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REKAYASA GENETIKA
IBD 131

By Seprianto S.Pi, M.Si

Pertemuan 13

DNA Polimorfisme 2

Universitas Esa Unggul

Sasaran Perkuliahan

- Mahasiswa memahami dan menjelaskan tentang pemanfaatan DNA polimorfisme dalam menentukan keragaman genetik makhluk hidup (Biodivesitas)
- Dapat menjelaskan Tentang RAPD dan kegunaaan dalam Keanekaragaman genetik
- Menjelaskan dan memahami proses pengunaan AFLP dalam menentukan keragaman genetik tanaman
- Pemanfaatan SCAR sebagai marka melekuler

DNA Polimorfisme

- Random Amplified Polymorphic DNA (RAPD)
- Amplified Fragment Length Polymorphism (AFLP)
- Sequence Characterized Amplified Region (SCAR)
- DNA barkoding (Barcoding DNA)

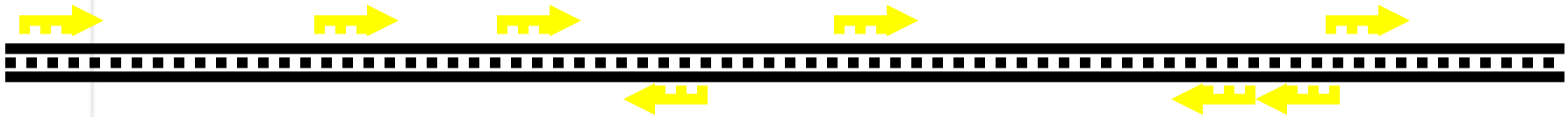
What is RAPD?

RAPD is a PCR-based method which employs single primers of arbitrary nucleotide sequence with 10 nucleotides to amplify anonymous PCR fragments from genomic template DNA

PCR product occurs when:

- The primers anneal in a particular orientation (such that they point towards each other)
- The primers anneal within a reasonable distance of one another (150 -3000 bp)

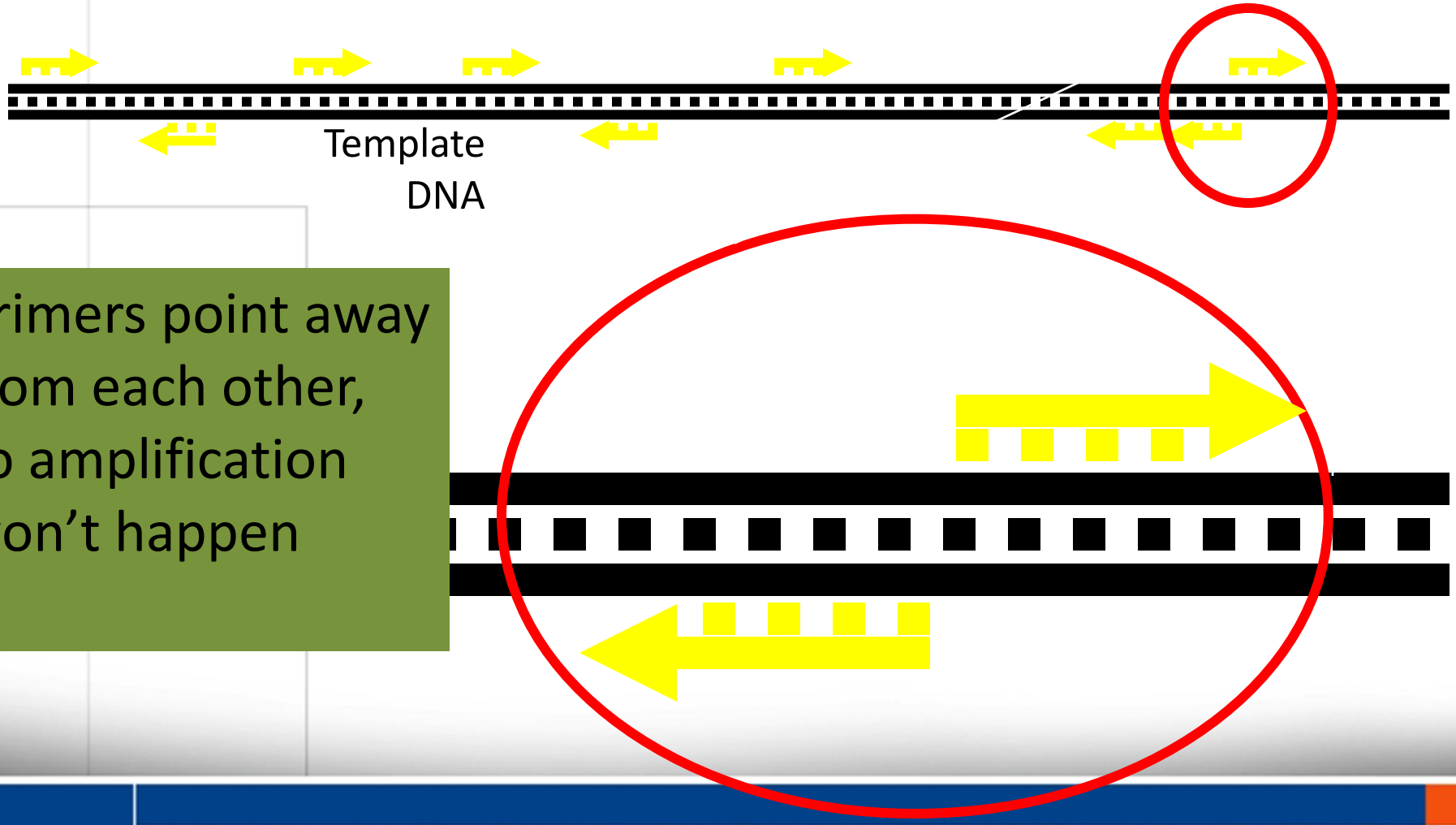
RAPD



Template DNA

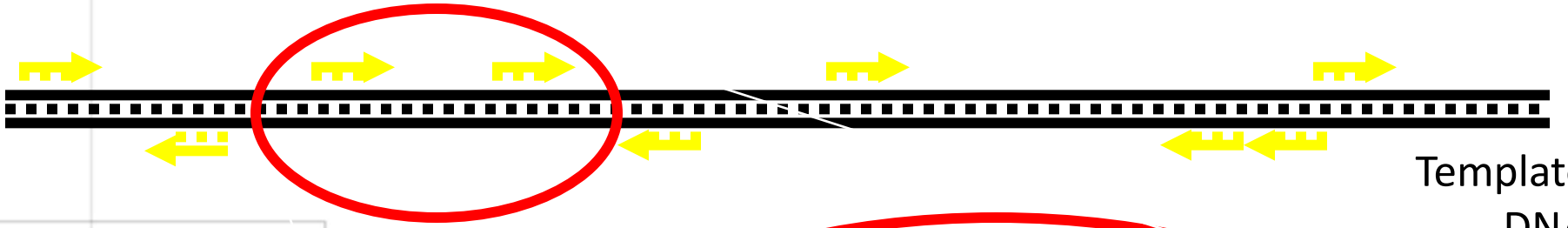
- Primer binds to many locations on the template DNA
- Only when primer binding sites are close and oriented in opposite direction (the primers point toward each other) will amplification take place

