# Polymerase Chain Reaction (PCR)

A Level

Polymerase Chain Reaction (PCR) is a laboratory technique used to "amplify" (replicate) a selected region of DNA. It can therefore be thought of as an artificial form of DNA replication.

## Part A The process

The first step of PCR is called [ ] and takes place at $\approx 95\,^{\circ}\mathrm{C}$. [ ] causes the DNA double-helix to unwind and [ ] bonds between strands to break.

The second step of PCR is called [ ] and takes place at $\approx 55\,^{\circ}\mathrm{C}$. [ ] (single-stranded DNA sequences, usually $18$ to $30$ bases long) bind to opposite strands at opposite ends of the selected region.

The third step of PCR is called [ ] and takes place at $\approx 72\,^{\circ}\mathrm{C}$. [ ] catalyses the sequential addition of free DNA nucleotides along the strands.

The whole process is repeated $25$ to $35$ times to produce a large yield of the selected region of DNA.

Items:

DNA ligase | The high temperature | unwinding | Okazaki fragments | DNA polymerase | denaturation | annealing | preparation | hydrogen | extension | DNA primers | finalisation | DNA helicase

## Part B DNA replication vs PCR

Which of the following statements are true of DNA replication in living cells? Select all that apply.

- [ ] The two original DNA strands are broken apart by DNA helicase
- [ ] The two original DNA strands are broken apart by extremely high temperatures.
- [ ] For each original DNA molecule, the two new strands are both synthesised continuously.
- [ ] For each original DNA molecule, one new strand is synthesised continuously and the other new strand is synthesised in short fragments.
- [ ] Nucleotides are added to the $3'$ end of each new strand.
- [ ] Nucleotides are added to the $5'$ end of each new strand.
- [ ] All of the DNA present is replicated.
- [ ] Only a specific region of DNA is replicated.

Which of the following statements are true of PCR? Select all that apply.

- [ ] The two original DNA strands are broken apart by DNA helicase
- [ ] The two original DNA strands are broken apart by extremely high temperatures.
- [ ] For each original DNA molecule, the two new strands are both synthesised continuously.
- [ ] For each original DNA molecule, one new strand is synthesised continuously and the other new strand is synthesised in short fragments.
- [ ] Nucleotides are added to the $3'$ end of each new strand.
- [ ] Nucleotides are added to the $5'$ end of each new strand.
- [ ] All of the DNA is replicated.
- [ ] Only a specific region of DNA is replicated.

## Part C Taq polymerase

One of the most commonly used DNA polymerases in PCR is called *Taq* polymerase, which comes from the bacterium *Thermus aquaticus*. Why do scientists use this DNA polymerase instead of DNA polymerase from humans or other mammals?

- *Thermus aquaticus* has evolved to have a very fast cell cycle, and so its DNA polymerase works faster than human/mammal DNA polymerases.
- *Taq* polymerase breaks apart the original strands as well as adding nucleotides to the new strands.
- *Thermus aquaticus* has evolved to cope with extremely hot temperatures, and so its DNA polymerase is not denatured at the high temperatures used in PCR.
- *Taq* polymerase is able to add nucleotides to both ends of the new strand, whereas human/mammal DNA polymerases can only add nucleotides to the $3'$ end of a new strand.
- Scientists only ever amplify bacterial DNA, so it is better to use a bacterial polymerase.

## Part D How many molecules?

Each PCR "cycle" doubles the number of DNA molecules you have.

If you started with only $10$ DNA molecules, how many DNA molecules would you have after $25$ PCR cycles?

Give your answer to 1 significant figure.

# Gel Electrophoresis

A Level

Gel electrophoresis is a laboratory technique used to separate DNA fragments based on their size.

An agarose gel is made by adding agarose powder to a buffer solution. The mixture is heated to ensure that the powder fully dissolves. A fluorescent "tag" (e.g. Ethidium Bromide) is also added which will bind to DNA and fluoresce under UV light.

Wells (indentations) are created in the gel, and once the gel has solidified it is placed in a tank and submerged under buffer solution.

