Blast

Basic Local Alignment Search Tool

Adapted from the courses of the Bonsai team,

CRIStAL UMR 9189

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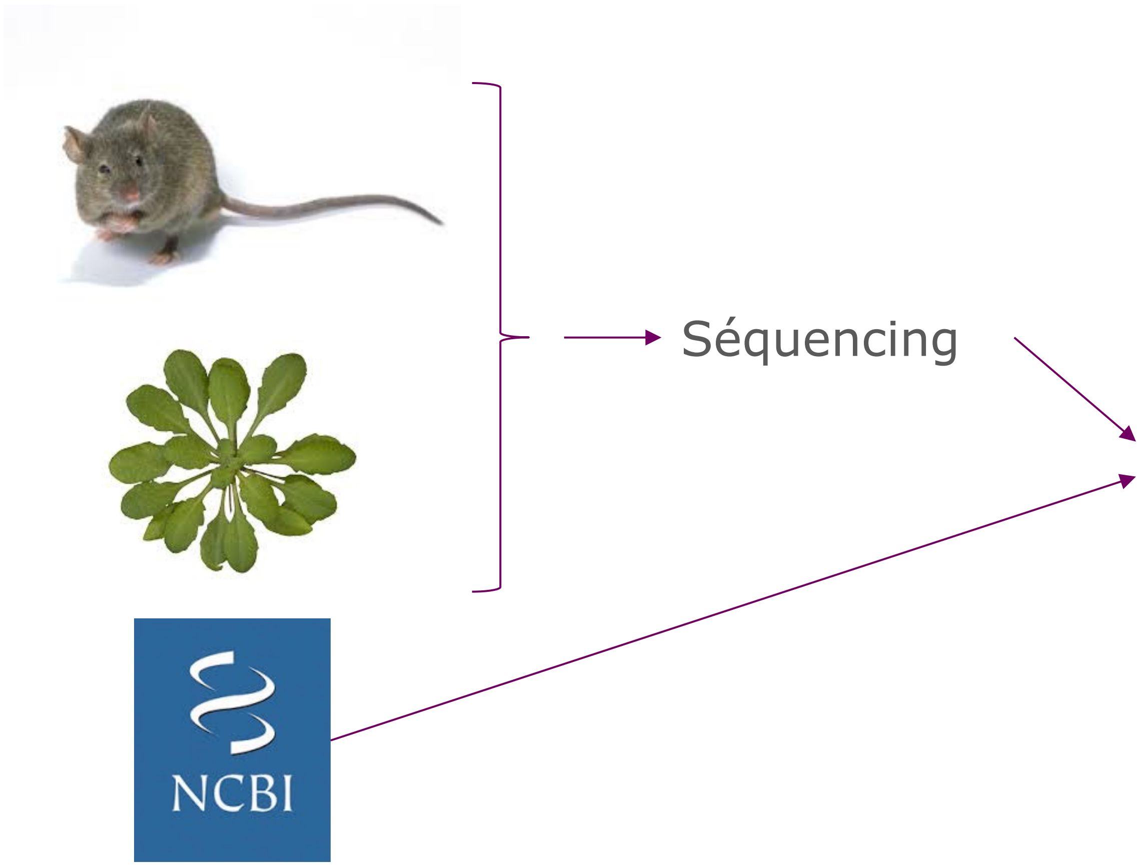
Introduction

challenge

- We assumed that you have a **nucleotide or protein sequence** obtained from a biological sample or from a database



Séquencing

>Your sequence
ACGTCGTAGCATGCA
GCGATTAGCTACGAT
CAGCTAGCATCGATC
GATGGGCATTACGTA
CGTAGCTATGTCAGC
GATCGA…

challenge

- You want to know if the sequence you have obtained is **already known or is similar** to other sequences in the databases

- A **sequence similarity searc**h often provides the first information about a new nucleotide or protein sequence

→ **inferring the function** from similar sequences

challenge

- We have:

  a **query** sequence *q*

  a **database** *T* = {*t1*, ..,*tn*}

- What we want: to find **significant alignments** between *q* and *ti*

- Classical algorithms (e.g. Smith and Waterman's local alignment) do not work: too time consuming, need to find **workarounds**

Blast, generalities

- Blast (NCBI definition) : The Basic Local Alignment Search Tool (BLAST) finds regions of **local similarity between sequences**. The program compares nucleotide or protein sequences to sequence databases and calculates the **statistical significance** of the alignments

- Blast can be used to infer **functional and evolutionary relationships** between sequences and can also help identify **members of a gene family**

- Blast uses **heuristics*** to deliver results quickly

*A heuristic is a computational method that quickly provides a feasible solution, not necessarily an optimal one, it may miss some results

Blast, generalities

- Since most proteins are **modular** (composed of functional domain(s)), Blast is made to find these domains between different sequences
- The algorithm also allows the alignment between **mRNA and genomic sequences**

- However, if 2 sequences are expected to be aligned to their full length (global alignment), it is possible that Blast will only return the most conserved parts of this alignment

History

- First version released by NCBI in 1990 (Altschul et al. 1990)

- This version only performs **ungapped alignments**, but provides a p-value that allows the user to assess the significance of the results

- A version allowing **gaps** (Blast2) appeared in 1997 (Altschul et al. 1997) and included the **PSI Blast** (see below)

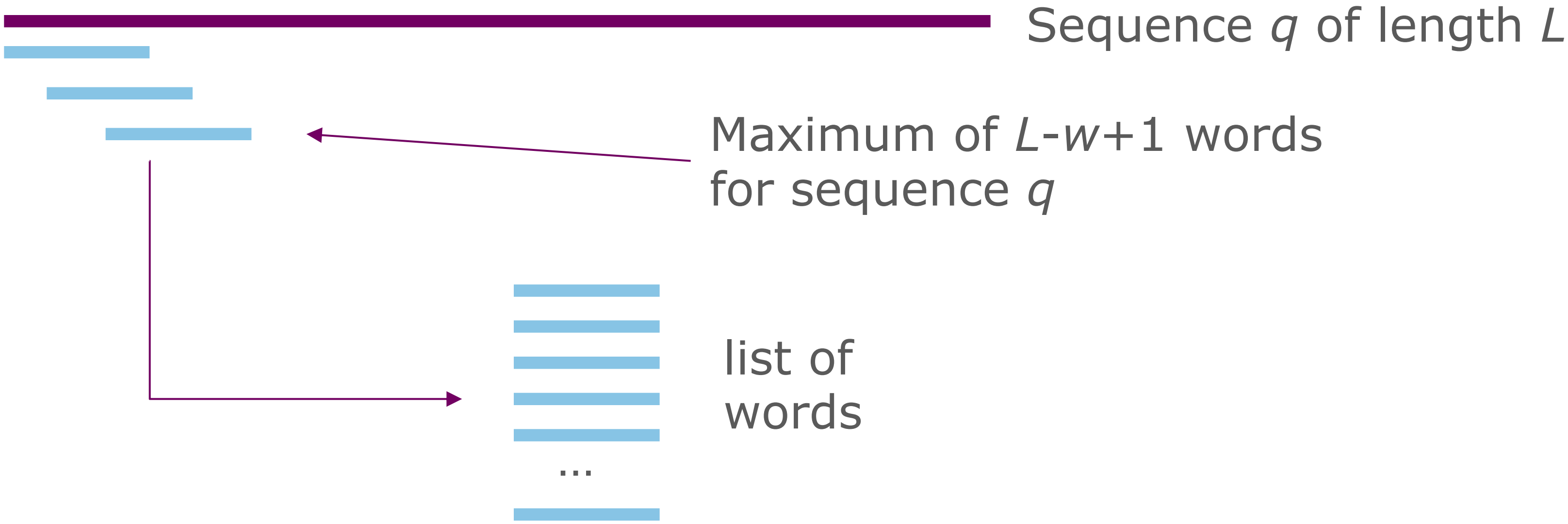
- In 2009, NCBI released a new version of Blast (**BLAST+**) (Camacho et al. 2009)

- **Currently BLAST+2.11.0 released (April 2021)**

Algorithm

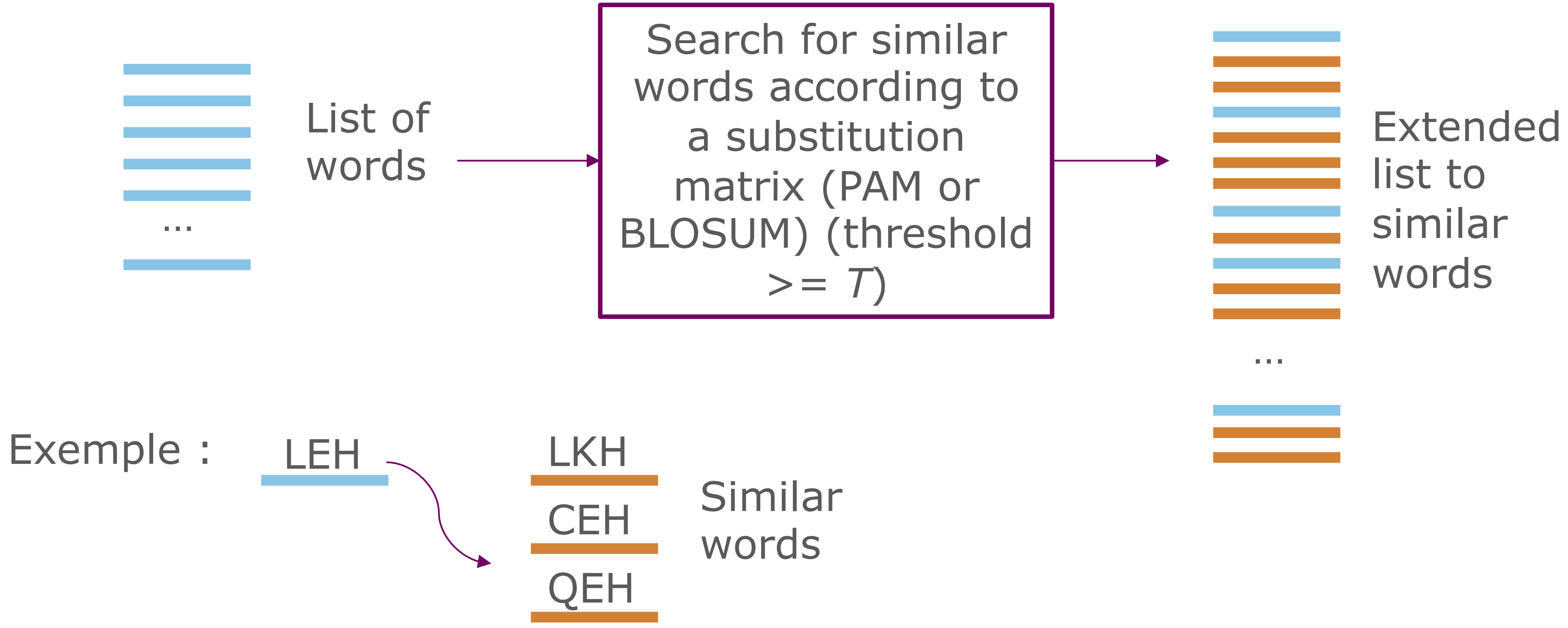
Algorithm

- **1st step**: define from the query sequence *q* **a list of words** (seeds) of defined size *w* (default size of 11 for DNA and 3 for proteins)


Sequence *q* of length *L*
Maximum of *L*-*w*+1 words for sequence *q*
...
list of words

Algorithm

- Particularity for **proteins**



List of words

…

Search for similar words according to a substitution matrix (PAM or BLOSUM) (threshold >= *T*)

Extended list to similar words

…

Exemple : LEH

LKH

CEH

QEH

Similar words

Algorithm

- Clarification on **similar words**

  - For each word of size *w*=3, Blast generates the neighbouring words using a BLOSUM62 matrix with a score threshold *T*=11

  - Words with 3 amino acids: 20^3 possible alignments !

