Genetic Engineering & Biotechnology

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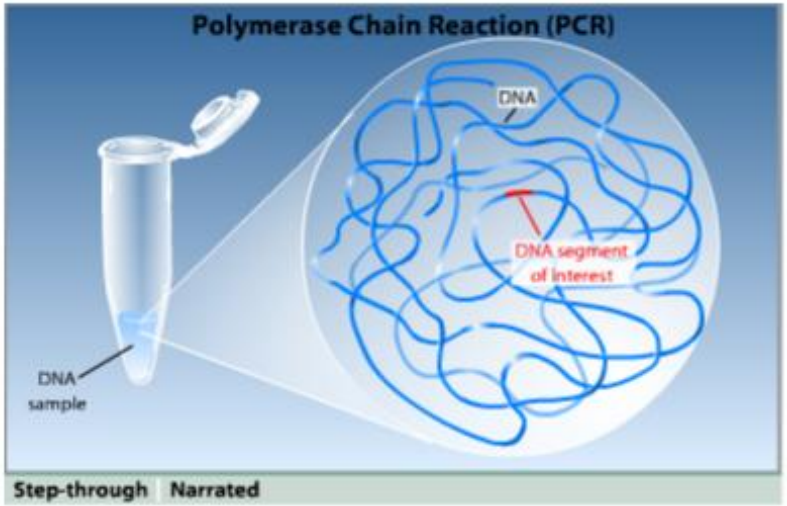
Polymerase Chain Reaction

If we collect a small sample of DNA, we can use PCR to amplify it - by using DNA polymerase, we can make more copies of the target section.

1. DNA is denatured
2. Primers are annealed to the start and end of the required section of DNA
3. DNA polymerase uses complementary base pairing to make more copies of the DNA.



http://spine.rutgers.edu/cellbio/flash/pcr.htm

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

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

This cycle is repeated up to 35 times to yield sufficient DNA for use in the lab.

Often, PCR is used to amplify a small sample found at a crime scene so it can be used as evidence in DNA profiling.

The PCR Song

There was a time when to amplify DNA,
You had to grow tons and tons of tiny cells.
(Oooh) Then along came a guy named Dr. Kary Mullis,
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*.

http://www.youtube.com/watch?v=7uafUVNkuzg

[Chorus]
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)

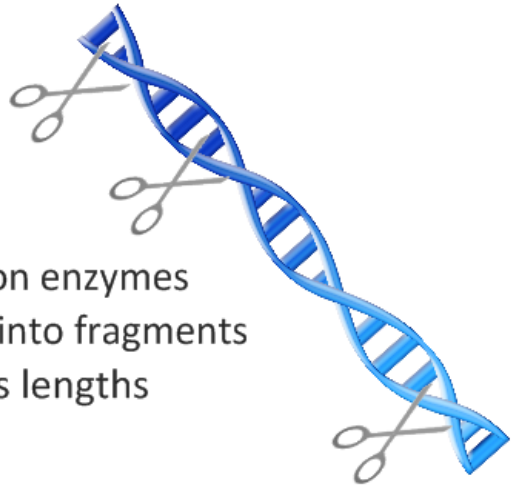
And here's the hilarious follow-up about PCR enzymes:

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

DNA Profiling: Gel Electrophoresis

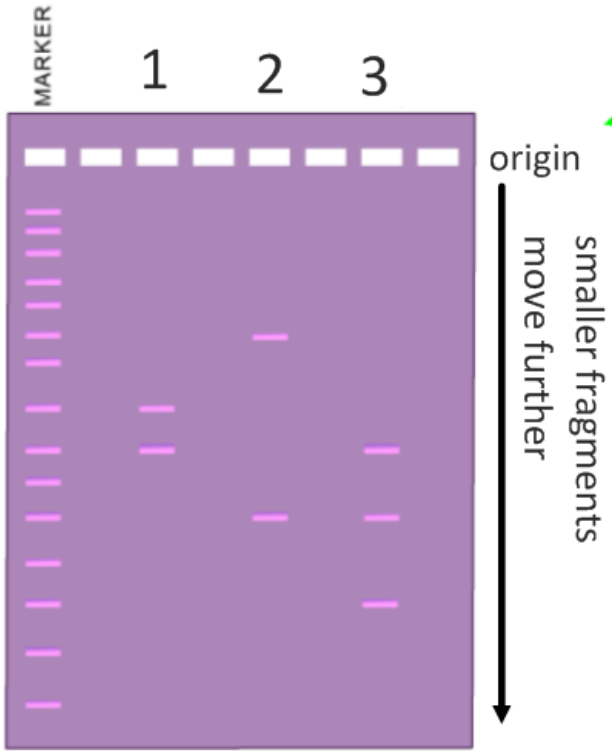
DNA sample is taken

PCR amplifies DNA to get a useful amount



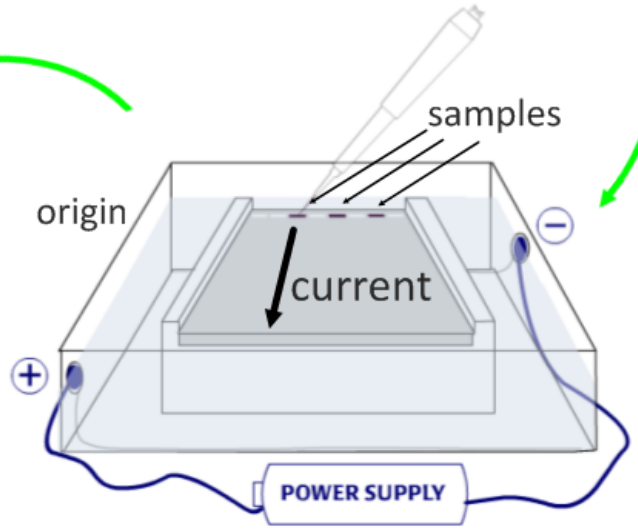
Restriction enzymes cut DNA into fragments of varous lengths

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



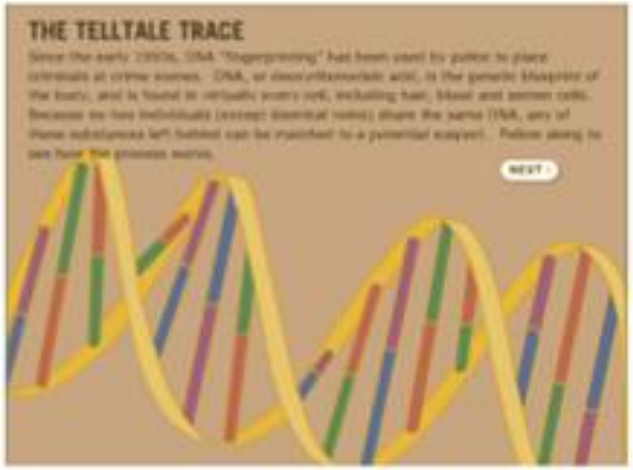
A 'tag' can be added to bind to fragments at certain base sequences. This will glow under fluorescent light and gives a series of bands which can be compared as the results of the DNA profile.

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.

