Chapter 9 DNA-Based Information Technologies

Multiple Choice Questions

1. DNA cloning: the basics
Page: 307 Difficulty: 1 Ans: C
Restriction enzymes:

A) act at the membrane to restrict the passage of certain molecules into the cell.
B) are highly specialized ribonucleases that degrade mRNA soon after its synthesis.
C) are sequence-specific DNA endonucleases.
D) are very specific proteases that cleave peptides at only certain sequences.
E) catalyze the addition of a certain amino acid to a specific tRNA.

2. DNA cloning: the basics
Pages: 307-308 Difficulty: 2 Ans: B
The biological role of restriction enzymes is to:

A) aid recombinant DNA research.
B) degrade foreign DNA that enters a bacterium.
C) make bacteria resistant to antibiotics.
D) restrict the damage to DNA by ultraviolet light.
E) restrict the size of DNA in certain bacteria.

3. DNA cloning: the basics
Page: 308 Difficulty: 1 Ans: A
The size of the DNA region specifically recognized by type II restriction enzymes is typically:

A) 4 to 6 base pairs.
B) 10 to 15 base pairs.
C) 50 to 60 base pairs.
D) 200 to 300 base pairs.
E) about the size of an average gene.

4. DNA cloning: the basics
Page: 308 Difficulty: 2 Ans: D
Which of the following statements about type II restriction enzymes is *false*?

A) Many make staggered (off-center) cuts within their recognition sequences.
B) Some cut DNA to generate blunt ends.
C) They are part of a bacterial defense system in which foreign DNA is cleaved.
D) They cleave and ligate DNA.
E) They cleave DNA only at recognition sequences specific to a given restriction enzyme.

5. DNA cloning: the basics
Page: 308 Difficulty: 2 Ans: C
Certain restriction enzymes produce cohesive (sticky) ends. This means that they:

A) cut both DNA strands at the same base pair.
B) cut in regions of high GC content, leaving ends that can form more hydrogen bonds than ends of high AT content.
C) make a staggered double-strand cut, leaving ends with a few nucleotides of single-stranded DNA protruding.
D) make ends that can anneal to cohesive ends generated by any other restriction enzyme.
E) stick tightly to the ends of the DNA they have cut.

6. DNA cloning: the basics
Page: 311 Difficulty: 3 Ans: E
In the laboratory, recombinant plasmids are commonly introduced into bacterial cells by:

A) electrophoresis – a gentle low-voltage gradient draws the DNA into the cell.
B) infection with a bacteriophage that carries the plasmid.
C) microinjection.
D) mixing plasmids with an extract of broken cells.
E) transformation – heat shock of the cells incubated with plasmid DNA in the presence of $CaCl_2$.

7. DNA cloning: the basics
Page: 311 Difficulty: 2 Ans: B
The *E. coli* recombinant plasmid pBR322 has been widely utilized in genetic engineering experiments. pBR322 has all of the following features *except*:

A) a number of conveniently located recognition sites for restriction enzymes.
B) a number of palindromic sequences near the *Eco*RI site, which permit the plasmid to assume a conformation that protects newly inserted DNA from nuclease degradation.
C) a replication origin, which permits it to replicate autonomously.
D) resistance to two different antibiotics, which permits rapid screening for recombinant plasmids containing foreign DNA.
E) small overall size, which facilitates entry of the plasmid into host cells.

8. DNA cloning: the basics
Page: 311 Difficulty: 2 Ans: E
Which of the following statements regarding plasmid cloning vectors is correct?

A) Circular plasmids do not require an origin of replication to be propagated in *E. coli*.
B) Foreign DNA fragments up to 45,000 base pairs can be cloned in a typical plasmid.
C) Plasmids do not need to contain genes that confer resistance to antibiotics.
D) Plasmid vectors must carry promoters for inserted gene fragments.
E) The copy number of plasmids may vary from a few to several hundred.

9. DNA cloning: the basics
Page: 311 Difficulty: 1 Ans: C
A convenient cloning vector with which to introduce foreign DNA into *E. coli* is a(n):

A) *E. coli* chromosome.
B) messenger RNA.
C) plasmid.
D) yeast "ARS" sequence.
E) yeast transposable element.

10. DNA cloning: the basics
Pages: 312-313 Difficulty: 2 Ans: C
In genetic engineering, in vitro packaging is used to:

A) cut a desired region out of the host bacterium's chromosome.
B) ensure that genetically engineered bacteria are not accidentally released into the environment.
C) incorporate recombinant DNA into infectious bacteriophage particles.
D) place an antibiotic resistance gene in a plasmid.
E) splice a desired gene into a plasmid.

11. From genes to genomes
Page: 318 Difficulty: 2 Ans: B
Which of the following does *not* apply to the construction or use of a DNA library?

