RAPD PCR

Random amplification of polymorphic DNA

By

Assist. Prof. Ali Aldeewan

RAPD reactions are PCR reactions but they amplify segments of DNA which are essentially unknown to the scientist (Random).

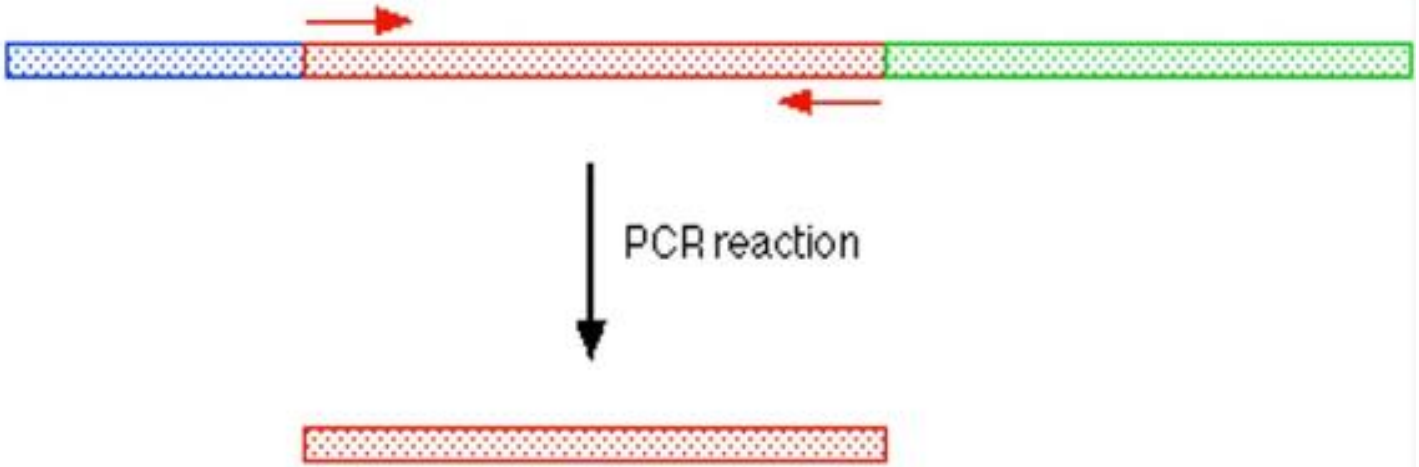
Often, PCR is used to amplify a known sequence of DNA.

Thus, the scientists chooses the sequence he or she wants to amplify, then designs and makes primers which will anneal to sequences flanking the sequence of interest for the amplification of a particular segment of DNA.

PCR

This DNA fragment contains 3 genes. A scientist is interested in amplifying only *gene B*:

The scientist prepares 2 primers which will anneal to each end of *gene B*:



Only *gene B* is amplified, and can then be purified for further analysis.

However, in RAPD analysis, the target sequence(s) (to be amplified) unknown. The scientist will design primer with an **arbitrary** sequence.

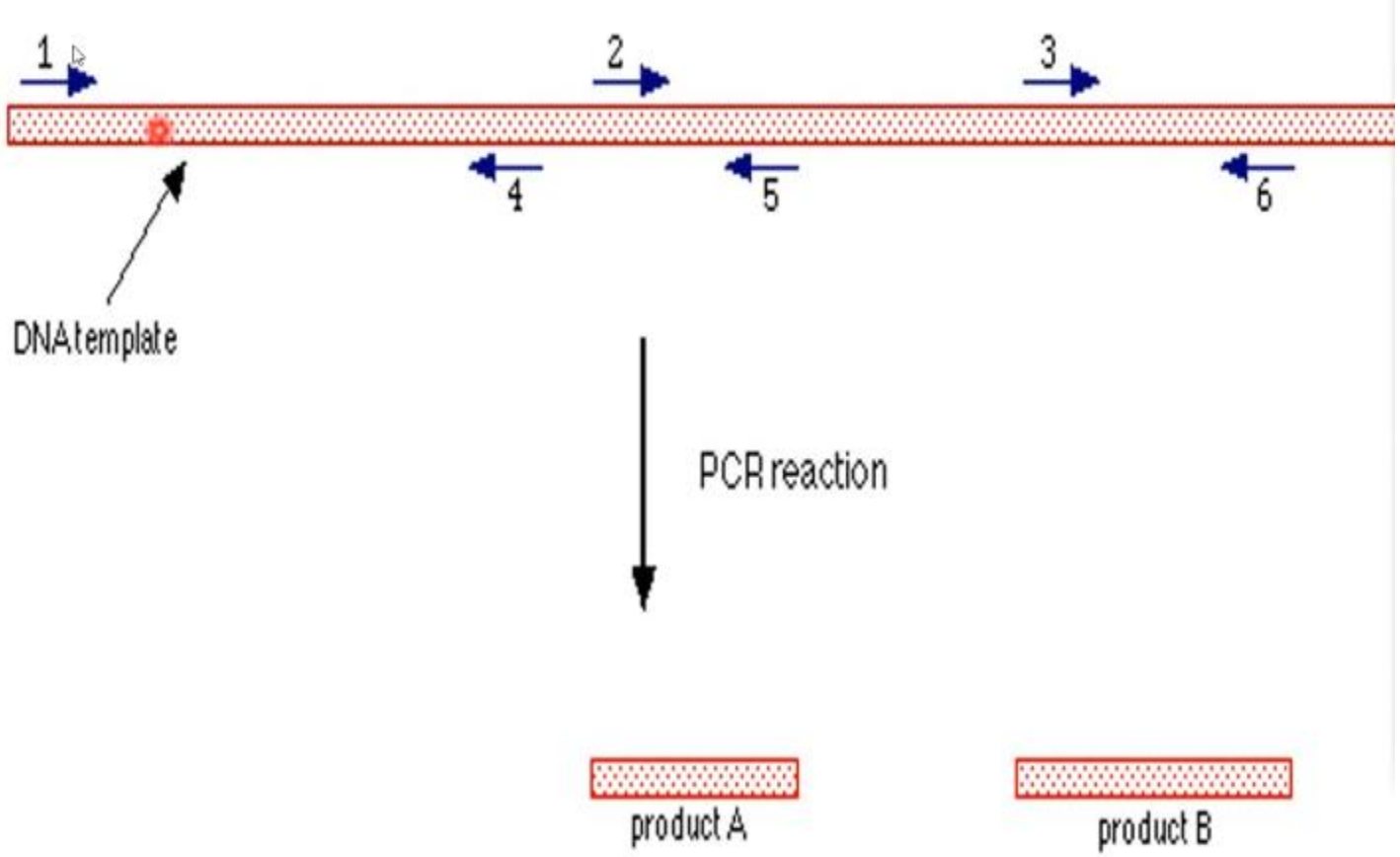
In other words, the scientist simply makes up a 10 base pair sequence (or may have a computer randomly generate a 10 bp sequence), then synthesizes the primer.

