**Spring 2018**

**BIOL241 – Introduction to Genetics**

**and Molecular Biology**

**Midterm III (105 points)**

**4/3/2018**

**Name:\_\_\_\_\_\_\_\_\_\_\_\_KEY\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Purdue ID:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

Question A: (52) Question B: (6)

Question C: (7) Question D: (7)

Question E: (16) Question F: (6)

Question G: (11)

**Total:**

**A. Multiple-choice questions (52 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 a decrease in gene transcription?**

1. Methylation of cytosines in CG islands near a transcription unit.
2. Acetylation of lysine residues in histone tails near a transcription unit.
3. The binding of TBP to TATA box
4. The phosphorylation of C-terminal tail of RNA polymerase by TFIIH.
5. The binding the transcription factors to enhancer elements.

**2) Regarding the zinc finger motif, which of the following statements is correct?**

1. This motif is characterized by two alpha helices joined by a short linker.
2. This motif is found in microRNAs, allowing them to bind to targets.
3. This motif is typically found in RNA-binding proteins, allowing them to regulate alternative splicing.
4. This motif is typically found in transcription factors, allowing them to be imported into the nucleus.
5. This motif is found in some transcription factors, allowing them to bind to specific regulatory DNA sequences.

**3) While analyzing the function of an unknown eukaryotic gene, you noticed that it contains leucine zippers. Based on this information, this gene is likely to:**

1. Code for a microRNA.
2. Regulate alternative splicing.
3. Code for a protein that forms dimers.
4. Code for a protein that binds to translational regulatory elements.
5. Code for a protein that repairs DNA.

**4) Bisulfite sequencing is typically used to determine:**

1. The methylation state of histone proteins in eukaryotic chromosomes.
2. The genomic DNA sequence of different species.
3. The acetylation state of histone proteins in eukaryotic chromosomes.
4. The DNA methylation state near promoter regions in eukaryotic genomes.
5. The relative abundance of different transcripts in eukaryotic genomes.

**5) Which of the following statements accurately describes the basis for bisulfite sequencing?**

1. Dideoxynucleotides are used to terminate DNA elongation.
2. Antibodies staining are used to detect modified histone tails.
3. Cytosines methylated at the 5’ position are resistant to bisulfite-dependent modification.
4. Promoter regions bound by transcription factors are protected from DNaseI-dependent cleavage.
5. Cytosines in GATC are methylated by specific methylases.

**6) The Ames test is a convenient assay capable of demonstrating that:**

1. Spontaneous mutations allowing bacteria to become antibiotic-resistant are random.
2. Certain chemicals, after processed by liver enzymes, are mutagenic.
3. Certain transcription factors bind to DNA at specific sequences.
4. Nucleosomes in eukaryotic chromosomes can be remodeled.
5. Certain proteins are capable of interacting with each other in vivo.

**7) Functional *transformer* (*tra*) proteins are made in *Drosophila* females, but not in males. This is accomplished by:**

1. The binding of female-specific Dsx (double sex) transcription activator to enhancers near the Tra promoter.
2. The binding of male-specific Dsx transcription repressor to silencer elements near the Tra promoter.
3. The binding of Sxl protein to tra mRNA in females to regulate alternative splicing.
4. The binding of Sxl protein to tra mRNA in males to regulate alternative splicing
5. The binding of Sxl mcroRNA to Tra mRNA in males.

**8) In RNA-mediated gene silencing, the dicer gene product can:**

1. Export the microRNA from the nucleus into the cytoplasm.
2. Bind to the dsRNA directly and form RISC complex.
3. Bind to target mRNA directly and facilitate its degradation.
4. Bind to target mRNA at the 3’ UTR directly and inhibits its translation.
5. Cleave injected siRNA into 21 bps dsRNA.

**9) 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:**

1. 1 contig.
2. 2 contigs
3. 3 contigs.
4. 4 contigs.
5. 9 contigs.

