Master Biomedizin 2016

Phylogeny

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*Images from: Wikipedia

11

a. Using the proteins “P13056” and “P49116”, find their orthologs in UniProt in the following organisms: mouse (*Mus musculus*), platypus (*Ornithorhynchus anatinus*), frog (*Xenopus laevis*) and chicken (*Gallus gallus*). Choose reviewed entries whenever possible. Save the sequences in a fasta file.

b. Which phylogenetic relations can you describe using these sequences? Use Trex.

Mouse
(*Mus musculus*)

Chicken (rooster)
(*Gallus gallus*)

Frog
(*Xenopus laevis*)

Platypus
(*Ornithorhynchus anatinus*)

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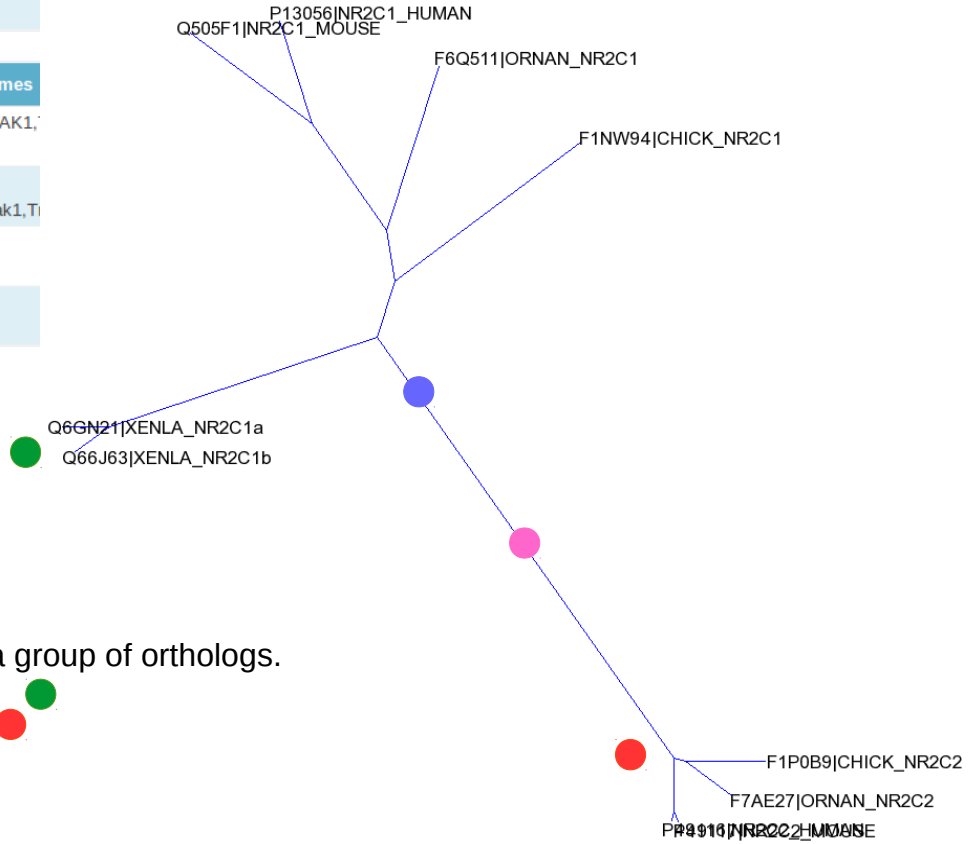
Homology + Phylogeny

*Images from: UniProt, Trex

11

Your list:...AXKGJ	Entry	Entry name		Protein names	Gene names	Organism	Length
P13056	P13056	NR2C1_HUMAN		**Nuclear receptor subfamily 2 group ...**	**NR2C1** TR2	Homo sapiens (Human)	603
Q505F1	Q505F1	NR2C1_MOUSE		**Nuclear receptor subfamily 2 group ...**	**Nr2c1** Tr2,Tr2-11	Mus musculus (Mouse)	590
F6Q511	F6Q511	F6Q511_ORNAN		**Uncharacterized protein**	**NR2C1**	Ornithorhynchus anatinus (Duckbill platypus)	652
Q6GN21	Q6GN21	N2C1A_XENLA		**Nuclear receptor subfamily 2 group ...**	**nr2c1-a** dor2	Xenopus laevis (African clawed frog)	637
Q66J63	Q66J63	N2C1B_XENLA		**Nuclear receptor subfamily 2 group ...**	**nr2c1-b** dor2	Xenopus laevis (African clawed frog)	637
F1NW94	F1NW94	F1NW94_CHICK		**Uncharacterized protein**	**NR2C1**	Gallus gallus (Chicken)	562

