codecrush

Bioinformatics Session - March 1, 2014 - 9:00am – 12:00pm

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PROGRAM SCHEDULE:

9:00AM-9:15AM	INTRODUCTION
9:15AM-9:30AM	WHAT IS BIOINFORMATICS?
9:30AM-10:25AM	**ACTIVITY**: EXTRACTING DNA FROM A STRAWBERRY
10:25AM-10:45AM	HOW IS BIOINFORMATICS USEFUL?
10:45AM-11:50AM	**ACTIVITY**: FINDING DISEASE GENES, BLAST, and NETWORKS
11:50AM-12:00PM	CONCLUSIONS

ACTIVITY 1: DNA EXTRACTION

Materials:

- 1 plastic zip top bag
- 3 strawberries
- DNA extraction buffer
  - 2 Tb dish soap or shampoo
  - ½ tsp salt (NaCl)
  - ½ c water (H_20)
- Filtering apparatus
  - 1 8oz. clear plastic cup
  - 1 coffee filter or a 3x3in square piece of cheesecloth
- 2 Tb rubbing alcohol
- Toothpick, bamboo skewer, or wooden craft stick

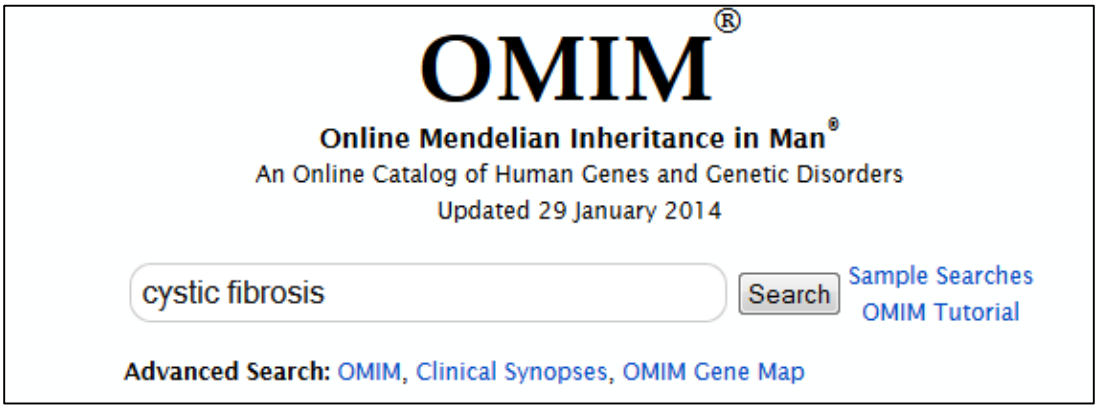
Methods:

1. Obtain strawberries. Remove top stem and any leaves and discard.
2. Place strawberry in zip top baggie. Squeeze the air out of the bag and seal it. *Gently* mash the strawberry into a pulp; try not to create and bubbles and be careful not to tear the bag.
3. Add the DNA extraction buffer to the bag (dish soap, salt, water). Continue to mash the strawberry and mix the pulp with the extraction buffer. Try not to create bubbles.
4. Using the coffee filter or cheesecloth, filter the strawberry solution into the plastic cup. This will take some time, so be patient as the liquid filters through – do not squeeze the filter and try not to tear it or you will have to start over! Discard the plastic bag and filter when you are done.
5. *Gently* and *slowly* pour the 2Tb of rubbing alcohol down the inside of the cup so it forms a layer on top of the strawberry mixture.
6. Observe what happens at the interface between the two liquids. This may take a few minutes. The white material that forms is the strawberries DNA.

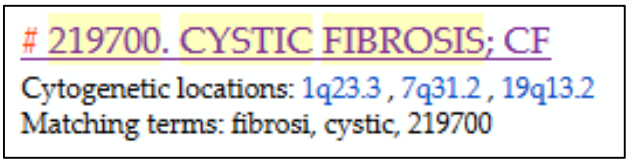
7. Use the toothpick/skewer/craft stick to pull the DNA out of the solution so you can have a closer look. When you are finished with your observations, clean up your area.

ACTIVITY 2: DISEASES GENES AND BIOINFORMATICS TOOLS

1. Go to the Online Mendelian Inheritance in Men website at: http://www.omim.org.
2. In the search box, type "Cystic Fibrosis" and hit the "Search" button.

