This week. Week 3

- Objective: To obtain the mapping of epitopes to complete reference proteomes

Next week. Week 4.

- Objectives:
  - To learn about protein disorderness → Tue @2pm Lilia Iakuscheva will be given a presentation on this topic
  - Start calculating sequence conservation for epitopes
  - Start calculating disorderness for epitopes

Week 3

- Objective: To obtain the mapping of epitopes to complete reference proteomes
- Algorithm:
  - Input (SA – source antigen):
    - {epitope ID; epitope sequence; SA ID}
    - {SA ID; SA sequence; source organism ID; parent taxonomic ID}
    - {parent taxonomic ID; reference proteome sequences}
  - TODO….
  - Output:
    - {epitope ID; ref. sequence ID; ref. sequence; epitope positions in ref sequence}

What you will learn today

- Sequence databases for genes and proteins: NCBI, UniProt
- FASTA format for sequences
- Pairwise sequence alignment: a bit of theory & major algorithms: BLAST

The NAR online Molecular Biology Database Collection

www.oxfordjournals.org/nar/database/c/

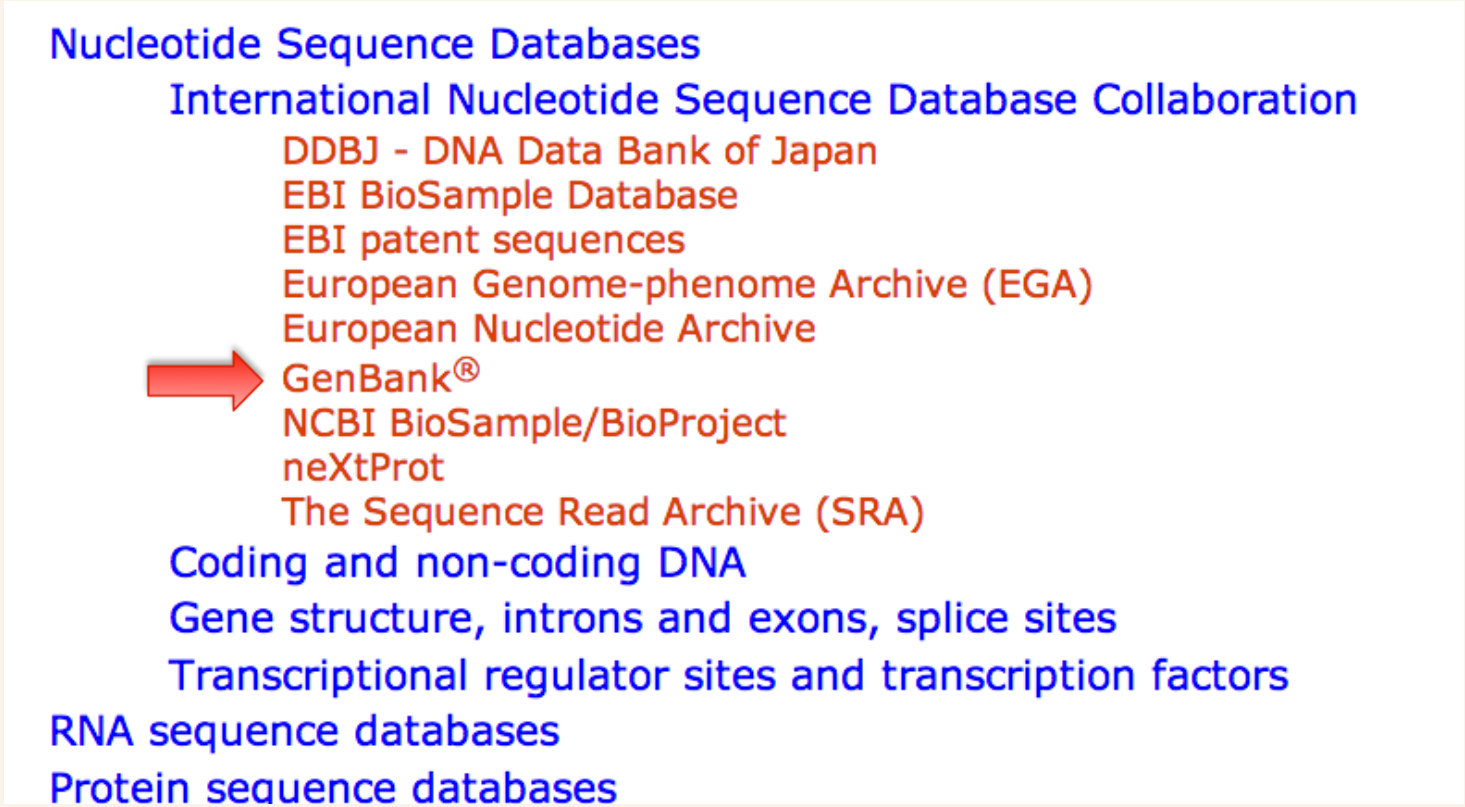
1512 online databases

2013 NAR Database Summary Paper Category List

- Nucleotide Sequence Databases
- RNA sequence databases
- Protein sequence databases
- Structure Databases
- Genomics Databases (non-vertebrate)
- Metabolic and Signaling Pathways
- Human and other Vertebrate Genomes
- Human Genes and Diseases
- Microarray Data and other Gene Expression Databases
- Proteomics Resources
- Other Molecular Biology Databases
- Organelle databases
- Plant databases
- Immunological databases
- Cell biology

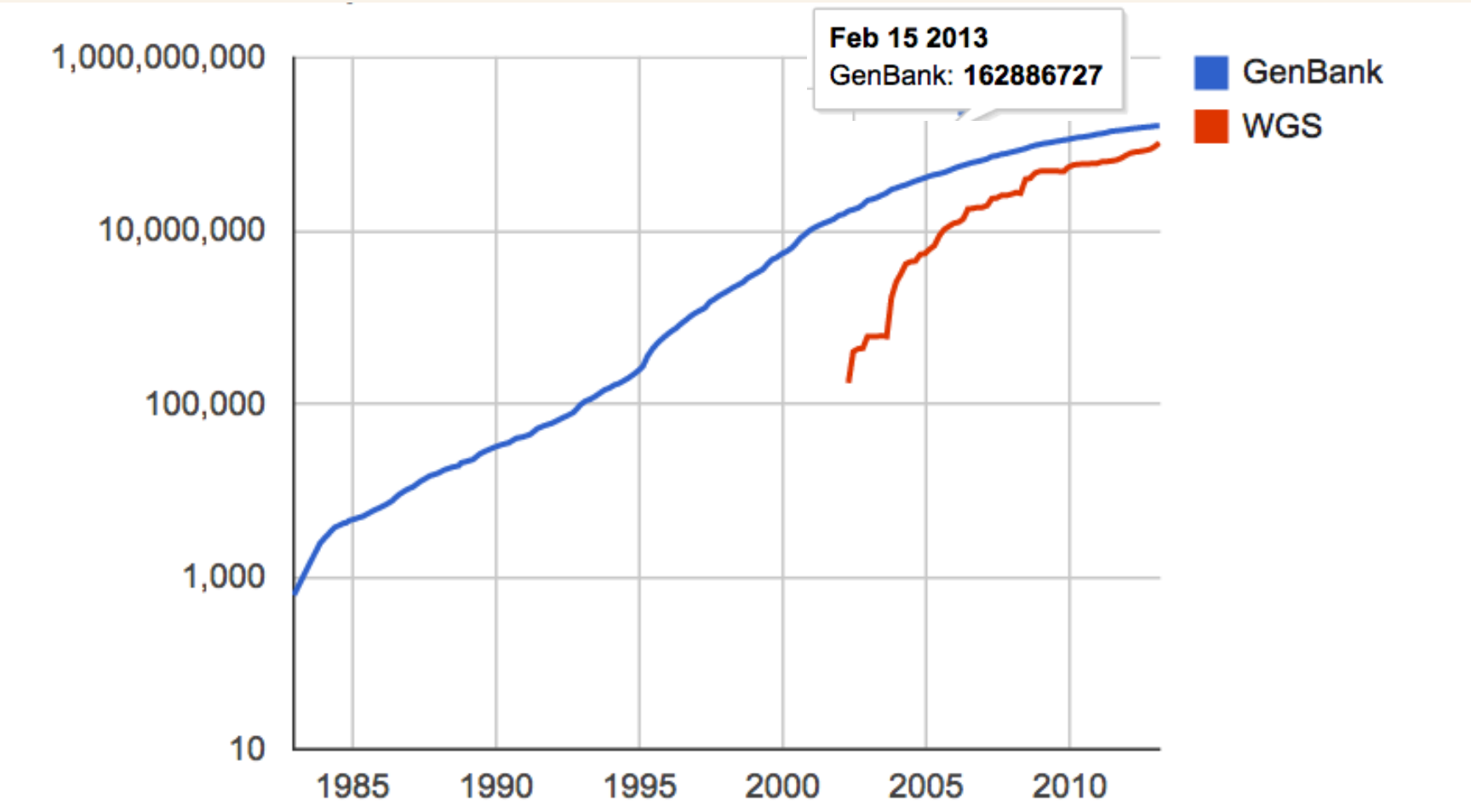
- Compilation Paper
- Category List
- Alphabetical List
- Category/Paper List
- Search Summary Papers

Primary nucleotide sequence databases



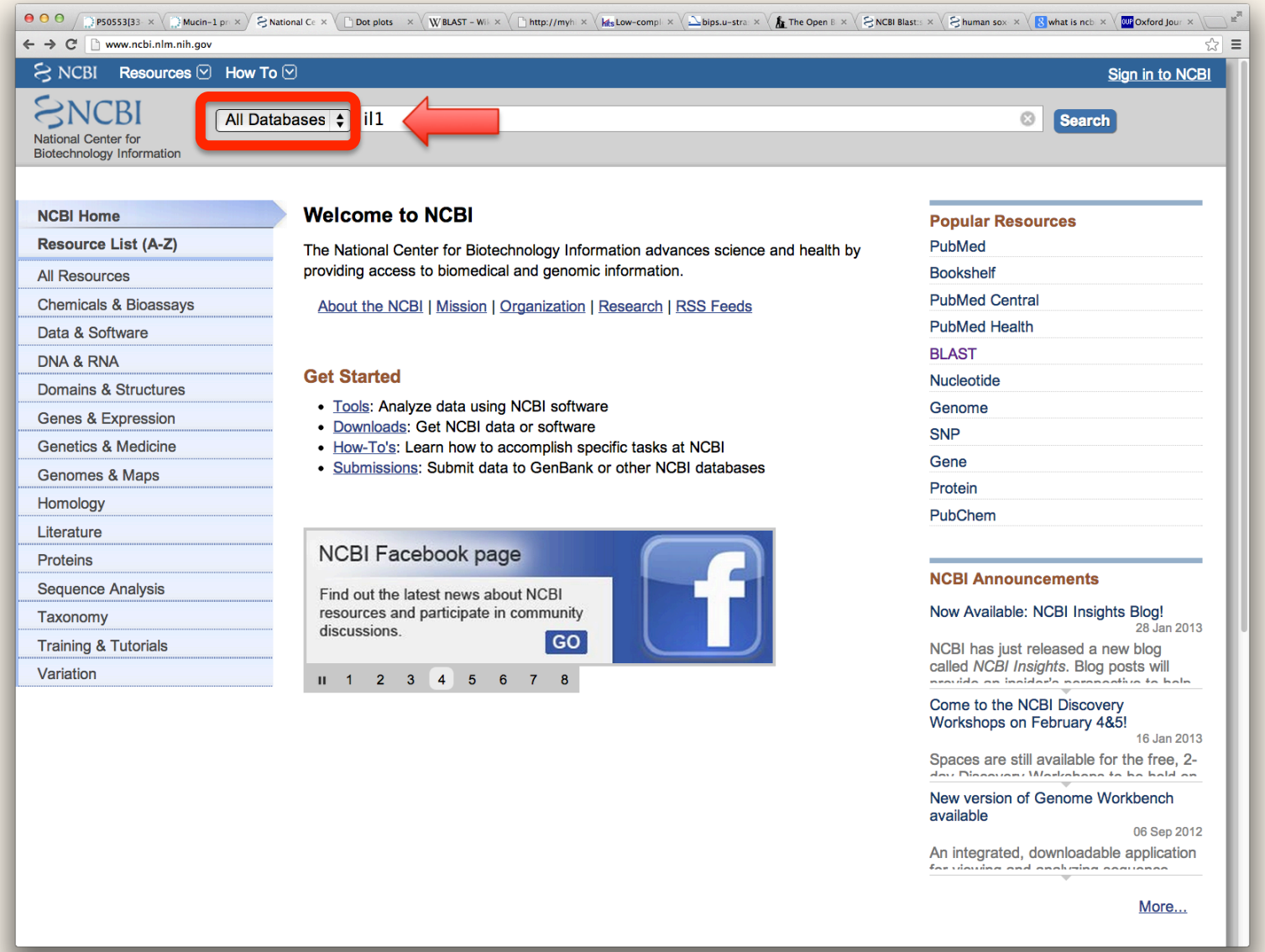
GenBank is part of the International Nucleotide Sequence Database Collaboration , which comprises the DNA DataBank of Japan (DDBJ), the European Molecular Biology Laboratory (EMBL), and GenBank at NCBI. These three organizations exchange data on a daily basis.

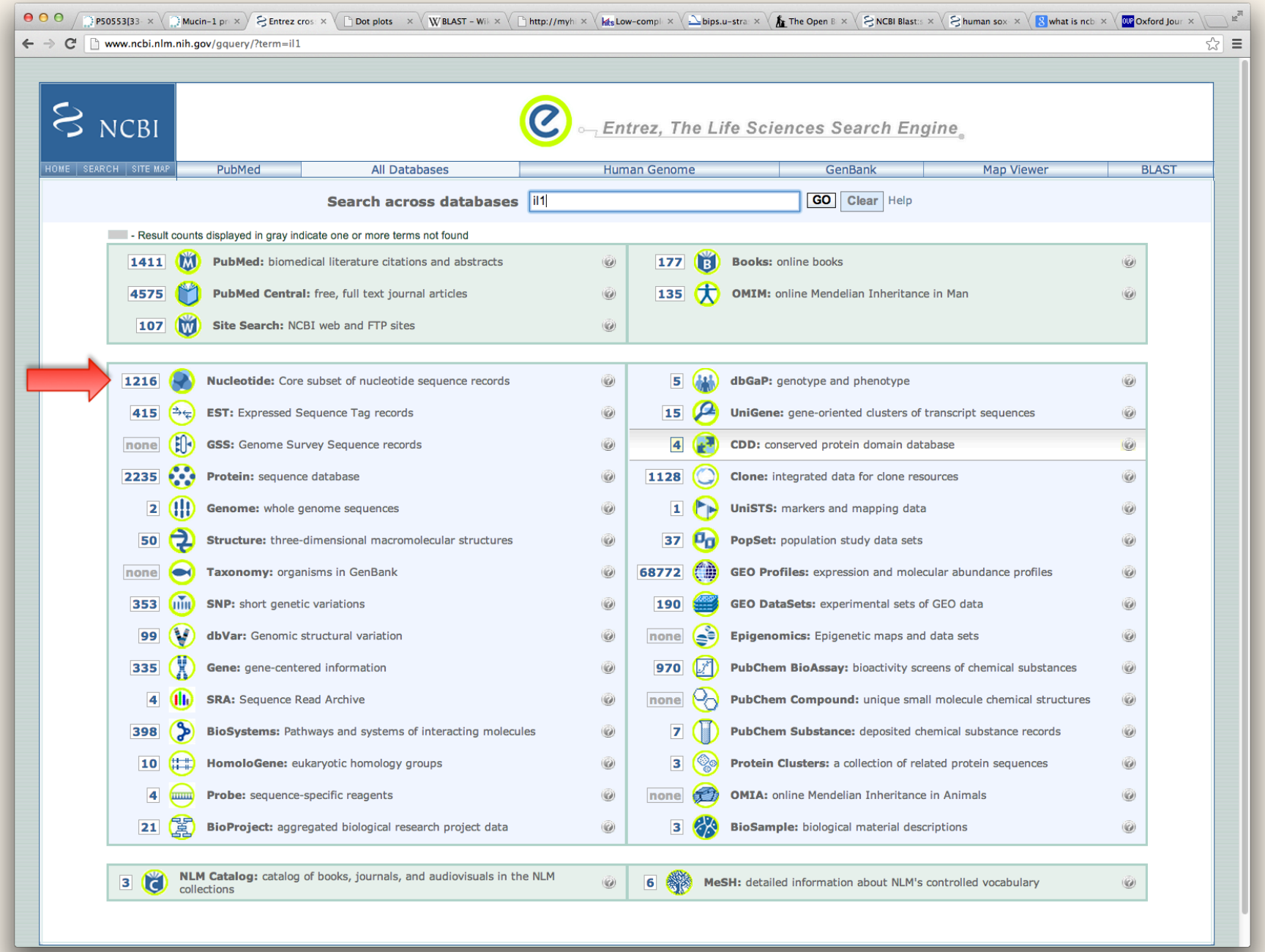
Growth of the number of sequences in NCBI GenBank



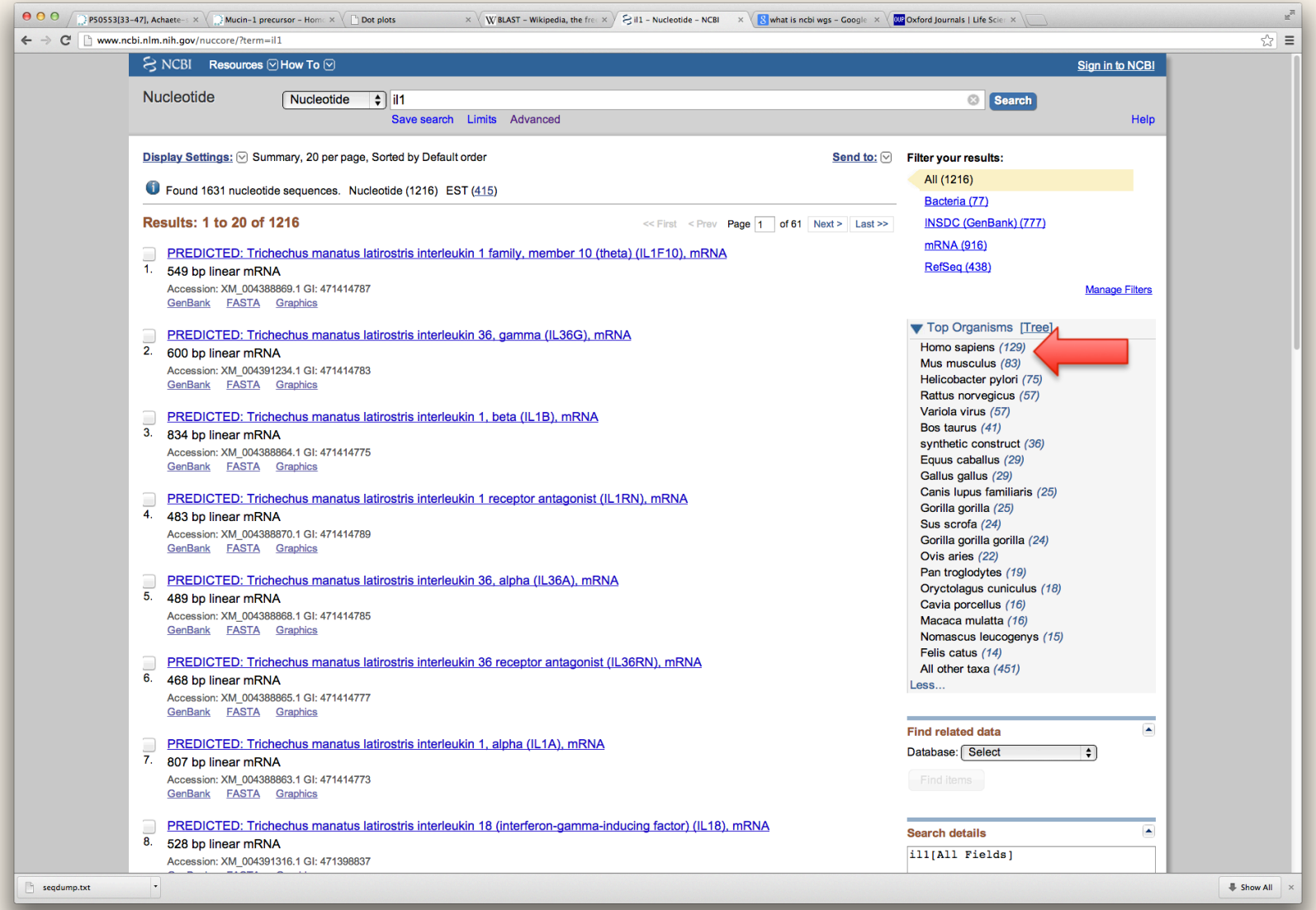
Whole Genome Shotgun (WGS) sequencing projects are incomplete genomes or incomplete chromosomes that are being sequenced by a whole genome shotgun strategy. WGS projects may be annotated, but annotation is not required.

Search for il1 in Entrez: must select “All Databases”

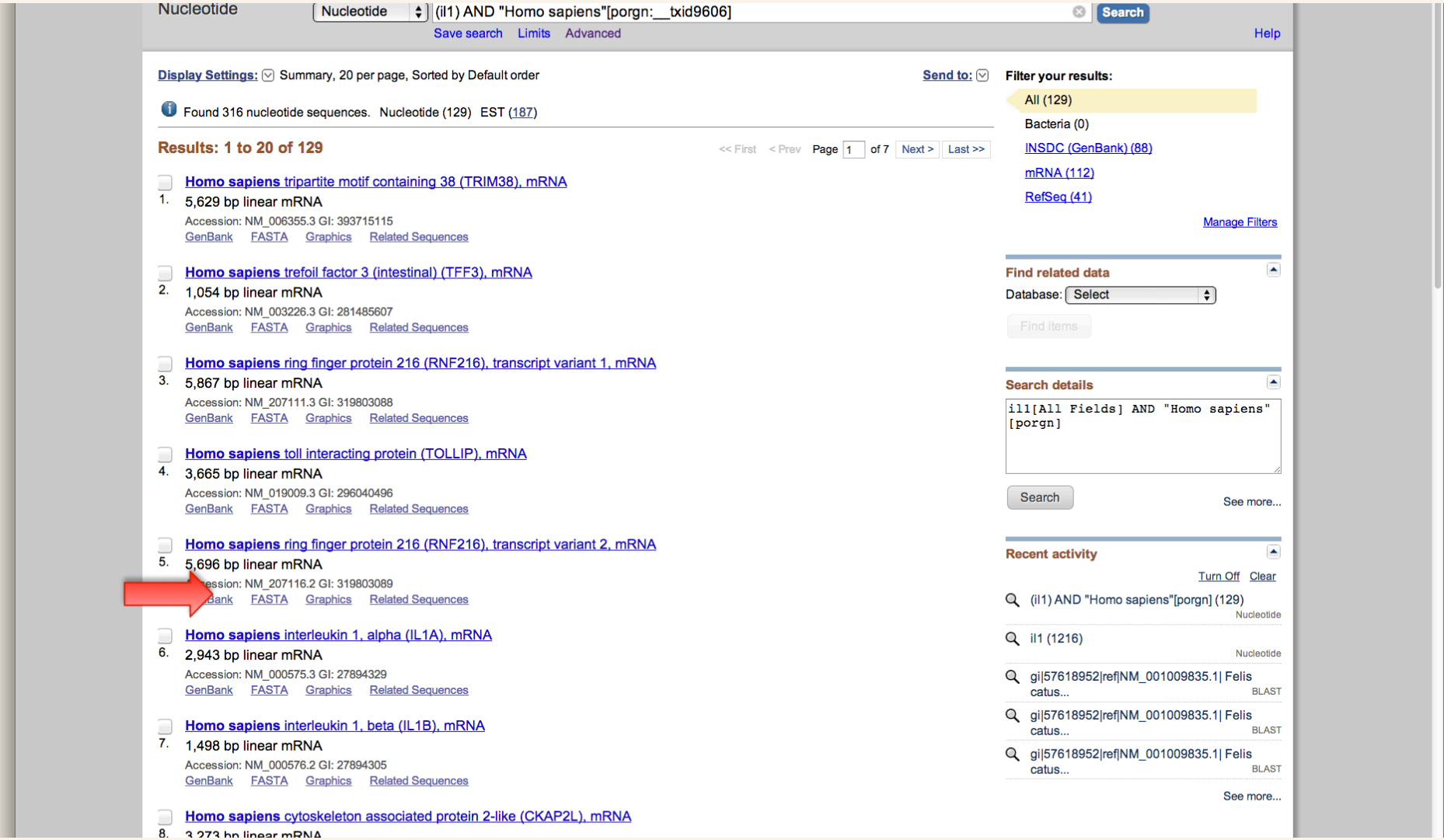



www.ncbi.nlm.nih.gov/gquery/?term=il1
NCBI
Entrez, The Life Sciences Search Engine
HOME
SEARCH
SITE MAP
PubMed
All Databases
Human Genome
GenBank
Map Viewer
BLAST
Search across databases
il1
GO
Clear
Help
- Result counts displayed in gray indicate one or more terms not found
1411 PubMed: biomedical literature citations and abstracts
4575 PubMed Central: free, full text journal articles
107 Site Search: NCBI web and FTP sites
177 Books: online books
135 OMIM: online Mendelian Inheritance in Man
1216 Nucleotide: Core subset of nucleotide sequence records
415 EST: Expressed Sequence Tag records
none GSS: Genome Survey Sequence records
2235 Protein: sequence database
2 Genome: whole genome sequences
50 Structure: three-dimensional macromolecular structures
none Taxonomy: organisms in GenBank
353 SNP: short genetic variations
99 dbVar: Genomic structural variation
335 Gene: gene-centered information
4 SRA: Sequence Read Archive
398 BioSystems: Pathways and systems of interacting molecules
10 HomoloGene: eukaryotic homology groups
4 Probe: sequence-specific reagents
21 BioProject: aggregated biological research project data
5 dbGaP: genotype and phenotype
15 UniGene: gene-oriented clusters of transcript sequences
4 CDD: conserved protein domain database
1128 Clone: integrated data for clone resources
1 UniSTS: markers and mapping data
37 PopSet: population study data sets
68772 GEO Profiles: expression and molecular abundance profiles
190 GEO DataSets: experimental sets of GEO data
none Epigenomics: Epigenetic maps and data sets
970 PubChem BioAssay: bioactivity screens of chemical substances
none PubChem Compound: unique small molecule chemical structures
7 PubChem Substance: deposited chemical substance records
3 Protein Clusters: a collection of related protein sequences
none OMIA: online Mendelian Inheritance in Animals
3 BioSample: biological material descriptions
3 NLM Catalog: catalog of books, journals, and audiovisuals in the NLM collections
6 MeSH: detailed information about NLM's controlled vocabulary

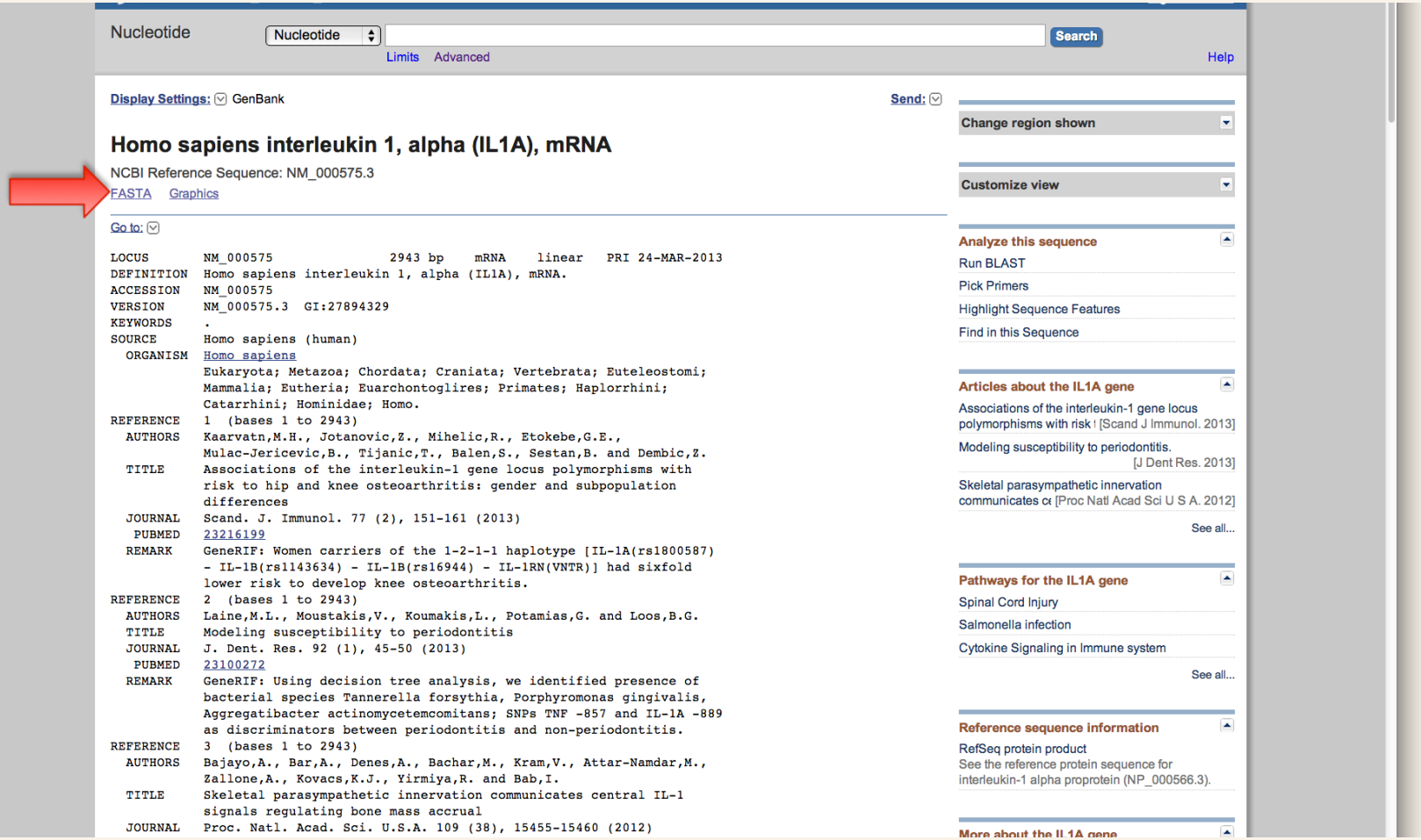
Select human



Select mRNA for IL1 alpha



Get FASTA-formatted sequence



FASTA format: the legacy of the a DNA and protein sequence alignment software package first described (as FASTP) by Lipman & Pearson in 1985

