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Biotechnology: DNA Techniques, Cloning, CRISPR, and Bioproducts

Study Guide — Biotechnology

IB/Pre-med style biotechnology MCQs covering core lab techniques (PCR, gels, restriction enzymes, cloning, sequencing, blots), genetic engineering (vectors, reporters, selection), CRISPR basics, DNA profiling, and classic applications (recombinant insulin, GM crops, monoclonal antibodies, ELISA). Emphasis on conceptual traps and correct interpretation of results.

62 items — Study Guide with Answers

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1 **A student performs PCR but forgets to add primers. What is the most likely outcome?**

A Amplification still occurs because DNA polymerase can start anywhere

B **No amplification occurs because DNA polymerase requires a primer with a free 3 -OH** ✓

C Amplification occurs but only for GC-rich regions

D Only RNA will be amplified

E The product will be single-stranded DNA only

▶ **Explanation:** DNA polymerase cannot initiate DNA synthesis de novo; it needs a primer providing a free 3 -OH. Without primers, PCR cannot amplify any specific region.

2 **In PCR, why is Taq polymerase commonly used?**

A It can synthesize RNA and DNA

B **It is stable at high temperatures used for DNA denaturation** ✓

C It does not require nucleotides

D It repairs DNA mismatches perfectly

E It binds DNA only at promoters

▶ **Explanation:** PCR involves repeated heating to ~95°C to denature DNA. Taq polymerase (from thermophilic bacteria) survives these temperatures, unlike many other polymerases.

3 **A PCR protocol uses 95°C, 55°C, and 72°C steps. The 55°C step is primarily for:**

A Denaturation of DNA strands

B Extension by DNA polymerase

C **Annealing of primers to the template** ✓

D Ligation of DNA fragments

E Cutting DNA with restriction enzymes

▶ **Explanation:** Around 50–65°C is used for primer annealing (binding) to complementary sequences. Denaturation occurs at high temperature (95°C) and extension typically around 72°C for Taq polymerase.

4 **A student designs two primers for PCR but accidentally makes both primers bind to the same DNA strand in the same orientation. What is the most likely result?**

A Normal exponential amplification of the target region

B Only one strand is copied, but still exponentially

C **No defined product because primers do not face each other to bracket a region** ✓

D A single product of exactly 3 base pairs

E PCR will amplify RNA instead of DNA

▶ **Explanation:** PCR requires primers that bind on opposite strands with 3 ends facing inward, so they define (bracket) the region to amplify. Same-strand/same-direction primers won't produce a defined amplicon.

5 **During agarose gel electrophoresis of DNA, which fragment generally migrates the farthest from the wells?**

A The largest fragment

B **The smallest fragment** ✓

C The fragment with the most GC content

D The fragment with the most A-T base pairs

E All fragments travel the same distance because DNA has the same charge

▶ **Explanation:** DNA fragments have a similar charge-to-mass ratio, so separation is mainly by size: smaller fragments move more easily through the gel matrix and migrate farther.

6 Why does DNA move toward the positive electrode during gel electrophoresis?

A DNA is positively charged due to nitrogenous bases

B DNA is negatively charged due to phosphate groups ✓

C DNA becomes neutral in buffer

D DNA is pushed by heat from the power supply

E DNA is carried by enzymes toward the anode

▶ **Explanation:** The phosphate backbone gives DNA a net negative charge, so it migrates toward the positive electrode (anode) in an electric field.

7 A restriction enzyme produces 'sticky ends.' What is the main advantage of sticky ends for cloning?

A They prevent DNA ligase from working

B They allow complementary base pairing between insert and vector, increasing ligation efficiency ✓

C They make DNA fragments migrate faster on gels

D They convert DNA into RNA

E They destroy plasmid origin of replication

► **Explanation:** Sticky ends have single-stranded overhangs that can base-pair with complementary overhangs on a vector, helping align insert and plasmid for ligase to seal the backbone efficiently.

8 **Which enzyme directly seals the sugar-phosphate backbone to join DNA fragments during cloning?**

A DNA helicase

B **DNA ligase** ✓

C Reverse transcriptase

D RNA polymerase

E Restriction endonuclease

► **Explanation:** DNA ligase forms phosphodiester bonds between adjacent nucleotides, sealing nicks and joining DNA fragments into a continuous backbone.

