DUE: Friday Feb26th at the BEGINNING of class.
Hand In: Answer the questions on paper, number your answers, show all work (as necessary), identify key assumptions, and indicate all collaborations and assistance received or given.

Note that showing work means that if you utilize software for assistance (programs you write or stock software such as Excel or R), you should indicate as such and provide sufficient details so that I can judge the work. That may mean sending me (by email) source code or associated Excel files.

Your work must be legible -- if your handwriting isn't great, type it up and print it.

Questions

1. You will need the homework.fa file on D2L for this question. This fasta file contains a segment of an *archaeal* genome that is not quite finished. The goal is to analyze this segment. Using NCBI Blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi), follow the directions and answer the following questions.

a) Let's first assume there that are NO closely-related archaeal species in the database. Which flavor of BLAST is most sensitive for comparing your sequence to species that are NOT closely related? Explain in one sentence.

You are starting with a nucleotide sequence, so your options are BlastN, BlastX or TBlastX. TBlastX will be the most sensitive because both the query and database sequences will be translated into all six reading frames and compared on the protein level (6 frames x 6 frames = 36 different sequence comparisons).

Alternatively, if you manually translate all six reading frames and run PSI-BLAST or discontinuous megaBlast against just the protein databases (probably not practical, but possible) on those, you could get additional sensitivity.

(b) Given the computational demands of the most sensitive form of BLAST, you decide to instead use BLASTX. Search only the first 6000 nucleotides against the non-redundant (nr) database, limited to "Archaea" and "Bacteria" organisms only. (Note you can adjust all of these parameters from the standard NCBI BlastX interface). You are only interested in the top hits, so set the "Max target sequences" parameter to 50 and word size to 6. (Note that these are “Algorithm Paramters” and can be adjusted by expanding the form at the bottom.) What species contains a sequence that is the most similar by overall score? What fraction of the query is included in this alignment? Include the query coordinates in your answer.

Ignisphaera aggregans DSM 17230 27% coverage [5548 - 3881] (so this is on the negative strand)

(c) Looking at the "Taxonomy Report" (find by first clicking on the "Taxonomy Reports" link), which species had the most hits to this sequence?

Sulfolobus islandicus with 13 hits.

(d) Given the protein similarity of your top hit, if you repeated your search and wanted to have the most accurate scoring matrix for the top hit, which BLOSUM matrix would you choose? (explain)

It is 100% identical so a BLOSSUM90 matrix makes sense.

(e) Do you think you know exactly what species this sequence is taken from? (any web search or bioinformatics tool is fair game) If yes, give your evidence.

Nope -- clearly we did not find any long hits with 100% identity to a database sequence, so we cannot determine the species identity from this particular sequence and analysis method.

2. Answer the following questions using PubMed, the NCBI biomedical literature index/search engine. Provide the accession number(s) (PMID) for all information you utilize to answer these questions.

a) Prof. David Haussler has been a prolific, leading scientist in genome research, although he did not start in biomedical research. In what year did Prof. Haussler score his first publication in the journal "Science"?

1974

b) Prof. Haussler's brother, Mark R. Haussler, is also a noted scientist. How many publications were they co-authors on, and what molecule did they study together?

3 co-authored; 1 alpha,25-dihydroxyvitamin D3

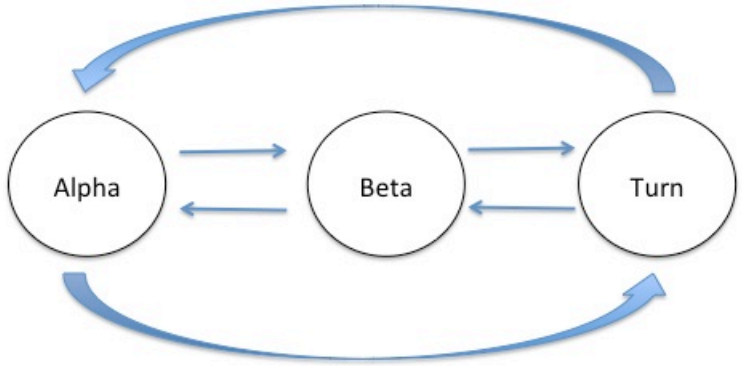
c) Using only papers published between 2004 and 2008, name two parts of the human body that have been found to contain archaea?

Any two of mouth/oral cavity, gut or intestines, and vagina; pubmed search with words “human” and “archaea” in title, and years 2004 or 2008 gives 3 papers. The first with PMID: 18644435 describes in the abstract and first paragraph of the introduction.

3. You consider using an HMM approach to model protein secondary structure prediction. The straight-forward approach uses three secondary structure confirmations: “**α**-helix”, “**β**-strand”, and “turn” as the hidden states emitting observable amino acids. It is assumed that the frequencies/probabilities of each

of the twenty amino acids can be determined from experimental data for each of those confirmations.

a) Draw the state diagram (circles and arrows) of the HMM.



Perfectly acceptable alternatives include a start state with arrows to each of the above three states and/or a stop state (with transitions from each of the above states). Note that the correct answer to c depends on what you drew here. Including a start state adds 3 more transitions. Adding both start and stop adds 6 more transitions.

b) How many emission parameters are needed to describe this model?
20 emissions per state x 3 states = 60 total emissions
c) How many transition parameters are needed to describe this model?
Each state has 3 possible outgoing transitions x 3 states = 9 total transitions

4. You are excited about being able to use the human genome browser to look more closely at the molecular basis of human genetic diseases in the news. To start with, you decide to investigate one of the genes mentioned in the NY Times article "Disease Cause is Pinpointed with Genome" by Nicholas Wade (http://www.nytimes.com/2010/03/11/health/research/11gene.html?_r=0).