RAPD

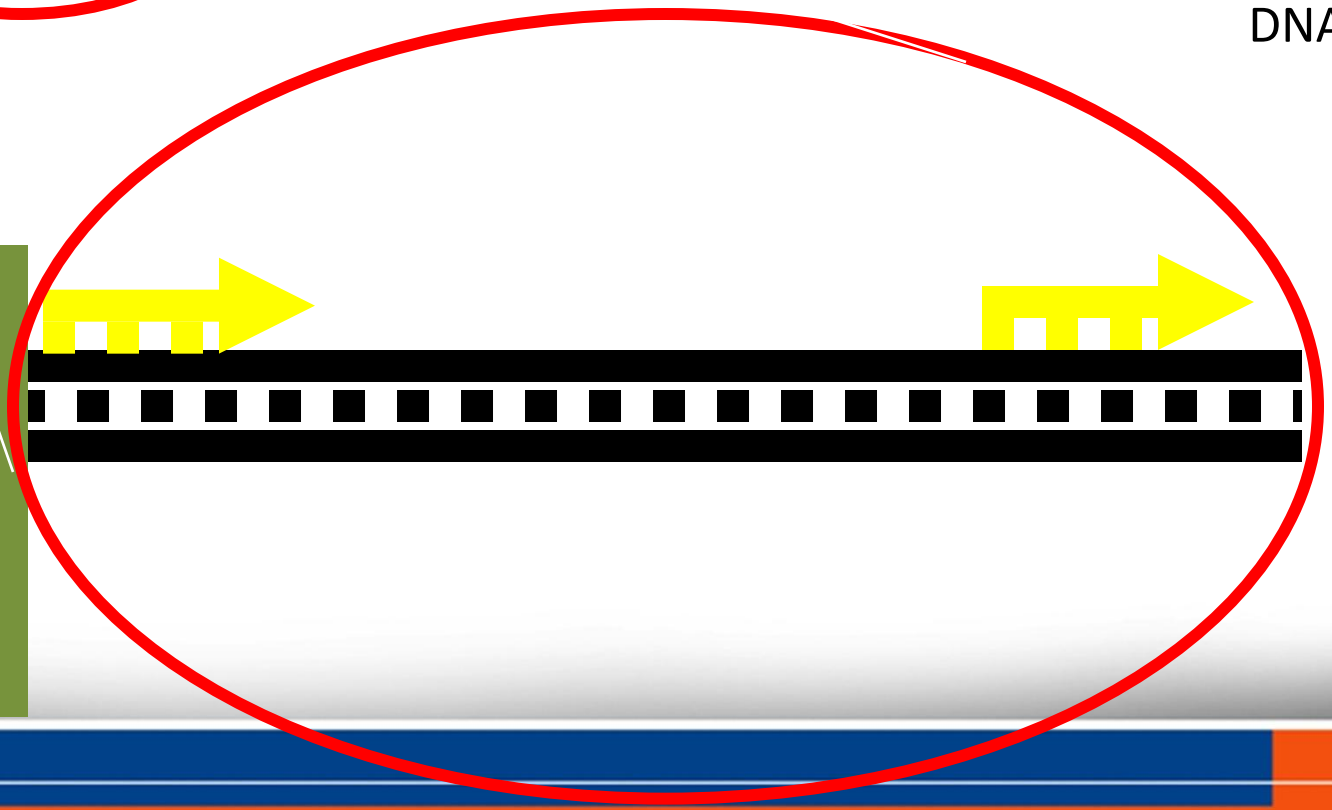


Primers point away from each other, so amplification won't happen

RAPD

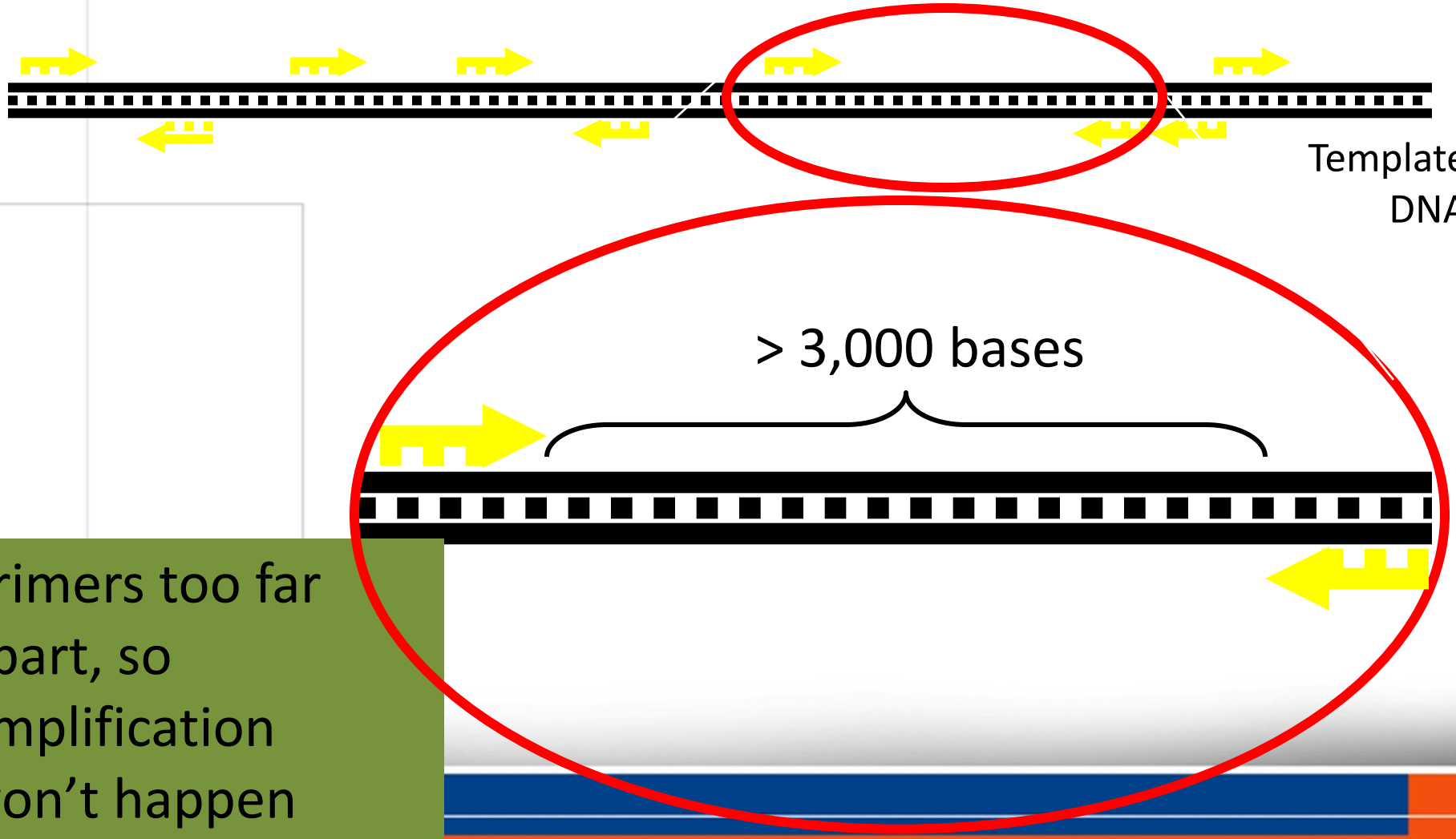


Template DNA



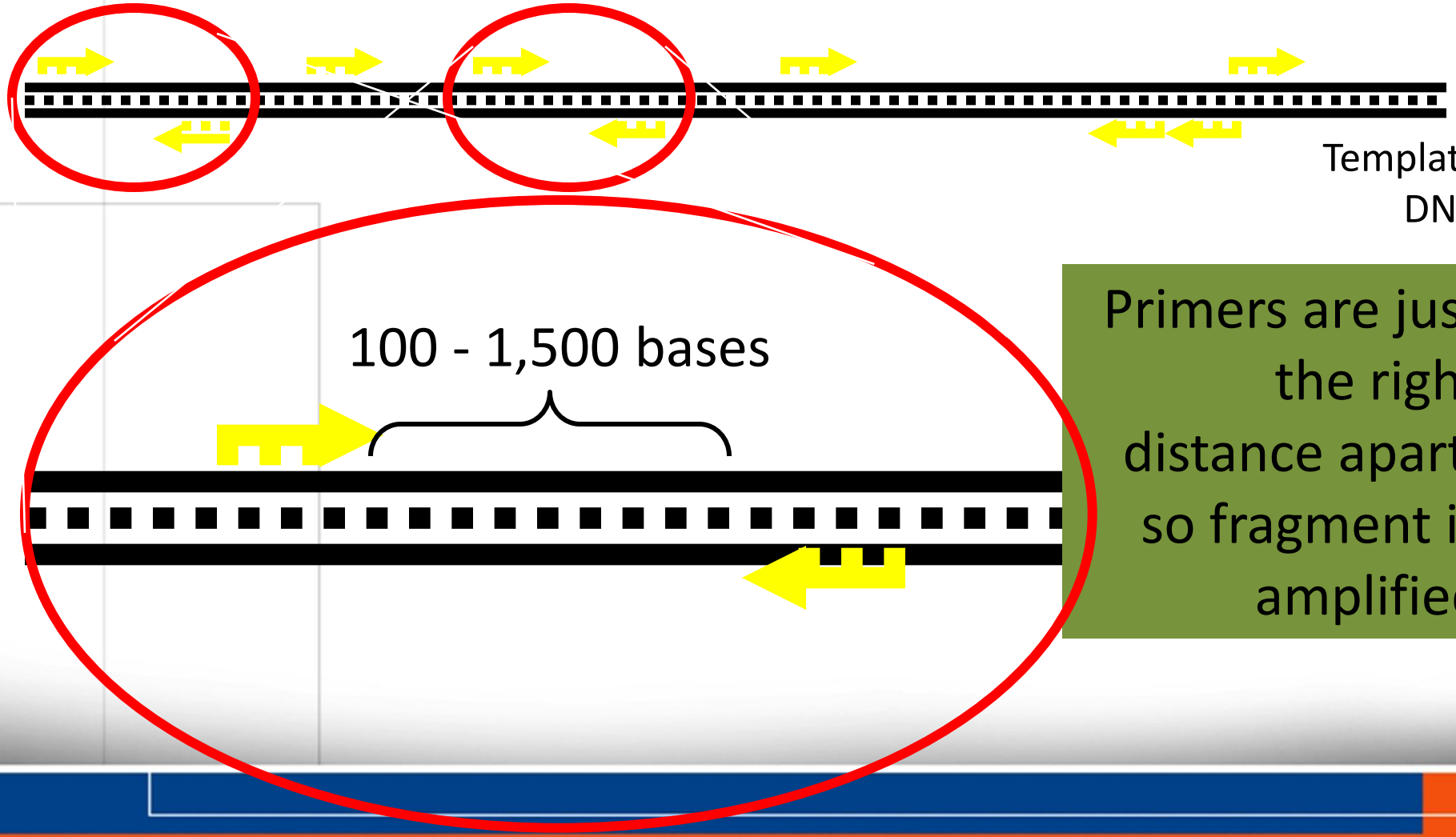
Primers point in the same direction, so amplification won't happen

