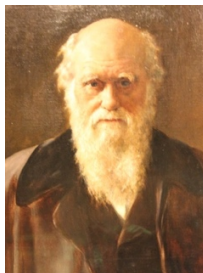
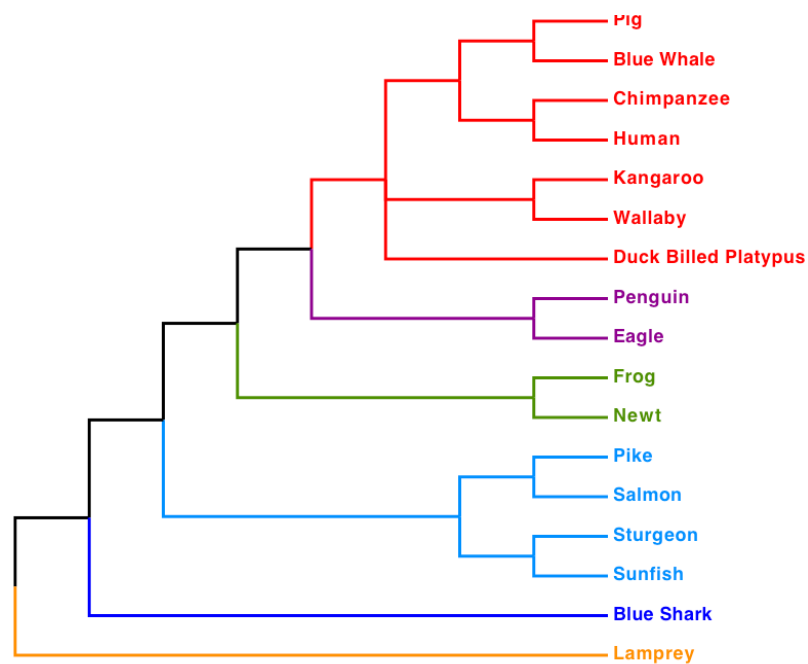


# Investigating Vertebrate Evolutionary Trees

## Student Guide



# Investigating Vertebrate Evolutionary Trees

Appropriate for

**Opportunities for CPAC assessment:** 1(a), 2(a), (b), 4(a), 5(a)

Aim: To use computer modelling software to investigate evolutionary relationships between major vertebrate groups

## Objectives

- ✓ Using protein sequences to determine evolutionary distances between species
- ✓ To create evolutionary trees to study the relationships between vertebrate species and groups of species

## Purpose/Relevance

Modern evolutionary trees are created from amino acid sequences taken from important proteins. Shared amino acids will lead to animals being clustered together, while differences between species lead to them being separated on branches.

Evolution often occurs in a regular, clocklike, fashion. This means closely related animals have very similar sequences and the number of differences in amino acids increases over evolutionary time.

We will use the protein **NADH dehydrogenase 3** to study how vertebrates (animals with backbones) have evolved. The dataset provided will show the closest relatives of humans and also how major vertebrate groups are related to each other.

## Notes

- All protein sequences have been downloaded from the NCBI database
- Sequences will be aligned using ClustalX 2.1
- Evolutionary trees will be generated in ClustalX 2.1 and viewed using FigTree 1.4.3

## Health and Safety

- Do not eat or drink near the computer

## Exercise 1

- In this first experiment we will compare the human protein with the same protein from a chimpanzee
- Copy the protein sequences, including the title line, for human and chimpanzee and paste into a Notepad++ document.
- Save the document as a .txt file (e.g. HumanChimp.txt)
- Open ClustalX and click on **Load Sequences** from the **File** Menu
- Open your HumanChimp.txt file
- From the **Alignment** menu click on **Do Complete Alignment** – Clustal will now match the amino acids from humans with the corresponding amino acids from the chimpanzee.
- Clustal shows matches of amino acids with \*. Non-matches are shown by :, . or a **gap**
- How many amino acids differ between the human and chimpanzee? Make a note of this number.

