Centurion University

Biotechnological tools: DNA markers

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What is marker ?

- Heritable traits that can be assayed

Markers are of different kind:-

- Morphological
- Auxotrophic
- Molecular

Types of marker

- Morphological :-

Based on phenotypic characteristics or qualitative character that can be scored visually.

e.g. colour, height, maturity etc.

- Auxotrophic:- based on growth requirements

e.g. colony colour, morphology

Constraints of using morphological marker

- Influenced by environment
- Stage specificity
- Inadequacy of polymorphic morphological markers

Molecular markers

Characteristics of biomolecule can serve as marker on genetic chromosome.

It is of different kinds-

- Antigenic :- blood group antigens are carbohydrates
- Enzymatic:- isoenzymes are protein
- DNA based :- RFLP, RAPD, SSR etc.

Properties of molecular marker

- It must be polymorphic
- It must have codominant inheritance
- It should be reproducible
- It should be cheap, easy and fast

Advantages of molecular marker

- Not affected by environment
- Not stage specific
- Knowledge of dominance and recessive relationship is not necessary for a trait
- Abundant polymorphism
- Mendelian inheritance

DNA based marker

Molecular marker which detect polymorphism at DNA level

these are grouped into following categories:-

- Hybridization based :- RFLP (Restriction Fragment Length Polymorphism)
- PCR based :- based on amplification of a particular genome region using polymerase chain reaction (PCR) e.g. RAPD, ISSR, AFLP, SSR etc.

Several Molecular Markers

1. RFLP= Restriction Fragment Length Polymorphism
2. RAPD= Randomly Amplified Polymorphic DNA
3. ISSR= Inter Simple Sequence Repeat
4. AFLP= Amplified Fragment Length Polymorphism
5. DAF= DNA Amplification Fingerprinting
6. STS= Sequence Tagged Sites
7. SCAR= Sequence Characterized Amplified Region for Amplification of Specific Bands
8. CAPs= Cleaved Amplified Polymorphic Sequences
9. RAMPs= Randomly Amplified Microsatellite Polymorphisms
10. STMs= Sequence Tagged Microsatellites
11. SSCP= Sequence Strand Conformation Polymorphism
12. SSR= Simple Sequence Repeat
13. SAP= Specific Amplicon Polymorphism
14. ALP= Amplicon Length Polymorphism
15. AP-PCR= Arbitrary primed PCR

Several Molecular Markers

RFLP – Botstein et al. (1980)

RAPD – Wells & McClelland (1991)

ISSR – Zietkiewicz et al. (1994)

AFLP – Zabeau et al. (1993)

SCAR – Martin et al. (1991)

EST – Adams et al. (1991)

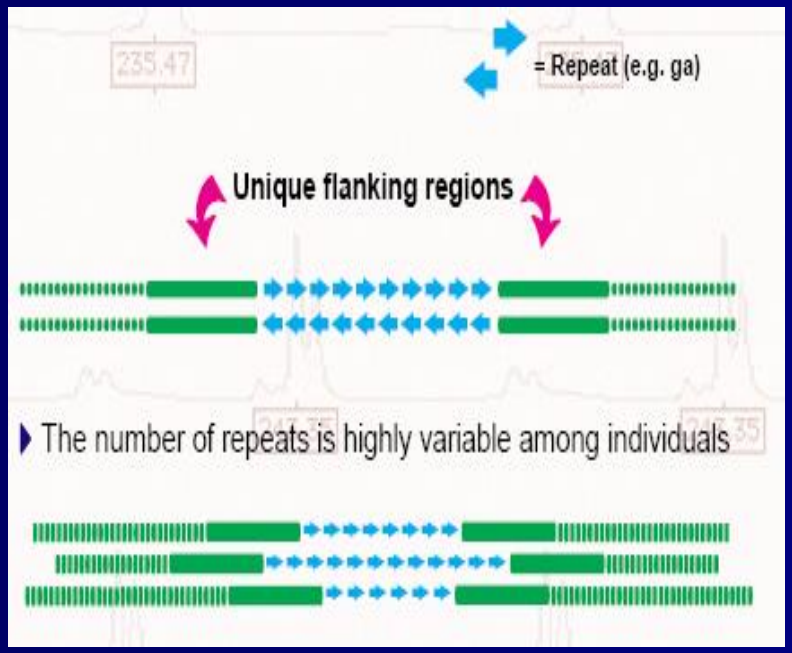
Microsatellite – Litt & Lutty (1989)

