For written notes on this lecture, please read chapter 19 of *The Practical Bioinformatician*
and *Hawkins & Kihara, JBCB 5(1):1-30, 2007*

CS2220: Introduction to Computational Biology

# Lecture 6: Sequence Homology Interpretation

**Limsoon Wong**

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# Plan

- **Recap of sequence alignment**
- **Guilt by association**
- **Active site/domain discovery**
- **What if no homology of known function is found?**
  - Genome phylogenetic profiling
  - SVM-Pairwise
  - Protein-protein interactions
- **Key mutation site discovery**

# Very Brief Recap of Sequence Comparison/Alignment

# Motivations for Sequence Comparison

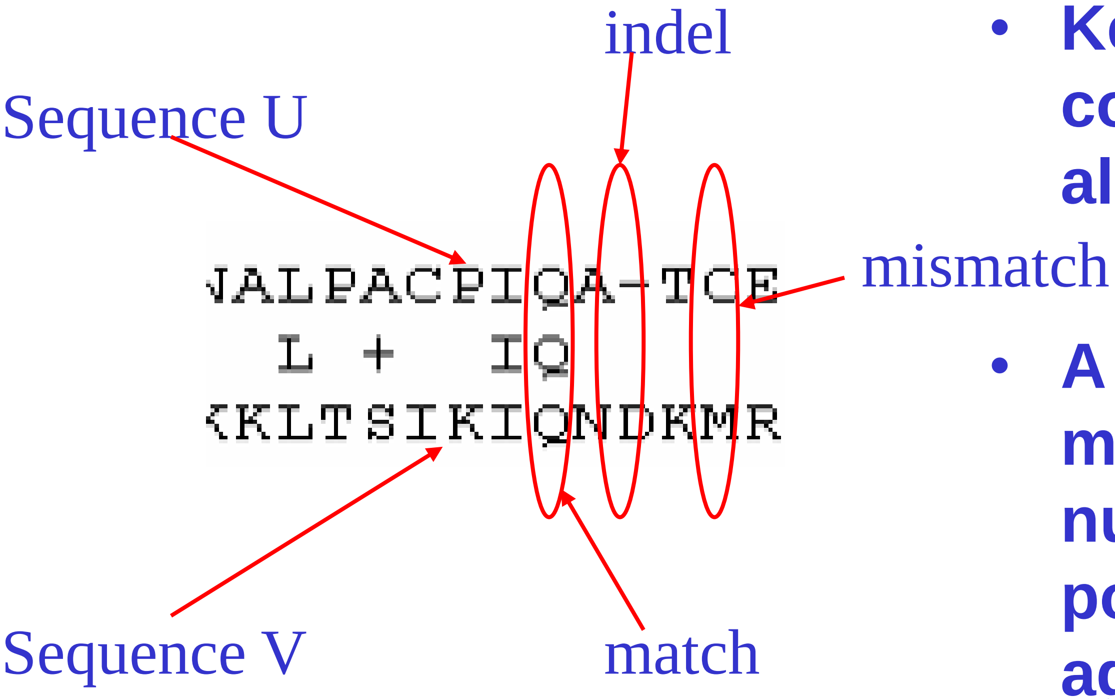
- **DNA is blue print for living organisms**

**⇒ Evolution is related to changes in DNA**

**⇒ 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



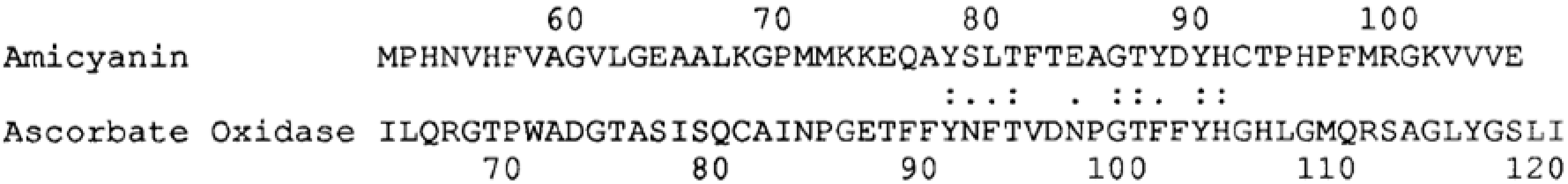
- **Key aspect of seq comparison is seq alignment**
- **A seq alignment maximizes the number of positions that are in agreement in two sequences**

# Sequence Alignment: Poor Example

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

**⇒ The two proteins are not likely to be homologous**

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



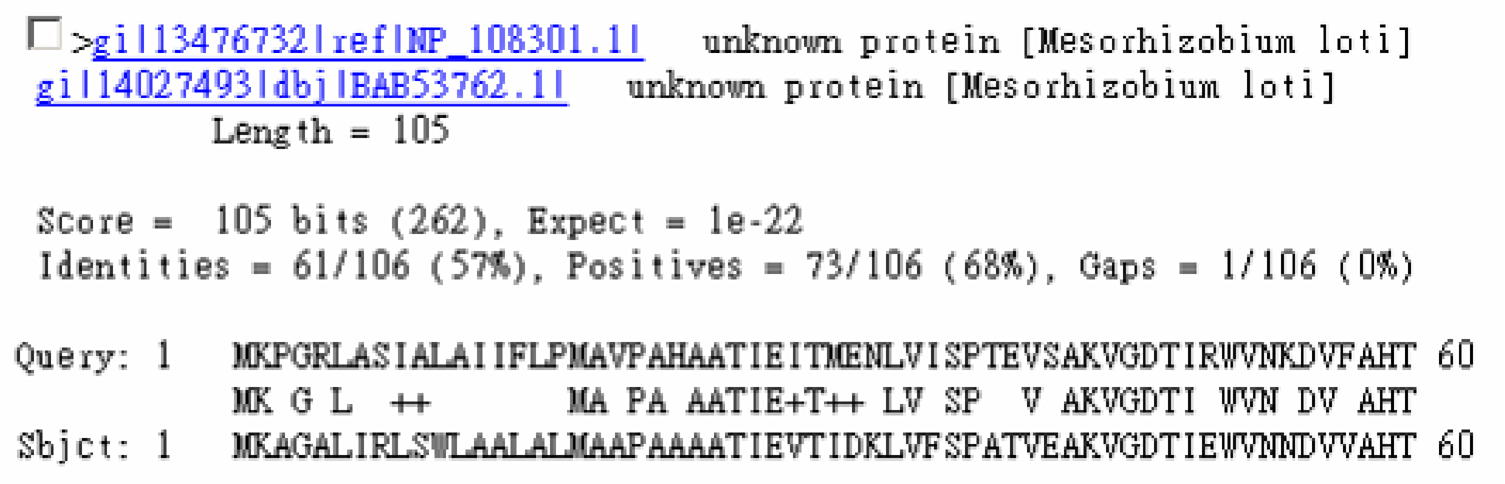
```
                          60        70        80        90       100
Amicyanin         MPHNVHFVAGVLGEAALKGPMMKKEQAYSLTFTEAGTYDYHCTPHPFMRGKVVVE
                                             :..:  . ::. ::
Ascorbate Oxidase ILQRGTPWADGTASISQCAINPGETFFYNFTVDNPGTFFYHGHLGMQRSAGLYGSLI
                 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

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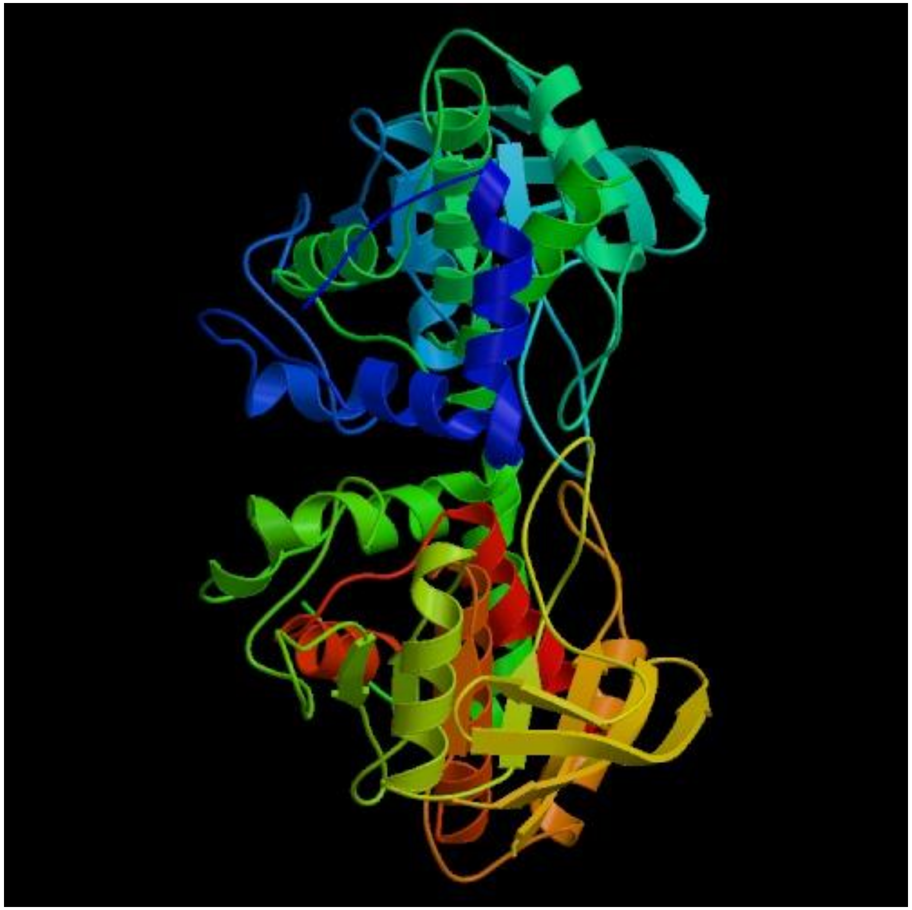
# A protein is a ...

- **A protein is a large complex molecule made up of one or more chains of amino acids**
- **Protein performs a wide variety of activities in the cell**



# Function Assignment to Protein Sequence

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

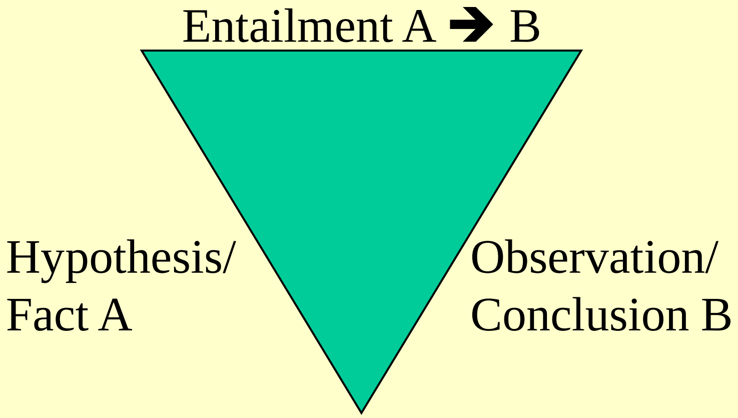
- **How do we attempt to assign a function to a new protein sequence?**

# Invariant and Abductive Reasoning

- **Function is determined by 3D struct of protein & environment protein is in**
- **Constraints imposed by 3D struct & environment give rise to "invariant" properties observed in proteins having the ancestor with that function**

⇒ **Abductive reasoning**

– If those invariant properties are seen in a protein, then the protein is homolog of this protein



⇒ **"Guilt by association"**

# Guilt-by-Association

- Compare the target sequence T with sequences $S_1$, …, $S_n$ of known function in a database

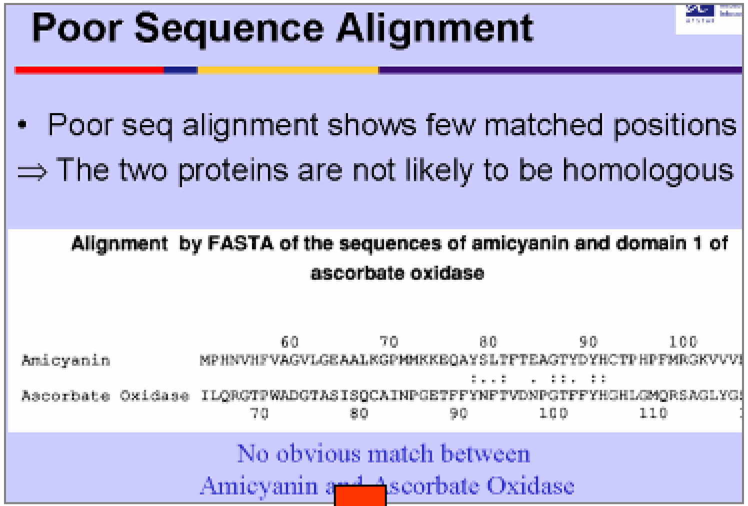
- Determine which ones amongst $S_1$, …, $S_n$ are the mostly likely homologs of T

- 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



## Poor Sequence Alignment

- Poor seq alignment shows few matched positions

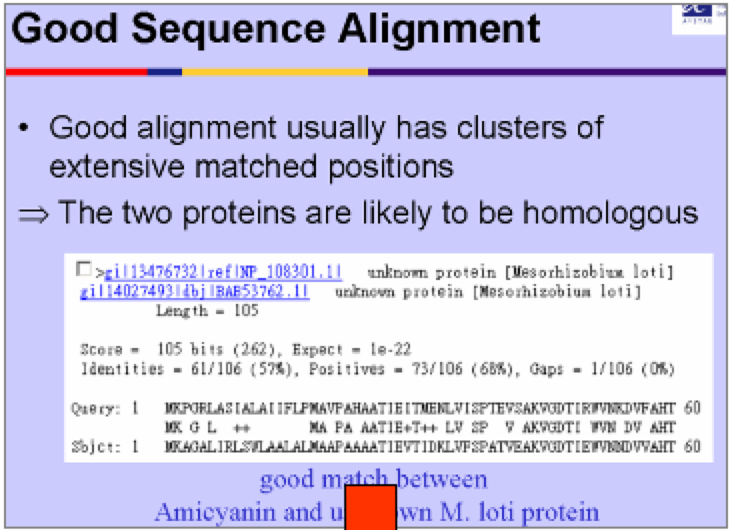
⇒ The two proteins are not likely to be homologous

**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



## Good Sequence Alignment

- Good alignment usually has clusters of extensive matched positions

⇒ The two proteins are likely to be homologous

