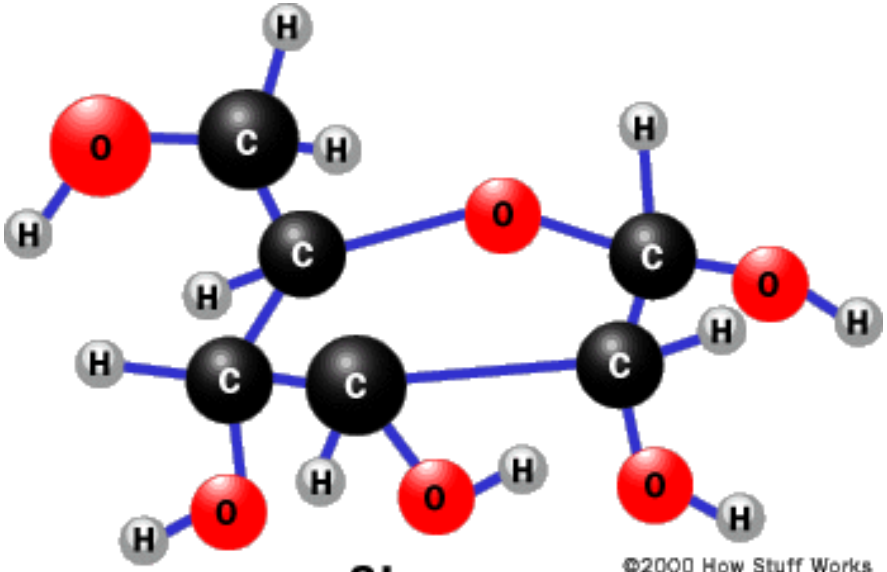
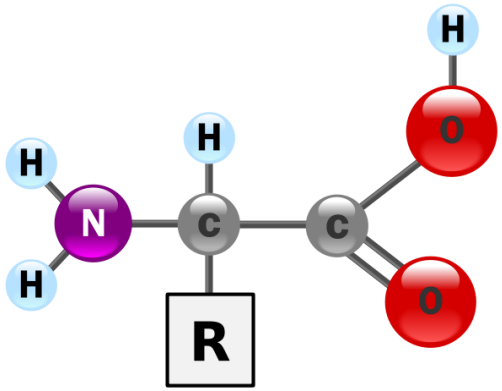
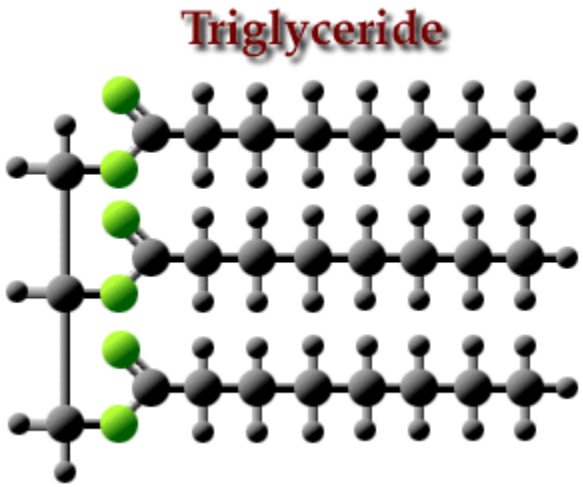
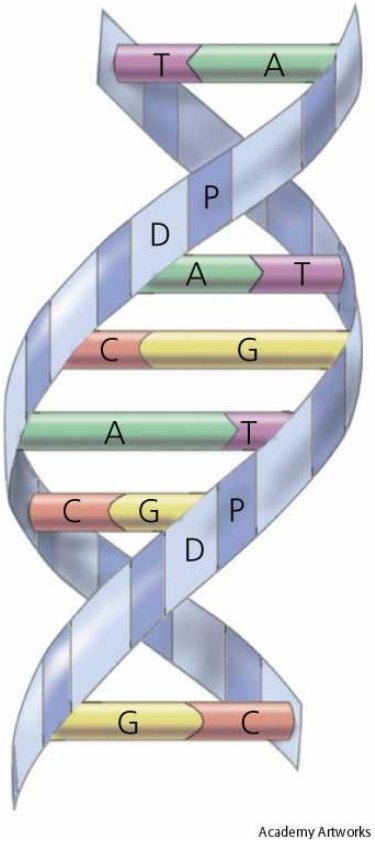
The Macromolecules





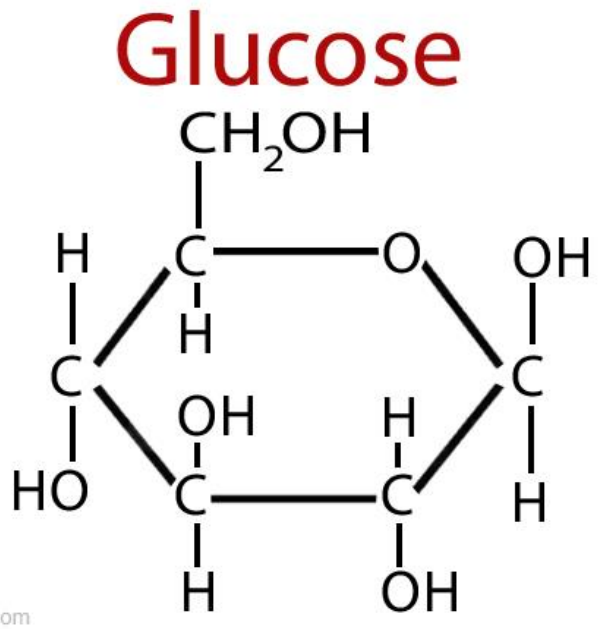




Carbohydrate Review (C,H,O)

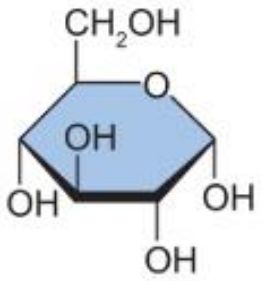
Carb Facts

- All carbs are made of sugar.
- They are polymers of many sugar units.
- Carbs are the bodies most important energy source.
- Carbs can be classified according to the number of sugar units they contain.

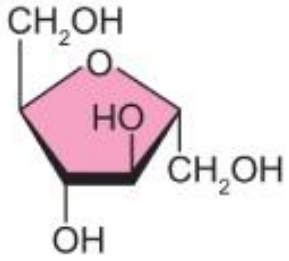


Monosaccharides

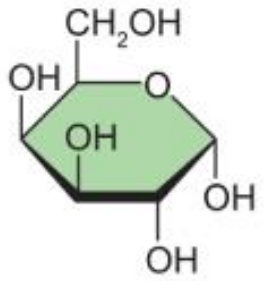
- These contain only a single sugar unit.
- Glucose, Fructose, galactose and Deoxyribose are examples.



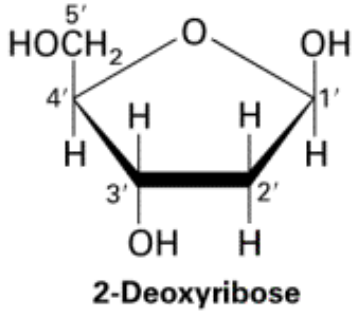
Glucose



Fructose



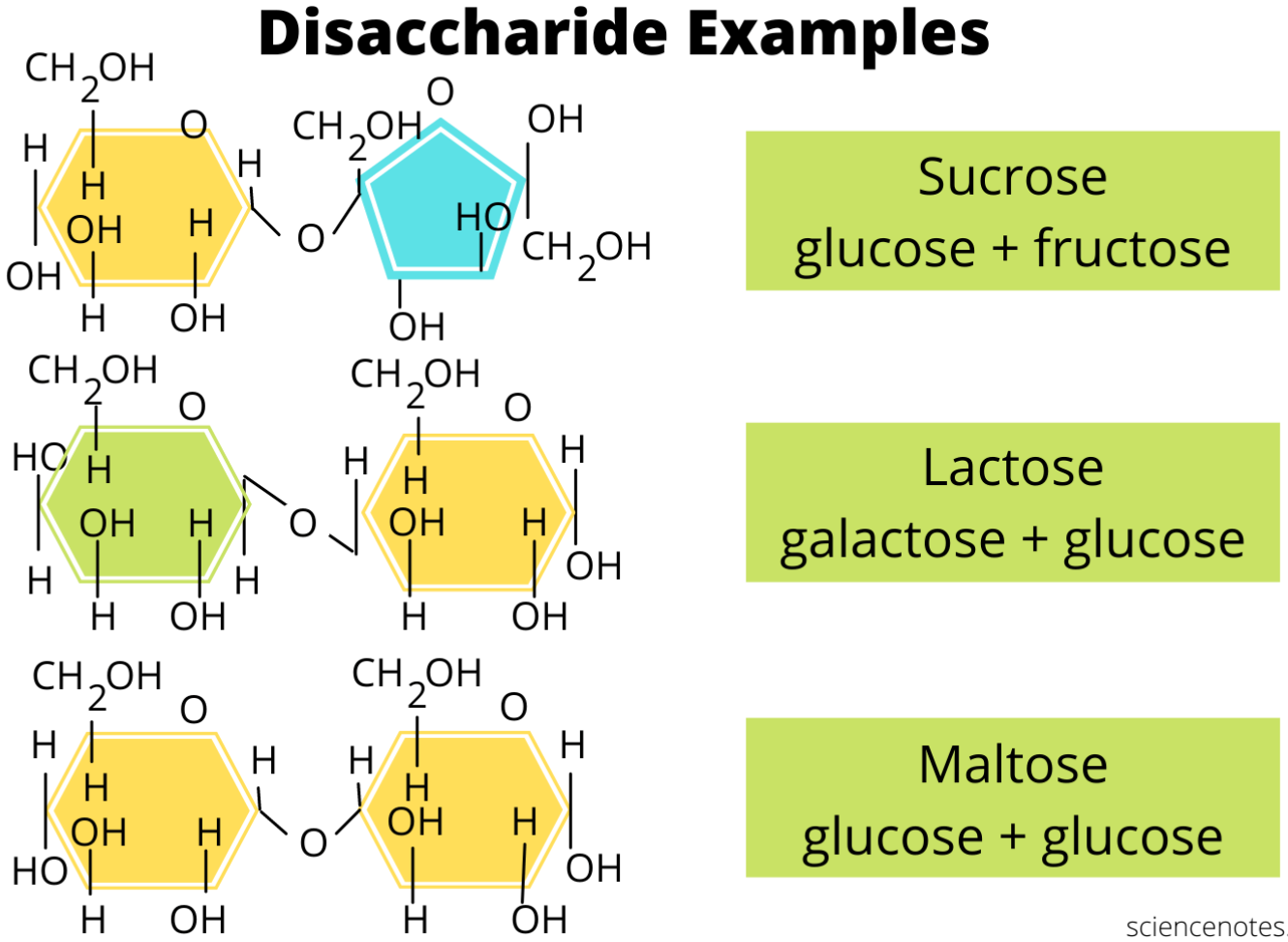
Galactose



2-Deoxyribose

Disaccharides

- Are a combination of two monosaccharides.
- Sucrose, Maltose, and Lactose are examples.

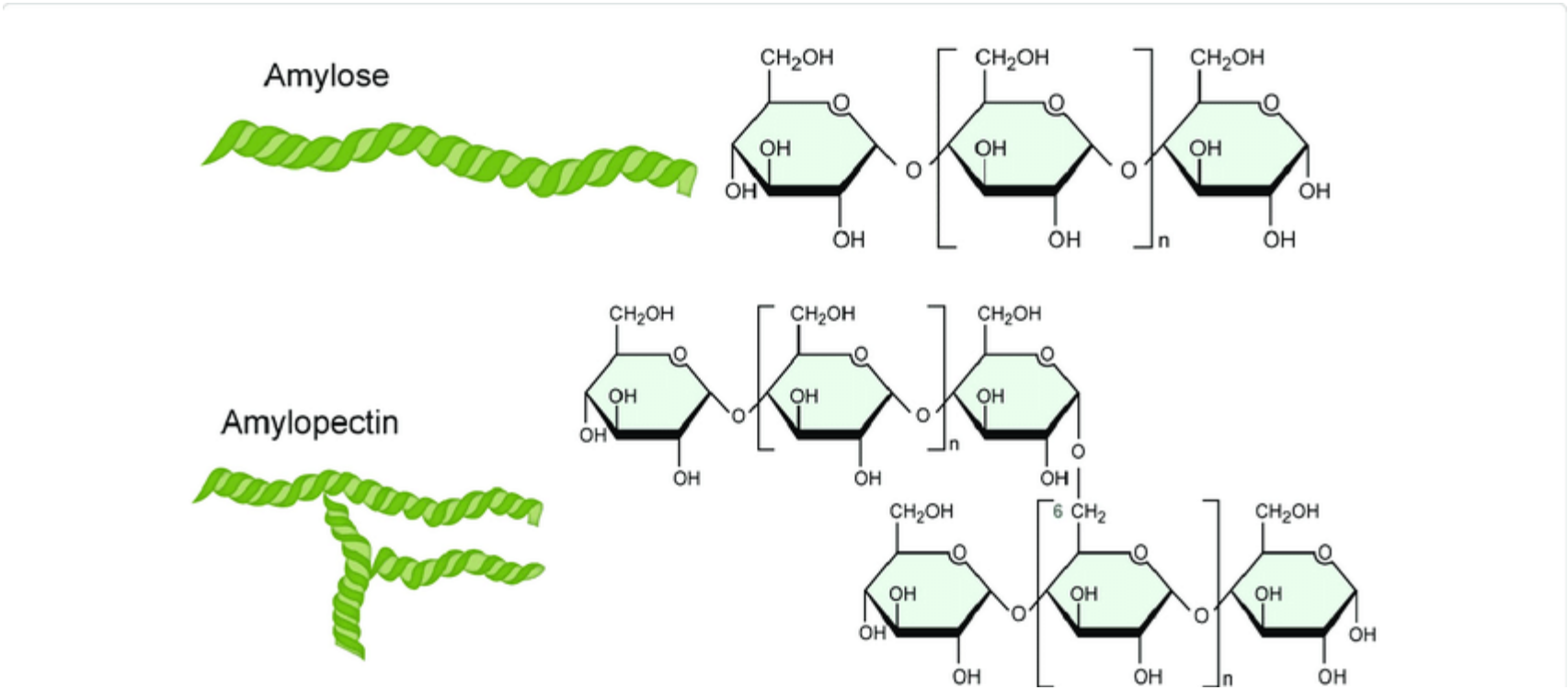


Polysaccharides

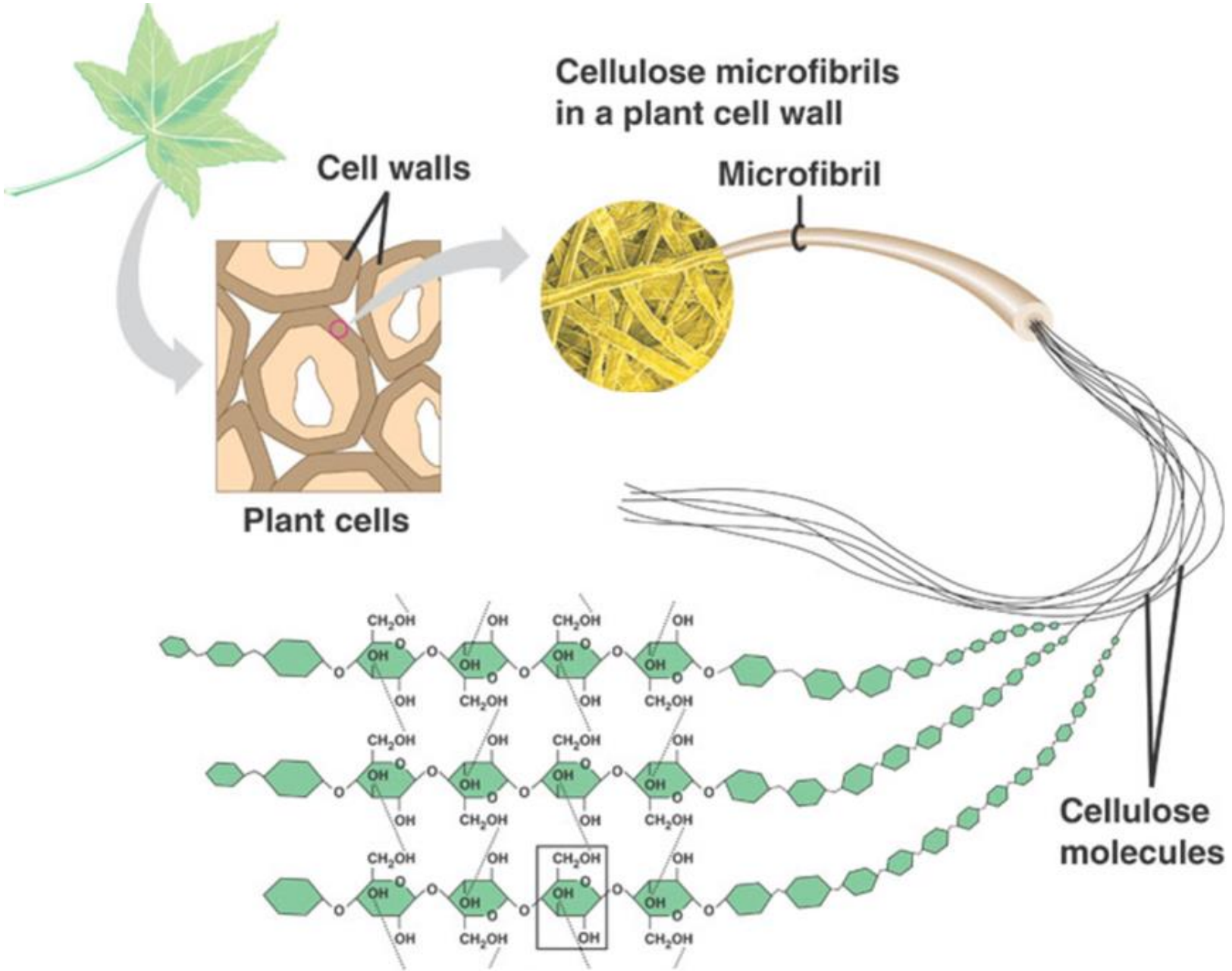
- A carb formed when 3 or more sugars are linked together.
- There are 3 main examples of polysaccharides.
- 1) Starch – a sugar storage molecule found only in plants
- 2) Cellulose – a carb based building molecule plants use to construct cell walls.
- 3) Glycogen – a sugar storage molecule found mainly in animal cells (muscle and liver).

Starch

- Starch is a sugar storage molecule found only in plants.
- Starch can be composed of 1000-6000 sugar units linked together.

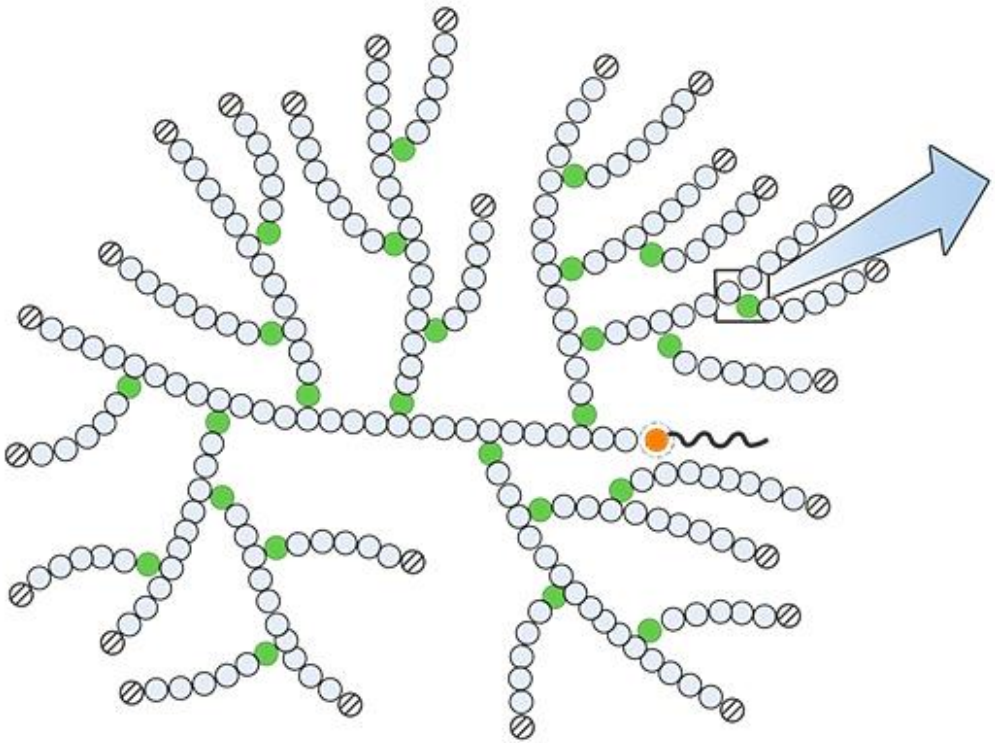


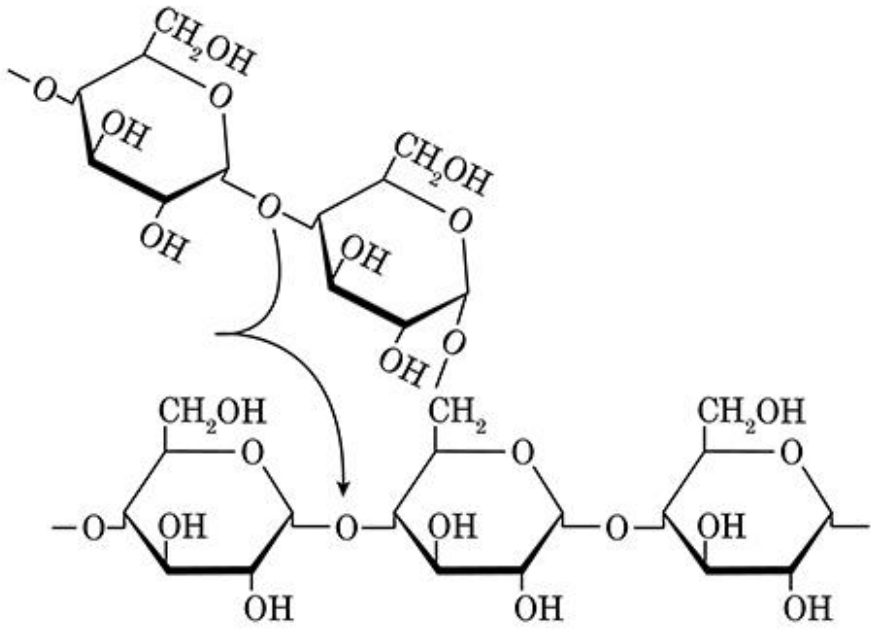
Cellulose


Cellulose microfibrils
in a plant cell wall
Cell walls
Microfibril
Plant cells
Cellulose
molecules

Glycogen

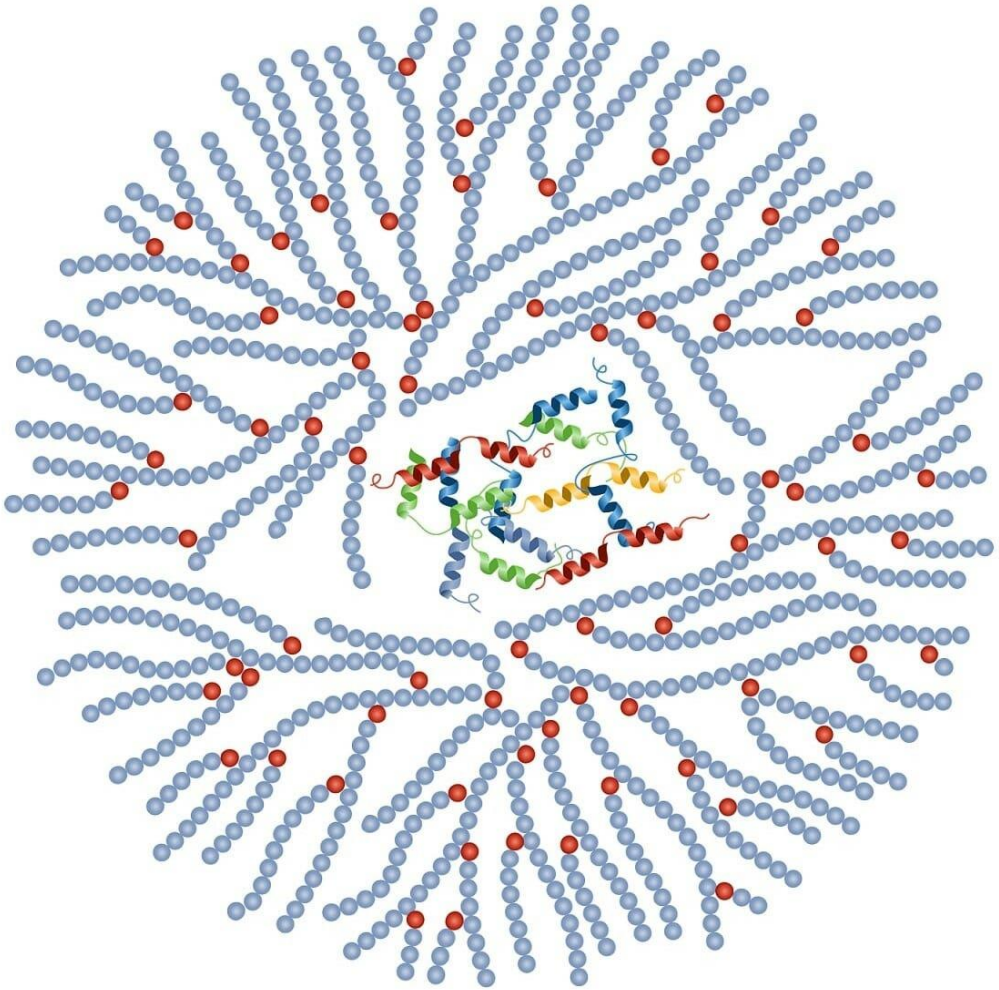
- The main sugar storing molecule in our bodies.

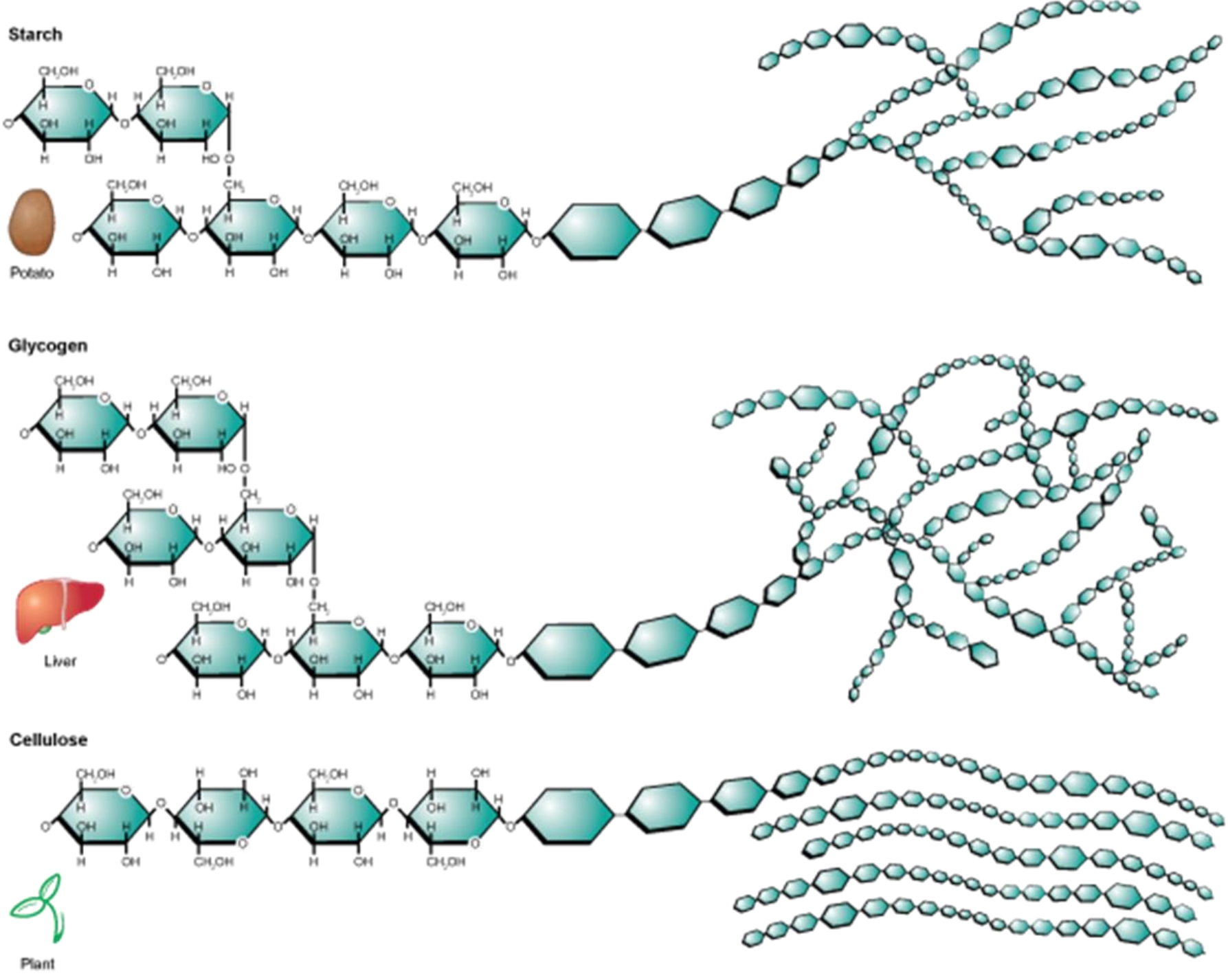


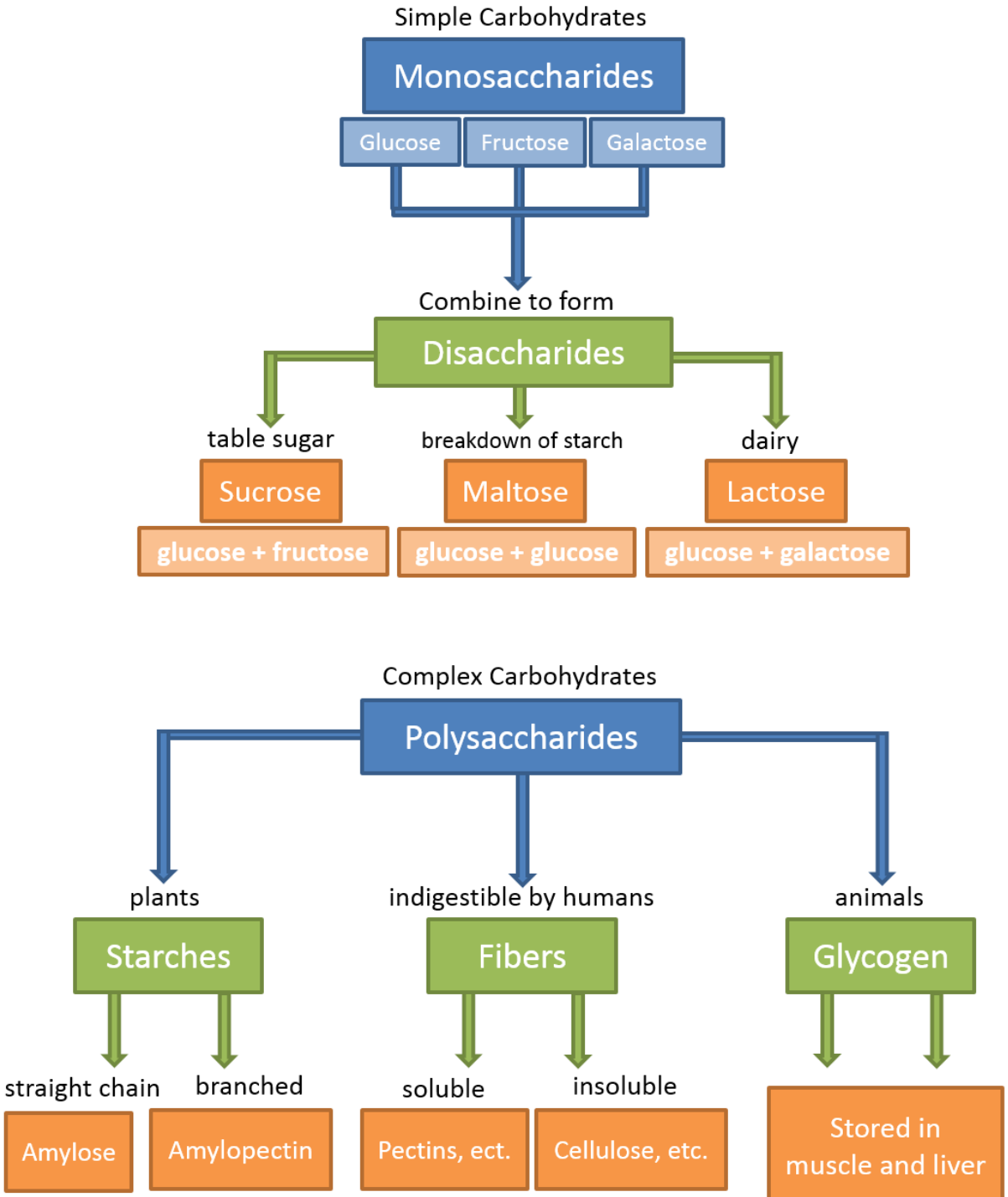


Glycogen

- Glycogen is formed when excess sugars are linked together in our muscle and liver cells forming huge chains of sugars.




Starch
Potato
Glycogen
Liver
Cellulose
Plant

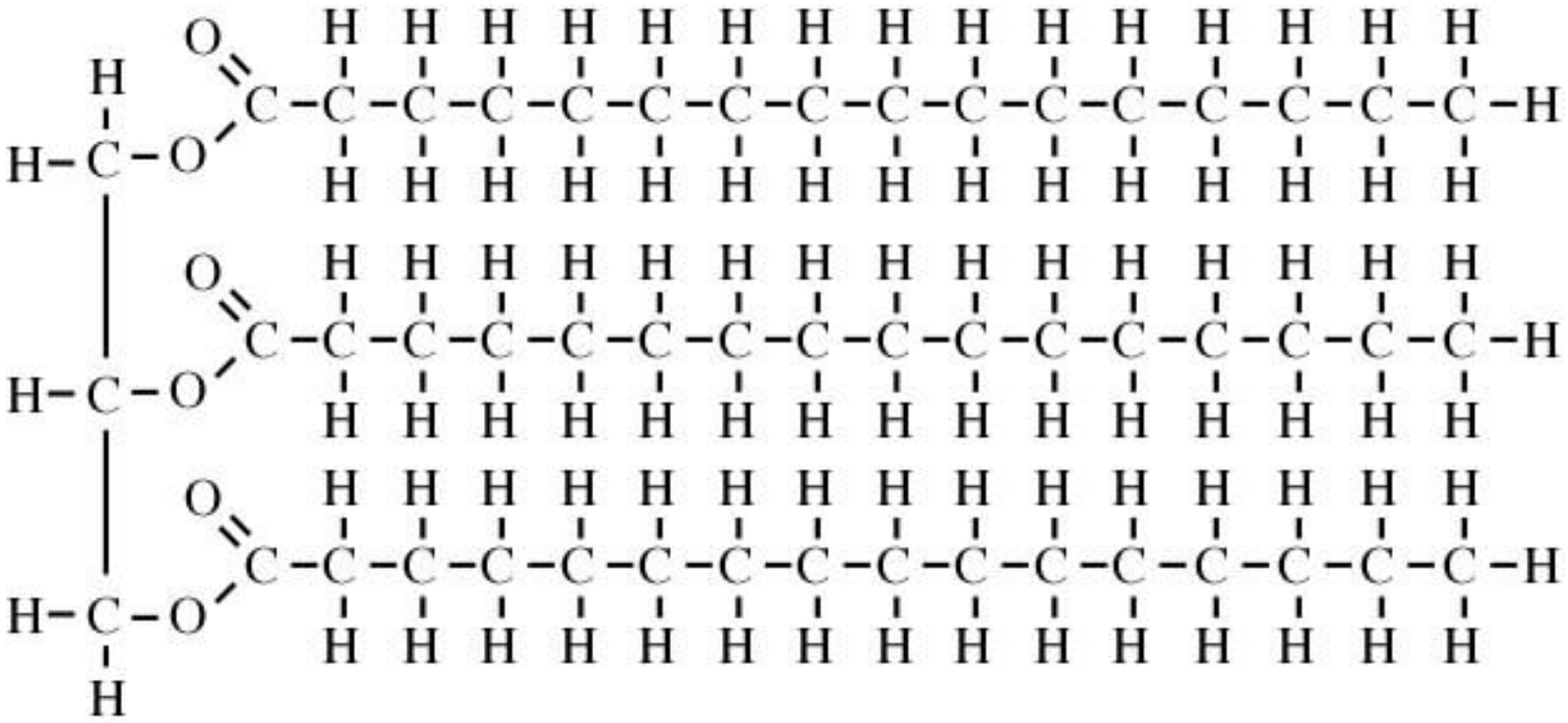

Simple Carbohydrates
Monosaccharides
Glucose
Fructose
Galactose
Combine to form
Disaccharides
table sugar
breakdown of starch
dairy
Sucrose
Maltose
Lactose
glucose + fructose
glucose + glucose
glucose + galactose
Complex Carbohydrates
Polysaccharides
plants
indigestible by humans
animals
Starches
Fibers
Glycogen
straight chain
branched
soluble
insoluble
Amylose
Amylopectin
Pectins, ect.
Cellulose, etc.
Stored in muscle and liver

Lipid Review (C,H,O)

- Lipids are the only molecule organisms can use to store vast quantities of energy (calories).
- Fat is also important as an insulator, and it protects and cushions internal organs.
- Fat can be modified into hormones, phospholipids (for cell membranes), waxes.

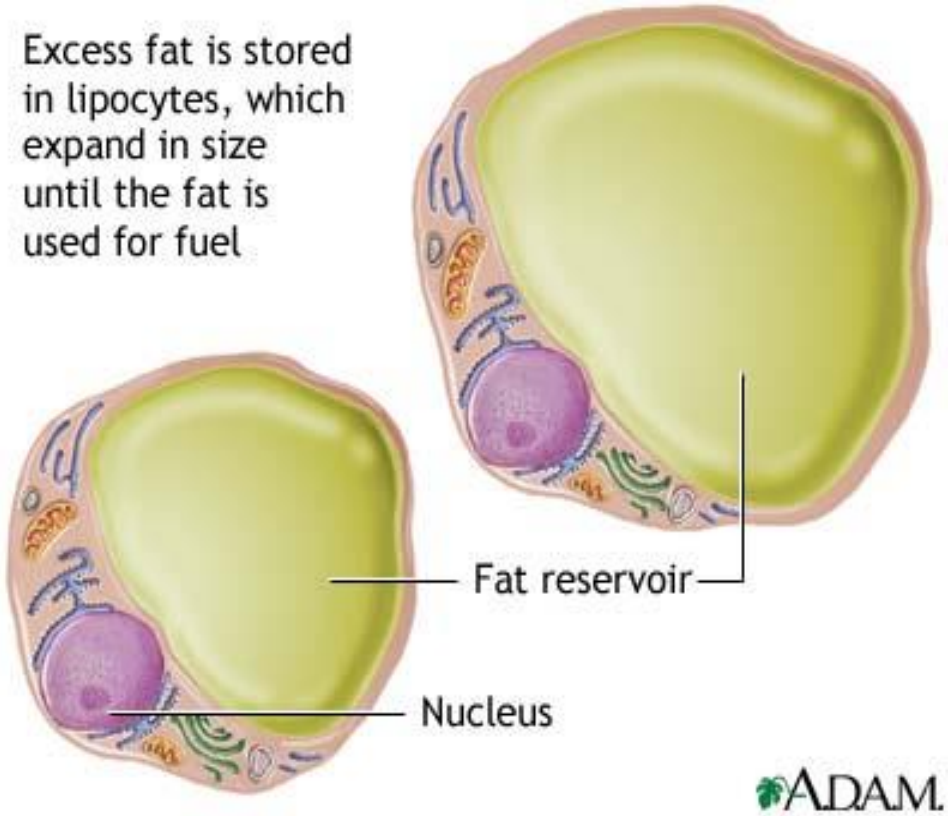
Triglycerides

- Triglycerides are formed by the union of glycerol (a 3 carbon chain) and three fatty acids (themselves long chains of carbon atoms).
- Excess carbs are converted into fatty acids and then into triglycerides before being sent into the blood to a fat cell.



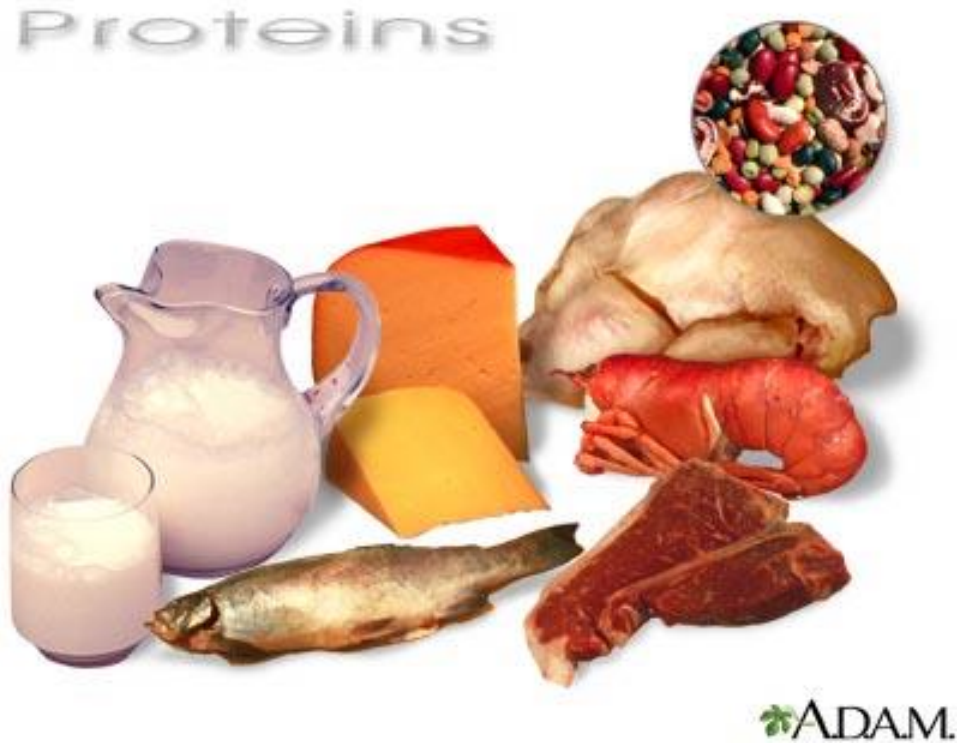
- We are born with ~10 billion fat cells.
- Depending on the quality and quantity of the foods we eat and our activity levels as children, we will build the 20-30 billion fat cells that we keep as adults.
- Those fat cells can hold between 20-30 pounds.
- Overweight people might build 75 billion fat cells.
- For the extremely obese we build up to 300 billion fat cells.

Fat Cells

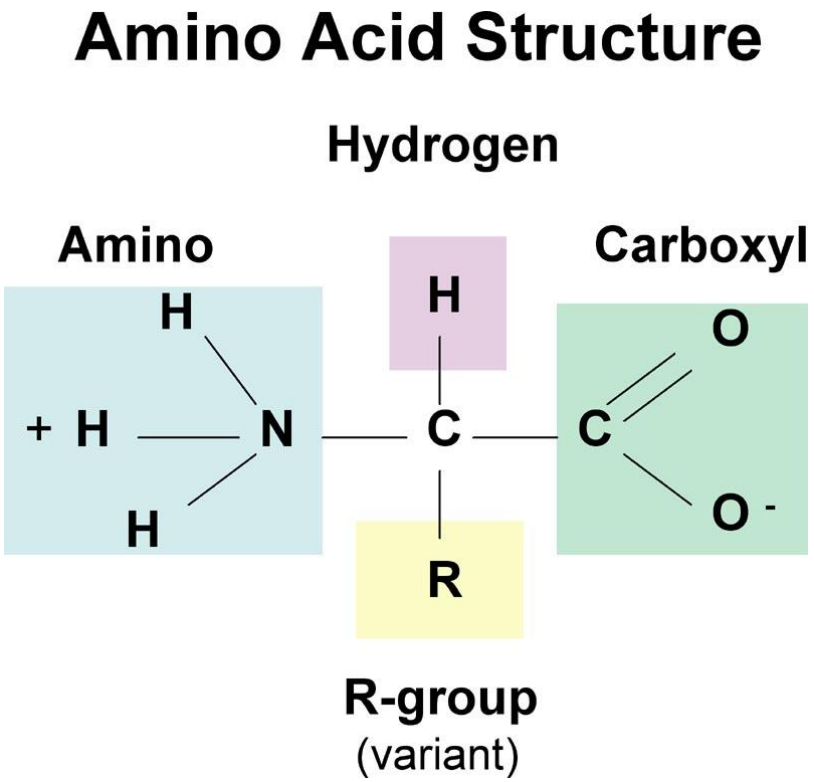


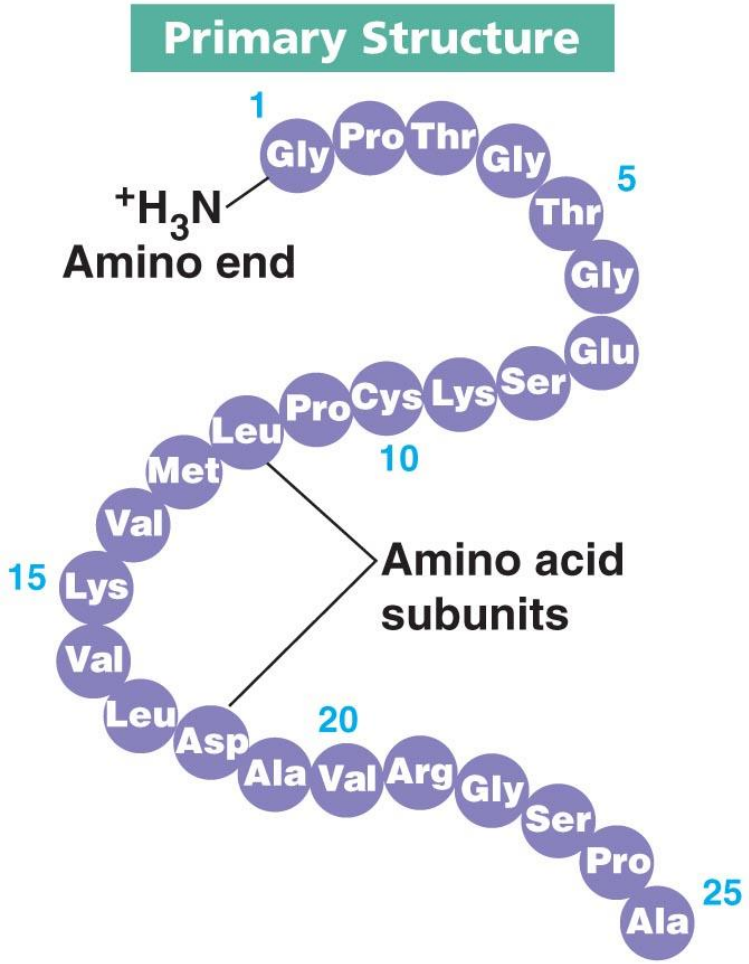
Protein Review (C,H,O,N)

- Proteins are **structural** molecules that give most cells, and therefore organisms their shape and appearance.
- **Enzymes** are protein molecules that help almost every chemical reaction in the body take place.

