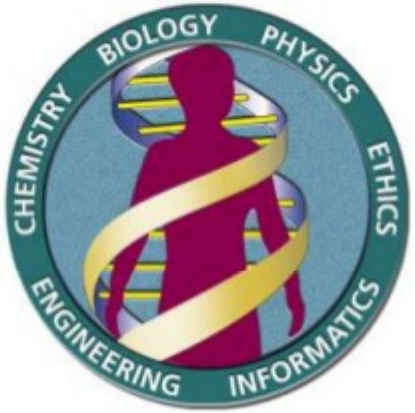


Genetic Engineering & Biotechnology

Genetic Engineering & Biotechnology

'The Genetic Code is Universal'

- Genetic Engineering: The directed alteration of genetic material by intervention in genetic processes.
- Biotechnology: The industrial use of living organisms or their components to improve human health and food production. Often includes genetic engineering.

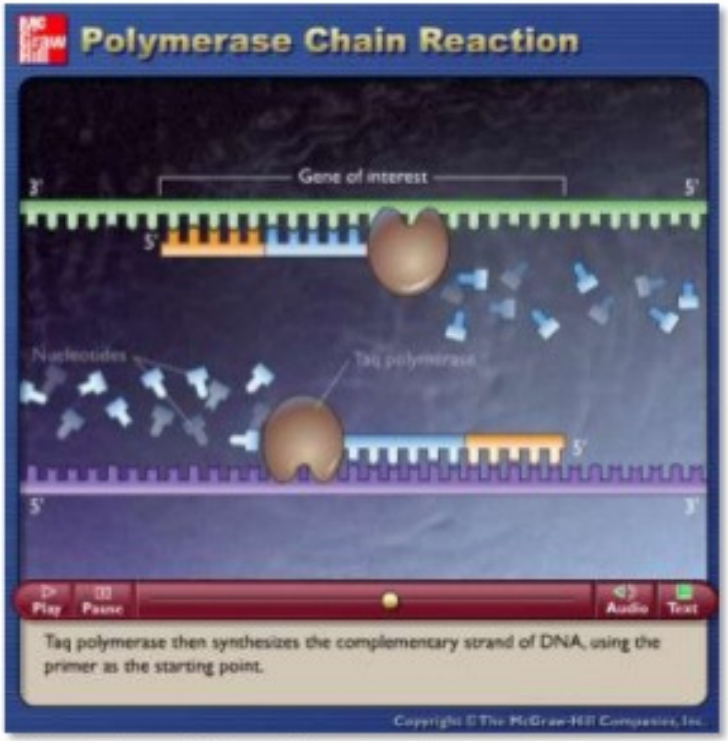
Polymerase Chain Reaction

- *Used to amplify small samples of DNA*
- *In order to use them for DNA profiling, recombination, species identification or other research.*

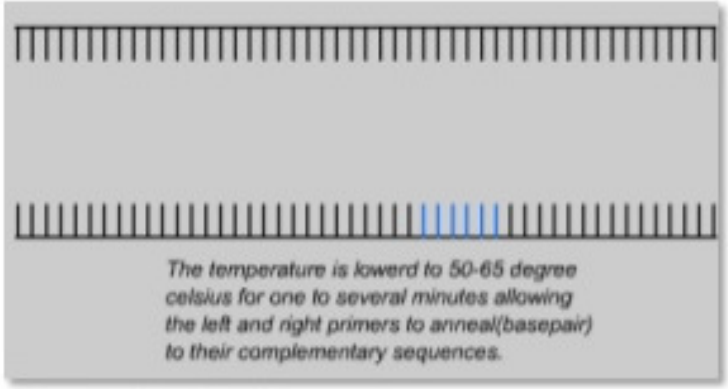
The process needs a thermal cycler, primers, free DNA nucleotides and DNA polymerase.

1. Heating in the thermal cycler denatures hydrogen bonds, exposing bases.
2. The mixture cools. Primers are added to the start of the target genes.
3. DNA Polymerase replicates the DNA using complementary base pairing.
4. This cycle is repeated many times, until there are thousands of copies – enough to amplify even tiny samples found at a crime scene!

You do not need to know details of this method, but can you see how the technology has mimicked the natural process of DNA replication?



Animation from McGraw Hill:
http://highered.mcgraw-hill.com/olc/dl/120078/micro15.swf



Animation from DNAi.org::
http://www.dnai.org/text/mediashowcase/index2.html?id=582

http://highered.mcgraw-hill.com/olc/dl/120078/micro15.swf

PCR Song

There was a time when to amplify DNA,
You had to grow tons and tons of tiny cells.
Then along came a guy named Dr. Kary Mullis*
Who said you can amplify *in vitro* just as well.

Just mix your template with a buffer and some primers,
Nucleotides and polymerases too.
Denaturing, annealing, and extending,
Well it's amazing what heating and cooling and heating will *do -o-o-o*.

PCR: when you need to detect mutation
(detect mutation)
PCR: when you need to recombine
(recombine)
PCR: when you need to find out who the daddy is
(who's your daddy?)
PCR: when you need to solve a crime
(solve a crime)

**Won a Nobel for this (the process, not the song)*

http://www.youtube.com/watch?v=x5yPkxCLads

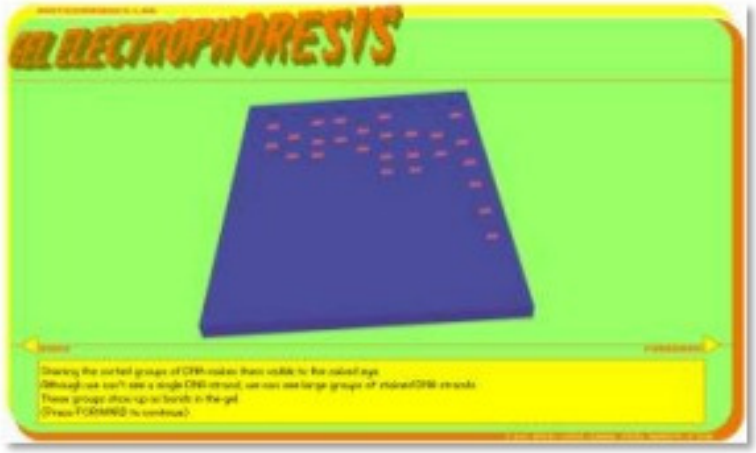
http://www.youtube.com/watch?v=CQEaX3MiDow

- http://www.youtube.com/watch?v=x5yPkxCLads
- http://www.youtube.com/watch?v=CQEaX3MiDow

DNA Profiling

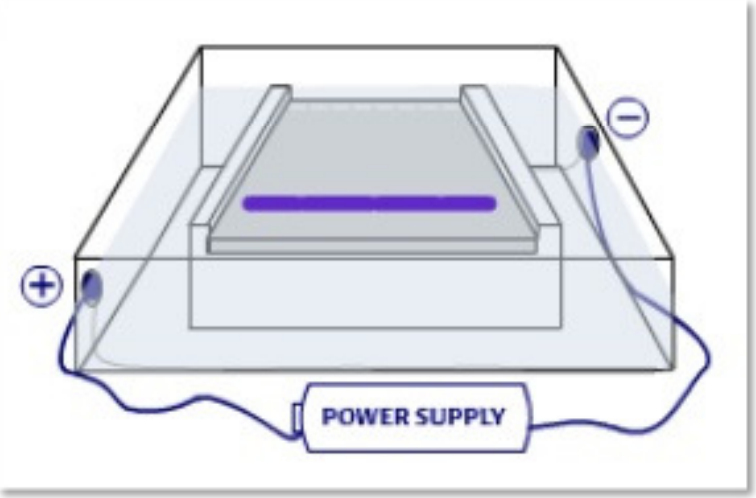
Compares sections of DNA between individuals in order to determine paternity or relationships, as evidence in criminal cases or to identify species.

Through gel electrophoresis, fragments of DNA are moved through an electric field and separated based on their size.



Animation from Learn.Genetics:
http://learn.genetics.utah.edu/content/labs/gel/

1. DNA samples are taken and amplified with PCR.
2. **Restriction enzymes** cut DNA into fragments at specific base sequences in each sample.
3. A fluorescent marker binds to a triplet in the DNA fragments, so that results can be seen.
4. Samples are added to a gel electrophoresis chamber. **Electric current** is passed through, pushing the fragments along.
5. Heavier fragments stay closer to the origin and smaller fragments go further.
6. A banding pattern shows up for each DNA sample and can be compared.



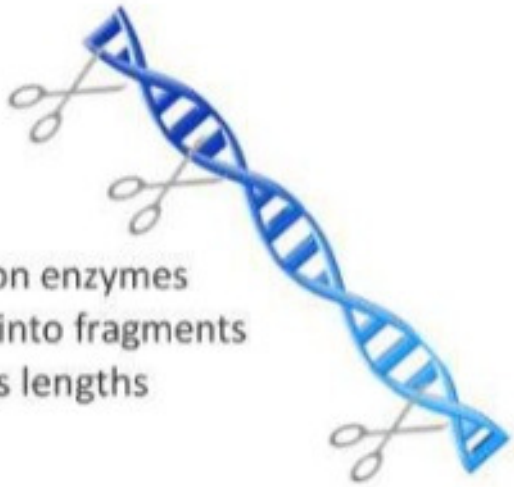
Animation from Dolan DNA Learning Centre::
http://www.dnalc.org/resources/animations/gelelectrophoresis.html

- http://learn.genetics.utah.edu/content/labs/gel/
- www.DNAIC.org

DNA Profiling: Gel Electrophoresis

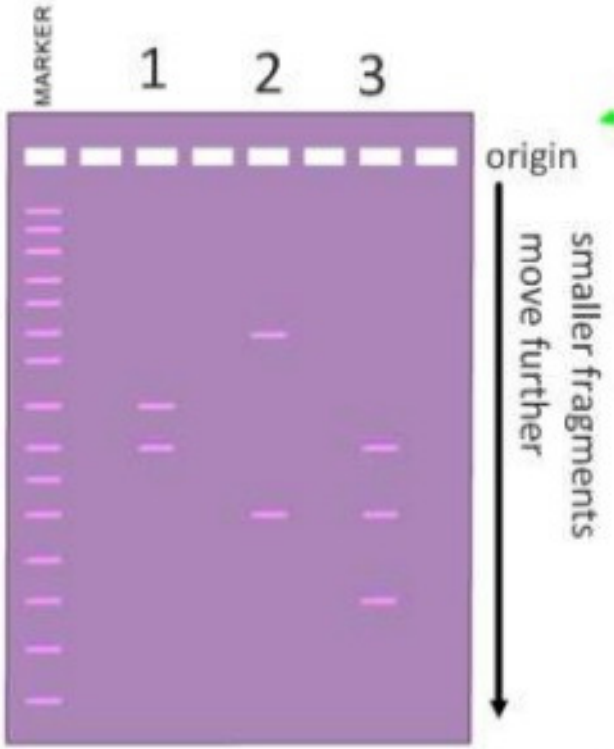
DNA sample is taken

PCR amplifies DNA to get a useful amount



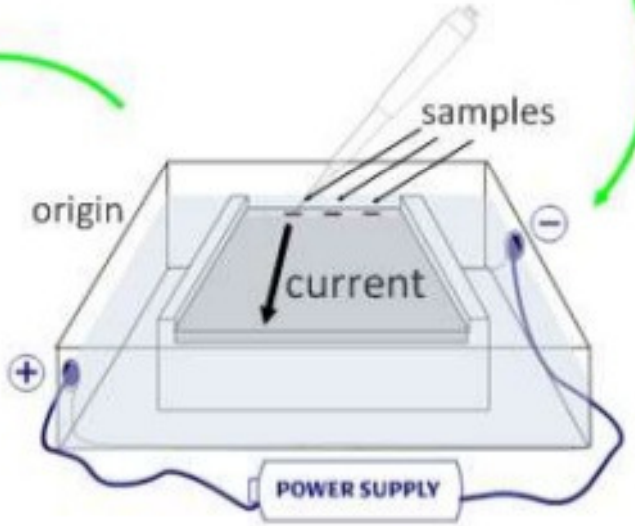
Restriction enzymes cut DNA into fragments of varous lengths

Marker (or standard) is used to show all the possible DNA fragments.