LEH→ score= 17
LKH→ score= 13
CEH→ score= 12
Threshold QEH→ score= 11
LMP→ score= 10
LFH→ score= 9
LER→ score= 9
SEH→ score=9
…

- The neighbouring words are aligned with LEH and the alignment score is calculated from the BLOSUM matrix62

- Only words with a score ≥ to the threshold *T* are conserved

Substitution matrix

- A substitution matrix is used to **associate a score to each pair of residues** in an alignment

- For **nucleotide** sequences, **identical penalti**es are generally used for all substitutions

	A	**C**	**G**	**T**
A	1			
C	-3	1		
G	-3	-3	1	
T	-3	-3	-3	1

- For a given alignment, **the score is the sum** of the scores of each pair of residues

```
        A  C  G  C  A  T  G  C  A  T  C
        A  G  G  C  A  T  C  G  A  T  T
Score:  1 -3  1  1  1  1 -3 -3  1  1  1 = -1
```

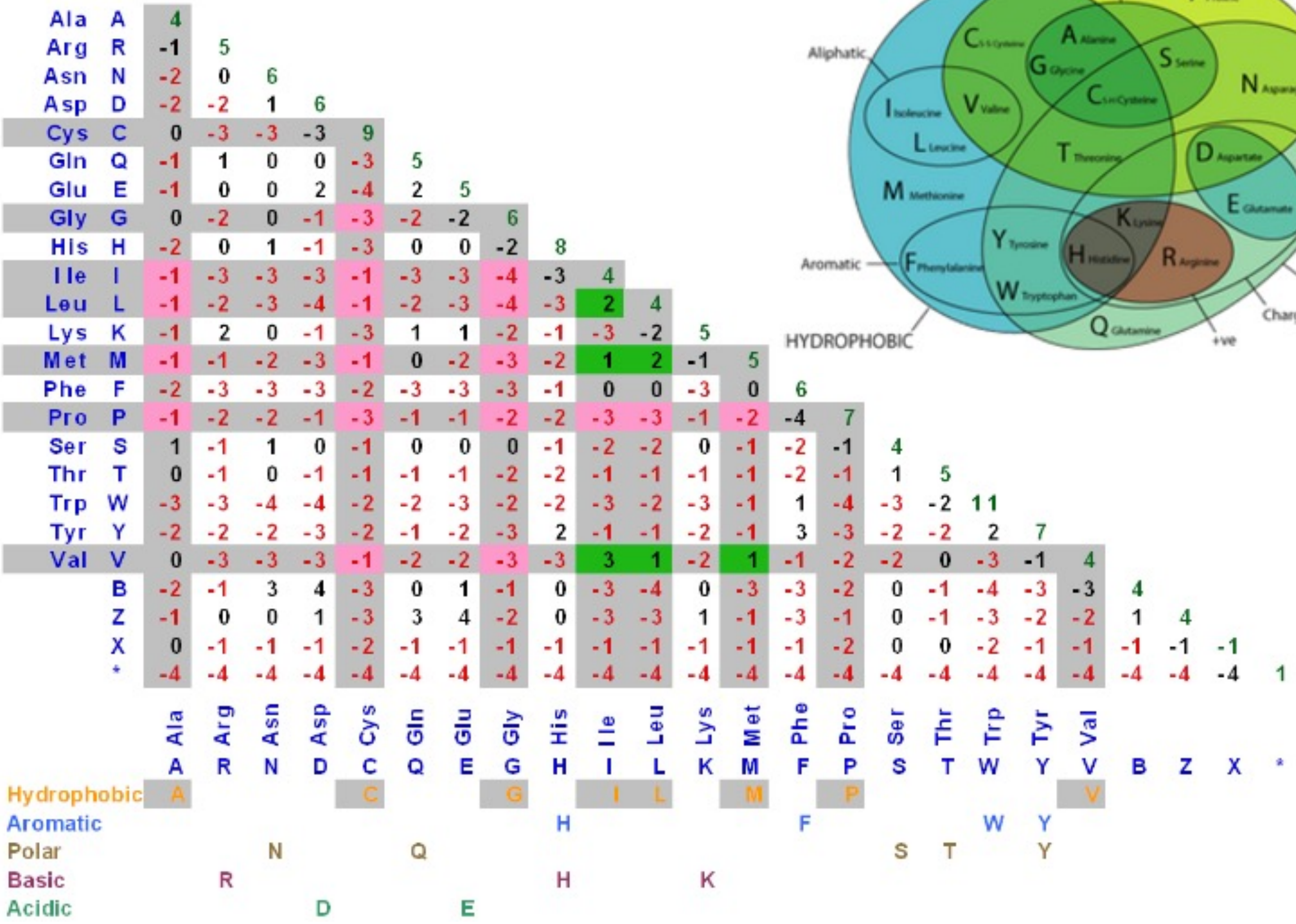
Substitution matrix

- For protein sequences, **BLOSUM or PAM** matrices are used. They provide different scores depending on the substitutions

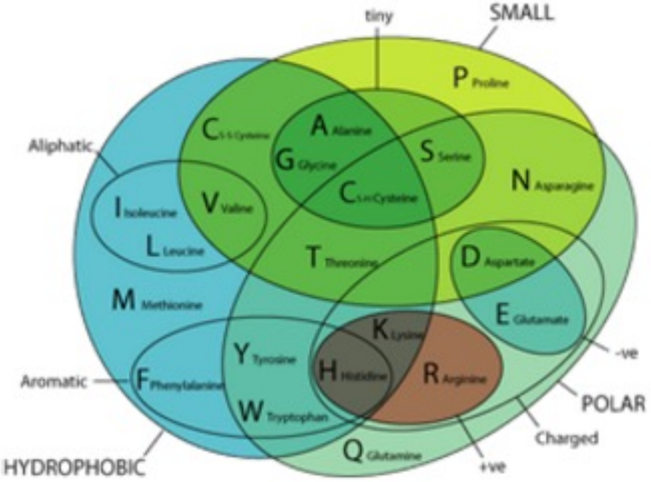
- **Positive scores** indicate frequent ("accepted") substitutions, i.e. substitutions observed more frequently than would be expected by chance

- **Negative values** indicate rare mutations, which are observed less frequently than at random. This is an indication of negative selection, suggesting that these mutations are unfavourable to the function of the protein

BLOSUM62 matrix



		Ala A	Arg R	Asn N	Asp D	Cys C	Gln Q	Glu E	Gly G	His H	Ile I	Leu L	Lys K	Met M	Phe F	Pro P	Ser S	Thr T	Trp W	Tyr Y	Val V	B	Z	X	*
Ala	A	4																							
Arg	R	-1	5																						
Asn	N	-2	0	6																					
Asp	D	-2	-2	1	6																				
Cys	C	0	-3	-3	-3	9																			
Gln	Q	-1	1	0	0	-3	5																		
Glu	E	-1	0	0	2	-4	2	5																	
Gly	G	0	-2	0	-1	-3	-2	-2	6																
His	H	-2	0	1	-1	-3	0	0	-2	8															
Ile	I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4														
Leu	L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4													
Lys	K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5												
Met	M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5											
Phe	F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6										
Pro	P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7									
Ser	S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4								
Thr	T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5							
Trp	W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11						
Tyr	Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7					
Val	V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4				
	B	-2	-1	3	4	-3	0	1	-1	0	-3	-4	0	-3	-3	-2	0	-1	-4	-3	-3	4			
	Z	-1	0	0	1	-3	3	4	-2	0	-3	-3	1	-1	-3	-1	0	-1	-3	-2	-2	1	4		
	X	0	-1	-1	-1	-2	-1	-1	-1	-1	-1	-1	-1	-1	-1	-2	0	0	-2	-1	-1	-1	-1	-1	
	*	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	1
Hydrophobic		A				C			G		I	L		M		P					V				
Aromatic										H					F				W	Y					
Polar				N			Q										S	T		Y					
Basic			R							H			K												
Acidic					D			E																	



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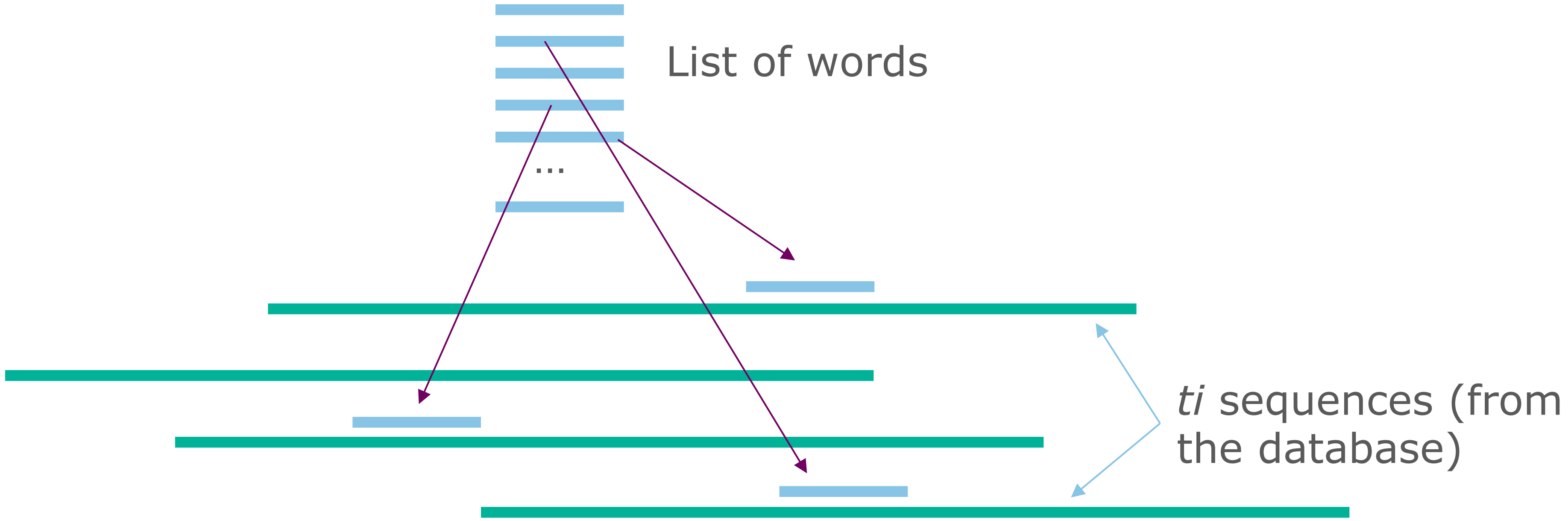
From J. van Helden, Université d'Aix-Marseille

Algorithm

- **Step 2**: Search for exact matches between the words in the list (DNA) or the extended list (proteins) and the *ti* sequences in the database

- These alignments are *hits*

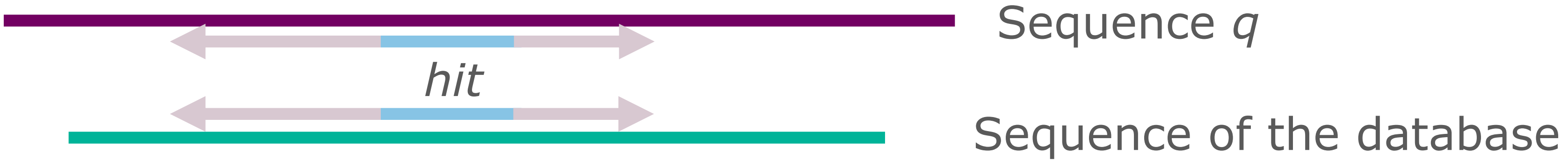
- A *hit* is therefore a "common" word of size *w* (and of score greater than *T* in the case of proteins) between the sequences *q* and *ti*



Algorithm

- **3rd step:** each hit is extended to the left and to the right: the extension is stopped when the *hit* score decreases by more than *X* (*X-drop*)

- Schematically



- Each extended hit forms an **LMSP**: Localy Maximal scoring Segment Pair

- Blast conserved only LMPSPs with a score higher than a given threshold score: the **HSPs**: High scoring Segment Pairs

- The most significant HSP is called **MSP**: Maximum scoring Segment Pair

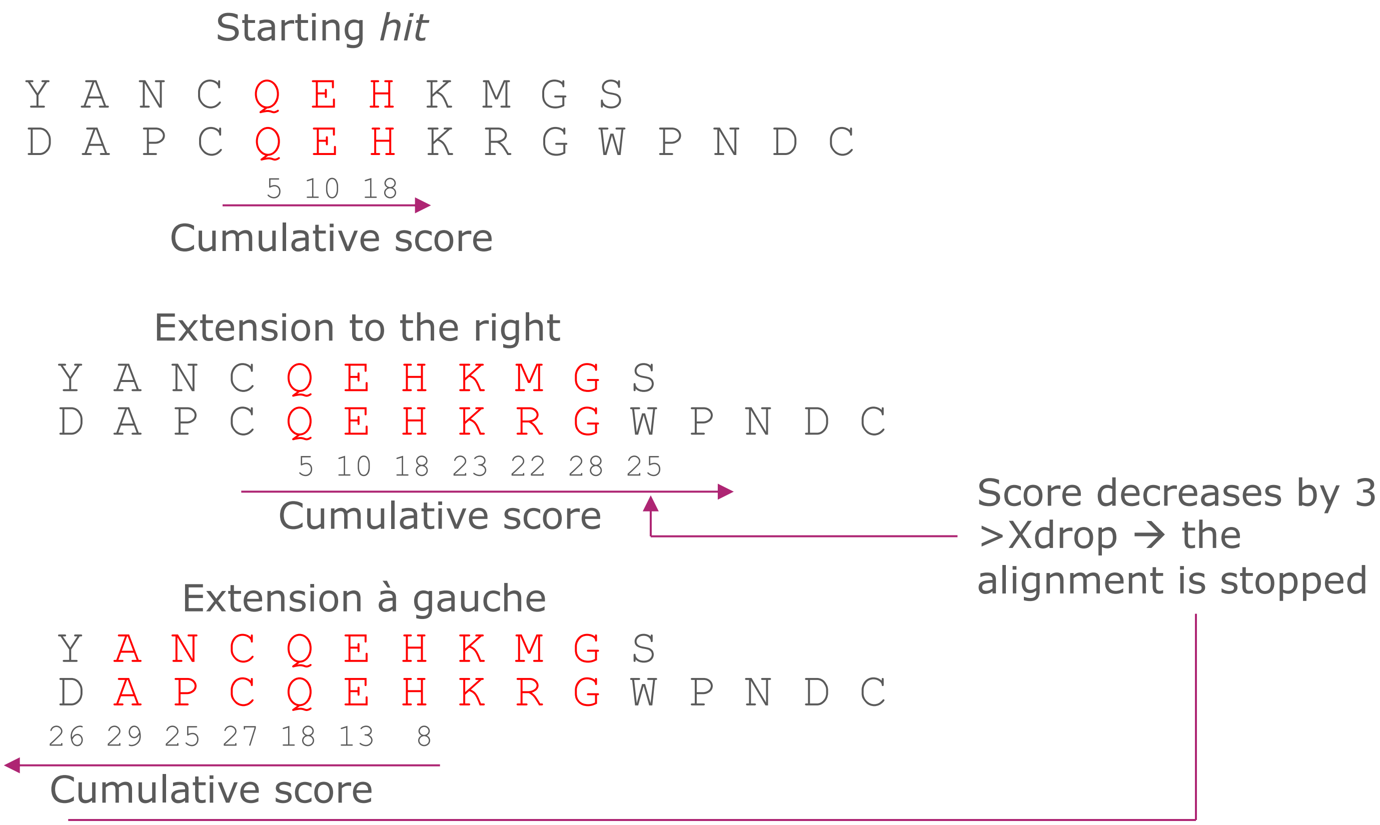
Algorithm

- Clarification on X-drop

Query *q* : Y A N C Q E H K M G S

Subject ti : D A P C Q E H K R G W P N D C

Xdrop=2
Score calculated
from BLOSUM62



Starting *hit*

```
Y A N C Q E H K M G S
D A P C Q E H K R G W P N D C
        5 10 18
```

