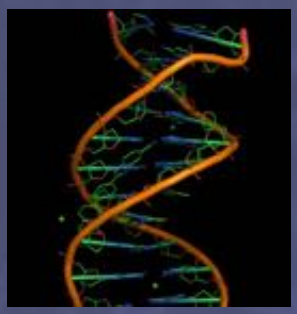
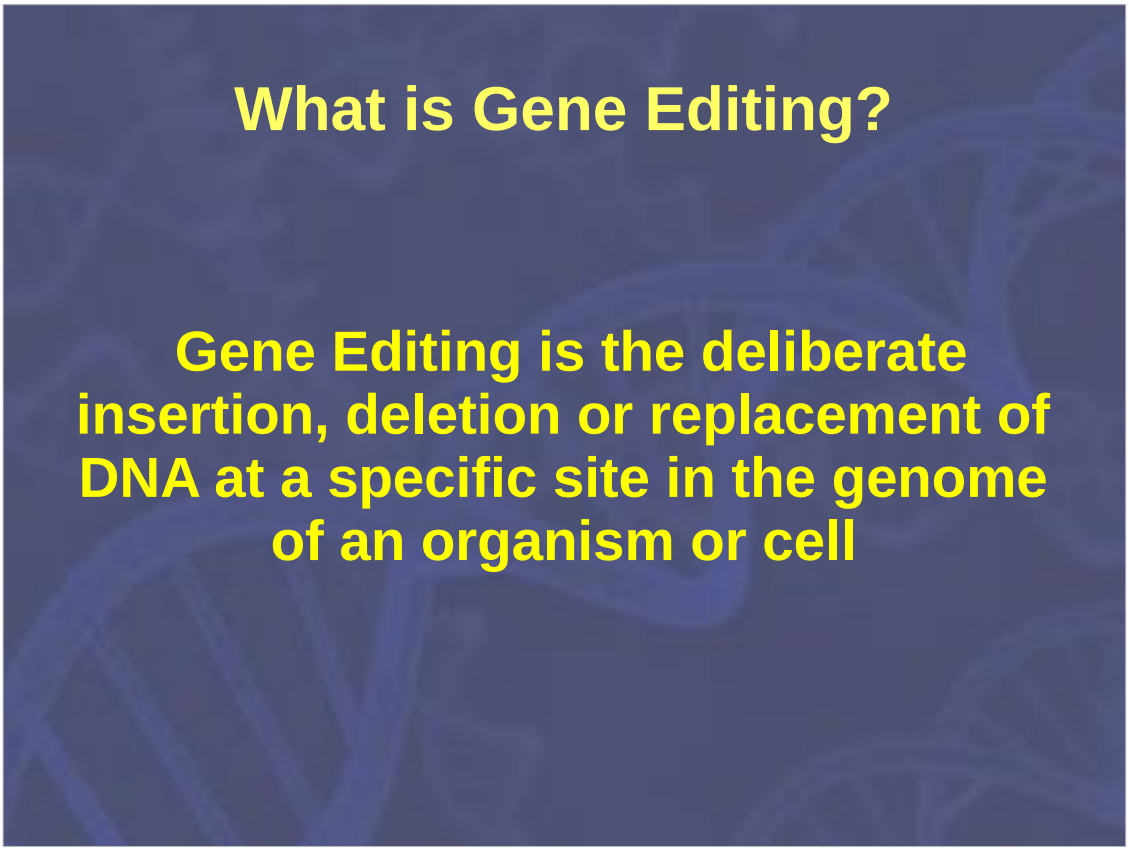
# GENE EDITING

**Science & Technology**
**May 2017**






What is gene editing? Why is it important?
Gene editing is the deliberate insertion, deletion or replacement of DNA at a specific site in the genome of an organism or cell.

This technique is new and quite different from established techniques like selective breeding (cattle, dogs etc).

As we will see the availability of this techniques has implications for the future of all life on the planet - including us.

This is not a “far way in the future” technology, this is real now and is beginning to have implications for us all.

