EXERCISE 2: PAIRWISE ALIGNMENTS

Program is available

DOTLET
http://myhits.isb-sib.ch/cgi-bin/dotlet
Alignment based on dot representation (use NetScape)
https://dotlet.vital-it.ch/

Use the following sequences in FastA format:

DATASET 1 Two terminal oxidases from the same family

>gi|13449404|ref|NP_085587.1| cytochrome c oxidase subunit 1 [Arabidopsis thaliana]
MKNLVRWLFSTNHKDIGTLYFIFGAIAGVMGTCFSVLIRMELARPGDQILGGNHQLYNVLITAHAFLMIFFMVMPAMIGG
FGNWFVPILIGAPDMAFPRLNNISFWLLPPSLLLLLSSALVEVGSGTGWTVYPPLSGITSHSGGAVDLAIFSLHLSGVSS
ILGSINFITTIFNMRGPGMTMHRLPLFVWSVLVTAFLLLLSLPVLAGAITMLLTDRNFNTTFFDPAGGGDPILYQHLFWF
FGHPEVYILILPGFGIISHIVSTFSGKPVFGYLGMVYAMISIGVLGFLVWAHHMFTVGLDVDTRAYFTAATMIIAVPTGI
KIFSWIATMWGGSIQYKTPMLFAVGFIFLFTIGGLTGIVLANSGLDIALHDTYYVVAHFHYVLSMGAVFALFAGFYYWVG
KIFGRTYPETLGQIHFWITFFGVNLTFFPMHFLGLSGMPRRIPDYPDAYAGWNALSSFGSYISVVGICCFFVVVTITLSS
GNNKRCAPSPWALELNSTTLEWMVQSPPAFHTFGELPAIKETKSYVK

>gi|461786|sp|P33517|COX1_RHOSH Cytochrome c oxidase polypeptide I (Cytochrome AA3 subunit 1)
MADAAIHGHEHDRRGFFTRWFMSTNHKDIGVLYLFTGGLVGLISVAFTVYMRMELMAPGVQFMCAEHLESGLVKGFFQSL
WPSAVENCTPNGHLWNVMITGHGILMMFFVVIPALFGGFGNYFMPLHIGAPDMAFPRMNNLSYWLYVAGTSLAVASLFAP
GGNGQLGSGIGWVLYPPLSTSESGYSTDLAIFAVHLSGASSILGAINMITTFLNMRAPGMTMHKVPLFAWSIFVTAWLIL
LALPVLAGAITMLLTDRNFGTTFFQPSGGGDPVLYQHILWFFGHPEVYIIVLPAFGIVSHVIATFAKKPIFGYLPMVYAM
VAIGVLGFVVWAHHMYTAGLSLTQQSYFMMATMVIAVPTGIKIFSWIATMWGGSIELKTPMLWALGFLFLFTVGGVTGIV
LSQASVDRYYHDTYYVVAHFHYVMSLGAVFGIFAGSTSGIGKMSGRQYPEWAGKLHFWMMFVGANLTFFPQHFLGRQGMP
RRYIDYPEAFATWNFVSSLGAFLSFASFLFFLGVIFYSLSGARVTANNYWNEHADTLEWTLTSPPPEHTFEQLPKREDER
APAH

DATASET 2 Two terminal oxidases from a different family

>gi|13449404|ref|NP_085587.1| cytochrome c oxidase subunit 1 [Arabidopsis thaliana]
MKNLVRWLFSTNHKDIGTLYFIFGAIAGVMGTCFSVLIRMELARPGDQILGGNHQLYNVLITAHAFLMIFFMVMPAMIGG
FGNWFVPILIGAPDMAFPRLNNISFWLLPPSLLLLLSSALVEVGSGTGWTVYPPLSGITSHSGGAVDLAIFSLHLSGVSS
ILGSINFITTIFNMRGPGMTMHRLPLFVWSVLVTAFLLLLSLPVLAGAITMLLTDRNFNTTFFDPAGGGDPILYQHLFWF
FGHPEVYILILPGFGIISHIVSTFSGKPVFGYLGMVYAMISIGVLGFLVWAHHMFTVGLDVDTRAYFTAATMIIAVPTGI
KIFSWIATMWGGSIQYKTPMLFAVGFIFLFTIGGLTGIVLANSGLDIALHDTYYVVAHFHYVLSMGAVFALFAGFYYWVG
KIFGRTYPETLGQIHFWITFFGVNLTFFPMHFLGLSGMPRRIPDYPDAYAGWNALSSFGSYISVVGICCFFVVVTITLSS
GNNKRCAPSPWALELNSTTLEWMVQSPPAFHTFGELPAIKETKSYVK

>gi|2114418|gb|AAB58264.1| cbb3-type cytochrome oxidase component FixN [Rhizobium leguminosarum bv. viciae]
MNYTTETMVIAVAAFLALLVAAFAHDHLFAVHMGILCLCLVMGAVLMVRKVDFSPAGQQRNVDRSGYFDEVIRYGLIATV
FWGVVGFLVGVIIALQLAFPDLNIAPYLNFGRLRPVHTSAVIFAFGGNALIMTSFYVVQRTCRARLFGGNLAWFVFWGYQ
LFIVMAATGYVLGITQGREYAEPEWYVDLWLTIVWVAYLAVYLGTILKRKEPHIYVANWFYLSFIVTIAMLHVVNNLAVP
ASFLGSKSYSVSSGVQDALTQWWYGHNAVGFFLTAGFLGMMYYFVPKQANRPVYSYRLSIIHFWALIFMYIWAGPHHLHY
TALPDWAQTLGMVFSIMLWMPSWGGMINGLMTLSGAWDKIRTDPIIRMMIVAIAFYGMSTFEGPMMSVKTVNSLSHYTEW
TIGHVHSGALGWVGMITFGAIYYLTPKLWGRERLYSLRMVNWHFWLATFGIVVYAAVLWVAGIQQGLMWREYNSQGFLVY
SFAETVAAMFPYYVLRAVGGTLYLAGGLVMAWNVFMTIRGHLRDEAAIPTTFVPQAQPAE