**10) A microarray (gene-chip) experiment is useful for:**

1. Detecting restriction fragment length polymorphism between two homologous chromosomes.
2. Finding homologous genes from related species.
3. Detecting phosphorylation targets of a particular kinase.
4. Determining the possible alternative sliced isoforms from a particular gene.
5. Comparing the level of mRNA of different genes between cells at two different states.

**11) Which of the following statements best describes how expressed sequence tags (ESTs) are generated?**

1. Sequencing the entire genomic DNA clones.
2. Sequencing the entire cDNA clones.
3. Sequencing the ends of genomic DNA clones.
4. Sequencing the ends of cDNA clones.
5. Sequencing alternatively spliced cDNA clones.

**12) Which of the following statements best describes the ChIP (chromatin immunoprecipitation) assays?**

1. Using antibody to precipitate histones that are acetylated.
2. Using antibody to precipitate histones that are methylated.
3. Using DNaseI to detect chromatin regions that have open conformations.
4. Using antibody to precipitate DNA fragments bound to specific transcription factors.
5. Using antibody to precipitate remodeling complexes.

**13) Comparing genomes from various prokaryotic species, which of the following statements is correct?**

1. Prokaryotic organisms contain mostly linear chromosomes.
2. Different prokaryotic organisms contain similar amount of genomic DNA.
3. Genes in prokaryotic organisms are always expressed as single-gene mRNAs.
4. Genes in prokaryotic organisms contain introns.
5. Expression of prokaryotic genes is often regulated as polycistronic mRNAs.

**14) The genome size of *Staphylococcus aureus*, a gram-positive bacterium, is 2.88mb (106 bases). Based on the comparison of prokaryotic genomes, the number of genes in *Staphylococcus aureus* is expected to be:**

1. 28000.
2. 14000.
3. 4000.
4. 2800.
5. 1400.

**15) Horizontal gene transfer is likely facilitated by:**

1. The transformation by DNA from closely related species.
2. Transposon-mediated DNA movement from one chromosome to a non-homologous chromosome.
3. The transfer of DNA from agarose gel onto nitrocellulose membrane.
4. The detection of clones containing specific DNA on nitrocellulose membrane with radioactively labeled probes.
5. The mating between F+ and F- bacterial strains.

**16) 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:**

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

**17) You are asked to sequence a DNA template using Sanger’s method. The sequence of the template is:**

5’- GTCGAGTCACGCGGACCTGGTCTCGAACATTGTCCGAATTGCCGATCGGATC -3’

3’- CAGCTCAGTGCGCCTGGACCAGAGCTTGTAACAGGCTTAACGGCTAGCCTAG -5’

**The result is shown in the gel below.**

**Which the following could be a primer for this reaction?**

1. 5’- GTCGAGTCACGC -3’
2. 5’- GCCGATCGGATC -3’
3. 5’- GATCCGATCGGC -3’
4. 5’- CAGCTCAGTGCG -3’
5. 5’- CTAGGCTAGCCG -3’

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

****

1. 5’-CTGGTCGAATATCA-3’ and 5’-GTCCATAGTATAGTG-3’
2. 5’-CTGGTCGAATATCA-3’ and 5’-CAGGTATCATATCAC-3’
3. 5’-CTGGTCGAATATCA-3’ and 5’-GTGATATGATACCTG-3’
4. 5’-GACCAGCTTATAGT-3’ and 5’-GTCCATAGTATAGTG-3’
5. 5’-GACCAGCTTATAGT-3’ and 5’-CAGGTATCATATCAC-3’

**19) Which of the following techniques will be useful for identifying proteins that physically bind to protein-of-interest?**

1. Yeast 2-hybrid screen.
2. Luria and Delbruck fluctuation test.
3. Gel mobility shift assay.
4. Microarray analysis.
5. Epitasis test.