Sequences in this format are accepted by almost any bioinformatics program

Homo sapiens interleukin 1, alpha (IL1A), mRNA

NCBI Reference Sequence: NM_000575.3

GenBank Graphics

```
>gi|27894329|ref|NM_000575.3| Homo sapiens interleukin 1, alpha (IL1A), mRNA
ACCAGGCAACACCATTGAAGGCTCATATGTAAAAATCCATGCCTTCCTTTCTCCCAATCTCCATTCCCAA
ACTTAGCCACTGGCTTCTGGCTGAGGCCTTACGCATACCTCCCGGGGCTTGCACACACCTTCTTCTACAG
AAGACACACCTTGGGCATATCCTACAGAAGACCAGGCTTCTCTCTGGTCCTTGGTAGAGGGCTACTTTAC
TGTAACAGGGCCAGGGTGGAGAGTTCTCTCCTGAAGCTCCATCCCCTCTATAGGAAATGTGTTGACAATA
TTCAGAAGAGTAAGAGGATCAAGACTTCTTTGTGCTCAAATACCACTGTTCTCTTCTCTACCCTGCCCTA
ACCAGGAGCTTGTCACCCCAAACTCTGAGGTGATTTATGCCTTAATCAAGCAAACTTCCCTCTTCAGAAA
AGATGGCTCATTTTCCCTCAAAAGTTGCCAGGAGCTGCCAAGTATTCTGCCAATTCACCCTGGAGCACAA
TCAACAAATTCAGCCAGAACACAACTACAGCTACTATTAGAACTATTATTATTAATAAATTCCTCTCCAA
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TCCCTTTTTCTAAGACTACATGTTTGTCATCTTATAAAGCAAAGGGGTGAATAAATGAACCAAATCAATA
ACTTCTGGAATATCTGCAAACAACAATAATATCAGCTATGCCATCTTTCACTATTTTAGCCAGTATCGAG
TTGAATGAACATAGAAAAATACAAAACTGAATTCTTCCCTGTAAATTCCCCGTTTTGACGACGCACTTGT
AGCCACGTAGCCACGCCTACTTAAGACAATTACAAAAGGCGAAGAAGACTGACTCAGGCTTAAGCTGCCA
GCCAGAGAGGGAGTCATTTCATTGGCGTTTGAGTCAGCAAAGAAGTCAAGATGGCCAAAGTTCCAGACAT
GTTTGAAGACCTGAAGAACTGTTACAGTGAAAATGAAGAAGACAGTTCCTCCATTGATCATCTGTCTCTG
AATCAGAAATCCTTCTATCATGTAAGCTATGGCCCACTCCATGAAGGCTGCATGGATCAATCTGTGTCTC
TGAGTATCTCTGAAACCTCTAAAACATCCAAGCTTACCTTCAAGGAGAGCATGGTGGTAGTAGCAACCAA
CGGGAAGGTTCTGAAGAAGAGACGGTTGAGTTTAAGCCAATCCATCACTGATGATGACCTGGAGGCCATC
GCCAATGACTCAGAGGAAGAAATCATCAAGCCTAGGTCAGCACCTTTTAGCTTCCTGAGCAATGTGAAAT
ACAACTTTATGAGGATCATCAAATACGAATTCATCCTGAATGACGCCCTCAATCAAAGTATAATTCGAGC
CAATGATCAGTACCTCACGGCTGCTGCATTACATAATCTGGATGAAGCAGTGAAATTTGACATGGGTGCT
TATAAGTCATCAAAGGATGATGCTAAAATTACCGTGATTCTAAGAATCTCAAAAACTCAATTGTATGTGA
CTGCCCAAGATGAAGACCAACCAGTGCTGCTGAAGGAGATGCCTGAGATACCCAAAACCATCACAGGTAG
TGAGACCAACCTCCTCTTCTTCTGGGAAACTCACGGCACTAAGAACTATTTCACATCAGTTGCCCATCCA
```
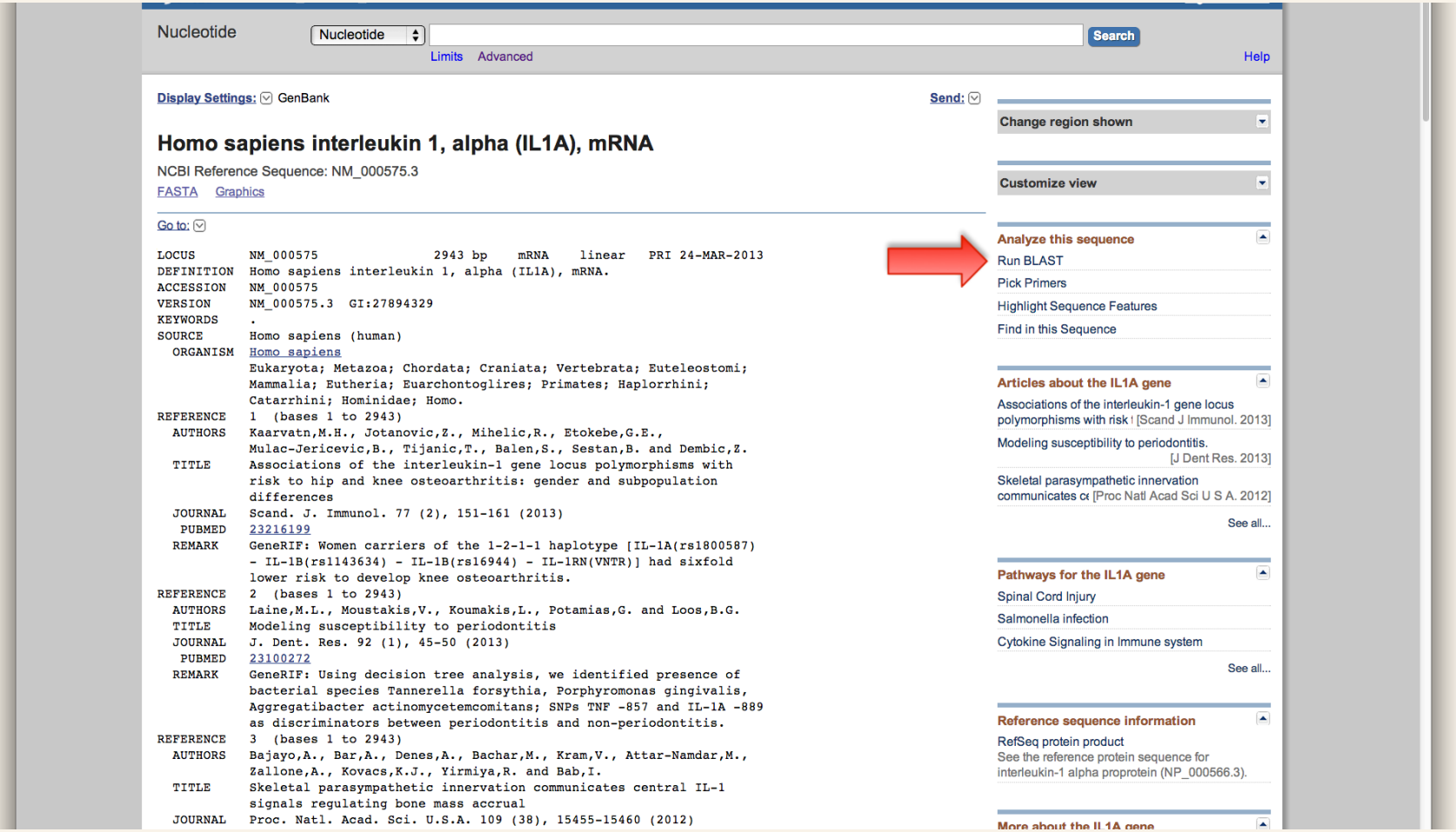
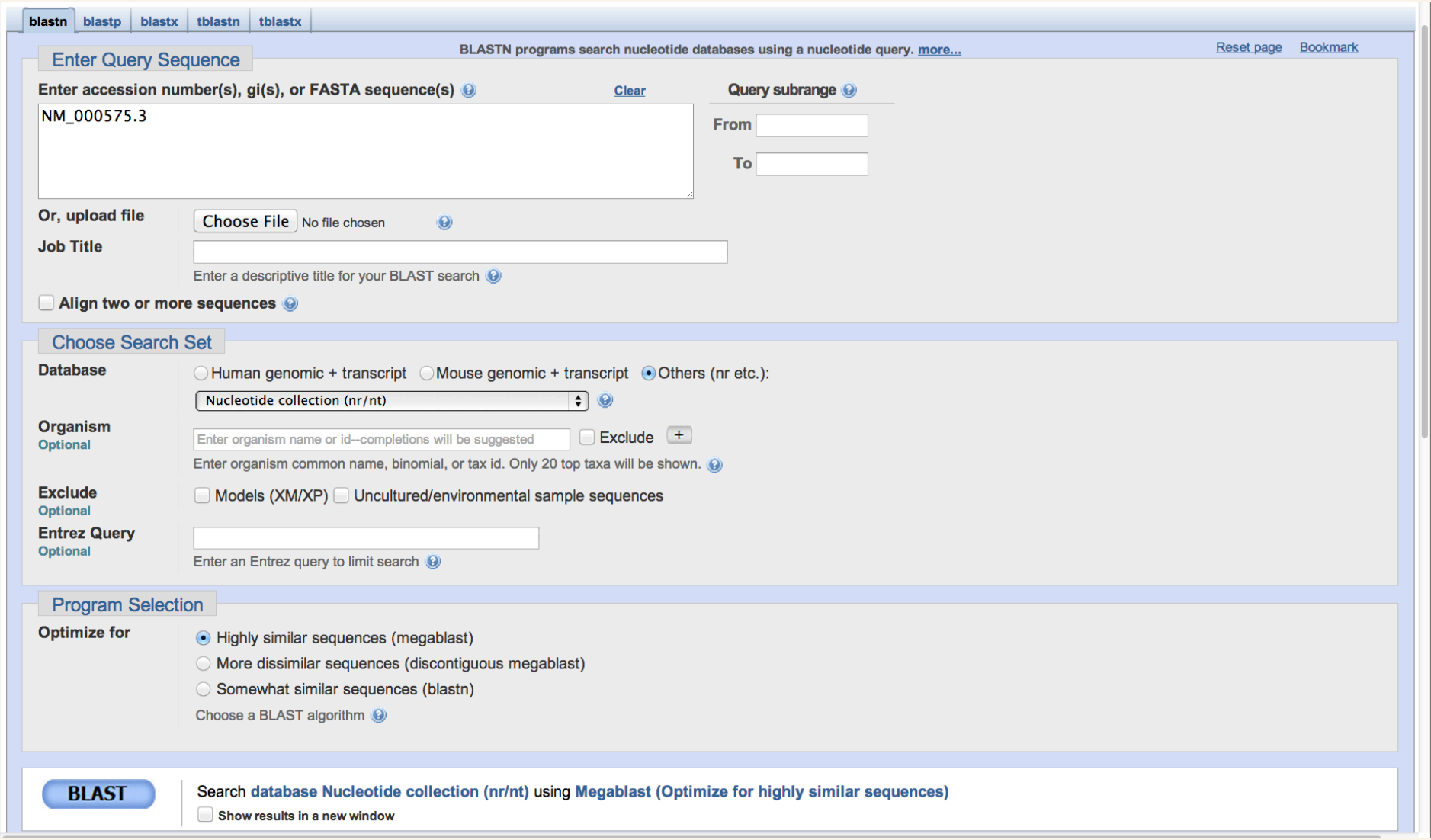
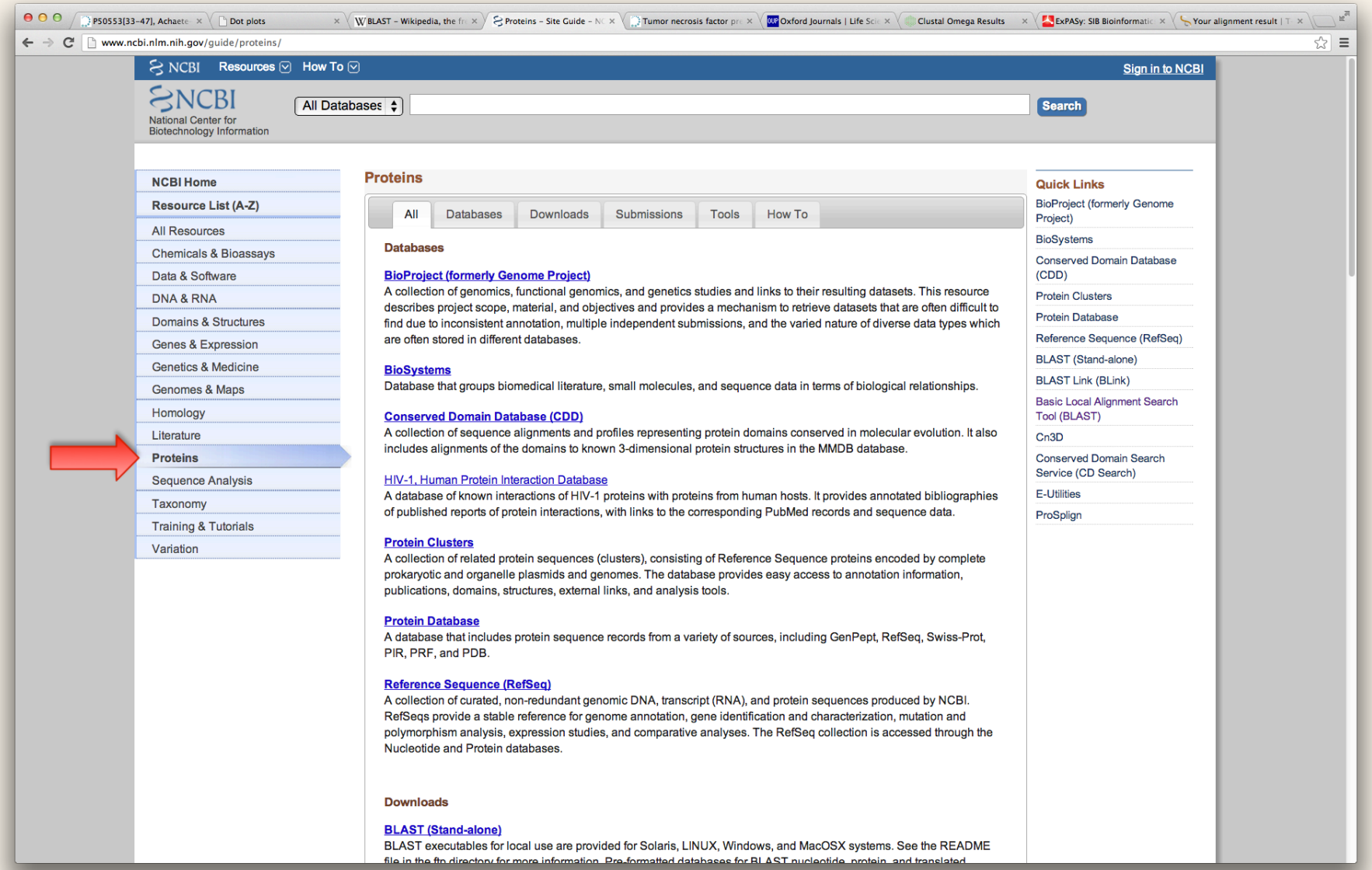

You can run BLAST to search for similar sequences from any sequence record in NCBI databases



NCBI BLAST to search NCBI databases

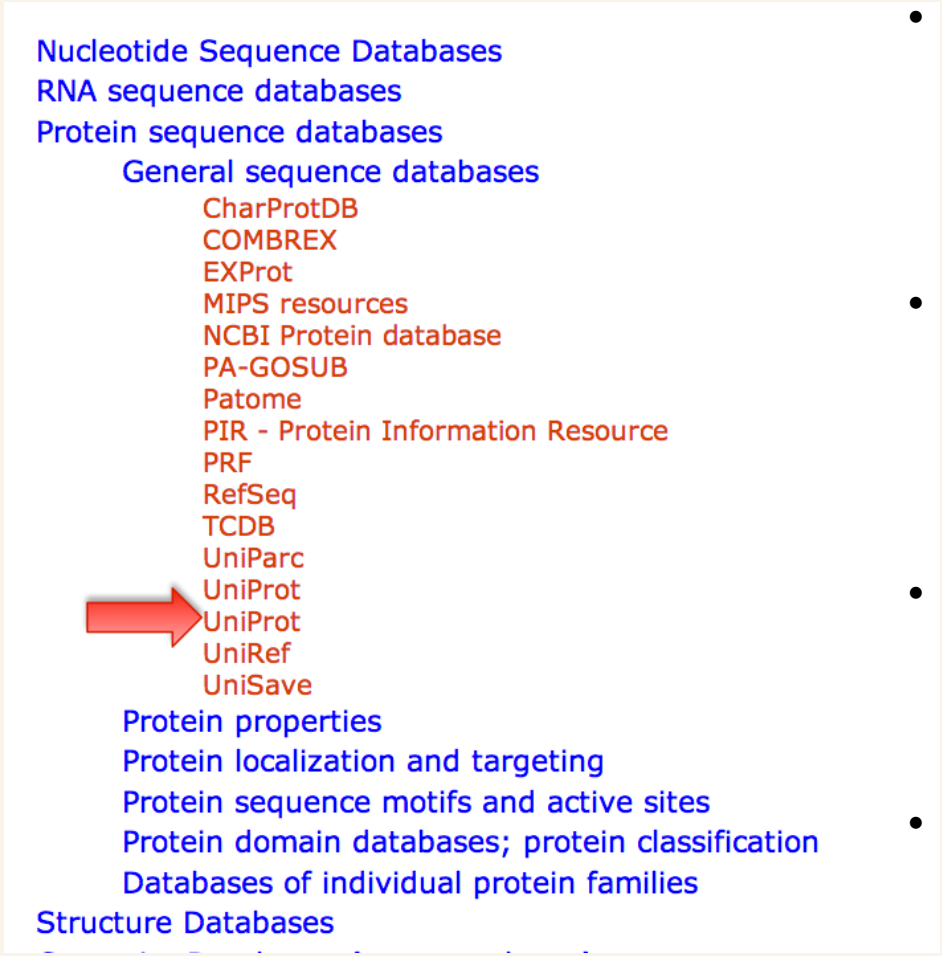


Here is the list of NCBI protein databases



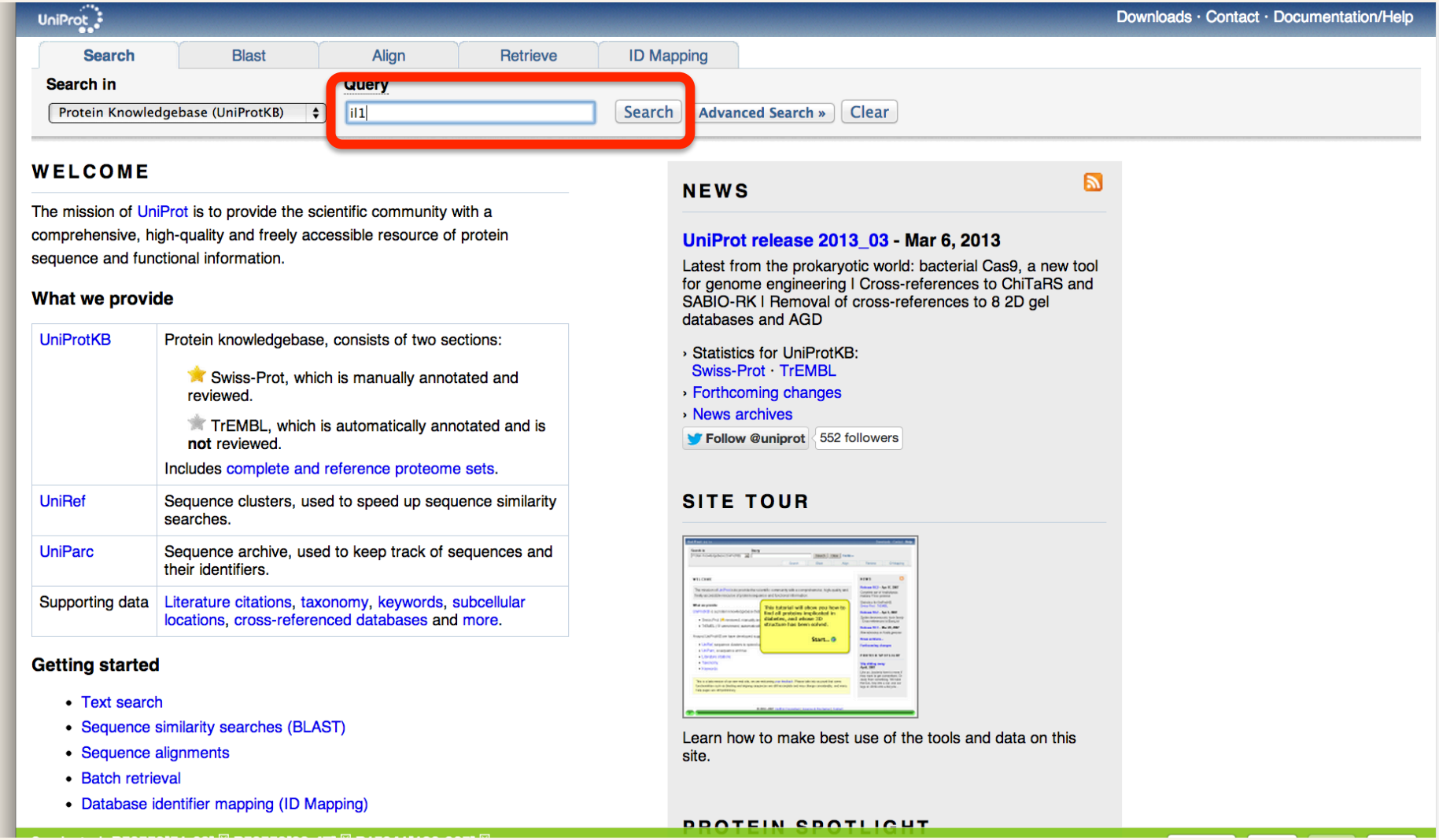
The NAR online Molecular Biology Database Collection

www.oxfordjournals.org/nar/database/c/

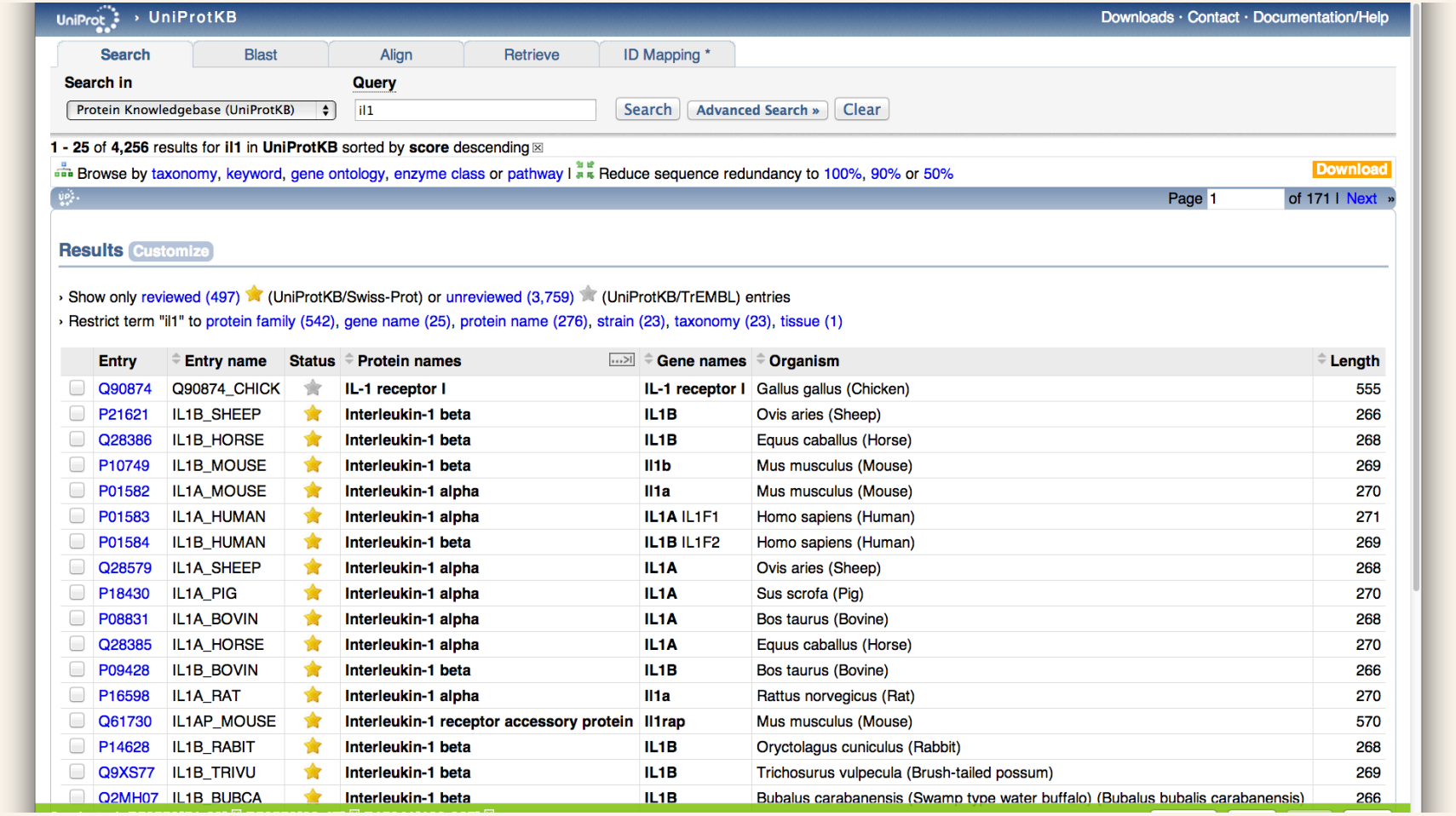


- The Swiss-Prot, TrEMBL, and PIR protein database activities have united in 2004 to form **the Universal Protein Resource (UniProt)**, a central resource on protein sequences and functional annotation.
- **UniProtKB** is a "hub" for all available protein products produced by a each gene. It has two parts: manually annotated **UniProtKB /Swiss-Prot** and automatically annotated **UniProtKB/TrEMBL.**
- **UniRef** speed similarity searches via sequence space compression by merging sequences that are 100% (UniRef100), 90% (UniRef90), or 50% (UniRef50) identical.
- **UniProt Archive (UniParc)** stores all publicly available protein sequences, containing the history of sequence data with links to the source databases.

UniProt databases can be searched at uniprot.org via keyword or doing sequence search via BLAST



Output: sequences can be selected for download, further blasting or aligning using Clustal Omega



UniProt › UniProtKB — Downloads · Contact · Documentation/Help

Search | Blast | Align | Retrieve | ID Mapping *

Search in: Protein Knowledgebase (UniProtKB) — Query: il1 — Search | Advanced Search » | Clear

1 - 25 of 4,256 results for il1 in UniProtKB sorted by score descending

Browse by taxonomy, keyword, gene ontology, enzyme class or pathway | Reduce sequence redundancy to 100%, 90% or 50% — Download

Page 1 of 171 | Next »

Results Customize

› Show only reviewed (497) (UniProtKB/Swiss-Prot) or unreviewed (3,759) (UniProtKB/TrEMBL) entries

› Restrict term "il1" to protein family (542), gene name (25), protein name (276), strain (23), taxonomy (23), tissue (1)

Entry	Entry name	Status	Protein names	Gene names	Organism	Length
Q90874	Q90874_CHICK		**IL-1 receptor I**	**IL-1 receptor I**	Gallus gallus (Chicken)	555
P21621	IL1B_SHEEP	★	**Interleukin-1 beta**	**IL1B**	Ovis aries (Sheep)	266
Q28386	IL1B_HORSE	★	**Interleukin-1 beta**	**IL1B**	Equus caballus (Horse)	268
P10749	IL1B_MOUSE	★	**Interleukin-1 beta**	**Il1b**	Mus musculus (Mouse)	269
P01582	IL1A_MOUSE	★	**Interleukin-1 alpha**	**Il1a**	Mus musculus (Mouse)	270
P01583	IL1A_HUMAN	★	**Interleukin-1 alpha**	**IL1A** IL1F1	Homo sapiens (Human)	271
P01584	IL1B_HUMAN	★	**Interleukin-1 beta**	**IL1B** IL1F2	Homo sapiens (Human)	269
Q28579	IL1A_SHEEP	★	**Interleukin-1 alpha**	**IL1A**	Ovis aries (Sheep)	268
P18430	IL1A_PIG	★	**Interleukin-1 alpha**	**IL1A**	Sus scrofa (Pig)	270
P08831	IL1A_BOVIN	★	**Interleukin-1 alpha**	**IL1A**	Bos taurus (Bovine)	268
Q28385	IL1A_HORSE	★	**Interleukin-1 alpha**	**IL1A**	Equus caballus (Horse)	270
P09428	IL1B_BOVIN	★	**Interleukin-1 beta**	**IL1B**	Bos taurus (Bovine)	266
P16598	IL1A_RAT	★	**Interleukin-1 alpha**	**Il1a**	Rattus norvegicus (Rat)	270
Q61730	IL1AP_MOUSE	★	**Interleukin-1 receptor accessory protein**	**Il1rap**	Mus musculus (Mouse)	570
P14628	IL1B_RABIT	★	**Interleukin-1 beta**	**IL1B**	Oryctolagus cuniculus (Rabbit)	268
Q9XS77	IL1B_TRIVU	★	**Interleukin-1 beta**	**IL1B**	Trichosurus vulpecula (Brush-tailed possum)	269
Q2MH07	IL1B_BUBCA	★	**Interleukin-1 beta**	**IL1B**	Bubalus carabanensis (Swamp type water buffalo) (Bubalus bubalis carabanensis)	266

Whole proteomes:
UniProt: complete vs. reference

What are complete proteomes?

Last modified March 21, 2012

UniProt provides 'complete proteome' sets of proteins thought to be expressed by organisms whose genomes have been completely sequenced.

What is a complete proteome?

A complete proteome is the entire set of proteins expressed by a specific organism. The majority of the UniProt complete proteomes are based on the translation of a completely sequenced genome, and will normally include sequences that derive from extra-chromosomal elements such as plasmids or organellar genomes in organisms where these occur. Some complete proteomes may also include protein sequences based on high quality cDNAs that cannot be mapped to the current genome assembly due to sequencing errors or gaps. These are only included in the complete proteome following manual review of the supporting evidence, including careful analysis of homologous sequences from closely related organisms.

What is the curation status of UniProt complete proteomes?

UniProt complete proteomes may include both manually reviewed (UniProtKB/Swiss-Prot) and unreviewed (UniProtKB/TrEMBL) entries. The proportion of reviewed entries varies between proteomes, and is obviously greater for the proteomes of intensively curated model organisms: some complete proteomes, such as those of *Saccharomyces cerevisiae* 288C and *Escherichia coli* strain K12 consist entirely of reviewed entries. Curation is a continuing process, and complete proteomes are updated in a regular manner as new information becomes available: pseudogenes and other dubious uncharacterized ORFs may be removed, other newly identified and characterized sequences may be added

1. Ensembl sequences are first mapped to their UniProtKB counterparts under stringent conditions, requiring 100% identity over 100% of the length of the two sequences. These entries are tagged with the keyword `'Complete proteome'` and updated with an Ensembl cross-reference.
2. Ensembl sequences that are absent from UniProtKB are imported into UniProtKB/TrEMBL. These entries are tagged with the keyword `'Complete proteome'` and have an Ensembl cross-reference.
3. All other UniProtKB/Swiss-Prot entries within the proteome that do not map to Ensembl are tagged with the keyword `'Complete proteome'`.

Therefore, a complete proteome is formed from all UniProtKB/Swiss-Prot entries (irrespective of whether they map to Ensembl) plus those UniProtKB/TrEMBL entries mapping to Ensembl for that proteome.

To date this pipeline has been used to populate UniProtKB with additional sequences for the human and mouse proteomes (see headline Complete proteomes for *Homo sapiens* and *Mus musculus*) and many other vertebrata.

See also: Where do the UniProtKB protein sequences come from?

How to retrieve complete proteomes?

Complete proteomes for specific taxa can be retrieved by searching for the taxonomic identifier in the `organism` field together with the keyword `'Complete proteome'`. For example, to retrieve the complete proteome for *Escherichia coli* (strain K12), which has the taxonomic identifier 83333, the required query would be:

- Query: organism:83333 AND keyword:"Complete proteome"

The taxonomic identifier can also be used to query the `taxonomy` field rather than the `organism` field. This will result in the retrieval of all complete proteome sequences at or below the taxonomic rank specified by the identifier. For example, to retrieve the complete proteome for *Escherichia coli* (strain K12) and all complete proteomes at lower taxonomic nodes (substrains such as *Escherichia coli* (strain K12 / DH10B)), then the required query would be:

- Query: taxonomy:83333 AND keyword:"Complete proteome"

www.uniprot.org/uniprot/?query=organism:83333+AND+keyword:"Complete+proteome+[KW-0181]"

UniProt › UniProtKB Downloads · Contact · Documentation/Help

Search | Blast | Align | Retrieve | ID Mapping *

Search in Protein Knowledgebase (UniProtKB)

Query organism:83333 AND keyword:"Complete proteome [KW-0181]"

Search | Advanced Search »

1 - 25 of 4,305 results for **organism:"Escherichia coli (strain K12) [83333]"** ☒ **AND keyword:"Complete proteome [KW-0181]"** ☒ in **UniProtKB**

Browse by taxonomy, keyword, gene ontology, enzyme class or pathway | Reduce sequence redundancy to 100%, 90% or 50% Download

Page 1 of 173 | Next »

Results Customize

› Show only reviewed (4,303) (UniProtKB/Swiss-Prot) or unreviewed (2) (UniProtKB/TrEMBL) entries

› Expand search to "Escherichia coli (strain K12) [83333]" to include lower taxonomic ranks

› Show only entries from a reference proteome set (4,303)

› Add columns: Keywords

Entry	Entry name	Status	Protein names	Gene names	Organism	Length
P05100	3MG1_ECOLI	★	**DNA-3-methyladenine glycosylase 1**	**tag** b3549 JW3518	Escherichia coli (strain K12)	187
P04395	3MG2_ECOLI	★	**DNA-3-methyladenine glycosylase 2**	**alkA** aidA b2068 JW2053	Escherichia coli (strain K12)	282
P00350	6PGD_ECOLI	★	**6-phosphogluconate dehydrogenase, decarboxyla...**	**gnd** b2029 JW2011	Escherichia coli (strain K12)	468
				pgl ybhE b0767	Escherichia coli	

CROProgram....docx | CRO-CID 2mts....pptx | letter_NSF_UC....docx | iedbProgRpt20....pdf | ppat.1003390.s....pdf | Show all downloads...

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www.iedb.org/sourceOrgId/467144

IMMUNE EPITOPE DATABASE AND ANALYSIS RESOURCE

Keyword Searc

Home | Browse | Advanced Search | Tools | Support | More

Modified Vaccinia Ankara virus epitopes

Source Organism	
Source Organism:	Modified Vaccinia Ankara virus
Source NCBI Taxonomy ID:	467144
Parent NCBI Taxonomy ID:	10245

Structure (93) | Reference (10) | Source Antigen (57) | MHC Binding (38) | T Cell Assay (127) | MHC Ligand Elution (6)

93 item(s) found, displaying 1 to 25 (Click the column headers to adjust the sorting)

« previous | 1 | 2 | 3 | 4 | next » | Go To » | 1

Export all results: (full)

Epitope ID	Structure	Source Antigen	Source Organism
1780	AHINALEY	Protein A47	Modified Vaccinia Ankara virus
4868	ATAAVCLLFIQGYSIYENYGN	putative 16.3k protein	Modified Vaccinia Ankara virus
5364	AVHLIIYYQLAGYILTVLGLG	putative 31.5k protein	Modified Vaccinia Ankara virus
6630	CLTEYILWV	putative 21.7k protein (2 more)	Vaccinia virus (2 more)
6637	CLVFEPFVNQSGIEILLLYFK	nucleoside triphosphate phosphohydrolase I, DNA helicase	Modified Vaccinia Ankara virus
7566	DAKNNAAKLAVDKL	dsRNA dependent PK inhibitor	Modified Vaccinia Ankara virus
7892	DDYMFFVIKNLGR	protein kinase	Modified Vaccinia Ankara virus
8263	DFFKFSFMYIESIKVDRIGDN	putative 49.1k protein	Modified Vaccinia Ankara virus
9432	DNCILANRCFVKI	putative 18.6k protein	Modified Vaccinia Ankara virus
11202	EATKLCDVL	Thymidine kinase (1 more)	Modified Vaccinia Ankara virus (1 more)

CROProgram....docx | CRO-CID 2mts....pptx | letter_NSF_UC....docx | iedbProgRpt20....pdf | ppat.1003390.s....pdf | Show all downloads...

