Chapter 20: Biotechnology

The AP Biology exam has reached into this chapter for essay questions on a regular basis over the past 15 years. Student responses show that biotechnology is a difficult topic. This chapter requires a strong conceptual understanding of the technological processes and the underlying biology that guides the procedure. With a little careful work, this chapter will give you insights into the incredible advancements already made and a basis for understanding the new marvels yet to be discovered in biotechnology.

Overview

1. It is important to understand the meaning of the three terms in bold to start this chapter.
   - **recombinant DNA:** A DNA molecule made in vitro with segments from different sources
   - **biotechnology:** The manipulation of organisms or their components to produce useful products. It includes selective breeding, genetic engineering, and methods of DNA analysis.
   - **genetic engineering:** The direct manipulation of genes for practical purposes

**Concept 20.1 DNA cloning yields multiple copies of a gene or other DNA segment**

2. Plasmids are important in biotechnology. Give a full and complete definition of **plasmid**.
   
   A small, circular, double-stranded DNA molecule that carries accessory genes separate from those of a bacterial chromosome; in DNA cloning, used as vectors carrying up to about 10,000 base pairs (10 kb) of DNA. Plasmids are also found in some eukaryotes, such as yeasts.

3. The production of multiple copies of a single gene is called **gene cloning**.

4. Using Figure 20.2 in your text, label and explain the four steps in this preview of **gene cloning**.

   See page 397 of your text for the labeled figure.

5. Read the description of **restriction enzymes** on page 398 in your text carefully. Then draw and explain each step of Figure 20.3. When you finish, you should have recreated Figure 20.3 in following space.

   See page 398 of your text for the labeled figure.

6. What is a **cloning vector**? Why are bacterial plasmids widely used as cloning vectors?

   In genetic engineering, a DNA molecule that can carry foreign DNA into a host cell and replicate there. Cloning vectors include plasmids and bacterial artificial chromosomes (BACs), which move recombinant DNA from a test tube back into a cell, and viruses that transfer recombinant DNA by infection. Bacterial plasmids are widely used as cloning vectors because they can be readily obtained...
from commercial suppliers, manipulated to form recombinant plasmids, and then introduced into bacterial cells, and they multiply rapidly owing to the high reproductive rate of their host cells.

7. Figure 20.4 in your text is a more detailed discussion of the gene cloning procedure shown in Figure 20.2. Explain the following key points.

a. What is the source of the gene of interest?

A particular species of hummingbird

b. Explain why the plasmid is engineered with \( \text{amp}^R \) and \( \text{lac}Z \).

\( \text{Amp}^R \) is used because it makes the E. coli cells resistant to the antibiotic ampicillin. \( \text{Lac}Z \) encodes the enzyme which hydrolyzes lactose. The \( \text{amp}R \) gene will be a selective marker, as only cells that have taken up the plasmid will be able to grow on agar containing ampicillin.

c. Why are both the gene of interest and the plasmid cut with the same restriction enzyme?

They are cut with the same restriction enzyme so that both the gene and the plasmid will have the same sticky ends and be able to be annealed by ligase.

d. What is the role of DNA ligase in this process?

The DNA ligase covalently bonds the sugar-phosphate backbones of the fragments, whose sticky ends have base-paired.

e. After transformation has occurred, why are some colonies blue?

Colonies with nonrecombinant plasmids will be blue because they can hydrolyze X-gal, forming a blue product.

f. Why are some colonies white? Why is this important?

Colonies with recombinant plasmids, in which \( \text{lac}Z \) is disrupted, will be white because they cannot hydrolyze X-gal. These plasmids did not take up the hummingbird DNA fragments.

8. The cloning procedure described in question 7 and Figure 20.4 will produce many different fragments of hummingbird DNA. These fragments may be stored in a genomic library.

a. What is the purpose of a genomic library?

