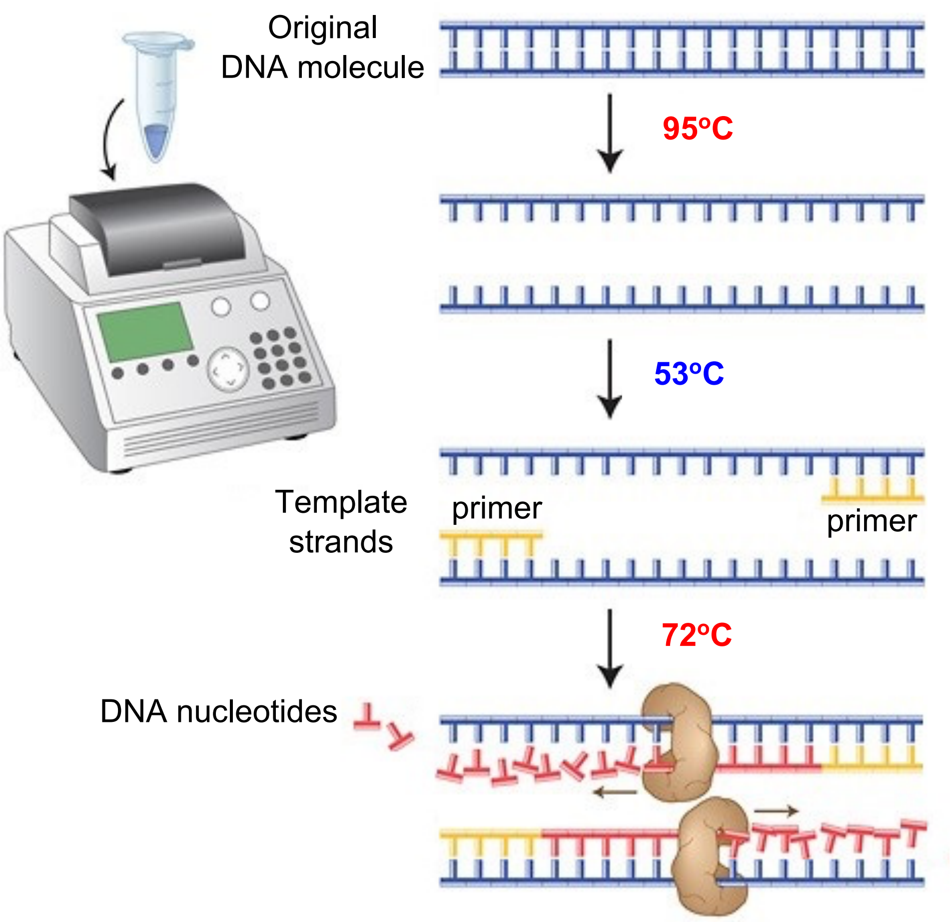
A. POLYMERASE CHAIN REACTION (PCR)

- Used to **amplify** the **amount** of **DNA**.
- A machine **replicates DNA** over **many cycles**.
- Used to amplify DNA for **forensic science, paternity cases** and **diagnosing disease**.

Ingredients needed

- **DNA molecule**
- **DNA nucleotides**
- **Primers**
- **Taq DNA polymerase**



Denaturation = strand separation

Temperature is increased to **95°C** to separate the DNA strands

Binding of primers

Temperature is reduced to **53°C** to allow **primers** to bind to the ends of the template strands by complementary base pairing.

Primers allow **Taq DNA polymerase** to start replication.

Extension

Temperature is increased to **73°C** as this is the optimum temperature for **Taq DNA polymerase**.

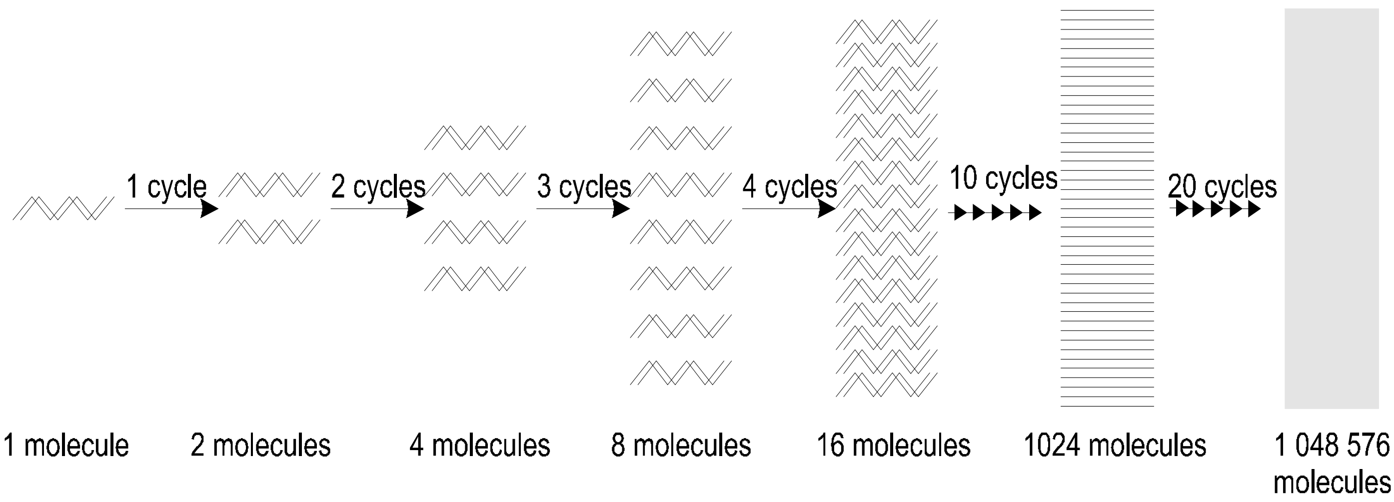
Taq DNA polymerase adds new complementary nucleotides to the new DNA strand.