```
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            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

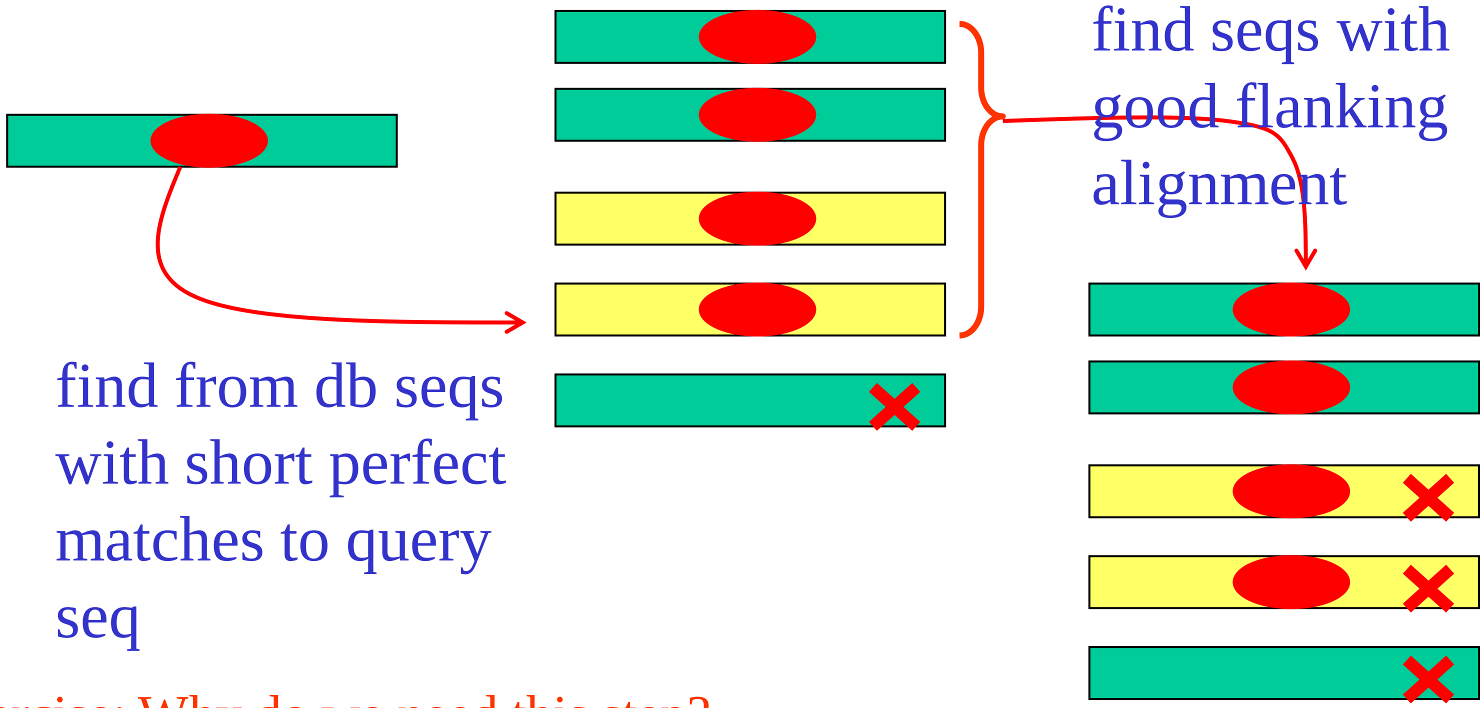
Assign to *T* same function as homologs

Confirm with suitable wet experiments

# 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**

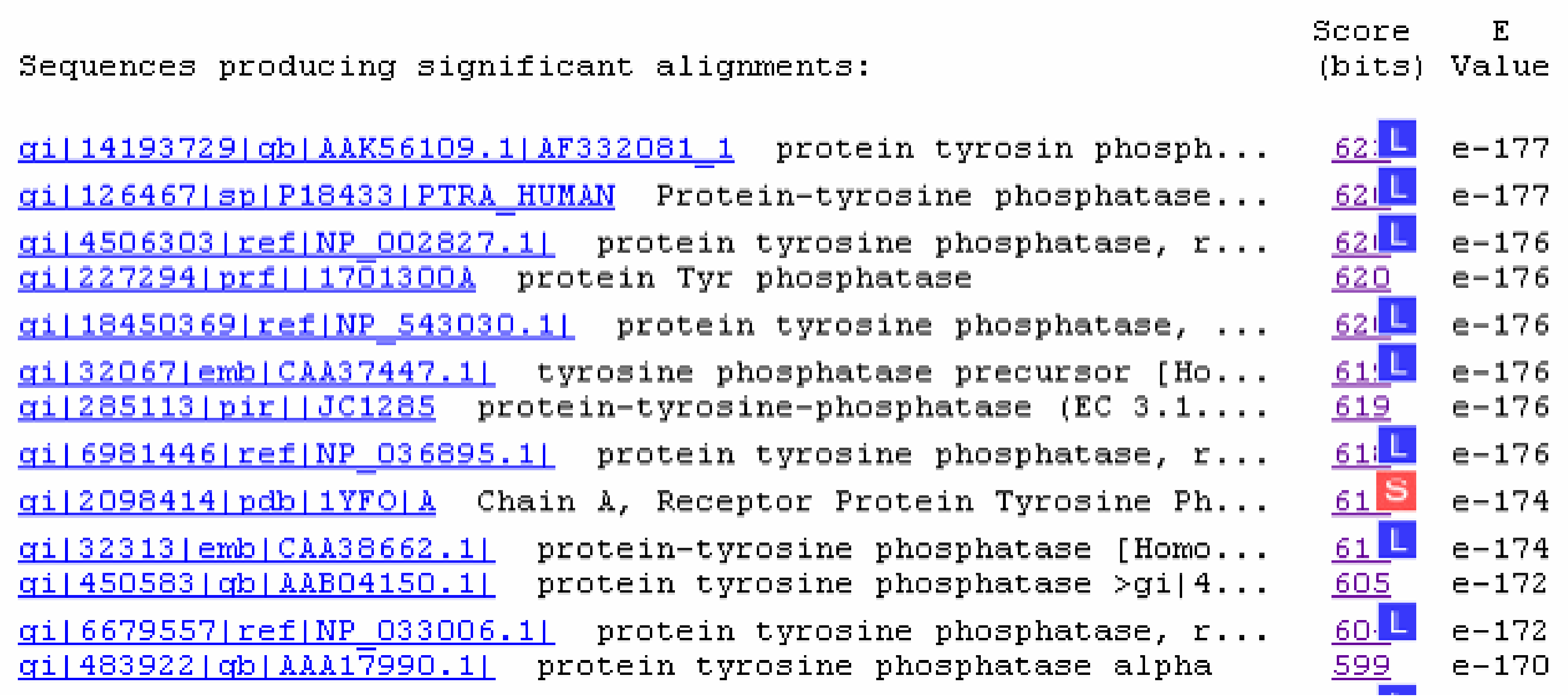


find seqs with good flanking alignment

find from db seqs with short perfect matches to query seq

Exercise: Why do we need this step?

# Homologs obtained by BLAST

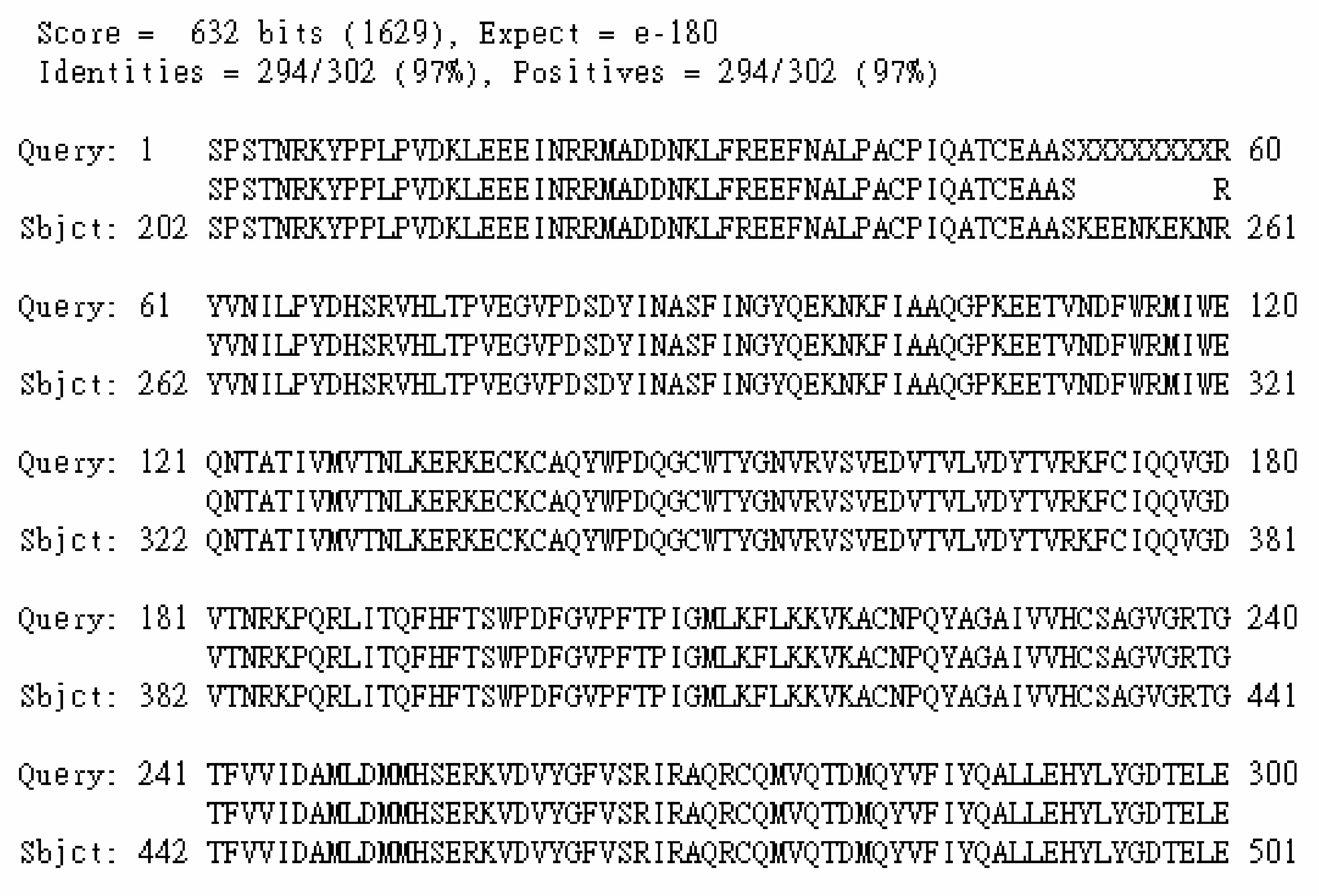


```
                                                                 Score    E
Sequences producing significant alignments:                     (bits) Value

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
```

# Guilt-by-Association: Caveats

- Ensure that the effect of database size has 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

⇒ Need to correct for database size if your seq comparison prog does not do that!

Note: $P = 1 - e^{-E}$

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

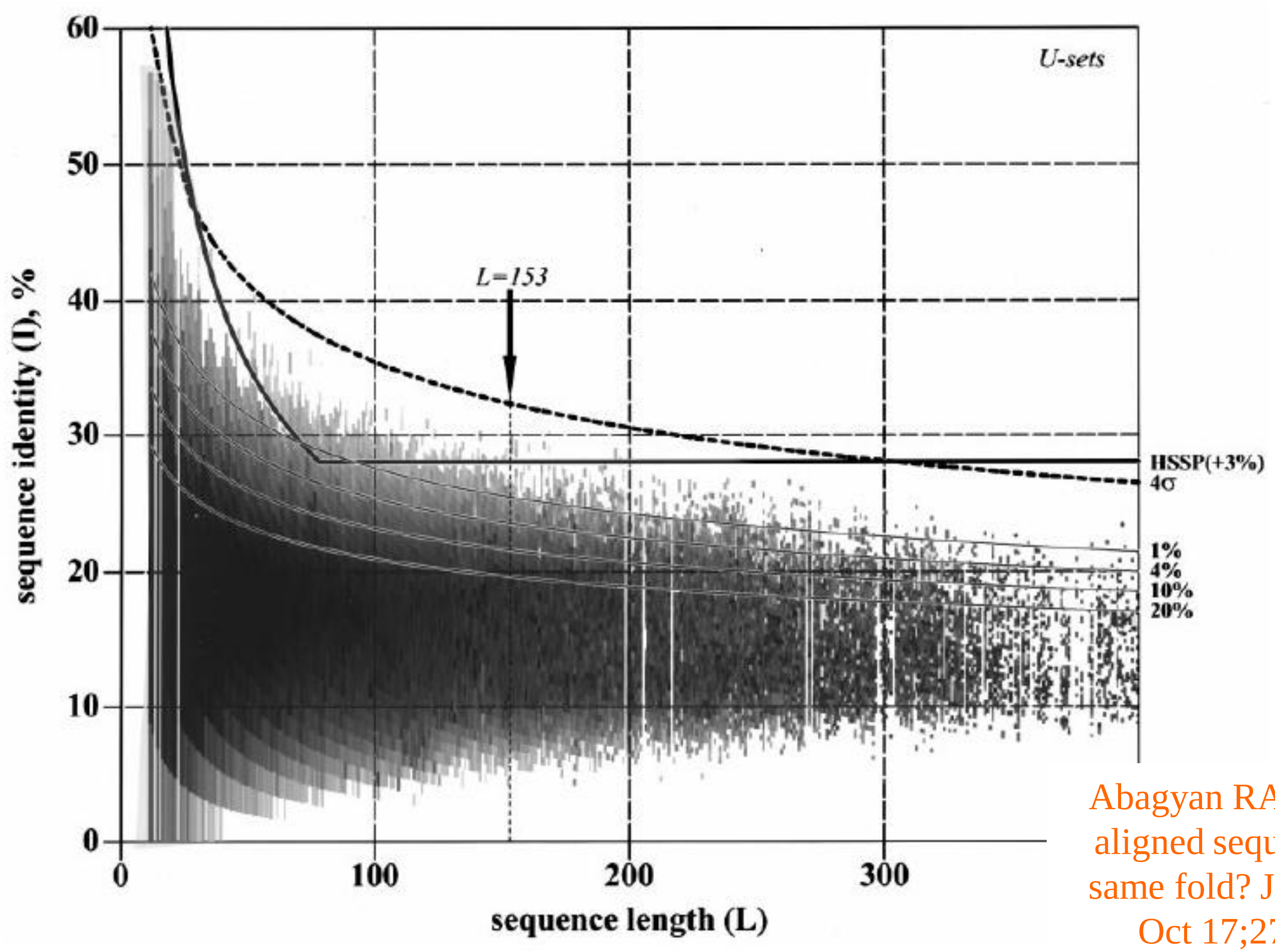
- **One fourth of all residues in protein seqs occur in regions with biased amino acid composition**
- **Alignments of two such regions achieves high score purely due to segment composition**