Cumulative score

Extension to the right

```
Y A N C Q E H K M G S
D A P C Q E H K R G W P N D C
        5 10 18 23 22 28 25
```

Cumulative score

Score decreases by 3
>Xdrop → the
alignment is stopped

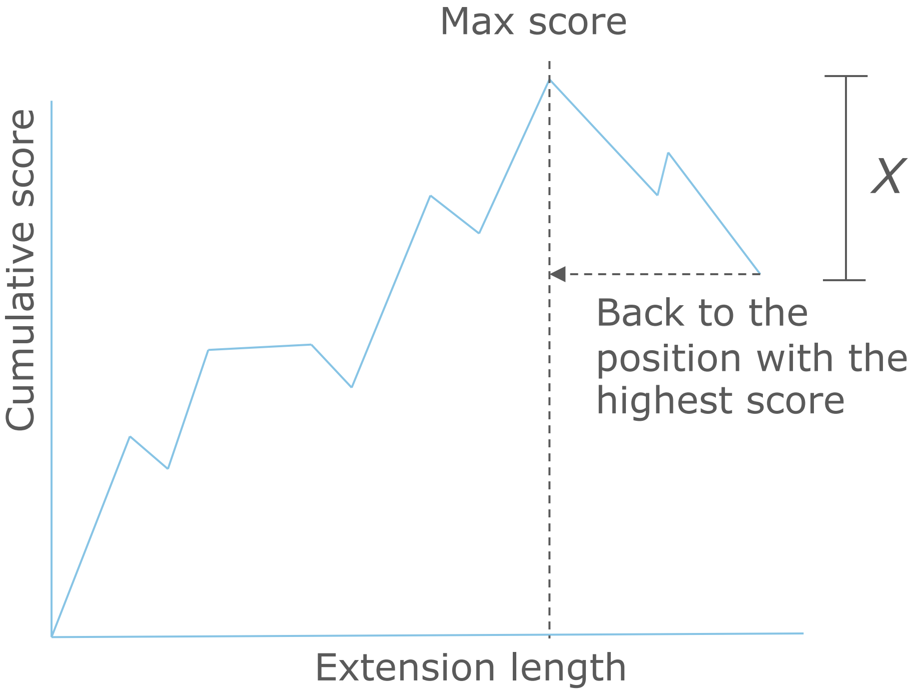
Extension à gauche

```
 Y  A  N  C  Q  E  H  K  M  G  S
 D  A  P  C  Q  E  H  K  R  G  W  P  N  D  C
26 29 25 27 18 13  8
```

Cumulative score

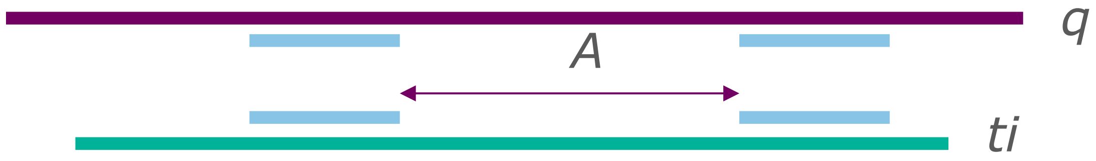
Algorithm

- Clarification on X-drop



Gapped-Blast (BLAST2)

- Based on 2 hits with a maximum distance of *A* (BLASTP). To keep a good sensitivity, *T* is lowered from 13 to 11



- Extend the hits by allowing gaps

- This method is **faster** than the previous one

Significance of alignments

Significance of alignments

- **Two sequences** can **always be aligned**

- There is always one (at least) **best *S*-score** alignment between two sequences (an MSP)

- **Issues**

- Is this score high enough to prove homology?

- Can we find a MSP with a better score in two random sequences?

Significance of alignments

- S is the score obtained by the alignment of 2 sequences

- The **p-value** measures the **probability** that 2 random sequences of the same length and composition have an MSP of score ≥ S

- The **E-value** measures the esperance E of the number n of MSPs of score ≥ S in 2 random sequences of the same length and composition

→*For example, if the E-value is equal to 10 for a HSP with score S, it means that 10 HSPs with score ≥ S can be found by chance! So probably your alignment is not significant!*

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Calculation of the E-value

- According to Karlin and Altschul, 1991

$$E = Kmne^{-\lambda s} \qquad p = 1 - e^{-E}$$

With *m* the size of the sequence `q`, `n` the size of the database, `S` the score of the HSP, `K` and `lambda` depend on the score matrix, `K` can be adjusted according to the cost of the gaps

- If `S` is the score for a hit
- The bit-score (normalized score) is: $S' = \frac{\lambda s - lnK}{ln2}$
- The E-value is then: $E = mn2^{-S'}$

Variation in E-value

- If the size of the query sequence increases: the E-value ...

- If the size of the database is divided by two: the E-value ...

- If the score increases: the E-value ...

- What bit-score to obtain an E-value of 0.05 for a sequence of length 250 and a bd of length 50000000 ?

- If we increase the E-value to 0.01, what will be the bit-score?

Variation in E-value

- If the size of the query sequence increases: the E-value increases

- If the size of the database is divided by two: the E-value decreases

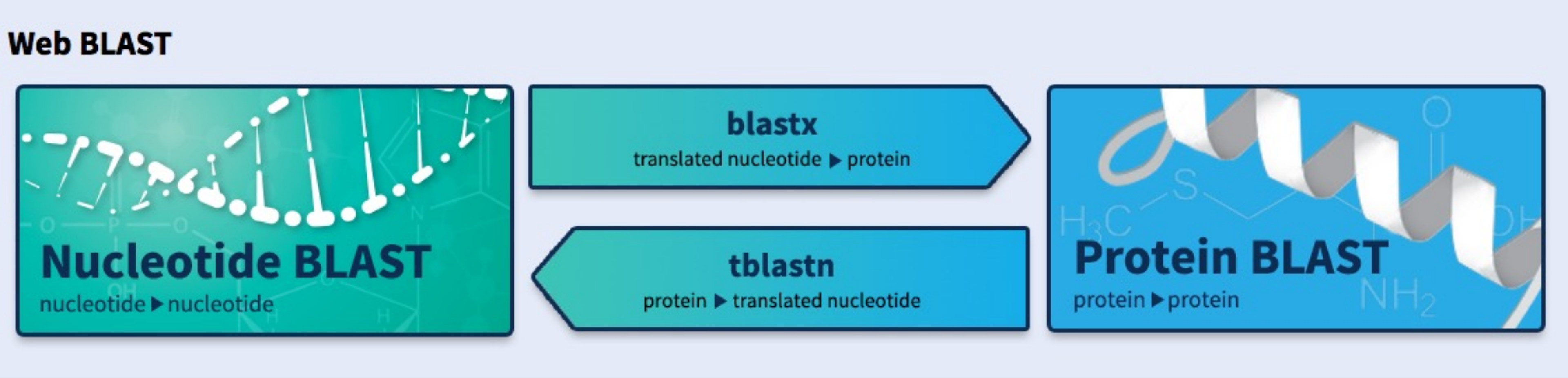
- If the score increases: the E-value decreases

- What bit-score to obtain an E-value of 0.05 for a sequence of length 250 and a bd of length 50000000 ? 38 bits

- If we increase the E-value to 0.01, what will be the bit-score? 40 bits

Run Blast!

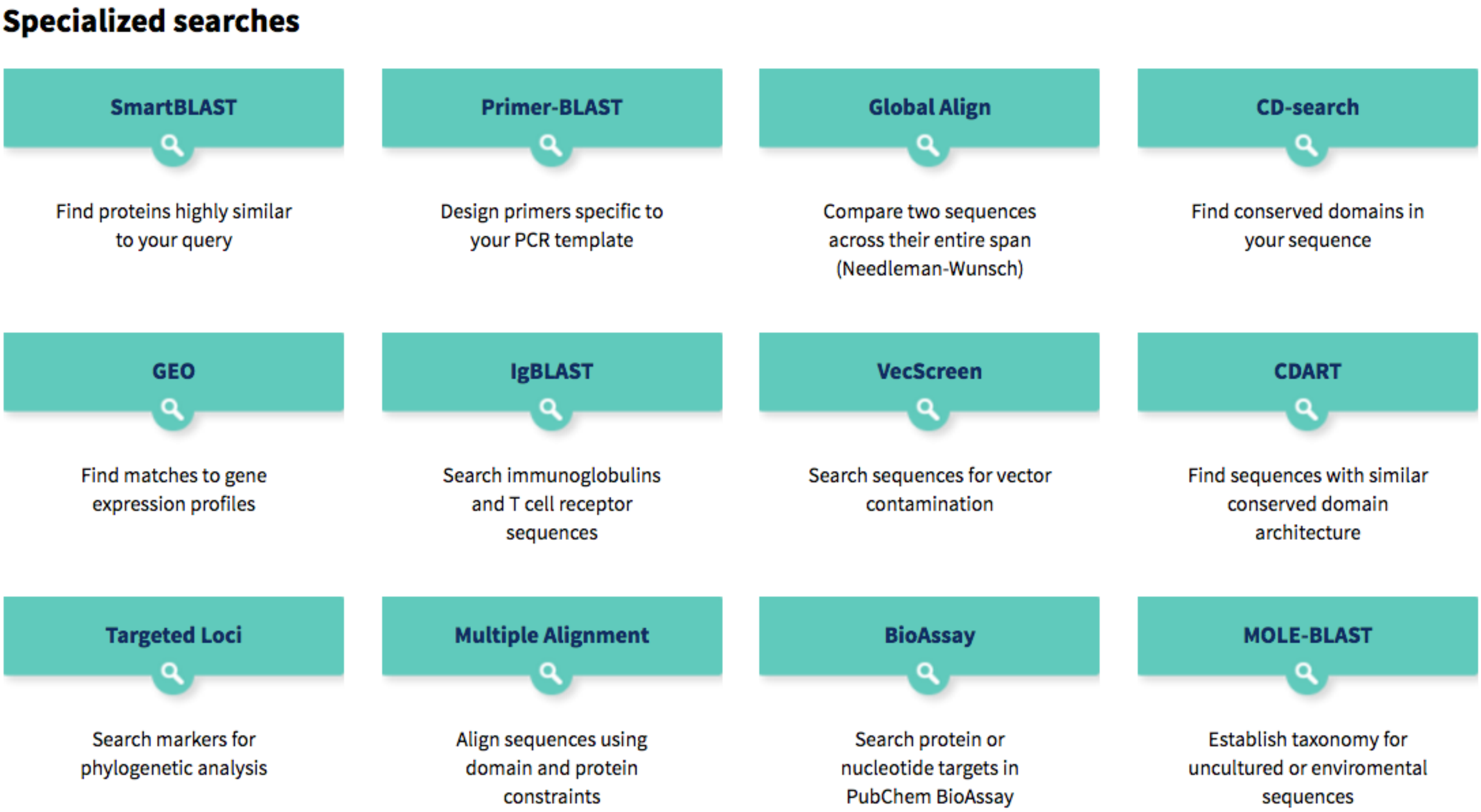
Home page



Query \ Database	nucléique	protéique	nucléique traduit
nucléique	blastn	x	x
protéique	x	blastp	tblastn
nucléique traduit	x	blastx	tblastx

ftp://ftp.ncbi.nlm.nih.gov/pub/factsheets/HowTo_BLASTGuide.pdf

Home page



Specialized searches

SmartBLAST

Find proteins highly similar to your query

Primer-BLAST

Design primers specific to your PCR template

Global Align

Compare two sequences across their entire span (Needleman-Wunsch)

