# Bioinformatics

## Sequence Analysis: Part II. Database Searching

Fran Lewitter, Ph.D.
Head, Biocomputing
Whitehead Institute

# Course Syllabus

| | |
|---|---|
| Jan 7 | Sequence Analysis I. Pairwise alignments, database searching including BLAST (FL) [1, 2, 3] |
| Jan 14 | Sequence Analysis II. Database searching (continued), Pattern searching(FL)[7] |
| Jan 21 | No Class - Martin Luther King Holiday |
| Jan 28 | Sequence Analysis III. Hidden Markov models, gene finding algorithms (FL)[8] |
| Feb 4 | Computational Methods I. Genomic Resources and Unix (GB) |
| Feb 11 | Computational Methods II. Sequence analysis with Perl. (GB) |
| Feb 18 | No Class - President's Birthday |
| Feb 25 | Computational Methods III. Sequence analysis with Perl and BioPerl (GB) |
| Mar 4 | Proteins I. Multiple sequence alignments, phylogenetic trees (RL) [4, 6] |
| Mar 11 | Proteins II. Profile searches of databases, revealing protein motifs (RL) [9] |
| Mar 18 | Proteins III.Structural Genomics:structural comparisons and predictions (RL) |
| Mar 25 | Microarrays: designing chips, clustering methods (FL) |

# Topics to Cover

- Introduction
- Scoring alignments
- Alignment methods
  - Dot matrix analysis
  - Exhaustive methods; Dynamic programming algorithm (Smith-Waterman (Local), Needleman-Wunsch (Global)
  - Heuristic methods; Approximate methods; word or k-tuple (FASTA, BLAST)
- Significance of alignments
- Database searching methods
- Demo

# Questions

- Why do a database search?
- What database should be searched?
- What alignment algorithm to use?
- What do the results mean?

# Issues affecting DB Search

- Substitution matrices
- Statistical significance
- Filtering
- Database choices

# BLASTP Results

Sequences producing significant alignments:

| | | Score (bits) | E Value |
|---|---|---|---|
| gi\|17647947\|ref\|NP_523565.1\| | (NM_078841) spellchecker1 [Dro... | 613 | e-175 |
| gi\|17508445\|ref\|NP_491202.1\| | (NM_058801) DNA mismatch repai... | 392 | e-108 |
| gi\|17462805\|ref\|XP_003992.5\| | (XM_003992) similar to mutS (E... | 230 | 5e-60 |
| gi\|17545870\|ref\|NP_519272.1\| | (NC_003295) PROBABLE DNA MISMA... | 223 | 9e-58 |
| gi\|17934261\|ref\|NP_531051.1\| | (NC_003304) DNA mismatch repai... | 218 | 4e-56 |
| gi\|17988084\|ref\|NP_540718.1\| | (NC_003317) DNA MISMATCH REPAI... | 211 | 4e-54 |
| gi\|17508447\|ref\|NP_491163.1\| | (NM_058762) DNA mismatch repai... | 189 | 2e-47 |
| gi\|17534743\|ref\|NP_495451.1\| | (NM_063050) recombiation prote... | 159 | 2e-38 |
| gi\|17539736\|ref\|NP_502531.1\| | (NM_070130) Yeast DNA mismatch... | 152 | 1e-36 |
| gi\|13182948\|gb\|AAK15001.1\|AF233343_4 | (AF233343) Msh4p-relat... | 134 | 7e-31 |
| gi\|17535283\|ref\|NP_495458.1\| | (NM_063057) T02G5.6.p [Caenorh... | 70 | 2e-11 |
| gi\|17485036\|ref\|XP_037759.2\| | (XM_037759) KIAA0376 protein [... | 36 | 0.21 |
| gi\|17933948\|ref\|NP_530738.1\| | (NC_003304) ATP-dependant DNA ... | 32 | 4.3 |

**Alignments**

>gi|17647947|ref|NP_523565.1| (NM_078841) spellchecker1 [Drosophila melanogaster]
gi|7298155|gb|AAF53392.1| (AE003643) spell1 gene product [Drosophila melanogaster]
Length = 854

Score = 613 bits (1582), Expect = e-175
Identities = 352/854 (41%), Positives = 508/854 (59%), Gaps = 86/854 (10%)

# Topics to Cover

- Introduction
- Scoring alignments
- Alignment methods
  - Dot matrix analysis
  - Exhaustive methods; Dynamic programming algorithm (Smith-Waterman (Local), Needleman-Wunsch (Global)
  - Heuristic methods; Approximate methods; word or k-tuple (FASTA, BLAST)
- Significance of alignments
- Database searching methods
- Demo

# Significance of Alignment

# Significance of Alignment

How strong can an alignment be expected by chance alone?