EN 5:15 PM 7/8/2013


www.uniprot.org/uniprot/?query=taxonomy%3A467144+AND+keyword%3A"complete+proteome"&sort=score
UniProt › UniProtKB
Downloads · Contact · Documentation/Help
Search
Blast
Align
Retrieve
ID Mapping
Search in
Protein Knowledgebase (UniProtK
Query
taxonomy:467144 AND keyword:"complete proteome"
Search
Advanced Search »
0 result for taxonomy:"Modified Vaccinia Ankara virus [467144]" AND keyword:"complete proteome" in UniProtKB
Results
Customize
› Can't find what you are looking for? Please contact us.
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EMBL-EBI
PIR
SIB
CROProgram....docx
CRO-CID 2mts....pptx
letter_NSF_UC....docx
iedbProgRpt20....pdf
ppat.1003390.s....pdf
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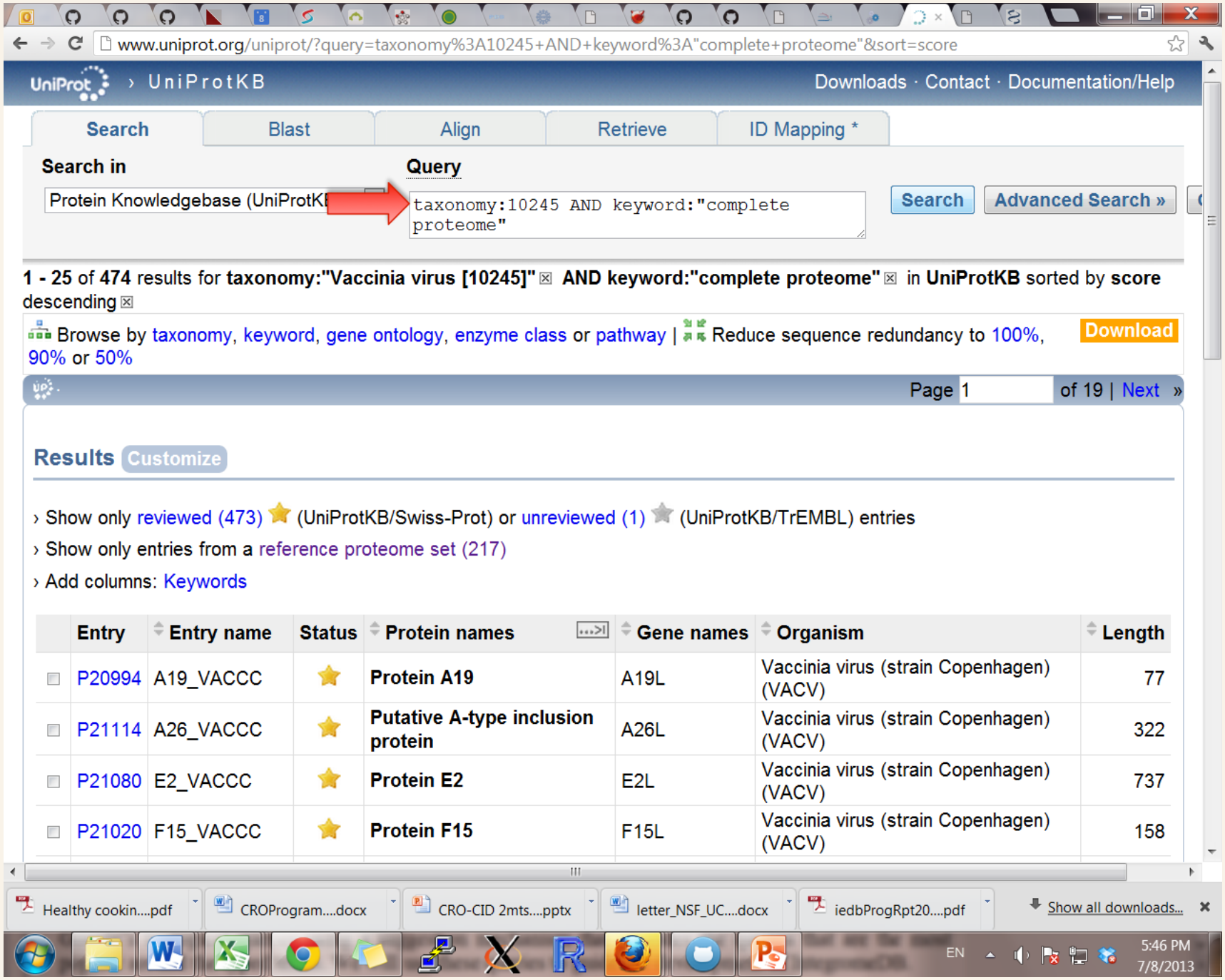
7/8/2013


www.uniprot.org/uniprot/?query=taxonomy%3A10245+AND+keyword%3A"complete+proteome"&sort=score
UniProt › UniProtKB
Downloads · Contact · Documentation/Help
Search
Blast
Align
Retrieve
ID Mapping *
Search in
Protein Knowledgebase (UniProtKB)
Query
taxonomy:10245 AND keyword:"complete proteome"
Search
Advanced Search »
1 - 25 of 474 results for taxonomy:"Vaccinia virus [10245]" AND keyword:"complete proteome" in UniProtKB sorted by score descending
Browse by taxonomy, keyword, gene ontology, enzyme class or pathway | Reduce sequence redundancy to 100%, 90% or 50%
Download
Page 1 of 19 | Next »
Results Customize
› Show only reviewed (473) (UniProtKB/Swiss-Prot) or unreviewed (1) (UniProtKB/TrEMBL) entries
› Show only entries from a reference proteome set (217)
› Add columns: Keywords
Entry | Entry name | Status | Protein names | Gene names | Organism | Length
P20994 | A19_VACCC | Protein A19 | A19L | Vaccinia virus (strain Copenhagen) (VACV) | 77
P21114 | A26_VACCC | Putative A-type inclusion protein | A26L | Vaccinia virus (strain Copenhagen) (VACV) | 322
P21080 | E2_VACCC | Protein E2 | E2L | Vaccinia virus (strain Copenhagen) (VACV) | 737
P21020 | F15_VACCC | Protein F15 | F15L | Vaccinia virus (strain Copenhagen) (VACV) | 158
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CRO-CID 2mts....pptx
letter_NSF_UC....docx
iedbProgRpt20....pdf
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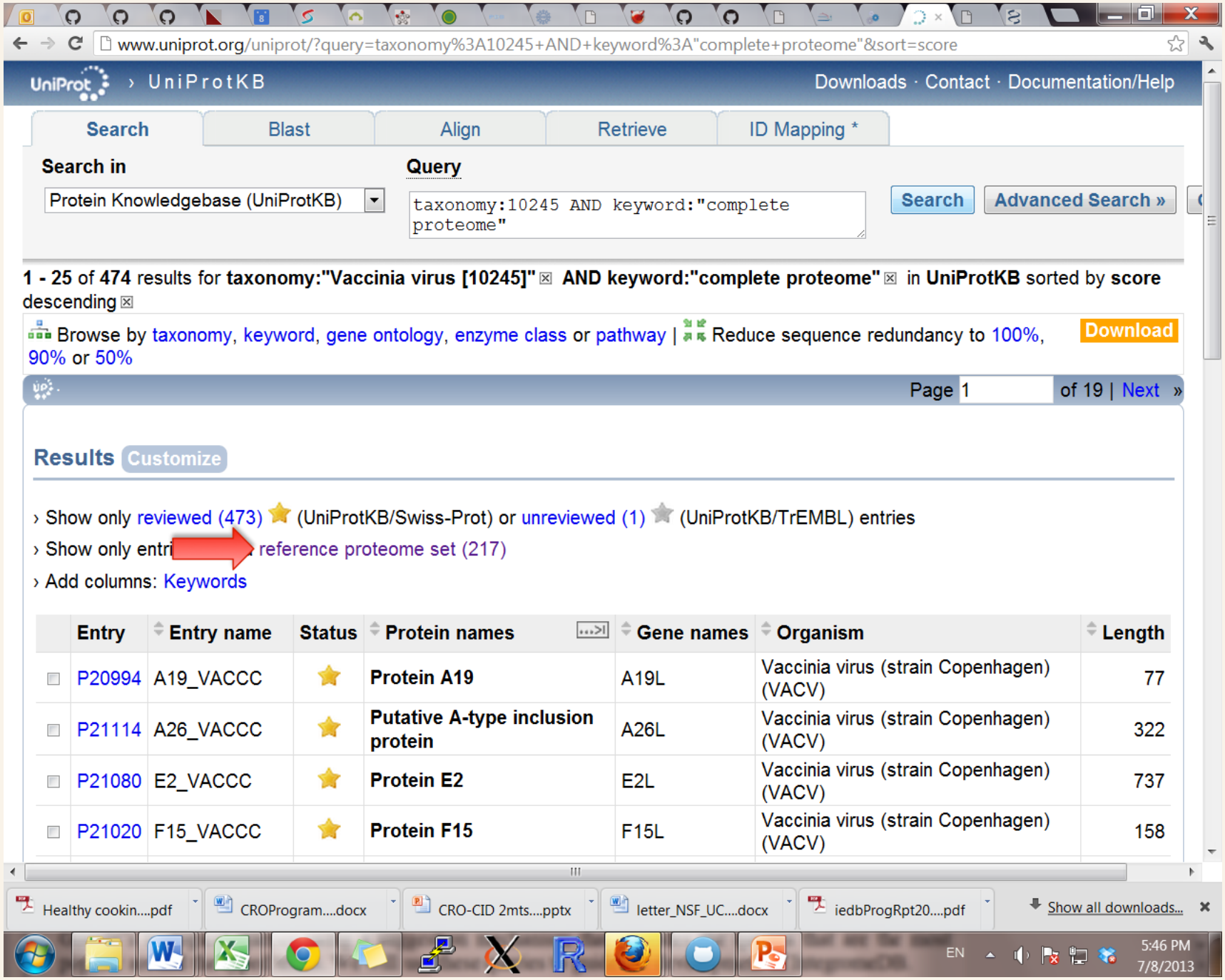
7/8/2013

☐	P21020	F15_VACCC	★	**Protein F15**	F15L	Vaccinia virus (strain Copenhagen) (VACV)	158
☐	P24764	SEMA_VACCW	★	**Semaphorin-like protein A39**	VACWR163 A39R	Vaccinia virus (strain Western Reserve) (VACV) (Vaccinia virus (strain WR))	295
☐	P20841	SPI2A_VACCC	★	**Putative serine proteinase inhibitor 2 homolo...**	B13R	Vaccinia virus (strain Copenhagen) (VACV)	116
☐	P20842	SPI2B_VACCC	★	**Putative serine proteinase inhibitor 2 homolo...**	B14R	Vaccinia virus (strain Copenhagen) (VACV)	222
☐	P21100	VC16_VACCC	★	**Protein C16/B22**	B22R C16L	Vaccinia virus (strain Copenhagen) (VACV)	181
☐	P21103	VC19_VACCC	★	**Protein C19/B25**	B25R C19L	Vaccinia virus (strain Copenhagen) (VACV)	259
☐	P21096	A31_VACCC	★	**Protein A31**	A31R	Vaccinia virus (strain Copenhagen) (VACV)	124
☐	P24760	A31_VACCW	★	**Protein A31**	VACWR154 A31R	Vaccinia virus (strain Western Reserve) (VACV) (Vaccinia virus (strain WR))	124
☐	P21060	A37_VACCC	★	**Protein A37**	A37R	Vaccinia virus (strain Copenhagen) (VACV)	263
☐	P24762	A37_VACCW	★	**Protein A37**	VACWR160 A37R	Vaccinia virus (strain Western Reserve) (VACV) (Vaccinia virus (strain WR))	263
☐	P21067	A47_VACCC	★	**Protein A47**	A47L	Vaccinia virus (strain Copenhagen) (VACV)	244
☐	P26673	A47_VACCW	★	**Protein A47**	VACWR173 A47L	Vaccinia virus (strain Western Reserve) (VACV) (Vaccinia virus (strain WR))	252
☐	P21068	A49_VACCC	★	**Protein A49**	A49R	Vaccinia virus (strain Copenhagen) (VACV)	162
☐	P21069	A51_VACCC	★	**Protein A51**	A51R	Vaccinia virus (strain Copenhagen) (VACV)	334


www.uniprot.org/uniprot/?query=taxonomy%3A10245+AND+keyword%3A"complete+proteome"&sort=score
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Blast
Align
Retrieve
ID Mapping *
Search in
Protein Knowledgebase (UniProtKB)
Query
taxonomy:10245 AND keyword:"complete proteome"
Search
Advanced Search »
1 - 25 of 474 results for taxonomy:"Vaccinia virus [10245]" AND keyword:"complete proteome" in UniProtKB sorted by score descending
Browse by taxonomy, keyword, gene ontology, enzyme class or pathway | Reduce sequence redundancy to 100%, 90% or 50%
Download
Page 1 of 19 | Next »
Results Customize
› Show only reviewed (473) (UniProtKB/Swiss-Prot) or unreviewed (1) (UniProtKB/TrEMBL) entries
› Show only entri reference proteome set (217)
› Add columns: Keywords
Entry | Entry name | Status | Protein names | Gene names | Organism | Length
P20994 | A19_VACCC | Protein A19 | A19L | Vaccinia virus (strain Copenhagen) (VACV) | 77
P21114 | A26_VACCC | Putative A-type inclusion protein | A26L | Vaccinia virus (strain Copenhagen) (VACV) | 322
P21080 | E2_VACCC | Protein E2 | E2L | Vaccinia virus (strain Copenhagen) (VACV) | 737
P21020 | F15_VACCC | Protein F15 | F15L | Vaccinia virus (strain Copenhagen) (VACV) | 158
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www.uniprot.org/uniprot/?query=taxonomy%3A10245+AND+keyword%3A"complete+proteome"+AND+keyword%3A1185&sort=score

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Search | Blast | Align | Retrieve | ID Mapping *

Search in Protein Knowledgebase (UniProtKB)

Query taxonomy:10245 AND keyword:"complete proteome" AND keyword:1185

Search | Advanced Search »

1 - 25 of 217 results for **taxonomy:"Vaccinia virus [10245]"** ☒ **AND keyword:"complete proteome"** ☒ **AND keyword:"Reference proteome [1185]"** ☒ in **UniProtKB** sorted by **score** descending ☒

Browse by taxonomy, keyword, gene ontology, enzyme class or pathway | Reduce sequence redundancy to 100%, 90% or 50% Download

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Results Customize

› Show only reviewed (216) (UniProtKB/Swiss-Prot) or unreviewed (1) (UniProtKB/TrEMBL) entries

Entry	Entry name	Status	Protein names	Gene names	Organism	Length
P24764	SEMA_VACCW	★	**Semaphorin-like protein A39**	VACWR163 A39R	Vaccinia virus (strain Western Reserve) (VACV) (Vaccinia virus (strain WR))	295
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P26673	A47_VACCW	★	**Protein A47**	VACWR173 A47I	Vaccinia virus (strain Western Reserve) (VACV) (Vaccinia virus	252

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www.uniprot.org/taxonomy/complete-proteomes

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Search | Blast | Align | Retrieve | ID Mapping

Search in Taxonomy

Query * AND complete:yes

Search | Advanced Search » | Clear

COMPLETE PROTEOMES AND REFERENCE PROTEOMES

A complete proteome consists of the set of proteins thought to be expressed by an organism whose genome has been completely sequenced.

A reference proteome is the complete proteome of a representative, well-studied model organism or an organism of interest for biomedical research.

These organisms can be searched via the taxonomy pages, which provide links to download complete and reference proteome sets when available, as well as links to the HAMAP web site.

Browse or list organisms with:

Complete proteomes	Reference proteomes
Browse by hierarchy	Browse by hierarchy
List all Bacteria	List all Bacteria
List all Archaea	List all Archaea
List all Eukaryota	List all Eukaryota
List all Viruses	List all Viruses

Search organisms with complete proteomes:

FAQ

› What are complete proteome sets?

› What are reference proteome sets?

› How to retrieve sets of protein sequences?

› What is HAMAP?
HAMAP is a system, based on manual protein annotation, that identifies and semi-automatically annotates proteins... More

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www.uniprot.org/taxonomy/complete-proteomes

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Search | Blast | Align | Retrieve | ID Mapping

Search in Taxonomy

Query * AND complete:yes

Search | Advanced Search » | Clear

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Complete proteomes	Reference proteomes
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List all Bacteria	List all Bacteria
List all Archaea	List all Archaea
List all Eukaryota	List all Eukaryota
List all Viruses	List all Viruses

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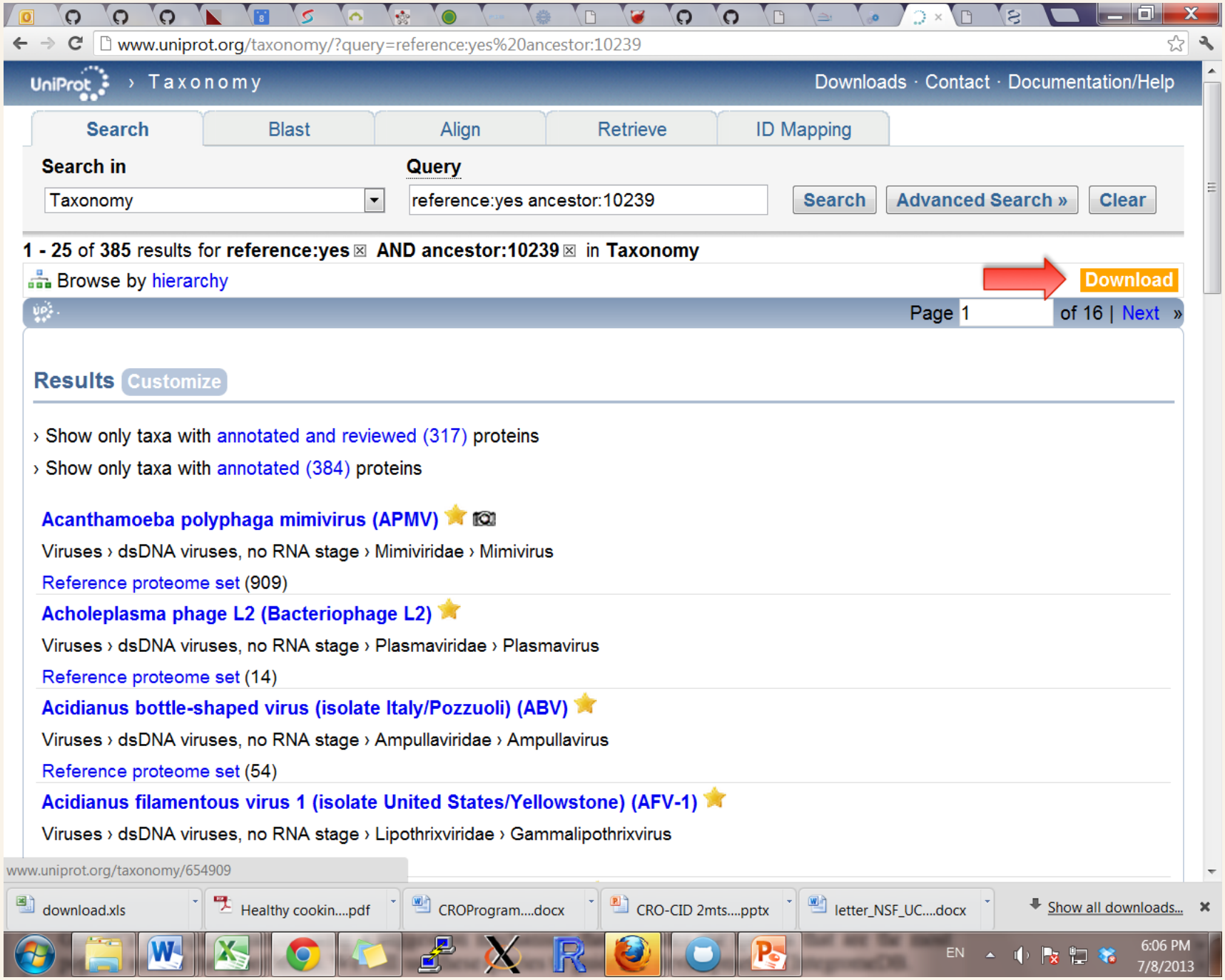
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ID Mapping
Search in
Taxonomy
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reference:yes ancestor:10239
Search
Advanced Search »
Clear
1 - 25 of 385 results for reference:yes ⊠ AND ancestor:10239 ⊠ in Taxonomy
Browse by hierarchy
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Results Customize
› Show only taxa with annotated and reviewed (317) proteins
› Show only taxa with annotated (384) proteins
Acanthamoeba polyphaga mimivirus (APMV)
Viruses › dsDNA viruses, no RNA stage › Mimiviridae › Mimivirus
Reference proteome set (909)
Acholeplasma phage L2 (Bacteriophage L2)
Viruses › dsDNA viruses, no RNA stage › Plasmaviridae › Plasmavirus
Reference proteome set (14)
Acidianus bottle-shaped virus (isolate Italy/Pozzuoli) (ABV)
Viruses › dsDNA viruses, no RNA stage › Ampullaviridae › Ampullavirus
Reference proteome set (54)
Acidianus filamentous virus 1 (isolate United States/Yellowstone) (AFV-1)
Viruses › dsDNA viruses, no RNA stage › Lipothrixviridae › Gammalipothrixvirus
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Query reference:yes ancestor:10239 Search Advanced Search » Clear

385 results for **reference:yes** ☒ **AND ancestor:10239** ☒ in **Taxonomy**

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Summary information from the result view.

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List

List of NCBI taxonomy identifiers.

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* Estimate on the basis of the average entry size.

EMBL-EBI PIR SIB

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We need to map each epitope to a reference proteome

- Algorithm:
  - Input (SA – source antigen):
    - {epitope ID; epitope sequence; SA ID}
    - {SA ID; SA sequence; source organism ID; parent taxonomic ID}
    - **{parent taxonomic ID; reference proteome sequences}**
  - TODO….
  - Output:
    - {epitope ID; ref. sequence ID; ref. sequence; epitope positions in ref sequence}

To get {parent taxonomic ID; reference proteome sequences}

- For each Epitope → Source Organism → Source NCBI Taxonomy ID → "Parent NCBI Taxonomy ID"
- Scenario #1:
  - Get a list of Taxonomy IDs for all complete reference proteomes (UniProt or NCBI)
  - Look up if for "Parent NCBI Taxonomy ID" a reference proteome is available. If not, delete the epitope from the list.
  - Get the list of "Parent NCBI Taxonomy ID" from the filtered epitope list.
  - Get complete reference proteomes for this list.
  - The mapping program will work with filtered epitope list and a set of proteomes.
- Scenario #2 (more general):
  - Get the sequences of all complete reference proteomes.
  - The mapping program will work with initial list of all epitopes and all proteomes.

- You need to figure out how to get all complete proteome sequences for bacteria and viruses
- Or, alternatively, but worse for reproduction and slow approach, to implement Scenario #1.

Sequence alignment

Why to align sequences?

- To search sequences in the databases.

- To annotate (assign function to) genes, proteins, genomes by means of transferring curated annotation of **homologous** -- orthologous (*conserved across species and direct descendants of a sequence in a common ancestor*) and paralogous *(conserved within species and separated by the event of gene duplication)* -- genes/proteins.

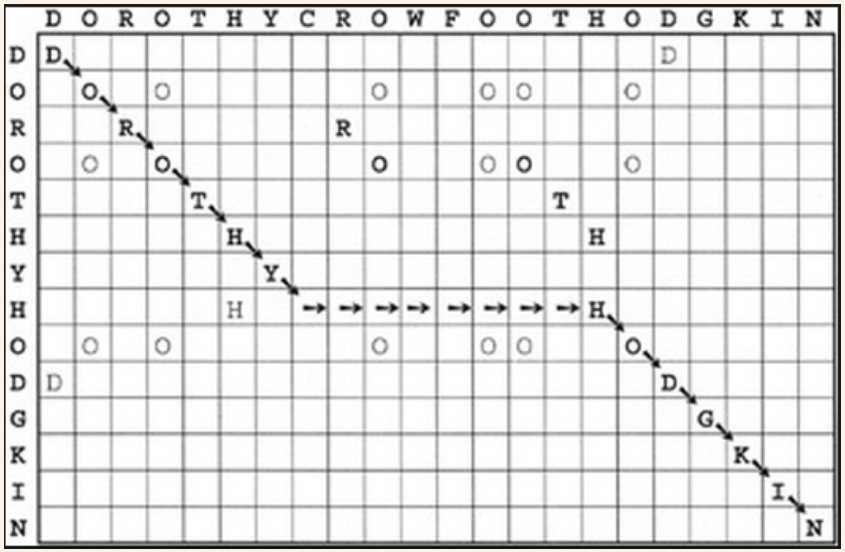
- The underlining hypothesis is that all genes and proteins are a product of evolution.
  - Therefore, conserved along evolution gene and protein sequences (*forming a functional family*) might have a similar function.
  - Likewise, a conserved region or a sequence motif might suggest that this region or motif has structural or functional importance (*e.g., active sites of enzymes, binding sites of protein receptors, cis-regulatory elements in DNA*).

Pairwise sequence alignment methods

- **Dot matrix methods**: applets Dotlet, DNADot, Dottup (*can be used for comparing entire genomes*); EBI LALIGN program generates dotplots
- **Global:**
  - **The dynamic programming algorithms**:
    - The Needleman-Wunsch algorithm (1970; NCBI BLAST web-site, also Needle at EBI www.ebi.ac.uk/Tools/psa/
- **Local:**
  - **The dynamic programming algorithms**:
    - LALIGN (uses modified Smith-Waterman algorithm of 1981); EBI
  - **Word or k-tulip methods**:
    - NCBI BLAST server (Altschuls et al., 1991); used to search NCBI databases and UniProt
    - FASTA (Lipman & Pearson, 1985) - available at EBI to search for UniProt protein databases

- Wikipedia lists 15 methods for database search and 41 for pairwise alignment (some for protein or DNA only, some for both, 25 are available via web sites)

Pairwise sequence comparison: Dotplots

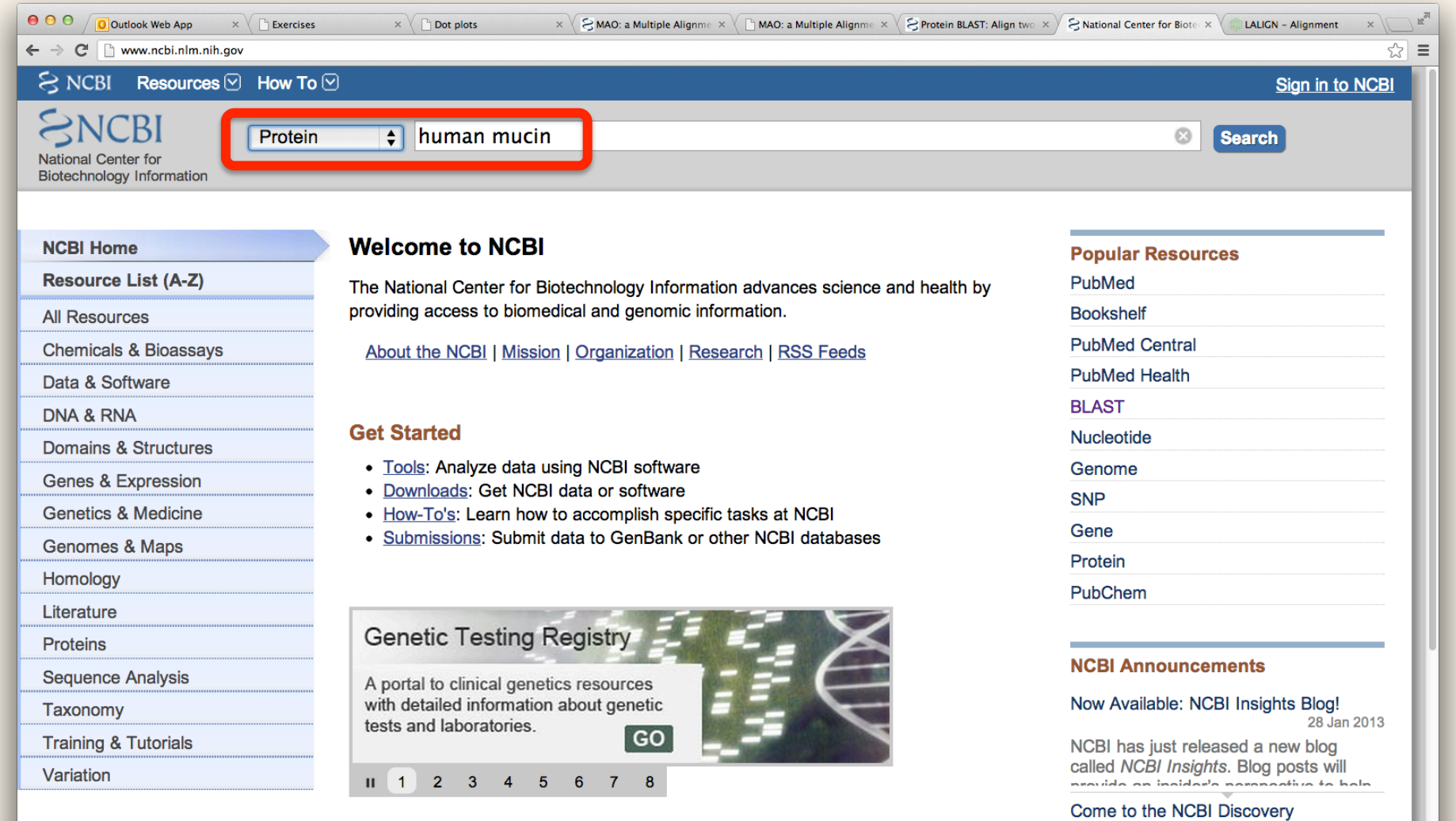


If two sequences are closely related, the alignment can be read directly from the dotplot:

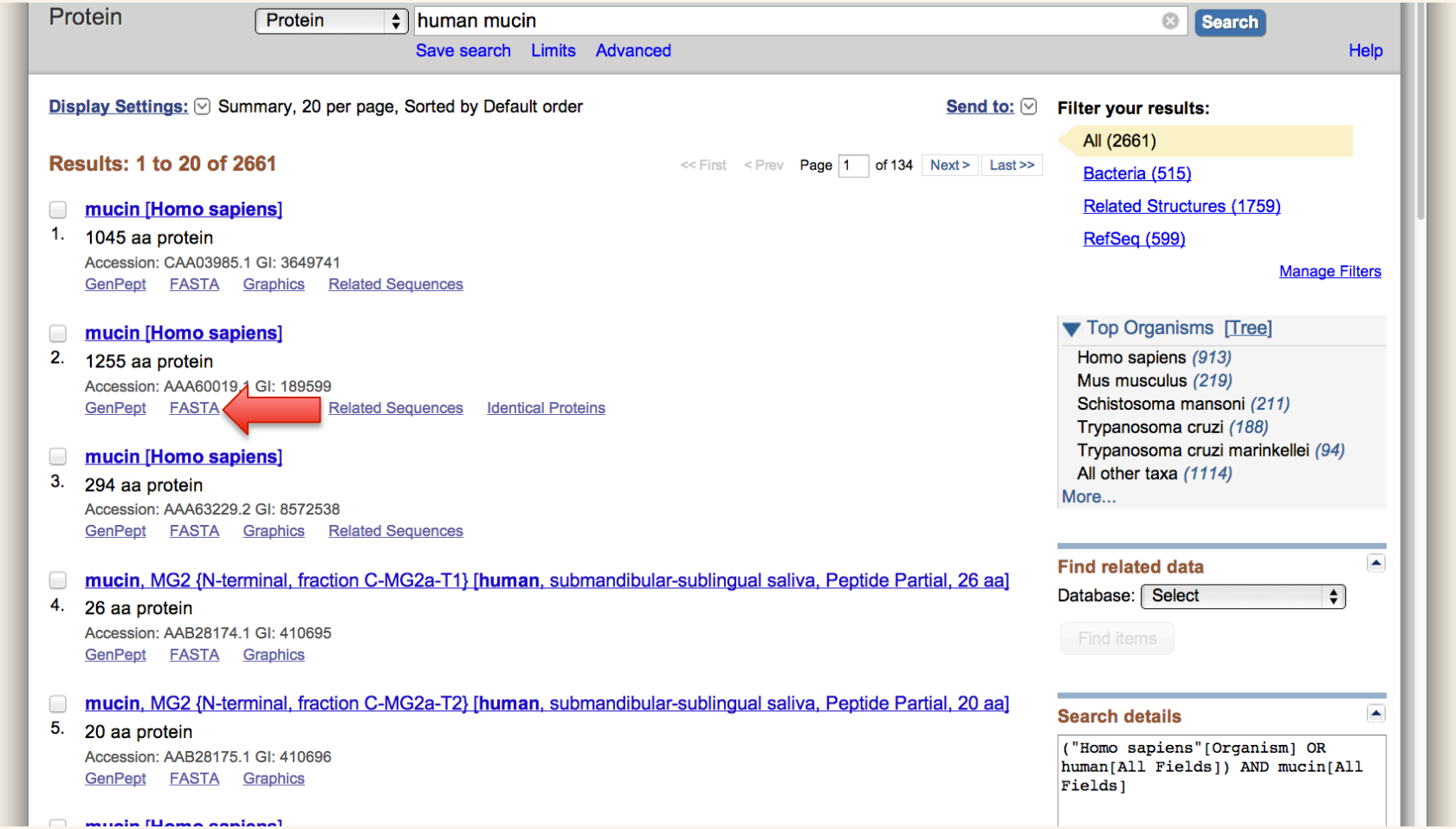
```
DOROTHYCROWFOOTHODGKIN
DOROTHY--------HODGKIN
```

Let’s generate a dotplot for the human mucin using LALIGN

Open NCBI main page www.ncbi.nlm.nih.gov
Type “human mucin” and select “protein” database

