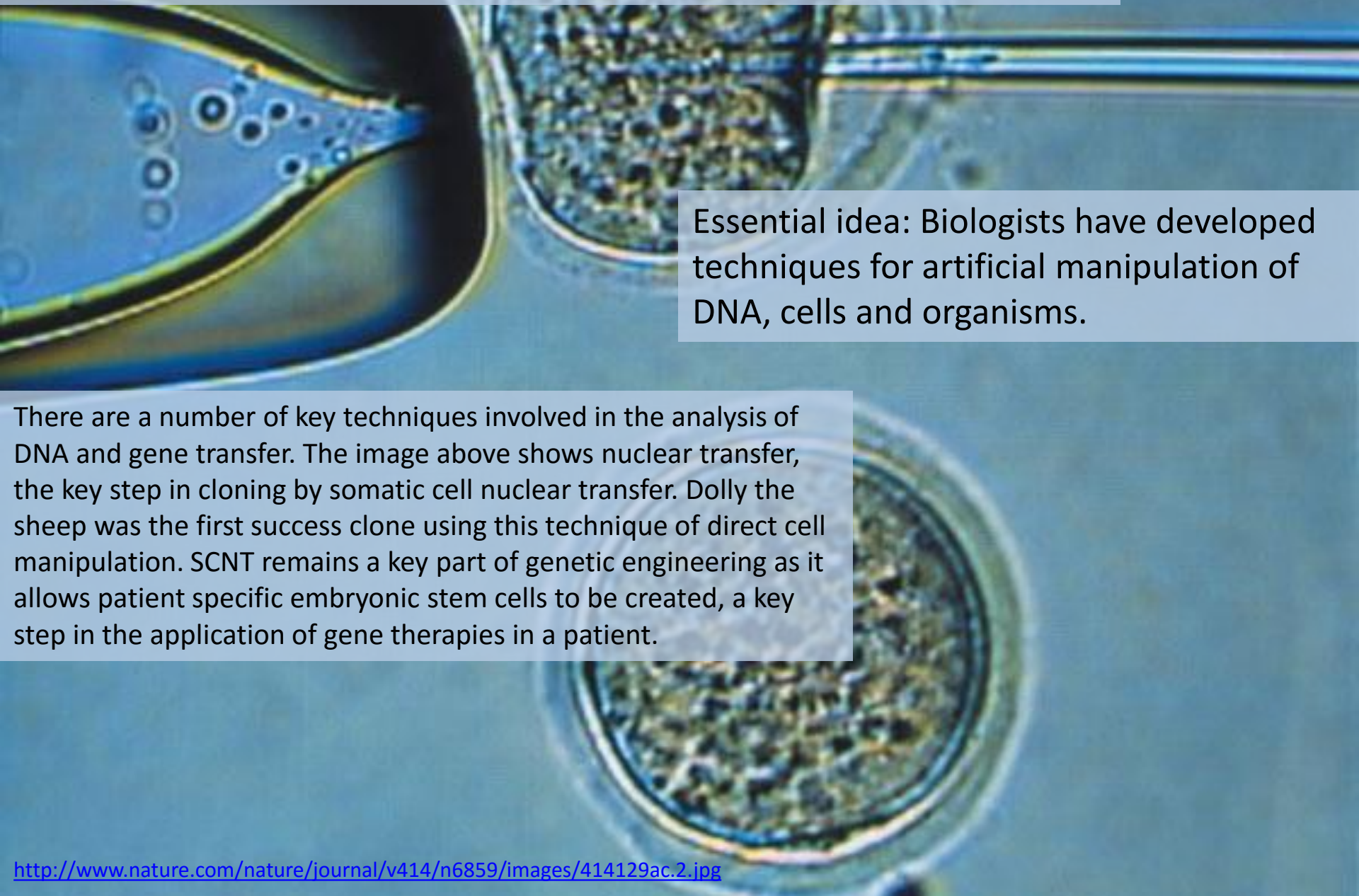
3.5 Genetic modification and biotechnology



Essential idea: Biologists have developed techniques for artificial manipulation of DNA, cells and organisms.

There are a number of key techniques involved in the analysis of DNA and gene transfer. The image above shows nuclear transfer, the key step in cloning by somatic cell nuclear transfer. Dolly the sheep was the first success clone using this technique of direct cell manipulation. SCNT remains a key part of genetic engineering as it allows patient specific embryonic stem cells to be created, a key step in the application of gene therapies in a patient.

http://www.nature.com/nature/journal/v414/n6859/images/414129ac.2.jpg

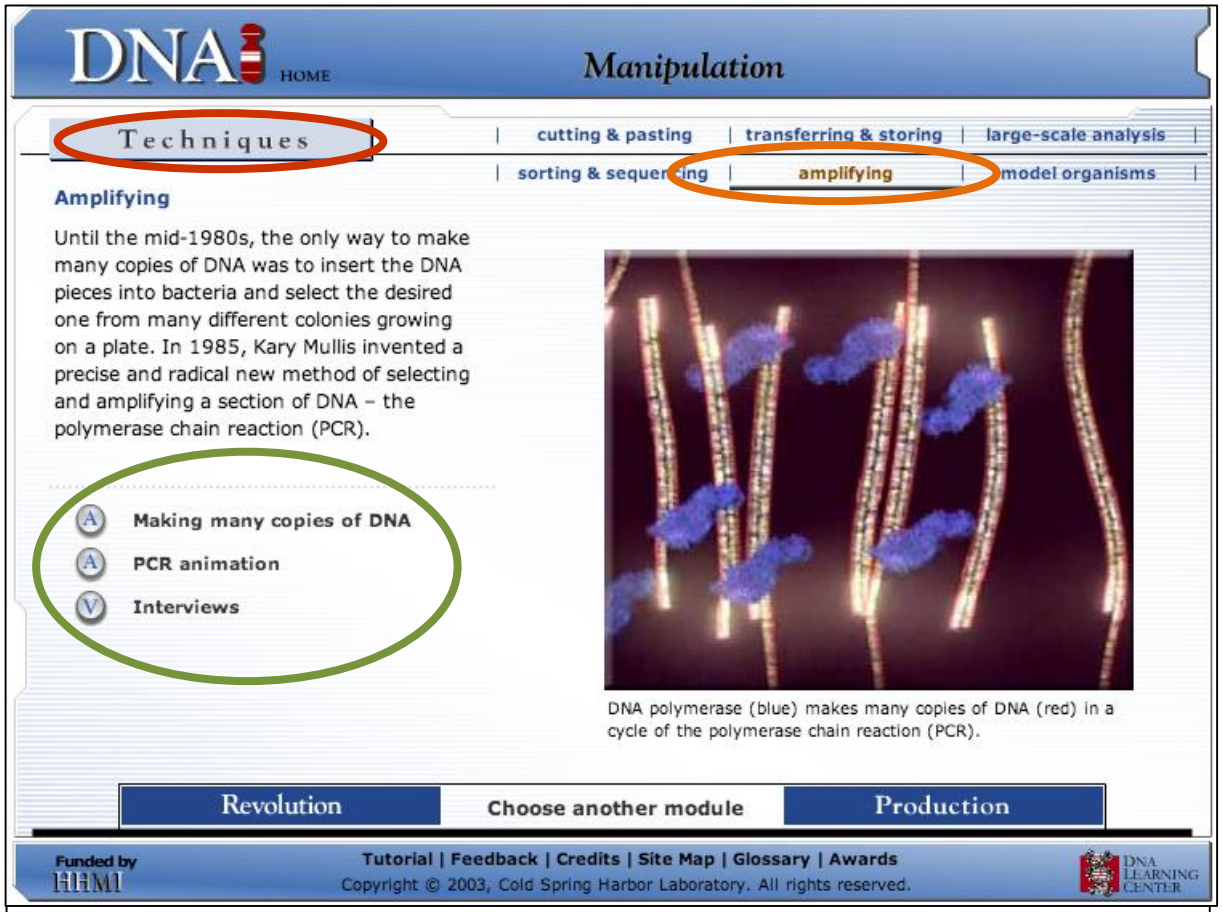
Understandings

	Statement	Guidance
3.5.U1	Gel electrophoresis is used to separate proteins or fragments of DNA according to size.	
3.5.U2	PCR can be used to amplify small amounts of DNA.	
3.5.U3	DNA profiling involves comparison of DNA.	
3.5.U4	Genetic modification is carried out by gene transfer between species.	
3.5.U5	Clones are groups of genetically identical organisms, derived from a single original parent cell.	
3.5.U6	Many plant species and some animal species have natural methods of cloning.	
3.5.U7	Animals can be cloned at the embryo stage by breaking up the embryo into more than one group of cells.	
3.5.U8	Methods have been developed for cloning adult animals using differentiated cells.	

Applications and Skills

	Statement	Guidance
3.5.A1	Use of DNA profiling in paternity and forensic investigations.	
3.5.A2	Gene transfer to bacteria using plasmids makes use of restriction endonucleases and DNA ligase.	
3.5.A3	Assessment of the potential risks and benefits associated with genetic modification of crops.	
3.5.A4	Production of cloned embryos produced by somatic-cell nuclear transfer.	Dolly can be used as an example of somatic-cell transfer.
3.5.S1	Design of an experiment to assess one factor affecting the rooting of stem-cuttings.	A plant species should be chosen for rooting experiments that forms roots readily in water or a solid medium.
3.5.S2	Analysis of examples of DNA profiles.	Students should be able to deduce whether or not a man could be the father of a child from the pattern of bands on a DNA profile.
3.5.S3	Analysis of data on risks to monarch butterflies of Bt crops.	

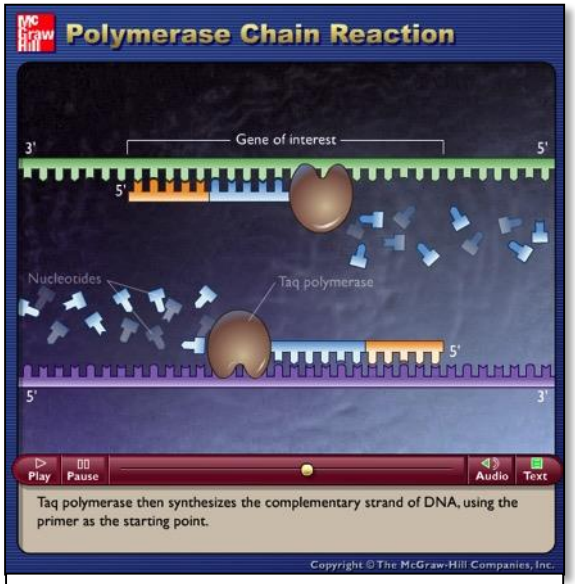
Review: 2.7.A1 Use of Taq DNA polymerase to produce multiple copies of DNA rapidly by the polymerase chain reaction (PCR).



http://www.dnai.org/b/index.html

After clicking on the myDNA link choose techniques and then amplifying to access the tutorials on the polymerase chain reaction (PCR).

