Genetic Engineering

Learner outcomes…

What you need to know!

- explain, in general, how restriction enzymes cut DNA molecules into smaller fragments and how ligases reassemble them
- explain, in general, how cells may be transformed by inserting new DNA sequences into their genomes

Terms you need to know

Recombinant DNA technology
Recombinant Enzymes
Plasmid
Restriction Enzymes
Ligase
PCR – Polymerase Chain Reaction
Gel Electrophoresis
DNA Fingerprinting-Southern Blotting

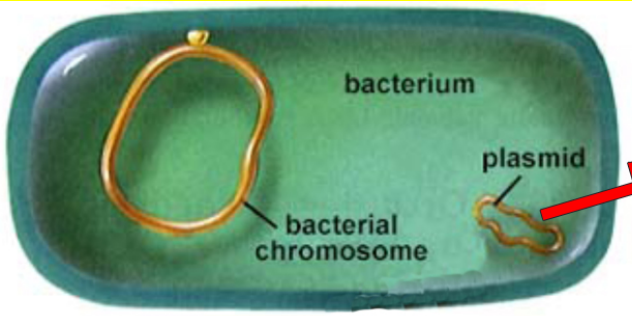
Genetic Engineering

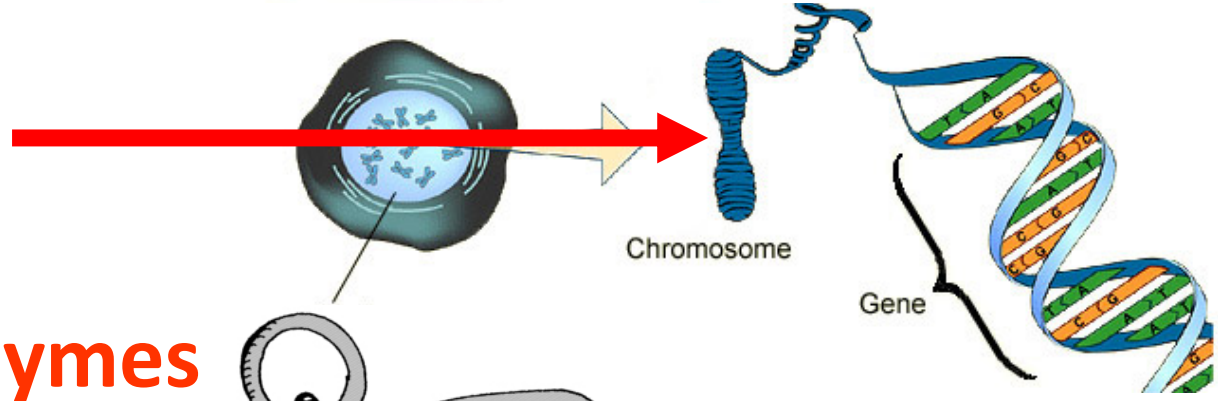
- Building **new** DNA by **manipulating** the DNA of organisms
- Biotechnologists **splice** new pieces of DNA (genes) from one chromosome to another
- Refinement of traditional breeding practices
- Also called **recombinant DNA technology**
- **TRANSGENIC: Genome of an organism that has been altered by introducing foreign genetic material**

Recombinant DNA Technology

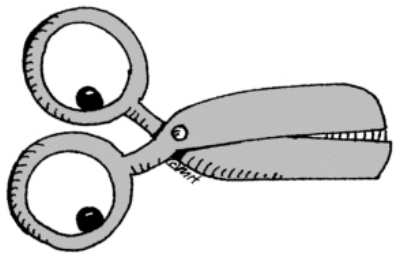
<u>Requires 4 things</u>

1. Plasmid from E. coli bacteria
2. Required Gene





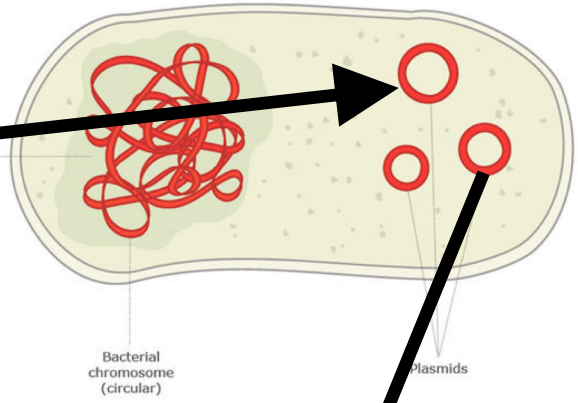
3. **Restriction Enzymes** (for cutting)



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
- **<u>Plasmid</u> = 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**



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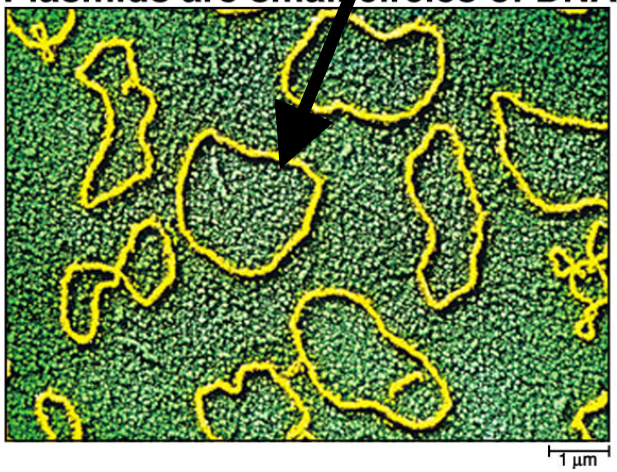
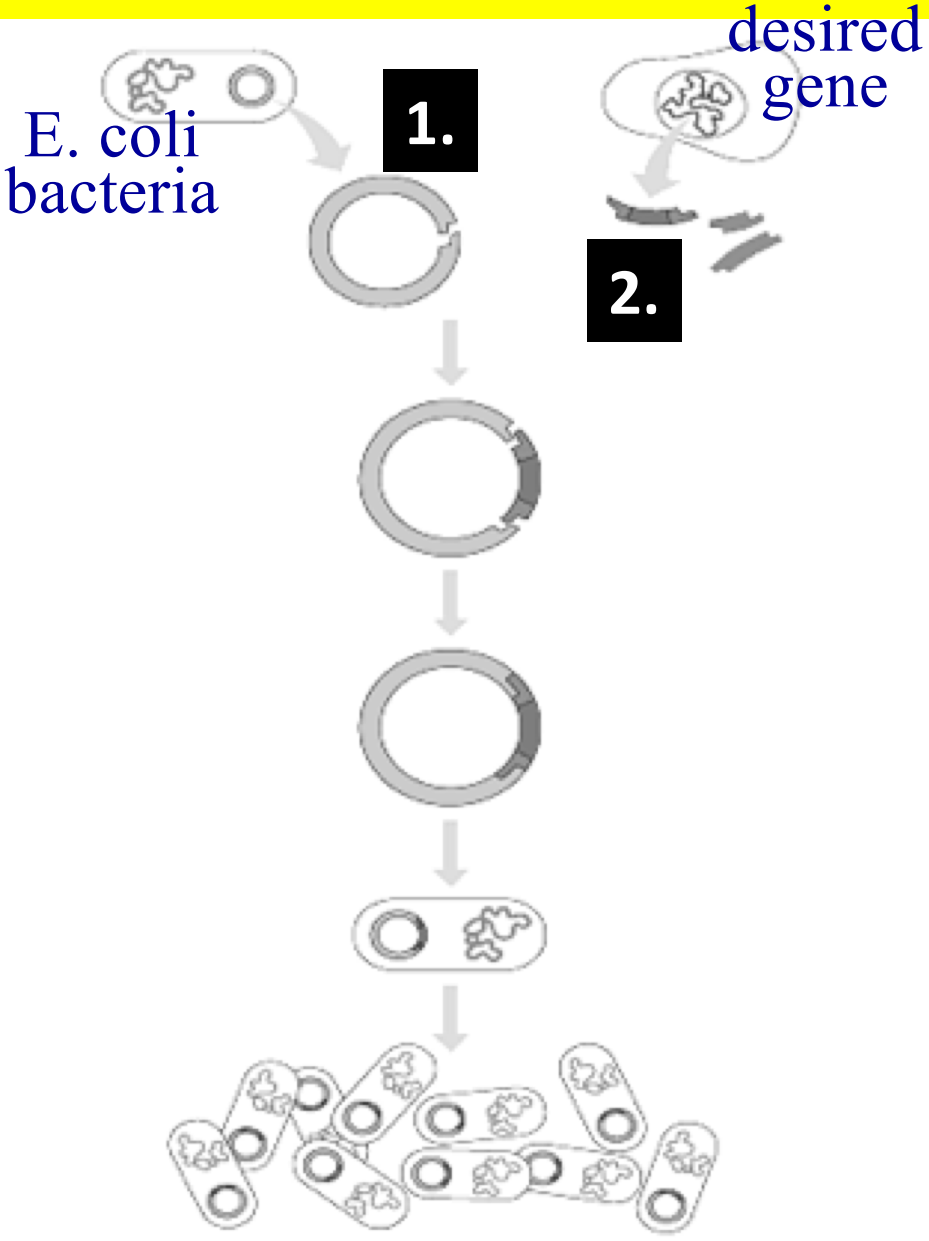
Plasmids are small circles of DNA



Recombinant DNA Process



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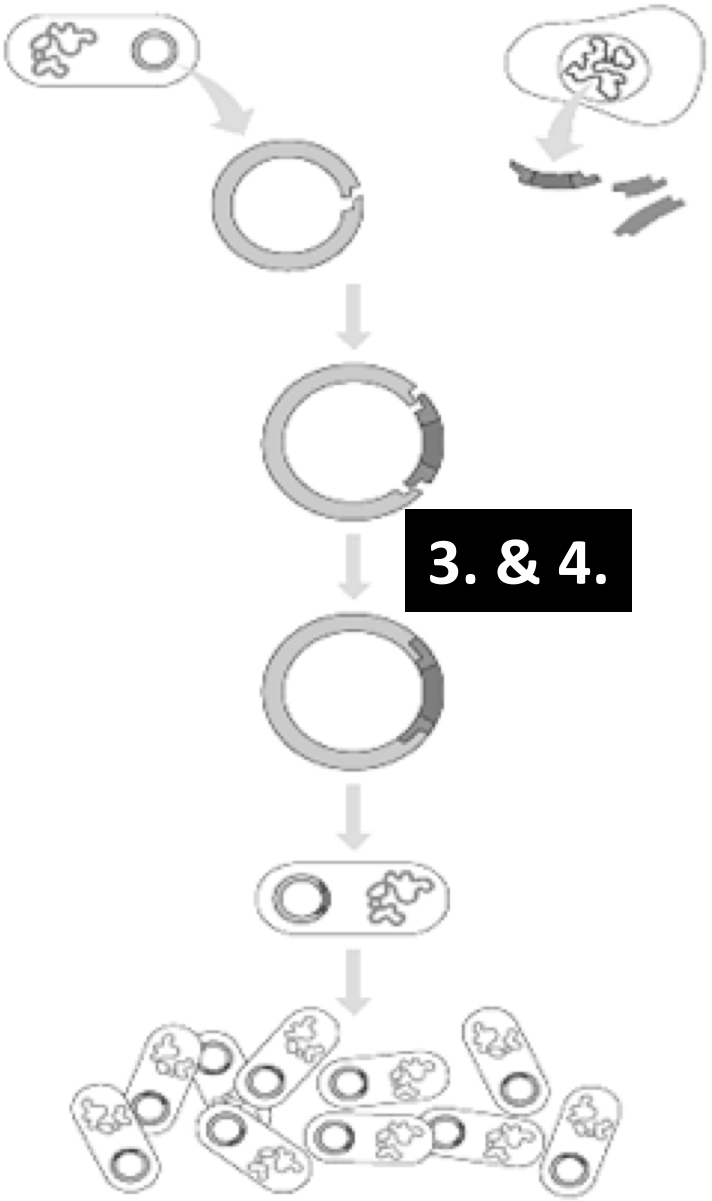
1. A bacterial plasmid is opened using a restriction enzyme (scissors).

