Exercise 1 – Multiple Alignment using NCBI BLAST online

Objectives:

- become familiar with the NCBI BLAST online system
- make use of some very basic multiple alignments options online.
- learn to download the aligned proteins for future analysis.

Our Test Protein:

This protein sequence comes from a 1000-year old skeleton, found at a monastery in Germany. The protein sequence is the translation of a short open reading frame found on a piece of DNA from that skeleton. More precisely, the DNA was from calcified dental calculus ("tooth-plaque"). We'll work on this short protein for the next couple of exercises. You can use 'copy-and-paste' to copy it from here:

```
GFFGDRVGRKFIIWFSILGTAPFALWLPYADADTTAILVILIGFIISSAFASILVYSQELLPKKIGMISGV
FYGFAFGMGGLASALLGKLIDLTDITFVYKVCSFLPLMGLIAYFLPNLRKVKMKE
```

1) submit the protein for a BLAST search

when confronted with an unknown protein, a good idea is to first search a sequence database online, to find out whether similar proteins have been described already.

- open your browser (Chrome/Firefox), and take it to the NCBI website: http://www.ncbi.nlm.nih.gov
- towards the right side of the page, under "Popular Resources", click "BLAST".
- Under the "Web BLAST" section, click on "Protein Blast".
- now, copy-and-paste our test-protein from above, and paste it into the big search box. Scroll down the page, and click the "BLAST" button – database and algorithm choices are already correct.
- wait until the results show up. Scroll down the page a bit, to get to the detailed overview of 'similar' proteins that are already known.
- let's see what we can find out about our query protein. Click on the first similarity hit that is reported ('MFS transporter […]'). This will bring up your first protein alignment … so far, it is not a multiple alignment but just an alignment between two proteins (our "query", and a similar protein found in the database of known proteins ["Subject"]).
- From which organism is the Subject protein? Is that an organism that we would expect to see on the surface of a tooth? Can you translate its Latin name, or at least parts of it ? … ☺
- how long is the subject protein? Longer or shorter than our test protein? Go back and look at a few other subject proteins. What seems to be a typical

first hit: Capnocytophaga gingivalis

length subject: 406
length test: 126
others are slightly above 400

it is shorter, since it was in suboptimal conditoins being conserved, so some stuff might be destroyed or not sequenced well enough in the lab

length for this protein family? Can you think of any reason why our protein might be shorter than the typical family member? Remember under what circumstances we found it …

transporter for membranes

- by reading the annotations of the various subject proteins, try to find out what the function of our test protein might be. Why would this be an interesting protein to study?

fosmodycin resistance proteins gives antibiotic resistance against it, so it makes sense to transport it out

[=> some proteins further down the result list are annotated as 'fosmidomycin resistence proteins' … what is fosmidomycin? ask Wikipedia … would you have expected such a function in a 1000-year old skeleton?]

- now go back to the top of the page. There should be a link called "Multiple Alignment" … follow that one now (you may have to wait some time for the results to show up) and then scroll down a bit to the section "Alignment". Voilà – this is your first multiple sequence alignment. Our test protein is shown at the very top … try to look around and understand what you see. What do the positions labeled '-' mean? Sometimes there are square brackets with numbers '[20]', what might those mean?
- Is our protein the only protein that is shorter than the others? It looks like there are some other shorter proteins known as well. Is there a part that in the alignment that every protein indeed covers?

  [indeed there is, towards the end. This is the most 'conserved' part, and hence likely the most important and characteristic part of the family. It forms part of a protein 'domain', as we will see in the next exercise.]

2) download the aligned sequences.

