FROM DNA TO PROTEIN

PROMISE

SANFORD RESEARCH

WATCH THIS!

Can you find a diagnosis for this family?

Start by reviewing Blake's info and filling in his Patient Chart.

PATIENT CHART

PATIENT NAME: Blake Fugere	AGE: 5	SEX AT BIRTH: ☒ Male ☐ Female ☐ Unassigned
WEIGHT: 40	BLOOD PRESSURE: 120/80	HEART RATE: 95 bpm

KEY SYMPTOMS:

- stumbling
- vision worsening
- seizures
- not recognizing mother

INITIAL DIAGNOSIS: Unknown

ORDER TESTS

To diagnose Blake, you must conduct clinical observations and order initial tests.

TEST RESULTS

Vision Test: Irregular - significant vision loss

Blood Test: Normal - no sign of infection or kidney/liver failure

Genetic Test: Irregular - recessive for both copies of the CLN6 gene (Homozygous)

Clinical Observations: Irregular - disrupted gait, myoclonic jerks

INVESTIGATING DNA

Deoxyribonucleic acid is a molecule made up of deoxyribose sugar, phosphate, and nitrogenous bases.

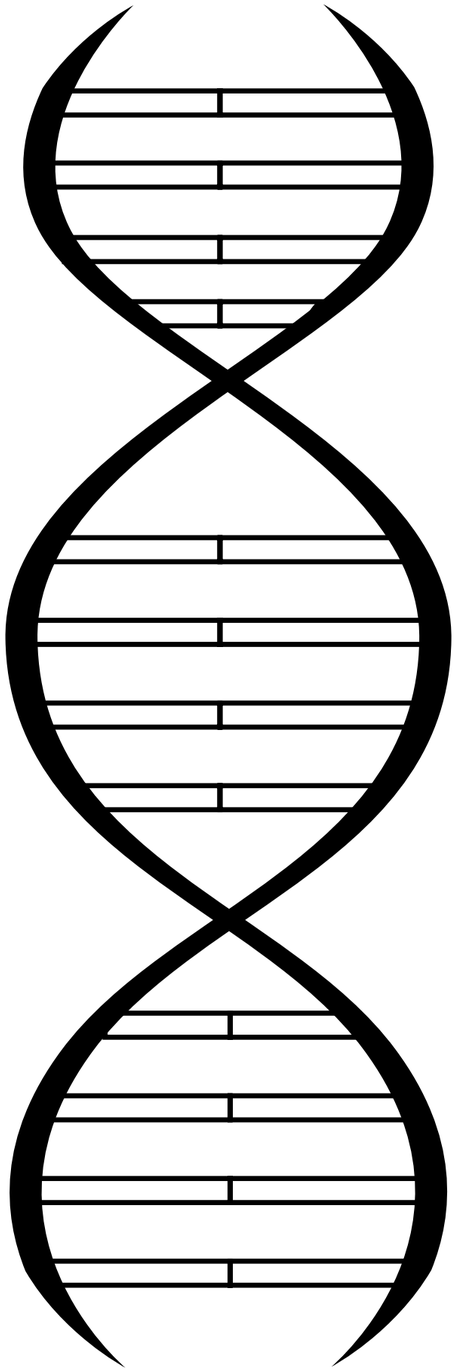
There are 4 types of nitrogenous bases that make up the whole human genome.

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

INVESTIGATING DNA

Each somatic cell has 46 chromosomes (23 from each parent).

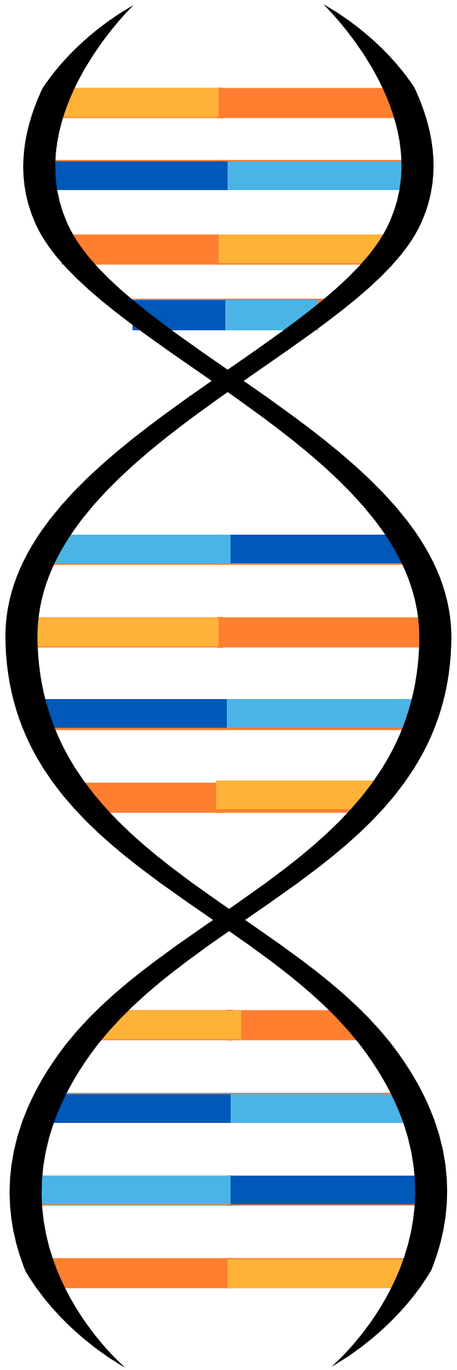
The human genome is 3.2 billion bases long. Multiply that by 2 of each chromosome. That equals 6.4 billion bases.



