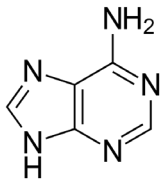
Molecular Methods and Applications Exam
Study Guide

Molecular Biology and Genetics

1. Label the nitrogenous bases:



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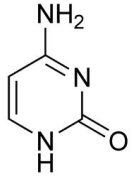

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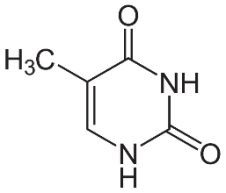

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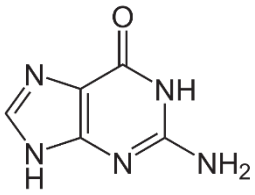

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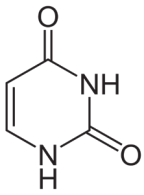
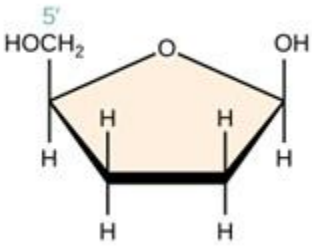


_____

2. Label the sugar molecules:



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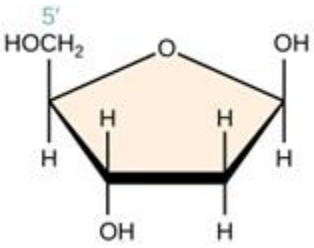

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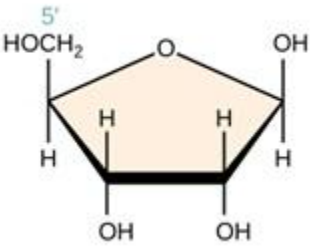


_____

3. Single-ring nitrogenous bases are called _____, while double-ring nitrogenous bases are called _____.

4. Nucleic acids are read by polymerase in the _____ direction, but new strands are synthesized in the _____ direction.

5. What chromosome shape does each of the below images represent?

_____

_____

_____

6. Differentiate the following kinds of genetic disorders/disease:

   Single-Gene Disorder -

   Polygenic (Multifactorial) Disorder -

   Chromosomal Disorder -

   Somatic (Acquired) Disorder -

   Constitutional Disorder -

7. Chromosomes are at their most highly condensed during the _____ stage of mitosis.

8. Define the following terms, as they relate to gene structure:
   a. Promoter:

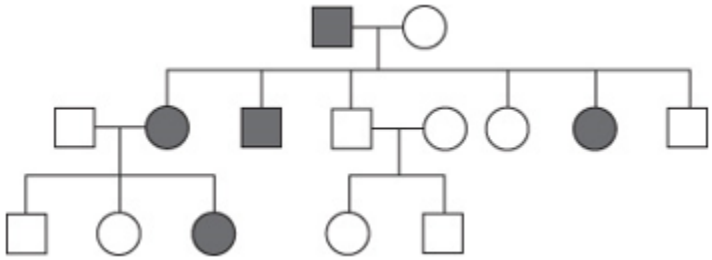
   b. Intron:

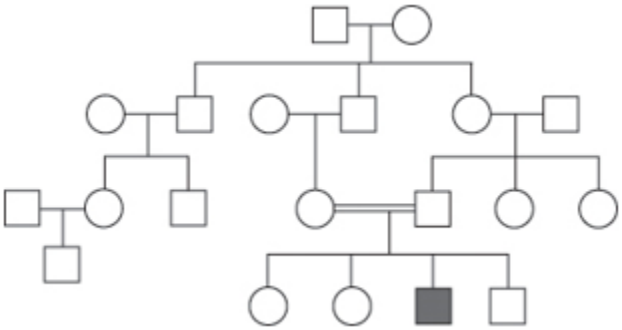
   c. Exon:

