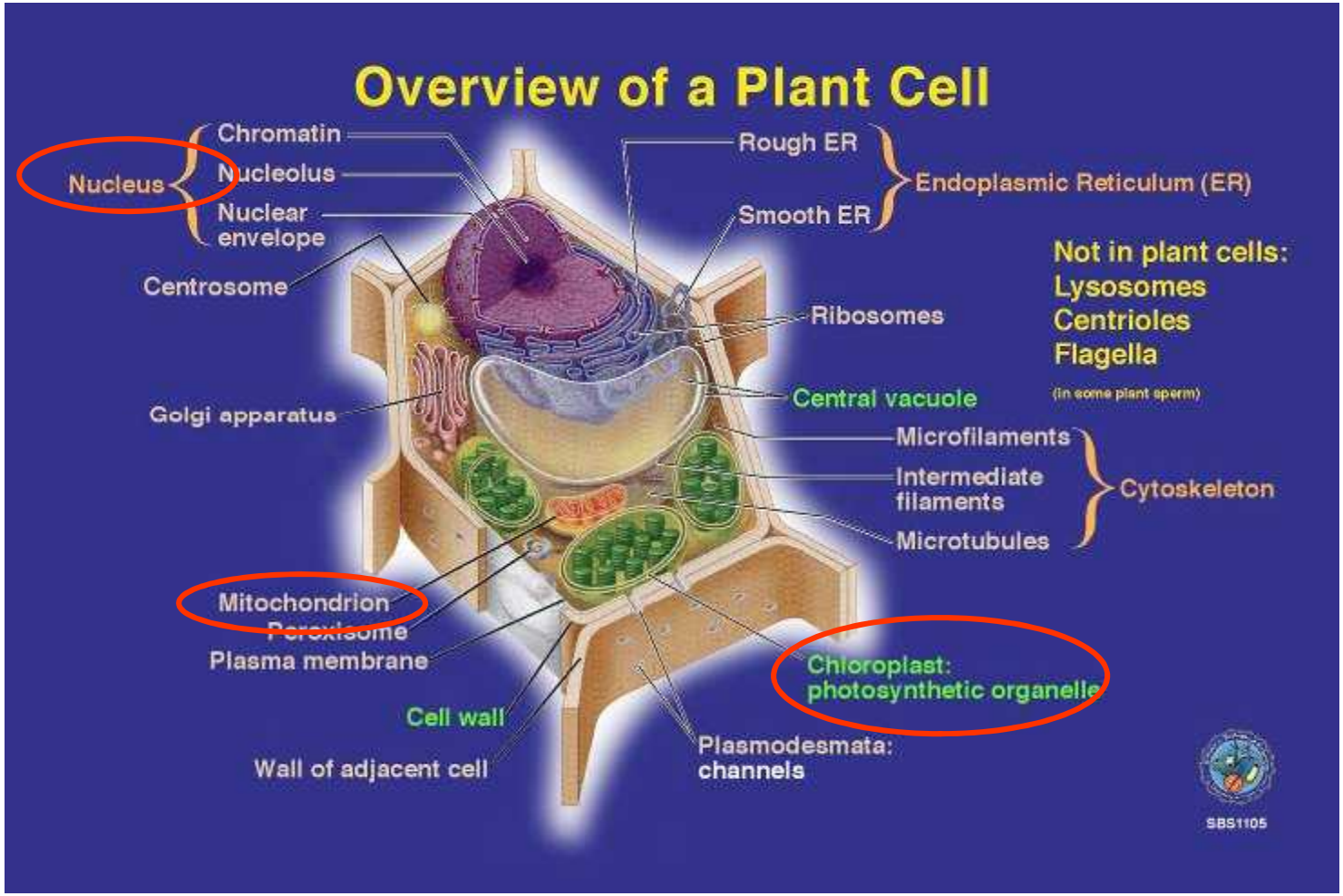
Molecular studies (SSR) for screening of genetic variability among direct regenerants of sugarcane clone NIA-98