DATASET 3 Random sequences

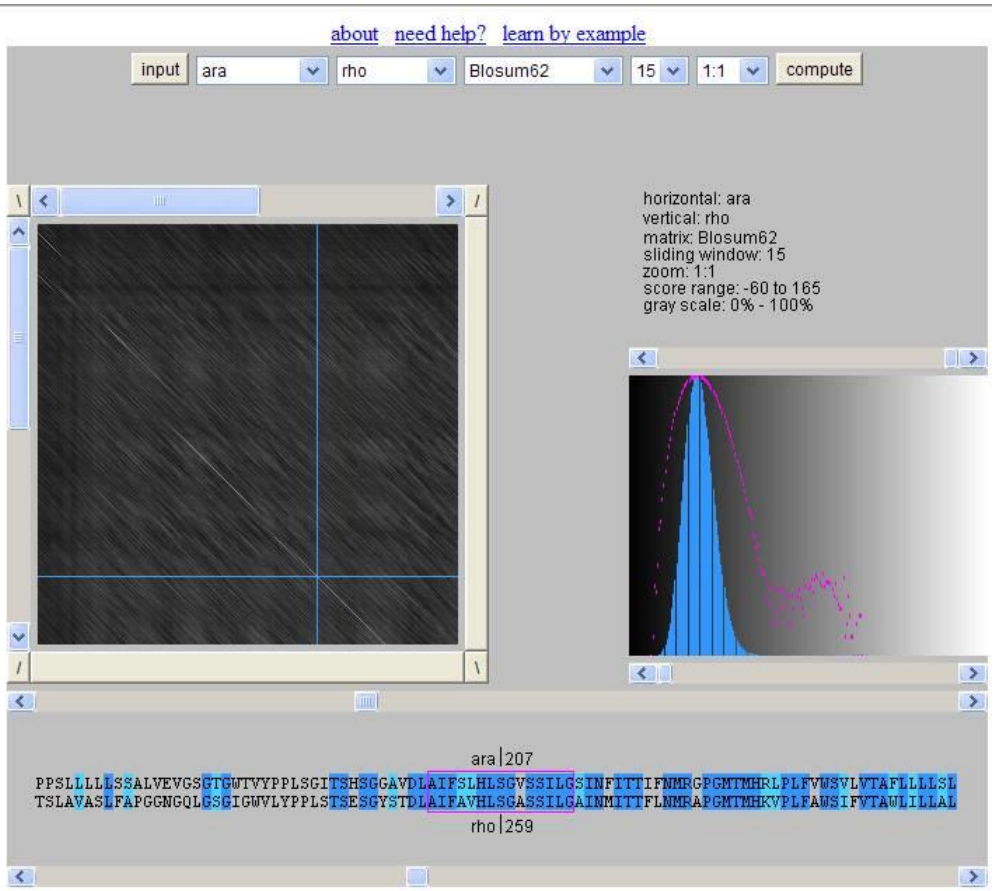
>gi|13449404|ref|NP_085587.1| cytochrome c oxidase subunit 1 [Arabidopsis thaliana]
MKNLVRWLFSTNHKDIGTLYFIFGAIAGVMGTCFSVLIRMELARPGDQILGGNHQLYNVLITAHAFLMIFFMVMPAMIGG
FGNWFVPILIGAPDMAFPRLNNISFWLLPPSLLLLLSSALVEVGSGTGWTVYPPLSGITSHSGGAVDLAIFSLHLSGVSS
ILGSINFITTIFNMRGPGMTMHRLPLFVWSVLVTAFLLLLSLPVLAGAITMLLTDRNFNTTFFDPAGGGDPILYQHLFWF
FGHPEVYILILPGFGIISHIVSTFSGKPVFGYLGMVYAMISIGVLGFLVWAHHMFTVGLDVDTRAYFTAATMIIAVPTGI
KIFSWIATMWGGSIQYKTPMLFAVGFIFLFTIGGLTGIVLANSGLDIALHDTYYVVAHFHYVLSMGAVFALFAGFYYWVG
KIFGRTYPETLGQIHFWITFFGVNLTFFPMHFLGLSGMPRRIPDYPDAYAGWNALSSFGSYISVVGICCFFVVVTITLSS
GNNKRCAPSPWALELNSTTLEWMVQSPPAFHTFGELPAIKETKSYVK

>gi|16121653|ref|NP_404966.1| transport ATP-binding protein [Yersinia pestis]
MQTSHLMNKTRQYELIRWLKKQSAPAQRWLRLSMLLGLLSGLLIIAQAWLLATLLQSLIIDKLPRATLTTEFSLLAGAFA
LRAVISWLRERVGFICGMRVRQQIRKVVLDRLEQLGPSWVKGKPAGSWATIILEQIEDMQEYYSRYLPQMYLAVFIPVLI
LIAVFPINWAAGLILFVTAPLIPIFMILVGMGAADANRRNFVALARLSGNFLDRLRGLDTLRLFNRAKAETDQIRDSSED
FRSRTMEVLRMAFLSSAVLEFFAAISIAVVAVYFGFSYLGELNFGSYGLGVTLFAGFLVLILAPEFFQPLRDLGTFYHAK
AQAVGAAESLVTFLSSEGEAIGQGEKQLDGKEAIALEANELEILAPNGTRLAGPLNFSLPAGKRVAIVGQSGAGKSSLLN
LLLGFLPYRGSLKVNGIELRELEPQVWRSQLSWVGQNPHLPEQTLATNILLRQPDASEHQLQQAVERAYINEFLKDLPQG
LNTEIGDHSARLSVGQAQRIAVARALLNPCRLLLLDEPTASLDAHSEQLVMKALEEASRAQSTLLVTHQLEDTLGYDQIW
VMDNGRLIQQGDYSTLSQSAGSFANLLSQRNEEL

1.1 Make an alignment using dotlet

https://dotlet.vital-it.ch/
Import the sequences
1) Compare a sequence to itself e.g. Arabidopsis to Arabidopsis
2) Align the sequence of Rhodobacter (COX1_RHOS) with the one of Arabidopsis (Dataset 1)
3) Align the sequence of Arabidopsis to the one of Rhizobium (dataset 2)
4) Align the sequence of Arabidopsis to the sequence from dataset 3

Try out the effect of different substitution matrices and of the sequence length used to put a dot.



