Biology 113 Closed Book Take-Home Final Exam

There is no time limit on this test, though I have tried to design one that you should be able to complete within 3 hours. There are 7 pages in this final exam, including this cover sheet. There are two supplementary files as well: "RawData_Fall2020.xlsx" and "Sequencing Results Exam3 F2020.docx". You are not allowed to look at someone else's test, nor use your notes, old tests, the internet, any books, nor are you allowed to discuss the test with anyone until all exams are turned in by noon EST Tuesday December 8. **EXAM IS DUE NO LATER THAN NOON TUESDAY DECEMBER 8th**. If you turn in your exam late, then you lose a letter grade for each day you are late unless you communicate with me in advance. The **answers to the questions must be typed directly under the questions** unless the question specifically says to write the answer in different place. If you do not write your answers in the appropriate location, I may not find them.

I have provided you with a "Data Gallery" in the form of figures and tables. To choose a figure in support of your answer, state Figure #x and do NOT move the image on your test. Do not assume how many of the data images you will use, or not use. Simply choosing the data is not sufficient support for your answer, however. You must explain the significance of the data and how they support your answer. I have given you word limits so be concise.

-3 pts if you do not follow this direction.
Please do not write or type your name on any page other than this cover page.
Staple all your pages together when finished with the exam. Do not print test pages without answers. I only want to see your answers. You can type your answers right under each question.

Name (please type here):

Read the pledge and sign if you can do so with honor:

_____

On my honor I have neither given nor received unauthorized information regarding this work, I have followed and will continue to observe all regulations regarding it, and I am unaware of any violation of the Honor Code by others.

How long did this exam take you to complete?

Lab Questions

10 pts.

1) This question is holistic. You must perform several steps to reach the final conclusion and answer this question.

Step 1: Open the file "Sequencing Results Exam4 F2020.docx" and perform a clustal omega alignment on these DNA sequences. (https://www.ebi.ac.uk/Tools/msa/clustalo/) Capture a screenshot of your results and paste it here: 2 pts

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CLUSTAL O(1.2.4) multiple sequence alignment


X1_cloned_promoter                NNNNNNNNNGATCNNNANANNNNNCTGAGACGACTCCCTATCAGTGATAGAGATTGACAT       60
X3_cloned_promoter                ----------------------NNNCTGAGACGACTCCCTATCAGTGATAGAGATTTACAT       39
intended_promoter_no_stickyends   -----------------------------------TCCCTATCAGTGATAGAGATTGACAT       26
X2_cloned_promoter                --AACCCCNNNNNNANTNNNNNNNCTGAGACGACTCCCTATCAGTGATAGAGATTGACAT       58
                                                                     ******************** ****


X1_cloned_promoter                CCCTATCAATGATAGAGATACTGAGCACGCGGGAAGACAACTAGGGGCCCAAGTTCACTT       120
X3_cloned_promoter                CCCTATCAGTGATAGAGATACTGAGCACGCGGGAAGACAACTAGGGGCCCAAGTTCACTT       99
intended_promoter_no_stickyends   CCCTATCAGTGATAGAGATACTGAGCAC--------------------------------       54
X2_cloned_promoter                CCCTATCAGTGATAGAGATACTGAGCACGCGGGAAGACAACTAGGGGCCCAAGTTCACTT       118
                                  ******** ******************
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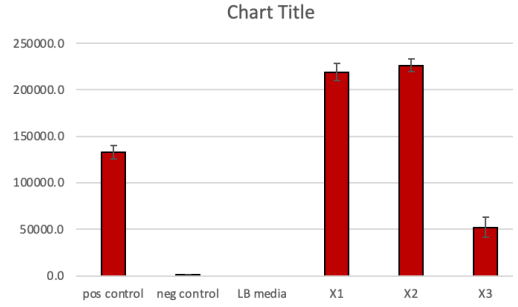
Step 2: Notice that the -35 and -10 sites have been underlined in the intended promoter sequence you used for the alignment.

Step 3: Process the raw data in the file called "RawData_Fall2020.xlsx". These data include RFP values and absorbance values at 600 nm, as indicated. Calculate the average relative fluorescence for the positive control, the negative control and the 3 experimentally cloned promoters labeled X1 – X3.

Step 4: Produce a bar graph with standard error of the mean for error bars (SEM formula is "=STDEV(start:end)/SQRT(n)". Note that start:end is the data range and n = sample size.

