For written notes on this lecture, please read chapter 19 of *The Practical Bioinformatician*

KI1972: Applied Bioinformatics & Computational Biology

Sequence Homology Interpretation

Limsoon Wong

June 2006

Karolinska Institutet

Plan

- **Recap of sequence alignment**
- **Guilt by association**
- **Active site/domain discovery**
- **Key mutation site discovery**

- **Guilt by other types of association**
  - Genome phylogenetic profiling
  - Protfun
  - SVM-Pairwise

Very Brief Recap of Sequence Comparison/Alignment

Karolinska Institutet

Motivations for Seq Comparison

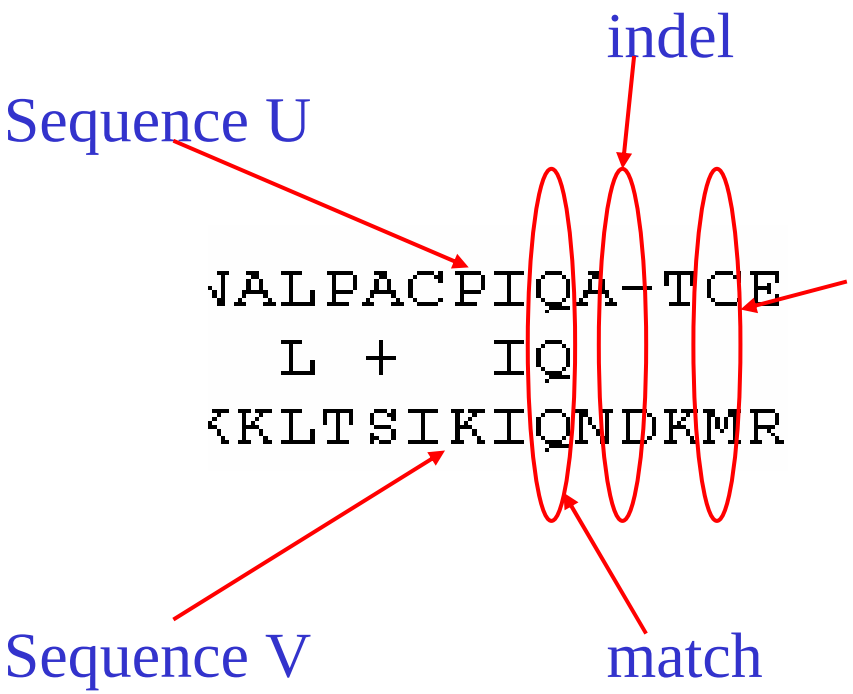
- **DNA is blue print for living organisms**

$\Rightarrow$ **Evolution is related to changes in DNA**

$\Rightarrow$ **By comparing DNA sequences we can infer evolutionary relationships between the sequences w/o knowledge of the evolutionary events themselves**

- **Foundation for inferring function, active site, and key mutations**

Sequence Alignment



- **Key aspect of seq comparison is seq alignment**
- **A seq alignment maximizes the number of positions that are in agreement in two sequences**

Sequence Alignment: Poor Example

- **Poor seq alignment shows few matched positions**

$\Rightarrow$ **The two proteins are not likely to be homologous**

Alignment by FASTA of the sequences of amicyanin and domain 1 of ascorbate oxidase

```
                          60         70         80         90         100
Amicyanin          MPHNVHFVAGVLGEAALKGPMMKKEQAYSLTFTEAGTYDYHCTPHPFMRGKVVVE
                                                :..:   . ::. ::
Ascorbate Oxidase ILQRGTPWADGTASISQCAINPGETFFYNFTVDNPGTFFYHGHLGMQRSAGLYGSLI
                        70         80         90         100        110        120
```

No obvious match between
Amicyanin and Ascorbate Oxidase

Sequence Alignment: Good Example

- **Good alignment usually has clusters of extensive matched positions**

⇒ **The two proteins are likely to be homologous**

```
>gi|13476732|ref|NP_108301.1|   unknown protein [Mesorhizobium loti]
gi|14027493|dbj|BAB53762.1|   unknown protein [Mesorhizobium loti]
          Length = 105

 Score =  105 bits (262), Expect = 1e-22
 Identities = 61/106 (57%), Positives = 73/106 (68%), Gaps = 1/106 (0%)

Query: 1   MKPGRLASIALAIIFLPMAVPAHAATIEITMENLVISPTEVSAKVGDTIRWVNKDVFAHT 60
           MK G L  ++       MA PA AATIE+T++ LV SP  V AKVGDTI WVN DV AHT
Sbjct: 1   MKAGALIRLSWLAALALMAAPAAAATIEVTIDKLVFSPATVEAKVGDTIEWVNNDVVAHT 60
```

good match between
Amicyanin and unknown M. loti protein

Multiple Alignment: An Example

- **Multiple seq alignment maximizes number of positions in agreement across several seqs**
- **seqs belonging to same "family" usually have more conserved positions in a multiple seq alignment**

```
gi|126467|      FHFTSWPDFGVPFTPIGMLKFLKKVKACNP--QYAGAIVVHCSAGVGRTGTFVVIDAMLD
gi|2499753      FHFTGWPDHGVPYHATGLLSFIRRVKLSNP--PSAGPIVVHCSAGAGRTGCYIVIDIMLD
gi|462550|      YHYTQWPDMGVPEYALPVLTFVRRSSAARM--PETGPVLVHCSAGVGRTGTYIVIDSMLQ
gi|2499751      FHFTSWPDHGVPDTTDLLINFRYLVRDYMKQSPPESPILVHCSAGVGRTGTFIAIDRLIY
gi|1709906      FQFTAWPDHGVPEHPTPFLAFLRRVKTCNP--PDAGPMVVHCSAGVGRTGCFIVIDAMLE
gi|126471|      LHFTSWPDFGVPFTPIGMLKFLKKVKTLNP--VHAGPIVVHCSAGVGRTGTFIVIDAMMA
gi|548626|      FHFTGWPDHGVPYHATGLLSFIRRVKLSNP--PSAGPIVVHCSAGAGRTGCYIVIDIMLD
gi|131570|      FHFTGWPDHGVPYHATGLLGFVRQVKSKSP--PNAGPLVVHCSAGAGRTGCFIVIDIMLD
gi|2144715      FHFTSWPDHGVPDTTDLLINFRYLVRDYMKQSPPESPILVHCSAGVGRTGTFIAIDRLIY
                 ..* *** ***      . *                ..****** ****... ** ..
```

Conserved sites

Application of Sequence Comparison: Guilt-by-Association

Karolinska Institutet

Function Assignment to Protein Seq

```
SPSTNRKYPPLPVDKLEEEINRRMADDNKLFREEFNALPACPIQATCEAASKEENKEKNR
YVNILPYDHSRVHLTPVEGVPDSDYINASFINGYQEKNKFIAAQGPKEETVNDFWRMIWE
QNTATIVMVTNLKERKECKCAQYWPDQGCWTYGNVRVSVEDVTVLVDYTVRKFCIQQVGD
VTNRKPQRLITQFHFTSWPDFGVPFTPIGMLKFLKKVKACNPQYAGAIVVHCSAGVGRTG
TFVVIDAMLDMMHSERKVDVYGFVSRIRAQRCQMVQTDMQYVFIYQALLEHYLYGDTELE
VT
```

- **How do we attempt to assign a function to a new protein sequence?**

Guilt-by-Association

- **Compare the target sequence T with sequences S_1, …, S_n of known function in a database**
- **Determine which ones amongst S_1, …, S_n are the mostly likely homologs of T**
- **Then assign to T the same function as these homologs**
- **Finally, confirm with suitable wet experiments**

Guilt-by-Association

Compare *T* with seqs of known function in a db

Good Sequence Alignment

- Good alignment usually has clusters of extensive matched positions

⇒ The two proteins are likely to be homologous

```
>gi|134767321|ref|NP_108301.1|  unknown protein [Mesorhizobium loti]
gi|14027493|dbj|BAB53762.1|  unknown protein [Mesorhizobium loti]
          Length = 105

 Score =  105 bits (262), Expect = 1e-22
 Identities = 61/106 (57%), Positives = 73/106 (68%), Gaps = 1/106 (0%)

Query: 1   MKPGRLASIALAIIFLPMAVPAHAATIEITMENLVISPTEVSAKVGDTIRWVNKDVFAHT 60
           MK G L  ++       MA PA AATIE+T++ LV SP  V AKVGDTI WVN DV AHT
Sbjct: 1   MKAGALIRLSWLAALALMAAPAAAATIEVTIDKLVFSPATVEAKVGDTIEWVNNDVVAHT 60
```

good match between Amicyanin and u[illegible]wn M. loti protein

Assign to *T* same function as homologs

Confirm with suitable wet experiments

Poor Sequence Alignment

- Poor seq alignment shows few matched positions

⇒ The two proteins are not likely to be homologous

Alignment by FASTA of the sequences of amicyanin and domain 1 of ascorbate oxidase

```
                    60        70        80        90       100
Amicyanin          MPHNVHFVAGVLGEAALKGPMMKKEQAYSLTFTEAGTYDYHCTPHPFMRGKVVV
                                                :.:  . ::. ::
Ascorbate Oxidase ILQRGTPWADGTASISQCAINPGETFFYNFTVDNPGTFFYHGHLGMQRSAGLYG
                    70        80        90       100       110
```

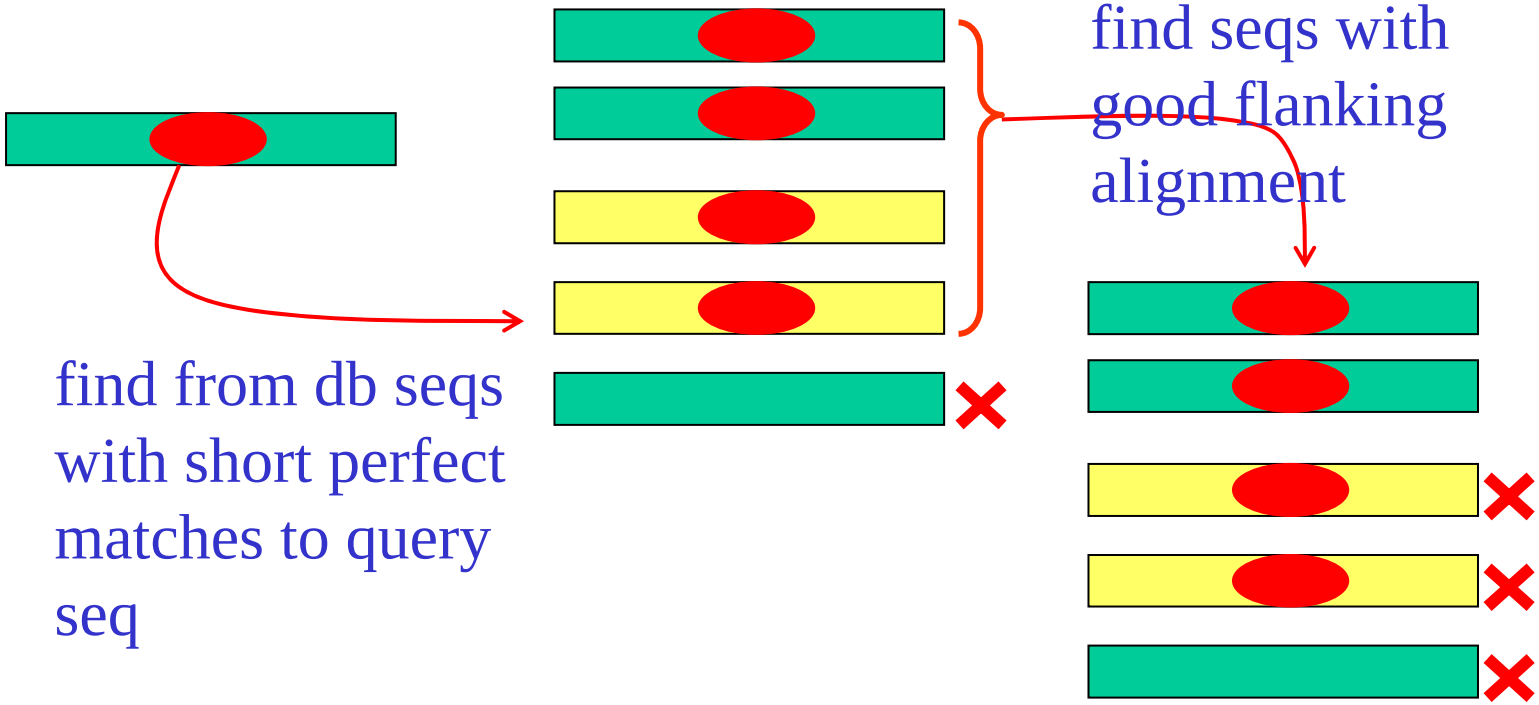
No obvious match between Amicyanin and Ascorbate Oxidase

