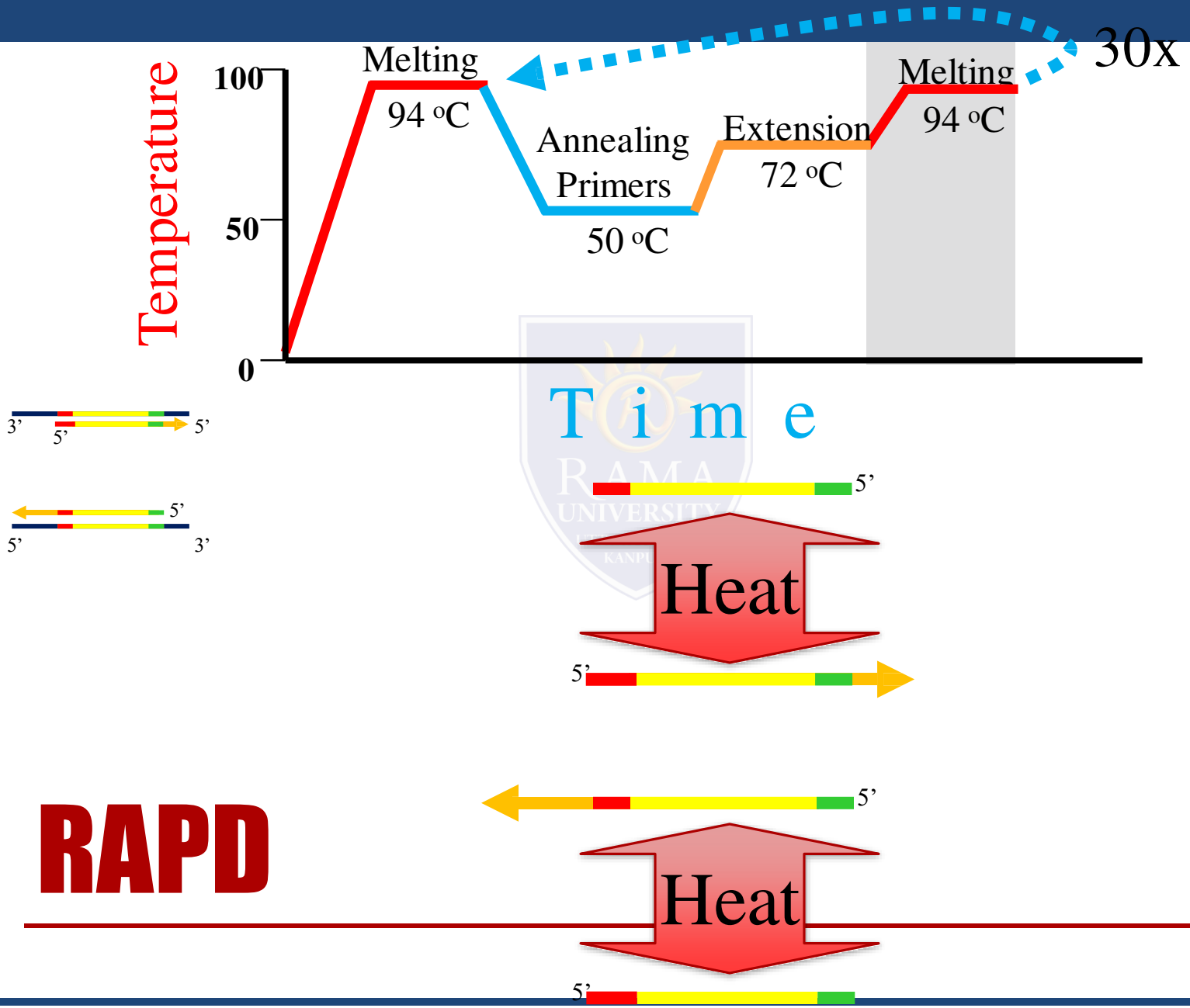
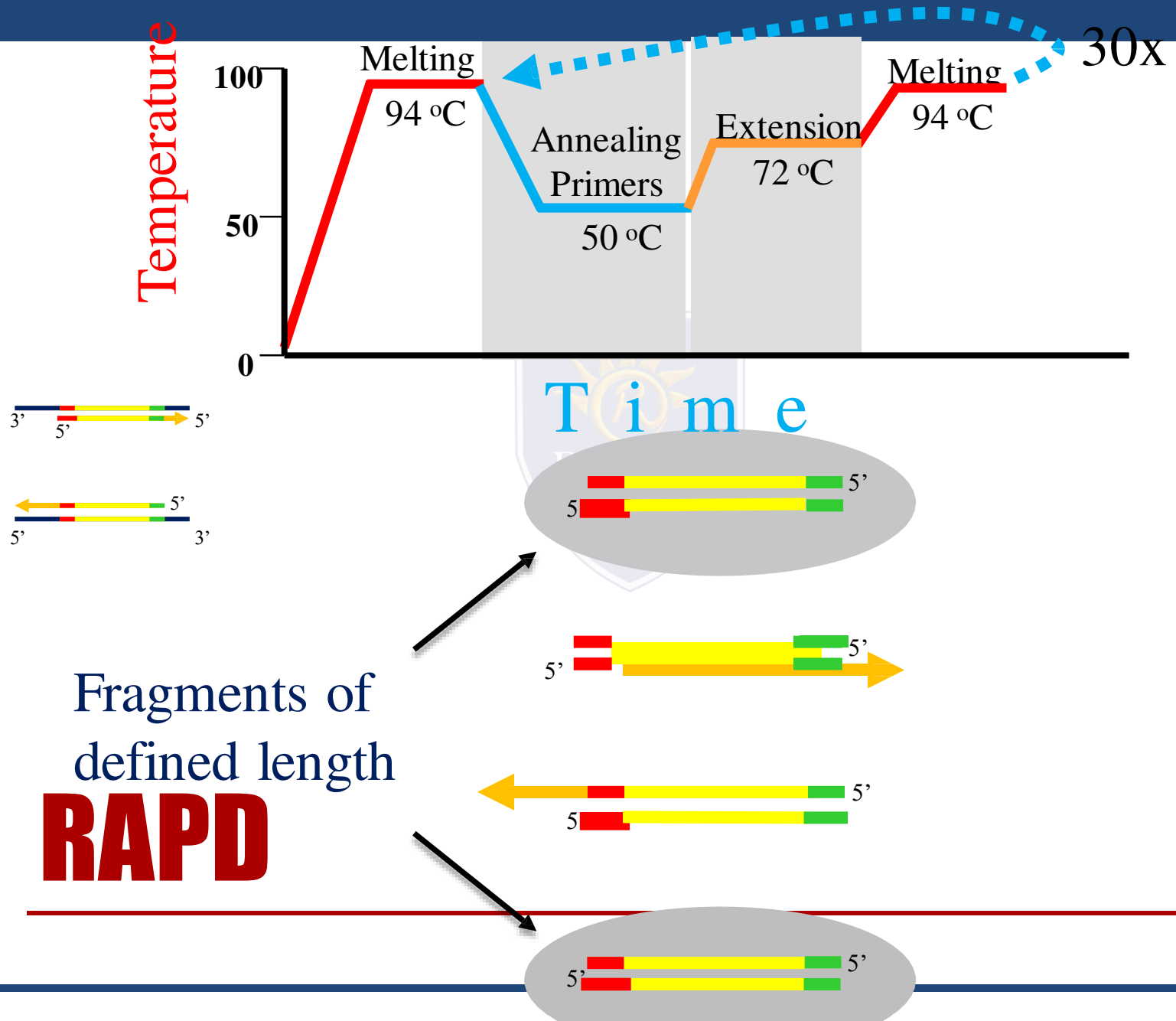
FACULTY OF ENGINEERING &TECHNOLOGY