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

Recombinant DNA Process



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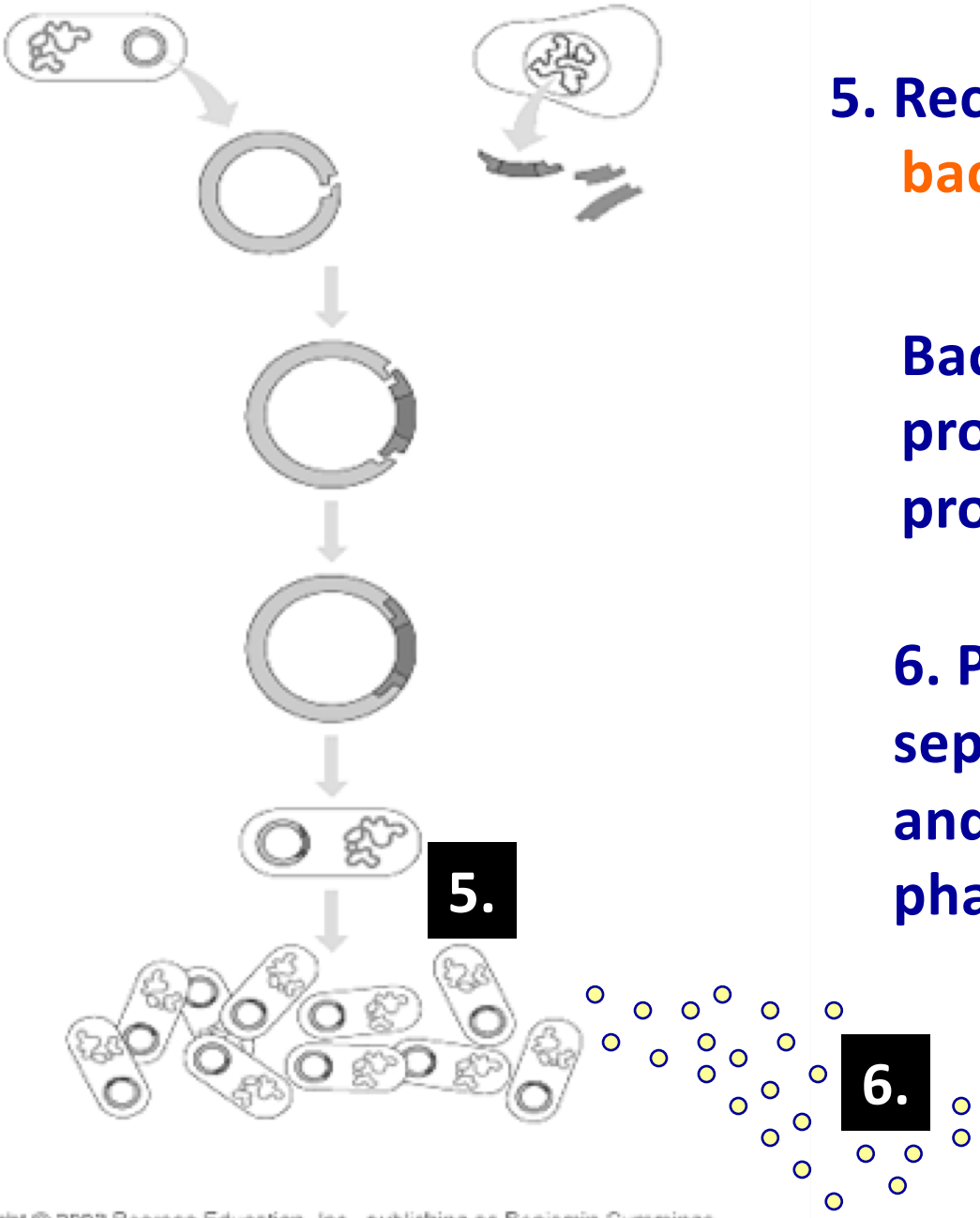
3. Both DNA samples are combined in a petri dish or a test tube.

4. Ligase is added to glue DNA together.

Recombinant DNA Process



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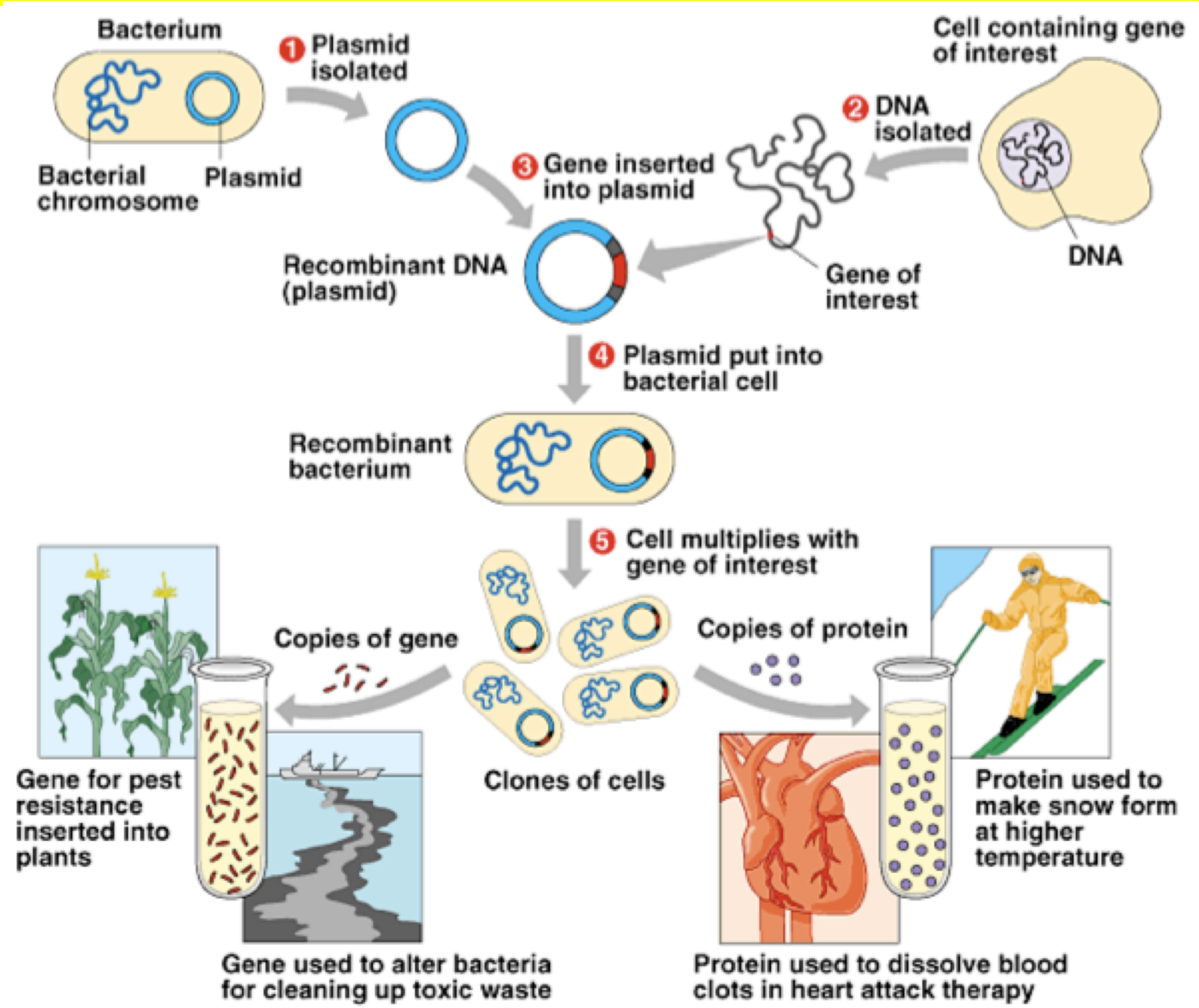
5. Recombined plasmid inserted back into E. coli bacterial cell.

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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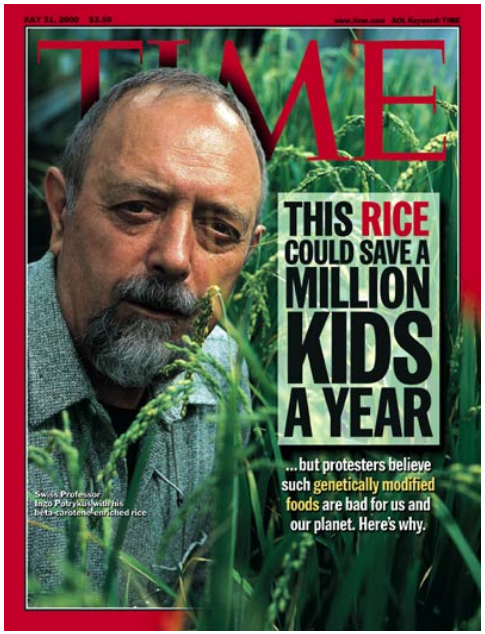
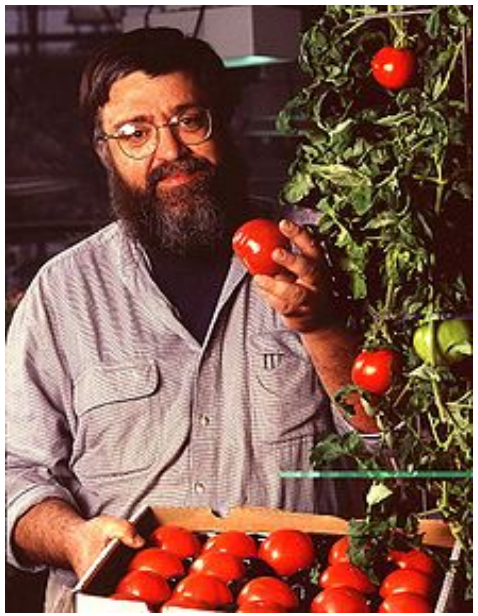
The miracle of genetic engineering

search ID: lfjn49

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)