- BLAST has done an alignment for us, but what if we want to use a different algorithm and/or different parameter settings? For that, we need to download the protein sequences and work with them locally on our computer.
- in the BLAST multiple alignment view, towards the top, there is a link labeled 'Download'. Follow that link, and then select "Fasta plus gaps". You will be offered to save the alignment into a file. Choose your home directory, and give the file the name "`input_proteins_1.fa`" (the file-ending 'fa' stands for 'fasta', a frequently used file format for storing biological sequences).
- take a look at the file you just saved: open the "Terminal" application (remember the Unix tutorial). In the terminal, go to your home directory, and look at the first few lines of the file:

```
cd                                  [brings you to your home directory, if not already the case]
head -n 30 input_proteins_1.fa      [prints the first 30 lines of the file]
```

Exercise 2 – Domain Analysis using 'Interpro' online

Objectives:

- roughly understand what a 'protein domain' is
- learn to discover and browse domain information using InterPro.
- learn how to download the 'seed' alignment sequences for a given domain.

Our test protein:

same protein as in exercise 1, from the 1000-year old skeleton:

```
GFFGDRVGRKFIIWFSILGTAPFALWLPYADADTTAILVILIGFIISSAFASILVYSQELLPKKIGMISGV
FYGFAFGMGGLASALLGKLIDLTDITFVYKVCSFLPLMGLIAYFLPNLRKVKMKE
```

1) What is a 'protein domain':

in short, a *protein domain* is a short stretch of multiple sequence alignment that somehow seems 'important' enough to be given its own name and annotation. Most proteins typically consist of one *or more than one* protein domain, but also of non-domain sections that align less well and may perhaps be less important or at least less diagnostic of function.
In practice, protein domains are discovered by experts staring at alignments all day. As part of describing and naming a new domain, these experts will usually provide a summary on where the domain can be found, and what function it might have. Within the actual proteins, domains often represent autonomously folding structural subunits. Many proteins are consisting of multiple different domains, which can be rearranged and exchanged over evolutionary timescales ('domain shuffling'). As of today, most domains in existence have probably been discovered and described already.

2) submit the test protein for a domain search

after exploring an unknown protein against sequence databases (exercise 1), the next typical strategy is to search it for previously described domains. This may provide a broader idea of its function, and often also some three-dimensional structure information.

- open your browser (Chrome/Firefox), and take it to the EBI website: https://www.ebi.ac.uk/
- Click on "Services", then on "Proteins" and then on to "InterPro" (fifth entry under Data Resources).
- Use copy-and-paste to enter our test protein into the box 'Analyse your protein sequence', and click 'Submit'.

- after a while, a graphical summary will show up, indicating domains and features that have been found for our test protein. The sets of four grey lines towards the bottom indicate that the protein likely has transmembrane sections, so it may be sitting in a membrane. The colored lines are the actual domains. They refer to the very same domain several times, but some of the classifications are more generic ('superfamily'), whereas other ones are more specific ('domain').
- now, click on second line from the top (under "Domains and Repeats", domain 'IPR020846'). This brings up the so-called 'Interpro-abstract', which provides an excellent summary of the general function of this protein family. As you will see, it is a transporter, which the cell uses to transport a variety of 'small molecules' across the membrane. Now we know a lot more already, but of course the annotation is too generic to let us know which is the preferred 'small molecule' that our protein wants to transport.
- now, notice that in the annotation, it said that these proteins typically have 12 trans-membrane sections, but we only found four … this is another indication that our test protein from the skeleton is indeed incomplete. Hence, let's repeat the analysis with the closest relative it has in the sequence databases (this is the 'best hit' we found in exercise 1)
- Ccpy-and-paste the first best hit found in the file "input_proteins_1.fa. It should be the second entry in that file. Submit this protein to InterPro, as before. How many transmembrane sections do you find now? (take care to remove the gap characters ["-"] before submitting)
- Next, let's find out a bit more about this domain. Proceed to the Interpro abstract again, like before ('IPR020846').
- towards the left of the abstract, click on 'Species' … this will tell us in which organisms the domain is found. If you scroll down a bit (section "Taxa"), you will find that it occurs virtually everywhere (Bacteria, Eukaryotes, Archaea, even viruses).
- next, proceed to 'Domain architectures' … this will tell us which other domains can sometimes be seen together with our domain in a protein. Hover over some of them to see their function. Our case is the most boring, most frequent case: only the domain, and nothing else (first row).
- finally, click on the 'Structures' section. This will provide examples of known three-dimensional structures. Select one entry form the 'PDBe' section, and look at the protein structure. Now we know how our protein generally looks like … but again no useful information about the substrate (in this case, the proteins with the solved structure transport mainly sugars).

