3000788 Intro to Comp Molec Biol

Lecture 6: Applications of sequence alignment

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- Research Affairs
- Center of Excellence in Computational Molecular Biology (CMB)
- Center for Artificial Intelligence in Medicine (CU-AIM)

Sequence homology

Evolution occurs at the sequence level

Histone H1 (residues 120-180)

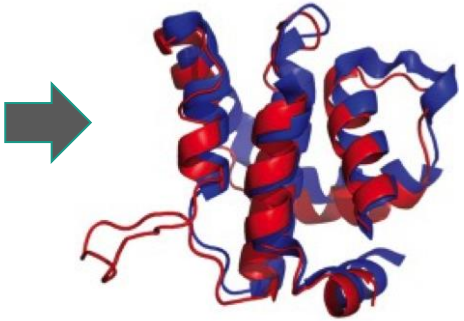
```
HUMAN  KKASKPKKAASKAPTKKPKATPVKKAKKLAATPKKAKKPKTVKAKPVKASKPKKAKPVK
MOUSE  KKAAKPKKAASKAPSKKPKATPVKKAKKPAATPKKAKKPKVVKVKPVKASKPKKAKTVK
RAT    KKAAKPKKAASKAPSKKPKATPVKKAKKPAATPKKAKKPKIVKVKPVKASKPKKAKPVK
COW    KKAAKPKKAASKAPSKKPKATPVKKAKKPAATPKKTKKPKTVKAKPVKASKPKKTKPVK
CHIMP  KKASKPKKAASKAPTKKPKATPVKKAKKLAATPKKAKKPKTVKAKPVKASKPKKAKPVK
       ***:**********:************** ******:**** **.**********:* **
```

https://en.wikipedia.org/wiki/Homology_(biology)

- Genes / proteins originating from the same ancestor will have similar sequence
- High sequence similarity → functional similarity, structural similarity, etc.

Sequence alignment enables inference

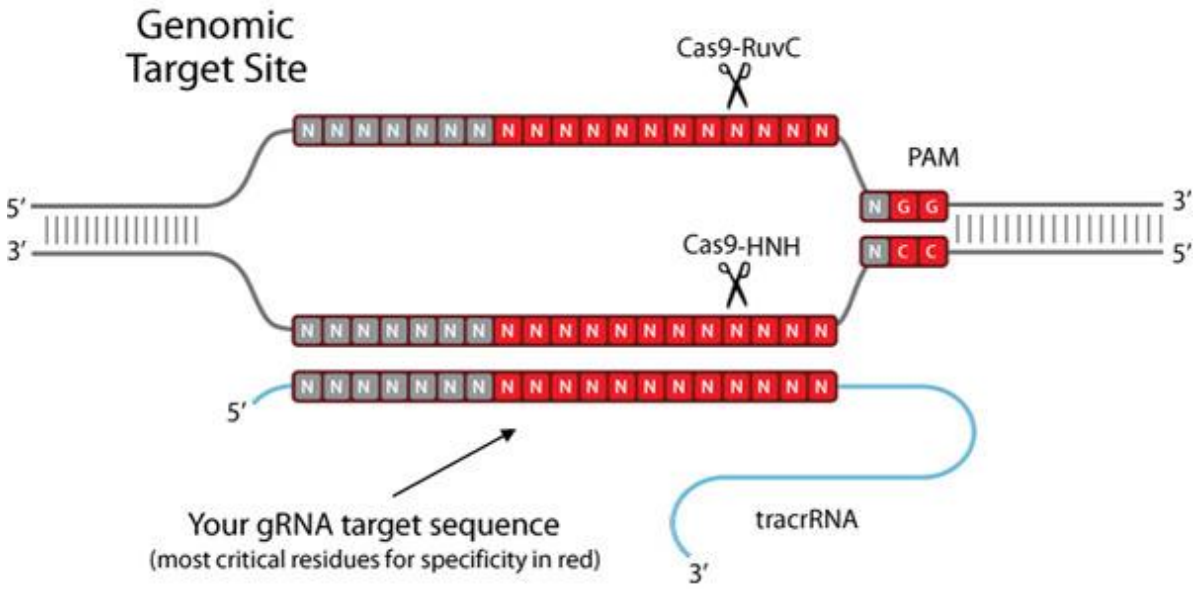
```
                α1                          α2                 α3
N1   1    MRTLLIRYILWRNDNDQTQQNDDFKKLMLLDELVDDGDVCTLIKNMRMTL
N2   53   IIAILNRFLTMNKDELNNTQCHIIKEFMTYEQMAIDHYGEYVNAILYQIR
                α4                                α5
N1   51   SDGPLLDRLN------------QPVNNIEDAKRMIAISAKVARDIGERSE
N2   103  KRPNQHHTIDLFKKIKRTPYDTFKVDPVEFVKKVIGFVSILNKYKPVYSY
                α6                α7
N1   90   IRWEESFTILFRMIETYFDDLMIDLYG
N2   153  VLYENVLYDEFKCKINYVETKYF----
```



Ferguson et al. J General Virology, 94: 2070-2081 (2013)

- Same amino acid residue positions are involved in similar secondary structure
- Properties of amino acid side chains are important

Molecular probe design

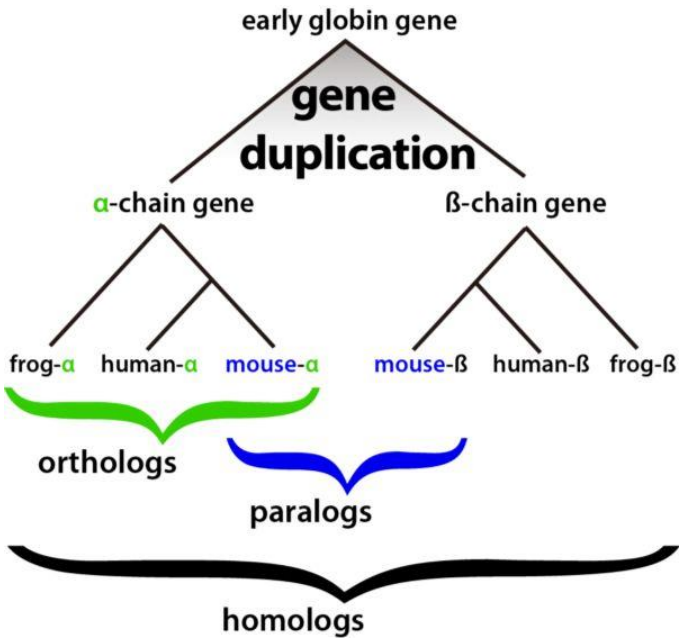


http://www.sigmaaldrich.com/technical-documents/articles/biology/crispr-cas9-genome-editing.html

- Sequence alignment can check the specificity of your probes

Broad applications of sequence homology

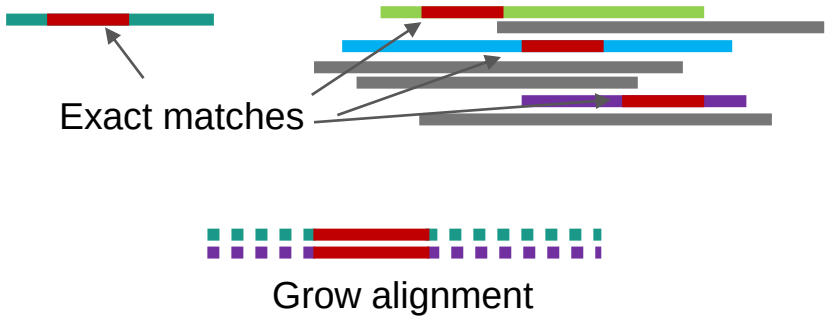
- Infer evolutionary relationship across species
  - Many-to-many alignment between gene lists
- Identify the species of origin for a sequence
  - One-to-many alignment against a reference database
  - Host vs pathogen
- Predict function and structure
  - Partial similarity is good enough
  - Locate conserved functional domain / motif
- Check the specificity of designed probes



https://sites.google.com/site/jkim339n/part2a

Components of sequence alignment

Starting from exact match (seed / word)



- Input sequence length = 300
- Expected similarity between input and reference = 95% (genome re-sequencing)
- Expected 15 mismatches
- If mismatches are random, there should be a run of 285/16 ~ 18 positions with matches
  - MM…MEM…MEM……MEM…MM
  - NCBI's MEGABLAST searches for a run of 28 matches