RAPD



Primers too far apart, so amplification won't happen

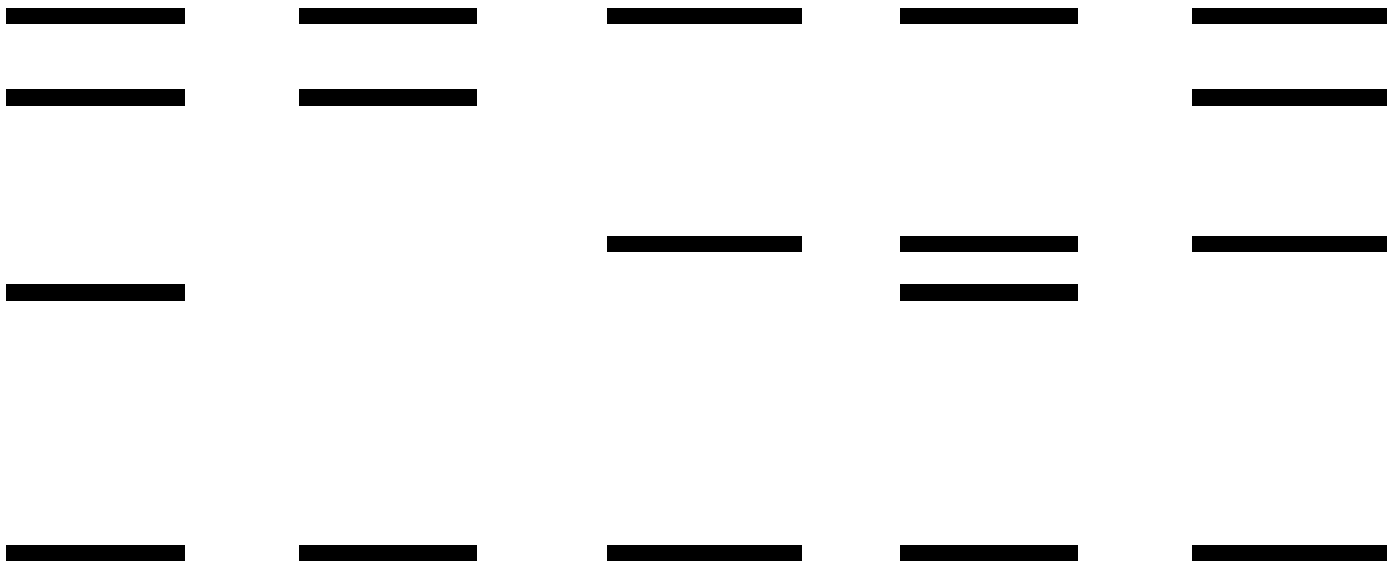
RAPD



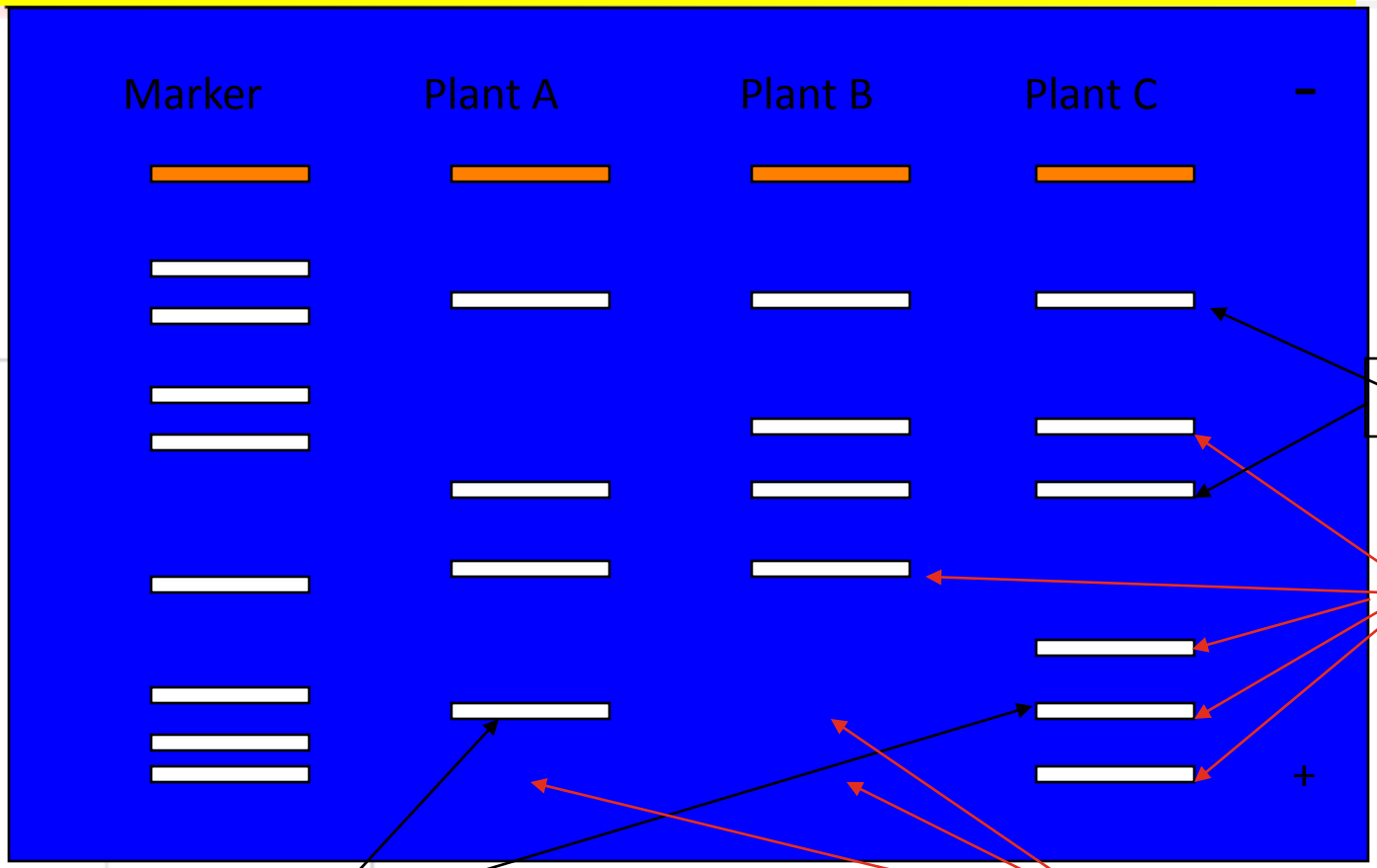
Primers are just the right distance apart, so fragment is amplified

The nature of RAPD polymorphism

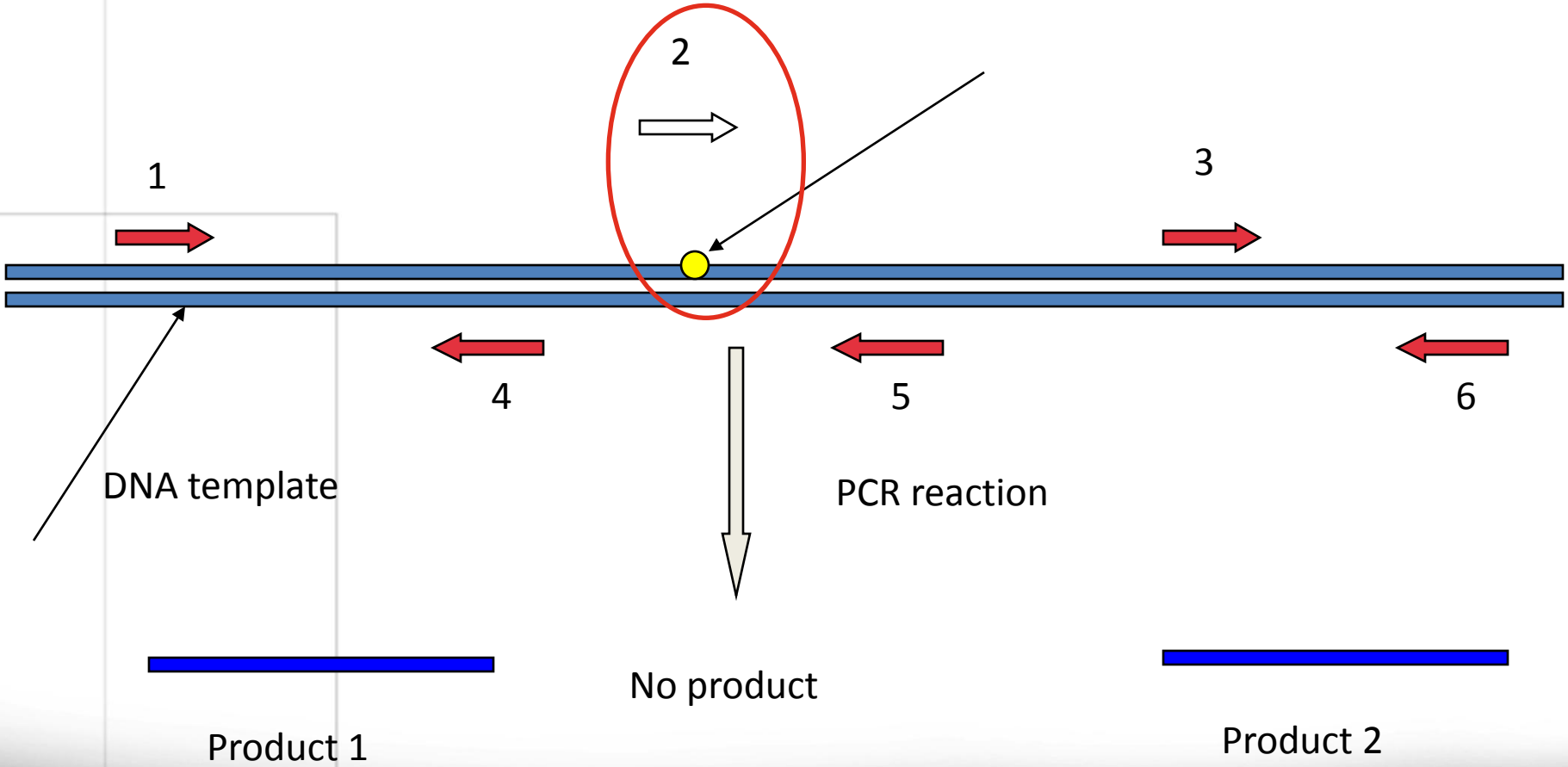
Polymorphic ?