**20) In using the CRISPR/Cas system for genomic editing, the specificity of Cas9 is controlled by?**

1. Palindromic restriction sites.
2. MicroRNA.
3. Flanking direct repeats.
4. Flanking inverted repeats.
5. Guide RNA.

**21) The “chain terminator” nucleotides used in Sander sequencing differ from dNTP in:**

1. The absence of nitrogen base at the 1’ position.
2. The absence of triphosphate at the 5’ position.
3. The presence of diphosphate at the 5’ position.
4. The absence of hydroxyl group at the 2’ position.
5. The absence of hydroxyl group at the 3’ position.

**22) Cytosines in DNA damaged by deamination will be recognized by:**

1. Cytosine methyl transferase.
2. Cytosine acetyl transferase.
3. Uracil DNA glycosylase.
4. MutS.
5. DNA polymerase I.

**23) In DNA repair, the “SOS” response itself is mutagenic because:**

1. The double-stranded DNA breaks are not repaired via homologous recombination.
2. The response forms a template-independent error-prone DNA polymerase.
3. The response generates toxic byproducts, which can damage DNA bases.
4. The response forms a nuclease, which cuts the genomic DNA into fragments.
5. The response generates additional thymine dimers.

**24) In DNA repair, *E. coli* over-expressing *dam* methylase will:**

1. Have a lower mutation rate because the system can differentiate the newly synthesized DNA from the template more efficiently.
2. Have a lower mutation rate because the system can better remove the incorrectly inserted nucleotides.
3. Have a higher mutation rate because the system has less time to differentiate the newly synthesized DNA from the template.
4. Have a higher mutation rate because the system has more time to differentiate the newly synthesized DNA from the template.
5. Have a higher mutation rate because more adenines are methylated.

**25) You have been asked to perform the gel mobility shift assay to analyze yeast GAL regulation. A UAS-containing DNA fragment, incubated with or without GAL4 protein and galactose (indicated by the – and + below), was subjected to native gel electrophoresis. Which of the following gels will be the expected result? B**

**26) Regarding yeast GAL regulation, which of the following statements is correct?**

1. GAL1, GAL7, and GLA10 are regulated as a polycistronic mRNA.
2. GAL4 acts as a transcriptional repressor, and UAS is the operator.
3. In the absence of galatose, GAL4 binds to the UAS, and in the presence of galactose, GAL80 binds to the UAS.
4. In the absence of galactose, GAL4 activation domain is masked by GAL80.
5. In the presence of galactose, GAL80 is degraded by a protease.

**B)** From screens of embryonic patterning mutants, loss-of-function (lof) and gain-of-function (gof) mutations in *tube*, *cactus*, and *dorsal* were isolated. The normal function of *tube* and *dorsal* is to specify ventral structures, whereas the function of *cactus* is to inhibit ventral structure formation.

1. Based on the above description, what is the phenotype (missing ventral structures or excessive ventral structures) of **(4 points**):

Gain-of-function mutations in *tube* (*tubegof*):

excessive ventral structures

Loss-of-function mutations in *cactus* (*cactuslof*):

excessive ventral structures

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

tubelof, cactuslof double mutants contain excessive ventral structures.

tubegof, dorsallof double mutants contain missing ventral structures.

cactusgof, dorsalgof double mutants contain excessive ventral structures.

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

tube – cactus - dorsal

**C) Based on the lectures, please complete the following table (7 points):**

|  |  |  |
| --- | --- | --- |
| **Type of mutagen** | **Example of the mutagen** | **Genetic lesion(s) induced** |
| Alkylating agents | EMS | Base substitutions |
| Ionizing radiation | X ray | Double-stranded breaks |
| Intercalating agents | Acridine Orange | Insertions/deletions |
| UV light | UV light | Thymine dimers |
| Base analogs | 5-bromo-uracil | Base substitutions |

**D) Based on the lectures, please complete the following table (7 points):**

|  |  |  |
| --- | --- | --- |
| **Type of genetic lesion** | **Repair system** | **Specific component of the system** |
| Mismatched nucleotides | Mismatch repair (MMR) | *dam* methylase |
| Thymine dimers | Nucleotide excision (NER) | UvrABC |
| Apurinic sites | Base excision (BER) | AP endonuclease |
| Thymine dimers | Photoreactivation | Photolyase |
| Mismatched nucleotides | Proofreading | 3’-5’ exonuclease |

**E)** As discussed in class, *lin-14* and *lin-4* are two *C. elegans* mutants, in which the timing of developmental stages is disrupted. In *lin-14-* mutants, the embryos skip the L1 stage. In *lin-14gof* (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).

