E1. A. Cytogenetic mapping

B. Linkage mapping

C. Physical mapping

D. Cytogenetic mapping

E. Linkage mapping

F. Physical mapping

E2. They are complementary to each other.

E3. *In situ* hybridization is a cytological method of mapping. A probe that is complementary to a chromosomal sequence is used to locate the gene microscopically within a mixture of many different chromosomes. Therefore, it can be used to cytologically map the location of a gene sequence. When more than one probe is used, the order of genes along a particular chromosome can be determined.

E4. Because normal cells contain two copies of chromosome 14, one would expect that a probe would bind to complementary DNA sequences on both of these chromosomes. If a probe recognized only one of two chromosomes, this means that one of the copies of chromosome 14 has been lost, or it has suffered a deletion in the region where the probe binds. With regard to cancer, the loss of this genetic material may be related to the uncontrollable cell growth.

E5. The term *fixing* refers to procedures that chemically freeze cells and prevent degradation. After fixation has occurred, the contents within the cells do not change their morphology. In a sense, they are frozen in place. For a FISH experiment, this keeps all the chromosomes within one cell in the vicinity of each other; they cannot float around the slide and get mixed up with chromosomes from other cells. Therefore, when we see a group of chromosomes in a FISH experiment, this group of chromosomes comes from a single cell.

It is necessary to denature the chromosomal DNA so that the probe can bind to it. The probe is a segment of DNA that is complementary to the DNA of interest. The strands of chromosomal DNA must be separated (i.e., denatured) so that the probe can bind to complementary sequences.

E6. After the cells and chromosomes have been fixed to the slide, it is possible to add two or more different probes that recognize different sequences (i.e., different sites) within the genome. Each probe has a different fluorescence emission wavelength, so it can be identified by its color. Usually, a researcher will use computer imagery that recognizes the wavelength of each probe and then assigns that region a bright color. The color seen by the researcher is not the actual color emitted by the probe; it is a secondary color assigned by the computer. This can be done with two or more different probes, as a way to color the regions of the chromosomes that are recognized by the probes. In a sense, the probes, with the aid of a computer, are "painting" the regions of the chromosomes that are recognized by a probe. An example of chromosome painting is shown in Figure 20.4. In this example, human chromosome 5 is painted with six different colors.

E7. If the sample was from a normal individual, two spots (one on each copy of chromosome 21) would be observed. Three spots would be observed if the sample was from a person with Down syndrome because the person has three copies of chromosome 21.

E8. A contig is a collection of clones that contain overlapping segments of DNA that span a particular region of a chromosome. To determine if two clones are overlapping, one could conduct a Southern blotting experiment. In this approach, one of the clones is used as a probe. If it is overlapping with the second clone, it will bind to it in a Southern blot. Therefore, the second clone is run on a gel and the first clone is used as a probe. If the band corresponding to the second clone is labeled, this means that the two clones are overlapping.

E9. A YAC vector can contain extremely large pieces of DNA, so they are used as a first step to align the segments of DNA in a physical mapping study. However, it is difficult to work with them in subcloning and DNA sequencing experiments. Cosmids, by comparison, contain smaller segments of the genome. The locations of cosmids can be determined by hybridizing them to YACs. The cosmids can then be used for subcloning and DNA sequencing.

E10. YAC cloning vectors have the replication properties of a chromosome and the cloning properties of a plasmid. To replicate like a chromosome, the YAC vector contains an origin of replication and centromeric and telomeric sequences. Therefore, in a yeast cell, a YAC can behave as a chromosome. Like a plasmid, YACs also contain selectable markers and convenient cloning sites for the insertion of large segments of DNA. The primary advantage is the ability to clone very large pieces of DNA.

