Faculty of Science
Department of Botany,
Telangana University
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Paper: **Plant Molecular Biology, and Genetic Engineering**
Internal Assessment: II
Question Bank

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1. The molecular markers that are used to initiate chromosome walks are [c]
a. cDNA
b. Genomic Clones of genes
c. cDNA and Genomic Clones of genes
d. RNA
e. mRNA
2. Polymorphisms are simply variations in the length of [b]
a. RNA fragments
b. DNA fragments
c. Mitochondria
d. Ribosome
e. None of these
3. RFLP stands for [b]
a. Record of Fragment Length Polymorphisms
b. Restriction Fragment Length Polymorphisms
c. Restriction Frequency Length Polymorphisms
d. Restriction Fraternity Length Polymorphisms
e. Rearrangement of Fragment Length Polymorphisms
4. The small size of the ***Azabdiopsis thaliana*** genome makes it possible to construct complete physical maps for [a]
a. 5 chromosomes
b. 10 chromosomes
c. 15 chromosomes
d. 25 chromosomes
e. 55 chromosomes
5. RAPD stands for [b]
a. Rapid Action of PCR of di nucleotide
b. Random Amplification of Polymorphic DNA
c. Random Application of Polymorphic DNA
d. Random Activation of Polymorphic DNA
e. Random Action of Polymorphic DNA
6. Random Amplification of Polymorphic DNA is a type of [a]
a. PCR Reaction, but the segments of DNA that are amplified are random
b. PCR Reaction, but the segments of DNA that are amplified are not random
c. PCR Reaction, but the segments of RNA that are amplified are random
d. PCR Reaction, but the segments of RNA that are amplified are not random
e. None of these
7. The scientists performing RAPD creates several arbitrary short primers the proceeds with the PCR using [a]
a. A large template of genomic DNA
b. A small template of genomic DNA
c. A large template of genomic RNA
d. A small template of genomic RNA
e. None of these
8. RAPD is used to characterize and trace the phylogeny of diverse [c]
a. Plant species
b. Animal species
c. Plant and animal species
d. Bacteria species
e. Virus species
9. RAPD markers are [b]
a. Recessive
b. Dominant
c. Natural
d. Artificial
e. None of these
10. PCR is a cell free amplification technique for [a]
a. Synthesizing multiple identical copies
b. Systematic multiple identical copies
c. Synthetic multiple identical copies
d. Simulative multiple identical copies
e. Synthesizing multiple individual copies
11. From a single molecule of DNA PCR can make [d]
a. One additional copy
b. Hundred of copies

c. Thousands of copies
d. Millions of copies
e. None of these

12. Double stranded DNA is denaturated with specific temperature of [a]
a. 94°C
b. 44°C
c. 74°C
d. 54°C
e. 64°C

13. PCR is a [e]
a. DNA degradation technique
b. DNA apmplication technique
c. DNA sequencing technique
d. Polymerase chain reaction
e. All of the given

14. ***Thermos aquaticus*** is the source of [a]
a. Taq polymerase
b. Vent polymerase
c. DNA polymerase-III
d. RNA polymerase-III
e. None of these

15. The basic requirements of PCR reaction include [e]
a. DNA segmentary to amplified
b. Two aligo nucleotide primers
c. A heat stable DNA polymerase
d. dNTPs
e. all of these

16. The first step of PCR is [a]
a. denaturation
b. renaturation
c. annealing
d. primer extention
e. none of these

17. The last step of PCR is [d]
a. denaturation
b. renaturation
c. annealing
d. primer extention
e. none of these

18. Denaturation involves [a]
a. heating between 90°C to 98°C
b. heating between 50°C to 60°C
c. heating between 70°C to 80°C
d. heating between 20°C to 38°C
e. heating between 60°C to 70°C

19. Renaturation involves [b]
a. heating between 94°C to 95°C
b. heating between 50°C to 60°C
c. heating between 72°C to 75°C
d. heating between 90°C to 98°C
e. heating between 60°C to 70°C

20. DNA synthesis involves [c]
a. heating between 94°C to 95°C
b. heating between 50°C to 60°C
c. heating between 70°C to 80°C
d. heating between 90°C to 98°C
e. heating between 60°C to 70°C

21. The process of binding of primers to the denatured strand is called [b]
a. Denaturation
b. Annealing
c. Synthesis
d. Primer
e. None of these

22. PCR is used to [e]
a. Amplify gene of interest
b. Construct RAPD maps
c. Construct RFLP maps
d. Detect the presence of transgenic in an organism
e. All of these

23. Reverse transcriptase PCR uses [a]
a. mRNA as a template to from cDNA
b. RNA as a template to from DNA
c. mRNA as a template to from DNA

d. RNA as a template to from cDNA
e. None of these
24. Chemical synthesis of DNA was coined by [a]
a. Har Gobind Khorana
b. Letsinger
c. Ogilvie
d. Gilbert
e. Singar
25. Har Gobind Khorana DNA synthesis method is [a]
a. Diester method
b. Phosporamidite method
c. Phospodiester method
d. All of these
e. None of these
26. Phosporamidite method was coined by [b]
a. Lestinger and Ogilvie
b. Beaucage and Caruthers
c. Bentham and Hooker
d. Hooker and Ogilvie
e. None of these
27. Phospodiester method was coined by [a]
a. Lestinger and Ogilvie
b. Beaucage and Caruthers
c. Bentham and Ogilvie
d. Hooker and Hooker
e. None of these
28. The basic haploid chromosome set of an individual genes is [a]
a. Genome
b. Plerome
c. Codan
d. Plasmid
e. All of these
29. The transcription process of RNA nucleotide sequence is [a]
a. Reverse transcriptase
b. Transduction
c. Transcription
d. Adapter
e. None of these
30. The cell cycle phase during which DNA synthesis occurs is [a]
a. S phase
b. G phase
c. DNA phase
d. RNA phase
e. None of these
31. Conversion of a DNA information sequence into a mRNA sequence is known as [a]
a. Transcription
b. Transduction
c. Reverse transcriptase
d. Adapter
e. None of these
32. Introduction of foreign genetic material into a cell by means of a virus is called as [a]
a. Transduction
b. Transcription
c. Reverse transcriptase
d. Adapter
e. All of these
33. A synthetic double stranded oligo nucleotide used to attach sticky ends to blunt ended molecules is known as [a]
a. Adapter
b. Transduction
c. Transcription
d. Reverse transcriptase
e. All of these
34. The primers length of nucleotide sequence is [a]
a. 10-15 bases
b. 10-20 bases
c. 10-30 bases
d. 30-45 bases
e. 50-65 bases
35. Plant molecular markers are [a]
a. RFLP and RAPD
b. RFLP
c. RAPD