# Significance of Alignment

How strong can an alignment be expected by chance alone?

- Real but non-homologous sequences

# Significance of Alignment

How strong can an alignment be expected by chance alone?

- Real but non-homologous sequences
- Real sequences that are shuffled to preserve compositional properties

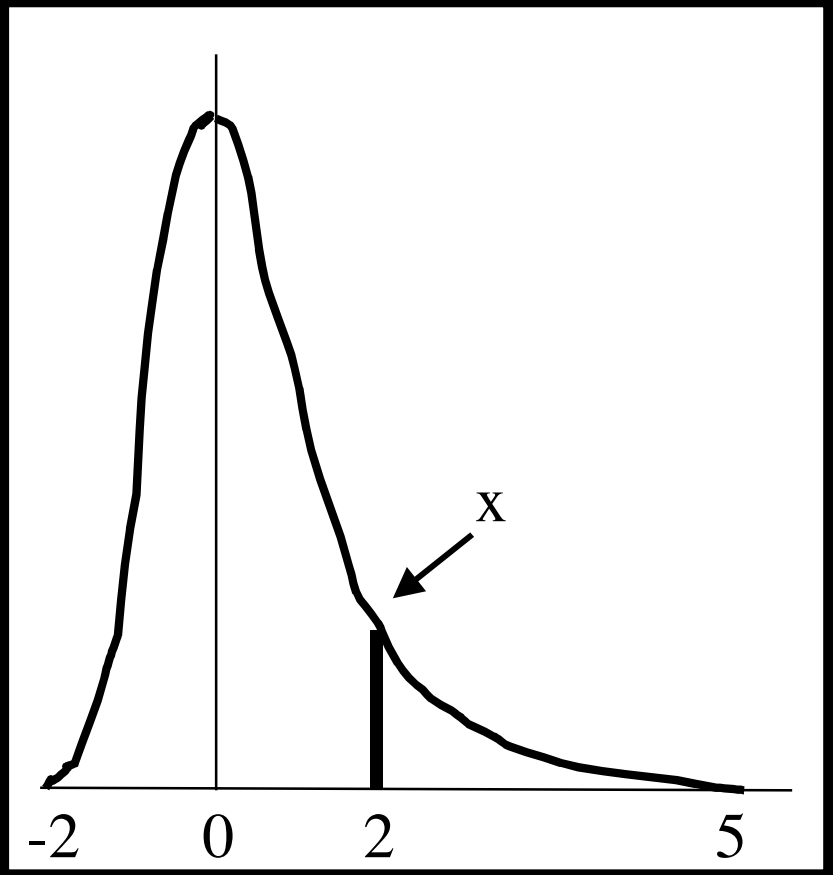
# Significance of Alignment

How strong can an alignment be expected by chance alone?

- Real but non-homologous sequences
- Real sequences that are shuffled to preserve compositional properties
- Sequences that are generated randomly based upon a DNA or protein sequence model

# Extreme Value Distribution

- When 2 sequences have been aligned optimally, the significance of a local alignment score can be tested on the basis of the distribution of scores expected by aligning two random sequences of the same length and composition as the two test sequences.



# Statistical Significance

# Statistical Significance

- Raw Scores - score of an alignment equal to the sum of substitution and gap scores.

# Statistical Significance

- Raw Scores - score of an alignment equal to the sum of substitution and gap scores.
- Bit scores - scaled version of an alignment's raw score that accounts for the statistical properties of the scoring system used.

# Statistical Significance

- Raw Scores - score of an alignment equal to the sum of substitution and gap scores.
- Bit scores - scaled version of an alignment's raw score that accounts for the statistical properties of the scoring system used.
- E-value - expected number of distinct alignments that would achieve a given score by chance. Lower E-value => more significant.