Dr. Imtiaz A. Khan
Pr. Scientist /
PI sugarcane and molecular marker group

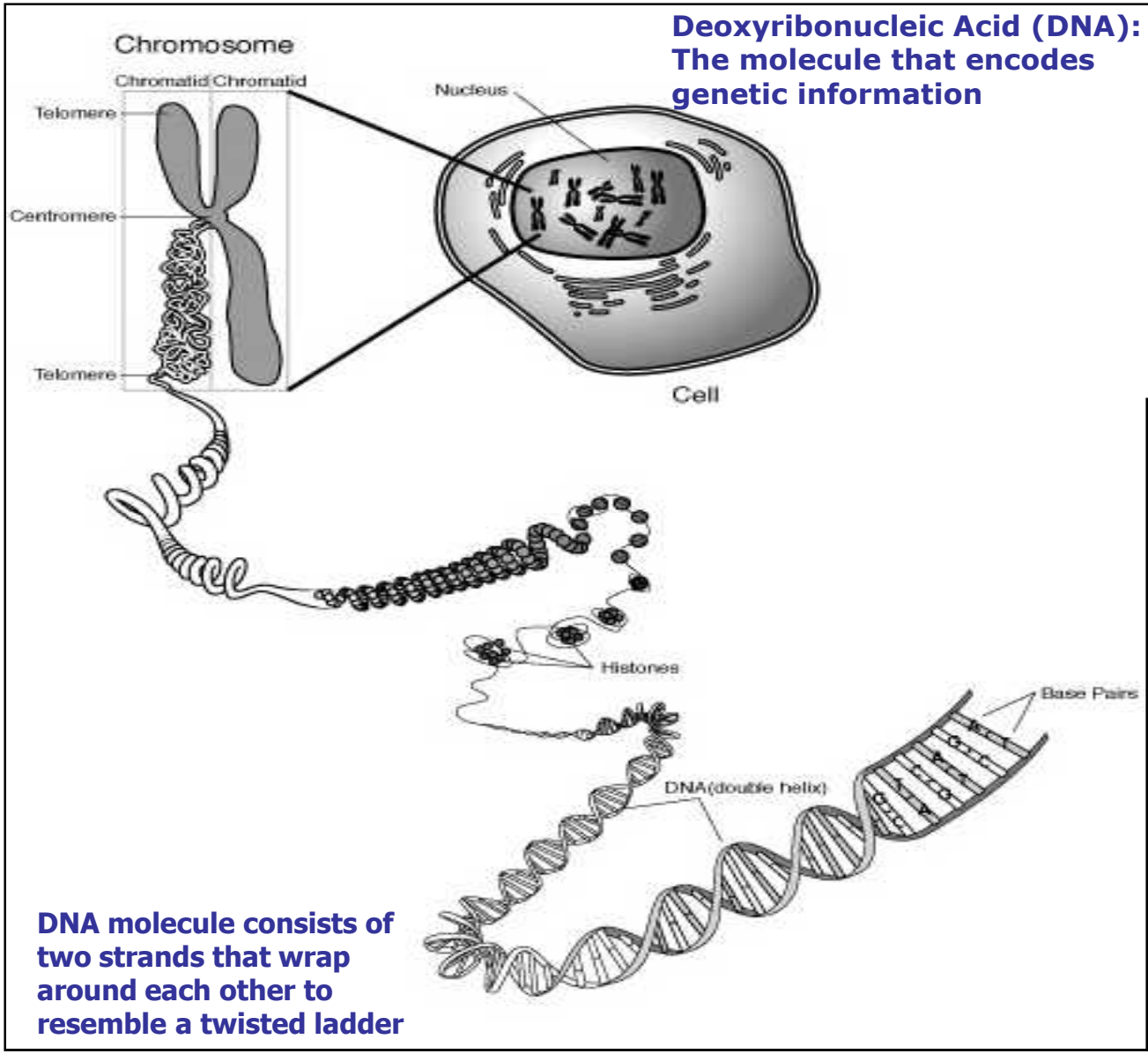
Outlines

- ✓ Organization and flow of genetic information
- ✓ Molecular techniques to reveal genetic variation
- ✓ Type of molecular markers
- ✓ Which marker for what purpose
- ✓ Microsatellite marker
- ✓ Case study 1: using microsatellites to estimate gene flow via pollen
- ✓ Case study 2: using microsatellites for individual-specific DNA fingerprints

FLOW OF GENETIC INFORMATION



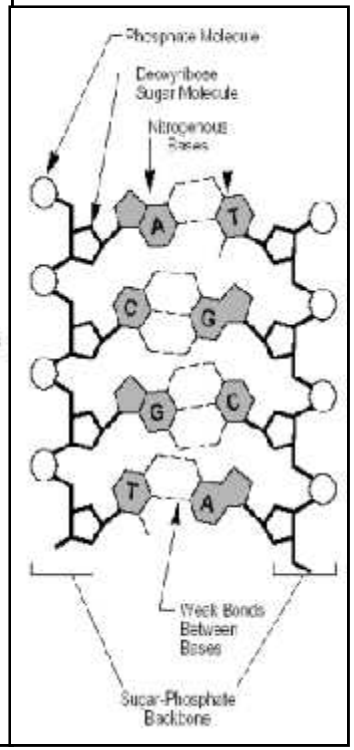
Deoxyribonucleic Acid (DNA): The molecule that encodes genetic information



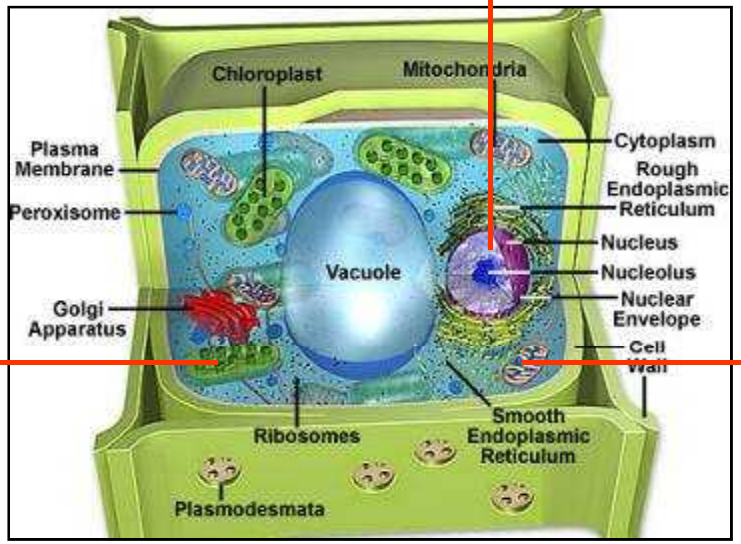
DNA molecule consists of two strands that wrap around each other to resemble a twisted ladder

A pairs with T

C pairs with G



Nuclear DNA: Diploid; biparental inherited; recombination occur; can be viewed as a huge ocean of largely nongenic DNA, with some tens of thousands of genes and gene clusters scattered around like small islands and archipelagos. A high proportion of this apparently nonfunctional DNA consists of repeated motifs and may be considered as junk DNA or selfish DNA



Choroplast DNA: Haploid; usually maternally inherited in angiosperms and paternally inherited in gymnosperms; typically ranging from 135 to 160 kb in size, is packed with genes and thus resembles the streamlined configuration of its cyanobacterial ancestral genome

Mitochondrial DNA: Haploid; typically maternally inherited; about 370 to 490 kb, about 10% of these sequences represent genes, another 10 to 26% were found to be made up of repetitive DNA, including retrotransposons. Thus, the majority of plant mtDNA sequences lack any obvious features of information

- Organism's genomic DNAs are subjected to **mutation** as a result of normal cellular operations or interactions with environment

- The rates of mutation are depending on:
  - Biology of organism
  - Genomes under consideration
  - Types of mutations

- **Mutations in genomic DNA can be classified into several categories:**

Base substitution

GATCCGAGTATCGCAATTAGCA
GATCCGAGTGTCGCAATTAGCA

Deletion

GATCCGAGTATCGCAATTAGCA
GATCCGAGTAATTAGCA

Insertion

GATCCGAGTATCGCAATTAGCA
GATCCGAGTATCGCAGCATTAGCA

Duplication

GATCCGAGTATCGCAATTAGCA
GATCCGAGTATCTCGCAATTAGCA

Inversion

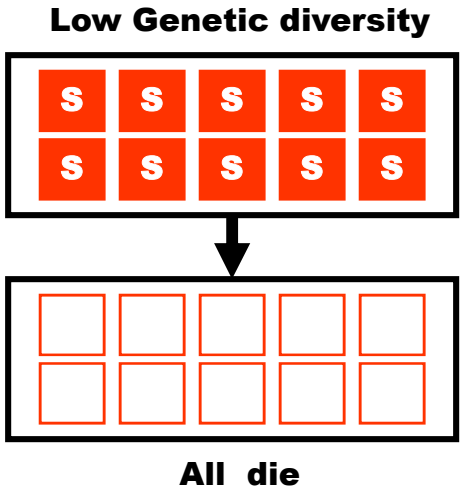
GATCCGAGTATCGCAATTAGCA
GATGCCAGTATCGCAATTAGCA

Through long evolutionary accumulation, many different instances of mutation as mentioned above should exist in any given species

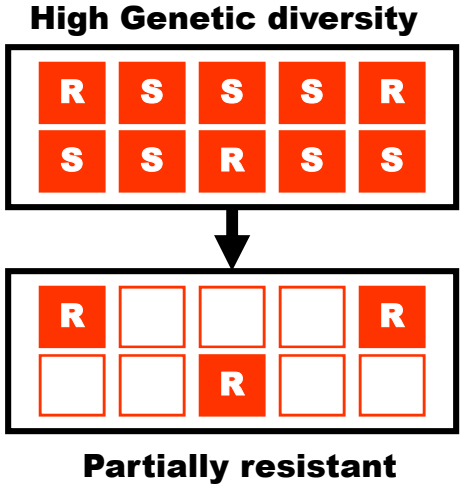
The number and degree of the various types of mutations define the genetic diversity within a species

