MDCAT Biology Unit 16: Biotechnology, study guide with practice MCQs

What MDCAT asks

Unit 16 covers the tools of genetic engineering and their uses in medicine, agriculture, forensics and cloning, with the ethical questions they raise. Its two subtopics carry equal weight and together account for about 2 to 3 of the 81 Biology questions. Most are recall of named enzymes, organisms and steps, plus the occasional PCR calculation or gel question.

B16.1 Recombinant DNA techniques

B16.1.1 Restriction enzymes, vectors, ligase and the steps of gene cloning

Recombinant DNA is DNA assembled from two or more sources, for example a human gene joined to a bacterial plasmid. **Gene cloning** is the production of many identical copies of a chosen gene inside host cells.

Restriction enzymes (restriction endonucleases) are bacterial enzymes that cut double-stranded DNA at specific short sequences called **recognition sites**. Bacteria use them against bacteriophage DNA; each is named after its source bacterium.

- Recognition sites are usually 4 to 8 base pairs long and **palindromic**: they read the same 5′ → 3′ on both strands. EcoRI recognises 5′-GAATTC-3′.
- A staggered cut leaves single-stranded overhangs called **sticky ends** (EcoRI cuts G/AATTC, leaving 5′-AATT). A cut at the same point on both strands leaves **blunt ends** (SmaI, CCC/GGG).
- Fragments cut by the same enzyme have complementary sticky ends and pair by hydrogen bonding, whatever their source. This is why gene and vector must be cut with the **same** enzyme.

A **vector** carries the foreign gene into a host cell and replicates there. The usual vector is the bacterial **plasmid**, a small circular DNA molecule separate from the chromosome. A good plasmid vector has an **origin of replication**, a **selectable marker** (an antibiotic resistance gene) and a **unique restriction site** for the insert; pBR322 carries ampicillin and tetracycline resistance.

DNA ligase is the joining enzyme. After the sticky ends have paired by hydrogen bonds, each backbone still has a gap. Ligase forms the missing **phosphodiester bonds**, using ATP: hydrogen bonds hold the ends temporarily, ligase makes the covalent bond.

Steps of gene cloning. Learn the order.

1. **Isolate the gene:** cut it from chromosomal DNA with a restriction enzyme, or make it from the gene's mRNA with **reverse transcriptase**. The mRNA route gives **complementary DNA (cDNA)**, which has no introns. Bacteria cannot remove introns, so a human gene to be expressed in *E. coli* (insulin, for example) is cloned as cDNA.
2. **Cut the plasmid** with the same restriction enzyme.

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3. **Join gene and plasmid** with DNA ligase to make the recombinant plasmid.
4. **Transform the host.** Uptake of naked DNA by a bacterium is **transformation**; cells are made competent with calcium chloride and a heat shock, or by an electric pulse (electroporation).
5. **Select** on a medium containing the antibiotic: only cells carrying the plasmid survive.
6. **Grow the clones** in a fermenter. Every division copies the plasmid; with a suitable promoter the host also **expresses** the gene, and the protein is harvested.

Enzyme	Source organism	Site (5′ → 3′)	Cut	Ends
EcoRI	*Escherichia coli* RY13	GAATTC	G/AATTC	Sticky
BamHI	*Bacillus amyloliquefaciens* H	GGATCC	G/GATCC	Sticky
HindIII	*Haemophilus influenzae* Rd	AAGCTT	A/AGCTT	Sticky
SmaI	*Serratia marcescens*	CCCGGG	CCC/ GGG	Blunt

B16.1.2 PCR, gel electrophoresis and DNA sequencing

The **polymerase chain reaction (PCR)** copies a chosen stretch of DNA in a test tube, with no living cell. The mixture contains the **template** DNA; two **primers**, short single-stranded DNA pieces complementary to the two ends of the target, one for each strand; the four nucleotides (dNTPs); **Taq polymerase**, a heat-stable DNA polymerase from *Thermus aquaticus*, a hot-spring bacterium, which survives the denaturation step; and a buffer with Mg^{2+}. A **thermal cycler** repeats three steps:

Step	Temperature (approx.)	What happens
1. Denaturation	94 to 95 °C	Hydrogen bonds break; the two strands separate
2. Annealing	50 to 65 °C	Primers base-pair with their complementary sequences
3. Extension	72 °C	Taq polymerase adds nucleotides to the 3′ end of each primer

Each cycle doubles the target, so after *n* cycles there are **starting number × 2^n** molecules. Because it needs only a trace of DNA, PCR is the first step in forensic work and in detecting a viral genome before antibodies appear.