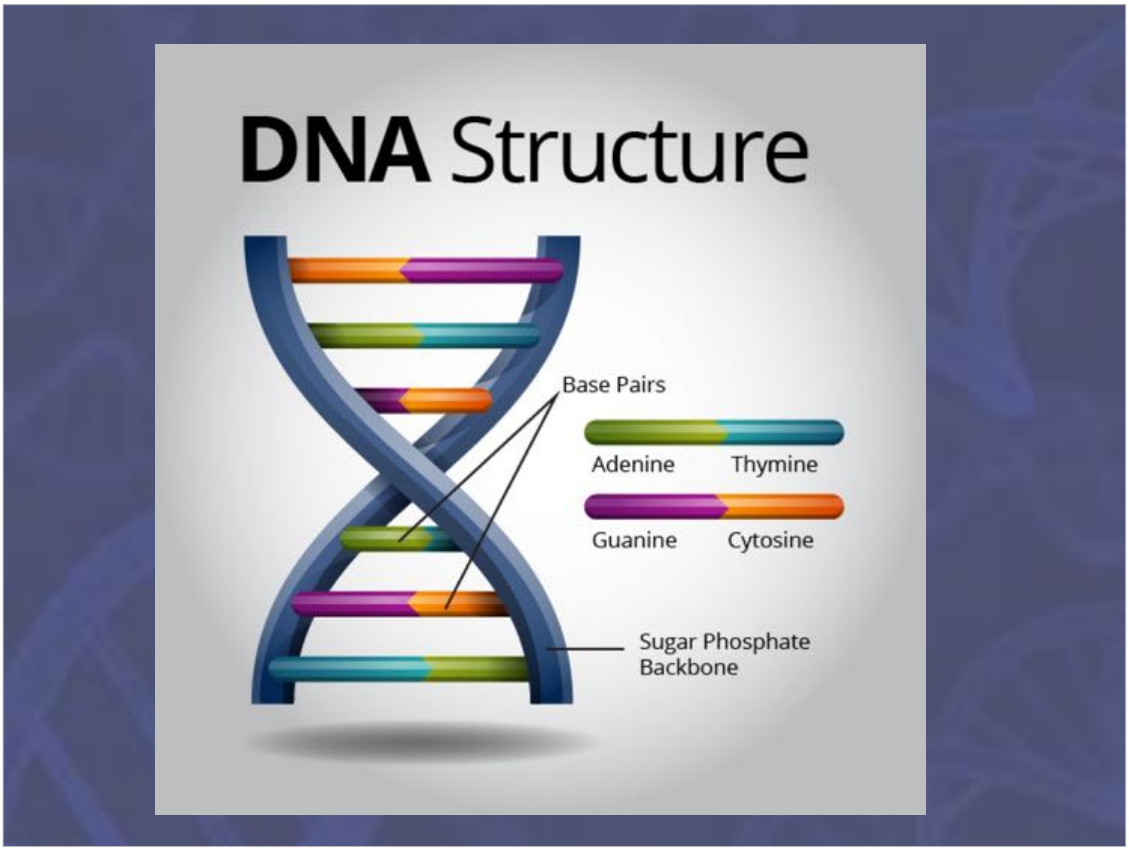
## DNA - Deoxyribonucleic acid

- **Large complex molecule which determines the characteristics of an organism and is passed on to its offspring.**
- **Comprises a sequence of nucleotide bases: Adenine, Thymine, Guanine and Cytosine on a sugar phosphate backbone.**
- **The sequence codes for amino acids and RNA molecules which create proteins and control cell function.**
- **Formed in a double helix first described by Watson and Crick in 1953.**

Human genome is a sequence of base pairs assembled in a double helix. In simple terms the sequence of bases specifies the sequence of amino acids which when assembled in the **right order** make a protein.

Why only proteins? Why not fats or carbohydrates?
Two types of proteins:
- Structural
- Enzymes which enable the synthesis of everything else.

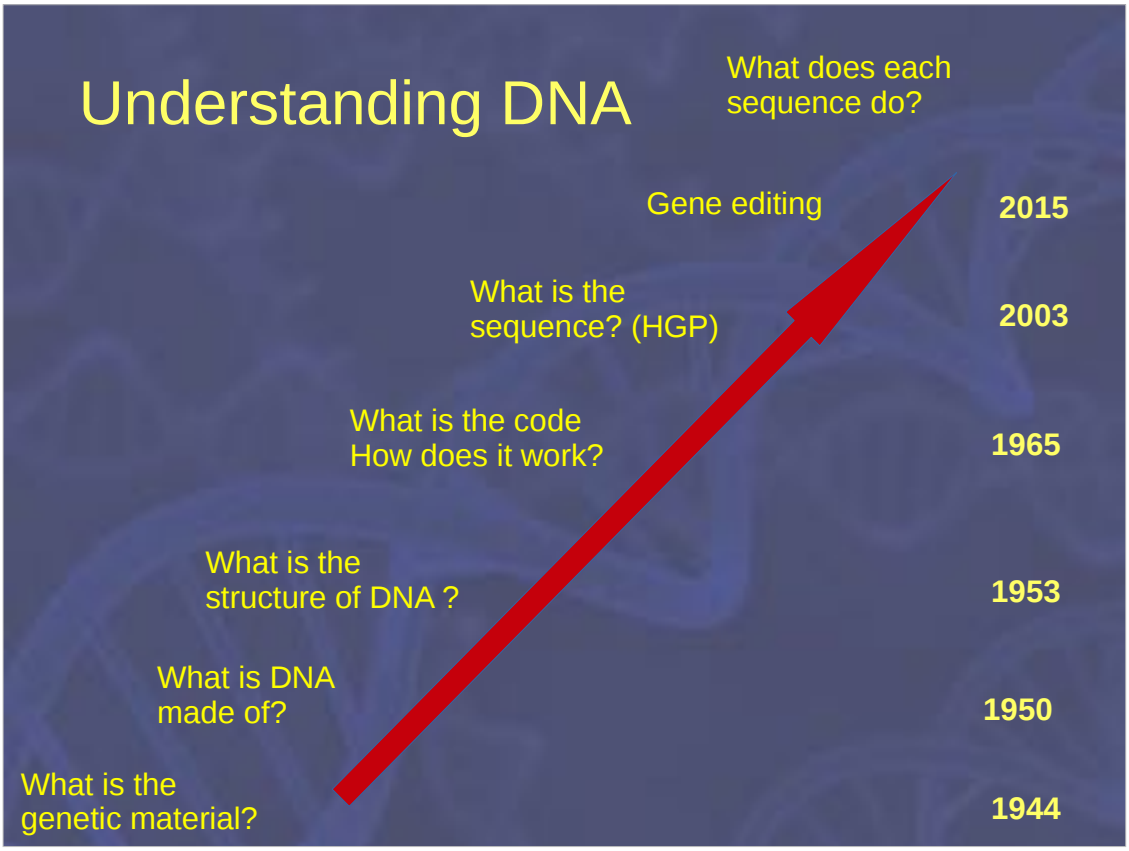
The DNA sequence is now known to specify much of the control mechanism for biological processes as well as the protein blueprint. In fact proteins account for <2% of the genome code.




Watson and Crick had published the structure of DNA in 1953 but this only told us the chemical structure – **not the code or how it worked.**

They worked out from xray crystallography data generated by Rosalind Franklin that DNA is a double helix of nucleotide bases assembled on a sugar phosphate backbone.

Important things about DNA Structure:
- Two strands
- Made up of only four bases which link the strands
- A links to T and C links to G
- base order is “the code”
- built in redundancy
- Starting points at both ends



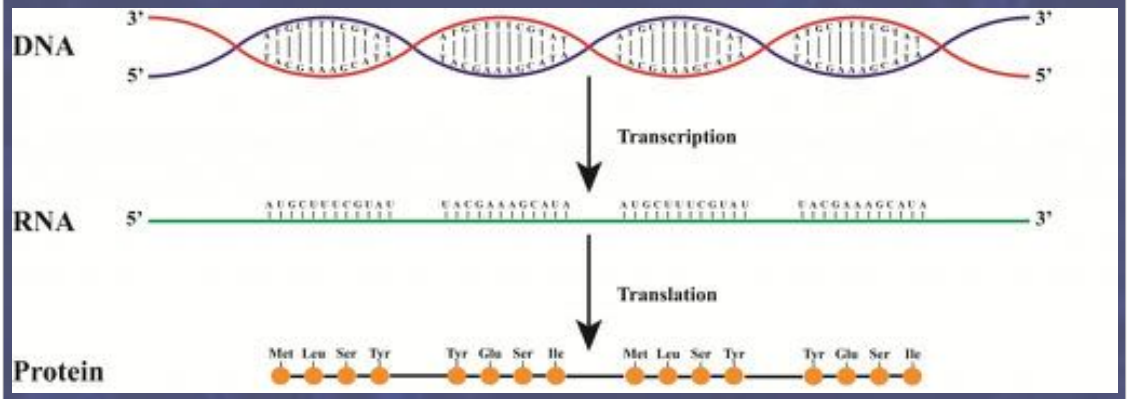

Understanding DNA is much more than just the double helix structure

In order to make use of technology which allows us to edit the sequence we need to know:

- What the sequence is
- How function maps onto the sequence
- How the cell translates the sequence into useful things
- What controls the process

1944 Oswald Avery identifies DNA as genetic material
1950 Erwin Chargaff discovered basic DNA composition
1953 Watson and Crick published structure of DNA
1965 Marshall Nirenberg discovered the genetic code
2003 HGP completed
2015 CRISPR and the ability to change the code

# Code for Proteins

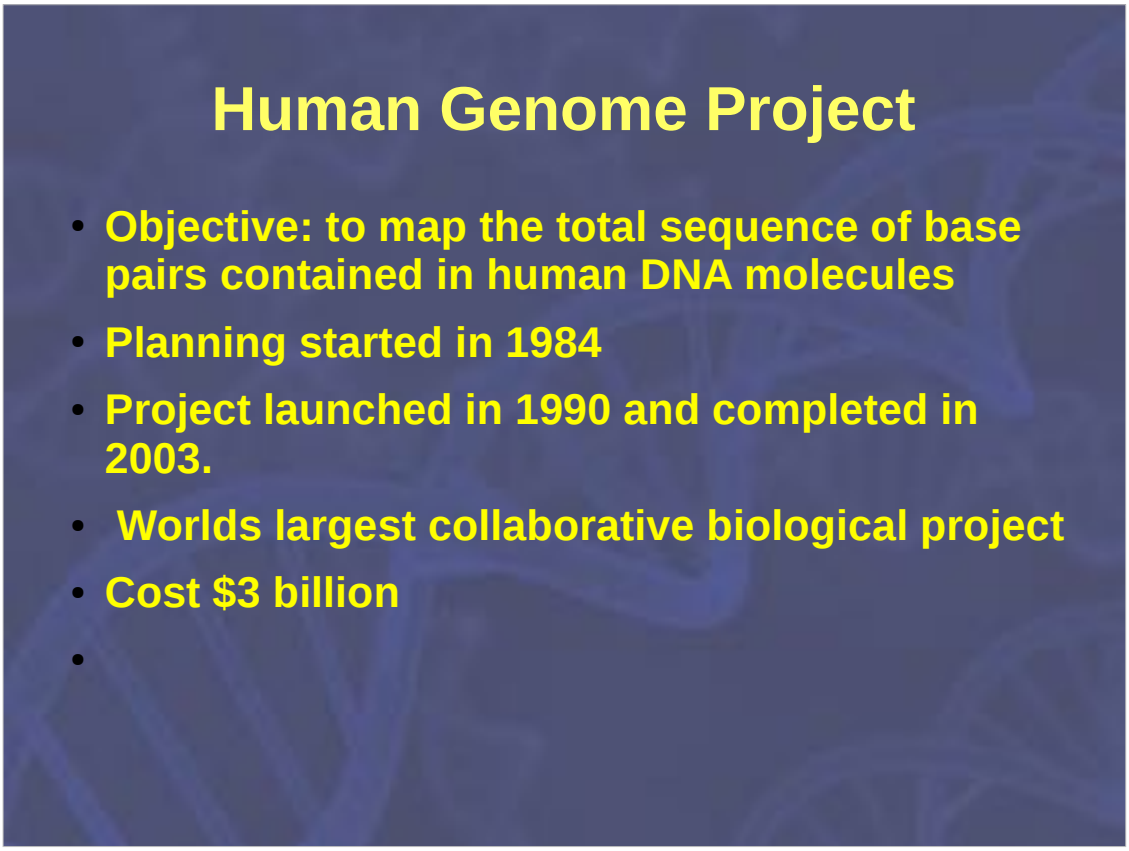



How DNA codes for proteins

Two strands 5’ and 3’ (5 prime and 3 prime)
DNA has a direction or sense

mRNA reads from the 3’ end producing a sequence equivalent to the 5’ strand.

AUG (mRNA) or ATG (DNA) codes for the amino acid Methionine



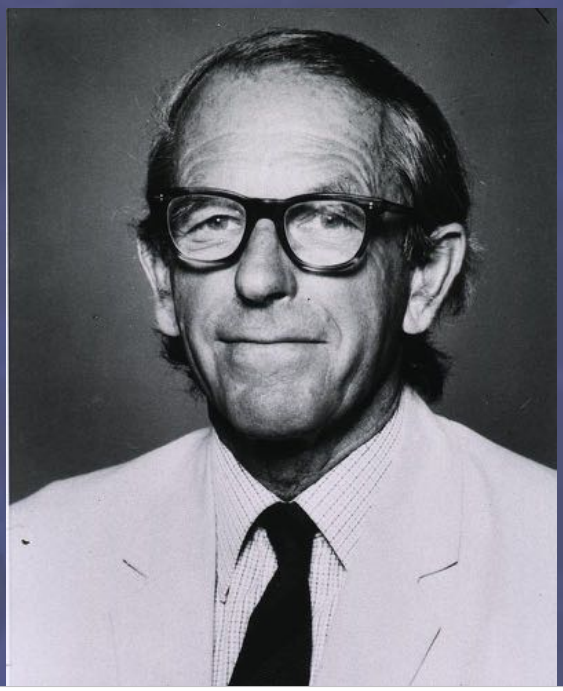

I think we have all heard of the Human Genome Project and this is a good place to start since it forms one of the major stepping stones in developing gene editing. No point knowing how to changing things unless you know what is going to happen.

The Human Genome Project. Planning started in 1984, project launched in 1990 and completed in 2003.

Aim was to map the nucleotide base pairs contained in human chromosomes – that is the total sequence of base pairs in human DNA molecules.

No single human genome so original result is a composite of a few individuals.

## Frederick Sanger



The success of the project was due to a large number of people but this man developed the initial techniques for sequencing. Frederick Sanger (1918-2013) Rencombe Gloucestershire.

Awarded two Nobel Prizes.
The first for being the first man to sequence a protein - bovine insulin in 1952, and the
The second for his work on methods of sequencing DNA.