## Exercise 2

- We now want to compare the human protein with the protein from a Neanderthal. The Neanderthals were a species of human who are now extinct. Do you think the human protein will be more similar to the chimpanzee or Neanderthal?
- Copy the protein sequences, including the title line, for human and Neanderthal and paste into a new Notepad<sup>++</sup> document.
- Save the document as a .txt file (e.g. HumanNeanderthal.txt)
- Open ClustalX and click on **Load Sequences** from the **File** Menu
- Open your HumanNeanderthal.txt file
- From the **Alignment** menu click on **Do Complete Alignment**.
- How many amino acids differ between the human and Neanderthal? Make a note of this number.
- From the evidence of NADH dehydrogenase 3 can you say if humans are more closely related to Neanderthals or chimpanzees?

## Exercise 3

- In this exercise we will look at deeper evolutionary relationships by studying three well known mammals.
- Using the protocol from above determine the number of differences between the cow protein and the pig protein.
- In a new text file now compare the cow protein with the giraffe protein.
- Do you think cows are more closely related to pigs or giraffes?

## Exercise 4

- Comparing two protein sequences can give us important information on the divergence between two species.
- We can gain more information by aligning multiple sequences and creating an evolutionary tree.

- In this exercise we will create an evolutionary tree of the primates. We will use this to test the results we gained in the first two exercises.
- Copy and paste all of the primate proteins, along with their titles, into a new Notepad<sup>++</sup> document and save as Primates.txt.
- Load your primate file into Clustal and align the amino acids. You should notice now there are fewer asterisks – this means there are more evolutionary changes in this alignment.
- We can now create our evolutionary tree of primates. Go to the **Trees** menu and click on **Draw Trees**.
- Open the program FigTree to view your tree. Click on **Open** in the **File** menu and open the file name Primates.ph
- To read the tree correctly we need to 'root' it. This means to place the oldest group at the bottom of the tree. In this tree the root is between the howler and woolly monkeys and the other primates.
- With the cursor click on the branch leading to the howler and woolly monkeys. Now press Reroot.
- Closely related organisms share more branches, so we can see that humans are more closely related to Neanderthals than chimpanzees. Is this what you found before?
- FigTree allows us to highlight different groups. Click on the branch that leads to the five species of ape.
- Click on the **Clade** button. This should highlight all of the branches in the group of apes.
- Press Colour and choose a colour to denote the apes on your tree.
- You can save your tree by going to **File** and clicking **Export PDF**. This will give you an image file of your evolutionary tree.

### Exercise 5

- We will now create an evolutionary tree of all of the mammal proteins.
- Paste the protein sequences, along with their titles into a new Notepad<sup>++</sup> document. Save as **Mammals.txt**. Do not paste in the subheadings, e.g. **Primates**.
- Align your sequences in Clustal.
- We can now create our evolutionary tree of mammals. Go to the **Trees** menu and click on **Draw Trees**.
- Open the program FigTree to view your tree. Click on **Open** in the **File** menu and open the file named Mammals.ph
- To read the tree correctly we need to 'root' it. In this tree the root is between the marsupials (kangaroo, wombat and Tasmanian Devil) and the other mammals.
- With the cursor click on the branch leading to the marsupials. Now press Reroot.
- Colour code the different mammal groups using **Clade** and **Colour**.

### Questions

- Have all of the groups been correctly recovered as intact groups?
- What mammals are the closest relatives of the primates?
- Are the marine mammals all closely related to each other or do they form different groups? You will need to identify the marine mammals in the tree to answer this.
- From your tree can you determine if the giant panda is more closely related to the red panda or bears?

### Exercise 6

In the last exercise we will create an evolutionary tree using all of the proteins in our dataset. This will give us a broad picture on how the vertebrates have evolved for the last 470 million years.

