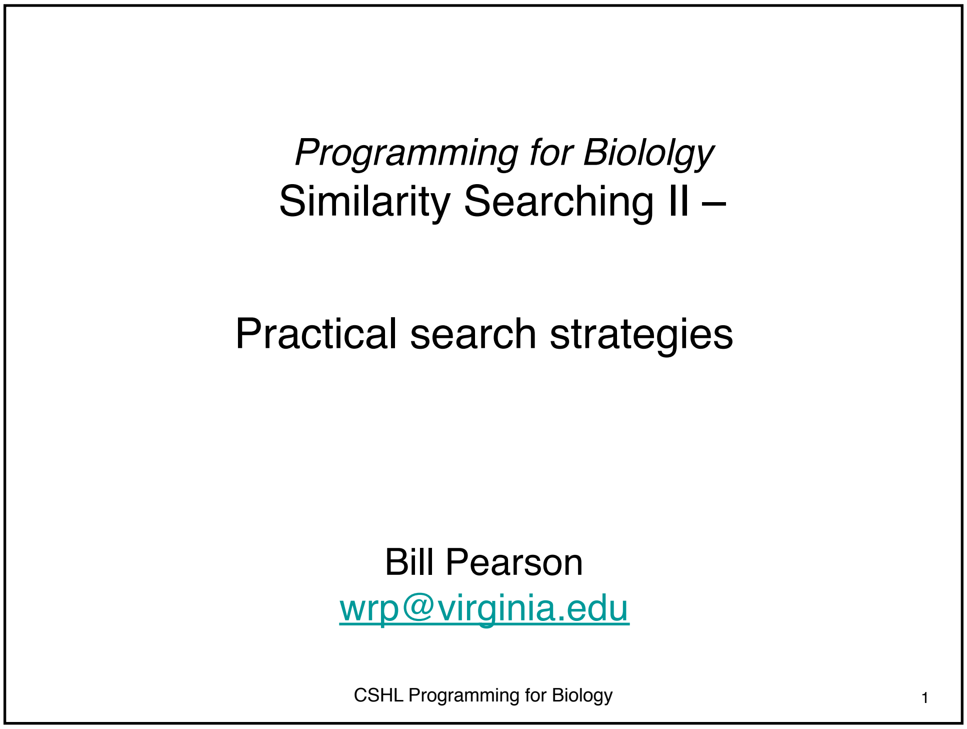
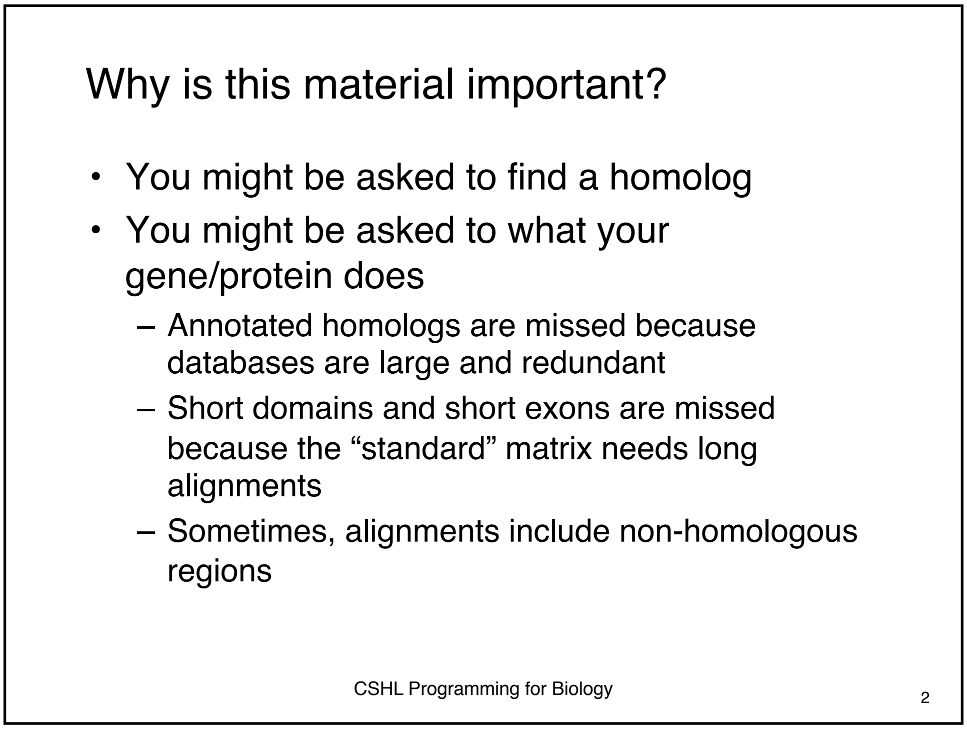


Programming for Biololgy

Similarity Searching II –

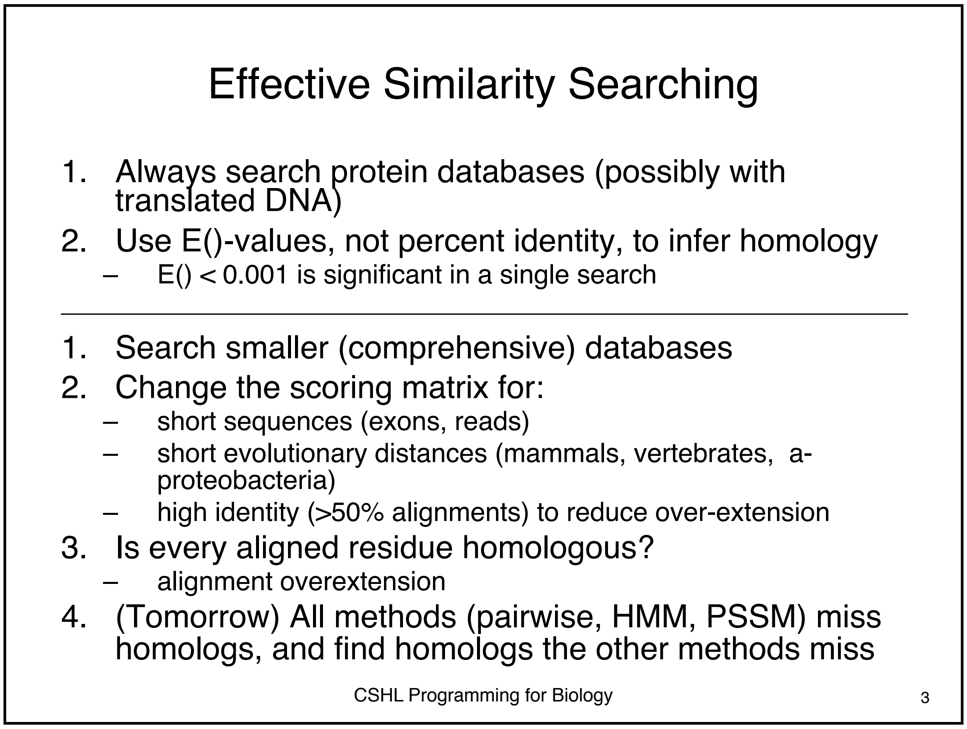
Practical search strategies

Bill Pearson
wrp@virginia.edu



Why is this material important?

- You might be asked to find a homolog
- You might be asked to what your gene/protein does
  - Annotated homologs are missed because databases are large and redundant
  - Short domains and short exons are missed because the “standard” matrix needs long alignments
  - Sometimes, alignments include non-homologous regions



Effective Similarity Searching

1. Always search protein databases (possibly with translated DNA)
2. Use E()-values, not percent identity, to infer homology
   - E() < 0.001 is significant in a single search

