Moleküler Markörler ve Kullanım alanları

Definition

Bir moleküler işaret, kolaylıkla tespit edilen ve kalıtımı kolaylıkla izlenebilen bir DNA dizisidir.

Moleküler Belirtec Nedir?

- . Tüm genom içinde tanımlanabilen spesifik DNA parçaları.

- Moleküler belirteçler, iki veya daha fazla birey arasındaki sekans farklılıklarının saptanmasına izin veren genel testlerdir.

- Moleküler belirteçler, genomun belirli yerlerinde bulunur.

- Belirli bir genin konumunu veya belirli bir özelliğin veya istenen özelliklerin kalıtımını 'işaretlemek' için kullanılırlar.

Marker systems are tools which is used to mark a trait in living organism

MORPHOLOGICAL MARKER:

Classical markers

MOLECULAR MARKERS:

Variation in macro-molecules

BIOCHEMICAL MARKERS

- ISOENZYME

- Low polymorphism
- Requires expression of trait / gene
- Dominance effect
- Expression sex limited
- Expressed late in life

They are protein produced by expression of gene

GENETIC MARKERS

- RFLP
- AFLP
- RAPD

Depend upon sequence of DNA

DNA markers

Non-PCR Based,

- **RFLP-** Restriction fragment lengthpolymorphism.

PCR Based

- **RAPD-** Random amplification of polymorphic DNA**.**
- **AFLP**-Amplified fragment lengthpolymorphism.
- **SSR**-Simple sequence repeats

Properties of Ideal Genetic Marker

- polimorfik olmalıdır.
- Eş baskın olmalıdır.
- Genom boyunca eşit aralıklarla ve sıklıkla dağılmalıdır.
- Tespit edilmesi kolay, hızlı ve ucuz olmalıdır..
- Yeniden üretilebilir olmalıdır.
- Bir çok çalışmada ortak kullanıma sahip olmalıdır.

Table 1 : Comparision of the most broadly used techniques of molecular markers

Feature	RFLP	RAPD	AFLP	SSR or Microsatellite
DNA required (µg)	10	0.02	0.5-1.0	0.05
DNA quality	High	High	Moderate	Moderate
PCR-based	No	Yes	Yes	Yes
No. of polymorphic loci analysed	1.0-3.0	1.5-50	20-100	1.0-3.0
Ease of use	Not easy	Easy	Easy	Easy
Reproductibily	High	Unreliable	High	High
Development cost	Low	Low	Moderate	High
Cost per analysis	High	Low	Moderate	Low

Cont…..

RFLP

Definition

Restriksiyon endonukleazlar kullanılarak genomik DNA nın spesifik bölgelerden kesilerek DNA fragmanlarının uzunluklarındaki varyasyonlar belirlenir.

Principle

Belirli parça uzunluğu polimorfizmi (RFLP) teknolojisi ilk olarak 1980'lerde insan genetik uygulamalarında kullanılmak üzere geliştirilmiş ve daha sonra bitkilere uygulanmıştır..

Total DNa spesifik enzimlerle kesilerek sınırsız sayıda belirli parça uzunluğunda polimorfik Dna elde edilebilir.

RFLP'ler boyut olarak nispeten küçüktür ve doğaları gereği birlikte baskındır..

İki birey, enzimlerin kesim alanında tek bir nükleotid farklılık gösterirse, restiksiyon enzimi birinin DNA'sını kesecek, diğerini kesmeyecektir. Böylece farklı uzunlukta RFLP parçaları oluşacaktır.

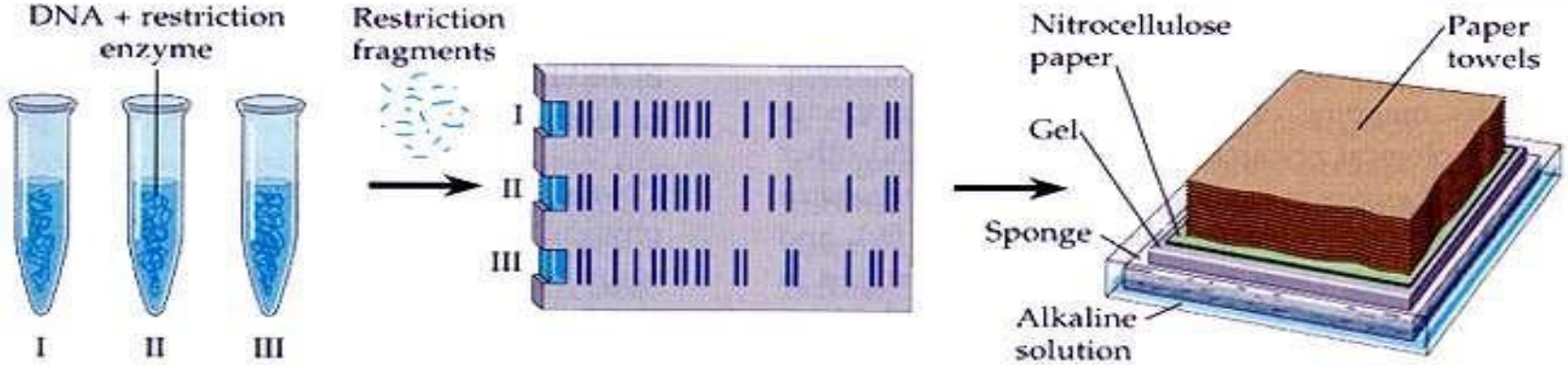
Buna karşılık RFLP analizleri karmaşık,zaman alıcı ve pahalı bir işlem süresine sahiptir.

Principle

The hybridization results can be visualized by

1. Autoradiography (if the probes are radioactively labeled), or
2. Chemiluminesence (if non-radioactive, enzyme-link methods are used for probe labeling and detection).

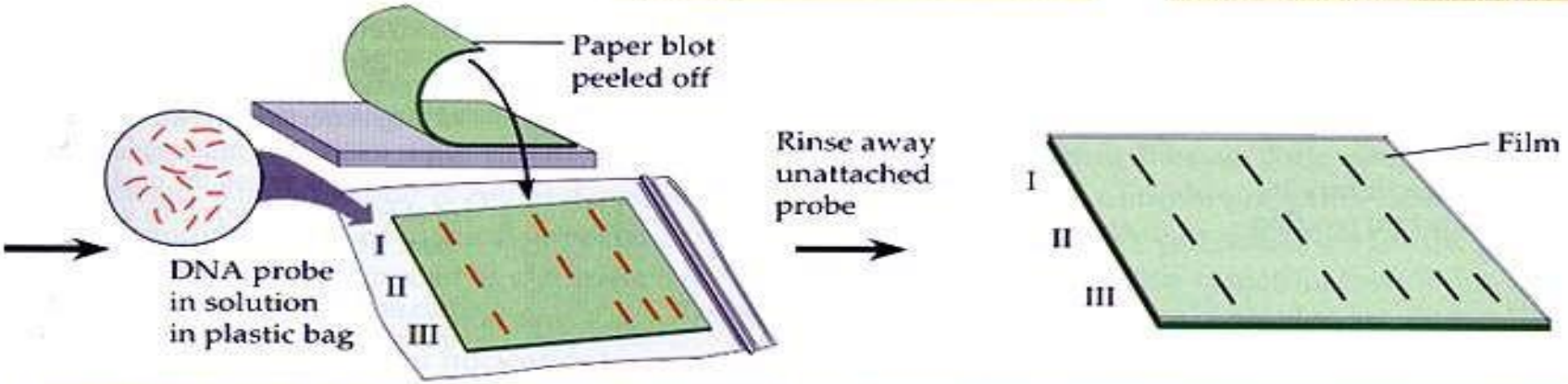
Any of the visualization techniques will give the same results. The visualization techniques used will depend on the laboratory condition



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.