1.2 Perform a global alignment (Needleman Wunsch)

http://www.ebi.ac.uk/Tools/emboss/align/index.html

a) Use the Default settings (EMBOSS global alignment)
Write down the score for
Use dataset 1: 1715
Use dataset 2: 169
Use dataset 3: 31
And visually inspect the alignments
Question: Can you visually make a distinction between the relevant and spurious alignments?
Question: How is the score derived? Can it help you making a distinction between the relevant and spurious alignments?

Question: Is there much difference between the scores of the different datasets?
Question: Can you compare the score between the different datasets?
In principle you cannot because you are comparing different sequence sets that might not have exactly the same length. However, because the sequence lengths between the datasets here are quite comparable the score level gives a hint.

b) Try different gap parameter on the second dataset
Lower the gap opening penalty term: Increase the gap extension parameter.
What changes do you expect? Can you see them in the alignment? Use dataset2 as here the changes in parameter setting will most severely affect the results

Dataset2 13

Gap open 1
Gap extend 10
Dataset 2 656
Dataset 3 437
(many small gaps)

Gap open 100
Gap extend 10
Dataset 2 6
Dataset 3 2
(one big gap)

Question: Explain why you find each time you use different parameter setting a different score.
Because if you use a different scoring scheme different scores are obtained and these scores can not be compared mutually. As soon as you change the gap scoring systems alignment scores become incomparable (and a higher score does not reflect as better alignment)

c) change the substitution scoring matrices:
Question: From which score matrices can you choose?
Answer: a whole series of PAM and BLosum matrices

Try on dataset2 a different scoring scheme (lower blosum matrix 30).
Question: Why would this scoring scheme be more appropriate? –
Answer: Because we deal with evolutionary distant sequences (646.5)

Question: How is the score influenced by choosing a lower numbered BLOSUM matrix?
Answer: The score should improve (# Score: 643.5 instead of 169)! Note that this setting in which you have a global alignment, use the same gap penalty parameter and substitution scoring system (except for the numbers) and the same sequence set, the obtained alignment scores can be compared.

1.3 Perform a local alignment (Smith Waterman)

Use first the smith waterman procedure of Emboss
http://www.ebi.ac.uk/Tools/psa/emboss_water/

Default parameter settings used: (Blosum 60)

Write down the score?
Dataset 1: 1725
Dataset 2: 189
Dataset 3: 46

Question: For which dataset would you use the local alignments instead of the global ones? Why?
Answer: dataset 2 as this contain evolutionary quite distinct sequences that cannot well be aligned globally but that must contain local stretches that are conserved during evolution (in this case those that are involved in the binding of heme groups that are of relevance to the functioning of the protein)

Conclusion:

- Scores of alignments are length dependent and dependent on the scoring system so they can never be compared when trying to align different sequences (so no comparison of scores between datasets is possible)
- Scores of datasets of the same length (sequences to be aligned have the same length) can be compared as the used scoring system is the same (the gap penalty scoring and the substitution matrices).
- Scores obtained with different PAMs or BLOSUMs on the same pairwise alignment will differ and here it is assumed that the highest score is the result of the matrix being more adapted to the phylogenetic distance of the sequences to be compared. This does not tell anything about whether the alignment obtained with the respective different scoring systems will be the same.

Having a statistical meaning of the scores would help us in distinguishing between good and bad alignments

1.4 Statistical significance of local alignments

Now we will as a local alignment procedure, the procedure
http://www.ebi.ac.uk/Tools/services/web_lalign/

Use the BLOSUM62:

Default gap parameters are then: -7 open, -1 extension

```
>>gb|AAB58264.1| cbb3-type cytochrome oxidase              (540 aa)
 Waterman-Eggert score: 198;  29.7 bits; E(1) <  0.00033
24.6% identity (49.7% similar) in 455 aa overlap (23-423:74-451)

                  30        40             50         60
ref|NP FGAIA----GVMGTCFSVLIRMELARPGDQI-----LGGNHQLY-NVLITA---HAFLMI
       .: ::    ::.:   .:.: ..:: :  .:     .:  . .. ...: :   .:..:
gb|AAB YGLIATVFWGVVGFLVGVIIALQLAFPDLNIAPYLNFGRLRPVHTSAVIFAFGGNALIMT
            80        90       100       110       120       130

      70            80          90       100       110       120
ref|NP FFMVMP----AMIGGFGN--WFVPILIGAPDMAFPRLNNISFWLLPPSLLLLLSSALVEV
        : :.     : . : ::  :::                  ::          .  :  :
gb|AAB SFYVVQRTCRARLFG-GNLAWFV-----------------FW----------GYQLFIV
           140        150                                  160

           130       140            150       160       170
```

The advantage of this approach is that it calculates the significance of the alignments by shuffling.

