

Questions:

1. Assume that the recognition site of a restriction enzyme is GAATTC and that the cuts/beaks the sugar-phosphate backbone between the G and the A. Therefore, each time the enzyme encounters this recognition site, it will cut the DNA in the same place as is illustrated below:



How many pieces/fragments of DNA would result from the cut shown above? _____

2. DNA fragment size can be expressed as the number of base pairs in the fragment. Indicate the size of the fragments [mention any discrepancy you may detect]. Note that there will be a region of single-bases (i.e. not base pairs) at the region of the cut. Therefore, these would formally not be included in the determination of the fragment size. Normally, the number of unpaired bases is insignificant compared to the total size of the double-stranded region (frequently a hundred to several thousand bases). However, in such small fragments shown in the example, the effect is exaggerated.

a) The smaller fragment is _____ base pairs (bp).

b) The larger fragment is _____ bp.

3. Consider the two samples of DNA shown below single strands are shown for simplicity:

~~Sample #1: C A G T G A T C T C G A A T T C G C T A G T A A C G T T~~

~~Sample #2: T C A T G A A T T C C T G G A A T C A G C A A A T G C A~~

If both samples are treated with a restriction enzyme [recognition sequence GAATTC], indicate the number of fragments and the size of each fragment from each sample of DNA that would result.

~~Sample #1: No. of fragments: _____ Sample #2: No. of fragments: _____~~

~~List fragment sizes for each sample in order of largest (bp) to smallest (bp):~~

~~Sample #1:~~

~~Sample #2:~~

4. If you could not see the original sequences but could only compare the number and sizes of the fragments, would you say that these samples come from the same person or two different people? Explain your answer:

D. Transcription

Within the cell, DNA serves as a set of instructions that help with the assembly of proteins. As we shall see, the specific order of the base pairs in the DNA is critical to the proper construction of the huge variety of proteins a cell can make. DNA resides within the nucleus of eukaryotic cells and does not leave. However, the proteins are assembled outside the nucleus in the cytoplasm. This means that the information stored in the specific order of the base pairs on the DNA must be passed from the nucleus to the cytoplasm. This is the job of **messenger RNA (mRNA)**. Messenger RNA

is assembled in a process know as **transcription**. During transcription, a region of the DNA double helix unwinds and unzips and a single strand of mRNA is constructed using the nucleotide sequence of one strand of the DNA as a template. This mRNA strand then leaves the nucleus via pores in the nuclear membrane and is used to build proteins in the cytoplasm. The DNA double helix reforms its hydrogen bonds (“re-zips” itself) and can thus be read over and over again to make more mRNA as it (and the corresponding proteins) are needed by the cell. Two other types of RNA are also produced by transcription (tRNA and rRNA). While these RNAs are not directly translated, as is mRNA, they do function in the process of protein synthesis.

Materials:

- 1 DNA Puzzle Kit

Before starting this exercise, check that all the pieces in your puzzle kit have the same number written on the back and that the number matches the one on the box lid. If you have numbered pieces that don’t belong to your puzzle, return them to the proper box. Then check to make sure your puzzle has the following pieces:

- 24 deoxyribose sugars (dark pink)
- 12 ribose sugars (light pink)
- 24 phosphate groups (yellow)
- 4 adenine bases
- 8 guanine bases
- 8 cytosine bases
- 4 thymine bases
- 2 uracil bases
- 4 transfer RNA (tRNA) (tan and brown)
- 4 amino acids (tan and brown)
- 4 activating units (tan and brown)
- 1 ribosome template sheet (white paper)

Procedure:

1. Using the appropriate puzzle pieces, construct the nucleotides necessary to build one half of a DNA molecule with the base sequence **A-C-C-T-G-C-A-C-C-T-G-C**. Remember to include the deoxyribose sugars and the phosphate groups in the “backbone”. Lay the strand on the table horizontally with the exposed bases pointing away from you with the A on the left.

2. Now build twelve RNA nucleotides that are complimentary to the exposed DNA bases, which are referred to as the template strand. Remember to review the differences between DNA and RNA when you build these nucleotides. Build the RNA strand by pairing the bases between the RNA and DNA and connect the sugar (ribose) and phosphates on the RNA. Write the base sequences of the two strands below:

mRNA strand sequence: UGGACGUGGACG ___ ___ ___

DNA template strand sequence: ACCTGCACCTGC ___ ___ ___

Separate the DNA template and the mRNA strands. In a cell, the mRNA strand would undergo some “editing” including the addition of the 5’ CAP, the 3’ poly-A tail, and removal of the introns. Then it would leave the nucleus, and the DNA strand would rejoin with its complementary half by reforming the hydrogen bonds between complimentary base pairs.

