Modern Trends in Plant Taxonomy

Dr. M. Sathiyabama
Associate Professor
Department of Botany

Taxonomists consider: morphological characters are not always sufficient to differentiate

To achieve this: evidences from anatomy, embryology, palynology, cytology, ecology, paleobotany, biochemistry were included.

Anatomy in relation to taxonomy

- Anatomical features cannot constitute the basis of classification but can be used with supplement to morphology
- Vegetative anatomy: Stomata: Stomata have been classified according to the position of subsidiary cells, guard cells in relation to the aperture (anomocytic, anisocytic, paracytic, diacytic, graminaceous type)

- Crystal: Crystals of calcium oxalate are found in many families like apocyanaceae, begoniaceae, cactaceae, euyphorbiaceae, caricaceae etc.,
- Latex tissue: Laticeiferous tissue is found in a number of families: Butomaceae, Araceae, Musaceae, Nymphaeceae, etc.,
- Cystoliths: Crystals of calcium carbonate eg. Acanthaceae, Urticaeae etc.,

- Vascular bundle: types (Scattered – monocot: in ring – Dicot)
- Bicollateral vascular bundles (cucurbitaceae, gentianaceae), Cortical bundles (Bombaceae, Cactaceae, Asteraceae), Medullary phloem (Amaranthaceae, Euphorbiaceae), Interxylary phloem (Menispermaceae).
- Ring of cambium (Amaranthaceae, Menispermaceae)

Embryology in relation to taxonomy

- Cyperaceae: (Formation of Microspore): Four microspore nuclei produced after meiosis, only the fourth one develps to form the generative cell and then the male gametes.
- Centrospermales: glandular anther tapetum- cells become 2-4 nucleate, Microspore mother cells – two meiotic divisions are succeeded by a simultaneous quadripartition into the microspores, trinucleate pollen-grains, Campylotropus ovules, functioning of the chalazal megaspore of the tetrad, formation of a monosporic eight-nucleate embryosac, functioning of the perisperm as main storage region.

- Loranthaceae: mode of development of embryosac, endosperm, embryo
- Onagraceae: A monosporic tetranuceleate embryosac is characeteristic feature.

Palynology in relation to taxonomy

- Pollen characters: Caryophyllales recognized by centrospermous type of pollen with spinulose tectum.
- Malavaceae and compositae- typically spinulose exine
- Graminaceae: smooth.
- Number of nuclei in pollen: number of nuclei in the pollen at the time of dispersal (polypetalae- binuleate; gamopetalae- trinucleate).