A genomic library is set of cell clones containing all the DNA segments from a genome, each within a plasmid, BAC, or other cloning vector.

b. Explain how a bacterial artificial library (BAC) and a cDNA library are formed.

A bacterial artificial library (BAC) is formed by trimming down the large plasmid to contain just the genes necessary for replication. cDNA is made in vitro using mRNA as a template for the first
strand. Because the mRNA contains only exons, the resulting double-stranded cDNA carries the complete coding sequence of the gene but no introns.

9. Once the hummingbird DNA is cloned, we have the problem of finding the piece of DNA that holds our gene of interest. Explain how nucleic acid hybridization will accomplish this task.

Nucleic acid hybridization is used to detect the gene’s DNA by its ability to base-pair with a complementary sequence on another nucleic acid molecule.

10. Describe how a radioactively labeled nucleic acid probe can locate the gene of interest on a multiwell plate. (Use Figure 20.7 in your text to guide your response.)

Plate by plate, cells from each well, representing one clone, are transferred to a defined spot on a special nylon membrane. The nylon membrane is treated to break open the cells and denature their DNA; the resulting single-stranded DNA molecules stick to the membrane.

The membrane is then incubated in a solution of radioactive probe molecules complementary to the gene of interest. Because the DNA immobilized on the membrane is single-stranded, the single-stranded probe can base-pair with any complementary DNA on the membrane. Excess DNA is then rinsed off.

The membrane is laid under photographic film, allowing any radioactive areas to expose the film. Black spots on the film correspond to the locations on the membrane of DNA that has hybridized to the probe. Each spot can be traced back to the original well containing the bacterial clone that holds the gene of interest.

11. Getting a cloned eukaryotic gene to function in bacterial host cells can be difficult. What are two problems with bacterial gene expression systems, and how is each solved?

1. Certain aspects of gene expression are different in eukaryotes and bacteria. To overcome differences in promoters and other DNA control sequences, scientists usually employ an expression vector, a cloning vector that contains a highly active bacterial promoter just upstream of a restriction site where the eukaryotic gene can be inserted in the correct reading frame.

2. Bacterial cells are unable to edit mRNA, so genomic DNA presents a problem because of the presence of noncoding regions (introns) in most eukaryotic genes. This problem can be surmounted by using a cDNA form of the gene, which includes only the exons.

12. Why are the advantages of using yeasts as host for cloning and/or expressing genes of interest?

Yeasts offer two advantages: They are as easy to grow as bacteria, and they have plasmids, a rarity among eukaryotes.

13. What are two techniques besides use of cloning vectors that can be used to introduce recombinant DNA into eukaryotic cells?

1. Electroporation: A brief electrical pulse applied to a solution containing cells creates temporary holes in their plasma membrane, through which DNA can enter.
2. Scientists can inject DNA directly into single eukaryotic cells using microscopically thin needles.

14. The **polymerase chain reaction (PCR)** is a Nobel Prize–winning idea that is used by scientists to amplify DNA, particularly when the quantity of DNA is very small or contaminated. Explain the three initial steps that occur in cycle 1 of PCR.

   See page 404 of your text for the labeled figure.

   1. Denaturation: Heat briefly to separate DNA strands.
   2. Annealing: Cool to allow primers to form hydrogen bonds with ends of target sequence.
   3. Extension: DNA polymerase adds nucleotides to the 3' ends of each primer.

15. How many molecules will be produced by four PCR cycles? Number of molecules equals $2^n$, where $n$ is the number of cycles. $2^4 = 16$

### Concept 20.2 DNA technology allows us to study the sequence, expression, and function of a gene

This section begins with a discussion of **gel electrophoresis**, a technique covered in AP Biology Lab 6. It is important to understand the principles of gel electrophoresis.

16. **Gel electrophoresis** is a technique used to separate nucleic acids or proteins that differ in size or electrical charge.

17. Why is the DNA sample to be separated by gel electrophoresis always loaded at the cathode or negative end of the power source?

