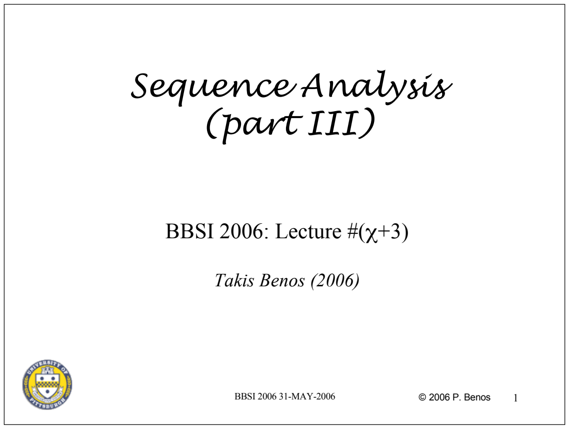
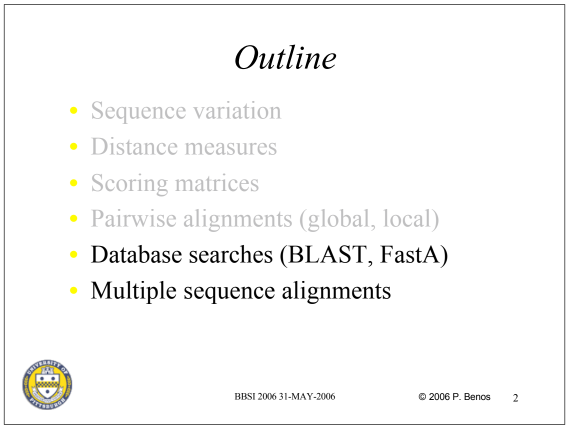


Sequence Analysis (part III)

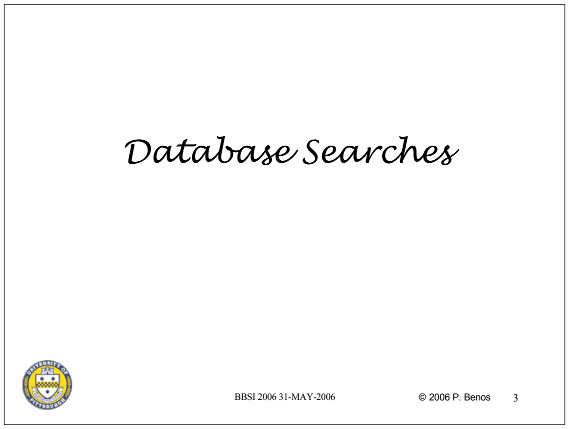
BBSI 2006: Lecture #(χ+3)

Takis Benos (2006)

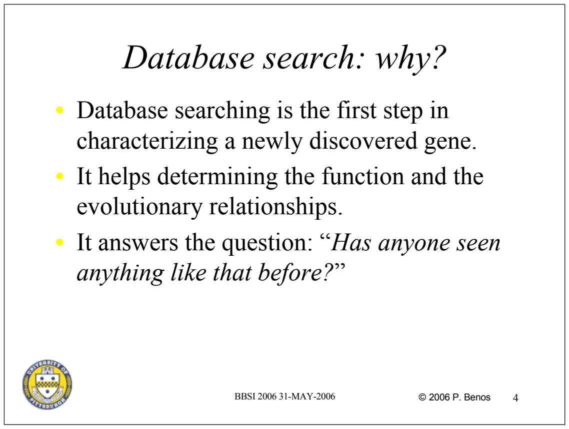


Outline

- Sequence variation
- Distance measures
- Scoring matrices
- Pairwise alignments (global, local)
- Database searches (BLAST, FastA)
- Multiple sequence alignments

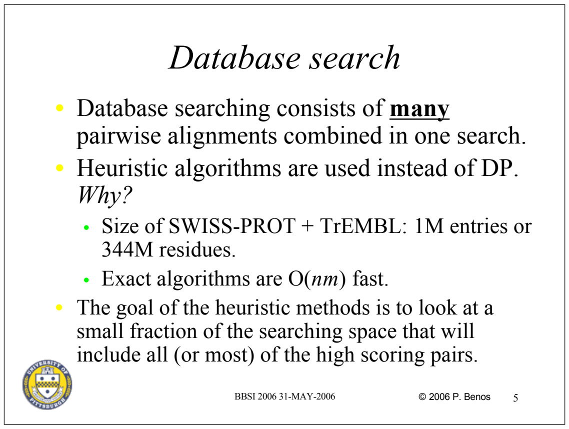


Database Searches



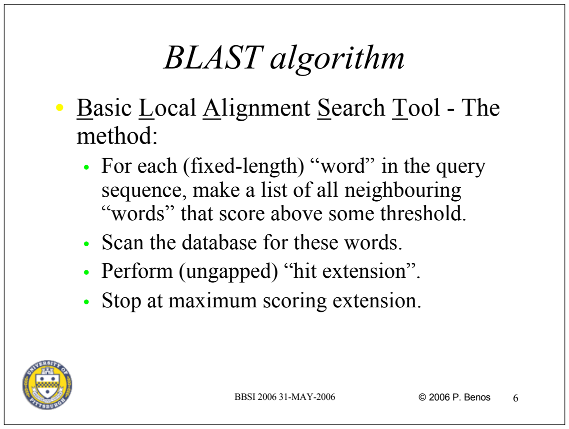
Database search: why?

- Database searching is the first step in characterizing a newly discovered gene.
- It helps determining the function and the evolutionary relationships.
- It answers the question: "*Has anyone seen anything like that before?*"



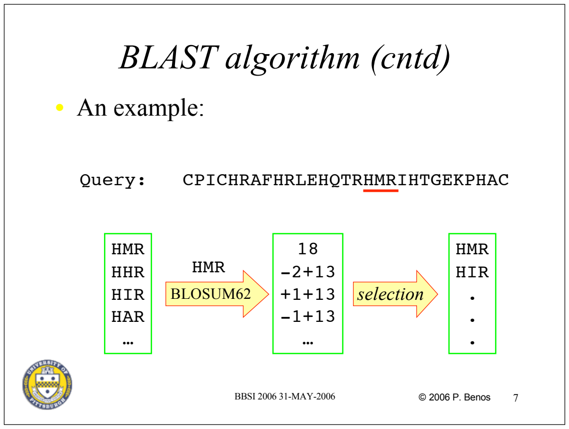
Database search

- Database searching consists of **many** pairwise alignments combined in one search.
- Heuristic algorithms are used instead of DP. *Why?*
  - Size of SWISS-PROT + TrEMBL: 1M entries or 344M residues.
  - Exact algorithms are O(*nm*) fast.
- The goal of the heuristic methods is to look at a small fraction of the searching space that will include all (or most) of the high scoring pairs.



