**Spring 2015**

**BIOL241 – Introduction to Genetics**

**and Molecular Biology**

**Midterm III (105 points)**

**4/8/2015**

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

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

Question A: (68) Question B: (11)

Question C: (5) Question D: (6)

Question E: (5) Question F: (10)

**Total:**

**A. Multiple-choice questions (68 points, 2 points each, please use a scantron sheet for part A).**

1. **If the cytosines in CpG sequences near the promoter of a eukaryotic gene are highly methylated, this suggests that:**
2. This gene is expressed at a high level.
3. This gene encodes a non-coding RNA.
4. This gene has multiple alternatively spliced variants.
5. The expression of this gene is repressed.
6. This gene is located in euchromatin.

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

1. The methylation state of histone proteins in eukaryotic chromosomes.
2. The DNA sequence of whole eukaryotic genomes.
3. The acetylation state of histone proteins in c eukaryotic chromosomes.
4. The methylation state of DNA in eukaryotic chromosomes.
5. This DNA sequence of actively transcribed genes in eukaryotic genomes.

**3) While analyzing the function of an unknown eukaryotic gene, you noticed that it contains a zinc finger motif. 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 transcriptional regulatory elements.
5. Code for a protein that repairs DNA.

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

1. Transcription initiation from alternative promoters.
2. Translation inhibition by small non-coding RNA.
3. Alternative splicing.
4. Duplication of the DSCAM gene onto different chromosomes.
5. The usage of alternative transcription termination sites.

**5) The Luria-Delbruck fluctuation test demonstrated that:**

1. Spontaneous mutations are random.
2. Certain chemicals, after processed by liver enzymes, are mutagenic.
3. 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.

**6) Regarding siRNA, which of the following statements is correct?**

1. Injecting single-stranded antisense RNA into cells can efficiently degrade target mRNA.
2. siRNA mediates gene silencing by predominantly inhibiting translation.
3. The RISC complex cleaves injected-dsRNA into short 21 base pairs of dsRNA.
4. siRNA usually binds to target mRNA at specific sites in the 3’ UTR.
5. siRNA mediates gene silencing by degrading target mRNA.

**7) The most common spontaneous mutations are caused by:**

1. DNA polymerase inserting nucleotides mistakenly.
2. Template slippages during DNA replication.
3. Tautomeric shifts, which alter base-pairing properties of affected nucleotides.
4. Depurinations.
5. Double-stranded breaks.

**8) Spontaneous mutations caused by slippage during DNA replication are likely to be?**

1. Insertions or deletions.
2. Translocations.
3. Base substitutions.
4. Missense mutations.
5. Inversions.

**9) Which of the following enzymes will recognize and directly repair the lesions generated by UV light in E. coli?**

1. AP endonuclease.
2. Uracil DNA glycosylase.
3. 3’-5’ exonuclease of DNA polymerase 1.
4. Photolyase.
5. Alkyltransferase.

**10) Which of the following enzymes allows the *E. coli* mismatch repair system to distinguish the newly synthesized strand from the old template?**

1. Restriction endonuclease.
2. AP endonuclease.
3. dam methylase.
4. Alkyltransferase.
5. Cytosine methyltransferase.

**11) Which of the following mutagens is most likely to cause large chromosomal aberrations?**

1. Ethylmethane sulfonate.
2. 5-bromouracil.
3. Acridine orange.
4. UV light.
5. Ionizing irradiation.

**12) Which of the following repair mechanisms can fix T^T dimers:**

1. Photo-reactivation repair.
2. Base excision repair.
3. Nucleotide excision repair.
4. A and B only.
5. A and C only.

**13) Regarding acridine orange, which of the following statements is correct?**

1. It effectively induces insertion and deletion mutations.
2. It is an example of alkylating agent.
3. It undergoes tautomeric shift at high frequency.
4. It causes the formation of thymine dimers at high frequency.
5. It has the unusual property of base-pairing with thymines.