Advantages

-Özel sekans bölgesine ihtiyaç duymadığı için basit bir metoddur

--Ko-dominant markerler vardır

-PCR a ihtiyaç duymaz

Disadvantages

-Yüksek miktarda saf DNA ihtiyacı vardır.

-Sürekli prob ihtiyacı varıdr

-Uygun endonukleazları belirlemek zahmetli bir iştir.

-Çok vakit gerektirir.

-Autoradyografide özel uzmanlık alanı gerektirir.

Applications of molecular markers

- GENETİK ÇEŞİTLİLİĞİN ÖLÇÜSÜ
- DBA PARMAK İZİ
- GENOTİPİK SEÇİLİM
- MARKER YARDIMI İLE SEÇME
- GENOTİP TESPİTİ

RAPD

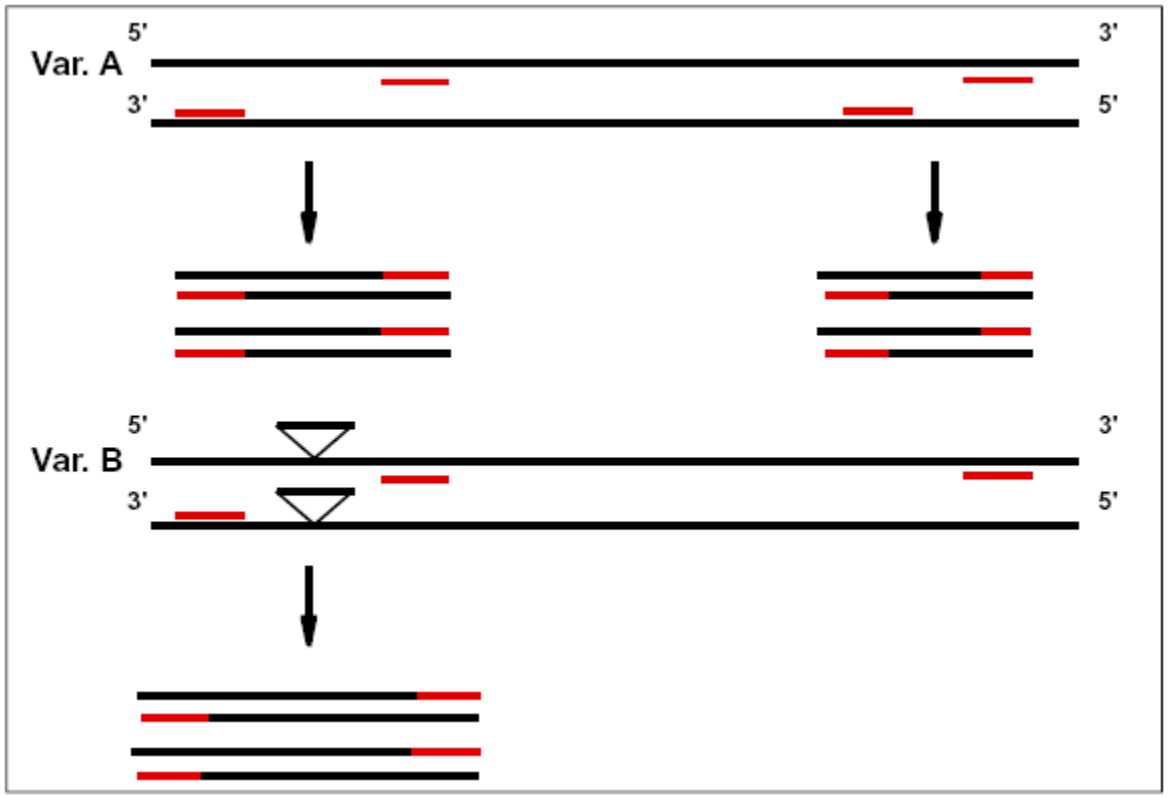
Definition

Rasgele amplifiye edilmiş polimorfik DNA kısa DNA primerleri kullınalrak PCR vasitasiti ile rastgele çoğaltma yapan bir sistemdir.