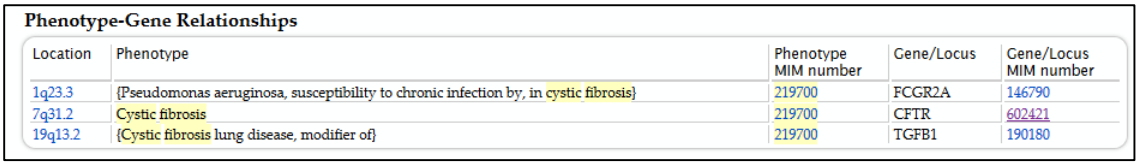


3. Click on the result "#219700 Cystic Fibrosis". If you do not see this result, raise your hand and let one of the assistants know.



4. Examine the Phenotype-Genotype relationships table at the top of the page. *Can you spot the most well-known cystic fibrosis related gene?*

5. Click the 'Gene/Locus MIM Number" for the CFTR gene in the table.

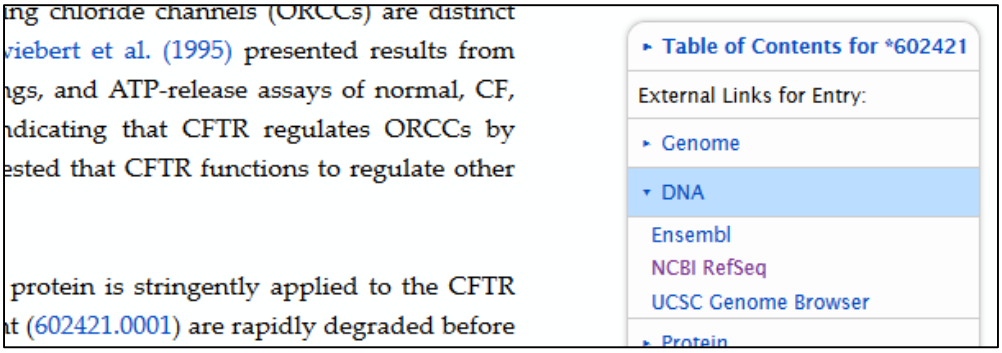


Phenotype-Gene Relationships

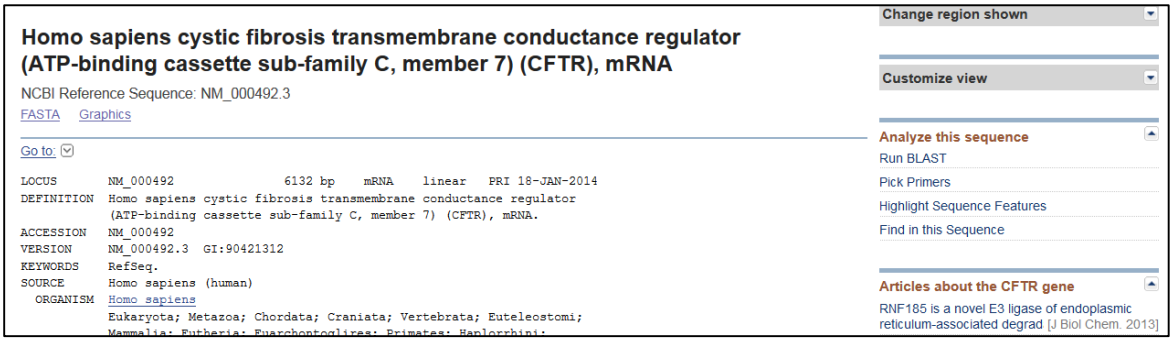
Location	Phenotype	Phenotype MIM number	Gene/Locus	Gene/Locus MIM number
1q23.3	{Pseudomonas aeruginosa, susceptibility to chronic infection by, in cystic fibrosis}	219700	FCGR2A	146790
7q31.2	Cystic fibrosis	219700	CFTR	602421
19q13.2	{Cystic fibrosis lung disease, modifier of}	219700	TGFB1	190180

This will take you to a new page that lists common conditions or "phenotypes" observed when there is a mutation in the CFTR gene in humans, in addition to its location and a link to the phenotype. *What are some of the other phenotypes associated with mutations in the CFTR gene?*