9 **A plasmid vector is most accurately described as:**

A A linear piece of human chromosomal DNA

B **A circular, independently replicating DNA molecule used to carry foreign DNA into cells** ✓

C A protein that cuts DNA at specific sites

D A type of ribosome used in bacteria

E A lipid vesicle that stores enzymes

► **Explanation:** Plasmids are typically circular DNA molecules found in bacteria that replicate independently and can be engineered as vectors to deliver genes into host cells.

10 **Which plasmid feature is essential to ensure it can be copied inside a bacterial host?**

A A promoter recognized by human RNA polymerase II only

B **An origin of replication (ori)** ✓

C A telomere

D Centromere

E Spliceosome binding sites

▶ **Explanation:** The origin of replication is the DNA sequence where replication begins, allowing the plasmid to be copied in the host. Telomeres/centromeres are features of eukaryotic chromosomes, not typical bacterial plasmids.

11 **Why is an antibiotic-resistance gene commonly included in a cloning plasmid?**

A To make the plasmid replicate faster

B **To allow selection of bacteria that successfully took up the plasmid** ✓

C To force bacteria to make ATP faster

D To cut the DNA at specific restriction sites

E To stop transcription of the inserted gene

▶ **Explanation:** If bacteria are grown on antibiotic-containing media, only those containing the plasmid (with the resistance gene) survive. This selects for transformed cells.

12 **A researcher inserts a human gene containing introns into bacteria using a plasmid. They get no functional human protein. The most likely reason is that bacteria:**

A Cannot replicate plasmids

B **Do not have spliceosomes to remove introns from pre-mRNA** ✓

C Cannot translate mRNA into protein

D Always degrade human DNA immediately

E Use mitochondria for transcription

▶ **Explanation:** Bacteria generally lack the splicing machinery needed to remove introns. For bacterial expression, scientists often use cDNA (made from mRNA) that lacks introns.

13 **To express a eukaryotic gene in bacteria, a common strategy is to clone which version of the gene?**

A Genomic DNA including introns

B **cDNA synthesized from mature mRNA using reverse transcriptase** ✓

C Only the promoter region without coding sequence

D Only the introns

E The protein sequence directly

▶ **Explanation:** cDNA is made from mature mRNA and lacks introns, so it can be expressed in bacteria without needing splicing.

14 **Which enzyme is required to make cDNA from an mRNA template?**

A DNA ligase

B **Reverse transcriptase** ✓

C Restriction endonuclease

D Topoisomerase

E Protease

▶ **Explanation:** Reverse transcriptase synthesizes DNA from an RNA template. It's commonly used to make cDNA from mRNA for cloning and RT-PCR.

15 **A gene is cloned into a plasmid, but the host bacteria produce no protein from it. Which missing element is most likely responsible?**

- A **A bacterial promoter upstream of the coding sequence** ✓
- B A phosphate group on the DNA backbone
- C A chloroplast transit peptide
- D A telomere at each end of the plasmid
- E A cell wall around the plasmid

▶ **Explanation:** To transcribe a gene in bacteria, the plasmid must include a promoter recognized by bacterial RNA polymerase. Telomeres/chloroplast targeting are irrelevant to bacterial expression.

16 **In blue-white screening (lacZ), colonies with an insert disrupting lacZ typically appear:**

- A Blue because -galactosidase is active
- B **White because -galactosidase is inactive** ✓
- C Green because GFP is expressed
- D Red because hemoglobin is produced
- E No colonies grow at all

▶ **Explanation:** If the insert disrupts lacZ, -galactosidase is not produced, so X-gal is not cleaved and colonies remain white. Blue colonies typically indicate intact lacZ (no insert).

17 **A plasmid is cut with a restriction enzyme that produces sticky ends. The insert DNA is cut with a different restriction enzyme producing incompatible sticky ends. What is the most likely result?**

A Insert and vector ligate efficiently

B **Insert and vector cannot base-pair at ends, so ligation is strongly reduced or fails** ✓

C The insert becomes RNA and ligates anyway

D The plasmid automatically repairs itself with the insert

E Bacteria will splice the ends to match

▶ **Explanation:** Sticky ends must be complementary to base-pair and align properly. Incompatible overhangs do not anneal, making ligation very inefficient or impossible without additional steps (e.g., blunting or adapters).

