Mapping and Tagging of gene and Marker Assisted Selection

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Gene Mapping

- Gene mapping determines the order and the relative distances between genes on a chromosome in map units.

- 1 map unit=1cM (centimorgan)

- Its divided into two categories:
  - Genetic mapping
  - Physical mapping

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- Gene mapping methods use recombination frequencies between alleles in order to determine the relative distances between them
- Recombination frequencies between genes are inversely proportional to their distance apart.
- Distance measurement: 1 map unit = 1 percent recombination (true for short distances)

Genetic mapping

- The first steps of building a genetic map are the development of genetic markers and a mapping population.
- Since the closer the two markers are on the chromosome, the more likely they are to be passed on to the next generation together, therefore the "co-segregation" patterns of all markers can be used to reconstruct their order.
- The quality of the genetic maps is largely dependent upon these two factors:
  - the number of genetic markers on the map and
  - the size of the mapping population.

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- The two factors are interlinked, as a larger mapping population could increase the "resolution" of the map and prevent the map being "saturated".
- In genetic mapping, any sequence feature that can be faithfully distinguished from the two parents can be used as a genetic marker.
- Genes, in this regard, are represented by "traits" that can be faithfully distinguished between two parents.

Restriction fragment length polymorphism (RFLP) markers

- RFLP markers are co-dominance and high reproducibility.
- The requirement of relatively large amounts of pure and intact DNA.

RFLPs are detected by cutting genomic DNA with restriction enzymes.

Restriction enzymes has a specific recognition sequence which is typically palindromic

Restriction enzymes leads to restriction fragments of certain length.

These fragments are size-separated with agarose gel electrophoresis

Analyzed by Southern blots using either locus-specific or multilocus probes.

Randomly-amplified polymorphic DNA (RAPD) markers

- RAPD markers are based on the PCR amplification of random DNA segments with single, typically short primers of arbitrary nucleotide sequence.
- Dominant marker

The primers bind to complementary sample DNA sequences

Forward and reverse primers are used

Followed by PCR and the DNA is amplified

The DNA amplification products are visualized by gel electrophoresis

Amplified fragment length polymorphism (AFLP) markers

- The AFLP technique combines elements of RFLP and RAPD.
- It is based on the selective PCR amplification of restriction fragments.
- AFLP markers are dominant rather than co-dominant.

Genomic DNA is digested and oligonucleotide adapters are ligated to both ends of the resulting restriction fragments.

Fragments are selectively amplified, using the adapter and restriction site sequences as primer binding sites for subsequent PCR reactions.

Only a certain portion of the restriction fragments is amplified whose ends are perfectly complementary to the 3′ends of the selective primers.

Amplified fragments are resolved by gel electrophoresis and visualized by either autoradiography, silver staining or fluorescence

Microsatellite markers

Microsatellites are also known as simple sequence repeats (SSRs).

- SSRs are DNA stretches, consisting of tandemly repeated short nucleotide units (1-5 bases per unit). Such repeats are distributed throughout the genomes of all eucaryotic species.

In microsatellite analysis, sequence information of the regions flanking the repeats is used for creating locus specific PCR primer pairs.

The resulting amplification products are separated on polyacrylamide gels and visualized.

- The differences in the numbers of repeated units cause differences in band size, which are locus-specific, co-dominantly inherited and highly polymorphic.

Single nucleotide polymorphism (SNP) markers

SNP markers are based on sequence differences at single-base pair positions in genomes.

- SNP is not a gel-based technology.

For the large-scale genotyping required in marker assisted breeding programs, technologies based on gel electrophoresis are often too labor intensive and time consuming.

- The high density of SNP markers increases the probability to find polymorphisms in a target gene.

Populations used for genetic mapping

- Backcross double haploid
- F2
- Recombinant inbred
- Near isogenic lines
- F1 between heterogygous parents

- There are a number of different kinds of genome maps,
- e.g. cytogenetic, linkage, physical, etc.
- Genetic linkage mapping allows the localization of inherited markers relative to each other. As with cytogenetic maps, linkage maps examine chromosomes as they are in cells.

Physical Mapping

- Physical maps are actually constructed by first shattering the genome into hierarchically smaller pieces.

The fragmentation of the genome by restriction enzyme cutting or by physically shattering the genome by processes like sonication.

DNA fragments are separated by electrophoresis.

