Genetic Engineering

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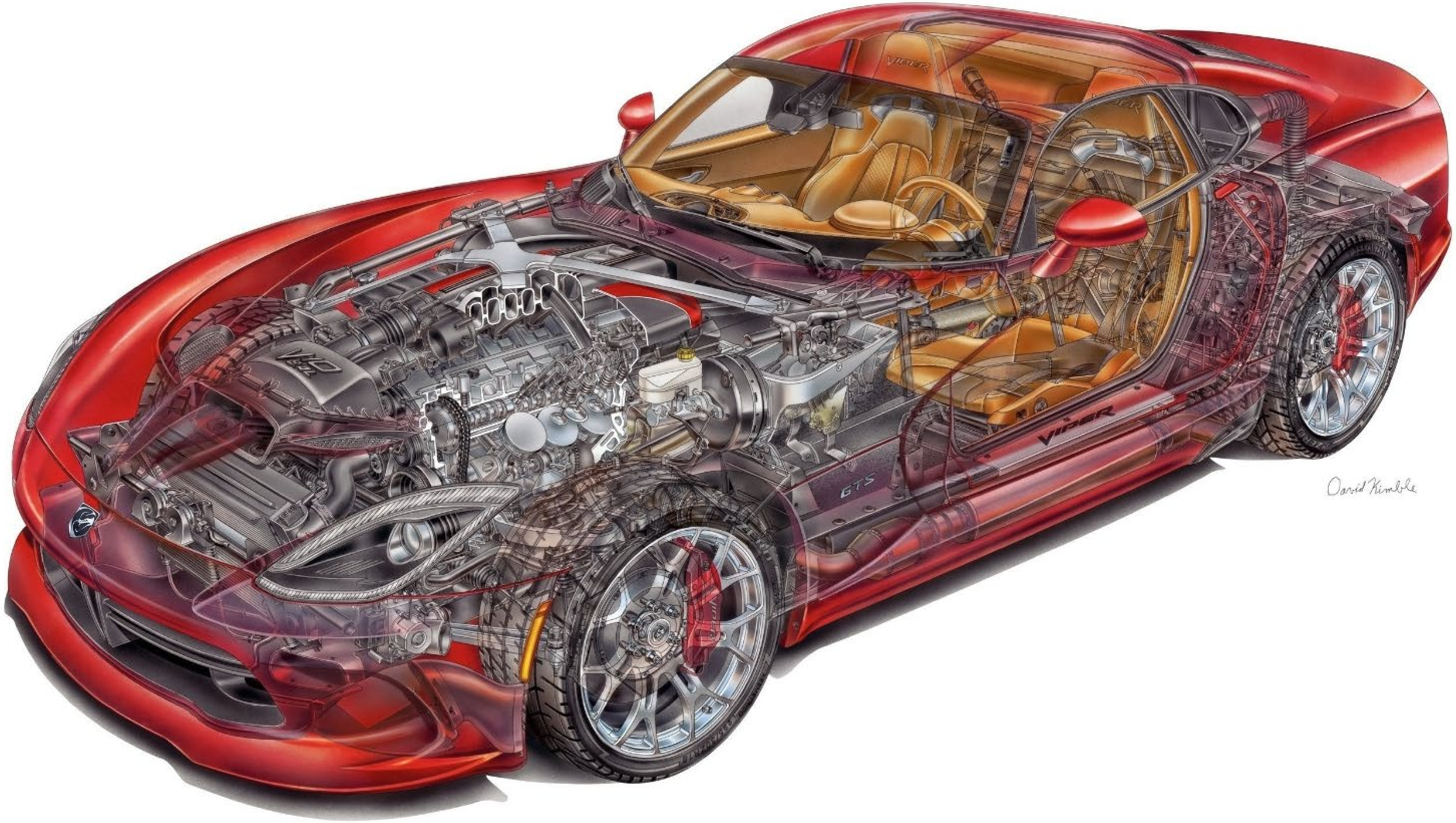
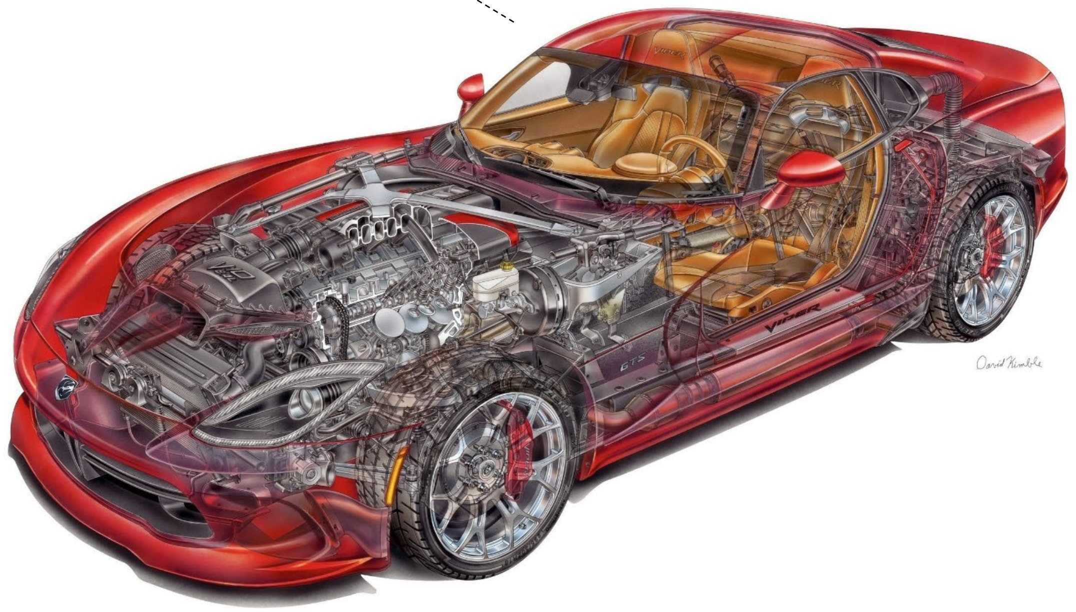
Engineering



Engineering

Parts



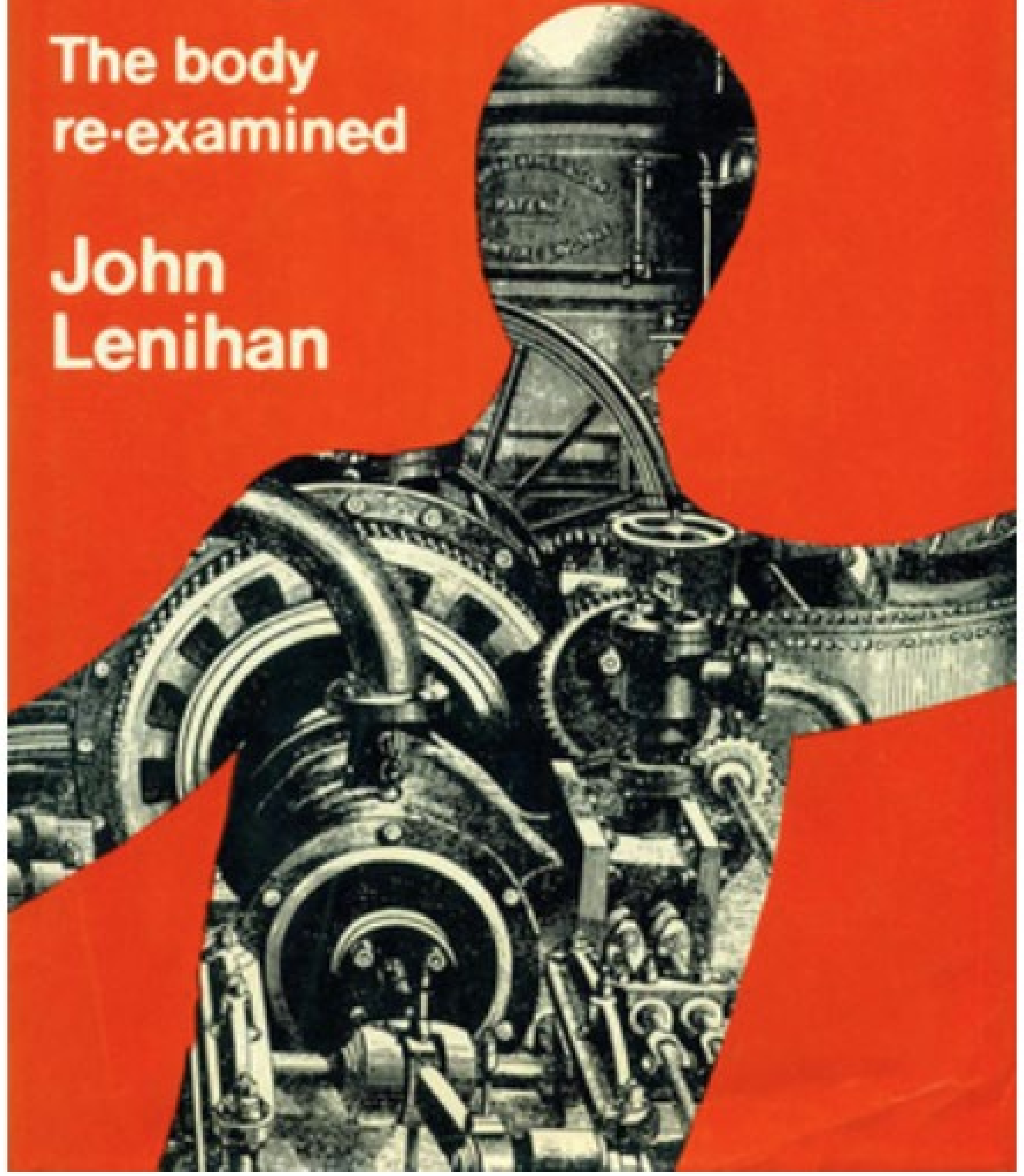
Tools

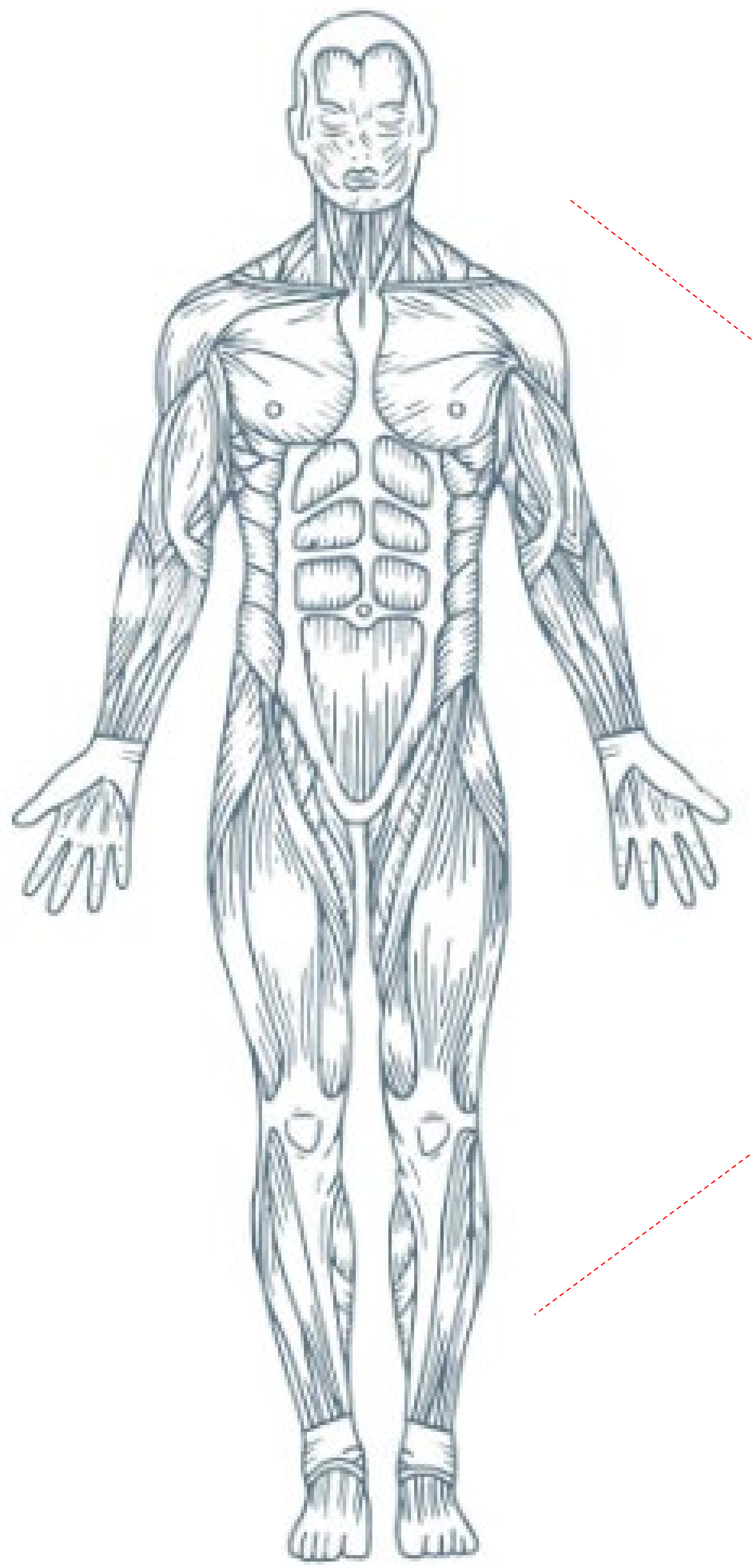
Equipment

Human Engineering

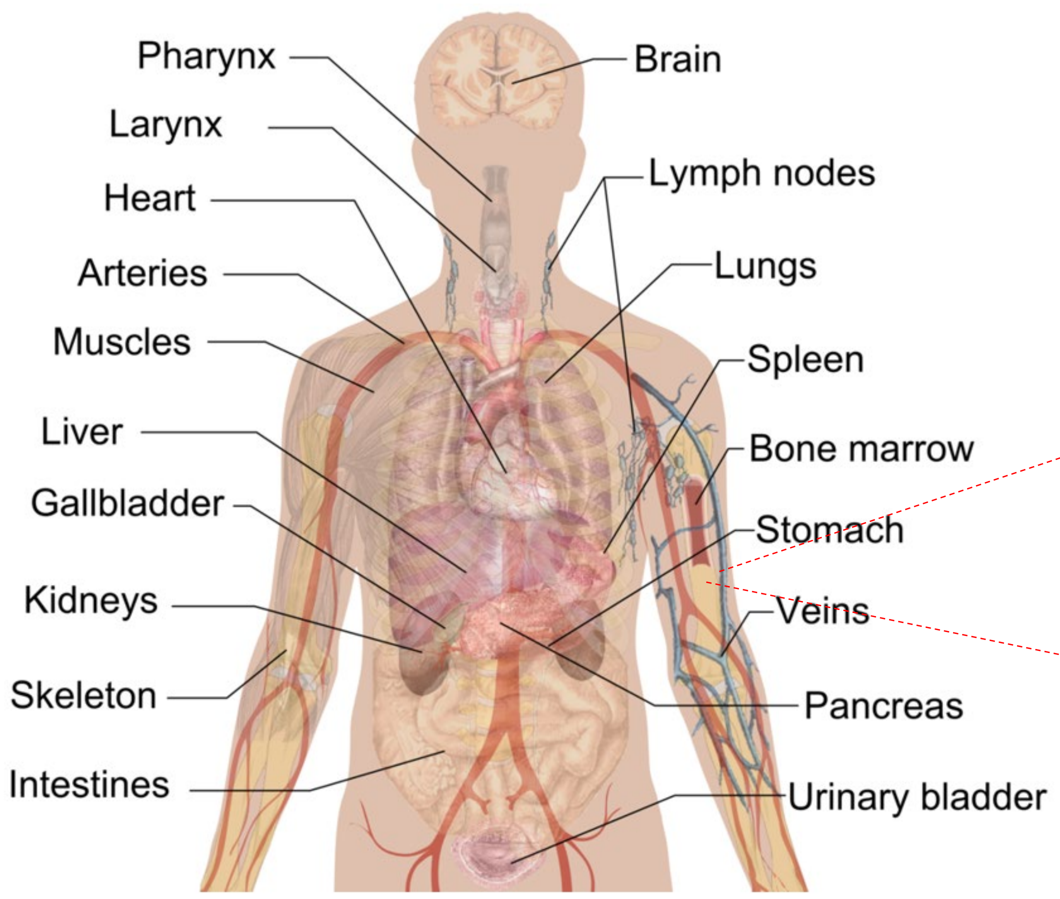
The body re·examined

John Lenihan





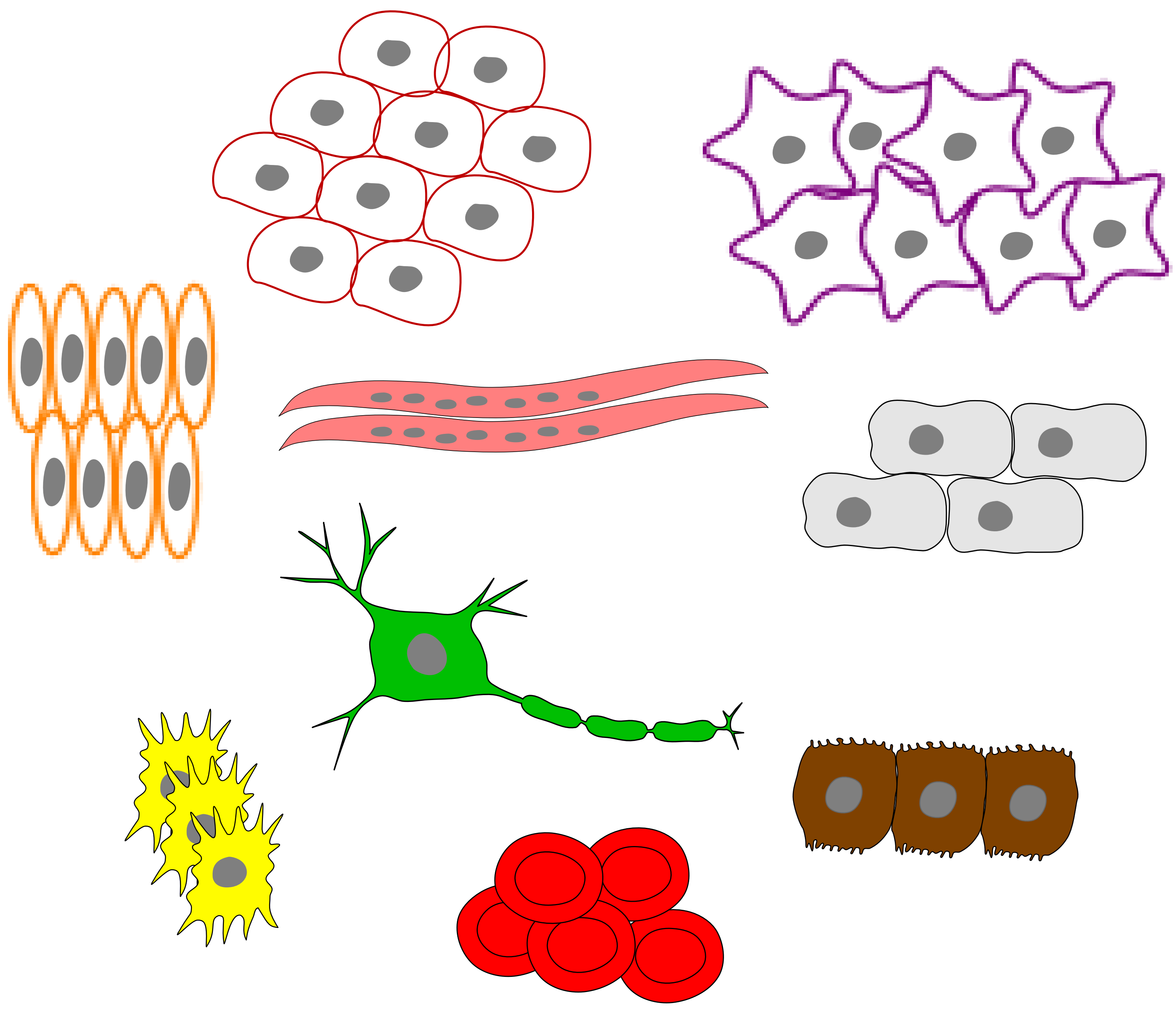
Human are made from hundreds of different tissues



Each tissue is made from millions cells

A human has approximately
100 trillion (10^{14}) cells
100,000,000,000,000 cells

Each cell is
a separate entity / **building block**
of a human



eg. heart (cardiac) cells, liver (hepatic) cells, kidney cells, red blood cells, lymphocytes, neurons, epithelial cells, etc.

The Cell is very complex

It is highly **organised**

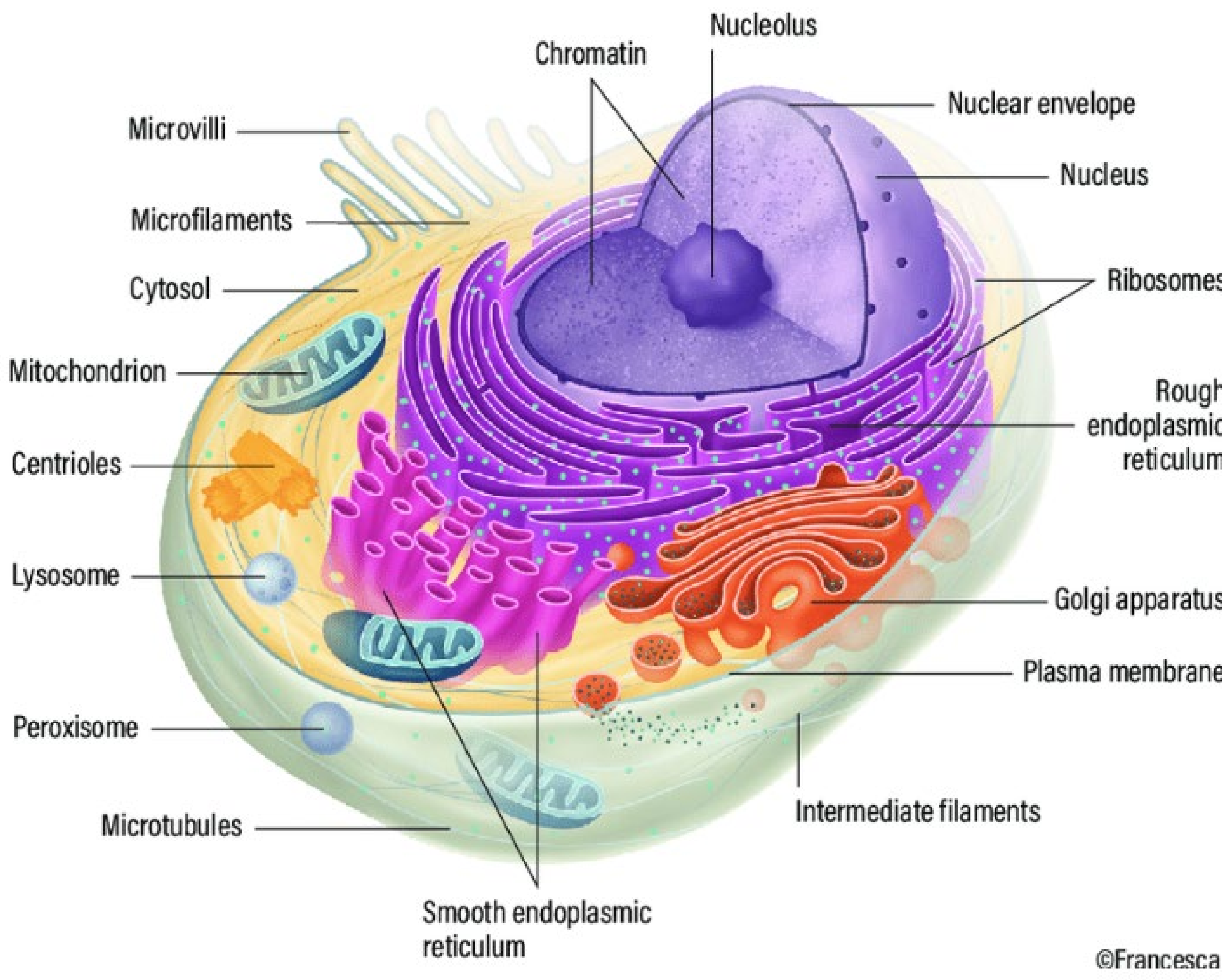
It has **multiple compartments**

It has **multiple structures**

The majority of the **items** in the cell are **made** by machines called **proteins**

A human has over **21,000** protein machines

Each protein does a different **job**



Structure of a mammalian cell

Proteins carry out the majority of the key activities in cells

Each cell has thousands of proteins and each protein does a different job in the cell. These jobs include

- **importing food** into the cell
- **exporting waste** out of the cell

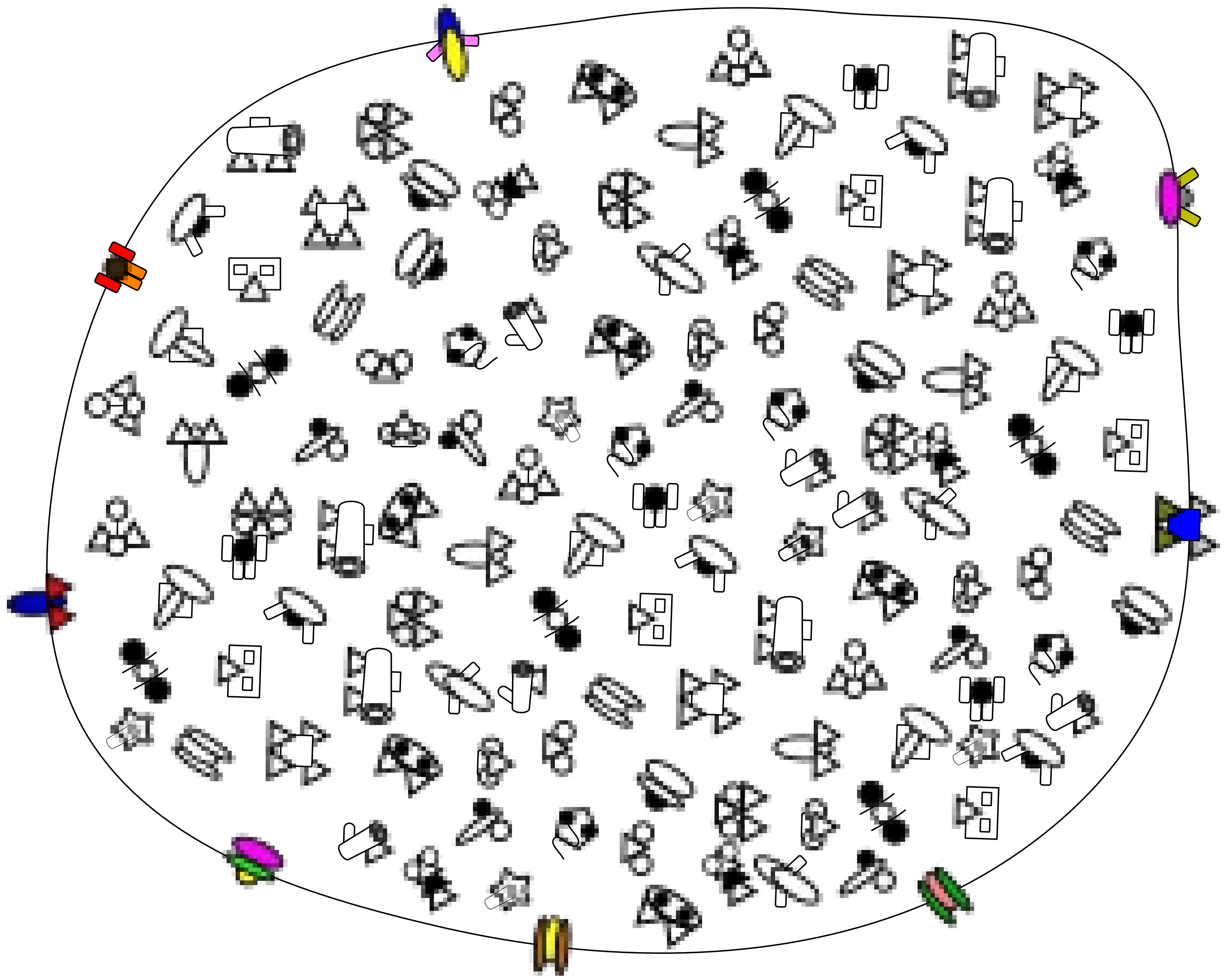
- **breaking** down the food into tiny bits (molecules)
- **converting** the food into energy

- **making** or importing **all the components** in the cells
- **transporting** them to the right place
- **duplicating** DNA
- **upkeep, maintenance** and **repair** DNA

Each protein can be considered a **tiny nano machine**

One millionth of a millimeter in size!