Minisatellite – Jeffrey (1985)

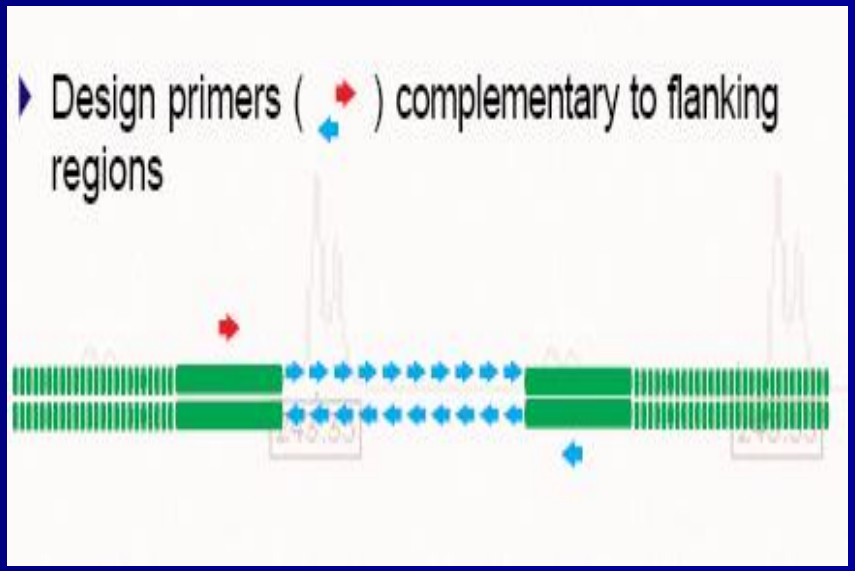
Simple Sequence Repeat (VNTR,Microsatellite)

- Microsatellites are short tandem repeat (1-10bp) of mono,di,tri,tetra nucleotide
- Number of repetition varies among individuals
- They are uniformly distributed through out the eukaryotic genome
- It reflects polymorphism of repetitive sequences.

SSR



Structure of SSR



Primer Designing

SSR

Advantages

- Very reliable, highly polymorphic
- Highly reproducible
- Inherited as codominant marker
- Simple and ready to use
- Small amount of DNA is required

