This examination is worth 35 points. Take your time, read the questions carefully, and answer them concisely. **Be sure to answer the question I am asking and all parts of the question**. Many questions have more than one possible correct answer. Be sure to explain your logic in arriving at the answer you give since explaining your thinking helps me to decide whether a wrong answer still might deserve partial credit. Good luck!

1. (9 points) You have recently been recruited to SpaceX where you will be part of a team that characterizes the many potential biological materials on Mars. SpaceX has established a colony at the South Pole of Mars. Unknown to anyone else, SpaceX scientists returned samples from the liquid water found under the polar CO2 ice cap to Earth for characterization. SpaceX's CEO, Elon Musk, is an entrepreneurial guy who is looking to exploit his investment in this Mars colony to fund future expansion and establishment of other colonies.

a) (3 points) To get the ball rolling, you put a few drops of the water on an agar plate. To your's, and everyone else's surprise, colonies of some sort of microorganism grew on the plate. Since you know that many microorganisms cannot be cultured, you expect that there are many more in the sample. **1) How should you go about determining how many types of microorganisms are present in the sample? 2) What is the main assumption you must make in order for this to be successful?**

1) I was looking for 16S rRNA sequencing here to determine the number of microorganisms, although, an approach like the Venter paper in the first half of the quarter is acceptable.

2) the main assumption you must make is that these organisms have DNA and RNA that is sufficiently similar to that of Earth organisms for this to work, use the same 5 bases as Earth (A,C,G,T,U) and that rRNA sequencing will work.

b) (3 points) Luck is on your side and your sample yields 11,412 different sequences. **How will you determine whether these sequences are bona fide residents of Mars vs. contaminants introduced on Earth by a sloppy technician (not you, of course) ?**

Simple. Just compare the sequences you obtain with those of Earth organisms in the Genbank database. If they are not identical to any Earth organisms, then the chances are that they will be substantially different. Any contaminants will probably match sequences in the database exactly.

c) (3 points) Unfortunately, first one, and then several of your colleagues on the project develop a strange illness – they grow orange fur all over their bodies. After two weeks, they develop high fevers, become delirious and demand to be BioSci majors at UCI. Sadly, they die shortly afterward (after developing fevers, that is). You suspect that they have contaminated themselves with Martian microorganisms but there is no evidence that this might have happened from surveillance videos. **How might you prove that skin from the affected individuals contains microorganisms from the Martian sample?**

You will want to isolate skin samples from the affected individuals and perform 16S or metagenomic sequencing to compare with the database of Martian sequences and the Earth DNA database. If you find Martian organisms on the skin of patients, then you will have proven that they came from the Martian sample.

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2. (3 point) You are the kind of person who makes lemonade out of lemons, instead of complaining that they are not sweet enough. While it is tragic that your colleagues have died, their illness, together with the identification of Martian DNA sequences in their skin, strongly suggests that these microorganisms can be pathogenic. Patients, doctors and nurses at UCIMC begin to develop orange fur. Suddenly Anderson Cooper is broadcasting live from in front of the hospital, bravely not wearing hazadardous materials protective gear. He is muy macho but not very bright (and does not look good in a hazmat suit anyway). As time passes and more data are collected, you notice that there are 3 phenotypes among people who show evidence of Martian microorganisms on their skin: a) orange fur, delirious, then dead, b) orange fur but otherwise healthy and c) unaffected. This sounds to you like a classic case of a single gene that differs when it is homozygous for one allele (sensitive, ie. dead), or the other allele (resistant), or heterozygous (orange but alive). You perform whole genome association studies and quickly discover that one region on chromosome 20 (about 1 megabase long) is highly associated with resistance to the Martian microorganisms. **How might you <u>prove</u> that this region is transcribed into mRNA?**

The most direct way to do this would be to hybridize this region with RNA and determine whether it hybridizes with transcripts. Other forms of RNA detection could work, but bioinformatic analysis would not be acceptable because it cannot prove that mRNA is transcribed from this region.