Proteins



Each cell has thousands of different proteins

Proteins examples

Lactase

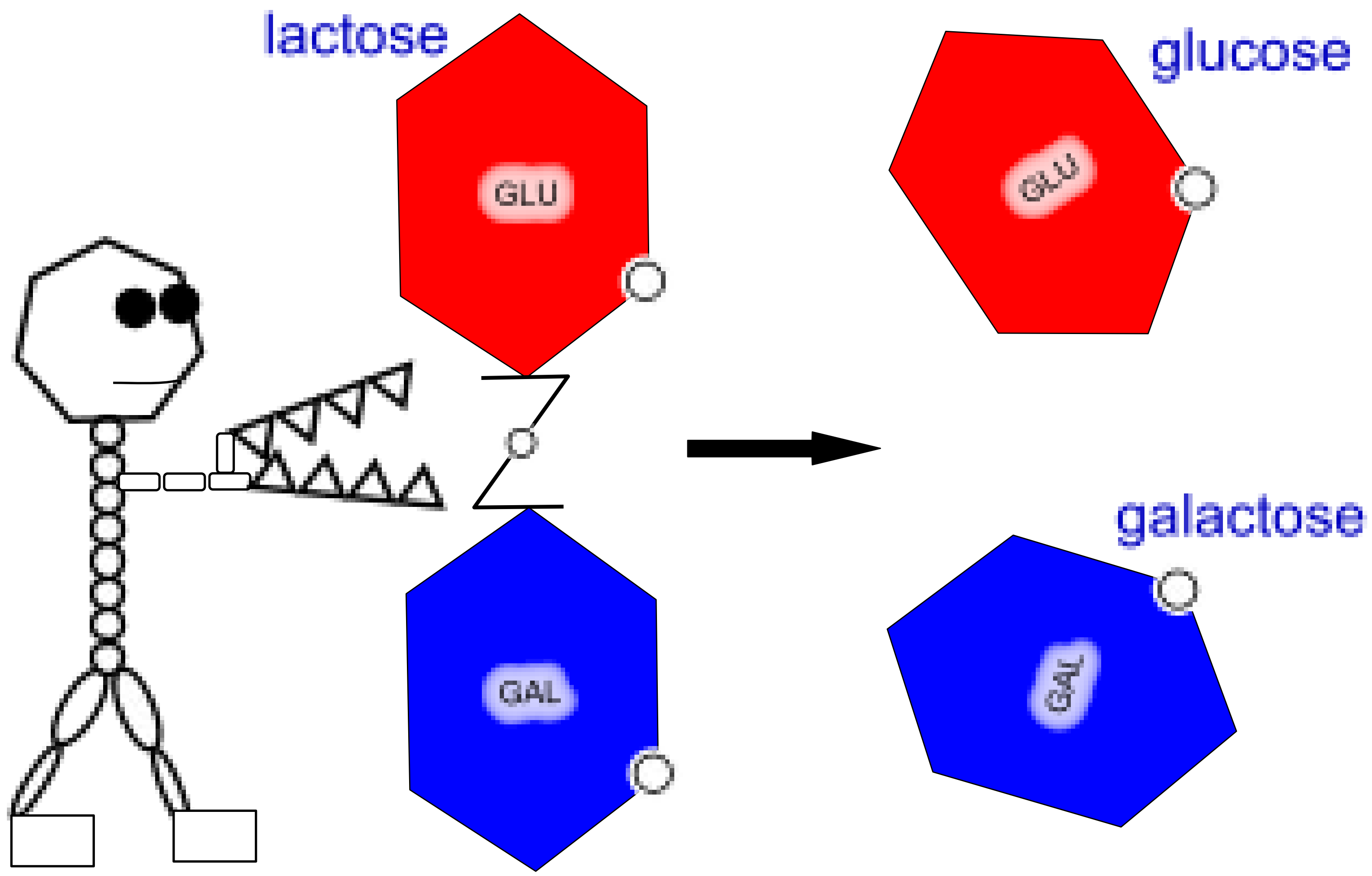
The lactase protein breaks down lactose into galactose and glucose.

Lactose is the main sugar in milk. All babies produce lactase to break down lactose into its component sugars, these are subsequently used to make energy.

Most humans have reduced lactase when they reach adulthood. Thus most humans (about 4 billion) are lactose intolerant (~90% of East Asia).

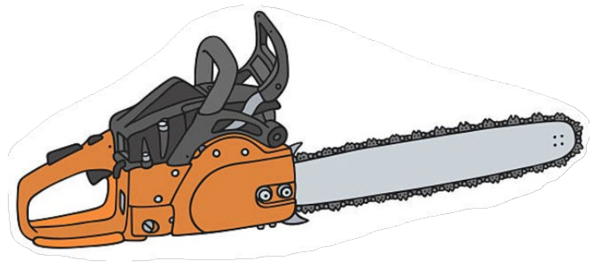
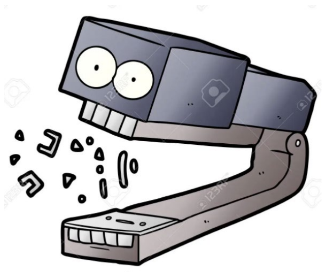
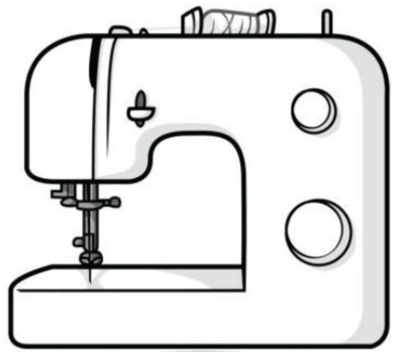
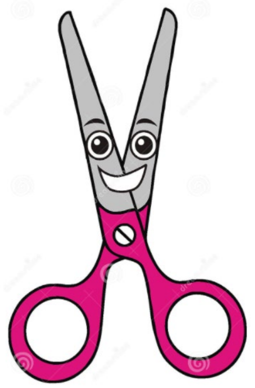
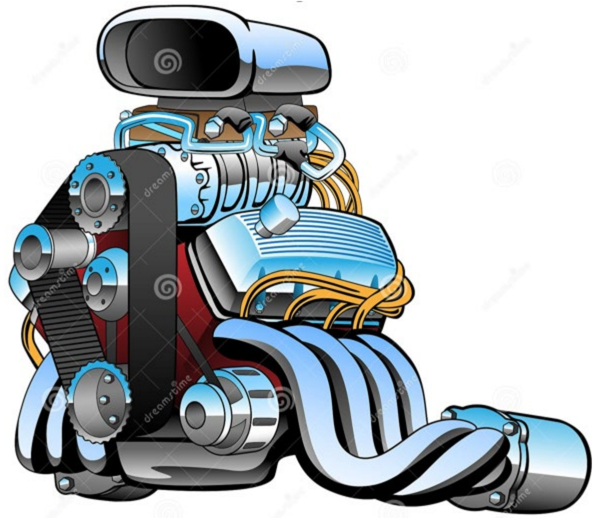
A mutation is present in most Caucasians that causes them to produce lactase throughout their life - consequently they are lactose tolerant (~95% of Europeans).

Each protein does a different job in the cell



The lactase protein
cleaves lactose into glucose and galactose

Proteins are miniature robots / miniature machines

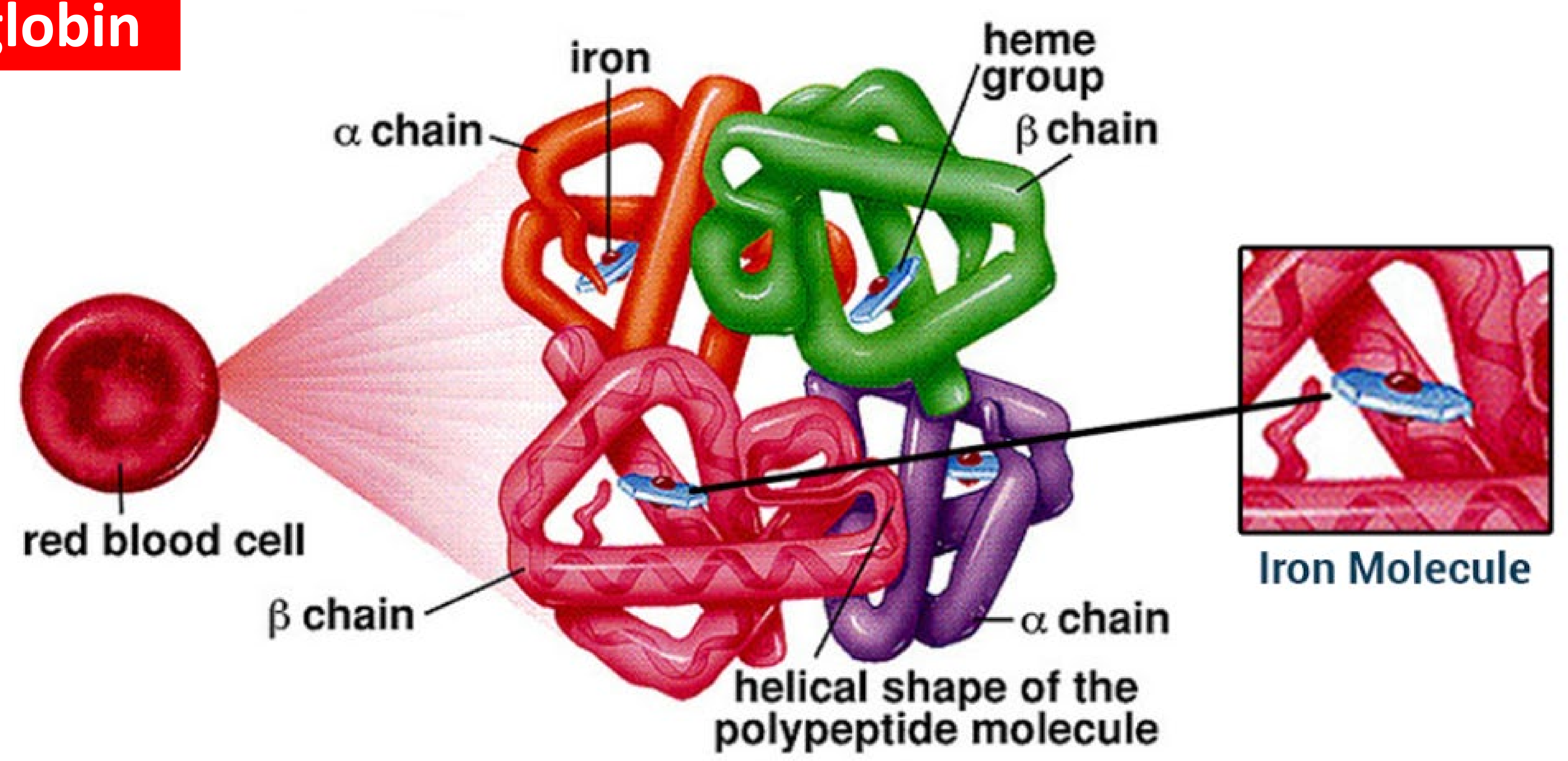
     

Proteins essentially
-make all the components found in the cell
-do all the jobs in the cell
-do all the jobs in the body

What exactly does a protein look like?

What exactly does a protein look like?

Haemoglobin

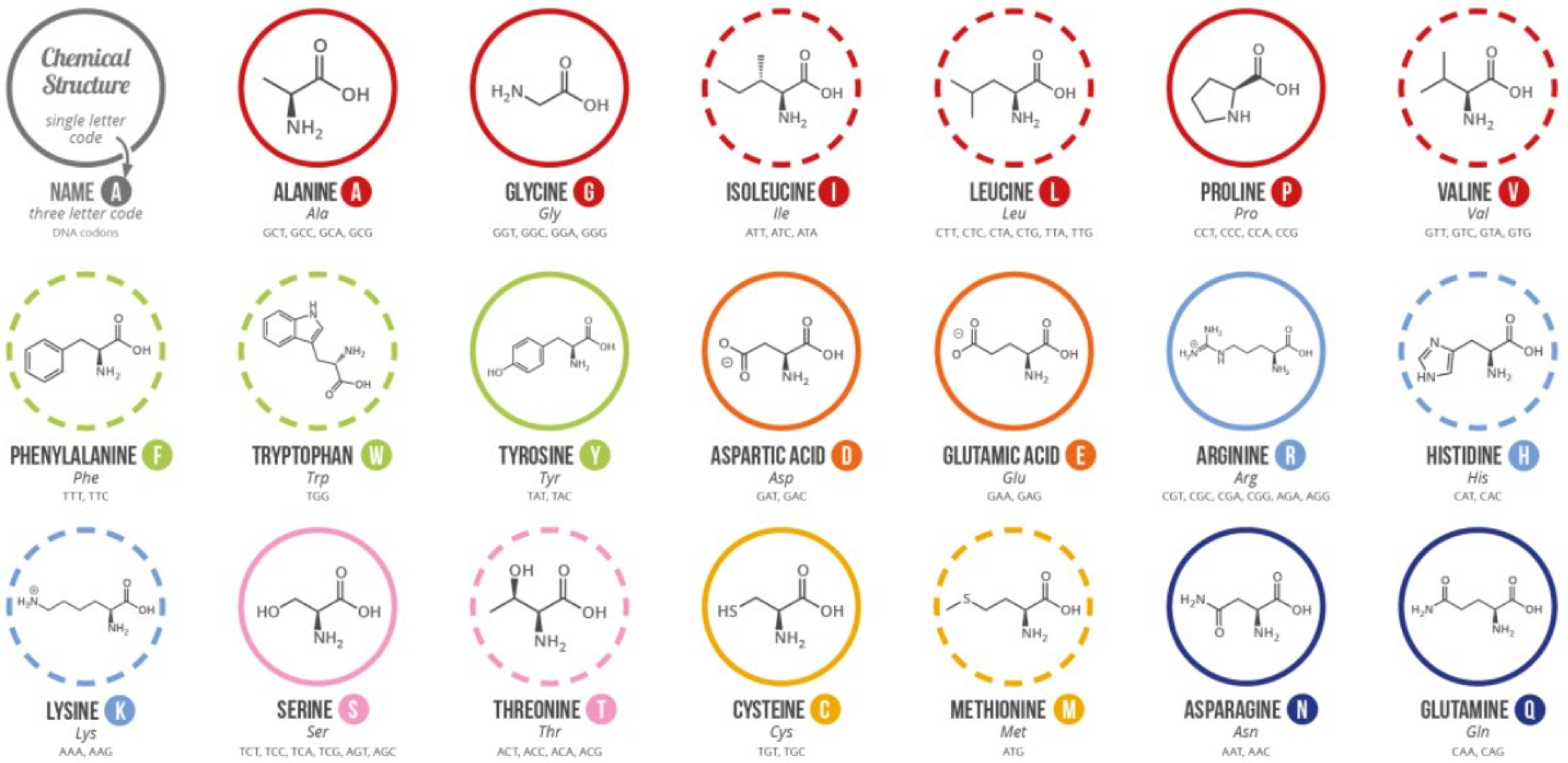


What are the haemoglobin chains made of?

The haemoglobin chains are made of strings of amino acids

There are 20 different amino acids

Amino acid building blocks



What are proteins made of?

Proteins are made of **amino acids**

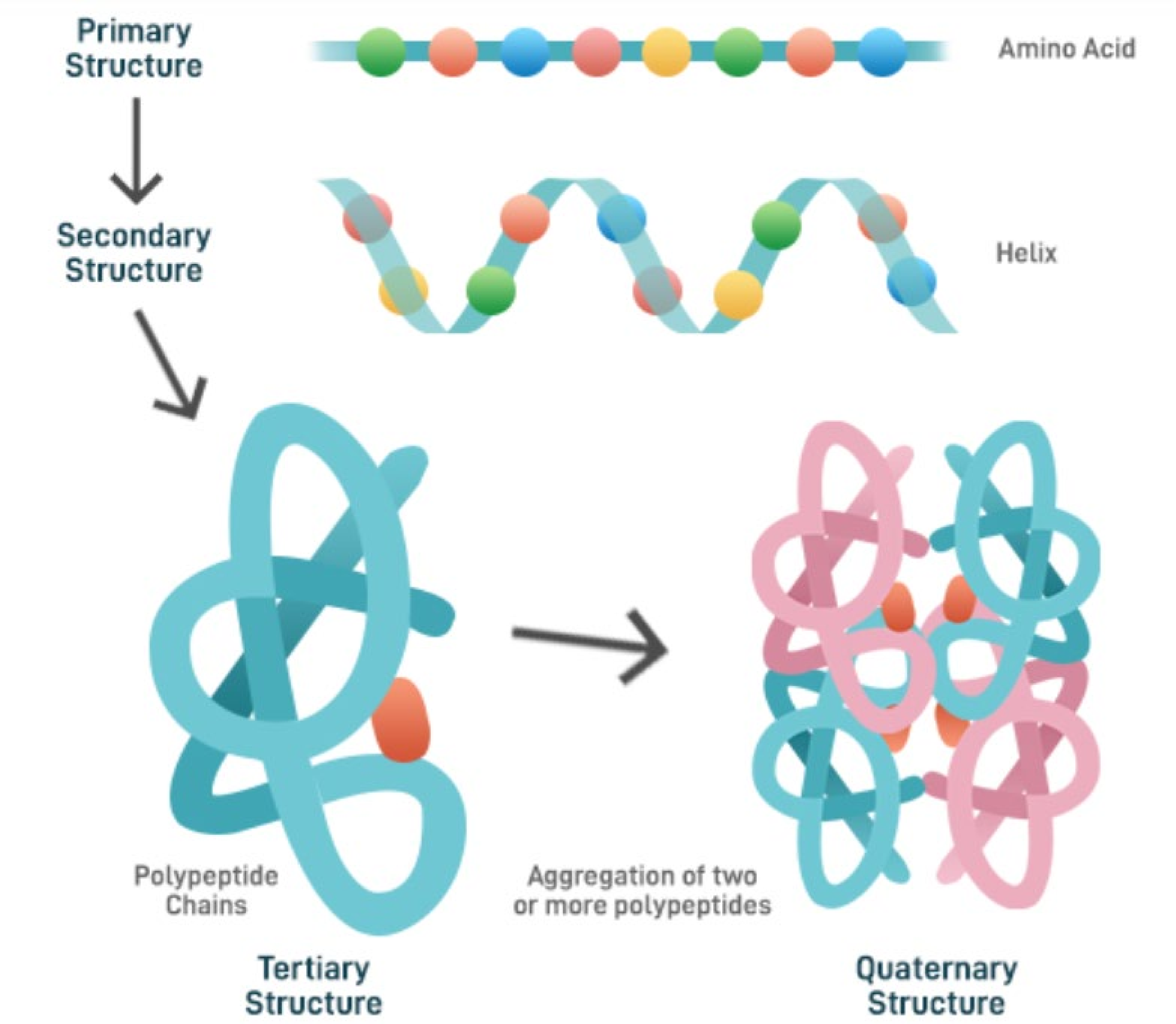
There are **20 amino acids**
Consider each amino acid as a **building block**

Each protein is made of a string of amino acid building blocks
The **smallest** protein has about **5 amino acids**
The **largest** protein has **27,000 amino acids**

Sections of the amino acid string in the protein fold into motifs

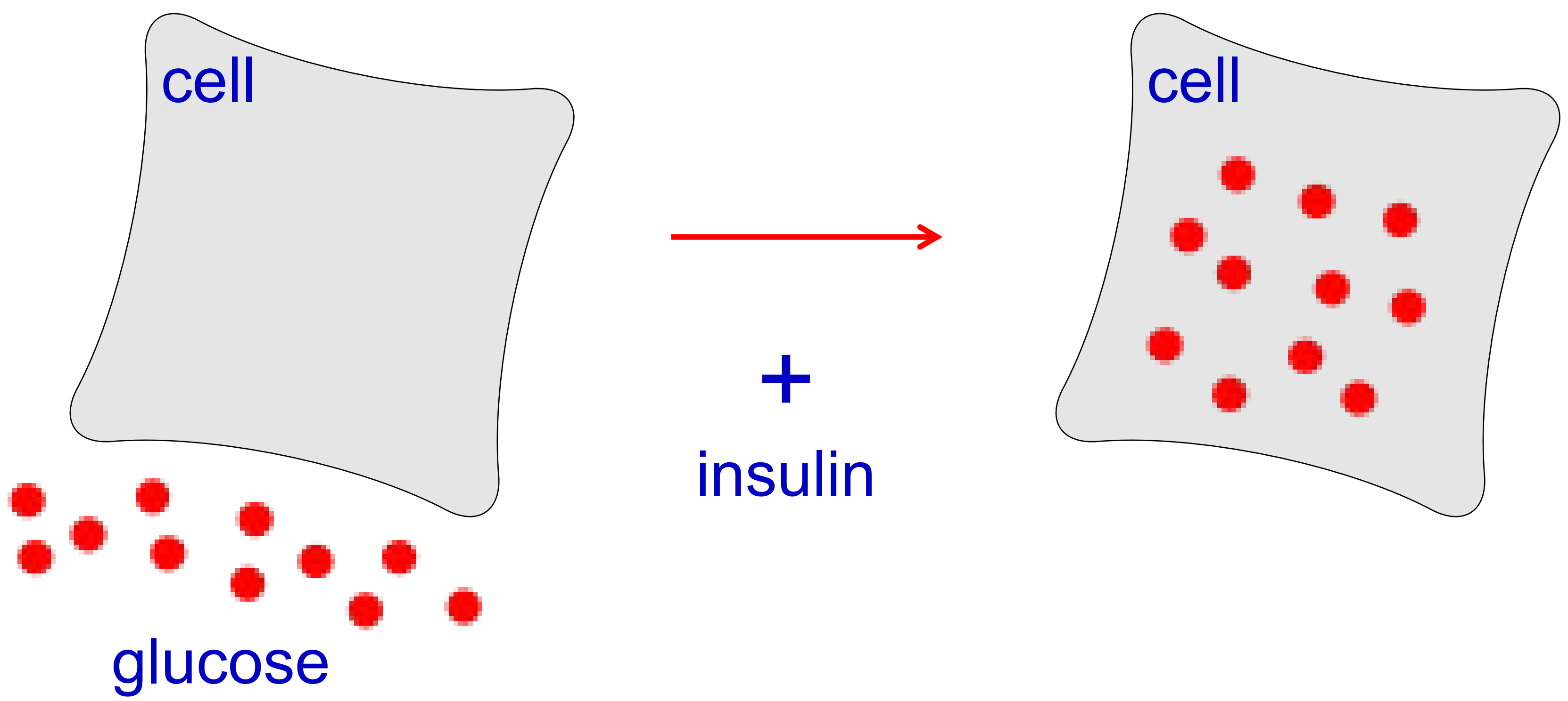
The whole string of amino acids fold into a highly complex 3D structure generating a complex protein

The protein then goes and does it job



AlphaFold, an AI tool that **predicts protein structures**, was awarded the **2024 Nobel Prize in Chemistry**.
The prize was given to **John Jumper and Demis Hassabis of Google DeepMind**, and **David Baker** of the University of Washington.