Your list:...UZYS3	Entry	Entry name		Protein names	Gene names
P49116	P49116	NR2C2_HUMAN		**Nuclear receptor subfamily 2 group ...**	**NR2C2** TAK1,
P49117	P49117	NR2C2_MOUSE		**Nuclear receptor subfamily 2 group ...**	**Nr2c2** Mtr2r1,Tak1,T
F7AE27	F7AE27	F7AE27_ORNAN		**Uncharacterized protein**	**NR2C2**
F1P0B9	F1P0B9	F1P0B9_CHICK		**Uncharacterized protein**	**NR2C2**



a. 10 sequences.

b. 1) All NR2C1 proteins together in one branch, as well as the NR2C2 proteins in another branch.
2) All of them are homologs, and each branch contains a group of orthologs.
3) In relation to NR2C1, *X.laevis* suffered a duplication.
4) In relation to NR2C2, *X.laevis* suffered a gene loss.

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14

Consider the following sequences: P50225, P50226, P0DMM9, P0DMN0, O43704, O00338, Q6IMI6, O75897. All of them are part of a human paralog family (sulfotransferases).

a. Look for the coordinates of the substrate binding region in the UniProt entry “P50225”. Do you expect all the paralogs to share this substrate binding region? Why? Check it.

b. Using the previous sequences, could you determine which human paralog is the ortholog of the sequence in “*file4.fasta*” (https://cbdm.uni-mainz.de/mb16/)? Do not use BLAST.

c. Which protein is the one found in “file4.fasta”?

MSA + Phylogeny + Homology

*Images from: UniProt, Trex

14

(P50225)

