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LAB ____: PROTEIN SYNTHESIS — TRANSCRIPTION AND TRANSLATION

DNA is the molecule that stores the genetic information in your cells. That information is coded in the four **bases** of DNA: C (cytosine), G (guanine), A (adenine), and T (thymine). The DNA directs the functions of the cell on a daily basis and will also be used to pass on the genetic information to the next generation. Because of its critical role in all the functions of the cell, DNA is kept protected in the nucleus of your cells.

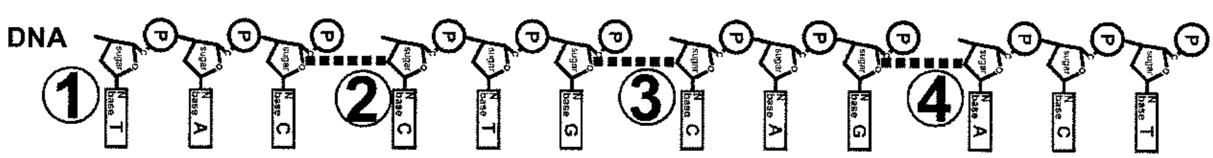
DNA is organized in sections called **genes**. Genes code for **proteins**, and it is proteins that do all the work in the cell. They function as **structural proteins** — serving as the building blocks of cells and bodies. And they function as **enzymes** — directing all the chemical reactions in living organisms.

Proteins are made in the **cytoplasm** by **ribosomes**. Since DNA cannot leave the nucleus, the *information* from DNA must be transmitted from the nucleus to the cytoplasm. During **transcription**, each gene on the DNA is read and codes directly for a **messenger RNA** (**mRNA**) molecule. The mRNA is made by matching its complementary bases — C, G, A, and **U** (**uracil**) — to the DNA bases. This process is called *transcription*, because the message is going from one version of nucleic acid language (DNA code) to another version of nucleic acid language (RNA code), so it is like transcribing from the key of G to the key of C in music. Before leaving the nucleus, this primary mRNA transcript is modified in several ways. **Introns** (intervening non-coding units) are edited out and **exons** (expressed coding sequences) are spliced together. In addition, a **5′ GTP cap** and a **3′ poly-A tail** are added to the mRNA to protect it from RNase enzymes in the cytoplasm. This mature mRNA transcript then leaves the nucleus and carries the code for making the protein from the DNA gene in the nucleus to the ribosome in the cytoplasm.

During **translation**, the ribosome reads the sequence of bases on the mRNA in sets of three — the triplet **codons**. Another type of RNA — **transfer RNA** (**tRNA**) — brings the protein building blocks — **amino acids** — to the ribosome as they are needed. The ribosome bonds the amino acids together to build the protein coded for by the gene back in the nucleus. This process is called *translation*, because the message is going from nucleic acid language (DNA/RNA code) to the completely different amino acid language (protein code), so it is like translating from English to Chinese.

PROCEDURE

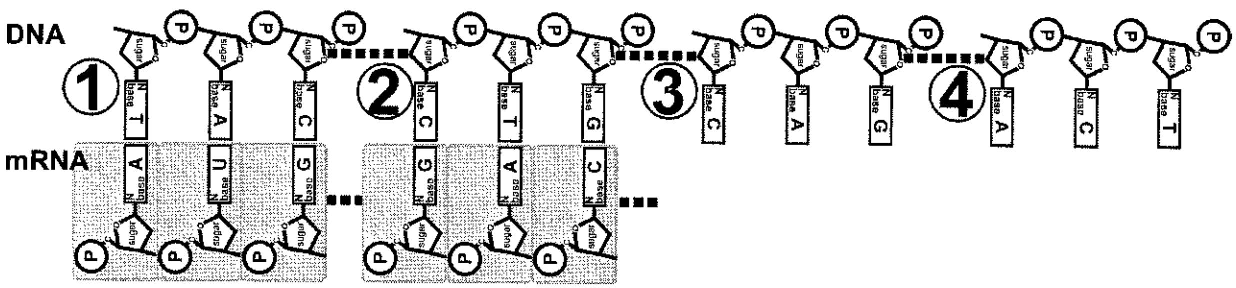
1. Obtain the cardstock with 4 sections of DNA. Cut the strips out along straight lines and tape them together to make a long one-sided DNA molecule. Each section is numbered. Lay them out on the desk from left (#1) to right (#4). See the diagram below. This will form one long strand of DNA and will serve as the **template strand** of our gene.



