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First Name	
Last Name	

Student Number

MCB*2050 Molecular Biology of Cell

Midterm Examination, Winter 2013
Saturday Feb. 9, 11:30 – 12:30

Instructors: Dr. Ray Lu
Dr. George van der Merwe

Time: 60 minutes

The midterm consists of two parts: Part A is worth 18 marks. Part B is worth 19 marks.

Part A consists of 18 multiple choice questions. Answer these questions on the computer test scoring sheet. Mark your answers on the test scoring sheet in **PENCIL ONLY**. Please note that only the test score sheet is graded for questions 1 to 18 inclusive.

Choose only one and the best answer among the choices given.

PART B consists of FOUR short answer questions, 19 to 22 inclusive. Answer these questions in the space provided. **Questions answered in pencil will not be re-graded.** Please do not use red ink.

Short Answer	Q19	Q20	Q21	Q22	Total
Marks					

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PART A (Choose only one and the best answer among the choices given.)

1. After cutting DNA, which of the following enzymes is used to join the ends of two DNA fragments?

   restriction endonucleases
   DNA polymerase
   DNA gyrase
   DNA ligase
   Helicase

2. Which of the following is a DNA palindrome?

   5′-AATGCCGGCATT-3′
   3′-TTACGGCCGTAA-5′

   5′-AATGCCCCGTAA-3′
   3′-TTACGGGGCATT-5′

   5′-AATGCCAATGCC-3′
   3′-TTACGGTTACGG-5′

   5′-AATGCCTTACGG-3′
   3′-TTACGGAATGCC-5′

   None of the above

3. Which of the following enzymes is required in a Sanger's DNA sequencing reaction?

   RNA polymerase
   DNA polymerase
   Restriction endonuclease
   DNA ligase
   More than one of the above

4. Reverse transcriptase is required for the synthesis of:

   cDNA
   sDNA
   gDNA
   rDNA
   miRNA

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5. Which of the following techniques can be used to determine if a genomic DNA clone has a particular STS?

   Southern Blotting
   Northern Blotting
   RT-PCR
   RFLP
   More than one of the above

6. Which of the following is a common feature in all cloning vectors?

   YAC sequence
   The ability to carry very large sequences
   A centromere
   ori
   Shuttling sequences

7. The transcription factor Luman is bound to the endoplasmic reticulum via a transmembrane domain near its C-terminus. In response to stress, Luman undergoes proteolytic cleavage and moves into the nucleus. What form of regulation does the gene encoding Luman exhibit?

   transcriptional regulation
   regulation by alternate splicing
   regulation through RNA transport
   translational regulation
   post-translational regulation

8. Which of the following statement regarding mRNA alternate splicing may be correct?

   It happens in both prokaryotic and eukaryotic cells
   The splicing variant that has more exons may encode a shorter polypeptide
   Some of the splicing variants may not encode functional proteins
   All eukaryotic genes have more than one transcripts
   More than one of the above

9. Which of the following statements regarding post-transcriptional gene silencing by small RNAs is correct?

   a) RISC base-pairs with its target mRNA followed by cleavage of the target mRNA by Dicer.

miRNAs usually result in the repression of translation of the target mRNA.
miRNAs are usually exogenous in origin.
miRNAs target the open reading frame region of an mRNA.
More than one of the above is correct.

10. The correct chronological order of the following events, beginning with the earliest, is: (1) splicing of an RNA molecule, (2) migration of an mRNA molecule into the cytoplasm, (3) transcription of a gene, (4) degradation of an mRNA molecule, (5) polypeptide synthesis.

3 – 2 – 1 – 5 – 4
1 – 3 – 2 – 5 – 4
3 – 1 – 2 – 5 – 4
3 – 2 – 4 – 5 – 1
None of the above is correct.