Step 5: Insert a screenshot of your graph here: 2pts -0.5 for graphing LB media above zero



Step 5: Evaluate the 3 experimental promoters using all the data provided to you. Limit your evaluations to a maximum of 45 words each. 2 pts each -0.5 for no use of error bars

X1: mutation between -10 and -35 does not affect function, as good as X2 (wt)

X2: no mutations, functions very well, better than + control

X3: mutation in -35, weaker than wt, stronger than - control

Step 6: Include in your email to me the Excel file and this Word file so I can grade both.

Class Questions:

15 pts. 5 pts each

2) "Out damn spot!" Macbeth, Act 5, Scene 1.

a) As you know, I recently donated blood to the Red Cross because there is a national need and I am O negative blood type (universal donor). I biked to the donation site and my blood had a pH of 7.1. After doing the paperwork, my blood had pH 7.5 and the oxygen concentration was 30 mmHg oxygen the entire time. Quantify the **percentage** of my hemoglobin that contained the indicated number of oxygen molecules. (type your numerical answers in the boxes)

	pH 7.1	**pH 7.5**
0 oxygen bound	60	35
1 oxygen bound	0	0
2 oxygen bound	0	0
3 oxygen bound	0	0
4 oxygen bound	40	65 -2 for 90%

b) Describe the two emergent properties that influence your answers in the table above. Limit your answer to a maximum of 40 words for each emergent property.

Property 1: pH affects affinity for O_2

Property 2: cooperativity in hemoglobin -1 cooperativity

c) What common mechanism is responsible for these emergent properties. Support your answer with one figure. Limit your answer to a maximum of 40 words. -3 if pH, -2 if cooperativity

Figure 1, movement of histidine opposite the O_2, or allosteric modulation

15 pts.

3) There's a light, over at the Frankenstein place…

a) Use figure 33 to explain how *V. fischeri* turns on light production. Limit your answer to a maximum of 50 words. +feedback loop -1

luciferase transcription starts and positive feedback loop amplifies small amount of autoinducer diffusing into cells.

b) Can bacteria communicate with each other about more than just cell density? Support your answer using 2 figures. Limit your answer to a maximum of 40 words for each figure. -2.5 if figure 9 and strains or fig 11

Figure 1: #15 two shapes of autoinducers

Figure 2: #6 about food abundance

15 pts.

4) pregnancy

a) Integrate figures 8 and 18 to describe a mother's immune system during a successful pregnancy. Limit your answer to a maximum of 40 words for each figure.
Figure 8: retains robust immunity and embryos protected, not babies -1 only embryos
Figure 18: MHC IG produced in cytotrophoblasts to suppress mother's immunity

b) Summarize the function of MHC I. Support your summary with two figures. Limit your answer to a maximum of 40 words for each figure.
Figure 1: 2 present peptides made inside every cell
Figure 2: 21 identify cell as self from MHC I

15 pts.
5) You cannot wait on your weight.
a) What tissues produce the encoded proteins that are mutated in *ob* and *db* animals? For each answer describe the function of the protein. Limit your answer to a maximum of 40 words for each gene.
ob: fat cells make leptin, ligand that signals too much fat
db: every cell, receptor for signal transduction

b) Explain to your family, using simple vocabulary, what is likely to happen to their bodies if they over-eat on Thanksgiving. Include the aspects of 1) fat accumulation and 2) changes in appetite as part of your answer. Support your answer with one figure. Limit your answer to a maximum of 50 words.
figure 23 - lipostat
1) overeating leads to more fat, more leptin and signal to stop eating and burn energy
2) appetite suppression leads to less food, reduced fat, reduced leptin to set point

15 pts.
6) True story – advice given during a press conference with a researcher who discovered the cause of a genetic disease: "Choose your parents carefully."
a) Look at figures 4 and 16 and explain why the atypical individuals look the way they do. Limit your answer to a maximum of 40 words for each figure.
4: homeotic gene turned on in the wrong cell types and second thorax produced
16: bicoid protein removed and suppressed so bicaudal phenotype, embryonic lethal -1 mRNA

b) Connect figures 7, 12 and 19 to explain what happens in the cells of typical human females. Limit your answer to a maximum of 40 words for each figure.
7: Xist mRNA binds to inactive X (Xi), the same chromosome that transcribed Xist mRNA
12: cells with more than one X chromosome transcribe Xist to inactivate all but one X chromosome
19: epigenetic mechanisms such as methylation help silence Xi. -1 epigenetics

15 pts.

7) Don't let politicians make claims about sex determination that contradict scientific evidence.

a) Connect the genotypes and phenotypes for the atypical individuals in figures 25B, 26 and 29. Limit your answer to a maximum of 40 words for each figure.