Pollen Wall Structure

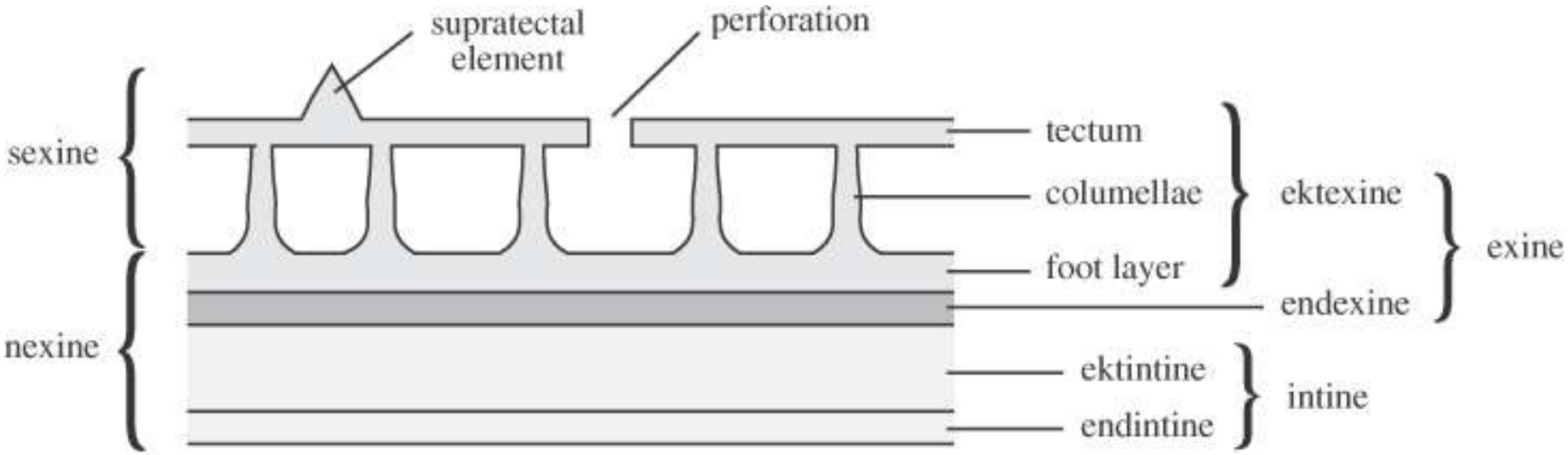
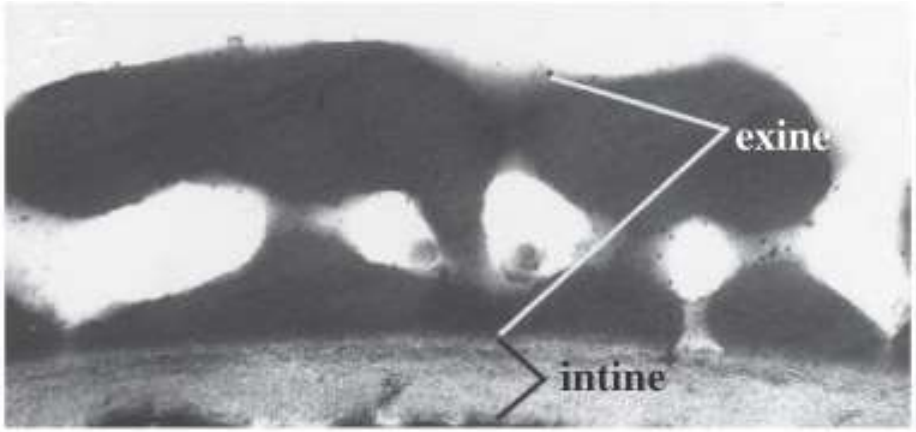
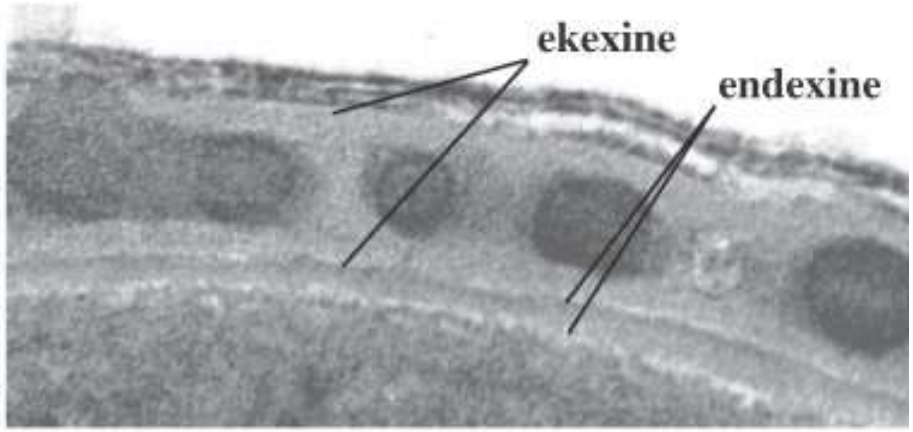


Figure 12.8 Pollen wall structure.

tectate-columellate

Pollen Wall Structure





tectate-columellate

Numerical Taxonomy

Numerical evolution of similarity between groups of organisms and the ordering of these groups into higher ranking taxa on the basis of these similarities (Heywood,1967).

A synonym “Taxometrics” proposed by Rogers (1963).

- Binnary character: yes/no; +/-; 1/0
- Multistate character: three or more states. (length, height, weight)

Chemotaxonomy

- The use of chemical characters of plants in classification or in solving taxonomic problems.
- Chemical compounds such as – alkaloids, caroteinoids, tannins, terpenoids, flavonoids, polysaccharide, fatty acid, amino acid etc.,
- Alkaloids (solanaceae); tannins (Anacardiacea, casuarinaceae, sapindaceae,etc.,)

- Steriods (apocyananceae, asclepiadaceae)
- Terpenoids (mint, eucalyptus, citrus, rubber)
- Flavonoids (fabaceae, arecaceae)
- Alkaloids (papavareceae, fabaceae, solanaceae)
- Waxes: (palmceae,)
- Sulphur compounds (Allium sp.)

serotaxonomy

- Application of serum in taxonomy
- Protein extracted form one plant - injected into animal system – antibody mixed with protein of another plant : precipatition (similarity) No precipitation (not related)

Cladistics (Phylogenetic)

- **Cladistics** is an approach to biological classification in which organisms are categorized based on shared derived characteristics that can be traced to a group's most recent common ancestor and are not present in more distant ancestors. Therefore, members of a group are assumed to share a common history and are considered to be closely related. (evolutionary biology)

- Species divergence from common ancesteroal line.
- All species in a grouping must share a common ancestor.
- All species derived from a common ancestor must be included in the taxon.

Biosystematics

- The statistical analysis of data obtained from genetic, biochemical, and other studies to assess the taxonomic relationships of organisms or populations, especially within an evolutionary framework.

What are Molecular Markers?

- Specific fragments of DNA that can be identified within the whole genome. Molecular markers are the general assays that allow detection of the sequence differences between two or more individual. Molecular markers are found at specific locations of the genome. They are used to 'flag' the position of a particular gene or the inheritance of a particular characteristic or desired characteristics
- molecular marker is a DNA sequence that is readily detected and whose inheritance can easily be monitored .