A) Determining the location of a particular DNA sequence in a DNA library requires a suitable hybridization probe.
B) Genomic libraries are better for expressing gene products than cDNA libraries.
C) Many segments of DNA from a cellular genome are cloned.
D) Specialized DNA libraries can be made by cloning DNA copies of mRNAs.
E) The DNA copies of mRNA found in a cDNA library are made by reverse transcriptase.

12. From genes to genomes
Pages: 319-321 Difficulty: 2 Ans: C
The PCR reaction mixture does *not* include:

A) all four deoxynucleoside triphosphates.
B) DNA containing the sequence to be amplified.
C) DNA ligase.
D) heat-stable DNA polymerase.
E) oligonucleotide primer(s).

13. From genes to genomes
Pages: 319-321 Difficulty: 2 Ans: B
Which of the following statements about the polymerase chain reaction (PCR) is *false*?

A) DNA amplified by PCR can be cloned.
B) DNA is amplified at many points within a cellular genome.
C) Newly synthesized DNA must be heat-denatured before the next round of DNA synthesis begins.
D) The boundaries of the amplified DNA segment are determined by the synthetic oligonucleotides used to prime DNA synthesis.
E) The technique is sufficiently sensitive that DNA sequences can be amplified from a single animal or human hair.

14. From genes to genomes
Page: 322 Difficulty: 1 Ans: E
RFLP is a:

A) bacteriophage vector for cloning DNA.
B) genetic disease.
C) plasmid vector for cloning DNA.
D) protein.
E) variation in DNA base sequence.

15. From genes to genomes
Page: 324 Difficulty: 1 Ans: C
Current estimates indicate that humans have about _____ genes.

A) 3,000
B) 10,000
C) 30,000
D) 100,000
E) 300,000

16. From genomes to proteomes
Pages: 326-330 Difficulty: 2 Ans: C
Which one of the following analytical techniques does *not* help illuminate a gene's cellular function?

A) DNA microarray analysis
B) Protein chip analysis
C) Southern blotting
D) Two-dimensional gel electrophoresis
E) Two-hybrid analysis

17. From genomes to proteomes
Pages: 329-330 Difficulty: 2 Ans: E
The technique known as two hybrid analysis for detecting interacting gene products depend on:

A) activation of DNA polymerase by the nearby binding of hybridizing protein complexes.
B) direct binding of a Gal4p activation domain to a DNA sequence in the promoter region.
C) having a promoter that responds directly to one of the two proteins whose interactions is being measured.
D) hybridization of DNA segments corresponding to the two genes being examined.
E) stimulation of trasncription by interaction of two Gal4p domains via fused protein sequences.

18. Genome alterations and new products of biotechnology
Page: 332 Difficutly: 2 Ans: B
A common cloning strategy for introducing foreign genes into plants with *Agrobacterium* employs all the following features except:

A) a selectable antibiotic marker such as kanamycin resistance.
B) a shuttle vector with 25 bp T-DNA repeats flanking the foreign gene of choice.
C) a Ti plasmid lacking its T-DNA segment.
D) active *vir* gene products from the altered Ti plasmid.
E) an ability to induce crown gall formation in infected leaves.

Short Answer Questions

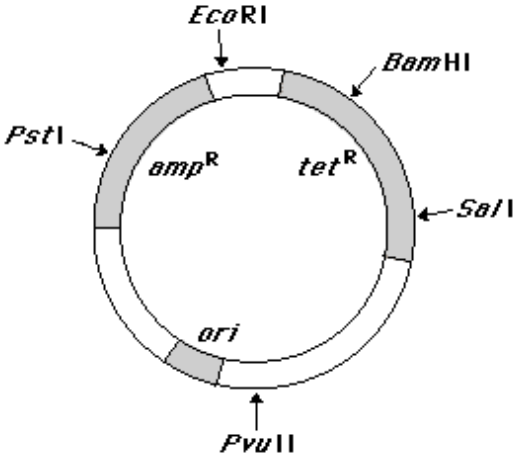
19. DNA cloning: the basics
Pages: 310-312 Difficulty: 2
A plasmid that encodes resistance to ampicillin and tetracycline is digested with the restriction enzyme *Pst*I, which cuts the plasmid at a single site in the ampicillin-resistance gene. The DNA is then annealed with a *Pst*I digest of human DNA, ligated, and used to transform *E. coli* cells. (a) What antibiotic would you put in an agar plate to ensure that the cells of a bacterial colony contain the plasmid? (b) What antibiotic-resistance phenotypes will be found on the plate? (c) Which phenotype will indicate the presence of plasmids that contain human DNA fragments?

Ans: (a) tetracycline; (b) tet^R amp^R and tet^R amp^S; (c) The tet^R amp^S phenotype indicates that the gene for ampicillin resistance has been interrupted by the insertion of a human DNA fragment.

