

Genetic Engineering



Organ Grown in Dish

- Organ Grown on Mouse
- Ear Grown on Arm

Genetic Engineering

- Building new DNA by **manipulating** the DNA of organisms
- Biotechnologists **splice** new pieces of DNA (genes) from one chromosome to another
- Refinement of traditional breeding practices
- Also called **recombinant DNA technology (rDNA)**

Close to Home By John McPherson



Recombinant DNA Technology

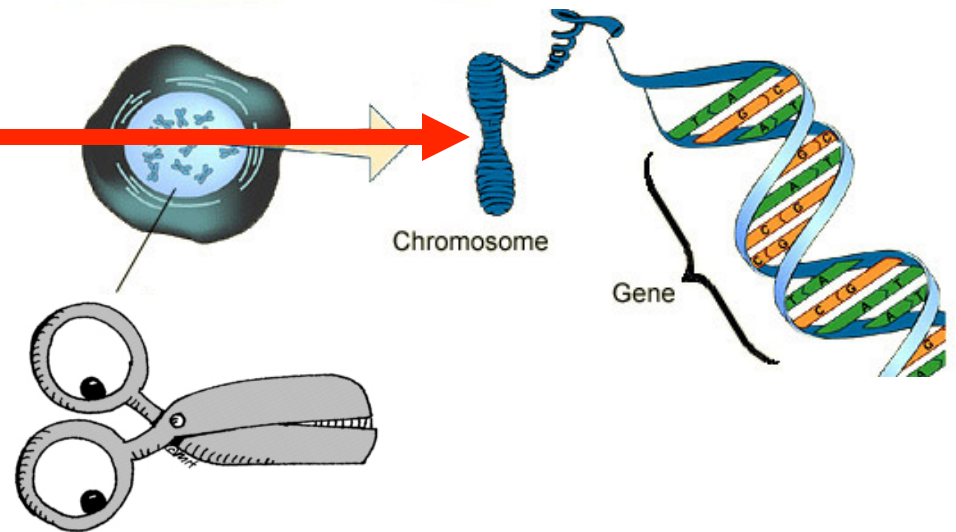
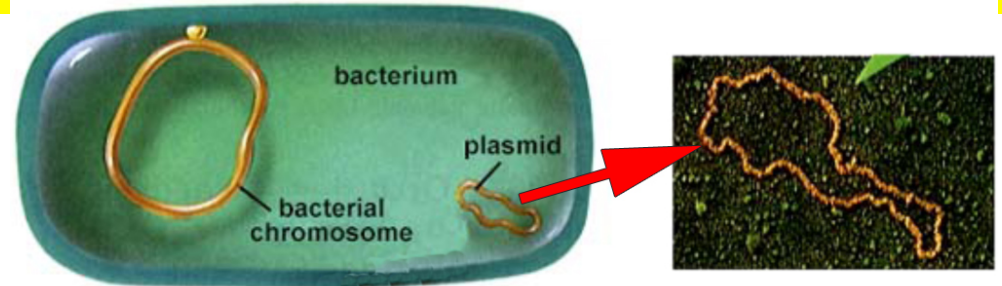
Requires 4 things

1. Plasmid from E. coli bacteria

2. Required Gene

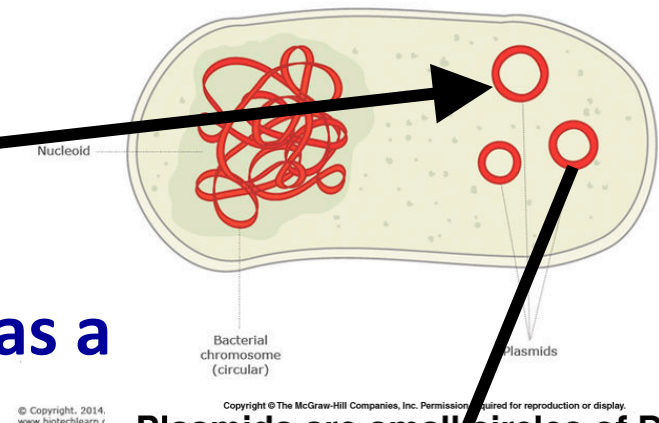
3. Restriction Enzymes
(for cutting)

4. Ligase (glue) (for
attaching required
gene into host DNA)

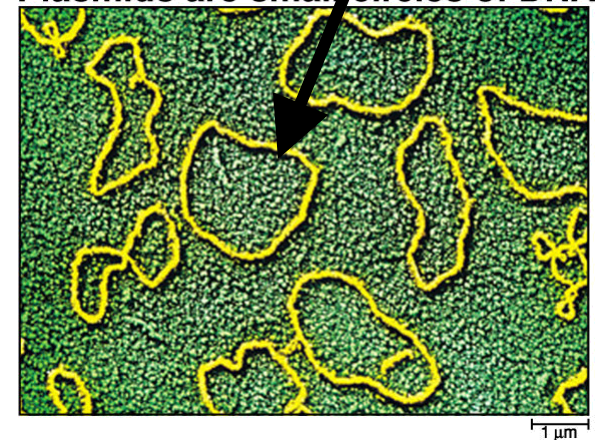


Why is E. coli bacteria used?

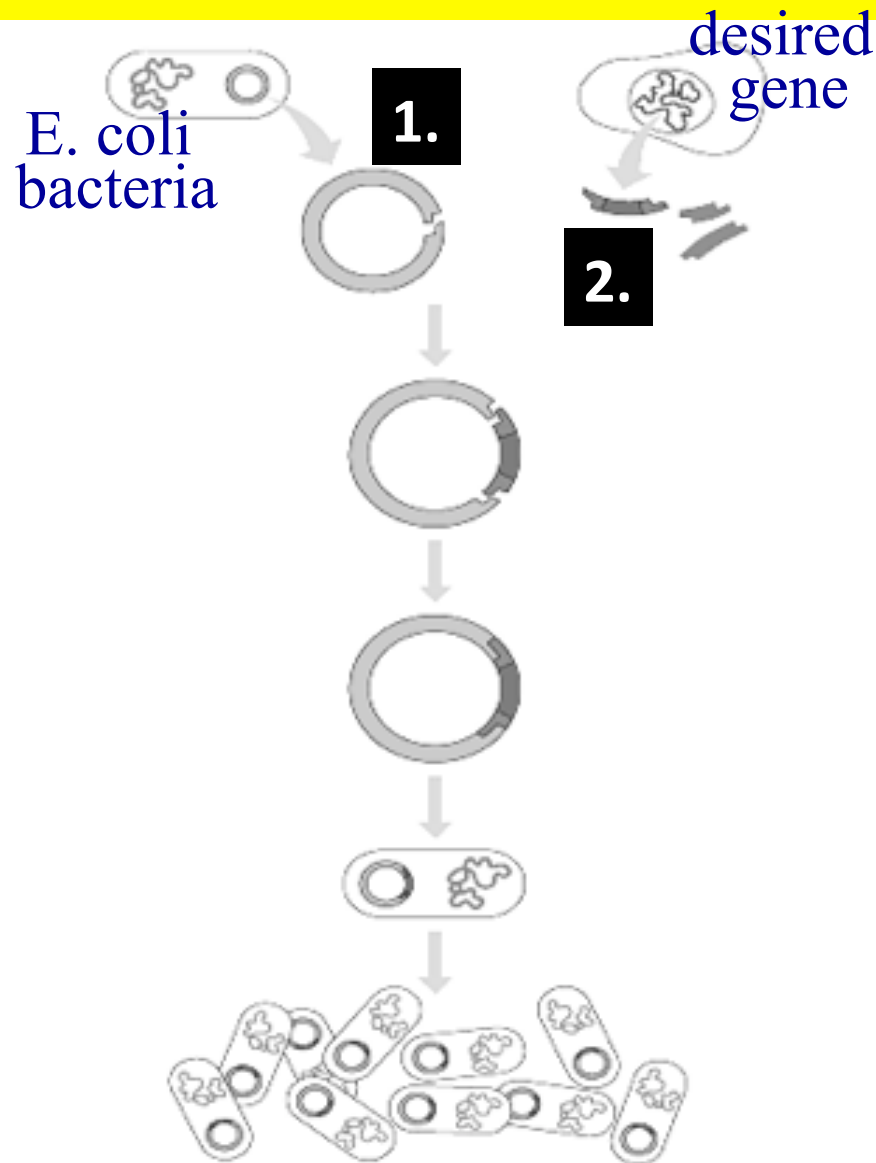
- E.coli is plentiful, easy to manipulate and cheap
- Have a short life span
- Contains circular piece of DNA called a **plasmid**
- **Plasmid = small ring of DNA** used as a “host” to clone a gene
 - In bacteria, plasmid can be replicated separately from bacterial DNA
 - Replication of the plasmid doesn't affect the bacterium's genetic make-up



