MCB 102
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Online Document

Problem Set 6

Answer Key

1. **A double-stranded DNA molecule contains 20% adenine. Determine the number of cytosine bases if the DNA molecule is:**

   (a) 1000bp long
   600 Cytosine residues

   (b) 1cm long
   The DNA molecule has 29411765 bp and 17647058 will be Cytosine residues.

2. **DNA Sequences**

   Write the DNA base sequences that complement each of the following DNA strands.

   a) 5' AATCGCCCATTGCAGTTC 3' b) 5' CGATTGGCTTA 3'

   a) 5' GAA CTG CAA TGG GCG ATT 3' b) 5' TAA GCC AAT CG 3'

3. **Writhe and Twist**

   I was a horrible problem and all calculations were wrong! :(
   Please forget me.....

4. **Eucaryotic Chromosomes**

   In eukaryotic chromosomes, 150 bp of DNA are wrapped 1.8 times around the nucleosome core proteins, and there are ∼100 bp of DNA between each nucleosome.

   (a) Calculate the diameter of a nucleosome core particle.
   If 150 bp wraps around the nucleosome 1.8 times, then 150/1.8 bp = 83 bp wraps around the nucleosome once. The length of DNA is (83 bp)(0.34 nm/bp) = 28.2 nm = circumference. Recall that the circumference of a circle = D, therefore, D = 28.2 nm/ = 9.0 nm.
   (You may know that the nucleosome core particle is called the 11 nm particle. Thats because our calculation has not taken into account the diameter of the DNA duplex itself, which is 2.2 nm. When we add the DNA diameter to the length diameter, then the diameter of the nucleosome core particle is 9.0 nm + 2.2 nm = 11.2 nm.)

(b) Calculate the superhelical density of eukaryotic DNA.
W = -1.8 turns for 150 bp + 100 bp. Therefore, σ= W/N = –1.8/250 = –0.0072

5. **DNA packing**

A human mitochondrion (about 1.5μm in size) contains 16569 base pairs of DNA.

(a) Calculate the packing ratio for DNA in the mitochondria.
DNA length: 16569bp x 0.34nm/bp = 5633nm = 5.6μm
packing ratio: 5.6μm/1.5μm = 3.8

(b) Does this suggest that mitochondria have nucleosomes (briefly explain your answer)?
Bacteria have no nucleosomes. The packing ratio for E.coli is for example 3600 (you can find the length of the genomic DNA and the avarage cell size in your book) whereas for eucaryotic cells, which do have nucleosomes >10000. Thus, this suggests that mitochondria do not have nucleosomes as they do not need this high packing ratio.

6. **DNA Replication**

The following are some examples of problems that can occur during DNA replication. Identify the defective enzyme or enzymes that would cause each problem.

(a) Mismatched base pairs are found in the newly synthesized DNA.
3' → 5' Exonuclease of the DNA polymerase

(b) RNA bases are found in the newly synthesized DNA.
DNA polymerase I

(c) Replication on the leading strand is not initiated.
HU, FIS, and/or IHF, all of Table 25-3

(d) Replication forks are not formed.
dnaA protein

7. **DNA Replication II**

In the fruit fly Drosophila melanogaster, DNA replication at a single replication fork occurs at a rate of about 2600 nucleotide pairs per minute. The DNA molecule occurring in one of the largest chromosomes of this species has been estimated to contain $6*10^7$ nucleotide pairs.

(a) If replication of this molecule was initiated at a single origin in the middle of the chromosome, estimate the time, in days, required for complete replication of the chromosome.
$\frac{6 \cdot 10^7}{2600 \cdot min^{-1}} = 23,076.92\ min = 384.62\ hours = 16\ days$
→ for bidirectional replication: 8 days

(b) Estimates based on living cells indicate that this chromosome replicates in about four minutes. Assuming that the origins are spaced equally along the DNA, how many of them would be required to completely replicate this chromosome in four minutes?

