BIO 2133 – Midterm #2 Answers

The following provides answers to the questions of midterm #2. Note that the TAs have received this document to guide them in their evaluation of answers. The TAs have also received a copy of the grading rubric provided on the course website. If you have not consulted the grading rubric, it essentially outlines my perspectives on the assessment of the **quality of answers** . As the objective of the assessment is to evaluate the level at which you are attaining the learning outcomes, this grading rubric serves **as a guide** for the assessment of your answers. If your answers provide different information, it will be considered and assessed accordingly vis-a-vis the learning outcomes. On that note, the answer key provides the key points for each question and I understand that other information may merit marks. As I indicated, these will be considered.

The following learning outcomes were assessed during this examination:

1) Define key terms and concepts

2) Describe methodologies for genetic analysis and interpret observations they provide.

3) Justify the use of different methodologies for genetic analysis.

4) Analyse phenotypic and/or results of genetic screens and/or molecular analyses (markers) data to deduce possible methods of expression/inheritance from family histories and experimental crosses.

5) Extract information about genes, alleles and gene function from genetic crosses and pedigree analysis.

Note: You will notice that I have included the learning outcomes (L.O.) in each of the questions. This is to indicate which learning outcome(s) is being assessed.

(15 pts) Part 1. Explain what the following terms means or the biological significance. For each, include a relevant example to which the term applies. For this part, provide answers for 5 of the following 8 terms. (Note: if you explain more than 5 terms, the first 5 will be evaluated). **L.O.#1**

Bioinformatics: L.O.#1

The design and application of software and computer science for the storage, analysis, and management of biological information such as nucleotide or amino acid sequences.

Ex: Using BLAST search to retrieve DNA or protein sequences from the gene bank (NCBI).

Recombinant DNA technology: L.O.#1

A tool that allows DNA to be produced by adjoining heterologous DNA molecules produced by in vitro ligation.

Ex: Cloning a gene – inserting a DNA fragment of interest in a plasmid vector

Site directed mutagenesis: L.O.#1

A process that uses a synthetic oligonucleotide containing a mutant base (mutation) or sequence as a primer for inducing a mutation at a specific site in a cloned gene.

Ex: Introduce a point mutation in the insulin gene. This is then used to produce a transgenic organism containing the point mutation (an illustration is accepted in this case, for example)

Complimentary gene interaction: L.O.#1

This type of interaction between genes occurs when there is the need of at least 1 dominant allele of each of two genes pairs is essential for the expression of a given phenotype. Ex. Cross AaBb X AaBb and obtain a 9 (A-B-):7(aaB- or A-bb or aabb) phenotypic ratio.

Penetrance: L.O.#1

The frequency, expressed as a percentage, with which individuals of a given phenotype manifest at least some degree of a specific mutant phenotype associated with a trait.

Ex: 70% of individual with the AA genotype actually expresses the A phenotype.

Incomplete dominance: L.O.#1

Expressing a heterozygous phenotype that is distinct from the phenotype of either homozygous parent. The heterozygotes express a blended phenotype that is achieved by the expression of different alleles of the same gene. In a cross between heterozygotes, you would obtain a 1 (A^1 A^1):2 (A^1A^2 blended phen.): 1(A^2A^2) ratio.

Ex. Cross between a white and red flower and obtain a heterozygote pink flower.

Hemizygous: L.O.#1

Having a gene present in a single dose in an otherwise diploid cell. Usually applied to genes on the X-chromosome in heterogametic males.

Ex: White eyed drosophila males are said to be hemizygous white because the allele for the white mutation is located on the X. Males in this case only have 1 X chromosome therefore referred to as hemizygous.

Y chromosome: L.O.#1

Chromosome containing the genes (SRY gene) responsible for directing the development of the male characteristics during foetal life in humans and other organisms. Also referred to the sex chromosome is species where the male is heterogametic.
Ex. Human males XY

(15 pts) Part 2. Answer 3 of the following 4 questions in the space provided.

2.1 List the steps you would perform to produce a mouse that is homozygous for a rat growth-hormone transgene? (5 pts) L.O.#2

1) Produce a recombinant DNA molecule containing the rat GH sequence to be replaced in the mouse.
2) Introduce the molecule to an embryonic stem cell from an agouti mouse.
3) Through genetic screens, select embryonic stem cells that had homologous recombination involving the recombinant DNA molecule.
4) Introduce embryonic stem cell to a blastocyst from a black mouse thus producing a chimera.
5) Cross chimera with a black mouse.
6) Self-cross the agouti mouse (presumed heterozygote) and screen for the homozygous mice for the transgene.

