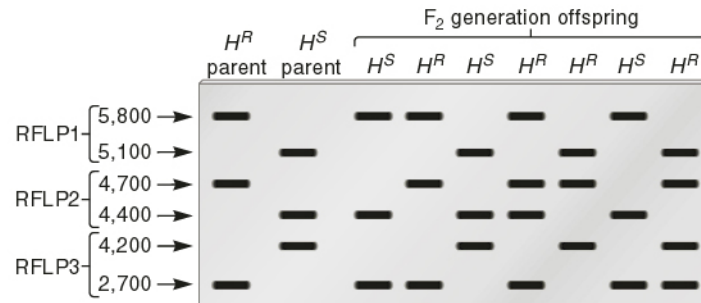


S1. An RFLP marker is located 1 million bp away from a gene of interest. Your goal is to start at this RFLP marker and walk to this gene. The average insert size in the library is 55,000 bp and the average overlap at each end is 5,000 bp. Approximately how many steps will it take to get there?

Answer: Each step is only 50,000 bp (i.e., 55,000 minus 5,000) because you have to subtract the overlap between adjacent fragments, which is 5,000 bp. Therefore, it will take about 20 steps to go 1 million bp.

S2. When many RFLPs have been mapped within the genome of a plant, an RFLP analysis can be used to map a herbicide-resistance gene. For example, let's suppose that an agricultural geneticist has two strains, one that is herbicide resistant and one that is herbicide sensitive. The two strains differ with regard to many RFLPs. The sensitive and resistant strains are crossed, and the F_1 offspring are allowed to self-fertilize. The F_2 offspring are then analyzed with regard to their herbicide sensitivity and RFLP markers. The following results were obtained:



To which RFLP might the herbicide-resistance gene be linked?

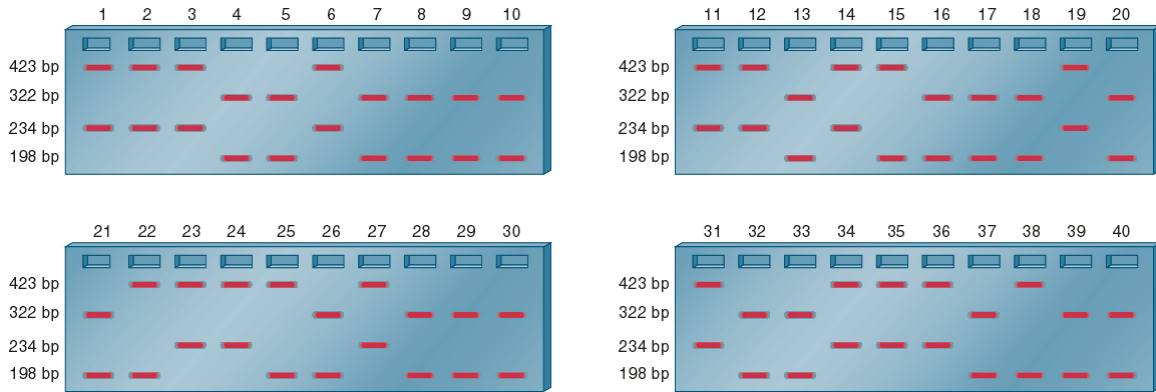
Answer: The aim of this experiment is to correlate the presence of a particular RFLP with the herbicide-resistant phenotype. As shown in the data, the herbicide-resistant parent and all the herbicide-resistant offspring have an RFLP that is 4,700 bp in length. In an actual experiment, a more thorough lod analysis would be conducted to determine if linkage is considered likely. If so, the 4,700 bp RFLP may either contain the gene that confers herbicide resistance or, as is more likely, the two may be linked. If the 4,700 bp RFLP has already been mapped to a particular site in the plant's genome, the herbicide-resistance gene also maps to the same site or very close to it. This information may then be used by a plant breeder when making future crosses to produce herbicide-resistant plant strains.

S3. Does a molecular marker have to be polymorphic to be useful in mapping studies? Does a molecular marker have to be polymorphic to be useful in linkage mapping (i.e., involving family pedigree studies or genetic crosses)? Explain why or why not.

Answer: A molecular marker does not have to be polymorphic to be useful in mapping studies. Many sequence-tagged sites that are used in physical or cytogenetic studies are monomorphic. Monomorphic markers can provide landmarks in mapping studies.

