

18 pts 6. Genes and Gene Organization:

(4) a. Define a gene.

A Gene is the entire DNA sequence necessary for the synthesis of a polypeptide or RNA molecule. Hence, it includes coding regions plus introns for eukaryotic genes.

Lodish includes transcription control regions in his definition of a gene.

(8) b. Provide the two most significant differences between gene structure in prokaryotes and in eukaryotes.

1. Genes in eukaryotes often have an intron-exon structure, where Introns are sequences in the genome that are absent from the final mRNA and exons are the sequences that get expressed into amino acid sequence in proteins. Prokaryotic genes have no intron-exon structure.
2. Prokaryotic genes are often found in clusters called Operons, where the genes are expressed contiguously (all turned on, or off, simultaneously). Eukaryotic genes are expressed singly (usually), with one mRNA per gene (usually), and are not present in Operons.

(6) c. What are the major DNA sequence features in human DNA that account for the complex Cot curve observed for human DNA (these features are rather similar to those of calf DNA)?

Human Cot curve renaturation kinetics shows three classes of human DNA: [Lodish, p. 317]

1. Highly repetitive DNA: STRs etc ... Simple DNA sequences that renature at low Cot
2. Middle repetitive DNA: UNTRs such as Alu sequences ... renature at middle Cot
3. Unique sequence DNA: DNA sequences present once per genome ... renature at high Cot

18 pts 7. Cloning Vehicles:

(6) a. What are the REQUIRED features of a DNA molecule for it to be a cloning vehicle?

1. An Origin of DNA replication (DNA must be a replicon)
2. A selectable marker, e.g. antibiotic resistance gene (see Lodish, Fig 7-1)
3. At least one cloning site (restriction site) NOT in the origin or selection gene.

(4) b. What features must a YAC cloning vehicle have that are NOT required in a plasmid cloning vehicle?

1. SEPARATE selection markers for BOTH the right and left YAC arms.
2. A CEN marker (centromere sequence) for stable inheritance
3. TELomeric sequences at the ends of BOTH YAC arms, for replication of linear artificial chromosome.

(3) c. What are the major advantages of use of a cosmid as a cloning vehicle?

1. In vitro packaging permits maintenance of cosmids as Lambda virions.
2. In vitro packaging provides strong selection for cloning large 35-45 kb DNA frags
3. Cosmids present as Lambda virions means efficient transfection of host via infection.
4. Cosmid as plasmid means very little of the total DNA packaged in lambda virions need be cloning vehicle DNA.

(5) d. Briefly describe the step in a phage lambda cloning experiment during which the recombinant DNA is actually "cloned".

The recombinant DNA is "cloned" (identical DNA copies are made) during the step when progeny lambda phage are made. This is typically during the step on plates when lambda plaques are made: one plates  $10^8$  bacteria with about 100 phage in "soft agar" on an agar plate. During growth, the bacteria form a "lawn" on the plate everywhere except where the phage repeatedly go through the lytic cycle, yielding progeny phage with cloned DNA, and lysing the bacteria, thereby yielding a Plaque.

35 pts 5. The following sizes are obtained from single and double BamHI and PstI R. enzyme digests of a 1700 bp EcoRI fragment cloned into the MCS of plasmid cloning vehicle pBS:

BamHI: 4200, 600, 400 bp PstI: 3700, 1000, 300, 200 bp

Double digest sizes (-> means "yields", to indicate double digest fragments from single digest frags):

from BamHI frags: 4200 -> 3700, 500 bp; 600 -> 200, 300, 100 bp; 400 -> 400 bp

from PstI frags: 3700 -> 3700 bp; 1000 -> 500, 400, 100 bp; 300 -> 300 bp; 200 -> 200 bp

The order of restriction sites in the MCS is: - PstI - BamHI - EcoRI -

(2) a. What is the size of this recombinant DNA plasmid?

From BamHI frags: size = 4200 + 600 + 400 = 5200 bp

From PstI frags: size = 3700 + 1000 + 300 + 200 = 5200 bp, in agreement

(2) b. What is the size of the cloning vehicle pBS?

Size of cloned EcoRI fragment = 1700 bp

Size of cloning vehicle pBS is then: 5200 - 1700 = 3500 bp

(15) c. Determine a BamHI and PstI restriction map for the recombinant DNA plasmid from the above data.

See Figure in Soft Reserves copy of Key

(5) d. Is the restriction map a unique map or not? If not, why not?

The restriction map is NOT unique.

Both the position and the orientation of the 200 bp and the 300 bp PstI fragments are not uniquely determined: positions can be either 200-300-100 or 300-200-100 within the 600 bp BamHI fragment.

(3) f. What important advantage does restriction mapping by partial digestion have over restriction mapping by complete digestion?

Restriction mapping by partial digestion immediately provides position of each complete fragment on the map relative to every other complete fragment.

For example, a partial PstI mapping for the above DNA would give information on the position of the 200 bp PstI frag relative to the 300 bp fragment.

(2) g. What is the ultimate physical map of a DNA molecule?

The nucleotide sequence.

(6) h. In the Burke-Olson YAC paper, partial digestion restriction mapping of the BamHI sites of YAC YY1 was done. Briefly describe how this experiment was done, including results obtained.

1. YAC YY1 was digested with several concentrations of BamHI in separate aliquots.
2. DNA from each reaction was run in a separate lane of a pulse-field gradient gel.
3. The DNA was denatured and transferred to a nitrocellulose filter via capillary action ala Southern gel analysis.
4. The transferred DNA was "probed" via hybridization to a P-32 labeled probe from one end or the other of the YAC vector (left end results are shown in the paper, Fig. 5)
5. The P-32 is visualized via autoradiography.
6. The positions of the BamHI R.sites in YY1 are determined from the positions of the P-32 bands seen in the lanes in the gel.