BLAST algorithm

- Basic Local Alignment Search Tool - The method:
  - For each (fixed-length) "word" in the query sequence, make a list of all neighbouring "words" that score above some threshold.
  - Scan the database for these words.
  - Perform (ungapped) "hit extension".
  - Stop at maximum scoring extension.



BLAST algorithm (cntd)

- An example:

```
Query:    CPICHRAFHRLEHQTRHMRIHTGEKPHAC
```

HMR
HHR
HIR
HAR
…

HMR
BLOSUM62

18
-2+13
+1+13
-1+13
…

selection

HMR
HIR
.
.
.

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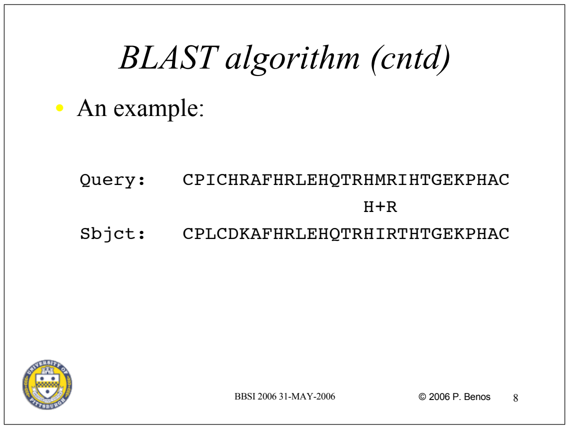

BLAST algorithm (cntd)

- An example:

```
Query:    CPICHRAFHRLEHQTRHMRIHTGEKPHAC
                          H+R
Sbjct:    CPLCDKAFHRLEHQTRHIRTHTGEKPHAC
```

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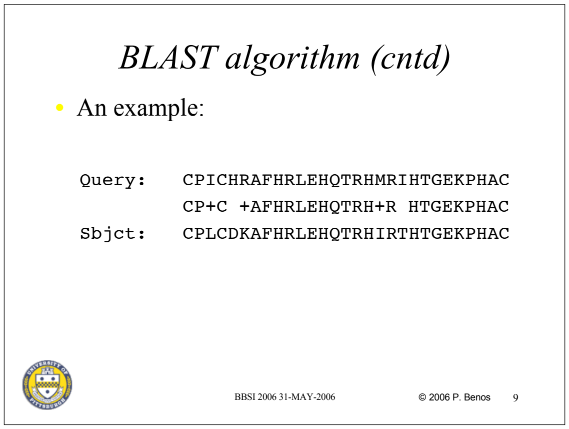

BLAST algorithm (cntd)

- An example:

```
Query:    CPICHRAFHRLEHQTRHMRIHTGEKPHAC
          CP+C +AFHRLEHQTRH+R HTGEKPHAC
Sbjct:    CPLCDKAFHRLEHQTRHIRTHTGEKPHAC
```

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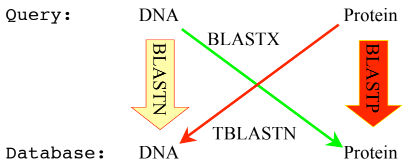
BLAST algorithm (cntd)

- The idea: a high scoring match alignment is very likely to contain a short stretch of very high scoring matches.
- Word length: 3 (proteins) and 11 (DNA).
- HSSP: multiple HSSPs can be reported for each database entry.
- Gapped alignments: more recently, BLAST versions perform gapped alignments.

BLAST flavours



Query: DNA Protein

BLASTX

BLASTN

BLASTP

TBLASTN

Database: DNA Protein

TBLASTX: DNA Query to DNA Database *via* translation

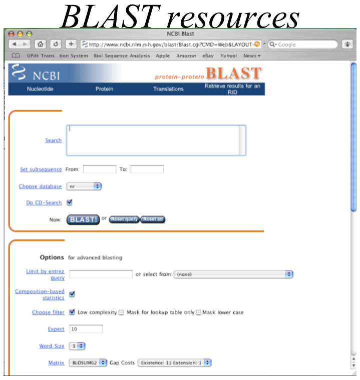
Database searching programs

	Program	Query	Database	Examples of usage
1.	BLASTN	DNA	DNA	identical/closely related genes
2.	BLASTP	Prot	Prot	general use program *(recom.)*
3.	BLASTX	DNA(*)	Prot	find exons in your genomic seq.
4.	TBLASTN	Prot	DNA(*)	find the location/structure of your gene in the genome
5.	TBLASTX	DNA(*)	DNA(*)	*nothing really….*

(*) translated query/database

BLAST tutorial:
http://www.ncbi.nlm.nih.gov/Education/BLASTinfo/information3.html

BLAST resources



Search

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: (none)

Composition-based statistics ☑

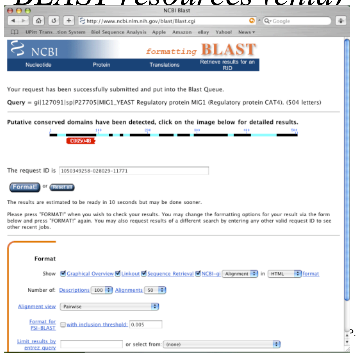
Choose filter ☑ Low complexity □ Mask for lookup table only □ Mask lower case

Expect 10

Word Size 3

Matrix BLOSUM62 Gap Costs Existence: 11 Extension: 1

BLAST resources (cntd)



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

Query = gi|127091|sp|P27705|MIG1_YEAST Regulatory protein MIG1 (Regulatory protein CAT4). (504 letters)

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

The request ID is 1050349258-028029-11771

Format! or Reset all

The results are estimated to be ready in 10 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!" again. You may also request results of a different search by entering any other valid request ID to see other recent jobs.