Gel electrophoresis separates DNA fragments by size. DNA is loaded into wells in an **agarose gel** and a voltage is applied. Phosphate groups give DNA a uniform **negative charge**, so every fragment moves towards the **positive electrode** (anode). The gel is a mesh: small fragments pass through its pores and travel far, large fragments are held back near the wells. Fragments of one size form a **band**, seen after staining, and a **DNA ladder** of known sizes run alongside gives each band's size.

DNA sequencing reads the order of bases. In the **Sanger chain-termination (dideoxy) method**, a polymerase extends a primer on the template in a mixture that also contains a little of each **dideoxynucleotide (ddNTP)**. A ddNTP has no hydroxyl group on the 3′ carbon of its sugar, and the next phosphodiester bond needs that 3′-OH, so once a ddNTP is added the chain stops. Termination happens by chance at every position, giving fragments differing by one nucleotide, each labelled by its ddNTP. The fragments are separated by electrophoresis, shortest first, and the label on each successive band gives the next base, so the sequence is read from the smallest fragment to the largest.

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MDCAT trap: which band goes where in the gel?

Students regularly pick the **largest** fragment as the one that travels furthest, perhaps because it "has the most charge". Charge per unit length is the same for every fragment, so charge does not decide the race; the gel's pores do. The smallest fragment threads through the mesh fastest and ends up furthest from the well, nearest the positive electrode; the largest stays closest to the well. Fix the linked rules too: DNA moves to the **positive** electrode because of its negative phosphate backbone, and in Sanger sequencing the sequence is read from the **shortest** fragment at the bottom of the gel upwards.

Worked example: a restriction digest on a gel

A linear DNA molecule of 3,000 base pairs has EcoRI sites 500 bp and 1,800 bp from its left end. It is cut completely with EcoRI and run on an agarose gel. How many bands appear, and which fragment lies nearest the positive electrode?

1. Two cuts in a linear molecule give three fragments: 500 bp, 1,300 bp (500 to 1,800) and 1,200 bp (1,800 to 3,000). Check: 500 + 1,300 + 1,200 = 3,000.
2. Three different sizes give three bands.
3. The smallest travels furthest, so the 500 bp fragment is nearest the positive electrode; the 1,300 bp fragment stays closest to the well.

Answer: three bands; the 500 bp fragment is nearest the positive electrode.

B16.2 Applications of biotechnology

B16.2.1 Applications in medicine (insulin, vaccines, gene therapy) and agriculture (transgenic crops)

An organism that carries and expresses a gene from another species is **transgenic**; "genetically modified (GM)" is the broader term for any engineered genome.

Recombinant human insulin. Before the 1980s insulin for diabetes mellitus came from the pancreases of cattle and pigs. Because pig and cow insulin differ from human insulin in one and three amino acids respectively, some patients developed allergic reactions or antibodies, and supply was limited. In 1982 recombinant human insulin (Humulin) became the first genetically engineered medicine approved. The insulin gene, as cDNA, is placed in a plasmid next to a strong bacterial promoter and put into *E. coli*. The product is identical to human insulin and made in unlimited amounts in fermenters. **Human growth hormone** for pituitary dwarfism is made the same way.

Recombinant vaccines. A traditional vaccine contains a weakened or killed pathogen or an inactivated toxin; a **subunit vaccine** contains only one protein of the pathogen, the antigen the immune system must learn. The **hepatitis B vaccine** is the model: the gene for the hepatitis B surface antigen (HBsAg) is expressed in the yeast *Saccharomyces cerevisiae* and the purified protein is injected. With no viral DNA and no whole virus it cannot cause hepatitis.