In linkage studies, a marker must be polymorphic to be useful. Polymorphic molecular markers can be RFLPs or microsatellites. To compute map distances in linkage analysis, individuals must be heterozygous for two or more markers (or genes). For experimental organisms, heterozygotes are testcrossed to homozygotes, and then the number of recombinant offspring and nonrecombinant offspring are determined. For markers that do not assort independently (i.e., linked markers), the map distance is computed as the number of recombinant offspring divided by the total number of offspring, times 100.

S4. The distance between two molecular markers that are linked along the same chromosome can be determined by analyzing the outcome of crosses. This can be done in humans by analyzing the members of a pedigree. However, the accuracy of linkage mapping in human pedigrees is fairly limited because the number of people in most families is pretty small. As an alternative, researchers can analyze a population of sperm, produced from a single male, and compute linkage distance in this manner. As an example, let's suppose a male is heterozygous for two polymorphic sequence-tagged sites. STS-1 exists in two sizes: 234 bp and 198 bp. STS-2 also exists in two sizes: 423 bp and 322 bp. A sample of sperm was collected from this man, and individual sperm were placed into 40 separate tubes. In other words, there was one sperm in each tube. Believe it or not, one sperm has enough DNA to conduct PCR! Into each of the 40 tubes were added the primers that amplify STS-1 and STS-2, and then the samples were subjected to PCR. The following results were obtained.



- A. What is the arrangement of these two sequence-tagged sites in this individual?
 B. What is the linkage distance between STS-1 and STS-2?

Answer: Keep in mind that mature sperm are haploid, so they will have only one copy of STS-1 and one copy of STS-2.

- A. If we look at the 40 lanes, most of the lanes (i.e., 36 of them) have either the 234 bp and 423 bp STSs or the 198 bp and 322 bp. This is the arrangement of STSs in this male. One chromosome has STS-1 that is 234 bp and STS-2 that is 423 bp and the homologous chromosome has STS-1 that is 198 bp and STS-2 that is 322 bp.
 B. There are four recombinant sperm, shown in lanes 15, 22, 25, and 38.

$$\text{Map distance} = \frac{4}{40} \times 100$$

$$= 10.0 \text{ mu}$$

Note: This is a relatively easy experiment compared to a pedigree analysis, which would involve contacting lots of relatives and collecting samples from each of them.

S5. The technique of chromosome walking involves the stepwise cloning of adjacent DNA fragments until the gene of interest is reached. This method was used to clone the gene that (when mutant) causes cystic fibrosis, an autosomal recessive disorder in humans. Linkage mapping studies first indicated that the *CF* gene was located between two markers designated MET and D7S8 (see next). Later mapping studies put the *CF* gene between IRP and D7S8. Walking was initiated from the D7S122 marker.

MET D7S122 IRP CFgene D7S8
 (from *Science* 245, 1059–69)

In this study, the researchers restriction mapped this region using two enzymes, *Not I* and *Xho I*. The locations of sites are shown here:

MET D7S122 IRP CFgene D7S8
 ↑A↑ B↑C↑ D↑E↑F↑
 N X X X X X N
 N = *Not I*, X = *Xho I*

How would the researchers know they were walking toward the *CF* gene and not away from it?

Answer: The general answer is that they had to determine if they were walking toward the IRP marker or toward the MET marker.

As depicted in the previous diagram, *Not I* would produce a large DNA fragment (including regions A–F) that contained both the D7S122 and IRP markers. Since the D7S122 and IRP markers were available as probes, they could confirm that this region contained both markers by Southern hybridization of this *Not I* fragment to both markers. *Xho I* cuts this region into smaller pieces. The piece labeled B carried the D7S122 marker, and the piece labeled D carried the IRP marker. Again, this could be confirmed by hybridization. This map enabled the researchers to determine which direction to walk. During their walking, they began at fragment B (which contained the D7S122 marker) and walked toward the adjacent fragment labeled C. They then walked to the fragment labeled D, which contains the IRP marker. In contrast, if they had walked toward the fragment labeled A, they would then have walked to the MET marker. This is the incorrect direction, because they knew from their linkage mapping that IRP is closer to the *CF* gene compared to MET.