2) download a 'seed' alignment from Pfam

the Pfam database (= "Protein families") is similar to InterPro, but is actually one of the original databases for protein domains (in contrast, InterPro is a 'meta-resource' bundling several original databases such as Pfam).
For many of its domains, Pfam maintains a 'seed' alignment, which is the original alignment that was made by the discoverer of the domain (or an updated version

thereof). It is made up of non-redundant, representative sequences, and often has been quality-checked manually. For further work on alignments and trees, we'll get one such seed alignment from Pfam now.

- open your browser (Chrome/Firefox), and take it to the EBI website: https://pfam.xfam.org
- towards the middle of the page, click on "SEQUENCE SEARCH", and enter the longer version of our test protein, as we did for Interpro before.
- in case the systems complains about "too many search jobs", you can also try your search here: https://www.ebi.ac.uk/Tools/pfa/pfamscan/ (but you would need to additionally change the output format there, to "plain text").
- this should discover the same domain as before ('Major Facilitator Superfamily', here called 'MFS_1').
- click on MFS_1 to bring up the annotation page. After having a look around, click on the Alignment tab located on the left. Now, under the section "Format an alignment", choose the format "FASTA", and set Gaps as "Gaps as '-' (dashes). Under Download/View choose 'Download', and then click on "Generate".
- when offered the file for download, save it into your home directory, and give it the name "`input_proteins_2.fa`".

Exercise 3 – Multiple Alignment on your Computer

Objectives:

- perform a multiple alignment of proteins using three different programs.
- prepare the input files, bringing them into the right format.
- check whether the results are identical.

1) Prepare the input files.

In the last two exercises, we've made two files with protein sequences already. One of them came from BLAST, it will form the basis for an 'easy' alignment because the proteins are very similar. The other is from a domain database, this will be the 'hard' alignment because the proteins span a very large area in sequence space. We'll use those input files to create six different multiple alignments; but first we'll have to properly prepare the input. Both input files should be un-aligned, and for one of them we also still need to add our test protein.

Prior to the course, the files "`input_proteins_1.fa`" and "`input_proteins_2.fa`" were unaligned using regular expressions. The character "-" was replaced with empty character "" and empty lines were removed. This removed all the gaps, and hence destroyed the alignment. This was saved into "`unaligned1.fa`" and "`unaligned2.fa`" respectively; the files are available on OLAT.

- download the files "`unaligned1.fa`" and "`unaligned2.fa`" from OLAT and story them in your home directory (you may have to use "right-click" in the browser to indicate where you want the files stored.
- now, to deal with our test protein: in one file, it simply needs to be renamed … and in the other file it is missing, so we need to add it.
- open the File '`unaligned1.fa`' in an editor of your choice, and change the name of the very first protein, to read 'query_protein'. Then save the file. The first lines of the file should now look something like this:

```
>query_protein
GFFGDRVGRKFIIWFSILGTAPFALWL
PYADADTTAILVILIGFIISSAFASILVYSQELLPKKIGMISGVFYGFAFGMGGLASAL
LGKLIDLTDITFVYKVCSFLPLMGLIAYFLPNLRKVKMKE
>gi|488743029|ref|WP_002666381.1| fosmidomycin resistance protein [Capnocytophaga gingivalis]
METKQRTQYLIIIL
ISLSHCLNDLLQGVLPSIYPALQSKFALSMAQIGLITFCYQIAASILQPIVGAYTDKHPK
PYAQVVGMAFSALGIGLLSWVDSYTLVLCSVVFVGIGSSIFHPEASRISFLASGGKRSFA
[…]
```

- open the second File '`unaligned2.fa`' in an editor of your choice, and add the query protein to the beginning of the file (simply copy-and-paste from the example output above).
- *optional extra task for the geeks among you: can you do the above file manipulations using the unix-editor "`vi`" as your editor? If you can't, you're not a geek … ☺*

2) Multiple alignment using ClustalX.