Feature key	Position(s)	Length	Description
Region [i]	106 – 108	3	Substrate binding

```
        10         20         30         40         50
MELIQDTSRP PLEYVKGVPL IKYFAEALGP LQSFQARPDD LLISTYPKSG
        60         70         80         90        100
TTWVSQILDM IYQGGDLEKC HRAPIFMRVP FLEFKAPGIP SGMETLKDTP
       110        120        130        140        150
APRLLKTHLP LALLPQTLLD QKVKVVYVAR NAKDVAVSYY HFYHMAKVHP
       160        170        180        190        200
EPGTWDSFLE KFMVGEVSYG SWYQHVQEWW ELSRTHPVLY LFYEDMKENP
       210        220        230        240        250
KREIQKILEF VGRSLPEETV DFVVQHTSFK EMKKNPMTNY TTVPQEFMDH
       260        270        280        290
SISPFMRKGM AGDWKTTFTV AQNERFDADY AEKMAGCSLS FRSEL
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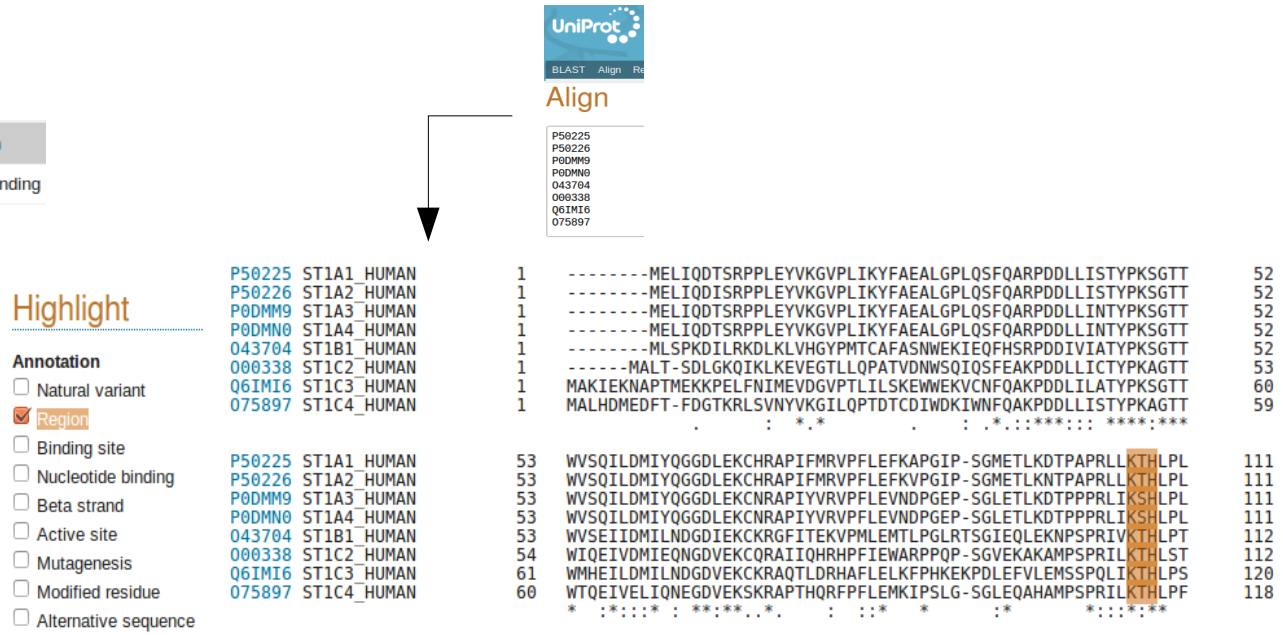
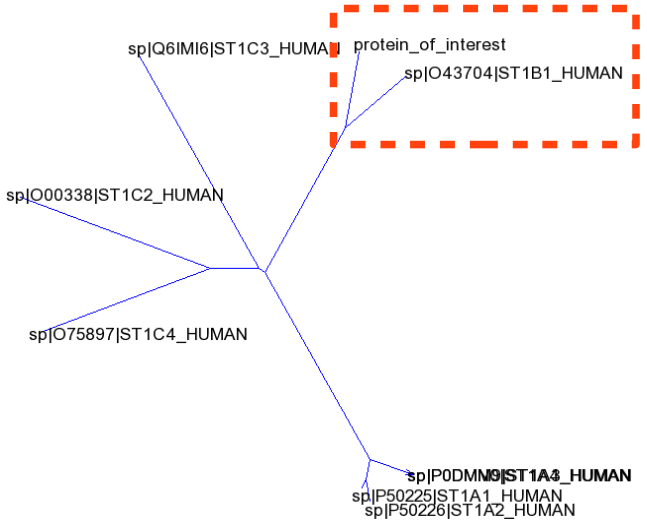


a. Yes, because they share the motif “K[ST]H”.

b. The ortholog of “protein_of_interest” is ST1B1_HUMAN.

c. ST1B1_CANLF, from *Canis familiaris* (dog).

Entry	Protein names	Match hit (200 400 600 800 1k)	Identity
Q95JD5	Sulfotransferase family cytosolic 1B member 1 (Canis lupus familiaris)		100.0%