Insulin protein causes cells to take up glucose from the blood

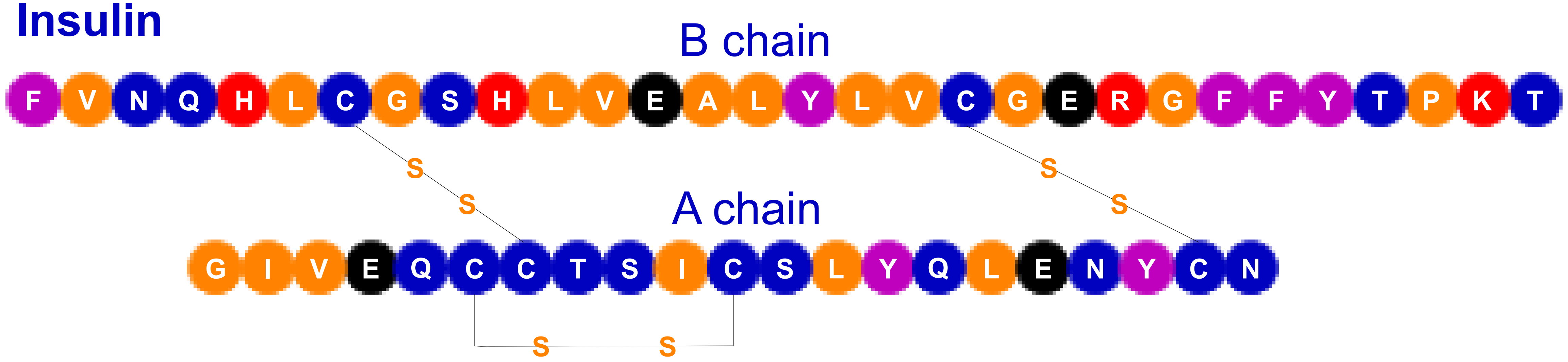


Insulin protein is deficient in diabetes type 1

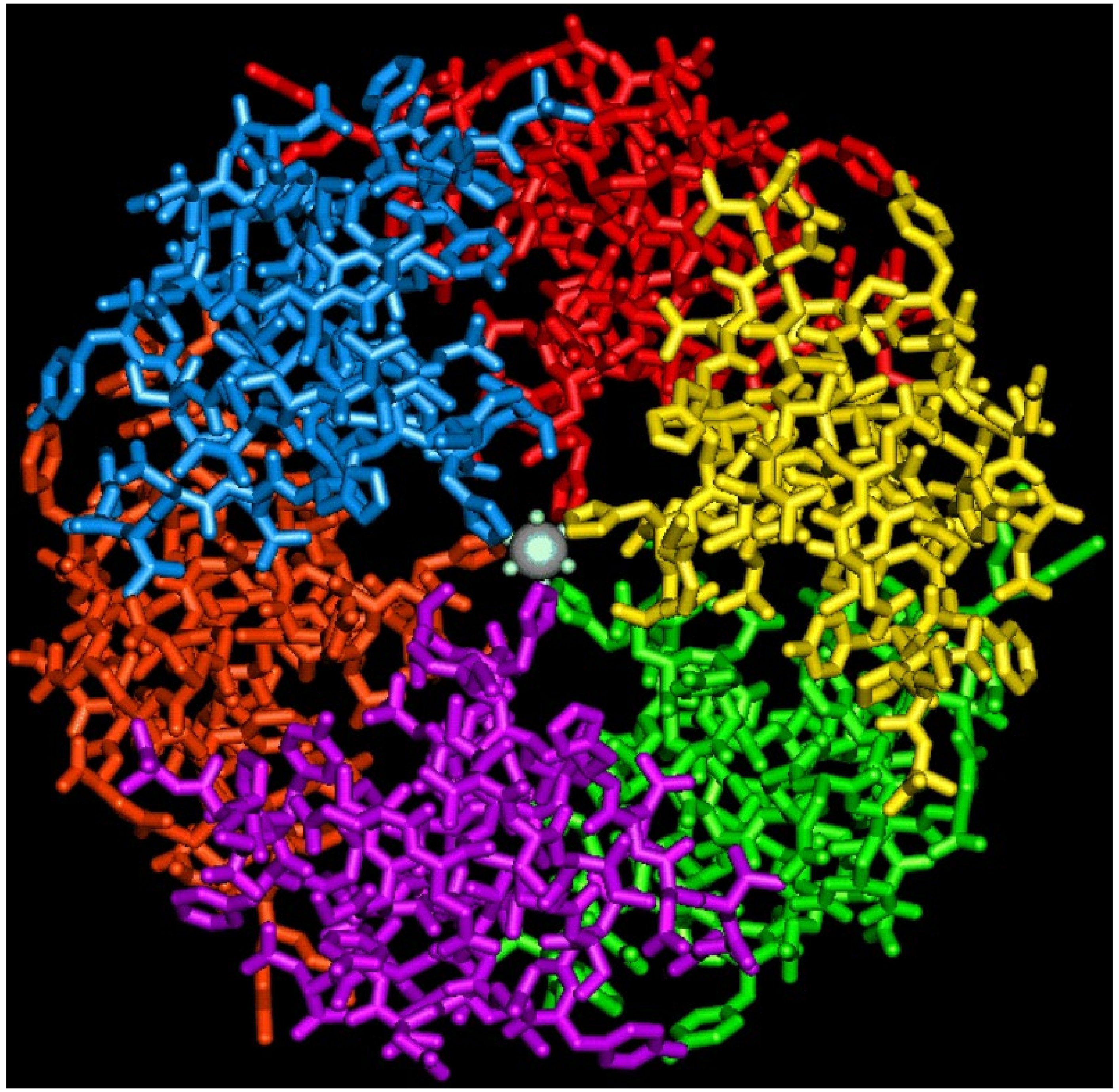
Solution – inject insulin into the body!

Insulin

Insulin
String of
amino acids



Insulin
3D structure



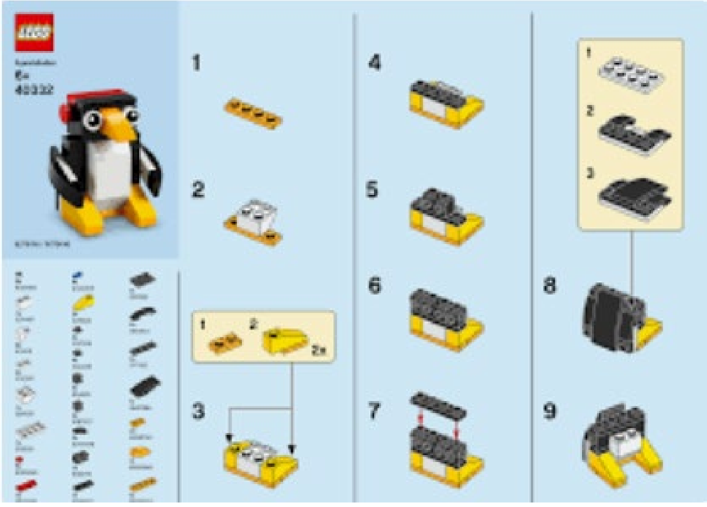
Engineering

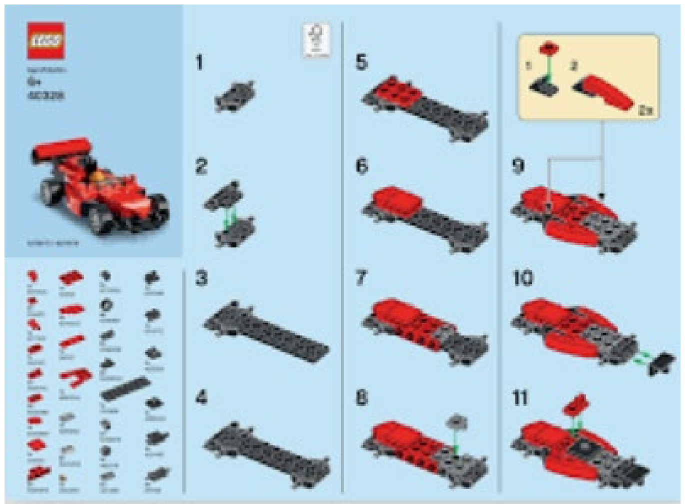
Parts

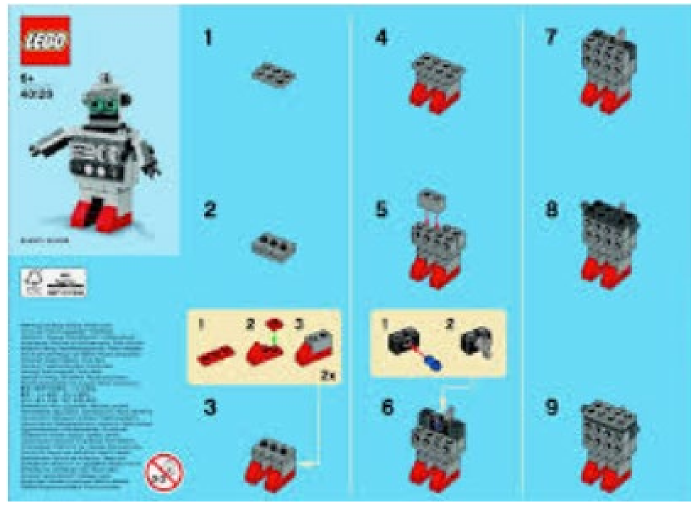
(amino acids)

Instructions

/ Blueprint

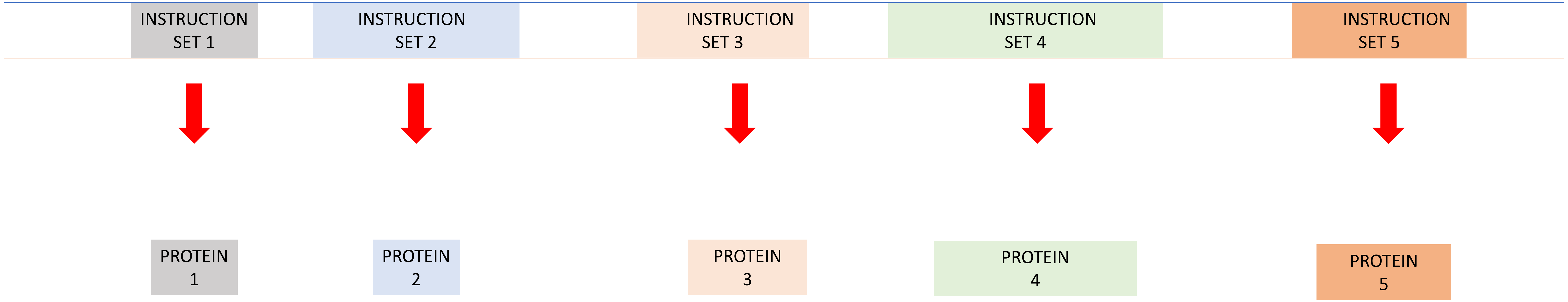


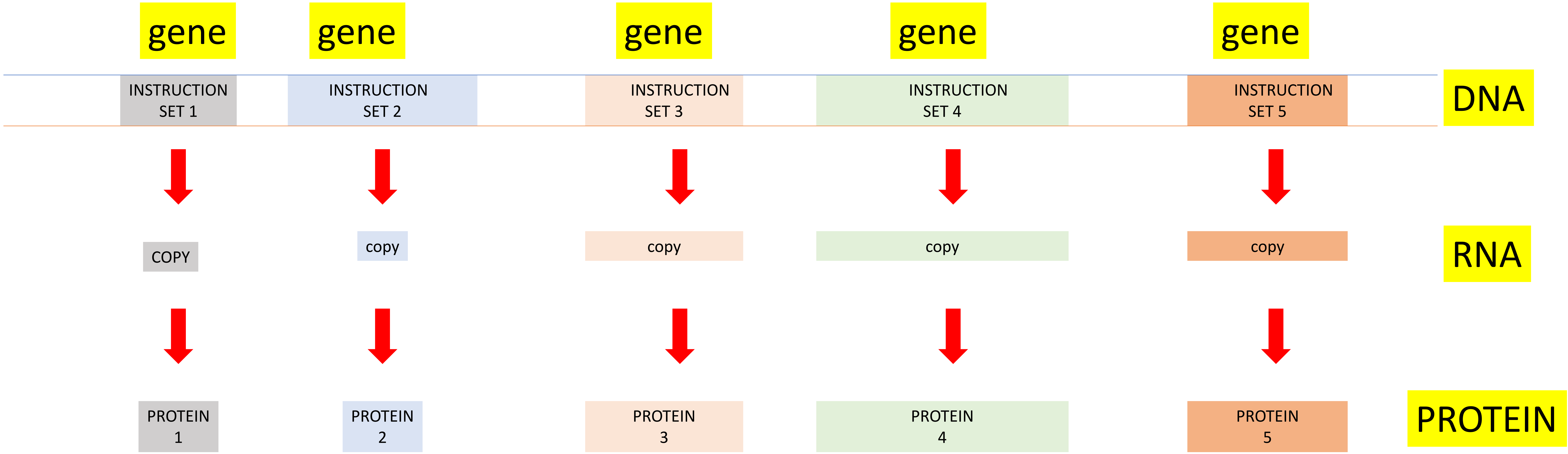




Product

(protein)


INSTRUCTION SET 1
INSTRUCTION SET 2
INSTRUCTION SET 3
INSTRUCTION SET 4
INSTRUCTION SET 5
PROTEIN 1
PROTEIN 2
PROTEIN 3
PROTEIN 4
PROTEIN 5


gene
gene
gene
gene
gene
INSTRUCTION SET 1
INSTRUCTION SET 2
INSTRUCTION SET 3
INSTRUCTION SET 4
INSTRUCTION SET 5
DNA
COPY
copy
copy
copy
copy
RNA
PROTEIN 1
PROTEIN 2
PROTEIN 3
PROTEIN 4
PROTEIN 5
PROTEIN

-DNA is a double stranded molecule made up of a sequence of **4 letters**
-DNA is **transcribed** into messenger RNA (mRNA) by **specialised transcription proteins**

-mRNA bases are the same as DNA
EXCEPT Uracil (U) is in place of T and
mRNA is single stranded

mRNA is translated into protein by **specialised translation proteins**

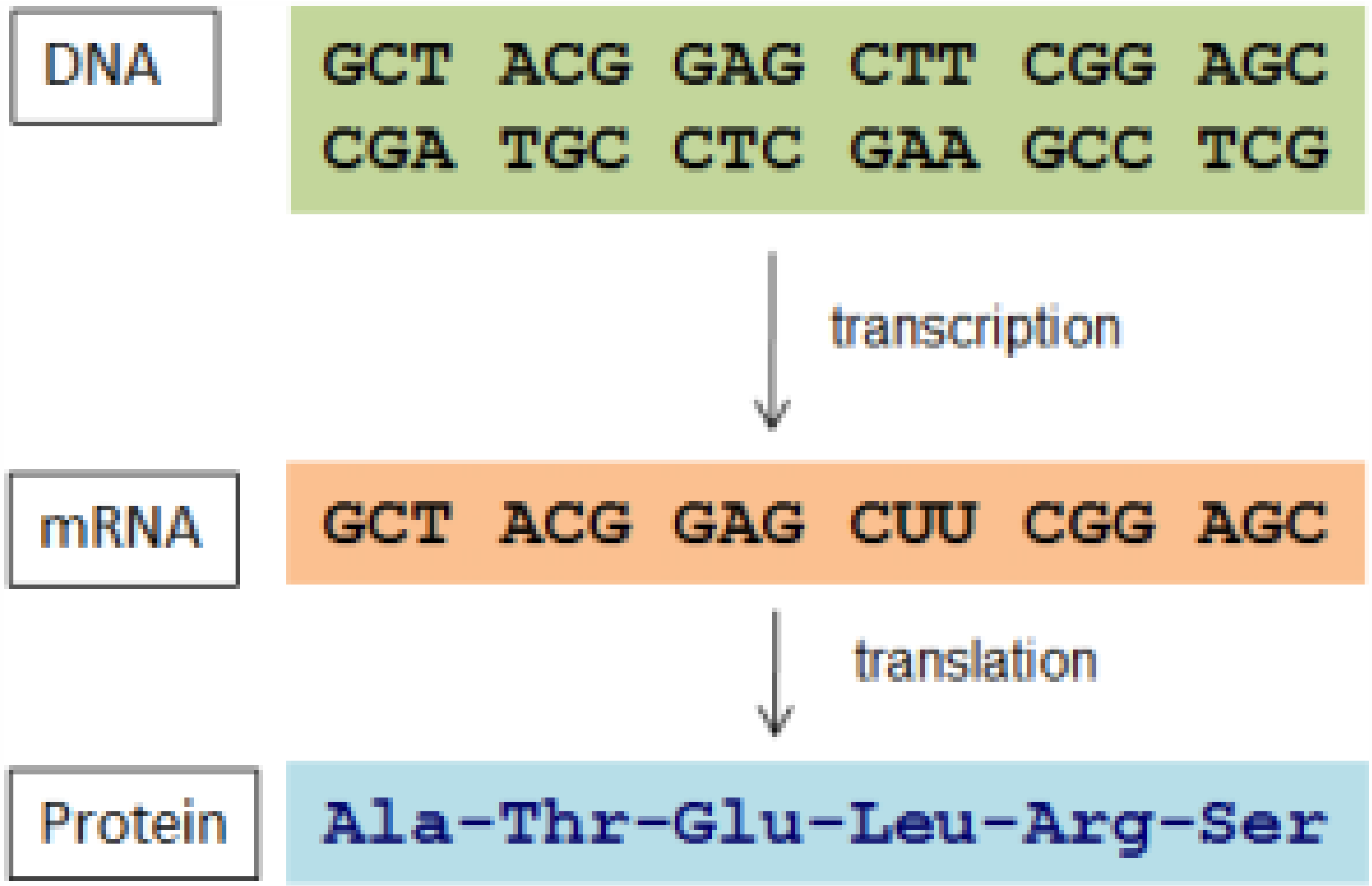
Combinations of letters in DNA code for the different amino acids and is called the **genetic code**.

There are 4 letters (**bases**) in DNA. Each amino acid is specified by a **3 letter word** e.g. **GCT**=**Alanine** amino acid
The 3 letter words are called **codons**
There are **64** possible words

Some amino acids are encoded by only one codon eg. **methionine** (Met) is encoded by **ATG**

Other amino acids are encoded by **up to** six different codons eg. **Serine** (Ser) is encoded by TCC, TCG, TCA, TCT, AGT and AGC

A stop codons specifies the end of the protein – there are 3 stop codons



DNA has 4 bases (letters) ACGT

Each amino acid is specified by a 3 letter DNA word called a codon
There are 64 codons in the genetic code including 3 stop codons

The genetic code for 6 of the 20 amino acids is shown below along with the code for the stop codons

alanine	cysteine	phenylalanine	histidine	methionine	serine	
A	C	F	H	M	S	STOP
GCT	TGT	TTT	CAT	ATG	TCT	TAG
GCC	TGC	TTC	CAC		TCC	TAA
GCA	ACA		GTA		TCA	TGA
GCG	ACG		GTG		TCG	
					AGT	
					AGC	

Test your understanding

DNA - the genetic code

3 bases of DNA code for an amino acid

-Each 3 bases of DNA that codes for an amino acid is called a **codon (word)**

-There are **64** possible **codons (words)**

-61 codons code for amino acids

-3 codons don't code for any amino acids, these are called stop codons

Amino Acid	Coding DNA Strand Base Triplets Not Transcribed
alanine	GCT, GCC, GCA, GCG
arginine	CGT, CGC, CGA, CGG AGA, AGG
asparagine	AAT, AAC
aspartic acid	GAT, GAC
cysteine	TGT, TGC
glutamic acid	GAA, GAG
glutamine	CAA, CAG
glycine	GGT, GGC, GGA, GGG
histidine	CAT, CAC
isoleucine	ATT, ATC, ATA
leucine	TTA, TTG, CTT, CTC CTA, CTG
lysine	AAA, AAG
methionine (start)	ATG
phenylalanine	TTT, TTC
proline	CCT, CCC, CCA, CCG
serine	TCT, TCC, TCA, TCG AGT, AGC
stop	TAA, TAG, TGA
threonine	ACT, ACC, ACA, ACG
tryptophan	TGG
tyrosine	TAT, TAC
valine	GTT, GTC, GTA, GTG

T in DNA = U in RNA

The Genetic Code

2nd base in codon

1st base in codon	U	C	A	G	3rd base in codon
U	Phe	Ser	Tyr	Cys	U
U	Phe	Ser	Tyr	Cys	C
U	Leu	Ser	STOP	STOP	A
U	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
C	Leu	Pro	His	Arg	C
C	Leu	Pro	Gln	Arg	A
C	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
A	Ile	Thr	Asn	Ser	C
A	Ile	Thr	Lys	Arg	A
A	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
G	Val	Ala	Asp	Gly	C
G	Val	Ala	Glu	Gly	A
G	Val	Ala	Glu	Gly	G

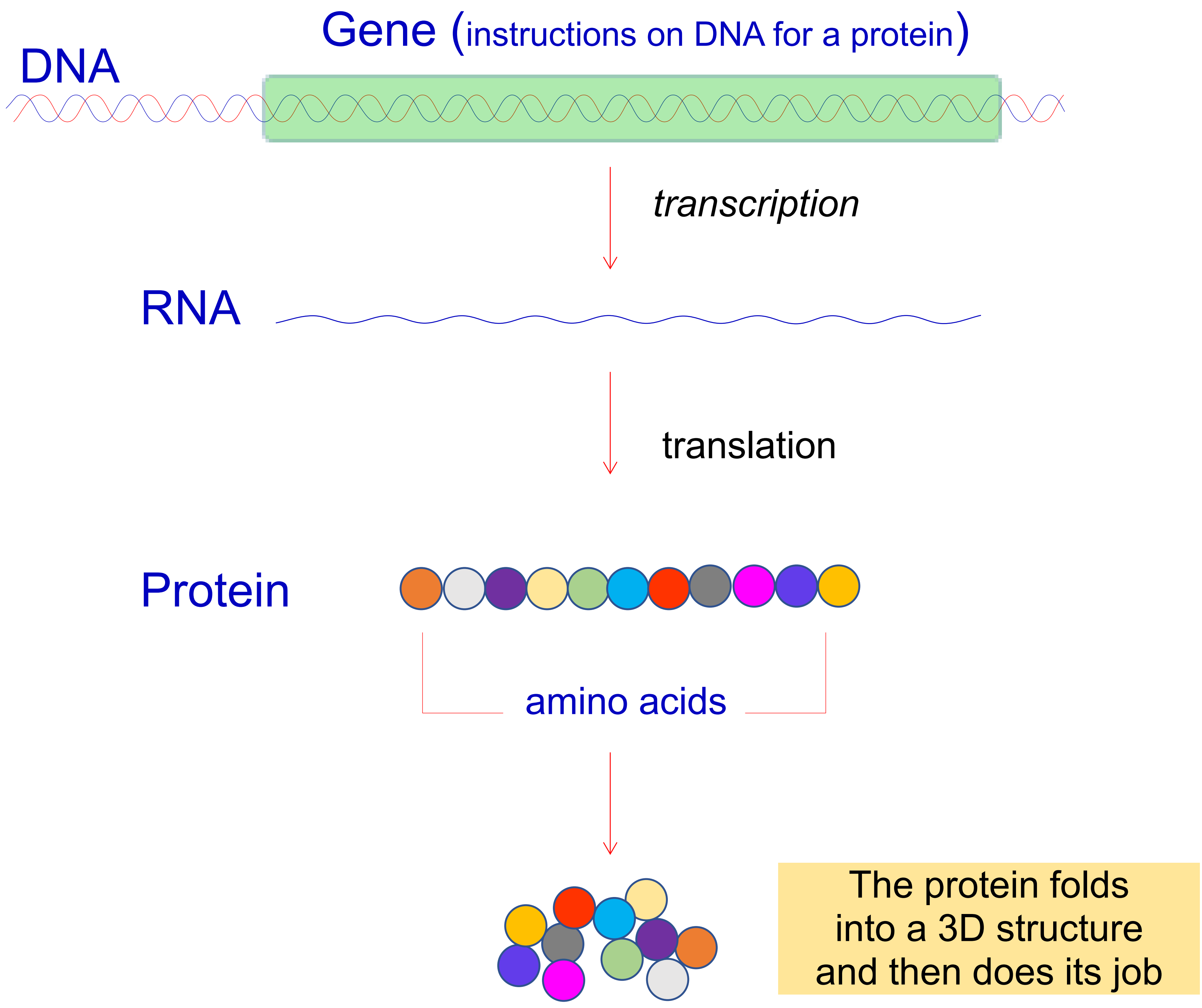
Test yourself!
Translate the piece of mRNA below into protein

auggcuacaggcucccuag

mRNA letters/bases are the same as DNA except Uracil (U) is in place of T

A gene includes all the code necessary to make the string of amino acids in a protein*

DNA is transcribed into RNA
RNA is translated into protein



* There are other genes which encode RNA molecules which are not translated into proteins

Human have 3 Billion DNA bases (ACGT)
These are broken into 23 separate string
Each string is a chromosome
(human cells carry 2 copies of the DNA)

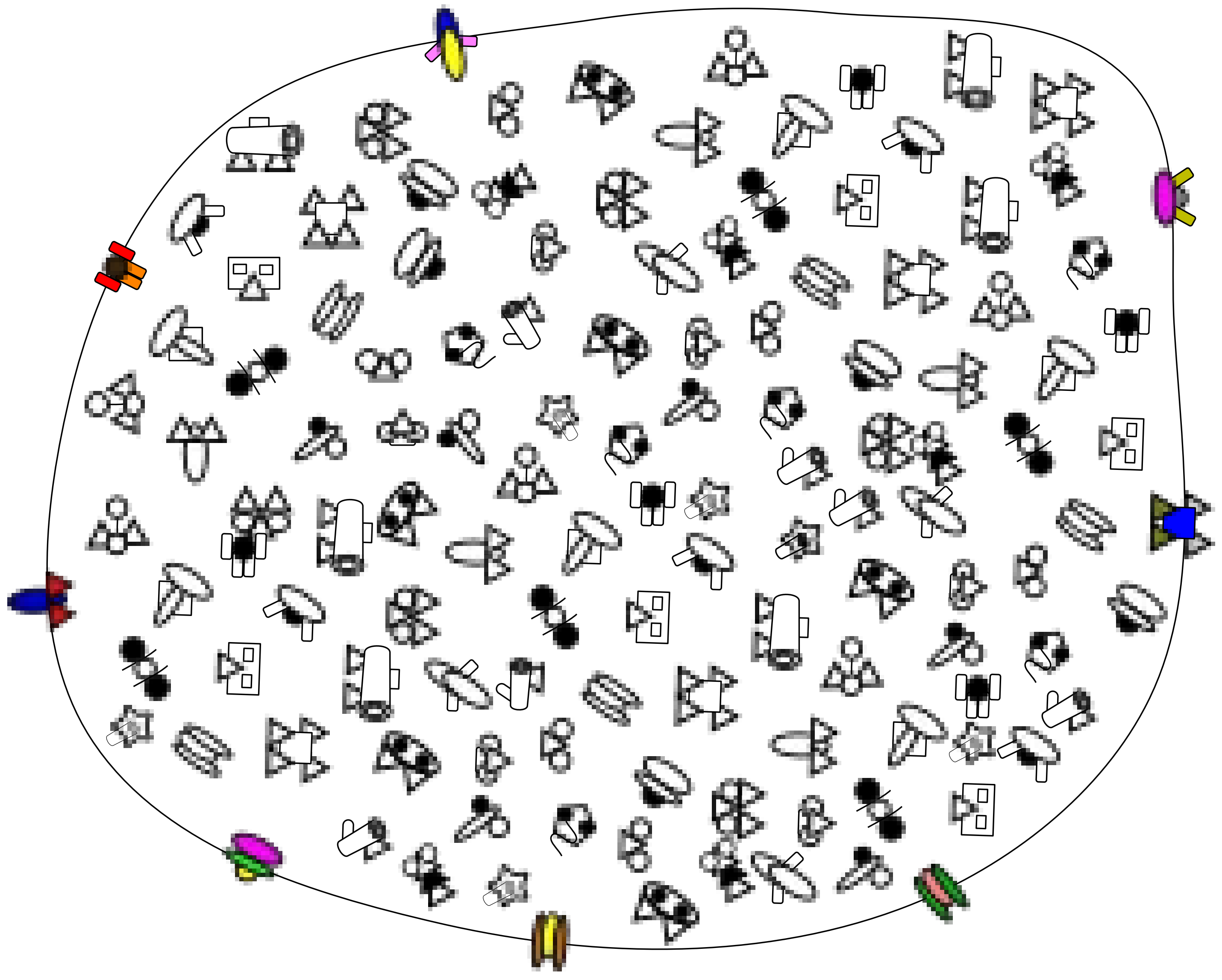
Human DNA encodes about 21,000 proteins
i.e. it has 21,000 sets of instructions

Proteins carry out the majority of the key activities in cells

Almost all diseases can be potentially be cured with the right protein!