Plasmids are small circles of DNA



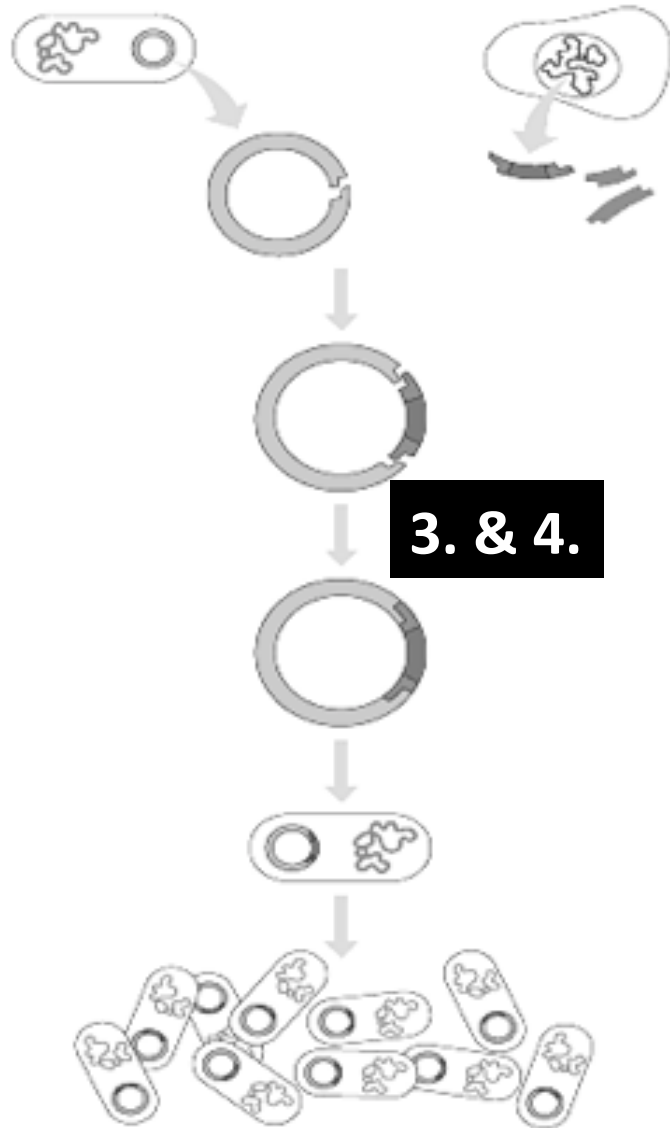
Recombinant DNA Process



1. A bacterial plasmid is opened using a restriction enzyme (scissors).

2. Isolation of the desired gene using the same Restriction Enzyme (scissors).

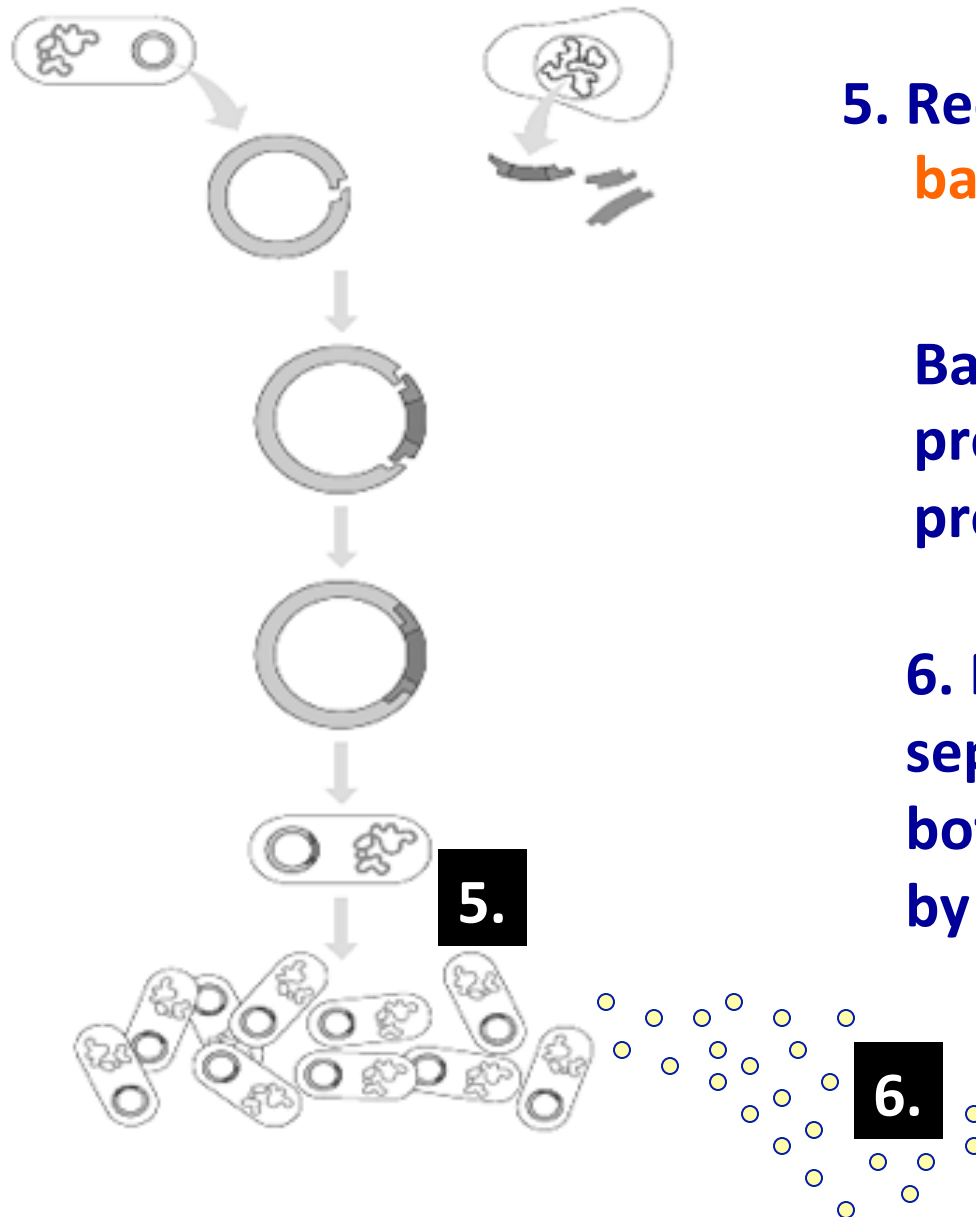
Recombinant DNA Process



3. Both DNA samples are combined in a petri dish or a test tube.

4. **Ligase** is added to glue DNA together.

Recombinant DNA Process

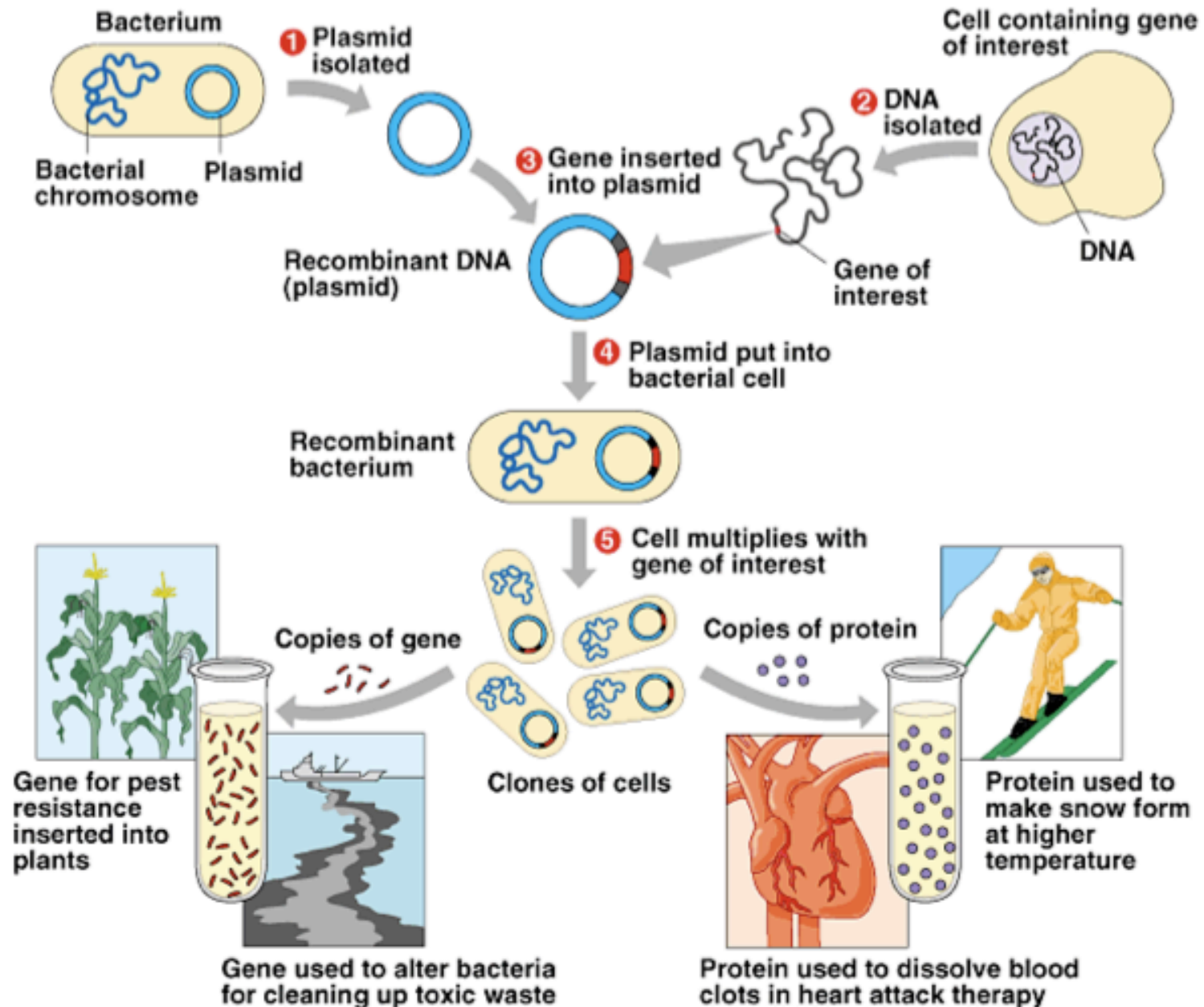


5. Recombined **plasmid** inserted **back into** E. coli bacterial cell.

Bacteria divides, mass producing the desired product, for example, insulin

6. Product (eg. insulin) separated, sterilized, bottled and eventually sold by pharmaceutical industry

Recombinant DNA Process



Review Questions

1. What is a gene?

A small section of DNA that codes for a specific trait.

