Spring 2017

BIOL241 – Introduction to Genetics and Molecular Biology

Midterm III (105 points)

4/4/2017

Name:_____

Purdue ID:_____

Question A:_____(38)	Question B:_____(7)
Question C:_____(4)	Question D:_____(4)
Question E:_____(5)	Question F:_____(12)
Question G:_____(5)	Question H:_____(10)
Question I:_____(10)	Question J:_____(10)

Total: _____

A. Multiple-choice questions (38 points, 2 points each, please use a scantron sheet for part A).

1) In eukaryotic gene regulation, which of the following events is likely to be associated with an increase in gene transcription?

a. Methylation of cytosines in CG islands near a transcription unit.
b. Deacetylation of lysines in histone tails near a transcription unit.
c. Acetylation of lysines histone tails near a transcription unit.
d. The expression of microRNAs with sequences complementary to the promoter regions.
e. The binding the transcription factors to silencer elements.

2) Regarding the helix-turn-helix motif, which of the following statements is correct?

a. This motif allows proteins to repair specific DNA damages.
b. This motif allows microRNAs to bind to their targets.
c. This motif allows RNA-binding proteins to regulate alternative splicing.
d. This motif allows proteins to be imported into the nucleus.
e. This motif allows proteins to bind to specific regulatory DNA sequences.

3) After analyzing the sequence of a gene, you have found that it contains leucine zippers. Based on this, which of the following statements is correct?

a. This gene encodes a microRNA.
b. This gene encodes a protein that forms dimers.
c. The gene encodes a protein that binds to RNA.
d. This domain allows the protein to bind to specific DNA sequences.
e. This domain allows the protein to cleave dsRNA into smaller pieces.

4) The *Drosophila DSCAM* locus is capable of expressing multiple isoforms. This is accomplished by:

a. Duplication of the DSCAM gene onto different chromosomes.
b. Transcription initiation from alternative promoters.
c. Translation inhibition by small non-coding RNA.
d. The usage of alternative transcription termination sites.
e. Alternative splicing.

5) Regarding siRNA, which of the following statements is incorrect?

a. Dicer cleaves injected-dsRNA into short 21 base pairs of dsRNA.
b. siRNA usually binds to target mRNA at specific sites in the 3' UTR.
c. siRNA is typically perfectly complementary to target mRNA.
d. siRNA typically negatively regulates gene expression.
e. siRNA mediates gene silencing by degrading target mRNA.

6) The Luria-Delbrück fluctuation test demonstrated that:

a. Spontaneous mutations are random.
b. Certain chemicals, after processed by liver enzymes, are mutagenic.
c. Transcription factors bind to DNA at specific sequences.
d. Nucleosomes in eukaryotic chromosomes can be remodeled.
e. Certain proteins are capable of interacting with each other in vivo.

7) Regarding xeroderma pigmentosum (XP), which of the following statements is incorrect?

a. XP is caused by mutations in the components of nucleotide excision repair pathway.
b. XP mutations are typically autosomal recessive.
c. Individuals with XP are sensitive to UV light.
d. Individuals with XP are often freckled and have high disposition for skin cancers.
e. XP is caused by mutation in the components of mismatch repair pathway.

8) Which of the following events is most likely to cause translocations?

a. Tautomeric shifts.
b. Treating cells with X rays.
c. Depurinations.
d. Treating cells with base analogs.
e. Treating cells with intercalating agents.

9) Sequencing the ends of clones from cDNA library is known as?

a. Sanger sequencing.
b. Maxam-Gilbert sequencing.
c. Map-based sequencing.
d. Expressed sequence tag sequencing.
e. Shotgun sequencing.

10) Bisulfite sequencing is typically used to determine:

a. The methylation state of histone proteins in eukaryotic chromosomes.
b. The DNA sequence of whole eukaryotic genomes.
c. The acetylation state of histone proteins in c eukaryotic chromosomes.
d. The methylation state of DNA in eukaryotic chromosomes.
e. This DNA sequence of actively transcribed genes in eukaryotic genomes.

