BIOC4004 - Industrial Biochemistry
Lecture 13 - Mon Feb 22, 04

Topics for the Day:

- Searching molecular databases
- BLAST
  - how it works
  - how to use
  - how to interpret
- Bioinformatics resources

Searching of Molecular Databases

- Why ?

  To look for similarities between a sequence of interest and and the sequences in the database

  - could help elucidate the function of an unknown sequence
  - could help find conserved motifs shared by different sequences

- How ?

  Need to compare the sequence to every sequence in the DB

  - need to align the query sequence to every sequence in the DB
  - need to calculate some metric to assess the quality of the match
  - need to report the "good" matches

- Early sequence alignment programs were meant to perform the best possible alignment of a pair of, at most, a few (dozen) sequences
  - good results but computationally expensive
  - no way could you use these to screen a DB of thousands of sequences

- Design of "heuristic" methods to perform the searches:
  - an initial quick and dirty alignment between query and all the sequences
  - assessment of potentially matching sequences worth "revisiting"
  - a second, more accurate, alignment on the worthwhile sequences

- These heuristic methods are computationally economical:
  - fast and good
  - can handle lots of queries !!!
  - Provide a statistical assessment of the match (more on this later)

- The program used for searching the GenBank DB is BLAST (Basic Local Alignment Tool)

  [Some of the European DBs use a program called FASTA, however, GenBank cross-references entries in the European DBs anyway, so we'll mostly talk about GB]

BLAST (Basic Local Alignment Tool)

- find it at http://www.ncbi.nlm.nih.gov/BLAST/
  also mirrored at the Canadian Bioinformatics Resource
  http://www.cbr.ca/blast/
- uses an approach based on:
  - matching short sequence fragments (HSPs: High Scoring Segment Pairs)
    - HSPs can be perfect matches or highly homologous
  - looks for clusters of HSPs that are close to each other
  - a "cut-off alignment score" is calculated to determine the minimum score that should be met by an alignment in order to be significant
  - the best local alignments between the query sequence and the database sequence(s) are calculated and reported

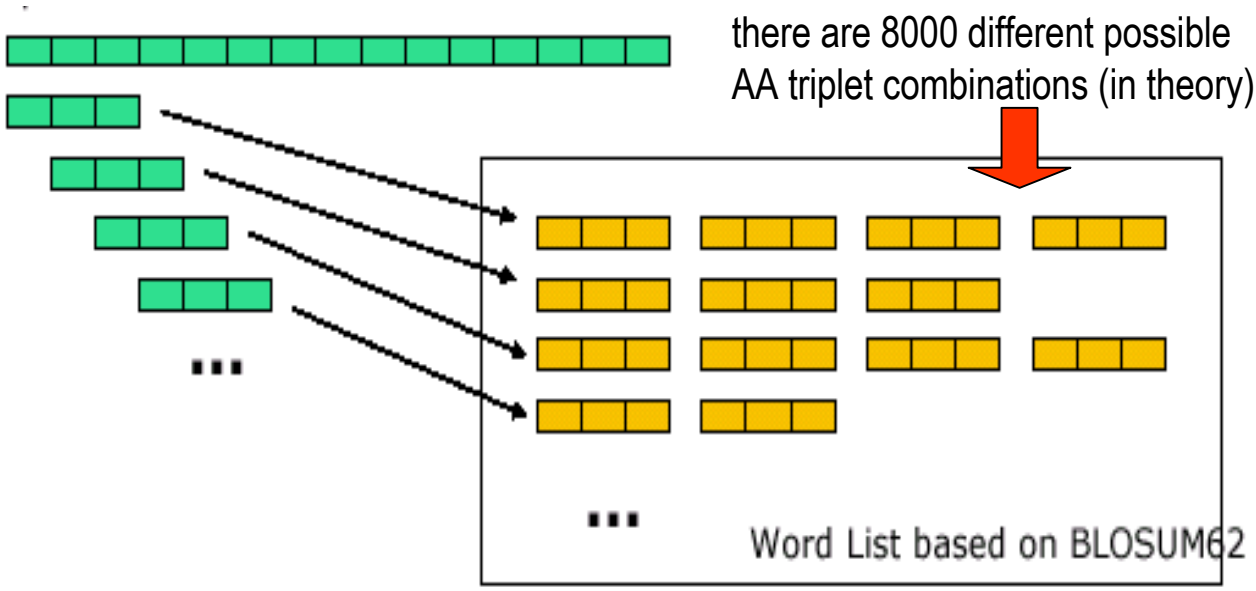
BLAST VARIANTS

PROGRAM	QUERY	DB	COMMENTS
BLASTP	protein	protein	compares amino acid query against protein sequences
BLASTN	DNA	DNA	compares nucleotide query against DNA sequences
BLASTX	DNA	protein	compares 6X translations of nucleotide query against protein sequences
TBLASTN	protein	DNA	compares protein query against 6X translations of DNA sequences
TBLASTX	DNA	DNA	compares 6X translations of nucleotide query against 6X translations of DNA sequences

- also :
  - PSI-BLAST: protein query and protein DB - different statistics used to detect weak similarities
  - PHI-BLAST: protein query and protein DB - used to look for protein patterns (small regions of conservation)
  - MEGA-BLAST: larges sets of long DNA sequences
  - CD Search - conserved domain detection
  - BLAST2: pairwise alignment of two sequences
  - Genome BLAST : alignment of DNA sequences to genome data
  - VecScreen: used to detect vector sequence within sequence data

How does BLAST work ? (part I)

- A list of words of length 3 in the query protein sequence is made.
- Using BLOSUM62, the query words are evaluated for an exact match with a word of any database sequence.(cf. 20 x 20 x 20 = 8000)



• each triplet is searched against all of the triplets generated from the database
• use scoring matrix to get a score for each potential match

How does BLAST work ? (part II)

Let's look at one example:

Query: TPQGQRQGQ....

