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Canadian Bioinformatics Workshops

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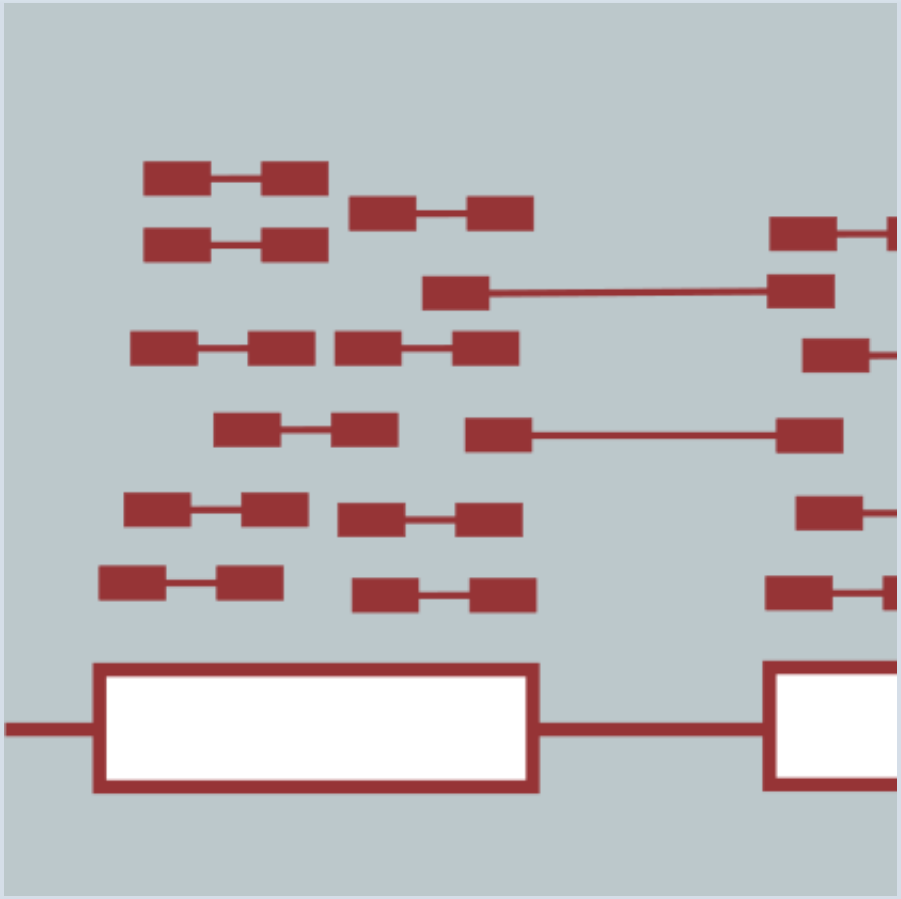
Functional Annotation and Analysis of Transcripts

Brian Haas

Informatics for RNA-Seq Analysis

May 28-30, 2018

bioinformatics.ca



Learning Objectives of Module

- Explore methods to glean biological function from transcript sequences.
- Differentiate between homology-based and sequence composition-based functional inference.

Transcript Functional Annotation

GGAGCTGGAGGCCCCCAGGCAACTACACCGTCCACGTACCCAGAGGGGCTGGGCCCTCCC
ACCAGAGACCACGCCCTGGTGTGCCTTAGGGGCCCTGGTTTGTTAGTCTCTGAGTGTGCA
GTTGCTGCACATGGGGCCCTGGCGCTTGCTGCACCAACTTCCTGTTGGGCCCGTGGTCCT
TGGAGGCATGCAGTTCAGCAGACAGTGACTCAGCCATCCACCCAACATGCGGAACGTGTC
TCTTCTGCAGGTCCCGGTCCACAGCAGGATTCCCCCTCTGTGAAAAGGCACGCTGATCTG
TCTGGA TCGAC
TCTCCC TCCCA
AAAGAC CCTGG
GGCTTC CCTAA
TGACCT TGCTG
GAAAAC CAGCC
TTGTCA TTCCA
GGAAGCACATAATTGAAGGACTGAAAGCGTCCCTGGAGCGGCTGCAGCTGGAGTACGTGG
ATGTGGTTTTTGCCAACCGCCCAGACCCCAACACGCCCATGGAAGAGACCGTGCGGGCCA
TGACCCATGTCATCAACCAGGGGATGGCCATGTACTGGGGCACATCACGCTGGAGCTCCA
TGGAGATCATGGAGGCCTACTCGGTGGCTCGGCAGTTCAACCTGATCCCGCCCATCTGCG
AGCAAGCGGAATATCACATGTTCCAGAGGGAGAAGGTGGAGGTCCAGCTGCCAGAGCTGT
TCCACAAGATAGGAGTAGGTGCCATGACCTGGTCCCCTCTGGCGTGCGGCATCGTCTCAG
GGAAGTATGACAGCGGGATCCCACCCTACTCCAGAGCCTCCCTGAAGGGCTACCAGTGGT
TGAAGGACAAGATCCTGAGTGAGGAGGGTCGCCGCCAGCAGGCCAAGCTGAAGGAACTGC

Can we gather hints of biological function from sequence?

Methods used to predict function from sequence

- Sequence homology

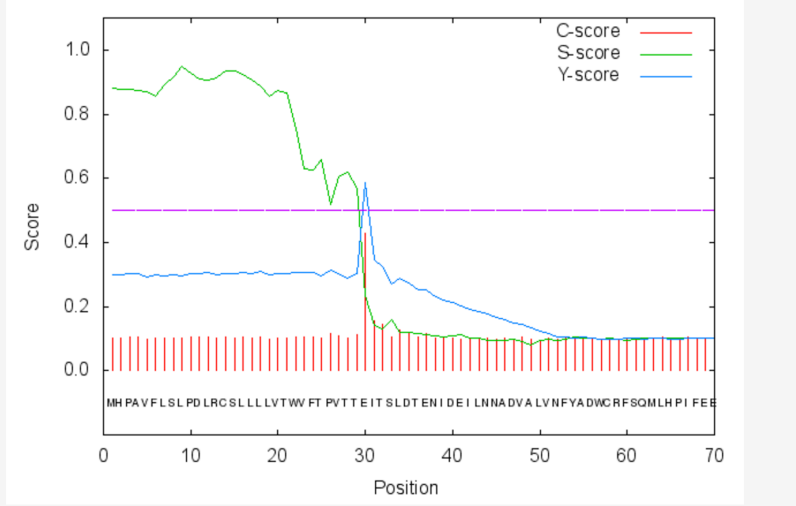
Searching protein database for sequence similarity

```
Query           THVHRPYNEHKSLSGTARYMSINTHLGREQSRRDDLESMGHVFMYFLRGSLPW--QGLKA
                T    P +  K   GT  Y S + HLG    RR DLE +G      L   LPW  Q L A
Database Match  TGDFKP-DPKKMHNGTIEYTSRDAHLG-VPTRRADLEILGYNLIEWLGAELPWVTQKLLA
```

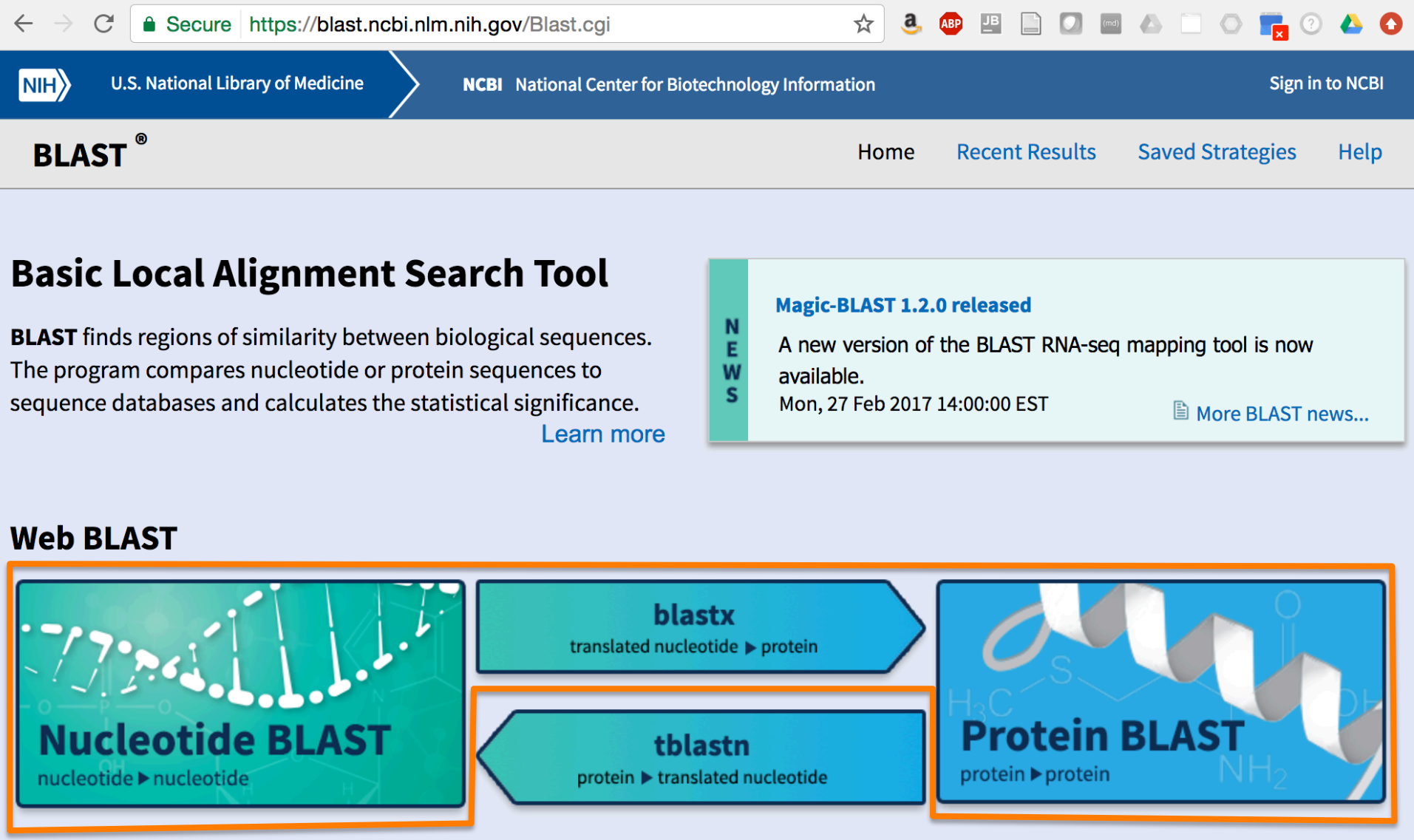
- Sequence composition

Predict functions of sequence using machine learning methods for pattern recognition.