DNA STRUCTURE

DNA has two strands that wind together in a helix shape.

If laid flat, DNA would look like a ladder, with bases meeting in the middle.



DNA STRUCTURE

Bases are paired together. **Adenine** always bonds to **Thymine**. **Cytosine** always bonds to **Guanine**.

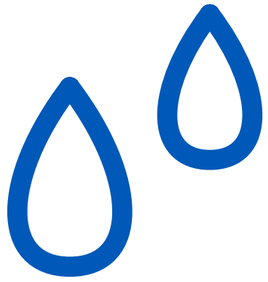
The sequence of **ATCG** is what gives every organism a unique code.

What does the DNA code do?

Segments of DNA that hold the code to make a protein are called genes.

Each gene can make an average of three proteins.

What human proteins do you know about?



HEMOGLOBIN

A protein with 4 chains that carries oxygen for your red blood cells

MELANIN

A protein that is found in skin cells that help protect your cells from UV radiation

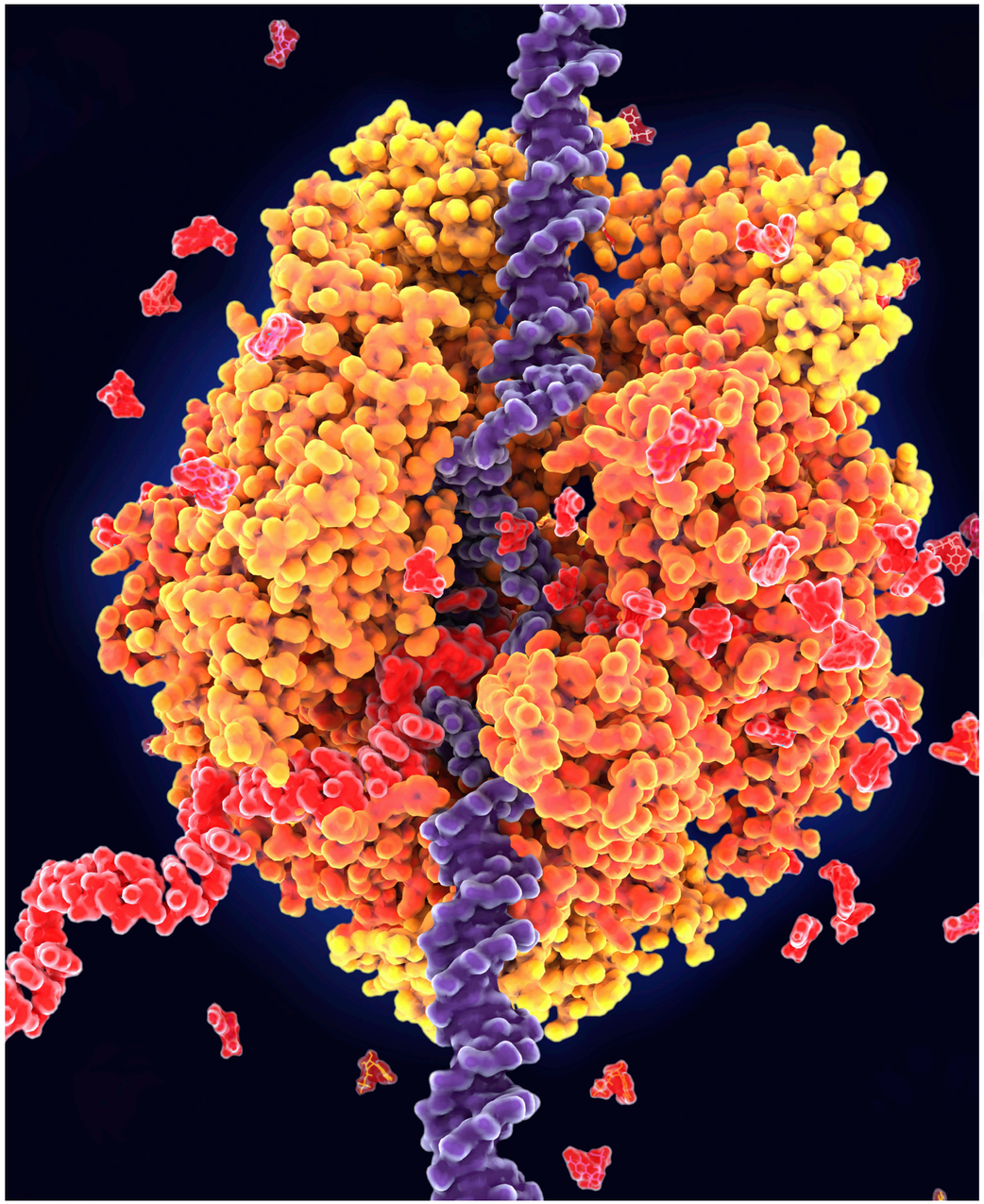
LACTASE

An enzyme that helps your digestive system to break down lactose-the sugar found in milk

DNA CODE TO PROTEIN

DNA needs to be transcribed into messenger RNA.

