Name	Date started	Target end date

GCE A LEVEL — BIOLOGY UNIT 4 QUESTION PACK

1075 (Legacy BY5) · New spec Unit 4 Topic 8 · A2 unit, first sat 2017, 90 marks, 2h paper

REVISE
.wales

BIOLOGY — UNIT 4 · RECOMBINANT DNA, GENE THERAPY & BIOTECH

4.5 Applications of reproduction and genetics — recombinant DNA, gene therapy, PCR, gel electrophoresis and GM crops

Restriction enzymes and DNA ligase to construct recombinant plasmids, gel electrophoresis to size DNA fragments, PCR to amplify regions of interest, gene therapy delivery (illustrated by cystic fibrosis), and the construction and ethical evaluation of herbicide-resistant GM crops including the use of micro-propagation.

LEGACY 2008 SPECIFICATION

Estimated time for entire question pack: ~1 h 18 min

Derived from the legacy BY5 papers' pace of ~1.6 min/mark, padded for long-prose answers (49 marks over 4 questions).

*You are advised to **not** attempt to complete all of this in one sitting.*

ABOUT THIS QUESTION PACK

This is a **comprehensive practice question pack**, not a single mock paper. It contains every question from the legacy WJEC BY5 papers (2008 modular spec, 2011–2017) that maps onto new-spec A2 Unit 4 Topic 8 (4.5).

Questions are ordered by source paper date.

INSTRUCTIONS

Use black ink or black ball-point pen. Show all working — quality of written communication will affect marks. A calculator is allowed. Diagrams included in answers must be fully annotated.

For Examiner's use only

Q	Source	Max	Mark
1	BY5 Jun 12 Q5	17	
2	BY5 Jun 12 Q7	8	
3	BY5 Jun 14 Q5	12	
4	BY5 Jun 15 Q4	12	
Total		**49**	

Recombinant DNA, Gene Therapy & Biotech — what the new spec asks

WJEC GCE A Level Biology (from 2015) · Unit 4: Variation, Inheritance & Options · Topic 4.5.

Restriction enzymes

- Recognise specific palindromic sequences and cut DNA.
- Many leave sticky ends (e.g. EcoRI cuts GAATTC).
- Same enzyme on plasmid + target gene gives complementary sticky ends.

DNA ligase & plasmids

- DNA ligase seals nicks between insert and plasmid.
- Plasmids carry origin of replication + selectable marker.
- Recombinant plasmid transformed into bacteria for cloning.

Gel electrophoresis

- Agarose gel; DNA migrates to + electrode.
- Smaller fragments travel further; ladder calibrates size.
- Visualised with ethidium bromide / SYBR safe.

PCR

- Cycle of denaturation (95°C), annealing (~55°C), extension (72°C).
- Taq polymerase (thermostable from *Thermus aquaticus*).
- Doubles target DNA each cycle → 2^n.

Gene therapy

- Insert functional copy of faulty gene into patient's cells.
- Vectors: liposomes (CF inhaler), adenovirus, lentivirus.
- Somatic (treats patient only) vs germ-line (heritable, ethically contentious).

GM crops

- Roundup-resistant crops — herbicide kills weeds but not crop.
- Bt-corn — engineered to produce insecticidal Bt toxin.
- Concerns: gene flow to wild relatives, monopolies, ecological impacts.

Crib sheet »

Recombinant DNA, Gene Therapy & Biotech in one page

Quick-reference notes — revisit before each question.

Restriction enzymes

Cut DNA at specific recognition sequences.

Often palindromes; sticky or blunt ends.

EcoRI cuts G^AATTC; HindIII cuts A^AGCTT.

DNA ligase

Forms phosphodiester bonds.

Seals plasmid + insert into a recombinant.

Plasmids

Small circular bacterial DNA — carry genes outside the main chromosome.

Used as vectors; include selectable markers (antibiotic resistance).

Gel electrophoresis

Agarose gel + electric field.

DNA moves to + electrode (phosphate −).