---

1. Search smaller (comprehensive) databases
2. Change the scoring matrix for:
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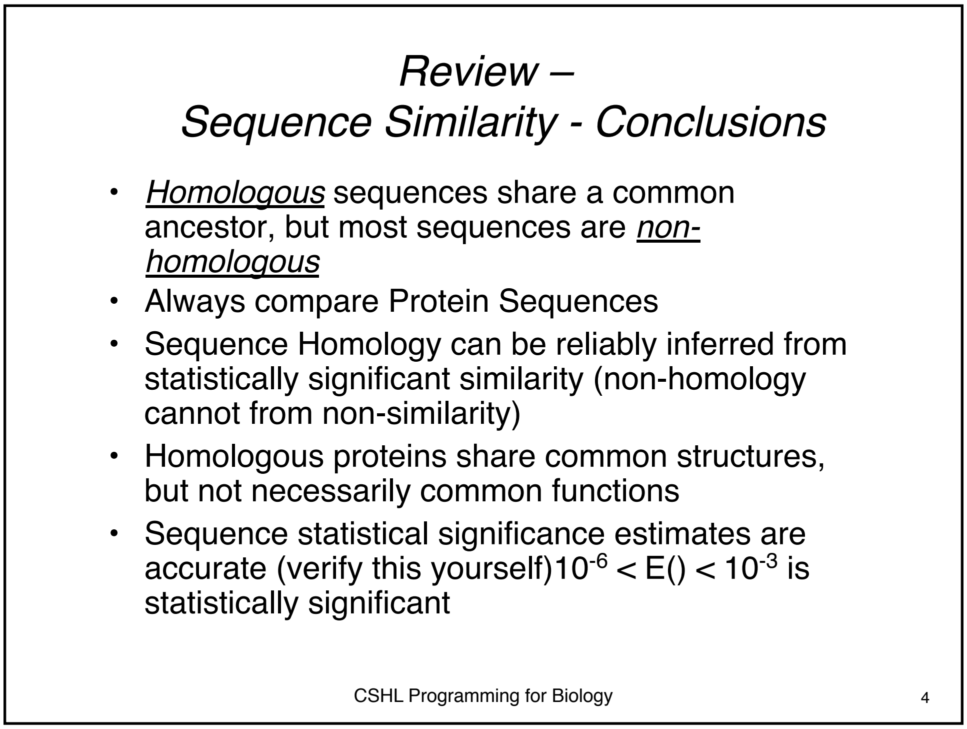
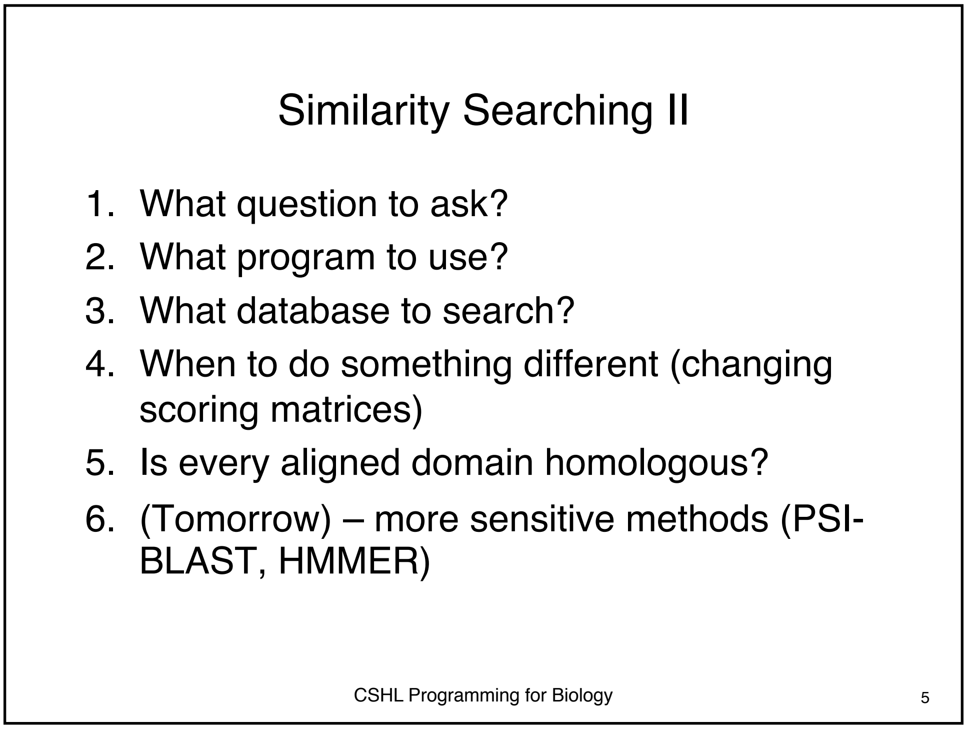
   - high identity (>50% alignments) to reduce over-extension
3. Is every aligned residue homologous?
   - alignment overextension
4. (Tomorrow) All methods (pairwise, HMM, PSSM) miss homologs, and find homologs the other methods miss



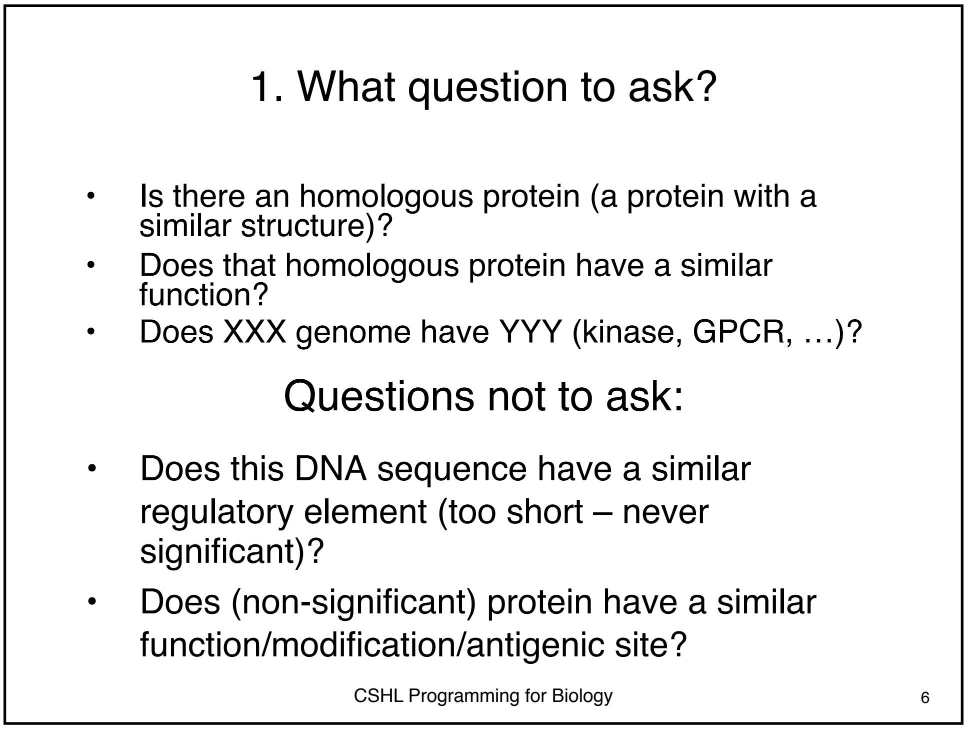
Review – Sequence Similarity - Conclusions

- *Homologous* sequences share a common ancestor, but most sequences are *non-homologous*
- Always compare Protein Sequences
- Sequence Homology can be reliably inferred from statistically significant similarity (non-homology cannot from non-similarity)
- Homologous proteins share common structures, but not necessarily common functions
- Sequence statistical significance estimates are accurate (verify this yourself)$10^{-6} < E() < 10^{-3}$ is statistically significant



Similarity Searching II

1. What question to ask?
2. What program to use?
3. What database to search?
4. When to do something different (changing scoring matrices)
5. Is every aligned domain homologous?
6. (Tomorrow) – more sensitive methods (PSI-BLAST, HMMER)



1. What question to ask?

- Is there an homologous protein (a protein with a similar structure)?
- Does that homologous protein have a similar function?
- Does XXX genome have YYY (kinase, GPCR, …)?

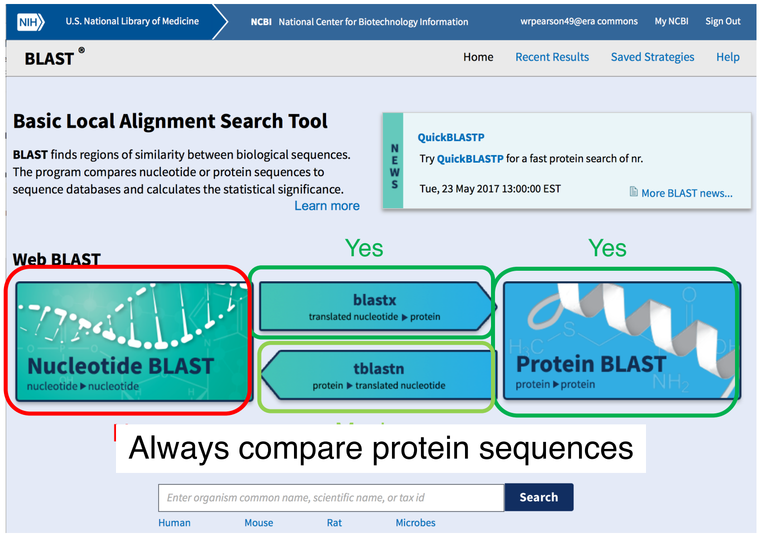
Questions not to ask:

- Does this DNA sequence have a similar regulatory element (too short – never significant)?
- Does (non-significant) protein have a similar function/modification/antigenic site?

2. What program to run?

- What is your query sequence?
  - protein – BLASTP (NCBI), SSEARCH (EBI)
  - protein coding DNA (EST) – BLASTX (NCBI), FASTX (EBI)
  - DNA (structural RNA, repeat family) – BLASTN (NCBI), FASTA (EBI)
- Does XXX genome have YYY (protein)?
  - TBLASTN YYY vs XXX genome
  - TFASTX YYY vs XXX genome
- Does my protein contain repeated domains?
  - LALIGN (UVa http://fasta.bioch.virginia.edu, EBI)

NCBI BLAST Server
blast.ncbi.nlm.nih.gov



Basic Local Alignment Search Tool

BLAST finds regions of similarity between biological sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance.
Learn more

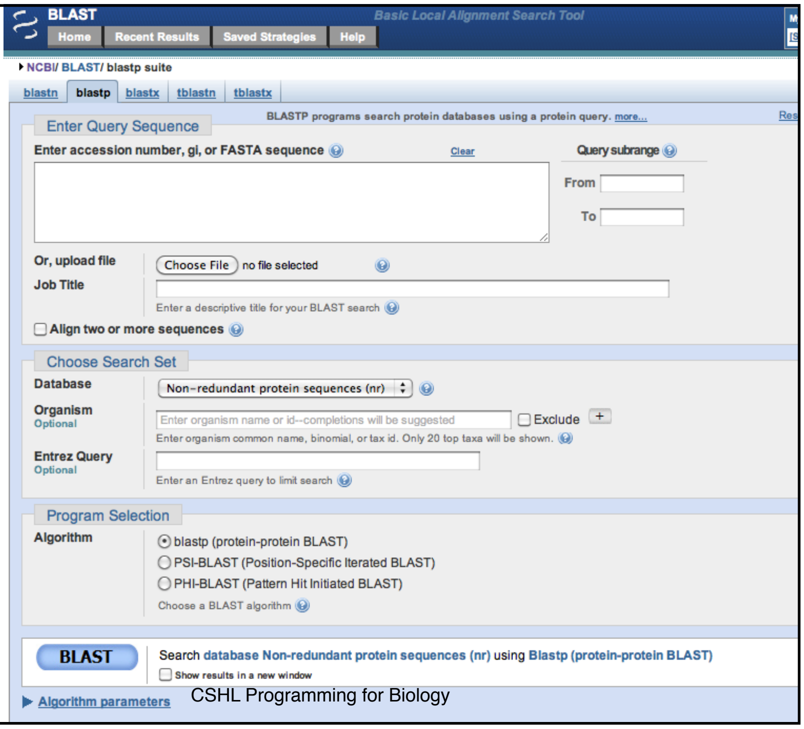
NEWS

QuickBLASTP
Try **QuickBLASTP** for a fast protein search of nr.
Tue, 23 May 2017 13:00:00 EST
More BLAST news...