25B: Nr5a1 -/+ heterozygous XY sex reversal to female phenotype

26: XX sox9 +/+/+ sex reversal to male phenotype

29: XX Sry+ sex reversal to male phenotype

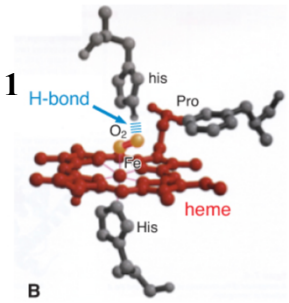
b) Explain to a politician why a law requiring people to use public toilets based on "their sex as determined at birth" is a misguided law. Support your answer using figures 22 and 31. Limit your answer to a maximum of 50 words for each figure.

22: ambiguous or truly hermaphrodite (intersex) people

31: many, many genes involved and no simple test to know birth sex

Data Gallery

1



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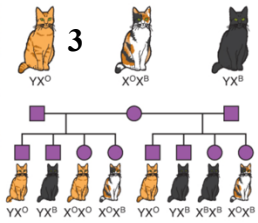

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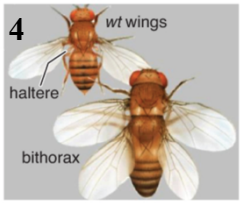
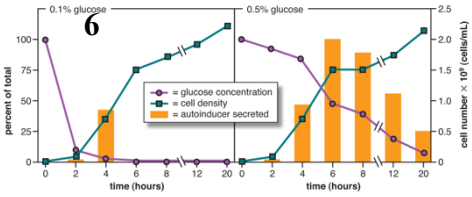


5

genotype	age (days)	free-feeding mice			pair-fed mice		
		body weight (g)	weight change (g)	percent fat	body weight (g)	weight change (g)	percent fat
wt	20	14.6 ± 0.5*	–	9.5 ± 0.4	15.1 ± 0.6	–	n.d.
wt	48	26.1 ± 0.9	+ 11.5	9.1 ± 0.8	21.4 ± 0.8	+ 6.3	14.9 ± 0.8
ob/ob	20	17.0 ± 0.5	–	23.8 ± 1.1	17.1 ± 0.4	–	n.d.
ob/ob	48	38.6 ± 0.4	+ 21.6	42.3 ± 1.4	25.6 ± 1.0	+ 8.5	43.7 ± 1.0
db/db	20	16.8 ± 0.4	–	24.3 ± 0.9	16.6 ± 0.5	–	n.d.
db/db	48	38.2 ± 0.5	+ 21.4	36.8 ± 0.8	24.3 ± 1.2	+ 7.7	41.7 ± 0.7

*mean values ± standard error of the mean with 4 mice in each group.

6



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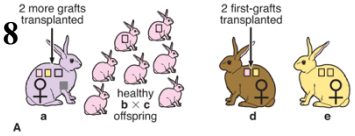


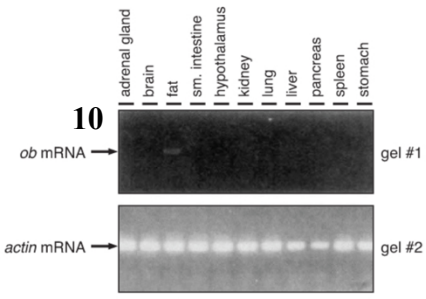
experimental conditions	baby skin transplanted to:		rabbit E skin transplanted to:	
	foster mother A	unrelated rabbit D	foster mother A	unrelated rabbit D
average days graft survived	4.0*	6.5	6.0*	7.0

*indicates $p < 0.01$; experiment replicated 5 times

9

treatment	strain 1 cells	strain 2 cells
negative control media	delayed	delayed
strain 1 media	immediate	delayed
strain 1 media, filtered	immediate	nt
strain 1 media, boiled	delayed	nt
strain 2 media	delayed	immediate
strain 2 media, filtered	nt	immediate
strain 2 media, boiled	nt	immediate

10



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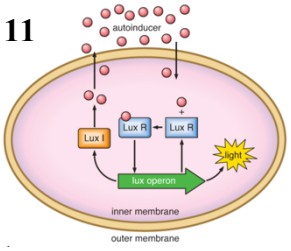