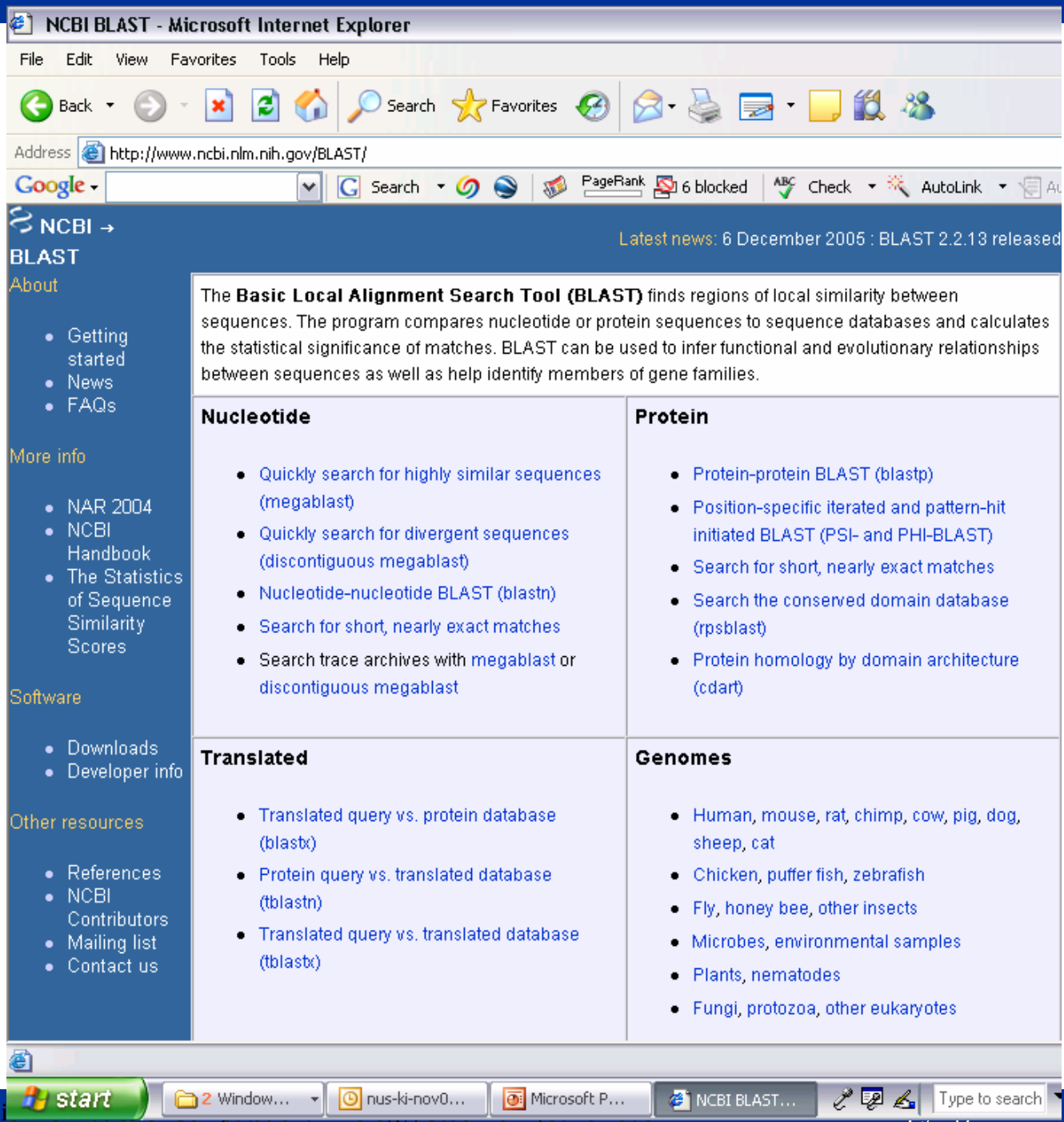
Discard this function as a candidate

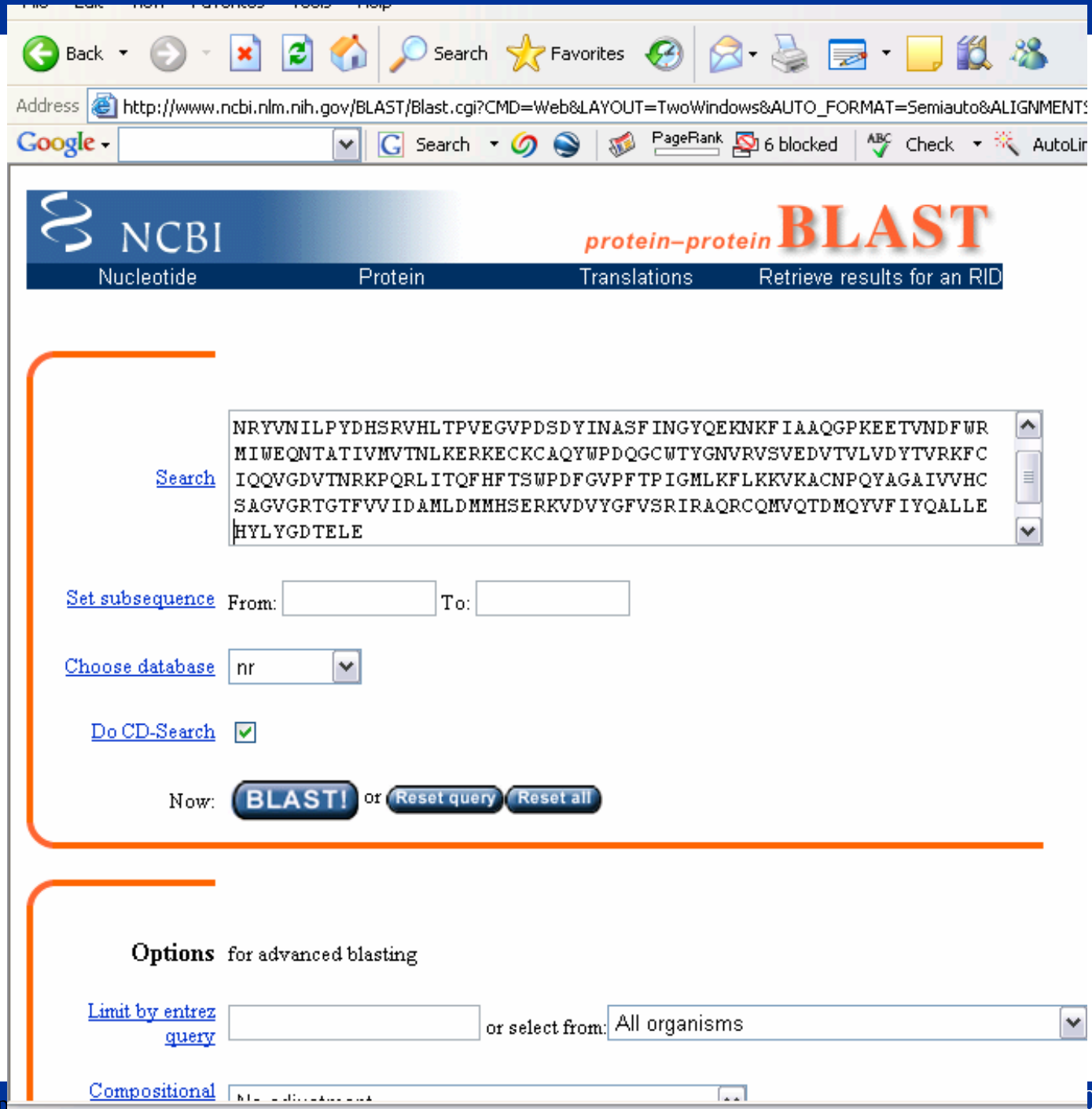
BLAST: How It Works

Altschul et al., *JMB*, 215:403--410, 1990

- **BLAST is one of the most popular tool for doing "guilt-by-association" sequence homology search**






Back
Search
Favorites
Address
http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi?CMD=Web&LAYOUT=TwoWindows&AUTO_FORMAT=Semiauto&ALIGNMENT
Google
Search
PageRank
6 blocked
Check
NCBI
protein–protein BLAST
Nucleotide
Protein
Translations
Retrieve results for an RID
Search
NRYVNILPYDHSRVHLTPVEGVPDSDYINASFINGYQEKNKFIAAQGPKEETVNDFWR
MIWEQNTATIVMVTNLKERKECKCAQYWPDQGCWTYGNVRVSVEDVTVLVDYTVRKFC
IQQVGDVTNRKPQRLITQFHFTSWPDFGVPFTPIGMLKFLKKVKACNPQYAGAIVVHC
SAGVGRTGTFVVIDAMLDMMHSERKVDVYGFVSRIRAQRCQMVQTDMQYVFIYQALLE
HYLYGDTELE
Set subsequence
From:
To:
Choose database
nr
Do CD-Search
Now:
BLAST!
or
Reset query
Reset all
Options
for advanced blasting
Limit by entrez query
or select from:
All organisms
Compositional

NCBI

formatting **BLAST**

Nucleotide Protein Translations Retrieve results for an RID

Your request has been successfully submitted and put into the Blast Queue.

Query = (302 letters)

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

The request ID is 1146357911-10937-56421952334.BLASTQ4

Format! or Reset all

The results are estimated to be ready in 9 seconds but may be done sooner.

Please press "FORMAT!" when you wish to check your results. You may change the formatting options for your result via the form below and press "FORMAT!" aga
request results of a different search by entering any other valid request ID to see other recent jobs.