It has been widely recognized that loss of genetic diversity is a major threat for the maintenance and adaptive potential of species

- **Example** - if low genetic diversity, when a virulent form of a disease arises, many individuals may be susceptible and die



- But as a result of natural genetic diversity within local plant populations, there may be some individuals that are at least partially resistant and there are able to survive and thus perpetuate the species



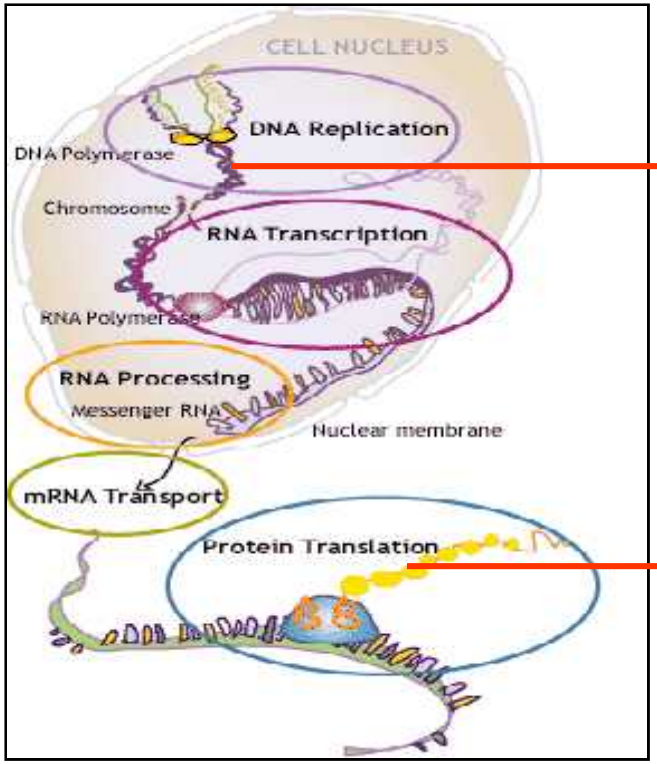
- For many plant species, ex situ and in situ **conservation strategies** have been developed to **safeguard** the extant of **genetic diversity**

- To manage this genetic diversity effectively the ability to identify genetic diversity is indispensable

- In addition, for this variation to be useful, it must be **heritable** and **discernable**; as recognizable phenotypic variation or as genetic mutation distinguishable through **molecular marker technologies**

Definition of molecular markers

A sequence of DNA or protein that can be screened to reveal key attributes of its state or composition and thus used to reveal genetic variation