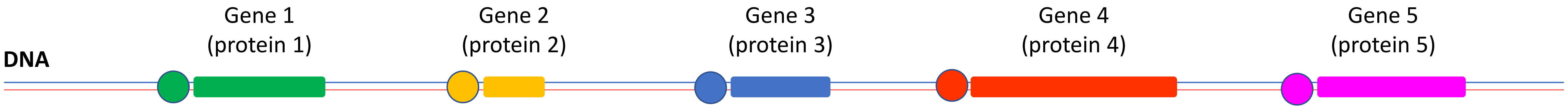
Almost all activity in the environment carried out by living organisms is done by proteins

Proteins

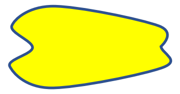


Each cell has thousands of different proteins

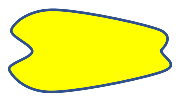
Genes have control regions



A protein called **RNA polymerase binds** to the **control** region before each gene
with the help of other proteins called transcription factors
And
makes RNA copies of the **gene** from the DNA **template**



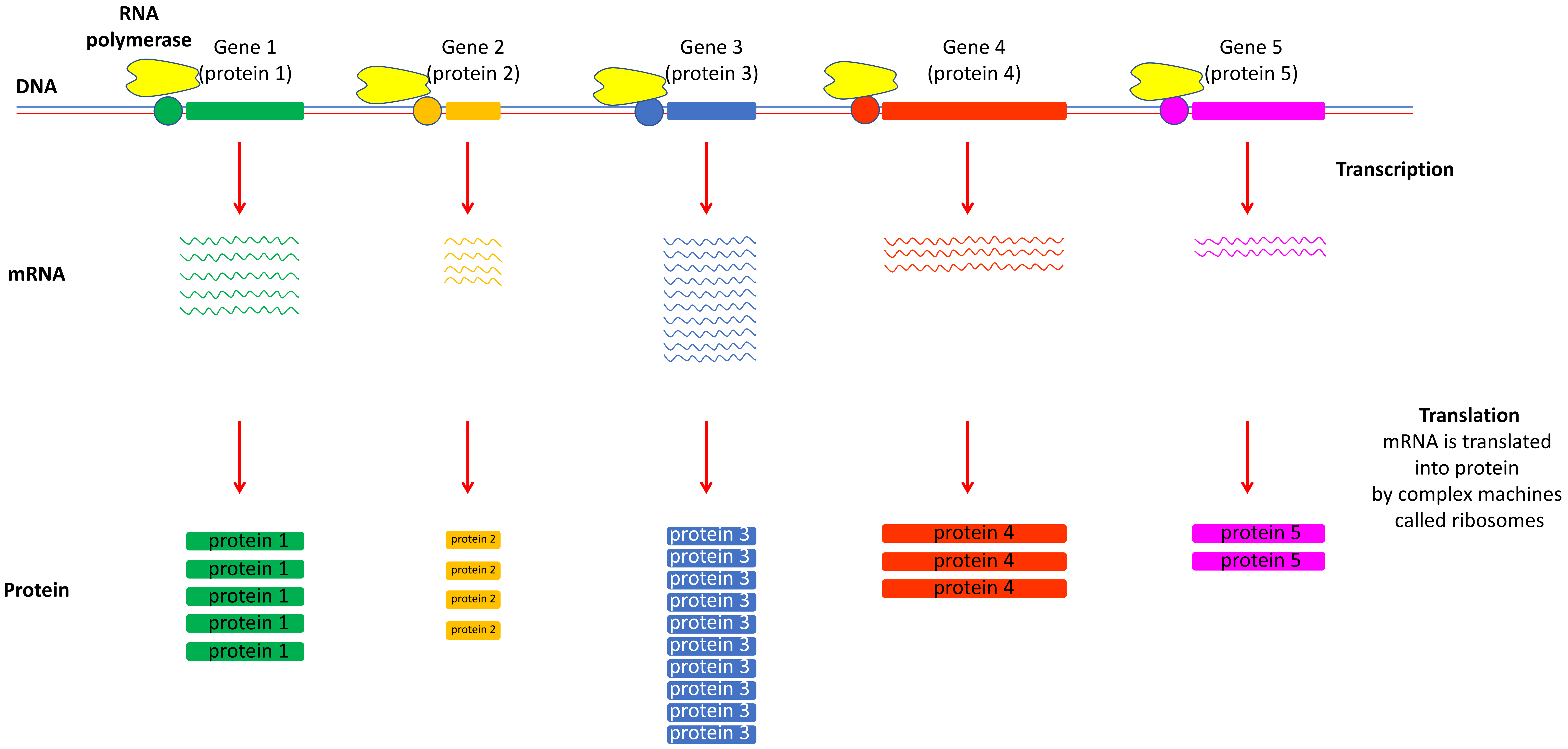
RNA polymerase binds to the control region and makes copies of RNA (mRNA) from the DNA template

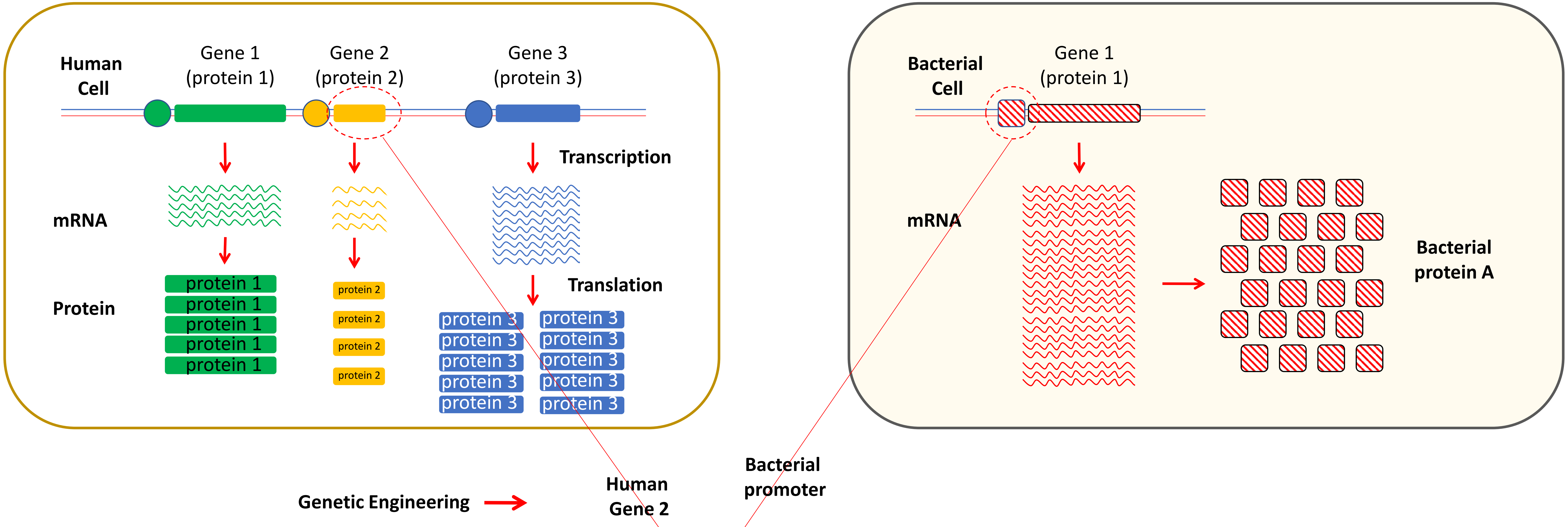


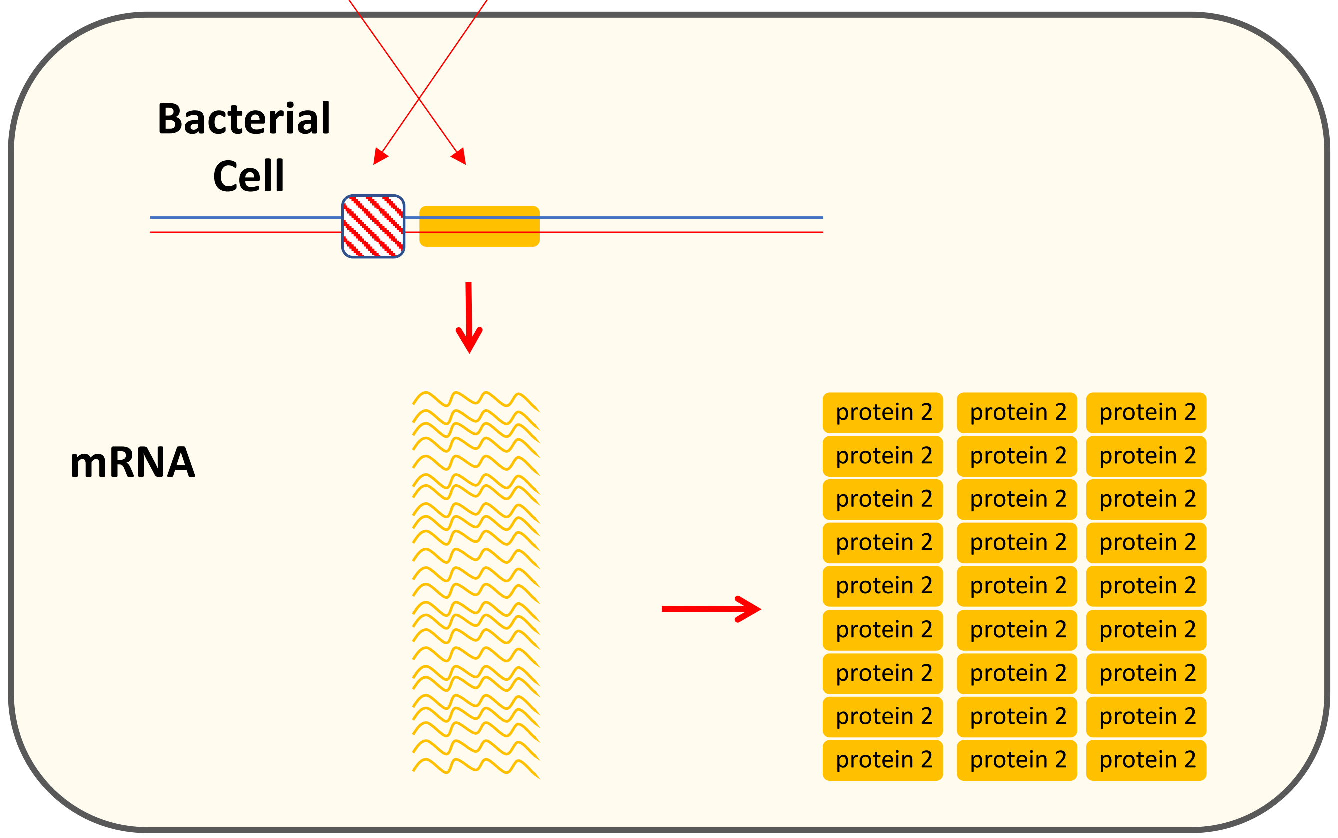
This process is called **transcription**

Control regions **vary** in strength – some are strong – some are weak

These control regions are called **promoters**







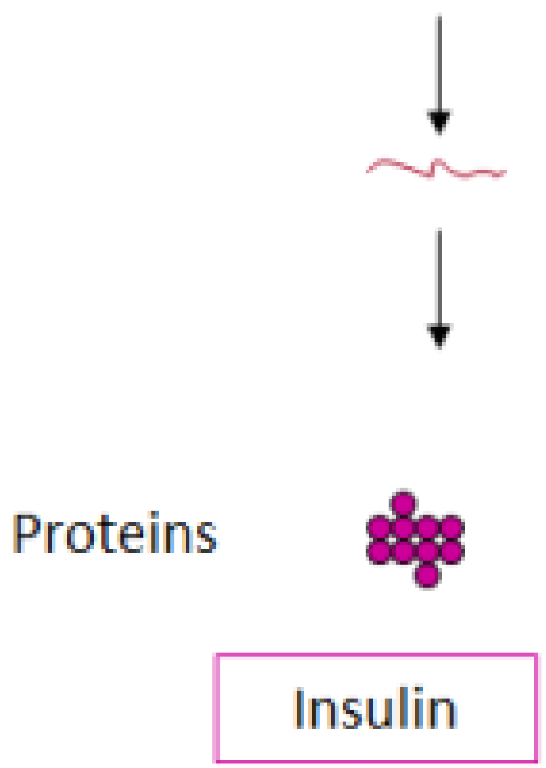
A simple concept requiring elaborate tools

- Take the DNA for a strong bacterial promoter
- Attach the DNA of a human gene to the promoter
- Put the promoter/human gene into a bacterial
- The bacteria will transcribe the human gene into mRNA
- The bacteria will translate the mRNA into protein
- Grow up lots of the bacteria
- Break them open
- Harvest the protein
- Use for medicine or for other applications

Protein production

Human cells vs Yeast cells vs Bacterial cells

	BACTERIAL CELL	YEAST CELL	HUMAN CELL
Cost of producing a protein	Cheap	Cheap	Expensive 1000 fold higher
Ease of use	Easy	Easy	Complex
Cost of raw materials	Low	Low	High
Expertise required	Medium	Medium	High
Infrastructure and equipment required	Medium	Medium	High



A cure for Diabetes (>50m affected worldwide)

-insulin protein is deficient because body destroys own insulin producing cells in pancreas

-insulin is needed to facilitate uptake of glucose in blood into cells in tissues

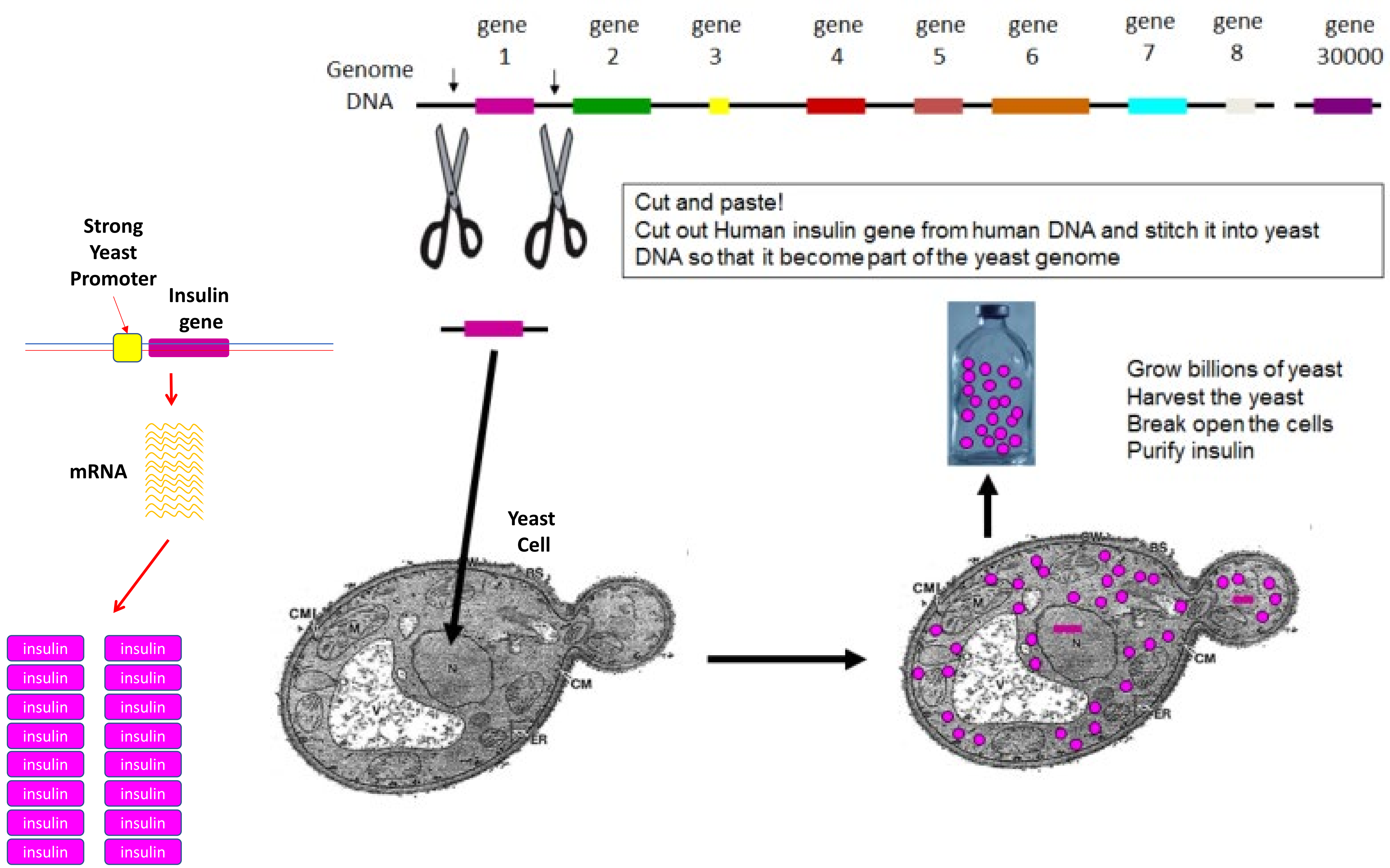
-replacing insulin protein artificially cures Diabetes

-Where can we get insulin protein to use for treatment???????????

?Purify protein from human blood? – not feasible
?Purify protein from animals – yes - feasible but difficult (pigs used in past)

IDEA: could we put the human gene into bacteria or yeast and use them to produce insulin and then purify the insulin?
Very attractive since bacteria and yeast are very cheap to grow!.

HOW?



Genome DNA
gene 1
gene 2
gene 3
gene 4
gene 5
gene 6
gene 7
gene 8
gene 30000
Cut and paste!
Cut out Human insulin gene from human DNA and stitch it into yeast DNA so that it become part of the yeast genome
Strong Yeast Promoter
Insulin gene
mRNA
insulin
Yeast Cell
Grow billions of yeast
Harvest the yeast
Break open the cells
Purify insulin


HULTS BRUK


BAHCO FORCE


Husqvarna


LXT
makita

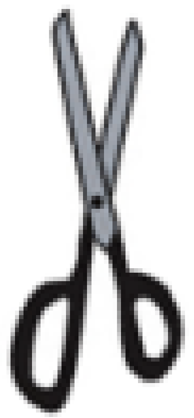

EVO-STIK
WOOD
GLUE
FAST SETTING FORMULA
INTERIOR
D1
W

A major breakthrough in the 70s

Genome DNA

gene 1 | gene 2 | gene 3 | gene 4 | gene 5 | gene 6 | gene 7 | ... | gene ...000

The tools



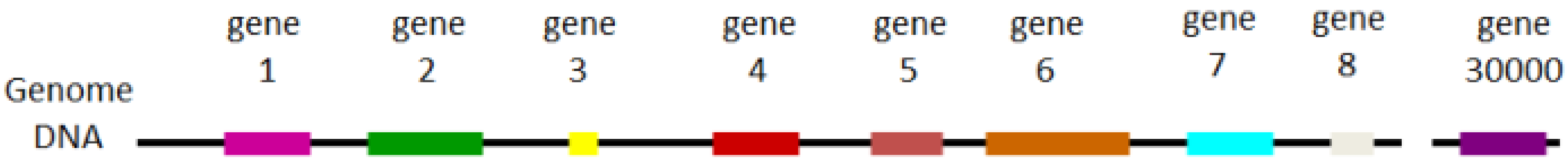
Restriction enzym...
to cut DNA a...

...ligase can be used
to glue bits of DNA together

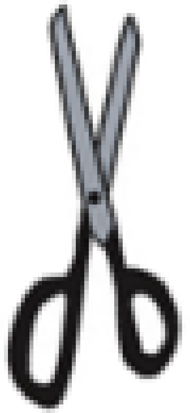
...erted into cells by chemical and electrical processes

...eering of genes = genetic engineering = recombinant DNA technology

In the example, the human insulin gene was "cloned" into yeast.
i.e. a single copy of the human insulin gene was inserted into yeast
= human gene clone in yeast



The tools



Restriction enzymes can be used to cut DNA at specific sequences

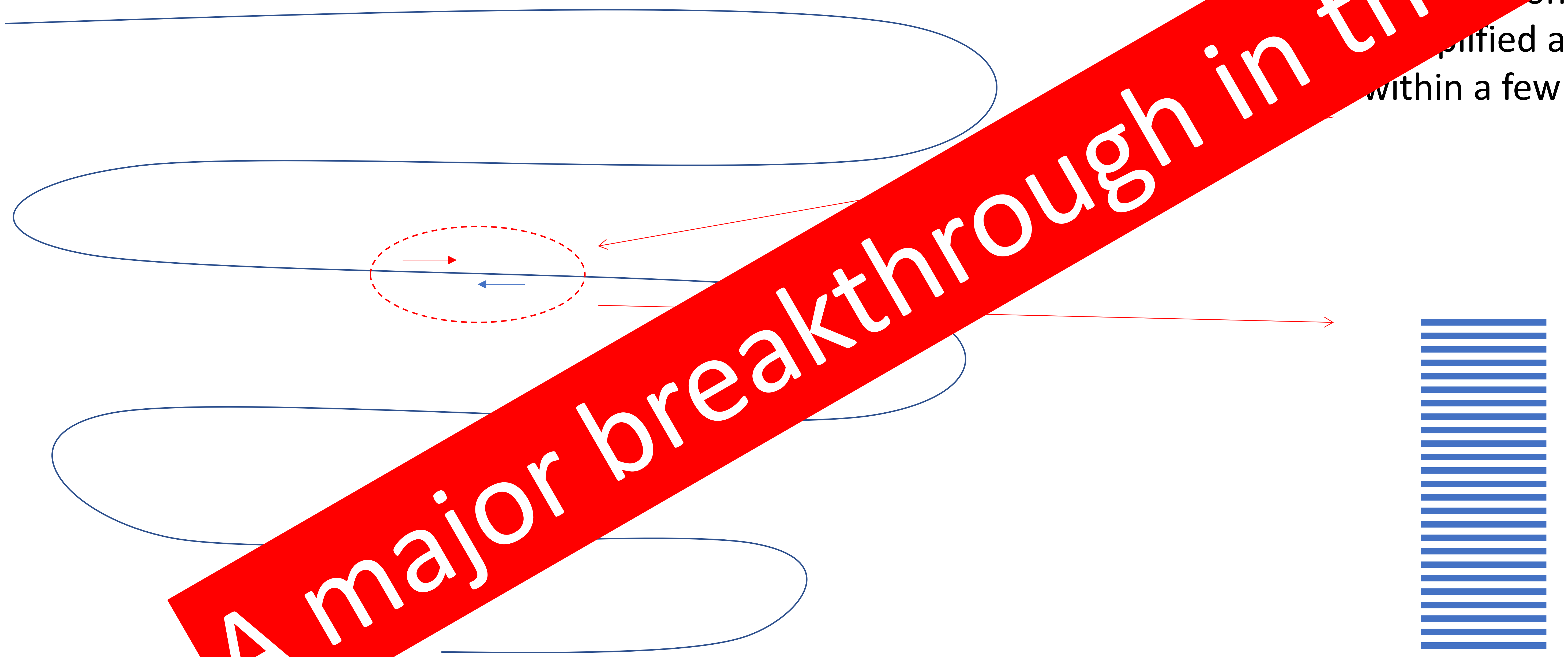
DNA ligase can be used to glue bits of DNA together

DNA can be inserted into cells by chemical and electrical processes

Engineering of genes = genetic engineering = recombinant DNA technology

In the example, the human insulin gene was "cloned" into yeast.
i.e. a single copy of the human insulin gene was inserted into yeast
= human gene clone in yeast

The Human Genome
3 billion base pairs of DNA

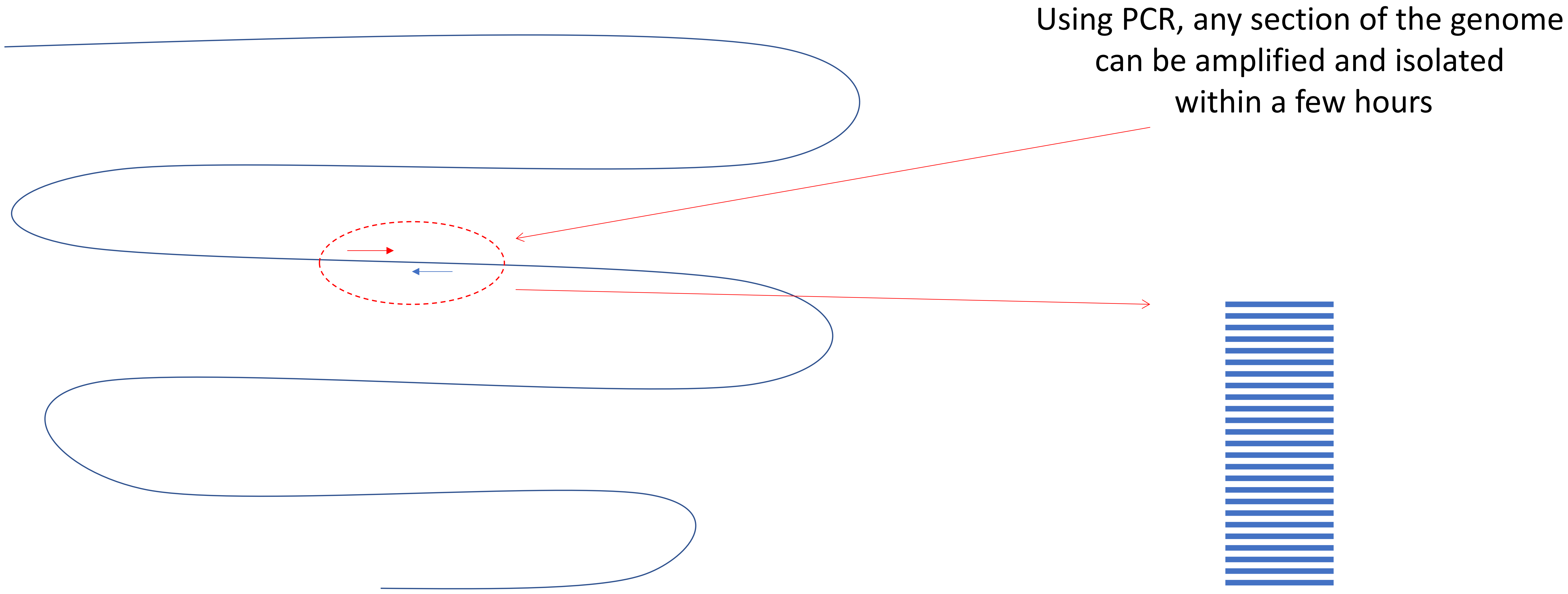


A major breakthrough in the 80s

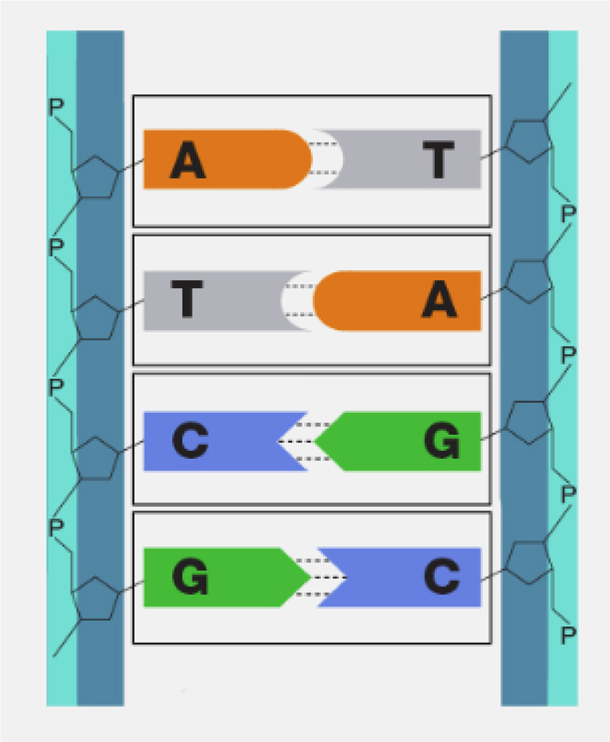
... of the genome
... and isolated
within a few hours