Worth understanding the magnitude of the task. - - -Worlds largest collaborative biological project carried out by research centres in US, UK, Japan, France, Germany, and China.
-Finance came from mainly government sources ($3bn).
- Human chromosome contains more than 3bn base pairs. At the start of the project, the largest DNA sequence that had been decoded was ~48000

## DNA Code looks like this - 3bn bases

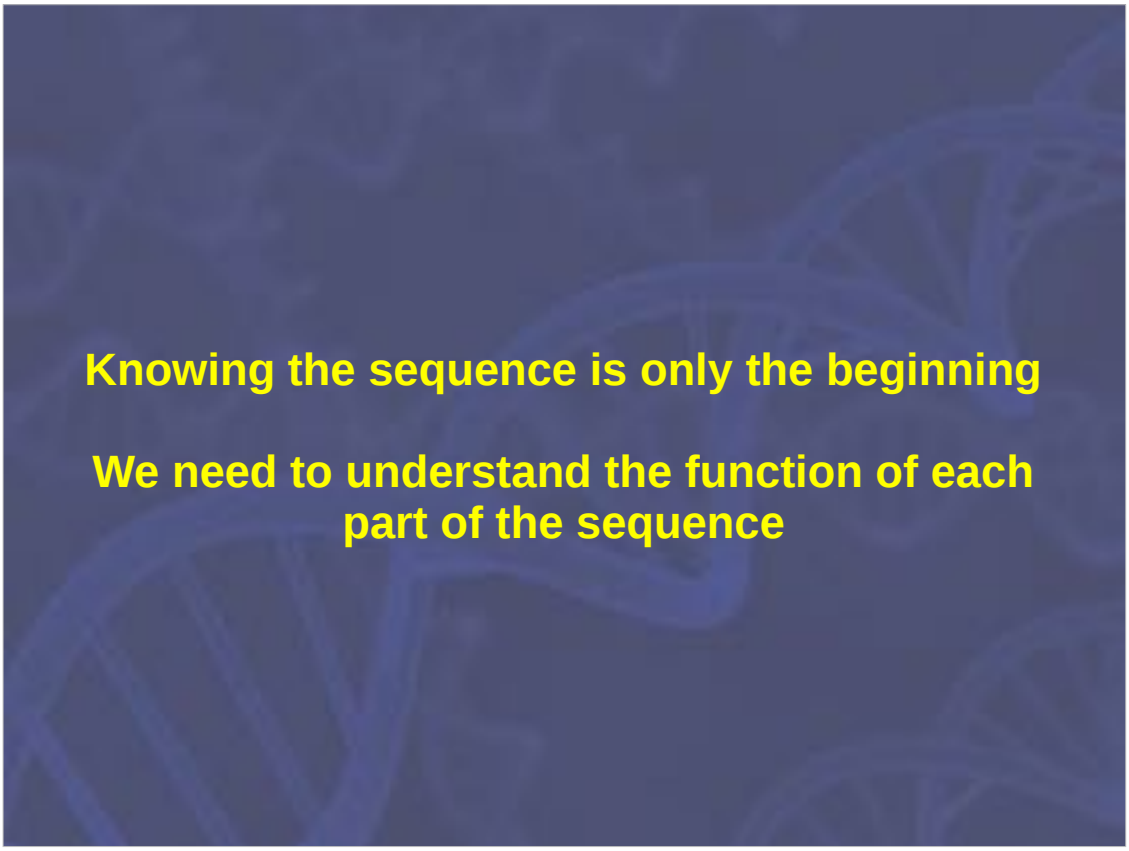
What did the HGP tell us?

- The complete sequence of 3bn bases.
- Sequence of 22,000 protein coding genes and their locations
- Protein coding genes represent less than 2% of the DNA sequence.
- There were more areas of duplication than expected
- Less than 7% of the proteins appeared to be vertebrate specific. Hundreds of genes appear to have come from bacteria.
- Most mutations occur in males
- Overall humans are 99.8% genetically similar.
- The purpose of “junk DNA” - (98%) is being discovered – but it is definitely not “junk”

- The human body has 100 trillion cells – each contains a copy of the entire Genome

Cost of sequencing has dropped dramatically now approx $0.1 / million bases.

- Ancestry service

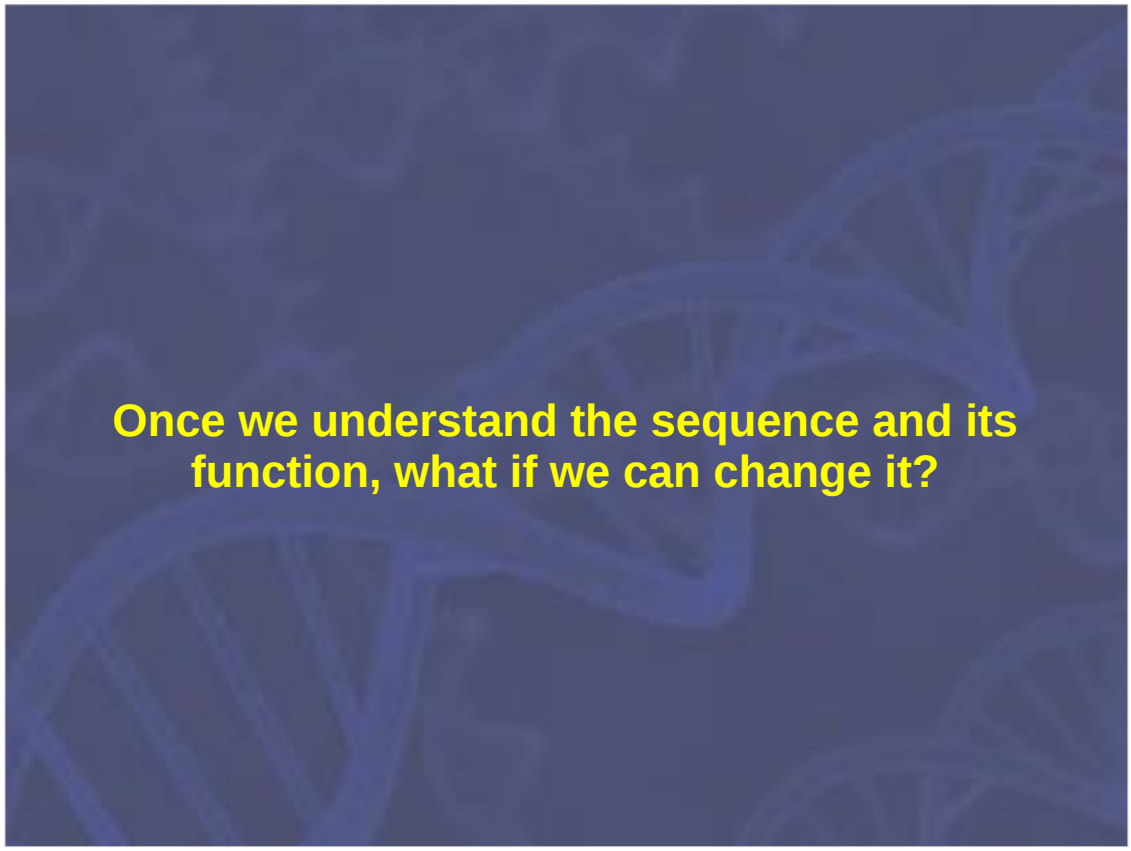



Knowing the sequence is only the start:
Necessary to understand the location and function of each base sequence.