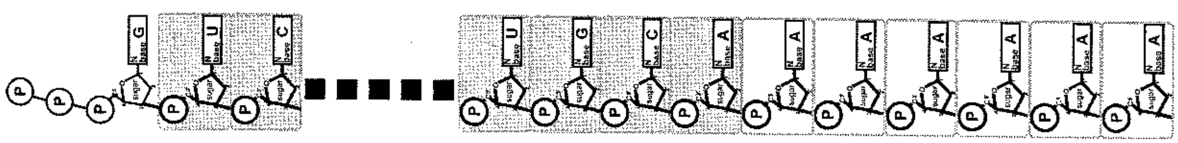
2. We are going to use this section of our DNA as a gene to be transcribed and then translated into a protein the cell needs. Remember it used to be part of a double-stranded DNA

molecule. But it has already been unzipped and now will be used as the template to build mRNA, one base at a time. So first design an **<u>RNA polymerase enzyme</u>** to do this mRNA synthesis job.

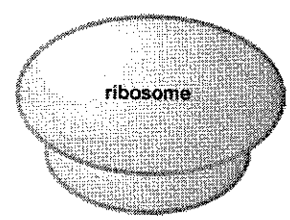
3. You have also been supplied with mRNA nucleotides. Build a mRNA molecule, one base at a time, from this gene by transcribing your DNA template. Don't forget to only start transcribing downstream from the **<u>TATA box promoter sequence</u>**. As you are transcribing, tape this mRNA molecule along its length to simulate the covalent bonds between bases. This way, it will be a stable molecule and can be moved off of the DNA to the ribosome for translation in the cytoplasm. Do ***<u>not</u>*** tape the mRNA to the DNA! Remember it has to leave the DNA in the nucleus and travel to the ribosome in the cytoplasm. Follow the diagram below.



4. You have just made a **<u>primary transcript</u>**. It must be processed so it successfully travels to the ribosome in the cytoplasm. Although we will not be simulating **<u>intron</u>** and **<u>exon</u>** splicing in this lab, you do need to add a **<u>5′ GTP cap</u>** and a **<u>3′ poly-A tail</u>** to the mRNA to protect the mRNA. Although poly-A tails may be 20-100 bases long, add 6 adenine bases for your simulated poly-A tail.

