

Name: Answer Key

Recitation Section or TA: \_\_\_\_\_

## 7.013 Exam Two -- 2007

Exam starts at 11:05 am and ends at 11:55 am.

There are 10 pages including this cover page.

Please write your name on each page.

Only writing on the **FRONT** of every page will be graded.  
(You may use the backs, but only as scratch paper.)

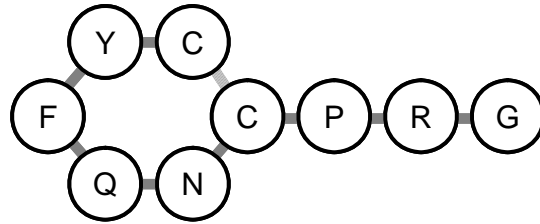
Questions that call for short answers should be limited to 15 words or fewer.

|                |                     |                |                     |
|----------------|---------------------|----------------|---------------------|
| <b>Topic 1</b> | <b>10 pts</b> _____ | <b>Topic 5</b> | <b>14 pts</b> _____ |
| <b>Topic 2</b> | <b>24 pts</b> _____ | <b>Topic 6</b> | <b>20 pts</b> _____ |
| <b>Topic 3</b> | <b>8 pts</b> _____  | <b>Topic 7</b> | <b>12 pts</b> _____ |
| <b>Topic 4</b> | <b>12 pts</b> _____ |                |                     |

**TOTAL** out of 100 \_\_\_\_\_

**Topic 1.**

Vasopressin is a small peptide secreted by the hypothalamus (part of the brain) into the bloodstream. Vasopressin has the following amino acid structure.



The DNA sequence and the mature transcript from the human gene that encodes vasopressin are depicted on Page 9 of this exam. Based on the information provided here and on Page 9, answer the following questions.

1a. (2 pts.) How many exons does this gene have? 3

Name two processes that modify the primary vasopressin transcript to form the mature vasopressin transcript.

1b. (2 pts.) intron splicing

1c. (2 pts.) polyadenylation (also: 5' capping)

Name one post-translational modification that must be occurring if the primary translation product from the gene on Page 9 forms the vasopressin depicted above.

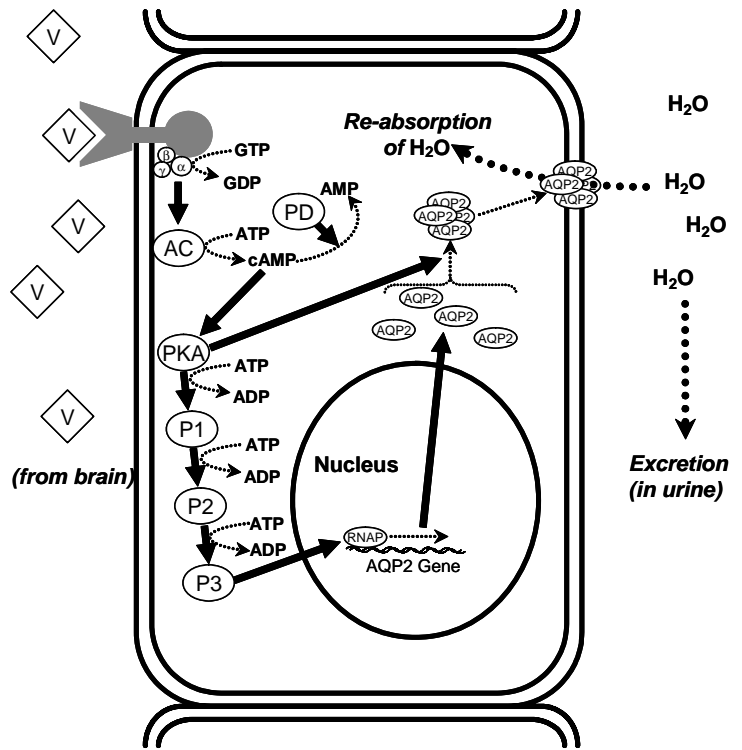
1d. (4 pts.) Proteolytic processing (cleavage of the primary polypeptide)

or Formation of disulfide bond between C (cysteine) residues

**Topic 2.**

Binding of brain-derived vasopressin to receptors on the surfaces of cells lining the kidneys will activate a signal transduction cascade as depicted here.