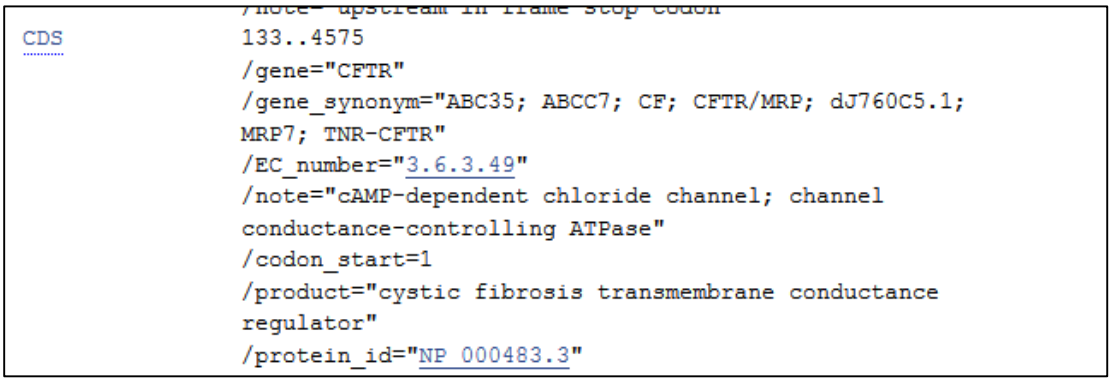
6. In the "External Links" table on the right, click "DNA" and then click "NCBI Refseq." This will take you to the National Center for Biotechnology Information's website, which is the US government's public biological data warehouse. The RefSeq for a gene is the trusted, normal copy of that gene.



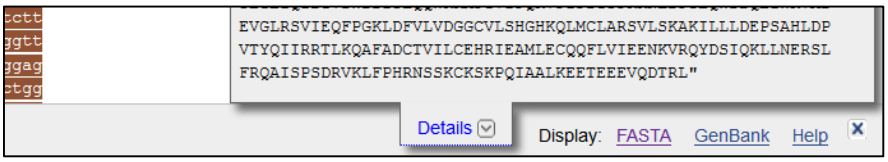
7. This link takes you to the Genbank entry for the *CFTR* gene.



This contains all the information about the gene, including the regulatory promoter region, and other non-coding sequence. To find where the actual protein-coding gene starts, we must scroll down to the "CDS." Clicking on "CDS" will highlight a portion of the genomic sequence at the bottom of the page; this highlighted portion is the protein-coding region of the *CFTR* gene.