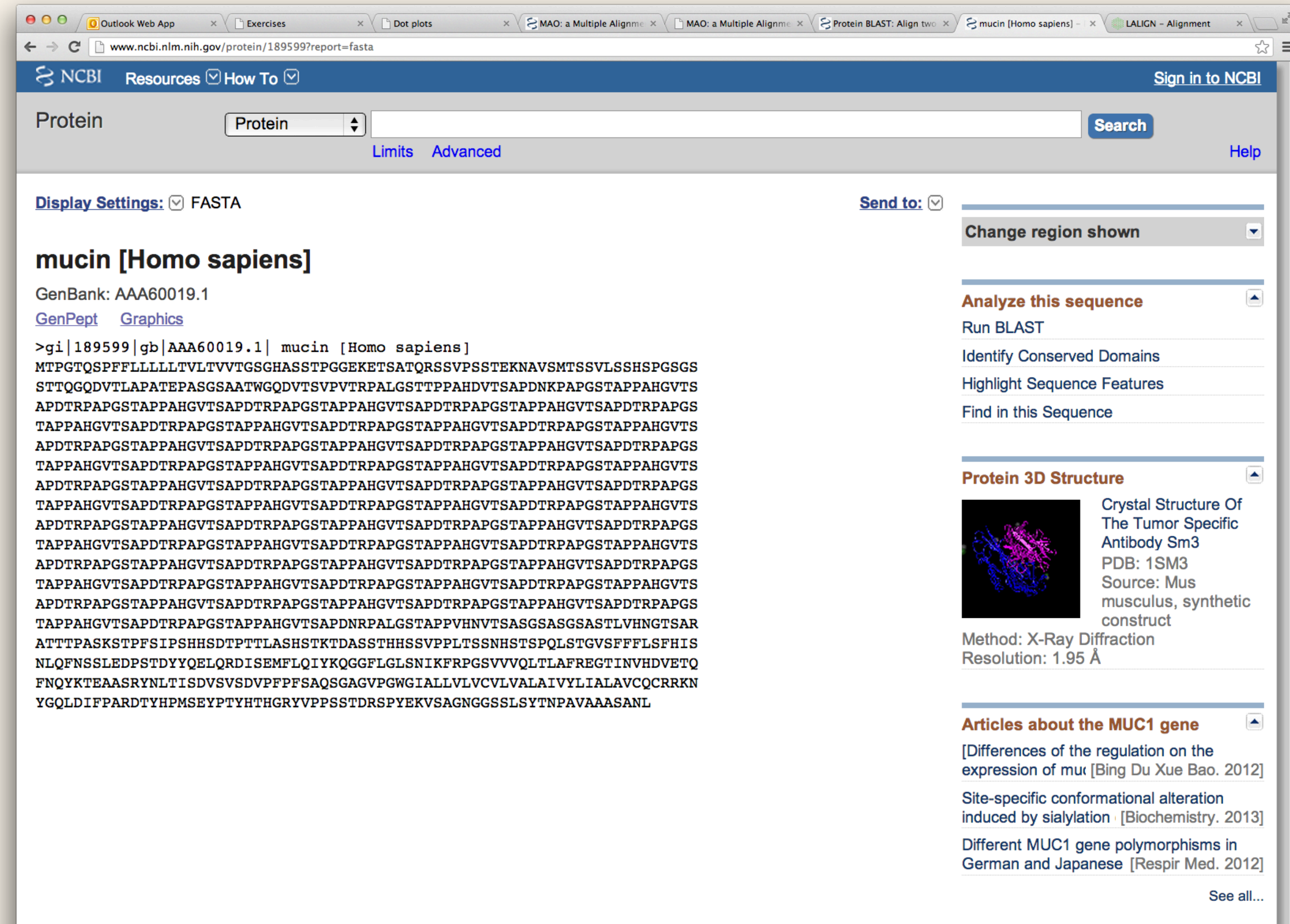


Select “FASTA” for the second protein

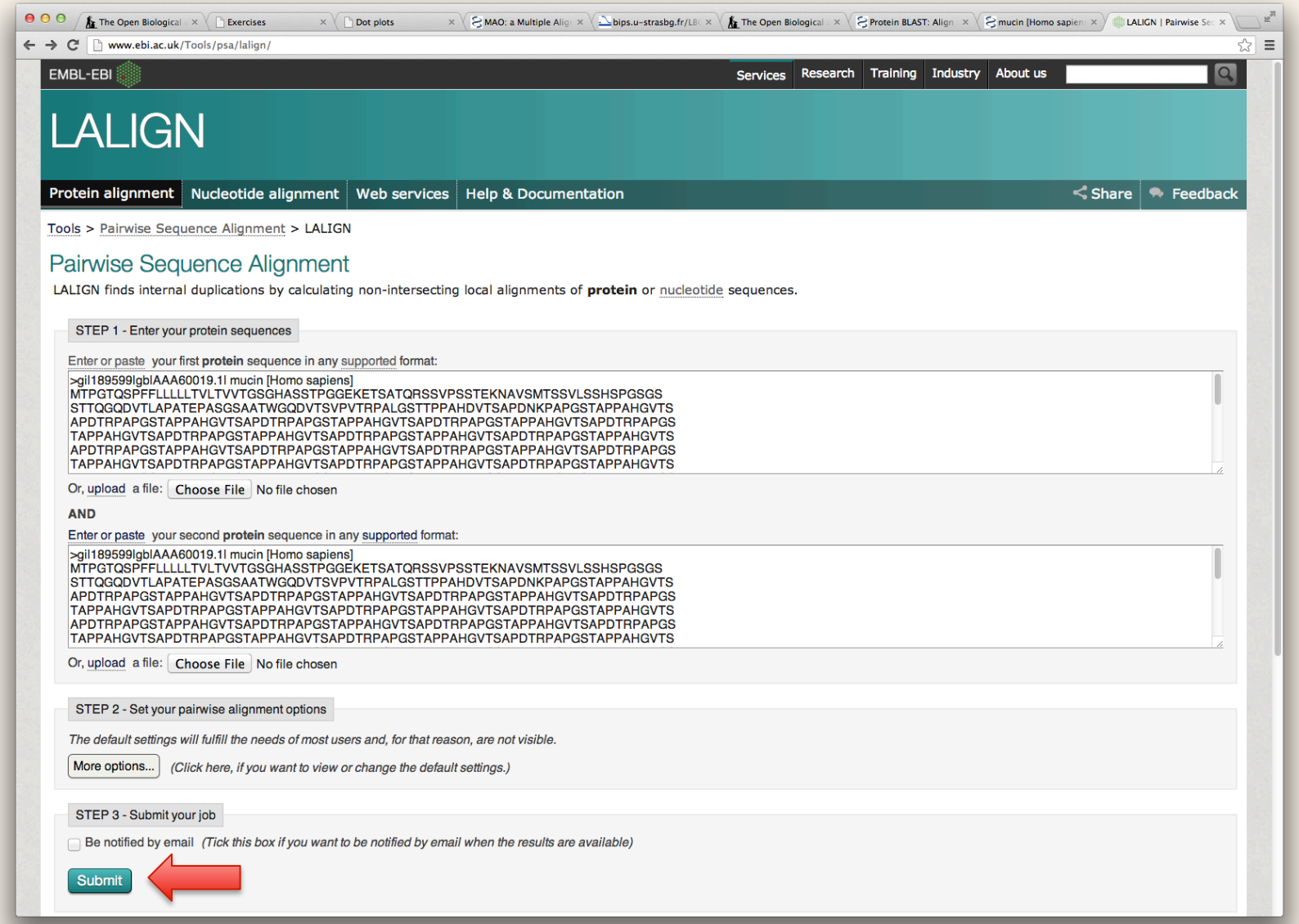


This is a sequence of the human musin:

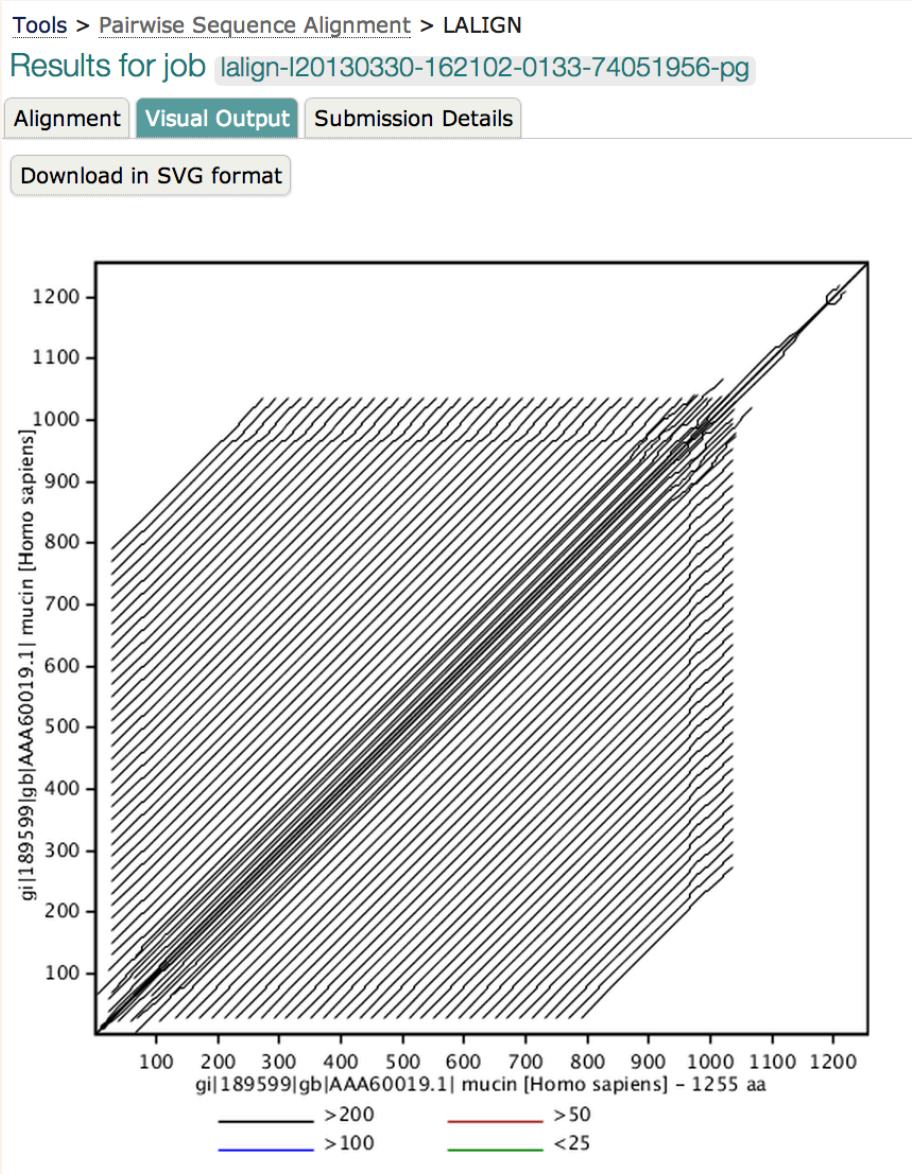
keep this window open and copy this sequence to clipboard



Open http://www.ebi.ac.uk/Tools/psa/lalign/



Dotplot for the human mucin compared to itself



What does it tell us about the protein sequence?

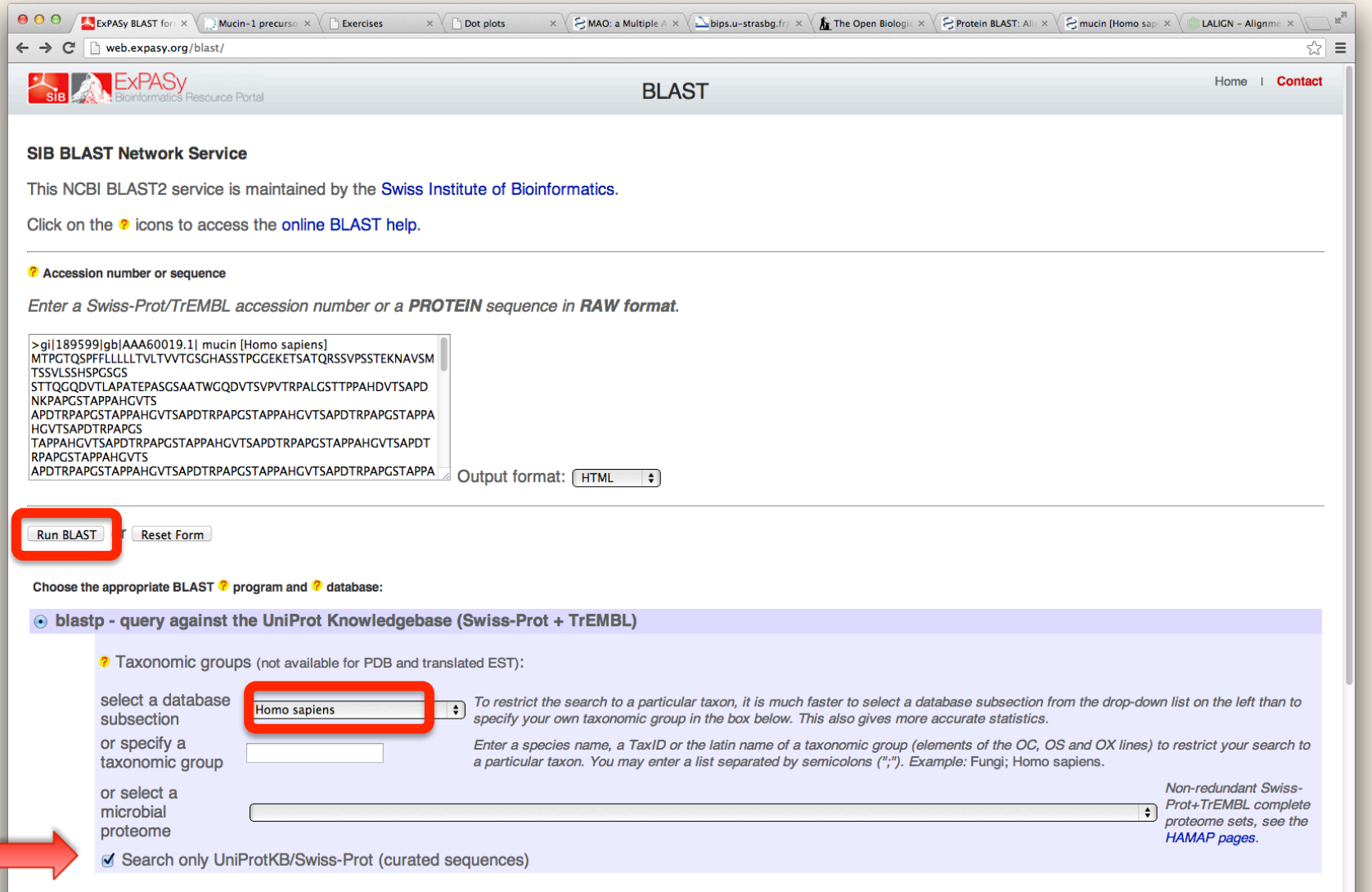
This sequence contains repeats!
Dotplots are useful to identify repeats!

Where to find information about repeats?

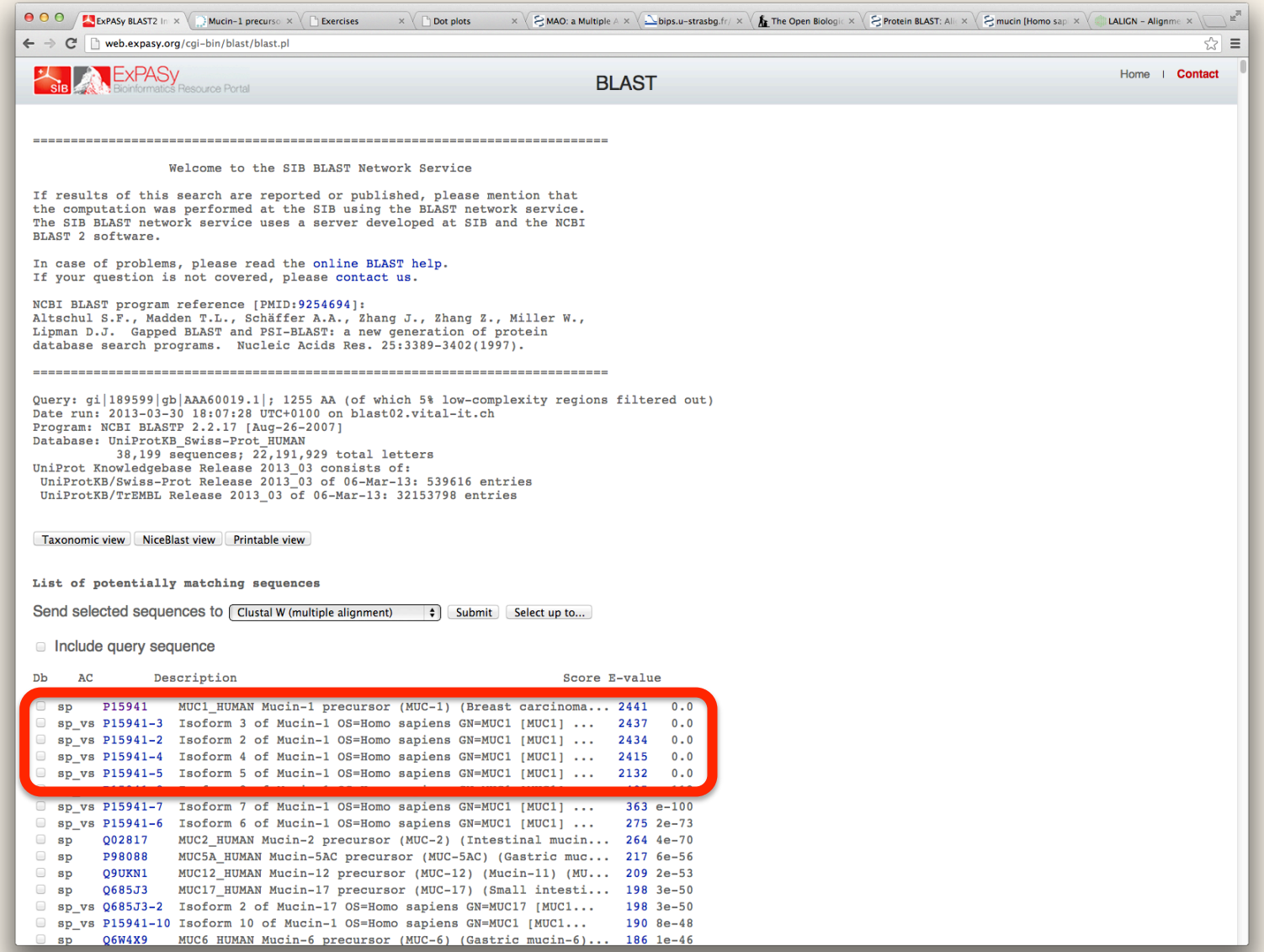
- NCBI doesn't provide information on repeats.
- Let's see what UniProt provides.
- Since there is no direct link between the two and IDs do not match, the only option to search UniProt by sequence.

Go to http://web.expasy.org/blast/

paste the sequence, select blastp, Homo Sapience and search for curated sequences to speed up the search



There are five hits with E-value 0.0



web.expasy.org/cgi-bin/blast/blast.pl

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In case of problems, please read the online BLAST help.
If your question is not covered, please contact us.

NCBI BLAST program reference [PMID:9254694]:
Altschul S.F., Madden T.L., Schäffer A.A., Zhang J., Zhang Z., Miller W.,
Lipman D.J. Gapped BLAST and PSI-BLAST: a new generation of protein
database search programs. Nucleic Acids Res. 25:3389-3402(1997).

==========

Query: gi|189599|gb|AAA60019.1|; 1255 AA (of which 5% low-complexity regions filtered out)
Date run: 2013-03-30 18:07:28 UTC+0100 on blast02.vital-it.ch
Program: NCBI BLASTP 2.2.17 [Aug-26-2007]
Database: UniProtKB_Swiss-Prot_HUMAN
38,199 sequences; 22,191,929 total letters
UniProt Knowledgebase Release 2013_03 consists of:
UniProtKB/Swiss-Prot Release 2013_03 of 06-Mar-13: 539616 entries
UniProtKB/TrEMBL Release 2013_03 of 06-Mar-13: 32153798 entries

Taxonomic view | NiceBlast view | Printable view

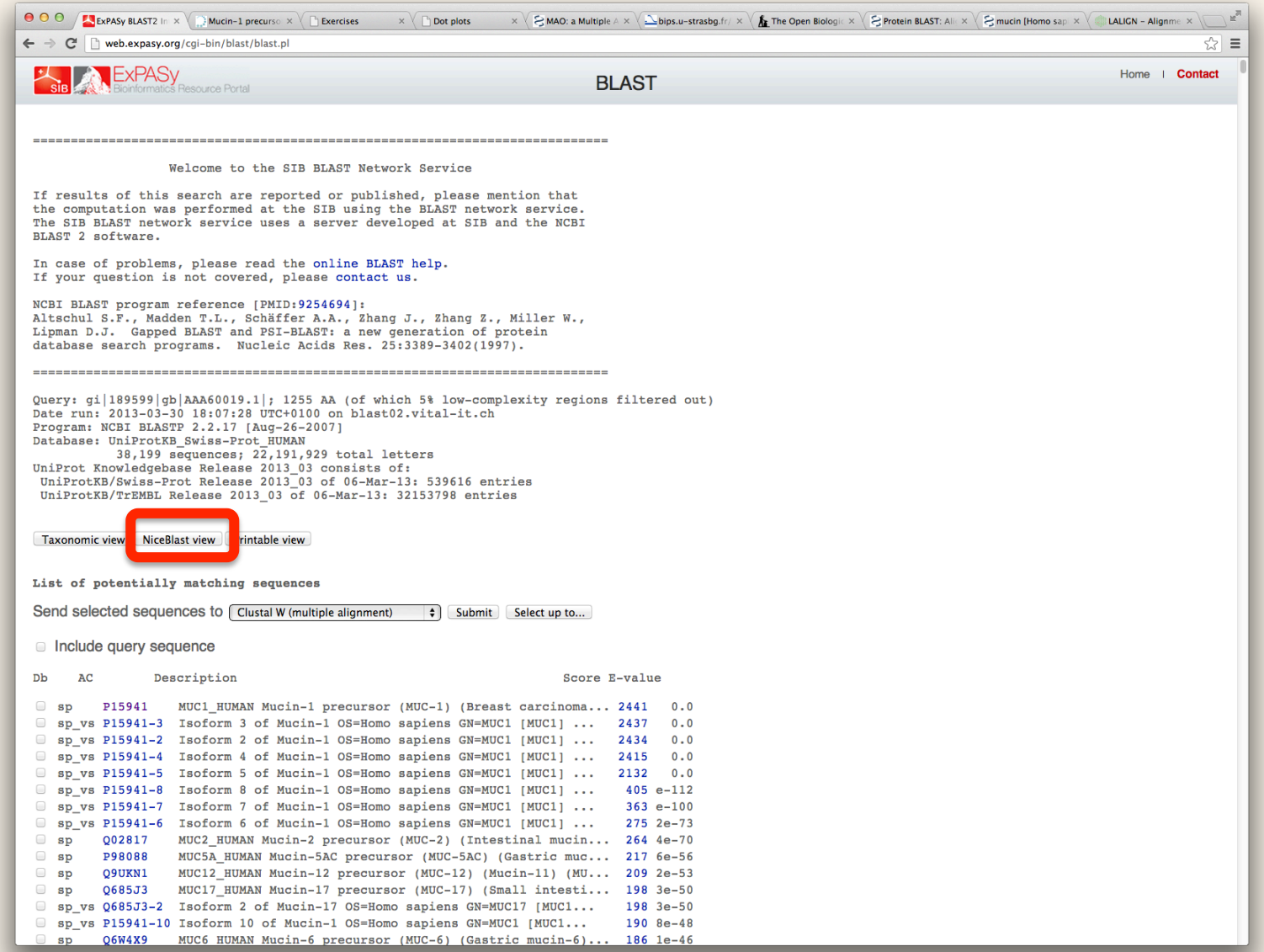
List of potentially matching sequences

Send selected sequences to Clustal W (multiple alignment) Submit Select up to...

Include query sequence

Db	AC	Description	Score	E-value
sp	P15941	MUC1_HUMAN Mucin-1 precursor (MUC-1) (Breast carcinoma...	2441	0.0
sp_vs	P15941-3	Isoform 3 of Mucin-1 OS=Homo sapiens GN=MUC1 [MUC1] ...	2437	0.0
sp_vs	P15941-2	Isoform 2 of Mucin-1 OS=Homo sapiens GN=MUC1 [MUC1] ...	2434	0.0
sp_vs	P15941-4	Isoform 4 of Mucin-1 OS=Homo sapiens GN=MUC1 [MUC1] ...	2415	0.0
sp_vs	P15941-5	Isoform 5 of Mucin-1 OS=Homo sapiens GN=MUC1 [MUC1] ...	2132	0.0
sp_vs	P15941-7	Isoform 7 of Mucin-1 OS=Homo sapiens GN=MUC1 [MUC1] ...	363	e-100
sp_vs	P15941-6	Isoform 6 of Mucin-1 OS=Homo sapiens GN=MUC1 [MUC1] ...	275	2e-73
sp	Q02817	MUC2_HUMAN Mucin-2 precursor (MUC-2) (Intestinal mucin...	264	4e-70
sp	P98088	MUC5A_HUMAN Mucin-5AC precursor (MUC-5AC) (Gastric muc...	217	6e-56
sp	Q9UKN1	MUC12_HUMAN Mucin-12 precursor (MUC-12) (Mucin-11) (MU...	209	2e-53
sp	Q685J3	MUC17_HUMAN Mucin-17 precursor (MUC-17) (Small intesti...	198	3e-50
sp_vs	Q685J3-2	Isoform 2 of Mucin-17 OS=Homo sapiens GN=MUC17 [MUC1...	198	3e-50
sp_vs	P15941-10	Isoform 10 of Mucin-1 OS=Homo sapiens GN=MUC1 [MUC1...	190	8e-48
sp	Q6W4X9	MUC6_HUMAN Mucin-6 precursor (MUC-6) (Gastric mucin-6)...	186	1e-46

We are looking for the protein of 1255 aa, to see the length, change view to "Nice Blast view"



web.expasy.org/cgi-bin/blast/blast.pl

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NCBI BLAST program reference [PMID:9254694]:
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Query: gi|189599|gb|AAA60019.1|; 1255 AA (of which 5% low-complexity regions filtered out)
Date run: 2013-03-30 18:07:28 UTC+0100 on blast02.vital-it.ch
Program: NCBI BLASTP 2.2.17 [Aug-26-2007]
Database: UniProtKB_Swiss-Prot_HUMAN
38,199 sequences; 22,191,929 total letters
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UniProtKB/TrEMBL Release 2013_03 of 06-Mar-13: 32153798 entries

Taxonomic view | NiceBlast view | rintable view

List of potentially matching sequences

Send selected sequences to Clustal W (multiple alignment) Submit Select up to...

Include query sequence

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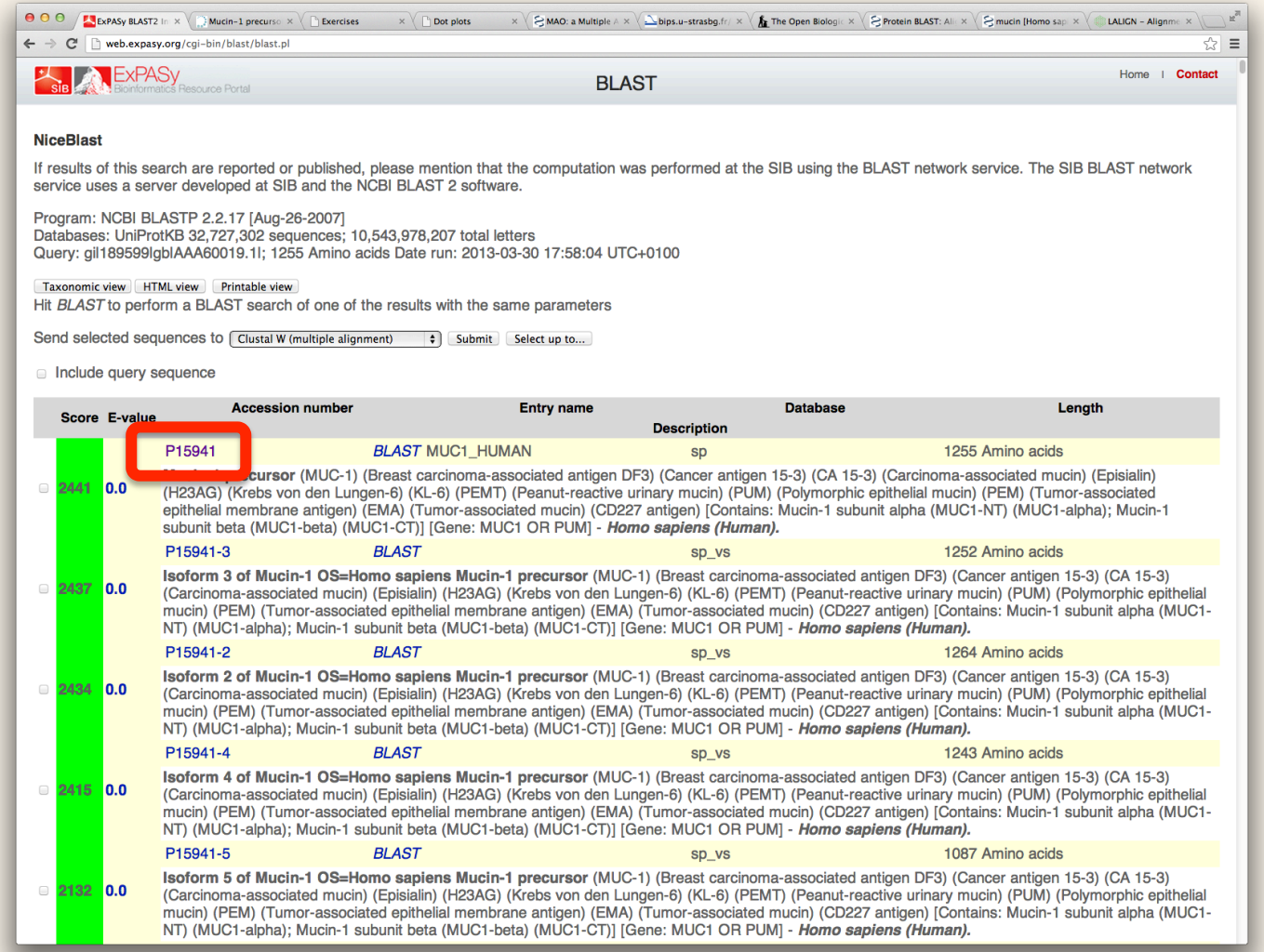
sp_vs	P15941-3	Isoform 3 of Mucin-1 OS=Homo sapiens GN=MUC1 [MUC1] ...	2437	0.0
sp_vs	P15941-2	Isoform 2 of Mucin-1 OS=Homo sapiens GN=MUC1 [MUC1] ...	2434	0.0
sp_vs	P15941-4	Isoform 4 of Mucin-1 OS=Homo sapiens GN=MUC1 [MUC1] ...	2415	0.0
sp_vs	P15941-5	Isoform 5 of Mucin-1 OS=Homo sapiens GN=MUC1 [MUC1] ...	2132	0.0
sp_vs	P15941-8	Isoform 8 of Mucin-1 OS=Homo sapiens GN=MUC1 [MUC1] ...	405	e-112
sp_vs	P15941-7	Isoform 7 of Mucin-1 OS=Homo sapiens GN=MUC1 [MUC1] ...	363	e-100
sp_vs	P15941-6	Isoform 6 of Mucin-1 OS=Homo sapiens GN=MUC1 [MUC1] ...	275	2e-73
sp	Q02817	MUC2_HUMAN Mucin-2 precursor (MUC-2) (Intestinal mucin...	264	4e-70
sp	P98088	MUC5A_HUMAN Mucin-5AC precursor (MUC-5AC) (Gastric muc...	217	6e-56
sp	Q9UKN1	MUC12_HUMAN Mucin-12 precursor (MUC-12) (Mucin-11) (MU...	209	2e-53
sp	Q685J3	MUC17_HUMAN Mucin-17 precursor (MUC-17) (Small intesti...	198	3e-50
sp_vs	Q685J3-2	Isoform 2 of Mucin-17 OS=Homo sapiens GN=MUC17 [MUC1...	198	3e-50
sp_vs	P15941-10	Isoform 10 of Mucin-1 OS=Homo sapiens GN=MUC1 [MUC1...	190	8e-48
sp	Q6W4X9	MUC6_HUMAN Mucin-6 precursor (MUC-6) (Gastric mucin-6)...	186	1e-46

Our protein has UniProt Accession number P15941. Click on it!



web.expasy.org/cgi-bin/blast/blast.pl

ExPASy Bioinformatics Resource Portal

BLAST

Home | Contact

NiceBlast

If results of this search are reported or published, please mention that the computation was performed at the SIB using the BLAST network service. The SIB BLAST network service uses a server developed at SIB and the NCBI BLAST 2 software.