- Paste the protein sequences, along with their titles into a new Notepad<sup>++</sup> document.
- Save as **Vertebrates.txt**. Do not paste in the subheadings, e.g. **Primates**.
- Align your sequences in Clustal.
- We can now create our evolutionary tree of vertebrates. Go to the **Trees** menu and click on **Draw Trees**.
- Open the program FigTree to view your tree. Click on **Open** in the **File** menu and open the file named Vertebrates.ph.
- To root this evolutionary tree correctly you will need to place the root between the sharks and rays and the other vertebrates.
- With the cursor click on the branch leading to the sharks and rays. Now press Reroot.
- Colour code the different vertebrate groups using **Clade** and **Colour**.

### Questions

- Have all of the vertebrate groups been correctly recovered as intact groups in the tree?
- Using your tree can you tell if the bony fish called the coelocanth are more closely related to land vertebrates or other bony fish?
- Your tree shows the evolutionary origin of birds. Which group of vertebrates did the birds evolve from?
- If you look at the Clustal alignment for all vertebrates you will see very few columns have asterisks. This means that most amino acids in NADH dehydrogenase 3 have changed during vertebrate evolution – can you think why some amino acids have not changed?

## Glossary

**Alignment:** An ordered manner of writing out amino acid or nucleotide sequences. Columns in the alignment should contain characters with shared evolutionary history.

**Branch:** Lines in a *phylogenetic tree* which show evolutionary history. Branch length is proportional to time, e.g. long branches represent long evolutionary time. Branches split at points called *nodes*.

**Clade:** An evolutionary group with a single origin. None of the organisms in the group have evolved into other types of species.

**Node:** A point in a *phylogenetic tree* where a *branch* splits into two. This represents a point where one species diverges into two.

**Phylogenetic Tree:** A graphical representation of how organisms or genes have evolved. Phylogenetic trees are estimates based upon a particular dataset, so different datasets may give different trees.

**Root:** The oldest point in a *phylogenetic tree*. All species have evolved from the organism that existed at the time of the root.

# NADH Dehydrogenase 3 Protein Sequences

## Mammals

### *Primates*

>Human

MNFALILMINTLLALLLMIITFWLPQLNGYMEKSTPYECGFDPMSPARVPFSMKFFLVAITFLLFDLEIA  
LLLPLPWALQTTNPLPLMVMSLLLIILALSLAYEWLQKGLDWTE

>Neanderthal

MNFALILMINTLLALLLMIITFWLPQLNGYMEKSTPYECGFDPMSPARVPFSMKFFLVAITFLLFDLEIA  
LLLPLPWALQTTNPLPLMVTSSLLLIILALSLAYEWLQKGLDWAE

>Chimpanzee

MNFVLIILMTNTLLALLLMIITFWLPQLNSYMEKSTPYECGFDPMSPARVPFSMKFFLVAITFLLFDLEIA  
LLLPLPWALQTTANPLPLMVTSSLLLITILALSLAYEWLQKGLDWTE

