HOW CAN GENETIC VARIATION BE MEASURED?

Information Provided by the Molecular Tools

- Genetic identification and discrimination of aquaculture stocks;
- Monitoring of inbreeding or other changes in the genetic composition of the stocks that may result from such phenomena as breeding programmes, founder events and genetic drift;
- Comparisons between hatchery and wild stocks;
- Assessment of the impact on natural populations of escaped or released cultured fish;
- Assignment of progeny to parents through genetic tags, so that animals from different families can be reared together in breeding programmes;
- Identification of marker genetic loci associated with quantitative trait loci (QTL) and use of these markers in selection programmes (marker assisted selection);
- Assessment of successful implementation of genetic manipulations such as induction of polyploidy and gynogenesis

DNA fragment size variation

Genetic variation can be measured and quantified at several levels

Differences between sizes of DNA fragments can be identified and used to address particular genetic questions.

Nuclear DNA markers (nDNA);

Mitochondrial DNA markers (mtDNA);

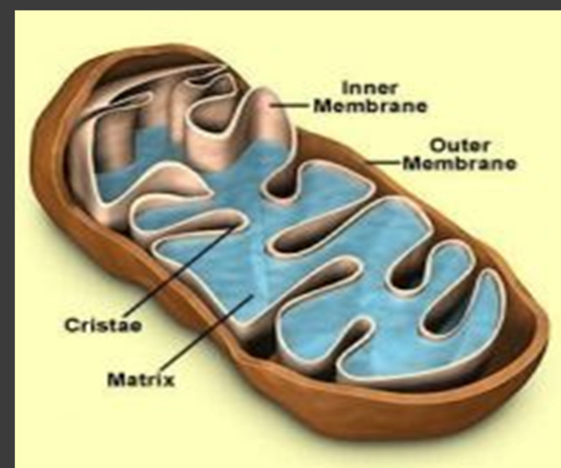
Nuclear DNA Markers

Main Characteristics:

- abundant in genome;
- Mendelian inheritance;
- potential to detect high levels of polymorphism;
- suitable for assessment of genetic structuring at the species or lower level.

Mitochondrial DNA Markers

Fast sequence divergence accumulation, when compared with nDNA, due to:
- faster mutation rate, that may result from a lack of repair mechanisms during replication;
- smaller effective population size because of the strictly maternal inheritance of the haploid mitochondrial genome.



Applications of mtDNA:

- to investigate stock structure
- used in identification of broodstocks

Mitochondrial DNA Markers

Advantages:

• High levels of polymorphism (compared to allozymes);

• More sensitive indicator of population phenomena, e. g. bottlenecks and hybridizations;

• Multiple copies in each cell;

• Transmitted uniparentally (it does not recombine);

Compared with nDNA

Disadvantage:

• inability to detect male mediated genetic mixing of stocks.