F6V3V8	Sulfotransferase (Canis lupus familiaris)		98.3%
D2I2Q5	Sulfotransferase (Ailuropoda melanoleuca)		94.3%
U6CQE2	Sulfotransferase (Neovison vison)		93.6%



15

Using T-Coffee, align the protein sequences found in "*file3.fasta*" (https://cbdm.uni-mainz.de/mb16/). All of them are paralogs, but one ancestor protein to be used as outgroup.

a. Could you infer from the alignment which sequence should be used as outgroup?

b. In what order were the paralogs originated after diverging from the ancestor?

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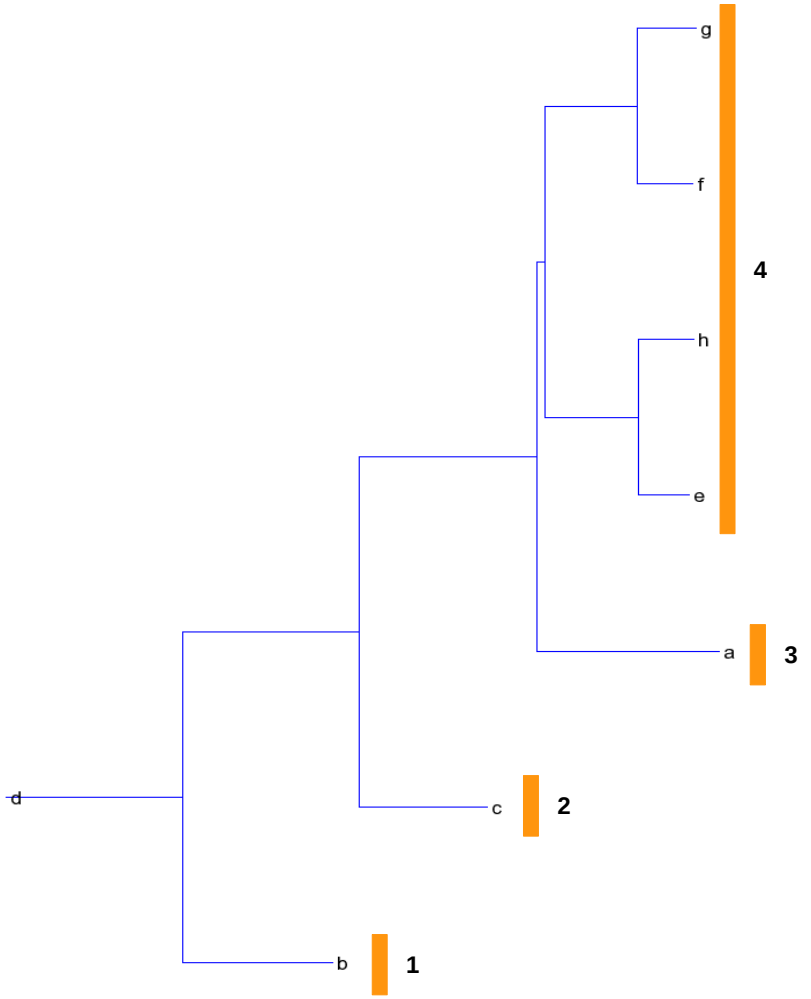
MSA + Phylogeny

*Images from: Tcoffee, Trex

15

a. Outgroup: “d”. Hints in red boxes.

b. Order: b, c, a, ancestor of [e+h] & [f+g].



16

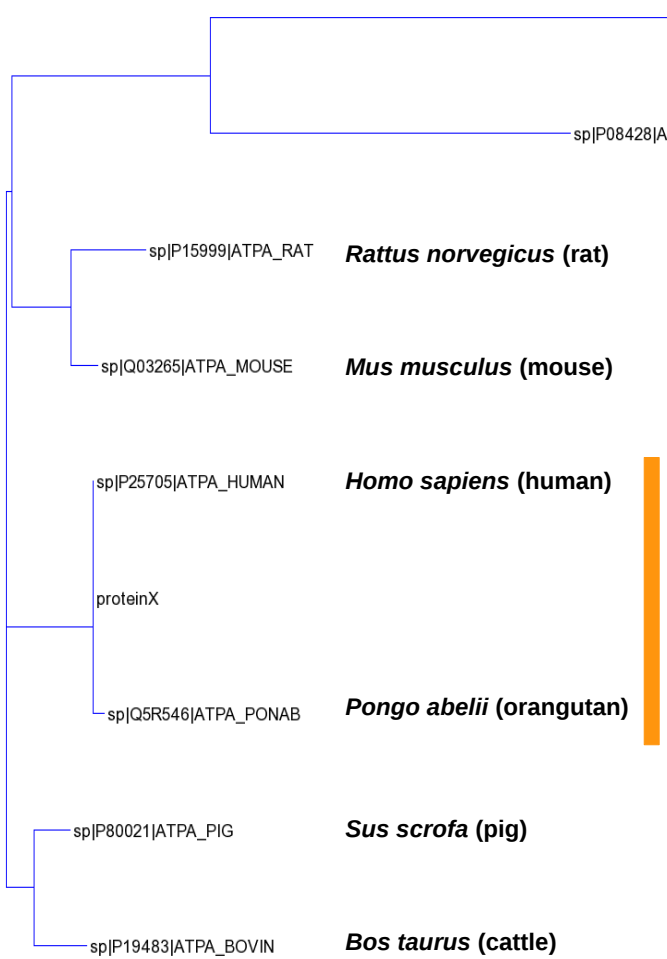
a. Using "*file2.fasta*" (https://cbdm.uni-mainz.de/mb16/) and Trex, can you approximate to which taxonomic division belongs "proteinX"?

b. From which organism could it be? After guessing, check it.

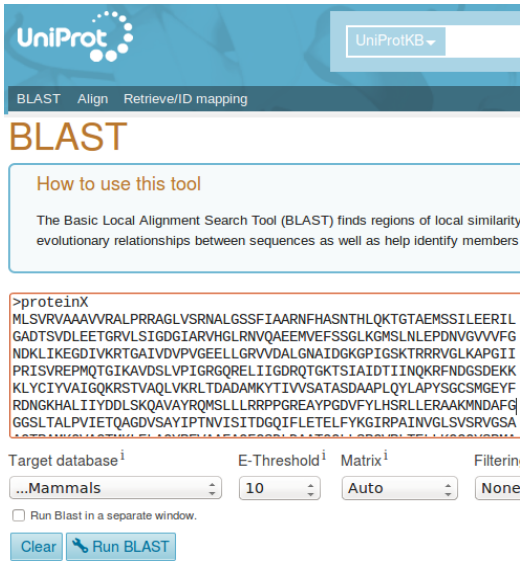
Phylogeny

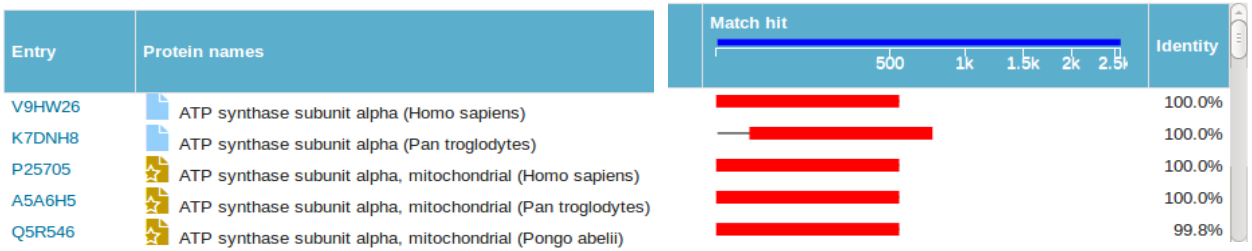
*Images from: Trex, UniProt

16