A 'tag' can be added to bind to fragments at certain base sequences. This will glow under fluorescent light and gives a series of bands which can be compared as the results of the DNA profile.

We may be looking for a number of shared bands (e.g. paternity), or a total match (crime scene evidence)



Samples are added to wells at the origin end of the electrophoresis gel.

Images from Dolan DNA Learning Centre::
http://www.dnalc.org/resources/animations/gelelectrophoresis.html

- http://www.dnalc.org/resources/animations/gelelectrophoresis.html

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.

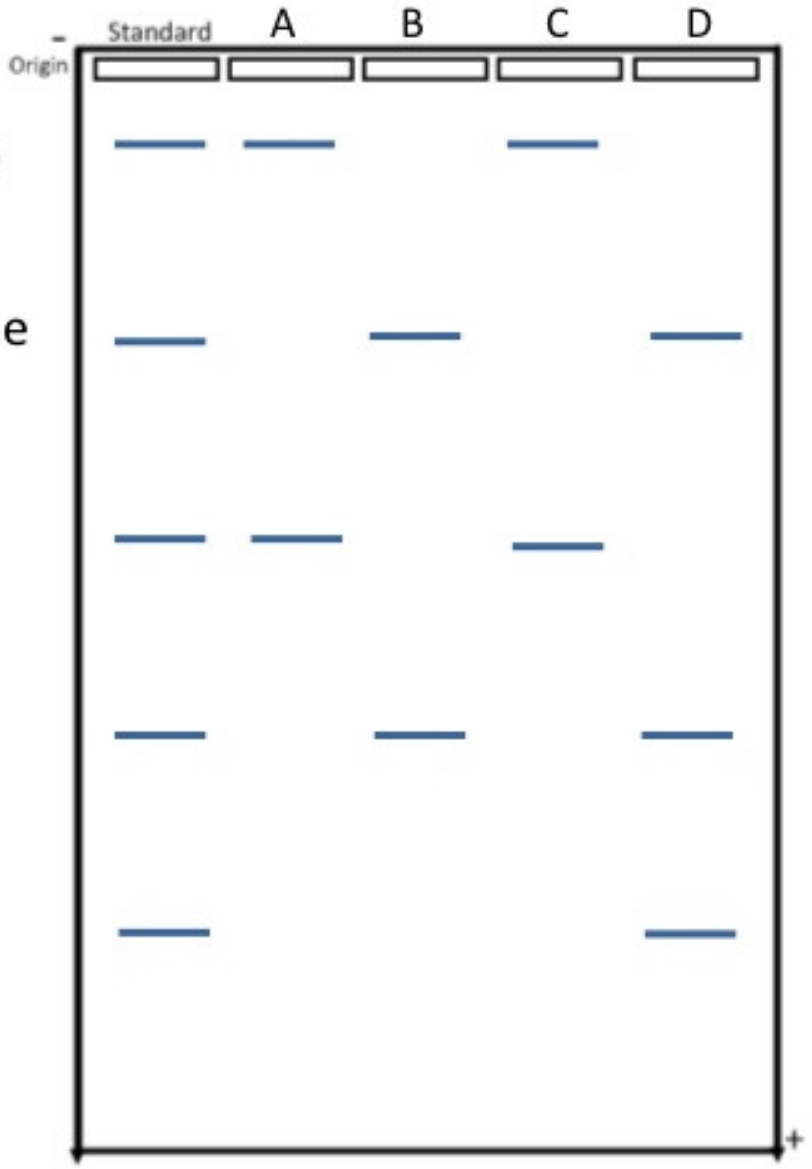
In this case, a hair follicle was left at a scene of a crime. Who was the perpetrator?

A = trace evidence

B = homeowner

C = suspect 1

D = suspect 2



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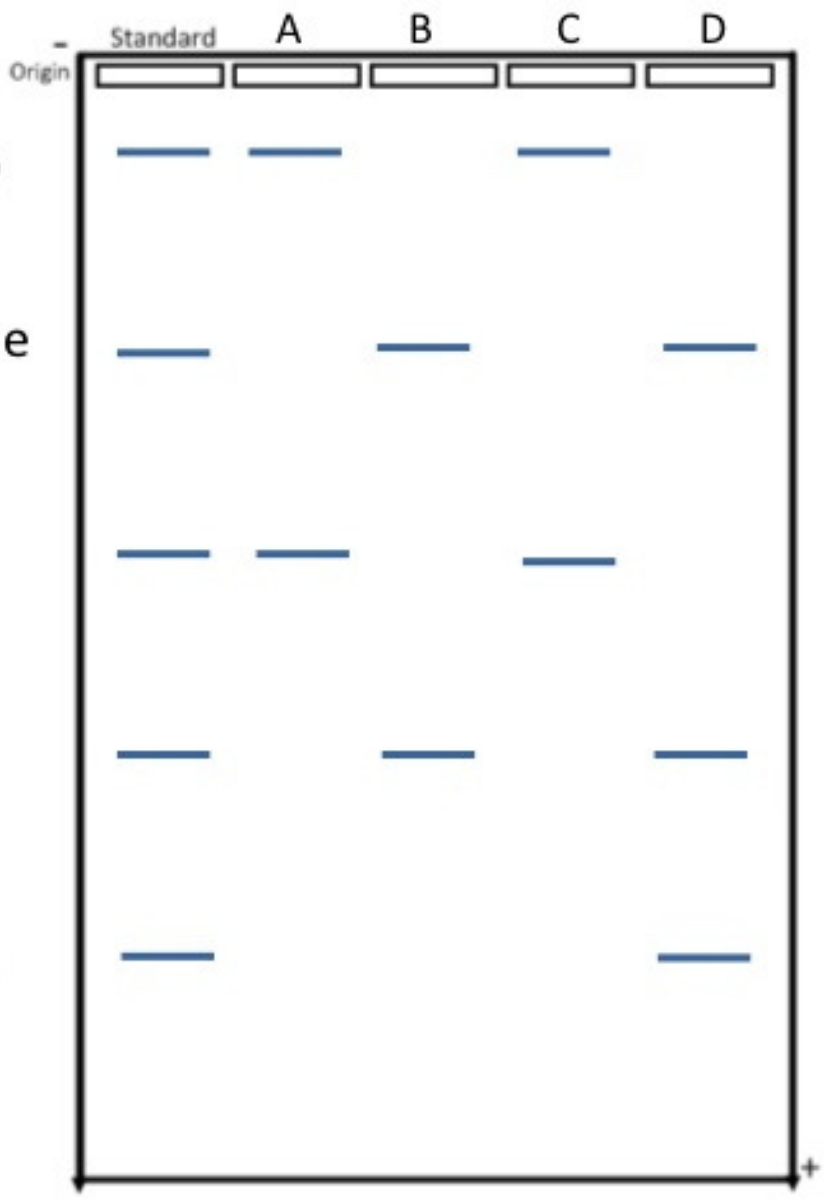
D = suspect 2

Explanation:

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



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.

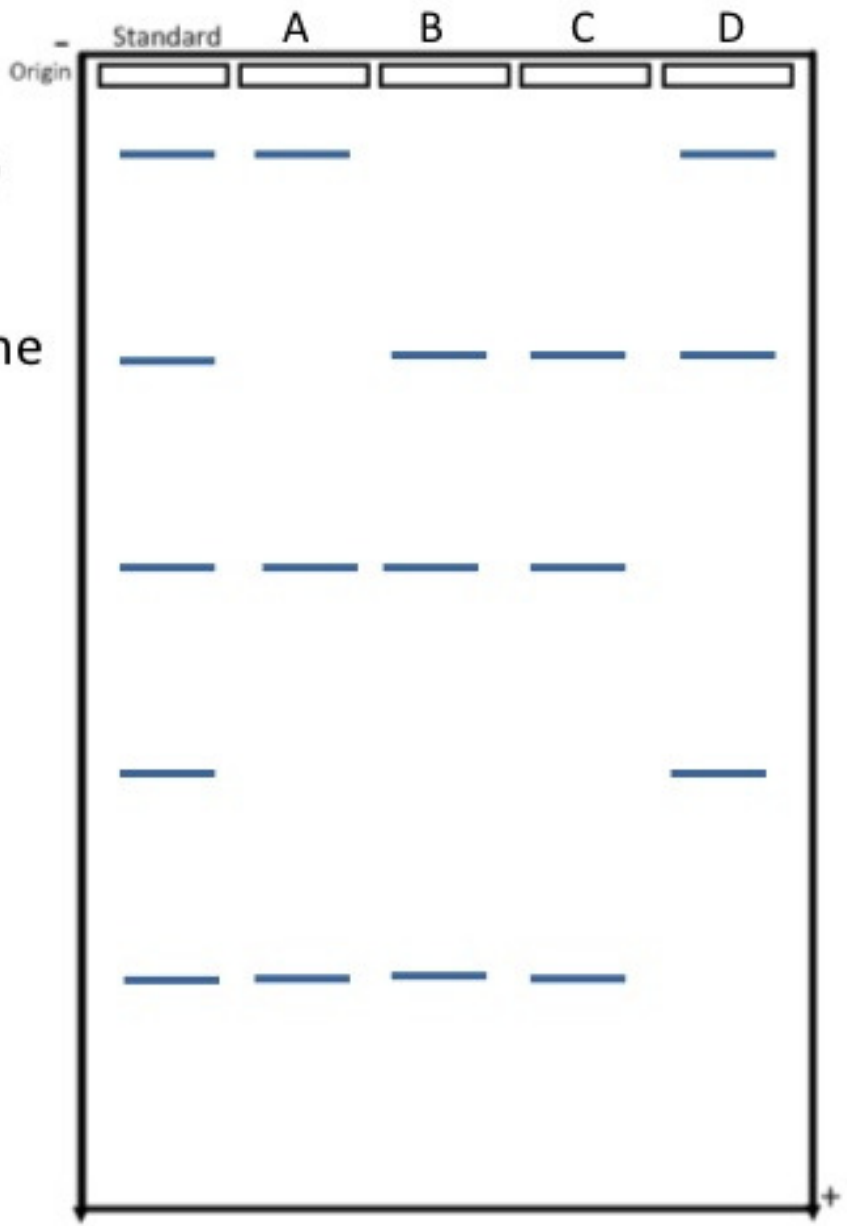
In this case, a lot of blood was left at a crime scene. Who was the perpetrator?