- DNA markers Non-PCR Based, RFLP- Restriction fragment length polymorphism. PCR Based RAPD- Random amplification of polymorphic DNA . AFLP -Amplified fragment length polymorphism. SCAR -Sequence characterize amplified region. STS - Sequence tagged sites. EST- Express sequence tags. SNP- Single nucleotide polymorphism. SSR -Simple sequence repeats, ISSR, ITS CAPS -Cleaved amplified polymorphic sequences etc.

Molecular Taxonomy

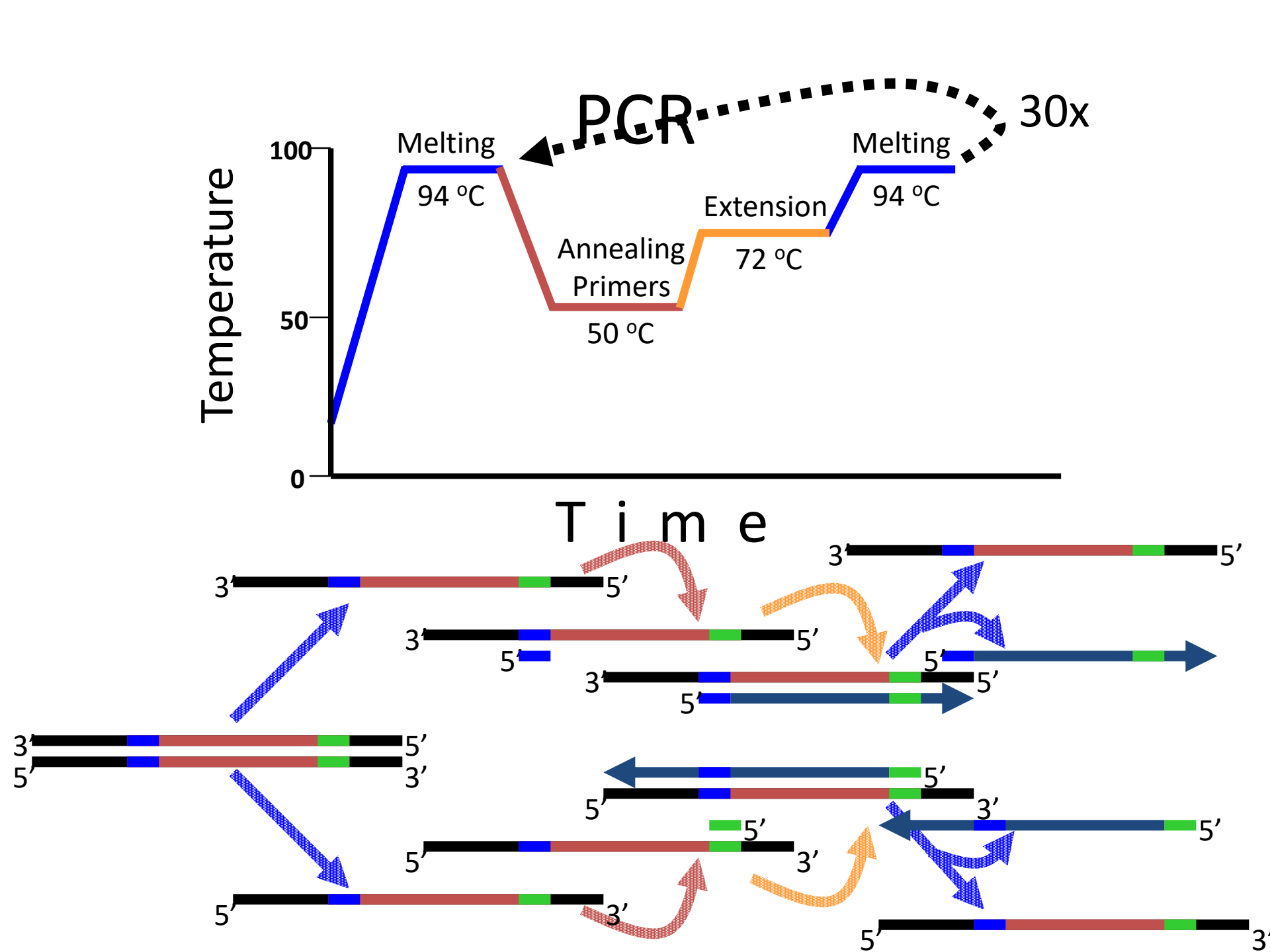
- RAPD (Random Amplified Polymorphic DNA)
- RFLP (restriction fragment length polymorphism)
- ISSR (Inter-simple sequence repeat)
- SSR (simple sequence repeat)
- ITS (Internal transcribed spacer)