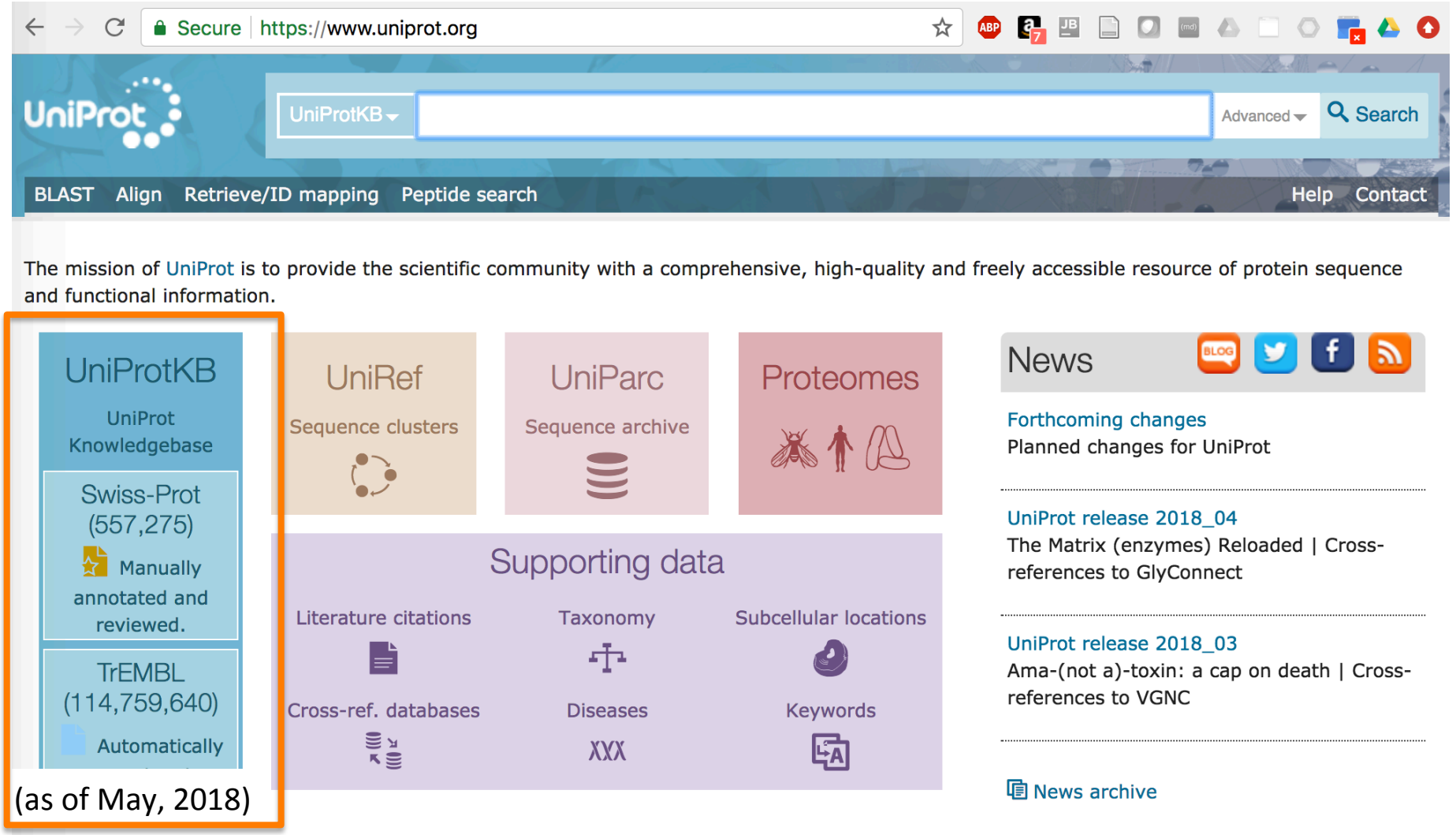
- Neural Networks
- Hidden Markov Models



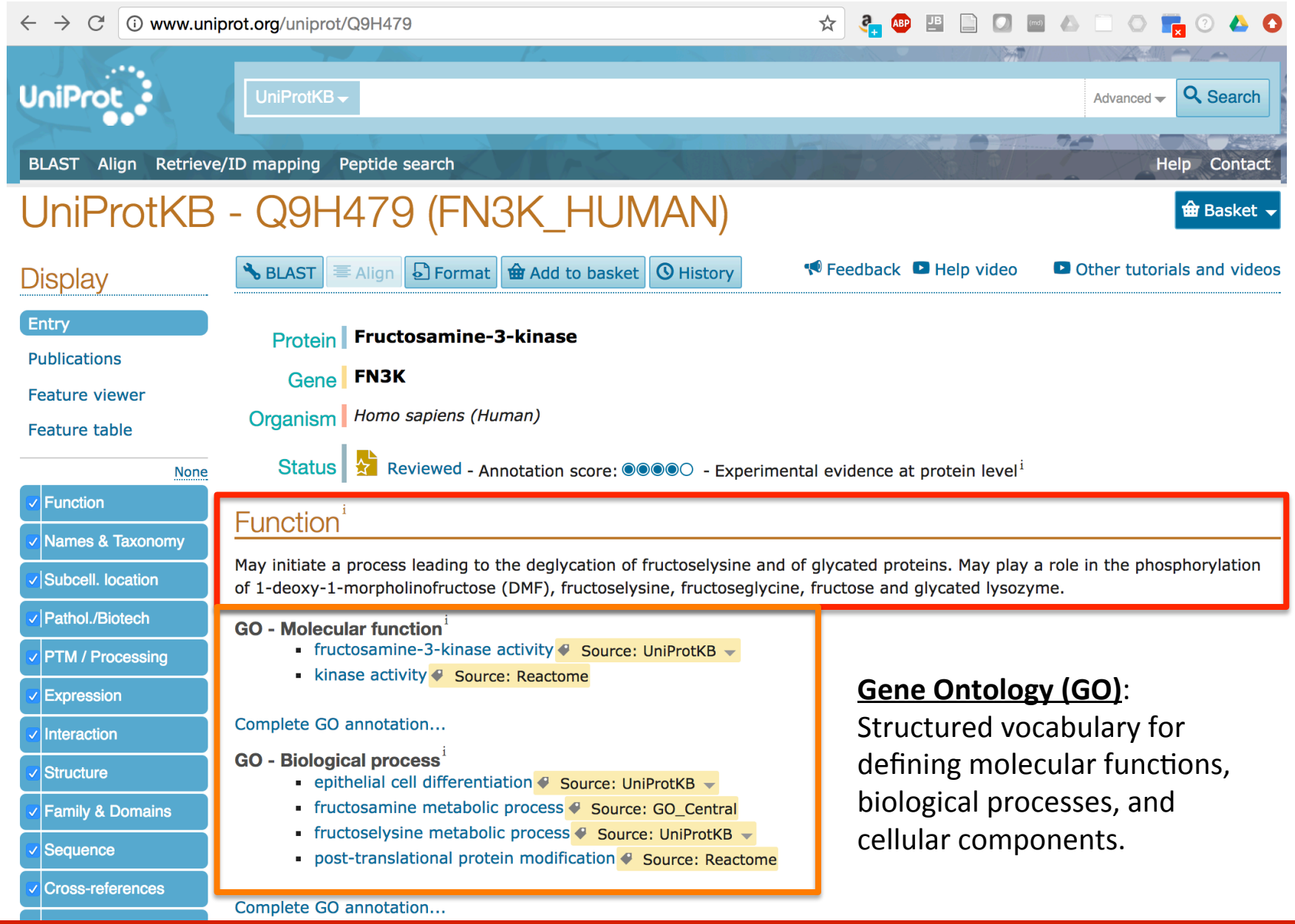
Use BLAST to search for sequence similarity to known proteins