Query Wordlist

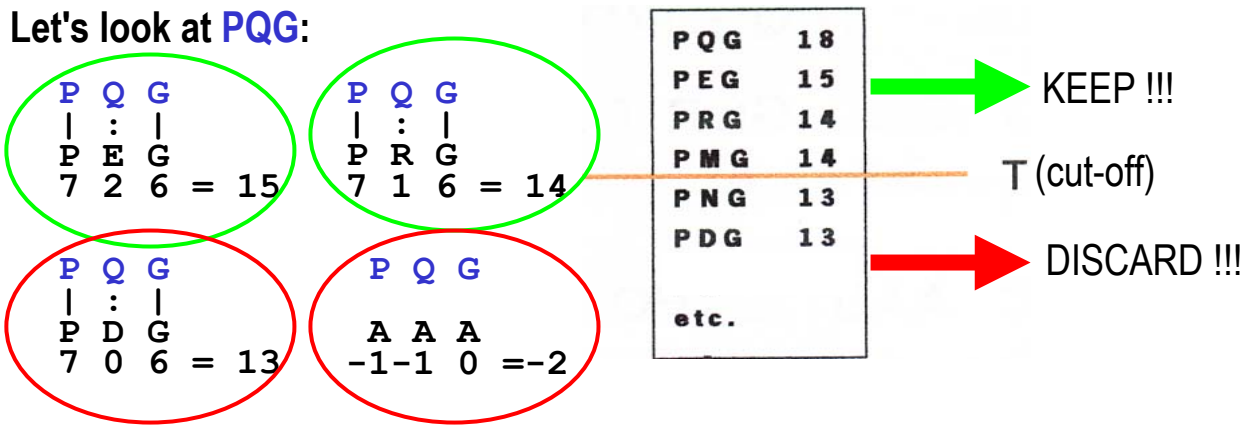
```
TPQ PQG QGQ
GQR QRQ RQG
QGQ etc etc
...
```

Dbase Wordlist

```
AAA AAC AAD ... PQG QGQ YYY
AGA AGC AAN ... PEG QGM ...
AAG CAC AAE ... PRG MGQ ...
GAA ACC AAQ ... PMG QAQ ...
```

- A cutoff score called neighborhood word score threshold(T) is used to pull out low-score matches.

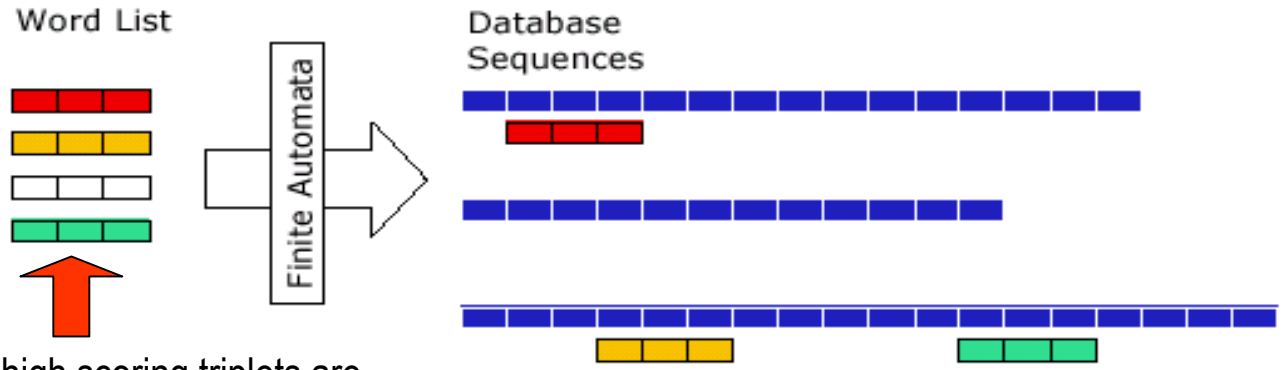
Let's look at PQG:



1. Select all Dbase triplets that score higher than cut-off (using BLOSUM62 score)
2. Discard low scoring Dbase triplets (ie.lower than cut-off)
3. Most triplets will be discarded:
   - less data to sift through in subsequent (computationally intensive) steps

How does BLAST work ? (part III)

- You now have a "restricted data-set" of all the high scoring triplets
- You scan every sequence in the database for an exact match to each of the high-scoring triplets (ie. do this with tens or hundreds of triplets, not 8000 !!!)
- Wherever there is a perfect match ⇒ SEED



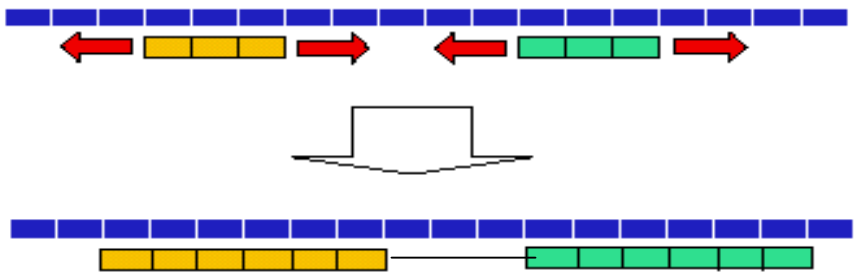
only high scoring triplets are run against the Dbase !