Web BLAST

Always compare protein sequences

NCBI BLAST Server



BLASTP programs search protein databases using a protein query. more...

Enter Query Sequence

Enter accession number, gi, or FASTA sequence

Query subrange

From

To

Or, upload file: no file selected

Job Title

Enter a descriptive title for your BLAST search

□ Align two or more sequences

Choose Search Set

Database: Non-redundant protein sequences (nr)

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.

Entrez Query (Optional)

Enter an Entrez query to limit search

Program Selection

Algorithm

● blastp (protein-protein BLAST)

○ PSI-BLAST (Position-Specific Iterated BLAST)

○ PHI-BLAST (Pattern Hit Initiated BLAST)

Choose a BLAST algorithm

Search database Non-redundant protein sequences (nr) using Blastp (protein-protein BLAST)

□ Show results in a new window

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3. What database to search?

- Search the smallest comprehensive database likely to contain your protein
  - vertebrates – human proteins (40,000)
  - NCBI Landmark sequences (human, mouse, no rat)
  - Quest for Orthologs reference proteomes (1,000,000)
- Search a richly annotated protein set (SwissProt, 500,000)
- Always search NR (> 50 million) *LAST*
- Never Search “GenBank” (DNA)

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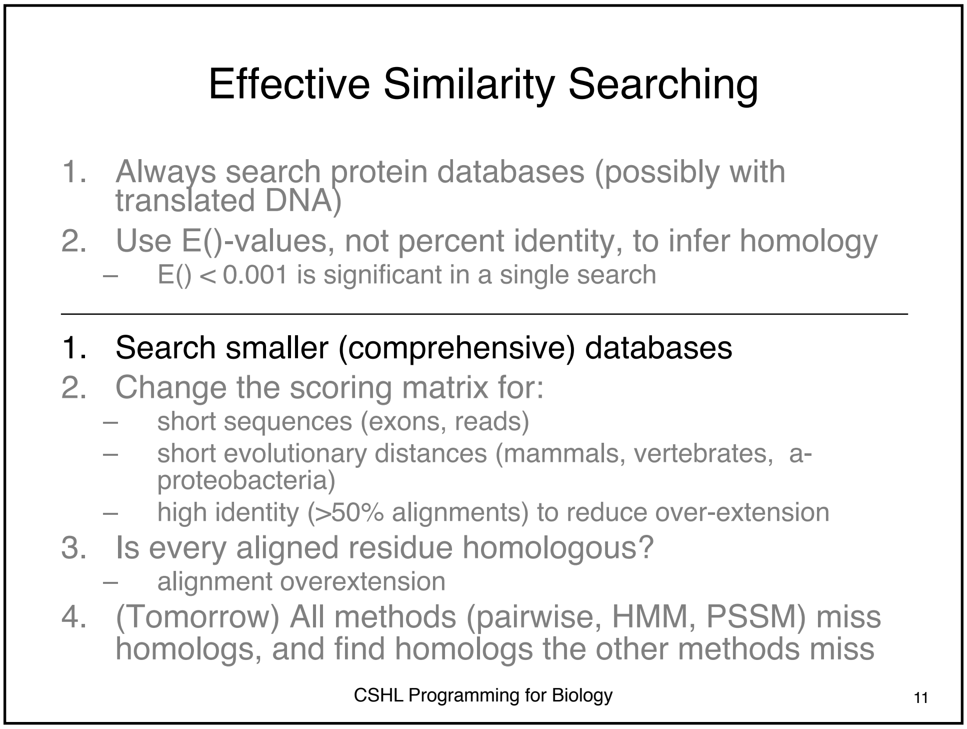

Effective Similarity Searching

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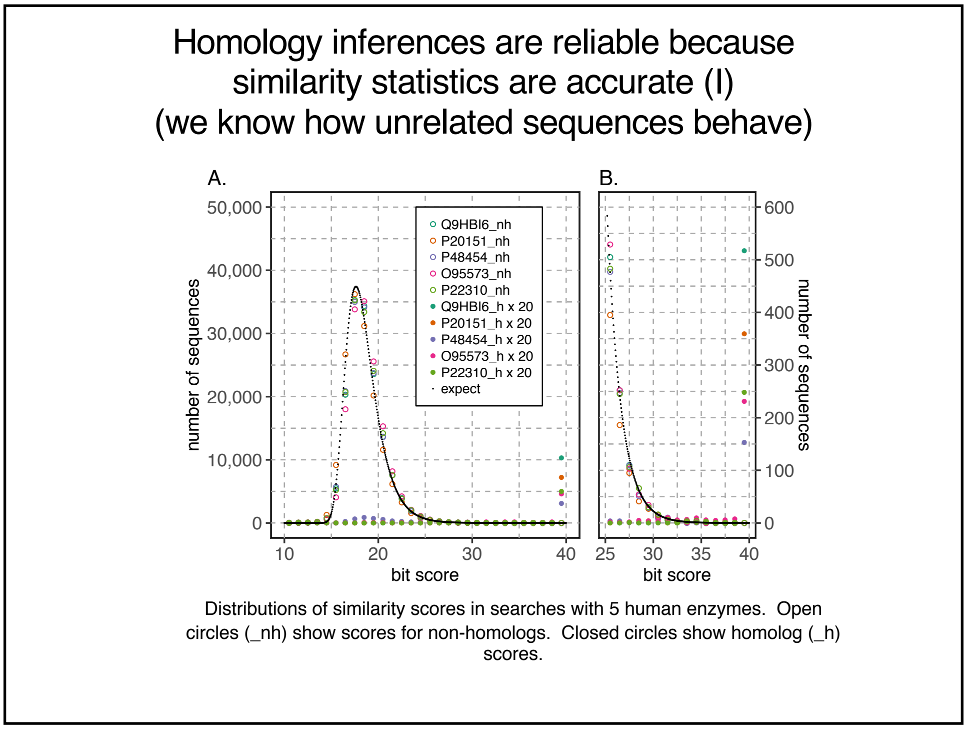

Homology inferences are reliable because similarity statistics are accurate (I) (we know how unrelated sequences behave)

Distributions of similarity scores in searches with 5 human enzymes. Open circles (_nh) show scores for non-homologs. Closed circles show homolog (_h) scores.

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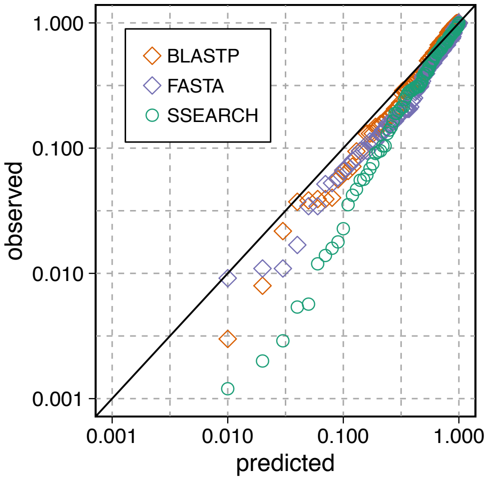
Homology inferences are reliable because similarity statistics are accurate (II) (we know how unrelated sequences behave)



Reported (observed) and expected probabilities of the highest scoring unrelated sequence in searches with 100 human enzymes vs 78 complete proteomes (~1 million sequences).

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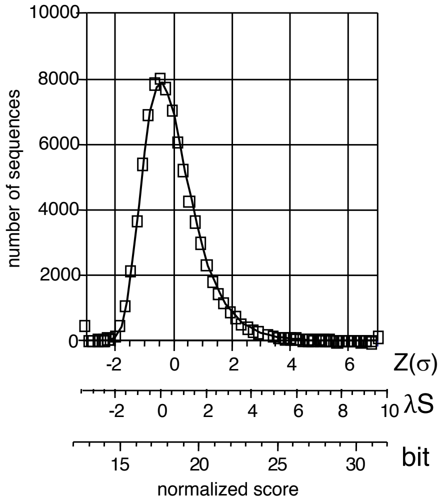
Why smaller databases are better – statistics



