Chapter 9: Biotechnology: Principles and Processes

Exercises — Chapter-end Exercises

Q1 Can you list 10 recombinant proteins which are used in medical practice? Find out where they are used as therapeutics (use the internet).

SOLUTION

Ten recombinant proteins used in medical practice are:

1. Insulin (Humulin) — treatment of diabetes mellitus.
2. Human Growth Hormone (somatropin) — treatment of growth hormone deficiency and short stature.
3. Erythropoietin (EPO) — treatment of anaemia, especially in chronic kidney disease.
4. Interferon-alpha — antiviral therapy; treatment of hepatitis B and C, and certain cancers.
5. Interferon-beta — treatment of multiple sclerosis.
6. Tissue Plasminogen Activator (tPA) — dissolves blood clots in myocardial infarction and stroke.
7. Factor VIII — treatment of haemophilia A (clotting disorder).
8. Factor IX — treatment of haemophilia B.
9. Granulocyte-Colony Stimulating Factor (G-CSF, filgrastim) — stimulates white blood cell production; used during chemotherapy.
10. Hepatitis B surface antigen (HBsAg) — used as a recombinant subunit vaccine against hepatitis B.

These proteins are produced by inserting the corresponding human gene into a suitable host (bacteria, yeast, or mammalian cell lines), which then expresses and secretes the functional protein. The recombinant protein is subsequently purified and formulated for therapeutic use.

Answer: Ten examples: insulin (diabetes), human growth hormone (growth disorders), erythropoietin (anaemia), interferon-alpha (hepatitis/cancer), interferon-beta (multiple sclerosis), tPA (clot dissolution), Factor VIII (haemophilia A), Factor IX (haemophilia B), G-CSF (neutropenia during chemotherapy), recombinant HBsAg (hepatitis B vaccine).

Q2 Make a chart (with diagrammatic representation) showing a restriction enzyme, the substrate DNA on which it acts, the site at which it cuts DNA and the product it produces.

SOLUTION

Example: Restriction enzyme EcoRI (from Escherichia coli RY 13)

Substrate DNA — double-stranded DNA containing the palindromic recognition sequence:

5' — G A A T T C — 3'
3' — C T T A A G — 5'

Recognition site — EcoRI recognises the hexanucleotide palindromic sequence 5'-GAATTC-3'.

Cut site — EcoRI cuts between G and A on each strand, but not at exactly the centre of the

palindrome. It cuts the top strand between G↓AATTC and the bottom strand between CTTAA↑G (staggered cuts):

5' — G AATTC — 3'
3' — CTTAA G — 5'

Products — Two DNA fragments each bearing single-stranded overhanging 'sticky ends':

Fragment 1: Fragment 2:
5'— G 3' 5'— AATTC — 3'
3'— CTTAA — 5' 3'— G — 5'

The 4-nucleotide 5' overhangs (—AATT—) on each fragment are called 'sticky ends' because they are complementary to each other and can form hydrogen bonds, facilitating ligation by DNA ligase.

Note: A labelled chart should depict (i) the intact double-stranded DNA with the GAATTC recognition site highlighted, (ii) arrows showing the staggered cut positions on each strand, and (iii) the two resulting fragments with their sticky ends labelled.

Answer: EcoRI recognises 5'-GAATTC-3' (palindrome), cuts staggered between G and A on both strands, producing two fragments with 5' sticky ends: —AATT overhangs. These sticky ends facilitate joining by DNA ligase.

Q3 From what you have learnt, can you tell whether enzymes are bigger or DNA is bigger in molecular size? How did you know?

SOLUTION

DNA is bigger in molecular size than enzymes (proteins).

Reasoning:

Enzymes are proteins made of amino acid chains. Even large enzymes typically consist of a few hundred to a few thousand amino acids, giving them a molecular weight in the range of tens of thousands to a few hundred thousand Daltons.

DNA, on the other hand, is a polynucleotide that can be extremely long. In humans, each chromosome contains a single continuous DNA molecule containing millions to hundreds of millions of base pairs. The human genome has approximately 3.2×10^9 base pairs, distributed across 23 chromosomes. Even a small bacterial chromosome (e.g., E. coli) has about 4.6×10^6 base pairs.