- Each SEED is extended in either direction to get high-scoring segment pair (HSP)

```
Query:  325 LNKCKTPQGQRQGQQWIKQPLMDKN 350
            L    TPQGQR++++W+  P+ D
Sbjct:  290 LDCTVTPQGQREAERWLHMPVRDTR 315
```

An HSP is obtained when the score can neither be improved by extending or trimming

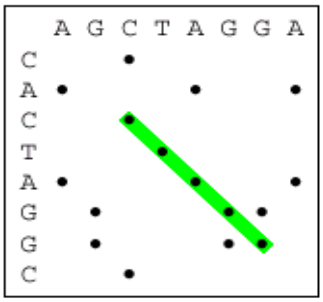
- HSPs that are in close physical proximity are merged into one long HSP
  - ie. a gapped alignment !

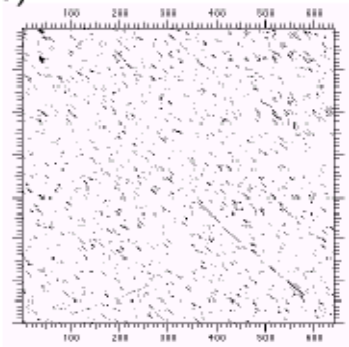


How does BLAST work ? (part IV)

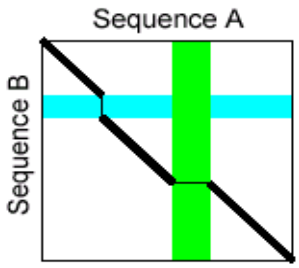
Dot Matrix Analysis

- A.J. Gibbs and G.A. McIntyre (1970)

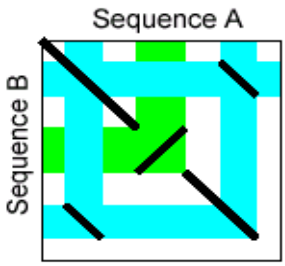




- Insertions or deletions

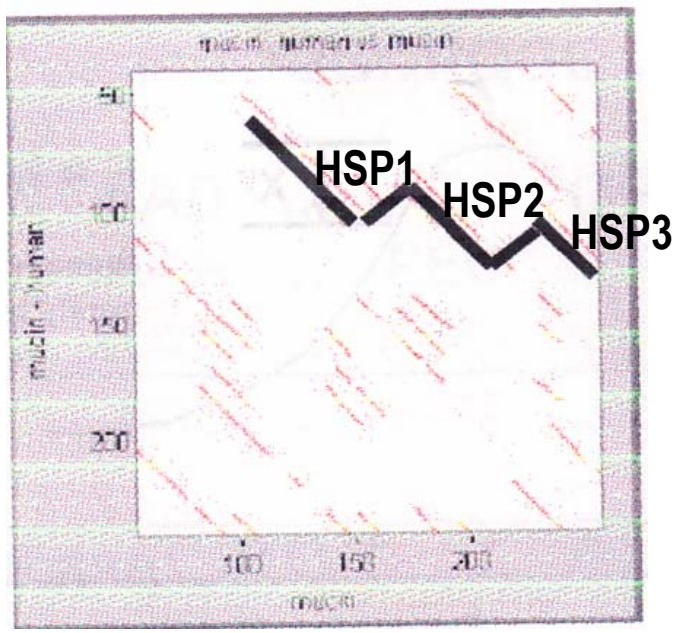


- Direct repeat and inverted region



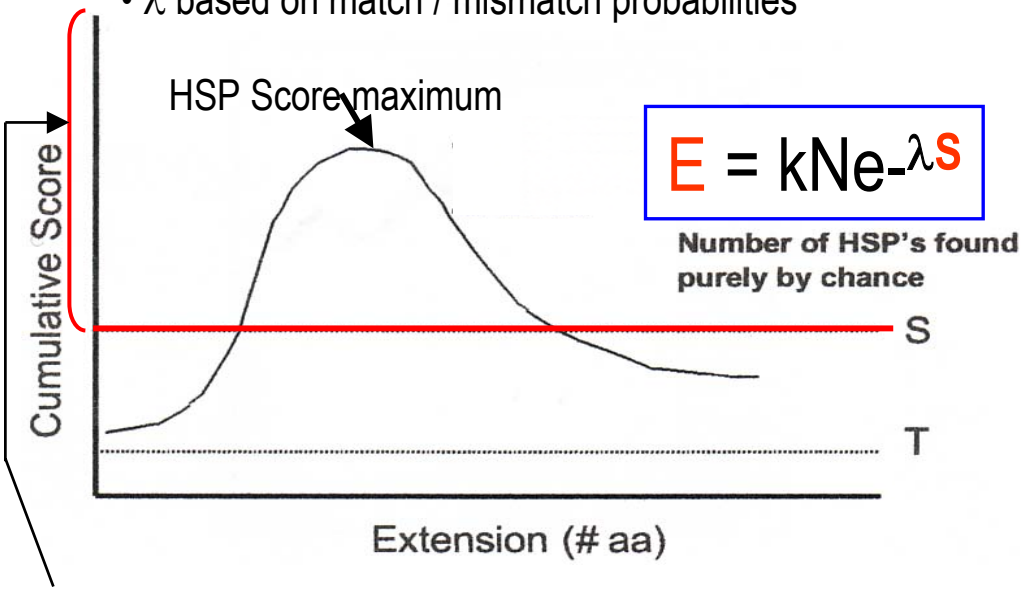
- HSPs in the same diagonals(ungapped) or in near diagonals(gapped) are merged.