→ for bidirectional replication in 4 minutes:
$2 \cdot 4 \cdot 2600 = 20800$
$\frac{6 \cdot 10^7}{20800} = 2885$

Exam Questions from 2008

8. ***Pelagibacter ubique***

   Pelagibacter ubique has the smallest genome yet found in a free-living organism. The contour length of the relaxed circular chromosome is 445μm. In your analysis you find the chromosome has a superhelical density of –0.33. What is its Lk? What is the Twist and the Writhe? Describe in one sentence its topological properties.

   445000nm / 0.34nm/bp = 1308823.5 bp
   Lk_o = Twist = 124649.85bp
   $\Delta Lk = \sigma$ x Lk_o = Writhe = – 41134.45 bp
   Lk = 1308823.5 bp – 41134.45 bp = 83515.4 bp
   The chromosome is negatively supercoiled.

9. **Sanger sequencing**

   (a) Write down the sequence of this DNA from 5' to 3' (cluster the letters into threes please):
   5' – CTC CTA GGG GCC C ATG GCT CGA GCT TAA GCA TTA GTA CCA GTA TC – 3'

   (b) Find the ORF and indicate its location on your sequence.
   ORF = ATG.....TAA 5 - CTC CTA GGG GCC C *ATG GCT CGA GCT TAA* GCA TTA GTA CCA GTA TC 3

   (c) Translate this cDNA sequence into the Protein sequence corresponding to the ORF. Translate as much as you can.
   5' – AUG GCU CGA GCU UAA – 3'
   Met Ala ArgAla stop

   (d) A new student rotating through a lab attempts to sequence this strand of DNA again using the Sanger method with the proper radioactively labeled primer:

   ←———PRIMER———→
   5'- CTT AAG CTC GAG CCA -3'-OH

   Unsure how to use the dideoxynucleotides the student uses the following mixtures in each separate lane:
   T-lane: dATP, ddTTP, dCTP, dGTP
   G-lane: dATP, dTTP, dCTP, dGTP, ddGTP
   C-lane: dATP, dTTP, dCTP, dGTP
   A-lane: dATP, dTTP, dCTP, dGTP, ddATP (excess)
   The resulting bands where separated by electrophoresis on a gel. Draw what the gel would look like, labeling the four lanes (A, T, G, C). Use therefore the pre-drawn gel in table.
   (In the old exam: 12 points, 3 points each lane)

Anode (-)

A	T	C	G
		full length	full length
			band
			band
			band
band			
			band
			band
			band
	band		

Cathode (+)

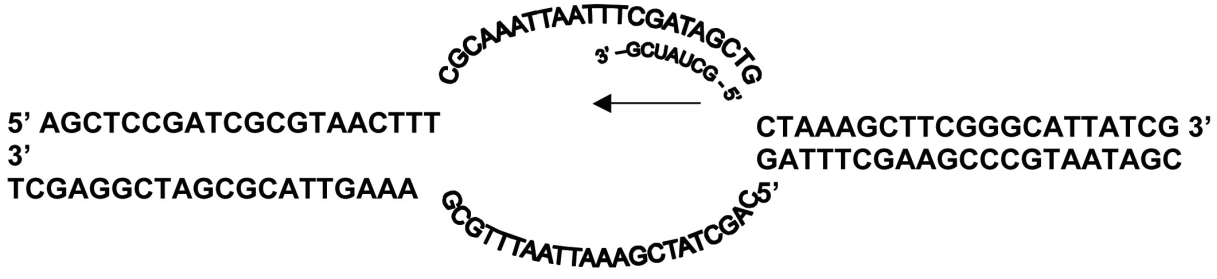
A lane = ATCCCCGGGTACCGAGCTCGAATTC (25bp)
Because ddATP is in excess, the sequence will always terminate after the first A, leaving only 1 band *(3points if in the right position, markings of 1 light and 1 strong band if in right position got also full credit; 1.5 points if only 1 band anywhere)*
T lane = TACCGAGCTCGAATTC (16bp)
Because the only T is ddTTP, the sequenceing will always terminate after the first T, leaving only 1 band *(3 points if in the right position, 1.5 points if indicated anywhere)*
C lane = GAGGATCCCCGGTACCGAGCTCGAATTC (19bp)
Because no dd-nucleotide, the only product seen on the gel will be full length*: 3 points. If someone did not take into account that the primer is labeled and therefore thought there would be no bands: 1.5points.*
G lane = ddGTP is included, but also GTP – this is a normal mix
All right bands (3 points) would have been:
GAGGATCCCCGGGTACCGAGCTCGAATTC (29bp – full length)
GGATCCCCGGGTACCGAGCTCGAATTC (27bp)
GATCCCCGGGTACCGAGCTCGAATTC (26bp)
GGGTACCGAGCTCGAATTC (19bp)
GGTACCGAGCTCGAATTC (18bp)
GTACCGAGCTCGAATTC (17bp)