A = victim

B = unknown blood at scene

C = suspect 1

D = suspect 2



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

A = victim

B = unknown blood at scene

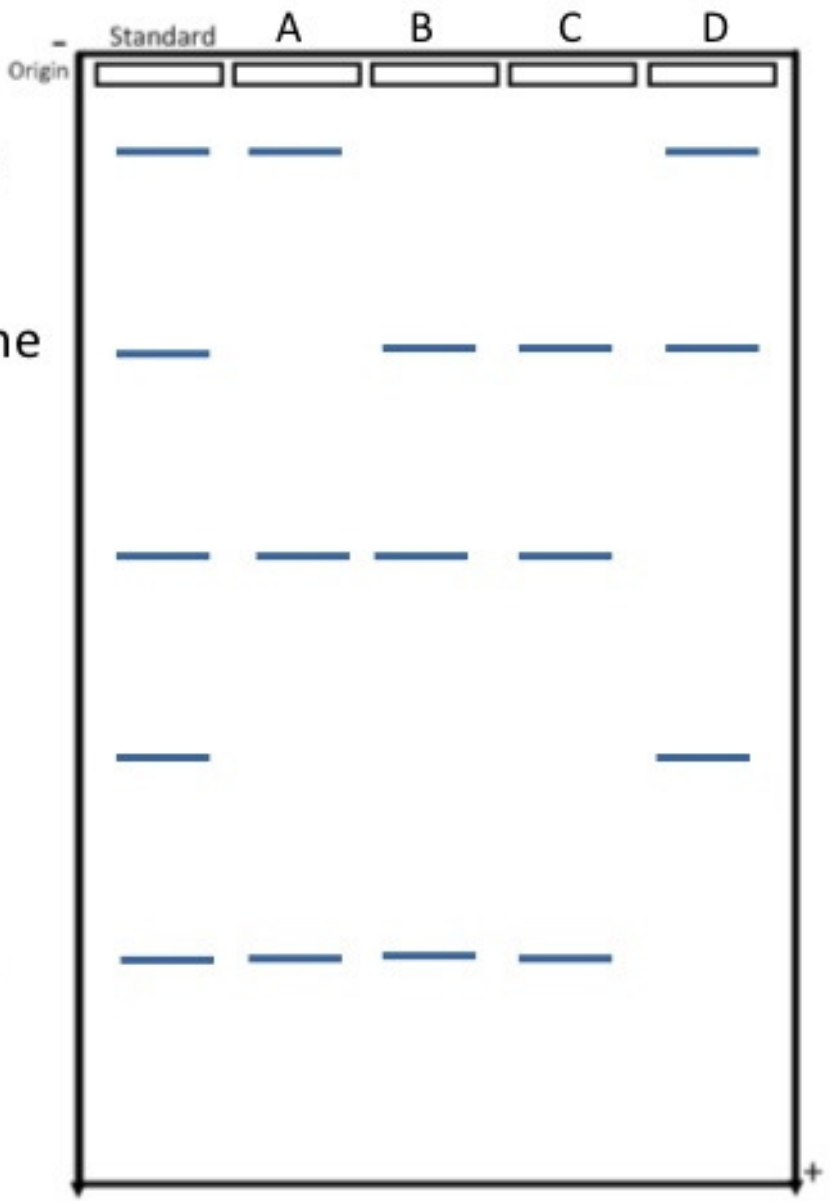
C = suspect 1

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



DNA Profiling in forensics

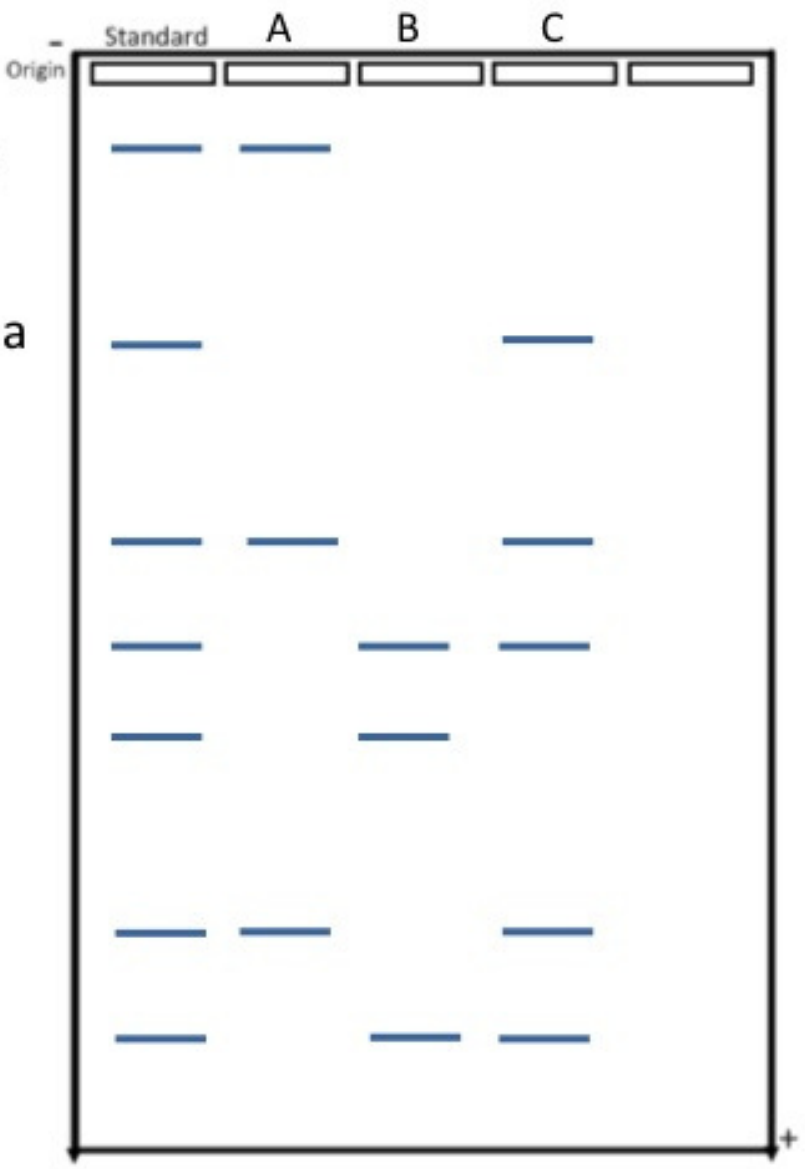
DNA Profiling can be used to identify suspects from trace DNA evidence. It can also be used to eliminate the innocent from the investigation.

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



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

A = trace evidence

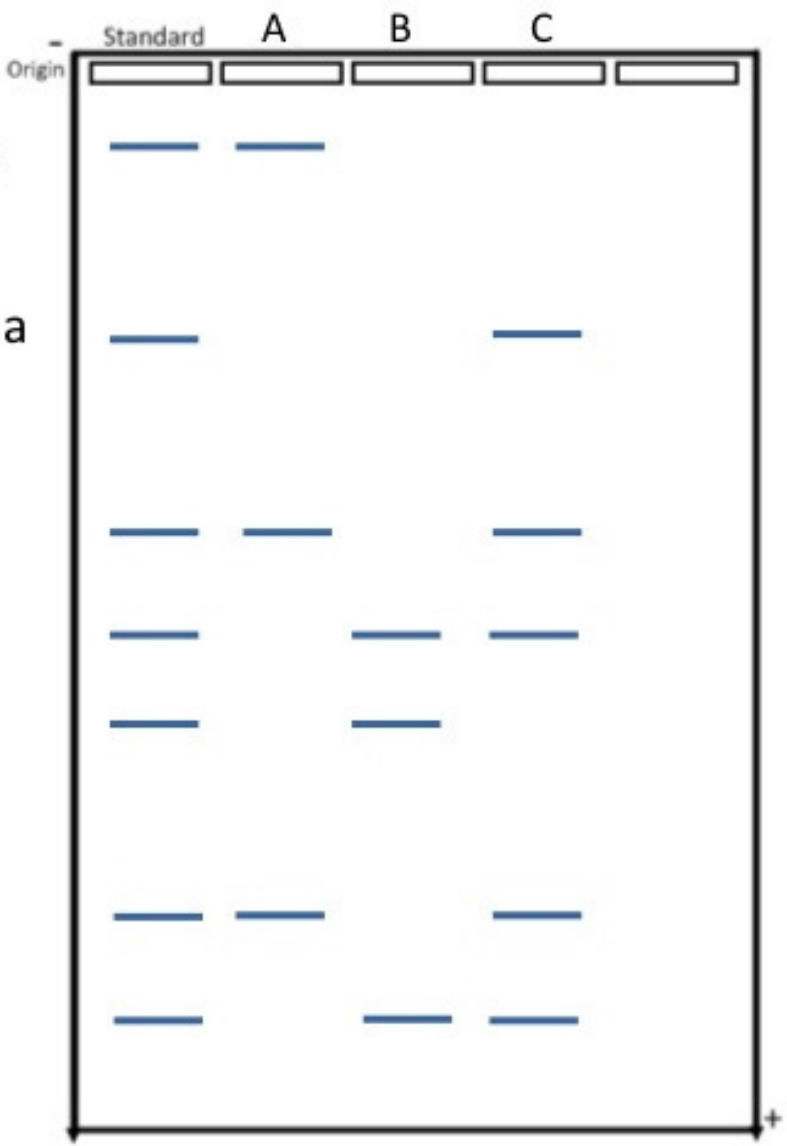
B = homeowner

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.