Homologs obtained by BLAST

Sequences producing significant alignments:	Score (bits)	E Value
gi|14193729|gb|AAK56109.1|AF332081_1 protein tyrosin phosph...	62 L	e-177
gi|126467|sp|P18433|PTRA_HUMAN Protein-tyrosine phosphatase...	62 L	e-177
gi|4506303|ref|NP_002827.1| protein tyrosine phosphatase, r...	62 L	e-176
gi|227294|prf||1701300A protein Tyr phosphatase	620	e-176
gi|18450369|ref|NP_543030.1| protein tyrosine phosphatase, ...	62 L	e-176
gi|32067|emb|CAA37447.1| tyrosine phosphatase precursor [Ho...	61 L	e-176
gi|285113|pir||JC1285 protein-tyrosine-phosphatase (EC 3.1....	619	e-176
gi|6981446|ref|NP_036895.1| protein tyrosine phosphatase, r...	61 L	e-176
gi|2098414|pdb|1YFO|A Chain A, Receptor Protein Tyrosine Ph...	61 S	e-174
gi|32313|emb|CAA38662.1| protein-tyrosine phosphatase [Homo...	61 L	e-174
gi|450583|gb|AAB04150.1| protein tyrosine phosphatase >gi|4...	605	e-172
gi|6679557|ref|NP_033006.1| protein tyrosine phosphatase, r...	60 L	e-172
gi|483922|gb|AAA17990.1| protein tyrosine phosphatase alpha	599	e-170

- **Thus our example sequence could be a protein tyrosine phosphatase α (PTPα)**

Example Alignment with PTPα

```
 Score =  632 bits (1629), Expect = e-180
 Identities = 294/302 (97%), Positives = 294/302 (97%)

Query: 1   SPSTNRKYPPLPVDKLEEEINRRMADDNKLFREEFNALPACPIQATCEAASXXXXXXXXR 60
           SPSTNRKYPPLPVDKLEEEINRRMADDNKLFREEFNALPACPIQATCEAAS        R
Sbjct: 202 SPSTNRKYPPLPVDKLEEEINRRMADDNKLFREEFNALPACPIQATCEAASKEENKEKNR 261

Query: 61  YVNILPYDHSRVHLTPVEGVPDSDYINASFINGYQEKNKFIAAQGPKEETVNDFWRMIWE 120
           YVNILPYDHSRVHLTPVEGVPDSDYINASFINGYQEKNKFIAAQGPKEETVNDFWRMIWE
Sbjct: 262 YVNILPYDHSRVHLTPVEGVPDSDYINASFINGYQEKNKFIAAQGPKEETVNDFWRMIWE 321

Query: 121 QNTATIVMVTNLKERKECKCAQYWPDQGCWTYGNVRVSVEDVTVLVDYTVRKFCIQQVGD 180
           QNTATIVMVTNLKERKECKCAQYWPDQGCWTYGNVRVSVEDVTVLVDYTVRKFCIQQVGD
Sbjct: 322 QNTATIVMVTNLKERKECKCAQYWPDQGCWTYGNVRVSVEDVTVLVDYTVRKFCIQQVGD 381

Query: 181 VTNRKPQRLITQFHFTSWPDFGVPFTPIGMLKFLKKVKACNPQYAGAIVVHCSAGVGRTG 240
           VTNRKPQRLITQFHFTSWPDFGVPFTPIGMLKFLKKVKACNPQYAGAIVVHCSAGVGRTG
Sbjct: 382 VTNRKPQRLITQFHFTSWPDFGVPFTPIGMLKFLKKVKACNPQYAGAIVVHCSAGVGRTG 441

Query: 241 TFVVIDAMLDMMHSERKVDVYGFVSRIRAQRCQMVQTDMQYVFIYQALLEHYLYGDTELE 300
           TFVVIDAMLDMMHSERKVDVYGFVSRIRAQRCQMVQTDMQYVFIYQALLEHYLYGDTELE
Sbjct: 442 TFVVIDAMLDMMHSERKVDVYGFVSRIRAQRCQMVQTDMQYVFIYQALLEHYLYGDTELE 501
```

HSPs, E-Value, Bits, & P-Value

- **HSPs**
  - A local alignment without gaps consists simply of a pair of equal length segments, one from each of the two sequences being compared.
  - A segment pair whose score cannot be improved by extension or trimming is called high-scoring segment pairs or HSPs
- **E-Value**
  - For large seq lengths *m* and *n*, the stats of HSP scores are characterized by two params, *K* and λ
  - Expected number of HSPs with score > *S* is given by $E = Kmne^{-\lambda S}$

Source: NCBI

HSPs, E-Value, Bit Score, & P-Value

- **Bit Score**
  - "Citing a raw score alone is like citing a distance without specifying feet, meters, or light years"
  - Normalize raw score to $S' = (\lambda S - \ln K) / \ln 2$ to get "bit score", which has a standard set of units
  - E-value corresponds to bit score as $E = mn2^{-S'}$
- **P-Value**
  - Number of random HSPs with score $\geq S$ is described by a Poisson distribution

  $\Rightarrow$ Chance of finding no HSPs with score $\geq S$ is e^{-E}

  $\Rightarrow$ Prob of finding ≥ 1 such HSP is $P = 1 - e^{-E}$

Source: NCBI

Guilt-by-Association: Caveats

- **Ensure that the effects of database size and composition have been accounted for**
- **Ensure that the function of the homology is not derived via invalid "transitive assignment''**
- **Ensure that the target sequence has all the key features associated with the function, e.g., active site and/or domain**

Law of Large Numbers

- **Suppose you are in a room with 365 other people**
- **Q: What is the prob that a specific person in the room has the same birthday as you?**
- **A: 1/365 = 0.3%**
- **Q: What is the prob that there is a person in the room having the same birthday as you?**
- **A: $1 - (364/365)^{365} = 63\%$**
- **Q: What is the prob that there are two persons in the room having the same birthday?**
- **A: 100%**

Interpretation of P-value

- **Seq. comparison progs, e.g. BLAST, often associate a P-value to each hit**
- **P-value is interpreted as prob that a random seq has an equally good alignment**
- **Suppose the P-value of an alignment is 10^{-6}**
- **If database has 10^7 seqs, then you expect $10^7 * 10^{-6} = 10$ seqs in it that give an equally good alignment**

$\Rightarrow$ **Need to correct for database size if your seq comparison prog does not do that!**

Exercise: Name a commonly used method for correcting p-value for a situation like this

Lightning Does Strike Twice!

- **Roy Sullivan, a former park ranger from Virgina, was struck by lightning 7 times**
  - 1942 (lost big-toe nail)
  - 1969 (lost eyebrows)
  - 1970 (left shoulder seared)
  - 1972 (hair set on fire)
  - 1973 (hair set on fire & legs seared)
  - 1976 (ankle injured)
  - 1977 (chest & stomach burned)
- **September 1983, he committed suicide**

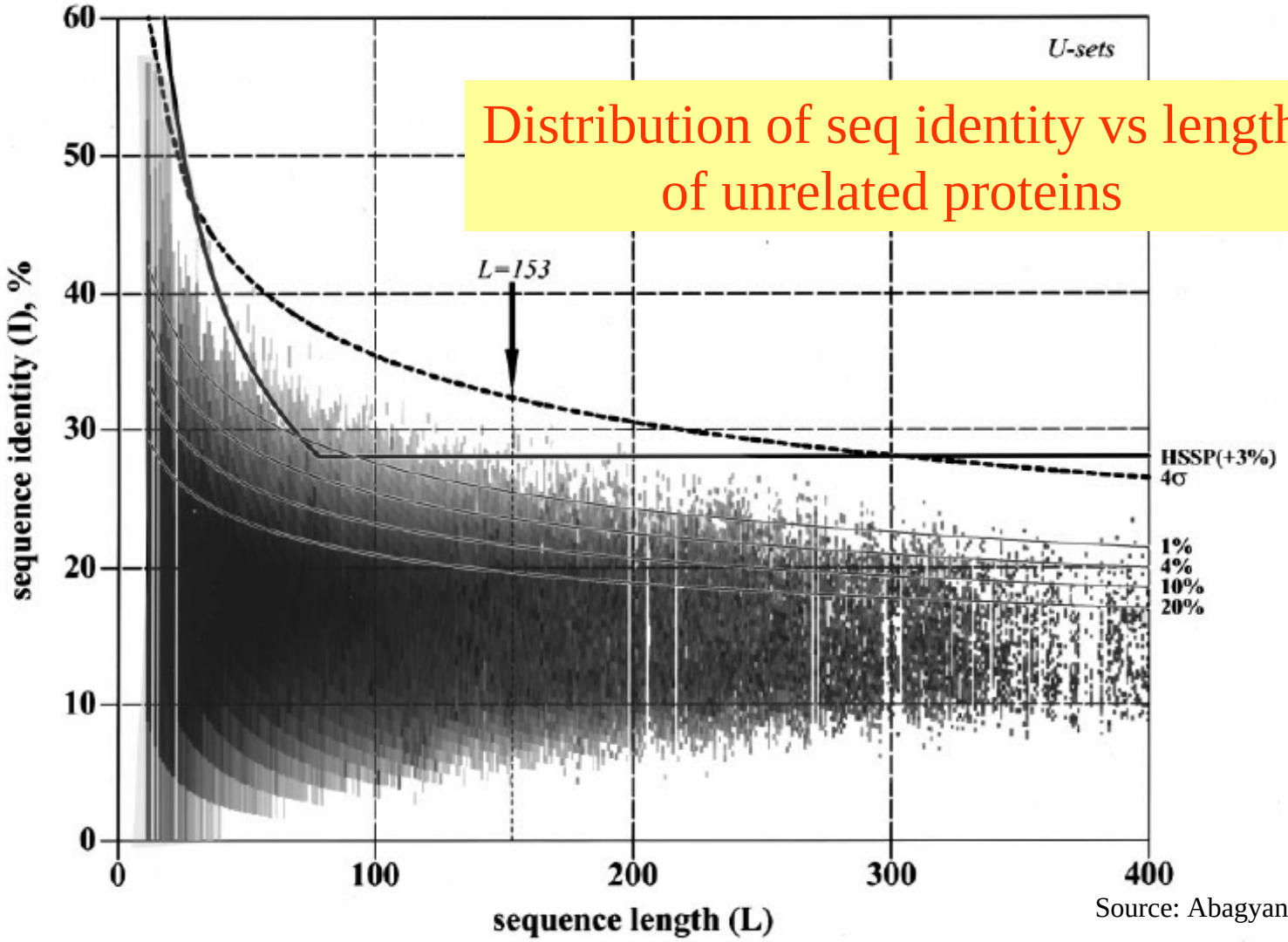
Cartoon: Ron Hipschman
Data: David Hand

Effect of Seq Compositional Bias

- One fourth of all residues in protein seqs occur in regions with biased amino acid composition
- Alignments of two such regions achieves high score purely due to segment composition
- While it is worth noting that two proteins contain similar low complexity regions, they are best excluded when constructing alignments
- BLAST employs the SEG algorithm to filter low complexity regions from proteins before executing a search

Source: NCBI

Effect of Sequence Length



Distribution of seq identity vs length of unrelated proteins

Source: Abagyan & Batalov

Examples of Invalid Function Assignment:

The IMP Dehydrogenases (IMPDH)