E. Translation

In the cell, the mRNA leaves the nucleus through a pore in the nuclear membrane. Once in the cytoplasm, it attaches to a ribosome and is “read” three base pairs at a time to determine the appropriate amino acid that should be inserted in the growing protein. A block of three consecutive nucleotides in the mRNA is called a codon. The ribosome (which is made of ribosomal RNA or rRNA) is comprised of two subunits named the 40S subunit and the 60S subunit (You can think of the “S” as a unit of weight measurement). The transfer RNA (tRNA) function to bring specific amino acids to the ribosome to be used in building the specific protein. Each tRNA has a three base sequence on one end called the anticodon and a sequence CCA on the other which serves as an attachment site for the tRNA’s specific activating enzyme. The activating enzyme is responsible for catalyzing the chemical reaction of joining the amino acid to the tRNA. The anticodon on the tRNA is complementary to the codon on the mRNA, which explains how the correct amino acid is brought to the site of translation.

Procedure:

1. Use the mRNA strand that was created in the transcription activity. You will now translate this mRNA into an amino acid sequence of a protein.

2. Place the large, white sheet of paper from the puzzle kit on the desk in front of you. The black outline on the paper (template) represents the ribosome. Carefully slide your mRNA strand onto the mRNA binding site of the 40 S subunit of the ribosome template. The phosphate attached to the G on the right end of the mRNA should line up with the 5’ on the template. The exposed bases should point toward the P and A sites.

3. Find the four tRNA puzzle pieces and attach their specific activating enzymes to them. Then attach the specific amino acid to each activating unit.

4. The order in which the amino acids are connected to build the protein is determined by the sequence of the bases on the mRNA. Recall that this sequence was ultimately determined by the sequence of the bases on the DNA strand from which it was transcribed. Pick up the t-RNA/amino acid unit whose anticodon complements the mRNA codon above the P site of the 60S subunit. Place it on the P site with the codon and anticodon paired. Do the same for the A site.

5. The amino acids form their peptide bonds with each other when their t-RNAs are base paired with the mRNA in the A and P sites. Detach the P site amino acid from its tRNA activating unit and slide it over the tRNA, attaching it to the A site amino acid. Move the mRNA to the right until the tRNA activating unit amino acid chain occupies the P site (the tRNA-activating unit amino acid chain is moved from the A site to the P site). Carefully lift the empty tRNA-activating unit complex, slide it out from under the amino acid chain, and place it to one side. Fill the A site with another tRNA-amino acid unit and repeat the process until you have produced a chain of four amino acid units. Then complete the table below and on your group lab report form:

Template DNA base sequence:	^{5'} A C C	T G C	A C C	T G C ^{3'}	READ mRNA FROM 5' TO 3' FOR TRANSLATION
mRNA codons:	^{3'} UGG	ACG	UGG	ACG ^{5'}	
tRNA anticodons:	ACC	UGC	ACC	UGC	
Amino acid sequence:	GLY	ALA	GLY	ALA	

6. In the previous lab on DNA replication, we discussed that errors can occur during the replication process. Typically, these errors are corrected by proofreading enzymes, but sometimes mistakes persist. A mistake that results in a single base change is called a point mutation and may affect the amino acid composition of the resulting protein.

a) How would the protein have been different if the DNA template sequence was G-C-C-T-C-C-A-G-C-T-G-A?
 Note: Use the table of the genetic code included at the end of the lab:

Template DNA base sequence:	<u>G</u> C C	T C C	A <u>G</u> C	T G <u>A</u>	
mRNA codons:	<u>CGG</u>	<u>AGG</u>	<u>UCG</u>	<u>ACU</u>	READ mRNA FROM 5' TO 3' FOR TRANSLATION
tRNA anticodons:	<u>GCC</u>	<u>UCC</u>	<u>AGC</u>	<u>UGA</u>	
Amino acid sequence:	<u>GLY</u>	<u>GLY</u>	<u>ALA</u>	<u>SER</u>	

b) Do all point mutations cause a different amino acid to be inserted?