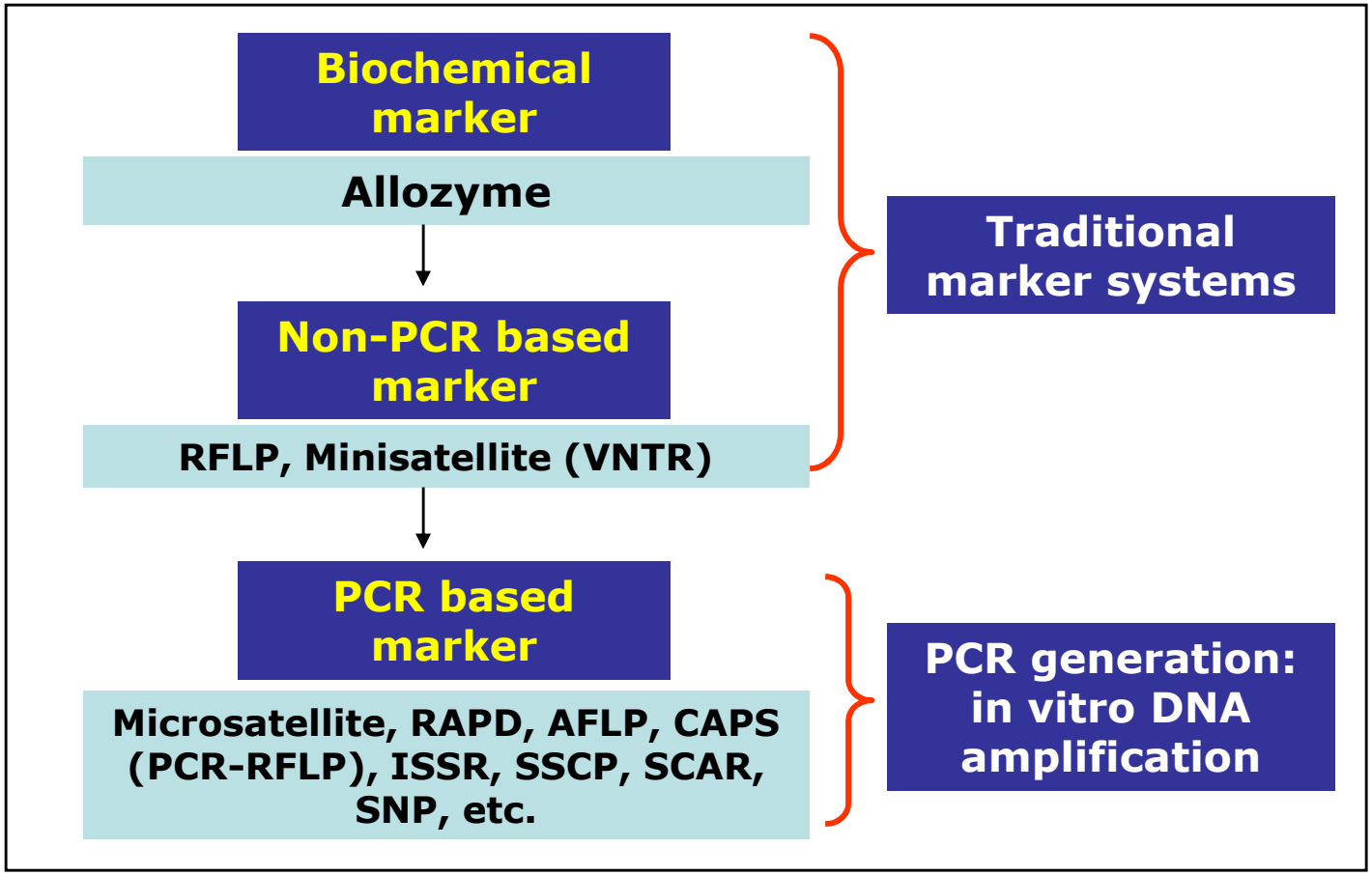
Mutation

Mutation arises genetic variation at the DNA level → **DNA markers**

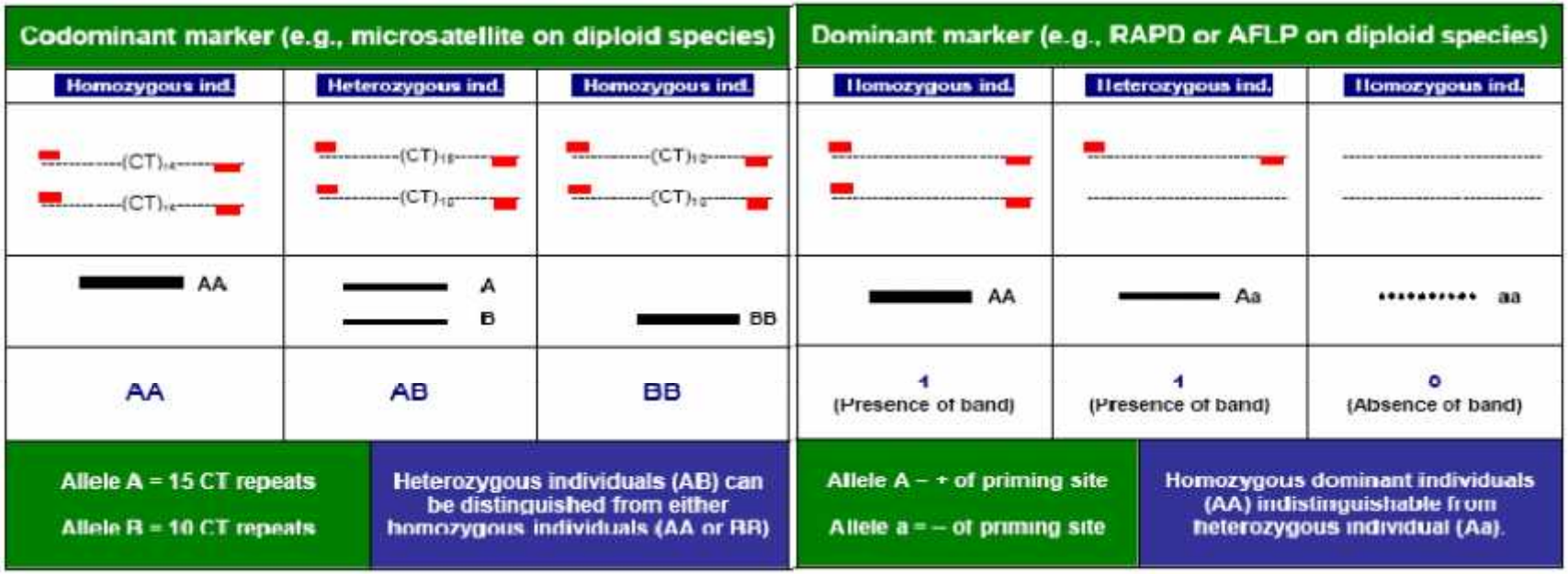
Subsequently, mutation arises genetic variation at DNA will cause variation at the protein level → **Protein markers**

TYPES OF MOLECULAR MARKERS

- Due to rapid developments in the field of molecular genetics, a variety of molecular markers has emerged during the last few decades



• Codominance or dominace



Codominant marker:
A marker in which both alleles are expressed, thus heterozygous individuals can be distinguished from either homozygous state

Dominant marker:
A marker shows dominant inheritance with homozygous dominant individuals indistinguishable from heterozygous individuals

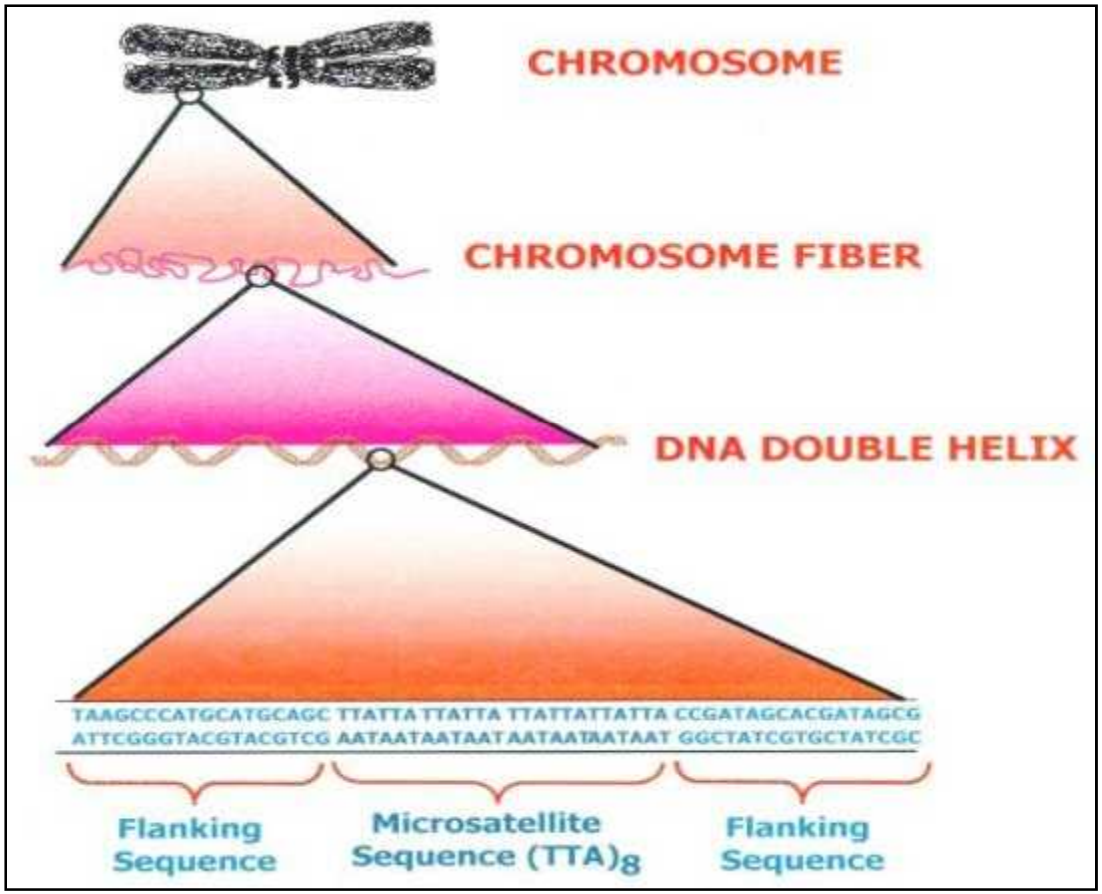
What are microsatellite?

- **Tandem repeated sequences with a 1-6 repeat motif**
  - **Dinucleotide** (CT) 6 - CTCTCTCTCTCT
  - **Trinucleotide** (CTG) 4 - CTGCTGCTGCTG
  - **Tetranucleotide** (ACTC) 4 - ACTCACTCACTCACTC

- **Synonymous to SSR and STR; Depending on nature of repeat tract, SSR can further divided into four categories:**

Perfect repeat when repeat tract pure for one motif	CTCTCTCTCTCT
Compound SSR when repeat tract pure for two motifs	CTCTCTCACACA
Imperfect SSR if single base substitution	CTCTCTACTCTCT
Region of cryptic simplicity if complex but repetitive structure	GTGTCACAGAGT

Where are microsatellites found?