- download the 'ClustalX' application, from here:

  http://www.clustal.org/download/current/

  (choose the file `clustalx-2.1-macosx.dmg`, mount the disk image, and copy the application to your home directory. Make sure you choose 'clustalx', not 'clustalw').
- launch clustalx with a double-click on the application icon.
- click 'File' -> 'Load Sequences', and choose the File '`unaligned1.fa`'.
- go to 'Alignment' -> 'Output Format Options', and set the 'Output Order' to 'Input'. This way, the order of sequences will not be changed. Also, choose the output format 'FASTA'.
- now, to align, choose 'Alignment' -> 'Do Complete Alignment'. You can keep the suggested filename for the guide-tree, but please change the output filename to '`aligned.clustalx.easy.aln`'. Then click OK (the alignment takes a few seconds).
- now let's make a nice graphical overview (for example to print it out later). Choose 'File' -> 'Write Alignment As Postscript'. On the dialog that comes up, choose the filename '`aligned.clustalx.easy.ps`', set the 'block length' to 300, and choose 'OK' (you can ignore the warning). This will create a postscript graphics file … open the Mac "Preview" application ("Vorschau" in german), locate the file you just made and open it. This should give you a nice illustration of the alignment.
- repeat the exact same procedure for the second input file. This time, name the outputfile '`aligned.clustalx.hard.fa`'. The alignment is more difficult; it should take around five to ten minutes. Again, create a nice graphical overview ('`aligned.clustalx.hard.ps`').

3) Multiple Alignment with Muscle on the Command Line

the program 'muscle' is somewhat faster and often produces better alignments than ClustalX in benchmarks, so it is often preferred for larger and/or more difficult alignments.

- to install Muscle, proceed to this website:
  http://www.drive5.com/muscle/downloads.htm
  locate the file '`muscle3.8.31_i86darwin64.tar.gz`', and save it to your home directory.

- then, in the Terminal, decompress and unpack the file:

```
cd                                          [to go to your home directory]
gunzip muscle3.8.31_i86darwin64.tar.gz      [decompresses the file, removes ending 'gz']
tar xfvp muscle3.8.31_i86darwin64.tar       [unpacks the file]
ls -lart                                    [list files; did everything work as expected?]
```

- now, we can run muscle on both of our input files:

```
./muscle3.8.31_i86darwin64 -in unaligned1.fa -out aligned.muscle.easy.fa
./muscle3.8.31_i86darwin64 -in unaligned2.fa -out aligned.muscle.hard.fa
```

- ok, let's use ClustalX to format this new alignment in a pretty way again: go back to the ClustalX window, and simply overwrite the old sequences with the new alignment, by choosing 'File' -> 'Load Sequences', and selecting the file we just made ('`aligned.muscle.easy.fa`'). Then, choose 'File' -> 'Write Alignment As Postscript', and proceed as before. Do the same for the other file ('`aligned.muscle.hard.fa`')

4) Multiple Alignments using HMMAlign

the program HMM-align produces very good alignments, but it can only be used if the proteins to be aligned have a previously known domain.

- to install HMMAlign, download the following file from OLAT: '`hmmer-3.2.macosx.precompiled.tar.gz`' and save it into your home directory.