Program: NCBI BLASTP 2.2.17 [Aug-26-2007]
Databases: UniProtKB 32,727,302 sequences; 10,543,978,207 total letters
Query: gil189599lgblAAA60019.1l; 1255 Amino acids Date run: 2013-03-30 17:58:04 UTC+0100

Taxonomic view | HTML view | Printable view

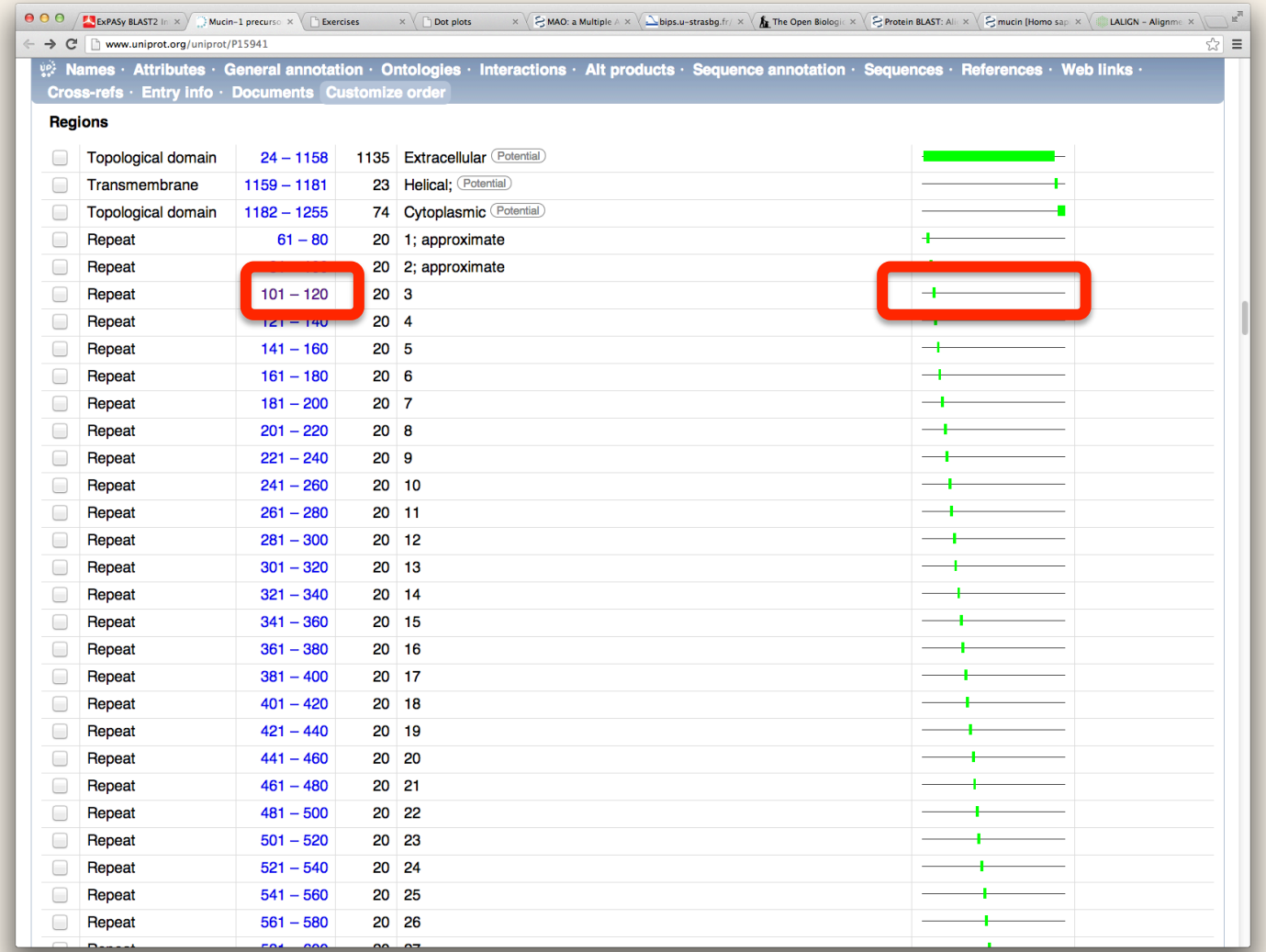
Hit *BLAST* to perform a BLAST search of one of the results with the same parameters

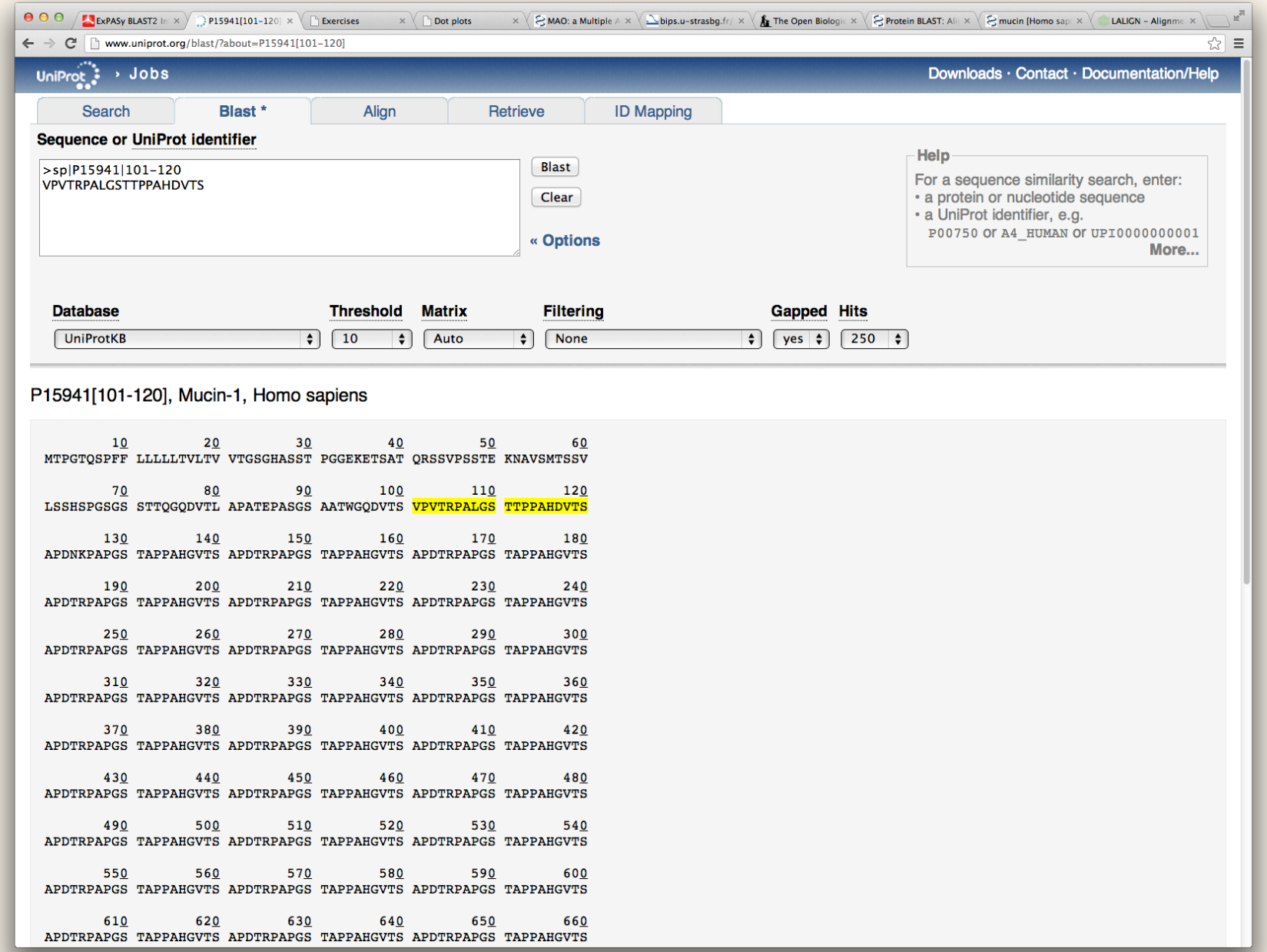
Send selected sequences to Clustal W (multiple alignment) Submit Select up to...

Include query sequence

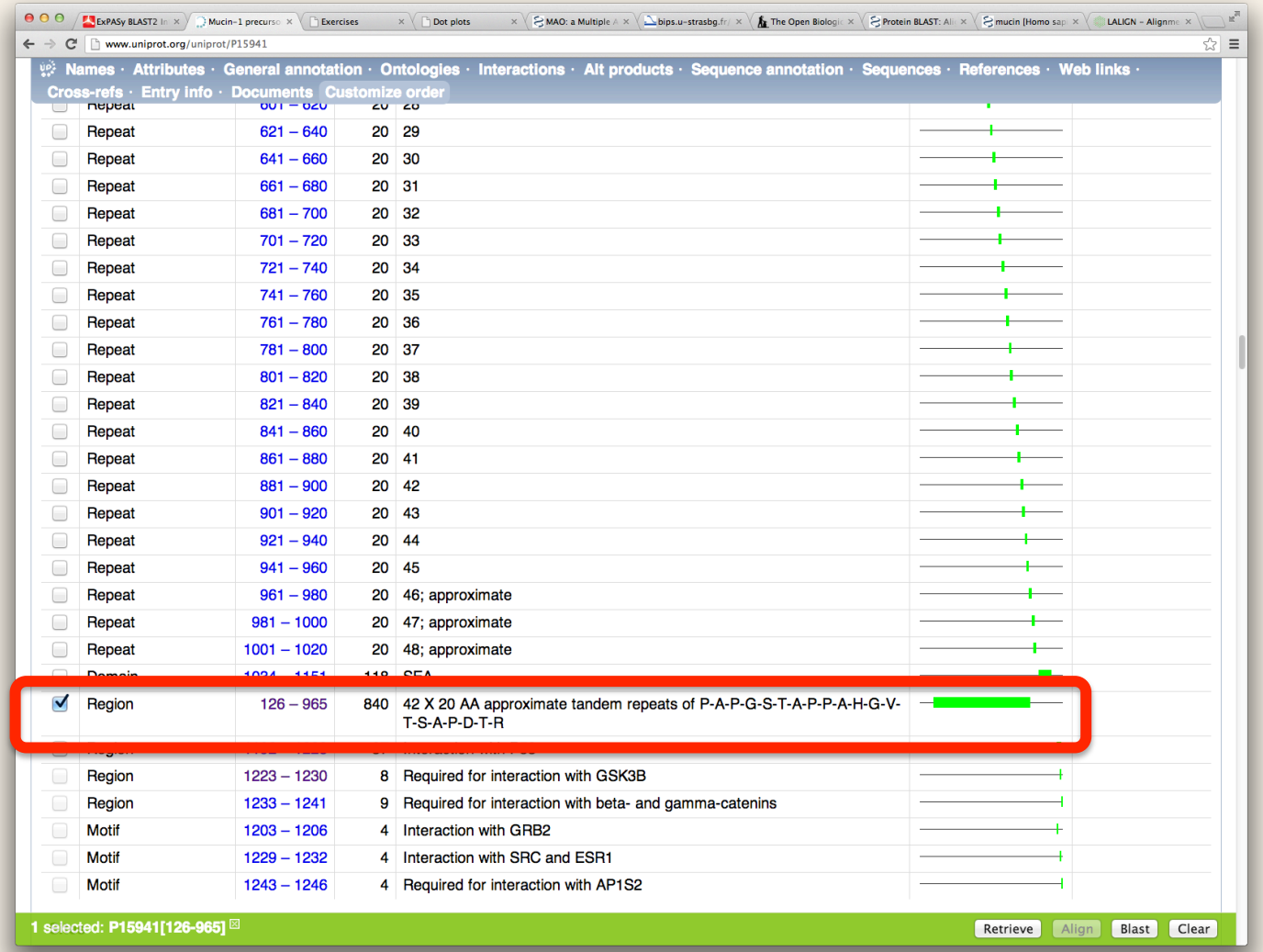
Score	E-value	Accession number	Entry name	Database	Length	Description
		P15941	*BLAST* MUC1_HUMAN	sp	1255 Amino acids	
2441	0.0					**cursor** (MUC-1) (Breast carcinoma-associated antigen DF3) (Cancer antigen 15-3) (CA 15-3) (Carcinoma-associated mucin) (Episialin) (H23AG) (Krebs von den Lungen-6) (KL-6) (PEMT) (Peanut-reactive urinary mucin) (PUM) (Polymorphic epithelial mucin) (PEM) (Tumor-associated epithelial membrane antigen) (EMA) (Tumor-associated mucin) (CD227 antigen) [Contains: Mucin-1 subunit alpha (MUC1-NT) (MUC1-alpha); Mucin-1 subunit beta (MUC1-beta) (MUC1-CT)] [Gene: MUC1 OR PUM] - ***Homo sapiens (Human).***
		P15941-3	*BLAST*	sp_vs	1252 Amino acids	
2437	0.0					**Isoform 3 of Mucin-1 OS=Homo sapiens Mucin-1 precursor** (MUC-1) (Breast carcinoma-associated antigen DF3) (Cancer antigen 15-3) (CA 15-3) (Carcinoma-associated mucin) (Episialin) (H23AG) (Krebs von den Lungen-6) (KL-6) (PEMT) (Peanut-reactive urinary mucin) (PUM) (Polymorphic epithelial mucin) (PEM) (Tumor-associated epithelial membrane antigen) (EMA) (Tumor-associated mucin) (CD227 antigen) [Contains: Mucin-1 subunit alpha (MUC1-NT) (MUC1-alpha); Mucin-1 subunit beta (MUC1-beta) (MUC1-CT)] [Gene: MUC1 OR PUM] - ***Homo sapiens (Human).***
		P15941-2	*BLAST*	sp_vs	1264 Amino acids	
2434	0.0					**Isoform 2 of Mucin-1 OS=Homo sapiens Mucin-1 precursor** (MUC-1) (Breast carcinoma-associated antigen DF3) (Cancer antigen 15-3) (CA 15-3) (Carcinoma-associated mucin) (Episialin) (H23AG) (Krebs von den Lungen-6) (KL-6) (PEMT) (Peanut-reactive urinary mucin) (PUM) (Polymorphic epithelial mucin) (PEM) (Tumor-associated epithelial membrane antigen) (EMA) (Tumor-associated mucin) (CD227 antigen) [Contains: Mucin-1 subunit alpha (MUC1-NT) (MUC1-alpha); Mucin-1 subunit beta (MUC1-beta) (MUC1-CT)] [Gene: MUC1 OR PUM] - ***Homo sapiens (Human).***
		P15941-4	*BLAST*	sp_vs	1243 Amino acids	
2415	0.0					**Isoform 4 of Mucin-1 OS=Homo sapiens Mucin-1 precursor** (MUC-1) (Breast carcinoma-associated antigen DF3) (Cancer antigen 15-3) (CA 15-3) (Carcinoma-associated mucin) (Episialin) (H23AG) (Krebs von den Lungen-6) (KL-6) (PEMT) (Peanut-reactive urinary mucin) (PUM) (Polymorphic epithelial mucin) (PEM) (Tumor-associated epithelial membrane antigen) (EMA) (Tumor-associated mucin) (CD227 antigen) [Contains: Mucin-1 subunit alpha (MUC1-NT) (MUC1-alpha); Mucin-1 subunit beta (MUC1-beta) (MUC1-CT)] [Gene: MUC1 OR PUM] - ***Homo sapiens (Human).***
		P15941-5	*BLAST*	sp_vs	1087 Amino acids	
2132	0.0					**Isoform 5 of Mucin-1 OS=Homo sapiens Mucin-1 precursor** (MUC-1) (Breast carcinoma-associated antigen DF3) (Cancer antigen 15-3) (CA 15-3) (Carcinoma-associated mucin) (Episialin) (H23AG) (Krebs von den Lungen-6) (KL-6) (PEMT) (Peanut-reactive urinary mucin) (PUM) (Polymorphic epithelial mucin) (PEM) (Tumor-associated epithelial membrane antigen) (EMA) (Tumor-associated mucin) (CD227 antigen) [Contains: Mucin-1 subunit alpha (MUC1-NT) (MUC1-alpha); Mucin-1 subunit beta (MUC1-beta) (MUC1-CT)] [Gene: MUC1 OR PUM] - ***Homo sapiens (Human).***

Clicking on any repeat or region highlights it on the sequence

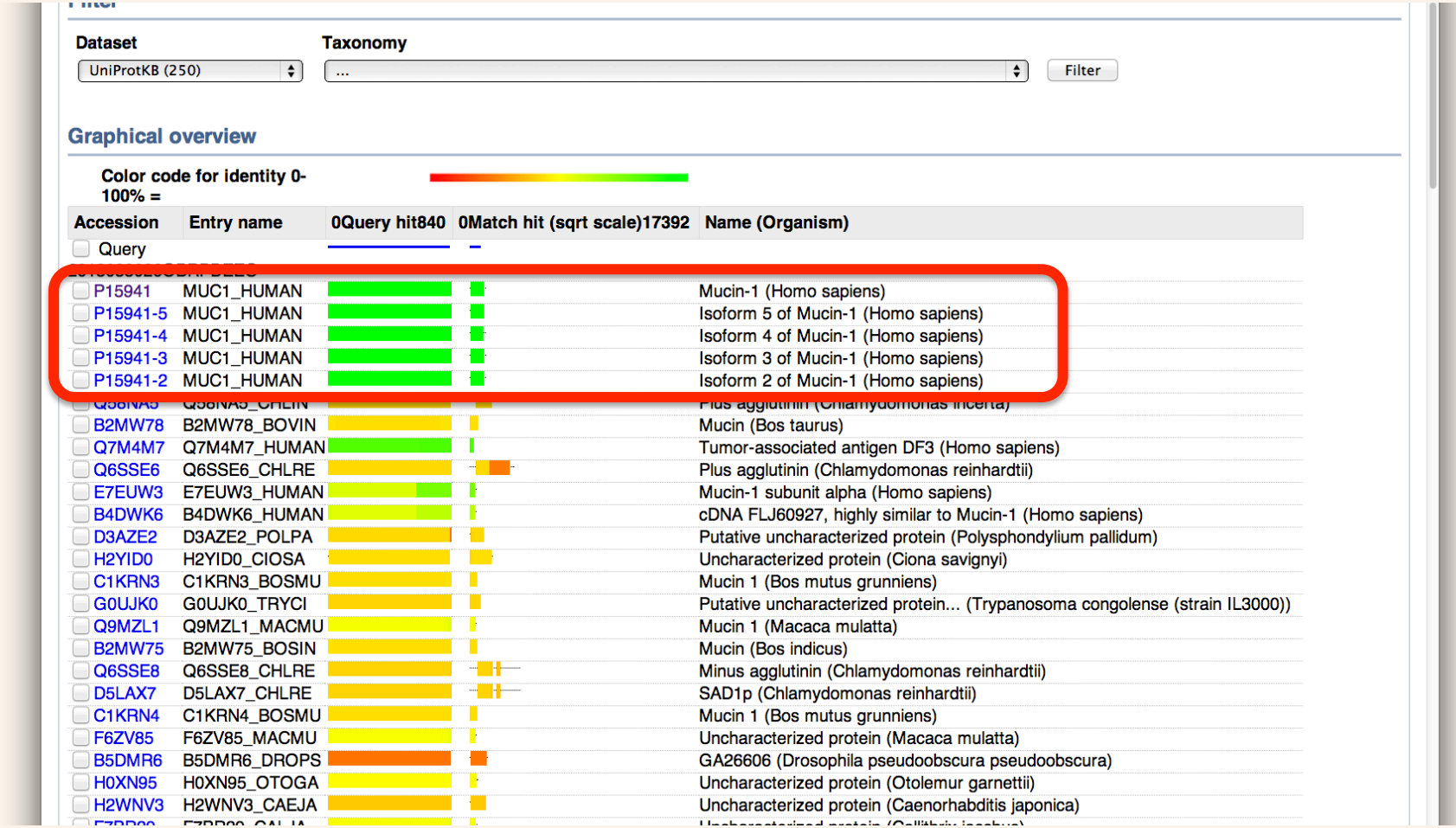



ExPASy BLAST2 In
P15941[101-120]
Exercises
Dot plots
MAO: a Multiple A
bips.u-strasbg.fr/
The Open Biologic
Protein BLAST: Ali
mucin [Homo sap
LALIGN – Alignme
www.uniprot.org/blast/?about=P15941[101-120]
UniProt
› Jobs
Downloads · Contact · Documentation/Help
Search
Blast *
Align
Retrieve
ID Mapping
Sequence or UniProt identifier
>sp|P15941|101-120
VPVTRPALGSTTPPAHDVTS
Blast
Clear
« Options
Help
For a sequence similarity search, enter:
• a protein or nucleotide sequence
• a UniProt identifier, e.g.
P00750 or A4_HUMAN or UPI0000000001
More...
Database
Threshold
Matrix
Filtering
Gapped
Hits
UniProtKB
10
Auto
None
yes
250
P15941[101-120], Mucin-1, Homo sapiens
10 20 30 40 50 60
MTPGTQSPFF LLLLLTVLTV VTGSGHASST PGGEKETSAT QRSSVPSSTE KNAVSMTSSV
70 80 90 100 110 120
LSSHSPGSGS STTQGQDVTL APATEPASGS AATWGQDVTS VPVTRPALGS TTPPAHDVTS
130 140 150 160 170 180
APDNKPAPGS TAPPAHGVTS APDTRPAPGS TAPPAHGVTS APDTRPAPGS TAPPAHGVTS
190 200 210 220 230 240
APDTRPAPGS TAPPAHGVTS APDTRPAPGS TAPPAHGVTS APDTRPAPGS TAPPAHGVTS
250 260 270 280 290 300
APDTRPAPGS TAPPAHGVTS APDTRPAPGS TAPPAHGVTS APDTRPAPGS TAPPAHGVTS
310 320 330 340 350 360
APDTRPAPGS TAPPAHGVTS APDTRPAPGS TAPPAHGVTS APDTRPAPGS TAPPAHGVTS
370 380 390 400 410 420
APDTRPAPGS TAPPAHGVTS APDTRPAPGS TAPPAHGVTS APDTRPAPGS TAPPAHGVTS
430 440 450 460 470 480
APDTRPAPGS TAPPAHGVTS APDTRPAPGS TAPPAHGVTS APDTRPAPGS TAPPAHGVTS
490 500 510 520 530 540
APDTRPAPGS TAPPAHGVTS APDTRPAPGS TAPPAHGVTS APDTRPAPGS TAPPAHGVTS
550 560 570 580 590 600
APDTRPAPGS TAPPAHGVTS APDTRPAPGS TAPPAHGVTS APDTRPAPGS TAPPAHGVTS
610 620 630 640 650 660
APDTRPAPGS TAPPAHGVTS APDTRPAPGS TAPPAHGVTS APDTRPAPGS TAPPAHGVTS

Scrolling down shows summary on repeats. Any region can be sent to blast by selecting it! If more than one region selected they can be aligned!

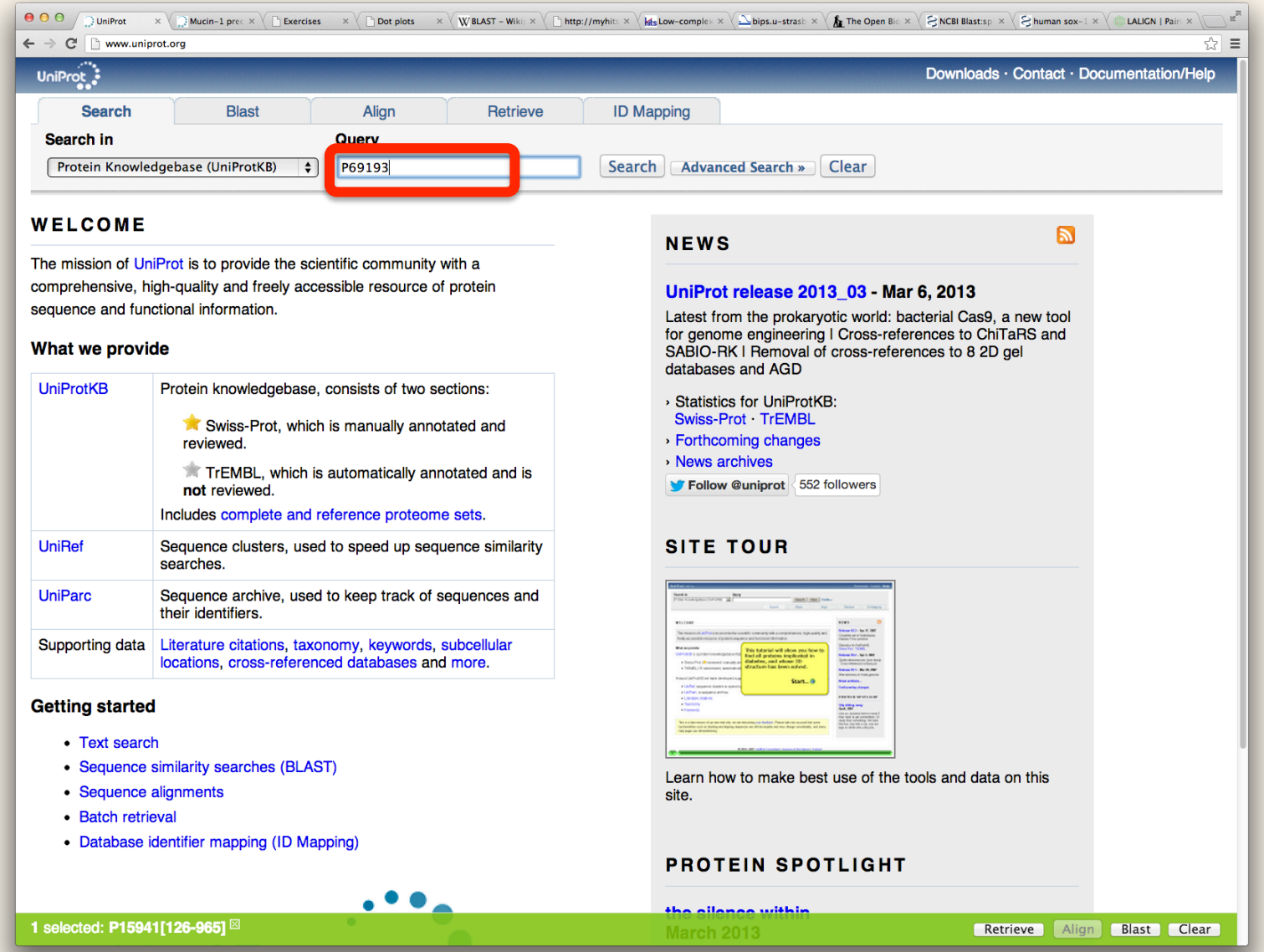


For example, from the blast results it can be seen that this region of 42 tandem repeats is present in isoforms 2, 3, 4, and 5 of human mucin, but not in other reported in the UniProt entry isoforms 6, 7, 8, 9, 10, without looking at the description of each isoform

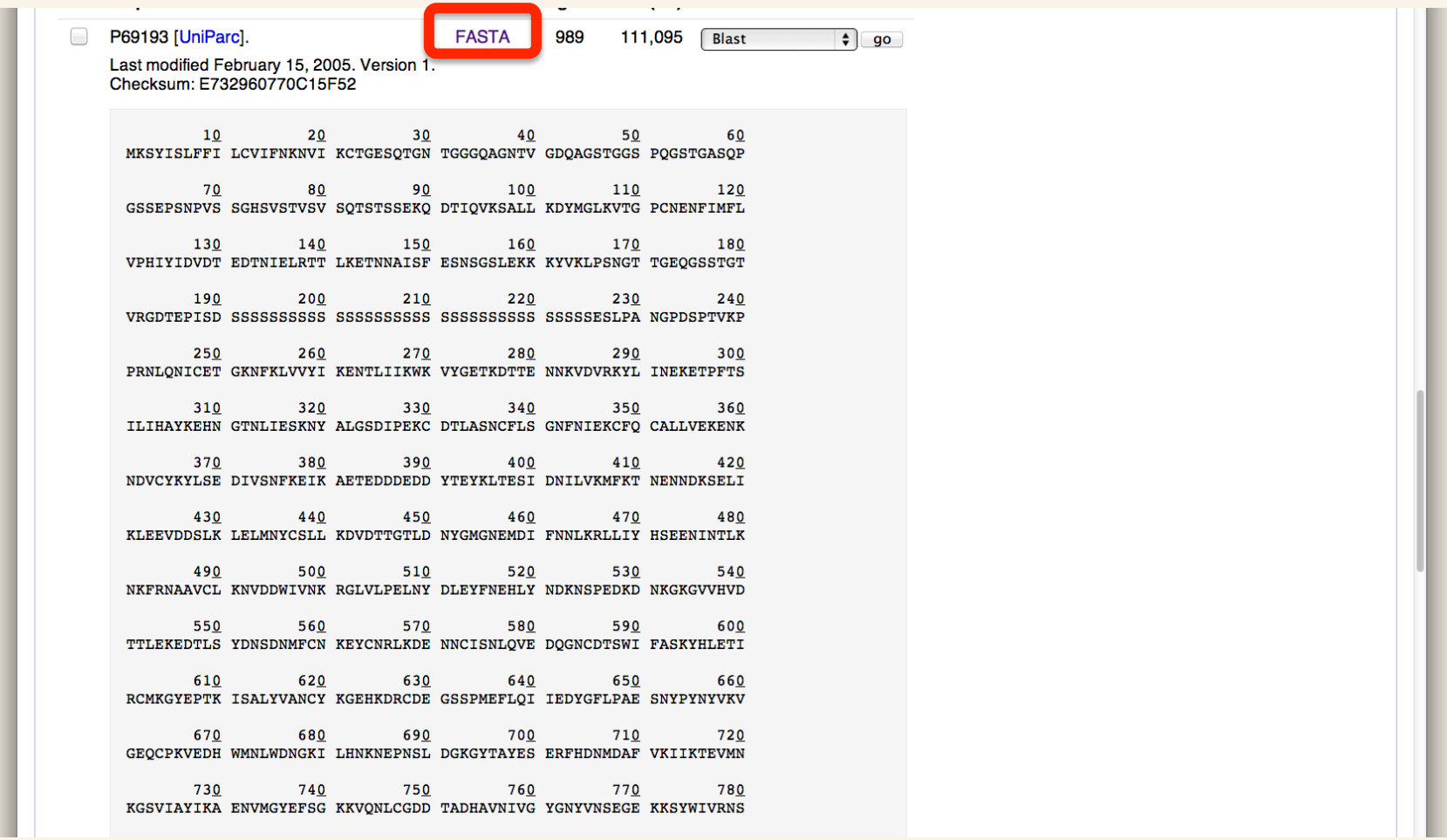


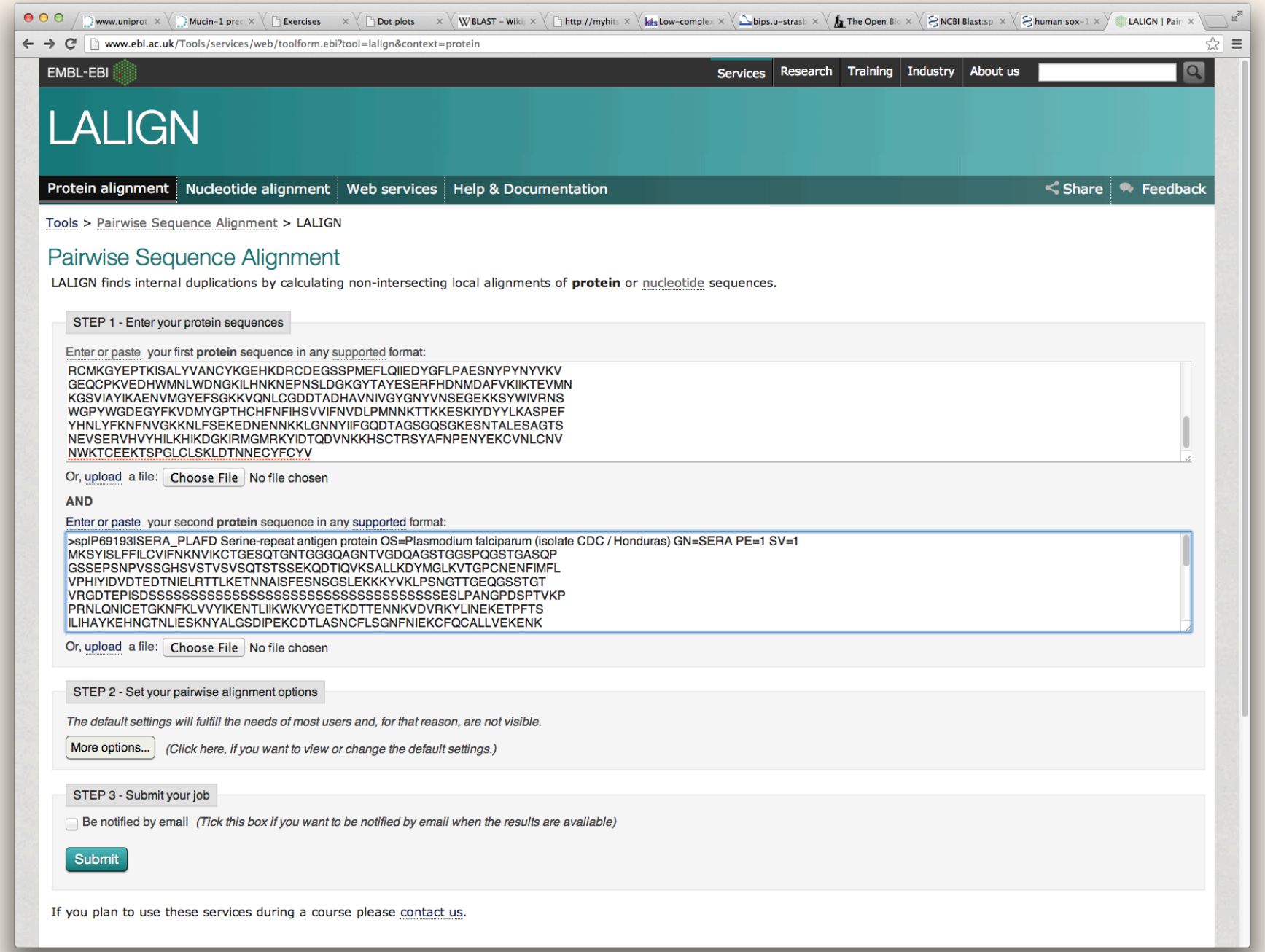
Let's generate a dotplot, using ALIGN for P69193 comparing it against itself