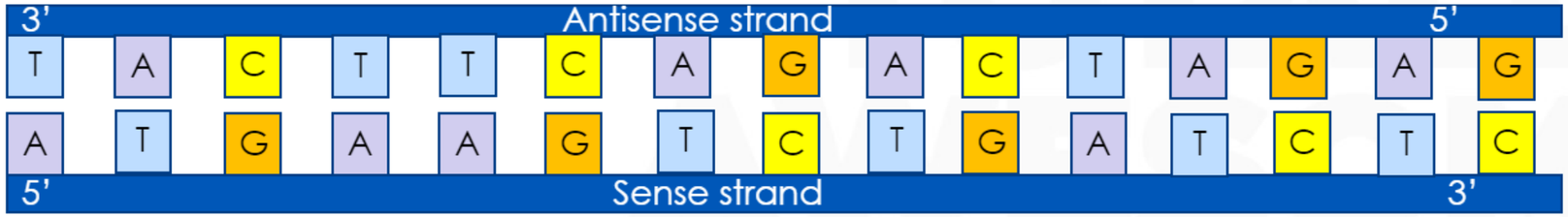
DNA can’t leave the nucleus, so mRNA takes the code out to the cytoplasm.



Enzymes transcribe DNA into mRNA.

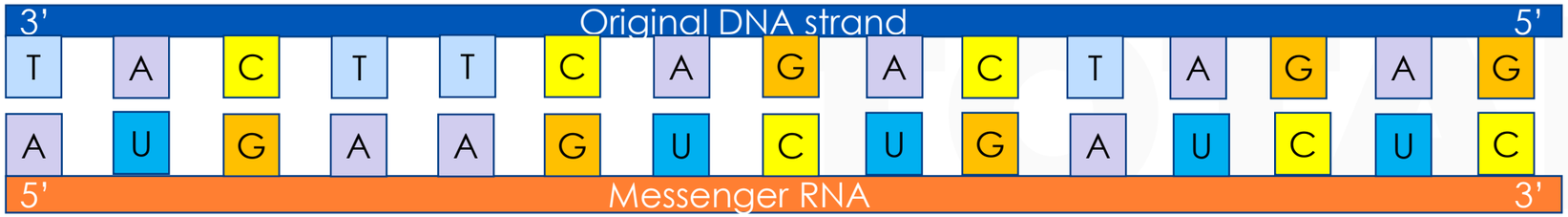
- **DNA helicase:** This enzyme unzips the DNA at the start of the gene.
- **RNA polymerase:** This enzyme assembles RNA nucleotides and makes the complementary code to DNA.

OPEN READING FRAME



- An ORF is the coding part of a gene.
- It starts with a start codon - AUG
- It ends with a stop codon - Several different options
- The antisense strand serves as the template for RNA.
- RNA nucleotides are assembled from the template.