a. Primates





b. *Homo sapiens* (human) or *Pan troglodytes* (chimpanzee), they are 100% identical.

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17

Human hemoglobin consists of four protein subunits: two from the alpha globin gene cluster (located on chromosome 16) and two more from the beta globin gene cluster (located on chromosome 11). But there are at least nine different globin genes in these clusters, which are: zeta, alpha, gamma2, theta1, mu, beta, gamma1, epsilon, alpha, delta.

a. Find the protein sequence for each one of them.

b. Sort them either in cluster alpha or cluster beta.

c. Why do you think they are clustered in either cluster alpha or cluster beta?

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Phylogeny

*Images from: UniProt, Trex, UCSC

17

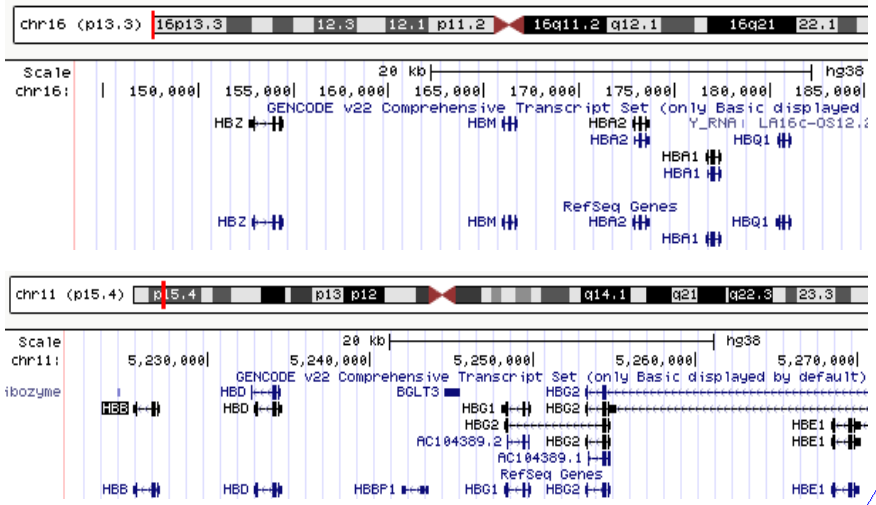
	Your list:...ITXRE	Entry	Entry name		Protein names
☐	HBAZ_HUMAN	P02008	HBAZ_HUMAN		Hemoglobin subunit zeta
☐	HBM_HUMAN	Q6B0K9	HBM_HUMAN		Hemoglobin subunit mu
☐	HBA_HUMAN	P69905	HBA_HUMAN		Hemoglobin subunit alpha
☐	HBAT_HUMAN	P09105	HBAT_HUMAN		Hemoglobin subunit theta-1
☐	HBE_HUMAN	P02100	HBE_HUMAN		Hemoglobin subunit epsilon