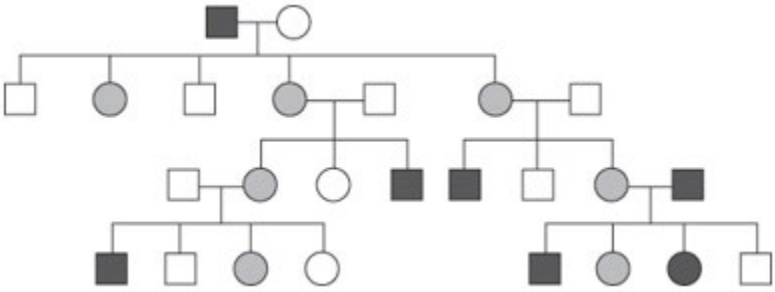
   d. Alternative splicing:

   e. CpG Islands/CpG site:

9. Identify the likely pattern of inheritance for the following pedigrees. Dark-grey shading indicates "affected" patients while light-grey shading indicates "carrier" patients.

a.  _____

b.  _____

c.  _____

10. List the codon sequence(s) associated with each of the following:
    a. Translation initiation: __ __ __
    b. Translation termination: __ __ __ __ __ __ __ __ __

Specimen Collection and Processing

11. _____ is the anticoagulant of choice for most molecular diagnostics tests. Explain how this anticoagulant both preserves DNA/RNA quality while also potentially inhibiting PCR:

12. How do specimen handling and storage requirements differ for samples requiring DNA or RNA extraction?
    DNA Extraction:

    RNA Extraction:

13. Formalin-fixed paraffin embedded tissue requires _____ prior to DNA/RNA extraction and FISH probing.

14. Is formalin-fixed paraffin embedded tissue an acceptable specimen type for cell culturing, karyotyping, and metaphase FISH? Why?

15. _____ are enzymes ubiquitous in the environment that degrade DNA/RNA.

Nucleic Acid Extraction and Quantification

16. Comparatively, would **peripheral blood** or **FFPET** produce higher quality and less fragmented DNA from extraction? Why?

17. The _____ extraction method uses the highly toxic chemicals _____ and _____.

18. The solid-phase isolation method uses silica in the form of _____ or _____ to bind and retain DNA/RNA during wash steps.

19. The organic and inorganic isolation methods use a _____ pH and _____ salt solution to precipitate nucleic acid.

20. Nitrogenous bases have peak absorbance at _____ nm.

21. Why do DNA/RNA quants from fluorometry tend to be lower than quants from spectrophotometry?

22. What would a 260/280 ratio of 1.35 indicate for an RNA extraction?

23. When performing a manual DNA extraction, a technologist failed to perform the alcohol drying step prior to adding hydration solution. What affect might this have on a spectrophotometric quant/qualification?

24. A DNA sample run on an agarose gel appears as a distinct, clearly-visible smear that extends all the way from the loading well to the anode. How might the fluorometric and spectrophotometric quants differ for this sample?

25. A DNA sample has a spectrophotometric quant of 256.5 ng/µL and a fluorometric quant of 225 ng/µL. How might this sample appear when run on an agarose gel?

26. Spectrophotometric readings for an RNA extraction hydrated in 0.5mL of TE buffer were as follows:

    A260 = 2.944
    A280 = 1.595
    A230 = 1.85

    Calculate the following:
    a. Concentration

    b. Total yield

    c. 260/280 (indicate acceptability)

    d. 260/230 (indicate acceptability)

27. Supposing the A260 value in the previous question was derived from measurement of 1:10 dilution of RNA in 0.5mL, how would you re-calculate the following?
    a. Concentration

    b. Total yield

28. The _____ method of quantification uses lab-on-a-chip technology to generate a gel-like quantification reading and uses as little as 1uL of patient DNA.

