the appropriate sources. A restriction enzyme is added to the three samples of DNA to produce restriction fragments.

2 Electrophoresis. The mixtures of restriction fragments from each sample are separated by electrophoresis. Each sample forms a characteristic pattern of bands. (There would be many more bands than shown here, and they would be invisible unless stained.)

3 Blotting. Capillary action pulls an alkaline solution upward through the gel and through a sheet of nitrocellulose paper laid on top of it, transferring the DNA to the paper and denaturing it in the process. The single strands of DNA stick to the paper, positioned in bands exactly as on the gel.

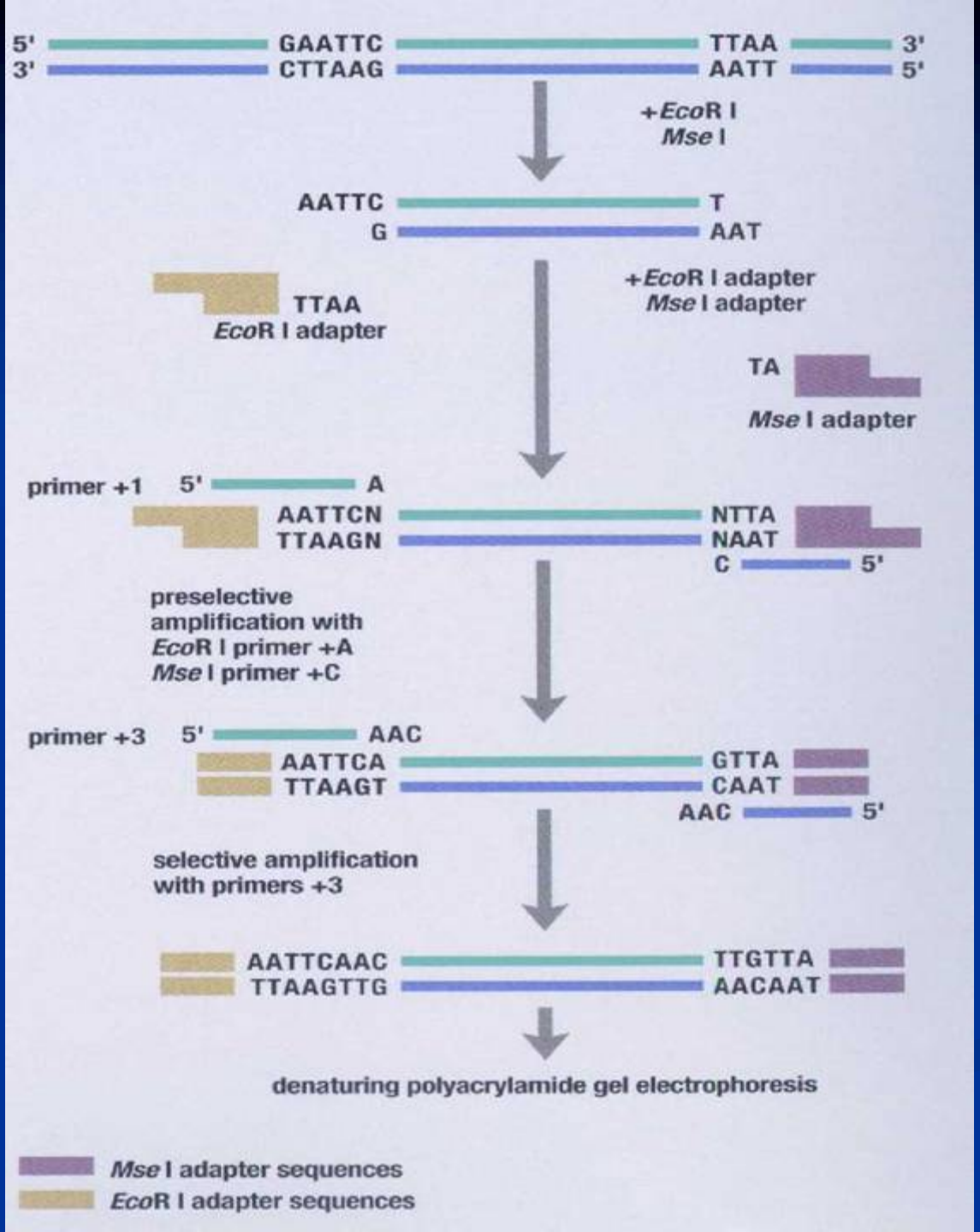


4 Hybridization with radioactive probe. The paper blot is exposed to a solution containing radioactively labeled probe. The probe is single-stranded DNA complementary to the DNA sequence of interest, and it attaches by base pairing to restriction fragments of complementary sequence.