d. AFLP
e. None of these
36. RAPD can be used for identification of [e]
a. Somatic hybrids
b. Genome mapping
c. Genetic resources
d. monocytogenes
e. All of these
37. The PCR primers needs to be length of [a]
a. 18-30 nucleotides long
b. 28-38 nucleotides long
c. 48-60 nucleotides long
d. 68-80 nucleotides long
e. 90-98 nucleotides long
38. Required dNTPs for DNA synthesis [e]
a. ATP
b. GTP
c. TTP
d. CTP
e. All of these
39. The range of dNTPs normally used in DNA synthesis [a]
a. 20- 200µm
b. 20- 300µm
c. 20- 400µm
d. 20- 500µm
e. 20- 600µm
40. ELISA stands for [a]
a. Enzyme Linked Immuno Sorbent Assay.
b. Enzyme Liked Immuno Sorbent Assay.
c. Enzyme Linked Indo Sorbent Assay.
d. Enzyme Linked Immuno Servant Assay.
e. None of these
41. QTLs stands for [a]
a. Quantitative Trait Loci
b. Qualitative Trait Loci
c. Quantitative Treat Loci
d. Quantitative Trait Loading
e. Quantitative Target Loci
42. ESTs stands for [a]
a. Expressed Sequence Tags
b. Extracted Sequence Tags
c. Experienced Sequence Tags
d. Experiment Sequence Tags
e. None of these
43. Insulin hormone synthesized by β cells of [a]
a. Pancreas
b. Liver
c. Kidney
d. Lungs
e. All of the given
44. Klenow fragmrent of DNA polymerase-I that lack [b]
a. 3' exonuclease activity
b. 5' exonuclease activity
c. 3' endonuclease activity
d. 5' endonuclease activity
e. 7' exonuclease activity
45. The smallest functional unit of DNA that encodes a polypeptide chain is called [a]
a. Citron
b. Muten
c. Recon
d. Intron
e. Peptron
46. A segment of DNA that is tagged with a label is called [a]
a. DNA probe
b. RNA probe
c. Probe of mRNA
d. Probe of tRNA
e. None of these
47. A short sequence of oligonucleotides that hybridizes with template stand and provides initiation for the nuclic acid synthesis is [a]
a. Primer
b. Provider
c. Probe

d. Fragment
e. Template
48. A labeled molecule used in hybridization technique is [a]
a. Probe
b. Primer
c. Template
d. Fragment
e. None of these
49. An expressed sequence tag is a [a]
a. Short sub-sequence of cDNA
b. short sub-sequence of RNA
c. short sub-sequence of DNA
d. short sub-sequence of RNA and DNA
e. None of these
50. A quantitative trait is done using molecular tags such as [a]
a. AFLP
b. RFLP
c. RAPD
d. AMP
e. None of these

1. The agency responsible for regulating products of biotechnology is (A)
A. CFIA B. BSI C. ICRISAT D. AIIMS E. None of these
2. When an organ can be taken from transgenic animal bearing homology of man, then it is called as (B)
A. Xanthotrasplantation B. Xenotransplantation C. Zenotransplantation D. Orthotransplantation E. All of these
3. Antibodies present in transgenic plants are called as (A)
A. Plantibodies B. Aminoibodies C. Pleurobodies D. Probodies E. None of these
4. The reporter gene that glows in dark when transferred into plants is (B)
A. Chloramphenicol acetyl transferase B. Luciferase C. Neomycin phosphotransferase D. Glucoronidase E. None of these
5. To express human milk protein, beta casein can be introduced into a plant which could be edible such as in (A)
A. Potato B. Brinjal C. Tomato D. Carrot E. All of these
6. The gene that is responsible to convert cellulose to ethanol is (B)
A. Nopaline synthase B. cellulose synthase C. octapine synthase D. phosphosynthase E. All of these
7. Through transgenics agronomic traits can be improved by producing plants with increased resistance to (E)
A. Pests B. disease C. Stress D. drought E. All of these
8. The reporter gene that breaks down glucoronides giving a coloured reaction is (A)
A. Gus B. Npt C. Cat D. lux E. All of these
9. The disarmed Ti plasmid which is used as vector for gene transfer in dicots is obtained from (A)
A. Agrobacterium tumifaciens B. Bacillus thuringenesis C. Salmonella D. Vibrio E. None of these
10. One of the best studied plant promoter is (A)
A. 35S B. 37S C. 39S D. 32S E. None of these
11. Biolistics is shooting of DNA into plant cells coated with (A)
A. Tungsten or gold B. palladium or gold C. copper or gold D. silver or gold E. none of these
12. Gene transfer method for generating transgenic plants is (E)
A. Agrobacterium mediated B. electroporation C. Biolistics D. particle bombardment. E. All of these
13. The disease caused by Agrobacterium tumifaciens is (A)
A. Crown gall B. hairy root C. red rot D. wilt E. smut
14. The size of Ti plasmid is (A)
A. 210kb B. 220kb C. 240kb D. 430kb E. 480kb
15. The phenolic compound that acts as inducer of vir genes is (A)
A. Acetosyringone B. acetoorcein C. flavonoids D. acetoflavone E. None of these
16. The antibiotic which is most widely used as selective agent in plants is (A)
A. Kanamycin B. ampicillin C. rifamycin D. tetracycline E. erthromycine
17. The gene that is involved in catabolism of nopaline is (A)
A. Noc B. Nos C. Tum D. Ori E. Vir
18. The target cell for gene transfer method in plants is (E)
A. Protoplasts B. meristem cells C. shoots D. pollen E. All of these
19. The selectable marker gene imparting resistance to kanamycin is (B)
A. Cat B. Npt II C. Npt I D. Gus E. None of these
20. Cereals that are generated in vitro by somatic embryogenesis are (E)
A. Wheat B. barley C. sorghum D. Corn E. All of these
21. The major abiotic stress in agriculture is (B)
A. Water stress B. Salt stress C. Temp. stress D. Heat stress E. None of these
22. Identify salt stress plant from the following (A)
A. Carrot B. potato C. brinjal D. onion E. None of these

23. Transformation of chloroplast genome by organogenesis was achieved in (C)
A. Arabidopsis B. potato C. tomato D. tobacco E. All of these
24. The explants utilized for gene transfer are (E)
A. Tissue slices B. leaf discs C. section of roots D. floral tissue E. All of these
25. The disease caused by Agrobacterium rhizogenes is (A)
A. Hairy root B. wilt C. smut D. red-rot E. None of these

1. For the first time in 1955, Michelson chemically synthesized a dinucleotide in laboratory.

2. 1970, Har Govind Khorana and K.L. Agarwal for the first time chemically synthesized gene coding for tyrosine tRNA of yeast.

