Gel electrophoresis is a fundamental technique used to separate macromolecules—proteins, DNA, or RNA—based on size and charge. Samples are loaded into wells of a polyacrylamide (for proteins or small nucleic acids) or agarose (for large DNA) gel, and an electric field is applied. Negatively charged molecules migrate toward the positively charged anode. Smaller molecules navigate the gel matrix more easily and thus travel farther than larger ones. A molecular “ladder” of known sizes is run alongside samples to estimate fragment lengths.

Native-PAGE preserves protein tertiary and quaternary structure, separating proteins by size under non-denaturing conditions. In contrast, SDS-PAGE denatures proteins: sodium dodecyl sulfate binds uniformly (one SDS per two amino acids), conferring a constant charge-to-mass ratio so proteins separate solely by mass. Reducing SDS-PAGE adds a reducing agent (e.g., β-mercaptoethanol) to break disulfide bonds, fully denaturing multi-subunit proteins into individual polypeptides.

Isoelectric focusing separates proteins by their isoelectric points (pI) using a gel with an immobilized pH gradient. Proteins migrate until they reach the gel region where pH equals their pI, at which point they carry no net charge and stop moving.

Western blotting combines SDS-PAGE with immunodetection. After separating proteins by SDS-PAGE, they are transferred onto a membrane and probed with a primary antibody specific to the target protein. A labeled secondary antibody binds the primary, and the complex is visualized (often via chemiluminescence or autoradiography), identifying both the presence and approximate size of the protein of interest.

**1. Which feature of SDS-PAGE ensures that proteins separate based solely on mass?**  
**Answer:** SDS confers a uniform charge-to-mass ratio.  
**Explanation:** SDS binds at a constant rate (one SDS per two amino acids), giving all proteins the same negative charge per unit mass so that their migration through the gel depends only on size.

**2. During reducing SDS-PAGE, which type of bond is specifically broken by the reducing agent?**  
**Answer:** Disulfide (S–S) bonds.  
**Explanation:** Reducing agents such as β-mercaptoethanol cleave disulfide bridges between cysteine residues, fully denaturing multi-subunit proteins into separate polypeptide chains.

**3. In isoelectric focusing, why do proteins accumulate at their pI rather than migrating to the anode?**  
**Answer:** At their pI, proteins carry no net charge and thus no longer experience an electric force.  
**Explanation:** Migration in an electric field depends on net charge; when the local pH equals a protein’s isoelectric point, its net charge is zero, so it stops moving.

**4. Which of the following correctly orders steps in a Western blot?  
A. Transfer → SDS-PAGE → antibody probing → detection  
B. SDS-PAGE → transfer → antibody probing → detection  
C. Antibody probing → SDS-PAGE → transfer → detection  
D. SDS-PAGE → antibody probing → transfer → detection**  
**Answer:** B. SDS-PAGE → transfer → antibody probing → detection  
**Explanation:** First proteins are separated by SDS-PAGE, then transferred onto a membrane, probed with primary and then secondary antibodies, and finally visualized.

**5. A researcher runs a native-PAGE and observes a single band at 60 kDa, but on SDS-PAGE the same sample shows two bands at 25 kDa and 35 kDa. Which is the most likely explanation?**  
**Answer:** The native protein is a heterodimer composed of 25 kDa and 35 kDa subunits.  
**Explanation:** Under non-denaturing conditions the intact heterodimer migrates as ~60 kDa; denaturing SDS-PAGE separates it into its individual 25 kDa and 35 kDa polypeptides.

**6. In gel electrophoresis, a 100 bp DNA fragment and a 500 bp fragment are run on the same agarose gel. Which fragment migrates farther and why?**  
**Answer:** The 100 bp fragment migrates farther because smaller fragments move more easily through the gel matrix.  
**Explanation:** Gel pores impose resistance that slows larger molecules more; thus shorter DNA fragments travel farther toward the anode.

**7. What is the primary purpose of including a molecular ladder when running gels?**  
**Answer:** To provide reference bands of known size for estimating the molecular weight of sample bands.  
**Explanation:** Comparing sample migration distances to the ladder’s defined fragment sizes allows accurate determination of unknown sample sizes.

**8. A protein with a pI of 8.0 is subjected to isoelectric focusing on a pH 3–10 gradient. At roughly what point in the gel will it focus?**  
**Answer:** Near the region of the gel where pH ≈ 8.0 (toward the basic end).  
**Explanation:** In an immobilized pH gradient, proteins migrate until they reach the zone matching their pI, then stop.

**9. Which statement about reducing versus non-reducing SDS-PAGE is true?  
A. Only reducing SDS-PAGE uses SDS.  
B. Only reducing SDS-PAGE breaks peptide bonds.  
C. Only non-reducing SDS-PAGE preserves disulfide-linked subunits.  
D. Only non-reducing SDS-PAGE uses a reducing agent.**  
**Answer:** C. Only non-reducing SDS-PAGE preserves disulfide-linked subunits.  
**Explanation:** Non-reducing SDS-PAGE omits the reducing agent, leaving disulfide bonds intact, whereas reducing SDS-PAGE cleaves them.

**10. During Western blot detection via autoradiography, what role does the secondary antibody play?**  
**Answer:** It binds to the primary antibody and carries the detectable label.  
**Explanation:** The secondary antibody amplifies the signal by providing the tag (e.g., enzyme or radiolabel) used for visualization.

**Passage**

Gel electrophoresis separates macromolecules—proteins, DNA, or RNA—by size and charge. Samples are loaded into wells of a polyacrylamide gel (for proteins or small nucleic acids) or agarose gel (for DNA >500 bp), and an electric field drives negatively charged molecules toward the positively charged anode. Smaller species navigate the gel matrix more easily and thus travel farther. Gels are stained (e.g., Coomassie Blue), and a molecular ladder of known sizes allows estimation of fragment lengths .

**Native-PAGE** runs under non-denaturing conditions, preserving protein structure and separating complexes by size. In **SDS-PAGE**, SDS denatures proteins and binds one SDS per two amino acids, equalizing charge-to-mass ratios so migration reflects mass alone. **Reducing SDS-PAGE** adds a reducing agent (e.g., β-mercaptoethanol) to break disulfide bonds, fully dissociating multi-subunit proteins into individual polypeptides .

**Isoelectric focusing** uses a polyacrylamide gel with an immobilized pH gradient. Proteins migrate until they reach the region where pH equals their isoelectric point (pI), at which point they carry no net charge and stop moving .

**Western blotting** couples SDS-PAGE with immunodetection. Separated proteins are transferred to a membrane, probed with a primary antibody specific to the target, then with a labeled secondary antibody. The complex is visualized (e.g., via autoradiography) to identify both presence and approximate size of the protein .

**Southern** (DNA) and **Northern** (RNA) blotting detect specific sequences. After restriction digest (DNA only), single-strand denaturation (DNA), and electrophoresis, fragments are transferred to nitrocellulose and probed with a radiolabeled complementary oligonucleotide. Autoradiography reveals bands of interest; Northern blot omits the digest and denaturation steps for RNA .

**Sanger dideoxynucleotide sequencing** determines DNA sequence by synthesizing fragments terminated by chain-blocking ddNTPs. Four reactions—each with one type of ddNTP—yield fragment ladders. Electrophoresis and autoradiography of each lane allow reading of the sequence from shortest (5′ end) to longest .

