Master Biomedizin 2016

Genomics
Homology

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*Images from: Wikipedia

1

a. Get the fasta sequence of the human (*Homo sapiens*) protein P53 from UniProt (http://www.uniprot.org/). Which one of all the isoforms should you download?

b. Find the P53 protein from mouse (*Mus musculus*). As you see, there are more than one entry for mouse. Which UniProt entry should you select?

c. BLAT the human P53 using “hg19” as database (in UCSC, http://genome.ucsc.edu/), and answer:

How many amino acids has the query sequence?
And how many nucleotides?
Is it a perfect alignment?
Which is the genomic locus of the target?

d. Visualize and navigate through the P53 genome region, and answer:

Which genes are around?
How many exons does it have?

e. BLAT the mouse P53 against the human genome “hg19”. What do you observe?

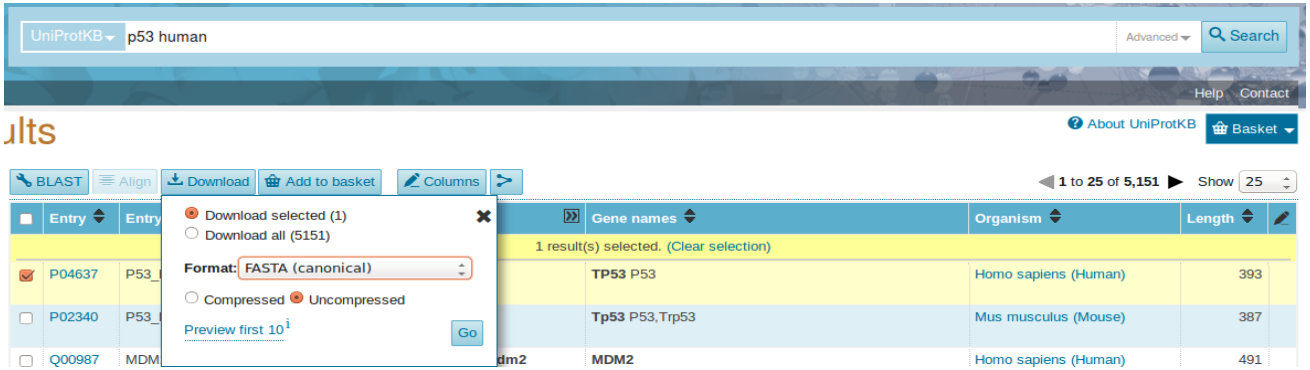
Human
(*Homo sapiens*)

Mouse
(*Mus musculus*)

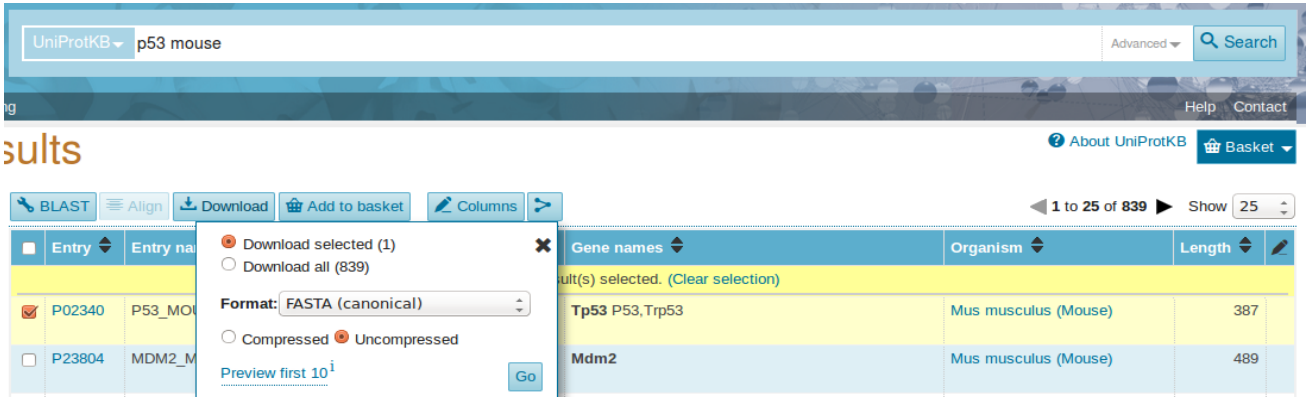
Genomics

*Images from: UniProt

1



a. P04637 (P53_HUMAN). The canonical.



b. P02340 (P53_MOUSE).

Genomics

*Images from: UCSC

1

BLAT Search Genome

Genome:	Assembly:	Query type:	Sort output:	Output type:
Human	Feb. 2009 (GRCh37/hg19)	BLAT's guess	query,score	hyperlink

```
>sp|P04637|P53_HUMAN Cellular tumor antigen p53 OS=Homo sapiens GN=TP53 PE=1 SV=4
MEEPQSDPSVEPPLSQETFSDLWKLLPENNVLSPLPSQAMDDLMLSPDDIEQWFTEDPGP
DEAPRMPEAAPPVAPAPAAPTPAAPAPAPSWPLSSSVPSQKTYQGSYGFRLGFLHSGTAK
SVTCTYSPALNKMFCQLAKTCPVQLWVDSTPPPGTRVRAMAIYKQSQHMTEVVRRCPHHE
RCSDSDGLAPPQHLIRVEGNLRVEYLDDRNTFRHSVVVPYEPPEVGSDCTTIHYNYMCNS
SCMGGMNRRPILTIITLEDSSGNLLGRNSFEVRVCACPGRDRRTEEENLRKKGEPHHELP
PGSTKRALPNNTSSSPQPKKKPLDGEYFTLQIRGRERFEMFRELNEALELKDAQAGKEPG
GSRAHSSHLKSKKGQSTSRHKKLMFKTEGPDSD
```

submit | I'm feeling lucky | clear

ACTIONS	QUERY	SCORE	START	END	QSIZE	IDENTITY	CHRO	STRAND	START	END	SPAN
browser details	P53_HUMAN	1149	1	393	393	100.0%	17	+-	7572930	7579912	6983