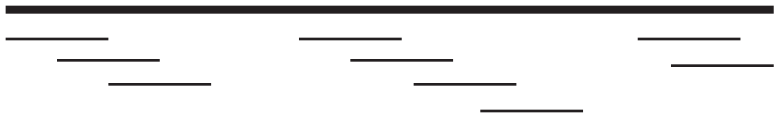
11) Which of the following techniques can detect the difference between two homologous chromosomes?

a. Chromatin immunoprecipitation.
b. Screening cDNA libraries.
c. Chromosome walking.
d. Restriction fragment length polymorphism.
e. Screening genomic libraries.

12) The ChIP (chromatin immunoprecipitation) assays will identify:

a. The differences in mRNA abundances of different genes between cells at two different states.
b. The differences in restriction sites between two homologous chromosomes.
c. The protein-protein interactions.
d. The sequences of transcription enhancer elements.
e. The sequences of intron/exon boundaries of different genes

13) You are entrusted with the task of sequencing the genome of a new species. After screening through a genomic library (made from this species) and analyzing the clones by map-based approach, you have assigned 9 clones (represented by thin lines in the figure below) to a particular chromosome (represented by the thick line). After compiling the sequences from all 9 clones, you should establish:



a. 1 contig.
b. 2 contigs
c. 3 contigs.
d. 4 contigs.
e. 9 contigs.

14) The dideoxynucleotides (ddNTP) differ from dNTP in:

a. The absence of nitrogen base at the 1' position.
b. The absence of triphosphate at the 5' position.
c. The presence of diphosphate at the 5' position.
d. The absence of hydroxyl group at the 2' position.
e. The absence of hydroxyl group at the 3' position.

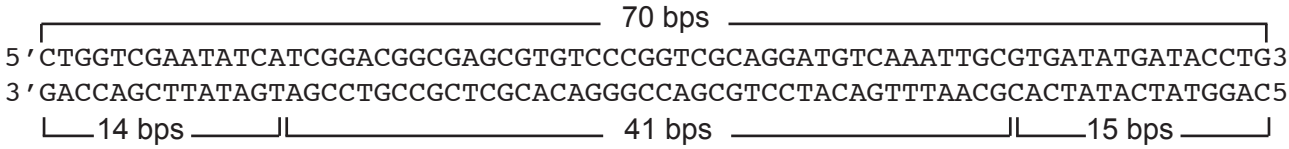
15) Which of the following statements accurately describes the advantage of using RNAi in a genetic screen?

a. RNAi completely inhibits the expression of targeted genes.
b. The identification of genes affected by RNAi is instantaneous.
c. RNAi can only be used in haploid cells.
d. RNAi does not cause off-target effects.
e. RNAi can only be used in diploid cells.

16) Which of the following approaches can be used to introduce specific changes in *Drosophila* genome?

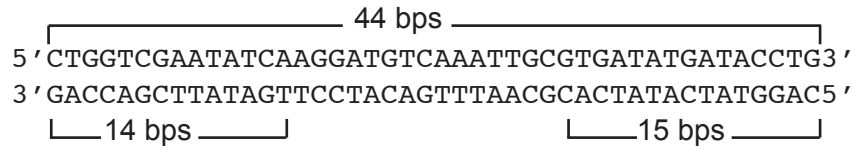
a. Feeding male flies with EMS.
b. Irradiating male flies with X rays.
c. Mobilizing P element transposons by expressing functional transposase.
d. Injecting embryos with specific gRNA and Cas9 nuclease.
e. Injecting embryos with dsRNA.

17) The following linear DNA template needs to be amplified using PCR, which of the following primer pairs should be used?