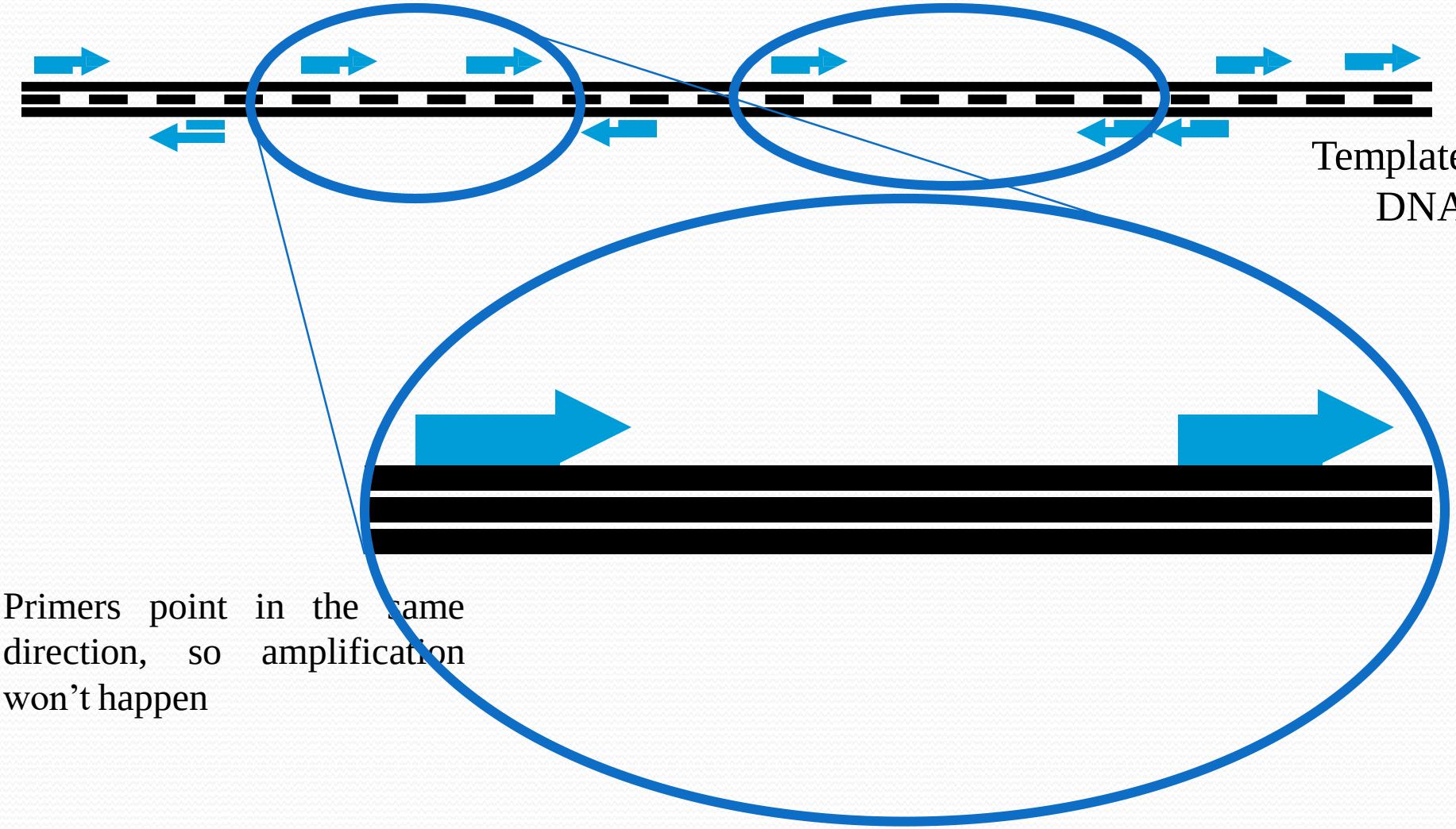
RAPD sistemi dominant bir marker sistemidir.

Principle

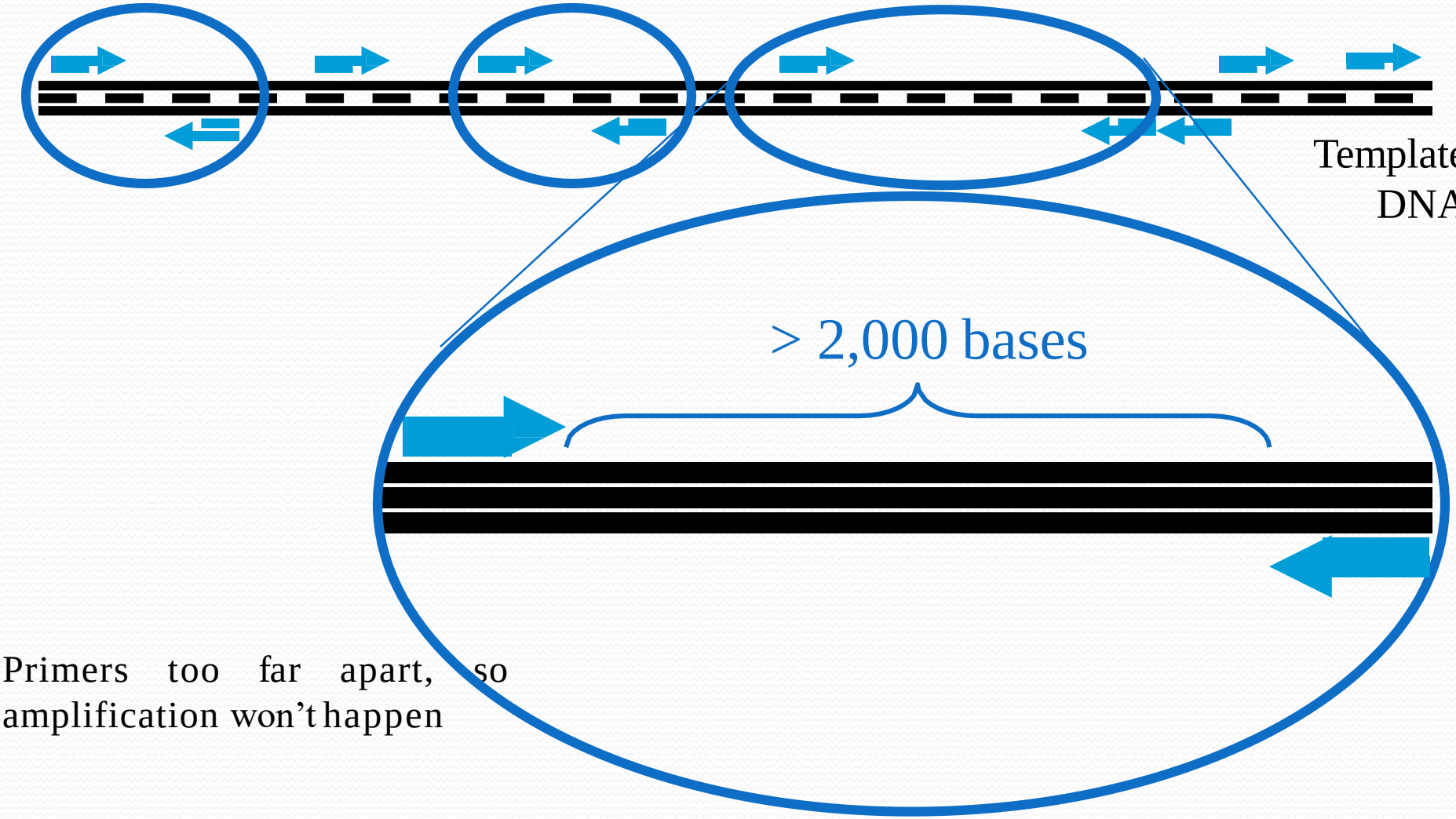
1. RAPD'ler, genomik DNA ve rastgele primerler kullanılarak PCR ile üretilir
2. 2. Taq polimeraz, yakın aralıklı sekans (<2kb) ve kısa rastgele oligomerleri tamamlayıcı (tipik olarak 10-mer) arasındaki DNA segmentini amplifiye etmek için kullanılır.
3. RAPD polimorfizmi, DNA sekansındaki primer bağlanma sahasındaki değişiklikten kaynaklanır.