uniprot.org P69193

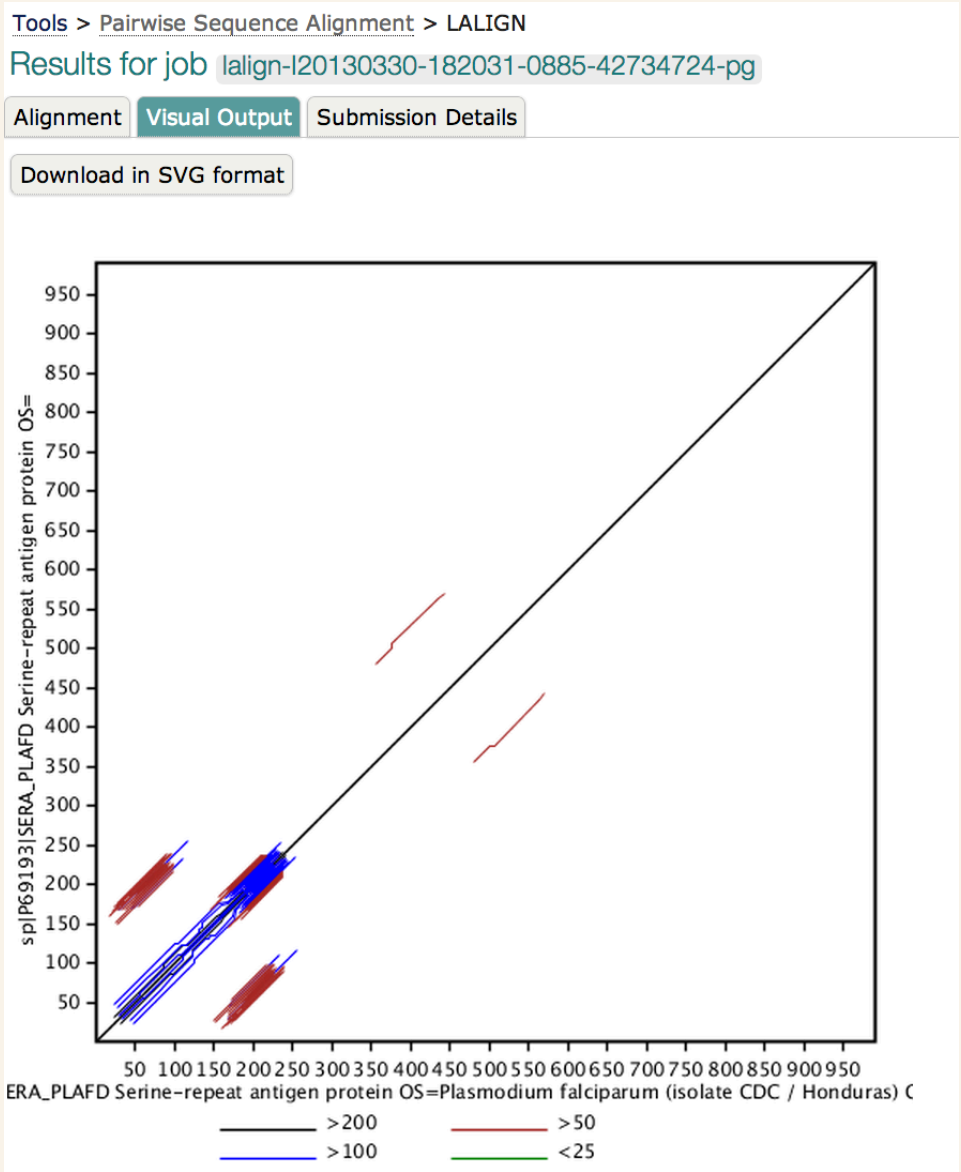


Get FASTA sequence, copy it and paste in ALIGN




www.ebi.ac.uk/Tools/services/web/toolform.ebi?tool=lalign&context=protein
EMBL-EBI
Services
Research
Training
Industry
About us
LALIGN
Protein alignment
Nucleotide alignment
Web services
Help & Documentation
Share
Feedback
Tools > Pairwise Sequence Alignment > LALIGN
Pairwise Sequence Alignment
LALIGN finds internal duplications by calculating non-intersecting local alignments of protein or nucleotide sequences.
STEP 1 - Enter your protein sequences
Enter or paste your first protein sequence in any supported format:
RCMKGYEPTKISALYVANCYKGEHKDRCDEGSSPMEFLQIIEDYGFLPAESNYPYNYVKV
GEQCPKVEDHWMNLWDNGKILHNKNEPNSLDGKGYTAYESERFHDNMDAFVKIIKTEVMN
KGSVIAYIKAENVMGYEFSGKKVQNLCGDDTADHAVNIVGYGNYVNSEGEKKSYWIVRNS
WGPYWGDEGYFKVDMYGPTHCHFNFIHSVVIFNVDLPMNNKTTKKESKIYDYYLKASPEF
YHNLYFKNFNVGKKNLFSEKEDNENNKKLGNNYIIFGQDTAGSGQSGKESNTALESAGTS
NEVSERVHVYHILKHIKDGKIRMGMRKYIDTQDVNKKHSCTRSYAFNPENYEKCVNLCNV
NWKTCEEKTSPGLCLSKLDTNNECYFCYV
Or, upload a file: Choose File No file chosen
AND
Enter or paste your second protein sequence in any supported format:
>sp|P69193|SERA_PLAFD Serine-repeat antigen protein OS=Plasmodium falciparum (isolate CDC / Honduras) GN=SERA PE=1 SV=1
MKSYISLFFILCVIFNKNVIKCTGESQTGNTGGGQAGNTVGDQAGSTGGSPQGSTGASQP
GSSEPSNPVSSGHSVSTVSVSQTSTSSEKQDTIQVKSALLKDYMGLKVTGPCNENFIMFL
VPHIYIDVDTEDTNIELRTTLKETNNAISFESNSGSLEKKKYVKLPSNGTTGEQGSSTGT
VRGDTEPISDSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSESLPANGPDSPTVKP
PRNLQNICETGKNFKLVVYIKENTLIIKWKVYGETKDTTENNKVDVRKYLINEKETPFTS
ILIHAYKEHNGTNLIESKNYALGSDIPEKCDTLASNCFLSGNFNIEKCFQCALLVEKENK
Or, upload a file: Choose File No file chosen
STEP 2 - Set your pairwise alignment options
The default settings will fulfill the needs of most users and, for that reason, are not visible.
More options...
(Click here, if you want to view or change the default settings.)
STEP 3 - Submit your job
Be notified by email (Tick this box if you want to be notified by email when the results are available)
Submit
If you plan to use these services during a course please contact us.

Dotplot for P69193 compared to itself



What does it tell us about the protein sequence?

This sequence contains a low-complexity region around position 200!

Dotplots are useful to identify low-complexity regions!

- "**Low-complexity region**" means a region of compositional bias; that is a sequence composed of few kinds of elements:

  homopolymeric runs,
  short-period repeats,
  overrepresention of several residues

P69193

Names and origin

Protein names	*Recommended name:* **Serine-repeat antigen protein** *Alternative name(s):* 111 kDa antigen p126
Gene names	Name:**SERA**
Organism	**Plasmodium falciparum (isolate CDC / Honduras)**

Regions

☐	Region	571 – 989	419	Thiol-protease-like	
☐	Compositional bias	27 – 227	201	Ser-rich	
☐	Compositional bias	191 – 225	35	Poly-Ser	

```
        10         20         30         40         50         60
MKSYISLFFI LCVIFNKNVI KCTGESQTGN TGGGQAGNTV GDQAGSTGGS PQGSTGASQP

        70         80         90        100        110        120
GSSEPSNPVS SGHSVSTVSV SQTSTSSEKQ DTIQVKSALL KDYMGLKVTG PCNENFIMFL

       130        140        150        160        170        180
VPHIYIDVDT EDTNIELRTT LKETNNAISF ESNSGSLEKK KYVKLPSNGT TGEQGSSTGT

       190        200        210        220        230        240
VRGDTEPISD SSSSSSSSSS SSSSSSSSSS SSSSSSSSSS SSSSSESLPA NGPDSPTVKP

       250        260        270        280        290        300
PRNLQNICET GKNFKLVVYI KENTLIIKWK VYGETKDTTE NNKVDVRKYL INEKETPFTS
```

P50553

Names and origin

Protein names	*Recommended name:* **Achaete-scute homolog 1** Short name=ASH-1 Short name=hASH1 *Alternative name(s):* Class A basic helix-loop-helix protein 46 Short name=bHLHa46
Gene names	Name: **ASCL1** Synonyms:ASH1, BHLHA46, HASH1
Organism	**Homo sapiens (Human)** [Reference proteome]

Regions

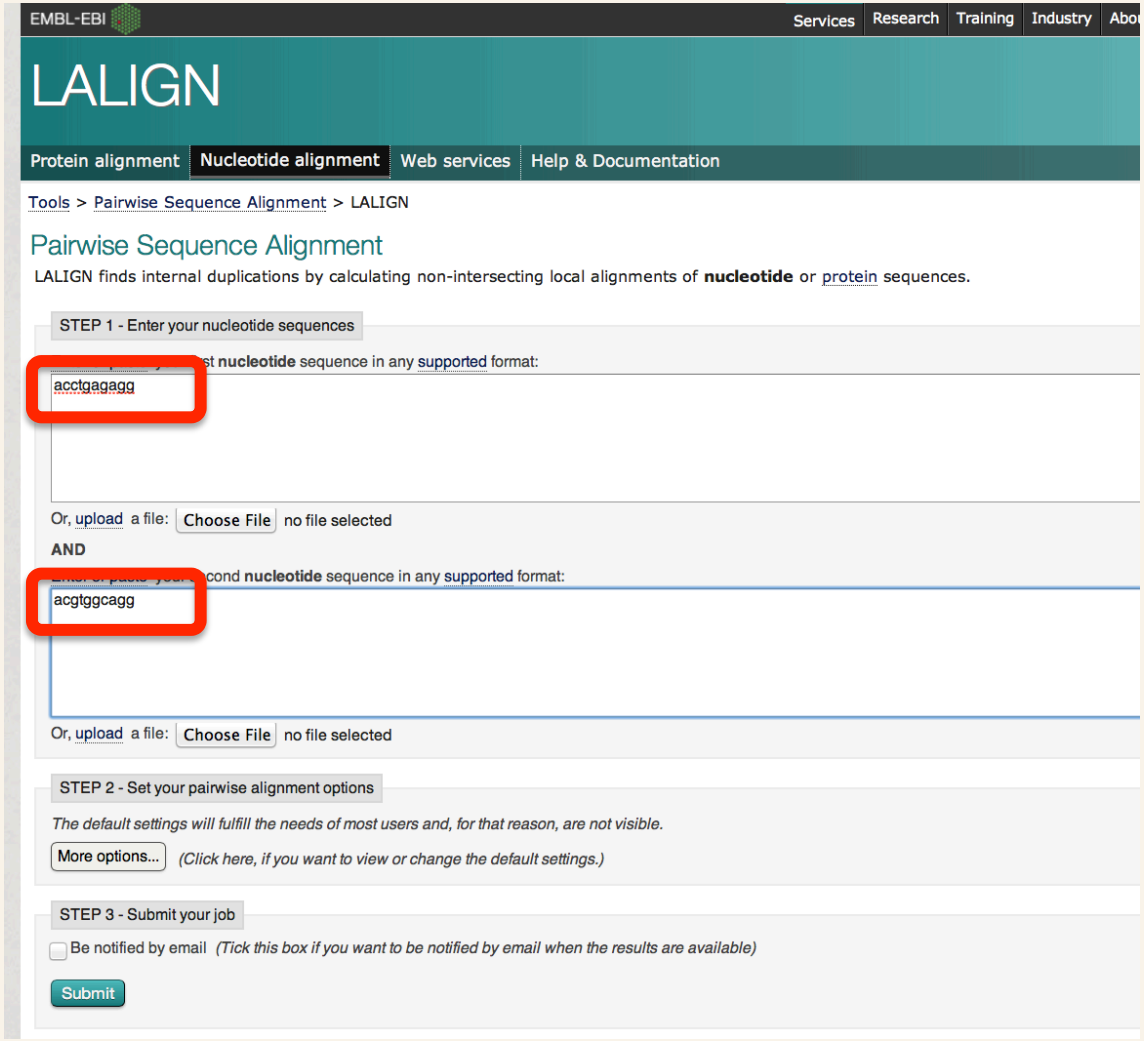
Domain	118 – 170	53	bHLH	
Compositional bias	33 – 47	15	Poly-Ala	
Compositional bias	51 – 62	12	Poly-Gln	

```
        10         20         30         40         50         60
MESSAKMESG GAGQQPQPQP QQPFLPPAAC FLATAAAAAA AAAAAAQSA QQQQQQQQQQ

        70         80         90        100        110        120
QQAPQLRPAA DGQPSGGGHK SAPKQVKRQR SSSPELMRCK RRLNFSGFGY SLPQQQPAAV

       130        140        150        160        170        180
ARRNERERNR VKLVNLGFAT LREHVPNGAA NKKMSKVETL RSAVEYIRAL QQLLDEHDAV

       190        200        210        220        230
SAAFQAGVLS PTISPNYSND LNSMAGSPVS SYSSDEGSYD PLSPEEQELL DFTNWF
```

Let's try to align two nucleotide sequences with LALIGN which implements a modified Smith-Waterman algorithm of 1981

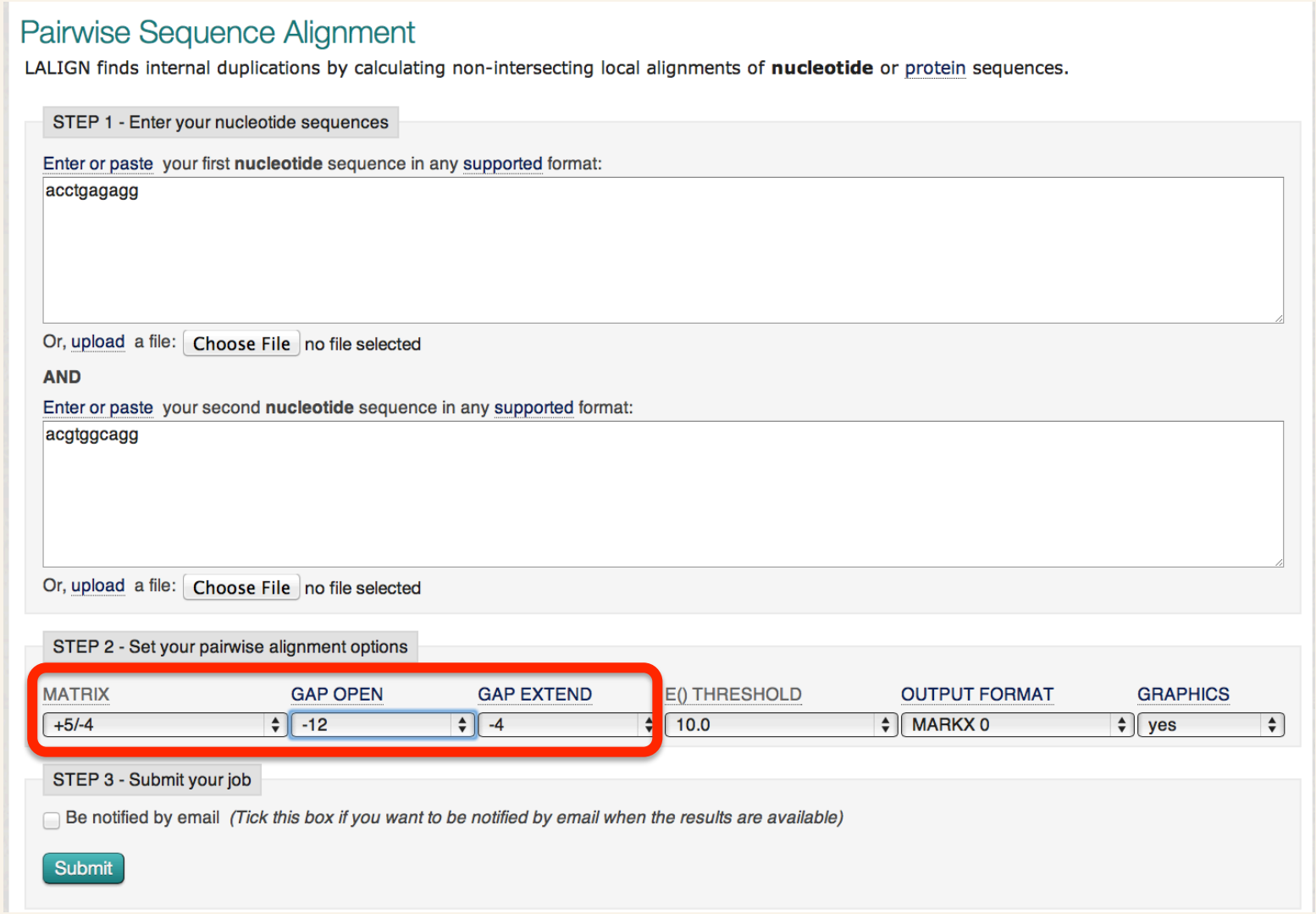
- ✓ Scoring of the alignment
- ✓ Match/mismatch
- ✓ Penalty on a gap opening
- ✓ Penalty on a gap extension
- ✓ E-value

LALIGN at EBI
www.ebi.ac.uk/Tools/psa/lalign/nucleotide.html



```
>>EMBOSS_001                                                    (10 nt)
 Waterman-Eggert score: 23;  9.3 bits; E(1) <  0.15
70.0% identity (70.0% similar) in 10 nt overlap (1-10:1-10)

               10
EMBOSS ACCTGAGAGG
       :: ::  :::
EMBOSS ACGTGGCAGG
               10
```

Let's look at parameters



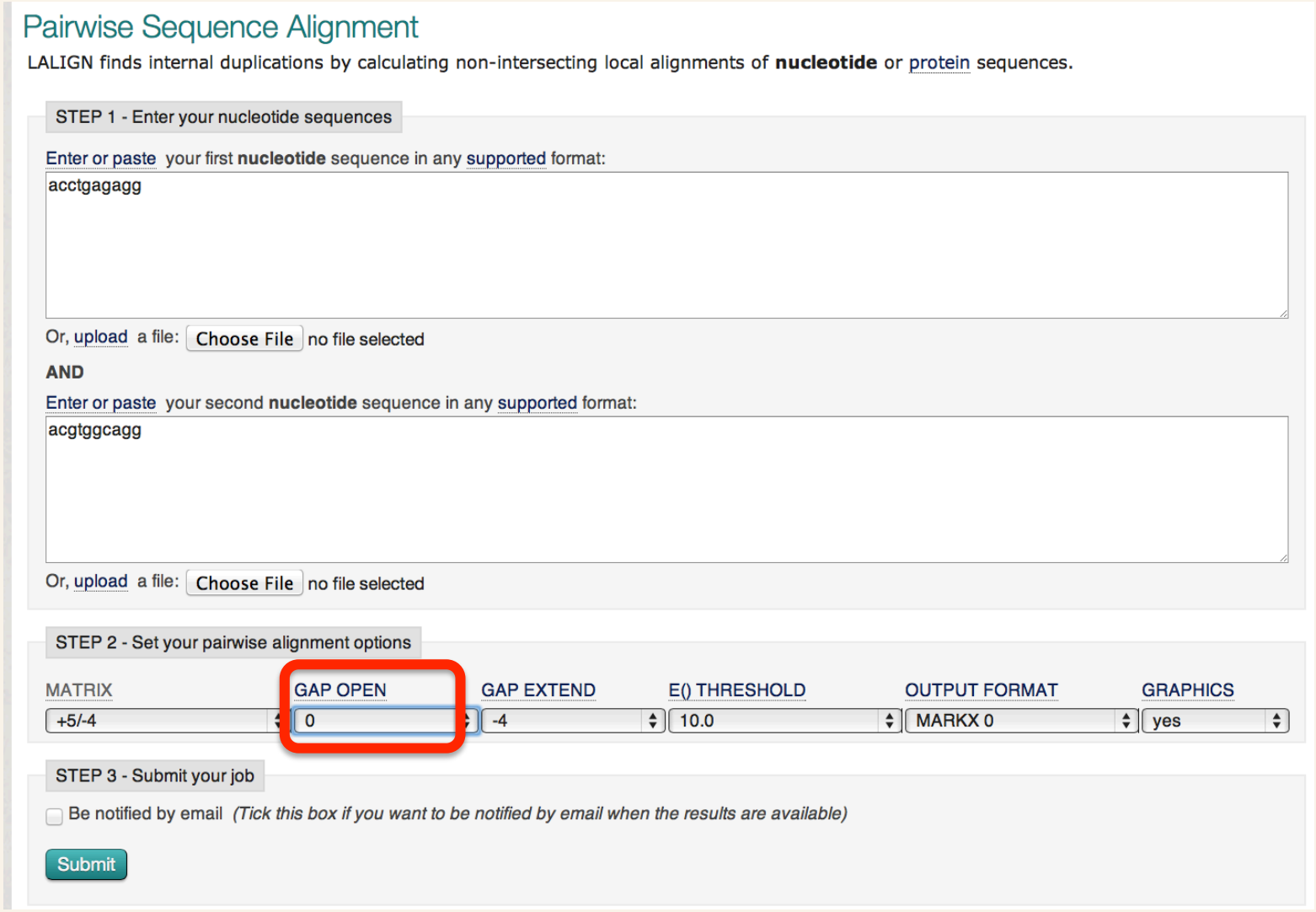
Default parameters: score 5 for match, -4 for mismatch, -12 for gap opening, -4 for a gap extension

```
>>EMBOSS_001                                                    (10 nt)
 Waterman-Eggert score: 23;  9.3 bits; E(1) <  0.15
70.0% identity (70.0% similar) in 10 nt overlap (1-10:1-10)

               10
EMBOSS ACCTGAGAGG
       :: ::  :::
EMBOSS ACGTGGCAGG
               10
```

Score = 5 * {number of matches} – 4 * {number of mismatches} – 12 * {number of gap openings} – 4 * {number of positions gaps extended} →

Score is 23 = 5 * **7** - 4 * **3** -12 * **0** – 4 * **0**

Let's "not punish" the appearance of a gap



```
>>EMBOSS_001                                                    (10 nt)
 Waterman-Eggert score: 28;  10.2 bits; E(1) <  0.08
72.7% identity (72.7% similar) in 11 nt overlap (1-10:1-10)

                10
EMBOSS ACCTGAG-AGG
       :: :: : :::
EMBOSS ACGTG-GCAGG
                10
```

Score = 5 * {number of matches} – 4 * {number of mismatches} – 12 * {number of gap openings} – 4 * {number of positions gaps extended} →

Score is 28 = 5 * **8** - 4 * **3** - 0 * **2** – 4 * **0**

Which alignment is “better”?

```
>>EMBOSS_001                                          (10 nt)
 Waterman-Eggert score: 23;  9.3 bits; E(1) <  0.15
70.0% identity (70.0% similar) in 10 nt overlap (1-10:1-10)

               10
EMBOSS ACCTGAGAGG
       :: ::  :::
EMBOSS ACGTGGCAGG
               10
```

```
>>EMBOSS_001                                          (10 nt)
 Waterman-Eggert score: 28;  10.2 bits; E(1) <  0.08
72.7% identity (72.7% similar) in 11 nt overlap (1-10:1-10)

               10
EMBOSS ACCTGAG-AGG
       :: :: : :::
EMBOSS ACGTG-GCAGG
               10
```

The second one: it has higher score and lower E-value, although it was obtained with changed from default parameters. While both E-values are not much significant as they above 1e-6.

What is E-value?

- The E-value of the alignment is the **expected number of sequences** that give the same **Z-score** or better if the database is probed with a random sequence.

- **Z-score** of the score *S* of the alignment = {*(S – mean)/SD*}.

- **The E-value is not the probability!** If *P* is the probability to obtain by chance an alignment with the same Z-score or better, then *P* and *E* (E-value) are related as *P*= {*1- e^(-E)*}.

- **The E-value depends on the size of the database** and it ranges from 0 to the number of sequences in the databases.

Interpretation of E-value

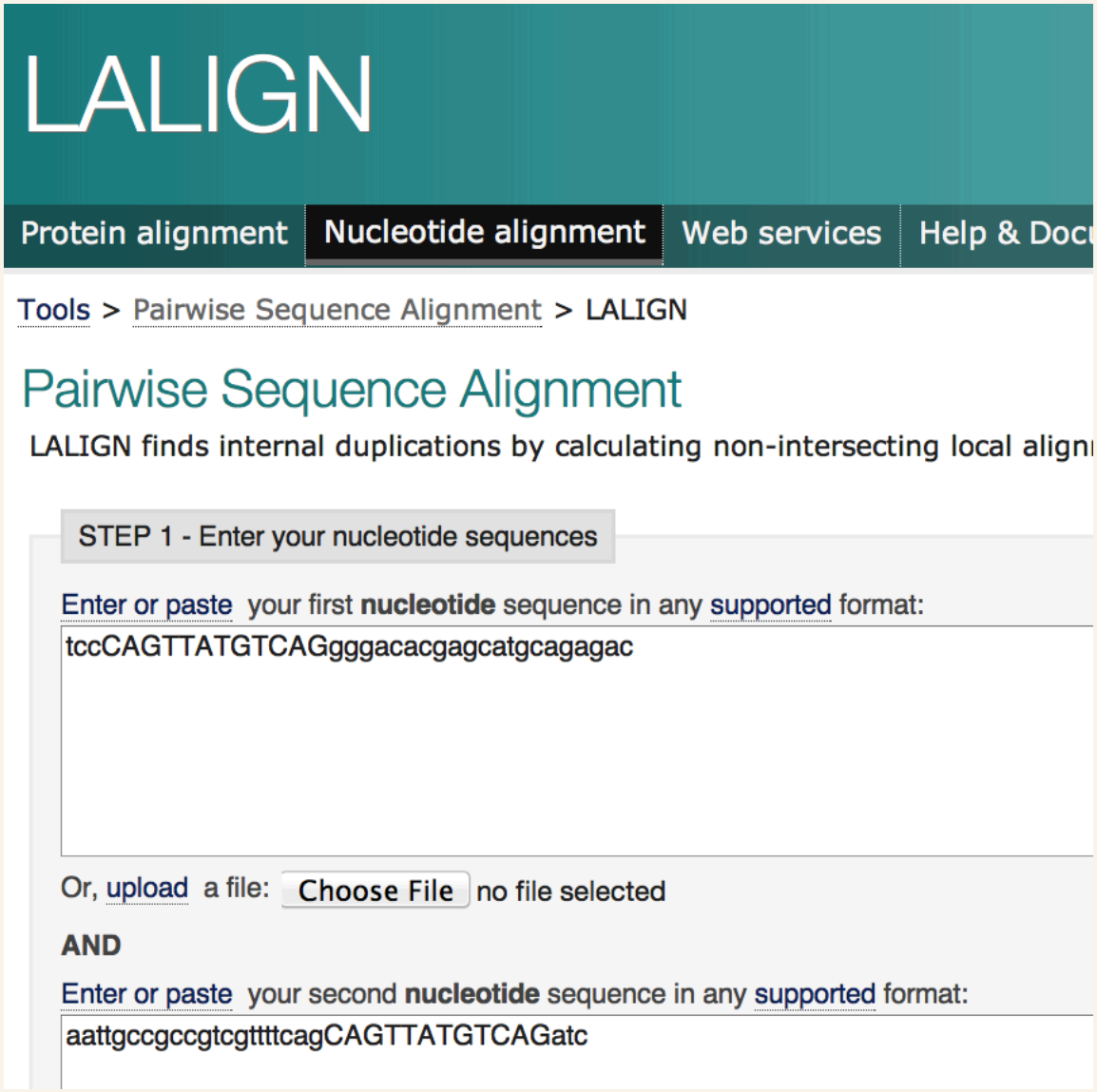
- In LALIGN, E-values have been calculated only with the default parameters on a set of 500 randomly selected sequences. Therefore it is much less meaningful than the E-value reported by BLAST search of a database.
- A rough interpretation of E-value:
  - ✓ E > 1 – This match can be expected by chance
  - ✓ 1 > E > 0.001 - Homology cannot be ruled out
  - ✓ E < 0.001 (1e-3) – Probable homology, while different researches reported different E-values for different algorithms and databases to be significant ranging from 1e-3 to 1e-6
- The E-value is not the only measure of the alignment significance. For proteins (as a lot of research has been done): If two proteins share 25% and above of sequence identity at 150 aa or 40% at 70 aa intervals they might be homologous.

Local vs Global alignment

- Global alignment compares two sequences along the entire length.

- Local alignment searches for the most similar regions in the two sequences.

Let's try longer sequences in LALIGN

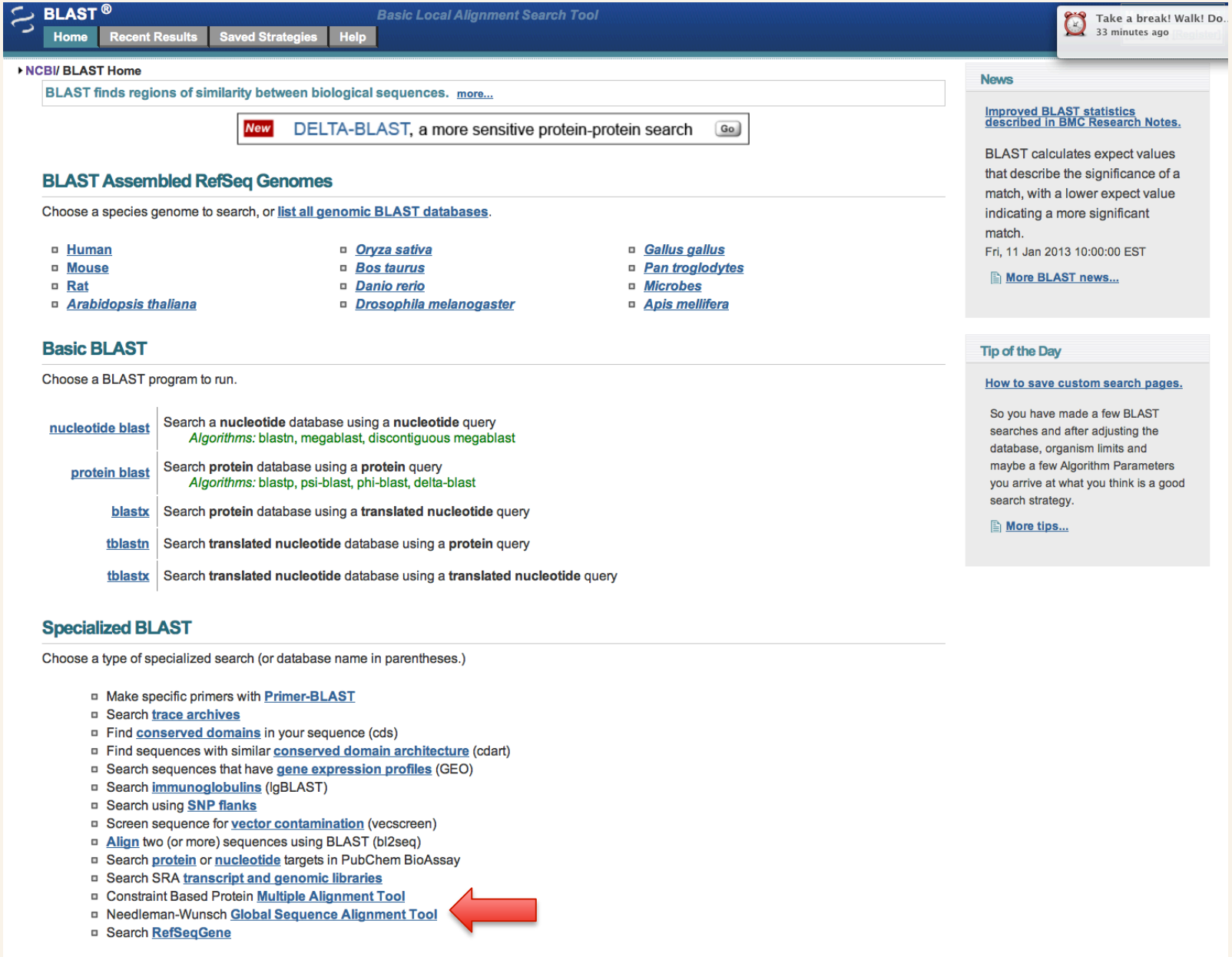


LALIGN result

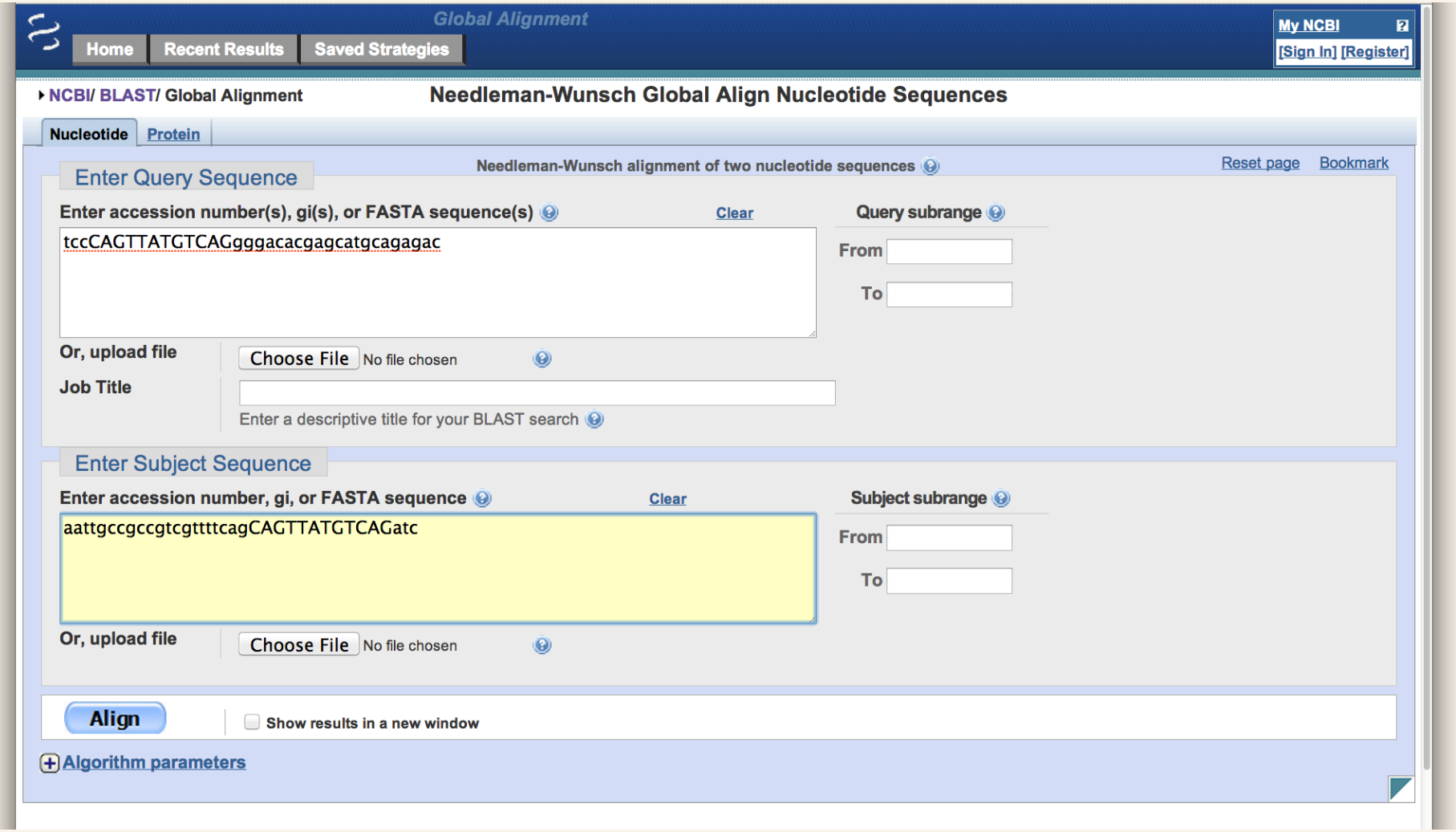
```
>>EMBOSS_001                                                    (36 nt)
 Waterman-Eggert score: 60;  21.0 bits; E(1) <  0.00063
100.0% identity (100.0% similar) in 12 nt overlap (4-15:22-33)

            10
EMBOSS CAGTTATGTCAG
       ::::::::::::
EMBOSS CAGTTATGTCAG
               30
```

Go back to the input page of LALIGN and keep it open.

