Our Animal DNA:
Comparing genes across the Tree of Life

Practical 2: align sequences with Clustal

Step 1: Fetch human sequence

First, we need to export our sequences from Ensembl. We already have our human sequence from Practical 1, which looks like this:

Header

```
>ENSG00000139648:ENST00000267119.6 peptide: ENSP00000267119 pep:protein_coding
MSRQFTCKSGAAAKGGFSGCSAVLSGGSSSSFRAGSKGLSGGFGSRSLYSLGGVRSLNVA
SGSGKSGGYGFGRGRASGFAGSMFGSVALGPVCPTVCPPGGIHQVTVNESLLAPLNVELD
PEIQKVRAQEREQIKALNNKFASFIDKVRFLEQQNQVLETKWELLQQLDLNNCKNNLEPI
LEGYISNLRKQLETLSGDRVRLDSELRNVRDVVEDYKKRYEEEINKRTAAENEFVLLKKD
VDAAYANKVELQAKVESMDQEIKFFRCLFEAEITQIQSHISDMSVILSMDNNRNLDLDSI
IDEVRTQYEEIALKSKAEAEALYQTKFQELQLAAGRHGDDLKNTKNEISELTRLIQRIRS
EIENVKKQASNLETAIADAEQRGDNALKDARAKLDELEGALHQAKEELARMLREYQELMS
LKLALDMEIATYRKLLESEECRMSGEFPSPVSISIISSTSGGSVYGFRPSMVSGGYVANS
SNCISGVCSVRGGEGRSRGSANDYKDTLGKGSSLSAPSKKTSR
```

Amino acid 1-letter codes

If you have lost this sequence, follow steps 1 and 2 of the first practical to find it again

Copy and paste this into a document. To make it easier to see what's what, change the header (the line beginning >) to:
>human_KRT71

Make sure the header line starts with >, otherwise Clustal won't know it's the header and will try to read it as amino acid sequence. The amino acid lines should not have > at the beginning. Make sure you don't remove the newline between the header and the first line of sequence. Do not add any extra newlines between the header and the first line of the sequence. Do not have multiple lines starting with >.

Step 2: Fetch other species sequences

Now you need to find the sequences from sea otter, black swan and Atlantic cod.

Go back to the tab with your BLAST results. Click on the identifier in the first column of the first row in the results table – this is the protein identifier.