29. Where is sample DNA/RNA found following each step of liquid-phase organic extraction? Will it be solubilized or precipitated?
    a. After cell lysis step:
    b. After protein precipitation/centrifugation step:
    c. After phenol-chloroform/centrifugation step:
    d. After isopropanol/centrifugation step:

30. Where is sample DNA/RNA found following each step of liquid-phase inorganic extraction? Will it be solubilized or precipitated?
    a. After cell lysis step:
    b. After protein precipitation/centrifugation step:
    c. After isopropanol/centrifugation step:

Molecular Diagnostic Techniques

31. Connect the steps of PCR to their relative temperatures and order:

Step 1	**Extension**	50°C – 70°C
Step 2	**Annealing**	90°C – 96°C
Step 3	**Denaturation**	68°C – 75°C

32. PCR amplifies DNA (circle one→) **linearly** / **exponentially** / **logarithmically** / **inversely**. This means that a PCR program performed on a single template strand of DNA with 40 cycles will produce (circle one→) **2(40)** / **2^{40}** / **40^2** copies.

33. Define the following terms and provide examples:

Target amplification –

Probe amplification –

Signal amplification –

34. Explain the purpose of each component of a traditional PCR master mix:
    a. Molecular Biology Grade Water:

    b. Buffer:

    c. Mg2+:

    d. Nucleotides (dNTPs):

    e. Primers:

    f. DNA Polymerase:

35. Examine the following forward and reverse primer designs:
    Forward: 5'-GAGTCAACGGATTTGGTCGT-3'
    Reverse: 5'-GACAAGCTTCCCGTTCTCAG-3'

    a. Are these primers long enough? **Yes/No**

    b. Is the GC/AT content of these primers acceptable? **Yes/No**

    c. What are the melt temperatures of each of these primers:
    Forward (Tm) =
    Reverse (Tm) =

    d. Based on the above calculated Tms, would this be acceptable for co-hybridization of primers at a common annealing temperature (Ta)? **Yes/No**

    e. Is there any risk of cross-primer-dimer interactions at the 3' ends? **Yes/No**

    f. When hybridized to their targets, the 3' ends of the primers are 100bp apart. Assuming no mutation is present in the template sequence, what size (bp) would an amplicon produced from these primers be? _____

36. Define the following terms associated with PCR modifications, and explain how they improve upon traditional PCR:
    a. Reverse transcriptase/transcription PCR (RT-PCR):

    b. Multiplex PCR:

    c. Nested/Semi-Nested PCR:

    d. Hot-Start PCR

    e. Touchdown PCR

    f. Digital PCR

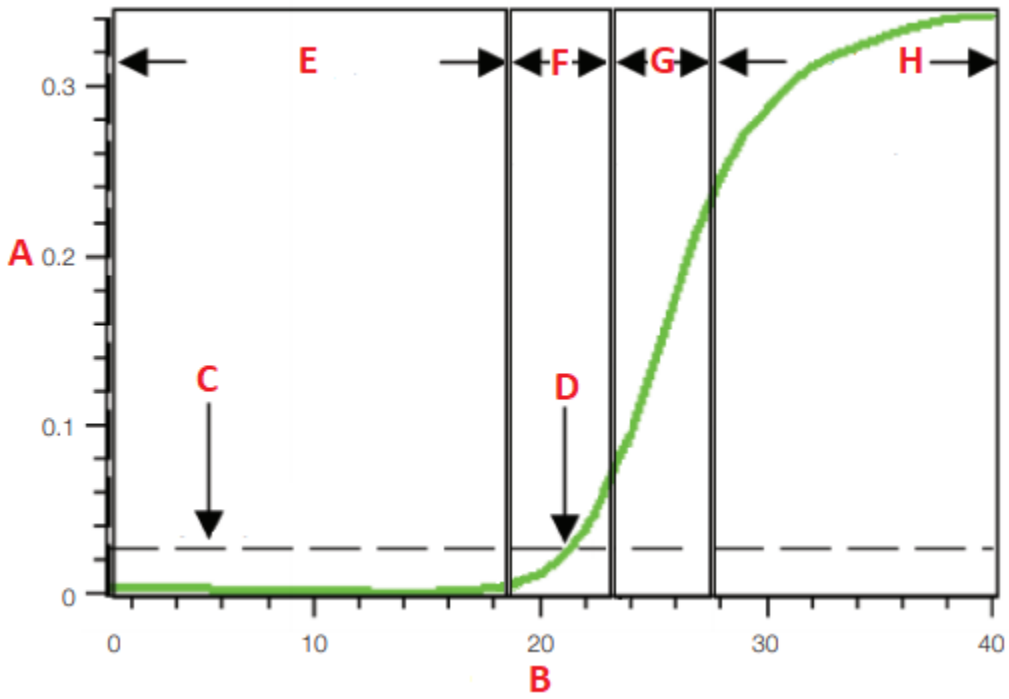
37. Which gel electrophoretic method is best suited for resolution of very small nucleic acids? Which electrophoretic method is best suited for very large molecules (50,000 to 250,000+ bps)?