⇒ **While it is worth noting that two proteins contain similar low complexity regions, they are best excluded when constructing alignments**

- **E.g., by default, BLAST employs the SEG algo to filter low complexity regions from proteins before executing a search**

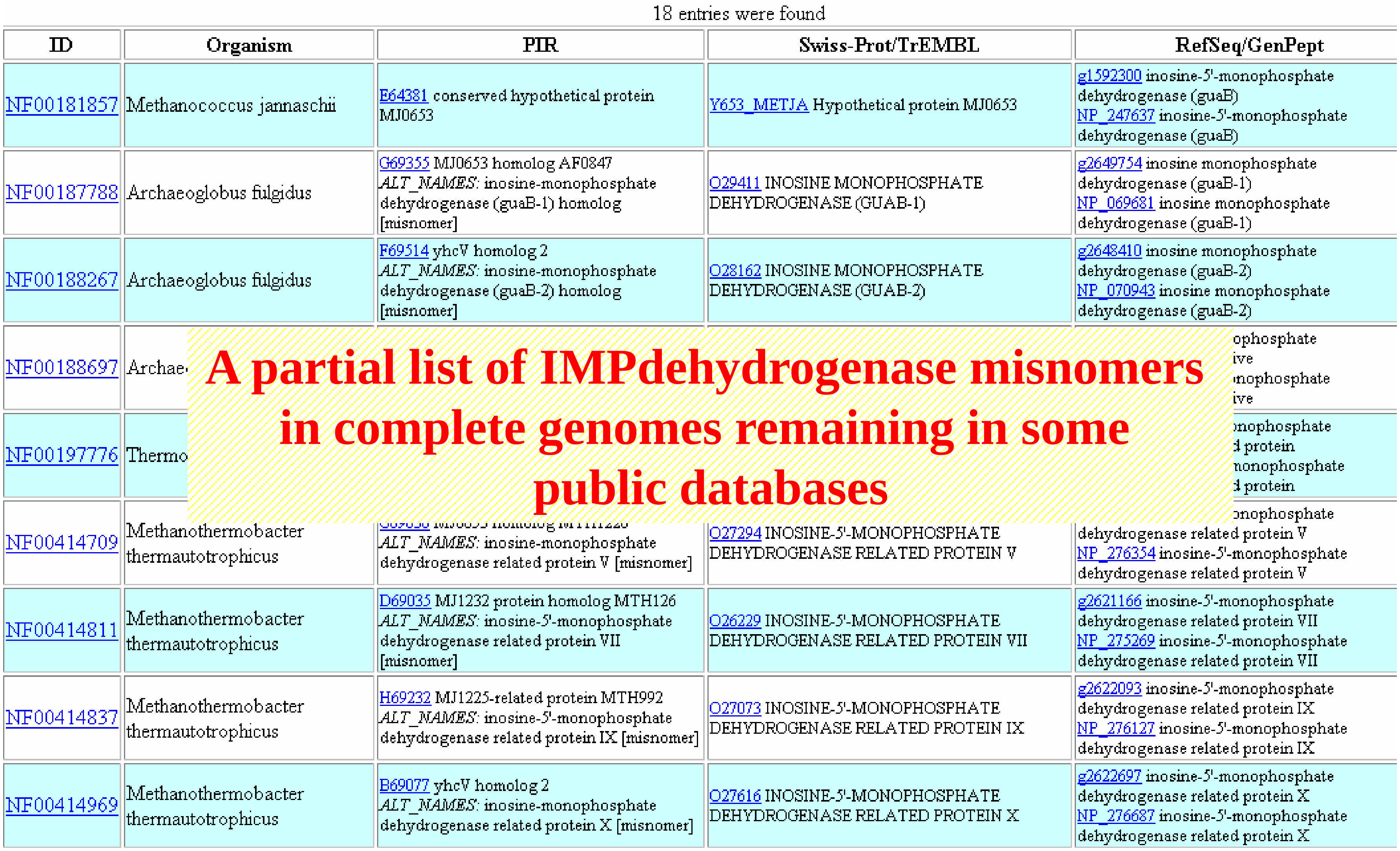
Source: NCBI

# Effect of Sequence Length



Abagyan RA, Batalov S. Do aligned sequences share the same fold? J Mol Biol. 1997 Oct 17;273(1):355-68

# Examples of Invalid Function Assignment:
# The IMP Dehydrogenases (IMPDH)

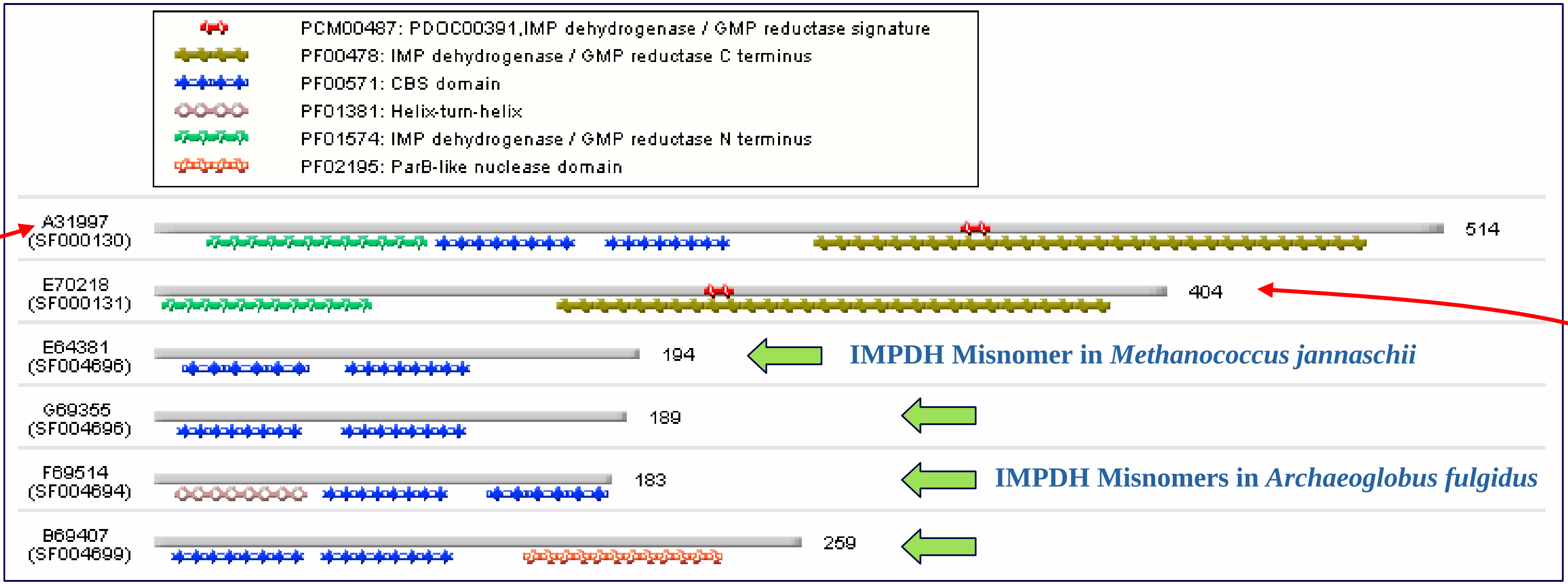


18 entries were found

| 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)<br>NP_247637 inosine-5'-monophosphate dehydrogenase (guaB) |
| NF00187788 | Archaeoglobus fulgidus | G69355 MJ0653 homolog AF0847<br>*ALT_NAMES*: inosine-monophosphate dehydrogenase (guaB-1) homolog [misnomer] | O29411 INOSINE MONOPHOSPHATE DEHYDROGENASE (GUAB-1) | g2649754 inosine monophosphate dehydrogenase (guaB-1)<br>NP_069681 inosine monophosphate dehydrogenase (guaB-1) |
| NF00188267 | Archaeoglobus fulgidus | F69514 yhcV homolog 2<br>*ALT_NAMES*: inosine-monophosphate dehydrogenase (guaB-2) homolog [misnomer] | O28162 INOSINE MONOPHOSPHATE DEHYDROGENASE (GUAB-2) | g2648410 inosine monophosphate dehydrogenase (guaB-2)<br>NP_070943 inosine monophosphate dehydrogenase (guaB-2) |
| NF00188697 | Archae | | | ophosphate<br>ive<br>nophosphate<br>ive |
| NF00197776 | Thermo | | | onophosphate<br>d protein<br>nonophosphate<br>d protein |
| NF00414709 | Methanothermobacter thermautotrophicus | [illegible]<br>*ALT_NAMES*: inosine-monophosphate dehydrogenase related protein V [misnomer] | O27294 INOSINE-5'-MONOPHOSPHATE DEHYDROGENASE RELATED PROTEIN V | onophosphate dehydrogenase related protein V<br>NP_276354 inosine-5'-monophosphate dehydrogenase related protein V |
| NF00414811 | Methanothermobacter thermautotrophicus | D69035 MJ1232 protein homolog MTH126<br>*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<br>NP_275269 inosine-5'-monophosphate dehydrogenase related protein VII |
| NF00414837 | Methanothermobacter thermautotrophicus | H69232 MJ1225-related protein MTH992<br>*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<br>NP_276127 inosine-5'-monophosphate dehydrogenase related protein IX |
| NF00414969 | Methanothermobacter thermautotrophicus | B69077 yhcV homolog 2<br>*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<br>NP_276687 inosine-5'-monophosphate dehydrogenase related protein X |

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

# IMPDH Domain Structure


PCM00487: PDOC00391,IMP dehydrogenase / GMP reductase signature
PF00478: IMP dehydrogenase / GMP reductase C terminus
PF00571: CBS domain
PF01381: Helix-turn-helix
PF01574: IMP dehydrogenase / GMP reductase N terminus
PF02195: ParB-like nuclease domain
A31997 (SF000130)
514
E70218 (SF000131)
404
E64381 (SF004696)
194
G69355 (SF004696)
189
F69514 (SF004694)
183
B69407 (SF004699)
259

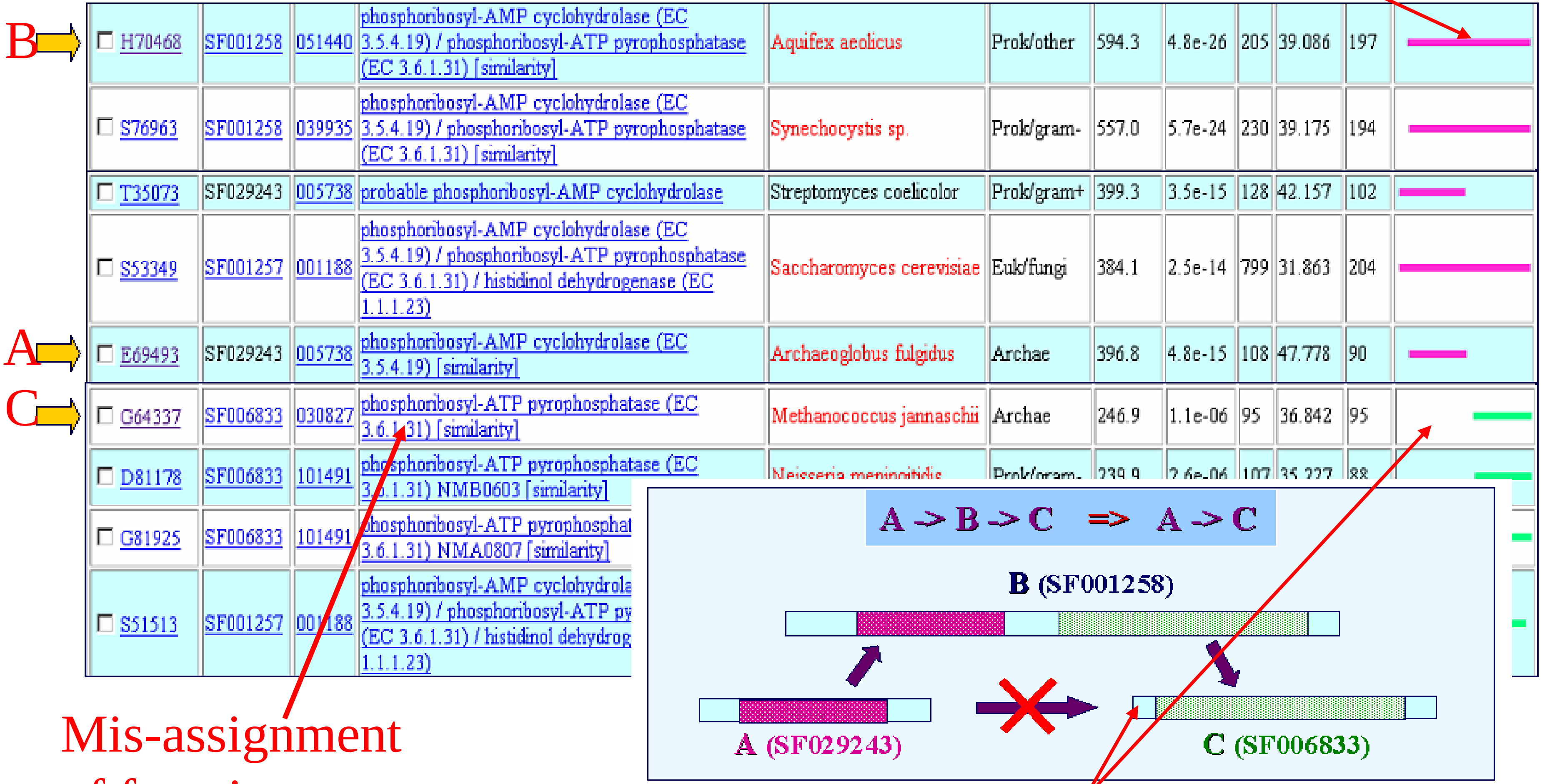
IMPDH Misnomer in *Methanococcus jannaschii*