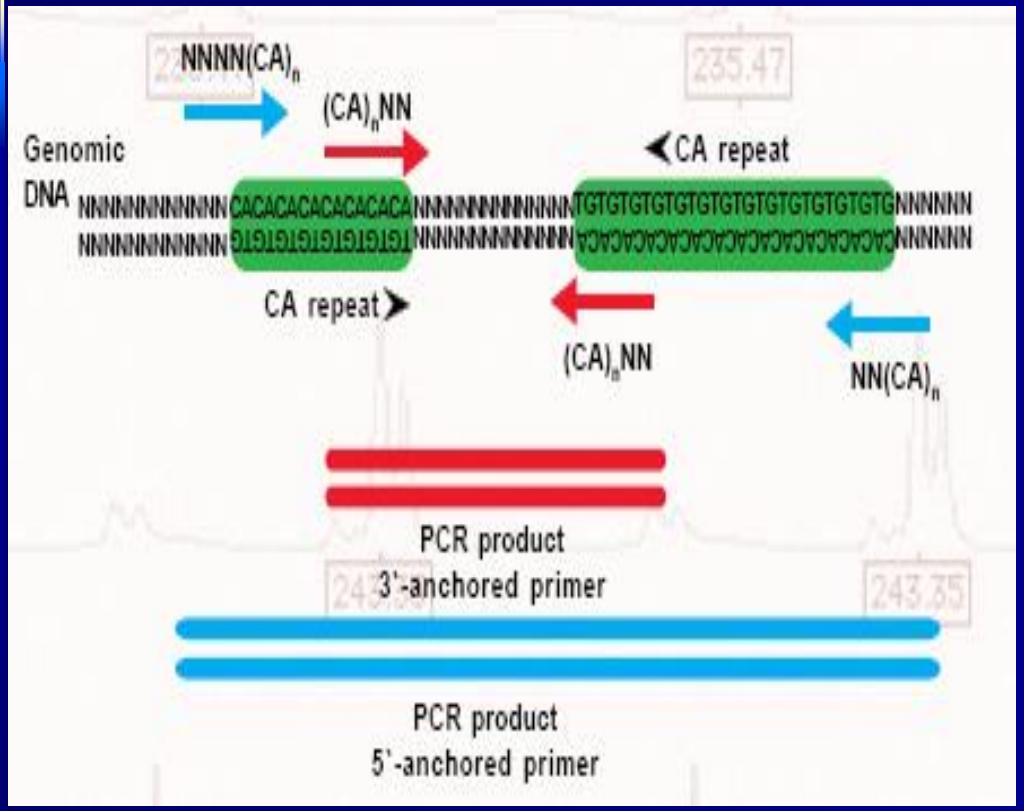
Limitations

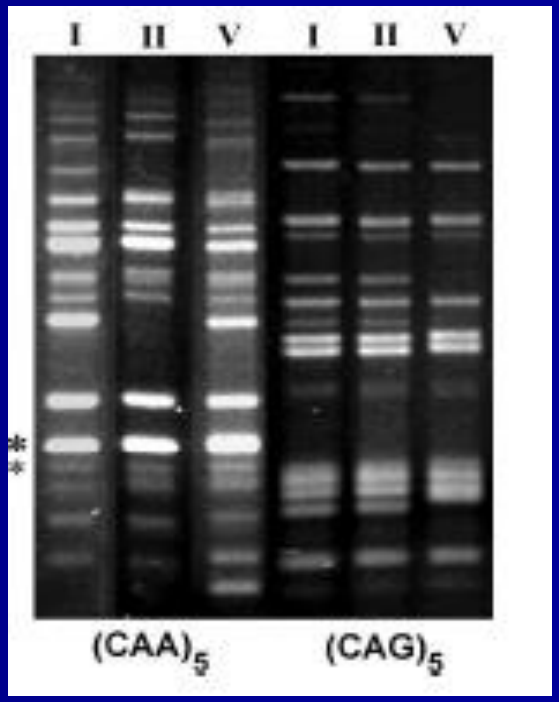
- Prior sequence knowledge is necessary for primer development
- Locus specific primer development is time consuming expensive

Inter Simple Sequence Repeat

- They are the region found in between microsatellite repeat
- This technique is based on PCR amplification of inter microsatellite repeat
- Because of the abundance of repeat sequences spread all over the genome it targets multiple loci

Designing of primer for ISSR polymorphism





Gel picture (cauliflower)

ISSR

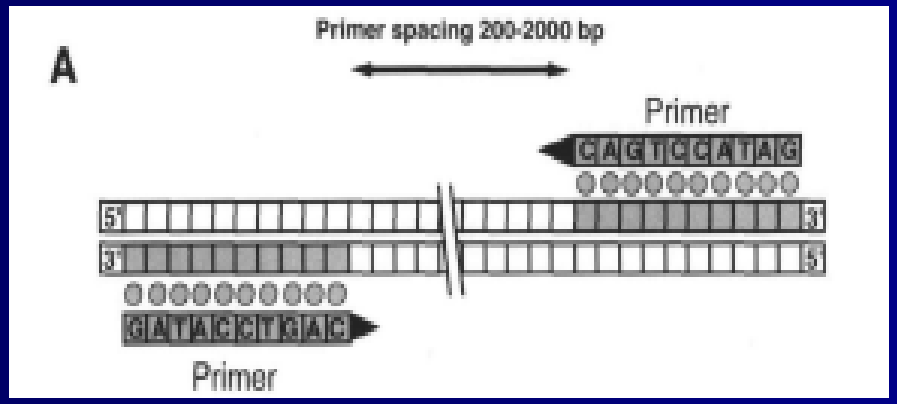
- Advantages
- Do not require prior sequence knowledge
- Very polymorphic
- Very useful for DNA profiling especially for closely related species
- Variation within unique regions of genome can be found at several loci simultaneously.