DEPARTMENT OF BIOTECHNOLOGY


30x
Melting
94 °C
Annealing
Primers
50 °C
Extension
72 °C
Melting
94 °C
Temperature
100
50
0
Time
3'
5'
5'
5'
5'
3'
5'
Heat
5'
5'
Heat
5'
RAPD

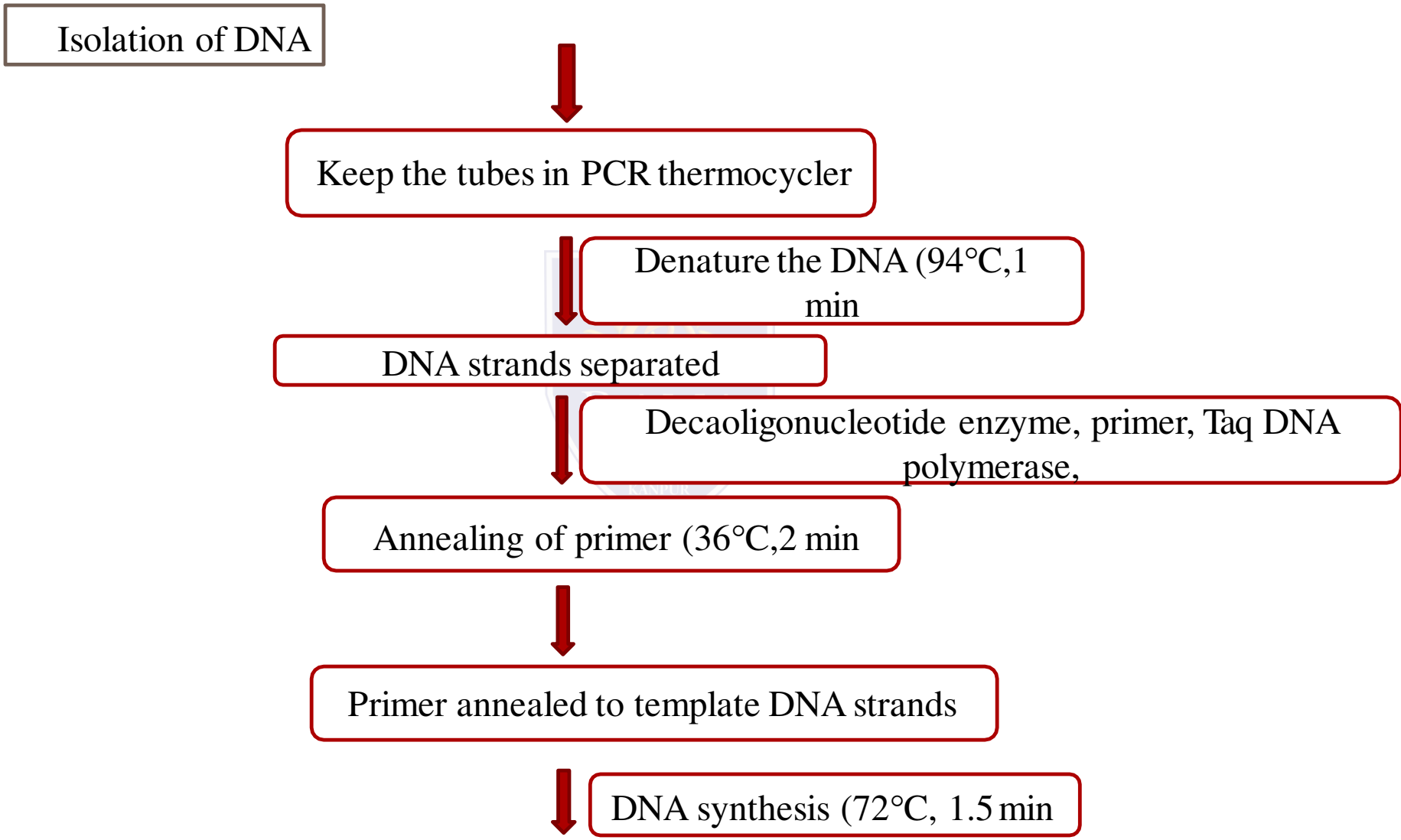


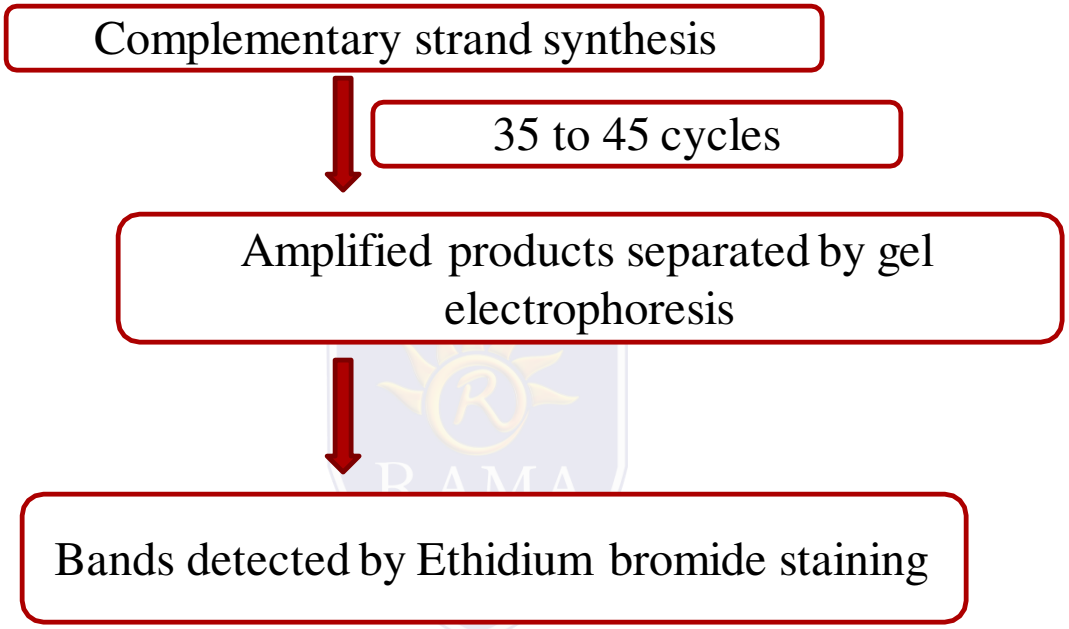