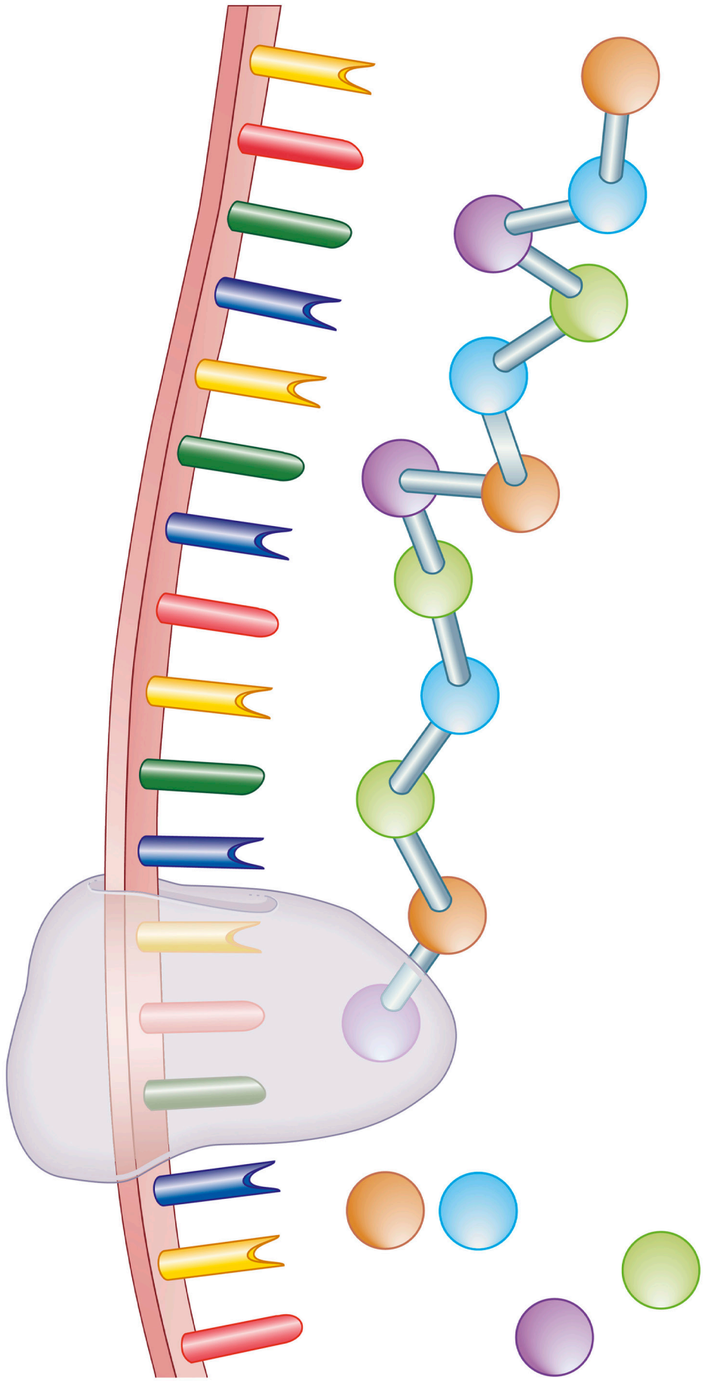
OPEN READING FRAME



In DNA the base pairing rules include Adenine-Thymine; Guanine-Cytosine. RNA contains Uracil instead of Thymine.

The pairing is as follows:

- Adenine → Uracil
- Thymine → Adenine
- Cytosine → Guanine
- Guanine → Cytosine



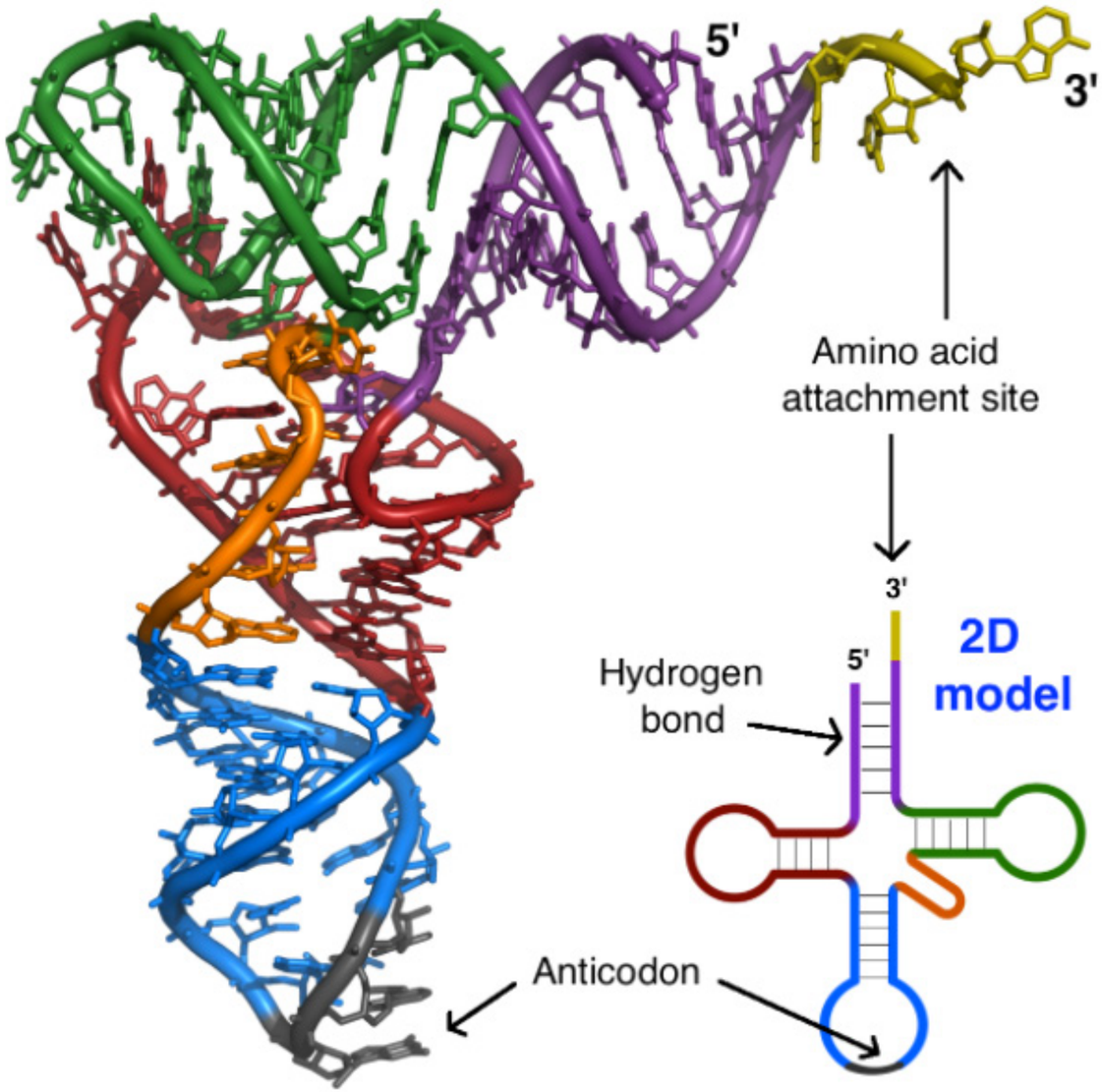
TO THE CYTOPLASM

- Messenger RNA leaves the nucleus through nuclear pores and DNA winds back up.
- There are portions of the RNA that are non-coding regions (Introns)
- Introns are spliced to leave only the coding region of mRNA- (Exons)
- mRNA moves through the cytoplasm and docks on a ribosome.
- At the ribosome, the next step of protein synthesis happens.

TRANSLATION

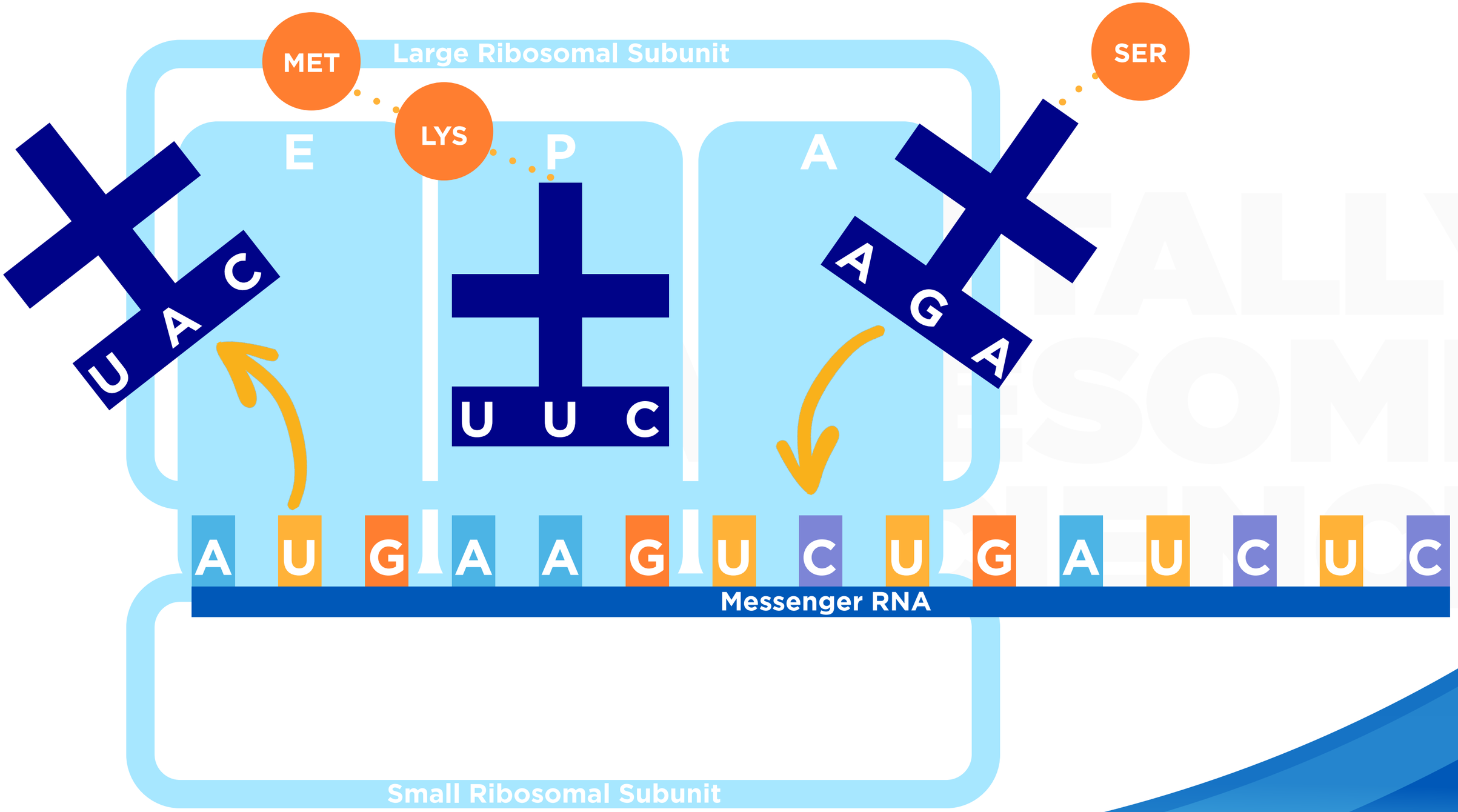
	U	C	A	G	
U	UUU, UUC } Phe - F UUA, UUG } Leu - L	UCU, UCC, UCA, UCG } Ser - S	UAU, UAC } Tyr - Y **UAA Stop** **UAG Stop**	UGU, UGC } Cys - C **UGA Stop** UGG } Trp - W	U C A G
C	CUU, CUC, CUA, CUG } Leu - L	CCU, CCC, CCA, CCG } Pro - P	CAU, CAC } His - H CAA, CAG } Gln - Q	CGU, CGC, CGA, CGG } Arg - R	U C A G
A	AUU, AUC, AUA } Ile - I **AUG Met - M**	ACU, ACC, ACA, ACG } Thr - T	AAU, AAC } Asn - N AAA, AAG } Lys - K	AGU, AGC } Ser - S AGA, AGG } Arg - R	U C A G
G	GUU, GUC, GUA, GUG } Val - V	GCU, GCC, GCA, GCG } Ala - A	GAU, GAC } Asp - D GAA, GAG } Glu - E	GGU, GGC, GGA, GGG } Gly - G	U C A G