Dynamic programming algorithm

Dynamic programming matrix:

j → (sequence **y**)

i (sequence **x**)		0	1 T	2 G	3 C	4 T	5 C	6 G	7 T	8 = N A
0		0	−6	−12	−18	−24	−30	−36	−42	−48
1	T	−6	5	−1	−7	−13	−19	−25	−31	−37
2	T	−12	−1	3	−3	−2	−8	−14	−20	−26
3	C	−18	−7	−3	8	2	3	−3	−9	−15
4	A	−24	−13	−9	2	6	0	1	−5	−4
5	T	−30	−19	−15	−4	7	4	−2	6	0
M = 6	A	−36	−25	−21	−10	1	5	2	0	11

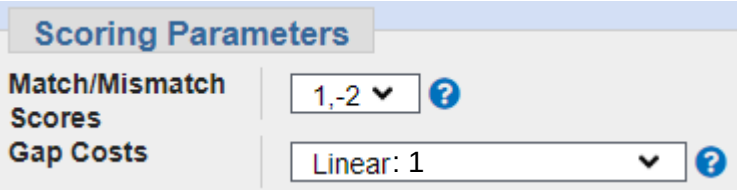
Optimum alignment scores 11:

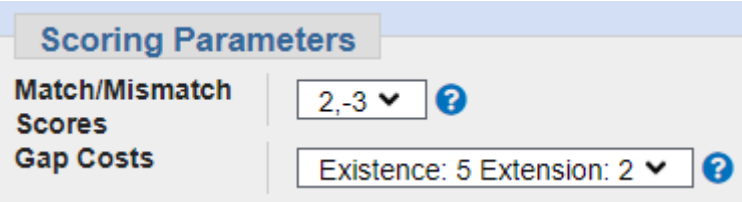
T	–	–	T	C	A	T	A
T	G	C	T	C	G	T	A
+5	−6	−6	+5	+5	−2	+5	+5

- The best alignment for TTCATA vs TGCTCGTA is either
  - T/T + best alignment for TCATA vs GCTCGTA
  - T/– + best alignment for TCATA vs TGCTCGTA
  - –/T + best alignment for TTCATA vs GCTCGTA
- Rely on the score function

Eddy, S.R. Nature Biotechnology 22:909-10 (2004)

Alignment scores






```
Ref:   ACCGTATCG
       ||    ||||
Query: AC---ATCG
```

Score = +1+1-1-1-1+1+1+1+1
= +3

Score = +2+2-5-2-2-2+2+2+2+2
= +1

- Gap cost models
  - Constant = Same penalty regardless of length
  - Linear = Penalty x Length
  - Affine = Existence + (Extension x Length)

Alignment score interpretation

- **Match / Mismatch = +1 / –2**
  - To permit a mismatch, there must be >2 matches afterward to gain score
  - Want hits with high identity

- **Match / Mismatch = +2 / –3**
  - A mismatch followed by two matches = net +1 score
  - Want hits with intermediate identity

- **Gap cost**
  - Constant = An insertion/deletion can be of any length
  - Linear = Long indel is less likely than short indel
  - Affine = Existence + (Extension x Length)
    - Balance between constant and linear

Global and local alignment

Global vs local alignment

Local Alignment

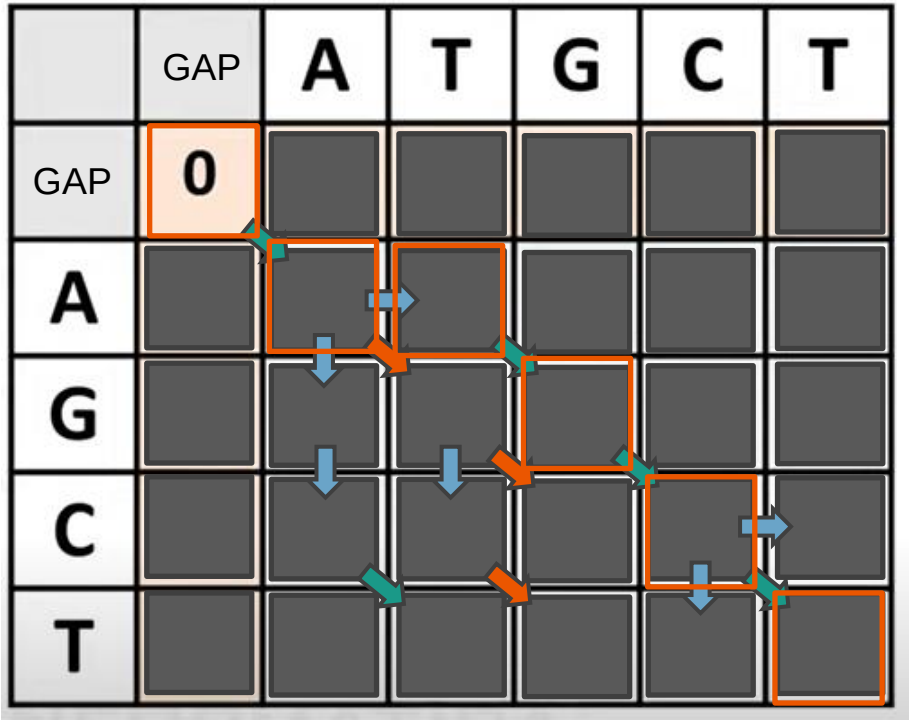
```
Target Sequence
5' ACTACTAGATTACTTACGGATCAGGTACTTTAGAGGCTTGCAACCA 3'
             |||| |||||| |||||||||||||||
Query Sequence 5' TACTCACGGATGAGGTACTTTAGAGGC 3'
```

Global Alignment

```
Target Sequence
5' ACTACTAGATTACTTACGGATCAGGTACTTTAGAGGCTTGCAACCA 3'
   |||||||||||    |||||||  ||||||||||||||| |||||||
5' ACTACTAGATT----ACGGATC--GTACTTTAGAGGCTAGCAACCA 3'
Query Sequence
```

Images generated from BABA http://baba.sourceforge.net/

Global alignment



Match : 1
Mismatch : -1
GAP : -2

```
Seq1:  ATGCT
        | |||
Seq2:  A-GCT
```

https://www.youtube.com/watch?v=ipp-pNRIp4g

Local alignment

		A	T	G	C	T
	0	0	0	0	0	0
A	0	1	0	0	0	0
G	0	0	0	1	0	0
C	0	0	0	0	2	0
T	0	0	0	0	0	3

Match : 1
Mismatch : -1
GAP : -2

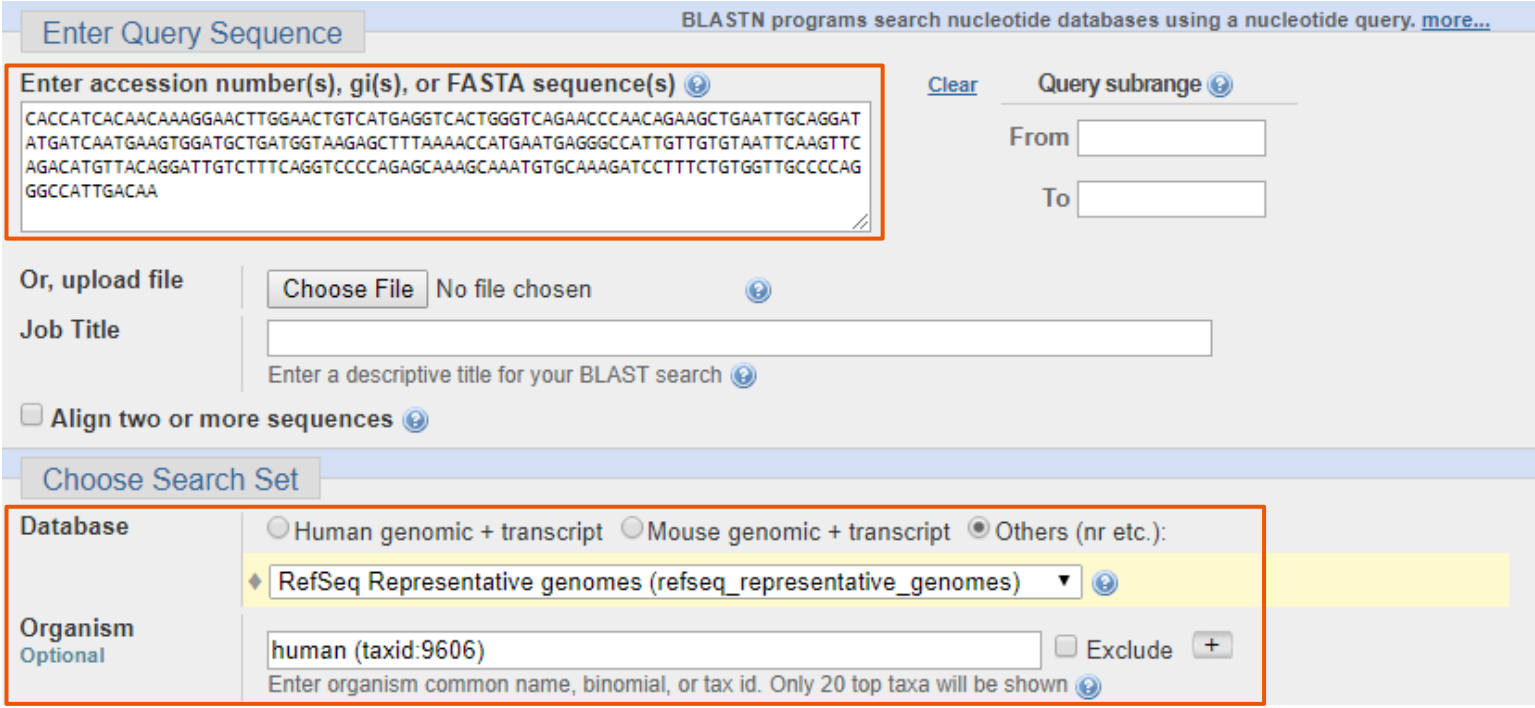
```
Seq1:  ATGCT
          |||
Seq2:   AGCT
```

