CRISPR AND CAS-9
GENE EDITING TOOLS

COMPILED BY HOWIE BAUM
CRISPR-Cas9
Clustered Regularly Interspaced Short Palindromic Repeats
CRISPR associated protein 9
THE MIRACLE OF HOW OUR BODY WORKS

THE FIRST PART OF THIS PRESENTATION IS ABOUT DNA, CHROMOSOMES, GENES, THE DIVISION OF BODY CELLS AND OTHER AMAZING THINGS THAT ARE GOING ON IN OUR BODIES ALL OF THE TIME, TO KEEP US AS HEALTHY AS POSSIBLE.

AS THEY ARE DISCUSSED, PLEASE CONSIDER:

**WHAT** MIRACULOUS PROCESSES ARE TAKING PLACE, TO MAKE ALL OF THEM HAPPEN?

**HOW** DO ALL OF THESE MINIATURE BODY CELLS AND PARTS, KNOW HOW TO MAKE THIS HAPPEN?
The human body contains 37.2 trillion cells. There is a nucleus inside each human cell (except red blood cells).

Each nucleus contains 46 chromosomes, arranged in 23 pairs. One chromosome of every pair is from each parent.

The chromosomes are filled with tightly coiled strands of DNA. Genes are segments of DNA that contain instructions to make proteins—the building blocks of life.

A chromosome contains hundreds to thousands of genes.

Note that there are 2 very special things that DNA does in each of in our cells—

1) It makes each of the 42 million proteins that are in each of our cells that our body needs

2) The ability to be duplicated accurately, to make new cells that replace old ones that die.
Cytoplasm

Cell

Nucleus
Acts as control centre of cell and contains chromosomes

Chromosome
X-like structure composed of DNA molecule

Supercoiled DNA
Coils of DNA double-helix are themselves twisted into a supercoil

Helical repeat
DNA helix twists once for every 10.4 rungs of base pairs

Histone
Ball-shaped protein; eight histones compose a core unit, or nucleosome

Core unit
Package of proteins around which 2-5 turns of DNA is wrapped; also known as a nucleosome

DNA backbone
Constructed of alternating units of deoxyribose (a form of sugar) and phosphate chemicals

Adenine-thymine link
Adenine always forms a base pair with thymine

Guanine-cytosine link
Guanine always forms a base pair with cytosine

DNA
The double helix of DNA consists of two corkscrew-like backbones joined by cross-rungs, which are pairs of chemical bases, of four kinds:

- Adenine (A)
- Thymine (T)
- Guanine (G)
- Cytosine (C).

The bases always pair in a specific way - A to T and G to C and are called a base pair.

There are almost 3 billion base pairs in each strand of DNA!

https://www.youtube.com/watch?v=egVws2sZdd0&t=48s 50 sec
Stated Clearly
All humans have 99.9% identical genetic makeup.

The remaining 0.1% difference may provide useful information about diseases.
DNA REPLICATION IS PROBABLY ONE OF THE MOST AMAZING TRICKS THAT DNA DOES.

- We start out from a single cell and end up with 37.2 trillion cells.

- Each cell with a nucleus, contains all the DNA you need to make the other cells.

- During that process of cell division, all the information in a cell, has to be copied, and it has to be copied perfectly, which takes about 8 hours.

We have our own miniature manufacturing process going on to create a duplicate copy of new DNA, for each cell!

https://www.youtube.com/watch?v=O6f3ZbKaL7A  1.4 min

Animation created by Drew Berry
Most of the time, DNA looks like a tangled ball of yarn or big bowl of noodles in each cell—diffuse, disordered, chaotic.

This is the time that copies of the long DNA strand is copied.

In preparation, it tidies up by packing the DNA into dense, sausage-like rods, called Chromatids, with 2 identical ones together to make up the chromosomes’ most familiar form, in the shape of an X.

They are bonded at the center with a part called the Centromere.

https://www.youtube.com/watch?v=gbSIBhFwQ4s
GO TO 1.12 MINUTES

https://www.youtube.com/watch?v=57Q5V0HcIWU
A genome is the full set of genetic instructions for a living thing, controlling its development from a single cell into a complex, adult body.

It consists of an estimated 20,000–25,000 genes, carried on the double set of 46 chromosomes, found in nearly every kind of body cell.

Except for the sex chromosomes X and Y, the rest are called Autosomes.
CHROMOSOMES

There are 46 chromosomes in every human cell, except for red blood cells which don’t have a nucleus.

These consist of 22 equivalent pairs, one of each pair derived from the mother and one from the father and are numbered from 1 (largest) to 22 (smallest).

The 23rd pair is the sex chromosomes, XX signifying female and XY (as here) male.

Men determine the sex of a baby depending on whether their sperm is carrying an X or Y chromosome. An X chromosome combines with the mother's X chromosome to make a baby girl (XX) and a Y chromosome will combine with the mother’s, to make a boy (XY).

When colored by chemical stains, dark and pale stripes called banding patterns show up on each chromosome.