Format

Show ☑ Graphical Overview ☑ Linkout ☑ Sequence Retrieval ☑ NCBI-gi Alignment in HTML format

Number of: Descriptions 100 Alignments 50

Alignment view Pairwise

Format for PSI-BLAST □ with inclusion threshold: 0.005

Limit results by entrez query or select from: (none)

BLAST output

```
BLASTP 2.2.5 [Nov-16-2002]
Reference :Altschul, Stephen F., Thomas L. Madden,
Alejandro A. Schäffer, Jinghui Zhang, Zheng Zhang, Webb
Miller, and David J. Lipman (1997),
"Gapped BLAST and PSI-BLAST: a new generation of protein
database search
programs",  Nucleic Acids Res. 25:3389-3402.

RID: 1050176602-03205-14137

Query= gi|127091|sp|P27705|MIG1_YEAST Regulatory protein
MIG1 (Regulatory protein CAT4).
         (504 letters)

Database: All non-redundant GenBank CDS
translations+PDB+SwissProt+PIR+PRF
           1,411,415 sequences; 454,141,287 total letters
```

BLAST output (cntd)

```
                                                              Score    E
Sequences producing significant alignments:                   (bits)  Value
gi|6321403|ref|NP_011480.1| Transcription factor involved i... 549   e-15
gi|3437|emb|CAA39084.1| MIG1 [Saccharomyces cerevisiae]        441   e-12
gi|1709031|sp|P52288|MIG1_KLUMA Regulatory protein MIG1 >gi... 106   1e-2
gi|1709030|sp|P50898|MIG1_KLULA Regulatory protein MIG1 >gi... 104   3e-2
gi|416840|sp|Q01981|CREA_EMENI DNA-binding protein creA (Ca... 99   1e-19
gi|101802|pir||A41694 regulatory protein creA - Emericella ... 99   1e-19
gi|544095|sp|Q05620|CREA_ASPNG DNA-binding protein creA (Ca... 99   2e-19
gi|2293072|emb|CAA04425.1| carbon catabolite repressor CRES... 99   2e-19
gi|12229763|sp|Q9P889|CREA_ASPOR DNA-binding protein creA (... 99   2e-19
gi|12229746|sp|O94130|CREA_BOTCI DNA-binding protein creA (... 99   2e-19
```

BLAST output (cntd)

```
>gi|3437|emb|CAA39084.1| MIG1 [Saccharomyces cerevisiae]
Length = 386 Score =  441 bits (1133), Expect = e-122 Identities = 262/380
(68%), Positives = 262/380 (68)%

Query: 1   MQSPYPMTQVSNVDDGXXXXXXXXXXXXXXXXXXPRPHACPICHRAFHRLEHQTRHMRIH 60
            MQSPYPMTQVSNVDDG                  PRPHACPICHRAFHRLEHQTRHMRIH
Sbjct: 1   MQSPYPMTQVSNVDDGSLLKESKSKSKVAAKSEAPRPHACPICHRAFHRLEHQTRHMRIH 60

Query: 61  TGEKPHACDFPGCVKRFSRSDELTRHRRIHTNSHPXXXXXXXXXXXXXXXXXXXXXXXXX 120
            TGEKPHACDFPGCVKRFSRSDELTRHRRIHTNSHP
Sbjct: 61  TGEKPHACDFPGCVKRFSRSDELTRHRRIHTNSHPRGKRGRKKKVVGSPINSASSSATSI 120

Query: 121 XDLNTANFXXXXXXXXXXXXXXXAIAPKENXXXXXXXXXXXXXFEIGESGGNDPYMVSSP 180
             DLNTANF               AIAPKEN             FEIGESGGNDPYMVSSP
Sbjct: 121 PDLNTANFSPPLPQQHLSPLIPIAIAPKENSSRSSTRKGRKTKFEIGESGGNDPYMVSSP 180
```

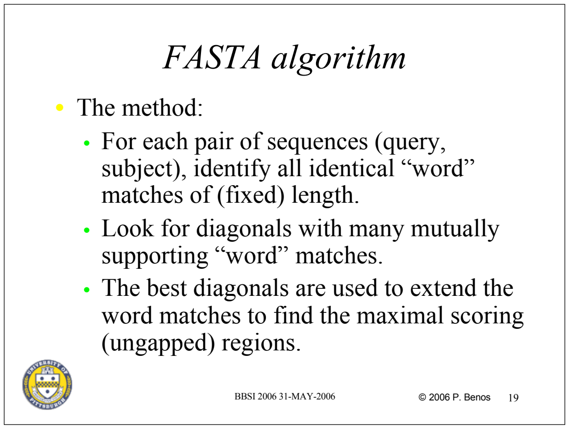
```
>gi|8926704|emb|CAB96530.1| MIG repressor [Pichia jadinii]
Length = 345 Score = 85.9 bits (211), Expect = 2e-15 Identities = 42/58
(72%), Positives = 49/58 (84%), Gaps = 2/58 (3%)

Query: 36 RPHACPICHRAFHRLEHQTRHMRIHTGEKPHACDFPGCVKRFSRSDELTRHRRIHTNS 93
           RP+ C +C++AFHRLEHQTRHMRIHTGEKP  C F  C K+FSRSDELTRH RIH+N+
Sbjct: 17 RPYVCTVCNKAFHRLEHQTRHMRIHTGEKPFQCTF--CSKKFSRSDELTRHTRIHSNT 72
```

Other database searching programs

- MEGABLAST.

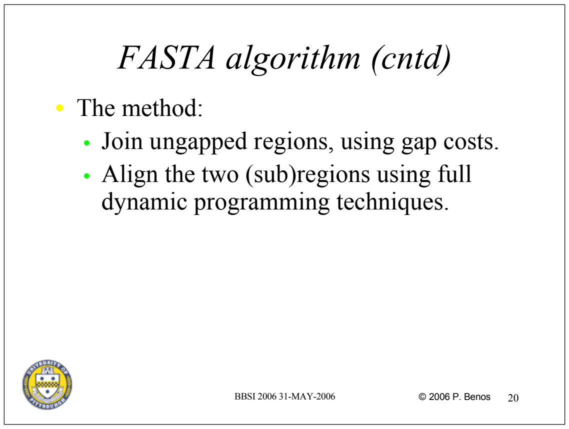
  It can be used for comparing two large sets of sequences with each other.
- PSI-BLAST (*Position-Specific Iterated*).

  It performs iterative searches; the sequences found in one searching round are used to build models for searching in the next round. To be used when seeking increased sensitivity.



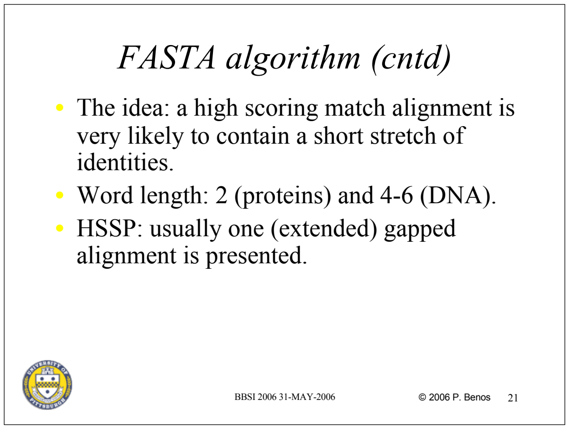
FASTA algorithm

- The method:
  - For each pair of sequences (query, subject), identify all identical “word” matches of (fixed) length.
  - Look for diagonals with many mutually supporting “word” matches.
  - The best diagonals are used to extend the word matches to find the maximal scoring (ungapped) regions.