Write down the scores:

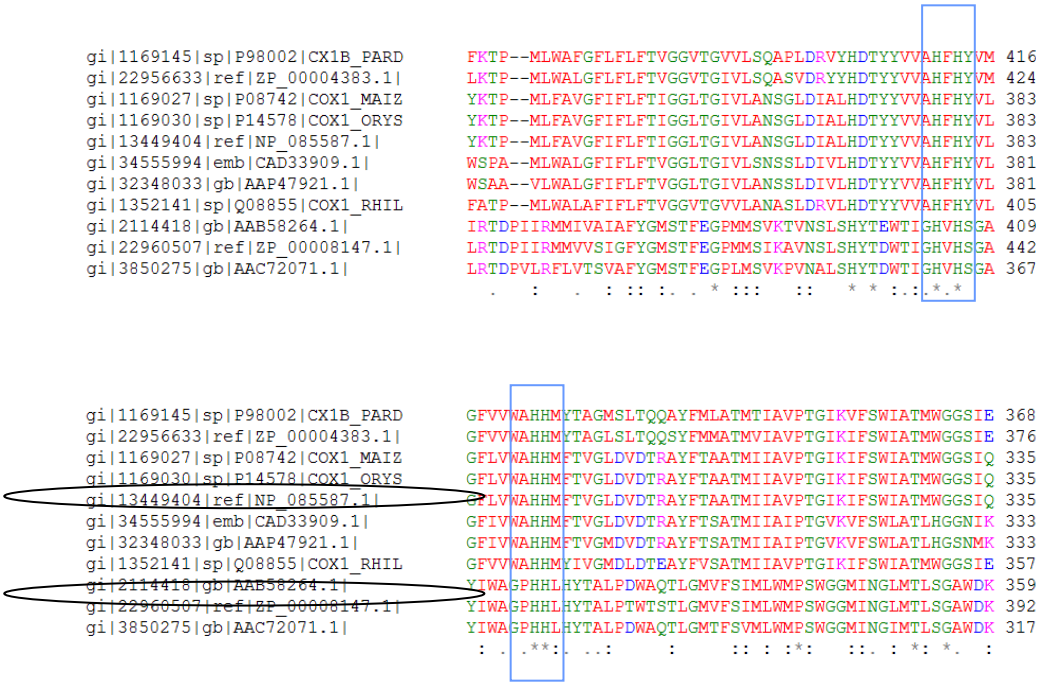
1) Dataset 1: `Waterman-Eggert score: 1720;  195.3 bits; E(1) <  5e-54`

2) Dataset 2 `Waterman-Eggert score: 181;  36.6 bits; E(1) <  2.8e-06`

3) Dataset 3: `Waterman-Eggert score: 47;  17.6 bits; E(1) <  0.8`

What is the meaning of the scores?
Can you compare the E-values between alignments?

Especially for 2 sequences that are not very similar anymore it is difficult to assess whether the alignment is still biologically true. Introducing more sequences and making use of a multiple sequence alignment can increase the information (see exercise 2)



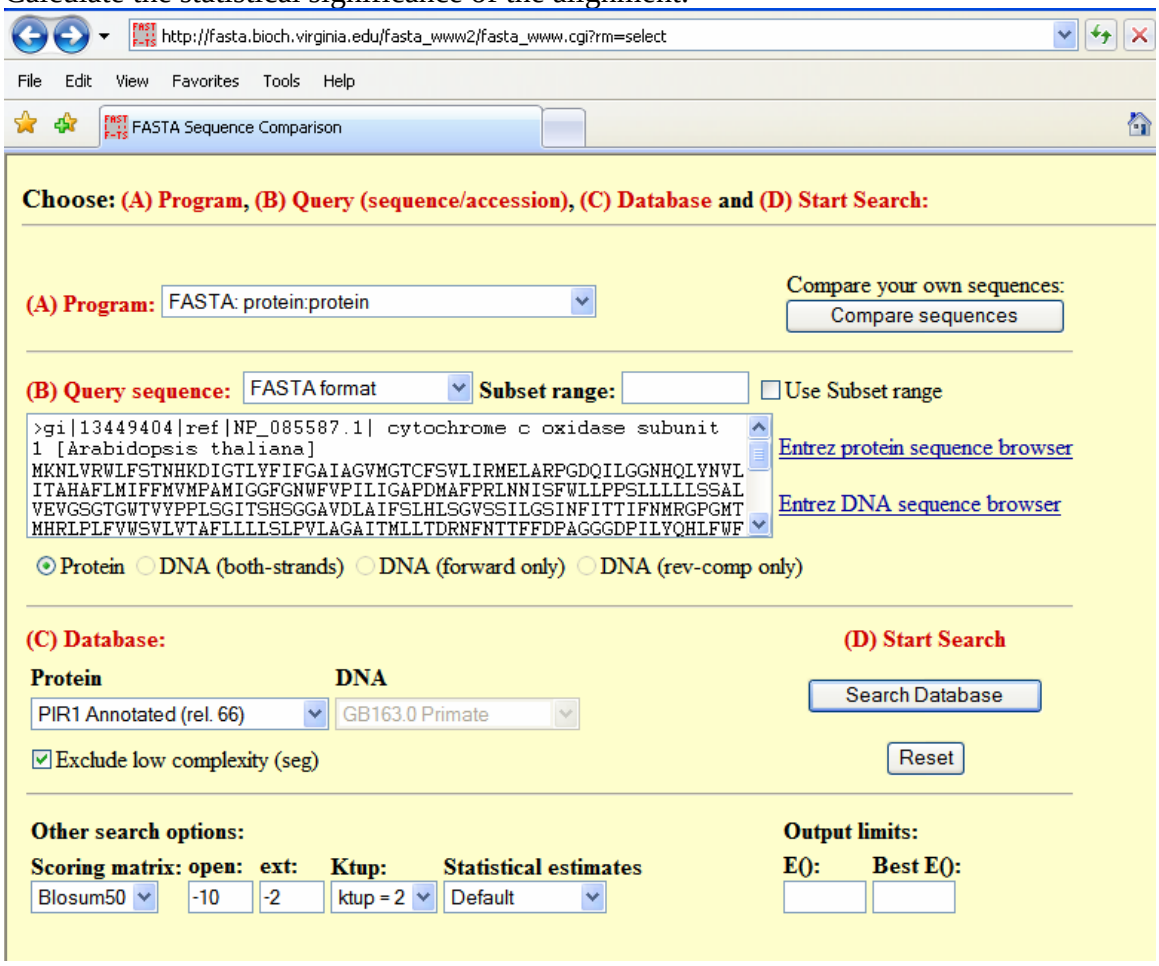
Tuning the parameters of the pairwise alignment allows you to get a better alignment of the relevant residues in case of data set 2 (see below for the parameters). The aminoacids required for the enzymatic reaction get better aligned.