IMPDH Misnomers in *Archaeoglobus fulgidus*

- **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**

# Invalid Transitive Assignment

Root of invalid transitive assignment



| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| □ 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 | ▬ |
| □ E69493 | SF029243 | 005738 | phosphoribosyl-AMP cyclohydrolase (EC 3.5.4.19) [similarity] | Archaeoglobus fulgidus | Archae | 396.8 | 4.8e-15 | 108 | 47.778 | 90 | ▬ |
| □ 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) | | | | | | | | ▬ |

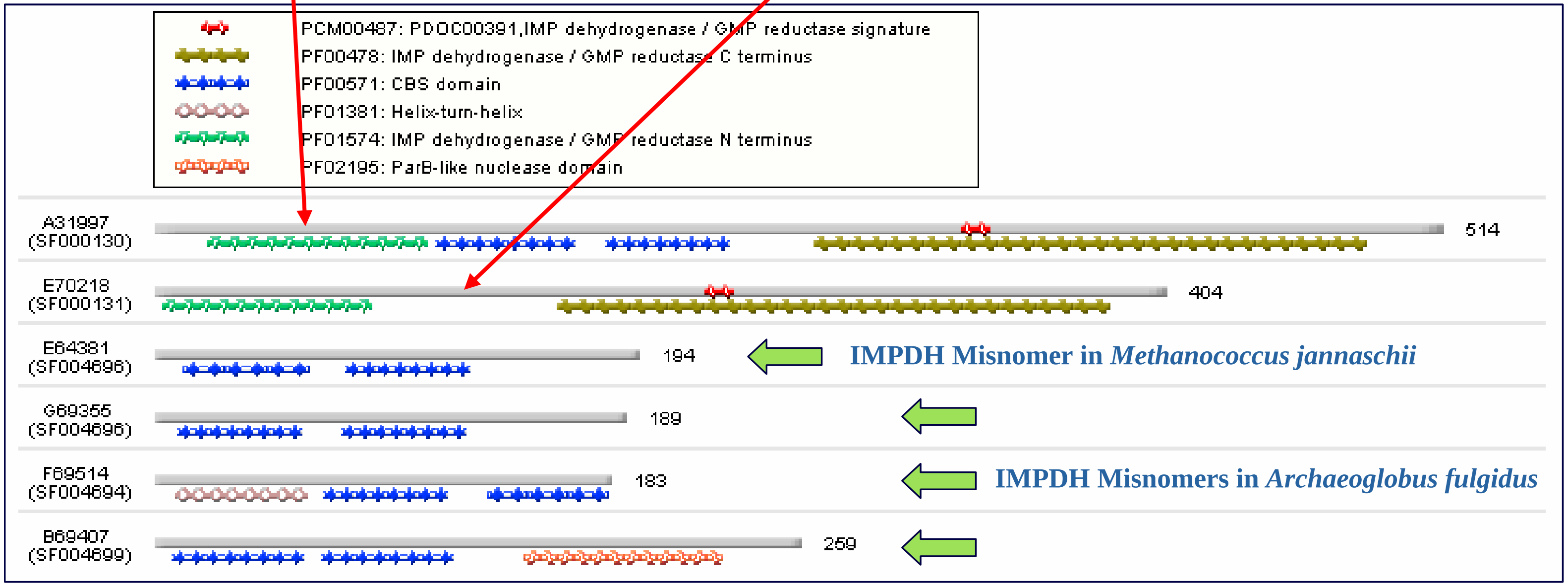
Mis-assignment of function

No IMPDH domain

# Emerging Pattern

Typical IMPDH

Functional IMPDH w/o CBS


PCM00487: PDOC00391,IMP dehydrogenase / GMP reductase signature
PF00478: IMP dehydrogenase / GMP reductase C terminus
PF00571: CBS domain
PF01381: Helix-turn-helix
PF01574: IMP dehydrogenase / GMP reductase N terminus
PF02195: ParB-like nuclease domain
A31997 (SF000130)
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G69355 (SF004696)
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F69514 (SF004694)
183
B69407 (SF004699)
259

IMPDH Misnomer in *Methanococcus jannaschii*

IMPDH Misnomers in *Archaeoglobus fulgidus*

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

⇒ **IMPDH domain is the emerging pattern**

# Application of
# Sequence Comparison:
# Active Site/Domain Discovery

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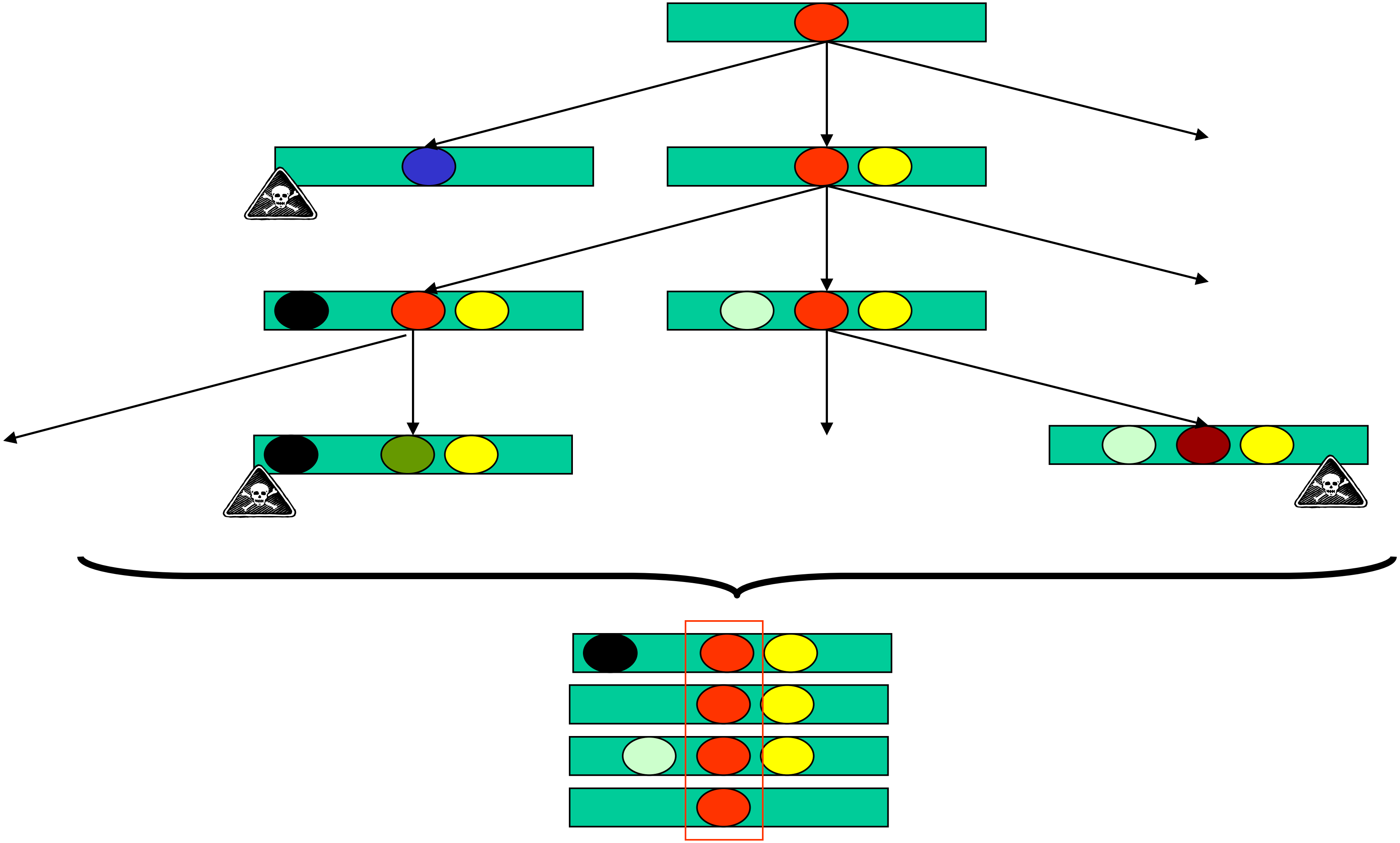
# Discover Active Site and/or Domain

- **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?

# In the course of evolution…



# 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**

⇒ **They are candidate active sites**

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

# What if there is no useful seq homolog?

- **Guilt by other types of association!**
  - – Domain modeling (e.g., HMMPFAM)
  - ✓ Similarity of phylogenetic profiles
  - ✓ Similarity of dissimilarities (e.g., SVM-PAIRWISE)
  - – Similarity of subcellular co-localization & other physico-chemico properties(e.g., PROTFUN)
  - – Similarity of gene expression profiles
  - ✓ Similarity of protein-protein interaction partners
  - – …
  - – Fusion of multiple types of info

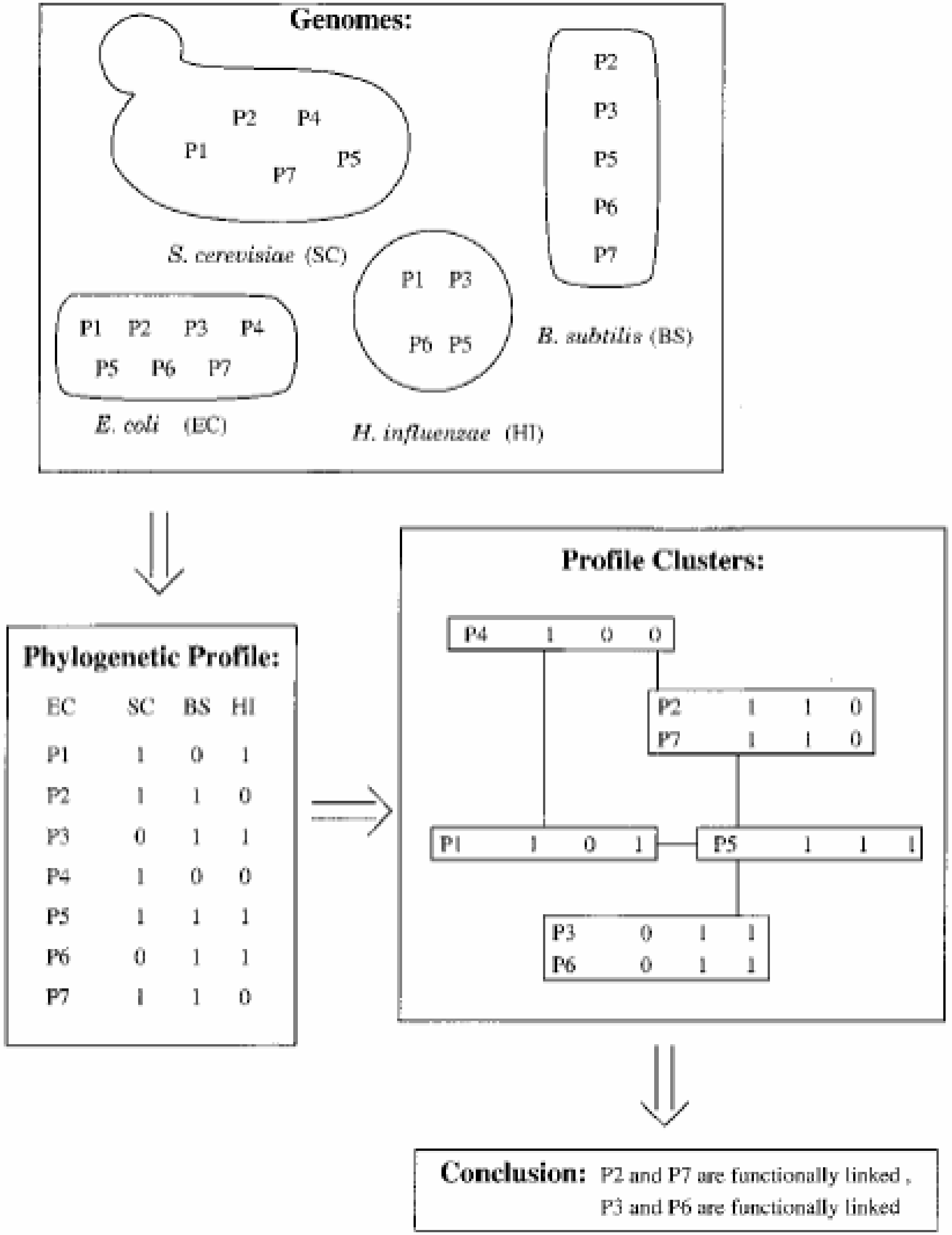
# Phylogenetic Profiling

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

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

**⇒ 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 Profile:**

| | EC | SC | BS | HI |
|---|---|---|---|---|
| P1 | | 1 | 0 | 1 |
| P2 | | 1 | 1 | 0 |
| P3 | | 0 | 1 | 1 |
| P4 | | 1 | 0 | 0 |
| P5 | | 1 | 1 | 1 |
| P6 | | 0 | 1 | 1 |
| P7 | | 1 | 1 | 0 |