Easy Introduction:

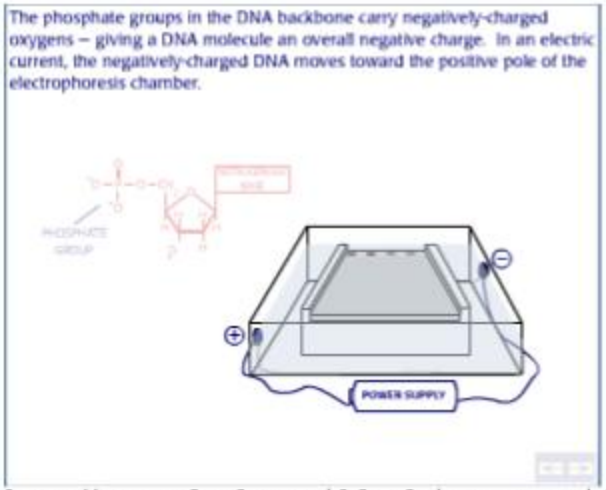


http://www.courttv.com/graphics/dna_anniv/50_dna.swf

More in-depth knowledge:

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

Gel-electrophoresis:

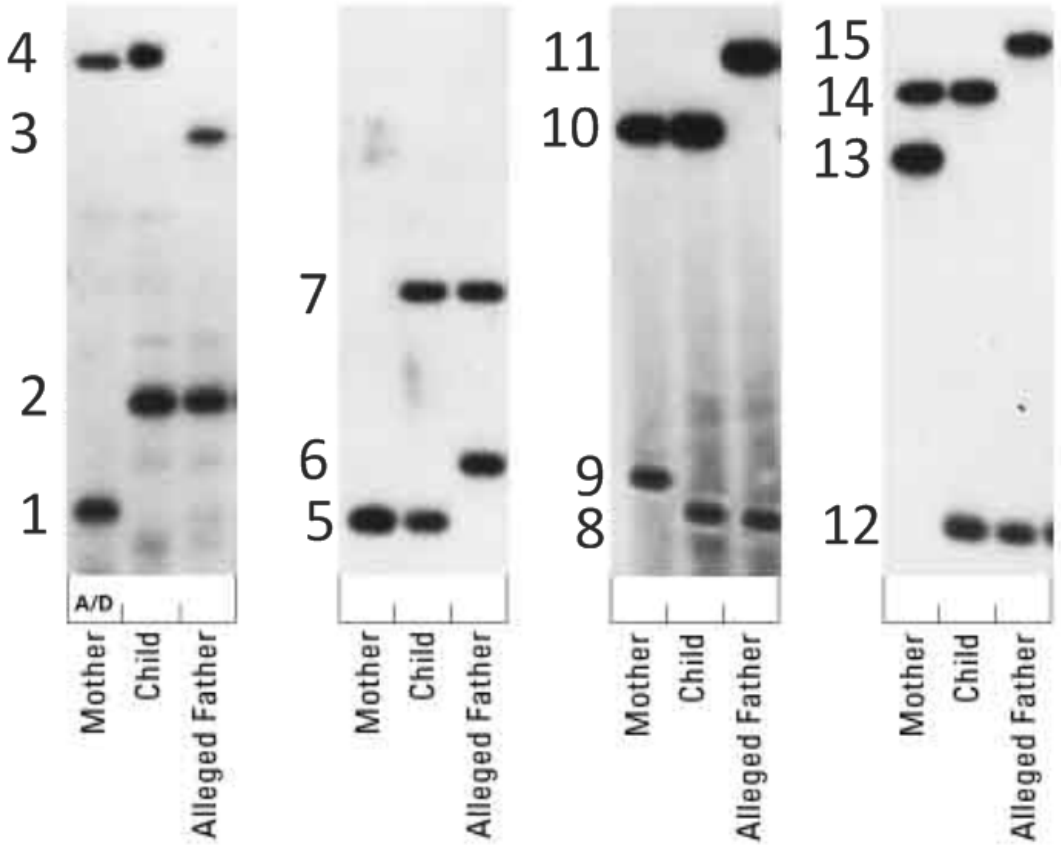


http://www.dnalc.org/ddnalc/resources/electrophoresis.html

Gel-electrophoresis:

http://learn.genetics.utah.edu/units/biotech/gel/

A paternity case: is he the father?



These four strips are actually sections of the same gel electrophoresis run.

Fragment 1 is the largest (closest to the origin). Fragment 15 is smallest.

Does the man share sufficient bands with the baby which the mother does not share in order for him to be considered the father?

What do you understand by the expression: "*DNA profiling is better at proving innocence than guilt.*"

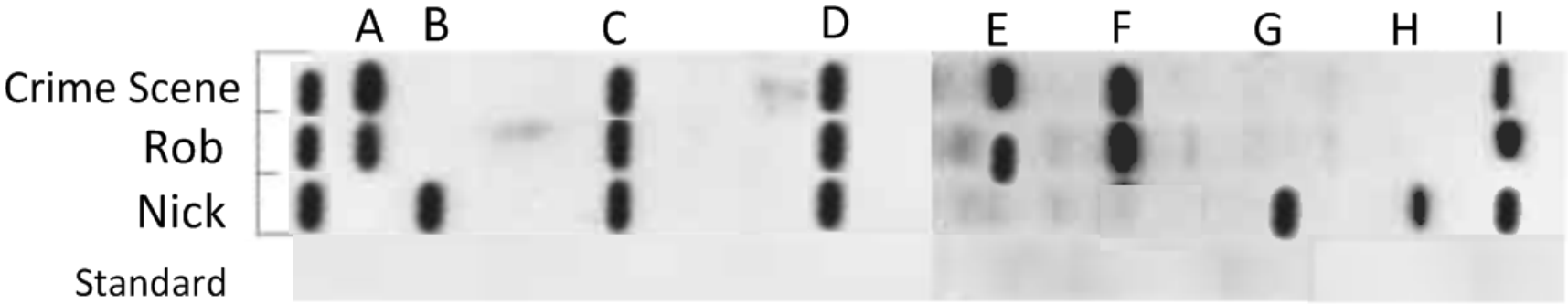
Case 7286224: The Green Street Hooligans

The case: a little old lady got caught in a brawl between two rival groups of football supporters at Green Street Park and was badly hurt. She was hit by one man, who she said was smoking a cigarette and threw it on the floor.

Other witnesses identified two men, Rob McCarr (a Green Street supporter) and Nick Allott (a Blue Docks supporter) as both being at the scene and smoking, though they couldn't be sure which one hit the lady.

Crime scene investigators found the cigarette where she had been hit, and arrested both suspects. They were submitted to DNA profiling to determine the culprit.

DNA Profile Results: Case 7286224

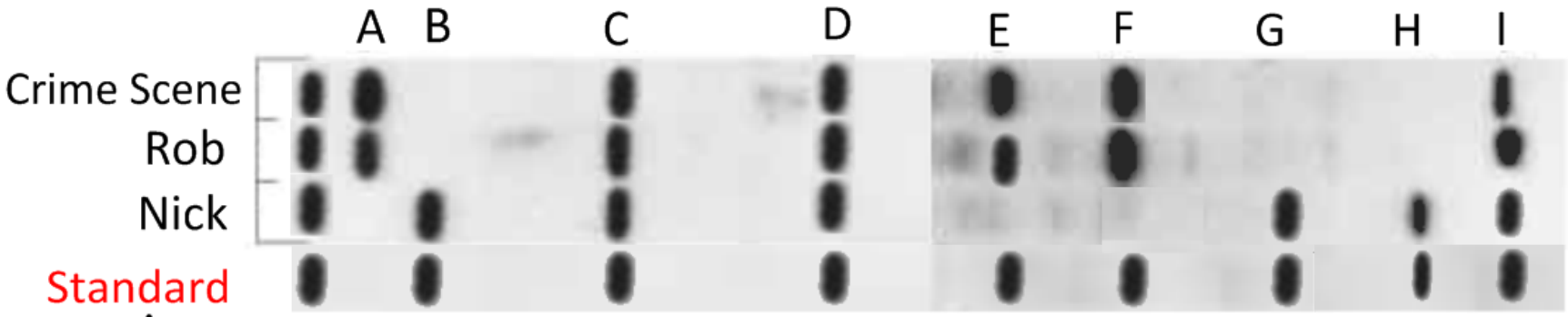


Add a row of fragments that would represent the Standard sample.