Fragments of defined length

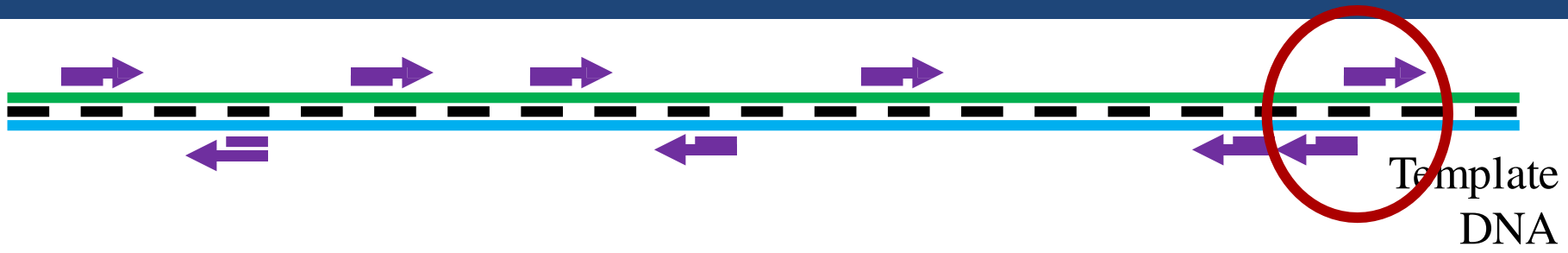
RAPD

- Primers are commercially available from various source (ex.. Opéron Technologies Inc.,California; Biosciences, Bangalore; Euro Finns, Bangalore; GCC Biotech, Kolkata).