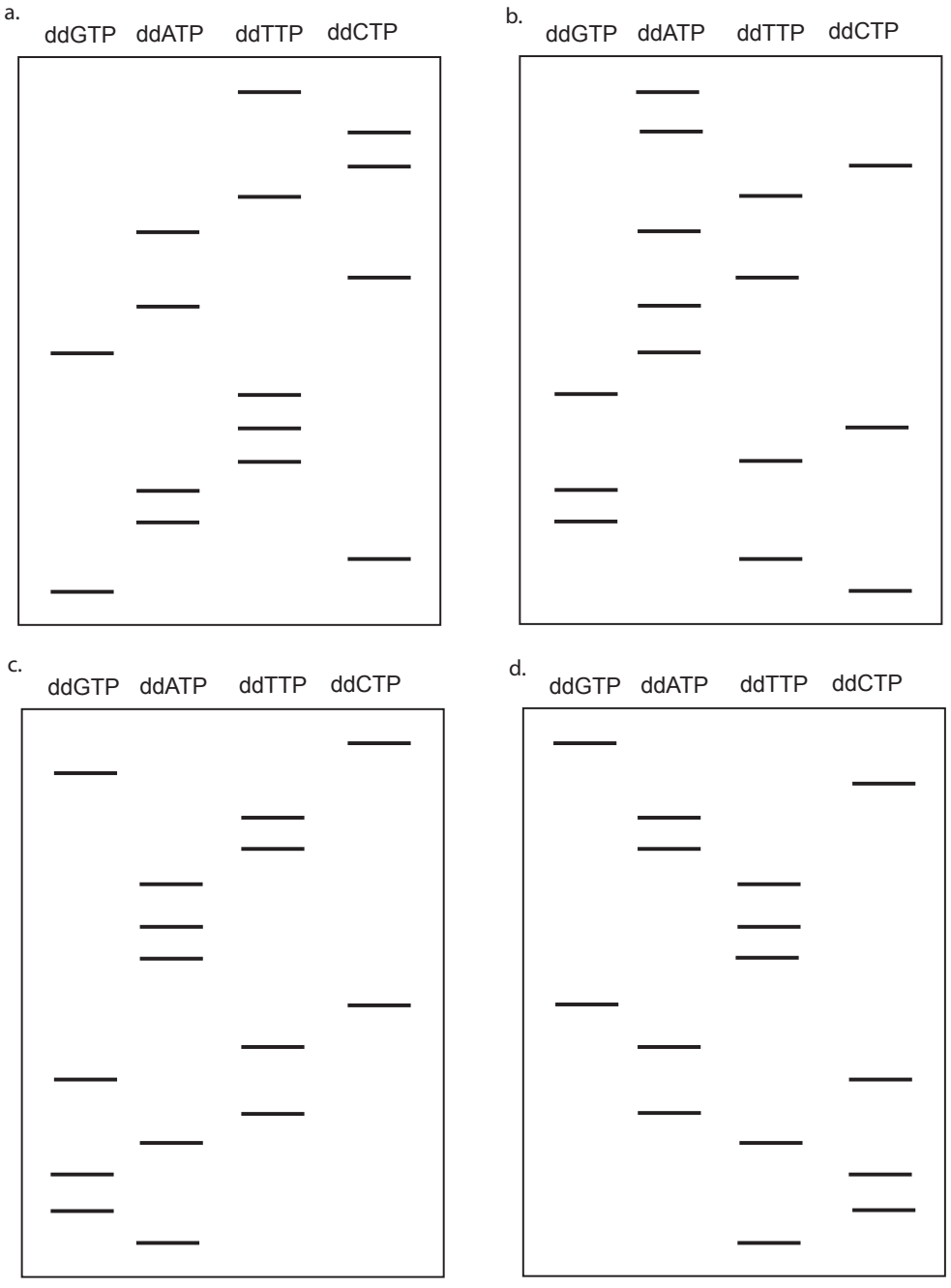


a. 5'-CTGGTCGAATATCA-3' and 5'-GTCCATAGTATAGTG-3'
b. 5'-CTGGTCGAATATCA-3' and 5'-CAGGTATCATATCAC-3'
c. 5'-CTGGTCGAATATCA-3' and 5'-GTGATATGATACCTG-3'
d. 5'-GACCAGCTTATAGT-3' and 5'-GTCCATAGTATAGTG-3'
e. 5'-GACCAGCTTATAGT-3' and 5'-CAGGTATCATATCAC-3'

18) The following linear DNA template was analyzed by Sanger's sequencing method using a primer 5'-CTGGTCGAATATCA-3'.