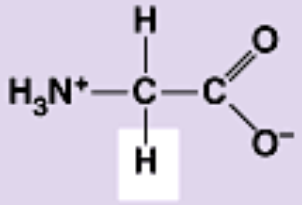


The building blocks of proteins are Amino Acids.

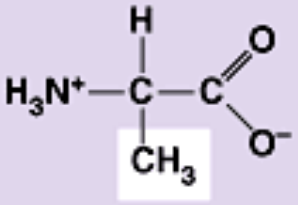




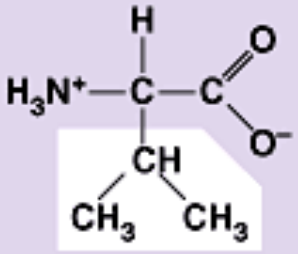
“R” Groups of a few Amino Acids



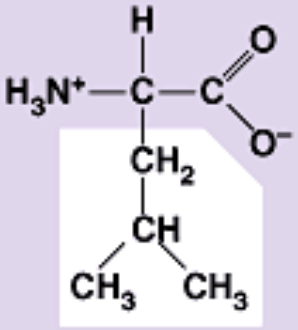
Glycine (Gly)



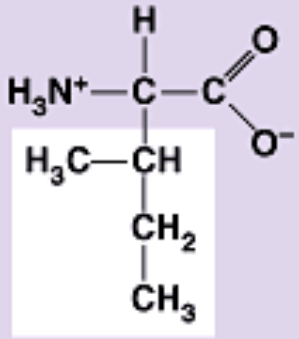
Alanine (Ala)



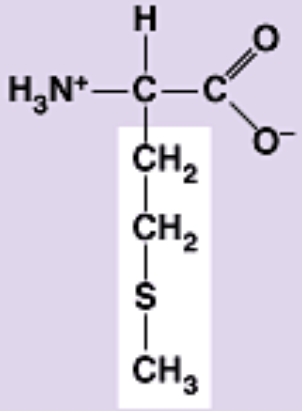
Valine (Val)



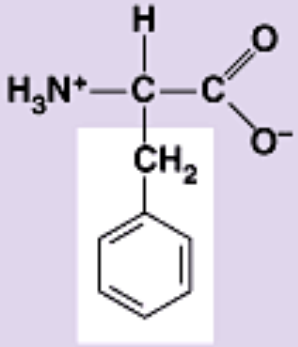
Leucine (Leu)



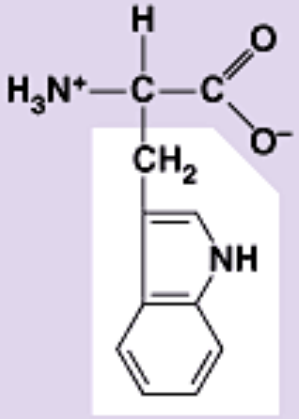
Isoleucine (Ile)



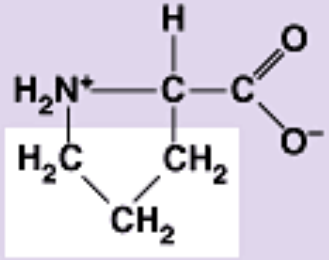
Methionine (Met)



Phenylalanine (Phe)

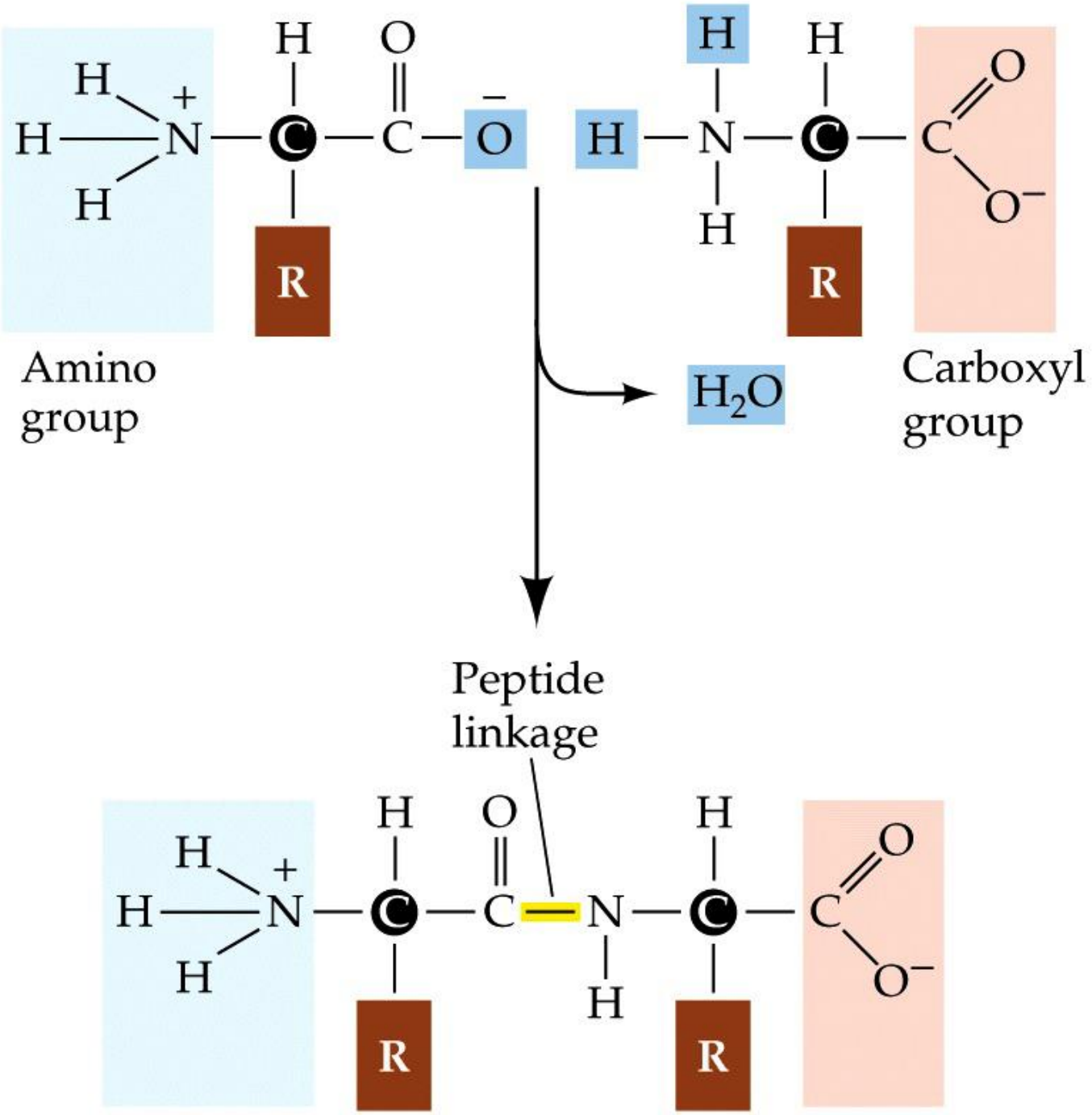


Tryptophan (Trp)



Proline (Pro)

Peptide Bond

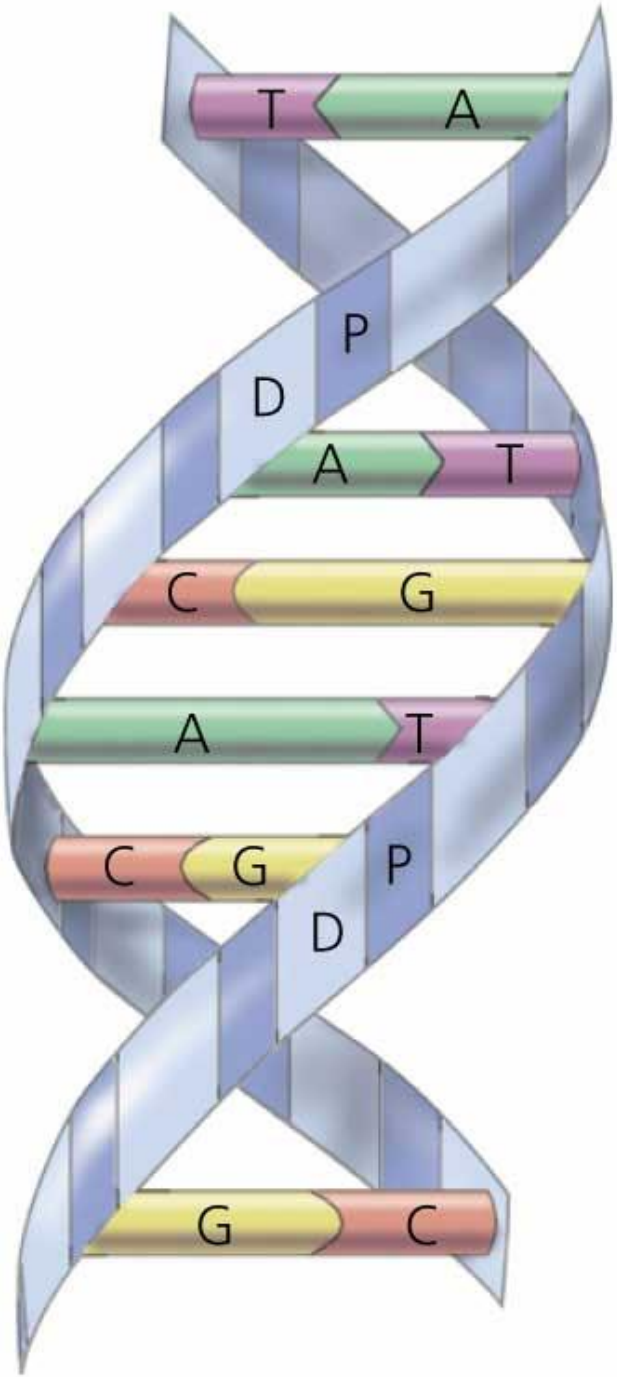


The Order of the Amino Acids

- Letters are to words as amino acids are to proteins.
- In what 2 ways this analogy might hold true.
- 1) Letters are the building blocks of words – Amino Acids are the building blocks of proteins.
- 2) The precise order of the letters spells out particular words – the precise order of the Amino Acids spells out particular proteins.

Nucleic Acids (C,H,O,N,P)

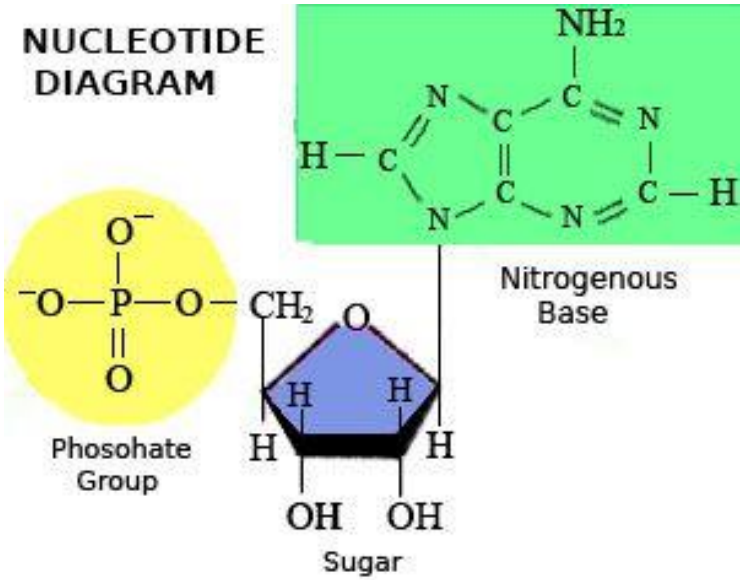
- **Nucleic acids** are biological molecules essential for life.
- Examples include DNA and RNA.
- They function in encoding, transmitting and expressing genetic information.
- The functional units (or building blocks) of nucleic acids are Nucleotides.

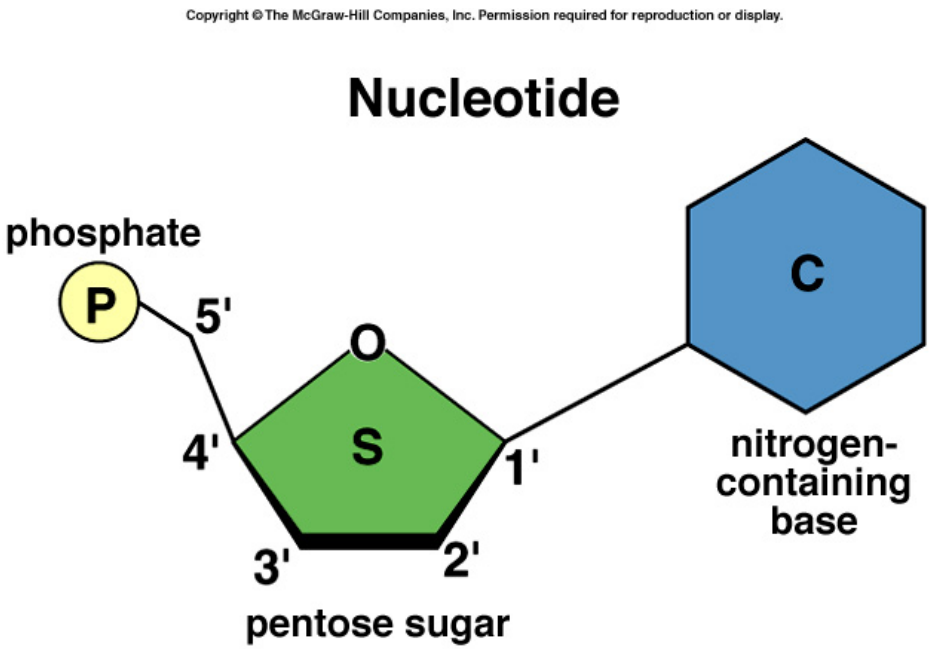


Academy Artworks

Nucleotides

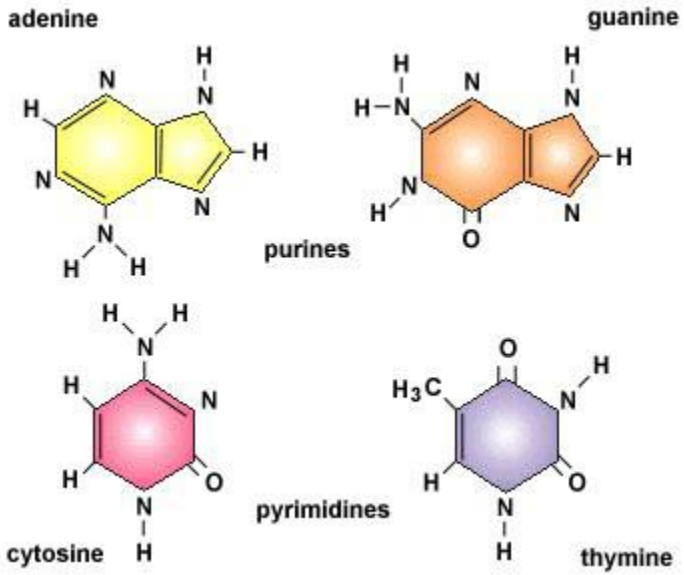
- They are the functional units (or building blocks) of nucleic acids .
- Each nucleotide contains;
- - a 5 carbon sugar
- - a phosphate group
- - a nitrogen base

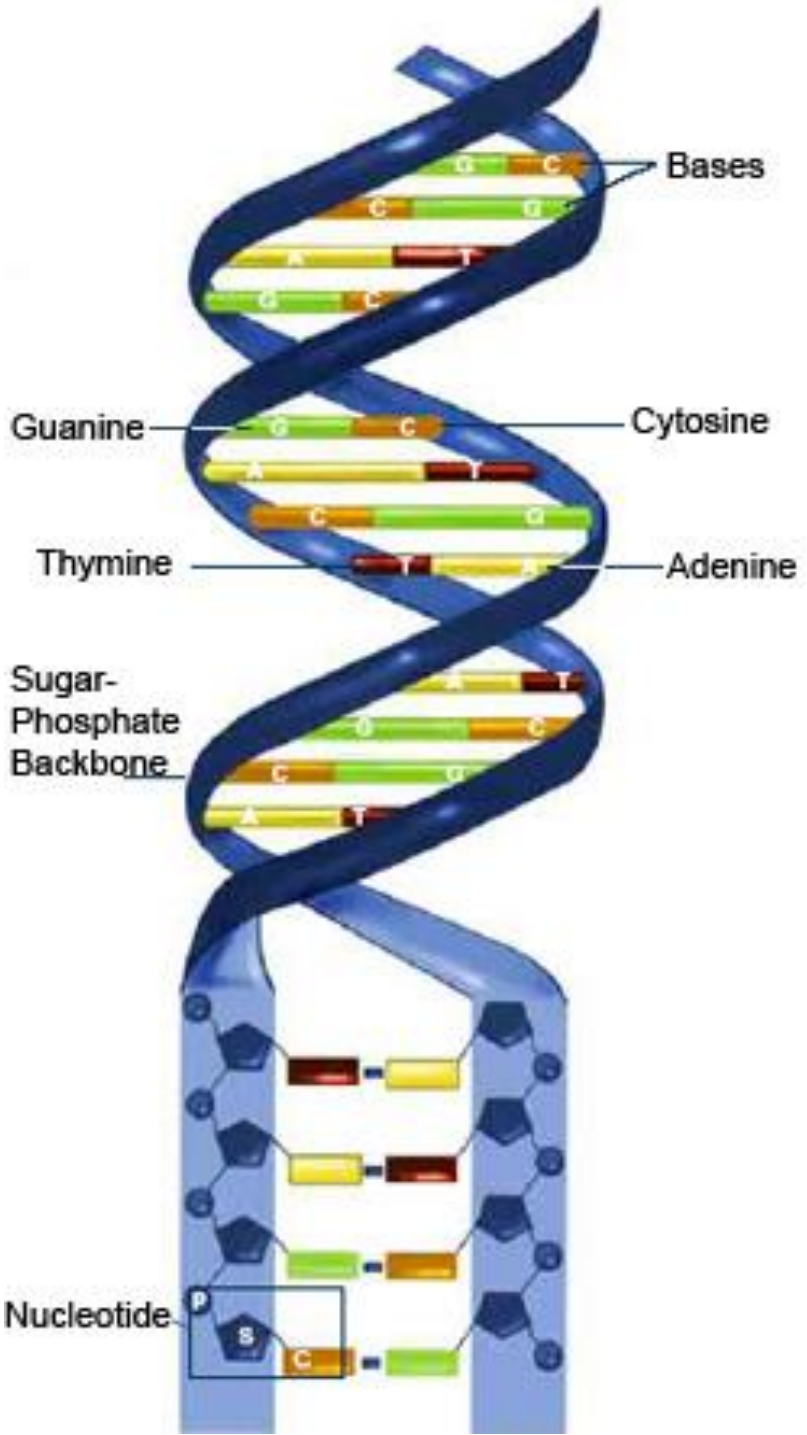




Nucleotides are linked together to form Nucleic Acids.

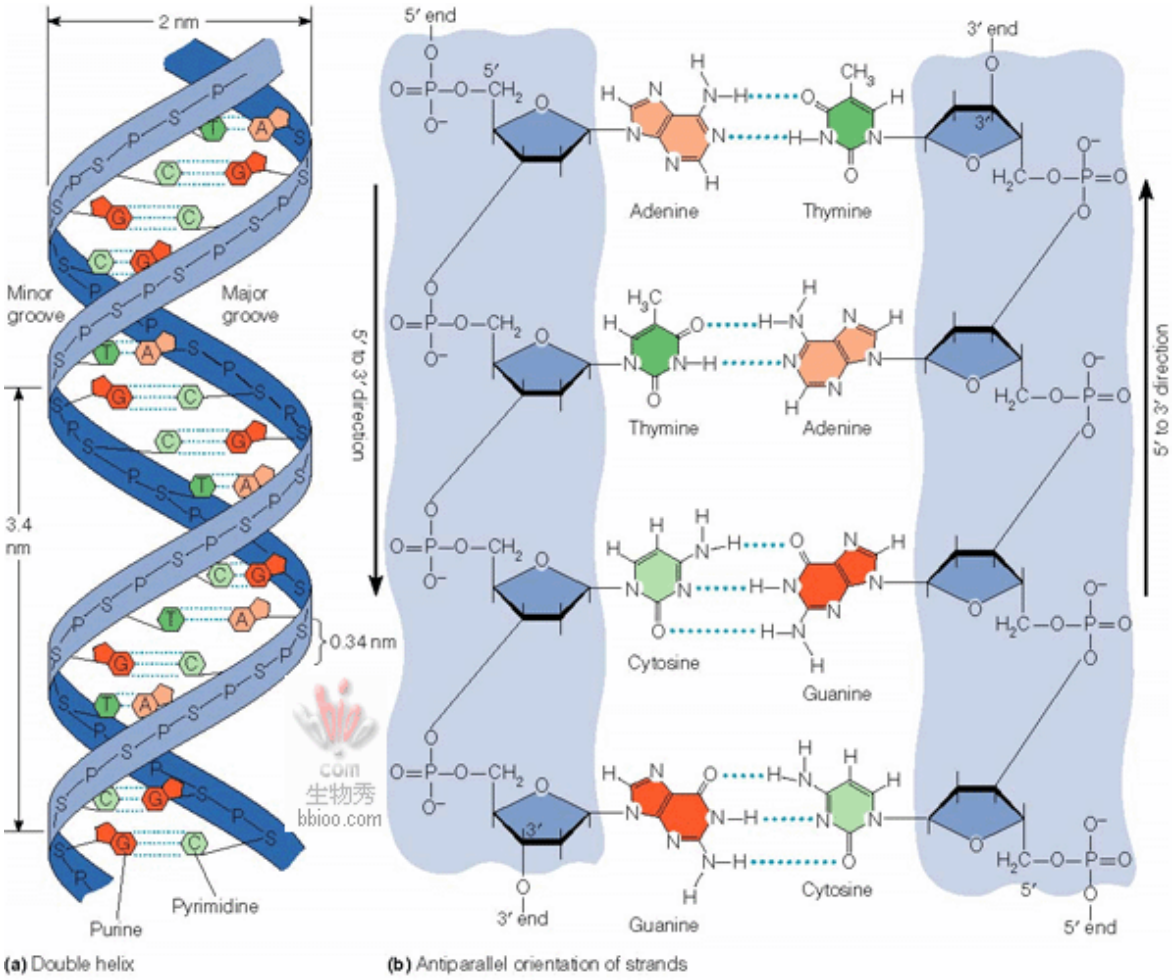
- These are the 4 DNA nucleotides.
- A pairs with T
- G pairs with C





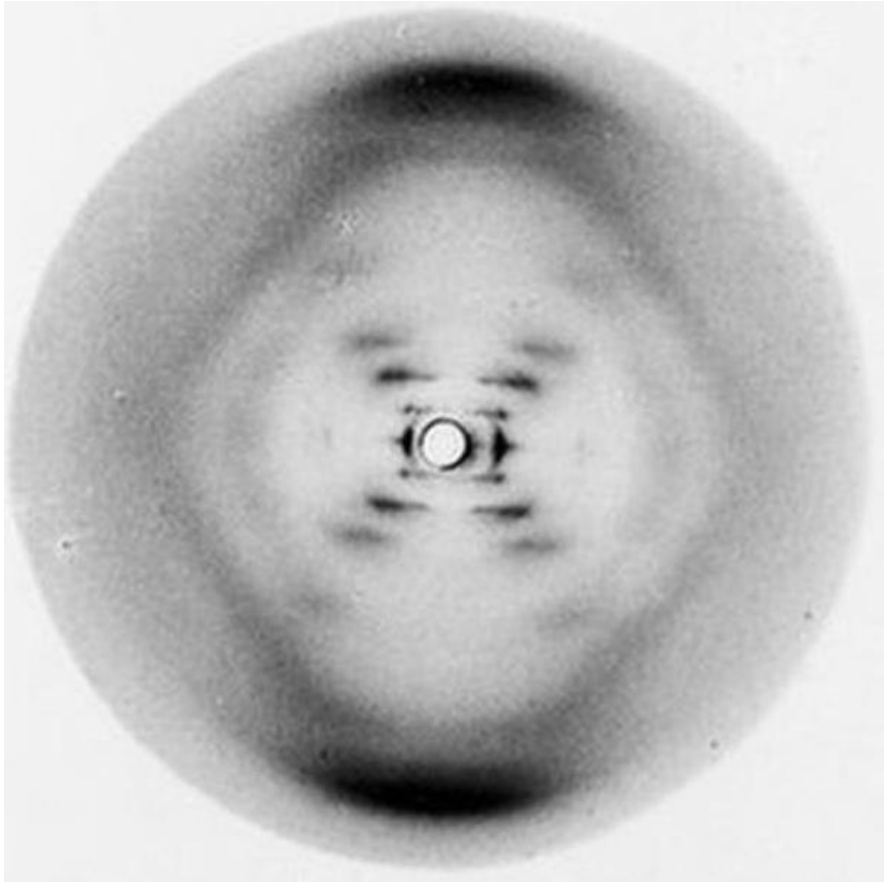
Structure of DNA

- First described by James Watson and Francis Crick who won the Nobel prize in 1953 (although they stole the work of Rosa Franklin who used X-ray diffraction to work out the exact structure.



Rosalind Franklin – the unsung hero of DNA

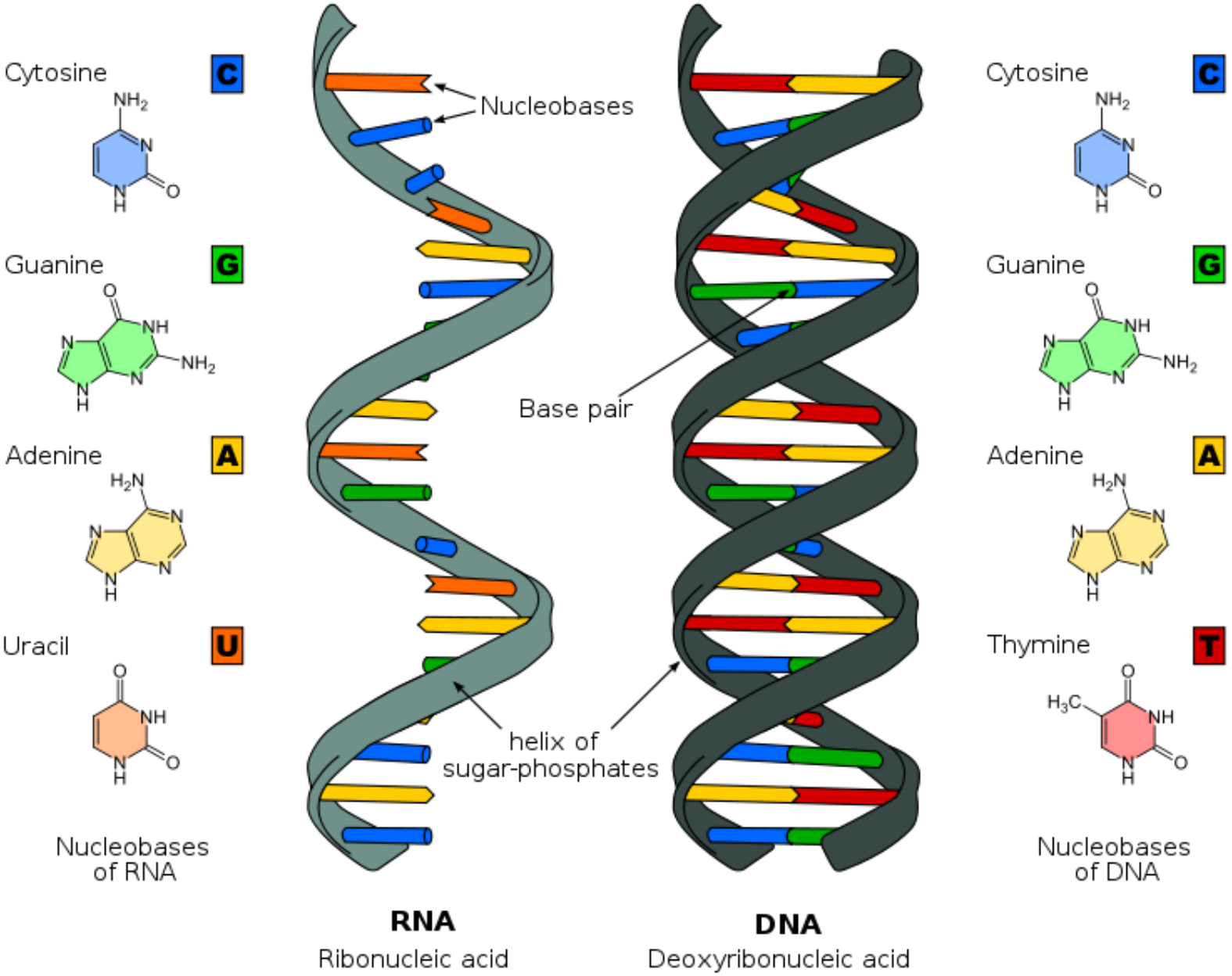
Many refer to Photo 51 as the most important photo ever taken in science!!!



RNA

- RNA is similar to DNA in that it is a string of nucleotides.
- There are however 3 key differences:
- 1) RNA is single stranded.
- 2) RNA has no Thymine (T).
  A pairs with U (Uracil).
- 3) RNA has a ribose sugar.
- RNA plays a role in 3 processes we'll examine:
- 1) As RNA primer in DNA replication
- 2) As mRNA in protein synthesis
- 3) As tRNA in protein synthesis

Comparing DNA to RNA



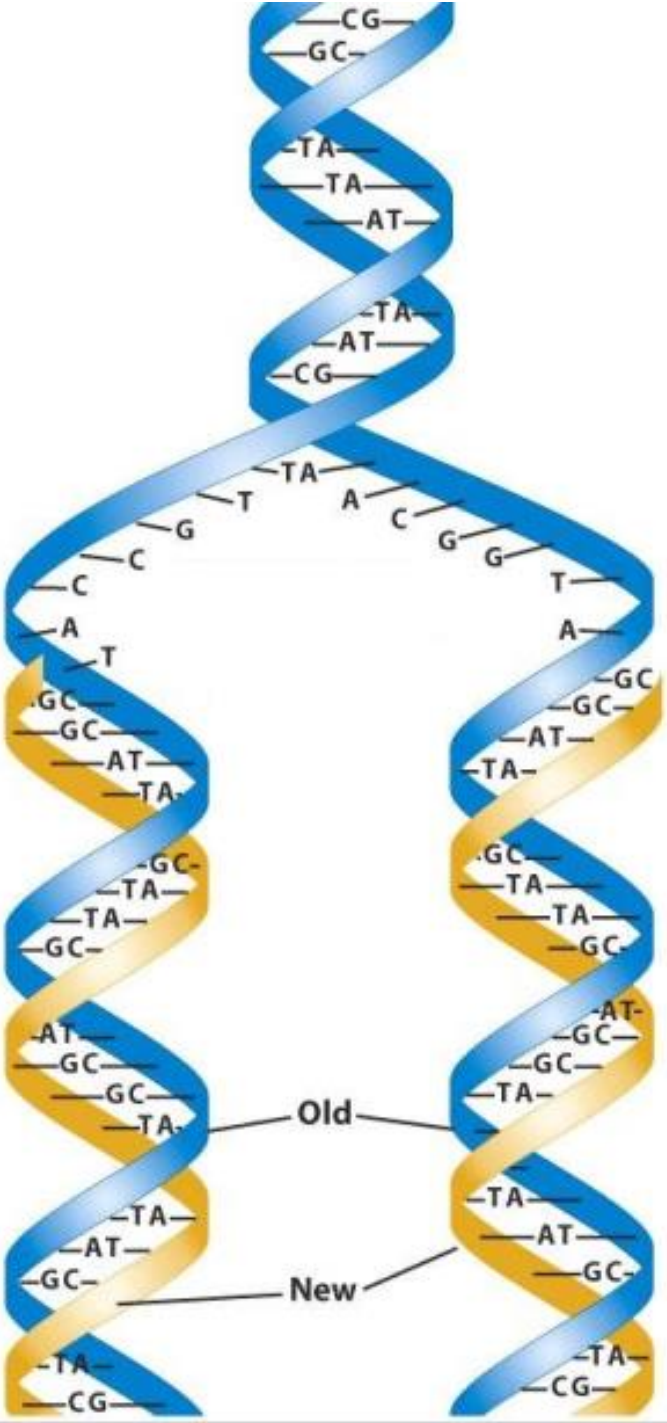
DNA Replication

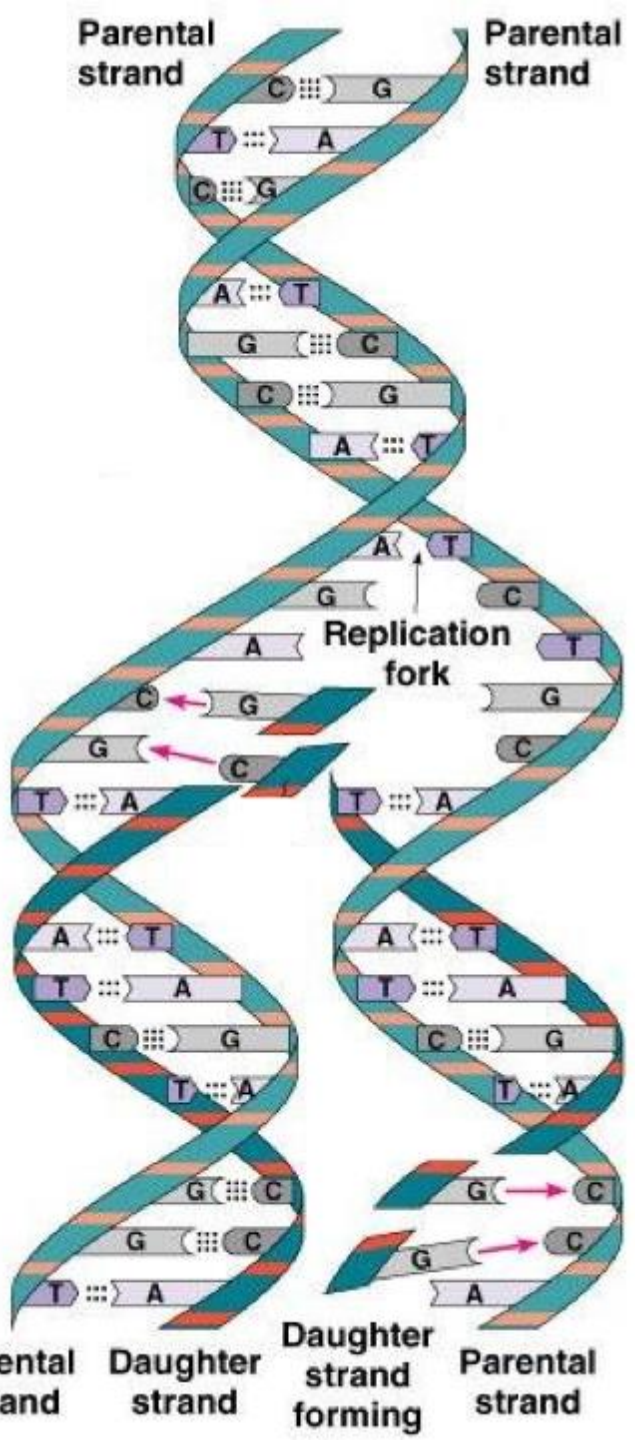
- DNA is unique in it's ability to replicate itself.
- A chromosome (an average DNA strand has 150 million base pairs) can replicate itself in less than 1 hour.
- Of the 80-100 trillion cells in your body, which cells need to replicate their DNA?
- So how is it done?
- Given a DNA model, what would you need in order to replicate it?
- Given that a nucleus has no eyes, hands, or brain, how might it replicate itself?

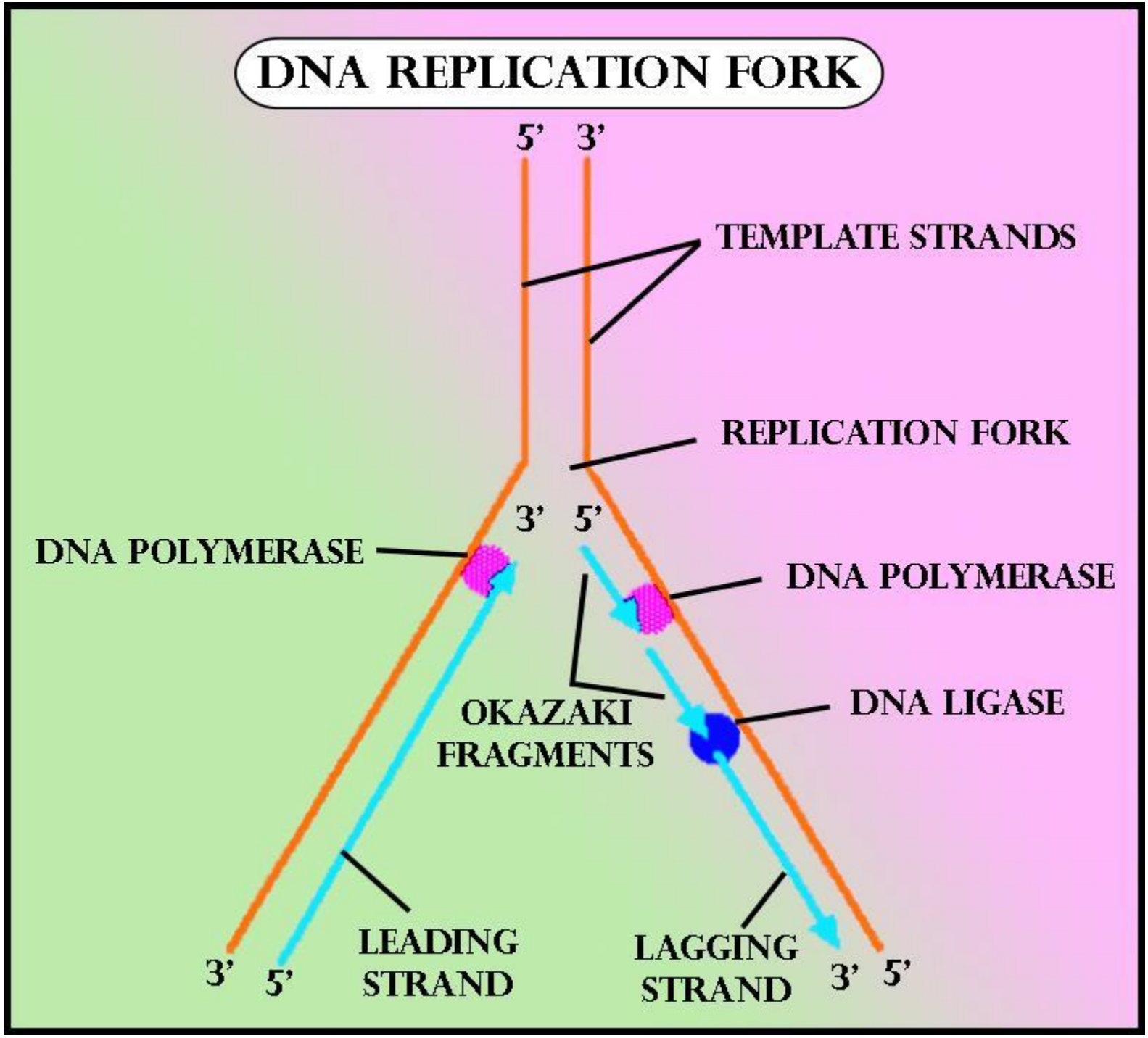
DNA Replication

- Looking at the DNA model, can you think of a method in which DNA replication could be accomplished?
- Hint: What happens when a T approaches a G. When a C approaches an A.
- Now what happens when we bring a T and an A together?
- And a G and a C?

DNA Replication


Old
New

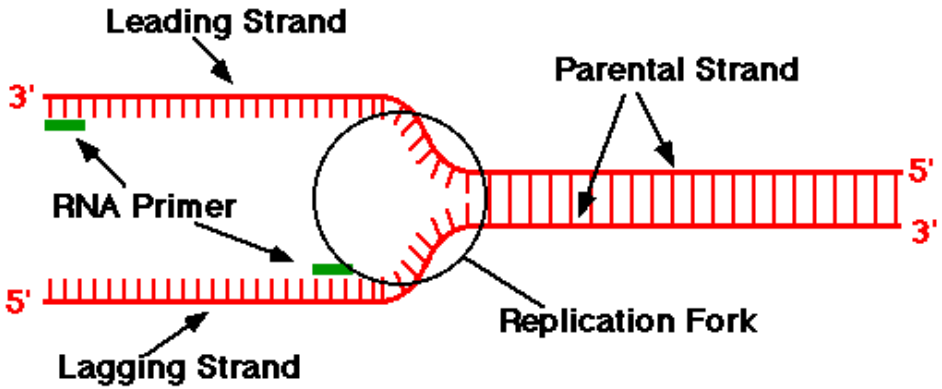

Parental strand
Parental strand
Replication fork
Parental strand
Daughter strand
Daughter strand forming
Parental strand

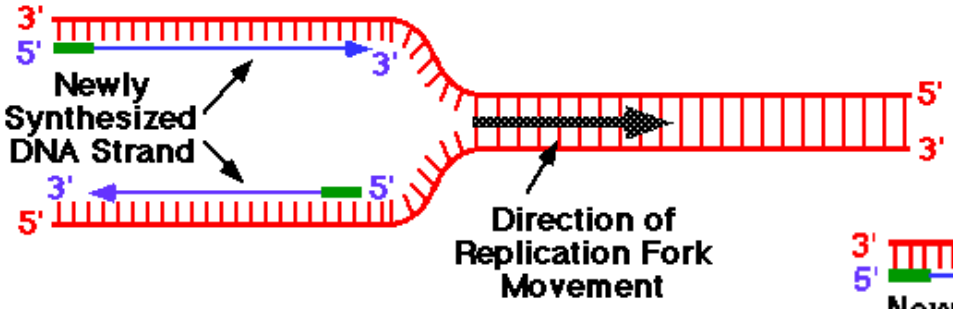

DNA REPLICATION FORK
5' 3'
TEMPLATE STRANDS
REPLICATION FORK
3' 5'
DNA POLYMERASE
DNA POLYMERASE
OKAZAKI FRAGMENTS
DNA LIGASE
3' 5'
LEADING STRAND
LAGGING STRAND
3' 5'

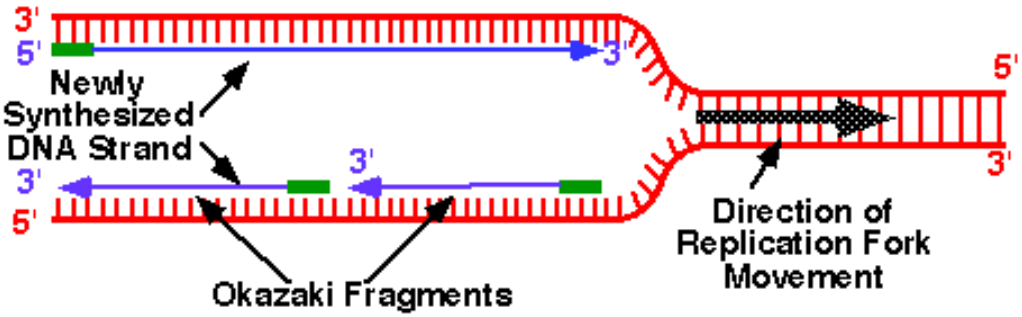
DNA Replication – 6 steps

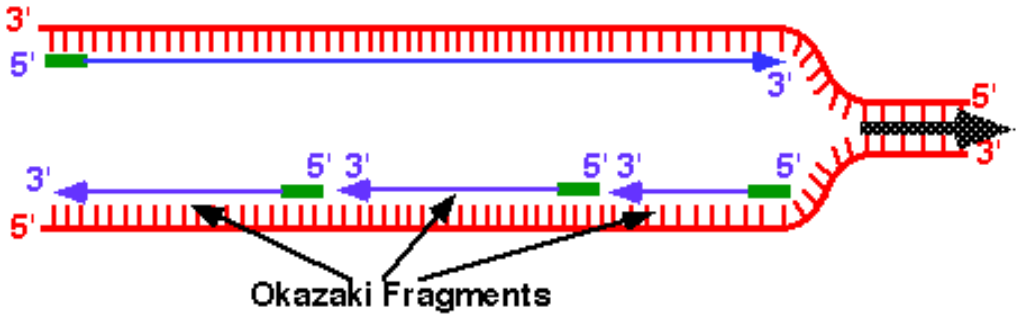
1. A portion of the DNA unwinds and unzips.
2. An RNA primer is added to the parent strand.
3. An enzyme **DNA polymerase** binds to one side of the open DNA (**leading strand**) and works **towards the fork** fitting in new complementary nucleotides (A-T and C-G).
4. On the second side of the open DNA (**lagging strand**), the RNA primer is again followed by **DNA polymerase** which again binds to the open DNA and works **away from the fork** fitting in new complementary nucleotides. On the lagging strand smaller fragments called "Okazaki fragments" are formed.
5. RNA primers are replaced with DNA nucleotides.
6. Okazaki fragments are stitched together by the enzyme **DNA ligase**.