A solution containing a mixture of DNA fragments of known sizes is usually added to one of the wells. This solution is called the "DNA ladder", and is used as a reference of DNA molecule size.

The solutions containing the DNA fragments of interest are added to the other wells.

An electric current is applied to the tank.

## Part A The process

DNA is [ ] charged due to its [ ]. This means that, when the electric current is applied, DNA fragments will move towards the [ ] electrode.

Larger DNA fragments will move [ ] through the agarose gel than smaller DNA fragments. This allows fragments of different sizes (i.e. lengths) to be separated from each other, as they will appear as separate "bands" under UV fluorescence.

If a DNA ladder is added to the same gel, the size of each DNA fragment can be estimated by comparing the position of that band with the DNA ladder bands. This is useful for checking that a region of DNA has been amplified correctly during [ ].

Items:

positive | slower | positively | phosphate groups | faster | bases | PCR | deoxyribose sugars | negative | negatively

## Part B Analysing a gel

A scientist is trying to insert a gene (gene Z) from a jellyfish into *E. coli*, in order to get the *E. coli* to produce the protein that gene Z codes for.

The scientist extracted DNA from the jellyfish, and used PCR to try to amplify the gene. They ran five different PCR reactions: each using a different set of primers. To check whether they have managed to amplify the correct DNA region in any of their PCR reactions, they run the PCR products on a gel.

The results are shown in **Figure 1**.

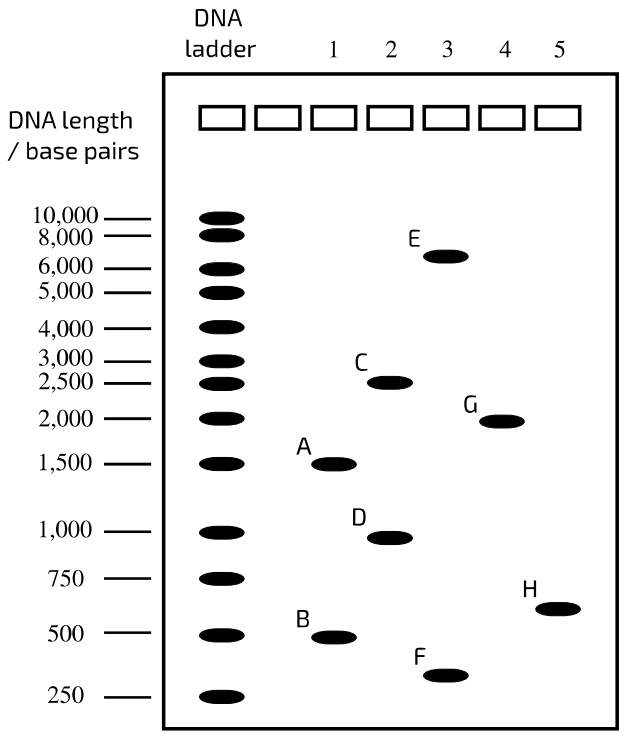


**Figure 1:** A gel electrophoresis schematic. Seven wells are shown at the top. The leftmost well was filled with a DNA ladder, and the sizes of each DNA ladder fragment are shown on the left. The next well was left empty, and the remaining five wells (1-5) were filled with PCR products from different PCR attempts. An electric current was applied to the gel tank for 45 minutes, and then the gel was removed and illuminated with UV light to visualise the bands. Each band is labelled (A-H).

Gene Z is approximately 2.5 kbp (kilobase pairs) long.

Which band, if any, contains gene Z?

- A
- B
- C
- D
- E
- F
- G

- H
- none of the above

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In **Figure 1**, where would the positive electrode be positioned?

- Top
- Right side
- Bottom
- Left side

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# Genetic Engineering

A Level

Genetic engineering (also called "genetic modification") refers to the process of artificially modifying an organism's genome. This may involve "**recombination**": combining DNA from two different individuals into one genome.

Genetic engineering can be used to engineer bacteria that will produce a certain protein (e.g. insulin). To do this, a "recombinant" plasmid is created that contains the human gene for insulin. This plasmid is then introduced into host *E. coli* cells in a process called "**transformation**".