$S' = \lambda S_{raw} - \ln K\, m\, n$

$S_{bits} = (\lambda S_{raw} - \ln K)/\ln(2)$

$P(S'>x) = 1 - \exp(-e^{-x})$

$P(S_{bits} > x) = 1 - \exp(-mn2^{-x})$

$E(S'>x\ |D) = P\ D$

Bonferroni correction

$P(B \text{ bits}) = m\ n\ 2^{-B}$

$P(40 \text{ bits}) = 1.5\text{x}10^{-7}$

$E(40\ |\ D=4000) = 6\text{x}10^{-4}$

$E(40\ |\ D=60E6) = 9$

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Local similarity statistics

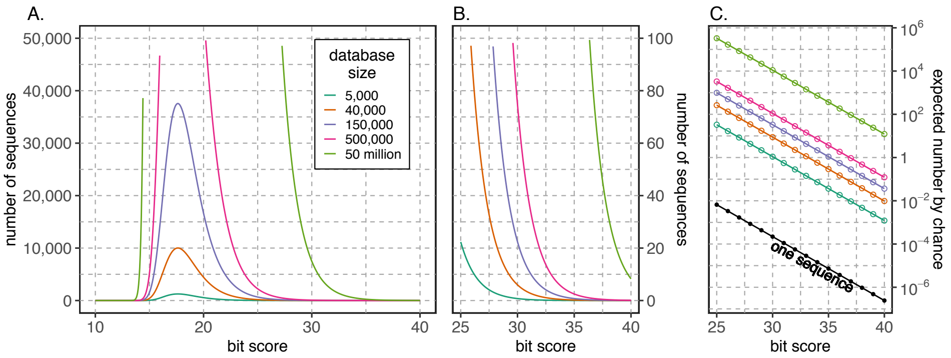
$S' = \lambda S_{raw} - \ln K\, m\, n$ m: query length, n: subj length
$S_{bit} = (\lambda S_{raw} - \ln K)/\ln(2)$
$P(S'>x) = 1 - \exp(-e^{-x})$
$P(S'>x) = e^{-x}$ (for $P < 0.1$)

$P(S_{bits} > bits) = 1 - \exp(-mn2^{-x})$
$P(S_{bits} > bits) = mn2^{-bits}$ (for $P < 0.1$)

$E(S', S_{bits}\ ID) = PD$
$E(S_{bits}\ ID) = D\ mn2^{-bits}$ Bonferroni correction

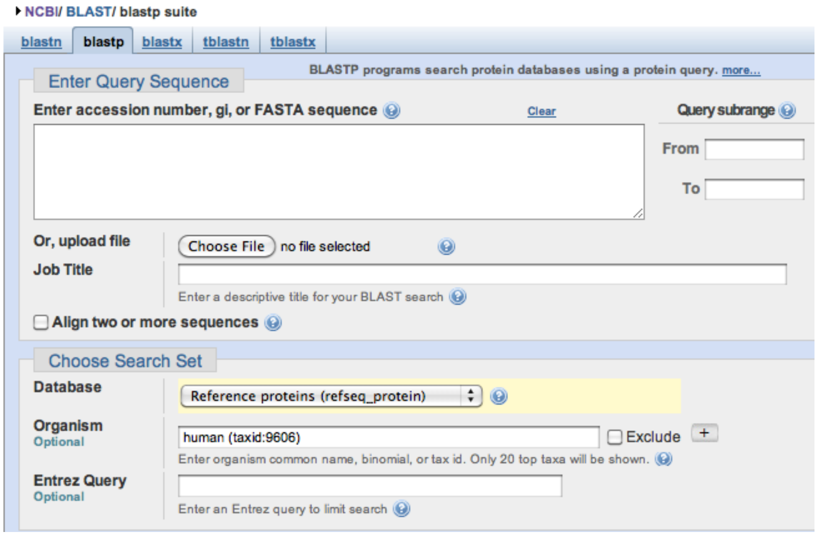
$dblength = D\ n$
$E(S_{bit}) = m\ dblength\ 2^{-bits}$ (BLAST)

Smaller databases increase sensitivity



stat_chk/hist_probs4b_xy.r

NCBI – selecting sequences with Entrez



▸ NCBI/ BLAST/ blastp suite

blastn | blastp | blastx | tblastn | tblastx

BLASTP programs search protein databases using a protein query. more...

Enter Query Sequence

Enter accession number, gi, or FASTA sequence Clear

Query subrange

From

To

Or, upload file: Choose File no file selected

Job Title

Enter a descriptive title for your BLAST search

□ Align two or more sequences

Choose Search Set

Database: Reference proteins (refseq_protein)

Organism (Optional): human (taxid:9606) □ Exclude +

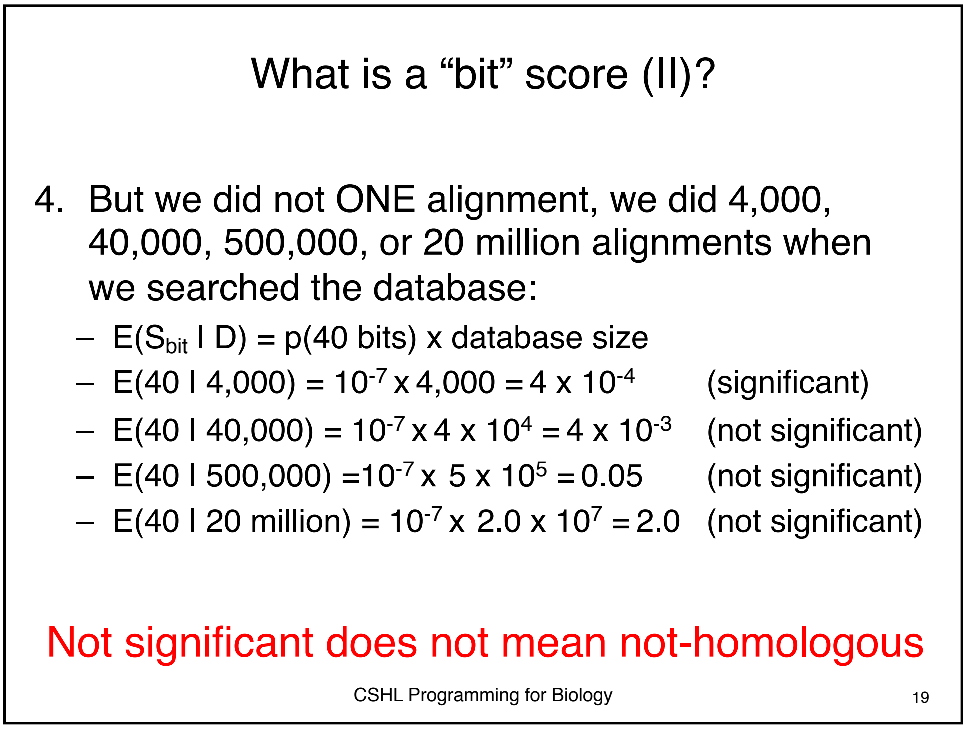
Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown.

Entrez Query (Optional)

Enter an Entrez query to limit search

What is a “bit” score (I)?

1. Scoring matrices (PAM250, BLOSUM62, VTML40) contain “log-odds” scores:
   - $s_{i,j}$ (bits) = $\log_2(q_{i,j}/p_i p_j)$ ($q_{i,j}$ freq. in homologs / $p_i p_j$ freq. by chance)
   - $s_{i,j}$ (bits) = 2 -> a residue is 2^2=4-times more likely to occur by homology compared with chance (at one residue)
   - $s_{i,j}$ (bits) = -1 -> a residue is 2^{-1} = 1/2 as likely to occur by homology compared with chance (at one residue)
2. An alignment score is the maximum sum of $s_{i,j}$ bit scores across the aligned residues.
   - A 40-bit score is 2^{40} more likely to occur by homology than by chance.
3. How often should a score occur by chance? In a 400 * 400 alignment, there are ~160,000 places where the alignment could start by chance, so we expect a score of 40 bits would occur: $P(S_{bit} > x) = 1 - \exp(-mn2^{-x}) \sim mn2^{-x}$
   - 400 x 400 x 2^{-40} = 160,000 / 2^{40} ($10^{13.3}$) = 1.5 x 10^{-7} times
   - Thus, the probability of a 40 bit score in ONE alignment is ~ 10^{-7}



What is a “bit” score (II)?

4. But we did not ONE alignment, we did 4,000, 40,000, 500,000, or 20 million alignments when we searched the database:
   - $E(S_{bit} | D) = p(40 \text{ bits}) \times \text{database size}$
   - $E(40 | 4{,}000) = 10^{-7} \times 4{,}000 = 4 \times 10^{-4}$ (significant)
   - $E(40 | 40{,}000) = 10^{-7} \times 4 \times 10^{4} = 4 \times 10^{-3}$ (not significant)
   - $E(40 | 500{,}000) = 10^{-7} \times 5 \times 10^{5} = 0.05$ (not significant)
   - $E(40 | 20 \text{ million}) = 10^{-7} \times 2.0 \times 10^{7} = 2.0$ (not significant)

Not significant does not mean not-homologous

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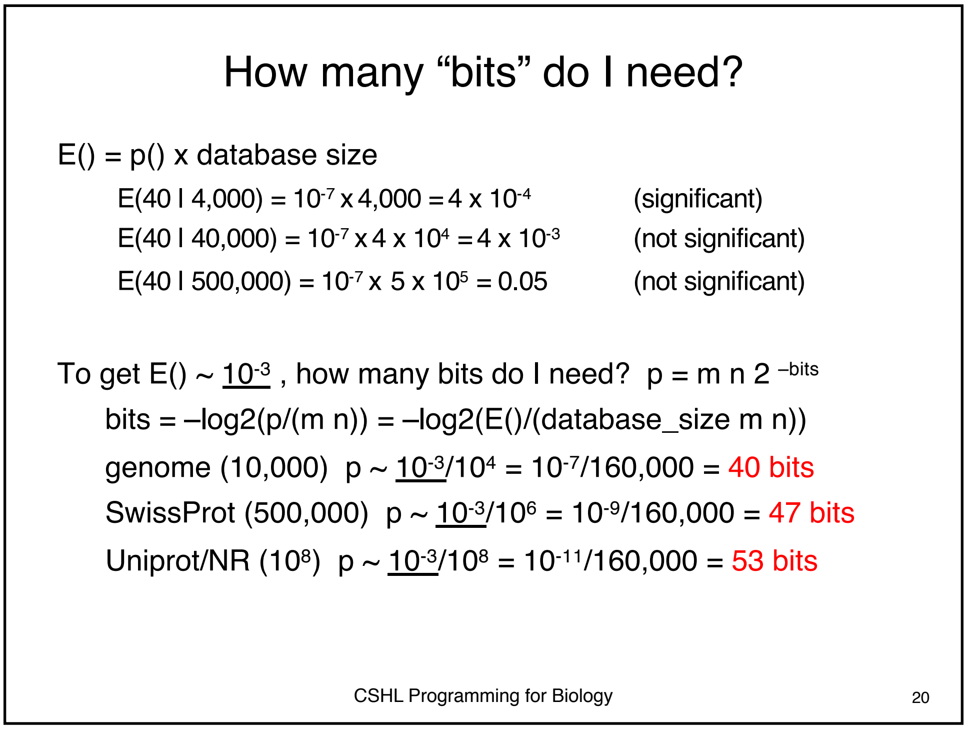

How many “bits” do I need?