Smaller fragments travel further; ladder calibrates.

PCR

Denature (95°C), anneal primers (~55°C), extend (72°C).

Taq polymerase — heat-stable.

Each cycle doubles target sequence.

Gene therapy

Insert functional copy of gene into patient's cells.

Vectors: liposome (CF inhalation), adenovirus, lentivirus.

Somatic (treats individual) vs germ-line (heritable, controversial).

GM crops

Add genes for resistance to herbicide / pests / drought.

Used *Agrobacterium* or particle gun delivery.

Ethical / ecological concerns: gene flow, biodiversity, monopoly.

Cystic fibrosis

CFTR gene mutation → faulty chloride channel.

Liposome / adenovirus deliver normal CFTR cDNA to airway cells.

Clinical trials limited durability so far.

Examiner only

5. *(a)* (i) What is meant by the term 'gene therapy'? [1]

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(ii) The following statements describe the two different types of gene therapy, somatic cell therapy and germ line therapy.

1. Targets cells in affected tissues
2. Introduces genes into the egg
3. Inherited
4. Not inherited.

Under the two headings below write the appropriate **numbers** of the statements that describe the two forms of gene therapy. [2]

Somatic cell therapy	Germ line therapy

(b) (i) Cystic fibrosis is caused by a mutation of the gene producing the protein CFTR. Explain how the presence of this altered protein results in the production of thick, sticky mucus and how this accounts for the respiratory symptoms of the disease. [4]

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(ii) Describe **one** technique that could be used to introduce functional CFTR genes into someone with cystic fibrosis. [3]

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Examiner only

(c) (i) Explain why the replication of DNA is described as semi-conservative. [1]

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(ii) The polymerase chain reaction (PCR) is used to make many copies of a section of DNA. Each stage of the reaction takes place at different temperatures. [3]
Explain why during each stage

I. the DNA is first heated to 95°C

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II. the temperature is then reduced to 55°C

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III. the temperature is then increased to 70°C

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(iii) About twenty percent of the DNA produced by the polymerase chain reaction (PCR) is copied inaccurately. Suggest and explain why it is not safe to use the PCR to clone the CFTR gene for use in treating cystic fibrosis. [3]

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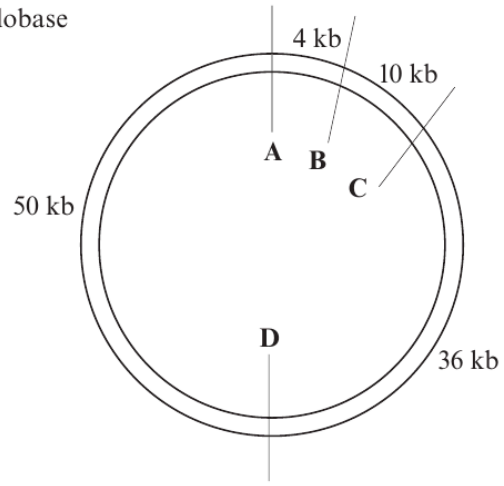
(Total 17 marks)

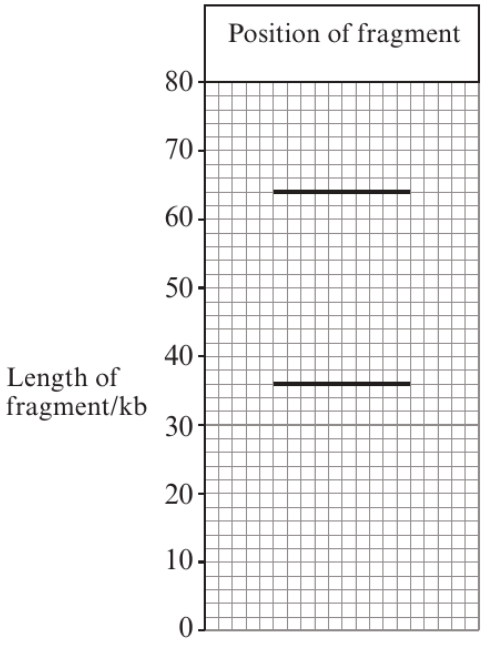
Examiner only

7. *(a)* In the formation of recombinant DNA many different restriction endonuclease enzymes are used. Each enzyme cuts the DNA of a plasmid at a specific base sequence called a restriction site.
The diagram shows the position of restriction sites, **A**, **B**, **C** and **D**, for each of four different enzymes on a plasmid.
The distance between these sites is measured in kilobases of DNA.