PCR allows amplification and isolation of any region of DNA that you desire

The Human Genome
3 billion base pairs of DNA



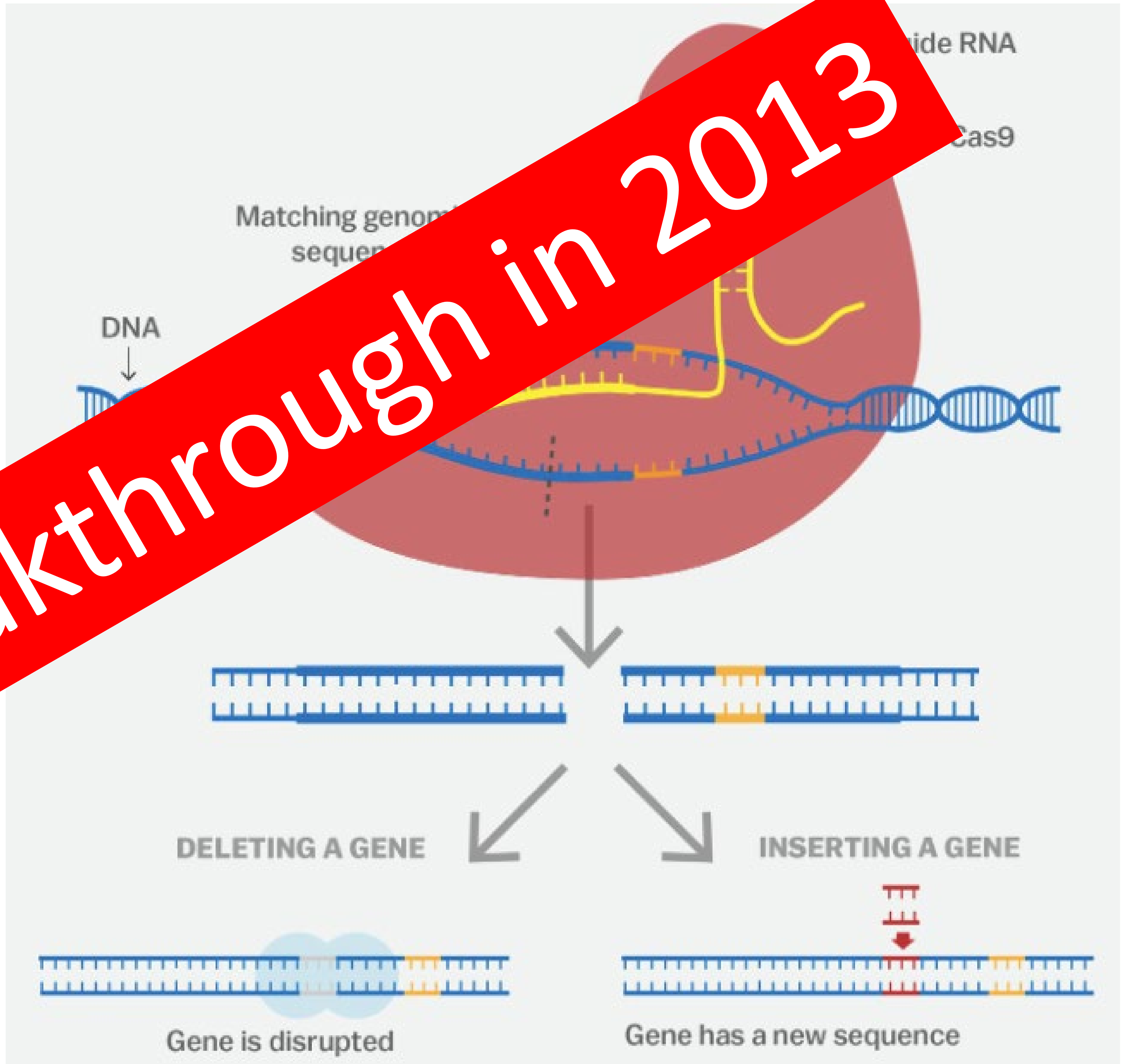
PCR allows amplification and isolation of any region of DNA that you desire



A:T

G:C

A major breakthrough in 2013



tttaaacccggg

ACGTGACGCATCGCATGACAAATTTGGGCCCGATACGCGCGACGAC

Harry Potter and The Sorcerer's Stone

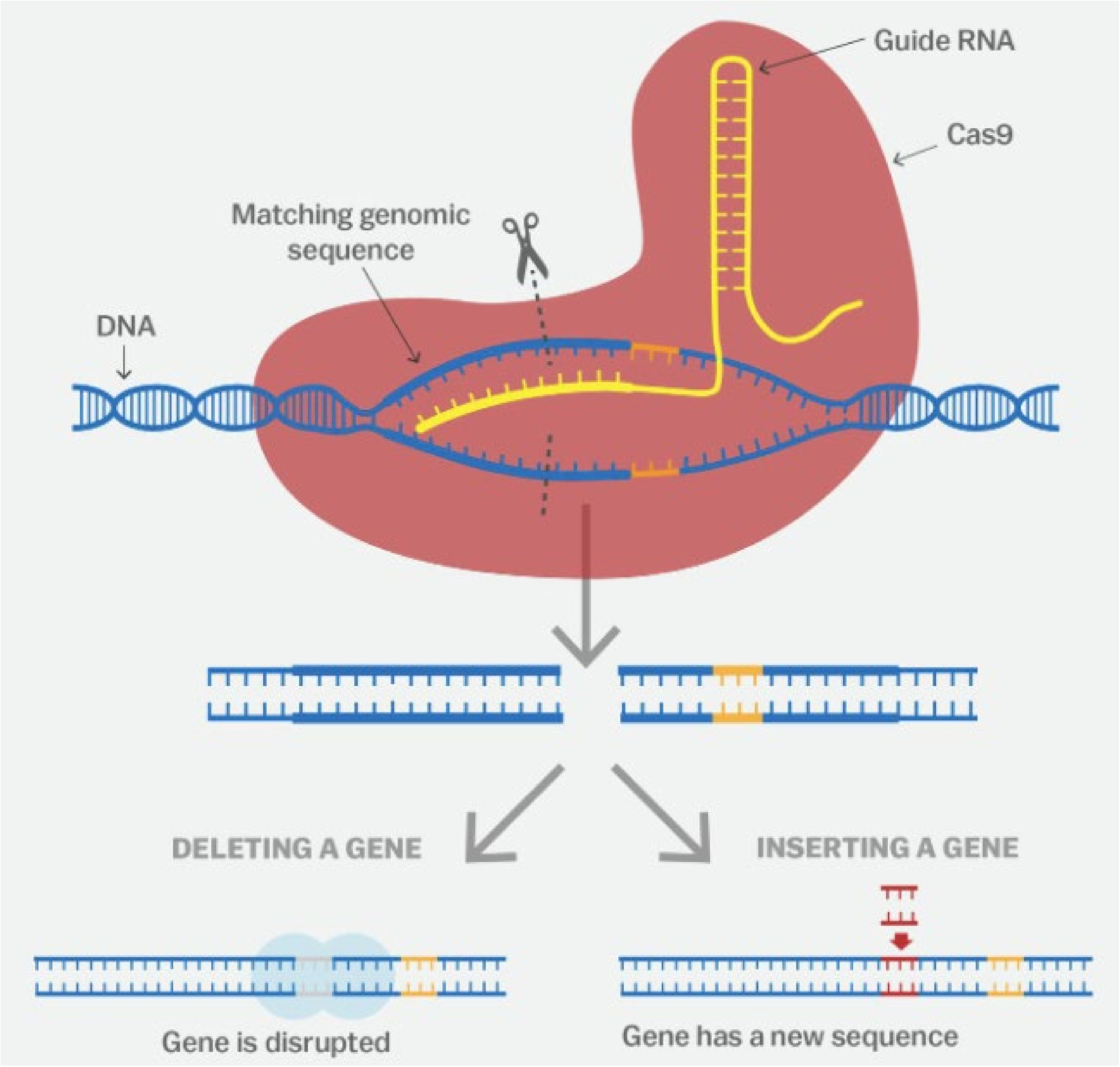
Nearly ten years had passed since the Dursleys had woken up to find their nephew on the front step, but Privet Drive had hardly changed at all. The sun rose on the same tidy front gardens and lit up the brass number four on the Dursleys' front door; it crept into their living room, which was almost exactly the same as it had been on the night when Mr. Dursley had seen that fateful news report about the owls.

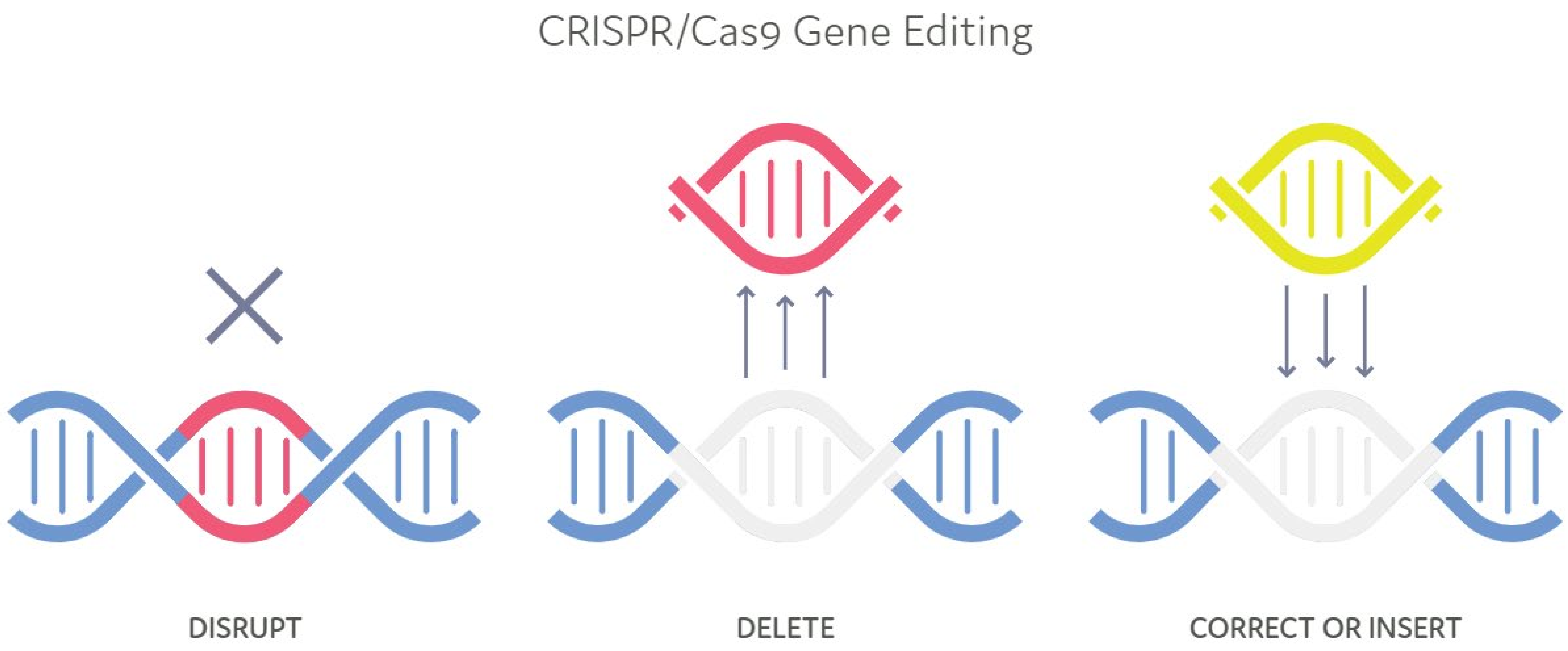
brass number four

312 pages

aaatttgggccc

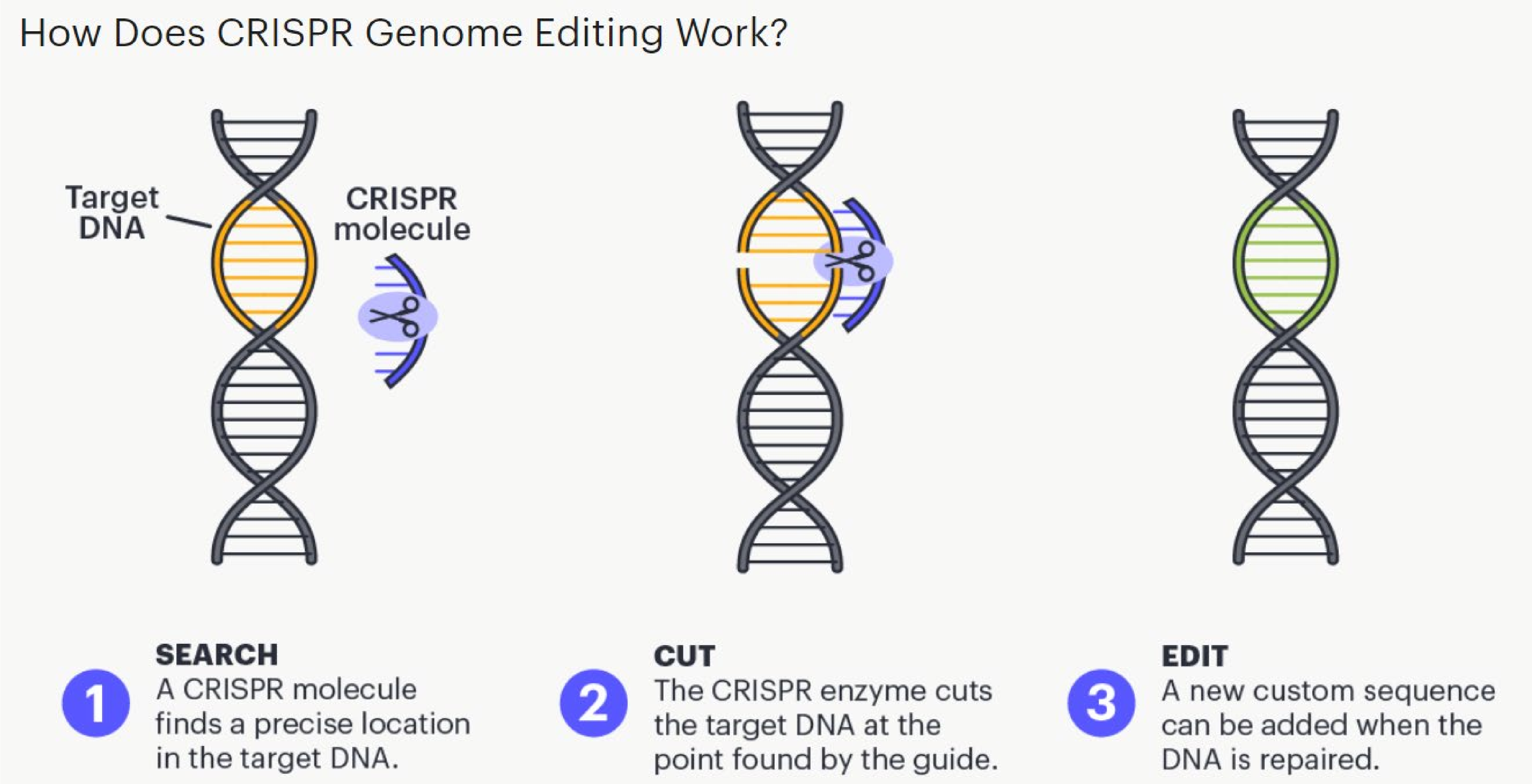
CATCGCATGACAAATTTGGGCCCGATACGCGCGA





The CRISPR system disruption, deletion, correction or insertion of any DNA sequence in the genome

The CRISPR system enables cutting of DNA at any location desired



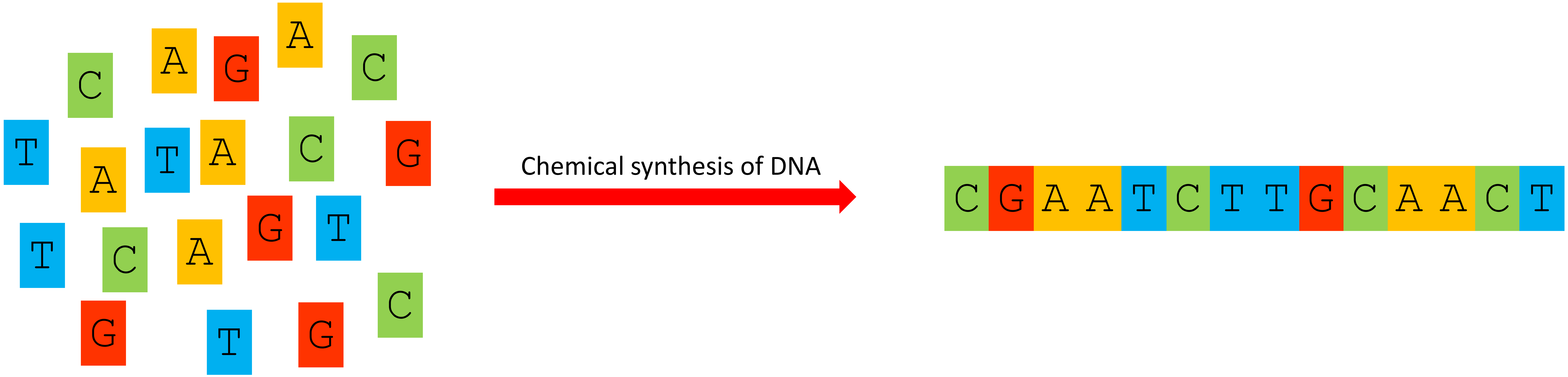
and modification of the gene at the cut site
-edit gene – remove gene - or add new gene-

THE HUMAN GENOME

At your fingertips!

Plus 40,000 other genomes!

Strings of DNA can be synthesised chemically



Genetic engineering allows

- **cloning** of genes
- **over production of proteins** encoded by genes
- **alteration of genes** as desired
- **gluing different bits of genes together** to make new proteins
- **removing existing** genes from organisms
- **introduction of new genes** into organisms
- **modification of genes** in organisms

In a nutshell - Genetic engineering was developed by scientists through the following discoveries

1. how to **isolate** DNA

2. how to **put DNA molecules back** into an organism
especially through the use of naturally occurring
small circular DNA molecules found in bacteria called **plasmids**

3. how to **grow** numerous copies of a plasmid
in bacteria and then isolate it from the bacteria

4. how to **cut** DNA at specific sequences

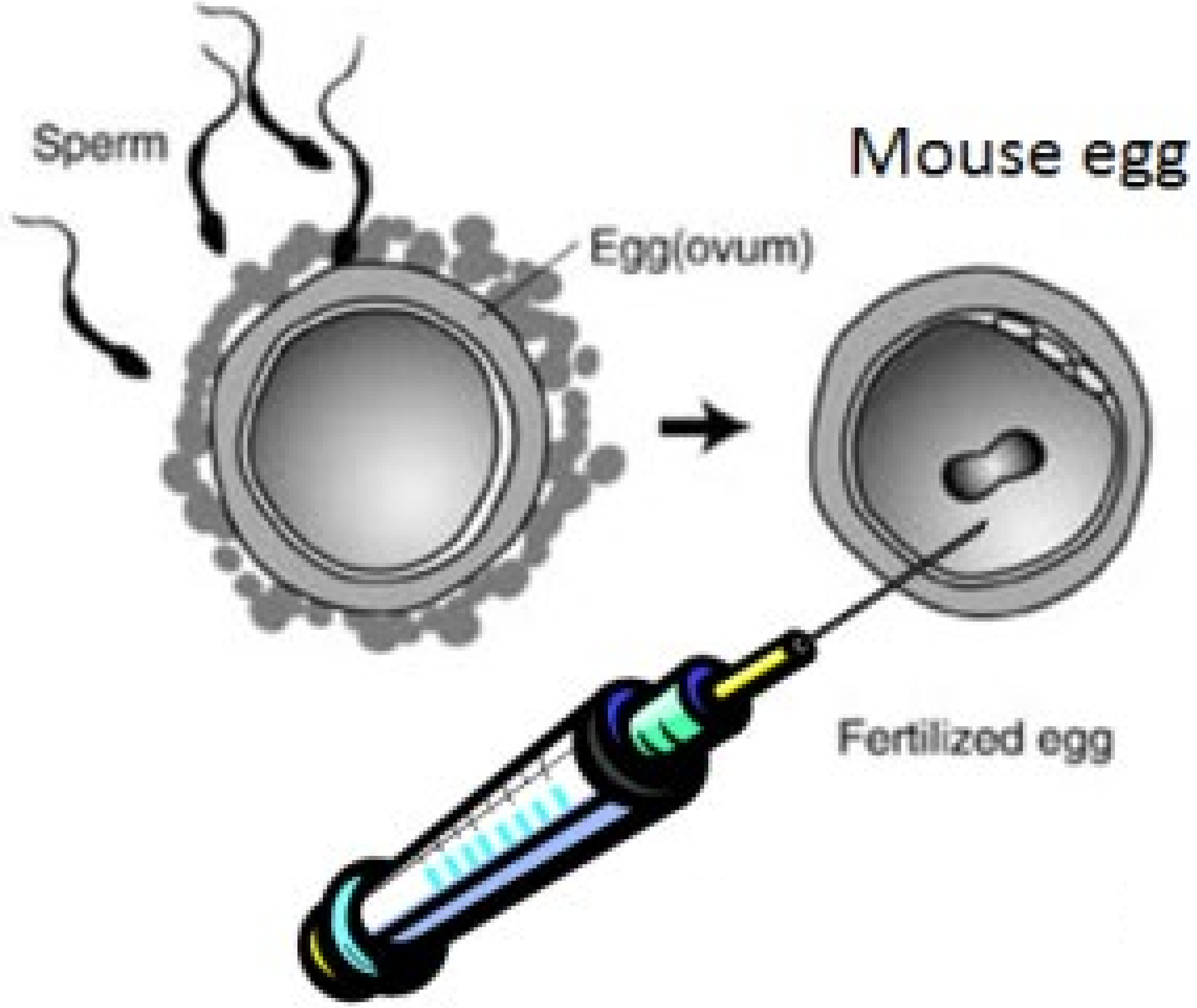
5. how to **glue** bits of DNA together

6. how to **chemically synthesise** DNA

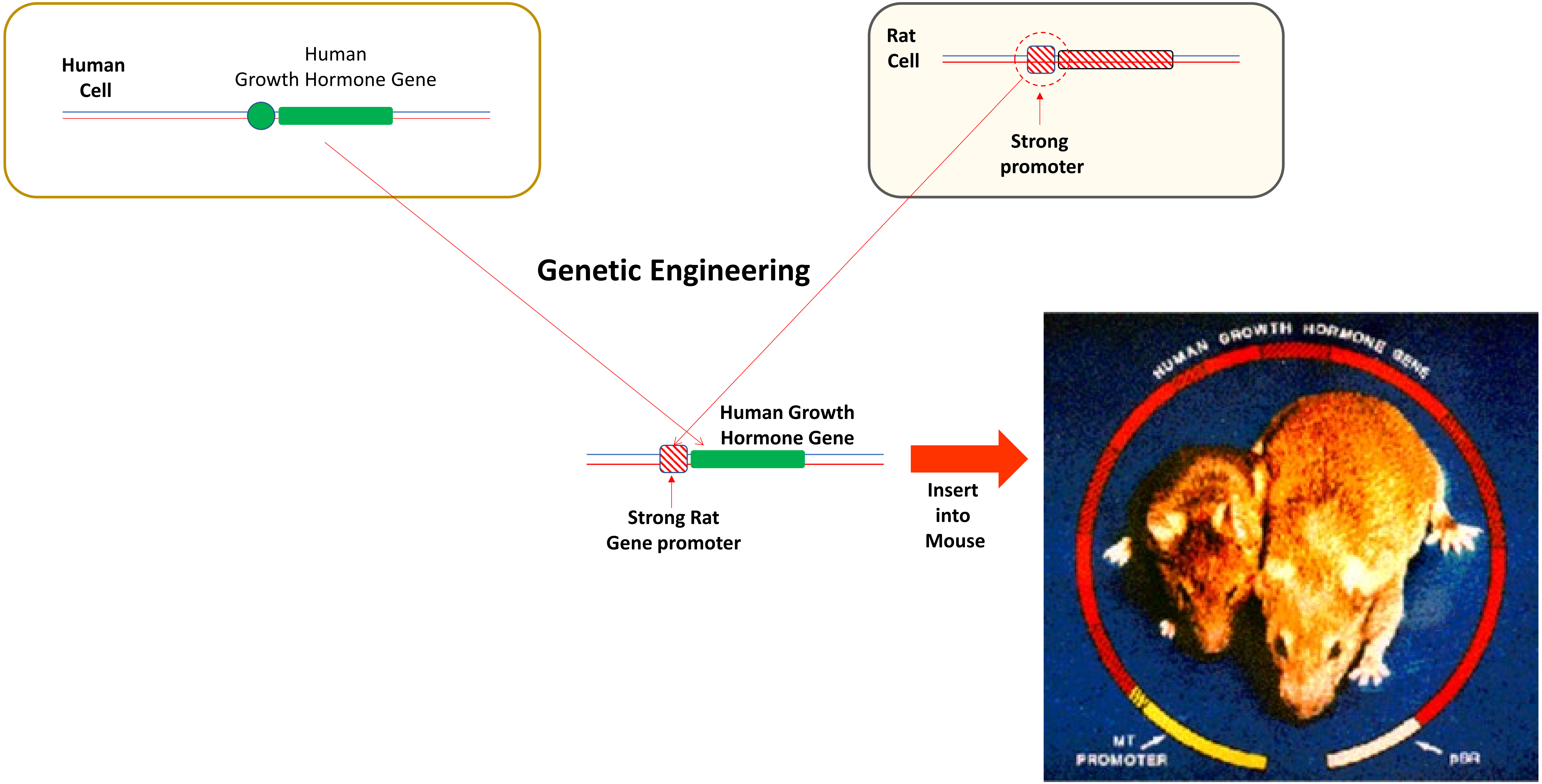
7. how to **sequence** DNA

8. how to **modify** any piece of DNA

9. how to **amplify** any section of DNA



Implant in surrogate mother



Mouse with growth hormone grew as big as a rat

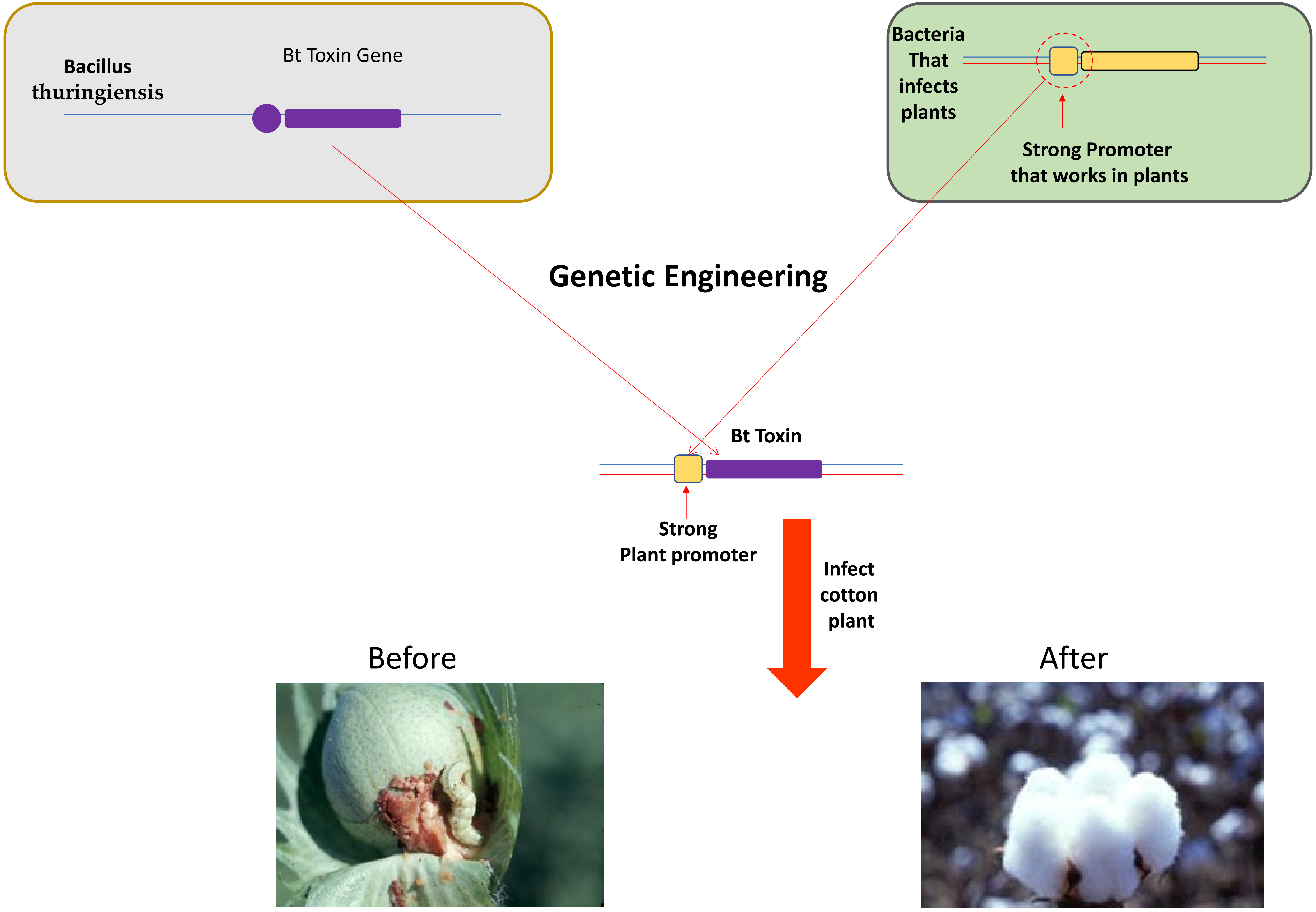
Genetic engineering of cotton to resist the bollworm

Scientists produced a genetically engineered form of cotton that is resistant to the bollworm.