CD-search

Find conserved domains in your sequence

GEO

Find matches to gene expression profiles

IgBLAST

Search immunoglobulins and T cell receptor sequences

VecScreen

Search sequences for vector contamination

CDART

Find sequences with similar conserved domain architecture

Targeted Loci

Search markers for phylogenetic analysis

Multiple Alignment

Align sequences using domain and protein constraints

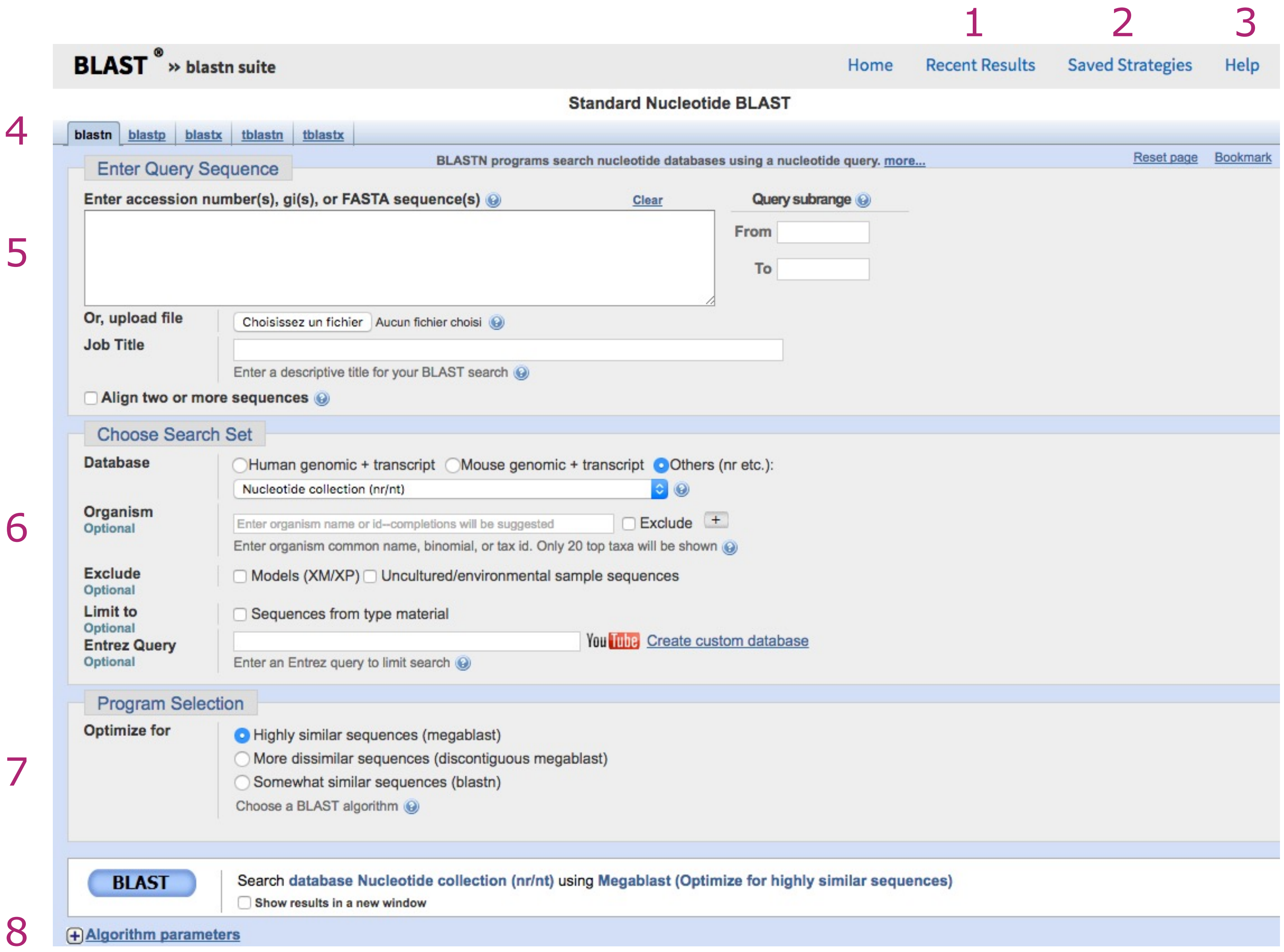
BioAssay

Search protein or nucleotide targets in PubChem BioAssay

MOLE-BLAST

Establish taxonomy for uncultured or enviromental sequences

Graphical user interface



BLAST® » blastn suite

Home | Recent Results | Saved Strategies | Help

Standard Nucleotide BLAST

blastn | blastp | blastx | tblastn | tblastx

BLASTN programs search nucleotide databases using a nucleotide 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 Choisissez un fichier Aucun fichier choisi

Job Title

Enter a descriptive title for your BLAST search

□ **Align two or more sequences**

Choose Search Set

Database ○ Human genomic + transcript ○ Mouse genomic + transcript ● Others (nr etc.):

Nucleotide collection (nr/nt)

Organism Optional

Enter organism name or id--completions will be suggested □ Exclude +

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

Exclude Optional □ Models (XM/XP) □ Uncultured/environmental sample sequences

Limit to Optional □ Sequences from type material

Entrez Query Optional YouTube Create custom database

Enter an Entrez query to limit search

Program Selection

Optimize for

● Highly similar sequences (megablast)

○ More dissimilar sequences (discontiguous megablast)

○ Somewhat similar sequences (blastn)

Choose a BLAST algorithm

BLAST Search database Nucleotide collection (nr/nt) using Megablast (Optimize for highly similar sequences)

□ Show results in a new window

+ Algorithm parameters

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Graphical user interface

- **1 Recent Results:** The results of your searches over the last 36 hours. If you are registered on MyNCBI, you can access your results from any machine. If not, only searches from the active browser session are kept

- **2 Saved Strategies :** allows you to save the parameters of a Blast search in order to restart a search with the same parameters later (connection to MyNCBI required)

- **3 Help :** documentations, links and tutorials

- **4 :** type of Blast

Graphical user interface

- **5 Enter Query Sequence**

Copy/paste or upload your query sequence(s). You can also define a search range in your sequences. You can give a title to your search.

The "Align two or more sequences" function allows you to compare sequences between them without using a database

- **6 Choose Search Set**

Select your database. You can limit your search to specific organisms or exclude organisms. You can exclude sequences produced from genome annotation projects or from non-cultured/bred organisms. You can limit your search to model specimens and strains

- **7 Program Selection**

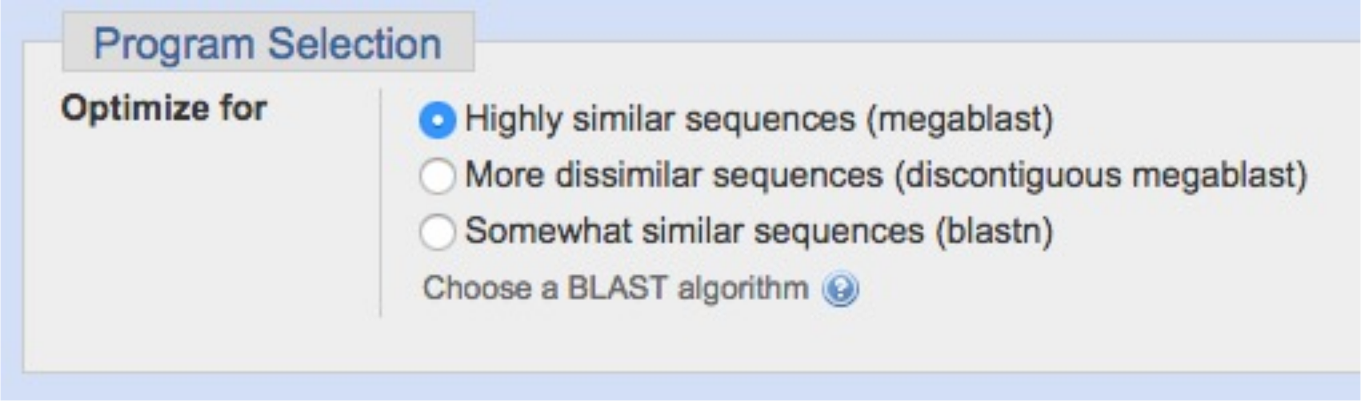
Allows you to optimise your search for different scenarios (e.g. intra or inter species searches)

Graphical user interface

- **8 Algorithm parameters**

This is the place to modify the parameters of the BLAST agoritm that has been selected (see dedicated section)

Nucleotide Blast



Program Selection

Optimize for

● Highly similar sequences (megablast)

○ More dissimilar sequences (discontiguous megablast)

○ Somewhat similar sequences (blastn)

Choose a BLAST algorithm

- **Megablast**:

- a Faster Blast when searching for high similarity

- Implementation: use larger word sizes (28 *vs* 11)

- To be reserved when searching for very similar sequences or when we want to know if our sequence is in the database

Nucleotide Blast

- **Discontigous megablast**:

 - Use a spaced seed rather than an exact word (contiguous seed)

- Useful for inter-species comparisons

- Example of contiguous seed: 1 1 1 1 1 1: an exact word (without mismatch) of 6 nucleotides

 - Example of spaced seed: 1 1 1 0 1 0 1 1 : a word of 8 nucleotides, with 6 interrogated positions (represented by ”1”). So, in this example, positions 4 and 6 may represent mismatches since they are not interrogated (represented by ”0”)

Spaced seeds *vs* contiguous seeds

- We consider a sequence *q* of length *l*=26

- A seed (word) of size 6

- We can therefore define a maximum of 26-6+1=21 seeds

- The sequence *ti* is identical to *q*: therefore all seeds can be aligned with *ti*

```
ATCTGATCGATCGATCGATCGATCGA : q
||||||||||||||||||||||||||
ATCTGATCGATCGATCGATCGATCGA : ti
111111
 111111
  111111
   111111
    111111
     111111
      111111
       111111
        111111
         111111
          111111
           111111
            111111
             111111
              111111
               111111
                111111
                 111111
                  111111
                   111111
                    111111
```

Spaced seeds *vs* contiguous seeds

```
ATCTGATCGATCGATCGATCGATCGA
||||||||||||||||||||||||||
ATCTGATCGATCGATCGATCGATCGA
111111
 111111
  111111
   111111
    111111
     111111
      111111
       111111
        111111
         111111
          111111
           111111
            111111
             111111
              111111
               111111
                111111
                 111111
                  111111
                   111111
                    111111
```

```
ATCTGATCGATCGATCGATCGATCGA
||||||||||||||||||||||||||
ATCTGATCGATCGATCGATCGATCGA
11101011
 11101011
  11101011
   11101011
    11101011
     11101011
      11101011
       11101011
        11101011
         11101011
          11101011
           11101011
            11101011
             11101011
              11101011
               11101011
                11101011
                 11101011
                  11101011
```

- Spaced seeds behave in the same way as contiguous seeds in this example

Spaced seeds *vs* contiguous seeds

- Now let's introduce a mismatch between *q* and *ti*…

```
ATCTGATCGATCGATCGATCGATCGA
|||||.||||||||||||||||||||
ATCTGCTCGATCGATCGATCGATCGA
111111
 111111
  111111
   111111
    111111
     111111
      111111
       111111
        111111
         111111
          111111
           111111
            111111
             111111
              111111
               111111
                111111
                 111111
                  111111
                   111111
                    111111
```

```
ATCTGATCGATCGATCGATCGATCGA
|||||.||||||||||||||||||||
ATCTGCTCGATGGATGGATCGTTCGA
11101011
 11101011
  11101011
   11101011
    11101011
     11101011
      11101011
       11101011
        11101011
         11101011
          11101011
           11101011
            11101011
             11101011
              11101011
               11101011
                11101011
                 11101011
                  11101011
```

Some seeds (in red) are lost

Spaced seeds can be permissive

Spaced seeds *vs* contiguous seeds

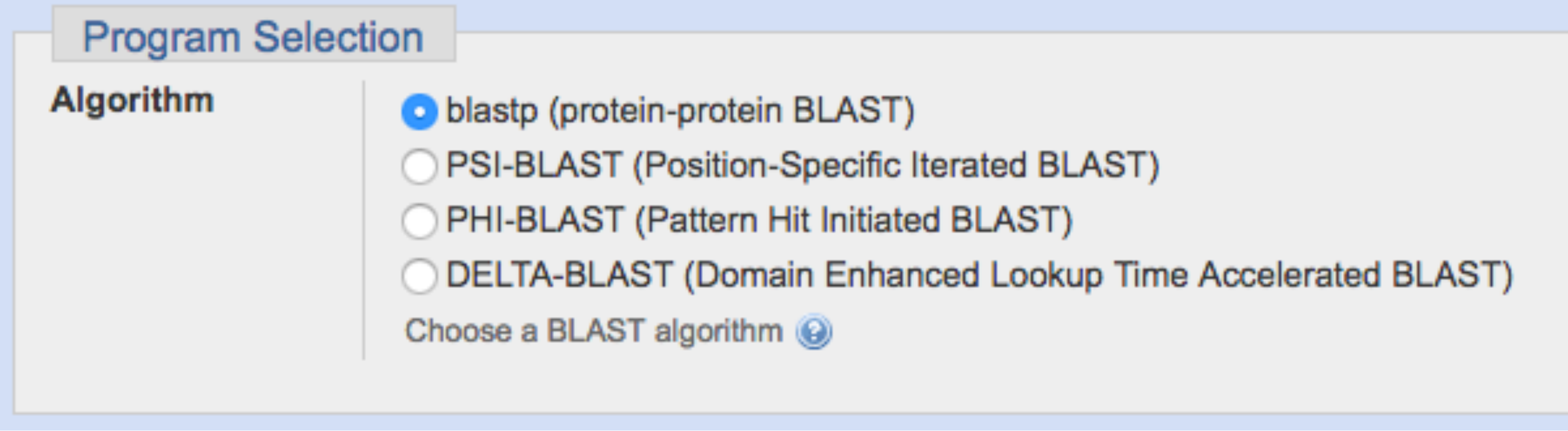
- Then, Let's introduce more mismatches between q and ti . …

```
ATCTGATCGATCGATCGATCGATCGA
|||||.|||||.|||.|||||.||||
ATCTGCTCGATGGATGGATCGTTCGA
111111
 111111
  111111
   111111
    111111
     111111
      111111
       111111
        111111
         111111
          111111
           111111
            111111
             111111
              111111
               111111
                111111
                 111111
                  111111
                   111111
                    111111
```

```
ATCTGATCGATCGATCGATCGATCGA
|||||.|||||.|||.|||||.||||
ATCTGCTCGATGGATGGATCGTTCGA
11101011
 11101011
  11101011
   11101011
    11101011
     11101011
      11101011
       11101011
        11101011
         11101011
          11101011
           11101011
            11101011
             11101011
              11101011
               11101011
                11101011
                 11101011
                  11101011
```

In that case the ti sequence could only have been found by a spaced seed!

Protein Blast



Program Selection

Algorithm

● blastp (protein-protein BLAST)
○ PSI-BLAST (Position-Specific Iterated BLAST)
○ PHI-BLAST (Pattern Hit Initiated BLAST)
○ DELTA-BLAST (Domain Enhanced Lookup Time Accelerated BLAST)

Choose a BLAST algorithm ?