The scientist then carries out a PCR reaction and runs an agarose gel to see if any DNA segments were amplified in the presence of the arbitrary primer.

Remember! In order for PCR to occur:

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

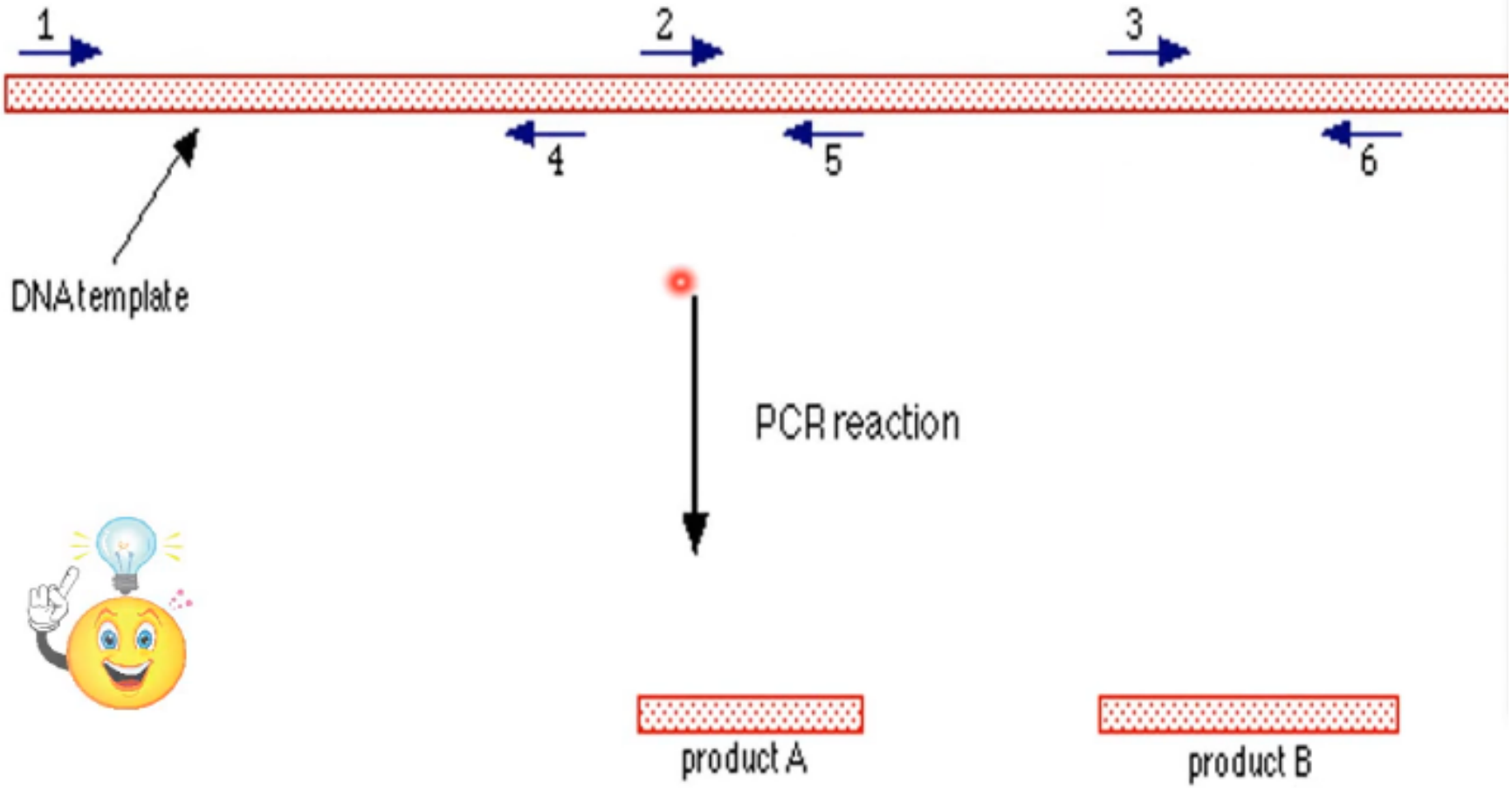
In this figure which depicts a RAPD reaction, a large fragment of DNA is used the template in a PCR reaction containing many copies of a single arbitrary primer