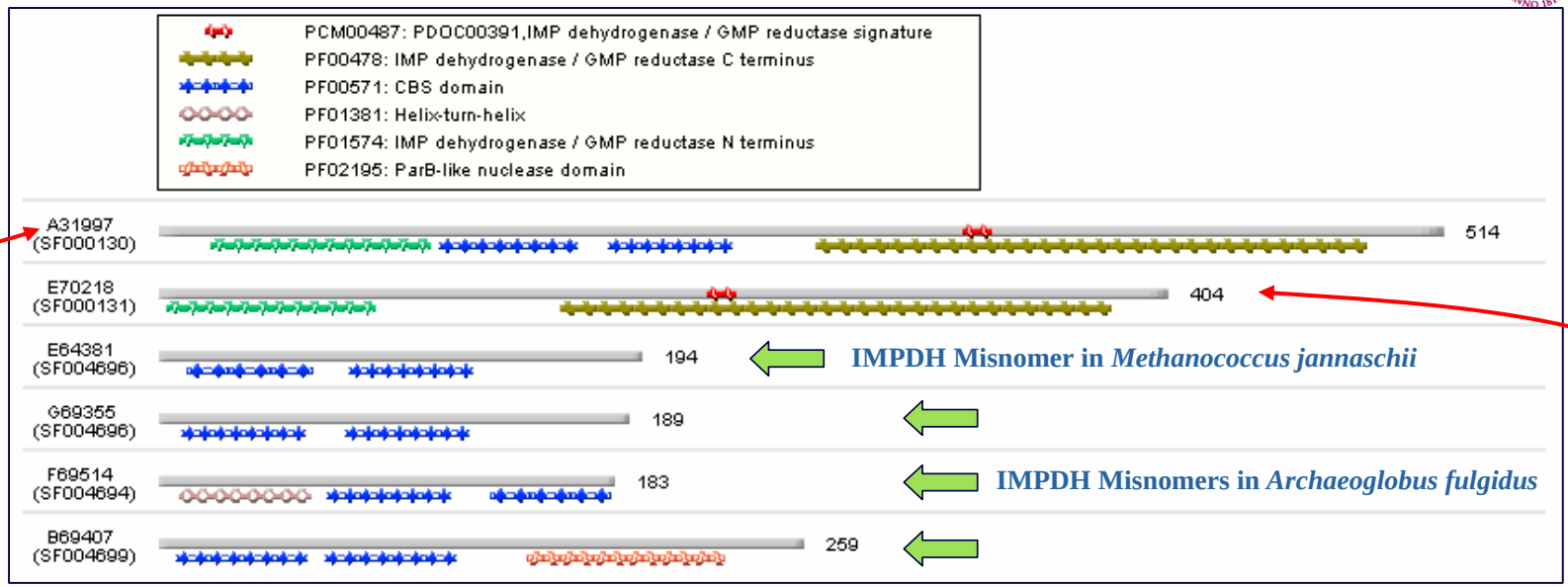
18 entries were found

ID	Organism	PIR	Swiss-Prot/TrEMBL	RefSeq/GenPept
NF00181857	Methanococcus jannaschii	E64381 conserved hypothetical protein MJ0653	Y653_METJA Hypothetical protein MJ0653	g1592300 inosine-5'-monophosphate dehydrogenase (guaB) NP_247637 inosine-5'-monophosphate dehydrogenase (guaB)
NF00187788	Archaeoglobus fulgidus	G69355 MJ0653 homolog AF0847 *ALT_NAMES:* inosine-monophosphate dehydrogenase (guaB-1) homolog [misnomer]	O29411 INOSINE MONOPHOSPHATE DEHYDROGENASE (GUAB-1)	g2649754 inosine monophosphate dehydrogenase (guaB-1) NP_069681 inosine monophosphate dehydrogenase (guaB-1)
NF00188267	Archaeoglobus fulgidus	F69514 yhcV homolog 2 *ALT_NAMES:* inosine-monophosphate dehydrogenase (guaB-2) homolog [misnomer]	O28162 INOSINE MONOPHOSPHATE DEHYDROGENASE (GUAB-2)	g2648410 inosine monophosphate dehydrogenase (guaB-2) NP_070943 inosine monophosphate dehydrogenase (guaB-2)
NF00188697	Archae			ophosphate ive nophosphate ive
NF00197776	Thermo			nophosphate d protein nonophosphate d protein
NF00414709	Methanothermobacter thermautotrophicus	*ALT_NAMES:* inosine-monophosphate dehydrogenase related protein V [misnomer]	O27294 INOSINE-5'-MONOPHOSPHATE DEHYDROGENASE RELATED PROTEIN V	onophosphate dehydrogenase related protein V NP_276354 inosine-5'-monophosphate dehydrogenase related protein V
NF00414811	Methanothermobacter thermautotrophicus	D69035 MJ1232 protein homolog MTH126 *ALT_NAMES:* inosine-5'-monophosphate dehydrogenase related protein VII [misnomer]	O26229 INOSINE-5'-MONOPHOSPHATE DEHYDROGENASE RELATED PROTEIN VII	g2621166 inosine-5'-monophosphate dehydrogenase related protein VII NP_275269 inosine-5'-monophosphate dehydrogenase related protein VII
NF00414837	Methanothermobacter thermautotrophicus	H69232 MJ1225-related protein MTH992 *ALT_NAMES:* inosine-5'-monophosphate dehydrogenase related protein IX [misnomer]	O27073 INOSINE-5'-MONOPHOSPHATE DEHYDROGENASE RELATED PROTEIN IX	g2622093 inosine-5'-monophosphate dehydrogenase related protein IX NP_276127 inosine-5'-monophosphate dehydrogenase related protein IX
NF00414969	Methanothermobacter thermautotrophicus	B69077 yhcV homolog 2 *ALT_NAMES:* inosine-monophosphate dehydrogenase related protein X [misnomer]	O27616 INOSINE-5'-MONOPHOSPHATE DEHYDROGENASE RELATED PROTEIN X	g2622697 inosine-5'-monophosphate dehydrogenase related protein X NP_276687 inosine-5'-monophosphate dehydrogenase related protein X

A partial list of IMPdehydrogenase misnomers in complete genomes remaining in some public databases

Source: Cathy Wu

IMPDH Domain Structure



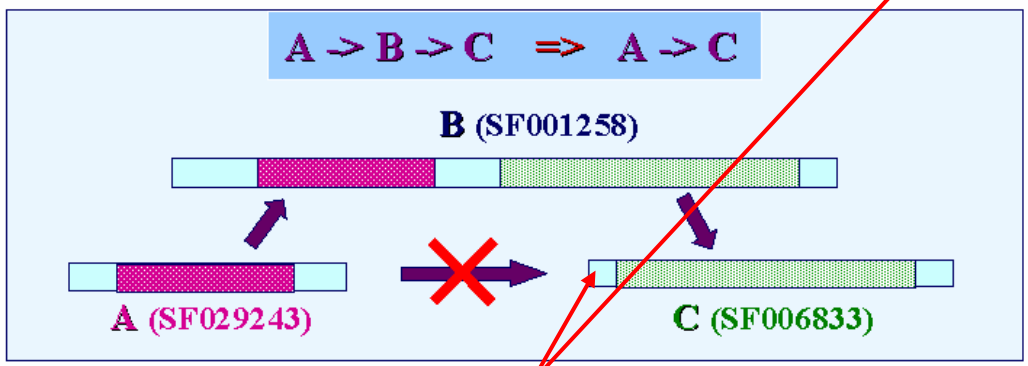
- **Typical IMPDHs have 2 IMPDH domains that form the catalytic core and 2 CBS domains.**
- **A less common but functional IMPDH (E70218) lacks the CBS domains.**
- **Misnomers show similarity to the CBS domains**

Source: Cathy Wu

Invalid Transitive Assignment

Root of invalid transitive assignment

B	H70468	SF001258	051440	phosphoribosyl-AMP cyclohydrolase (EC 3.5.4.19) / phosphoribosyl-ATP pyrophosphatase (EC 3.6.1.31) [similarity]	Aquifex aeolicus	Prok/other	594.3	4.8e-26	205	39.086	197
	S76963	SF001258	039935	phosphoribosyl-AMP cyclohydrolase (EC 3.5.4.19) / phosphoribosyl-ATP pyrophosphatase (EC 3.6.1.31) [similarity]	Synechocystis sp.	Prok/gram-	557.0	5.7e-24	230	39.175	194
	T35073	SF029243	005738	probable phosphoribosyl-AMP cyclohydrolase	Streptomyces coelicolor	Prok/gram+	399.3	3.5e-15	128	42.157	102
	S53349	SF001257	001188	phosphoribosyl-AMP cyclohydrolase (EC 3.5.4.19) / phosphoribosyl-ATP pyrophosphatase (EC 3.6.1.31) / histidinol dehydrogenase (EC 1.1.1.23)	Saccharomyces cerevisiae	Euk/fungi	384.1	2.5e-14	799	31.863	204
A	E69493	SF029243	005738	phosphoribosyl-AMP cyclohydrolase (EC 3.5.4.19) [similarity]	Archaeoglobus fulgidus	Archae	396.8	4.8e-15	108	47.778	90
C	G64337	SF006833	030827	phosphoribosyl-ATP pyrophosphatase (EC 3.6.1.31) [similarity]	Methanococcus jannaschii	Archae	246.9	1.1e-06	95	36.842	95
	D81178	SF006833	101491	phosphoribosyl-ATP pyrophosphatase (EC 3.6.1.31) NMB0603 [similarity]	Neisseria meningitidis	Prok/gram-	239.9	2.6e-06	107	35.227	88
	G81925	SF006833	101491	phosphoribosyl-ATP pyrophosphat 3.6.1.31) NMA0807 [similarity]							
	S51513	SF001257	001188	phosphoribosyl-AMP cyclohydrola 3.5.4.19) / phosphoribosyl-ATP py (EC 3.6.1.31) / histidinol dehydrog 1.1.1.23)							



Mis-assignment of function

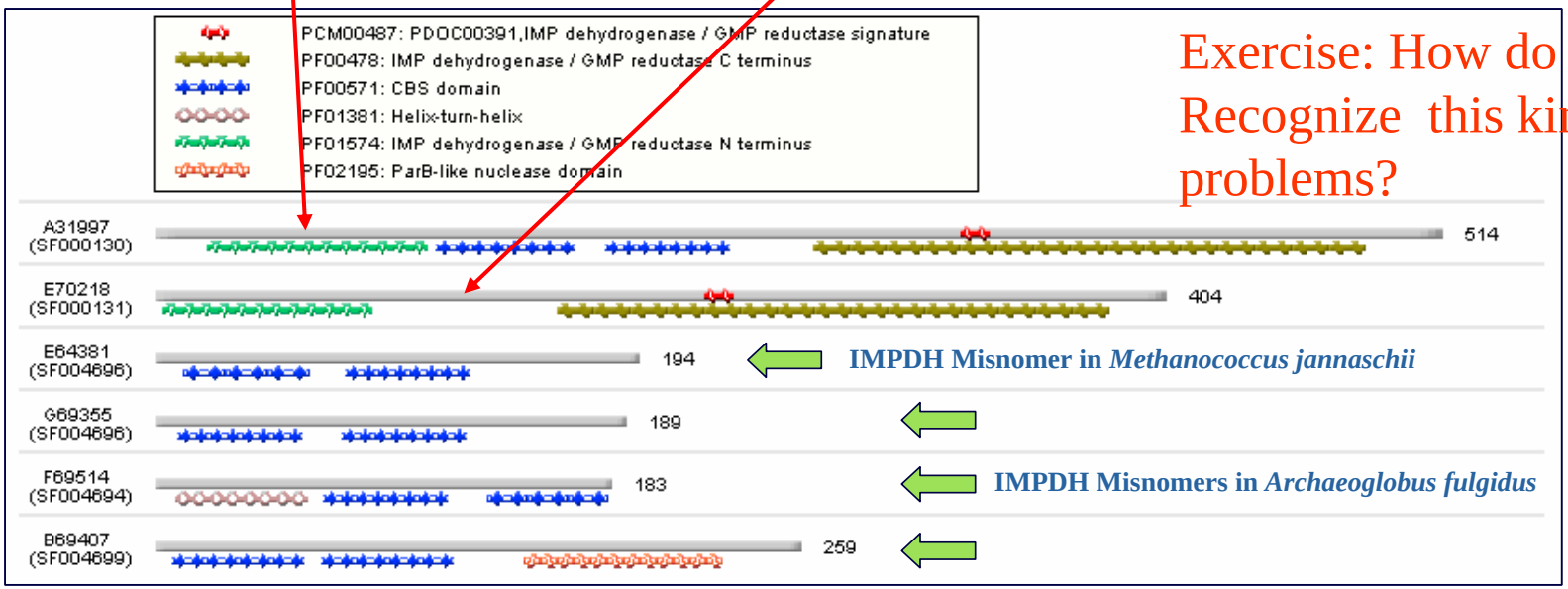
No IMPDH domain

Source: Cathy Wu

Emerging Pattern

Typical IMPDH

Functional IMPDH w/o CBS



Exercise: How do you Recognize this kind of problems?