18 **Which statement best describes a restriction enzyme?**

A A protein that joins DNA fragments by forming phosphodiester bonds

B **A DNA-cutting enzyme that recognizes specific nucleotide sequences** ✓

C A carbohydrate that stores genetic information

D An RNA that carries amino acids

E A lipid that forms the nuclear membrane

▶ **Explanation:** Restriction enzymes (restriction endonucleases) recognize specific DNA sequences (often palindromes) and cut DNA at or near those sites.

19 **A DNA sample is run on a gel without loading dye. What is the main practical issue?**

A DNA will migrate toward the wrong electrode

B DNA becomes positively charged

C **The sample will not sink into the wells easily and migration progress cannot be tracked** ✓

D DNA fragments will permanently ligate together

E The gel will dissolve

▶ **Explanation:** Loading dye increases sample density so it sinks into the wells and provides tracking dyes to monitor electrophoresis. DNA still runs without dye, but handling and monitoring become difficult.

20 **In DNA fingerprinting using STRs (short tandem repeats), the major reason STRs are useful is that they:**

A Are identical in all people, making comparisons easy

B **Have highly variable repeat numbers between individuals** ✓

C Are found only on the Y chromosome

D Are copied only during meiosis II

E Cannot be amplified by PCR

▶ **Explanation:** STR loci vary in repeat number among individuals, producing different fragment lengths that can be compared. They are easily amplified by PCR, making them highly useful in profiling.

21 **A forensic sample shows two bands for an STR locus, while another sample shows one band at the same locus. The most likely interpretation is:**

A The first sample is homozygous and the second is heterozygous

B **The first sample is heterozygous and the second is homozygous** ✓

C The first sample contains no DNA

D The second sample must be bacterial

E Both samples must be from the same person

▶ **Explanation:** At one STR locus, a heterozygote typically shows two different allele lengths (two bands/peaks). A homozygote shows one allele length (one band/peak).

22 Which statement about PCR is correct?

A PCR requires living cells because replication occurs only in nuclei

B **PCR can amplify DNA from a very small starting amount** ✓

C PCR directly produces proteins from DNA

D PCR works only for circular DNA

E PCR cannot be used on degraded DNA

▶ **Explanation:** PCR can amplify tiny amounts of DNA in vitro. It does not require living cells, and short targets can sometimes still be amplified from partially degraded DNA.

23 A PCR reaction produces many products of different sizes (smearing on gel). Which is the most likely cause?

A Primers are too specific and bind only once

B **Annealing temperature is too low, causing primers to bind nonspecifically** ✓

C Too little DNA template was used

D dNTPs were not added

E Gel concentration is too high, making all bands overlap

▶ **Explanation:** Low annealing temperature allows primers to bind imperfectly at multiple sites, generating nonspecific amplicons and a smear or many bands of different sizes.

24 **A scientist wants to detect a specific protein in a mixture. Which technique is most appropriate?**

A Northern blot

B **Western blot** ✓

C Southern blot

D PCR

E DNA fingerprinting

▶ **Explanation:** Western blot detects specific proteins using antibodies. Southern blot detects DNA; Northern blot detects RNA; PCR amplifies DNA.

25 **A scientist wants to detect whether a particular gene sequence is present in a DNA sample without amplifying it first. Which technique fits best?**

A Western blot

B Northern blot

C **Southern blot using a labeled DNA probe** ✓

D ELISA

E Chromatography

▶ **Explanation:** Southern blot detects specific DNA sequences by hybridization with a labeled probe after DNA fragments are separated and transferred to a membrane.

26 **Which technique detects RNA transcripts to measure gene expression levels?**

A **Northern blot** ✓

B Southern blot

C Western blot

D Restriction digest

E Gram stain

▶ **Explanation:** Northern blot detects RNA and can be used to assess gene expression by measuring transcript presence/size. Southern is DNA, Western is protein.

27 **An ELISA test is most commonly used to detect:**

A DNA fragments by size

B ATP production in mitochondria

C **Antigens or antibodies using enzyme-linked antibodies and a color change** ✓

D RNA splicing errors

E Chromosome number in meiosis

▶ **Explanation:** ELISA uses antibodies and an enzyme-linked detection system that produces a measurable signal (often color). It's widely used to detect antigens or antibodies (e.g., infection markers).