3. (6 points) As you might expect, Anderson Cooper has now developed orange fur. CNN is desperate to know whether or not he will survive and the ongoing drama is generating big ratings. They need to know how long the ratings bonanza will continue so that they can project how much advertising revenue they can expect. Meanwhile, Larry King has come out of retirement to anchor this news story (while Anderson is hospitalized) since he is so old that no one really cares whether he will contract the disease.

a) (3 points) You have blood samples from 50 people with orange fur who died and from 50 orange furred people who survived. **Other than waiting to see what happens, how might you quickly determine which group Anderson would belong to?**

I was thinking of a metabolomic analysis to identify what might be common among those that survived compared with those that died.

b) (3 points) Elon Musk has a wild idea. He hypothesizes that the Martian microorganisms infect skin cells and become intracellular parasites (like *Chlamydia*) and grow in as intracellular parasites in skin stem cells. Your analysis suggests that the infected skin stem cells are instead transformed into a cell type like hair follicle stem cells activating the promoter of an endogenous skin gene(s)**. Assuming that you can isolate both cell types in pure form, outline how would you identify which genes might show altered accessibility to transcription factors in infected vs. normal skin stem cells?**

The key clue here is altered accessibility to transcription factors. You will want to use one of the methods that is specialized to detect this such as ATAC-seq, DNAse-seq, MNase-seq, etc. I'd use ATAC-seq since it is the easiest method (you can buy a kit). Isolate nuclei from normal vs. infected cells, treat them with the kit reagents, construct libraries for Illumina sequencing, deep sequence and compare accessibility in infected vs. normal skin cells.

4. (6 points) Anderson survived and is back to work! You notice that when he gives a report from the beaches of Miami, his fur turns blue. However, when he is broadcasting from his home state of New York, his fur remains orange. The CNN producers are having a lot of fun sending Anderson back and forth between cold and warm climates to watch him slowly change color, leaving his hair a succession of orange and blue stripes. You feel badly for Anderson and want to help. You suspect that changes in temperature might trigger alterations in the epigenome of infected melanocyte stem cells, changing the color of the new fur. Your previous work identified a gene, *pelz*, expressed only in stem cells infected by Martian microorganisms (but note that *pelz* is a human gene). You hypothesize that changes in *pelz* expression levels are responsible for fur color.

a) (3 points) **How could you identify epigenetic differences between the *pelz* gene in infected vs. uninfected skin stem cells that are responsible for increased *pelz* expression?**

I left this question broad so that you could pick from several methods. One possibility would be to do methylation analysis. Since I specified only the pelz gene, you don't need to do whole genome analysis, but can focus on the gene and surrounding areas. Limited bisulfite sequencing would be the best approach but you could use MeDIP-seq or MBD-seq if you wanted to spend more money. You could assess histone methylation using a variety of antibodies against methylated and acetylated histones and do ChIP-seq to assess differences in the pelz gene region. Prepare DNA or chromatin from infected vs. uninfected skin cells and evaluate differences in methylation or histone occupation and or methylation/acetylation in the pelz gene region.

b) (3 points) **How can you determine which epigenetic changes are affected by warm vs cold temperature that might be related to the changes in fur color?**

Now this is a bit more complicated but still straightforward. You are going to need to culture cells both at normal warm temperatures, and in reduced temperatures, prepare DNA and/or chromatin and evaluate any differences in epigenetic changes noted above between warm vs. cold. If you were extra careful, you might want to do whole genome methylation analysis here to test whether regions outside the pelz gene were important.