These allow researchers to “map” the locations of particular genes within the chromosome.

Chromosome pair #7
Chromosome seven

One of the first chromosomes to be sequenced, it contains more than 5 per cent of the genome’s total DNA, with about 159 million pairs of bases.

Almost 60 million are in the short arm, 7p, with the rest in the longer arm, 7q.

The conventions of labelling a chromosome make it possible to find the site of the gene if you know its “address”.

The cystic fibrosis gene (CFTR), for example, is located at 7q31.32.
Myotonic Dystrophy
Form of adult muscular dystrophy

Amyloidosis
Accumulation in the tissues of an insoluble fibrillar protein

Neurofibromatosis (NF1)
Benign tumors of nerve tissue below the skin

Breast Cancer
5% of all cases

Polycystic Kidney Disease
Cysts resulting in enlarged kidneys and renal failure

Tay-Sachs Disease
Fatal hereditary disorder involving lipid metabolism often occurring in Ashkenazi Jews

Alzheimer Disease
Degenerative brain disorder marked by premature senility

Retinoblastoma
Childhood tumor of the eye

Human chromosome number

Spinocerebellar Ataxia
Abnormally high absorption of iron from the diet

Cystic Fibrosis
Mucus in lungs, interfering with muscle control

Werner Syndrome
Premature aging

Melanoma
Tumors originating in the skin

Multiple Endocrine Neoplasia, Type 2
Tumors in endocrine gland and other tissues

Sickle-Cell Anemia
Chronic inherited anemia, in which red blood cells sickle, clogging arterioles and capillaries

Phenylketonuria (PKU)
An inborn error of metabolism; if untreated, results in mental retardation
DOMINANT AND RECESSIVE GENES THAT CREATE OUR PHYSICAL TRAITS

Physical traits are observable characteristics that children inherit from their parents – eye and hair color, height, etc.

Individuals receive two versions of each gene, known as alleles, from each parent.

The allele that will be expressed is called the dominant gene.

The other type that is expressed is called recessive.
WHAT ARE PHYSICAL TRAITS?
Physical traits are observable characteristics that children inherit from their parents.

Some physical traits, such as freckles, are expressed completely due to dominant or recessive inheritance of a single gene.

Other traits are expressed in varying degrees because they are influenced by multiple genes, such as left or right-handedness.

<table>
<thead>
<tr>
<th>Dominant Traits</th>
<th>Recessive Traits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black or brown hair</td>
<td>Blond hair</td>
</tr>
<tr>
<td>Full lips</td>
<td>Short thin lashes</td>
</tr>
<tr>
<td>Free earlobes</td>
<td>Curly hair</td>
</tr>
<tr>
<td>Attached earlobes</td>
<td>Thin lips</td>
</tr>
<tr>
<td>Dimples in cheeks</td>
<td>Freckles</td>
</tr>
<tr>
<td>Feet with normal arches</td>
<td>Attached earlobes</td>
</tr>
<tr>
<td>High and narrow nose</td>
<td>No dimples in cheeks</td>
</tr>
<tr>
<td>Brown eyes</td>
<td>Broad nose</td>
</tr>
<tr>
<td>Farsightedness and astigmatism</td>
<td>Blue eyes</td>
</tr>
</tbody>
</table>

1-5 This chart shows some of the inherited traits that dominate over recessive traits.
Widow's peak or not?
Can roll the tongue or not?
Which thumb is on the top?
Length of second toe? Is it longer than your big toe or not?

Little finger straight or crooked?
Ear lobe hangs free
Darwin's point
Ear lobe attached
No Darwin's point

Front teeth close together
Front teeth with a definite gap
CRISPR

WHAT DOES IT STAND FOR?
CRUNCHY
RECTUMS
IN
SASSY
INK
RAY-BANS
THIS IS ONE OF SEVERAL BEAGLES THAT HAVE BEEN CHANGED USING CRISPR TO BE LARGER AND MORE MUSCULAR

I WONDER WHO THE SCIENTISTS WERE, WHO MADE THIS CHANGE?
ONE OF THE MAIN SCIENTISTS WHO HAVE WORKED ON DEVELOPING CRISPR IS GEORGE CHURCH, SHOWN AT THE RIGHT, DURING AN INTERVIEW.

“GEORGE, IS IT POSSIBLE TO USE CRISPR TO CREATE A UNICORN?”

HE ANSWERED POSSIBLY USING CELLS FROM A HORNED RHINOCEROS
HE WAS IMAGINING THAT IT COULD CREATE A BEAUTIFUL UNICORN THAT LOOKED LIKE THIS.

IT ALSO COULD TURN OUT LIKE THE ONE ON THE NEXT SLIDE......
BACTERIOPHAGE (PHAGE)

They are a virus that:

1) Parasitizes a bacterium by infecting it
2) Reproduce inside it
3) Makes multiple copies
4) The copies break out of it, so they can infect other bacteria or Archaea.

There are over 10,000,000 types of phages and the one shown here is described as T4 which is very common.