The Swiss-Prot database is a valuable source of proteins with known functions

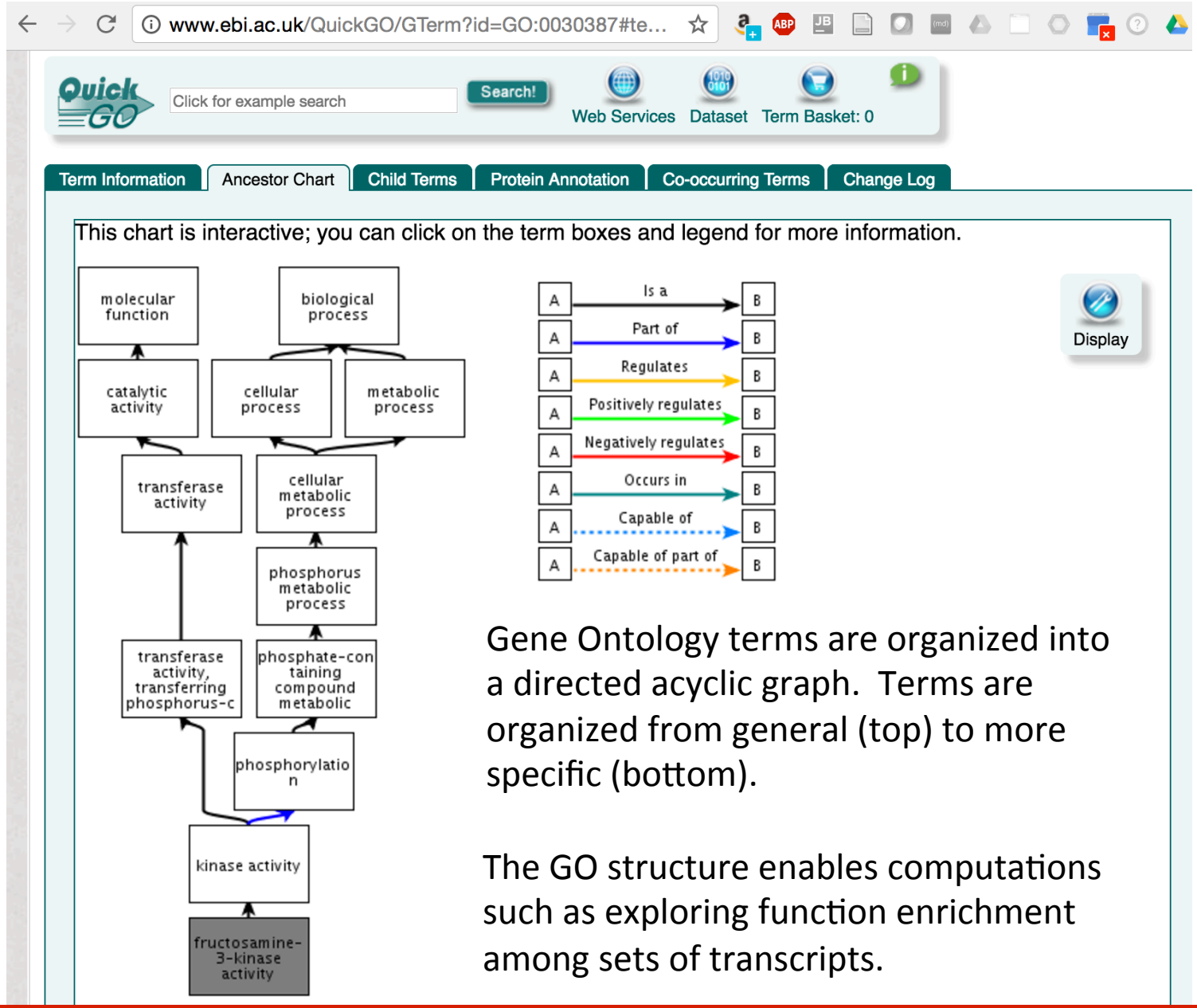


Example of a Swiss-Prot Record



Gene Ontology (GO):
Structured vocabulary for defining molecular functions, biological processes, and cellular components.

Gene Ontology: a structured relational vocabulary for describing biological functions



Gene Ontology terms are organized into a directed acyclic graph. Terms are organized from general (top) to more specific (bottom).

The GO structure enables computations such as exploring function enrichment among sets of transcripts.

Gene ontology functional enrichment

	(+) Differentially Expressed	(-) Not Differentially Expressed	Totals
+ Gene Ontology	50	200	250
- Gene Ontology	1950	17800	19750
Totals	2000	18000	20000

	drawn	not drawn	total
green marbles	k	$K - k$	K
red marbles	$n - k$	$N + k - n - K$	$N - K$
total	n	$N - n$	N

The probability of drawing exactly k green marbles can be calculated by the formula

$$P(X = k) = f(k; N, K, n) = \frac{\binom{K}{k}\binom{N-K}{n-k}}{\binom{N}{n}}.$$

No significant sequence similarity… What else?

GGAGCTGGAGGCCCCCAGGCAACTACACCGTCCACGTACCCAGAGGGGCTGGGCCCTCCC
ACCAGAGACCACGCCCTGGTGTGCCTTAGGGGCCCTGGTTTGTTAGTCTCTGAGTGTGCA
GTTGCTGCACATGGGGCCCTGGCGCTTGCTGCACCAACTTCCTGTTGGGCCCGTGGTCCT
TGGAGGCATGCAGTTCAGCAGACAGTGACTCAGCCATCCACCCAACATGCGGAACGTGTC
TCTTCTGCAGGTCCCGGTCCACAGCAGGATTCCCCCTCTGTGAAAAGGCACGCTGATCTG
TCTGGATAAGTGTGGCCGGCCCCATGTATCCGGAATCAACCACGGGGTCCCCAGCTCGAC
TCTCCCTGCGGCAGACAGGCTCCCCCGGGATGATCTACAGTACTCGTTATGGGAGTCCCA
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GGCTTGGAACATGGGTGACCTTCGGGGGCCAGATCACGGATGAGATGGCAGAGCACCTAA
TGACCTTGGCCTACGATAATGGCATCAACCTGTTCGATACGGCGGAGGTCTACGCTGCTG
GAAAAGCTGAAGTGGTATTAGGGAACATCATTAAGAAGAAGGGATGGAGACGGTCCAGCC
TTGTCATCACCACCAAGATCTTCTGGGGTGGAAAAGCGGAGACTGAGAGAGGCCTTTCCA
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TGACCCATGTCATCAACCAGGGGATGGCCATGTACTGGGGCACATCACGCTGGAGCTCCA
TGGAGATCATGGAGGCCTACTCGGTGGCTCGGCAGTTCAACCTGATCCCGCCCATCTGCG
AGCAAGCGGAATATCACATGTTCCAGAGGGAGAAGGTGGAGGTCCAGCTGCCAGAGCTGT
TCCACAAGATAGGAGTAGGTGCCATGACCTGGTCCCCTCTGGCGTGCGGCATCGTCTCAG
GGAAGTATGACAGCGGGATCCCACCCTACTCCAGAGCCTCCCTGAAGGGCTACCAGTGGT
TGAAGGACAAGATCCTGAGTGAGGAGGGTCGCCGCCAGCAGGCCAAGCTGAAGGAACTGC

Is there an ORF for a potential Coding Region?

GGAGCTGGAGGCCCCCAGGCAACTACACCGTCCACGTACCCAGAGGGGCTGGGCCCTCCC
ACCAGAGACCACGCCCTGGTGTGCCTTAGGGGCCCTGGTTTGTTAGTCTCTGAGTGTGCA
GTTGCTGCACATGGGGCCCTGGCGCTTGCTGCACCAACTTCCTGTTGGGCCCGTGGTCCT
TGGAGGCATGCAGTTCAGCAGACAGTGACTCAGCCATCCACCCAACATGCGGAACGTGTC
TCTTCTGCAGGTCCCGGTCCACAGCAGGATTCCCCCTCTGTGAAAAGGCACGCTGATCTG
TCTGGATAAGTGTGGCCGGCCCCATGTATCCGGAATCAACCACGGGGTCCCCAGCTCGAC
TCTCCCTGCGGCAGACAGGCTCCCCCGGGATGATCTACAGTACTCGTTATGGGAGTCCCA
AAAGACAGCTCCAGTTTTACAGGAATCTGGGCAAATCTGGCCTTCGGGTCTCCTGCCTGG
GGCTTGGAACATGGGTGACCTTCGGGGGCCAGATCACGGATGAGATGGCAGAGCACCTAA
TGACCTTGGCCTACGATAATGGCATCAACCTGTTCGATACGGCGGAGGTCTACGCTGCTG
GAAAAGCTGAAGTGGTATTAGGGAACATCATTAAGAAGAAGGGATGGAGACGGTCCAGCC
TTGTCATCACCACCAAGATCTTCTGGGGTGGAAAAGCGGAGACTGAGAGAGGCCTTTCCA
GGAAGCACATAATTGAAGGACTGAAAGCGTCCCTGGAGCGGCTGCAGCTGGAGTACGTGG
ATGTGGTTTTTGCCAACCGCCCAGACCCCAACACGCCCATGGAAGAGACCGTGCGGGCCA
TGACCCATGTCATCAACCAGGGGATGGCCATGTACTGGGGCACATCACGCTGGAGCTCCA
TGGAGATCATGGAGGCCTACTCGGTGGCTCGGCAGTTCAACCTGATCCCGCCCATCTGCG
AGCAAGCGGAATATCACATGTTCCAGAGGGAGAAGGTGGAGGTCCAGCTGCCAGAGCTGT
TCCACAAGATAGGAGTAGGTGCCATGACCTGGTCCCCTCTGGCGTGCGGCATCGTCTCAG
GGAAGTATGACAGCGGGATCCCACCCTACTCCAGAGCCTCCCTGAAGGGCTACCAGTGGT
TGAAGGACAAGATCCTGAGTGAGGAGGGTCGCCGCCAGCAGGCCAAGCTGAAGGAACTGC

Is there an ORF for a potential Coding Region?

GGAGCTGGAGGCCCCCAGGCAACTACACCGTCCACGTACCCAGAGGGGCTGGGCCCTCCC
ACCAGAGACCACGCCCTGGTGTGCCTTAGGGGCCCTGGTTTGTTAGTCTCTGAGTGTGCA
GTTGCTGCAC**ATGGGGCCCTGGCGCTTGCTGCACCAACTTCCTGTTGGGCCCGTGGTCCT**
TGGAGGCATGCAGTTCAGCAGACAGTGACTCAGCCATCCACCCAACATGCGGAACGTGTC
TCTTCTGCAGGTCCCGGTCCACAGCAGGATTCCCCCTCTGTGAAAAGGCACGCTGATCTG
TCTGGATAAGTGTGGCCGGCCCCATGTATCCGGAATCAACCACGGGGTCCCCAGCTCGAC
TCTCCCTGCGGCAGACAGGCTCCCCCGGGATGATCTACAGTACTCGTTATGGGAGTCCCA
AAAGACAGCTCCAGTTTTACAGGAATCTGGGCAAATCTGGCCTTCGGGTCTCCTGCCTGG
GGCTTGGAACATGGGTGACCTTCGGGGGCCAGATCACGGATGAGATGGCAGAGCACCTAA
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GAAAAGCTGAAGTGGTATTAGGGAACATCATTAAGAAGAAGGGATGGAGACGGTCCAGCC
TTGTCATCACCACCAAGATCTTCTGGGGTGGAAAAGCGGAGACTGAGAGAGGCCTTTCCA
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TGACCCATGTCATCAACCAGGGGATGGCCATGTACTGGGGCACATCACGCTGGAGCTCCA
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GGAAGTATGACAGCGGGATCCCACCCTACTCCAGAGCCTCCCTGAAGGGCTACCAGTGGT
TGAAGGACAAGATCCTGAGTGAGGAGGGTCGCCGCCAGCAGGCCAAGCTGAAGGAACTGC

Find all ORFs using ORFfinder

Secure https://www.ncbi.nlm.nih.gov/orffinder/

NCBI Resources How To Sign in to NCBI

ORFfinder PubMed Search

Open Reading Frame Finder

ORF finder searches for open reading frames (ORFs) in the DNA sequence you enter. The program returns the range of each ORF, along with its protein translation. Use ORF finder to search newly sequenced DNA for potential protein encoding segments, verify predicted protein using newly developed SMART BLAST or regular BLASTP.

This web version of the ORF finder is limited to the subrange of the query sequence up to 50 kb long. Stand-alone version, which doesn't have query sequence length limitation, is available for Linux x64.