- **Most IMPDHs have 2 IMPDH and 2 CBS domains**
- **Some IMPDH (E70218) lacks CBS domains**

⇒ **IMPDH domain is the emerging pattern**

Source: Cathy Wu

Application of Sequence Comparison: Active Site/Domain Discovery

Karolinska Institutet

Discover Active Site and/or Domain

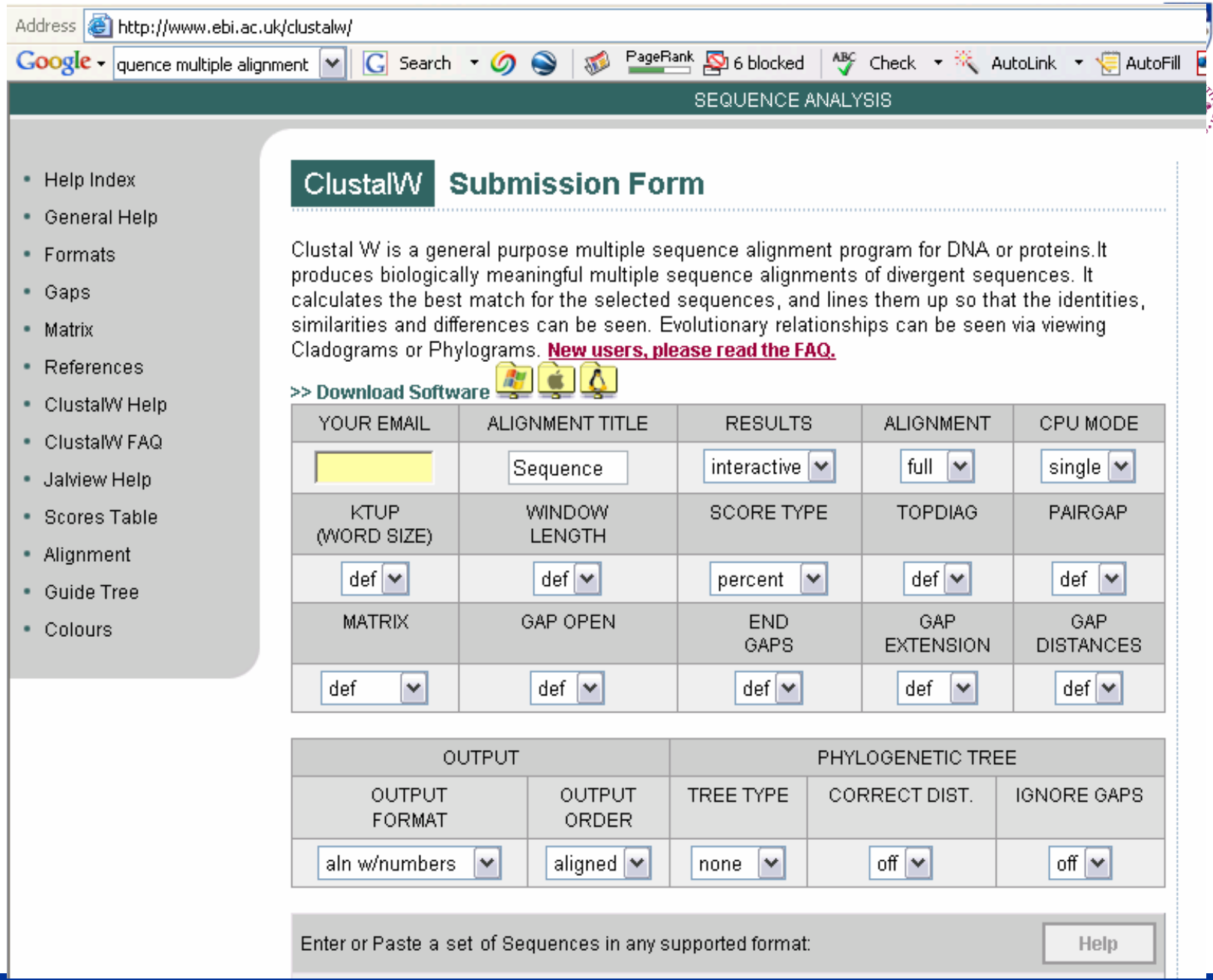
- **How to discover the active site and/or domain of a function in the first place?**
  - Multiple alignment of homologous seqs
  - Determine conserved positions

  ⇒ Emerging patterns relative to background

  ⇒ Candidate active sites and/or domains

- **Easier if sequences of distance homologs are used**

Exercise: Why?



Multiple Alignment of PTPs

```
gi|126467|      FHFTSWPDFGVPFTPIGMLKFLKKVKACNP--QYAGAIVVHCSAGVGRTGTFVVIDAMLD
gi|2499753      FHFTGWPDHGVPYHATGLLSFIRRVKLSNP--PSAGPIVVHCSAGAGRTGCYIVIDIMLD
gi|462550|      YHYTQWPDMGVPEYALPVLTFVRRSSAARM--PETGPVLVHCSAGVGRTGTYIVIDSMLQ
gi|2499751      FHFTSWPDHGVPDTTDLLINFRYLVRDYMKQSPPESPILVHCSAGVGRTGTFIAIDRLIY
gi|1709906      FQFTAWPDHGVPEHPTPFLAFLRRVKTCNP--PDAGPMVVHCSAGVGRTGCFIVIDAMLE
gi|126471|      LHFTSWPDFGVPFTPIGMLKFLKKVKTLNP--VHAGPIVVHCSAGVGRTGTFIVIDAMMA
gi|548626|      FHFTGWPDHGVPYHATGLLSFIRRVKLSNP--PSAGPIVVHCSAGAGRTGCYIVIDIMLD
gi|131570|      FHFTGWPDHGVPYHATGLLGFVRQVKSKSP--PNAGPLVVHCSAGAGRTGCFIVIDIMLD
gi|2144715      FHFTSWPDHGVPDTTDLLINFRYLVRDYMKQSPPESPILVHCSAGVGRTGTFIAIDRLIY
                 ..* *** ***      . *                ..****** ****... ** ..
```

- **Notice the PTPs agree with each other on some positions more than other positions**
- **These positions are more impt wrt PTPs**
- **Else they wouldn't be conserved by evolution**

$\Rightarrow$ **They are candidate active sites**

Application of Sequence Comparison: Key Mutation Site Discovery

Karolinska Institutet

Identifying Key Mutation Sites

K.L.Lim et al., *JBC*, 273:28986--28993, 1998

Sequence from a typical PTP domain D2

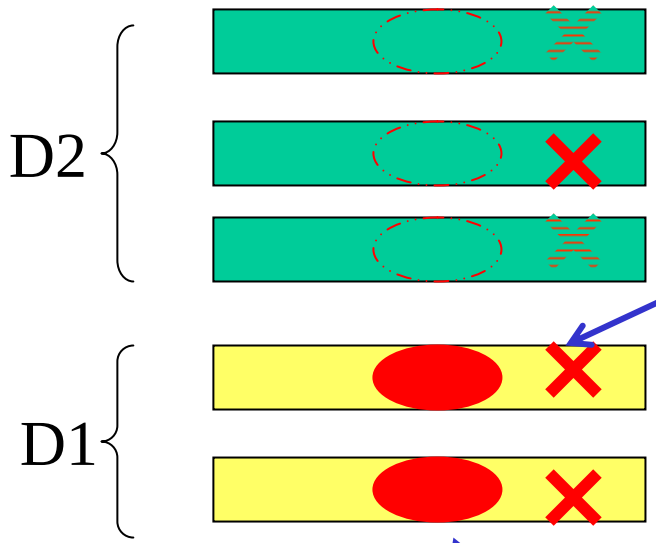
```
>gi|OOOOO|PTPA-D2
EEEFKKLTSIKIQNDKMRTGNLPANMKKNRVLQIIPYEFNRVIIPVKRGEENTDYVNASF
IDGYRQKDSYIASQGPLLHTIEDFWRMIWEWKSCSIVMLTELEERGQEKCAQYWPSDGLV
SYGDITVELKKEEECESYTVRDLLVTNTRENKSRQIRQFHFHGWPEVGIPSDGKGMISII
AAVQKQQQQSGNHPITVHCSAGAGRTGTFCALSTVLERVKAEGILDVFQTVKSLRLQRPH
MVQTLEQYEFCYKVVQEYIDAFSDYANFK
```

- **Some PTPs have 2 PTP domains**
- **PTP domain D1 is has much more activity than PTP domain D2**
- **Why? And how do you figure that out?**

Emerging Patterns of PTP D1 vs D2

- **Collect example PTP D1 sequences**
- **Collect example PTP D2 sequences**
- **Make multiple alignment A1 of PTP D1**
- **Make multiple alignment A2 of PTP D2**
- **Are there positions conserved in A1 that are violated in A2?**
- **These are candidate mutations that cause PTP activity to weaken**
- **Confirm by wet experiments**

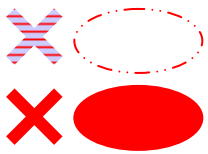
Emerging Patterns of PTP D1 vs D2



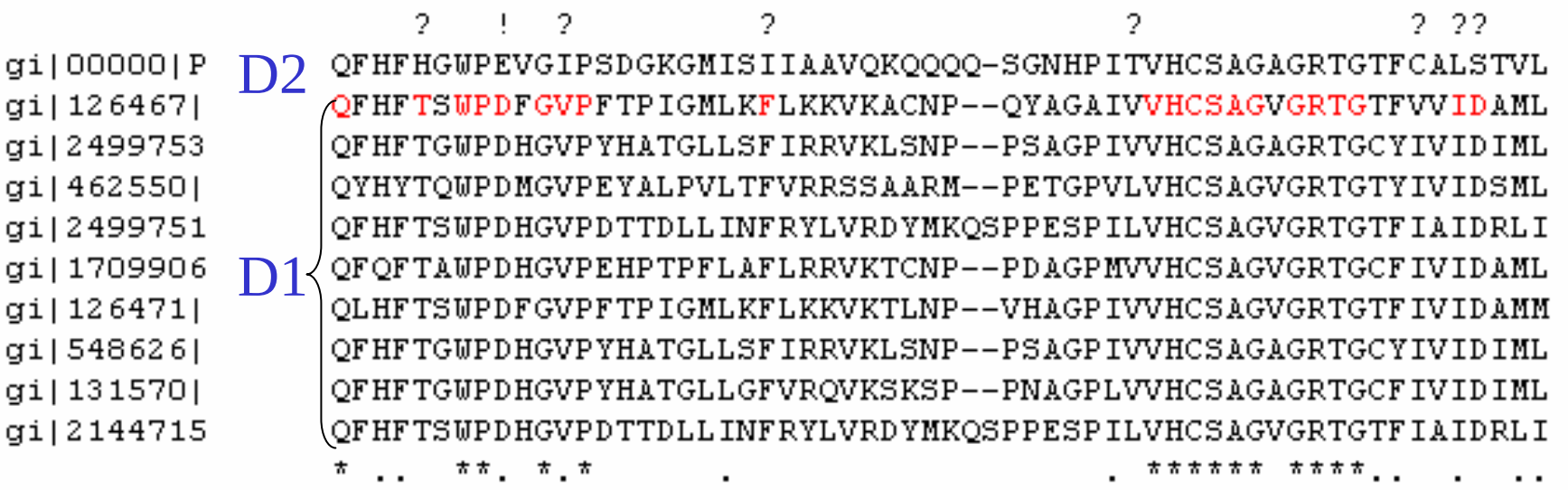
This site is consistently conserved in D1,
but is not consistently missing in D2
⇒ it is not an EP
⇒ not a likely cause of D2's loss of function

Exercise: Why?