☐	HBG1_HUMAN	P69891	HBG1_HUMAN		Hemoglobin subunit gamma-1
☐	HBG2_HUMAN	P69892	HBG2_HUMAN		Hemoglobin subunit gamma-2
☐	HBD_HUMAN	P02042	HBD_HUMAN		Hemoglobin subunit delta
☐	HBB_HUMAN	P68871	HBB_HUMAN		Hemoglobin subunit beta

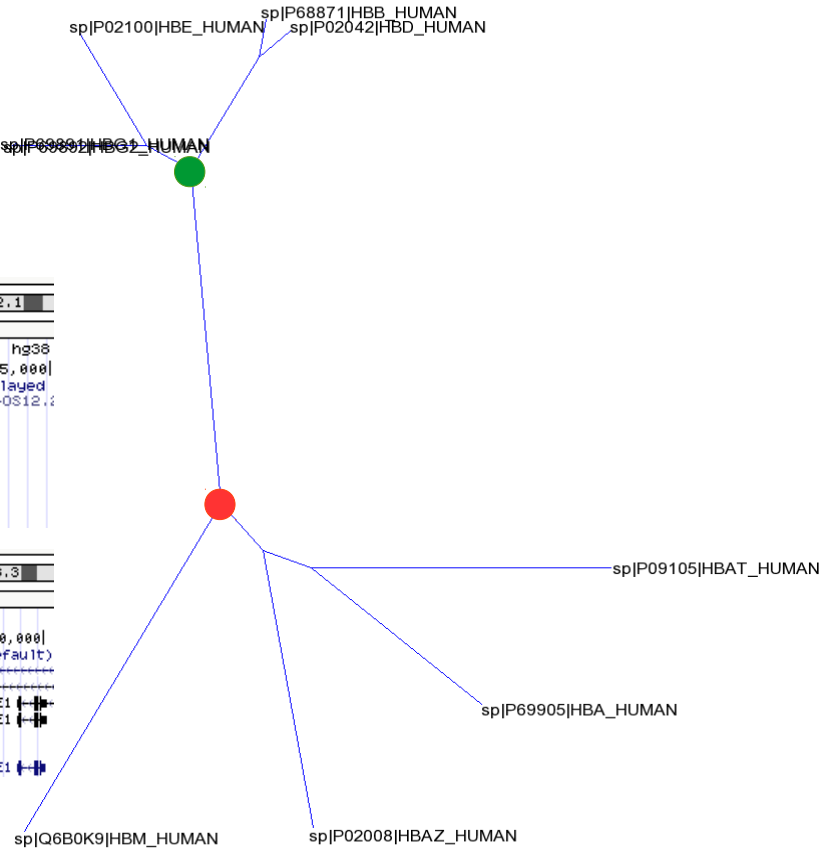
a. In UniProt.

b. Using Trex:

Cluster alpha

Cluster beta





c. Paralogous expansion from one ancestral alpha and one ancestral beta.

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18

a. All of the sequences in "*file1.fasta*" (https://cbdm.uni-mainz.de/mb16/) are homologs. How many groups of orthologs would you say there are in this file?

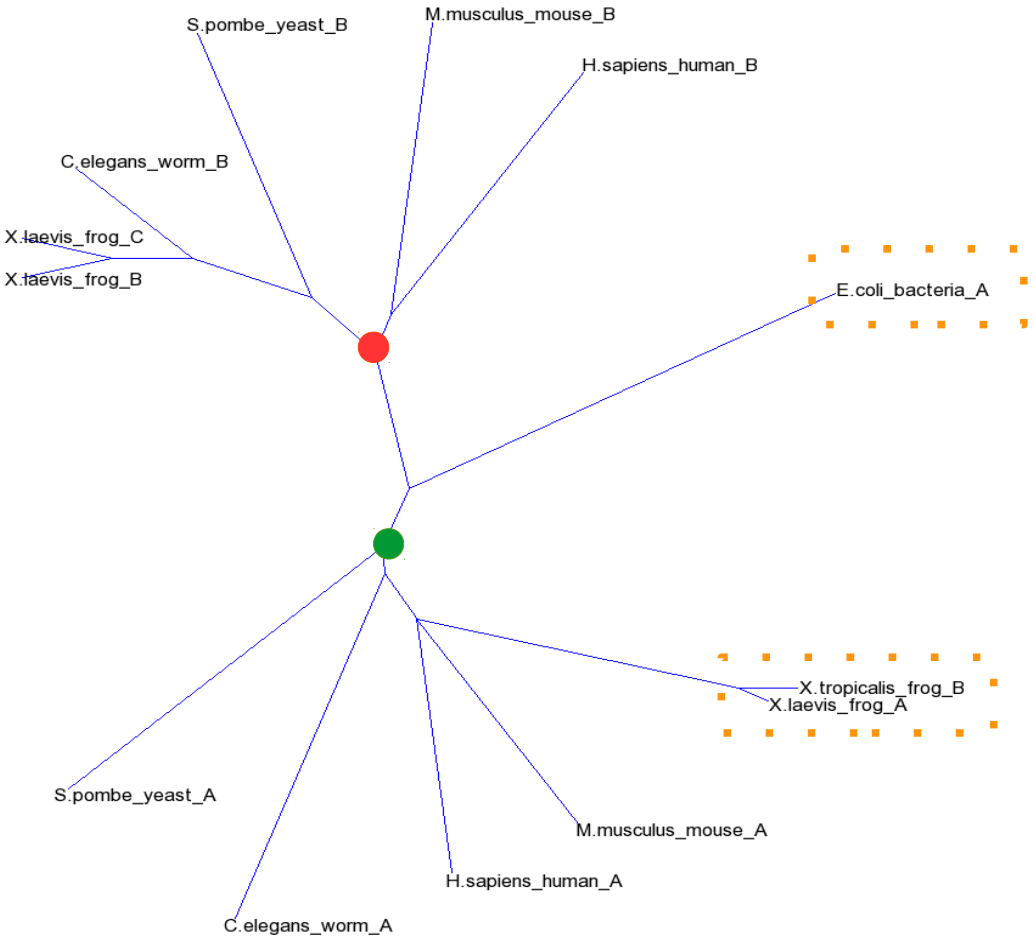
b. What could you say about the history of this protein family?

c. Would you say there is any wrongly annotated sequence?

Phylogeny

*Images from: Trex

18



a. Two groups of orthologs:

- Proteins “A”
- Proteins “B”

b. E.coli has only one protein, and then it duplicated to form A and B. It is possible that X.laevis_B duplicated later to form B and C.

c. X.tropicalis_B is wrongly annotated. It should be X.tropicalis_A, because they are in the same branch. The actual X.tropicalis_B is not in the dataset, or was lost during evolution.