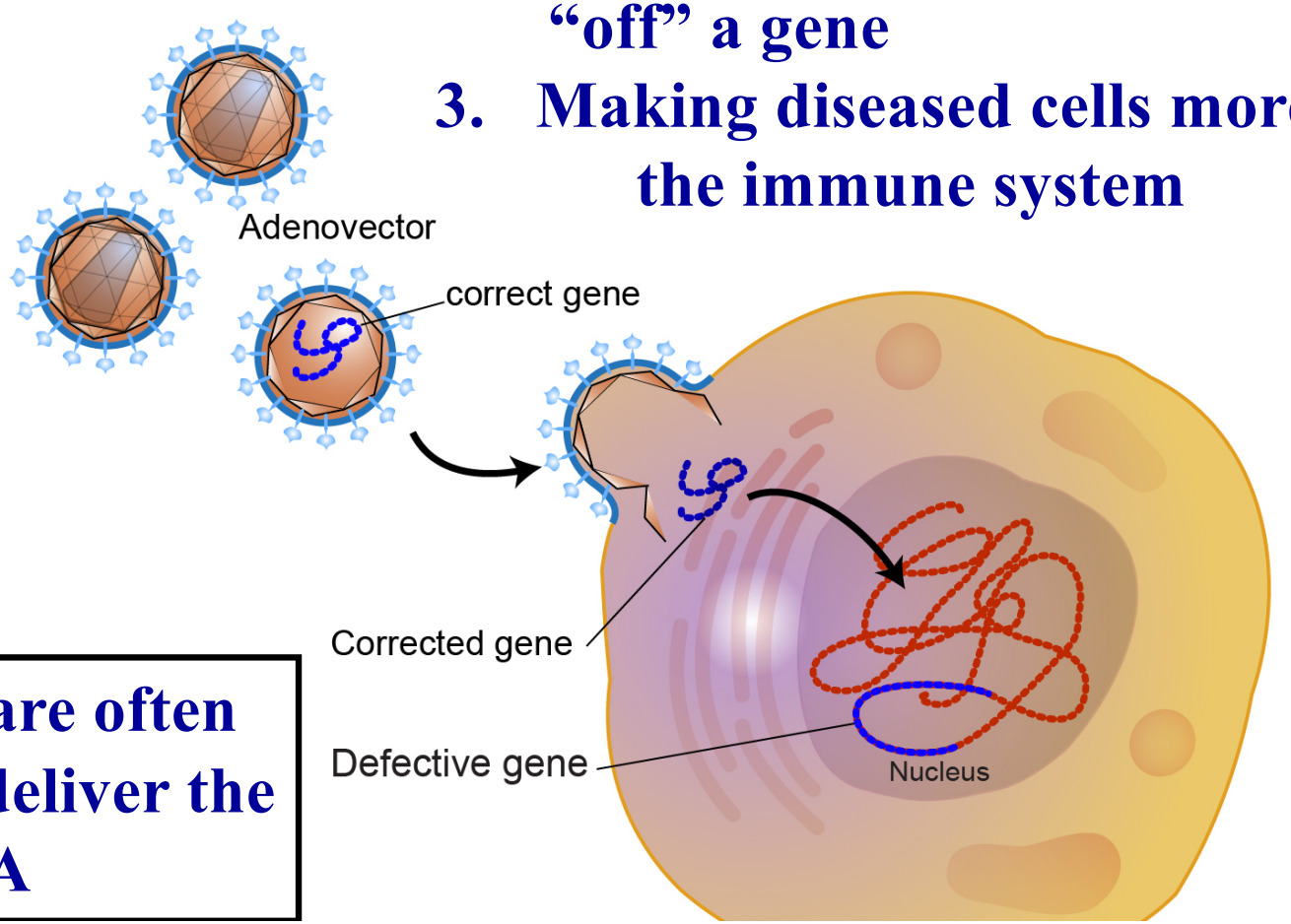
4. Production of BST

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

GENE THERAPY

5. Gene therapy- used to correct defective genes in order to cure a disease or help your body better fight disease.

1. **Replacing mutated genes**
2. **Fixing mutated genes – turn "on" or "off" a gene**
3. **Making diseased cells more evident to the immune system**



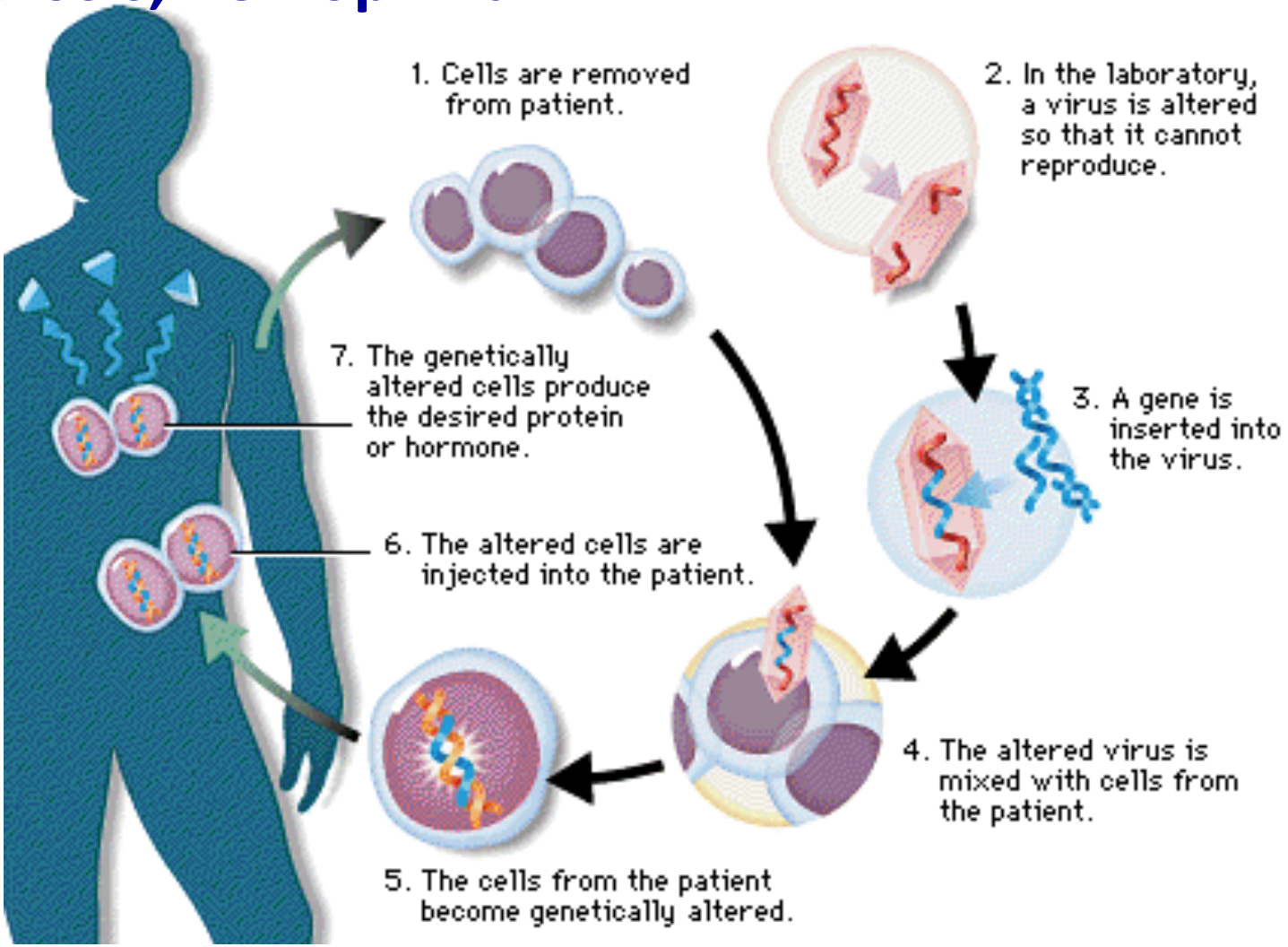
Viruses are often used to deliver the new DNA

Applications of rDNA Technology

5. Gene Therapy

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

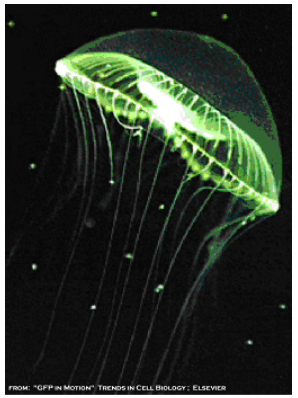
Issues: How do we get the genes in the Body and control them?



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.

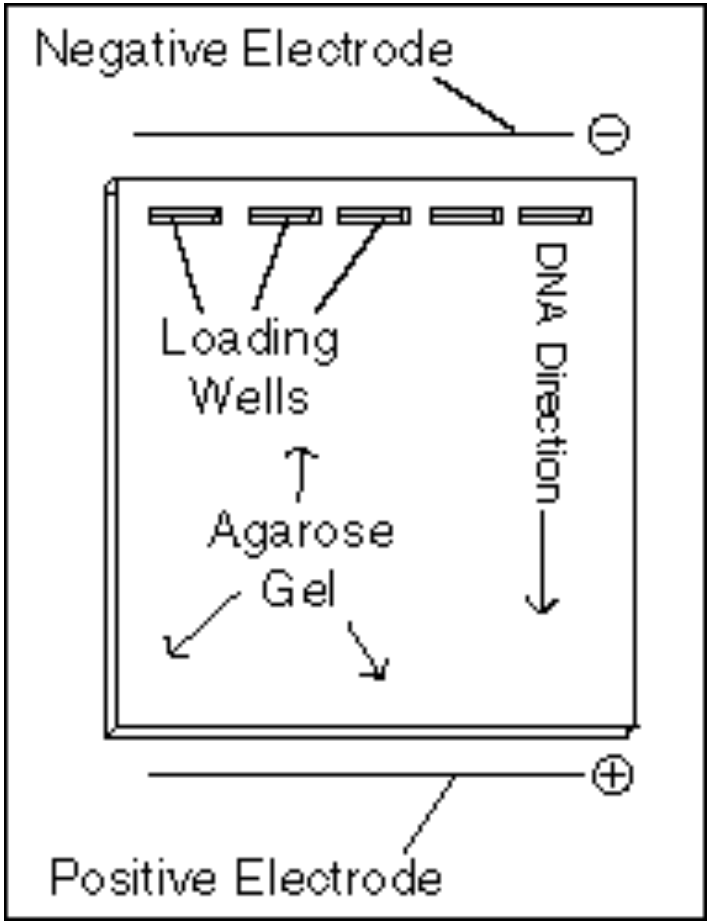
Pushing science to the brink of altering humans, researchers have created the world's first genetically modified primate, a baby rhesus monkey with jellyfish DNA that glows green in the dark.

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.

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.

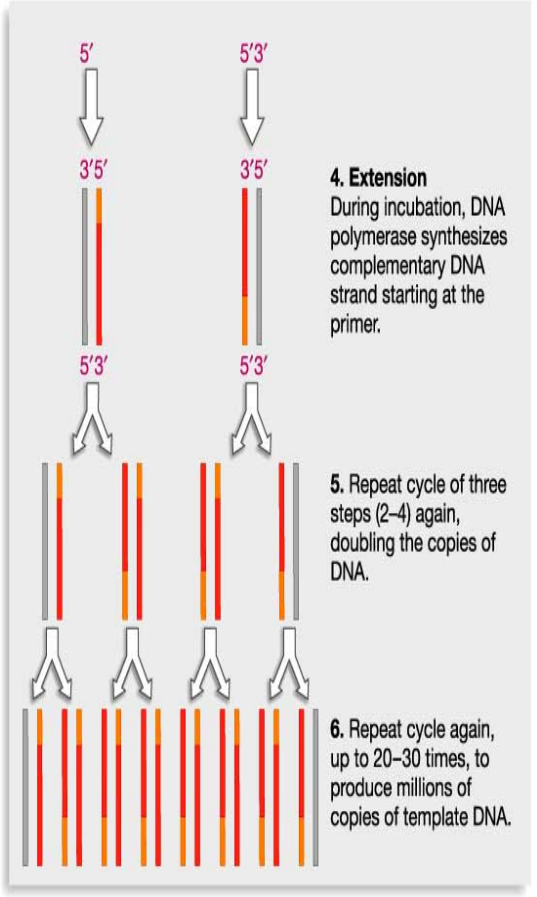
Technologies to Analyze DNA

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!)**
- 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!)**



HOW?