11. Which of the following is involved in chromatin remodeling

HAT complex
HAD complex
Histone transferase
DNase I
More than one of the above

12. Which of the following statements is correct?

Helix-turn-helix domains are protein dimerization domains.
Helix-loop-helix domains are DNA-binding domains.
Zn finger domains are DNA-binding domains.
Leucine zippers are DNA-binding domains.
More than one of the above is correct.

13. Contigs are -

overlapping genomic DNA clones
overlapping cDNA clones
overlapping RNA clones
continuous tandem coding sequences
none of the above

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14. When using the DNA microarray technology for transcription profiling, one would

    hybridize fluorescently labeled cDNA samples to DNA probes on the array
    hybridize fluorescently labeled cDNA samples to RNA probes on the array
    hybridize cDNA samples to fluorescently labeled DNA probes on the array
    hybridize cDNA samples to fluorescently labeled RNA probes on the array
    none of the above

15. The SNPs on a chromosome segment tend to be inherited together and define a genetic unit, which is called a:

    phenotype
    genotype
    haplotype
    maplotype
    allotype

16. Based on the sequencing data acquired from the Human Genome Project, approximately what percentage of the human genome is comprised of exons that code for proteins?

    1%
    25%
    50%
    75%
    99%

17. Which of the following statements about chimeras is correct? Chimeras are -

    a) heterozygous transgenic animals
    b) the animals that have two types of cells that have contributed to the formation of adult tissues
    c) the animals whose germ line cells do not have a transgene
    d) usually bred with other chimeras to produce true transgenic animals
    e) none of the above

18. Gene imprinting is primarily caused by

    DNA methylation
    RNA methylation
    RNA interference
    X-linked genes
    none of the above

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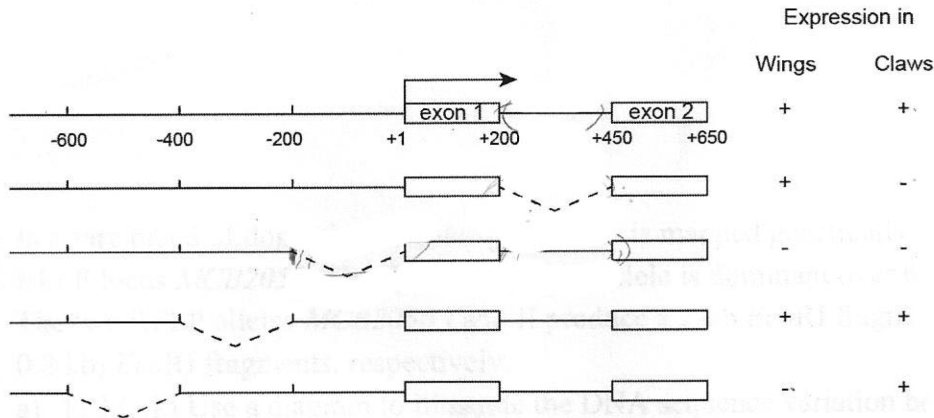
PART B

19. In *Drosophila*, expression of the *yellow* gene is necessary for pigmentation in wings and claws; without its expression, wings and claws appear yellow in color. The following is the experimental results to map regulatory DNA sequences of this gene. The numbers are nucleotide positions from the transcription initiation site. The sequences between the dashed lines are deleted, with the flanking sequences rejoined. The results of the *yellow* gene expression are shown on the right.



a) [1 Mark] Where is the promoter sequence of the yellow gene? Why?

b) [1 Mark] Where is the Wing enhancer? Why?

c) [1 Mark] What is the outcome if you switch the positions of DNA fragment (+200 to +450) and fragment (-600 to -400)? Why?

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d) [2 Marks] What are the two techniques (covered in this course) that you may use to check the *yellow* gene expression? Briefly explain the techniques

20. In a rare breed of dogs, the fur color gene locus is mapped genetically 10 cM from the closest RFLP locus *MCB2050.* The *Golden* fur color allele is dominant over the *white* color allele. The two RFLP alleles *MCB2050* I and II produce a 2-kb *Eco*RI fragment and two (1.2 kb and 0.8 kb) *Eco*RI fragments, respectively.

a) [1 Mark] Use a diagram to illustrate the DNA sequence variation between these two RFLP alleles.

b) [3 Marks] Outline the experimental procedure used to detect these two RFLP alleles.