- Shortly after Kary Mullis invented the Polymerase Chain Reaction (PCR) it was realized that short primers would bind to several locations in a genome and thus could produce multiple fragments
- Williams *et al*. (1990) developed Random Amplified Polymorphic DNA (RAPD) a technique using very short 10 base primers to generate random fragments from template DNAs

- RAPD fragments can be separated and used as genetic markers or a kind of DNA fingerprint
- RAPD polymorphism result from change in the primer-binding site in the DNA sequence
- In variety A there are 4 primer binding sites resulting in two RAPD products, variety B lacks one of the binding sites resulting in only one RAPD marker being produced

- Template DNA Primers point in the same direction, so amplification won’t happen
- Template DNA Primers too far apart, so amplification won’t happen > 2,000 bases
- Template DNA Primers are just the right distance apart, so fragment is amplified 100 - 1,500 bases

1. Buffer (containing Mg^{++}) - usually high Mg^{++} concentrations are used lowering annealing stringency
2. Template DNA
3. 1 short primer (10 bases) not known to anneal to any specific part of the template DNA (2 Primers that flank the fragment of DNA to be amplified as in the case of PCR)
4. dNTPs
5. *Taq* DNA Polymerase (or another thermally stable DNA polymerase)

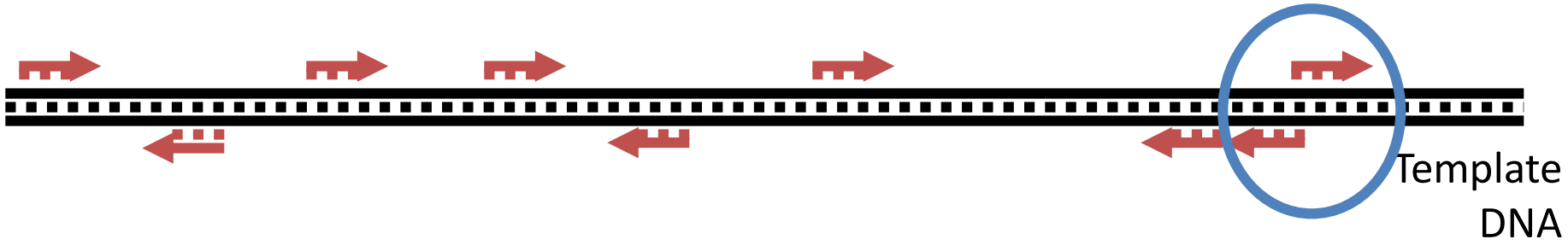

PCR
30x
Melting
94 °C
Annealing
Primers
50 °C
Extension
72 °C
Melting
94 °C
100
50
0
Temperature
T i m e
3’
5’

Modifying Thermal Cycling

- Two modifications made to typical thermal cycling when RAPD is being done:

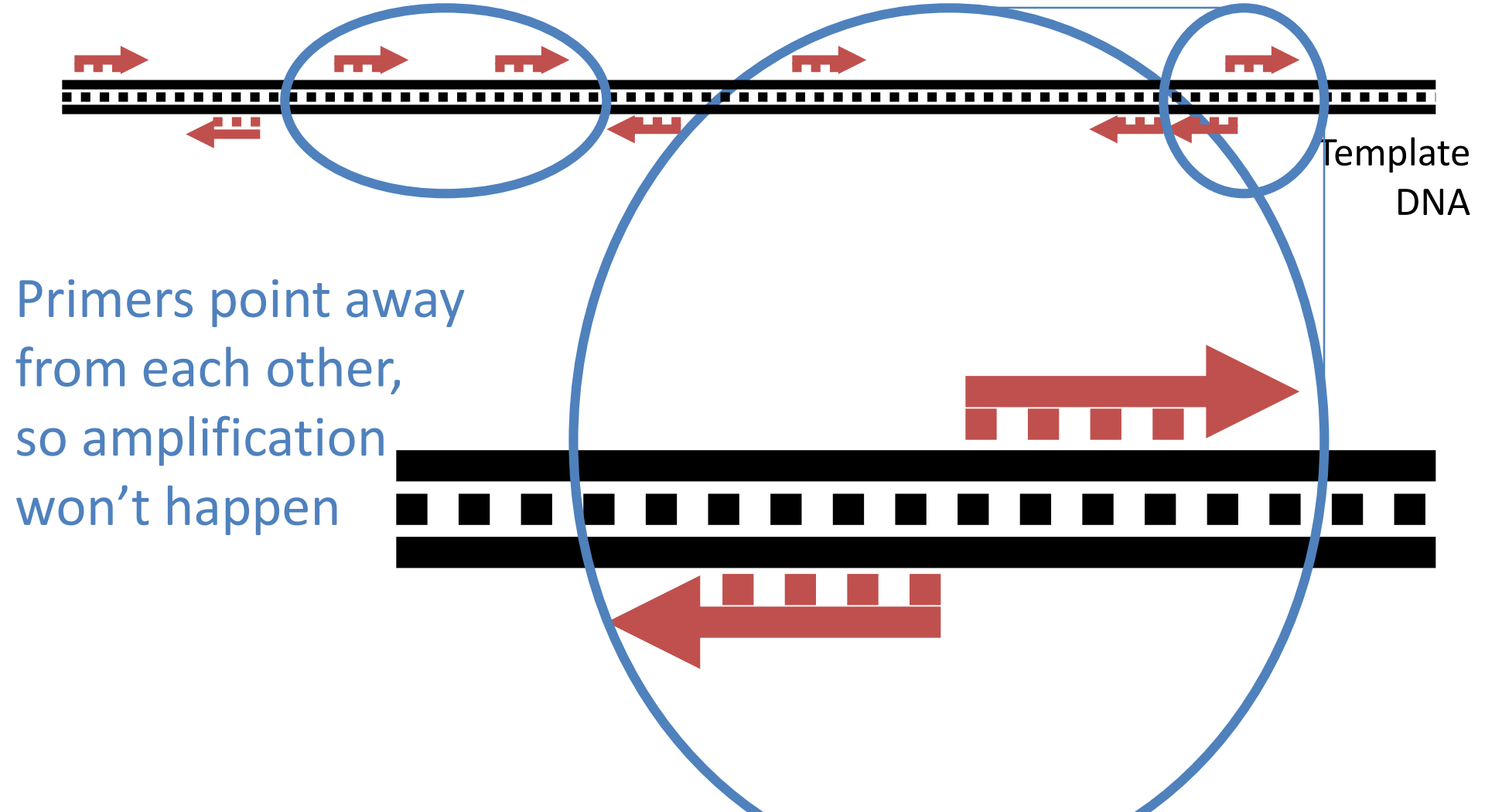
1. Annealing temperatures are generally very low, around 36 ^{o}C - This allows very short primers to anneal to template DNA
2. More thermal cycles are used, typically 45 - This compensates for the inefficiency which results from using such short primers.