Protein

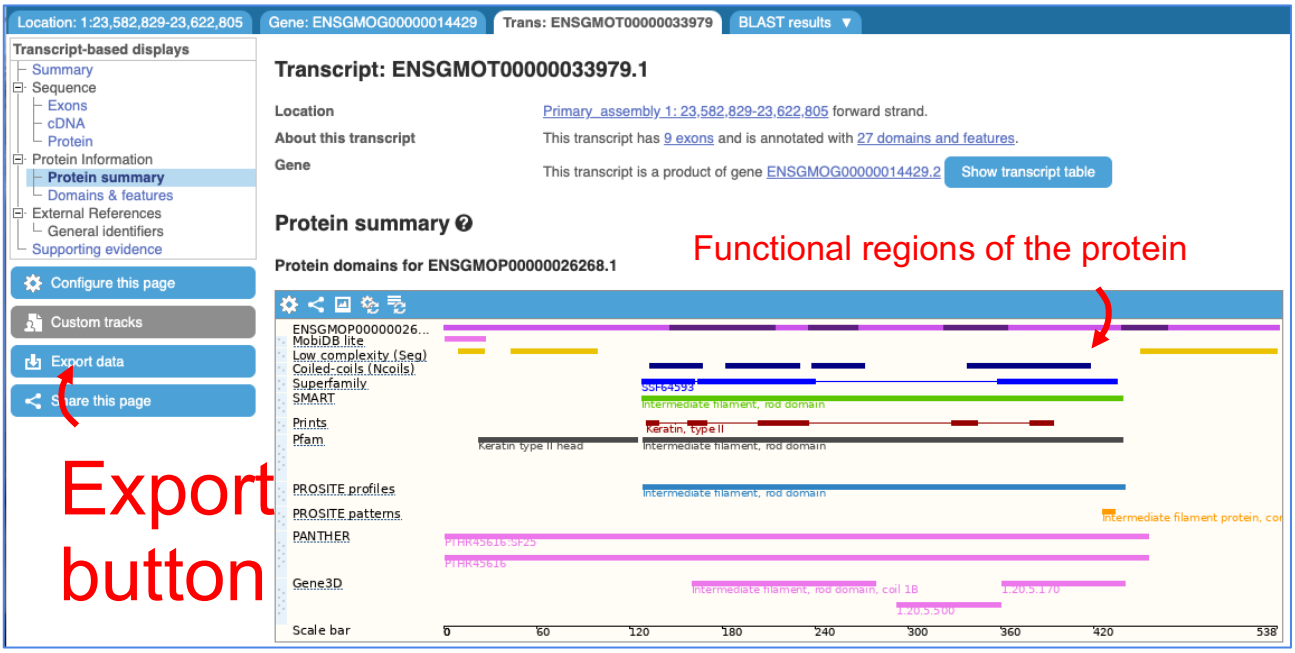
Results table

Show All entries | Show/hide columns (2 hidden) | Filter

Subject name	Gene hit	Subject start	Subject end	Subject ori	Genomic Location	Orientation	Query start	Query end	Length	Score	E-val	%ID
ENSGMOP00000026268	ENSGMOG00000014429	101	441	Forward	1:23583241-23621465 [Sequence]	Forward	102	444	343 [Sequence]	424	9e-142	58.31 [Alignment]
ENSGMOP00000039893	ENSGMOG00000014429	239	578	Forward	1:23583241-23621462 [Sequence]	Forward	102	443	342 [Sequence]	424	3e-140	58.48 [Alignment]
ENSGMOP00000038280	ENSGMOG00000014429	239	578	Forward	1:23583241-23621462 [Sequence]	Forward	102	443	342 [Sequence]	424	3e-140	58.48 [Alignment]
ENSGMOP00000043242	ENSGMOG00000014429	239	578	Forward	1:23583241-23621462 [Sequence]	Forward	102	443	342 [Sequence]	424	3e-140	58.48 [Alignment]

If you no longer have the BLAST results open, you can search for the IDs you noted down instead.

This will take you to a protein page. This page shows us the regions of the protein that are known to be involved in particular functions. These have been computed from the sequence, based on those functions in other proteins.



As before, we can export the sequence by clicking on the Export data button and selecting Peptide sequence only, ensuring that you select None under Genomic sequence. Refer back to step 2 of Practical 1 for more details and a screenshot of what to select.

Step 3: Make your sequence file

Copy and paste the sequence you get into the same document as the human protein sequence and change the name to give you the species again, making sure that you have > at the beginning of the header line and not at the beginning of the sequence lines.

Now do the same for the other two species. You should end up with a document containing four protein sequences, like this:

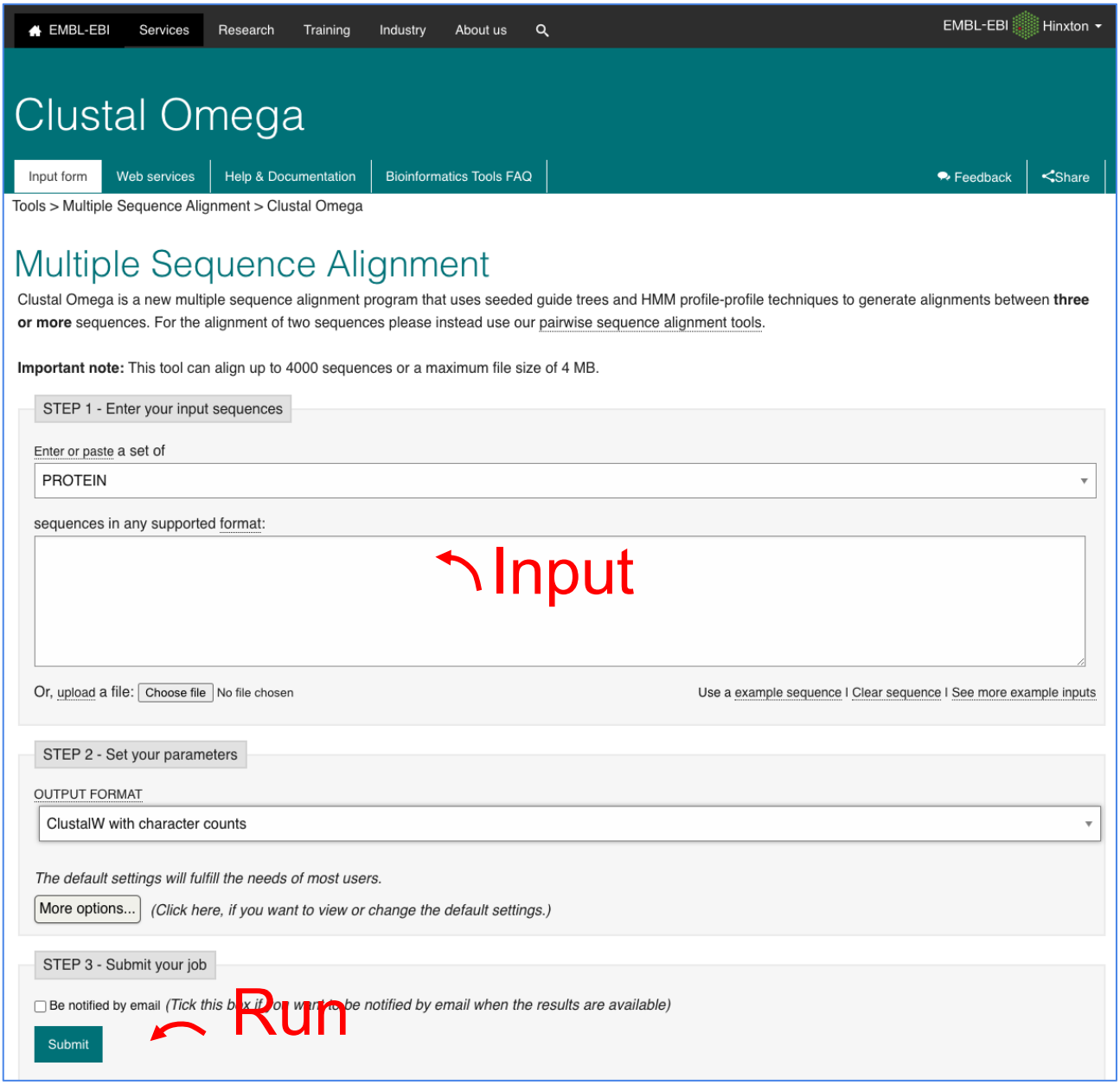
```
>human_KRT71
MSRQFTCKSGAAAKGGFSGCSAVLSGGSSSSFRAGSKGLSGGFGSRSLYSLGGVRSLNVA
SGSGKSGGYGFGRGRASGFAGSMFGSVALGPVCPTVCPPGGIHQVTVNESLLAPLNVELD
PEIQKVRAQEREQIKALNNKFASFIDKVRFLEQQNQVLETKWELLQQLDLNNCKNNLEPI
LEGYISNLRKQLETLSGDRVRLDSELRNVRDVVEDYKKRYEEEINKRTAAENEFVLLKKD
VDAAYANKVELQAKVESMDQEIKFFRCLFEAEITQIQSHISDMSVILSMDNNRNLDLDSI
IDEVRTQYEEIALKSKAEAEALYQTKFQELQLAAGRHGDDLKNTKNEISELTRLIQRIRS
EIENVKKQASNLETAIADAEQRGDNALKDARAKLDELEGALHQAKEELARMLREYQELMS
LKLALDMEIATYRKLLESEECRMSGEFPSPVSISIISSTSGGSVYGFRPSMVSGGYVANS
SNCISGVCSVRGGEGRSRGSANDYKDTLGKGSSLSAPSKKTSR

>swan
MSRQSTVRIQRGRSGFSAASAIVPNTCRTSFSSRSVTRVGSCNAGSGFSRVGGGFGSKSL
YNVGGCKRISVAGRGGGFYGPAGFGGGAGSVYGCGGGFGMPANLVSVNQSLLKPLNLEID
PSIQRIRKEEKEQIKTLNNKFASFIDKVRFLEQQNKVLETKWSLLQEQGMKTVKNNLEPL
FETYINNLRMQLNSLLSDKGRLEGELVNTQYLVEDFKKKYEDEINRRTVAENEFVTLKKD
VDASYMNKVELQAKVDALTEEINFLRALYEAELSQMQTQISDTSVVLTMDNNRNLDLDSI
ISEVKAQYEDIANRSRAEAESWYQTKYEELQATAGRHGDDLRNTKQEISELNRHVQRLRS
EIDSVKKQCANLKAAIADAEERGELALKDAKAKLAELEDALQQAKADLARQLREYQELMN
VKLALDIEIATYRKLLEGEECRWVGNTTLLVLVLEDPTQVLVMLGISEMHMLNGVSRGRG
KGCLVNSVTL