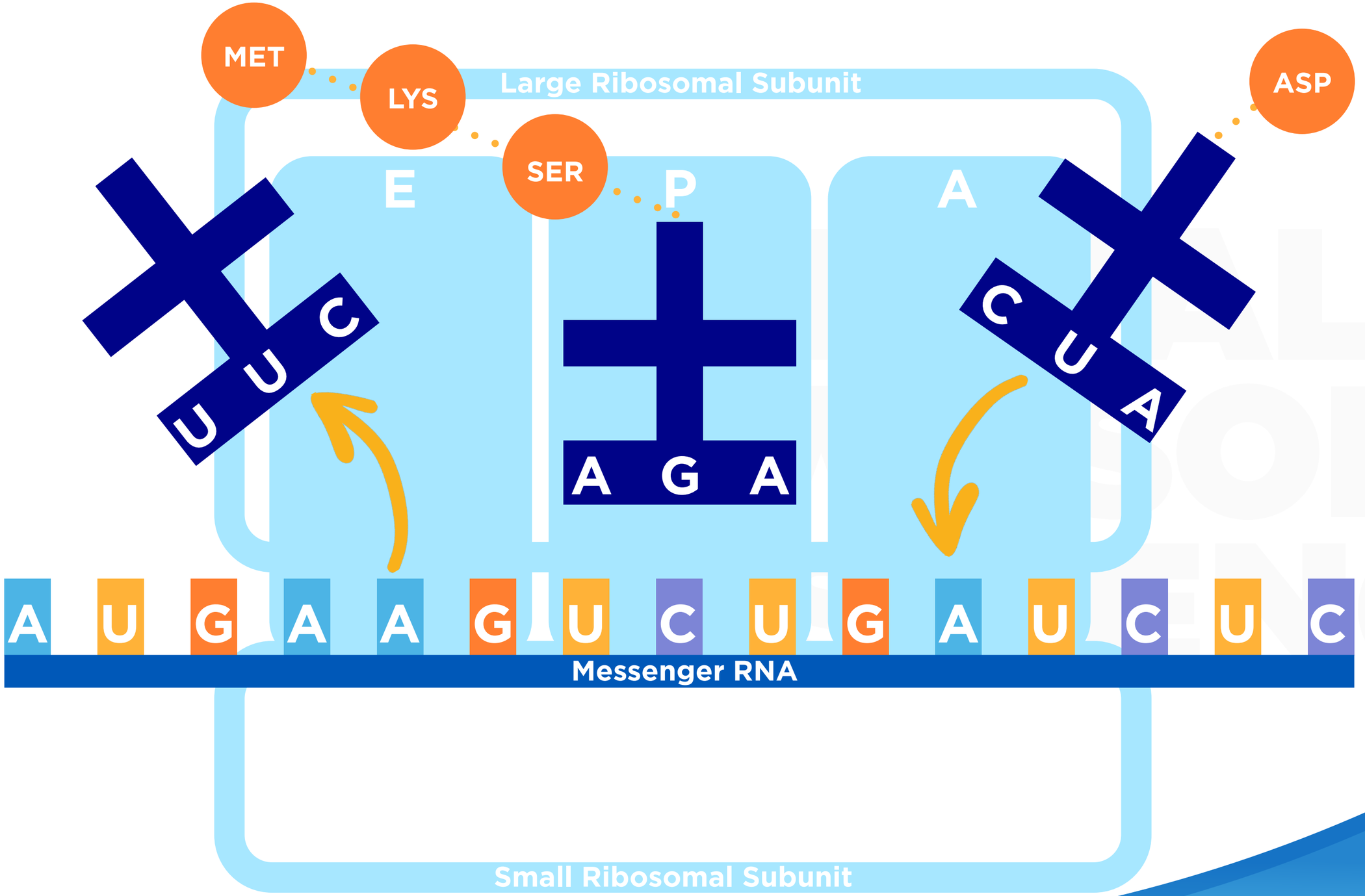
- A group of 3 nucleotides is called a codon.
- Each codon corresponds to one amino acid.
- There are 20 amino acids.
- A sequence of amino acids is what makes up a protein.