2.2 A comprehensive cDNA library is based on mRNA samples from different tissues, different developmental stages, or from organisms grown in different environmental conditions. List the steps involved in producing a cDNA library starting from an mRNA sample. (5 pts) L.O.#2

1) Isolate mRNA from cells or tissue
2) Add oligo-dT primer and nucleotides (dNTPs)
3) Add reverse transcriptase to synthesize a complementary DNA strand using the mRNA as a template.
4) Digest the mRNA.
5) Add polymerase to synthesize complementary strand thus completing the double strand.
6) Clone cDNA into a plasmid vector (insert into plasmid using restriction enzymes and ligases)
7) Introduce plasmid vectors into bacteria and grow on an agar plate.

2.3 Polymerase chain reactions (PCR) are used to amplify regions of DNA. Describe what is needed to perform a PCR reaction.(5 pts) L.O.#2

You need the following:

1) DNA (genomic, cDNA, PCR products – you need template)
2) Two primers (forward and reverse primers)
3) Nucleotides (dATP, dTTP, dGTP, dCTP)
4) Taq polymerase
5) Buffer with $MgCl_2$
6) Thermocycler

2.4 List the steps involved in performing DNA sequencing. (5 pts) L.O.#2

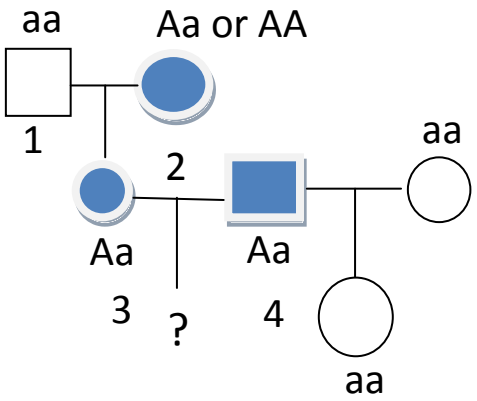
There are two techniques, but both are essentially the same.

1) Add template – DNA stranded in the 3' to 5' direction
2) Add primer to begin the synthesis of strands using the template
3) Primer extension using a polymerase. Chain is terminated with a ddNTP is added inserted.
4) Newly synthesized strands are recovered, loaded on gel for electrophoresis. Strands migrate according to their size.
5) Strands are then visualized based on the ddNTP that terminated the chain during extension.
6) DNA sequence is determined based on the size of the strands and their identifying ddNTP. (read from shortest strand to longest strand in the 5' to 3' direction)

(30 pts) Part 3. Problem Solving Questions. **Answer ALL questions.**

Note: To receive full marks for each answer, you must demonstrate your work towards the final answer.

3.1 The ability to taste the chemical phenylthiocarbamide is an autosomal dominant phenotype, and the inability to taste is recessive. If a taster woman with a nontaster father marries a taster man who in a previous marriage had a nontaster daughter, what is the probability that their first child will be: **L.O. #4, #5**



A) A nontaster girl (2 pts): ____1/8____

Chances of having a girl (XX) = ½

Chances of nontasting (aa) = ¼

Therefore chances of a nontasting girl= ½ x ¼ = 1/8

B) A taster girl (2 pts): __3/8_____

Chances of having a girl (XX) = ½

Chances of tasting (A-) = ¾

- (cross Aa x Aa → ¼ AA, 2/4 Aa → tasters)

Therefore chances of a tasting girl= ½ x 3/4 = 3/8

C) A taster boy (2 pts): ___3/8_____

Chances of having a boy (XY) = ½

Chances of tasting (Aa) = ¾

- (cross Aa x Aa → ¼ AA, 2/4 Aa → tasters)