RAPD



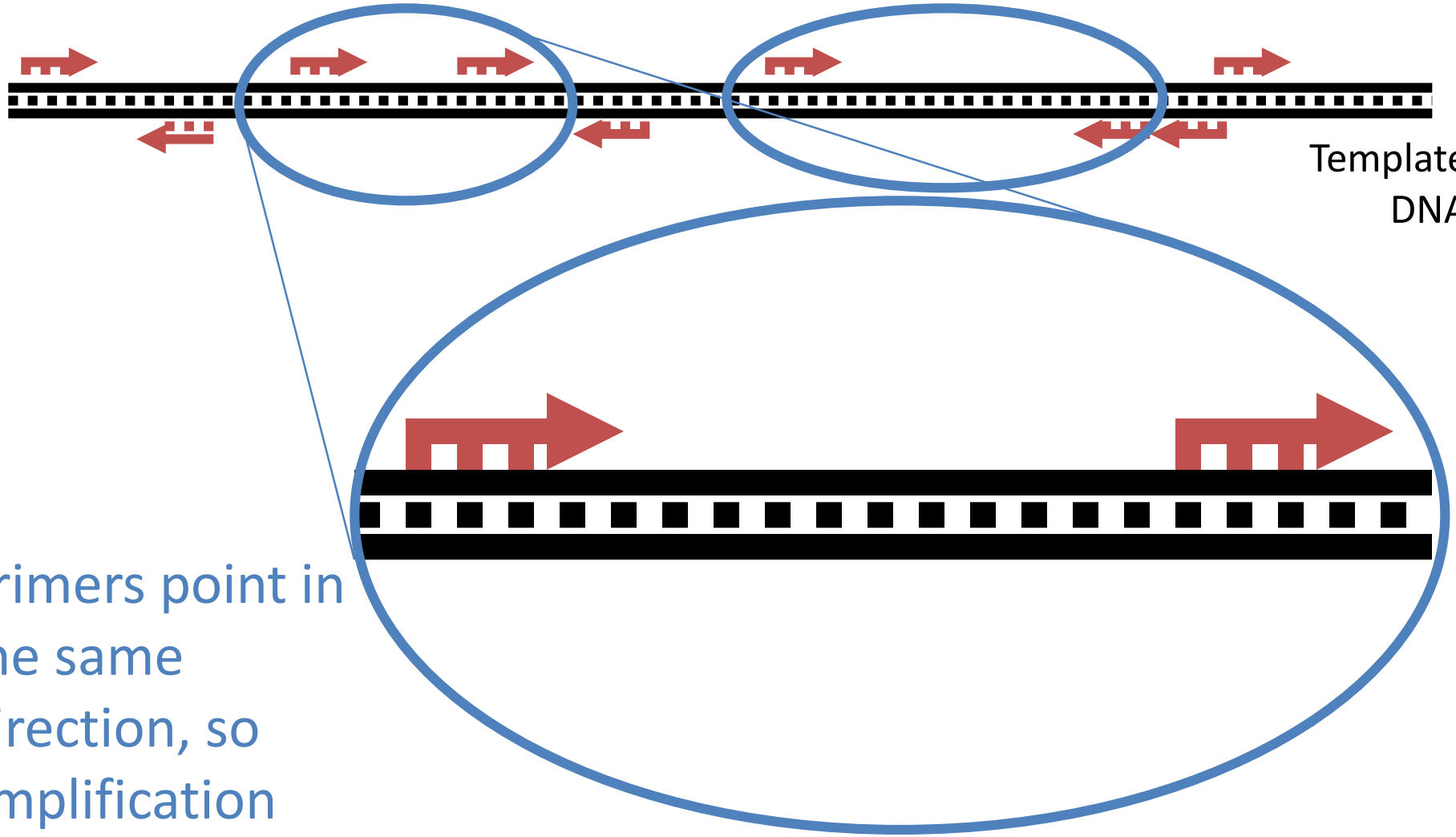
- Primer binds to many locations on the template DNA
- Only when primer binding sites are close and oriented in opposite direction so the primers point toward each other will amplification take place