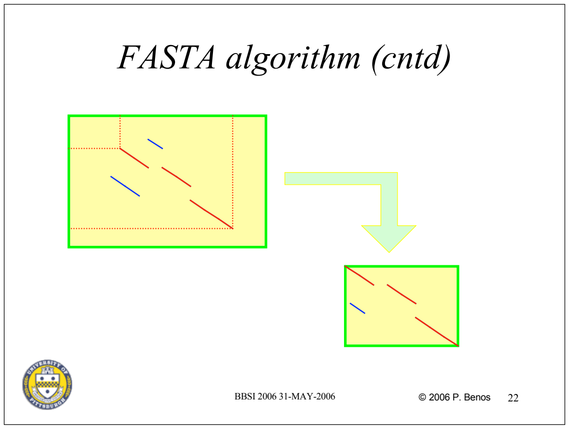
FASTA algorithm (cntd)

- The method:
  - Join ungapped regions, using gap costs.
  - Align the two (sub)regions using full dynamic programming techniques.

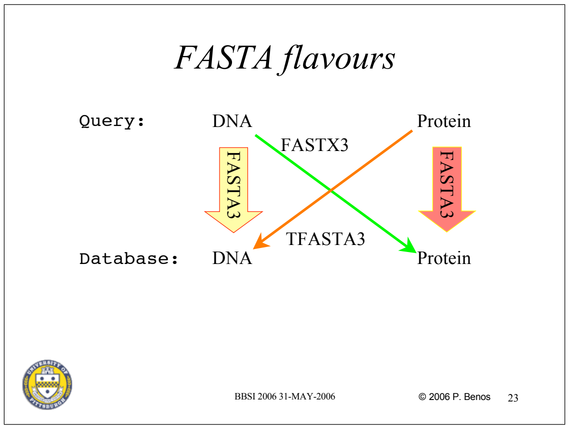


FASTA algorithm (cntd)

- The idea: a high scoring match alignment is very likely to contain a short stretch of identities.
- Word length: 2 (proteins) and 4-6 (DNA).
- HSSP: usually one (extended) gapped alignment is presented.



FASTA algorithm (cntd)



FASTA flavours

Query: DNA Protein

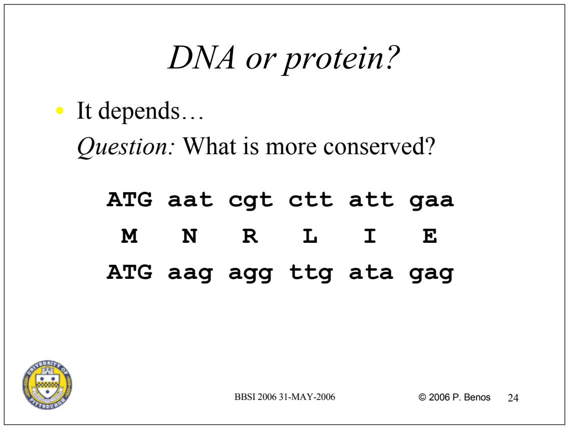
FASTX3

FASTA3

FASTA3

TFASTA3

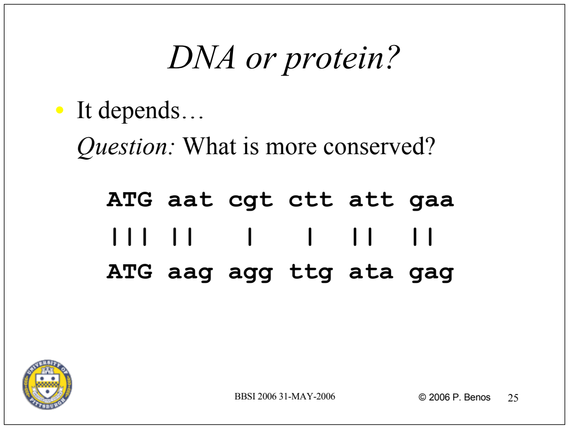
Database: DNA Protein



DNA or protein?

- It depends…
  Question: What is more conserved?

```
ATG aat cgt ctt att gaa
 M   N   R   L   I   E
ATG aag agg ttg ata gag
```

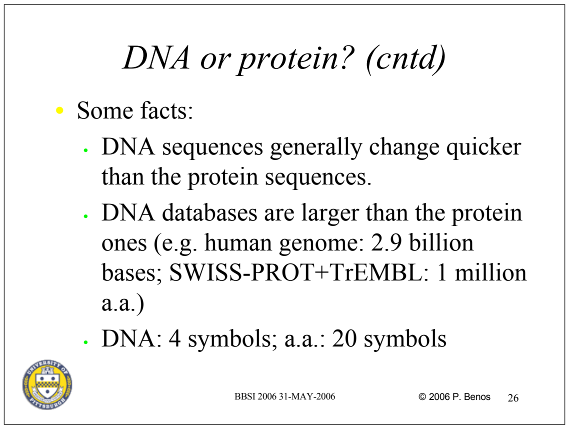


DNA or protein?

- It depends…

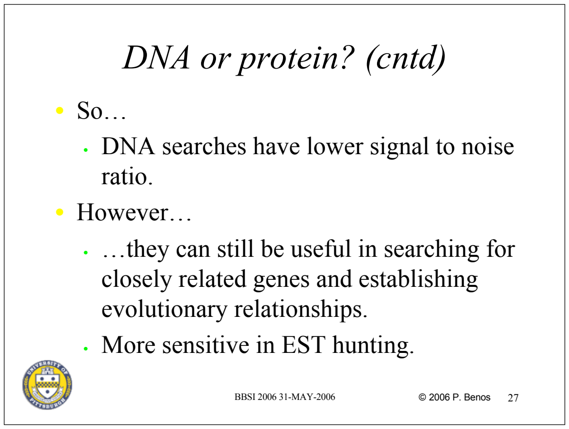
  Question: What is more conserved?

```
ATG aat cgt ctt att gaa
||| ||   |   |  ||  ||
ATG aag agg ttg ata gag
```