- Disadvantages
- Dominant inheritance
- Poor reproducibility

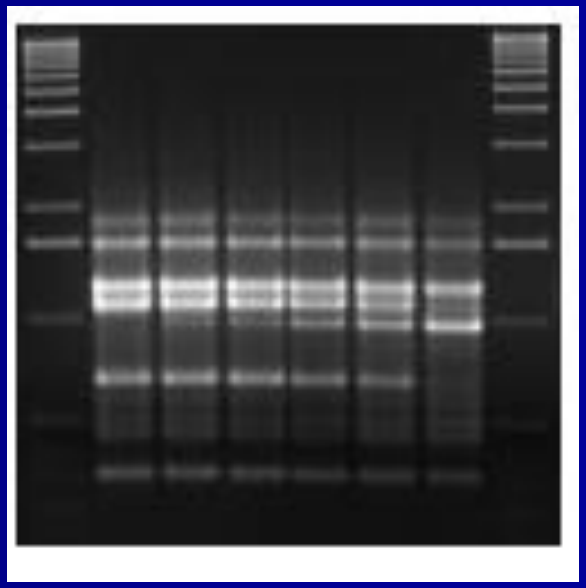
RAPD (Random Amplified Polymorphic DNA)

- Identify polymorphism at random
- Short (~10 nt) , single oligonucleotide primer is used
- Same primer bind at 2 sites allowing DNA synthesis to proceed as normal PCR
- Due to short primer, priming site occur more often

Primer designing in RAPD



Primer designing



RAPD Gel picture

RAPD

Advantages :-

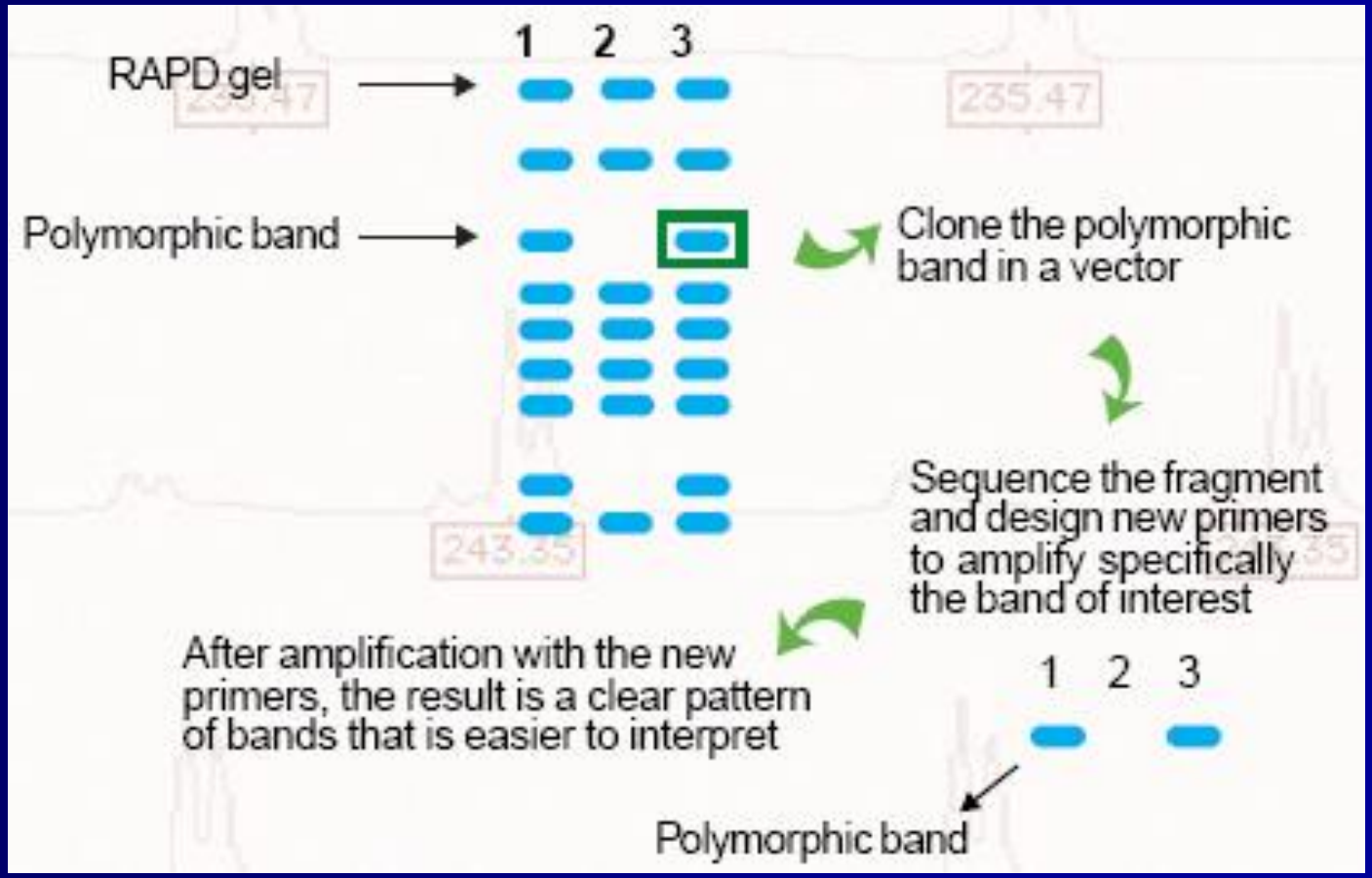
- Bands are highly polymorphic
- Many bands are produced per locus
- Involves nonradioactive assays
- Cheap, easy and fast
- Does not require sequence knowledge