This was achieved by **cloning the gene for a toxin called the Bt toxin from the soil bacterium Bacillus thuringiensis and inserting it into the cotton plant.**

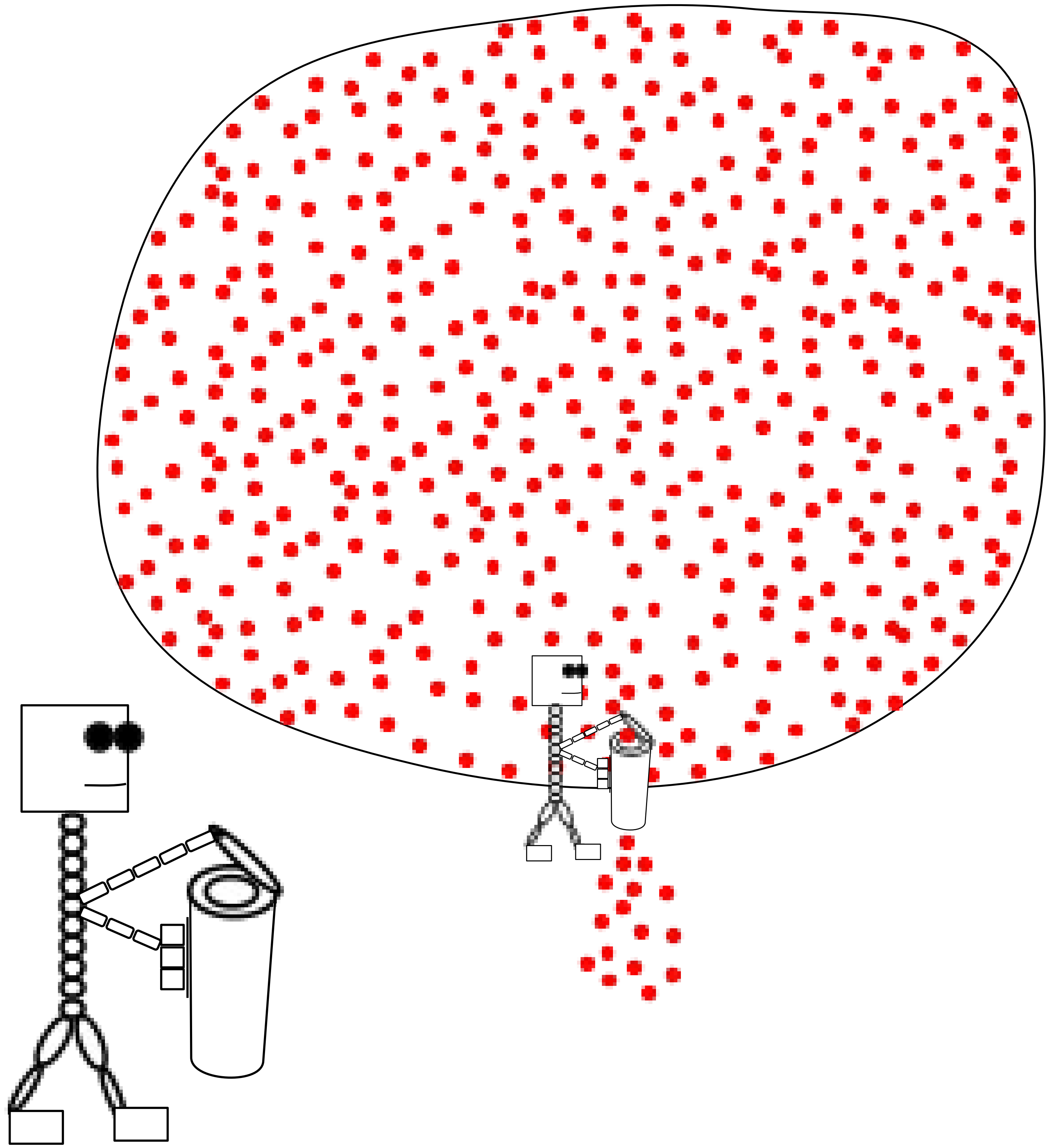
The cotton plant **produces** the Bt toxin in its leaves. Consequently when the bollworm feeds on a cotton leaf, it is **killed** by the **Bt toxin**.

Net production effect: cotton yield increase by up to **30%**


Bacillus thuringiensis
Bt Toxin Gene
Bacteria That infects plants
Strong Promoter that works in plants
Genetic Engineering
Bt Toxin
Strong Plant promoter
Infect cotton plant
Before
After

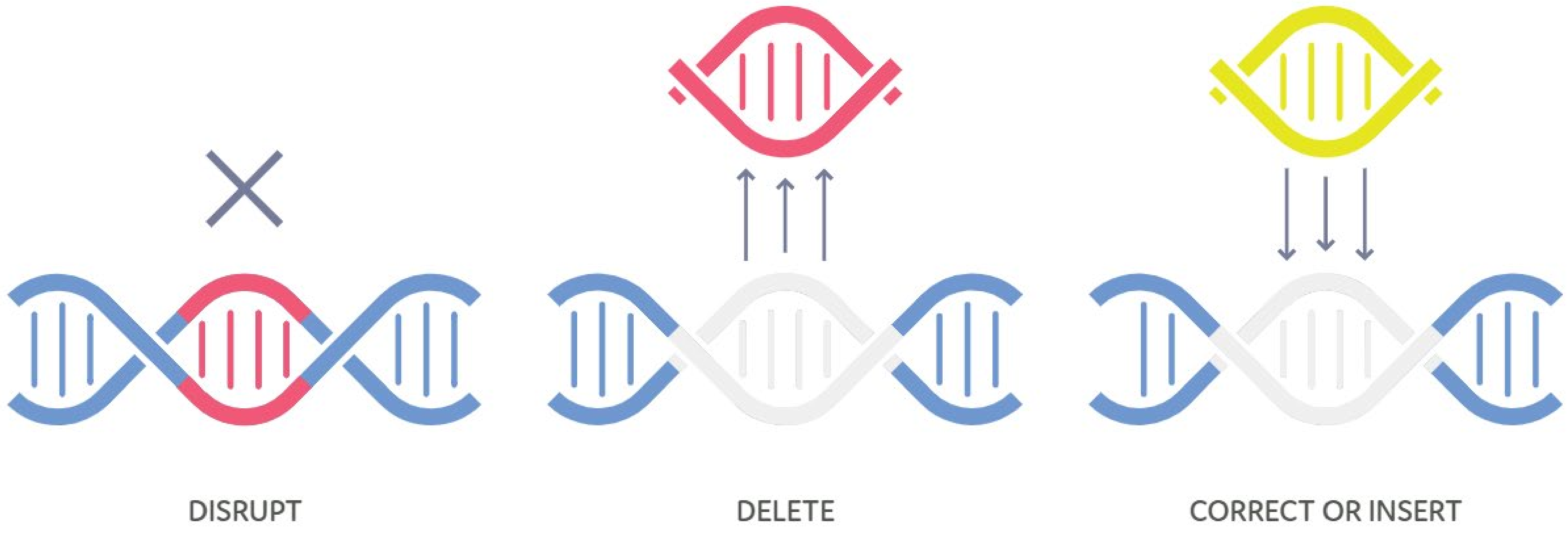
The Cystic Fibrosis Transmembrane Regulator (CFTR) protein transports chloride ions out of and into cells by transporting them across the cell membrane.

This protein is defective in cystic fibrosis due to a mutation in the CFTR gene.



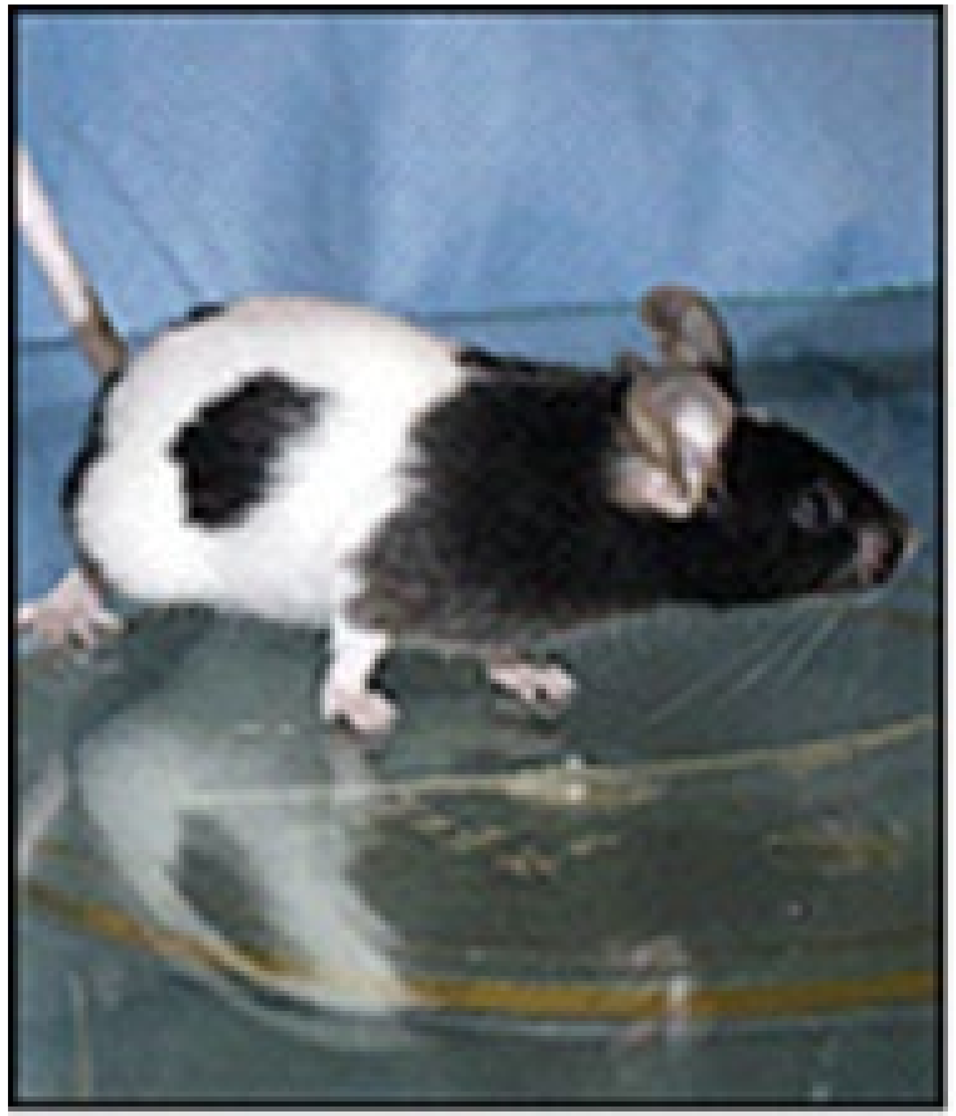
The **cystic fibrosis transmembrane regulator (CFTR)** protein
The CFTR protein is essentially a valve channel for letting chloride into and out of the cell

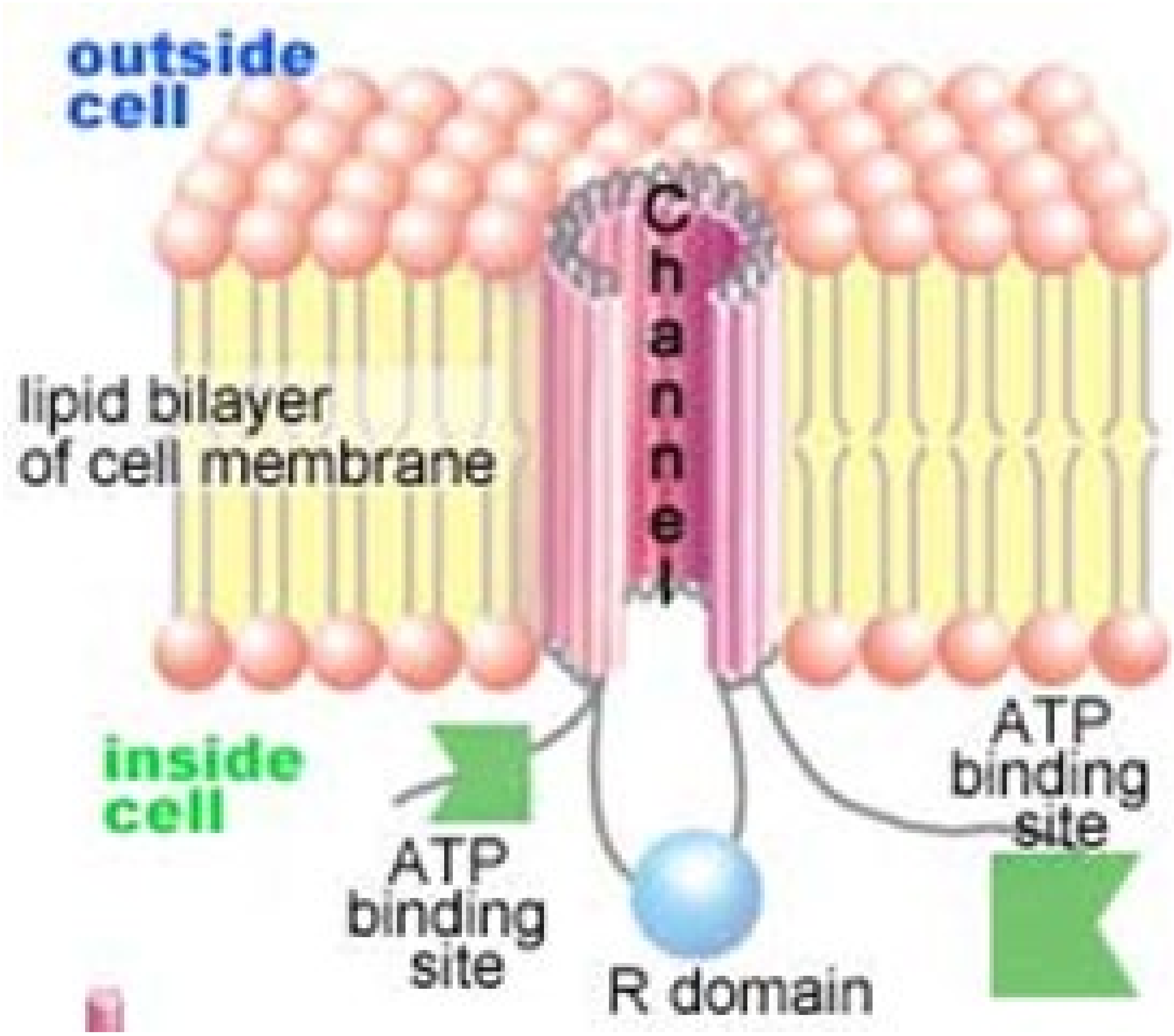
Genetic Engineering



Subtracting Genes
Creation of a cystic fibrosis mouse by genetic engineering

The Cystic Fibrosis Mouse





Deleting Genes

Creation of a cystic fibrosis mouse by genetic engineering

The Cystic Fibrosis Mouse

The CFTR gene encodes a protein stopcock nano-valve designed to let chloride ions in and out of cells.

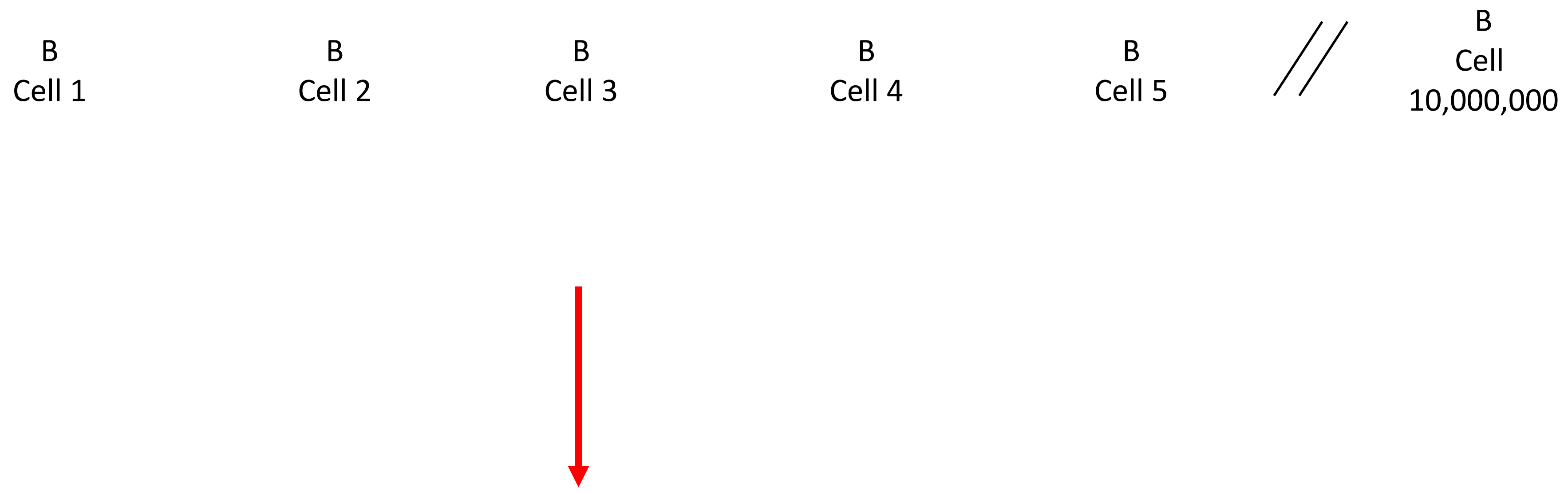
– the CFTR gene is inactive in humans with cystic fibrosis

- **Scientists inactivated the CFTR gene in a mouse by removing a bit of the CFTR gene.**

-This mouse has cystic fibrosis just like humans

-This mouse is used for **developing and testing new drugs** for cystic fibrosis and has speeded up CF therapy.

B Cell 1 | B Cell 2 | B Cell 3 | B Cell 4 | B Cell 5 // B Cell 10,000,000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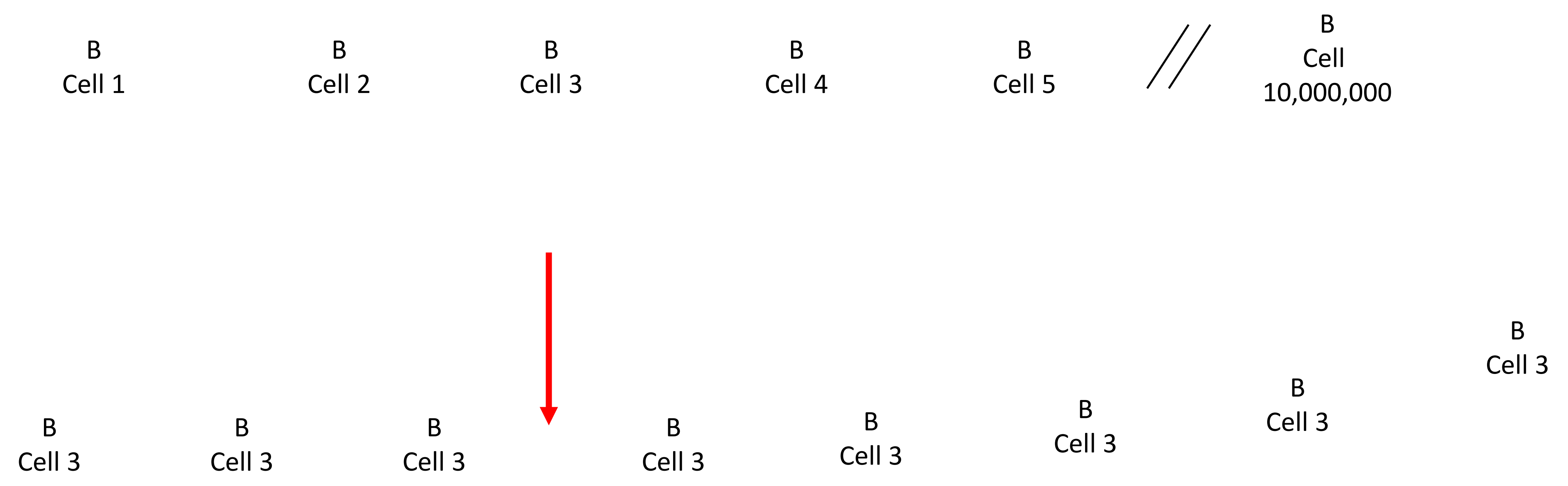
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ANTIBODY 3

ANTIBODY 3

1. Antibodies are proteins that bind to specific shapes present on a protein
2. An antibody protein will only bind to another protein if the antibody shape is complimentary to the shape on the protein
3. We have about 10 million B-cells in our blood
4. Each B-cell has a different antibody protein on its surface
5. When a foreign protein gets into our blood system, it is likely that it will have a shape complimentary to one on the antibodies on a B-cell and will be bound by the "matching" antibody
6. This cause the B-cell with the matching antibody to grow and divide and produce many more of the same B-cell.
7. The B-cell mature and secrete the antibodies into the blood
8. The secreted antibody bind to the foreign protein and directs the immune system to destroy it.

9. T-cell work is much the same way except that they recognise proteins stuck on to cells and when they bind the protein, the are activated and essentially kill the cell with the foreign protein.

Antibody drugs

1. Antibodies can be made against any protein.

Many human diseases are caused by abnormal proteins or production of too much of certain proteins.

Antibodies can be developed against any disease causing protein and can be used to stop the disease causing protein from working.

Antibody drugs are particularly effective against certain cancers and inflammatory diseases.

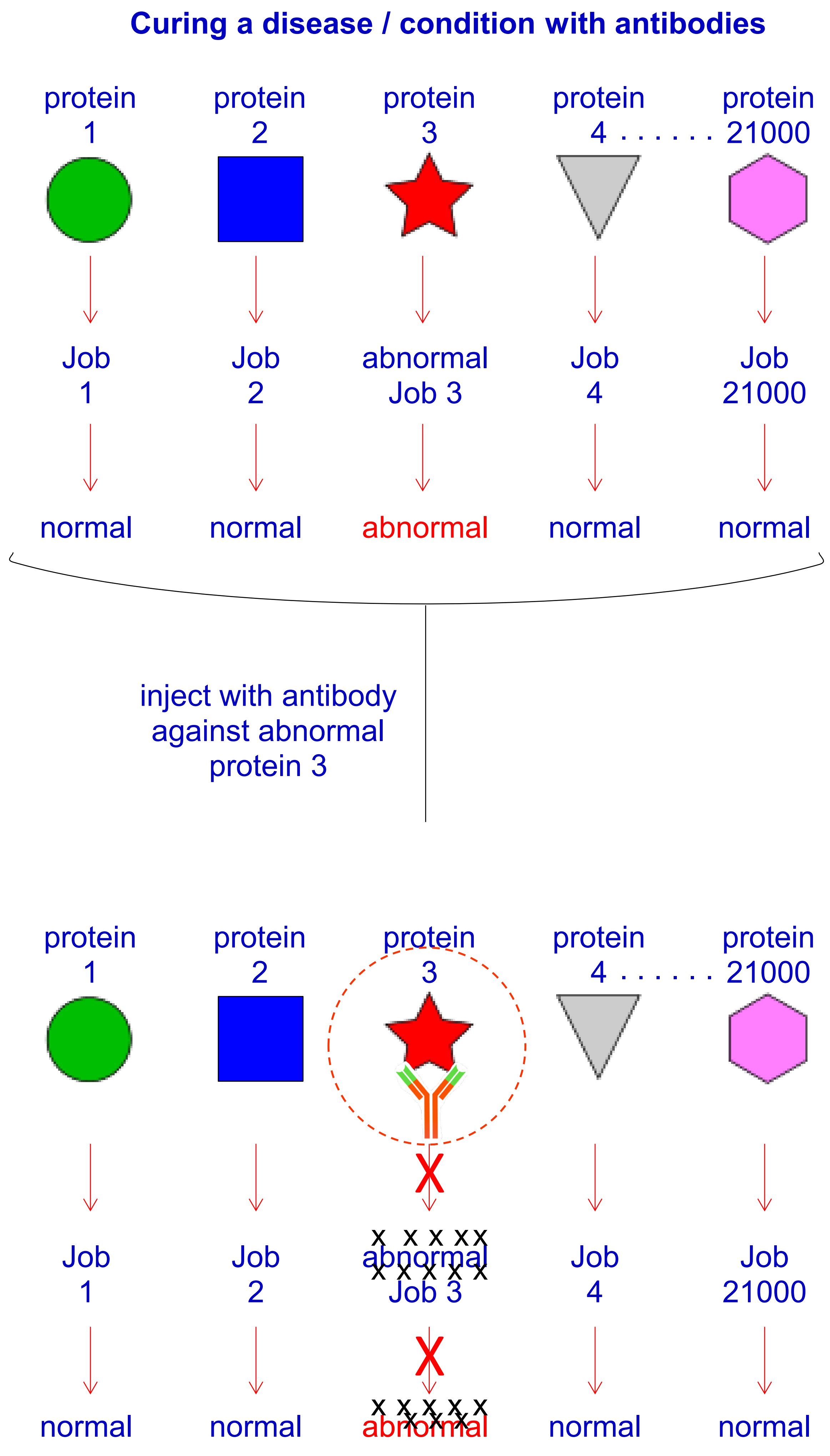
Antibodies are also used extensively as detection tools for detecting proteins.