- Ignore possibilities with negative score
  - Start over is better

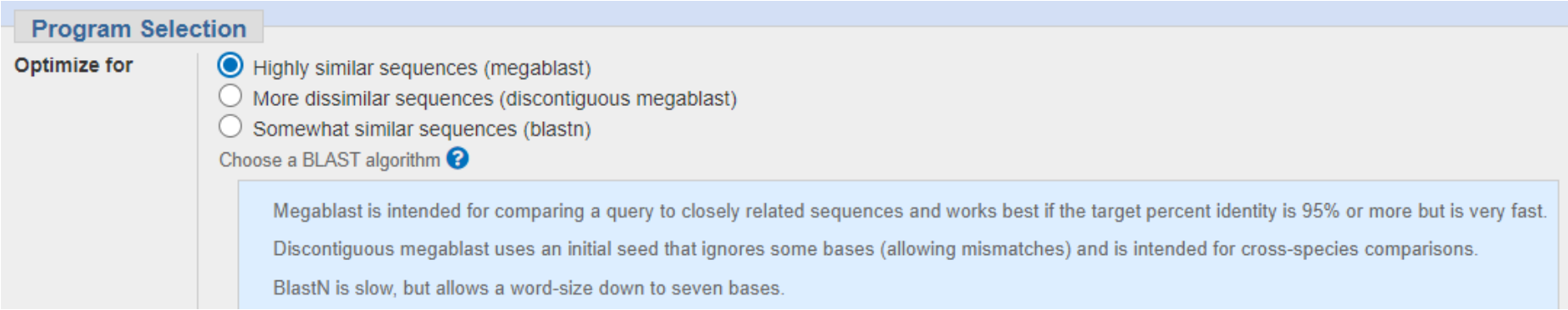
https://www.youtube.com/watch?v=lu9ScxSejSE

Basic Local Alignment Search Tool BLAST

NCBI's nucleotide BLAST interface



Nucleotide BLAST algorithms



- MEGABLAST: word size = 28, match/mismatch score = +1/−2, linear gap
- BLASTN: word size = 11, match/mismatch score = +2/−3, affine gap

MEGABLAST vs BLASTN

MEGABLAST = few, high-identity hits

Job title: Nucleotide Sequence (240 letters)

RID VCC9FM9501R (Expires on 09-12 14:46 pm)

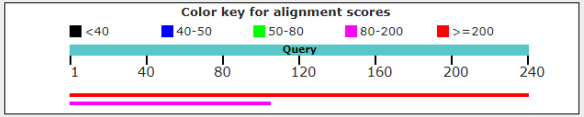
Query ID lcl|Query_58243
Description None
Molecule type nucleic acid
Query Length 240

Database Name refseq_representative_genomes (GPIPE/9606/108/ref_top_level)
Description ▹See details
Program BLASTN 2.7.0+ ▹Citation

Other reports: ▹Search Summary [Taxonomy reports] [Distance tree of results] [MSA viewer]

Graphic Summary

Distribution of the top 2 Blast Hits on 2 subject sequences
Mouse over to see the title, click to show alignments



Descriptions

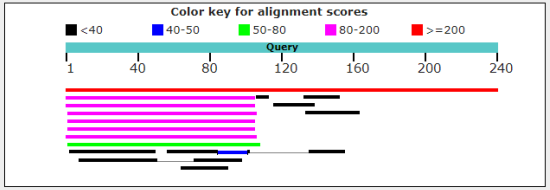
Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Homo sapiens chromosome 14, GRCh38.p7 Primary Assembly	444	444	100%	1e-122	100%	NC_000014.9
Homo sapiens chromosome X, GRCh38.p7 Primary Assembly	91.6	91.6	43%	2e-16	84%	NC_000023.11

BLASTN = lots of intermediate-identity hits



escriptions

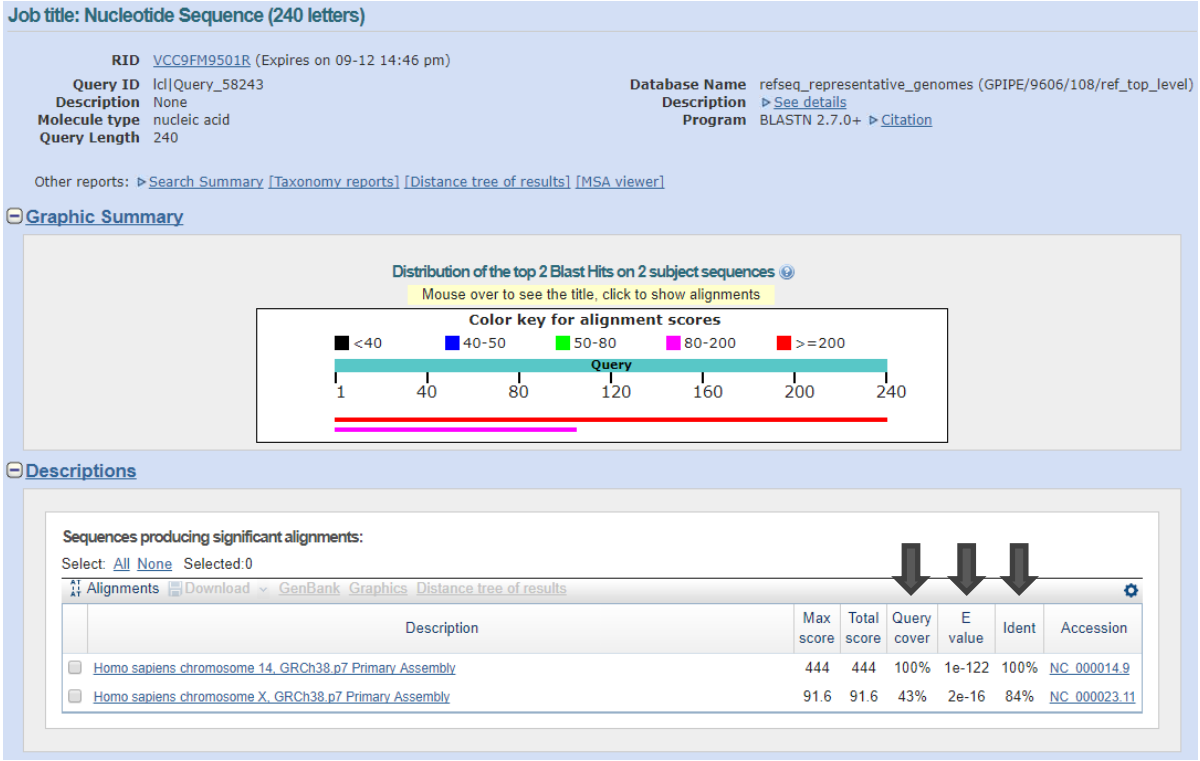
Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Homo sapiens chromosome 14, GRCh38.p7 Primary Assembly	434	434	100%	1e-119	100%	NC_000014.9
Homo sapiens chromosome 7, GRCh38.p7 Primary Assembly	154	231	43%	2e-35	92%	NC_000007.14
Homo sapiens chromosome X, GRCh38.p7 Primary Assembly	118	118	43%	1e-24	84%	NC_000023.11
Homo sapiens chromosome 2, GRCh38.p7 Primary Assembly	113	150	43%	6e-23	84%	NC_000002.12
Homo sapiens chromosome 10, GRCh38.p7 Primary Assembly	109	109	43%	7e-22	84%	NC_000010.11
Homo sapiens chromosome 13, GRCh38.p7 Primary Assembly	104	193	43%	3e-20	83%	NC_000013.11
Homo sapiens chromosome 19, GRCh38.p7 Primary Assembly	102	102	44%	1e-19	81%	NC_000019.10
Homo sapiens chromosome 17, GRCh38.p7 Primary Assembly	71.6	71.6	44%	2e-10	75%	NC_000017.11
Homo sapiens chromosome 3, GRCh38.p7 Primary Assembly	41.0	153	27%	0.32	88%	NC_000003.12
Homo sapiens chromosome 5, GRCh38.p7 Primary Assembly	39.2	78.3	29%	1.1	82%	NC_000005.10
Homo sapiens chromosome 8, GRCh38.p7 Primary Assembly	39.2	39.2	10%	1.1	92%	NC_000008.11
Homo sapiens chromosome Y, GRCh38.p7 Primary Assembly	39.2	39.2	20%	1.1	81%	NC_000024.10
Homo sapiens chromosome 9, GRCh38.p7 Primary Assembly	37.4	37.4	8%	3.8	100%	NC_000009.12
Homo sapiens chromosome 11, GRCh38.p7 Primary Assembly	37.4	37.4	10%	3.8	92%	NC_000011.10
Homo sapiens chromosome 12, GRCh38.p7 Primary Assembly	37.4	37.4	9%	3.8	96%	NC_000012.12
Homo sapiens chromosome 18, GRCh38.p7 Primary Assembly	37.4	37.4	12%	3.8	87%	NC_000018.10