28 **In a typical sandwich ELISA used to detect an antigen, the correct sequence is best described as:**

A Antigen → enzyme substrate → capture antibody → color

B **Capture antibody on plate → antigen binds → enzyme-linked detection antibody binds → substrate produces signal** ✓

C DNA probe binds RNA → antibody binds DNA → substrate changes color

D Enzyme binds antigen directly without antibodies

E Antigen is amplified by PCR before detection antibody binds

▶ **Explanation:** Sandwich ELISA uses a plate-bound capture antibody to immobilize antigen, then a second antibody (often enzyme-linked) binds the antigen; the enzyme substrate generates a measurable signal.

29 **Monoclonal antibodies differ from polyclonal antibodies because monoclonal antibodies:**

A Recognize multiple epitopes on the same antigen

B Are a mixture produced by many B cell clones

C Are identical and bind the same epitope, produced by a single B cell clone (or hybridoma) ✓

D Cannot be used in diagnostics

E Are made only in plants

▶ **Explanation:** Monoclonal antibodies come from one clone, so they are identical and bind one epitope. Polyclonal antibodies are a mixture from many B cell clones and recognize multiple epitopes.

30 **Hybridoma technology produces monoclonal antibodies by fusing:**

A A T cell with a red blood cell

B A B cell producing a desired antibody with a myeloma (cancer) cell ✓

C Two bacterial cells

D A mitochondrion with a ribosome

E A neuron with a muscle cell

▶ **Explanation:** A B cell provides antibody specificity, and the myeloma cell provides unlimited division. The hybrid (hybridoma) can produce large quantities of one antibody type.

31 **Which of the following best describes a 'reporter gene' in genetic engineering?**

A A gene that cuts DNA at specific sequences

B **A gene inserted to produce an easily detectable product, indicating successful expression or insertion** ✓

C A gene that prevents plasmid replication

D A gene that always causes disease

E A gene found only in viruses

▶ **Explanation:** Reporter genes (e.g., GFP, lacZ) help identify cells where a construct has been taken up or expressed by producing a visible/measureable signal.

32 **A researcher adds a plasmid to bacteria. Only a small fraction survive on antibiotic plates. Which process most directly explains why only a fraction survive?**

A Transcription

B Translation

C **Transformation efficiency is low: only some cells take up the plasmid** ✓

D Meiosis produces genetic variation

E The plasmid is converted to RNA in most cells

▶ **Explanation:** In bacterial transformation, not all cells take up plasmid DNA. Antibiotic selection kills non-transformed cells, leaving only those that acquired the resistance plasmid.

33 **Why is DNA ligase particularly useful after using restriction enzymes in cloning?**

A Restriction enzymes already seal DNA, so ligase is redundant

B **Ligase repairs and joins cut DNA backbones to form a stable recombinant plasmid** ✓

C Ligase cuts DNA at palindromic sites

D Ligase replaces primers in PCR

E Ligase converts DNA into amino acids

▶ **Explanation:** Restriction enzymes cut DNA, producing fragments. DNA ligase seals the sugar-phosphate backbone to create stable recombinant DNA molecules.

34 **A student runs a restriction digest but forgets to add the restriction enzyme. On a gel, the plasmid most likely appears as:**

A Multiple bands matching predicted fragment sizes

B No DNA at all

C **Uncut plasmid forms (often supercoiled/nicked), not the expected fragment pattern** ✓

D A ladder with evenly spaced bands

E Only RNA bands

▶ **Explanation:** Without enzyme, the DNA is not cut, so it runs as uncut plasmid conformations rather than discrete restriction fragments at predicted sizes.

35 **CRISPR-Cas9 is best described as a tool that:**

A Amplifies DNA using thermal cycling

B Separates proteins based on size and charge

C Cuts DNA at a targeted sequence guided by an RNA molecule ✓

D Transcribes DNA into mRNA

E Transfers antibodies between organisms

▶ **Explanation:** CRISPR-Cas9 uses a guide RNA to direct the Cas9 nuclease to a specific DNA sequence, where it makes a double-strand break that cells repair (enabling edits).

36 A key reason CRISPR-Cas9 can cause unintended ('off-target') edits is that:

A Cas9 cuts RNA instead of DNA

B Guide RNA may bind imperfectly to similar DNA sequences, leading Cas9 to cut at similar sites ✓

C DNA polymerase replaces Cas9 randomly

D Restriction enzymes always cut before Cas9

E CRISPR only works in bacteria and never in eukaryotes

▶ **Explanation:** If the guide RNA partially matches other genomic regions, Cas9 can bind and cut there, producing off-target edits. This is a key limitation and design challenge.