Alternatively watch the McGraw-Hill tutorial



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

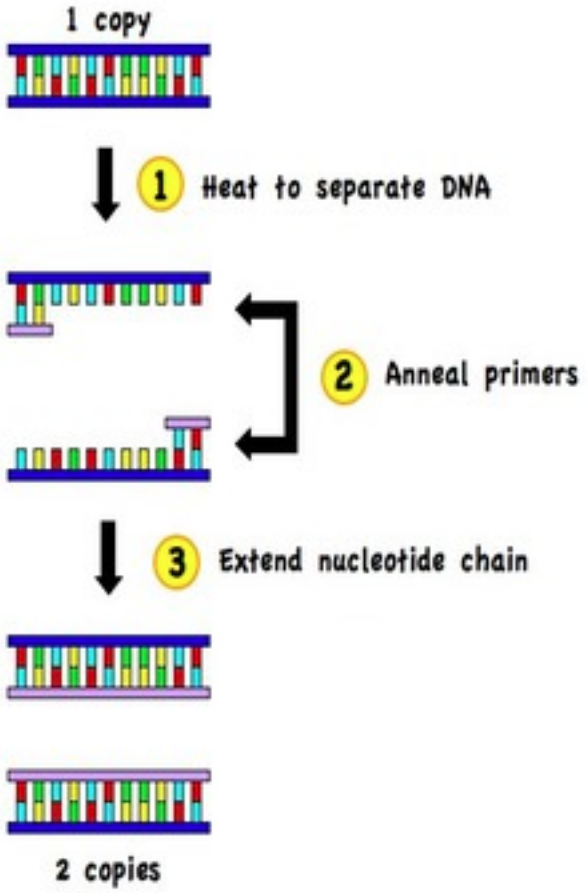
Review: 2.7.A1 Use of Taq DNA polymerase to produce multiple copies of DNA rapidly by the polymerase chain reaction (PCR).

To summarise:

PCR is a way of producing large quantites of a specific target sequence of DNA. It is useful when only a small amount of DNA is avaliable for testing e.g. crime scene samples of blood, semen, tissue, hair, etc.

PCR occurs in a thermal cycler and involves a repeat procedure of 3 steps:

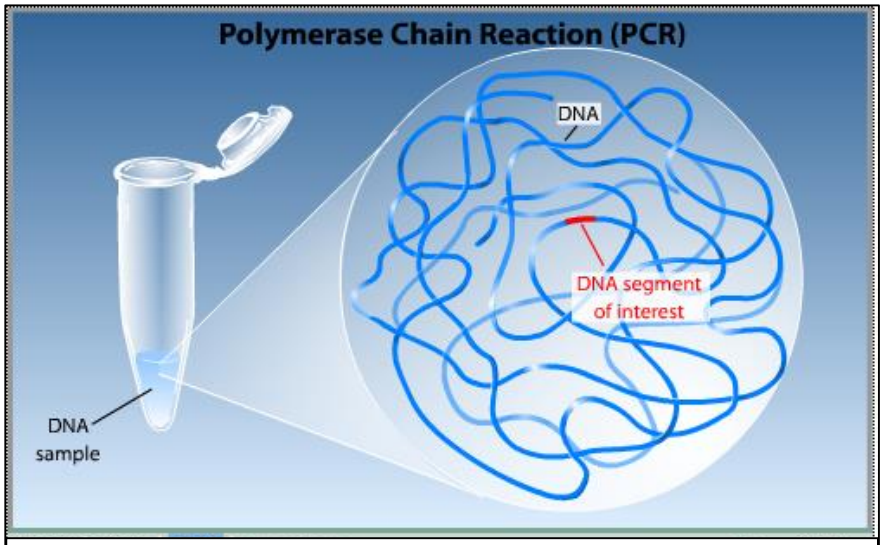
1. **Denaturation**: DNA sample is heated to separate it into two strands
2. **Annealing**: DNA primers attach to opposite ends of the target sequence
3. **Elongation**: A heat-tolerant DNA polymerase (Taq) copies the strands



- One cycle of PCR yields two identical copies of the DNA sequence
- A standard reaction of 30 cycles would yield 1,073,741,826 copies of DNA (2^{30})

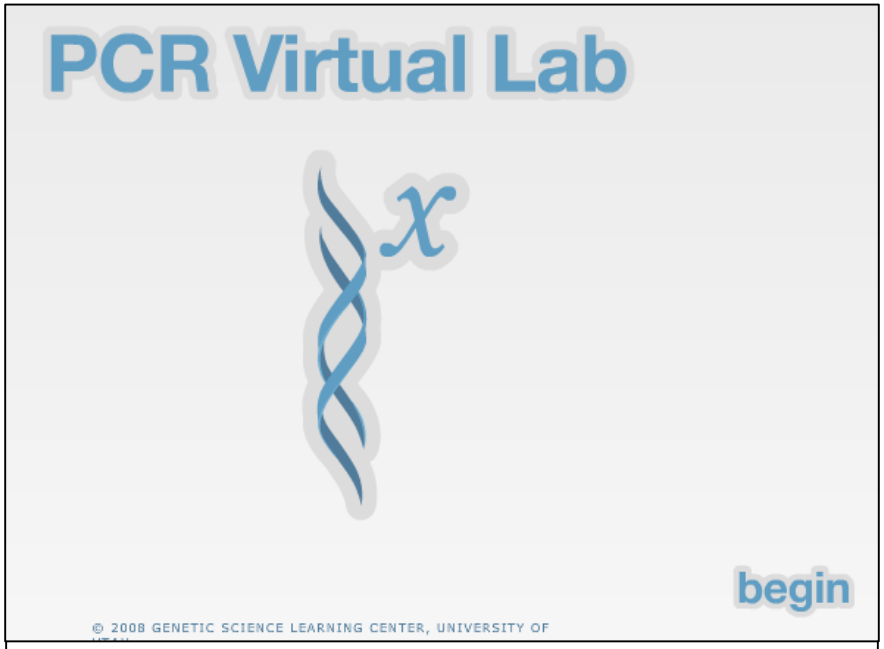
Polymerase Chain Reaction (PCR)

- *Typically used to copy a segment of DNA – not a whole genome*
- *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.



http://www.sumanasinc.com/webcontent/animations/content/pcr.html

Learn the detail using the virtual lab and/or the animation:



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

Can you see how the technology has mimicked the natural process of DNA replication?

http://www.slideshare.net/gurustip/genetic-engineering-and-biotechnology-presentation

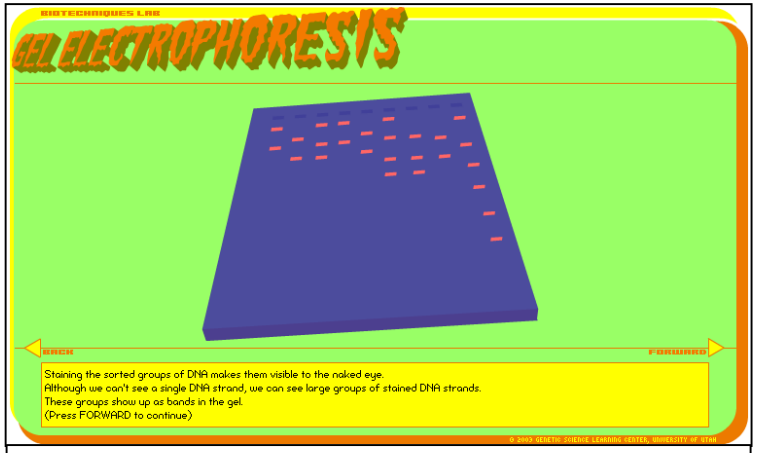
DNA Profiling

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

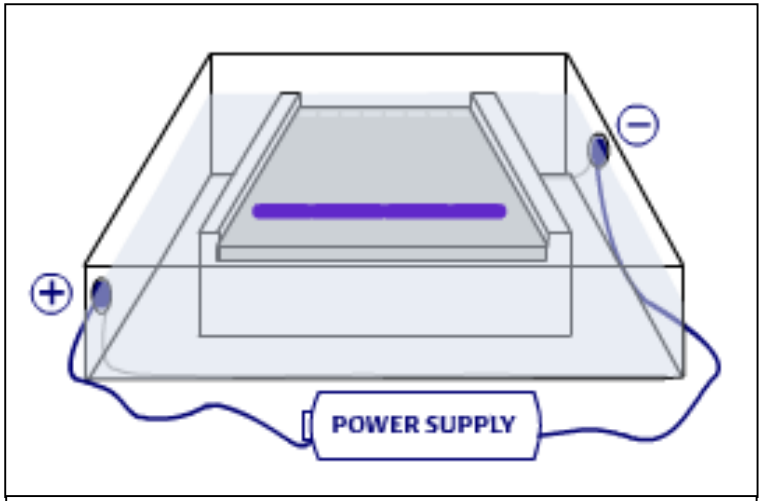
Through gel electrophoresis, fragments of DNA are moved through an electric field and separated based on their size.

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.

Learn the detail using the virtual lab and/or the animation:



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



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

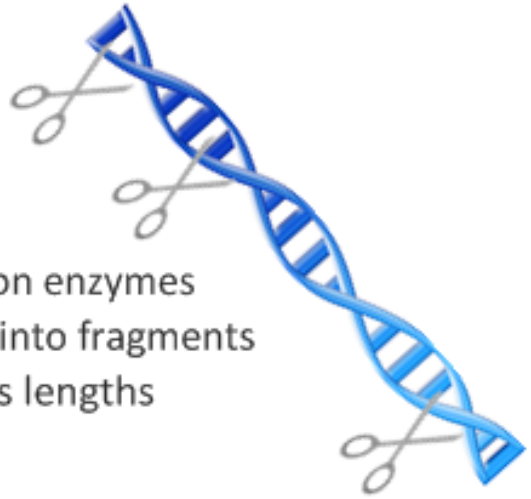
http://www.slideshare.net/gurustip/genetic-engineering-and-biotechnology-presentation

DNA Profiling: Gel Electrophoresis

DNA sample is taken

PCR amplifies DNA to get a useful amount

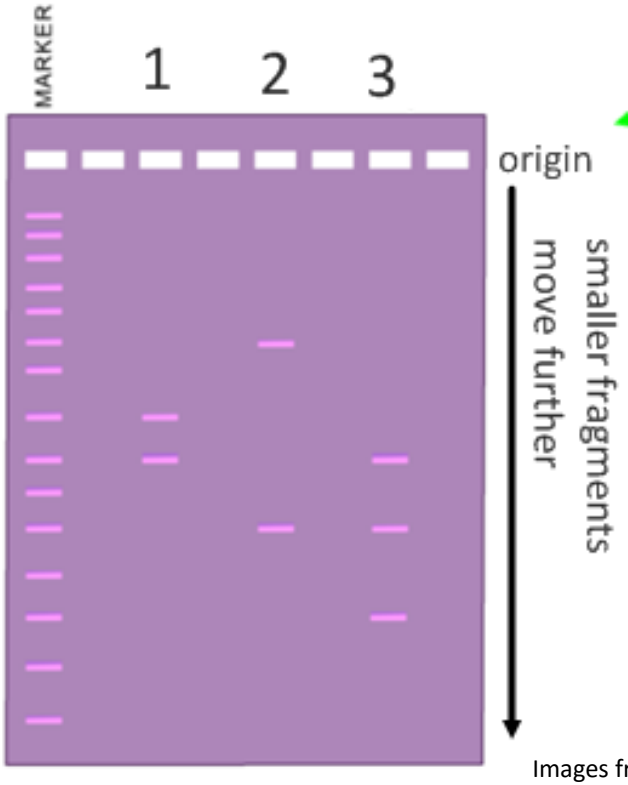
Restriction enzymes cut DNA into fragments of varous lengths

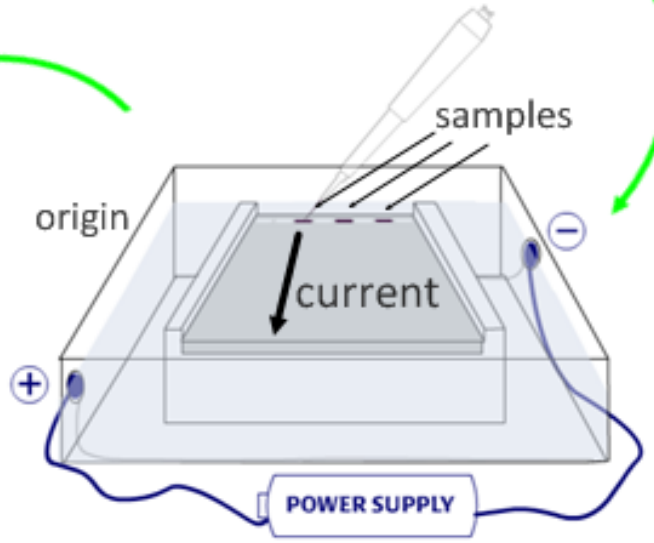


Marker (or standard) is used to show all the possible DNA fragments.