5 Autoradiography. A sheet of photographic film is laid over the paper. The radioactivity in the bound probe exposes the film to form an image corresponding to specific DNA bands—the bands containing DNA that base pairs with the probe. The band patterns for samples I and II are identical, but III is different.

Amplified Fragment Length Polymorphism (AFLP)

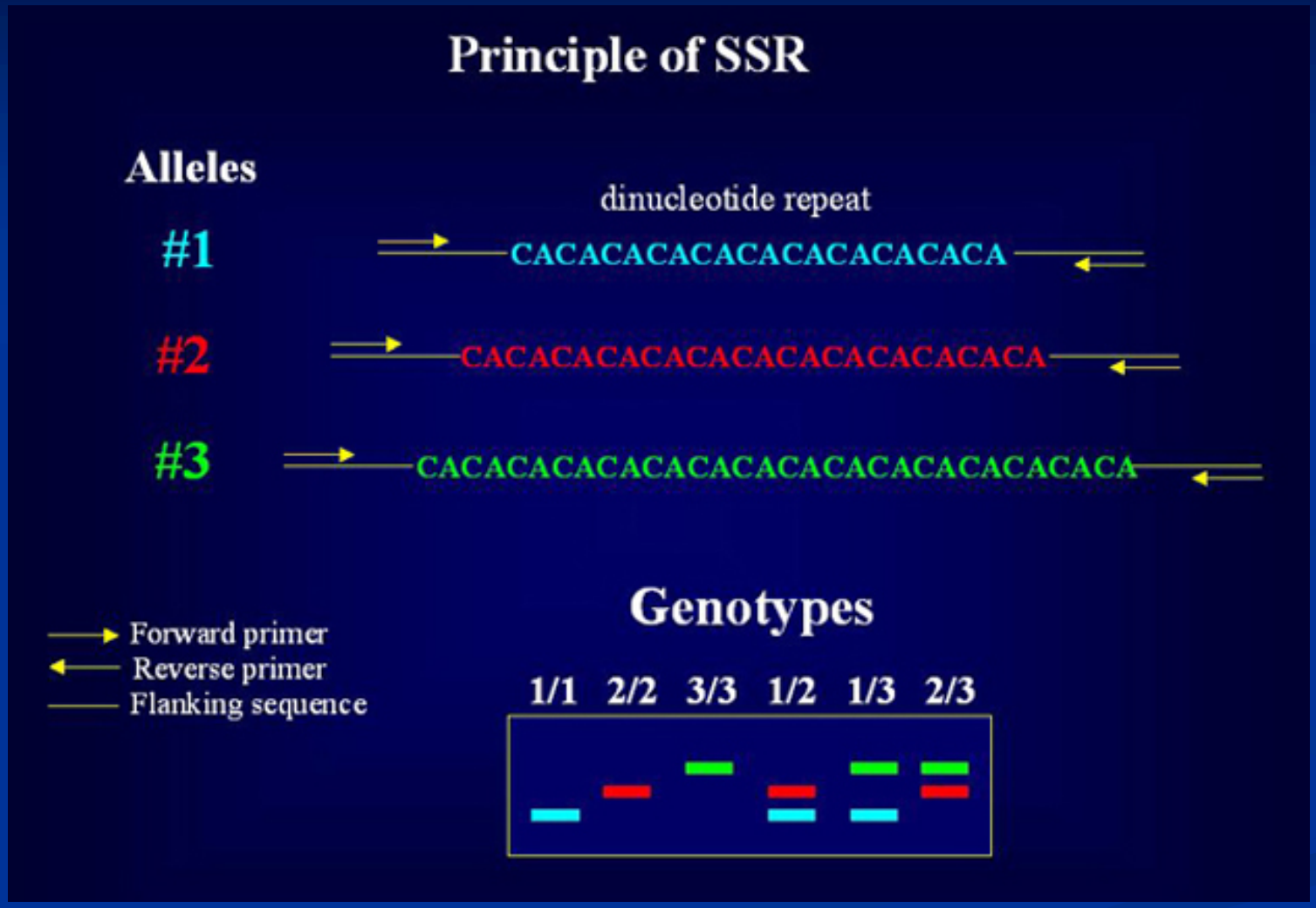
- Restriction endonuclease digestion of DNA
- Ligation of adaptors
- Amplification of ligated fragments
- Separation of the amplified fragments via electrophoresis and visualization
- AFLPs have stable amplification and good repeatability


