Questions and Answers for Genetics and Genomics in Medicine Chapter 3

Question 1
What are the two broad classes of experimental method used to amplify (increase the copy number of) a DNA sequence of interest? Explain the essential difference between the two approaches.

Answer
- DNA cloning: amplifying DNA by replicating DNA sequences within cells, using a host cell's DNA polymerase.
- The polymerase chain reaction: amplifying DNA by replicating DNA sequences within a test tube, using a purified DNA polymerase.

Question 2
What is a restriction endonuclease and what is its natural function?

Answer
A restriction endonuclease is a class of enzyme found in bacteria that cleaves both strands of a DNA molecule at, or close to, a short defined target sequence that it specifically recognizes. Different bacterial strains have specific types of restriction endonuclease that recognize different target sequences.

The natural function of a restriction endonuclease is to protect the bacterial cell in which it is made from virus infection, by selectively cleaving double-stranded viral DNA sequences. The DNA of the bacterial host cell is protected from cleavage by its own restriction endonuclease by a preemptive methylation of the target sequences. At an early stage, a strain-specific DNA methylase adds methyl groups to the target sequences to block cleavage of the host cell DNA by its own restriction endonuclease. With the genome of the host cell protected, the restriction endonuclease helps protect the cell from subsequent viral infection by cleaving unmethylated viral DNA.

Question 3
Restriction nucleases often recognize palindromic DNA sequences and often cut the recognition sequence asymmetrically. For example, the restriction endonuclease *Eco*RI cleaves the sequence GGATCC by breaking the bond connecting the two guanines in the recognition sequence. What is meant by a palindromic DNA sequence, and why does asymmetric cutting of a palindromic DNA sequence generate fragments with 'sticky ends'?

Answer
In a palindromic DNA sequence the 5'→3' sequence on one DNA strand is identical to that of the complementary DNA sequence on the opposing strand, as for the GGATCC recognition sequence. The enzyme *Eco*RI cleaves DNA at the position marked by arrows:

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5'-------------G↓AATC C-----------------3'
3'-------------C CTAA↑G-----------------5'
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The term 'sticky end' is used of fragments with overhanging, palindromic ends because the overhanging sequences are complementary in sequence to any other end of the same sequence. If a vector DNA and a test sample DNA are both cut by *Eco*RI, for example, all the cut molecules will have a 5'-AATC overhanging end and base pairing between the 5'-AATC overhang on the vector and the complementary 5'-AATC sequence on a test sample DNA helps stick the fragments together so that they can be ligated to form recombinant DNA (see Figure 2 of Box 3.1).

Question 4

When compared with cloning DNA in cells, PCR has multiple advantages as a method of amplifying DNA. Describe four advantages.

Answer

- Speed. A typical PCR reaction can be carried out in an hour or less, but cloning DNA in cells typically takes days and sometimes weeks.
- Simplicity. The PCR reaction is largely automated and is practically very simple, so that hundreds (and sometimes thousands or more) of PCR assays can be conveniently carried out in parallel, whereas DNA cloning is not simple and multiple DNA cloning efforts carried out in parallel can be very laborious.
- Sensitivity. PCR is so sensitive that it can amplify a single-copy DNA sequence from a single cell, which makes it valuable in forensic science and allows diagnostic assays of a single cell or a few cells, as occurs in preimplantation diagnosis.
- Robustness. PCR can be used successfully to amplify DNA from degraded tissue, formalin-fixed tissue sections, and so on.

Question 5

When compared with cloning DNA in cells, PCR has two major disadvantages. What are they?

Answer

- Short product lengths. Standard PCR is excellent at amplifying short pieces of DNA, hundreds of nucleotides long, but it is much more difficult to use PCR to amplify sequences that are several kilobases long. Cloning DNA in cells readily allows the amplification of sequences up to several kilobases long, and specialized DNA cloning allows the cloning of inserts that are hundreds of kilobases long, and sometimes megabases of DNA in length.
- Low product amounts. The PCR reaction reaches saturation after a limited number of cycles (Figure 3.4) and it is impractical to produce very large amounts of amplified product. Cloning DNA in bacterial cells, by contrast, allows potentially huge amounts of purified DNA, which can be beneficial when there is a need to make very large amounts of a therapeutic human protein, for example.

Question 6

Labeling of nucleic acids is an important way of tracking them in different types of reaction, such as in DNA hybridization, in DNA sequencing, and in quantitative PCR. That typically involves incorporating nucleotides that have been labeled in some way into the nucleic acid. The nucleotides are usually labeled by covalently attaching to them a specific chemical group that can be detected in some specific way. Two popular methods involve attaching some type of fluorescent dye or a biotin group. Explain how these labels can be specifically detected.

Answer

Fluorescent dyes (also called fluorophores) are chemicals that can absorb light of a specific wavelength and then emit light at a longer wavelength. Often the light that is absorbed is in the ultraviolet range (and so is invisible to the naked eye) and the light that is emitted is in the visible range. To detect a fluorophore, therefore, the sample is exposed to a source of light of the wavelength that is known to be absorbed by it, and some kind of recorder, such as a laser scanner, detects the emitted light.