  - **close physical proximity**
  - **gapped or ungapped**



How does BLAST work ? (part V)

- **E-value (or Expect value):**
  - Expected # of HSP alignments with a **Score > S** by chance alone
  - S is computed for each query / dbase based on the other parameters
    - BLAST uses a default E = 10
    - k is based on the word length (default = 3)
    - N based on the query sequence length and the dbase size
    - λ based on match / mismatch probabilities



- All HSPs with score greater than cut-off score(S) are identified and returned as hits
- All others discarded

How do we distinguish between good HSPs and poor HSPs ?

- For every HSP an E-value statistic is calculated based on the HSPs score

$$P = 1 - e^{(-E)}$$

When E< 0.01, P~E (ie. E approximates the probability of match occurring by chance alone as E gets smaller)

- **So the E value is a good measure of the significance of the alignment**
  - the lower the E-value(closer to 0), the more likely that the match is real
  - the higher the E-value (closer to 1), the more chance the match is bogus

Running a BLAST search at NCBI

- **Paste in sequence (FASTA format or type in GI or accession number)**

```
>Mysequence MT0895
KIQIYGTGCANCQMLEKNAREAVKELGIDAE
FEKIKEMDQILEAGLTALPGLAVDGELKIDS
```

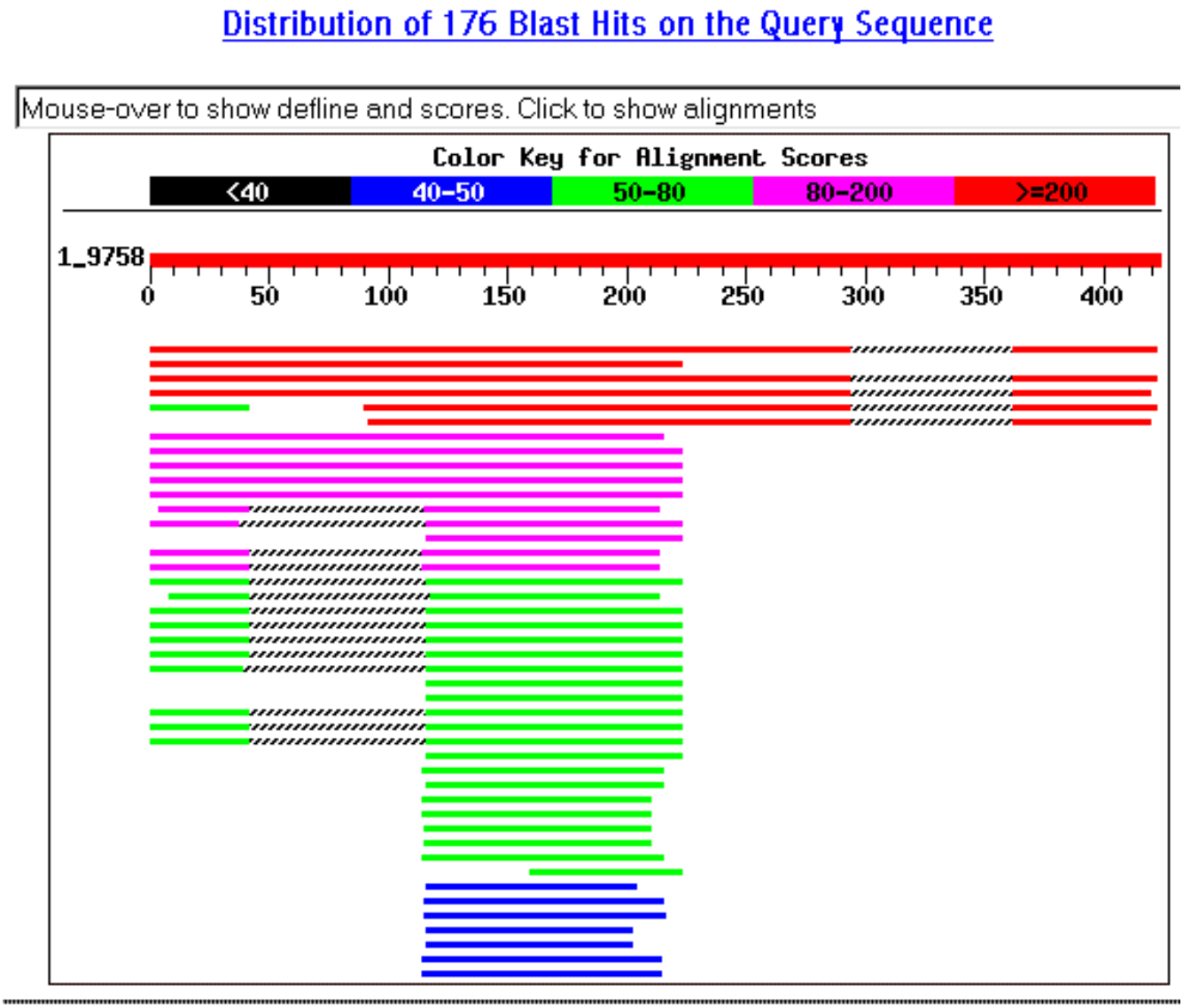
OR

```
>
KIQIYGTGCANCQMLEKNAREAVKELGIDAE
FEKIKEMDQILEAGLTALPGLAVDGELKIDS
```

- Choose a range of interest in the sequence "set subsequences" (not usually used)
- Select the database from pull-down menu (usually choose nr = non-redundant)
- Keep CD Search "check box" on ⟶ Conserved Domain
- Leave "Options" unchanged (use defaults)
- Go to "Format" menu and adjust Number of descriptions and alignments as desired

C'est tout !!!