- (3) f. What are the L, T, and W values of the DNA AFTER treatment with both enzymes?

6000 bp =>  $T = 6000 / 10 = 600$  For 10.5 bp/turn:  $T = 6000 / 10.5 = 571$

normal cccDNA =>  $W/T = -0.05$

$W = -0.05 \times 600 = -30$

$L = T + W = 600 - 30 = 570$

$W = -0.05 \times 571 = -28$

$L = T + W = 571 - 28 = 543$

**18 pts 3. Nucleosomes:**

- (4) a. What are the supposed two main functions of histone H1 in chromatin structure?

1. H1 functions in the Nucleosome to "lock" the DNA to the Nucleosome core as the DNA wraps around the outside of the nucleosome core. (see Lodish, Fig 9-50a)
2. H1 functions in formation and structure of the 30 nm fiber with the H1 histones interacting with each other to form the core of the solenoid. (Lodish, Fig 9-50 b)

- (4) b. Define the packing ratio. What is the approximate packing ratio for metaphase chromosomes?

Packing Ratio (PR): ratio of DNA length in condensed state to that of DNA in free state

Packing Ratio for metaphase chromosomes is 1000 - 8000.

- (4) c. What are the histones present in a nucleosome, and how many copies of each?

$(H2A - H2B)_2 (H3 - H4)_2$

or 8 histones, 2 copies each of H2A, H2B, H3, H4; H2A-H2B and H3-H4 each as 4 pairs

Also: 1 copy of H1 ... but H1 is not always considered part of the core nucleosome

- (6) d. What is the superhelical content of the DNA in a nucleosome and briefly how was this determined?

Superhelical content is approximately 1 negative supercoil per nucleosome.

Experiment:

1. Isolate viral SV40 DNA as chromatin and use as substrate
2. Treat with Topoisomerase I to remove supercoils NOT associated with nucleosomes.
3. Deplete the chromatin of histones, to disassociate the nucleosomes.
4. Determine supercoils now present...these came from the disassociated nucleosomes.
5. Use this number of supercoils and the number of nucleosomes per SV40 chromatin molecule (determined by others) to calculate the Number of supercoils per nucleosome.

**12 pts 4. Restriction Fragment Length Polymorphisms (RFLPs):**

- (4) a. Briefly explain what an RFLP is.

An RFLP is a variation among individuals in a population of the length of a restriction fragment that comes from identical parts of the genome.

RFLPs arise from mutations in a restriction site. Such mutations result in "alleles" at this chromosome position, such that some individuals in the population will have the R.site (one allele) or have a mutated R.site (a second allele), as seen in RFLPs.

- (4) b. In using an RFLP locus in a genetics experiment, what is the "phenotype" that is used?

The "phenotype" is a methodology to measure the length of the restriction fragment in question. This methodology is usually to isolate the DNA, restrict it, and run it on an agarose gel, using EtBr fluorescence to determine lengths of the R.fragments. (see Lodish, Fig. 8-22)

Note how this "phenotype" differs from the usual genetic phenotype of an organism ..

- (4) c. Why has use of RFLP loci been of such great value in human genetics?

Before RFLPs, human genetics was limited to relatively few naturally occurring genetic disease and other traits with obvious phenotypes; one can not "cross" human individuals.

RFLPs occur naturally very often in human DNA. They provide a very large source of new genetic markers for human genetics and for getting a high resolution human genetic map. These are used for mapping new loci whose map positions are unknown.

20 pts 1. Draw the structure of an A:U base pair from an RNA molecule. Label the parts of each molecule and label the 5' and 3' ends.

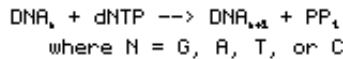
See Lodish, Figs 4-2, 4-3, 4-4, 4-5, 4-6, and 4-7  
Also see Figure in Soft Reserves copy of Key

19 pts 2. The DNA shown, an open-circular 6000 bp dsDNA with a gap, is treated with E. coli DNA polymerase I (PolI).

(3) a. Label the DNA to indicate the 3 properties this DNA must have to be a substrate for the PolI polymerization reaction.

3 properties: Template strand, Primer strand, 3-OH Primer Terminus  
See Figure in Soft Reserves copy of Key

(4) b. Write down the basic polymerization reaction catalyzed by PolI.



(3) c. Assume this DNA is a substrate for PolI and that the polymerization reaction goes as far as it can go. Draw the final product DNA, using a squiggly line for the newly synthesized DNA.

See Figure in Soft Reserves copy of Key

"Klenow fragment" is a mutant E. coli DNA polymerase I that lacks the 5'→3' exonuclease reaction.  
(3) d. Draw the final product DNA, using a squiggly line for the newly synthesized DNA, assuming that the original gapped ocDNA is treated with Klenow fragment.

See Figure in Soft Reserves copy of Key

This product DNA is now converted into normal supercoiled DNA by treatment with DNA ligase and DNA gyrase, the E. coli topoisomerase II, in the presence of ATP.

(3) e. What are the L, T, and W values of the ocDNA BEFORE treatment with ligase and gyrase?

$$\begin{aligned} 6000 \text{ bp} &\Rightarrow T = 6000 / 10 = 600 & \text{For } 10.5 \text{ bp/turn: } T &= 6000 / 10.5 = 571 \\ \text{ocDNA} &\Rightarrow W = 0 & W &= 0 \\ L = T + W &\Rightarrow L = 600 & L &= 571 + 0 = 571 \end{aligned}$$