Primers

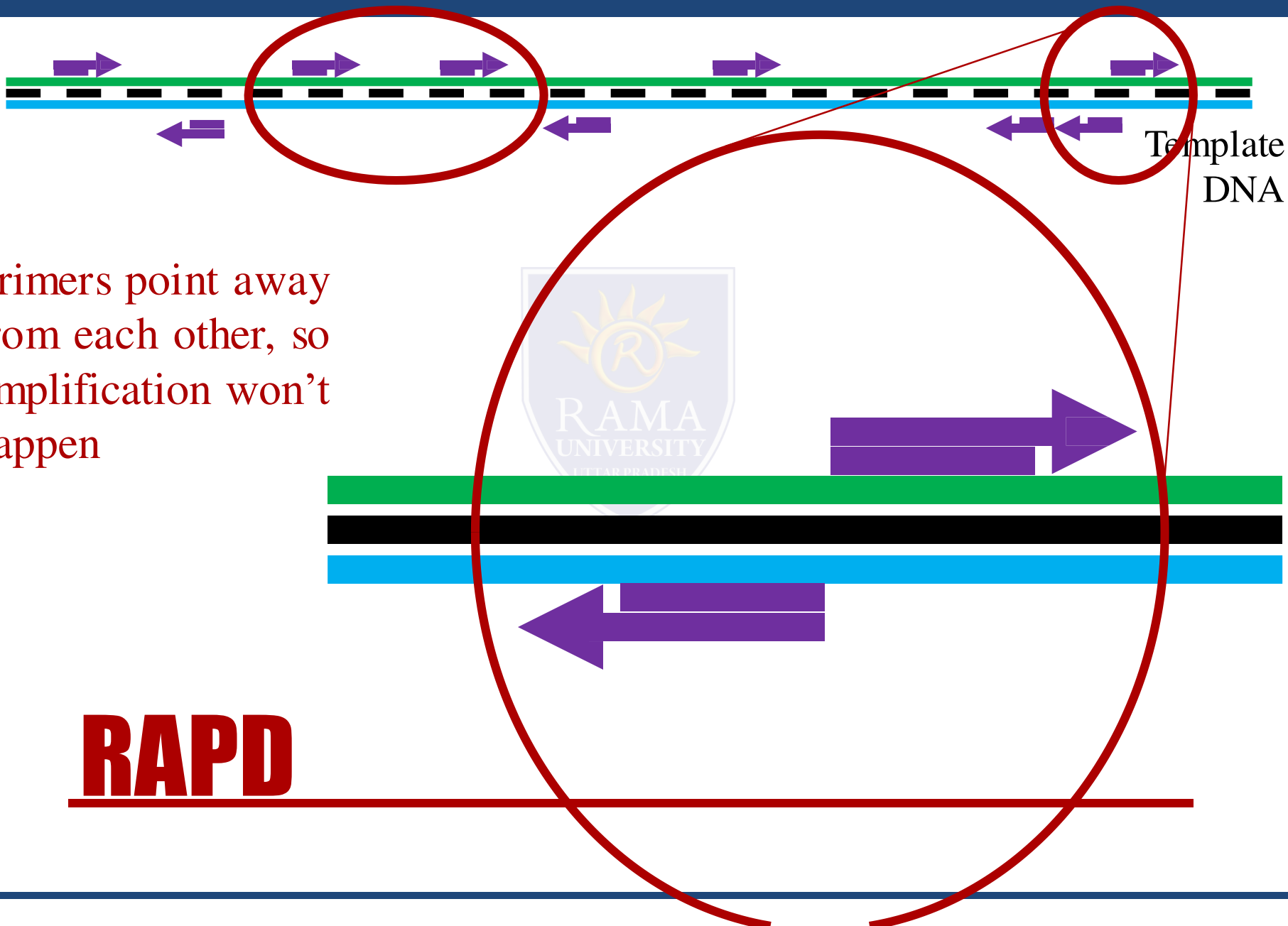

Isolation of DNA
Keep the tubes in PCR thermocycler
Denature the DNA (94°C,1 min
DNA strands separated
Decaoligonucleotide enzyme, primer, Taq DNA polymerase,
Annealing of primer (36°C,2 min
Primer annealed to template DNA strands
DNA synthesis (72°C, 1.5 min


Complementary strand synthesis
35 to 45 cycles
Amplified products separated by gel electrophoresis
Bands detected by Ethidium bromide staining



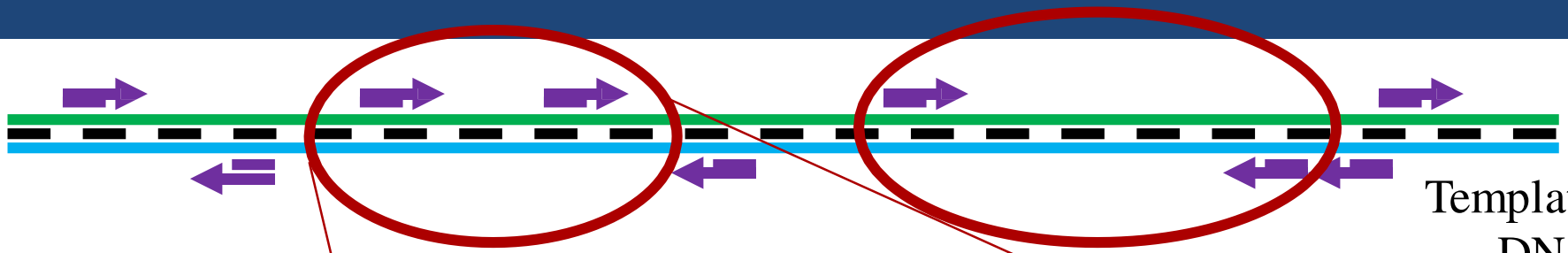
- Primer binds to many locations on the template DNA
- Only when primer binding sites are close and oriented in opposite direction so the primers point toward each other will amplification take place