Examples (click to set values, then click Submit button) :

- NC_011604 Salmonella enterica plasmid pWES-1; genetic code: 11; 'ATG' and alternative initiation codons; minimal ORF length: 300 nt
- NM_000059; genetic code: 1; start codon: 'ATG only'; minimal ORF length: 150 nt

Enter Query Sequence

Enter accession number, gi, or nucleotide sequence in FASTA format:

```
GGAGCTGGAGGCCCCCAGGCAACTACACCGTCCACGTACCCAGAGGGGCTGGGCCCTCCC
ACCAGAGACCACGCCCTGGTGTGCCTTAGGGGCCCTGGTTTGTTAGTCTCTGAGTGTGCA
GTTGCTGCACATGGGGCCCTGGCGCTTGCTGCACCAACTTCCTGTTGGGCCCGTGGTCCT
TGGAGGCATGCAGTTCAGCAGACAGTGACTCAGCCATCCACCCAACATGCGGAACGTGTC
TCTTCTGCAGGTCCCGGTCCACAGCAGGATTCCCCCTCTGTGAAAAGGCACGCTGATCTG
TCTGGATAAGTGTGGCCGGCCCCATGTATCCGGAATCAACCACGGGGTCCCCAGCTCGAC
TCTCCCTGCGGCAGACAGGCTCCCCCGGGATGATCTACAGTACTCGTTATGGGAGTCCCA
AAAGACAGCTCCAGTTTTACAGGAATCTGGGCAAATCTGGCCTTCGGGTCTCCTGCCTGG
GGCTTGGAACATGGGTGACCTTCGGGGGCCAGATCACGGATGAGATGGCAGAGCACCTAA
TGACCTTGGCCTACGATAATGGCATCAACCTGTTCGATACGGCGGAGGTCTACGCTGCTG
```

From: To:

ORFfinder finds all open reading frames and provides translations

Secure https://www.ncbi.nlm.nih.gov/orffinder/

NCBI Resources How To Sign in to NCBI

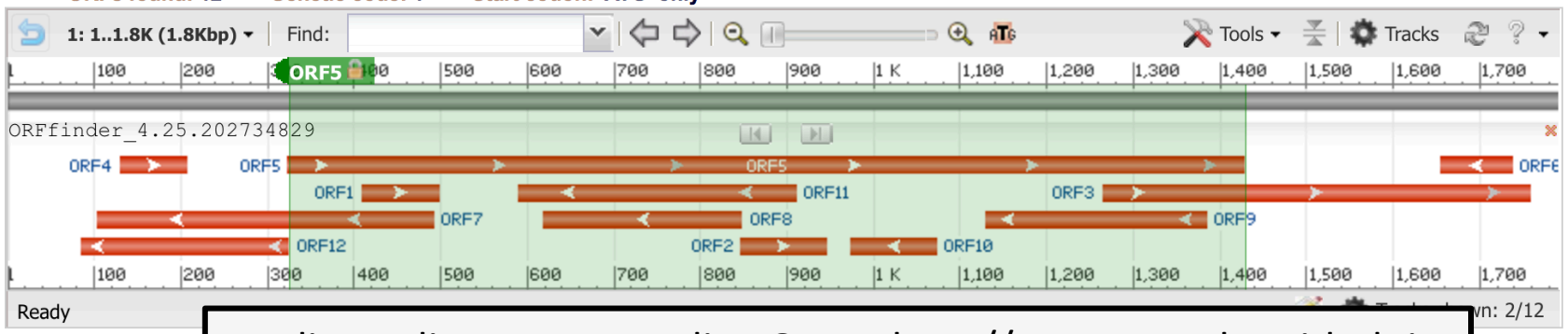
ORFfinder PubMed Search

Open Reading Frame Viewer

Sequence

ORFs can appear in random sequence – so further analysis is required

ORFs found: 12 Genetic code: 1 Start codon: 'ATG' only



Predict coding vs. non-coding ORFs: http://TransDecoder.github.io

Add six-frame translation track

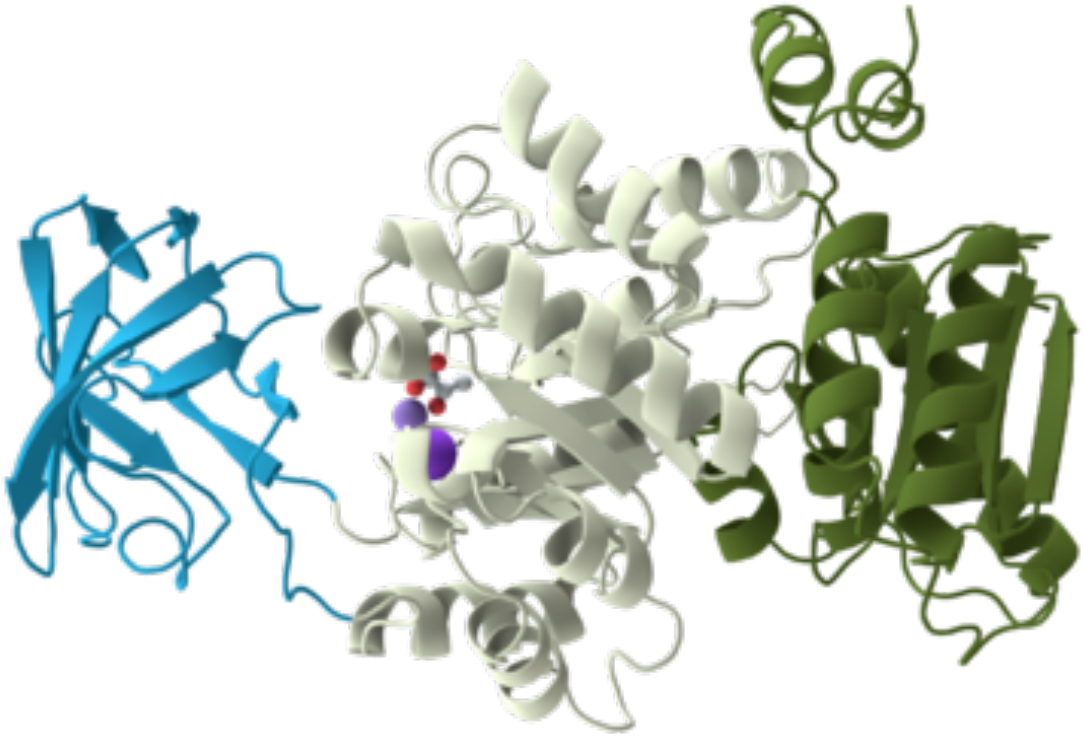
ORF5 (367 aa) Display ORF as... Mark

```
>lcl|ORF5
MYPESTTGSPARLSLRQTGSPGMIYSTRYGSPKRQLQFYR
NLGKSGLRVSCLGLGTWVTFGGQITDEMAEHLMTLAYDNG
INLFDTAEVYAAGKAEVVLGNIIKKKGWRRSSLVITTKIF
WGGKAETERGLSRKHIIEGLKASLERLQLEYVDVVFANRP
DPNTPMEETVRAMTHVINQGMAMYWGTSRWSSMEIMEAYS
VARQFNLIPPICEQAEYHMFQREKVEVQLPELFHKIGVGA
MTWSPLACGIVSGKYDSGIPPYSRASLKGYQWLKDKILSE
EGRRQQAKLKELQAIAERLGCTLPQLAIAWCLRNEGVSSV
LLGASNAEQLMENIGAIQVLPKLSSSIVHEIDSILGNKPY
SKKDYRS
```

Mark subset... Marked: 0 Download marked set as Protein FA

Label	Strand	Frame	Start	Stop	Length (nt |
ORF5	**+**	**3**	**324**	**1427**	**1104 | 36**
ORF3	+	1	1264	1758	495 | 16
ORF7	-	1	492	103	390 | 12
ORF11	-	3	910	590	321 | 10
ORF9	-	3	1384	1130	255 | 8
ORF12	-	3	325	86	240 | 7

Can we recognize functional domains in putative coding regions?

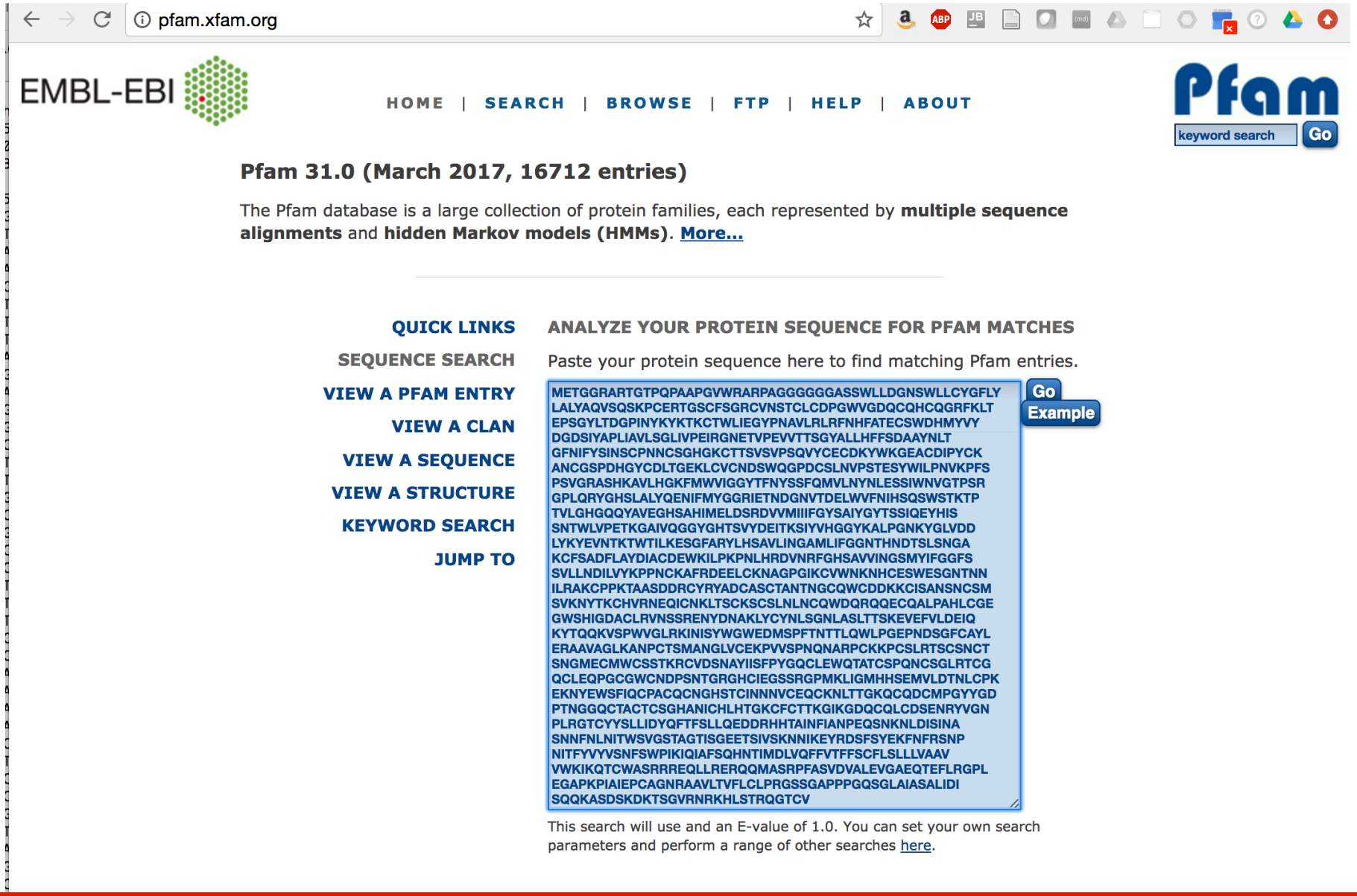


Hints at substrate binding or catalytic activity

DNA, RNA, calcium, phoshate, etc.