**14) Expressed sequence tags (ESTs) are generated from:**

1. Sequencing of the ends of cDNA clones.
2. Sequencing of genomic DNA clones from shotgun libraries.
3. Sequencing of RFLP between homologous chromosomes.
4. Sequencing of gaps between contigs.
5. Sequencing of genomic DNA clones from chromosome-specific (map-based) libraries.

**15) The Chromatin immunoprecipitation assay can be useful for:**

1. Detecting the difference in mRNA level of different genes between cells at two different states.
2. Detecting the difference in restriction sites between two homologous chromosomes.
3. Detecting protein-protein interactions.
4. Identify the enhancer elements recognized by a sequence-specific transcription activator.
5. Determining the isoforms generated from alternative splicing from a particular gene.

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

1. Prokaryotic organisms contain mostly circular chromosomes.
2. Different prokaryotic organisms contain similar amount of genomic DNA.
3. Genes in prokaryotic organisms are frequently expressed as poly-cistronic mRNA.
4. Genes in prokaryotic organisms do not contain introns.
5. The functions of about 40% of the prokaryotic genes are not known.

**17) The genome size of *Bacillus subtilis*, a gram-positive bacterium, is 4.2mb (106 bases). Based on the comparison of prokaryotic genomes, the number of genes in *Bacillus subtilis* is expected to be:**

1. 25,000.
2. 8,400.
3. 4,200.
4. 840.
5. 420.

**18) Comparing different eukaryotic genomes, which of the following organisms has the highest gene density?**

1. Human.
2. Corn (Maize).
3. Drosophila.
4. Yeast.
5. C. elegans.

**19) Horizontal gene transfer refers to:**

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

**20) Conservation in the order of genes along the chromosomes from different species is known as:**

1. Orthologs.
2. Synteny.
3. Homologs.
4. Paralogs.
5. Contigs.

**21) The condition that a cell has mitochondria of different genotypes is known as:**

1. Hemizygous.
2. Homozygous.
3. Heterozygous.
4. Heteroplasmy.
5. Homoplasmy.

**22) Comparing human and Drosophila genomes, which of the following statements is correct?**

1. The number of genes is similar.
2. The number of introns per gene is similar.
3. The average size of introns is similar.
4. The number of identified protein domains is similar.
5. The amount of DNA is similar.

**23) When a haploid *petite* mutant mates with haploid normal yeast, the resulting diploid zygote exhibits the normal phenotype. After sporulation (meiosis), 50% of the haploid progeny are normal and the other 50% are petite. This pattern of inheritance describes:**

1. Segregational petite.
2. Neutral petite.
3. Suppressive petite.
4. Codominance.
5. Incomplete dominance.

**24) The explanation for the petite mutation in 23) is that**:

1. A mutation disrupting a nuclear gene that participates in mitochondrial function.
2. A gain-of-function mutation in a maternal-effect gene that participates mitochondrial function.
3. A loss-of-function mutation in a mitochondrial gene, which disrupts mitochondrial function.
4. A deletion in mitochondrial genome, which gives defective mitochondria proliferation advantage.
5. None of above.

**25) A different yeast *petite* mutant is isolated. To determine the type of mutation causing this phenotype, the haploid *petite* and wild type strains are crossed. The diploid zygotes are petite, and all the haploid ascospores after sporulation are petite. This *petite* mutation is:**

1. Recessive.
2. Segregational.
3. Neutral.
4. Suppressive.
5. Dominant.

**26) Which of the following reagents is useful for genomic editing?**

1. Double-stranded RNA.
2. Cas9 endonuclease and guide RNA.
3. MicroRNA.
4. AP endonuclease.
5. 3’-5’ exonuclease activity of DNA polymerases.

**27) 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? C**

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

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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’

**29) If the primers in the previous question were used to amplify the above linear DNA template by PCR, what will be the size of the PCR product?**

1. There will be no PCR product.
2. 140 bps.
3. 70 bps.
4. 56 bps.
5. 41 bps.

**30) Which of the following techniques will be useful for comparing the gene expression at the mRNA levels between cells at two different states?**

1. Yeast 2-hybrid screen.
2. Luria and Delbruck fluctuation test.
3. 2-dimentional gel.
4. Microarray analysis.
5. Epitasis test.