Gene therapy treats a genetic disease by putting a working copy of the gene into the patient's cells instead of giving the missing protein. The normal allele is delivered by a disabled retrovirus or adenovirus. The first success, in 1990, treated a girl with **severe combined immunodeficiency**

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(SCID) caused by lack of adenosine deaminase (ADA): her white blood cells were removed, given the ADA gene by a retrovirus, and returned. Cystic fibrosis (CFTR gene delivered to lung cells by aerosol) is another target.

- **Somatic gene therapy** alters body cells only. It is **not inherited**, because the patient's gametes still carry the faulty allele. It is the only kind permitted in humans.
- **Germ-line gene therapy** would alter gametes or an early embryo, so every cell of the person and their descendants would carry the change. It is banned in humans.

Transgenic crops. Plant cells are usually transformed with the Ti plasmid of *Agrobacterium tumefaciens*, the soil bacterium that causes crown gall; its tumour genes are replaced by the wanted gene, which the bacterium inserts into a plant chromosome.

Crop	Gene introduced	Source	Benefit
Bt cotton, Bt maize	*cry* gene for Cry protein	*Bacillus thuringiensis*, a soil bacterium	Kills caterpillars (bollworm, corn borer); active only in the alkaline insect gut, so harmless to humans. Widely grown in Pakistan
Herbicide-tolerant soybean, maize, cotton	Enzyme that glyphosate cannot block	A soil bacterium	Field sprayed with glyphosate; weeds die, crop survives
Golden rice	Enzymes that make beta-carotene in the endosperm	A daffodil gene and a bacterial gene	Body converts beta-carotene to vitamin A; prevents deficiency blindness
Flavr Savr tomato	Antisense polygalacturonase gene	The tomato's own gene, reversed	Softening enzyme switched off; fruit stays firm longer

B16.2.2 DNA fingerprinting, cloning and the ethical issues of biotechnology

DNA fingerprinting (DNA profiling) was developed by Alec Jeffreys in 1984. It looks not at genes but at **non-coding repeated sequences** whose number of repeats varies from person to person: **VNTRs** (variable number tandem repeats) in the classic method, shorter **STRs** (short tandem repeats) amplified by PCR in modern profiling. The pattern over several regions is practically unique. Only **identical twins** share a profile. The classic procedure: extract DNA → cut with a restriction enzyme that cuts outside the repeats, so fragment length depends on repeat number (RFLPs) → gel electrophoresis → transfer the single-stranded fragments to a nylon membrane (Southern blotting) → add a radioactive or fluorescent **DNA probe** complementary to the repeat → detect as a bar-code-like pattern of bands. Uses: forensics, identifying bodies and **paternity testing**: a child gets one allele of each repeat from each parent, so every band in the child must appear in the mother or the father.

Cloning is the production of genetically identical copies of a gene, a cell or a whole organism. Animals are cloned by **somatic cell nuclear transfer**: the nucleus of a body cell from the animal to be copied is placed in an egg whose own nucleus has been removed (an **enucleated** egg); an electric pulse starts division, and the embryo is implanted in a surrogate. **Dolly the sheep**, born in 1996 at the Roslin Institute, Scotland, was the first mammal cloned from an adult cell: a nucleus from a mammary gland (udder) cell of a Finn Dorset ewe went into an enucleated egg from a Scottish Blackface ewe, and a Blackface surrogate carried the embryo. Dolly was a Finn Dorset, identical in nuclear DNA to the

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udder-cell donor; only her mitochondrial DNA came from the egg donor.

Feature	Reproductive cloning	Therapeutic cloning
Aim	A whole organism identical to the donor; embryo implanted in a surrogate	Embryonic stem cells matched to a patient, for tissue that will not be rejected; embryo cultured to the blastocyst stage, never implanted
In humans	Banned	Allowed under strict rules in some countries, debated in others

Ethical and safety issues. MDCAT asks you to recognise these, not to take sides.