An Example of a Typical BLAST search (part I)



- The first thing you get is the distribution of Blast hits on the query
  - evaluate if the match occurs along the whole length of the query

    vs
  - matches on restricted are of query
- How good is the match ? The higher the score !!!!

An Example of a Typical BLAST search (part II)

Probability of match appearing by chance

```
Query= Pbpp58b (423 letters)

Database: nr (493,611 sequences; 154,780,071 total letters)

                                                                    Score     E
Sequences producing significant alignments:                         (bits)  Value

sp|Q08168|HRP_PLABE 58 KD PHOSPHOPROTEIN (HEAT SHOCK-RELATED PRO...   334  1e-90
gb|AAC37300.1| (L21710) 58 kDa phosphoprotein [Plasmodium berghei]    329  3e-89
pir||T10455 heat shock related protein - Plasmodium berghei >gi|...   250  2e-65
sp|P50503|HIP_RAT HSC70-INTERACTING PROTEIN >gi|4379408|emb|CAA5...   106  5e-22
sp|P50502|HIP_HUMAN HSC70-INTERACTING PROTEIN (PROGESTERONE RECE...    87  3e-16
gb|AAF45894.1| (AE003429) CG2947 gene product [Drosophila melano...    87  4e-16
pir||T24865 hypothetical protein T12D8.8 - Caenorhabditis elegan...    86  5e-16
pir||T04562 hypothetical protein T12H17.60 - Arabidopsis thalian...    81  2e-14
.
.
.
.
gb|AAC60555.2| (S59774) STI1 stress-inducible protein homolog [S...    48  2e-04
gb|AAD33401.1|AF129086_1 (AF129086) carboxy terminus of Hsp70-in...    48  2e-04
pir||A56534 P58 protein - bovine >gi|468012|gb|AAA17795.1| (U046...    48  2e-04
gb|AAB49720.1| (U89984) transformation-sensitive protein homolog...    47  4e-04
pir||T16689 hypothetical protein R05F9.10 - Caenorhabditis elega...    47  4e-04
gb|AAD33400.1|AF129085_1 (AF129085) carboxy terminus of Hsp70-in...    46  6e-04
.
.
.
.
.
emb|CAA61595.1| (X89416) protein phosphatase 5 [Homo sapiens]          43  0.007
pdb|1A17|    Tetratricopeptide Repeats Of Protein Phosphatase 5       43  0.007
ref|NP_006238.1|| protein phosphatase 5, catalytic subunit >gi|1...    43  0.007
pir||S52570 phosphoprotein phosphatase (EC 3.1.3.16) 5, catalyti...    43  0.007
gb|AAB18614.1| (U12203) phosphoprotein phosphatase [Rattus norve...    43  0.007
sp|P53042|PPP5_RAT SERINE/THREONINE PROTEIN PHOSPHATASE 5 (PP5) ...    43  0.007
gb|AAB60384.1| (U25174) serine-threonine phosphatase [Homo sapiens]    43  0.007
sp|Q60676|PPP5_MOUSE SERINE/THREONINE PROTEIN PHOSPHATASE 5 (PP5...    42  0.009
gb|AAB70573.1| (AF018262) protein phosphatase 5; PP5 [Mus musculus]    42  0.009
```

Good !

CRAP

CRAP

Entries removed for clarity

Alignment Score

Entries removed for clarity

Identifier Line:
database | accession # | name or locus

- Note that the first few hits have very low E-values (1e-90)
  - very close to 0; very good match
- I wouldn't trust anything with an E-value higher that ~1e-10 (if that…)
  - as E gets closer to 1, you get closer to a 100% probability that the match could have occurred by chance alone