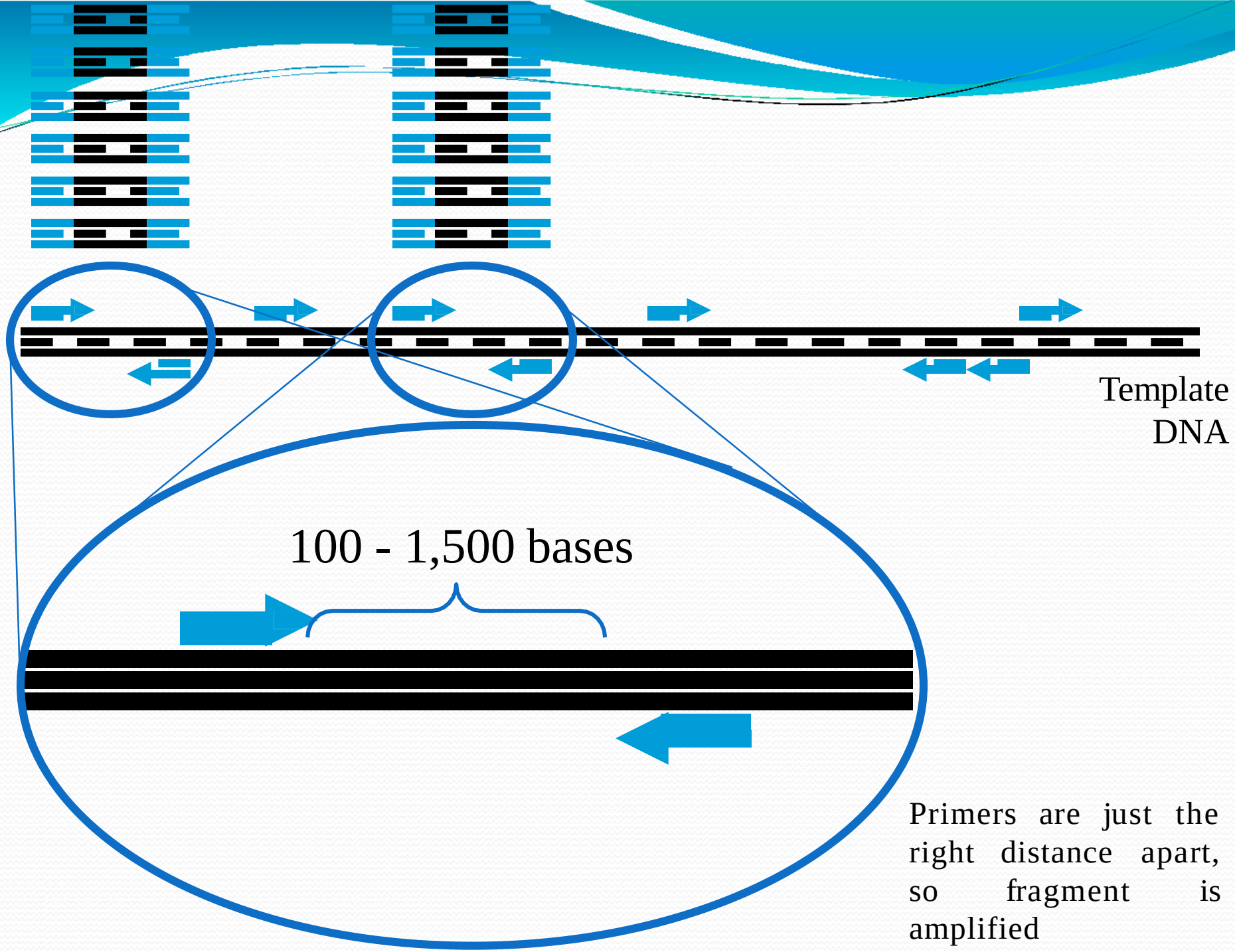
A çeşidinde, iki RAPD ürünü ile sonuçlanan 4 primer bağlama bölgesi vardır.B, bağlanma yerlerinden birine sahip değildir ve yalnızca bir RAPD işaretinin üretilmesine neden olur.



Primers point in the same direction, so amplification won't happen



Primers too far apart, so amplification won't happen



Primers are just the right distance apart, so fragment is amplified

Protocol

1) Master Stock Mixture

	Conc. stock solution	Vol	Final concentration
PCR-buffer + $MgCl_2$	10x (15 mM)	3µl	1x (1.5 mM)
Primer	15 µM	0.4 µl	0.2 µM
dNTP-mix	10 mM	0.51 µl	0.17 mM
Taq-polymerase	5 U/µl	0.25 µl	1.25 U
ddH_20		20.84 µl	
Final volume		25 µl	

2) Add 25µl of master mix to 5µl of your DNA in a sterile tube

Note: In each PCR run you conduct, include 2 sample, one of control DNA without primer (3µl DNA), and one sample without DNA (5µl ddH_2O)

Advantages

-need small amount of DNA

-it involves non-radioactive assay

-it does not required specific probe libraries

-it provide quick and efficient screening for DNA sequence based on polymorphism at many loci

Disadvantages

-it is inherited as dominant traits

-there is a bands due to relatively short primer

-the production of non-parental bands in the offspring of known pedigree warrants its use with extreme care

-it is sensitive to change in PCR conditions

Application of RAPD

Genetic maps
F_1 identification
Varietal/line identification (multiplexing of primers necessary)
Breeding
Bulk segregant analysis
Diversity studies
Marker-assisted selection
Seed testing
Map-based gene cloning

AFLP

Definition

Any difference between corresponding DNA fragment from two organisms A & B that is detected by amplified restriction length polymorphism technique