E() = p() x database size

$E(40 | 4{,}000) = 10^{-7} \times 4{,}000 = 4 \times 10^{-4}$ (significant)

$E(40 | 40{,}000) = 10^{-7} \times 4 \times 10^{4} = 4 \times 10^{-3}$ (not significant)

$E(40 | 500{,}000) = 10^{-7} \times 5 \times 10^{5} = 0.05$ (not significant)

To get E() ~ $\underline{10^{-3}}$, how many bits do I need? $p = m\, n\, 2^{-\text{bits}}$

bits = –log2(p/(m n)) = –log2(E()/(database_size m n))

genome (10,000) $p \sim \underline{10^{-3}}/10^{4} = 10^{-7}/160{,}000$ = 40 bits

SwissProt (500,000) $p \sim \underline{10^{-3}}/10^{6} = 10^{-9}/160{,}000$ = 47 bits

Uniprot/NR (10^{8}) $p \sim \underline{10^{-3}}/10^{8} = 10^{-11}/160{,}000$ = 53 bits

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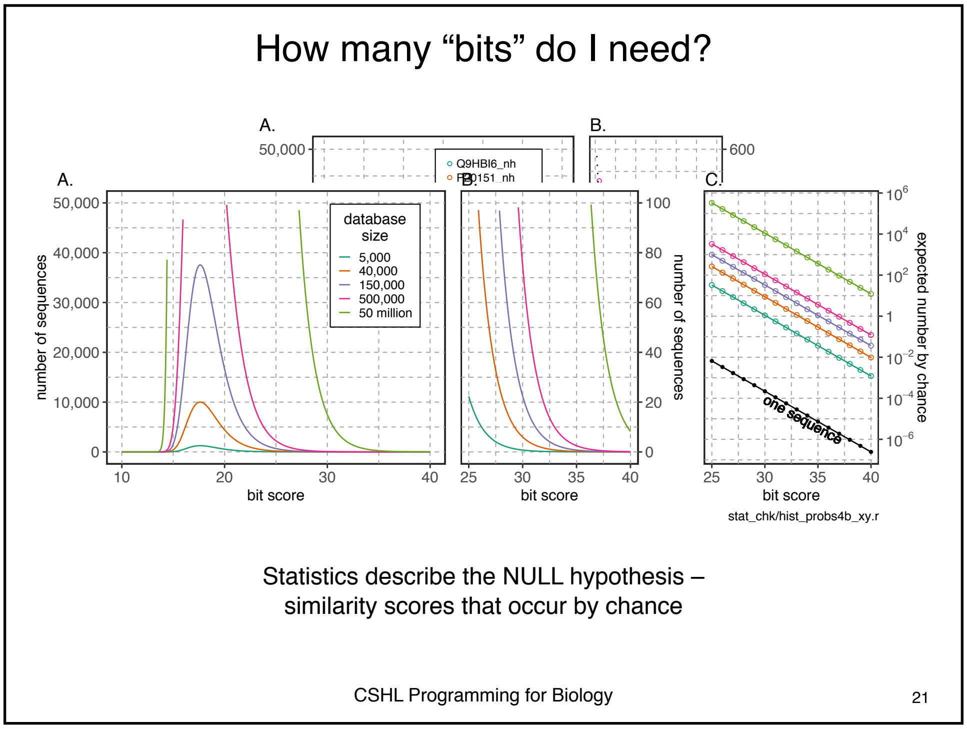
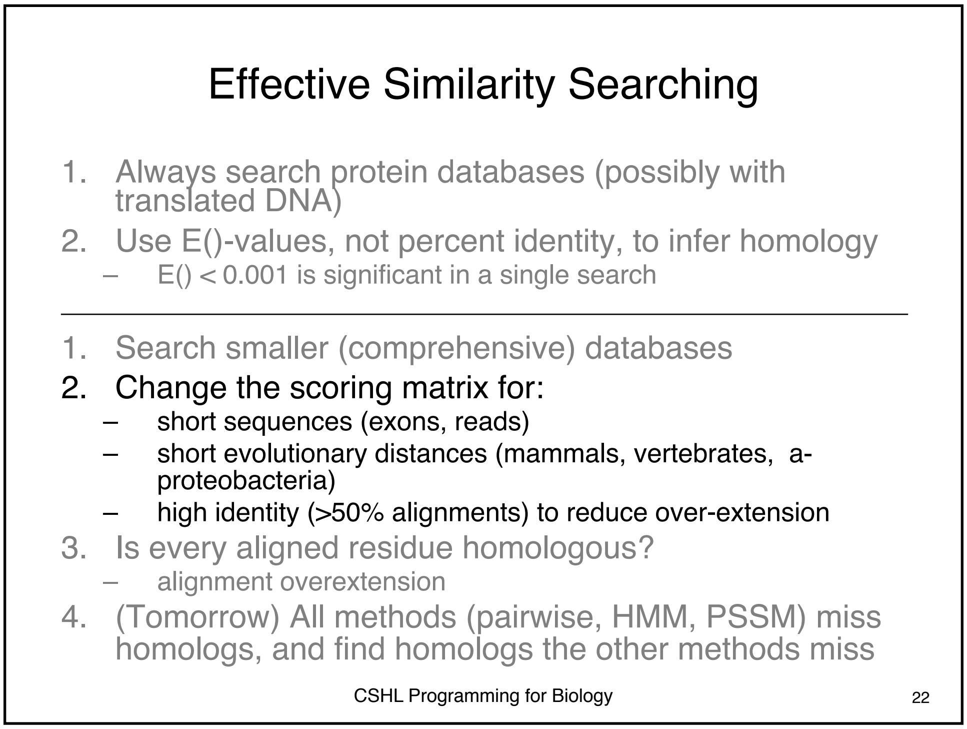


How many "bits" do I need?

Statistics describe the NULL hypothesis –
similarity scores that occur by chance



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Scoring matrices

- Scoring matrices can set the evolutionary look-back time for a search
  - Lower PAM (PAM10/VT10 … PAM/VT40) for closer (10% … 50% identity)
  - Higher BLOSUM for higher conservation (BLOSUM50 distant, BLOSUM80 conserved)
- Shallow scoring matrices for short domains/short queries (metagenomics)
  - Matrices have “bits/position” (score/position), 40 aa at 0.45 bits/position (BLOSUM62) means 18 bit ave. score (50 bits significant)
- Deep scoring matrices allow alignments to continue, possibly outside the homologous region

Scoring matrices and alignment length

Pam40

	A	R	N	D	E	I	L
A	8						
R	-9	12					
N	-4	-7	11				
D	-4	-13	3	11			
E	-3	-11	-2	4	11		
I	-6	-7	-7	-10	-7	12	
L	-8	-11	-9	-16	-12	-1	10

Pam250

	A	R	N	D	E	I	L
A	2						
R	-2	6					
N	0	0	2				
D	0	-1	2	4			
E	0	-1	1	3	4		
I	-1	-2	-2	-2	-2	5	
L	-2	-3	-3	-4	-3	2	6

$$\boxed{\lambda S_{i,j} = \log_b\left(\frac{q_{i,j}}{p_i p_j}\right)}$$

q_{ij} : homolog frequency wat PAM40, 250

$q_{R:N\,(\ 40)} = 0.000435$ $p_R = 0.051$

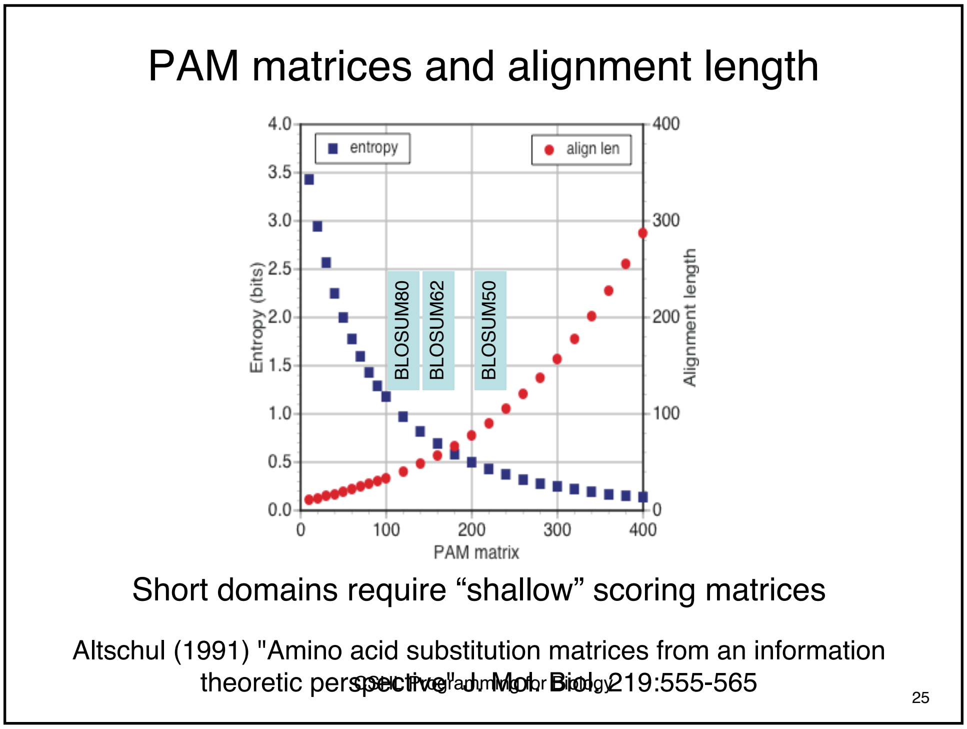
$q_{R:N\,(250)} = 0.002193$ $p_N = 0.043$