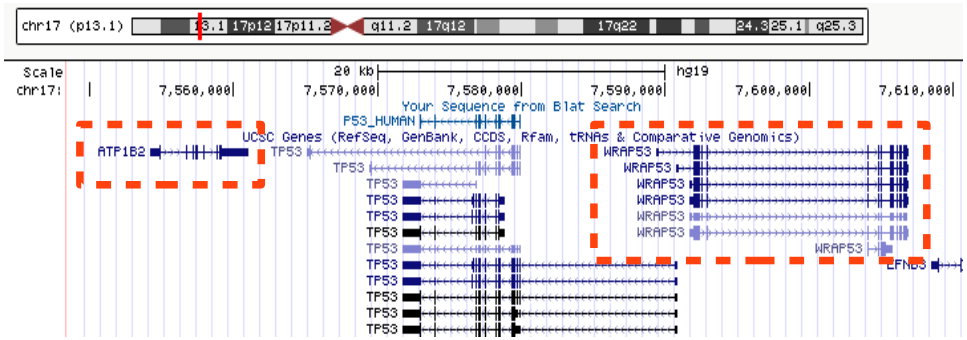
c. 393 amino acids
393*3 = 1179 nucleotides

Not a perfect alignment
(“*lpennvl*”)

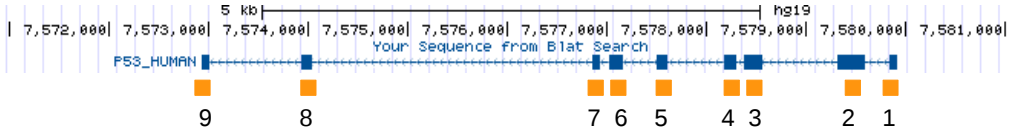
chr17
7572930-7579912

P53_HUMAN

```
MEEPQSDPSV EPPLSQETFS DLWKLlpenn vlSPLPSQAM DDLMLSPDDI EQWFTEDPGP  60
DEAPRMPEAA PPVAPAPAAP TPAAPAPAPS WPLSSSVPSQ KTYQGSYGFR LGFLHSGTAK  120
SVTCTYSPAL NKMFCQLAKT CPVQLWVDST PPPGTRVRAM AIYKQSQHMT EVVRRCPHHE  180
RCSDSDGLAP PQHLIRVEGN LRVEYLDDRN TFRHSVVVPY EPPEVGSDCT TIHYNYMCNS  240
SCMGGMNRRP ILTIITLEDS SGNLLGRNSF EVRVCACPGR DRRTEEENLR KKGEPHHELP  300
PGSTKRALPN NTSSSPQPKK KPLDGEYFTL QIRGRERFEM FRELNEALEL KDAQAGKEPG  360
GSRAHSSHLK SKKGQSTSRH KKLMFKTEGP DSD
```



d. ATP1B2 and WRAP53. 9 exons (9 blocks).



BLAT Search Genome

Genome:	Assembly:	Query type:	Sort output:	Output type:
Human	Feb. 2009 (GRCh37/hg19)	BLAT's guess	query,score	hyperlink

```
>sp|P02340|P53_MOUSE Cellular tumor antigen p53 OS=Mus musculus GN=Tp53 PE=1 SV=3
MEESQSDISLELPLSQETFSGLWKLLPPEDILPSPHCMDDLLLPQDVEEFFEGPSEALRV
SGAPAAQDPVTETPGPVAPAPATPWPLSSFVPSQKTYQGNYGFHLGFLQSGTAKSVMCTY
SPPLNKLFCQLAKTCPVQLWVSATPPAGSRVRAMAIYKKSQHMTEVVRRCPHHERCSDGD
GLAPPQHLIRVEGNLYPEYLEDRQTFRHSVVVPYEPPEAGSEYTTIHYKYMCNSSCMGGM
NRRPILTIITLEDSSGNLLGRDSFEVRVCACPGRDRRTEEENFRKKEVLCPELPPGSAKR
ALPTCTSASPPQKKKPLDGEYFTLKIRGRKRFEMFRELNEALELKDAHATEESGDSRAHS
SYLKTKKGQSTSRHKKTMVKKVGPDSD
```

submit | I'm feeling lucky | clear

ACTIONS	QUERY	SCORE	START	END	QSIZE	IDENTITY	CHRO	STRAND	START	END	SPAN
browser details	P53_MOUSE	592	74	387	387	84.3%	17	+-	7572930	7579449	6520

e. The result is worse (84.3%).