**Questions**

1. Which gel electrophoresis component provides a size reference for estimating fragment lengths?
2. What characteristic of SDS ensures proteins separate by mass in SDS-PAGE?
3. During reducing SDS-PAGE, which bond does β-mercaptoethanol specifically cleave?
4. In isoelectric focusing, a protein will stop migrating when it reaches the gel region where:  
   A. its net charge is zero  
   B. the pH equals the lower limit of the gradient  
   C. SDS concentration is highest  
   D. the gel’s porosity changes
5. Which step is unique to Northern blotting compared to Southern blotting?
6. What is the primary purpose of the secondary antibody in a Western blot?
7. In Sanger sequencing, ddNTPs cause termination of DNA synthesis because they lack:  
   A. a 5′ phosphate  
   B. a 3′ hydroxyl  
   C. a base  
   D. a 2′ deoxyribose
8. A protein with multiple subunits linked by disulfide bonds will appear as a single band in SDS-PAGE but multiple bands in reducing SDS-PAGE because:
9. Why do smaller DNA fragments migrate farther than larger ones in an agarose gel?
10. Which technique separates proteins based on their isoelectric points rather than size or charge alone?

**Answer Key and Explanations**

1. **Answer:** A molecular ladder.  
   **Explanation:** A ladder contains DNA or protein fragments of known sizes that run alongside samples, enabling size estimation by comparison .
2. **Answer:** Uniform charge-to-mass ratio conferred by SDS.  
   **Explanation:** SDS binds one molecule per two amino acids, giving proteins a constant negative charge proportional to their mass so that mobility depends on size alone .
3. **Answer:** Disulfide (S–S) bonds.  
   **Explanation:** Reducing agents like β-mercaptoethanol cleave disulfide bridges between cysteines, fully denaturing multi-subunit proteins .
4. **Answer:** A. its net charge is zero  
   **Explanation:** Proteins focus at their pI, where they carry no net charge and thus no longer migrate in the electric field .
5. **Answer:** Restriction enzyme digestion.  
   **Explanation:** Southern blotting begins with restriction digest of DNA; Northern blotting uses intact RNA, so the digest step is omitted .
6. **Answer:** To bind the primary antibody and carry the detectable label.  
   **Explanation:** The secondary antibody amplifies signal by providing an enzyme or radiolabel that enables visualization of the primary antibody–protein complex .
7. **Answer:** B. a 3′ hydroxyl  
   **Explanation:** ddNTPs lack the 3′–OH required for phosphodiester bond formation, terminating DNA strand elongation on incorporation .
8. **Answer:** Disulfide bonds keep subunits linked under non-reducing conditions.  
   **Explanation:** SDS alone denatures but does not break disulfide bonds, so multi-subunit proteins remain intact in SDS-PAGE but separate into individual chains when reduced .
9. **Answer:** Because smaller fragments experience less resistance moving through the gel matrix.  
   **Explanation:** Gel pores impede larger molecules more, so shorter DNA migrates farther toward the anode under an electric field .
10. **Answer:** Isoelectric focusing.  
    **Explanation:** This method separates proteins by their pI in a pH gradient gel, rather than by size or uniform charge alone

**Passage**

Chromatography separates components of a mixture based on differential affinities between a stationary phase and a mobile phase. Molecules with greater interaction with the stationary phase elute more slowly, while those favoring the mobile phase elute more quickly . In liquid chromatography, silica (polar) serves as the stationary phase and a non-polar solvent (e.g., toluene) as the mobile phase . High-performance liquid chromatography (HPLC) uses high pressure to force the mobile phase through a finely ground stationary phase, enhancing resolution; in reverse-phase HPLC, the stationary phase is non-polar and the mobile phase is polar . Gas chromatography vaporizes analytes into an inert carrier gas (e.g., helium), passing them through a liquid/polymer-coated column; more polar compounds interact longer with the stationary phase and exhibit longer retention times . Gel-filtration (size-exclusion) chromatography separates molecules by size: small molecules enter porous beads and elute later, whereas large molecules are excluded and elute first . Ion-exchange chromatography employs charged resin beads to separate proteins by net charge: in cation exchange (negatively charged beads), positively charged proteins bind and elute later, while negatively charged proteins elute first; anion exchange (positively charged beads) functions inversely . Affinity chromatography leverages specific ligand–protein interactions: target proteins bind ligand-coated beads and are eluted by competition with free ligand . In thin-layer chromatography (TLC), a silica-coated plate is the stationary phase; a non-polar solvent ascends by capillary action, and compounds separate by polarity. The Rf value (distance traveled by compound ÷ distance traveled by solvent front) quantifies migration .

**Questions with Answers and Explanations**

1. **In chromatography, the primary factor determining the elution order of compounds is their relative**  
   A. molecular weights  
   B. affinities for the stationary versus mobile phase  
   C. boiling points  
   D. optical activity

**Answer:** B. affinities for the stationary versus mobile phase  
**Explanation:** Elution order reflects how strongly compounds interact with each phase: stronger stationary-phase interactions retard elution, while stronger mobile-phase affinities accelerate it .

1. **In normal-phase liquid chromatography, which phase is polar?**  
   A. Mobile phase  
   B. Stationary phase  
   C. Both phases  
   D. Neither phase

**Answer:** B. Stationary phase  
**Explanation:** In normal-phase LC, the stationary phase (silica) is polar, and the mobile phase (toluene) is non-polar .

1. **Which change distinguishes reverse-phase HPLC from normal-phase HPLC?**  
   A. Use of gas instead of liquid  
   B. Inversion of mobile- and stationary-phase polarity  
   C. Elution by size rather than polarity  
   D. Addition of a reducing agent

**Answer:** B. Inversion of mobile- and stationary-phase polarity  
**Explanation:** Reverse-phase HPLC switches to a non-polar stationary phase and a polar mobile phase, enhancing separation of non-polar analytes .

1. **In gel-filtration chromatography, which molecule elutes first?**  
   A. Small molecules, because they diffuse into gel pores  
   B. Large molecules, because they are excluded from gel pores  
   C. Charged molecules, because they bind the stationary phase  
   D. Hydrophobic molecules, because they partition into beads

**Answer:** B. Large molecules, because they are excluded from gel pores  
**Explanation:** In size-exclusion chromatography, large molecules cannot enter bead pores and therefore elute before smaller molecules that diffuse into the beads .

1. **In anion-exchange chromatography, which proteins elute last?**  
   A. Neutral proteins  
   B. Positively charged proteins  
   C. Negatively charged proteins  
   D. Hydrophobic proteins

**Answer:** C. Negatively charged proteins  
**Explanation:** In anion-exchange (positively charged resin), negatively charged proteins bind strongly and elute later, while positively charged proteins elute first .

1. **How are target proteins typically eluted in affinity chromatography?**  
   A. By lowering the pH to precipitate them  
   B. By applying high pressure  
   C. By adding excess free ligand to compete off the beads  
   D. By increasing salt concentration to disrupt ionic bonds

**Answer:** C. By adding excess free ligand to compete off the beads  
**Explanation:** Affinity chromatography elutes bound proteins by flooding the column with free ligand, which competes for the binding site and displaces the target proteins .