**Vasopressin (V) signal transduction in kidney cells.** Heavy arrows indicate activation of an enzyme, pathway or process. Dotted arrows indicate chemical pathways, processes or movements. Abbreviations: AC = adenylyl cyclase; PD = phosphodiesterase; PKA, P1, P2 and P3 = individual enzymes; RNAP = RNA polymerase; AQP2 = aquaporin



- 2a. (2 pts.) Is the vasopressin/receptor interaction an example of an autocrine, endocrine, paracrine, or amacrine pathway?  
**endocrine**
- 2b. (2 pts.) What name would best describe the enzyme that metabolizes GTP (indicated by the small circles labeled  $\alpha$ ,  $\beta$ ,  $\gamma$ )?  
**G-protein or GTPase**
- 2c. (4 pts.) What is the advantage of having multiple enzymes in a protein kinase cascade?  
**Signal amplification**
- 2d. (4 pts.) Of all the labeled proteins in the figure, which would best be described as a possible transcriptional activator?  
**P3**
- 2e. (2 pts.) Only AQP2 tetramers (not monomers) in the cytoplasm form active water channels. The assembly of AQP2 tetramers is a regulated process. Would a chemical that inhibits the phosphorylation of P1 *inhibit*, *enhance* or *have no effect* on the expression of new AQP2 monomers?  
**inhibit**
- 2f. (2 pts.) Would a chemical that inhibits the phosphorylation of P1 *inhibit*, *enhance* or *have no effect* on the assembly of AQP2 tetramers from previously synthesized monomers?  
**have no effect**
- 2g. (4 pts.) Of the enzymes and proteins depicted in the above diagram, which one acts to suppress (or weaken) the vasopressin response? Explain in 15 words or fewer.  
**PD weakens the vasopressin response by lowering the concentration of (metabolizing) cAMP in the cell**
- 2h. (4 pts.) Diabetes insipidus is a disorder in which the hypothalamus fails to produce vasopressin. Based on the diagram above, what would be the most obvious symptom in someone who suffers from diabetes insipidus? Explain in 15 words or fewer.  
**Frequent or excessive urination; excessive thirst/dehydration**

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### Topic 3.

In order to develop a treatment for patients who do not make sufficient vasopressin, you set out to use cultured *E. coli* to produce recombinant vasopressin. To begin, you will use PCR to amplify a portion of the vasopressin gene (underlined sequences on page 9).

- 3a. (4 pts.) Using the vasopressin gene sequence as presented on Page 9 of this exam, design two primers, each 10 bases long, that could be used to precisely amplify only the underlined portion of the gene (recall that your primers will become part of the resulting PCR product). Label the 5' and 3' ends of your primer sequences.

Primer 1 sequence: 5'-CTCGGCCTAC-3'

Primer 2 sequence: 5'-CTGTCTCAGC-3'

- 3b. (4 pts.) To express the recombinant vasopressin in *E. coli*, select the most appropriate promoter from the following list. Explain your selection (15 words or fewer).

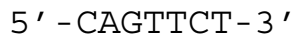
- The promoter from the uromodulin gene, which directs kidney-specific transcription
  - The promoter from the beta-actin gene, which is expressed in nearly every mammalian cell type
  - A constitutive promoter from the lambda phage, a virus that infects bacterial cells
  - The original promoter from the human vasopressin gene
- Of these promoters, only the lambda phage promoter will be active in the E. coli (bacterial) cells that you are using to produce the recombinant vasopressin*

**Topic 4.**

You now have the vasopressin PCR product cloned in a plasmid vector. A portion of the expected plasmid sequence is shown below.