RAPD



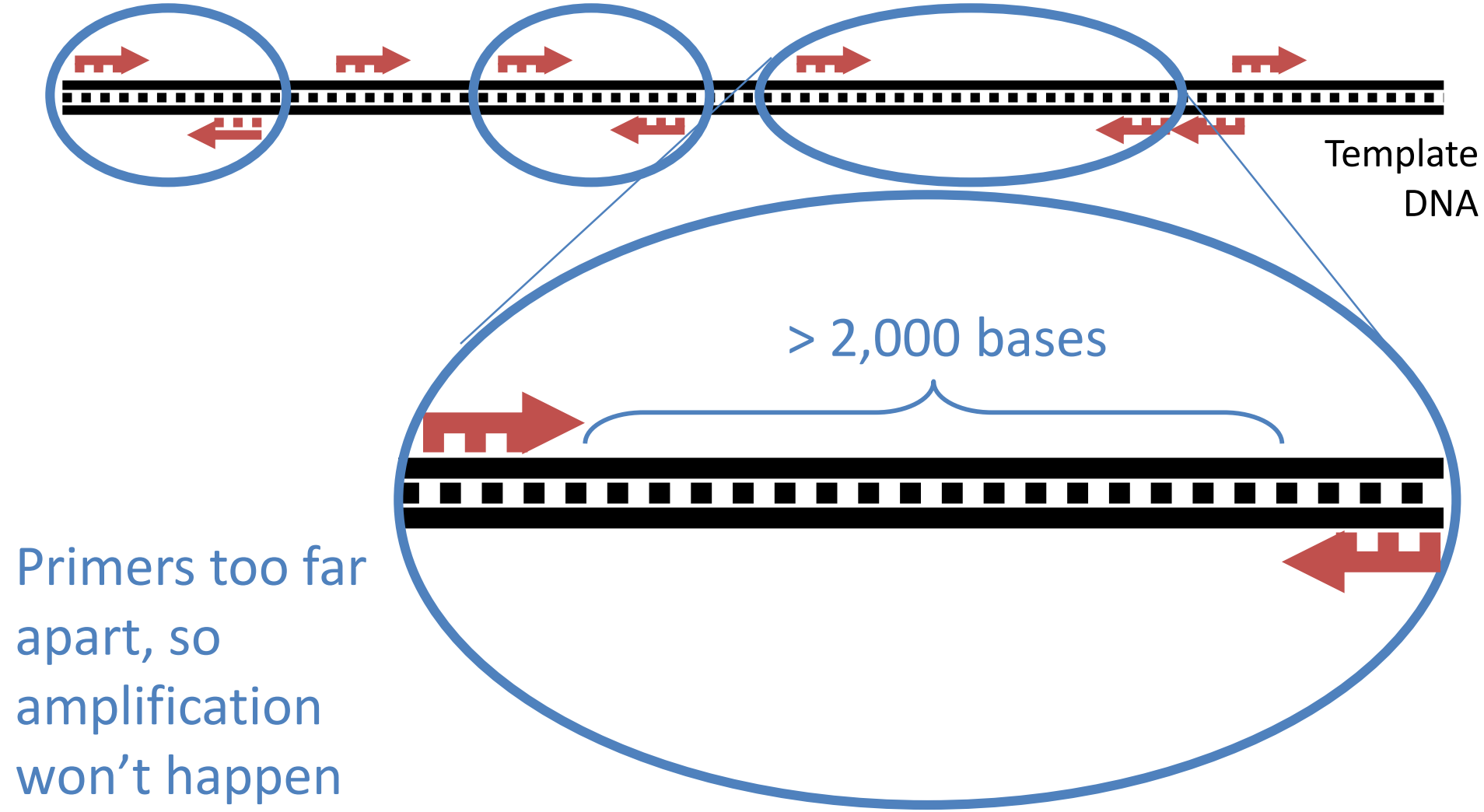
Primers point away from each other, so amplification won't happen