Interpreting BLAST result



Query coverage = % of input sequence used in the alignment

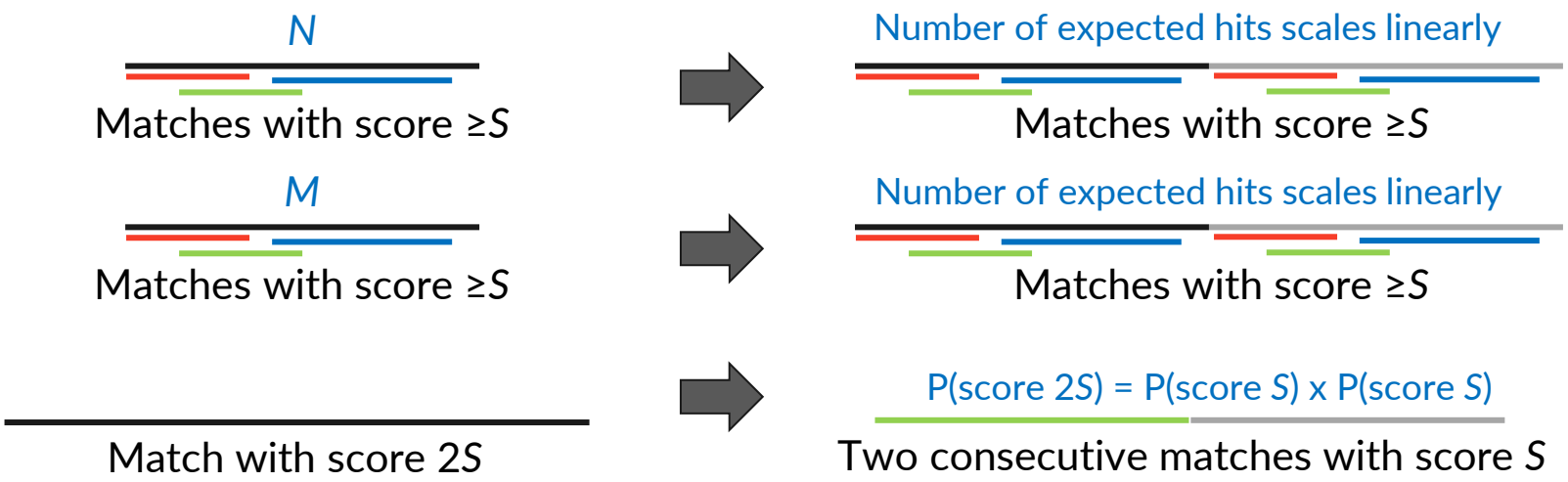
Identity = % of identity between input and matched sequences in the aligned region

E value = expected number of hits with the same or higher score by chance (given input length and database size)

Typical cutoff is 1e-5

Understanding E value

- Given an input sequence of length *N* and a reference sequence of length *M*
- E value for a hit with score *S* is proportional to *N* x *M* x $e^{-\lambda S}$



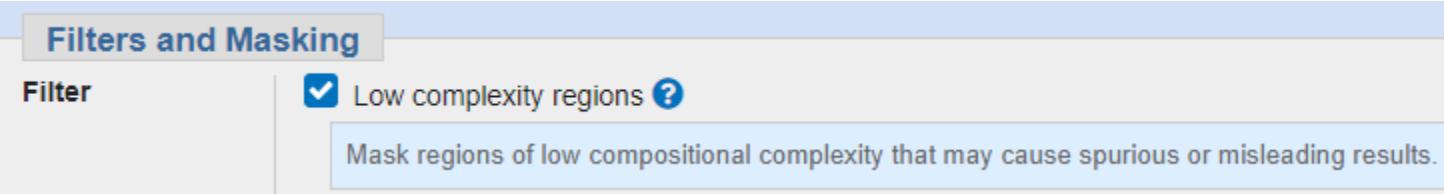
E value as Poisson distribution

- Event of interest = hits with score >*S* occurs on the sequence of length *N*
- Expected value = E value
- Probability of observing *k* hits with score >*S* = $\frac{E^k e^{-E}}{k!}$

Low complexity region

CG island

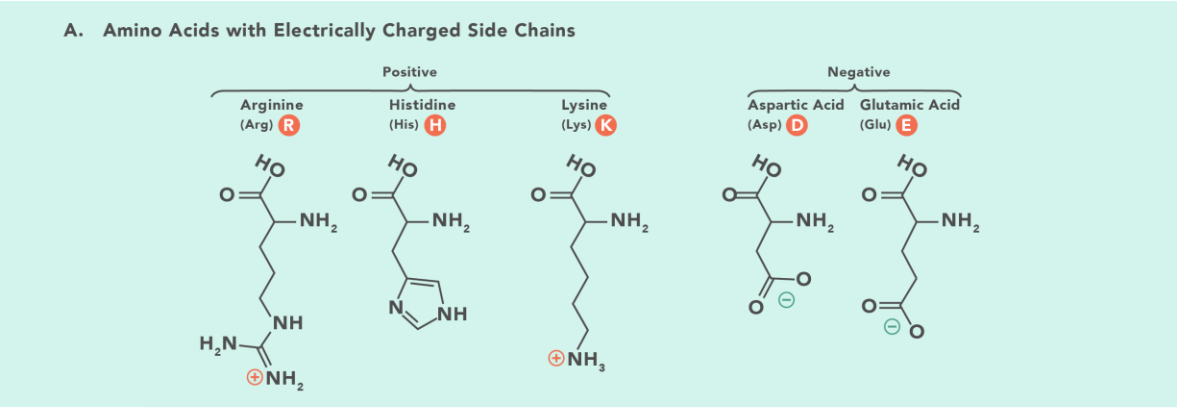
CCCGCGCGCCCCGGCGCCCGATGCAACTAGC

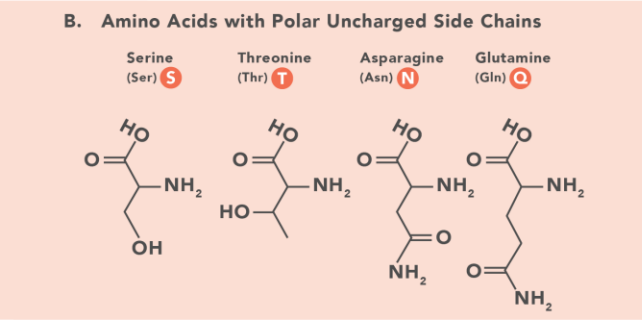


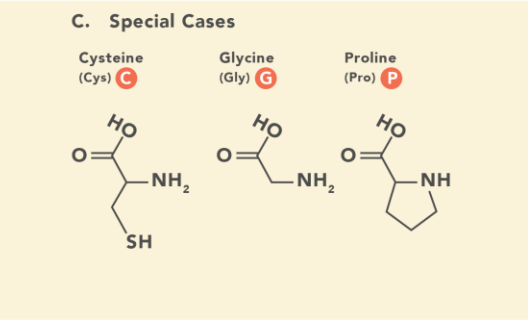
- Probability of getting a hit with score >*S* will be high if both sequences contain only C's and G's
- BLAST withholds these regions from score calculation