Not all. Mutations in the last nucleotide of mRNA codons will sometimes have no affect on what amino acid is coded

c) Notice from the table of the genetic code (included at the end of this lab) that 61 codons represent the 20 different amino acids. Why do you think it is advantageous, from a genetic perspective, to have this redundancy (i.e. the same amino acid is represented by more than one codon)?

(see answer above)

7. Once translation is complete, the protein, the mRNA, and the two subunits of the ribosome separate. In a cell, the resulting protein would be passed into the endoplasmic reticulum where it would undergo further modifications before being used within the cell or exported to other locations within an organism.

Questions:

1. If the base sequence on a strand of DNA is TACCCGTATACT, what would be the sequence of the codons on the mRNA transcribed from this DNA?

AUGGGCAUAUGA

2. What would the sequence of bases of the anticodons on the tRNA be?

UACCCGUAUACU

3. What would be the sequence of amino acids that would be translated from this DNA?

MET GLY ILE STOP

4. If a mistake (point mutation) is made during replication of this strand so that the new DNA strand had the base sequence TACACGTATACT, what would be the resulting change in the protein built from this strand?

MET CYS ILE STOP

*Don't
worry
about 5'-3'
for these...

5. Complete the following table using the information provided:

Template DNA Strand	ATG	TCG	GAG	GGG	GGA
Non-Template DNA Strand	TAC	AGC	CTC	CCC	CCT
mRNA	UAC	AGC	CUC	CCC	CCU
tRNA anticodon	AUG	UCG	GAG	GGG	GGA
Amino Acid	TYR	SER	Leucine	GLY	GLY

F. Review Questions

- The electrophoresis apparatus creates an electrical field with positive and negative poles at the ends of the gel. DNA molecules have a _____ charge. To which electrode pole of the electrophoresis field would you expect DNA to migrate? (+ or -)?
-
- After DNA samples are loaded into the sample wells, they are “forced” to move through the gel matrix. What size fragments (large vs. small) would you expect to move toward the opposite end of the gel most quickly?
SMALL
- What is the difference, with respect to transcription, between the template and non-template DNA strands?
only the template codes for the mRNA
- What are the DNA-DNA rules of complementary base pairing?
A-T; C-G
- What are the DNA-RNA rules of complementary base pairing?
A-U; C-G
- What are the RNA-RNA rules of complementary base pairing?
A-U; C-G
- Distinguish between a codon and an anticodon. How are they similar? How are they different?
codon is three sequential nucleotides in mRNA that codes for a specific amino acid. Anticodon is on tRNA and is complimentary to codon.
- What are the functions of the mRNA, tRNA, and rRNA in translation.
mRNA delivers DNA code to ribosome; tRNA delivers amino acid to ribosome; rRNA makes up ribosome
- In the genetic code table, three codons (UAA, UAG, and UGA) are associated with “STOP”. What does this mean with regards to translation?
this is the end of the new polypeptide. No more amino acids to be added.

	U	C	A	G		
First position (5' end)	U	UUU } Phe UUC } UUA } Leu UUG }	UCU } UCC } Ser UCA } UCG }	UAU } Tyr UAC } UAA } Stop UAG } Stop	UGU } Cys UGC } UGA } Stop UGG } Trp	U C A G
	C	CUU } CUC } Leu CUA } CUG }	CCU } CCC } Pro CCA } CCG }	CAU } His CAC } CAA } Gln CAG }	CGU } CGC } Arg CGA } CGG }	U C A G
	A	AUU } AUC } Ile AUA } AUG }	ACU } ACC } Thr ACA } ACG }	AAU } Asn AAC } AAA } Lys AAG }	AGU } Ser AGC } AGA } Arg AGG }	U C A G
	G	GUU } GUC } Val GUA } GUG }	GCU } GCC } Ala GCA } GCG }	GAU } Asp GAC } GAA } Glu GAG }	GGU } GGC } Gly GGA } GGG }	U C A G

Amino acid names:

Ala = alanine	Gln = glutamine	Leu = leucine	Ser = serine
Arg = arginine	Glu = glutamate	Lys = lysine	Thr = threonine
Asn = asparagine	Gly = glycine	Met = methionine	Trp = tryptophan
Asp = aspartate	His = histidine	Phe = phenylalanine	Tyr = Tyrosine
Cys = cysteine	Ile = Isoleucine	Pro = proline	Val = valine