# Some formulas

# Some formulas

$$E = Kmn\, e^{-\lambda S}$$

# Some formulas

$$E = Kmn\, e^{-\lambda S}$$

This is the Expected number of high-scoring segment pairs (HSPs) with score at least S for sequences of length m and n.

# Some formulas

$$E = Kmn\, e^{-\lambda S}$$

This is the Expected number of high-scoring segment pairs (HSPs) with score at least S for sequences of length m and n.

This is the E value for the score S.

# Topics to Cover

- Introduction
- Scoring alignments
- Alignment methods
- Significance of alignments
- Database searching methods
  - BLAST - ungapped and gapped
  - BLAST vs. FASTA
  - PSI-BLAST
  - PHI-BLAST
  - Pattern searching
- Demo

# Low Complexity Regions

- **Local regions of biased composition**
- **Common in real sequences**
- **Generate false positives on BLAST search**

- **DUST for BLASTN (n's in sequence)**
- **SEG for other programs (x's in sequence)**

**Filtering is only applied to the query sequence (or its translation products), not to database sequences.**

# Filtered Sequence

>HUMAN MSH2

MAVQPKETLQLESAAEVGFVRFFQGMPEKPTTTVRLFDRGDFYTAHGEDALLAAR
EVFKTQGVIKYMGPAGAKNLQSVVLSKMNFESFVKDLLLVRQYRVEVYKNRAGNK
ASKENDWYLAYKASPGNLSQFEDILFGNNDMSASIGVVGVKMSAVDGQRQVGVGY
VDSIQRKLGLCEFPDNDQFSNLEALLIQIGPKECVLPGGETAGDMGKLRQIIQRG
GILITERKKADFSTKDIYQDLNRLLKGKKGEQMNSAVLPEMENQVAVSSLSAVIK
FLELLSDDSNFGQFELTTFDFSQYMKLDIAAVRALNLFQGSVEDTTGSQSLAALL
NKCKTPQGQRLVNQWIKQPLMDKNRIEERLNLVEAFVEDAELRQTLQEDLLRRFP
DLNRLAKKFQRQAANLQDCYRLYQGINQLPNVIQALEKHEGKHQKLLLAVFVTPL
TDLRSDFSKFQEMIETTLDMDQVENHEFLVKPSFDPNLSELREIMNDLEKKMQST
LISAARDLGLDPGKQIKLDSSAQFGYYFRVTCKEEKVLRNNKNFSTVDIQKNGVK
FTNSKLTSLNEEYTKNKTEYEEAQDAIVKEIVNISSGYVEPMQTLNDVLAQLDAV
VSFAHVSNGAPVPYVRPAILEKGQGRIILKASRHACVEVQDEIAFIPNDVYFEKD
KQMFHIITGPNMGGKSTYIRQTGVIVLMAQIGCFVPCESAEVSIVDCILARVGAG
DSQLKGVSTFMAEMLETASILRSATKDSLIIIDELGRGTSTYDGFGLAWAISEYI
ATKIGAFCMFATHFHELTALANQIPTVNNLHVTALTTEETLTMLYQVKKGVCDQS
FGIHVAELANFPKHVIECAKQKALELEEFQYIGESQGYDIMEPAAKKCYLEREQG
EKIIQEFLSKVKQMPFTEMSEENITIKLKQLKAEVIAKNNSFVNEIISRIKVTT