Therefore chances of a tasting boy = ½ x 3/4 = 3/8

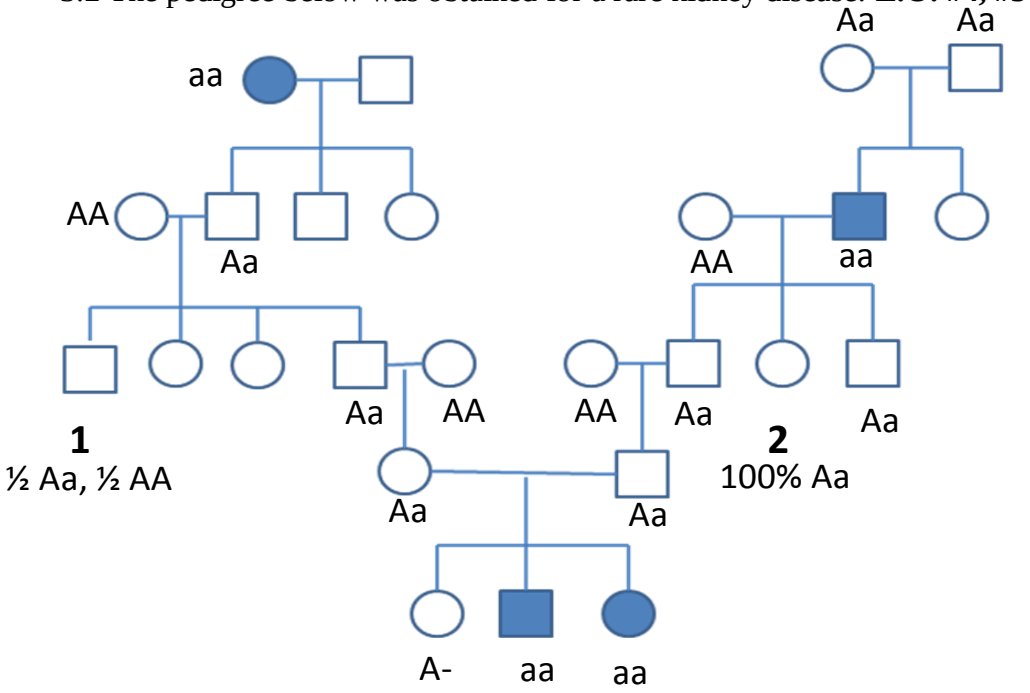
Reason for the answers:

Tasting is caused by an autosomal dominant allele. Therefore, anyone who is a taster must have the dominant allele (A). Therefore, the mother(3) and father (4) have the A allele. Because the mom (3) is a taster and had a dad (1) who is a nontaster, her mom (2) must be a taster. Therefore, this makes mother (3) 100% heterozygote, regardless if her mother is heterozygote or homozygous dominant.

Father (4) had a nontaster girl. Therefore he must also be 100% heterozygote – because his girl must have inherited a recessive allele from both parents.

Given that we know the genotypes of the parents, then we can answer the questions A, B, C

3.2 The pedigree below was obtained for a rare kidney disease: **L.O. #4, #5**



A) Deduce the inheritance of this condition, stating your reasons for your answer. (3 pts)

The mode of inheritance shown in this pedigree is **autosomal recessive**. This is caused by a recessive allele. The evidence for this is shown where 2 unaffected parents have an affected child (seen in generation 1 family on the right, and generation 4, family in the middle).

Both sets of parents have affected and unaffected children. This suggests the parents are heterozygous. (Note: there is no evidence supporting the possibility of sex-linked inheritance , sex-limited and mitochondrial inheritance)

Note: *For the next question, knowledge of the fact it's a rare autosomal disease allows us to determine with certainty the genotypes of a number of individuals, and for others the probability of being certain genotypes. Also, in this case, outsider rules apply – normal outsiders are homozygous AA unless there is information showing otherwise.*

The genotypes indicated in the pedigree are those that help you, in this case, determine the genotypes (and their odds) for parent 1 and 2 (for the next question).

Italics = information not part of the answer

B) If person 1 and 2 have a child, what is the probability that their child will have the kidney disease. (3 pts) (You may use the next page to show your work, but transfer your final answer on this page).

Final answers:_____1/8_____

Dad (1) can be either ½ Aa or ½ AA; Mom (2) is 100% Aa

The only way that this couple can have an affected child is if the father is heterozygote. In this case, the dad has ½ chance of being heterozygote.

If Dad is ½ Aa and Mom is Aa, then:

= ½ (dad heteroz.) X 1 (mom has 100% chance of being Aa) X ½ (the chance of dad giving the recessive allele) X ½ (chances of mom giving the recessive allelle) = chances of having an affected child (male or female)

= 1/8 chances of having an affected child.

3.3 Consider the following cross: **L.O. #4**

Note: You may use the next few pages as draft paper. You must, however, transfer your work and final answer in the space provided on this page.

\ A/a ; B/b ; C/c ; D/d ; E/e X a/a ; B/b ; c/c ; D/d ; e/e

A) What proportion of progeny will phenotypically resemble the first parent? (2 pts)

Therefore the chances of having: A- B- C- D- E-

= ½ (phen. A) X ¾ (phen. B) X ½ (phen. C) X ¾ (phen. D) X 1/9 (phen. E) =

= **9/128** = 0.0703 (not needed) = 70.3 % (not needed)

B) What proportion of progeny will be genotypically the same as the second parent? (2 pts)

Therefore: aa Bb cc Dd ee

½ x 1 x [(½ X ½) + (½ x ½)] x ½ x 1 x [(½ X ½) + (½ x ½)] x ½ x 1 = **4/ 128** = 1/32

3.4 Most flour beetles are black, but several color variants are known. Crosses of pure-breeding parents produced the following results (see table) in the F1 generation, and intercrossing the F1 from each cross gave ratios shown for the F2 generation. The phenotypes are abbreviated: Bl, Black; Br, brown; Y, yellow; and W, white. **L.O. #4, #5**