- **DNA Polymerase is added to separated(unzipped) DNA strands**
- **DNA Polymerase duplicates the DNA using complementary base pairing**
- **This process is done many times**

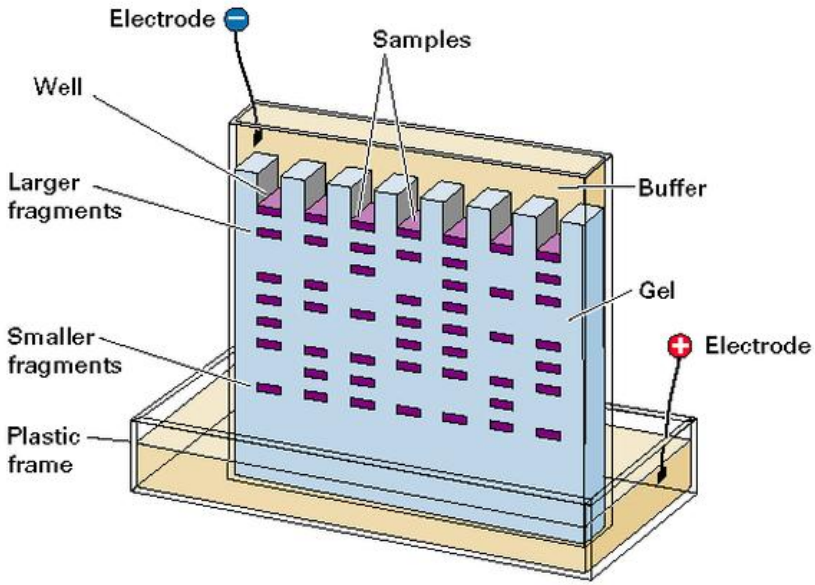
PCR song

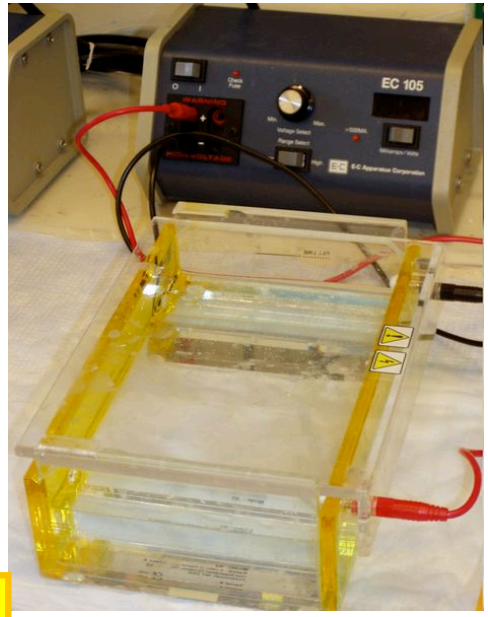
PCR video

Click here to make your c

Gel electrophoresis

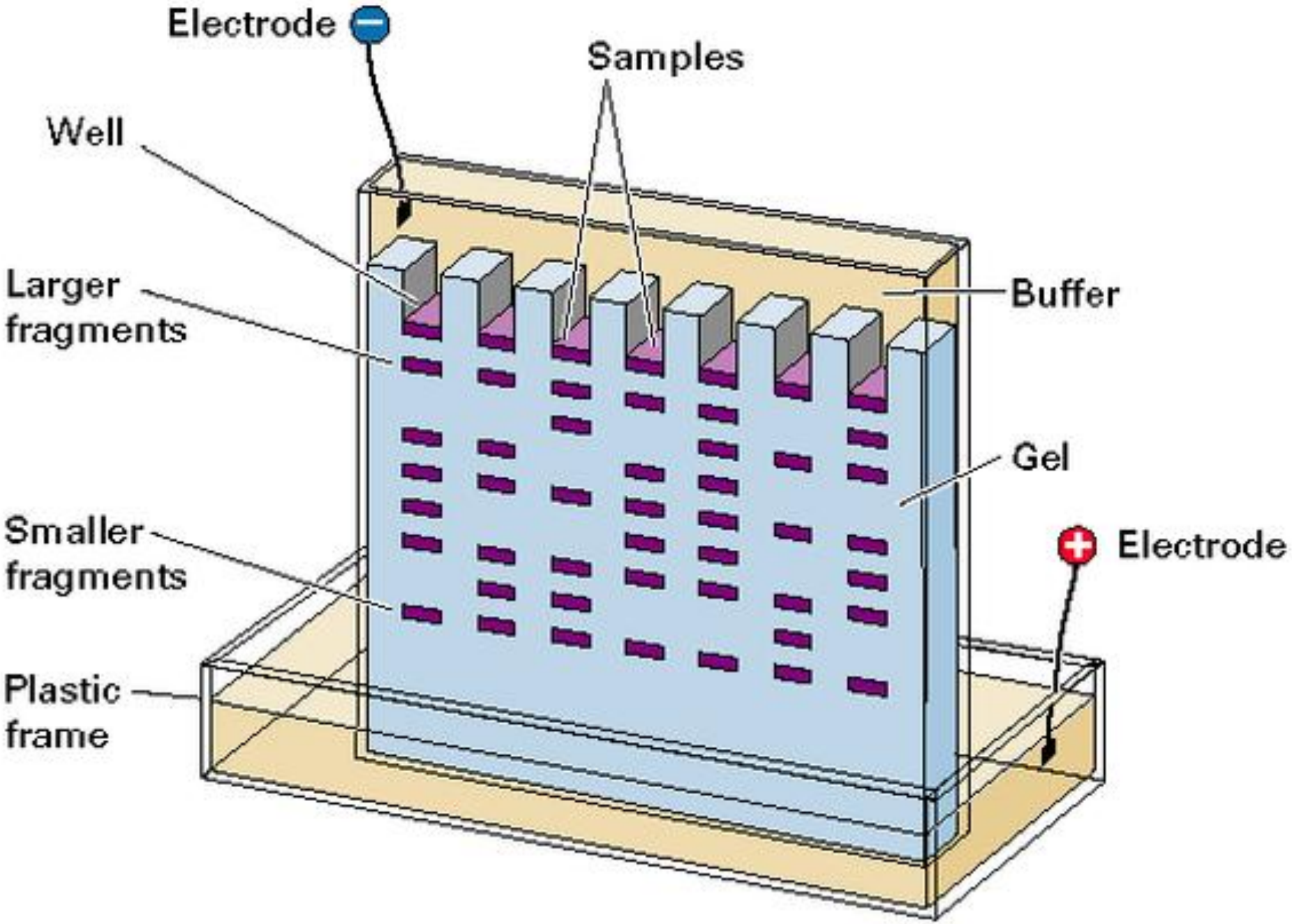
- ***Ethanol*** **added to solution of ruptured cells**
- **DNA *precipitates* out and spooled onto a glass rod**
- **Recombinant Enzymes used to cut DNA into fragments**
- **DNA loaded into wells on electrophoresis plate with dye**
- **Current applied and negatively charged DNA moves up the plate to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





Gel Electrophoresis Animation

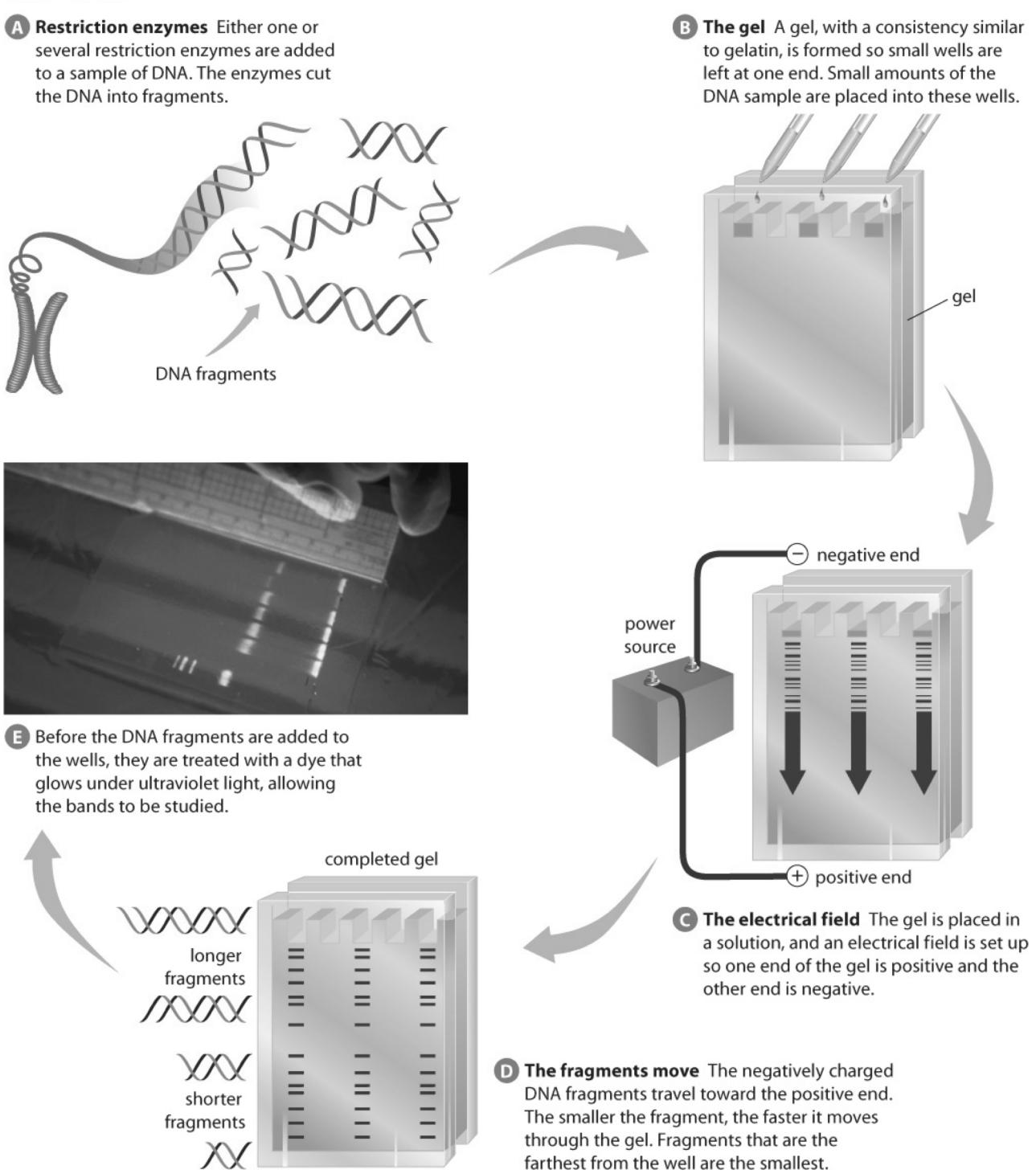
Gel electrophoresis video- amoeba sisters