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

http://www.slideshare.net/gurustip/genetic-engineering-and-biotechnology-presentation

DNA profiling in paternity and forensic investigations

DNA is often left behind at a crime scene. It is present in all kinds of evidence, including blood, hair, skin, saliva, and semen.

In 1986 forensic DNA analysis was first used. Originally known as "DNA fingerprinting," this type of analysis is now called "DNA profiling" or "DNA testing" to distinguish it from traditional skin fingerprinting.

It is easier to exclude a suspect than to convict someone based on a DNA match. The FBI estimates that one-third of initial rape suspects are excluded because DNA samples fail to match.

Forensic investigators take many precautions to prevent mistakes, but human error can never be eliminated.

http://learn.genetics.utah.edu/content/science/forensics/

DNA can also be used in paternity investigations.

DNA samples are needed from the mother, (potential) father and child in question.

Reasons for investigations include:

- Inheritance of property, savings etc.
- Father is required to pay maintenance to support his biological child

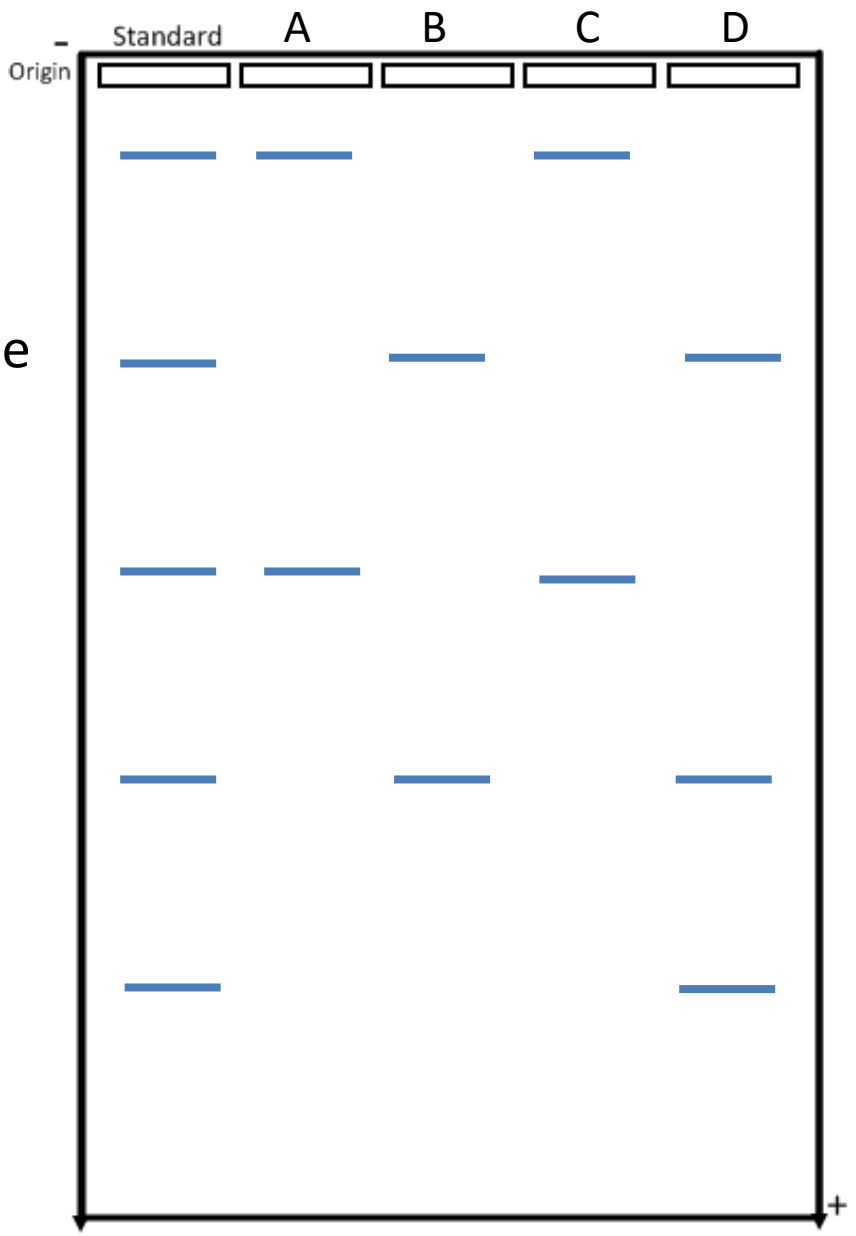
https://commons.wikimedia.org/wiki/File:Father_and_Son_%286330243602%29.jpg

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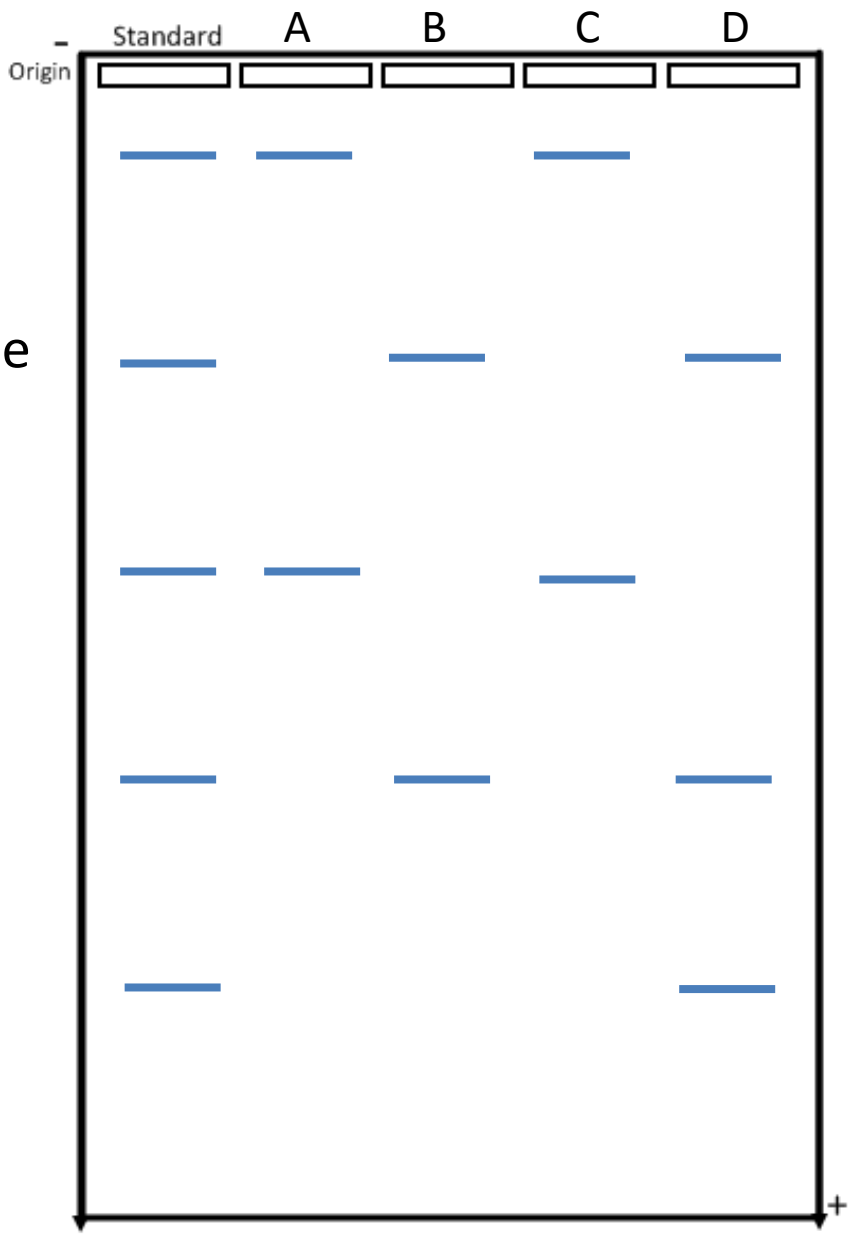
Explanation:
We expect 100% match as the cells left behind are the perpetrator's own cells.