- **PSI-BLAST :**

- Initial search with blastp

- Construction of a multiple alignment and then a profile from the best hits → position-specific score matrix (PSSM)

- New search with the profile

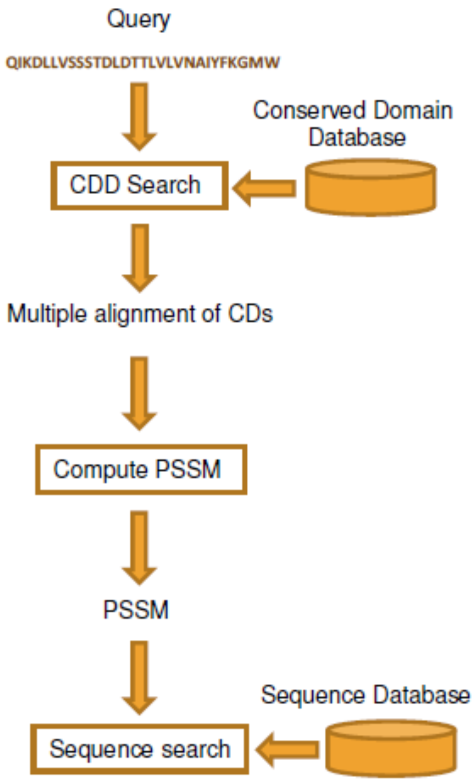
Protein Blast

- **PHI-BLAST :**

- Input: a protein sequence and a motif (regular expression)
- Restriction of the library to sequences for which the motif is found

- **DELTA-BLAST**

- Use of PSSMs built from a NCBI CDD (conserved domain database)
- Faster than PSI-BLAST, also more sensitive

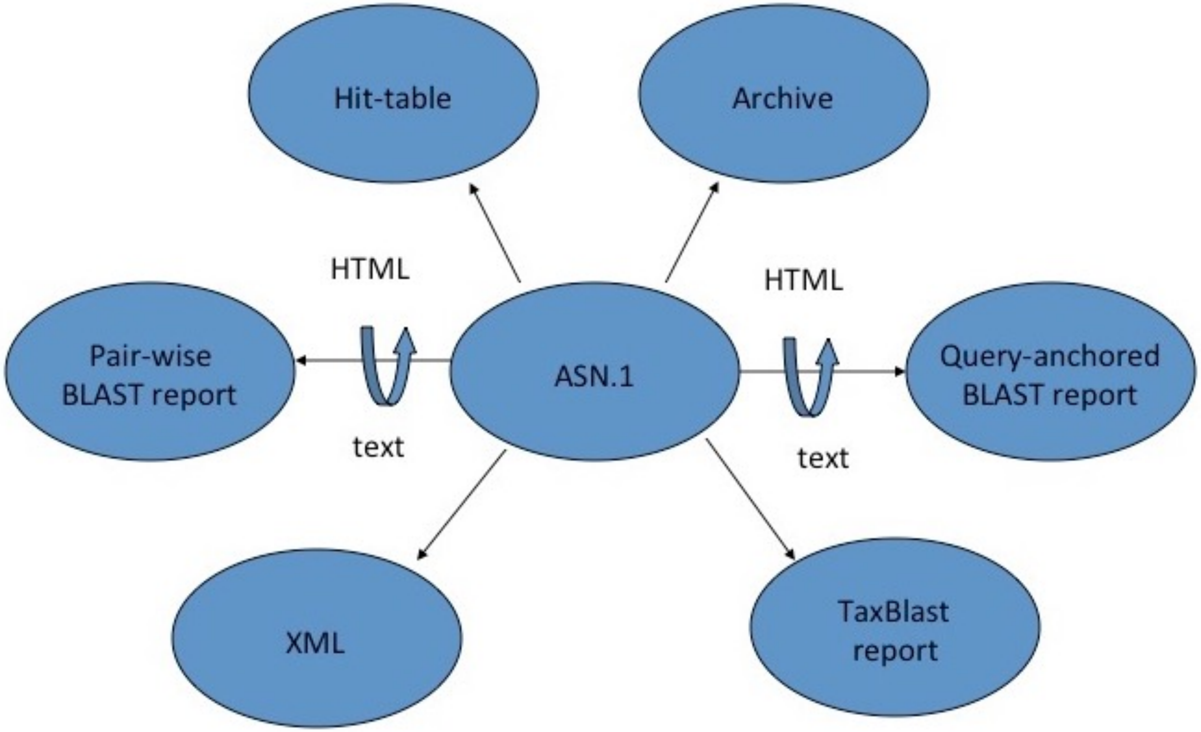

Query
QIKDLLVSSSTDLDTTLVLVNAIYFKGMW
Conserved Domain Database
CDD Search
Multiple alignment of CDs
Compute PSSM
PSSM
Sequence Database
Sequence search

Boratyn et al 2012

Blast results

Results

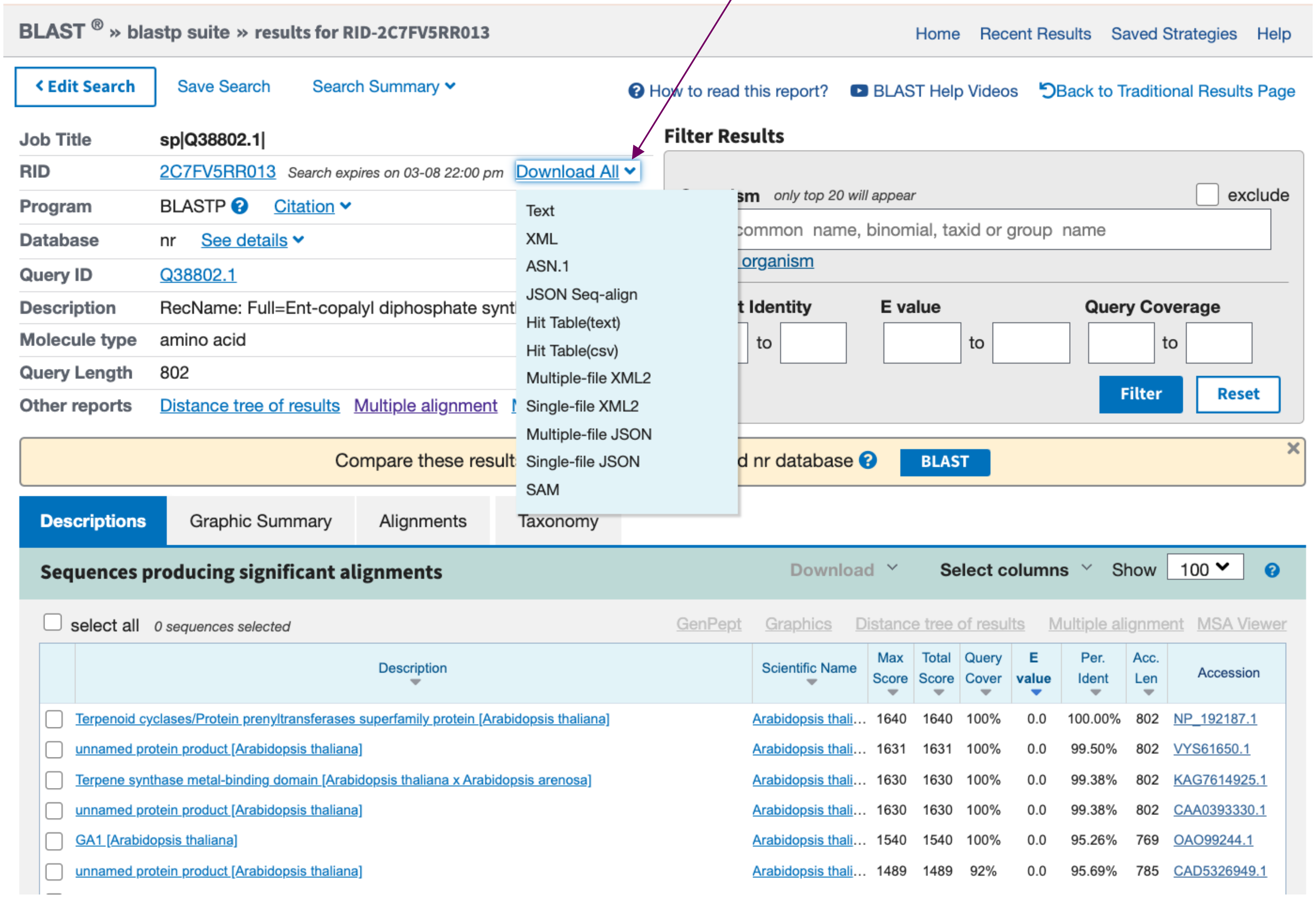
- Structured results: a flexible output



Madden, 2013

Results

Download and format results



BLAST ® » blastp suite » results for RID-2C7FV5RR013

Job Title	sp|Q38802.1|
RID	2C7FV5RR013 *Search expires on 03-08 22:00 pm* Download All
Program	BLASTP Citation
Database	nr See details
Query ID	Q38802.1
Description	RecName: Full=Ent-copalyl diphosphate synt
Molecule type	amino acid
Query Length	802
Other reports	Distance tree of results Multiple alignment M

Filter Results

sm *only top 20 will appear* □ exclude

common name, binomial, taxid or group name

organism

t Identity to E value to Query Coverage to

Compare these result d nr database

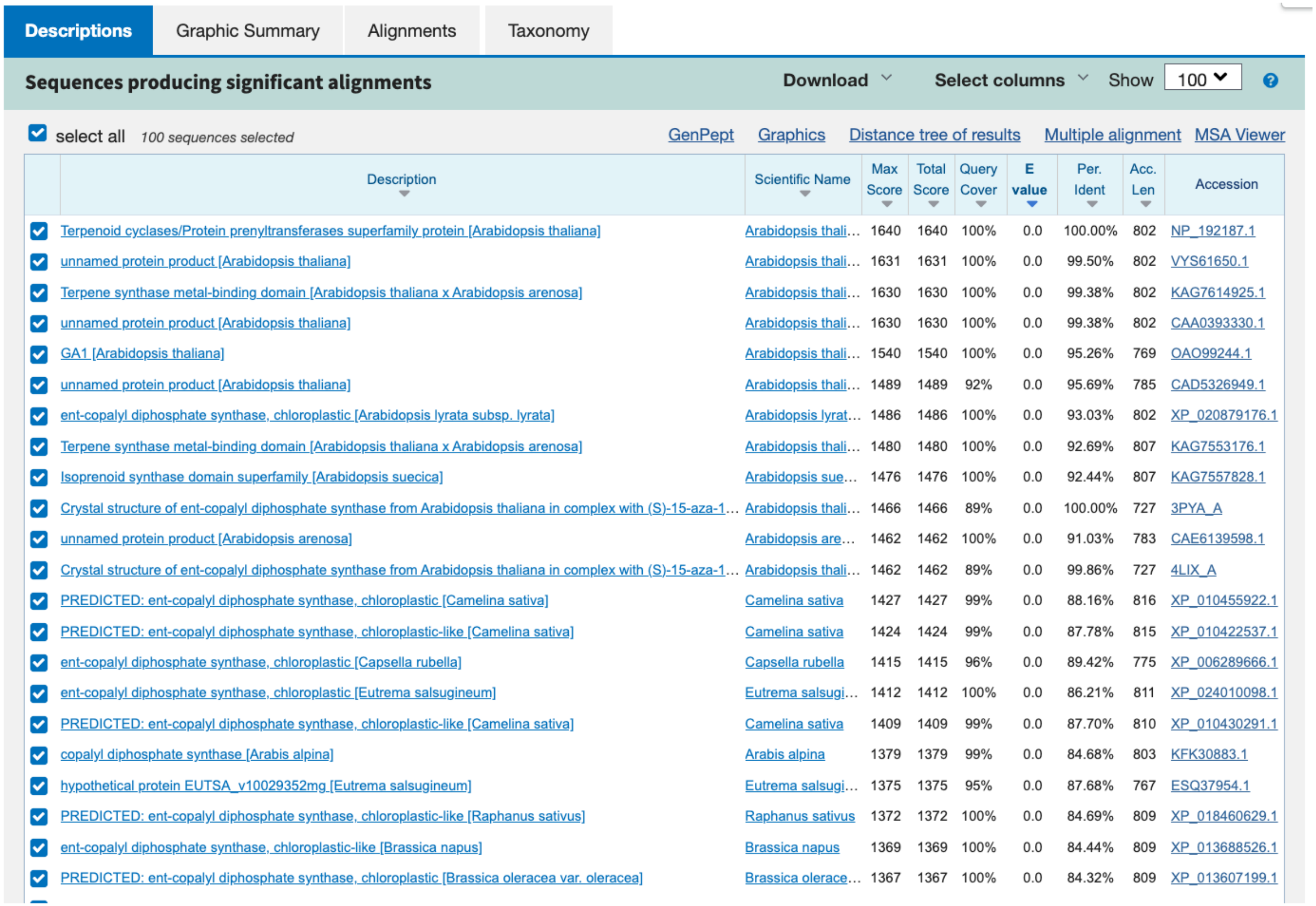
Sequences producing significant alignments

□ select all *0 sequences selected*

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
□	Terpenoid cyclases/Protein prenyltransferases superfamily protein [Arabidopsis thaliana]	Arabidopsis thali...	1640	1640	100%	0.0	100.00%	802	NP_192187.1
□	unnamed protein product [Arabidopsis thaliana]	Arabidopsis thali...	1631	1631	100%	0.0	99.50%	802	VYS61650.1
□	Terpene synthase metal-binding domain [Arabidopsis thaliana x Arabidopsis arenosa]	Arabidopsis thali...	1630	1630	100%	0.0	99.38%	802	KAG7614925.1
□	unnamed protein product [Arabidopsis thaliana]	Arabidopsis thali...	1630	1630	100%	0.0	99.38%	802	CAA0393330.1
□	GA1 [Arabidopsis thaliana]	Arabidopsis thali...	1540	1540	100%	0.0	95.26%	769	OAO99244.1
□	unnamed protein product [Arabidopsis thaliana]	Arabidopsis thali...	1489	1489	92%	0.0	95.69%	785	CAD5326949.1