>Gorilla

MNFALILMTNTLLALLLMIITFWLPQLNSYMEKSTNPYECGFDPVSPARIPFSMKFFLVAITFLLFDLEIA  
LLLPLPWALQTTNPLPLMVMSLLLIILTLAYEWLQKGLDWAE

>Orangutan

MNFVLALTINTLLALLLMIITFWLPQLNPYMEKSDPYECGFDPAYPARIPFSMKFFLVAITFLLFDLEIA  
LLLPLPWALQTTNPLPLMTTSSMLLIILALGLTYEWSQKGLDWAE

>Black\_Howler\_Monkey

MNLTTLTATNTLLALLLVTITFWLPQLNTYTEKFNPYECGFDPPTSAHLPFMSMKFFLVAITFLLFDLEIA  
LLLPLPWATQTNNMLTINTILTIIILALGLAYEWTQKGLDWVE

>Brown\_Woolly\_Monkey

MNLMLTTLTNTLLALLLVTITFWLPQLNIYTEKFNPYECGFDPPTTSARLPFSMKFFLIAITFLLFDLEIA  
LLLPLPWATQTNNMLTINMILALIVILAVGLAYEWTQKGLDWIE

>SnubNosed\_Monkey

MNLVLALTINTLLTLLLMIIMFWLPQLNPYAEKINPYECGFDPPLNSARIPFSMKFFLVAITFLLFDLEIA  
LLLPLPWALQTTSLPMMFKSSIMLIVILTLAYEWTQKGLEWIE

>Rhesus\_Monkey

MNLVLALTINTLLTSLLMIIMFWLPQLNPYTEKSTPYECGFDPPLNPARIPFSMKFFLVAITFLLFDLEIA  
LLLSLPWAIQTNNLPMMIKSTIAFIIILISLTYEWTQKGLDWAE

### *Rabbits & Hares*

>European\_Hare

MNLIIVLLINMTISLILVTIAFWLPQLNIYSEKSTPYECGFDPMG SARLPFSMKFFLVAITFLLFDLEIA  
LLLPLPWAAQFNNLNVLIMALMLISILALGLAYEWIQKGLEWVE

>Rabbit

MNLMLVLLINTTISLVLVTIAFWLPQLNIYSEKSSPYECGFDPMG SARLPFSMKFFLVAITFLLFDLEIA  
LLLPLPWAAQFNNLNVLIMALMLISILALGLAYEWIQKGLEWVE

### *Ungulates (Hoofed Animals)*

>Pig

MNIMLTLLTNVTLASLLVLIAFWLPQLNAYSEKSTPYECGFDPMG SARLPFSMKFFLVAITFLLFDLEIA  
LLLPLPWASQANNLKTMALFLILLLAASLAYEWTQKGLEWAE

>Deer

MNLMLALLTNFTLASLLVIAFWLPQLNVYSEKSTPYECGFDPMG SARLPFSMKFFLVAITFLLFDLEIA  
LLLPLPWASQTNNLGTMALFLILLLAASLAYEWTQKGLEWTE

>Giraffe

MNLMLTLLINLLLATLLVTIAFWLPQLNVYSEKSTPYECGFDPMG SARLPFSMKFFLVAITFLLFDLEIA  
LLLPLPWASQTNDLYTMLTMAALLLILLLAASLAYEWTQKGLEWTE

>Cow

MNLMLALLTNFTLATLLVIAFWLPQLNVYSEKSTPYECGFDPMG SARLPFSMKFFLVAITFLLFDLEIA  
LLLPLPWASQTANLNTMLTMAFLIILLAVSLAYEWTQKGLEWTE

>Killer\_Whale

MNLLSTLLTNTTLALLLMLIAFWLPQLNTYAEKSTPYECGFDPMG SARLPFSMKFFLVAITFLLFDLEIT  
LLLPLPWAIQTNNLTMLLMALFLISLLATSLAYEWTQKGLEWVK

>Blue\_Whale

MNLLLTLLTNTTLALLLVFIAFWLPQLNVYAEKSTPYECGFDPMG SARLPFSMKFFLVAITFLLFDLEIA  
LLLPLPWAIQSNNLNTMLTMAFLISLLAASLAYEWTQEGLEWAE

>Bottlenose\_Dolphin

MNLLLTLLTNTTLALLLMLIAFWLPQLNTYAEKSTPYECGFDPMG SARLPFSMKFFLVAITFLLFDLEIA  
LLLPLPWAIQTNNLTMLLMALFLISLLAASLAYEWTQKGLEWVK

>Sperm\_Whale

MNILLTLLTNTTLALLLMLIAFWLPQLNAYTEKSTPYECGFDPMSARLPFSMKFFLVAITFLLFDLEIA  
LLLPLPWATQTNNLKTMALFLISLLAASLAYEWTQEGLEWAE

## ***Carnivores***

>Fur\_Seal

MNMILALLTNTILASLLVLIAFWLPQLNIYSEKASPYECGFDPMG SARLPFSMKFFLVAITFLLFDLEIA  
LLLPLPWASHANNLT TTTLTMALMLISLLAASLAYEWTEKGLEWTE

>Walrus

MNMILTLLTNTALASLLMLIAFWLPQLNTYSEKASPYECGFDPMG SARLPFSMKFFLVAITFLLFDLEIA  
LLLPLPWASHANNMTTVLT TALMLISLLAASLAYEWA EKGLEWTE