2. What are restriction enzymes?

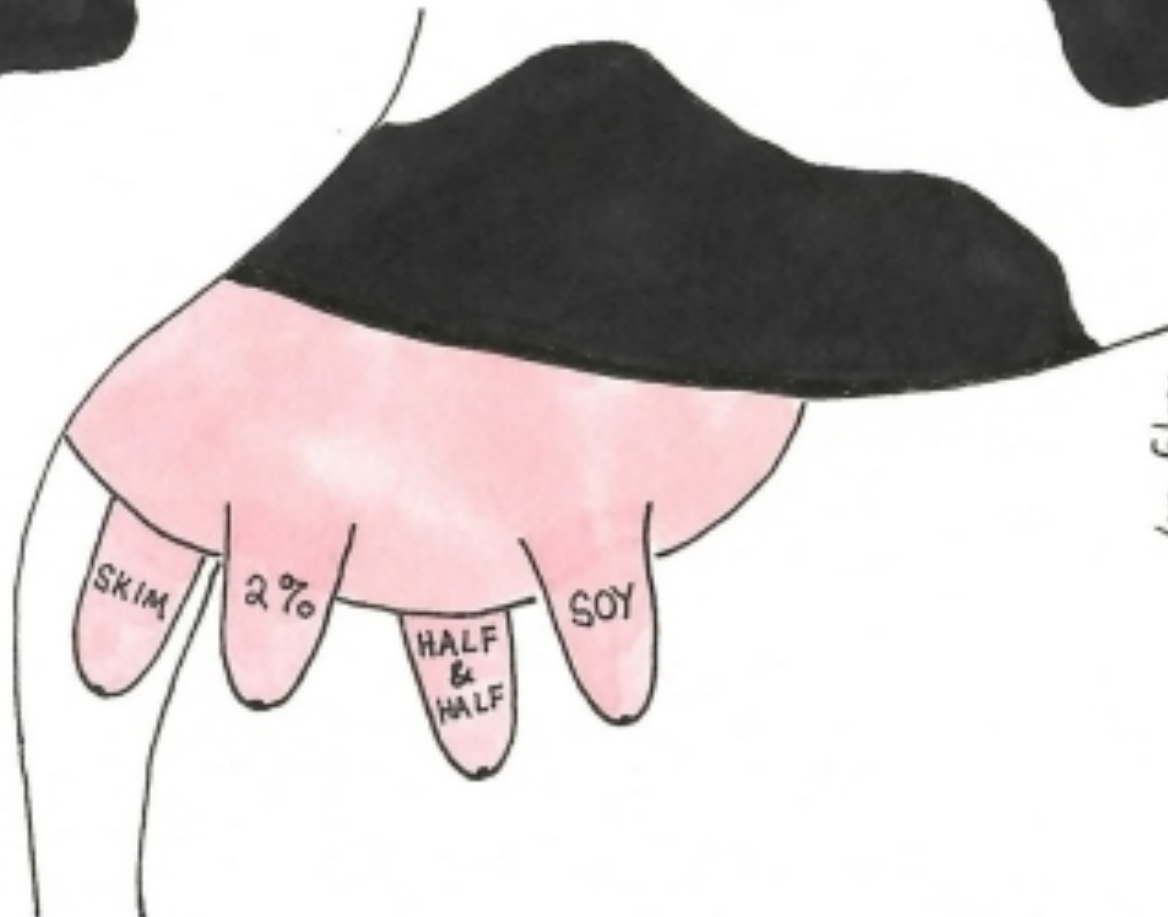
Enzymes that cut.

3. What is ligase used for?

To glue genes together.

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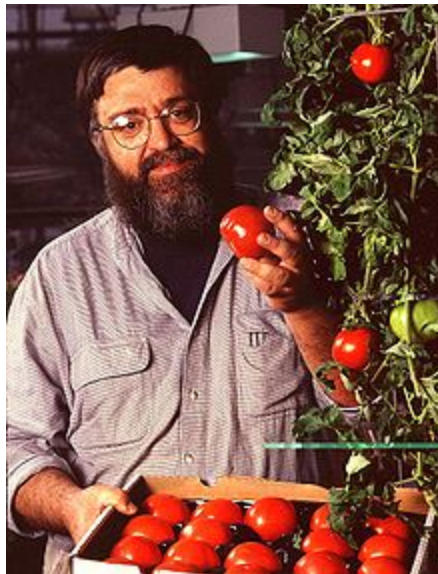
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heavy work

The miracle of genetic engineering

Applications of rDNA Technology

1. Agriculture

- Genetically modified Organisms (GMO's) such as tomatoes
- Roundup Ready Canola
 - Resistant to the herbicide Roundup
 - Increases crop yield and reduces the use of herbicides



Applications of rDNA Technology

2. Pharmaceuticals

e.g., antibodies, protein

3. Human Hormones and products

- Insulin
- Interferon (prevents molding)
- Somatotropin (HGH)

4. Production of BST

- also known as bovine somatotropin
- increases milk production in cows

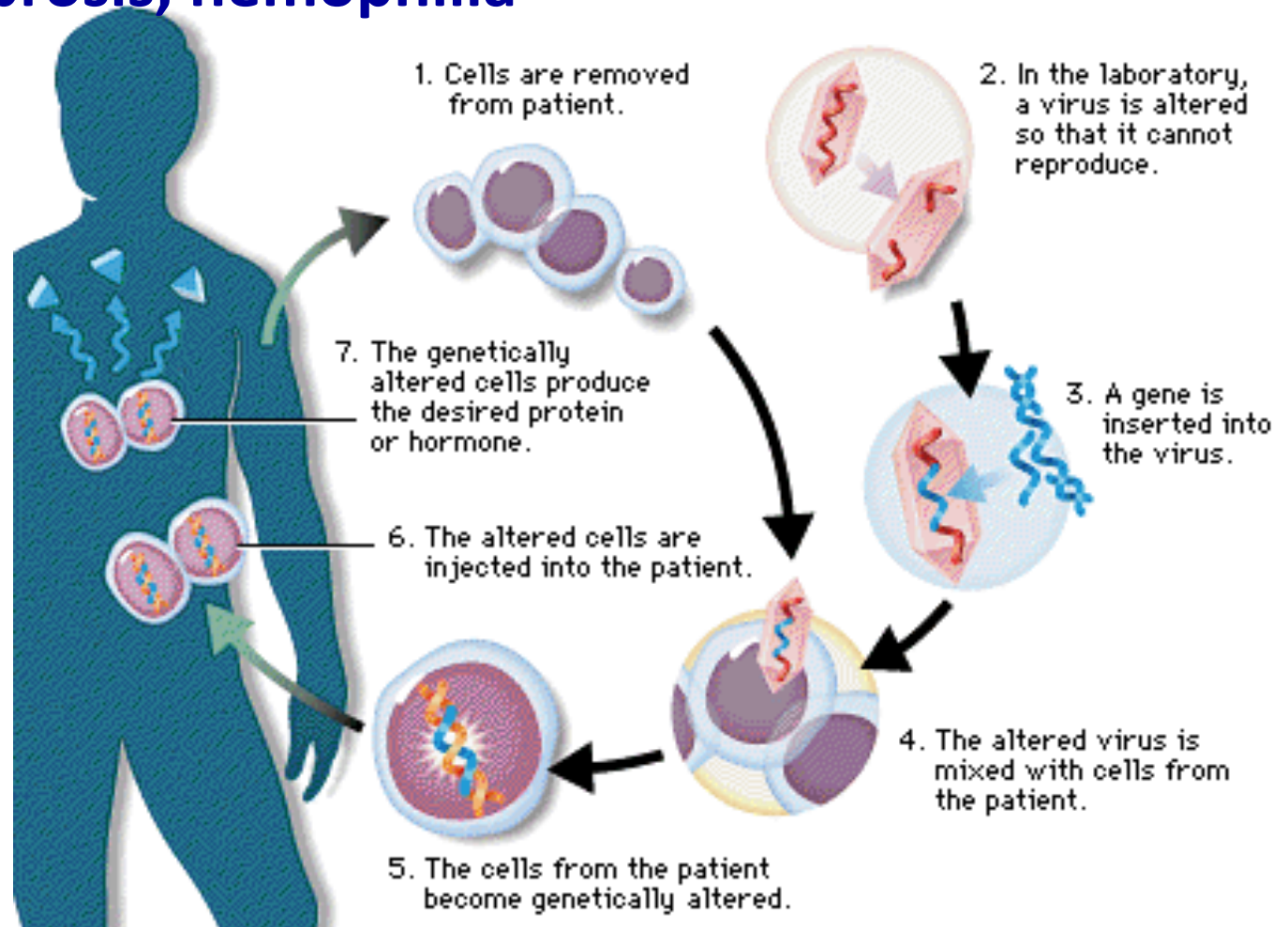


Applications of rDNA Technology

5. Gene Therapy

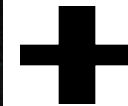
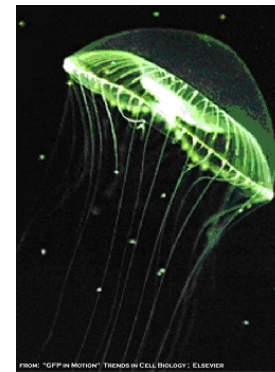
- replacing a defective gene with a non-defective gene
- e.g. Cystic fibrosis, hemophilia

Issues: How
do we get the
genes in the
Body and
control them?