RAPD

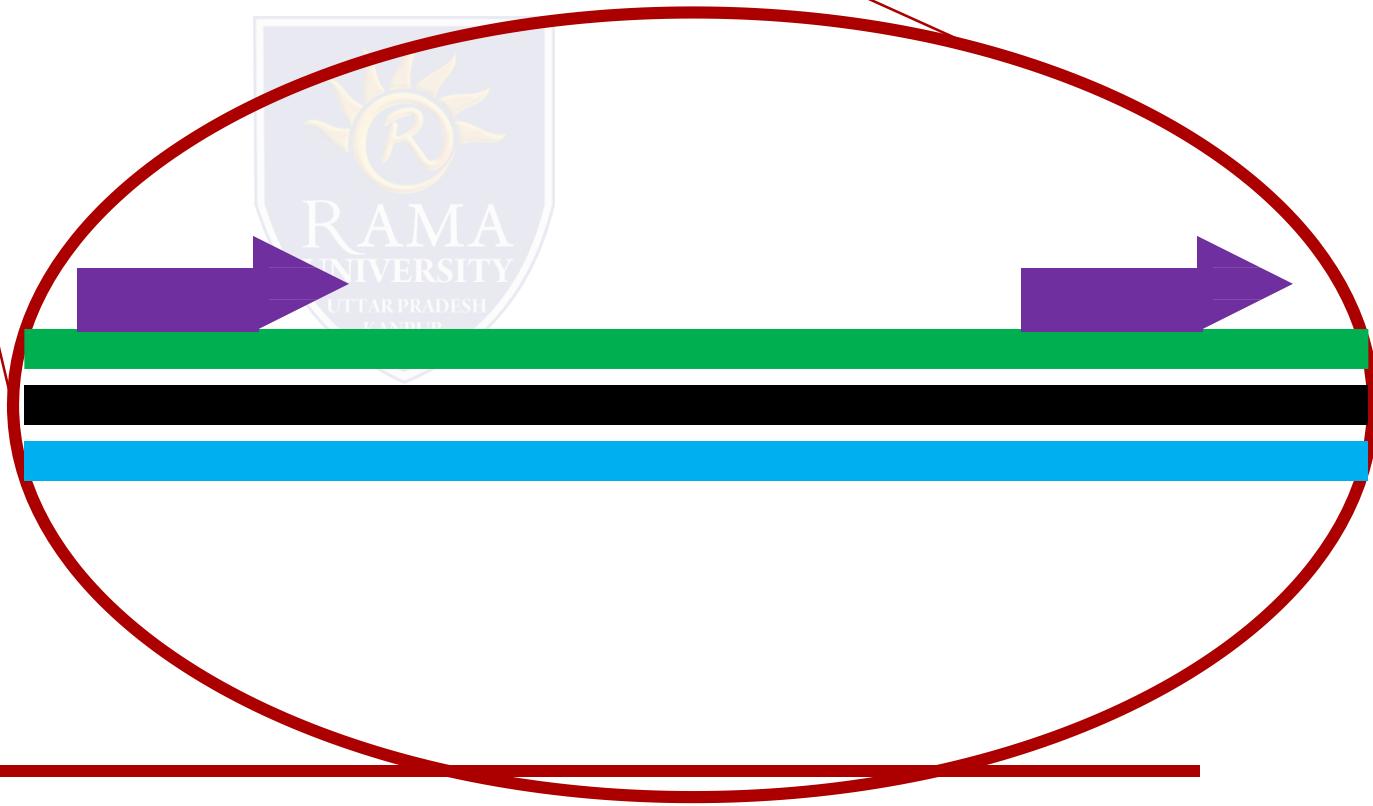


Primers point away from each other, so amplification won't happen

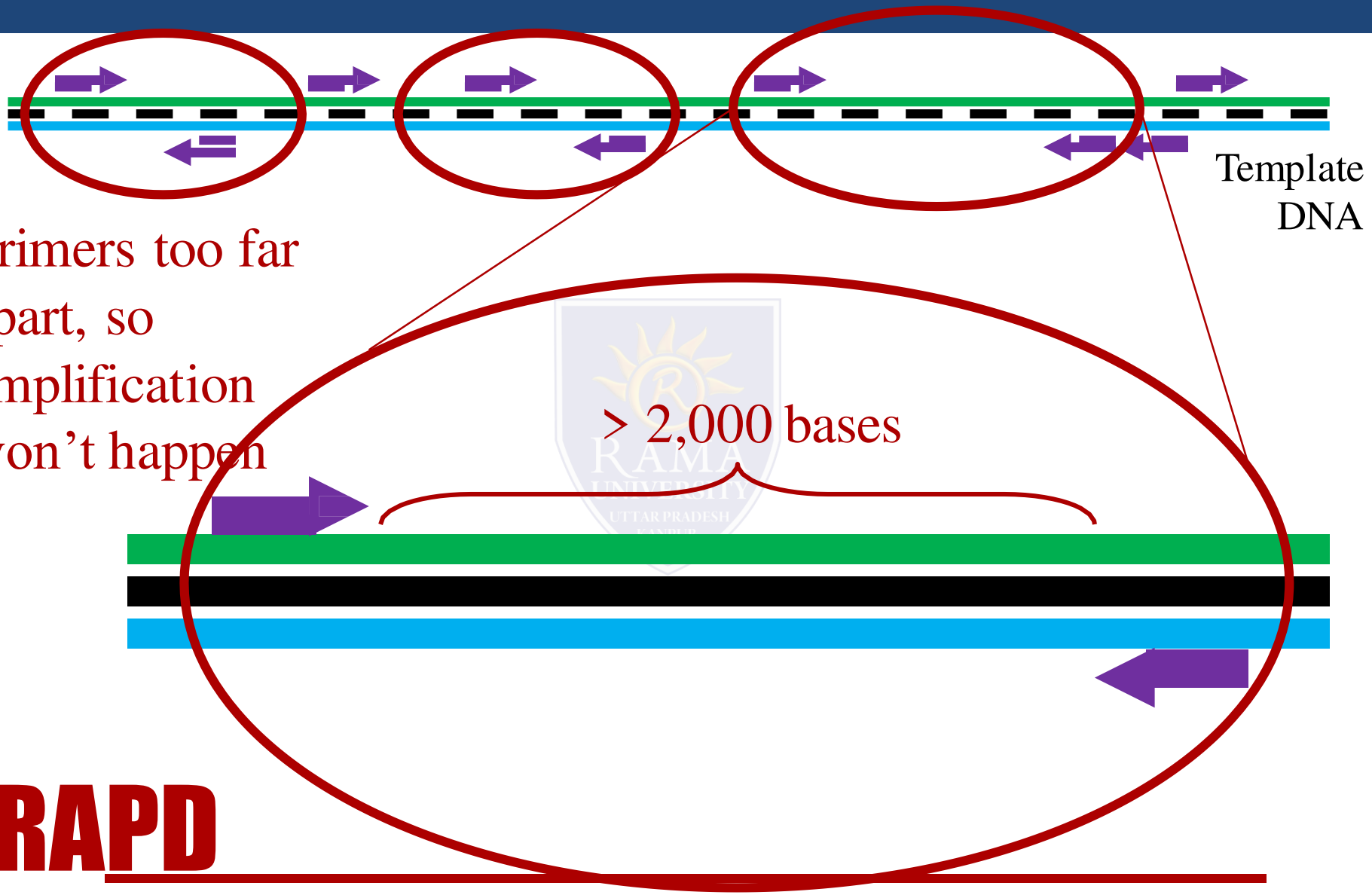