This site is consistently conserved in D1,
but is consistently missing in D2
⇒ it is an EP
⇒ possible cause of D2's loss of function



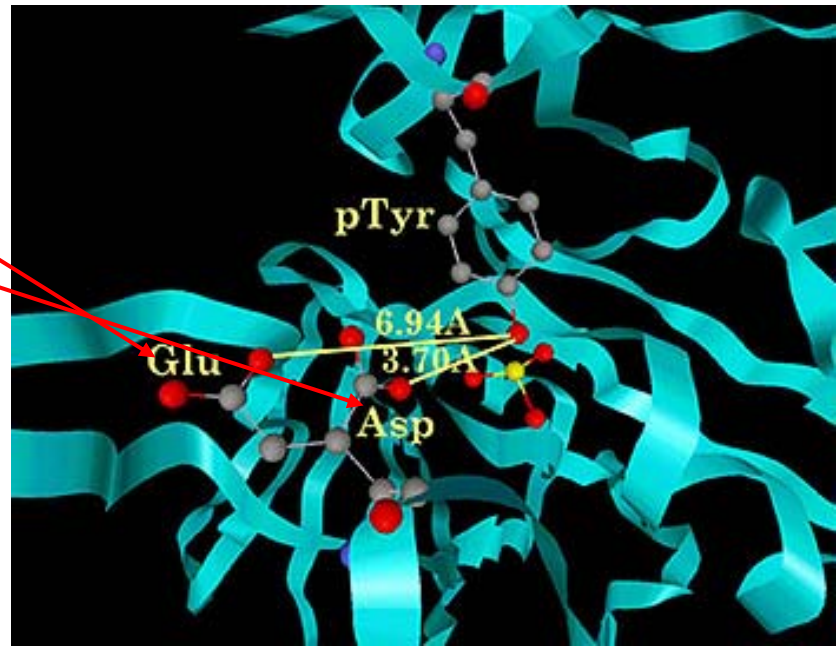
Key Mutation Site: PTP D1 vs D2



- **Positions marked by "!" and "?" are likely places responsible for reduced PTP activity**
  - All PTP D1 agree on them
  - All PTP D2 disagree on them

Key Mutation Site: PTP D1 vs D2

```
                                  ?   !   ?
gi|00000|P      D2   QFHFHGWPEVGIPSDGK(
gi|126467|           QFHFTSWPDFGVRFTPI(
gi|2499753           QFHFTGWPDHGVPYHAT(
gi|462550|           QYHYTQWPDMGVPEYAL]
gi|2499751           QFHFTSWPDHGVPDTTD]
gi|1709906      D1   QFQFTAWPDHGVPEHPT]
gi|126471|           QLHFTSWPDFGVPFTPI(
gi|548626|           QFHFTGWPDHGVPYHAT(
gi|131570|           QFHFTGWPDHGVPYHAT(
gi|2144715           QFHFTSWPDHGVPDTTD]
                     * ..  **. *.*
```



- **Positions marked by "!" are even more likely as 3D modeling predicts they induce large distortion to structure**

Confirmation by Mutagenesis Expt

- **What wet experiments are needed to confirm the prediction?**
  - Mutate E $\rightarrow$ D in D2 and see if there is gain in PTP activity
  - Mutate D $\rightarrow$ E in D1 and see if there is loss in PTP activity

Exercise: Why do you need this 2-way expt?

Guilt-by-Association:
What if no homolog of known function is found?

genome phylogenetic profiles

protfun's feature profiles

Karolinska Institutet

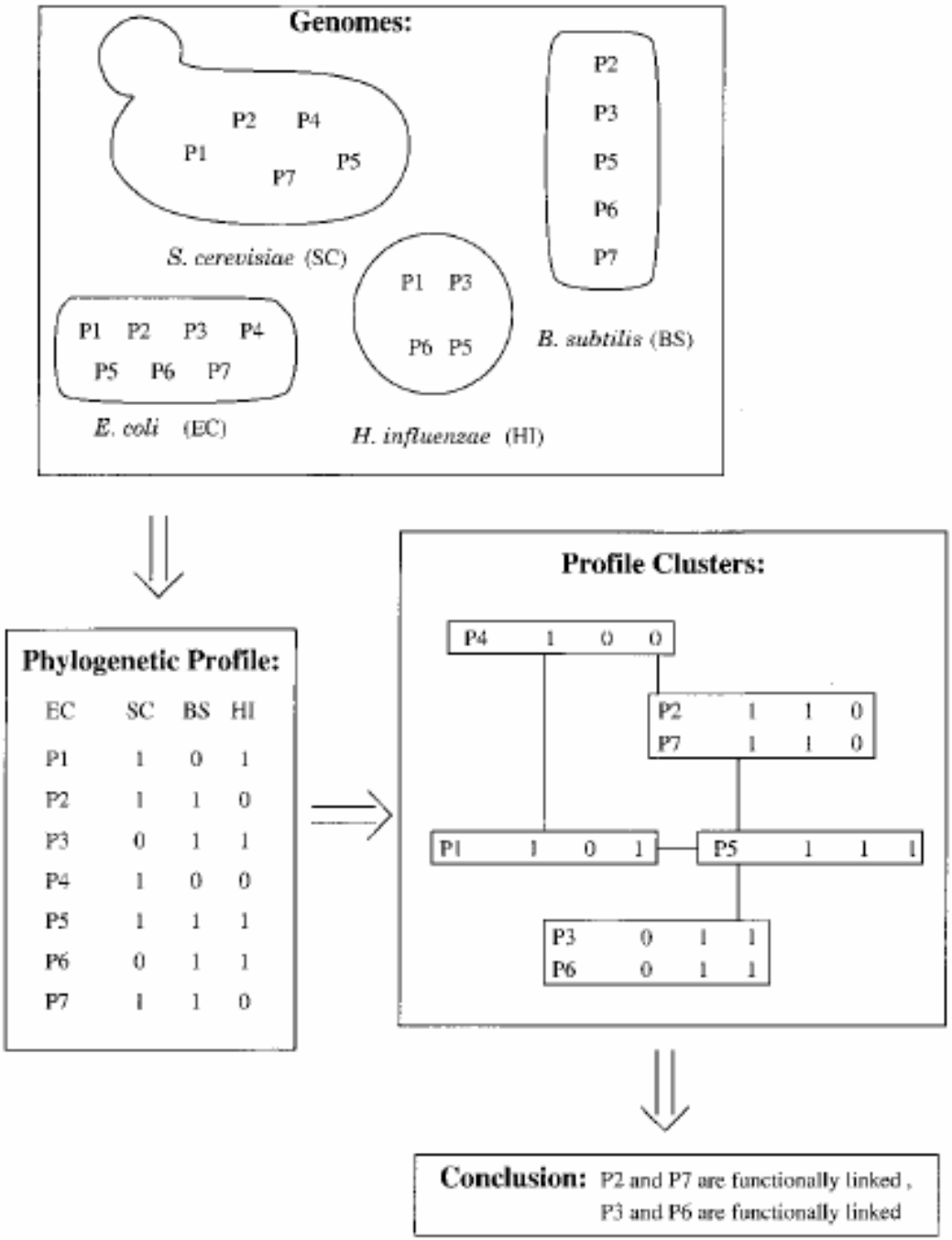
Phylogenetic Profiling

Pellegrini et al., *PNAS*, 96:4285--4288, 1999

- **Gene (and hence proteins) with identical patterns of occurrence across phyla tend to function together**

$\Rightarrow$ **Even if no homolog with known function is available, it is still possible to infer function of a protein**

Phylogenetic Profiling: How it Works



Phylogenetic Profiling: P-value

The probability of observing by chance z occurrences of genes X and Y in a set of N lineages, given that X occurs in x lineages and Y in y lineages is

$$P(z|N,x,y) = \frac{w_z * \overline{w_z}}{W}$$

where

$$w_z = \binom{N}{z}$$

$$\overline{w_z} = \binom{N-z}{x-z} * \binom{N-z}{y-z}$$

$$W = \binom{N}{x} * \binom{N}{y}$$

No. of ways to distribute *z* co-occurrences over *N* lineage's

No. of ways to distribute the remaining *x* – *z* and *y* – *z* occurrences over the remaining *N* – *z* lineage's

No. of ways of distributing *X* and *Y* over *N* lineage's without restriction

Phylogenetic Profiles: Evidence

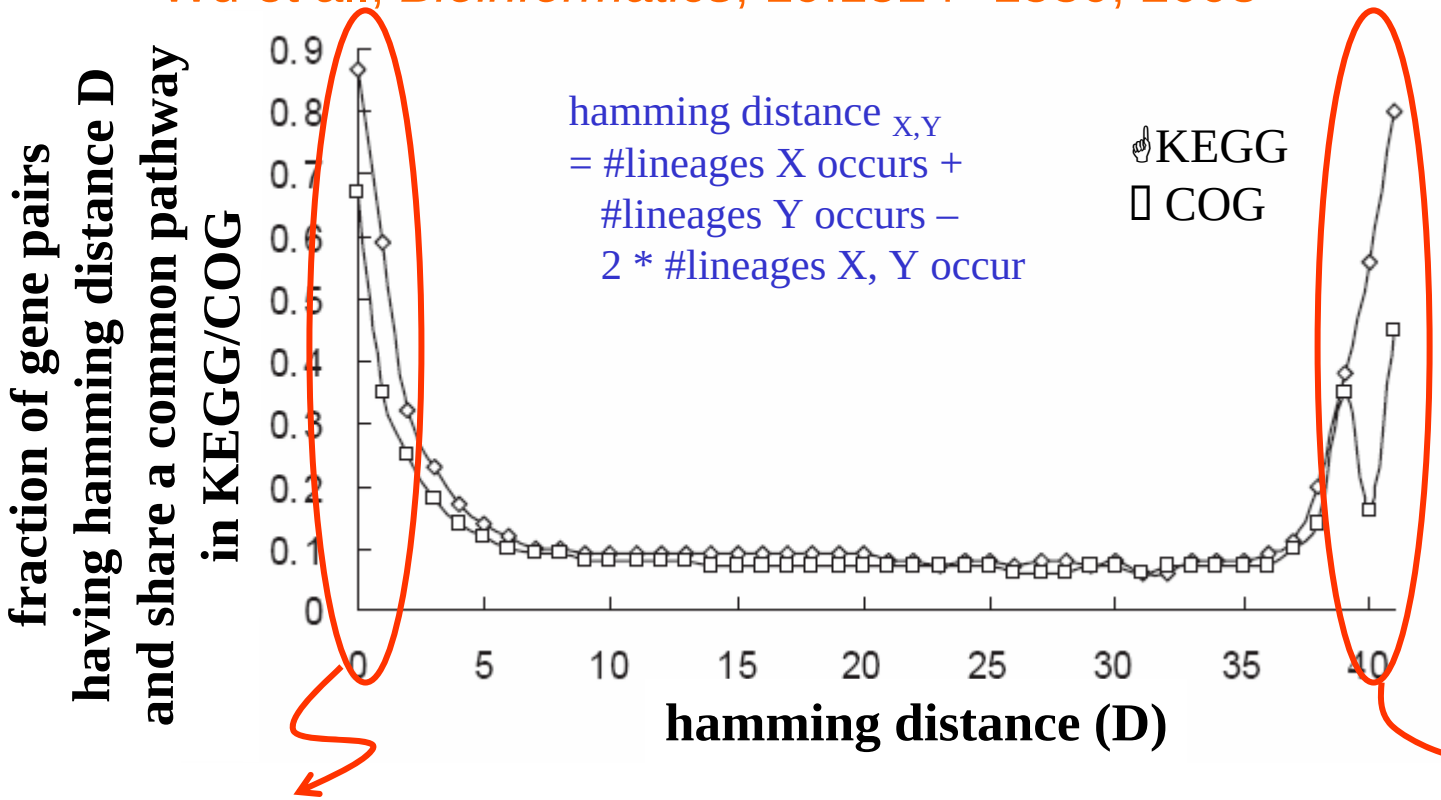
Pellegrini et al., *PNAS*, 96:4285--4288, 1999