We know this because restriction enzymes bind to specific short sequences (e.g., 4–8 bp recognition sites) on the much larger DNA molecule and cut it at those points. The fact that a single enzyme molecule acts on a vast DNA polymer — and that DNA must be packaged to fit within the cell nucleus — tells us that the DNA molecule is enormously larger than the enzyme.

Additionally, gel electrophoresis separates DNA fragments by size, and even small restriction fragments (a few hundred base pairs) are far larger in molecular weight than a typical enzyme.

Answer: DNA is far bigger in molecular size than enzymes. Human DNA contains billions of base pairs spanning enormous lengths, whereas enzymes are proteins of a few hundred to a few thousand amino acids. This is evident from the fact that a single enzyme recognises and cuts only a tiny specific sequence within a huge DNA molecule.

Q4 What would be the molar concentration of human DNA in a human cell? Consult your teacher.

SOLUTION

This is a calculation-based question to be worked out with the help of a teacher. The following approach provides the reasoning:

Each human diploid cell contains approximately 6.4 × 10^9 base pairs of DNA (3.2 × 10^9 bp per haploid set × 2).

The average molecular weight of a base pair is approximately 660 g/mol.

So the mass of DNA per cell ≈ 6.4 × 10^9 × 660 g/mol ÷ 6.022 × 10^{23} (Avogadro's number)
≈ (6.4 × 10^9 × 660) / (6.022 × 10^{23}) g per cell
≈ 4.224 × 10^{12} / 6.022 × 10^{23} g per cell
≈ 7.01 × 10^{-12} g = ~7 picograms per cell (this agrees with the known value of ~6–7 pg).

The volume of a human cell is approximately 1–4 picolitres (≈ 10^{-12} L).

If we treat the entire human genome (per cell) as a single DNA molecule, there is exactly 1 molecule of DNA per haploid set (or 46 molecules for 46 chromosomes per diploid cell).

Molar concentration = (number of molecules per litre) / Avogadro's number
≈ (46 / (2 × 10^{-12} L)) / 6.022 × 10^{23}
≈ 2.3 × 10^{13} / 6.022 × 10^{23} mol/L
≈ 3.8 × 10^{-11} mol/L (≈ 38 picomolar), treating each chromosome as one molecule.

This is an extremely low molar concentration because each DNA molecule is gigantic.

Answer: The molar concentration of human DNA in a human cell is extremely low — on the order of ~10^{-11} mol/L (tens of picomolar) — because each DNA molecule is enormous. Consult your teacher for the precise calculation using cell volume and genome size.

Q5 Do eukaryotic cells have restriction endonucleases? Justify your answer.

SOLUTION

No, eukaryotic cells do not have restriction endonucleases.

Justification:

Restriction endonucleases are enzymes found in prokaryotes (bacteria). They serve as a natural defence mechanism against foreign DNA, particularly bacteriophage (viral) DNA. When a bacteriophage injects its DNA into the bacterium, the restriction enzyme recognises specific sequences in the foreign DNA and cleaves it, thereby restricting the growth of the phage. The

bacterium's own DNA is protected from cleavage because the recognition sites in its DNA are methylated by a companion methylase enzyme, preventing the restriction enzyme from cutting its own DNA.

Eukaryotic cells do not possess such restriction-modification systems. Their DNA is protected differently — it is packaged into chromatin with histones and enclosed within the nucleus. Eukaryotes use other mechanisms (e.g., immune responses, interferon production) to defend against viral infections.

Furthermore, in genetic engineering and recombinant DNA technology, restriction endonucleases are isolated exclusively from prokaryotic organisms (bacteria). Over 900 restriction enzymes have been identified from more than 230 strains of bacteria, confirming that these enzymes are a prokaryotic feature.

Therefore, eukaryotic cells lack restriction endonucleases because they lack the prokaryotic restriction-modification defence system.

Answer: No, eukaryotic cells do not have restriction endonucleases. These enzymes are a prokaryotic defence mechanism against foreign (phage) DNA. Eukaryotes protect their DNA via chromatin packaging and other immune mechanisms, not restriction-modification systems.

Q6 Besides better aeration and mixing properties, what other advantages do stirred tank bioreactors have over shake flasks?