>Cat

MNVMLALLTNTLLSTLLVLIAFWLPQLNIYAEKASPYECGFDPMG SARLPFSMKFFLVAITFLLFDLEIA  
LLLPLPWASQTDKLP TMLTMALLLISLLAASLAYEWTQKGLEWTE

>Lion

MNVMLALLTNTLLSTLLALIAFWLPQLNISAEKASPYECGFDPMG SARLPFSMKFFLVAITFLLFDLEIA  
LLLPLPWASQTNKLSTMLTMALLLISLLAASLAYEWTQKGLEWTE

>Tiger

MNMMLALLTNTLLSTLLMLIAFWLPQLNTYAEKASPYECGFDPMG SARLPFSMKFFLVAITFLLFDLEIA  
LLLPLPWASQTNKLSTMLITALLLISLLAASLAYEWTQKGLEWTE

>Dog

MNVMLTLMTNVTLASLLVLIAFWLPQLNIYTDKTS PYECGFDPMG SARLPFSMKFFLVAITFLLFDLEIA  
LLLPLPWASQTNKLTTMLIMALLLISLLAASLAYEWTEKGLEWTE

>Wolf

MNVMLTLMTNVTLASLLVLIAFWLPQLNIYTDKTS PYECGFDPMG SARLPFSMKFFLVAITFLLFDLEIA  
LLLPLPWASQTNKLTTMLIMALLLISLLAASLAYEWTEKGLEWTE

>Fox

MNMMLTLMTNITLASLLVMIAFWLPQLNIYADKTS PYECGFDPMG SARLPFSMKFFLVAITFLLFDLEIA  
LLLPLPWASQTNKLTTMLIMALLLISLLAASLAYEWTEKGLEWTE

>Badger

MNMLLTLLINVS LASLLVLIAFWLPQLNIYTEKASPYECGFDP LGSARLPFSMKFFLVAITFLLFDLEIA  
LLLPLPWASQSSNLNTTLATALLLILLATS LAYEWAEEGLEWNE

>Otter

MNMMLTLFTNVSLASLLILIAFWLPQLNIYTEKAGPYECGFDP LGSARLPFSMKFFLVAITFLLFDLEIA  
LLLPLPWASQSINLKTTLTALT LVSLAYEWTEEGLEWNE

>Brown\_Bear

MNLVLA LFTNMLLASLLVLIAFWLPQLNIYAEKASPYECGFDPMG SARLPFSMKFFLVAITFLLFDLEIA  
LLLPLPWASQTDNLM TMLTMALLLISLLAVSLAYEWA EKGLEWAE

>Polar\_Bear

MNLILA LFTNMLLASLLVLIAFWLPQLNIYAEKASPYECGFDPMG SARLPFSMKFFLVAITFLLFDLEIA  
LLLPLPWASQTDNLM TMLTMALLLISLLAVSLAYEWTEKGLEWAE

>Giant\_Panda

MNLILA LLTNVLLASLLVLIAFWLPQLNIYAEKVSPYECGFDPMG SARLPFSMKFFLVAITFLLFDLEIA  
LLLPLWALQTNNLM TMLIMALLLISLLAASLAYEWTEKGLEWAE

>Red\_Panda

MNLIMTLFINITLTSLVLIAFWLPQLNIYTEKTS PYECGFDPMG SARMPFSMKFFLVAITFLLFDLEIA  
LLLPLPWATQTVNLT TMLTTALLLISLLAVSLAYEWTEKGLEWTE

## ***Afrotheria (Mammals Who Evolved From Africa)***

>African\_Elephant

MNLMTTLLTNTMLTSLMVLIAFWLPQTYTYSEKTS PYECGFDPMG SARLPFSMKFFLVAITFLLFDLEIA  
LLLPLPWAIQANDTNLTLLMSFMLI ILLAIGLAYEWLQKGLEWTK