DNA fragment size variation

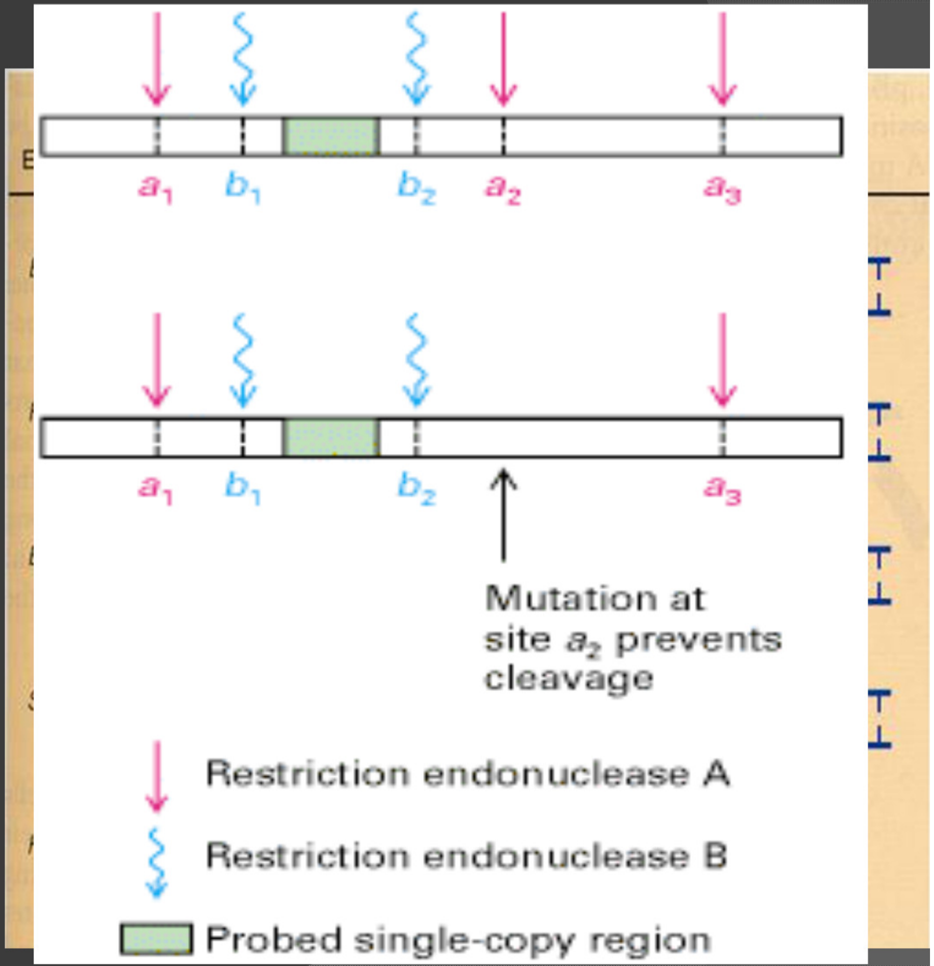
- restriction fragment length polymorphism (RFLP)
- variable number tandem repeats (VNTR)
- random amplified polymorphic DNA (RAPD)
- amplified fragment length polymorphism (AFLP)
- inter simple sequence repeat (ISSR)
- Single Nucleotide Polymorphism (SNP)
- Protein variation (allozymes)

restriction fragment length polymorphism (RFLP)

PCR product of a particular fragment is incubated with a number of different restriction endonucleases (REs) which will cleave it into a number of lengths depending on the position of the RE recognition sites.

The various lengths produced can be separated by size and stained on an agarose gel.

The same piece of DNA from different individuals will produce different sets of restricted fragments if there have been point mutations affecting the RE recognition sequences.



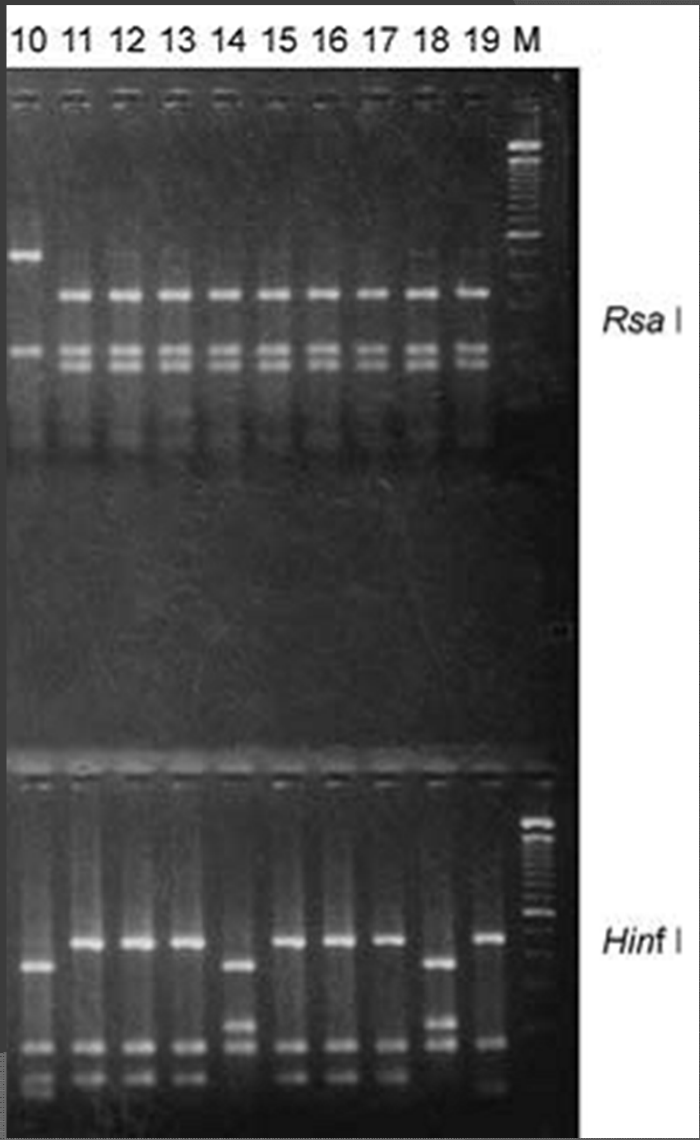
restriction fragment length polymorphism (RFLP)