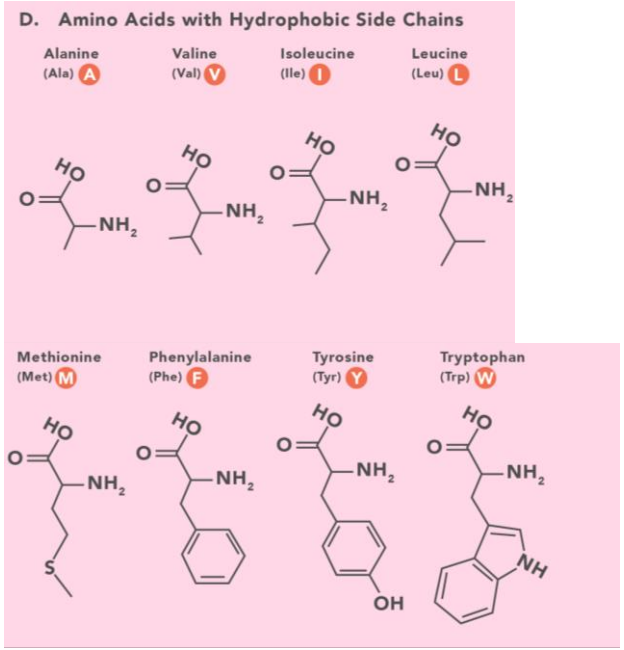
Protein sequence alignment

Amino acid side chains









https://www.technologynetworks.com/applied-sciences/articles/essential-amino-acids-chart-abbreviations-and-structure-324357

Similar side chains are interchangeable

wikipedia.com

- Amino acids with similar properties can replace each other with minimal impact on protein function

- D, E have –COOH groups
- K, R have positively charged $-NH_2$ groups
- A, V, I, L have small hydrocarbon side chains
- F, Y, W have benzene rings

- Alignment score must reflect these!

Block Substitution Matrix (BLOSUM)

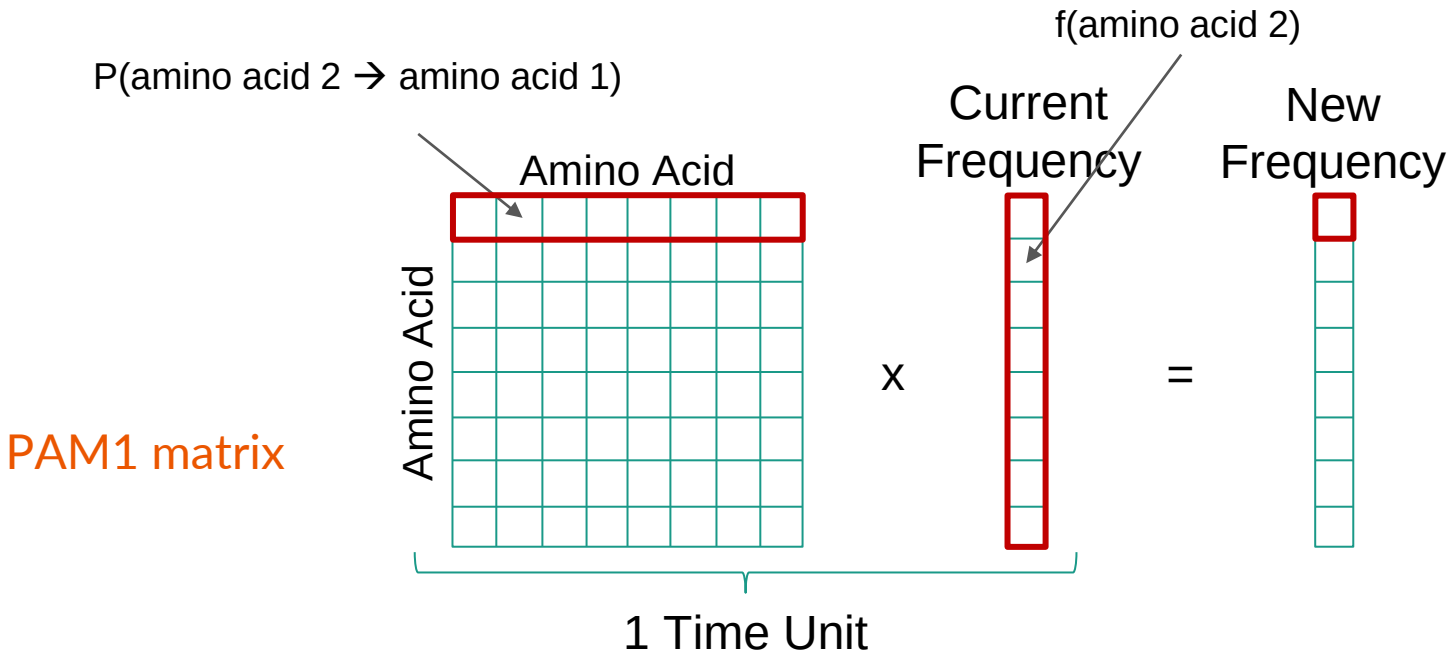
	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
Ala	4																			
Arg	-1	5																		
Asn	-2	0	6																	
Asp	-2	-2	1	6																
Cys	0	-3	-3	-3	9															
Gln	-1	1	0	0	-3	5														
Glu	-1	0	0	2	-4	2	5													
Gly	0	-2	0	-1	-3	-2	-2	6												
His	-2	0	1	-1	-3	0	0	-2	8											
Ile	-1	-3	-3	-3	-1	-3	-3	-4	-3	4										
Leu	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4									
Lys	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5								
Met	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5							
Phe	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6						
Pro	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7					
Ser	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4				
Thr	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5			
Trp	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11		
Tyr	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	
Val	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

No change = highest scores

- Constructed using substitution rates from actual protein families
- BLOSUM45 was constructed using protein families with >45% conservation