**Conclusion:** P2 and P7 are functionally linked ,
P3 and P6 are functionally linked

# 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

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

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

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

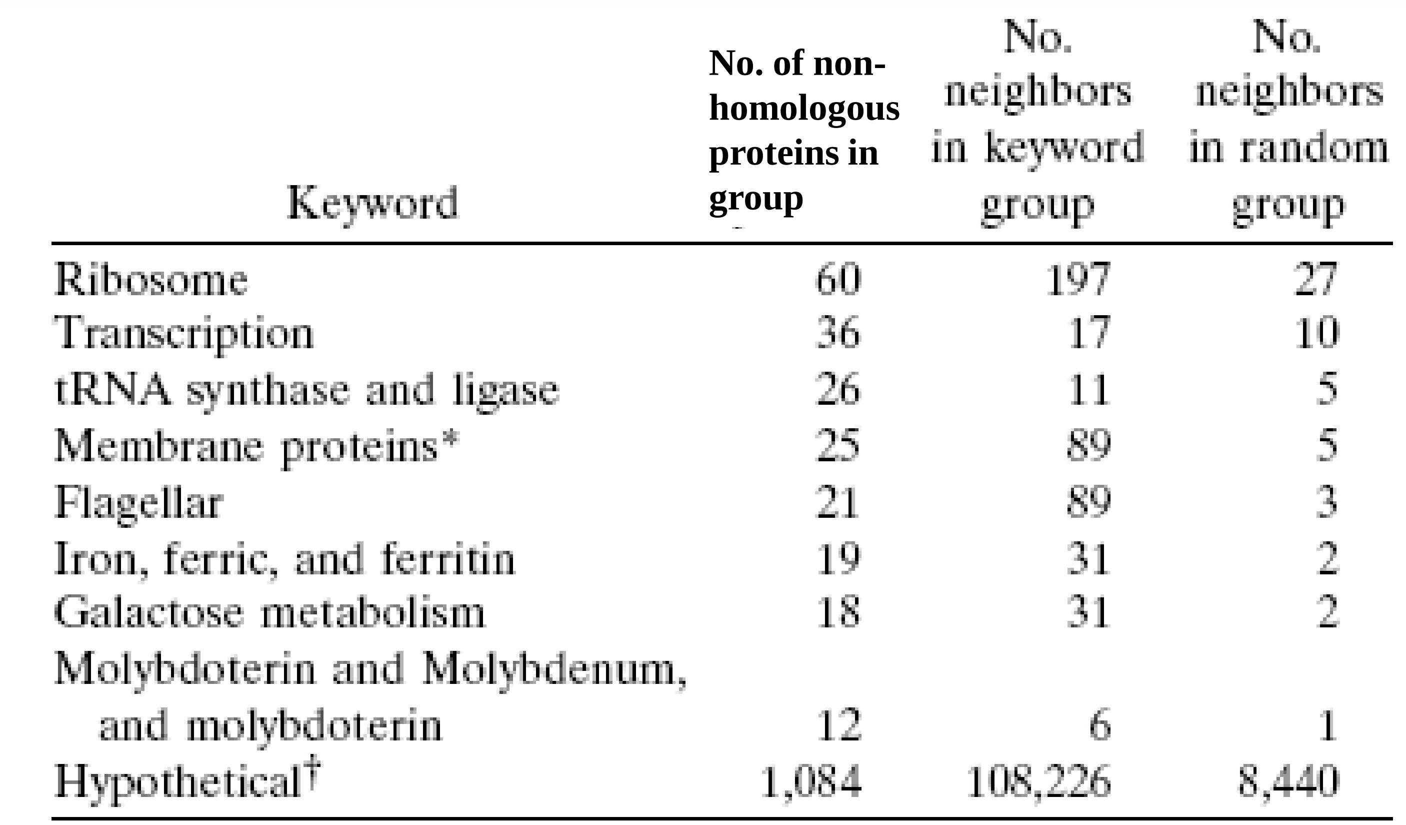
**No. of ways to distribute the remaining *x* – *z* and *y* – *z* occurrences over the remaining *N* – *z* lineage's**

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

**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

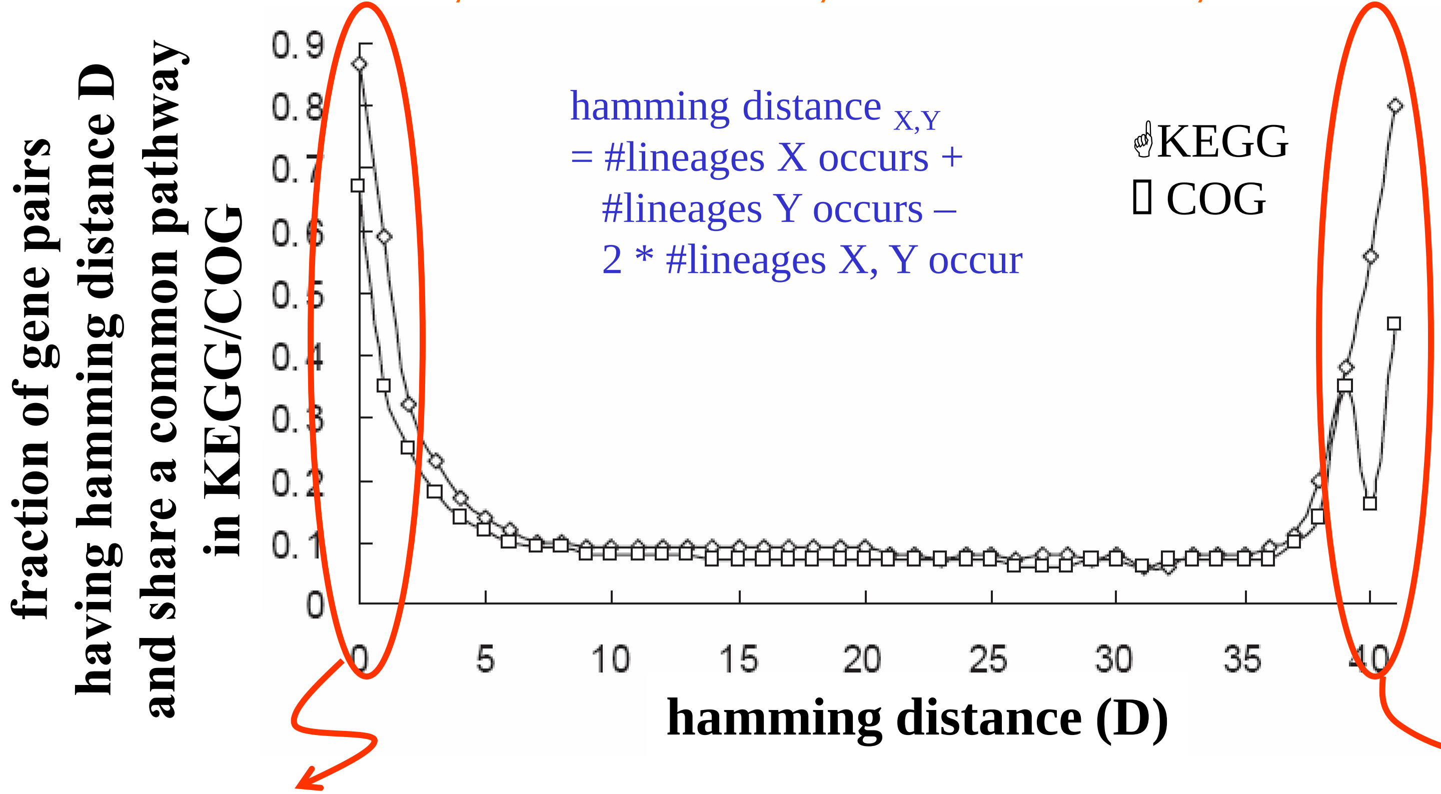


| Keyword | No. of non-homologous proteins in group | No. neighbors in keyword group | No. neighbors in random group |
|---|---|---|---|
| Ribosome | 60 | 197 | 27 |
| Transcription | 36 | 17 | 10 |
| tRNA synthase and ligase | 26 | 11 | 5 |
| Membrane proteins* | 25 | 89 | 5 |
| Flagellar | 21 | 89 | 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

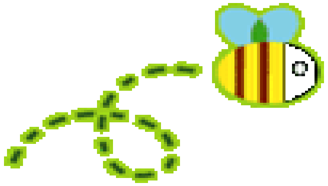


hamming distance $_{X,Y}$
= #lineages X occurs +
#lineages Y occurs –
2 * #lineages X, Y occur

- **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?

# Guilt by Association of Dissimilarities

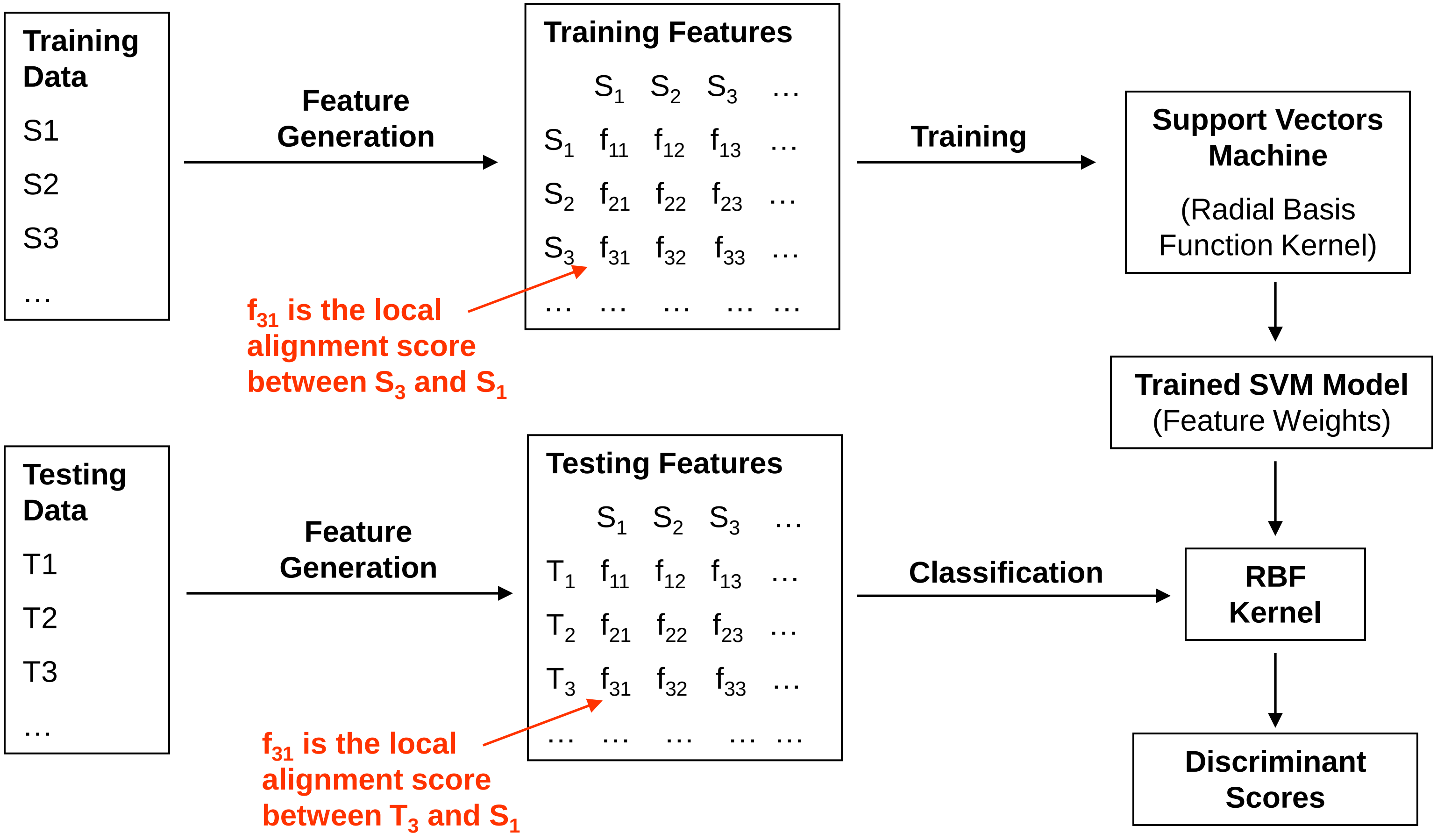


Differences of "unknown" to other fruits are same as "apple" to other fruits

"unknown" is an "apple"!

| | Orange$_1$ | Banana$_1$ | … |
|---|---|---|---|
| Apple$_1$ | Color = red vs orange<br>Skin = smooth vs rough<br>**Size = small vs small**<br>**Shape = round vs round** | Color = red vs yellow<br>**Skin = smooth vs smooth**<br>**Size = small vs small**<br>Shape = round vs oblong | … |
| Orange$_2$ | **Color = orange vs orange**<br>**Skin = rough vs rough**<br>**Size = small vs small**<br>**Shape = round vs round** | Color = orange vs yellow<br>**Skin = rough vs smooth**<br>**Size = small vs small**<br>Shape = round vs oblong | … |
| Unknown$_1$ | **Color = red vs orange**<br>**Skin = smooth vs rough**<br>Size = small vs small<br>Shape = round vs round | **Color = red vs yellow**<br>Skin = smooth vs smooth<br>Size = small vs small<br>**Shape = round vs oblong** | … |
| … | … | … | … |

# SVM-Pairwise Framework



**Training Data**

S1

S2

S3

…

**Feature Generation**

**Training Features**

|  | $S_1$ | $S_2$ | $S_3$ | … |
|---|---|---|---|---|
| $S_1$ | $f_{11}$ | $f_{12}$ | $f_{13}$ | … |
| $S_2$ | $f_{21}$ | $f_{22}$ | $f_{23}$ | … |
| $S_3$ | $f_{31}$ | $f_{32}$ | $f_{33}$ | … |
| … | … | … | … | … |

**$f_{31}$ is the local alignment score between $S_3$ and $S_1$**

**Training**

**Support Vectors Machine**

(Radial Basis Function Kernel)

**Trained SVM Model**

(Feature Weights)