P53_MOUSE

```
meesqsdisl elplsqetfs glwkllpped ilpsphcmdd lllpqdveef fegpsealrv  60
sgapaaqdpv tetPgPvAPA PAtpWPLSSf VPSQKTYQGn YGFhLGFLqS GTAKSVmCTY  120
SPpLNKlFCQ LAKTCPVQLW VsaTPPaGsR VRAMAIYKkS QHMTEVVRRC PHHERCSDgD  180
GLAPPQHLIR VEGNLypEYL eDRqTFRHSV VVPYEPPEaG SeyTTIHYkY MCNSSCMGGM  240
NRRPILTIIT LEDSSGNLLG RdSFEVRVCA CPGRDRRTEE ENfRKKevlc pELPPGSaKR  300
alptctsasp pqkkkpldge yftLkIRGRk RFEMFRELNE ALELKDAhAt eEsGdSRAHS  360
SyLKtKKGQS TSRHKKtMvK kvGPDSD
```

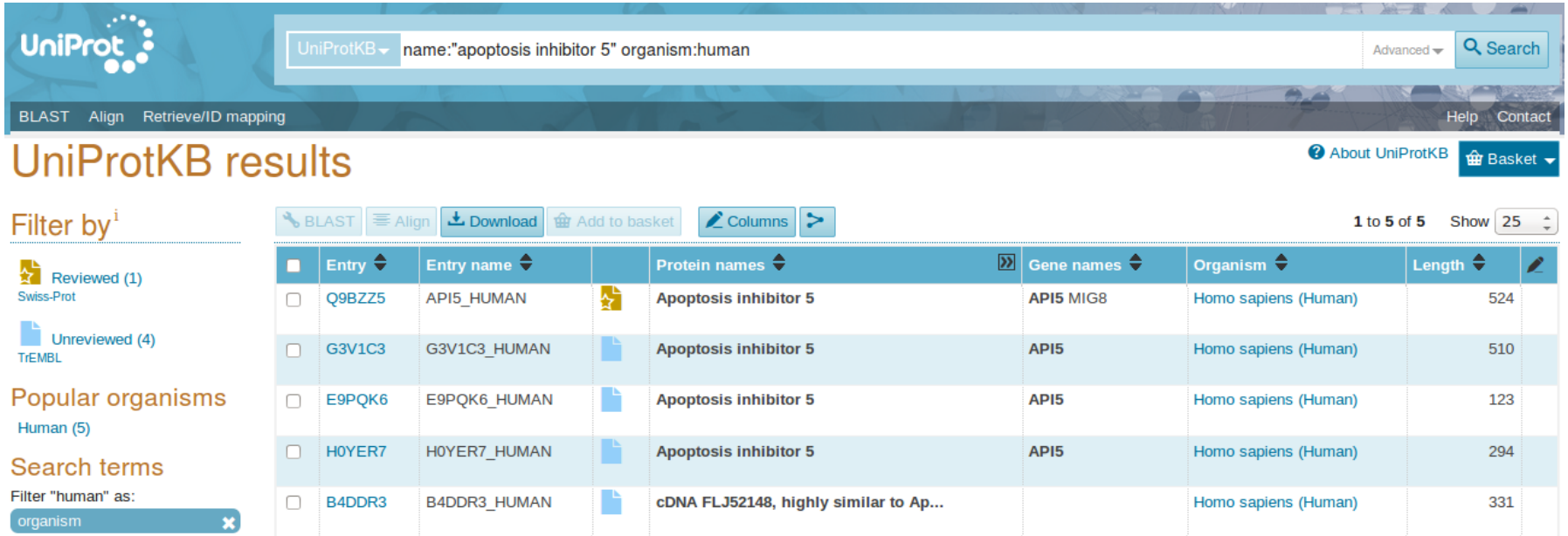
UniProt database

2

a. How many “Apoptosis inhibitor 5” (api5) proteins are there in human (*Homo sapiens*)? Use UniProt.

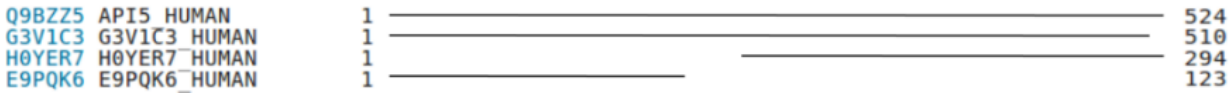
b. And how many UniProt entries?

2



a. One protein (SwissProt): Q9BZZ5.

b. At least four UniProt entries.



*Images from: Wikipedia

3

a. Using the human protein “P21741”, find its orthologous proteins in frog (*Xenopus laevis*) and get their UniProt AC.

b. Check the identity between the orthologs (human – frog proteins).

c. Check the identity between the paralogs (frog – frog proteins).