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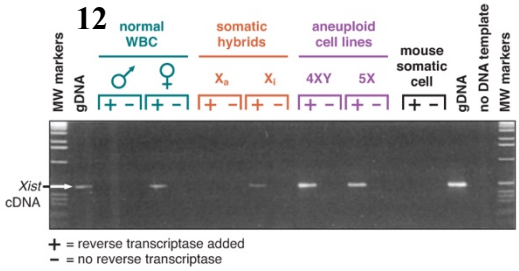

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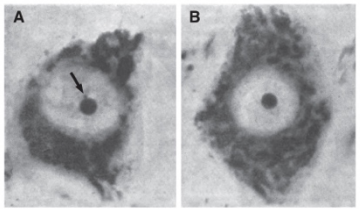

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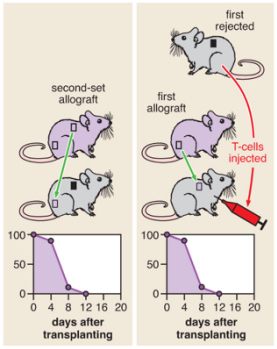

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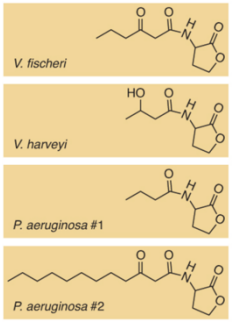

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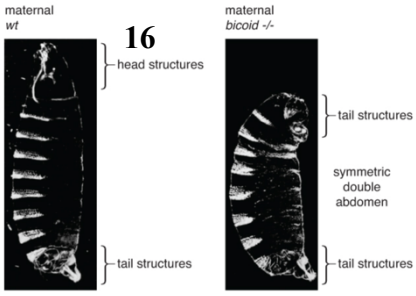

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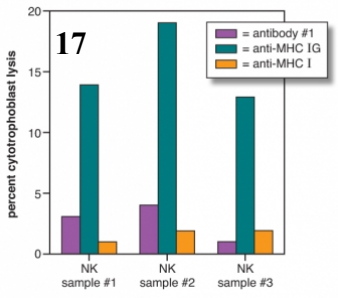

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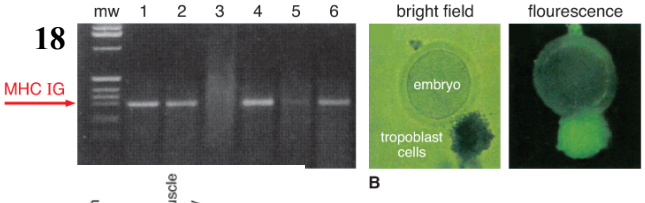
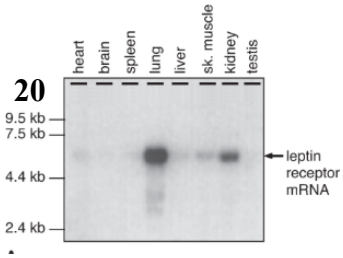


19

Xist RNA	DNA methylation	histone hypoacetylation	relative X^{GFP}	relative X^{Hprt}
wt	*wt*	*wt*	1X	1X
deleted	*wt*	*wt*	2.5X	100X
wt	blocked	*wt*	19X	12X
wt	*wt*	blocked	1X	ND
deleted	blocked	*wt*	30X	4,800X
wt	blocked	blocked	29X	ND
deleted	blocked	blocked	60X	ND
wt	*Dnmt1* deleted	*wt*	1,500X	ND
deleted	*Dnmt1* deleted	*wt*	2,500X	ND

20



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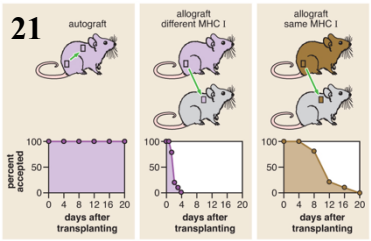

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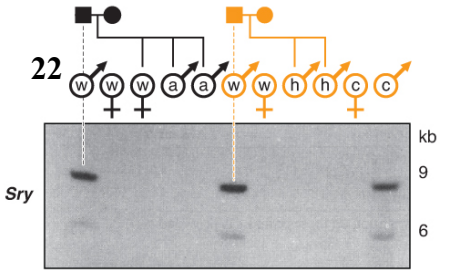

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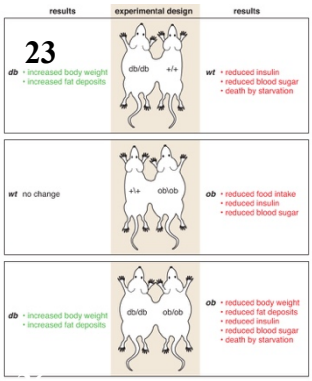