Which of the following autoradiograms would represent the result of this sequencing reaction?



19) To generate mouse mutants by homologous recombination, researchers often make a targeting construct, which contains a flanking thymidine kinase gene. The purpose of this thymidine kinase gene is to:

a. Serve as a marker to selecting ES cells with constructs integrated by homologous recombination.
b. Increase the efficacy of construct integration into the embryonic stem (ES) cell genome.
c. Increase the likelihood that transfected ES cells become germ cells when injected into blastocyst.
d. Serve as a selectable marker for the presence of the targeting construct in the ES cell genome.
e. Mediate the transposition of the targeting construct when it is integrated into the ES cell genome.

B) Based on our discussion of GAL regulation in yeast, complete the table below describing GAL7 expression in various genotypes under different conditions ("+" for presence of GAL7 expression; "-" for absence of GAL7 expression) **(4 points).**

Genotype	GAL7 expression galactose present	galactose absent
$GAL4^{+}$ $GAL80^{+}$		
$GAL4^{-}$ $GAL80^{+}$		
$GAL4^{+}$ $GAL80^{-}$		
$GAL4^{-}$ $GAL80^{-}$		

If a mobility shift assay was performed using DNA containing UAS and purified GAL4 protein, predict the expected results in lanes 3 and 4 in the panel below **(3 points).**

	1	2	3	4
GAL4 protein:	-	+	+	-
Galactose:	-	+	-	+
UAS-containing DNA:	+	+	+	+

C) Based on the lectures, please complete the following table (4 points):

Type of mutagen	Example of the mutagen	Genetic lesion(s) induced
	γ (gamma) ray	
Alkylating agent		
		Insertion/deletion
	5-bromo-uracil	

D) Based on the lectures, please complete the following table (4 points):

Type of genetic lesion	Repair system	Specific component of the system
		dam methylase
		UvrABC
		Uracil DNA glycosylase
Depurination		

E) From screens for *C. elegans* cell death mutants, loss-of-function (lof) and gain-of-function (gof) mutations in *egl-1*, *ced-4*, and *ced-9* were isolated. The function of *egl*-1 and *ced-4* is to promote apoptosis (programmed cell death), whereas the function of *ced-9* is to inhibit cell death.

a. Based on the above description, what is the phenotypes (no cell death or excessive cell deaths) of **(3 points**):

   Goss-of-function mutations in *egl-1* (*egl-1*^{gof}):

   Loss-of-function mutations in *ced-9* (*ced-9*^{lof}):

b. To order these genes along a pathway, you examined the phenotypes of double mutants and found that –

   ced-9^{lof}*, egl-1*^{lof} double mutants contain excessive cell deaths.

   ced-9^{lof}*, ced-4*^{lof} double mutants contain no cell death.

   Based on these observations, please order the function of these genes (from upstream to downstream) (**2 points**).

F) As discussed in class, *lin-14* and *lin-4* are two *C. elegans* genes that regulate the timing of developmental stages. In *lin-14*$^{-}$ mutants, the embryos skip the L1 stage. In *lin-14*^{gof} (gain-of-function) mutants, the embryos repeat the L1 stage. The lin-14 proteins are present in L1 embryonic stage, but absent in subsequent larval stages (see the table below).

a. What is the nature of lin-4 gene product (**1.5 points**)?

b. How does lin-4 regulate lin-14 expression (**1.5 points**)?

c. If an epistasis analysis is performed using *lin-4* and *lin-14* loss-of-function mutations, which will be the epistatic gene (**1 point**)?

d. If a "sponge" for lin-4 (a synthetic mRNA with multiple binding sites for lin-4) is over-expressed in wild type *C. elegans*, how would this construct affect the developmental timing (skip L1 stage or repeat L1 stage, **1 point**)? Explain briefly how this "sponge" construct would generate the observed phenotype (**2 points**)?

e. Based on our discussion in class, please complete the table below ("+" for present and "-" for absent) (**5 points**).