SOLUTION

Stirred tank bioreactors offer several advantages over shake flasks:

1. Large volume capacity — Bioreactors can process large volumes of culture (100–1000 litres or more), enabling large-scale industrial production of recombinant proteins, enzymes, vaccines, and antibiotics. Shake flasks are limited to small volumes (up to a few litres).

2. Controlled growth conditions — Bioreactors are equipped with control systems for temperature, pH, oxygen levels, and nutrient supply. This maintains cells in optimal physiological condition throughout the process, which is not possible with shake flasks.

3. Foam control — Bioreactors have a foam control (foam breaker) system to prevent excessive foaming during culture, which can disrupt cell growth. Shake flasks have no such mechanism.

4. Continuous culture capability — Bioreactors can be operated in a continuous mode, where used medium is drained out and fresh medium is continuously added. This keeps cells in the log/exponential growth phase for maximum productivity. Shake flasks are used only for batch culture.

5. Sampling ports — Bioreactors have sampling ports that allow periodic withdrawal of small culture volumes for monitoring cell growth, product yield, and quality without disrupting the main culture.

6. Sterility maintenance — Bioreactors are designed for strict sterile (contamination-free)

operation at large scale, with steam sterilisation and sterile air sparging systems. Maintaining strict sterility at scale is much harder with shake flasks.

7. Higher biomass and product yield — The controlled conditions and continuous culture capability result in higher biomass and greater yields of the desired product compared to shake flasks.

Answer: Stirred tank bioreactors offer large volume processing (100–1000 L), precise control of temperature/pH/oxygen, foam control systems, continuous culture mode (higher yields), sampling ports for monitoring, and rigorous sterility maintenance — all of which are impossible or impractical in shake flasks.

Q7 Collect 5 examples of palindromic DNA sequences by consulting your teacher. Better try to create a palindromic sequence by following base-pair rules.

SOLUTION

A palindromic DNA sequence reads the same on both strands in the 5'→3' direction. The following are five well-known examples, each the recognition site of a restriction enzyme:

1. EcoRI recognition sequence:
   5'— G A A T T C —3'
   3'— C T T A A G —5'
   (Reads GAATTC on the top strand 5'→3' and GAATTC on the bottom strand 5'→3')

2. HindIII recognition sequence:
   5'— A A G C T T —3'
   3'— T T C G A A —5'

3. BamHI recognition sequence:
   5'— G G A T C C —3'
   3'— C C T A G G —5'

4. SalI recognition sequence:
   5'— G T C G A C —3'
   3'— C A G C T G —5'

5. PstI recognition sequence:
   5'— C T G C A G —3'
   3'— G A C G T C —5'

Creating a palindromic sequence:
Using base-pair rules (A pairs with T, G pairs with C), write one strand and fill in the complementary strand. For a sequence to be palindromic, the complement read 5'→3' must equal the original 5'→3'.

Example of a student-created 6 bp palindrome:
5'— A T G C A T —3'
3'— T A C G T A —5'

(Bottom strand 5'→3': A T G C A T — same as the top strand)

Answer: Five palindromic sequences (recognition sites): EcoRI (GAATTC), HindIII (AAGCTT), BamHI (GGATCC), SalI (GTCGAC), PstI (CTGCAG). A palindromic sequence reads the same on both strands in the 5'→3' direction because the complementary strand is also its own reverse complement.

Q8 Can you recall meiosis and indicate at what stage a recombinant DNA is made?

SOLUTION

In the context of meiosis, recombinant DNA (in the natural, chromosomal sense) is formed during Prophase I of Meiosis I — specifically during the substage called Pachytene.

During Pachytene, homologous chromosomes (bivalents) are tightly paired in synapsis. At this stage, non-sister chromatids of homologous chromosomes undergo crossing over at points called chiasmata. Crossing over involves breakage and rejoining of DNA segments between non-sister chromatids, resulting in new combinations of alleles — this is the natural process by which recombinant DNA (recombinant chromosomes) is produced.

The recombinant chromosomes are then distributed to the daughter cells through Meiosis I and Meiosis II, ultimately producing gametes that carry novel genetic combinations.

