A-LEVEL BIOLOGY • EXERCISE SHEET

19.1 Principles of genetic technology

Name: _____ Class: _____ Date: _____ **Total: 34 marks**

KEY WORDS gene 基因 • plasmid 质粒 • polymerase 聚合酶 • polymerase chain reaction 聚合酶链式反应 • gel electrophoresis 凝胶电泳

Objective

Build the skills to answer exam questions on the **principles of genetic technology** — **recombinant DNA** 重组 DNA, the tools of **genetic engineering** 基因工程, **PCR** 聚合酶链式反应 and **gel electrophoresis** 凝胶电泳.

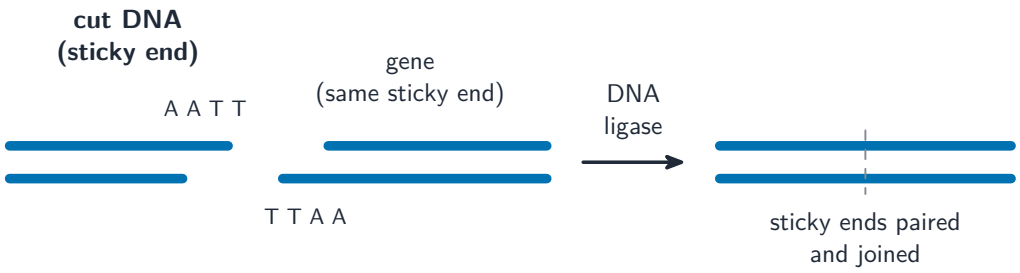
You must be able to:

- define recombinant DNA and explain the roles of the key enzymes and a plasmid vector
- explain why a promoter and a marker gene are transferred with a gene
- describe PCR and gel electrophoresis

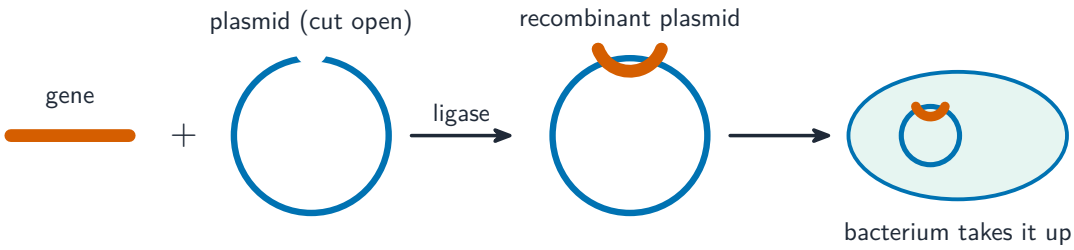
1 Worked examples

The tools

Recombinant DNA is DNA joined from two different sources. The gene can be cut from a **donor** 供体, made from its mRNA by **reverse transcriptase** 逆转录酶, or built from **nucleotides** 核苷酸.



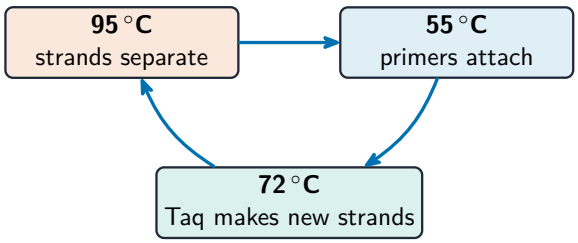
A restriction endonuclease leaves matching sticky ends; DNA ligase joins the gene to the cut DNA



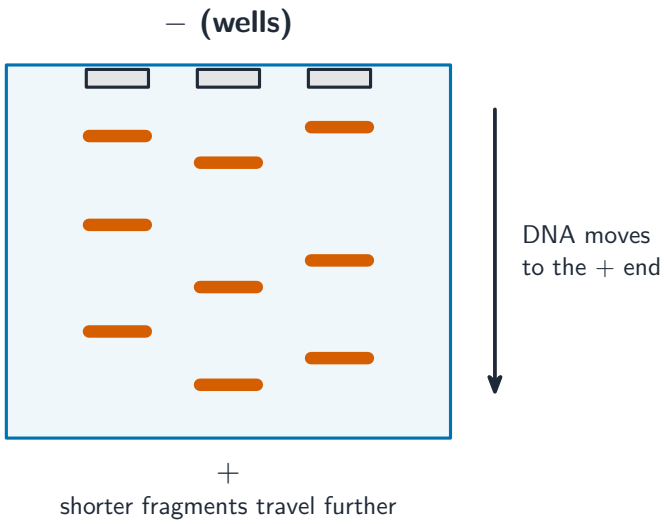
A plasmid is a cloning vector: the gene is joined into it, and the recombinant plasmid enters a bacterium

- **restriction endonuclease** 限制性内切酶 — cuts DNA at a specific sequence (sticky ends); **DNA ligase** — joins DNA; **plasmid** 质粒 — a vector to carry the gene; **DNA polymerase** — copies DNA.
- a **promoter** 启动子 (the gene's "switch") must be transferred so the gene is transcribed; a **marker gene** 标记基因 (e.g. fluorescent) confirms the transfer worked.

