Spring 2012 Genomics Exam #2
Sequence Variations and Microarrays

There is no time limit on this test, though I don't want you to spend too much time on it. I have tried to design an exam that will take less time that exams in the past. You do not need to read any additional papers other than the ones I send to you. There are 6 pages, including this cover sheet, for this test. You are not allowed discuss the test with anyone until all exams are turned in no later than 12:30 am on Wednesday March 28. **ELECTRONIC COPIES OF YOUR EXAM ANSWERS ARE DUE AT 12:30 pm ON WEDNESDAY MARCH 28**. You may use a calculator, a ruler, your notes, the book, and the internet. You may take this exam in as many blocks of time as you want. Submit your electronic version before 12:30 pm (eastern time zone).

The **answers to the questions must be typed in a Word file and emailed to me as an attachment**. Be sure to backup your test answers just in case (I suggest a thumb drive or other removable medium). You will need to capture screen images as a part of your answers which you may do without seeking permission since your test answers will not be in the public domain. Remember to explain your thoughts in your own words and use screen shots to support your answers. Screen shots without ***your*** words are worth very few points. Support your answers with data using screen shots liberally (no permission required since your exam is a private document).

DO NOT READ or DOWNLOAD ANY PAPERS FOR THIS EXAM. RELY ON YOUR EXPERIENCE, AND YOUR SKILLS.

-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 (INCLUDING THE TEST PAGES) together when finished with the exam.

Name (please type):

Write out the full pledge and sign (electronic signature is ideal):

How long did this exam take you to complete?

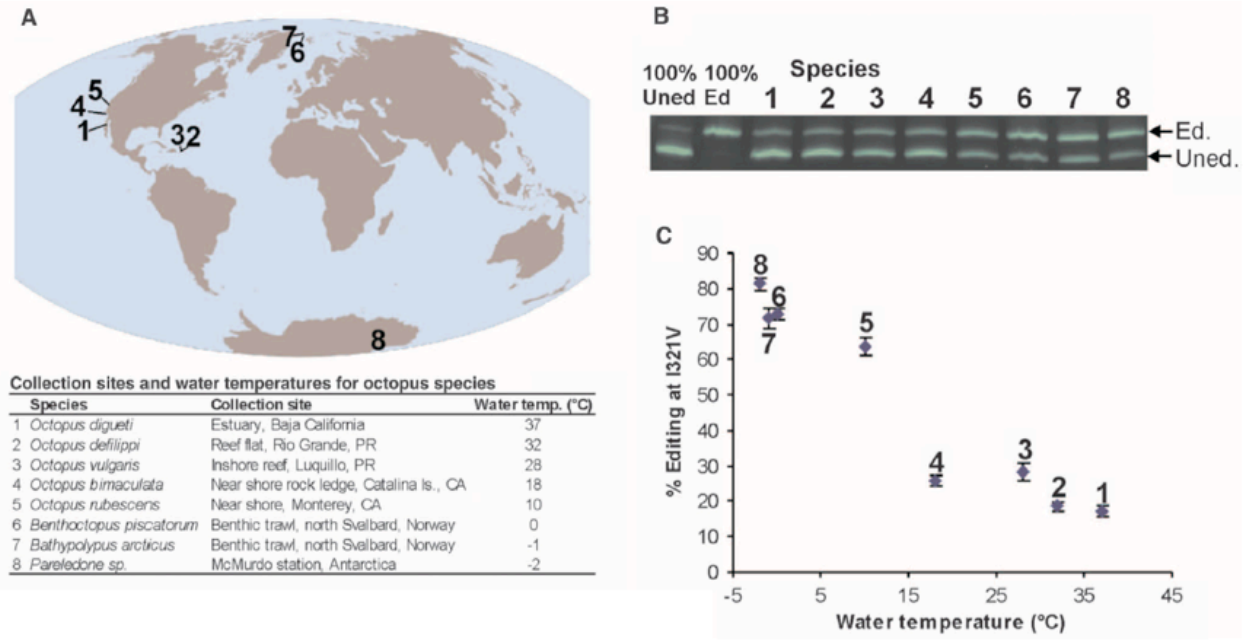
30 points

1) This question is about variation but in a form we have not studied. I want to see how you can synthesize what we have already covered as well as transfer previous information to a new situation. This is very much like what you will do for the rest of your life outside of school.

a) Summarize what adenosine deaminase does biochemically? *Limit your answer two 2 sentences*. To get credit for this answer, you must show me a screen shot of the database you used to determine the function. (Hint, do not use Wikipedia.) Your screen shot should include all three forms of "function" to get full credit for this answer.

b) How is adenosine deaminase responsible for variation in proteomes that is not detectable in genomes? Cite your source for this answer. *Limit your answer two 2 sentences*.

c) The figure below is from a 2012 paper trying to figure out how K^+ channels in neurons of different species can have different functional properties based on different amino acid sequences when their DNA alleles do not vary in sequences. They worked with octopi from around the world as shown. In panel B, they analyzed sequence mature mRNAs using a PCR-based method. QUESTION: How do the different species of octopi produce K^+ channels with different properties given their alleles are identical? **Use only your answers to a) and b) plus the data in this figure. Do not search for any papers to answer this question.** *Limit your answer two 2 sentences*.