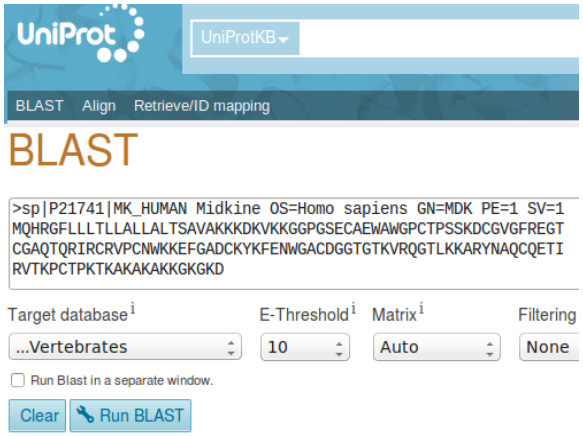
Human
(*Homo sapiens*)

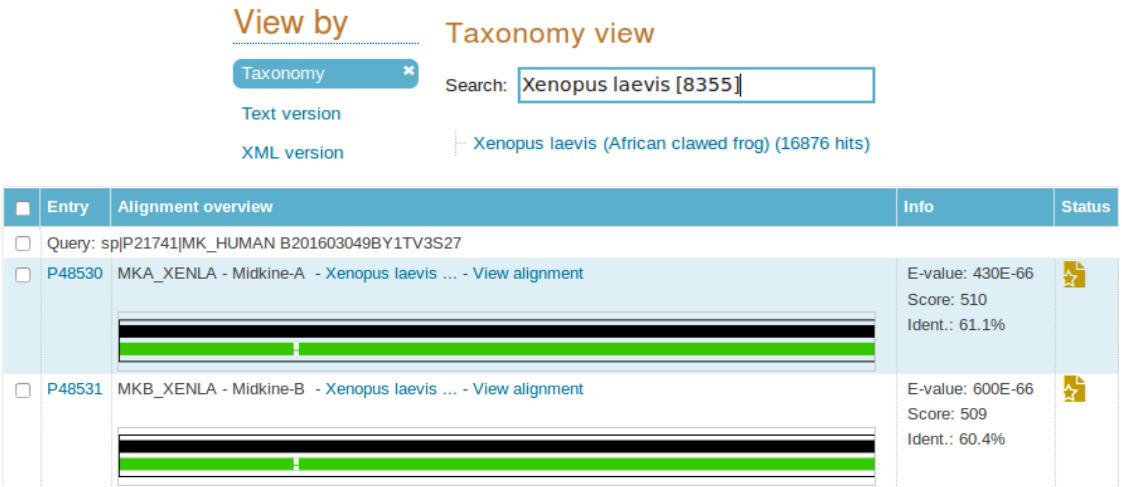
Frog
(*Xenopus laevis*)

Homology

*Images from: UniProt

3

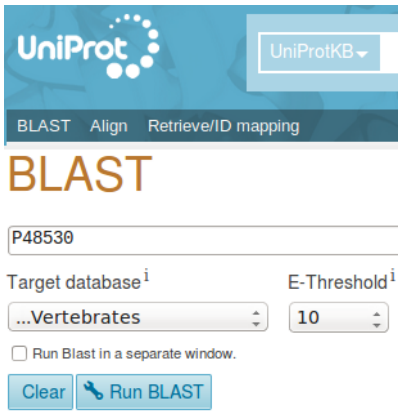


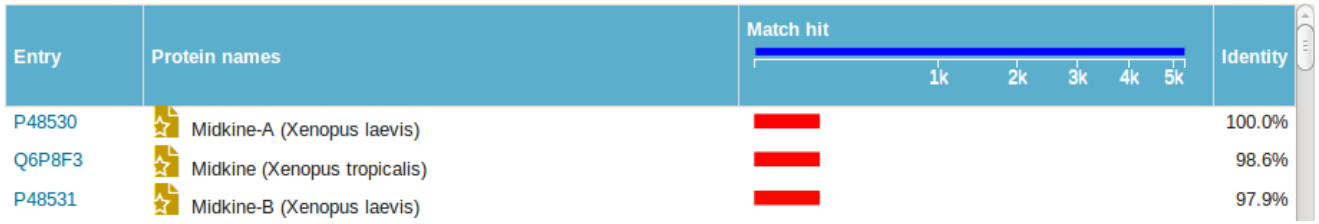


a. Query: P21741.
Ortholog1: P48530.
Ortholog2: P48531.

b. P21741-P48530 = 61.1%
P21741-P48531 = 60.4%

c. P48530-P48531 = 97.9%
Note: may also be done with “alignments”.





Homology

4

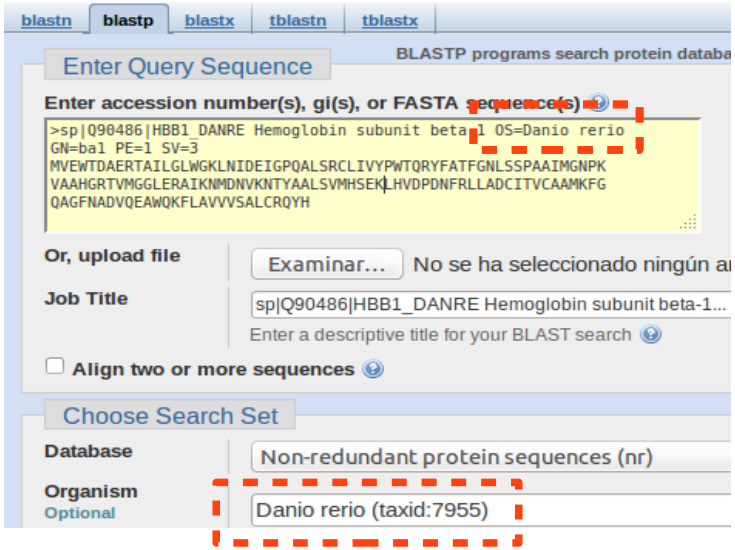
a. Using the protein “Q90486”, find its paralog/s using NCBI BLAST.

b. Check the identity between each paralog and the query.

