E1. The human gene that encodes the hormone is manipulated in vitro and then transformed into a bacterium. In many cases, the coding sequence of the human hormone gene is fused with a bacterial gene to prevent the rapid degradation of the human hormone. The bacteria then express the fusion protein and the hormone is separated by cyanogen bromide cleavage.

E2. The plasmid with the wrong orientation would not work because the coding sequence would be in the wrong direction relative to the promoter sequence. Therefore, the region containing the somatostatin sequence would not be transcribed into RNA.

E3. One possibility is to clone the toxin-producing genes from *B. thuringiensis* and introduce them into *P. syringae.* This bacterial strain would have the advantage of not needing repeated applications. However, it would be a recombinant strain and might be viewed in a negative light by people who are hesitant to use recombinant organisms in the field. By comparison, *B. thuringiensis* is a naturally occurring species.

E4. To construct the coding sequence for somatostatin, the researchers synthesized eight oligonucleotides, labeled A–H. When these oligonucleotides were mixed together, they would hydrogen bond to each other due to their complementary sequences. The addition of ligase would covalently link the DNA backbones. The *left* side of the coding sequence had an *Eco*RI site and the *right* side had a *Bam*HI site; these sites made it possible to insert this sequence at the end of the *ß*-galactosidase gene. You may also notice that an ATG codon (AUG in the mRNA) precedes the first alanine codon in somatostatin. This AUG codon specifies methionine, which allows the somatostatin to be cleaved from *ß*-galactosidase using cyanogen bromide. This was necessary because somatostatin, by itself, is rapidly degraded in *E. coli,* whereas the fusion protein is not.

E5. Basically, one can follow the strategy described in Figure 19.6. If homologous recombination occurs, only the $Neo^R$ gene is incorporated into the genome. The cells will be neomycin resistant and also resistant to gancyclovir. If gene addition occurs, the cells will be sensitive to gancyclovir. By growing the cells in the presence of neomycin and gancyclovir, one can select for homologous recombinants. The chimeras are identified by the observation that they have a mixture of light and dark fur.

E6. A kanamycin-resistance gene is contained within the T DNA. Exposure to kanamycin selects for the growth of plant cells that have incorporated the T DNA into their genome. The carbenicillin kills the *A. tumefaciens.* The phytohormones promote the regeneration of an entire plant from somatic cells. If kanamycin were left out, it would not be possible to select for the growth of cells that had taken up the T DNA.

E7. 1. *A. tumefaciens:* Genes are cloned into the T DNA of the Ti plasmid. This T DNA from this plasmid is transferred to the plant cell when it is infected by the bacterium.

2. Microinjection: A microscopic needle containing a solution of DNA is used to inject DNA into plant cells.

3. Electroporation: An electrical current is used to introduce DNA into plant cells.

4. Biolistic gene transfer: DNA is coated on microprojectiles that are “shot” into the plant cell.

5. Also, DNA can be introduced into protoplasts by treatment with polyethylene glycol and calcium phosphate. This method is similar to bacterial transformation procedures.

E8. The term *gene knockout* refers to an organism in which the function of a particular gene has been eliminated. For autosomal genes in eukaryotes, a gene knockout is a homozygote for a defect in both copies of the gene. If a gene knockout has no phenotypic effect, perhaps the gene is redundant. In other words, there may be multiple genes within the genome that can carry out the same function. Another reason why a gene knockout may not have a phenotypic effect is because of the environment. As an example, let’s say a mouse gene is required for the synthesis of a vitamin. If the researchers were providing food that contained the vitamin, the knockout mouse that was lacking this gene would have a normal phenotype; it would survive just fine. Sometimes, researchers have trouble knowing the effects of a gene knockout until they modify the environmental conditions in which the animals are raised.

E9. In Mendel’s work, and the work of many classical geneticists, an altered (mutant) phenotype is the initial way to identify a gene. For example, Mendel recognized a gene that affects plant height by the identification of tall and dwarf plants. The transmission of this gene could be followed in genetic crosses, and eventually, the gene could be cloned using molecular techniques. Reverse genetics uses the opposite sequence of steps. The gene is cloned first, and a phenotype for the gene (based on the creation of a gene knockout) is discovered later, by making a transgenic animal with a gene knockout.