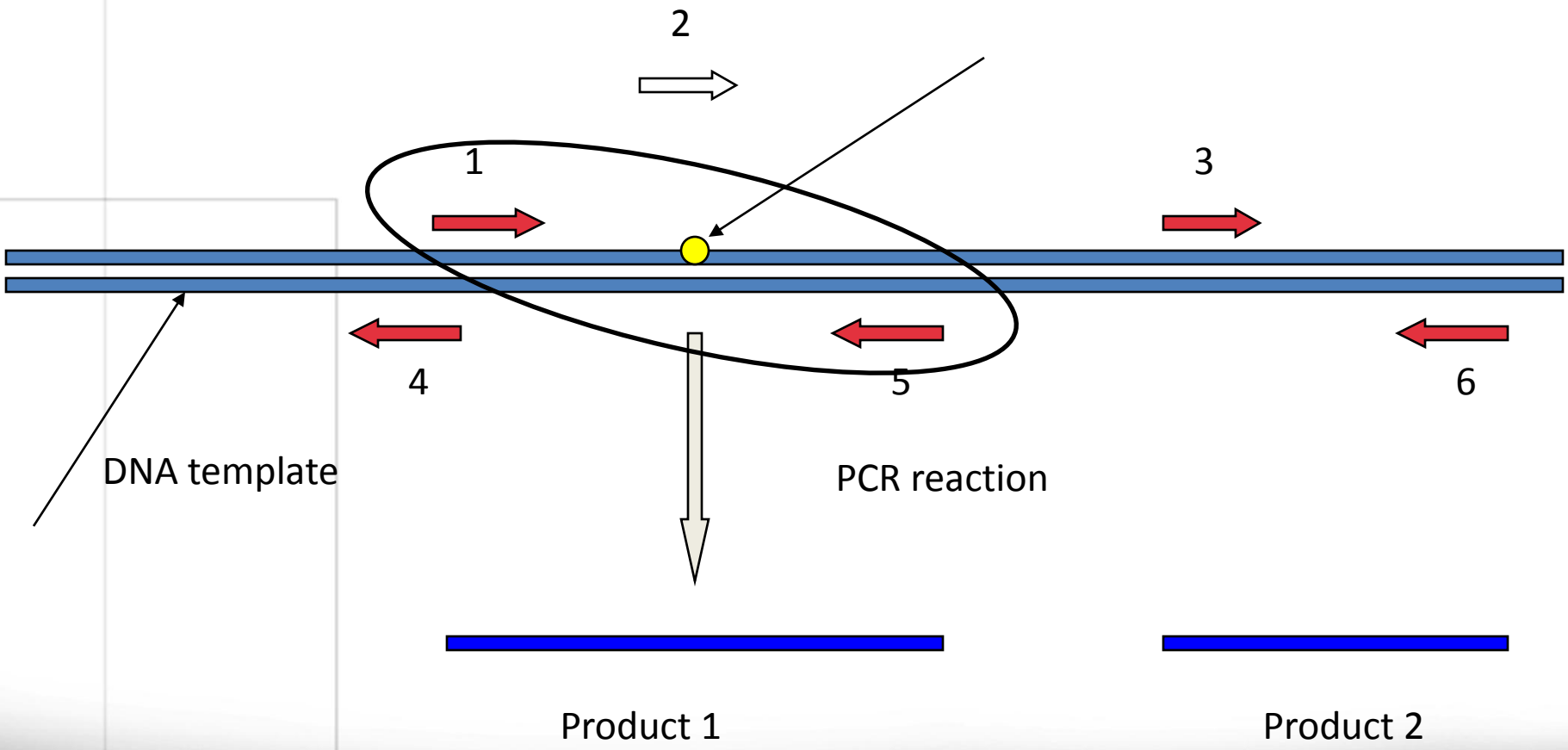
A schematic picture of an agarose gel



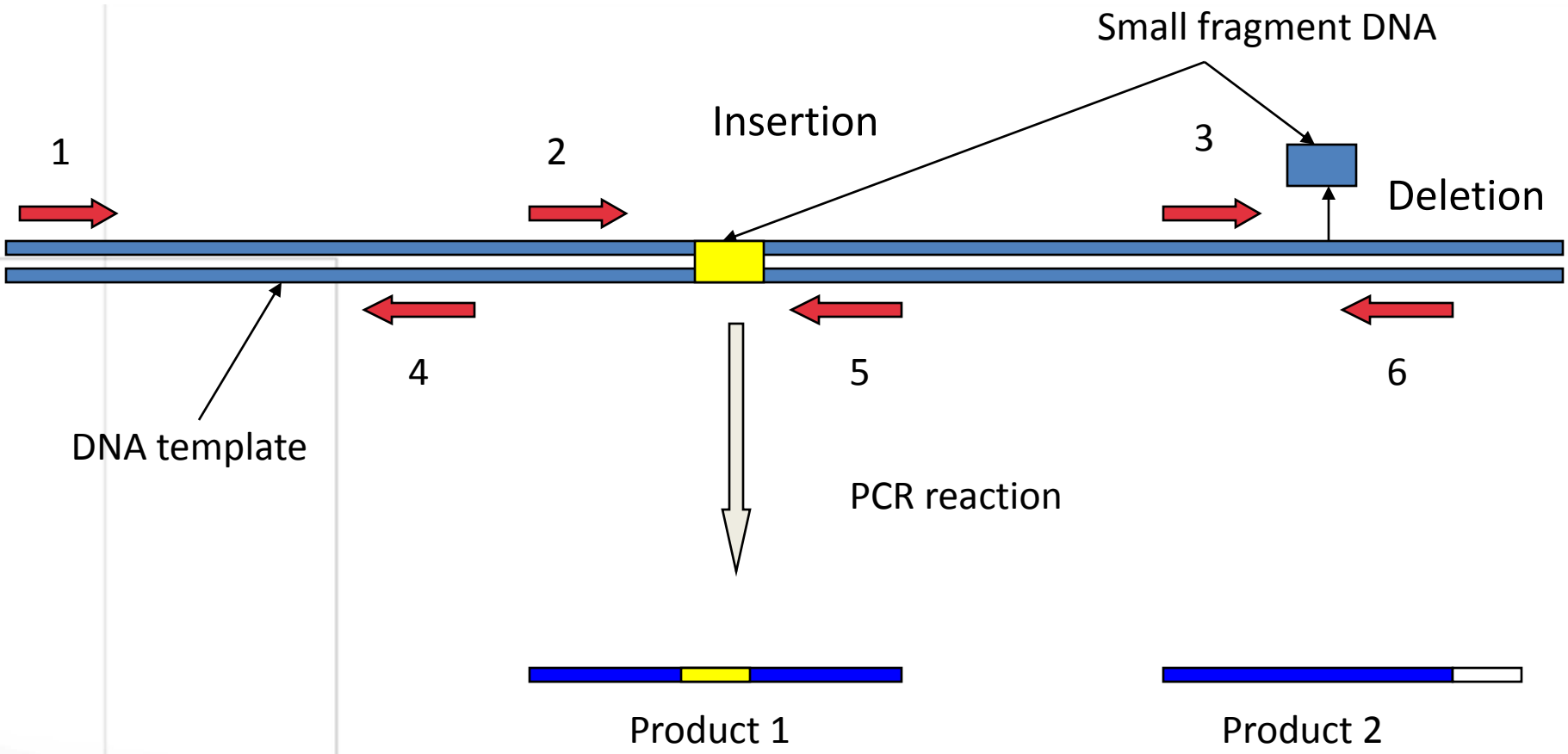
a) nucleotide substitution within target sites may affect the annealing process - either no fragment is detected



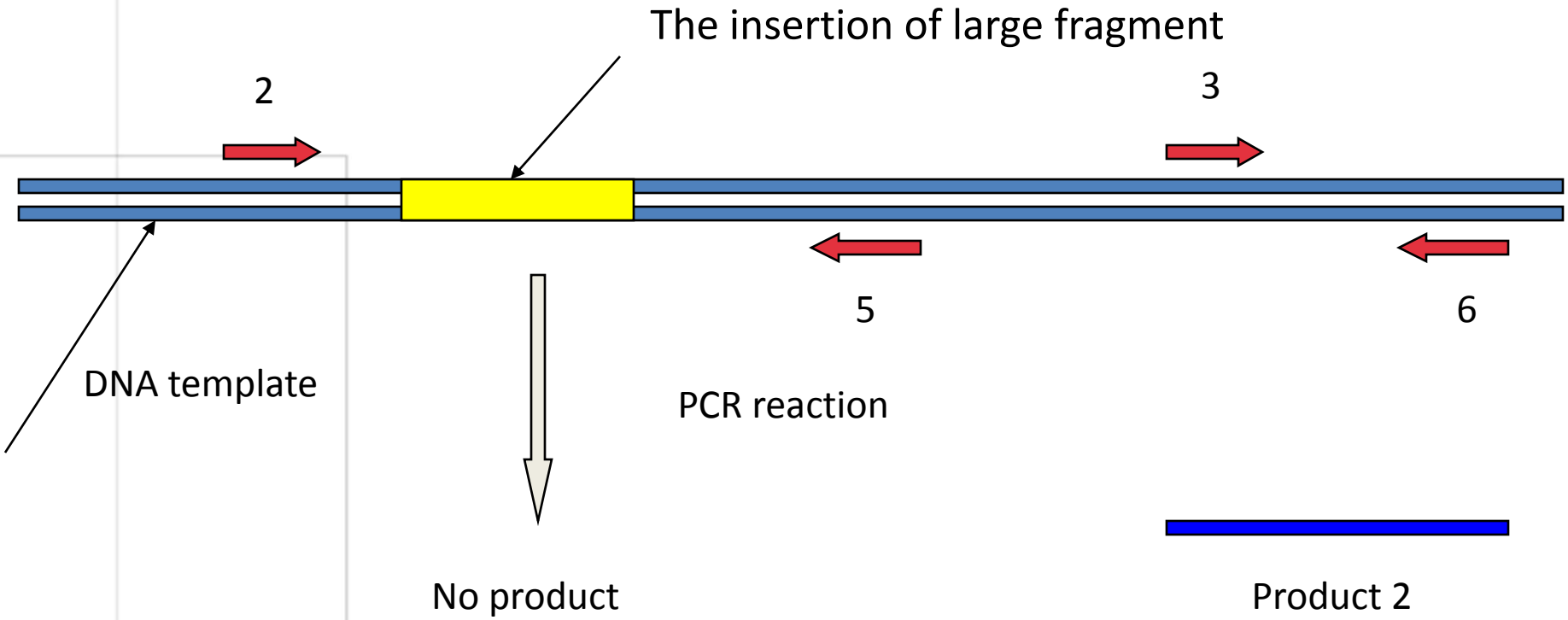
or detected fragment is of increased size



b) insertion or deletion of a small fragment of DNA - the amplified fragments are changed in size

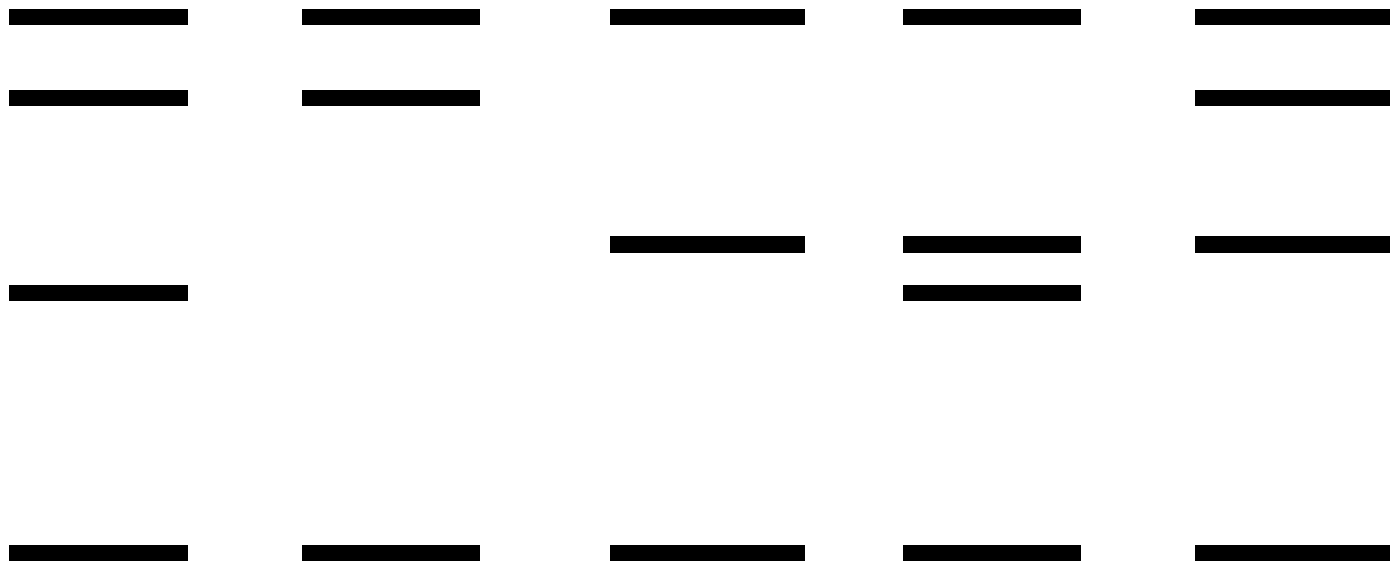


c) insertion of a large piece of DNA between the primer -binding sites may exceed the capacity of PCR - no fragment is detected