Analysis of the DNA pattern.

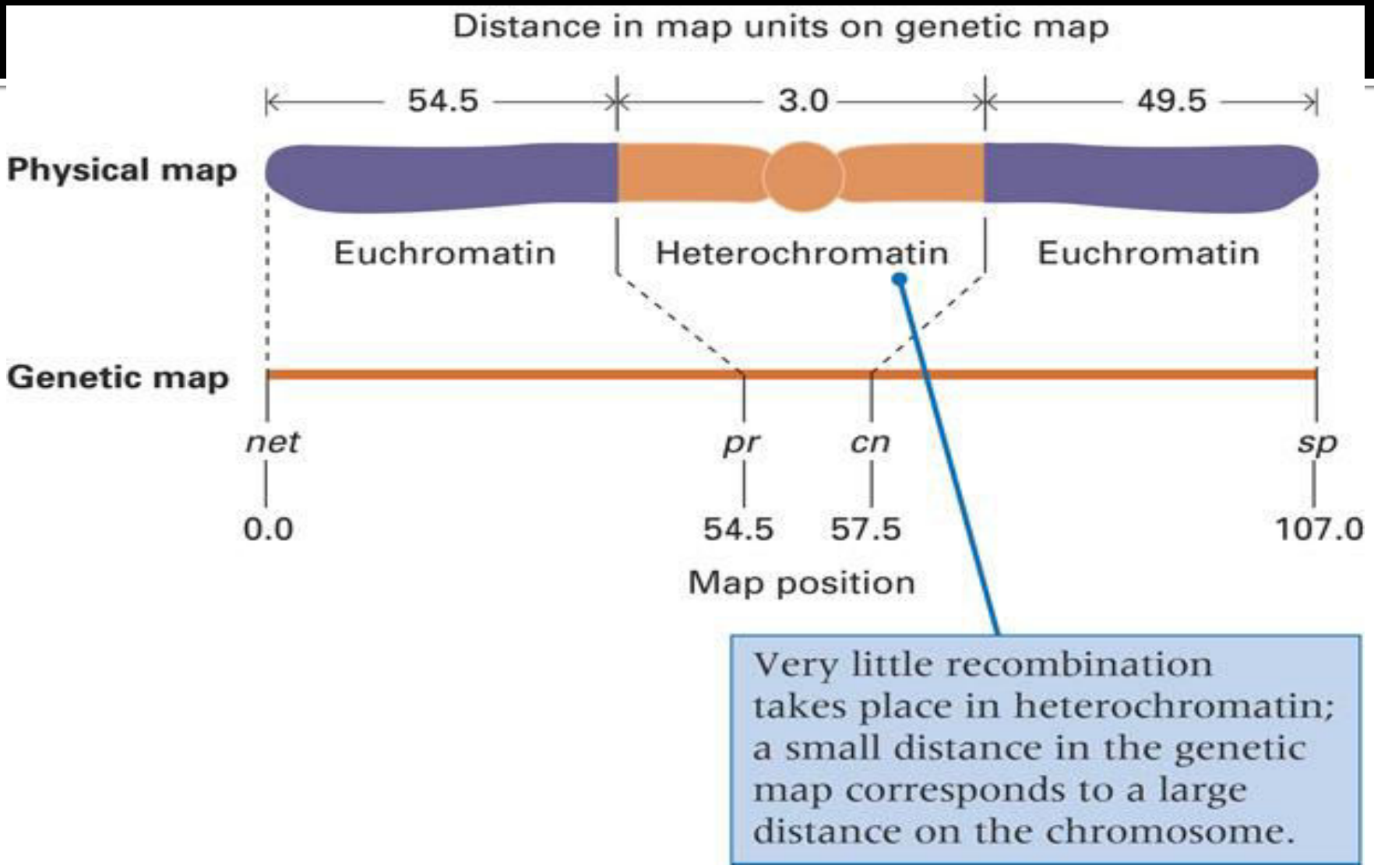
By analyzing the fingerprints, contigs are assembled by automated (FPC) or manual means (Pathfinders) into overlapping DNA stretches.

Now a good choice of clones can be made to efficiently sequence the clones to determine the DNA sequence of the organism under study.

