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MCB 142 second midterm: Molecular Genetics

Please write your name, ID, and TA's name on the top of ALL 8 pages of this exam.

Please put all your answers in the spaces below.

Please use pen to write your answers. *If you use pencil, you cannot request a re-grade.*

You may not use any calculator, cellular phone, internet connection, playstation, or other electronic device during this exam.

Please double check that you have written your name, ID, and TA's name on top of every page in the exam.

Exam format: There are ten short answers (4-7 points each, total of 45 points), and five problems (10-15 points each, total of 60 points). The whole exam is worth 100 points. You have 90 minutes to complete this exam. Please pace yourself, and don't get bogged down in any one problem (especially the short answers, where typically you will either know the right answer, or you won't). Good luck.

For Grader's Use Only

Page 2 score (out of 24) _____
Page 3 score (out of 21) _____
Page 4 score (out of 15) _____
Page 5 score (out of 10) _____
Page 6 score (out of 10) _____
Page 7 score (out of 10) _____
Page 8 score (out of 10) _____

TOTAL SCORE (out of 100) _____

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1. Replication (4 points). DNA replication is "semiconservative." This means
(a) one strand watches Fox News, the other CNN
(b) in a given double helix, one strand is inherited directly from the parental helix and the other strand is newly synthesized as its complement
(c) both strands are inherited from the parent, but DNA repair removes half of the sequence randomly, keeping ("conserving") the other half.
(d) If a (previously unlabeled) bacteria is grown in the presence of labeled nucleotide precursors, half of all future descendents will have labeled DNA

ANSWER: B.

2. Human genome (4 points). True or False: "Exons make up only a small fraction (<5%) of the human genome."

ANSWER: TRUE

3. Restriction enzymes (4 points). Restriction enzyme recognition sites are often palindromic because
(a) DNA is an antiparallel double helix, so the two strands are the equivalent
(b) In a recognition site the number of A's is the same as the number of T's, and the number of G's is the same as the number of C's.
(c) Restriction enzymes typically bind to DNA as dimers
(d) Processing an mRNA requires the spliceosome, which "restricts" the genomic contribution to a gene by removing introns

ANSWER: C

4. Start codon (4 points). True or False: "Every mRNA begins with the "start codon" AUG."

ANSWER: FALSE. Only the coding sequence starts with AUG – there is typically additional 5' untranslated (UTR) sequence

5. Translation (4 points). Which of the following element(s) are essential for the process of translation from a mature mRNA? For full credit, be sure to identify ALL elements.
(a) RNA polymerase
(b) DNA polymerase
(c) Spliceosome
(d) 5' capping enzyme
(e) Ribosome
(f) Reverse transcriptase
(g) Transfer RNA

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ANSWER: E AND G. Note that the A, C, and D are involved in transcription and mRNA processing, but the question asked about translation from a mature (i.e., already transcribed and processed mRNA). F is not involved in either transcription or translation

6. Ribosome (4 points). True or False: "The ribosome is a ribonucleoprotein complex that catalyses the covalent ribose-phosphate linkages in mRNA and tRNA."

ANSWER: FALSE. Ribosome catalyses formation of peptide bonds in protein synthesis, guided by mRNA.

7. Human variation (4 points). If two randomly selected human genomes are compared, a single nucleotide polymorphism is typically found in autosomal DNA

(a) Every ~hundred base pairs
(b) Every ~thousand base pairs
(c) Every ~ten thousand base pairs
(d) It depends on which mitochondrial haplotypes are present

ANSWER: B

8. Principle of gel electrophoresis (4 points). Which one of the following statements is true?

(a) Longer DNA molecules move more rapidly through a gel because they carry more charge, and so are pulled to the anode more rapidly.
(b) Shorter DNA molecules move more readily through a gel. So shorter molecules move faster.
(c) Movement of DNA through a gel is sequence-dependent – that's how DNA sequencing works!
(d) Radioactively labeled double-stranded DNA can be separated from non-radioactively labeled DNA using agarose gel electrophoresis.