<u>Advantages</u>:

- Numerous
- co-dominant
- reproducible
- representative
- relatively polymorphic

Disadvantages:

- laborious
- complex patterns
- large amount DNA required
- automation is difficult



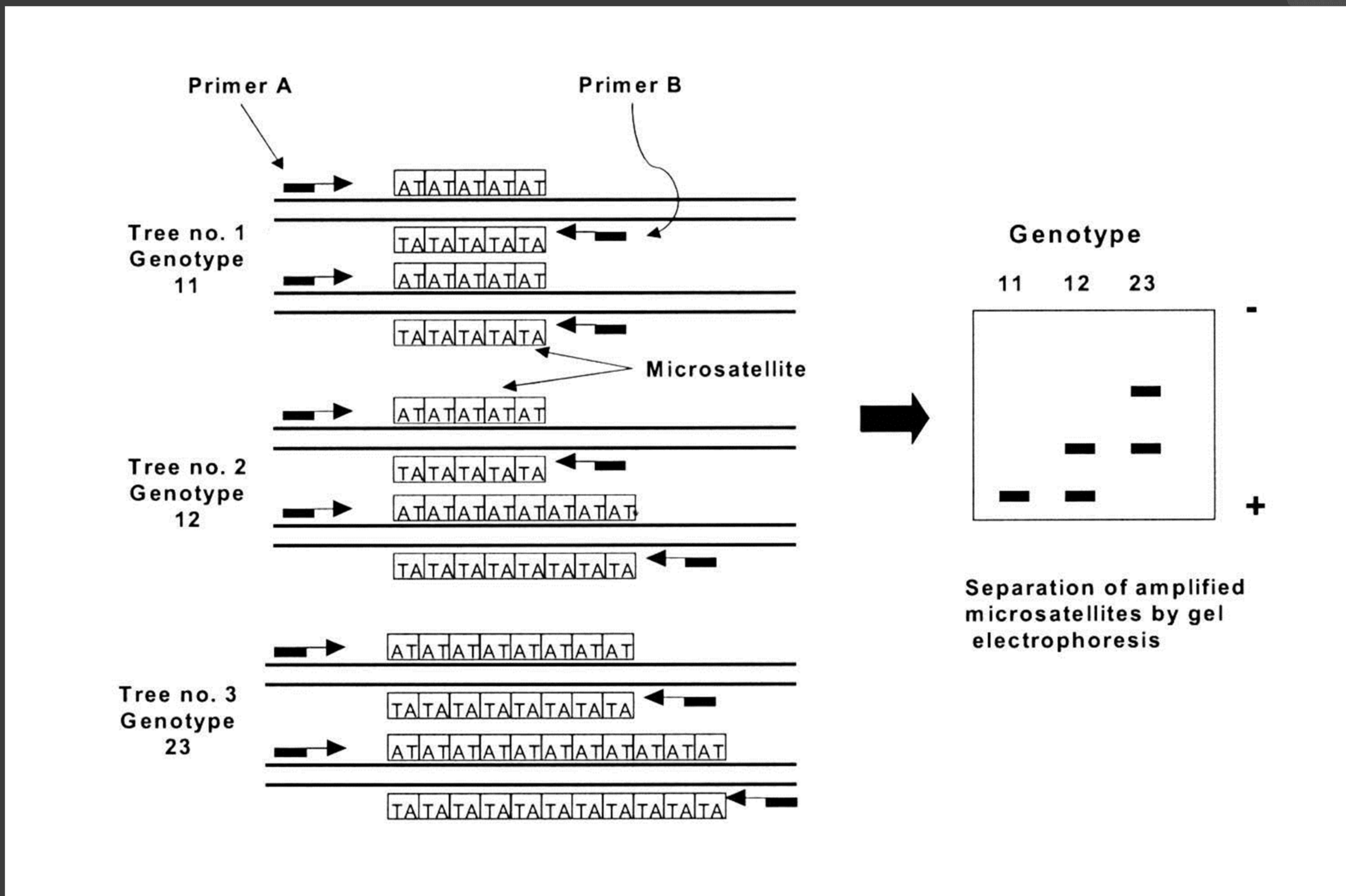
Variable number tandem repeats (VNTR)