Pro' s of Recombinant DNA

1. Production of human products without side effects ex. **insulin**
2. Production of **disease resistant** strains of plants for agriculture
3. **Combines** characteristics of 2 different organisms into one
4. Very specialized form of **selective breeding**
5. Cure human disorders (**gene therapy**)





Pigs - Taiwan (Jan 2006)

National Taiwan University cloned 3 pigs which are green inside out inclusive their internal organs.

**Animals that have the GFP
(green fluorescent protein)
gene inserted** Video 2:49

<https://www.youtube.com/watch?v=eCPtDVnaQ1w>

Top 7 Genetically Modified Animals



Normal rice vs. Golden rice. Golden rice contains 4 foreign genes:

1. a gene from beans to increase iron content;
2. a gene from a fungus which helps with iron absorption;
3. a gene from wild rice to help with iron uptake;
4. a gene from a daffodil to synthesize beta-carotene.

Cons of Recombinant DNA

1. Some believe that we are **playing “God”**
eg. **Eugenics** - we may use it to select genetic information in offspring
2. **Expensive** procedure
3. Not sure about **long term effects** on other organisms





Two featherless chickens peck around in some grass May 22, 2002 at the Hebrew University in Rehovot. Israeli scientists at the Agriculture department of the university have genetically engineered bare-skinned chickens as part of a research project to develop succulent, low fat poultry that is environmentally friendly. The naked chicken, as the bird has been dubbed, would also save poultry farmers large amounts of money on ventilation to prevent their chickens from overheating. REUTERS/Havakuk Levison

دجاجتان بلا ريش في الجامعة العبرية في ريهوفوت. العلماء الإسرائيليون في وزارة الزراعة عندهم دجاج ذو بشرة عاري مهندس وراثيا كجزء من مشروع بحث لتطوير الدواجن القليلة الدسم، يوفر كميات كبيرة من المال لمزارعي الدواجن

Genetic Engineering

Genetically modified monkey created

Jellyfish DNA used; researchers hope method will help in fight against diseases

The Associated Press
PORTLAND, ORE.

Pushing science to the brink of altering humans, researchers have created the world's first genetically modified primate, a baby rhesus monkey with jellyfish DNA that glows green in the dark.

The Oregon Health Science University researchers who created ANDi — for “inserted DNA,” spelled backward — said their goal is not to tinker with the human blueprint but to use monkeys in the laboratory to advance medical research and wipe out diseases.

The researchers hope to introduce other



Pushing science to the brink of altering humans, researchers have created the world's first genetically modified primate, a baby rhesus monkey with jellyfish DNA that glows green in the dark.

Technologies to Analyze DNA

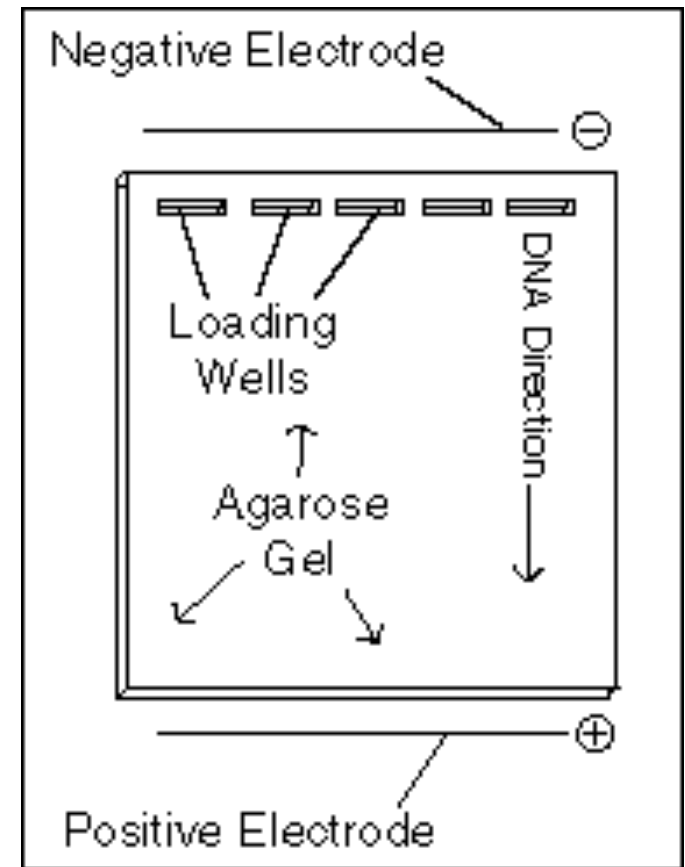
1. **PCR-** polymerase chain reaction

2. **Gel Electrophoresis:**

- Separates segments of DNA or specific genes

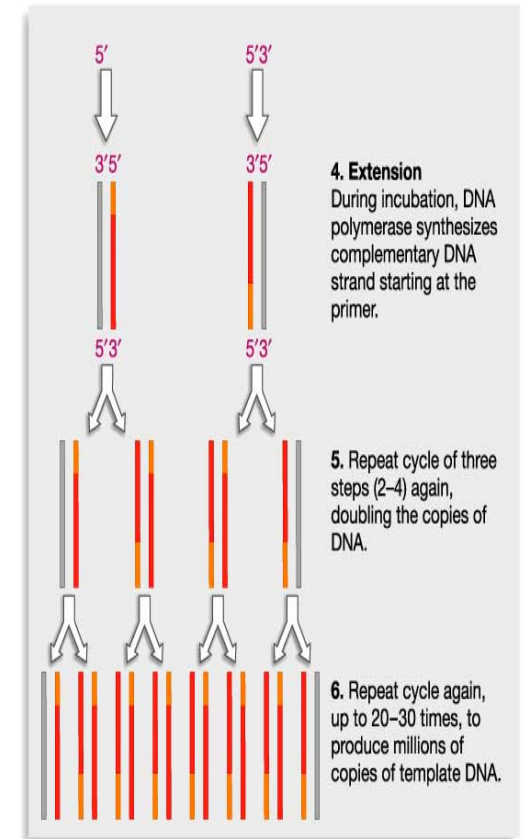
3. **DNA Fingerprinting:**

- A method of producing an image of cut segments of DNA
- DNA is cut into several pieces of different sizes using **restriction enzymes**



Polymerase Chain Reaction (PCR)

- Used to make **millions of copies** of a specific DNA segment in a **test tube**. **(Amplify DNA!)**
- It is an **automated** process.
- Multiple copies of DNA fragments are needed to complete the mapping process to **detect mutations or recombine**.
- From a **very tiny amount** of DNA, the polymerase chain reaction can be used to produce **more copies** of the DNA for analysis.
- PCR can be applied for **forensic** purposes as well. **(Solve a crime!)**



[PCR song](#)

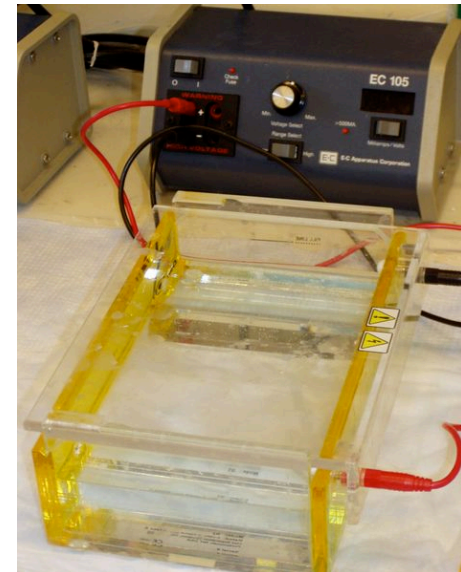
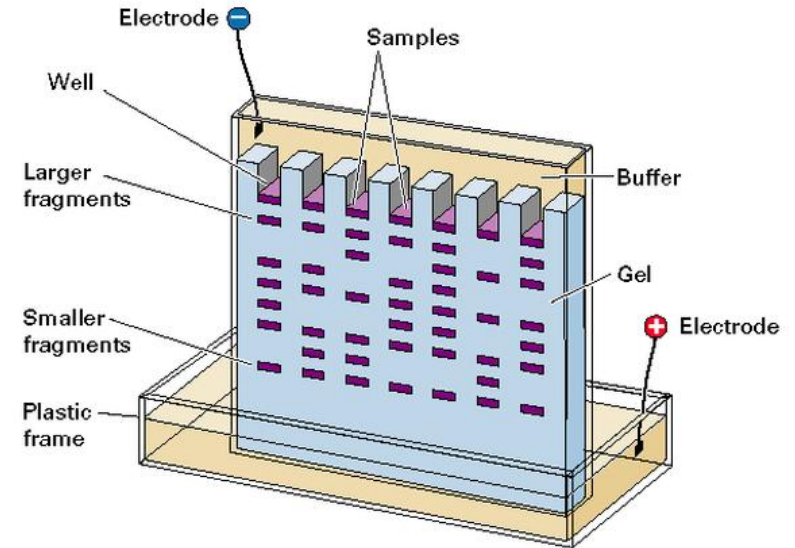
[Click here to make your own DNA](#)