The bacterial cells multiply and express the gene, and the protein can be harvested from the bacterial populations.

## Part A Recombination

In order to create a recombinant plasmid, [ ] are used to cut open the plasmid at a specific site (sequence). A solution containing the desired gene is added to a solution containing the cut plasmid, and [ ] is added to this to catalyse the binding of the gene to both ends of the cut plasmid.

There are two main types of [ ]: those that create "blunt ends" and those that create "sticky ends".

Blunt ends are ends in which both strands stop at the same point, whereas sticky ends are ends in which there is an "overhang" i.e. one strand continues past the other strand. [ ] ends make it easier to insert the desired gene, because there are exposed bases that can bind by complementary base pairing before DNA ligase catalyses the formation of [ ] bonds. However, this does require complementary sequences to be added to either side of the desired gene.

Items:

**restriction enzymes** **Blunt** **phosphodiester** **DNA ligase** **hydrogen** **Sticky**

**proteolytic enzymes** **DNA polymerase**

## Part B Transformation

After a [ ] plasmid has been made, it is introduced into bacterial cells by adding a solution containing the plasmid to a solution containing bacterial cells. The bacterial cells that take up the plasmid are described as "[ ]".

Because not all bacterial cells will take up the plasmid, scientists need to be able to select only the transformed cells. Usually this is done by using a plasmid that contains a gene for a specific [ ]. The cells are then left to grow on agar that contains the matching [ ]. This ensures that only the transformed cells will survive and form colonies, which can then be used in the rest of the process.

Items:

| | | | | | |
|---|---|---|---|---|---|
| antibiotic resistance | transformed | antigen | recombinant | antibiotic | antibody |

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# Factor VIII Transgenesis

A Level

Factor VIII is a protein that is necessary for blood clotting. **Figure 1** shows how the human gene that codes for this protein can be incorporated into bacterial DNA and inserted into a bacterium.

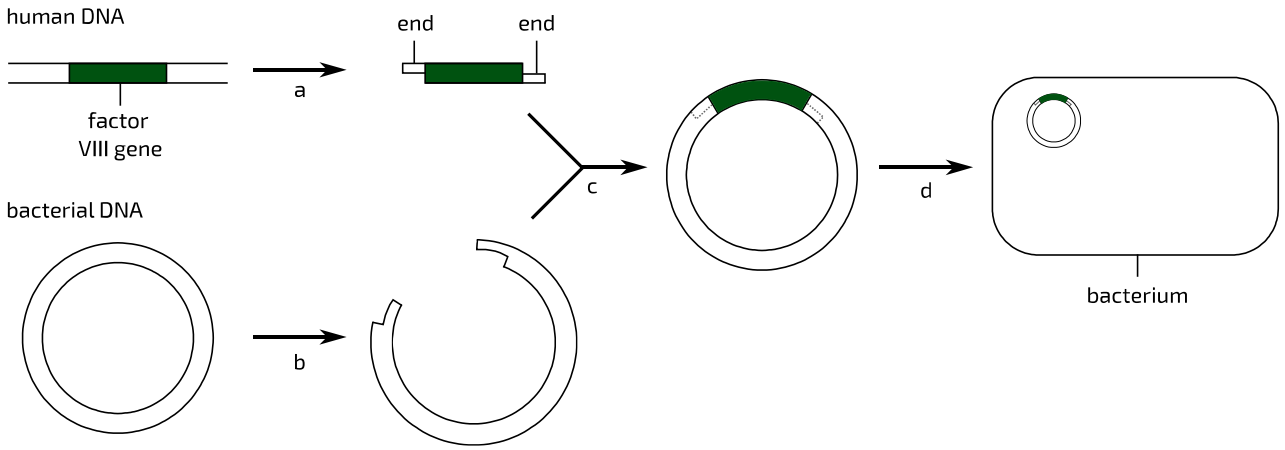


**Figure 1:** Genetic engineering of a bacterium to contain the human factor VIII gene. The human factor VIII gene is cut out from human DNA and inserted into a bacterial plasmid that has been cut. This plasmid is then introduced into a bacterium.

