

GENES to PROTEINS - MOLECULAR BASIS : **ANSWERS**

Framework

The goal of today's exercise is for you to look at RNA, its structure, its transcription, and its function in making proteins. The triplet code instructions of DNA are transcribed into a sequence of codons in mRNA. In eukaryotes mRNA is processed before it leaves the nucleus to produce a mature functional cytoplasmic mRNA. Complexed with ribosomes, mRNA is translated into a linear sequence of amino acids in a polypeptide as tRNAs match their anticodons to the codons of the mRNA. Have one member, in turn, of your Learning Community answer one part of each of the questions or problems, then let the next member go on to the next part in the materials below.

Part 1. Transcription of DNA & Genetic Code

- a. In your text book is a copy of the genetics code. Practice using the dictionary of the genetic code by determining the proper amino acid sequence for the polypeptide coded by the following DNA. Have one group of 3 members first make the mRNA first, then have another group of 3 make the correct polypeptide. remember the proper polarity.

5'- ATGCCTGACTTTAAGTGA -3'
3'- TACGGACTGAAATTC ACT -5'

mRNA... **5'-AUG CCU GAC UUU AAG UGA-3'**

Polypeptide... **H₂N- MET-PRO-ASP-PHE-LYS-stop-COOH**

- b. Using the codons and amino acids you identified in part 1a. above have one member of your group, in turn, fill in the following table.

DNA Triplet 3' → 5'	mRNA codon 5' → 3'	Anticodon 5' → 3'	Amino acid
TAC	AUG	UAC	methionine
GGA	CCU	GCA	PROLINE
TTC	AAG	UUC	LYSINE
ATC	UAG	AUC	STOP

- Part 2. a. How does a mature cytoplasmic, eukaryotic mRNA differ physically from its primary transcript?**
A mature cytoplasmic mRNA has a 5'-cap, a reversed G-ppp nucleotide attached to the 5'-end of the message, which prevents digestion of the mRNA by 5' nuclease enzymes of the nucleus. In addition, a mature mRNA would have a poly-A tail at the 3'-end of the molecule. Poly-A polymerase would add between 20 and 200 adenines to the 3' end to protect the mRNA from enzymatic digestion by nucleases.
- b. Have one member of your group, in turn, define the function of each of the following types of RNAs.
- mRNA – carries the information of the DNA coded sequences and eventually specify the unique sequence of amino acids in a polypeptide.**
 - tRNA – carries a specific amino acid, attached to its 3'-end, to the site of protein synthesis, the amino-acyl site on the large subunit of the ribosome.**
 - rRNA – is the RNA that makes up 60% of structure of the ribosome. It is copied from rDNA as a primary transcript & is then processed by cutting out unused segments & nucleotides.**
 - snRNA – small nuclear RNA is part of the spliceosome and plays a structural and catalytic role in the splicing of eukaryotic genes, removing introns and assembling exons.**
 - snRNP RNA – is the RNA that is part of the signal recognition particle that binds to the signal peptides of the polypeptides targeted to the endoplasmic reticulum.**

- c. Define the differences between each of the following: non-sense mutation and missense mutation.
- A mis-sense mutation is a point mutation in which a single nucleotide replaces another in the wild-type genotype and results in a different amino acid being substituted for the normal amino acid in the wild-type polypeptide.**
- A non-sense mutation occurs when the nucleotide that substitutes in the wild-type genotype results in a stop codon replacing a normal amino acid in the affected polypeptide.**

Part 3. Have one member of your group fill in each of the following boxes in the table below. Define or explain the role of the listed function in Transcription and Translation.

	Transcription	Translation
Template	DNA	RNA
Location	Nucleus (cytoplasm in prokaryotes)	Cytoplasm; ribosome – free or on ER
Molecules Involved	RNA nucleotides, DNA template strand, RNA polymerase, transcription factors, activator proteins.	Amino acids, tRNA, ribosomes, ATP, GTP, enzymes, initiation/elongation/termination factors
Enzymes Involved	RNA polymerase, ribozymes, RNA processing enzymes	Aminoacyl-tRNA synthetases, ribozymes
Control start stop	Transcriptions factors at TATA box Terminator sequences	Initiation factors, initiator sequence (AUG), stop codons, release factor
Product	Primary transcript (pre-mRNA molecule)	Polypeptides
Energy source	Ribonucleoside triphosphates	ATP & GTP

Part 4. In the figure to the right which details protein synthesis:

<ol style="list-style-type: none"> 1. codon recognition – an elongation factor helps an aminoacyl-tRNA into the A-site, where the codon-anticodon pair; one GTP is required. 2. Peptide bond formation – the ribosomes peptidyl transferase (possibly a ribozyme) catalyzes peptide bond formation between the new amino acid and the polypeptide held in the P-site 3. Translocation-elongation- the tRNA in the P-site move to the E-site and is released; the tRNA now holding the growing polypeptide moves from the A-site to the P-site; one GTP is used 4. Termination – release factor protein binds to the stop codon in the A-site; the free polypeptide and the tRNA is released; the initiation complex disassembles. 	<ol style="list-style-type: none"> a. amino end of growing polypeptide b. aminoacyl-tRNA c. large subunit of ribosome d. A-site e. small subunit of ribosome f. 5' end of mRNA g. peptide bond formation by peptidyl transferase h. E-site i. termination factor j. mRNA codons k. peptidyl site – growing polypeptide l. newly made polypeptide
---	--

Part 5. Label the components of the diagram below of the formation of an initiation complex in eukaryotes.

