Master Biomedizin 2018

1) UCSC & UniProt
2) Homology
3) MSA
4) Phylogeny

Pablo Mier
munoz@uni-mainz.de

*Images from: Wikipedia

3

a. Using the human protein “P21741”, find its orthologous proteins in frog (*Xenopus laevis*) and get their UniProt AC. P48530, P48531

b. Check the identity between the orthologs (human – frog proteins).
P21741-P48530 = 61.1%, P21741-P48531 = 60.4%

c. Check the identity between the paralogs (frog – frog proteins). P48530-P48531 = 97.9%

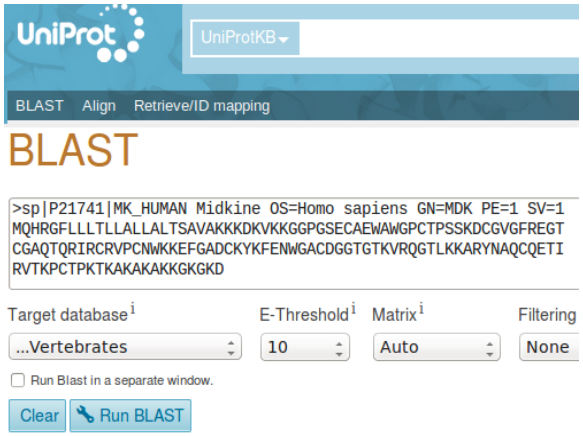
Human
(*Homo sapiens*)

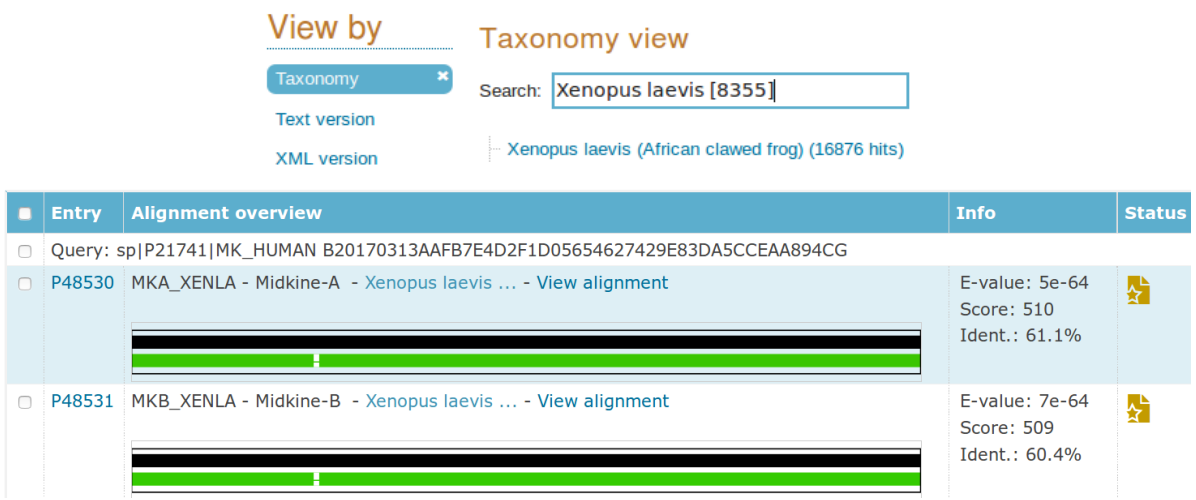
Frog
(*Xenopus laevis*)