3. Genes of tRNA are the smallest genes containing about 80 nucleotides

4. In 1965, Robert W. Holley and coworkers worked out first the molecular structure of yeast alanine tRNA

5. Presence of transposable elements was first predicted by Barbara McClintock in maize (corn)

6. The controlling elements were later on called as transposable elements by Alexander Brink.

7. A transposon may be defined as: "a DNA sequence that is able to move or insert itself at a new location in the genome.

8. Transposon tagging is based on the use of mobile genetic elements to disrupt gene function through insertion in or near coding sequences.

9. The polymerase chain reaction **(**PCR) is used to amplify a precise fragment of DNA from a complex mixture of starting material usually termed as template DNA

10. Helix De-stabilisers / Additives of PCR: Dimethyl sulphoxide (DMSO), Dimethyl form amide (DMF), Urea or Form amide

11. Cycle Number is the number of amplification cycles necessary to produce a band visible on a gel depends largely on the starting concentration of the target DNA.

12. Elongation Temperature and Time is normally 70-72°C, for 0.5-3 min.

13. The reverse primer sequence was as follows TCGAATTCNCCYAAYTGNCCNT where Y = T + C, and N = A + G + C + T

14. Formula for calculation of the T_m is T_m = 4(G + C) + 2(A + T)°C

15. The specific complementary association due to hydrogen bonding of single-stranded nucleic acids is referred to as **"**annealing"

16. Randomly amplified polymorphic DNA (RAPD) was developed by William et al. (1990) to detect genetic polymorphism in crop plants.

17. RAPD reaction is based on the principle of polymerase chain reaction (PCR), an enzymatic assay for amplification of specific DNA segments in the target DNA

18. Quantitative trait locus (QTL) mapping is a genome-wide inference of the relationship between genotype at various genomic locations and phenotype for a set of quantitative traits in terms of the number, genomic positions, effects and interactions

19. The primary purpose of QTL mapping is to localize chromosomal regions that significantly affect the variation of quantitative traits in a population.

20. Data for mapping QTL consist of types of a number of polymorphic genetic markers and quantitative trait values for a number of individuals.

21. Based on segregation analysis, these markers can be ordered in linkage groups or linearly on chromosomes to represent a genetic linkage map.

22. Traditional experimental designs for locating QTL start with two parental lines differing both in trait values and in the marker variants they carry.

23. QTL mapping is a method utilized to define the general chromosomal position of genes or genetic variants that influence the magnitude of a measurable trait.

24. Most measurable or quantitative traits are considered complex in that they are determined by the influence of multiple genes that function independently and that also interact with each other and with the environment.

25. Molecular markers have been employed for genetic diversity studies in many crop species

26. Markers have provided information on the ecological or geographic patterns of diversity distribution in numerous crops and their wild relatives

27. Molecular markers have also been used to evaluate the effectiveness of different conservation strategies on the genetic structure of populations

28. Molecular markers have provided insight into the identification of crop progenitors, origins of domestication, and the molecular changes underlying domestication traits

29. Introgression between wild populations and cultivated plants is a widespread phenomenon with 12 of the 13 most important food crops of the world hybridizing with wild relatives in some regions of their agricultural distribution

30. Molecular markers are of considerable value in first identifying populations for collecting by developing optimum sampling strategies for gene banks

31. Currently, less than 30% of the 7.4 million plant germplasm accessions held in gene banks worldwide

32. Periodic regeneration and multiplication are essential features of gene bank management

33. Molecular markers can also be employed to verify accession identity, detect inadvertent seed mixtures, and monitor changes in alleles/allele frequencies as well as gene flow between accessions

34. The Germplasm Resource Information System Global Project, a partnership between the Global Crop Diversity Trust, Bioversity International and the Agricultural Research Service of the United States Department of Agriculture

35. Plastid transgenes often give high expression levels, can be stacked in operons and are largely excluded from pollen transmission

36. Plant cells have three genomes and, in some seed plants, two of these genomes are transformable: the nuclear genome and the genome of the plastids (chloroplasts).

37. The plastid genome of photosynthetically active seed plants is a small circularly mapping genome of 120–220 kb, encoding 120–130 genes.

38. Transgene integration into the plastid genome occurs exclusively by homologous recombination, making plastid genome engineering a highly precise genetic engineering technique for plants

39. The plastid genetic system is devoid of gene silencing and other epigenetic mechanisms that interfere with stable transgene expression.

40. Many plastid genes are arranged in operons offering the possibility to stack transgenes by arranging them in artificial operons.

41. Plastid transformation has received significant attention as a superb tool for transgene containment due to the maternal mode of plastid inheritance in most angiosperm species, which drastically reduces transgene transmission through pollen

42. The transplastomic technology has been extensively used to insert resistance genes into the plastid genome (making plants tolerant to herbicides or resistant to insect pests), express recombinant proteins for molecular farming (e.g. vaccines) and engineer metabolic pathways.

43. Chloroplasts are ideal hosts for the expression of transgenes

44. Chloroplast DNA sequences that flank transgenes, higher plants have efficiently and stably integrated transgenes imbuing important agronomic traits, including herbicide, insect and disease resistance, drought and salt tolerance, and phytoremediation.

45. Highly efficient, soybean, carrot and cotton plastid transformation have been accomplished via somatic embryogenesis using species-specific vectors

46. Chloroplast transgenic carrot plants withstand salt concentrations that only halophytes could tolerate

47. Vaccines preventing anthrax, plague, tetanus and cholera, and pharmaceuticals like human somatotropin, serum albumin, interferons and insulin-like growth factor have been produced in transgenic chloroplasts.

48. The chloroplast also contains machinery that allows for correct folding and disulfide bond formation, resulting in fully functional human blood proteins or vaccine antigens.

49. *Agrobacterium tumefaciens* are used to generate transgenics

50. Plant transformation used as a means of isolating genes from plants by gene tagging and this may provide a means of isolating plant genes of agronomic value.

Applications of Transgenic Plants

- **Resistance to biotic and abiotic stress:** Biotic stress is imposed on plants as a result of the action of living beings such as viruses, bacteria, pests and pathogens. To relieve the plants from such stress they are incorporated with disease-resistant genes, which gives a better yield and quality to the crops.
- Abiotic stress, as a result of changes in the environment, causes great damage to the plants. Soil composition, humidity, water level, and temperature are important factors for plant growth. Due to changes in the climate, all the factors seem to be altered. Thus, plants are incorporated with stress-tolerant genes for better production.
- **Increased nutritional value:** Biofortification is the process of increasing the nutritional value of a crop. Malnutrition is a common problem in developing countries. As a solution, plants are engineered to produce crops of better nutritional value.
- **Factories for production of recombinant proteins:** Recombinant human proteins have been produced using animal and microorganism systems, but due to some shortcomings it has been shifted to the plant system. Vaccines and antibiotics have been obtained from transgenic plants. However, this application is still in the development stage and has not been commercialised yet.