ANSWER: B.

9. Polymerase chain reaction (4 points). What primers could you use to amplify the target DNA sequence indicated by the dots? Be sure to specify both primers in the 5'-to-3' direction.

```
5′ GGCTAAGATCTGAATTTTCCAAG … TTGGGCAATAATAATGTAGCGCCTT 3′
```

ANSWER:
Primer 1: 5′ GGCTAAGATCTGAATTTTCCAAG 3′
Primer 2: 5′ AAGGCGCTACATTATTATTGCCCAA 3′ = reverse complement of the right hand sequence
(Any appreciable subsequences of these two sequences is also acceptable.)

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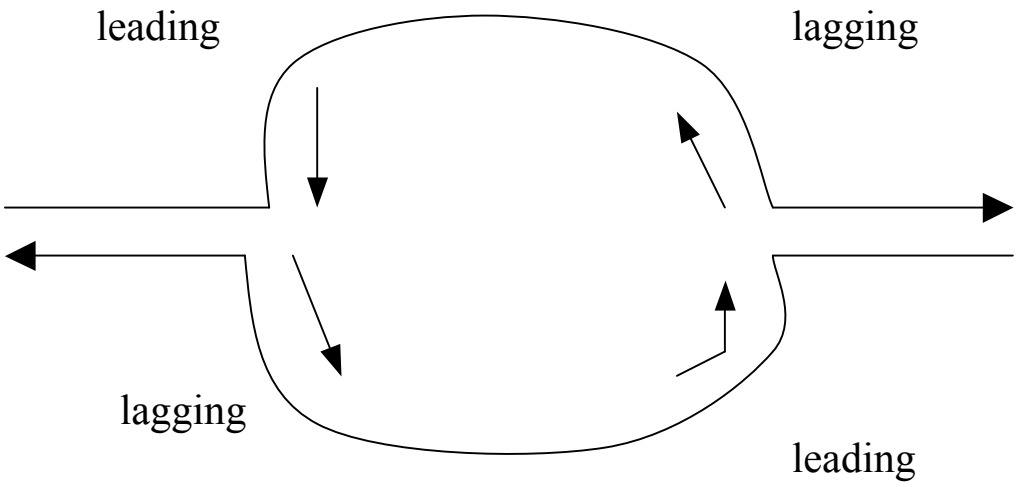
10. Replication bubble (7 points). On the diagram of a bidirectional replication bubble below

(a) draw arrows to indicate the 5'-to-3' directionality of all newly synthesized strands **ANSWER: You're given strand direction on the templates; strand direction on the newly synthesized strands are, as always, antiparallel.**
(b) label each strands as "leading" or "lagging" **ANSWER: "leading" strands point into the fork; they get extended as the fork moves "forward". Lagging strands point away from the fork.**



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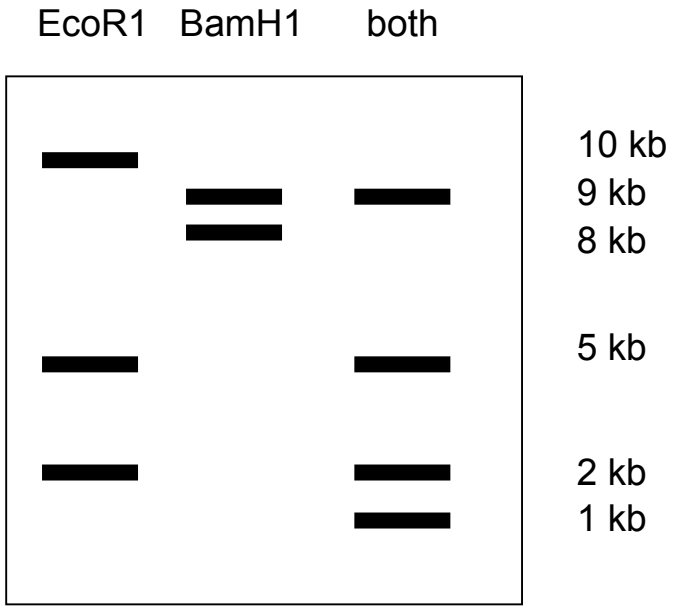
11. Restriction digests II (15 points). A linear DNA molecule is subjected to complete restriction digestion by (1) EcoR1 alone, (2) BamHI alone, and (3) both enzymes together. The fragments are run on a gel. Results are shown below.