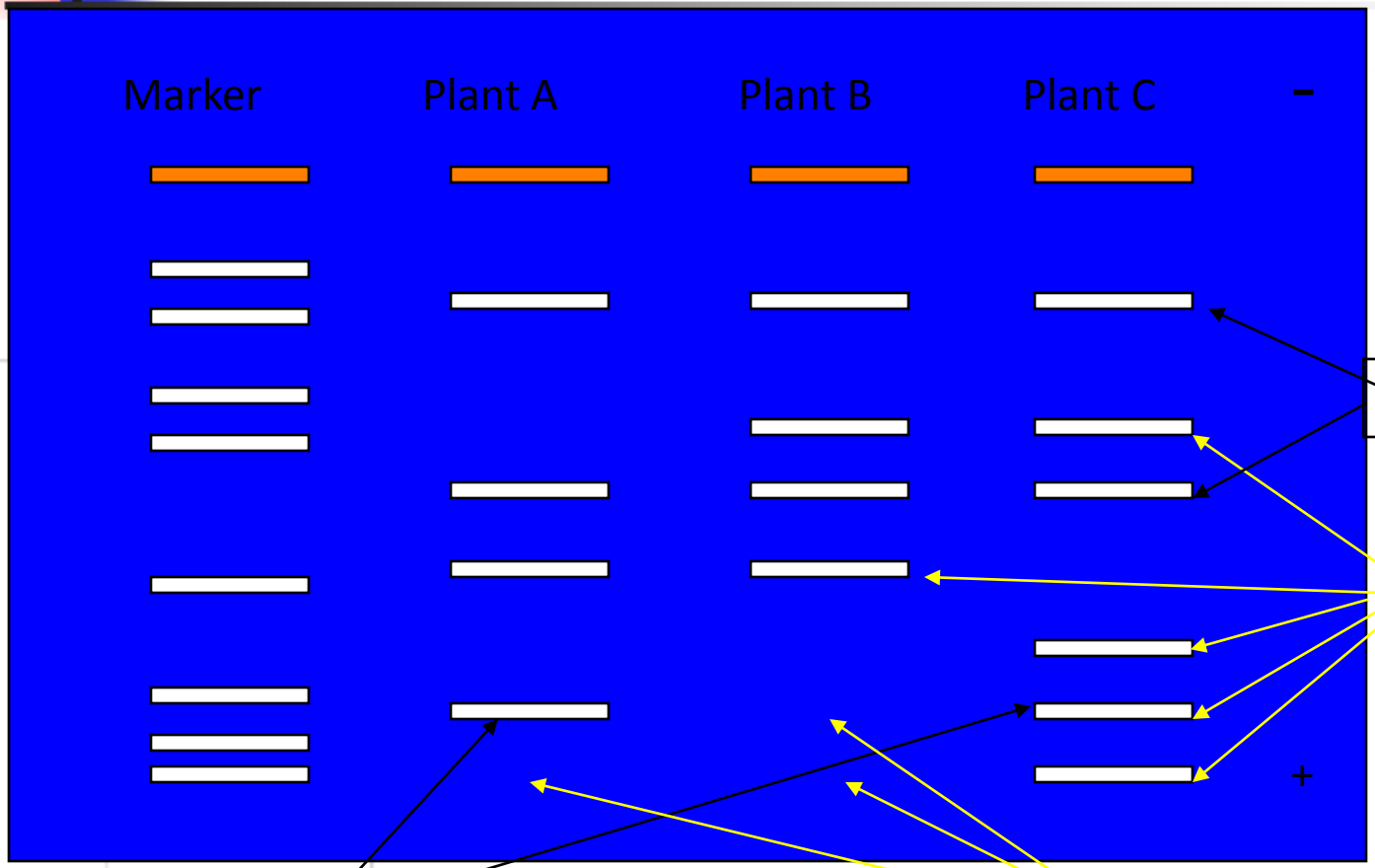


Data analysis

Bands Number; Locus Number



Polymorphic VS Monomorphic



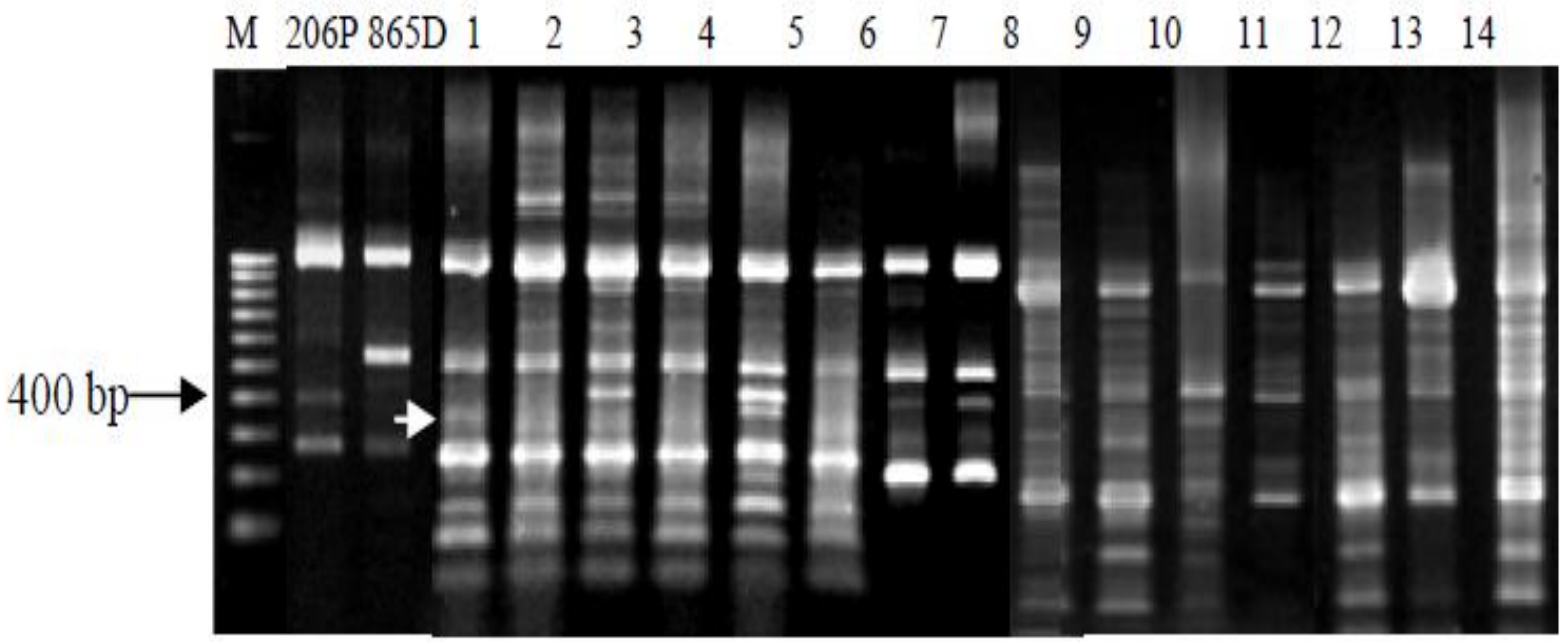
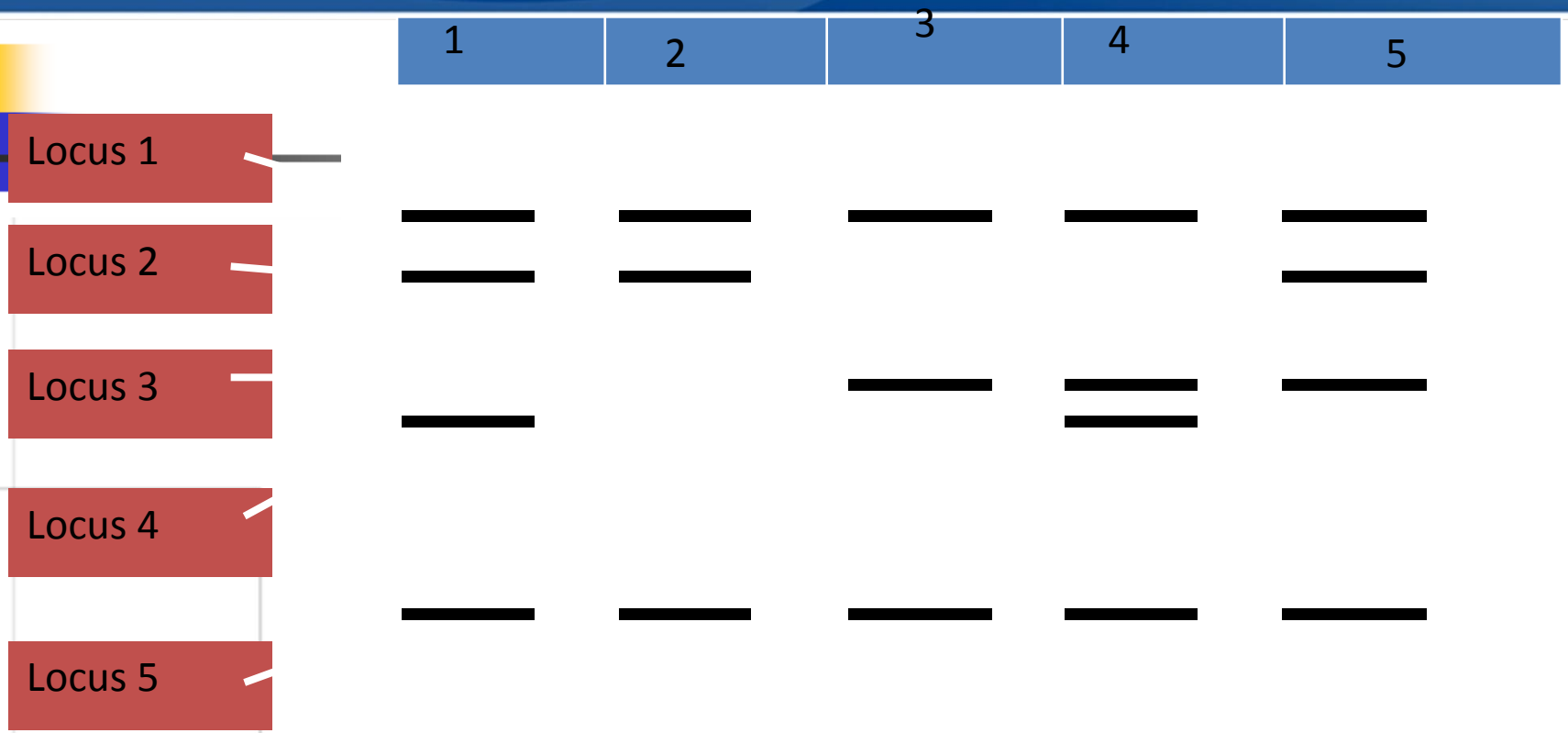