Results

- Descriptions



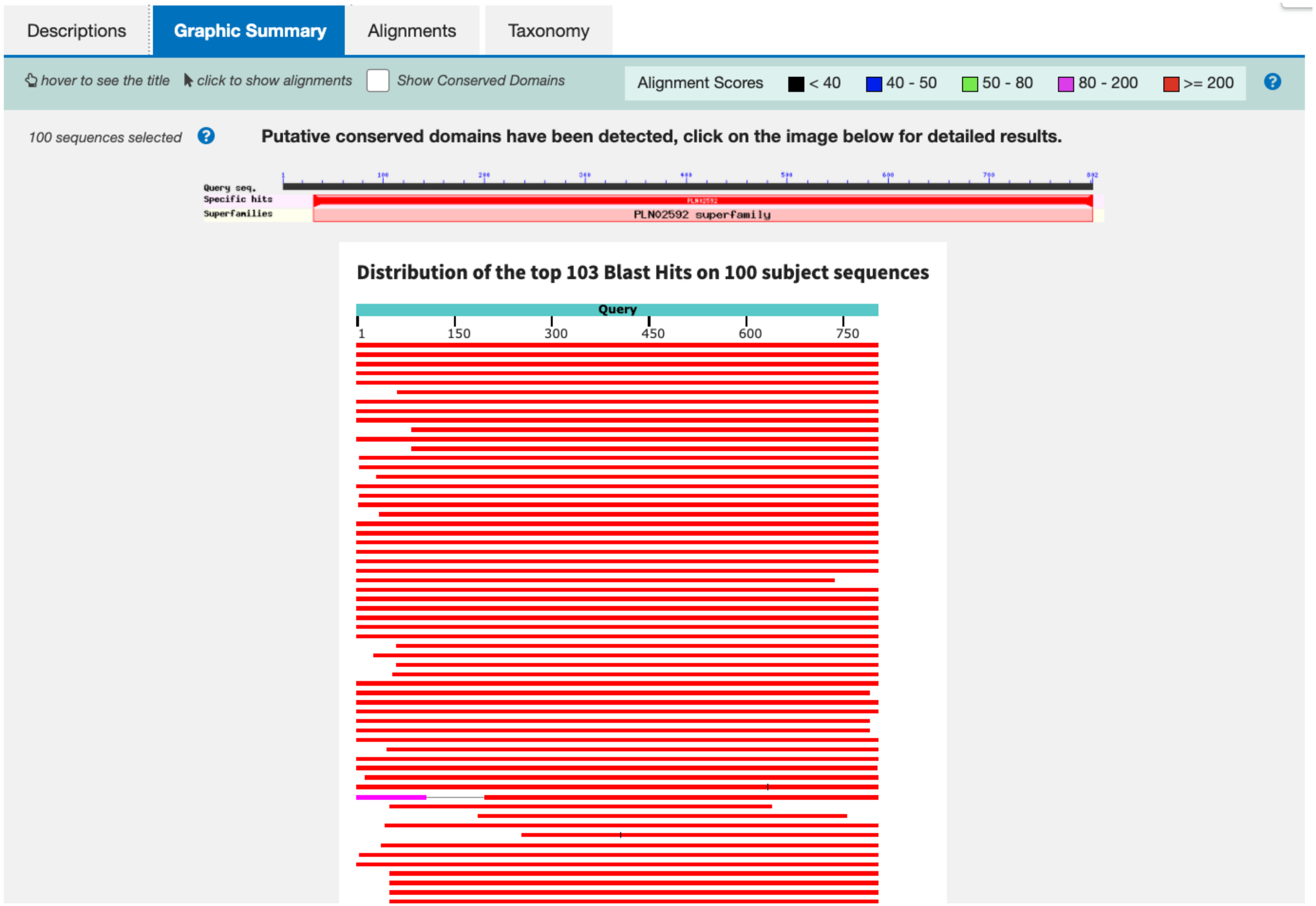
Sequences producing significant alignments

☑ select all *100 sequences selected*

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Terpenoid cyclases/Protein prenyltransferases superfamily protein [Arabidopsis thaliana]	Arabidopsis thali...	1640	1640	100%	0.0	100.00%	802	NP_192187.1
☑	unnamed protein product [Arabidopsis thaliana]	Arabidopsis thali...	1631	1631	100%	0.0	99.50%	802	VYS61650.1
☑	Terpene synthase metal-binding domain [Arabidopsis thaliana x Arabidopsis arenosa]	Arabidopsis thali...	1630	1630	100%	0.0	99.38%	802	KAG7614925.1
☑	unnamed protein product [Arabidopsis thaliana]	Arabidopsis thali...	1630	1630	100%	0.0	99.38%	802	CAA0393330.1
☑	GA1 [Arabidopsis thaliana]	Arabidopsis thali...	1540	1540	100%	0.0	95.26%	769	OAO99244.1
☑	unnamed protein product [Arabidopsis thaliana]	Arabidopsis thali...	1489	1489	92%	0.0	95.69%	785	CAD5326949.1
☑	ent-copalyl diphosphate synthase, chloroplastic [Arabidopsis lyrata subsp. lyrata]	Arabidopsis lyrat...	1486	1486	100%	0.0	93.03%	802	XP_020879176.1
☑	Terpene synthase metal-binding domain [Arabidopsis thaliana x Arabidopsis arenosa]	Arabidopsis thali...	1480	1480	100%	0.0	92.69%	807	KAG7553176.1
☑	Isoprenoid synthase domain superfamily [Arabidopsis suecica]	Arabidopsis sue...	1476	1476	100%	0.0	92.44%	807	KAG7557828.1
☑	Crystal structure of ent-copalyl diphosphate synthase from Arabidopsis thaliana in complex with (S)-15-aza-1...	Arabidopsis thali...	1466	1466	89%	0.0	100.00%	727	3PYA_A
☑	unnamed protein product [Arabidopsis arenosa]	Arabidopsis are...	1462	1462	100%	0.0	91.03%	783	CAE6139598.1
☑	Crystal structure of ent-copalyl diphosphate synthase from Arabidopsis thaliana in complex with (S)-15-aza-1...	Arabidopsis thali...	1462	1462	89%	0.0	99.86%	727	4LIX_A
☑	PREDICTED: ent-copalyl diphosphate synthase, chloroplastic [Camelina sativa]	Camelina sativa	1427	1427	99%	0.0	88.16%	816	XP_010455922.1
☑	PREDICTED: ent-copalyl diphosphate synthase, chloroplastic-like [Camelina sativa]	Camelina sativa	1424	1424	99%	0.0	87.78%	815	XP_010422537.1
☑	ent-copalyl diphosphate synthase, chloroplastic [Capsella rubella]	Capsella rubella	1415	1415	96%	0.0	89.42%	775	XP_006289666.1
☑	ent-copalyl diphosphate synthase, chloroplastic [Eutrema salsugineum]	Eutrema salsugi...	1412	1412	100%	0.0	86.21%	811	XP_024010098.1
☑	PREDICTED: ent-copalyl diphosphate synthase, chloroplastic-like [Camelina sativa]	Camelina sativa	1409	1409	99%	0.0	87.70%	810	XP_010430291.1
☑	copalyl diphosphate synthase [Arabis alpina]	Arabis alpina	1379	1379	99%	0.0	84.68%	803	KFK30883.1
☑	hypothetical protein EUTSA_v10029352mg [Eutrema salsugineum]	Eutrema salsugi...	1375	1375	95%	0.0	87.68%	767	ESQ37954.1
☑	PREDICTED: ent-copalyl diphosphate synthase, chloroplastic-like [Raphanus sativus]	Raphanus sativus	1372	1372	100%	0.0	84.69%	809	XP_018460629.1
☑	ent-copalyl diphosphate synthase, chloroplastic-like [Brassica napus]	Brassica napus	1369	1369	100%	0.0	84.44%	809	XP_013688526.1
☑	PREDICTED: ent-copalyl diphosphate synthase, chloroplastic [Brassica oleracea var. oleracea]	Brassica olerace...	1367	1367	100%	0.0	84.32%	809	XP_013607199.1

Results

- Graphic summary



Alignment Scores ■ < 40 🟦 40 - 50 🟩 50 - 80 🟪 80 - 200 🟥 >= 200

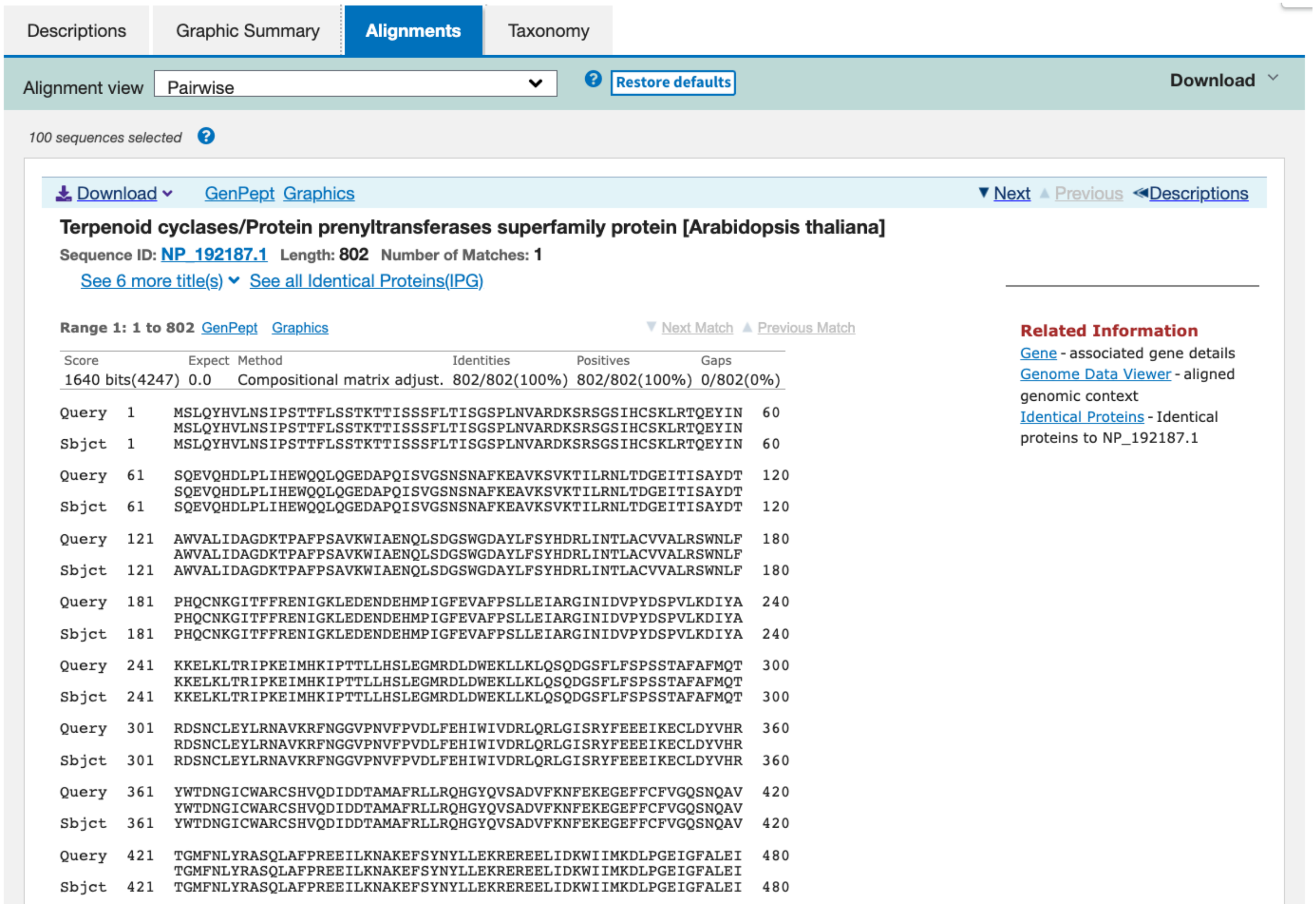
100 sequences selected

Putative conserved domains have been detected, click on the image below for detailed results.

Distribution of the top 103 Blast Hits on 100 subject sequences

Results

- Alignments



Alignment view: Pairwise

100 sequences selected

Terpenoid cyclases/Protein prenyltransferases superfamily protein [Arabidopsis thaliana]

Sequence ID: NP_192187.1 **Length:** 802 **Number of Matches:** 1

See 6 more title(s) See all Identical Proteins(IPG)

Range 1: 1 to 802 GenPept Graphics

Score	Expect	Method	Identities	Positives	Gaps
1640 bits(4247)	0.0	Compositional matrix adjust.	802/802(100%)	802/802(100%)	0/802(0%)

```
Query  1    MSLQYHVLNSIPSTTFLSSTKTTISSSFLTISGSPLNVARDKSRSGSIHCSKLRTQEYIN  60
            MSLQYHVLNSIPSTTFLSSTKTTISSSFLTISGSPLNVARDKSRSGSIHCSKLRTQEYIN
Sbjct  1    MSLQYHVLNSIPSTTFLSSTKTTISSSFLTISGSPLNVARDKSRSGSIHCSKLRTQEYIN  60

Query  61   SQEVQHDLPLIHEWQQLQGEDAPQISVGSNSNAFKEAVKSVKTILRNLTDGEITISAYDT  120
            SQEVQHDLPLIHEWQQLQGEDAPQISVGSNSNAFKEAVKSVKTILRNLTDGEITISAYDT
Sbjct  61   SQEVQHDLPLIHEWQQLQGEDAPQISVGSNSNAFKEAVKSVKTILRNLTDGEITISAYDT  120

Query  121  AWVALIDAGDKTPAFPSAVKWIAENQLSDGSWGDAYLFSYHDRLINTLACVVALRSWNLF  180
            AWVALIDAGDKTPAFPSAVKWIAENQLSDGSWGDAYLFSYHDRLINTLACVVALRSWNLF
Sbjct  121  AWVALIDAGDKTPAFPSAVKWIAENQLSDGSWGDAYLFSYHDRLINTLACVVALRSWNLF  180

Query  181  PHQCNKGITFFRENIGKLEDENDEHMPIGFEVAFPSLLEIARGINIDVPYDSPVLKDIYA  240
            PHQCNKGITFFRENIGKLEDENDEHMPIGFEVAFPSLLEIARGINIDVPYDSPVLKDIYA
Sbjct  181  PHQCNKGITFFRENIGKLEDENDEHMPIGFEVAFPSLLEIARGINIDVPYDSPVLKDIYA  240

Query  241  KKELKLTRIPKEIMHKIPTTLLHSLEGMRDLDWEKLLKLQSQDGSFLFSPSSTAFAFMQT  300
            KKELKLTRIPKEIMHKIPTTLLHSLEGMRDLDWEKLLKLQSQDGSFLFSPSSTAFAFMQT
Sbjct  241  KKELKLTRIPKEIMHKIPTTLLHSLEGMRDLDWEKLLKLQSQDGSFLFSPSSTAFAFMQT  300

Query  301  RDSNCLEYLRNAVKRFNGGVPNVFPVDLFEHIWIVDRLQRLGISRYFEEEIKECLDYVHR  360
            RDSNCLEYLRNAVKRFNGGVPNVFPVDLFEHIWIVDRLQRLGISRYFEEEIKECLDYVHR
Sbjct  301  RDSNCLEYLRNAVKRFNGGVPNVFPVDLFEHIWIVDRLQRLGISRYFEEEIKECLDYVHR  360

Query  361  YWTDNGICWARCSHVQDIDDTAMAFRLLRQHGYQVSADVFKNFEKEGEFFCFVGQSNQAV  420
            YWTDNGICWARCSHVQDIDDTAMAFRLLRQHGYQVSADVFKNFEKEGEFFCFVGQSNQAV
Sbjct  361  YWTDNGICWARCSHVQDIDDTAMAFRLLRQHGYQVSADVFKNFEKEGEFFCFVGQSNQAV  420

Query  421  TGMFNLYRASQLAFPREEILKNAKEFSYNYLLEKREREELIDKWIIMKDLPGEIGFALEI  480
            TGMFNLYRASQLAFPREEILKNAKEFSYNYLLEKREREELIDKWIIMKDLPGEIGFALEI
Sbjct  421  TGMFNLYRASQLAFPREEILKNAKEFSYNYLLEKREREELIDKWIIMKDLPGEIGFALEI  480
```