Isolating DNA in the lab

- *Ethanol* added to solution of ruptured cells
- DNA *precipitates* out and spooled onto a glass rod
- Recombinant Enzymes used to cut DNA into fragments
- DNA loaded into wells on electrophoresis plate with dye
- Current applied and negatively charged DNA moves up the plate to the positive pole
- Heavier strands move **slowly** and the less dense strands end up moving **further away**

Gel Electrophoresis Animation

Gel electrophoresis video

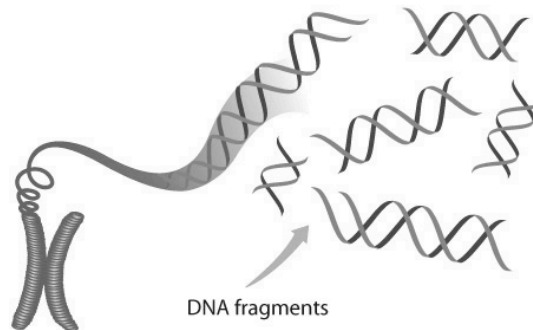


Isolating DNA in the lab

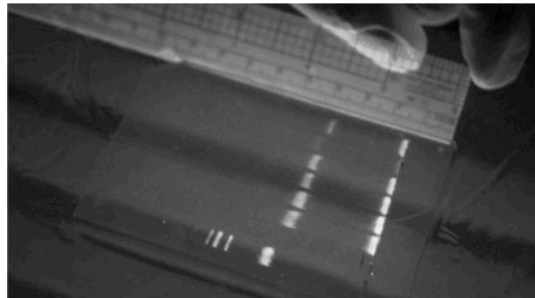
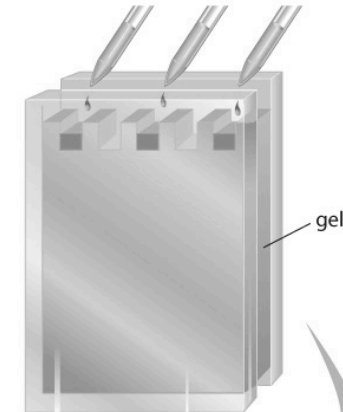
University of Utah Gel Electrophoresis Lab

[http://
learn.genetics.utah.edu/
content/labs/gel/](http://learn.genetics.utah.edu/content/labs/gel/)

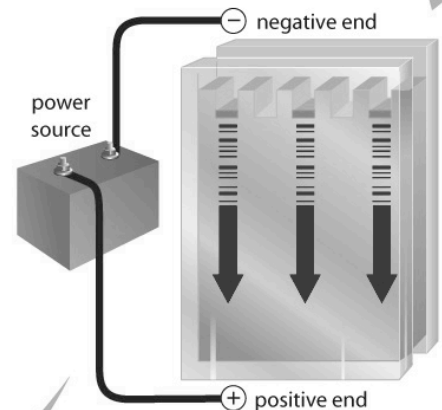
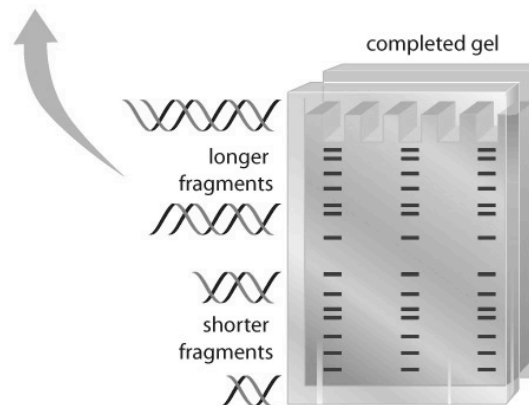
A Restriction enzymes Either one or several restriction enzymes are added to a sample of DNA. The enzymes cut the DNA into fragments.



B The gel A gel, with a consistency similar to gelatin, is formed so small wells are left at one end. Small amounts of the DNA sample are placed into these wells.

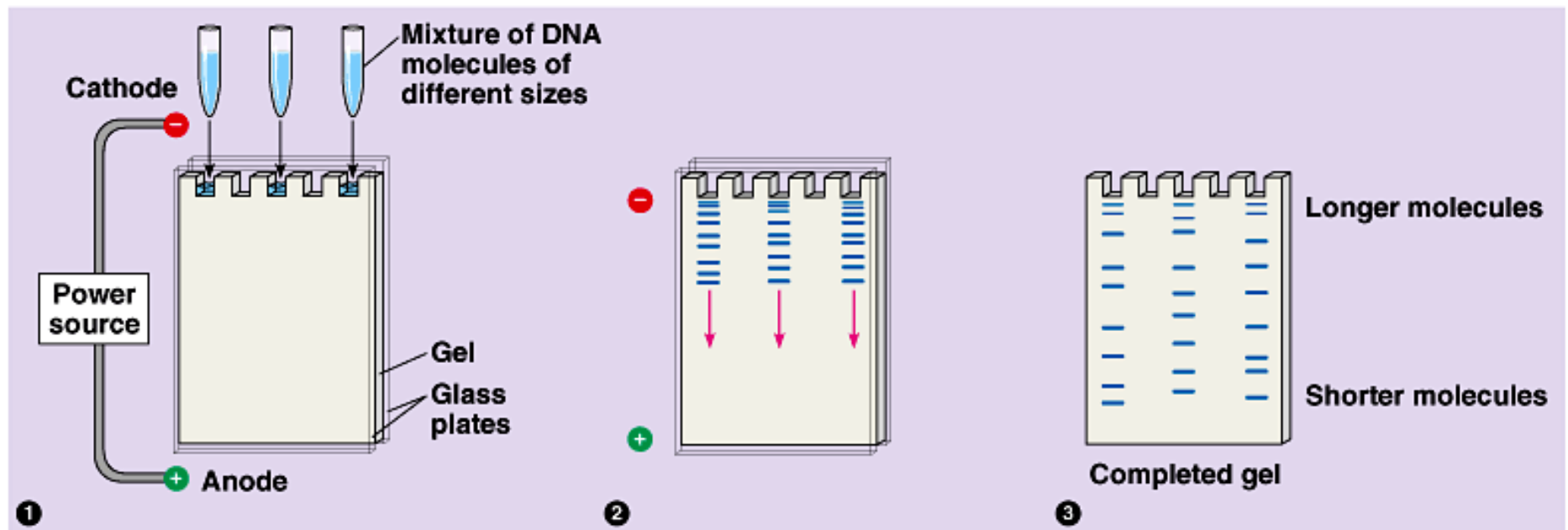


E Before the DNA fragments are added to the wells, they are treated with a dye that glows under ultraviolet light, allowing the bands to be studied.

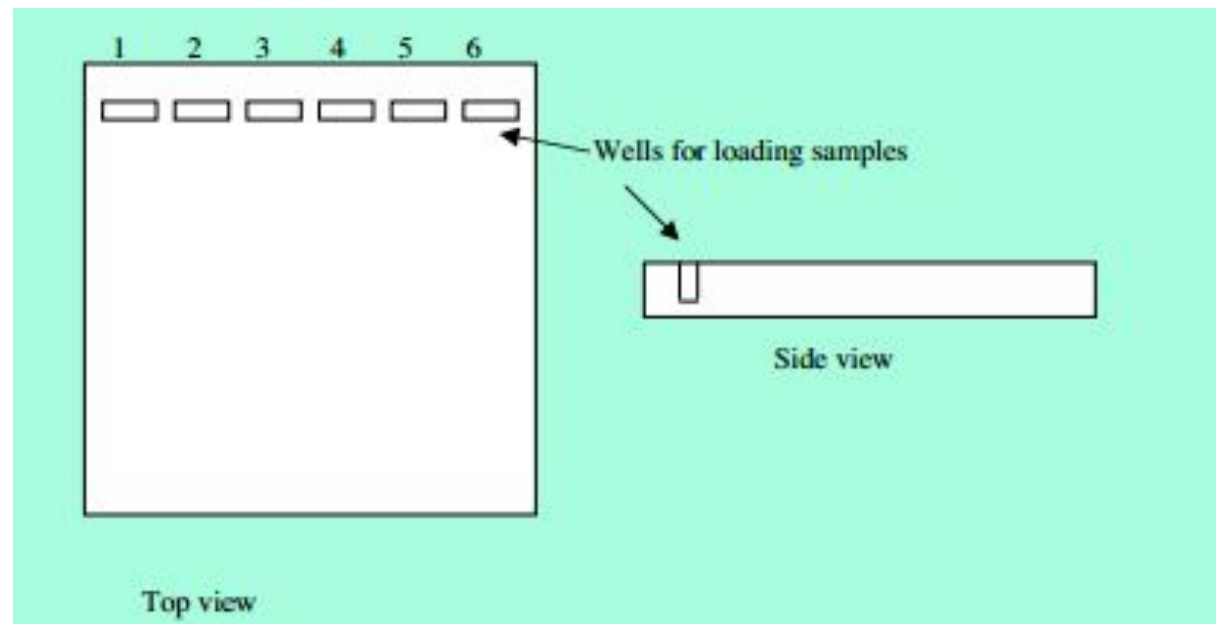


C The electrical field The gel is placed in a solution, and an electrical field is set up so one end of the gel is positive and the other end is negative.

D The fragments move The negatively charged DNA fragments travel toward the positive end. The smaller the fragment, the faster it moves through the gel. Fragments that are the farthest from the well are the smallest.