# Filtered Sequence

>HUMAN MSH2

NEEYTKNKTEYEE

QGMPEKPTTTVRLFDRGDFYTAHGEDALLAAR
VLSKMNFESFVKDLLLVRQYRVEVYKNRAGNK
ILFGNNDMSASIGVVGVKMSAVDGQRQVGVGY
ALLIQIGPKECVLPGGETAGDMGKLRQIIQRG
GILITERKHADFSTKDIYQDLNRLLKGKKGEQMNSAVLPEMENQVAVSSLSAVIK
FLELLSDDSNFGQFELTTFDFSQYMKLDIAAVRALNLFQGSVEDTTGSQSLAALL
NKCKTPQGQRLVNQWIKQPLMDKNRIEERLNLVEAFVEDAELRQTLQEDLLRRFP
DLNRLAKKFQRQAANLQDCYRLYQGINQLPNVIQALEKHEGKHQKLLLAVFVTPL
TDLRSDFSKFQEMIETTLDMDQVENHEFLVKPSFDPNLSELREIMNDLEKKMQST
LISAARDLGLDPGKQIKLDSSAQFGYYFRVTCKEEKVLRNNKNFSTVDIQKNGVK
FTNSKLTSLNEEYTKNKTEYEEAQDAIVKEIVNISSGYVEPMQTLNDVLAQLDAV
VSFAHVSNGAPVPYVRPAILEKGQGRIILKASRHACVEVQDEIAFIPNDVYFEKD
KQMFHIITGPNMGGKSTYIRQTGVIVLMAQIGCFVPCESAEVSIVDCILARVGAG
DSQLKGVSTFMAEMLETASILRSATKDSLIIIDELGRGTSTYDGFGLAWAISEYI
ATKIGAFCMFATHFHELTALANQIPTVNNLHVTALTTEETLTMLYQVKKGVCDQS
FGIHVAELANFPKHVIECAKQKALELEEFQYIGESQGYDIMEPAAKKCYLEREQG
EKIIQEFLSKVKQMPFTEMSEENITIKLKQLKAEVIAKNNSFVNEIISRIKVTT

# Filtered Sequence

# Example Alignment w/o filtering

Score = 29.6 bits (65), Expect = 1.8
Identities = 22/70 (31%), Positives = 32/70 (45%), Gaps = 12/70 (17%)

```
Query: 31   PPPTTQGAPRTSSFTPTTLT-----------NGTSHSPTALNGAPSPPNGFS 71
            PPP+ Q   R  S +  T T            NG+S S ++ + + S  +  S
Sbjct: 1221 PPPSVQNQQRWGSSSVITTTCQQRQQSVSPHSNGSSSSSSSSSSSSSSSTS 1273

Query: 72   NGPSSSSSSSLANQQLP 88
            +  SSSS+SS    Q P
Sbjct: 1274 SNCSSSSASSCQYFQSP 1290
```

# Example BLAST w/ filtering

```
 Score = 36.6 bits (83), Expect = 0.67
 Identities = 21/58 (36%), Positives = 25/58 (42%), Gaps = 1/58 (1%)

Query: 471 AEDALAVINQQEDSSESCWNCGRKASETCSGCNTARYCGSFCQHKDWE-KHHHICGQT 527
           A D   V  Q +  +  C  CG  A  TCS C  A YC    Q  DW+  H   C Q+
Sbjct: 61  ASDTECVCLQLKSGAHLCRVCGCLAPMTCSRCKQAHYCSKEHQTLDWQLGHKQACTQS 118
```

```
 Score = 37.0 bits (84), Expect = 0.55
 Identities = 18/55 (32%), Positives = 22/55 (39%)

Query: 483 DSSESCWNCGRKASETCSGCNTARYCGSFCQHKDWEKHHHICGQTLQAQQQGDTP 537
           D    C  CG  A++ C+ C  ARYC    Q  DW  H   C +        D P
Sbjct: 75  DGPGLCRICGCSAAKKCAKCQVARYCSQAHQVIDWPAHKLECAKAATDGSITDEP 129
```

# WU-BLAST vs NCBI BLAST

# WU-BLAST vs NCBI BLAST

- WU-BLAST first for gapped alignments

# WU-BLAST vs NCBI BLAST

- WU-BLAST first for gapped alignments
- Use different scoring system for gaps

# WU-BLAST vs NCBI BLAST

- WU-BLAST first for gapped alignments
- Use different scoring system for gaps
- Report different statistics

# WU-BLAST vs NCBI BLAST

- WU-BLAST first for gapped alignments
- Use different scoring system for gaps
- Report different statistics
- WU-BLAST does not filter low-complexity by default

# WU-BLAST vs NCBI BLAST

- WU-BLAST first for gapped alignments
- Use different scoring system for gaps
- Report different statistics
- WU-BLAST does not filter low-complexity by default
- WU-BLAST looks for and reports multiple regions of similarity

# WU-BLAST vs NCBI BLAST

- WU-BLAST first for gapped alignments
- Use different scoring system for gaps
- Report different statistics
- WU-BLAST does not filter low-complexity by default
- WU-BLAST looks for and reports multiple regions of similarity
- Results will be different

# BLAT

# BLAT

- Developed by Jim Kent at UCSC

# BLAT

- Developed by Jim Kent at UCSC
- BLAT is not BLAST

# BLAT

- Developed by Jim Kent at UCSC
- BLAT is not BLAST
- For DNA it is designed to quickly find sequences of >= 95% similarity of length 40 bases or more.