**Testing Data**

T1

T2

T3

…

**Feature Generation**

**Testing Features**

|  | $S_1$ | $S_2$ | $S_3$ | … |
|---|---|---|---|---|
| $T_1$ | $f_{11}$ | $f_{12}$ | $f_{13}$ | … |
| $T_2$ | $f_{21}$ | $f_{22}$ | $f_{23}$ | … |
| $T_3$ | $f_{31}$ | $f_{32}$ | $f_{33}$ | … |
| … | … | … | … | … |

**$f_{31}$ is the local alignment score between $T_3$ and $S_1$**

**Classification**

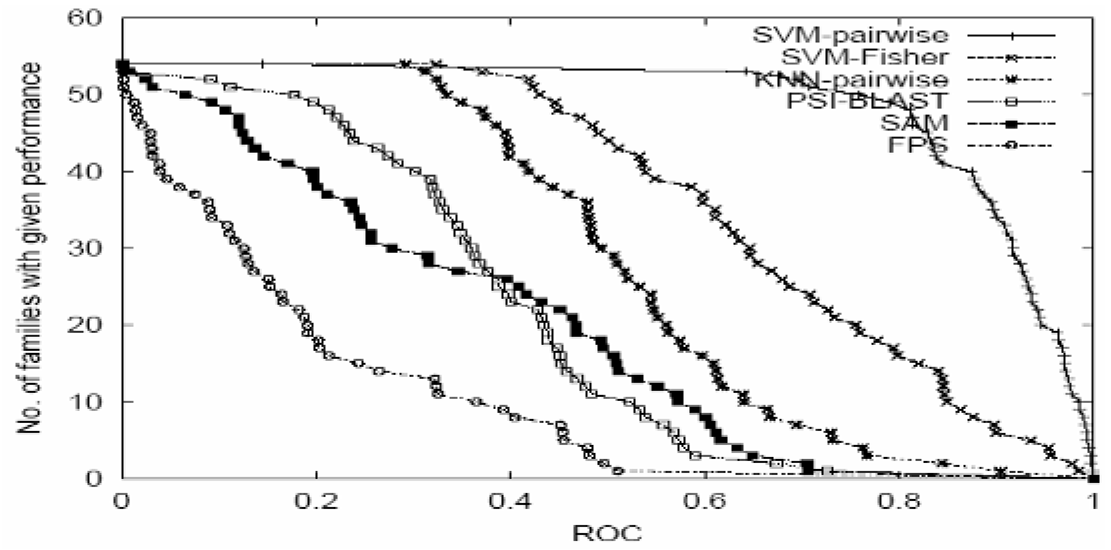
**RBF Kernel**

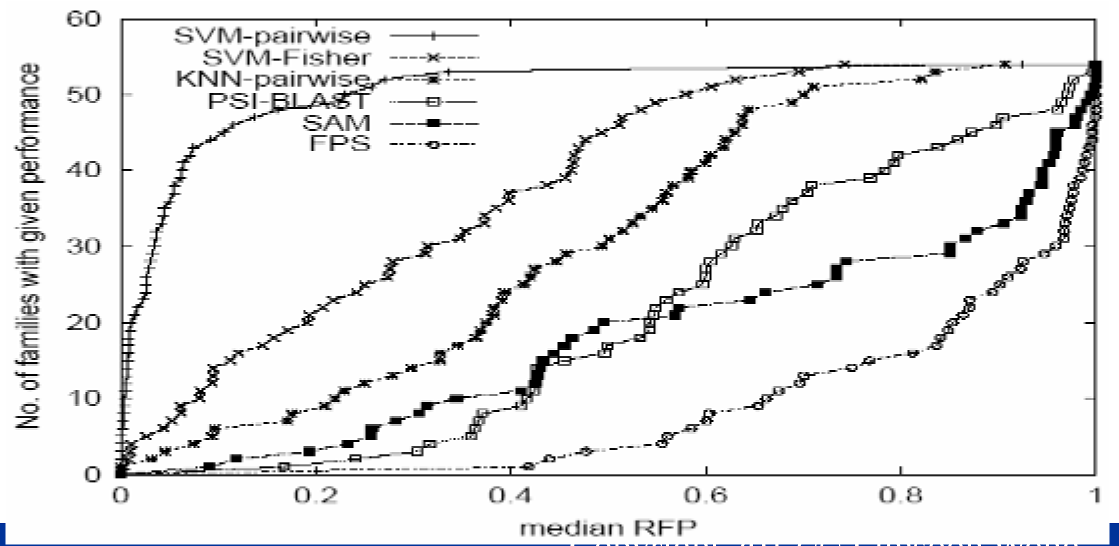
**Discriminant Scores**

Image credit: Kenny Chua

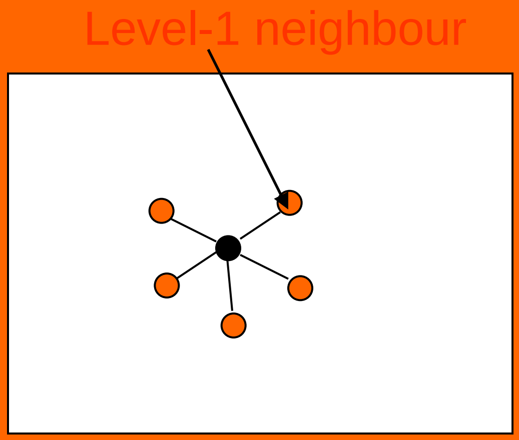
# Performance of SVM-Pairwise

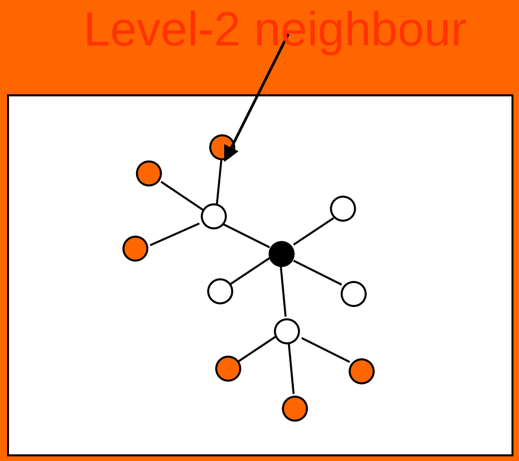
- **Receiver Operating Characteristic (ROC)**
  - The area under the curve derived from plotting true positives as a function of false positives for various thresholds.
- **Rate of median False Positives (RFP)**
  - The fraction of negative test examples with a score better or equals to the median of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





# Protein Function Prediction from Protein Interactions



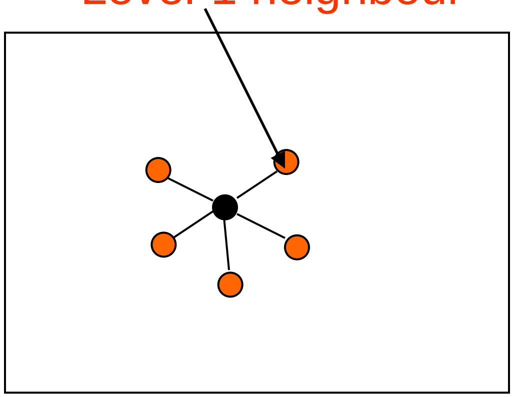


NUS
National University
of Singapore

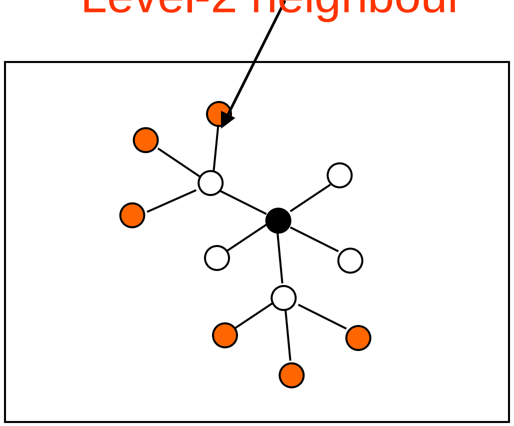
# Functional Association Thru Interactions

- **Direct functional association:**
  - Interaction partners of a protein are likely to share functions w/ it
  - Proteins from the same pathways are likely to interact
- **Indirect functional association**
  - Proteins that share interaction partners with a protein may also likely to share functions w/ it
  - Proteins that have common biochemical, physical properties and/or subcellular localization are likely to bind to the same proteins

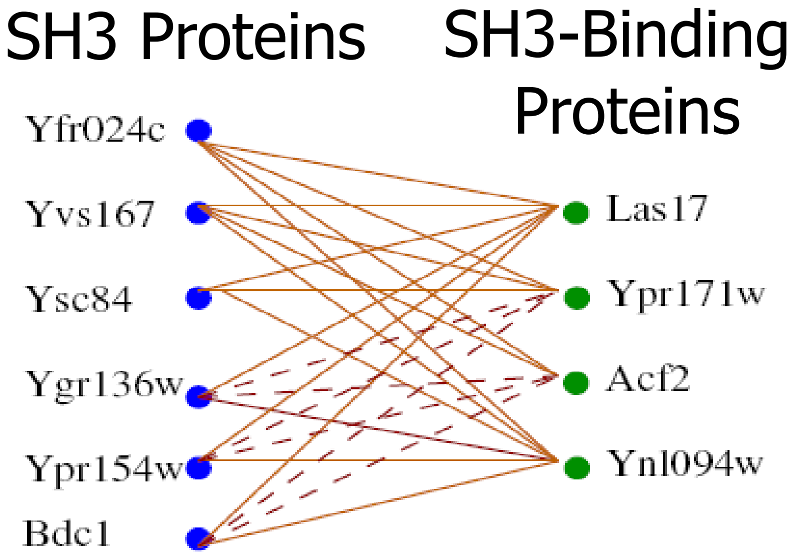
Level-1 neighbour



Level-2 neighbour

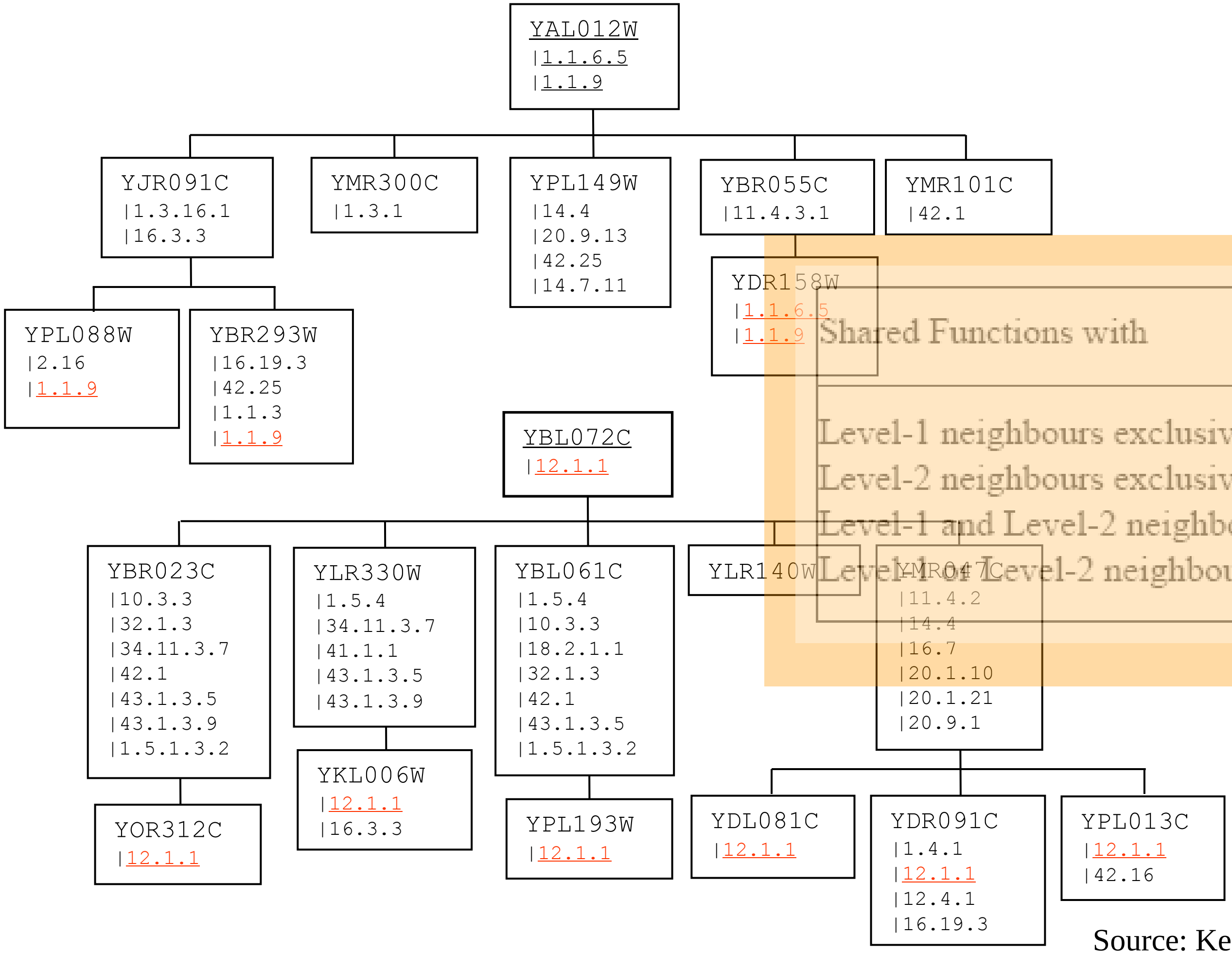


# An illustrative Case of Indirect Functional Association?



- Is *indirect functional association* plausible?
- Is it found often in real interaction data?
- Can it be used to improve protein function prediction from protein interaction data?