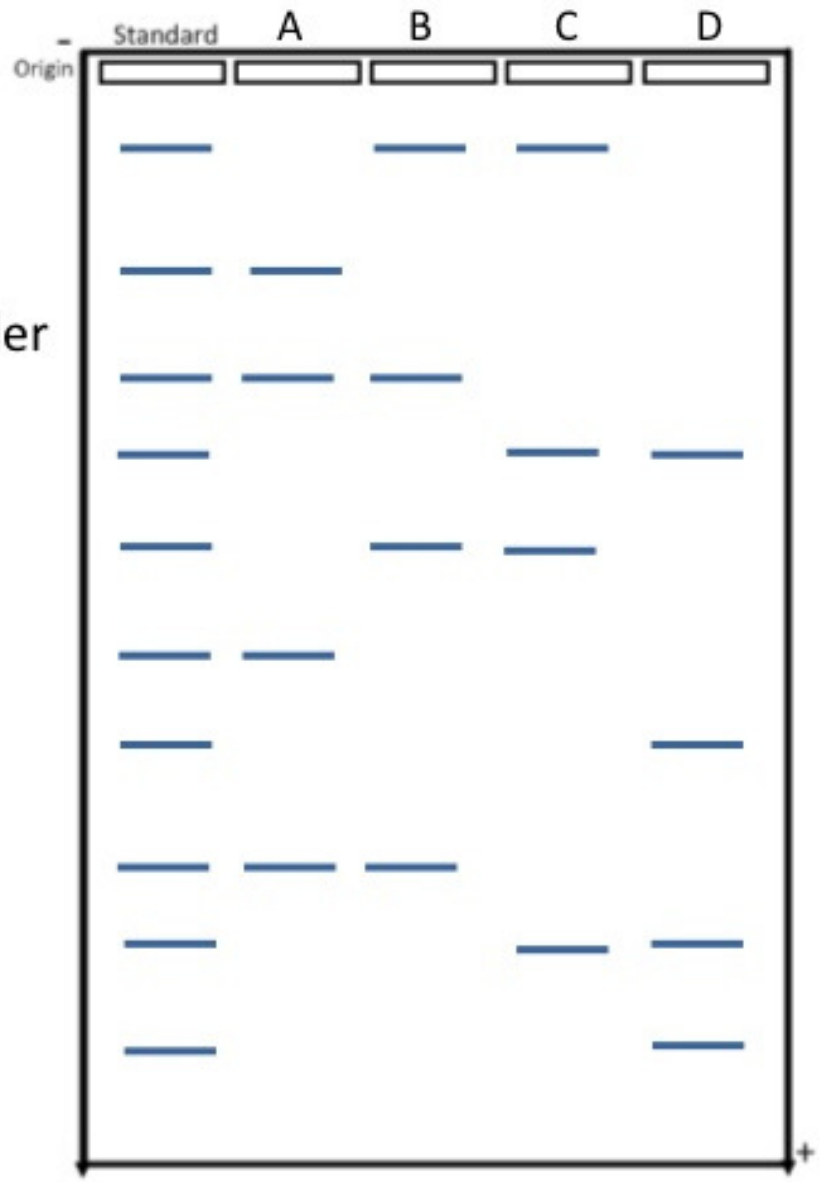
In this case, the parentage of a child is under question. Who's the daddy?

A = mother

B = child

C = man 1

D = man 2



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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

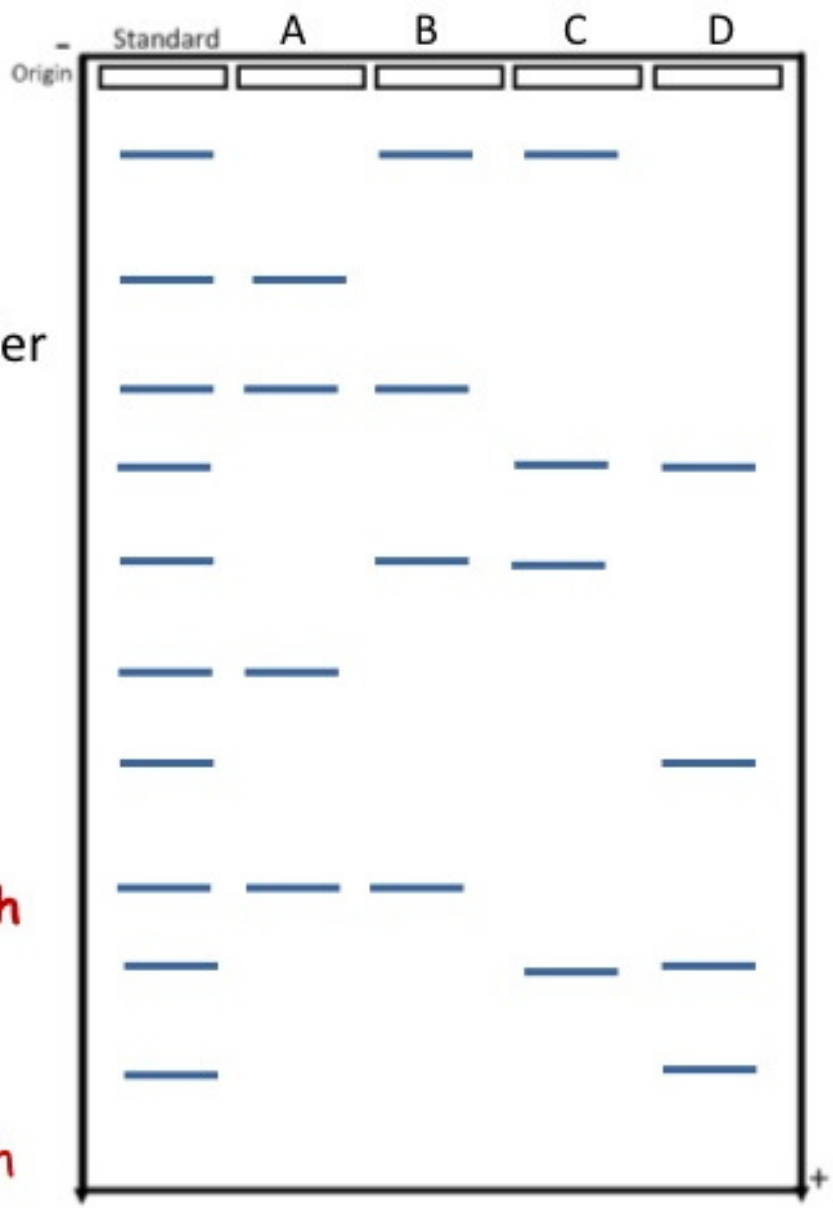
B = child

C = man 1

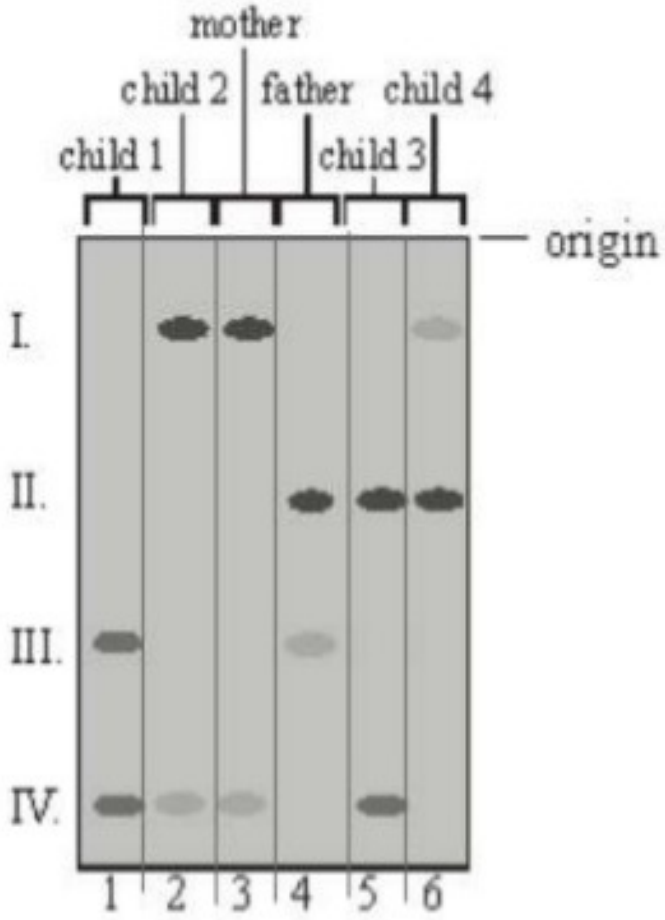
D = man 2

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.

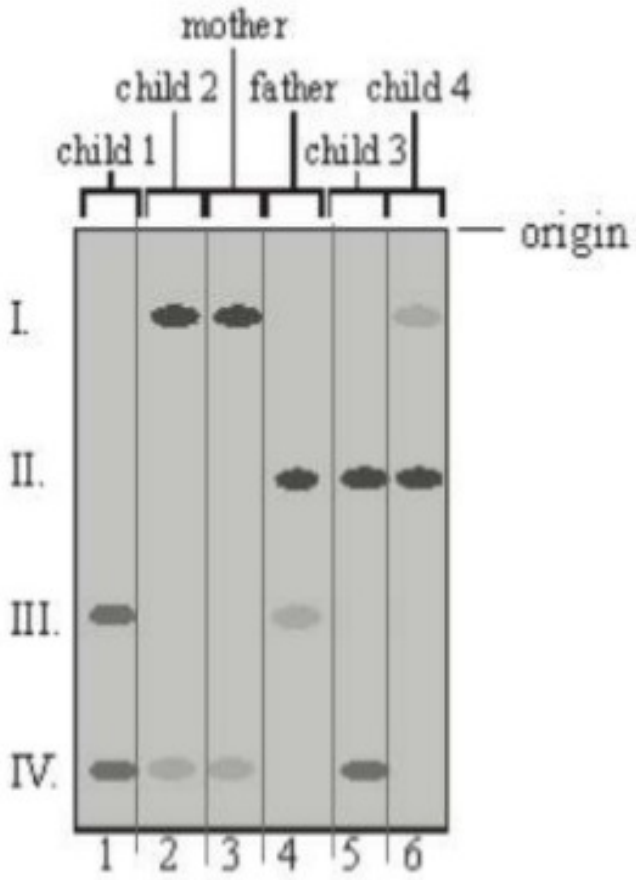
2. State the number of bands that would appear in the 'standard' lane.

