Genetics and Genomics in Medicine Chapter 3

Questions & Answers

Multiple Choice Questions

Question 3.1

Which of the following statements, if any, is false?

a) Amplifying DNA means making many identical copies of one or more starting DNA sequences.
b) The object of DNA cloning is to amplify DNA.
c) The object of PCR is to amplify DNA
d) The object of DNA sequencing is to amplify DNA

Answer 3.1

d) The object of DNA sequencing is to amplify DNA

Explanation 3.1

In most DNA sequencing methods, the template DNA does get amplified. However, the object is not so much to amplify DNA but to get enough of it to work out its structure at the base level. The object is usually either to get functional information (or allow studies that will provide functional information), or to enable studies of genetic variation. In any case, some DNA sequencing methods, known collectively as single-molecule sequencing, do not involve amplifying DNA.

Question 3.2

Which of the following statements, if any, is false?

a) Amplifying DNA always requires a DNA polymerase.
b) Amplifying DNA must be carried out in bacterial or yeast cells.
c) DNA cloning in cells involves attaching the DNA to be cloned to a vector DNA to form a recombinant DNA that may be circular or linear.
d) Vector molecules in DNA cloning must have a replication origin that confers the ability to replicate extrachromosomally.

Answer 3.2

b) Amplifying DNA must be carried out in bacterial or yeast cells.
d) Vector molecules in DNA cloning must have a replication origin that confers the ability to replicate extrachromosomally.

Explanation 3.2

b) Is false because PCR is carried out in a test tube, not in cells.
c) Is true because in some DNA cloning systems (using lambda phage, yeast artificial chromosomes, and so on) the recombinant DNA is linear.
d) Is false because in yeast artificial chromosomes, the replication origin is a chromosomal one.

Question 3.3

Which of the following statements, if any, is false?

a) Cloning DNA in bacteria typically requires a plasmid or a bacteriophage vector.
b) Plasmids and bacteriophages are both small circular double-stranded DNA molecules.
c) Plasmids and bacteriophages each contain a replication origin that allows them to replicate independently of the bacterial chromosome.
d) To be useful as vectors plasmids and bacteriophages need to be genetically modified.

Answer 3.3

b) Plasmids and bacteriophages are both small circular double-stranded DNA molecules.

Explanation 3.3

Some bacteriophages that are used for DNA cloning are naturally single-stranded (but can be converted to a replicative form, such as M13 phages) or are linear (such as lambda phages)

Question 3.4

Which of the following statements, if any, is false?

a) The polymerase chain reaction (PCR) is a cell-free method of DNA amplification.
b) PCR is usually used to amplify a specific DNA sequence of interest using oligonucleotide primers that bind to closely flanking sequences.
c) PCR is superior to cell-based DNA cloning for two major reasons: it is much quicker and it allows much greater DNA amplification.
d) PCR requires the use of a heat-stable DNA polymerase to make copies of the template DNA.

Answer 3.4

c) PCR is superior to cell-based DNA cloning for two major reasons: it is much quicker and it allows much greater DNA amplification.

Explanation 3.4

Cell-based DNA cloning allows much greater DNA amplification than PCR

Question 3.5

With respect to nucleic acid hybridization, which, if any, of the following statements is false?

a) The probe is a labeled nucleic acid or oligonucleotide that is expected to hybridize to a target DNA sequence within an unlabelled test nucleic acid population.
b) The probe is a known single-stranded nucleic acid or oligonucleotide that is intended to hybridize to complementary target sequences in a poorly understood nucleic acid test sample.
c) The object of a hybridization assay is to identify target sequences within a test sample that are related to the probe so that some new information is gained about the target sequences.
d) During a hybridization assay, a heteroduplex is formed by un-natural base pairing between complementary probe and target sequences that show a sufficiently high degree of base pairing across part or all of their lengths.

Answer 3.5

a) The probe is a labeled nucleic acid or oligonucleotide that is expected to hybridize to a target DNA sequence within an unlabelled test nucleic acid population.