>otter
MSRQFTCKSGAATKGGFSGCSAVLSGGSSSSYRAGGKGISGGFGSRSLYSLGGIRNISFN
MTSGSGKSGGYGFGRGRASGFAGSMFGSVALGPVCPSVCPPGGIHQVTVNESLLAPLNVE
LDPEIQKVRAQEREQIKALNNKFASFIDKVRFLEQQNQVLETKWELLQQLDLNNCKNNLE
PILEGYISNLRKQLETLSGDRVRLDSELRSVRDVVEDYKKKYEEEINRRTAAENEFVLLK
KDVDAAYANKVELQAKVDSMDQEIKFFKCLYEAEIAQIQSHISDMSVILSMDNNRDLNLD
SIIDEVRAQYEEIALKSKAEAEALYQTKFQELQLAAGRHGDDLKNTKNEISELTRLIQRI
RSEIENVKKQASNLETAIADAEQRGDNALKDARAKLDELESALHQAKEELARMLREYQEL
MSLKLALDMEIATYRKLLESEECRMSGEFPSPVSISIISSTSGSGGYGFRPSSVSGGYVA
SSSSCISGVCSVRGGDVRGRGSSSDYKDTLGRGSSQSTSSKKASR

>cod
MSRQFTCKSGAATKGGFSGCSAVLSGGSSSSYRAGGKGISGGFGSRSLYSLGGIRNISFN
MTSGSGKSGGYGFGRGRASGFAGSMFGSVALGPVCPSVCPPGGIHQVTVNESLLAPLNVE
LDPEIQKVRAQEREQIKALNNKFASFIDKVRFLEQQNQVLETKWELLQQLDLNNCKNNLE
PILEGYISNLRKQLETLSGDRVRLDSELRSVRDVVEDYKKKYEEEINRRTAAENEFVLLK
KDVDAAYANKVELQAKVDSMDQEIKFFKCLYEAEIAQIQSHISDMSVILSMDNNRDLNLD
SIIDEVRAQYEEIALKSKAEAEALYQTKFQELQLAAGRHGDDLKNTKNEISELTRLIQRI
RSEIENVKKQASNLETAIADAEQRGDNALKDARAKLDELESALHQAKEELARMLREYQEL
MSLKLALDMEIATYRKLLESEECRMSGEFPSPVSISIISSTSGSGGYGFRPSSVSGGYVA
SSSSCISGVCSVRGGDVRGRGSSSDYKDTLGRGSSQSTSSKKASR
```