Majority are in non-coding region

Abundance in genome

- Microsatellites have been found in every organism studied so far
- Most frequent in human > insect > plant > yeast > nematode
- Most common dinucleotide:

➢ Human	CA/GT
➢ Plant	GC content

Genotyping procedure

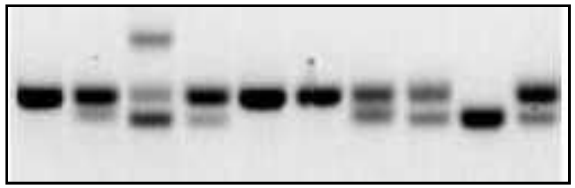
PCR

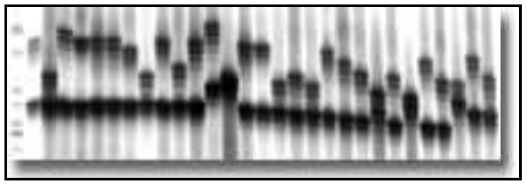
Electrophoresis

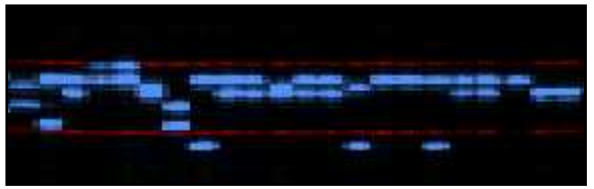
- **Agarose**
- **PAGE**
- **Denaturing PAGE**
- **Capillary**

Visualization

- **Silver staining**
- **SybrGreen staining**
- **Autoradio-graphy**
- **Fluorescent dyes**