E11. A polymorphism refers to genetic variation at a particular locus within a population. If the polymorphism occurs within gene sequences, this is allelic variation. A polymorphism can also occur within genetic markers such as RFLPs. The molecular basis for an RFLP is that two distinct individuals will have variation in their DNA sequences and some of the variation may affect the relative locations of restriction enzyme sites. Since this occurs relatively frequently between unrelated individuals, many RFLPs can be identified. They can be detected by restriction digestion and agarose gel electrophoresis and then Southern blotting. They are useful in mapping studies because it is relatively easy to find many of them along a chromosome. They can be used in gene cloning as a starting point for a chromosomal walk.

E12. The resistance gene appears to be linked to RFLP 4B.

E13. If the genes were unlinked, we would expect a 1:1:1:1 ratio among the four combinations of offspring. Since there are a total of 272 offspring, there are expected to be 68 in each category according to independent assortment.

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

$$\chi^2 = \frac{(40 - 68)^2}{68} + \frac{(98 - 68)^2}{68} + \frac{(97 - 68)^2}{68} + \frac{(37 - 68)^2}{68}$$

$$\chi^2 = 51.2$$

With 3 degrees of freedom, this high chi square value would be expected to occur by chance less than 1% of the time. Therefore, we reject the hypothesis that the RFLPs are independently assorting.

In this example, the recombinant offspring contain the 5,200, 4,500, and 2,100 and 4,500, 2,100, and 3,200 RFLPs.

$$\text{Map distance} = \frac{40 + 37}{40 + 98 + 97 + 37} \times 100$$

Map distance = 28.3 mu

E14. For most organisms, it is usually easy to locate many RFLPs throughout the genome. The RFLPs can be used as molecular markers to make a map of the genome. This is done using the strategy described in Figure 20.8. To map a functional gene, one would also follow the same general strategy described in Figure 20.8, except that the two strains would also have an allelic difference in the gene of interest. The experimenter would make crosses, such as dihybrid crosses, and determine the number of parental and recombinant offspring based on the alleles and RFLPs they had inherited. If an allele and RFLP are linked, there will be a much lower percentage (i.e., less than 50%) of the recombinant offspring.

E15. Child 1 and child 3 belong to father 2.

Child 2 and child 4 belong to father 1.

Child 5 could belong to either father.

E16.

Deduced Outcome

STSs

4 6 7 9 3 2 8 5 1

YAC

3 <——————>

1 <————————————>

5 <——————>

4 <——————>

2 <————————————>

E17. An explanation is that the rate of recombination between homologous chromosomes is different during oogenesis compared to spermatogenesis. Physical mapping measures the actual distance (in bp) between markers. The physical mapping of chromosomes in males and females reveals that they are the same lengths. Therefore, the sizes of chromosomes in males and females are the same. The differences obtained in linkage maps are due to differences in the rates of recombination during oogenesis versus spermatogenesis.

E18. A. One homologue contains the STS-1 that is 289 bp and STS-2 that is 422 bp while the other homologue contains STS-1 that is 211 bp and STS-2 that is 115 bp. This is based on the observation that 28 of the sperm have either the 289 bp and 422 bp bands or the 211 bp and 115 bp bands.

B. There are two recombinant sperm; see lanes 12 and 18. Since there are two recombinant sperm out of a total of thirty:

$$\text{Map distance} = \frac{2}{30} \times 100$$
$$= 6.7 \text{ mu}$$

C. In theory, this method could be used. However, there is not enough DNA in one sperm to carry out an RFLP analysis unless the DNA is amplified by PCR.

E19. They appear to be linked. If they were not linked, we would expect equal amounts of the four types of offspring. However, as seen in the data, there is a much higher proportion of parental combinations (red, small and purple, big) compared to nonparental combinations. The map distance is

$$\text{Map distance} = \frac{111+109}{725+111+109+729} \times 100 = 13.1 \text{ mu}$$

With regard to RFLP inheritance, the following results are expected:

725 red, small flowers with the 4,000 bp and 1,600 bp RFLPs

111 red, big flowers with the 4,000 bp and 7,200 bp RFLPs

109 purple, small flowers with the 3,400 bp and 1,600 bp

729 purple, big flowers with the 3,400 bp and 7,200 bp

E20. One possibility is that the geneticist could try a different restriction enzyme. Perhaps there is sequence variation in the vicinity of the pesticide-resistance gene that affects the digestion pattern of a restriction enzyme other than *Eco*RI. There are hundreds of different restriction enzymes that recognize a myriad of different sequences.