- *for 2 or more bands anywhere: 1.5 points*
- *for indicating the full length band: 1.5 points*
- *for indicating the full length band plus 2 or more bands on random position: 2.5 points*

10. **Replication Bubble**

(a) Place the following primer on the diagram above: 5'– GCUAUCG –3' and represent the direction of replication by an arrow.

(b) Is the primer made out of DNA or RNA?
RNA



(c) If the replication fork moves to the right, will the primer be used to create the leading strand of replication or the lagging strand? Explain your answer.

Lagging Strand. Since DNA polymerase moves from 5-¿ 3, and the primers 3 end faces the left replication fork, DNA polymerase can only proceed towards the left. Thus, for the case of the replication fork moving to the right, the direction of replication is opposite of the direction of fork movement, which is consistent with lagging strand replication.

(d) If you answered lagging strand, explain why this leads to discontinuous replication. If you answered leading strand, explain how this leads to continuous replication.

In lagging strand synthesis, multiple RNA primers need to be made as the DNA at the replication fork is unwound (because the direction of replication is opposite the direction of DNA unwinding). Thus, DNA polymerase must detach from the old primer and re-attach to the new primer during lagging strand replication. Since the polymerase cannot replicate the strand without detaching, lagging strand synthesis is considered 'discontinuous. This is in contrast to leading strand synthesis in which DNA polymerase attaches to one RNA primer and replicates the entire strand without detaching.

11. ***Saccharomyces cerevisiae***

The size of the Saccharomyces cerevisiae (yeast, the one you use to bake cakes!) genome is about $1.5*10^7$ base pairs. Yeast, which uses 400 replication origins to replicate its 17 chromosomes, takes 30 minutes to complete S phase (replicate its genome). Assume all replicons are the same size, initiate replication at the same time during the cell cycle and are replicated at the same rate.

(a) How many base pairs will be replicated every minute per replicon?

$1.5x10^7$ bp / 30min = 500000 bp / min
500000 bp/ min / 400 replicon = 1250 bp / min /replicon
From each origin of replication replication can start in both directions: 37500 bp / 2 = 18750 bp

(b) How fast does a yeast replication fork move (give your answer in micrometer)?

1250 bp/ min / replicon, but each replicon has two replication forks so, (1250 bp/ min / replicon) x (replicon / 2 replication fork) =625 bp/min/replication fork 625bp/min/fork x 0.34nm/bp x 1 μm/1000nm = 0.2125 μm/min/fork.

(c) Telomeres are special and necessary for the proper replication of eukaryotic chromosomes. Telomeres are not required for the replication of bacterial chromosomes. What important role do telomeres serve and what replaces the function of the telomere in bacteria?

Telomeres protect the ends of chromosomes for its destruction; they compensate for incomplete semiconservative replication at the chromosomal ends. In addition, they protect for homologues recombination and non–homologues end–joining (You will learn the latter next week). Bacteria have mostly circular chromosomes which do not suffer from in–mature replication.