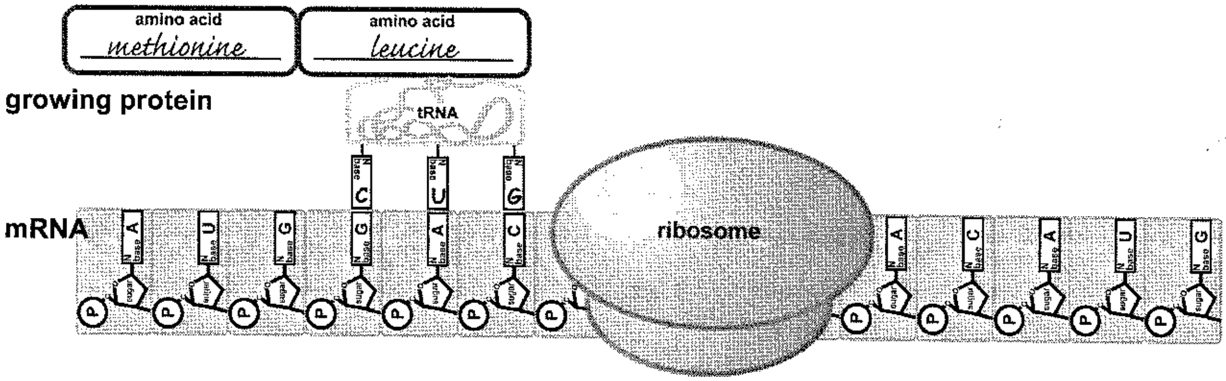


5. To be ready for the mRNA in the cytoplasm, design a ribosome to use in your simulation. Be sure to distinguish the small and large ribosomal subunits and mark the A, P, and E sites of the ribosome.

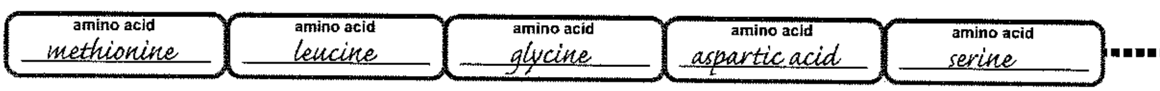


6. To help the ribosome do its job, use a pencil to draw lines which divide your mRNA into triplet codons. Now obtain tRNA molecules and write in the complementary anticodons to match your mRNA codons so that the tRNAs bring the correct amino acid to the ribosome.

7. Label the name of the amino acid that each tRNA is carrying. To help you with this, use the mRNA codon chart and the amino acid code chart supplied by your teacher. Start reading the mRNA at the **<u>START</u>** codon and end at the **<u>STOP</u>** codon. Follow the diagram below.



8. As the tRNA molecules match the mRNA codons, cut off the amino acid and bond them together in a chain to simulate the action of the ribosome — covalently bonding the amino acids in a polypeptide chain. Show your completed mRNA and your polypeptide to your teacher for credit.



Teacher's Initials ____________________

9. Use your DNA, your mRNA, and your polypeptide to answer the Summary Questions.

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AMINO ACID CODES

	Alanine	ALA
	Cysteine	CYS
	AsparticAcid	ASP
	GlutamicAcid	GLU
	Phenylalanine	PHE
	Glycine	GLY
	Histidine	HIS
	Isoleucine	ILE
	Lysine	LYS
	Leucine	LEU
	Methionine	MET
	Asparagine	ASN
	Proline	PRO
	Glutamine	GLN
	Arginine	ARG
	Serine	SER
	Threonine	THR
	Valine	VAL
	Tryptophan	TRP
	Tyrosine	TYR

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SUMMARY QUESTIONS

1. Record the gene sequence (*downstream from the TATA box*) of the **DNA strand** (from 3′ to 5′) that coded for your mRNA.

3′ 5′

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47

2. Record the sequence of the **mRNA strand** (from 5′ to 3′) that you built from the DNA in this lab.

5′ 3′

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47

3. Divide the **mRNA** sequence into the triplet **codons** and list them in order below.

5′ 3′

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47

4. Record the **amino acid** sequence that this mRNA coded for.

		1	2	3	4	5	6	7	8	9	10	11	12

5. Record the **tRNA anti-codons** that carried the amino acids to the ribosome.

		1	2	3	4	5	6	7	8	9	10	11	12

Name ______________________________

MUTATIONS

Sometimes when DNA is copied (replicated) errors occur. We call these **mutations**. When these mutations occur in gametes, they have the potential of being passed on to offspring and therefore will affect the next generation. Sometimes mutations cause only minor changes to a gene and therefore make only minor changes in the protein produced from that gene. These types of mutations may cause only minor effects to the phenotype of an organism. But sometimes mutations can cause great changes to the gene and therefore greatly alter the protein that is made from that gene. This will likely have great effects on the organism, since the protein will not be able to perform its normal function. This may lead to the inheritance of a genetic disease.