Examples of Transgenic Plants

- **Golden rice:** Golden rice was produced to overcome the deficiency of vitamin A in children. Using the gene gun methods, rice species were incorporated with the phytoene synthase genes, which increases the vitamin A content of the rice grains.
- **Bt cotton:** Bt cotton is a genetically modified crop that is resistant to pest bollworm.
- **Flavr Savr:** Flavr Savr is a genetically modified tomato crop that has a longer shelf life due to delays in ripening and softening.

Transgenic plant and Methods Used for Gene Transfer

A transgenic plant is a modified organism where genes are transferred from one organism to another through genetic engineering techniques".

The purpose of producing a transgenic plant is to obtain a species that has ideal traits, high yield and quality.

Methods Used for Gene Transfer

There are two methods majorly which are used to transfer genes in plants. The two methods include:

1. *Agrobacterium* mediates gene transfer

Agrobacterium tumefaciens is a plant pathogen. It is known to cause crown gall disease, which is swelling in plants just above the soil level. After infecting the plants, they transfer their genetic material to them, which eventually gets incorporated into the plant genome.

For genetic engineering, the bacterium is incorporated with a Ti plasmid with desirable genes and made to infect the plant.

The Ti plasmid is a tumour inducing circular plasmid that transfers the host chromosomes to the plants and is also responsible for causing the swelling.

2. Particle bombardment / Gene gun method

As the name suggests, in this method, the desired gene is coated in a gold or tungsten particle and bombarded into the plant cells. Once bombarded, the sequence is incorporated into the plant cells, which can be proliferated by tissue culture methods.

Advantages of Chloroplast genetic engineering

Advantages of Chloroplast genetic engineering offers a number of unique advantages, including a high-level of transgene expression, multi-gene engineering in a single transformation event, transgene containment via maternal inheritance, lack of gene silencing, position and pleiotropic effects, and undesirable foreign DNA. Over forty transgenes have been stably integrated and expressed via the tobacco chloroplast genome to confer important agronomic traits, as well as express industrially valuable biomaterials and therapeutic proteins. The hyperexpression of recombinant proteins within plastid engineered systems offers a cost effective solution for using plants as bioreactors.

Chloroplast Genome:

Most of the higher plants have about 100 chloroplasts per leaf cell. Each chloroplast contains approximately 100 copies of chloroplast DNA genome. The chloroplast genome (the plastome) is a circular double-stranded DNA molecule (or chromosome) located in the stroma. Majority of chloroplast genomes are in the size of 120-1 60 kbp and contain about 120-140 genes. About 100 chloroplast genes are known to code for proteins. The protein synthesis in chloroplasts resembles that of prokaryotes.

Chloroplast Engineering:

Genetic engineering of chloroplast that leads to chloroplast (plastid) transformation is an important and exciting field in modern biotechnology as it offers the following advantages:

1. Chloroplasts are maternally inherited; hence there is no danger of gene transfer through pollen to related weeds. This is because pollen does not contain transgenes.

2. Multi-gene transfer can be conveniently carried out in chloroplasts which is rather difficult with nuclear genome.

3. Chloroplasts genome is functionally comparable to prokaryotic genome. A single promoter can control the expression of group of genes (transgenes). It is therefore possible to introduce desirable multiple genes which can be expressed under the control of a single promoter.
4. High level of transgene expression is possible with chloroplasts. There are about 100 chloroplasts per cell, each containing about 100 copies of genome. Thus, there is possibility of 10,000 copies of transgenes per cell! This is a tremendous number of transgenes carried by transformed chloroplasts. There is a tremendous potential for a very high level of gene expression and large scale production of active proteins.
5. Chloroplast transformation is not associated with gene silencing which is a major problem with nuclear genome transformation.
6. Antibiotic resistance genes need not be used as selectable markers. Even if used, they can be easily excised.
7. Toxicity associated with foreign protein production in chloroplasts is much less when compared to nuclear-controlled foreign proteins.

Design of Vectors for Chloroplast Transformation:
A diagrammatic representation of two vector constructs for chloroplast transformation is depicted in Fig. 49.13.
1. A construct for expression of a single gene:
The vector for chloroplast transformation is based on the selectable marker gene aadA that provides resistance to antibiotic spectinomycin. The single foreign (desirable) gene is fused to regulatory sequences (promoter and terminator) which in turn is flanked on either side by chloroplast DNA (Cp DNA) (Fig. 49.13A).
2. A construct for expression of multiple genes:
In this case, -the selectable marker is the betaine-aldehyde dehydrogenase (badh) gene. It is flanked by a promoter and the multiple transgenes are flanked by a terminator. At both ends chloroplast DNA sequences are present. In between the transgenes, these are ribosome-binding sites (one between two transgenes) to ensure efficient translation (Fig. 49.13B).
Introduction of Foreign Genes into Chloroplast Genome:
Most of the methods used for introducing the foreign genes into nuclear genome are not useful for chloroplast transformation. The most successful method for inserting foreign genes into chloroplasts is particle gun bombardment.
After the bombardment, homologous recombination occurs between the chloroplast DNA sequences on the vector and those of on the genome. This is a site-specific integration and thus avoids the frequent problems associated with random insertion of foreign genes into nuclear genome. The regenerated plants derived from the modified plastome (chloroplast genome) are regarded as transplastomic plants.

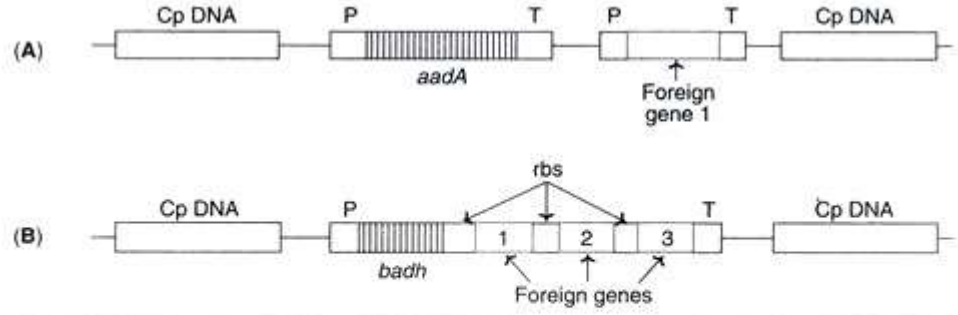

*Fig. 49.13 : A diagrammatic representation of vectors for chloroplast transformation (**A**) A construct designed for a single foreign gene (**B**) A construct designed for multiple foreign genes (Cp DNA-Chloroplast DNA; P-Promoter; aadA-A selectable marker gene that confers resistance to antibiotic spectinomycin; T-Terminator; badh-A selectable marker gene encoding betaine aldehyde dehydrogenase; rbs-Ribosome binding site).*

The Future of Chloroplast Transformation:
The technology of chloroplast transformation is in the developing stages. In fact, it has not become as routine as transformation of nuclear genomes of plants. Chloroplast engineering, however, holds a great promise in plant biotechnology being an efficient, clean and environmental-friendly approach for the production of transgenic plants.