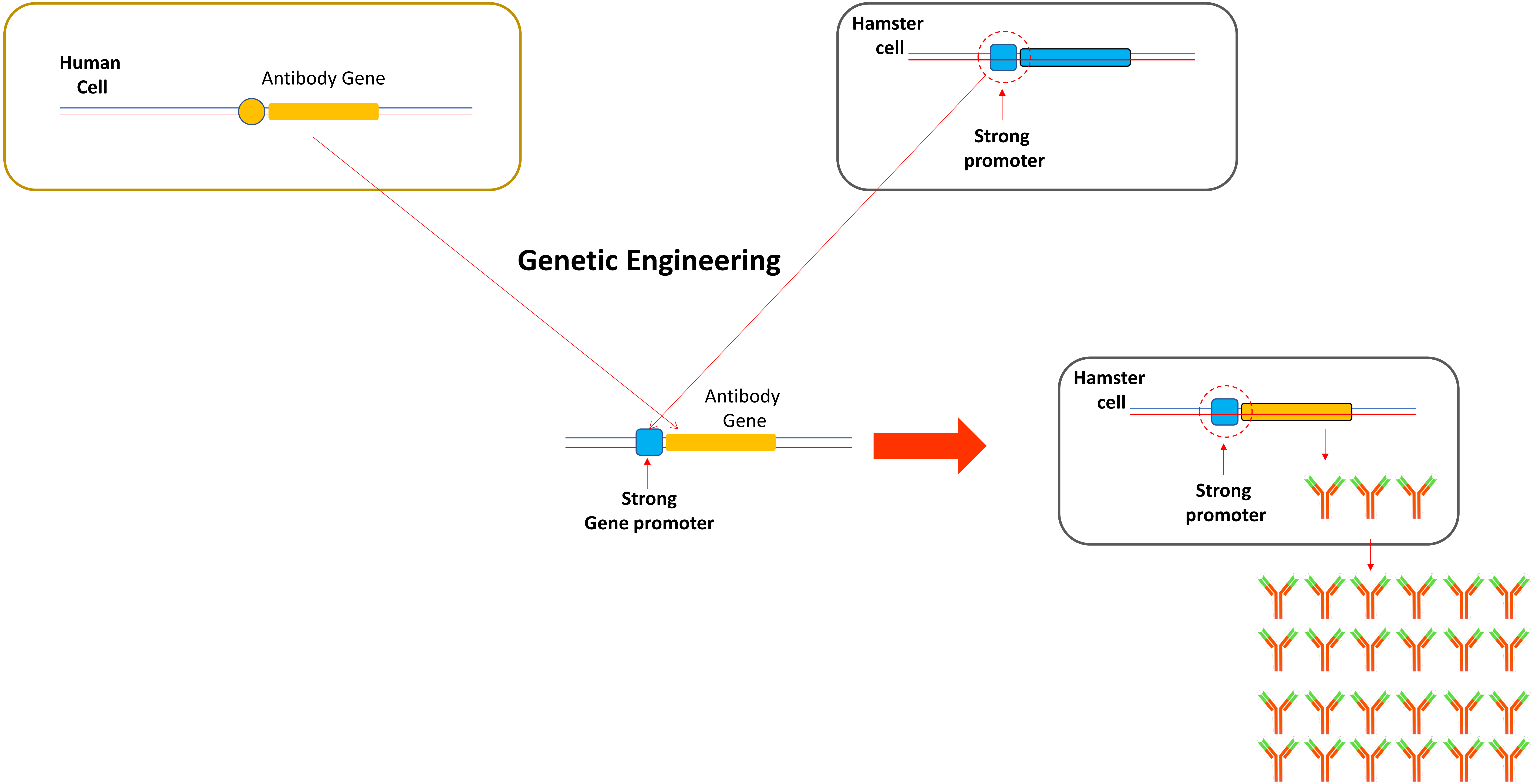
Antibody proteins can be used to treat many conditions.

Antibodies are protein produced by B-cells of the immune system.

Antibodies can bind specifically to specific proteins and stop them from working.

Humans can make up to 100 trillion different antibodies from about 500 gene segments by mixing them up and joining them together.




Human Cell
Antibody Gene
Hamster cell
Strong promoter
Genetic Engineering
Antibody Gene
Strong Gene promoter
Hamster cell
Strong promoter

Anti-TNF antibodies

When our immune system is stimulated, many processes are initiated. One of these involves production of tumour necrosis factor (TNF). TNF is a that mediates important i) responses in inflammation, ii) responses to tumours and iii) responses to infection.

TNF is a type of protein called a cytokine and actually has several biological activities including stimulation of i) cell proliferation, ii) differentiation, iii) cell death (apoptosis), iv) cell infiltration v) cachexia lipid metabolism and vi) fever.

While production of some TNF over a short period of time is good for fighting tumours and infection, prolonged production of TNF can lead to chronic inflammation which results in tissue damage.

Reducing the activity of TNF has been found to be a very good approach to reducing the symptoms of several chronic autoimmune diseases.

Companies have **developed antibodies** that can be injected into the blood where they bind and **inactivate TNF.**

Antibodies against TNF have been found to be **effective treatments** for several diseases including

1. rheumatoid arthritis
2. psoriatic arthritis (arthritis that affects up to 30% of people with psoriasis)
3. Crohn's disease
4. Ankylosing spondylitis (inflammatory disease that can cause fusion of vertebrae)

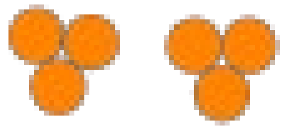
Antibody Drugs

Tumour necrosis factor (TNF) Protein

normal immune response

↓

production of TNF



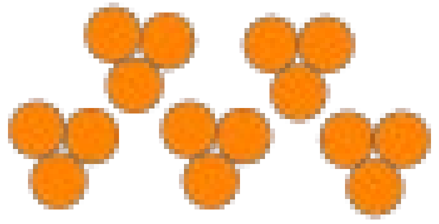
↓

inflammation
repair
recovery

abnormal immune response

↓

Increased production of TNF



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chronic inflammation

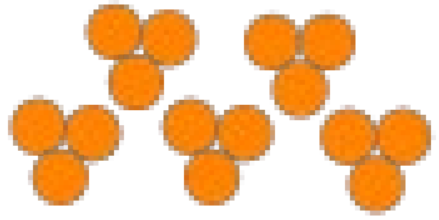
↓

chronic tissue damage

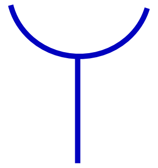
abnormal immune response

↓

Increased production of TNF



plus a limited amount of antibody that binds and inactivates TNF



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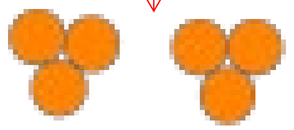
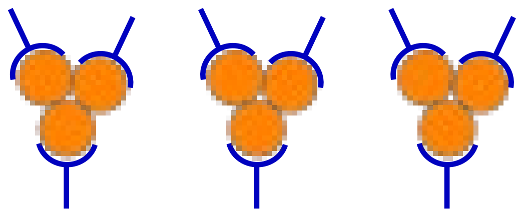




|

inflammation
repair
recovery

The **chimeric antigen receptor is a** special receptor created by genetic engineering that is designed to bind to certain proteins on cancer cells.

The chimeric antigen receptor is then added to immune cells called T cells.

This helps the T cells find and kill cancer cells that have the specific protein that the receptor is designed to bind.

These changed T cells called chimeric antigen receptor T (CAR-T) cells are then grown in large numbers in the laboratory and given to cancer patients.

Chimeric antigen receptor T cells are being developed and used in the treatment of some types of cancer.

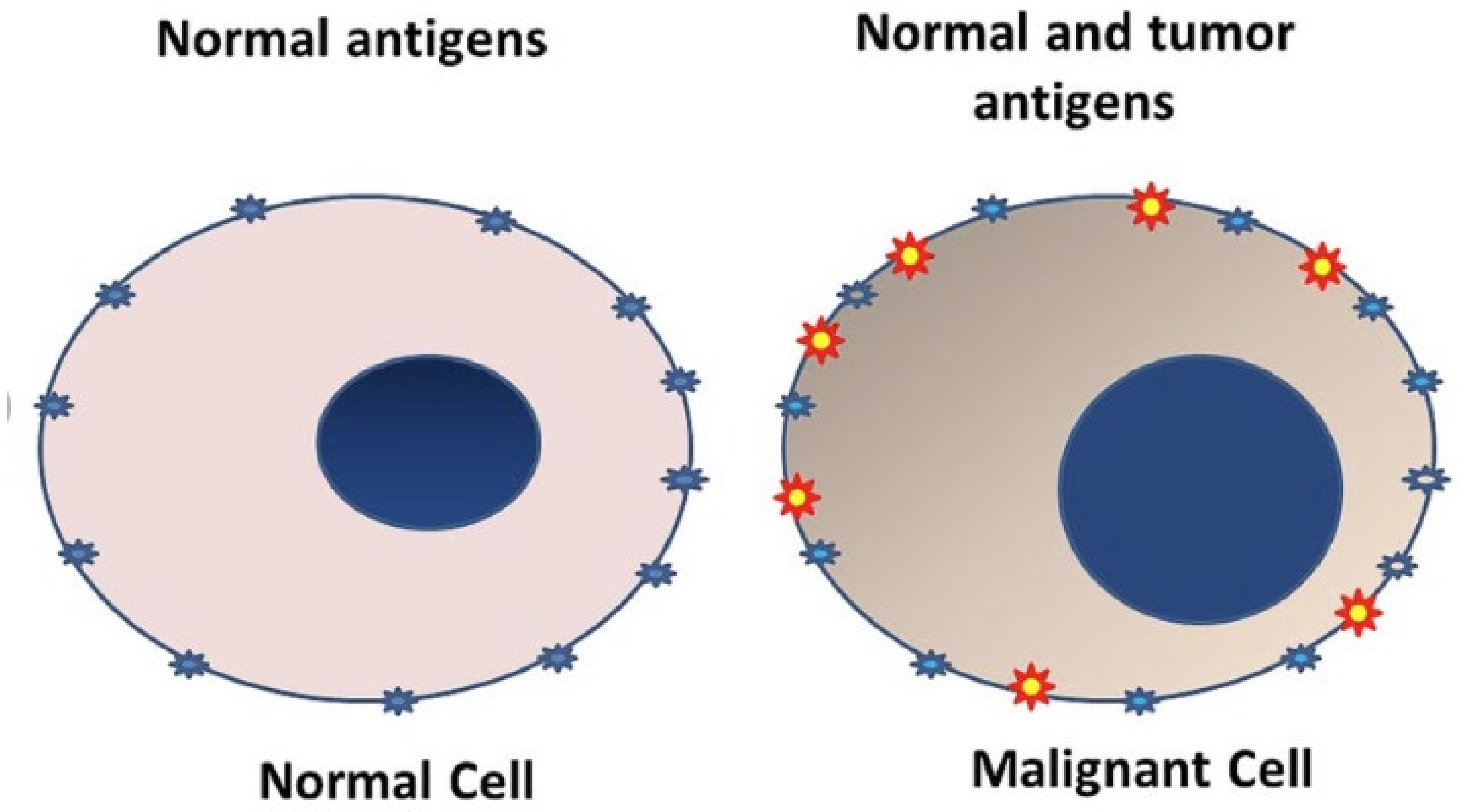
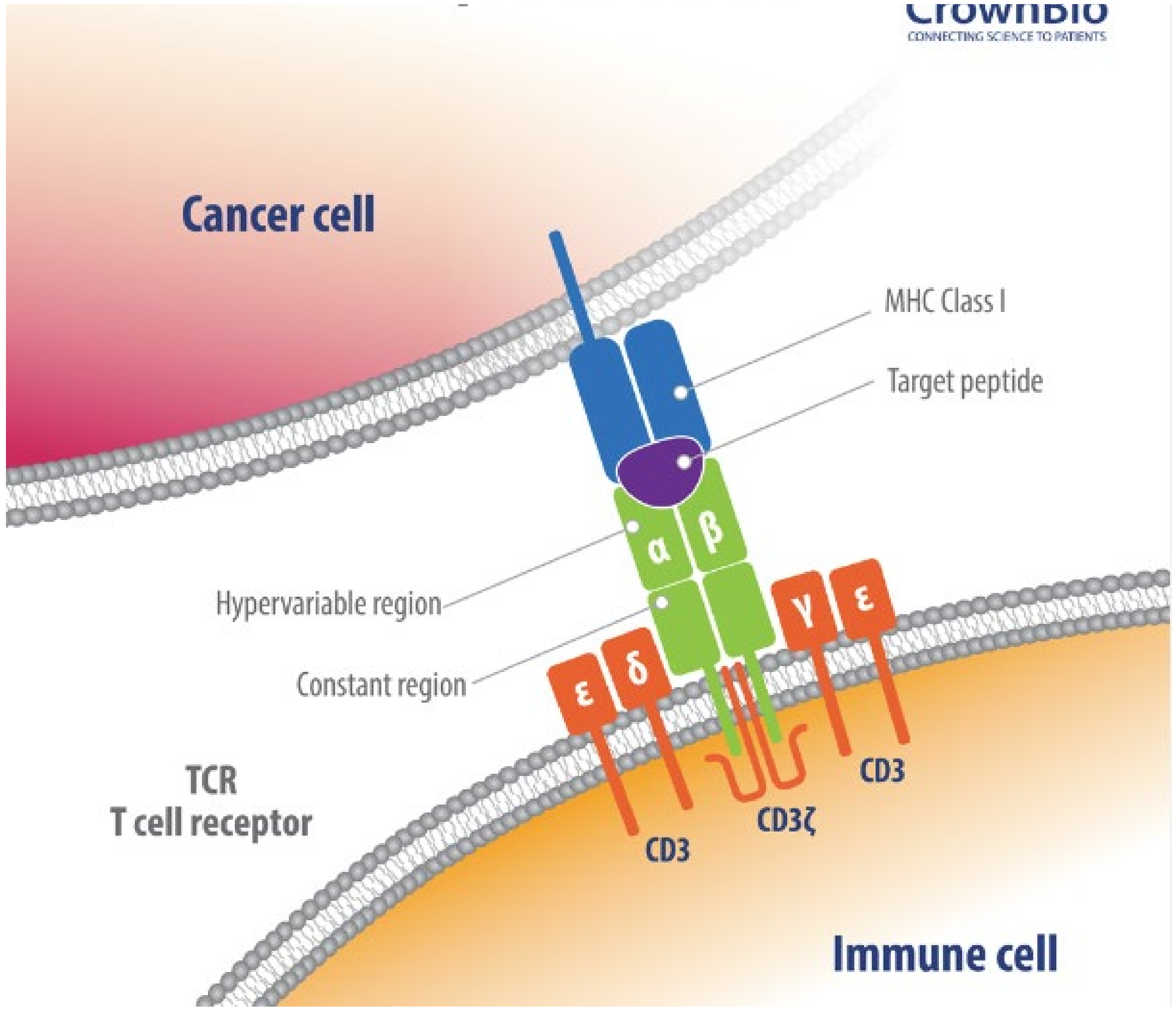
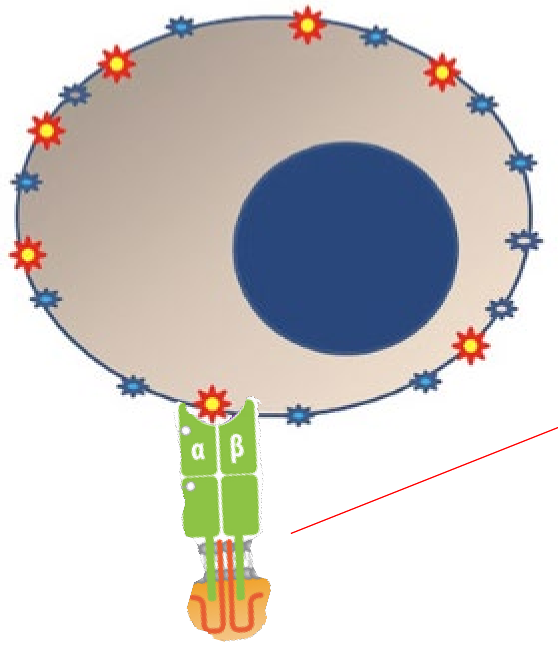
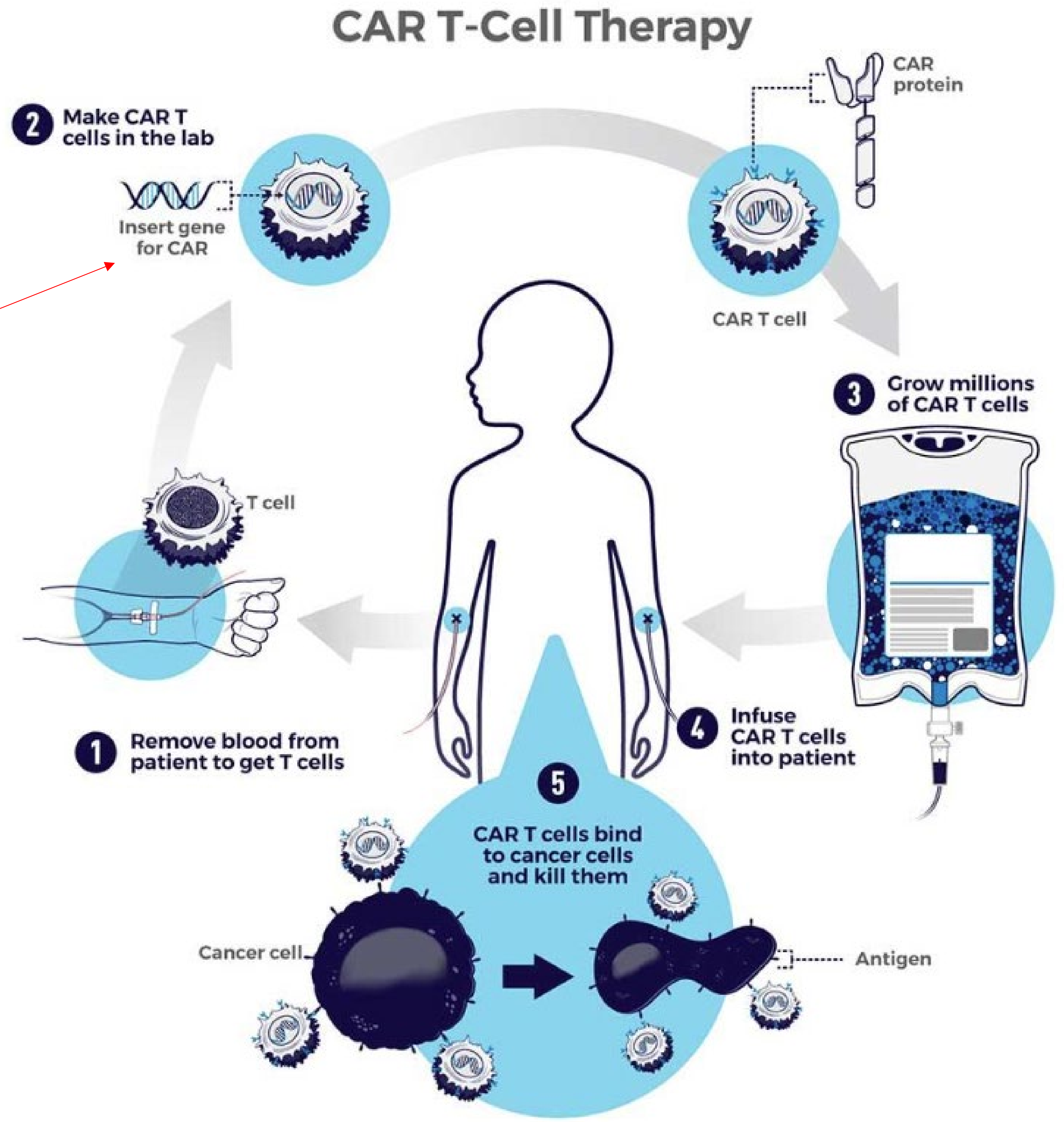


Diagram showing surface protein antigens on a normal cell, compared with a cancer cell which has the normal protein antigens as well as additional protein antigens only found on the specific cancer cells (shown as yellow-red)