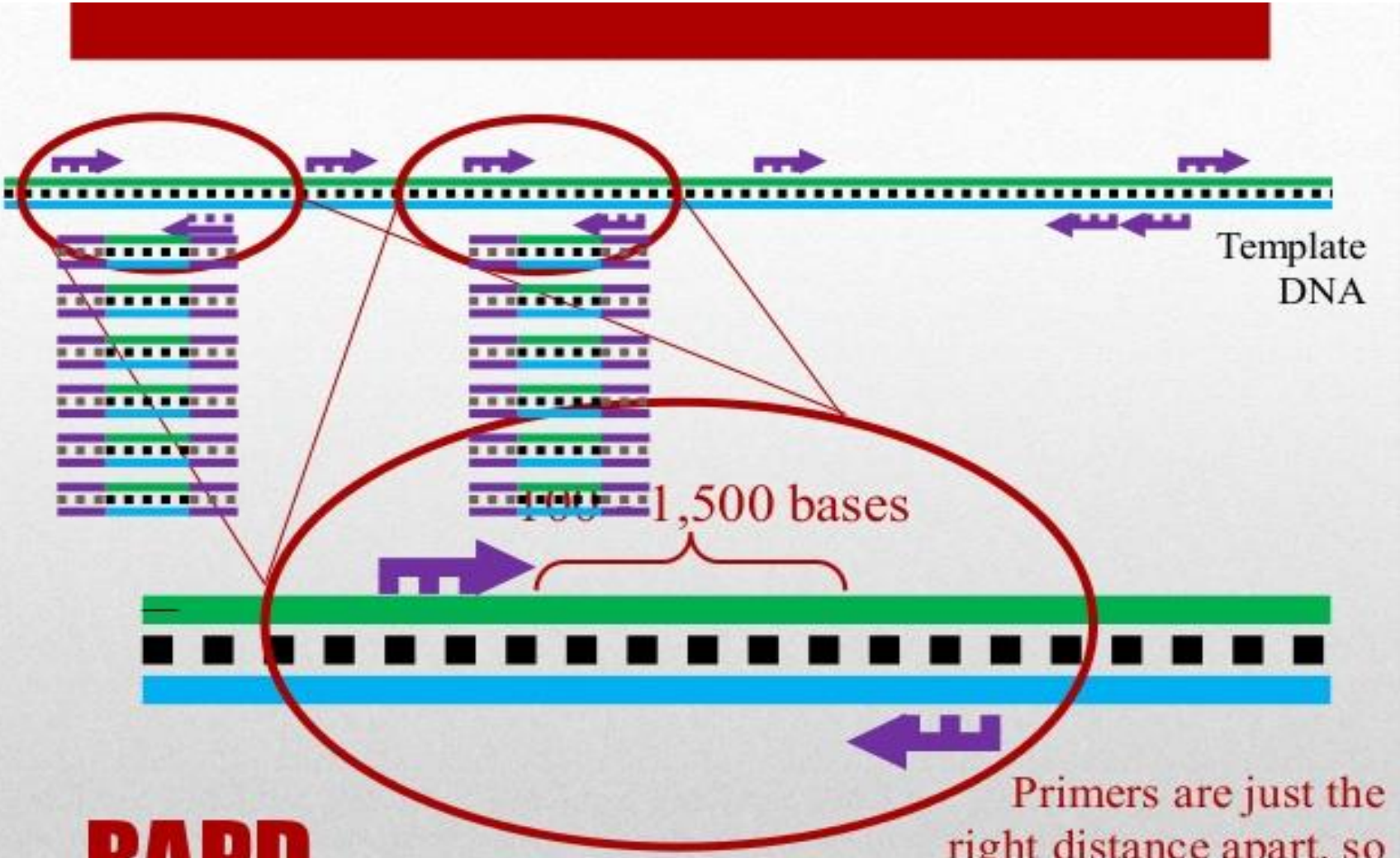


In this example, only 2 RAPD PCR product

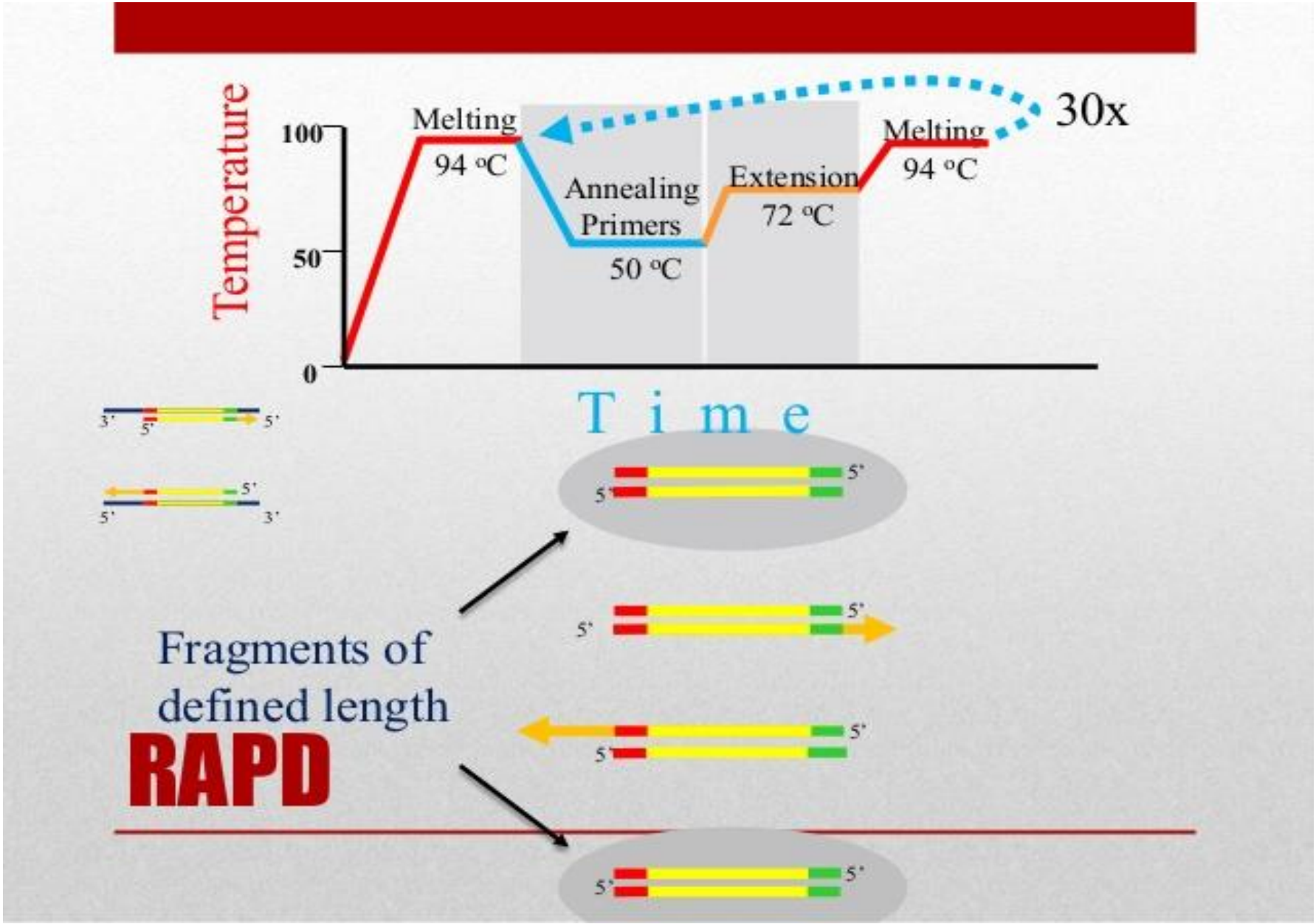
1) Product A is produced by PCR amplification of the DNA sequence which lies in between the primers bound at positions 2 and 5.