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c) [2 Marks] A breeding dog is heterozygous at both loci, with the dominant *Golden* allele linked to the *MCB2050 I* allele on the same chromosome. If this dog is mated with a dog that is homozygous for the *white* fur color allele and the *MCB2050 II* allele, what is the percentage of Golden-colored dogs in F1 progenies that have two (1.2 kb and 0.8 kb) *Eco*RI fragments but not the 2 kb fragment? Why?

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21. [3 Marks] Suppose that you are working on aging research using a novel model organism. The genome sequence of this organism is known. You have isolated a sub-population of animals which can live 2-3 times longer than the average under the same conditions. Design an experimental strategy to conduct a genome-wide screen for genes, the expression of which may contribute to the observed longevity in these animals. Outline the main steps of your procedure.

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22. The following is a STR locus, with the DNA sequence of only one strand shown. The underlined regions are used to design 6-nucleotide PCR primers to detect the DNA polymorphism.

5′-----------AAGTCC---[cag]$_n$---CAGCCG---------- 3′

a) [1 mark] Write down the DNA sequences of the 6-nucleotide primers and label the 5' and 3' ends.

b) [1 mark] What experimental technique will you use to separate the PCR products of different alleles? Explain the working principle behind this technique.

c) [3 marks] Use a diagram to illustrate the steps of two PCR cycles. Be sure to write down the nucleotide sequences of the primers and their targeting sequences, and label the 5' or 3' ends of all DNAs.

(PART A)

GREY EXAM

1. B	2. A	3. B	4. B	5. C
6. B	7. A	8. B	9. C	10. E
11. E	12. D	13. A	14. C	15. C
16. D	17. (skipped)	18. A		

PINK EXAM

1. D	2. A	3. B	4. A	5. A
6. D	7. E	8. E	9. B	10. C
11. A	12. C	13. A	14. A	15. C
16. A	17. (skipped)	18. A		

*** These 17 questions still weighs 18 marks towards your final grades.**

(PART B)

19a)
The promoter should be essential for the *yellow* gene expression in both wings and claws. **[0.5]** Deletion of -200 to +1 disrupted the expression in both wings and claws, so it has the promoter sequence. **[0.5]**

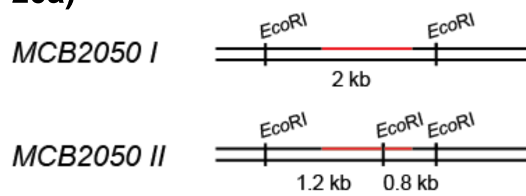
19b)
Deletion of the Wing enhancer should only affect the expression in wings, but not in claws. **[0.5]** Only fragment -600 to -400 has the Wing enhancer. **[0.5]**

19c)
Switching the locations of the Wing enhancer (-600 to -400) and the Claw enhancer (+200 to +450) will not change the expression pattern of the *yellow* gene **[0.5]**, as enhancers are position-independent. **[0.5]**

19d)
Northern blotting **[0.5]:** Isolate RNA, perform gel electrophoresis and transfer RNA in the gel to a membrane. Hybridize the membrane with a radioactively or fluorescently labeled *yellow* gene probe. **[0.5]**

RT-PCR **[0.5]:** Reversely transcribe the *yellow* mRNA to cDNA, using a primer base-paired to the 3' end of the mRNA and reverse transcriptase. Then PCR amplify the cDNA using a pair of *yellow* gene-specific primers, and check the PCR products by gel electrophoresis **[0.5]**