This knowledge allows us the potential to understand the root cause of genetic mutations and genetic differences between people relating to disease or physical attributes. Comparison of DNA in genetic diseases with healthy genome.

Understanding function is made more difficult because 98% of DNA does not code for protein and is involved in other functions such as control of expression.

Control sequences are not necessarily located next to the protein sequences they control.

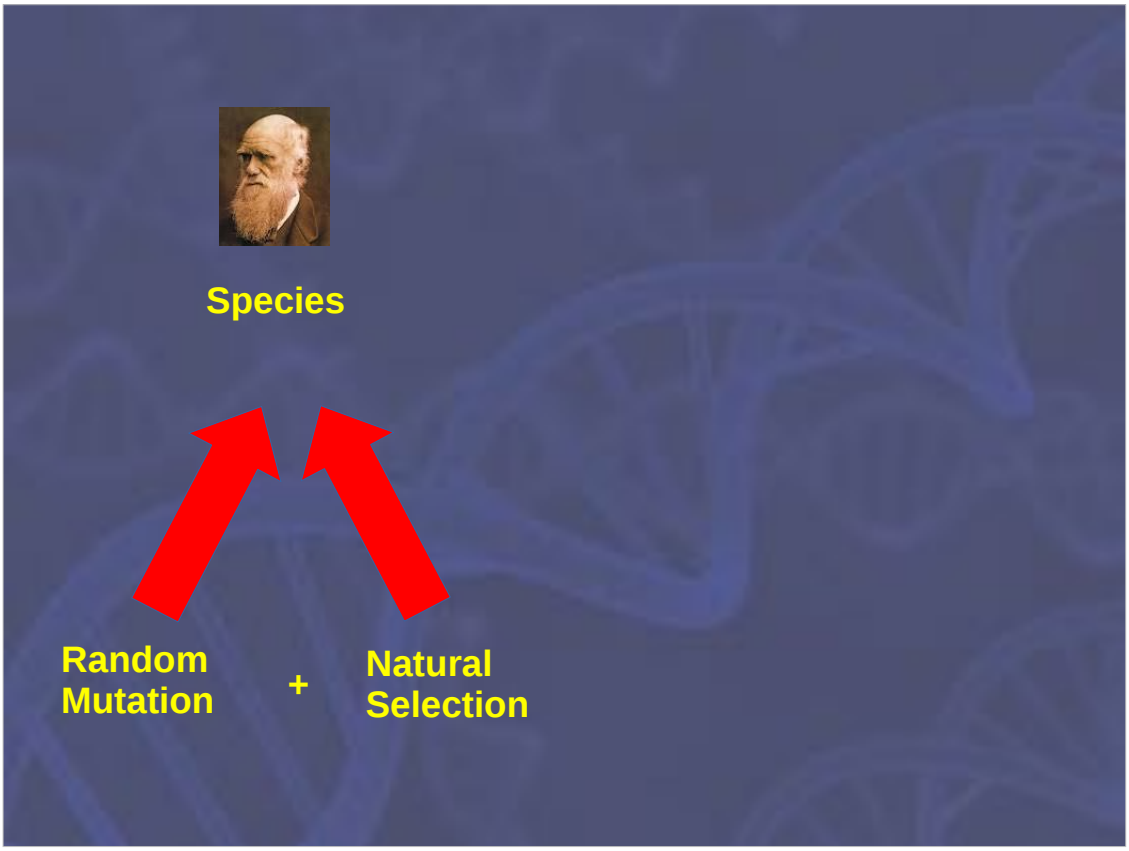



We can now change the sequence – this is gene editing

Gene editing gives us the possibility of making "educated" changes to the the DNA sequence - not just in humans but animals, plants in fact any living organism.

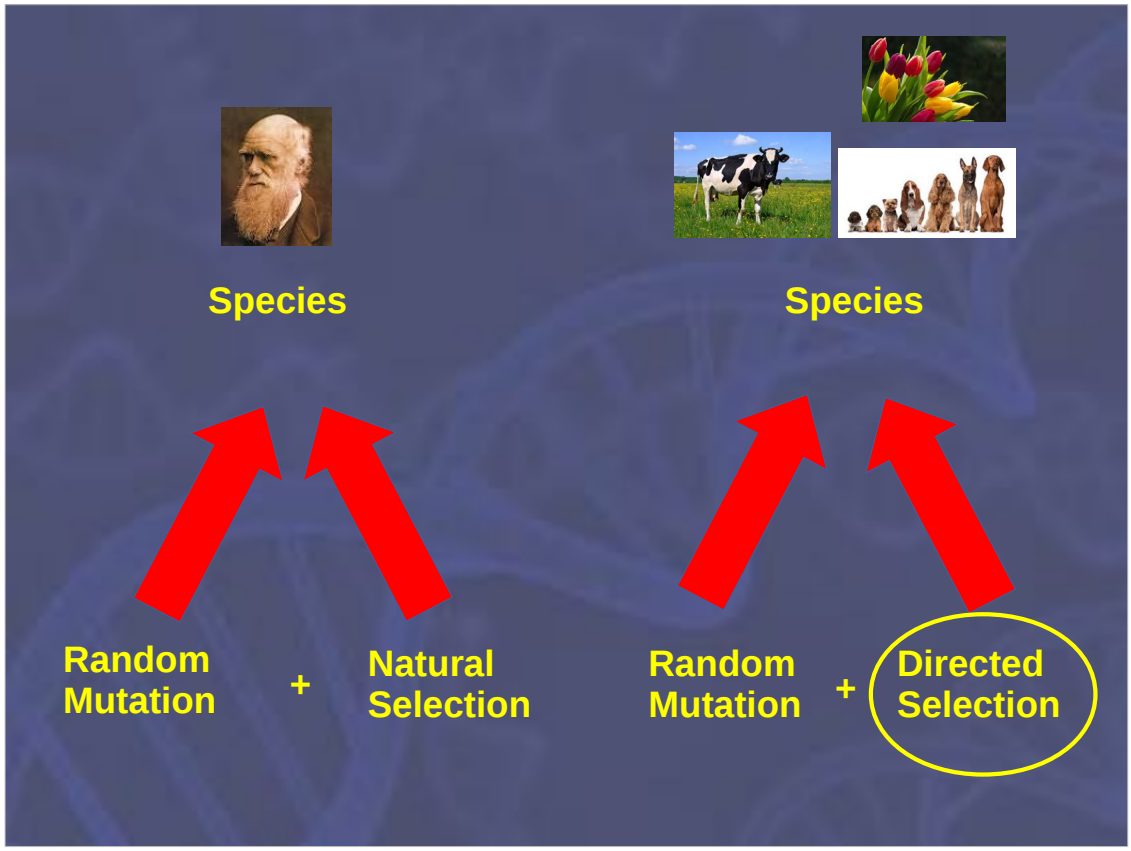
You can think of gene editing as using a word processor on a very large document. The analogy works if if you consider what happens when you want to change a specific piece of text.