Two identical DNA molecules are produced.

The process is then repeated.

- **Taq DNA polymerase** is from a bacterium (*Thermophilus aquaticus*) that lives in volcanoes. This enzyme does not denature at high temperatures.

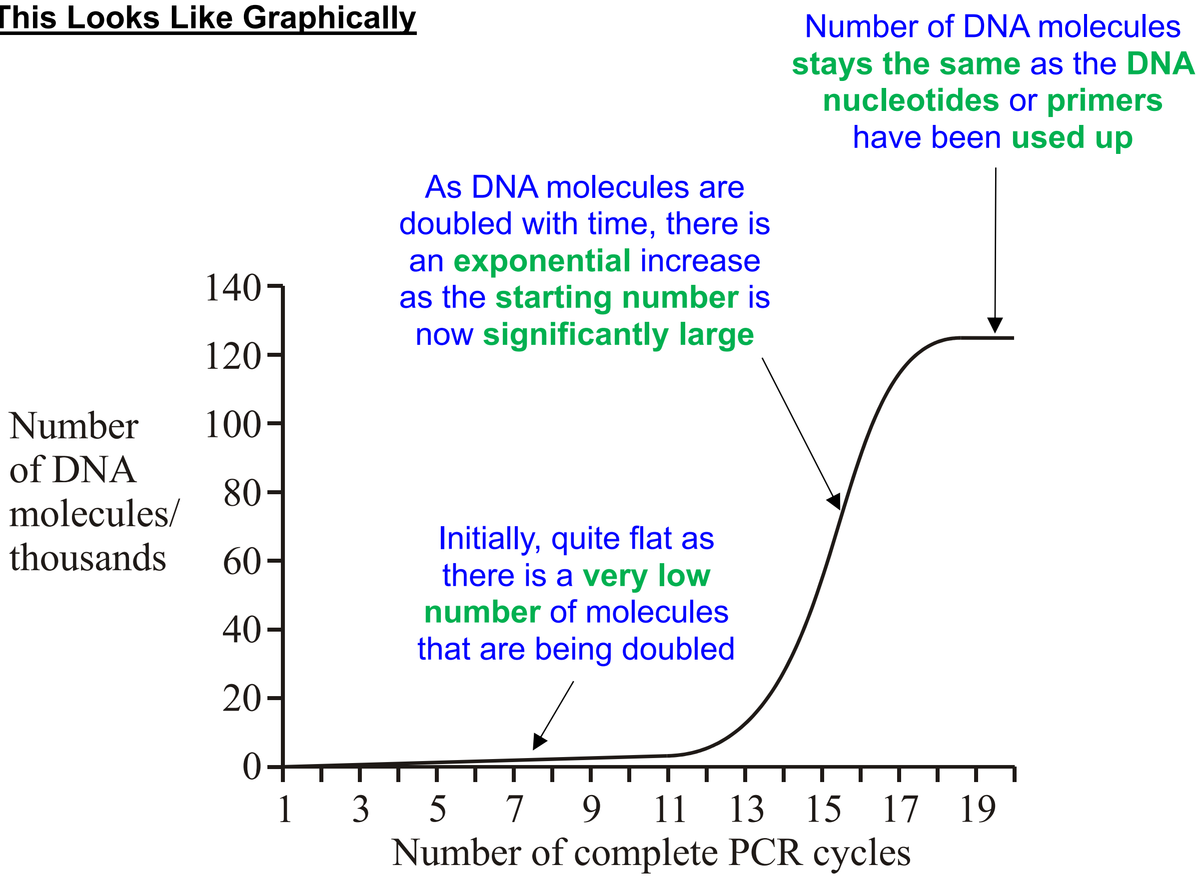
What Each Cycle Produces



Number of DNA molecules produced = 2^n

Where **n** is the number of cycles

What This Looks Like Graphically



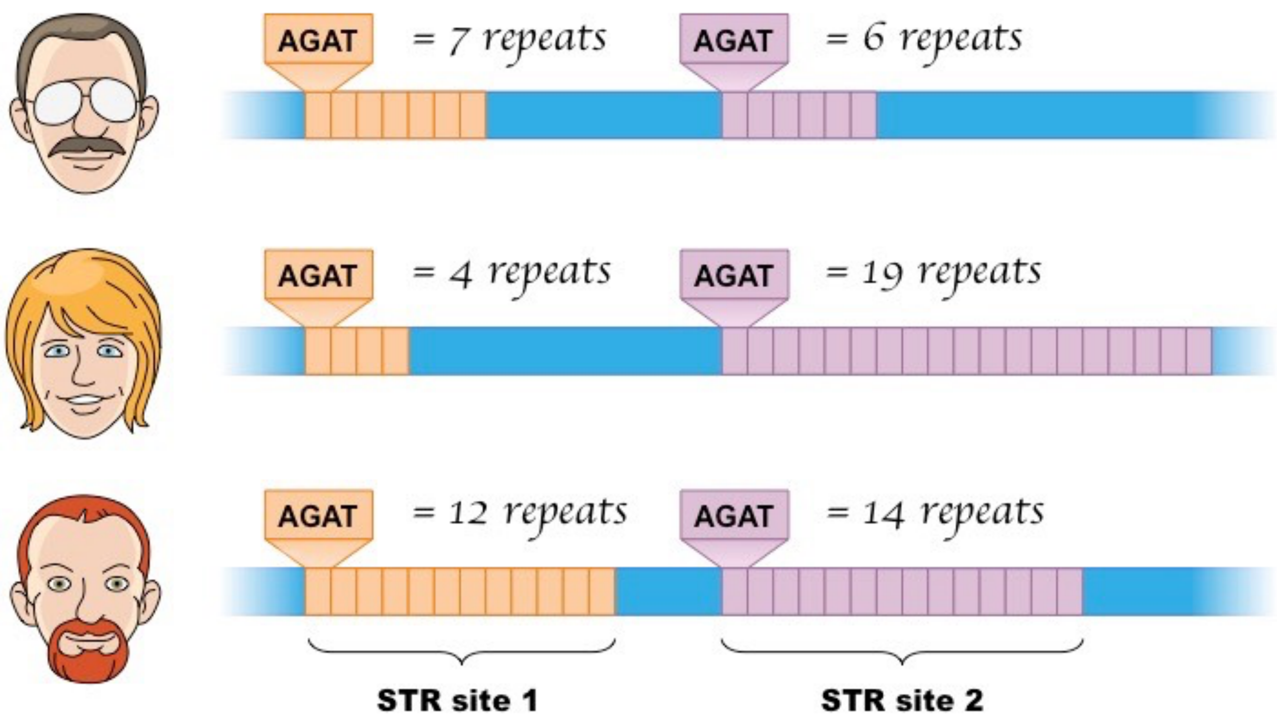
B. DNA FINGERPRINTING

Principle Behind It

- Some of our DNA base sequences **do not code** for **proteins** (they are called 'introns').
- Some are just **short base sequences** that are **repeated many times**.
- These are called **short tandem repeats** (**STRs**).