1 kb = 1 kilobase



The plasmid was cut using only **two** of the restriction enzymes. The resulting fragments were separated by gel electrophoresis. The positions of the fragments are shown in the chart.



Examiner only

(i) Which of the restriction sites were cut? [1]

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(ii) Explain your answer. [1]

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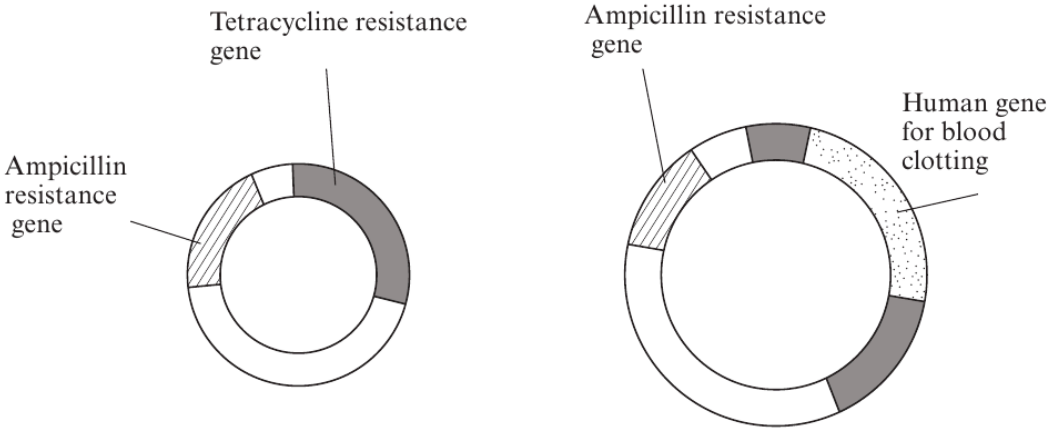
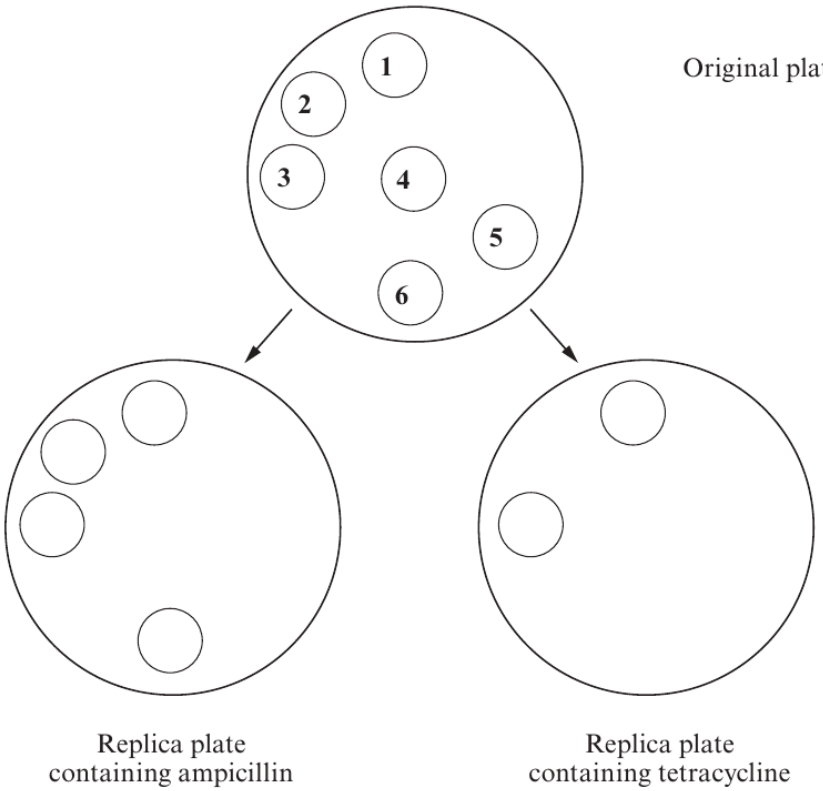
(b) In genetic engineering, genes for antibiotic resistance in bacterial plasmids can be used as genetic markers. Scientists used a plasmid containing genes for resistance to two antibiotics, ampicillin and tetracycline. A human gene for blood clotting was inserted in the plasmid in the position shown in diagram below.