- Radiation hybrid mapping
- Happy mapping
- Optical mapping
- Radiation hybrid (RH) mapping involves screening of randomly broken fragments of DNA for specific markers.
- HAPPY mapping is a more versatile variation on RH mapping.
- Optical mapping is undertaken on single DNA molecules. Optical mapping involves the imaging of single DNA molecules during restriction enzyme digestion.

Genetic Mapping VS Physical Mapping

- Both maps are a collection of genetic markers and gene loci.
- Genetic maps distances are based on the genetic linkage information measured in centimorgans (cM), while physical maps use actual physical distances usually measured in number of base pairs.
- While the physical map could be a more "accurate" representation of the genome, genetic maps often offer insights into the nature of different regions of the chromosome,
- e.g. the genetic distance to physical distance ratio varies greatly at different genomic regions which reflects different recombination rates, and such rate is often


Distance in map units on genetic map
54.5
3.0
49.5
Physical map
Euchromatin
Heterochromatin
Euchromatin
Genetic map
net
pr
cn
sp
0.0
54.5
57.5
107.0
Map position
Very little recombination takes place in heterochromatin; a small distance in the genetic map corresponds to a large distance on the chromosome.

Gene tagging

Gene tagging refers to identification of existing DNA or introduction of new DNA that can function as a tag or label for the gene of interest.

- Molecular breeding involves primarily gene tagging, followed by marker-assisted selection of desired genes or genomes.
- In order for the DNA sequences to be conserved as a tag, important prerequisites have been identified.