(a) [5 points] How long is the original DNA molecule? **ANSWER: all lanes adds up to 10+5+2 = 9+8 = 9+5+2+1 = 17 kb**

(b) [5 points] How many EcoR1 recognition sites does it have? **ANSWER: Since the DNA is given as initially linear, three fragments are generated by two cuts.**

(c) [5 points] Does the longest EcoR1 fragment contain a BamH1 restriction site? **ANSWER: Yes. The longest EcoRI fragment is 10 kb. Since there is no 10 kb fragment in the combined EcoRI/BamHI digest, the 10 kb EcoRI fragment must be cut further by BamH1.**



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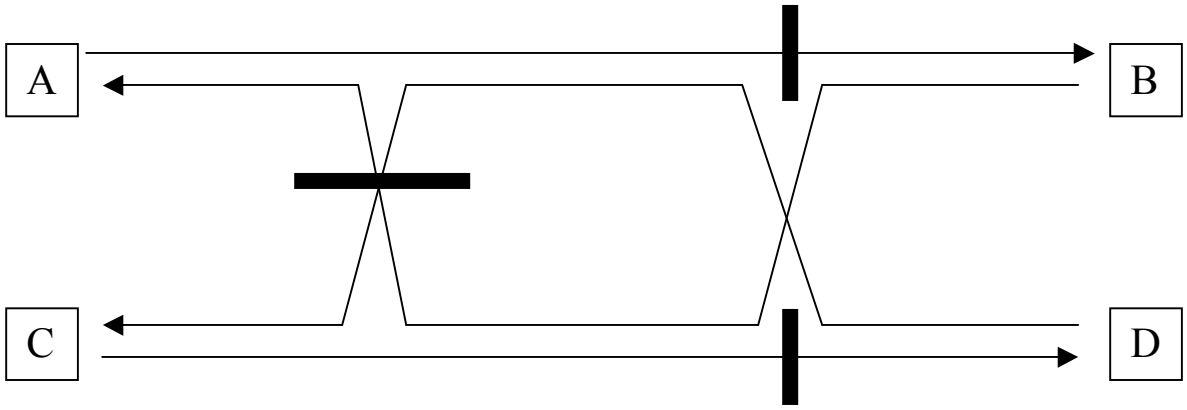
12. Crossing over or gene conversion (10 points)? Consider the following double Holliday junction. (The 5'-to-3'directionality of the individual strands of DNA is shown, but not the helical structure.) If the Holliday junctions are resolved by cutting the strands as shown by the thick black bars, will the result be crossing over or gene conversion? For full credit, explain your logic. For convenience, the rest of the maternal chromosome is shown with shaded boxes at the ends, and the ends of the paternal chromosome is shown with open boxes.

ANSWER: If cuts are made on the (four) strands as shown by the thick black bars, then box "A" remains connected to box "D" through either double –stranded or (near the breaks) single-stranded sequence. Similarly, box "B" is connected to "C". After repairing single-stranded regions, this will result in crossing over in this region, since the left hand side of the maternal chromosome will be joined with the right hand side of the paternal chromosome, and vice versa.



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13. Transcription and translation (10 points). The partial sequence

```
5′ TCTAGCCTGAACTAATGC 3′
3′ AGATCGGACTTGATTACG 5′
```

is found in the middle of the protein-coding region of a gene from a bacterial genome. That is, the stop codon of the gene is found outside of the sequence shown.

(a) Which strand is the mRNA-like strand? Please explain your logic.