5' GAATTC TTAA 3'
3' CTTAAG AATT 5'
+EcoR I
Mse I
AATTC T
G AAT
+EcoR I adapter
Mse I adapter
TTAA
EcoR I adapter
TA
Mse I adapter
primer +1 5' A
AATTCN NTTA
TTAAGN NAAT
C 5'
preselective
amplification with
EcoR I primer +A
Mse I primer +C
primer +3 5' AAC
AATTCA GTTA
TTAAGT CAAT
AAC 5'
selective amplification
with primers +3
AATTCAAC TTGTTA
TTAAGTTG AACAAT
denaturing polyacrylamide gel electrophoresis
Mse I adapter sequences
EcoR I adapter sequences

SSR: Simple Sequence Repeat or Microsatellite

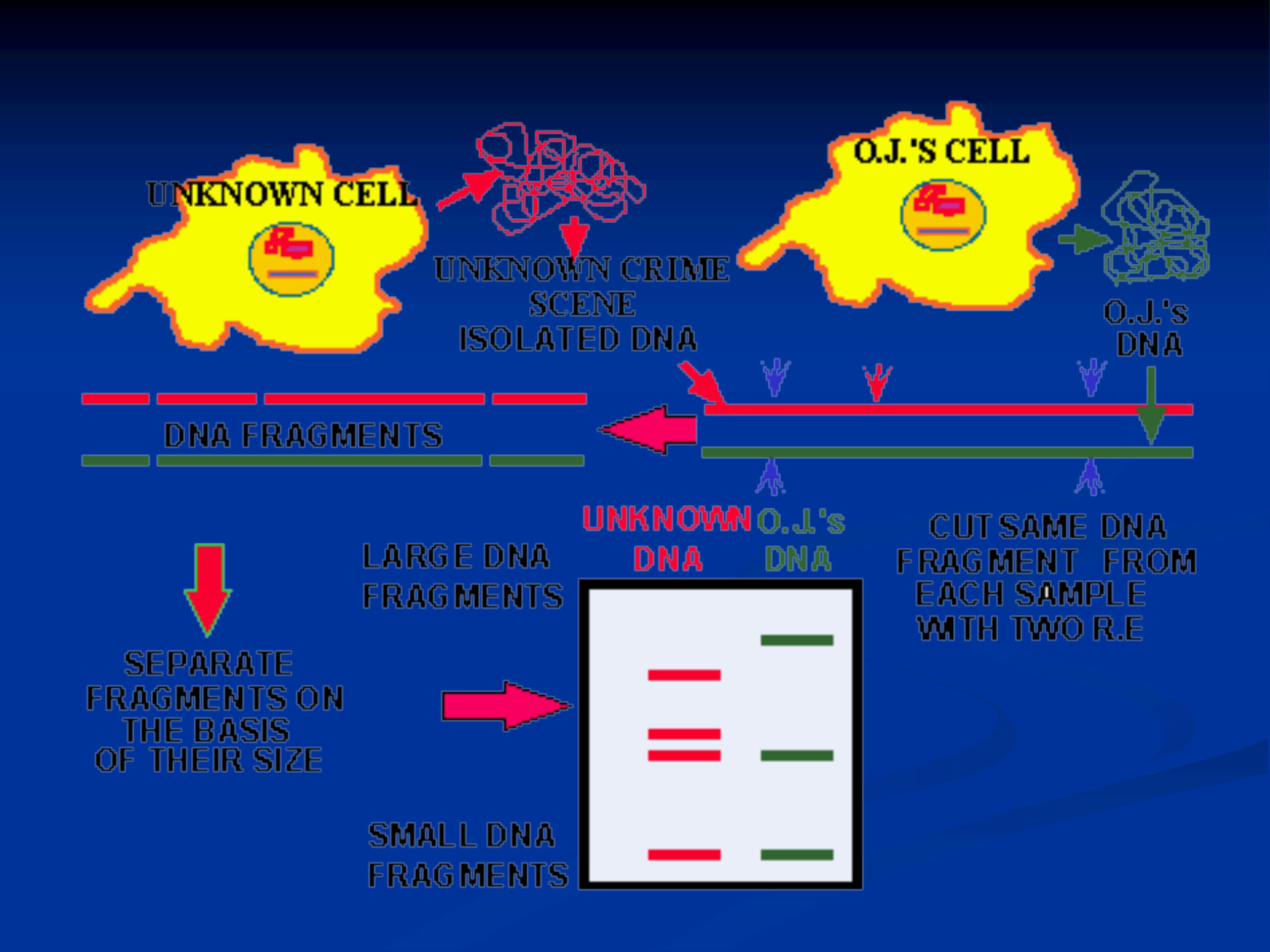
- PCR based markers with 18-25 base pair primers
- SSR polymorphisms are based on no. of repeat units and are hypervariable
- SSRs have stable amplification and good repeatability
- SSR are easy to run and automate