Question 3.6

With respect to nucleic acid hybridization, which, if any, of the following statements is false?

a) The strength of base pairing between a probe and a complementary target sequence depends on the number of stable base pairs that are formed.
b) Among other parameters, the hybridization stringency depends on the salt concentration and temperature of the hybridization reaction.
c) To identify a target sequence that is distantly related to the probe, high stringency hybridization needs to be used.
d) Under conditions that favor low hybridization stringency long heteroduplexes with significant base mismatching may be stable.

Answer 3.6

c) To identify a target sequence that is distantly related to the probe, high stringency hybridization needs to be used.

Question 3.7

Nucleic acid hybridization assays are normally carried out under relaxed hybridization stringency (to maximize the chances of heteroduplex formation) but afterwards, washes are carried out that can be designed to favor perfectly matched sequences only by changing some parameter. Which, if any, of the following changes would be consistent with that aim?

a) An increase in temperature.

b) An increase in salt concentration.
c) An increase in the concentration of a polar molecule, such as urea or formamide.

Answer 3.7

a) An increase in temperature.
c) An increase in the concentration of a polar molecule, such as urea or formamide.

Fill in the Blanks Questions

Question 3.8

Fill in the blanks below.

In cell-based DNA cloning, a DNA population of interest (which consists of very long DNA fragments) needs to be cleaved by a _____1_____ ____2_____ into manageably short DNA pieces that can be transported more easily into cells. The resulting DNA fragments are joined by a ___3____ ____4_____ to a ____5____ DNA, resulting in the formation of a ____6____ ___3___. The ___5___ carries a replication origin that allows it, and the ____6____ ___3____ , to replicate within a suitable host dell, usually some type of ____7____ or ____8____ cell.

Answer 3.8

1. restriction. 2. nuclease. 3. DNA. 4. Ligase. 5. vector. 6. recombinant. 7. bacterial. 8. yeast.

Question 3.9

Fill in the blanks below.

In cell-based DNA cloning a key step is ____ 1____ , the stage when the DNA of interest enters the provided host dells. The ____1_____ efficiency is usually very low, but when ____ 1____ occurs just a ___2____ DNA molecule usually enters the cell. As a result, a complex starting DNA population can be fractionated by the cells (which effectively act as sorting offices). A second key step allows identification of ____3_____ cells (in the case of bacteria, the host cells are genetically modified to be sensitive to some ____4____ , and the vector carries a gene conferring ____5____ to the ____4____) . Many of the transformed cells contain just the vector DNA instead of the desired ____5____ DNA. To identify a specific ____5____ DNA, a more specific assay is required that often involves ____6_____ using a closely related labelled DNA or RNA ____7____.

Answer 3.9

1. transformation. 2. single. 3. transformed. 4. antibiotic. 5. vector. 6. hybridization. 7. probe.

Essay and Lists Questions

Question 3.10

List four parameters that affect the stability of a heteroduplex and describe how they have an effect

Answer 3.10

1) Length of the region of base matching. Longer regions of base matching provide more hydrogen bonds and confer greater heteroduplex stability
2) Base composition. Higher GC composition confers greater heteroduplex stability (GC base pairs have three hydrogen bonds; AT base pairs have just two hydrogen bonds)
3) Temperature. Higher temperatures lead to breakage of hydrogen bonds and increased heteroduplex instability
4) Salt concentration. Lower [NaCl] increases heteroduplex instability.

There is often also the effect of polar molecules such as urea, formamide and so on which serve to disrupt hydrogen bonds and so increase heteroduplex instability.

Question 3.11

During PCR, each cycle has three defined steps. What are they, and what is involved?

Answer 3.11

1) Denaturation – heating of the DNA to disrupt hydrogen bonding so that complementary sequences are separated into single strands.
2) Annealing – cooling of the DNA to allow oligonucleotide primers to bind to desired target sequences in the starting DNA,
3) Synthesis – the bound oligonucleotides prime the synthesis of new DNA strands

Question 3.12

In cell-based DNA cloning two types of enzyme are critical for making recombinant DNA. What are they, and what roles do they carry out?