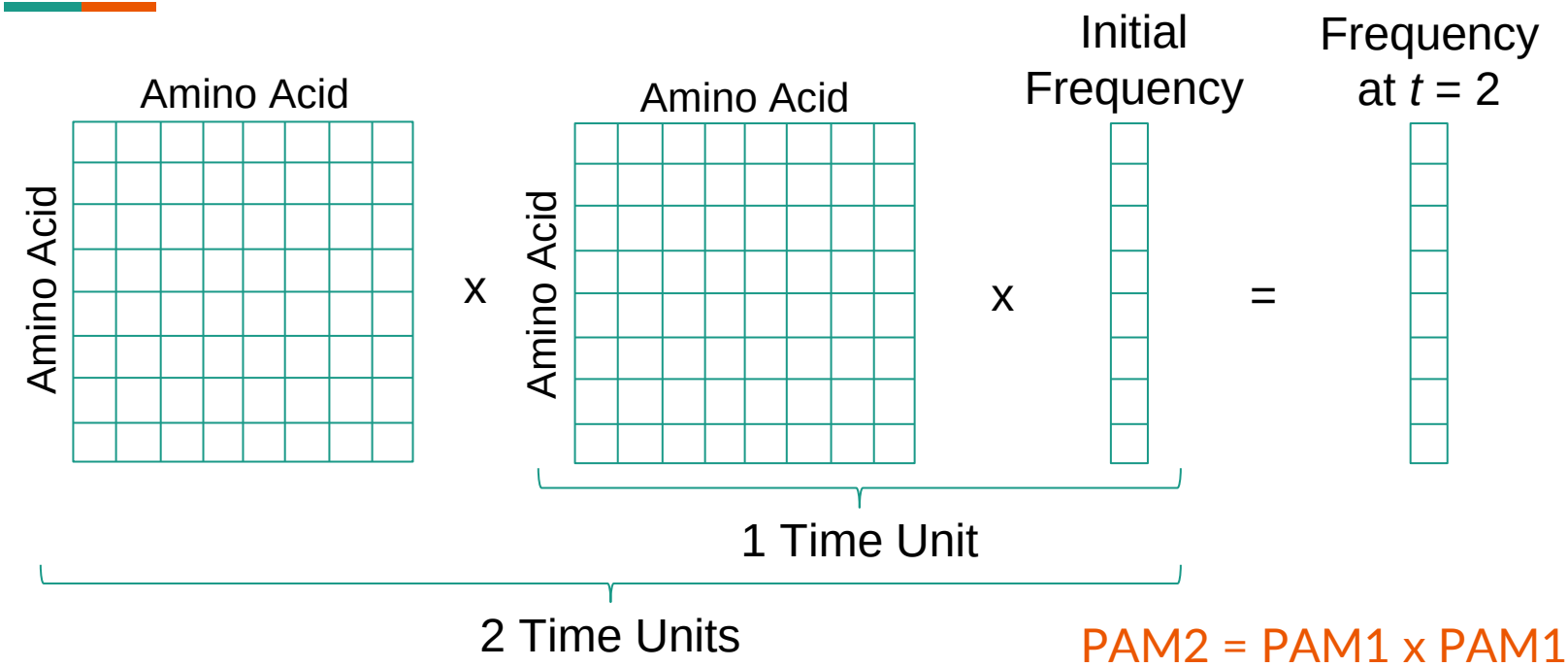
https://en.wikipedia.org/wiki/BLOSUM

Point Accepted Mutation (PAM)



- Estimate amino acid substitution rate between highly similar proteins (>85%)

Point Accepted Mutation (PAM)



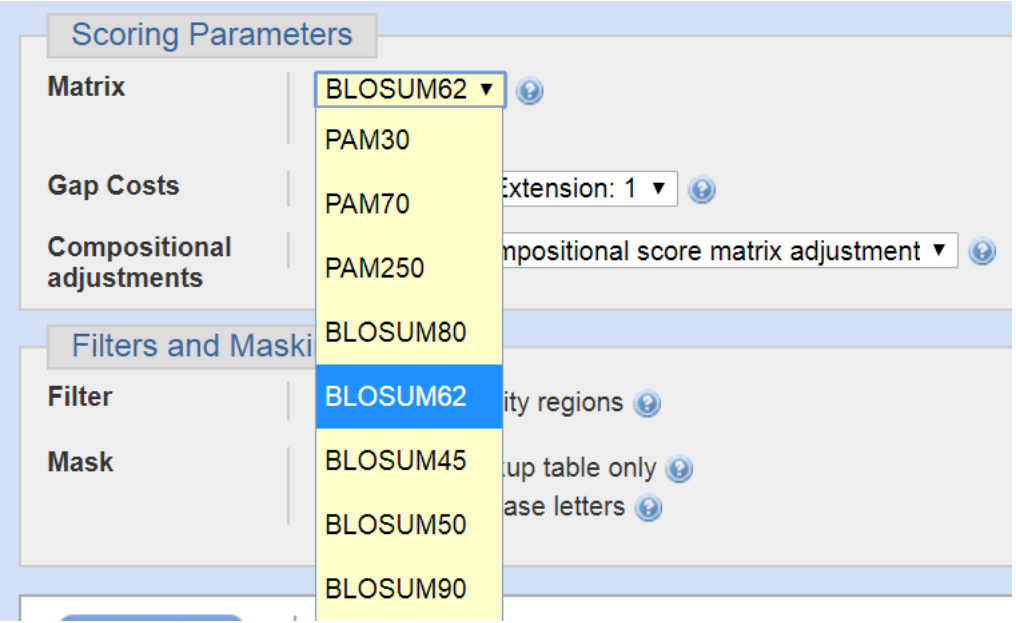
PAM2 = PAM1 x PAM1

- Extrapolate substitution rates for more distant proteins

PAM vs BLOSUM

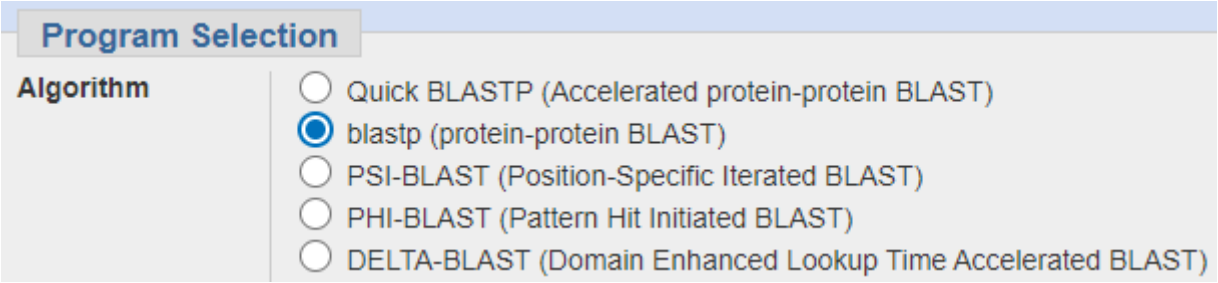
PAM	BLOSUM
PAM100	BLOSUM90
PAM120	BLOSUM80
PAM160	BLOSUM60
PAM200	BLOSUM52
PAM250	BLOSUM45

Data from https://en.wikipedia.org/wiki/BLOSUM



- BLOSUM for low identity, PAM for high identity

Protein BLAST algorithms



- Standard BLASTP assumes that all amino acid residue positions are the same
- But there are protein domains & motifs with specific patterns

Position-specific scoring matrix (PSSM)

www.nemates.org/uky/520/Lecture/Lect6/BIO520_2010_Lect6.pp

weblogo.berkeley.edu

- Different scoring matrix for each position in the motif
- But how do we know the position-specific amino acid profile?

Pattern hit initiated (PHI-BLAST)

x = any amino acid

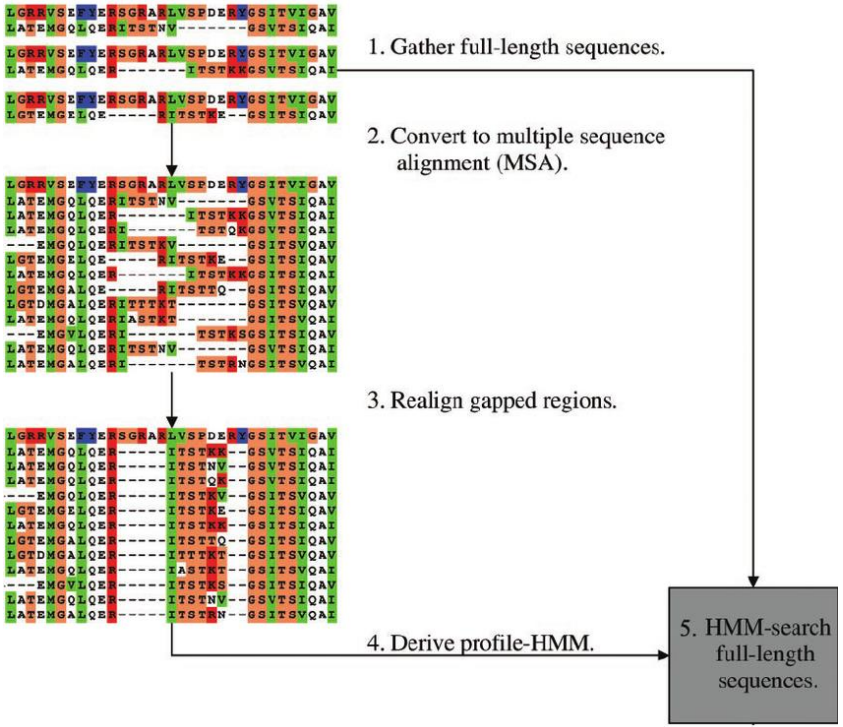
[LIVMF]-G-E-x-[GAS]-[LIVM]-x(5,11)-R-[STAQ]

L, I, V, M, or F

any sequences of 5-11 amino acids

- Combine regular BLASTP with user-specified pattern
- Hits must be similar to the input sequence AND match the pattern
- Search for known protein domain

Position-specific iteratred (PSI-BLAST)



- Start from user inputs
- First round of BLASTP
- Construct PSSM from hits
- Re-search using the PSSM
- Repeat