Homology

*Images from: UniProt

3

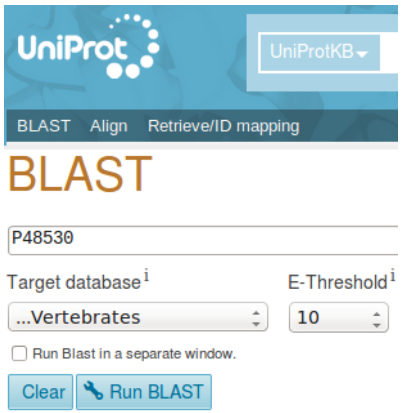


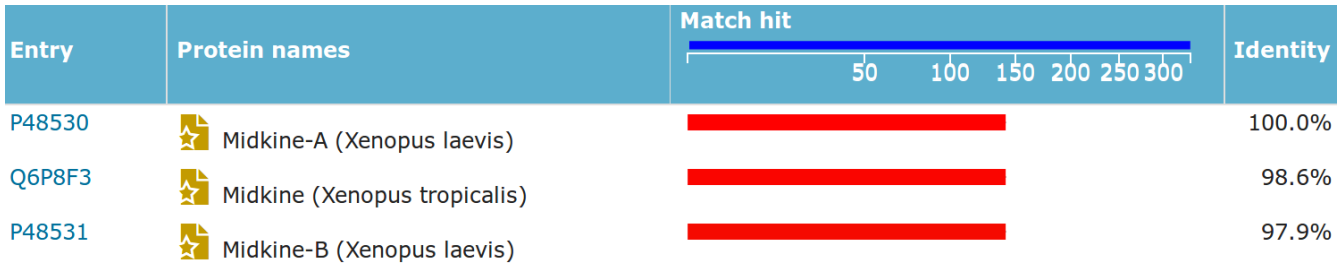


a. Query: P21741.
Ortholog1: P48530.
Ortholog2: P48531.

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

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





*Images from: Wikipedia

4

a. Based on the sequence of the "ATP synthase subunit a" protein from the extinct mammoth (*Mammuthus primigenius*) [Q38PR7], was the mammoth closer to the asian elephant (*Elephas maximus*) or to the african elefant (*Loxodonta africana*)? Use only SwissProt proteins.

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

b. Is there evidence enough to conclude if they are / are not closer? No.

c. Could you check with the "cytochrome b" protein too? [P92658] Use only SwissProt proteins.

M.primigenius (P92658) – *E.maximus* (O47885) = 96.3%
M.primigenius (P92658) – *L.africana* (P24958) = 97.9%

Woolly mammoth
(*Mammuthus primigenius*)

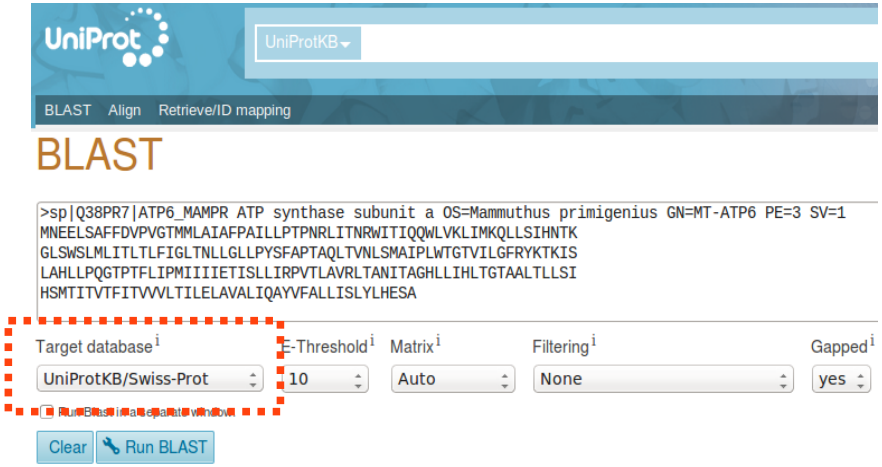
Asian elephant
(*Elephas maximus*)

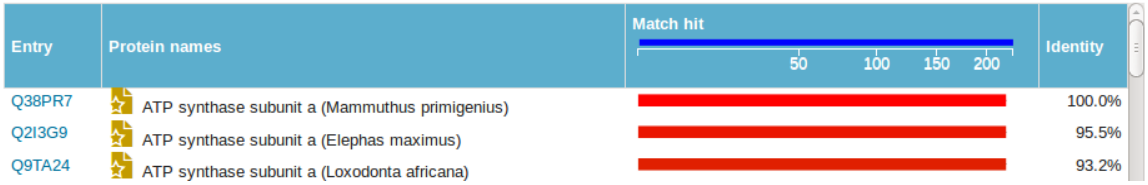
African elephant
(*Loxodonta africana*)