DNA Replication

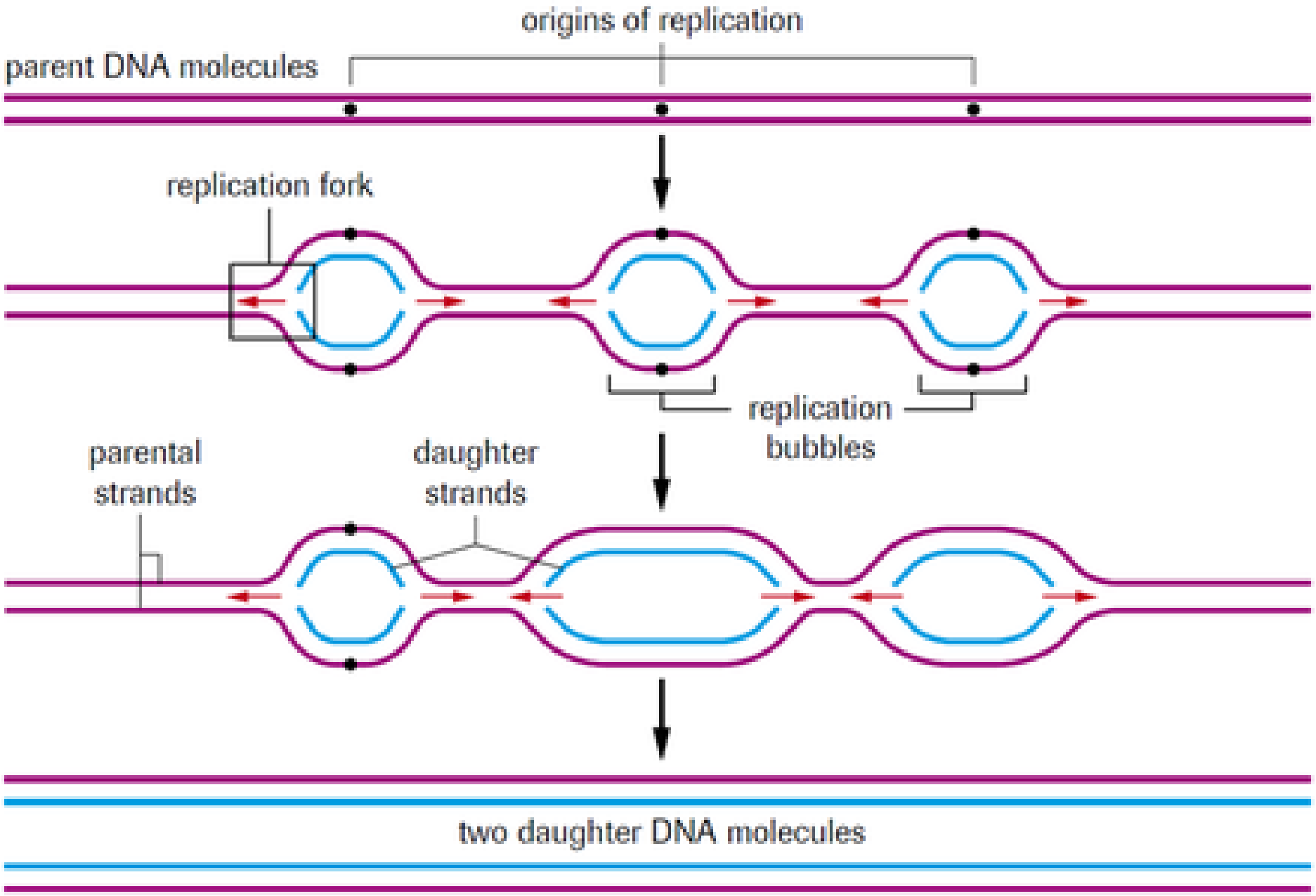




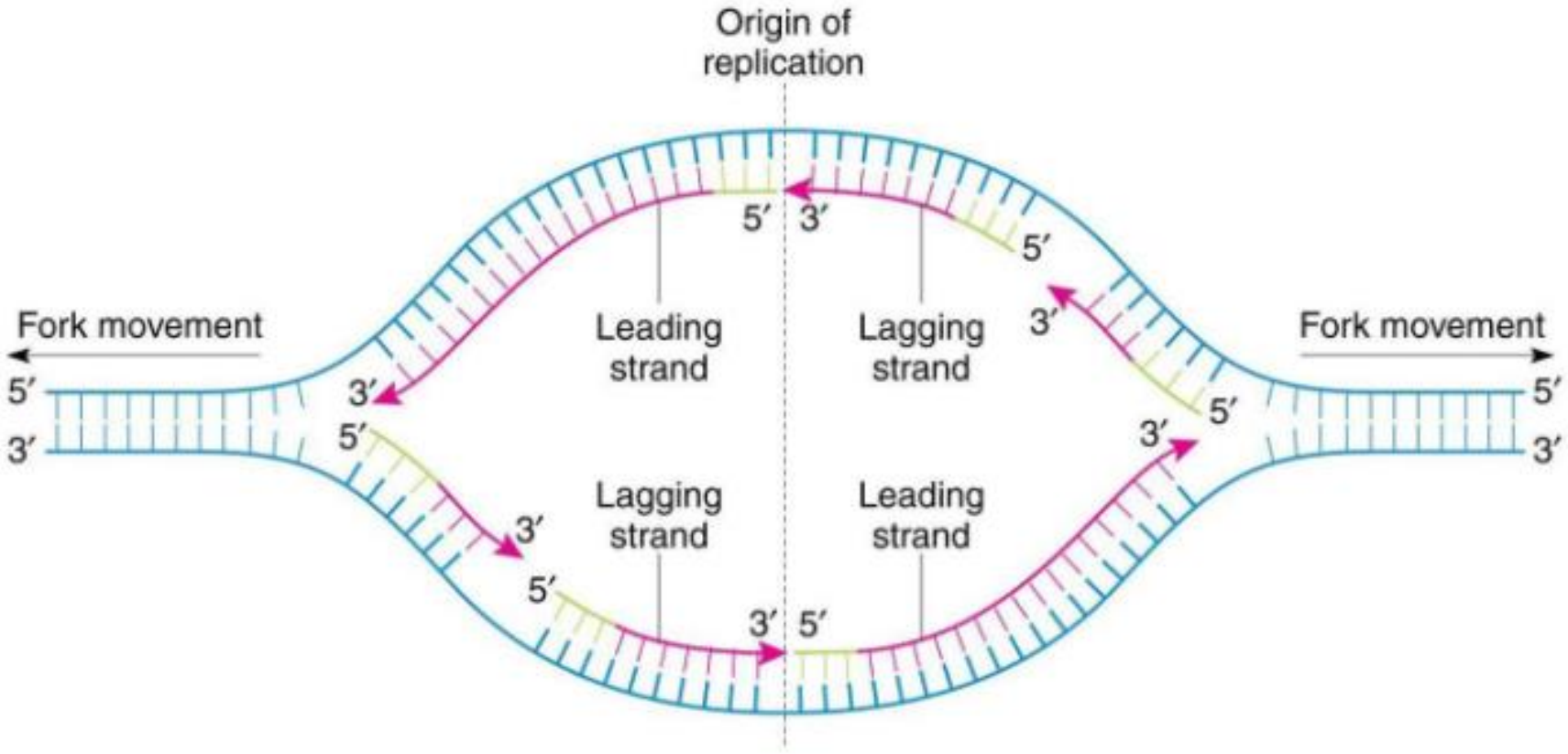




Replication Bubbles

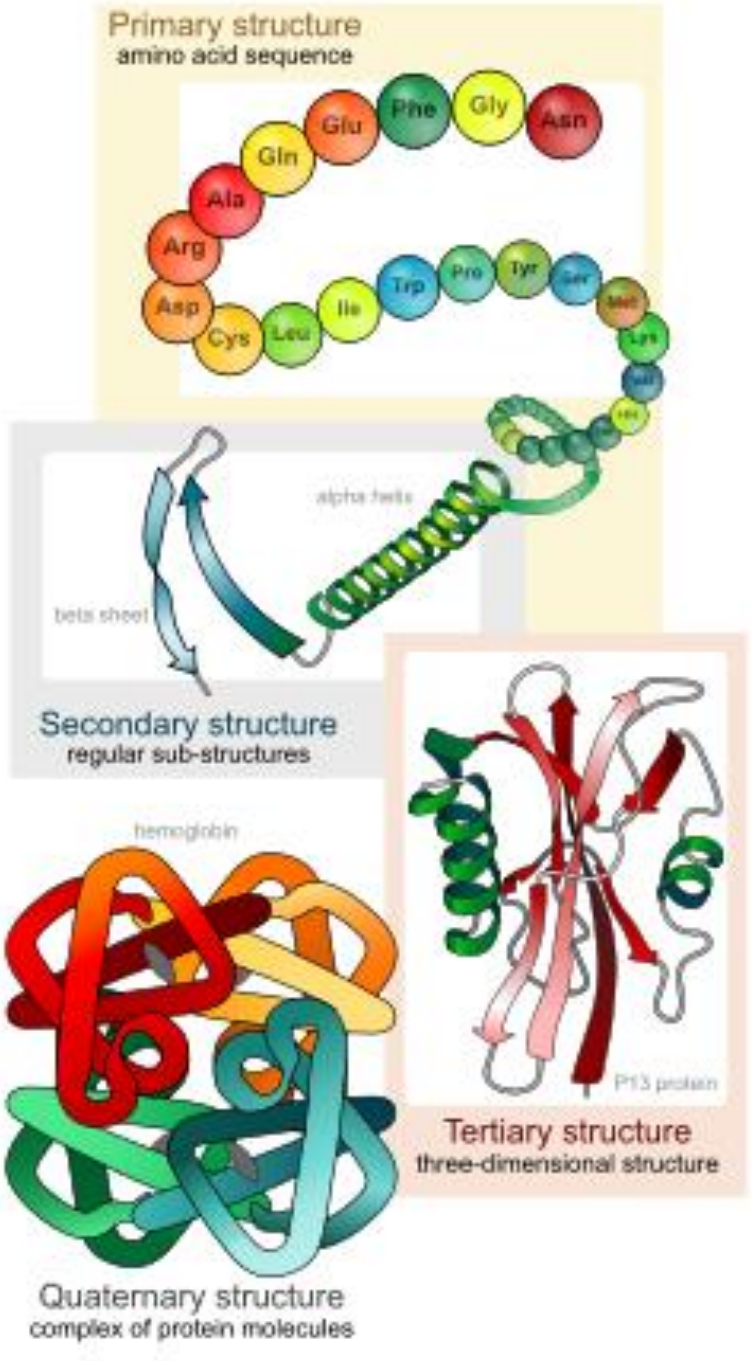


There is a Replication Fork on each side of the Replication Bubble

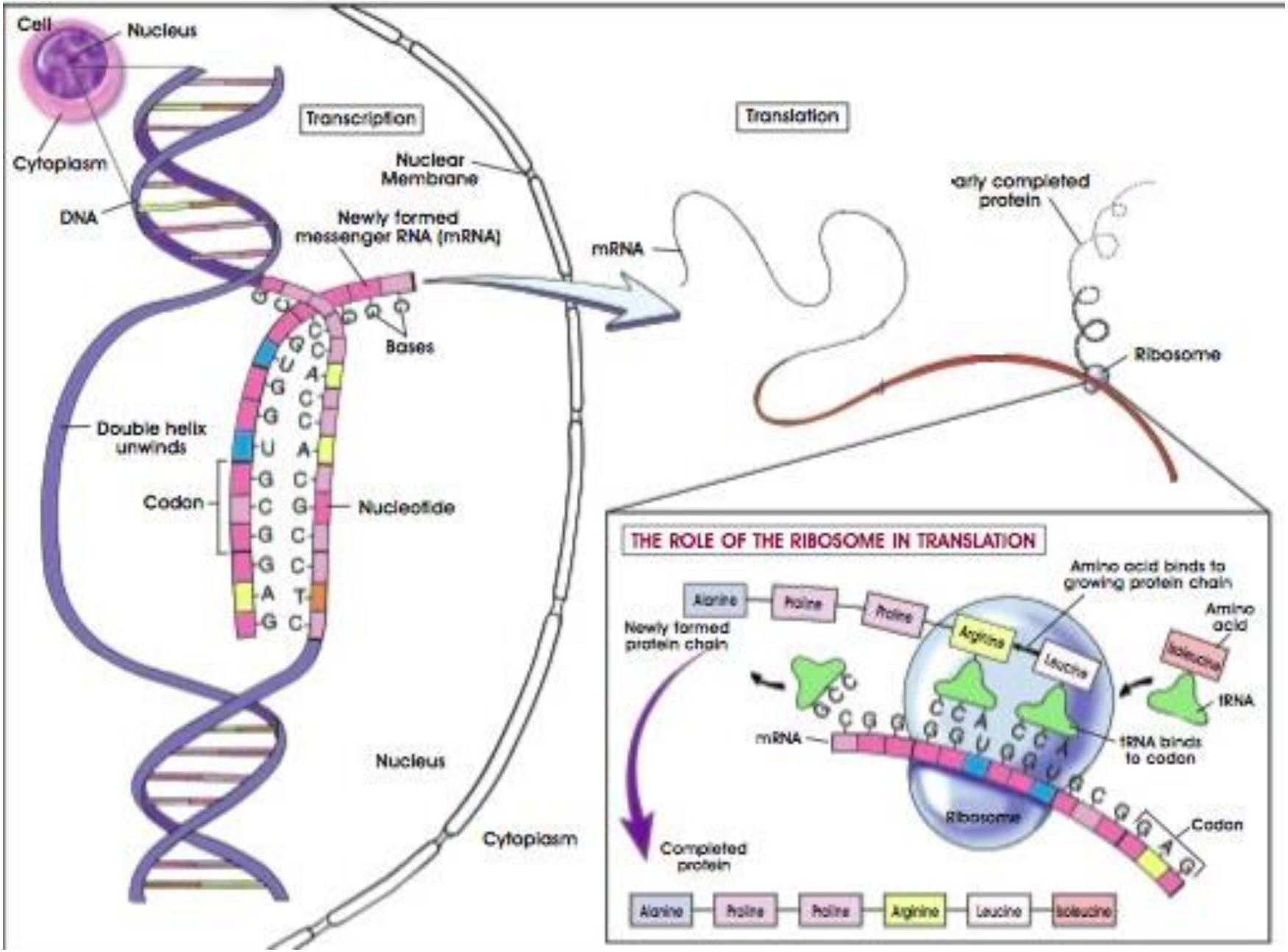


Protein Synthesis

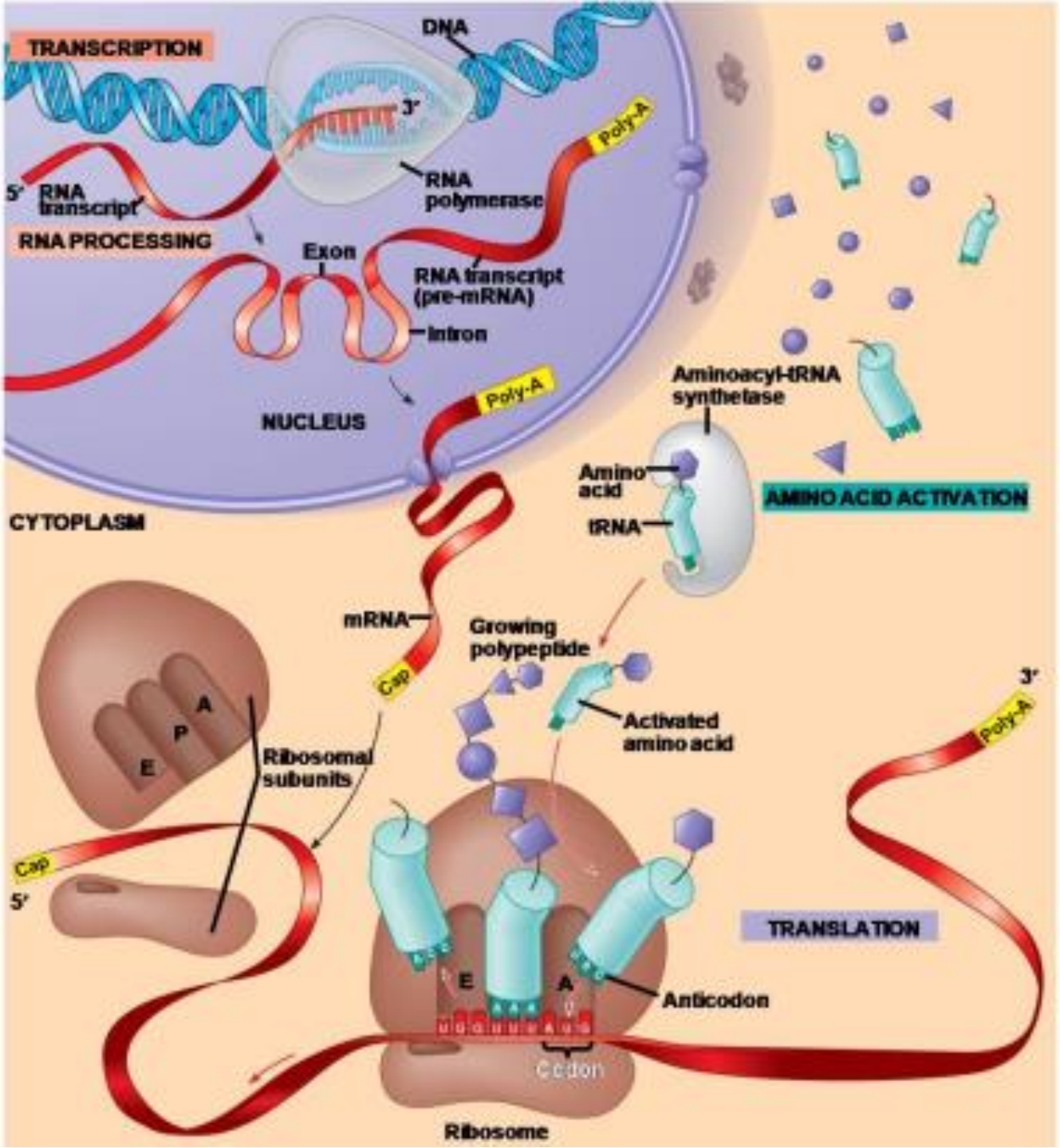
- Literally "making protein".
- The process is divided into two parts:
- 1) Transcription
- 2) Translation



Protein Synthesis – Overview

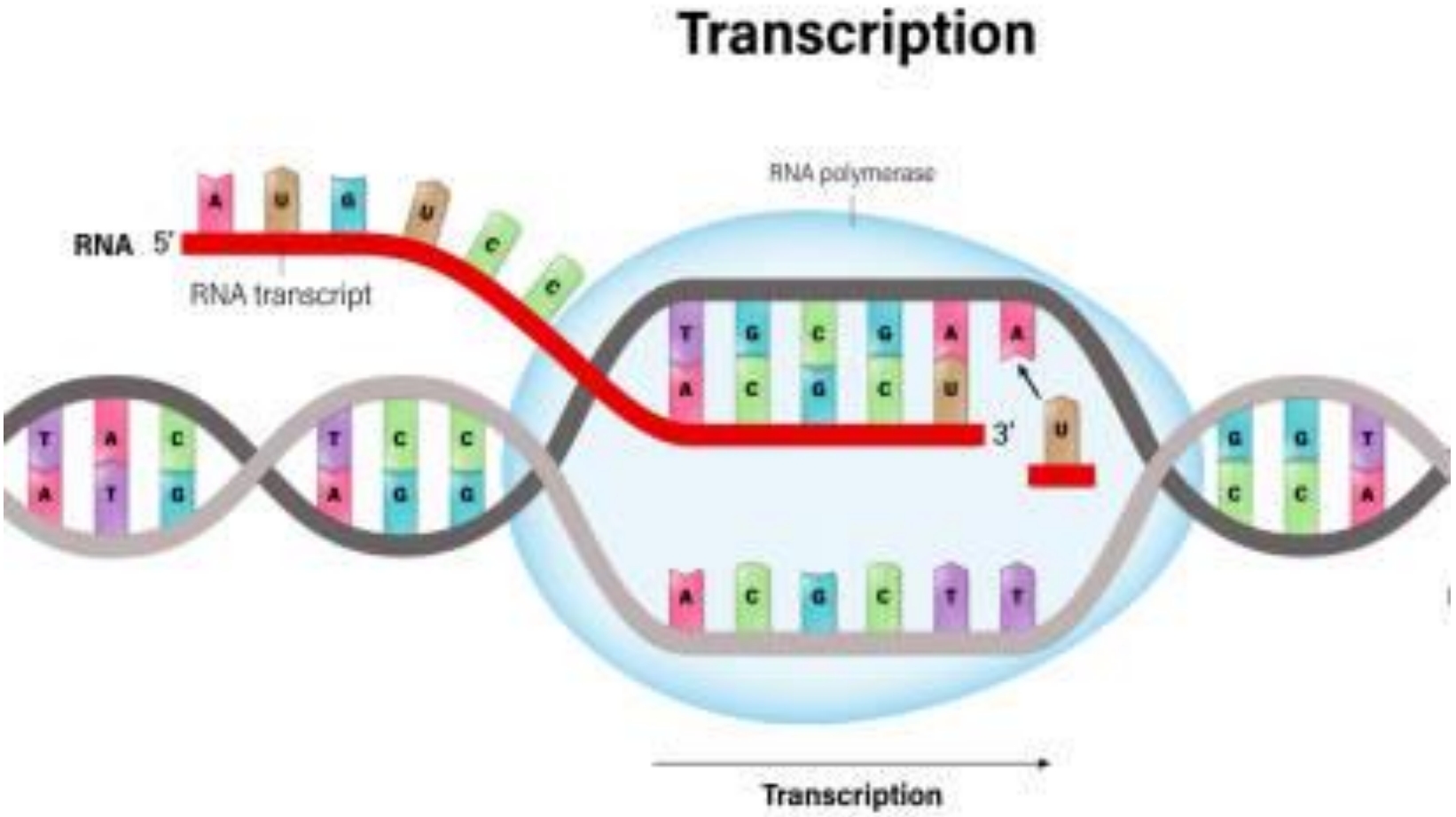


Protein Synthesis in detail



Transcription - Overview

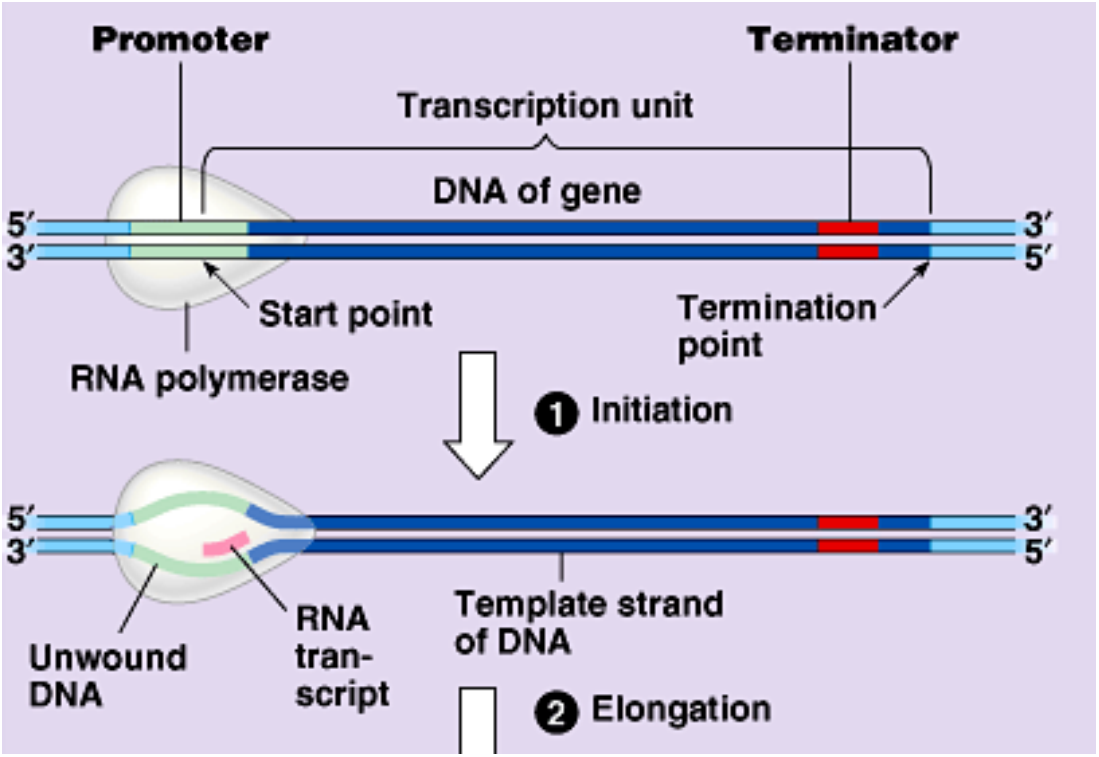
- the process wherein a molecule of mRNA (messenger RNA) is made using a template strand of DNA.
- occurs inside nucleus in the nucleolus.



Transcription – The Steps

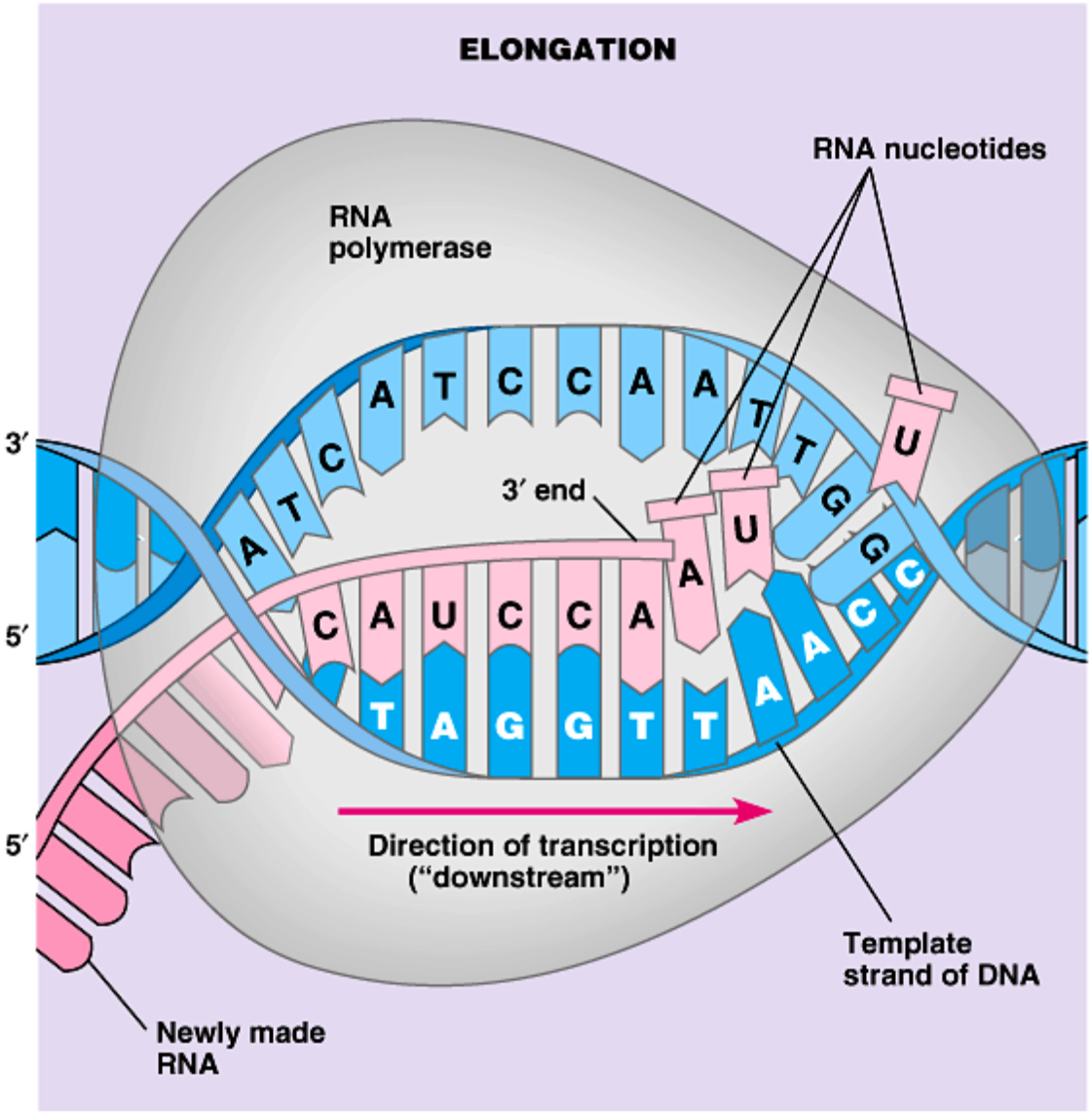
- Step 1 Initiation
  a section of DNA (called a gene) opens up and a promoter sequence allows an enzyme **RNA Polymerase II** to attach to 1/2 the parent DNA.
- Step 2 Elongation
  pre-mRNA forms using open DNA as template
  RNA Polymerase II assembles the RNA nucleotides complementary to the DNA template strand.
- Step 3 Termination
  when **RNA Polymerase II** reaches a terminator sequence of base pairs along the DNA template, transcription halts.
- before it leaves the nucleus, the pre-mRNA is processed by:
  1) having its ends capped to protect it.
  2) having introns (non coding sections) removed while leaving exons (coding sections) in place.
- mRNA moves to cytoplasm to find a ribosome.

Transcription Step 1

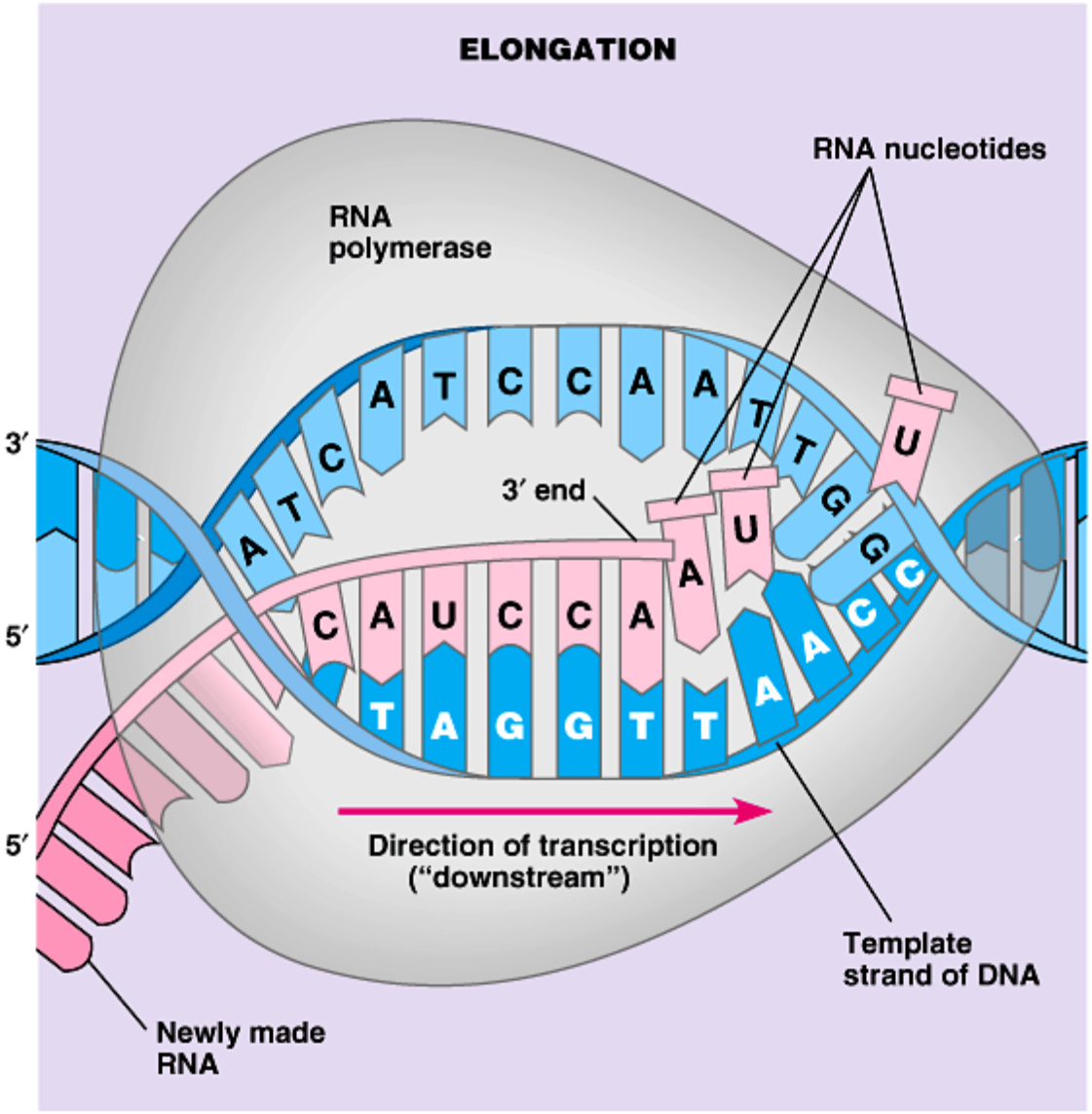


- <u>Step 1 Initiation</u>
  a section of DNA (called a gene) opens up and a promoter sequence allows an enzyme **RNA Polymerase II** to attach to 1/2 the parent DNA.

Transcription Step 2

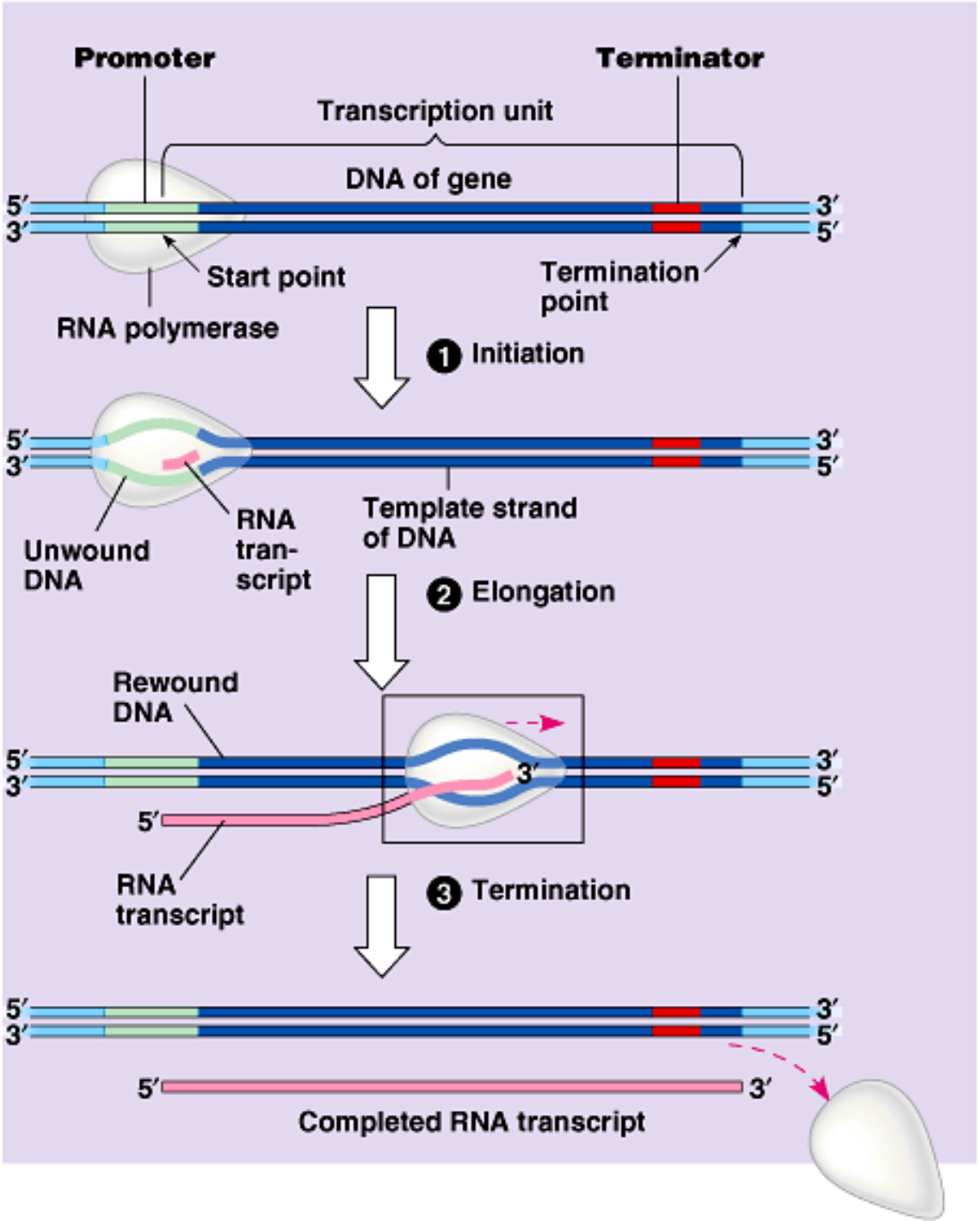


- Step 2 Elongation
  pre-mRNA forms using open DNA as template **RNA Polymerase II** assembles the RNA nucleotides complementary to the DNA template strand.



Transcription Step 3

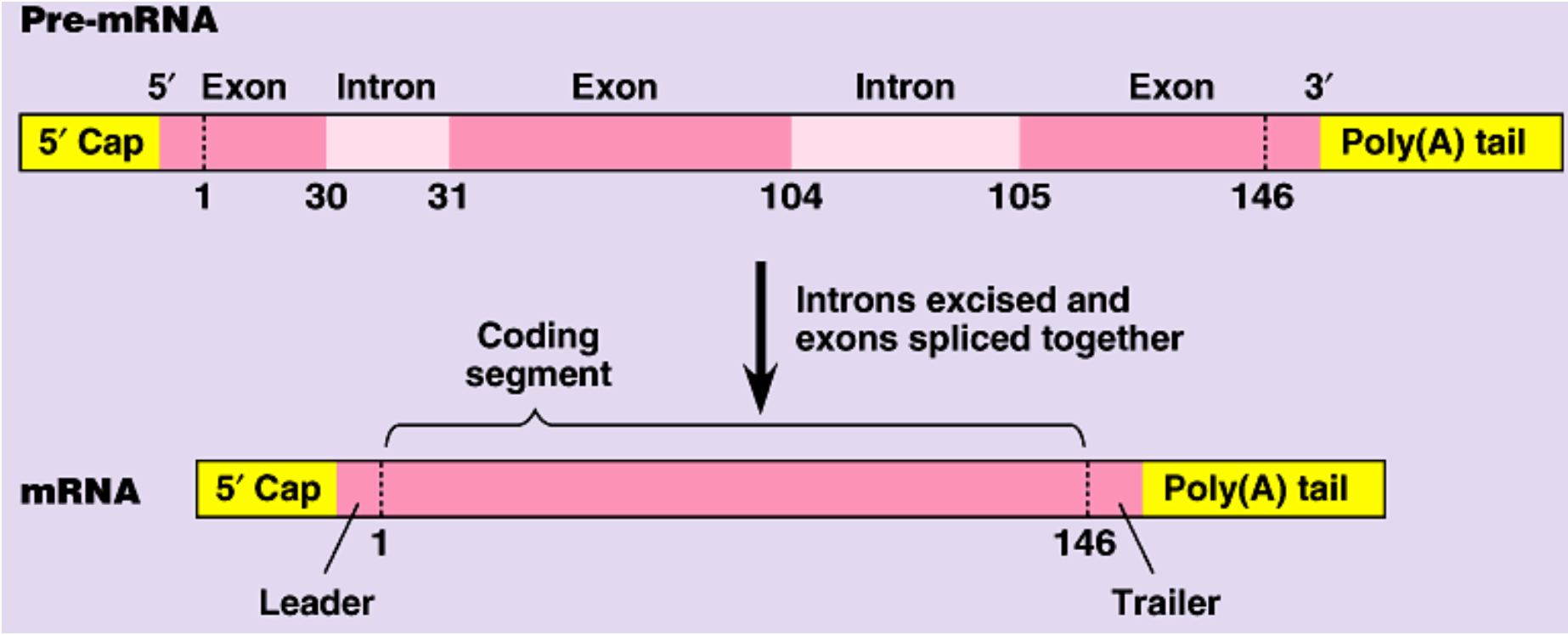
- Step 3 Termination when **RNA Polymerase II** reaches a terminator sequence of base pairs along the DNA template, transcription halts.



Transcription Putting it Together

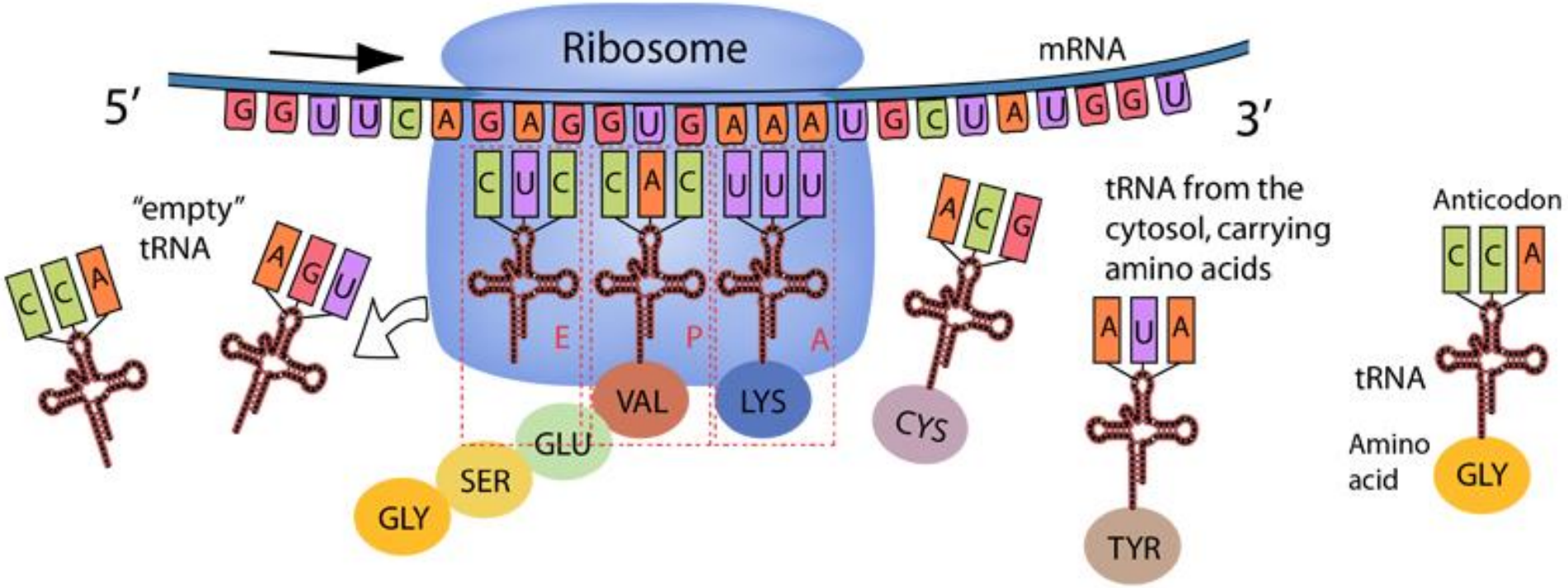
Transcription - mRNA Processing

- before it leaves the nucleus, the pre-mRNA is processed by:
- 1) having its ends caped to protect it.
- 2) having introns (non coding sections) removed while leaving exons (coding sections) in place.



Translation - Overview

- the process of creating a polypeptide (protein) using the genetic information present in the mRNA molecule.
- occurs in cytoplasm at a ribosome.

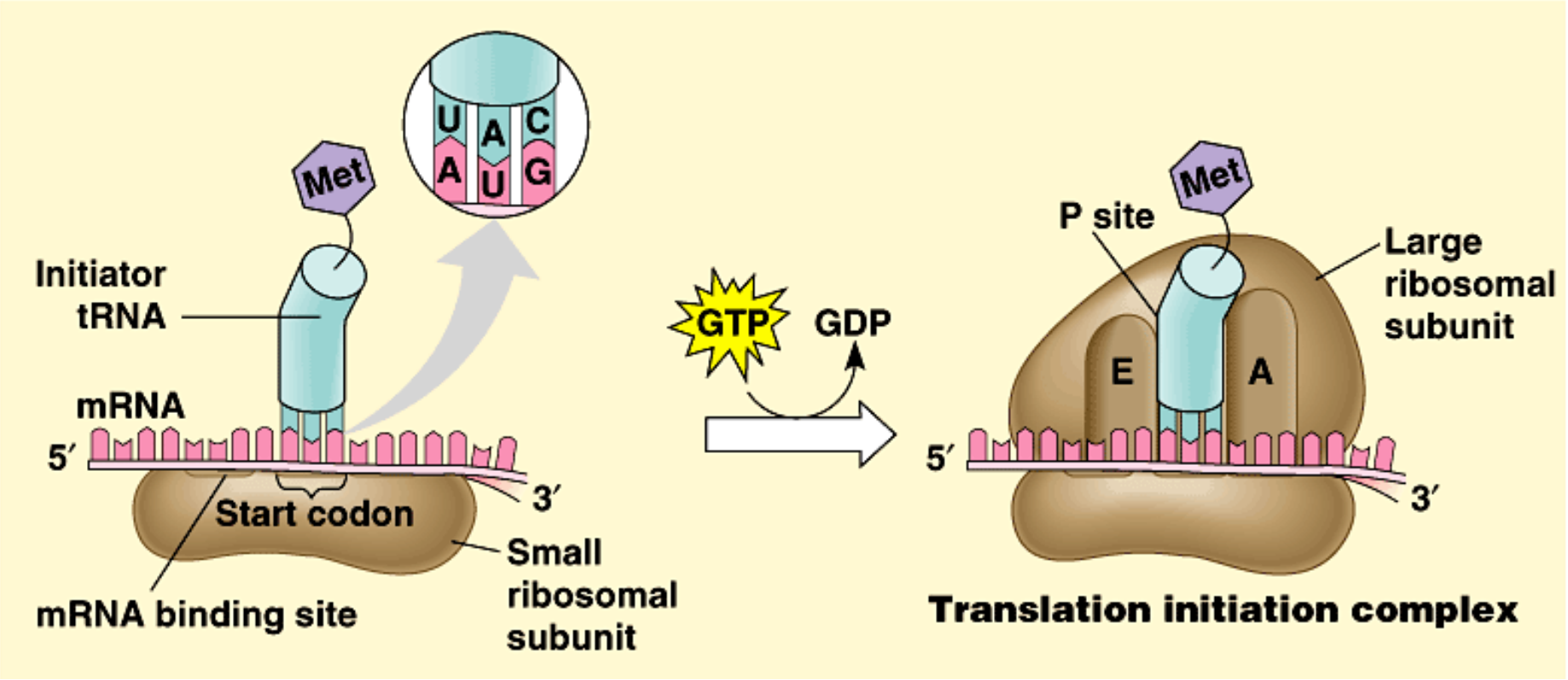


Translation – The Steps

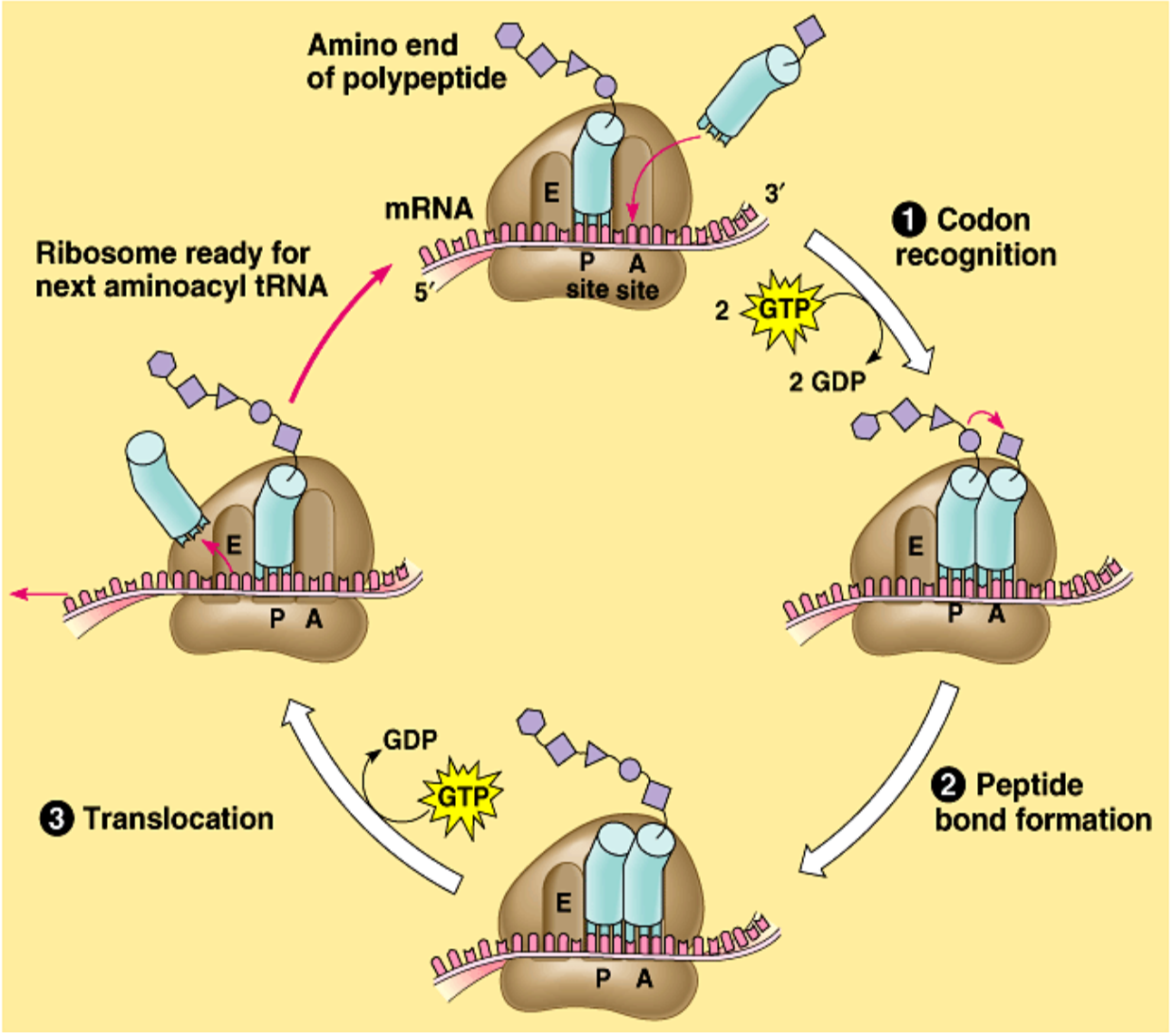
- Step 1 Initiation
  when the mRNA attaches itself to both the ribosome and the tRNA at the “AUG” initiator sequence.
- Step 2 Elongation
  Every 3 nucleotides of mRNA called a **"codon"** codes for a particular amino acid. Transfer RNA (tRNA) carrying an amino acid, binds its **"anticodon"** to the complementary mRNA codon.
- A peptide bond forms between adjacent amino acids and the “empty” tRNA is released to find another amino acid. This continues as the mRNA slides along the ribosome.
- Step 3 Termination
  Translation is terminated when a “stop codon” is reached in the mRNA strand. The completed polypeptide (now called a protein) is released.