Related Information

Gene - associated gene details

Genome Data Viewer - aligned genomic context

Identical Proteins - Identical proteins to NP_192187.1

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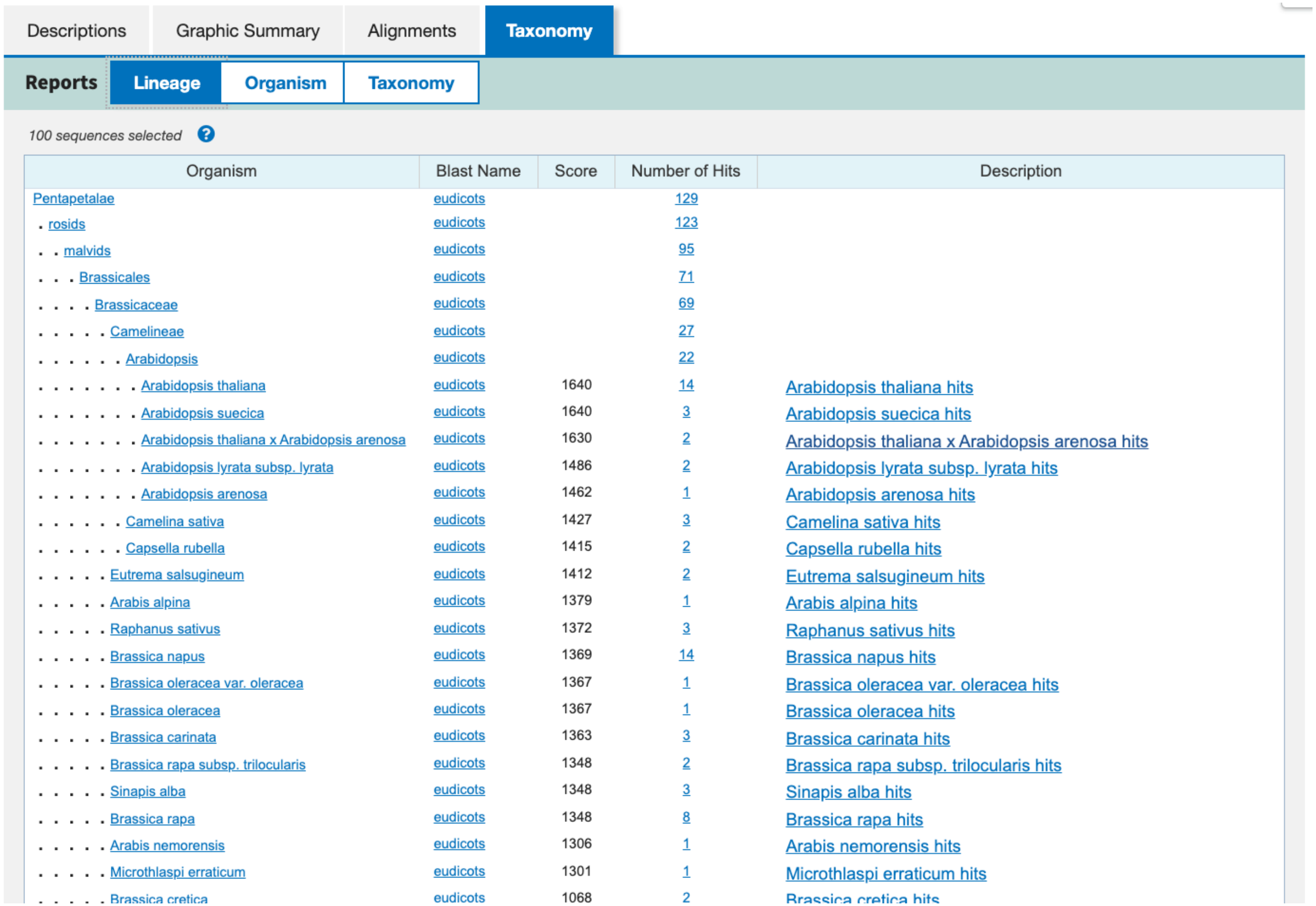
Results

- Taxonomy



100 sequences selected ?

Organism	Blast Name	Score	Number of Hits	Description
Pentapetalae	eudicots		129	
. rosids	eudicots		123	
. . malvids	eudicots		95	
. . . Brassicales	eudicots		71	
. . . . Brassicaceae	eudicots		69	
. . . . . Camelineae	eudicots		27	
. . . . . . Arabidopsis	eudicots		22	
. . . . Arabidopsis thaliana	eudicots	1640	14	Arabidopsis thaliana hits
. . . . Arabidopsis suecica	eudicots	1640	3	Arabidopsis suecica hits
. . . . Arabidopsis thaliana x Arabidopsis arenosa	eudicots	1630	2	Arabidopsis thaliana x Arabidopsis arenosa hits
. . . . Arabidopsis lyrata subsp. lyrata	eudicots	1486	2	Arabidopsis lyrata subsp. lyrata hits
. . . . Arabidopsis arenosa	eudicots	1462	1	Arabidopsis arenosa hits
. . . . . . Camelina sativa	eudicots	1427	3	Camelina sativa hits
. . . . . . Capsella rubella	eudicots	1415	2	Capsella rubella hits
. . . . . Eutrema salsugineum	eudicots	1412	2	Eutrema salsugineum hits
. . . . . Arabis alpina	eudicots	1379	1	Arabis alpina hits
. . . . . Raphanus sativus	eudicots	1372	3	Raphanus sativus hits
. . . . . Brassica napus	eudicots	1369	14	Brassica napus hits
. . . . . Brassica oleracea var. oleracea	eudicots	1367	1	Brassica oleracea var. oleracea hits
. . . . . Brassica oleracea	eudicots	1367	1	Brassica oleracea hits
. . . . . Brassica carinata	eudicots	1363	3	Brassica carinata hits
. . . . . Brassica rapa subsp. trilocularis	eudicots	1348	2	Brassica rapa subsp. trilocularis hits
. . . . . Sinapis alba	eudicots	1348	3	Sinapis alba hits
. . . . . Brassica rapa	eudicots	1348	8	Brassica rapa hits
. . . . . Arabis nemorensis	eudicots	1306	1	Arabis nemorensis hits
. . . . . Microthlaspi erraticum	eudicots	1301	1	Microthlaspi erraticum hits
. . . . . Brassica cretica	eudicots	1068	2	Brassica cretica hits

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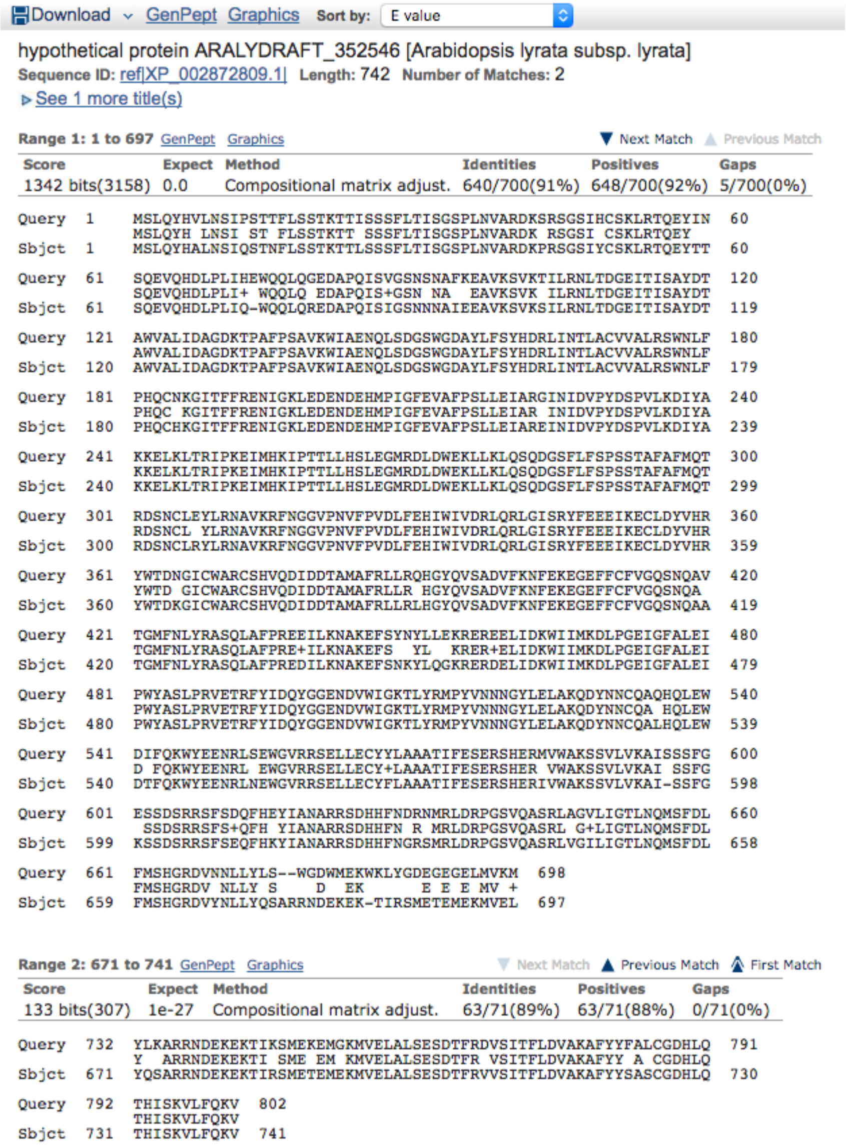
Results

- Alignments

HSPs



hypothetical protein ARALYDRAFT_352546 [Arabidopsis lyrata subsp. lyrata]

Sequence ID: ref|XP_002872809.1| Length: 742 Number of Matches: 2

▷ See 1 more title(s)