- The **same STRs** are found on the **same chromosome position** in **different people**, but:

Different people have **STRs** of **different lengths** – some have **more repeats** than **others**

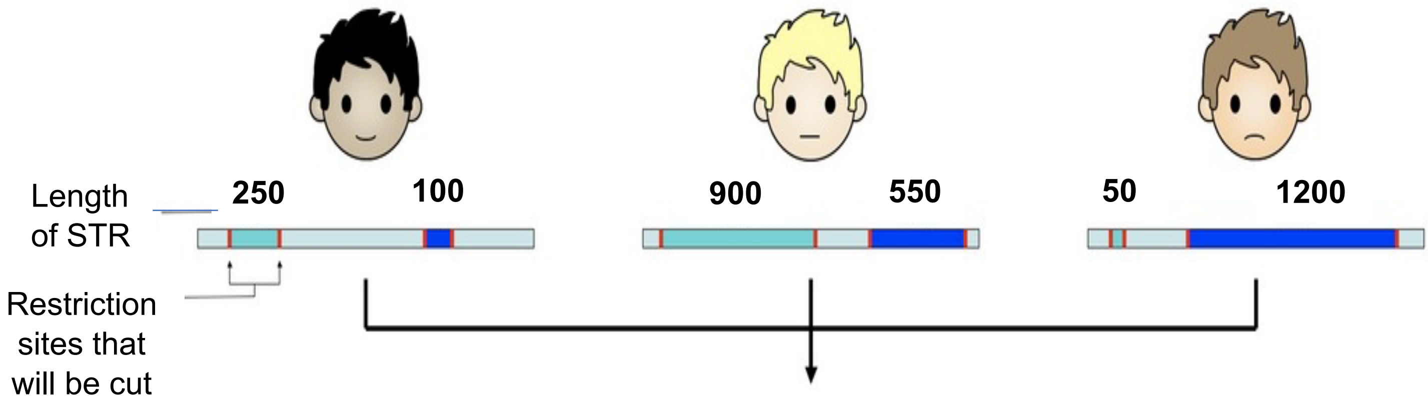
DNA fingerprinting exploits the fact that STRs are different base lengths in different people



How It Is Done

1. DNA Extraction & Digestion

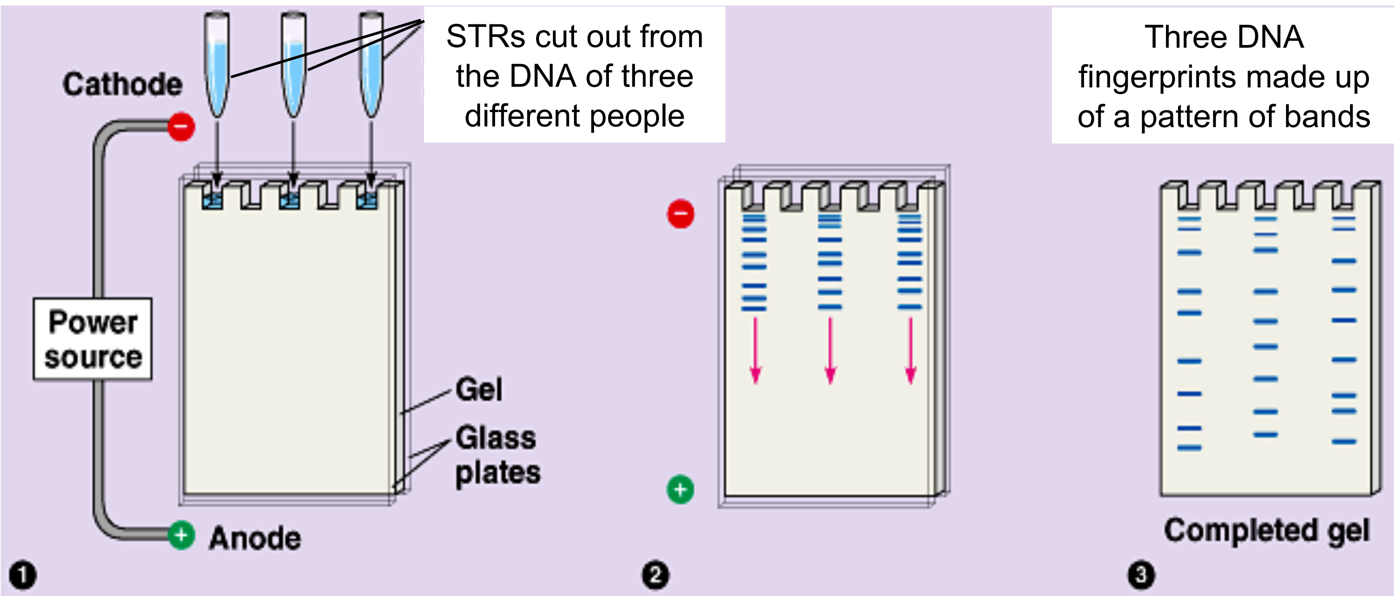
- **Extract DNA** from cheek cells/hair/blood.
- **Amplify** the **DNA** (make more copies) using **PCR** (polymerase chain reaction).
- **Restriction enzymes** are used to **cut out** the **STR base sequences** from the DNA.
- **Restriction enzymes** are **specific** – different ones **cut out different base sequences**.