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

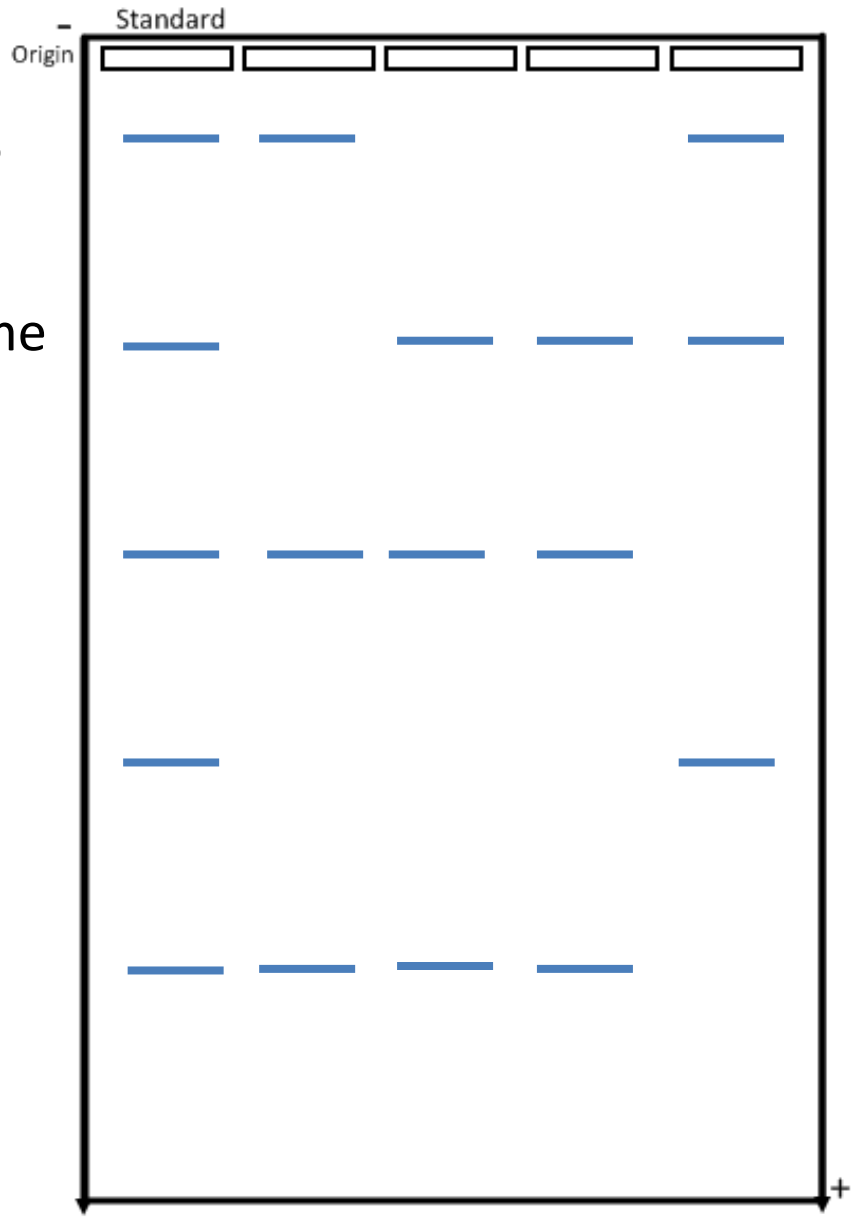
In this case, a lot of blood was left at a crime scene. Who was the perpetrator?

A = victim

B = unknown blood at scene

C = suspect 1

D = suspect 2



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A = victim

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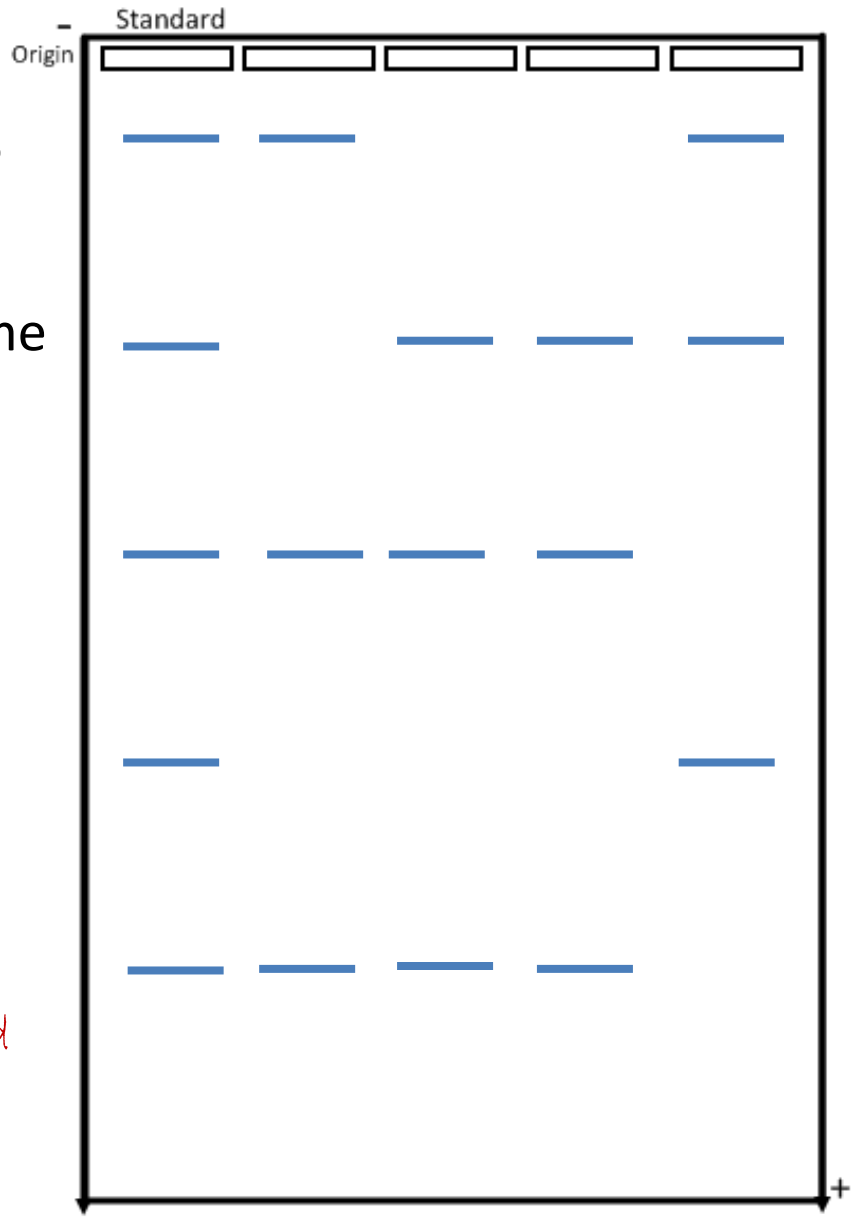
D = suspect 2

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.



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DNA Profiling in forensics

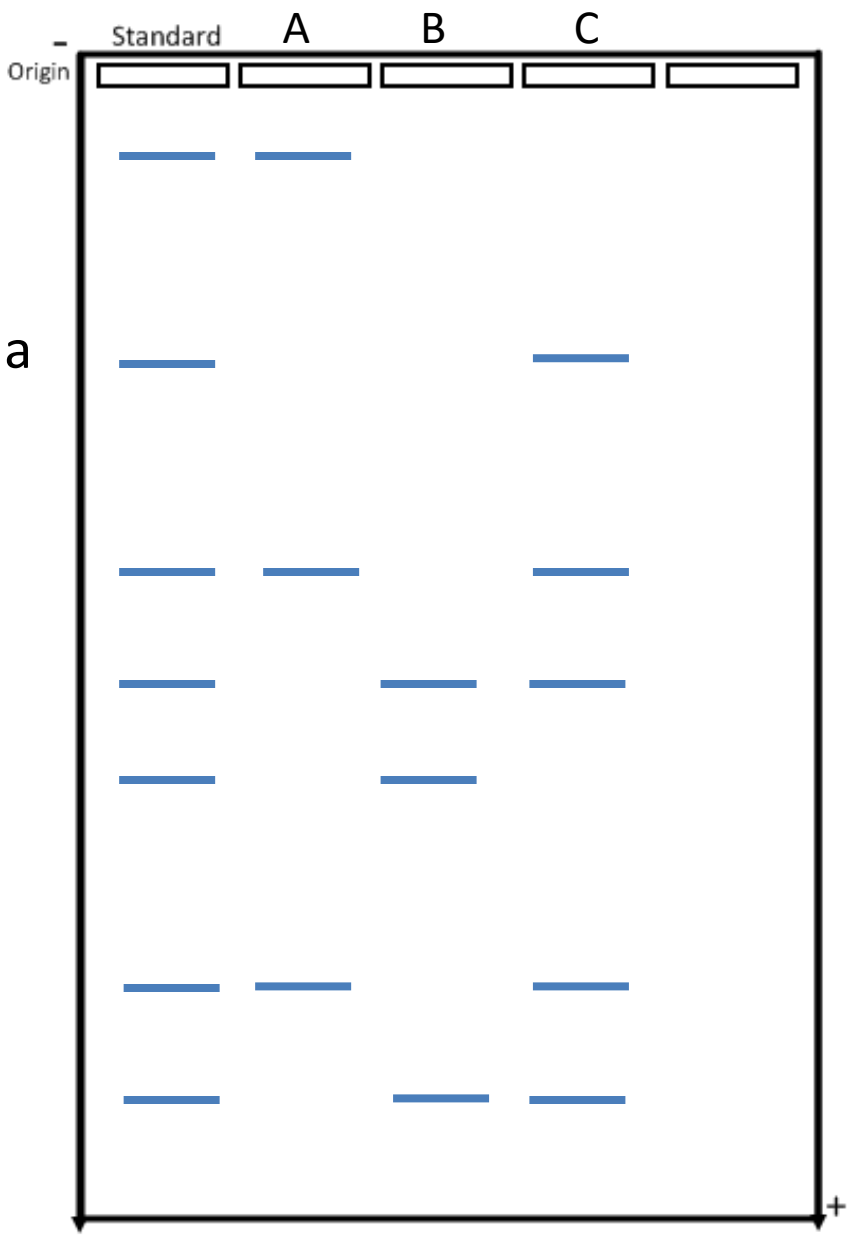
DNA Profiling can be used to identify suspects from trace DNA evidence. It can also be used to eliminate the innocent from the investigation.

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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DNA Profiling in forensics

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

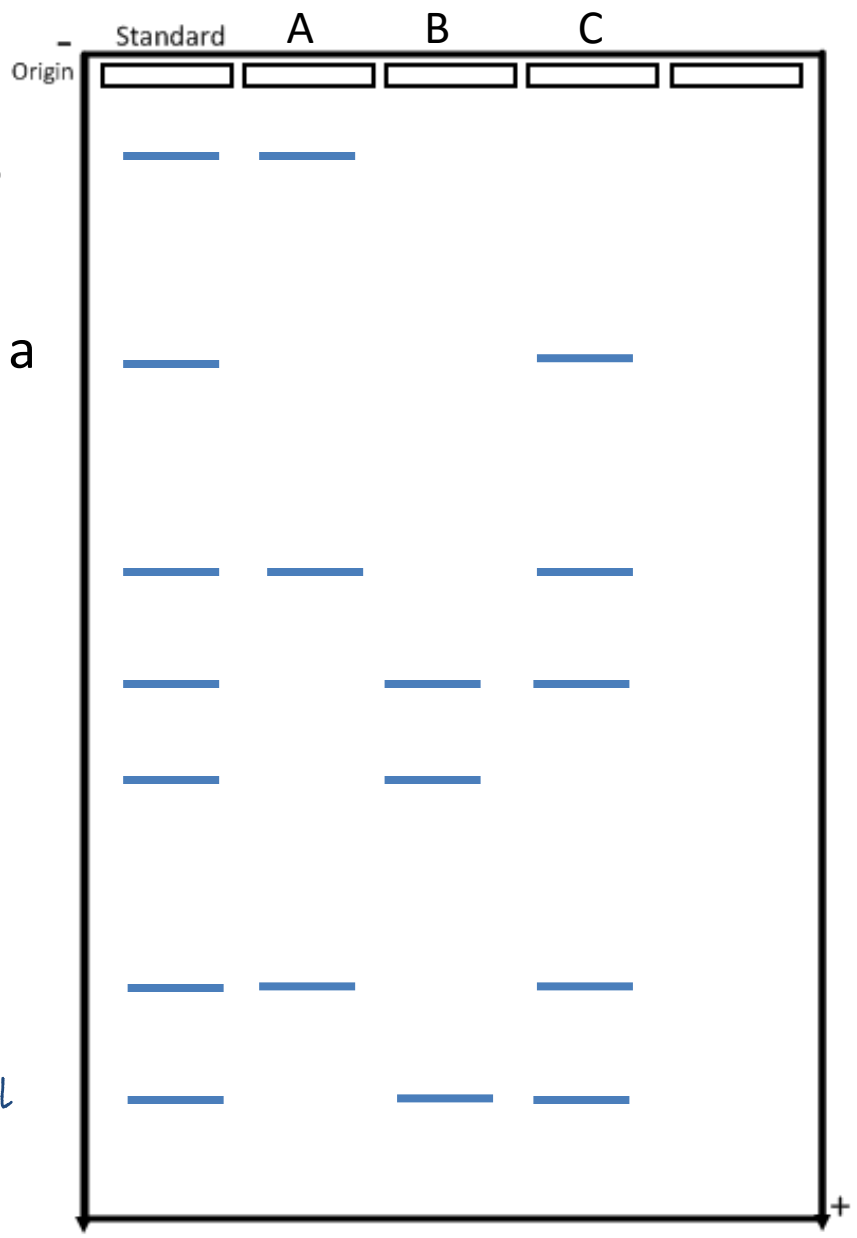
A = trace evidence
B = homeowner
C = prisoner

No

Explanation:

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.