- Disadvantages :-
- Reproducibility poor
- Dominant inheritance

Sequence characterised amplified region (SCAR)

- This technique converts a band prone to difficulties in interpretation and or reproducibility into a very reliable marker
- They use 16- 24 bases primer designed from ends of cloned RAPD marker

Diagram of SCAR procedure



Advantages (SCAR)

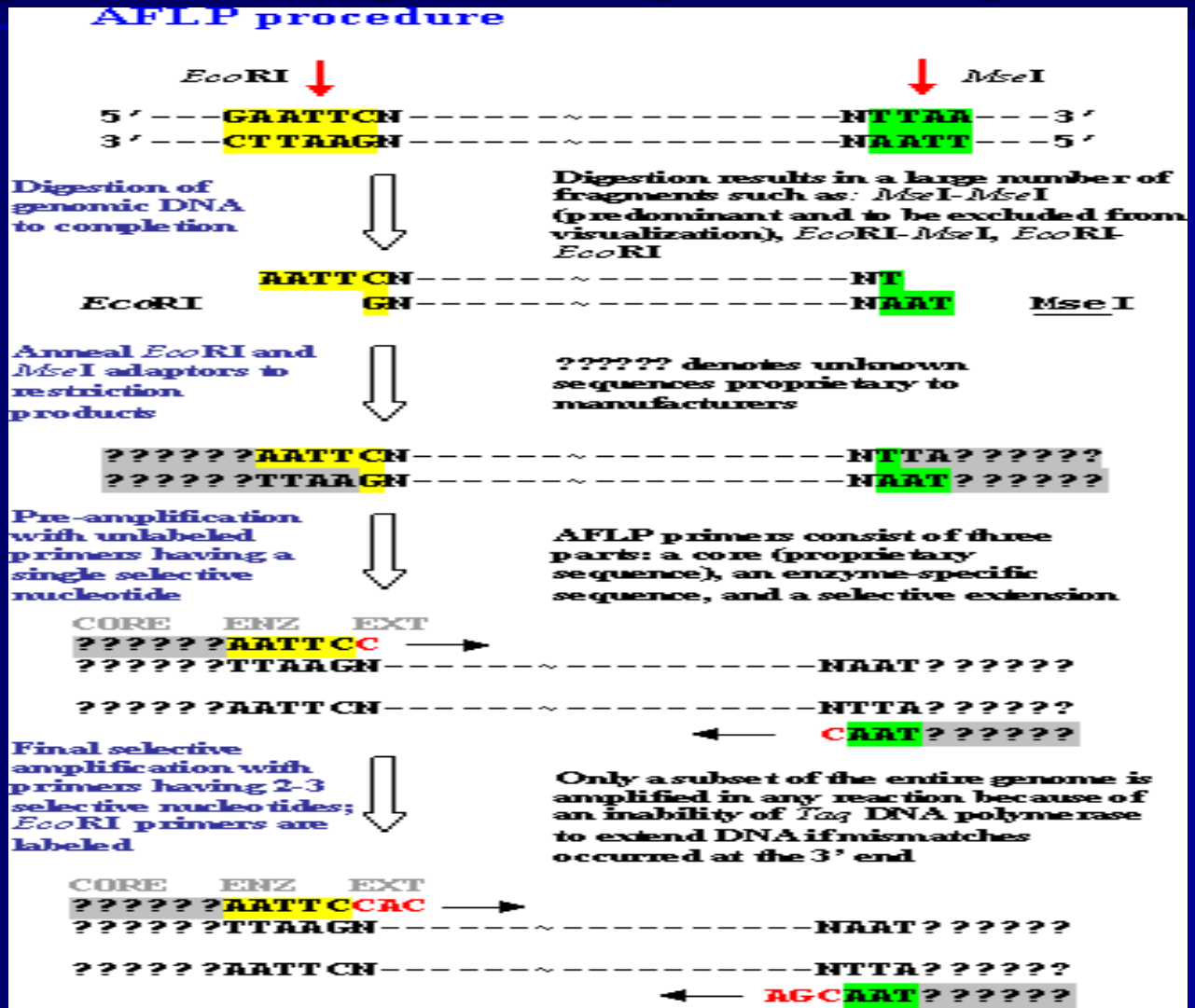
Advantages

- It is a codominant marker
- It detects only one locus
- High reproducibility

Disadvantages

- Requires small degree of sequence knowledge
- Requires effort and expense in designing specific primer for each locus

AFLP (Amplified Fragment Length Polymorphism)



AFLP

Advantages

- It has high reproducibility
- No prior sequence of knowledge is essential
- It detects more polymorphism per reaction than RFLP and RAPD
- Non arbitrary priming