- This will produce **different lengths** of **DNA fragments** (STRs) for **different people**.
- (As) different people have a **different number** of **repeating units** for **the same STR**.

2. Separation Of DNA Fragments By Gel Electrophoresis

- **Gel electrophoresis** is used to **separate** the **STRs/DNA fragments**.



Mixture put into separate wells on gel and an **electric current** is switched on

DNA fragments are **negatively charged**, so they move **down** to the positive electrode.

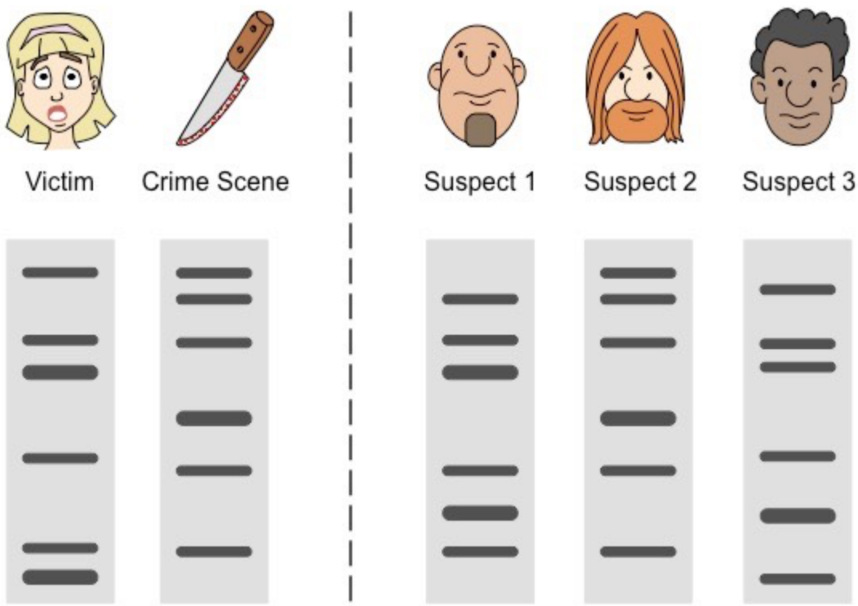
Shorter DNA fragments move faster and **further**.

3. Addition Of Radioactive Probes

- The **bands** on the **gel** (which show the different STRs) are **not yet visible** to the **eye**.
- Special man-made lengths of DNA called **probes** are added to the gel.
- **Probes** have **complementary base sequences** to the **STRs** and are **radioactively-labelled**.
- All probes are given time to **attach** to their specific **STRs**
- Any **unbound probes** are then **washed away**.

4. Autoradiography

- An **X-ray** film is taken and the **radioactive probes** show up as **dark bands**.
- This **indirectly** shows us where the corresponding **STRs** are located.
- The **pattern** of **bands** (DNA profile) is **unique** to an individual.

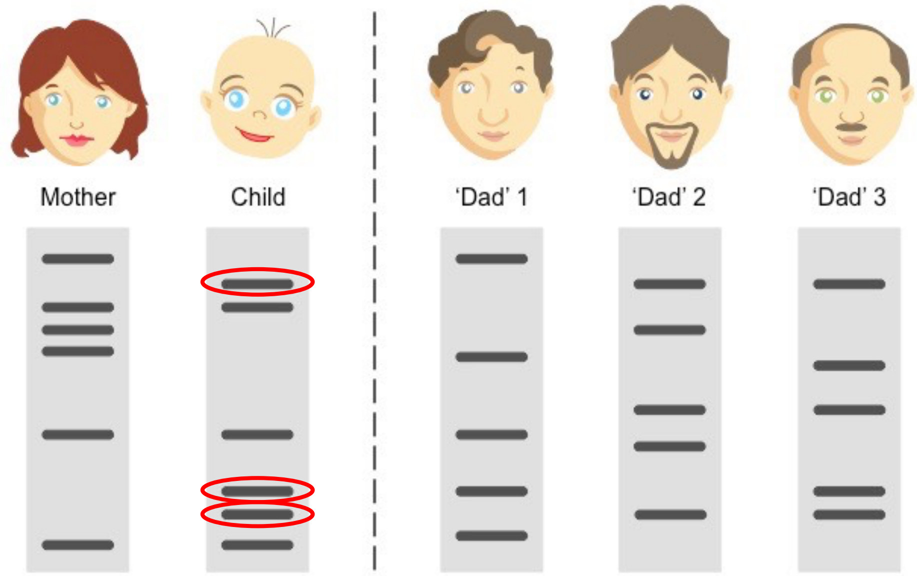


- In this example, we can deduce that **Suspect 2 was present at the crime scene**.
- However, **more evidence** is needed to **prove** that he **committed the crime**.
- The **pattern** of **bands** can also be **compared** for **paternity cases.**

How DNA fingerprinting is used in paternity cases

A child can only inherit its STRs from its natural mother and father

Look at the **bands** that the child did **not** receive from its **mother**
Only the **real father** could have passed **these bands** on
Half of the **child's bands** will match the **real father**.