Homology

*Images from: NCBI

4



a. Query + four paralogs.

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☑	hemoglobin subunit beta-1 [Danio rerio]	312	312	100%	6e-113	100%	NP_571095.1
☐	Ba1 globin [Danio rerio]	311	311	100%	2e-112	99%	AAI15159.1
☑	hemoglobin subunit beta-2 [Danio rerio]	310	310	100%	4e-112	98%	NP_001005403.1
☐	Ba2 globin [Danio rerio]	308	308	100%	2e-111	97%	AAH53176.1
☐	beta globin [Danio rerio]	224	224	70%	1e-78	100%	AAB05405.1
☐	novel protein similar to zebrafish ba2 globin (ba2) [Danio rerio]	222	222	70%	5e-78	98%	CAE30439.1
☑	hemoglobin beta embryonic-1.1 [Danio rerio]	216	216	100%	3e-75	69%	NP_932339.1
☑	hemoglobin beta embryonic-2 [Danio rerio]	214	214	100%	3e-74	68%	NP_998011.1
☐	PREDICTED: uncharacterized protein LOC445037 [Danio rerio]	204	204	99%	2e-70	61%	XP_005164394.1
☐	uncharacterized protein LOC445037 [Danio rerio]	203	203	99%	4e-70	61%	NP_001003431.1
☑	hemoglobin beta embryonic-3 [Danio rerio]	194	194	99%	2e-66	61%	NP_001015058.1

b. Hemoglobin subunit beta-1 (query) to:

Subunit beta-2 = 98%
Beta embryonic-1.1 = 69%
Beta embryonic-2 = 68%
Beta embryonic-3 = 61%

We consider only the RefSeq protein entries “NP”.

*Images from: Wikipedia

5

a. Based on the sequence of the “ATP synthase subunit a” protein from the extinct mammoth (*Mammuthus primigenius*), was the mammoth closer to the asian elephant (*Elephas maximus*) or to the african elefant (*Loxodonta africana*)? Use only SwissProt proteins.

b. Is there evidence enough to conclude if they are / are not closer?

c. Could you check with the “cytochrome b” protein too? Use only SwissProt proteins.

Woolly mammoth
(*Mammuthus primigenius*)

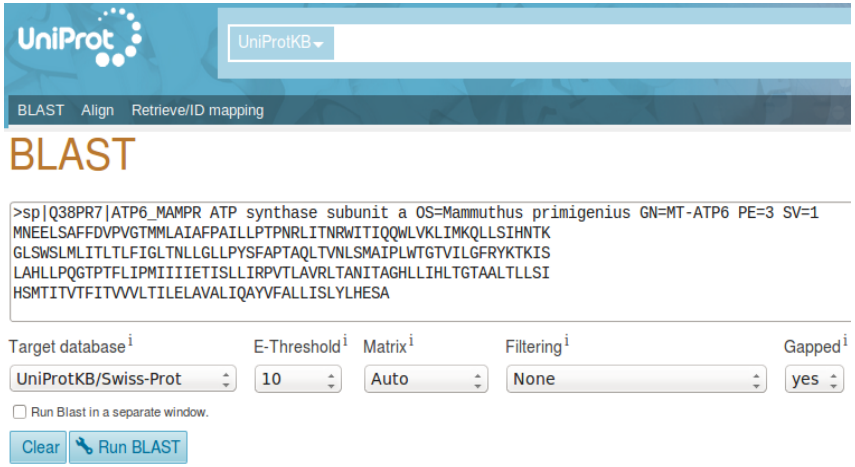
Asian elephant
(*Elephas maximus*)

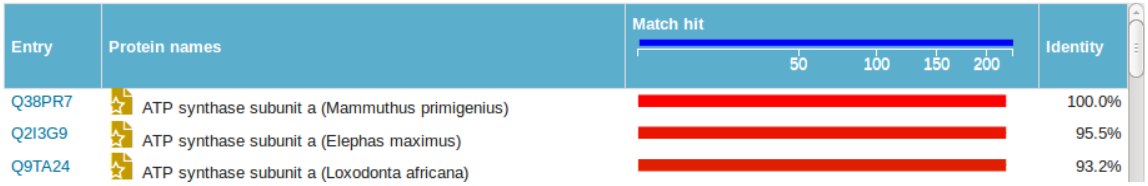
African elephant
(*Loxodonta africana*)

Homology

*Images from: UniProt

5





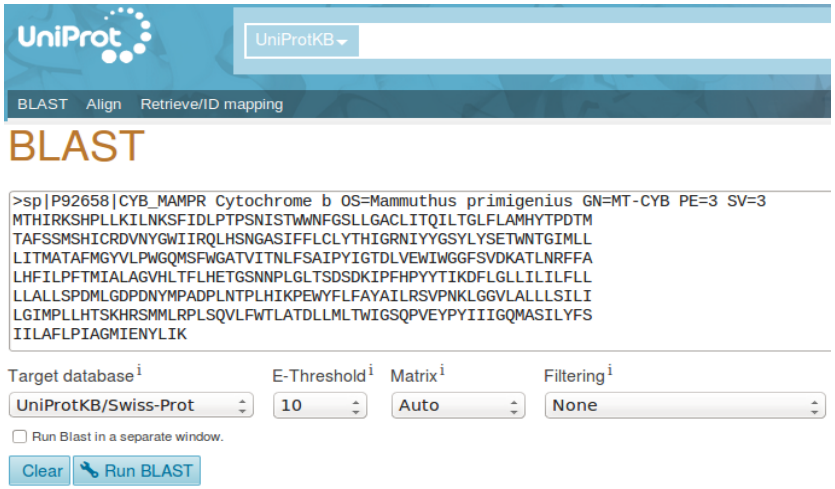
a. *M.primigenius* (Q38PR7) – *E.maximus* (Q2I3G9) = 95.5%
M.primigenius (Q38PR7) – *L.africana* (Q9TA24) = 93.2%