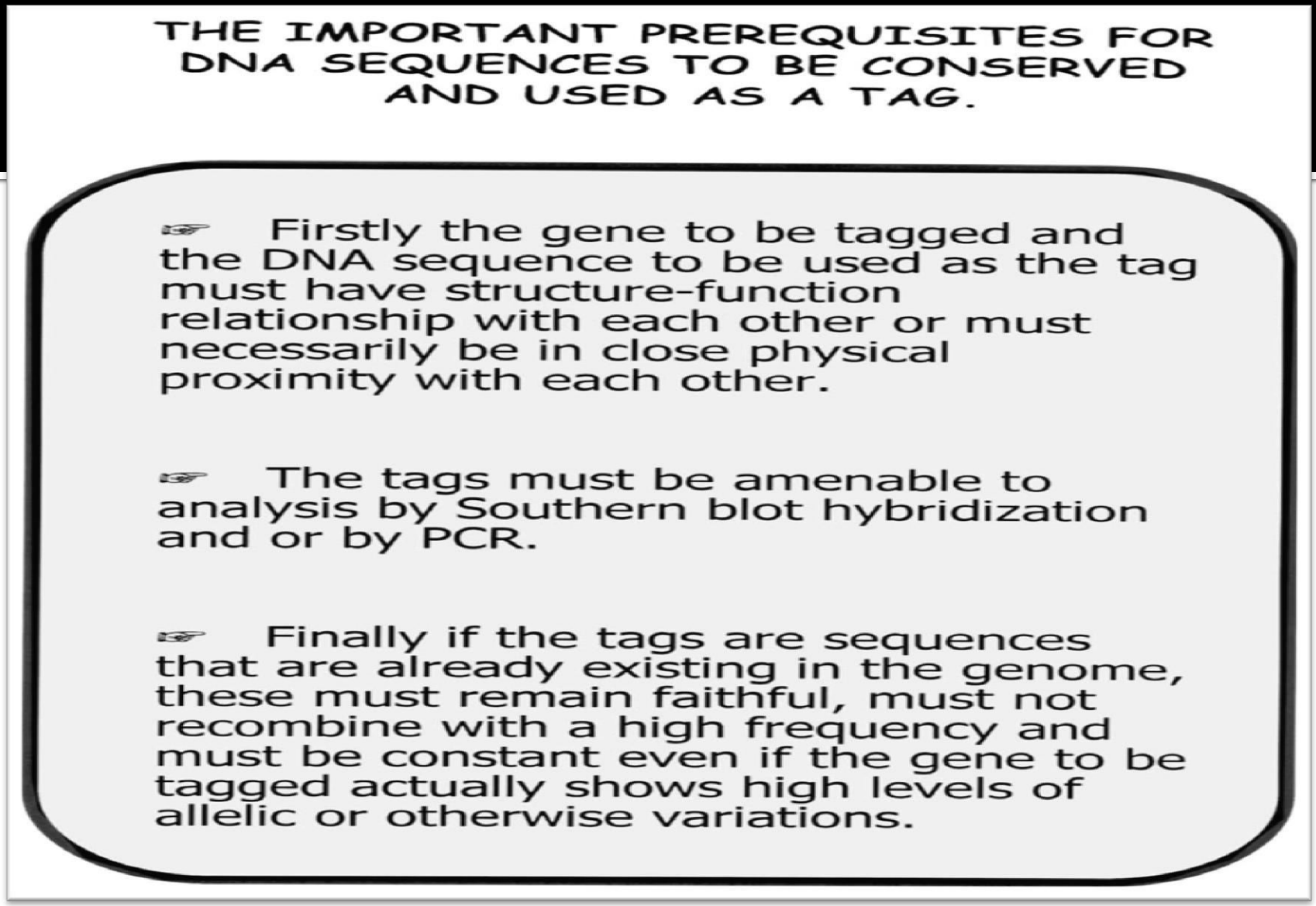


FIGURE 1. The important prerequisites for the use of DNA sequences as gene tags .

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- A number of molecular tags have now been determined for many genes in most of the important plants.
- These molecular tags include cloned restriction fragment length polymorphism (RFLP) probes, oligonucleotide RFLP probes, variable number tandem repeats (VNTR), microsatellite, minisatellite, and other DNA fingerprint loci, and specific as well as arbitrary sequence primers.

MARKERS USED FOR GENE TAGGING

Gene tagging using RAPD markers has at least three major advantages over other methods:

First, a universal set of primers can be used and screened in a short period

second, isolation of cloned DNA probes or preparation of hybridization filters is not required

third, only a small quantity of genomic DNA is needed for each Analysis

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- They can detect more number of loci and alleles that are phenotypically neutral, and can be scored at any stage of plant development.
- RFLP markers have been employed extensively to tag useful genes in several crop plants and trees.
- Isozymes have been used as markers and genetic characters.
- The isozymes have found lesser applications in gene tagging and marker assisted selection programs, despite being among the earliest to be developed for molecular breeding work.
- The efforts that are involved in tagging a gene can be used further as a part of marker-assisted selection program.

Marker Assisted Selection (MAS)

- *Marker assisted selection (MAS) is a combined product* of traditional genetics and molecular biology.
- Marker assisted selection or marker aided selection (MAS) is a process whereby a marker (morphological, biochemical or one based on DNA/RNA variation) is used for indirect selection of a genetic determinant or determinants of a trait of interest (e.g. productivity, disease resistance, abiotic stress tolerance, and quality). This process is used in plant and animal breeding.

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- MAS allows for the selection of genes that control traits of interest.
- Its combined with traditional selection techniques.
- MAS has become a valuable tool in selecting organisms for traits of interest, such as color, meat quality, or disease resistance.

Application of MAS in plant breeding

- **(a) Marker-assisted backcrossing**
- Backcrossing is a plant breeding method most commonly used to incorporate one or a few genes into an adapted or elite variety.
- In the first level, markers can be used in combination with or to replace screening for the target gene or QTL. This is referred to as ‘foreground selection’.
- The second level involves selecting BC progeny with the target gene and recombination events between the target locus and linked flanking markers.

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- **(b) Marker-assisted pyramiding**
- Pyramiding is the process of combining several genes together into a single genotype. The most widespread application for pyramiding has been for combining multiple disease resistance genes (i.e. combining qualitative resistance genes together into a single genotype).
- **(c) Early generation marker-assisted selection**
- MAS is a great advantage in early generations because plants with undesirable gene combinations can be eliminated.
- **(d) Combined marker-assisted selection**
- Combined MAS' may have advantages over phenotypic screening or MAS alone in order to maximize genetic gain.

Conclusion

- Markers opened up the possibility of identifying, mapping, tagging, and even isolating or transferring quantitative trait loci (QTLs).
- Molecular mapping and tagging of agronomically important genes using RFLP and RAPD markers have been carried out in three different crops: rice, mustard and chickpea.
- The use of markers can therefore greatly accelerate a breeding program, allowing us to the first to market with the best genetics.
- Gene tagging and marker-assisted selection is an essential component of molecular breeding and is based on saturation mapping of the genomes.
- In MAS Large numbers of samples can be screened for the gene(s) conferring resistance to a given


Thank you!