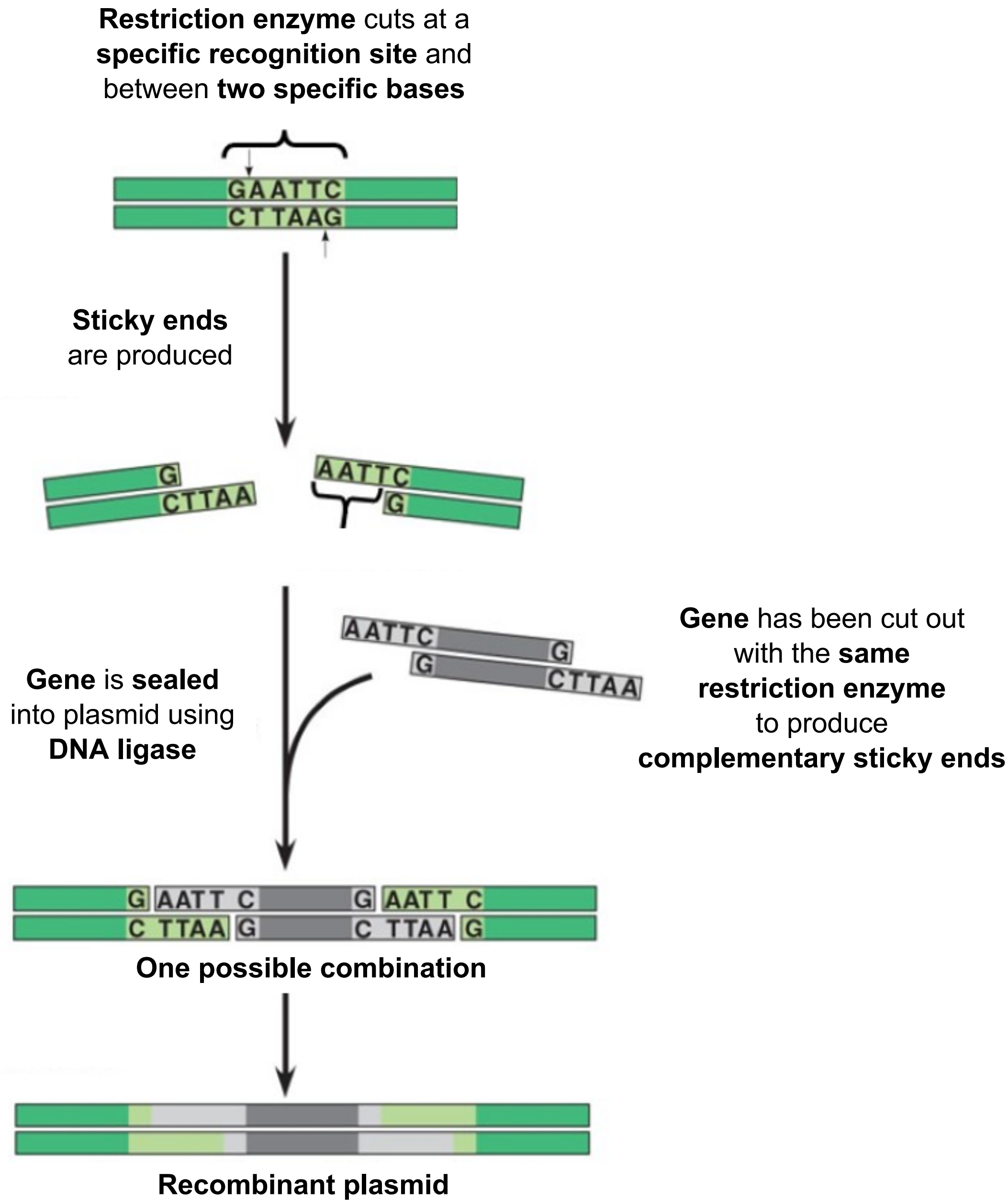


- Dad **3** is the biological father of this child as he has **all STRs** that **the child did not receive from its mother**.
- Scientists look at **many STRs** (usually 13) when doing this - not just five or six!

C. RESTRICTION ENZYMES

- Each **restriction enzyme** cuts a **specific DNA base sequence**.

- They **break phosphodiester bonds** between the **deoxyribose** sugar and **phosphate** (of the sugar-phosphate backbone).



- Bacteria can be infected with **virus**.

- **Restriction enzymes** occur **naturally** in **bacteria** and they are designed to **cut viral DNA** to **prevent infection**.