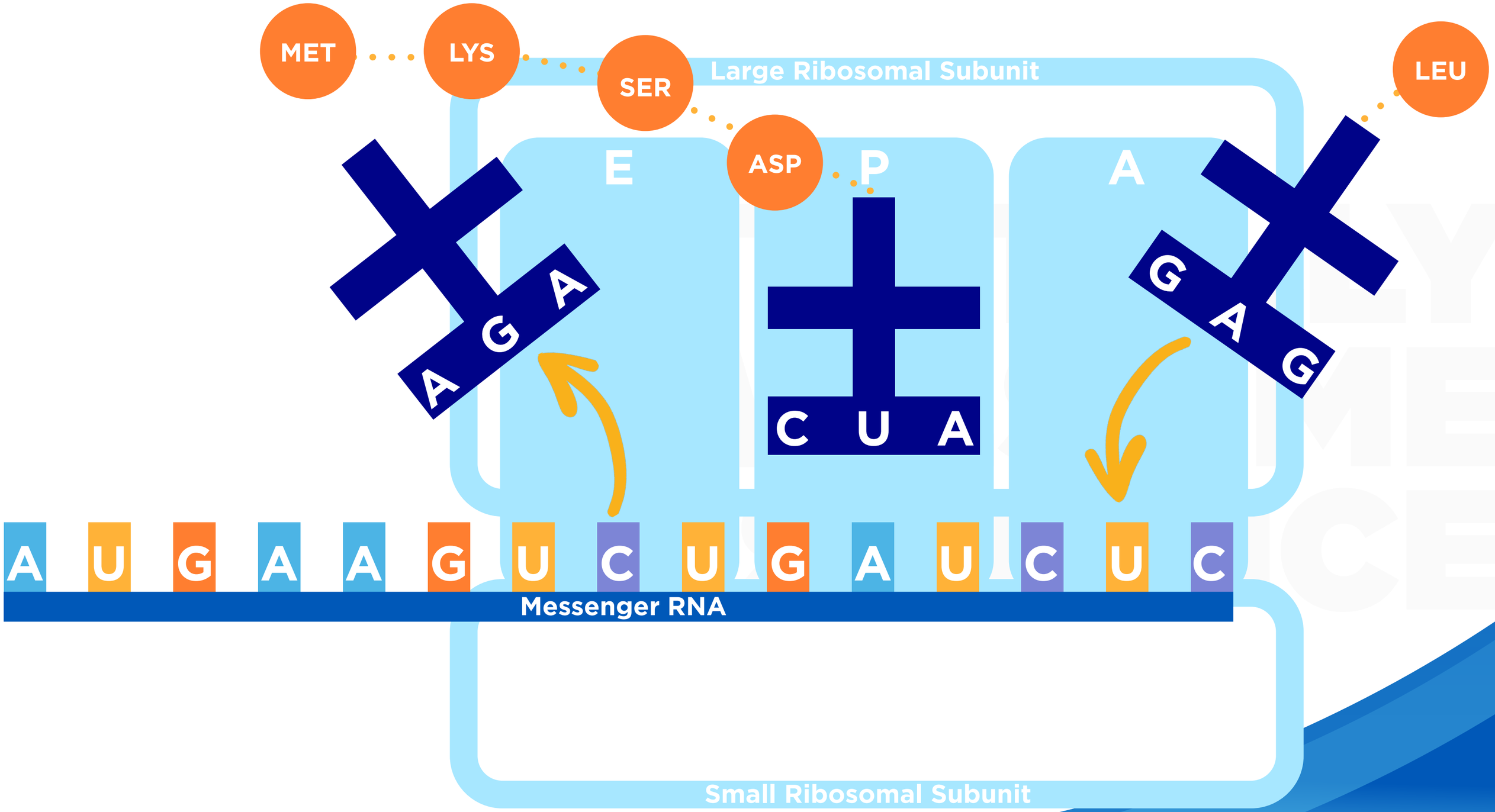


In order to change RNA code to amino acid sequence, a “translator” is needed. This is the function of transfer RNA.

tRNA contains an anticodon on one end and an amino acid on the other.


MET
LYS
SER
Large Ribosomal Subunit
E
P
A
U A C
U U C
A G A
A U G A A G U C U G A U C U C
Messenger RNA
Small Ribosomal Subunit


MET
LYS
SER
ASP
Large Ribosomal Subunit
E
P
A
U U C
A G A
C U A
A U G A A G U C U G A U C U C
Messenger RNA
Small Ribosomal Subunit


MET
LYS
SER
ASP
LEU
Large Ribosomal Subunit
E
P
A
A G A
C U A
G A G
A U G A A G U C U G A U C U C
Messenger RNA
Small Ribosomal Subunit

FINISHING STEPS

A chain of amino acids is called a polypeptide.

Polypeptides need to be folded. Some proteins (cytosolic) are folded in the cytosol. Some proteins are sent to the Rough Endoplasmic Reticulum to be folded.

Signal sequences are added as a tag so they go to the right place.

FINISHING STEPS

Folded proteins are sent to the Golgi Apparatus to be packaged in a vesicle and sent to the correct location.

Any protein that is not folded correctly is labeled for destruction.

Name: _____

PROMISE SANFORD RESEARCH

TRANSCRIPTION & TRANSLATION

The goal of this activity is to transcribe and translate this DNA sequence to a polypeptide (protein). This DNA sequence belongs to a zebrafish. Zebrafish are important to research because they share 70% of their genes with humans.