**31) The dideoxynucleotides (ddNTP) differ from dNTP in:**

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

**32) If a male with MERRF (Myoclonic Epilepsy with Ragged Red Fibers, a mitochondria disorder) syndrome marries a normal female, what will be the phenotypic ratio of the progeny?**

1. All progeny will be normal.
2. All progeny will have MERRF.
3. The sons will have MERRF, but the daughters will be normal.
4. The daughters will have MERRF, but the sons will be normal.
5. 50% of the progeny will be normal and the other 50% will have MERRF.

**33) If a female with MERRF syndrome marries a normal male, what will be the phenotypic ratio of the progeny?**

1. All progeny will be normal.
2. All progeny will have MERRF.
3. The sons will have MERRF, but the daughters will be normal.
4. The daughters will have MERRF, but the sons will be normal.
5. 50% of the progeny will be normal and the other 50% will have MERRF.

**34) Regarding human genome, which of the following statements is correct?**

1. The gene densities of different autosomes are similar.
2. The number of genes in the genome is estimated to be around 25,000 to 30,000.
3. Most of the human DNA contains protein-coding sequences.
4. Human genes are frequently expressed as poly-cistronic operons.
5. The number of introns is fairly constant among human genes.

**B)** As discussed in class, lin-14 and lin-4 gene products control the timing of *C. elegans* development. In lin-14- (loss-of-function) 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 embryonic stage, but absent in larval stages (see the table below). The lin-4 gene product inhibits the expression of lin-14 proteins.

1. What is the phenotype of *lin-4-* (loss-of-function) mutants (skip or repeat the L1 stage) (**2 points**)?

Repeat L1

1. What is the phenotype of *lin-4-*, *lin-14-* double mutants (skip or repeat the L1 stage) (**2 points**)?

Skip L1

1. Please briefly describe how lin-4 inhibits the expression of lin-14 protein (**4 points**)?

The lin-4 gene encodes a microRNA, which binds to the 3’UTR of lin-14 mRNA through imperfect pairing and inhibits its translation.

1. Based on our discussion in class, please complete the table below (“+” for present and “-“ for absent) (**3 points**).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | In wild type embryo (L1 stage) | In wild type larvae (L4 stage) | In lin-4- embryo | In lin-4- larvae |
| lin-14 mRNA | + | + | + | + |
| lin-14 protein | **+** | **-** | + | + |

**C)** 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.

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

Loss-of-function mutations in egl-1 (egl-1lof):

No cell death

Gain-of-function mutations in ced-4 (ced-4gof):

Excessive cell death

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

ced-9lof, egl-1lof double mutants contain excessive cell deaths.

ced-9lof, ced-4lof double mutants contain no cell death.

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

**Egl-1 - Ced-9 - Ced-4**

**D)** The genome size of *Drosophila simulans* (a close relative of *Drosophila melanogaster*) is 120mb (106 bases). If *Drosophila simulans* genomic DNA is digested with EcoRI (recognizes GAATTC), what is the average size of the restriction fragments (**3 points**)?

46=4096 bps.

If a *Drosophila simulans* genomic DNA library was constructed using cosmids, which on average contain DNA inserts of 40kb (103 bases) in length. If you want to have 90% chance of recovering a cosmid clone containing a particular sequence, how many distinct clones from this library should you screen through **(3 points**)?

N=ln(1-0.9)/ln(1-40/120000)=6906

**E)** If you are given the task of generating a mouse knockout mutant (inactivating a gene of interest) by homologous recombination, please briefly describe (use diagrams if necessary) the components of your targeting construct and the selection procedure for ES cells containing disrupted gene **(5 points).**

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The targeting construct contains a neomycin resistance gene cassette disrupting the gene-of-interest and a flanking thymidine kinase gene.



To select for ES cells containing disrupted genes, ES cells transfected with this construct will be selected for the presence of neomycin resistance gene and selected against thymidine kinase gene (or selected for the absence of TK gene).

**F)** Digestion of a circular plasmid 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 (**6 points**).



If the 2kb fragment from the HindIII digest 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; **4 points)**?