Homology

*Images from: UniProt

4





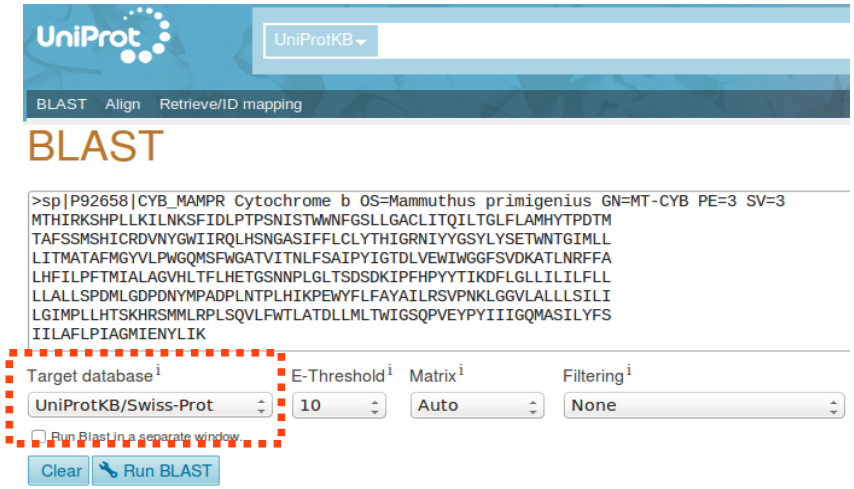
a. *M.primigenius* (Q38PR7) – *E.maximus* (Q2I3G9) = 95.5%
M.primigenius (Q38PR7) – *L.africana* (Q9TA24) = 93.2%

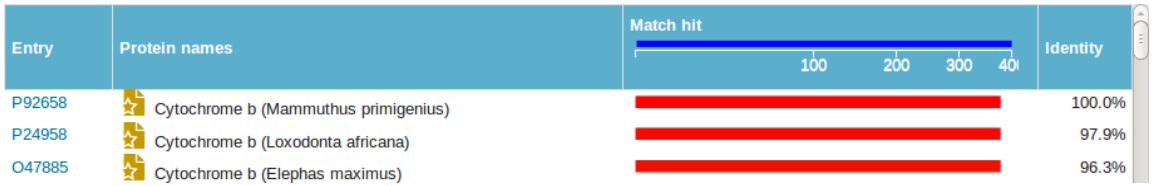
b. Just this sequence similarity is not evidence enough for claiming the mammoth is closer to the asian elephant than to the african elephant,

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 4a.

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

5

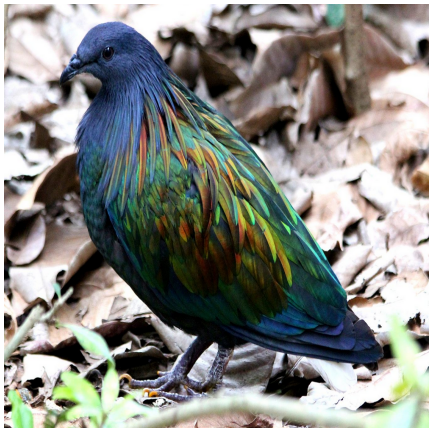
a. Based solely on the sequence of the "Cytochrome b" protein (Q8SG72) from the extinct dodo (*Raphus cucullatus*), was the dodo closer to the Nicobar pigeon "*Caloenas nicobarica*" or to the chicken (*Gallus gallus*)? Use NCBI Blast.