ANSWER: There are six possible reading frames. As noted, since this is the middle of a protein-coding region, the correct reading frame should not have a stop codon (UGA, UAA, UAG)

If top strand were the mRNA-like strand, there are three possible mRNA reading frames as indicated:

5′ UCU AGC CUG AAC UAA UGC 3′
5′ U CUA GCC UGA ACU AAU GC 3′
5′ UC UAG CCU GAA CUA AUG C 3′

but all three frames have a stop codon (underlined). So none of them is the mRNA-like strand.

If the bottom strand is mRNA-like, then the three reading frames are (reading as always from 5' to 3')

5′ GCA UUA GUU CAG GCU AGA 3′
5′ G CAU UAG UUC AGG CUA GA 3′
5′ GC AUU AGU UCA GGC UAG A 3′

and as noted the bottom two have stop codons. So by elimination the correct answer must be that the bottom strand is mRNA-like.

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(b) What is the amino acid sequence of the peptide encoded by this (partial) gene? Indicate which is the amino terminal end of the peptide.

ANSWER: mRNA is translated from 5' to 3', producing a corresponding peptide from the amino terminal to the carboxy terminal.

5′ GCA UUA GUU CAG GCU AGA 3′
Amino Ala Leu Val Gln Ala Ser Carboxy

Note: The genetic code table is found below

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

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14. Complementation groups (10 points). In a haploid yeast strain, eight mutants were found in a haploid yeast require the amino acid lysine for survival. (Wild type yeast does not require this amino acid.) All mutations were found to revert at a frequency of 1×10^{-6}, except for mutants 5 and 6.

Matings were made between strains carrying these mutations. The ability of the resultant diploid strains to grow in the absence of lysine is shown in the table: "+" means growth and "-" means no growth.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	-	+	+	+	+	-	+	-
2	+	-	+	+	+	+	+	+
3	+	+	-	-	-	-	-	+
4	+	+	-	-	-	-	-	+
5	+	+	-	-	-	-	-	+
6	-	+	-	-	-	-	-	-
7	+	+	-	-	-	-	-	+
8	-	+	+	+	+	-	+	-

(a) How many complementation groups are revealed by this data?
ANSWER: Complementation groups are collections of alleles that <u>do not</u> complement each other in pairwise crosses. From the table, we have the following groups that do not complement each other in all pairwise crosses:

- **(1,6,8) (all have mutually "-" diploid offspring),**
- **(2) (only not complemented by itself, and so a complementation "group" all by itself),**
- **(3,4,5,6,7) (all have mutually "-" diploid offspring).**

Note that 6 is unusual in that it is in two of these groups.

(b) Which point mutations are found in which complementation groups?
ANSWER: As noted in the statement of the question, Mutants 5 and 6 <u>don't revert</u>. As discussed in the text and in class, these must be deletions or some other essentially irreversible change in the genes. If we exclude these deletions from consideration, there are three non-overlapping complementation groups, with the following membership:

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- **(1,8)**
- **(2)**
- **(3,4,7)**

The interpretation is that deletion 5 is likely a deletion in the gene corresponding to complementation group (3,4,7), since it behaves in the same way as these point mutatios.
Deletion 6 is somewhat more complicated – it is not complemented by the genes corresponding to BOTH (1,8) AND (3,4,7). It is therefore a deletion that involves BOTH of these genes. This explains why it appears in two group in part (a).

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15. Human variation [10 points]. A polymorphic microsatellite locus is PCR amplified in four individuals from the same family, and the resulting products are run on a gel. The alleles are labeled "1", "2" , "3", and "4" from longest to shortest.

(a) (5 points). From which parent did "Kid1" inherit allele 2?
ANSWER: Mom is heterozygous, carring alleles 1 and 4; similarly, Dad has alleles 2 and 3. Kid1 has alleles 2 and 4, and so got the "2" allele from Dad.

(b) (5 points). Kid1 and Kid2 don't share any alleles. Can we infer that Kid2 is not related to Kid1? Why or why not? **ANSWER: Kid1 and Kid2 have genotypes at this locus that are consistent with Mom and Dad being the parents of both. So we can't rule out that they're related. But its also possible that they had different parents – only one locus is not enough to**