1. What is the effect (positive or negative) of lin-4 gene product on lin-14 protein expression (**1 point**)?

The effect of lin-4 on lin-14 protein expression is negative.

1. Please explain how lin-4 regulates lin-14 expression (**4 points**).

The lin-4 gene product is a microRNA (1 point), which binds to the 3’UTR (1 point) of lin-14 mRNA via imperfect pairing (1 point) and inhibits the translation of lin-14 mRNA (1 point).

1. To analyze lin-14 function, you have generated a siRNA, targeting the coding region of lin-14 mRNA. Please predict the phenotype (skip or repeat the L1 stage) of animals injected with this siRNA at embryonic stage (**1 point**).

As the siRNA is designed to silence lin-14 expression, the phenotype will be to skip the L1 stage.

1. Please explain how this siRNA regulates lin-14 expression (**3 points**).

The siRNA binds (1 point) to lin-14 mRNA via perfect pairing (1 point) and facilitate the degradation of lin-14 mRNA (1 point).

1. A deletion in the 3’UTR of lin-14 (hereafter referred as *lin-14Δ3UTR*) causes the mutant embryos to repeat L1. Propose a mechanism how this mutation generates the observed phenotype (**2 points**)?

Deletion of the 3’UTR in lin-14 mRNA may not allow lin-4 microRNA to bind (lin-4 cannot bind). As a result, lin-14 mRNA will be translated and the animal remain in the L1 stage.

1. 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 larvae + siRNA against lin-14 | In *lin-14Δ3UTR* larvae + siRNA against lin-14 |
| lin-14 mRNA | **+** | **+** | **+** | **+** | **-** | **-** |
| lin-14 protein | **+** | **-** | **+** | **+** | **-** | **-** |

**F)** The human genome size is 3,000mb (106 bases). If human genomic DNA is digested with NotI (recognizes GCGGCCGC), what is the average size (in kb) of the restriction fragments (**2 points**)?

The average fragment size will be 48 = 65,536 bps or 65.5 kb

How many restriction fragments will likely be generated (**2 points**)?

The number of fragments will be 3000000/65.536= 45776.4

If a human genomic DNA library was constructed using bacterial artificial chromosomes, which are capable of carrying DNA inserts of 300kb (103 bases) in length. If you want to have 80% chance of recovering a BAC clone containing a particular sequence, how many distinct clones from this library should you screen through **(2 points**)?

N=ln(1-P)/ln(1-f)=ln(1-0.8)/ln(1-300/3000000)=16093.5

**G)** Digestion of a 16kb linear DNA with restriction enzymes yields the following fragments (sizes indicated in kb). Please draw a restriction map for this DNA, and indicate the location of the restriction sites and the distance between them (**5 points**).





You are have obtained two clones from screening a cDNA library: clone A and clone B. When cDNA clone A is made radioactive and used as a probe in a Southern blot of this gel, the following bands (circled) appear on an autoradiogram.

When cDNA clone B is made radioactive and used as a probe in a Southern blot of this gel, the following bands (circled) appear on an autoradiogram.



Please indicate the positions of cDNA clones A and B on your map (**4 points**).

See previous page

After sequencing, it is revealed that cDNA clones A and B, while not identical, are closely related. Please use appropriate terminology to explain how these two clones might be related (**2 points**).

Since both clones are cDNA, it is possible that they are isoforms generated by alternative splicing.