- Small amount of DNA is required

Limitations

- Highly expensive
- Technically more demanding
- It is a dominant marker

COMPARISON BETWEEN RFLP & RAPD

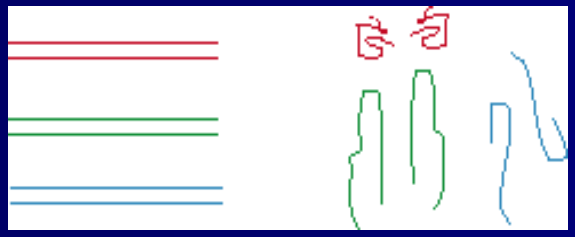
	FEATURES	RFLP	RAPD
1	Inheritance pattern	Codominant	Dominant
2	Detection of multiple alleles of a marker	Yes	No
3	Quality of DNA needed for study	Pure	Crude
4	Amount of DNA needed	2-10 μg	>10 ng
5	Radioisotopes	Must be used	Not used
6	Restriction enzymes	Must be used	Not used
7	Type of probe used	Species specific probes, generally, low copy genomic or cDNA	Randon base sequence, 9-12 base nucleotides
8	Time required	About 5-times more than RAPDs	1/5th of that for RFLPs

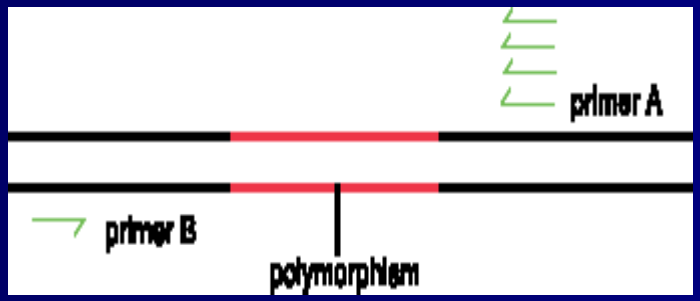
SSCP (Single Strand Conformation Polymorphism)

Mobility of double stranded DNA is independent of nucleotide sequences but mobility of single strand vary.