1. **A compound with a high Rf value in TLC is most likely:**  
   A. Very polar, because it travels farther  
   B. Very non-polar, because it travels farther  
   C. Very large, because it is retained by silica  
   D. Very small, because it ascends faster

**Answer:** B. Very non-polar, because it travels farther  
**Explanation:** In TLC with a polar stationary phase, non-polar compounds interact weakly and travel farther up the plate, yielding a higher Rf .

1. **What is the mobile phase in gas chromatography?**  
   A. Helium or another inert gas  
   B. Toluene  
   C. Silica gel  
   D. Water

**Answer:** A. Helium or another inert gas  
**Explanation:** Gas chromatography uses an inert carrier gas (e.g., helium or nitrogen) as the mobile phase to transport vaporized analytes through the column .

1. **Compared to standard liquid chromatography, HPLC’s main advantage is:**  
   A. Use of an inert gas for better resolution  
   B. Ability to separate proteins by isoelectric point  
   C. Higher resolving power due to finely packed stationary phase under high pressure  
   D. Elimination of the need for a stationary phase

**Answer:** C. Higher resolving power due to finely packed stationary phase under high pressure  
**Explanation:** HPLC employs high pressures to pass solvent through small stationary-phase particles, increasing surface interactions and resolution .

1. **Which chromatography method separates proteins primarily by size?**  
   A. Ion-exchange chromatography  
   B. Affinity chromatography  
   C. Gel-filtration chromatography  
   D. Thin-layer chromatography

**Answer:** C. Gel-filtration chromatography  
**Explanation:** Also known as size-exclusion chromatography, this method separates molecules solely by size, with large molecules eluting before small ones

**Passage**

Distillation is used to separate two or more molecules from a solution . It exploits differences in boiling points to accomplish separation.

* **Simple Distillation** is used when the boiling points of the two components differ by 25 °C or greater .
* **Fractional Distillation** is used when the boiling points differ by less than 25 °C .
* **Vacuum Distillation** lowers the pressure to reduce boiling points, making it suitable for high-boiling or thermally sensitive compounds that risk chemical change at atmospheric pressure .

**Questions with Answers and Explanations**

1. **What property does distillation exploit to separate mixture components?**  
   A. Density  
   B. Boiling point  
   C. Polarity  
   D. Solubility

**Answer:** B. Boiling point  
**Explanation:** Distillation separates compounds based on differences in their boiling points, allowing one component to vaporize and be collected before the other .

1. **In simple distillation, the minimum boiling-point difference required between two compounds is:**  
   A. 10 °C  
   B. 25 °C  
   C. 50 °C  
   D. 100 °C

**Answer:** B. 25 °C  
**Explanation:** Simple distillation is effective only when the boiling points differ by at least 25 °C .

1. **Fractional distillation is most appropriate when the boiling-point difference between two components is:**  
   A. Less than 25 °C  
   B. 25 °C or greater  
   C. Greater than 50 °C  
   D. Exactly 100 °C

**Answer:** A. Less than 25 °C  
**Explanation:** Fractional distillation uses a fractionating column to improve separation when boiling points are within 25 °C of each other .

1. **Vacuum distillation is used when:**  
   A. Boiling points are high and risk chemical change  
   B. Boiling points differ by ≥25 °C  
   C. Boiling points differ by <25 °C  
   D. Components are immiscible

**Answer:** A. Boiling points are high and risk chemical change  
**Explanation:** By reducing pressure, vacuum distillation lowers boiling points, preventing decomposition of heat-sensitive compounds .

1. **A mixture has components with boiling points of 120 °C and 150 °C. Which distillation technique is best?**  
   A. Fractional distillation  
   B. Simple distillation  
   C. Vacuum distillation  
   D. Steam distillation

**Answer:** B. Simple distillation  
**Explanation:** The 30 °C difference exceeds 25 °C, making simple distillation sufficient .

1. **To separate ethanol (78 °C) and water (100 °C), which method is optimal?**  
   A. Fractional distillation  
   B. Simple distillation  
   C. Vacuum distillation  
   D. Steam distillation

**Answer:** A. Fractional distillation  
**Explanation:** The 22 °C boiling-point gap is less than 25 °C, requiring fractional distillation for effective separation .

1. **Which technique lowers boiling points via reduced pressure to prevent thermal decomposition?**  
   A. Simple distillation  
   B. Fractional distillation  
   C. Vacuum distillation  
   D. Steam distillation

**Answer:** C. Vacuum distillation  
**Explanation:** Vacuum distillation reduces external pressure, lowering boiling points for heat-sensitive or high-boiling compounds .

1. **Why is fractional distillation preferred over simple distillation when boiling-point differences are small?**  
   A. It uses a vacuum to reduce boiling points  
   B. It employs repeated vaporization-condensation cycles for finer separation  
   C. It operates at much higher temperatures  
   D. It requires no specialized apparatus

**Answer:** B. It employs repeated vaporization-condensation cycles for finer separation  
**Explanation:** Fractional distillation’s column enhances separation when boiling points differ by less than 25 °C by providing multiple theoretical plates .

1. **For compounds with boiling points above 300 °C that decompose at high temperature, which method is ideal?**  
   A. Simple distillation  
   B. Fractional distillation  
   C. Vacuum distillation  
   D. Steam distillation

**Answer:** C. Vacuum distillation  
**Explanation:** Vacuum distillation is designed for high-boiling or heat-labile compounds by lowering the boiling point via reduced pressure .

1. **Which of the following is NOT described as a distillation technique in this section?**  
   A. Simple distillation  
   B. Fractional distillation  
   C. Steam distillation  
   D. Vacuum distillation

**Answer:** C. Steam distillation  
**Explanation:** Only simple, fractional, and vacuum distillations are detailed in the distillation section

Polymerase chain reaction (PCR) is a molecular technique used to amplify minute quantities of DNA by repeated thermal cycling. In each cycle, double-stranded DNA is first **denatured** at 95 °C for 15 s, separating the strands. The temperature is then lowered to 54 °C to allow **DNA primers**—short, complementary oligonucleotides—to **anneal** to their target sequences. Next, the mixture is heated to 72 °C, the optimal temperature for **Taq DNA polymerase** to synthesize new complementary strands by extending from the primers. This three-step cycle is **repeated** until the desired amount of DNA is generated .

**Questions with Answers and Explanations**

1. **What is the primary purpose of PCR?**  
   A. To sequence RNA  
   B. To amplify a small quantity of DNA by several orders of magnitude  
   C. To cleave DNA at specific sites  
   D. To label proteins with radioactive probes

**Answer:** B. To amplify a small quantity of DNA by several orders of magnitude  
**Explanation:** PCR uses repeated cycles of denaturation, annealing, and extension to exponentially increase the amount of a target DNA sequence .

1. **Which temperature step denatures the DNA strands in PCR?**  
   A. 54 °C  
   B. 72 °C  
   C. 95 °C  
   D. 37 °C

**Answer:** C. 95 °C  
**Explanation:** Heating to 95 °C breaks the hydrogen bonds between DNA strands, yielding single-stranded templates .