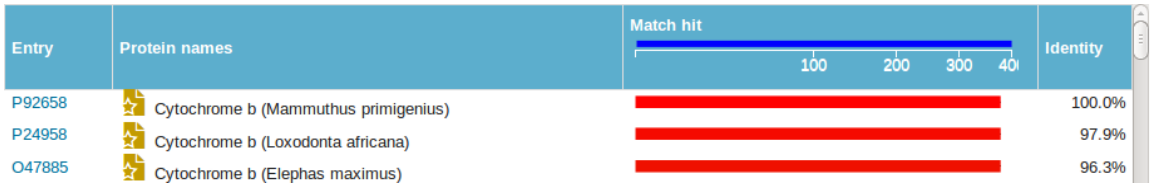
b. Just this sequence similarity is not evidence enough for claiming the Mammoth is closer to the asian elephant than to the african elephant,

BUT

the last genome sequencing works on the Woolly Mammoth (PMID: 19020620), in 2008, provides evidence enough to determine that it is really closer to the asian elephant; corroborating the similarity shown in exercise 5a.

c. Different results! (read “b” again...)
M.primigenius (P92658) – *E.maximus* (O47885) = 96.3%
M.primigenius (P92658) – *L.africana* (P24958) = 97.9%





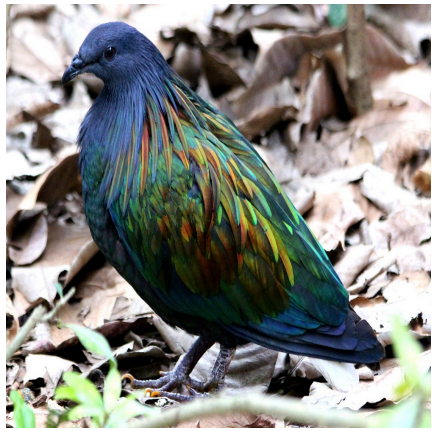
*Images from: Wikipedia

6

a. Based solely on the sequence of the “Cytochrome b” protein from the extinct dodo (*Raphus cucullatus*), was the dodo closer to the Nicobar pigeon “*Caloenas nicobarica*” or to the chicken (*Gallus gallus*)? Use NCBI Blast.

b. There are more than 300 species of pigeons. Do the results differ if you consider the street pigeon (*Columba livia*)?

Dodo
(*Raphus cucullatus*)



Nicobar pigeon
(*Caloenas nicobarica*)

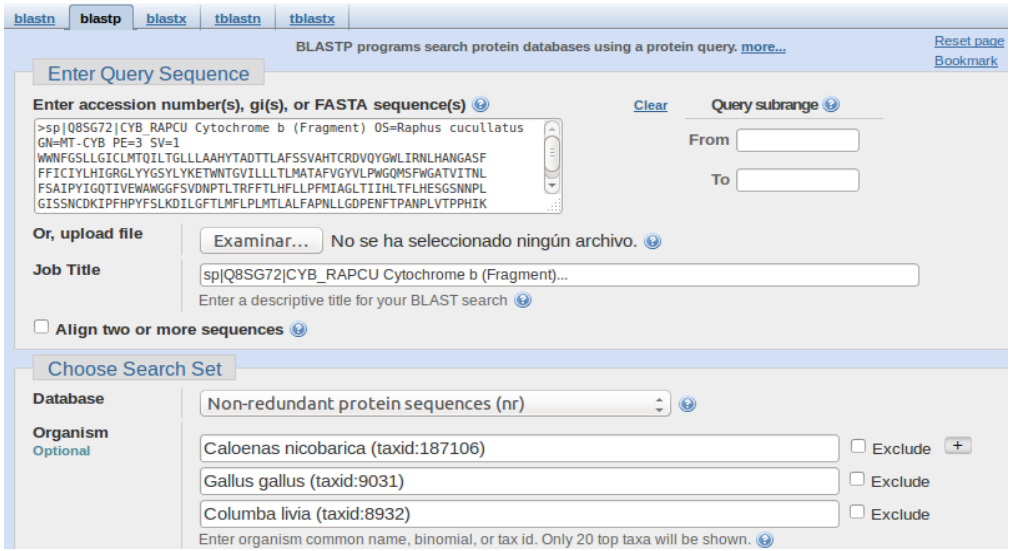
Chicken (rooster)
(*Gallus gallus*)

Pigeon
(*Columba livia*)