   Because nucleic acid molecules carry negative charges on their phosphate groups, they all travel toward the positive pole in an electric field.

18. Explain why shorter DNA molecules travel farther down the gel than larger molecules.

   As the nucleic acid molecules move, the thicket of agarose fibers impedes the longer molecules more than it does the shorter ones, separating them by length.

19. To the right of the $\beta$-globin alleles, show the results of electrophoresis of the DNA patterns obtained from a patient with the normal $\beta$-globin alleles and a sickle-cell patient. Label each lane. For help, examine Figure 20.10. Refer to Figure 20.10(b) on page 406.

20. A patient who is a carrier for sickle-cell anemia would have a gel electrophoresis pattern showing four bands. Add this pattern to your gel in number 19 and explain why the gel shows a four-band pattern.

   The carrier is heterozygous and therefore has both alleles. The normal allele will produce three fragments. The abnormal allele will produce two fragments, one of which is the same as the normal allele. Therefore, there will be four different size fragments.
21. What is the purpose of a *Southern blot*?

Southern blotting is a technique that enables specific nucleotide sequences to be detected in samples of DNA. It involves gel electrophoresis of DNA molecules and their transfer to a membrane (blotting), followed by nucleic acid hybridization with a labeled probe.

22. What two techniques discussed earlier in this chapter are used in performing a Southern blot?

Gel electrophoresis and nucleic acid hybridization

In working toward the general idea of how DNA sequencing was mechanized, look at Figure 20.12 in your text to answer the following general questions about the *dideoxy chain termination method* for sequencing DNA

23. The following chart shows the materials that are added to the reaction tube to sequence a piece of DNA. Give the purpose of each.

<table>
<thead>
<tr>
<th>Molecules Added to the Reaction Tube</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Template strand</td>
<td>Provides pattern for the DNA strand to be synthesized</td>
</tr>
<tr>
<td>Primer</td>
<td>Will bind to the DNA and serve as the point where DNA polymerase can begin synthesis</td>
</tr>
<tr>
<td>DNA polymerase</td>
<td>Catalyzes the elongation of new DNA</td>
</tr>
<tr>
<td>Deoxyribonucleotides</td>
<td>These are the raw materials for synthesizing the new strand.</td>
</tr>
<tr>
<td>Dideoxyribonucleotides</td>
<td>These nucleotide forms are added randomly, and when this occurs, prevent further elongation of the strand.</td>
</tr>
</tbody>
</table>

24. Why does a dideoxyribonucleotide terminate a growing DNA strand? (You may need to refer to Figure 16.14, as suggested in the text, to answer this question.)

New DNA elongates in a 5' → 3' direction, and dideoxyribonucleotides lack the 3' —OH group to continue the chain.

25. Why are the four nucleotides in DNA each labeled with a different color of fluorescent tag?

The color of the fluorescent tag on each strand indicates the identity of the nucleotide at its end.

26. Use Figure 20.15 in your text to explain the four steps of *DNA microarray assays*.

See page 411 of your text for the labeled figure.

1. Isolate mRNA.

2. Make cDNA by reverse transcription, using fluorescently labeled nucleotides.
3. Apply cDNA mixture to a microarray with different genes in each spot. The cDNA will hybridize with any complementary DNA on the microarray.

4. Rinse off excess cDNA; scan microarray for fluorescence. Each fluorescent spot represents a gene expressed in the tissue sample.

27. Explain how microarrays are used in understanding patterns of gene expression in normal and cancerous tissue.

Different genes are “on” in different tissue types or under different conditions. Microarrays can be used to determine what genes are “on” or “off” in a particular type of tumor, and assist in both diagnosis and treatment.

28. What are SNPs? How are they used to screen for various diseases?

SNPs are single base-pair sites in a genome where nucleotide variation is found in at least 1% of the population. Scientists use SNPs to screen for various diseases by searching for SNP markers usually inherited with the disease-causing allele.