   2 3 4 5 6

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

   1 2 3 4

Sample Questions



1. Identify the smallest DNA fragment.

   I. II. III. (IV.)

2. State the number of bands that would appear in the 'standard' lane.

   2 3 (4) 5 6

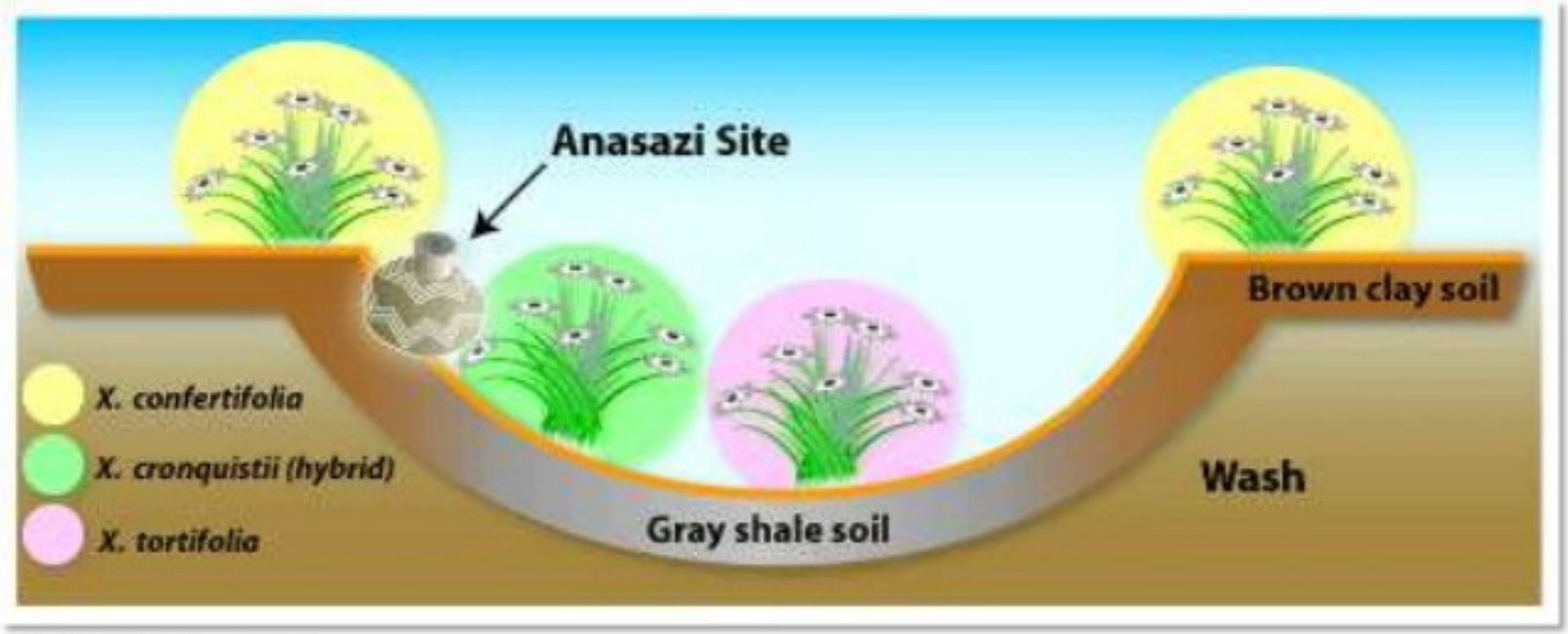
3. Identify the child which is most likely to be from the mother's previous marriage.

   1 (2) 3 4

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

Mystery of the Pot Hunters

Try this interesting case study in another way in which DNA profiling evidence can be used in a criminal case.



Case Study from Learn.Genetics:
http://learn.genetics.utah.edu/archive/mystery/

- http://learn.genetics.utah.edu/archive/mystery/

Explain how biotechnology is used to determine the identity of a criminal from a small blood sample left at a crime scene.

(8 marks)

“DNA evidence is better at proving innocence than guilt.”

Discuss.

Human Genome Project

Completed in April 2003, the HGP was an international, collaborative effort to record the entire base sequence of the human genome.
Aside from international cooperation and information sharing, the HGP achieved many landmark feats in Science:

- The number and loci of all the genes in our genome were found ('only' 30,000 or so) - which has led to targeted research in diagnostics, treatment and pharmacology.
- Many new proteins and their functions were discovered.
- DNA comparisons can be made with other species and we can find out a lot more about the evolutionary history of us and other species.
- Bioinformatics was born - high-tech way to collect, collate and access information from genetic databases.

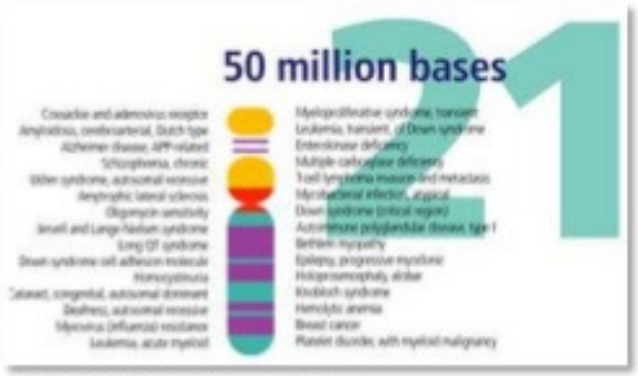
Videos & resources from Genome.gov:
http://www.genome.gov/19519278

- http://www.genome.gov/19519278

Human Genome Project

has pushed forwards medical and pharmacological research
- by helping us see the 'real' cause of many illnesses.

Many more diseases than we first thought are rooted in genetic causes
- including lung cancer, obesity and learning disabilities.



Free genome poster from ORNL:
http://www.ornl.gov/besc_lims/

By opening up the human genome and locating the genes which are at fault in the case of many of these diseases, we can know who is more at risk of certain conditions.

This allows for faster diagnosis, genetic 'report cards' to show potential risk of future illnesses and provides genetic targets for new medicines.

In pharmacogenomics, blood samples are taken from patients with the same conditions and tested for gene variants linked to positive or negative reactions to a drug - then the healthcare professionals can make a safer decision on who should be given a certain treatment.

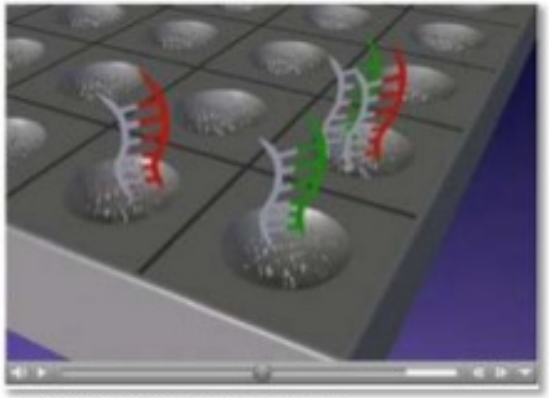
Pharmacogenomics, from Genome.gov:
http://www.ornl.gov/besc_lims/

Human Genome Project

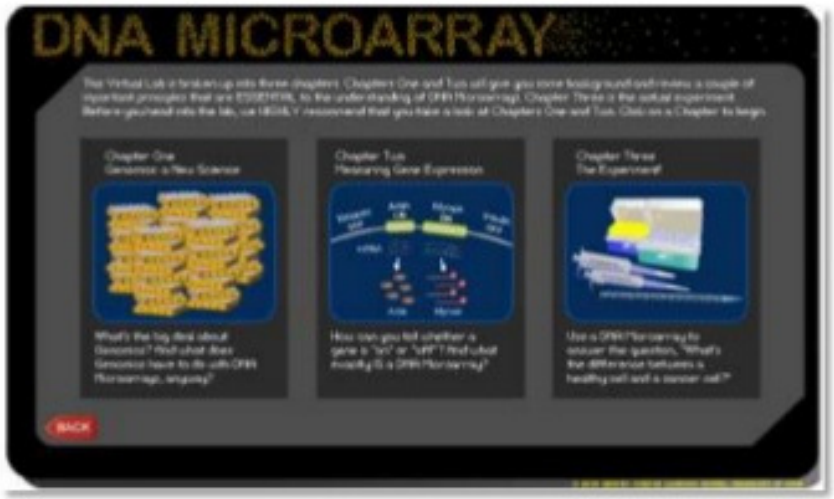
has given rise to Bioinformatics (or *Genomics*)
- looking at the whole genome at once!

By scanning a whole set of genetic markers, research and diagnosis can now be completed much more quickly.

This allows for:
- early detection of illnesses
- testing multiple genes at the same time
- information about potential future illnesses by identifying alleles associated with risk (such as Alzheimer's, Parkinson's disease, stroke).



Find out more about genomics::
http://www.learner.org/courses/biology/units/genom/index.html

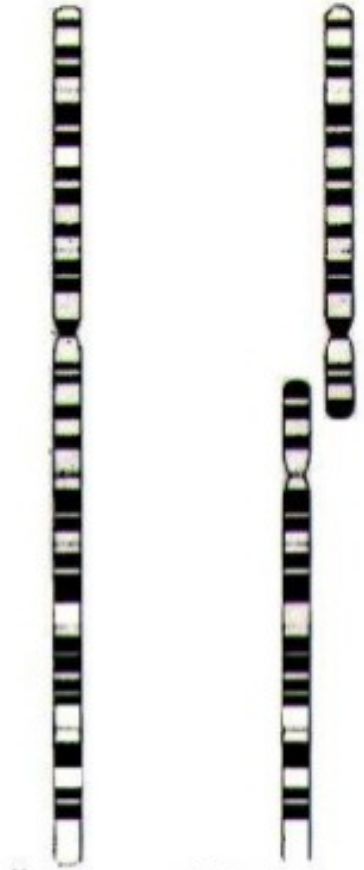


Try this virtual lab from Learn.Genetics:
http://learn.genetics.utah.edu/content/labs/microarray/

- http://learn.genetics.utah.edu/content/labs/microarray/
- http://www.learner.org/courses/biology/units/genom/index.html

Human Genome Project

has given us greater insights into evolutionary relationships.



Human Chromosome 2

Chimp Chromosome 2p and 2q

What separates us from chimps? Not much, really:
http://www.evolutionpages.com/images/hum_ape_chrom_2.gif

By sequencing and databasing genes, we can see similarities and differences between species.

The closer the genome matches between species, the closer their relationship in terms of evolution. As mutations accumulate over time, genomes become more divergent.

Human chromosome number 2 is actually a fusion of two ancestral chromosomes.