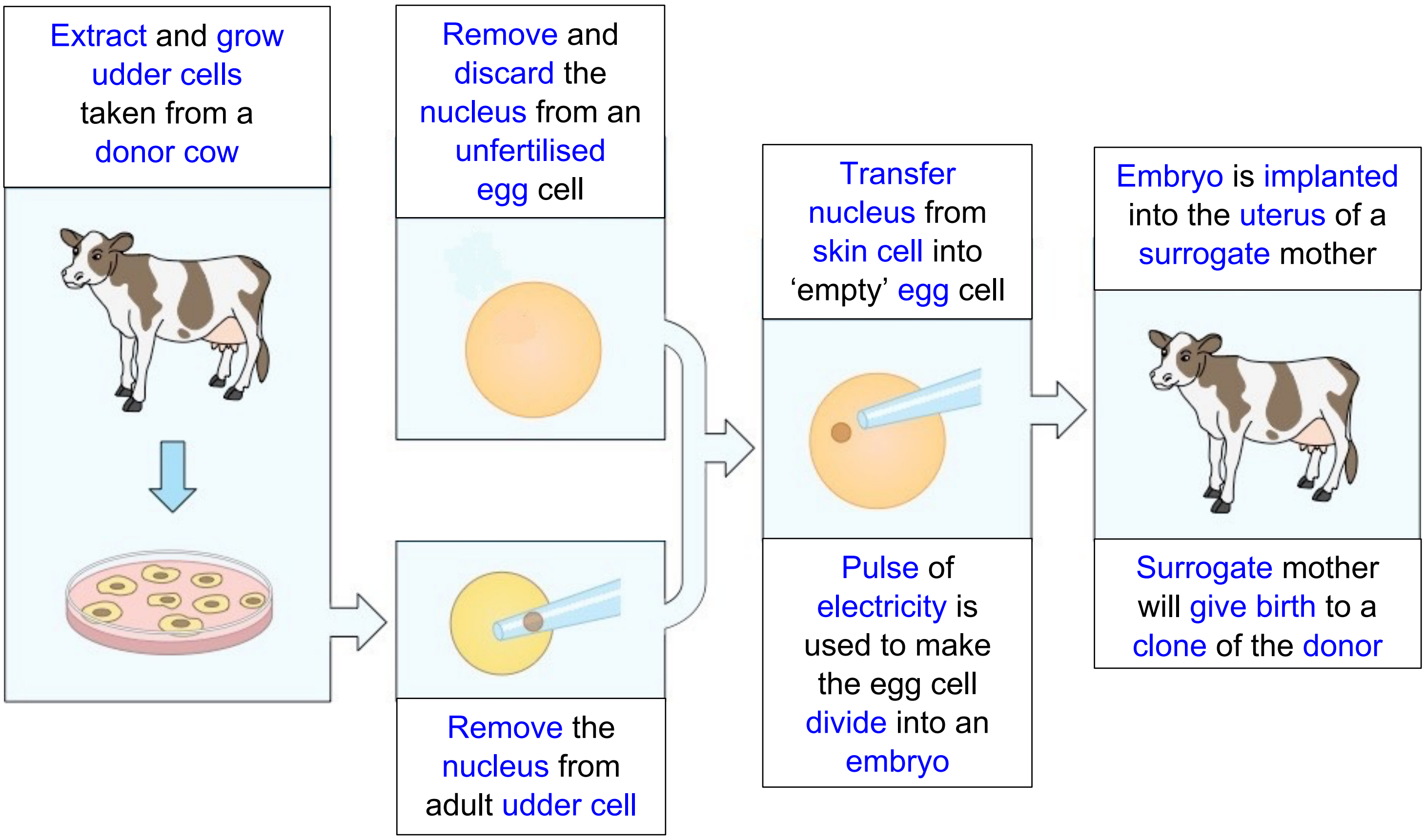
- We have **exploited** their natural use in **genetic engineering**.

Outline the application of DNA fingerprinting to determine paternity.

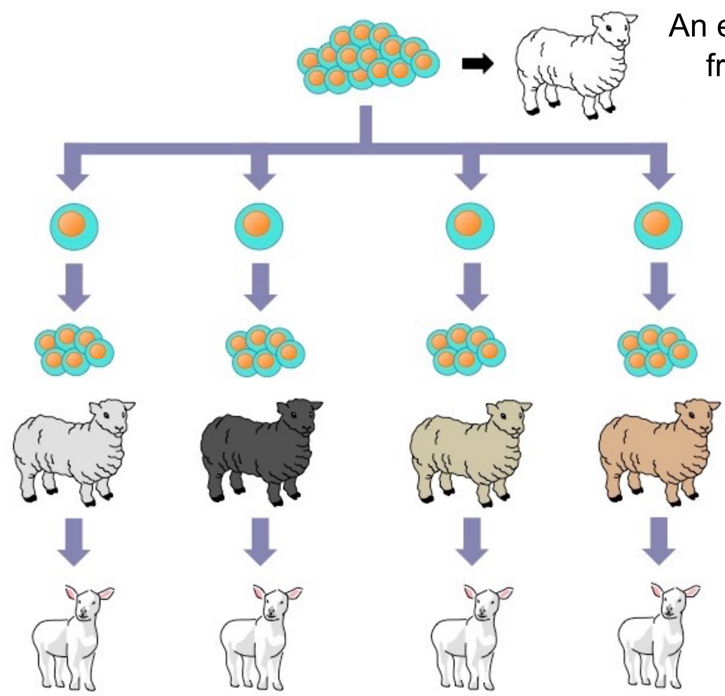
- DNA from child, mother and possible father(s) used
- DNA copied/amplified using PCR
- DNA cut using restriction enzymes
- To cut out short tandem repeats/STRs
- (Gel) electrophoresis used to separate DNA fragments/STRs
- Pattern of bands is produced (in gel)
- (Bands) analysed for matches between child with mother and possible father
- (About) half the child's bands will match the father
- (Biological) father will have all bands the child did not receive from its mother

D. CLONING ANIMALS USING DIFFERENTIATED BODY CELLS

Clones are groups of **genetically identical organisms**, derived from a **single original parent cell**



- Be aware that an **older**, and **less reliable** technique, called ‘**embryo splitting**’, was **first** used to produce clones.
- **All** cells in an **embryo** are **genetically identical**.