38. As gel concentration _____, pore size _____.

39. A PCR-amplified target sequence from human DNA contains two restriction sites for the *Bam*H1 restriction enzyme. Assuming no mutation is present, how many fragments can be expected to be visualized on an agarose gel following *Bam*H1 digestions? _____

40. A common single nucleotide polymorphism alters only one of the two restriction sites in the previous example. Following *Bam*H1 digestion, what would be the expected number of fragments for the following genotypes?
    Homozygous mutant (both alleles have the SNP) _____
    Heterozygous mutant (one allele with, one without SNP) _____
    Homozygous wildtype (no SNP on either allele) _____

41. Identify what each letter indicates in the below graphic of a real-time PCR reaction.



a. _____
b. _____
c. _____
d. _____
e. _____
f. _____
g. _____
h. _____

42. Define each phase of real-time PCR (qPCR):
    a. Lag:

    b. Exponential:

    c. Linear:

    d. Plateau:

43. For dual-hybridization probes, the _____ molecule transfers energy to the _____ molecule to generate detectable fluorescence.

44. For hydrolysis probes, molecular beacons, and scorpion probes, the _____ molecule prevents the generation of detectable fluorescence from the _____ molecule.

45. A real-time PCR run yielded the following results:

    Patient 1: C_T = 25
    Patient 2: no amplification
    Patient 3: C_T = 15

    Which patient had the highest amount of target template in their original specimen? Justify your answer:

46. Connect the following data presentations with their associated test methodology:

Luminescence vs. Nucleotide Added	Real-Time PCR
Change in Fluorescence vs. Temperature	Capillary electrophoresis
Intensity vs. Mass	Melt Curve Analysis
Fluorescence vs. Base Pair Size	Pyrosequencing
Fluorescence vs. Cycle	MALDI-TOF

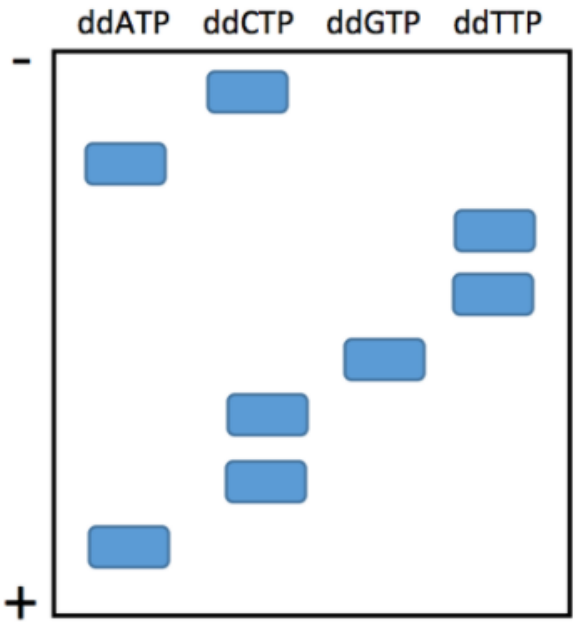
47. Explain the use of ddNTPs in Sanger Sequencing:

48. In MALDI-TOF, how does the "time of flight" relate to fragment mass? Would heavier DNA fragments travel faster or slower?