Check that:

1. All header lines start with >.
2. Each sequence has only one line of header.
3. The sequence lines do not start with >.
4. There are no extra lines between the header and sequence.

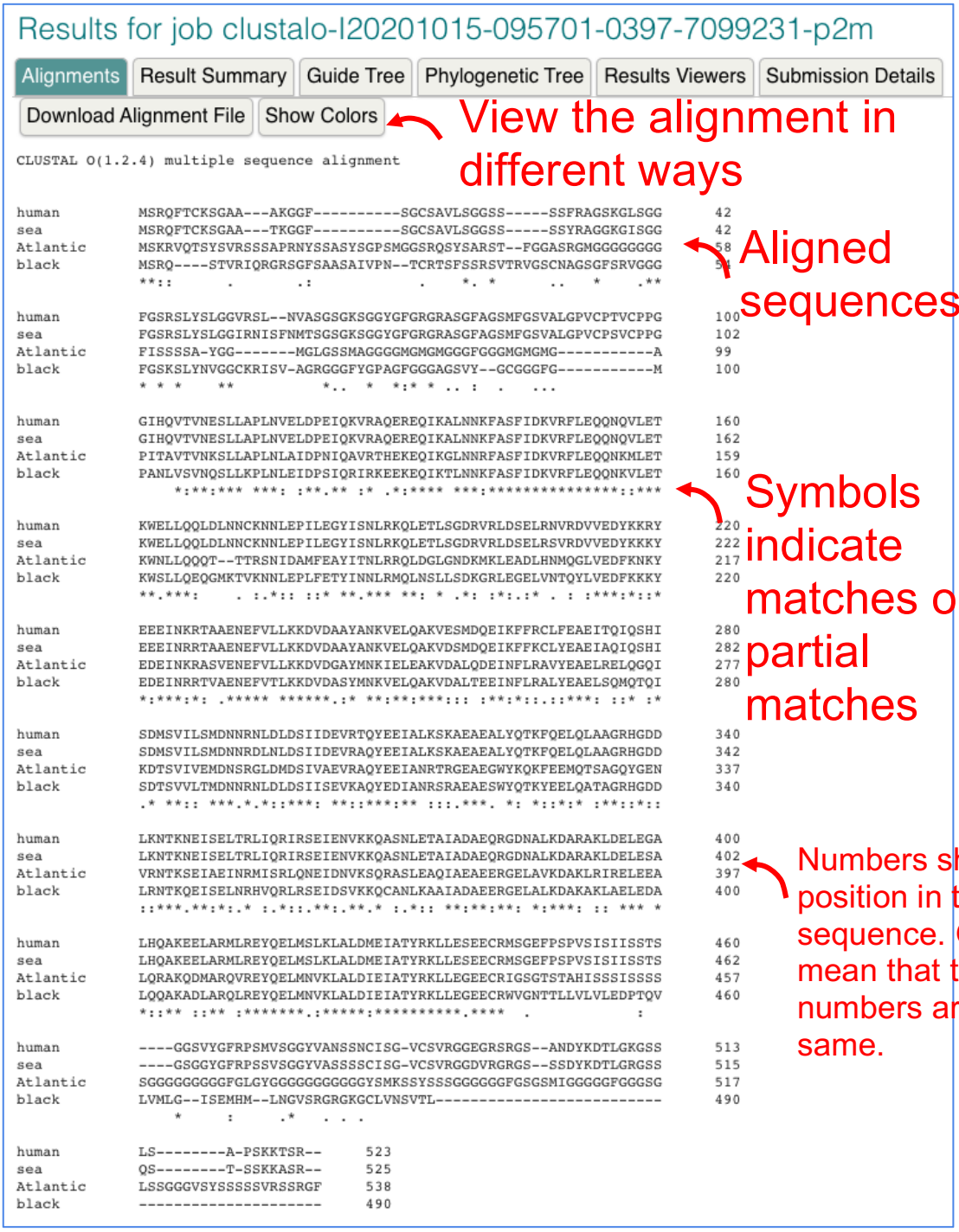
Step 4: Clustal input

You need to put these sequences into Clustal Ω. Go to https://www.ebi.ac.uk/Tools/msa/clustalo/.