Gene Synthesis
The complete procedure of synthesizing gene for yeast alanine tRNA is discussed in the following steps:
1. Synthesis of Oligonucleotides:
In the first approach, fifteen oligonucleotides ranging from penta-nucleotide (i.e. oligodeoxynucleotide of five bases) to an icosanucleotide (i.e. oligodeoxynucleotide of twenty bases) were synthesized.
The chemical synthesis was brought about through condensation between the -OH group at 3′ position of one deoxynucleotide and the $-PO_4$ group at 5′ position of the second deoxynucleotide. All other functional groups of deoxyribonucleotides not taking part in condensation processes were protected so that the condensation could be brought about.
2. Synthesis of Three Duplex Fragments of a Gene:
By using 15 single stranded oligonucleotides, three large double stranded DNA fragments were synthesized
3. Synthesis of a Gene from Three Duplex Fragments of DNA: The three segments (A, B, C) synthesized as above were joined by using the enzyme polynucleotide ligase to produce the complete gene for alanine tRNA

Gene Machine:

Recently, fully automated commercial instrument called automated polynucleotide synthesizer or gene machine is available in market which synthesizes predetermined polynucleotide sequence.

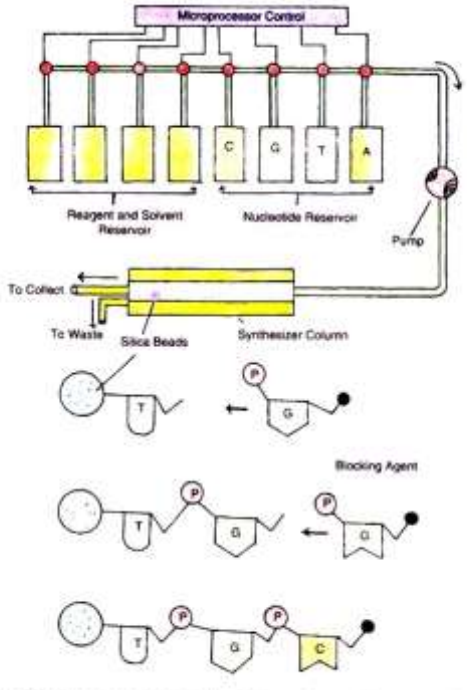

Fig. 6.9 : A gene machine and its working mechanism (diagrammatic)

Therefore, the genes can be synthesized rapidly and in high amount. For example, a gene for tRNA can be synthesized within a few days through gene machine. It automatically synthesizes the short segments of single stranded DNA under the control of microprocessor.

Working principle of a gene machine

The working principle of a gene machine includes:

(i) Development of insoluble silica-based support in the form of beads which provides support for solid phase synthesis of DNA chain, and

(ii) Development of stable deoxyribonucleoside phosphoramidites as synthons which are stable to oxidation and hydrolysis, and ideal for DNA synthesis.

The mechanism of a gene machine is shown in Fig. 6.9. Four separate reservoirs containing nucleotides (A, T, C and G) are connected with a tube to a cylinder (synthesiser column) packed with small silica beads. These beads provide support for assembly of DNA molecules. Reservoirs for reagent and solvent are also attached. The whole procedure of adding or removing the chemicals from the reagent reservoir in time is controlled by microcomputer control system i.e. microprocessor.

If one desires to synthesise a short polynucleotide with a sequence of nucleotides T, G, C, the cylinder is first filled with beads with a single 'T' attached. Thereafter, it is flooded with 'G' from the reservoir. The right hand side of each G is blocked by using chemicals from the reservoir so that its attachment with any other Gs can be prevented.

The remaining Gs which could not join with Ts are flushed from the cylinder. The other chemicals are passed from the reagent and solvent reservoirs so that these can remove the blocks from G which is attached with the T. In the same way this cycle is repeated by flooding with C from reservoir into the cylinder. Finally, the sequence TGC is synthesized on the silica beads which are removed chemically later on.

The desired sequence is entered on a key board and the microprocessor automatically opens the valve of nucleotide reservoir, and chemical and solvent reservoir. In the gene machine the nucleotides are added into a polynucleotide chain at the rate of two nucleotides per hour. By feeding the instructions of human insulin gene in gene machine insulin has been synthesized.

Stages in PCR:

It has three definite sets of times and temperature, termed as

Step I: Denaturation

Step II: Annealing and

Step III: Extension.

Each of the three steps are repeated 30-40 times or cycles. In first cycle the double stranded template DNA strand is first denatured by heating the reaction to above 90°C so that the region to be specifically amplified can be made accessible. The temperature is then cooled to between 40-60°C. The precise temperature is critical and each PCR system has to be defined and optimized. Reactions that are not optimized may give rise to other products along with amplified target sequence or even may not produce amplified product.

The second annealing step allows the hybridization of the two oligonucleotide primers, present in excess to bind to their complimentary sites which flank the DNA. The annealed oligonucleotide acts as a primer since they provide free 3'-end hydroxyl group to DNA polymerase. The third step, DNA synthesis or extension is carried out by a thermo stable DNA polymerase, most commonly Taq DNA polymerase.