- **GM crops:** gene flow by pollen to wild relatives, giving herbicide-resistant weeds; pests evolving resistance to the Bt protein; uncertainty about the long-term safety of GM food; farmers' dependence on companies that patent seed.
- **Medicine:** genetic privacy and discrimination by insurers or employers; "designer babies"; germ-line changes imposed on descendants who cannot consent.
- **Cloning:** human reproductive cloning is rejected almost everywhere; therapeutic cloning destroys an early embryo, which some regard as a human life; animal cloning has high failure rates.
- **Ownership and misuse:** patents on genes, unequal access for poor nations, engineered pathogens as weapons, irreversible release of organisms into ecosystems.

MDCAT trap: who is the clone a copy of?

Questions on Dolly test one point, and students get it wrong by picking the mother that gave birth. Three animals take part in nuclear transfer: the **nucleus donor** (whose body cell supplied the nucleus), the **egg donor** (whose enucleated egg supplied the cytoplasm) and the **surrogate** (who carried the pregnancy). Only the nucleus donor contributes chromosomes, so the clone is identical in nuclear DNA to that animal alone: Dolly was a Finn Dorset like her udder-cell donor, not a Scottish Blackface like the egg donor or the surrogate. The surrogate contributes nothing genetic; the egg donor contributes only mitochondrial DNA. Linked rules: the egg must be **enucleated**, or the embryo would carry the egg's own chromosomes as well as the donor's; and Dolly was a first because her nucleus came from an **adult body cell**.

Worked example: a paternity test

A child's DNA fingerprint shows bands at positions 1, 3, 5 and 7. The mother shows bands 1, 5, 8 and 9. Man X shows bands 2, 3, 6 and 7; man Y shows bands 2, 4, 6 and 8. Which man could be the father?

1. Every band in the child comes from one parent or the other. Bands 1 and 5 match the mother.
2. The remaining child bands, 3 and 7, must have come from the father, so the true father must show both.
3. Man X has 3 and 7, so he is not excluded. Man Y has neither, so he cannot be the father.

Answer: man X could be the father; man Y is excluded.

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Practice MCQs

1. After the sticky ends of a human gene and a cut plasmid have base-paired, which enzyme forms the phosphodiester bonds that join them permanently?
A. DNA polymerase
B. Restriction endonuclease
C. DNA ligase
D. Reverse transcriptase

2. A human gene that contains introns is to be expressed in *E. coli*. Which form of the gene should be inserted into the plasmid, and why?
A. Chromosomal DNA, because it is the natural form of the gene
B. cDNA made from the mRNA by reverse transcriptase, because it has no introns
C. The mRNA itself, because bacteria can translate it directly
D. The protein itself, because the bacterium will copy it

3. A sample contains 10 molecules of a target DNA. What is the smallest number of PCR cycles needed to produce more than 1,000 molecules, if every cycle works perfectly?
A. 4
B. 5
C. 6
D. 7

4. Why is Taq polymerase, rather than a human DNA polymerase, used in PCR?
A. It is not denatured at the temperature used to separate the template strands
B. It can start a new strand without a primer
C. It separates the two strands of the template by itself
D. It cuts the DNA at palindromic recognition sites

5. A mixture of DNA fragments of 5,000, 2,000, 800 and 200 base pairs is run on an agarose gel. Which band lies closest to the well?
A. 5,000 bp
B. 2,000 bp
C. 800 bp
D. 200 bp

6. In Sanger sequencing, a dideoxynucleotide stops the growth of the DNA chain because it lacks:
A. a phosphate group on the 5′ carbon
B. a hydroxyl group on the 3′ carbon of its sugar
C. a nitrogenous base
D. the sugar deoxyribose

7. The recombinant hepatitis B vaccine made in yeast contains:
A. live attenuated hepatitis B virus
B. the complete DNA genome of the virus
C. the surface antigen protein of the virus only
D. heat-killed whole virus

8. Bt cotton resists attack by bollworm because it carries:
A. a gene from *Agrobacterium* that makes it tolerant to glyphosate
B. a gene for the synthesis of beta-carotene

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C. an antisense gene that stops the boll from softening
D. a gene from *Bacillus thuringiensis* for a protein that is toxic to caterpillars

9. A man with cystic fibrosis is treated by delivering the normal CFTR allele to his lung cells. What can be said of his children?
A. They will all inherit the normal allele from him
B. They have the same chance of inheriting the faulty allele as if he had not been treated
C. They cannot inherit cystic fibrosis, because his gene has been corrected
D. They will inherit the normal allele only if their mother is also treated