1. **At 54 °C during PCR, what process occurs?**  
   A. DNA strand synthesis  
   B. Primer annealing  
   C. DNA denaturation  
   D. Probe hybridization

**Answer:** B. Primer annealing  
**Explanation:** Cooling to 54 °C allows primers to hybridize to their complementary sequences on the single-stranded DNA .

1. **Which enzyme synthesizes new DNA strands at 72 °C?**  
   A. DNA ligase  
   B. RNA polymerase  
   C. Taq DNA polymerase  
   D. Restriction endonuclease

**Answer:** C. Taq DNA polymerase  
**Explanation:** Taq DNA polymerase extends from annealed primers at 72 °C, adding dNTPs to build complementary strands .

1. **What is the correct order of temperature steps in the first three stages of PCR?**  
   A. 95 °C → 54 °C → 72 °C  
   B. 54 °C → 95 °C → 72 °C  
   C. 72 °C → 54 °C → 95 °C  
   D. 95 °C → 72 °C → 54 °C

**Answer:** A. 95 °C → 54 °C → 72 °C  
**Explanation:** PCR cycles through denaturation at 95 °C, annealing at 54 °C, and extension at 72 °C in that order .

1. **Which statement is FALSE regarding the PCR protocol described?**  
   A. Denaturation occurs at 95 °C  
   B. Primers anneal at 54 °C  
   C. DNA ligase is required to join fragments  
   D. New strands are synthesized at 72 °C

**Answer:** C. DNA ligase is required to join fragments  
**Explanation:** PCR relies on polymerase extension, not DNA ligase; no ligation step is included in the protocol .

1. **What is “Step 4” in the PCR cycle as described?**  
   A. Denaturation  
   B. Primer annealing  
   C. DNA synthesis  
   D. Repetition of the cycle

**Answer:** D. Repetition of the cycle  
**Explanation:** After extension, the cycle is repeated until sufficient DNA is generated .

1. **How many distinct steps are defined in one PCR cycle in the passage?**  
   A. 2  
   B. 3  
   C. 4  
   D. 5

**Answer:** C. 4  
**Explanation:** The cycle comprises denaturation (Step 1), annealing (Step 2), extension (Step 3), and repetition (Step 4) .

1. **Which component is NOT required for PCR as described?**  
   A. DNA primers  
   B. Taq DNA polymerase  
   C. Template DNA  
   D. Radiolabeled DNA probe

**Answer:** D. Radiolabeled DNA probe  
**Explanation:** Radiolabeled probes are used in Southern/Northern blotting, not in PCR .

1. **Immediately after the denaturation step, what occurs next in PCR?**  
   A. DNA synthesis  
   B. Cycle repetition  
   C. Primer annealing  
   D. Gel electrophoresis

**Answer:** C. Primer annealing  
**Explanation:** After denaturation at 95 °C, the reaction is cooled so primers can bind at 54 °C

**Passage**

Spectroscopy encompasses techniques used for molecular structure determination. In **^1H-NMR spectroscopy**, hydrogen nuclei resonate at chemical shifts (δ, ppm) reflecting electron shielding: 0–5 (alkanes), 3–5 (alkanes adjacent to heteroatoms), 5–7 (alkenes), 6–8 (aromatics), 9–10 (aldehydes), and 10–13 (carboxylic acids) . The **integration** of each peak is proportional to the number of equivalent hydrogens it represents, and **splitting patterns** follow the n+1 rule—singlet (0 neighbors), doublet (1), triplet (2), quartet (3), quintet (4), sextet (5), septet (6), multiplet (7+) .

**^13C-NMR spectroscopy** probes carbon-13 nuclei, with shifts indicating functional groups: 0–70 (alkanes), 90–120 (alkenes), 110–160 (aromatics), and 160–200 ppm (carbonyls) .

**IR spectroscopy** identifies molecular vibrations of polar bonds. Absorbance (y-axis) versus wavenumber (cm⁻¹, x-axis) highlights key regions:

* 1700–1750 (carbonyls)
* 1720–1740 (aldehydes)
* 1700–1725 (ketones, carboxylic acids)
* 1735–1750 (esters)
* 3200–3600 (O–H, broad)
* 3300–3400 (N–H, peak count indicates amine type) .

**UV-Vis spectroscopy** measures electronic transitions: increased conjugation lowers the HOMO–LUMO energy gap, shifting absorption to lower energy (longer wavelength)—e.g., absorption of green light yields a red appearance .

**Questions with Answers and Explanations**

1. **Which chemical shift range corresponds to aromatic hydrogens in ^1H-NMR?**  
   **Answer:** 6–8 ppm  
   **Explanation:** Aromatic protons are deshielded by the ring current and resonate in the 6–8 ppm region .
2. **A signal at 12.0 ppm in a ^1H-NMR spectrum most likely indicates which functional group?**  
   **Answer:** Carboxylic acid  
   **Explanation:** Carboxylic acid protons are highly deshielded and appear around 10–13 ppm, typically near 12 ppm .
3. **In ^1H-NMR, a peak with an integration of 6 corresponds to:**  
   **Answer:** Six equivalent hydrogen atoms  
   **Explanation:** Integration measures the relative number of equivalent protons contributing to that signal; an integral of 6 means six equivalent H’s .
4. **Which splitting pattern indicates one neighboring hydrogen in ^1H-NMR?**  
   **Answer:** Doublet  
   **Explanation:** According to the n+1 rule, one adjacent proton (n=1) produces a doublet (n+1=2) .
5. **In ^13C-NMR spectroscopy, which chemical shift range corresponds to carbonyl carbons?**  
   **Answer:** 160–200 ppm  
   **Explanation:** Carbonyl carbons (e.g., C=O) are strongly deshielded and resonate downfield between 160 and 200 ppm .
6. **A broad IR absorbance between 3200 and 3600 cm⁻¹ is characteristic of:**  
   **Answer:** O–H stretching in alcohols  
   **Explanation:** Hydroxyl groups form hydrogen bonds, creating broad O–H stretches in the 3200–3600 cm⁻¹ region .
7. **In an IR spectrum, a sharp peak around 1720 cm⁻¹ indicates the presence of which functional group?**  
   **Answer:** Aldehyde C=O bond  
   **Explanation:** Aldehydes exhibit strong, sharp C=O stretches between 1720 and 1740 cm⁻¹ .
8. **According to UV-Vis spectroscopy principles, as the number of conjugated π bonds increases, the absorption maximum (λmax) will:**  
   **Answer:** Shift to longer wavelengths  
   **Explanation:** Greater conjugation lowers the HOMO–LUMO gap, causing absorption of lower-energy (longer wavelength) light .
9. **Why do non-polar molecules typically show no absorbance in an IR spectrum?**  
   **Answer:** They lack a dipole moment required for IR activity  
   **Explanation:** IR absorption requires a change in dipole moment during vibration; non-polar bonds without dipole changes are IR inactive .
10. **In ^1H-NMR, a sextet splitting pattern corresponds to how many neighboring hydrogen atoms?**  
    **Answer:** Five neighboring hydrogens  
    **Explanation:** A sextet (six peaks) arises from n+1 splitting with n=5 adjacent protons

**Passage**

**Autoradiography** visualizes the location of radioactive substances within molecules or tissues. In MCAT contexts, it’s commonly used after blotting techniques (Southern, Northern, or Western blot). The sample containing radiolabeled atoms is placed against a photographic emulsion of silver halide crystals; emitted radiation reduces the crystals to metallic silver, producing a visible image corresponding to the radiolabel distribution .