Copying and sorting DNA



PCR doubles the DNA each cycle, making millions of copies



Gel electrophoresis sorts DNA fragments by length — shorter fragments move further

The tools, in the order a gene actually moves

Tool	What it does
restriction endonuclease 限制性内切酶	cuts DNA at a specific base sequence, often leaving **sticky ends** 黏性末端
DNA ligase DNA 连接酶	joins the sugar–phosphate backbones of two fragments
plasmid 质粒 or virus	the **vector** 载体 that carries the gene into the host cell
reverse transcriptase 逆转录酶	makes complementary DNA (cDNA) from an mRNA template
PCR 聚合酶链式反应	copies a DNA fragment millions of times
gel electrophoresis 凝胶电泳	separates fragments by length — small fragments travel furthest

The standard route, which is what an extended question asks for:

1. **Isolate the gene** — cut it out with a restriction enzyme, or build it from mRNA using reverse transcriptase.
2. **Cut the vector with the SAME restriction enzyme**, so its sticky ends are complementary to the gene's. This step is the one candidates omit, and it is always a mark.
3. **Anneal and join** — the complementary sticky ends pair by hydrogen bonding, and DNA ligase seals the backbones, giving **recombinant DNA** 重组 DNA.
4. **Transform the host** — the plasmid is taken up by bacteria, usually after treatment with calcium ions and heat shock, or by electroporation.
5. **Identify the transformed cells** with a **marker gene** — for example antibiotic resistance, or a fluorescent gene, so only cells that took up the plasmid grow or glow.
6. **Culture** the successful cells so the protein is produced in quantity.

Why sticky ends matter. They are short single-stranded overhangs. Because both the gene and the plasmid were cut with the same enzyme, their overhangs are complementary and pair specifically — which is what makes the joining reliable rather than random.

Worked example. Explain why human insulin made this way is better than insulin extracted from pigs. It is identical to human insulin, so it works properly and is far less likely to provoke an immune response or allergy; it can be produced in unlimited quantity from bacterial culture rather than depending on a supply of animal pancreases; and it avoids the religious and ethical objections some patients have to an animal product.

2 Practice

2.1 Name the enzyme that (a) cuts DNA at a specific sequence and (b) joins DNA fragments. [2]

2.2 State why a marker gene is transferred along with the desired gene. [1]

2.3 Name the enzyme that cuts DNA at a specific sequence, and the enzyme that joins two fragments. [2]

2.4 Explain why the gene and the plasmid must be cut with the same restriction enzyme. [2]

2.5 State the purpose of a marker gene. [2]

3 Exam-style questions

3.1 What is the role of a plasmid in genetic engineering? [1]

- **A** to cut the DNA
- **B** to act as a vector that carries the gene into a cell
- **C** to copy the DNA millions of times
- **D** to separate DNA fragments by length

3.2 In gel electrophoresis, the DNA fragments that travel **furthest** are the: [1]

- **A** longest
- **B** shortest
- **C** most positively charged

- **D** circular ones

3.3 (a) Explain why a promoter must often be transferred along with a gene. [2]

(b) Describe how the polymerase chain reaction (PCR) makes many copies of a DNA sample. [3]

3.4 Explain why restriction enzymes and DNA ligase are both needed to make recombinant DNA. [2]

3.3 A gene for a human protein is to be transferred into bacteria so that the protein can be produced commercially.

(a) Describe how the gene can be obtained from a human cell that expresses it, naming the enzyme used. [3]

(b) Explain the role of sticky ends in inserting the gene into a plasmid. [3]

(c) Name the enzyme that seals the gene into the plasmid, and state what the product

is called. [2]

(d) Describe how the bacteria that have taken up the plasmid can be identified. [3]

(e) Suggest two advantages of producing this protein in bacteria rather than extracting it from animals. [2]

(f) Explain how gel electrophoresis could be used to check that a fragment of the expected length has been inserted. [3]

Solutions

2.1 (a) restriction endonuclease; (b) DNA ligase.

2.2 to check (confirm) that the gene has been taken up and is expressed.

3.1 B. A plasmid is a vector carrying the gene into the cell.

3.2 B. Shorter fragments move further through the gel.

3.3 (a) the promoter is the switch that starts transcription; without it the transferred gene would not be transcribed / stay silent.
(b) heat to ~95 °C to separate the strands; cool to ~55 °C so primers attach; 72 °C lets Taq polymerase build new strands — repeating the cycle doubles the DNA each time.

3.4 the restriction enzyme cuts the gene and the plasmid, leaving matching sticky ends; DNA ligase joins the gene into the plasmid to make recombinant DNA.

2.3 Restriction endonuclease; DNA ligase.

2.4 Each restriction enzyme cuts at one specific sequence and leaves a particular sticky end; using the same enzyme on both means their overhangs are complementary, so the gene and the plasmid pair together specifically.

2.5 It allows the cells that have taken up the plasmid to be identified; for example an antibiotic-resistance gene lets only transformed cells grow on a medium containing that antibiotic.

3.3 (a) Extract the mRNA for the protein from a cell that expresses it; use reverse transcriptase to make complementary DNA from that mRNA template; this cDNA is the gene, and it has no introns. (Cutting the gene from the genome with a restriction endonuclease is also accepted.)
(b) Sticky ends are short single-stranded overhangs left when the restriction enzyme cuts; the gene and the plasmid are cut with the same enzyme, so their overhangs are complementary; the bases pair by hydrogen bonding, holding the gene in place in the plasmid.
(c) DNA ligase; the product is recombinant DNA.
(d) The plasmid also carries a marker gene, for example antibiotic resistance; the bacteria are grown on a medium containing that antibiotic; only the cells that took up the plasmid survive and form colonies, so those are the transformed cells. (A fluorescent marker viewed under UV is equally acceptable.)
(e) Any two of: the protein is identical to the human one, so it is less likely to cause an immune response; it can be produced in unlimited quantity by culturing bacteria; production does not depend on a supply of animal tissue; it avoids religious or ethical objections to an animal product.
(f) Cut the plasmid with the same restriction enzyme to release the insert; run the fragments through a gel under an electric field, where smaller fragments travel further; compare the distance travelled with a set of fragments of known length, and a band at the expected position shows the insert is the right size.