Translation Step 1

- <u>Step 1 Initiation</u>
  when the mRNA attaches itself to both the ribosome and the tRNA at the “AUG” initiator sequence.



Translation Step 2



- Step 2 Elongation
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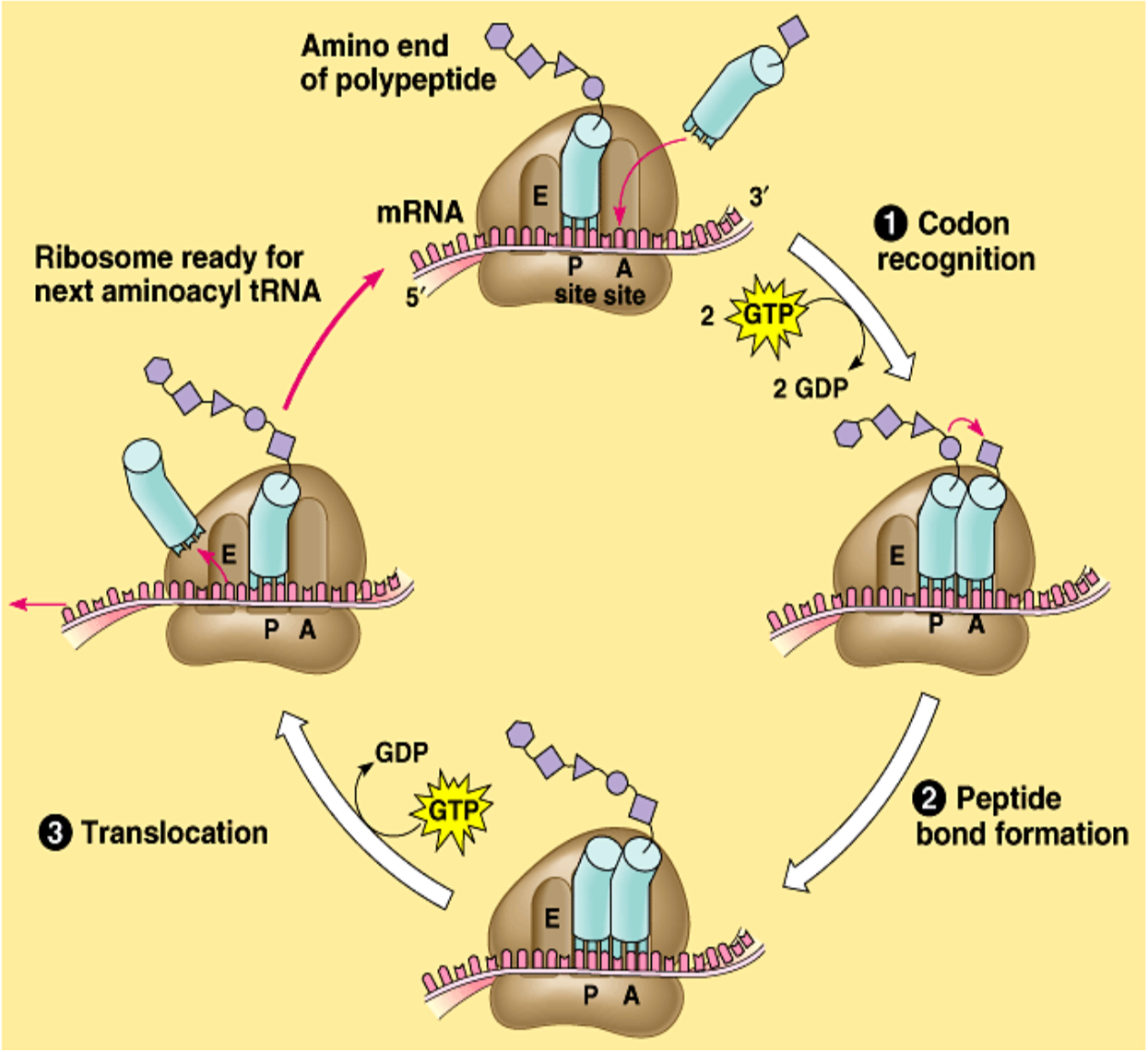
  Transfer RNA (tRNA) carrying an amino acid, binds its **"anticodon"** to the complementary mRNA codon.

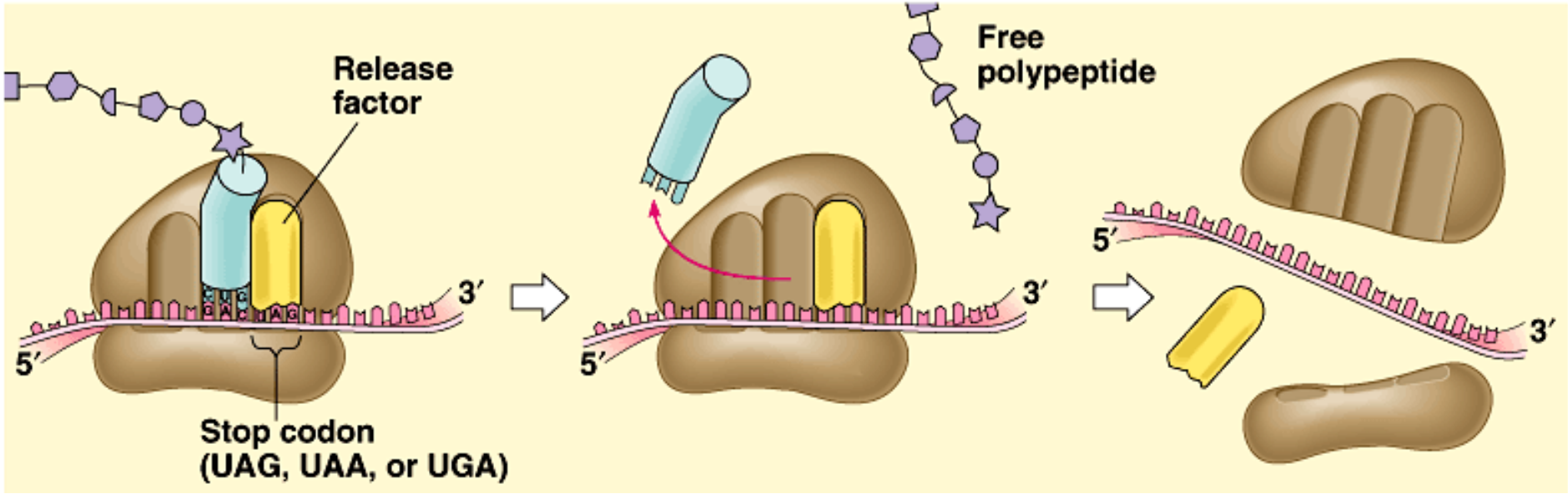
Translation Step 2

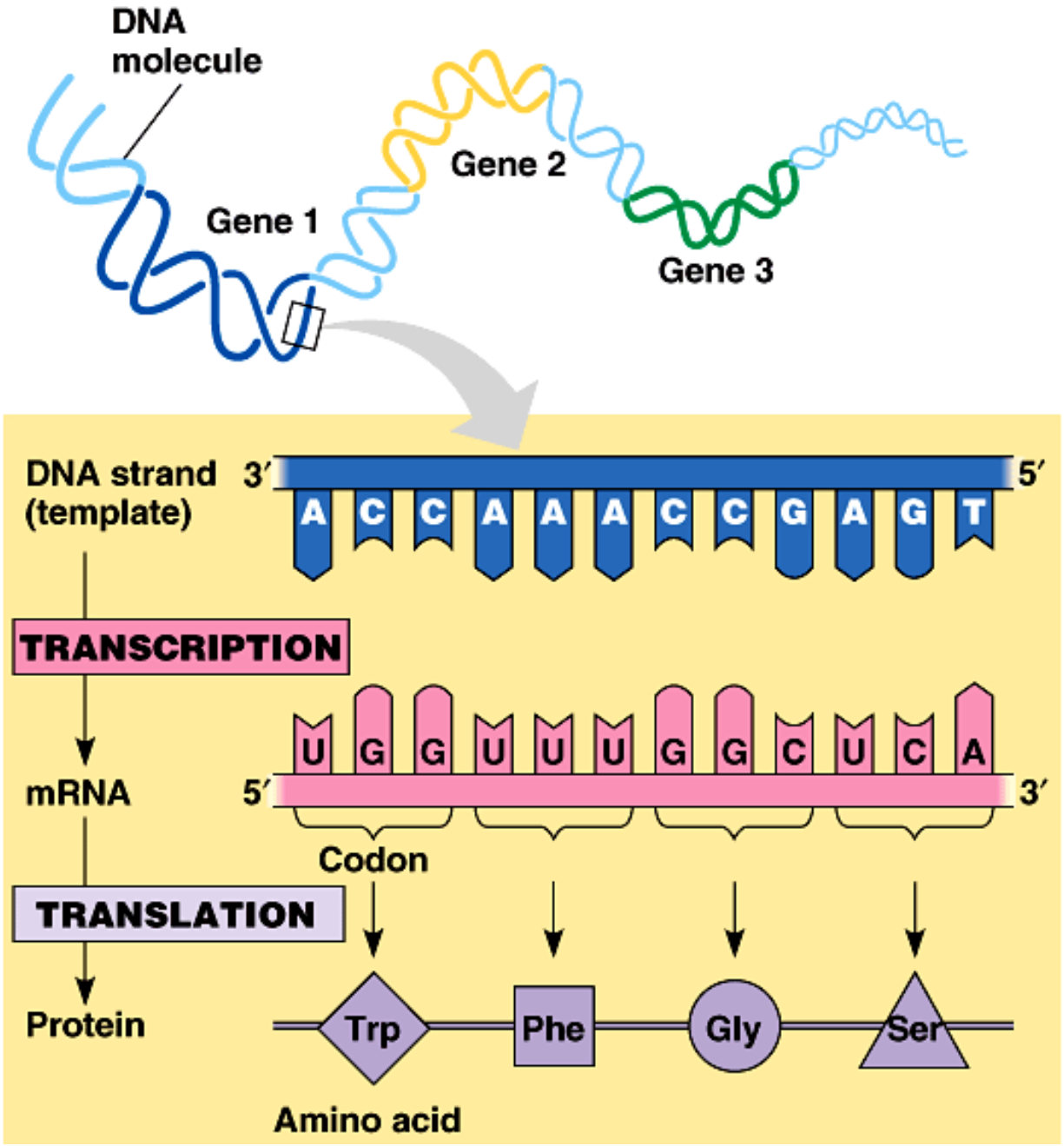


- A peptide bond forms between adjacent amino acids and the "empty" tRNA is released to find another amino acid.
  This continues as the mRNA slides along the ribosome.

Translation Step 3

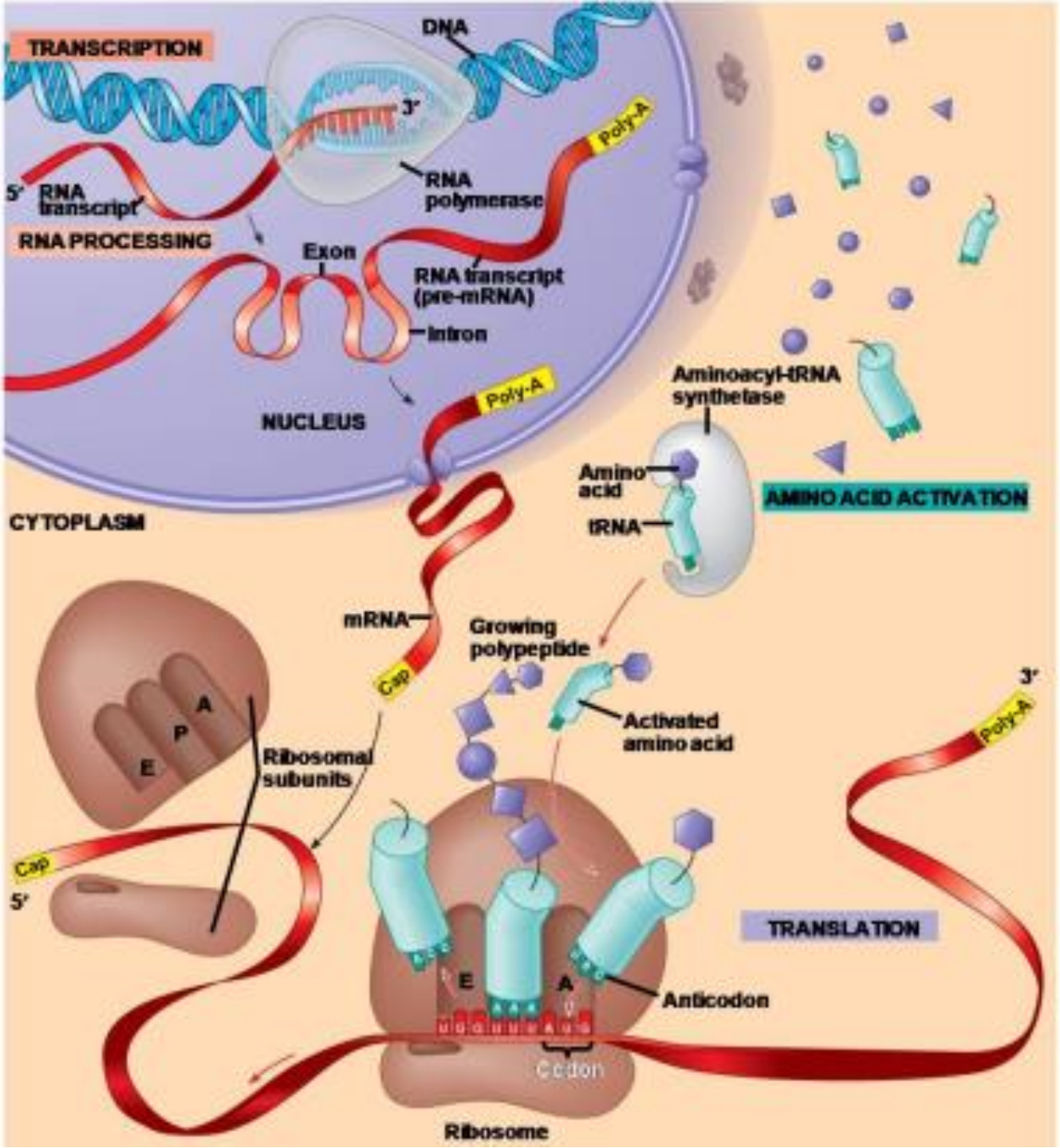
- <u>Step 3 Termination</u>
  Translation is terminated when a “stop codon” is reached in the mRNA strand.
  The completed polypeptide (now called a protein) is released.



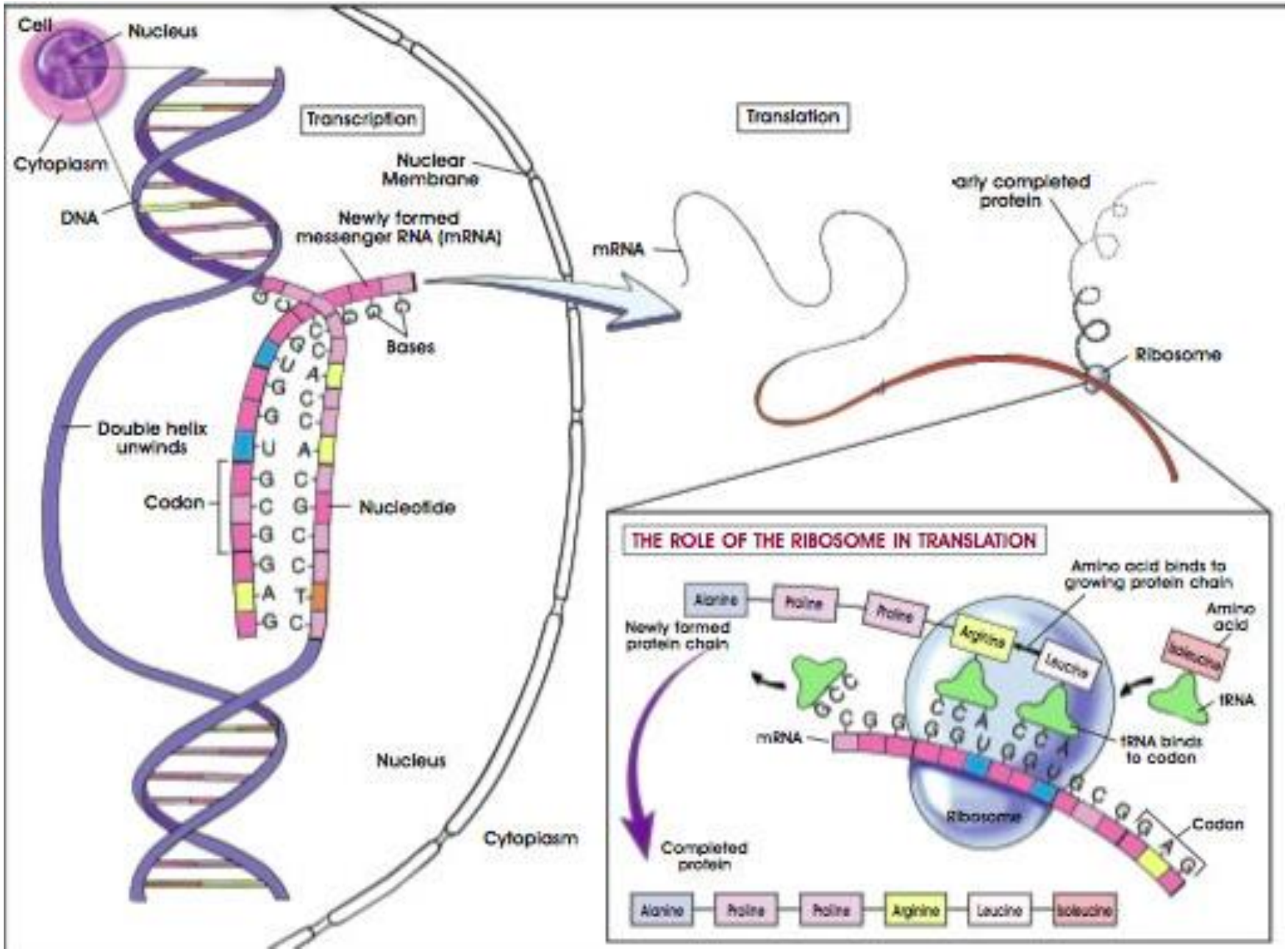


Transcription to Translation

Protein Synthesis in detail



Protein Synthesis - Recap



Recombinant DNA

- Also known as genetic engineering.
- What is Recombinant DNA?
- Genetically engineered DNA prepared by transplanting or splicing genes from one species into the cells of a host organism of a different species.

- The new DNA becomes part of the host's genetic makeup and is replicated.
- New proteins are made as a result.

Recombinant DNA – For the treatment of diseases

- 1) Insulin - Recombinant insulin has almost completely replaced insulin obtained from animal sources (e.g. pigs and cattle) for the treatment of insulin-dependent diabetes.
- 2) Human Growth Hormone - GH is administered to patients whose pituitary glands generate insufficient quantities to support normal growth and development. Before recombinant HGH became available, HGH for therapeutic use was obtained from pituitary glands of cadavers. This unsafe practice led to some patients developing serious diseases.

Recombinant DNA – For the treatment of diseases

- 3) Clotting Factors for hemophiliacs - Recombinant blood-clotting proteins are administered to patients with forms of the bleeding disorder hemophilia, who are unable to produce clotting factors in quantities sufficient to support normal blood coagulation.
- 4) Hepatitis B Vaccine Production - Prevention of HB infection is controlled through the use of a recombinant hepatitis B vaccine, which contains a form of the hepatitis B virus surface antigen that is produced in yeast cells.

Recombinant DNA – For the production of genetically modified foods

- 1) Herbicide Resistance Plants - Agricultural crops (including soy, corn, sorghum, canola, alfalfa, wheat and cotton) have been developed which incorporate a recombinant gene that results in resistance to herbicides.
- Work on Round-Up resistant Canola was done at the U of M in Winnipeg.
- 2) Insect Resistant Crops - adopted in agriculture and gardening, plants have been developed which express a recombinant form of the bacterial protein, which may effectively control some insect predators.

Recombinant DNA – For the production of genetically modified foods

- 3) Biosteel - a high-strength fiber material made of the recombinant spider silk protein extracted from the milk of recombinant goats.
- Biosteel is a lightweight, strong, and versatile materials being considered for a variety of medical and industrial applications (medical sutures and replacing Kevlar for bulletproof vests).
- 4) High Protein Wheat – after isolating a gene in wild wheat that increases the grain protein content by 15%, researchers at U of Cal Davis have successfully spliced the gene into domestic wheat.

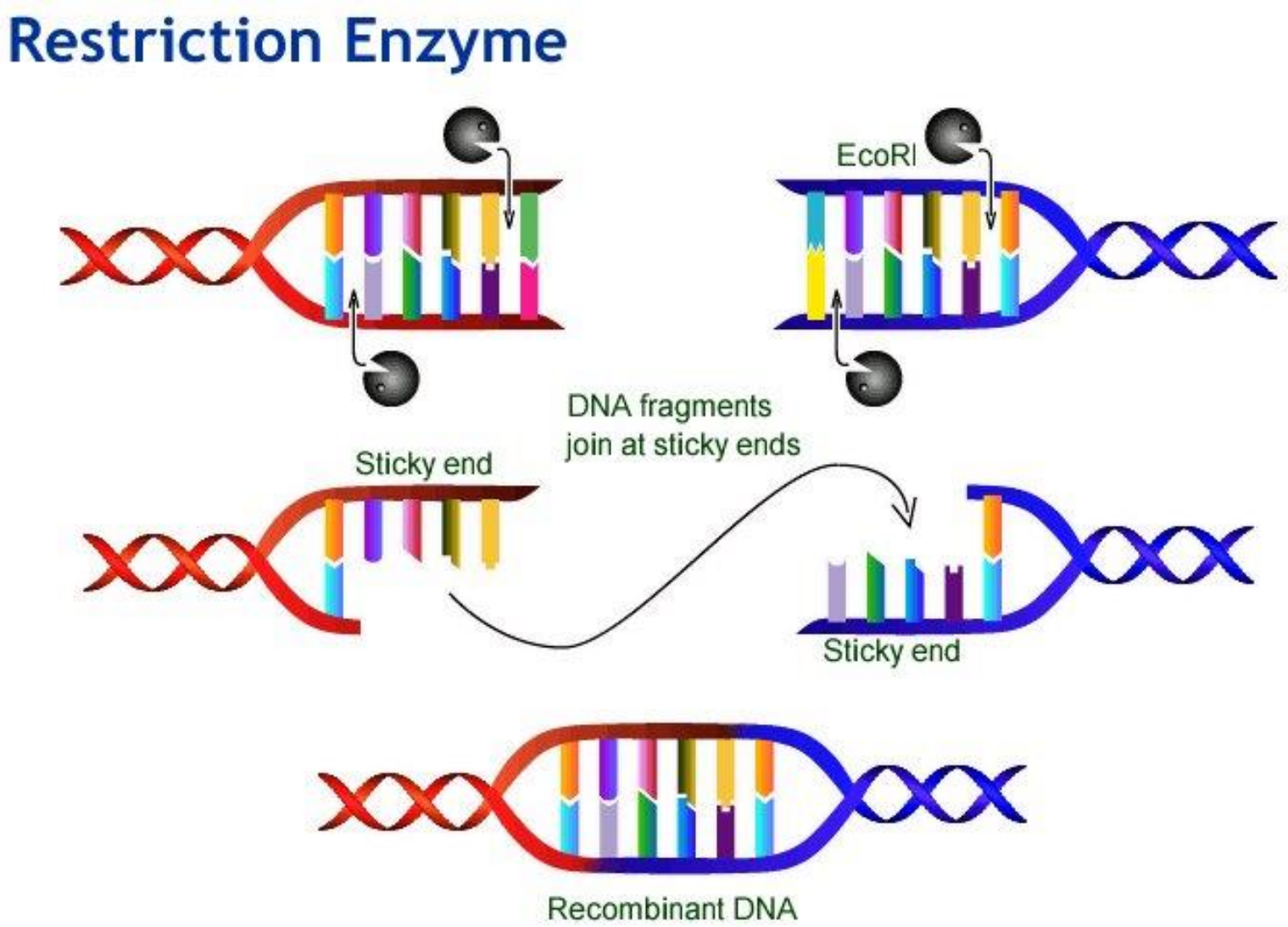
Recombinant DNA – For the production of genetically modified foods

- 5) Better Cheese – scientists have engineered a cheese making bacteria that has a gene that produces an enzyme (a protein) that eliminates the bitter taste created during ripening of certain cheeses.
- 6) Genetically modified tomatoes are 15% sweeter than regular tomatoes.
- 7) Rodent Altered Lettuce - by splicing rat genes into lettuce, Virginia Tech scientists have increased the vitamin C content of lettuce. They have yet to work out a name as “Rat Lettuce” seems somewhat unappetizing.

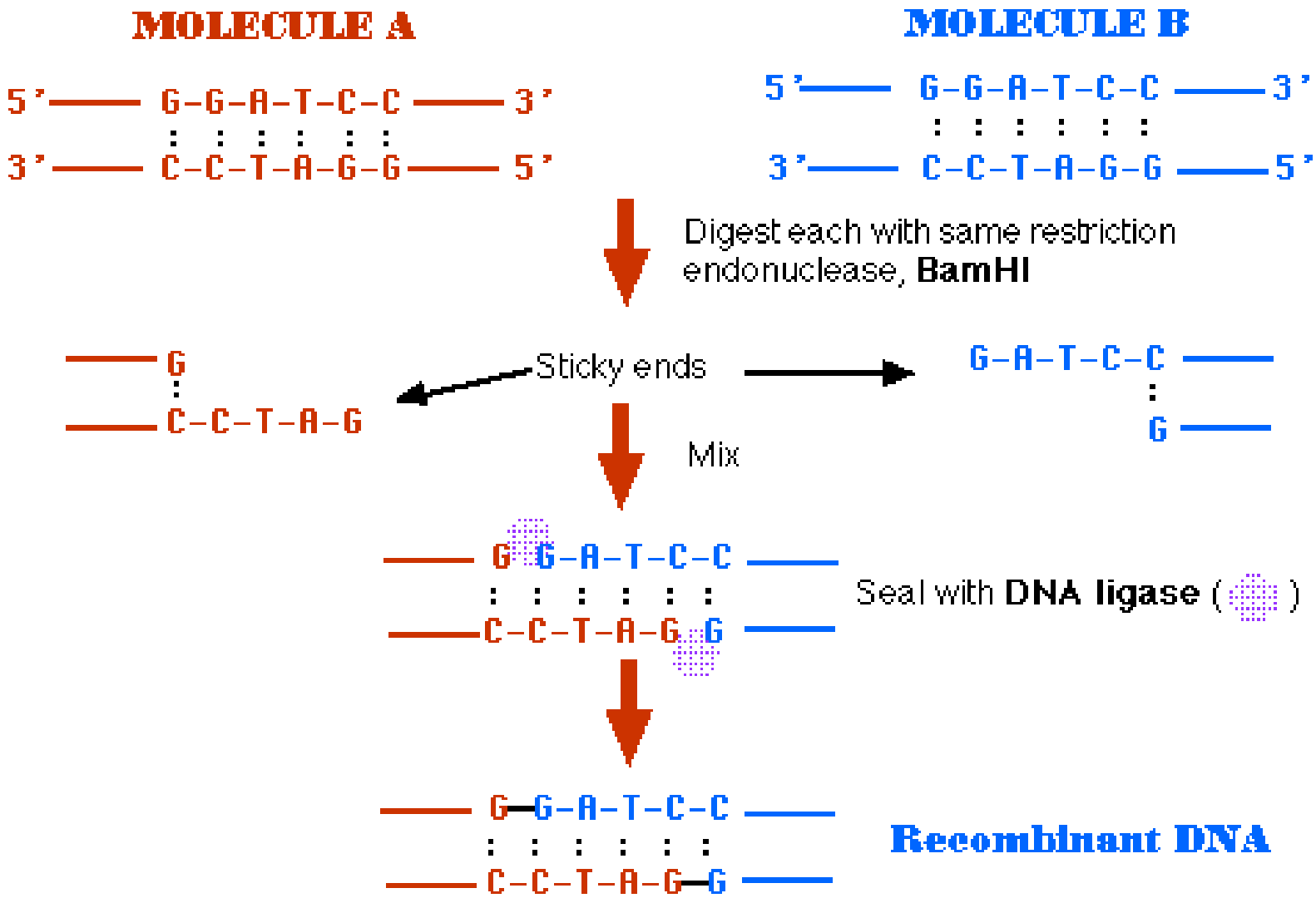
Recombinant DNA – How it Works

- The basic technique of recombinant DNA involves digesting a section of DNA with a restriction enzyme (a molecular scissors)
- The restriction enzyme cuts DNA at specific sites.
- A DNA molecule from the organism of interest is also digested, in a separate tube, with the same restriction enzyme.
- The two DNAs are then mixed together and joined, using an enzyme called DNA ligase (linker)

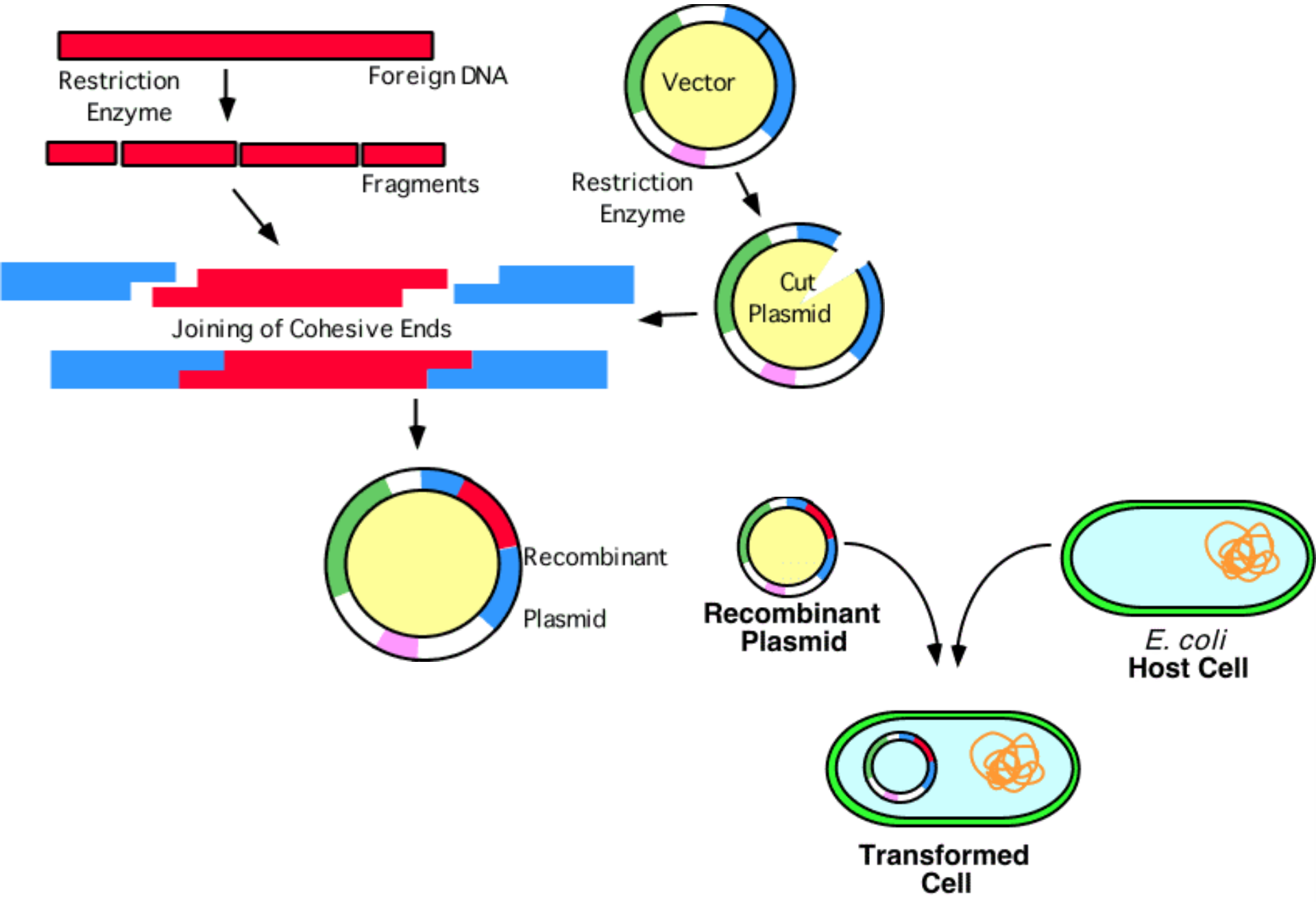
Recombinant DNA – How it Works



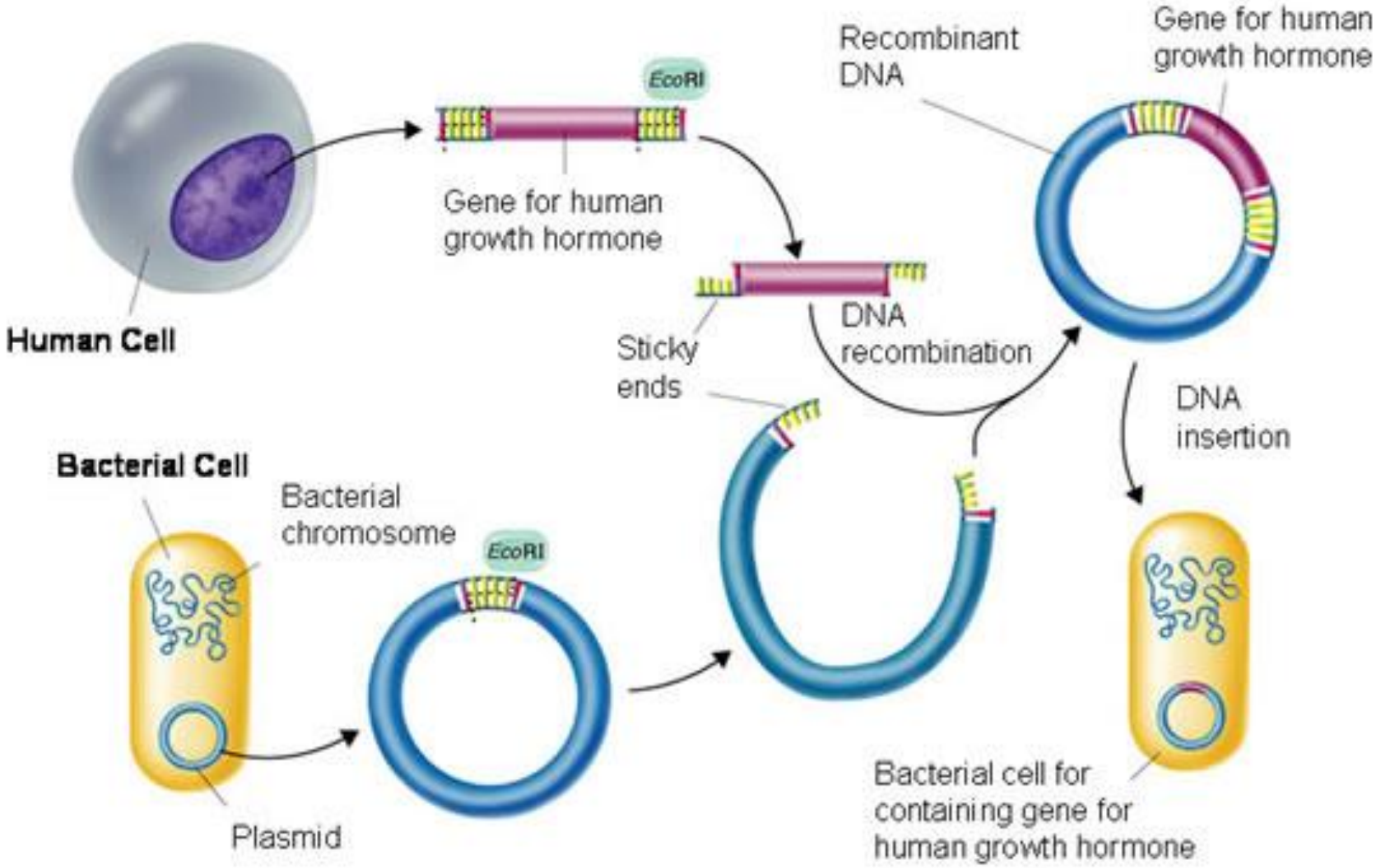
Recombinant DNA – How it Works



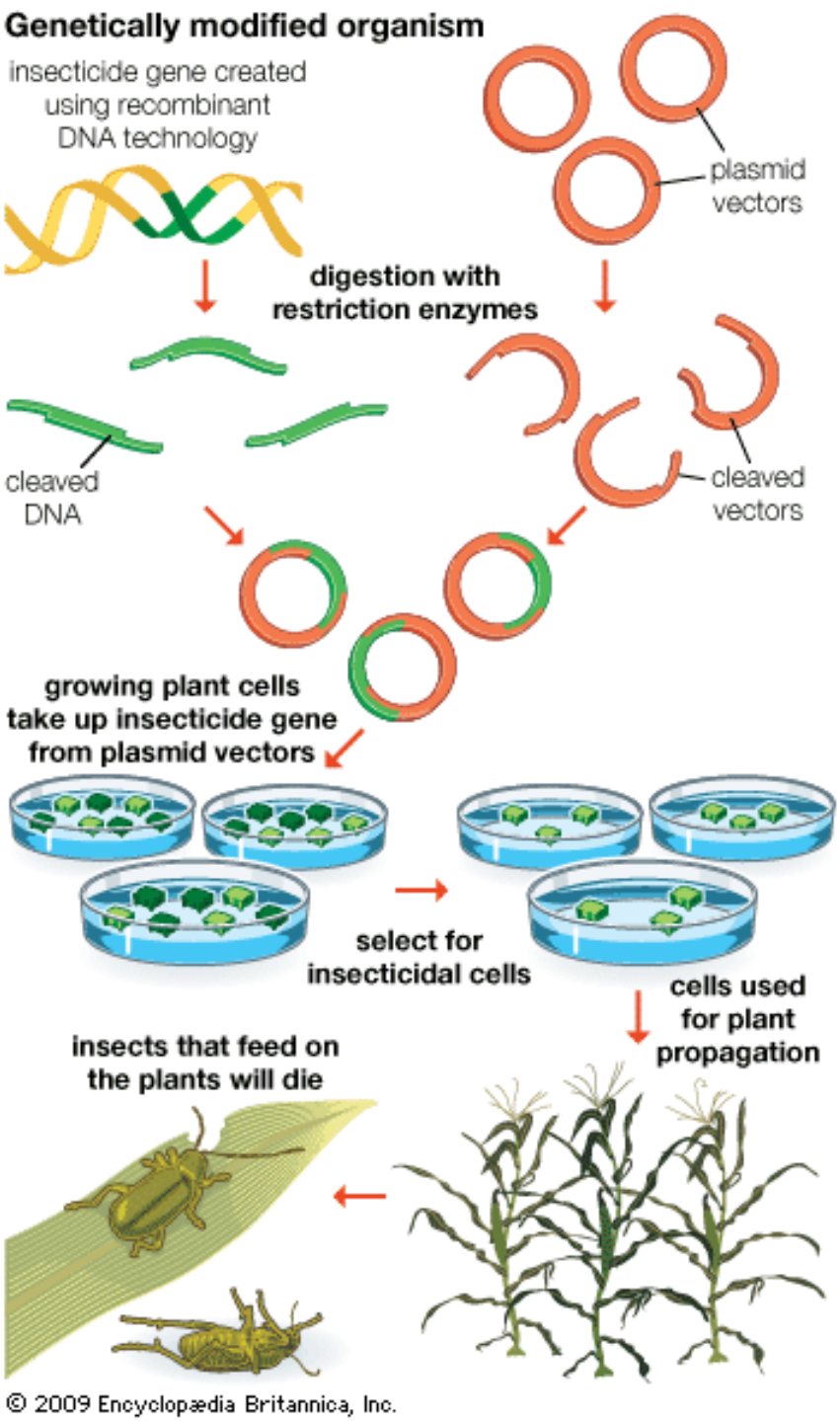
Recombinant DNA with Bacteria



Bacteria are used to Produce Human Growth Hormone

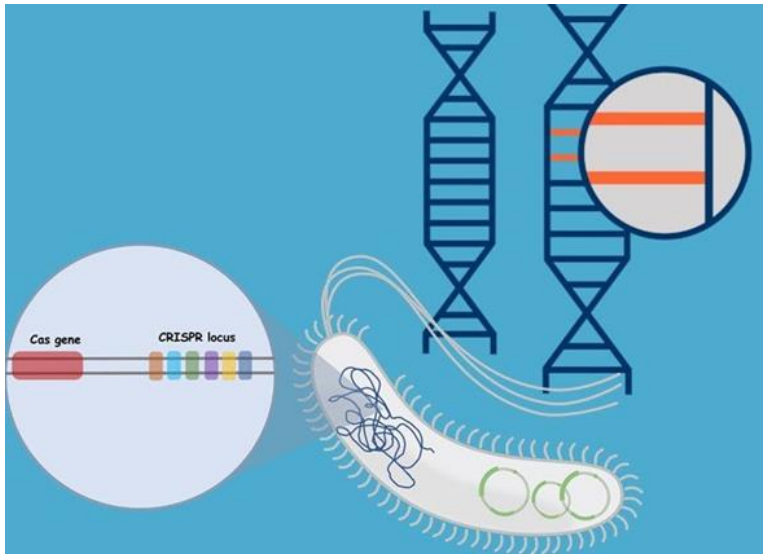


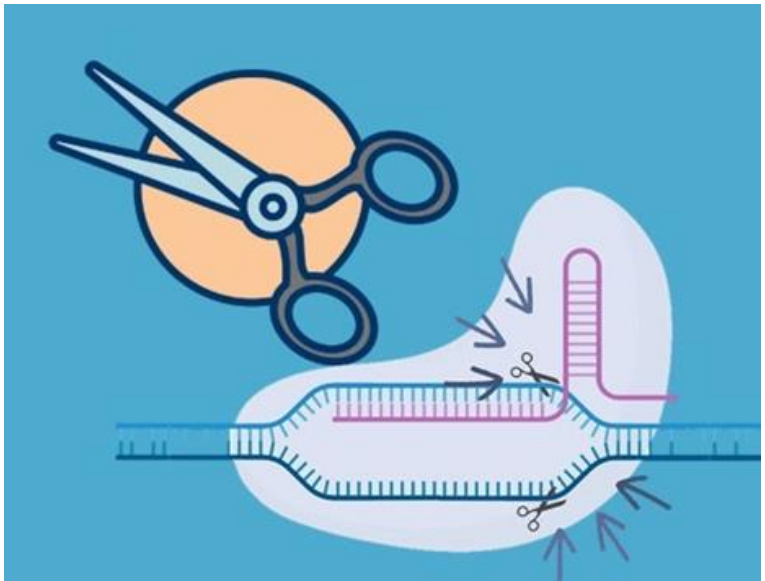
Genetically Modified Plants – can produce a protein insecticide



CRISPR Cas9 Gene Editing

- **C**lusters of
- **R**egularly
- **I**nterspaced
- **S**hort
- **P**alindromic
- **R**epeats





CRISPR Cas9 Gene Editing

- 2012 **Emmanuelle Charpentier** and **Jennifer Doudna** hypothesize CRISPR might be used as a "cut-and-paste" gene editing tool.
- They won the Nobel Prize for their work with CRISPR Cas9 in 2020.
- It's an extremely precise, technique for **GENE EDITING**.
- Regarded by MANY as the most significant scientific breakthrough in the last century. It's expected to lead the **genetic revolution**.
- CRISPR has decreased costs of gene editing by 99%.
- CRISPR has reduced research time for gene editing from 1-2 years to 1-2 weeks.

CRISPR Cas9 Gene Editing

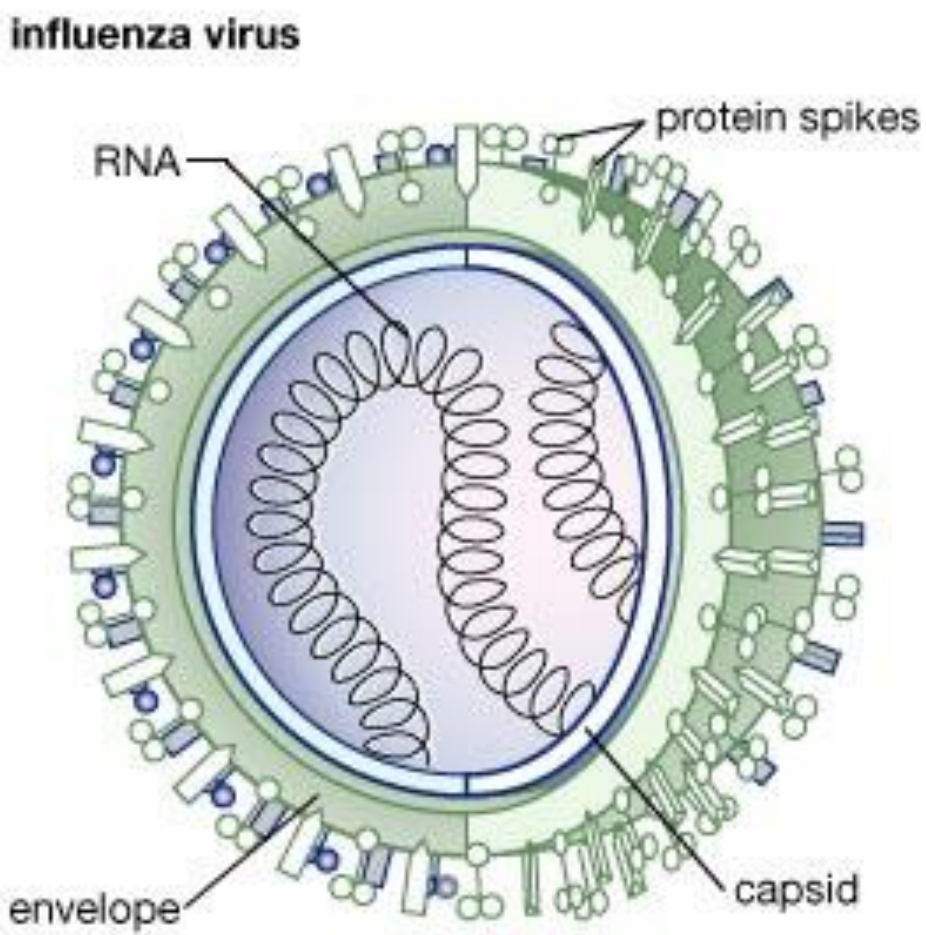
- CRISPR gene editing is analogous to how we can edit paragraphs using Microsoft Word.
- In the days of the typewriter, fixing mistakes was extremely slow and major editing was essentially impossible without retyping the entire document.
- With CRISPR the gene editing can be very precise, allowing for the addition or deletion of a specific gene and even the correction of a single base error along the genetic code. Consider the sentence below.
- In this analogy we have a that requires some **editing editing** in order for it **sentence** to make sense.