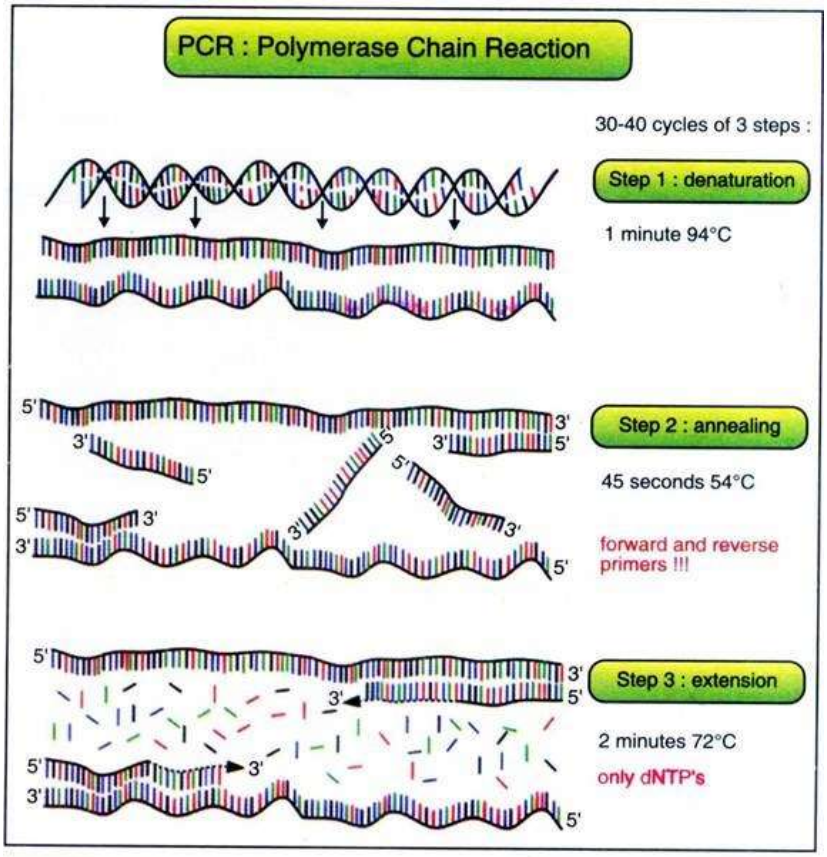

Fig. 3.10: Different Stages of PCR

DNA synthesis proceeds from both primers until new strands have been extended along and beyond the target DNA to be amplified. It is important to note that since the new strand extends beyond the target DNA, they will contain a region next to their 3′-end that is complimentary to the other primer. Thus in another round not only parental target strand but also newly synthesized strands also act as template DNA.

Primer Design in PCR:
The principle features o ideal primers are that these not only have to be complementary to the flanking sequence of the target DNA but must not be self-complementary otherwise they will form primer dimmers. Both primers have to be matched in their G+C content and should have similar annealing temperature.
It is also possible to design primer with additional sequences at their 5′-ends, such as restriction endonuclease target site or promoter sequences. However, these require alteration in annealing temperature to compensate for areas of non-homology.

PCR Reaction Buffer:
i. 10-50 mM Tris-HCl pH 8.3,
ii. Up to 50 mM KCl, 1,5mM or higher $MgCl_2$,
iii. Primers 0.2-1 µM each primer,
iv. 50-200 µM each dNTP,
v. Gelatin or BSA to 100 µg/ml,
vi. And/or non-ionic detergents such as Tween-20 or Nonidet P-40 or Triton X-100 (0.05- 0.10% v/v)

Applications for PCR:
1. The labelling of gene probes is one such area which has traditionally been undertaken by techniques such as nick translation. The nature of PCR makes it an ideal method for gene probe production and labelling.
2. Important modification of PCR known as quantitative PCR by which the initial concentration of template DNA can be estimated and is very useful for the measurement of, for example, a virus or an mRNA for protein expressed in abnormal amount in a disease process. Early quantitative PCR methods involved the comparison of a standard or control DNA template amplified with separate primers at the same time as the specific target DNA.
These kind of quantification rely on the reaction being exponential and so any factor affecting this may also affect the result. Other method involves the incorporation of radiolabel through primer and their detection in subsequent purification of PCR products.
3. One of the most general applications of development of PCR is direct PCR sequencing. This traditionally involves the cloning of sequences into vectors developed for chain termination sequencing. However, rapid accumulation of PCR products allows nucleotide sequence information to be obtained very quickly.

1. Allele-specific PCR:
AS-PCR is used to determine the genotype of single-nucleotide polymorphisms (SNPs) (single base differences in DNA) by using primers whose ends overlap the SNP and differ by that single base. PCR amplification is less efficient in the presence of a mismatch, so the differences in amplification resulting from different primers can be used to quickly determine which primer matches the sample genotype.

2. Assembly PCR:
Assembly PCR is the completely artificial synthesis of long gene products by performing PCR on a pool of long oligonucleotides with short overlapping segments. The oligonucleotides alternate between sense and antisense directions and the overlapping segments serve to order the PCR fragments so that they selectively produce their final product.

3. Asymmetric PCR:
Asymmetric PCR is used to preferentially amplify one strand of the original DNA more than the other. It finds use in some types of sequencing and hybridization probing where having only one of the two complementary stands is required. PCR is carried out as usual, but with a great excess of the primers for the chosen strand. Due to the slow (arithmetic) amplification later in the reaction after the limiting primer has been used up, extra cycles of PCR are required. A recent modification on this process, known as Linear-After-The-Exponential-PCR (LATE-PCR), uses a limiting primer with a higher melting temperature (TJ than the excess primer to maintain reaction efficiency as the limiting primer concentration decreases mid-reaction.

4. Colony PCR:
Bacterial clones (E. coli) can be rapidly screened for correct DNA vector constructs. Selected bacterial colonies are picked with a sterile toothpick from an agarose plate and dabbed into the master mix or sterile water. Primers (and the master mix) are added, and the PCR is started with an extended time at 95°C when standard polymerase is used or with a shortened denaturation step at 100°C and special chimeric DNA polymerase.

5. Helicase-dependent amplification:
Similar to traditional PCR, but maintains a constant temperature rather than cycling through denaturation and annealing/extension cycles. Helicase, an enzyme that unwinds DNA, is used in place of thermal denaturation.

6. Hot-start PCR:
Hot-start PCR is a technique that reduces non-specific amplification during the initial set up stages of the PCR. The technique may be performed manually by simply heating the reaction components briefly at the melting temperature (e.g., 95°C) before adding the polymerase.
Specialized enzyme systems have been developed that inhibit the polymerase's activity at ambient temperature, either by the binding of an antibody or by the presence of covalently bound inhibitors that only dissociate after a high-temperature activation step. Hot-start/cold-finish PCR is achieved with new hybrid polymerases that are inactive at ambient temperature and are instantly activated at elongation temperature.

7. Inter-sequence specific (ISSR) PCR:
A PCR method for DNA fingerprinting that amplifies regions between some simple sequence repeats to produce a unique fingerprint of amplified fragment lengths.

8. Inverse PCR:
Inverse PCR is a method used to allow PCR when only one internal sequence is known. This is especially useful in identifying flanking sequences to various genomic inserts. This involves a series of DNA digestions and self-ligation, resulting in known sequences at either end of the unknown sequence.

9. Ligation-mediated PCR.

10. Methylation Specific PCR:
Methylation Specific PCR (MSP) is used to detect methylation of CpG islands in genomic DNA. DNA is first treated with sodium bi-sulphite, which converts un-methylated cytosine bases to uracil, which is recognized by PCR primers as thymine. Two PCR reactions are then carried out on the modified DNA, using primer sets identical except at any CpG islands within the primer sequences.
At these points, one primer set recognizes DNA with cytosine's to amplify methylated DNA, and one set recognizes DNA with uracil or thymine to amplify un-methylated DNA. MSP using qPCR can also be performed to obtain quantitative rather than qualitative information about methylation.