**X-Ray Crystallography** determines molecular structures—especially of proteins—by analyzing X-ray diffraction patterns. A purified crystal is bombarded with X-rays, which diffract according to the atomic arrangement. Measuring the angles and intensities of diffracted beams allows reconstruction of a three-dimensional electron density map and thus atomic positions .

**Immunoprecipitation** purifies a specific protein from a solution. An antibody specific to the target protein is conjugated to solid beads (magnetic or agarose). When mixed with the sample, the antibody binds the protein, and subsequent centrifugation or magnetic separation pellets the bead–antibody–protein complex, isolating the protein of interest .

**Radioimmunoassay (RIA)** quantifies protein concentration using radiolabeled ligands. Wells are coated with a primary antibody specific to the target protein. A known amount of radiolabeled protein (e.g., ^125I-tyrosine labeled) binds the antibody; its gamma emission is measured. An unknown sample is then added to compete for antibody sites, displacing some radiolabeled protein; the reduction in radioactivity corresponds to the concentration of the unlabeled protein .

**Questions with Answers and Explanations**

1. **Autoradiography relies on which physical process to produce a visible image?**  
   **Answer:** Radiation-induced reduction of silver halide crystals to metallic silver.  
   **Explanation:** Emitted β or γ radiation from the radiolabel reduces silver halide in the emulsion, creating dark metallic silver grains that map the radiolabel distribution .
2. **In X-ray crystallography, what is directly measured to determine atomic positions?**  
   A. The fluorescence of the crystal  
   B. Angles and intensities of diffracted X-rays  
   C. Mass-to-charge ratios of ions  
   D. Absorbance at different wavelengths

**Answer:** B. Angles and intensities of diffracted X-rays  
**Explanation:** Diffraction patterns—angles and intensities of beams scattered by the crystal lattice—are used to compute the electron density map and infer atomic coordinates .

1. **What role do antibody-conjugated beads play in immunoprecipitation?**  
   A. They emit fluorescence upon binding target proteins.  
   B. They catalyze degradation of non-target proteins.  
   C. They provide a solid support to pellet the target protein–antibody complex.  
   D. They sequence the amino acids of the bound protein.

**Answer:** C. They provide a solid support to pellet the target protein–antibody complex.  
**Explanation:** Beads (magnetic or agarose) linked to specific antibodies allow the bound protein to be separated from solution by centrifugation or magnetism .

1. **Which feature distinguishes a radioimmunoassay from an ELISA?**  
   A. Use of a fluorescent tag instead of an enzyme  
   B. Competitive binding of radiolabeled versus unlabeled ligand  
   C. Requirement for gel electrophoresis separation  
   D. Measurement of light scattering instead of absorbance

**Answer:** B. Competitive binding of radiolabeled versus unlabeled ligand  
**Explanation:** RIA quantifies analyte by competition between a known radiolabeled ligand and the unlabeled sample for antibody binding sites, whereas ELISA uses enzyme-linked detection without radioactivity .

1. **During autoradiography, which component is essential for capturing the image?**  
   A. Agarose gel matrix  
   B. Photographic emulsion of silver halide crystals  
   C. Fluorescent secondary antibody  
   D. X-ray detector sensor

**Answer:** B. Photographic emulsion of silver halide crystals  
**Explanation:** Silver halide emulsion adjacent to the radiolabeled sample is reduced by radiation to form metallic silver grains that reveal the radiolabel’s location .

1. **A researcher obtains an X-ray diffraction pattern with very few spots. What is the most likely issue?**  
   A. The crystal is too large.  
   B. The crystal quality is poor (disordered).  
   C. The X-ray wavelength is too long.  
   D. The detector is saturated with signal.

**Answer:** B. The crystal quality is poor (disordered).  
**Explanation:** Well-ordered crystals produce sharp, numerous diffraction spots; poor or disordered crystals yield few or weak spots, impairing structure determination .

1. **Which washing step is critical in immunoprecipitation to reduce background?**  
   A. Washing beads with buffer to remove unbound proteins  
   B. Heating beads to denature non-specific binders  
   C. Exposing beads to X-rays  
   D. Incubating beads with silver stain

**Answer:** A. Washing beads with buffer to remove unbound proteins  
**Explanation:** After binding, beads are washed to eliminate non-specifically bound proteins, enhancing purity of the immunoprecipitated target .

1. **In a radioimmunoassay, if adding sample reduces measured radioactivity by 50%, what does that imply?**  
   A. The sample contains twice the concentration of radiolabeled ligand.  
   B. The sample’s unlabeled protein concentration equals the bound radiolabeled protein concentration.  
   C. The antibody is non-specific.  
   D. The radiolabel has decayed by 50%.

**Answer:** B. The sample’s unlabeled protein concentration equals the bound radiolabeled protein concentration.  
**Explanation:** A 50% reduction indicates equal competition by unlabeled and radiolabeled ligand for antibody sites, meaning equivalent concentrations .

1. **Which technique would you choose to determine the tertiary structure of a novel enzyme?**  
   A. Autoradiography  
   B. X-ray crystallography  
   C. Immunoprecipitation  
   D. Radioimmunoassay

**Answer:** B. X-ray crystallography  
**Explanation:** X-ray crystallography provides high-resolution, three-dimensional structures of proteins, revealing tertiary and quaternary arrangements .

1. **Why is a radiolabeled tyrosine residue commonly used in radioimmunoassays?**  
   A. Tyrosine residues enhance antibody binding specificity.  
   B. ^125I can be easily incorporated into tyrosine without disrupting protein function.  
   C. Tyrosine emits fluorescence upon excitation.  
   D. Tyrosine–silver halide interactions improve autoradiography.

**Answer:** B. ^125I can be easily incorporated into tyrosine without disrupting protein function.  
**Explanation:** Iodination of tyrosine residues labels proteins with γ-emitting ^125I, permitting sensitive detection without significantly altering protein conformation or antigenicity .

**Passage**

**Mass Spectrometry**  
Mass spectrometry determines the molecular weight of a compound and aids structural elucidation. The sample is vaporized and ionized—an electron is ejected, creating a radical cation. Fragments may form via α-cleavage, dehydration, or alkane fragmentation. The spectrum’s x-axis is the mass-to-charge ratio (m/z), representing molecular mass with the lightest isotopes (e.g., ¹²C, ¹H, ³⁵Cl), and the y-axis is relative abundance (%) of each ion. The tallest signal is the **base peak**, often a stable fragment. The **molecular ion peak (M)** corresponds to the intact radical cation’s m/z and thus the molecule’s molar mass. The **M+1 peak** arises from ^13C (1.1% abundance), allowing estimation of carbon count (relative abundance ≈ 1.1% × #C). The **M+2 peak** indicates halogens: a 3:1 M : M+2 ratio suggests chlorine (³⁵Cl/³⁷Cl), while a 1:1 ratio indicates bromine (⁷⁹Br/⁸¹Br) .