An early embryo is taken from a donor sheep

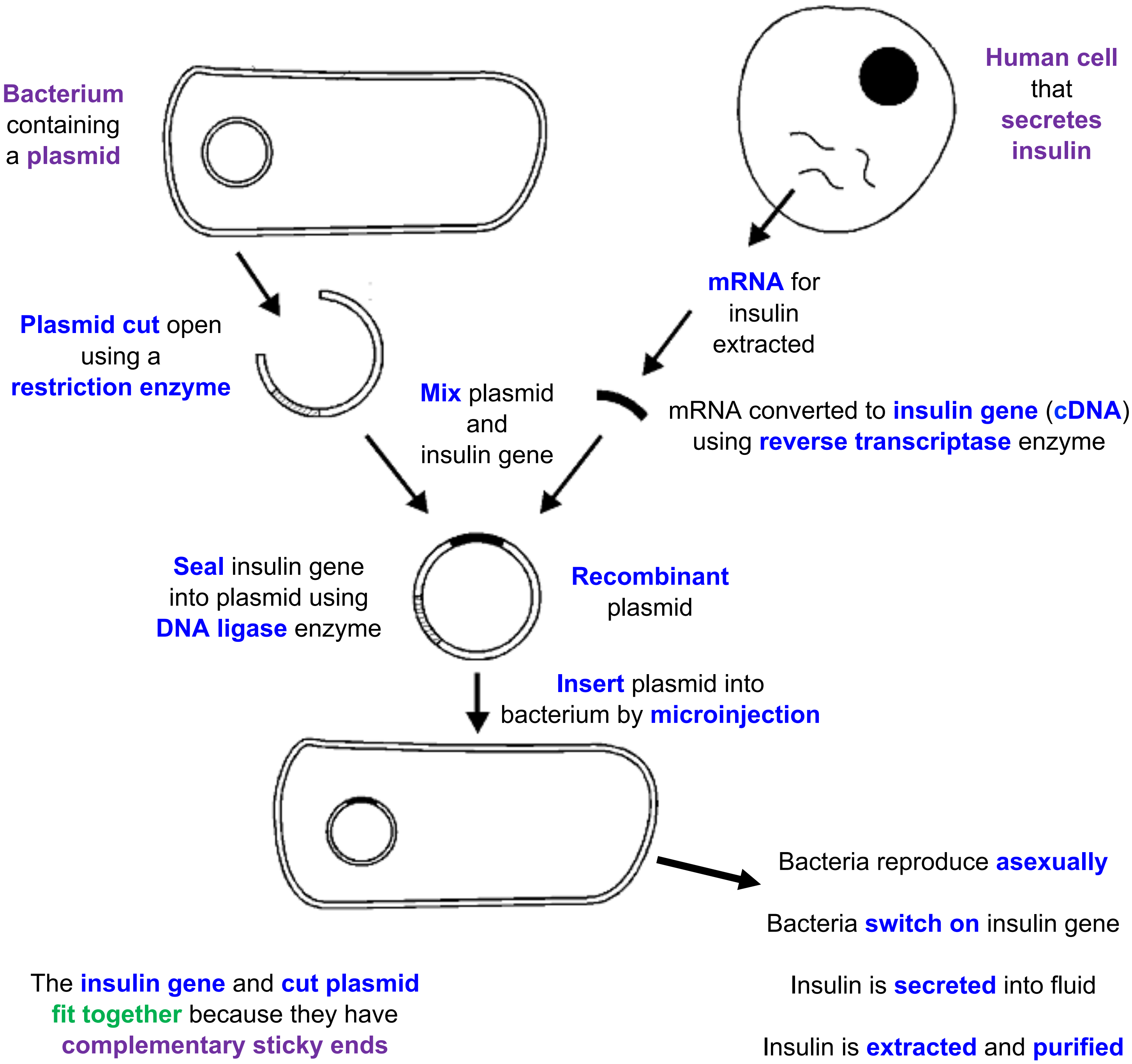
The embryo is split into separate cells that are genetically identical to each other.

The separate cells divide to form embryos. Each embryo is implanted into the uterus of a different surrogate mother.

All sheep born will be clones of each other but will not be clones of the donor sheep. She became pregnant naturally by sexual reproduction.

E. USING GENETIC ENGINEERING TO PRODUCE HUMAN INSULIN

- Human **insulin** is used to treat **diabetes**.
- Many years ago, it was extracted from the **dead** or **other animals**.
- This gave a **low yield** of insulin, which could contain **contaminants**.
- Nowadays, the **human insulin gene** is **transferred** into **bacteria**, so they produce human **insulin**.
- This works because the **genetic code** is **universal** – the **same codons** code for the **same amino acids** in **all organisms**.



- **Sticky ends** are made by adding extra **G nucleotides** to the **ends** of the **gene** and **extra C nucleotides** to the **ends** of the **cut plasmid**.