Glycoslase, methylase, kinase, nuclease, lipase, protease, etc.

Search the Pfam library of HMMs to identify potential functional domains



Example Pfam report illustrating modular domain architecture

pfam.xfam.org/search/sequence

EMBL-EBI

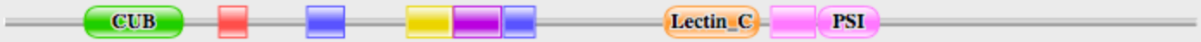
HOME | SEARCH | BROWSE | FTP | HELP | ABOUT

Pfam keyword search Go

Sequence search results

Show the detailed description of this results page.

We found **9** Pfam-A matches to your search sequence (**all** significant)



Show the search options and sequence that you submitted.

Return to the search form to look for Pfam domains on a new sequence.

Significant Pfam-A Matches

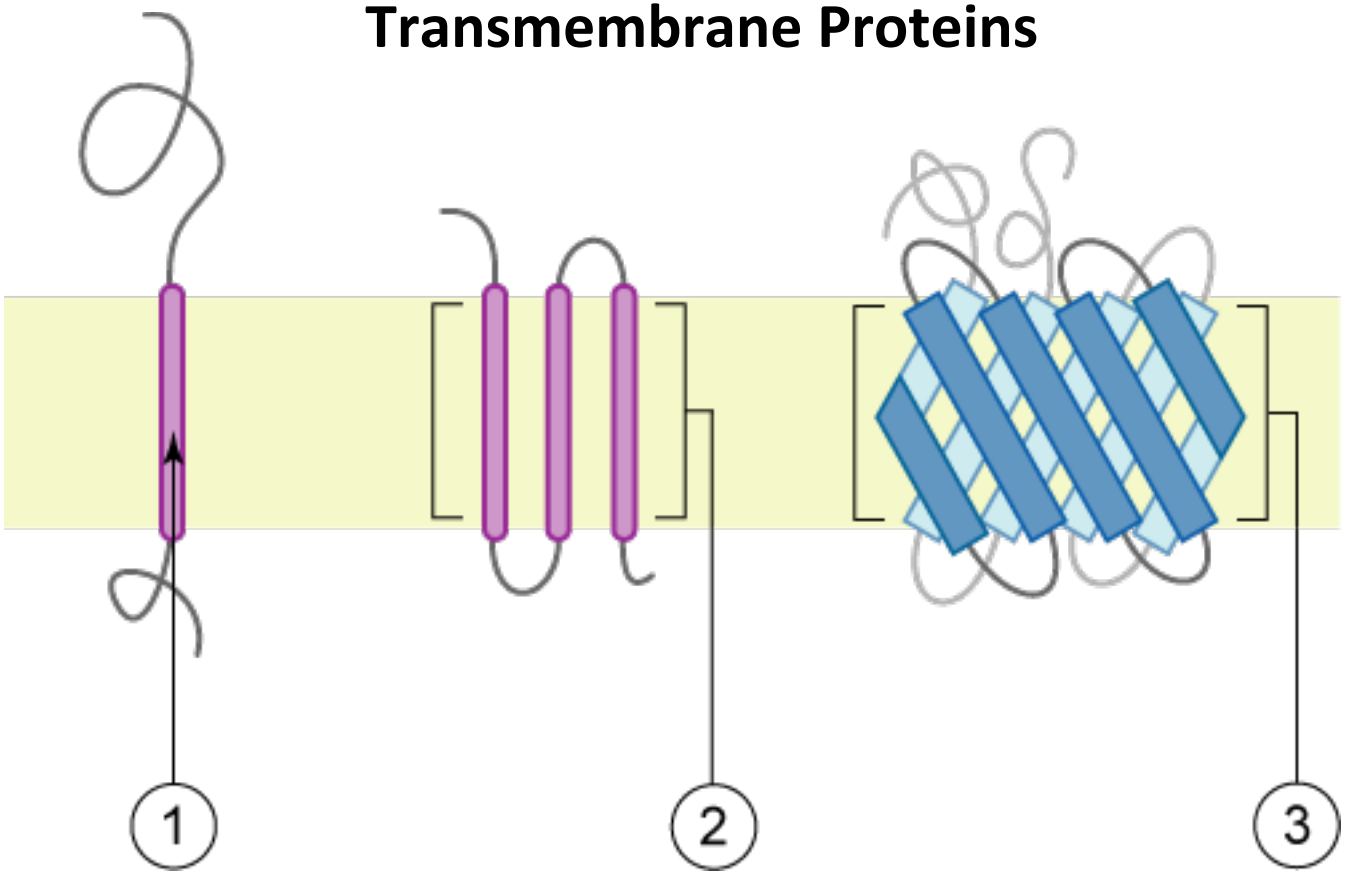
Show or hide all alignments.

Family	Description	Entry type	Clan	Envelope		Alignment		HMM		HMM length	Bit score	E-value	Predicted active sites	Show/hide alignment
				Start	End	Start	End	From	To					
CUB	CUB domain	Domain	CL0164	93	206	93	206	1	110	110	42.2	7.7e-11	n/a	Show
EGF_2	EGF-like domain	Domain	CL0001	249	280	249	280	1	32	32	22.5	0.0001	n/a	Show
Kelch_5	Kelch motif	Repeat	CL0186	351	393	352	392	**2**	**41**	42	33.7	2.2e-08	n/a	Show
Kelch_4	Galactose oxidase, central domain	Repeat	CL0186	466	518	468	514	**3**	**44**	49	20.6	0.0003	n/a	Show
Kelch_1	Kelch motif	Repeat	CL0186	520	574	520	573	1	**45**	46	20.0	0.00033	n/a	Show
Kelch_5	Kelch motif	Repeat	CL0186	579	614	581	613	**5**	**40**	42	25.3	9.7e-06	n/a	Show
Lectin_C	Lectin C-type domain	Domain	CL0056	765	874	766	874	**2**	108	108	70.2	2e-19	n/a	Show
PSI	Plexin repeat	Family	CL0630	889	939	890	938	**2**	**50**	51	27.8	2.5e-06	n/a	Show
PSI	Plexin repeat	Family	CL0630	942	1012	942	1012	1	51	51	50.0	2.9e-13	n/a	Show

Comments or questions on the site? Send a mail to **pfam-help@ebi.ac.uk**.

European Molecular Biology Laboratory

Transmembrane Proteins



Single transmembrane α-helix (bitopic membrane protein)

Polytopic transmembrane α-helical protein

Polytopic transmembrane β-sheet protein

Using TMHMM to identify putative transmembrane proteins

www.cbs.dtu.dk/services/TMHMM/

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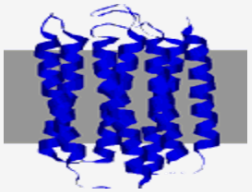
CBS >> CBS Prediction Servers >> TMHMM

TMHMM Server v. 2.0

Prediction of transmembrane helices in proteins



Instructions

SUBMISSION

Submission of a local file in FASTA format (HTML 3.0 or higher)

Choose File | No file chosen

OR by pasting sequence(s) in FASTA format:

```
MEILCEDNTSLSSIPNSLMQVDGDSGLYRNDFNSRDANSSDASNWTIDGENRTNLSFEG
YLPPTCLSILHLQEKNWSALLTAVVIILTIAGNILVIMAVSLEKKLQNATNYFLMSLAIADMLL
GFLVMPVSMLTILYGYRWPLPSKLCAVWIYLDVLFSTASIMHLCAISLDRYVAIQNPIHHSR
FNSRTKAFLKIIAVWTISVGVSMPIPVFGLQDDSKVFKQGSCLLADDNFVLIGSFVAFFIPLTI
MVITYFLTIKSLQKEATLCVSDLSTRAKLASFSFL
```