**Enzyme-Linked Immunosorbent Assay (ELISA)**  
ELISAs quantify target molecules via antigen–antibody binding and enzyme-mediated color change. In **Indirect ELISA**, antigen is coated on a microplate, a primary antibody binds the antigen, and an enzyme-linked secondary antibody binds the primary. Addition of substrate (e.g., H₂O₂) produces a colorimetric change measured by absorbance, compared against standards for concentration determination. In **Sandwich ELISA**, a “capture” antibody is coated on the plate, the sample antigen binds it, and a second enzyme-linked antibody binds a different epitope on the antigen. Signal generation and quantification proceed as above .

**Edman Degradation**  
Edman degradation sequences proteins by sequentially cleaving N-terminal residues. Phenyl isothiocyanate reacts with the free N-terminal amine, forming a phenylthiocarbamoyl derivative that cyclizes and detaches as a PTH-amino acid, which is identified chromatographically. The shortened peptide undergoes further cycles. This method is accurate only for peptides < 50 residues due to decreasing yield with each cycle .

**Questions with Answers and Explanations**

1. **In a mass spectrum, what does the m/z value of the molecular ion peak (M) represent?**  
   **Answer:** The molecular weight of the intact molecule (using the lightest isotopes)  
   **Explanation:** The M peak arises from the radical cation of the entire molecule; its mass-to-charge ratio corresponds to the molecule’s molar mass when z = 1 .
2. **What does the base peak in a mass spectrum indicate?**  
   **Answer:** The most abundant ion fragment  
   **Explanation:** The base peak is the tallest signal, representing the fragment (or molecular ion) with the greatest relative stability or formation frequency under ionizing conditions .
3. **A compound’s M+1 peak has a relative abundance of 5.5%. Approximately how many carbon atoms does it contain?**  
   **Answer:** Five carbons  
   **Explanation:** The M+1 peak abundance ≈ 1.1% per ^13C atom. Dividing 5.5% by 1.1% gives ~5 carbons .
4. **Which halogen is suggested by an M+2 peak equal in height to the M peak?**  
   **Answer:** Bromine  
   **Explanation:** Bromine’s two major isotopes (⁷⁹Br and ⁸¹Br) occur in ~1:1 ratios, producing an M+2 peak of similar abundance to M .
5. **In ELISA, what role does the secondary antibody play in an indirect assay?**  
   **Answer:** It binds the primary antibody and carries the enzyme that produces a detectable color change  
   **Explanation:** The enzyme-linked secondary amplifies signal by converting substrate to a colored product, enabling quantification via absorbance .
6. **What is coated on the microplate in a sandwich ELISA?**  
   A. Antigen  
   B. Capture (primary) antibody  
   C. Enzyme substrate  
   D. Secondary antibody  
   **Answer:** B. Capture (primary) antibody  
   **Explanation:** Sandwich ELISA immobilizes a specific antibody to “capture” antigen from the sample before detection by a second antibody .
7. **Which feature distinguishes sandwich from indirect ELISA?**  
   **Answer:** Sandwich ELISA requires two distinct antibodies binding different epitopes on the antigen, increasing specificity and sensitivity.  
   **Explanation:** Indirect ELISA binds antigen then one primary and one enzyme-linked secondary antibody; sandwich ELISA captures antigen between two antibodies .
8. **Edman degradation sequentially cleaves amino acids from which terminus of a peptide?**  
   **Answer:** N-terminus  
   **Explanation:** Phenyl isothiocyanate reacts with the free N-terminal amine, enabling one-by-one removal of residues .
9. **Which reagent is essential for Edman degradation?**  
   **Answer:** Phenyl isothiocyanate  
   **Explanation:** This reagent attaches to the N-terminus, cyclizes, and releases the PTH-amino acid for identification without disrupting the remaining peptide .
10. **Why is Edman degradation limited to peptides shorter than ~50 residues?**  
    **Answer:** Efficiency decreases with each cycle, leading to incomplete cleavage and loss of accuracy in longer peptides.  
    **Explanation:** Successive reaction and cleavage steps yield progressively lower yields, making sequencing of long peptides unreliable .

**Passage**

Gram staining differentiates bacteria into Gram-positive and Gram-negative based on cell‐wall structure. Steps: heat‐fix cells; apply **crystal violet**; add **iodine** (mordant); **decolorize** with alcohol/acetone; counterstain with **safranin**. Gram-positive retain crystal violet–iodine (purple) due to thick peptidoglycan; Gram-negative lose it and take up safranin (pink) .

1. **What is the purpose of the iodine step in Gram staining?**  
   **Answer:** B. To act as a mordant, forming a crystal violet–iodine complex  
   **Explanation:** Iodine binds crystal violet in the cell wall, creating a larger complex that is retained by thick peptidoglycan layers in Gram-positive bacteria .
2. **During the decolorization step, which cells lose the crystal violet stain?**  
   **Answer:** B. Gram‐negative only  
   **Explanation:** Alcohol or acetone disrupts the thin peptidoglycan layer and outer membrane of Gram-negative cells, allowing the dye–iodine complex to wash out, whereas Gram-positive cells retain it .
3. **Which of the following best explains why Gram-positive bacteria retain the crystal violet stain?**  
   **Answer:** B. They possess a thick peptidoglycan layer that retains the dye–iodine complex  
   **Explanation:** The multilayered peptidoglycan traps the complex, preventing decolorization .
4. **What color do Gram-negative bacteria appear after completion of the Gram stain?**  
   **Answer:** B. Pink  
   **Explanation:** After decolorization, Gram-negative cells take up the red safranin counterstain and appear pink .
5. **Which reagent is used as the counterstain in Gram staining?**  
   **Answer:** B. Safranin  
   **Explanation:** Safranin stains decolorized Gram-negative cells pink, providing contrast to purple Gram-positive cells .
6. **If the decolorizer is left on too long, what is the most likely result?**  
   **Answer:** B. Both Gram‐positive and Gram‐negative cells will appear pink  
   **Explanation:** Over‐decolorization removes dye from even Gram-positive cells, which then take up safranin .
7. **Heat fixation of bacterial smears serves primarily to:**  
   **Answer:** B. Kill and adhere cells to the slide  
   **Explanation:** Heat coagulates proteins, killing bacteria and fixing them for staining .
8. **Which structural feature distinguishes Gram-negative from Gram-positive bacteria in this test?**  
   **Answer:** B. Thickness of the peptidoglycan layer and presence of an outer membrane  
   **Explanation:** Gram-negative cells have a thin peptidoglycan plus an outer membrane; Gram-positive cells lack the outer membrane and have a thick peptidoglycan layer .
9. **In a mixed culture, you observe some bacteria stained purple and others pink. Which statement is correct?**  
   **Answer:** C. Purple cells are Gram-positive; pink cells are Gram-negative  
   **Explanation:** Gram-positive retain crystal violet (purple); Gram-negative take up safranin (pink) .
10. **Which step provides the most critical differentiation between Gram-positive and Gram-negative bacteria?**  
    **Answer:** C. Alcohol/acetone decolorization  
    **Explanation:** Decolorization selectively removes the dye–iodine complex from Gram-negative cells, distinguishing them from Gram-positive cells .