Fig. 3. RAPD patterns in hybrids and parents of cross #119 [865 (D) × 206 (P)]. The amplification products were compared on the basis of molecular size. Lane P and D were fragments from parents. Lane 1-15 were fragments from hybrids, obtained with primers OPT06.

Primer	Sequence fragments	Amplified fragments	Monomorphic fragments	Polymorphic fragments
OPB08	GTCCACACGG	13	5	8
OPT06	CAAGGGCAGA	8	1	7

RAPD bands are treated as independent loci:



	1	2	3	4	5
L1	AA/Aa	AA/Aa	AA/Aa	AA/Aa	AA/Aa
L2	AA/Aa	AA/Aa	aa	aa	AA/Aa
L3	aa	aa	AA/Aa	AA/Aa	AA/Aa
L4	AA/Aa	aa	aa	AA/Aa	aa
L5	AA/Aa	AA/Aa	AA/Aa	AA/Aa	AA/Aa

Binary Data

- RAPD bands are scored for presens ”1” and absens ”0”. Doubtful “999”
- Only clear, consistent and polymorphic bands are usually used to create a binary matrix for future statistical analyses

A binary matrix:

	Band 1	Band 2	Band 3	Band 4	Band 5	Band 6
Plant 1	1	0	0	1	1	1
Plant 2	0	1	0	1	1	1
Plant 3	1	1	1	0	0	0
Plant 4	1	1	0	1	1	1
Plant 5	0	1	0	999	1	1
...	...	...	...	...	...	...
Plant 20	1	0	1	0	0	0

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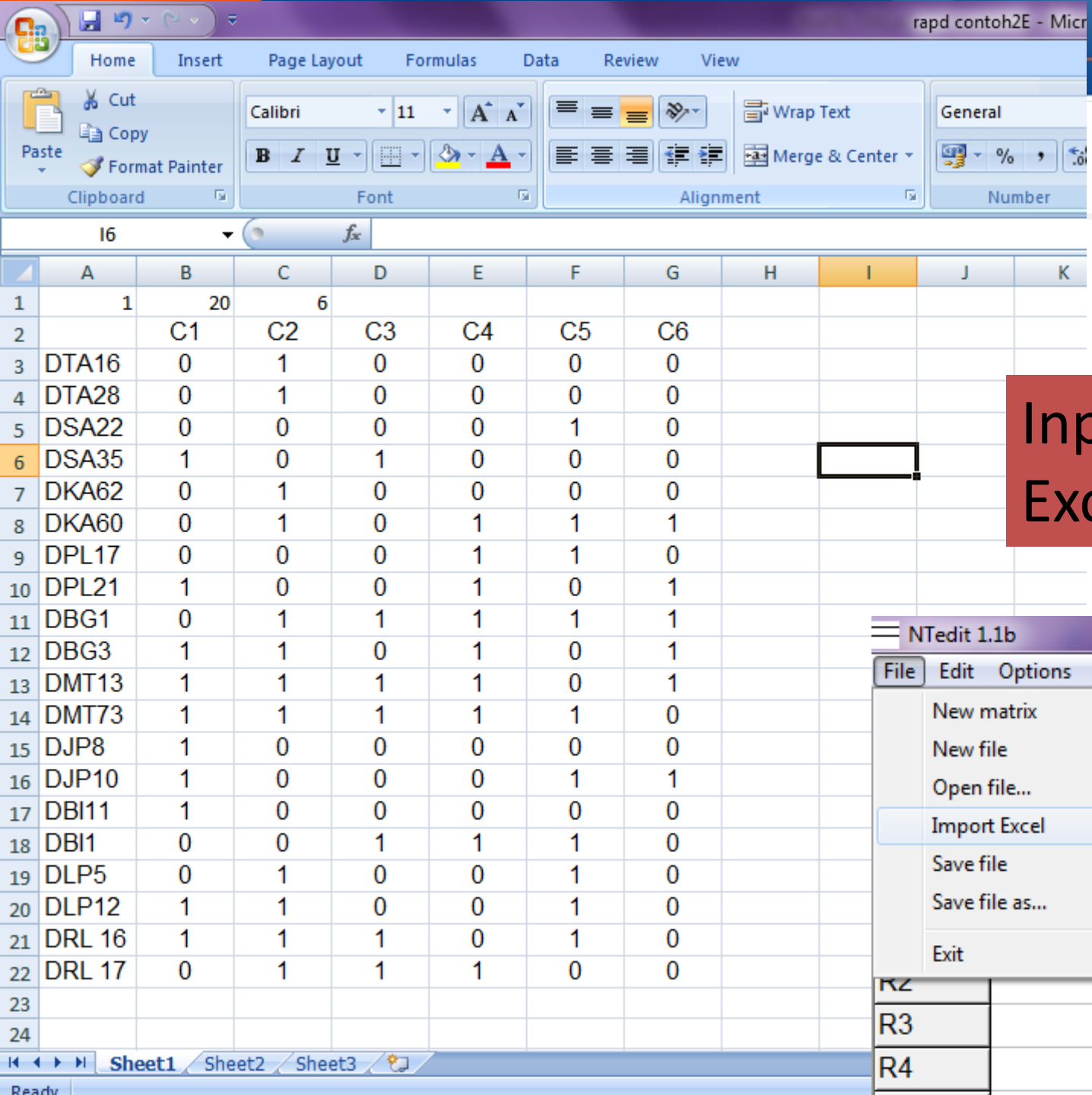
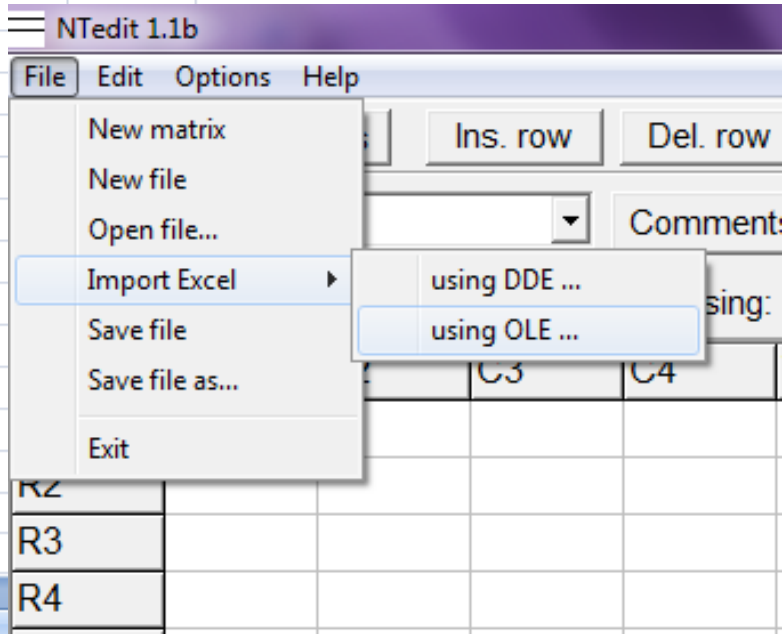
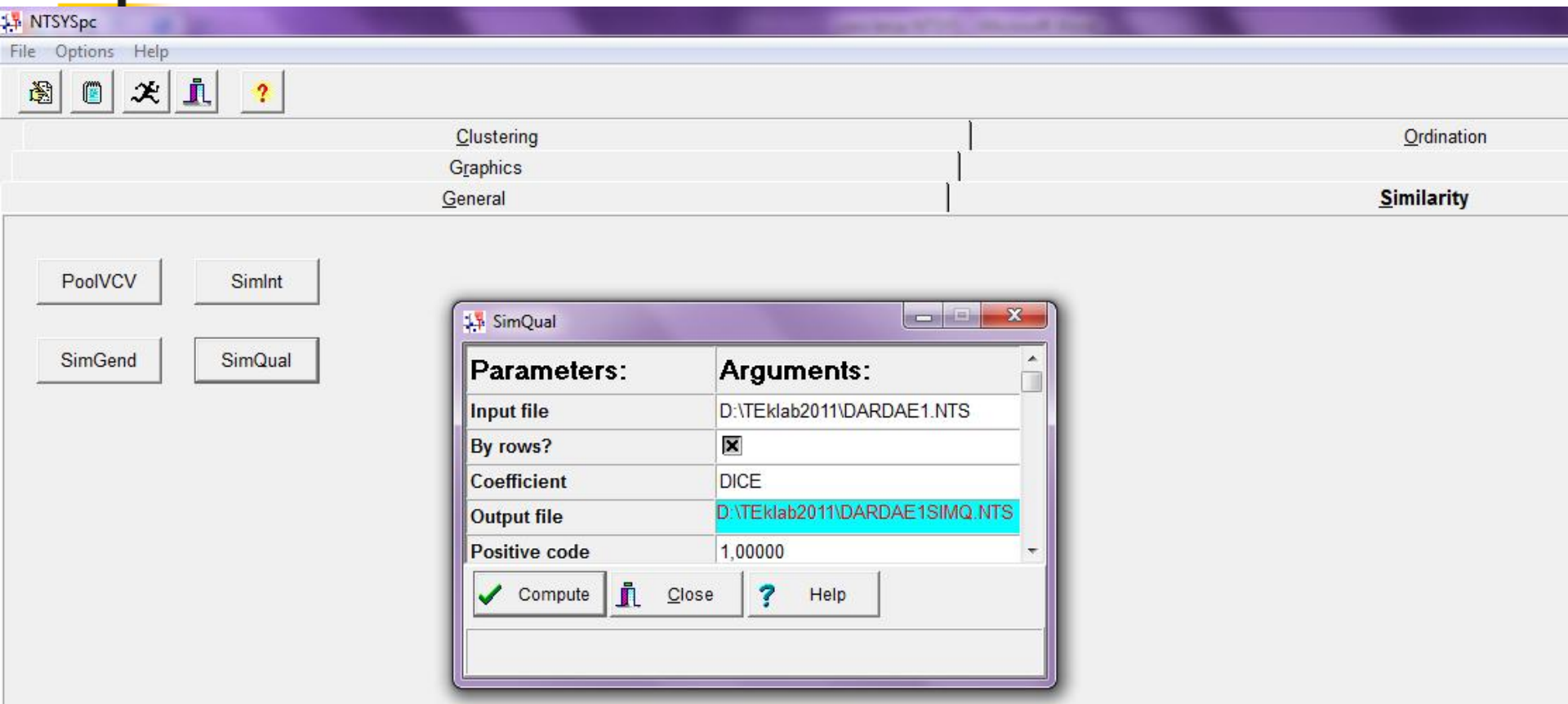
Software Development & IT Management Ltd.