Whose cigarette was found at the crime scene?

Is there any truth in the story that Rob and Nick are related?

DNA Profile Results: Case 7286224



Whose cigarette was found at the crime scene?
Rob's - all of the markers matched his blood sample.
Only some of Nick's matched, so he can be excluded.

Is there any truth in the story that Rob and Nick are related?
Possibly - they share lots of fragments. You would need to compare their samples with other known members of the family and some which are definitely not, to be more sure.

Learn.Genetics
Genetic Science Learning Center
at THE UNIVERSITY OF UTAH

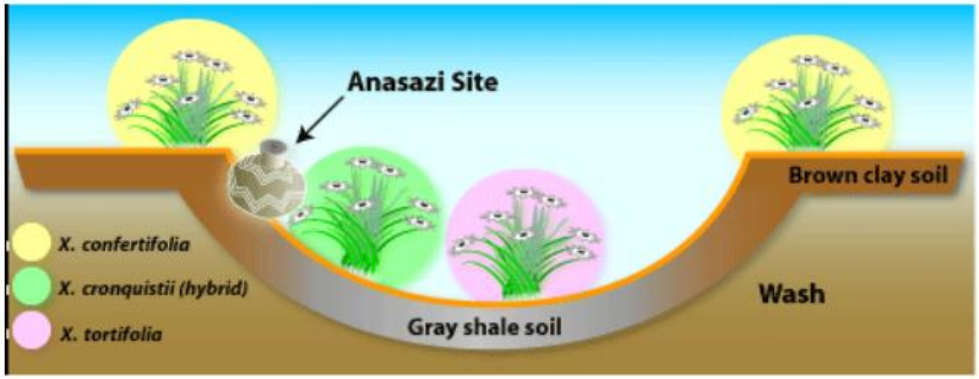
The case of the Pot-Hunters

http://learn.genetics.utah.edu/units/basics/mystery/evidence.cfm

Try this challenging case study to see how DNA fingerprinting is used in association with other evidence to investigate the case of a stolen archeological artifact.

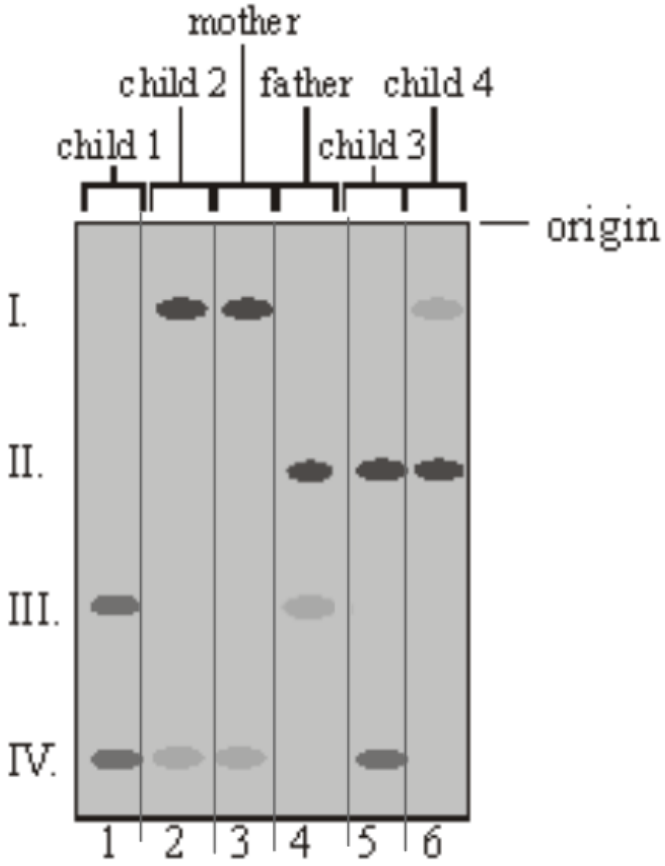
In the case, DNA evidence is taken from plants in and around the archaeological site and used to determine the origin of an ancient pot.

By reading the prepared evidence, analysing the gel electrophoresis results and reading the court transcript, you and your group should be able to decide whether or not the suspect is guilty of pot-theft.



Sample exam questions:

This DNA gel shows the profile for a man, a woman and their four children.



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

1. Which DNA fragment is smallest?

I. II. III. IV.

2. How many DNA fragments would be seen if the 'standard' column had been included?

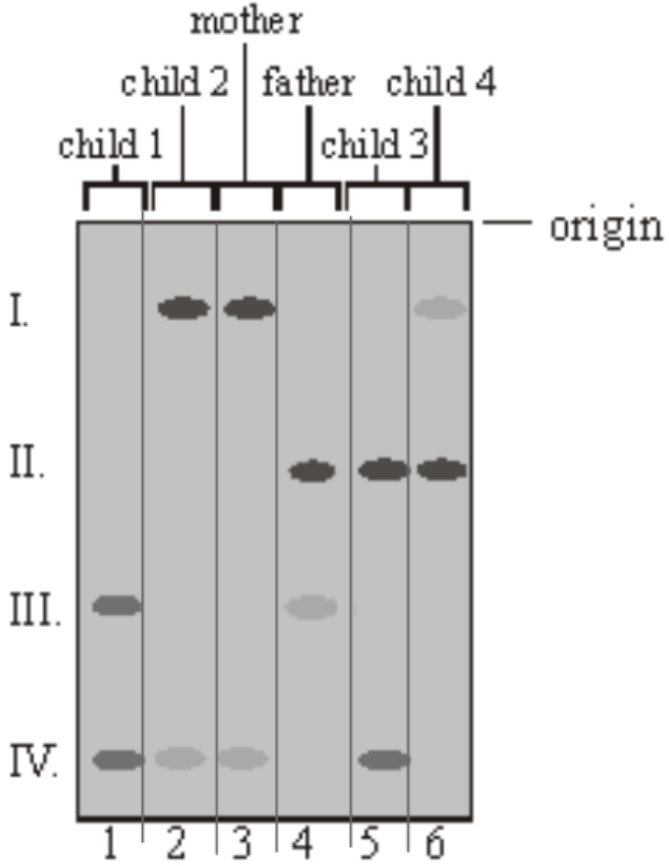
1 2 3 4

3. Which child is least likely to be the biological offspring of the father?

1 2 3 4

Sample exam questions:

This DNA gel shows the profile for a man, a woman and their four children.



1. Which DNA fragment is smallest?

I. II. III. (IV.)

2. How many DNA fragments would be seen if the 'standard' column had been included?

1 2 3 (4)

3. Which child is least likely to be the biological offspring of the father?

1 (2) 3 4

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

Human Genome Project

http://www.genome.gov/25019879

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:

Bioinformatics was born - high-tech way to collect, collate and access information from genetic databases.