2) Product B is the produced by PCR amplification of the DNA sequence which lies in between the primers bound at positions 3 and 6.




Template
DNA
1,500 bases
RAPD
Primers are just the
right distance apart, so
fragment is amplified


template DNA
heat PCR products to pull the strands apart
(melt or denature the DNA)
top strand
primer # 1
primer # 2
bottom strand
add DNA polymerase
and nucleotide subunits
primer # 1
primer # 2
heat PCR products to pull the strands apart
(melt or denature the DNA)
primer # 1
primer # 2
anneal more primers
primer # 2
primer # 1
primer # 2
primer # 1

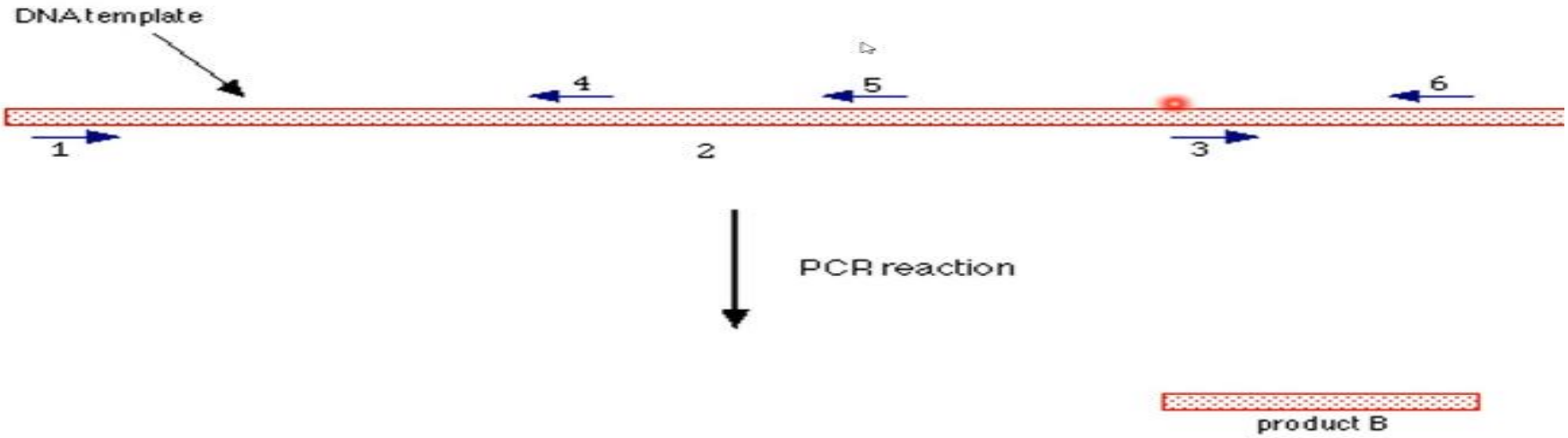

Temperature
100
50
0
Melting
94 °C
Annealing
Primers
50 °C
Extension
72 °C
Melting
94 °C
30x
T i m e
3'
5'
5'
5'
5'
3'
5'
5'
5'
5'
5'
5'
5'
5'
Fragments of
defined length
RAPD

Finding Differences Between Genomes Using RAPD Analysis

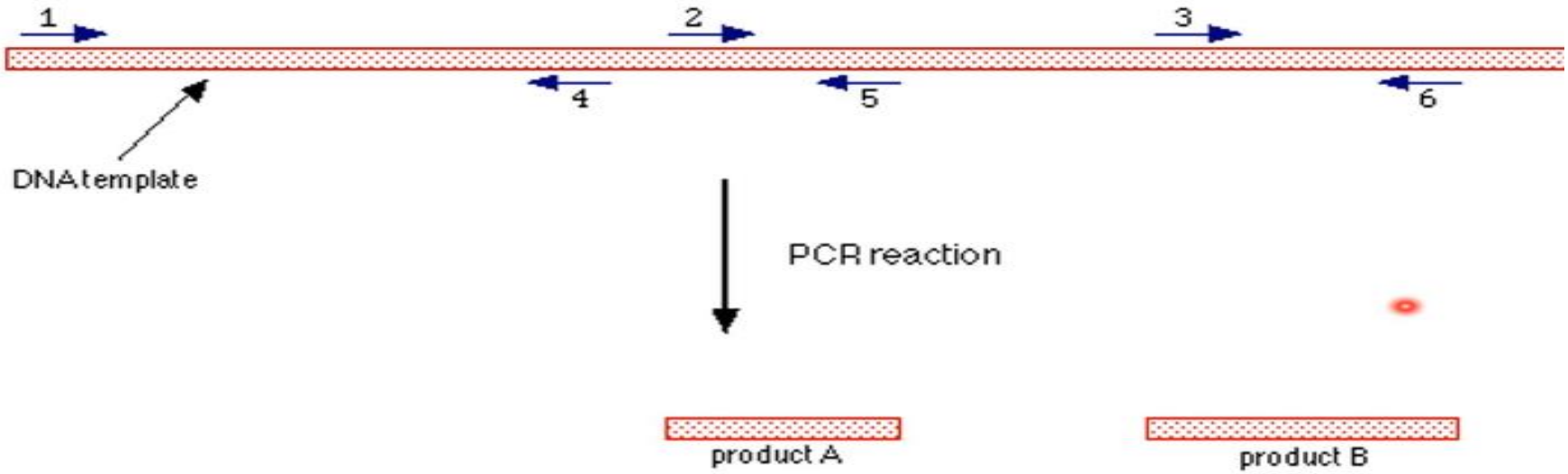
- Consider the figure above.

- If another DNA template (genome) was obtained from a different (yet related) source there would probably be some differences in the DNA sequence of the two templates.