Note: In the context of genetic engineering/recombinant DNA technology (the chapter topic), recombinant DNA is formed in vitro — outside a living cell — by cutting DNA from two different sources with the same restriction enzyme and joining the resulting fragments using DNA ligase. This is different from meiotic recombination but the question asks to connect the two concepts.

Answer: In meiosis, recombinant DNA is formed during Prophase I (specifically Pachytene substage) when crossing over occurs between non-sister chromatids of homologous chromosomes at chiasmata, generating chromosomes with novel combinations of genetic material.

Q9 Can you think and answer how a reporter enzyme can be used to monitor transformation of host cells by foreign DNA in addition to a selectable marker?

SOLUTION

A reporter enzyme is an enzyme whose activity can be easily detected and measured, and whose gene can be fused with the gene of interest or cloned into the vector. It provides a visible or measurable signal that directly indicates whether a host cell has been successfully transformed with foreign DNA.

Mechanism using β-galactosidase as a reporter enzyme (insertional inactivation method):

1. The gene encoding β-galactosidase (lacZ gene) is incorporated into the cloning vector at the site where the foreign DNA is to be inserted (the cloning site).

2. When the foreign (insert) DNA is successfully ligated into the vector, it disrupts the lacZ gene

— a process called insertional inactivation. The β-galactosidase enzyme is no longer produced (non-functional).

3. When the transformed host cells are plated on a growth medium containing a chromogenic substrate (e.g., X-gal — 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside):

— Non-transformants (cells with no plasmid): do not grow (if a selectable marker like ampicillin resistance is used).

— Transformants without insert (non-recombinants): β-galactosidase is active → cleaves X-gal → produces a blue colour → blue colonies.

— Transformants with insert (recombinants): β-galactosidase is inactivated → cannot cleave X-gal → no colour → white/colourless colonies.

4. White colonies are thus selected as recombinant transformants.

This method — blue-white screening — allows rapid, visual identification of recombinant colonies and acts as a reporter system complementary to the selectable marker (antibiotic resistance).

Other reporter enzymes used include luciferase (produces bioluminescence), chloramphenicol acetyltransferase (CAT), and green fluorescent protein (GFP), which are used in both bacterial and eukaryotic transformation studies.

Answer: A reporter enzyme (e.g., β-galactosidase) can be used via insertional inactivation: its gene is placed at the cloning site in the vector. Successful insertion of foreign DNA disrupts the reporter gene — transformed recombinant cells lose enzyme activity and remain colourless (white colonies) on chromogenic substrate (X-gal), while non-recombinant transformants produce blue colonies. This blue-white screening visually distinguishes recombinants from non-recombinants.

Q10 Describe briefly the following:
(a) Origin of replication
(b) Bioreactors
(c) Downstream processing

SOLUTION

(a) Origin of replication (ori):

The origin of replication is a specific DNA sequence present in a chromosome or in a plasmid/vector from which replication of DNA begins. It is the site recognised by the cell's replication machinery (DNA polymerase and associated proteins) to initiate DNA synthesis.

In the context of cloning vectors, the ori sequence is essential because any piece of foreign (alien) DNA must be linked to an origin of replication in order to multiply within the host cell. Without an ori, the foreign DNA cannot replicate independently and will be lost as the host cell divides.

The ori sequence also controls the copy number of the plasmid in the host cell — different ori sequences support different copy numbers (some plasmids have 15–100 copies per cell; others have only 1–2). If a high yield of the cloned gene is desired, a vector with an ori that supports a

high copy number should be chosen.

(b) Bioreactors:

Bioreactors are large vessels (typically 100–1000 litres in volume) in which raw materials are biologically converted into specific products — individual enzymes, recombinant proteins, vaccines, antibiotics, etc. — using microbial, plant, animal or human cells.

A bioreactor provides optimal growth conditions for the cultured organism by controlling temperature, pH, substrate concentration, salts, vitamins, and oxygen supply. The most commonly used type is the stirred-tank bioreactor, which is usually cylindrical with a curved base. It contains:
— An agitator system (flat-bladed impeller) for even mixing and oxygen distribution
— An oxygen delivery system (sterile air sparging)
— A foam control system (foam breaker)
— Temperature and pH control systems
— Sampling ports for periodic monitoring

Bioreactors enable continuous culture (used medium drained, fresh medium added), keeping cells in the exponential growth phase for maximum productivity and higher yields than small-scale shake flasks.