- a. **enhancers** – DNA sequences way upstream that bind activators proteins to help initiate transcription
- b. **activators** – specific protein factors that bind to activator sequences and facilitate formation of the transcription initiation complex.
- c. **transcription factors** – proteins that help in the formation of an initiation complex and allow RNA polymerase to bind to the promoter sequence.
- d. **promoter** – a unique nucleotide sequence in DNA that allows the binding of RNA polymerase to initiate transcription
- e. **TATA box** – a promoter region in eukaryotic DNA genes that facilitates the binding of RNA polymerase.
- f. **RNA polymerase** – the enzyme responsible for the complementary copying of a DNA template into an RNA product.

Part 6. The bloody crime scene....

The CSI collect blood sample and use the PCR reaction to make multiple copies of the collected DNAs. They then treat the DNA pieces with restriction endonuclease to cut it up into fragments that can be electrophoresed.

The samples from the crime scene blood show a band pattern that matches that of the victim and that of suspect 2. Thus there is evidence to suggest that suspect 2 was at the scene and left his/her blood there; good enough to issue an arrest warrant fro suspect 2.

Part 7. Fill in this table on the basic tools of gene manipulations used in DNA biotechnology.

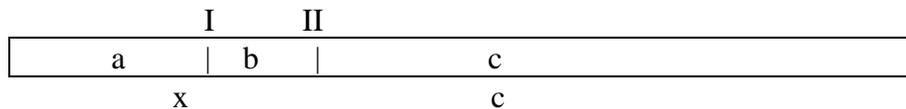
Technique or tool	Brief description	Some uses in DNA technology
Restriction enzymes	Bacterial enzymes that cut DNA at restriction sequence sites, creating complementary “sticky ends” that can base-pair with other DNA fragments with the same sticky ends.	Used to make recombinant DNA’s and form restriction fragments.
Gel electrophoresis	A mixture of molecules (proteins, DNA, or RNA) when applied to a gel within an electric field; the molecules separate at different rates within the gel due to difference in charge and size.	Used to separate restriction fragments into patterns of distinct and characteristic bands; the fragments may be removed from the gel retaining activity; often identified by binding with DNA probes.
cDNA	mRNA isolated from cells is treated with reverse transcriptase to produce a complementary DNA strand to the mRNA, which can then be made into double stranded DNA – minus its introns.	Used to create genes that are easier to clone in bacteria and to produce a library of active genes from cells.
Labeled probes	Radioactively or fluorescently tagged single stranded DNA or mRNA that can pair with complementary DNA or RNA.	Used to locate genes in a clone of bacteria; identify similar nucleic acid sequences; make a cytological map of genes via in situ hybridization.
Southern blots	DNA fragments separated by gel electrophoresis, transferred by blotting onto filter or membrane paper to which a labeled probe is added, rinsed, and autoradiographed.	Used to analyze DNA for homologous sequences; DNA fingerprinting.
DNA sequencing	Single stranded DNA fragments are incubated with the 4-deoxynucleotides, Taq DNA polymerase, and one of 4 dideoxy nucleotides that interrupts synthesis; samples are separated by high-resolution electrophoresis and the sequence of the nucleotides is read from the four sets of bands; use to sequence DNA	
PCR	Polymerase Chain Reaction: DNA is mixed with TAQ DNA polymerase, nucleotides, and primers having complementarity to the targeted DNA sections; mixture is repeatedly heated & cooled to allow multiple rounds of replication.	Used to rapidly produce multiple copies of DNA pieces <i>in vitro</i>
RFLP analysis	Restriction fragment analysis by Southern blotting to compare different band patterns caused by DNA differences in restriction sites.	Used for DNA fingerprinting in forensics; to map chromosomes using RFLP markers; to diagnose genetic diseases

Part 8. The next two questions deal with restriction fragment analysis.

- a. The segment of DNA has restriction sites I and II, which create restriction fragments a, b, and c. Which of the following gel electrophoresis patterns would represent the proper separation and identity of these fragments and why.

panel b... The DNA is cut into 3 pieces, the largest being fragment c. Since DNA is negatively charged, the DNA will migrate toward the positive pole in an electric field. The fragment c, due to its large size, migrates the slowest and shortest distance in the gel. The other fragments also migrate according to size, with the piece b moving the farthest and piece a not quite as far as b.

- b. This restriction fragment contains a gene whose recessive allele is lethal. The normal allele has restriction sites for the restriction enzyme PST-I at the points I and II. The recessive allele lacks restriction site I. An individual who had a sister with the lethal trait is being tested to determine if he is a carrier of that allele. Which of the following gel electrophoresis patterns would be produced if he is a carrier (heterozygous for the lethal gene).



panel d... this panel shows 4 fragments. The normal allele produces 3 fragments (a, b, and c) having been cut at its two restriction sites (I and II) – and as above in question 8.a, it will produce 3 bands in gel electrophoresis. So the presence of the normal allele results in the pattern of these 3 fragments; however, the recessive allele lacks the restriction site I, thus it will produce only 2 fragments, one being “c” and the other being “x”, which will be a combination of the old “b” and “a” pieces. This new piece “x” is smaller than the original “c” and bigger than either the “a” or “b” and thus should migrate to a point between the “c” and “a” bands (see below).