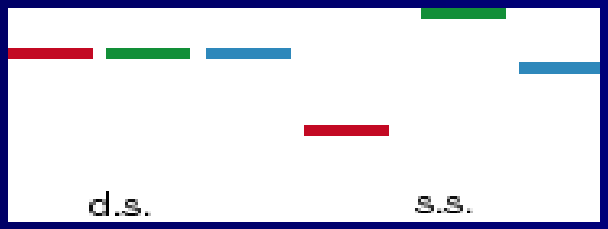
- It detects Polymorphism in sequence length as well as in nucleotide sequence
- Detects polymorphism at single loci

SSCP procedure





A : Asymmetric PCR



B : gel picture

SSCP

Advantages :-

- Inexpensive
- Inherited as codominant manner

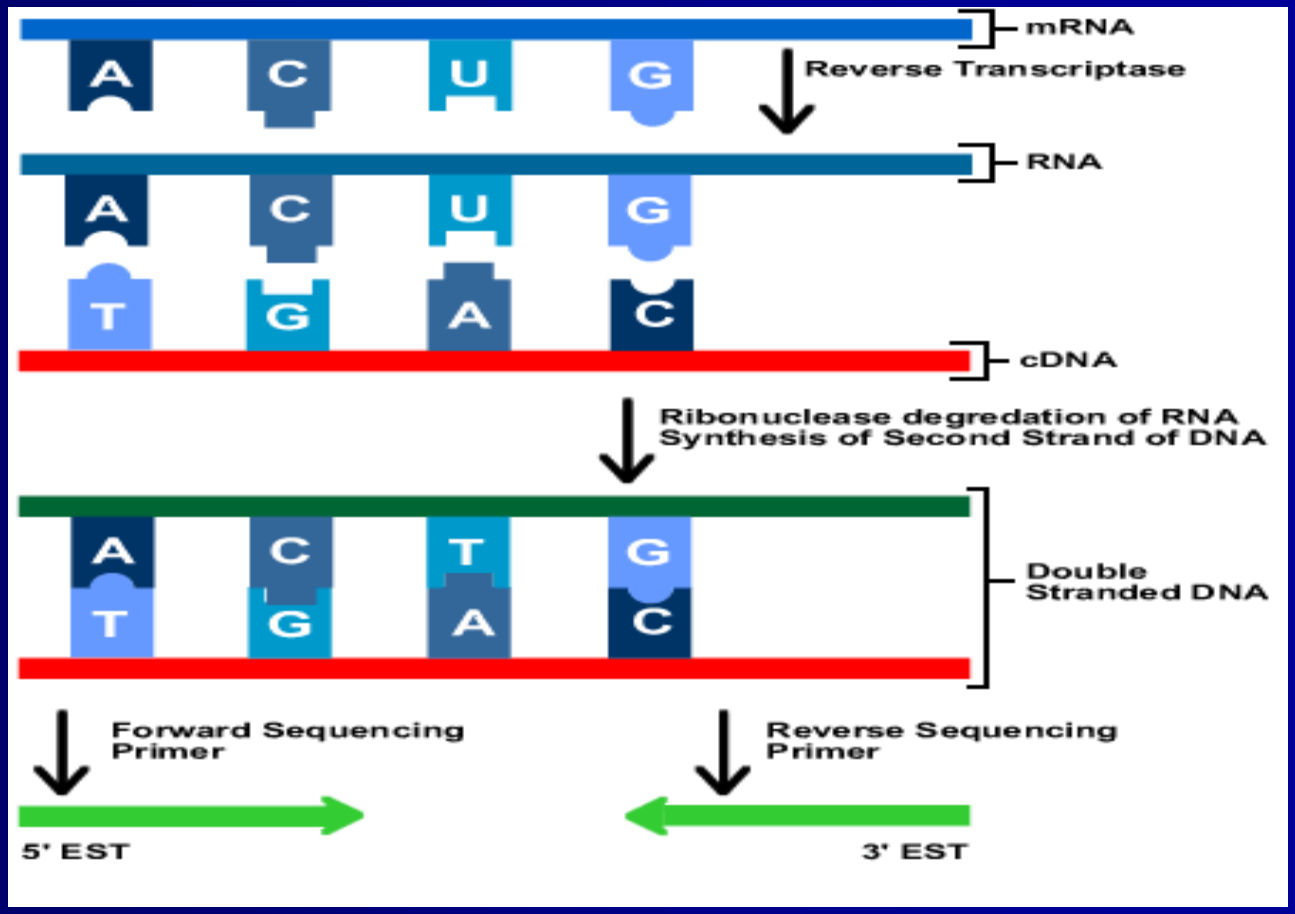
Limitations :-

- Mobility of single stranded DNA is affected by temperature and pH
- Fragment length (150-300bp) also affect sensitivity of SSCP