Variation in the sequence of DNA can occur at certain sites by a method which is not point mutation. Spread throughout the genome are regions called variable number tandem repeats (VNTR), also known as simple tandem repeats (STR), which contain tandem (i.e. linked in chains) repeats of DNA sequences.

Variation in the number of repeats at these satellite (repeated units 100 to 5000 bp), minisatellite (repeated units 5 to 100 bp) or microsatellite (repeated units 2 to 4 bp) loci can be very extensive in populations and provides a valuable tool for investigation of population genetic changes in the recent past.

The number of repeated units contained within a particular microsatellite locus can vary within a population, and this produces variation in the length of the locus. This variation can be detected by amplifying the locus using PCR, followed by electrophoresis.

Variable number tandem repeats (VNTR)



Variable number tandem repeats (VNTR)

Advantages:

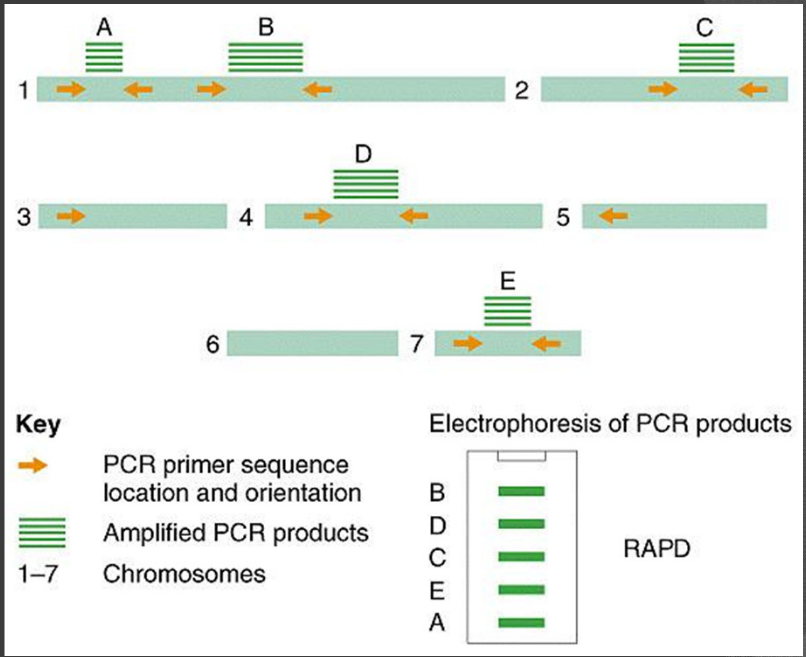
- co-dominant both alleles can be identified) and therefore can be analysed representative
- as ‘junk DNA’ they can usually be considered to be free of selective pressures
- the high number of both loci and alleles at each locus
- because automatic DNA sequencers can be used for automated genotyping at microsatellite loci

Disadvantages:

• Isolation and identification of microsatellites in a species is done by first producing a library of recombinant clones

random amplified polymorphic DNA (RAPD)

The RAPD method is based on the principle that the shorter the length of the primers which are used in PCR, the greater is the chance that non-target sequences will be amplified.



Using a single 10-mer oligonucleotide as the sole primer, PCR is conducted on raw DNA and the resulting fragments, which come from annealing of the primers all across the genome, are separated on agarose gel.

