

TEACHER LESSON PLAN FOR CLASSROOM USE

**Genetic Relationships of the  
Mustelidae Family.**

**WHO IS THE SEA OTTER RELATED TO?**

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**Designed by**

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**Background**

In this lesson students will gain insight into the genetic relationships between organisms within the Mustelidae family based on the genetic sequence of the protein Rhodopsin.

**Description of Audience:** This biotechnology/bioinformatics activity is designed for use by high school students enrolled in an advanced or AP Biology class.

**State Standards:** This biotechnology/bioinformatics activity fulfills the following State of California Science Standards:

Genetics:

- *4.e. Students know* proteins can differ from one another in the number and sequence of amino acids
- *4.f. Students know* why proteins having different amino acid sequences typically have different chemical properties.

Evolution:

- *8.f. Students know* how to use comparative embryology, DNA or protein sequence comparison from independent sources of data to create a branching diagram (cladogram) that shows evolutionary relationships.

**National Standards:** This biotechnology/bioinformatics activity fulfills the following National Science Standards:

- Content Standard A: Science as Inquiry
- Content Standard C: Life Science
- Content Standard E: Science and Technology

### **STEM Connection.**

This activity is related to the fields of criminology, medicine, genetic counseling, and population biology. Bioinformatic studies are allowing all of these disciplines to expand by providing new data and conclusions. There will obviously be a need for new individuals to assist in the analysis of this new information.

### **Goals(s):**

The goal of this lesson is to (List overall goals. What do YOU, as the teacher, set up for students to learn?):

- To reinforce the importance of genes and their translation into functional proteins.
- To demonstrate how amino acid sequences can be used to show evolutionary relationships.
- To expose students to online biological databases (NCBI) and other programs that are used in Bioinformatics studies.

### **Learning Objective(s)**

Upon completion of this lesson, students will be able to (Include process skills but be specific. What will the STUDENTS be able to do/demonstrate as a result of this lesson?):

- The expected out come is students will start to appreciate what molecular genetics and bioinformatics has done to help humans understand how animals are related.

### **Materials/Resources**

In order to complete this lesson, the following materials are needed:

- Computer with internet access
- Printer

### **Prior Teacher Preparation**

This lesson addresses basic biological concepts involving polypeptide sequences, protein synthesis evolution, and the diversity of life. Students should already have had instruction and lab experiences dealing with these biological concepts.

## **3-Step Procedure**

### **#1 Introduction**

- Make connections between prior knowledge and experiences with what is presented.
- Find out what students ideas are on this topic - uncover misconceptions!
- Review what was learned in prior lessons - then introduce content and vocabulary necessary for today's lesson.
- Use teaching charts, video clips, books, presentation software, instructional software, articles, tapes, overhead projector, handouts, models, etc. to accent instruction.

## #2 Exploration

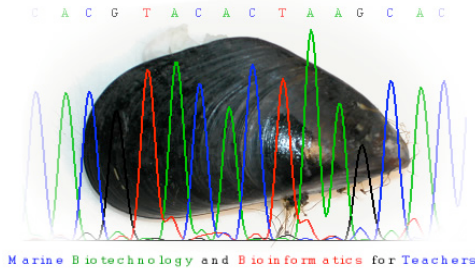
- Students will be given a list of animals within the Mustelidae Family for their research.
- Students will be given a tutorial on how to access and use the NCBI website, as well as ClustalW §Once students have their protein and organisms they will search the NCBI website for the amino acid sequence.
- A master file will be produced to allow students to clean up their titles and formatting.
- Once students have their sequences in the correct format, they will use the ClustalW website to align and execute a phylogenetic tree.

## #3 Application

- By analysing specific proteins that are common in several types of organisms, students and teachers can get a clearer idea of how those organisms relate.
- It is the intention of this lesson/activity that students may become excited about bioinformatics and would encourage them to pursue their own questions and ideas

## Conclusion/Lessons learned

- Students will understand the relationships between animals within the Mustelidae Family.
- Students will be able to access molecular genetic information through the websites used in this lesson
- Students will be able to construct their own phylogenetic tree based on organisms of their own interest.