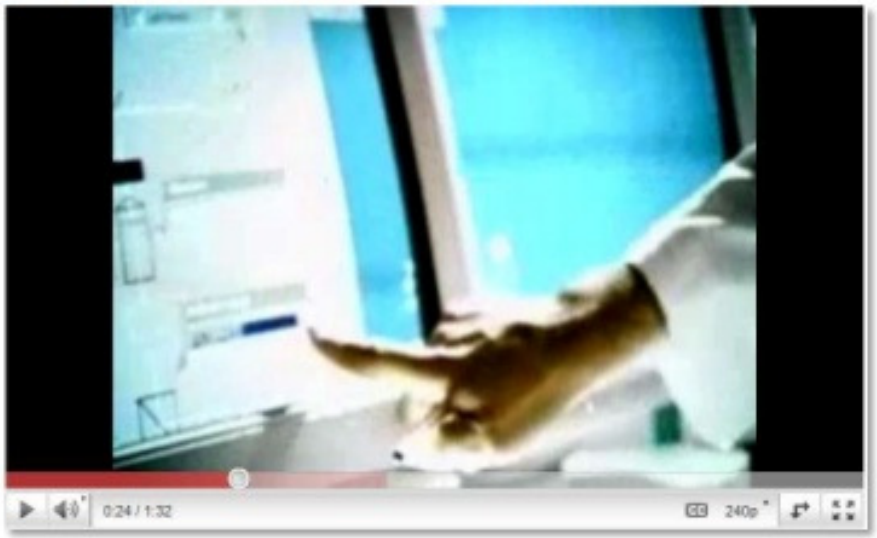
Ken Miller video clip:
http://www.youtube.com/watch?v=zi8FfMBYCkk

TIME TREE
THE TIMESCALE OF LIFE

When did we last share an ancestor with other taxa?
http://www.timetree.org/index.php

Human Genome Project

Ethical, Legal and Social Implications (ELSI).



ELSI of the Human Genome Project:
http://www.youtube.com/watch?v=gkQJ26DAxfs

Positive impacts:

- quick diagnosis through genomics
- identification of new diseases
- targeted production of new medicines

Ethical, Legal and Social Implications:

- genetic report cards of risk
- access and privacy issues
- insurance premiums

At this stage in research, it is important that research is regulated and protected, as well as the rights of those whose DNA is used.

Discussions:

- If you knew that a member of your family had a rare genetic disorder and that you could be tested for it quickly and easily, would you do it? Why?
- If you were invited to share your genome with researchers in the hope of finding cures for genetically-based illnesses, would you do it? Why?

What separates us from chimps? Not much, really:
http://www.evolutionpages.com/images/hum_ape_chrom_2.gif

- http://www.youtube.com/watch?v=gkQJ26DAxfs

Human Genome Project

Ethical, Legal and Social Implications (ELSI).

Gene Patenting?

Robert Cook-Deegan xplains a gene-patenting case in the news:
http://www.youtube.com/watch?v=R2RfApBy5jw

We have run into some sticky legal cases where companies have patented genes they have discovered, which limits the work of others who are researching the same genes.

Read more about these cases at BadScience.net:
http://www.badscience.net/2010/04/i-patent-your-ass-and-your-leg-and-your-nostril/

Debate it here:
http://debatepedia.idebate.org/en/index.php/Argument:_Gene_patent_monopolies_impair_research_and_development

2009: BRCA1 and BRCA2 Genes Patented

- Myriad aimed to block other companies working on their *intellectual property*
- What are the ethical, legal and social implications of this patent?
- Who are the stakeholders in this debate?

2010: Patent overturned

- Might open the gate to all researchers
- Should open research and market fields
- Still to be appealed

Discussions:

- Do you feel that gene patenting should be allowed? Why?

- http://www.youtube.com/watch?v=R2RfApBy5jw

Human Genome Project

Outline three outcomes of the sequencing of the complete human genome.

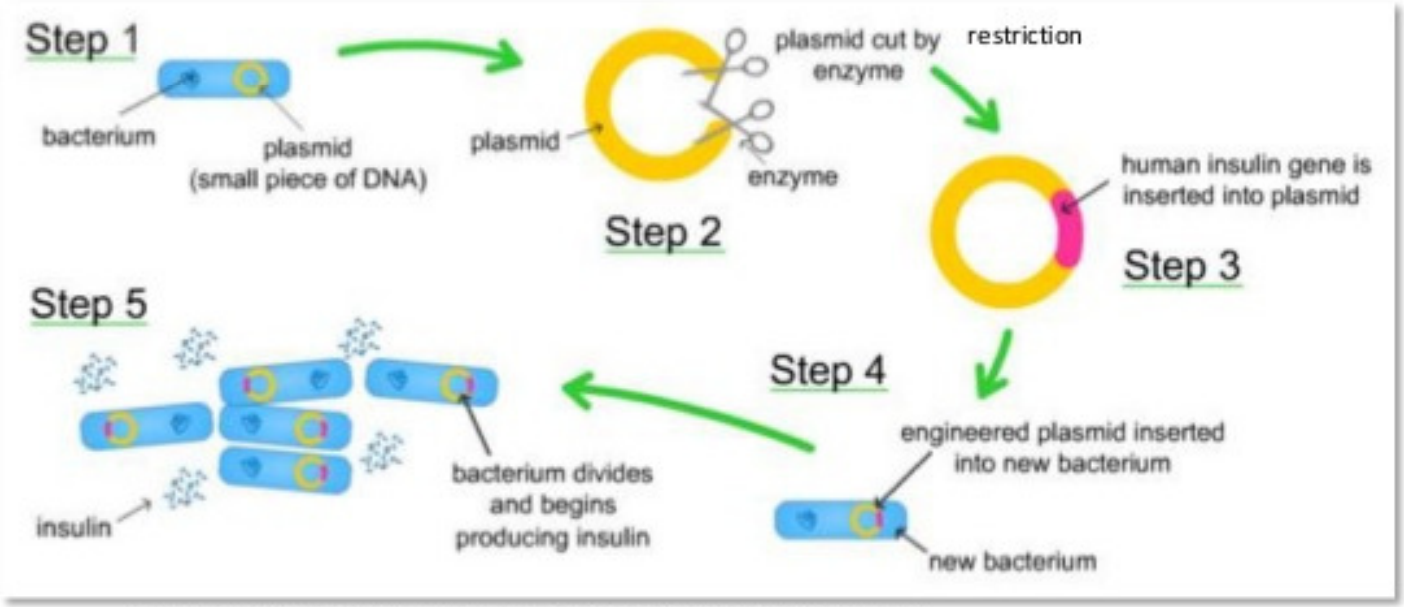
Outcome	Outline
Knowledge of the number and location of human genes	There are around 30,000 genes in the human genome. This is fewer than expected, so there must be more complex relationships between genes. Knowledge of gene loci allows for targeted research and diagnostics, as well as therapies.
Discovery of proteins and their functions	We know of more proteins and their functions, as well as the base sequences which code for them. Therefore, we can use transgenics to move beneficial genes from one species to another, or to use them to target research and medicine – even design new proteins.
Evolutionary relationships	Closely-related species share a lot of the base sequence of their genomes. Distant relatives share less. By sequencing genomes, we have further, more reliable evidence of evolution and are able to put species into more appropriate taxa.

Genetic Engineering

Also known as genetic modification, gene transfer or transgenics.

- All living things use the same bases and the same genetic code.
- Each codon produces the same amino acid in transcription and translation, regardless of the species.
- So the sequence of amino acids in a polypeptide remains unchanged.
- Therefore, we can take genes from one species and insert them into the genome of another species.

"The Genetic Code is Universal"



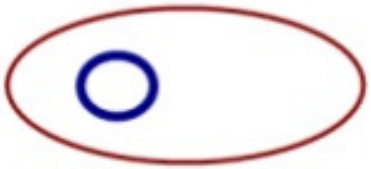
We already make use of gene transfer in industrial production of insulin:
http://www.abpischools.org.uk/res/coResourceImport/modules/hormones/en-flash/geneticeng.cfm

Gene Transfer *Requires plasmids, a host cell, restriction enzymes and ligase.*

Restriction enzymes 'cut' the desired gene from the genome.

E. coli bacteria contain small circles of DNA called **plasmids**.

These can be removed.



The **same** restriction enzyme cuts into the plasmid.

T C G A C G
A G C T G C A C C

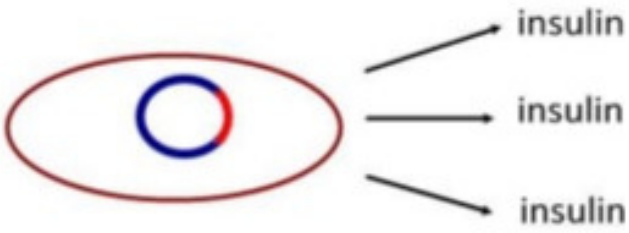
T G G A C G A G C
T G C T C G

Because it is the same restriction enzyme the same bases are left exposed, creating 'sticky ends'

T C G A C G T G G A C G A G C
A G C T G C A C C T G C T C G

Ligase joins the sticky ends, fixing the gene into the *E. coli* plasmid.

The **recombinant plasmid** is inserted into the host cell. It now expresses the new gene. An example of this is **human insulin production**.



Review question: *how and where is insulin produced in the cell and how is it exported from the cell?*

Genetically Modified Organisms

GMO's are already in circulation and have been produced for many uses, including agricultural and medical.

Plant examples	**Golden Rice** Enriched with beta-carotene, which is converted to vitamin A in the body. Can prevent malnutrition-related blindness in developing countries. **Insect-resistant corn** Produces proteins which pests do not like, therefore toxic insecticides are not needed on the farm. **Salt-resistant tomatoes** can be grown in saline soils
Animal examples	**Factor IX-producing sheep** Produce human clotting factors in their milk, for use in the treatment of hemophilia **Glowing pigs** Cells from these specimens are used to study transplants and grafts and the final destinations of transplanted cells in the host body

Golden rice image from Learner.org:
http://www.learner.org/courses/envsci/unit/unit_vis.php?unit=7

Genetically Modified Crops

The ethical debate over GMO's rages on, and as scientist we must always bear in the mind the precautionary principle:

"If an action is potentially harmful, the burden of proof of safety lies with those who propose to take the action."