20. DNA cloning: the basics
Pages: 311-312 Difficulty: 3
Explain how each of the following is used in cloning in a plasmid: (a) antibiotic resistance genes; (b) origin of replication; (c) polylinker region.

Ans: (a) Antibiotic resistance allows a researcher to select for a bacterial cell clone that carries the plasmid; loss of an antibiotic marker in a strain known to contain the plasmid can be used to infer the presence of a cloned DNA segment that interrupts the antibiotic resistance gene. (b) An origin of replication assures that the plasmid will replicate autonomously in the bacterium. (c) Polylinkers have cut sites for a variety of restriction enzymes, allowing insertion of DNA fragments produced with any of them.

21. DNA cloning: the basics
Page: 311 Difficulty: 2
Match each feature of the plasmid pBR322 (at left) with *one* appropriate description presented (at right) (see illustration of pBR322 below). Descriptions may be used more than once.



____ *amp*R sequence
____ *ori* sequence
____ *tet*R
____ *Bam*HI sequence
____ *Pst*I sequence

(a) permits selection of bacteria containing the plasmid
(b) a sequence required for packaging recombinant plasmids into bacteriophage
(c) origin of replication
(d) cleavage of the plasmid here does not affect antibiotic sequence resistance genes
(e) insertion of foreign DNA here permits identification of bacteria containing recombinant plasmids

Ans: a; c; a; e; e

22. DNA cloning: the basics
Pages: 311-312 Difficulty: 2
Explain briefly the properties of the plasmid pBR322 that make it so convenient as a vector for cloning fragments of foreign DNA.

Ans: pBR322 has two antibiotic resistance markers, so that its presence in a bacterium can be detected, bacteria that carry it can be selected, and insertion of cloned DNA into one of the resistance markers can be detected by loss of antibiotic resistance. The plasmid also has an origin of replication, so that it replicates autonomously in *E. coli*. Several convenient restriction sites allow easy insertion of restricted DNA fragments. Its small size facilitates its entry into *E. coli* by standard transformation protocols.

23. DNA cloning: the basics
Pages: 311-313 Difficulty: 2
When bacteriophage λ is used as a cloning vector, what limits the size of the DNA fragment that can be cloned?

Ans: The phage head can package only DNAs of a certain size (40,000 to 50,000 base pairs). DNAs larger than this cannot be contained in the phage. Up to about 23,000 base pairs of the λ genome are nonessential and can be replaced by a DNA fragment of similar length.

24. DNA cloning: the basics
Page: 313 Difficulty: 2
How does a Bacterial Artificial Chromosome (BAC) differ from a plasmid?

Ans: A BAC is a plasmid that is present in a small copy number in the bacterial cell. This allows it to carry a larger fragment of cloned DNA than a regular plasmid.

25. DNA cloning: the basics
Page: 313 Difficulty: 2
What is(are) the distinguishing feature(s) of a shuttle vector?

Ans: Shuttle vectors contain multiple sequences (such as replication origins) that allow their autonomous replication in two or more hosts, such as *E. coli* and yeast.

26. DNA cloning: the basics
Pages: 315-316 Difficulty: 2
What sequences are required in an expression vector (for use with *E. coli*) that are not essential in a cloning plasmid?

Ans: Regulated expression of the cloned gene requires: (1) a bacterial promoter and (2) its associated operator; (3) a transcription termination sequence; and (4) a ribosomal binding site. (Fig. 9-11, p. 316.)

27. DNA cloning: the basics
Pages: 315-316 Difficulty: 3
A scientist wishes to produce a mammalian protein in *E. coli*. The protein is a glycoprotein with a molecular weight of 40,000. Approximately 20% of its mass is polysaccharide. The isolated protein is usually phosphorylated and contains three disulfide bonds. The cloned gene contains no introns. (a) What sequences or sites will be required in the vector to get this gene regulated, transcribed, and translated in *E. coli*? (b) List two problems that might arise in producing a protein identical to that isolated from mammalian cells and describe each problem in no more than two sentences.

Ans: (a) The cloned gene must be preceded by a good *E. coli* promoter and its associated operator and by a ribosome-binding (Shine-Dalgarno) sequence. The other end of the gene should have a transcription terminator sequence. (b) Potential problems are that (1) *E. coli* enzymes may not glycosylate the protein, which may affect its folding and activity, and (2) the protein kinases that phosphorylate the protein in mammalian cells are probably absent in *E. coli*; therefore, the engineered protein will not be phosphorylated.

28. From genes to genomes
Page: 318 Difficulty: 2
What is the essential difference between a genomic library and a cDNA library?

