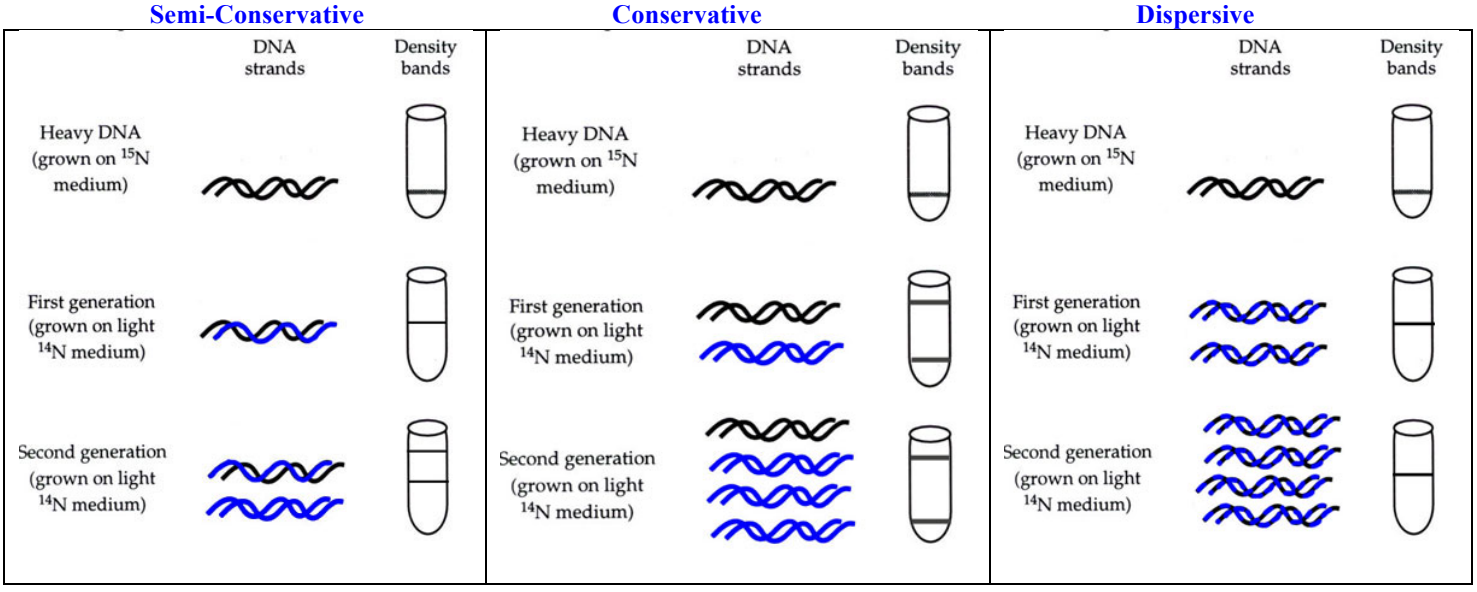
MOLECULAR BASIS OF INHERITANCE - ANSWERS

Part 1. Proof of DNA as the Genetic material

1. Hershey and Chase devised an experiment using radioactive isotopes to determine whether a bacteriophage's DNA or its proteins were transferred during viral replication.
   a) What and/or how did that label the phage protein?
      They grew the T4-bacteriophages in the presence of radioactive sulfur (^{35}S) to tag the cysteine amino acids of the phage proteins.
   b) How did they label the phage DNA?
      They grew the T4-bacteriophages in the presence of radioactive phosphorous (^{32}P) to tag the bases of the individual nucleotides of DNA.
   c) Where was the radioactivity found in the samples with labeled phage protein and why?
      Radioactive ^{35}S was found in the supernatant indicating that the bacteriophage proteins did not enter the E. coli cells.
   d) Where was the radioactivity found in the samples with labeled phage DNA and why?
      In the samples labeled with ^{32}P (DNA) most of the radioactivity was found in the pellet of the centrifuge tube along with the sedimented bacterial cells.
   e) What was Hershey & Chase's conclusion from their experiment?
      They concluded that viral DNA is injected into host bacterial cells during the process of viral replication, and thus DNA serves as the hereditary material for viral replication.

Part 2. Patterns of DNA Replication



Part 3. Structure of DNA

a. sugar-phosphate backbone
b. complementary base pair
c. adenine
d. pyrimidine bases
e. guanine
f. cytosine
g. purine bases
h. hydrogen bonds
i. adenine
j. nucleotide
k. deoxyribose
l. phosphate
m. 3.4nm
n. 0.34nm
o. 2nm diameter

Part 4. Enzymes of Replication.

a. helicase	b. binding proteins	c. DNA polymerase
d. leading strand	e. lagging strand	f. DNA ligase
g. Okazaki fragment	h. RNA primer	i. primase
j. replication fork	k. 3' end parental strand	l. 5' end parental strand

Part 5. Matching

1. F 2. C 3. D 4. G 5. E 6. A 7. B

Part 6. Transcription of DNA & Genetic Code

a. In you 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, than have another group of 3 make the correct polypeptide. remember the proper polarity.

5'- ATG CCT GAC TTT AAG TGA -3'
3'- TAC GGA CTG AAA TTC ACT -5'

mRNA… 5'- AUG CCU GAC UUU AAG UGA-3'

Polypeptide… H_2N – MET – PRO – ASP – PHE – LYS – stop - COOH

b. Using the codons and amino acids you identified in part 1a. 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**

c. 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.

d. Have one member of your group, in turn, define the function of each of the following types of RNAs.

1. **mRNA** – carries the information of the DNA coded sequences and eventually specify the unique sequence of amino acids in a polypeptide.
2. **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.
3. **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.
4. **snRNA** – small nuclear RNA is part of the splicesome and plays a structural and catalytic role in the splicing of eukaryotic genes, removing introns and assembling exons.
5. **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.

e. Define the differences between each of the following: non-sense mutation and missense mutation.

A redundant mutation results in the same amino acid ending up in the polypeptide due to a different, but redundant codon, while 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 7a,b,c. In the figure to the right which details protein synthesis:

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 aminoa cid 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 disassemblies.	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 7d. 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 8. 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 for suspect 2.

Part 9. 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 is 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 *in vitro*
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