Homology

*Images from: NCBI, Wikipedia

6

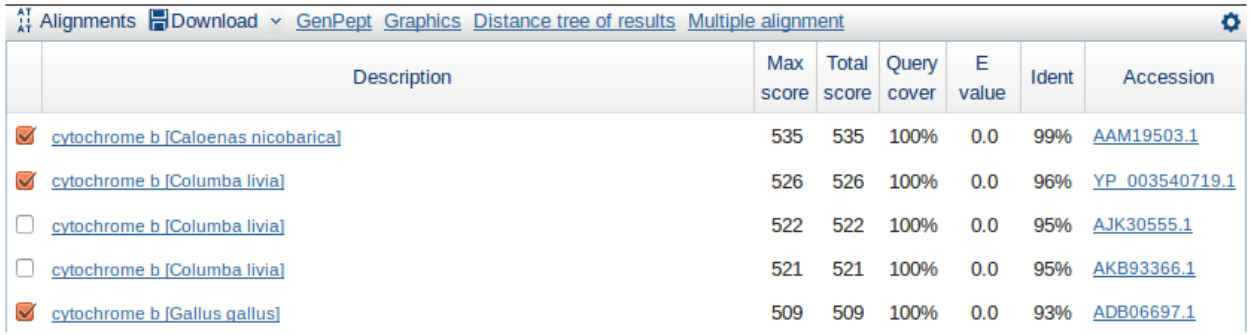


a. It seems that the dodo was closer to the pigeon than to the chicken.

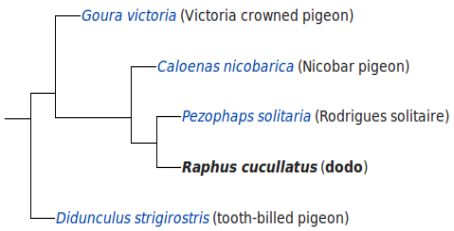
R.cucullatus – C.nicobarica = 99%
R.cucullatus – G.gallus = 93%

b. Same results for different pigeons.

R.cucullatus – C.livia = 96%
R.cucullatus – G.gallus = 93%



Description	Max score	Total score	Query cover	E value	Ident	Accession
cytochrome b [Caloenas nicobarica]	535	535	100%	0.0	99%	AAM19503.1
cytochrome b [Columba livia]	526	526	100%	0.0	96%	YP_003540719.1
cytochrome b [Columba livia]	522	522	100%	0.0	95%	AJK30555.1
cytochrome b [Columba livia]	521	521	100%	0.0	95%	AKB93366.1
cytochrome b [Gallus gallus]	509	509	100%	0.0	93%	ADB06697.1



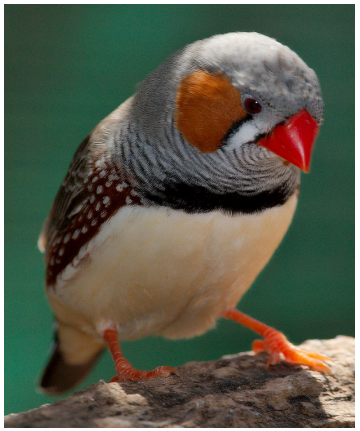
*Images from: Wikipedia

7

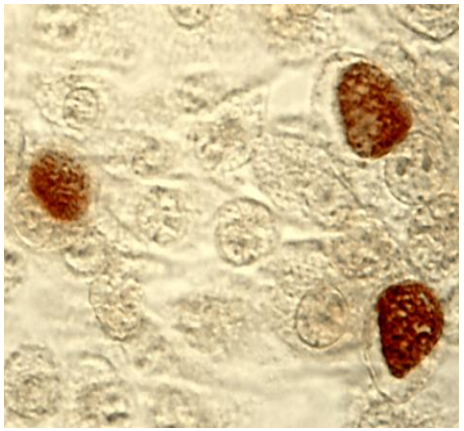
a. The UniProt entry “P04585” contains the Gag-Pol polyprotein from the virus HV1H2. Do you think it would resemble any protein in the proteome of the Zebra finch (*Taeniopygia guttata*)? Check it using NCBI Blast.

b. Discuss the results. What is the query coverage telling us?

c. The Gag-Pol polyprotein is composed of many proteins. Using only protein entries from the bacteria “*Chlamydia trachomatis*”, can you identify some of the individual proteins of the Gag-Pol polyprotein?



Zebra finch
(*Taeniopygia guttata*)