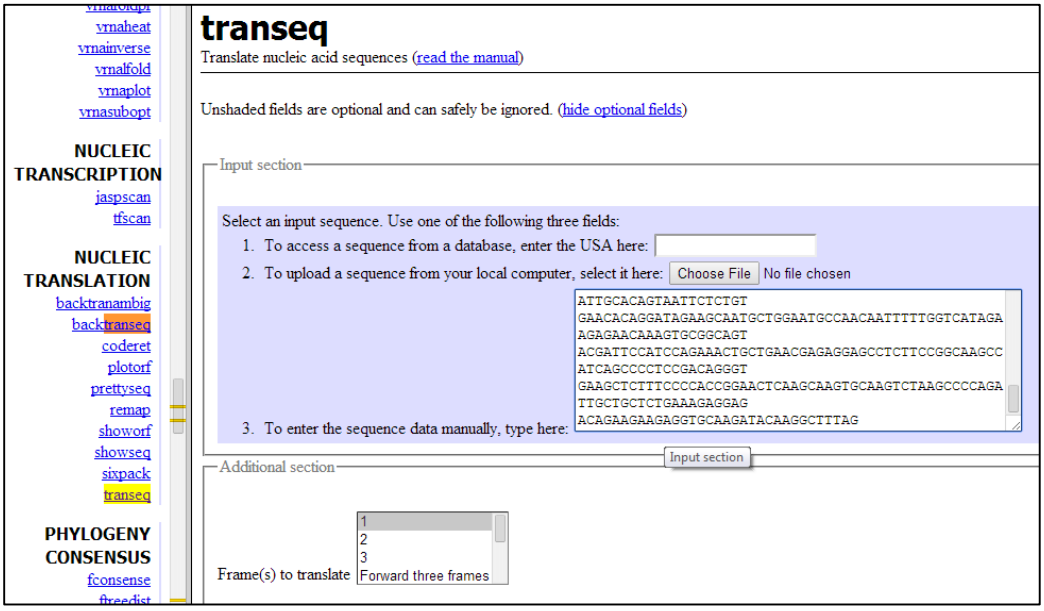


8. Once the "CDS" is clicked, it will highlight the CDS region and a toolbar should pop up at the bottom of the page. On the bottom right of the page, the toolbar should say Display: and then have multiple options. Click the "FASTA" option.



9. This will take us to another GenBank entry, which contains only the CDS portion of the gene. Next, we want to translate this gene into its protein sequence. To do this, highlight and copy the sequence data with your mouse.

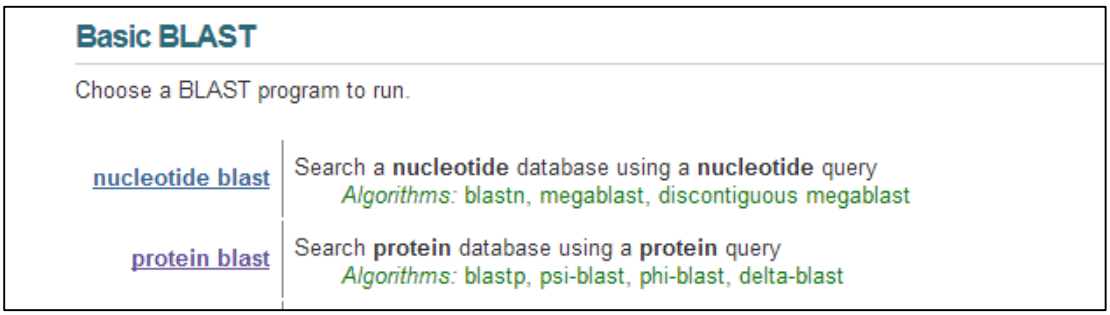
10. Next, we will visit UNO's in-house tool warehouse. In a new tab or window, go to http://biobase.ist.unomaha.edu/ee/ and scroll down to find the "transeq" tool. Click on the link and paste the sequence into the box. Then, scroll to the bottom and click "Run transeq."



11. The resulting protein sequence should be the non-mutated version of the CFTR protein. The deletion mutation that results in cystic fibrosis results in the deletion of a phenylalanine

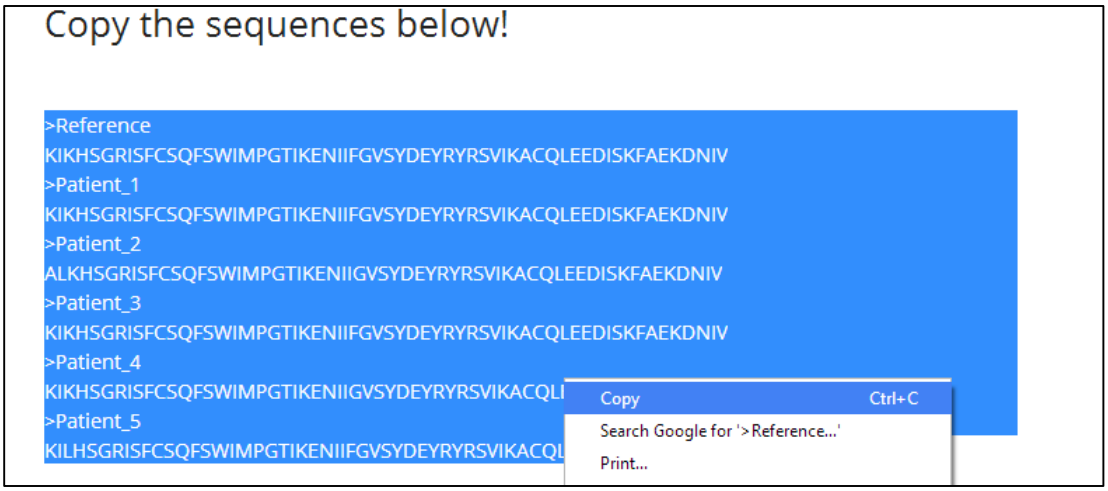
(amino acid symbol F) from the protein, surrounded by an "II" and a "GV." *Search for the "IIFGV" motif in your sequence – is it present or not? If it is, this is indeed the normal protein. If it is not (but "IIGV" is present) – this is the mutated version.*

12. We want to see if we can find similar genes to the *CFTR* gene that will have potential to be disease-causing. To do this, we can use a tool called BLAST that uses a heuristic to search the entire NCBI sequence database quickly and accurately. Go to http://blast.ncbi.nlm.nih.gov/Blast.cgi and click "protein_blast."