EST (Expressed Sequence Tag)

- EST are short (200-500nt) DNA sequences that can be used to identify a expressed gene in a cell at a particular time.
- EST are generated by sequencing either one or both ends of an expressed gene

EST procedure



EST

Advantages

- Inherited as codominant marker
- Good for mapping
- Used in discovery of genes associated with Quantitative Trait Loci

Disadvantages

- Require cloning and sequence information
- Design and creation of primer is expensive

Applications of marker

- Genome mapping
- Marker assisted selection
- Genetic diversity analysis
- Homogeneity check in breeding lines or variety
- Germplasm evaluation
- Genotype identification

Diversity analysis

Genetic diversity

It refers to the variation of genes within species

It covers both inter and intra population genetic variation

It is done by a software technology NTSYSpC (Numerical Taxonomy System version) - 2.2

Diversity analysis using molecular marker data

Band \ Var	V1	V2	V3	V4
A1	———	———	———	———
A2	———		———	
A3	———	———		———
A4	———	———	———	———

Conversion of Electrophoretic pattern into 1-0 matrix

Band \ var	V1	V2	V3	V4
A1	1	1	1	1
A2	1	0	1	0
A3	1	1	0	1
A4	1	1	1	1

Calculation of F-value and PIC value of band

- F – it refers to frequency of band
- PIC – refers to polymorphism information coefficient

PIC is calculated by following formula

$$PIC = 2F(1-F)$$

Table 1

Band	F value	PIC value
A1	4/4	0
A2	2/4	0.5
A3	3/4	0.38
A4	4/4	0

Average PIC value = 0.22

Calculation of pair wise similarity index (SI)

SI is calculated by using following formula

$SI = 2Nxy / Nx+Ny$

Where Nxy = bands present both in x and y

Nx = bands present in only x

Ny = bands present in only y

Table 2

Pair wise SI	Value
Between V1 and V2	0.86
Between V1 and V3	0.85
Between V1 and V4	0.86
Between V2 and V3	0.67
Between V2 and V4	1.00
Between V3 and V4	0.68

Clustering based on similarity index

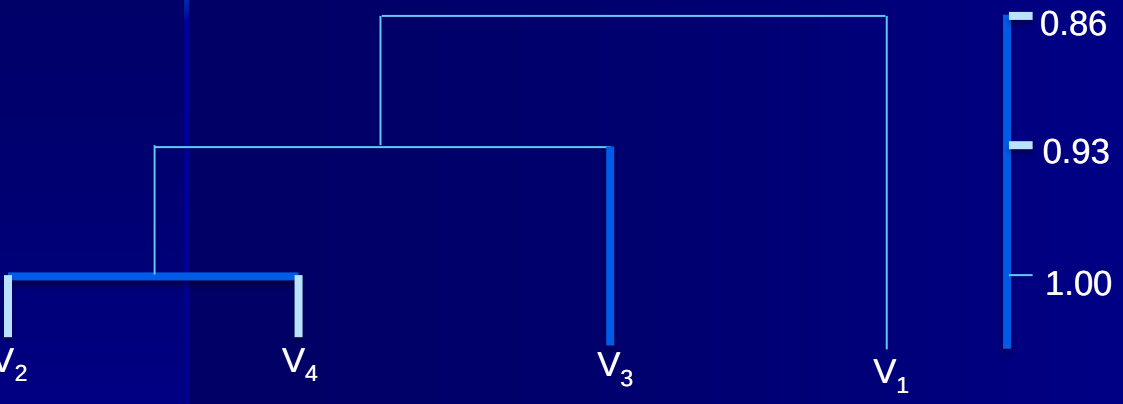
Pair wise SI in case of V2 and V4 is maximum i.e. 1

Arithmetic mean of pairwise SI of V1 & V2 & pairwise SI of V1 & V4 = 0.86

Arithmetic mean of pairwise SI of V2 & V3 & pairwise SI of V3 & V4 = 0.93

So V3 is more similar to V4 than V1

Drawing Of Dendogram



Probability of identity is calculated by the following formula :

PI = (average similarity coefficient)^{F} F = average number of bands

PI = $(.82)^4$ = 0.45

THANK YOU