

Study material.

This study guide covers material for the second exam: "Methods in molecular biology and the mechanics of replicating, repairing and copying genetic information," material from lectures 7-12 on the syllabus, Sept. 23 through Oct. 23.

If you have questions, please feel free to send email to me (smount at umd.edu).

I may be "releasing" edited versions of this in response to feedback. This is version 1 (10-21-08).

Be able to define, discuss, and explain the following:

molecular clone	host (for cloning)	vector (for cloning)
transformation	transduction	conjugation
F factor	Hfr	F'
lysogen	temperate bacteriophage	site-specific recombination
prophage	complexity (of DNA)	C ₀ t
PCR	cDNA	photolithography
preimplantation genetic diagnosis	polymorphism	SNP
RFLP	CAPs	allele-specific primer
microsatellite	CODIS	STR loci
contig	universal primer	
dideoxynucleotides	shotgun sequencing	raw sequence
coverage	DNA footprinting	EMSA
pseudogenes	LINES	SINES
satellite DNA	L1	Alu
retrotransposons	retroviruslike transposable elements	
target site duplications	Terminal inverted repeats.	provirus
homolog	ortholog	paralog
deamination	depurination	thymine dimer
direct repair	mismatch repair	AP endonuclease
topoisomerase II	base excision repair	nucleotide excision repair
recA	recBCD	Holliday junction
rho factor	sigma factor	core polymerase
consensus sequence	RNA polymerases I, II, III and IV.	
polycistronic	monocistronic	antitermination
operon	attenuation	TBP
helix-turn-helix motif	recognition helix	CTD
core promoter	enhancer	silencer
general transcription factors	preinitiation complex	zinc finger
YAC	BAC	

Review questions and suggested subjects for review.

Be sure that you understand the yeast two-hybrid assay for protein-protein interactions, both as a technique and as an illustration of principles behind transcriptional activation (see lecture 12).

1. Proteins with the helix-turn-helix motif interact with DNA using (pick one)
 - a) hydrogen bonds between amino acids in an alpha helix and bases in the minor groove of DNA
 - b) hydrogen bonds between amino acids in a beta sheet and bases in the minor groove of DNA
 - c) hydrogen bonds between amino acids in an alpha helix and bases in the major groove of DNA
 - d) hydrogen bonds between amino acids in a beta sheet and bases in the major groove of DNA
 - e) covalent bonds between amino acids in an alpha helix and bases in the minor groove of DNA
 - f) covalent bonds between amino acids in a beta sheet and bases in the minor groove of DNA
 - g) covalent bonds between amino acids in an alpha helix and bases in the major groove of DNA
 - h) covalent bonds between amino acids in a beta sheet and bases in the major groove of DNA

2. Transcription of a class II gene (a gene transcribed by RNA polymerase II) starts at a G 30 bp downstream of the first T in the TATA box. A deletion of 10 bp between the G and the TATA box would result in transcription starting where?

3. Be able to distinguish between natural and artificial transformation of bacteria.

4. Be able to describe each of the following vectors for molecular cloning (advantages, disadvantages, host, approximate size range for inserts, etc.): plasmids, lambda phage, BAC and YAC.

5. Be able to define compare and contrast microsatellites and SNPs as markers in human genetics, including information about frequency and degree of polymorphism.

6. Be able to select primers appropriate for the construction of mutations using PCR-based approaches.

7. For each of the polymerases we've discussed (DNA polymerase, E. coli RNA polymerase, each of the three eukaryotic RNA polymerases, primase, telomerase, reverse transcriptase) be able to state whether the product is DNA or RNA, whether the preferred template is DNA or RNA and whether the polymerase is primer-dependent).

8. There are several examples of genetic events which result from double-stranded breaks initiated by regulated endonuclease cleavage (as opposed to random DNA damage). Name at least two.

9. A species of bacteria has two chromosomes, A and B. A is 16 Mb. and B is 800 kb. The copy number of chromosome A is 2, meaning that on average, each cell has two of this chromosome. The copy number of chromosome B is 1, meaning that on average, each cell has one of this chromosome. You isolate DNA from the bacterium, shear the DNA to an average size of 10 kb. and denature the DNA by heating. DNA from which chromosome will renature first, and by what factor (give me the ratio of the time it takes for each DNA to reach 50% double-stranded)?

10. Multiplex PCR is accomplished by adding multiple primer pairs to a single reaction so that multiple PCR products are generated simultaneously. What are the practical and theoretical limitations on multiplex PCR? What is the (approximate) maximum number of primer pairs that would work under standard PCR conditions? (standard PCR conditions would be a 50 μ l. reaction with cycles of 45 seconds at 95°C, 45 seconds at 65°C and 1 minute at 72 °C; total concentration of all primers at 0.6 μ M).

Why?

11. Be able to explain the differences between various sequencing methods (Maxim/Gilbert, Sanger, 454/Roche, Solexa/Illumina).

12. AP endonuclease would be expected to act in which of the following repair events?

- a) depurination
- b) G:T mismatch
- c) deamination of cytosine
- d) double-strand break

What other enzymes function in other repair events?

13. Why are CG dinucleotides (the two nucleotide sequence with C 5' to G) rare in the human genome?

14. Which technique for detecting protein-DNA interaction (electrophoretic mobility shift assay or footprinting) requires that all (or nearly all) of the DNA present be bound by protein? Which technique generally involves an excess of DNA rather than protein? What would happen to a DNA footprinting reaction if the protein was not in excess?

15. Examine Figs. 9.20, 22.10 and 22.17. All of the genes shown are homologs. For each of the following pairs, state whether the term ortholog or paralog is most appropriate:

- a) human beta globin and mouse alpha globin
- b) human beta globin and mouse beta globin
- c) human delta globin and mouse beta globin
- d) human beta globin and mouse myoglobin
- e) human beta globin and human delta globin
- f) human beta globin and human myoglobin