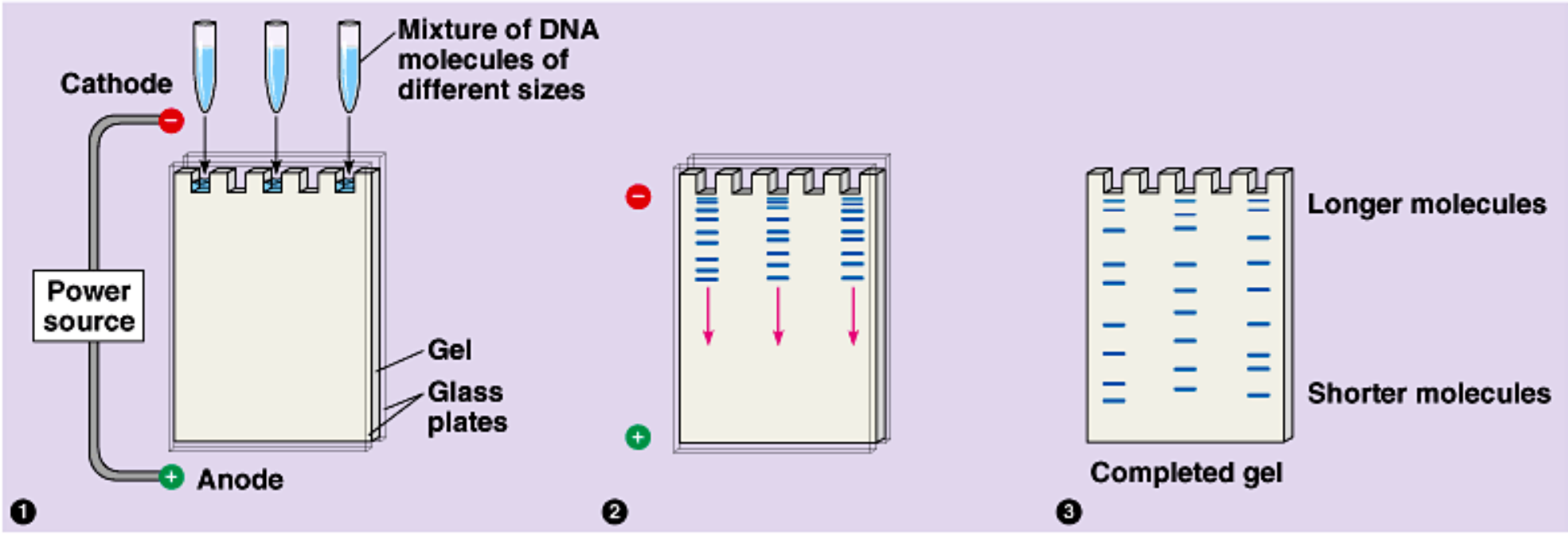

Electrode
Samples
Well
Larger fragments
Buffer
Gel
Smaller fragments
Electrode
Plastic frame

Isolating DNA in the lab

University of Utah Gel Electrophoresis Lab

http://learn.genetics.utah.edu/content/labs/gel/





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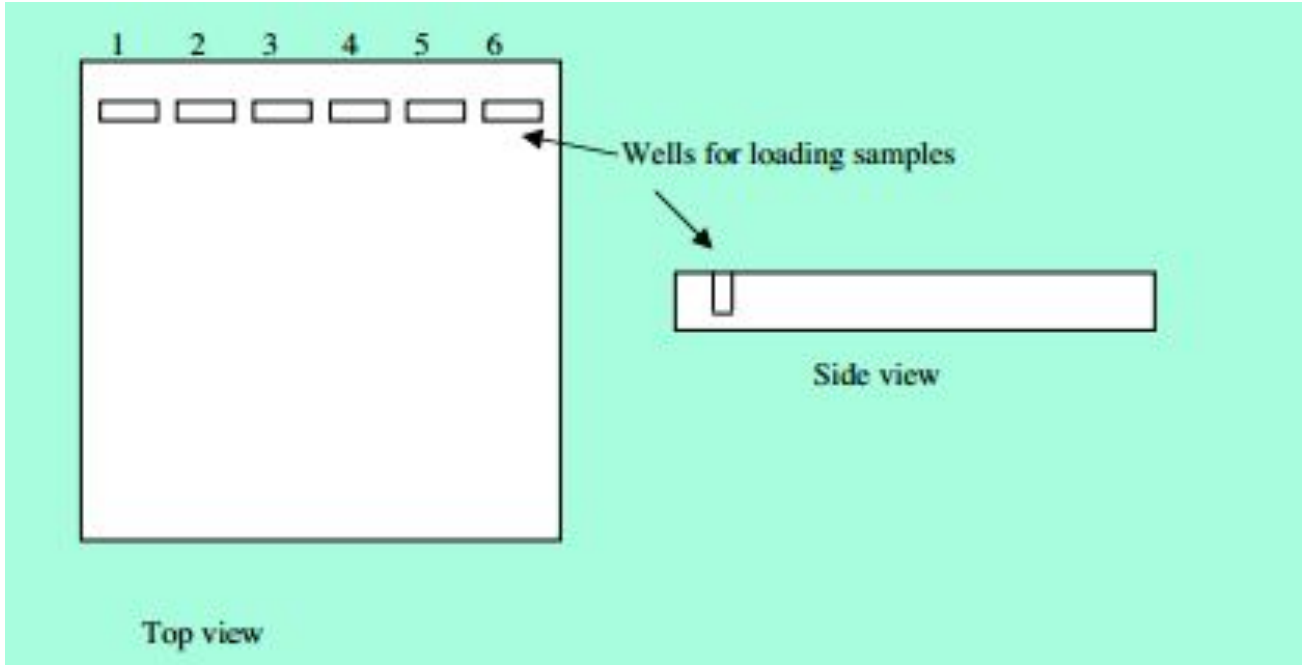


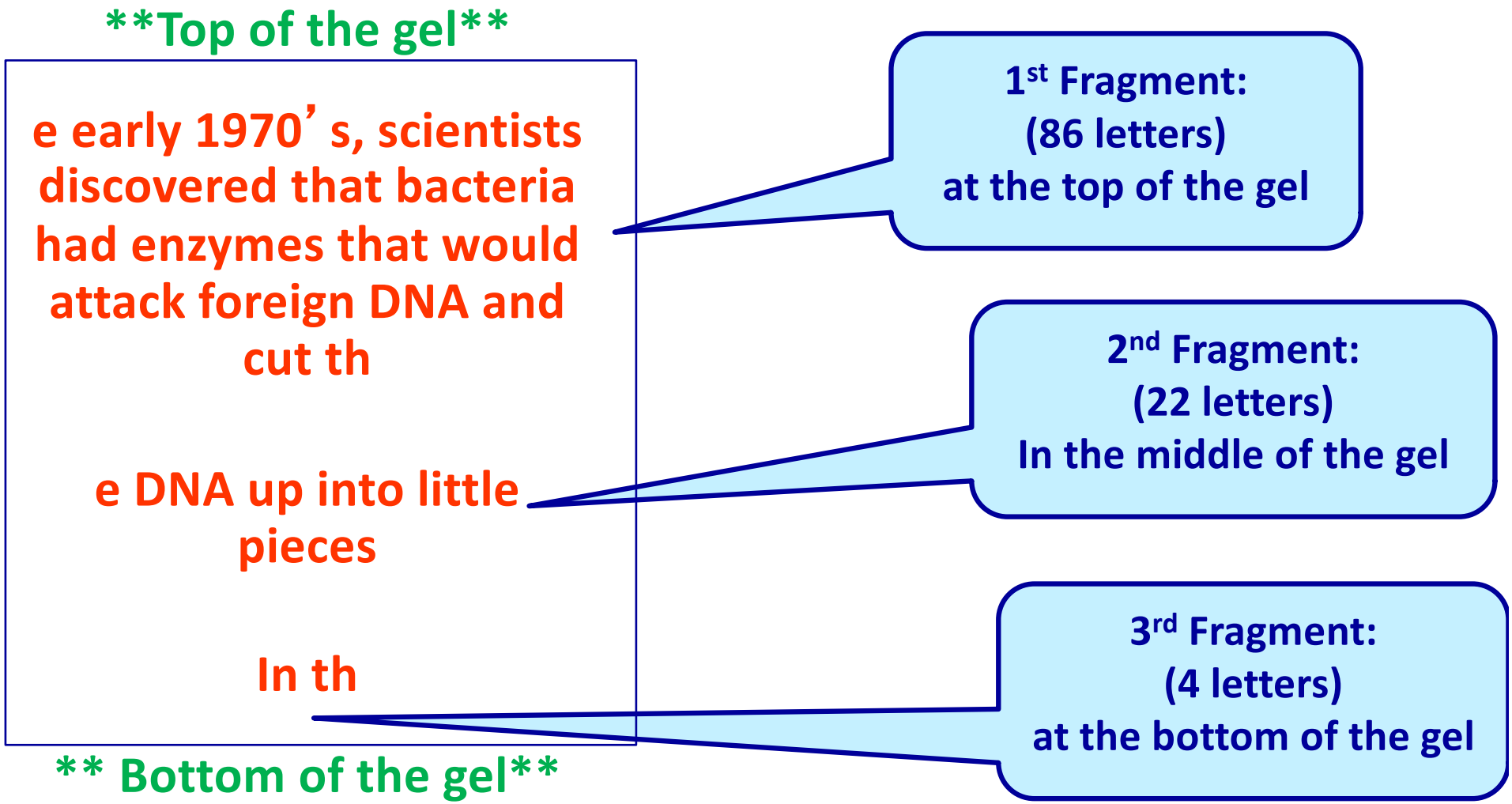
Gel Electrophoresis – An analogy

- 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):