Answer 3.12

1) A class II restriction endonuclease. Enzymes like this recognize short specific sequences, usually spanning a region of 4-8 nucleotides long, and then cut the DNA on both strands, either within this sequence or in the immediate vicinity. As a result of their sequence specificity, they allow cutting of the DNA at *specific* sites. The restriction endonuclease

that is chosen for use is one that is designed to cut the vector molecule at a *unique* location to produce *defined ends*. The DNA to be cloned is often a heterogeneous collection of very large DNA fragments with heterogeneous ends, but by cutting with a specific restriction nuclease the DNA population is reduced to DNA fragments that are both of manageable size and also have homogeneous ends that are compatible with the ends of the cut vector, allowing relatively easy joining of the cut vector to the DNA fragments.

2) A DNA ligase. Needed to covalently join the cut vector molecule to the DNA fragments produced by cutting the DNA sample with a restriction endonuclease.

Question 3.13

Some restriction endonucleases cut DNA to produce *sticky ends.* What is meant by "sticky ends", and why are the restriction endonucleases that produce them so valuable for DNA cloning.

Answer 3.13

Some restriction nucleases recognize a specific *palindromic* sequence of nucleotides (one that reads the same from 5' to 3' on both DNA strands), and then cut the two DNA strands at positions that are asymmetrical with respect to the centre of the target sequence. The cleavage produces fragments with overhanging ends that are complementary in base sequence. When a vector molecule and DNA to be cloned are both cut by the same restriction endonuclease, the vector DNA will have overhanging ends that are complementary in base sequence to the overhanging ends of the fragments of DNA from the DNA to be cloned. Hydrogen bonding between these ends increases ligation efficiency allows association of vector-target DNA hybrids in readiness for subsequent covalent joining of vector to target DNA using a DNA ligase.

Question 3.14

In cell-based DNA cloning using plasmids, it is usual to use a plasmid that will allow maximum amplification (increase in copy number) of a recombinant DNA. Sometimes, however, that is not the aim. Explain why.

Answer 3.14

The replication origins of some plasmids allow large number of copies of the plasmid to be made (sometimes 100 plasmid copies can be produced in a single host cell). That is a clear advantage when there is a need to produce as much recombinant DNA as possible.

A disadvantage of using plasmids is that they do not normally allow large inserts. Cloning human DNA fragments larger than 5 kb becomes increasingly difficult using normal plasmid vectors that allow high copy number of the plasmids. If the object is to clone very large DNA fragments a specialized class of plasmids is used that have a tight constraint on copy number (replication is regulated so that at most there are one or two plasmid copies per cell). That

happens in the case of bacterial artificial chromosome (BAC) cloning systems in which DNA fragments as large as 200 kb can be cloned.

Question 3.15

Most PCR reactions are intended to amplify a specific DNA sequence of interest from within a complex starting DNA, often a genomic DNA sample. In addition to a sample of starting DNA of this type in an appropriate buffer with the correct ions to sustain the reaction, list four additional key requirements of the reaction to be successful.

Answer 3.15

1) A heat-stable DNA polymerase.
2) Oligonucleotide primers that can hybridize to regions that flank the desired target DNA sequence.
3) Suitable quantities of the four deoxynucleoside triphosphates.
4) A thermal cycler to allow successive cycles that progressively sets different reaction temperatures for the steps of denaturation, primer annealing and DNA synthesis required in each cycle.

Question 3.16

Describe the three recognized phases of a PCR reaction.

Answer 3.16

The first phase is the *lag phase* where the amount of product increases slowly at first (in the first few cycles, many of the synthesized strands are not of the desired type (see Figure 3.3). From 16-18 cycles, the *exponential phase* begins and continues to about the 25^{th} cycle. This is when the amount of PCR product is taken to be proportional to the amount of input DNA (and so this is best time to take quantitative measurements during real-time PCR). After the 25^{th} cycle, the amount of product increases at first but then later tails off as the *saturation phase* begins. Here, the efficiency of the reaction diminishes as reaction products increasingly compete with the remaining primer molecules for hybridization to the template DNA.