(c) Downstream processing:

After the biosynthetic (fermentation) stage in a bioreactor is complete, the desired product must be isolated and purified before it can be marketed. The series of processes involved in this isolation and purification is collectively referred to as downstream processing.

It includes steps such as:
— Separation of cells from the culture medium (centrifugation, filtration)
— Cell disruption (if the product is intracellular)
— Purification of the target protein using chromatography, precipitation, ultrafiltration, etc.
— Formulation with suitable preservatives and stabilisers
— Quality control testing and clinical trials (for pharmaceutical products)

Downstream processing and quality control vary from product to product and are critical for ensuring the safety, efficacy, and shelf-life of the final product.

Answer: (a) Origin of replication: the specific DNA sequence from which replication initiates; essential in vectors for autonomous replication of foreign DNA and determines copy number. (b) Bioreactors: large vessels (100–1000 L) providing controlled conditions (temperature, pH, O_2, nutrients) for large-scale production of recombinant proteins/enzymes/antibiotics using living cells. (c) Downstream processing: post-fermentation steps — separation, purification, formulation, and quality control — to produce a marketable, safe final product.

Q11 Explain briefly
(a) PCR
(b) Restriction enzymes and DNA
(c) Chitinase

SOLUTION

(a) PCR (Polymerase Chain Reaction):

PCR is an in vitro technique used to amplify a specific segment of DNA (the gene of interest) to produce billions of copies in a short time.

The process uses:
— Two sets of primers: short, chemically synthesised oligonucleotides complementary to the two ends (flanking regions) of the target DNA sequence.
— Thermostable DNA polymerase: isolated from the bacterium Thermus aquaticus (Taq polymerase), which remains active at high temperatures.
— Deoxynucleotide triphosphates (dNTPs) as building blocks.

Each PCR cycle consists of three steps:
1. Denaturation: The double-stranded DNA is heated to ~94°C, causing the two strands to separate.
2. Primer annealing: The temperature is lowered to ~50–65°C so that the primers anneal (bind) to their complementary sequences on each strand.
3. Extension: The temperature is raised to ~72°C; Taq polymerase extends the primers by adding nucleotides in the 5'→3' direction, synthesising new complementary strands.

After repeated cycles (typically 25–35), the target DNA segment is amplified approximately 1 billion (10^9) times. The amplified fragment can then be used for cloning, sequencing, or diagnostic purposes.

(b) Restriction enzymes and DNA:

Restriction enzymes (restriction endonucleases) are enzymes isolated from bacteria that cut double-stranded DNA at specific sequences called recognition sequences or restriction sites. Each enzyme recognises a particular palindromic nucleotide sequence (typically 4–8 base pairs) and cleaves both strands of the DNA at or near this site.

The cuts are usually staggered (not at the same position on both strands), leaving short single-stranded overhangs called 'sticky ends'. Sticky ends are complementary to each other and can hydrogen-bond with complementary sticky ends on other DNA fragments cut by the same enzyme, facilitating ligation by DNA ligase.

For example, EcoRI recognises 5'-GAATTC-3' and cuts between G and A on both strands, producing 5' sticky ends (—AATT overhangs).

Restriction enzymes are the essential 'molecular scissors' of recombinant DNA technology. They allow scientists to cut DNA from different sources at precise locations and join the resulting fragments to create recombinant DNA molecules.

(c) Chitinase:

Chitinase is an enzyme that degrades chitin, the major structural polysaccharide found in the cell walls of fungi. In the process of isolating DNA from fungal cells, it is necessary to break open (lyse) the cell to release the intracellular contents including DNA.

Because fungal cell walls are composed of chitin (unlike bacterial cell walls which are made of peptidoglycan and are lysed by lysozyme, or plant cell walls made of cellulose and lysed by cellulase), the enzyme used to break down the fungal cell wall is chitinase.

Chitinase hydrolyses the β-1,4-glycosidic bonds in chitin, weakening and disrupting the cell wall so that the cell contents — DNA, RNA, proteins, polysaccharides, lipids — are released into the surrounding solution for further purification.