RAPD



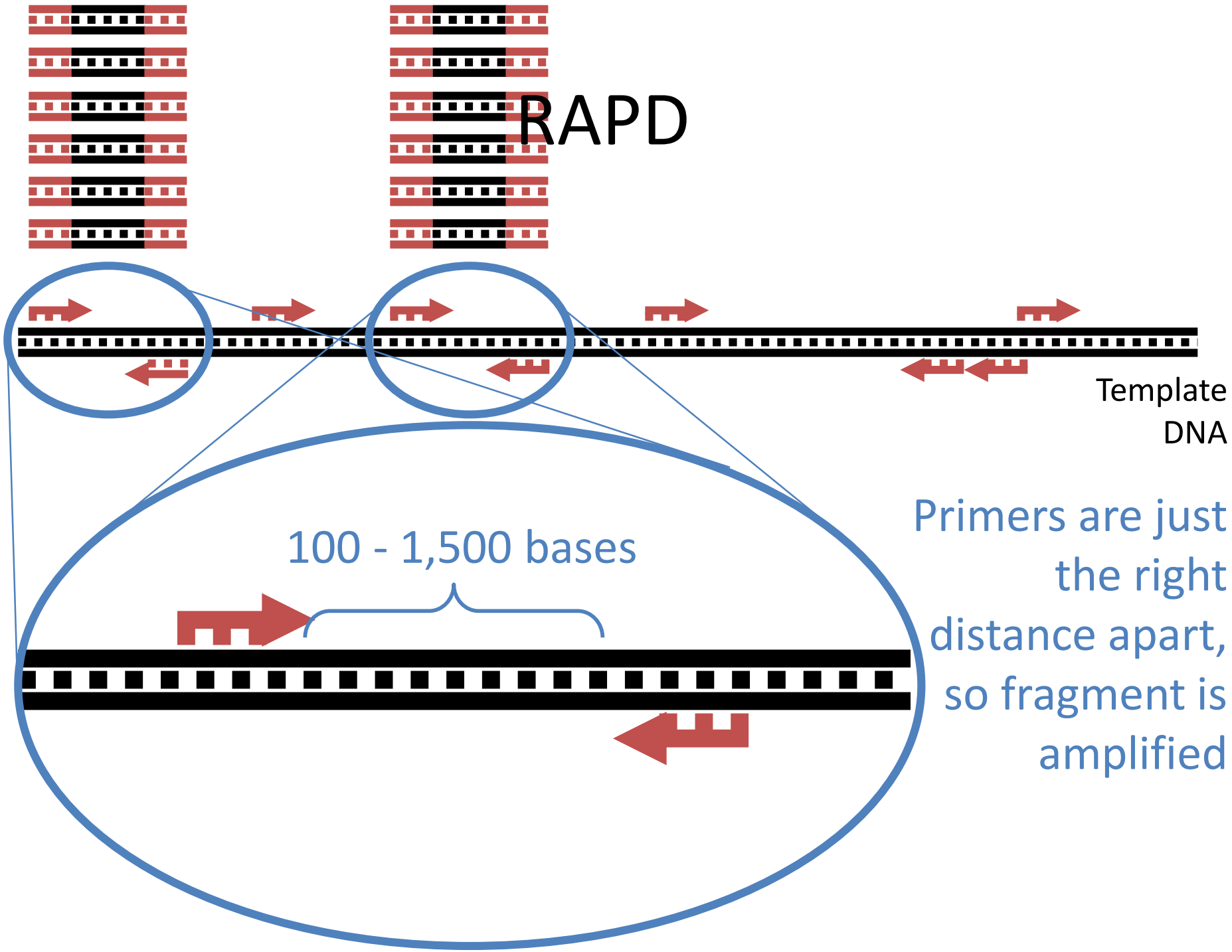
Primers point in the same direction, so amplification