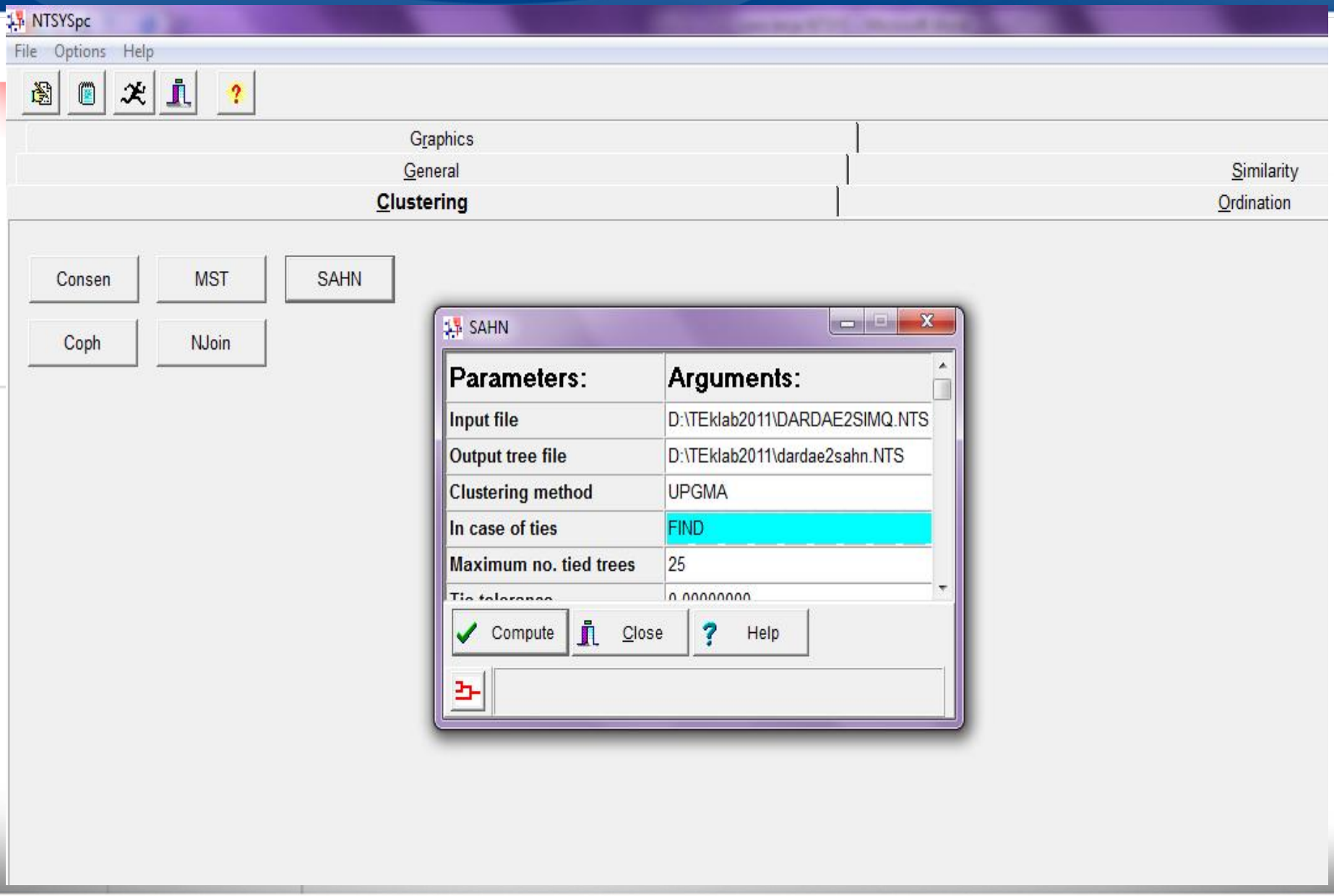


	A	B	C	D	E	F	G
1	1	20	6				
2		C1	C2	C3	C4	C5	C6
3	DTA16	0	1	0	0	0	0
4	DTA28	0	1	0	0	0	0
5	DSA22	0	0	0	0	1	0
6	DSA35	1	0	1	0	0	0
7	DKA62	0	1	0	0	0	0
8	DKA60	0	1	0	1	1	1
9	DPL17	0	0	0	1	1	0
10	DPL21	1	0	0	1	0	1
11	DBG1	0	1	1	1	1	1
12	DBG3	1	1	0	1	0	1
13	DMT13	1	1	1	1	0	1
14	DMT73	1	1	1	1	1	0
15	DJP8	1	0	0	0	0	0
16	DJP10	1	0	0	0	1	1
17	DBI11	1	0	0	0	0	0
18	DBI1	0	0	1	1	1	0
19	DLP5	0	1	0	0	1	0
20	DLP12	1	1	0	0	1	0
21	DRL 16	1	1	1	0	1	0
22	DRL 17	0	1	1	1	0	0
23							
24							

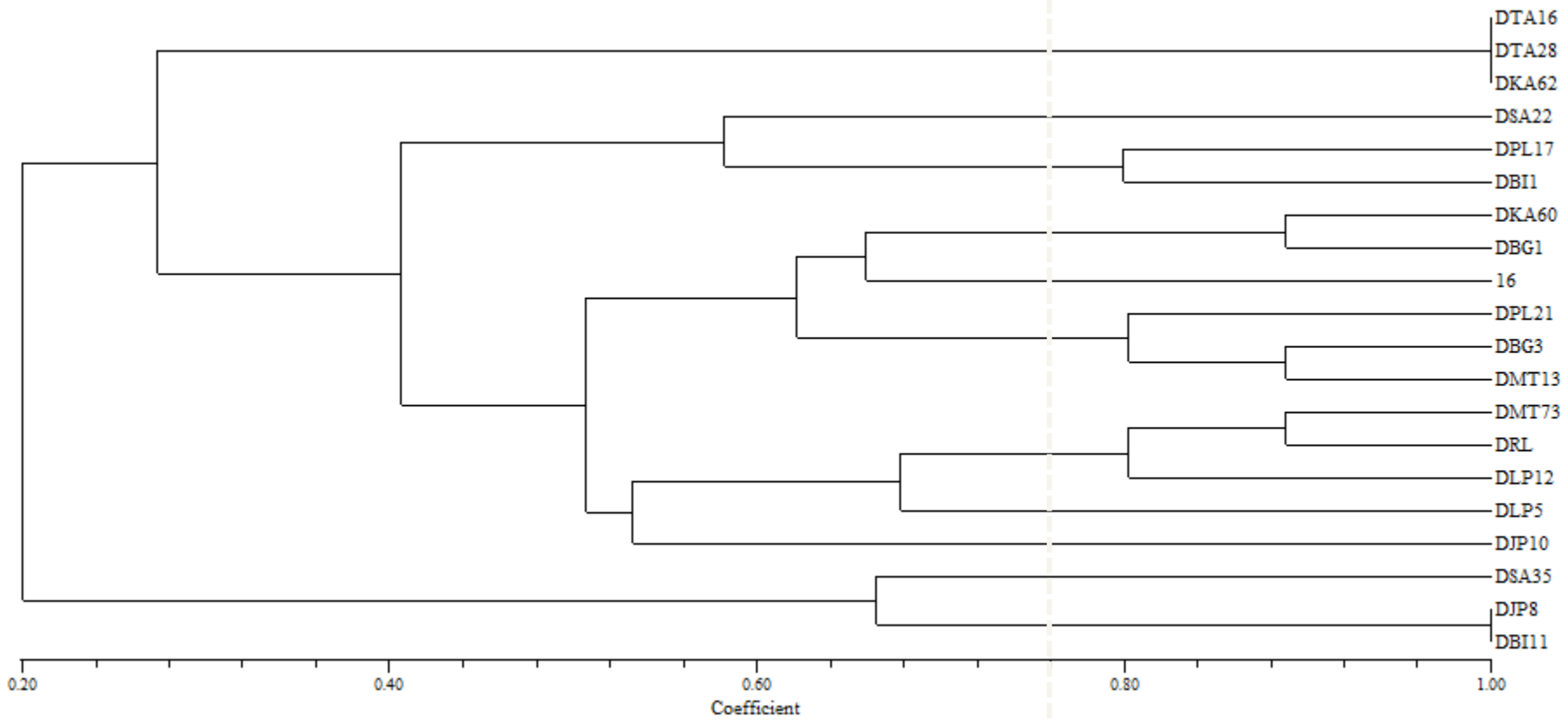
Input data di Excel




NTSYSpc
File Options Help
Clustering
Ordination
Graphics
General
Similarity
PoolVCV
SimInt
SimGend
SimQual
SimQual
Parameters:
Arguments:
Input file
D:\TEklab2011\DARDAE1.NTS
By rows?
Coefficient
DICE
Output file
D:\TEklab2011\DARDAE1SIMQ.NTS
Positive code
1,00000
Compute
Close
Help


NTSYSpc
File Options Help
Graphics
General
Similarity
Clustering
Ordination
Consen
MST
SAHN
Coph
NJoin
SAHN
Parameters:
Arguments:
Input file
D:\TEklab2011\DARDAE2SIMQ.NTS
Output tree file
D:\TEklab2011\dardae2sahn.NTS
Clustering method
UPGMA
In case of ties
FIND
Maximum no. tied trees
25
Compute
Close
Help

Dendrogram based on UPGMA analysis (Jaccard's coefficient) for RAPD data



Similarity %

PRINCIPLE OF AFLP

- The AFLP technique is based on the principle of selectively amplifying a subset of restriction fragments from a complex mixture of DNA fragments obtained after digestion of genomic DNA with restriction endonucleases.