Answer: (a) PCR amplifies a specific DNA segment in vitro using primers, thermostable Taq polymerase, and repeated cycles of denaturation, annealing, and extension — producing ~10^9 copies. (b) Restriction enzymes are bacterial 'molecular scissors' that cut dsDNA at specific palindromic recognition sequences, generating sticky ends used in constructing recombinant DNA. (c) Chitinase degrades chitin in fungal cell walls, enabling lysis of fungal cells to release DNA for isolation.

Q12 Discuss with your teacher and find out how to distinguish between
(a) Plasmid DNA and Chromosomal DNA
(b) RNA and DNA
(c) Exonuclease and Endonuclease

SOLUTION

(a) Plasmid DNA vs Chromosomal DNA:

Plasmid DNA:
— Small, circular, double-stranded DNA molecules.
— Found in the cytoplasm of bacterial cells (extrachromosomal), replicating independently of the main chromosome.
— Typically 1–200 kb in size.
— Present in multiple copies per cell (1–100 or more).
— Usually carry non-essential genes (e.g., antibiotic resistance).
— Not associated with histone proteins.
— Can be isolated separately by alkaline lysis and differential centrifugation (e.g., using caesium chloride density gradient — plasmid DNA forms a distinct band separate from chromosomal DNA due to its supercoiled, circular form having a higher buoyant density).

Chromosomal DNA:
— Very large, linear (in eukaryotes) or a single large circular molecule (in prokaryotes).
— Located in the nucleus (eukaryotes) or nucleoid region (prokaryotes).
— Carries all essential genetic information for the organism.
— In eukaryotes, tightly associated with histone proteins forming chromatin.
— Present as one copy (per haploid set) in the cell.

— Separated from plasmid DNA during isolation by its much larger size — it pellets under centrifugation conditions that leave plasmid DNA in solution.

(b) RNA vs DNA:

DNA (Deoxyribonucleic acid):
— Sugar: deoxyribose (lacks 2'-OH group).
— Bases: Adenine (A), Thymine (T), Guanine (G), Cytosine (C).
— Usually double-stranded (dsDNA).
— More stable; not easily hydrolysed.
— Reacts with Feulgen stain (specific for DNA); purple colour.
— Negative test with Bial's orcinol reagent.

RNA (Ribonucleic acid):
— Sugar: ribose (has 2'-OH group).
— Bases: Adenine (A), Uracil (U), Guanine (G), Cytosine (C) — no Thymine.
— Usually single-stranded.
— Less stable; the 2'-OH makes it susceptible to alkaline hydrolysis.
— Reacts with Bial's orcinol reagent (positive); green colour.
— Negative with Feulgen stain.
— Sensitive to ribonuclease (RNase); DNA is not degraded by RNase.
— DNA is sensitive to DNase; RNA is not.

(c) Exonuclease vs Endonuclease:

Both are types of nucleases — enzymes that degrade nucleic acids — but they differ in how they act:

Exonuclease:
— Removes nucleotides sequentially from the ends of a DNA (or RNA) strand.
— Acts from either the 5' end or the 3' end (depending on the specific enzyme).
— Requires a free end to start degradation.
— Examples: Exonuclease I (degrades single-stranded DNA from the 3' end), Bal31.

Endonuclease:
— Cleaves the phosphodiester bonds within (inside) the DNA or RNA chain — not at the ends.
— Does not require a free end; can act on circular DNA.
— Restriction endonucleases are a specific class that cut at defined recognition sequences.
— Examples: EcoRI, HindIII, BamHI (all restriction endonucleases); DNase I (non-specific endonuclease).

Answer: (a) Plasmid DNA: small, circular, extrachromosomal, multiple copies; chromosomal DNA: large, linear (eukaryotes), single copy, nuclear, histone-associated. Distinguished by size, shape, location, and density-gradient banding. (b) RNA has ribose sugar and uracil (no thymine), is single-stranded, and less stable; DNA has deoxyribose, thymine, is double-stranded, and more stable. (c) Exonucleases remove nucleotides from the ends of DNA strands; endonucleases (including restriction enzymes) cut within the DNA chain at internal phosphodiester bonds.