FACULTY OF ENGINEERING &TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

INTRODUCTION

- RAPD markers are decamer DNA fragments.
- RAPD is a type of PCR reaction, Segments amplified are Random.
- No knowledge of DNA sequence required. Hence a popular method.
- In recent years, RAPD is used to Characterize, & Trace, the phylogeny of diverse plant & animal species.
- Identical 10-mer primer will or will not amplify a segment of DNA, depending on positions that are complementary to the primer sequence.

How it Works ?

- The principle is that, a single, short oligonucleotide primer, which binds to many different loci, is used to amplify random sequences from a complex DNA template.
- This means that the amplified fragment generated by PCR depends on the length and size of both the primer and the target genome.
- These amplified products (of up to 3.0 kb) are usually separated on agarose gels (1.5-2.0%) and visualised by ethidium bromide staining.

- Nucleotide variation between different sets of template DNAs will result in the presence or absence of bands because of changes in the priming sites.
- Recently, sequence characterised amplified regions (SCARs) analysis of RAPD polymorphisms showed that one cause of RAPD polymorphisms is chromosomal rearrangements such as insertions/deletions.
- In order for PCR to occur:
  1)the primers must anneal in a particular orientation (such that they point towards each other) and,
  2)they must anneal within a reasonable distance of one another.

FINDING DIFFERENCES BETWEEN GENOMES USING RAPD ANAL

- Consider the Figure 2 (genome A). If another DNA template (genome B) was obtained from a different (yet related) source, there would probably be some differences in the DNA sequence of the two templates. Suppose there was a change in sequence at primer annealing site #2

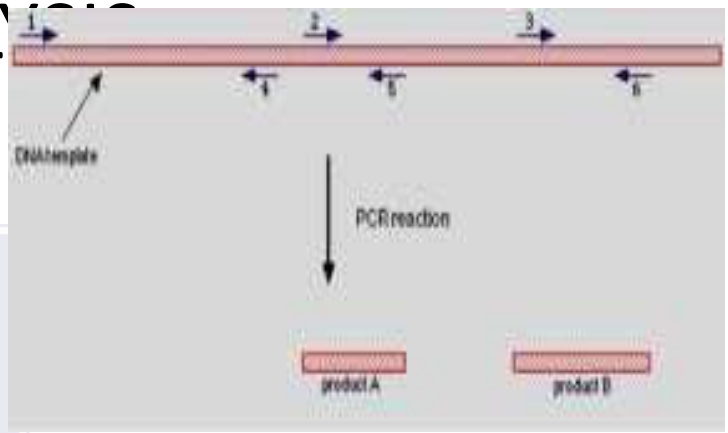


Figure 2 (genome A)

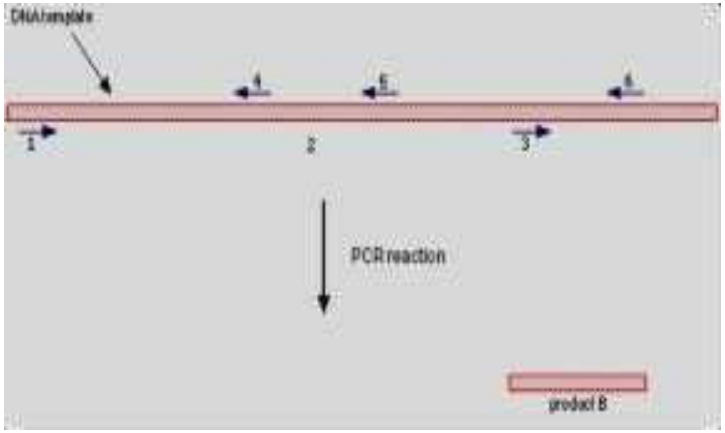
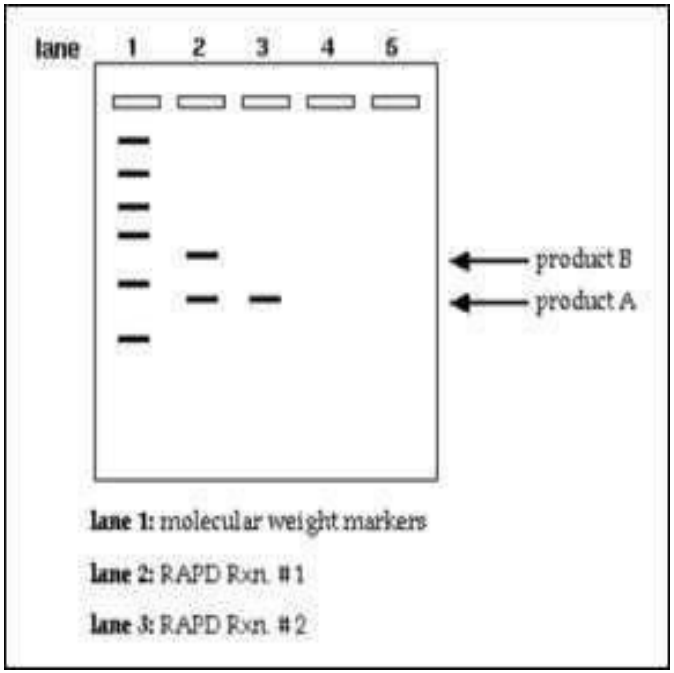