An Example of a Typical BLAST search (part III)

```
>sp|P50503|HIP_RAT  HSC70-INTERACTING  PROTEIN  >gi|4379408|emb|CAA57546.1|  (X82021)
Hsc70-interacting protein [Rattus norvegicus] (Length = 368)

 Score =  106 bits (261), Expect = 5e-22
 Identities = 60/224 (26%), Positives = 97/224 (42%)

Query: 1   MDIEKIEDLKKFVASCEENPSILLKPELSFFKDPIESPGGKIKKDKMGYXXXXXXXXXX 60
           MD  K+ +L+ FV  C ++PS+L   E+ F ++++E+ GGK+
Sbjct: 1   MDPRKVSELRAFVKMCRQDPSVLHTEEMRFLRRWVESMGGKVPPATHKAKEENTKEEKR 60

           (SDEEEEDEEEEEEEEEDDDPEKLE)
Query: 61  XXXXXXXXXXXXXXXXXXXXXXXXXXXXXAVECPPLAPXXXXXXXXXXXXXXCKLKEEA 120
                                        + P                  + K  A
Sbjct: 61  DKTTEDNIKTEEPSSEESDLEIDNEGVIEADTDAPQEMGDENAEITEAMMDEANEKKGAA 120

Query: 121 VDLVENKKYEEALEKYNKIISFGNPSAMIYTKRASILLNLKRPKACIRDCTEALNLNVDS 180
           +D + + + ++A++ +   I      A++Y KRAS+ + L++P A IRDC  A+ +N DS
Sbjct: 121 IDALNDGELQKAIDLFTDAIKLNPRLAILYAKRASVFVKLQKPNAAIRDCDRAIEINPDS 180

Query: 181 ANAYKIRAKAYRYLGKWEFAHADMEQGQKIDYDENLWDMQKLIQ 224
           A  YK R KA+R LG WE A  D+    K+DYDE+   M + +Q
Sbjct: 181 AQPYKWRGKAHRLLGHWEEAARDLALACKLDYDEDASAMLREVQ 224
```

Middle row = matches and similar residues (+)

```
>pir||T24865 hypothetical protein T12D8.8 -Caenorhabditis elegans (Length = 422)

 Score = 86.2 bits (210), Expect = 5e-16
 Identities = 44/101 (43%), Positives = 60/101 (58%), Gaps = 2/101 (1%)

Query: 119 EAVDLVENKKYEEALEKYNKIISFGNPSAMIYTKRASILLNLKRPKACIRDCTEALNLNV 178
           +A +   N  ++ AL  +   I     SAM++ KRA++LL LKRP A I DC +A+++N
Sbjct: 121 KAQEAFSNGDFDTALTHFTAAIEANPGSAMLHAKRANVLLKLKRPVAAIADCDKAISINP 180

Query: 179 DSANAYKIRAKAYRYLGKWEFAHADMEQGQKIDYDE--NLW 217
           DSA  YK R +A R LGKW  A  D+    K+DYDE  N W
Sbjct: 181 DSAQGYKFRGRANRLLGKWVEAKTDLATACKLDYDEAANEW 221

 Score = 41.4 bits (95), Expect = 0.016
 Identities = 16/34 (47%), Positives = 23/34 (67%)

Query: 9   LKKFVASCEENPSILLKPELSFFKDPIESPGGKI 42
           LK+FV  C+ NP++L  PE  FFKD++ S G  +
Sbjct: 7   LKQFVGMCQANPAVLHAPEPGFFKDYLVSLGATL 40
```

Filtering--Glu rich

A second high-scoring segment

Gaps to maximize alignment

- note that low complexity regions have been filtered out
  - can generate bogus matches

Bioinformatics Analysis (revisited)

- Sequence Alignments
    - Sequence Assembly
    - Database Searching
        - Function Prediction
        - Domain Identification
    - Molecular phylogenetics

- Motif-Finding
    - DNA:
        - restriction sites
        - gene structure
            - Transcription/Translation signals
            - Intron/Exon boundaries
        - gene prediction

    - Proteins
        - PTM prediction (phosphorylation, glycosylation, …)
        - structural motifs (zinc-finger, ATP-binding cassette…)
        - transmembrane domains
        - export sequences

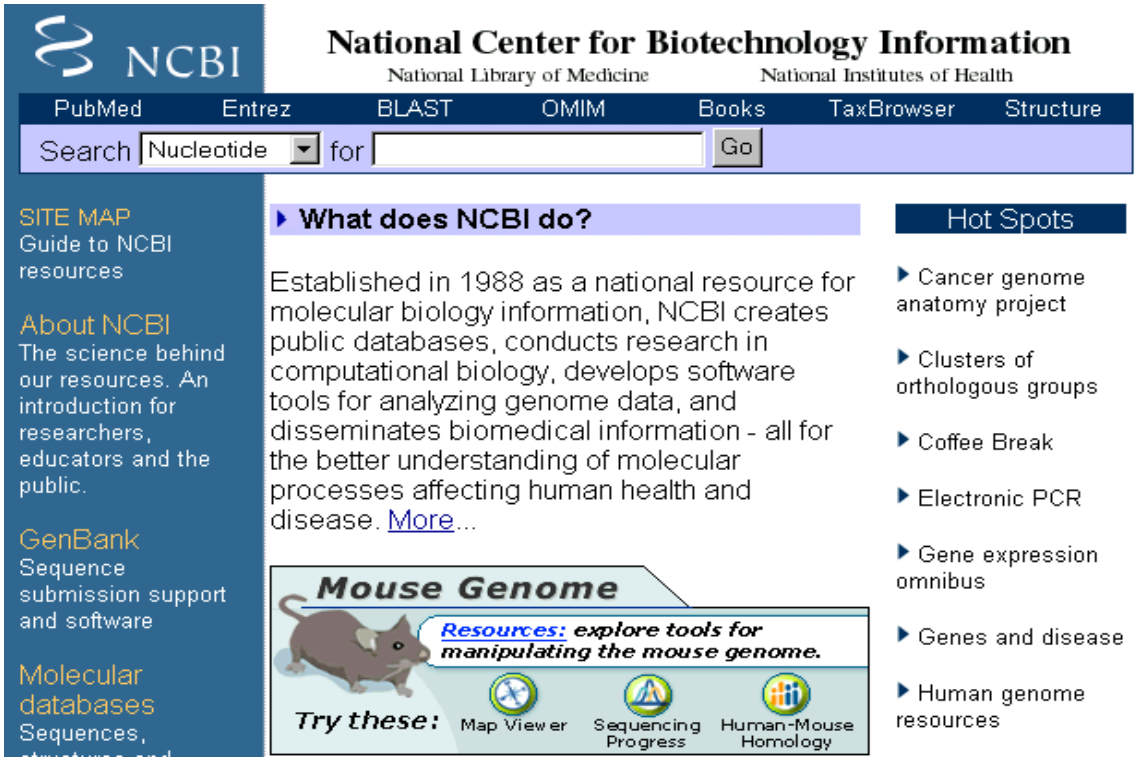
- Protein Structure Analysis
    - physical characteristics (hydrophobicity, α-helix/β-sheet potential…)
    - protein structure comparison
    - protein structure prediction (protein-fold prediction or threading)
    - X-ray crystallography and NMR data analysis