RAPD



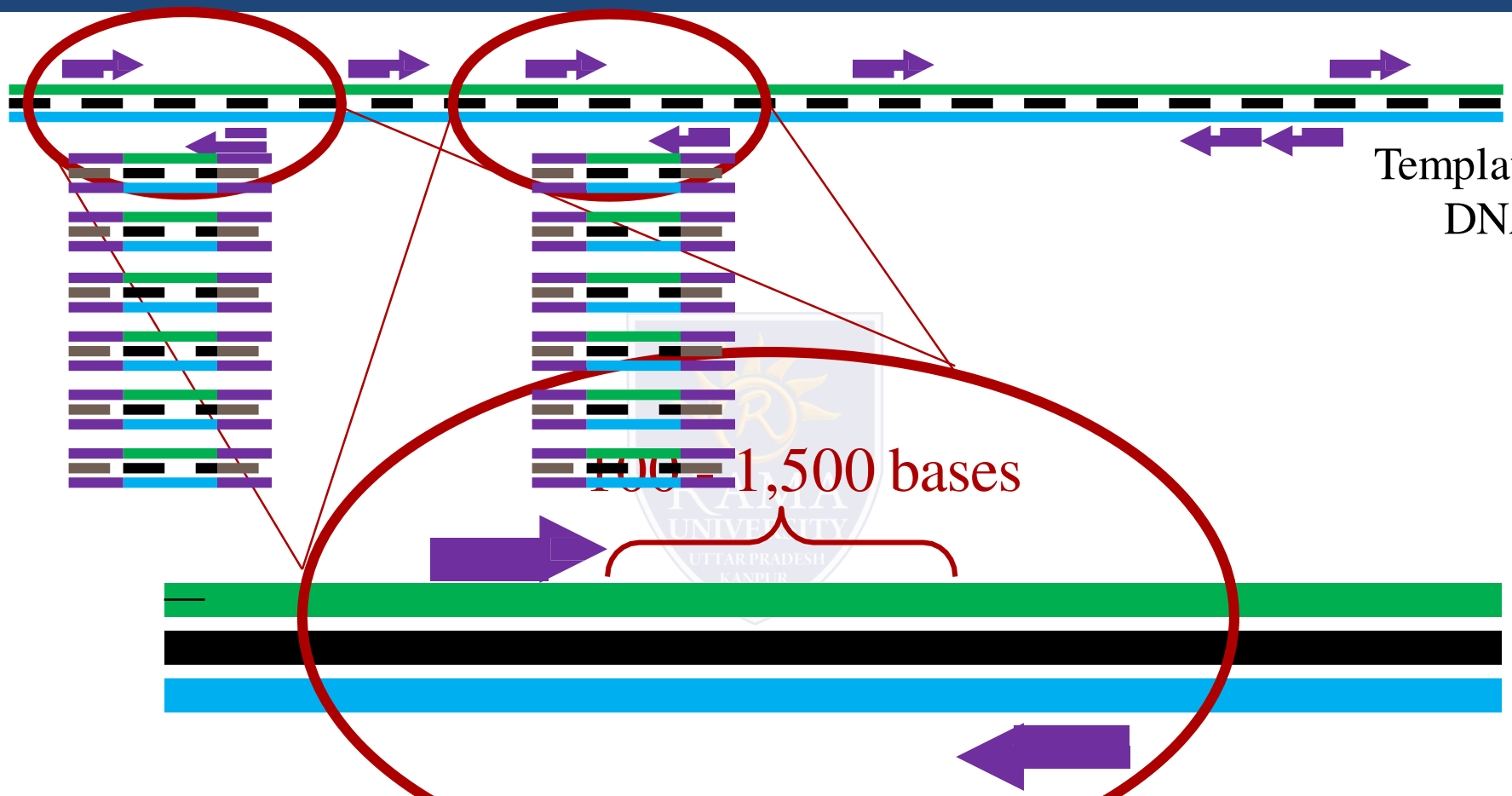
Primers point in the same direction, so amplification won't happen