37 What is the immediate DNA-level effect of Cas9 in CRISPR editing?

A It methylates cytosine bases to silence genes

B It introduces a double-strand break at a targeted site ✓

C It adds introns into coding sequences

D It converts DNA into RNA

E It joins two DNA fragments without cutting

▶ **Explanation:** Cas9 is a nuclease that creates a double-strand break. Cellular DNA repair pathways then fix the break, which is how edits (knockouts/insertions) are achieved.

38 **A gene knockout using CRISPR often relies on error-prone repair that introduces small insertions/deletions. Which repair process is typically responsible?**

A DNA replication by helicase

B **Non-homologous end joining (NHEJ)** ✓

C Transcription by RNA polymerase

D RNA splicing

E Translation by ribosomes

▶ **Explanation:** NHEJ repairs double-strand breaks quickly but can introduce small indels, often disrupting the reading frame and 'knocking out' the gene.

39 **A gene knock-in (precise insertion) using CRISPR is more likely when the cell repairs the cut using:**

A Non-homologous end joining

B **Homology-directed repair using a donor template** ✓

C RNA interference

D Chromosome segregation

E Glycolysis

▶ **Explanation:** Precise insertion is promoted by homology-directed repair (HDR) when a donor DNA template is provided. NHEJ is more error-prone and typically yields knockouts rather than precise insertions.

40 **In Sanger sequencing, DNA fragments of different lengths are generated primarily because:**

A Restriction enzymes cut DNA randomly

B **dideoxynucleotides (ddNTPs) terminate chain elongation when incorporated** ✓

C Primers cannot bind to the template

D ATP synthase removes nucleotides

E RNA polymerase changes DNA into RNA fragments

▶ **Explanation:** ddNTPs lack a 3 -OH, so once incorporated, DNA polymerase cannot add the next nucleotide. This creates a set of fragments ending at each base position.

41 **Why do ddNTPs in Sanger sequencing stop DNA synthesis?**

A They contain uracil instead of thymine

B They bind to primers and block annealing

C **They lack a 3 -OH group needed to form the next phosphodiester bond** ✓

D They destroy the DNA template

E They are proteins, not nucleotides

▶ **Explanation:** DNA elongation requires a free 3 -OH to attach the incoming nucleotide. ddNTPs lack this, causing chain termination.

42 **A researcher wants to compare the sizes of proteins and identify a specific one with an antibody. The best combination is:**

A Agarose gel electrophoresis + Southern blot

B **SDS-PAGE + Western blot** ✓

C PCR + Northern blot

D Restriction digest + ELISA

E Gram stain + PCR

▶ **Explanation:** SDS-PAGE separates proteins mainly by size, and Western blot uses antibodies to detect the specific protein among separated bands.

43 **SDS in SDS-PAGE is used mainly to:**

A Cut DNA into fragments

B **Add a uniform negative charge and denature proteins so separation depends mainly on size** ✓

C Make proteins fluorescent automatically

D Convert proteins into RNA

E Join proteins together into longer chains

▶ **Explanation:** SDS denatures proteins and coats them with negative charge, minimizing shape/charge differences so migration is mostly size-dependent.

44 **A student uses a DNA probe in a Southern blot. The probe must be:**

A A protein that binds DNA nonspecifically

B **Single-stranded and complementary to the target DNA sequence** ✓

C Double-stranded and identical to the target sequence

D A restriction enzyme

E An antibody against DNA polymerase

▶ **Explanation:** Hybridization requires complementary base pairing, so the probe is typically single-stranded DNA (or RNA) complementary to the target sequence and labeled for detection.

45 **Which is the best reason scientists separate DNA fragments by gel electrophoresis before doing a Southern blot?**

A So DNA becomes RNA

B So the probe can cut the DNA

C So fragments are separated by size, letting you identify the size of the fragment containing the target sequence ✓

D So plasmids can replicate faster

E So proteins can be detected instead of DNA

▶ **Explanation:** Electrophoresis separates DNA by size. After transfer and probing, the position of the hybridized band reveals the approximate fragment size containing the target sequence.