# Freq of Indirect Functional Association



| Shared Functions with | Fraction |
|---|---|
| Level-1 neighbours exclusively | 0.016338 |
| Level-2 neighbours exclusively | 0.226574 |
| Level-1 and Level-2 neighbours | 0.463960 |
| Level-1 or Level-2 neighbours | 0.706872 |

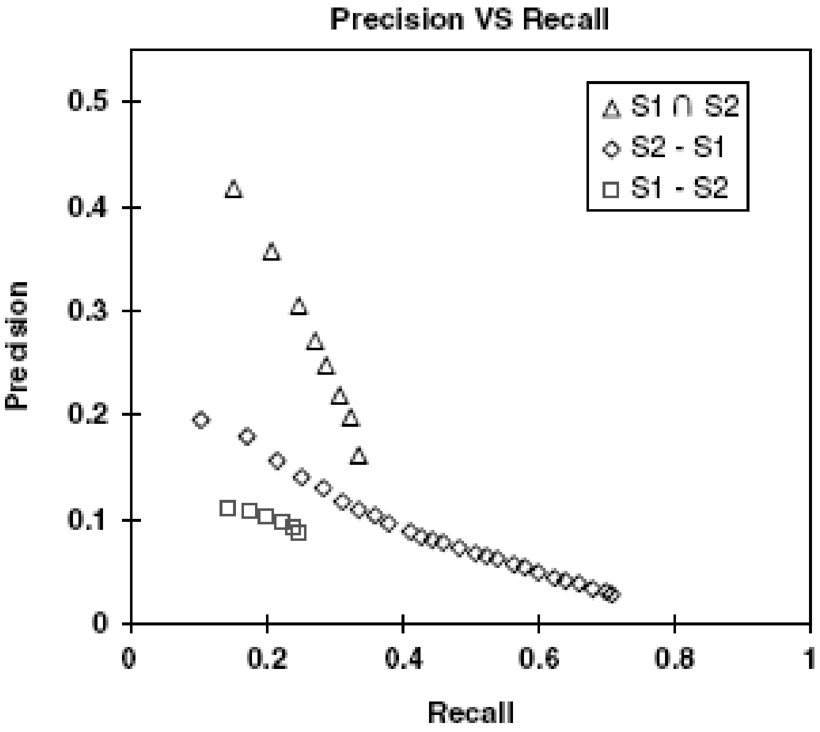
Source: Kenny Chua

# Prediction Power By Majority Voting

- Remove overlaps in level-1 and level-2 neighbours to study predictive power of “level-1 only” and “level-2 only” neighbours
- Sensitivity vs Precision analysis

$$PR = \frac{\sum_i^K k_i}{\sum_i^K m_i} \qquad SN = \frac{\sum_i^K k_i}{\sum_i^K n_i}$$

- $n_i$ is no. of fn of protein i
- $m_i$ is no. of fn predicted for protein i
- $k_i$ is no. of fn predicted correctly for protein i



⇒ “level-2 only” neighbours performs better

⇒ L1 ∩ L2 neighbours has greatest prediction power

# Functional Similarity Estimate: Czekanowski-Dice Distance

- **Functional distance between two proteins** **(Brun et al, 2003)**

$$D(u,v) = \frac{|N_u \Delta N_v|}{|N_u \cup N_v| + |N_u \cap N_v|}$$

- **$N_k$ is the set of interacting partners of k**
- **X Δ Y is symmetric diff betw two sets X and Y**
- **Greater weight given to similarity**

**Is this a good measure if u and v have very diff number of neighbours?**

**⇒ Similarity can be defined as**

$$S(u,v) = 1 - D(u,v) = \frac{2X}{2X + (Y + Z)}$$

# Functional Similarity Estimate: FS-Weighted Measure

- **FS-weighted measure**

$$S(u,v)=\frac{2|N_u \cap N_v|}{|N_u - N_v| + 2|N_u \cap N_v|} \times \frac{2|N_u \cap N_v|}{|N_v - N_u| + 2|N_u \cap N_v|}$$

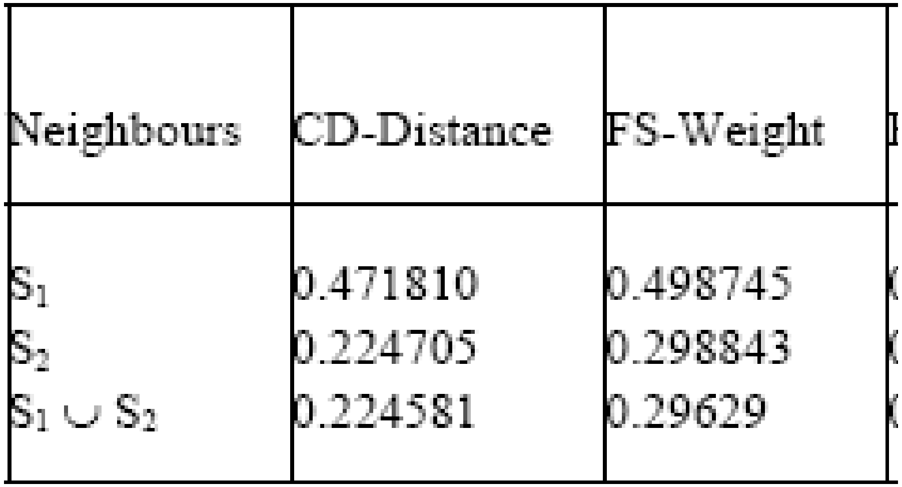
  - **$N_k$ is the set of interacting partners of k**
  - **Greater weight given to similarity**

⇒ **Rewriting this as**

$$S(u,v)=\frac{2X}{2X+Y} \times \frac{2X}{2X+Z}$$

# Correlation w/ Functional Similarity

- **Correlation betw functional similarity & estimates**



| Neighbours | CD-Distance | FS-Weight | I |
|---|---|---|---|
| $S_1$ | 0.471810 | 0.498745 | ( |
| $S_2$ | 0.224705 | 0.298843 | ( |
| $S_1 \cup S_2$ | 0.224581 | 0.29629 | ( |

- **Equiv measure slightly better in correlation w/ similarity for L1 & L2 neighbours**

# Reliability of Expt Sources

- **Diff Expt Sources have diff reliabilities**
  - Assign reliability to an interaction based on its expt sources (Nabieva et al, 2004)
- **Reliability betw u and v computed by:**

$$r_{u,v} = 1 - \prod_{i \in E_{u,v}} (1 - r_i)$$

  - **$r_i$ is reliability of expt source i,**
  - **$E_{u,v}$ is the set of expt sources in which interaction betw u and v is observed**

| Source | Reliability |
|---|---|
| **Affinity Chromatography** | **0.823077** |
| **Affinity Precipitation** | **0.455904** |
| **Biochemical Assay** | **0.666667** |
| **Dosage Lethality** | **0.5** |
| **Purified Complex** | **0.891473** |
| **Reconstituted Complex** | **0.5** |
| **Synthetic Lethality** | **0.37386** |
| **Synthetic Rescue** | **1** |
| **Two Hybrid** | **0.265407** |

# Functional Similarity Estimate: FS-Weighted Measure with Reliability

- **Take reliability into consideration when computing FS-weighted measure:**

$$S_R(u,v)=\frac{2\sum\limits_{w\in(N_u\cap N_v)} r_{u,w}r_{v,w}}{\left(\sum\limits_{w\in N_u-N_v} r_{u,w}+\sum\limits_{w\in(N_u\cap N_v)} r_{u,w}(1-r_{v,w})\right)+2\sum\limits_{w\in(N_u\cap N_v)} r_{u,w}r_{v,w}}\times\frac{2\sum\limits_{w\in(N_u\cap N_v)} r_{u,w}r_{v,w}}{\left(\sum\limits_{w\in N_v-N_u} r_{v,w}+\sum\limits_{w\in(N_u\cap N_v)} r_{v,w}(1-r_{u,w})\right)+2\sum\limits_{w\in(N_u\cap N_v)} r_{u,w}r_{v,w}}$$

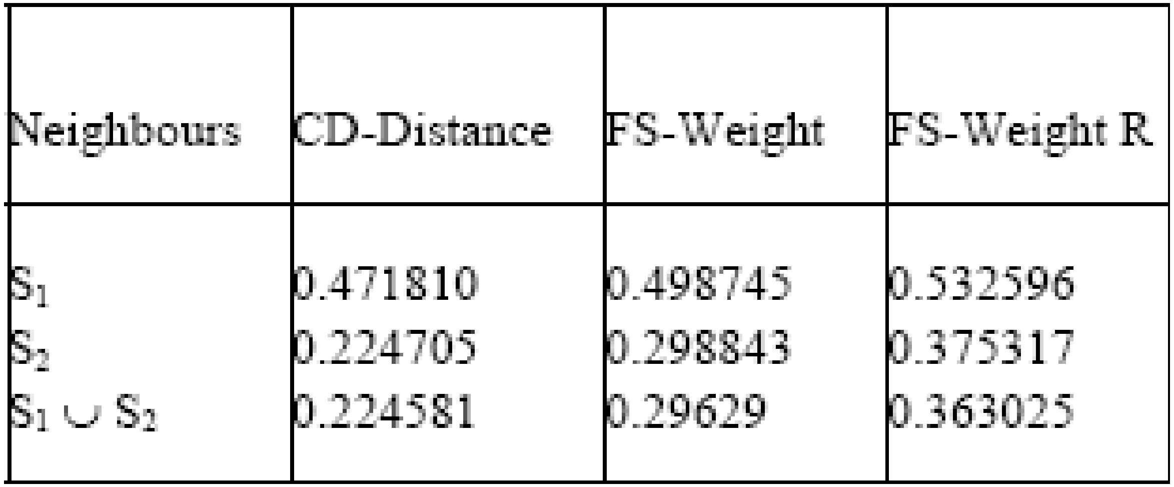
  - **$N_k$ is the set of interacting partners of k**
  - **$r_{u,w}$ is reliability weight of interaction betw u and v**

$\Rightarrow$ **Rewriting**

$$S(u,v)=\frac{2X}{2X+Y}\times\frac{2X}{2X+Z}$$

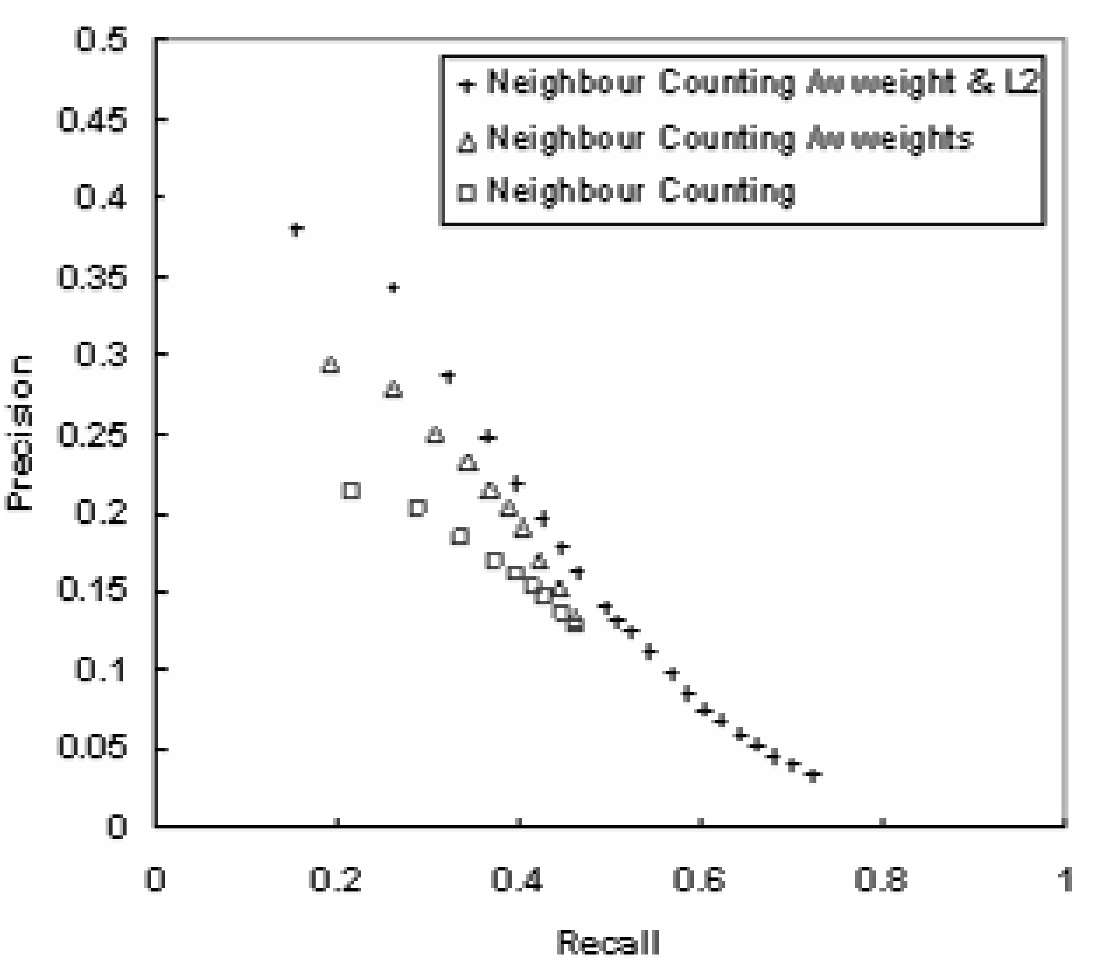
# Integrating Reliability

- **Equiv measure shows improved correlation w/ functional similarity when reliability of interactions is considered:**