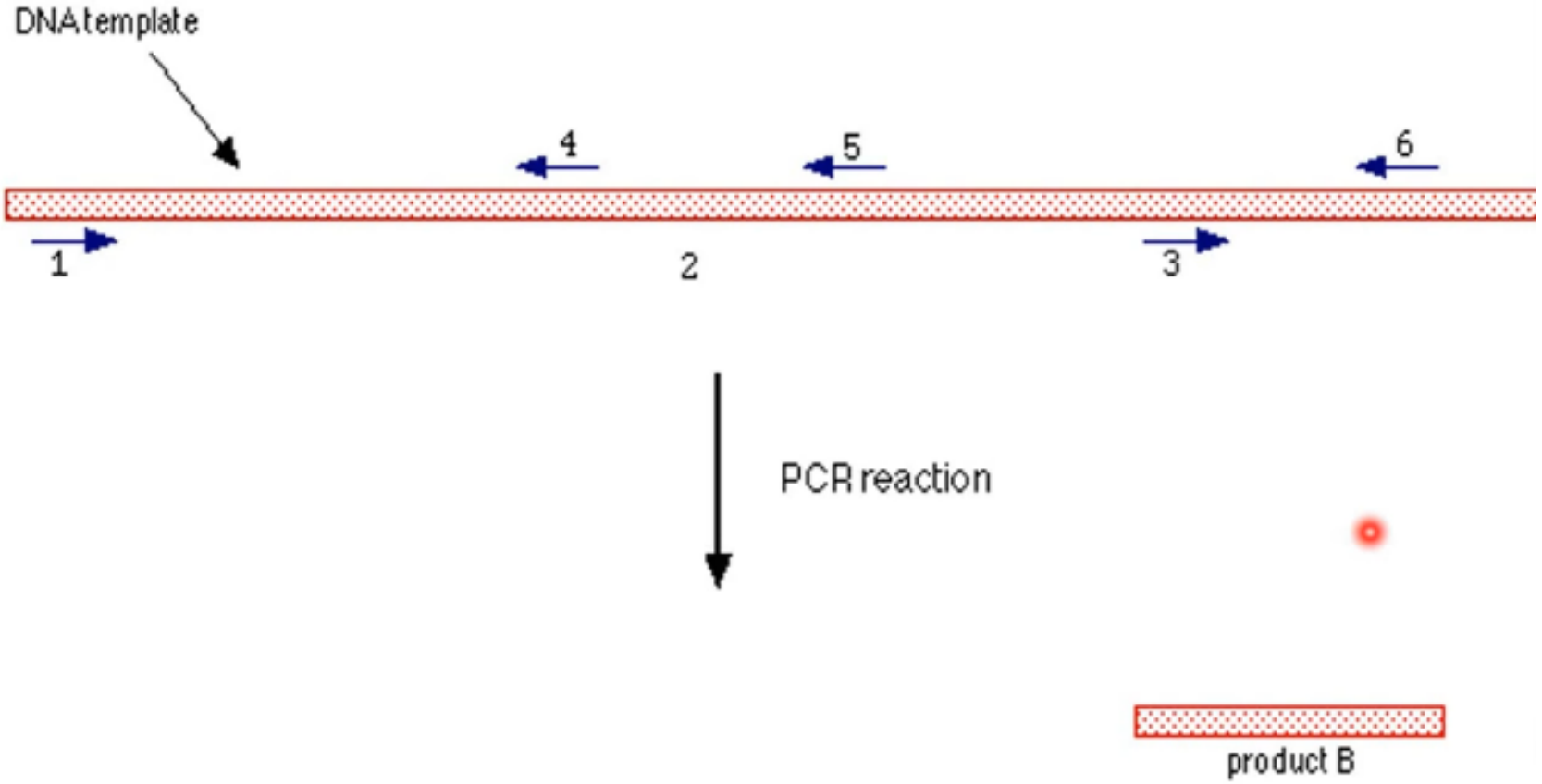
Sample 2

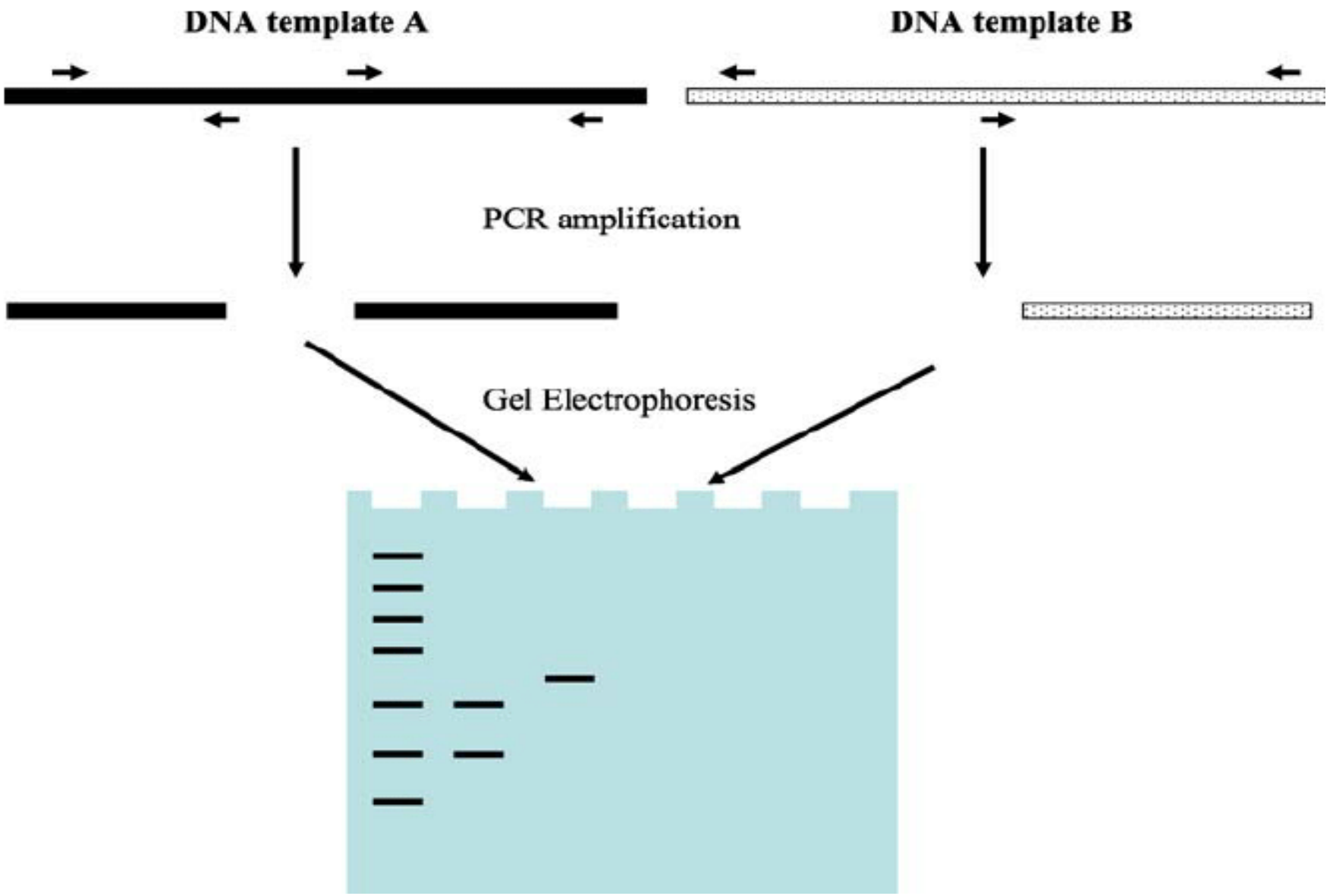


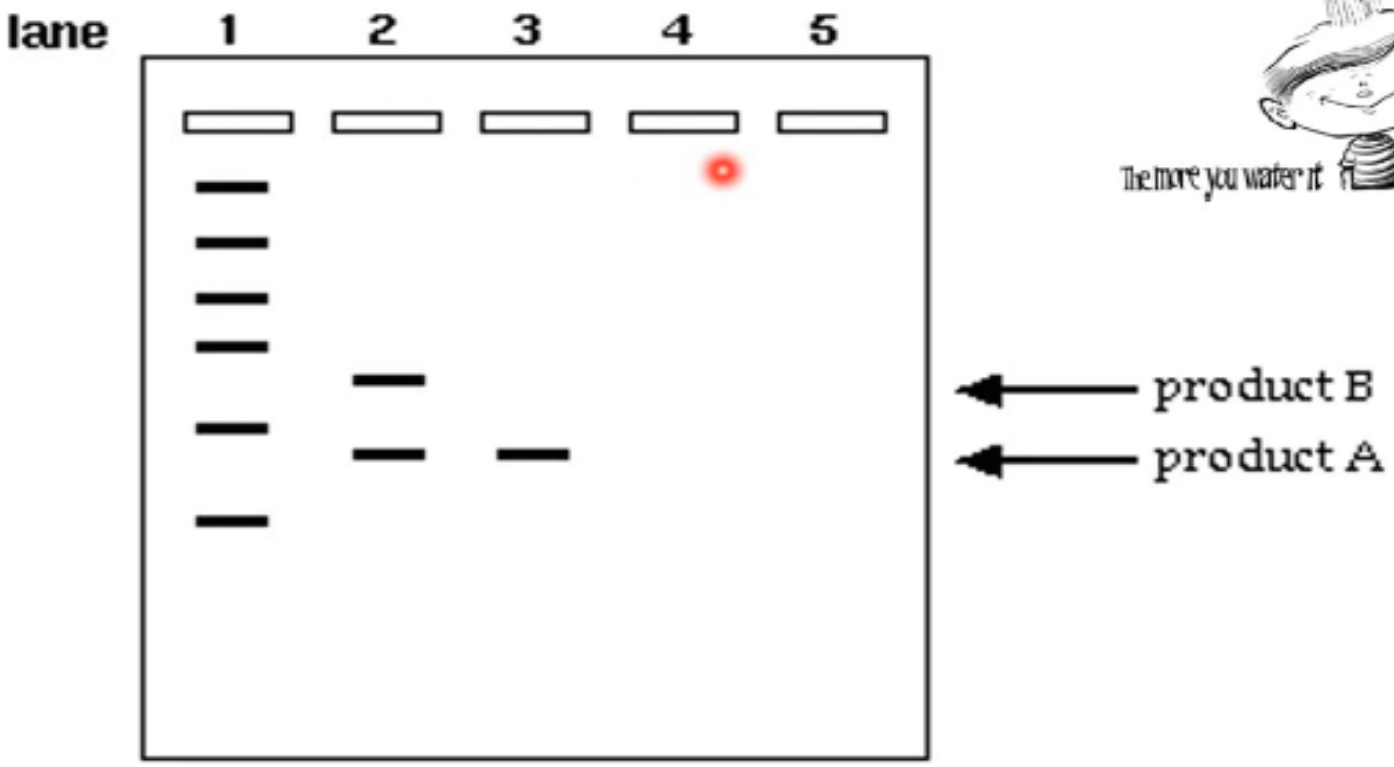
Sample 1



Suppose there was a change in sequence at primer annealing site #2:
As shown in this figure, the primer is no longer able to anneal to site #2, and thus the PCR product A is not produced. Only product B is produced.