The different kinds of bacteriophages only target one or a few types of bacteria. The T4 type targets mainly the E. Coli bacteria.

https://www.youtube.com/watch?v=5AAR7bNSM_s go to 40 seconds
A teaspoon of seawater typically contains about fifty million (50,000,000) marine viruses. They are the most abundant life form on earth.

They are a virus that infects, replicates in bacteria, which then kills it.

They target the dangerous bacteria, without harming human or animal cells, due to how specific they are.

It turns out that CRISPR is a defense mechanism of bacteria, to fight off infecting viruses.

A T4 Bacteriophage

Once a bacteria has been attacked by a phage, within 1 hour, more than 100 new phages are released.

https://www.youtube.com/watch?v=uFXuxGuT7H8 1.3 min

https://www.youtube.com/watch?v=B9rlBxqBgpU 4.5 min
NOW ITS TIME TO MOVE ON TO TALK ABOUT WHAT CRISPR REALLY IS AND WHAT IT CAN DO FOR GENE EDITING

CRISPR

(Clustered Regularly Interspaced Short Palindromic Repeats) is a method of gene editing that uses the Cas9 protein and specific guide RNAs to either disrupt host genes or insert sequences of interest.

Initially used in bacteria as an adaptive immunity response, CRISPR has been since used in the biological field as a new alternative to genome engineering.

https://www.youtube.com/watch?v=UKbrwPL3wXE&t=16s 1.5 min
The last 3 letters – **SPR** describe a specific sequence of DNA

**Short** means the sequence is about 20 to 40 base pairs long.

An example of a Palindrome is KAYAK. **Palindromic** means those 20 to 40 base pairs can be read the same way backward or forward.

**Repeats** means that identical copies of this sequence are repeated over and over again.

The first 3 letters CRI tell us where these short palindromic repeats are located.

**Clustered** means they are found together in the genome and **Regularly interspaced** refers to the fact that between these repeats, are unique pieces of DNA.
Within the genome, in the bacteria, there are repeating strands of DNA comprised of a palindromic (same phrase backward) sequence of nucleotides (the 4 letters A,T,C, and G.

These are our short palindromic repeats.
However, there are spaces between these repeats of DNA within the bacterium’s genetic sequence.

These are filled with **spacer DNA**, the material that’s important.

Additionally, each segment of this type of DNA is different.

And so we have our ‘Clustered Regularly Interspaced Short Palindromic Repeats’

Our spacer DNA segments are what make our palindromic repeats ‘interspaced’.
These spacer DNA genes match up with bacteriophage DNA.

Further genes exist in a bacterium’s genome that are CRISPR-associated and are therefore called cas genes.

These cas genes make Cas proteins — which are helicases (for unzipping the DNA) and nucleases (for cutting the DNA).

This means that these proteins have the capability to unzip and splice genetic material.
Let’s discuss what this would look like with DNA

First, we make a gRNA (guide RNA) so that it can bond with our target DNA sequence.

This is how we specify our Cas9 protein where to splice precisely.

Guanine (G) always pairs with cytosine (C), and adenine (A) always pairs with thymine (T).

So, if our target segment had the code ATCG we’d make a gRNA (guide RNA) with the code TAGC.
Because our Cas9 protein is a helicase, it unzips our DNA strand.

Eventually, our guide RNA (gRNA) will pair with the intended sequence, specifying where the strand needs to be cleaved.
The ‘pincers’ in this diagram are where the protein’s nuclease functions come out.

They’re in charge of splicing the DNA.
We now have an inactive gene.

The cell can add or delete DNA to try and repair and mutate the gene naturally.

If we wanted to choose what the gene should look like, we can create a custom host RNA to be added.
Our cell will repair the rest of the DNA manually, and therefore our gene has been modified to our liking.
These are the main people who have developed various aspects of the CRISPR process. Jennifer Doudna and Emmanuelle Charpentier are the 2 who received the Nobel prize for their discoveries of CRISPR. A patent challenge is going on to decide who and what organization owns the process.
1989 - First application of gene transfer in humans

1990 - First gene delivery for therapeutic intent in ADA-SCID patients

1993 - CRISPR locus described

1999 - Jessie Gelsinger death from gene therapy-induced immunotoxicity

2000 - Gene therapy-induced leukemia in ADA-SCID patients

2002 - "CRISPR" term first coined

2005 - Cas9 identified as a single-effector endonuclease

2008 - spacers transcribed as crRNAs identified

2010 - CRISPR/Cas9 identified as the adaptive immune system in prokaryotes

2011 - trRNA identified to form duplex with crRNA to guide Cas9

2012 - sgRNA construct developed for simplification

2013 - CRISPR/Cas9 gene editing achieved in mammalian cells

2017 - First CRISPR clinical trial for treatment against HIV-1 (China)

2017 - First CRISPR germline editing in implanted human embryos (China)

2018 - First CRISPR clinical trial for cancer immunotherapy (USA)

2019 - First in vivo CRISPR clinical trial for treatment against blindness (USA)
THE END
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