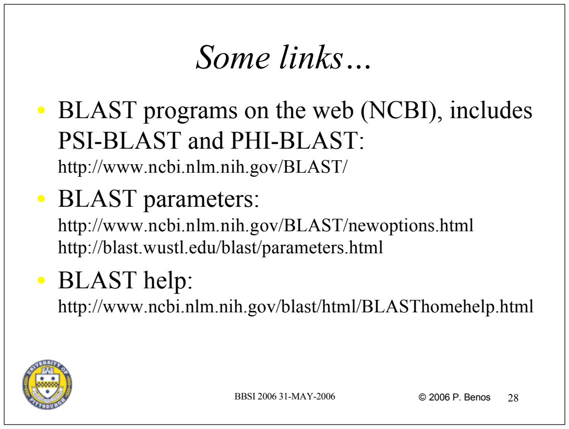
DNA or protein? (cntd)

- Some facts:
  - DNA sequences generally change quicker than the protein sequences.
  - DNA databases are larger than the protein ones (e.g. human genome: 2.9 billion bases; SWISS-PROT+TrEMBL: 1 million a.a.)
  - DNA: 4 symbols; a.a.: 20 symbols



DNA or protein? (cntd)

- So…
  - DNA searches have lower signal to noise ratio.
- However…
  - …they can still be useful in searching for closely related genes and establishing evolutionary relationships.
  - More sensitive in EST hunting.



Some links…

- BLAST programs on the web (NCBI), includes PSI-BLAST and PHI-BLAST:
  http://www.ncbi.nlm.nih.gov/BLAST/
- BLAST parameters:
  http://www.ncbi.nlm.nih.gov/BLAST/newoptions.html
  http://blast.wustl.edu/blast/parameters.html
- BLAST help:
  http://www.ncbi.nlm.nih.gov/blast/html/BLASThomehelp.html

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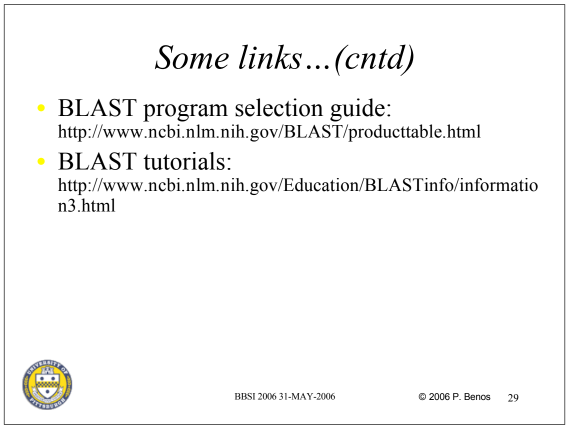

Some links…(cntd)

- BLAST program selection guide:
  http://www.ncbi.nlm.nih.gov/BLAST/producttable.html
- BLAST tutorials:
  http://www.ncbi.nlm.nih.gov/Education/BLASTinfo/information3.html

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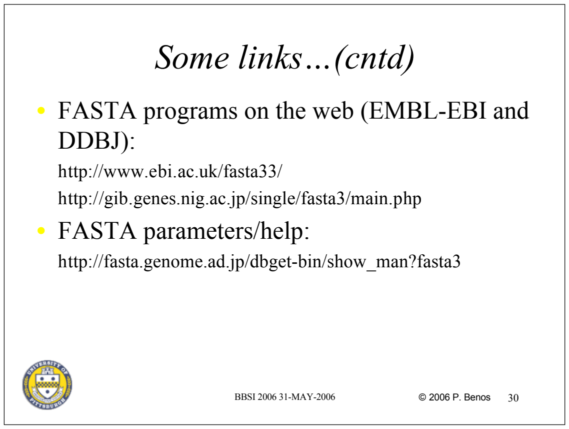

Some links…(cntd)

- FASTA programs on the web (EMBL-EBI and DDBJ):
  http://www.ebi.ac.uk/fasta33/
  http://gib.genes.nig.ac.jp/single/fasta3/main.php
- FASTA parameters/help:
  http://fasta.genome.ad.jp/dbget-bin/show_man?fasta3

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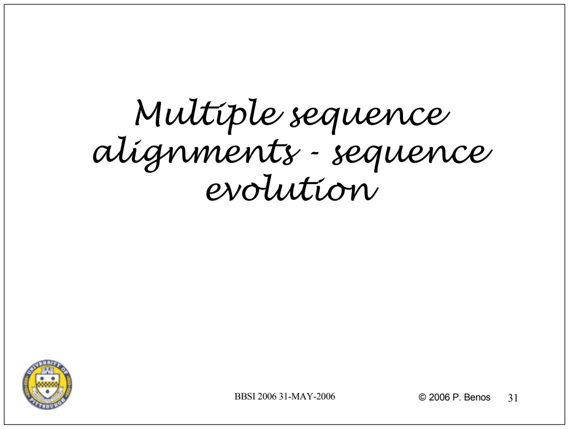
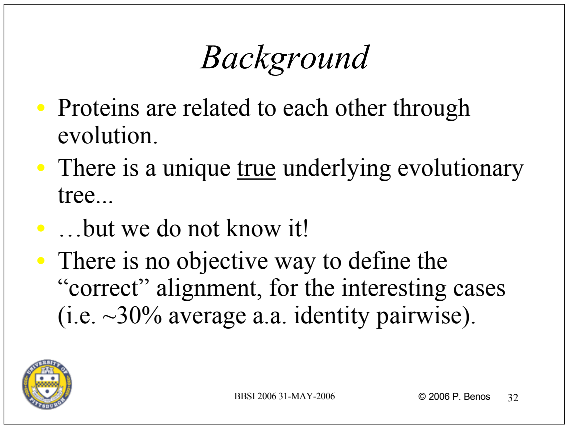
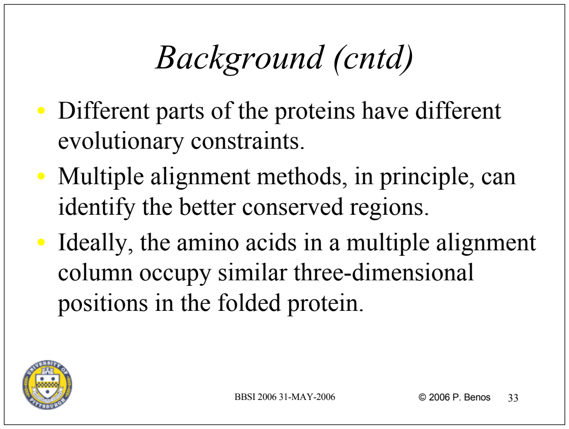