Keyword	No. of non-homologous proteins in group	No. neighbors in keyword group	No. neighbors in random group
Ribosome	60	197	27
Transcription	36	17	10
tRNA synthase and ligase	26	11	5
Membrane proteins*	25	89	5
Flagellar	21	89	3
Iron, ferric, and ferritin	19	31	2
Galactose metabolism	18	31	2
Molybdoterin and Molybdenum, and molybdoterin	12	6	1
Hypothetical†	1,084	108,226	8,440

- **E. coli proteins grouped based on similar keywords in SWISS-PROT have similar phylogenetic profiles**

Phylogenetic Profiling: Evidence

Wu et al., *Bioinformatics*, 19:1524--1530, 2003



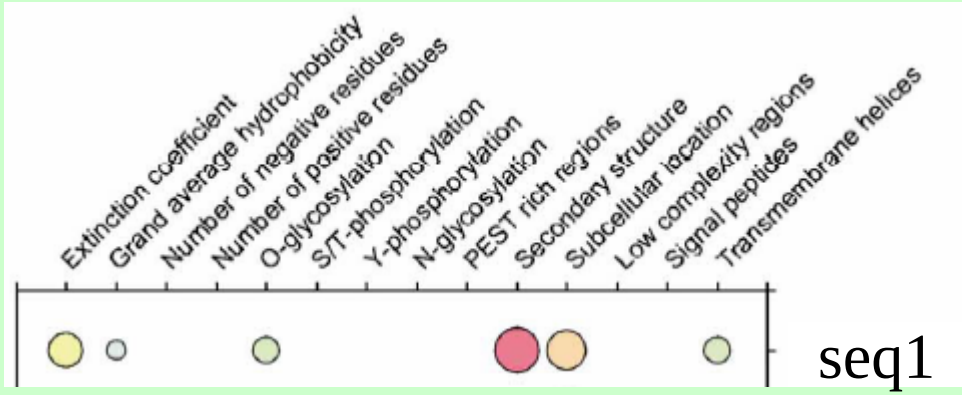
- **Proteins having low hamming distance (thus highly similar phylogenetic profiles) tend to share common pathways**

Exercise: Why do proteins having high hamming distance also have this behaviour?

The ProtFun Approach

Jensen, *JMB*, 319:1257--1265, 2002

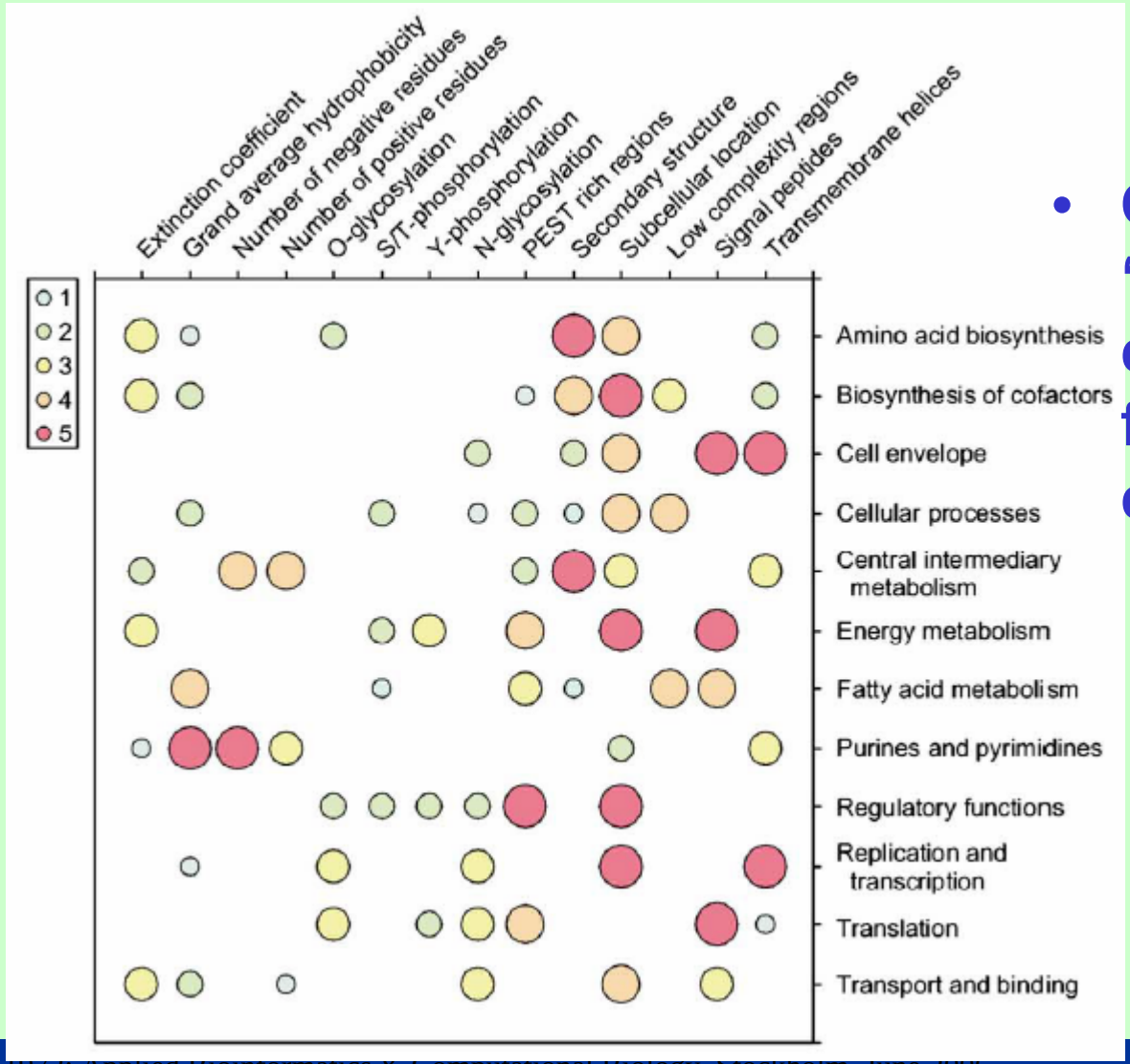
- **A protein is not alone when performing its biological function**
- **It operates using the same cellular machinery for modification and sorting as all other proteins do, such as glycosylation, phospharylation, signal peptide cleavage, …**
- **These have associated consensus motifs, patterns, etc.**



- **Proteins performing similar functions should share some such "features"**

⇒ **Perhaps we can predict protein function by comparing its "feature" profile with other proteins?**

ProtFun: Evidence



- **Combinations of "features" seem to characterize some functional categories**

ProtFun: How it Works

Abbriviation	Encoding	Description
ec	single value	Extinction coefficient predicted by ExPASy ProtParam
gravy	single value	Hydrophobicity predicted by ExPASy ProtParam
nneg	single value	Number of negatively charged residues counted by ExPASy ProtParam
npos	single value	Number of positively charged residues counted by ExPASy ProtParam
nglyc	potential in 5 bins	N-glycosylation sites predicted by NetNGlyc
oglyc	potential-threshold in 10 bins	GalNAc O-glycosylations predicted by NetOGlyc
pest	fraction in 10 bins	PEST rich regions identified by PESTfind
phosST	potential in 10 bins	Serine and threonine phosporylations predicted by NetPhos
phosY	potential in 10 bins	Tyrosine phosporylations predicted by NetPhos
psipred	helix, sheet, coil in 5 bins	Predicted secondary structure from PSI-Pred
psort	20 probabilities	Subcellular location predtions by PSORT
seg	fraction in 10 bins	Low-complexity regions identified by SEG
signalp	meanS, maxY, log(cleavage pos)	Signal peptide predictions made by SignalP
tmhmm	inside, outside, membrane in 5 bins	Transmembrane helix predictions made by TMHMM

Extract feature profile of protein using various prediction methods

Category	Hidden units	Input features
Amino acid biosynthesis	30	ec psipred psort tmhmm
	30	ec psipred tmhmm
	30	ec netoglyc psipred psort
	30	gravy psipred psort
	30	oglyc psipred psort

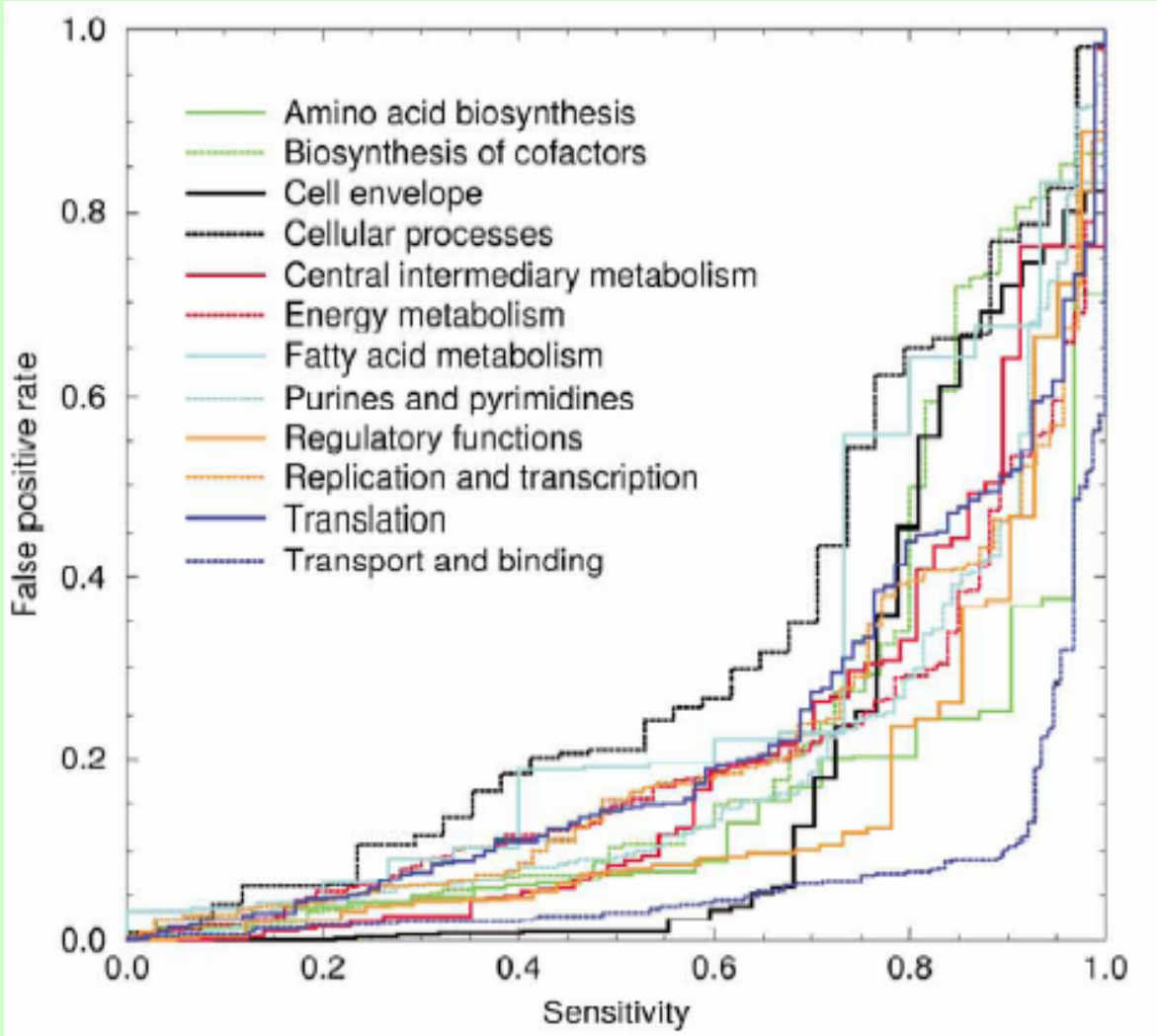
Average the output of the 5 component ANNs