RAPD


Template DNA
Primers too far apart, so amplification won't happen
> 2,000 bases

RAPD



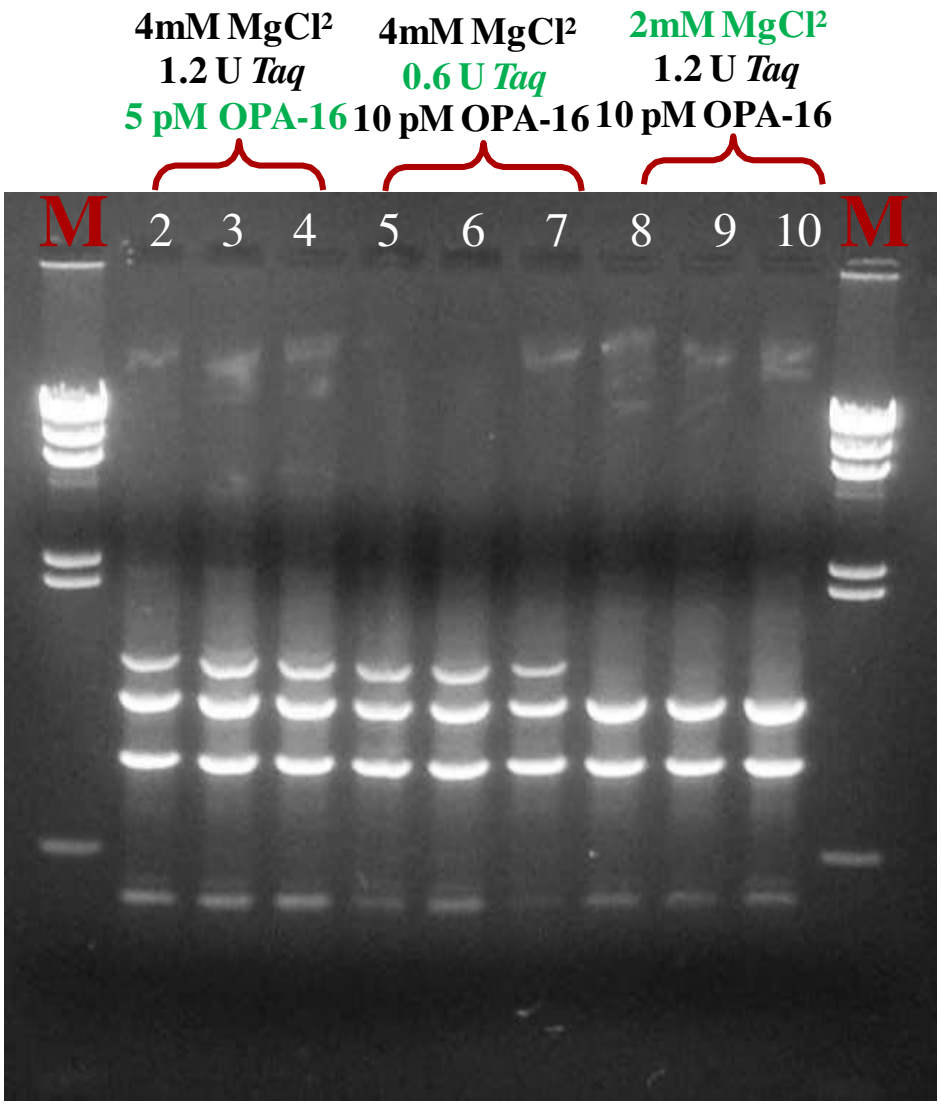
RAPD

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

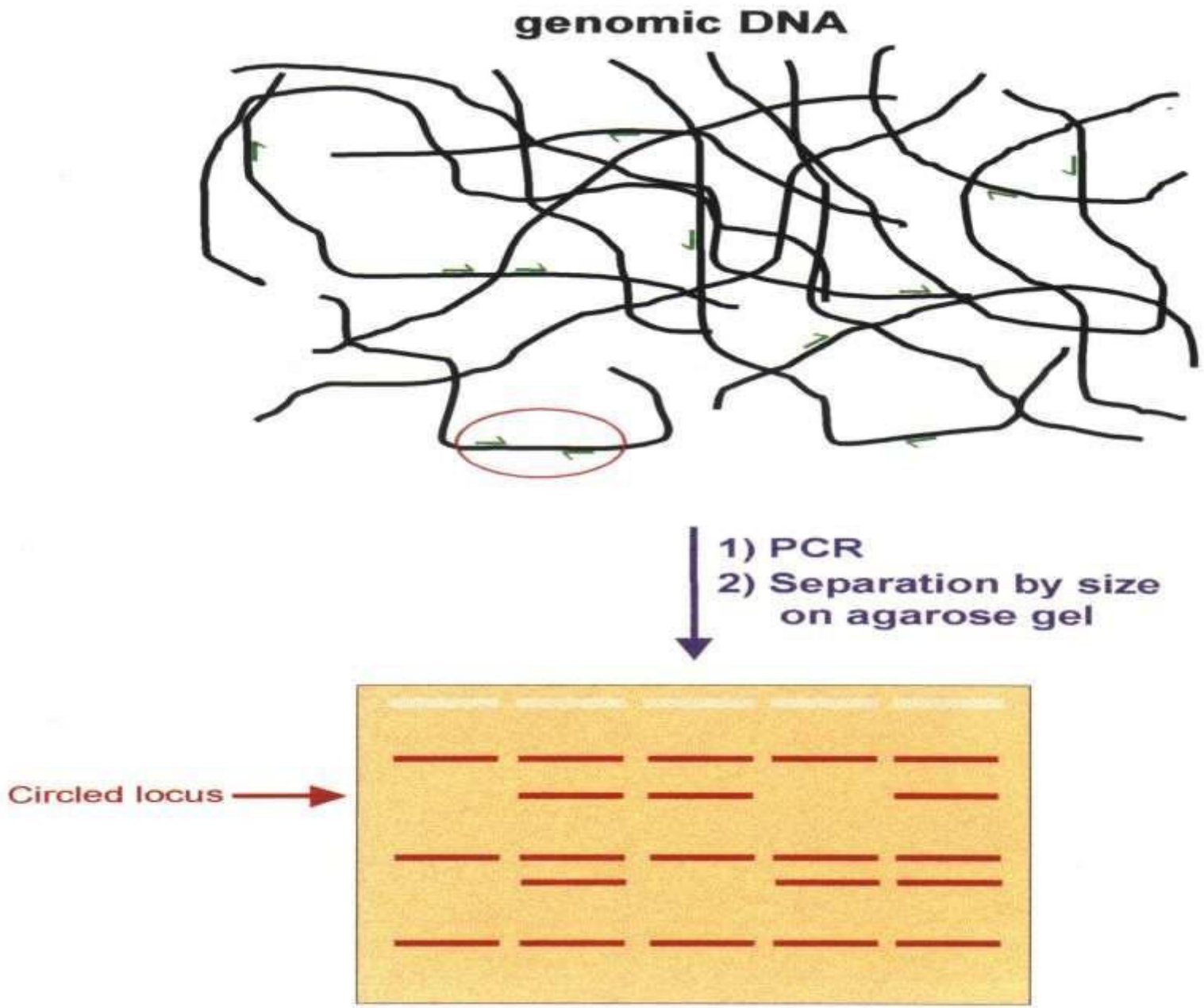
Separated RAPD Fragments

RAPD reactions were run in groups of 3 using the same template and primer, but varying Magnesium, polymerase and primer concentrations

Normal concentrations are shown in green text.
M = A size standard



Lowering Magnesium ion concentration results in loss of the largest fragment visible in lanes 2-7


genomic DNA
1) PCR
2) Separation by size
on agarose gel
Circled locus

Advantages

- It requires no DNA probes and sequence information for the design of specific primers.
- It involves no blotting or hybridisation steps, hence, it is quick, simple and efficient.
- It requires only small amounts of DNA (about 10 ng per reaction) and the procedure can be automated.
- High number of fragments.
- Arbitrary primers are easily purchased.
- Unit costs per assay are low compared to other marker technologies.

Disadvantages

- Nearly all RAPD markers are dominant,
- PCR is an enzymatic reaction, therefore, the quality and concentration of template DNA, concentrations of PCR components, and the PCR cycling conditions may greatly influence the outcome.
- Mismatches between the primer and the template may result in the total absence of PCR product as well as in a merely decreased amount of the product.
- Lack of a prior knowledge on the identity of the amplification products.
- Problems with reproducibility.
- Problems of co-migration.

DEVELOPING LOCUS-SPECIFIC, CO-DOMINANT MARKERS FROM RAPDs