In order to verify that your PCR-product does not contain any errors (misincorporated bases) you choose to determine its sequence. You obtain the following primer:



You carry out a Sanger sequencing reaction using DNA polymerase, high levels of dATP, dCTP, dGTP, dTTP, low levels of labeled-ddATP, the primer, the DNA template (depicted above), some magnesium, and a buffer to control pH. After the reaction has gone to completion, you separate the reaction products on a sequencing gel and detect the labeled DNA.

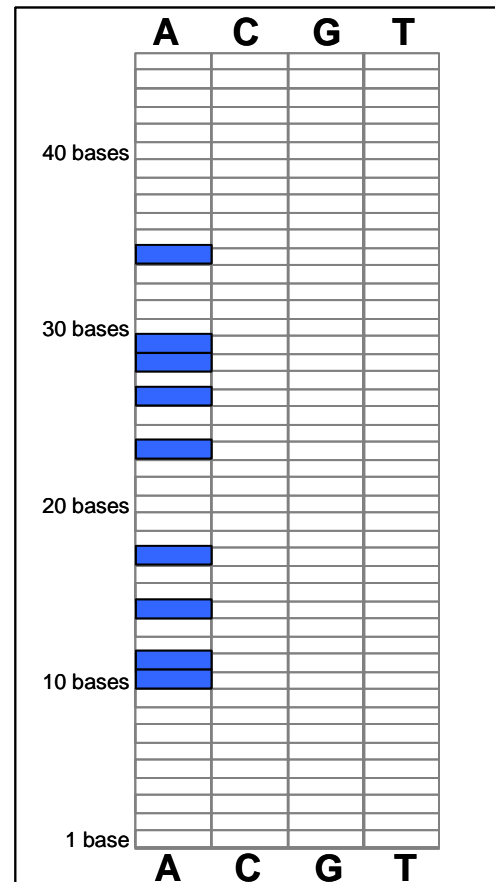
**4a. (12 pts.)** Use the diagram to the right to indicate where the labeled reaction products would appear on the gel.

For each reaction product, fill a single box in the diagram to indicate where each product will appear.

The distances at which molecules of different sizes (in bases) would migrate are indicated to the left of the diagram.

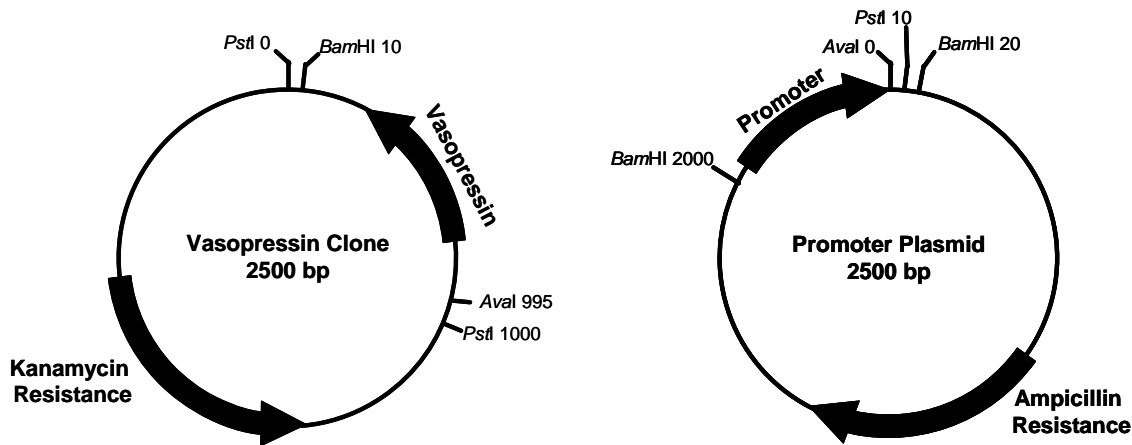
*(Note: limit your answer to the sequence shown above; you shouldn't need to refer to the vasopressin gene sequence shown on page 9.)*