Collection sites and water temperatures for octopus species

	Species	Collection site	Water temp. (°C)
1	*Octopus digueti*	Estuary, Baja California	37
2	*Octopus defilippi*	Reef flat, Rio Grande, PR	32
3	*Octopus vulgaris*	Inshore reef, Luquillo, PR	28
4	*Octopus bimaculata*	Near shore rock ledge, Catalina Is., CA	18
5	*Octopus rubescens*	Near shore, Monterey, CA	10
6	*Benthoctopus piscatorum*	Benthic trawl, north Svalbard, Norway	0
7	*Bathypolypus arcticus*	Benthic trawl, north Svalbard, Norway	-1
8	*Pareledone sp.*	McMurdo station, Antarctica	-2

d) The table below summarizes a lot of data. Look at row AZIN1 and tell me about each column for this one gene, starting with the function of this one gene. Please produce a numbered list (1-12) to help me keep track of the grading - a number for each column. *Limit one sentence per column*.

e) Tell me how much AZIN1 mRNA is produced in the 3 cell types listed in the table below. Support your answer with a link from the page or pages where you got your information. *Limit of 1 sentence for your answer*.

Table 2. Sanger sequencing of RDD sites.

Gene	Chr.	Position (bp)*	Type	Location	Amino acid change	B cells†		Primary skin fibroblast†		Brain (cortex)†	
						No. of informative individuals	No. of individuals showing RDD	No. of informative individuals	No. of individuals showing RDD	No. of informative individuals	No. of individuals showing RDD
EIF2AK2	2	37,181,512	A-to-G	3′ UTR	Not applicable	8	8	8	0	10	10
	2	37,181,517	A-to-G	3′ UTR	Not applicable	8	8	8	3	10	10
	2	37,181,520	A-to-G	3′ UTR	Not applicable	8	8	8	3	10	10
	2	37,181,538	A-to-G	3′ UTR	Not applicable	8	8	8	6	10	10
AZIN1‡	8	103,910,812	A-to-G	Coding, exonic	S to G	2	2	10	0	9	8
DPP7	9	139,128,755	C-to-T	Coding, exonic	Synonymous (P)	9	2	8	1	10	0
PPWD1	5	64,894,960	G-to-A	Coding, exonic	E to K	2	2	8	8	8	8
HLA-DQB2	6	32,833,537	G-to-A	Coding exonic	G to S	2	2	10	10	NE§	NE
	6	32,833,545	G-to-A	Coding, exonic	R to H	2	2	10	10	NE	NE
	6	32,833,550	C-to-T	Coding, exonic	Synonymous (I)	2	2	10	10	NE	NE
BLCAP#	20	35,580,977	A-to-G	Coding, exonic	Q to R	6	4	10	4	6	6
NDUFC2	11	77,468,303	C-to-G	Coding, exonic	L to V	10	0	10	0	10	0

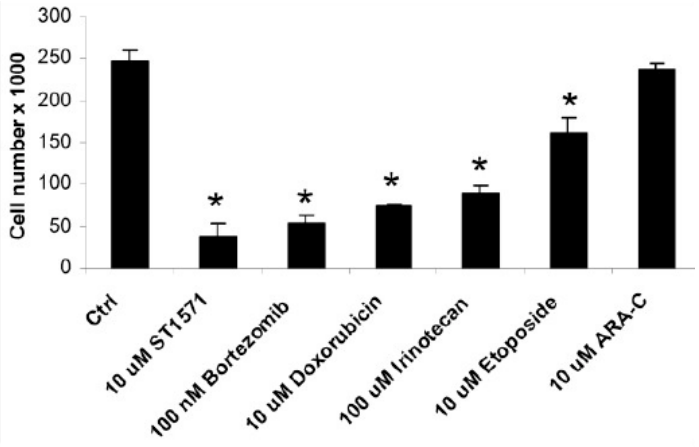
*hg18 build of the human genome. †In all cases, matched DNA and RNA samples from the same individuals were sequenced. ‡Also reported by Li *et al.* (6). §NE, not expressed. #Known site that we used as a positive control.

f) This question allows you to synthesize what you have learned. Use what we have covered in class to construct a numbered list of sources of phenotype variation that you would recommend someone measure who is interested in determining the multiple sources of phenotypic variation in a population. *Limit of 1 sentence for each number in your list*.