6. **POINT MUTATION 1:** One mutation is called a **point mutation** where only one base in the DNA is copied incorrectly during **DNA replication**. Here is your original DNA sequence from this lab and the amino acid sequence that was translated from it:

3′ ... 5′

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
C	T	G	A	G	C	T	A	C	T	G	A	G	C	T	G	A	G	C	T	G	C	A	G	A	G	C	C	G	A	G	C	T	C	C	T	G	T	G	T	A	A	A	C	T	T	G
X			X			MET			THR			ARG			LEU			ASP			VAL			SER			ALA			ARG			GLY			HIS			ILE			STOP				

a. Let's simulate a point mutation at the **24th base**. It was accidentally changed during replication from a **G** to a **C**. Now transcribe this new DNA strand into **mRNA**, and then translate it into its **amino acid sequence**.

3′ ... 5′

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
C	T	G	A	G	C	T	A	C	T	G	A	G	C	T	G	A	G	C	T	G	C	A	C	A	G	C	C	G	A	G	C	T	C	C	T	G	T	G	T	A	A	A	C	T	T	G

b. Did this change in the DNA sequence cause any significant change to the protein produced? Explain.

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Name ______________________________

c. What is the name of this type of point mutation and why is it referred to by this terminology?

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7. **POINT MUTATION 2:** Here is your original DNA sequence from this lab and the amino acid sequence that was translated from it:

3′ … 5′

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
C	T	G	A	G	C	T	A	C	T	G	A	G	C	T	G	A	G	C	T	G	C	A	G	A	G	C	C	G	A	G	C	T	C	C	T	G	T	G	T	A	A	A	C	T	T	G
X			X			MET			THR			ARG			LEU			ASP			VAL			SER			ALA			ARG			GLY			HIS			ILE			STOP				

a. Now, let's simulate a point mutation at the **13th base**. It was accidentally changed during replication from a **G** to an **A**. Now transcribe this new DNA strand into **mRNA**, and then translate it into its **amino acid sequence**.

3′ … 5′

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
C	T	G	A	G	C	T	A	C	T	G	A	**A**	C	T	G	A	G	C	T	G	C	A	G	A	G	C	C	G	A	G	C	T	C	C	T	G	T	G	T	A	A	A	C	T	T	G

b. Did this change in the DNA sequence cause any significant change to the protein produced? Explain.

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Name ______________________

c. What is the name of this type of point mutation and why is it referred to by this terminology?

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8. **POINT MUTATION 3:** Here is your original DNA sequence from this lab and the amino acid sequence that was translated from it:

3′ → 5′

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
C	T	G	A	G	C	T	A	C	T	G	A	G	C	T	G	A	G	C	T	G	C	A	G	A	G	C	C	G	A	G	C	T	C	C	T	G	T	G	T	A	A	A	C	T	T	G
X			X			MET			THR			ARG			LEU			ASP			VAL			SER			ALA			ARG			GLY			HIS			ILE			STOP				

a. Finally, let's simulate a point mutation at the **21st base**. It was accidentally changed during replication from a **G** to an **T**. Now transcribe this new DNA strand into **mRNA**, and then translate it into its **amino acid sequence**.

3′ → 5′

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
C	T	G	A	G	C	T	A	C	T	G	A	G	C	T	G	A	G	C	T	T	C	A	G	A	G	C	C	G	A	G	C	T	C	C	T	G	T	G	T	A	A	A	C	T	T	G

b. Did this change in the DNA sequence cause any significant change to the protein produced? Explain.

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Name ______________________

c. What is the name of this type of point mutation and why is it referred to by this terminology?

d. Why could a mutation in a gamete have more profound biological consequences than a mutation in a somatic cell?

9. Sickle cell anemia is an example of a genetic disease caused by a point mutation.
   a. Describe the specific DNA changes that produce the abnormal sickle cell hemoglobin.

   b. Explain the structural effect that this point mutation has on the hemoglobin protein.

   c. Explain why the sickle cell mutation is selected for in certain areas of the world.