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DNA Profiling in paternity

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

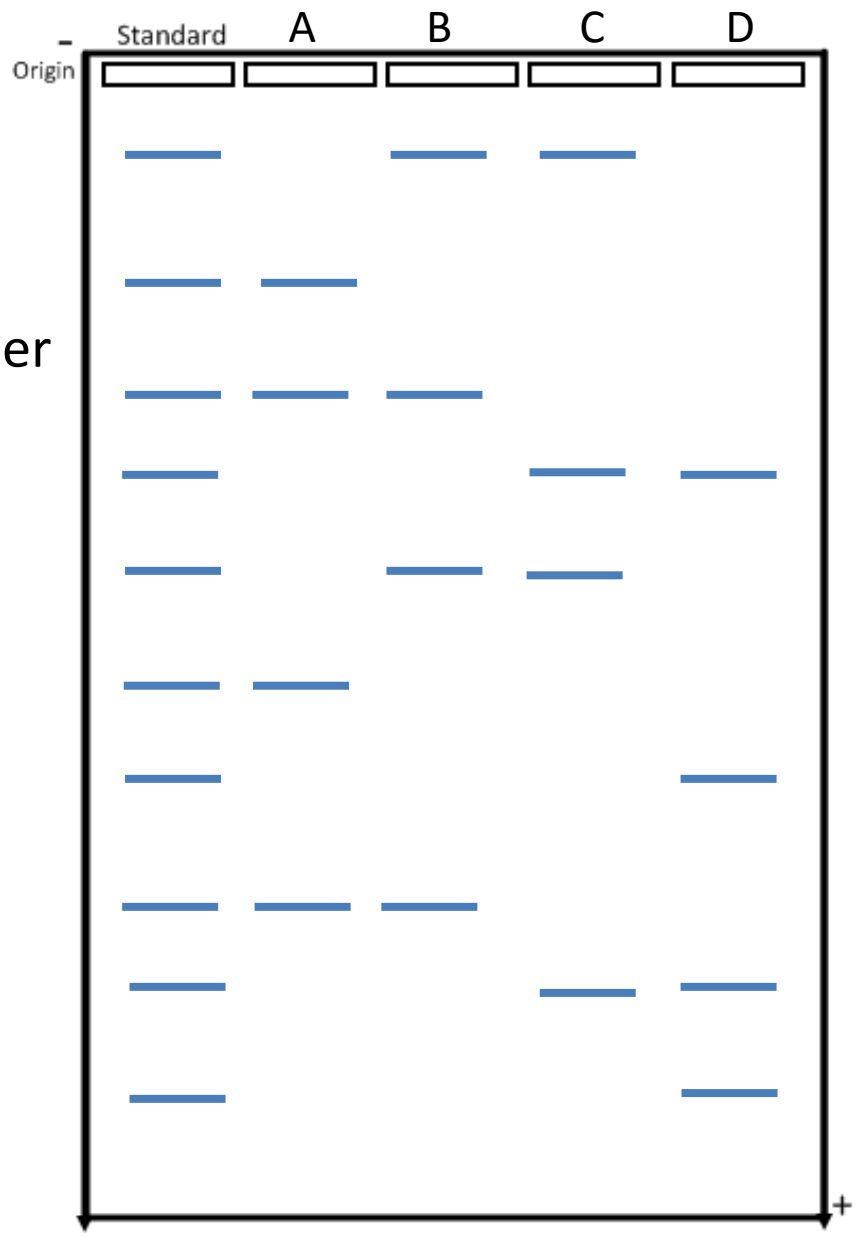
In this case, the parentage of a child is under question. Who's the daddy?

A = mother

B = child

C = man 1

D = man 2



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DNA Profiling in paternity

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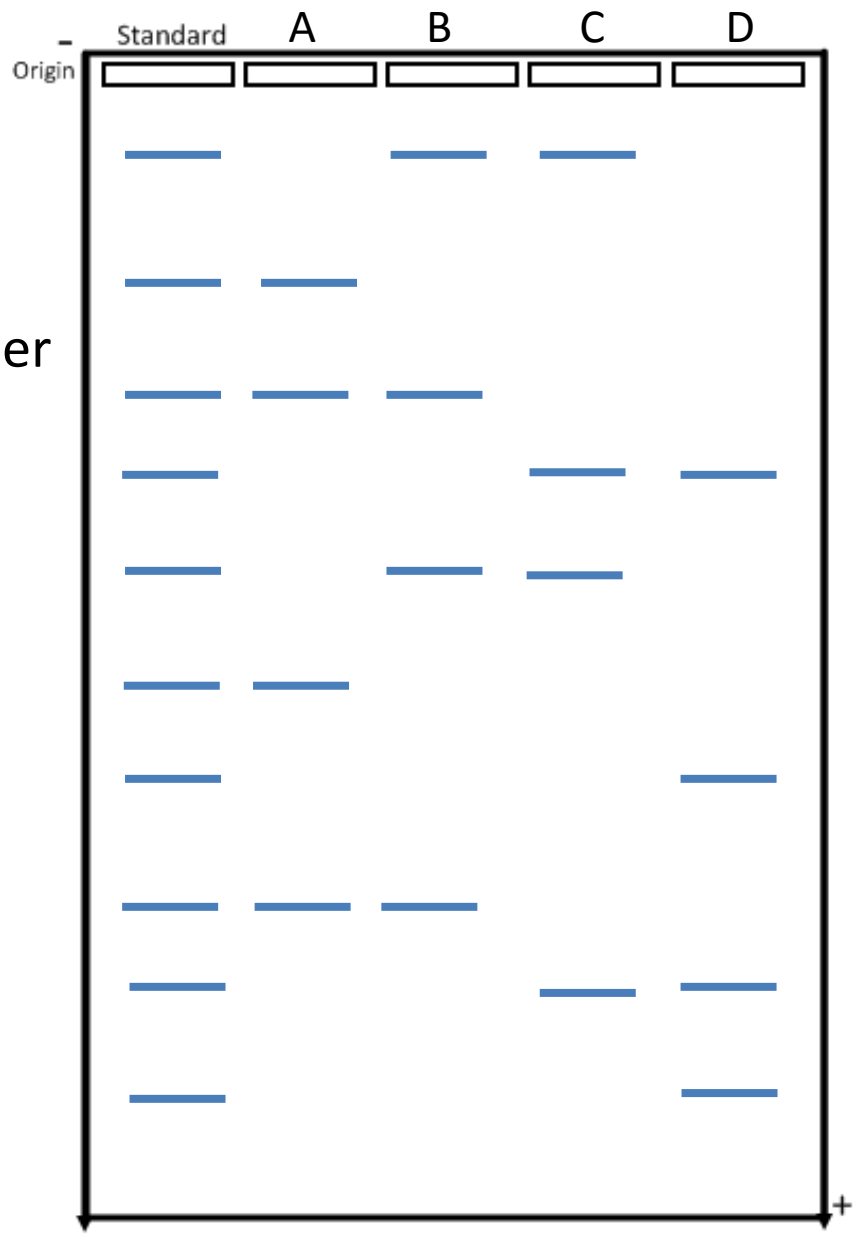
In this case, the parentage of a child is under question. Who's the daddy?

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.



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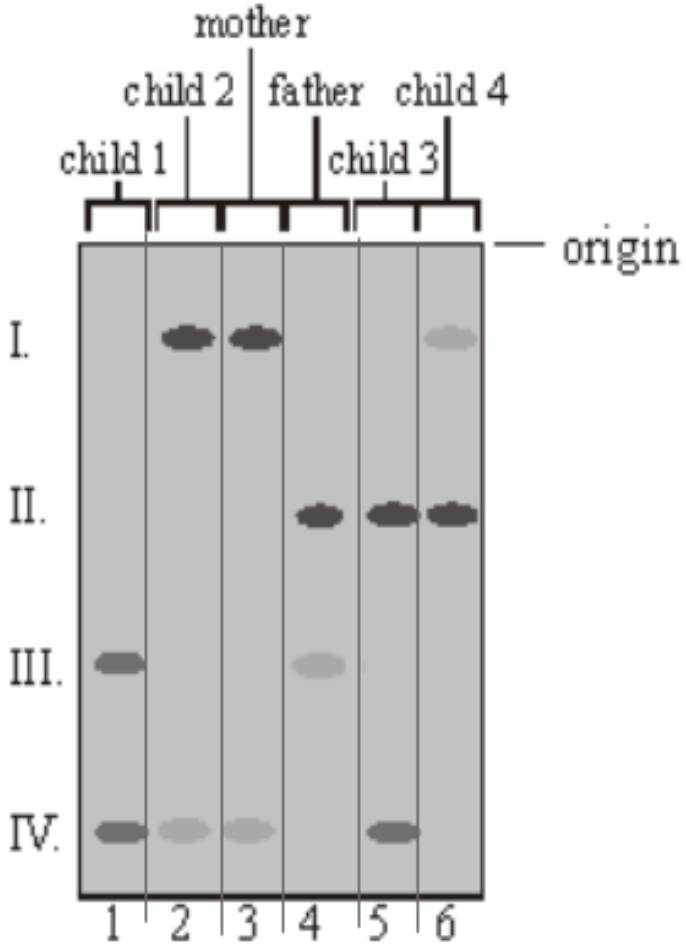
Sample Questions