$\lambda_2\ S_{ij} = \mathrm{lg}_2\ (q_{ij}/p_ip_j)\ \lambda_e\ S_{ij} = \ln(q_{ij}/p_ip_j)$ $p_Rp_N = 0.002193$

$\lambda_2\ S_{R:N(\ 40)} = \mathrm{lg}_2\ (0.000435/0.00219) = -2.333$

$\lambda_2 = 1/3;\ S_{R:N(\ 40)} = -2.333/l_2 = -7$

$\lambda\ S_{R:N(250)} = \mathrm{lg}2\ (0.002193/0.002193) = 0$



PAM matrices and alignment length

Short domains require “shallow” scoring matrices

Altschul (1991) "Amino acid substitution matrices from an information theoretic perspective" J. Mol. Biol. 219:555-565

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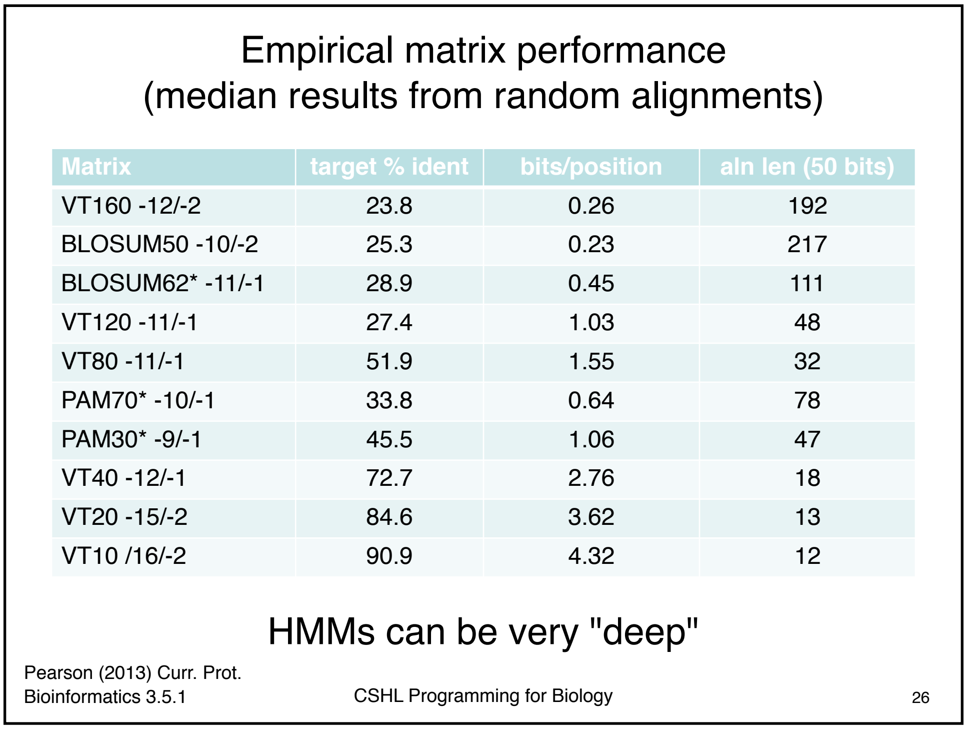

Empirical matrix performance (median results from random alignments)

Matrix	target % ident	bits/position	aln len (50 bits)
VT160 -12/-2	23.8	0.26	192
BLOSUM50 -10/-2	25.3	0.23	217
BLOSUM62* -11/-1	28.9	0.45	111
VT120 -11/-1	27.4	1.03	48
VT80 -11/-1	51.9	1.55	32
PAM70* -10/-1	33.8	0.64	78
PAM30* -9/-1	45.5	1.06	47
VT40 -12/-1	72.7	2.76	18
VT20 -15/-2	84.6	3.62	13
VT10 /16/-2	90.9	4.32	12

HMMs can be very "deep"

Pearson (2013) Curr. Prot. Bioinformatics 3.5.1

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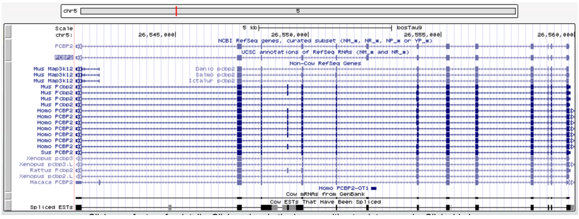
Scoring matrices, alignment length, and exon detection – bovine PCBP2



```
       [       10        20  ][    30][      40 ][     50        60        70        80
human  MDTGVIEGGLNVTLTIRLLMHGKEVGSIIGKKGESVKKMREESGARINISEGNCPERIITLAGPTNAIFKAFAMIIDKLE
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
bovin  MDTGVIEGGLNVTLTIRLLMHGKEVGSIIGKKGESVKKMREESGARINISEGNCPERIITLAGPTNAIFKAFAMIIDKLE
       [       10        20  ][    30][      40 ][     50        60        70        80
       ][      90       100       110       120    ][ 130       140       150       160
human  EDISSSMTNSTAASRPPVTLRLVVPASQCGSLIGKGGCKIKEIRESTGAQVQVAGDMLPNSTERAITIAGIPQSIIECVK
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
bovin  EDISSSMTNSTAASRPPVTLRLVVPASQCGSLIGKGGCKIKEIRESTGAQVQVAGDMLPNSTERAITIAGIPQSIIECVK
       ][      90       100       110       120    ][ 130       140       150       160
              ][0       180       190      ][00       210       220       ][0       240
human  QICVVMLETLSQSPPKGVTIPYRPKPSSSPVIFAGGQDRYSTGSDSASFPHTTPSMCLNPDLEGPPLEAYTIQGQYAIPQ
       ::::::::    ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
bovin  QICVVMLE----SPPKGVTIPYRPKPSSSPVIFAGGQDRYSTGSDSASFPHTTPSMCLNPDLEGPPLEAYTIQGQYAIPQ
              ]   1[0       180       190  ][   200       210       220   ][  230
        ][    250       260     ][270       28][      290      ][00       310       320
human  PDLTKLHQLAMQQSHFPMTHGNTGFSGIESSSPEVKGYWGLDASAQTTSHELTIPNDLIGCIIGRQGAKINEIRQMSGAQ
       ::::::::::::::::::::::::::             :::::::::::::::::::::::::::::::::::::::::
bovin  PDLTKLHQLAMQQSHFPMTHGNTGFSA------------GLDASAQTTSHELTIPNDLIGCIIGRQGAKINEIRQMSGAQ
        ][0       250       260 ][                270       280][     290       300
              330       340       350   ][  360    ]
human  IKIANPVEGSTDRQVTITGSAASISLAQYLINVRLSSETGGMGSS
       :::::::::::::::::::::::::::::::::::::::::::::
bovin  IKIANPVEGSTDRQVTITGSAASISLAQYLINVRLSSETGGMGSS
          310       320       330       ][0        ]
```

Scoring matrices, alignment length, and exon detection – bovine PCBP2

name	start	end	len	MD10	bits	BP62	bits
ex_1	1	23	23	=	58	+5	45
ex_2	24	31	8	=	30	-	<25
ex_3	32	42	11	=	36	+1	27
ex_4	43	81	39	=	88	=	61
ex_5	82	125	44	=	96	=	66
ex_6	126	168	43	=	96	+5	65
ex_7	169	197	29	=	69	+2	50
ex_8	198	228	31	=	76	+43	53
ex_9	229	242	14	+4	40	+4	32
ex_10	243	266	24	+5	60	+87	45
ex_11	267	280	14	=	42	+38	32
ex_12	281	297	17	=	49	+2	34
ex_13	298	354	57	=	120	=	78
ex_14	355	365	11	=	37	=	32

MD10

```
qRegion: bits=71.5; Id=1.000; exon_8~8

       ][00       210       220       ]
sp|Q15 QDRYSTGSDSASFPHTTPSMCLNPDLEGPPLE
       ::::::::::::::::::::::::::::::::
chr5:2 QDRYSTGSDSASFPHTTPSMCLNPDLEGPPLE
```