29. What is a totipotent cell?

A totipotent cell is one that can give rise to all parts of the embryo and adult, as well as extraembryonic membranes in species that have them.

30. How is nuclear transplantation performed in animals?

Scientists remove the nucleus of an unfertilized or fertilized egg, and replace it with the nucleus of a differentiated cell.

31. Label the six steps in reproductive cloning for mammals and briefly explain each step.

See page 414 of your text for the labeled figure and explanations.

32. Describe three problems associated with animal cloning.

1. Many cloned animals exhibit birth defects

2. In the nuclei of fully differentiated cells, a small subset of genes is turned on and expression of the rest is repressed.

3. DNA in cells from cloned embryos, like that of differentiated cells, often has more methyl groups than does the DNA in equivalent cells from normal embryos of the same species.
33. What are *stem cells*?

A stem cell is any relatively unspecialized cell that can produce, during a single division, one identical daughter cell and one more specialized daughter cell that can undergo further differentiation.

34. What is the major difference between *embryonic stem cells (ES)* and *adult stem cells*?

Adult stem cells are not able to give rise to all cell types in the organism, though they can generate multiple types. Embryonic stem cells are capable of giving rise to differentiated embryonic cells of any type.

35. How might *induced pluripotent stem cells (iPS)* resolve the debate about using stem cells for medical treatments?

Researchers have been able to reprogram differentiated cells to act like ES cells, resolving the ethical and political issues surrounding harvesting ES cells from human embryos.

36. What are two potential uses for human iPS cells?

Cells from patients suffering from diseases can be reprogrammed to become iPS cells, which can act as model cells for studying the disease and potential treatment.

In the field of regenerative medicine, a patient’s own cells could be reprogrammed into iPS cells and then used to replace nonfunctional tissues.

**Concept 20.4 The practical applications of DNA technology affect our lives in many ways**

37. Explain the idea of *gene therapy*, and discuss the problems with this technique as demonstrated in the treatment of SCID.

Gene therapy is the introduction of genes into an afflicted individual for therapeutic purposes. The problems with this treatment were demonstrated during the trial treatment of SCID, when three patients developed leukemia. Two factors may have contributed to the development of leukemia: the insertion of the retroviral vector near a gene involved in the proliferation of blood cells and an unknown function of the replacement gene itself.

38. Explain how *transgenic* “pharm” animals might be able to produce human proteins.

Scientists can introduce a gene from an animal or one genotype into the genome of another individual, often of a different species. Assuming that the introduced gene encodes a protein desired in large quantities, these transgenic animals can act as pharmaceutical “factories.”
39. Describe how short tandem repeats (STRs) can produce a sensitive genetic profile.

In short tandem repeats, simple sequence DNA containing multiple tandemly repeated units of two to five nucleotides. Variations in STRs act as genetic markers in STR analysis, used to prepare genetic profiles. The number of repeats present in these regions is highly variable from person to person, creating a more sensitive genetic profile.

40. How does the Ti plasmid make genetic engineering in plants a possibility?

For many plant species, a single tissue cell grown in culture can give rise to an adult plant, meaning that genetic manipulation can be performed on an ordinary somatic cell and the cell then used to generate an organism with new traits. The Ti plasmid integrates a segment of its DNA, known as T DNA, into the chromosomal DNA of its host plant cells.

41. What are genetically modified organisms, and why are they controversial?

An organism that has acquired one or more genes by artificial means; also known as a transgenic organism. Advocates of cautious approach toward GM crops fear that transgenic plants might pass their new genes to close relatives in nearby wild areas.

Test Your Understanding Answers

Now you should be ready to test your knowledge. Place your answers here:

1. b  2. c  3. d  4. c  5. d  6. b  7. a  8. c

9. Use the space below for the drawing.

See page A-19 (Appendix) for the answer.