20 points

2) Below you will see part of a partial abstract and one figure from a paper published in 2011. Feel free to look up any words you don't know **but don't look up the full abstract or paper.**

ACC [don't worry about the name] is an uncommon malignancy, accounting for less than 1% of all pancreatic cancers. Because of its rarity, only a few retrospective studies are available to help guide management. We report the case of a patient with metastatic ACC who achieved prolonged survival as a result of personalized treatment designed in part on the basis of molecular and *in vitro* data collected on analysis of the tumor and a cell line developed from the liver metastasis. To our knowledge, this represents the first human cell line of ACC.



a) Describe how this research represents personalized medicine. *Limit your answer to a maximum of 2 sentences*.

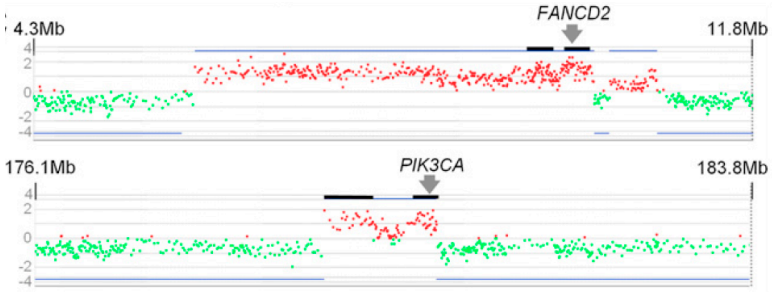
b) Summarize the type of "molecular data" you think were used to characterize this patient's cancer. *Limit your answer to a maximum of 2 sentences*.
c) Which treatment seems to be the most effective of the drugs tested *in vitro* (all of them are already FDA-approved) based on the figure above. *Limit your answer to a maximum of 1 sentence*.
d) Use the case study above to formulate a generalizable method to treat every cancer patient in a personalized way. *Limit your answer to a maximum of 3 sentences*.

20 pts

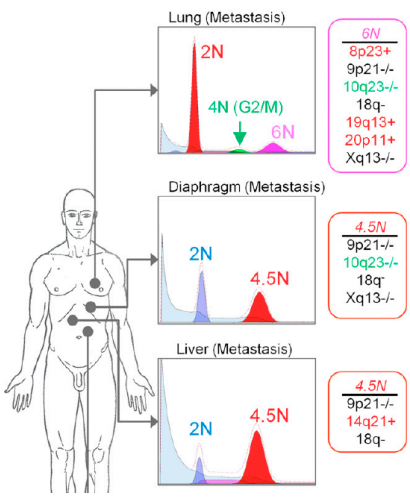
3) The data below show what happened in one patient when his cancer became metastatic and spread to the indicated organs. This study used PCR of selected genes to measure the amount of chromosomal DNA in cells isolated from tumor biopsies. They used FACS to isolate single cells from the tumors (lots of data!). **Panel A** quantifies the PCR products from short segments from particular chromosomes taken from one tumor biopsy. **Panel B** shows the FACS data from individual cells isolated from several different tumor biopsies as indicated – DAPI stain was used to quantify the DNA (X-axis). Green chromosomal text on the right indicates segments of DNA with shared abundance in non-cancerous liver cells; red text are segments of DNA unique to the indicated tumor biopsy; black indicates segments of DNA shared in all cancer biopsies. (You may use the site below to remind yourself what FACS is, and feel free to remind yourself what DAPI stains by searching the web.
<http://media.pearsoncmg.com/bc/bc_campbell_genomics_2/medialib/method/FACS.html>
Figure 2 of this web page is the most informative for this question.)

a) What chromosome(s) is/are being tested in the top and bottom panels of panel A? *Limit your answer to a maximum of 1 sentence*.
b) Summarize the results shown in panel A. *Limit your answer to a maximum of 2 sentences*.
c) What can you conclude from panel B about this man's cancer that has spread to several different organs in his body? *Limit your answer to a maximum of 3 sentences*.
d) Based on what you have learned this semester, what procedure would you recommend in the form of ideal, personalized cancer treatment of this patient (hospice and similar recommendations are not a valid answer for this test, though it might be in real life). *Limit your answer to a maximum of 3 sentences*.

A.