**Passage**

**Restriction Fragment Length Polymorphism (RFLP)**  
Restriction Fragment Length Polymorphism (RFLP) detects sequence variations by comparing the lengths of DNA fragments generated by restriction enzyme digestion. A restriction endonuclease recognizes a specific palindromic sequence (identical in the 5′→3′ direction on both strands) and cleaves both DNA strands at that site. Differences such as point mutations can abolish or create restriction sites, altering fragment sizes. The digested DNA is separated by gel electrophoresis, and band patterns are compared between wild-type and mutant samples to reveal polymorphisms .

**Salting Out and Dialysis**  
Salting out selectively precipitates proteins by adding high concentrations of salt, which compete with proteins for water molecules, reducing protein solubility. Each protein precipitates at a characteristic salt concentration. After precipitation, proteins are redissolved and the excess salt removed via dialysis: the solution is placed in a semipermeable membrane (dialysis bag) and immersed in a hypotonic buffer. Small ions diffuse out through the membrane pores, while larger protein molecules remain inside .

**Questions with Answers and Explanations**

1. **Which of the following best describes the principle of RFLP analysis?**  
   A. DNA fragments are ligated and sequenced.  
   B. Differences in fragment lengths generated by restriction enzyme cleavage reflect underlying sequence variation.  
   C. DNA is amplified by PCR and quantified.  
   D. Proteins bound to DNA are immunoprecipitated.

**Answer:** B  
**Explanation:** RFLP relies on restriction enzymes cutting at specific palindromic sites; sequence changes that alter these sites produce fragments of different lengths, detectable by gel electrophoresis .

1. **Restriction enzymes used in RFLP recognize which type of sequence?**  
   A. Repeating trinucleotide sequences  
   B. Palindromic sequences  
   C. Single-stranded loops  
   D. Promoter regions

**Answer:** B  
**Explanation:** Restriction endonucleases target palindromic DNA motifs—identical in the 5′→3′ direction on both strands—where they cleave both strands .

1. **A point mutation that removes a restriction site will most likely cause which change in an RFLP pattern?**  
   A. Appearance of an extra smaller fragment  
   B. Loss of all fragments on the gel  
   C. Formation of a larger fragment spanning the two original fragments  
   D. No change in fragment sizes

**Answer:** C  
**Explanation:** Abolishing a cut site prevents cleavage at that position, so two adjacent fragments merge into one larger fragment, shifting the band pattern .

1. **Why is gel electrophoresis essential in RFLP analysis?**  
   A. To digest DNA at specific temperatures  
   B. To separate DNA fragments by size so polymorphisms can be visualized  
   C. To amplify DNA fragments before analysis  
   D. To label DNA with fluorescent probes

**Answer:** B  
**Explanation:** After restriction digestion, gel electrophoresis resolves fragments by length; differences in migration patterns reveal sequence polymorphisms .

1. **Comparing wild-type and mutant samples, you observe one extra band in the mutant lane. What does this suggest?**  
   A. The mutant has an additional restriction site not present in wild type  
   B. The mutant lacks all restriction sites  
   C. The wild type sample was not digested  
   D. The gel ran at too high voltage

**Answer:** A  
**Explanation:** A novel restriction site in the mutant produces an extra cleavage, generating an additional fragment and band .

1. **What drives protein precipitation in the salting-out process?**  
   A. Reduction of pH below the protein’s pI  
   B. Competition between salt ions and protein molecules for water  
   C. Increase in temperature above protein melting point  
   D. Addition of organic solvents

**Answer:** B  
**Explanation:** High salt concentrations strip hydration water from proteins by competing for water molecules, reducing solubility and causing selective precipitation .

1. **Why do different proteins precipitate at different salt concentrations during salting out?**  
   A. Their tertiary structures differ in temperature sensitivity  
   B. Their unique hydrophilic and hydrophobic surface properties alter water-binding affinities  
   C. Their molecular weights determine solubility  
   D. Their isoelectric points are identical

**Answer:** B  
**Explanation:** Variations in amino acid composition and surface characteristics change each protein’s interaction with water and salt, so distinct salt concentrations are required for precipitation .

1. **What is the primary purpose of dialysis following protein precipitation?**  
   A. To concentrate the protein by reducing volume  
   B. To remove precipitated proteins  
   C. To separate proteins by size  
   D. To remove excess salt ions while retaining proteins

**Answer:** D  
**Explanation:** Dialysis employs a semipermeable membrane that allows small ions and molecules to diffuse out into buffer, leaving larger protein molecules inside the bag .

1. **During dialysis, which species will pass through the membrane pores into the external solution?**  
   A. Proteins larger than the membrane’s molecular-weight cutoff  
   B. Salt ions and small metabolites  
   C. Intact cells  
   D. Macromolecular protein complexes

**Answer:** B  
**Explanation:** Dialysis membranes exclude large biomolecules (like proteins) but permit diffusion of small ions and solutes, removing unwanted salts .

1. **Equilibrium in dialysis is reached when:**  
   A. Protein concentration inside equals salt concentration outside  
   B. All proteins have precipitated  
   C. Concentrations of diffusible species are equal on both sides of the membrane  
   D. The membrane disintegrates

**Answer:** C  
**Explanation:** Dialysis continues until chemical potentials equalize, at which point small solute concentrations are uniform across the membrane while proteins remain retained .

**Passage**

Reducing sugar tests detect the presence of free aldehyde or ketone groups in carbohydrates. A **reducing sugar** can act as a reducing agent because it can adopt an open-chain form exposing a free aldehyde or ketone; thus all monosaccharides are reducing sugars, and some disaccharides (e.g., maltose) are reducing, whereas others (e.g., sucrose) are non-reducing due to a 1→2 glycosidic bond between anomeric carbons that prevents mutarotation .

* **Tollen’s Test** employs Tollens’ reagent, Ag(NH3)2Ag(NH₃)₂Ag(NH3​)2​⁺, to oxidize aldehydes; a positive result yields a silver “mirror” or precipitate of elemental silver. Ketoses do not react unless they are α-hydroxy-ketones .
* **Benedict’s Test** uses a mixture of sodium carbonate, sodium citrate, and Cu²⁺ (from copper(II) sulfate); aldehydes reduce Cu²⁺ to Cu₂O, producing a brick-red precipitate as the solution changes from blue to red. Ketoses react only if they’re α-hydroxy-ketones .
* **Fehling’s Test** consists of two solutions—A (Cu²⁺) and B (potassium sodium tartrate + NaOH)—which mix to form a blue complex; aldehydes reduce Cu²⁺ to Cu₂O, yielding a brick-red precipitate. Ketoses react only if α-hydroxy-ketones are present .

**Questions with Answers and Explanations**

1. **Which functional group must a sugar possess to be classified as a reducing sugar?**  
   **Answer:** A free aldehyde or ketone group  
   **Explanation:** Reducing sugars can open to expose an aldehyde (or α-hydroxy ketone) that donates electrons in redox tests .
2. **Why are all monosaccharides considered reducing sugars?**  
   **Answer:** They undergo mutarotation to form an open-chain aldehyde or ketone  
   **Explanation:** Monosaccharides exist in equilibrium between ring and open-chain forms; the latter exposes a reactive carbonyl .
3. **Which disaccharide is non-reducing due to its glycosidic linkage?**  
   A. Maltose  
   B. Lactose  
   C. Sucrose  
   D. Cellobiose

**Answer:** C. Sucrose  
**Explanation:** Sucrose’s 1→2 bond links two anomeric carbons, preventing mutarotation and blocking a free carbonyl .