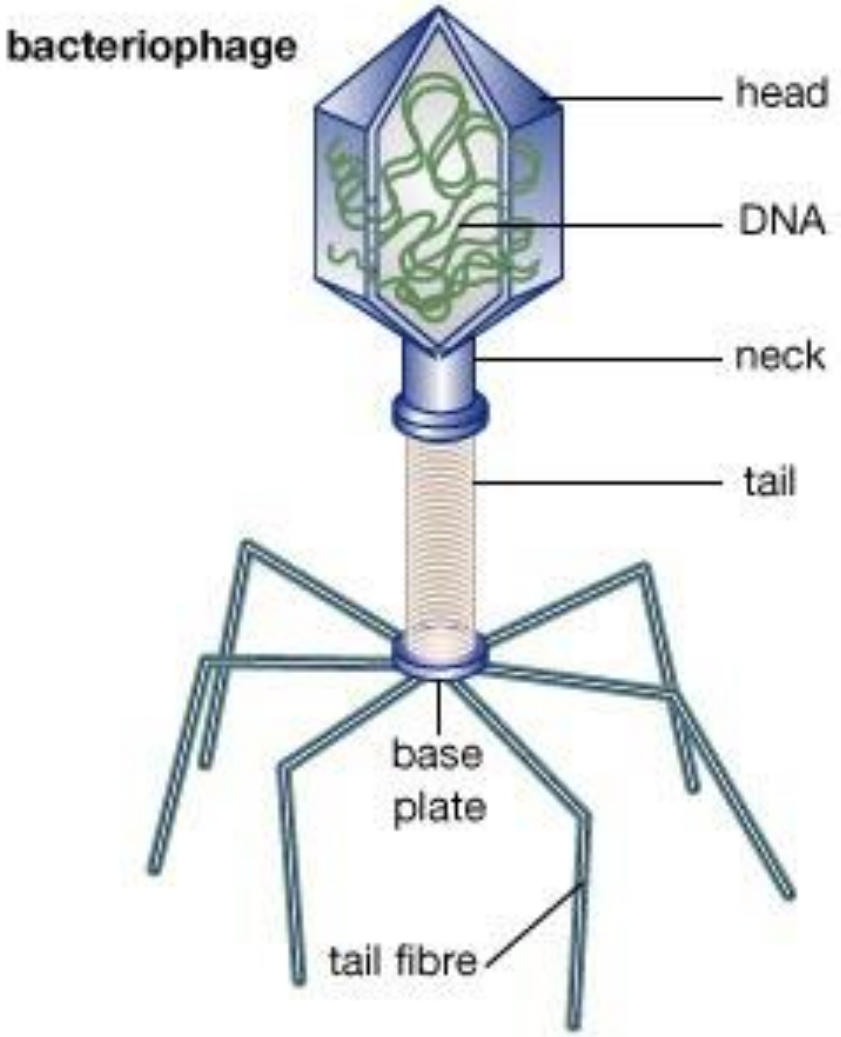
The 3.5 Billion Year Old War

- There is a war being waged on earth that has been going on for over 3.5 billion years.
- It is the battle between bacteria and viruses. (Viruses kill 40% of bacteria)

The 3.5 Billion Year Old War

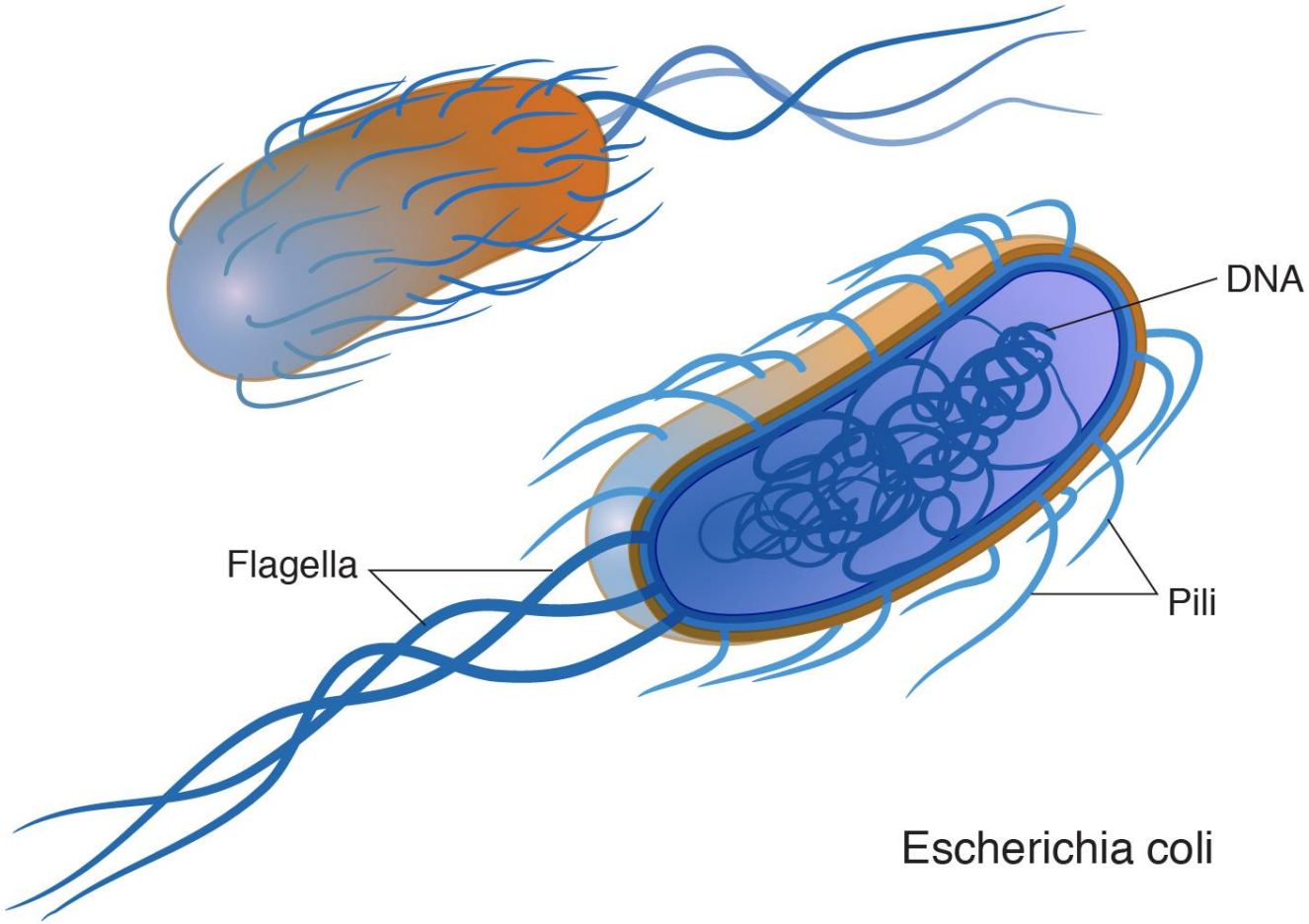
- Below is the morphology of 2 typical viruses.





The 3.5 Billion Year Old War

- Below is the morphology of a typical bacteria.



1987 Japanese scientist **Yoshizumi Ishino** discovered the following interesting and perplexing pattern when studying gene sequencing in E. Coli (bacteria).

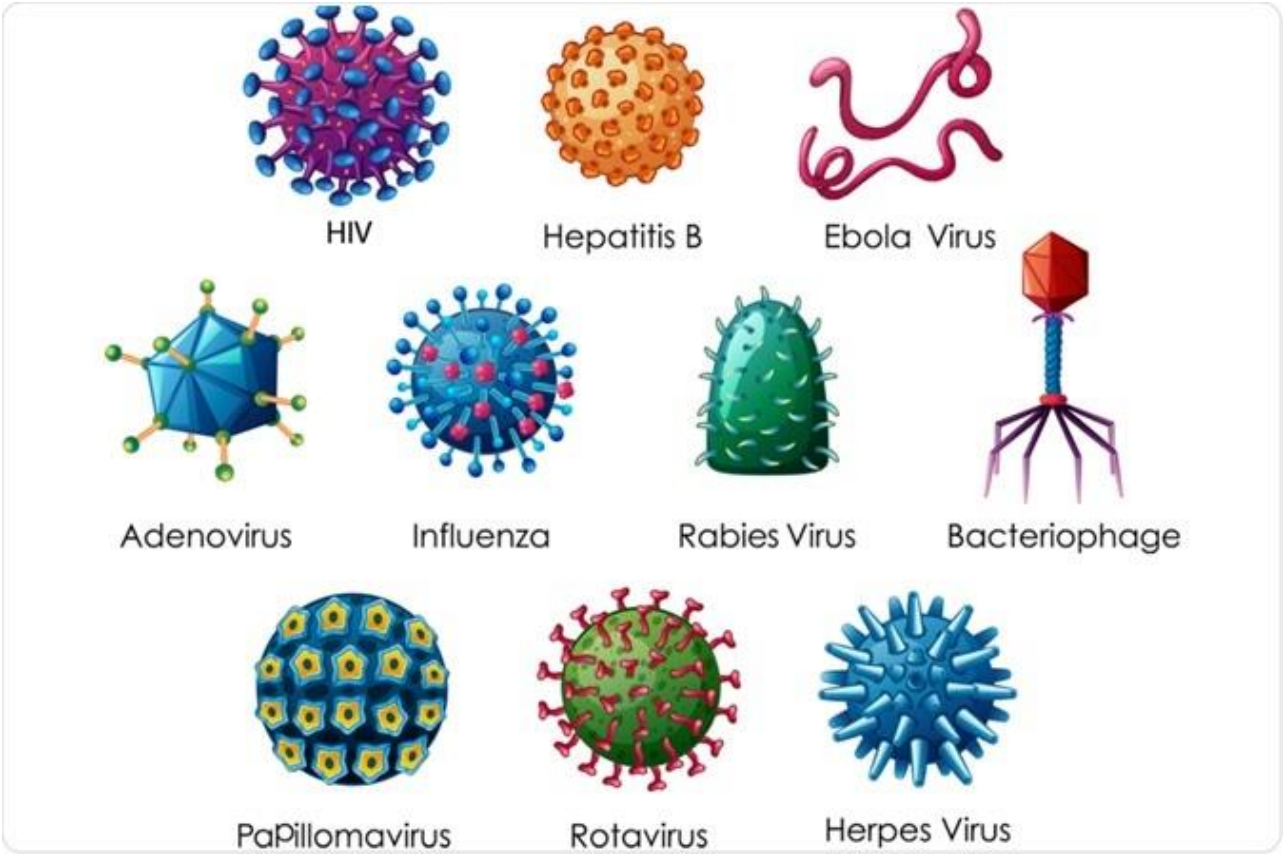
….ATCTATCAGAACTTGTTCATCTGAGCTACACGGGATACTTGTTCAATCACAGATA

TACAGACTTGTTCACTGATTAGATACGATAGACTTGTTCACTAAAACAGATAGACAC

CATAGACTTGTTCACTAACCAACATACGATACTTGTTCAATCAACACATTATATCAG

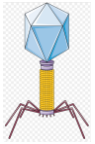
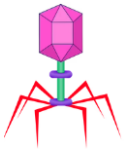
AGAAACACTTG…

2000 advancements in gene sequencing and the availability of gene sequence databases allow **Francisco Mojica** to discover that the “interspaced random DNA segments” **Yoshizumi Ishino** discovered match those of viruses.

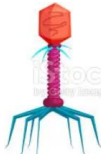


But what did this mean…

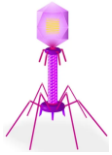
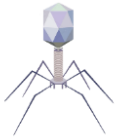
….ATCTATCAGAACTTGTTCATCTGAGCTACACGGGATACTTGTTCAATCACAGATA

TACAGACTTGTTCACTGATTAGATACGATAGACTTGTTCACTAAAACAGATAGACAC

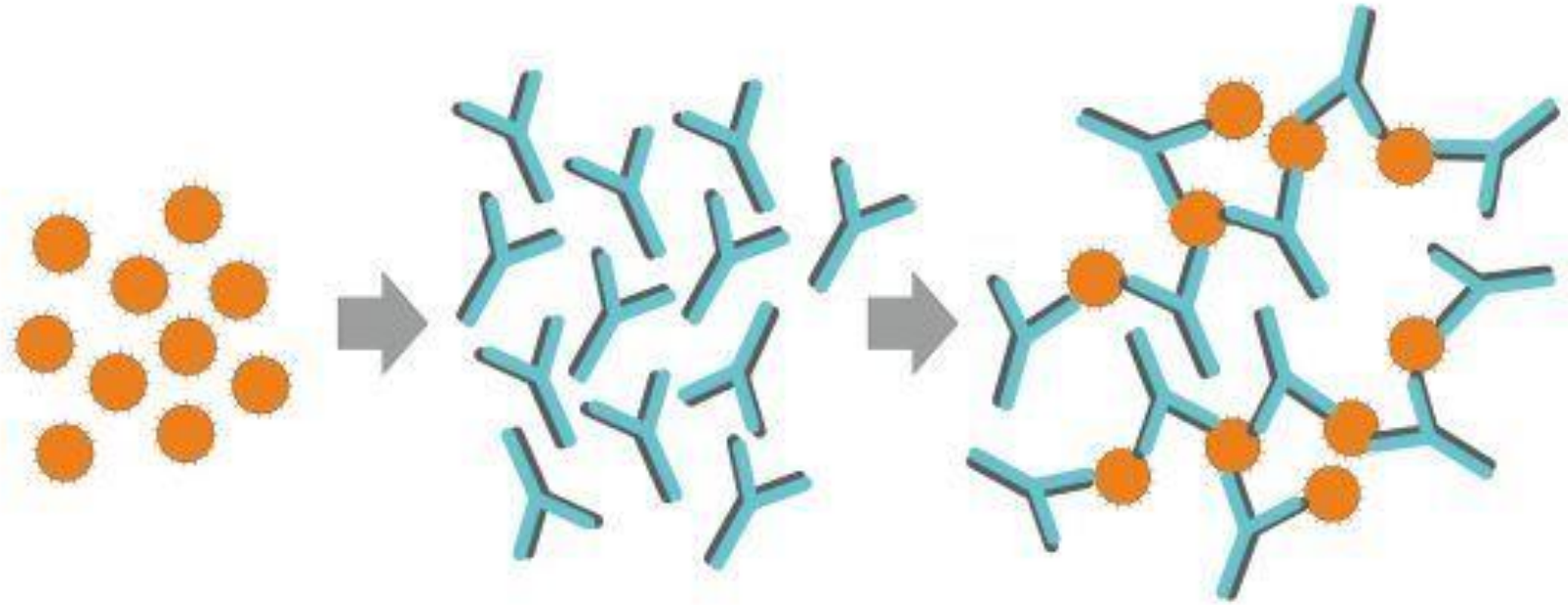



CATAGACTTGTTCACTAACCAACATACGATACTTGTTCAATCAACACATTATATCAG

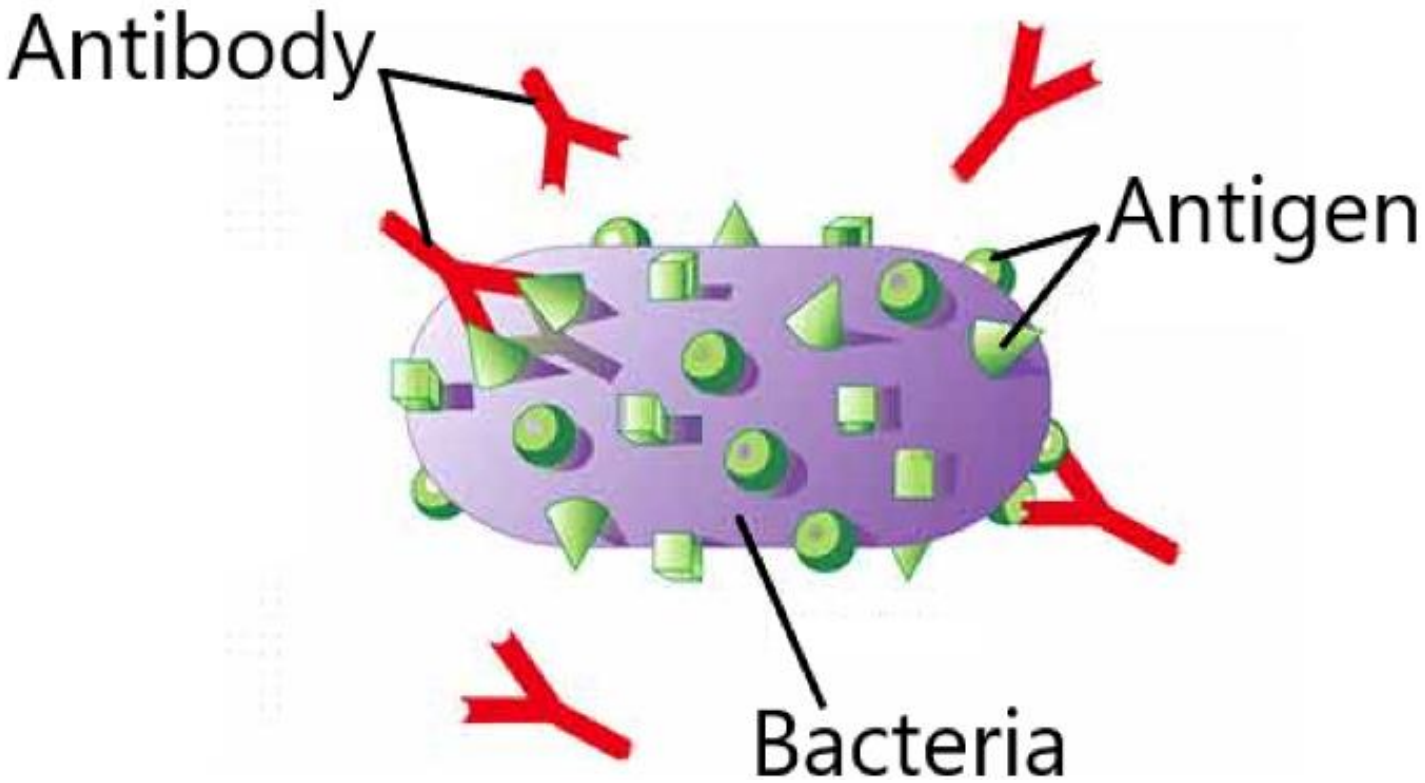
AGAAACACTTG…

Francisco Mojica hypothesized that this CRISPR system was some type of bacterial defense system…possibly similar to our antigen antibody immune system.



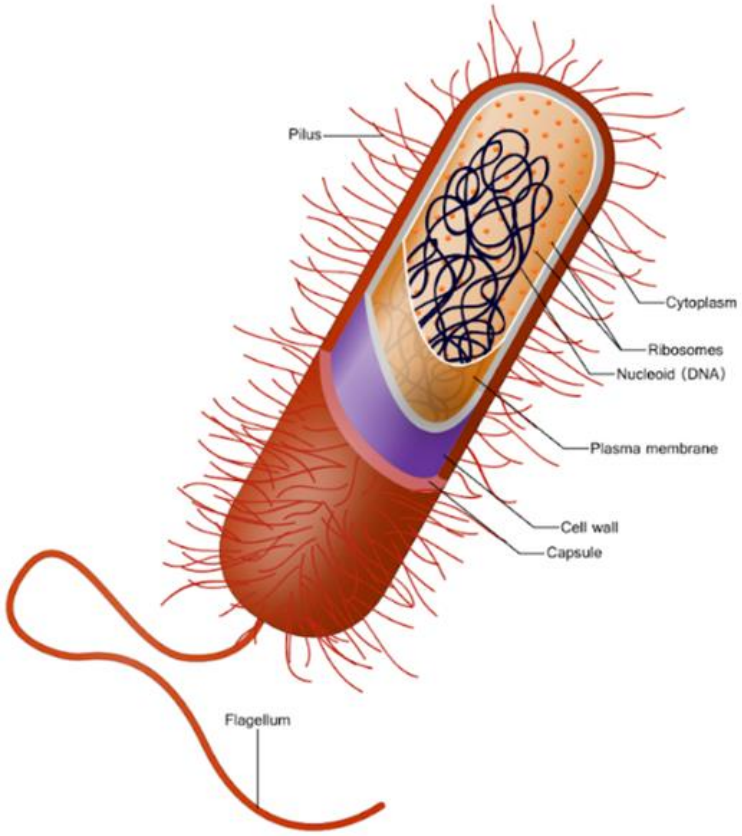
CRISPR Cas9 - Bacterial Defence

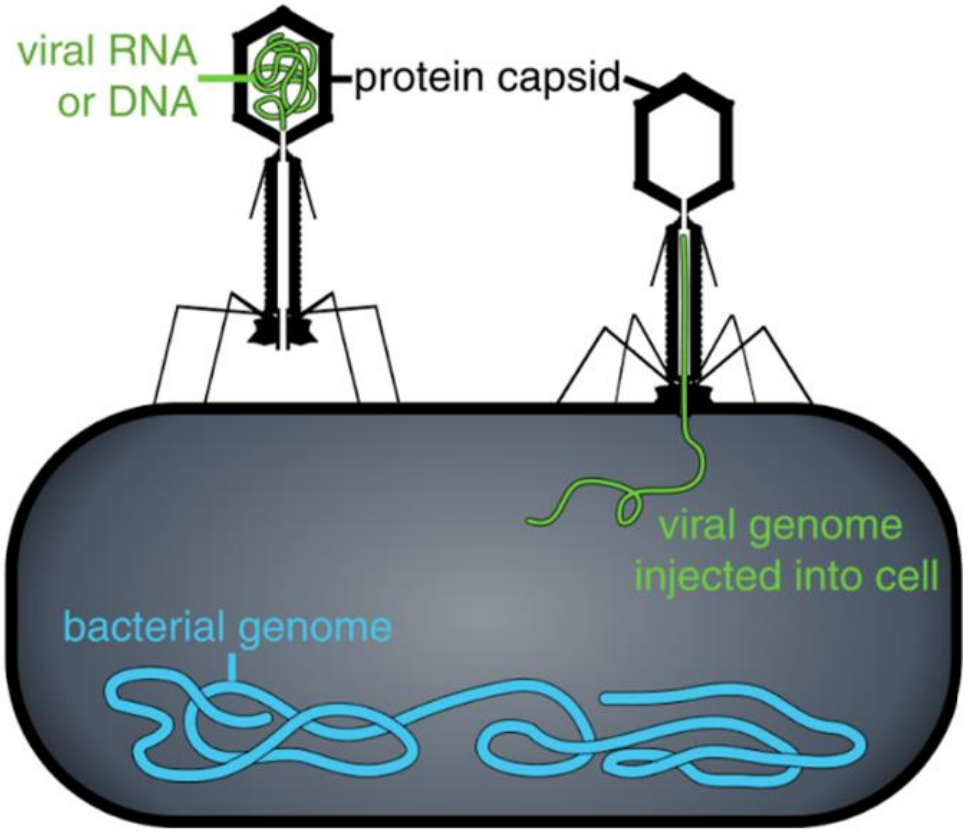
- CRISPR systems were discovered in bacteria as an **acquired** and **hereditary** means to help defend against viral attacks.
- Bacterial CRISPR systems are like a vault that stores pieces of viral DNA as a means to help identify future invading viruses (similar to our ANTIGIN-ANTIBODY response seen below).



CRISPR Cas9 – Battle to the DEATH

- If the same virus enters the bacteria again a Cas9 protein would quickly identify it and destroy it by cutting it up.
- In other words the bacteria would then be immune to that virus.





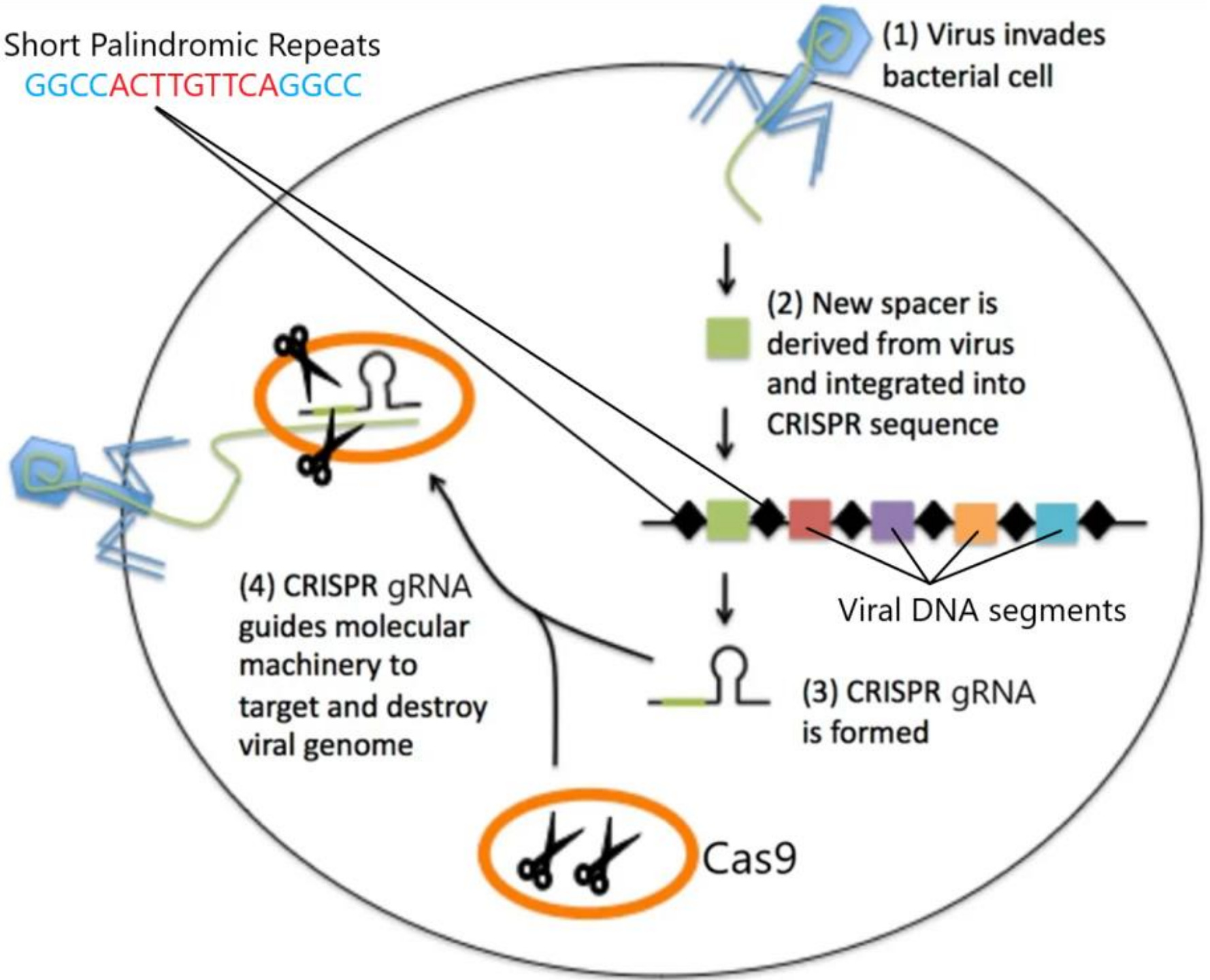
CRISPR Cas9 - Bacterial Defence

- CRISPR System is a section of DNA that holds sections of CRISPR spacers (viral DNA) and short palindromic repeats that store segments of virus DNA that have been captured.

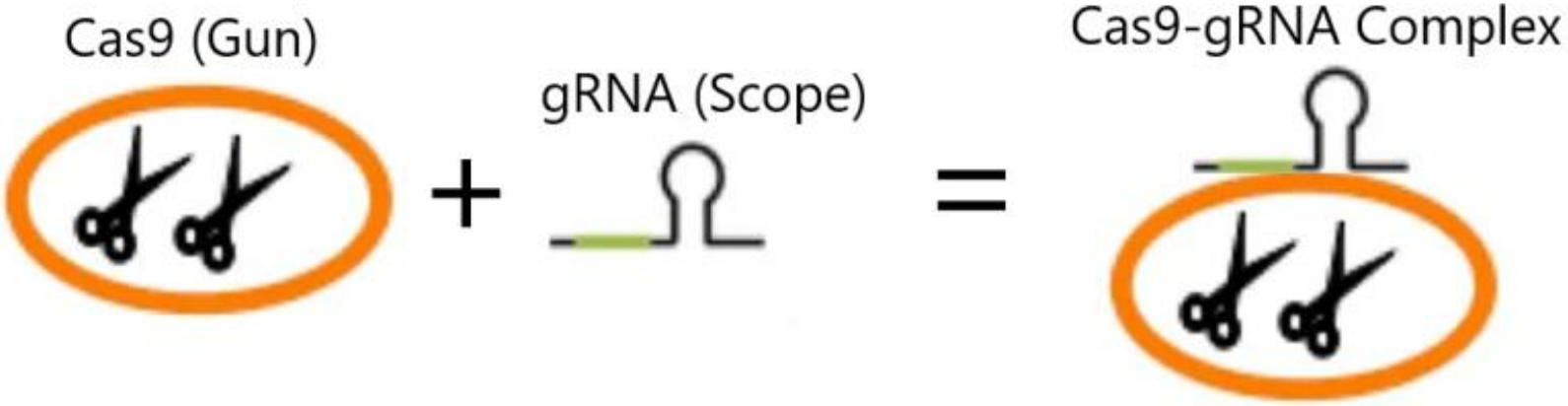
- Guide RNA (gRNA) is a section of RNA synthesized by the CRISPR spacers that are used to identify future invading viruses.

- Cas9 is a protein that is guided by the gRNA to seek out and cut the specific viral DNA if it enters the cell again.

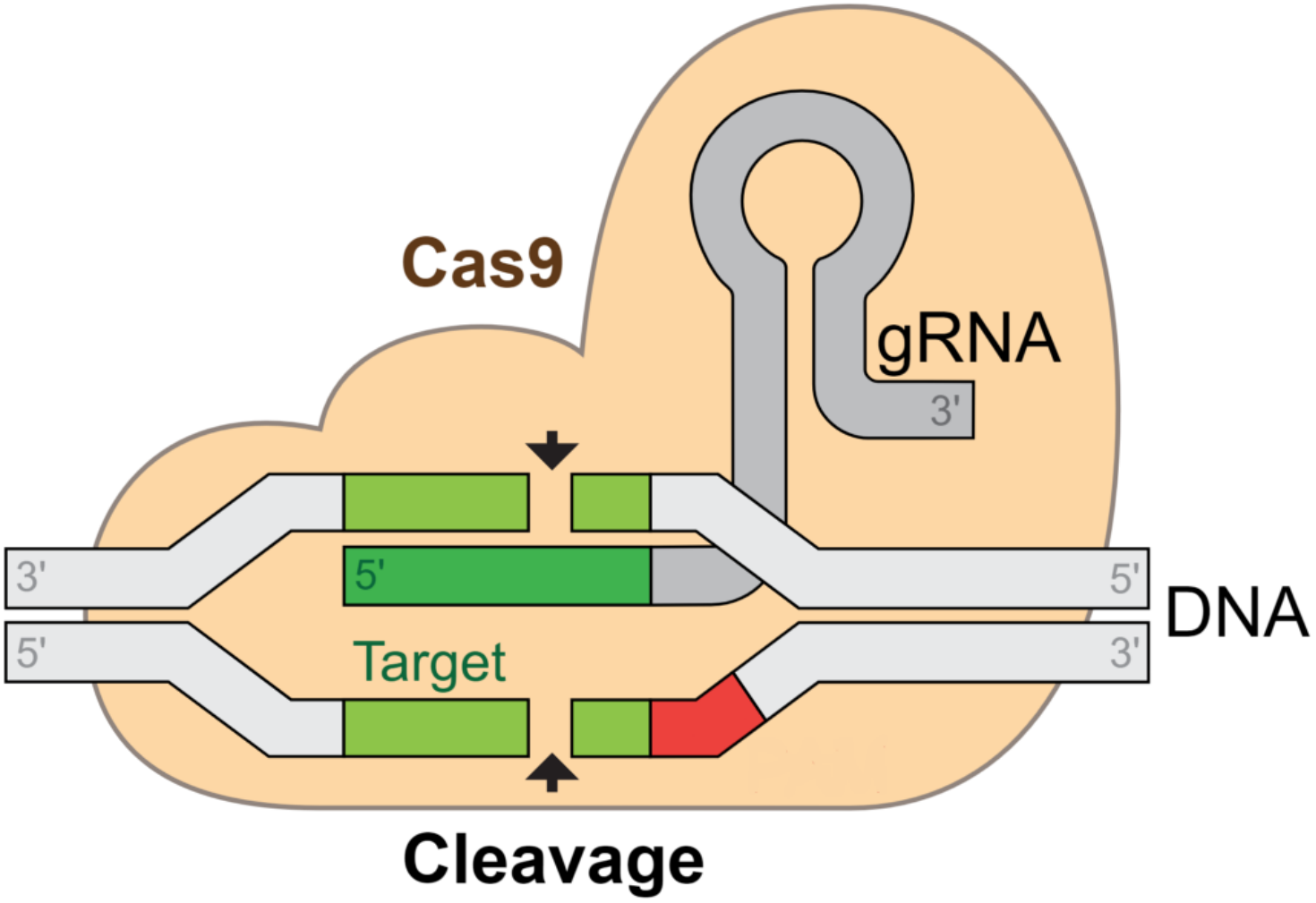
- **Charpentier** and **Doudna** realized that this CRISPR - Cas9 - gRNA system could be harnessed by genetic engineers to conduct very precise gene editing (inserting, deleting and moving genes of interest).


Short Palindromic Repeats
GGCCACTTGTTCAGGCC
(1) Virus invades bacterial cell
(2) New spacer is derived from virus and integrated into CRISPR sequence
Viral DNA segments
(3) CRISPR gRNA is formed
(4) CRISPR gRNA guides molecular machinery to target and destroy viral genome
Cas9

Cas9-gRNA Complex Is Like a Gun with Laser Guided Scope



Cas9 – gRNA Complex



CRISPR Cas9 Gene Editing

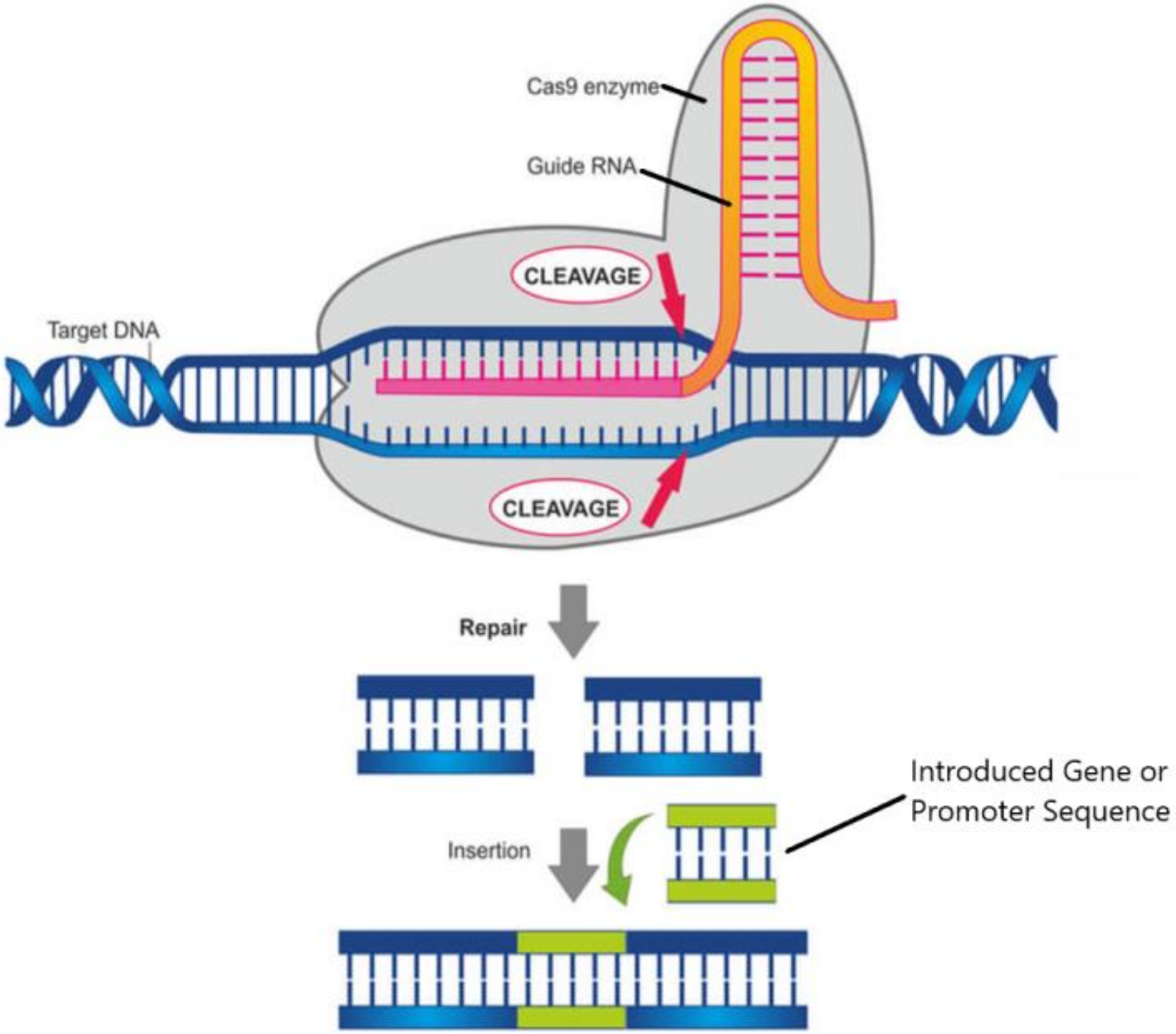
- Genetic engineers use this CRISPR system to edit DNA. This is similar to the cut and paste feature in Microsoft word.

- Scientists engineer a specific gRNA segment that will target a precise section of DNA like a laser guided scope.

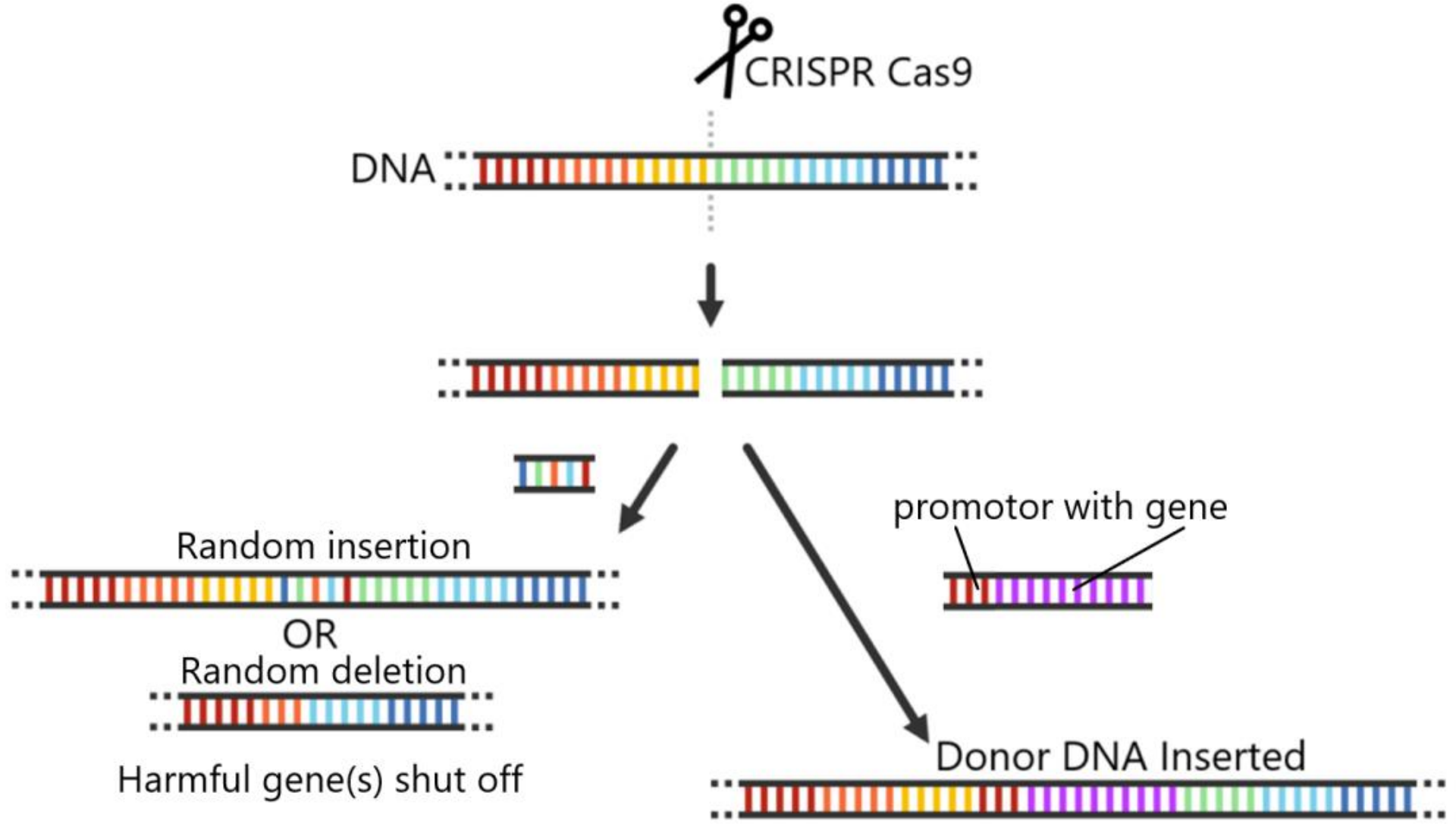
- These engineered gRNA segments are then mounted into a Cas9 protein capable of cutting DNA.

- The Cas9 (along with its gRNA guidance system) called the **Cas9-gRNA complex**, are introduced into the cell of interest where the gRNA leads the Cas9 to the DNA section of interest (typically a promotor or gene).

- The Cas9 then surgically cuts the DNA in the precise location allowing the gene to be deleted, replaced, repaired etc.


Cas9 enzyme
Guide RNA
CLEAVAGE
Target DNA
CLEAVAGE
Repair
Introduced Gene or
Promoter Sequence
Insertion

CRISPR Cas9 Gene Editing



CRISPR Cas9 - Some Potential Uses

- CRISPR Antibiotics - Creating a new class of antibiotics that encourages bacterial Cas9 proteins to chop up their own DNA killing the bacteria.

- CRISPR Disease Diagnosing/Treatment – using Cas proteins that cause wide spread collateral cleavage damage to a select region of disease causing DNA.

- CRISPR Organ Donor Animals – using GMO pigs (and other mammals) to create human organs for organ replacement.

- CRISPR Disease Spread Reduction – engineering the genome of vector species to make them immune to disease preventing them from transmitting to humans. For example lyme disease moves from mice to ticks to humans. CRISPR modified mice genome that is immune to lyme disease protects humans.

- Curing genetic diseases like certain forms of cancer, heart disease, diabetes, HIV, cystic fibrosis and so many more!!!

- Designer babies, muscle bound pigs and cows, and so much more!!!

Gene Drives – Population Engineering

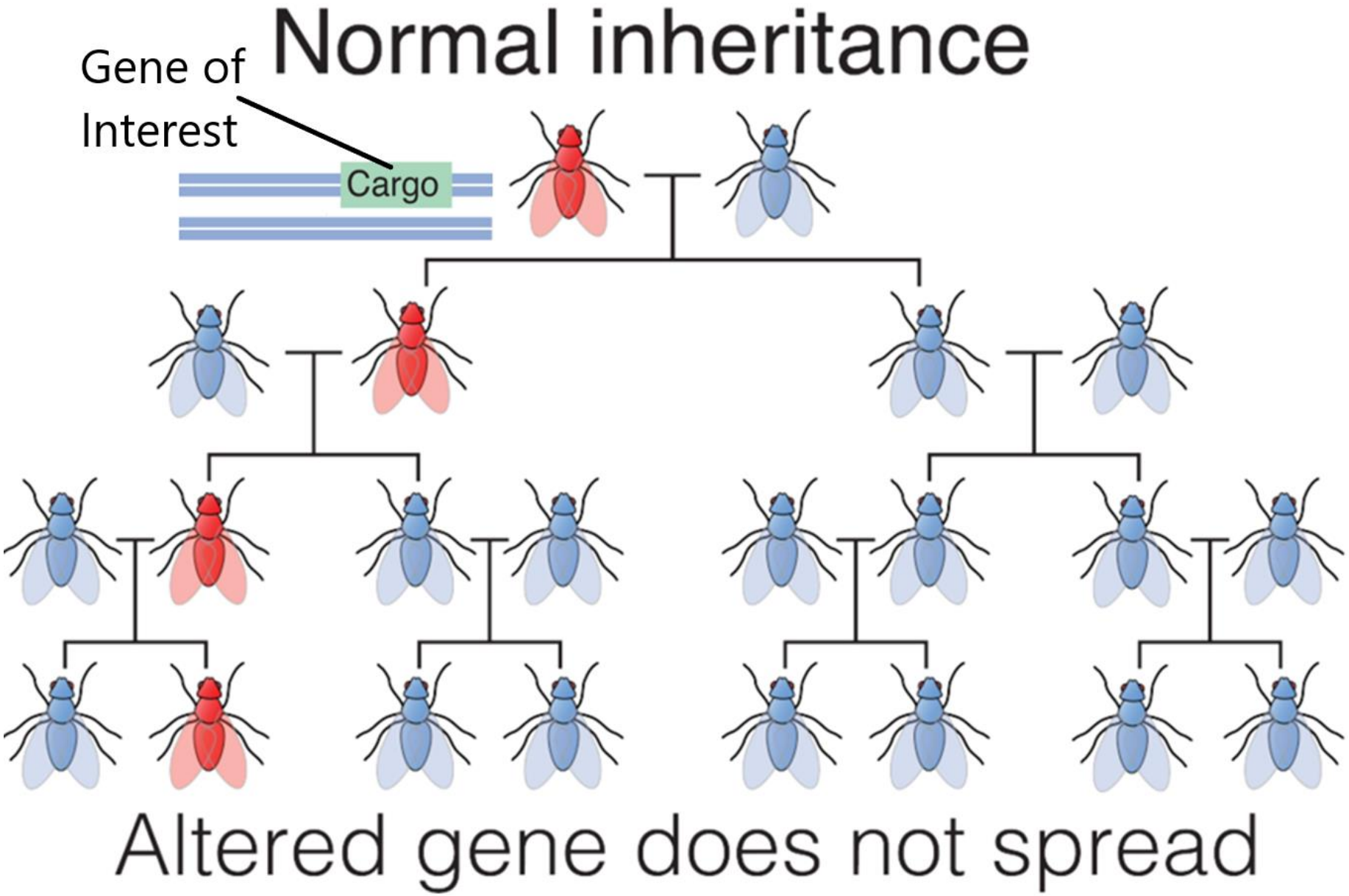
- A gene drive is a type of genetic engineering technique that modifies genes so that they don't follow the typical rules of heredity.