Principle

1. The amplified fragment length polymorphism technique combines components of RFLP analysis with PCR technology.

2. Total genomic DNA is digested with a pair of restriction enzymes normally a frequent and rare cutter.

3. Adaptors of known sequence are then ligation to the DNA fragments.

4. Primer complementary to the adaptors are used to amplify the restriction fragments.

5. The PCR amplified fragments can then separated by gel electrophoresis and banding patterns visualized.

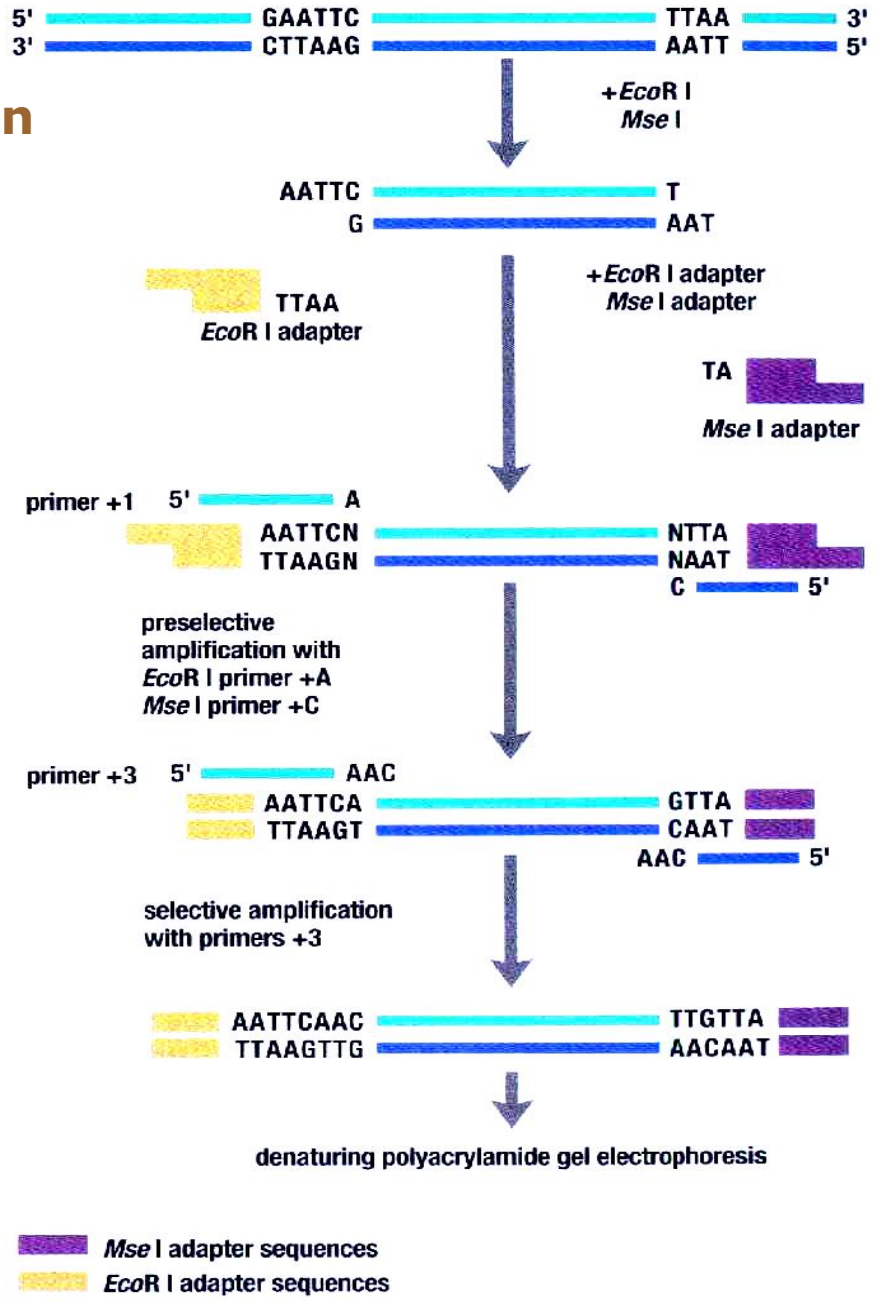
6. A range of enzymes and primer are available to manipulate the complexity of AFLP fingerprint to suitapplication

Restriksiyon-Digestion

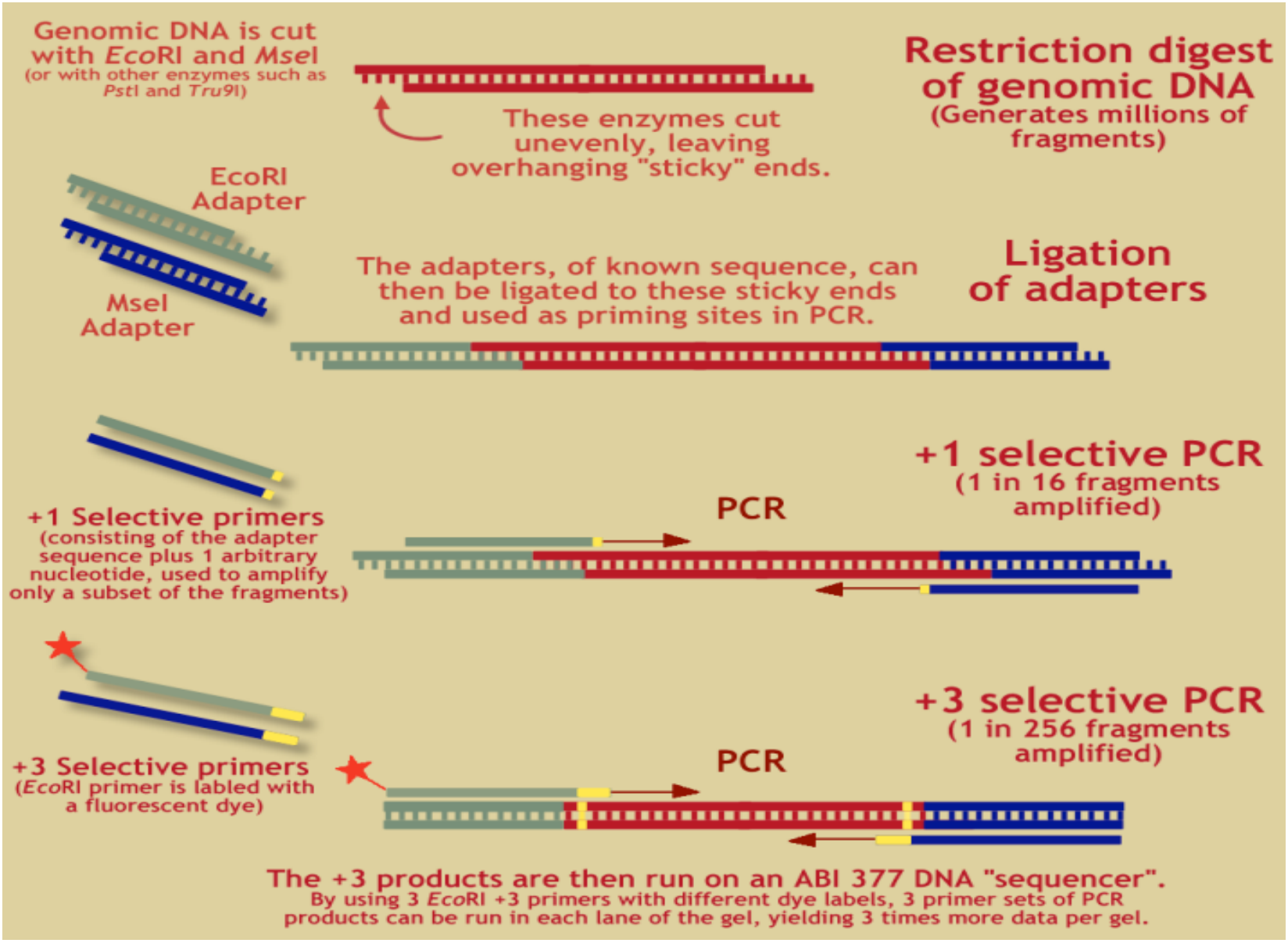
Adaptor Ligation

Amplification

Electrophoresis



AFLP (Amplified Fragment Length Polymorphisms)
=Çoğaltılmış parça uzunluğu polimorfizmi