## Genetic Relationships of the Mustelidae Family.

### WHO IS THE SEA OTTER RELATED TO?

Bioinformatics is emerging as a hugely important field affecting all areas of biology. While bioinformatics is formally the application of computer technologies to biological sciences - ranging from automated analysis of microarrays containing thousands of individual experiments to the development of browser tools for looking at whole genomes - students in all areas of biology need to be familiar with software tools developed by bioinformaticians to accomplish routine tasks in biology.

#### Skills developed in this lab:

- Use of National Center for Biotechnology Information (NCBI) databases
- Retrieval of sequences from NCBI
- Alignment of homologous protein sequences using ClustalX
- Using ClustalX output to prepare phylogenetic networks (trees)
- Testing evolutionary hypotheses

To answer that question we will use the resources of the:

#### [National Center for Biotechnology Information \(NCBI\)](http://www.ncbi.nlm.nih.gov)

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Finding your sequences:

To see what is available for *the Sea Otter* enter that search term. Refine the search by clicking "Protein" and adding the search modifier for "organism" like this:

### **Enhydra lutris [orgn]**

That should reduce the number of hits a bit. Adding "rhodopsin" with quotes like this should help a lot:

### **Enhydra lutris [orgn] " Rhodopsin "**

Finally, if you add the search modifier for "protein" like this:

### **Enhydra lutris [orgn] " Rhodopsin " [prot]**

The resulting sequence you should use:

>**Sea Otter [Enhydra lutris]** VIFFCYGQLVFTVKEAAAQQQESATTQKAEKEVTRMVIIMVIAFLICWVPYASVAFYIFTH

In order to save time I have downloaded an additional **eight sequences** in FASTA format and saved them into a single master file for us to use in this exercise.