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Gel Electrophoresis – An analogy

- Imagine we have a restriction enzyme that recognizes the word “the”
- The R.E. cuts between the letters “h” and “e” (th/e)
- We apply the restriction enzyme to the following sentence (which represents a DNA molecule):

In the early 1970's, scientists discovered that bacteria had enzymes that would attack foreign DNA and cut the DNA up into little pieces.

What happens? Where does the restriction enzyme cut our “DNA” molecule?

This is what the gel would look like:

****Top of the gel****

In the early 1970's, scientists discovered that bacteria had enzymes that would attack foreign DNA and cut it

cut the DNA up into little pieces

In the

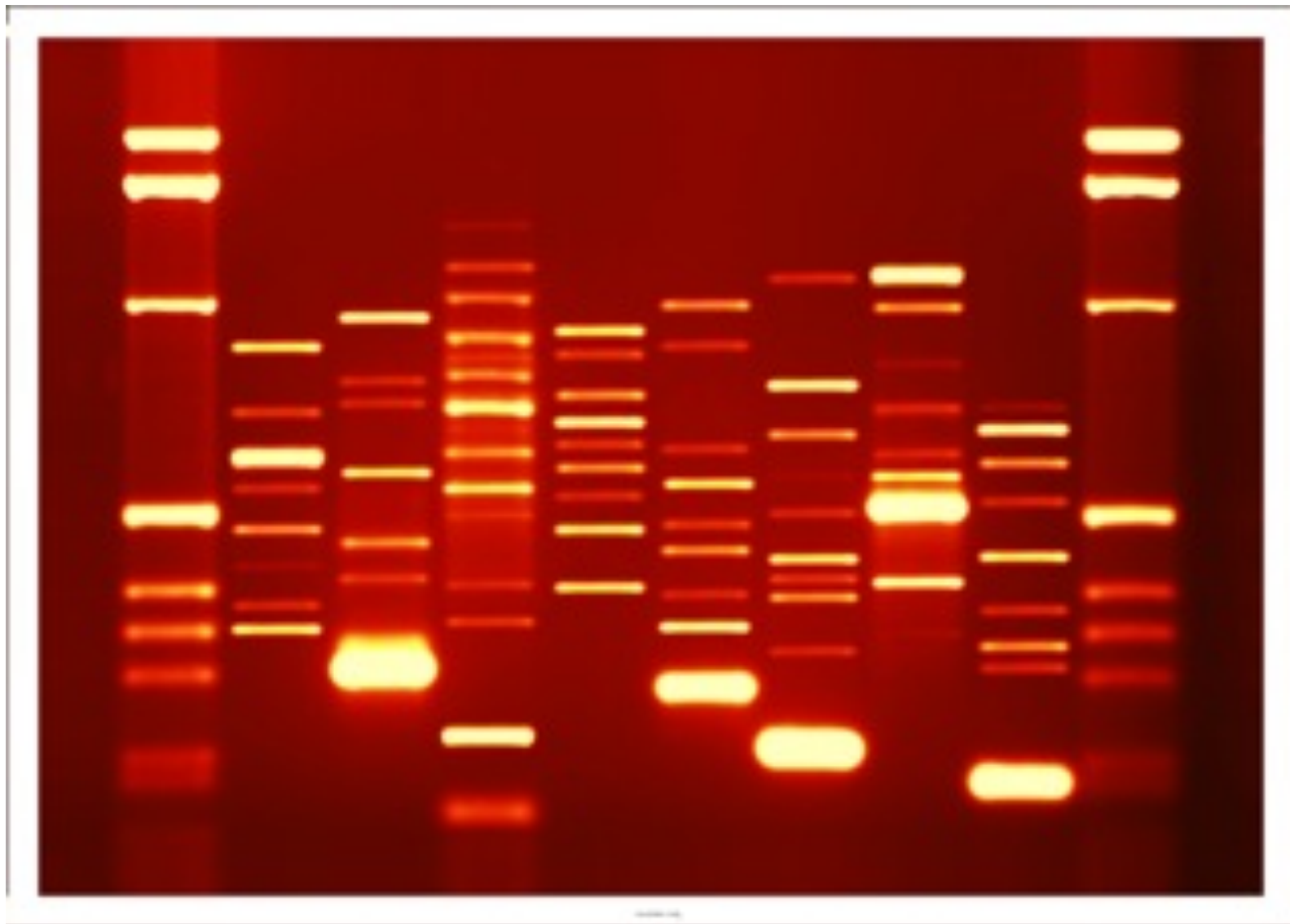
**** Bottom of the gel****

1st Fragment:
(86 letters)
at the top of the gel

2nd Fragment:
(22 letters)
In the middle of the gel

3rd Fragment:
(4 letters)
at the bottom of the gel

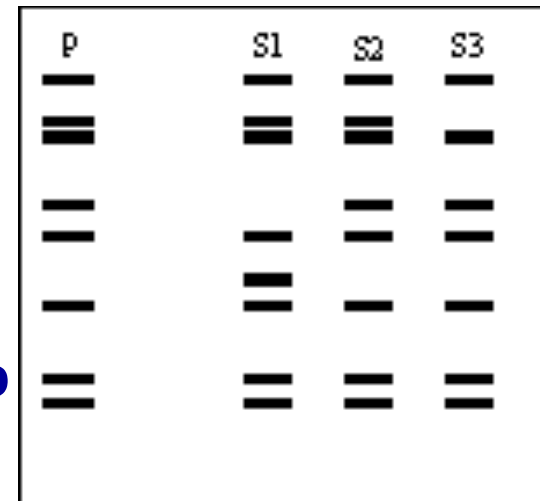
An Actual Gel Electrophoresis



DNA Fingerprinting

Southern Blotting

- Each person has their **own** print
- Used to solve **crimes**, to prove **paternity** and diagnose inherited disorders
- **Identical twins** are the only ones with identical prints
 - They come from the same sperm & egg
 - Zygote formed after fertilization splits into 2 accidentally
 - 2 cell masses formed are **identical**



Practical Applications of DNA Fingerprinting

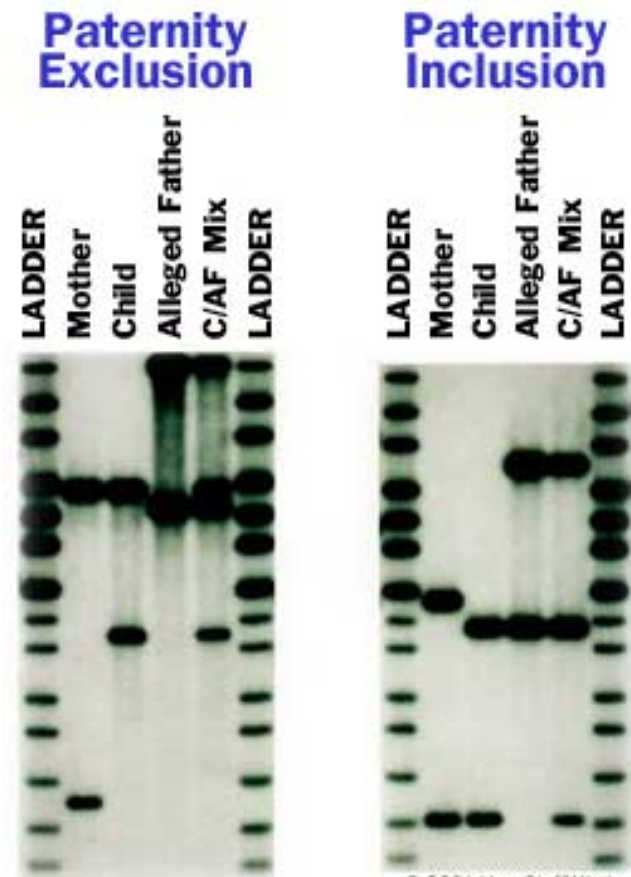
1. Paternity and Maternity Determination

- The child's banding pattern will be a combination of $\frac{1}{2}$ the mother's and $\frac{1}{2}$ the father's


















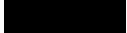


































2. Criminal Identification and Forensics

- DNA isolated from blood, hair, skin

3. Personal Identification



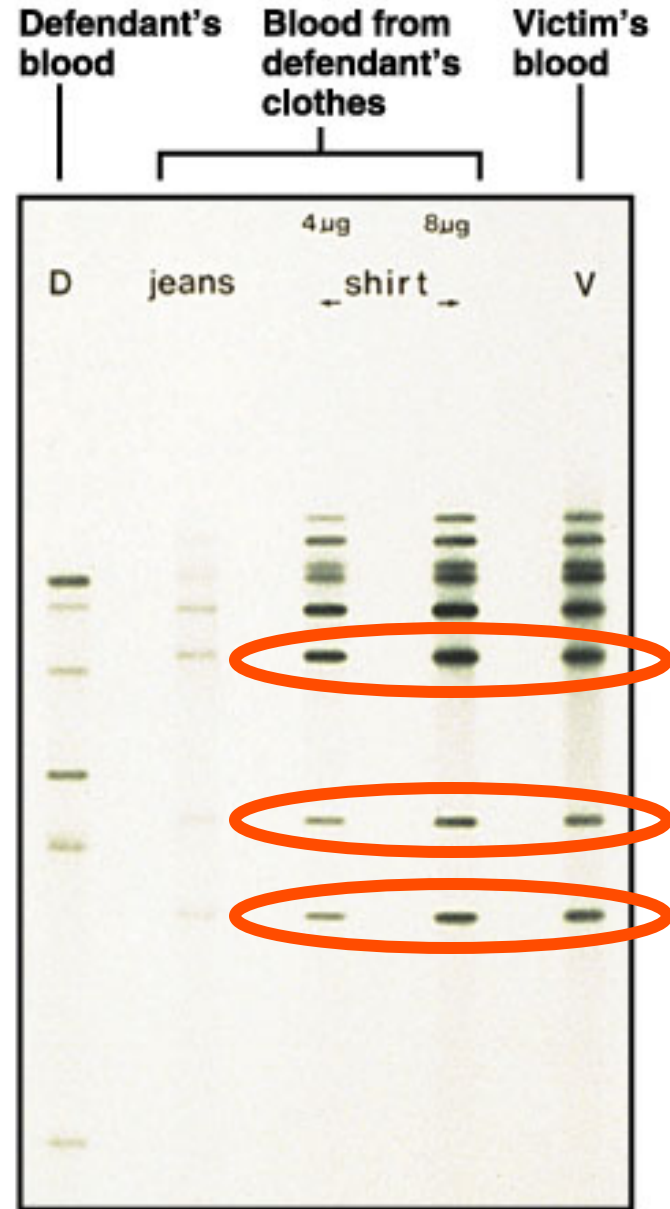
© 2001 How Stuff Works

	Parents A		Parents B		Parents C		Parents D	
Child	♀	♂	♀	♂	♀	♂	♀	♂
								
								
								
								
								
								
								
								
								
								
								

- Which parental DNA matches the child's DNA? How do you know?