Amplified Fragment Length Polymorphism (AFLP)

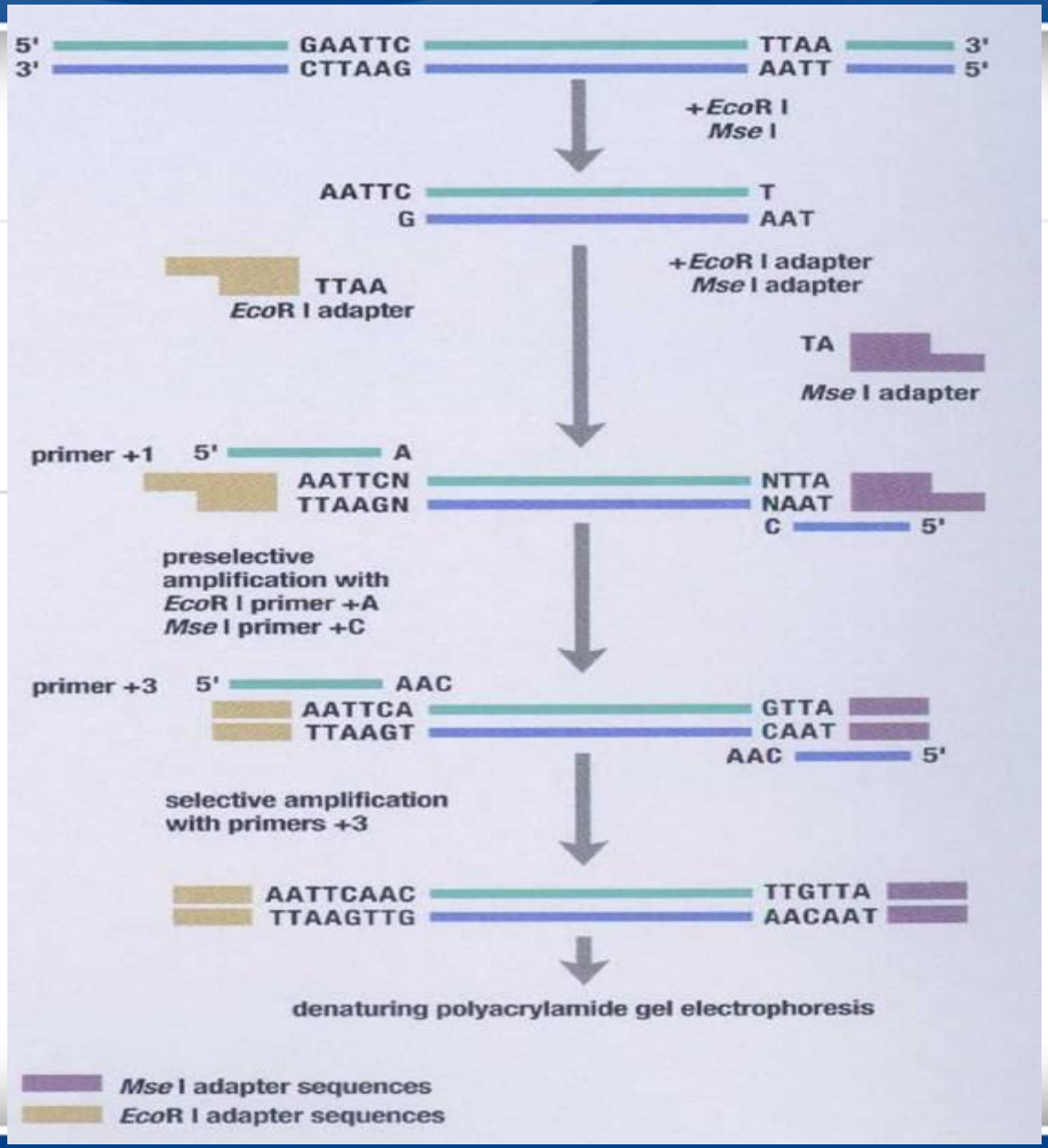
- Restriction endonuclease digestion of DNA
- Ligation of adaptors with PCR adaptor
- Amplification of ligated fragments
- Separation of the amplified fragments via electrophoresis and visualization
- AFLPs have stable amplification and good repeatability

CHARACTERISTICS OF AFLP -

- dominant marker.
- DNA variation is detected by presence/absence of DNA bands due to:

a) presence/absence of restriction sites

b) additional bases (insertion) between two restriction sites are too large


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

Sequence Characterized Amplified Region (SCAR)

- SCARs are DNA fragments amplified by the PCR using specific 15-30 bp primers, designed from nucleotide sequences established from cloned RAPD fragments linked to a trait of interest.
- By using longer PCR primers, SCARs do not face the problem of low reproducibility generally encountered with RAPDs

Strengths

- The main advantage of SCARs is that they are quick and easy to use. In addition, SCARs have a high reproducibility and are locus-specific. Due to the use of PCR, only low quantities of template DNA are required

Applications

SCARs are locus specific and have been applied in gene mapping studies and marker assisted selection

Weaknesses

Disadvantages include the need for sequence data to design the PCR primers

Introduction **DNA barcoding**

- DNA barcoding is a technology using gene sequences to differentiate species, similar to the way retail stores rely on short, standardized barcodes to differentiate the hundreds of thousands of items they sell

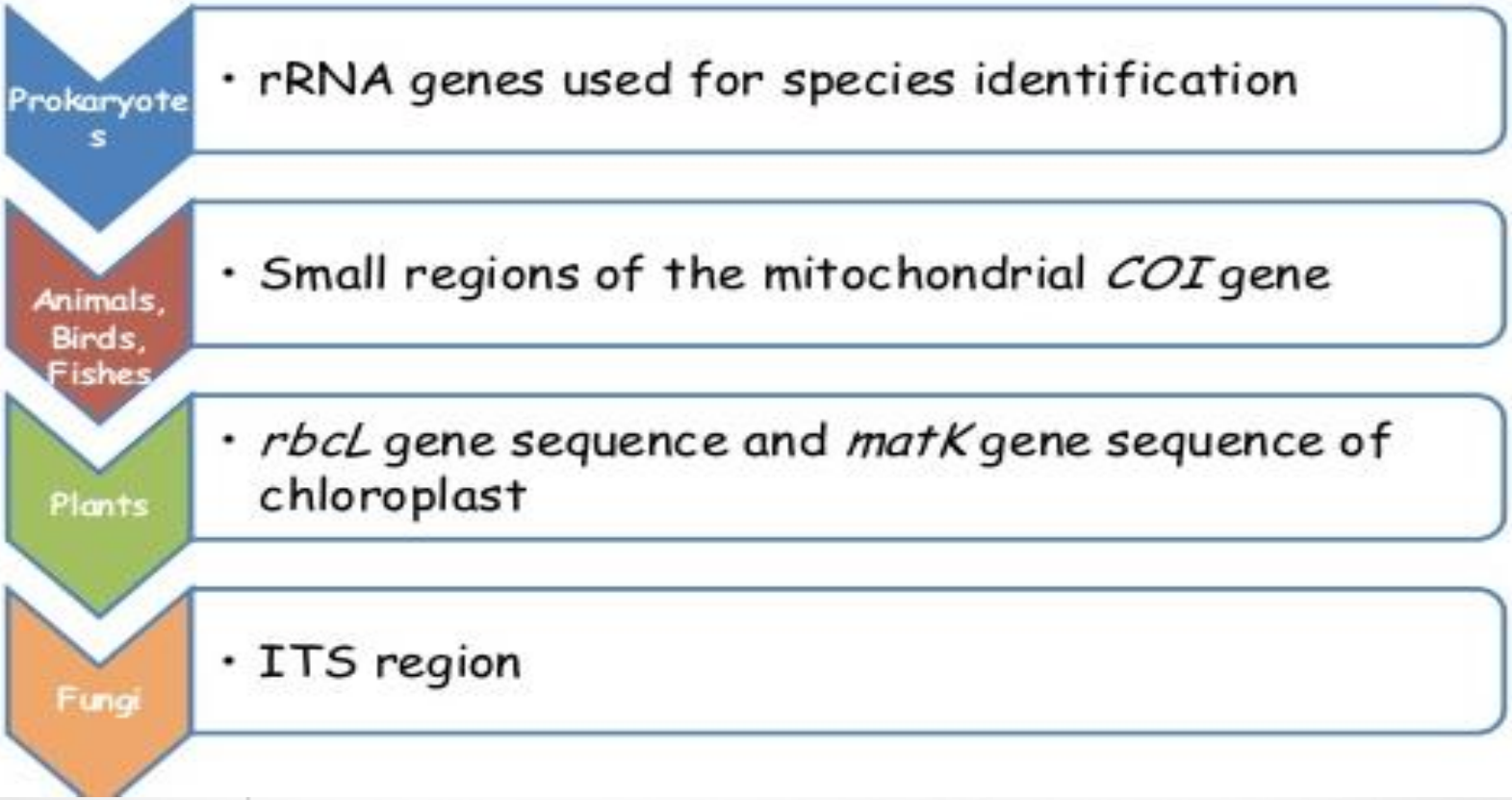
What???

- DNA barcoding is a technique in which species identification is performed by using DNA sequences from a small fragment of the genome,
- with the aim of contributing to a wide range of ecological and conservation studies in which traditional taxonomic identification is not practical
- Short DNA sequences are called as DNA barcodes- ranging from 400- 800 bp.

Successful DNA barcode

- a) Short enough to be quickly sequenced,
- b) Easily identified in all species of organisms.
- c) Variable enough to provide a unique sequence for each species
- d) Size of sequence : 600 – 700 bp
- e) Universality

Which gene fragments?

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