46 **A bacterial plasmid with an antibiotic-resistance marker is introduced into bacteria. Which control best checks whether the antibiotic plate is working correctly?**

A Plate bacteria with plasmid on non-antibiotic agar

B Plate bacteria without plasmid on antibiotic agar ✓

C Plate only water on antibiotic agar

D Plate bacteria with plasmid on agar with no nutrients

E Plate bacteria without plasmid on agar containing primers

▶ **Explanation:** A no-plasmid control on antibiotic agar should show no growth if the antibiotic selection is functioning. If it grows, the antibiotic is ineffective or absent.

47 **A scientist wants to amplify DNA from RNA viruses. Which additional step is required before PCR?**

A Use restriction enzymes to cut RNA into fragments

B **Convert RNA to cDNA using reverse transcriptase (RT)** ✓

C Translate RNA into protein first

D Run SDS-PAGE on RNA

E Add DNA ligase to RNA

▶ **Explanation:** PCR requires DNA as a template. For RNA viruses, reverse transcriptase is used to synthesize cDNA first (RT-PCR).

48 **Which statement best describes why DNA fingerprinting usually uses multiple STR loci rather than just one?**

A One STR locus is always identical in everyone

B **Multiple loci greatly increase the power to distinguish individuals (lower chance of a random match)** ✓

C Using one locus makes gels run backward

D STRs cannot be amplified by PCR

E Multiple loci are needed only to detect mitochondrial DNA

▶ **Explanation:** Each additional independent STR locus multiplies discrimination power. Matching many loci makes random matching extremely unlikely, improving identification reliability.

49 **Which is the most accurate statement about recombinant insulin production in bacteria?**

A Bacteria are given human pancreatic tissue to digest

B **A human insulin gene (often as cDNA) is inserted into a bacterial plasmid for expression** ✓

C Bacteria naturally have the human insulin gene

D Insulin is made by PCR directly

E Insulin is made only in chloroplasts

▶ **Explanation:** Recombinant insulin is produced by inserting the insulin coding sequence (commonly cDNA without introns) into a bacterial expression vector so bacteria produce the insulin protein.

50 **A scientist wants bacteria to produce a human protein with correct intron removal. Which choice is most appropriate?**

A Insert the human genomic DNA (with introns) into bacteria and rely on bacterial splicing

B **Insert the cDNA version of the gene (introns removed) into bacteria** ✓

C Insert only introns so they can be removed

D Use SDS-PAGE to remove introns

E Use ELISA to convert introns to exons

▶ **Explanation:** Bacteria generally cannot splice introns. Using cDNA (from mature mRNA) avoids introns and allows correct translation of the coding sequence.

51 **A GM plant is engineered to produce Bt toxin to resist insect pests. The most direct intended effect is that:**

A The plant makes antibodies that kill insects

B **Insects feeding on the plant are harmed, reducing crop damage** ✓

C The plant performs photosynthesis faster by increasing chlorophyll

D The plant becomes resistant to all viruses automatically

E The plant's mitochondria stop respiration

▶ **Explanation:** Bt crops express a bacterial toxin that is harmful to certain insects, reducing pest damage. It is not an antibody and does not directly increase photosynthesis.

52 Which statement best explains why antibiotic-resistance markers in GM crops are ethically debated?

A They always cause the crop to become poisonous to humans

B There is concern (even if low probability) about transfer of resistance genes to microbes, potentially affecting antibiotic effectiveness ✓

C They stop plants from reproducing

D They prevent photosynthesis

E They guarantee that no insects can survive anywhere

▶ **Explanation:** A key concern is horizontal gene transfer potentially spreading resistance markers. This is why alternative markers or marker-free methods are often preferred.

53 Which biotechnology method best matches the goal: 'Determine whether a person has a specific single-base mutation in a gene'?

A Sanger sequencing of the PCR-amplified region ✓

B Western blot of blood plasma

C Gram staining

D ELISA for antibodies

E Meiosis karyotyping

▶ **Explanation:** A single-base change is directly detected by sequencing the relevant DNA region (often after PCR). Protein or antibody tests may not reliably reveal a silent or subtle DNA mutation.