**The reaction composition described above would cause polymerization to terminate (due to incorporation of the dideoxyATP) only at positions corresponding to A residues in the newly synthesized "top" strand. Since the primer becomes part of the newly synthesized strand, a molecule that terminates at the first "A" position would be 10 bases long, etc.**



**Topic 5.**

Creating a recombinant vasopressin expression vector requires you to move the cloned PCR product from one plasmid into a second plasmid that already carries the appropriate promoter.



Above are maps of the two plasmids, with total plasmid lengths indicated in the center of each, and the relative positions of enzyme cleavage sites (in basepairs) indicated. You excise the vasopressin PCR product from the plasmid (“vasopressin clone”) as a *PstI* fragment, and you insert this into the *PstI* site of the promoter plasmid. You then introduce the resulting recombinant plasmid into *E. coli* cells via transformation.

- 5a. (2 pts.) What is the general name for enzymes such as *PstI*, *BamHI*, and *AvaI*, that cleave DNA in a sequence-specific manner?  
**Restriction enzymes**
- 5b. (2 pts.) What enzyme would you use to covalently bond the *PstI* fragment into the *PstI* site of the promoter plasmid?  
**DNA ligase**
- 5c. (2 pts.) After cloning, you introduce the new plasmid into *E. coli* cells via transformation. With which antibiotic or antibiotics should you select the transformants?  
**Ampicillin**

You recover plasmid DNA from 4 candidate colonies. To examine the composition of these plasmids, you digest each with either *PstI*, *AvaI* or *BamHI*. After separating these on agarose gels, you tabulate the approximate sizes (in kilobasepairs) of the DNA fragments produced. (Fragments smaller than 100 basepairs are not shown.)

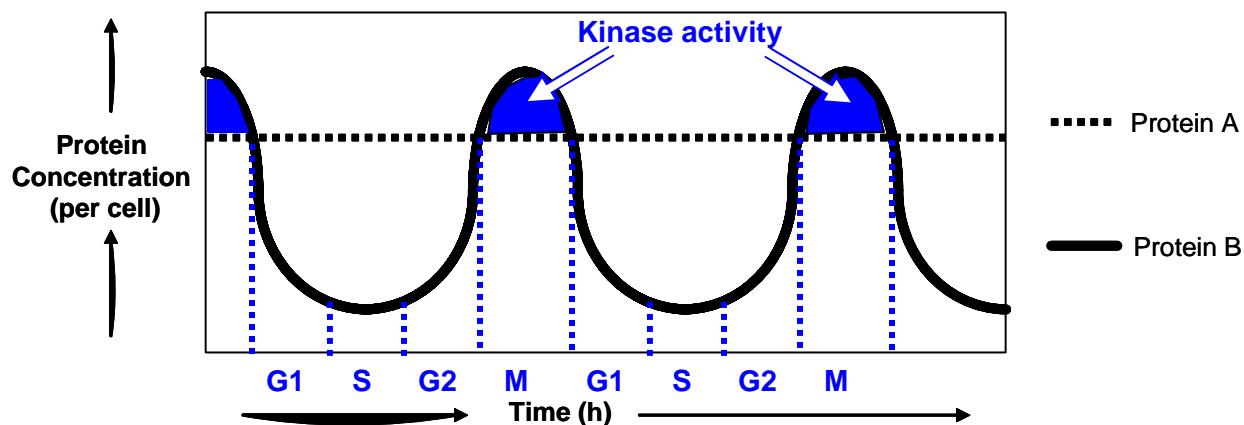
| Candidate #          | Enzyme used for test digests |     |          |          |             |     |     |          |              |          |          |               |
|----------------------|------------------------------|-----|----------|----------|-------------|-----|-----|----------|--------------|----------|----------|---------------|
|                      | <i>PstI</i>                  |     |          |          | <i>AvaI</i> |     |     |          | <i>BamHI</i> |          |          |               |
|                      | A                            | B   | C        | D        | A           | B   | C   | D        | A            | B        | C        | D             |
| Fragment sizes (kbp) | 2.5<br>1                     | 2.5 | 2.5<br>1 | 2.5<br>1 | 2.5<br>1    | 2.5 | 3.5 | 2.5<br>1 | 3<br>0.5     | 2<br>0.5 | 2<br>1.5 | 3<br>1<br>0.5 |