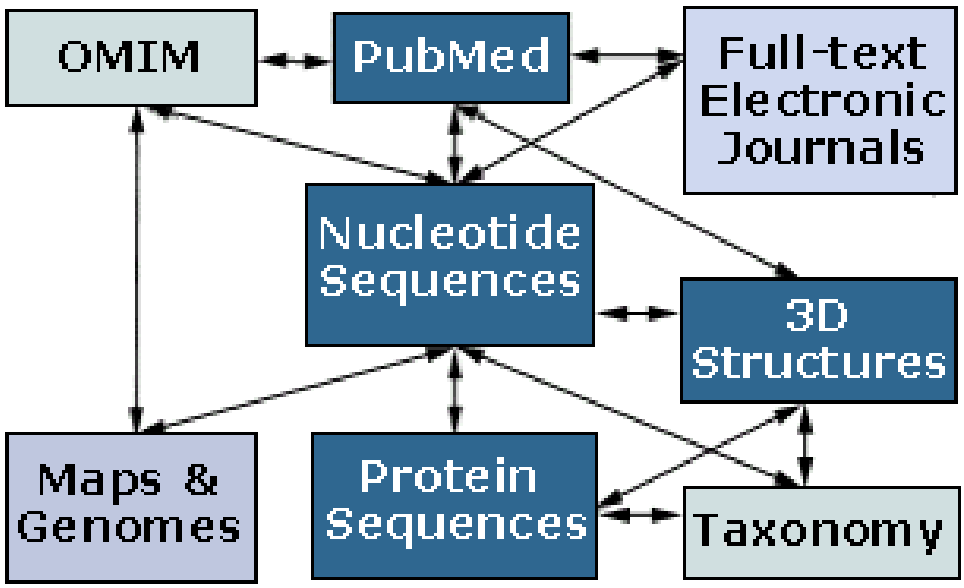
- Gene Expression Analysis
    - microarray data analysis (image analysis, data clustering)
    - proteomics data analysis (2-D gel analysis, Mass Spec data analysis)
    - metabolomics data analysis (Mass Spec data analysis)

- Pathway Analysis
    - metabolic pathway reconstruction
    - elucidation of regulatory networks

The original bioinformatics resource…..