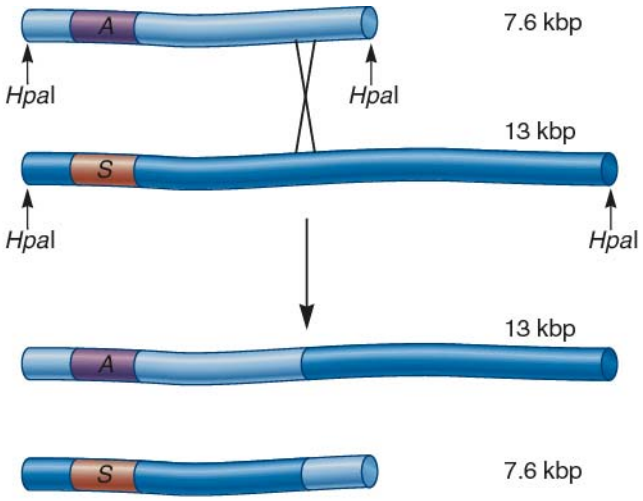
Alternatively, the geneticist could give up on the RFLP approach and try to identify one or more sequence-tagged sites that are in the vicinity of the pesticide-resistance gene. In this case, the geneticist would want to identify STSs that are also microsatellites. As described in Figure 20.12, the transmission of microsatellites can be followed in genetic crosses. Therefore, if the geneticist could identify microsatellites in the vicinity of the pesticide-resistance gene, this would make it possible to predict the outcome of crosses. For example, let's suppose a microsatellite linked to the pesticide-resistance gene existed in three forms: 234 bp, 255 bp, and 311 bp. And let's also suppose that the 234 bp form was linked to the high-resistance allele, the 255 bp form was linked to the moderate-resistance allele, and the 311 bp form was linked to the low-resistance allele. According to this hypothetical example, the geneticist could predict the level of resistance in an alfalfa plant by analyzing the inheritance of these microsatellites.

E21. You would have expected equal amounts of the four patterns of RFLPs. There would have been about 25 of each of the four patterns.

E22. When chromosomal DNA is isolated and digested with a restriction enzyme, this produces thousands of DNA fragments of different sizes. This makes it impossible to see any particular band on a gel, if you simply stained the gel for DNA. Southern blotting allows you to detect one or more RFLPs that are complementary to the radioactive probe that is used.

E23. Besides a selectable marker and an origin that will replicate in *E. coli*, YAC vectors also require two telomere sequences, a centromere sequence, and an ARS sequence. The telomeres are needed to prevent the shortening of the artificial chromosome from the ends. The centromere sequence is needed for the proper segregation of the artificial chromosome during meiosis and mitosis. The ARS sequence is the yeast equivalent of an origin of replication, which is needed so that the YAC DNA can be replicated.

E24. Based on these results, it is likely that the sickle-cell allele originated in an individual with the 13.0 kbp RFLP. This would explain why the Hb^S allele is usually transmitted with the 13.0 kbp RFLP. On occasion, however, a crossover could occur in the region between the ß-globin gene and the distal *Hpa*I site.



After crossing over, the Hb^S allele is now linked to a 7.6 kbp RFLP.

E25. The 13.0 kbp fragment is not always linked to the Hb^S allele because, on rare occasions, a crossover can occur between the restriction site and the allele. Nevertheless, within the human population, the 13.0 kbp fragment is usually linked to the Hb^S allele. Therefore, if a person is heterozygous for the 13.0 kbp fragment, he/she has a much higher probability of being a heterozygous carrier for the Hb^S allele. This information can be useful in predicting the likelihood of having an affected child.

E26. PFGE is a method of electrophoresis that is used to separate small chromosomes and large DNA fragments. The electrophoresis devices used in PFGE have two sets of electrodes. The two sets of electrodes produce alternating pulses of current, and this facilitates the separation of large DNA fragments.

