**A) Multiple-choice questions (8 points, 2 points each)**.

**1) You are asked to amplify the following DNA template by PCR.**

5’GATCGCCGCAT--------------------------------------AATTGCGAGCGGC3’

3’CTAGCGGCGTA--------------------------------------TTAACGCTCGCCG5’

**Which the following primer sets is appropriate for the task?**

1. 5’ATGCGGCGATC3’ and 5’AATTGCGAGCGGC3’
2. 5’GATCGCCGCAT3’ and 5’CGGCGAGCGTTAA3’
3. 5’GATCGCCGCAT3’ and 5’AATTGCGAGCGGC3’
4. 5’GATCGCCGCAT3’ and 5’GCCGCTCGCAATT3’
5. 5’CTAGCGGCGTA3’ and 5’CGGCGAGCGTTAA3’

**2) In generating a cDNA library, total RNA prepared from a tissue is subjected to poly-dT selection (total RNA is passed through a column containing poly-dT oligos). The main purpose of this procedure is to:**

1. Generate primers for the synthesis of second strand DNA.
2. Separate mRNA from rRNA and tRNA.
3. Protect RNA from exonuclease-mediated degradation.
4. Generate restriction sites for cloning the cDNA into vector.
5. Separate mRNA from genomic DNA.

**3) Which of the following depicts the chain terminator used in Sanger sequencing? D**



**4) If a primer 5’GCTAGCTCG3’ was used in a sequencing reaction and the following result (in right) was obtained. Which the following is the likely template for the sequencing reaction (the primer sequence is underlined)?**

1. 5’TGGATCTCTGAACGGCTAGCTCG3’

3’ACCTAGAGACTTGCCGATCGAGC5’

1. 5’GCAAGTCTCTAGGTGCTAGCTCG3’

3’CGTTCAGAGATCCACGATCGAGC5’

1. 5’CGATCGAGCGCAAGTCTCTAGGT3’

3’GCTAGCTCGCGTTCAGAGATCCA5’

1. 5’GCTAGCTCGGCAAGTCTCTAGGT3’

3’CGATCGAGCCGTTCAGAGATCCA5’

1. 5’GCTAGCTCGTGGATCTCTGAACG3’

3’CGATCGAGCACCTAGAGACTTGC5’

**B) The *D. melanogaster* genome is about 150,000kb. If *D. melanogaster* genomic DNA is digested with EcoRI, which recognizes and cleaves the sequence GAATTC, what is the average size of DNA fragments generated by this digest (2 points)?**

Because EcoRI is a 6-cutter, the average DNA fragment size will be 46=4096 bps.

**Also, what is the expected number of DNA fragments generated by this digest (1 point)?**

The expected number will be 150,000/4.096=36621

**C) If a *D. melanogaster* genomic DNA library was constructed using cosmids, which are capable of carrying DNA inserts of 40kb in length. If you want to have 80% chance of recovering a plasmid clone containing a particular sequence, how many distinct clones from this library should you screen through (2 points)?**

N=ln(1-P)/ln(1-f)=ln(1-0.8)/ln(1-40/100000)=6035

**D)** A 10kb circular DNA digested with restriction enzymes yields the following fragments. Based on these results, please make a restriction map (please label the restriction sites and fragment sizes clearly, **4 points**)



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**E)** If the 2.5kb fragment from the BamHI-HindIII digest (indicated in circle) is made radioactive and used as a probe in a Southern blot of this gel, which bands do you expect to appear radioactive on an autoradiogram **(**circle the bands that will be seen on an autoradiogram; **3 points)**?