# BLAT

- Developed by Jim Kent at UCSC
- BLAT is not BLAST
- For DNA it is designed to quickly find sequences of >= 95% similarity of length 40 bases or more.
- For proteins it finds sequences of >= 80% similarity of length 20 amino acids or more.

# BLAT

- Developed by Jim Kent at UCSC
- BLAT is not BLAST
- For DNA it is designed to quickly find sequences of >= 95% similarity of length 40 bases or more.
- For proteins it finds sequences of >= 80% similarity of length 20 amino acids or more.
- DNA BLAT works by keeping an index of the entire genome in memory - non-overlapping 11-mers (< 1 GB of RAM)

# BLAT

- Developed by Jim Kent at UCSC
- BLAT is not BLAST
- For DNA it is designed to quickly find sequences of >= 95% similarity of length 40 bases or more.
- For proteins it finds sequences of >= 80% similarity of length 20 amino acids or more.
- DNA BLAT works by keeping an index of the entire genome in memory - non-overlapping 11-mers (< 1 GB of RAM)
- Protein BLAT uses 4-mers (~ 2 GB)

# FASTA

# FASTA

- Index "words" and locate identities

# FASTA

- Index "words" and locate identities
- Rescore best 10 regions

# FASTA

- Index "words" and locate identities
- Rescore best 10 regions
- Find optimal subset of initial regions that can be joined to form single alignment

# FASTA

- Index "words" and locate identities
- Rescore best 10 regions
- Find optimal subset of initial regions that can be joined to form single alignment
- Align highest scoring sequences using Smith-Waterman

# PSI-BLAST

# PSI-BLAST

- Position specific iterative BLAST uses a profile (or position specific scoring matrix, PSSM) that is constructed (automatically) from a multiple alignment of the highest scoring hits in an initial BLAST search.

# PSI-BLAST

- Position specific iterative BLAST uses a profile (or position specific scoring matrix, PSSM) that is constructed (automatically) from a multiple alignment of the highest scoring hits in an initial BLAST search.
- The PSSM is generated by calculating position-specific scores for each position in the alignment. Highly conserved positions receive high scores and weakly conserved positions receive scores near zero.