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10. **FRAMESHIFT MUTATION 1:** Another group of mutations is called **frameshift mutations** where at least one base is either added to or deleted from the DNA as it is copied during **DNA replication**. Let's investigate the effects of these.

a. Here is your original DNA sequence from this lab and the amino acid sequence that was translated from it:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
C	T	G	A	G	C	T	A	C	T	G	A	G	C	T	G	A	G	C	T	G	C	A	G	A	G	C	C	G	A	G	C	T	C	C	T	G	T	G	T	A	A	A	C	T	T	G
X			X			MET			THR			ARG			LEU			ASP			VAL			SER			ALA			ARG			GLY			HIS			ILE			STOP				

b. Let's simulate a frameshift mutation by **adding** an additional base between the 36th & 37th bases. The base **A** was accidentally added to the sequence of the gene. Now transcribe this new DNA strand into **mRNA**, and then also translate it into its **amino acid sequence**.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36		37	38	39	40	41	42	43	44	45	46	47
C	T	G	A	G	C	T	A	C	T	G	A	G	C	T	G	A	G	C	T	G	C	A	G	A	G	C	C	G	A	G	C	T	C	C	T	A	G	T	G	T	A	A	A	C	T	T	G

c. Did this change in the DNA sequence cause any significant change to the protein produced? Explain.

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d. Why are insertions and deletions called "frameshift" mutations, and what is meant by the "reading frame" of a gene?

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Name ______________________________

11. **FRAMESHIFT MUTATION 2**: Here is your original DNA sequence from this lab and the amino acid sequence that was translated from it:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
C	**T**	**G**	**A**	**G**	**C**	**T**	**A**	**C**	**T**	**G**	**A**	**G**	**C**	**T**	**G**	**A**	**G**	**C**	**T**	**G**	**C**	**A**	**G**	**A**	**G**	**C**	**C**	**G**	**A**	**G**	**C**	**T**	**C**	**C**	**T**	**G**	**T**	**G**	**T**	**A**	**A**	**A**	**C**	**T**	**T**	**G**
X			X			**MET**			**THR**			**ARG**			**LEU**			**ASP**			**VAL**			**SER**			**ALA**			**ARG**			**GLY**			**HIS**			**ILE**			**STOP**				

a. Now let's simulate a frameshift mutation by **deleting** the 10^{th} base. Now transcribe this new DNA strand into **mRNA**, and then also translate it into its **amino acid sequence**.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
C	**T**	**G**	**A**	**G**	**C**	**T**	**A**	**C**	X	**G**	**A**	**G**	**C**	**T**	**G**	**A**	**G**	**C**	**T**	**G**	**C**	**A**	**G**	**A**	**G**	**C**	**C**	**G**	**A**	**G**	**C**	**T**	**C**	**C**	**T**	**G**	**T**	**G**	**T**	**A**	**A**	**A**	**C**	**T**	**T**	**G**

b. Did this change in the DNA sequence cause any significant change to the protein produced? Explain.

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c. Which do you think would cause a more profound biological impact: (1) a deletion/insertion near the beginning of a gene, or (2) a deletion/insertion towards the end of a gene? Explain.

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mRNA CODONS

	Second base: U	C	A	G	
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	**U**
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	**C**
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	**A**
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	**G**
C	CUU Leu	CCU Pro	CAU His	CGU Arg	**U**
	CUC Leu	CCC Pro	CAC His	CGC Arg	**C**
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	**A**
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	**G**
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	**U**
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	**C**
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	**A**
	AUG Met or start	ACG Thr	AAG Lys	AGG Arg	**G**
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	**U**
	GUC Val	GCC Ala	GAC Asp	GGC Gly	**C**
	GUA Val	GCA Ala	GAA Glu	GGA Gly	**A**
	GUG Val	GCG Ala	GAG Glu	GGG Gly	**G**