Dataset 2

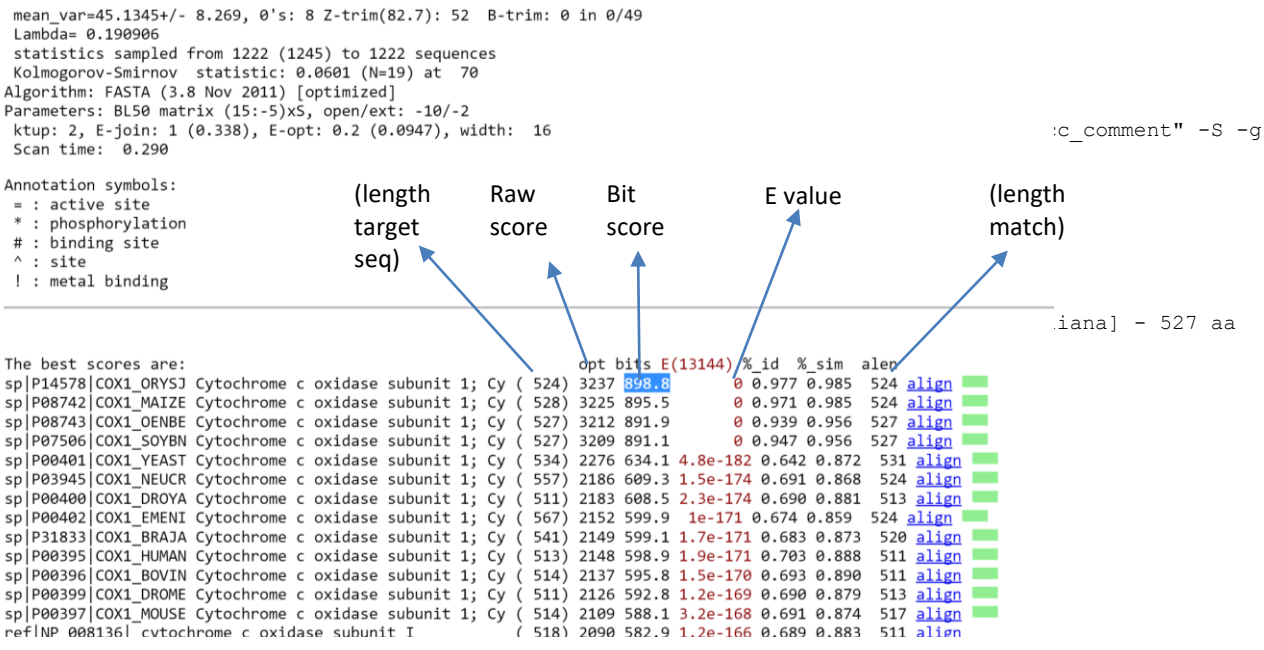
```
/seqprg/slib/bin/lalign -N 5000   -s P250  -f -16 -g -4 -w 75 -q @ @

 LALIGN finds the best local alignments between two sequences
 version 2.1u05 August 2003
Please cite:
 X. Huang and W. Miller (1991) Adv. Appl. Math. 12:373-381

alignments <  E(  0.05):score: 57 (50 max)
 Comparison of:
(A) @          gi|13449404|ref|NP_085587.1| cytochrome c oxidase  - 527 aa
(B) @          gi|2114418|gb|AAB58264.1| cbb3-type cytochrome oxi - 576 aa
 using matrix file: PAM250, gap penalties: -16/-4 E(limit)   0.05

  17.3% identity in 231 aa overlap (198-423:260-487); score:  147 E(10000): 5.3e-07

       200       210       220       230       240       250       260       270
gi|134 LLLSLPVLAGAITMLLTDRNFNTTFFDPAGGGDPILYQHLFWFFGHPEVYILILPGF-GIISHIVSTFSGKPVFG
       .......:  . .. .... .... .. ..: .  : :   :..:: .: ... .:: :.. ..:.. ...::..
gi|211 FIVTIAMLHVVNNLAVPASFLGSKSYSVSSGVQDALTQ---WWYGHNAVGFFLTAGFLGMMYYFVPKQANRPVYS
     260       270       280       290          300       310       320       330

            280       290       300       310       320       330       340
gi|134 YLGMVYAMISIGVLGFLVWAHHMFTVGLDVDTRAYFTAATMIIAVPTGIKIFSWIATMWGGSIQYKTPMLFAVGF
       :   .  . .. . . . .::.  ..:  ...   . .... .:.   ... . :. :.  . .:  .. . .
gi|211 YRLSIIHFWALIFMYIWAGPHHLHYTALPDWAQTLGMVFSIMLWMPSWGGMINGLMTLSGAWDKIRTDPIIRMMI
            340       350       360       370       380       390       400

          350       360       370       380       390       400       410
gi|134 --IFLFTIGGLTGIVLANSGLDIALHDTYYVVAHFHYVLSMGAVFALFAGFYYWVGKIFGRT--YPETLGQIHFW
         : ........: ... ....   : : ....: :      . .  :...:: . :..::.  :.  . . :::
gi|211 VAIAFYGMSTFEGPMMSVKTVNSLSHYTEWTIGHVHSGALGWVGMITFGAIYYLTPKLWGRERLYSLRMVNWHFW
        410       420       430       440       450       460       470       480

       420
gi|134 ITFFGV
       .. ::.
gi|211 LATFGI
```

1.5 Database search using FastA

Take a protein sequence from dataset 1
Use the FASTA program: http://fasta.bioch.virginia.edu/fasta/cgi/searchx.cgi?pgm=fa
What does this program do?
What is the meaning of ktup?
Calculate the statistical significance of the alignment.