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*Images from: Wikipedia

19

Consider the following sequences: Q9T4B2 (CYB_DELDE, dolphin), P00156 (CYB_HUMAN, human), P00157 (CYB_BOVIN, cattle), Q9MIX8 (CYB_DANRE, zebrafish), P18946 (CYB_CHICK, chicken), Q36461 (CYB_ORNAN, platypus) and P00160 (CYB_XENLA, frog).

a. Do you think that a phylogenetic tree built using these sequences would be coherent with the evolution?

b. Check with the taxonomy provided by UniProt (http://www.uniprot.org/taxonomy/).

Human
(*Homo sapiens*)

Dolphin
(*Delphinus delphis*)

Cattle
(*Bos taurus*)

Chicken (rooster)
(*Gallus gallus*)

Zebrafish
(*Danio rerio*)

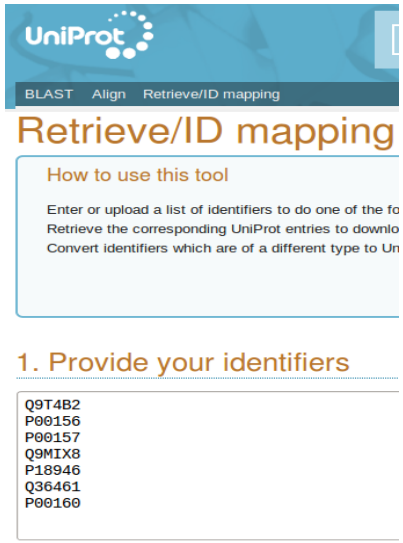
Platypus
(*Ornithorhynchus anatinus*)

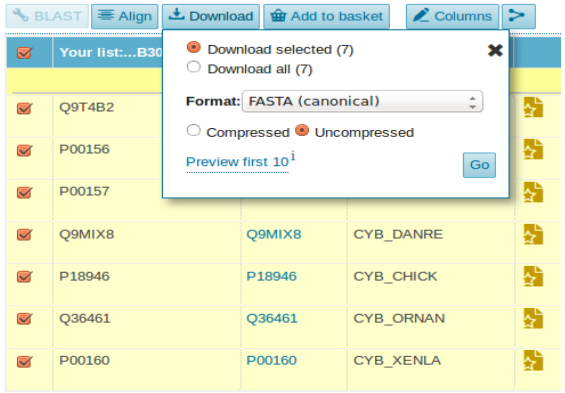
Frog
(*Xenopus laevis*)

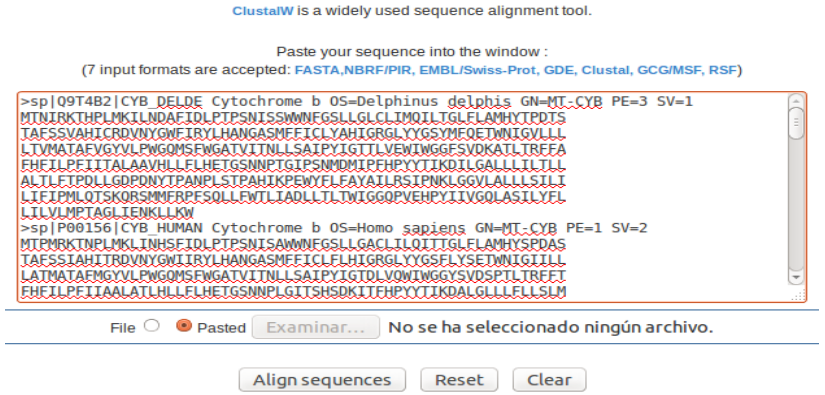
Phylogeny

*Images from: UniProt, Trex

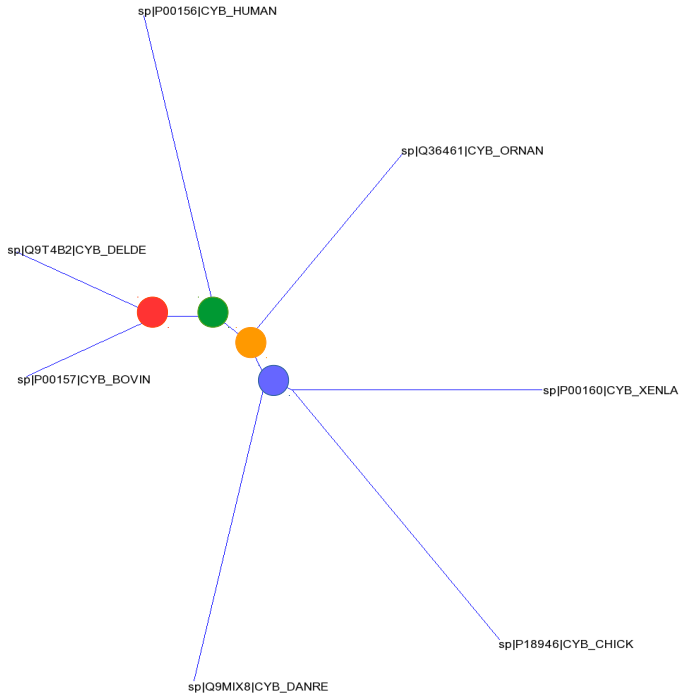
19







Dolphin → Eukaryota › Metazoa › Chordata › Craniata › Vertebrata › Euteleostomi › Mammalia › Eutheria › Laurasiatheria › Cetartiodactyla › Cetacea › Odontoceti › Delphinidae › Delphinus