Multiple sequence alignments - sequence evolution



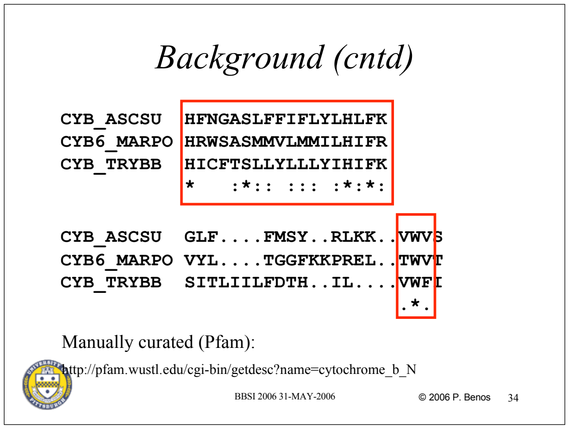
Background

- Proteins are related to each other through evolution.
- There is a unique true underlying evolutionary tree...
- …but we do not know it!
- There is no objective way to define the “correct” alignment, for the interesting cases (i.e. ~30% average a.a. identity pairwise).



Background (cntd)

- Different parts of the proteins have different evolutionary constraints.
- Multiple alignment methods, in principle, can identify the better conserved regions.
- Ideally, the amino acids in a multiple alignment column occupy similar three-dimensional positions in the folded protein.



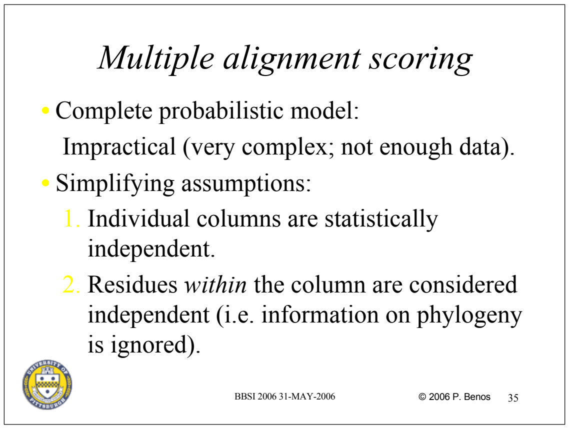
Background (cntd)

```
CYB_ASCSU   HFNGASLFFIFLYLHLFK
CYB6_MARPO  HRWSASMMVLMMILHIFR
CYB_TRYBB   HICFTSLLYLLLYIHIFK
            *   :*:: ::: :*:*:

CYB_ASCSU   GLF....FMSY..RLKK..VWVS
CYB6_MARPO  VYL....TGGFKKPREL..TWVT
CYB_TRYBB   SITLIILFDTH..IL....VWFI
                               .*.
```

Manually curated (Pfam):

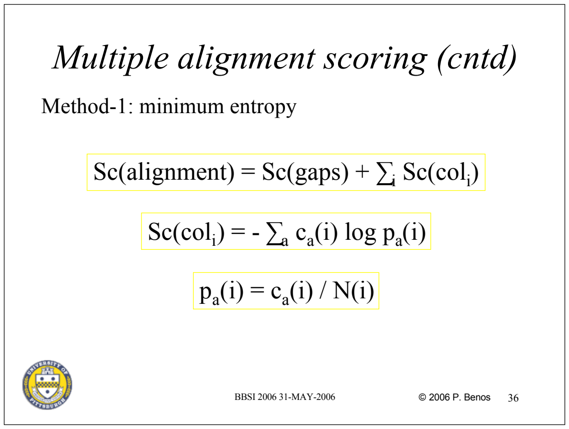
http://pfam.wustl.edu/cgi-bin/getdesc?name=cytochrome_b_N



Multiple alignment scoring

- Complete probabilistic model:

  Impractical (very complex; not enough data).
- Simplifying assumptions:
  1. Individual columns are statistically independent.
  2. Residues *within* the column are considered independent (i.e. information on phylogeny is ignored).



Multiple alignment scoring (cntd)

Method-1: minimum entropy

$$\boxed{\mathrm{Sc}(\mathrm{alignment}) = \mathrm{Sc}(\mathrm{gaps}) + \sum_i \mathrm{Sc}(\mathrm{col}_i)}$$

$$\boxed{\mathrm{Sc}(\mathrm{col}_i) = - \sum_a c_a(i) \log p_a(i)}$$

$$\boxed{p_a(i) = c_a(i) / N(i)}$$

Multiple alignment scoring(cntd)

Method-2: sum of pairs (SP)

$$SP(i) = Sc(col_i) = \sum_l \sum_{k<l} Sc_i(k,l)$$

BLOSUM

Problem: Compare SP scores

- N sequences with *Arg* at position *i*.
- N-1 sequences with *Arg* and one with *Lys*.

Multiple alignment scoring(cntd)

N sequences with *Arg* at position *i*.

- BLOSUM62: Sc(*Arg*, *Arg*) = 5
- SP1 = 5 x N (N - 1) / 2

N-1 sequences with *Arg,* one with *Lys.*

- BLOSUM62: Sc(*Arg*, *Lys*) = 2
- SP2 = SP1 - 3 x (N - 1)
- (SP1-SP2)/SP1 = 6/5N !!

Methods

- A naïve approach:

  Use dynamic programming to calculate all possible alignments of the *N* sequences of length *L* and choose the best.
- Problem:
  - Memory complexity $O(L^N)$, time complexity $O(2^N L^N)$.

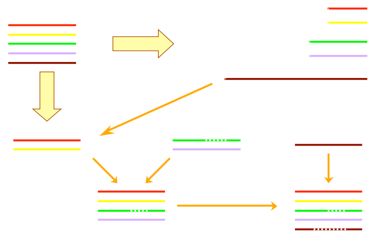
Methods (cntd)

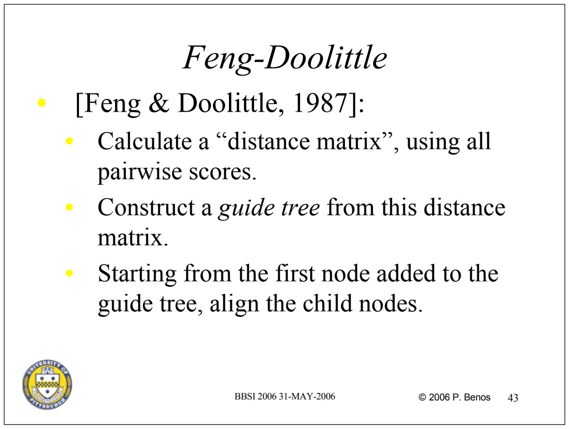
- Example:
  - Aligning N sequences requires $(2L)^{N\text{-}2}$ pairwise comparisons.
  - You have 15 sequences, 50 a.a. long.
  - Your computer needs 1 sec for each pairwise comparison.
  - How many sequences you'll align until the end of our sun? (i.e. approx. 5 billion years)

Progressive algorithms

- General idea:
  - Calculate all pairwise alignments.
  - Cluster the sequences according to some scoring scheme.
  - Align the two closest sequences; fix their alignment.
  - Continue with next sequence and/or alignment, until all sequences are aligned.