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

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

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

Dodo
(*Raphus cucullatus*)



Nicobar pigeon
(*Caloenas nicobarica*)

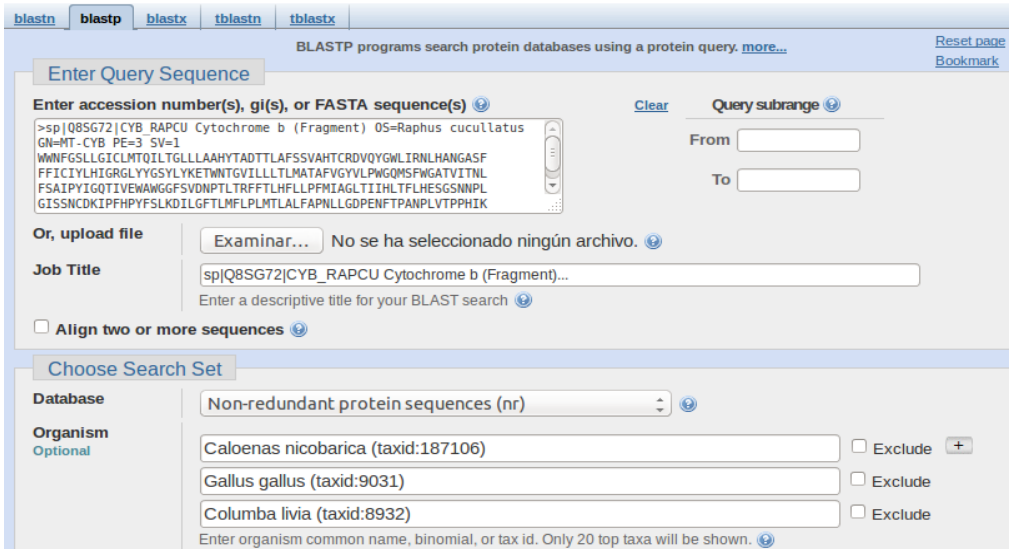
Chicken (rooster)
(*Gallus gallus*)

Pigeon
(*Columba livia*)

Homology

*Images from: NCBI, Wikipedia

5

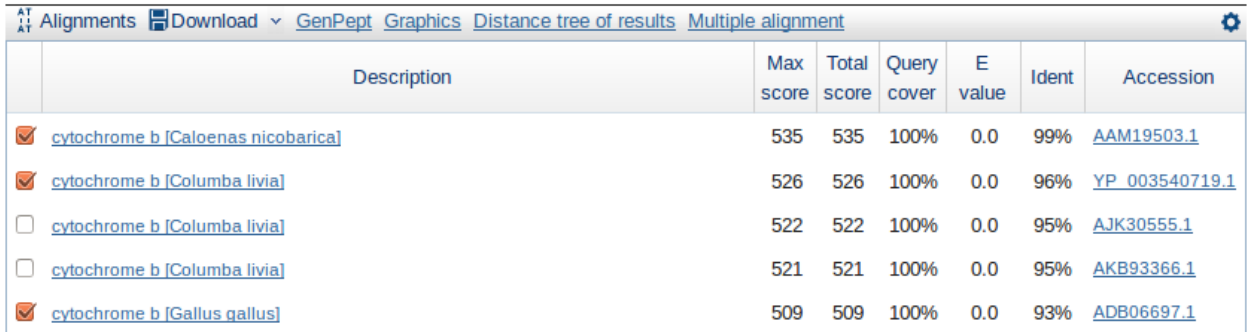


a. It seems that the dodo was closer to the pigeon than to the chicken.

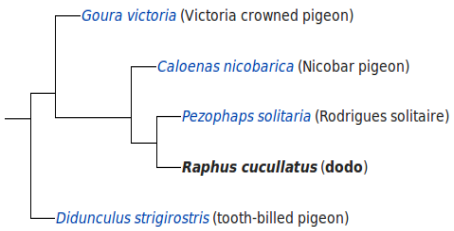
R.cucullatus – C.nicobarica = 99%
R.cucullatus – G.gallus = 93%