- (a) use "Find" to search for the required piece of text
- (b) use "paste" to replace the selected text with different characters.



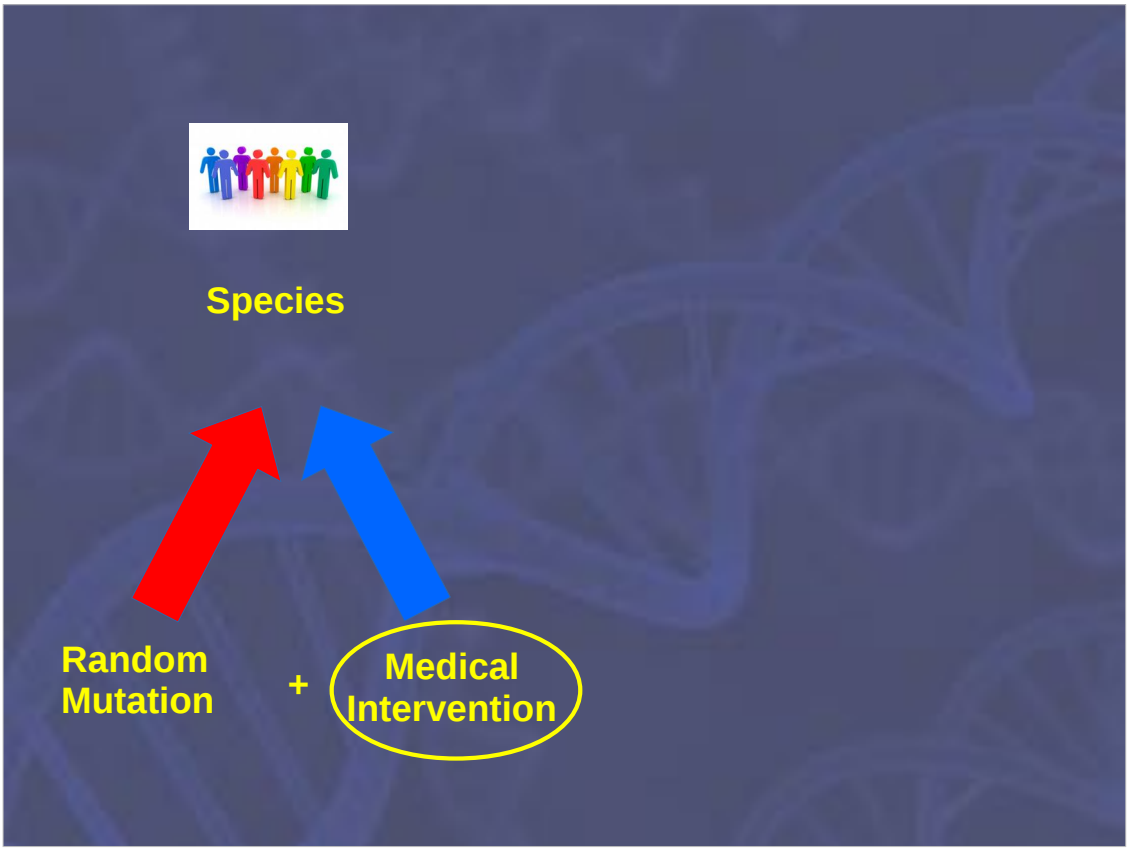

Lets look at Darwin's theory today.

Humans are the result of Random mutation and natural selection. "Survival of the fittest".




In plants and animals we have already been modifying genetic material for years using selective breeding. “Survival of the fattest” However the outcome is always uncertain because of genetic recombination.

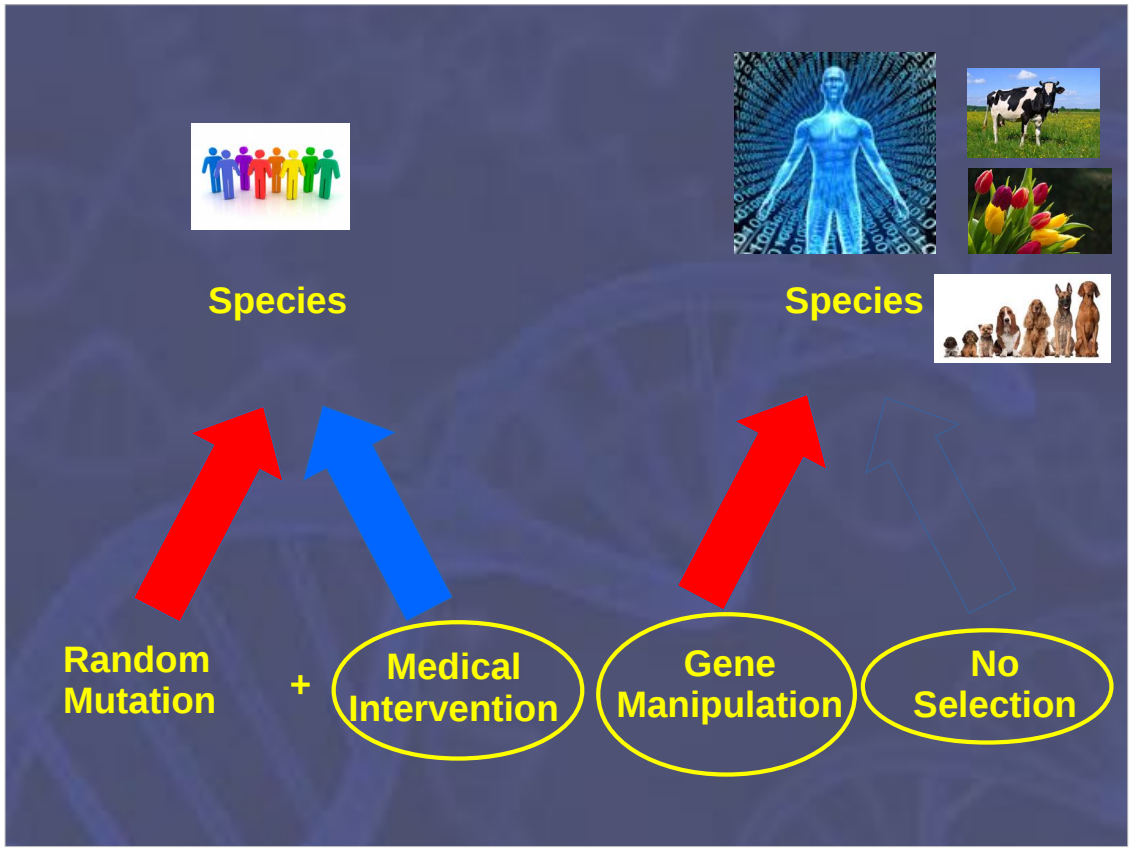
What is Genetic recombination?