	In wild type embryo (L1 stage)	In wild type larvae (L4 stage)	In *lin-4*$^{-}$ embryo	In *lin-4*$^{-}$ larvae	In wild type embryo + siRNA against lin-14	In wild type larvae + siRNA against lin-14
lin-14 mRNA						
lin-14 protein	**+**	**-**				

G) The genome size of *Bacillus subtilis* is 4.2 mb (10^6 bases). If *B. subtilis* genomic DNA is digested with BamHI (recognizes GGATCC), how many restriction fragments will likely be generated (**2.5 points**)?

If a *B. subtilis* genomic DNA library was constructed using plasmids, which are capable of carrying DNA inserts of 15kb (10^3 bases) 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 **(2.5 points**)?

H) Identify the protein(s) responsible for each of the following reactions **(10 points).**

a. This complex uses energy from ATP hydrolysis to alter chromatin conformation.

b. This complex represses transcription by removing acetyl groups from the histone tails in the nucleosome core.

c. This *E. coli* enzyme can use blue light energy to directly repair T^T dimers.

d. This *E. coli* enzyme can directly repair damaged DNA bases caused by ethylmethane sulfonate

e. This complex incorporates one of the strand from 21bps dsRNA and facilitates its binding to target mRNA.

I) Identify the technique(s) we have covered in class that can address the following questions **(10 points).**

a. To assess mutagenicity of different chemicals.

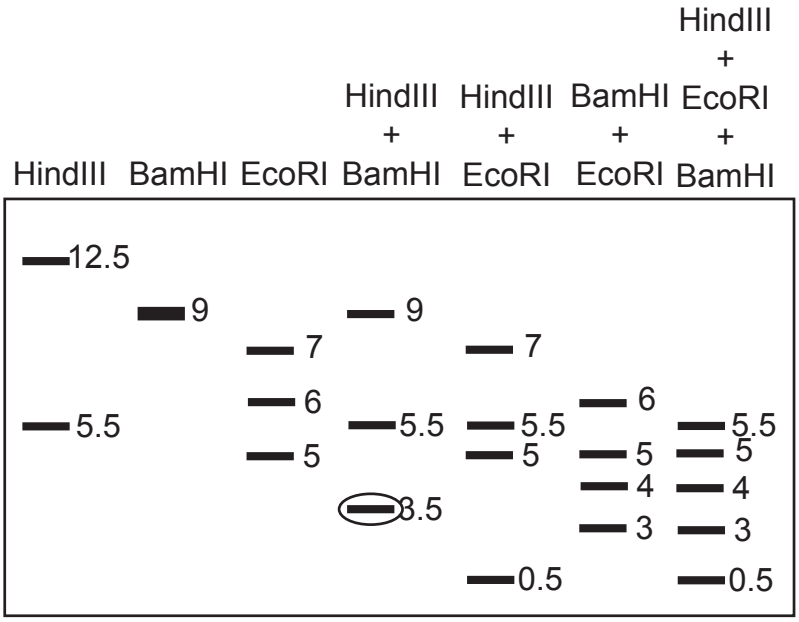
b. To identify genes based on their distributions in specific tissues.

c. To identify genes based on their abilities to physically interact with bait proteins.

d. To determine whether two mutants with similar phenotypes are allelic.

e. To perform a genome-wide comparison of mRNA levels between cells at two different states.

J) Digestion of a 18kb linear genomic DNA with restriction enzymes yields the following fragments (sizes indicated in kb). Please draw a restriction map for this plasmid, and indicate the location of the restriction sites and the distance between them (**4 points**).



If the 3.5kb fragment from the HindIII+BamHI digest (circled above) 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; **6 points)**?