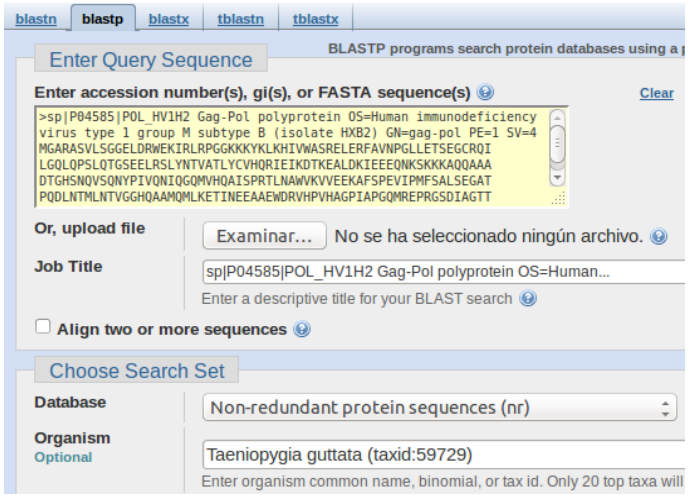


Chlamydia trachomatis

Homology

*Images from: NCBI

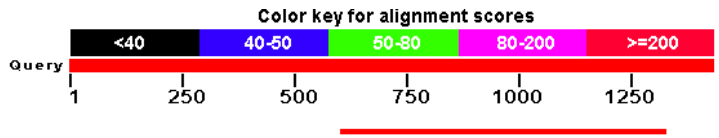
7



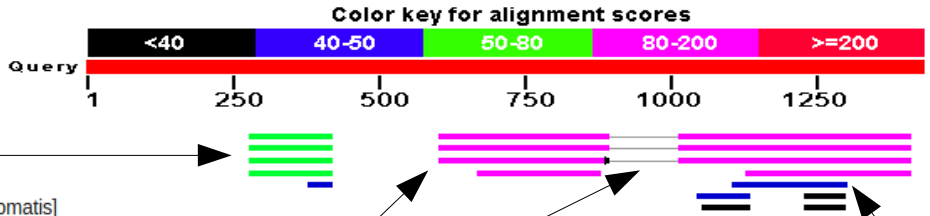
	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	PREDICTED: endogenous retrovirus group K member 18 Pol protein-like [Taeniopygia guttata]	240	240	50%	2e-65	27%	XP_012432209.1

a. XP_012432209.1. It has 27% identity with an endogenous retrovirus in *T.guttata*'s genome.

b. The query coverage is 50%, meaning that the viral "pol" protein (C-terminal) is integrated, while the "gag" protein (N-terminal) is not.



c. Database: protein entries from "*Chlamydia trachomatis*".



P24, 282-426

gag gene protein p24 (core nucleocapsid protein) [Chlamydia trachomatis]
Sequence ID: emb|CRH58881.1| Length: 382 Number of Matches: 1

Range 1: 176 to 314 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
74.3 bits(181)	2e-13	Compositional matrix adjust.	52/152(34%)	73/152(48%)	20/152(13%)

Ribonuclease H, 1109-1306

ribonuclease H [Chlamydia trachomatis]
Sequence ID: emb|CRH57958.1| Length: 831 Number of Matches: 1

Range 1: 368 to 589 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
40.8 bits(94)	0.007	Compositional matrix adjust.	57/222(26%)	92/222(41%)	24/222(10%)

Reverse transcriptase, 608-899 + 1018-1416

Reverse transcriptase (RNA-dependent DNA polymerase) [Chlamydia trachomatis]
Sequence ID: emb|CRH45814.1| Length: 867 Number of Matches: 2

Range 1: 3 to 292 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
164 bits(414)	6e-41	Compositional matrix adjust.	105/295(36%)	157/295(53%)	8/295(2%)

Range 2: 423 to 828 GenPept Graphics ▼ Next Match ▲ Previous Match ▲ First Match

Score	Expect	Method	Identities	Positives	Gaps
97.1 bits(240)	4e-20	Compositional matrix adjust.	105/431(24%)	178/431(41%)	57/431(13%)

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8

Using the protein “P38398”, perform a “tblastn” search in NCBI against human entries.

a. What would be this search used for?

b. Is there any difference between the first and the second result?

8

a. Query: protein. Database: nucleotide. To look for the gene encoding the query protein.

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	Homo sapiens breast cancer 1 (BRCA1), transcript variant 1, mRNA	3576	3576	100%	0.0	94%	NM_007294.3
☐	Homo sapiens breast and ovarian cancer susceptibility (BRCA1) mRNA, complete cds	3576	3576	100%	0.0	94%	U14680.1

Homo sapiens breast cancer 1 (BRCA1), transcript variant 1, mRNA

Sequence ID: ref|NM_007294.3| Length: 7224 Number of Matches: 1

Range 1: 233 to 5821 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps	Frame
3576 bits(9273)	0.0	Compositional matrix adjust.	1863/1863(100%)	1863/1863(100%)	0/1863(0%)	+2

```
Query  1    MDLSALRVEEVQNVINAMQKILECPICLELIKEPVSTKCDHIFCKFCMLKLLNQKKGPSQ  60
            MDLSALRVEEVQNVINAMQKILECPICLELIKEPVSTKCDHIFCKFCMLKLLNQKKGPSQ
Sbjct  233  MDLSALRVEEVQNVINAMQKILECPICLELIKEPVSTKCDHIFCKFCMLKLLNQKKGPSQ  412
```

Homo sapiens breast and ovarian cancer susceptibility (BRCA1) mRNA, complete cds

Sequence ID: gb|U14680.1|HSU14680 Length: 5711 Number of Matches: 1

Range 1: 120 to 5708 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps	Frame
3576 bits(9273)	0.0	Compositional matrix adjust.	1863/1863(100%)	1863/1863(100%)	0/1863(0%)	+3

```
Query  1    MDLSALRVEEVQNVINAMQKILECPICLELIKEPVSTKCDHIFCKFCMLKLLNQKKGPSQ  60
            MDLSALRVEEVQNVINAMQKILECPICLELIKEPVSTKCDHIFCKFCMLKLLNQKKGPSQ
Sbjct  120  MDLSALRVEEVQNVINAMQKILECPICLELIKEPVSTKCDHIFCKFCMLKLLNQKKGPSQ  299
```

b.

First result: NM_007294.3
7224 bp, transcript variant 1, mRNA

Query: 1 ——— 1863

Subject: 233 ——— 5821

Second result: U14680.1
5711bp, complete CDS

Query: 1 ——— 1863

Subject: 120 ——— 5708