One of the two papers described in this article has two authors "Lupski JR" and "Gibbs RA", and titled " **Whole-genome sequencing in a patient with Charcot-Marie-Tooth neuropathy"**. [The article is downloadable on D2L.]

(a) What is the human gene name, and abbreviation (six letters) that was found to causative of Charcot-Marie-Tooth neuropathy in this subject's genome (you can use this gene name to find the gene quickly, use the "gene" window).
Homo sapiens SH3 domain and tetratricopeptide repeats 2 (SH3TC2)

(b) Use the genome.ucsc.edu human genome browser (version NCBI36/hg18) to answer the following questions. How many exons does this gene have (use "RefSeq Genes" track, or dark blue "UCSC Genes" track)? What is the "genomic size" (full length, including exons and introns)?

17 exons; +/- 81,024 (Depending on where you look this answer could probably be +/- 100ish. If you see a number in this ballpark (26580) that means you looked at the mRNA and not the genome sequence.

(c) Which DNA sequencing technology was used?

SOLID

(d) The paper describes following two independent mutations through the extended family, and showed only those who inherited both mutations had the disease. What are these mutations? (i.e. Q340R)

Y169H and R954X

(e) For family members with only one bad allele (haploinsufficiency), what were two typical symptoms?

Symptom 1: "confers predisposition to a mild polyneuropathy" (kind of a general neural disfunction).

Symptom 2: "particular susceptibility to the carpal tunnel syndrome"

5. **Extra Credit** This question also refers to Charcot-Marie-Tooth paper discussed in question #4. It is extra credit for ALL students (graduate and undergraduate).
Use the genome.ucsc.edu human genome browser (version NCBI36/hg18)

(a) Using coordinates and/or protein sequence from Figure 2 from the paper you found in question 6 (you need the full text, not just the abstract), and its legend, find the position in the UCSC genome browser of the mutation that normally codes for Tyrosine. Figure 2C gives this alignment, but it does NOT mention that there are two species that have the precise mutation variant responsible for this disease. What are these two species?
(Hint: in Muliz alignment of 44-vertebrates, click on the settings bar (grey vertical bar on left), and select "+" at the top to select and see all species in the Multiz alignment track. Another hint: note that the gene is on the reverse/minus strand, so to "turn it around" with 5' end on the left, click on the "reverse" button just below the browser window (between the "configure" and "refresh" buttons).

Mouse lemur and Bush Baby have the mutation discussed in the paper. Cat and Microbat also have mutations at this position, but these aren't the same Y->H mutations discussed in the paper.

(b) You want to develop a genetic test for this mutation, so you need to find the closest "SNP" (single nucleotide polymorphism). You notice there is a SNP in

the "Simple Nucleotide Polymorphisms" track right next to your mutation. What is the dbSNP id # (starts with "rs"), and the chromosome coordinate (i.e. chrX:12345443).

Name: rs17722293, Coordinate: chr5:148402467

(c) You notice that this mutation is found in a relatively small exon. If you were to go looking in the largest exon for other genetic mutations, which exon would that be? Give the exon number and first five nucleotides (on the 5' end) of this exon.
Exon number 17, GATGC = Full credit;

CTACG, TTCCA, AAGGT = Partial Credit. Note: CTACG = right location but not the right strand, TTCCA = Wrong strand and wrong side of exon (but right exon), AAGGT= Right strand, right exon, but wrong side.

6. (Advanced) Consider the two state HMM describing DNA sequence that was discussed in class. Namely where one state was GC-poor (we will call this state L) and one state is GC-rich (we will call this state H).

Consider the following parameters of the model:
T(H,H) = 0.5 T(H,L) = 0.5 T(L,H) = 0.4 T(L,L) = 0.6
Emissions:

	A	C	G	T
H	.2	.3	.3	.2
L	.3	.2	.2	.3

The probability of starting in H or L is 0.5 => T(0,L) = 0.5 T(0,H) = 0.5

a) What algorithm is used to calculate the most likely path for a sequence?
Viterbi

b) What is the most likely path for the sequence GGCACTGAA?

$V(j,i) = E(j, b) * \max_k \{V(k,i-1) * T(l,k)\}$
$E(j,b) = P(s_i = b \mid h_i = j)$ (i.e. emit character 'b' from state 'j')
$T(l,k) = P(h_i=k \mid h_{i-1} = l)$ (i.e. transition from state 'k' to 'l')
$V(0,0) = 1$ (initiation case)

	G	G	C	A	C	T
H (.5)	.5*.3 = .15	.15*.5*.3 = .0225 (HH)	.00338 (HH)	3.38×10^{-4} (HH)	6.08×10^{-5} (LH)	6.08×10^{-6} (HH)
L	.5*.2	.1*.5*.2	.00225	5.063×10^{-4}	6.08×10^{-5}	1.09x

(.5)	= .10	= .015 (HL)	(HL)	(HL)	(LL)	10^{-5} (LL)

	G	A	A
H (.5)	$1.13x10^{-6}$ (LH)	$1.31x10^{-7}$ (HH)	$1.9x10^{-8}$ (LH)
L (.5)	$1.13x10^{-6}$ (LL)	$2.36x10^{-7}$ (LL)	$4.2x10^{-8}$ (LL)

Note in the above I've noted which transition is taken. The max value in the last position is in the L state, therefore the correct path is: HHHLLLLLL
The path is marked with grey background.

This can also be easily computed (and is better for longer sequences) in log space using summation:

$V(s,i) = \log(E(s, b)) + \max_k \{\log(V(k,i-1)) + \log(T(k,s))\}$