Biotin is a water-soluble vitamin (also called vitamin B_7 or vitamin H). It can be very specifically detected by binding with the bacterial protein streptavidin (so called because it was purified from *Streptomyces avidii*). The binding of streptavidin to biotin happens to be one of the very strongest noncovalent interactions in nature, and the streptavidin–biotin complex is resistant to extremes of both temperature and pH (and to chemical denaturants, detergents, and so on).

Question 7

In a hybridization assay what is meant by a probe, and what is the point of a hybridization assay?

Answer

A probe is a synthetic oligonucleotide of known sequence or a well-characterized nucleic acid that has been made single-stranded, as required. Single probe strands are intended to interrogate a test nucleic acid sample about which we wish to find information. The test nucleic acid sample must first be denatured so that it consists of single strands. By mixing single-stranded probe(s) with single strands of a test nucleic acid population, the probe strands will form heteroduplexes that can be identified and studied to give some information about the test sample. If the test sample is genomic DNA from a patient, for example, a hybridization assay can be used to monitor whether the patient has a deletion or duplication of some DNA sequence of interest.

Question 8

According to the conceptual design, there are standard and reverse hybridization assays. How do they differ, and in what class would you place microarray hybridization?

Answer

Standard hybridization assays use labeled probes and unlabeled test nucleic acid samples. Reverse hybridization assays use unlabeled probes and labeled test nucleic acids. Microarray hybridization is an example of a reverse hybridization assay.

Question 9

In some hybridization assays, the hybridization stringency is deliberately designed to be low; other hybridization assays depend on very stringent hybridization. What is meant by hybridization stringency, how can high and low stringency be achieved, and in what circumstances would very high and low hybridization stringencies be required for a hybridization assay?

Answer

Hybridization stringency means the degree to which incorrect base mismatching is tolerated. It may be very high (only perfect base matching is tolerated between a probe strand and a test sample DNA strand) or low (stable duplexes are formed between a probe strand and a test sample DNA strand that are partly complementary in sequence so that the duplex has occasional base mismatches.

The hybridization stringency depends on various factors, including the length of the region of base matching, the temperature, and the salt concentration. The heteroduplex is more stable when the region of base matching is long (more hydrogen bonds are holding the sequences together) and when the salt concentration is high but is less stable when the temperature increases significantly (hydrogen bonds are broken progressively).

To permit a low hybridization stringency, probes are typically many hundreds of nucleotides long and hybridization and/or washing conditions use high salt concentrations and modest temperatures to permit stable heteroduplexes with multiple base mismatches. To permit perfect base matching only, a high hybridization stringency is achieved by using a short (oligonucleotide) probe in which the heteroduplex is exposed to a low salt concentration and high temperatures.

High-stringency hybridization is important when it is required to find perfect matching, such as when designing hybridization probes that will bind with one allele of a gene but not with another allele that differs at single nucleotide position.

Low-stringency hybridization is important when we wish to use a probe to identify clearly related sequences that nevertheless have multiple nucleotide differences (using a human probe to identify the equivalent sequence in a mouse, for example).

Question 10

What function do dideoxynucleotides have in Sanger DNA sequencing?

Answer

Dideoxynucleotides are analogs of normal deoxynucleotides in which the 3' hydroxyl group has been replaced by a hydrogen. DNA synthesis occurs in the 5'→3' direction and a normal deoxynucleotide that has just been incorporated into the growing DNA chain will have a 3' hydroxyl group that can form a phosphodiester bond with the next incoming nucleotide. However, if a dideoxynucleotide is incorporated into a DNA chain, further DNA chain synthesis is impossible because there is no free hydroxyl group at the carbon 3' position.

The function of dideoxynucleotides in Sanger DNA sequencing is to act as chain terminators during DNA synthesis: once a dideoxynucleotide is incorporated into a growing DNA chain, further extension of the chain becomes impossible. By arranging base-specific reactions with four normal deoxynucleoside triphosphates (dNTPs) plus one of the four ddNTPs, there will be competition between the ddNTP and

its normal dNTP analog for insertion into the chain, sometimes causing chain termination and sometimes chain extension. A whole series of products can be made by DNA synthesis from a template DNA to produce nested sets of DNA fragments from which the DNA sequence can be deduced.

Question 11

Many types of DNA sequencing involve a DNA synthesis reaction in which labeled nucleotides are incorporated. Some of these methods also use some form of gel electrophoresis, but others are automated methods that do not use gel electrophoresis. When employed in DNA sequencing, what is the purpose of gel electrophoresis? Why is it not needed in some DNA sequencing methods?

Answer

In some DNA sequencing methods, such as classical Sanger (dideoxy) sequencing, the object is to make DNA strands that incorporate label in four base-specific reactions and allow DNA synthesis to produce a series of fragments of different lengths. The different DNA synthesis products need to be separated according to size by using gel electrophoresis so that they can be analyzed and interpreted. More modern methods often use some type of sequencing-by-synthesis in which the incorporation of labeled nucleotides is continuously followed while the DNA synthesis is taking place. In that case, there is no need for gel electrophoresis.