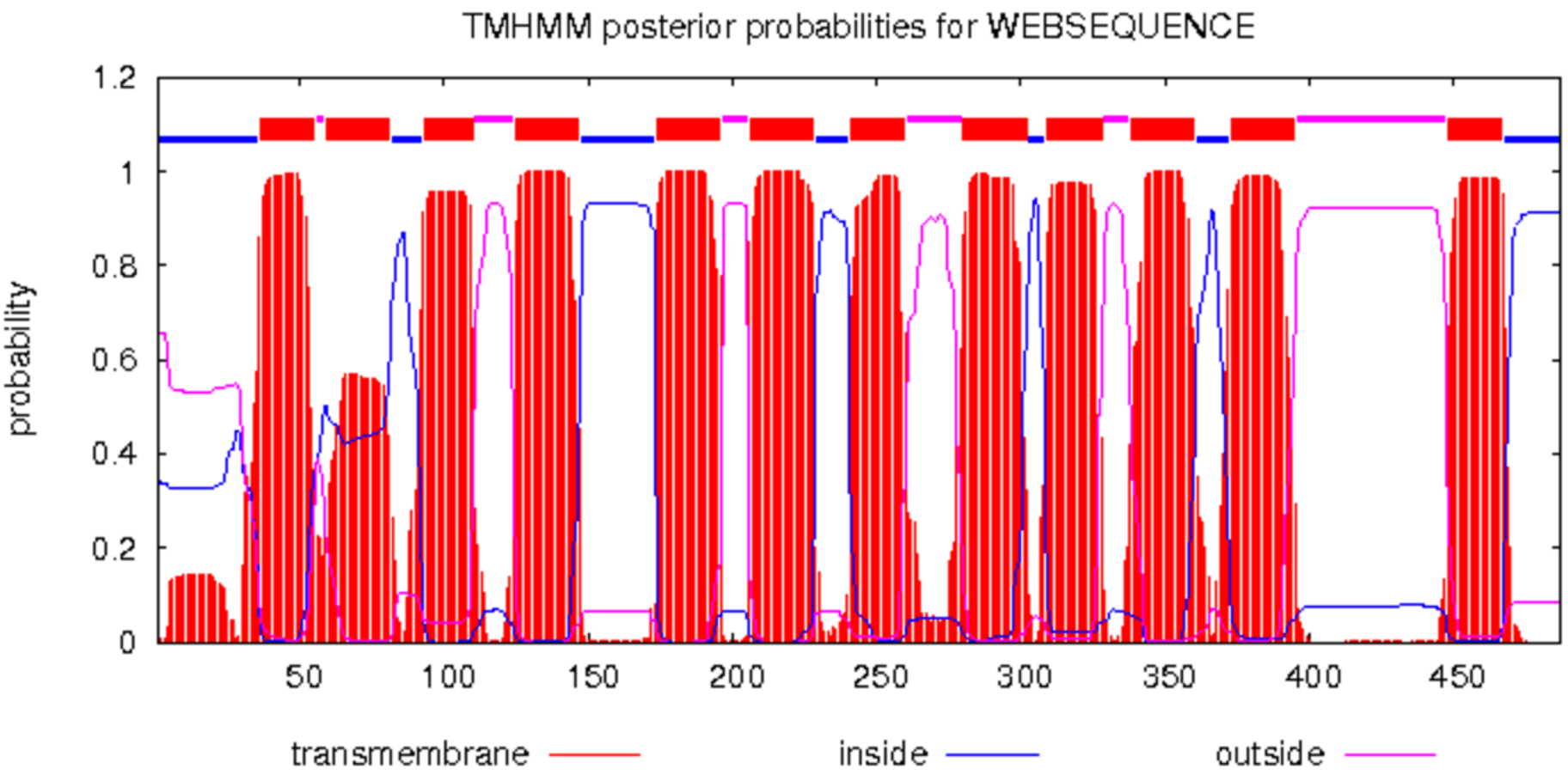
Output format:

- (•) Extensive, with graphics
- ( ) Extensive, no graphics
- ( ) One line per protein

Other options:

- [ ] Use old model (version 1)

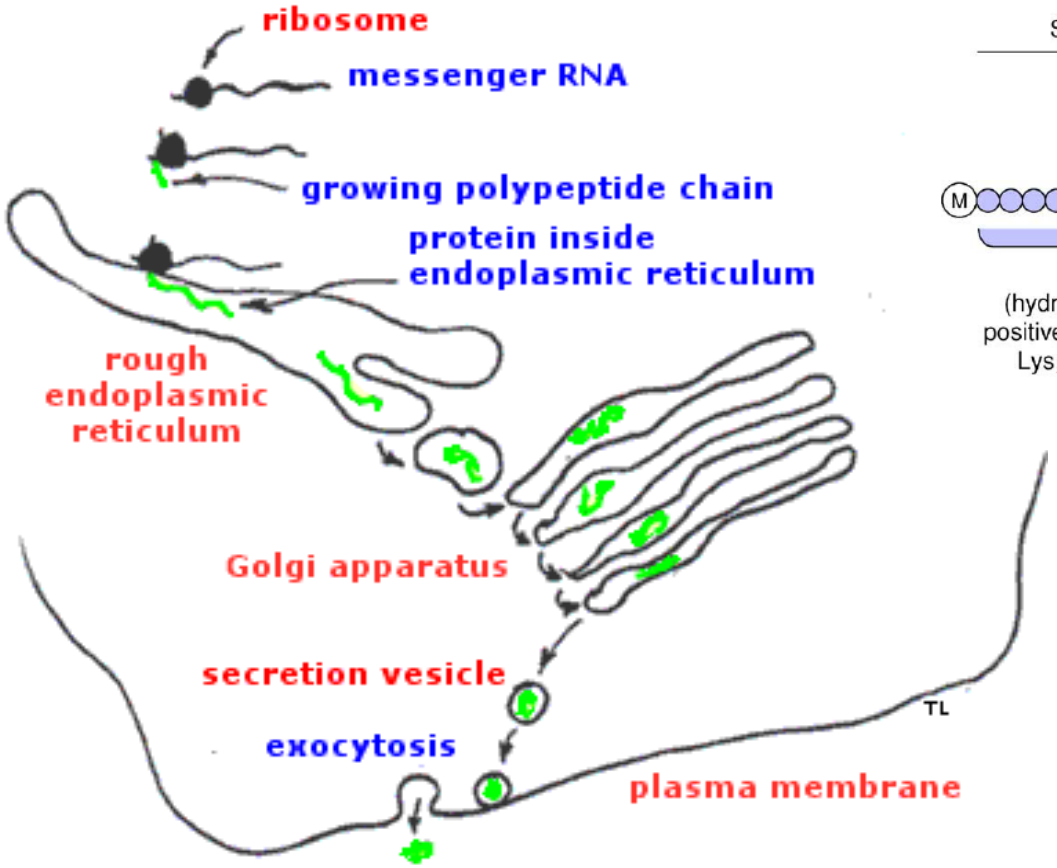
Trans-membrane Domains via TmHMM



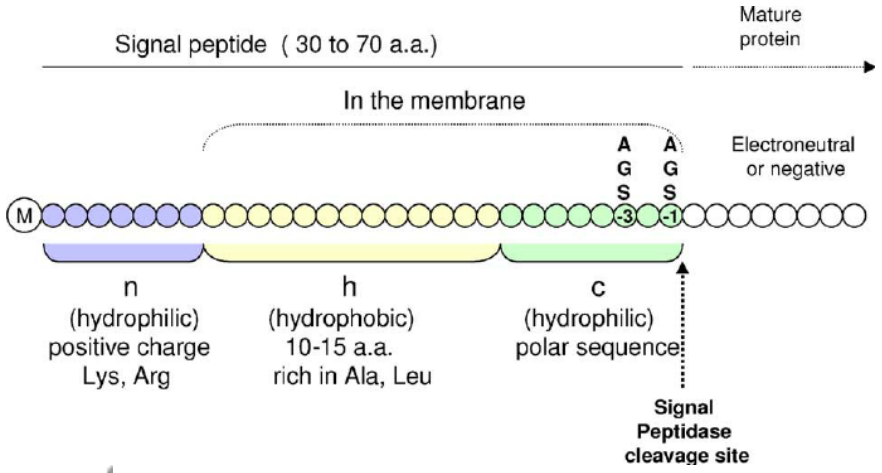
Topology=i36-55o59-81i93-110o125-147i174-196o206-228i241-260o280-302i309-328o338-360i373-395o448-467i

http://www.cbs.dtu.dk/services/TMHMM/

Predicting Secreted Proteins



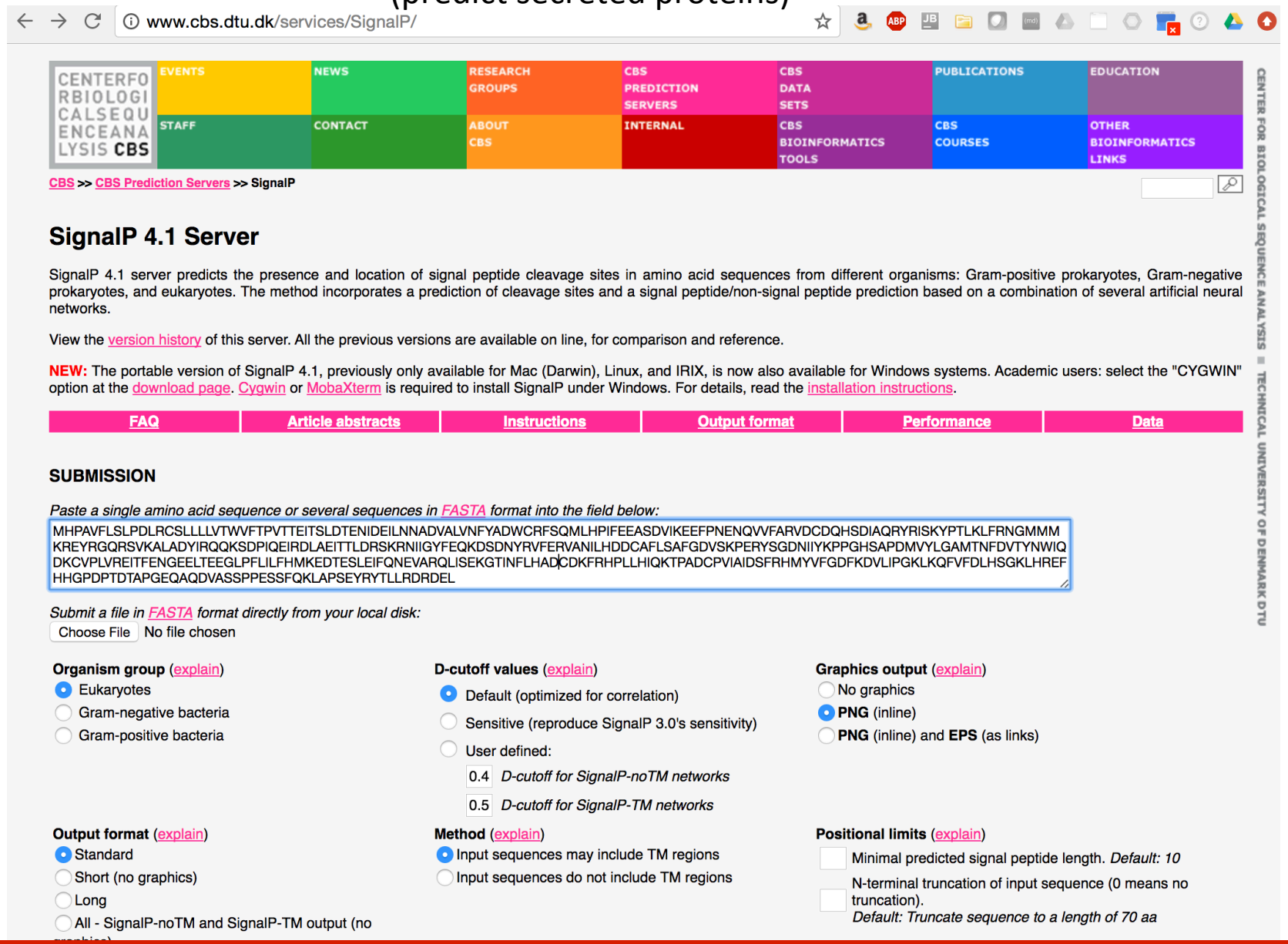
(from: https://courses.washington.edu/conj/cell/secretion.htm)