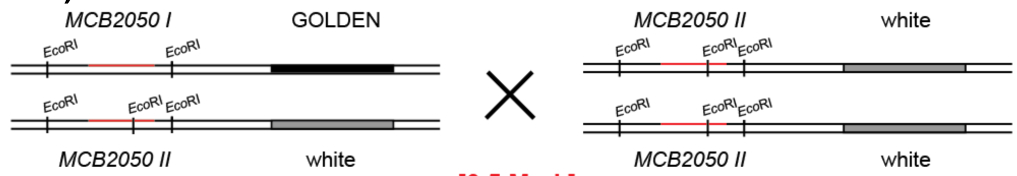
20a)



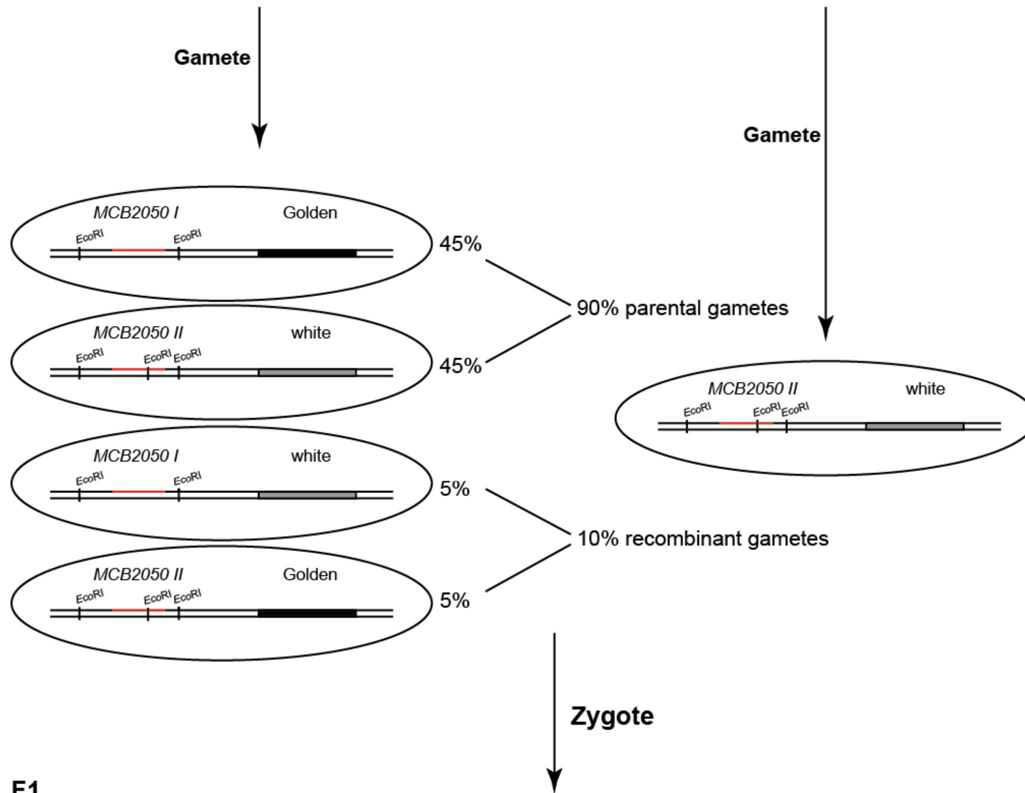
20b)

1. Isolate DNA from dogs; **[0.5]**
2. Digest DNA with restriction endonuclease/enzyme *Eco*RI; **[0.5]**
3. Separate DNA restriction fragments by gel electrophoresis; **[0.5]**
4. Transfer DNA restriction fragments to DNA-binding membrane; **[0.5]**
5. Hybridize DNA on the membrane to radioactively or fluorescently labeled probe (shown above in red); **[0.5]**
6. Wash the membrane/blot and detect the bands by exposing to x-ray file (autoradiography) or capture fluorescence by special cameras; **[0.5]**

20c)