Advantages

-extremely sensitive

-it has a wide scale applicability

-it discriminates heterozygotes from homozygotes when a gel scanner is used

-used for mapping

Disadvantages

-it is highly expensive

-it required more DNA than RAPD

-it required experience of sequencing gels

Application of AFLP

Fingerprinting
Very fast mapping
Region-specific marker saturation
Varietal identification
Genetic maps
F_1 identification
Gene tagging
Breeding
Bulk segregant analysis
Diversity studies
Marker-assisted selection
High-resolution mapping
Map-based gene cloning

(Microsatellite)

SSR

Principle

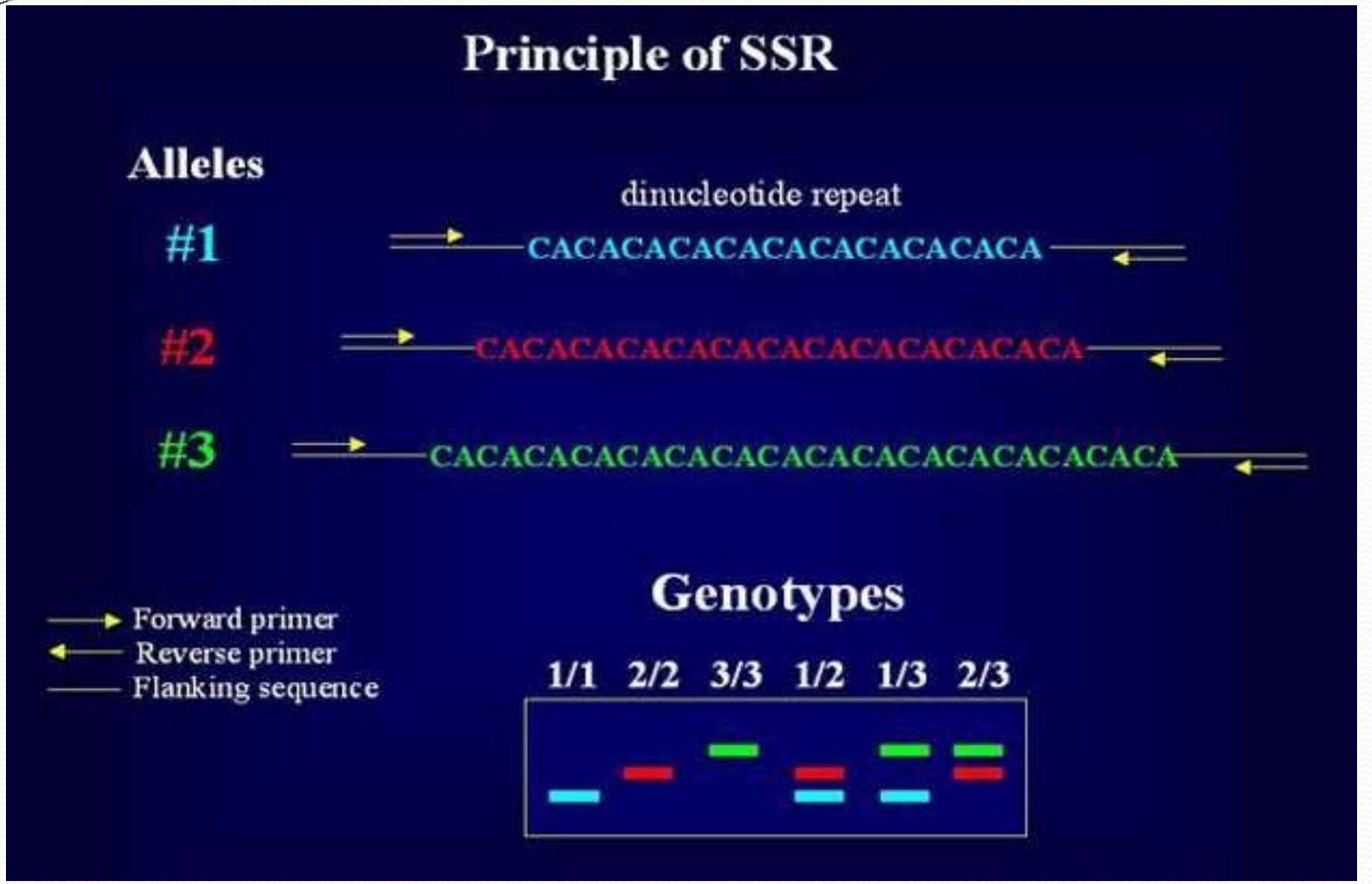
Mikrosatellitler, primer olarak çevreleyen bölgelerin benzersiz sekansları kullanılarak polimeraz zincir reaksiyonu (PCR) prosesi ile tanımlama için amplifiye edilebilir.

Çift ipliği ayırmak için DNA tekrar tekrar yüksek bir sıcaklıkta denatüre edilir, ardından primerlerin bağlanmasına ve nükleotid dizilerinin mikro uydu üzerinden uzatılmasına izin vermek için soğutulur.

Bu işlem, agaroz veya poliakrilamid jeller üzerinde görülebilecek yeterli DNA üretimiyle sonuçlanır.

PCR teknolojisinin bolluğuyla, mikro uydu lokuslarını çevreleyen primerlerin kullanımı basit ve hızlıdır, ancak doğru işleyen primerlerin geliştirilmesi genellikle zahmetli ve maliyetli bir süreçtir.

Principle of SSR



https://www.youtube.com/watch?v=lQSi84xFsrY&ab_channel=QuickBiochemistryBasics

Advantages

-simple and easy to use

-easy to detect via PCR

-co-dominant marker

-perfectly suited for used in map-based cloning

Disadvantages

-cost is higher for establishing polymorphic primer sites and investment in the synthesizing the oligonucleotides

-initial identification, DNA sequence information necessary

Application of SSR

- Assessment of genetic variability and characterization of germplasm.
- Identification and fingerprinting of genotypes.
- Estimation of genetic distances between population, inbreeds and breeding material.
- Marker assisted selection.
- Identification of sequence of useful candidate genes

Applications:-

F1 identification

An autoradiograph detecting parent (P1&P2) and homozygous and heterozygous (H) F_1 segregation

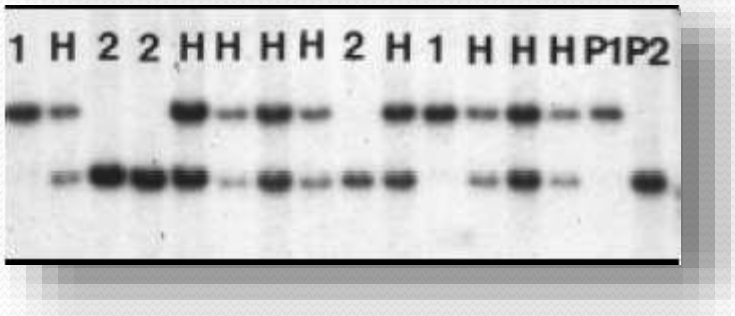


Table : Application of PCR-based marker MG3H001 for detecting resistant allele169 bp corresponds to the susceptible plant(s), alleles 146 bp and 150 bp to the ym4 or ym5 resistant plants, respectively.