Worth a thought... that our generation may represent the pinnacle of human development by natural selection.

“Recent” medical advances mean that we have been preserving faulty genes and allowing them to propagate - unchecked this approach will make humans more and more reliant on medical intervention. Perhaps “Survival of the richest”

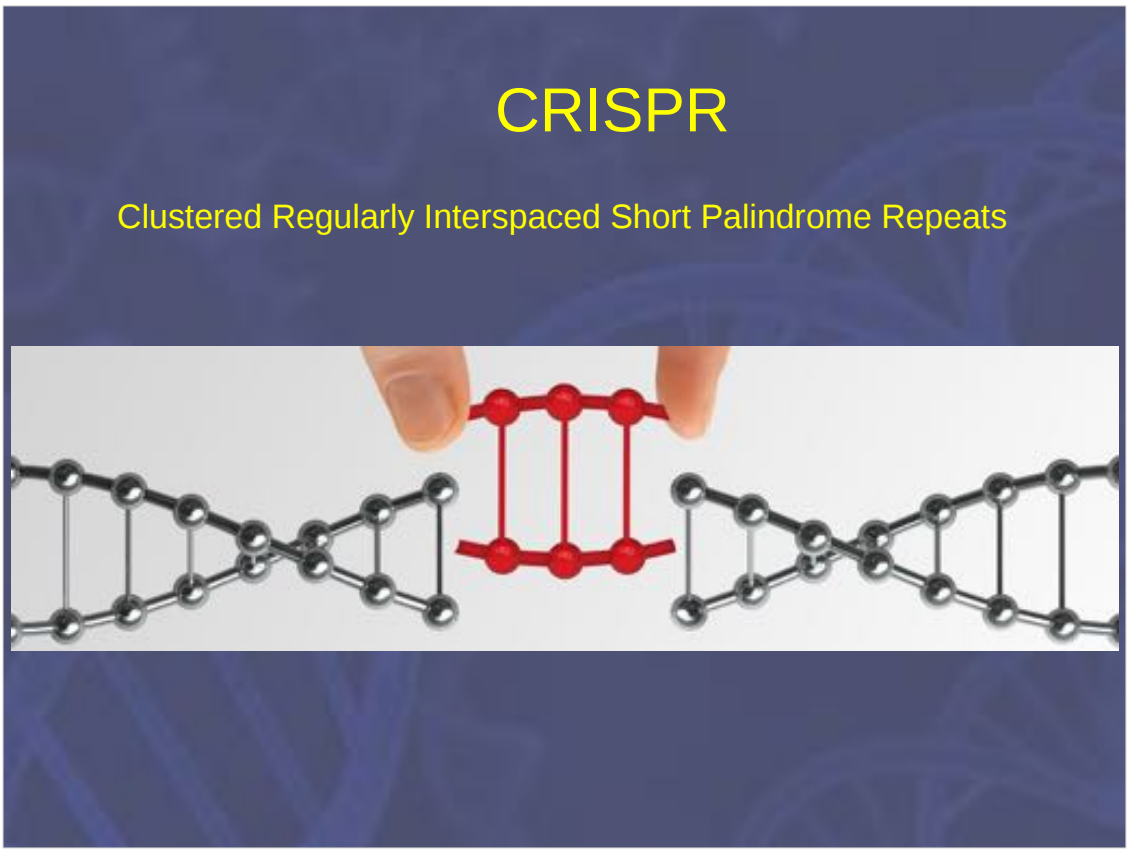
Not necessarily a bad thing just a consequence interfering in natural selection.




Gene editing offers the potential to modify genetic defects at source and effectively eliminate faulty genes or correct them for the benefit of future generations

Potentially removing the continued reliance on medical support.

However **we need to know what we are doing** to avoid unwanted genetic consequences of a change we don't fully understand.




So how is it done? And why is it difficult?

Think of the problem -….. once you think you know what you are doing ...
a) find a gene sequence somewhere in a string of many many millions (3 billion bases in human genome)
b) cut the DNA at an exact point and either remove some bases or add an additional string or both.
c) No room for error - a single base error can be critical.
d) All at a molecular level!

Amazingly a technique called CRISPR does this!!

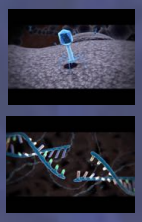
## Jennifer Doudna

CRISPR technique:

How was CRISPR discovered?
- Jennifer Doudna
- bacterial immune response

## CRISPR Development

- **Natural bacterial defence**
- **Modifying DNA**
- **Understanding this process allowed the creation of a method called CRISPR Cas9 which carries out precise modification of DNA.**



How CRISPR works.
- Based on bacterial immune response to virus infection
- makes use of cell DNA repair mechanism

To make use of CRISPR you have to know what you are doing. Prerequisites are the knowledge of what the sequence you are changing does and what the effects will be of making a change to that area.

Hence the importance of the HGP and the mapping of genes to function.


What sort of areas can CRISPR be a benefit?

Medical
- results of immune deficiency and lymphoma?
  Experiments Layla
- Modifying the immune response to things like tissue rejection, and HIV
- Genetic abnormalities:  Muscular Dystrophy
  Retinitis Pigmentosa, Haemophilia & many others
- Modifying cancer cells
- Improving agriculture yields
    - increasing plant yields and improving resistance to pests
    - increasing animal yields
- Malaria control

Malaria: Disease kills 1000 people a day, mostly in undeveloped countries.