Variations between individuals in the presence or absence of bands, reflect mutational difference at the primer sites.

random amplified polymorphic DNA (RAPD)

Advantages:

- numerous
- inexpensive
- easy to develop & assay
- very polymorphic

Disadvantages:

- low reproducibility
- dominant

amplified fragment length polymorphism (AFLP)

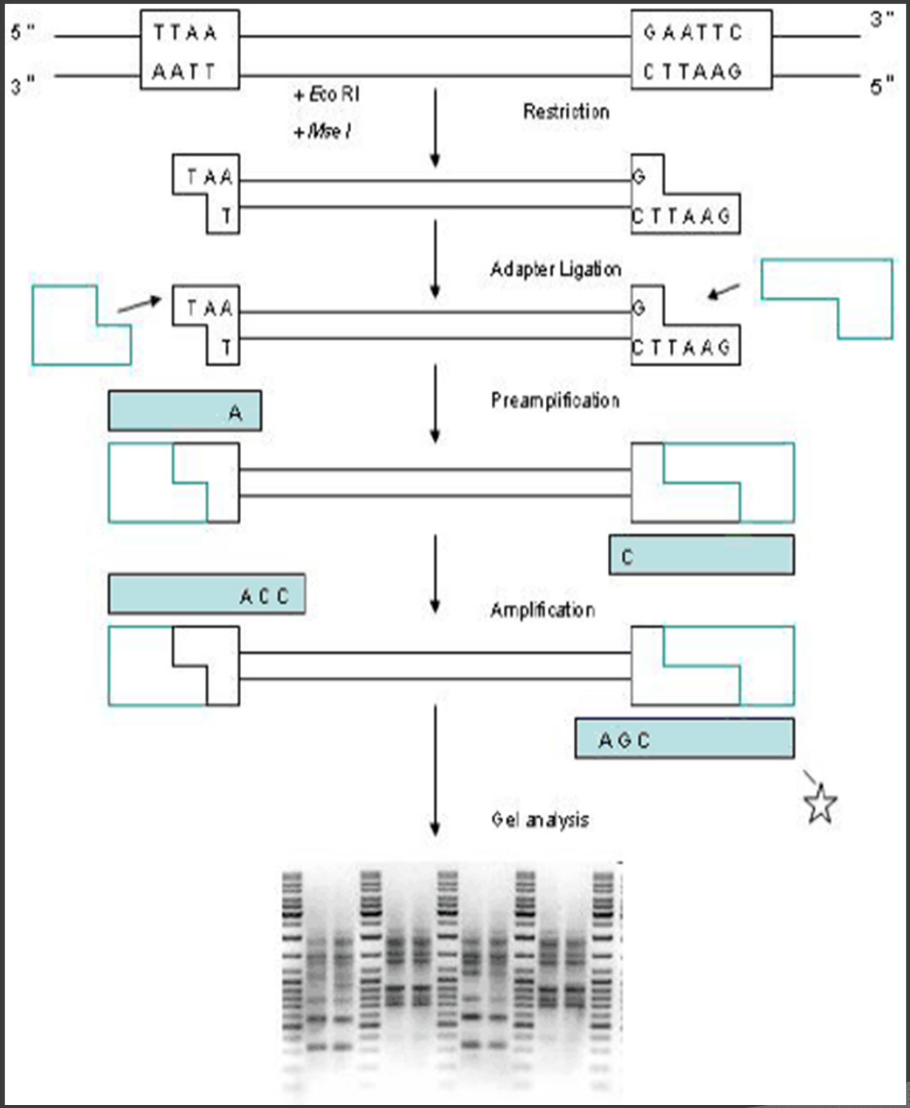
A method which amplifies randomly selected fragments of the genome much more reliably than RAPD is the AFLP method.

AFLP is almost an inverse form of RFLP – the genomic DNA is cut into fragments with restriction enzymes and then just a few of those fragments are selectively amplified using special radiolabelled PCR primers.

The resulting fragments are then visualized by separation on a polyacrylamide gel followed by autoradiography.