T CELL RECEPTOR PROTEIN BINDING TO A PROTEIN ON A CANCER CELL

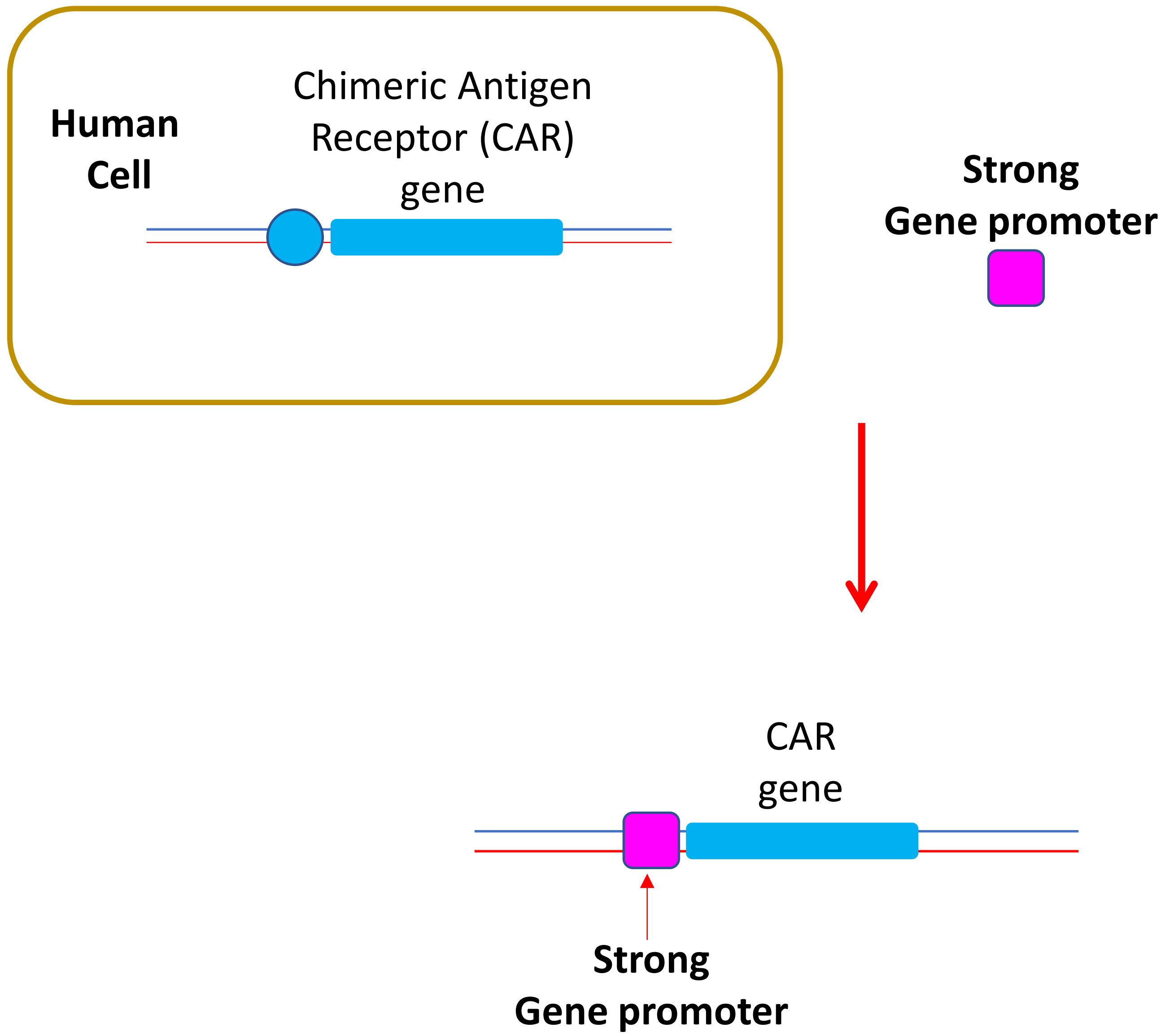


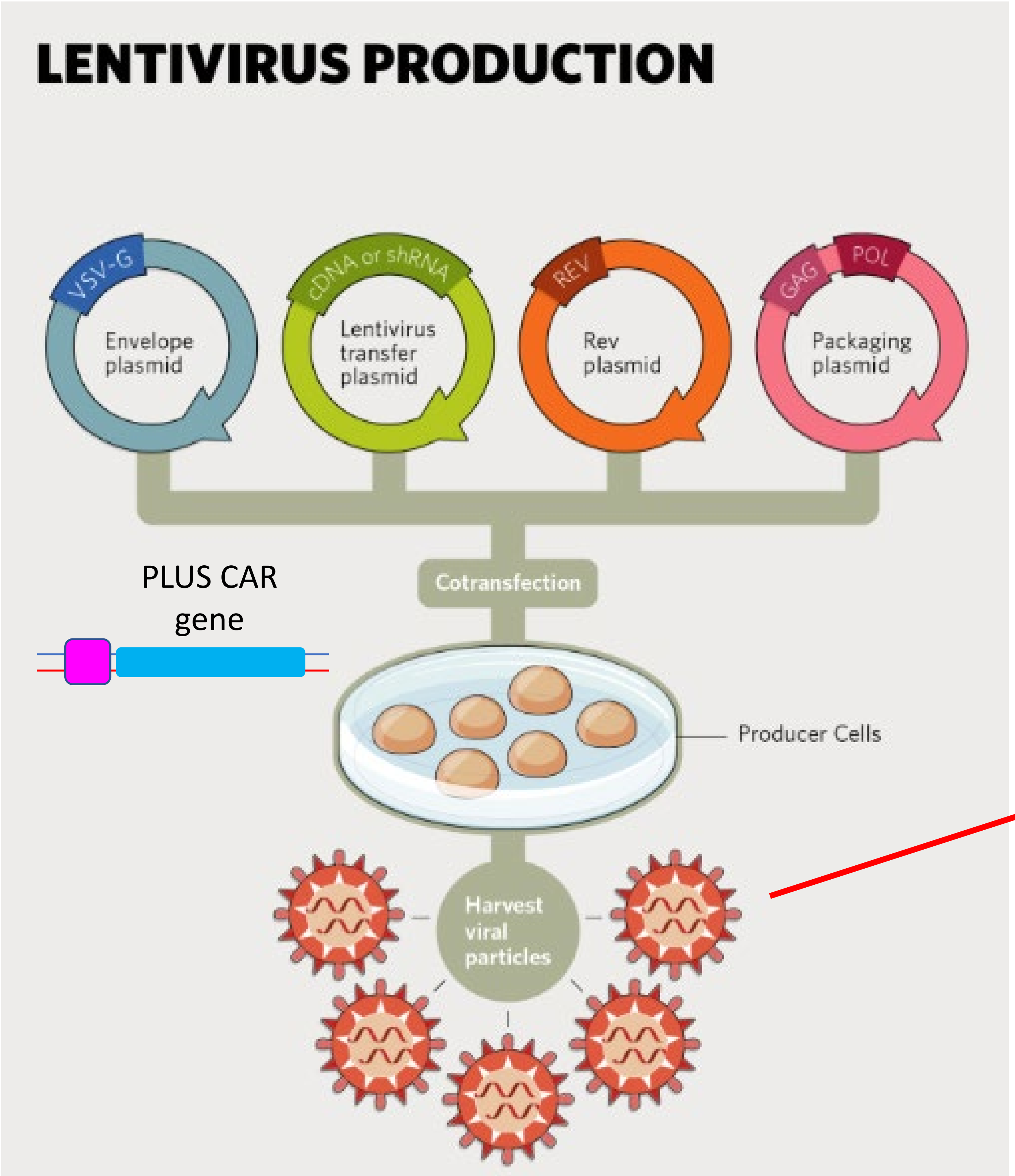


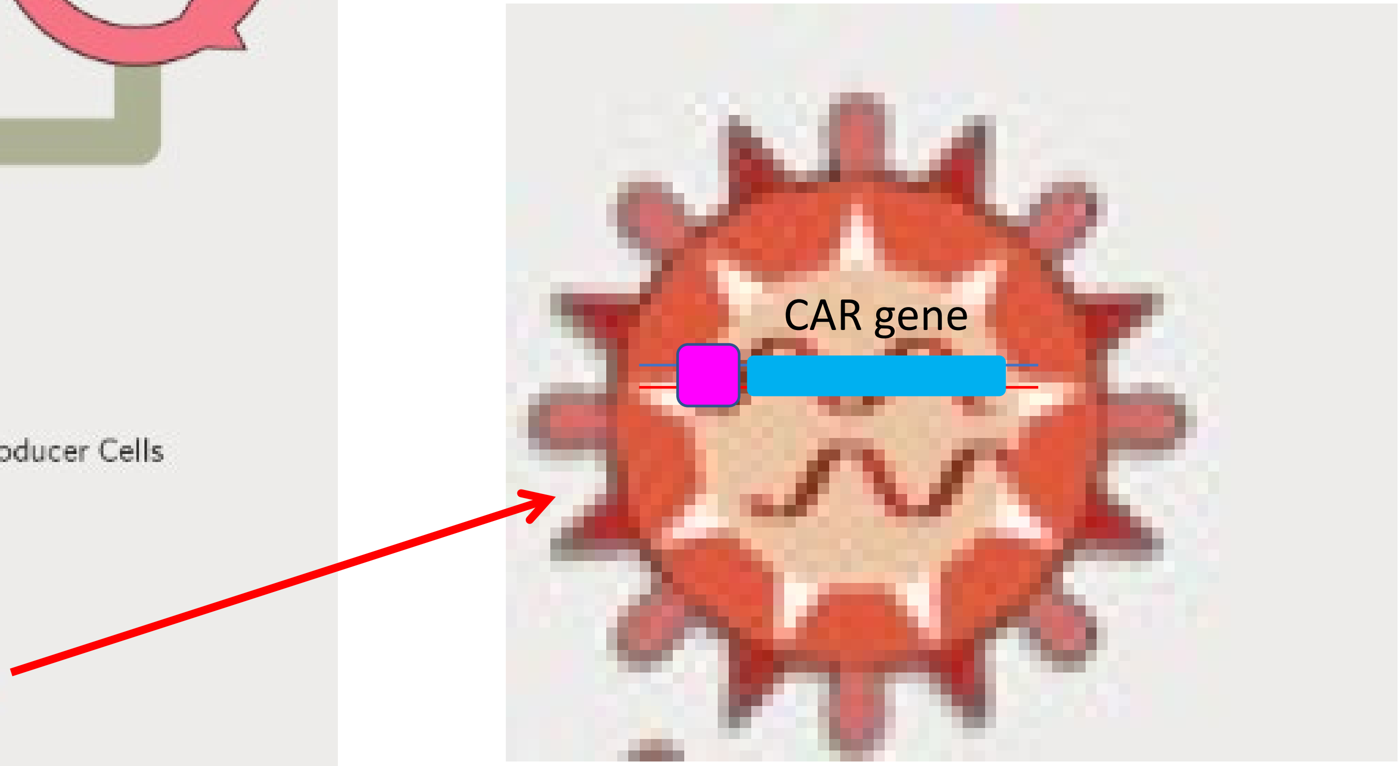


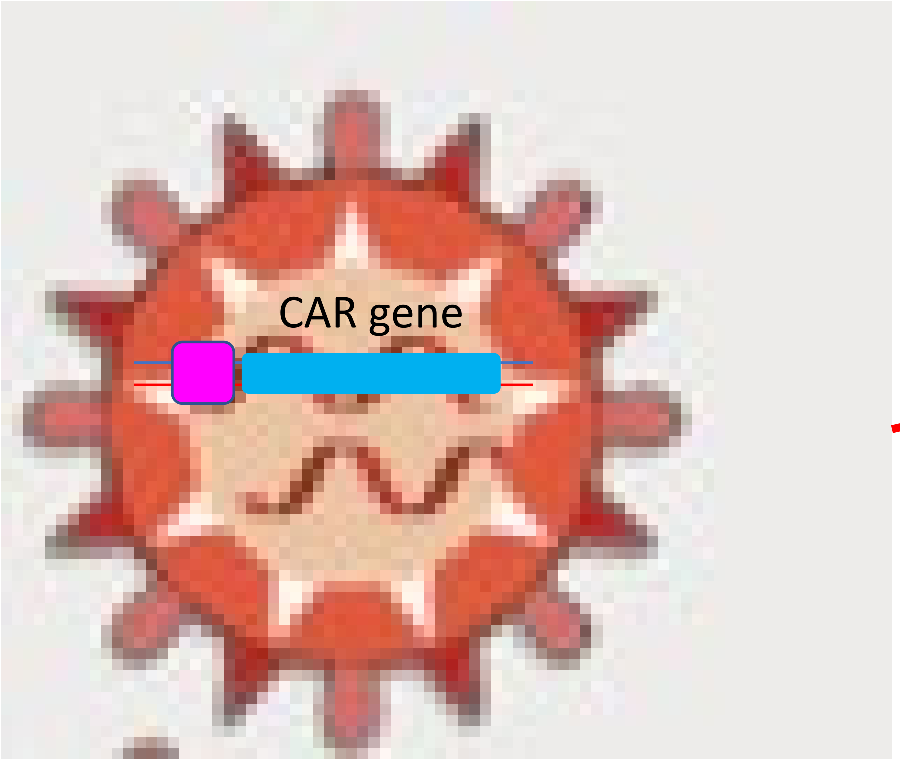
CAR T-cell therapy is a type of treatment in which a patient's T cells are genetically engineered in the laboratory so they will bind to specific proteins (antigens) on cancer cells and kill them. (1) A patient's T cells are removed from their blood. Then, (2) the gene for a special receptor called a chimeric antigen receptor (CAR) is inserted into the T cells in the laboratory. The gene encodes the engineered CAR protein that is expressed on the surface of the patient's T cells, creating a CAR T cell. (3) Millions of CAR T cells are grown in the laboratory. (4) They are then given to the patient by intravenous infusion. (5) The CAR T cells bind to antigens on the cancer cells and kill them.

Genetic Engineering

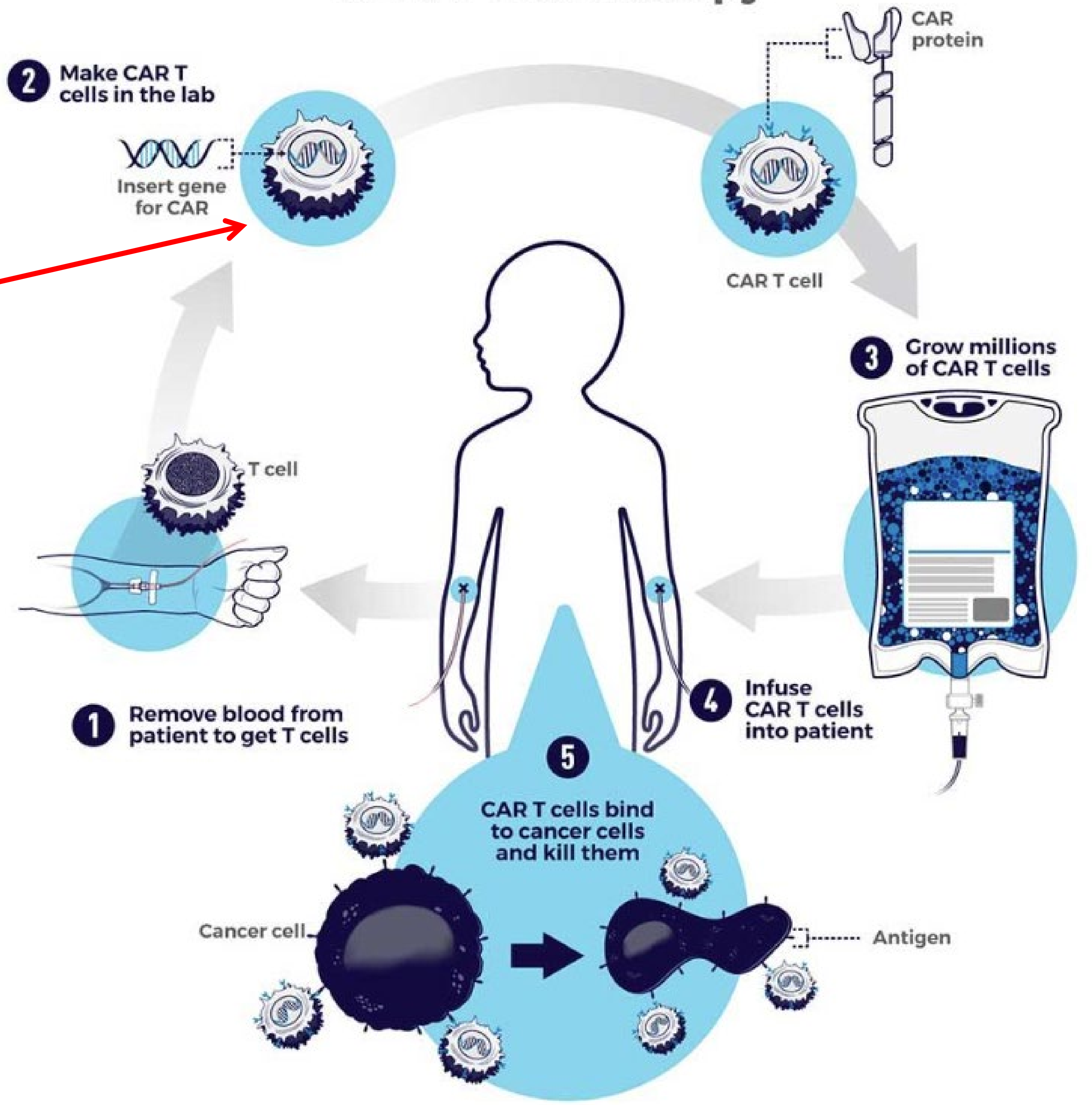








CAR T-Cell Therapy



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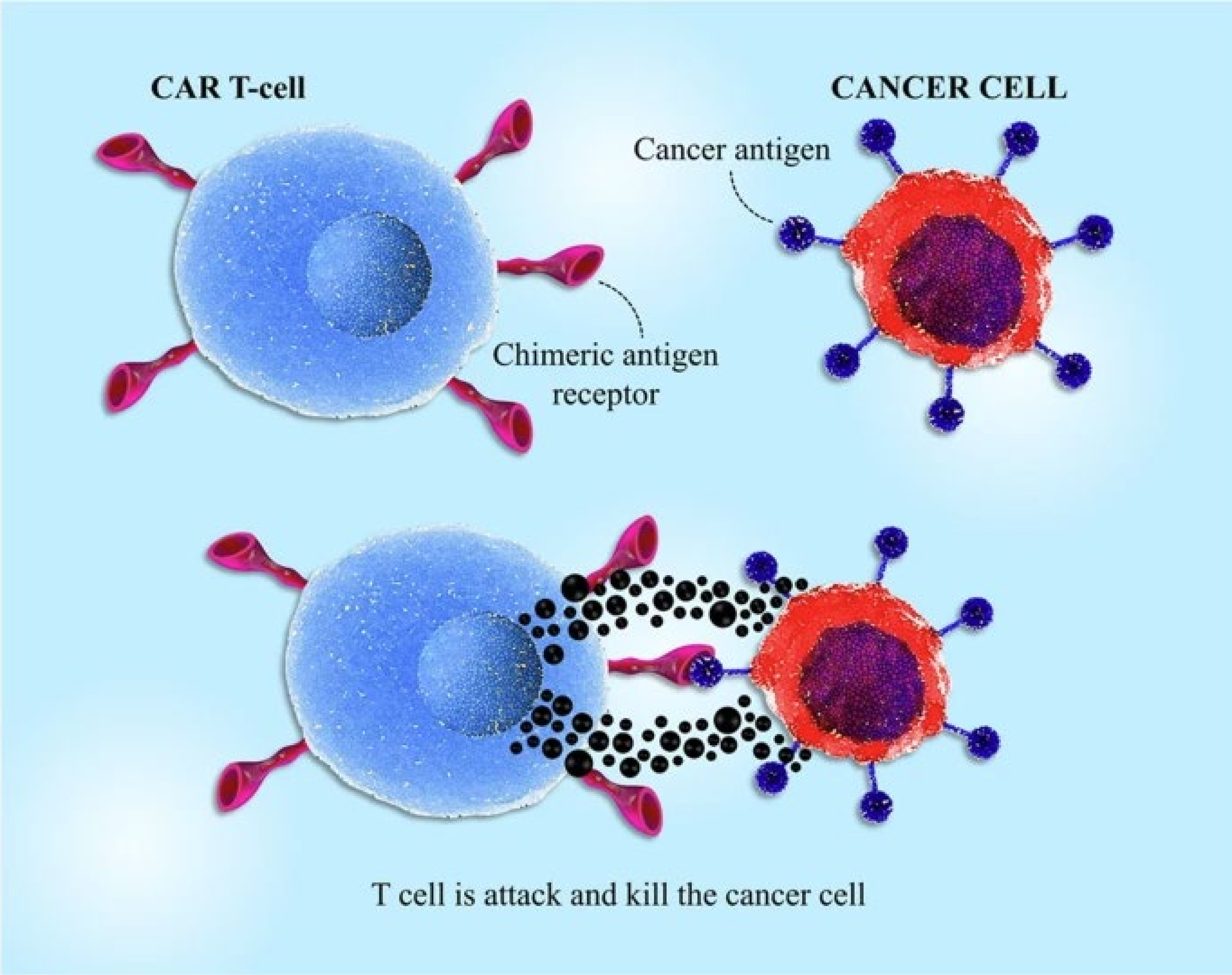


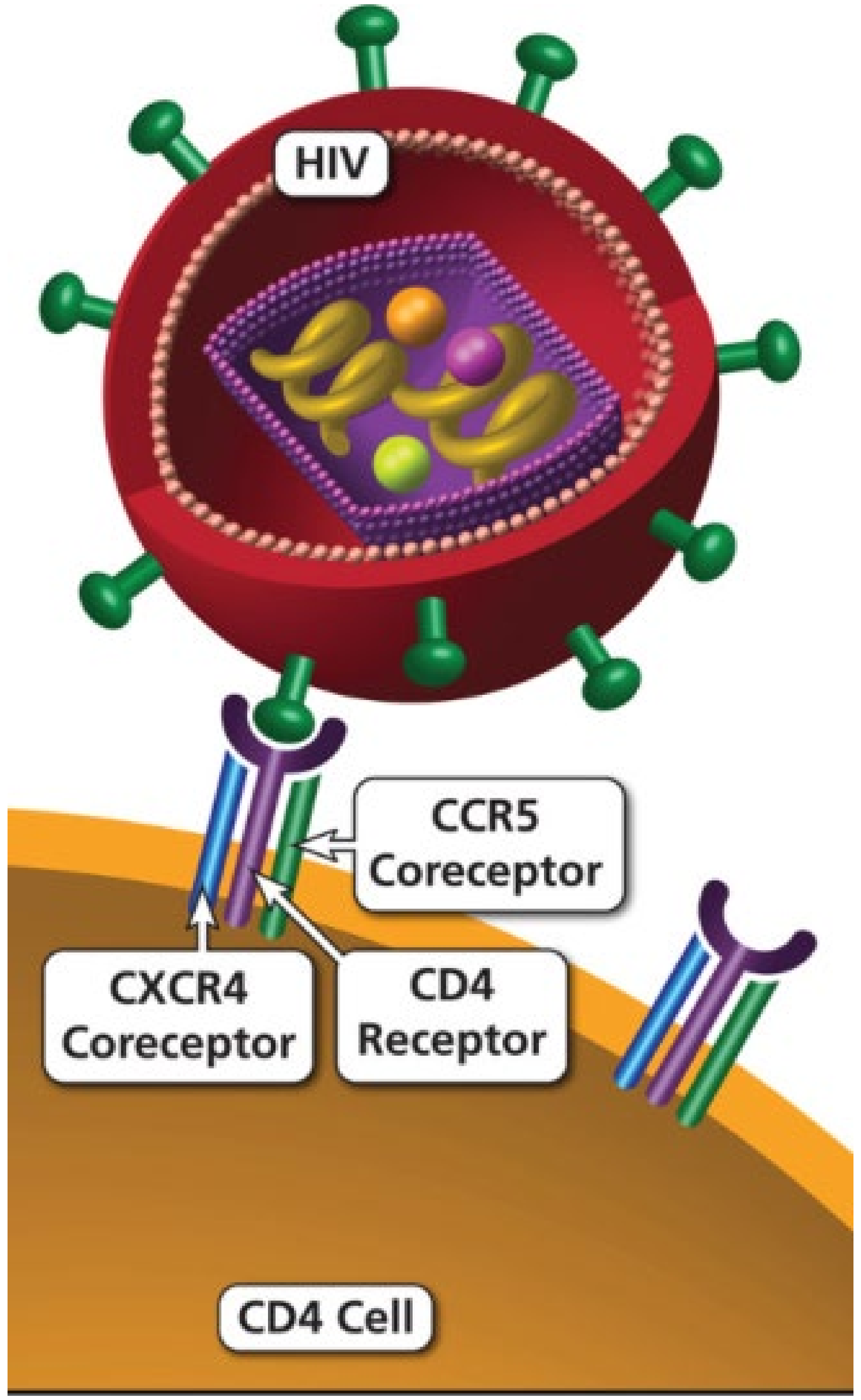
The genetically engineered chimeric antigen receptor (CAR) protein specifically recognises a protein antigen found on a specific cancer but not found on normal cells.
One end of the CAR protein is embedded in the T-cell and the other end recognises the cancer protein antigen. The CAR-T cells circulate throughout the body and when they stick to a cancer cell via the cancer protein antigen, the T-cell is activated to kill the cell to which it is attached.

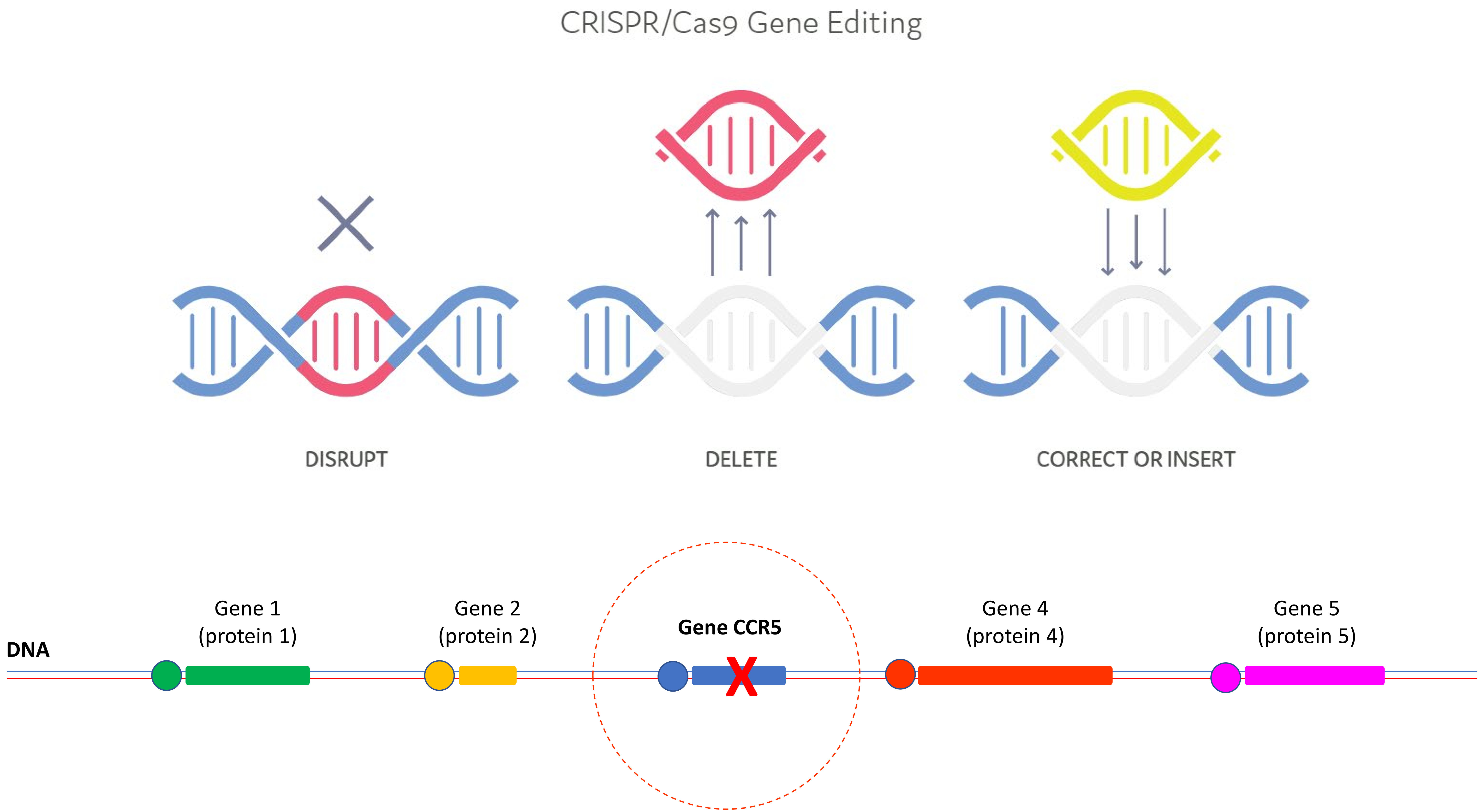
World's first gene-edited babies created in China, claims scientist

Unconfirmed scientific breakthrough sparks ethical and moral concerns

A scientist at work at a laboratory in Shenzhen in southern China. Many mainstream scientists have denounced the Chinese report as human experimentation. Photograph: Mark Schiefelbein/AP

A scientist in China claims to have created the world's first genetically edited babies, in a potentially ground-breaking and controversial medical first.

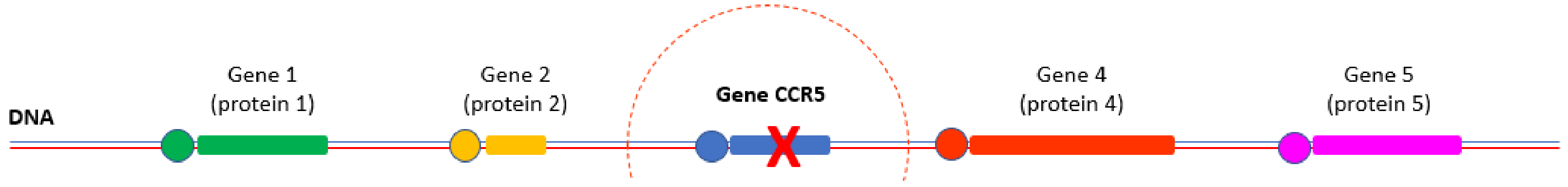


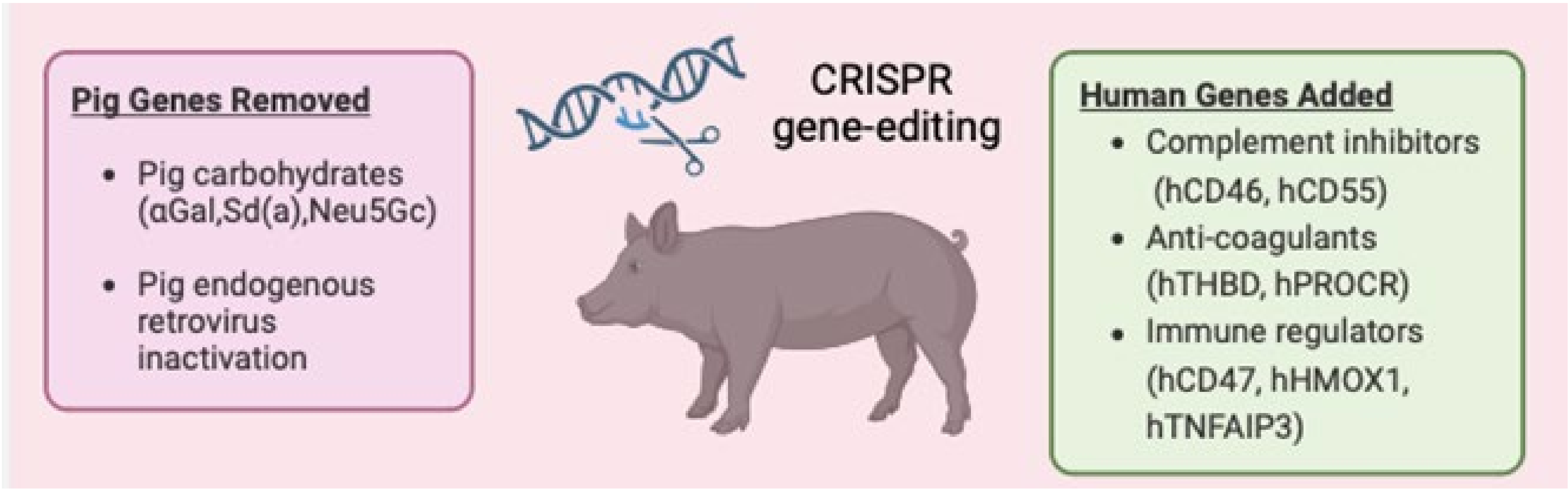

CRISPR/Cas9 Gene Editing
DISRUPT
DELETE
CORRECT OR INSERT
DNA
Gene 1
(protein 1)
Gene 2
(protein 2)
Gene CCR5
Gene 4
(protein 4)
Gene 5
(protein 5)

Genetic Engineering Examples

The pig with the jelly fish gene

CRISPR/Cas9 Gene Editing





PATIENT RECEIVES FIRST-EVER
PIG KIDNEY TRANSPLANT

First pig kidney transplant in a person: what it means for the future

The operation's early success has made researchers hopeful that clinical trials for xenotransplanted organs will start soon.

The recipient of the pig kidney is a 62-year-old man with end-stage renal failure named Richard Slayman. He is recovering well after the surgery on 16 March, according to his transplant surgeon. The kidney was taken from a miniature pig carrying a record 69 genomic edits, which were aimed at preventing rejection of the donated organ and reducing the risk that a virus lurking in the organ could infect the recipient. Nature, March 2024

https://www.youtube.com/watch?v=I7_BVEWhAXw

A pig kidney is unpacked for transplant into 62-year-old Richard Slayman of Massachusetts. Credit: Massachusetts General Hospital