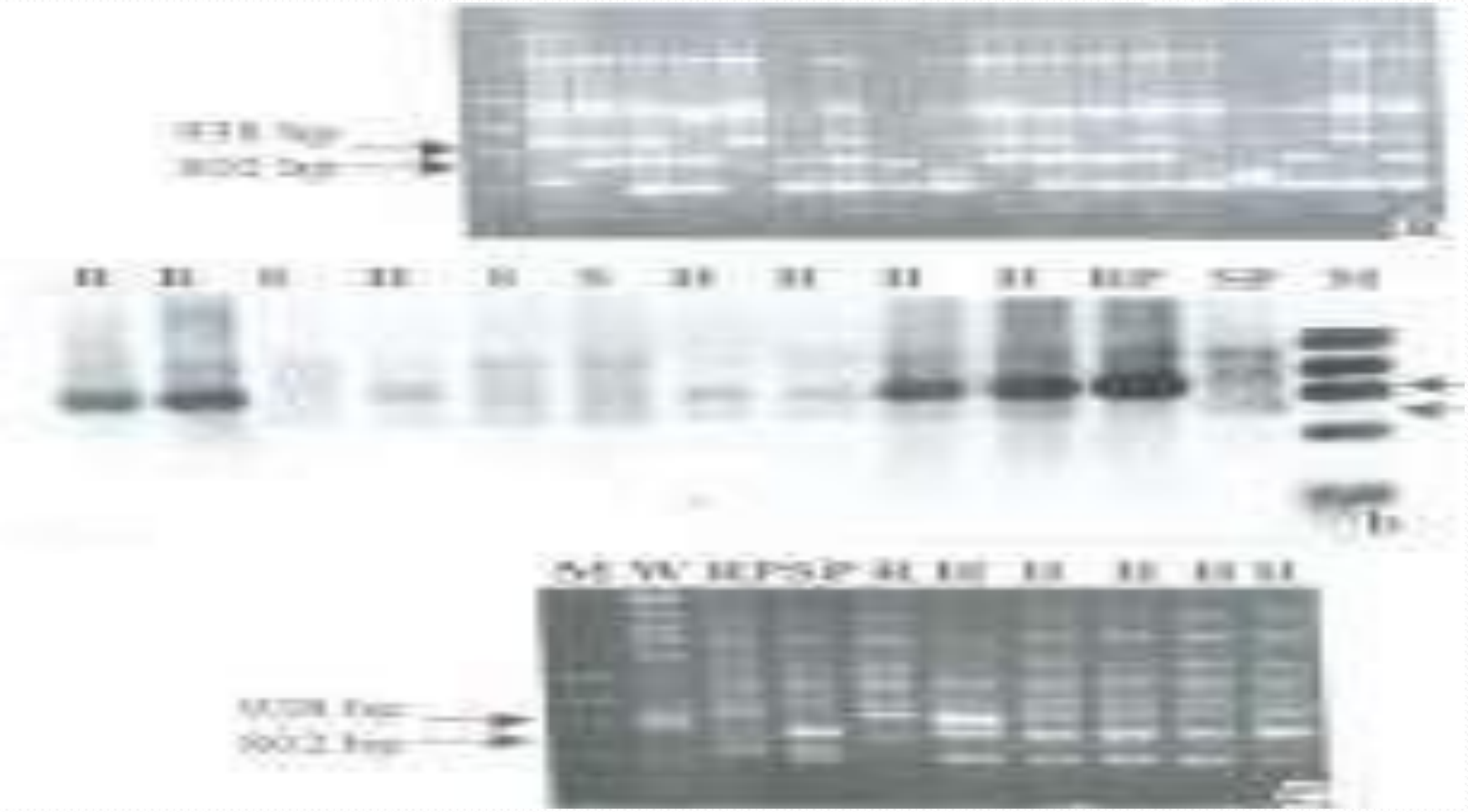
No.	Plants	Allele	MG3H1_146	MG3H_150	MH3H1_169	Resistance
1	Ge004 3-001	MG3H001_146	1	-	-	Resistence ym4
2	-002	MG3H001_169	-	-	1	Susceptible
3	-003	MG3H001_146	1	-	-	Resistence ym4
4	-004	MG3H001_169	-	-	1	Susceptible
5	-005	MG3H001_146	1	-	-	Resistence ym4
6	-006	MG3H001_146	1	-	-	Resistence ym4
7	-007	MG3H001_169	-	-	1	Susceptible
8	-008	MG3H001_169	-	-	1	Susceptible
9	-009	MG3H001_150	-	1	-	Resistence ym5
10	-010	MG3H001_150	-	1	-	Resistence ym5
11	-011	MG3H001_150	-	1	-	Resistence ym5
12	-012	MG3H001_169	-	-	1	Susceptible

Germany

Korzun, 2002

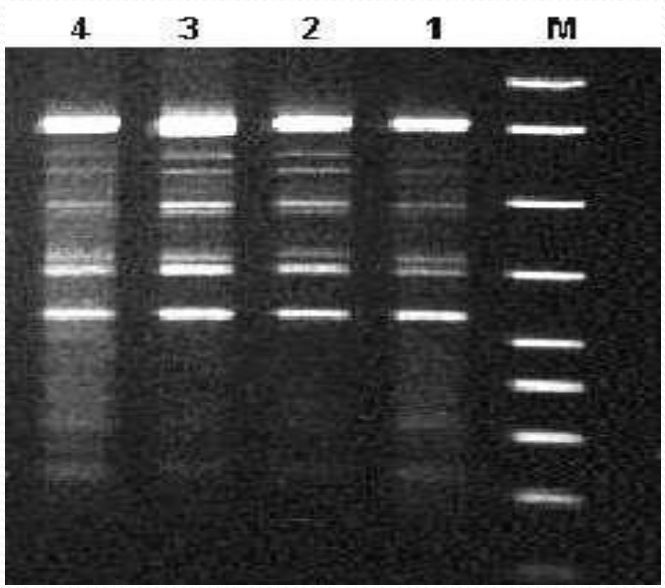
Fig: Identification of RAPD marker link to brown plant hoper resistance gene in rice