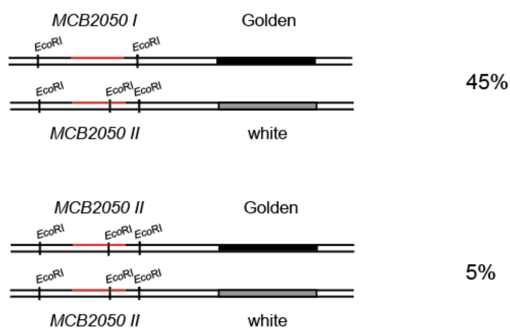


[0.5 Mark]



F1

[0.5 Mark]



In F1, 5% pups of all pups (or 10% Golden pups) are expected to be Golden-colored, homozyous at the *MCB2050 II* allele (1.2 kb and 0.8 kb bands ONLY).
[1 Mark]

21)

Use DNA microarray technology to perform genome-wide transcription profiling (or transcriptome analysis) **[1 Mark]**;

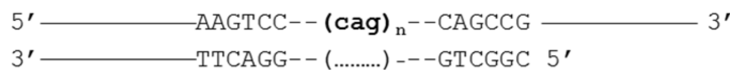
1. Prepare microarrays with DNAs specific to all the genes in this organism; **[0.5]**
2. Isolate RNAs from both long-lived and average/normal individuals, and labeled the RNAs (or the cDNAs reversely-transcribed from these RNA samples) differentially, e.g., red fluorescence for long-lived and green fluorescence for the average; **[0.5]**
3. Hybridize equal amount of labeled mRNAs or cDNAs to the DNA microarrays; **[0.5]**
4. Analyze the microarray results: The gene probes (spots) on the microarrays that are red indicate that those genes are expressed/transcribed at higher levels in long-lived animals than those with a normal lifespan. **[0.5]**

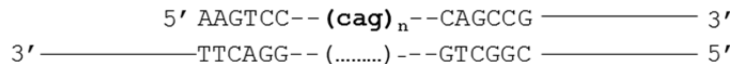
22a) **No partial marks**

Left/forward/upstream Primer: 5’ AAGTCC 3’ **[0.5 Mark]**
Right/reverse/downstream Primer: 5’ CGGCTG 3’ **[0.5 Mark]**

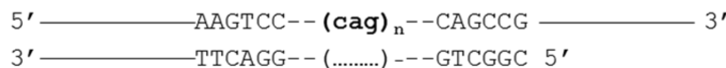
22b)
Gel electrophoresis **[0.5]**
Negatively charged DNAs will migrate to the anode (or positive electrode) in an electric field. When DNAs move in porous media (or gel), larger DNA fragments migrate slower so they are separated by size. **[0.5]**

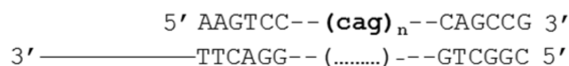
22c)
PCR Cycle 1:
Step 1: Denaturation at ~95 °C (or separation of the two DNA strands);
Step 2: Annealing at ~55 °C (or primers base pairing to their complementary sequences);
Step 3: Primer extension/DNA synthesis at ~70 °C, as shown below;

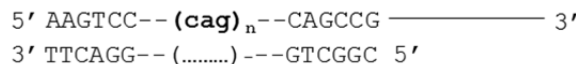


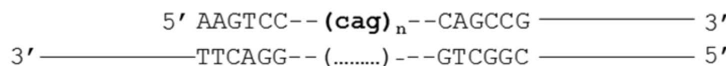


PCR Cycle 2
Same three steps as Cycle 1. The final DNA synthesis step is as follows:









- Three PCR steps, 0.5 Mark each, **[1.5 Mark]**
- Proper polarity and correct alignment of the two primers to their target sites **0.5 Mark each x 2 = [1 Mark]**
- Correct lengths of newly synthesized/nascent DNA strands **[0.5]**