Principle of SSR



DFP: DNA finger printing

- DNA extraction from individual
- Amplification of markers
- Electrophoresis separation of markers
- Visualization of markers
- Scoring of markers for each individual
- Data analysis

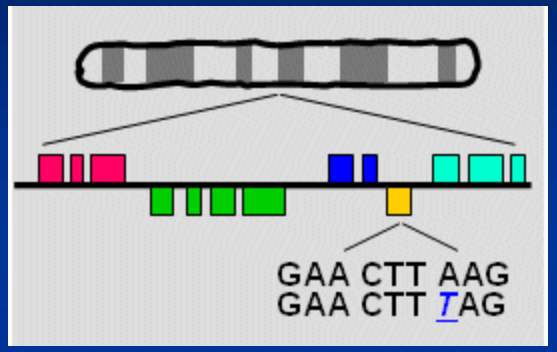

UNKNOWN CELL
UNKNOWN CRIME SCENE ISOLATED DNA
O.J.'S CELL
O.J.'s DNA
DNA FRAGMENTS
CUT SAME DNA FRAGMENT FROM EACH SAMPLE WITH TWO R.E
UNKNOWN DNA
O.J.'s DNA
LARGE DNA FRAGMENTS
SEPARATE FRAGMENTS ON THE BASIS OF THEIR SIZE
SMALL DNA FRAGMENTS

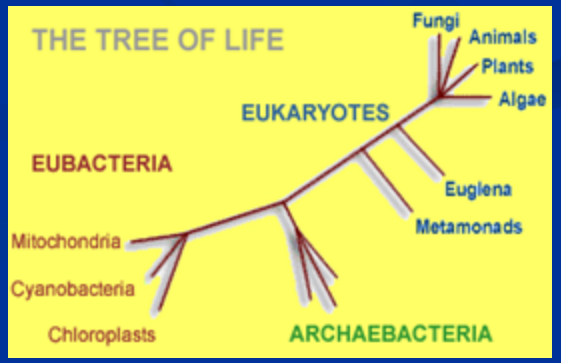
Properties of Different MM

Features	RFLP	PCR-RFLP	DFP	RAPD	Microsatellite	SNP
Detection method	Hybridization	PCR	Hybridization	PCR	PCR	PCR
Type of probe/primer used	g DNA/ cDNA sequence of structural genes	Sequence specific primers	Mini satellite synthetic oligos	Arbitrarily design primer	Sequence specific primers	Sequence specific primers
Requirement of radioactivity	Yes	No/Yes	Yes	No/Yes	No/Yes	No/Yes
Extant of genomic coverage	Limited	Limited	Extensive	Extensive	Extensive	Extensive
Degree of polymorphisms	Low	Low	High	Medium to High	High	High
Phenotype expression	Co dominant	Co dominant	Co dominant	Co dominant/Dominant	Dominant	Co dominant
Possibility of automation	No	Yes	No	Yes	Yes	Yes

Application of Molecular Markers

- Gene mapping
- Pre and post natal diagnosis of diseases
- Anthropological and molecular evolution studies