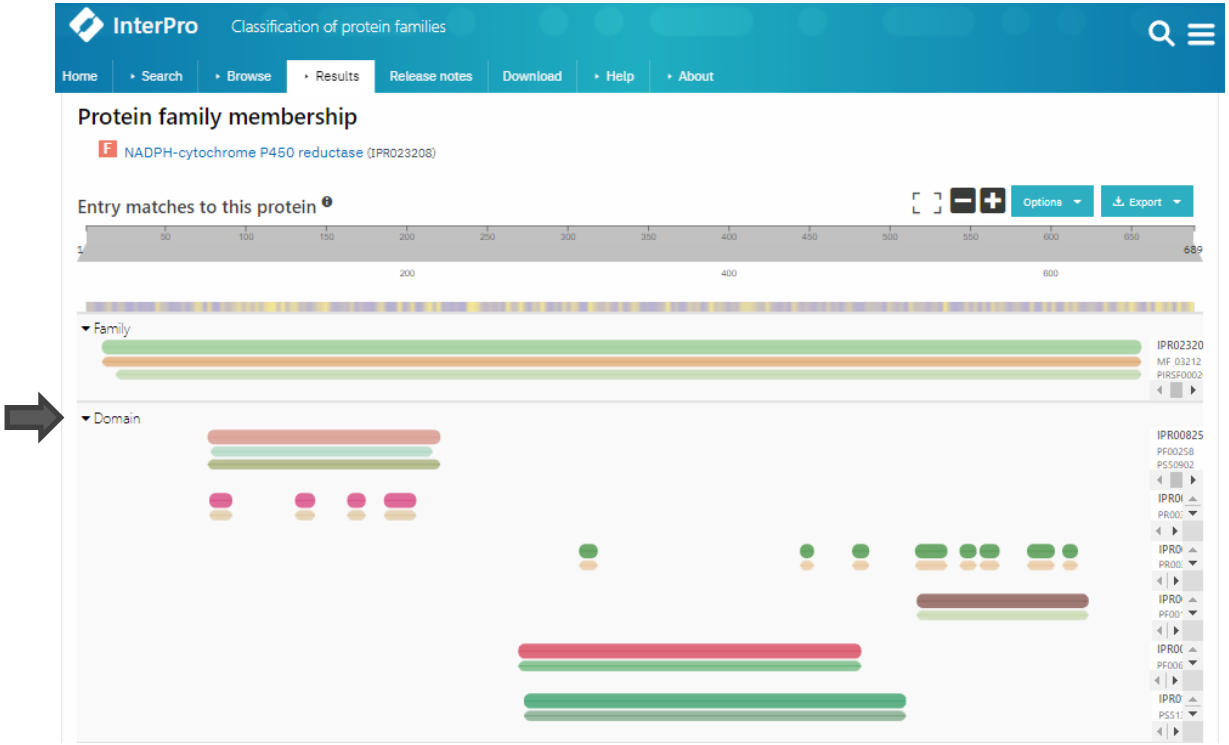
Frickey, T. and Lupas, A. NAR 32:5231-8 (2004)

Using BLASTP to annotate protein function

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
hypothetical protein JCGZ_15894 [Jatropha curcas]	Jatropha curcas	1161	1161	99%	0.0	89.37%	689	KDP41487.1
NADPH--cytochrome P450 reductase [Manihot esculenta]	Manihot esculenta	1159	1159	100%	0.0	86.98%	691	XP_021601058.2
NADPH--cytochrome P450 reductase [Manihot esculenta]	Manihot esculenta	1145	1145	100%	0.0	86.25%	690	XP_021601060.1
NADPH--cytochrome P450 reductase-like [Hevea brasiliensis]	Hevea brasiliensis	1130	1130	99%	0.0	85.59%	689	XP_021642755.1
NADPH--cytochrome P450 reductase [Ricinus communis]	Ricinus communis	1124	1124	99%	0.0	84.64%	692	XP_002514049.1
LOW QUALITY PROTEIN: NADPH--cytochrome P450 reductase-like [Hevea brasilien...	Hevea brasiliensis	1120	1120	100%	0.0	84.81%	698	XP_021660128.1
hypothetical protein COLO4_35252 [Corchorus olitorius]	Corchorus olitorius	1111	1111	100%	0.0	82.08%	1505	OMO57587.1
Flavodoxin [Corchorus capsularis]	Corchorus capsularis	1093	1093	100%	0.0	82.08%	692	OMO50775.1
NADPH--cytochrome P450 reductase-like [Hibiscus syriacus]	Hibiscus syriacus	1085	1085	100%	0.0	81.24%	693	XP_039050423.1
hypothetical protein CXB51_011412 [Gossypium anomalum]	Gossypium anomalum	1083	1083	100%	0.0	81.10%	694	KAG8494022.1
NADPH:cytochrome P450 reductase [Gossypium hirsutum]	Gossypium hirsutum	1083	1083	100%	0.0	81.24%	693	ACN54323.1
NADPH--cytochrome P450 reductase-like [Gossypium hirsutum]	Gossypium hirsutum	1083	1083	100%	0.0	81.10%	693	NP_001313876.2

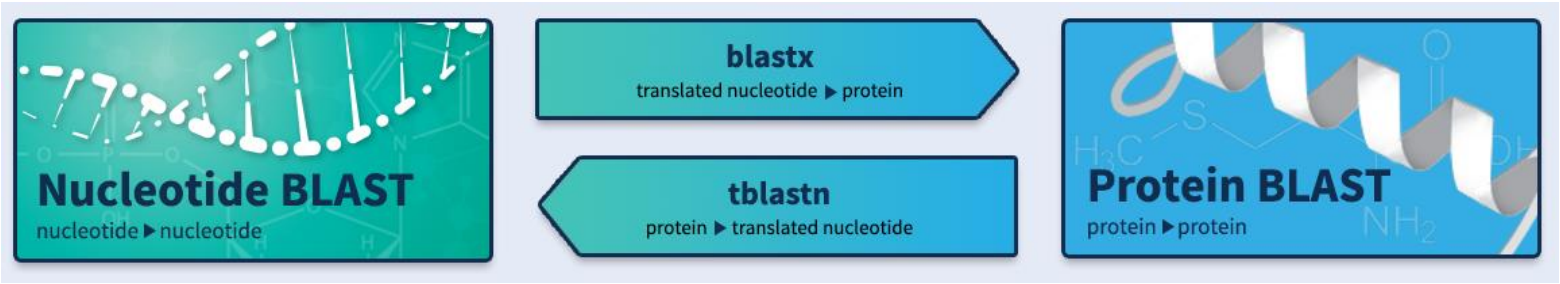
- Suspected novel CYP reductase from an indigenous plant
- BLASTP against plant sequences
- >80% similarity to known and predicted CYP reductase class I

InterPro: Protein domain search



Mixing protein-nucleotide alignment

BLASTX and TBLASTN



- For alignment of coding DNA sequence
  - Codon structure = not all nucleotide positions evolve in the same manner
  - Similarity in protein is more informative than similarity in DNA
- Align translated DNA to protein database
- Align protein to translated DNA database

Example use cases

- BLASTX = align translated DNA to protein database
  - You perform RNA-seq
  - Unsure which open reading frame is correct
  - Check whether this RNA translated to known protein or function

- TBLASTN = align protein to translated DNA database
  - You identified novel protein
  - No evidence in protein database
  - But there might be transcriptomics studies that identified the RNA of related proteins

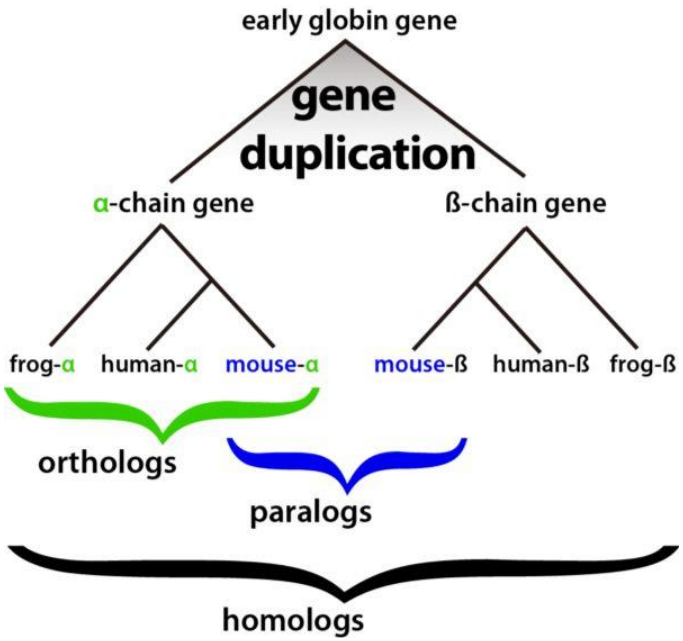
Beyond one-vs-all BLAST

All-vs-all BLAST

- Compare genes between related species to identify genes originated from a common ancestor
  - {Mouse-a, Human-a}, {Mouse-b, Human-b}