Question 3.17

What is the distinction, if any, between quantitative PCR and real-time PCR?

Answer 3.17

Quantitative PCR is any variant of PCR that seeks to quantify the amount of product. The amount of product can be measured after the end of a PCR reaction, but real-time PCR is designed to measure the amount of PCR product continuously during the reaction. That is, the

measurements are made in real time *while the reaction is progressing.* For that to happen, fluorescence labelling is used and specialized PCR machines are required that monitor the fluorescence throughout the PCR reaction.

Question 3.18

Nucleic acid hybridization assays require one of the interacting nucleic acid populations to be labelled in some way. Why is that required and what does it involve?

Answer 3.18

A hybridization assay involves using two nucleic acid populations: a well-understood *probe* population and a test sample population that is usually complex, and about which information is sought. The point of the hybridization assay is to allow the formation and detection of artificial heteroduplexes formed by base pairing between single nucleic acid strands from the probe and test sample populations.

To specifically detect the artificial heteroduplexes, one of the two nucleic acid populations needs to be labeled with distinctive nucleotides that can be identified in some way. Labeled heteroduplexes are usually physically separated in some way from labelled strands that have not formed heteroduplexes (including labeled strands that have reannealed to form homoduplexes) – often that is done by arranging that the unlabeled nucleic acid population is immobilized on a solid surface so that when washes are carried out after the hybridization reaction the only label that is trapped on the solid surface comes from labelled DNA strands that have formed heteroduplexes with complementary strands immobilized on the solid surface.

Labeling of a DNA or RNA population involves using a DNA or RNA polymerase in a DNA or RNA synthesis reaction in which at least one of the four deoxyribonucleoside triphosphates (dNTPs) or the respective ribonucleotides (rNTPs) carries a distinctive group that can be specifically detected in some way. In the past the labelled dNTPs/rNTPs would carry a radioisotope, but current methods usually employ a fluorescence label that can be specifically detected (ultraviolet light is used to induce the fluorescently labelled nucleotides to emit light of a specific wavelength that can be detected using appropriate detectors).

Question 3.19

What is the essential difference between the Sanger dideoxynucleotide sequencing method and massively parallel (= next generation) DNA sequencing?

Answer 3.19

The dideoxynucleotide sequencing method uses DNA synthesis reactions to make labelled copies of just one DNA template at a time; base-specific reactions are carried out with random-incorporation of one of the four chain-terminating dideoxynucleotides and the resulting nested series of fragments of increasing lengths is separated by gel electrophoresis and analysed.

Massively parallel DNA sequencing methods use different approaches, (such as sequencing-by-synthesis methods when the analysis occurs *during* base incorporation), but the essential difference is that a single run can simultaneously sequence millions or even billions of different DNA fragments in a complex population without any need for gel electrophoresis. These methods allow a huge amount of DNA sequencing to be carried out in one run and are designed for sequencing of complex DNAs. By contrast, dideoxynucleotide sequencing is better suited to sequencing purified DNA populations, such as PCR-amplified exons in mutation detection.

Question 3.20

Outline the different approaches to fractionating DNA using gel electrophoresis.

Answer 3.20

Gel electrophoresis can be used to separate DNA fragments according to size. Because of its multiple phosphates, DNA is negatively charged and can be induced to migrate through porous gels by fixing a negative electrode to one end of the gel assembly and a positive electrode to the other end. Because of frictional forces as the DNA migrates through the pores, larger DNA fragments migrate more slowly than smaller ones, allowing size fractionation.

Gels made of agarose are used for separating large DNA fragments but for small DNA fragments, polyacrylamide gels offer superior resolution and are used in standard dideoxynucleotide DNA sequencing methods. Slab gels have sometimes been used capillary gel electrophoresis is often used to separate DNA fragments in modern dideoxynucleotide DNA sequencing and in various mutation detection systems. It involves forcing the DNA fragments to migrate through ultrathin columns of polyacrylamide (as explained in Box 3.3).