- The polymorphic RAPD marker band is isolated from the gel.
- It is amplified in the PCR reaction. The PCR product is cloned and sequenced.
- New longer and specific primers are designed for the DNA sequence, which is called the sequenced characterized amplified region marker (SCAR).

Applications

- genetic diversity/polymorphism,
- germplasm characterization,
- genetic structure of populations,
- hybrid purity,
- genome mapping,
- developing genetic markers linked to a trait in question,
- population and evolutionary genetics,
- plant and animal breeding,
- animal-plant-microbe interactions,
- pesticide/herbicide resistance.

- RAPD is a lab technique used to amplify unknown(random) DNA segments

- It is a technique firstly DNA is isolated, which is then treated with decaoliganucleotide enzymes it act as a restriction enzymes which is used to cleave a short ten nucleotide segments of DNA.

- Then mixture is taken to PCR equipment and the process of DNA denaturation and the annealing of primer occcurs, then primer extension takes place for 35 to 45 cycles.

- DNA hybridizaion occurs at some segment of DNA amplification occurs at a particular site.

- DNA is subjected to gel electrophoresis,the amplified DNA will form distinct band detected by ethidium bromide staining and visible fluorescence's under U.V.light

Conclusion

- RAPD markers exhibit reasonable speed, cost and efficiency compared with other methods and,
- RAPD can be done in a moderate laboratory. Therefore, despite its reproducibility problem, it will probably be important until better techniques are developed in terms of cost, time and labour.