Interpret the results?



```
  50     5     5:=*
  52    22    15:====*===
  54    26    32:========= *
  56    67    55:==================*====
  58    73    79:======================= *
```

fasta36 -p -q -w 80 -m 9I -m 6 -m 9I -m 6 -H -f -10 -V "!./annot/ann_feats2ipr.pl --neg --acc_comment" -S -g -2 TMP.q A 2

```
FASTA searches a protein or DNA sequence data bank
 version 36.3.8c Dec, 2015(preload9)
Please cite:
 W.R. Pearson & D.J. Lipman PNAS (1988) 85:2444-2448

Query: TMP.q
  1>>>gi|13449404|ref|NP_085587.1| cytochrome c oxidase subunit 1 [Arabidopsis
thaliana] - 527 aa
Library: PIR1 Annotated (13K)
  5122066 residues in 13144 sequences
       opt      E()
< 40     8     0:===
  42     0     0:            one = represents 3 library sequences
  44     0     0:
  46     0     0:
  48     7     1:*==
  50     6     5:=*
  52    18    15:====*=
  54    28    32:==========*
  56    64    55:==================*===
  58    71    79:=======================  *
  60    48    99:================                 *
  62    87   111:============================       *
  64   113   115:====================================*
  66   121   111:====================================*====
  68    92   102:==============================  *
  70    89    91:==============================*
  72    87    78:=========================*===
  74    77    65:====================*====
  76    52    54:=================*
  78    35    44:============  *
  80    35    35:===========*
  82    33    28:==========*=
  84    24    22:=======*
  86    33    17:=====*=====
  88    26    14:====*====
  90    11    11:===*
  92     8     8:==*
  94    13     6:=*===
  96    10     5:=*==
  98     7     4:=*=
 100     3     3:*
 102     0     2:*
 104     1     2:*
 106     6     1:*=
 108     1     1:*           inset = represents 1 library sequences
 110     1     1:*
 112     2     1:*          :*=
 114     0     1:*          :*
 116     2     0:=          *==
 118     3     0:=          *===
 120     1     0:=          *=
 122     1     0:=          *=
 124     0     0:           *
```

```
 126      1      0:=          *=
 128      0      0:           *
 130      1      0:=          *=
 132      0      0:           *
 134      0      0:           *
 136      0      0:           *
 138      0      0:           *
>140     19      0:=======    *===================
  5122066 residues in 13144 sequences
Statistics:  Expectation_n fit: rho(ln(x))= 7.2005+/-0.00321; mu= 9.5405+/-
0.174
 mean_var=45.1345+/- 8.269, 0's: 8 Z-trim(82.7): 52  B-trim: 0 in 0/49
 Lambda= 0.190906
 statistics sampled from 1222 (1245) to 1222 sequences
 Kolmogorov-Smirnov  statistic: 0.0601 (N=19) at  70
Algorithm: FASTA (3.8 Nov 2011) [optimized]
Parameters: BL50 matrix (15:-5)xS, open/ext: -10/-2
 ktup: 2, E-join: 1 (0.338), E-opt: 0.2 (0.0947), width:  16
 Scan time:  0.290
```

```
The best scores are:                                          opt bits E(13143) %_id  %_sim  alen
sp|P14578|COX1_ORYSJ Cytochrome c oxidase subunit 1; Cy ( 524) 3237 898.8       0 0.977 0.985  524
align
sp|P08742|COX1_MAIZE Cytochrome c oxidase subunit 1; Cy ( 528) 3225 895.5       0 0.971 0.985  524
align
sp|P08743|COX1_OENBE Cytochrome c oxidase subunit 1; Cy ( 527) 3212 891.9       0 0.939 0.956  527
align
sp|P07506|COX1_SOYBN Cytochrome c oxidase subunit 1; Cy ( 527) 3209 891.1       0 0.947 0.956  527
align
sp|P00401|COX1_YEAST Cytochrome c o
```

Alignment score (bins)

Observed number of target sequences with a given match score

Expected number of target sequences with a given match score

19 sequences have a match with the query with a score higher than 140

```
Query: TMP.q
  1>>>gi|13449404|ref|NP_085587.1| cytochrome c oxidase subunit 1 [Arabidopsis thal
Library: PIR1 Annotated (13K)
  5122066 residues in 13144 sequences
       opt      E()
< 40     8     0:===
  42     0     0:           one = represents 3 library sequences
  44     0     0:
  46     0     0:
  48     7     1:*==
  50     6     5:=*
  52    18    15:====*=
  54    28    32:==========*
  56    64    55:=====================*==
  58    71    79:==============================  *
  60    48    99:================                 *
  62    87   111:=============================================
  64   113   115:=================================*
  66   121   111:====================================*====
  68    92   102:==============================  *
  70    89    91:==============================*
  72    87    78:=========================*===
  74    77    65:====================*====
  76    52    54:=================*
  78    35    44:============  *
  80    35    35:===========*
  82    33    28:=========*=
  84    24    22:=======*
  86    33    17:=====*=====
  88    26    14:====*====
  90    11    11:===*
  92     8     8:==*
  94    13     6:=*===
  96    10     5:=*==
  98     7     4:=*=
 100     3     3:*
 102     0     2:*
 104     1     2:*
 106     6     1:*=
 108     1     1:*          inset = represents 1 library sequences
 110     1     1:*
 112     2     1:*         :*=
 114     0     1:*         :*
 116     2     0:=         *==
 118     3     0:=         *===
 120     1     0:=         *=
 122     1     0:=         *=
 124     0     0:          *
 126     1     0:=         *=
 128     0     0:          *
 130     1     0:=         *=
 132     0     0:          *
 134     0     0:          *
 136     0     0:          *
 138     0     0:          *
>140    19     0:=======   *==================
  5122066 residues in 13144 sequences
Statistics:  Expectation_n fit: rho(ln(x))= 7.2005+/-0.00321; mu= 9.5405+/- 0.174
```

1.6 Database search using Blast

Go to the Blast homepage at NCBI
What is the difference between blastn, blastp,blastX
What does nr (on redundant mean)

Blast the unknown sequence

Interpret the result:
Explain E-value, bitscore, Identities, Positives, Query Subject
What does E-value = 0 mean?
What is the best match in the database?