49. Does high-Resolution Melt Curve Analysis use **hydrolysis** probes or **hybridization** probes? Why?

50. Read the following sequencing gel:

5'- _____ -3'



Based on the above sequencing gel, synthesize the complementary strand:
5'- _____ -3'

51. Detail the purpose of each of the following enzymes in pyrosequencing:

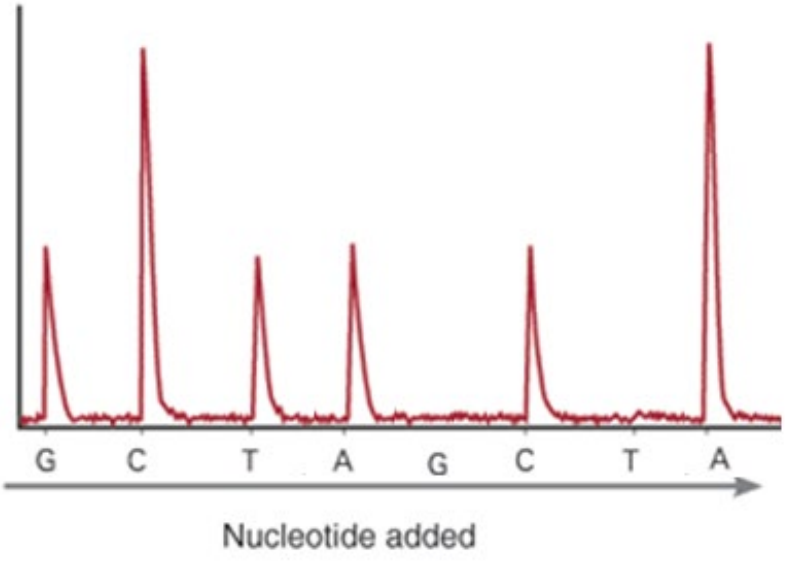
Polymerase –

Luciferase –

Sulfurylase –

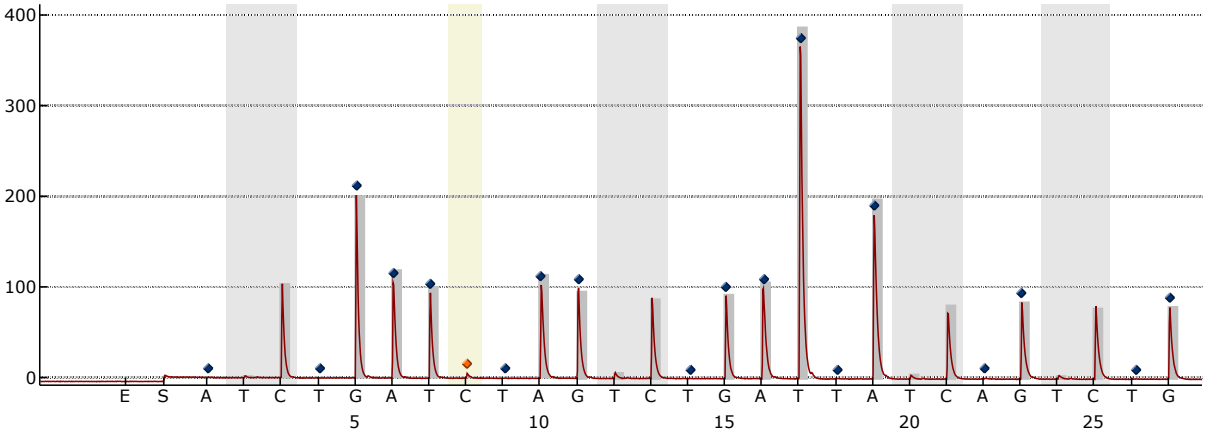
Apyrase –

52. Read the following pyrogram:



5'- _____ -3'

53. The following pyrogram represents a sample that underwent bisulfite conversion prior to pyrosequencing. They grey bins represent CpG islands important for gene regulation.