Plasmids were then inserted into bacteria, although some of the plasmids had not taken up the human gene. Plates were replicated to identify the bacteria with the human gene. The diagram overleaf shows the bacterial colonies that grew on the two replica plates.

Examiner only



(i) **Complete the above diagram** by writing the correct **numbers** for the bacterial colonies that grew on the replica plates. [1]

(ii) Explain the results of the replica plate containing ampicillin. [2]

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(iii) Explain the results of the replica plate containing tetracycline. [3]

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(Total 8 marks)

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Examiner only

5. The techniques of recombinant DNA technology and micro-propagation are used to produce Genetically Modified Crops. The following summary is adapted from an account given on the Food Standards Agency's web site [www.food.gov.uk]

1. A plant with the desired characteristic is identified – e.g. resistance to the herbicide 'Roundup'.

2. The specific gene that produces this characteristic is found in the plant's DNA and cut out.

3. To get the gene into the cells of the plant being modified, the gene needs to be attached to a carrier. A piece of bacterial DNA called a plasmid is joined to the gene to act as the carrier.

4. Once the gene is attached to the plasmid, a marker gene is also added to identify which plant cells take up the new gene.

5. The 'gene package' is put in a bacterium, which multiplies, to create many copies of the 'gene package'.

6. A copy of the 'gene package' is dried onto a gold or tungsten particle – and fired into a piece of tissue from the plant being modified. The particle carries the 'gene package' into the plant's cells.

7. The plant tissue is put into a selective growth medium so that only modified tissue develops into plants.

(a) Explain how different types of enzymes are used in stages 2 and 3 to produce the 'gene package'. [4]

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Examiner only

(b) Describe the steps involved in the culture of a large number of genetically identical plants from the plant tissue produced in stage 7. [3]

(c) (i) Explain the advantage to farmers of having crops resistant to 'Roundup'. [3]

(ii) Explain why environmentalists might have legitimate objections to using GM crops resistant to 'Roundup'. [2]

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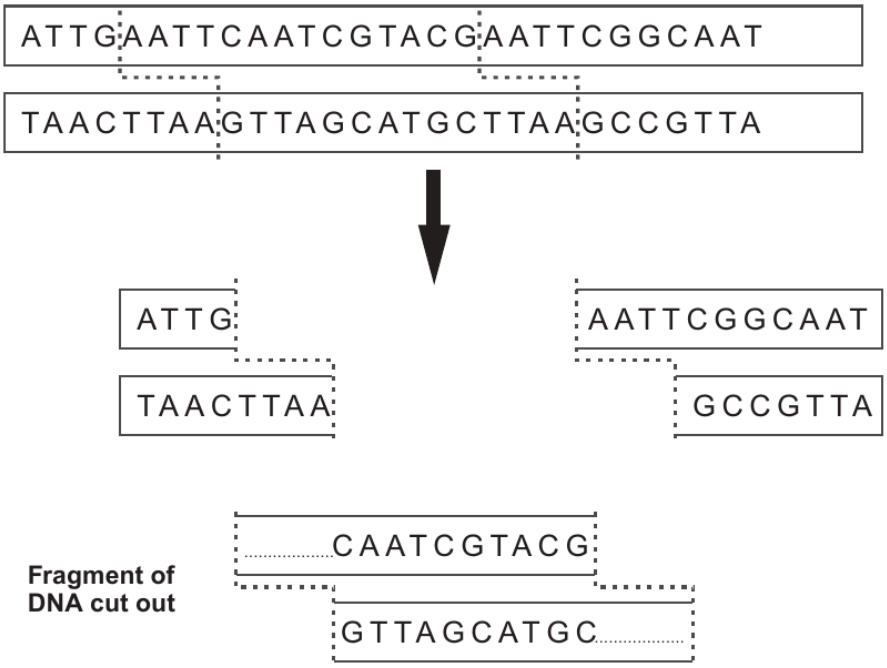
Examiner only