>Asian\_Elephant

MNLMTTLLTNTMLTSLMVLIAFWLPQTYTYSEKTS PYECGFDPMG SARLPFSMKFFLVAITFLLFDLEIA  
LLLPLPWAIQANNTNLTLLMSFMLI ILLAIGLAYEWLQKGLEWTK

>Woolly\_Mammoth

MNLMATLLTNTMLTSLMVLIAFWLPQTYTYSEKTS PYECGFDPMG SARLPFSMKFFLVAITFLLFDLEIA  
LLLPLPWAIQANNTNLTLLMSFMLI ILLAIGLAYEWLQKGLEWTK

>Seacow

MNLMTLFTNATLASLLILIAFWLPQSYAYAEKVTPYECGFDPMG SARLPFSMKFFLVAITFLLFDLEIA  
LLLPLPWAIQATNLNLVLFMALALITLLALSLAYEWIQKGLEWVE

## ***Marsupial Mammals***

>Kangaroo

MNLIITLIINTALSTIIVLIAFWLPQLYLYLEKSSPYECGFDP LGSARLPFSMKFFLIAITFLLFDLEIA  
LLLPLPWAIQLPTPNSTLILAYCLII LLTAGLAYEWIQKGLEWTE

>Tasmanian\_Devil

MNLMTLTLLINSTLATVVVLIAFWLPQLYLYLEKSSPYECGFDP LGSARLPFSMKFFLVAITFLLFDLEIA  
LLLPLPWAIQLPTANTILFLSYFII ILLTGGLAYEWFQKGLEWTE

>Wombat

MINLIITLIVNSLLSTIIVLIAFWLPQLYLYMEKSSPYECGFDP LGSARLPFSLKFFLIAITFLLFDLEI  
ALLLPLPWA VQLSNPNTMLILSYGLI LLLTAGLAYEWLQKGLEWTE