RAPD



Primers too far apart, so amplification won't happen

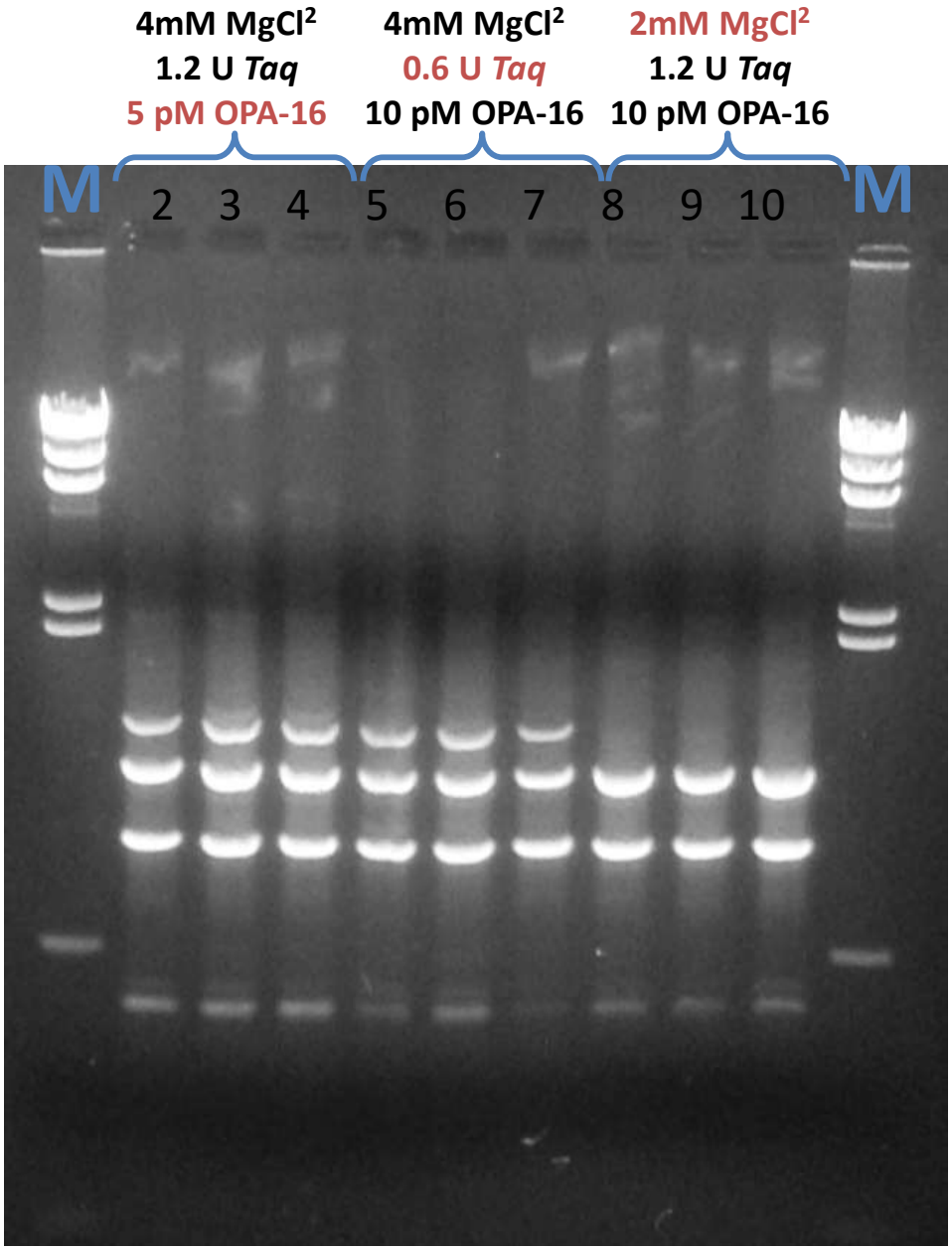
RAPD


Template DNA
100 - 1,500 bases
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 yellow text. M = A size standard



Which variable has the greatest impact on fragment patterns?

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

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 -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 Advantages Disadvantages

RFLP

- The variation(s) in the length of DNA fragments produced by a specific restriction endonuclease from genomic DNA s of two or more individuals of a species RFLP

- RFLP technology was first developed in the 1980s for use in human genetic applications and was later applied to plants. By digesting total DNA with specific restriction enzymes, an unlimited number of RFLPs can be generated. RFLPs are relatively small in size and are co-dominant in nature. If two individuals differ by as little as a single nucleotide in the restriction site, the restriction enzyme will cut the DNA of one but not the other. Restriction fragments of different lengths are thus generated. All RFLP markers are analyzed using a common technique. However, the analysis requires a relatively complex technique that is time consuming and expensive.

- The hybridization results can be visualized by Autoradiography (if the probes are radioactively labeled), or Chemiluminesence (if non-radioactive, enzyme-link methods are used for probe labeling and detection). Any of the visualization techniques will give the same results.

SSR

- Microsatellites can be amplified for identification by the polymerase chain reaction (PCR) process, using the unique sequences of flanking regions as primers DNA is repeatedly denatured at a high temperature to separate the double strand, then cooled to allow annealing of primers and the extension of nucleotide sequences through the microsatellite. This process results in production of enough DNA to be visible on agarose or polyacrylamide gels. With the abundance of PCR technology, primers that flank microsatellite loci are simple and quick to use, but the development of correctly functioning primers is often a tedious and costly process. Principle

- simple and easy to use -easy to detect via PCR -co-dominant marker -perfectly suited for used in map-based cloning -cost is higher for establishing polymorphic primer sites and investment in the synthesizing the oligonucleotides -initial identification, DNA sequence information necessary Advantages Disadvantages

- 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