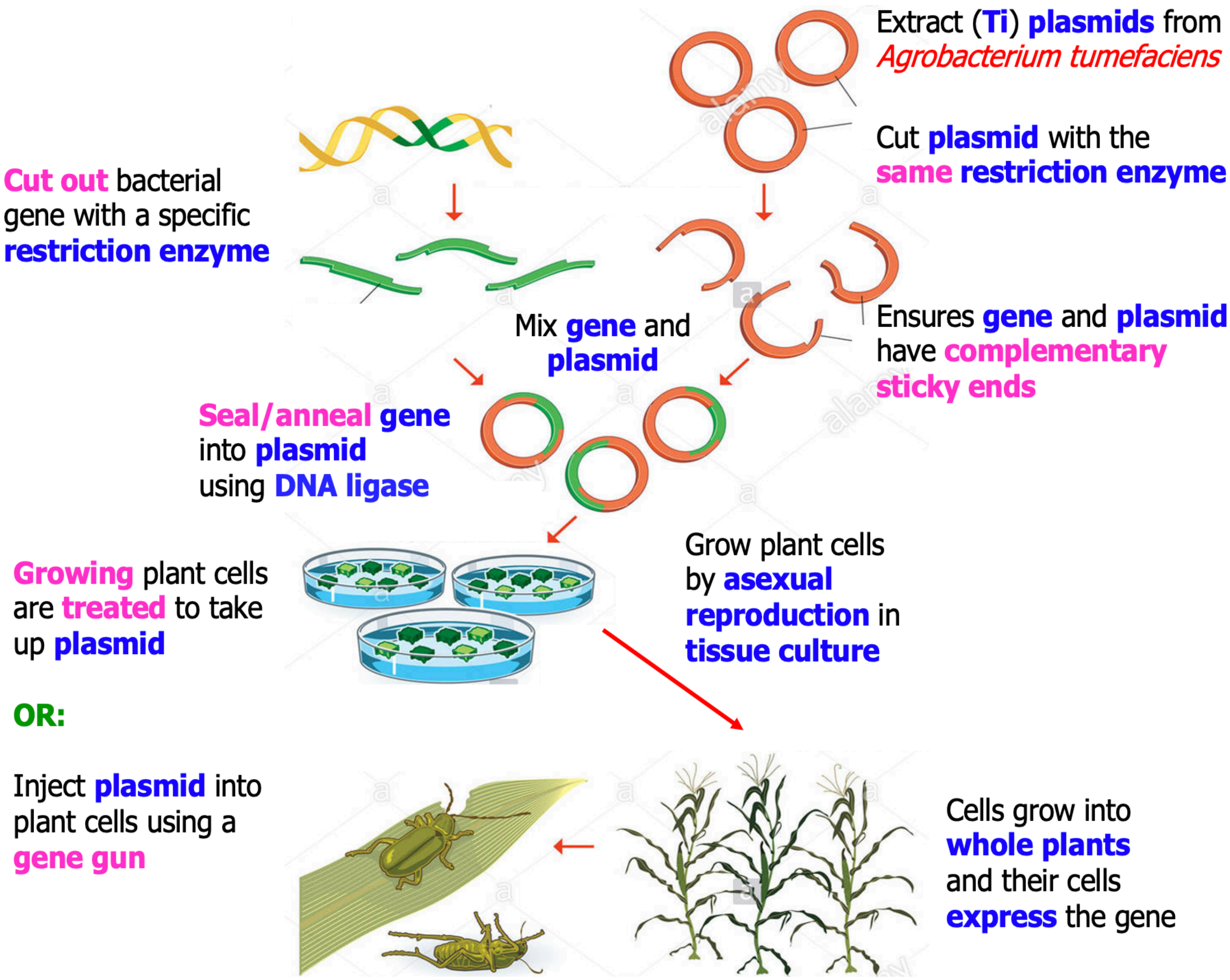
Why extract mRNA for insulin, rather than its gene (DNA)?

- There are **more copies** of **mRNA** than the gene (DNA) in the cell
- **Difficult to find one gene** among all the genes in the nucleus
- mRNA **already has the introns removed** so the **cDNA will not contain introns**

Why use bacteria?

- They **reproduce rapidly** – can double in number every 20 minutes!
- They can **reproduce asexually** – so **all offspring** will **contain** the human **insulin gene**.
- They are **easy** to **genetically manipulate**.

F. USING GENETIC ENGINEERING TO PRODUCE GENETICALLY MODIFIED (GM) PLANTS



G. BENEFITS AND RISKS OF GENETICALLY MODIFIED CROPS

The bt gene from a bacterium (*Bacillus thuringiensis*) that codes for the Bt toxin (poison) has been transferred into crop plants, such as corn or maize (*Zea Mays*).

It is designed to kill insects that feed on the crop plant.

Specific Example From The IB Syllabus

Possible benefits of Bt Maize	Possible harmful effects of Bt Maize
Higher crop yields and more food for humans	Insects that are not pests could be killed (e.g. bees are pollinators) Maize pollen containing the Bt toxin can be blown onto other plants, which monarch caterpillars eat. These caterpillars can be killed even though they do not feed on maize.
Less use of insecticides so: ○ less expensive ○ less disruption of food webs ○ less harm to wildlife	The gene may be transferred to unwanted plants such as weeds by cross-pollination
Less land needed for crop production so: some areas could be used for wildlife conservation	Insects many develop resistance to the toxin over time

Other General Points

Benefits	Possible Concerns
Increased resistance to herbicides/insects/disease/drought	Harmful effects if eaten by humans
Increased nutritional content e.g. golden rice: - has a high content of beta-carotene - which our body converts to vitamin A - prevents malnutrition in poorer countries (and night-blindness)	Long term effects are not yet known
Increased shelf life / less food spoilage	Reduced genetic variation so many may be killed by the same disease

H. THE HUMAN GENOME PROJECT

Scientists have found the **DNA base sequence** of **all human chromosomes**

This allows **identification** of **all human genes**

In the future, it may be possible to:

- find out the **structure** and **function** of **proteins** (that the genes code for)
- find **evidence** for **evolutionary relationships** with **other species**
- find **mutations** that cause **genetic diseases**
- find **mutations** that **increase the risk** of **getting a disease** (e.g. cancer or heart disease)
- develop **tests** that can **screen** for more **genetic diseases**
- develop **new drugs** that are **tailored** to the **specific genes** carried by a **person**