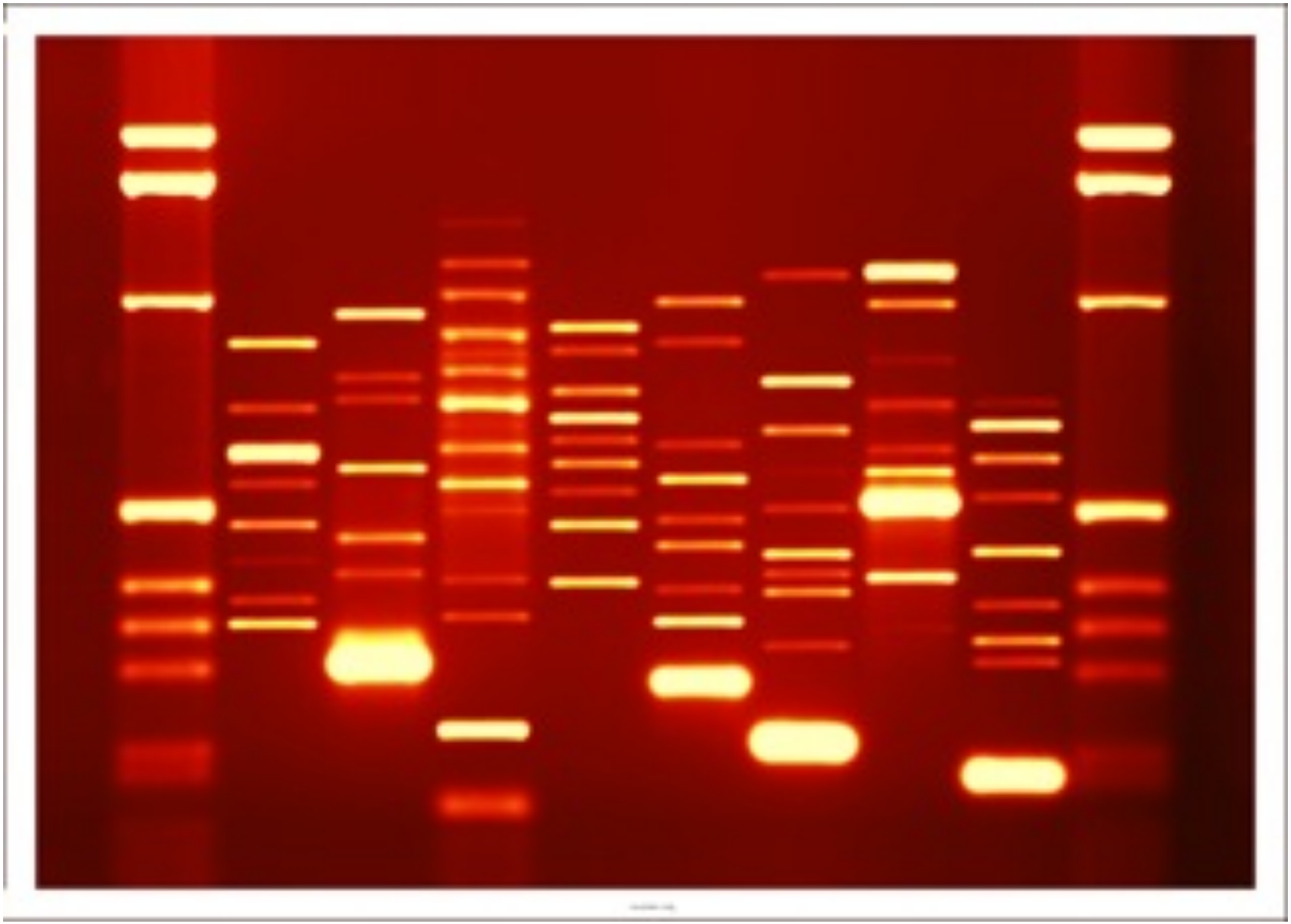
In the early 1970's, scientists discovered that bacteria had enzymes that would attack foreign DNA and cut the DNA up into little pieces.

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

This is what the gel would look like:



An Actual Gel Electrophoresis



DNA Fingerprinting

Southern Blotting

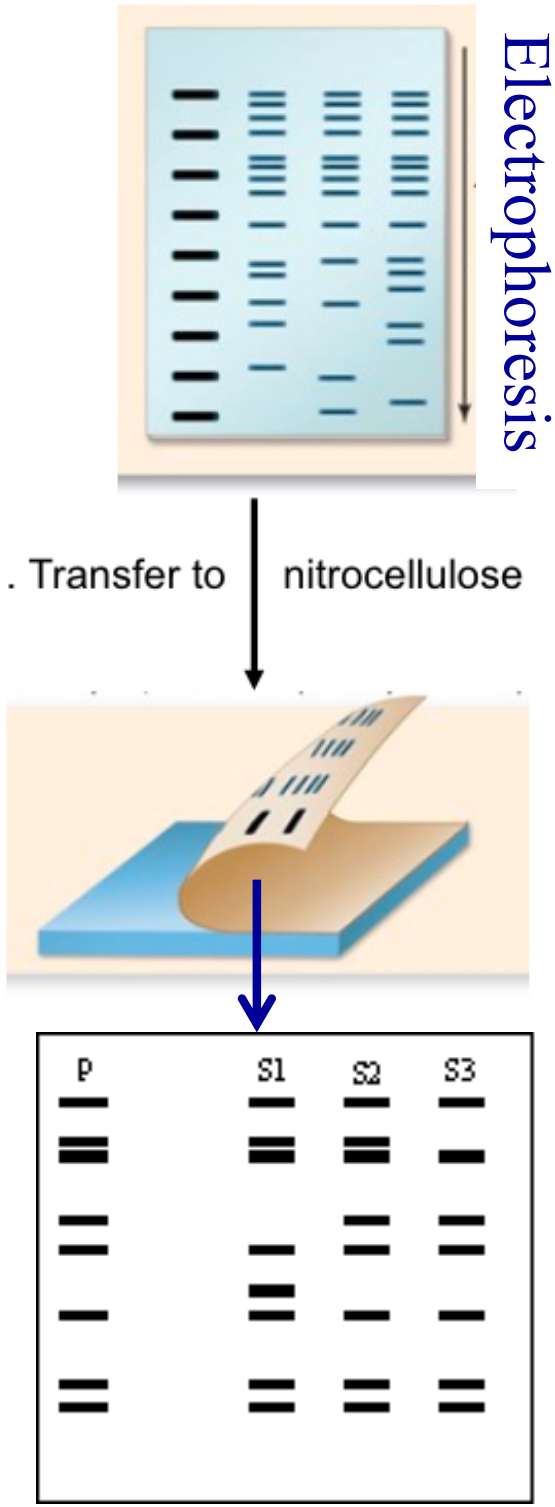
- Each person has their own print

STEPS

1. Electrophoresis used to separate DNA
2. The gel with the separated DNA fragments (now in bands) is placed on a filter
3. The fragments transfer onto the filter to be read

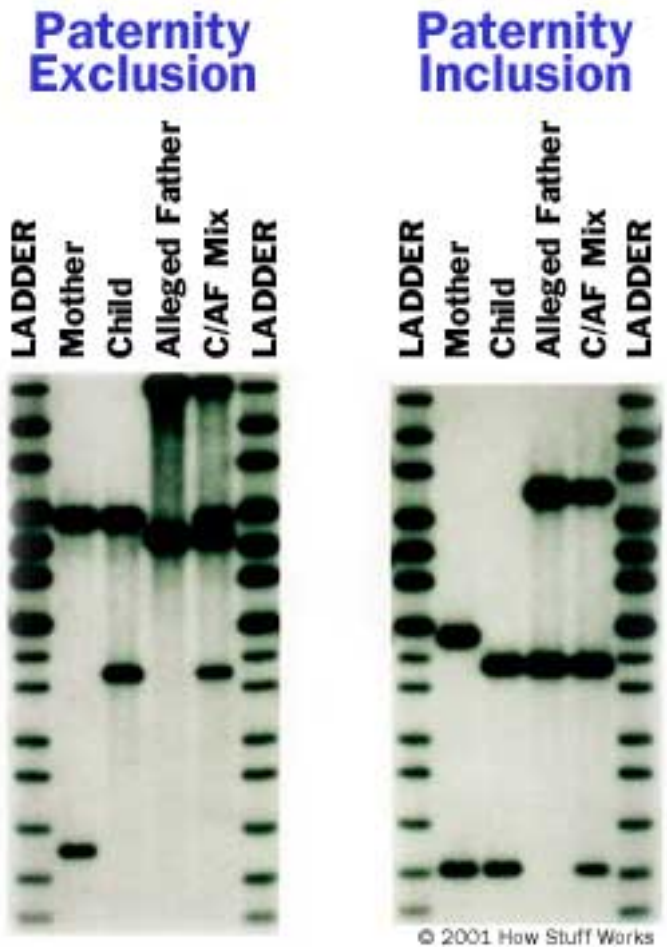
- Used to solve crimes, to prove paternity and diagnose inherited disorders
- Identical twins are the only ones with identical prints

Kahn Academy: Southern Blotting



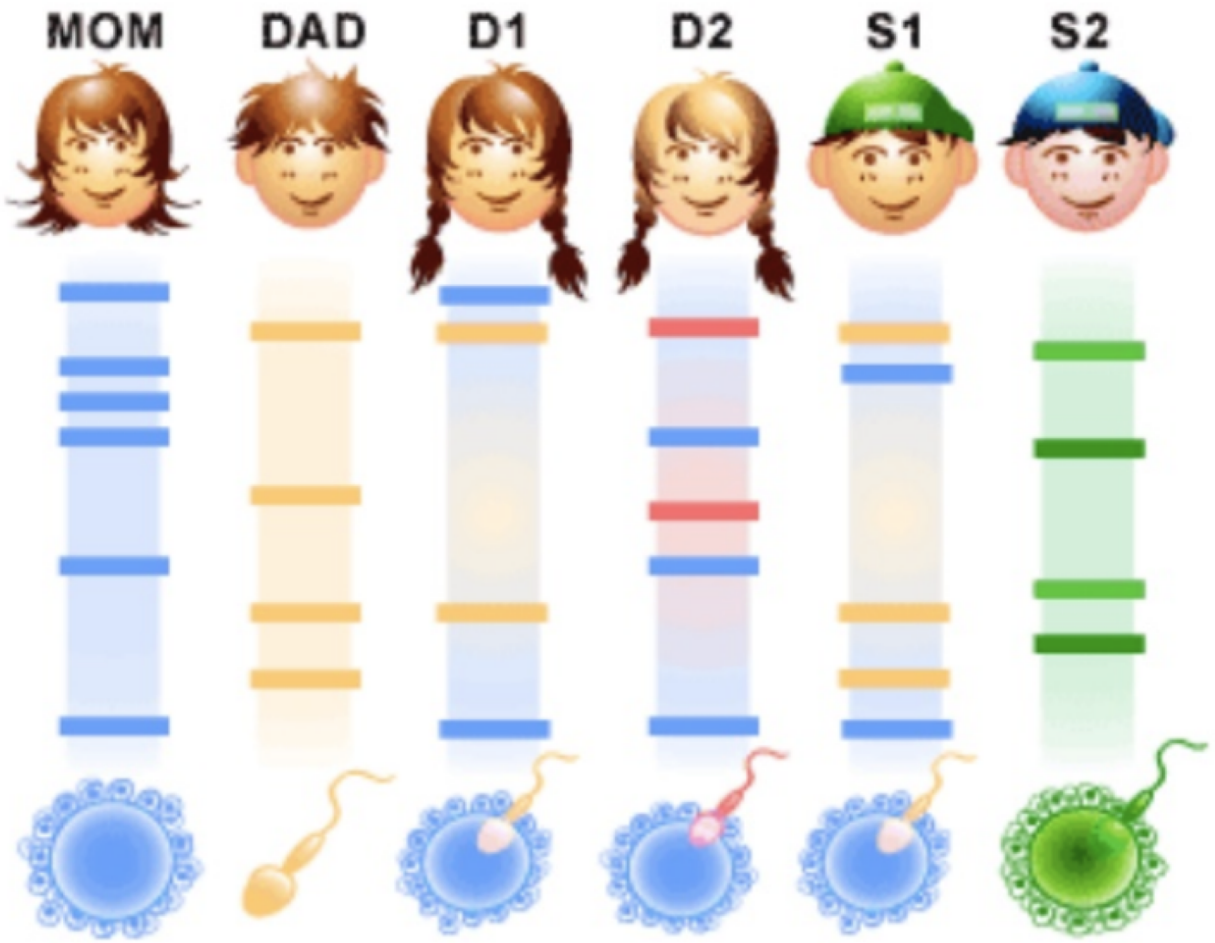
Practical Applications of DNA Fingerprinting