The products of AFLP – a series of bands of different sizes – are similar to the products of RAPDs and variation between individuals is based on the presence or absence of bands.

amplified fragment length polymorphism (AFLP)



Genomic DNA is cut with two restriction enzymes (here *Eco*RI and *Mse*I) and specific adapters are linked to both ends of all the resulting fragments. Two successive PCRs are then performed using specific primers complementary to the adapters and the restriction site with the 3'-ends extending by one or a few bases to effect selective amplification. Amplification products obtained by the second, selective PCR are separated on gels. Band detection can be through silver staining or labeling of one primer by a radioisotope or a fluorochrome (indicated by a star).

amplified fragment length polymorphism (AFLP)

Advantages:

- numerous
- moderately expensive
- very polymorphic

Disadvantages:

- dominant
- technically demanding
- requires high quality and quantity of DNA
- questionable reproducibility

Protein variation (Allozymes)

So far we have considered genetic variation at the level of the DNA. However, DNA sequence variation, when transcribed, can give rise to differences in the resulting proteins.

It is at this level that genetic variation begins to interact with the environment to affect the survivorship and reproduction of organisms and their genes.

Genetic variation at the level of proteins can be identified and quantified using Electrophoresis to separate the different protein products of alleles followed by staining to visualise these protein products.

Protein variation (Allozymes)

Advantages:

• co-dominant;

• ease to use;

• easily adjustable from species to species;

• low cost;

• general applicability.

Disadvantages:

• limited number of available allozyme loci precludes their use in large-scale genome mapping;

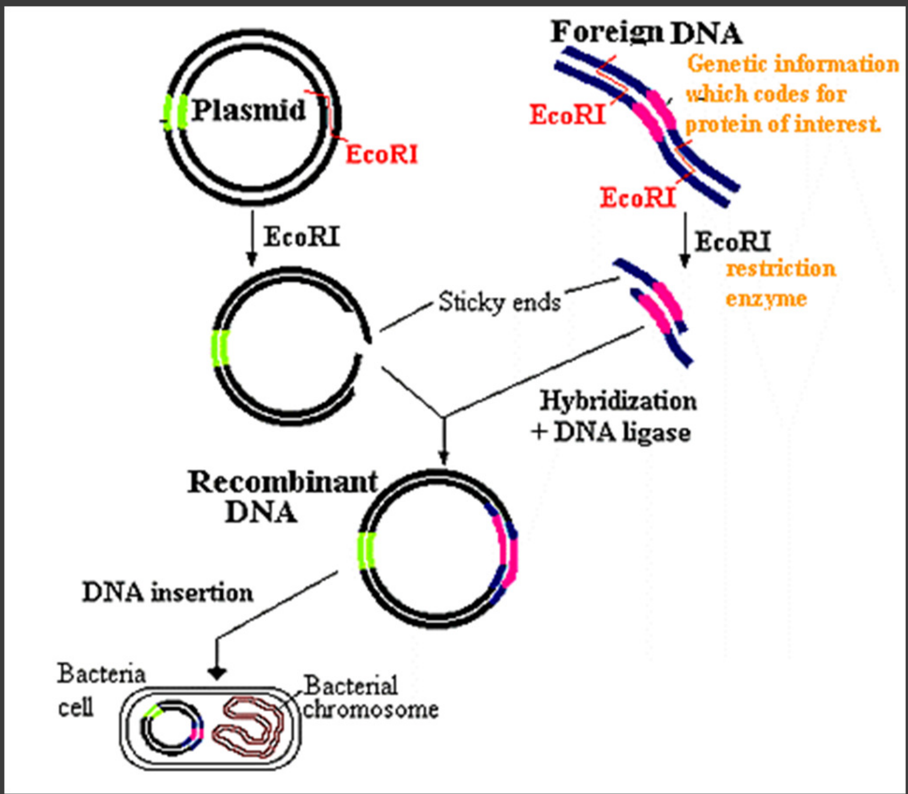
• reduce the level of detectable variation due to some changes in DNA sequence are masked at the protein level;

• only a small fraction of enzyme loci appear to be allozymically polymorphic;

There are several good PC programs available which could use to calculate genetic statistics

- MICROSAT
- GENEPOP
- ARLEQUIN
- GENETIX
- POPGENE
- PHYLIP

Gene Cloning



1. Remove bacterial DNA (plasmid).
2. Cut the Bacterial DNA with "restriction enzymes".
3. Cut the DNA from another organism with "restriction enzymes".
4. Combine the cut pieces of DNA together with another enzyme, ligase and insert them into bacteria.
5. Reproduce the recombinant bacteria.
6. The foreign genes will be expressed in the bacteria.