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DNA fingerprints from a murder



B

DNA Profiling in foren

DNA Profiling can be used to identify suspects from trace DN evidence. It can also be used to eliminate the innocent from the investigation.

In this case, a hair follicle was left at a scene of a crime. Who was the perpetrator?

A = trace evidence

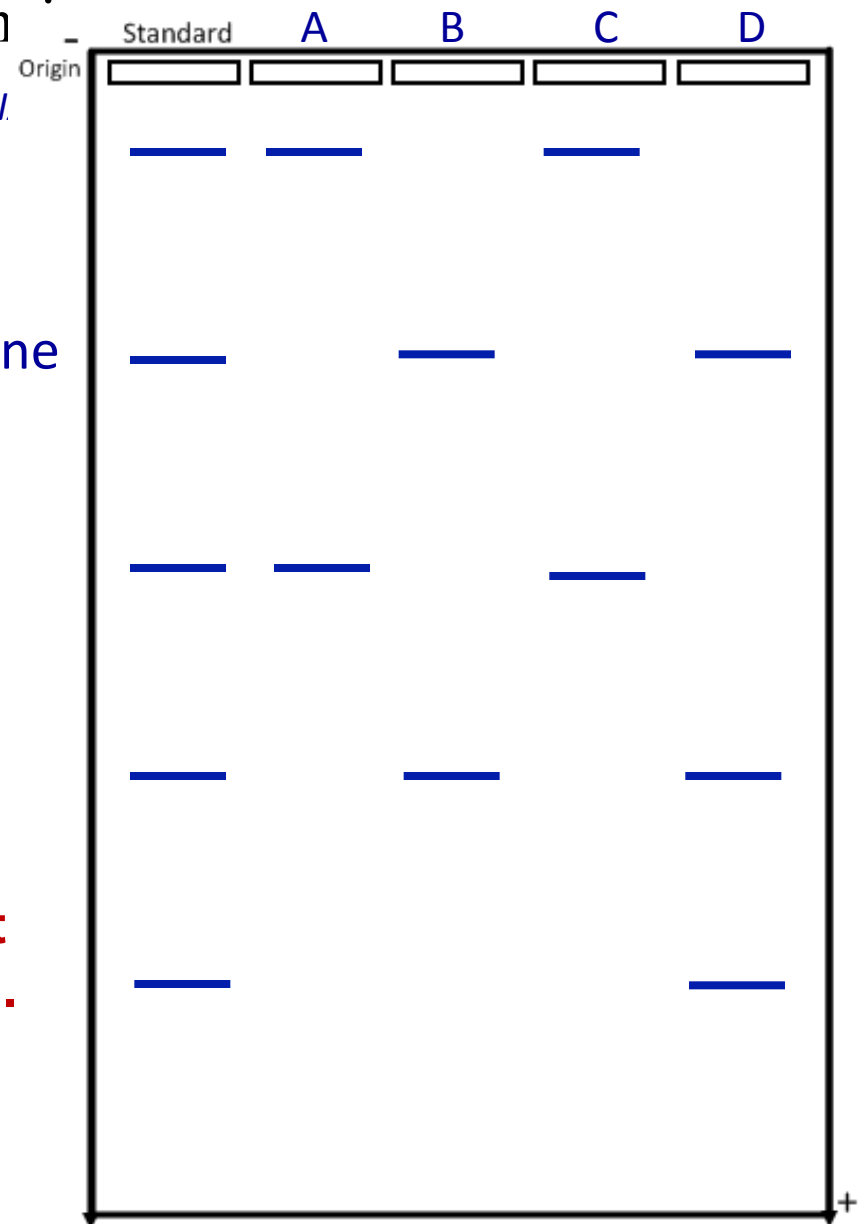
B = homeowner

C = suspect 1

D = suspect 2

Explanation:

We expect 100% match as the cells left behind are the perpetrator's own cells.



DNA Profiling in foren

DNA Profiling can be used to identify suspects from trace DNA evidence. It can also be used to eliminate the innocent from the investigation.

In this case, a lot of blood was left at a crime scene. Who was the perpetrator?

A = victim

B = unknown blood at scene

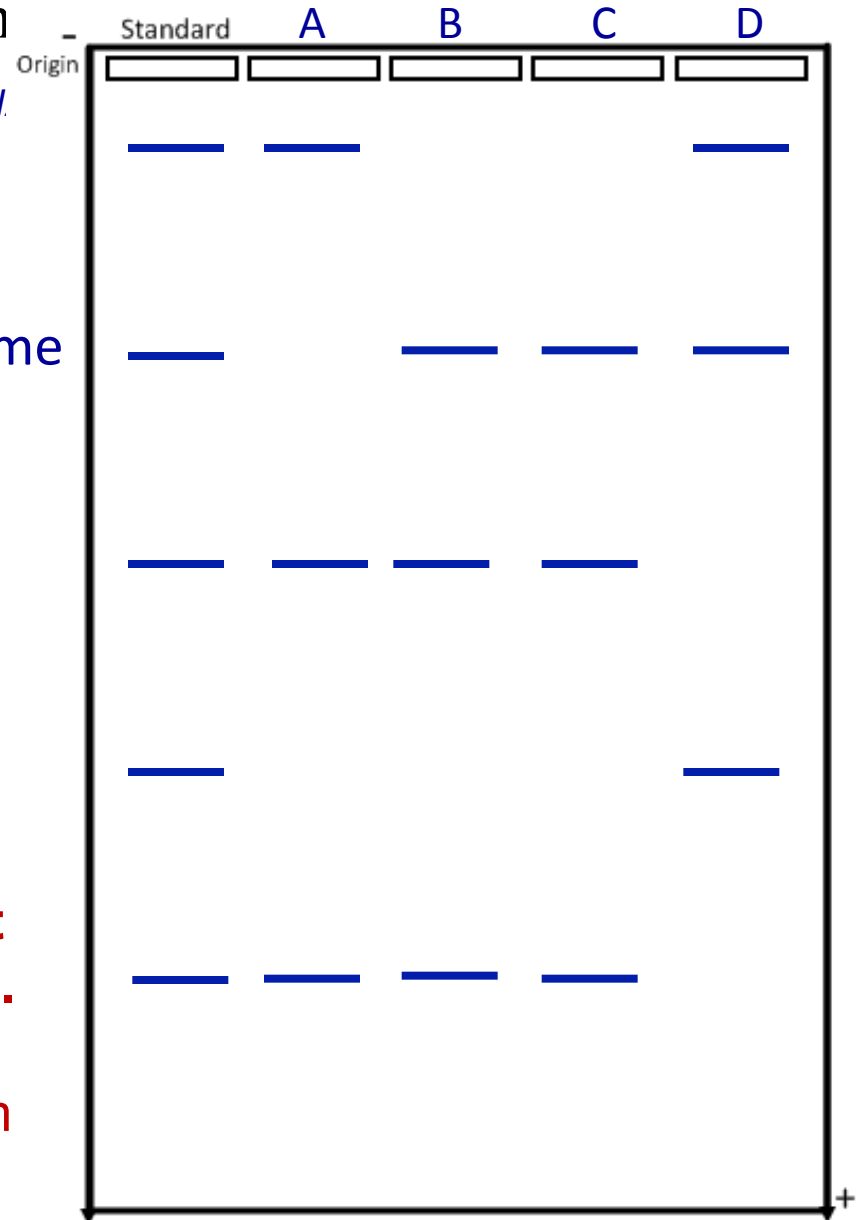
Ⓢ = suspect 1

D = suspect 2

Explanation:

We expect 100% match as the cells left behind are the perpetrator's own cells.

The overlapping bands between the victim and perpetrator suggest a close relationship.



DNA Profiling in forensics Standard

DNA Profiling can be used to identify suspects from trace DNA evidence. It can also be used to eliminate the innocent from the investigation.

In this case, DNA evidence is being used in a wrongful conviction case. Is the prisoner really guilty?