Ans: A genomic library contains (in principle) all of the sequences present in the chromosome(s), including DNA sequences that are not transcribed. Because a cDNA library is made as a DNA copy of mRNA, it contains only those DNA sequences that are expressed in the cell.

29. From genes to genomes
Pages: 318-319 Difficulty: 2
Name one enzyme that is always used to make a cDNA library, but is generally not used to make a

genomic DNA library. Describe its function briefly.
Ans: Reverse transcriptase is used to make first a single-stranded DNA complementary to mRNA, then a double-stranded DNA.

30. From genes to genomes
Pages: 319-320 Difficulty: 2
A DNA sequence that may be present as only a single copy in a large mammalian genome can be amplified and cloned using the polymerase chain reaction (PCR). Describe the steps and reaction components required in a PCR experiment. Illustrate the steps in just one round.

Ans: DNA with the desired sequence is heated to convert it to single strands and cooled in the presence of an excess of oligonucleotide primers that flank the sequence to be amplified. A heat-stable DNA polymerase extends the primers, replicating the desired sequence. (See Fig. 9-16.)

31. From genes to genomes
Page: 320 Difficulty: 1
Why must the DNA polymerase used in the polymerase chain reaction (PCR) be heat stable?

Ans: The PCR involves repeated heating of the reaction mixture (to denature the double-stranded DNA) and cooling (to allow hybridization of DNA with oligonucleotide primers). A heat-sensitive enzyme would be denatured by this procedure.

32. From genes to genomes
Page: 322 Difficulty: 2
What are RFLPs and how are they used in forensic DNA fingerprinting technology?

Ans: RFLPs (restriction fragment length polymorphisms) are minor variations among individuals in DNA base sequence that can be detected by variation in the patterns of fragments that are produced upon cleavage with restriction endonucleases. When several DNA regions are examined, these patterns are distinctive for an individual and can be used to determine the identity (or nonidentity) of two samples of DNA. One of these samples can be from a crime scene, the other from a known individual.

33. From genomes to proteomes
Page: 325 Difficulty: 2
Distinguish between protein function at the molecular, cellular, and phenotypic level.

Ans: Molecular function describes the precise biochemical activity of the protein (such as enzymatic reaction or ligand binding), cellular function depends on the network ofinteractions engaged in by the protein within a cell, and phenotypic function refers to the effects of the protein on the entire organism.

34. From genomes to proteomes
Pages: 326-327 Difficulty: 2
What is a DNA microarray? How does it resemble and how does it differ from a DNA library?

Ans: A DNA microarray is a solid surface upon which are placed DNA fragments from many thousands of genes. It is in essence a form of DNA library that is arranged physically to allow rapid simultaneous screening of many thousands of genes.

35. Genome alterations and new products of biotechnology
Pages: 330-332 Difficulty: 2
Match the molecules, plasmids, or genes involved in plant cell transformation by *Agrobacteria* (at left) with *one* appropriate description from the list (at right) - letters may be used more than once.

____ *vir*	(a) segment of the Ti plasmid transferred to the plant cell genome
____ cytokinin	(b) genes that encode proteins required for transfer of a segment of the Ti plasmid to the plant cell genome
____ opine	(c) a plant growth hormone
____ T DNA	(d) an unusual metabolite that can be metabolized only by *Agrobacteria*
	(e) encodes enzymes required to metabolize auxin

Ans: b; c; d; a

36. Genome alterations and new products of biotechnology
Page: 332 Difficulty: 2
Much time and money are currently being spent trying to genetically alter plants. Why? What benefits for humankind might be realized by altering the genetic material of plants?

Ans: The supply of food for humans and domestic animals might be increased through plants with increased resistance to disease, pests, or freezing. Plants with increased yield of fruit or tuber and plants richer in rare nutrient components (e.g., high-lysine corn) might also be developed through genetic alteration.

37. Genome alterations and new products of biotechnology
Page: 335 Difficulty: 2
Briefly describe how a transgenic animal is produced.

Ans: The gene to be transferred into the animal (e.g., a mouse) is injected into a fertilized mouse egg. In some cases, the gene is integrated into the mouse chromosome at a position where it can be expressed. When these eggs are reimplanted into the uteri of female mice, some will yield progeny containing one copy of the "new" gene. By repeated inbreeding, mouse strains homozygous for the gene are obtained.

38. Genome alterations and new products of biotechnology
Pages: 335-339 Difficulty: 3
Suppose a biochemist has just developed a technique for the efficient replacement of mutant genes with normal ones in human germ line cells. She proposes to use this technology to eliminate familial hypercholesterolemia from the human gene pool. Similar projects are being proposed in several other countries. Should the U.S. National Institutes of Health allow her to proceed? Why or why not? This question has no correct answer. Thoughtful responses should be defended in four sentences or less.

Ans: Answers will vary.