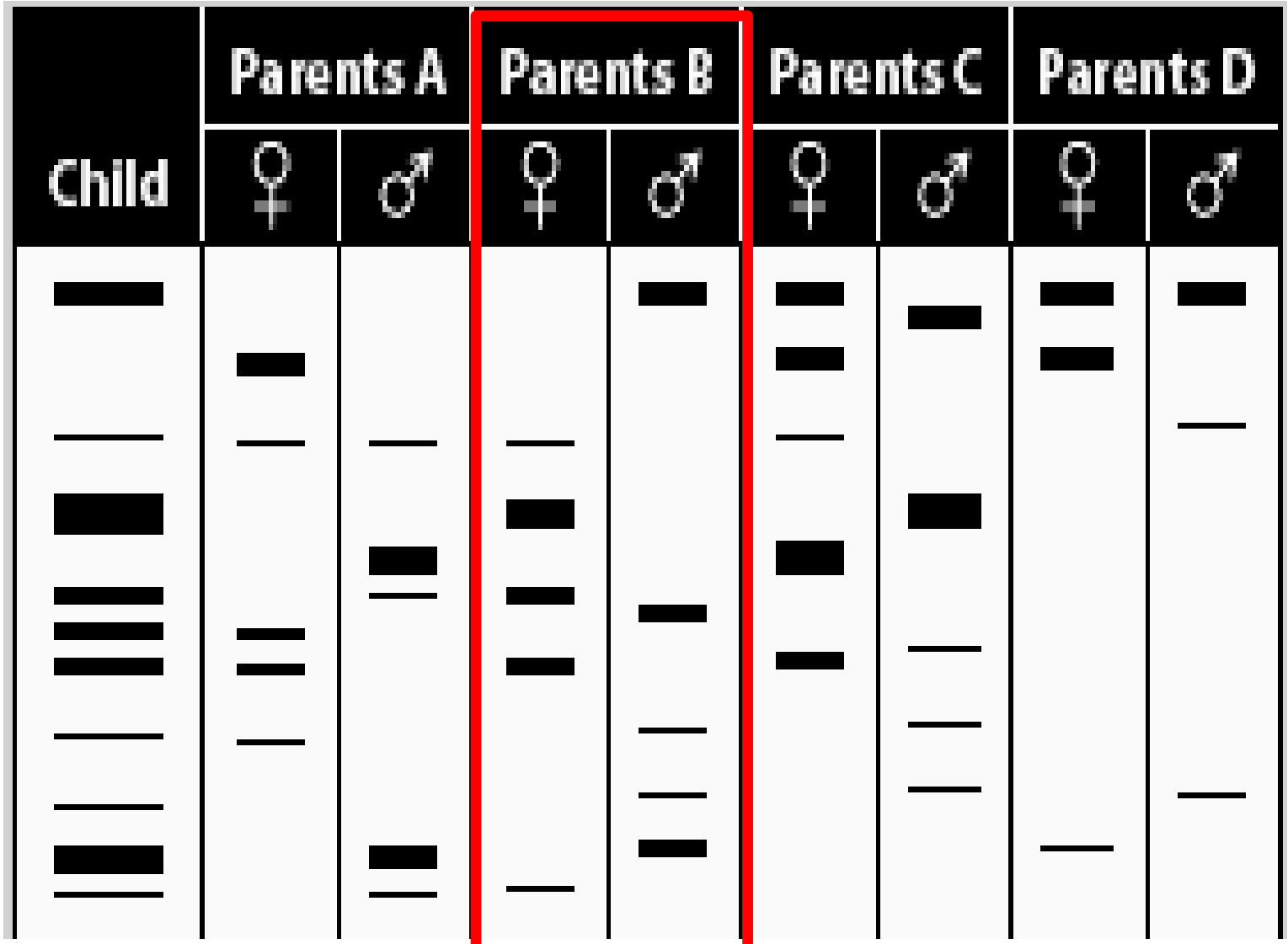
1. **Paternity and Maternity Determination**
   - The child's banding pattern will be a combination of ½ the mother's and ½ the father's
2. **Criminal Identification and Forensics**
   - DNA isolated from blood, hair, skin
3. **Personal** Identification



Who's the daddy?

- Used to identify a child's parents
- Each child inherits one set of chromosomes from each parent





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

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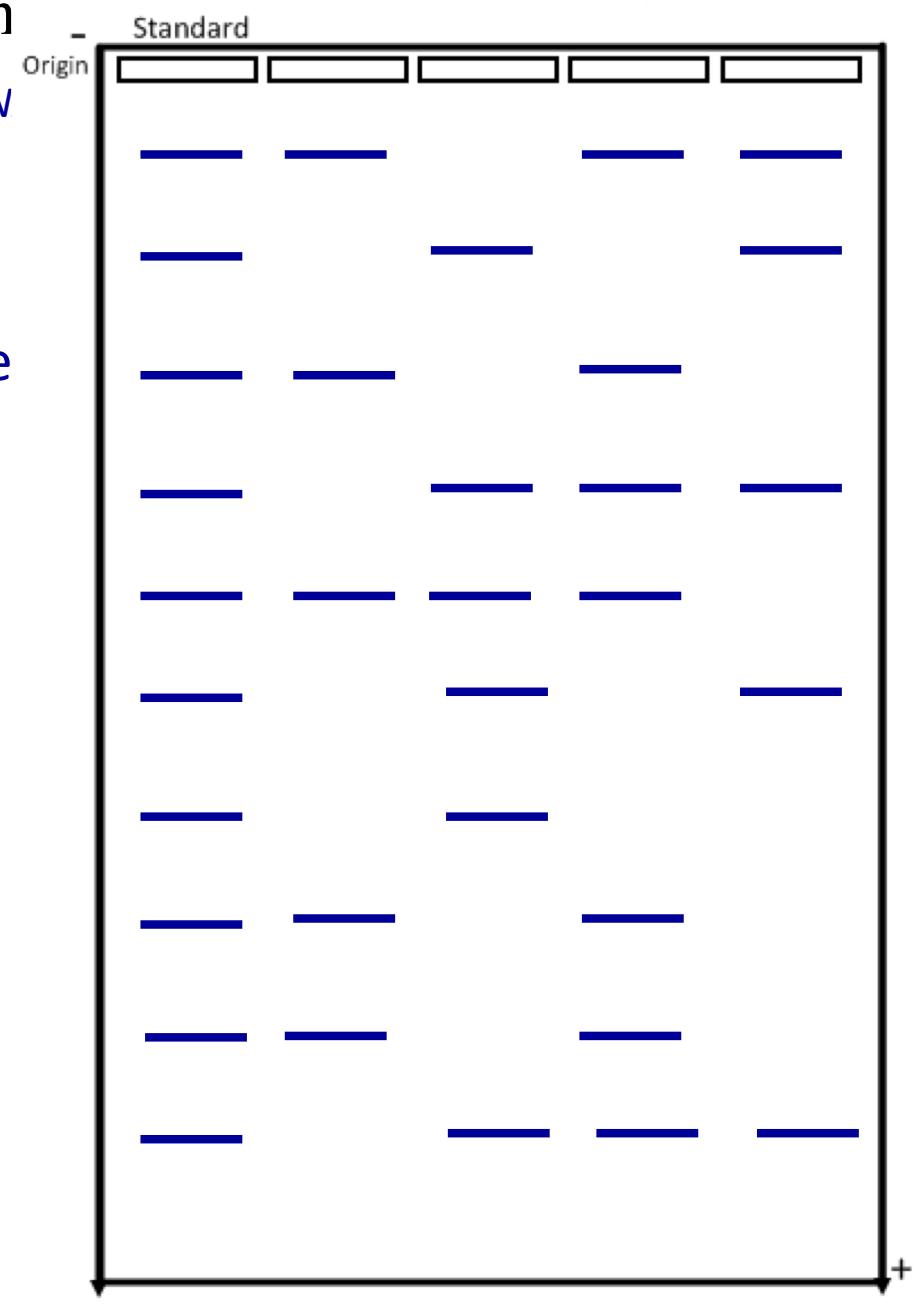
DNA fingerprints from a murder

The defendants bloods does not match the bands from the blood found on his cloths.

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 DN 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