Range 1: 1 to 697 GenPept Graphics

Score	Expect	Method	Identities	Positives	Gaps
1342 bits(3158)	0.0	Compositional matrix adjust.	640/700(91%)	648/700(92%)	5/700(0%)

```
Query  1    MSLQYHVLNSIPSTTFLSSTKTTISSSFLTISGSPLNVARDKSRSGSIHCSKLRTQEYIN  60
            MSLQYH LNSI ST FLSSTKTT SSSFLTISGSPLNVARDK RSGSI CSKLRTQEY
Sbjct  1    MSLQYHALNSIQSTNFLSSTKTTLSSSFLTISGSPLNVARDKPRSGSIYCSKLRTQEYTT  60

Query  61   SQEVQHDLPLIHEWQQLQGEDAPQISVGSNSNAFKEAVKSVKTILRNLTDGEITISAYDT  120
            SQEVQHDLPLI+ WQQLQ EDAPQIS+GSN NA  EAVKSVK ILRNLTDGEITISAYDT
Sbjct  61   SQEVQHDLPLIQ-WQQLQREDAPQISIGSNNNAIEEAVKSVKSILRNLTDGEITISAYDT  119

Query  121  AWVALIDAGDKTPAFPSAVKWIAENQLSDGSWGDAYLFSYHDRLINTLACVVALRSWNLF  180
            AWVALIDAGDKTPAFPSAVKWIAENQLSDGSWGDAYLFSYHDRLINTLACVVALRSWNLF
Sbjct  120  AWVALIDAGDKTPAFPSAVKWIAENQLSDGSWGDAYLFSYHDRLINTLACVVALRSWNLF  179

Query  181  PHQCNKGITFFRENIGKLEDENDEHMPIGFEVAFPSLLEIARGINIDVPYDSPVLKDIYA  240
            PHQC KGITFFRENIGKLEDENDEHMPIGFEVAFPSLLEIAR INIDVPYDSPVLKDIYA
Sbjct  180  PHQCHKGITFFRENIGKLEDENDEHMPIGFEVAFPSLLEIAREINIDVPYDSPVLKDIYA  239

Query  241  KKELKLTRIPKEIMHKIPTTLLHSLEGMRDLDWEKLLKLQSQDGSFLFSPSSTAFAFMQT  300
            KKELKLTRIPKEIMHKIPTTLLHSLEGMRDLDWEKLLKLQSQDGSFLFSPSSTAFAFMQT
Sbjct  240  KKELKLTRIPKEIMHKIPTTLLHSLEGMRDLDWEKLLKLQSQDGSFLFSPSSTAFAFMQT  299

Query  301  RDSNCLEYLRNAVKRFNGGVPNVFPVDLFEHIWIVDRLQRLGISRYFEEEIKECLDYVHR  360
            RDSNCL YLRNAVKRFNGGVPNVFPVDLFEHIWIVDRLQRLGISRYFEEEIKECLDYVHR
Sbjct  300  RDSNCLRYLRNAVKRFNGGVPNVFPVDLFEHIWIVDRLQRLGISRYFEEEIKECLDYVHR  359

Query  361  YWTDNGICWARCSHVQDIDDTAMAFRLLRQHGYQVSADVFKNFEKEGEFFCFVGQSNQAV  420
            YWTD GICWARCSHVQDIDDTAMAFRLLR HGYQVSADVFKNFEKEGEFFCFVGQSNQA
Sbjct  360  YWTDKGICWARCSHVQDIDDTAMAFRLLRLHGYQVSADVFKNFEKEGEFFCFVGQSNQAA  419

Query  421  TGMFNLYRASQLAFPREEILKNAKEFSYNYLLEKREREELIDKWIIMKDLPGEIGFALEI  480
            TGMFNLYRASQLAFPRE+ILKNAKEFS  YL  KRER+ELIDKWIIMKDLPGEIGFALEI
Sbjct  420  TGMFNLYRASQLAFPREDILKNAKEFSNKYLQGKRERDELIDKWIIMKDLPGEIGFALEI  479

Query  481  PWYASLPRVETRFYIDQYGGENDVWIGKTLYRMPYVNNNGYLELAKQDYNNCQAQHQLEW  540
            PWYASLPRVETRFYIDQYGGENDVWIGKTLYRMPYVNNNGYLELAKQDYNNCQA HQLEW
Sbjct  480  PWYASLPRVETRFYIDQYGGENDVWIGKTLYRMPYVNNNGYLELAKQDYNNCQALHQLEW  539

Query  541  DIFQKWYEENRLSEWGVRRSELLECYYLAAATIFESERSHERMVWAKSSVLVKAISSSFG  600
            D FQKWYEENRL EWGVRRSELLECY+LAAATIFESERSHER VWAKSSVLVKAI SSFG
Sbjct  540  DTFQKWYEENRLNEWGVRRSELLECYFLAAATIFESERSHERIVWAKSSVLVKAI-SSFG  598

Query  601  ESSDSRRSFSDQFHEYIANARRSDHHFNDRNMRLDRPGSVQASRLAGVLIGTLNQMSFDL  660
             SSDSRRSFS+QFH YIANARRSDHHFN R MRLDRPGSVQASRL G+LIGTLNQMSFDL
Sbjct  599  KSSDSRRSFSEQFHKYIANARRSDHHFNGRSMRLDRPGSVQASRLVGILIGTLNQMSFDL  658

Query  661  FMSHGRDVNNLLYLS--WGDWMEKWKLYGDEGEGELMVKM  698
            FMSHGRDV NLLY S    D  EK      E E E MV +
Sbjct  659  FMSHGRDVYNLLYQSARRNDEKEK-TIRSMETEMEKMVEL  697
```

Range 2: 671 to 741 GenPept Graphics

Score	Expect	Method	Identities	Positives	Gaps
133 bits(307)	1e-27	Compositional matrix adjust.	63/71(89%)	63/71(88%)	0/71(0%)

```
Query  732  YLKARRNDEKEKTIKSMEKEMGKMVELALSESDTFRDVSITFLDVAKAFYYFALCGDHLQ  791
            Y  ARRNDEKEKTI SME EM KMVELALSESDTFR VSITFLDVAKAFYY A CGDHLQ
Sbjct  671  YQSARRNDEKEKTIRSMETEMEKMVELALSESDTFRVVSITFLDVAKAFYYSASCGDHLQ  730

Query  792  THISKVLFQKV  802
            THISKVLFQKV
Sbjct  731  THISKVLFQKV  741
```

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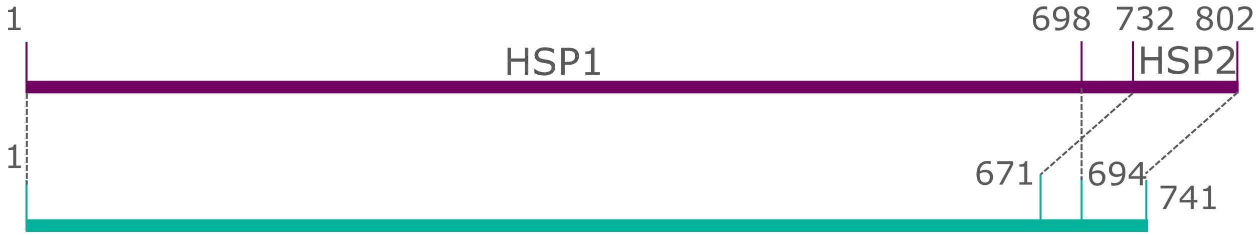
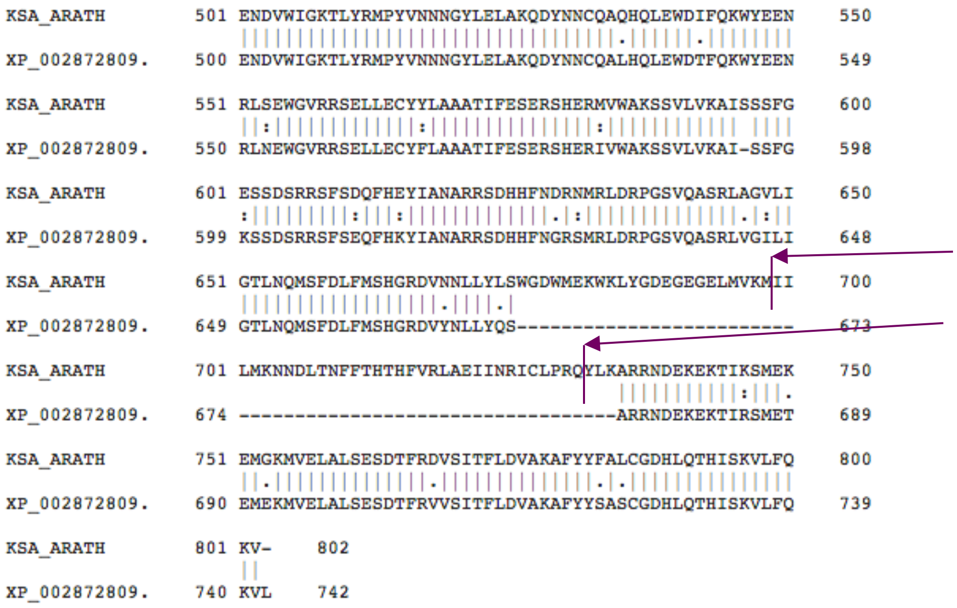
Blast vs global alignment

- Graphical overview of the Blast alignment



- Global alignment obtained using Needle



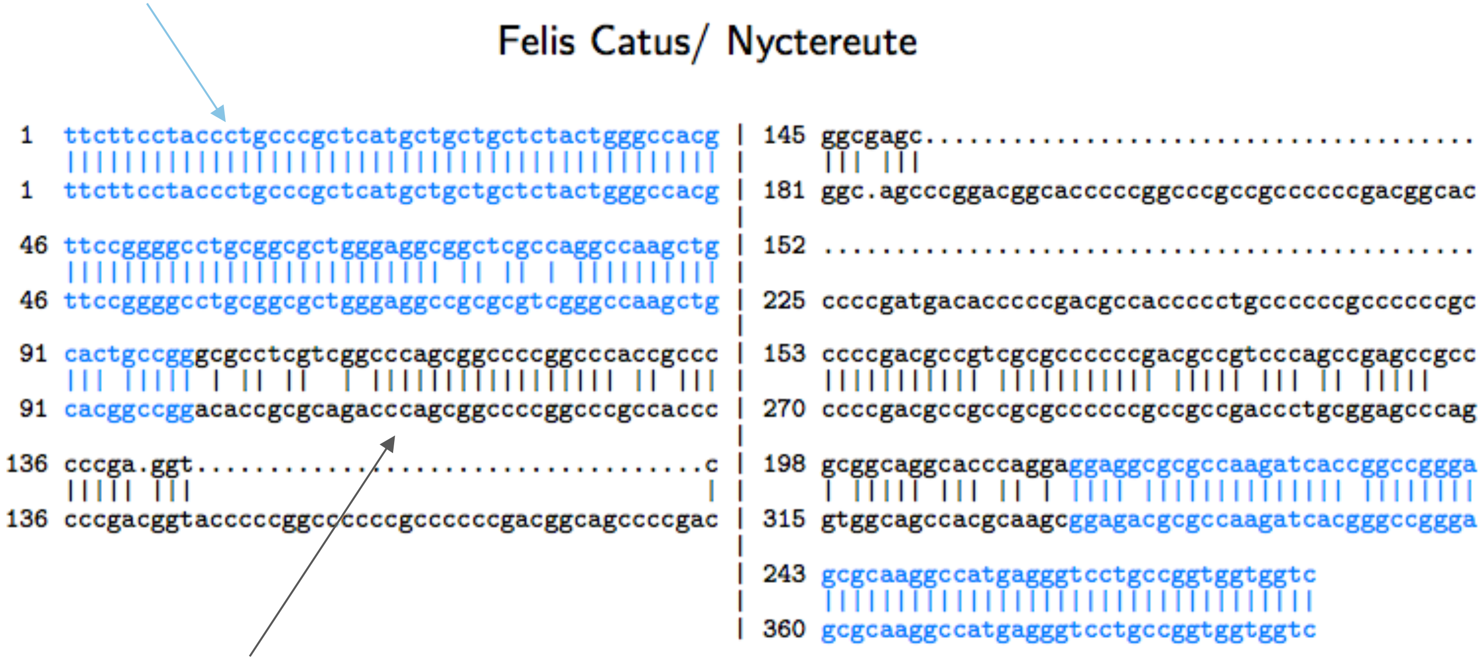
```
KSA_ARATH          501 ENDVWIGKTLYRMPYVNNNGYLELAKQDYNNCQAQHQLEWDIFQKWYEEN     550
                       ||||||||||||||||||||||||||||||||||.||||||.||||||||
XP_002872809.      500 ENDVWIGKTLYRMPYVNNNGYLELAKQDYNNCQALHQLEWDTFQKWYEEN     549

KSA_ARATH          551 RLSEWGVRRSELLECYYLAAATIFESERSHERMVWAKSSVLVKAISSSFG     600
                       ||:|||||||||||||:|||||||||||||||:|||||||||||| ||||
XP_002872809.      550 RLNEWGVRRSELLECYFLAAATIFESERSHERIVWAKSSVLVKAI-SSFG     598

KSA_ARATH          601 ESSDSRRSFSDQFHEYIANARRSDHHFNDRNMRLDRPGSVQASRLAGVLI     650
                       :|||||||||:|||:|||||||||||||.|:||||||||||||||.|:||
XP_002872809.      599 KSSDSRRSFSEQFHKYIANARRSDHHFNGRSMRLDRPGSVQASRLVGILI     648

KSA_ARATH          651 GTLNQMSFDLFMSHGRDVNNLLYLSWGDWMEKWKLYGDEGEGELMVKMII     700
                       ||||||||||||||||||.||||.|
XP_002872809.      649 GTLNQMSFDLFMSHGRDVYNLLYQS-------------------------     673

KSA_ARATH          701 LMKNNDLTNFFTHTHFVRLAEIINRICLPRQYLKARRNDEKEKTIKSMEK     750
                                                         |||||||||||:|||.
XP_002872809.      674 ----------------------------------ARRNDEKEKTIRSMET     689

KSA_ARATH          751 EMGKMVELALSESDTFRDVSITFLDVAKAFYYFALCGDHLQTHISKVLFQ     800
                       ||.||||||||||||||.||||||||||||||.|.|||||||||||||||
XP_002872809.      690 EMEKMVELALSESDTFRVVSITFLDVAKAFYYSASCGDHLQTHISKVLFQ     739

KSA_ARATH          801 KV-    802
                       ||
XP_002872809.      740 KVL    742
```

End HSP1 with Blast

Start HSP2 with Blast

Blast vs global alignment

Blast
alignment



Felis Catus/ Nyctereute

```
  1 ttcttcctaccctgcccgctcatgctgctgctctactgggccacg | 145 ggcgagc......................................
    ||||||||||||||||||||||||||||||||||||||||||||| |     ||| |||
  1 ttcttcctaccctgcccgctcatgctgctgctctactgggccacg | 181 ggc.agcccggacggcacccccggcccgccgccccccgacggcac
                                                  |
 46 ttccggggcctgcggcgctgggaggcggctcgccaggccaagctg | 152 .............................................
    |||||||||||||||||||||||||| || || | |||||||||| |
 46 ttccggggcctgcggcgctgggaggccgcgcgtcgggccaagctg | 225 ccccgatgacacccccgacgccaccccctgccccccgccccccgc
                                                  |
 91 cactgccgggcgcctcgtcggcccagcggccccggcccaccgccc | 153 ccccgacgccgtcgcgccccccgacgccgtcccagccgagccgcc
    ||| ||||| | || ||  | ||||||||||||||||| || ||| |     ||||||||||| ||||||||||| ||||| ||| || |||||
 91 cacggccggacaccgcgcagacccagcggccccggcccgccaccc | 270 ccccgacgccgccgcgccccccgccgccgaccctgcggagcccag
                                                  |
136 cccga.ggt...................................c | 198 gcggcaggcacccaggaggaggcgcgccaagatcaccggccggga
    ||||| |||                                   | |     | ||||| ||| || | |||| |||||||||||||| ||||||||
136 cccgacggtacccccggccccccgccccccgacggcagccccgac | 315 gtggcagccacgcaagcggagacgcgccaagatcacgggccggga
                                                  |
                                                  | 243 gcgcaaggccatgagggtcctgccggtggtggtc
                                                  |     ||||||||||||||||||||||||||||||||||
                                                  | 360 gcgcaaggccatgagggtcctgccggtggtggtc
```

Global
alignment
global

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