- uncompress and unpack the file, and create links to the executables in your home directory:

```
cd
gunzip hmmer-3.2.macosx.precompiled.tar.gz
tar xfvp hmmer-3.2.macosx.precompiled.tar
rm hmmer-3.2.macosx.precompiled.tar
ln -s hmmer-3.2.macosx.precompiled/bin/hmmsearch .
ln -s hmmer-3.2.macosx.precompiled/bin/hmmalign .
```

  - next, we need a so-called HMM-file, which describes all the knowledge that has been assembled for a given domain. In our case, follow the steps below:
  - Go to this link http://pfam.xfam.org/family/PF07690
  - Next, on the left, find Curation&Model tab, then go towards the bottom of that section and download the raw HMM file and store it into your home directory. Give it the filename "MFS_1.hmm"

- now, we can use the hmm-file to produce the alignments:

```
./hmmalign --outformat A2M MFS_1.hmm unaligned1.fa > aligned.hmmalign.easy.fa
./hmmalign --outformat A2M MFS_1.hmm unaligned2.fa > aligned.hmmalign.hard.fa
```

- as before, open the alignments you just created in ClustalX, and create nice visual representations for them.

5) compare the alignments

You should now have six different alignments: two each from the three different algorithms (one easy, one hard). Compare them side-by-side … are there differences? If so, which of these alignments looks 'better'? What criteria might be useful for deciding that?
As expected, the 'easy' alignments overall appear to be more similar to each other. With one exception: the hmmalign may put our query protein in the first half of the alignment, whereas the others usually put it in the second half (!). Any idea what this might mean?

Exercise 4 – cut out a domain of interest before aligning

Objectives:

- to learn how to identify domain positions in a protein with '`hmmsearch`'.
- to cut domains from the input proteins before aligning, using Python.

Introduction:

This exercise is somewhat more difficult than the first three, and it involves some serious Python coding. It should be stressed that there are much simpler ways to cut out domains from proteins, but we'll use Python here while the memory is still fresh from the Python introduction. Also, the generic task of parsing some large data files and manipulating them is frequently encountered in Bioinformatics, so this is a good 'real-world' training case for Python.

- on the course-website, there should be the two files below. Please download them and store them into your home directory

`mfs_domain_proteins.fa`	[this is a collection of 15 bacterial proteins, which have at least three domains each: one of our MFS-1 domains, and two enzymatic domains. From each, we want to cut out and align the MFS domain only, and discard the rest]
`MFS_1.hmm`	[this is a 'hidden markov model' describing how to identify "mfs" domains]

1) identify the MFS domains with 'hmmsearch':

- next, will use the 'hmm' file to find the positions of each MFS domain in the set of proteins. We'll run the '`hmmsearch`' utility to scan the hidden markov model along the sequences and to report any hit it finds. You should still have `hmmsearch` installed from the previous exercise.

```
./hmmsearch --domtblout domains_found.tsv MFS_1.hmm mfs_domain_proteins.fa
```

`ls -lart`	[list the contents of the directory, most recent last. there should be a new file]
`head domains_found.tsv`	[check the first few lines of that new file; the domain positions are in 'env coord']

2) use Python to cut out the domains:

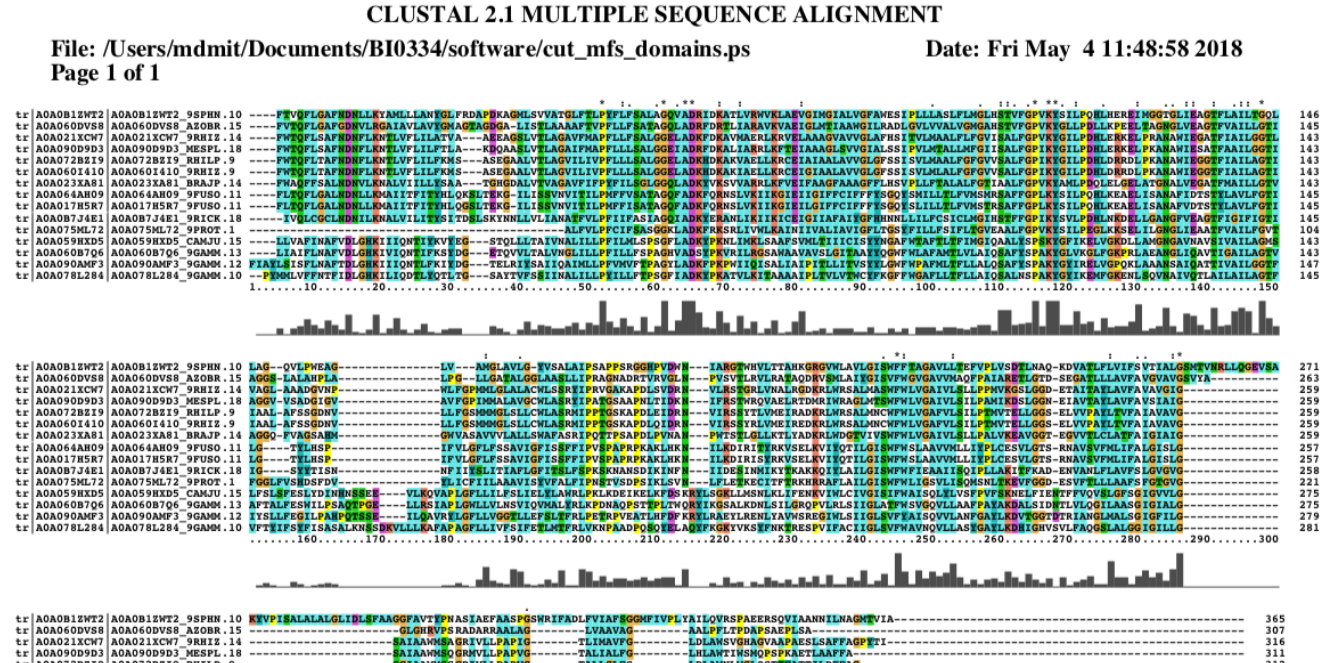
- now, we have all the information we need: one file with the protein sequences, and a second file containing the domain coordinates on these sequences.
- here is the challenge: can you write a Python script that uses this information to cut out the domain sequences and to print them into a third file?

hints: first, read the protein sequences, and store these into a dictionary (one 'string' of amino-acids per protein name). Then, read the second file and parse the coordinates. Whenever you've parsed one valid line from the second file, retrieve the corresponding protein sequence from the hash you've made earlier, and cut out the part that contains the domain. Use the 'substr' function in Python to cut out the relevant part of the string. Then, print the substring and proceed to the next line.

- the above task is about as difficult as it ever gets in terms of file-processing for Bioinformatics. So, do play around a bit with the task. Try to write a Python script that does at least part of it … and don't give up too easily … ;)
- when you do get stuck, on the course website you will find the solution (which you can download and run at any time):

```
python cut_domains_from_proteins.py > input_proteins_mfs_domain_only.fa
```

- finally, using `muscle` and `clustalx` as in the exercises before, align and visualize the proteins. You should get a very nice and very compact alignment:



Exercise 5 – Analysing and aligning newly discovered proteins

Objectives:

- to apply what has been learned today

Anonymous Test Proteins:

below, we provide 20 randomly chosen proteins. All have been derived from DNA on the teeth of ancient skeletons found in a german monastery (same as for the previous exercises). None of the proteins have been analyzed in detail before … Please select arbitrarily one of the proteins below, and analyze it like we did in exercises #1 through #3.