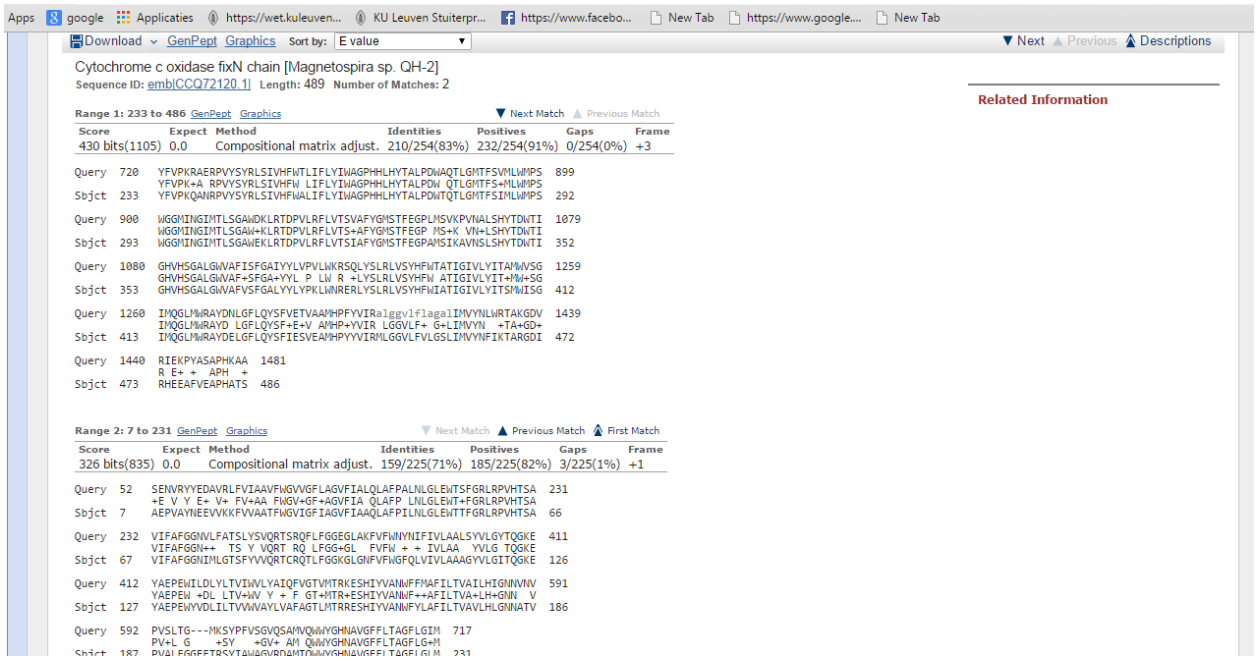
Do you have a clue on the function of the protein?
Go to the GenBank file of the best hit: (click on the link)
What information can you find in the GenBank file?

Suppose in the lab you sequenced the following sequence.
To learn more about the function of this gene, search for a homologue in the protein database.

```
>gene 1
ATGACATCAGCGACTCTGACGCCAGGGGCCGCCCTGGGCAGCCAGCGGGTGTCGGAAAATGTGCGTTACTACGAAGACGC
CGTCCGACTCTTCGTCATCGCTGCAGTGTTCTGGGGCGTCGTCGGCTTCCTCGCCGGCGTCTTCATCGCGCTGCAGCTGG
CTTTTCCGGCGCTGAATCTCGGCCTTGAGTGGACGAGCTTCGGGCGCCTGCGGCCGGTCCACACCTCGGCCGTGATCTTC
GCGTTTGGCGGCAACGTCCTGTTCGCCACCTCGCTCTACTCCGTGCAGCGCACCAGCCGCCAGTTCCTGTTCGGCGGCGA
GGGCCTCGCGAAGTTCGTCTTCTGGAACTACAACATCTTCATCGTCCTGGCGGCGCTCAGCTACGTGCTCGGCTACACCC
AGGGCAAGGAGTATGCAGAGCCGGAGTGGATCCTCGACCTCTACCTGACGGTCATCTGGGTCCTCTACGCCATCCAGTTC
GTCGGCACGGTGATGACCCGCAAGGAGTCGCACATCTACGTCGCCAACTGGTTCTTCATGGCGTTCATCCTGACCGTCGC
GATCCTCCACATCGGCAACAACGTCAACGTCCCGGTGTCGCTGACCGGGATGAAGTCCTACCCGTTCGTCTCGGGCGTGC
AGAGCGCCATGGTGCAGTGGTGGTACGGCCACAACGCGGTCGGCTTCTTCCTGACCGCCGGCTTCCTCGGCATCATGTCT
ACTTCGTTCCGAAGCGCGCGGAGCGGCCGGTCTATTCGTACCGCCTGTCGATCGTGCACTTCTGGACGCTGATCTTCCTC
TACATCTGGGCCGGCCCGCACCACCTGCACTACACGGCCCTGCCGGATTGGGCGCAGACGCTGGGCATGACCTTCTCGGT
CATGCTGTGGATGCCGTCCTGGGGCGGCATGATCAACGGCATCATGACCCTGTCGGGTGCCTGGGACAAGCTGCGCACCG
ACCCGGTCCTGCGCTTCCTCGTGACGTCGGTGGCCTTCTACGGCATGTCGACCTTCGAGGGCCCGCTGATGTCGGTGAAG
CCGGTCAACGCCCTGTCGCACTACACCGACTGGACGATCGGCCACGTGCACTCCGGTGCGCTCGGCTGGGTGGCCTTCAT
CTCCTTCGGCGCGATCTACTATCTGGTCCCGGTCCTGTGGAAGCGCTCGCAGCTCTACAGCCTGCGTCTGGTCAGCTACC
ACTTCTGGACCGCCACCATCGGCATCGTGCTCTACATCACCGCCATGTGGGTGTCGGGCATCATGCAGGGCCTGATGTGG
CGCGCCTACGACAACCTCGGCTTCCTCCAGTACTCGTTCGTCGAGACGGTCGCGGCCATGCATCCCTTCTACGTGATCCG
TGCGCTGGGCGGCGTCCTGTTCCTGGCTGGTGCCCTGATCATGGTCTACAACCTGTGGCGCACGGCCAAGGGTGACGTCC
GCATCGAGAAGCCCTATGCCTCCGCCCCGCACAAGGCGGCGGTCGGTGCGGCCTGA
```