Copy all four sequences into the input box on Clustal. Then click on Submit.

Step 5: Clustal results



The symbols underneath the aligned sequences indicate how similar the sequences are.

- - A dash indicates a gap, where there is no sequence.
- * An asterisk indicates that all the amino acids at that position are the same.
- : A colon indicates that they are very similar.
- . A dot indicates there is some similarity.
- A blank indicates no similarity at all.

The similarity is based on both chemical similarity between the amino acids at that position and also how many of them match between the different sequences.

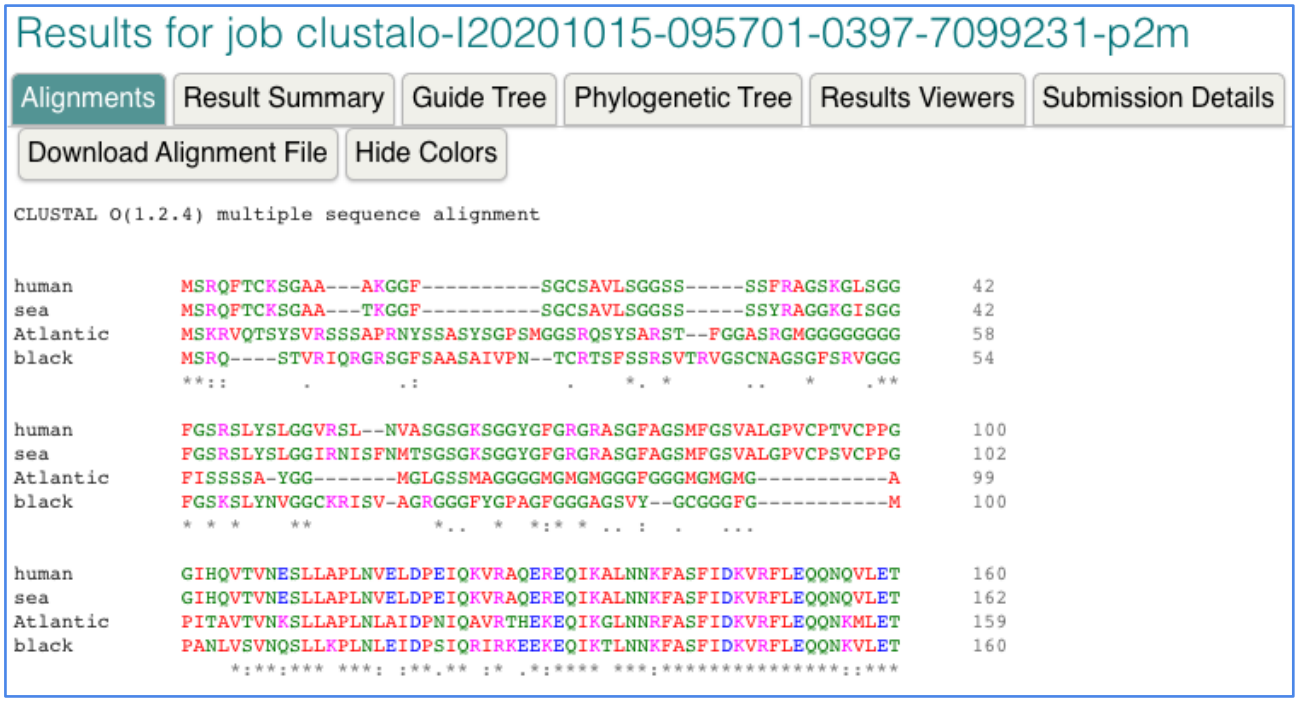
Questions:

1. Could you do this without a computer? How long do you think it would take to align all these sequences and spot similarities and differences between them by hand?

2. What if you had to do this for all 20,000 protein coding genes in a species?

Step 6: Change your view

There are other ways to see the alignments. Click on Show colors.