- 5d. (8 pts.) Based on these observations, which candidate plasmid carries the vasopressin PCR product oriented in the same direction as the promoter (based on the direction of the arrows in the above diagrams)?  
**Candidate C**

**Topic 6.**

You have identified two temperature-sensitive mutant yeast strains that cannot enter mitotic prophase at the non-permissive temperature. The mutants correspond to different genes. You perform multiple studies to try to understand the mechanism underlying this phenotype.

Your analyses show that the mutation in one strain leads to loss of Protein A expression. The mutation in the second strain leads to loss of Protein B expression. Examining the abundance of each protein in wild-type cells, you discover the following:



You further show that in wild type cells, Protein A is a kinase, that is, it can phosphorylate other proteins. However, it does this only when the concentration of Protein B exceeds that of Protein A.

- 6a. (2 pts.) Indicate on the diagram above when Protein A has kinase activity.  
(see diagram)
- 6b. (4 pts.) You isolate another temperature sensitive mutant that expresses a mutant Protein A lacking kinase activity. If kinase activity is required for cell cycle progression, then, at the non-permissive temperature, would the cell cycle progress normally, too quickly, or would it arrest at a specific stage, and if so, what stage?

*The cell cycle would arrest at the G2/M boundary*

- 6c. (4 pts.) Assuming that Protein B expression needs to reach a threshold concentration for the cell cycle to progress, what phenotype would you find in a mutant that expresses high levels of Protein B at all times?  
*The cell cycle would proceed through M excessively (i.e. regardless of whether the cell was "ready" for mitosis), perhaps leading to mitotic aberrations*
- 6d. (4 pts.) Assuming the cyclical expression of Protein B is important for cell cycle progression, label the above diagram with regard to the different stages of the cell cycle.  
(see diagram)
- 6e. (6 pts.) Describe two possible mechanisms of gene expression control that would result in increasing the concentration of protein B during the cell cycle.  
*Increased transcription of the gene for Protein B, and increased translation of the mRNA for Protein B (also, more efficient splicing of pre-mRNA; increased stability of Protein B; other reasonable answers possible)*

## Topic 7.

- 7a. (4 pts.) The functional kidney is the only organ that had been known to express the AQP2 protein. You are therefore surprised to find that a small group of cells in the embryo expresses AQP2, before any kidney is visible. Using the technique of fate mapping you ask whether these cells correspond to future kidney, or to something else. Describe what this technique involves (15 words or fewer).

*Injecting a non-diffusible dye; monitoring whether the dye can later be detected in kidney cells*

- 7b. (4 pts.) The HNF1B transcription factor is essential for kidney development. It turns out, however, that HNF1B is not expressed in the developing kidney, but in an adjacent group of cells. Suggest the most likely mechanism by which HNF1B influences kidney development. (15 words or fewer)

*HNF1B causes production of a signaling molecule that induces kidney-specific development in adjacent cells*

Functional kidney cells are present at 24 hours post fertilization (hpf). They express effector genes that encode proteins important for blood purification.

You perform an explant assay and find that cells are not committed (are undetermined) to a kidney fate at 6 hpf, but are committed (determined) at 12 hpf. However, at the time you begin the assay, neither the 6hpf nor 12hpf explanted group of cells expresses any of the effector genes that are active in older, functional kidney cells.