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 human evolutionary history.

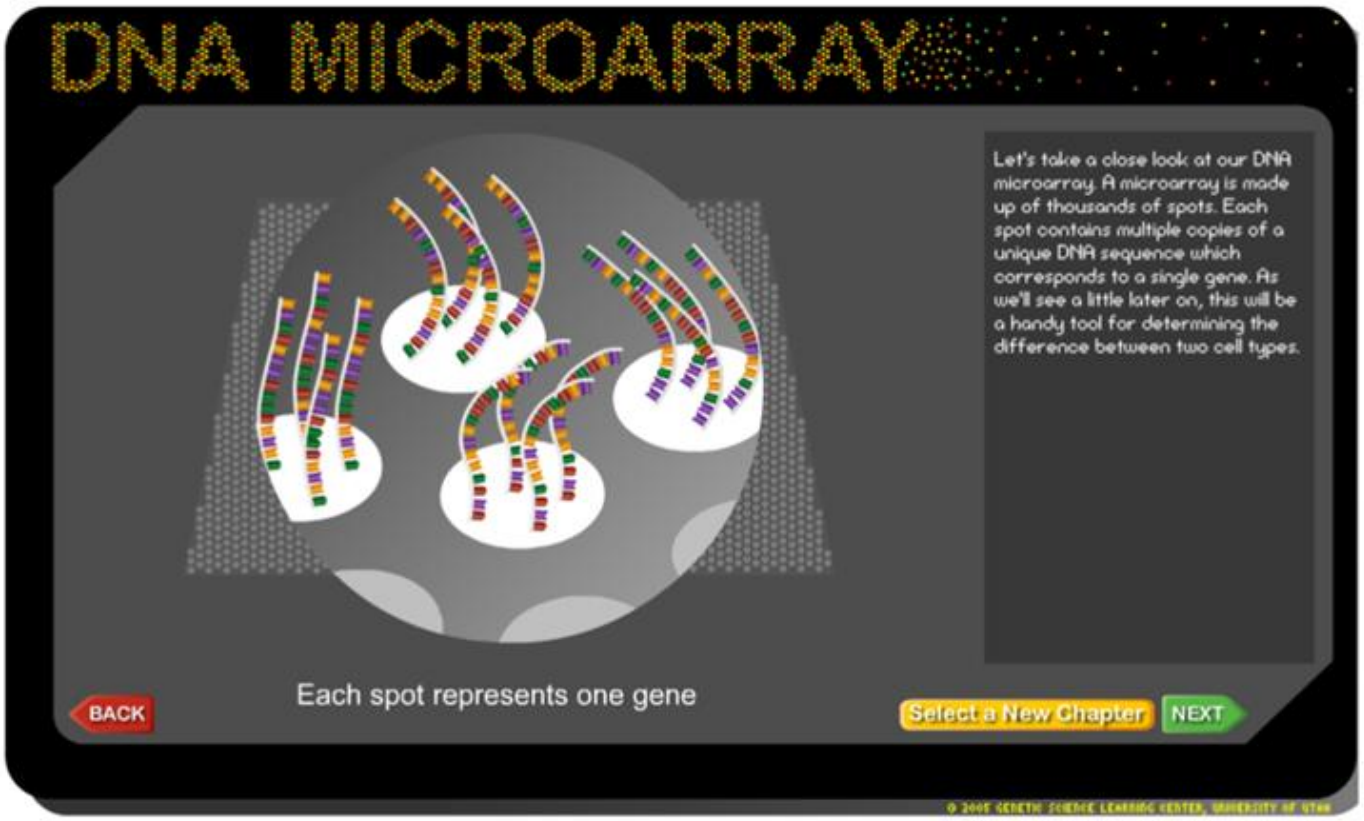

How to Sequence a Genome


Genes, Variation and Human History


Bioinformatics


Ethical, Legal and Social Implications (ELSI)

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



http://learn.genetics.utah.edu/units/biotech/microarray/

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

http://www.learner.org/channel/courses/biology/units/genom/images.html

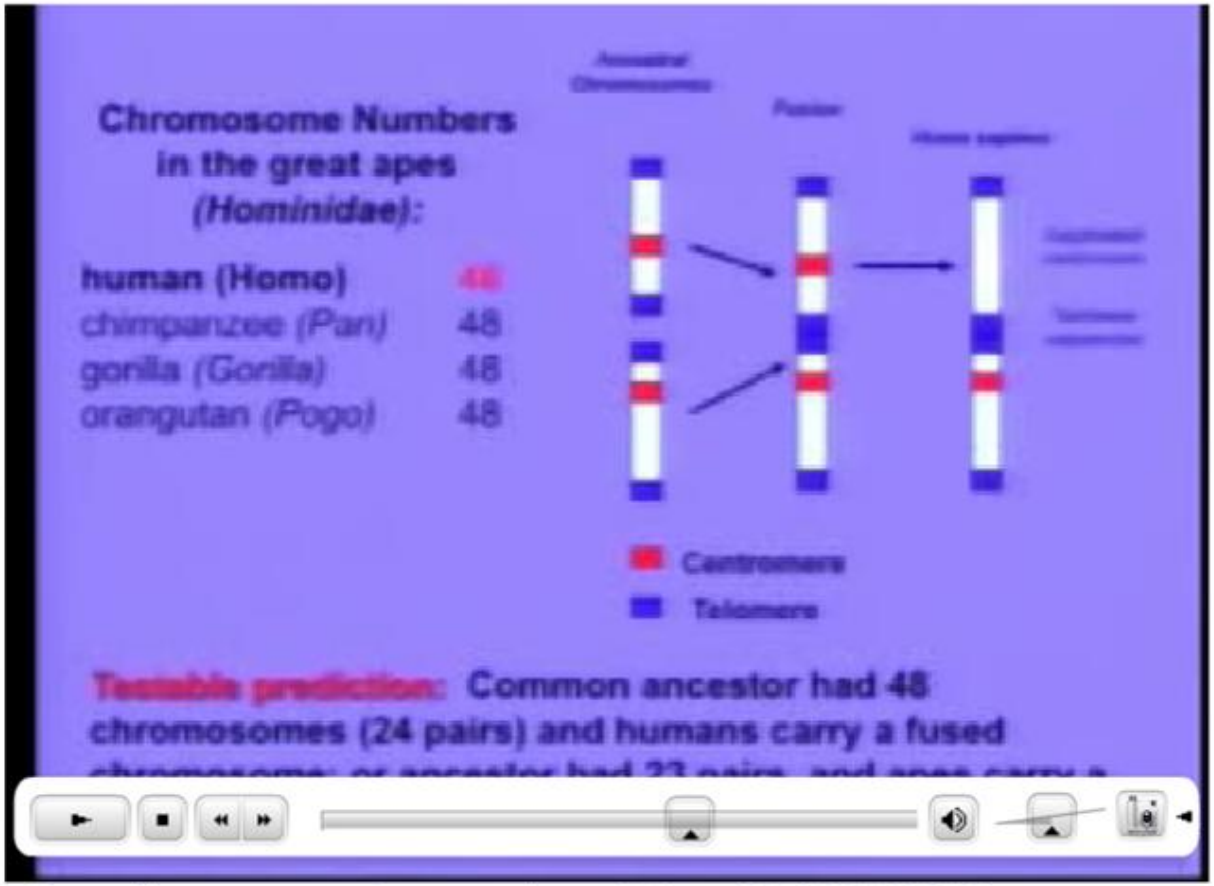
The Human Genome Project has pushed forwards medical and pharmacological research - by helping us see the 'real' cause.