5. (3 points) The orangutan genome has recently been sequenced and you notice that *pelz* is very similar to an orangutan gene that is reported to be associated with their beautiful orange fur. The human gene is quite similar, but is not normally expressed in humans because they have (luckily) lost expression of a key transcription factor required for *pelz* expression. You hypothesize that a protein from the Mars bacterium (*Mars andersoncooperii)* facilitates formation of interchromosomal connections that both activate the pelz promoter in some people and also disrupt thermal regulation in others. **How could you identify all possible intra- and interchromosomal interactions in normal and infected skin stem cells?**

Since I am asking you to identify intra- and interchromosmal interactions in normal and infected skin stem cells, you will need to employ a method that does this. The best choice will be a chromatin conformation capture method. If you assume that I am asking for contacts between pelz and anywhere else, then 3-C is teh way to go. If you want to assess all possible changes, including contacts in the pelz gene, Hi-C is probably best. I gave credit for any of these methods, including ChiA-PET. Culture normal and infected skin stem cells, isolate nuclei, perform the chosen method, deep-sequence and evaluate interactions using the appropriate computer programs.

6. (3 points) Recall that *pelz* is not expressed in normal humans. You had a wild idea and decided to test whether *pelz* is expressed in humans with the very rare condition called congenital generalized hypertrichosis (CGH), colloquially known as 'Werewolf syndrome'. Sure enough, *pelz* is expressed in the skin and hair follicles of affected individuals. This is interesting because although several candidate genomic regions for CGH have been identified, no specific gene has been associated with this syndrome to date. **Describe how you might make a mouse model to test whether *pelz* is sufficient to cause extreme hairiness and note what assumptions you must make in order to accomplish your goal.**

Since I am asking you to test whether pelz is sufficient to cause extreme hairiness, you will want to perform a gain-of-function experiment such as transgenesis. The method you use is not so important as long as you express pelz in skin and hair follicles. Perhaps the best way would be to attach the pelz coding region to a promoter that is active in skin and hair follicles, then perform standard transgenesis, or use CRISPR-cas9 based insertion of this gene into a place in the genome you know is not silenced in skin/hair follicle cells. The main assumption you must make is that you can detect the difference in hairiness between mice that don't express pelz and those that do. Maybe it would be a good idea to try this in a hairless mouse model? Another assumption that some of you noted is that pelz would not be expressed in mice and that you would want to test the effect of exprssing it.

7. (5 points) You have been extremely successful so far; your efforts identified *pelz* as a gene that can cause orange fur, you know which chromosomal interactions lead to expression of *pelz* and which also lead to lethality and SpaceX chemists identified an extract from Goji berries that can cause loss of orange fur in many people who eat at least 5 kg of these berries/day. When a purified compound from the Goji extract is tested in volunteers with orange fur 60% lose their orange fur within a month. Excellent. You also see something interesting the volunteers who lose their orange fur share a common feature – they are all runners, whereas those that do not respond all walk for exercise. **How could you identify which metabolic pathways might be responsible for the runners to lose their orange fur compared with the walkers who do not? How could you determine whether the key metabolites are from the patients themselves, or produced by their microbiomes?**

I was thinking a metabolomic approach here, but one that is detailed enough to discern metabolic pathways involved. Something like the analysis in the David paper combined with extensive non-targeted metabolomics could be sufficient. Isolate blood samples from runners vs. walkers and compare what you find and infer which metabolic pathways are altered based on the metabolites present. To determine whether these are patient vs. microbiome metabolites, you will want to perform metagenomic analysis on fecal samples and identify which of the metabolites found in the blood of runners that are not found in walkers (or are found at much lower levels) are microbial.

OPTIONAL – EXTRA CREDIT – 2 points.

Please show Riann that you have written something on this page so that she can record your 2 points, then tear it apart from the rest of the test to preserve your anonymity.

What suggestions do you have for improving the course in the future? I changed a fair amount of the lecture material, dropped some old papers and added some new ones for this year. I am particularly interested in knowing what parts of the course you found especially informative or beneficial, and which you think should be changed or replaced. Comments about the instructor or TA should be entered in the online evaluation, not here.