Questions:

- what protein family does your protein belong to?
- which domain(s), if any, does the protein contain?
- from which organism is it, likely?
- what function might it have?
- is it complete?
- how can it be best aligned to other members of its family?

```
>NODE_4178_length_1047_cov_6.240688_S6
SATSAAAMKLIAPSWPSRYVSPPRRRQGAAMAEWWSAQLVDRDGRIRGELPDIRGGSLEW
NISSAVRTGGSVEFAEPPSAGIDWVTTRIRILHHDGAEVRPMGVYRASWPNRKLRDGHTS
STLKLEAPTSRLRSQLGYWTQYEAGIVVTDRVAMTLRQLGESQLALTPSPQTLRTPLTWD
PDKTWGTLYSELLDAIGYGGIWCDANGWWRAAPYVAPMERPLAATYGGDPADYRCRTTYG
DEADWTDVPNRVLLYTRATSEAPALTSEVWITDPANPWHPDRVGPHTRCEAVEATSQEVL
DAKAKRLLAEGQERSRYITWTHPVDDTTLGDRVRIRRLGLDVAIEARK

>NODE_25515_length_1898_cov_8.371970_S6
RGGKMKIIIAGIGNIGAGLAGRLLNEGHDIVLVDRDIDRLEYNEETQDVMTVKGTCAAME
TLRKAGVEDADLLITATSSDEKNLLSCMTAHGMNPNIKTVARVRMQEYLETTSVFGEKFG
LSMIIDPGMYAAKDIEAILTYPGFLHRERFTKGMTDVVEAELHPESELCGKPVSAIQEIT
GSGALVCVVKRGDTAITPGRDFILREKDRIYVTAEEEDLSTLLRLFGKKKETVEKVMIVG
GGRIAKGLIPRLQKEGMEIIVIDTDKEICEELAMEFPKVNIVHGDGRKFALLERKNVREQ
DALICLTNDDESN

>NODE_60099_length_1027_cov_6.267770_S6
RVASVCLSTIVAVWMTSLIVPWASYSVKEGSRWAIESMYESRMVTKDDRDAFNWLAKQPH
AYDGIIFGNSADGYGWMYAYNKLPSLARHYDGVSAKPGAPSHVLRDSAYLIGAGNHGDPD
QRNRADLAAENLGVNFIMLSPPNFWWFQQSNLEMSAKLDKAPGLTLVYQKNSIRIYAVNA
KFKDAELTRMRASGPASNQLPVPQCPKDSADGKAAATAGETTQVEYDPDTGEQTTVTKPK
PCYHRPSKPDIPPRANDAKGKTPATPKSGGGDDKSNKYSEKTGLDLTEKEARRRRLDNGY
VHNEKATLRF
```

>NODE_77700_length_886_cov_21.930023_S6
WANMCPRKCKVMSMKRNQSAVHQLITYGMVIAAYILCQILVENGSMTRSLKGQLIPIAVY
IVMAVSLNLTVGISGELSLGHAGFMSVGAFSGIVVSQWMGTVYPNVHVYVRLVFAIVTGG
IAAGIAGVLIGIPVLRLRGDYLAIVTLAFGEIIRNIMNLLYVSVDQGRLRMAFNDGALPG
EQVIAGPKGAVGIEKIATFTMGFILVMITLFVVLNLINSRSGRAIMAIRDSRIAAESVGI
NVTKYKMMAFVISSVLAGMAGALFGLNYSTVSAGKFKFDMSILVLVFVVLGGIGNIRGSV

>NODE_87482_length_1095_cov_5.276712_S6
NPLIARTRGQQRDAHSVHARYGDKYLPFSDLENSMRDMEGLLNKVADLAVKAGSIMLSDS
DVEVGNKGTKENYVTSTDLKVQRFLREGLATLLPGAVFRGEEDDLPREDEGTRGEYVWIV
DPIDGTANYARGFGESAVSIALAKDDEPVLGVVRNPYARETYCAIKGRGAFLNGTPIHVS
GRSKENAMICLSWSAYDKSRSADCFRISQDLYAVCEDIRRTGSAAYELCLLARGSVDMHF
EIRLAPWDYAAGGLIIEEAGGRTGSLEGRLDMRRQCLVMAANSEKNFAFLKGVVSENLSL
RRRLAPVHV