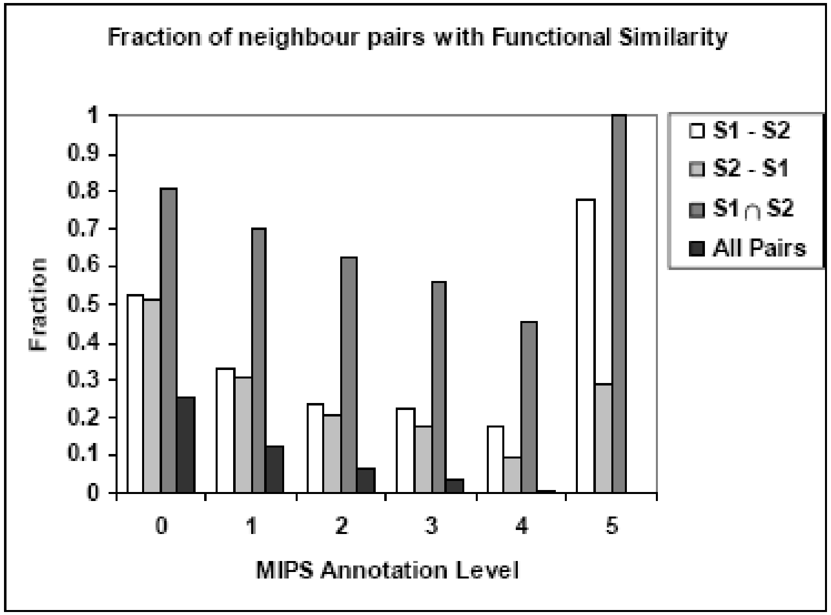
| Neighbours | CD-Distance | FS-Weight | FS-Weight R |
|---|---|---|---|
| $S_1$ | 0.471810 | 0.498745 | 0.532596 |
| $S_2$ | 0.224705 | 0.298843 | 0.375317 |
| $S_1 \cup S_2$ | 0.224581 | 0.29629 | 0.363025 |

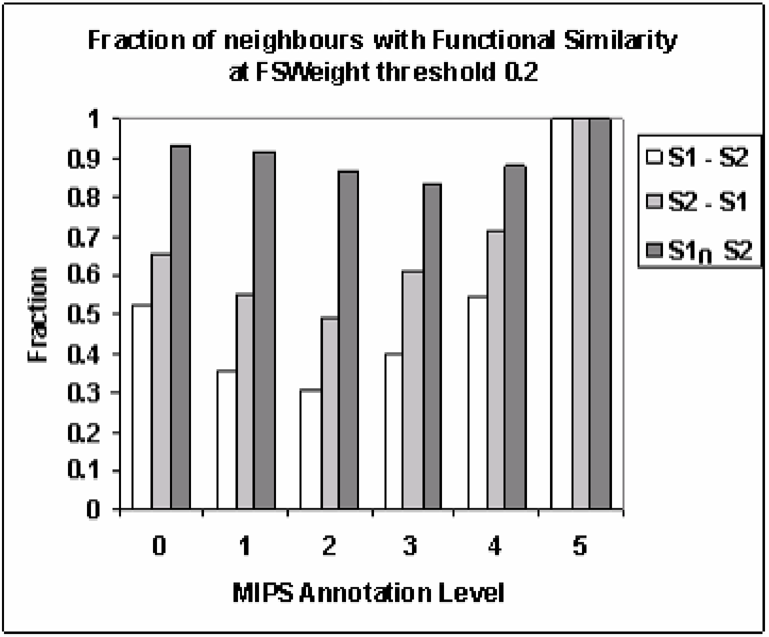
# Improvement to Prediction Power by Majority Voting



Considering only neighbours w/ FS weight > 0.2

# Improvement to Over-Rep of Functions in Neighbours





# Use L1 & L2 Neighbours for Prediction

- **FS-weighted Average**

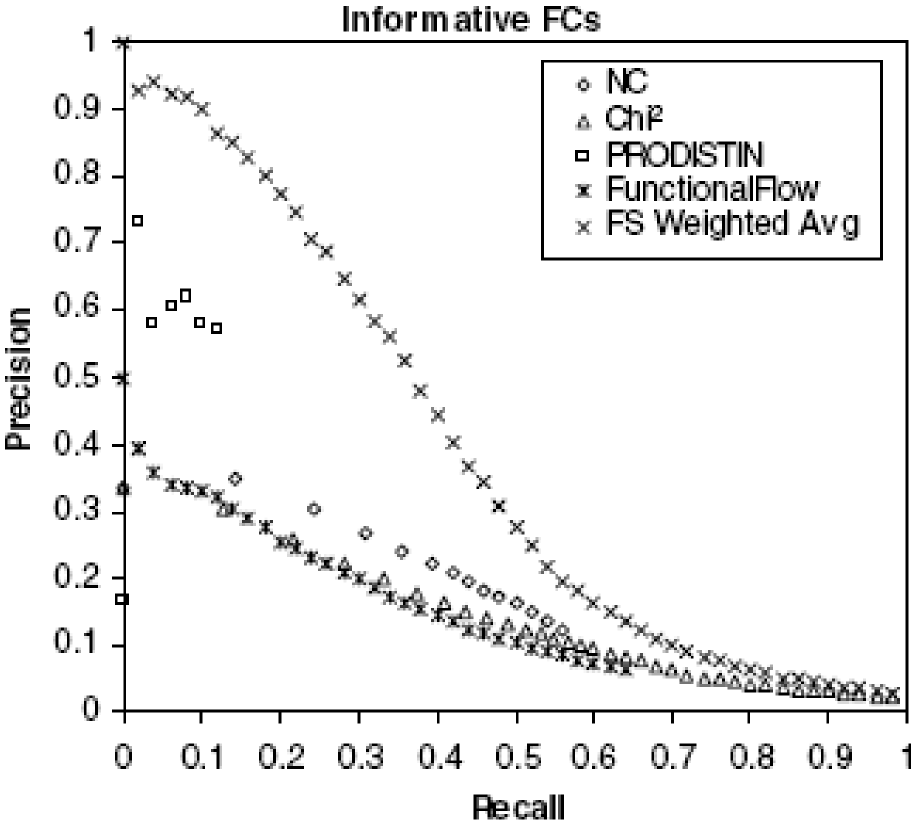
$$f_x(u) = \frac{1}{Z}\left[\lambda r_{\text{int}}\pi_x + \sum_{v\in N_u}\left(S_{TR}(u,v)\delta(v,x) + \sum_{w\in N_v} S_{TR}(u,w)\delta(w,x)\right)\right]$$

  - ***$r_{int}$*** **is fraction of all interaction pairs sharing function**
  - **λ is weight of contribution of background freq**
  - **δ(k, x) = 1 if k has function x, 0 otherwise**
  - **$N_k$ is the set of interacting partners of k**
  - **$\pi_x$ is freq of function x in the dataset**
  - **Z is sum of all weights**

$$Z = 1 + \sum_{v\in N_u}\left(S_{TR}(u,v) + \sum_{w\in N_v} S_{TR}(u,w)\right)$$

# Performance of FS-Weighted Averaging

- **LOOCV comparison with Neighbour Counting, Chi-Square, PRODISTIN**



# About the Inventor: Chua Hon Nian

- **Chua Hon Nian**
  - PhD, NUS, 2008
  - Postdoc at Harvard & Univ of Toronto
  - 49th hottest paper in Computer Science published in 2006
  - Winner, DREAM2 challenge PPI subnetwork, 2007

# Application of Sequence Comparison: Key Mutation Site Discovery

# Identifying Key Mutation Sites

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

Sequence from a typical PTP domain D2

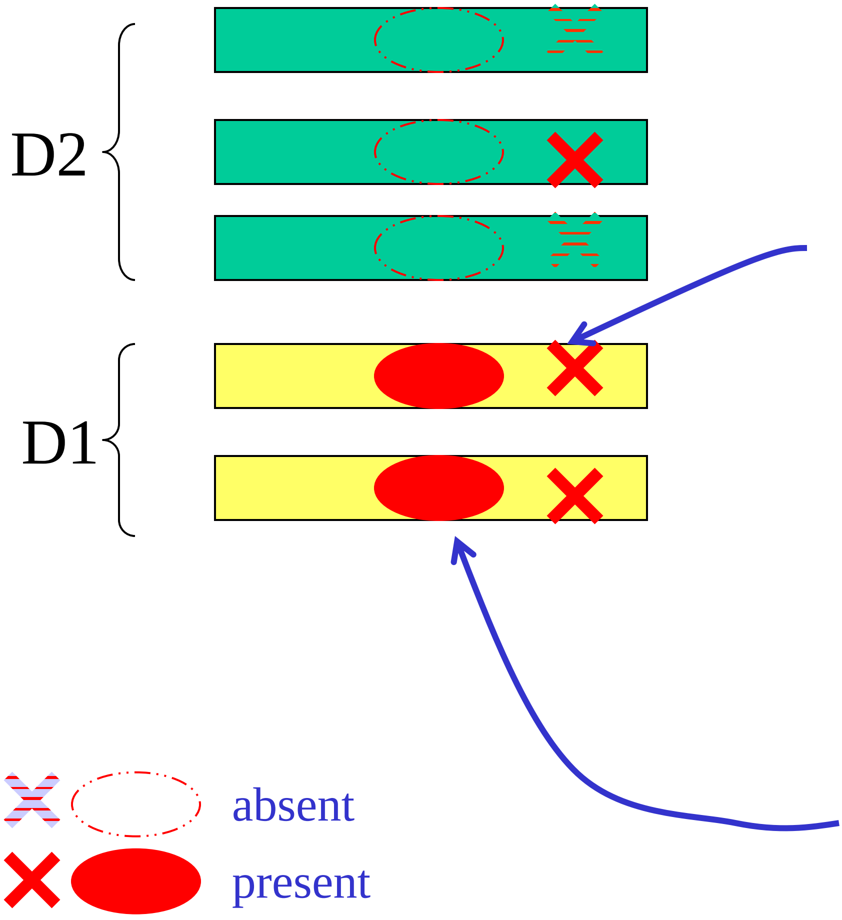
```
>gi|00000|PTPA-D2
EEEFKKLTSIKIQNDKMRTGNLPANMKKNRVLQIIPYEFNRVIIPVKRGEENTDYVNASF
IDGYRQKDSYIASQGPLLHTIEDFWRMIWEWKSCSIVMLTELEERGQEKCAQYWPSDGLV
SYGDITVELKKEEECESYTVRDLLVTNTRENKSRQIRQFHFHGWPEVGIPSDGKGMISII
AAVQKQQQQSGNHPITVHCSAGAGRTGTFCALSTVLERVKAEGILDVFQTVKSLRLQRPH
MVQTLEQYEFCYKVVQEYIDAFSDYANFK
```

- **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

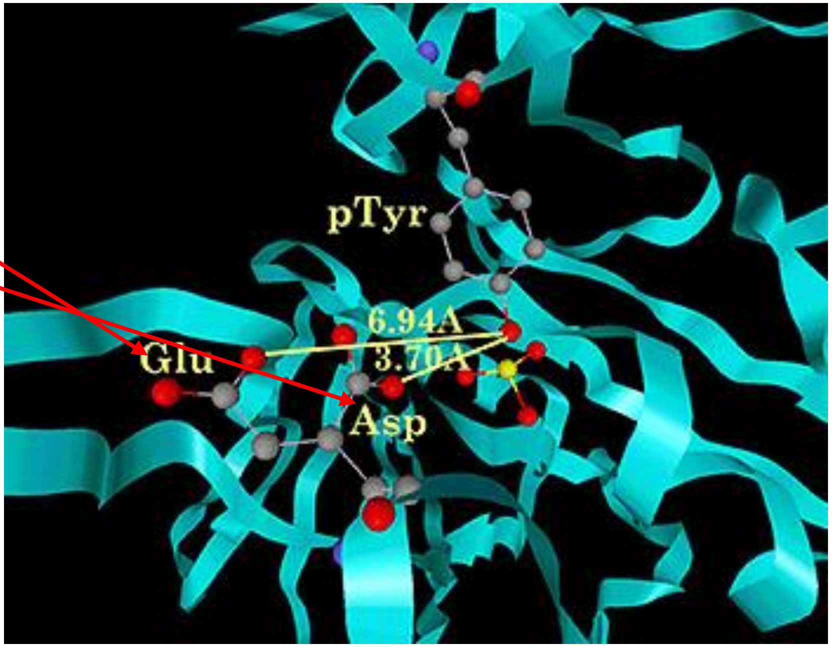
# Key Mutation Site: PTP D1 vs D2

```
                    ?   !  ?         ?                ?             ? ??
gi|00000|P  D2  QFHFHGWPEVGIPSDGKGMISIIAAVQKQQQ-SGNHPITVHCSAGAGRTGTFCALSTVL
gi|126467|      QFHFTSWPDFGVPFTPIGMLKFLKKVKACNP--QYAGAIVVHCSAGVGRTGTFVVIDAML
gi|2499753      QFHFTGWPDHGVPYHATGLLSFIRRVKLSNP--PSAGPIVVHCSAGAGRTGCYIVIDIML
gi|462550|      QYHYTQWPDMGVPEYALPVLTFVRRSSAARM--PETGPVLVHCSAGVGRTGTYIVIDSML
gi|2499751      QFHFTSWPDHGVPDTTDLLINFRYLVRDYMKQSPPESPILVHCSAGVGRTGTFIAIDRLI
gi|1709906  D1  QFQFTAWPDHGVPEHPTPFLAFLRRVKTCNP--PDAGPMVVHCSAGVGRTGCFIVIDAML
gi|126471|      QLHFTSWPDFGVPFTPIGMLKFLKKVKTLNP--VHAGPIVVHCSAGVGRTGTFIVIDAMM
gi|548626|      QFHFTGWPDHGVPYHATGLLSFIRRVKLSNP--PSAGPIVVHCSAGAGRTGCYIVIDIML
gi|131570|      QFHFTGWPDHGVPYHATGLLGFVRQVKSKSP--PNAGPLVVHCSAGAGRTGCFIVIDIML
gi|2144715      QFHFTSWPDHGVPDTTDLLINFRYLVRDYMKQSPPESPILVHCSAGVGRTGTFIAIDRLI
                * ..  **. *.*      .                 . ****** ****..  .  ..
```

- **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|       QFHFTSWPDFGVPFTPI
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 → D in D2 and see if there is gain in PTP activity
  - Mutate D → E in D1 and see if there is loss in PTP activity

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

# About the Inventor: Prasanna Kolatkar

- **Prasanna Kolatkar**
  - Research Fellow, BIC, NUS, 1997-1999
  - Currently Group Leader at GIS

# Concluding Remarks

# What have we learned?

- **General methodologies & applications**
  - Guilt by association for protein function inference
  - Invariants for active site discovery
  - Emerging patterns for mutation site discovery
- **Important tactics**
  - Genome phylogenetic profiling
  - SVM-Pairwise
  - Protein-protein interactions

Any Question?

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