Exercise walk-through

Principles of genetic technology ▪ 19.1

eXercise

You must be able to

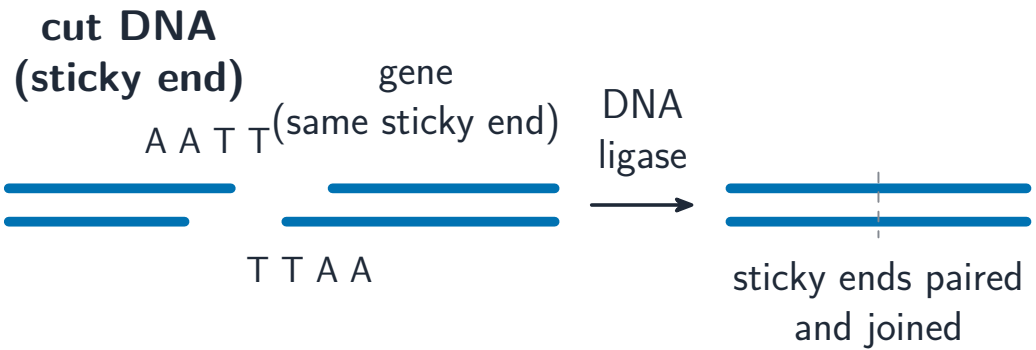
- define recombinant DNA and explain the roles of the key enzymes and a plasmid vector
- explain why a promoter and a marker gene are transferred with a gene
- describe PCR and gel electrophoresis

Method — 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.

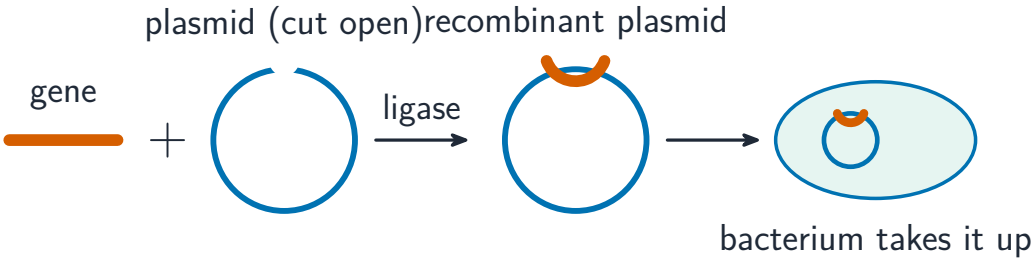
- **restriction endonuclease** 限制性内切酶 — cuts DNA at a specific sequence (sticky ends); **DNA ligase** — joins DNA; **plasmid** 质粒 — a vector to carry the gene; **DNA polymerase** — copies DNA.
- a **promoter** 启动子 (the gene's "switch") must be transferred so the gene is transcribed; a **marker gene**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

Method — The tools

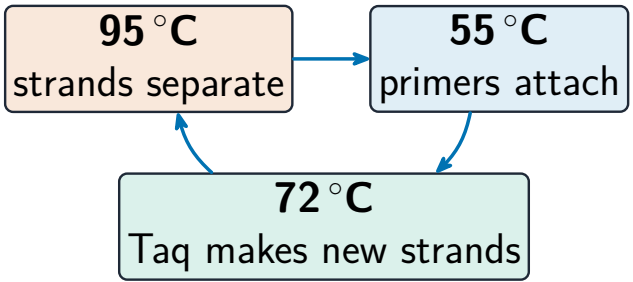


A restriction enzyme leaves matching sticky ends; DNA ligase joins a gene to the cut DNA

Method — The tools



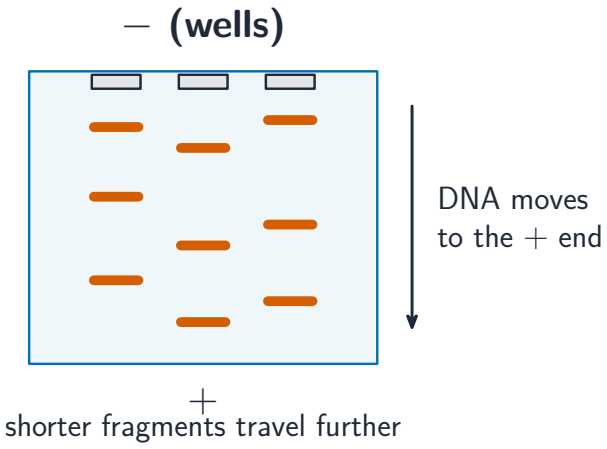
Method — Copying and sorting DNA



each cycle **doubles** the DNA → millions of copies

PCR repeats heat-and-cool cycles; each cycle doubles the DNA

Method — Copying and sorting DNA



Practice 2.1 ▪ 2 marks

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

Solution 2.1

Method: The tools

Worked steps

(a) restriction endonuclease B1; (b) DNA ligase B1.

Practice 2.2 ▪ 1 mark

State why a marker gene is transferred along with the desired gene.

Solution 2.2

Method: The tools

Worked steps

to check (confirm) that the gene has been taken up and is expressed B1.

Exam-style 3.1 ▪ 1 mark

What is the role of a plasmid in genetic engineering?

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

Solution 3.1

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

Worked steps

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

Exam-style 3.2 ▪ 1 mark

In gel electrophoresis, the DNA fragments that travel **furthest** are the:

A longest

B shortest

C most positively charged

D circular ones

Solution 3.2

Method: Copying and sorting DNA

A longest

B shortest ✓

C most positively charged

D circular ones

Worked steps

B. Shorter fragments move further through the gel.

Exam-style 3.3 ▪ 2 marks

(a) Explain why a promoter must often be transferred along with a gene.
(b) Describe how the polymerase chain reaction (PCR) makes many copies of a DNA sample.

Solution 3.3

Method: The tools

Worked steps

(a) the promoter is the switch that starts transcription **M1**; without it the transferred gene would not be transcribed / stay silent **A1**.
(b) heat to ~95 °C to separate the strands **B1**; cool to ~55 °C so primers attach **B1**; 72 °C lets Taq polymerase build new strands — repeating the cycle doubles the DNA each time **B1**.

Exam-style 3.4 ▪ 2 marks

Explain why restriction enzymes and DNA ligase are both needed to make recombinant DNA.

Solution 3.4

Method: The tools

Worked steps

the restriction enzyme cuts the gene and the plasmid, leaving matching sticky ends M1; DNA ligase joins the gene into the plasmid to make recombinant DNA A1.