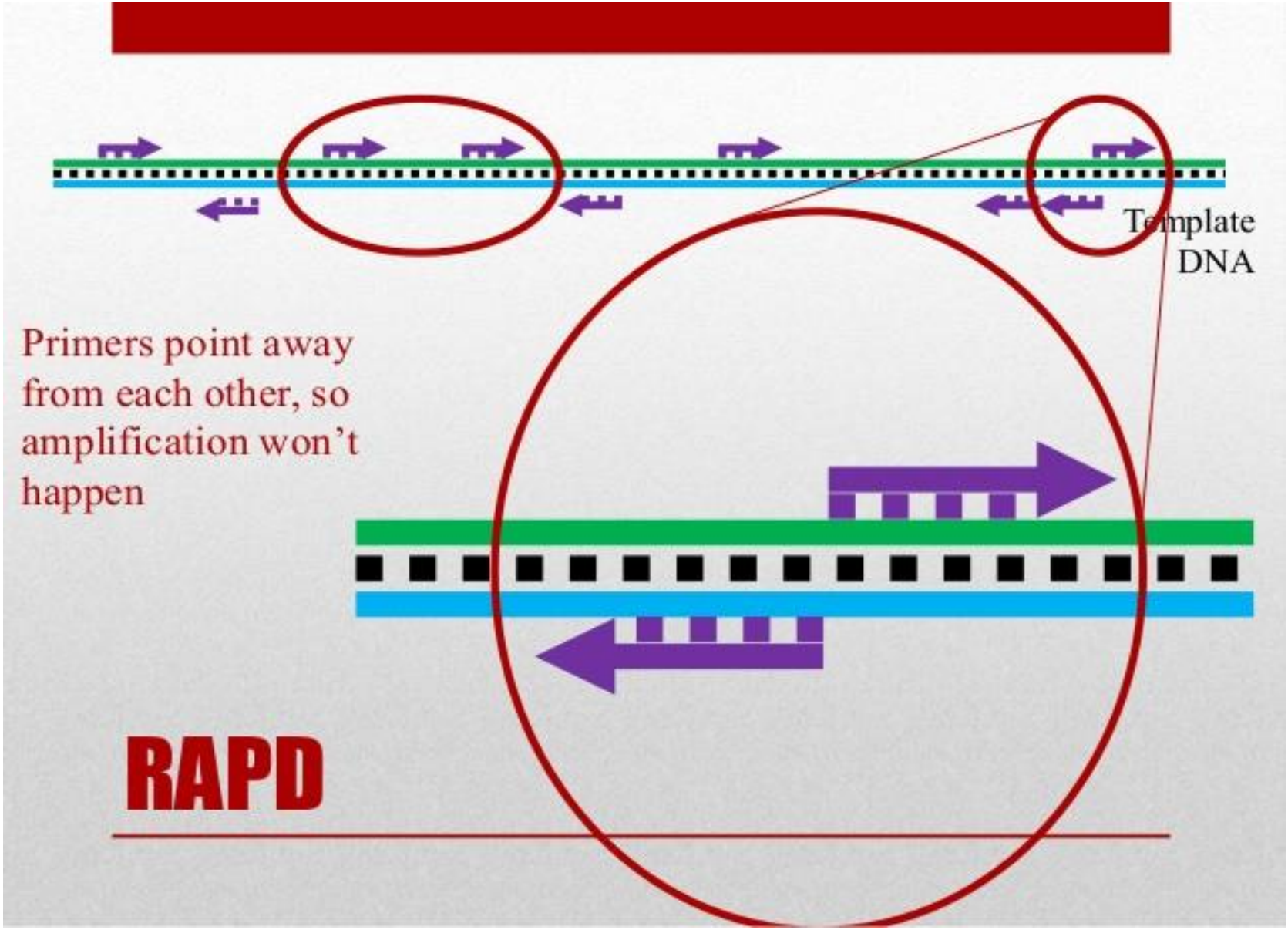

DNA template A
DNA template B
PCR amplification
Gel Electrophoresis

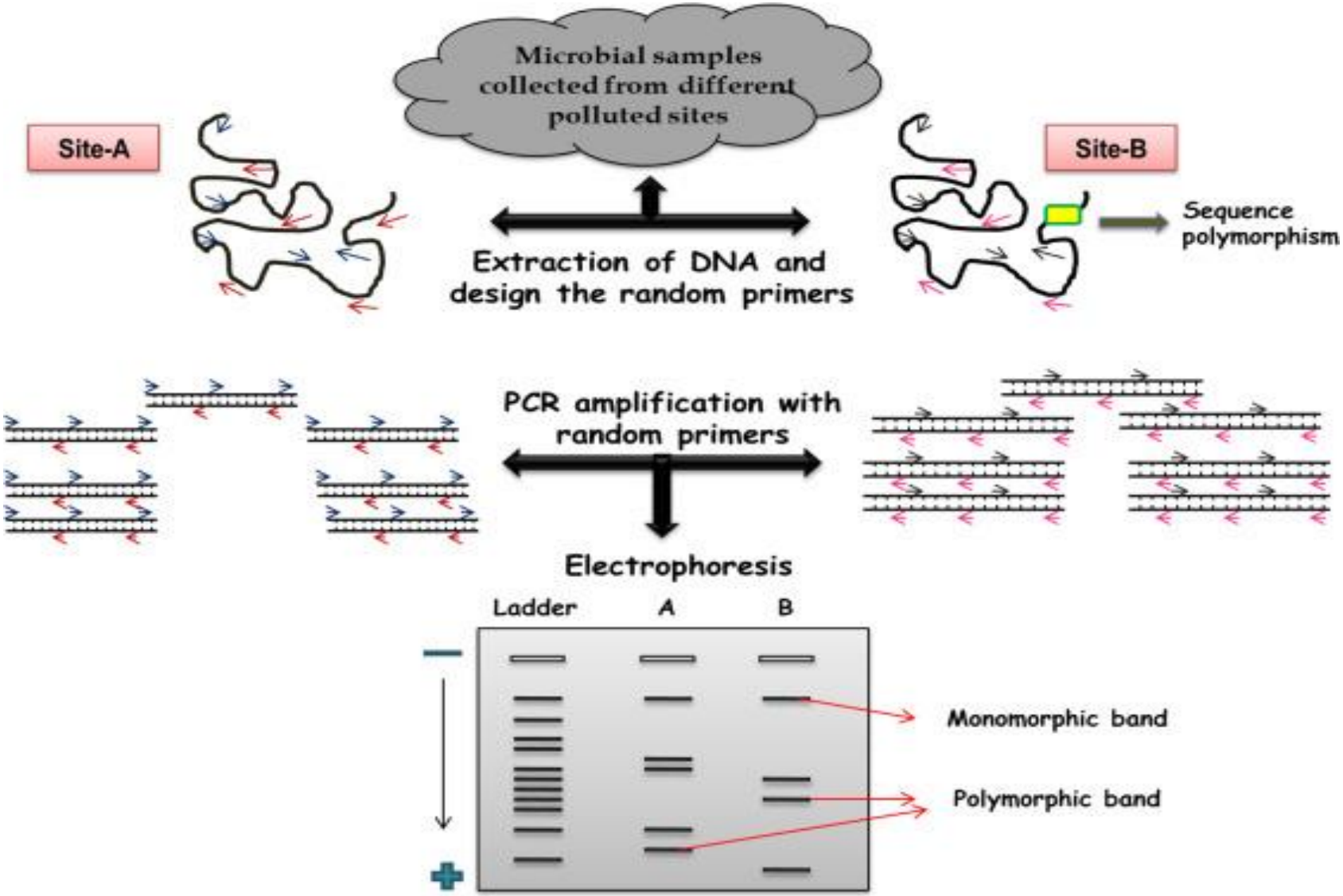


lane 1: molecular weight markers

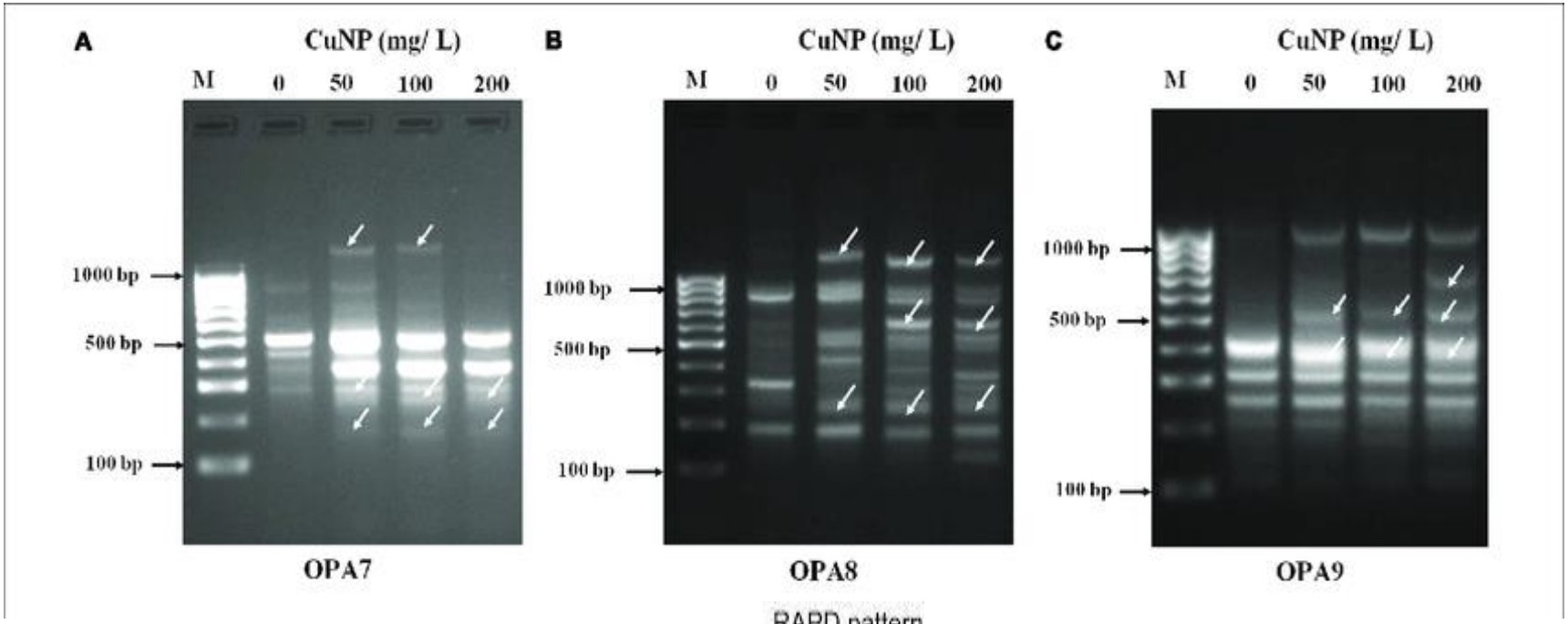
lane 2: RAPD Rxn. #1

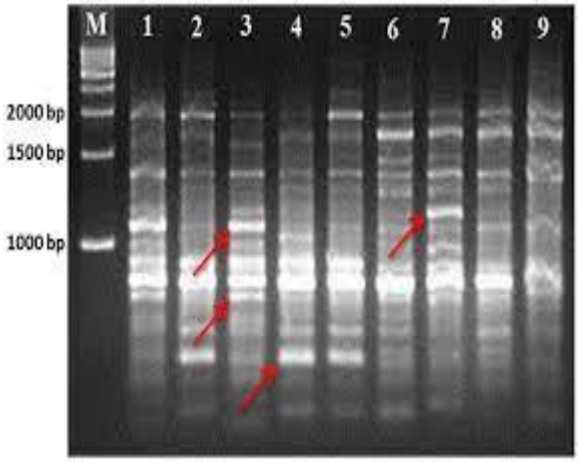
lane 3: RAPD Rxn. #2

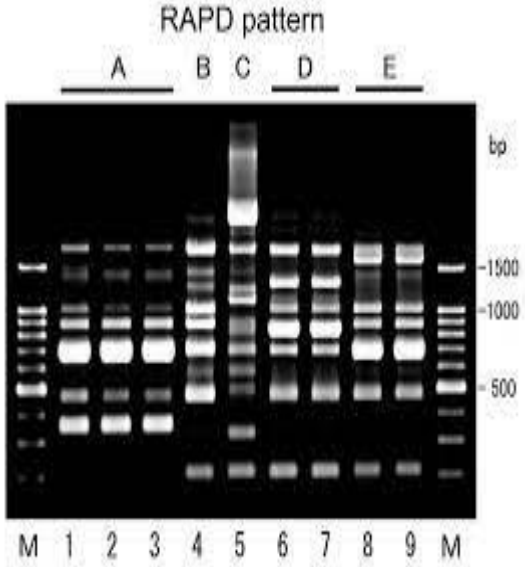

Template
DNA
Primers point away from each other, so amplification won't happen
RAPD