E10. Gene replacement occurs by homologous recombination. For homologous recombination to take place, two crossovers must occur, one at each end of the target gene. After homologous recombination, only the *Neo*$^R$ gene, which is inserted into the target gene, can be incorporated into the chromosomal DNA of the embryonic stem cell. In contrast, nonhomologous recombination can involve two crossovers anywhere in the cloned DNA. Since the *TK* gene and target gene are adjacent to each other, nonhomologous recombination usually transfers both the *TK* gene and the *Neo*$^R$ gene. If both genes are transferred to a stem cell, it will die because the cells are grown in the presence of gancyclovir. The product of the *TK* gene kills the cell under these conditions. In contrast, cells that have acquired the *Neo*$^R$ gene due to homologous recombination but not the *TK* gene will survive. Stem cells, which have not taken up any of the cloned DNA, will die because they will be killed by the neomycin. In this way, the presence of gancyclovir and neomycin selects for the growth of stem cells that have acquired the target gene by homologous recombination.

E11. A chimera is an organism that is composed of cells from two different individuals (usually of the same species). Chimeras are made by mixing together embryonic cells from two individuals, and allowing the cells to organize themselves and develop into a single individual.

E12. A. Dolly's chromosomes may seem so old because they were already old when they were in the nucleus that was incorporated into the enucleated egg. They had already become significantly shortened in the mammary cells. This shortening was not repaired by the oocyte.

B. Dolly's age does not matter. Remember that shortening does not occur in germ cells. However, Dolly's eggs are older than they seem by about 6 or 7 years, because Dolly's germ-line cells received their chromosomes from a sheep that was 6 years old, and the cells were grown in culture for a few doublings before a mammary cell was fused with an enucleated egg. Therefore, the calculation would be: 6 or 7 years (the age of the mammary cells that produced Dolly's germ-line cells) plus 8 years (the age of Molly), which equals 14 or 15 years. However, only half of Molly's chromosomes would appear to be 14 or 15 years old. The other half of her chromosomes, which she inherited from her father, would appear to be 8 years old.

C. Chromosome shortening is a bit disturbing, because it suggests that aging has occurred in the somatic cell, and this aging is passed to the cloned organism. If cloning was done over the course of many generations, this may eventually have a major impact on the life span of the cloned organism. It may die much earlier than a noncloned organism. However, chromosome shortening may not always occur. It does not seem to occur in mice, which were cloned for six consecutive generations.

E13. You would conduct a Southern blot to determine the number of gene copies. As described in Chapter 18, you will observe multiple bands in a Southern blot if there are multiple copies of a gene. You need to know this information to predict the outcome of crosses. For example, if there are four integrated copies at different sites in the genome, an offspring could inherit anywhere from zero to four copies of the gene. You would want to understand this so you could predict the phenotypes of the offspring.

You would use a Northern blot or a Western blot to monitor the level of gene expression. A Northern blot will indicate if the gene is transcribed into mRNA, and a Western blot will indicate if the gene is translated into protein.

E14. The term *molecular pharming* refers to the practice of making transgenic animals that will synthesize (human) products in their milk. It can be advantageous when bacterial cells are unable to make a functional protein product from a human gene. For example, some proteins are posttranslationally modified by the attachment of carbohydrate molecules. This type of modification does not occur in bacteria, but it may occur correctly in transgenic animals. Also, dairy cows produce large amounts of milk, which may improve the yield of the human product.

E15. Organismal cloning means the cloning of entire multicellular organisms. In plants, this is easy. Most species of plants can be cloned by asexual cuttings. In animals, cloning occurs naturally, as in identical twins. Identical twins are genetic replicas of each other because they begin from the same fertilized egg. (Note: There could be some somatic mutations that occur in identical twins that would make them slightly different.) Recently, as in the case of Dolly, organismal cloning has become possible by fusing somatic cells with enucleated eggs. The advantage, from an agricultural point of view, is that organismal cloning could allow one to choose the best animal in a herd and make many clones from it. Breeding would no longer be necessary. Also, breeding may be less reliable because the offspring inherit traits from both the mother and father.