(from: Vaccine 23(15):1770-8)

SignalP: Prediction of N-terminal signal peptides

(predict secreted proteins)



Example SignalP predicted signal peptide

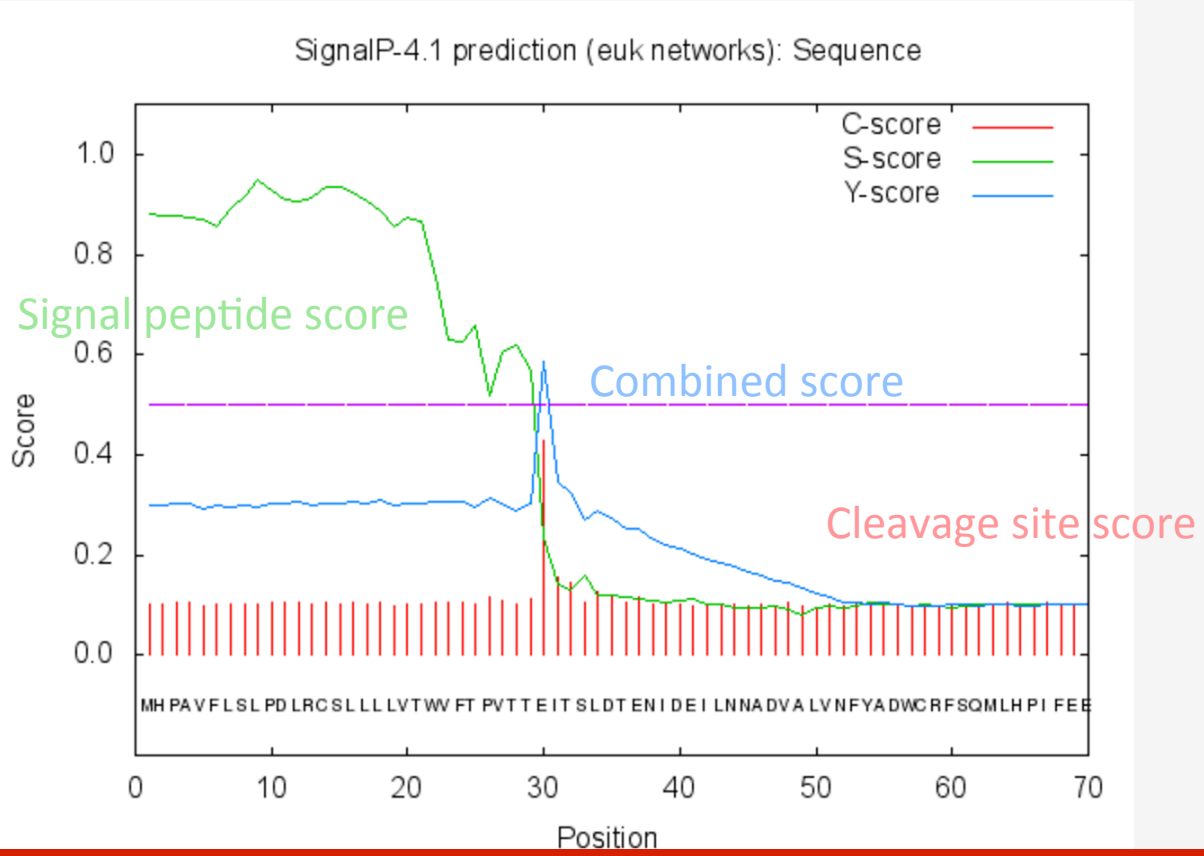
www.cbs.dtu.dk/cgi-bin/webface2.fcgi?jobid=58FFF29C00005F854B357EEA&w...

CENTERFOR BIOLOGICAL SEQUENCE ANALYSIS CBS

SignalP 4.1 Server - prediction results

Technical University of Denmark

```
# SignalP-4.1 euk predictions
>Sequence
```



Transcriptome-scale functional annotation using Trinotate

Trinotate: Transcriptome Functional Annotation and Analysis

Trinotate

TransDecoder

Trinity

Inchworm

Chrysalis

Butterfly

TMHMM

SignalP

NCBI BLAST

HMMER

Pfam

UniProt

eggNOG version 3.0

SQLite

the Gene Ontology

RNA-Seq ➡ Trinity ➡ Transcripts/Proteins ➡ Functional Data ➡ Discovery

There's no substitute for experimentally validating protein functions

nature protocols

Transcriptome Assembly is Just the End of the Beginning…

NATURE PROTOCOLS | PROTOCOL

De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis

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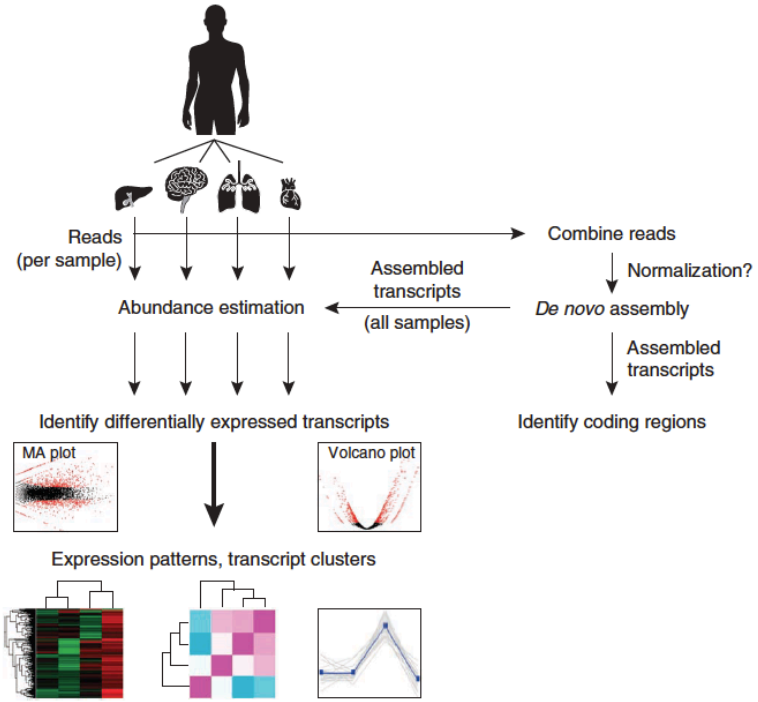
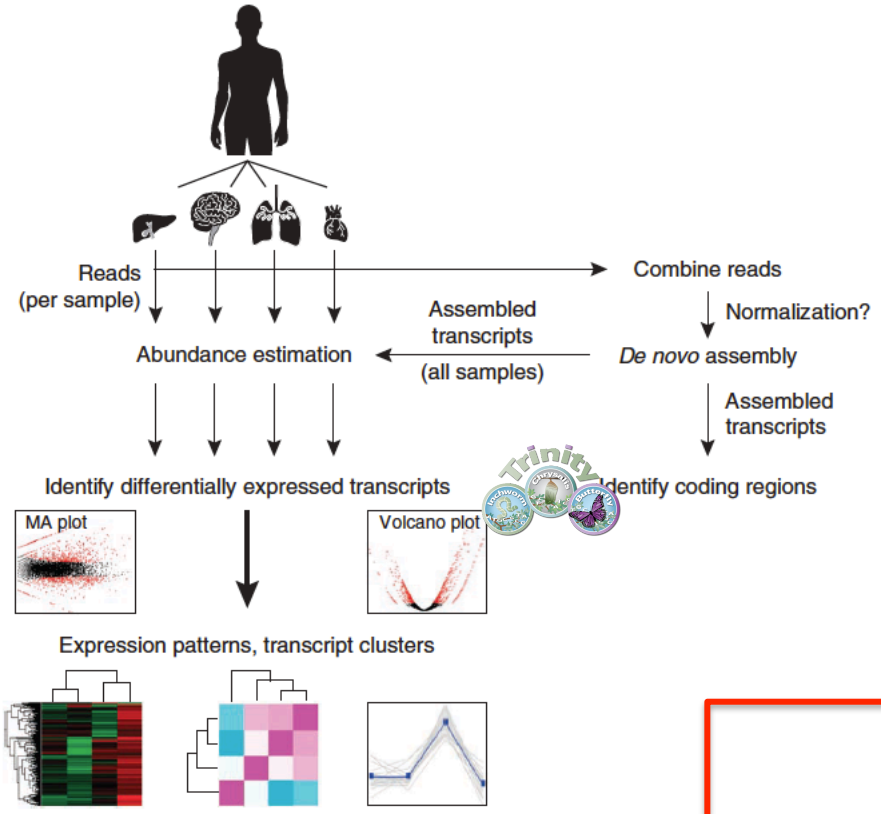
Nature Protocols **8**, 1494–1512 (2013) | doi:10.1038/nprot.2013.084