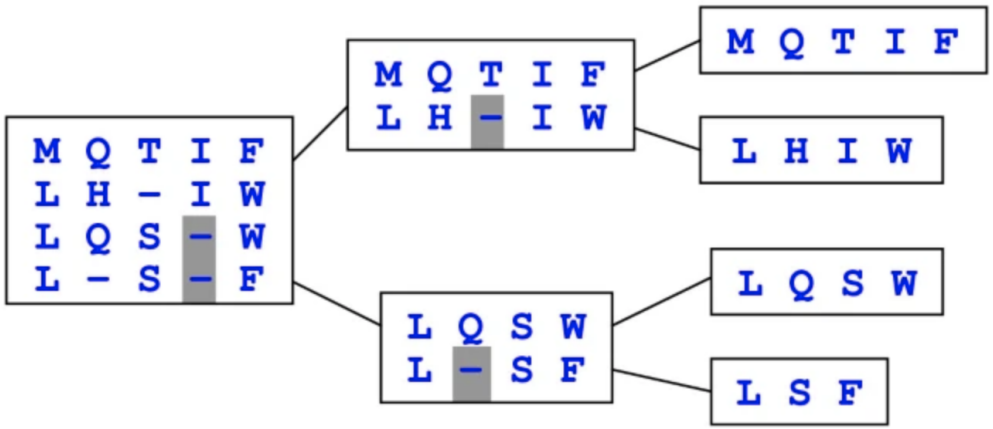
- BLAST mouse to human
- BLAST human to mouse

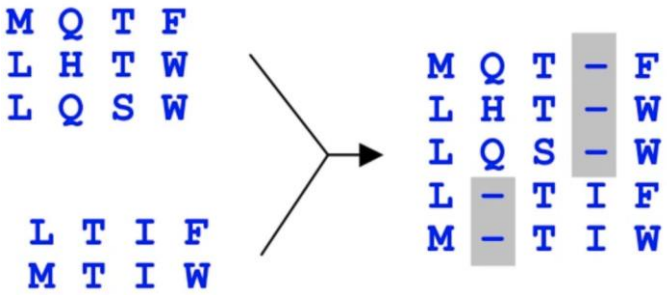
- Reciprocal best hit:
  - Human-a should be the best hit for Mouse-a
  - Mouse-a should be the best hit for Human-a



https://sites.google.com/site/jkim339n/part2a

Multiple sequence alignment (MSA)

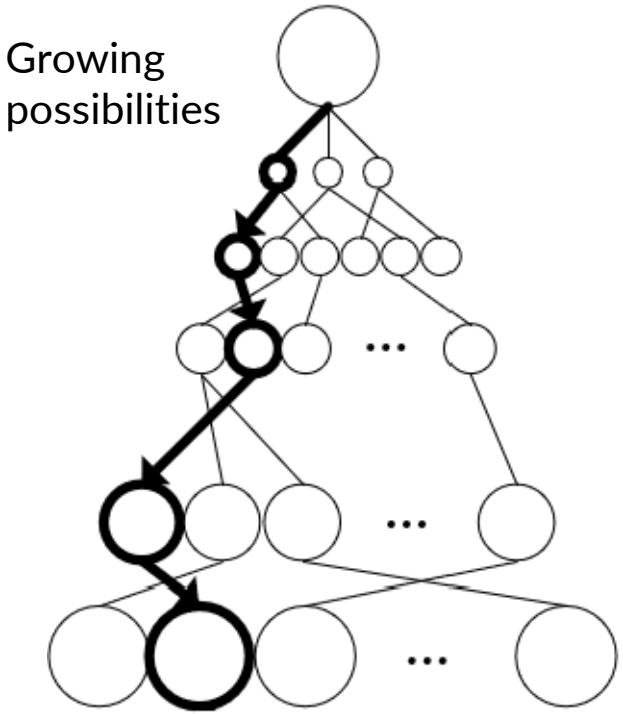




Edgar, BMC Bioinformatics, 5, 113 (2014)

- Dynamic programming is not feasible because of too many possibilities for grouping sequences
- Rely on heuristic algorithm

When the space of possible solutions is too large



- Heuristic algorithm makes a decision by estimating the cost of all future steps
- Greedy algorithm makes a decision by optimizing the cost of only the next step
- Randomized algorithm makes a lot of random decisions and keeps the best one found

Alignment output format

Aligned FASTA

```
>TRY2_RAT/24-239
------------------------IVGGYTCQENSVPYQVSLNSGY------------HFC
GGSLI------NDQ-WV-VSAAHCYKS----------RIQVRLGE-HNINVLEGN-----
-----EQFVNAAKIIKHPNFDRKT-L------------------------NNDIMLIKLS
SP--VKLNARVATVALPS---SCA---PAGTQCLISGWGN----------TLSSGV----
-------NEPDLLQ-CLDAP-LLPQADCEAS---YPGK--------ITDNMVCVGFL---
-EGG-KDSCQGDSGGPVVCNGE--------LQGIVSWG-YGCALPDN---PGVYTKVCNY
VDWI---------------

>Q16LB2_AEDAE/136-374
------------------------ILNGIEADLEDFPYLGALALLDNYT-----STVSYRC
GANLI------SDR-FM-LTAAHCLFG--------KQAIHVRMGTLSLTDNPDED-----
----APVIIGVERVFFHRNYTRRPIT------------------------RNDIALIKLN
RT---VVEDFLIPVCLYT---EQNDP-LPTVPLTIAGWGG----------NDSAS-----
-------LMSSSLM-KASVT-TYERDECNSL---LAKKI-----VRLSNDQLCALGRSEF
NDGLRNDTCVGDSGGPLELSIGR----RKYIVGLTSTG-IVCGNE-F---PSIYTRISQF
IDWI---------------
```

PHYLIP

```
      5    42
Turkey     AAGCTNGGGC ATTTCAGGGT GAGCCCGGGC AATACAGGGT AT
Salmo gairAAGCCTTGGC AGTGCAGGGT GAGCCGTGGC CGGGCACGGT AT
H. SapiensACCGGTTGGC CGTTCAGGGT ACAGGTTGGC CGTTCAGGGT AA
Chimp      AAACCCTTGC CGTTACGCTT AAACCGAGGC CGGGACACTC AT
Gorilla    AAACCCTTGC CGGTACGCTT AAACCATTGC CGGTACGCTT AA
```

ClustalW

```
Caballeronia_arvi          MNSRIDSHVKHLIFFCGHAGTGKTTLAKRLFAPLMQAAGEPFCLLDKDTLYGAYSAAAIG
Caballeronia_choica        --------MTHLVFFCGHAGTGKTTLAKRLFPRLMRATGEPFCLLDKDTLYGGYSAAAMG
Caballeronia_arationis     --------MTYLIFFCGHAGTGKTTLAKRLFPRLVRATGEPFCLLDKDTLYGAYSAAAMG
Caballeronia_telluris      --------MTHLIFFCGHAGTGKTTLAKRLFPRLAQASGEPFCLLDKDTLYGAYSAAAMN
                                   :.:*:******************. * .*:***************.*****:.

Caballeronia_arvi          ALTGDPHDRDSPLFIEHFRDPEYRCLVDTAAENLALGVSVVVVAPLTREVRSSRLFDRAW
Caballeronia_choica        ALTGDPNDRDSPLFLQHLRDPEYRALIDTARENLELGVSVAVVAPLSREVRDGRLFDRQW
Caballeronia_arationis     ALTGDPNDRDSPLFLQHFRDPEYRALIDTARENLDLGVSVAVVAPLTREVREERLFDRAW
Caballeronia_telluris      ALTGDPNDRDSPLFLQHLRDPEYRALIDTARENLDLGVSVAVVAPLTREVREGRLFDRTW
                           ******:*******::*:******.*:*** *** *****.*****:****. ***** *
```

Any question?

- See you on September 7