24 pts 1. DNA replication:

(8) a. The DNA of an E. coli dnaA mutant, a residual synthesis mutant, is labled with low radioactivity first at 30 C for 5 generations and then at 43 C for 0.8 generation. It then is labled at 43 C with high radioactivity for 1 more generation. Then it is labled at 30 C for 0.5 generations with low radioactivity, and finally pulselabled with high radioactivity. The DNA is analysed by autoradiography. Assuming intact chromosomes are examined, draw the radioactivity pattern expected for:

bidrectional replication unidirectional replication

See the Soft Reserves handout for figures ...

30 C, 5 gen, low activity: low level label of entire chromosomes
43 C, 0.8 gen, dnaA mutant: residual synthesis, completion of rounds of DNA rep
thus: most chroms finish rep and do not reinitiate; a few have not finished rounds
43 C, 1 gen, high activity: those that had not finished rounds of rep now finish ...
and receive high radioactivity at their Termini
30 C, 0.5 gen: all chroms reinitiate ... and replicate for 0.5 gen ...
30 C, pulse of high activity: all forks are highly radioactive ...

(6) b. All E. coli dna mutants are "conditional lethal" mutants. Why is this the case, and what does "conditional lethal" mean?

dna mutants are in the ESSENTIAL process of DNA replication. Mutants in essential processes are lethal. "Conditional lethal" means lethal under the 'restrictive' condition, e.g. high temperature, where the mutation is expressed, and NOT lethal under the 'permissive' condition, e.g. low temperature, where the mutation is not expressed.

(6) c. Suppose in the above experiment, radioactivity was not used, but rather the mutant was grown in heavy density medium until it was returned to 30 C for 0.5 generations. During this last growth for 0.5 generations, it was grown in light medium. DNA is isolated and analysed in CsCl gradients. Diagram below the expected bands, with correct relative heights, for:

bidrectional replication unidirectional replication

See the Soft Reserves handout for figures ...

Both bi- and uni-directional are the same ...
0.5 generation in light medium after several generations in heavy medium ...
Fragments isolated and banded in CsCl ... half of the parental DNA is converted into hybrid density, with half of the DNA (50%) newly synthesized ...
Remaining half is still heavy density ... thus have 3/2's original DNA, 1/2 is still heavy, 2/2 = 1 is hybrid ... 2:1 ratio of HL:HH DNA for relative peak hts

(4) d. Briefly explain why one sees bands of DNA in a CsCl gradient.

In a CsCl gradient, in which centrifugation is to equilibrium, the CsCl ions form an exponential gradient. The CsCl solution at the bottom has a higher density than the DNA molecules and the DNA "floats" up the tube. The CsCl solution at the top has a lower density than the DNA, and the DNA molecules "sink" down the tube. The DNA thus forms a band where the DNA neither rises nor sinks, i.e. at the CsCl density equal to the density of DNA in CsCl, the DNA buoyant density.

26 pts 2. Semidiscontinuous DNA replication:

(6) a. What 3 properties must a DNA molecule have to be a polymerization substrate for a DNA polymerase?

1) template strand
2) primer strand
3) 3'-OH primer terminus

(6) b. What is meant by "semidiscontinuous DNA replication" and what problem with DNA polymerases does this solve?

"Semidiscontinuous": one daughter DNA strand ("leading" strand) is synthesized continuously, the other DNA strand ("lagging" strand) is synthesized discontinuously.

Problem solved: all known DNA polymerases require only a 3'-OH primer terminus; none can synthesize DNA at the 5'-end of a DNA strand. Yet dsDNA is antiparallel. How then can the daughter DNA strand with its 5'-end in the replication fork be synthesized ????

(6) c. What is the "primer problem" in semidiscontinuous DNA replication and how is it solved?

Primer problem: lagging strand is synthesized via short pieces in 5' -> 3' direction. What can be the "primer" for this synthesis ????

Solution: primer is a short RNA oligonucleotide, synthesized via a DNA-dependent RNA polymerase called "Primase" (DNA dependent RNA polymerases do not require a primer in general).

(4) d. What structural features of the "DNA replicase" solve the problem in bacteria of simultaneous synthesis of leading and lagging DNA strands?

The "DNA replicase" in bacteria has two copies of the CoreDNAPolIII enzyme, that part of HoloPolIII that contains the active site for DNA polymerization. Each of these two copies is used for synthesis at the rep fork, one for the leading strand and one for the lagging strand.
These two copies are adjacent to each other, and the DNA is folded so as to bring the 3'-OH primer termini of the two daughter DNA strands into correct positioning in the two polymerization active sites.

(4) e. What differences in structural features of the "DNA replicase" in eukaryotes are used to solve this same problem (part d.)?

1) Rather than having two copies of the polymerization core enzyme, eukaryotes have two enzymes, Pol-alpha and Pol-delta. Pol-alpha is used for lagging strand synthesis (at least, initially) and Pol-delta is used for leading strand synthesis.
2) Rather than having separate polymerase and primase enzymes, Pol-alpha has both polymerase and primase activities.

12 pts 3. Diagram below a replicating bacterial genome as it would be visualized under the conditions indicated:

as seen in intact bacterium	gently isolated in high salt	gently isolated in low salt	usual isolation in low salt

See Soft Reserves key ...

Intact Bacterium: the highly condensed nuclear region
Gently isolated in high salt: Nucleoids ... Brown, Fig 6.12; Research Brief 6.1
Gently isolated in low salt: Circular DNA with 2 forks ... Brown, Fig 12.6
Usual isolation in low salt: DNA fragments; size depends on amount of shear force

13 pts 4. SINEs

(3) a. What is a SINE?