Culprit is a parasite called a plasmodium. Best known is plasmodium falciparum. Has a complicated life cycle involving two hosts.  Insect & vertebrate

In the case of human malaria this involves the mosquito. Also infect birds, reptiles,  rodents and primates.

- two options
a) remove the mosquito hosting ability or
b) remove mosquitoes.

Anthony James spent 20 years trying to find a way of making mosquitoes unable to host plasmodium. Finally achieved with the aid of CRISPR. **But how to spread the modification?**

## Gene Drive

- **CRISPR inserts a copy of itself along with a genetic modification**
- **Ensures every copy of the genome contains the new sequence**
- **Avoids genetic recombination**

Ethan Bier suggested a solution now known as the gene drive. This involves inserting the CRISPR system into the gene along with the required genetic modification ensuring that 100% of the mosquitoes will receive the gene”

Avoids the random effect of genetic recombination

## Main hurdles to CRISPR use

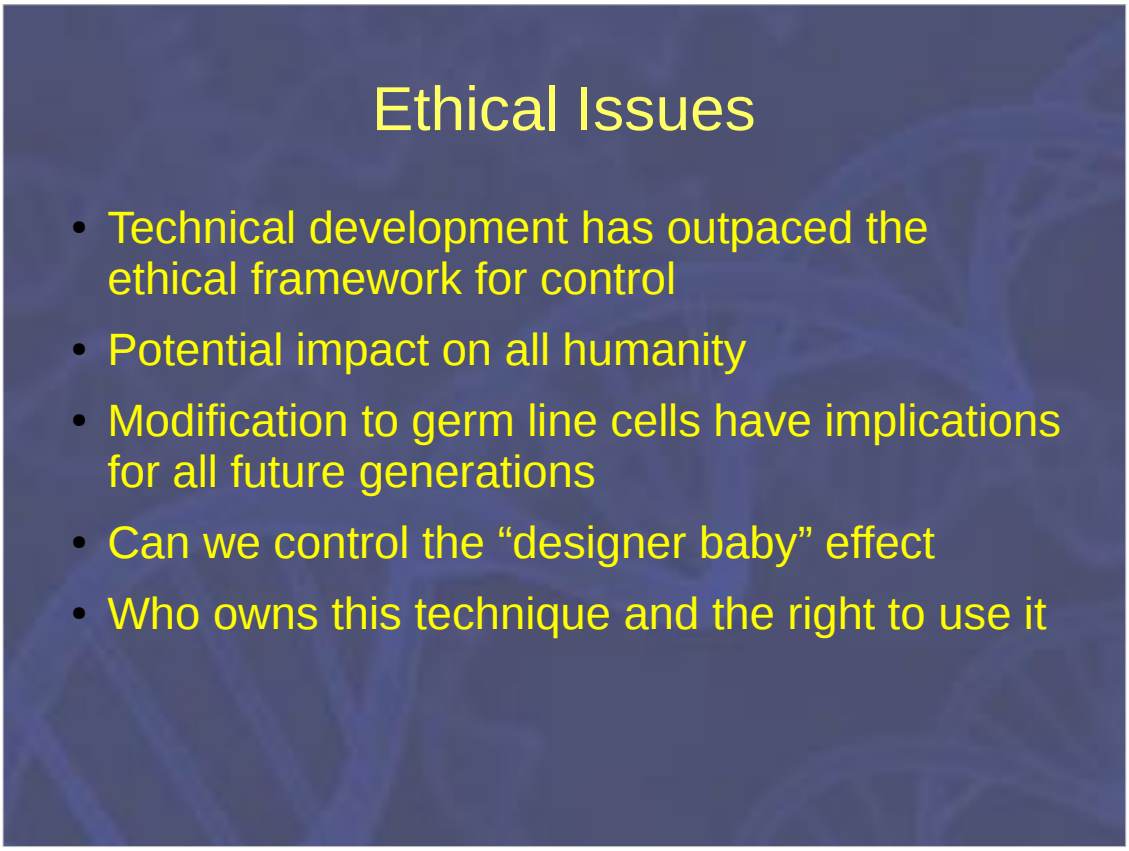
- **Access to the chromosome in vivo to make the changes**
- **Targeting specific organs or cells**
- **Delivery of CRISPR**
  - **Virus**
  - **Electroporation**
  - **Microsomes / liposomes**
- **Eliminating off target modifications**

CRISPR works in a test tube but how does it operate in real life.

- In cells DNA is inside the nucleus inside the cell
- How do we modify all the cells in an organism or all the cells in a target organ
- Modifying foetal cells
- Modifying stem cells
- Modifying germ cells

Delivery to cells
- Virus
- Lipid particles .. liposomes
- Electroporation
- Calcium phosphate

Can we increase specificity by eliminating changes in the genome other than the one we expect?




Ethical issues:
Technical development has outpaced the construction of an ethical framework to deal with the implications of the science.

- Who can decide to eliminate a species we don’t like – e.g. mosquitoes (human record not good)
- Implications for future generations
- Implications for designer babies
- Availability and control

## Difficult questions ….

- If one group can initiate the elimination of a whole species what can we do?
- Is it essentially “out of the box” and we can’t put it back in?
- Can we use it to improve the human race? And if so should we?
- Does it have the potential to destroy the human race?
- Who will have access to it? - only those that can afford it – or everyone?
- Could we stop it or is it inevitable that it will be used - ie like nuclear power
- Is it in fact essential to counter the proliferation of imperfect genes in humans that we cause by medical intervention
- Is it essential to allow the world to feed the 9 billion people due on the planet by 2050?
- Is it a natural part of human development to take control of our genes in the same way as we have taken control of transport, energy, manufacturing etc?

How do we control this type of technology?

-If one person or group can initiate the elimination of a whole species what can we do?

- Is it essentially “out of the box” and we can’t put it back in?
- Could we stop it or is it inevitable that it will be used - ie like nuclear power
- How do we control the application of this technique in humans
- Can we use it to improve the human race? And if so should we?
- Does it have the potential to destroy the human race?
- Who will have access to it

-Will we create different classes of humans

- Is it in fact essential to counter the proliferation of imperfect genes we cause by medical intervention
- Is it essential to allow the world to feed the inevitable 9 billion people due on the planet by 2050?
- Is it a natural part of human development to take control of our genes in the same way as we have taken control of transport, energy, manufacturing etc?