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

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.

What are the ethical, legal and moral implications of this knowledge?
Try to provide a balanced view by first identfying as many pro- or anti- genomics groups as possible and noting their arguments.

The Human Genome Project allows more insights into evolutionary relationships:

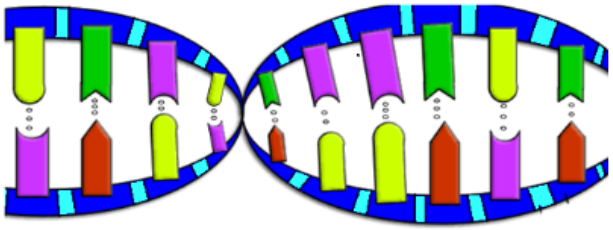


http://www.youtube.com/watch?v=zi8FfMBYCkk

Here is Ken Miller talking about genome evidence used to show how human chromosome number 2 is actually a fusion of two ancestral chromosomes, explaining why the other great apes have 24 pairs of chromosomes, but we only have 23.

The Genetic Code is Universal

All living organisms use the same bases:



This means that base sequences can be transferred from one organism to another without changing their function.

So...

we could take the gene for healthy insulin production from a human and insert it into a bacterial plasmid - and the bacteria will then be able to produce human insulin.

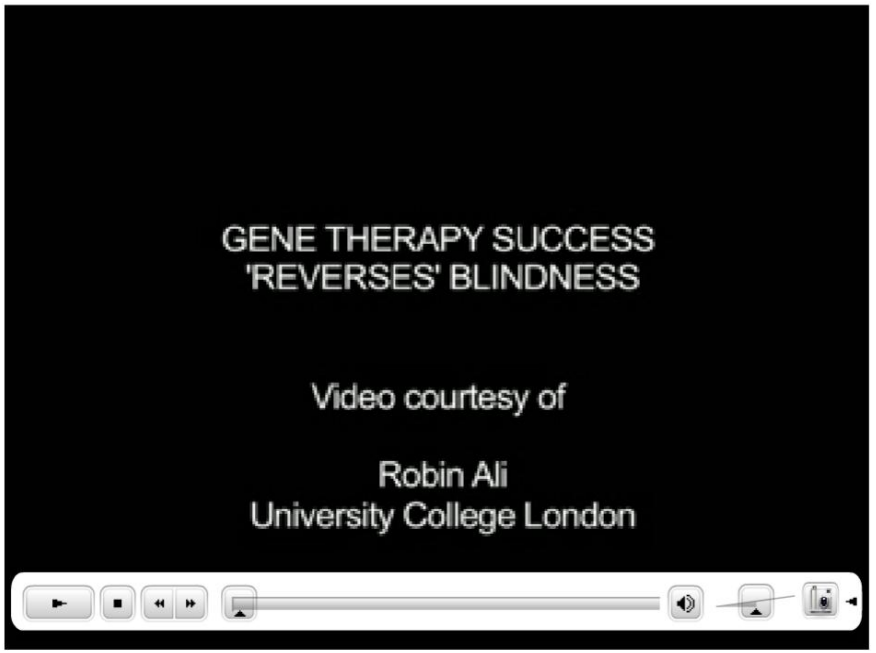
Gene Transfer:

find gene, get gene, stick it in

"Recombinant DNA Technology"

In gene transfer techniques, the gene for a favourable trait is identified in one organism and transferred to another.

Gene transfer can be used in:



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

GENE THERAPY

To repair a faulty gene in an organism (such as the SCID gene)

INDUSTRY/MEDICINE

To produce large volumes of a desired protein (such as insulin or clotting factor)

INDUSTRY/AGRICULTURE

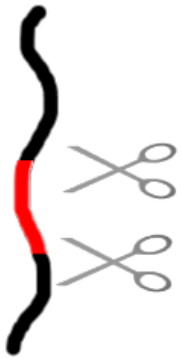
To genetically modify organisms for favourable characteristics (such as nutrient-rich golden rice)

Animations here:

http://highered.mcgraw-hill.com/sites/0072437316/student_view0/chapter16/animations.html

Gene Transfer: Basic Steps

Find gene

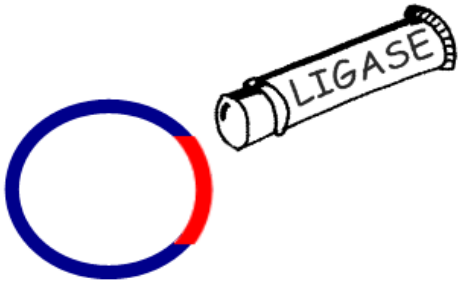


restriction enzyme cleaves (cuts out) the desired DNA fragment

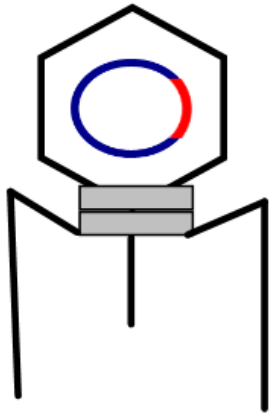
Get gene

the same restriction enzyme can be used to cut the same base sequence in the plasmid DNA.

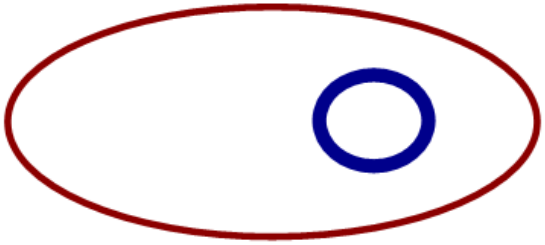
Stick it in



DNA ligase inserts the gene fragment into the plasmid. The modified plasmid is then inserted into a host cell (vector), e.g. virus, bacteria or fungi, which delivers the gene to the target cells.