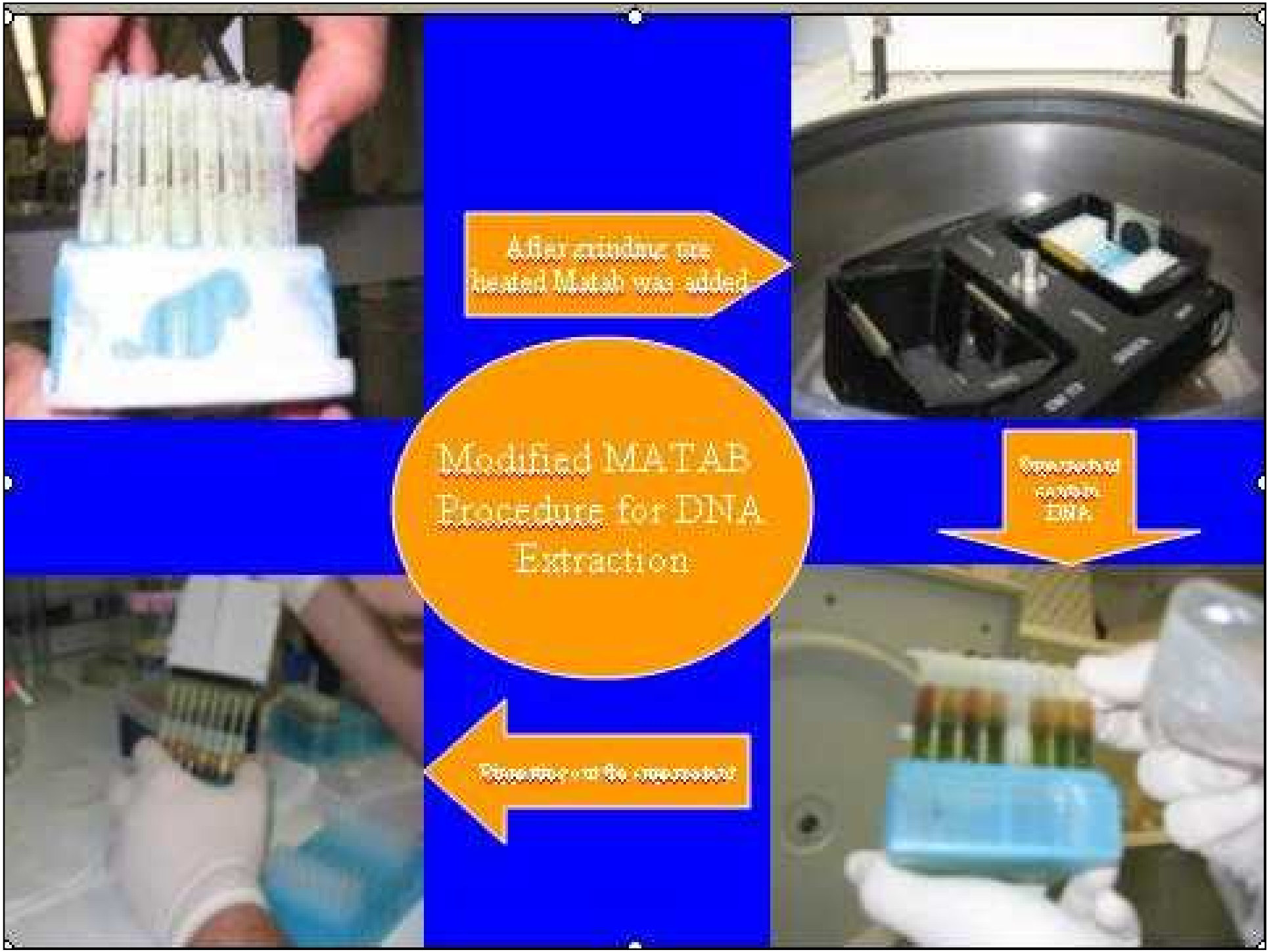


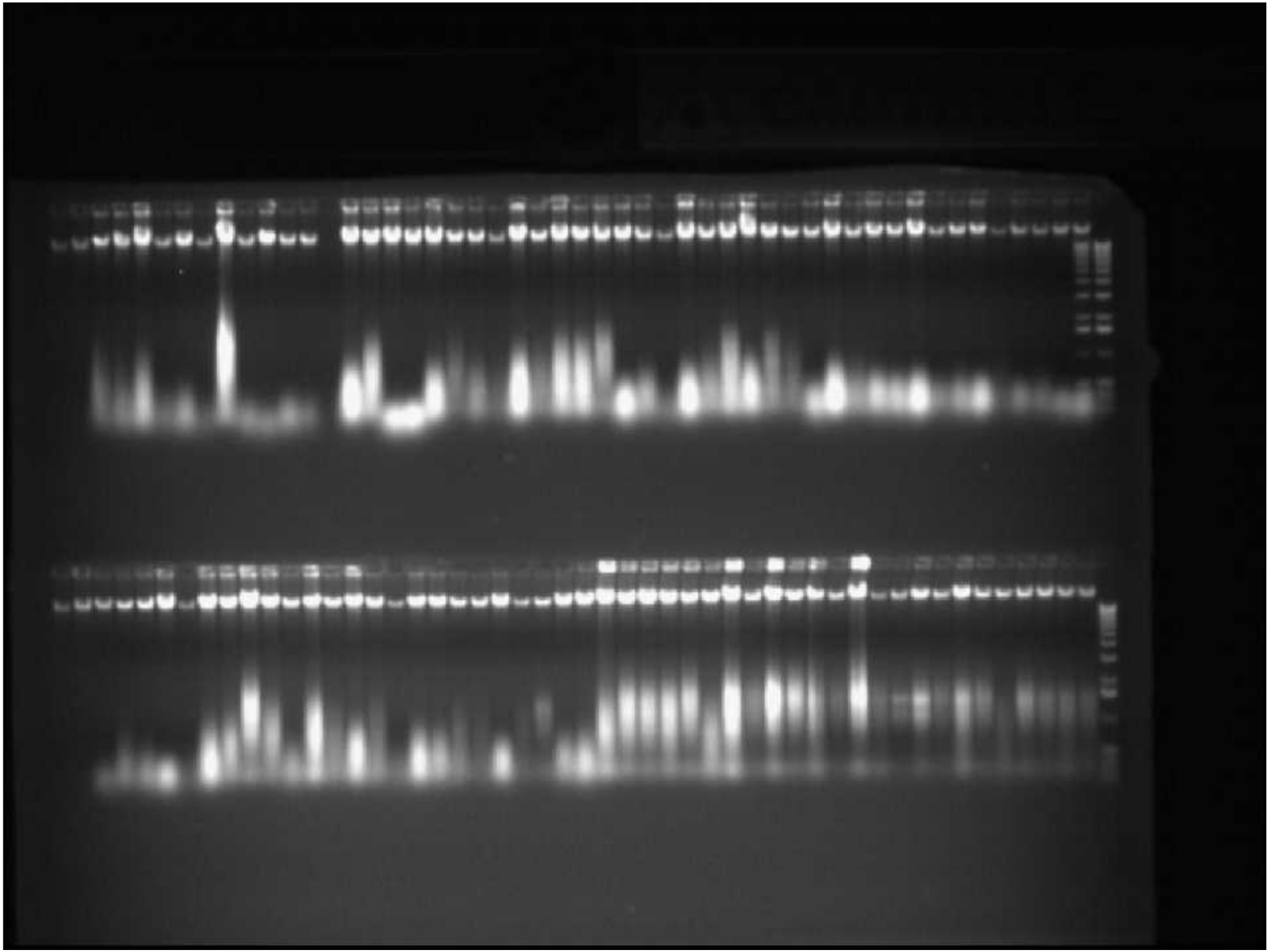




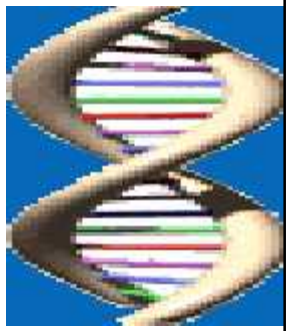
Advantages

- Low quantities of template DNA required (10-100 ng)
- High genomic abundance
- Random distribution throughout the genome
- High level of polymorphism
- Band profiles can be interpreted in terms of loci and alleles
- Codominance of alleles
- Allele sizes can be determined with an accuracy of 1 bp, allowing accurate comparison across different gels
- High reproducibility
- Different SSRs may be multiplexed in PCR or on gel
- Wide range of applications
- Amenable to automation


After grinding the heated Matab was added
Modified MATAB Procedure for DNA Extraction



PCR Programme

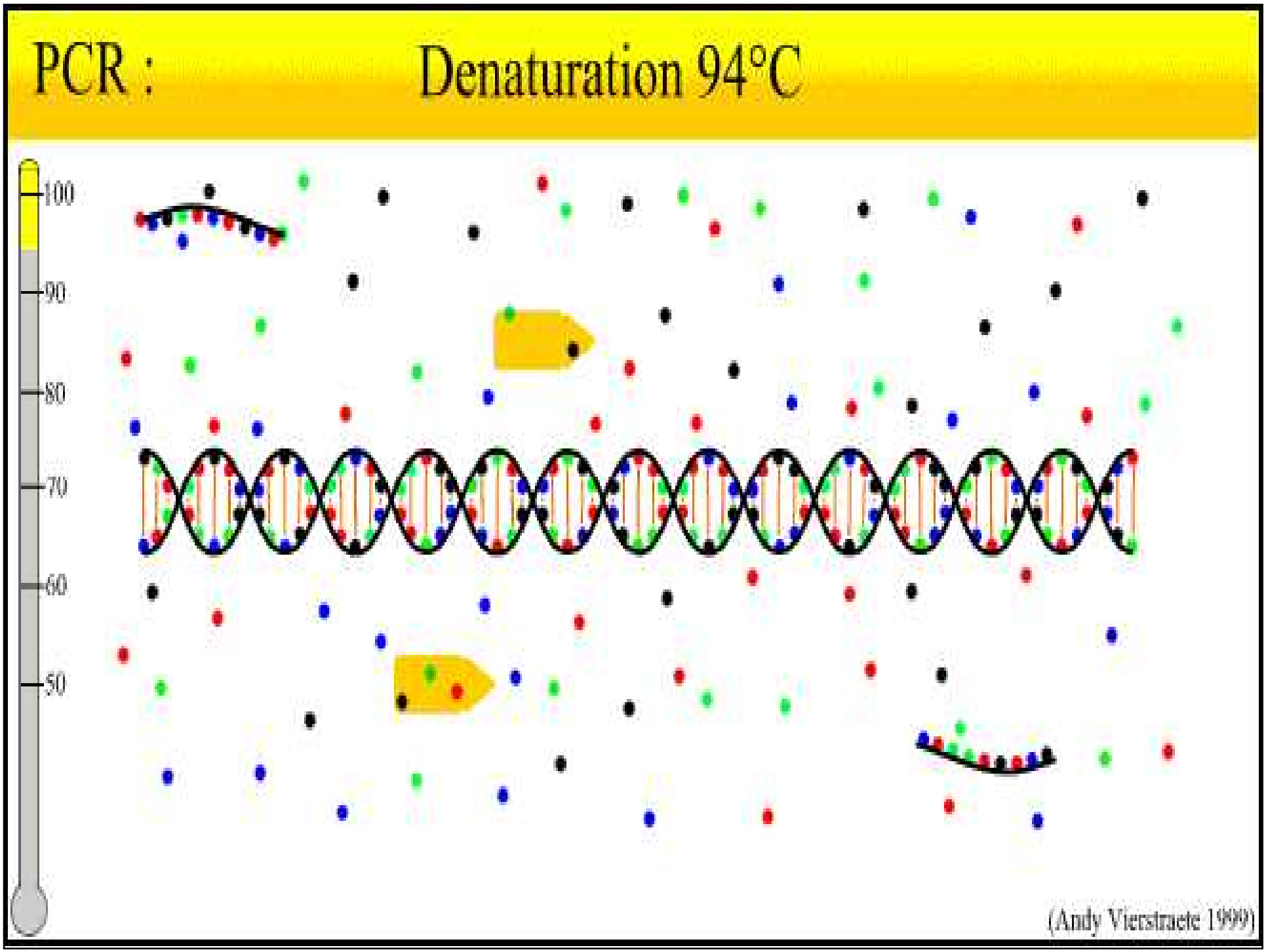


Initial denaturation	95 °C for 5 min
Denaturation at	94 °C for 60 sec
Primer annealing at,	52 °C for 60 sec
Extension at	72 °C for 1.30 min
Reaction was repeated for	32cycles
Final extension step at	72 ° C for 10 min
Hold at	4 °C