Cattle → Eukaryota › Metazoa › Chordata › Craniata › Vertebrata › Euteleostomi › Mammalia › Eutheria › Laurasiatheria › Cetartiodactyla › Ruminantia › Pecora › Bovidae › Bovinae › Bos
Human → Eukaryota › Metazoa › Chordata › Craniata › Vertebrata › Euteleostomi › Mammalia › Eutheria › Euarchontoglires › Primates › Haplorrhini › Catarrhini › Hominidae › Homo
Platypus → Eukaryota › Metazoa › Chordata › Craniata › Vertebrata › Euteleostomi › Mammalia › Monotremata › Ornithorhynchidae › Ornithorhynchus
Frog → Eukaryota › Metazoa › Chordata › Craniata › Vertebrata › Euteleostomi › Amphibia › Batrachia › Anura › Pipoidea › Pipidae › Xenopodinae › Xenopus › Xenopus
Chicken → Eukaryota › Metazoa › Chordata › Craniata › Vertebrata › Euteleostomi › Archelosauria › Archosauria › Dinosauria › Saurischia › Theropoda › Coelurosauria › Aves › … > Gallus
Zebrafish → Eukaryota › Metazoa › Chordata › Craniata › Vertebrata › Euteleostomi › Actinopterygii › Neopterygii › Teleostei › Ostariophysi › Cypriniformes › Cyprinidae › Danio



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