- 7c. (4 pts.) In terms of gene expression, what is different between the 6 and 12hpf explants? (15 words or fewer)

*12 hpf (but not 6 hpf) cells express regulatory genes that will cause expression of effector genes*

**Supplementary Information 1.****The Human Vasopressin Gene**

One strand of the DNA sequence from the vasopressin gene locus is shown below in **BOLD**. Where appropriate, the sequence of the mature transcript is shown directly beneath the corresponding DNA sequence (lower case and NOT BOLD). All sequences are written in the 5'→3' direction (left to right). Relative numbering of each is indicated to the left. "@" indicates 7-methyl-guanosine. Exclamation points (!!!) are positioned beneath the nucleotides that constitute the start codon; Asterisks (\*\*\*) are positioned beneath the nucleotides that constitute the stop codon.

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1  GCACCCGAGG ACCCCTCGGT GACCCCTCCC CCACCGCCGG CGCAGAGCGG AGGTGCCCTT
61  GTGGACGCCT GTATTTATCC GTCGGTCGTC TCCGTCGTCG ACAGAGCCAC CAAGCAGTGC
   1                                     @acagagccac caagcagugc

121 TGCATACGGG GTCCACCTGT GTGCACCAGG ATGCCTGACA CCATGCTGCC CGCCTGCTTC
   21 tgcatacggg gtccacctgt gtgcaccagg atgcctgaca ccatgctgcc cgctgcttc
   !!

181 CTCGGCCTAC TGGCCTTCTC CTCCGCGTGC TACTTCCAGA ACTGCCCGAG GGGCGGCAAG
   81 cucggccuac uggccuucuc cuccgcgugc uacuuccaga acugcccagag gggcggcaag

241 AGGGCCATGT CCGACCTGGA GCTGAGACAG GTACTTCCCA CTGTGGGCCA TCTCAGGGCT
   141 agggccaugu ccgaccugga gcugagacag

301 GCCATAGCGG GCAGTGCTGA CACCCTGGGT CAGGGGCTAG GAAAGAGGGA AGTCATGGGT
361 GGTGGTAGCC TTTAGGGGAA GTTCGGGGGA GGAAGAGGGA GGCATGGCAT GGCTGGGCAG
421 AGGAGCCAAT GGGGTGGGCC AGAGGGGACC AGGCTTTGGA GGAGGCTGGG AGAGGCTGAA
481 GGCCTCCTG GTCACTGTCG CCATCCAGAC AGGGATGCAG GGAATGAGG GATGCTTCCC
541 CGGTGACTGG GCTTGGGGCT GGATAGGGAG AACGGGGCAT CATGGCCTCC CCTGTGCCCA
601 (nucleotides 601-1500 not shown)
1501 GCGGCCGAG CGTGCCTGCA GCCGCAGCCC CGGTGTCCC CGCGACTCC GAGCCCTGGA
1561 CCCAGCATC CCCGCCTCGC TGCGTTCCTC TCCAACCCCT CGACTCCCAG CTCCCCTCCT
1621 CCCGCTACC CCGCCCGTCC CCGCAGTGCC TCCCCTGCGG CCCCGGGGGC AAAGGCCGCT
   171                                     ugcc ucccugcggg ccccgggggc aaaggccgcu

1681 GCTTCGGGCC CAGCATCTGC TGC GCGGACG AGCTGGGCTG CTTCTGGGGC ACGGCTGAGG
   205 gcuucggggc cagcaucugc ugcgcgagc agcugggcug cuucgugggc acggcugagg

1741 CGCTGCGCTG CCAGGAGGAG AACTACCTGC CGTCGCCCTG CCAGTCCGGC CAGAAGGCGT
   265 cgcugcgugc ccaggaggag aacuaccugc cgucgcccug ccaguccggc cagaagggcu