NCBI BLAST http://blast.ncbi.nlm.nih.gov/Blast.cgi



Needlman-Wunsch global alignment for nucleotide sequences: Let's run it for our two sequences



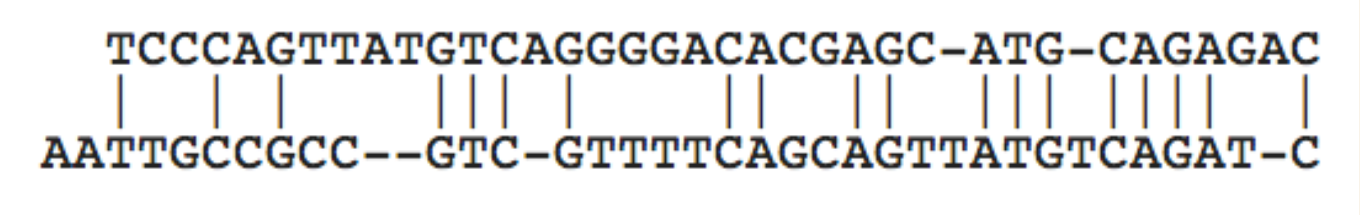
Needlman-Wunsch global alignment

NW Score	Identities	Gaps
-47	19/40(48%)	6/40(15%)

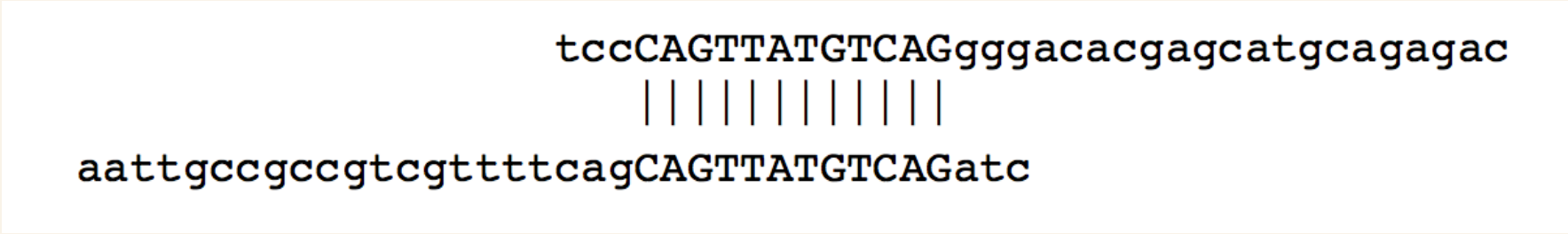
```
Query   1     TCCCAGTTATGTCAGGGGACACGAGC-ATG-CAGAGAC   36
              |   | |     |||| |     ||  ||  ||| ||||  |
Sbjct   1   AATTGCCGCC--GTC-GTTTTCAGCAGTTATGTCAGAT-C   36
```

Local vs Global alignment

- **Global alignment** compares two sequences along the entire length. It is therefore **best for highly similar sequences of approximately the same length**.



- **Local alignment** searches for the most similar regions in the two sequences. It can produce more than one alignment. It is best for sequences that share some degree of similarity or of different lengths. **Best for finding conserved elements**.



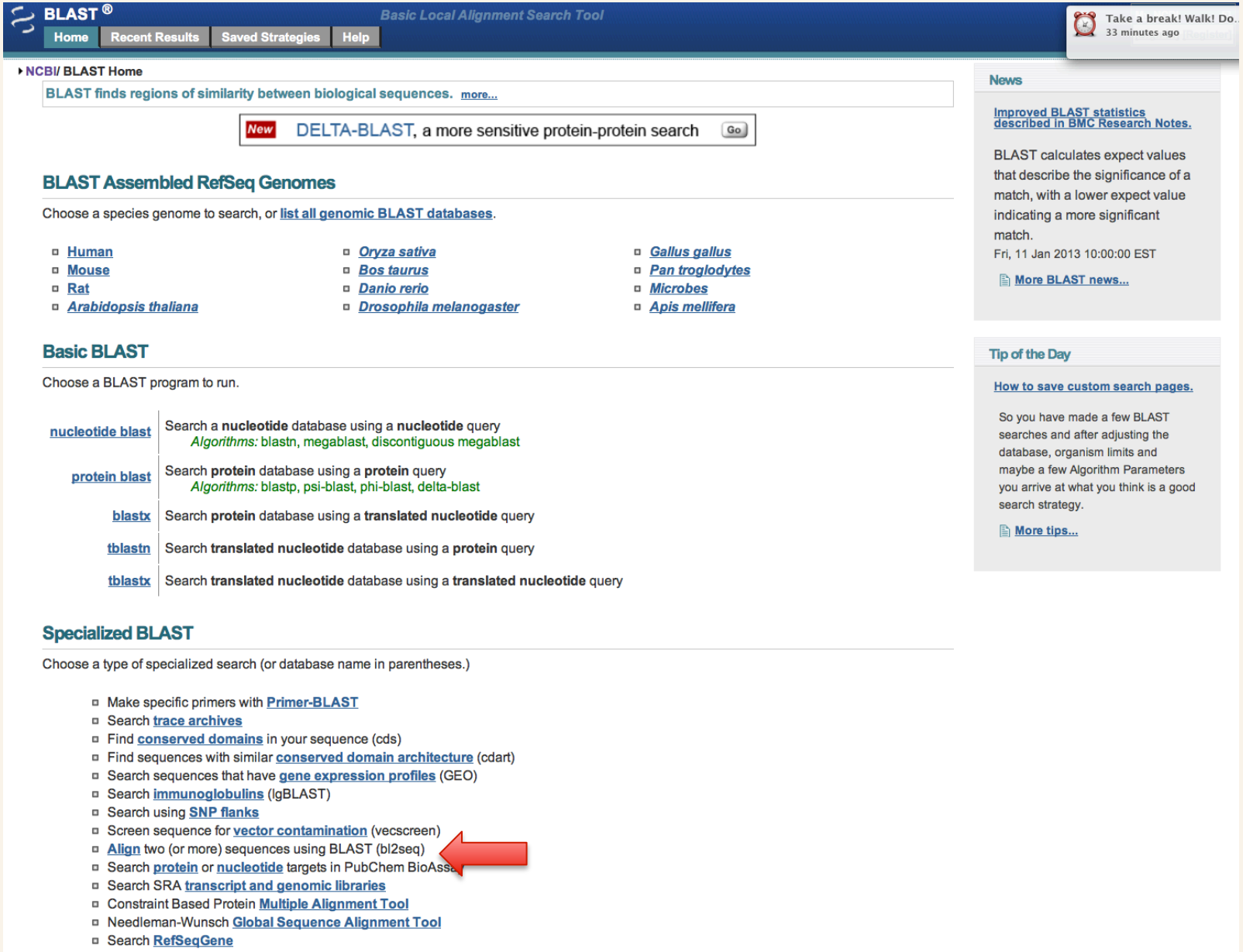
BLAST

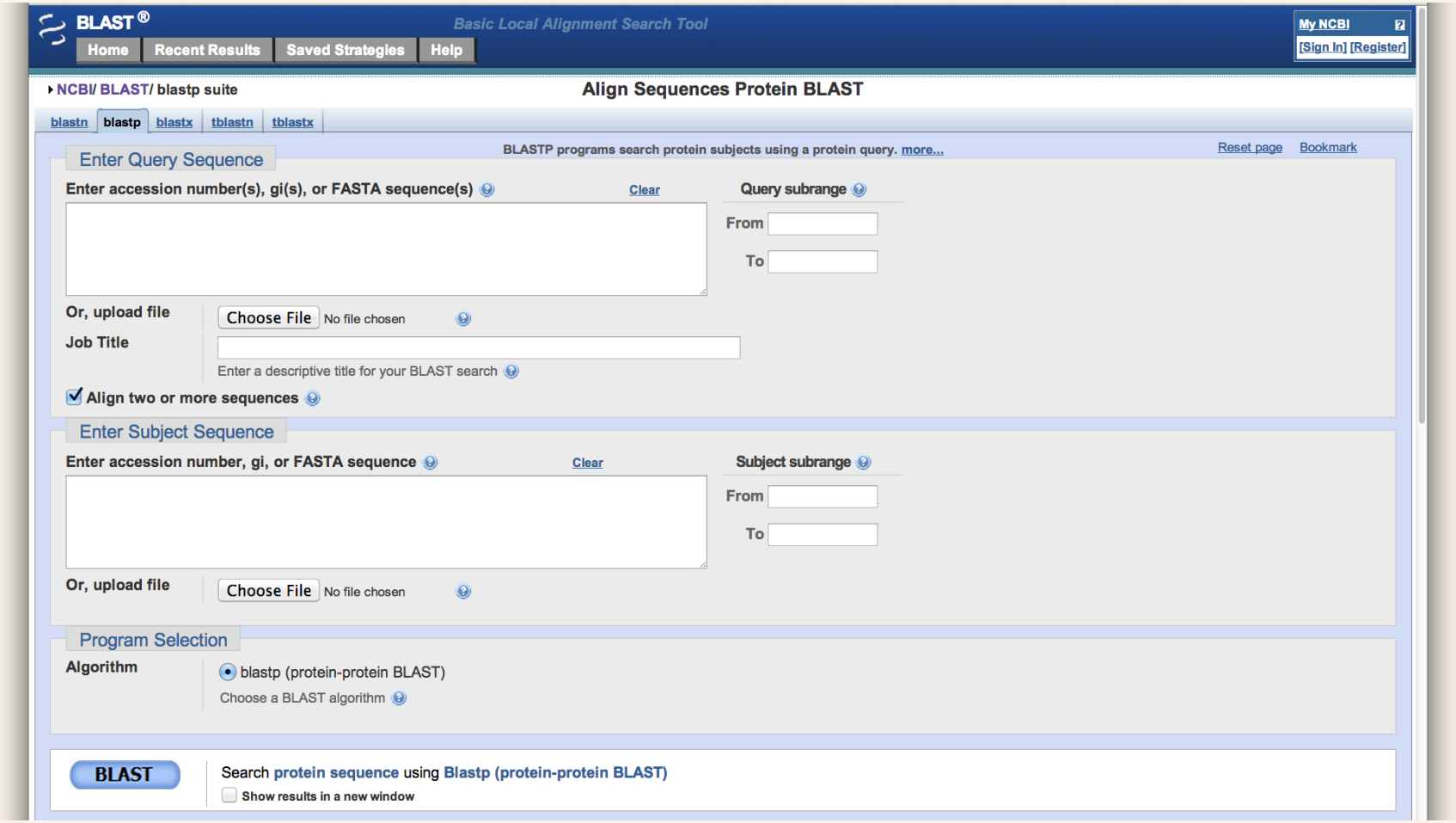
- BLAST algorithms belongs to the class of word or k-tulip methods for local alignment (Altschuls et al., 1991). This parameter, the word size is adjustable.

- It is used to search NCBI databases and UniProt: NCBI BLAST server, Entrez, ExPASy, UniProt web site.

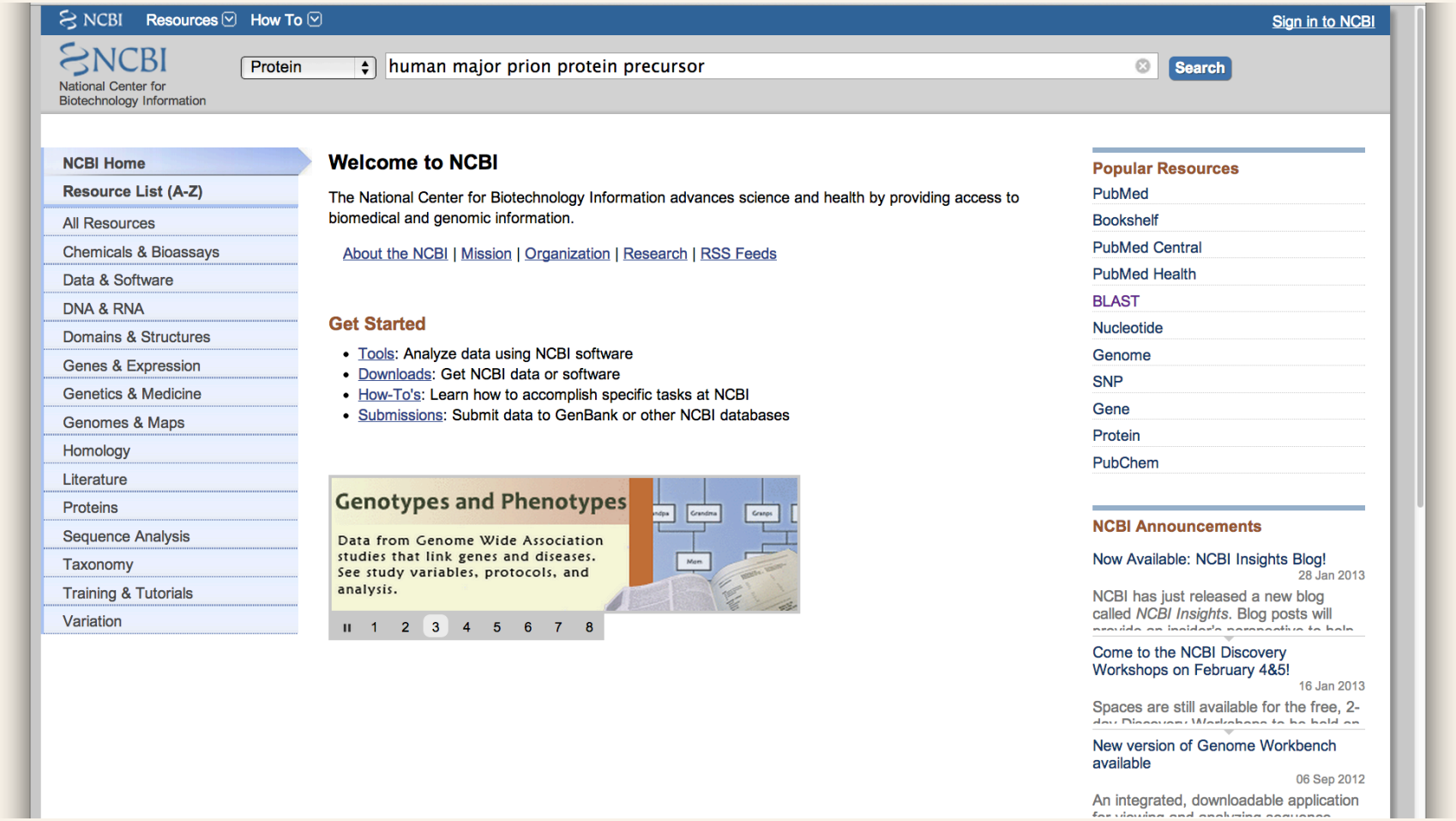
Let's use Bl2seq, the implementation of BLAST for the pairwise alignment, to see the result of masking low-complexity regions and to introduce scoring matrices: PAM, BLOSUM

NCBI BLAST http://blast.ncbi.nlm.nih.gov/Blast.cgi

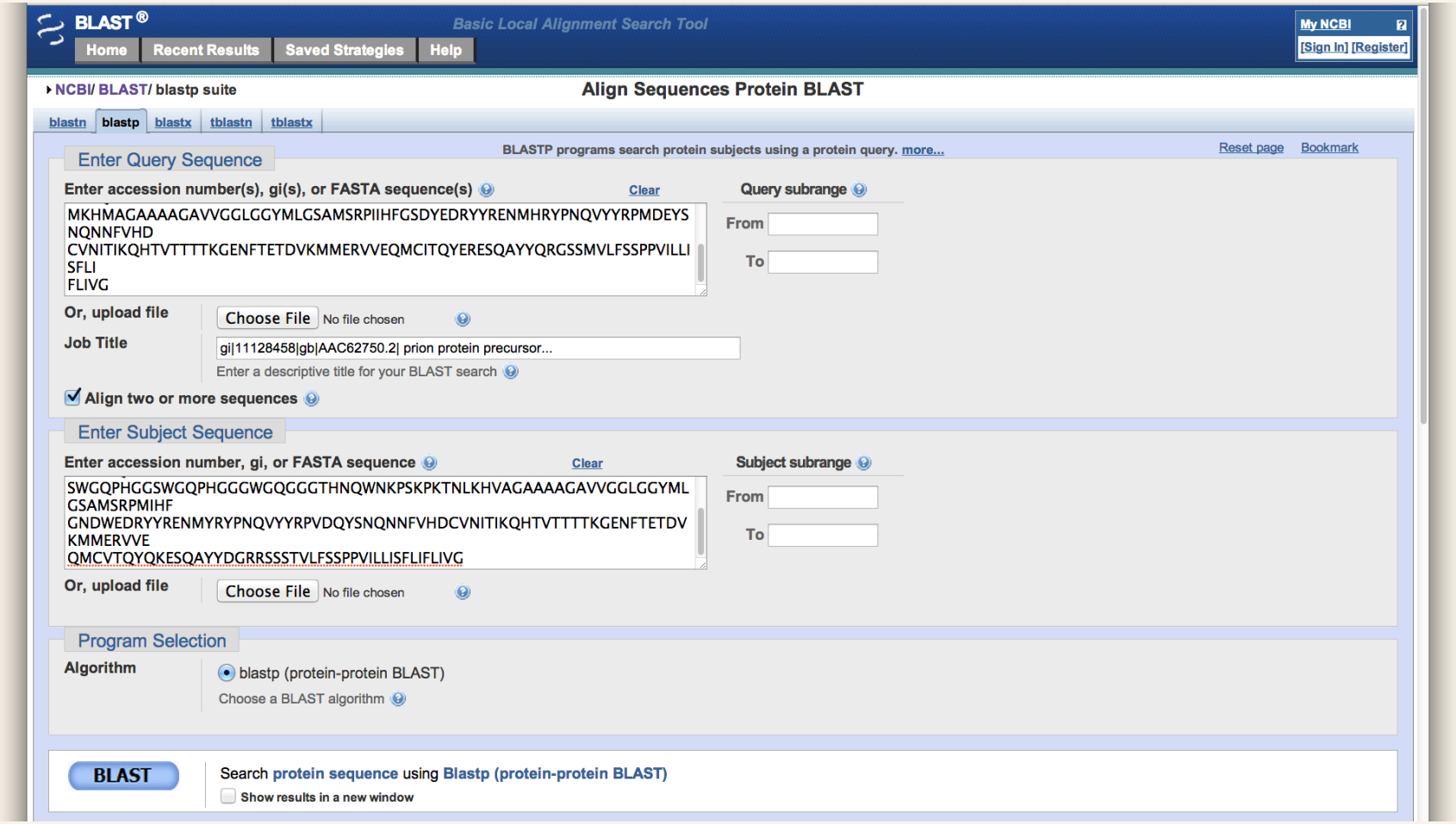



BLAST®
Basic Local Alignment Search Tool
Home
Recent Results
Saved Strategies
Help
My NCBI
[Sign In] [Register]
NCBI/ BLAST/ blastp suite
Align Sequences Protein BLAST
blastn
blastp
blastx
tblastn
tblastx
BLASTP programs search protein subjects using a protein query. more...
Reset page
Bookmark
Enter Query Sequence
Enter accession number(s), gi(s), or FASTA sequence(s)
Clear
Query subrange
From
To
Or, upload file
Choose File
No file chosen
Job Title
Enter a descriptive title for your BLAST search
Align two or more sequences
Enter Subject Sequence
Enter accession number, gi, or FASTA sequence
Clear
Subject subrange
From
To
Or, upload file
Choose File
No file chosen
Program Selection
Algorithm
blastp (protein-protein BLAST)
Choose a BLAST algorithm
BLAST
Search protein sequence using Blastp (protein-protein BLAST)
Show results in a new window

Search for “human major prion protein precursor” in protein NCBI databases



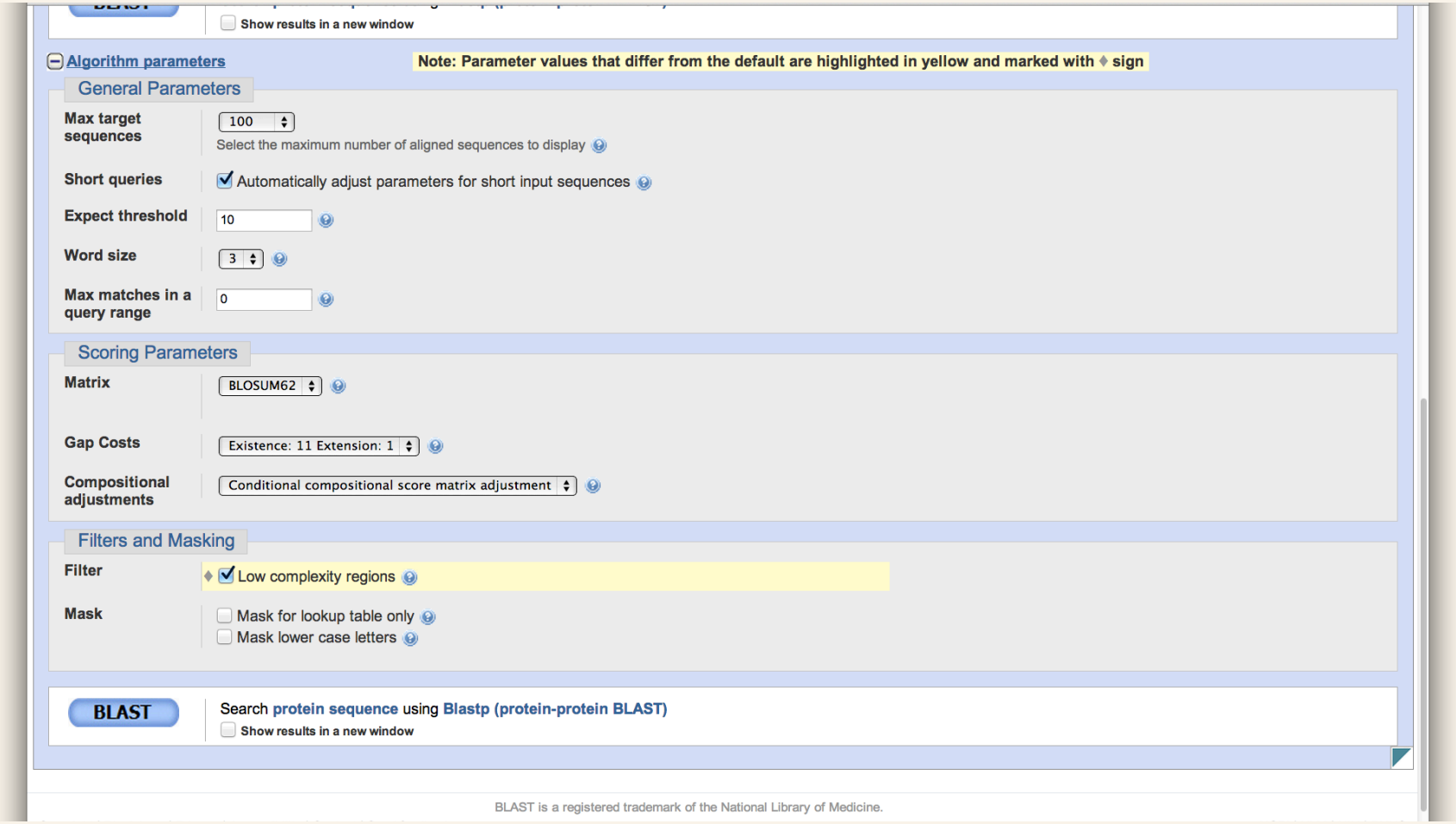
Get FASTA sequence of human and mouse proteins and paste them into bl2seq



Score	Expect	Method	Identities	Positives	Gaps
367 bits(941)	4e-132	Compositional matrix adjust.	227/287(79%)	243/287(84%)	35/287(12%)

```
Query  1    MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNRYPPQGGGGWGQP  60
            MANLG W+L LFV  W+D+GLCKKRPKPGGWNTGGSRYPGQGSPGGNRYPPQ GG WGQP
Sbjct  1    MANLGYWLLALFVTMWTDVGLCKKRPKPGGWNTGGSRYPGQGSPGGNRYPPQ-GGTWGQP  59

Query  61   HGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGG  120
            HGGGWGQPHGG WGQP                                 HGG WGQPHGGG
Sbjct  60   HGGGWGQPHGGSWGQP--------------------------------HGGSWGQPHGGG  87

Query  121  WGQGGGTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHFGSDYEDR  180
            WGQGGGTH+QWNKPSKPKTN+KH+AGAAAAGAVVGGLGGYMLGSAMSRP+IHFG+D+EDR
Sbjct  88   WGQGGGTHNQWNKPSKPKTNLKHVAGAAAAGAVVGGLGGYMLGSAMSRPMIHFGNDWEDR  147

Query  181  YYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVTTTTKGENFTETDVKMMER  240
            YYRENM+RYPNQVYYRP+D+YSNQNNFVHDCVNITIKQHTVTTTTKGENFTETDVKMMER
Sbjct  148  YYRENMYRYPNQVYYRPVDQYSNQNNFVHDCVNITIKQHTVTTTTKGENFTETDVKMMER  207

Query  241  VVEQMCITQYERESQAYY--QRGSSMVLFSSPPVILLISFLIFLIVG  285
            VVEQMC+TQY++ESQAYY  +R SS VLFSSPPVILLISFLIFLIVG
Sbjct  208  VVEQMCVTQYQKESQAYYDGRRSSSTVLFSSPPVILLISFLIFLIVG  254
```

Let's mask low-complexity regions



Score	Expect	Method	Identities	Positives	Gaps
182 bits(462)	6e-60	Compositional matrix adjust.	127/144(88%)	140/144(97%)	2/144(1%)

```
Query   127   THSQWNKPSKPKTNMKHMagaaaagavvgglggymlgsamsRPIIHFGSDYEDRYYRENM   186
              TH+QWNKPSKPKTN+KH+AGAAAAGAVVGGLGGYMLGSAMSRP+IHFG+D+EDRYYRENM
Sbjct   94    THNQWNKPSKPKTNLKHVAGAAAAGAVVGGLGGYMLGSAMSRPMIHFGNDWEDRYYRENM   153

Query   187   HRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHtvttttkgenftetDVKMMERVVEQMC   246
              +RYPNQVYYRP+D+YSNQNNFVHDCVNITIKQHTVTTTTKGENFTETDVKMMERVVEQMC
Sbjct   154   YRYPNQVYYRPVDQYSNQNNFVHDCVNITIKQHTVTTTTKGENFTETDVKMMERVVEQMC   213

Query   247   ITQYERESQAYY--QRGSSMVLFS   268
              +TQY++ESQAYY  +R SS VLFS
Sbjct   214   VTQYQKESQAYYDGRRSSSTVLFS   237
```

Range 2: 1 to 49 Graphics ▼ Next Match ▲ Previous Match ⍋ First Match

Score	Expect	Method	Identities	Positives	Gaps
98.2 bits(243)	1e-28	Compositional matrix adjust.	42/49(86%)	45/49(91%)	0/49(0%)

```
Query   1    MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNRY   49
             MANLG W+L LFV  W+D+GLCKKRPKPGGWNTGGSRYPGQGSPGGNRY
Sbjct   1    MANLGYWLLALFVTMWTDVGLCKKRPKPGGWNTGGSRYPGQGSPGGNRY   49
```

Score	Expect	Method	Identities	Positives	Gaps
367 bits(941)	4e-132	Compositional matrix adjust	227/287(79%)	243/287(84%)	35/287(12%)

```
Query  1    MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNRYPPQGGGGWGQP  60
            MANLG W+L LFV  W+D+GLCKKRPKPGGWNTGGSRYPGQGSPGGNRYPPQ GG WGQP
Sbjct  1    MANLGYWLLALFVTMWTDVGLCKKRPKPGGWNTGGSRYPGQGSPGGNRYPPQ-GGTWGQP  59

Query  61   HGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGG  120
            HGGGWGQPHGG WGQP                                  HGG WGQPHGGG
Sbjct  60   HGGGWGQPHGGSWGQP--------------------------------HGGSWGQPHGGG  87

Query  121  WGQGGGTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHFGSDYEDR  180
            WGQGGGTH+QWNKPSKPKTN+KH+AGAAAAGAVVGGLGGYMLGSAMSRP+IHFG+D+EDR
Sbjct  88   WGQGGGTHNQWNKPSKPKTNLKHVAGAAAAGAVVGGLGGYMLGSAMSRPMIHFGNDWEDR  147

Query  181  YYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVTTTTKGENFTETDVKMMER  240
            YYRENM+RYPNQVYYRP+D+YSNQNNFVHDCVNITIKQHTVTTTTKGENFTETDVKMMER
Sbjct  148  YYRENMYRYPNQVYYRPVDQYSNQNNFVHDCVNITIKQHTVTTTTKGENFTETDVKMMER  207

Query  241  VVEQMCITQYERESQAYY--QRGSSMVLFSSPPVILLISFLIFLIVG  285
            VVEQMC+TQY++ESQAYY  +R SS VLFSSPPVILLISFLIFLIVG
Sbjct  208  VVEQMCVTQYQKESQAYYDGRRSSSTVLFSSPPVILLISFLIFLIVG  254
```

182 bits(462)	6e-60	Compositional matrix adjust	127/144(88%)	140/144(97%)	2/144(1%)

```
Query  127  THSQWNKPSKPKTNMKHMagaaaagavvgglggymlgsamsRPIIHFGSDYEDRYYRENM  186
            TH+QWNKPSKPKTN+KH+AGAAAAGAVVGGLGGYMLGSAMSRP+IHFG+D+EDRYYRENM
Sbjct  94   THNQWNKPSKPKTNLKHVAGAAAAGAVVGGLGGYMLGSAMSRPMIHFGNDWEDRYYRENM  153

Query  187  HRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHtvttttkgenftetDVKMMERVVEQMC  246
            +RYPNQVYYRP+D+YSNQNNFVHDCVNITIKQHTVTTTTKGENFTETDVKMMERVVEQMC
Sbjct  154  YRYPNQVYYRPVDQYSNQNNFVHDCVNITIKQHTVTTTTKGENFTETDVKMMERVVEQMC  213

Query  247  ITQYERESQAYY--QRGSSMVLFS  268
            +TQY++ESQAYY  +R SS VLFS
Sbjct  214  VTQYQKESQAYYDGRRSSSTVLFS  237
```

Range 2: 1 to 49 Graphics ▼ Next Match ▲ Previous Match ◬ First Match

Score	Expect	Method	Identities	Positives	Gaps
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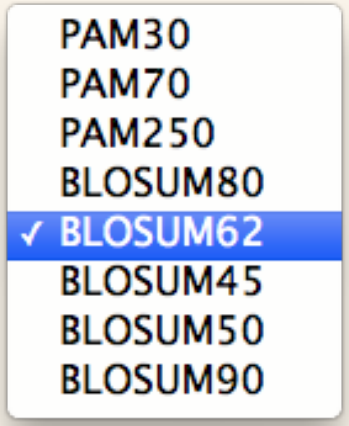
```
Query  1    MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNRY  49
            MANLG W+L LFV  W+D+GLCKKRPKPGGWNTGGSRYPGQGSPGGNRY
Sbjct  1    MANLGYWLLALFVTMWTDVGLCKKRPKPGGWNTGGSRYPGQGSPGGNRY  49
```



Protein similarity scoring matrices

- **PAM** (point accepted mutations) matrices (Dayhoff et al., 1978) depict amino acid substitution (1572 changes) patterns in 71 groups of closely related (sequence identity > 85%) proteins.
- Major assumptions:
  - Substitutions are independent!
  - All positions are equally mutable!
  - In 1978 most of known proteins were small and globular!
  - Speed of evolution and forces are constant!
- PAM1 corresponds to 1 aa change per 100 aa (1% divergence).
- PAM100 – 100 changes per 100 aa; made from PAM1
- PAM250 – 250 changes per 100 aa; made from PAM1

All PAM and BLOSUM matrices are log odds

$$S_{ij} = \log \frac{p_{ij}}{p_i p_j}$$

- S_{ij} is the log odd ratio of two probabilities: the probability that two residue types, i and j, are aligned by evolutionary descent and the probability that they are aligned by chance
- p_{ij} are the frequencies that residue i and j are observed to align in sequences known to be related.
- p_i and p_j are the frequencies of occurrence of residue i and j in all protein sequences (their abundance; or background probability)

Positive values mean that the substitution is observed to occur more often than would by chance, negative - the substitution is not observed to occur frequently and may arise more often than not by chance