Blast the following sequence to the non redundant protein database.

Which sequence has the highest score.
How do you explain the different HSPs observed for the same sequence?
What does the E-value tell you?


Apps
google
Applicaties
https://wet.kuleuven...
KU Leuven Stuiterpr...
https://www.facebo...
New Tab
https://www.google....
New Tab
Download
GenPept
Graphics
Sort by:
E value
Next
Previous
Descriptions
Cytochrome c oxidase fixN chain [Magnetospira sp. QH-2]
Sequence ID: emb|CCQ72120.1| Length: 489 Number of Matches: 2
Related Information
Range 1: 233 to 486 GenPept Graphics
Next Match
Previous Match
Score Expect Method Identities Positives Gaps Frame
430 bits(1105) 0.0 Compositional matrix adjust. 210/254(83%) 232/254(91%) 0/254(0%) +3
Query 720 YFVPKRAERPVYSYRLSIVHFWTLIFLYIWAGPHHLHYTALPDWAQTLGMTFSVMLWMPS 899
YFVPK+A RPVYSYRLSIVHFW LIFLYIWAGPHHLHYTALPDW QTLGMTFS+MLWMPS
Sbjct 233 YFVPKQANRPVYSYRLSIVHFWALIFLYIWAGPHHLHYTALPDWTQTLGMTFSIMLWMPS 292
Query 900 WGGMINGIMTLSGAWDKLRTDPVLRFLVTSVAFYGMSTFEGPLMSVKPVNALSHYTDWTI 1079
WGGMINGIMTLSGAW+KLRTDPVLRFLVTS+AFYGMSTFEGP MS+K VN+LSHYTDWTI
Sbjct 293 WGGMINGIMTLSGAWEKLRTDPVLRFLVTSIAFYGMSTFEGPAMSIKAVNSLSHYTDWTI 352
Query 1080 GHVHSGALGWVAFISFGAIYYLVPVLWKRSQLYSLRLVSYHFWTATIGIVLYITAMWVSG 1259
GHVHSGALGWVAF+SFGA+YYL P LW R +LYSLRLVSYHFW ATIGIVLYIT+MW+SG
Sbjct 353 GHVHSGALGWVAFVSFGALYYLYPKLWNRERLYSLRLVSYHFWIATIGIVLYITSMWISG 412
Query 1260 IMQGLMWRAYDNLGFLQYSFVETVAAMHPFYVIRalggvlflagalIMVYNLWRTAKGDV 1439
IMQGLMWRAYD LGFLQYSF+E+V AMHP+YVIR LGGVLF+ G+LIMVYN +TA+GD+
Sbjct 413 IMQGLMWRAYDELGFLQYSFIESVEAMHPYYVIRMLGGVLFVLGSLIMVYNFIKTARGDI 472
Query 1440 RIEKPYASAPHKAA 1481
R E+ + APH +
Sbjct 473 RHEEAFVEAPHATS 486
Range 2: 7 to 231 GenPept Graphics
Next Match
Previous Match
First Match
Score Expect Method Identities Positives Gaps Frame
326 bits(835) 0.0 Compositional matrix adjust. 159/225(71%) 185/225(82%) 3/225(1%) +1
Query 52 SENVRYYEDAVRLFVIAAVFWGVVGFLAGVFIALQLAFPALNLGLEWTSFGRLRPVHTSA 231
+E V Y E+ V+ FV+AA FWGV+GF+AGVFIA QLAFP LNLGLEWT+FGRLRPVHTSA
Sbjct 7 AEPVAYNEEVVKKFVVAATFWGVIGFIAGVFIAAQLAFPILNLGLEWTTFGRLRPVHTSA 66
Query 232 VIFAFGGNVLFATSLYSVQRTSRQFLFGGEGLAKFVFWNYNIFIVLAALSYVLGYTQGKE 411
VIFAFGGN++ TS Y VQRT RQ LFGG+GL FVFW + + IVLAA YVLG TQGKE
Sbjct 67 VIFAFGGNIMLGTSFYVVQRTCRQTLFGGKGLGNFVFWGFQLVIVLAAAGYVLGITQGKE 126
Query 412 YAEPEWILDLYLTVIWVLYAIQFVGTVMTRKESHIYVANWFFMAFILTVAILHIGNNVNV 591
YAEPEW +DL LTV+WV Y + F GT+MTR+ESHIYVANWF++AFILTVA+LH+GNN V
Sbjct 127 YAEPEWYVDLILTVVWVAYLVAFAGTLMTRRESHIYVANWFYLAFILTVAVLHLGNNATV 186
Query 592 PVSLTG---MKSYPFVSGVQSAMVQWWYGHNAVGFFLTAGFLGIM 717
PV+L G +SY +GV+ AM QWWYGHNAVGFFLTAGFLG+M
Sbjct 187 PVALFGGFFTRSYIAWGVRDAMTQWWYGHNAVGFFLTAGFLGLM 231