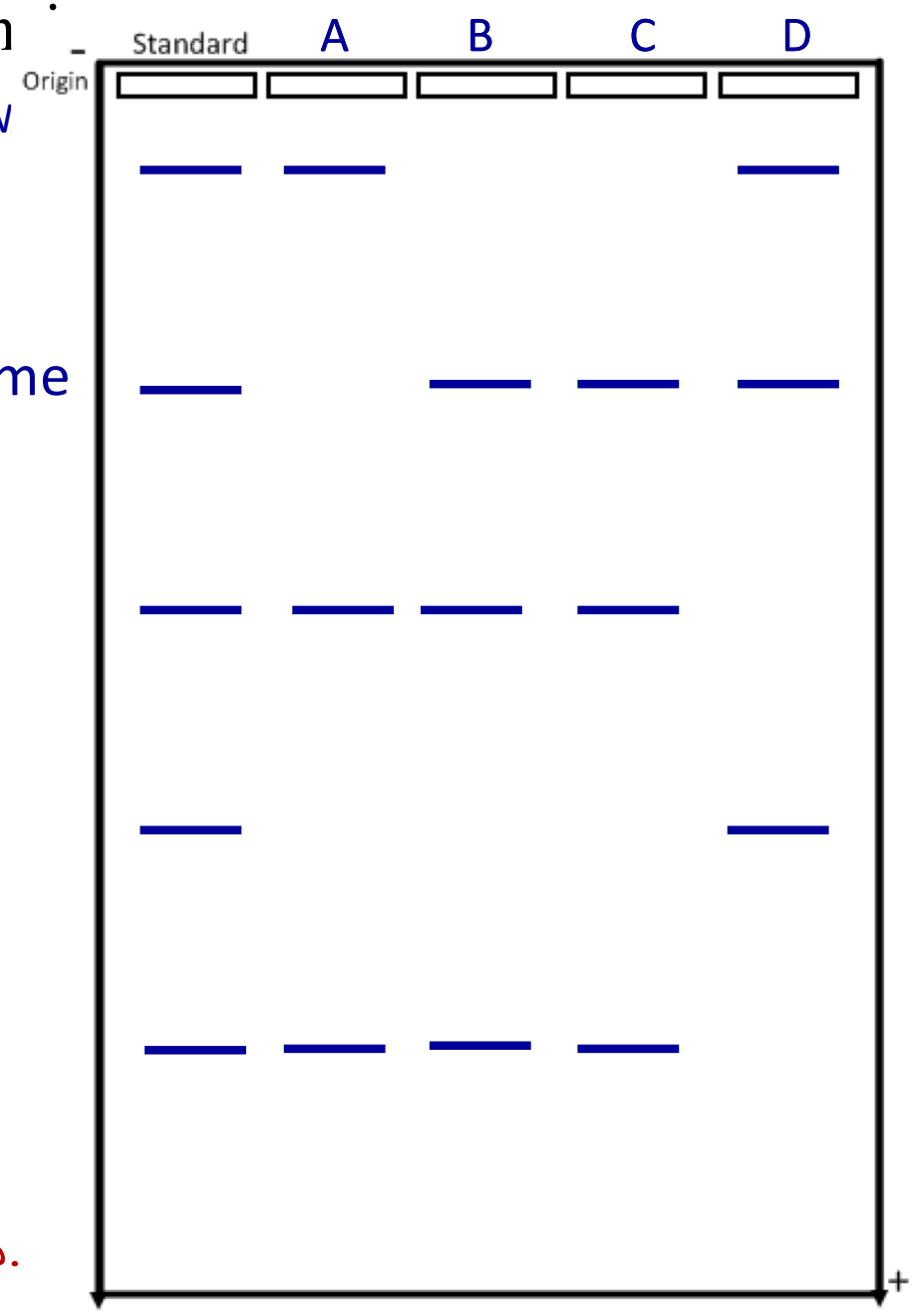
C = suspect 1

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

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

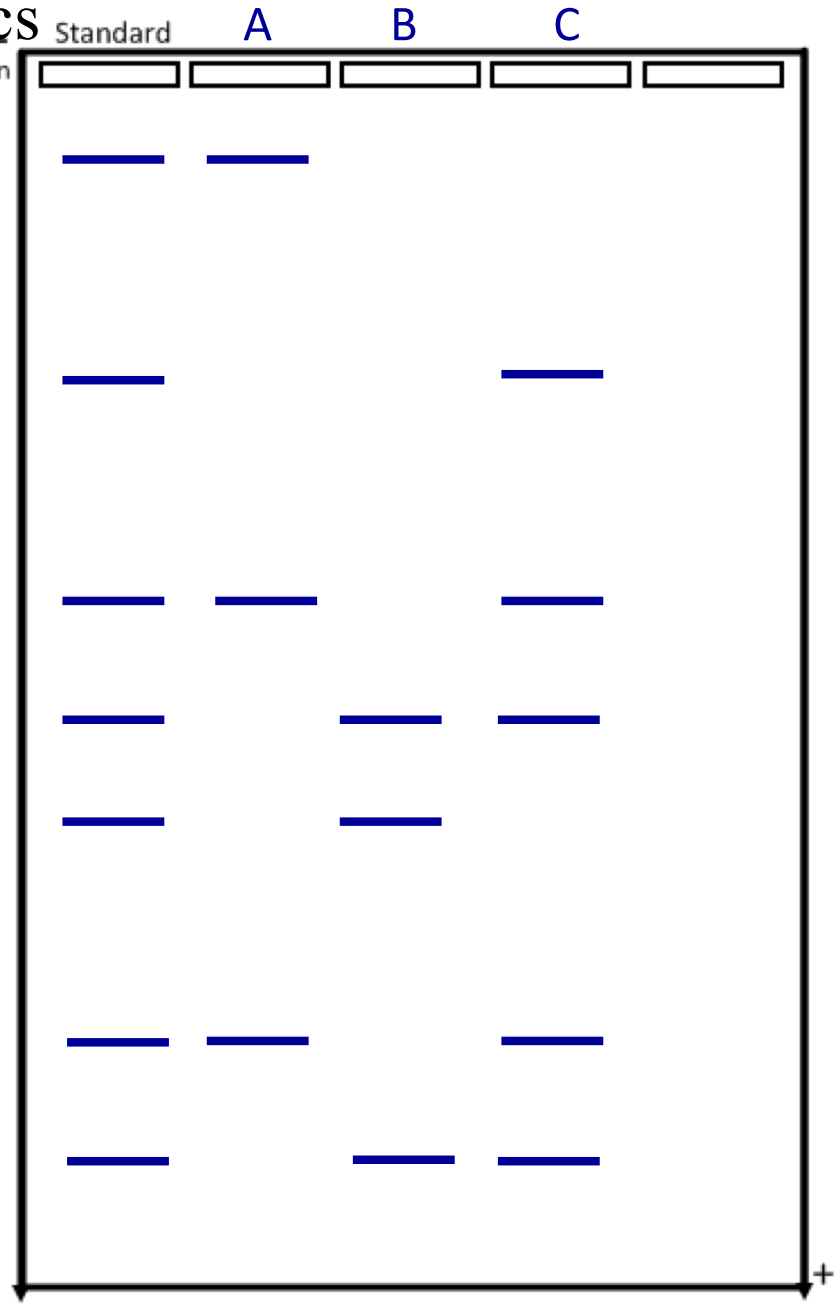
B = homeowner

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.

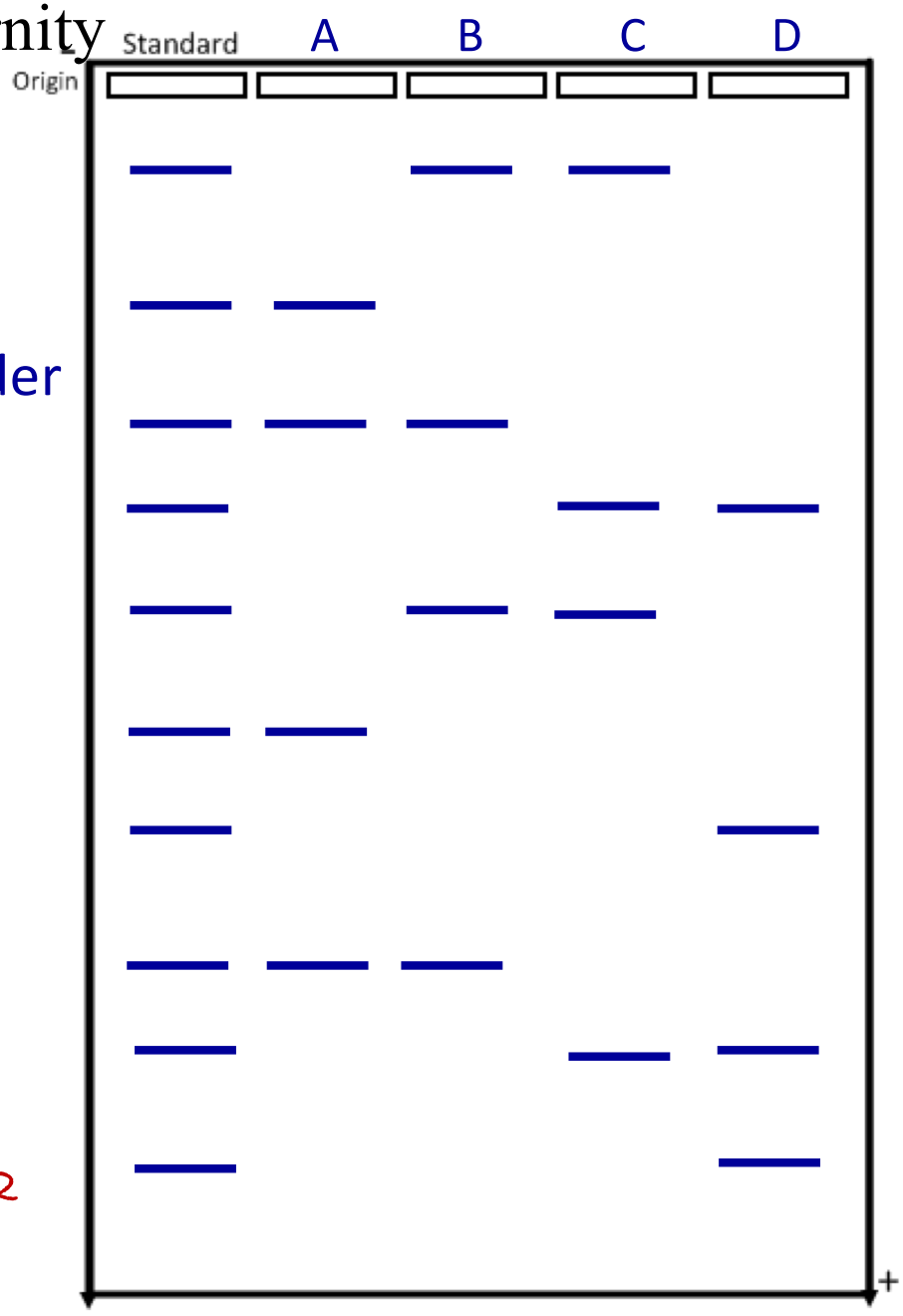
In this case, the parentage of a child is under question. Who's the daddy?

A = mother

B = child

C = man 1

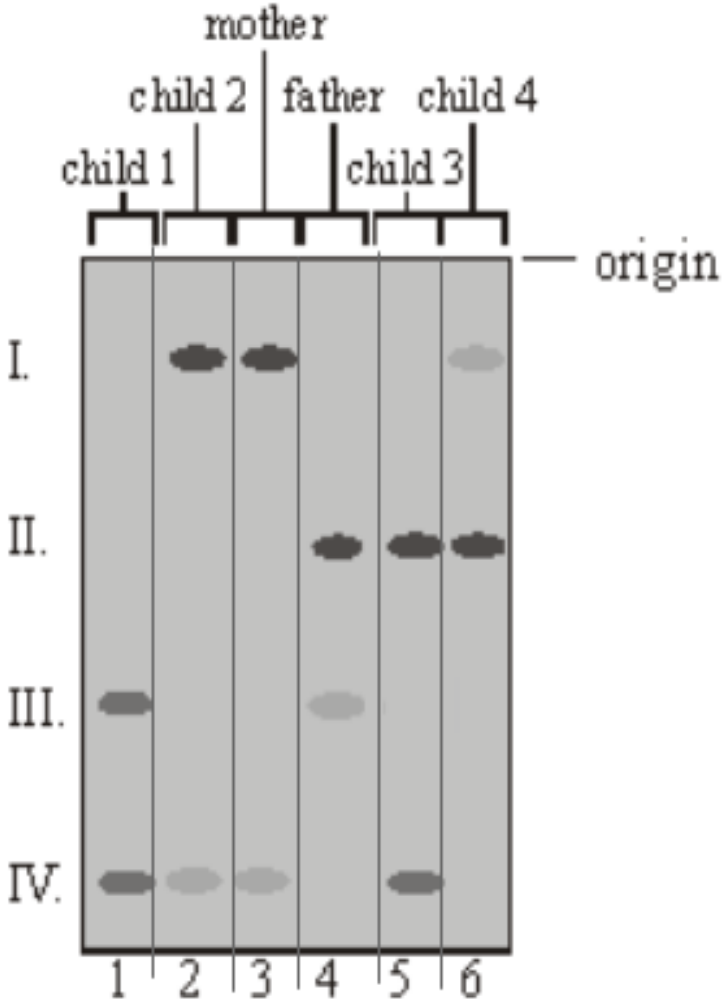
D = man 2



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



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

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

Workbook pg. 13, 14

Biotechnology: Some questions…

- **How safe is recombinant DNA research as we genetically engineer crops to withstand frost and insect pests or add genes for enzymes which prevent fruit from spoiling too fast?**

- **Will these genetically engineered crops be safe for consumption? If herbicide resistance is built into crops will the farmer use more herbicide to get rid of the weeds, and thereby threaten ground water drinking supplies?**

- **How should we care for frozen human embryos? What about parent's who decide that they have enough children and have frozen human embryos which remain? What if parents die and their embryos remain in freezers ?**

- **Will there come a time when we can select which genes will be found in our offspring once all genes are identified by the Human Genome Project?**

- Will employers discriminate against us based on our genetic make-up which might show a predisposition to Alzheimer's disease or cancer or alcoholism? Should our genetic make-up be considered personal information and be protected by Constitutional rights?
- How are we applying what we learn in terms of genetic technology? How are we to control genetic technology? Who will be the decision makers? What research needs to be held in check until ethical issues are studied? Will there be abuse and exploitation of the new technology?