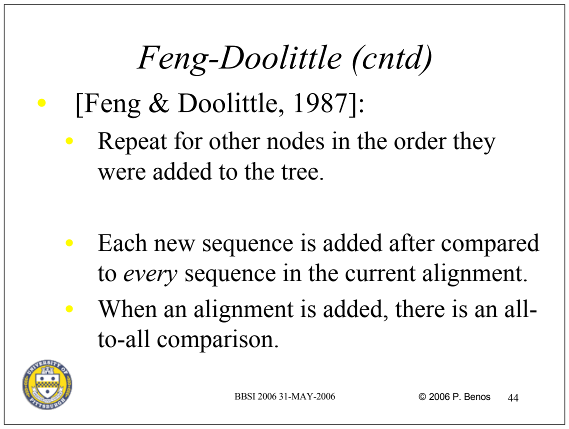
Progressive algorithms (cntd)





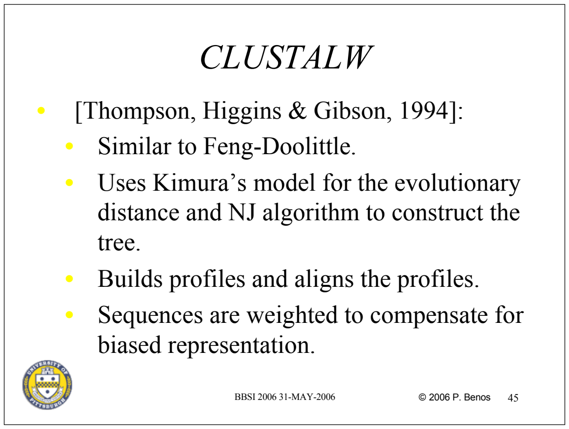
Feng-Doolittle

- [Feng & Doolittle, 1987]:
  - Calculate a “distance matrix”, using all pairwise scores.
  - Construct a *guide tree* from this distance matrix.
  - Starting from the first node added to the guide tree, align the child nodes.



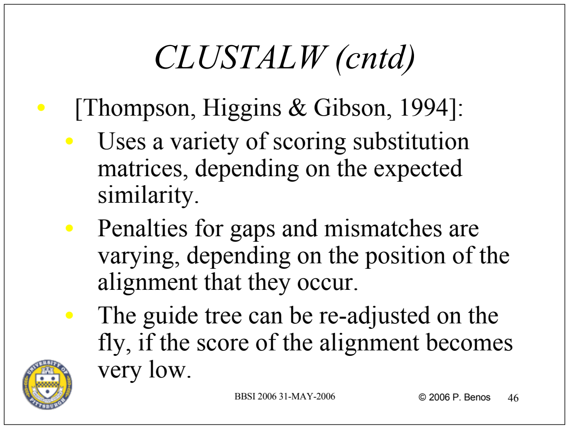
Feng-Doolittle (cntd)

- [Feng & Doolittle, 1987]:
  - Repeat for other nodes in the order they were added to the tree.
  - Each new sequence is added after compared to *every* sequence in the current alignment.
  - When an alignment is added, there is an all-to-all comparison.



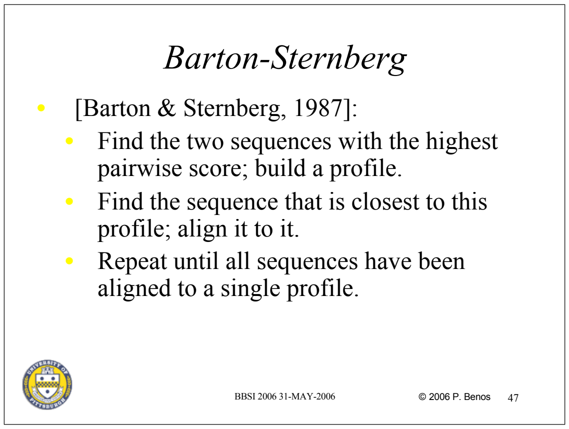
CLUSTALW

- [Thompson, Higgins & Gibson, 1994]:
  - Similar to Feng-Doolittle.
  - Uses Kimura’s model for the evolutionary distance and NJ algorithm to construct the tree.
  - Builds profiles and aligns the profiles.
  - Sequences are weighted to compensate for biased representation.



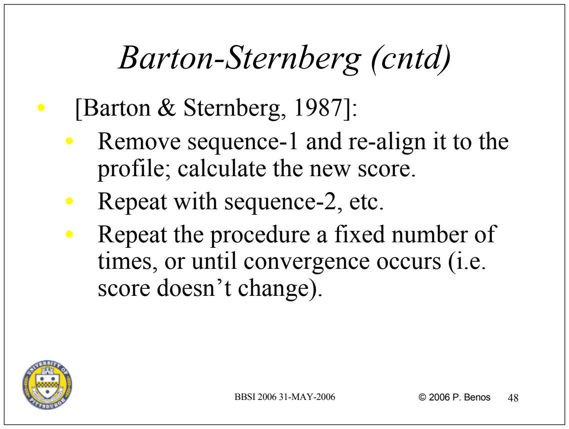
CLUSTALW (cntd)

- [Thompson, Higgins & Gibson, 1994]:
  - Uses a variety of scoring substitution matrices, depending on the expected similarity.
  - Penalties for gaps and mismatches are varying, depending on the position of the alignment that they occur.
  - The guide tree can be re-adjusted on the fly, if the score of the alignment becomes very low.