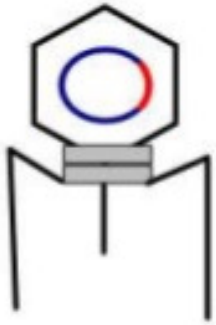
Watch this overview:
http://www.youtube.com/watch?v=B8p7M0WF_7A

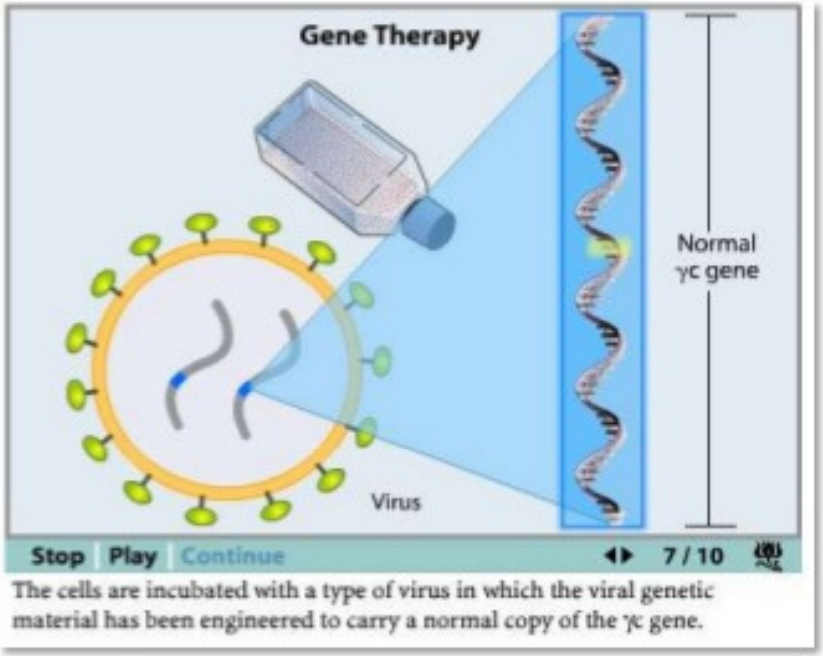
Benefits	• Increased **yields** of crops and faster breeing cycles. • Crops can be grown in **harsher environmental conditions**. • Reduced need for **pesticides** which can harm human and environmental health through biomagnification. • **Nutrient-enhanced** crops in areas of high food pressure or famine.
Potential harms	• Potential genetic pollution of organic crops through fertilisation by pollen of GM crops. • Unknown health risks of some crops. • Fear of monopoly-like behaviour as farmers need to buy expensive seeds annually. • Potential hybridisation of related species.

- http://www.youtube.com/watch?v=B8p7M0WF_7A

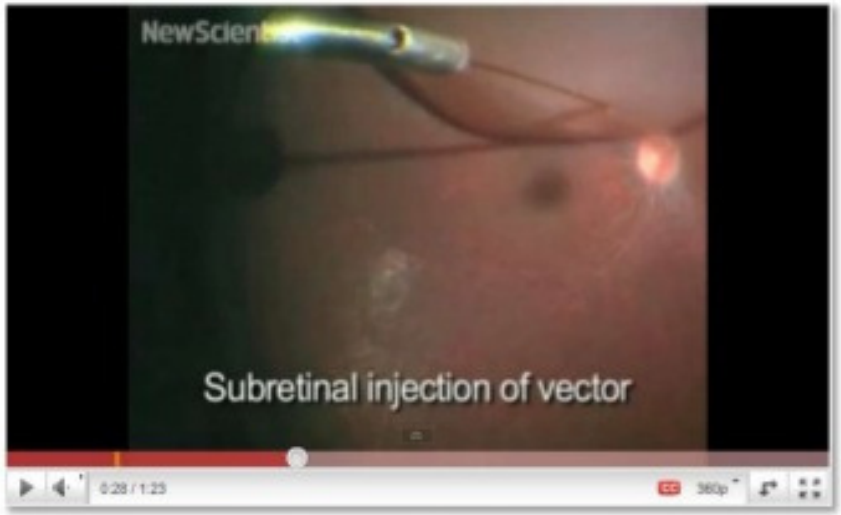
Gene Transfer *Can also be used in gene therapy.*

A virus vector is used to insert the recombinant plasmid into the genes of affected cells. The virus is chosen or designed to target only those specific cells.





Severe Combined Immune Deficiency can be treated this way:
http://www.sumanasinc.com/scienceinfocus/sif_genetherapy.html



Recently, hereditary blindness was treated with gene therapy:
http://www.youtube.com/watch?v=d_YJZn-ft_Q

Although very interesting, this is not in the IB Bio syllabus.

- http://www.sumanasinc.com/scienceinfocus/sif_genetherapy.html
- http://www.youtube.com/watch?v=d_YJZn-ft_Q

Clone

A group of genetically identical organisms.

A group of cells derived from a single parent cell.

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A group of genetically identical organisms.
A group of cells derived from a single parent cell.

Monozygotic twins are naturally-occurring clones. So why do they appear different?

Epigenetics has the answer…

Video from LearnGenetics:
http://www.youtube.com/watch?v=AV8FM_d1Leo

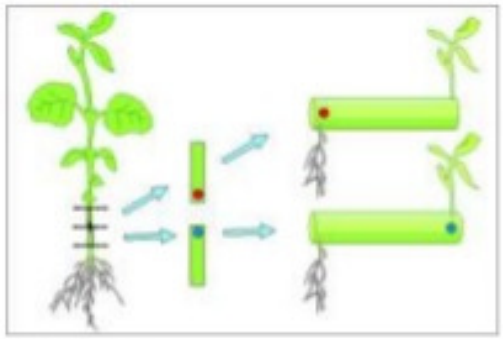
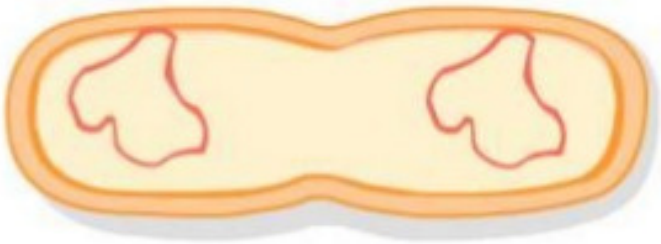


Image from:
http://www.mun.ca/biology/desmid/brian/BIOL3530/DB_Ch13/DBNRegen.html

Plant cuttings are also examples of clones.

So is **asexual reproduction**, such as binary fission in bacteria.



Binary fission in bacteria:
http://www.classzone.com/books/hs/ca/sc/bio_07/animated_biology/bio_ch05_0149_ab_fission.html

- http://www.youtube.com/watch?v=AV8FM_d1Leo

Reproductive Cloning

*Creating a **genetically identical organism** through **transfer** of a **differentiated diploid nucleus**.*

Reproductive cloning made simple:

1. Remove a differentiated diploid nucleus from individual to be cloned.
2. Enucleate a donor egg cell.
3. Insert the diploid nucleus into the enucleated
4. Implant into the endometrium of a surrogate and gestate.
5. The newborn will be genetically identical to tl nucleus parent.

Interactive tutorial from Learn.Genetics:
http://learn.genetics.utah.edu/content/tech/cloning/clickandclone/

Enucleation of an egg cell, from HHMI:
http://www.hhmi.org/biointeractive/stemcells/scnt_video.html

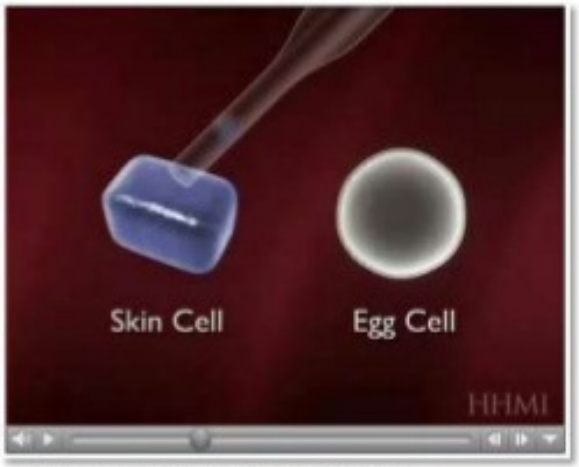
Dolly the sheep was the first successful cloning of a mammal from a differentiated somatic cell.
She was the result of many attempts. Interestingly, she dies young – but of age-related illnesses.

Human reproductive cloning is illegal.

- http://www.hhmi.org/biointeractive/stemcells/scnt_video.html

Therapeutic Cloning

*Creating an **embryo** as a source of **stem cells**, by transfer of a **differentiated diploid nucleus**.*



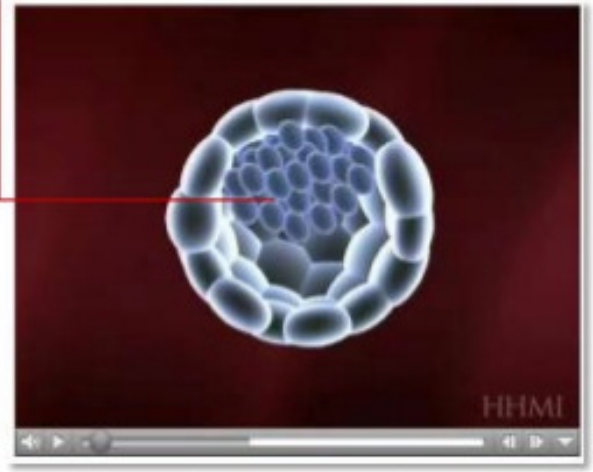
Nuclear transfer animation from HHMI:
http://www.hhmi.org/biointeractive/stemcells/scnt.html

Therapeutic cloning made simple:

1. Remove a differentiated diploid nucleus from the cell to be cloned.
2. Enucleate a donor egg cell.
3. Insert the diploid nucleus into the enucleated egg cell.
4. Stimulate it to divide and grow *in vitro*.
5. The resulting embryo is a rich source of stem cells which can be harvested or cultured.
6. The outer layer of cells is removed, so only the inner cell mass is used to culture the tissues needed.

Uses of therapeutic cloning:

- Create stem cells for transplants, such as in burns patients or leukemia.
- Replace other damaged tissues such as nerves, pancreas cells etc.
- Much reduced risk of rejection of cells are they are genetically identical to the recipient.



Creating stem cells animation from HHMI:
http://www.hhmi.org/biointeractive/stemcells/creating_lines.html

- http://www.hhmi.org/biointeractive/stemcells/scnt.html
- http://www.hhmi.org/biointeractive/stemcells/creating_lines.html

Ethics of Therapeutic Cloning

Therapeutic cloning is the centre of much debate.