11. Multiplex Ligation-dependent Probe Amplification (MLPA):
Permits multiple targets to be amplified with only a single primer pair, thus avoiding the resolution limitations of multiplex PCR (see below).

12. Multiplex-PCR:
The use of multiple, unique primer sets within a single PCR reaction to produce amplicons of varying sizes specific to different DNA sequences. By targeting multiple genes at once, additional information may be gained from a single test run that otherwise would require several times the reagents and more time to perform. Annealing temperatures for each of the primer sets must be optimized to work correctly within a single reaction, and amplicon sizes, i.e., their base pair length, should be different enough to form distinct bands when visualized by gel electrophoresis.

13. Nested PCR:
Nested PCR increases the specificity of DNA amplification, by reducing background due to non-specific amplification of DNA. Two sets of primers are being used in two successive PCR reactions. In the first reaction,

one pair of primers is used to generate DNA products, which besides the intended target, may still consist of non- specifically amplified DNA fragments.
The product(s) (sometimes after gel purification after electrophoresis of the PCR product) are then used in a second PCR reaction with a set of primers whose binding sites are completely or partially different from the primer pair used in the first reaction, but are completely within the DNA target fragment. Nested PCR is often more successful in specifically amplifying long DNA fragments than conventional PCR, but it requires more detailed knowledge of the target sequences.

14. Quantitative PCR:
Q-PCR (Quantitative PCR) is used to measure the quantity of a PCR product (preferably real-time). It is the method of choice to quantitatively measure starting amounts of DNA, cDNA or RNA. Q-PCR is commonly used to determine whether a DNA sequence is present in a sample and the number of its copies in the sample.
The method with currently the highest level of accuracy is Quantitative real-time PCR. It is often confusingly known as RT-PCR (Real Time PCR) or RQ-PCR. QRT-PCR or RTQ-PCR is more appropriate contractions. RT-PCR commonly refers to reverse transcription PCR (see below), which is often used in conjunction with Q- PCR.
QRT-PCR methods use fluorescent dyes, such as Sybr Green, or fluorophore- containing DNA probes, such as TaqMan, to measure the amount of amplified product in real time.

15. RT-PCR:
RT-PCR (Reverse Transcription PCR) is a method used to amplify, isolate or identify a known sequence from a cellular or tissue RNA. The PCR reaction is preceded by a reaction using reverse transcriptase to convert RNA to cDNA.
RT-PCR is widely used in expression profiling, to determine the expression of a gene or to identify the sequence of an RNA transcript, including transcription start and termination sites and, if the genomic DNA sequence of a gene is known, to map the location of exons and introns in the gene. The 5’ end of a gene (corresponding to the transcription start site) is typically identified by a RT-PCR method, named RACE-PCR, short for Rapid Amplification of cDNA Ends.

16. TAIL-PCR:
Thermal asymmetric interlaced PCR is used to isolate unknown sequence flanking a known sequence. Within the known sequence TAIL-PCR uses a nested pair of primers with differing annealing temperatures; a degenerate primer is used to amplify in the other direction from the unknown sequence.

17. Touchdown PCR:
Touchdown PCR is a variant of PCR that aims to reduce nonspecific background by gradually lowering the annealing temperature as PCR cycling progresses. The annealing temperature at the initial cycles is usually a few degrees above the T_m of the primers used, while at the later cycles, it is a few degrees below the primer T_m. The higher temperatures give greater specificity for primer binding, and the lower temperatures permit more efficient amplification from the specific products formed during the initial cycle.

Requirements of Random Amplified Polymorphic DNA (RAPD) Analysis:
1. RAPD random primers 5 p mol/1
2. dNTPs (dATP dGTP dCTP and dTTP) 10 mM
3. $MgCl_2$ 15 mM
4. Template DNA 25 ng/µl
5. PCR buffer10x (usually given with Taq polymerase).
6. Taq DNA polymerase. 5 U/µl
7. Agarose gel electrophoresis reagents.
8. Eppendorf tubes (1.5 and 0.5 ml).
9. PAGE or Agarose electrophoresis equipment.
10. Microcentrifuge.
11. Micropipettes (P_2, P_{20}, P_{200}, and P_{1000}).
12. 0.2 ml thin walled PCR tubes.
13. Gel drier.
14. —20°C deep freezer.
15. Refrigerator.
16. Photo documentation system.
17. Laminar clean air flow hood.
18. U.V. transilluminator.

Molecular Markers:
A molecular marker is a DNA sequence in the genome which can be located and identified. As a result of genetic alterations (mutations, insertions, deletions), the base composition at a particular location of the genome may be different in different plants.
These differences, collectively called as polymorphisms can be mapped and identified. Plant breeders always prefer to detect the gene as the molecular marker, although this is not always possible. The alternative is to have markers which are closely associated with genes and inherited together.
Molecular markers are of two types:
1. Based on nucleic acid (DNA) hybridization (non-PCR based approaches).
2. Based on PCR amplification (PCR-based approaches).

Restriction fragment length polymorphism (RFLP):

RFLP was the very first technology employed for the detection of polymorphism, based on the DNA sequence differences. RFLP is mainly based on the altered restriction enzyme sites, as a result of mutations and re-combinations of genomic DNA. An outline of the RFLP analysis is given in Fig. 53.2, and schematically depicted in Fig. 53.3.The procedure basically involves the isolation of genomic DNA, its digestion by restriction enzymes, separation by electrophoresis, and finally hybridization by incubating with cloned and labeled probes (Fig. 53.2).

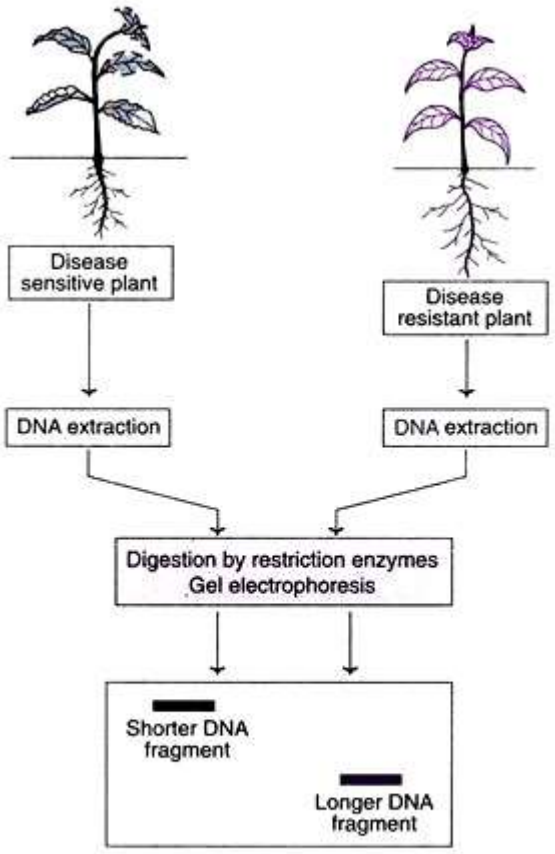

Fig. 53.1 : Basic principle of molecular marker detection (screening of genotypes for the identification of DNA markers).