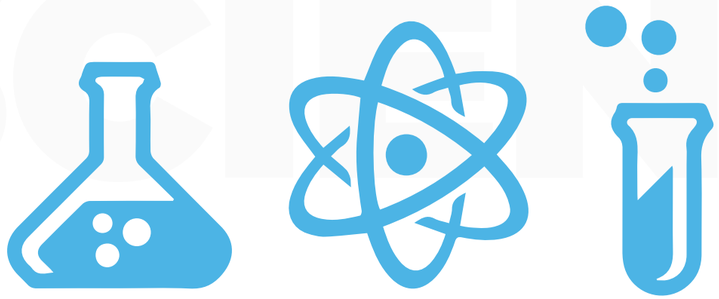
STEP 1: Transcribe DNA to RNA. A > U; T > A; C > G; G > C

STEP 2: Translate RNA to amino acids using the codon box below.

STEP 3: Use the table to decide which protein you made.

	U	C	A	G	
U	UUU, UUC – Phe - F UUA, UUG – Leu - L	UCU, UCC, UCA, UCG – Ser - S	UAU, UAC – Tyr - Y **UAA Stop** **UAG Stop**	UGU, UGC – Cys - C **UGA Stop** UGG – Trp - W	U C A G
C	CUU, CUC, CUA, CUG – Leu - L	CCU, CCC, CCA, CCG – Pro - P	CAU, CAC – His - H CAA, CAG – Gln - Q	CGU, CGC, CGA, CGG – Arg - R	U C A G
A	AUU, AUC, AUA – Ile - I **AUG Met - M**	ACU, ACC, ACA, ACG – Thr - T	AAU, AAC – Asn - N AAA, AAG – Lys - K	AGU, AGC – Ser - S AGA, AGG – Arg - R	U C A G
G	GUU, GUC, GUA, GUG – Val - V	GCU, GCC, GCA, GCG – Ala - A	GAU, GAC – Asp - D GAA, GAG – Glu - E	GGU, GGC, GGA, GGG – Gly - G	U C A G

CAN YOU TRANSCRIBE & TRANSLATE?



THINK - PAIR - SHARE

What would be the effect of the following scenarios?

- DNA is mutated and one base pair changes from a T to an A.
- Part of the DNA is deleted.
- The protein does not fold up correctly.
- An extra base is added into the original DNA sequence.
- A protein gets destroyed. This can happen through UV radiation or chemicals.

NORMAL DNA

DNA	TACATAAGAAAATCACCAGGGCCCCTTTAAGATGACGGGGGCGTTTGAGCACACACT
mRNA	
Amino Acids	

Each of the following DNA sequences is mutated. Circle or highlight the area of the DNA that is mutated. Complete transcription and translation on each sequence and determine the effect on the protein.

DNA	TACATAAGAAAGTCACCAGGGCCCCTTTAAGATGACGGGGGCGTTTGAGCACACACT
mRNA	
Amino Acids	

1. Which type of mutation(s) occurred above?

2. How did the mutation affect the amino acid sequence?

Complete the DNA mutation activity to see the effects of different types of mutations.

Let’s return to Blake’s case. Go back to the patient chart. Do some research on your own. Can you diagnose Blake’s condition? What treatment would you suggest?

Blake has CLN6-Batten Disease

AKA Neuronal Ceroid Lipofuscinoses (NCL)

BLAKE'S TREATMENT PLAN

With some versions of Batten Disease, the protein could be injected into cells. This is not possible with transmembrane proteins like CLN6.

Introduce the CLN6 gene into a neuron. This is called gene therapy. Gene therapy allows the gene to remain in cells so it can continually make proteins.

GENE THERAPY BASICS

1. Viral DNA is inserted into cells in a lab, which creates many viruses.
2. The viral genome is stripped of all the parts needed to replicate and the therapeutic gene is inserted.
3. The virus is injected into the neurons of a patient.
4. The patient cells take in the virus and the gene.
5. The gene moves to the nucleus where it remains to start producing the needed protein.
6. This DNA can stay within the nucleus for the entire life of the cell.

GENE THERAPY HURDLES

- Adeno-Associated Viruses (AAV) are commonly used for gene therapy. Most people are exposed to AAV during their life and have built up antibodies.
- In order to receive gene treatment, the patient should have a low antibody count to AAV.
- Gene therapy needs to prove effective and safe in mice and non-human primates before it can go through clinical trials.
- Patients need to be identified early because gene therapy prevents the condition from worsening, it doesn't repair damage.

CLINICAL TRIALS

Currently, gene therapy for various forms of Batten Disease are in clinical trials.

In a standard clinical trial, patients are enrolled and are either given treatment or a placebo. In pediatric rare disease clinical trials, patients who meet all the criteria are given gene therapy and studied for results.

The results are compared to a natural history cohort, which are people with similar conditions from the past.

Let’s return to Blake’s treatment. What would you tell Blake’s parents? What is the best treatment plan? Would you suggest Blake’s brother get genetic testing? Why or why not?