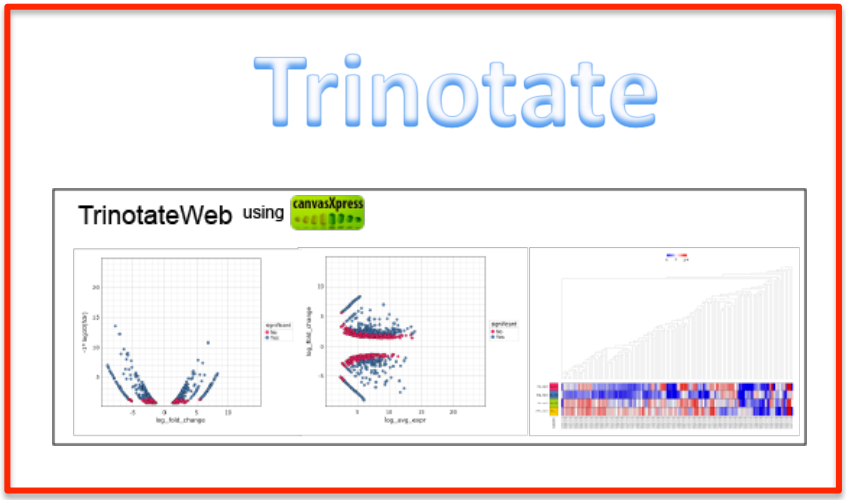
Published online 11 July 2013



Trinity Framework for De novo Transcriptome Assembly and Analysis



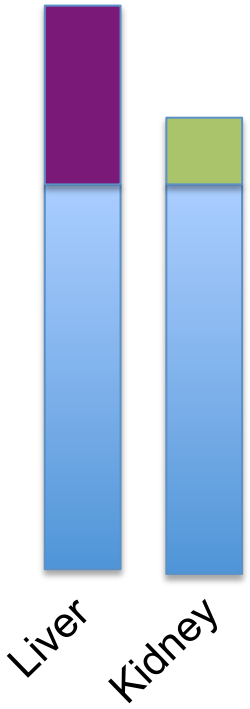
Bioconductor,
& Trinity



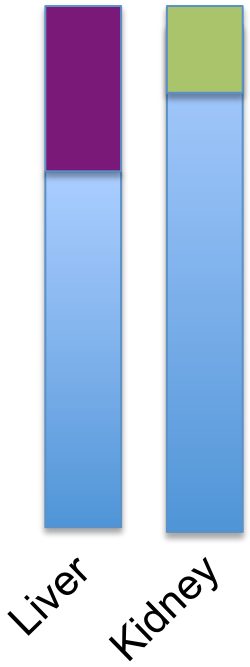
Trinotate Functional Annotation Lab

Why cross-sample normalization is important

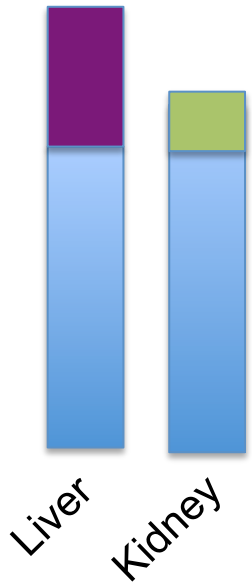
Absolute RNA quantities per cell



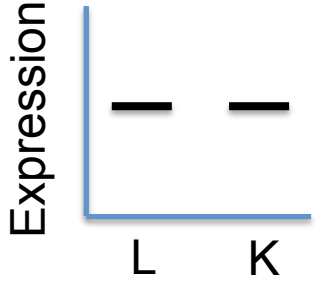
Measured relative abundance via RNA-Seq

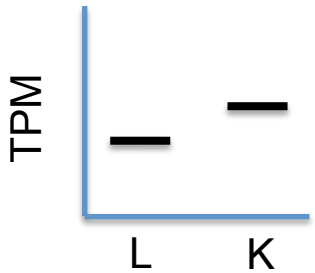


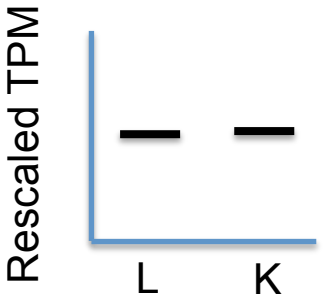
Cross-sample normalized (rescaled) relative abundance



eg. Some housekeeping gene's expression level:







Cross-sample Normalization Required
Otherwise, housekeeping genes look diff expressed due to sample composition differences

Subset of genes highly expressed in liver

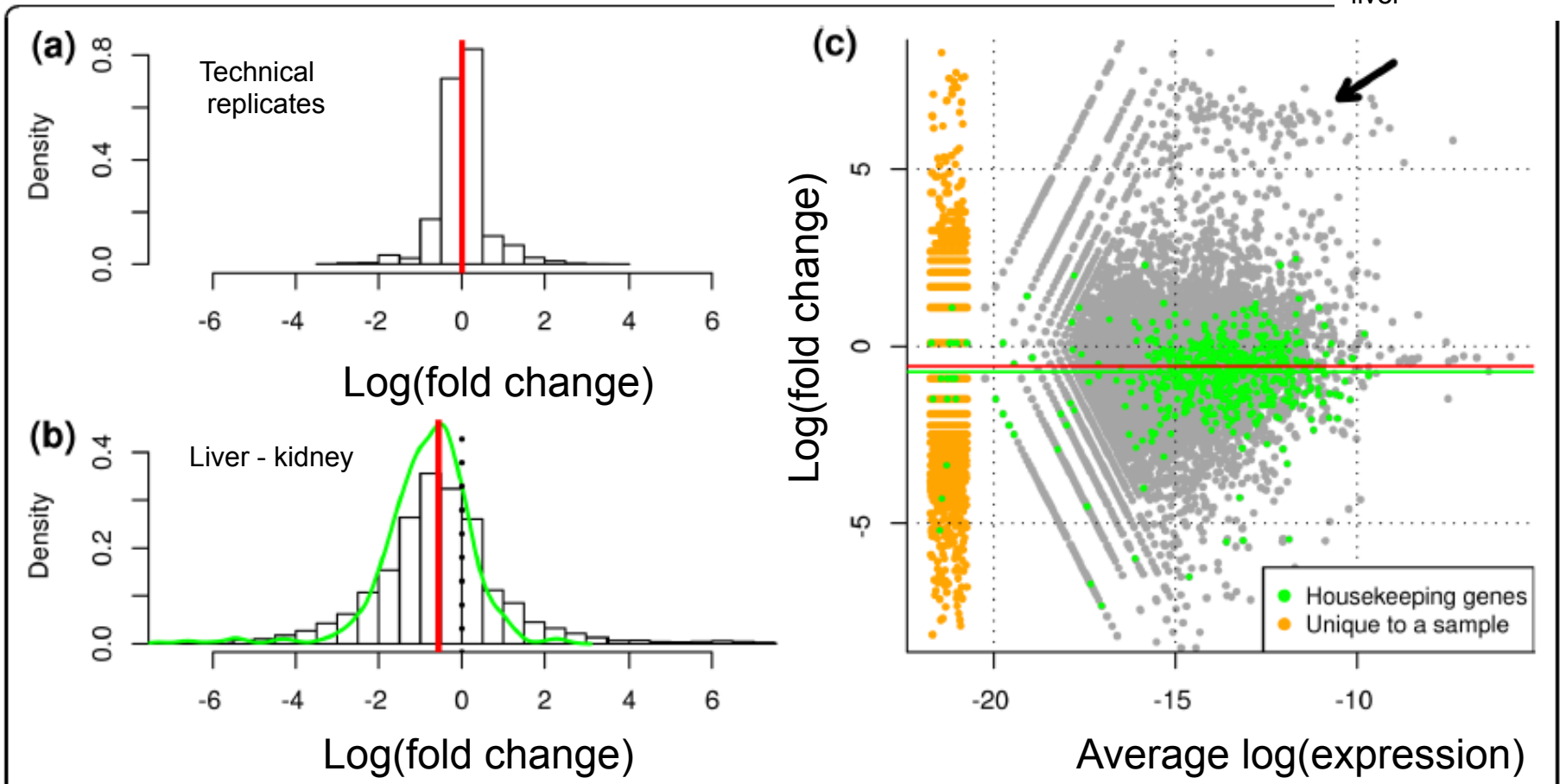
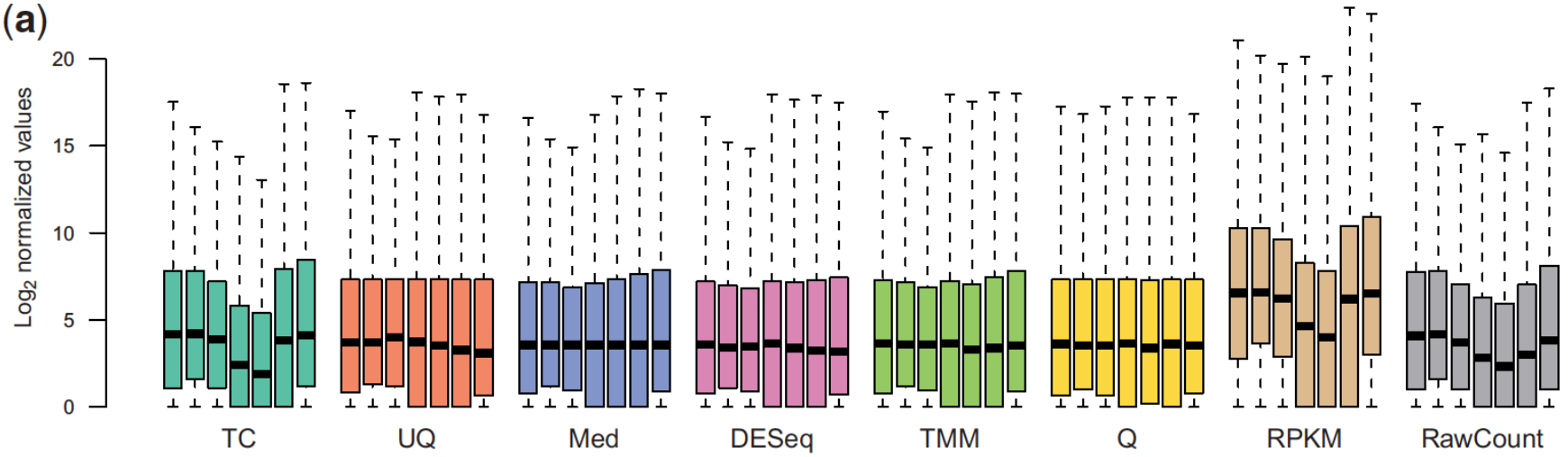


Figure 1 Normalization is required for RNA-seq data. Data from [6] comparing log ratios of **(a)** technical replicates and **(b)** liver versus kidney expression levels, after adjusting for the total number of reads in each sample. The green line shows the smoothed distribution of log-fold-changes of the housekeeping genes. **(c)** An M versus A plot comparing liver and kidney shows a clear offset from zero. Green points indicate 545 housekeeping genes, while the green line signifies the median log-ratio of the housekeeping genes. The red line shows the estimated TMM normalization factor. The smear of orange points highlights the genes that were observed in only one of the liver or kidney [illegible] bias in log-fold-changes.

Adapted from: Robinson and Oshlack, Genome Biology, 2010

Normalization methods for Illumina high-throughput RNA sequencing data analysis.



From “A comprehensive evaluation of normalization methods for Illumina high throughput RNA sequencing data analysis” Brief Bioinform. 2013 Nov;14(6):671-83
http://www.ncbi.nlm.nih.gov/pubmed/22988256