M R H H S R H H S R H H S H R R H R H



June *et al.*,2003

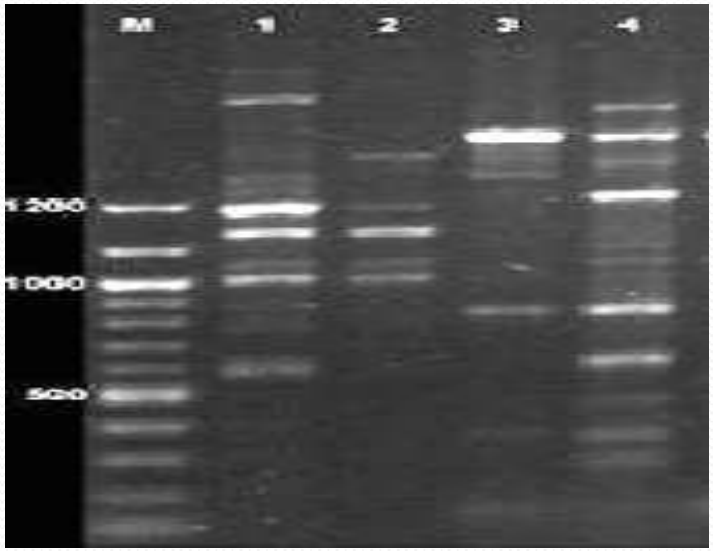
RAPD-ANALYSIS OF GENETIC VARIATION OF FOUR IMPORTANT RICE VARIETIES USING RAPD PRIMERS



Amplified RAPD patterns of OPR1

M - 1Kb DNA Ladder
1- ADT38
2 - ASD16
3 - IR20
4 - PONNI

Tamil Nadu

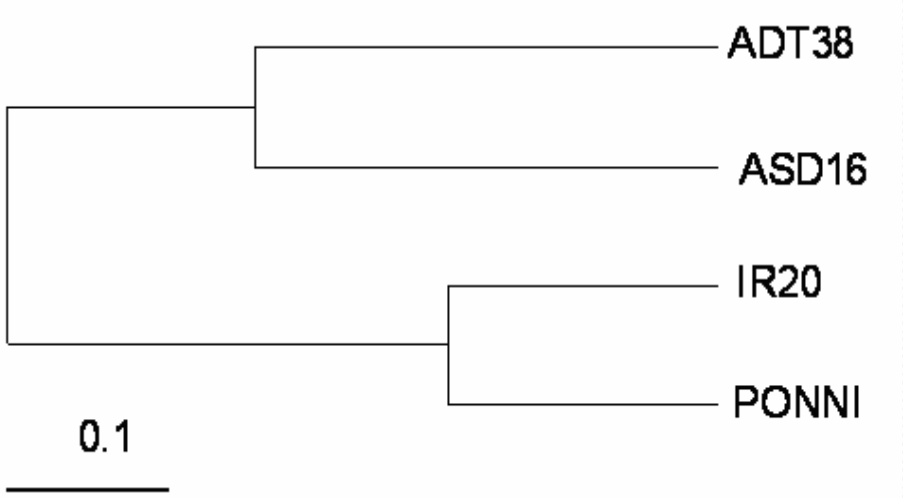


Amplified RAPD patterns of OPR2.

Mani *et al.* (2010)

Con..

Con..



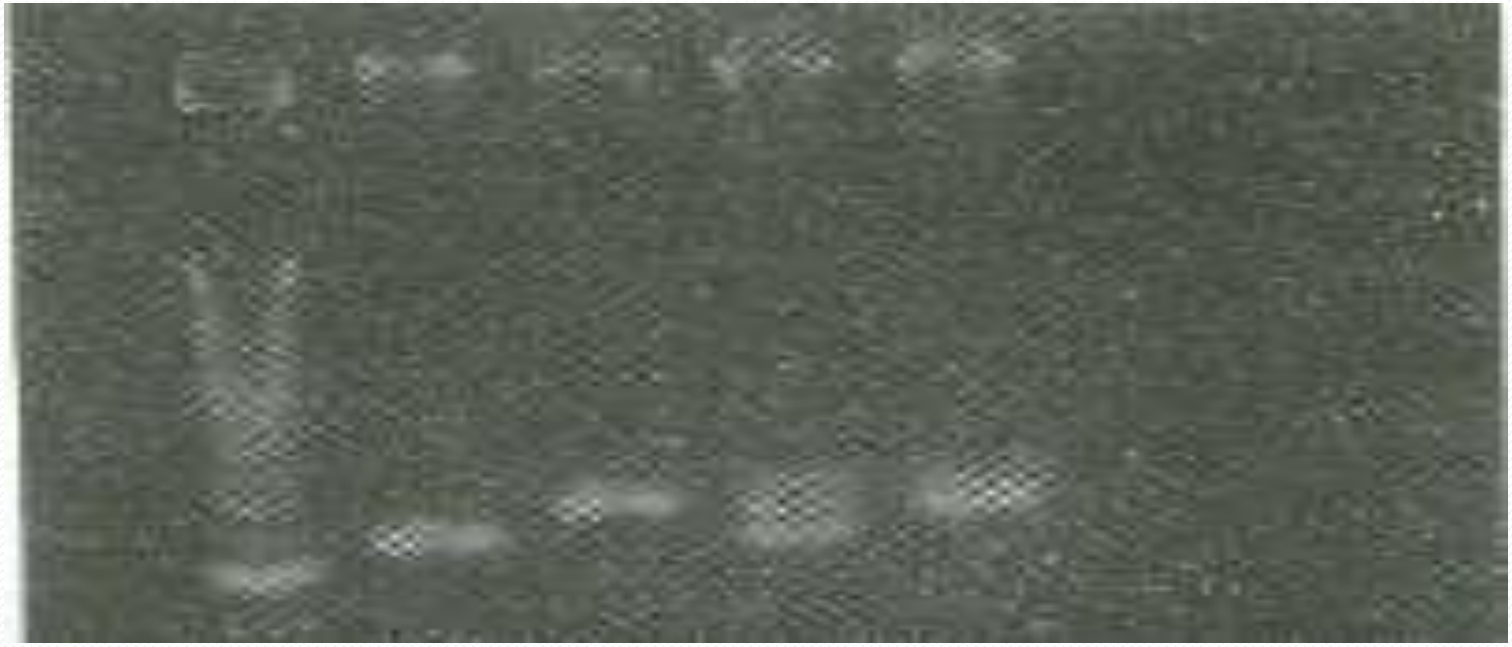
UPGMA dandogram based on Nei's (1978) original measure of genetic distance, summarizing the data on differentiation between four samples of *O. sativa genotypes according to RAPD analysis.*

Genetic distance between *O. sativa* populations of four different rice varieties based on Nei's 1978 measures of genetic distance.

	PONNI	**IR-20**	***ADT38***	***ASD16***
PONNI		**0.3913**	1.7776	1.02564
IR-20	0.3913		1.60944	**1.95601**
ADT38	1.77767	1.60944		0.8574
ASD16	1.02564	1.95601	0.8574	

Fig: Molecular mapping of fertility restorer gene in basmati rice using micro satellite marker.

1 2 3 4 5



A Rice microsatellite marker RM 258 identified to be linked with fertility restorer gene in PRR- 78 using bulk segregant analysis.

DNA marker (**lane 1**). Restorer line PRR 78 (**lane 2**).

CMS line IR 58025 (**lane 3**).

Fertile bulk showing heterozygous pattern (**lane 4**).

Sterile bulk showing homozygous pattern (**lane 5**)

Delhi

Mishra *et al.2001*