- Gene drives dramatically increase the likelihood that a particular gene will be passed onto the next generation, allowing the gene to rapidly spread through a population and override natural selection.

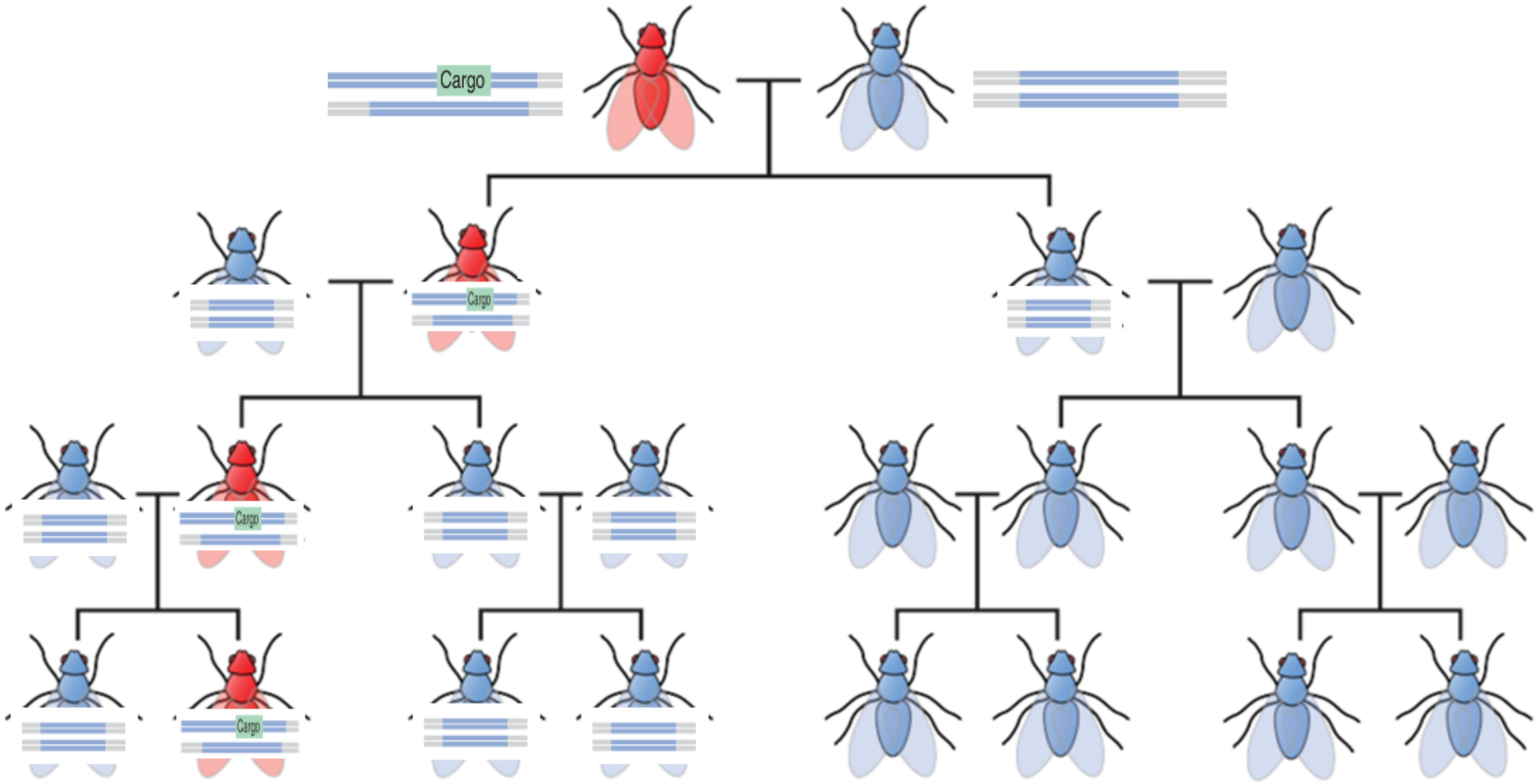
- CRISPR-Cas9 gene editing technology allows gene drives to be researched and built much easier.

- Austin Burt – Imperial College London - dreamt up the concept of a CRISPR gene drive while walking his dog in the park.
- 2015 George Church created the first gene drive modified yeast.
- 2016 Austin Burt developed gene drive modified mosquitos.

Gene Drives – Population Engineering



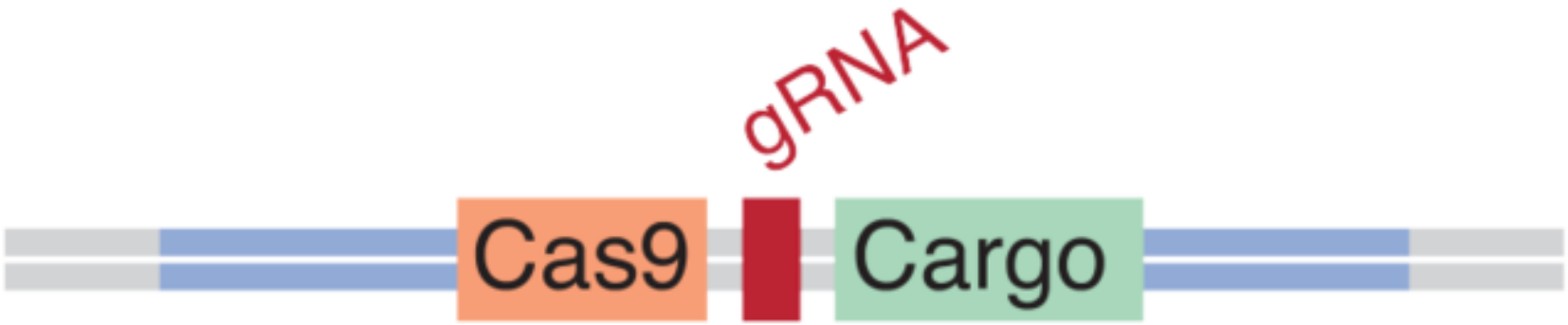
Normal inheritance



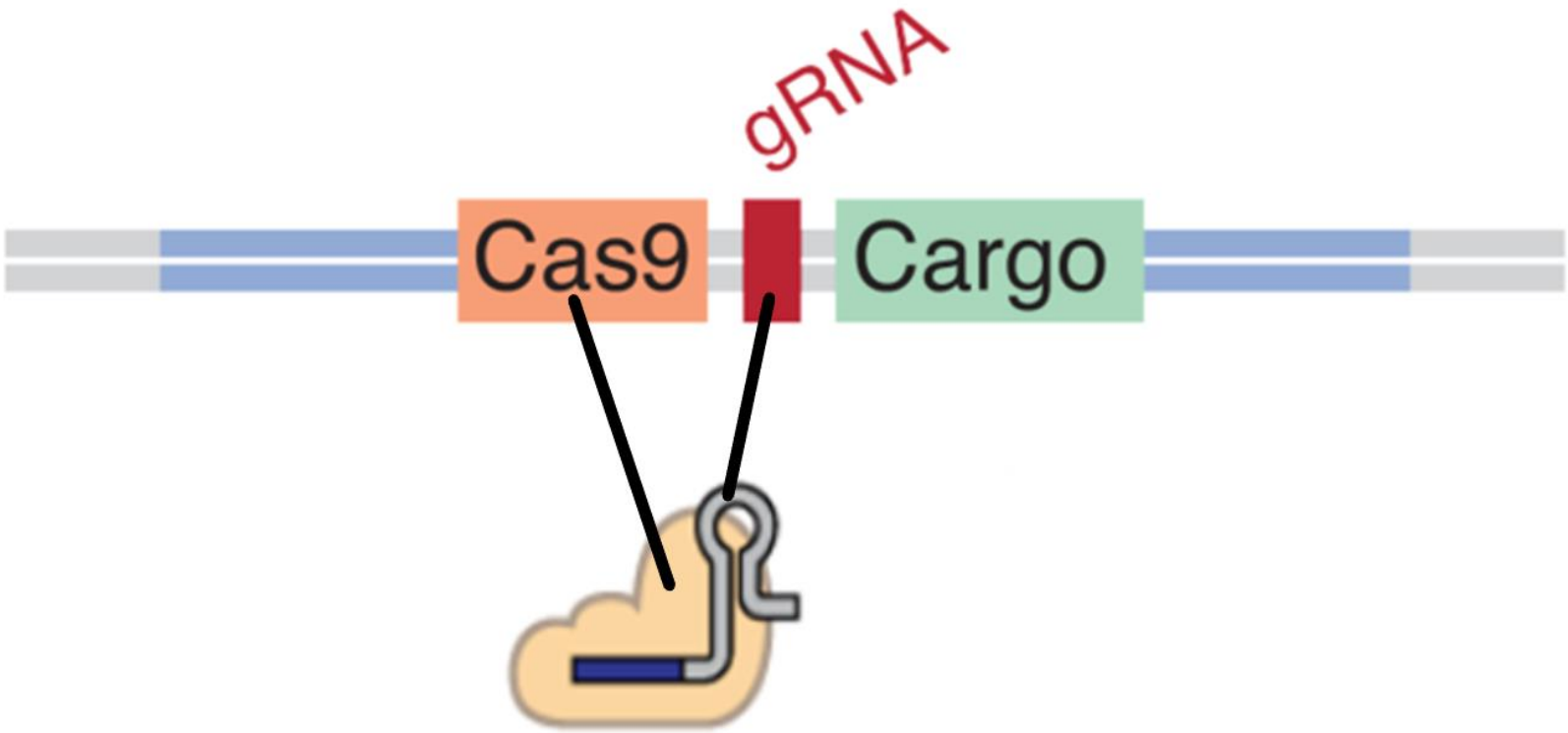
Altered gene does not spread

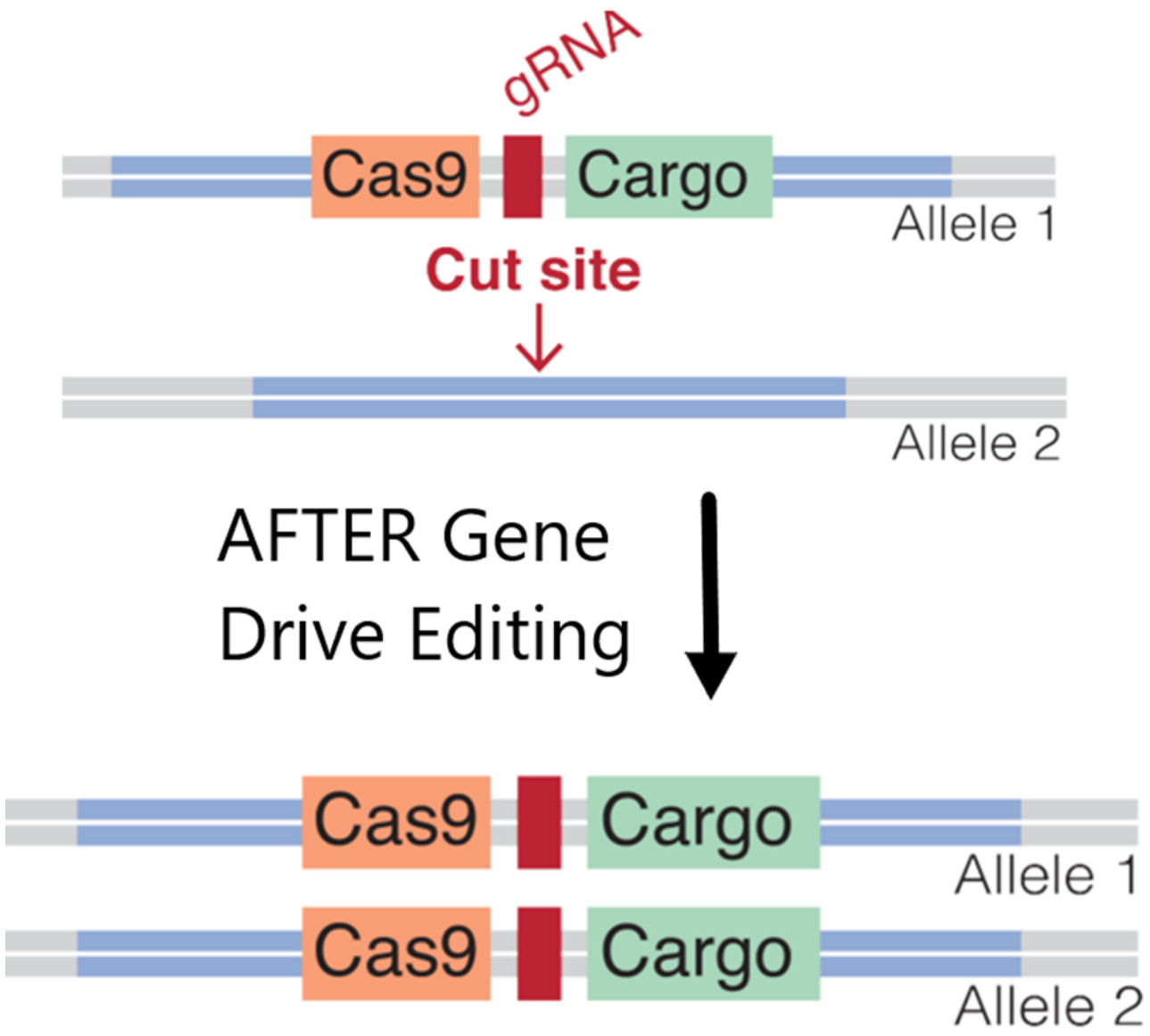
Cargo = gene(s) of Interest

Cargo + CRISPR System

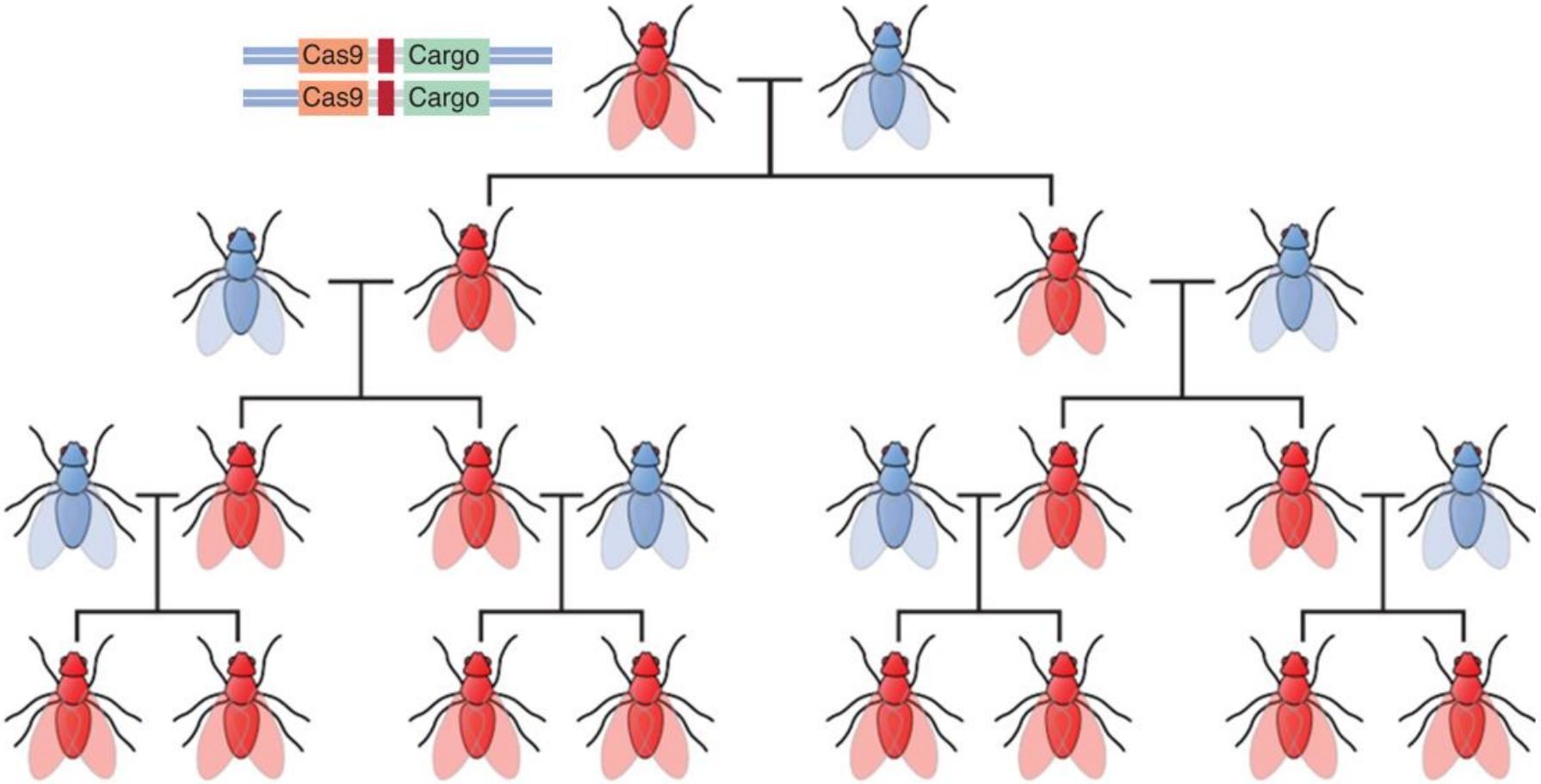


Cargo + CRISPR System



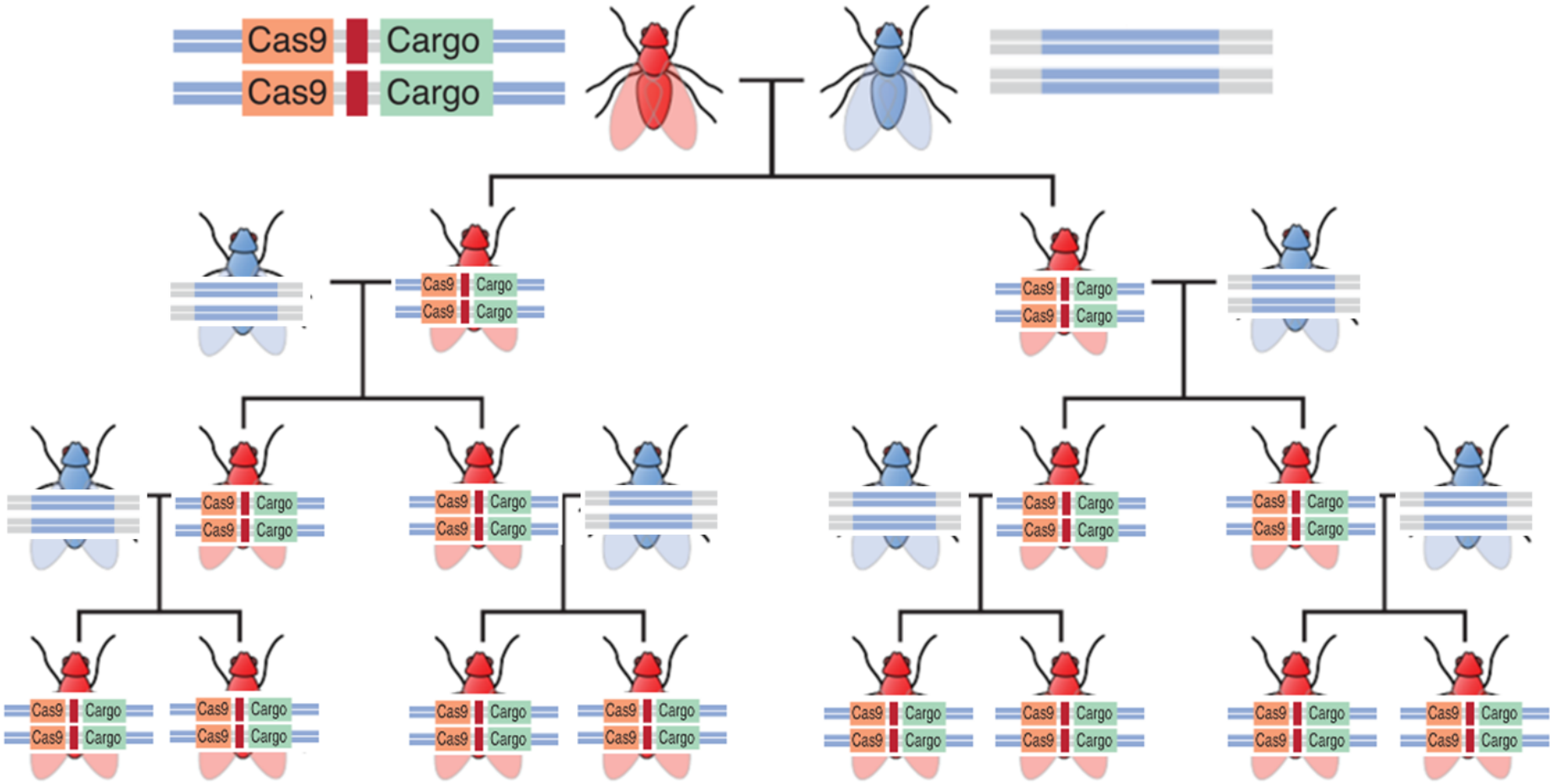

gRNA
Cas9
Cargo
Allele 1
Cut site
Allele 2
AFTER Gene
Drive Editing
Cas9
Cargo
Allele 1
Cas9
Cargo
Allele 2

Gene drive inheritance

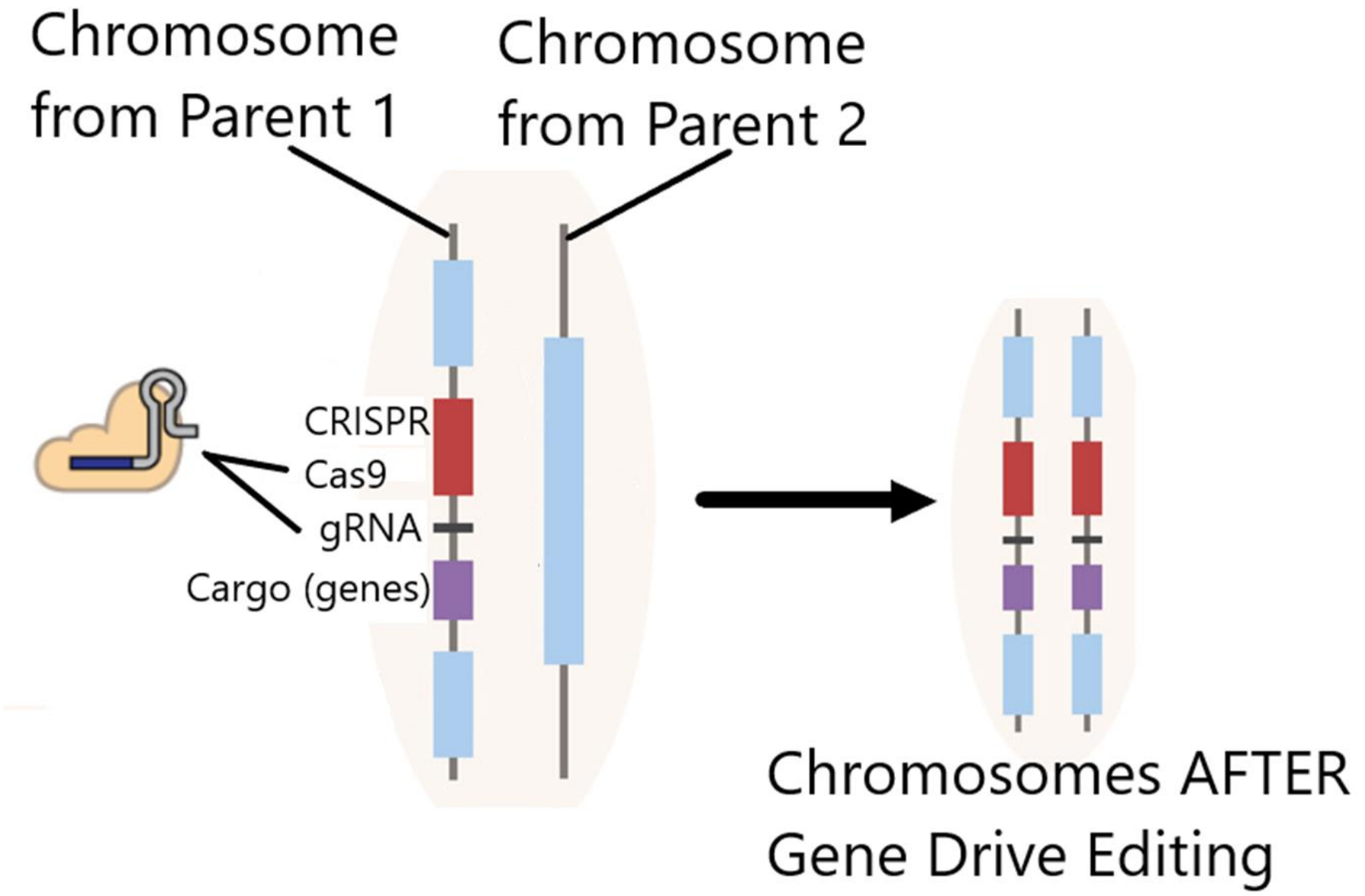


Altered gene is always inherited

Gene drive inheritance



Altered gene is always inherited


Chromosome from Parent 1
Chromosome from Parent 2
CRISPR
Cas9
gRNA
Cargo (genes)
Chromosomes AFTER Gene Drive Editing

Gene Drive Discussion

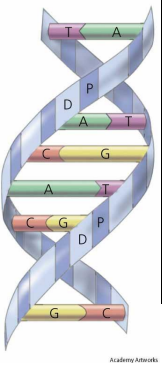
- Malaria kills 500,000 people (mainly kids) every year.
- Should CRISPR gene drives be used to engineer and release mosquitos that are immune to plasmodia (the microbes that cause malaria)?
- Would your view change if it was bears not malaria that was killing 500,000 children a year as they walked to school?
- Who decides if CRISPR gene drives are used in this way?
- Should CRISPR gene drives be used to engineer and release mosquitos in which only male offspring are produced?
- What would this do to the global mosquito population?
- What effect might this have on local and global ecosystems?

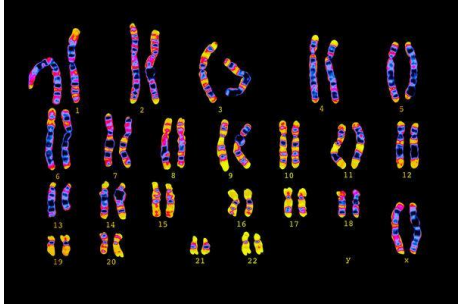
Gene Drive Discussion

- List of Mosquito Borne Disease:
- Malaria, West Nile, Zika, Dengue, Yellow fever, Chikungunya, several other Encephalitis causing diseases. 1 million deaths/year.
- How many people have lived on earth in total all time?
- 108 billion
- How many people have been killed by mosquito borne disease?
- 52 billion
- Who gets to decide if CRISPR gene drives are used in this way?

Gene Drive Discussion

- Should CRISPR gene drives be used to extirpate (locally extinct) various invasive species?
- Carp in Southern Saskatchewan lakes? Rabbits in Australia? Rats in the Galapagos Islands?
- Who gets to decide if CRISPR gene drives are used in this way?
- Should CRISPR gene drives be used in human populations?
- Could we and should we root out genes associated with all genetic disorders?
- What other genes/traits could be driven into a human population?
- Could this technology get into the wrong hands? What might result?
- Who gets to decide if CRISPR gene drives are used in this way?





DNA Typing

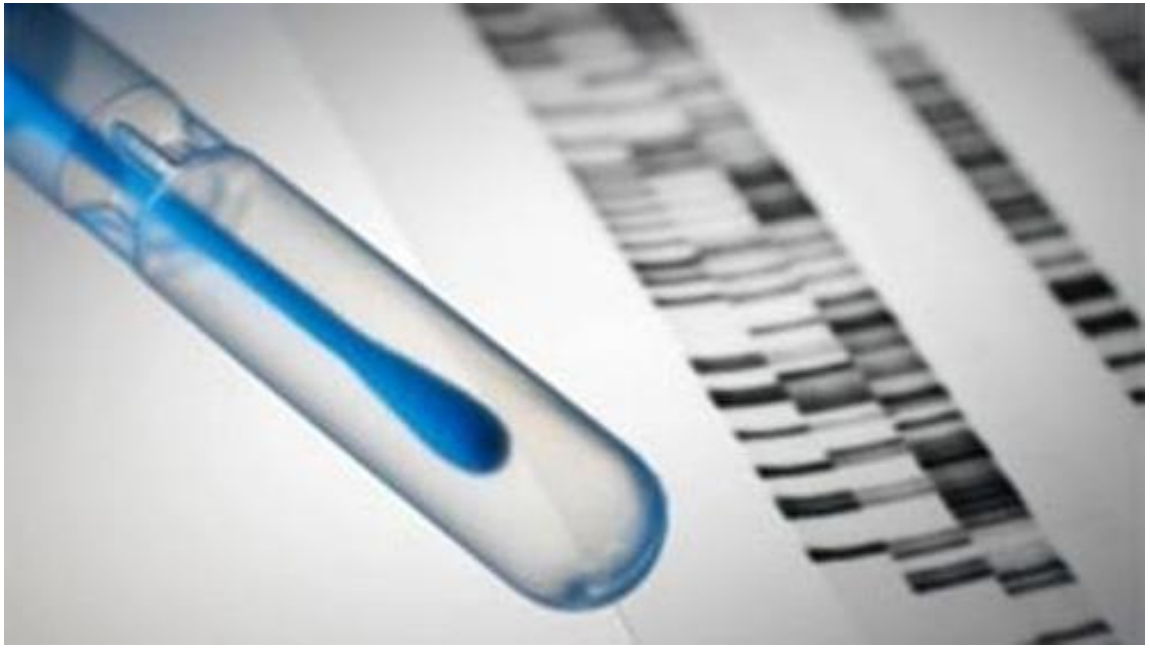
- What is it about fingerprints that allow them to function as a means of identifying criminals?
- Variability. No two people have the same fingerprint.
- What is it about DNA that allows it to function as a means of identifying criminals?
- Variability. No two people have the same DNA.
- What % of DNA do we share with all other humans?
- 99.5%
- The tiny differences in our DNA allow us to distinguish one person from another.

DNA Typing – Why it's Used.

1. Identifying rapists and other criminals.
2. Determining missing persons (human remains).
3. Determining paternity.
4. Determining if a hopeful immigrant is really a son or daughter of an already established resident.

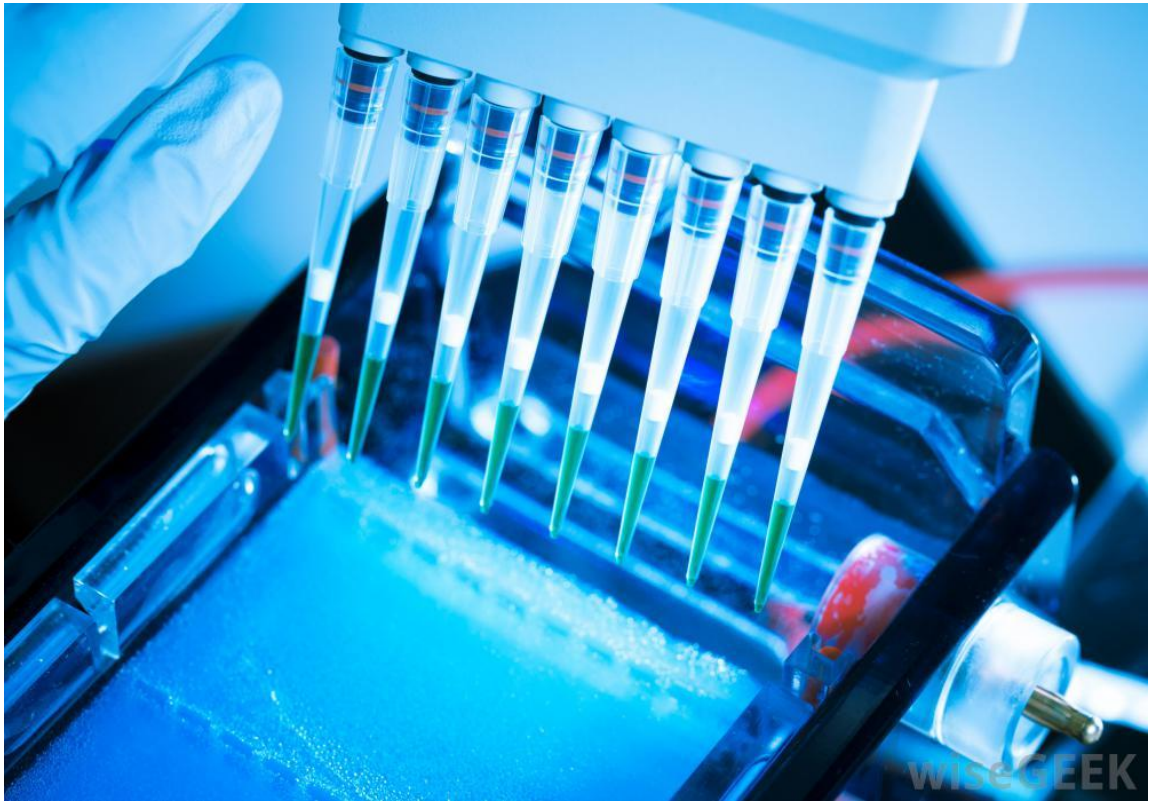
DNA Typing – How it Works

- Begins with a sample of an individual's DNA
- Usually a throat swab – but can be obtained from any cellular material - blood, saliva, semen, or other appropriate fluid or tissue from personal items (toothbrush, razor, etc.).
- Touch DNA is a new technique used to recover DNA left by simply touching objects such as a gun or knife handle.



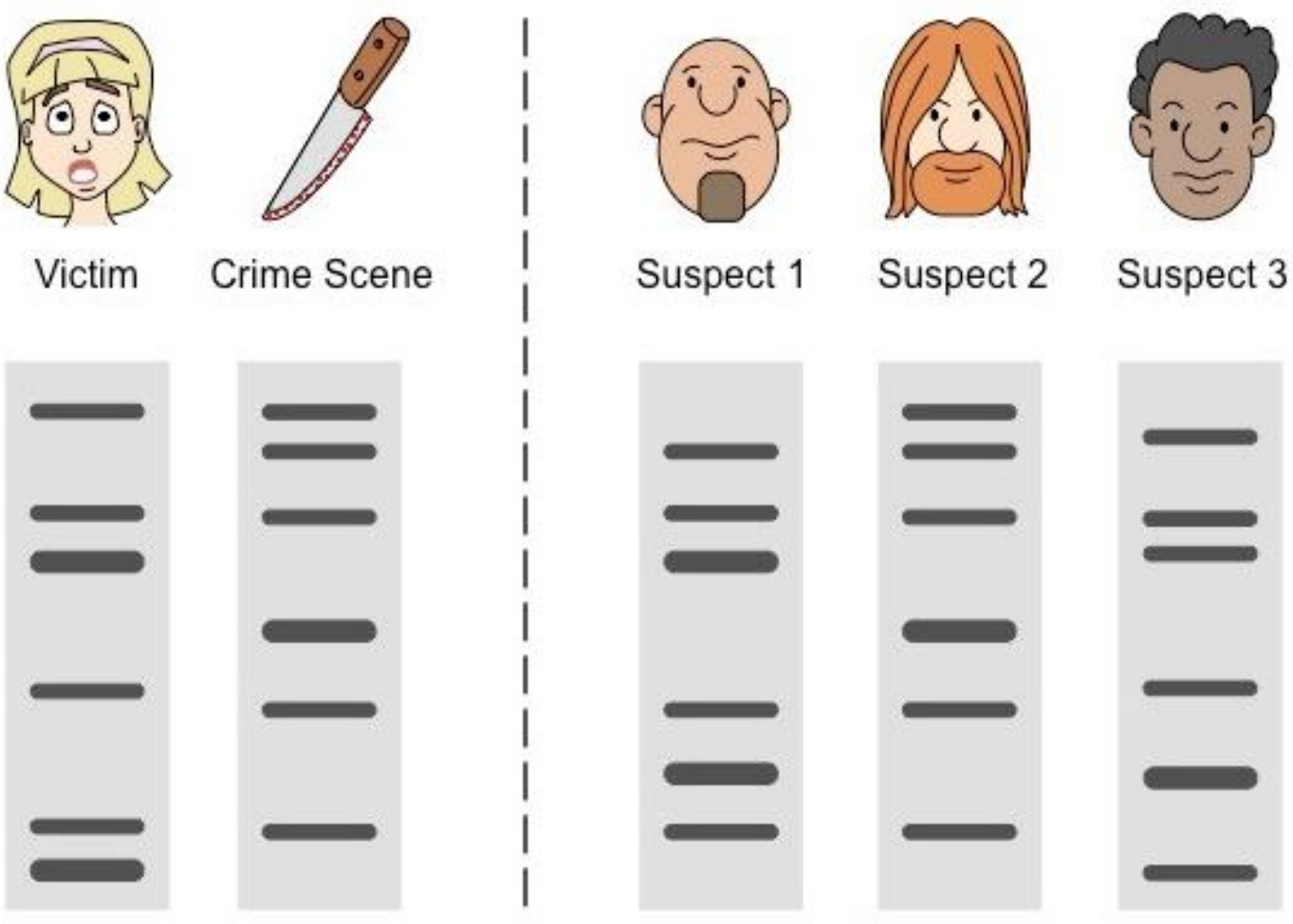
DNA Typing – How it Works

- The sample is then analyzed to create the individual's DNA profile.
- The DNA profile is then compared against another sample to determine whether there is a genetic match.



Who Dunnit?

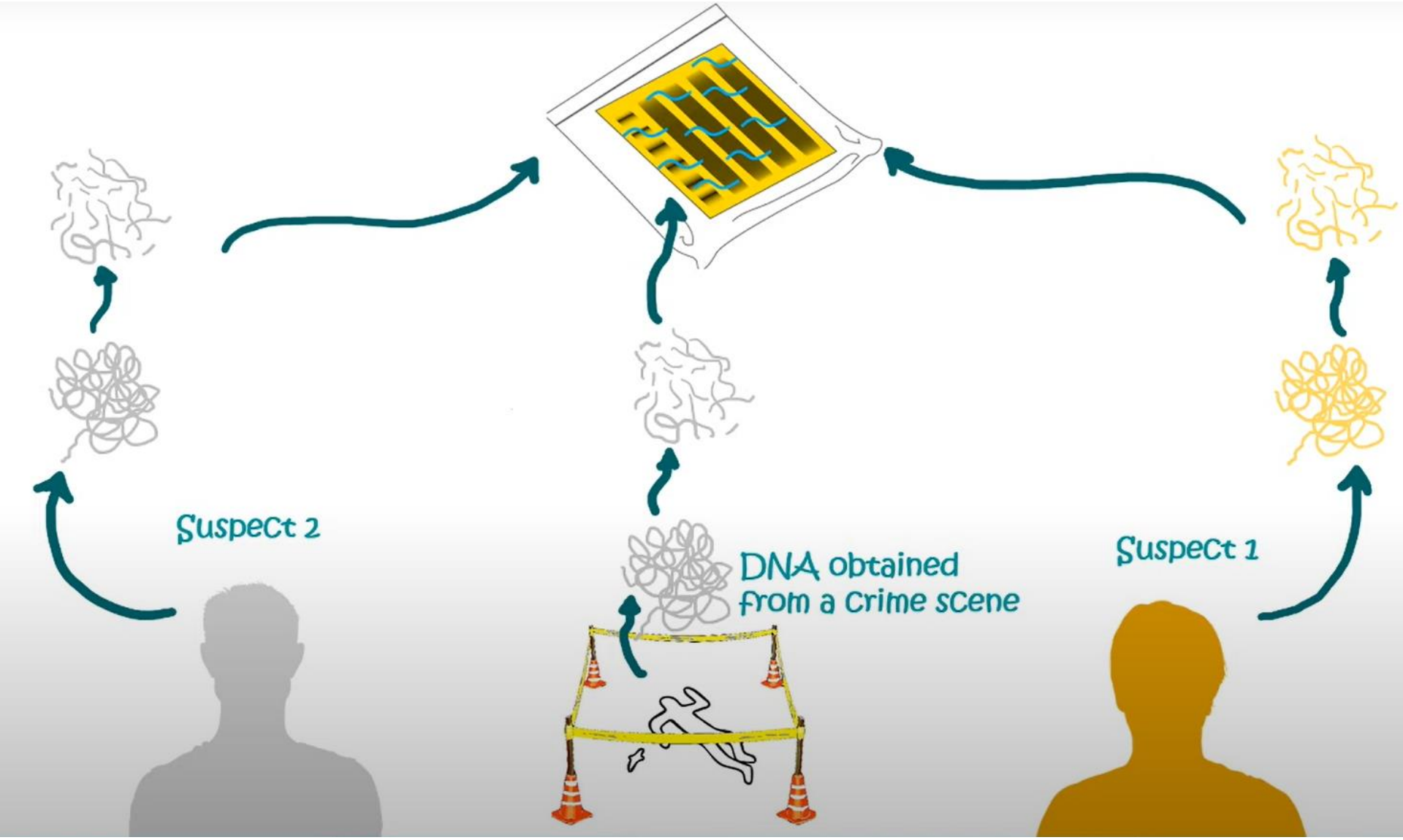
Even without knowing the science and what exactly we are looking at one can match evidence to a suspect.



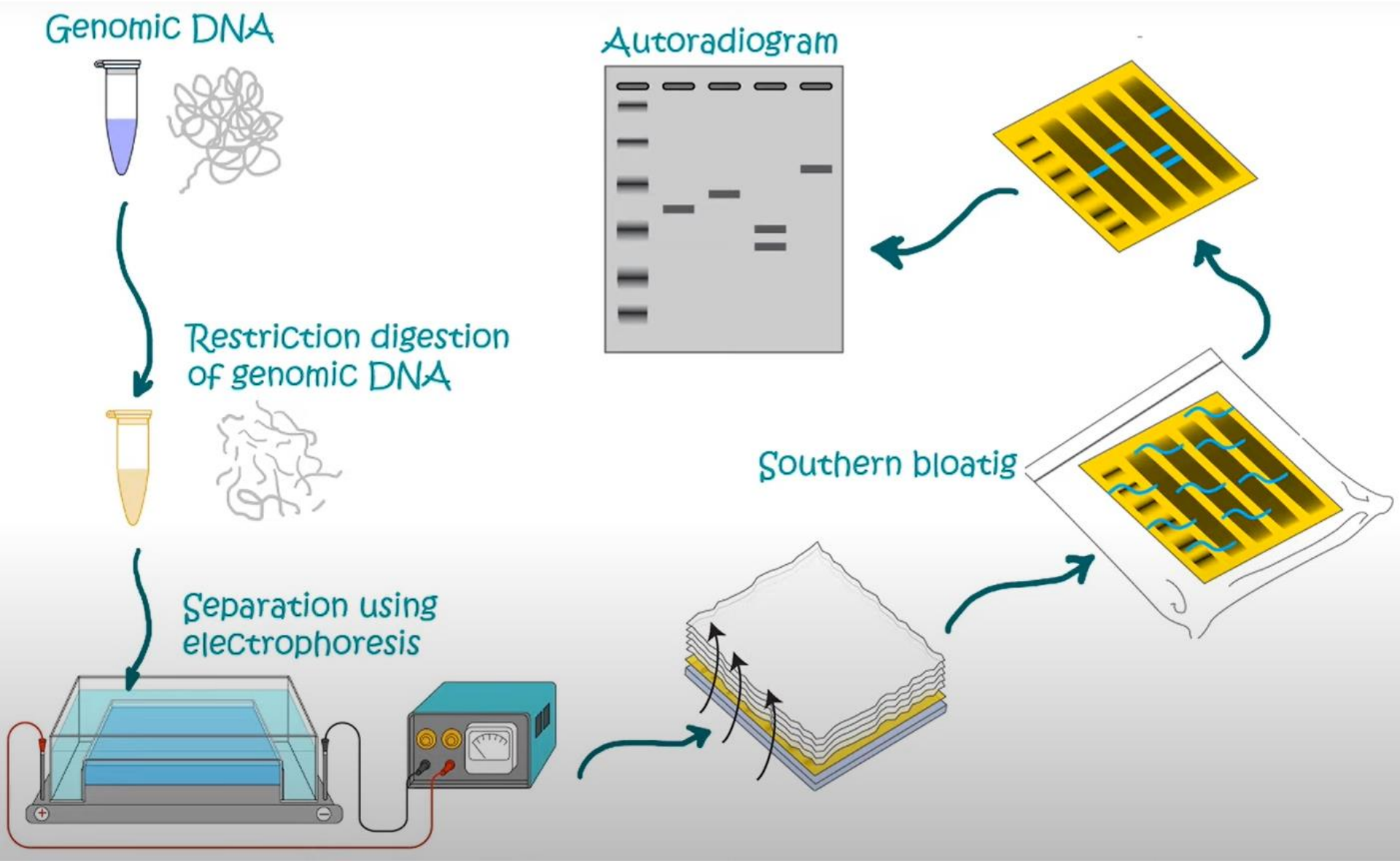
RFLP (Restriction Fragment Length Polymorphism)

- Alec Jeffreys credited with discovering the first technique referred to as RFLP (Restriction Fragment Length Polymorphism).
- A restriction enzyme (molecular scissors) was added to the sample of DNA effectively cutting it into fragments of various lengths.
- The fragments of DNA were then separated using a technique called gel electrophoresis.
- This separated DNA formed the persons “DNA profile”.