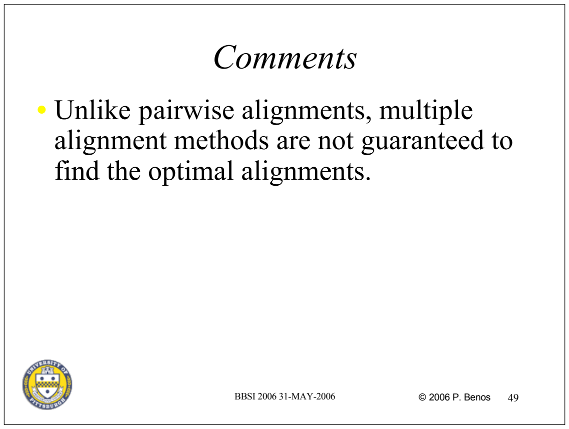
Barton-Sternberg

- [Barton & Sternberg, 1987]:
  - Find the two sequences with the highest pairwise score; build a profile.
  - Find the sequence that is closest to this profile; align it to it.
  - Repeat until all sequences have been aligned to a single profile.



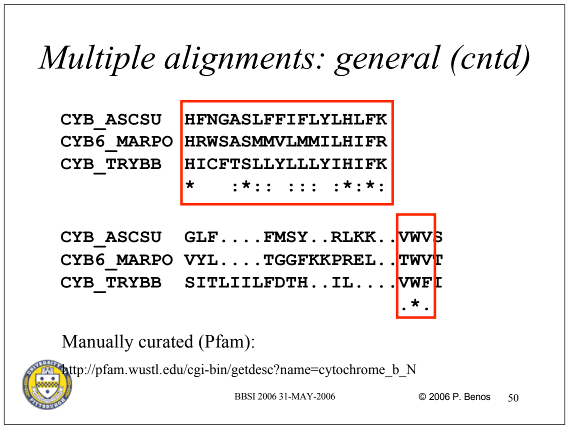
Barton-Sternberg (cntd)

- [Barton & Sternberg, 1987]:
  - Remove sequence-1 and re-align it to the profile; calculate the new score.
  - Repeat with sequence-2, etc.
  - Repeat the procedure a fixed number of times, or until convergence occurs (i.e. score doesn’t change).



Comments

- Unlike pairwise alignments, multiple alignment methods are not guaranteed to find the optimal alignments.



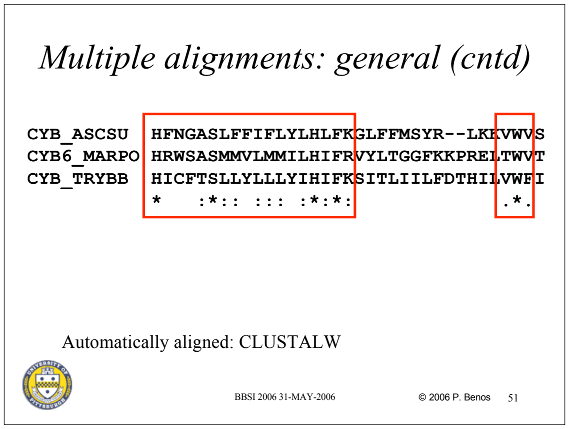
Multiple alignments: general (cntd)

```
CYB_ASCSU   HFNGASLFFIFLYLHLFK
CYB6_MARPO  HRWSASMMVLMMILHIFR
CYB_TRYBB   HICFTSLLYLLLYIHIFK
            *   :*:: ::: :*:*:

CYB_ASCSU   GLF....FMSY..RLKK..VWVS
CYB6_MARPO  VYL....TGGFKKPREL..TWVT
CYB_TRYBB   SITLIILFDTH..IL....VWFI
                               .*.
```

Manually curated (Pfam):

http://pfam.wustl.edu/cgi-bin/getdesc?name=cytochrome_b_N



Multiple alignments: general (cntd)

```
CYB_ASCSU   HFNGASLFFIFLYLHLFKGLFFMSYR--LKKVWVS
CYB6_MARPO  HRWSASMMVLMMILHIFRVYLTGGFKKPRELTWVT
CYB_TRYBB   HICFTSLLYLLLYIHIFKSITLIILFDTHILVWFI
            *   :*:: ::: :*:*:             .*.
```

Automatically aligned: CLUSTALW

Comments

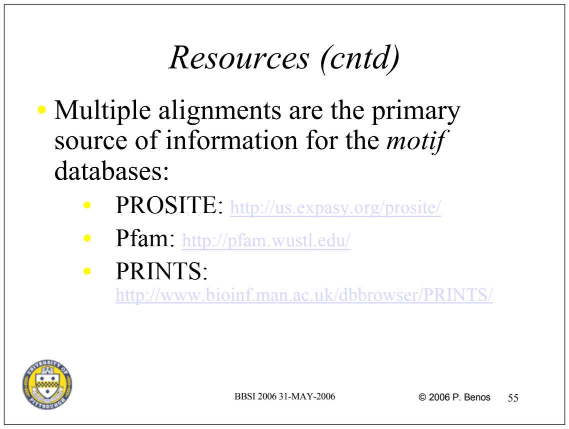
- Unlike pairwise alignments, multiple alignment methods are not guaranteed to find the optimal alignments.
- Multiple alignments are used to calculate profiles characteristic for protein families.
- These profiles can be used to identify new (distant) members of these families.

Comments (cntd)

- E.g., if your BLAST searches yield many poor(ish) results, profile searches might hint the function of your newly sequenced gene.
- Also, you can align all the top hits of your BLAST search, to create a profile and check if your sequence belongs to this profile.

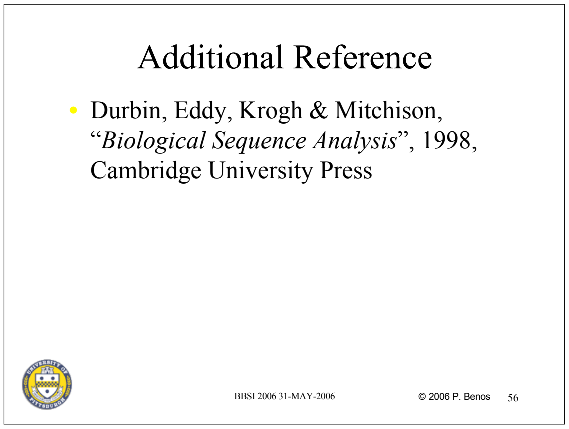
Resources

- CLUSTALW servers:
  - EBI: http://www.ebi.ac.uk/clustalw/
  - Baylor College of Medicine: http://searchlauncher.bcm.tmc.edu/multi-align/multi-align.html



Resources (cntd)

- Multiple alignments are the primary source of information for the *motif* databases:
  - PROSITE: http://us.expasy.org/prosite/
  - Pfam: http://pfam.wustl.edu/
  - PRINTS: http://www.bioinf.man.ac.uk/dbbrowser/PRINTS/



Additional Reference

- Durbin, Eddy, Krogh & Mitchison, "*Biological Sequence Analysis*", 1998, Cambridge University Press