## Part A Enzymes

What type of enzymes are used in process **a** and **b**?

What type of enzyme is used in process **c**?

## Part B Ends

What type of "ends" are formed in processes **a** and **b**?

## Part C Process c

What is the name of process **c**?

## Part D Process d

What is the name of process **d**?

Adapted with permission from OCR A Level January 2001, Biology Foundation, Question 6

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# Restriction Enzymes and Restriction Sites

A Level

Restriction enzymes cut DNA at specific sequences called restriction sites. Table 1 shows the restriction sites of a number of restriction enzymes, and how each enzyme cuts its specific sequence (green lines).

| restriction enzyme | restriction site |
|---|---|
| BamH I | 5' G\|G A T C C 3'<br>3' C C T A G\|G 5' |
| EcoR I | 5' G\|A A T T C 3'<br>3' C T T A A\|G 5' |
| Hae III | 5' G G\|C C 3'<br>C C\|G G<br>3' 5' |
| Hha I | 5' G\|C G C 3'<br>C G C\|G<br>3' 5' |
| Hind III | 5' A\|A G C T T 3'<br>3' T T C G A\|A 5' |

Table 1

## Part A Blunt ends

Which of the restriction enzymes described in Table 1 produce blunt ends? Select all that apply.

- [ ] BamH I
- [ ] EcoR I
- [ ] Hae III
- [ ] Hha I
- [ ] Hind III

## Part B Sticky ends

Which of the restriction enzymes described in Table 1 produce sticky ends? Select all that apply.

- [ ] BamH I
- [ ] EcoR I
- [ ] Hae III
- [ ] Hha I
- [ ] Hind III

## Part C Identify the enzymes

The image below shows how a particular DNA sequence will be cut by two of the restriction enzymes described in Table 1.


Which restriction enzyme is responsible for each cut?

- **a**:
- **b**:

Items:

EcoR I | BamH I | Hha I | Hind III | Hae III

## Part D Choosing restriction enzymes

A scientist wants to incorporate a particular gene region into a plasmid. This gene region is part of a larger sequence that has been amplified by PCR. The image below shows part of this sequence, with the gene region of interest shown in green.


Which of the restriction enzymes shown in Table 1 should the scientist use to cut out the gene region of interest?

- [ ] BamH I
- [ ] EcoR I
- [ ] Hae III
- [ ] Hha I
- [ ] Hind III

Adapted with permission from OCR A Level January 2003, Microbiology and Biotechnology, Question 4

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# Cloning

A Level

## Part A Natural clones

Cloning is the process of creating a genetically identical organism (i.e. a clone). This happens naturally in organisms that undergo [ ] reproduction.

- In bacteria, clones are single cells that are produced by [ ].
- In some plants (e.g. runners and tubers), clones can be produced by totipotent [ ] tissue that is able to differentiate into all other tissue types of the plant.
- In some animals (e.g. sponges) clones can be produced by budding. In mammals, the only way that natural clones are produced is if an early embryo splits in two, forming [ ] (identical) twins.

Items:

**sexual** **vascular** **meristematic** **binary fission** **meiosis** **monozygotic** **asexual** **mitosis** **dizygotic**

## Part B Artificial clones

Some plants (e.g. sugar cane) can be cloned by taking [ ] (sections of roots or stems) and planting them directly into soil. Other plants cannot be cloned in this way, and require micropropagation.

Micropropagation involves taking a small number of cells of [ ] tissue and growing them in a sterile culture medium. The cells divide, forming a [ ] (an undifferentiated mass of cells). This is then split up into separate clumps of cells, and each clump of cells is placed in a new culture medium containing hormones that will stimulate [ ] into the other tissue types. The new plantlets produced by this process can then be planted.

Animal clones can be produced by replacing the nucleus of an oocyte (egg cell) with the nucleus from a somatic cell of another individual. This process is called somatic cell nuclear transfer (SCNT). The cell then forms an embryo, which develops into an adult organism.

Items:

**cuttings** **seeds** **protoplast** **mutation** **vascular** **callus** **differentiation** **meristematic**

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