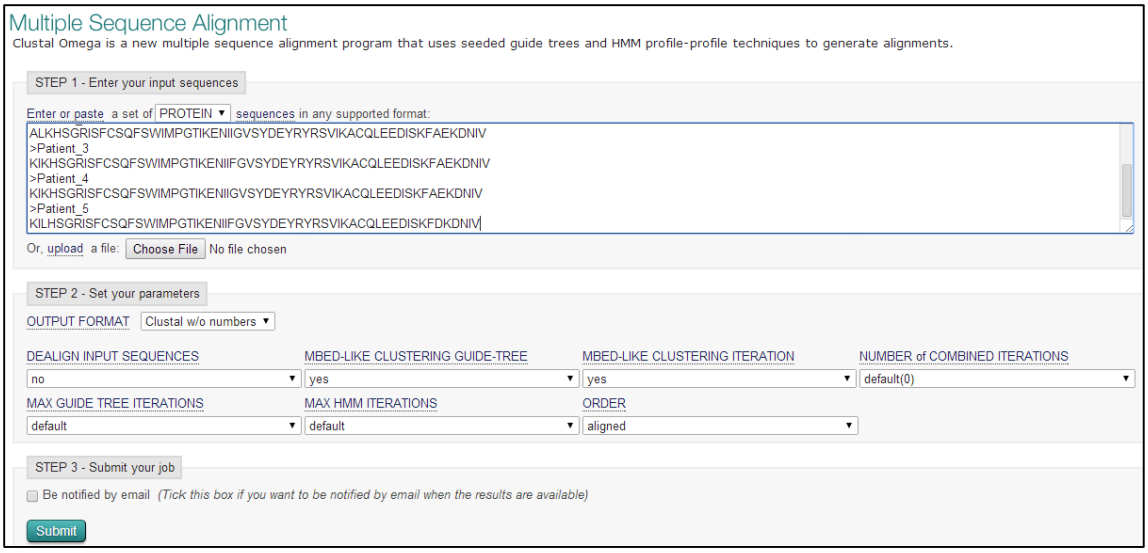


On the next page, paste the protein sequence into the box at the top, then in the "Organism" box, type in "Homo Sapiens." Scroll to the bottom of the page and click "BLAST." The results may take a few minutes to load.

13. Alignment: We want to take a set of personalized genome sequences and see who has the CFTR mutation and who does not. While your BLAST search is running, go to https://sites.google.com/site/sequences2014/ and copy the list of sequences for our five study participants.



Then, go to http://www.ebi.ac.uk/Tools/msa/clustalo/ and paste your sequences in the box, and then click "Submit"



On the alignment screen, click "Show Colors." *Which of the patients carries the CFTR mutation?*

ACTIVITY 3: NETWORKS AND DISEASE:

Materials:

- Index card
- Blue or pink highlighter
- Interaction number *X*

You will be given a number and a highlighter by the instructor. The number you are given is the number of marks you need to receive on your index card. For example, if your number is 3, when the instructor says "Go" you should go get highlighter marks on your index card from 3 other people. *However*, if you get a pink mark on your card, stop immediately and go exchange your blue highlighter for a pink one – all the marks you give out will now be pink. Then you can continue getting marks. Stop when you have the correct amount of marks on your index card. You can give out as many marks as you are asked for, but you should only have *X* marks on your card. *What was your interaction number? Did you get a pink mark? If the pink mark signified something negative, such as the plague, would it be better to have a high interaction number or a lower one? If the pink mark signified something positive, such as an invitation to your favorite concert, would it be better to have a high interaction number or a lower one?*

THANK YOU FOR COMING!!!! We hope you enjoyed your time with us. ☺