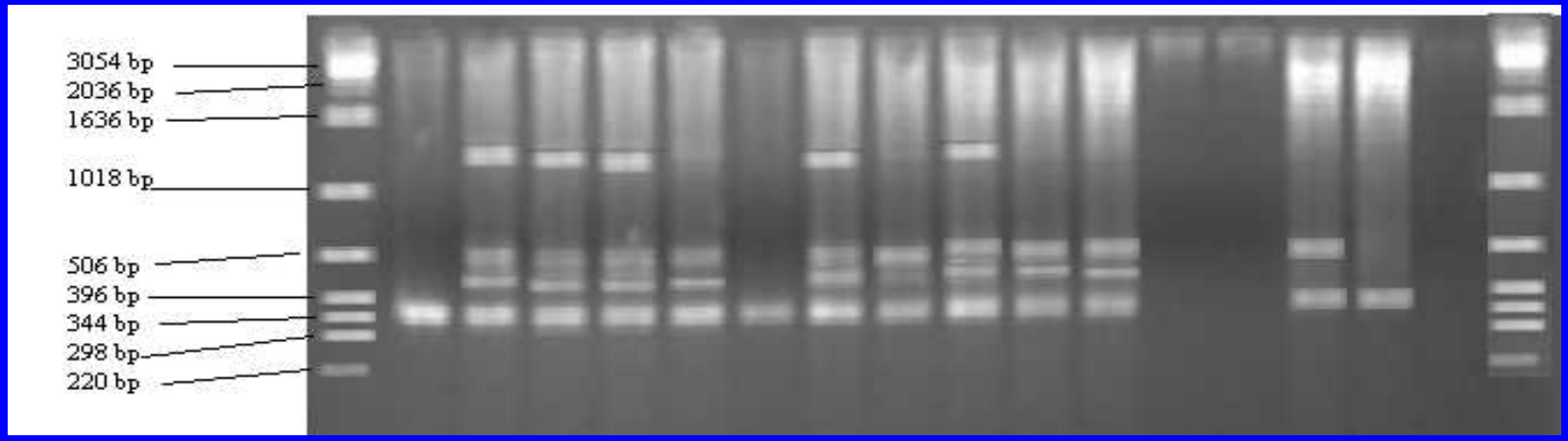
PCR reaction mixture for SSR

Reagent	Conc. 1	Conc. 2	Volume (µl)
PCR Buffer	10X	1X	1
Taq polymerase	1u/ µ	0.1u/ µ	1
dNTPs	10mM	0.2mM	0.2
Mg++	50mM	0.5mM	0.1
Forward Primer	10 µ M	0.08 µ M	0.08
Reverse Primer	10 µ M	0.10 µ M	0.1
Template DNA			
H_2O	25ng		5 µ l/reaction

Primer	Sequence	Primer	Sequence
07	AGCATAGTTTTTTTGGAC AGTTCTTTTCGTTCTCTGG	33	ACACCACTCACATCCACTTG TGATACACCATTGTTGATGC
12	AAAATCAGACAAACAGCAT AGAAGAAGCAGATACAGGT	36	TGGGGAGGGCTGACTAGA GGCGGTATATATGCTGTG
14	AATGGAAGGAGTTTTTGA CTGCTTTCTGTGAGTGTG	37	AGCATAGTTTTTGTGGAC AGTTCTTTTCGTTCTCTGG
18	CAACAATAACTTAACTGGTA CTGTCCTTTTTATTCTCTTT	55	ATATGTAGGAGTAGGACCAA CAACAGGTTTCAGTATATTT
27	CCCTATCACTGTTCTTCAT ATTTGTCCTTGCGAGAG	74	GCGCAAGCCACACTGAGA ACGCAACGCAAAACAACG

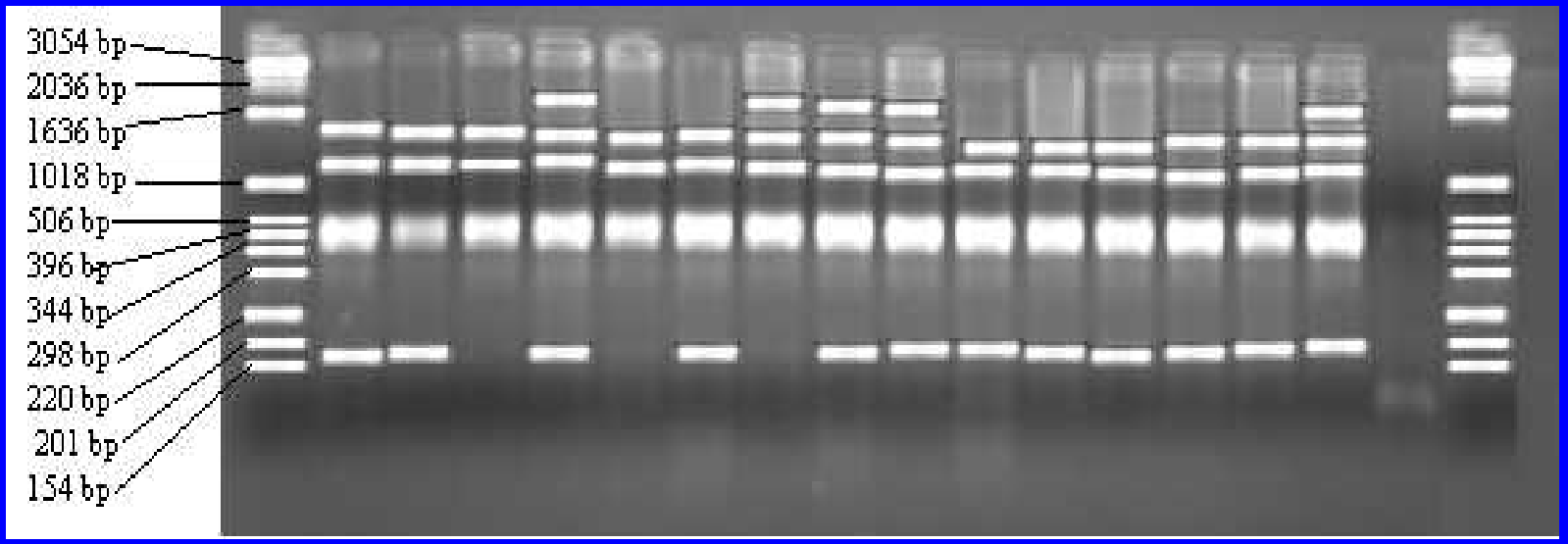

PCR : Denaturation 94°C
100
90
80
70
60
50
(Andy Vierstraete 1999)

Gel No.1 SSR primer MsCir 74 showing polymorphism in directly regenerated plantlets of NIA-98