The colours can make it easier to see similarities and differences – it is easy to spot a column of matching colours than to spot a column of matching letters. There are 20 different amino acids, and some are more similar than others. Chemically similar amino acids are coloured the same, so we can most easily identify changes that are likely to affect the protein.

For example, one column shows R, R, K and K, all in magenta. R and K are Arginine and Lysine, respectively, which are both alkaline. In contrast, another column shows E, E (blue), K (magenta) and Q (green), which represent Glutamic acid (acidic), Lysine (alkaline) and Glutamine (with an amide group).

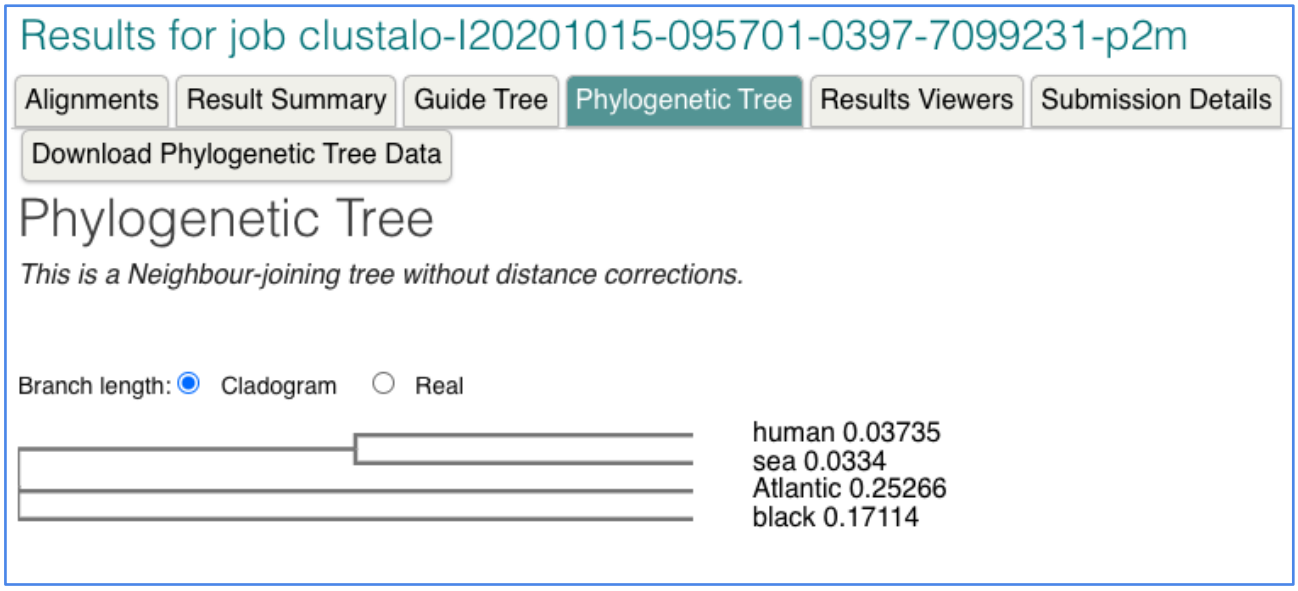
Questions:

3. Can you find any sections in the alignment with long runs of matching sequence? What is the longest you can find?

4. Look at position 5 in the alignment. What amino acids can you see there? Why have these been coloured differently? This is a great chart to look at for amino acids: https://www.compoundchem.com/2014/09/16/aminoacids/

5. Why do you think that some parts of the protein have lots of similarity between species, and other parts do not?

Another option is to see a tree constructed from the sequences, showing us which sequences are most similar to each other. Click on Phylogenetic tree at the top of the page.



Questions:

6. Which sequences are most similar to each other?

7. Why do you think this is?

Extended practical

Only if you have time or are particularly interested in this.

Why not see if you can find *KRT71* in more species and carry out an alignment of more sequences?

Why not find a different human gene and try searching for that?

Some genes to try:
ADAM30
NOTCH2NLR
PPIAL4A
LIX1L
GJA8