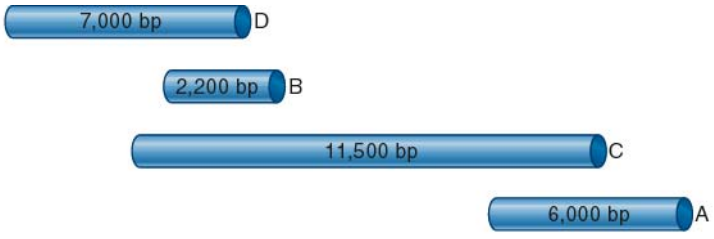
It is important to handle the sample gently to prevent the breakage of the DNA due to mechanical forces. Cells are first embedded in agarose blocks, and then the blocks are loaded into the wells of the gel. The agarose keeps the sample very stable and prevents shear forces that might mechanically break the DNA. After the blocks are in the gel, the cells within the blocks are lysed, and if desired, restriction enzymes can be added to digest the DNA. For PFGE, a restriction enzyme that cuts very infrequently might be used.

PFGE can be used as a preparative technique to isolate and purify individual chromosomes or large DNA fragments. PFGE can also be used, in conjunction with Southern blotting, as a mapping technique.

E27. The proper order is C, A, D, E, B.

1. Isolate whole chromosomes or large DNA fragments via pulsed-field gel electrophoresis or chromosome sorting.
2. Clone large fragments of DNA to make a YAC library.
3. Subclone YAC fragments to make a cosmid library.
4. Subclone cosmid fragments for DNA sequencing.
5. Determine the DNA sequence of subclones from a cosmid library.

E28. Note: The insert of cosmid B is contained completely within the insert of cosmid C.



E29. A sequence-tagged site is a segment of DNA, usually quite short (e.g., 100 to 400 bp in length), that serves as a unique site in the genome. STSs are identified using primers in a PCR reaction. STSs serve as molecular markers in genetic mapping studies. Sometimes, the region within an STS may contain a microsatellite. A microsatellite is a short DNA segment that is variable in length, usually due to a short repeating sequence. When a microsatellite is within an STS, the length of the STS will vary among different individuals, or even the same individual may be heterozygous for the STS. This makes the STS polymorphic. Polymorphic STSs can be used in linkage analysis, since their transmission can be followed in family pedigrees and through crosses of experimental organisms.

E30. A. The general strategy is shown in Figure 20.19. The researcher begins at a certain location and then walks toward the gene of interest. You begin with a clone that has a marker that is known to map relatively close to the gene of interest. A piece of DNA at the end of the insert is subcloned and then used in a Southern blot to identify an adjacent clone in a cDNA library. This is the first "step." The end of this clone is subcloned to make the next step. And so on. Eventually, after many steps, you will arrive at your gene of interest.

B. In this example, you would begin at STS-3. If you walked a few steps and happened upon STS-2, you would know that you were walking in the wrong direction.

C. This is a difficult aspect of chromosome walking. Basically, you would walk toward gene *X* using DNA from a normal individual and DNA from an individual with a mutant gene *X*. When you have found a site where the sequences are different between the normal and mutant individual, you may have found gene *X*. You would eventually have to confirm this by analyzing the DNA sequence of this region and determining that it encodes a functional gene.

E31. The first piece of information you would start with is the location of a gene or marker that is known to be close to the gene of interest by previous mapping studies. You would begin with a clone containing this marker (or gene) and follow the procedure of chromosome walking to eventually reach the gene of interest. A contig would make this much easier because you would not have to conduct a series of subcloning experiments to reach your gene. Instead, you could simply analyze the members of the contig.

E32. Two techniques to purify chromosomes are pulsed-field gel electrophoresis and chromosome sorting. They are useful because the isolation of a single chromosome can efficiently lead to the construction of a contig for that particular chromosome. Also, the isolation of a particular chromosome can lead to the mapping of markers or genes on that chromosome.