1. **Tollen’s reagent consists of which ion complex?**  
   A. Cu(NH3)4Cu(NH₃)₄Cu(NH3​)4​²⁺  
   B. Ag(NH3)2Ag(NH₃)₂Ag(NH3​)2​⁺  
   C. Fe(CN)6Fe(CN)₆Fe(CN)6​³⁻  
   D. Co(NH3)6Co(NH₃)₆Co(NH3​)6​³⁺

**Answer:** B. Ag(NH3)2Ag(NH₃)₂Ag(NH3​)2​⁺  
**Explanation:** Tollens’ reagent is the diamminesilver(I) complex that oxidizes aldehydes, precipitating metallic silver .

1. **What observation indicates a positive Tollen’s test?**  
   A. Formation of a blue complex  
   B. Silver “mirror” or black precipitate  
   C. Brick-red precipitate  
   D. Yellow solution

**Answer:** B. Silver “mirror” or black precipitate  
**Explanation:** Reduction of Ag⁺ to Ag⁰ coats the glassware with metallic silver .

1. **Which components make up Benedict’s reagent?**  
   **Answer:** Sodium carbonate, sodium citrate, and copper(II) sulfate  
   **Explanation:** The carbonate provides alkaline conditions, citrate complexes Cu²⁺, and copper(II) sulfate supplies the oxidizing ion .
2. **What color change signifies a positive Benedict’s test?**  
   **Answer:** Blue to brick-red precipitate  
   **Explanation:** Aldehydes reduce Cu²⁺ to Cu₂O, which precipitates as red/orange solids, while unreacted Cu²⁺ keeps the solution blue .
3. **Fehling’s solution differs from Benedict’s reagent in that it:**  
   A. Uses silver instead of copper  
   B. Is supplied in two separate solutions (A and B)  
   C. Detects ketones only  
   D. Operates under acidic conditions

**Answer:** B. Is supplied in two separate solutions (A and B)  
**Explanation:** Fehling’s A contains Cu²⁺; Fehling’s B has tartrate and OH⁻. Mixing them forms the active complex .

1. **Which of the following ketoses can give a positive result in these tests?**  
   A. Fructose, because it is an α-hydroxy-ketone  
   B. Sucrose, because it is a disaccharide  
   C. Ribulose, because it is non-polar  
   D. Cellulose, because it is a polymer

**Answer:** A. Fructose, because it is an α-hydroxy-ketone  
**Explanation:** α-Hydroxy-ketoses tautomerize to aldehydes under test conditions and reduce metal ions .

1. **Why must Fehling’s solution be freshly prepared before use?**  
   A. Tartrate precipitates over time  
   B. Copper(II) sulfate oxidizes rapidly  
   C. The alkaline complex decomposes, losing reactivity  
   D. Ammonia evaporates from solution

**Answer:** C. The alkaline complex decomposes, losing reactivity  
**Explanation:** The Cu²⁺–tartrate complex in basic solution slowly degrades, so fresh mixing of A and B ensures test sensitivity

**Passage**

cDNA libraries are collections of complementary DNA (cDNA) clones synthesized from mRNA transcripts, enabling expression of eukaryotic genes in bacterial vectors. Because eukaryotic genomic DNA contains introns that bacteria cannot splice, mRNA from the protein of interest is first isolated and primed with oligo-dT primers that anneal to the poly-A tail . Reverse transcriptase and dNTPs synthesize a cDNA–mRNA hybrid, which is then treated with alkaline solution to degrade the RNA strand. A second primer and DNA polymerase generate the complementary strand, yielding double-stranded cDNA that can be inserted into plasmid vectors via restriction enzymes for bacterial expression of the target protein .

**Questions with Answers and Explanations**

1. **What is the primary purpose of constructing a cDNA library?**  
   **Answer:** To clone and express eukaryotic genes (without introns) in bacterial cells.  
   **Explanation:** cDNA libraries provide intron-free copies of mRNA transcripts that bacteria can transcribe and translate into functional proteins, such as insulin .
2. **Why can’t eukaryotic genomic DNA be directly inserted into bacterial plasmids for protein expression?**  
   **Answer:** It contains introns that bacteria cannot splice out.  
   **Explanation:** Bacteria lack the splicing machinery to remove intronic sequences, so only cDNA—synthesized from processed mRNA—is suitable for expression .
3. **Which primer is used to initiate first-strand cDNA synthesis from mRNA?**  
   A. Random hexamer  
   B. Oligo-dT primer  
   C. Gene-specific primer  
   D. Poly-G primer

**Answer:** B. Oligo-dT primer  
**Explanation:** Oligo-dT primers anneal to the poly-A tail of mRNA, ensuring that reverse transcriptase copies full-length transcripts .

1. **What enzyme synthesizes the first cDNA strand from mRNA?**  
   **Answer:** Reverse transcriptase  
   **Explanation:** Reverse transcriptase uses mRNA as a template to generate a complementary DNA strand, forming a cDNA–mRNA hybrid .
2. **How is the RNA strand removed from the cDNA–mRNA hybrid?**  
   A. RNase H digestion  
   B. Alkaline hydrolysis  
   C. DNase I treatment  
   D. Heat denaturation

**Answer:** B. Alkaline hydrolysis  
**Explanation:** Treatment with a high-pH solution hydrolyzes RNA, leaving single-stranded cDNA for second-strand synthesis .

1. **Which enzyme synthesizes the second DNA strand to form double-stranded cDNA?**  
   **Answer:** DNA polymerase  
   **Explanation:** A DNA polymerase extends a primer annealed to the single-stranded cDNA, creating the complementary DNA strand .
2. **What feature of the primer used for first-strand synthesis ensures specificity for mRNA?**  
   A. Random sequence  
   B. Poly-T sequence  
   C. GC-rich region  
   D. Protein tag

**Answer:** B. Poly-T sequence  
**Explanation:** The oligo-dT primer’s thymine repeats anneal specifically to the poly-A tail found only on eukaryotic mRNAs .

1. **After generating double-stranded cDNA, how is it prepared for cloning into a plasmid?**  
   **Answer:** Digested with restriction enzymes and ligated into plasmid vectors.  
   **Explanation:** Restriction sites in both cDNA and vector allow directional insertion, enabling bacteria to propagate and express the cDNA .
2. **Which component is NOT required for first-strand cDNA synthesis?**  
   A. dNTPs  
   B. Reverse transcriptase  
   C. Oligo-dT primer  
   D. DNA ligase

**Answer:** D. DNA ligase  
**Explanation:** First-strand synthesis needs reverse transcriptase, dNTPs, and an oligo-dT primer; ligase joins DNA fragments but is not involved in strand synthesis .

1. **Why is cDNA less likely to contain non-coding sequences compared to genomic DNA?**  
   **Answer:** It is synthesized from processed mRNA, which has had introns spliced out.  
   **Explanation:** mRNA processing in eukaryotes removes introns, so cDNA reflects only the exonic coding regions .