## **Birds**

>Golden\_Eagle

MNMITFMLTTLTSLIILLITLNLWLAQTNPDSEKLSPYECGFDPLGSARLPFSIRFFLVAILFLLFDLEI  
ALLLPLPWAMQLQSPTTTLTWASIIISLLTLGLIYEWLQGGLEWAE

>Jackass\_Penguin

MNMITFMLILSLALSVALTTLNFWLAQTNPDSEKLSPYECGFDPLGSARLPFSIRFFLVAILFLLFDLEI  
ALLLPLPWAIQLQSPTTTLTWASAIILLTLGLIYEWMQGGLEWAE

>Puffin

MNMITFMLTSLALSIVILTTLNFWLAQMNPDEKLSPYECGFDPLGSARLPFSIRFFLVAILFLLFDLEI  
ALLLPLPWAVQLQSPTTTLIWTFTIIILLTLGLIYEWMQGGLEWAE

>Parrot

MNMIMFMLISSIILSMALTTLNFWLTQMPDSEKLSPYECGFDPLGSARLPFSIRFFLVAILFLLFDLEI  
ALLLPLPWATQLKHPPTTLIWASTIILLTLGLIYEWQGGLEWAE

>Peregrine\_Falcon

MNTILFMLTSLTSLIILISLNFWLAQTPDSEKLSPYECGFDPLGSARLPFSIRFFLVAILFLLFDLEI  
ALLLPLPWATQLQSPTTTLTWTLLIITLLTLGLIYEWLQGGLEWAE

>Swan

MNMLMFMFALSSILSAALIALNFWLAQMNPDEKLSPYECGFDPLGSARLPFSVRFFLVAILFLLFDLEI  
ALLLPLPWAIQLQSPLMTLAWTVAILLLTLGLAYEWVQGGLEWAE

>Duck

MNMLTFMFSLSLVLSAILTALNFWLAQMPDSEKLSPYECGFDPLGSARLPFSIRFFLVAILFLLFDLEI  
ALLLPLPWAIQLQSPLMTLAWTAAILSLTLGLAYEWAQGGLEWAE

## **Reptiles**

>Sand\_Lizard

MNLMTMFIITTLISTILILISFWLPQMPLDMEKLSPYECGFDPLGSARLPFSIRFFLVAILFLLFDLEIA  
LLLPTPWATNLPYPMVTVSWVYTIITLLTLGLIYEWQGGLDWAE

>Komodo\_Dragon

MNFVLMMLASFTISTLLIALSFWLPQTMPDMEKLSPYECGFDPLGSARLPFSLHFFLVAILFLLFDLEIA  
LLLPLPWATNLPNPTITLILTLIISLLTLGLIYEWKQGGLEWAI

>Turtle

MNMTISTMTIAITLSTILMVLNHWLTLMKPDTEKLSPYECGFDPLESARLPFSIRFFLVAILFLLFDLEI  
ALLLPLPWAIQLPSPTCTFTWALIILLLLTLGFMHEWIQGGLEWAE

>Terrapin

MNVTISTMTIAITLSTILMALNYWLTLMKPDTEKLSPYECGFDPLESARLPFSIRFFLVAILFLLFDLEI  
ALLLPLPWAIQLPSPIYFTFWALIILLLLTLGLIYEWVQGGLEWAE

>Tortoise

MNTTISIMIISLALSTILIMLNYWLTLMKPDNEKLSPYECGFDPLESARLPFSIRFFLVAILFLLFDLEI  
ALLLPLPWAIQLPHPTHSTWALTILSLLTLGLIYEWIQGGLEWAE

## **Amphibians**

>Japanese\_Gliding\_Frog

MMLVFTLMALTLSVILALISFWLPDTEKLSPYECGFDPLGSARLPYSMRFFLVAILFLLFDLEIAL  
LLPMPWAIQLTTPPSVMMWAMMVLLLLTMGFIYEWDAQGLEWAE

>Marsh\_Frog

MLLFFSIASLLSIIAAVSFWLPDTEKLSPYECGFDPLGSARLPYSMRFFLVAILFLLFDLEIAL  
LPTPWAIQLPNPLMTIIWASIIIVILLTLGFIYEWLQGGLEWAE

>Marine\_Toad

MSLFVFTLAIVLILATVSFWLPMPDSEKLSPYECGFDPLGSARLPYSMRFFLVAILFLLFDLEIAL  
LPTPWAAQLPHPTLSIFWASIIILILLTLGFIYEWLQGGLEWAE

>Tibetan\_Toad

MTLFVILTIVIVSILASISFWLPDTEKLSPYECGFDPLGSARLPYSMRFFLVAILFLLFDLEIAL  
LPTPWAAQLPYPTLSILLASVILILLTLGFVYEWLQGGLEWAE

## **Bony Fish**

>Coelocanth

MNLILAGLLIMSIILSMILAVIAFWLPNMPDTEKLSPYECGFDPLGSARLPFSLRFFLVAILFLLFDLEI  
ALLLPLPWADQLTNPTLVLTWTTTIIALLTLGLIHEWTQGGLEWAE

>Menado\_Coelocanth