A = trace evidence

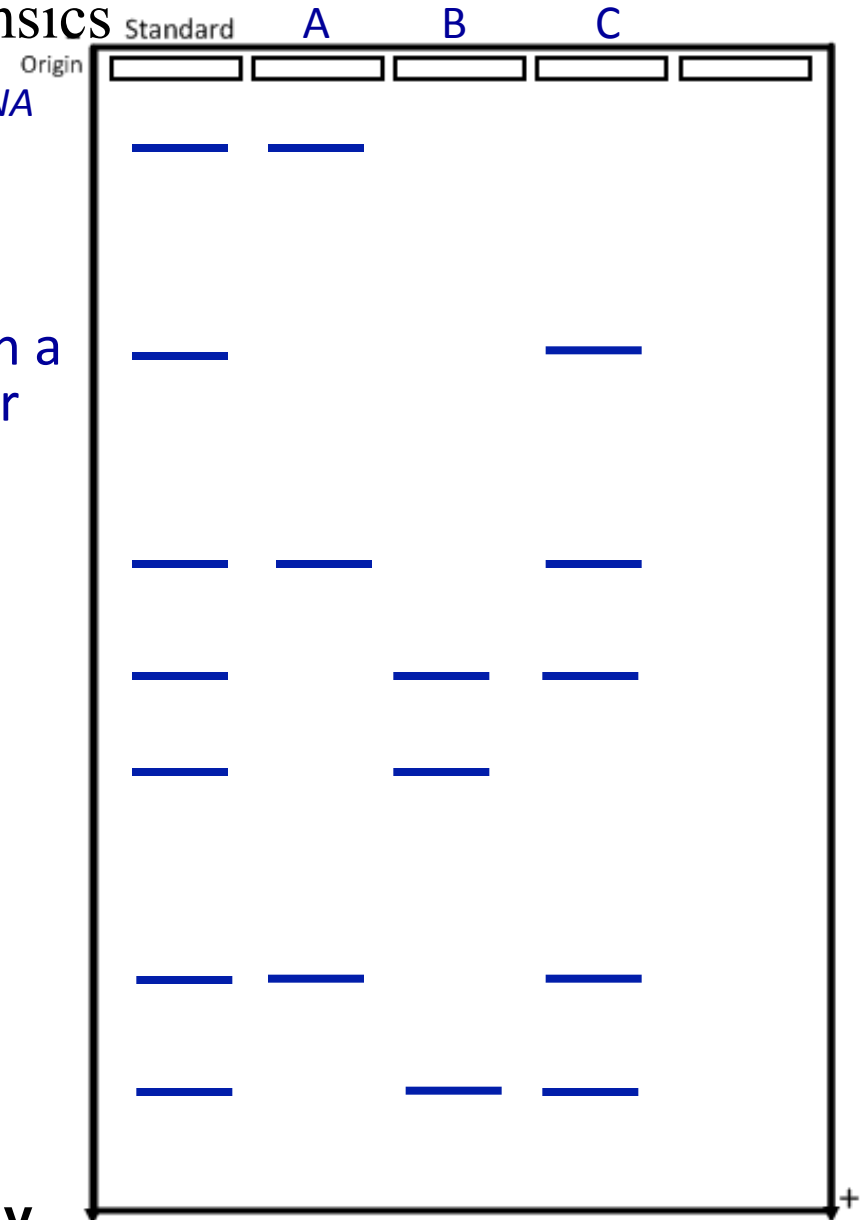
B = homeowner

C = prisoner

Explanation:

No. Without a stronger match, the evidence is insufficient to convict the suspect. He should be released and a new suspect found.

DNA evidence is being reviewed in many wrongful conviction lawsuits.



DNA Profiling in paternity

DNA Profiling can be used to identify relationships between people and to determine parentage.

In this case, the parentage of a child is under question. Who's the daddy?

A = mother

B = child

C = man 1

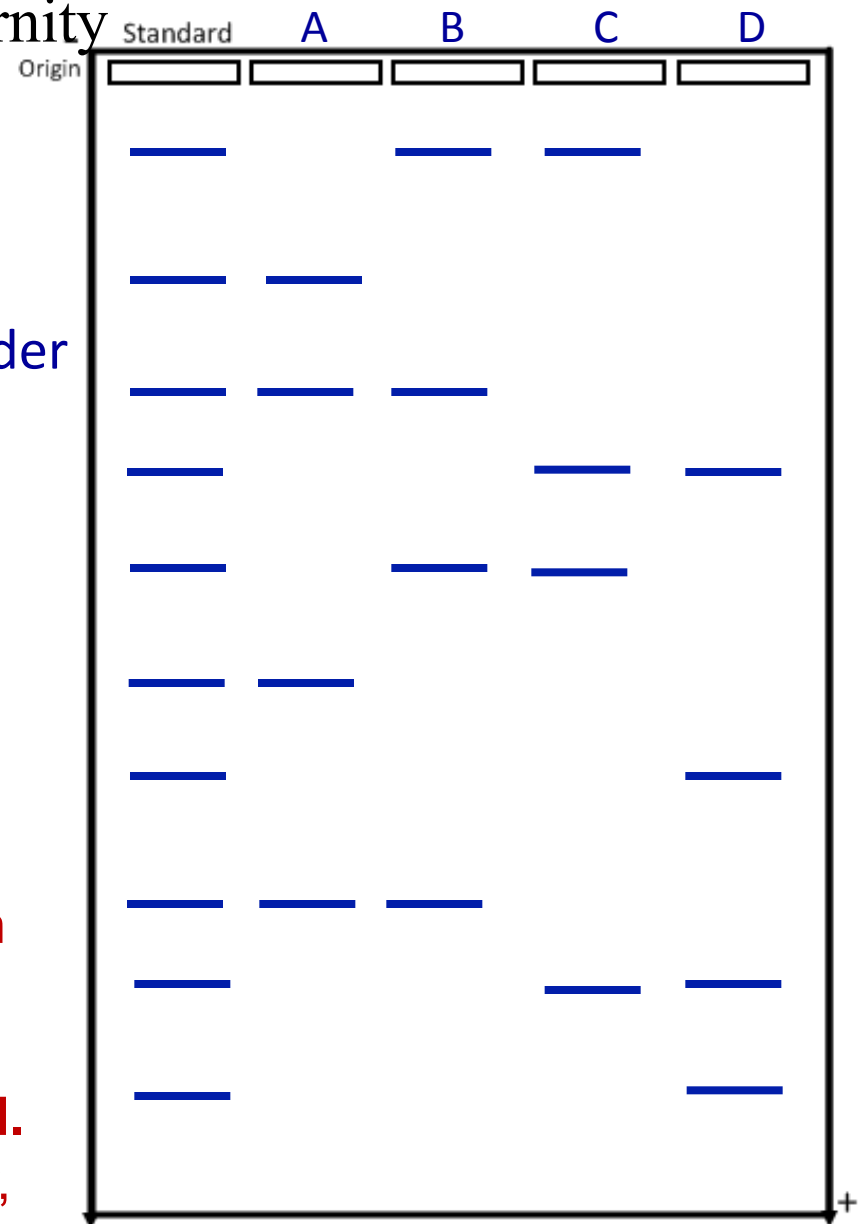
D = man 2

Explanation:

We expect some – around 50% - match between a parent and their own child.

The mother (A) and man 1 (B) each share two different bands with the child.

Man 1 and 2 share bands with each other, suggesting they might be related.



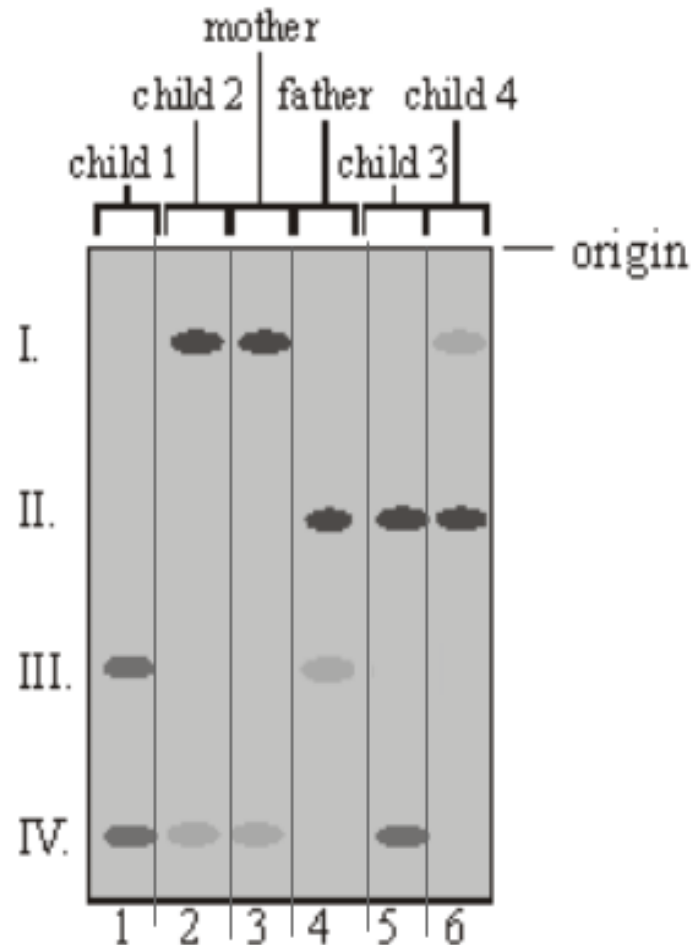
Sample Questions

1. Identify the smallest DNA fragment.

I. II. III. **IV.**

2. Identify the child which is most likely to be from the mother's previous marriage.

1 **2** 3 4



[Source: *The Biology Project*, University of Arizona]