Cross	Parents	F1	F2
1	Br X Y	Br	3 Br: 1Y
2	Bl X Br	Bl	3 Bl : 1Br
3	Bl X Y	Bl	3 Bl: 1Y
4	W X Y	Bl	9 Bl : 3Y : 4W
5	W X Br	Bl	9 Bl : 3 Br : 4W
6	Bl X W	Bl	9 Bl: 3Y: 4W

A) From these results, determine the mode of inheritance of these colours. (3 pts)

The mode of inheritance involves 2 genes – each involved in the expression of the colour. The first locus has multiple alleles (Bl, Br, Y; where Bl is more dominant than Br and Y, and Br is more dominant than Y. This is determined from the 3:1 ratios in crosses 1,2,3. ($C^{Bl} > C^{Br} > C^{Y}$). The second gene shows recessive epistasis to the colour expression. This is determined based on the phenotypic ratio in the F2 in crosses 4,5,6. Based on the phenotypic ratio (9:3:4), there is a recessive allele causing the white phenotype that when in the homozygous recessive state causes the white phenotype in the beetles regardless on the genotype at the first locus.

Determine the genotypes of the parents of the following crosses : #1, # 4, and #6

Genotypes of parents of cross #1:_____C^{Br} C^{Br} WW X C^{Y} C^{Y} WW__ (2 pts)

Genotypes of parents of cross #4:_____$C^{Bl}C^{Bl}$ww__X____$C^{Y}C^{Y}$WW_ (2 pts)

Genotypes of parents of cross #6:___$C^{Bl}C^{Bl}$WW X $C^{Y}C^{Y}$ww_____ (2 pts)

3.5 Are the following progeny numbers consistent with the results expected from selfing a plant presumed to be a dihybrid of two independently assorting genes, H/h; R/r? (5 pts) **L.O. #4, #5**

H = hairy leaves; h = smooth leaves; R = round ovary; r = elongated ovary

Explain your answer.

Results:

Smooth, round	99
Hairy , round	250
Smooth elongated	75
Hairy , elongated	24

A dihybrid cross: Hh Rr X Hh Rr

Expect the following ratio: 9 H-R- (hairy and round); 3 H-rr (hairy and elongated); 3 hhR- (smooth and round) and 1 hhrr (smooth and elongated)

Chi-square:

Expected numbers : chi-square

Phenotype Class H-R- = 9/16 (448) = 252.....χ^2 = (250-252)^2/252 = 0.016

Phenotype Class H-rr = 3/16 (448) = 84..... χ^2 = (24-84)^2/ 84 = 42.3

Phenotype Class hhR- = 3/16 (448) = 84..... χ^2 = (99-84)^2/84 = 2.68

Phenotype class hhrr = 1/16 (448) = 28..... χ^2 = (75-28)^2/28 = 78.9

Sum of the χ^2 = 123.9 df=4-1 = 3 p= lower than 5%

Conclusion: Reject the hypothesis. The results do not support a case of independent assortment of 2 genes.

Table 2-2 Critical Values of the χ^2 Distribution

	P									
df	0.995	0.975	0.9	0.5	0.1	0.05	0.025	0.01	0.005	df
1	.000	.000	0.016	0.455	2.706	3.841	5.024	6.635	7.879	1
2	0.010	0.051	0.211	1.386	4.605	5.991	7.378	9.210	10.597	2
3	0.072	0.216	0.584	2.366	6.251	7.815	9.348	11.345	12.838	3
4	0.207	0.484	1.064	3.357	7.779	9.488	11.143	13.277	14.860	4
5	0.412	0.831	1.610	4.351	9.236	11.070	12.832	15.086	16.750	5
6	0.676	1.237	2.204	5.348	10.645	12.592	14.449	16.812	18.548	6
7	0.989	1.690	2.833	6.346	12.017	14.067	16.013	18.475	20.278	7
8	1.344	2.180	3.490	7.344	13.362	15.507	17.535	20.090	21.955	8
9	1.735	2.700	4.168	8.343	14.684	16.919	19.023	21.666	23.589	9
10	2.156	3.247	4.865	9.342	15.987	18.307	20.483	23.209	25.188	10
11	2.603	3.816	5.578	10.341	17.275	19.675	21.920	24.725	26.757	11
12	3.074	4.404	6.304	11.340	18.549	21.026	23.337	26.217	28.300	12
13	3.565	5.009	7.042	12.340	19.812	22.362	24.736	27.688	29.819	13
14	4.075	5.629	7.790	13.339	21.064	23.685	26.119	29.141	31.319	14
15	4.601	6.262	8.547	14.339	22.307	24.996	27.488	30.578	32.801	15