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

1. Identify the smallest DNA fragment.

   I. II. III. IV.

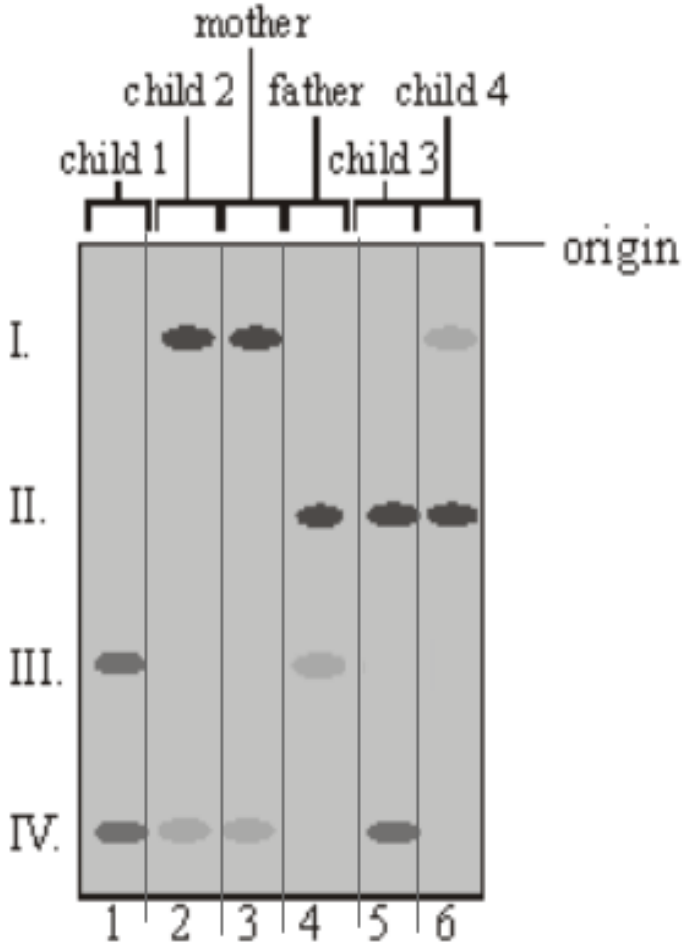
2. State the number of bands that would appear in the 'standard' lane.

   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



[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

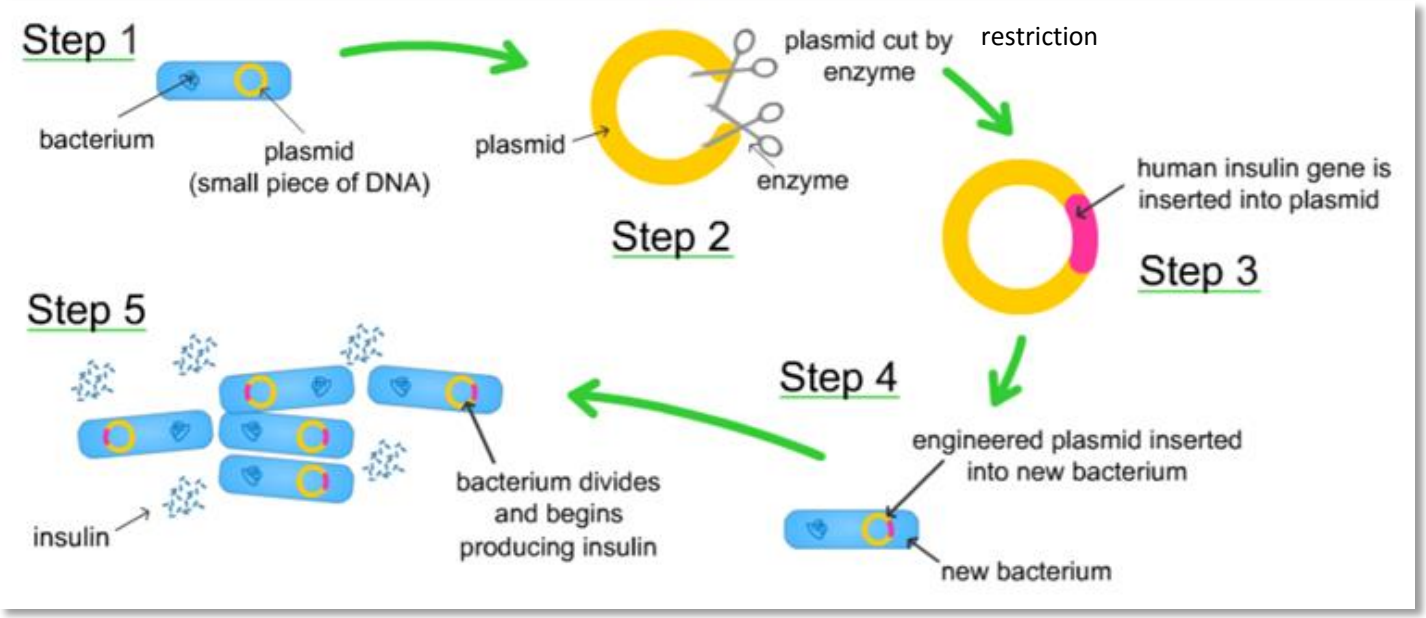
http://www.slideshare.net/gurustip/genetic-engineering-and-biotechnology-presentation

Genetic modification

Also known as genetic engineering, 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

Genetic modification

Also known as genetic engineering, gene transfer or transgenics.

milk containing spider silk protein *is produced by goats (spider silk is immensely strong)*

https://commons.wikimedia.org/wiki/File:Brown_female_goat.jpg

There are many different examples. Most processed food contains a GM dervied product

Salt tolerant tomato plants

https://commons.wikimedia.org/wiki/File:ARS_Ohio_processing_tomato.jpg

Golden rice *is coloured yellow as it contains β-carotene (a precursor to vitamin A)*

https://commons.wikimedia.org/wiki/File:Golden_Rice.jpg

Human insulin *produced by bacteria for diabetics*

https://en.wikipedia.org/wiki/File:E_coli_at_10000x,_original.jpg

Gene Transfer

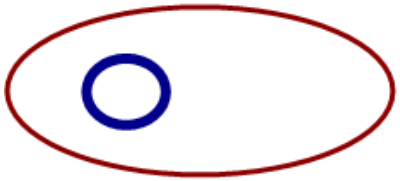
Requires plasmids, a host cell, restriction enzymes and ligase.

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

alternatively mRNA can treated with reverse transcriptase to produce short DNA segments

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

These can be removed.



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

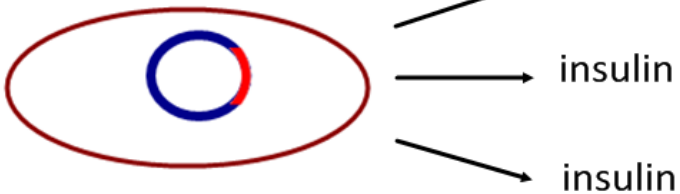
T C G A C G
A G C T G C A C C

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'

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**.



Fermenters are used to produce large quantities of bacteria. The human insulin is then separated from the bacteria and purified.

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

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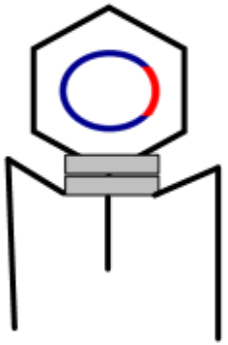
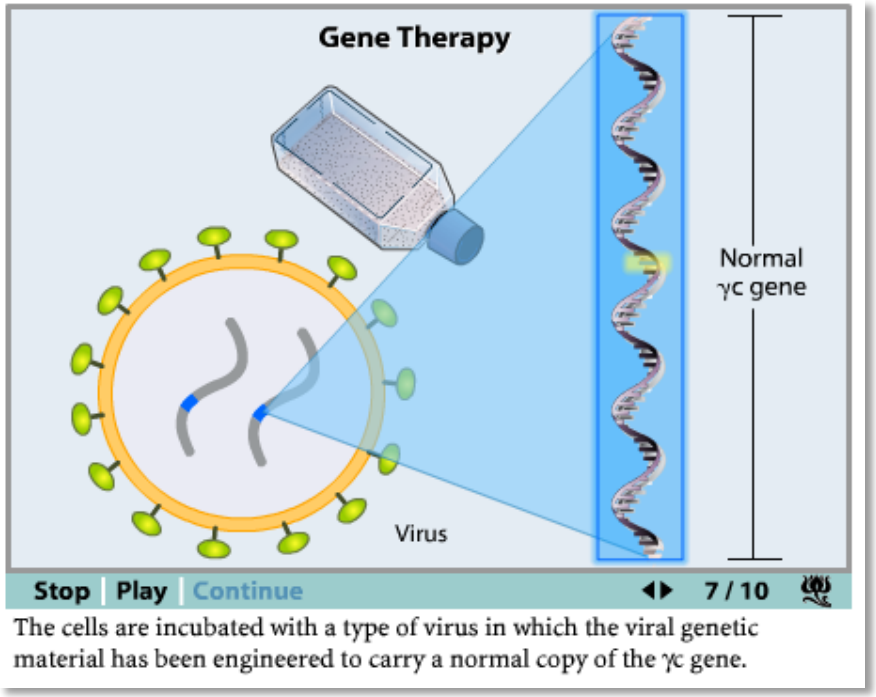
Gene Transfer *Can also be used in gene therapy.*



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_Ql

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

Bt Corn

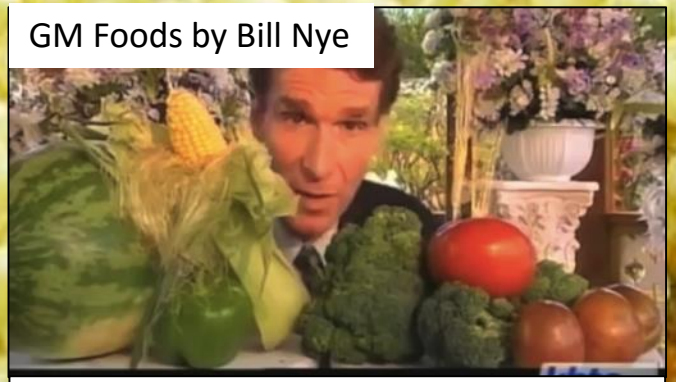
Genes from Bt have been inserted into maize so GM plants can produce an insecticidal toxin and therefore be resistant to pests, e.g. European Corn Borer.

Bacillus thuringiensis (Bt) is a soil bacterium that produces insecticidal toxins.

General Potential Benefits:

- **Introduction of a new trait** – Bt gene increases resistance to pests such as the *European Corn Borer*
- **Results in increased productivity** – less land used / greater yield / less crop damage
- **Less use of chemical pesticides** – reduced cost / ecological damage to wild the economic cost of farming
- **Increased disease resistance**
- **Less use of chemical herbicides**
- **Less use of chemical fetiliser**
- **Increased hardiness** – better drought/cold slinity tolerance and therefore can be grown in more locations / has a longer growing season
- **Increased nutritional content**

GM Foods by Bill Nye



https://youtu.be/8z_CqyB1dQo

Bt Corn FAQ by Colorado State University:
http://www.ext.colostate.edu/pubs/crops/00707.html

A hard look at GM crops by Nature:
http://www.nature.com/news/case-studies-a-hard-look-at-gm-crops-1.12907

Which benefits are **relevant** and need **assessing** for **Bt Corn**?

https://commons.wikimedia.org/wiki/File:VegCorn.jpg

Bt Corn

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GM Foods by Bill Nye

Nature of science: Assessing risks associated with scientific research - scientists attempt to assess the risks associated with genetically modified crops or livestock. (4.8)
This point is implicitly dealt with in the Bt Corn case study.