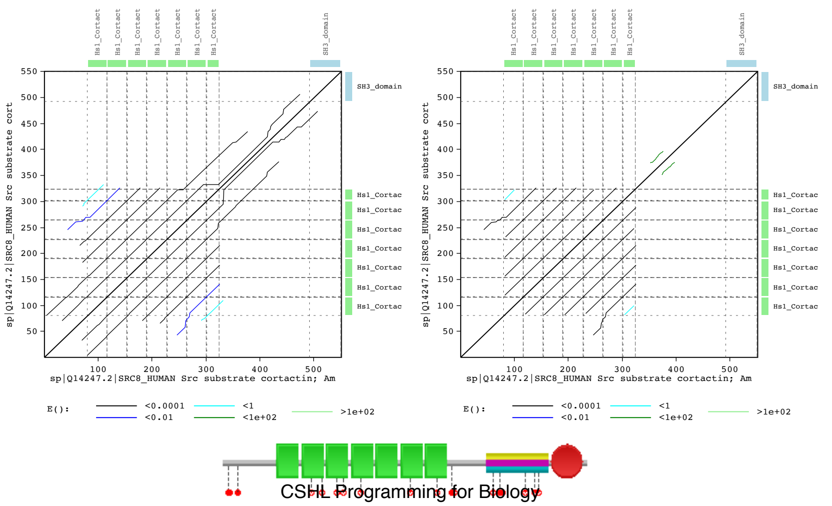
BP62

```
qRegion: 181-197 : bits=1.6;  Id=0.500:  exon_7~7
qRegion: 198-228 : bits=52.7; Id=1.000:  exon_8~8
qRegion: 229-242 : bits=0.0;  Id=0.333;  exon_9~9
qRegion: 243-255 : bits=5.4;  Id=0.286;  exon_10~10
              190      ][00       210       220       ][0       240 ][    250
sp|Q15 PYRPKPSSSPVIFAGGQDRYSTGSDSASFPHTTPSMCLNPDLEGPPLEAYTIQGQYAIPQPDLTKLHQLAMQQSH
       :.: : :  :      ::::::::::::::::::::::::::::::::   ..:.  . .: ::.  .:. . .:
chr5:2 PWRLKSSIYP------QDRYSTGSDSASFPHTTPSMCLNPDLEGPPLE---VRGD--VQSPRLTQSFRLSRDCQH
```

Scoring matrices affect alignment boundaries (homologous over-extension)

BLOSUM62 -11/-1

VTML80 -10/-1



Scoring Matrices - Summary

- PAM and BLOSUM matrices greatly improve the sensitivity of protein sequence comparison – low identity with significant similarity
- PAM matrices have an evolutionary model - lower number, less divergence – lower=closer; higher=more distant
- BLOSUM matrices are sampled from conserved regions at different average identity – higher=more conservation
- Shallow matrices set maximum look-back time
- Short alignments (domains, exons, reads) require shallow (higher information content) matrices

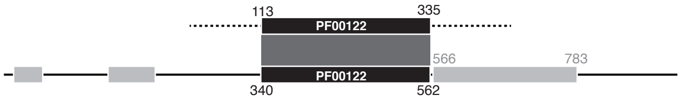
Effective Similarity Searching

1. Always search protein databases (possibly with translated DNA)
2. Use E()-values, not percent identity, to infer homology
   – E() < 0.001 is significant in a single search

---

1. Search smaller (comprehensive) databases
2. Change the scoring matrix for:
   – short sequences (exons, reads)
   – short evolutionary distances (mammals, vertebrates, a-proteobacteria)
   – high identity (>50% alignments) to reduce over-extension
3. Is every aligned residue homologous?
   – alignment overextension
4. (Tomorrow) All methods (pairwise, HMM, PSSM) miss homologs, and find homologs the other methods miss

Over-extension into random sequence

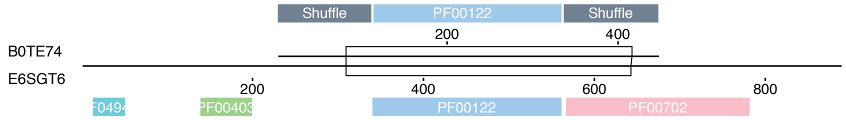


```
> pf26|15978520|E6SGT6|E6SGT6_THEM7 Heavy metal translocating P-type
ATPase EC=3.6.3.4
Length=888

 Score =  299 bits (766),  Expect = 1e-90, Method: Compositional matrix adjust.
 Identities = 170/341 (50%), Positives = 224/341 (66%), Gaps = 19/341 (6%)
                                       113
Query  84   FLFVNVFAALFNYWPTEGKILMFGKLEKVLITLILLGKTLEAVAKGRTSEAIKKLMGLKA  143
            +L+  V  A    +P+     +F  +  V++ L+ LG  LE  A+GRTSEAIKKL+GL+A
Sbjct  312  WLYSTVAVAFPQIFPSMALAEVFYDVTAVVVALVNLGLALELRARGRTSEAIKKLIGLQA  371
                                       340
Query  144  KRARVIRGGRELDIPVEAVLAGDLVVVRPGEKIPVDGVVEEGASAVDESMLTGESLPVDK  203
            + ARV+R G E+DIPVE VL GD+VVVRPGEKIPVDGVV EG S+VDESM+TGES+PV+
Sbjct  372  RTARVVRDGTEVDIPVEEVLVGDIVVVRPGEKIPVDGVVIEGTSSVDESMITGESIPVEM  431

Query  204  QPGDTVIGATLNKQGSFKFRATKVGRDTALAQIISVVEEAQGSKAPIQRLADTISGYFVP  263
            +PGD VIGAT+N+ GSF+FRATKVG+DTAL+QII +V++AQGSKAPIQR+ D +S YFVP
Sbjct  432  KPGDEVIGATINQTGSFRFRATKVGKDTALSQIIRLVQDAQGSKAPIQRIVDRVSHYFVP  491

Query  264  VVVSLAVITFFVWYFAVAPENFTRALLNFTAVLVIACPCALGLATPTSIMVGTGKGAEKG  323
             V+ LA++   VWY       +  AL+ F   L+IACPCALGLATPTS+ VG GKGAE+G
Sbjct  492  AVLILAIVAAVVWYVFGPEPAYIYALIVFVTTLIIACPCALGLATPTSLTVGIGKGAEQG  551
                      335
Query  324  ILFKGGEHLENAG---------GGAHTEGAENKAELLKTRATGISILVTLGLTAKGRDRS  374
            IL + G+ L+ A           G  T+G     +++   ATG    + L LTA
Sbjct  552  ILIRSGDALQMASRLDVIVLDKTGTITKGKPELTDVVA--ATGFDEDLILRLTA------  603
                      562 566
Query  375  TVAFQKNTGFKLKIPIGQAQLQREVAASESIVISAYPIVGV  415
              A ++ +   L   I +  L R +A  E+   +A P   GV
Sbjct  604  --AIERKSEHPLATAIVEGALARGLALPEADGFAAIPGHGV  642
```

Mills and Pearson (2013) Bioinformatics 29:3007

Sub-alignment scoring detects over-extension

```
>>sp|E6SGT6|E6SGT6_THEM7 Heavy metal translocating P-type ATPase EC=3.6.3.4   (888 aa)
 qRegion: 81-112:309-340 : score=15; bits=12.3; Id=0.219; Q=0.0 :  Shuffle
 qRegion: 113-335:341-563 : score=736; bits=232.8; Id=0.641; Q=644.7 :  PF00122
 qRegion: 336-415:564-642 : score=14; bits=12.0; Id=0.236; Q=0.0 :  Shuffle
  Region: 81-111:309-339 : score=11; bits=11.1; Id=0.194; Q=0.0 :  NODOM :0
  Region: 112-334:340-562 : score=736; bits=232.8; Id=0.641; Q=644.7 :  PF00122  Pfam
  Region: 338-415:566-642 : score=16; bits=12.6; Id=0.244; Q=0.0 :  PF00702  Pfam
 s-w opt: 632  Z-score: 1048.6  bits: 204.2 E(274545): 3.7e-51
Smith-Waterman score: 765; 49.7% identity (73.3% similar) in 344 aa overlap (81-415:309-642)
```



```
               50        60        70        80        90       100       110 ][    120
B0TE74 LPIVPGTMALGVQSDGKDETLLVALEVPVDRERVGPAIVDAFRFLFVNVFAALFNYWPTEGKILMFGKLEKVLITLILLG
                                               :  .:. .: .:. . .:. .   .:  .  :...:. ::
E6SGT6 ILGLLTLPVMLWSGSHFFNGMWQGLKHRQANMHTLISIGIAAAWLYSTVAVAFPQIFPSMALAEVFYDvtavvvalvnlg
      270       280       290       300       310       320       330        3][
                                          ...
              290       300       310       320       330    ][          340       350
B0TE74 APENFTRALLNFTAVLVIACPCALGLATPTSIMVGTGKGAEKGILFKGGEHLENAGG---------GAHTEGAENKAELL
           .  ::. :...:.::::::::::::::. :: :::::.:::...:. :. :.          :. :.:  . ....
E6SGT6 PEPAYIYALIVFVTTLIIACPCALGLATPTSLTVGIGKGAEQGILIRSGDALQMASRLDVIVLDKTGTITKGKPELTDVV
      510       520       530       540       550       560 ]   [ 570       580
             360       370       380       390       400       410       420       430
B0TE74 KTRATGISILVTLGLTAKGRDRSTVAFQKNTGFKLKIPIGQAQLQREVAASESIVISAYPIVGVVVDSLVTTAFLAVEEI
          :::..  . : :::        :.....   :   :..  : : .:  :.  ..: :  ::
E6SGT6 --AATGFDEDLILRLTA--------AIERKSEHPLATAIVEGALARGLALPEADGFAAIPGHGVEAQVEGHHVLVGNERL
            590       600               610       620       630       640       650
```