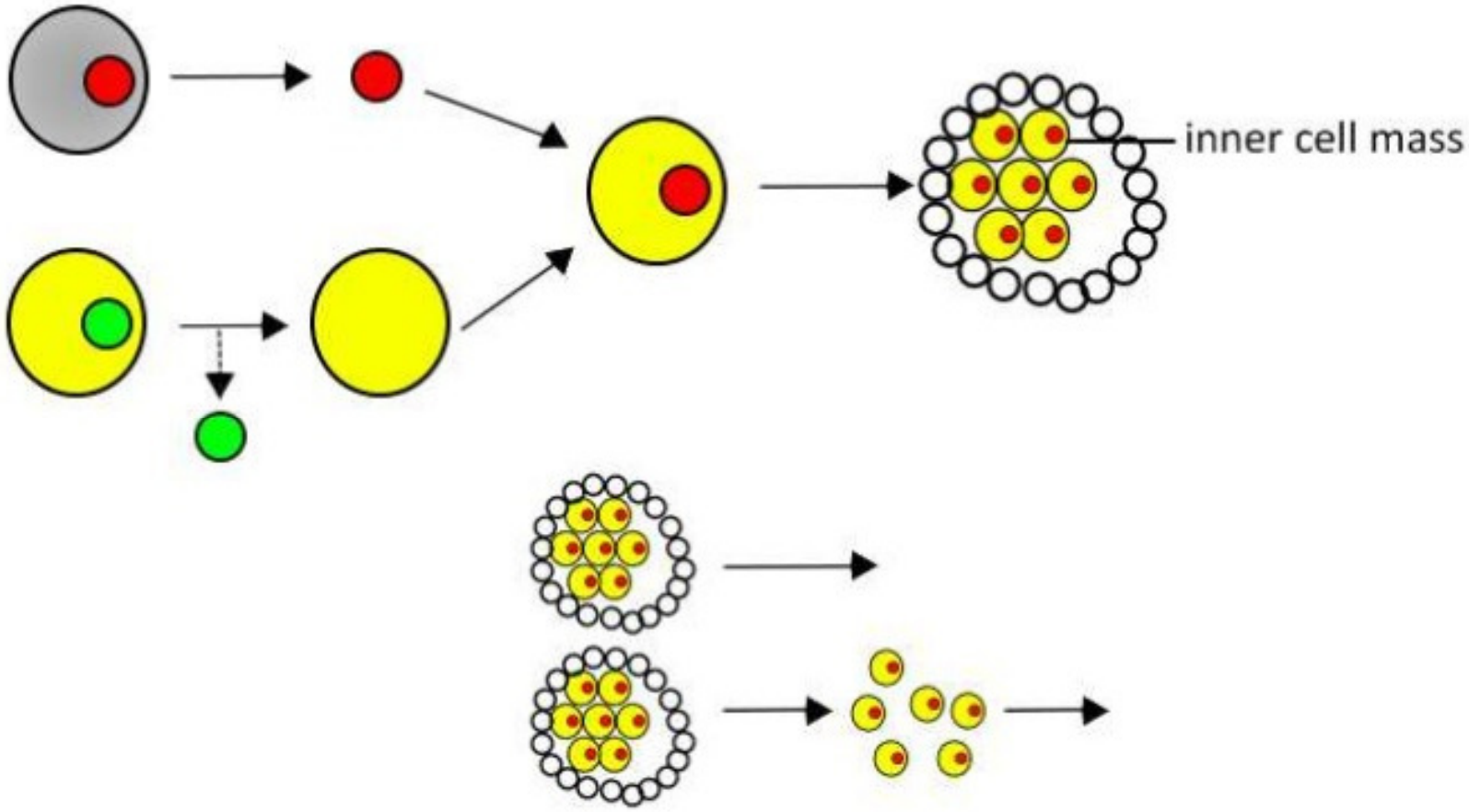
In part this is due to pre-conceived notions of cloning from the media and fiction. However, the root of much of the debate lies in the simple fact that the created embryos **could potentially** be implanted into a surrogate mother and **develop into human fetuses**.

NOT therapeutic cloning. What's your misconception?
http://www.youtube.com/watch?v=9uSfX6ljcRQ

Arguments in favour of therapeutic cloning	• Stem cells can be created without the need for fertilisation and destruction of 'natural' human embryos • Source of cells for stem cell transplants, such as in leukemia, diabetes, burns and many other medical cases. • Transplants do not require the death of another human. • Transplants are less likely to be rejected as they are cells which are genetically identical to the patient. • Embryos are not allowed to develop to the point where a nervous system forms, so there is no pain or perception.
Arguments against	• Religious or moral objections due to the 'playing God' argument. • The embryo which is created could potentially be used in IVF and develop into a human fetus, so are we creating human life to destroy it? • Although cloning humans reproductively is illegal, this has not been ratified by all nations. Potential for a race to clone the first human.

Reproductive vs Therapeutic Cloning

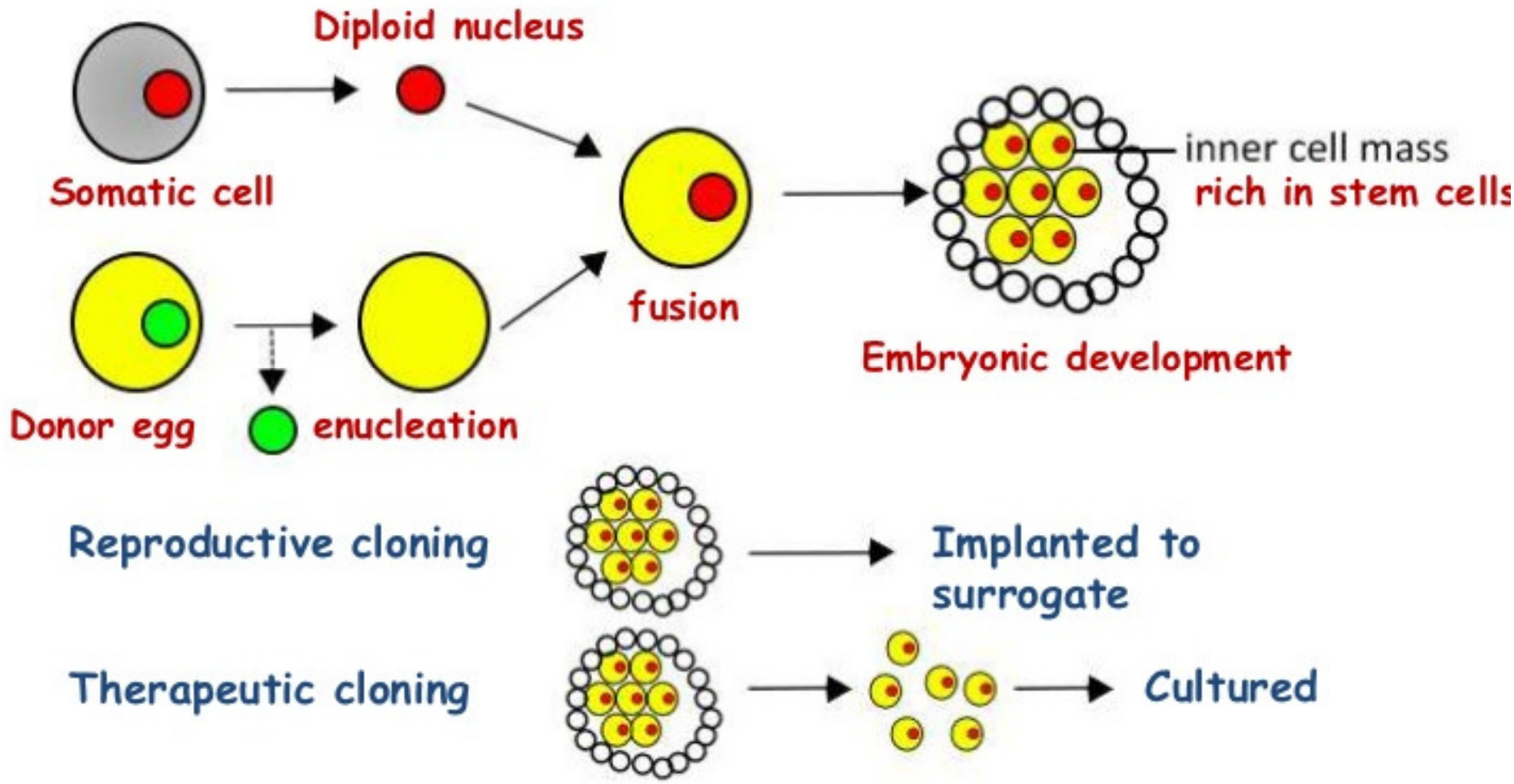
Annotate this flow chart to compare reproductive and therapeutic cloning.



Flow chart adapted from wikipedia
http://en.wikipedia.org/wiki/Therapeutic_cloning

Reproductive vs Therapeutic Cloning

Annotate this flow chart to compare reproductive and therapeutic cloning.



Flow chart adapted from wikipedia
http://en.wikipedia.org/wiki/Therapeutic_cloning

Induced Pluripotent Stem Cells

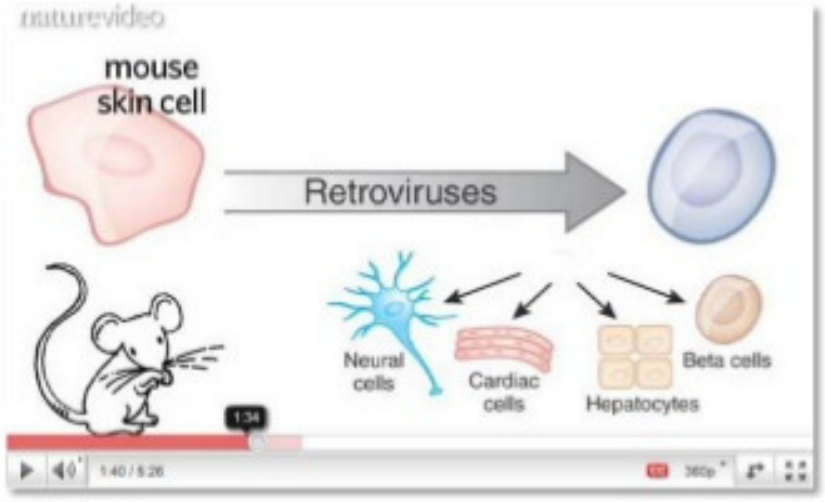
Is this new technology going to break the ethical deadlock?

By reprogramming regular differentiated cells back into a 'stem-like' state, scientists have been able to develop stem cell lines without the need for embryos.

Although beyond the scope of the syllabus, and very new, this is exciting research.

Visit Ed Yong's interactive timeline of iPS research to find out more!

Interactive timeline of IPS Stem Cell research:
http://blogs.discovermagazine.com/notrocketscience/2011/02/02/research-into-reprogrammed-stem-cells-an-interactive-timeline/



Watch this video!

TOK:

Does this represent a paradigm shift in Biology? How did the ability to replicate by other scientists reduce scepticism over the initial findings?

iPS Stem Cells – method of the year 2009:
http://www.youtube.com/watch?v=fGNchPdlaGU

- http://www.eurostemcell.org/resource/research-reprogrammed-stem-cells-interactive-timeline
- http://www.youtube.com/watch?v=fGNchPdlaGU&feature=related

Is it really Osama bin Laden?

In groups, explain how biotechnology will be used to make a positive ID on the body.

8 marks