General Potential Benefits:

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- **Increased disease resistance**
- **Less use of chemical herbicides**
- **Less use of chemical fetiliser**
- **Increased hardiness** – better drought/cold slinity tolerance and therefore can be grown in more locations / has a longer growing season
- **Increased nutritional content**

Bt Corn FAQ by Colorado State University:
http://www.ext.colostate.edu/pubs/crops/00707.html

A hard look at GM crops by Nature:
http://www.nature.com/news/case-studies-a-hard-look-at-gm-crops-1.12907

Which benefits are **relevant** and need **assessing** for **Bt Corn**?

https://commons.wikimedia.org/wiki/File:VegCorn.jpg

Bt Corn

Genes from Bt have been inserted into maize so GM plants can produce an insecticidal toxin and therefore be resistant to pests, e.g. European Corn Borer.

Bacillus thuringiensis (Bt) is a soil bacterium that produces insecticidal toxins.

Potential Benefits:

- **Introduction of a new trait** – Bt gene increases resistance to pests such as the *European Corn Borer*
- **Results in increased productivity** – less land used / greater yield / less crop damage
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Bt was successfully introduced and Bt Corn is significantly more resistant to pests

Maximum productivity has not increased, but losses in 'bad' years have been reduced.

Bt toxins are considered to be much more selective and safer for humans and non-target organisms than most conventional insecticides

Which benefits are **relevant** and need **assessing** for **Bt Corn**?

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All risks should be assessed as part of a comprehensive testing programme.

General Potential Risks:

- Could be toxic to or cause allergic reactions in humans
- Transferred genes could mutate after testing
- Non-target organisms affected by toxins
- Increases resistance to toxin evolves in pests
- Accidental release may result in competition with native plant species
- Super weeds - through cross-breeding the introduced gene could be transferred to wild varieties
- Biodiversity reduced – both plant populations by direct competition and animal populations directly and indirectly could be affected
- Patent laws prevent farmers producing locally suitable varieties – *this would lead to unregulated field tests, not a desirable situation*

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Bt toxin has been used for 30 years without toxicity in humans being detected, however some experimental transgenic plants have caused allergic responses.

Bt is a very common soil bacterium – therefore organisms have regularly exposed to Bt toxins previously. Negative affects on many non-target organisms thought to be minor …

… However many species of caterpillars occur in and around cornfields during the growing season, and might be affected by Bt corn. [see 3.5.S3]

https://commons.wikimedia.org/wiki/File:VegCorn.jpg

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Unknown, but there is a risk

Risk is known and there is evidence of superweeds evolved from other transgenic crops

Ecosystem wide risks are unknown, but specific examples [see 3.5.S3] have been examined.

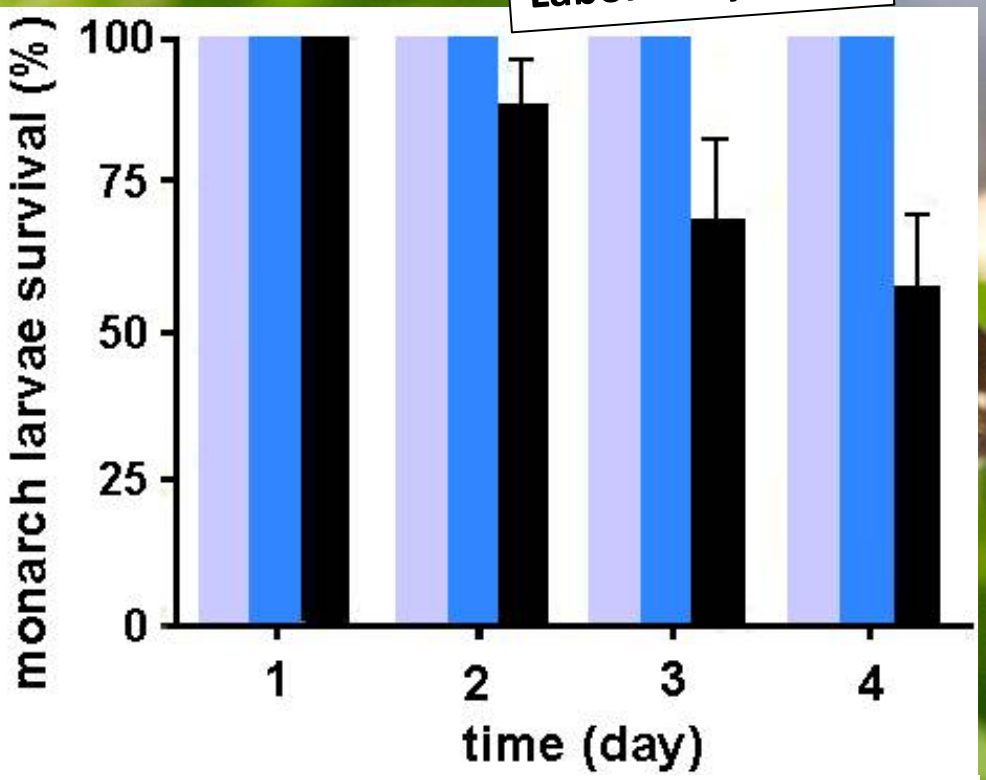
https://commons.wikimedia.org/wiki/File:VegCorn.jpg

Bt Corn *Risks to monarch butterflies*

- The caterpillar stage of the Monarch feeds on milkweed.
- Milkweed commonly grows on the edge of corn fields
- Studies show some mortality in Monarch caterpillars fed milkweed leaves covered with Bt corn pollen

http://www.news.cornell.edu/sites/chronicle.cornell/files/Losey1.72.JPG

Laboratory study



The survival of second to third-instar monarch larvae was tested. Three milkweed leaf treatments were conducted: leaves with no pollen (lavender), leaves treated with untransformed corn pollen (blue), and leaves dusted with pollen from Bt corn (black). The mean survival rate is based on the proportion of larvae surviving in five replicates of each treatment (from Losey, H. E., L. S. Rayor, and M. E. Carter. 1999. Transgenic pollen harms monarch larvae. Nature 399: 214, © 1999 Nature Publishing Group www.nature.com)

n.b. the error bars represent the standard error from the mean, i.e. the range within which the true mean is likely to be found.

http://www.esa.org/tiee/vol/v2/issues/figure_sets/biotech/figure2.html

https://commons.wikimedia.org/wiki/File:Monarch_Butterfly_Showy_Male_3000px.jpg

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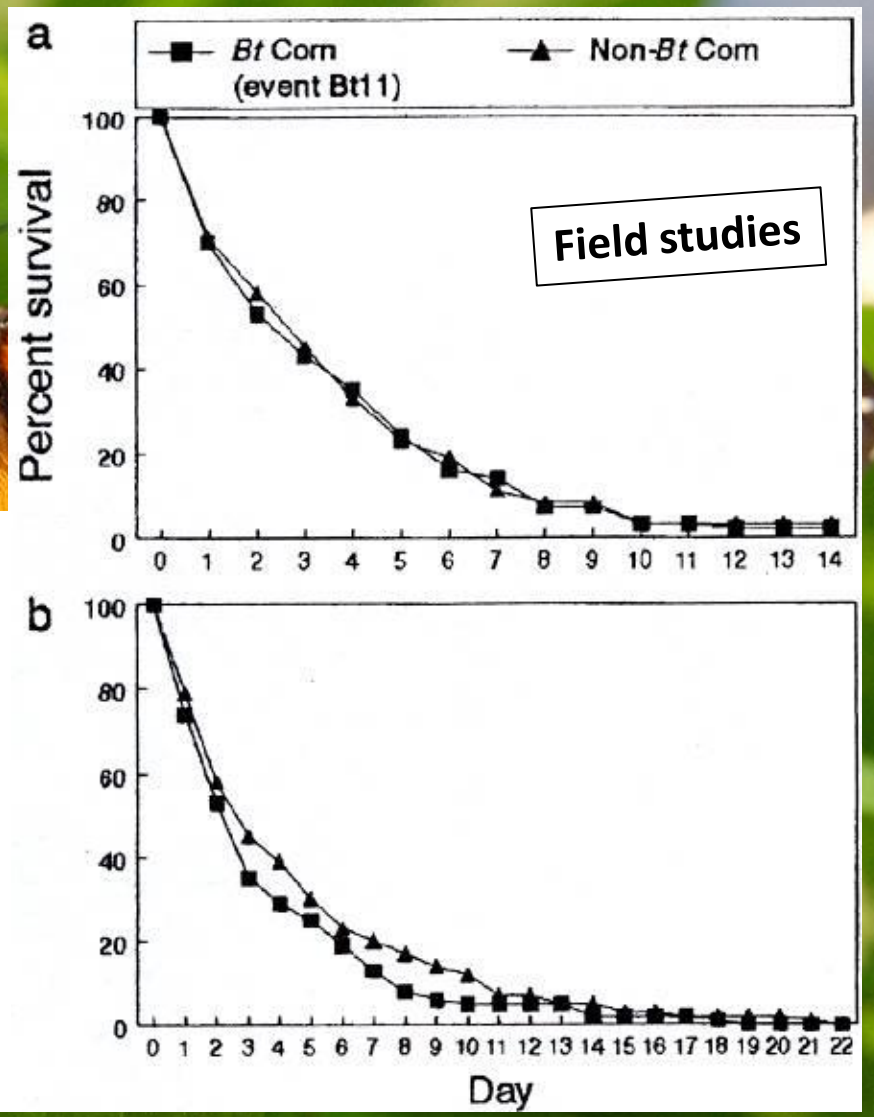

Survival curves for monarch larvae placed in and near Bt and non-Bt corn fields. Survival curve (a) is based on data from Iowa and survival curve (b) is based on data from New York (from Stanley-Horn, D. E. et al. 2001. Assessing the impact of Cry1Ab-expressing corn pollen on monarch butterfly larvae in field studies. *Proceedings of the National Academy of Sciences* 98: 11931-11936, © 2001 National Academy of Sciences, U.S.A.).

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Analyse the data:

1. Explain why untransformed corn was included in the study
2. Describe the trends seen in Laboratory study
3. Discuss whether there is evidence of Bt corn pollen affecting Monarch caterpillar survival. Are the error bars useful to the discussion?
4. Describe the trends seen in the field studies.
5. Suggest reasons why Monarch caterpillars' Bt toxicity is easier to detect in laboratory studies.
6. Evaluate the hypothesis that Bt Corn adversely affects Monarch butterfly populations.

Additional findings and questions:

- Sub-lethal affects of Bt toxins are not well known.
- Data does show reduced feeding levels on milkweed covered in Bt corn pollen.
- The use of most toxic variety of Bt corn has been discontinued and more modern varieties show little measurable effect.

http://www.news.cornell.edu/sites/chronicle.cornell/files/Losey1.72.JPG

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Clone

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?