MNLILAGLLIMSIILSMILAVIAFWLPNMPDTEKLSPYECGFDPLGSARLPFSLRFFLVAILFLLFDLEI  
ALLLPLPWADQLTNPTLVLTWTTTIIALLTLGLIHEWTQGGLEWAE

>Salmon

MNLITTTIAITITLSAVLATISFWLPQMPDPAEKLSPYECGFDPLGSARLPFSLRFFLIAILFLLFDLEI  
ALLLPLPWGDQLTTPALTALWASAAVLALLTLGLIYEWQGGLEWAE

>Cod



MNLISTVILIASALSLILILVSWFLPQLSPDYEKLSPYECGFDPLGSARLPFSLRFFLIAILFLLFDLEI  
ALLPLPWGDQLSNPTLTFMWATSVLALLTLGLIYEWLQGGLEWAE  
>Haddock  
MNLISTVILIASALSLILILVSWFLPQLSPDYEKLSPYECGFDPLGSARLPFSLRFFLIAILFLLFDLEI  
ALLPLPWGDQLSNPSLTFMWATSVLALLTLGLIYEWLQGGLEWAE  
>Trout  
MNLITTIITITITLSAVLATVSWFLPQITPDAEKLSPYECGFDPLGSARLPFSLRFFLIAILFLLFDLEI  
ALLPLPWGDQLATPALTLAWSAAVLALLTLGLIYEWLQGGLEWAE  
>Stickleback  
MNLVTTVTITITAALSIVLALVSWFLPQMTPDHEKLSPYECGFDPLGSARLPFSLRFFLVAILFLLFDLEI  
ALLPLPWGDQLATPLTLFWATAVLALLTLGLIYEWLQGGLEWAE  
>Pike  
MNLISTIFVITITITSAILATLSFWLPQMSPDTEKLSPYECGFDPLGTARLPFSLRFFLVAILFLLFDLEI  
ALLPLPWADQLSSPTLTLFWATTITLTLGLIYEWLQGGLEWAE  
>Goldfish  
MNLIMTILTITTALSLILATISFWLPQMNPDAEKLSPYECGFDPLGSARLPFSLRFFLVAILFLLFDLEI  
ALLPLPWGDQLNNPTGTFFWATTVLILLTLGLIYEWLQGGLEWAE  
>Barracuda  
MSLITTIILITLILSVILATVSWFLPQMNPDYEKLSPYECGFDPLGSARLPFSLRFFLIAILFLLFDLEI  
ALLPLPWGDQLASPLTLFWASAVLILLTLGLIYEWLQGGLEWAE  
>Herring  
MSLIMVVLAITLILSIILVIVSWFLPQMTPDHEKLSPYECGFDPRGSARMPFSLRFFLVAILFLLFDLEI  
ALLPLPWAYQLDNPMVTVVWAGAVLALLTLGLVYEWLQGGLEWAE  
>Sardine  
MSLIMVVLAITLILSIILVIVSWFLPQMTPDHEKLSPYECGFDPRGSARMPFSLRFFLVAILFLLFDLEI  
ALLPLPWAYQLANPLTTVAWATAVLVLLTLGLVYEWLQGGLEWAE

## **Sharks & Rays**

>Great\_White\_Shark  
MNLIMSSVAATALISLILVFIAFWLPSLNPNEKLSPYECGFDPLGNARLPFSLRFFLVAILFLLFDLEI  
ALLPLPWGNQLLSPLHTLFWATTILILLTLGLIYEWLQGGLEWAE  
>Tiger\_Shark  
MNLIMSSVVATALVSLMLALIAFWLPLLNPDNEKLSPYECGFDPLGNARLPFSLRFFLVAILFLLFDLEI  
ALLPLPWGNQSLTPLSTLFWATIILLILLILGLIYEWLQGGLEWAE  
>Dogfish  
MNLVMSSVAATALISLILAFVAFWLPLLNPDNEKLSPYECGFDPLGSARLPFSLRFFLVAILFLLFDLEI  
ALLPLPWGNQLLTPFISLLWATSIIILLTLGLIYEWLQGGLEWAE  
>Whale\_Shark  
MSLIMSSIAITALISLILATIAFWLPLLNPDNEKLSPYECGFDPLGNARLPFSLRFFLVAILFLLFDLEI  
ALLPLPWGDQLPMPSYTLWASIIILMLTLGLIYEWLQGGLEWAE  
>Basking\_Shark  
MSLITSSVAATALISLILVFITFWLPSLSPDNEKLSPYECGFDPLGSARLPFSLRFFLVAILFLLFDLEI  
ALLPLPWGDQLLSPLSTLLWAATILILLTLGLIYEWLQGGLEWAE  
>Manta\_Ray  
MNLITFIVALTALISLILAILAFWLPSLNPNEKMSPYECGFDPLGTARLPFSLRFFLIAILFLLFDLEI  
ALLPLPWGDQLTSPLITSLWAATILFLLTLGLIYEWLQGGLEWAE  
>Stingray  
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