1801 GCGGGAGCGG GGGCCGCTGC GCCGCCTTCG GCGTTTGCTG CAACGACGGT GCGCGGCGGG
   325 gcgggagcgg gggccgugc gccgcccugc gcguuugcug caacgagc

1861 GCGGGCCTG GGGCTGGGGG GGGCGCAGAC CGCTTGGGTG GGGGGACGC GGGCCTGCGG
1921 CGGGGTGGG GCTGCGTCGG GCCCGCAGG GAGGGTGTGG GCCCCCGCA CCCGAGCTG
1981 CGCCCGCCC AGGGCGCCG TGCTCACAG TCCTCCCGC AGAGAGCTGC GTGACCGAGC
   373                                     agagcugc gugaccgagc

2041 CCGAGTGCCG CGAGGGCTTT CACCGCCGCG CCCGCGCCAG CGACCGGAGC AACGCCACGC
   391 ccgagugccg cgagggcuuu caccgcccgg cccgcccag ccagccggagc aacgccacgc

2101 AGCTGGACGG GCCGGCCGGG GCCTTGCTGC TCGGCTGGT GCAGCTGGCC GGGGCGCCG
   451 agcuggacgg gccggcccgg gccuugcugc ugcggcuggu gcagcuggcc ggggcccgg

2161 AGCCCTTCGA GCCCGCCAG CCCGACGCT ACTGAGCCCC GCGCTCGCCC CACCGGCGCG
   511 agcccuucga gcccgcccag cccgacgcu acugagcccc gcgucgccc caccggcgcg
   ***

2221 CTCTTCGCGC CCGCCCTGC AGCACGGACA ATAAACCTCC GCCAATGCAC GGCCTCGCGT
   571 cucuucgugc ccgcccugc agcacggaca auaaacucc gccaaugcaa aaaaaaaaaa

2281 CTGTCTCAGT CTCTGGCGGG AAGAGGGAAA GGGAGAGAGG TGGGAGCGCG GACCCCGCC

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Name: Answer Key

**Supplementary Information 2. The Genetic Code**

|          | <b>U</b>  | <b>C</b>                               | <b>A</b>   | <b>G</b>   |
|----------|---|--|--|--|
| <b>U</b> | UUU Phe (F)<br>UUC “<br>UUA Leu (L)<br>UUG “        | UCU Ser (S)<br>UCC “<br>UCA “<br>UCG “ | UAU Tyr (Y)<br>UAC “<br>UAA <i>Stop</i><br>UAG <i>Stop</i> | UGU Cys (C)<br>UGC “<br>UGA <i>Stop</i><br>UGG Trp (W) |
| <b>C</b> | CUU Leu (L)<br>CUC “<br>CUA “<br>CUG “              | CCU Pro (P)<br>CCC “<br>CCA “<br>CCG “ | CAU His (H)<br>CAC “<br>CAA Gln (Q)<br>CAG “               | CGU Arg (R)<br>CGC “<br>CGA “<br>CGG “                 |
| <b>A</b> | AUU Ile (I)<br>AUC “<br>AUA “<br><b>AUG</b> Met (M) | ACU Thr (T)<br>ACC “<br>ACA “<br>ACG “ | AAU Asn (N)<br>AAC “<br>AAA Lys (K)<br>AAG “               | AGU Ser (S)<br>AGC “<br>AGA Arg (R)<br>AGG “           |
| <b>G</b> | GUU Val (V)<br>GUC “<br>GUA “<br>GUG “              | GCU Ala (A)<br>GCC “<br>GCA “<br>GCG “ | GAU Asp (D)<br>GAC “<br>GAA Glu (E)<br>GAG “               | GGU Gly (G)<br>GGC “<br>GGA “<br>GGG “                 |

**The Genetic Code.** Three-letter and one-letter abbreviations for each amino acid are listed alongside their corresponding codons. Ditto marks (“) indicate the same amino acid as above.