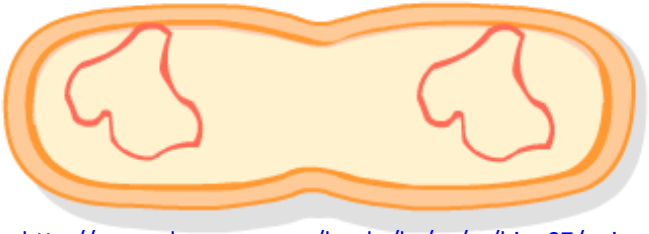
Epigenetics has the answer…

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

Starfish, if damaged, can regenerate a whole body from a single leg, another example of a natural clone.

http://subsinai.com/diving/img/underwaterguide/corals/c_a_00008.jpg

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



http://www.classzone.com/books/hs/ca/sc/bio_07/animated_biology/bio_ch05_0149_ab_fission.html

http://www.slideshare.net/gurustip/genetic-engineering-and-biotechnology-presentation

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

http://www.ohio.edu/people/braselto/readings/images/mint_runner.jpg

Runners are modified laterally growing stems used to reproduce asexually. Each new plantlet can separate to produce a new plant.

Clones allow plants to quickly propagate (produce copies) of successful plants.

Tubers, the swollen tips of underground stems, are storage organs in plants such as sweet potatoes. During winter the plant dies back, but in spring each tuber starts to grow producing separate plants, all clones of the parent plant.

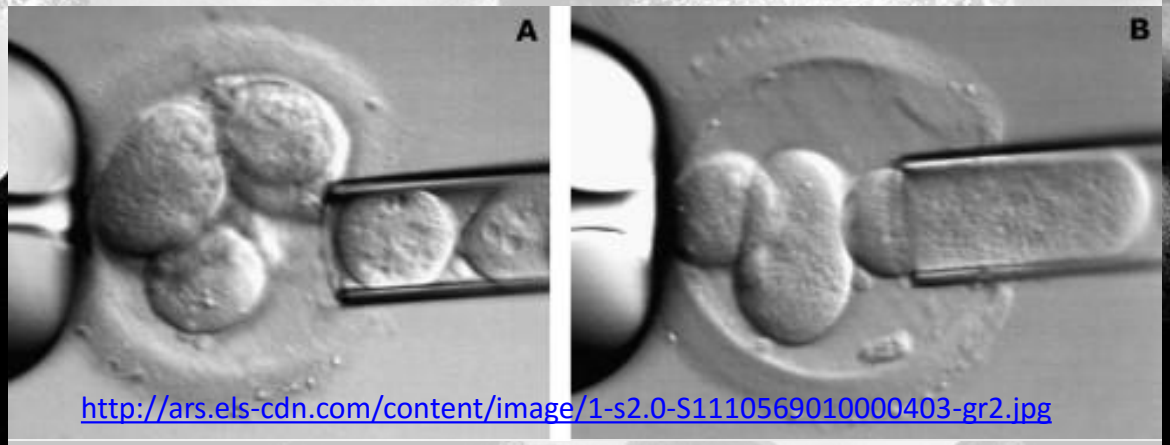
http://biobook.nerinxhs.org/bb/systems/plant_reproduction/Ipomoea_batatasL_ja01.jpg

Monozygotic twins

Embryos can split and then continue to develop separately to form identical twins.

This is possible because in embryonic development the cells are still unspecialised can become any type of cell.

Below shows this process being done artificially: using a pipettes cells are extracted from the embryo (A) and implanted into an embryo (B).



http://ars.els-cdn.com/content/image/1-s2.0-S1110569010000403-gr2.jpg

As an artificial process this has limited value as only very young embryonic cells can be used.

https://commons.wikimedia.org/wiki/File:Lee_Twins_profile.jpg

Investigating artificial propagation

Not all stem cuttings form roots and grow to become clones, why?

Many common plants root easily from stem cuttings producing full-grown (clone) plants quickly.

https://apps.rhs.org.uk/Advice/ACEImages//RHS_PUB0003119_679770.jpg

Examples of factors that can be investigated:

- Substrate - water, type of soil
- Numer of leaves on the stem
- Length of stem cut
- Effectiveness of commerical rooting powders
- Covering with a plastic bag (to reduce transpiration)
- How the stem is cut
- Proximity of a node (point of branching) to the base of the stem

Rigour of the design – things to consider:

- How will root growth/appearance be measured (you can expect multiple roots of variable length)?
- Which variables need to be controlled?
- What plant species/variety (or selection of) will be examined?
- How many cuttings are needed to ensure a reliable investigation?

http://www.richmondhillreflectionsmag.com/wp-content/uploads/2013/02/IMG_9289.jpg

Cloning differentiated cells

To clone an organism with **desired traits** is problematic as a developed organism consists of **specialised cells** which are multipotent, unipotent or cannot divide at all.

For a clone to develop **somatic (diploid body) cells** of the donor organism need to be **induced** to become **pluripotent** (cells capable of dividing to become any type of cell).

In 1958 John Gurdon **transplanted** the **nucleus** of a (specialised diploid) tadpole intestinal cell into an **enucleated** (nucleus removed) frog **egg**. In this way, he created tadpoles that were genetically identical to the one from which the intestinal cell was taken.

The method has been refined, but in essence remains the same. It is now termed **somatic-cell nuclear transfer**.

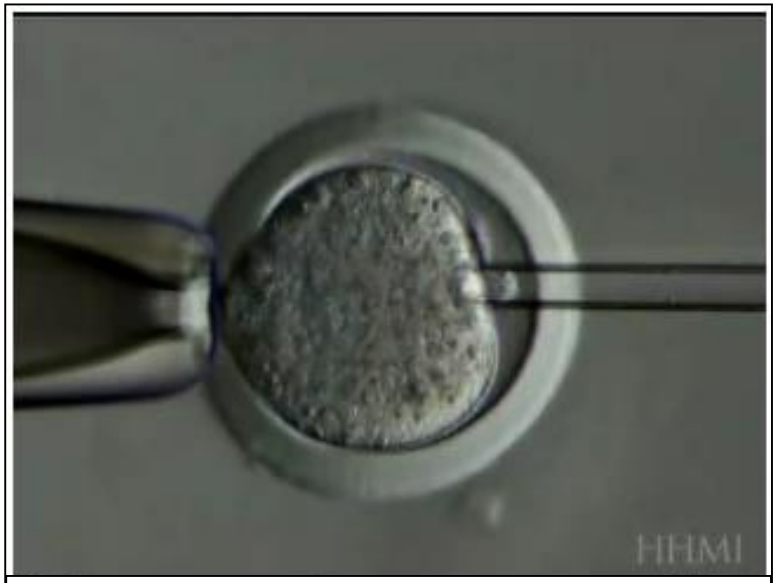
http://www.californiaherps.com/frogs/images/xlaevistadpolesocjh12103.jpg

Cloning by somatic-cell nuclear transfer (SCNT)

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

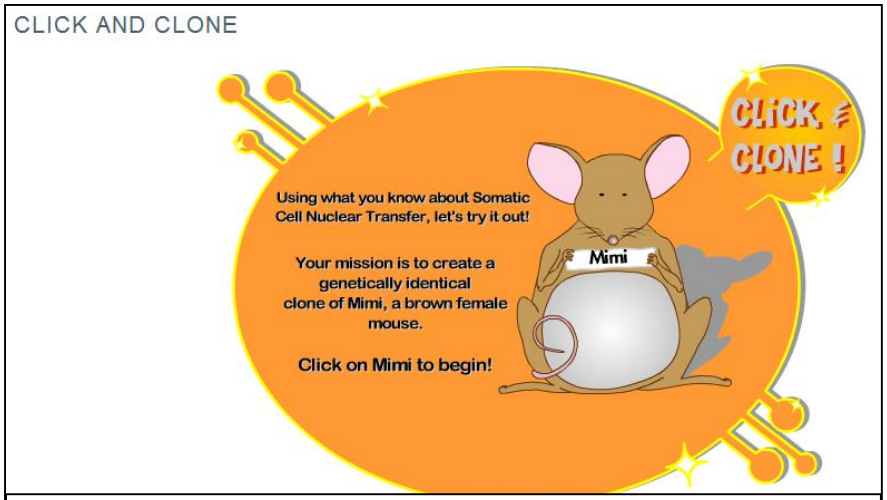
somatic-cell nuclear transfer made easy:

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



Video of enucleation of an egg cell:
http://www.hhmi.org/biointeractive/stemcells/scnt_video.html

Make your own cloned mice and learn the process of cloning animal cells



http://learn.genetics.utah.edu/content/cloning/clickandclone/

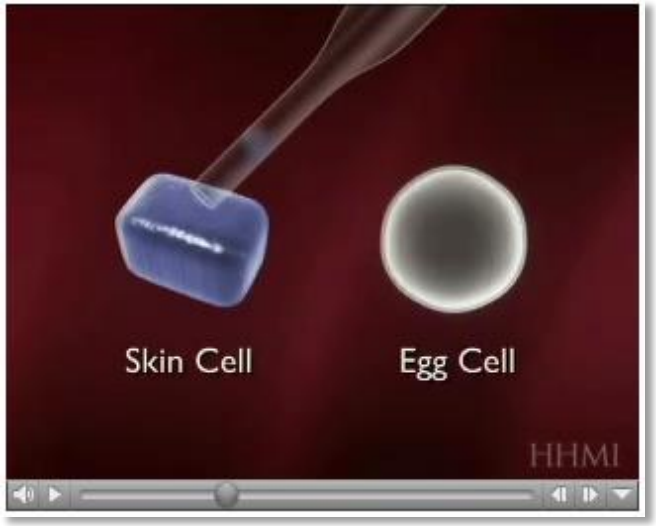
Dolly the sheep was the first successful cloning of a mammal from a differentiated somatic cell.
She was the result of many attempts. Interestingly, she dies young – but of age-related illness.

Human reproductive cloning is illegal

http://www.slideshare.net/gurustip/genetic-engineering-and-biotechnology-presentation

SCNT and Theraputic Cloning

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



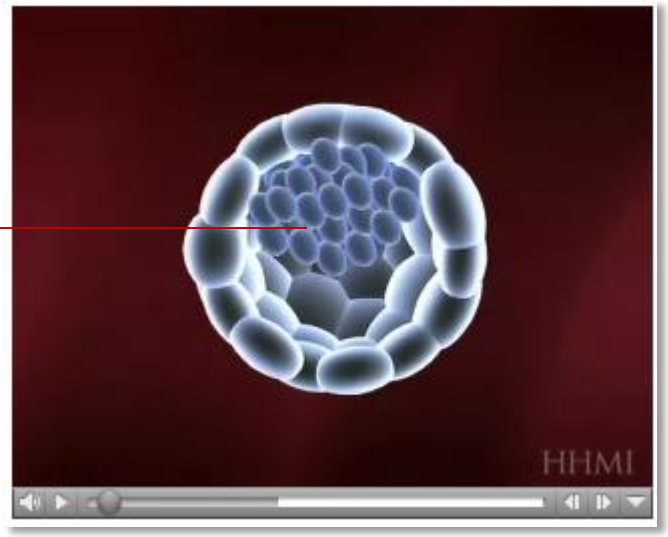
Nuclear transfer animation from HHMI:
http://www.hhmi.org/biointeractive/stemcells/scnt.html

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.slideshare.net/gurustip/genetic-engineering-and-biotechnology-presentation