#### **>Wolverine [Gulo gulo]**

VIFFCYGQLVFTVKEAAAQQQESATTQKAEKEVTRMVIIMVIAFLICWVPYASVAFYIFTH

#### **>Mink [Mustela nivalis]**

VIFFCYGQLVFTVKEAAAQQQESATTQKAEKEVTRMVIIMVIAFLICWVPYASVA

#### **>Chinese Ferret Badger [Melogale moschata]**

VIFFCYGQLVFTVKEAAAQQQESATTQKAEKEVTRMVIIMVIAFLICWLPYAGVAFYIFTH

#### **>Feret [Mustela putorius furo]**

VIFFCYGQLVFTVKEAAAQQQESATTQKAEKEVTRMVIIMVIAFLICWVPYASV

#### **>American Mink [Mustela vison]**

VIFFCYGQLVFTVKEAAAQQQESATTQKAEKEVTRMVIIMVIAFLICWVPYASVAFYIFTH

#### **>Polecat [Mustela putorius]**

VIFFCYGQLVFTVKEAAAQQQESATTQKAEKEVTRMVIIMVIAFLICWVPYASVA

#### **>Sloat [Mustela erminea]**

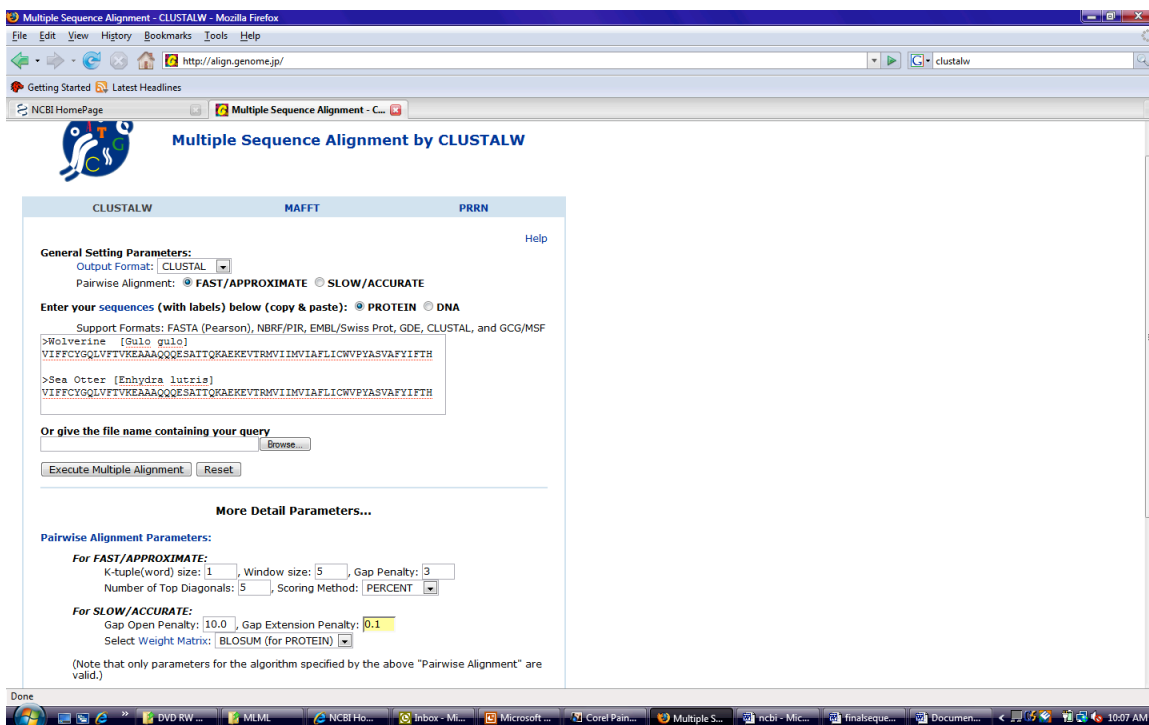
VIFFCYGQLVFTVKEAAAQQQESATTQKAEKEVTRMVIIMVIAFLICWVPYASVAFYIFTH

#### **>American Badger [Taxidea taxus]**

HHILLWPTCLHSQGGGCPAAGVGYHPEGGEGGHTHGHHHGHRIPELLGALRKCRILHHP

## Preparing sequences for comparison by aligning them using ClustalX

1. If you haven't already done so, create a folder called "Sequences" somewhere on your hard drive where you can find it again (Perhaps write down the pathname).
2. **Copy & Paste all nine sequences above into Notepad.** IT IS IMPORTANT THAT YOUR EDITOR BE ABLE TO SAVE THE FILE AS TEXT. ClustalX will only use text files. It will help a lot later if you insert a reasonable name in the space behind the ">". ClustalX reads names up to the first space.
3. Save the file as "all\_nine.txt" or something similar, maybe "seaotter" in your sequences folder, but leave it open.
4. Now open the website at: <http://align.genome.jp/> (Kyoto University Genome Net)
5. Copy and paste all nine sequences from the Notepad window into the Clustal window at the Kyoto University Genome Net website.



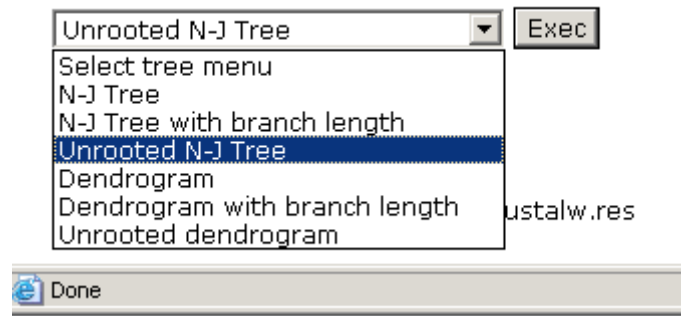
6. Before we ask the program to prepare the alignment for us, we should do some cleaning up of the title lines for the sequences... that will make the trees that are produced much more aesthetically pleasing. Edit each line so there is just the ">" and the name of the organism.
7. Click "Execute Multiple Alignment." Although there can be some machine errors... Clustal does a fairly good job of aligning the sequences. . Not surprisingly the software has been

able to identify the organisms that are most closely related on the basis of their Rhodopsin amino acid sequences.

## Preparing phylogenetic trees based on the sequence comparison

The program also compares the aligned sequences and measures how different they are from each other. The more differences, the less related they should be, and the more distant they should appear on a phylogenetic tree. The program first finds the two most related sequences then adds the next most related "neighbor" sequence. It calculates a difference score and outputs a little file of brackets and numbers that show the relationships and degree of relationship in the form of "branch lengths."

1. Scroll down to the bottom of the "results" web page. You should see a drop down menu like this one:



2. Select the Unrooted Neighbor Joining Tree and click "**Exec**" - and then try the others  
What do you think about the results?