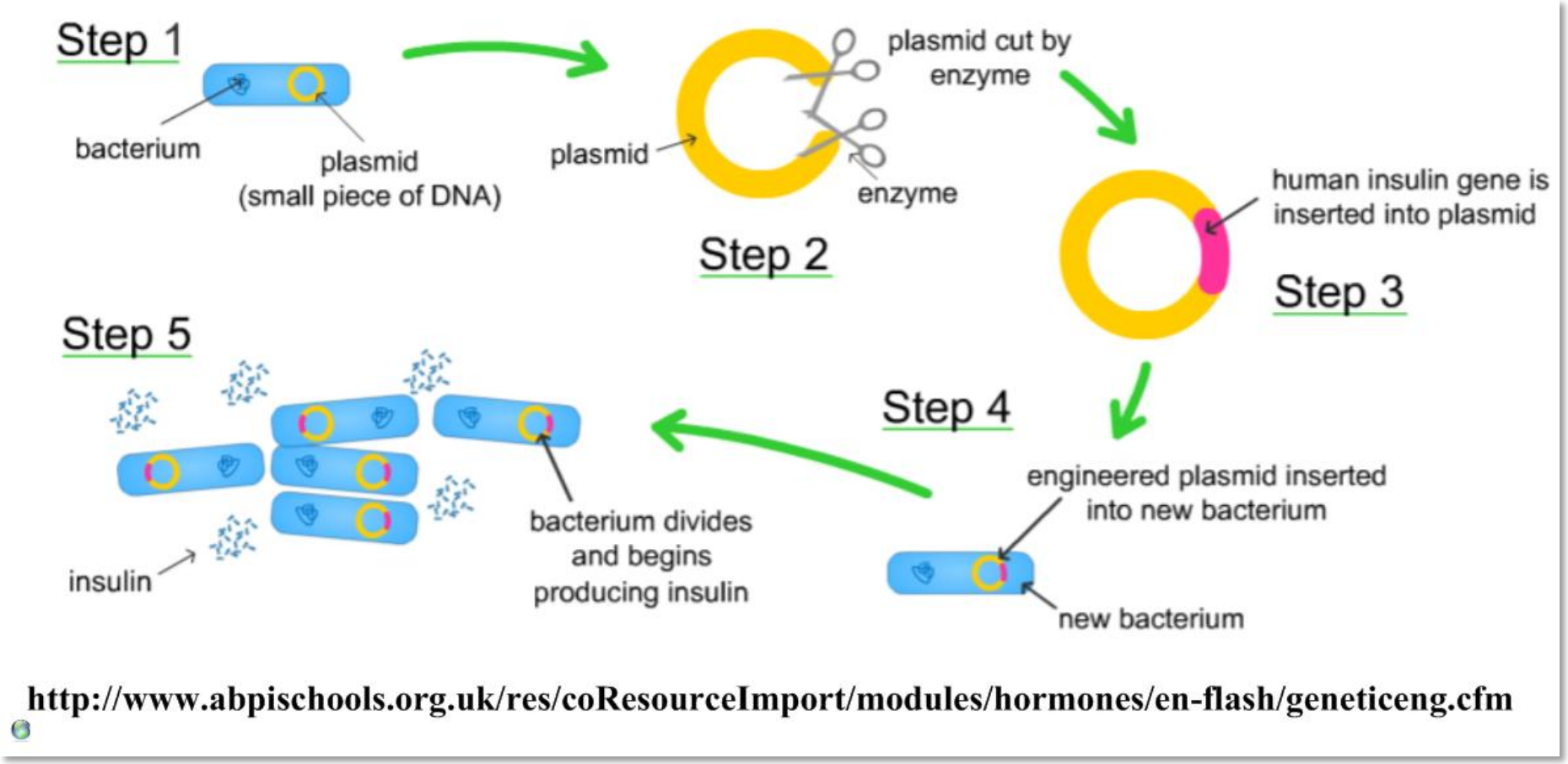


E. coli contains plasmids



circular sections of DNA

Gene Transfer in Insulin Production:



http://www.abpischools.org.uk/res/coResourceImport/modules/hormones/en-flash/geneticeng.cfm

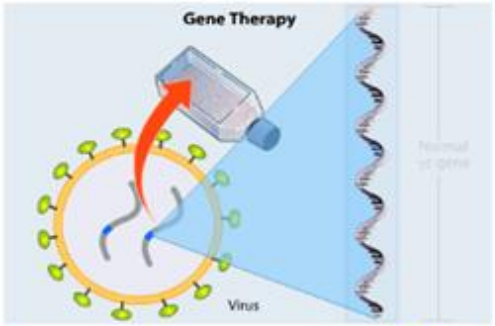
Gene Therapy and SCID: Severe Combined Immunodeficiency

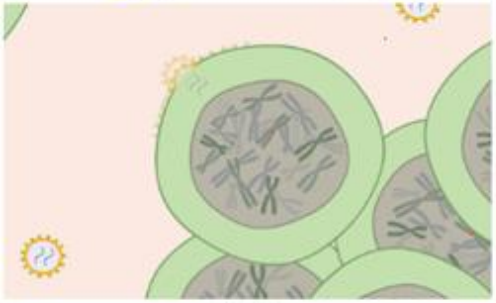
The lymphatic system fails to produce T-cells: essential for immune system function. Where this would once have been fatal to newborns as they cannot fight infection, now there is hope with early diagnosis (pre- or post-natal) and gene therapy.

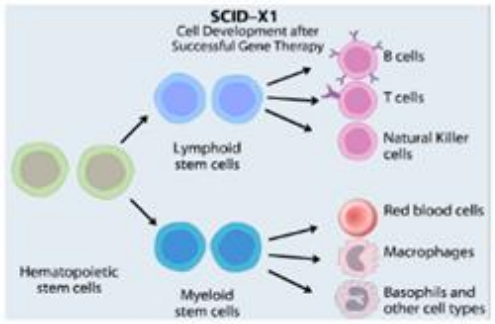
Bone marrow is taken from the patient and cultured with a virus that has been genetically modified to carry the working version of the gene.

The virus inserts the gene into the chromosomes of the bone marrow stem cells. In many of these cells, the gene will be inserted in the right position, and these cells are returned to the patient's bone marrow.

These re-inserted cells serve as progenitor cells - templates for building new, functional killer and helper T-cells.







Gene Therapy

A few years ago, a clinical trial began in France in the hope of curing children with a type of genetic immune deficiency called SCID-X1. Children with this disease have a defective gene, called gamma-c, which prevents a subset of the cells of the immune system from forming, and predisposes the children to life-threatening infections. In an attempt to cure the children—who would otherwise die at a young age—physicians used gene therapy to provide them with normal gamma-c genes.

This particular trial has had striking success as well as tragedy. Eight of the eleven children are currently thriving. However, in two cases the therapy successfully introduced gamma-c genes, but these children have since developed leukemia. In both children, a gamma-c gene inserted next to another gene, called *LMO2*. The *LMO2* gene has previously been linked to leukemia, and scientists speculate that the insertion of the gamma-c gene next to *LMO2* may have overstimulated the gene, causing T cells to proliferate in excess. An *LMO2* effect, in combination with the proliferation-inducing effects of the gamma-c gene itself, may be the cause of the leukemia in these two patients. Scientists are still investigating other possible causes.

From this single trial, it is clear that gene therapy holds significant promise, yet it is also clear that it poses significant risks. To learn more about the application of gene therapy in SCID, view the accompanying animation.

http://www.sumanasinc.com/scienceinfocus/sif_genetherapy.html

Genetically Modified Crops and Animals

Agriculture

Golden Rice is enriched with beta-carotene, which can be converted into vitamin A in the body and prevents blindness.

Insecticide sweet-corn is resistant to pests so the farmer does not have to spray potentially harmful insecticides.

Long-lasting, frost-resitant or salt-resistant tomatoes, strawberries etc. are able to grow in harsher conditions or remain fresh for longer for transport and consumption.

Medicine & Research

Factor IX-producing sheep produce human clotting factor in their milk, which is extracted and used to treat haemophiliacs.

Glow-in-the-dark pigs have been used in graft or transplant research to find out how far the donor cell DNA propagates in the recipient.

The Ethics of Genetic Modification

The idea of genetically modified organisms is hotly debated and can be divisive. The potential benefits of genetic engineering are great, though we must be willing to accept responsibility for the risks and possible consequences.

Benefits

Frost, flood, disease or pest resistant crops can increase yields in countries with food shortages. Aside from being a social boost, this can improve local and national economy.