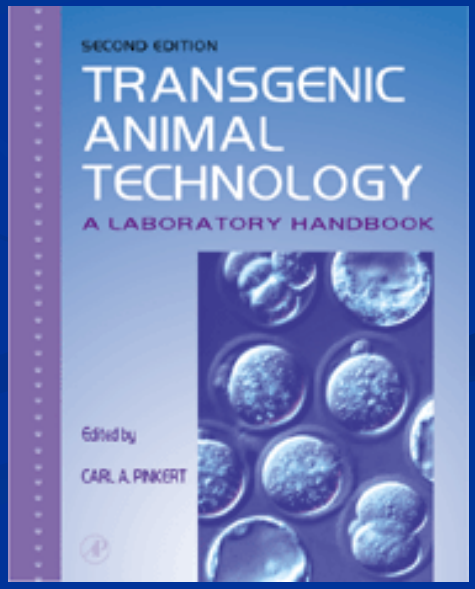


Animal breeding

A. Conventional breeding strategies

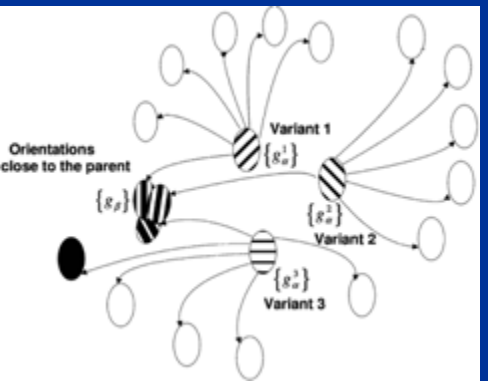
1. Short range
2. Long range

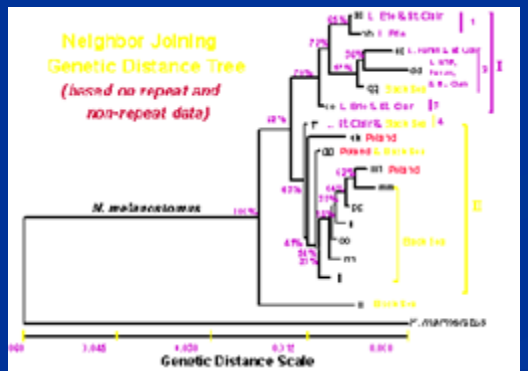
B. Transgenic breeding strategies

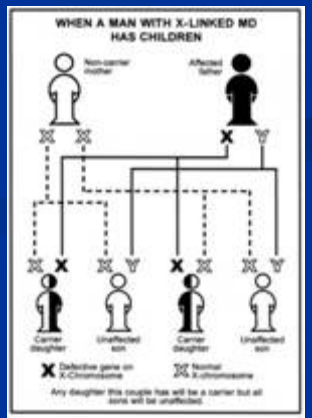


Short Range Application

- Parentage determination
- Genetic distance estimation
- Determination of twin zygosity & freemartins
- Sexing of pre-implanted embryos
- Identification of disease carries

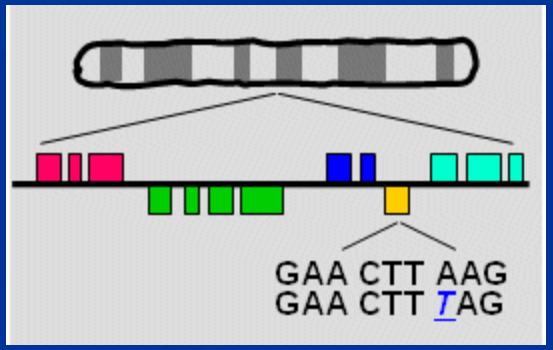






Long Range Applications

- **Gene mapping & mapping of QTL by linkage**
- **Marker assisted selection**



TRANSGENIC BREEDING STRATEGIES

- IDENTIFICATION OF ANIMALS CARRYING THE TRANSGENES

CONCLUSIONS

The genetic improvement of animals is a continuous and complex process. Ever since the domestication of animals by man, he has always remained busy in improving his animals. In this pursuit many methods have been developed and tested. In recent years, the demonstration of genetic polymorphism at the DNA sequence level has provided a large number of marker techniques with variety of applications. This has, in turn, prompted further consideration for the potential utility of these markers in animal breeding. However, utilization of marker-based information for genetic improvement depends on the choice of an appropriate marker system for a given application.


Thanks!