Scoring matrices, alignment length, and exon detection – bovine PCBP2

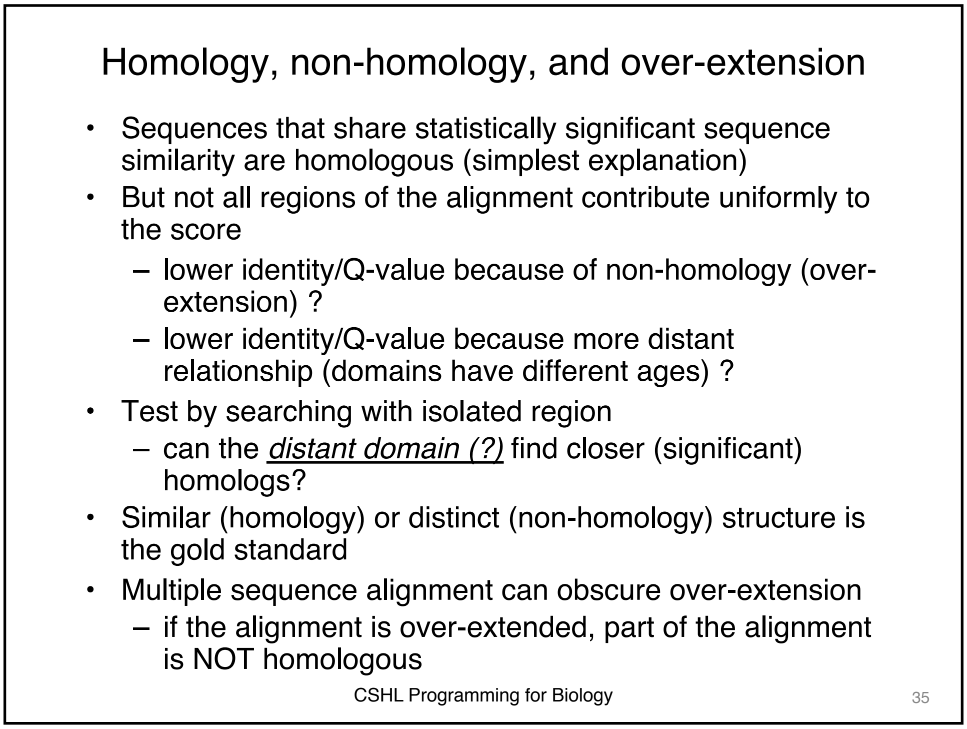
name	start	end	len	MD10	bits	BP62	bits
ex_1	1	23	23	=	58	+5	45
ex_2	24	31	8	=	30	-	<25
ex_3	32	42	11	=	36	+1	27
ex_4	43	81	39	=	88	=	61
ex_5	82	125	44	=	96	=	66
ex_6	126	168	43	=	96	+5	65
ex_7	169	197	29	=	69	+2	50
ex_8	198	228	31	=	76	+43	53
ex_9	229	242	14	+4	40	+4	32
ex_10	243	266	24	+5	60	+87	45
ex_11	267	280	14	=	42	+38	32
ex_12	281	297	17	=	49	+2	34
ex_13	298	354	57	=	120	=	78
ex_14	355	365	11	=	37	=	32

MD10

```
qRegion: bits=71.5; Id=1.000; exon_8~8

       ][00        210       220      ]
sp|Q15 QDRYSTGSDSASFPHTTPSMCLNPDLEGPPLE
       ::::::::::::::::::::::::::::::::
chr5:2 QDRYSTGSDSASFPHTTPSMCLNPDLEGPPLE
```

BP62

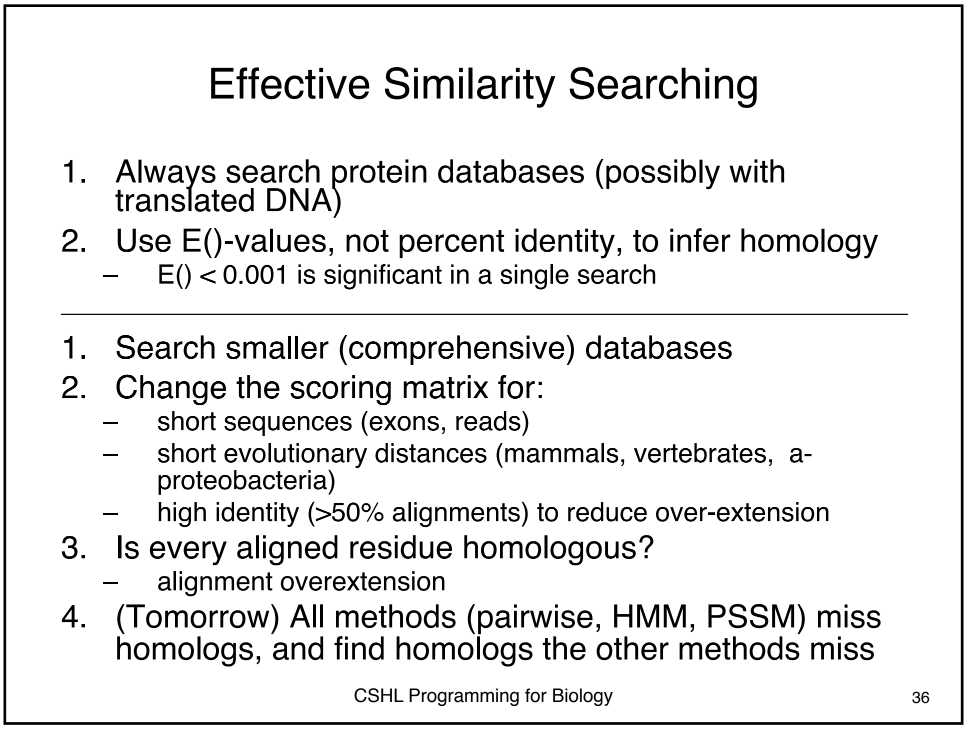
```
qRegion: 181-197 : bits=1.6;  Id=0.500:  exon_7~7
qRegion: 198-228 : bits=52.7; Id=1.000:  exon_8~8
qRegion: 229-242 : bits=0.0;  Id=0.333;  exon_9~9
qRegion: 243-255 : bits=5.4;  Id=0.286;  exon_10~10
              190      ][00       210       220       ][0       240 ][    250
sp|Q15 PYRPKPSSSPVIFAGGQDRYSTGSDSASFPHTTPSMCLNPDLEGPPLEAYTIQGQYAIPQPDLTKLHQLAMQQSH
       :.: : :  :      ::::::::::::::::::::::::::::::::   ..:.  . .: ::.  .:. . .:
chr5:2 PWRLKSSIYP------QDRYSTGSDSASFPHTTPSMCLNPDLEGPPLE---VRGD--VQSPRLTQSFRLSRDCQH
```



Homology, non-homology, and over-extension

- Sequences that share statistically significant sequence similarity are homologous (simplest explanation)
- But not all regions of the alignment contribute uniformly to the score
  - lower identity/Q-value because of non-homology (over-extension) ?
  - lower identity/Q-value because more distant relationship (domains have different ages) ?
- Test by searching with isolated region
  - can the *distant domain (?)* find closer (significant) homologs?
- Similar (homology) or distinct (non-homology) structure is the gold standard
- Multiple sequence alignment can obscure over-extension
  - if the alignment is over-extended, part of the alignment is NOT homologous

35



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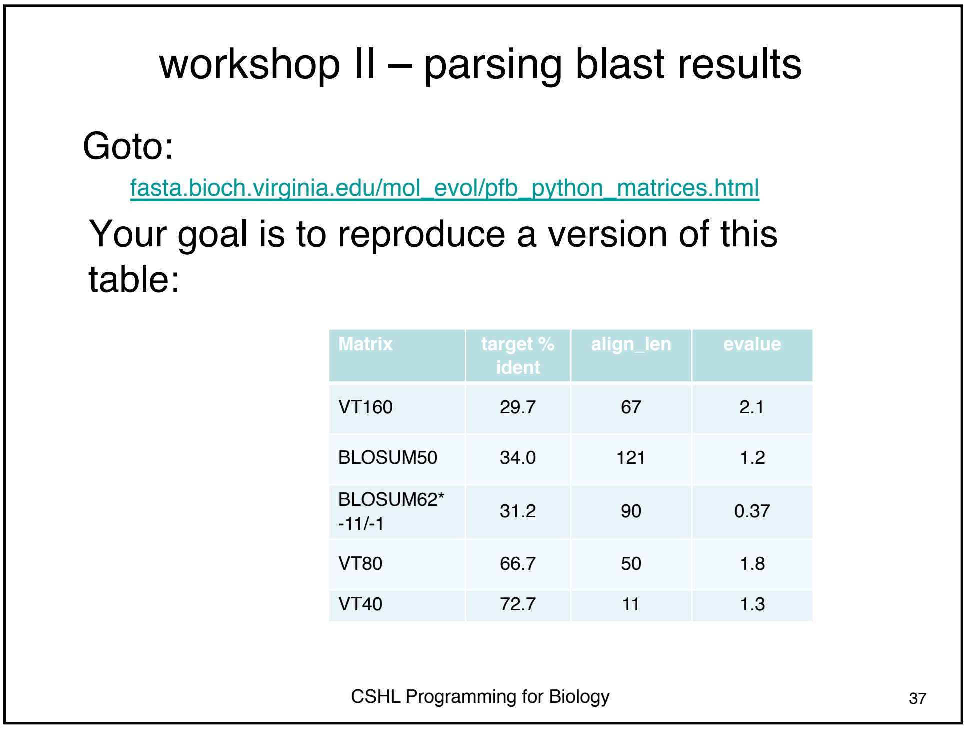
36



workshop II – parsing blast results

Goto:

fasta.bioch.virginia.edu/mol_evol/pfb_python_matrices.html

Your goal is to reproduce a version of this table:

Matrix	target % ident	align_len	evalue
VT160	29.7	67	2.1
BLOSUM50	34.0	121	1.2
BLOSUM62* -11/-1	31.2	90	0.37
VT80	66.7	50	1.8
VT40	72.7	11	1.3