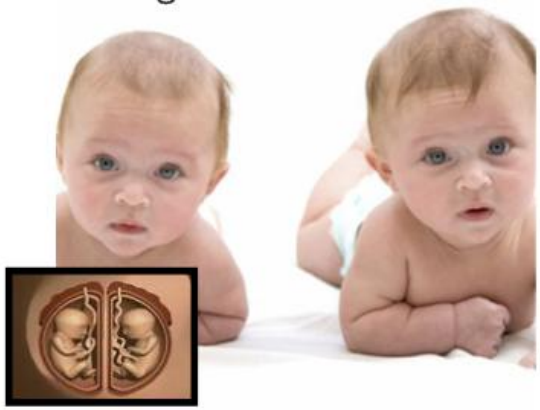
GM organisms can be used to produce human proteins, such as insulin or clotting factor, to be used as medical treatments.

Added nutrients (e.g. golden rice) can provide balanced nutrition in areas where food diversity is low.

Human genes can be inserted into other species to allow research into pathology and treatment of genetic illnesses - and help us find cures.

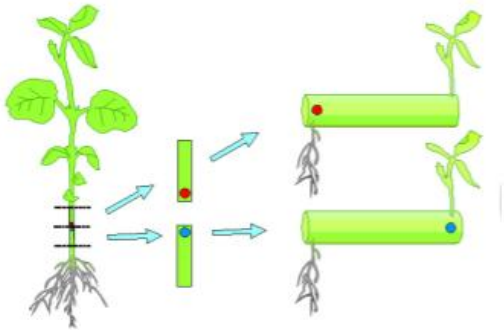
Cloning: a clone is a group of genetically identical organisms or cells

Including:



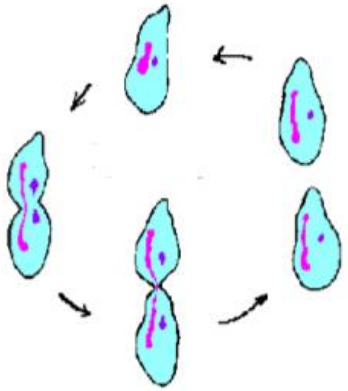
Identical (monozygotic) twins

http://science.howstuffworks.com/twin.htm



Plant cuttings

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



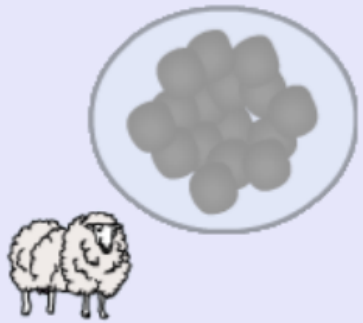
Asexual reproduction

http://en.wikipedia.org/wiki/Asexual_reproduction

http://www.youtube.com/watch?v=HcK-My1j9Hg

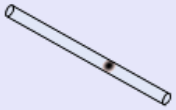
But of course, the most commonly understood meaning of the term 'clone' is the product of the transfer of a differentiated nucleus, and is the subject of many Holywood movies and junk novels.

Cloning by transfer of a differentiated nucleus:

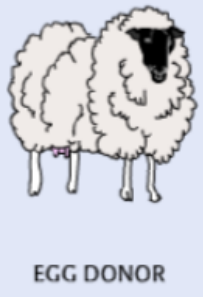


Udder cells removed from nuclear donor and kept in inactive G0 phase.

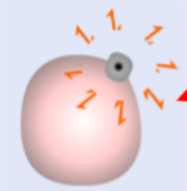
one cell taken



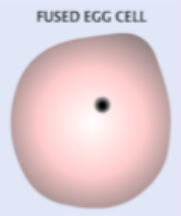
Nucleus removed



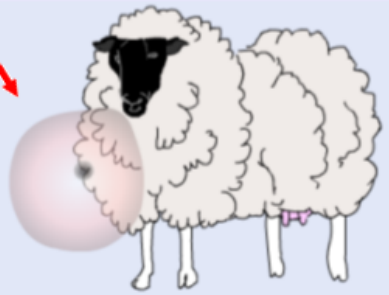
Egg cell taken from donor

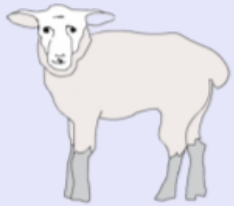


New nucleus fused with donor egg cell.



Fused egg cell implanted into uterus of a surrogate mother. Surrogate mother carries the fetus to term.

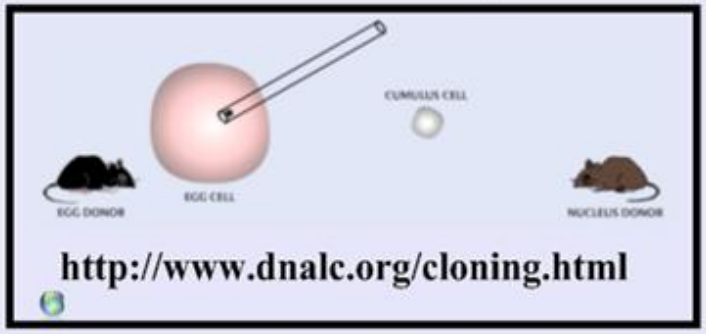




Dolly

Cloned sheep is born - DNA is exactly the same as the nuclear donor.

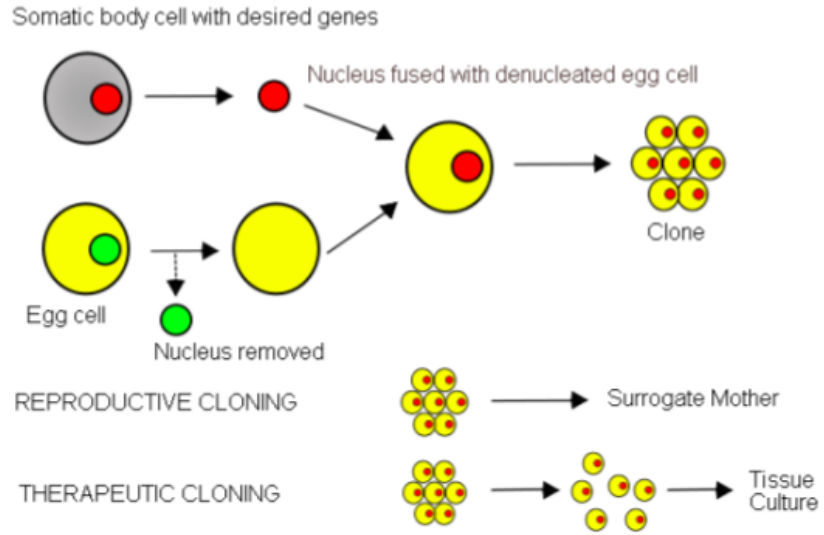
Some more examples:



Therapeutic Cloning:

the use of *embryonic stem cells* to produce healthy cells to be *transplanted back into a patient*.

This field of medicine, also known as *regenerative medicine*, is at the centre of ethical debates around the world.
At the crux of the issue is the creation of embryonic stem cells in the production of transplant tissues.



http://en.wikipedia.org/wiki/Therapeutic_cloning

Let's be clear on what therapeutic cloning is NOT:
- it is NOT creating a mini-me for use in evil global domination plots or to create armies
- it is NOT creating a copy of a human to use as an 'insurance policy' in case you need a transplant.

It IS:
- the creation of a 'new' egg cell using the DNA of a desired differentiated cell and a donor egg cell
- therefore the 'creation' of a human embryo via scientific means. Theoretically, this blastocyst could be implanted as in normal IVF and develop into a baby.