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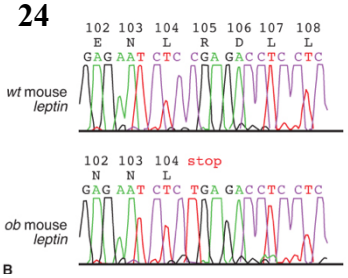

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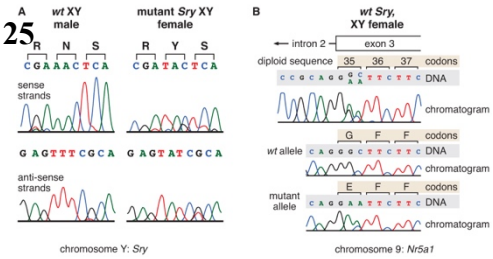

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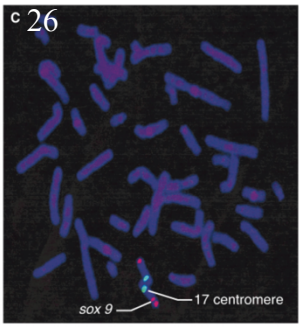

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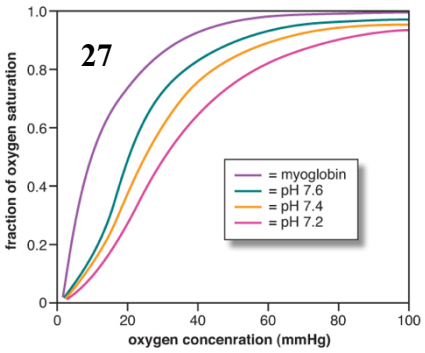

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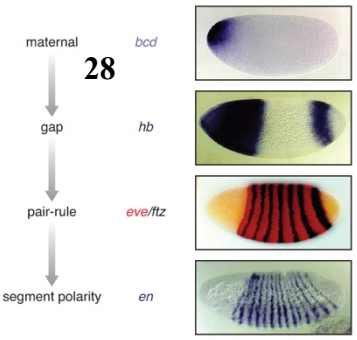

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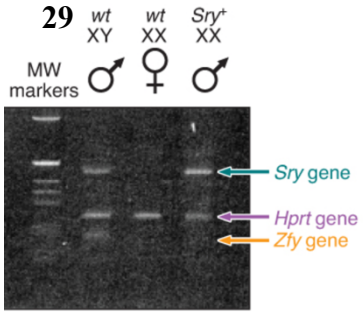

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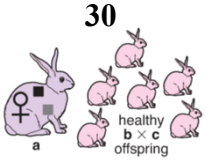
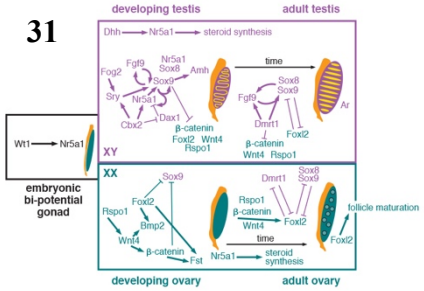


results	second graft transplanted	
	at transferral	mid-pregnancy
healthy litter	3	7
no litter	1	2

31



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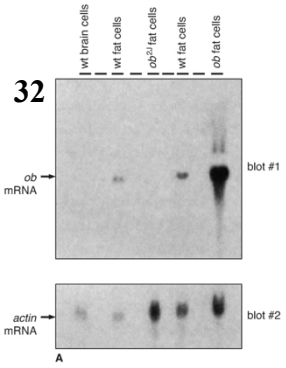

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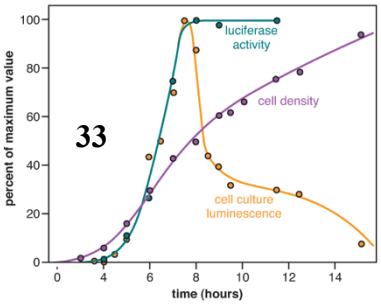
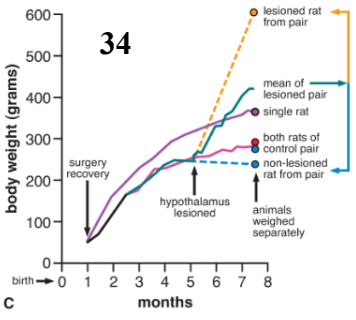
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