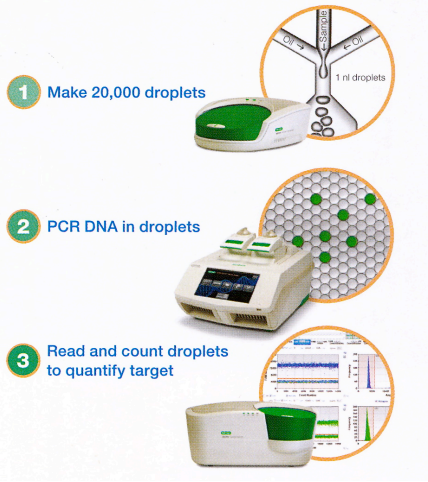
B.



30 pts

4) Here is another clinical case based on cancer. Sorry to be so morbid for this exam. There is a table (next page) and a figure (separate PDF file) associated with this question. Look up any terms you want, but focus on the questions I ask, not defining terms you don't need to know. **Do not look up this paper.**

> Small cell carcinoma of the ovary of the hypercalcemic type (SCCOHT) is a very rare tumor type that mainly affects young women. We report a 21-year old woman with SCCOHT. The patient initially presented with stage T3AN1MX disease and treated with surgery. The patient then received 8 cycles of multi-agent chemotherapy including cisplatin, bleomycin, cyclo-phosphamide, doxorubicin, and etoposide. Upon relapse, the patient underwent total abdominal hysterectomy, followed by chemotherapy with gemcitabine. The patient subsequently received radiation therapy and chemotherapy with bevacizumab, irinotecan and docetaxel. She passed away approximately 5 months after the second surgery and with her prior permission an immediate autopsy was performed. We examined the gene expression and copy number profiles of the tumor tissue samples obtained from the autopsy and com-pared them to normal ovary tissues. Our results indicated that although this tumor did not harbor chromosomal abnormalities nor gene copy number changes, there were significant gene expression changes in a number of genes/pathways. More than 5,000 genes showed significant differential expression in the tumor when compared to normal ovary tissue. Pathway enrichment analysis further identified several pathways/processes including the Vitamin D receptor signaling and the hedgehog signaling pathways to be significantly dysregulated. The gene expression profiling also suggests a number of agents such as pazopanib, bortezomib, 5-azacytidine, and PARP inhibitors as treatment options to possibly explore in future trials against this disease.



a) To measure the mRNAs, the research team used a new form of quantitative, reverse-transcriptase PCR (called digital PCR). The method is outlined in the figure to the right. Describe how digital PCR could be used to validate microarray data. You may read about this method from any web site you want as long as you cite your sources. *Limit your answer to a maximum of 2 sentences.*

b) What sort of control do you think you'd need to run with digital PCR? *Limit your answer to a maximum of 2 sentences.*

c) Summarize the function of VDR. *Limit your answer to a maximum of 2 sentences.*

d) How is VDR connected to chemotherapy? *Limit your answer to a maximum of 2 sentences.*

e) Do humans have VDR paralogs? Document how you reached your conclusion. *Limit your answer to a maximum of 2 sentences.*

f) Give me a link to a database showing some known VDR variant alleles.

g) Give me a link to a database showing VDR's expression level in various human tissues.

h) Look at the associated PDF file which shows a massive figure and associated figure legend. What problems do you foresee with trying to treat this type of cancer by targeting VDR. Base

your answer on what you have learned so far in class. Provide specific examples to support your answer. *Limit your answer to a maximum of 3 sentences*.

Table 1. Pathways significantly dysregulated in tumor.

Pathway Names	p-Value	Ratio
Transcription: Role of vitamin D receptor (VDR) in regulation of genes involved in osteoporosis	4.4E-05	28/43
Cytoskeleton remodeling: Keratin filaments	8.8E-05	24/36
Hedgehog signaling in gastric cancer	3.5E-04	19/28
Development: Pigment epithelium-derived factor (PEDF) signaling	5.4E-04	24/39
Development: TGF-beta-dependent induction of epithelial-to-mesenchymal transition (EMT) via SMADs	6.1E-04	22/35
Suppression of p53 signaling in multiple myeloma	1.2E-03	20/32
Development: Regulation of epithelial-to-mesenchymal transition (EMT)	1.3E-03	34/634
Cytoskeleton remodeling: Reverse signaling by ephrin B	1.3E-03	19/30
Neurophysiological process: Dopamine D2 receptor transactivation of PDGFR in CNS	2.6E-03	23/40
Regulation of lipid metabolism: G-alpha(q) regulation of lipid metabolism	2.7E-03	15/23

Ratio: Number of genes differentially expressed/ total number of genes in the pathway