Figure 3 (genome B)

- Genome A and B can represent genomic DNA from two individuals in the same species or possibly from two different species.
- Certain portions of genomic DNA tend to much conserved (very little variation) while other portions tend to vary greatly among individuals within a species or among different species.
- The trick in RAPD PCR analysis is to:

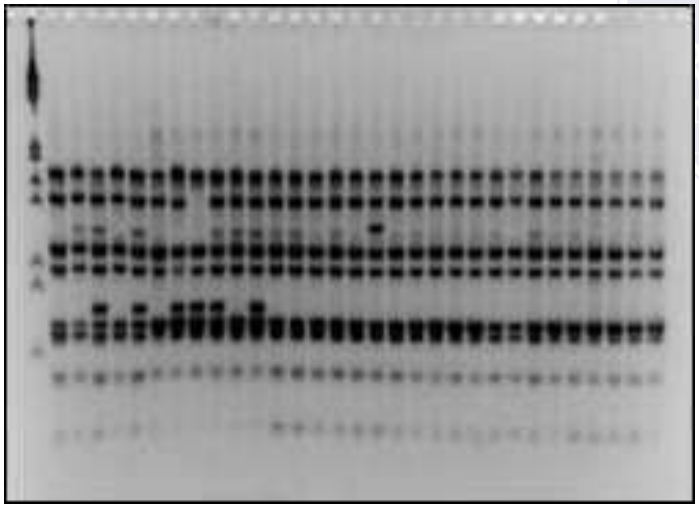
1. Find those sequences which have just enough variation to allow us to detect differences among the organisms that we are studying.
2. find the right PCR primers which will allow us to detect sequence differences.



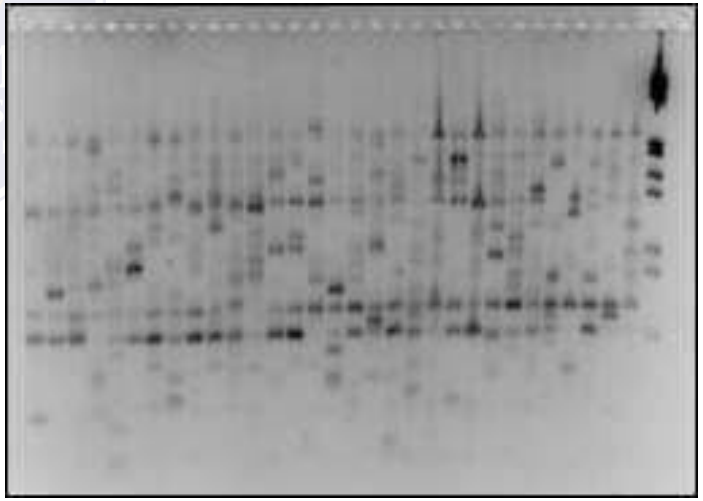
(genomes A and B) on a agarose gel

INTERPRETING RAPD BANDING PATTERNS

- Each gel is analysed by scoring the present (1) or absent (0) polymorphic bands in individual lanes. The scoring can be done based on the banding profiles which is clear and transparent (Fig. 5a) otherwise the scoring is very difficult (Fig. 5b).



(Fig. 5a)



(Fig. 5b)

- Criteria for selecting scoring bands:
  1) reproducibility—need to repeat experiments.
  2) thickness 3) size and,

4) expected segregation observed in a mapping population.

- DNA polymorphism among individuals can be due to:
  1) mismatches at the primer site.
  2) appearance of a new primer site and,
  3) length of the amplified region between primer sites.
- The NTSYS-pc software ver. 2.02 is used to estimate genetic similarities with the Jaccard's coefficient.

Components of a PCR and RAPD Reactions

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

RAPD involves following steps:-

1. The DNA of a selected species is isolated.

-quality influences the outcome of the PCR

-high molecular weight.

-No impure template

-less amount of RNA

- 2. An excess of selected decaoligonucleotide added.

-10 base primer for PCR, only 1 primer per reaction

- Short primer bind randomly on the chromosome.