SINE: Short INterspersed Element - These are genetic elements which are direct repeats of DNA of length about 300 bp, arising from nonviral retrotransposons. They are part of Medium Repetitive DNA, as determined by Cot curves.

(6) b. SINEs are an example of Satellite DNA. Briefly explain what is meant by Satellite DNA, and name a second class of Satellite DNA.

Satellite DNA is DNA which forms a DNA band in CsCl gradients that is at a different buoyant density from that of the major organismal DNA; hence, the band is a "satellite" to the band of the main DNA.

A second class of Satellite DNA: LINEs - Long INterspersed Elements (several kb long).

(4) c. SINEs are an example of Retrotransposons. What is a Retrotransposon?

Retrotransposon: a transposon that transposes (hops) via an RNA intermediate.

24 pts 5. DNA repair:

(12) a. Write down the steps for nucleotide excision used by the E. coli UvrABC system.

1) damage recognition and binding: 2x UvrA + UvrB
2) DNA distortion (bending): UvrA as ATPase
3) Incision: UvrB attaches, nicks 5 bases 3' to damgage; UvrB nicks 8 bases 5' to damage
4) Excision: helicase HelII removes 12-13 mer using ATP for energy; UvrC leaves
5) Repair DNA Synthesis: DNA PolI fills in the 12-mer gap ("Short Patch" repair)
6) Joining: DNA ligase seals the final nick.

(8) b. A "minimal" enzyme system of E. coli PolI plus 2 other enzymes is sufficient for nucleotide excision repair. What are these other 2 enzymes and their function, and what properties of PolI are needed?

The other 2 enzymes: 1) Nicking endonuclease DNase: nicks 5' to the damage, leaving a 3'-OH that functions as a primer terminus for PolI ...
This is also the "damage recognition" enzyme ...
2) DNA ligase, to seal the final nick.

PolI needed activities:
1) 5'->3' polymerization reaction and 2) 5'->3' exonuclease reaction
The two reactions, as a "nick translation" reaction, translate the nick from the endonuclease through the damage, thereby repairing the damage.

(4) c. What different steps are used in base excision repair than in nucleotide excision repair?

1) Base specific Glycosylase removes "bad" base
2) AP nuclease (A-Purinic or A-Pyrimiddinic DNA: base missing) recognizes DNA with missing base, introduces nick 5' to this damage

The remaining steps are the same as for Nucleotide Excision

17 pts 6. Genetic Mobile Elements:

(8) a. Diagram below a typical bacterial Composite Transposon, including all genes and sites.

See Tn10 figure on Web at:
http://www.biology.ucsd.edu/classes/bimm100.FA00/09.MobileElements.html#E2

More Tutorial at www.dumblittledoctor.com

(9) b. Briefly describe 3 ways in which this Transposon can generate mutations.

1) Insertion mutants: Tn hops into a gene ...
2) Deletion mutants: Recombination between two adjacent Tn elements as Direct Repeats generates Deletion of DNA between the two Transposons
3) Inversion mutants: Recombination between two adjacent Tn elements as Inverted Repeats generates Inversion of DNA between the two Transposons

14 pts 7. Homologous Recombination:
(6) a. Define a Heteroduplex region, and explain how such can arise during Homologous Recombination.

Heteroduplex region: region of a dsDNA molecule where one strand came from one source DNA molecule and the other strand came from a different source DNA molecule.

Heteroduplex regions arise during homologous recombination events mainly in the Branch Migration step: DNA between start and stop points of the Branch Migration is Heteroduplex DNA.

(4) b. For a gene A with alleles A and a in such a Heteroduplex region, show how a Gene Conversion segregation ratio of 5:3 could arise.

Gene Conversion arises is meiosis, when one has four chromatids ...
The Heteroduplex region will have one copy each of A and a alleles:
A:A ... A:a ... a:A ... a:a
If one Mismatch is repaired:
A:A ... A:A ... a:A ... a:A
This would now segregate in a 5:3 ratio of A to a ...

(4) c. What is the role of the RecA protein in prokaryotic Homologous Recombination?

RecA does the following:
1) establishes the structure for recombination via coating the DNA molecules, thereby stabilizing the recombination intermediates.
2) executes the Strand Assimilation and Strand Exchange reaction between the two DNA molecules, resulting in a D Loop being formed

20 pts 8. Eukaryotic Genome Anatomy:
(10) a. Briefly describe two similar experiments that indicate how much DNA is found 1) per nucleosome, and 2) between nucleosomes. Give approximate values for each of these quantities of DNA, in bp.

Experiment: 1) purify chromatin in low salt: nucleosomes
2) treat with DNase (micrococcal DNase or pancreatic DNase I)
3) remove histones via no salt, and size DNA on agarose gels

Two experiments:
1) low level of DNase: at most one double-strand break between nucleosomes ...
see "ladder" of DNA bands on gel, sizes multiples of 150-260 bp: DNA between nucleosome
2) high level of DNase: digest all DNA not wrapped around histones in nucleosome ...
see single band on gel, size ~ 145 bp: DNA per nucleosome

(6) b. What is the nucleosome Linking Number Paradox? Briefly describe the nucleosome structural feature that explains this paradox.

Linking Number Paradox: expect 2 supercoils per nucleosome, due to 1.75 "wraps" around histone core ... but experimentally find ONLY 1 supercoil per nucleosome

Reason: DNA wrapped around histones is "stretched" so that it loses one Watson-Crick turn during its 1.75 "wraps" around histone core ...

(4) c. Diagram the structure of chromatin gently isolated in the absence of histones.

This is essentially the same as the prokaryotic nucleoid, except that the electron dense material in the material resembles in shape the two chromatids joined at the centromere ... see Brown, Fig 8.3