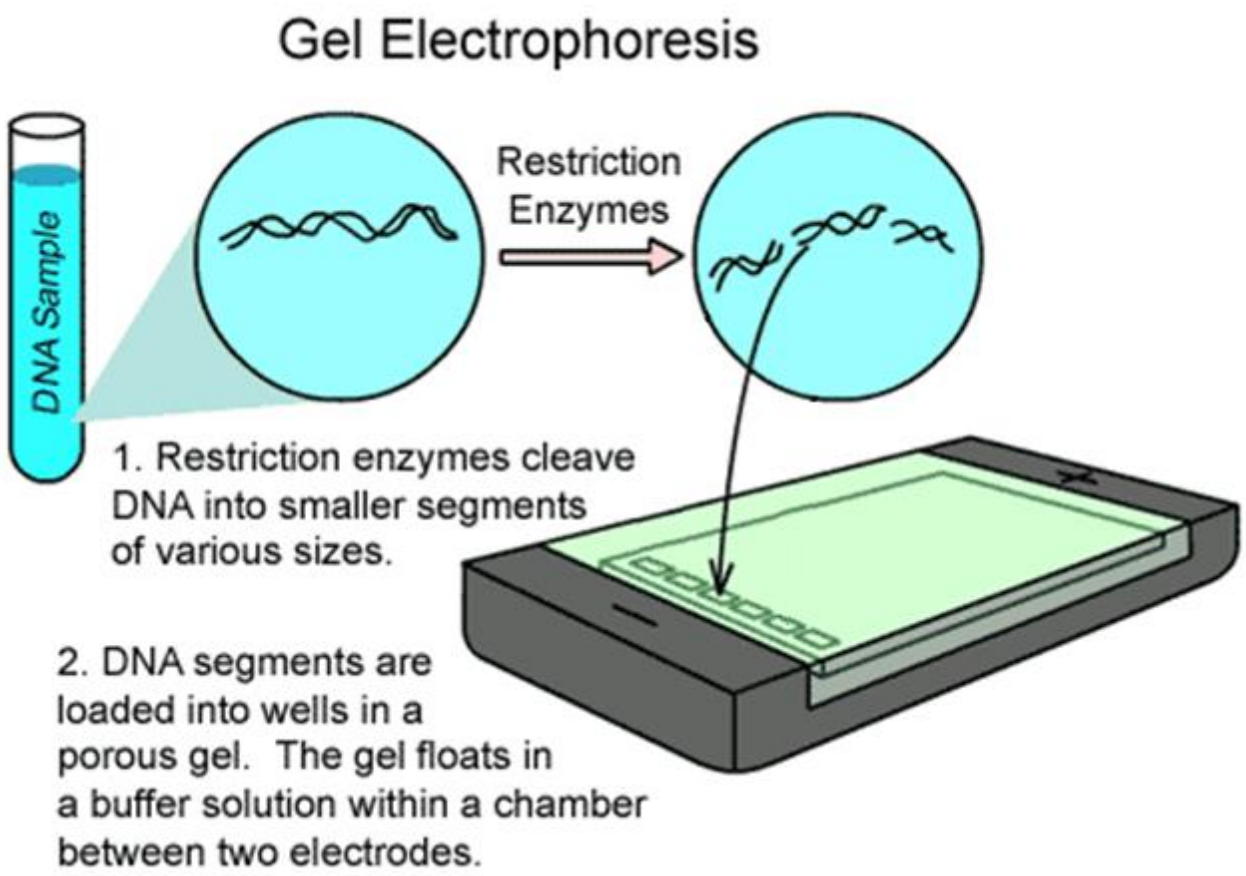
RFLP

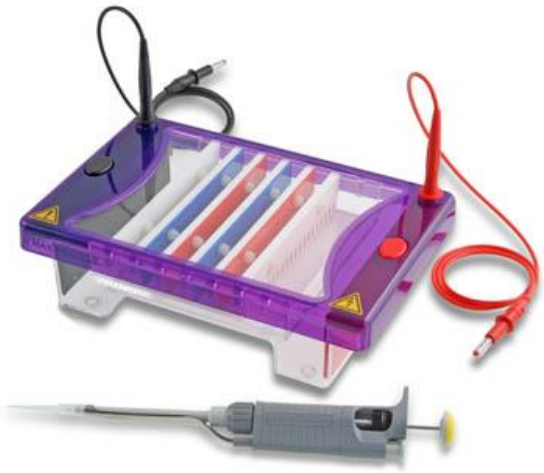


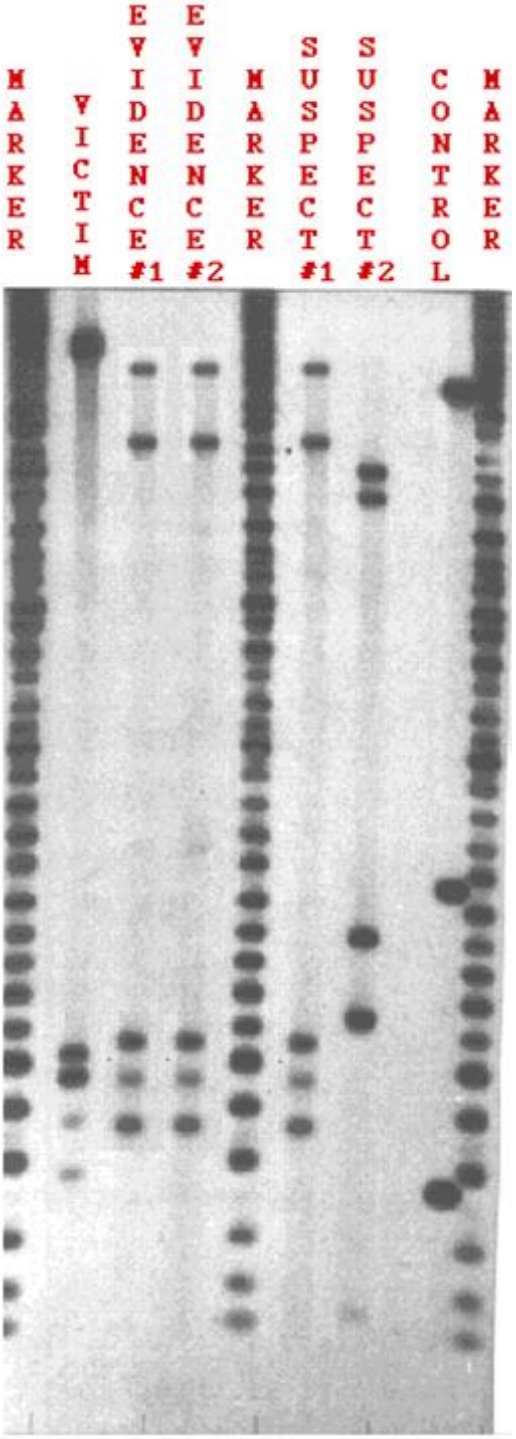
RFLP


Genomic DNA
Restriction digestion of genomic DNA
Separation using electrophoresis
Southern bloatig
Autoradiogram

How Gel Electrophoresis Works

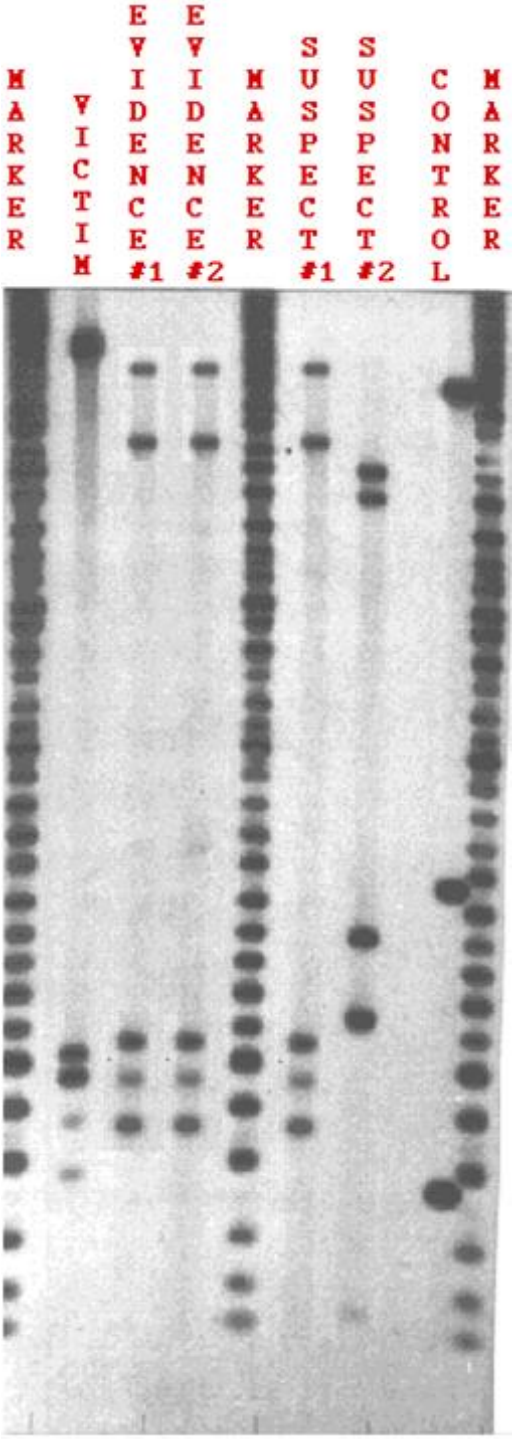






Results - An Actual Rape Case

- EVIDENCE #1 - semen stain left on the victim's clothing
- EVIDENCE #2 - semen removed from the vagina of the rape victim
- DNA from suspects #1 and #2
- DNA of the victim herself.

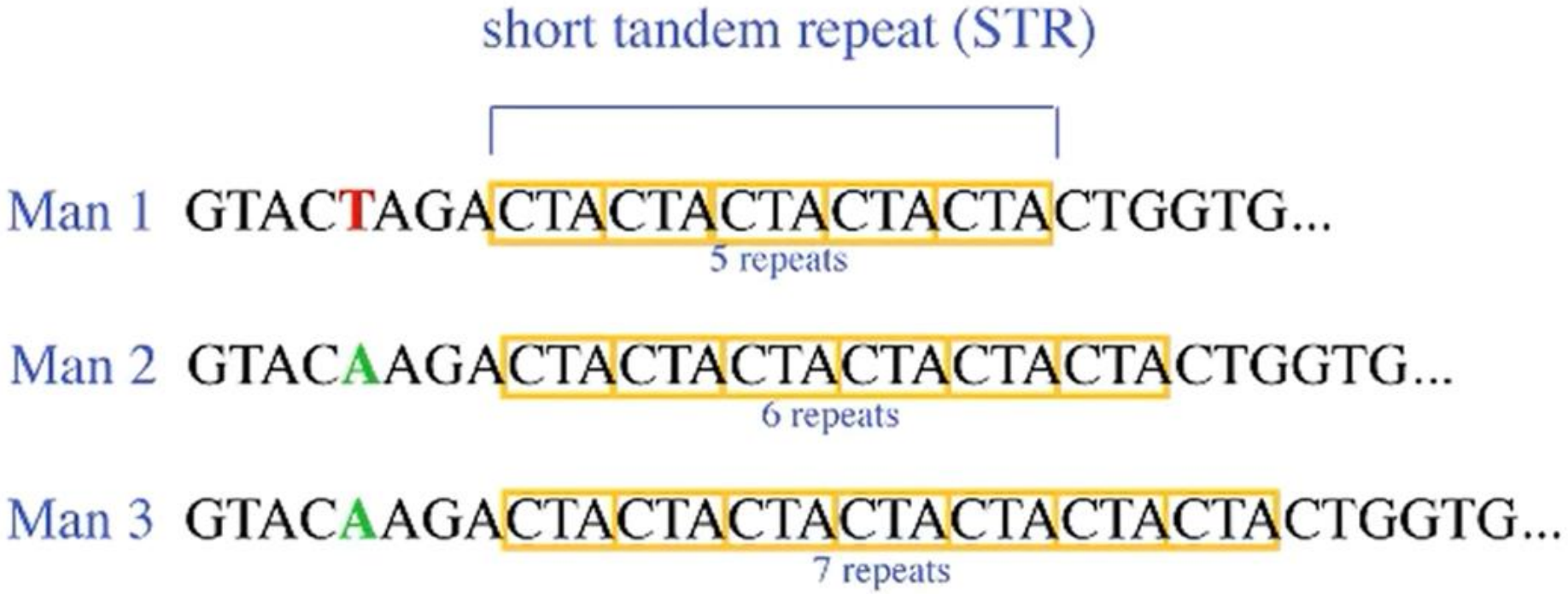


Results - An Actual Rape Case

- To further help remove all reasonable doubt, a statistical statement can be made regarding the probability that another random person would share that same DNA fingerprint.
- Each allele (band of DNA) has its own frequency in the population.
- These individual frequencies are multiplied together to fine the probability of having the same exact DNA fingerprint.
- In this particular case, the probability of having that exact DNA fingerprint is 1 in 268 million people.

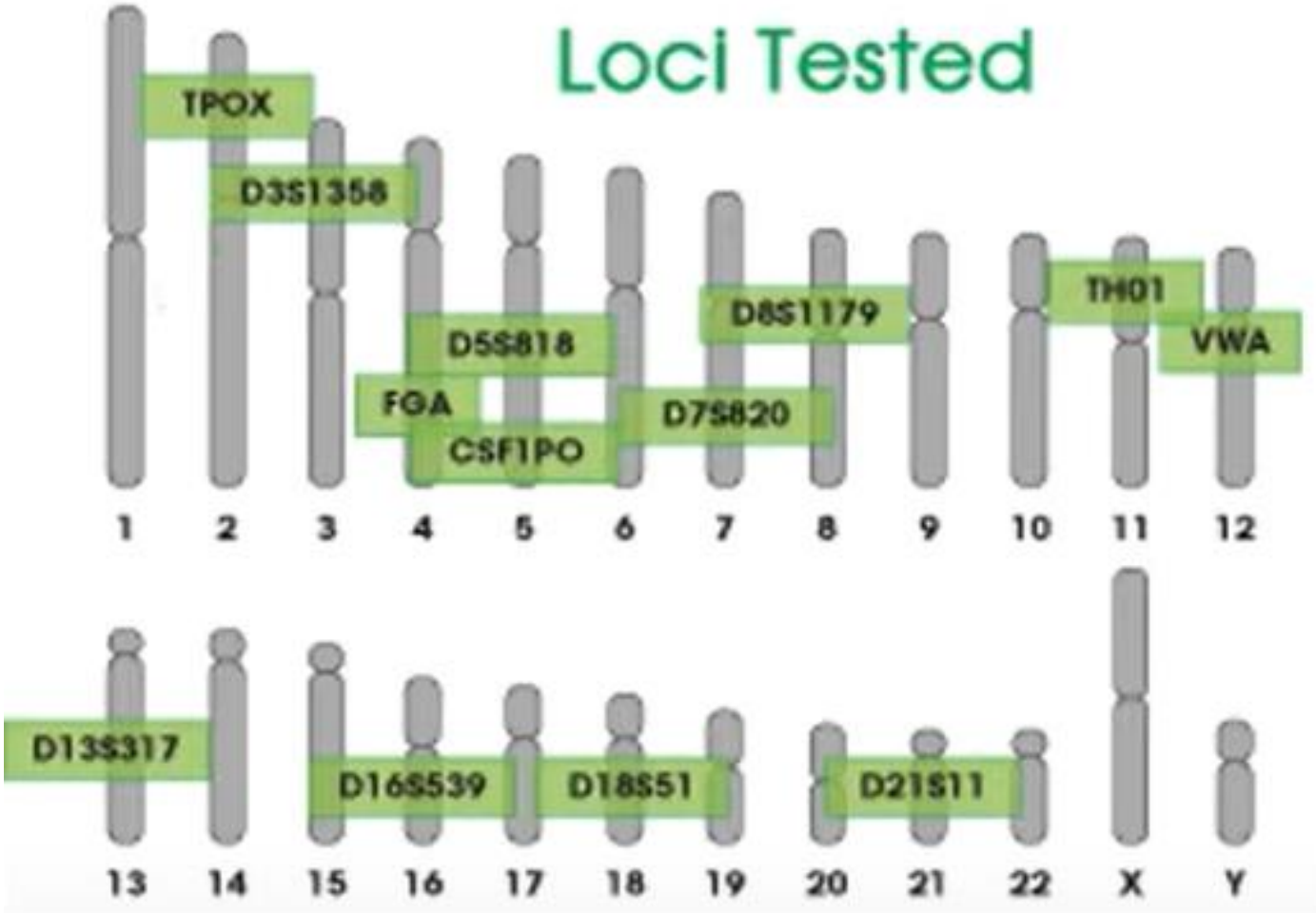
STR and VNTR Analysis

- The RFLP technique created by Jeffreys has now been improved upon and replaced by STR and VNRT Analysis.
- STR (Short Tanden Repeats) are repetitive units of 2-6 base pairs.
- VNTR (Variable Number Tanden Repeats) are repetitive units of 10-60 base pairs.



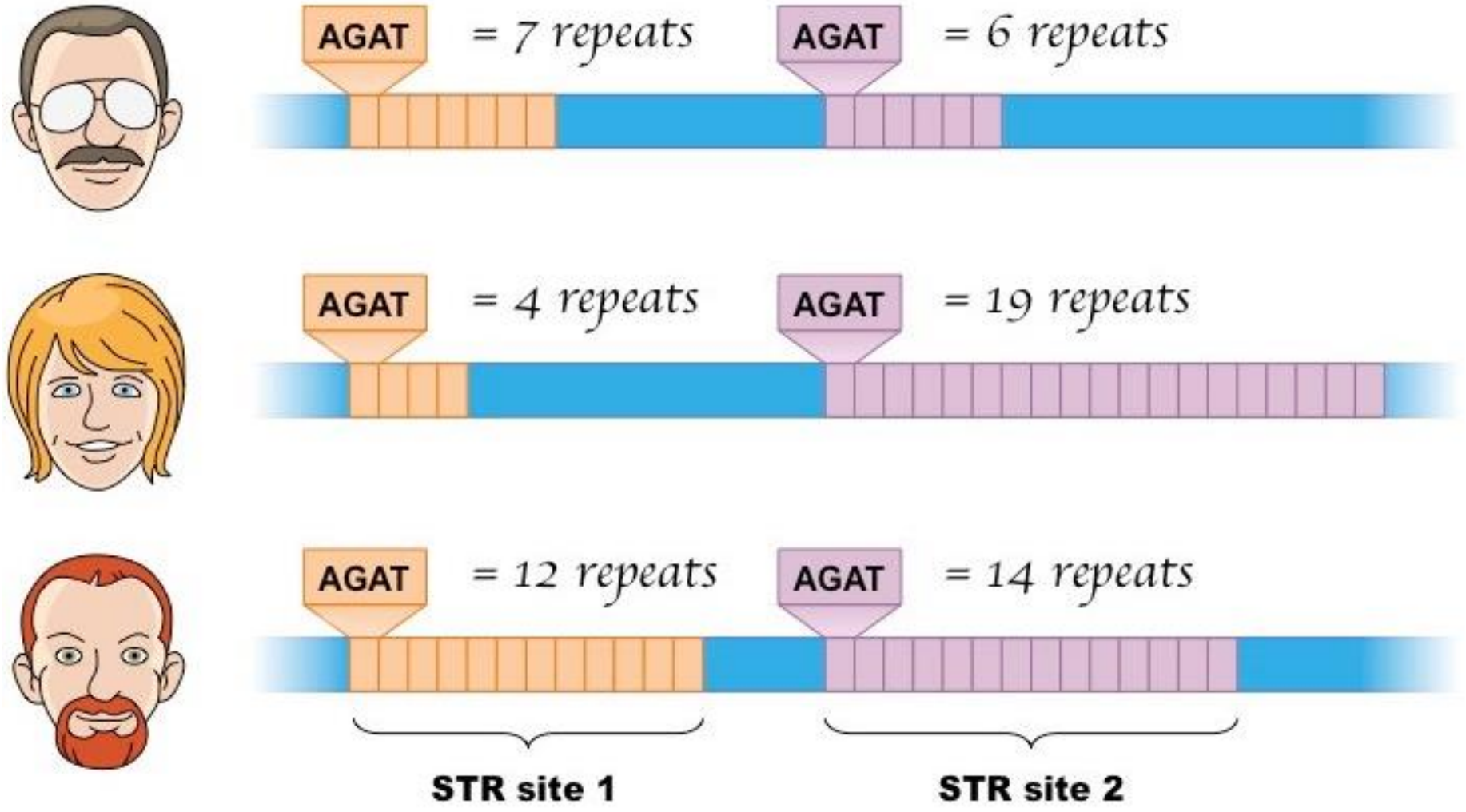
STR and VNTR Analysis

- These techniques compare the number of tandem repeats in 13 locations considered “junk DNA”.
- When all 13 sites match the odds of the DNA not coming from the same person are 1 in a billion.



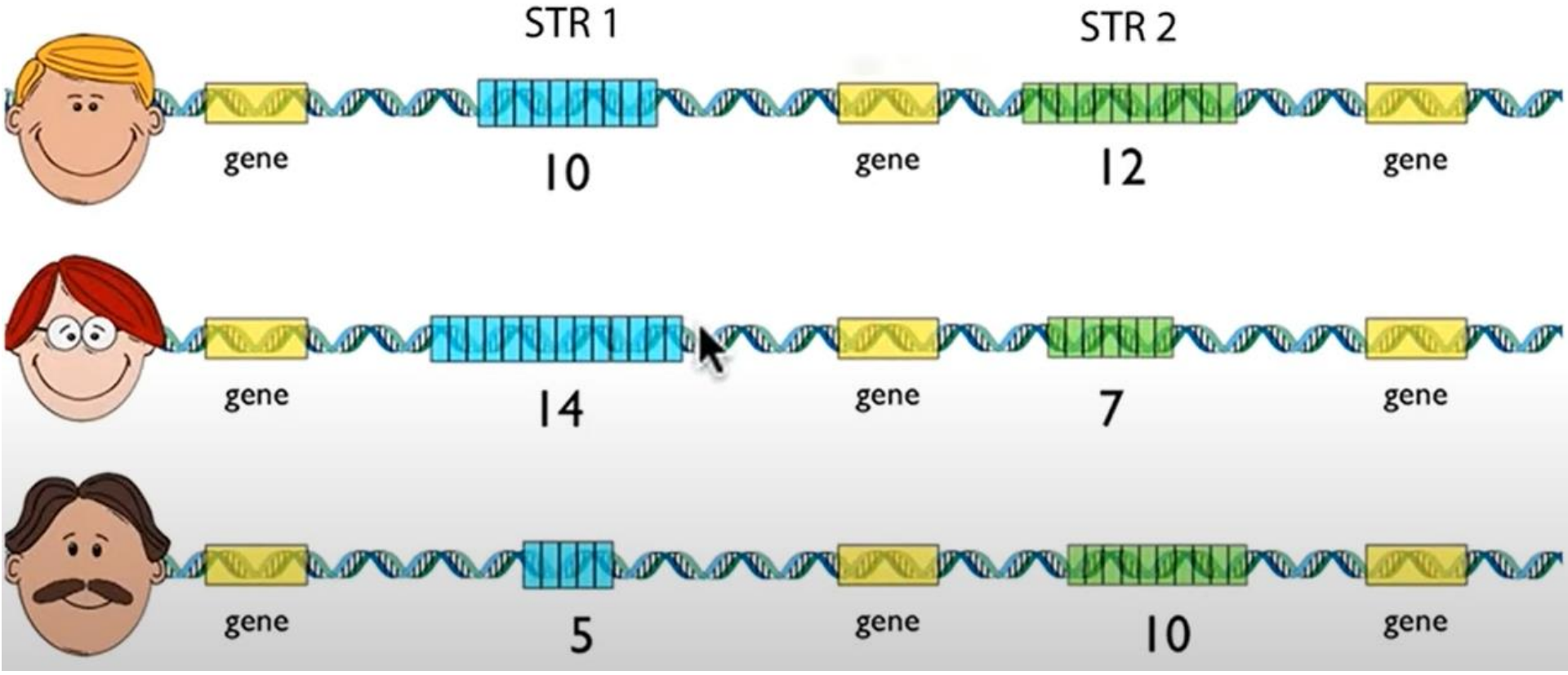
STR and VNTR Analysis

Essentially creates a barcode/profile for a person.

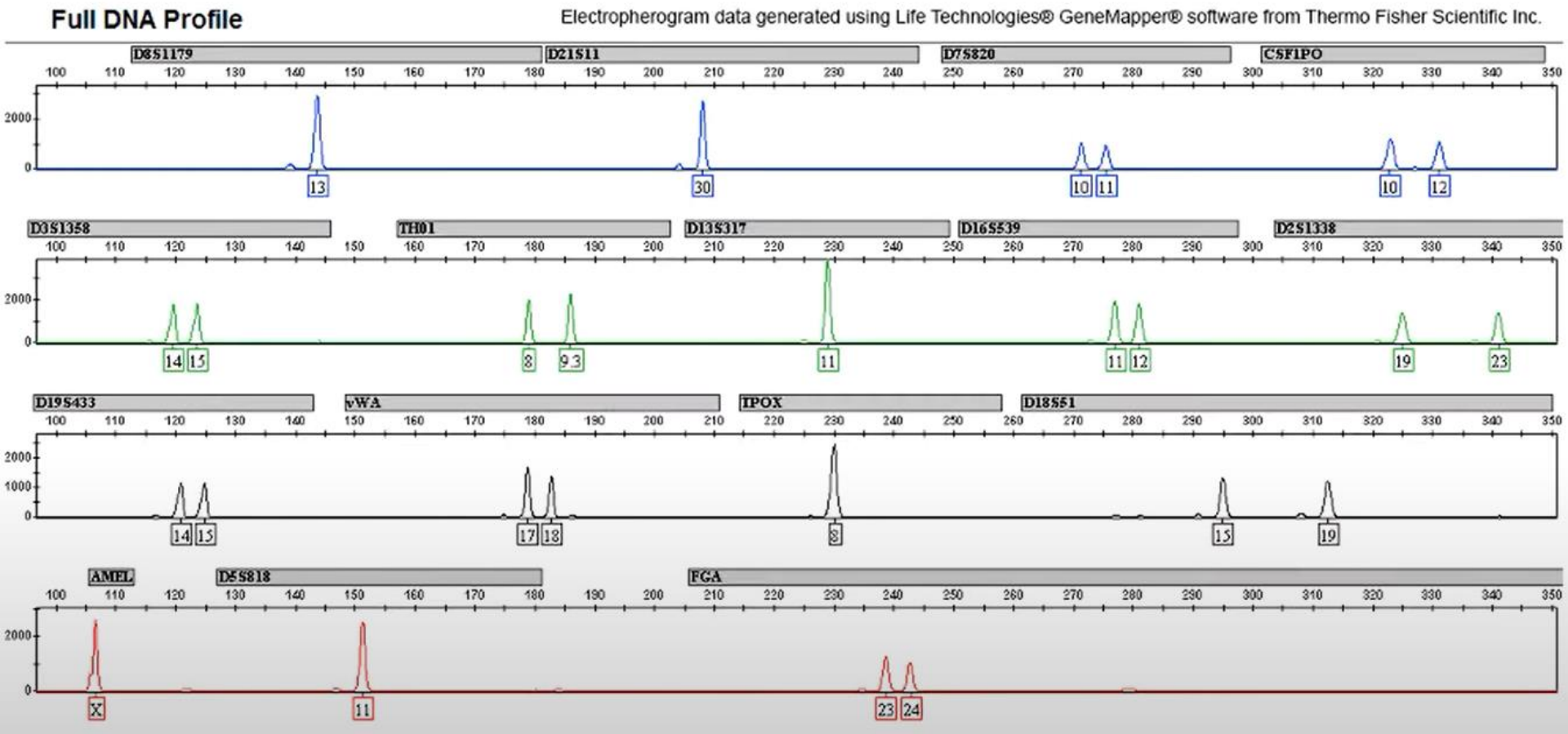


STR and VNTR Analysis

- Essentially creates a barcode/profile for a person.
- What is the advantage of creating a "barcode" for each person or piece of evidence?



STR and VNTR Analysis



What if you have no suspect to compare the DNA from the crime scene to?

- Forensic scientists will then compare the DNA from the crime scene to the DNA profile data base or CODIS (Combined DNA Index System ran by FBI).
- In 2024 there were over 20 million DNA profiles in CODIS.
- The vast majority of these profiles are those of convicted or suspected criminals (sexual assaults, murders etc).

What if you do not get a match with CODIS?

- In 2024 several states in the US are now allowing forensic scientists to perform Familial DNA Searches.
- Familial Searching grants forensic scientists' access to DNA profile bases that people have used to search out their ancestry using various companies/programs (AncestryDNA, 23andMe, FamilyTreeDNA and many others).
- In some cases, this expanded search matches someone in the database leading investigators to the suspect.
- In other cases, although no actual match can be found, they can sometimes identify an individual who is related to the perpetrator (sibling, first cousin, second cousin etc.). This points investigators in the direction of the suspect.
- Golden State killer – rapist and murderer convicted in 2018 for crimes committed from 1970s-2000s.

Universal DNA Database

- What would this do to crime rates?
- Could it act as a deterrent?
- What could it do to the rates of repeat offenders?
- On average, if not apprehended, sexual offenders will have 26.2 career victims.
- Could it drastically reduce time and cost of investigation?
- Kingdom of Bahrein created a Universal DNA Database.
- Pre-Universal DNA Databases - one of the highest rates of sexual assaults and murders.
- Post-Universal DNA Databases – second lowest rates of sexual assaults and murders in the world.

Universal DNA Database

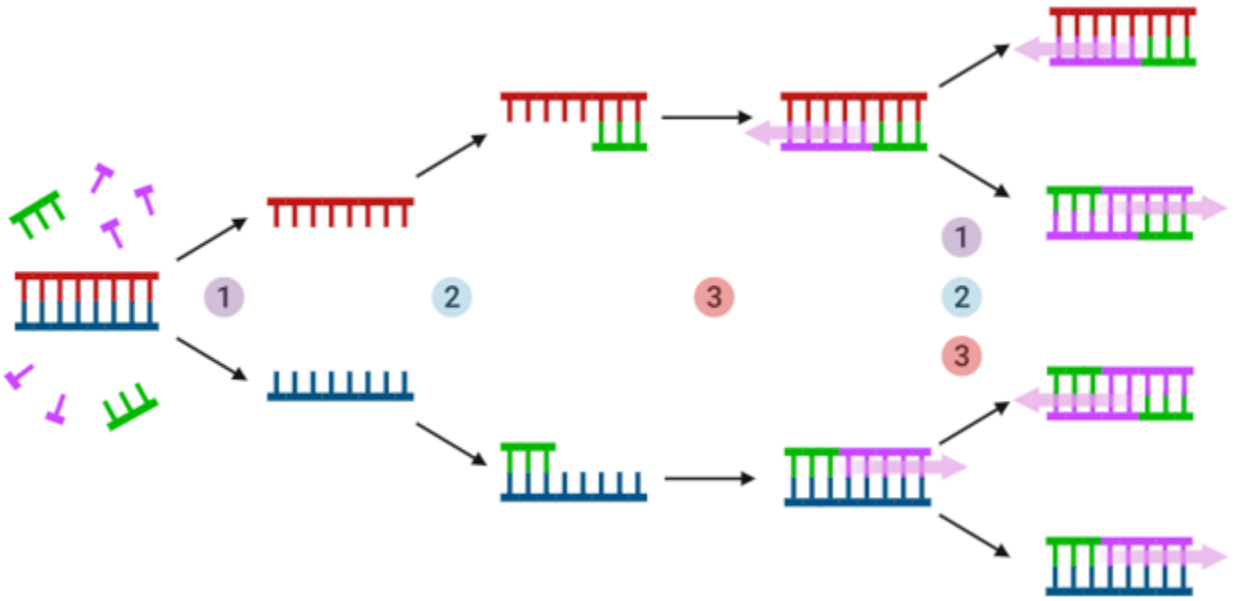
- How much pian and suffering could be saved?
- How many lives could be saved?
- Does it make sense financially to create a Universal DNA Database?
- The cost of a convicted sexual assault in 2023 is $435,000 USD?
- The cost of murder in 2023 is $17 million USD?
- This includes pain and suffering of the victims, lost wages, investigative costs, legal and trial costs, appeals, costs of incarceration etc).

Universal DNA Database

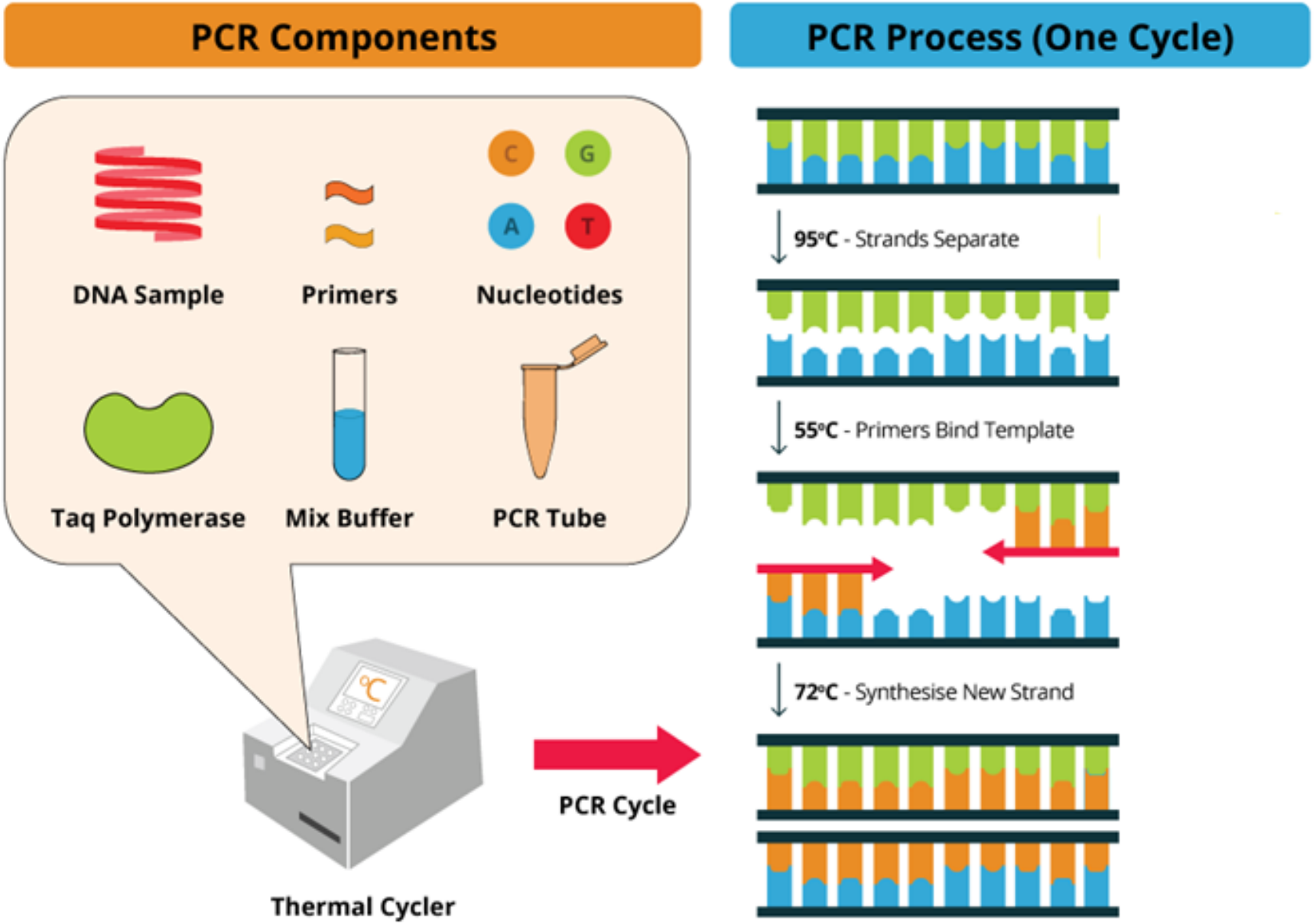
- A universal database might be viewed as more fair than current databases as all ethnic and socioeconomic groups would be represented based on their proportion of the population.
- A universal data base could protect individuals from wrongful convictions.
- Mark and Mark
- David Milgaard – 1969 at age 16 he was convicted of rape and murder of a nurse in Saskatoon – after 23 years in prison he was cleared by DNA evidence and awarded $10 million. Eventually the DNA evidence was used to convict the actual killer Larry Fisher.

PCR – Polymerase Chain Reaction

- A technique developed to amplify one DNA molecule to become over 1 billion molecules in less that 2 hours
- PCR is used for a variety of applications including gene mapping and expression and studying genetic diseases.
- PCR is also used to amplify DNA that was collected from a crime scene to levels sufficient for creating a DNA profile.

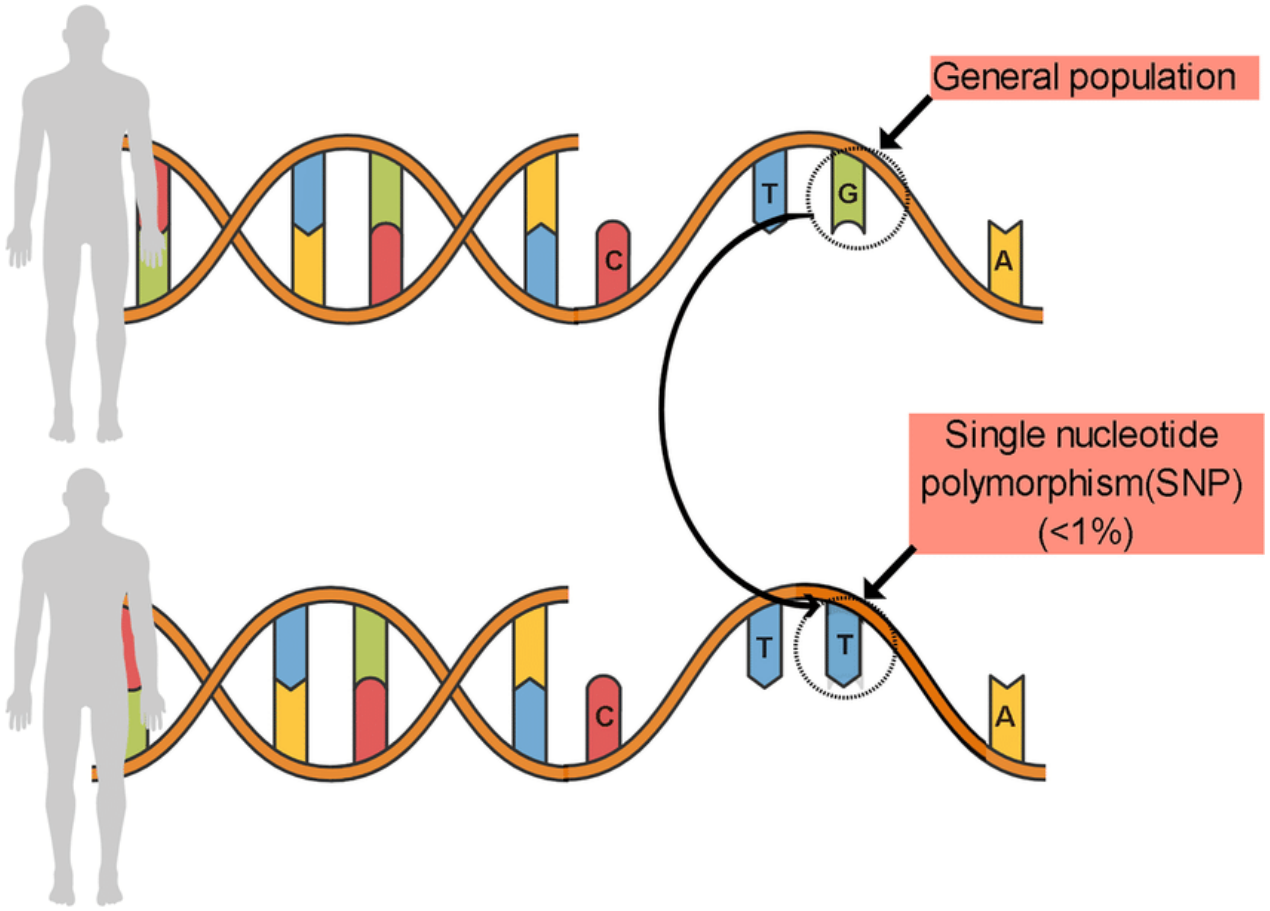


PCR – Polymerase Chain Reaction



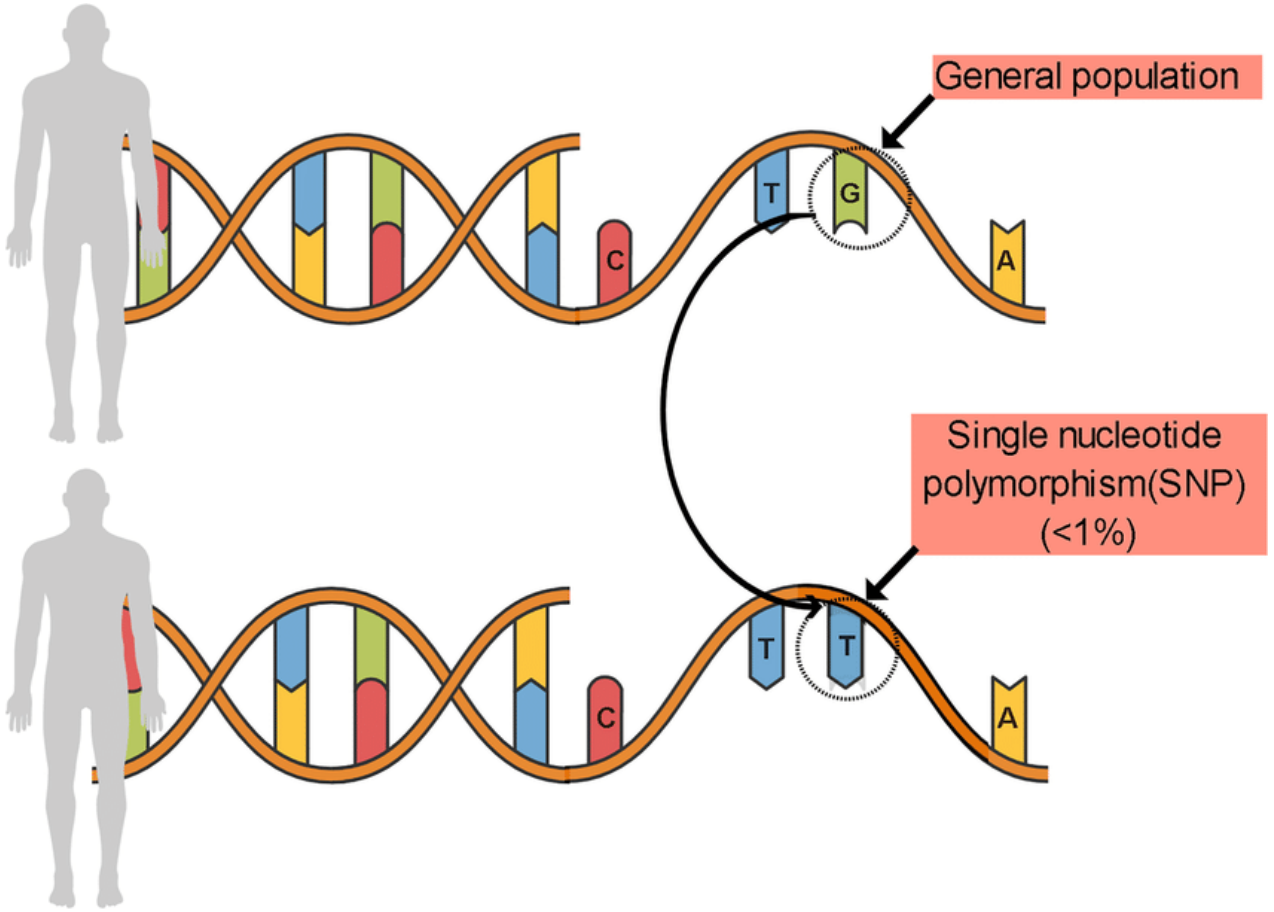
SNIPS Single Nucleotide Polymorphism AKA SNV Single Nucleotide Variant

- This is the latest technique use to analyze an individual's DNA.
- The DNA is analyzed to determine which of the 4 bases (A,T,C,G) is found in various precise key locations.



SNIPS Single Nucleotide Polymorphism AKA SNV Single Nucleotide Variant

- It turns out over 90% of the genetic difference we encounter are determined by which of the 4 nucleotide bases are found in that precise key location.

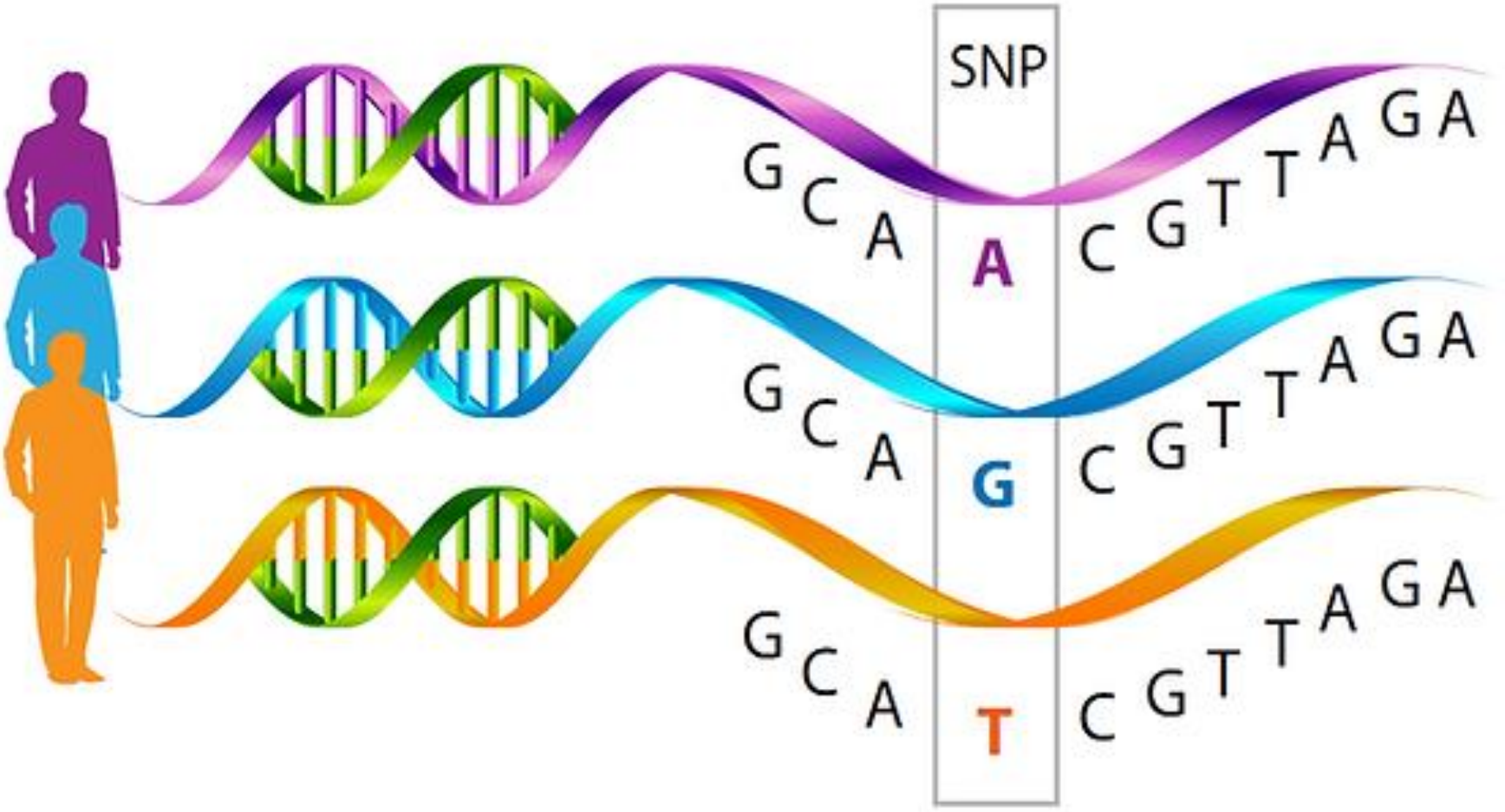


SNIPS Single Nucleotide Polymorphism
AKA SNV Single Nucleotide Variant

- In other words, for over 90% of the genes, how that gene is expressed, is determined by which of the 4 bases are found in that single precise location.
- For example, considering a gene that places you at risk for a particular disease such as cancer. Having an A in that precise key location might be beneficial and decrease your chances of that disease. Having a C might increase your chances of that disease, and a T or G might be neutral.