NCBI (http://ncbi.nlm.nih.gov/): BLAST, Medline, GenBank

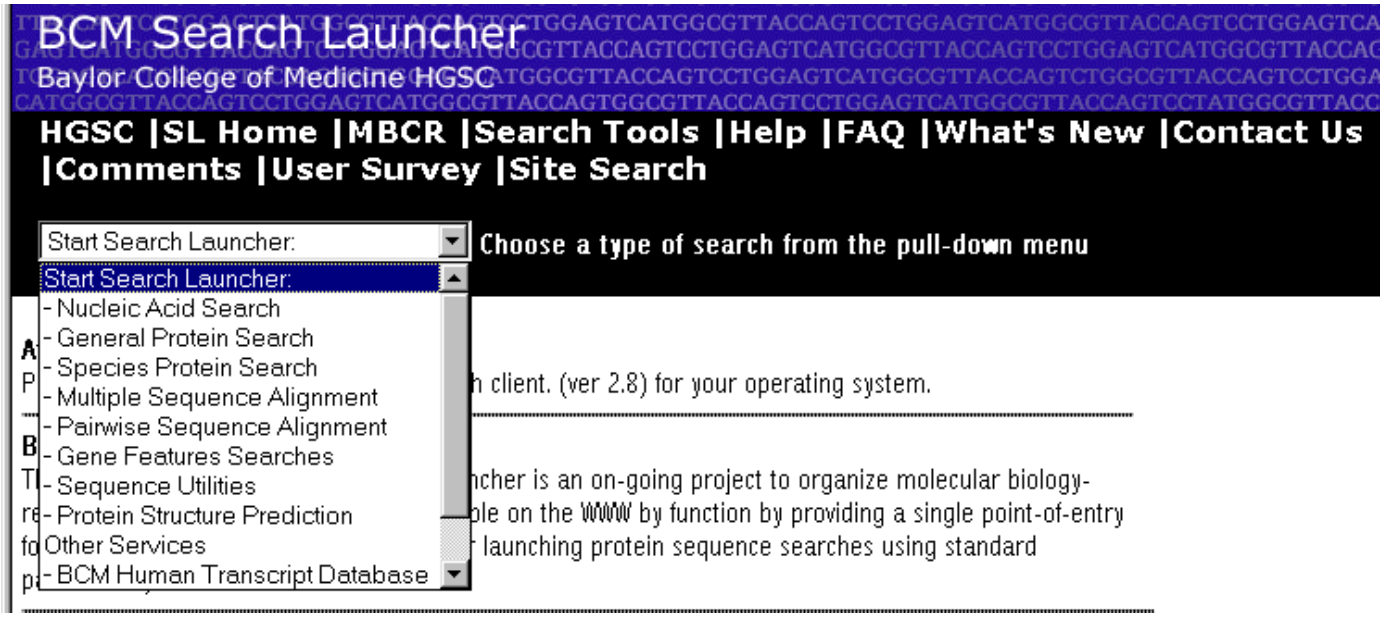




- as it's matured: more user friendly
- plenty of FAQs and tutorials !!!

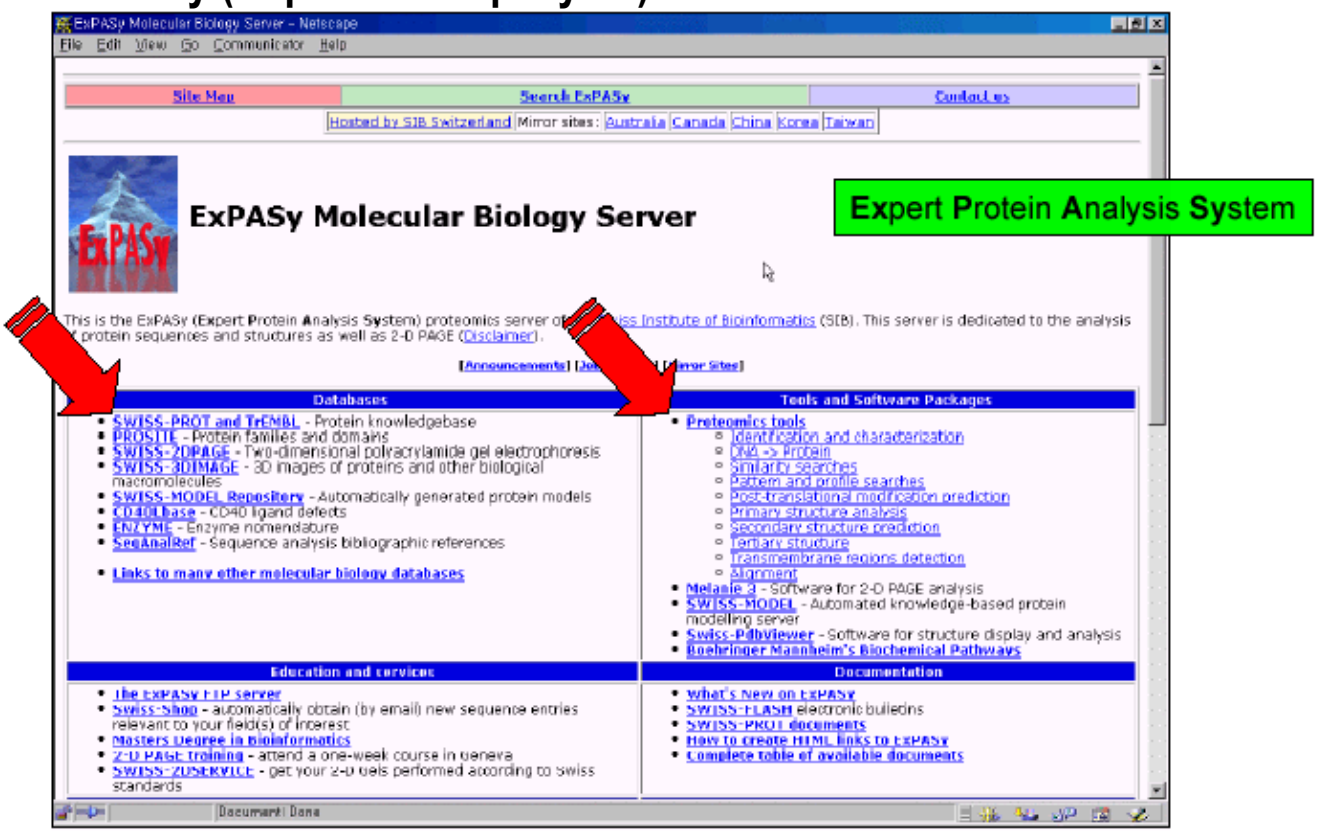
For a general sequence analysis tools

BCM Search launcher (http://searchlauncher.bcm.tmc.edu/)



For a myriad of protein analysis tools

ExPASy (http://www.expasy.ch)



Another excellent Site

CBR (http://www.cbr.nrc.ca/): lotsa good stuff !!!!!

- **A lot more sequence analysis tools than offered at NCBI**

- Mirror for other bioinformatics sites:
  - BLAST
  - ExPASy
  - CMS Molecular Biology resource (tons of stuff !!!)
    a full suite of protein and DNA analysis

- Also offers other analysis programs:
  - ClustalW: multiple sequence alignments
  - DNA fold: DNA and RNA folding
  - EMBOSS: a full suite of protein and DNA analysis
  - GeneMatcher: highly sensitive database and pattern searching
  - MAGPIE: genome data analysis
  - ReadSeq: sequence format conversion tool
  - PRIMER3: PCR primer design
  - WebPhylip: phylogenetic analysis