>NODE_107984_length_1345_cov_80.271378_S6
ERKAYSMGKRTIIPFGPQHPVLPEPVHLDLVIEDETVVEAIPSIGFIHRGLEKLVEKKEY
PEMVYVIERICGICSFGHGWGYCAAVEGAMNVEIPERAMYLRTILHELGRMHSHLLWLGL
LADGFGFESLFQHCWRIRETVLDLFEQTTGGRVIFSICKVGGLNKDIDNETLNKIVKTLR
GIEKEIREYTSVFINDTSVKNRLTGVGVLSREDAEALCTVGPMARASGLRQDMRLAGEGK
YLELGFEPVLEEAGDCMARCKVRIGELLQAIDIIEKAVAQIPDGDIAVAVKGNVDGEFIN
RLEQPRGEAFYYCKGQGTKFLERIRVRTPTNMNIPAMVKILQGCDLADVPMIVLTIDPCI
SCTER

>NODE_123020_length_4291_cov_7.623631_S6
AVFEERWGDRPFMRSYRIPSIPVRPIWICVSRQNRAVLCLKTYIQMEQAILGAKREPVCQ
AASHALGPSAEDSCLTARPDPMRVDYDTDVRAFAQRLLGGNVFEPVTFAGITLPLISFIL
FGAALAFLLIVQVARTMISNKLQNLFASKLYDEFLDTVDEPLTRFFIPAYNRTYLRLNAF
MAKGSVEKAMEAFDQLLAMRSTRAQRDDLLFKAFQFYMQQEDFKGAKAVLDEMQSYGRHE
KRVEECVQAYEIFGNNSYAYIDEMEAAFDEAPYALKVSYALMLAAQYTSKKDGEAAEKWQ
DTARELLENPPKKGPAETR

>NODE_182329_length_1939_cov_4.566271_S6
APDDPPRHRRREQREKLFRRTTCLHPWGRVLLRGDHGAAQRRSTRAYRTASQTARREKVR
DHRAAARSGRARPAARSGARRGATGGYRELGGKRAVRCRAVRRPRIRVLGRPRGRLRAHH
GRNRPSERALLPSRSRHLRSRVGRTPHRTRNALLYRAYRTGELHDCRPGSNGARVRGKHR
ATAQRGICRMTALRSIALAFTLFSRVPMPHVEWNPENMRYTMLAFPLVGCVIGTAVATWC
ALCATLGLNGAAFGAGTVLVPLFVTGGIHMDGFADVVDAQSSHAAPERKREILADPHIGA
FAAIGIGGYLLAWAALAS

>NODE_212586_length_1033_cov_30.919651_S6
ISKTDESYPDFLRPSDGALHPAVNEYRSLWISLSLKGALPGLYPIHIVVEQDGEECYRAT
LCVRVCTAPLEKQKLIHTEWLHADCLCSYYNVEAFSERHFALLENFIRAAVQDYGINMIL
TPVFTPPLDTQVGGERRTVQLVDIACDSRGYHFDFSKLARWADICKRCGVEYLEIAHLFT
QWGAQHCPKIIVTEKGRERKKFGWQSDAAGTEYRKFLEQFLPALRSALQGMGYPDEKVYY
HISDEPSEDNLEHYRRAKAQVADLLEGANVVDALSSYRFYQEGLVTEPIVSSDHIQAFLD
AGVPNLWVYYCCGQDKLVPNRFFAMPSPRNRVFGVLLYLSGVKGFLHWGYNFY

>NODE_238737_length_1166_cov_7.374785_S6
NRHQTMFKGEIVMNSLIIVSAALGLCALLFALVLAARVKSQDSGTERMTEIAAYIHQGAK
AFLMAEYRILVIFVAILFVLIGLGISWITAVCFLVGAAFSTVAGYIGMNVATAANVRTAA
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