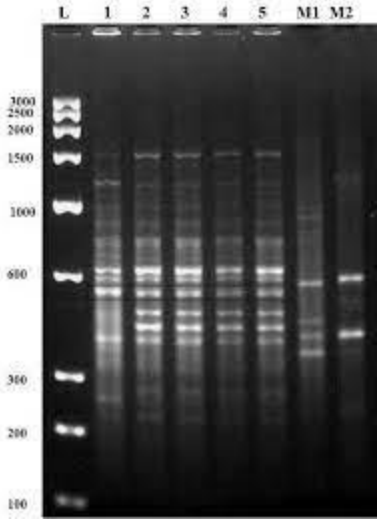


Furthermore, size of the amplified fragments were detected and compared


A
CuNP (mg/ L)
M 0 50 100 200
1000 bp
500 bp
100 bp
OPA7
B
CuNP (mg/ L)
M 0 50 100 200
1000 bp
500 bp
100 bp
OPA8
C
CuNP (mg/ L)
M 0 50 100 200
1000 bp
500 bp
100 bp
OPA9


M 1 2 3 4 5 6 7 8 9
2000 bp
1500 bp
1000 bp


RAPD pattern
A B C D E
bp
1500
1000
500
M 1 2 3 4 5 6 7 8 9 M


L 1 2 3 4 5 M1 M2
3000
2500
2000
1500
1000
600
300
200
100