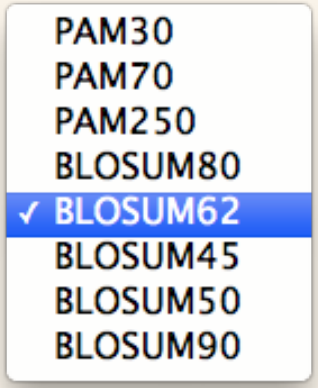
PAM 100 Matrix

```
#
# This matrix was produced by "pam" Version 1.0.7 [13-Aug-03]
#
# PAM 100 substitution matrix, scale = ln(2)/2 = 0.346574
#
# Expected score = -1.99, Entropy = 1.18 bits
#
# Lowest score = -9, Highest score = 12
#
   A  R  N  D  C  Q  E  G  H  I  L  K  M  F  P  S  T  W  Y  V  B  Z  X  *
A  4 -3 -1 -1 -3 -2  0  1 -3 -2 -3 -3 -2 -5  1  1  1 -7 -4  0 -1 -1 -1 -9
R -3  7 -2 -4 -5  1 -3 -5  1 -3 -5  2 -1 -6 -1 -1 -3  1 -6 -4 -3 -1 -2 -9
N -1 -2  5  3 -5 -1  1 -1  2 -3 -4  1 -4 -5 -2  1  0 -5 -2 -3  4  0 -1 -9
D -1 -4  3  5 -7  0  4 -1 -1 -4 -6 -1 -5 -8 -3 -1 -2 -9 -6 -4  4  3 -2 -9
C -3 -5 -5 -7  9 -8 -8 -5 -4 -3 -8 -8 -7 -7 -4 -1 -4 -9 -1 -3 -6 -8 -5 -9
Q -2  1 -1  0 -8  6  2 -3  3 -4 -2  0 -2 -7 -1 -2 -2 -7 -6 -3  0  5 -2 -9
E  0 -3  1  4 -8  2  5 -1 -1 -3 -5 -1 -4 -8 -2 -1 -2 -9 -5 -3  3  4 -2 -9
G  1 -5 -1 -1 -5 -3 -1  5 -4 -5 -6 -3 -4 -6 -2  0 -2 -9 -7 -3 -1 -2 -2 -9
H -3  1  2 -1 -4  3 -1 -4  7 -4 -3 -2 -4 -3 -1 -2 -3 -4 -1 -3  1  1 -2 -9
I -2 -3 -3 -4 -3 -4 -3 -5 -4  6  1 -3  1  0 -4 -3  0 -7 -3  3 -3 -3 -2 -9
L -3 -5 -4 -6 -8 -2 -5 -6 -3  1  6 -4  3  0 -4 -4 -3 -3 -3  0 -5 -4 -3 -9
K -3  2  1 -1 -8  0 -1 -3 -2 -3 -4  5  0 -7 -3 -1 -1 -6 -6 -4  0 -1 -2 -9
M -2 -1 -4 -5 -7 -2 -4 -4 -4  1  3  0  9 -1 -4 -3 -1 -6 -5  1 -4 -2 -2 -9
F -5 -6 -5 -8 -7 -7 -8 -6 -3  0  0 -7 -1  8 -6 -4 -5 -1  4 -3 -6 -7 -4 -9
P  1 -1 -2 -3 -4 -1 -2 -2 -1 -4 -4 -3 -4 -6  7  0 -1 -7 -7 -3 -3 -1 -2 -9
S  1 -1  1 -1 -1 -2 -1  0 -2 -3 -4 -1 -3 -4  0  4  2 -3 -4 -2  0 -2 -1 -9
T  1 -3  0 -2 -4 -2 -2 -2 -3  0 -3 -1 -1 -5 -1  2  5 -7 -4  0 -1 -2 -1 -9
W -7  1 -5 -9 -9 -7 -9 -9 -4 -7 -3 -6 -6 -1 -7 -3 -4 12 -2 -9 -6 -8 -6 -9
Y -4 -6 -2 -6 -1 -6 -5 -7 -1 -3 -3 -6 -5  4 -7 -4 -4 -2  9 -4 -4 -6 -4 -9
V  0 -4 -3 -4 -3 -3 -3 -3 -3  3  0 -4  1 -3 -3 -2  0 -9 -4  5 -4 -3 -2 -9
B -1 -3  4  4 -6  0  3 -1  1 -3 -5  0 -4 -6 -3  0 -1 -6 -4 -4  4  2 -2 -9
Z -1 -1  0  3 -8  5  4 -2  1 -3 -4 -1 -2 -7 -1 -2 -2 -8 -6 -3  2  5 -2 -9
X -1 -2 -1 -2 -5 -2 -2 -2 -2 -2 -3 -2 -2 -4 -2 -1 -1 -6 -4 -2 -2 -2 -2 -9
* -9 -9 -9 -9 -9 -9 -9 -9 -9 -9 -9 -9 -9 -9 -9 -9 -9 -9 -9 -9 -9 -9 -9  1
```

PAM 250 Matrix

```
#
# This matrix was produced by "pam" Version 1.0.7 [13-Aug-03]
#
# PAM 250 substitution matrix, scale = ln(2)/3 = 0.231049
#
# Expected score = -0.844, Entropy = 0.354 bits
#
# Lowest score = -8, Highest score = 17
#
   A  R  N  D  C  Q  E  G  H  I  L  K  M  F  P  S  T  W  Y  V  B  Z  X  *
A  2 -2  0  0 -2  0  0  1 -1 -1 -2 -1 -1 -3  1  1  1 -6 -3  0  0  0  0 -8
R -2  6  0 -1 -4  1 -1 -3  2 -2 -3  3  0 -4  0  0 -1  2 -4 -2 -1  0 -1 -8
N  0  0  2  2 -4  1  1  0  2 -2 -3  1 -2 -3  0  1  0 -4 -2 -2  2  1  0 -8
D  0 -1  2  4 -5  2  3  1  1 -2 -4  0 -3 -6 -1  0  0 -7 -4 -2  3  3 -1 -8
C -2 -4 -4 -5 12 -5 -5 -3 -3 -2 -6 -5 -5 -4 -3  0 -2 -8  0 -2 -4 -5 -3 -8
Q  0  1  1  2 -5  4  2 -1  3 -2 -2  1 -1 -5  0 -1 -1 -5 -4 -2  1  3 -1 -8
E  0 -1  1  3 -5  2  4  0  1 -2 -3  0 -2 -5 -1  0  0 -7 -4 -2  3  3 -1 -8
G  1 -3  0  1 -3 -1  0  5 -2 -3 -4 -2 -3 -5  0  1  0 -7 -5 -1  0  0 -1 -8
H -1  2  2  1 -3  3  1 -2  6 -2 -2  0 -2 -2  0 -1 -1 -3  0 -2  1  2 -1 -8
I -1 -2 -2 -2 -2 -2 -2 -3 -2  5  2 -2  2  1 -2 -1  0 -5 -1  4 -2 -2 -1 -8
L -2 -3 -3 -4 -6 -2 -3 -4 -2  2  6 -3  4  2 -3 -3 -2 -2 -1  2 -3 -3 -1 -8
K -1  3  1  0 -5  1  0 -2  0 -2 -3  5  0 -5 -1  0  0 -3 -4 -2  1  0 -1 -8
M -1  0 -2 -3 -5 -1 -2 -3 -2  2  4  0  6  0 -2 -2 -1 -4 -2  2 -2 -2 -1 -8
F -3 -4 -3 -6 -4 -5 -5 -5 -2  1  2 -5  0  9 -5 -3 -3  0  7 -1 -4 -5 -2 -8
P  1  0  0 -1 -3  0 -1  0  0 -2 -3 -1 -2 -5  6  1  0 -6 -5 -1 -1  0 -1 -8
S  1  0  1  0  0 -1  0  1 -1 -1 -3  0 -2 -3  1  2  1 -2 -3 -1  0  0  0 -8
T  1 -1  0  0 -2 -1  0  0 -1  0 -2  0 -1 -3  0  1  3 -5 -3  0  0 -1  0 -8
W -6  2 -4 -7 -8 -5 -7 -7 -3 -5 -2 -3 -4  0 -6 -2 -5 17  0 -6 -5 -6 -4 -8
Y -3 -4 -2 -4  0 -4 -4 -5  0 -1 -1 -4 -2  7 -5 -3 -3  0 10 -2 -3 -4 -2 -8
V  0 -2 -2 -2 -2 -2 -2 -1 -2  4  2 -2  2 -1 -1 -1  0 -6 -2  4 -2 -2 -1 -8
B  0 -1  2  3 -4  1  3  0  1 -2 -3  1 -2 -4 -1  0  0 -5 -3 -2  3  2 -1 -8
Z  0  0  1  3 -5  3  3  0  2 -2 -3  0 -2 -5  0  0 -1 -6 -4 -2  2  3 -1 -8
X  0 -1  0 -1 -3 -1 -1 -1 -1 -1 -1 -1 -1 -2 -1  0  0 -4 -2 -1 -1 -1 -1 -8
* -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8  1
```

http://www.bioinformatics.nl/tools/pam.html



Protein similarity scoring matrices

- **BLOSUM** (blocks substitution matrices) (henikoff & Henikoff, 1992) depict amino acid substitution in 2,000 blocks (corresponding to structural or functional motifs) in 500 groups of related proteins.
- Same major assumptions as for PAM matrices:
  - Substitutions are independent!
  - All positions are equally mutable!
  - Speed of evolution and forces are constant!
- In contrast to PAM matrices BLOSUM matrices are not derived but calculated directly.
- BLOSUM**62** represent the conservation level of sequences used to derive the matrix (seq. identity >62%)
- BLOSUM**90** ~ 90%, BLOSUM**45** ~ 45%, etc.

BLOSUM62 – the most often used matrix

	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W	
C	9																				C
S	-1	4																			S
T	-1	1	5																		T
P	-3	-1	-1	7																	P
A	0	1	0	-1	4																A
G	-3	0	-2	-2	0	6															G
N	-3	1	0	-2	-2	0	6														N
D	-3	0	-1	-1	-2	-1	1	6													D
E	-4	0	-1	-1	-1	-2	0	2	5												E
Q	-3	0	-1	-1	-1	-2	0	0	2	5											Q
H	-3	-1	-2	-2	-2	-2	1	-1	0	0	8										H
R	-3	-1	-1	-2	-1	-2	0	-2	0	1	0	5									R
K	-3	0	-1	-1	-1	-2	0	-1	1	1	-1	2	5								K
M	-1	-1	-1	-2	-1	-3	-2	-3	-2	0	-2	-1	-1	5							M
I	-1	-2	-1	-3	-1	-4	-3	-3	-3	-3	-3	-3	-3	1	4						I
L	-1	-2	-1	-3	-1	-4	-3	-4	-3	-2	-3	-2	-2	2	2	4					L
V	-1	-2	0	-2	0	-3	-3	-3	-2	-2	-3	-3	-2	1	3	1	4				V
F	-2	-2	-2	-4	-2	-3	-3	-3	-3	-3	-1	-3	-3	0	0	0	-1	6			F
Y	-2	-2	-2	-3	-2	-3	-2	-3	-2	-1	2	-2	-2	-1	-1	-1	-1	3	7		Y
W	-2	-3	-2	-4	-3	-2	-4	-4	-3	-2	-2	-3	-3	-1	-3	-2	-3	1	2	11	W
	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W	

N D E Q

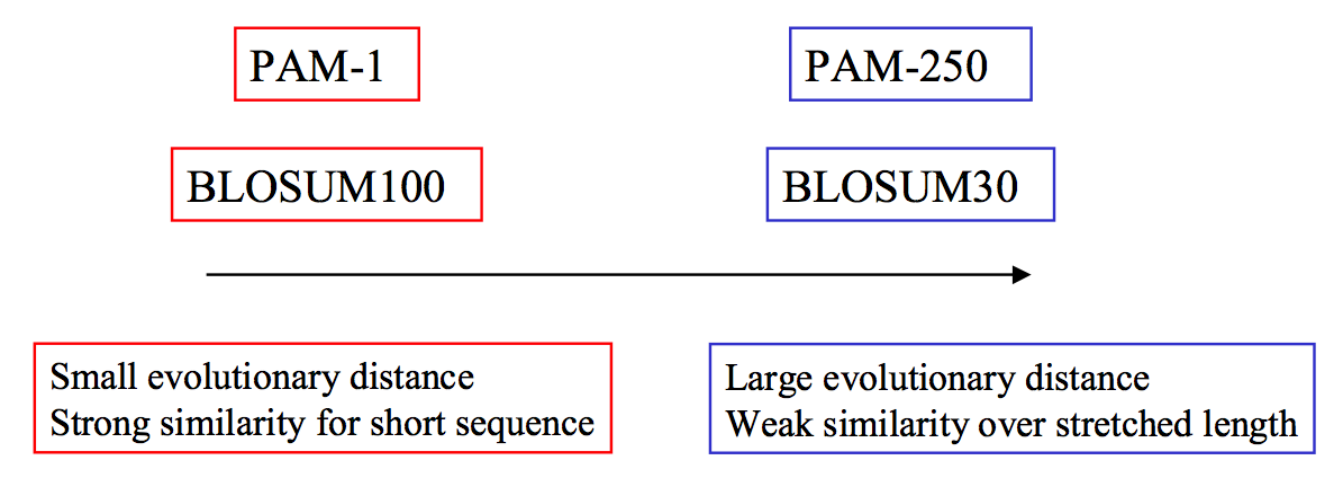
N T W Q

Score 6-1-3+5 = 7

PAM30
PAM70
PAM250
BLOSUM80
✓ BLOSUM62
BLOSUM45
BLOSUM50
BLOSUM90

What matrices should be used when?

Wheeler, 2003



- PAM40 & BLOSUM90 – Short highly significant alignments (70-90% similarity)
- PAM160 (~BLOSUM62) - Detecting members of a protein family (50-60%)
- BLOSUM80 (~PAM120) – Detecting members of a protein family (50-60%)
- **BLOSUM62 (~PAM160)** – Effective in finding all potential similarities (30-40%)
- PAM250 & BLOSUM30 – Longer alignments of divergent sequences (<30%)

The sequences we aligned using bl2seq have similarity >85%

Score	Expect	Method	Identities	Positives	Gaps
182 bits(462)	6e-60	Compositional matrix adjust.	127/144(88%)	140/144(97%)	2/144(1%)

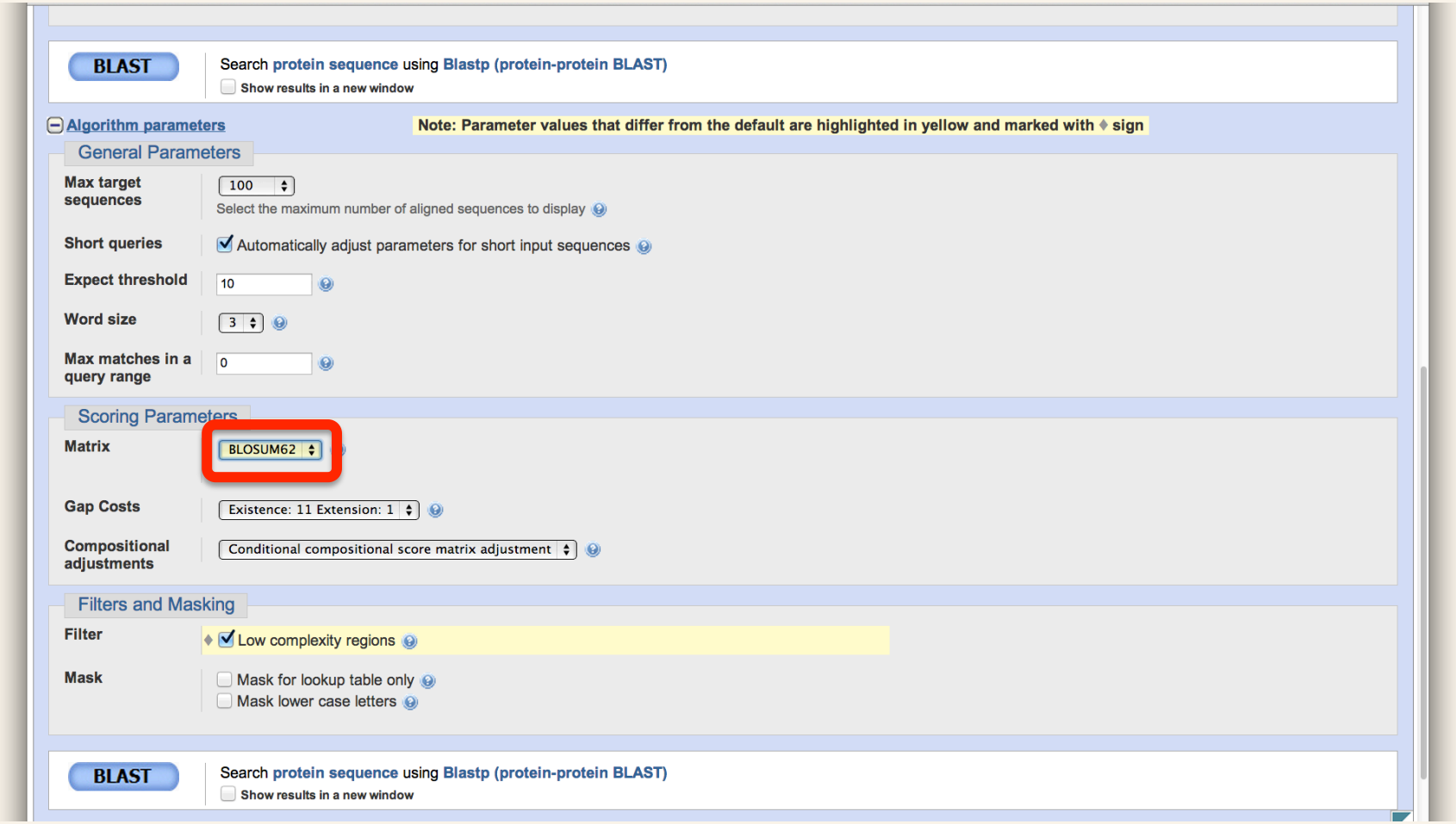
```
Query   127   THSQWNKPSKPKTNMKHMagaaaagavvgglggymlgsamsRPIIHFGSDYEDRYYRENM   186
              TH+QWNKPSKPKTN+KH+AGAAAAGAVVGGLGGYMLGSAMSRP+IHFG+D+EDRYYRENM
Sbjct   94    THNQWNKPSKPKTNLKHVAGAAAAGAVVGGLGGYMLGSAMSRPMIHFGNDWEDRYYRENM   153

Query   187   HRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHtvttttkgenftetDVKMMERVVEQMC   246
              +RYPNQVYYRP+D+YSNQNNFVHDCVNITIKQHTVTTTTKGENFTETDVKMMERVVEQMC
Sbjct   154   YRYPNQVYYRPVDQYSNQNNFVHDCVNITIKQHTVTTTTKGENFTETDVKMMERVVEQMC   213

Query   247   ITQYERESQAYY--QRGSSMVLFS   268
              +TQY++ESQAYY  +R SS VLFS
Sbjct   214   VTQYQKESQAYYDGRRSSSTVLFS   237
```

Range 2: 1 to 49 Graphics ▼ Next Match ▲ Previous Match ◮ First Match

Score	Expect	Method	Identities	Positives	Gaps
98.2 bits(243)	1e-28	Compositional matrix adjust.	42/49(86%)	45/49(91%)	0/49(0%)

```
Query   1    MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNRY   49
             MANLG W+L LFV  W+D+GLCKKRPKPGGWNTGGSRYPGQGSPGGNRY
Sbjct   1    MANLGYWLLALFVTMWTDVGLCKKRPKPGGWNTGGSRYPGQGSPGGNRY   49
```

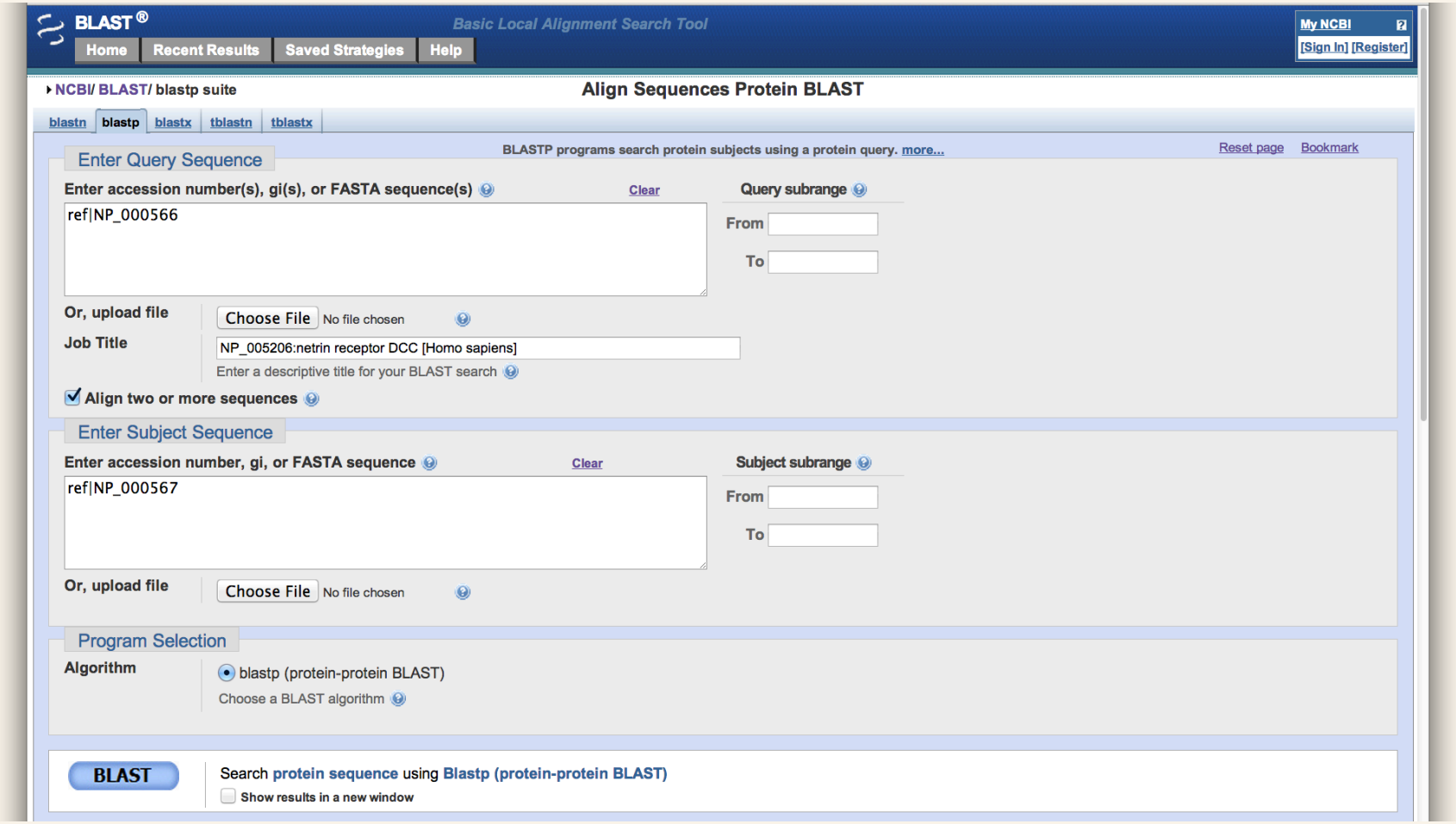
And which matrix we used (default)?



Let's try BLOSUM80 for higher sequence similarity

- The result is the same because there is no gaps in the alignment
- But let's try another sequences

NP_000566 vs NP_000567



BLOSUM62

Score	Expect	Method	Identities	Positives	Gaps
42.4 bits(98)	3e-09	Compositional matrix adjust.	71/286(25%)	118/286(41%)	42/286(14%)

```
Query  1    MAKVPDMFEDLKNCYSENEED-SSSIDHLSLNQKSFYHVSYGPLHEGCMDQSVSLSISET  59
            MA+VP++  ++   YS NE+D     D     + SF  +   PL     D  + L IS+
Sbjct  1    MAEVPELASEMMAYYSGNEDDLFFEADGPKQMKCSFQDLDLCPL-----DGGIQLRISDH  55

Query  60   SKTSKLTFKESMVVVATNGKVLKKRRLSLSQSITDDDL----------EAIANDSEEEII  109
              +    F+++  VV    K L+K  +   Q+  ++DL          E I  D+ +
Sbjct  56   HYSK--GFRQAASVVVAMDK-LRKMLVPCPQTFQENDLSTFFPFIFEEEPIFFDTWDNEA  112

Query  110  KPRSAPFSFLSNVKYNFMRIIKYEFILNDALNQSIIRANDQYLTAAAL--HNLDEAVKFD  167
             AP   L+               L D+  +S++ +    L A  L   ++++ V F
Sbjct  113  YVHDAPVRSLNCT----------LRDSQQKSLVMSGPYELKALHLQGQDMEQQVVFS  159

Query  168  MGAYKSSKDDAKITVILRISKTQLYVTA-QDEDQPVLLKEMPEIPKTITGSETNLLFFWE  226
            M   +  + + KI V L + +  LY++    +D+P L  E  + PK     +    F +
Sbjct  160  MSFVQGEESNDKIPVALGLKEKNLYLSCVLKDDKPTLQLESVD-PKNYPKKKMEKRFVFN  218

Query  227  THGTKNY--FTSVAHPNLFIATKQ--DYWVCLAG--GPPSITDFQI  266
                N   F S   PN +I+T Q  +  V L G  G   ITDF +
Sbjct  219  KIEINNKLEFESAQFPNWYISTSQAENMPVFLGGTKGGQDITDFTM  264
```

BLOSUM45

Score	Expect	Method	Identities	Positives	Gaps
37.7 bits(113)	8e-08	Compositional matrix adjust.	74/276(27%)	117/276(42%)	26/276(9%)

```
Query  1    MAKVPDMFEDLKNCYSENEED-SSSIDHLSLNQKSFYHVSYGPLHEGCMDQSVSLSISET  59
            MA+VP++  ++   YS NE+D     D     + SF  +   PL     D  + L IS+
Sbjct  1    MAEVPELASEMMAYYSGNEDDLFFEADGPKQMKCSFQDLDLCPL-----DGGIQLRISDH  55

Query  60   SKTSKLTFKESMVVVATNGKVLKKRRLSLSQSITDDDLEAI-ANDSEEEIIKPRSAPFSF  118
              +    F+++  VV    K L+K  +   Q+  ++DL        EEE I      F
Sbjct  56   HYSK--GFRQAASVVVAMDK-LRKMLVPCPQTFQENDLSTFFPFIFEEEPIF-----FDT  107

Query  119  LSNVKYNFMRIIK-YEFILNDALNQSIIRANDQYLTAAALHNLD--EAVKFDMGAYKSSK  175
               N  Y     ++     L D+  +S++ +    L A  L+  D  + V F M   +  +
Sbjct  108  WDNEAYVHDAPVRSLNCTLRDSQQKSLVMSGPYELKALHLQGQDMEQQVVFSMSFVQGEE  167

Query  176  DDAKITVILRISKTQLYVT-AQDEDQPVLLKEMPEIPKTITGS--ETNLLFFWETHGTKN  232
             + KI V L + +  LY++    +D+P L  E  + PK       E  ++F       K
Sbjct  168  SNDKIPVALGLKEKNLYLSCVLKDDKPTLQLESVD-PKNYPKKKMEKRFVFNKIEINNKL  226

Query  233  YFTSVAHPNLFIATKQ--DYWVCLAG--GPPSITDF  264
             F S   PN +I+T Q  +  V L G  G   ITDF
Sbjct  227  EFESAQFPNWYISTSQAENMPVFLGGTKGGQDITDF  262
```

BLOSUM90

Score	Expect	Method	Identities	Positives	Gaps
45.2 bits(99)	8e-10	Compositional matrix adjust.	74/278(27%)	112/278(40%)	26/278(9%)

```
Query  1    MAKVPDMFEDLKNCYSENEED-SSSIDHLSLNQKSFYHVSYGPLHEGCMDQSVSLSISET  59
            MA VP++  ++   YS NE+D     D     + SF      PL  G     + L IS+
Sbjct  1    MAEVPELASEMMAYYSGNEDDLFFEADGPKQMKCSFQDLDLCPLDGG-----IQLRISD-  54

Query  60   SKTSKLTFKESMVVVATNGKVLKKRRLSLSQSITDDDLEAI-ANDSEEEIIKPRSAPFSF  118
               SK   + + VVVA +   L+K      Q+  ++DL        EEE I      F
Sbjct  55   HHYSKGFRQAASVVVAMDK--LRKMLVPCPQTFQENDLSTFFPFIFEEEPIF-----FDT  107

Query  119  LSNVKYNFMRIIK-YEFILNDALNQSIIRANDQYLTAAALH--NLDEAVKFDMGAYKSSK  175
              N  Y     ++     L D+  +S++ +    L A  L+  ++++ V F M   +
Sbjct  108  WDNEAYVHDAPVRSLNCTLRDSQQKSLVMSGPYELKALHLQGQDMEQQVVFSMSFVQGEE  167

Query  176  DDAKITVILRISKTQLYVTA-QDEDQPVLLKEMPEIPKTITGSETNLLFFWETHGTKNY-  233
             + KI V L +    LY +    +D+P L  E  + PK          F        N
Sbjct  168  SNDKIPVALGLKEKNLYLSCVLKDDKPTLQLESVD-PKNYPKKKMEKRFVFNKIEINNKL  226

Query  234  -FTSVAHPNLFIATKQ--DYWVCLAG--GPPSITDFQI  266
             F S   PN +I+T Q  +  V L G  G   ITDF +
Sbjct  227  EFESAQFPNWYISTSQAENMPVFLGGTKGGQDITDFTM  264
```

PAM250

Score	Expect	Method	Identities	Positives	Gaps
31.9 bits(101)	3e-06	Compositional matrix adjust.	45/209(22%)	107/209(51%)	19/209(9%)

```
Query  1    MAKVPDMFEDLKNCYSENEEDSSSIDHLSLNQKSFYHVSYGPLHEGCMDQSVSLSISETS  60
            MA VP++  ++   YS NE+D     + + + K     S+  L+   +D ++ L IS+
Sbjct  1    MAEVPELASEMMAYYSGNEDDLFFEADGPKQMKC----SFQDLDLCPLDGGIQLRISDHH  56

Query  61   KTSKLTFKESMVVVATNGKVLKKRRLSLSQSITDDDLEAIAN---DSEEEIIKPRSAPFS  117
             +    F+++  VV + +K L+K  ++ +Q++ ++DL ++      + E  ++  +    +
Sbjct  57   YSK--GFRQAASVVVAMDK-LRKMLVPCPQTFQENDLSTFFPFIFEEEPIFFDTWDNE-A  112

Query  118  FLSNVKYNFMRIIKYEFILNDALNQSIIRANDQYLTAAALH--NLDEAVKFDMGAYKSSK  175
            ++ +     +R ++    L D+ ++S++ +    L A  L+  ++++ V F M+  ++
Sbjct  113  YVHDAP---VRSLNC--TLRDSQQKSLVMSGPYELKALHLQGQDMEQQVVFSMSFVQGEE  167

Query  176  DDAKITVILRISKTQLYVT-AQDEDQPVL  203
             + KI V L +   +LY++    +D+P L
Sbjct  168  SNDKIPVALGLKEKNLYLSCVLKDDKPTL  196
```

PAM30

Score	Expect	Method	Identities	Positives	Gaps
52.4 bits(116)	9e-12	Compositional matrix adjust.	74/277(27%)	96/277(34%)	28/277(10%)

```
Query  1    MAKVPDMFEDLKNCYSENEEDSSSIDHLSLNQKSFYHVSYGPLHEGCMDQSVSLSISETS  60
            MA VP++  ++   YS NE+D            K     S+  L    +D  + L IS+
Sbjct  1    MAEVPELASEMMAYYSGNEDDLFFEADGPKQMKC----SFQDLDLCPLDGGIQLRISD-H  55

Query  61   KTSKLTFKESMVVVATNGKVLKKRRLSLSQSITDDDLEA-IANDSEEEIIKPRSAPFSFL  119
              SK  F +   VV    K L K      Q   ++DL        EEE I      F
Sbjct  56   HYSK-GFRQAASVVVAMDK-LRKMLVPCPQTFQENDLSTFFPFIFEEEPI-----FFDTW  108

Query  120  SNVKYNF---MRIIKYEFILNDALNQSIIRANDQYLTAAAL--HNLDEAVKFDMGAYKSS  174
             N  Y      R       L D    S +      L A  L  +++++ V F M
Sbjct  109  DNEAYVHDAPVRSLNC--TLRDSQQKSLVMSGPYELKALHLQGQDMEQQVVFSMSFVQGE  166

Query  175  KDDAKITVILRISKTQLYVT-AQDEDQPVLLKEMPEIPKTITGS--ETNLLFFWETHGTK  231
             + KI V L      LY      +D P L  E  + PK       E    F       K
Sbjct  167  ESNDKIPVALGLKEKNLYLSCVLKDDKPTLQLESVD-PKNYPKKKMEKRFVFNKIEINNK  225

Query  232  NYFTSVAHPNLFIATKQDYW--VCLAG--GPPSITDF  264
             F S   PN +I T Q     V L G  G   ITDF
Sbjct  226  LEFESAQFPNWYISTSQAENMPVFLGGTKGGQDITDF  262
```

PAM250 (<30%) → BLOSUM45 → BLOSUM62 (30-40%) → BLOSUM90 (70-90%) → PAM30 (80-100%)

Score	Expect	Method	Identities	Positives	Gaps
31.9 bits(101)	3e-06	Compositional matrix adjust.	45/209(22%)	107/209(51%)	19/209(9%)

Score	Expect	Method	Identities	Positives	Gaps
37.7 bits(113)	8e-08	Compositional matrix adjust.	74/276(27%)	117/276(42%)	26/276(9%)

Score	Expect	Method	Identities	Positives	Gaps
42.4 bits(98)	3e-09	Compositional matrix adjust.	71/286(25%)	118/286(41%)	42/286(14%)

Score	Expect	Method	Identities	Positives	Gaps
45.2 bits(99)	8e-10	Compositional matrix adjust.	74/278(27%)	112/278(40%)	26/278(9%)

Score	Expect	Method	Identities	Positives	Gaps
52.4 bits(116)	9e-12	Compositional matrix adjust.	74/277(27%)	96/277(34%)	28/277(10%)

- In **BLAST** the suggested cut-offs to infer biologically significant similarity:
  - ✓ **For proteins: sequence identity >= 25%, E-value <= 1e-3**
  - ✓ **For nucleotides: sequence identity >= 70%, E-value <= 1e-6**

- **Do not use those cut-offs blindly!**