4. *(a)* Restriction enzymes are essential tools of genetic engineering. A restriction enzyme cuts the double-stranded DNA molecule at its specific **recognition site**. The diagram below shows how one such enzyme would cut out a DNA fragment.



(i) Draw in the bases which are missing from the ends of the fragment of DNA which has been cut out. [1]

(ii) Explain why the parts completed in (i) are known as 'sticky ends'. [1]

(iii) A number of different restriction enzymes are now available, some of which are shown in the table below:

Enzyme	Source	Recognition site
***Eco*RI**	*Escherichia coli* RY 13	GAATTC
***Bam*HI**	*Bacillus amyloliquefaciens* H	GGATCC
***Hind*III**	*Haemophilus influenzae* Rd	AAGCTT

Name the enzyme used in the example above. [1]

Examiner only

(b) In recombinant DNA technology, the piece of DNA which has been cut out is inserted into a plasmid which has been cut open using the same enzyme.

(i) Define the term 'plasmid'. [1]

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(ii) Why is it important to use the same enzyme? [1]

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(iii) Name the type of enzyme used to join the cut fragment into the plasmid. [1]

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(c) Restriction enzymes are also used to cut up DNA during DNA fingerprinting/profiling. Labelled DNA probes are then used to identify the positions of the fragments on an electrophoresis gel. The fragments used are sections cut from introns rather than exons.

Explain why introns are more useful for genetic fingerprinting than exons. [2]

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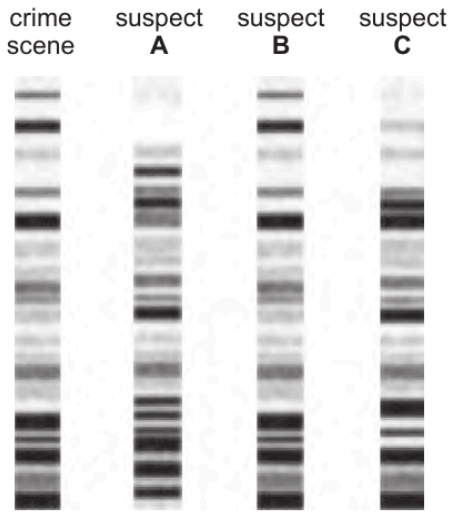
Examiner only

(d) DNA profiles of a sample of DNA taken from a crime scene and samples prepared from blood of three suspects are shown below.

DNA samples from:



Give **two** features of the DNA profiles which would lead to the identification of suspect **B** as being present at the scene of the crime. [1]

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(e) (i) DNA at crime scenes is often found in very small quantities. Polymerase Chain Reaction (PCR) is a technique that enables the analysis of these small samples of DNA. State how PCR makes this possible. [1]

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(ii) The enzyme used in the technique has an important function during interphase in both mitosis and meiosis.

Name: [2]

I. the enzyme used; .....

II. the enzyme's function in interphase. .....

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END OF QUESTION PACK

4 questions · 49 marks · ~1 h 18 min
Source: WJEC BY5 (2008 modular spec, 2011–2017)
Curated for WJEC Biology 2015 spec A2 Unit 4 — Topic 8 (4.5)