Find out more:

http://www.actionbioscience.org/biotech/mcgee.html

http://www.newscientist.com/channel/health/mg19826541.100-stem-cell-advances-could-lead-to-organ-regeneration.html

http://whyfiles.org/148clone_clash/4.html

The Ethics of Therapeutic Cloning

In a nutshell: create an embryo to provide a line of stem cells that are genetically identical to the patient, then reprogramme the cells to produce the desired tissue type. This is legal in some countries, including the UK and Australia.

http://en.wikipedia.org/wiki/Human_Fertilisation_and_Embryology_Act_1990

This is NOT reproductive cloning - cloning humans is illegal globally.

http://www.un.org/law/cloning/

Possible Benefits	Arguments Against
Rejection risk reduced in transplants	Religious obejctions to 'playing God' by creating what many consider to be human life
No need to wait for human donor to die to give organs	UN recommendations against reproductive cloning not ratified by all countries - possible risk of race to create the first human clone.
Some success stories already reported in therapeutic cloning.	

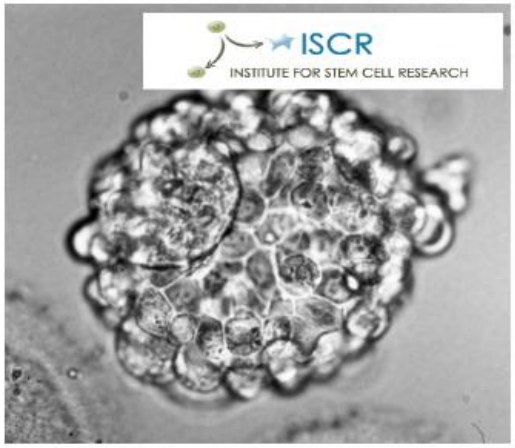
Therapeutic Cloning in the News

Therapeutic cloning cures Parkinsons mice

http://www.newscientist.com/channel/sex/mg19726493.000-therapeutic-cloning-cures-parkinsons-mice.html

Stem cell transplant 'cures' diabetic mice

http://www.newscientist.com/channel/sex/mg19726424.500-stem-cell-transplant-cures-diabetic-mice.html



Human Blastocyst
(embryonic stem cells)

http://www.iscr.ed.ac.uk/index.html

Stem cells 'reprogrammed' from human differentiated skin cells - does this mean embryonic stem cell cloning is no longer needed?

PubMed journal entry:

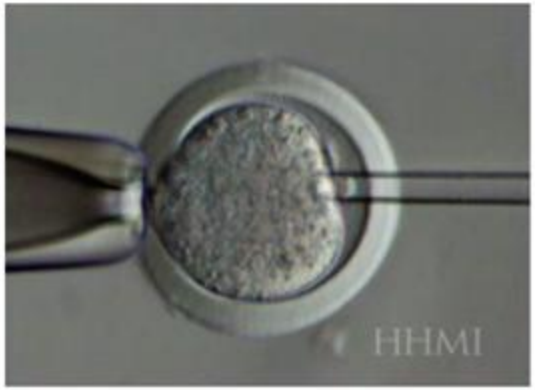
http://www.ncbi.nlm.nih.gov/pubmed/16904174

Ian Wilmut thinks so:

http://www.guardian.co.uk/science/2007/nov/21/stemcells

"*Stell Cems*" from a Fox News halfwit

http://www.youtube.com/watch?v=L-gDIA5RTds

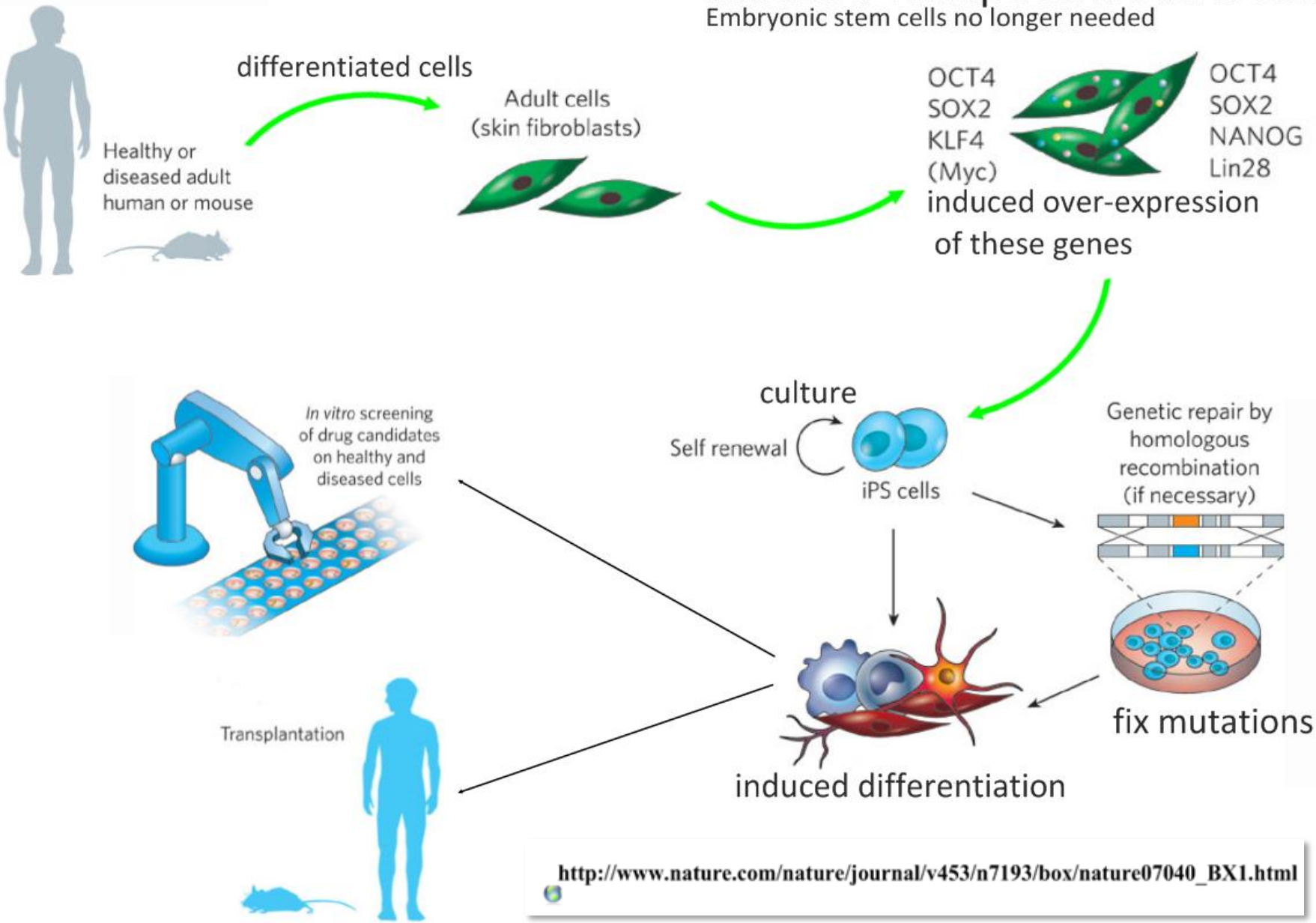


Enucleation of an egg cell

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

Induced Pluripotent Stem Cells

Embryonic stem cells no longer needed



http://www.nature.com/nature/journal/v453/n7193/box/nature07040_BX1.html

For more help and animations, visit:

http://sciencevideos.wordpress.com