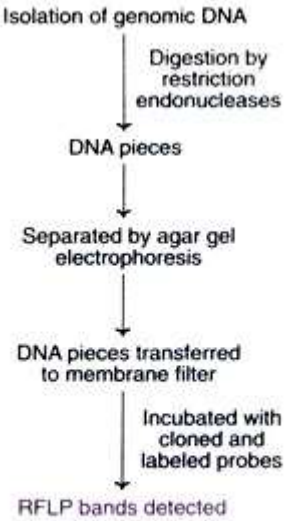

Fig. 53.2 : An outline of restriction fragment length polymorphism (RFLP) analysis as a molecular marker in plant breeding.

Based on the presence of restriction sites, DNA fragments of different lengths can be generated by using different restriction enzymes. In the Fig. 53.3, two DNA molecules from two plants (A and B) are shown. In plant A, a mutations has occurred leading to the loss of restriction site that can be digested by EcoRI.

Markers Based on PCR Amplification:

Polymerase chain reaction (PCR) is a novel technique for the amplification of selected regions of DNA .The advantage with PCR is that even a minute quantity of DNA can be amplified. Thus, PCR-based molecular markers require only a small quantity of DNA to start with.

PCR-based markers may be divided into two types:
1. Locus non-specific markers e.g. random amplified polymorphic DNA (RAPD); amplified fragment length polymorphism (AFLP).
2. Locus specific markers e.g. simple sequence repeats (SSR); single nucleotide polymorphism (SNP).

Random amplified polymorphic DNA (RAPD) markers:
RAPD is a molecular marker based on PCR amplification. An outline of RAPD is depicted in Fig. 53.4. The DNA isolated from the genome is denatured the template molecules are annealed with primers, and amplified by PCR.
Single short oligonucleotide primers (usually a 10-base primer) can be arbitrarily selected and used for the amplification DNA segments of the genome (which may be in distributed throughout the genome). The amplified products are separated on electrophoresis and identified.

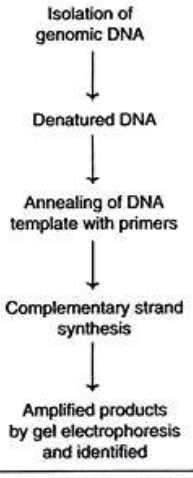

Fig. 53.4 : An outline of random amplified polymorphic DNA (RAPD) analysis as a molecular marker in plant breeding.

Amplified fragment length polymorphism (AFLP):
AFLP is a novel technique involving a combination of RFLP and RAPD. AFLP is based on the principle of generation of DNA fragments using restriction enzymes and oligonucleotide adaptors (or linkers), and their amplification by PCR. Thus, this technique combines the usefulness of restriction digestion and PCR.
The DNA of the genome is extracted. It is subjected to restriction digestion by two enzymes (a rare cutter e.g. MseI; a frequent cutter e.g. EcoRI). The cut ends on both sides are then ligated to known sequences of oligonucleotides (Fig. 53.5).

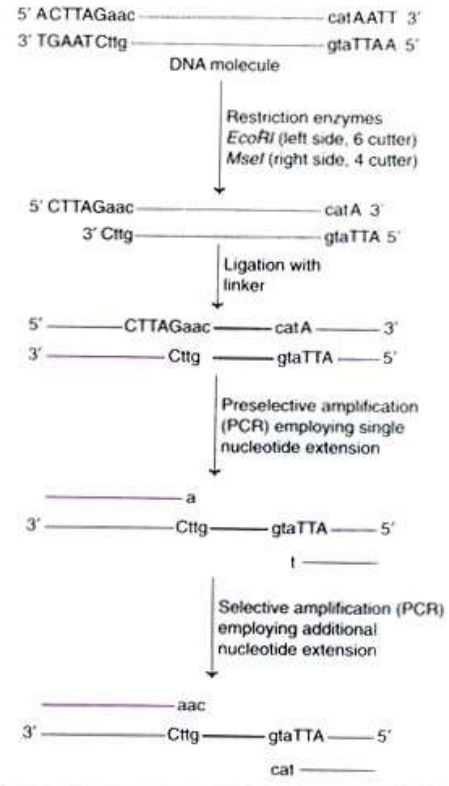

Fig. 53.5 : A diagrammatic representation of the amplified fragment length polymorphism (AFLP) (Note : The lower case letters represent the sequences found within the amplified region; the coloured lines indicate linkers).

Quantitative Trait Loci:
These are many characteristics controlled by several genes in a complex manner. Some good examples are growth habit, yield, adaptability to environment, and disease resistance. These are referred to as quantitative traits. The locations on the chromosomes for these genes are regarded as quantitative trait loci (QTL).

An expressed sequence tag (EST) is a short stretch of DNA sequence that is used to identify an expressed gene. Although EST sequences are usually only 200 to 500 nucleotides in length, this is generally sufficient to identify the full-length complementary DNA (cDNA). ESTs are generated by sequencing a single segment of random clones from a cDNA library. A single sequencing reaction and automation of DNA isolation, sequencing, and analysis have allowed the rapid determination of many ESTs. Now, the majority of the sequences in sequence databases are ESTs.

Generation of ESTs
The most important step in generating of ESTs is producing a cDNA library. First, messenger RNA (mRNA) is extracted from the material being studied and is used as a template by reverse transcriptase for cDNA synthesis. Then the DNA is cloned into a suitable vector to produce a cDNA library. Random clones are isolated from the library, and one or both ends are sequenced by single-pass sequencing.

Microprojectile Bombardment
Klein and colleagues (1987) discovered that naked DNA could be delivered to plant cells by "shooting" them with microscopic pellets to which DNA had been adhered. This is a crude but effective physical method of DNA delivery, especially in species such as corn, rice, and other cereal grains, which *Agrobacterium* does not naturally transform. Many GE plants in commercial production were initially transformed using microprojectile delivery.

Electroporation
In *electroporation,* plant protoplasts take up macromolecules from their surrounding fluid, facilitated by an electrical impulse. Cells growing in a culture medium are stripped of their protective walls, resulting in protoplasts. Supplying known DNA to the protoplast culture medium and then applying the electrical pulse temporarily destabilizes the cell membrane, allowing the DNA to enter the cell. Transformed cells can then regenerate their cell walls and grow to whole, fertile transgenic plants. Electroporation is limited by the poor efficiency of most plant species to regenerate from protoplasts.