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



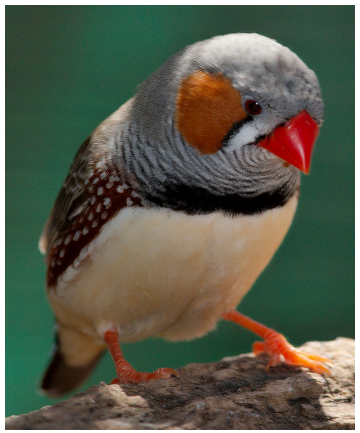
*Images from: Wikipedia

6

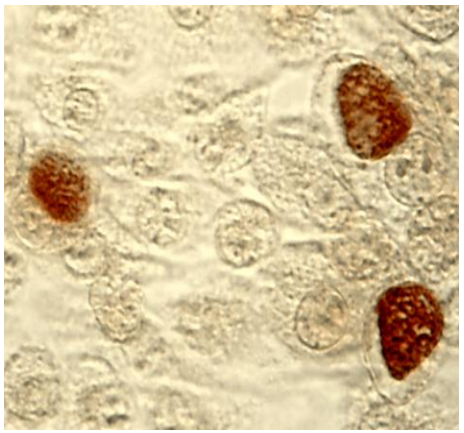
a. The UniProt entry “P04585” contains the Gag-Pol polyprotein from the virus HV1H2. Do you think it would resemble any protein in the proteome of the Zebra finch (*Taeniopygia guttata*)? Check it using NCBI Blast.
XP_012432209.1. It has 27% identity with an endogenous retrovirus in *T.guttata*'s genome.

b. Discuss the results. What is the query coverage telling us?
The query coverage is 50%, meaning that the viral “pol” protein (C-terminal) is integrated, while the “gag” protein (N-terminal) is not.

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?
P24, 282-426 Reverse transcriptase, 608-899 + 1018-1416 Ribonuclease H, 1109-1306



Zebra finch
(*Taeniopygia guttata*)



Chlamydia trachomatis

Homology

*Images from: NCBI

6

blastn | blastp | blastx | tblastn | tblastx

BLASTP programs search protein databases using a

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) — Clear

```
>sp|P04585|POL_HV1H2 Gag-Pol polyprotein OS=Human immunodeficiency
virus type 1 group M subtype B (isolate HXB2) GN=gag-pol PE=1 SV=4
MGARASVLSGGELDRWEKIRLRPGGKKKYKLKHIVWASRELERFAVNPGLLETSEGCRQI
LGQLQPSLQTGSEELRSLYNTVATLYCVHQRIEIKDTKEALDKIEEEQNKSKKKAQQAAA
DTGHSNQVSQNYPIVQNIQGQMVHQAISPRTLNAWVKVVEEKAFSPEVIPMFSALSEGAT
PQDLNTMLNTVGGHQAAMQMLKETINEEAAEWDRVHPVHAGPIAPGQMREPRGSDIAGTT
```

Or, upload file — Examinar... No se ha seleccionado ningún archivo.

Job Title — sp|P04585|POL_HV1H2 Gag-Pol polyprotein OS=Human...

Enter a descriptive title for your BLAST search

Align two or more sequences

Choose Search Set

Database — Non-redundant protein sequences (nr)

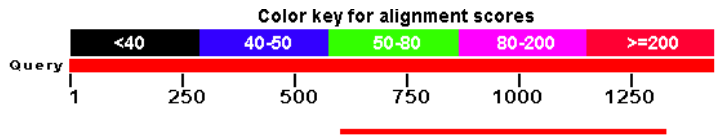
Organism (Optional) — Taeniopygia guttata (taxid:59729)

Enter organism common name, binomial, or tax id. Only 20 top taxa will

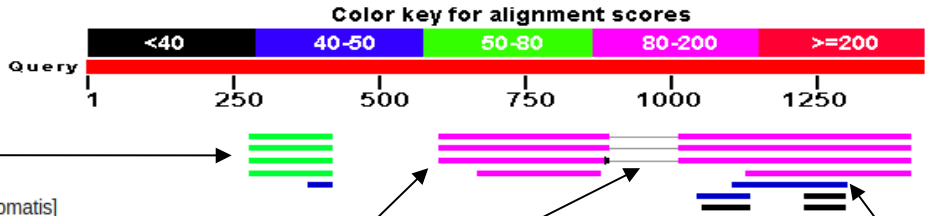
	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	PREDICTED: endogenous retrovirus group K member 18 Pol protein-like [Taeniopygia guttata]	240	240	50%	2e-65	27%	XP_012432209.1

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%)

Pablo Mier
munoz@uni-mainz.de

7

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

a. What would this search be used for? To look for the gene encoding the query protein.

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

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

Second result: U14680.1
5711bp, complete CDS

7

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