ProtFun: Example Output

	Prion	A4	TTHY
Amino acid biosynthesis	0.011	0.011	0.011
Biosynthesis of cofactors	0.041	0.161	0.034
Cell envelope	0.146	0.804	0.698
Cellular processes	0.027	0.027	0.051
Central intermediary metabolism	0.047	0.139	0.059
Energy metabolism	0.029	0.023	0.046
Fatty acid metabolism	0.017	0.017	0.023
Purines and pyrimidines	0.528	0.417	0.153
Regulatory functions	0.013	0.014	0.014
Replication and transcription	0.020	0.029	0.040
Translation	0.035	0.027	0.032
Transport and binding	0.831	0.827	0.812
Enzyme	0.233	0.367	0.227
Non-enzyme	0.767	0.633	0.773
Oxidoreductase (EC 1.–.–.–)	0.070	0.024	0.055
Transferase (EC 2.–.–.–)	0.031	0.208	0.037
Hydrolase (EC 3.–.–.–)	0.101	0.090	0.208
Isomerase (EC 4.–.–.–)	0.020	0.020	0.020
Ligase (EC 5.–.–.–)	0.010	0.010	0.010
Lyase (EC 6.–.–.–)	0.017	0.078	0.017

- **At the seq level, Prion, A4, & TTHY are dissimilar**
- **ProtFun predicts them to be cell envelope-related, tranport & binding**
- **This is in agreement w/ known functionality of these proteins**

ProtFun: Performance



SVM-Pairwise Framework

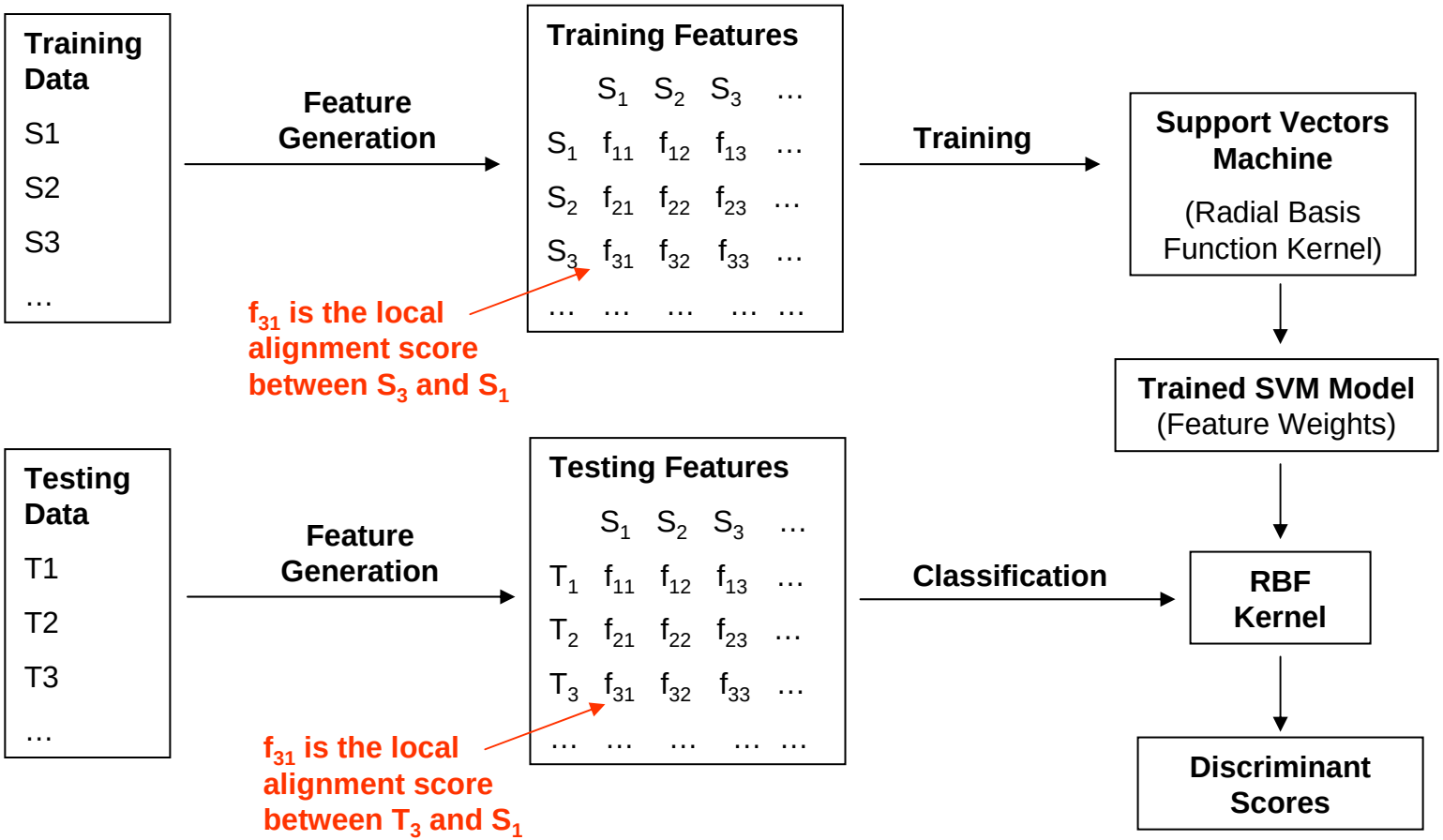
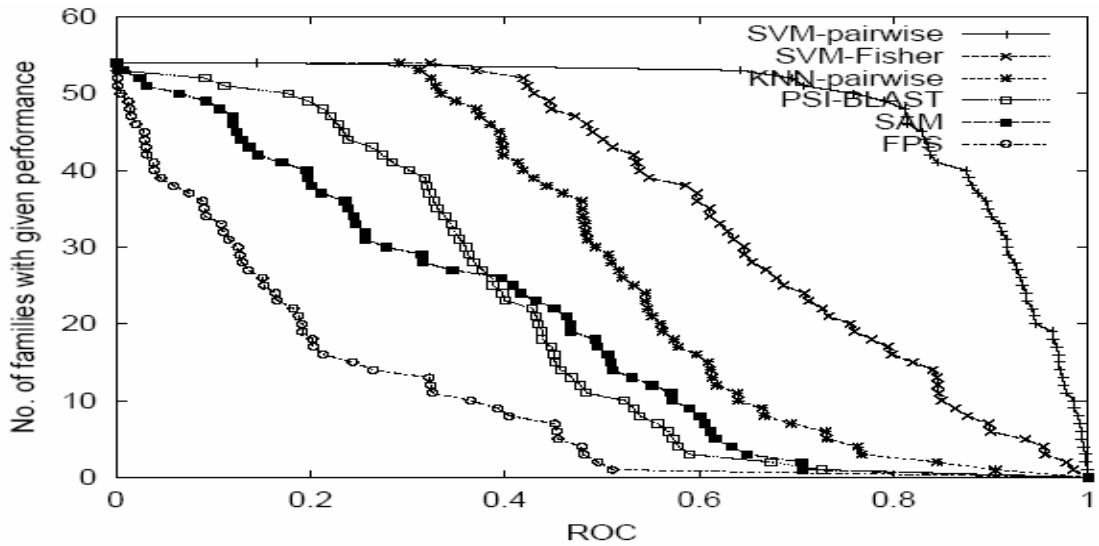
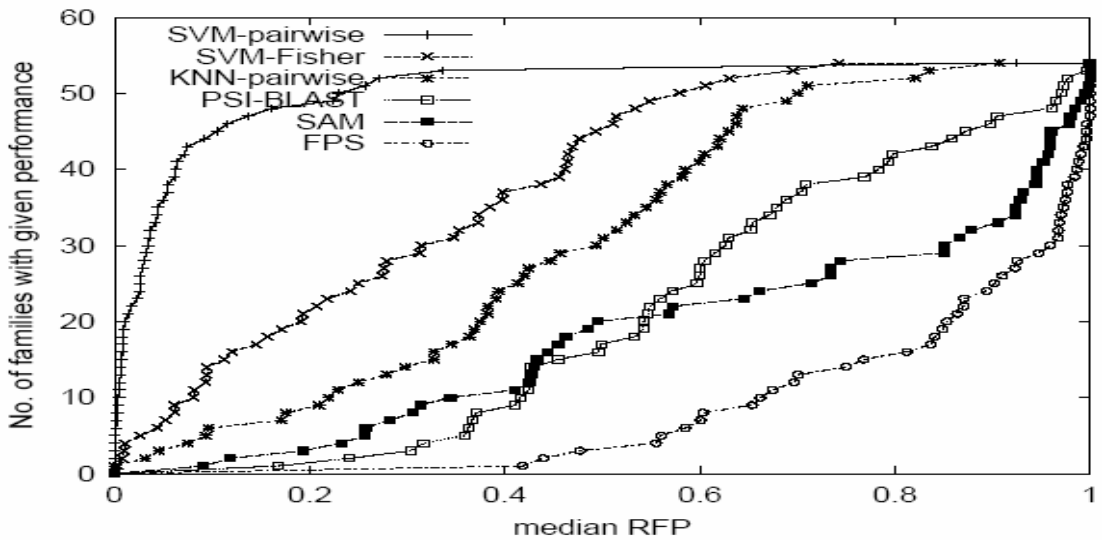


Image credit: Kenny Chua

Performance of SVM-Pairwise

- **Receiver Operating Characteristic (ROC)**
  - The area under the curve derived from plotting true positives as a function of false positives for various thresholds.
- **Rate of median False Positives (RFP)**
  - The fraction of negative test examples with a score better or equals to the median of the scores of positive test examples.





Any Questions?

Karolinska
Institutet

References

- T.F.Smith & X.Zhang. “The challenges of genome sequence annotation or `The devil is in the details’”, *Nature Biotech*, 15:1222--1223, 1997
- D. Devos & A.Valencia. “Intrinsic errors in genome annotation”, *TIG*, 17:429--431, 2001
- K.L.Lim et al. “Interconversion of kinetic identities of the tandem catalytic domains of receptor-like protein tyrosine phosphatase PTP-alpha by two point mutations is synergist and substrate dependent”, *JBC*, 273:28986--28993, 1998
- S.F.Altshcul et al. “Basic local alignment search tool”, *JMB*, 215:403--410, 1990
- S.F.Altschul et al. “Gapped BLAST and PSI-BLAST: A new generation of protein database search programs”, *NAR*, 25(17):3389--3402, 1997
- RA Abagyan, S Batalov. Do aligned sequences share the same fold? *JMB*, 273(1):355-68, 1997

References

- S.E.Brenner. “Errors in genome annotation”, *TIG*, 15:132--133, 1999
- M. Pellegrini et al. “Assigning protein functions by comparative genome analysis: Protein phylogenetic profiles”, *PNAS*, 96:4285--4288, 1999
- J. Wu et al. “Identification of functional links between genes using phylogenetic profiles”, *Bioinformatics*, 19:1524--1530, 2003
- C. Wu, W. Barker. “A Family Classification Approach to Functional Annotation of Proteins”, *The Practical Bioinformatician*, Chapter 19, pages 401—416, WSPC, 2004
- H.N. Chua, W.-K. Sung. A better gap penalty for pairwise SVM. Proc. APBC05, pages 11-20
- T. Jaakkola, M. Diekhans, and D. Haussler. A discriminative framework for detecting remote homologies. *JCB*, 7(1-2):95—11, 2000