L2= P-102, L3=P-105, L4=P-100, L5=P-110, L6=P-109, L7=P-108, L8=P-107, L9=Parent, L10=P-104, L11= P-101, L12= P-97, L13=P-98, L14=P-99, L15=P-96, L16=95, L17=Blank

Gel No.2 SSR primer MsCir 27 showing polymorphism in directly regenerated plantlets of NIA-98



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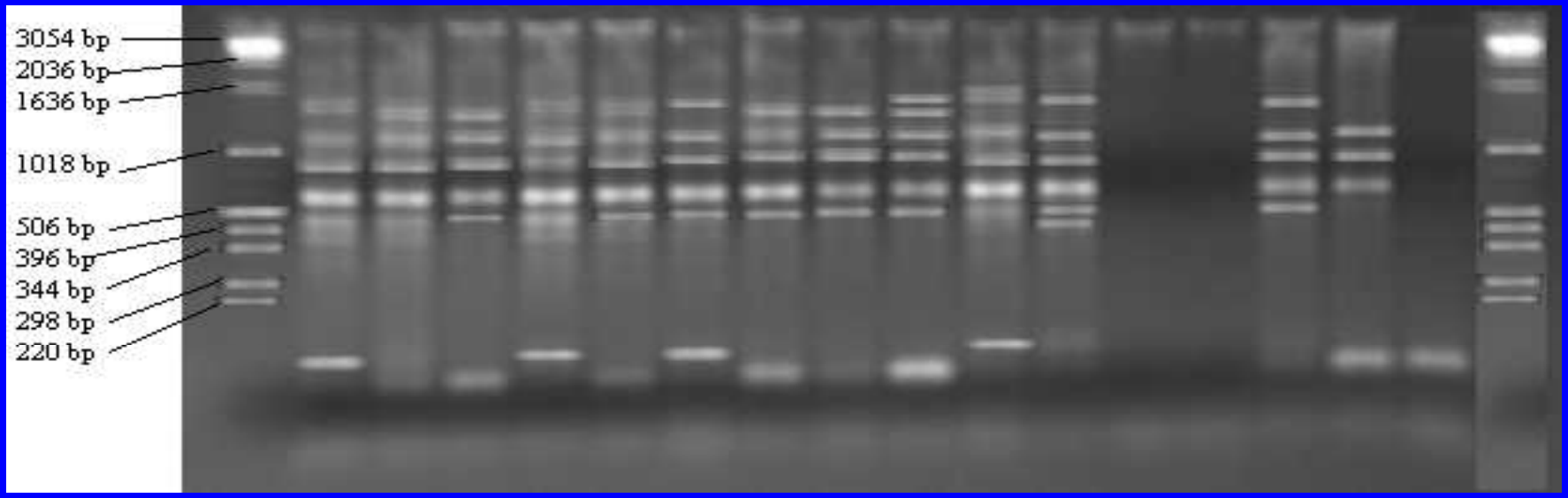

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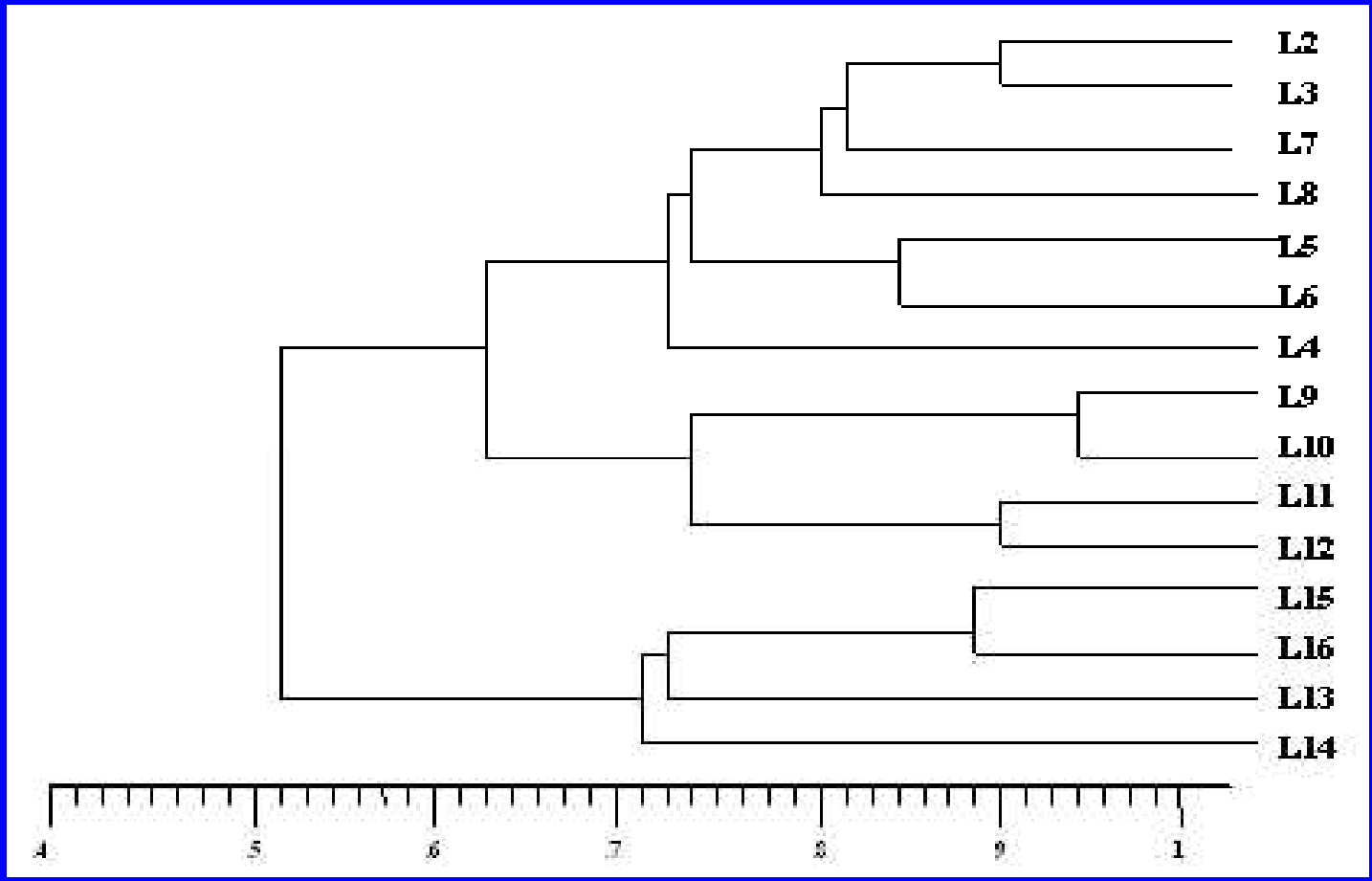

Dendrogram of fifteen sugarcane soma clones developed from SSR data using unweight pair group method of arithmetic means (UPGMA)

Thanks for patience listening
Your Questions Please