Does this pyrogram indicate the initial sequence was **methylated** or **un-methylated**? Why?

Is the gene associated with this sequence likely to be **expressed** or **under-expressed**? Why?

54. Define the following terms as they relate to reversible dye terminator sequencing:

Library Preparation –

Fragmentation –

End Repair –

Adapter–

Index–

Adapter –

Library Pooling –

Cluster Generation –

Bridge PCR –

Polony –

Bioinformatic pipeline –

FASTQ File –

BAM File –

VCF File –

55. Connect the following blotting techniques to their associated analytes:

Northern Blotting	DNA
Southern Blotting	RNA
Western Blotting	Protein

Cytogenomic Methods

56. Detail the purpose of each of the following cell harvesting steps and indicate any chemicals used:

    Mitotic Arrest -

    Hypotonic Treatment -

    Fixation -

57. A specific cell line can be encouraged to divide preferentially during cell culturing by the introduction of a _____. Cultures of this kind are called _____ cultures.

58. Explain how the following cytogenetic errors would affect the final metaphase spread:

    Colcemid concentration was too low –

    Hypotonic solution was too high –

    Room temperature was too high during slide dropping –

    Room humidity was too low during slide dropping –

    Trypsin concentration for G-banding was too high –

59. Connect the banding technique with the chromosomal regions they stain darkly:

G-Banding	Centromere
R-Banding	Heterochromatin
C-Banding	Euchromatin

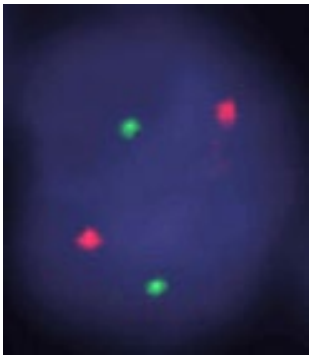
60. Would a G-banded chromosome from Group B have **more** or **less** bands than a chromosome from group F? Why?

61. In FISH, slides are counterstained with _____.

62. A FISH sample generates two red and two green signals following probing with a dual-color-dual-fusion FISH probe for the BCR gene (chromosome 22) and ABL gene (chromosome 9). Does this result indicate that a translocation has occurred?

63. A metaphase spread cultured from amniocentesis is probed using a green CEP probe for chromosome 18 and a red CEP probe for chromosome 13. If the fetus has Trisomy 18 (Edwards Syndrome), what signals would a cytogeneticist expect to see?

64. The ALK gene is located on the p-arm of chromosome 2. 2-7% of individuals with non-small cell lung carcinoma exhibit a rearrangement of the ALK gene, specifically an interstitial deletion and inversion of 2p resulting in an EML4/ALK fusion that is associated with positive prognosis. A FISH break-apart probe targeting ALK (green) and a highly conserved adjacent 2p sequence (red) generated the following signal pattern:



Does this signal pattern indicate that a rearrangement has occurred? **Yes** or **No**?

65. Compare interphase vs. metaphase FISH. Which is faster? Which is more sensitive? What specimen types are appropriate for each?

Quality Control and Assurance

66. Define the following kinds of controls as they relate to molecular diagnostic procedures:
    a. External Control -
        i. Positive Control -
        ii. Sensitivity Control -
        iii. Negative Control -
        iv. No template Control (NTC) -
    a. Internal Control -
        i. Intrinsic -
        ii. Extrinsic -

67. Would the failure of an internal control indicate a **sample failure** or a **run failure**? Explain:

68. Would the failure of an external control indicate a **sample failure** or a **run failure**? Explain:

69. Carryover from a previous PCR reaction can be prevented by maintaining a _____ workflow.

70. How does a dUTP-UNG system prevent carryover contamination?