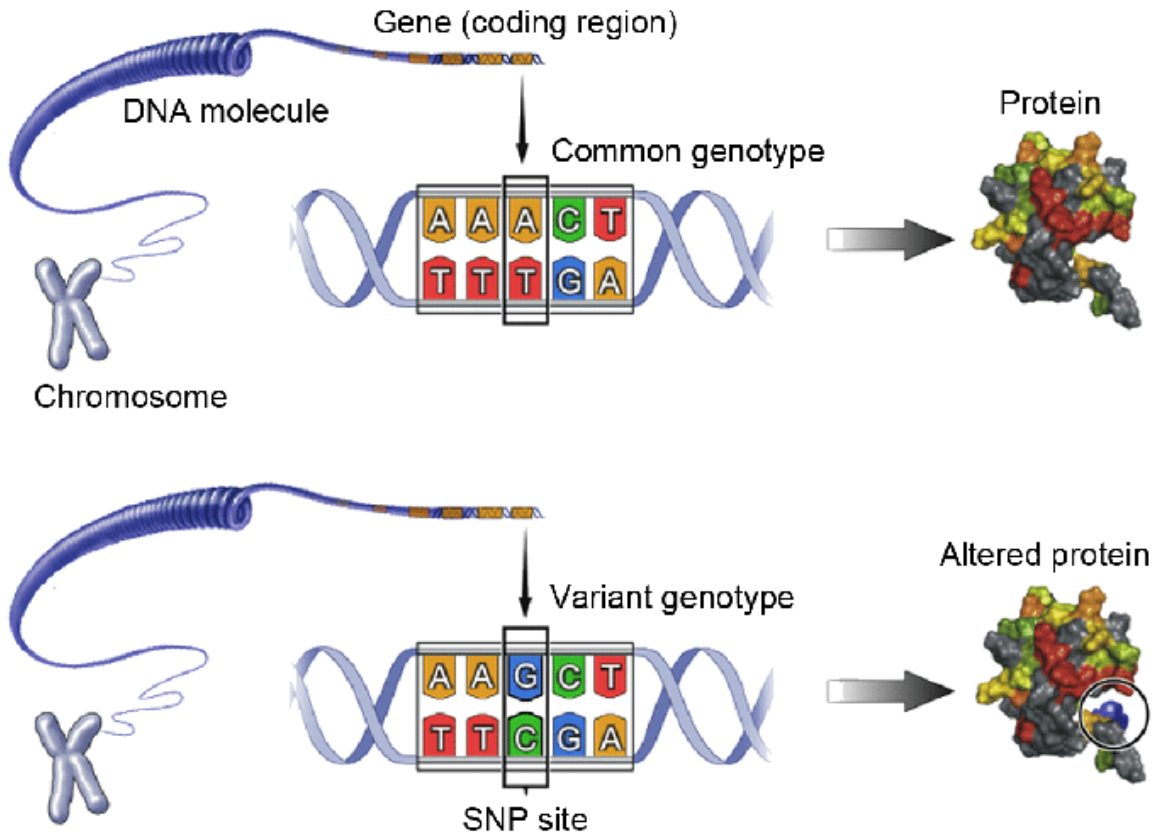
SNIPS Single Nucleotide Polymorphism
AKA SNV Single Nucleotide Variant

- Pictured below is 3 variants resulting form one SNP



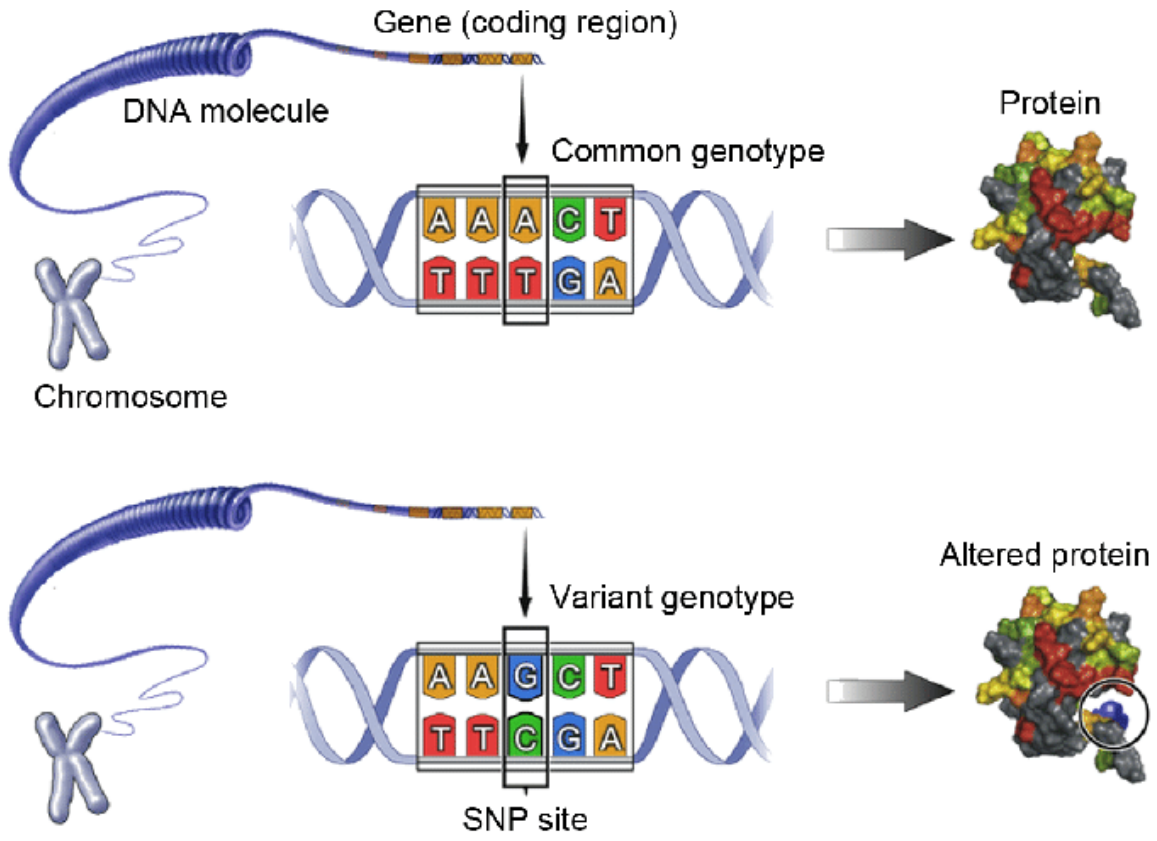
SNIPS Single Nucleotide Polymorphism AKA SNV Single Nucleotide Variant

- Your reported SNPs will look something like this, CYP1A2-rs762551 (A;A), indicating the name of the gene, the SNP, and what versions you inherited.



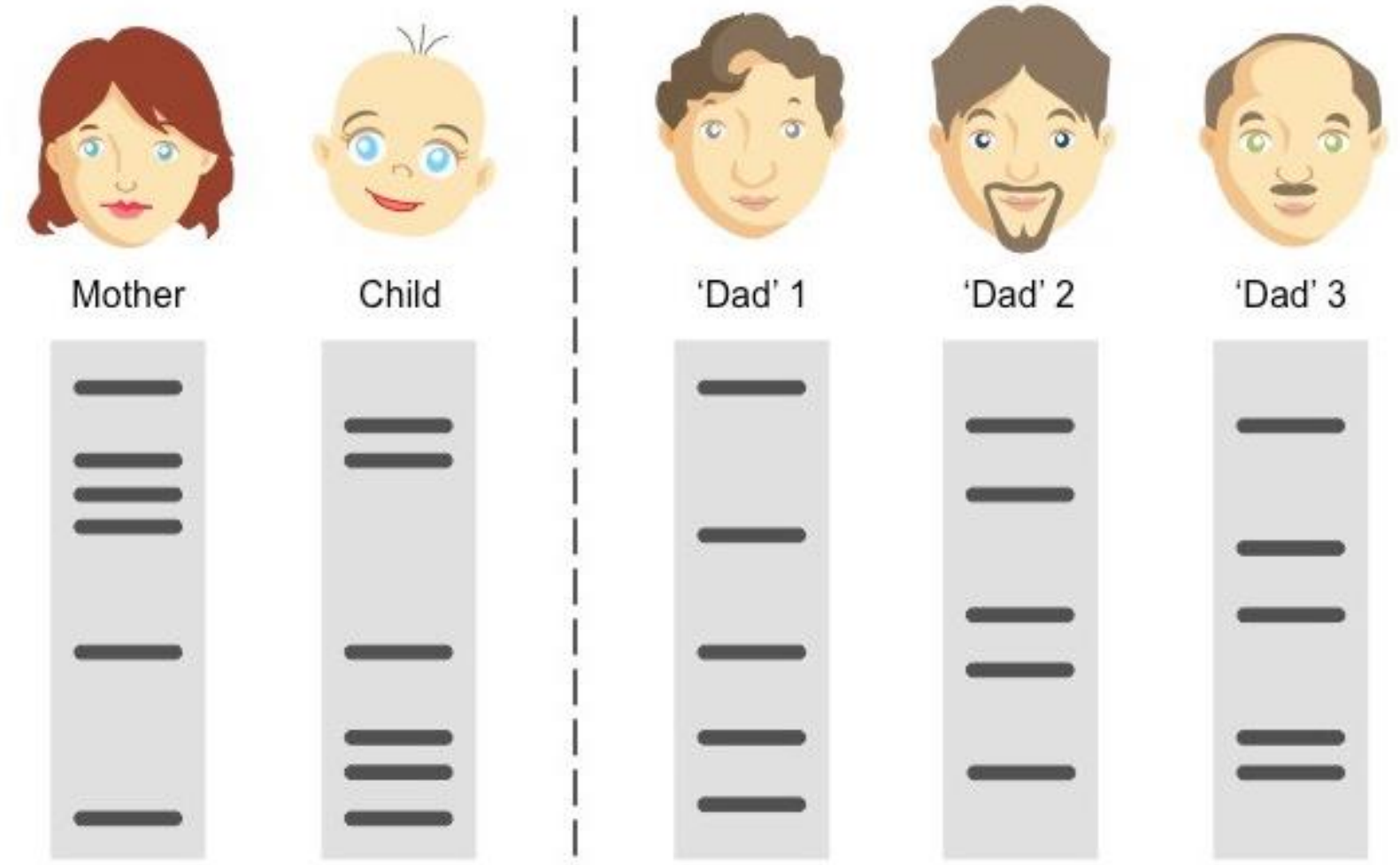
SNIPS Single Nucleotide Polymorphism
AKA SNV Single Nucleotide Variant

- Soon it might be possible for pharmaceutical companies and doctors to offer individuals specific and targeted treatments according to their genetic profile.

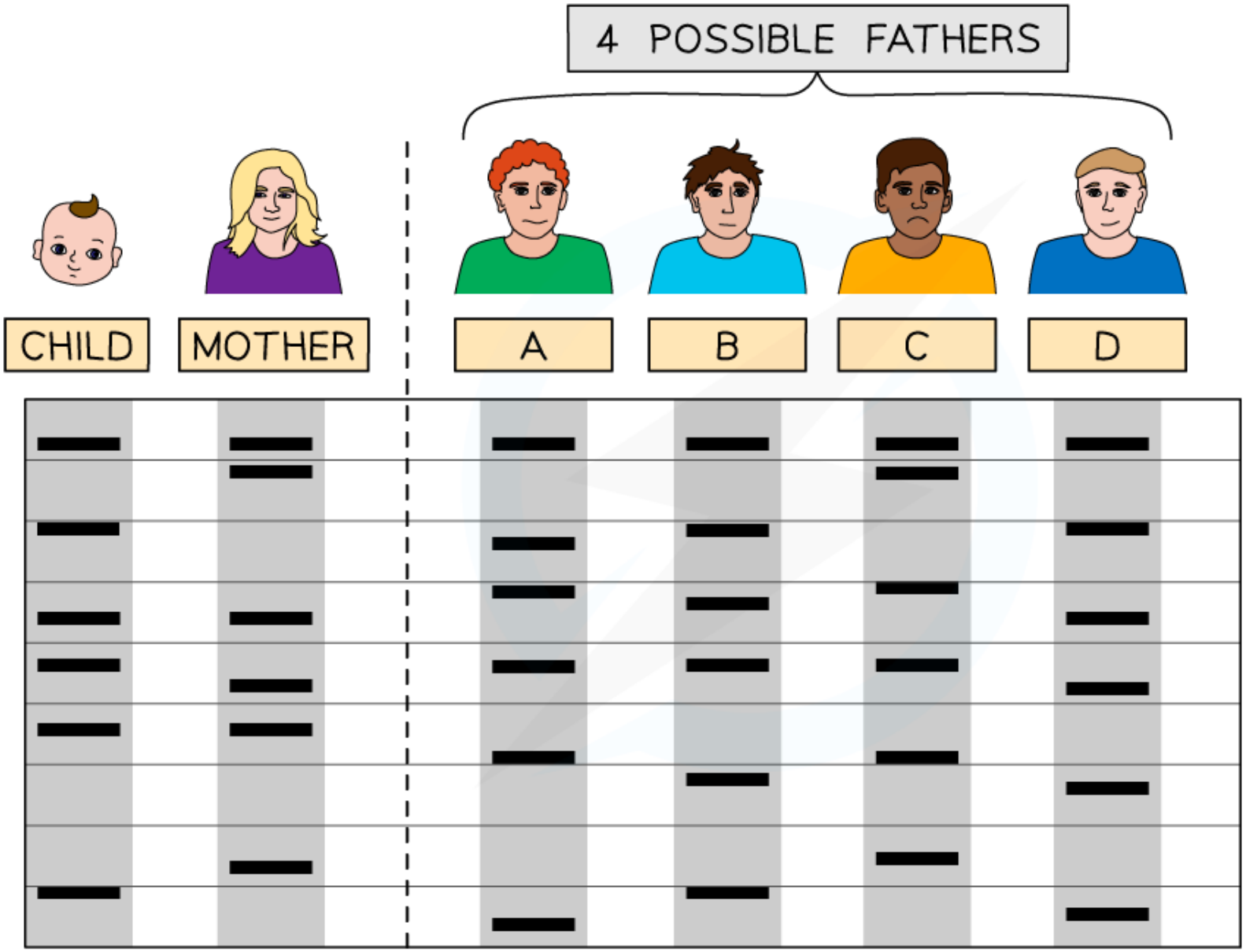


DNA Typing – Paternity Testing

- With paternity testing – would we expect the same type of exact DNA fingerprint matching as is the case with criminal investigations?
- NO. You get ½ your DNA from each parent.



Who's the father?



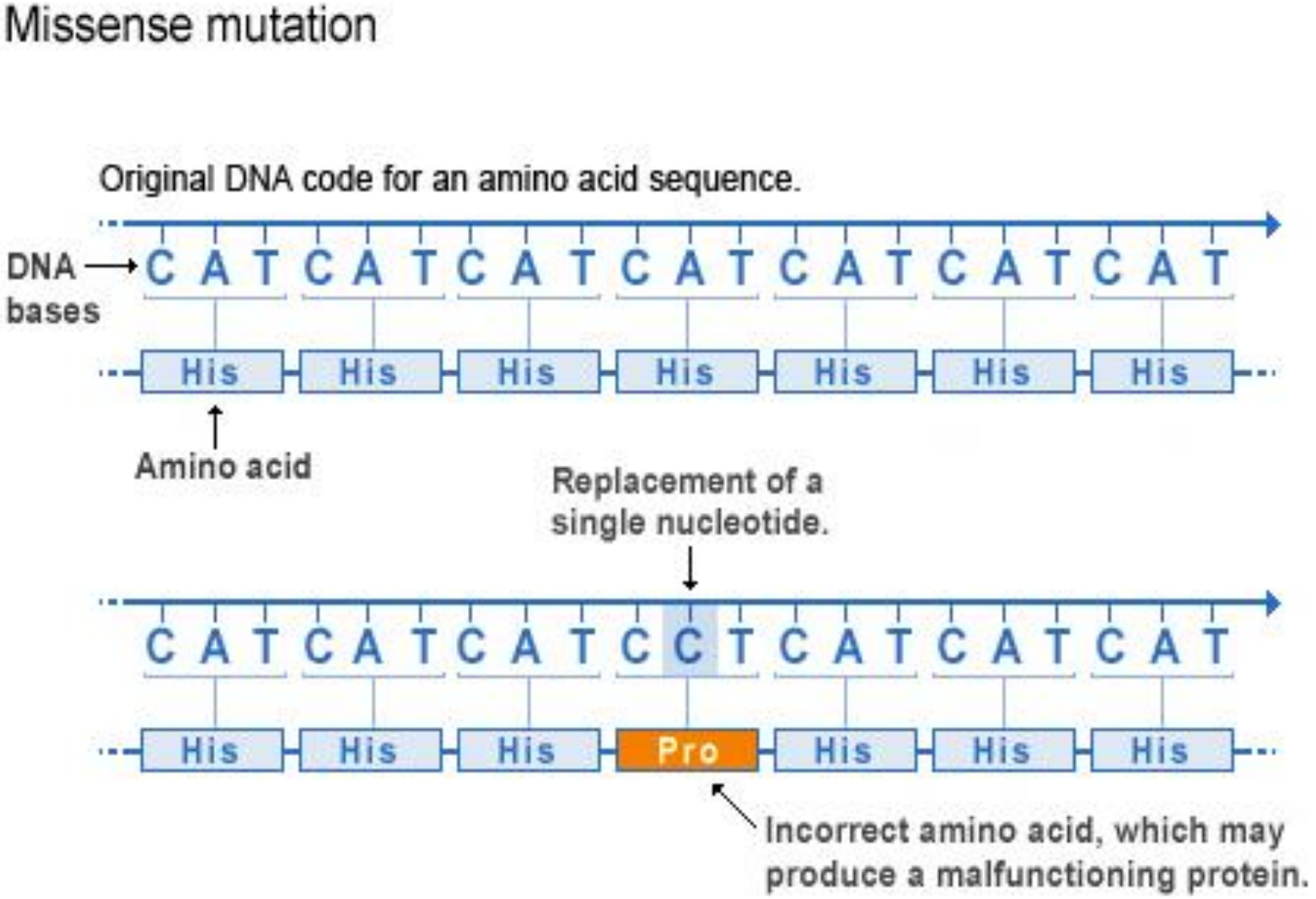
Mutations and Cancer

- A mutation is any change in the genetic code (a failure of DNA repair).
- There are 3 general types of mutations:
- 1) Single Base Substitutions
- 2) Insertions and Deletions
- 3) Translocations

Single Base Substitutions

- A single nucleotide (base) is replaced by another.
  Example: Adenine (A) replaced with Guanine (G)
- There are 3 types of single base substitutions.
- 1) Missense Mutation - alters the codon to produce different protein.
- 2) Nonsense Mutation - alters codon to one of the STOP codons resulting in a shortened protein.
- 3) Silent Mutation - the altered codon happens to code for the same amino acid as the original therefore no change in the protein produced.

Missense Mutation



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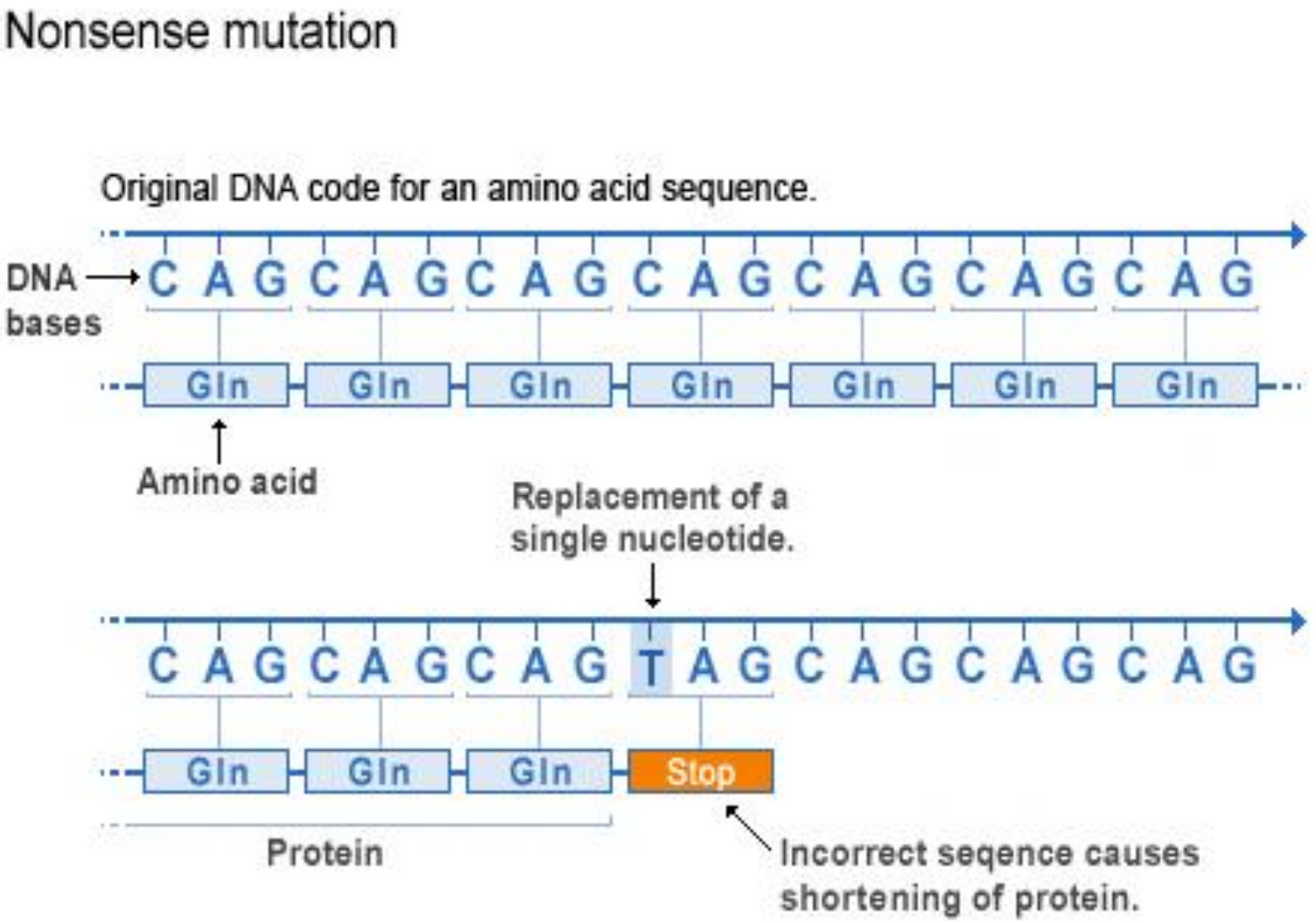
Nonsense Mutation



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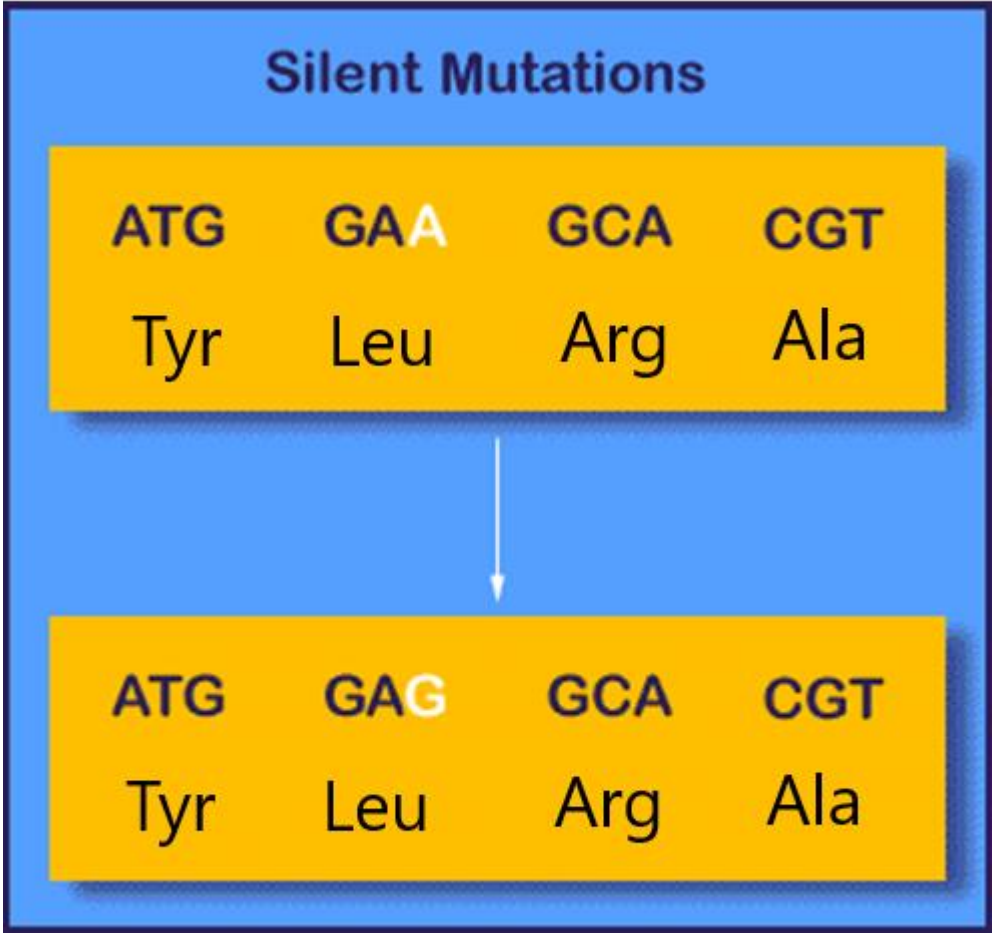
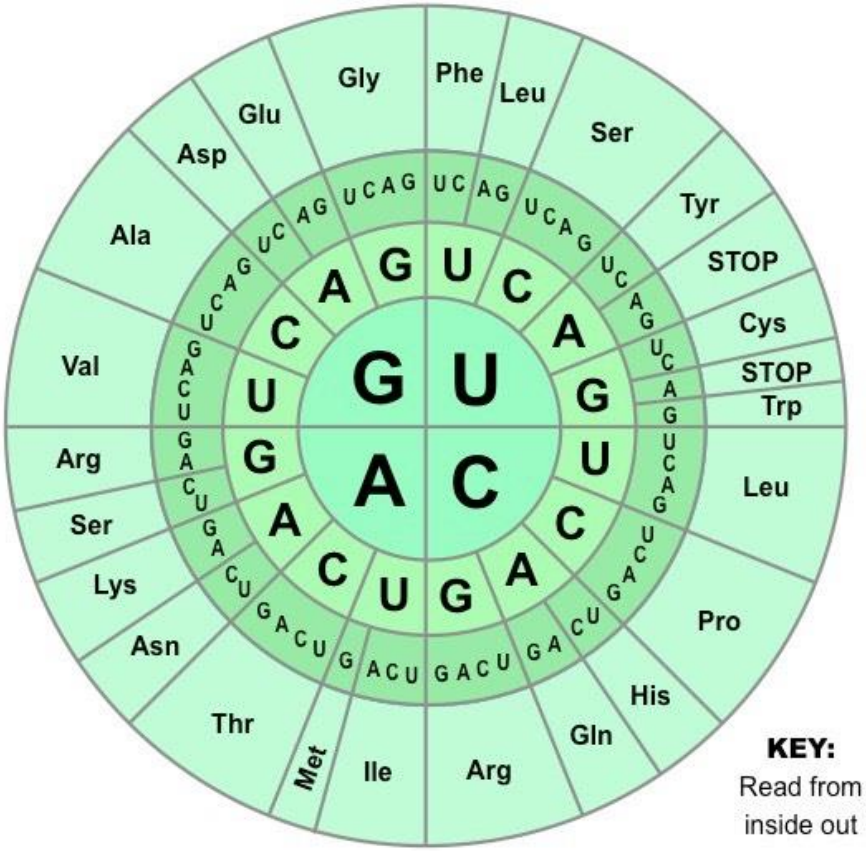


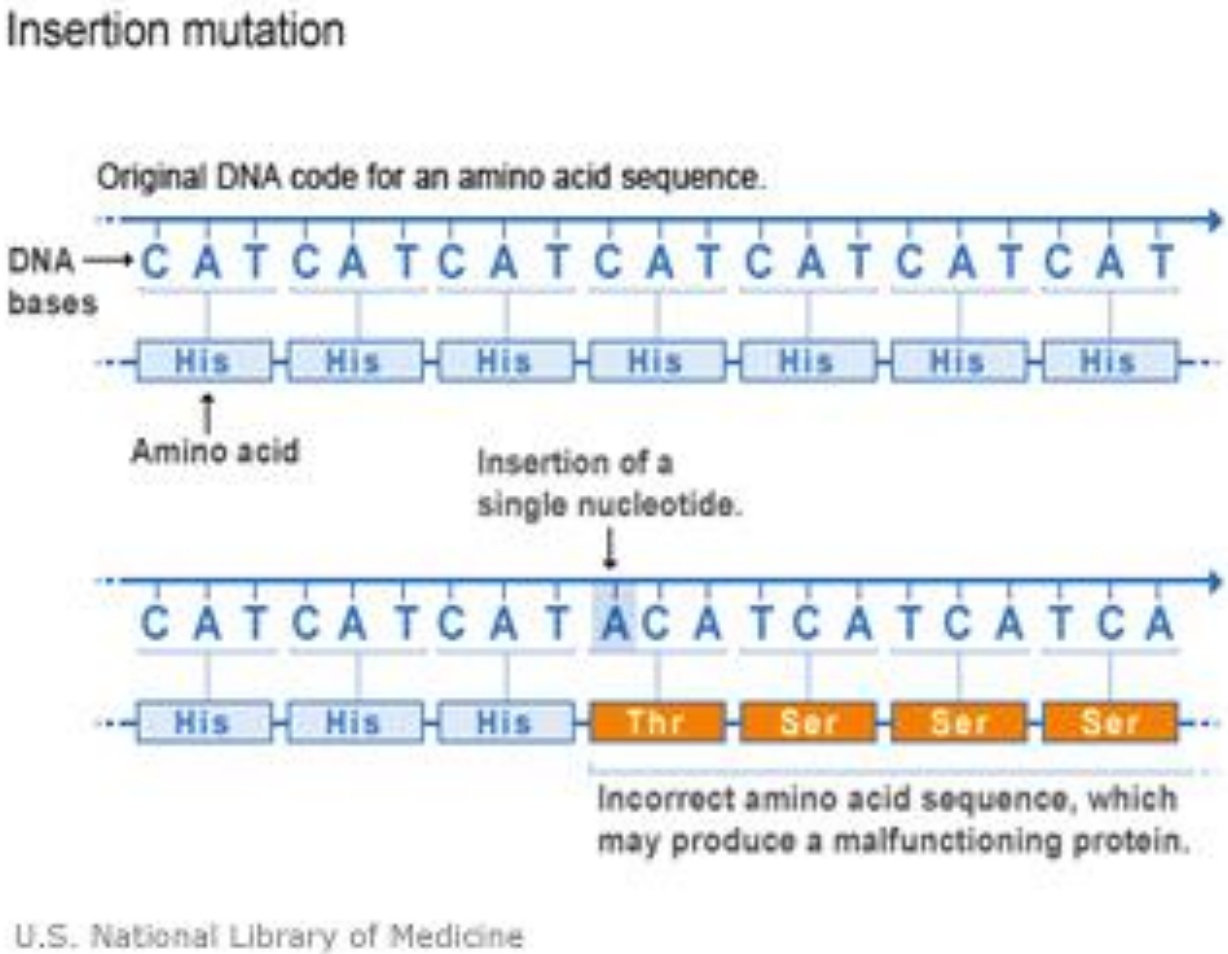
Silent Mutation



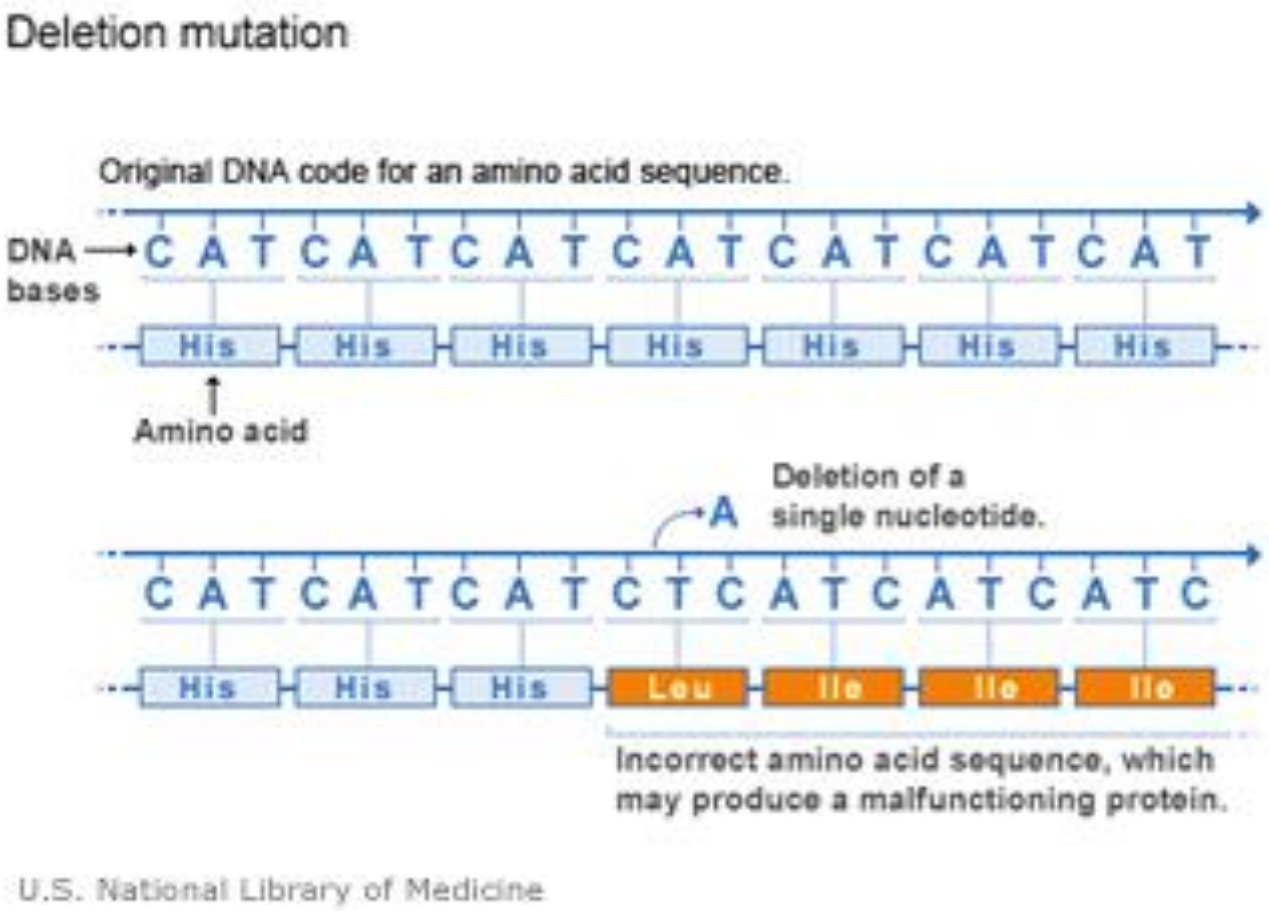
Insertions and Deletions

- Extra nucleotides (base pairs) are added or deleted from the DNA of a gene.
- If you had to choose between the following deletion mutations which would you choose?
  a) 1 nucleotide is deleted
  b) 3 nucleotides are deleted
- Insertions and deletions of 1 or 2 base pairs result in a "frameshift" and are particularly devastating.
- Insertions and deletions in multiples of 3 do not create a frameshift.

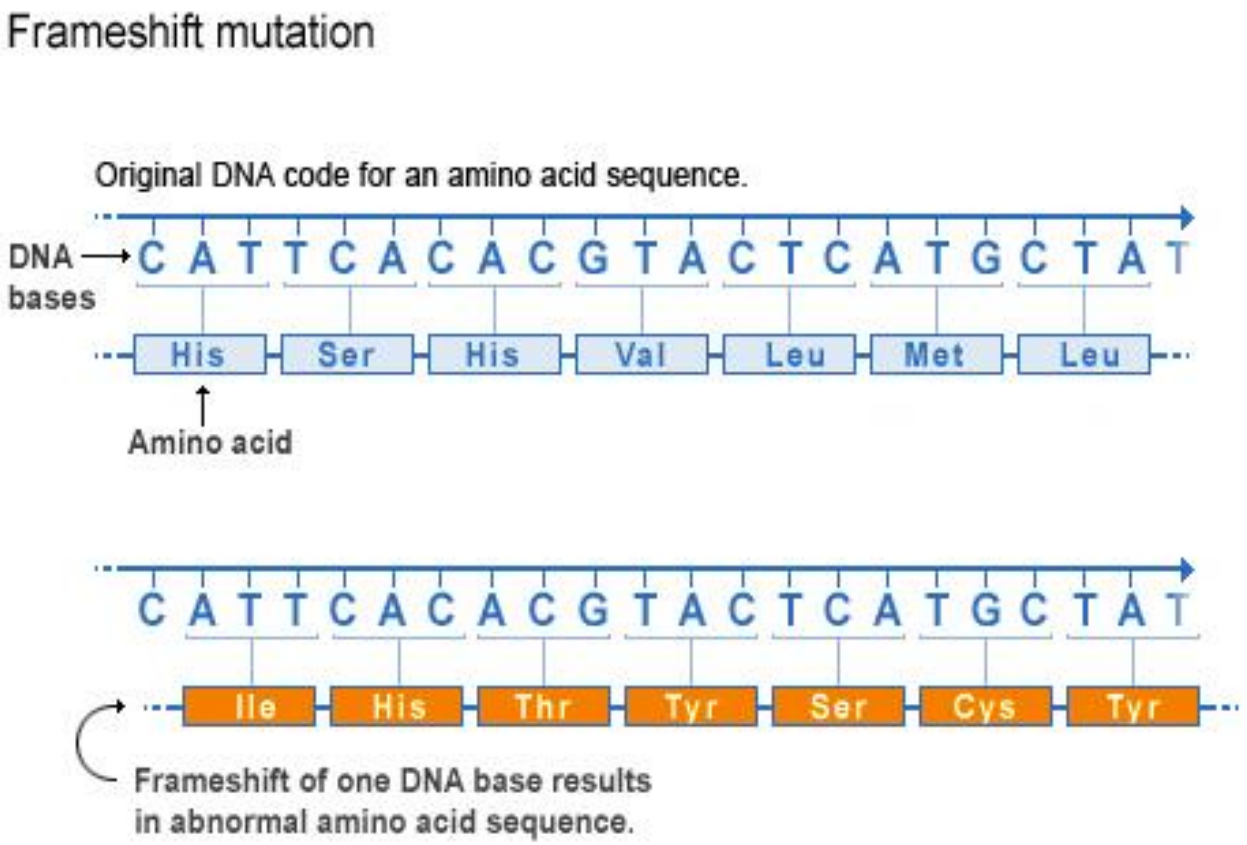
Insertion Mutation



Deletion Mutation

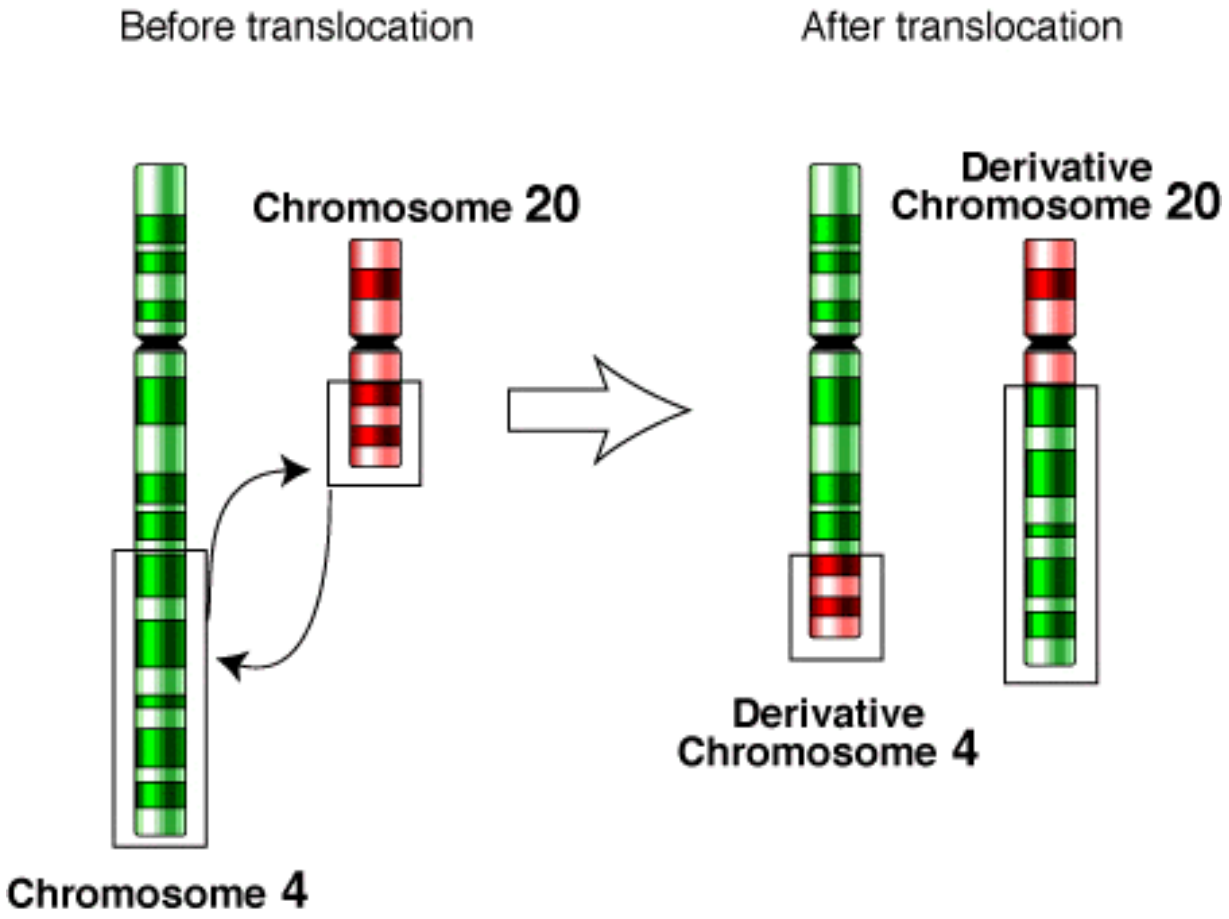


Frameshift Mutation



Translocation Mutation

- Transfers of an entire piece of one chromosome to a non-homologous chromosome.



Mutations and Cancer

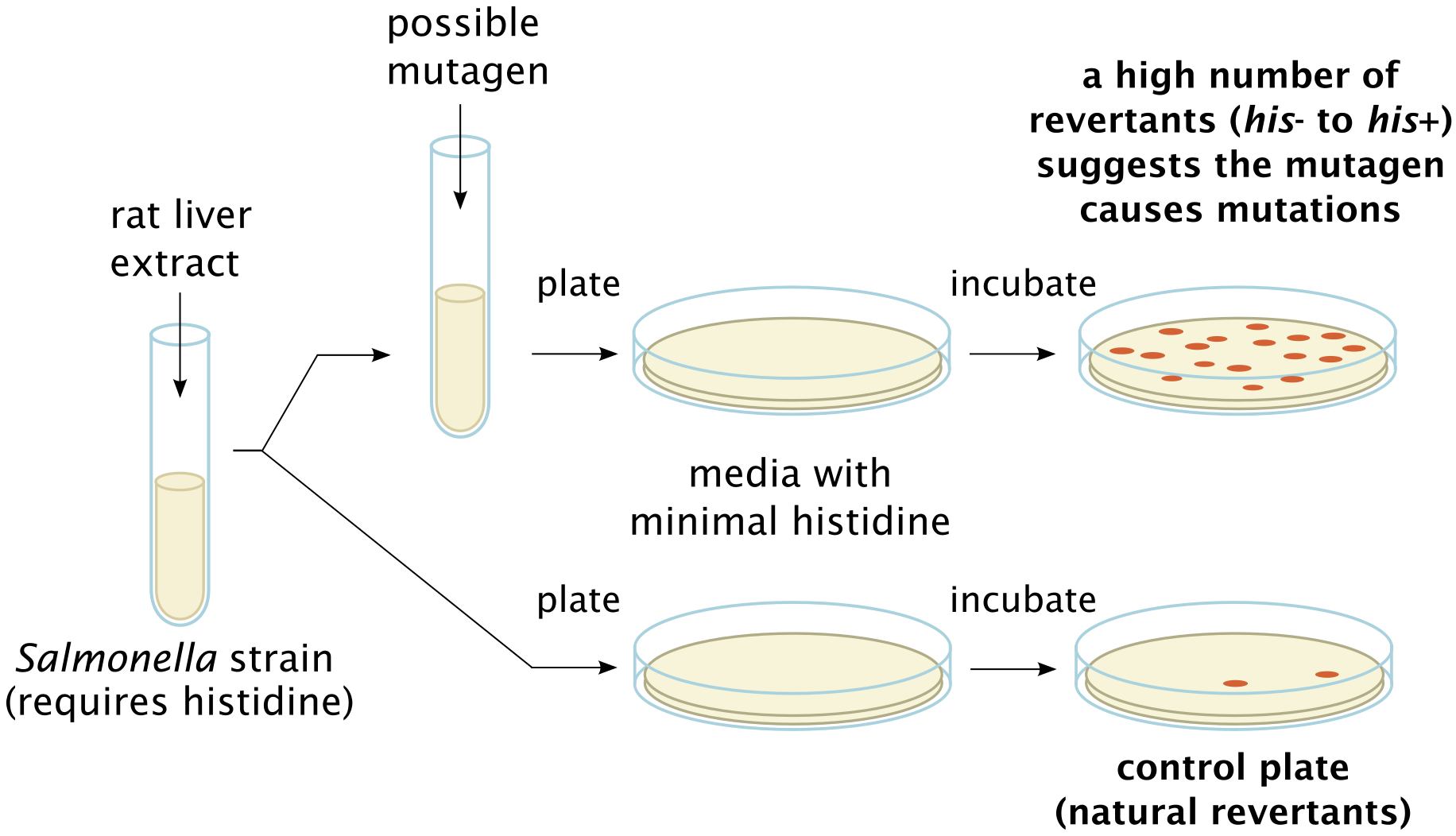
- Cancer is an uncontrolled growth of cells.
- The cell division can be fast or slow but the cells never stop dividing.
- Malign tumors are cancerous.
- Benign tumors are not.
- Cancers begin as a primary tumor and often establish metastases (moves through blood stream or lymph) in other body locations.
- Unfortunately these regions of secondary growth often end up being fatal.

Mutations and Cancer

- Anything that damages the DNA and causes a mutation can cause cancer.
- If the mutation is such that it "codes" for the cell to divide uncontrollably, then cancer has begun.
- Therefore chemicals that damage the DNA increasing the risk of cancer are called carcinogens.
- Other factors such as exposure to radiation can also damage the DNA and lead to cancer causing mutations.

The Ames Test

- An Ames test can be used to determine if a chemical is carcinogenic or not.
- The test uses a mutant strain of bacteria which suffered a point mutation such that it lacks the ability to produce a certain amino acid (histidine).
- If, after exposure to the "test chemical", colonies of bacteria begin to grow again, it is assumed that the "test chemical" caused a high number of mutations in the bacteria allowing it to again produce the amino acid it was lacking.
- Thus the "test chemical" is deemed a mutagen and might be eventually classified as "carcinogenic".


possible
mutagen
rat liver
extract
plate
incubate
a high number of
revertants (*his*- to *his*+)
suggests the mutagen
causes mutations
media with
minimal histidine
plate
incubate
Salmonella strain
(requires histidine)
control plate
(natural revertants)

- All plates lack histidine and are streaked with “histidine negative bacteria”.
- A and B are the control plates.
- Does Experimental Sample 1 pass the Ames test? Is it a mutagen/carcinogen?
- Does Experimental Sample 2 pass the Ames test? Is it a mutagen/carcinogen?