54 **A scientist wants to know whether a gene is being expressed in a tissue at the mRNA level. The most direct method is:**

A Southern blot

B Northern blot (or RT-PCR) for the transcript ✓

C Western blot

D Restriction digest only

E Simple staining of DNA with dye

▶ **Explanation:** Northern blot (or RT-PCR) measures RNA transcripts, giving direct evidence of gene expression at the mRNA level. Southern is DNA presence; Western is protein abundance.

55 **A student claims: 'PCR doubles DNA amount every cycle no matter what.' Which is the best correction?**

A True—PCR always doubles indefinitely

B False—early cycles can be near-doubling, but efficiency decreases later as reagents become limiting and products re-anneal ✓

C False—PCR triples DNA each cycle

D False—PCR can only amplify DNA once

E True—PCR depends only on restriction enzymes, so doubling is guaranteed

▶ **Explanation:** In ideal conditions PCR is exponential initially, but later cycles plateau because primers/dNTPs become limiting and polymerase activity decreases, so the amplification is not perfect doubling indefinitely.

56 **Which of the following best describes gene therapy at a high-school/pre-med level?**

A Replacing all chromosomes in every cell

B **Introducing functional genetic material into cells to compensate for a faulty gene** ✓

C Using antibiotics to kill mutated cells

D Using PCR to cure infections inside the body

E Using mitochondria to splice introns

▶ **Explanation:** Gene therapy aims to add or modify genetic material to restore function (e.g., supplying a working gene). It does not replace entire chromosomes in all cells.

57 **A major challenge of gene therapy is that:**

A DNA cannot enter cells under any circumstance

B **Delivering genes to the correct target cells safely and ensuring appropriate expression can be difficult** ✓

C All viruses are harmless vectors

D Genes always integrate perfectly into safe locations

E Gene therapy works only in bacteria

▶ **Explanation:** Key challenges include targeted delivery, avoiding immune reactions, controlling expression levels, and preventing harmful insertion effects. These practical issues define gene therapy limitations.

58 **Why are viruses sometimes used as gene therapy vectors?**

A **Viruses naturally deliver genetic material into host cells** ✓

B Viruses can perform photosynthesis

C Viruses translate proteins without ribosomes

D Viruses are made of plasmids only

E Viruses always integrate safely into the genome

▶ **Explanation:** Viruses are evolved to enter cells and deliver nucleic acids. Engineered viruses can be used to deliver therapeutic genes, though safety and control remain key concerns.

59 **Dolly the sheep was cloned using somatic cell nuclear transfer (SCNT). The nucleus used came from a:**

A Sperm cell

B Egg cell

C **Somatic (body) cell** ✓

D Bacterial cell

E Red blood cell

▶ **Explanation:** In SCNT, a nucleus from a somatic cell is transferred into an enucleated egg. The embryo developed is genetically (nuclear DNA) identical to the donor of the somatic nucleus.

60 **In SCNT cloning, which statement about genetic identity is most accurate?**

A The clone's nuclear DNA matches the egg donor, not the nucleus donor

B **The clone's nuclear DNA matches the nucleus donor, but mitochondrial DNA comes mainly from the egg donor** ✓

C The clone has no mitochondrial DNA

D The clone is always genetically identical to the surrogate mother

E The clone has half the chromosomes of the nucleus donor

▶ **Explanation:** Nuclear DNA is supplied by the transferred somatic nucleus, but mitochondria (and mitochondrial DNA) are largely from the enucleated egg's cytoplasm. This is a classic conceptual trap.

61 Which biotechnology technique is most directly used to produce many identical copies (clones) of a specific DNA fragment inside bacteria?

A **Transformation with a recombinant plasmid followed by bacterial replication** ✓

B Western blotting

C ELISA

D SDS-PAGE without antibodies

E Karyotyping

▶ **Explanation:** Molecular cloning inserts a DNA fragment into a plasmid, transforms bacteria, and uses bacterial replication to make many copies of the plasmid (and inserted DNA).

62 A scientist wants to separate DNA fragments of 50 bp to 200 bp very clearly. Compared with a low-percentage agarose gel, they should generally use:

A A lower percentage agarose gel for better small-fragment resolution

B **A higher percentage agarose gel for better small-fragment resolution** ✓

C A gel with no buffer

D A gel with reversed electrodes so DNA runs toward the negative side

E A gel made from lipids instead of agarose

▶ **Explanation:** Higher-percentage gels have smaller pores, providing better separation of small DNA fragments. Lower-percentage gels are better for separating larger fragments.