10. A nucleus from a mammary gland cell of a white-faced Finn Dorset ewe was placed in an enucleated egg from a black-faced Scottish Blackface ewe, and the embryo was carried by a second Blackface ewe. The lamb born was:
A. white-faced, with nuclear DNA identical to the Finn Dorset ewe
B. black-faced, with nuclear DNA identical to the egg donor
C. black-faced, like the ewe that gave birth to it
D. a mixture, with half its chromosomes from each ewe

1. C. DNA ligase forms the phosphodiester bonds of the backbone. Restriction enzymes cut; polymerases build new strands.

2. B. Bacteria cannot remove introns, so intron-free cDNA made from mature mRNA is used. mRNA and protein cannot be inserted into a plasmid or replicated.

3. D. Number = 10×2^n. After 6 cycles, $10 \times 64 = 640$, not enough; after 7 cycles, $10 \times 128 = 1{,}280$.

4. A. Taq polymerase, from *Thermus aquaticus*, survives the 94 to 95 °C denaturation step. It still needs a primer; heat, not the enzyme, separates the strands.

5. A. The largest fragment is held back most by the gel and stays nearest the well; the 200 bp fragment travels furthest.

6. B. The next nucleotide joins to the 3′-OH of the growing chain. A dideoxynucleotide has hydrogen instead of a hydroxyl group at the 3′ carbon, so no further bond can form.

7. C. It is a subunit vaccine: the HBsAg gene is expressed in yeast and only that protein is purified. With no viral genome or whole virus it cannot cause the disease.

8. D. Bt crops carry a *cry* gene from *Bacillus thuringiensis*; the Cry protein kills insect larvae. The other options are other GM traits.

9. B. Somatic gene therapy alters lung cells only; his sperm still carry the faulty allele, so inheritance is unchanged.

10. A. A clone is identical in nuclear DNA to the animal that supplied the nucleus. The egg donor gives only cytoplasm and mitochondria, and the surrogate gives nothing genetic.

Before the test: 12 facts to recite

1. Restriction endonucleases cut DNA at specific palindromic sites of 4 to 8 bp; EcoRI cuts G/AATTC and leaves sticky ends, SmaI leaves blunt ends.
2. Gene and vector are cut with the same enzyme so their sticky ends are complementary; DNA

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ligase then forms the phosphodiester bonds.

3. A plasmid vector needs an origin of replication, an antibiotic resistance marker and a unique restriction site.
4. Gene cloning: isolate gene → cut gene and plasmid → join with ligase → transform host → select on antibiotic → grow and express.
5. Eukaryotic genes go into bacteria as cDNA, made from mRNA by reverse transcriptase, because bacteria cannot remove introns.
6. PCR cycle: denaturation 94 to 95 °C, annealing 50 to 65 °C, extension by Taq polymerase (*Thermus aquaticus*) at 72 °C; copies after n cycles = starting number × 2^n.
7. In electrophoresis DNA moves to the positive electrode because of its phosphate groups; the smallest fragments travel furthest, the largest stay near the well.
8. Sanger sequencing uses dideoxynucleotides, which lack a 3′-OH and terminate the chain; the sequence is read from the shortest fragment upwards.
9. Recombinant human insulin (1982, *E. coli*) is identical to the human hormone, unlike cattle and pig insulin; hepatitis B vaccine is the viral surface antigen made in yeast.
10. Somatic gene therapy (first success 1990, ADA-SCID) corrects body cells and is not inherited; germ-line therapy would be inherited and is banned.
11. Bt crops carry a *cry* gene from *Bacillus thuringiensis* whose protein kills caterpillars; golden rice makes beta-carotene; plant genes are delivered by the Ti plasmid of *Agrobacterium tumefaciens*.
12. DNA fingerprinting (Jeffreys, 1984) compares non-coding repeats (VNTRs or STRs); only identical twins match, and every band in a child comes from a parent. Dolly (1996) was identical to her nucleus donor, not to the egg donor or the surrogate.

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