E16. You would first need to clone the normal mouse gene. Cloning methods are described in Chapter 18. After the normal gene was cloned, you would then follow the protocol shown in Figure 19.6. The normal gene would be inactivated by the insertion of the $Neo^R$ gene, and the *TK* gene would be cloned next to it. This DNA segment would be introduced into mouse embryonic stem cells and grown in the presence of neomycin and gancyclovir. This selects for homologous recombinants. The surviving embryonic stem cells would be injected into early embryos, which would then develop into chimeras. The chimeric mice would be identified by their patches of light and dark fur. At this point, if all has gone well, a portion of the mouse is heterozygous for the normal gene and the gene that has the $Neo^R$ insert. This mouse would be bred, and then brothers and sisters from the litter would be bred to each other. Southern blots would be conducted to determine if the offspring carried the gene with the $Neo^R$ insert. At first, one would identify heterozygotes that had one copy of the inserted gene. These heterozygotes would be crossed to each other to obtain homozygotes. The homozygotes are gene knockouts because the function of the gene has been "knocked out" by the insertion of the $Neo^R$ gene. Perhaps, these mice would be dwarf and exhibit signs of mental retardation. At this point, the researcher would have a mouse model to study the disease.

E17. Male 2 is the potential father, because he contains the bands that are found in the offspring but are not found in the mother. To calculate the probability, one would have to know the probability of having each of the types of bands that match. In this case, for example, male 2 and the offspring have four bands in common. As a simple calculation, we could eliminate the four bands that the offspring shares with the mother. If the probability of having each paternal band is 1/4, the chances that this person is not the father are $(1/4)^4$.

E18. DNA fingerprinting is a method of identification based on the properties of DNA. VNTR and STR sequences are variable with regard to size in natural populations. This variation can be seen when DNA fragments are subjected to gel electrophoresis. Within a population, any two individuals (except for identical twins) will display a different pattern of DNA fragments, which is called their DNA fingerprint.

E19. PCR is used to amplify DNA if there is only a small amount of it (e.g., a small sample at a crime scene). It is also used to amplify STRs. Southern blotting, using a probe that is complementary to a VNTR, is needed to specifically identify a limited number of bands (20 or so) that are variable within human populations.

E20. A VNTR is a sequence that is repeated several times within a genome and is variable in its length. In natural populations, it is common to find length variation. Therefore, any two individuals (who are not genetically identical) will differ with regard to the sizes of many of their VNTRs. When a gel is run and the VNTRs are seen in a Southern blot, the pattern of VNTR sizes provides a fingerprint of the individual's DNA. This fingerprint is a unique feature of each individual.

E21. This percentage is not too high. Based on their genetic relationship, we expect that a father and daughter must share at least 50% of the same bands in a DNA fingerprint. However, the value can be higher than that because the mother and father may have some bands in common, even though they are not genetically related. For example, at one site in the genome, the father may be heterozygous for a 4,100 bp and 5,200 bp VNTR, and the mother may also be heterozygous in this same region and have 4,100 bp and 4,700 bp VNTRs. The father could pass the 5,200 bp band to his daughter and the mother could pass the 4,100 bp band. The daughter would inherit the 4,100 bp and 5,200 bp bands. This would be a perfect match to both of the father's bands, even though the father transmitted only the 5,200 bp band to his daughter. The 4,100 bp band matches because the father and mother happened to have a VNTR in common. Therefore, the 50% estimate of matching bands in a DNA fingerprint based on genetic relationships is a minimum estimate. The value can be higher than that.

E22. The minimum percentage of matching bands is based on the genetic relationships.

A. 50%

B. 50% (on average, but occasionally it could be less)

C. 25% (on average, but occasionally it could be less)

D. 25% (on average, but occasionally it could be less)

E23. Ex vivo therapy involves the removal of living cells from the body and their modification after they have been removed. The modified cells are then reintroduced back into a person's body. This approach works well for cells such as blood cells that are easily removed and replaced. By comparison, this approach would not work very well for many cell types. For example, lung cells cannot be removed and put back again. In this case, in vivo approaches must be sought.

E24. In cystic fibrosis gene therapy, an aerosol spray, containing the normal *CF* gene in a retrovirus or liposome, is used to get the normal *CF* gene into the lung cells. The epithelial cells on the surface of the lung will take up the gene. However, these surface epithelial cells have a finite life span, so it is necessary to have repeated applications of the aerosol spray. Ideally, scientists hope to devise methods whereby the normal *CF* gene will be able to penetrate more deeply into the lung tissue.

E25. It is the gene product (i.e., the polypeptide) of an oncogene that causes cancerous cell growth. The antisense RNA (from the gene introduced via gene therapy) would bind to the mRNA from an oncogene. This would prevent the translation of the mRNA into polypeptides and thereby prevent cancerous cell growth.