# PSI-BLAST

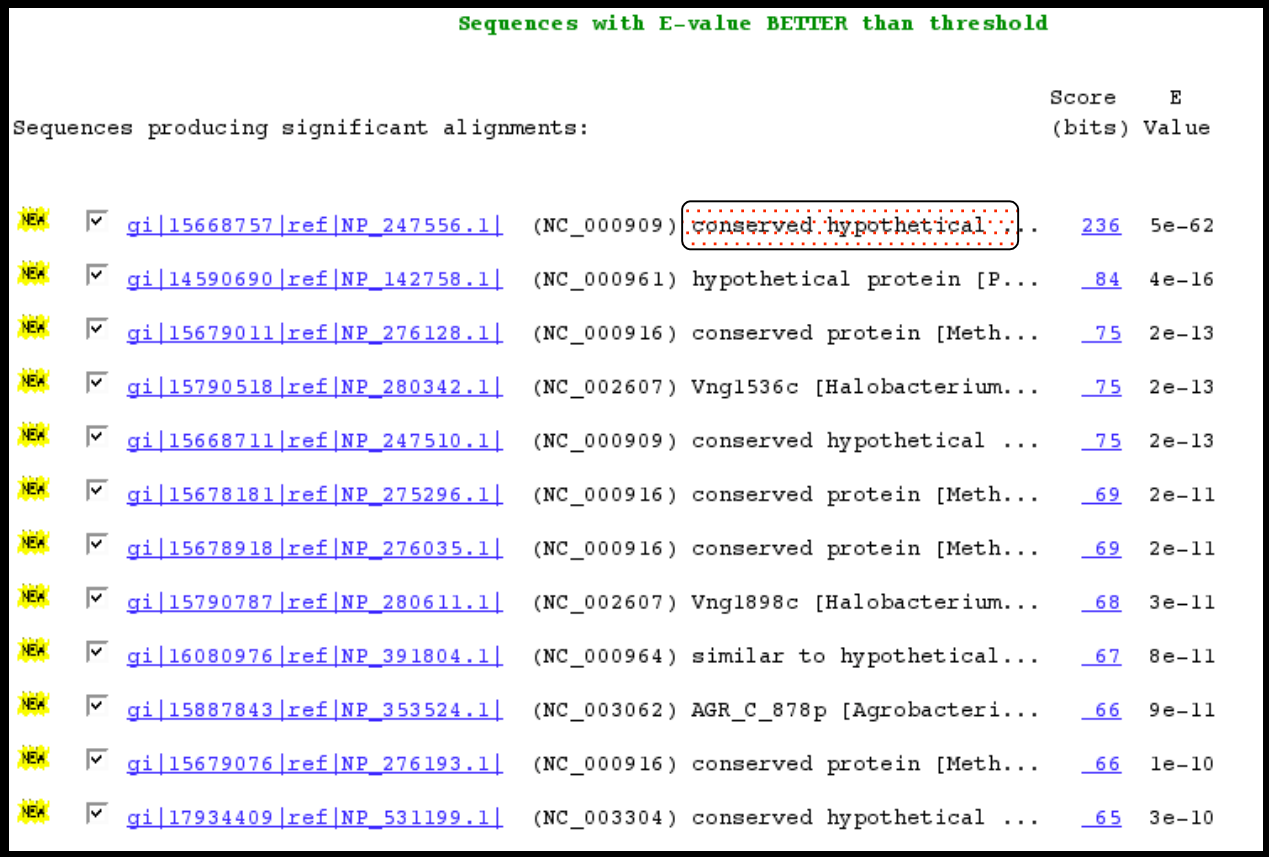
- Position specific iterative BLAST uses a profile (or position specific scoring matrix, PSSM) that is constructed (automatically) from a multiple alignment of the highest scoring hits in an initial BLAST search.
- The PSSM is generated by calculating position-specific scores for each position in the alignment. Highly conserved positions receive high scores and weakly conserved positions receive scores near zero.
- The profile is used to perform a second (etc.) BLAST search and the results of each "iteration" is used to refine the profile. This iterative searching strategy results in increased sensitivity.

# Start with a BLASTP search

**Sequences with E-value BETTER than threshold**

| Sequences producing significant alignments: | | Score (bits) | E Value |
|---|---|---|---|
| gi\|15668757\|ref\|NP_247556.1\| | (NC_000909) conserved hypothetical ... | 236 | 5e-62 |
| gi\|14590690\|ref\|NP_142758.1\| | (NC_000961) hypothetical protein [P... | 84 | 4e-16 |
| gi\|15679011\|ref\|NP_276128.1\| | (NC_000916) conserved protein [Meth... | 75 | 2e-13 |
| gi\|15790518\|ref\|NP_280342.1\| | (NC_002607) Vng1536c [Halobacterium... | 75 | 2e-13 |
| gi\|15668711\|ref\|NP_247510.1\| | (NC_000909) conserved hypothetical ... | 75 | 2e-13 |
| gi\|15678181\|ref\|NP_275296.1\| | (NC_000916) conserved protein [Meth... | 69 | 2e-11 |
| gi\|15678918\|ref\|NP_276035.1\| | (NC_000916) conserved protein [Meth... | 69 | 2e-11 |
| gi\|15790787\|ref\|NP_280611.1\| | (NC_002607) Vng1898c [Halobacterium... | 68 | 3e-11 |
| gi\|16080976\|ref\|NP_391804.1\| | (NC_000964) similar to hypothetical... | 67 | 8e-11 |
| gi\|15887843\|ref\|NP_353524.1\| | (NC_003062) AGR_C_878p [Agrobacteri... | 66 | 9e-11 |
| gi\|15679076\|ref\|NP_276193.1\| | (NC_000916) conserved protein [Meth... | 66 | 1e-10 |
| gi\|17934409\|ref\|NP_531199.1\| | (NC_003304) conserved hypothetical ... | 65 | 3e-10 |

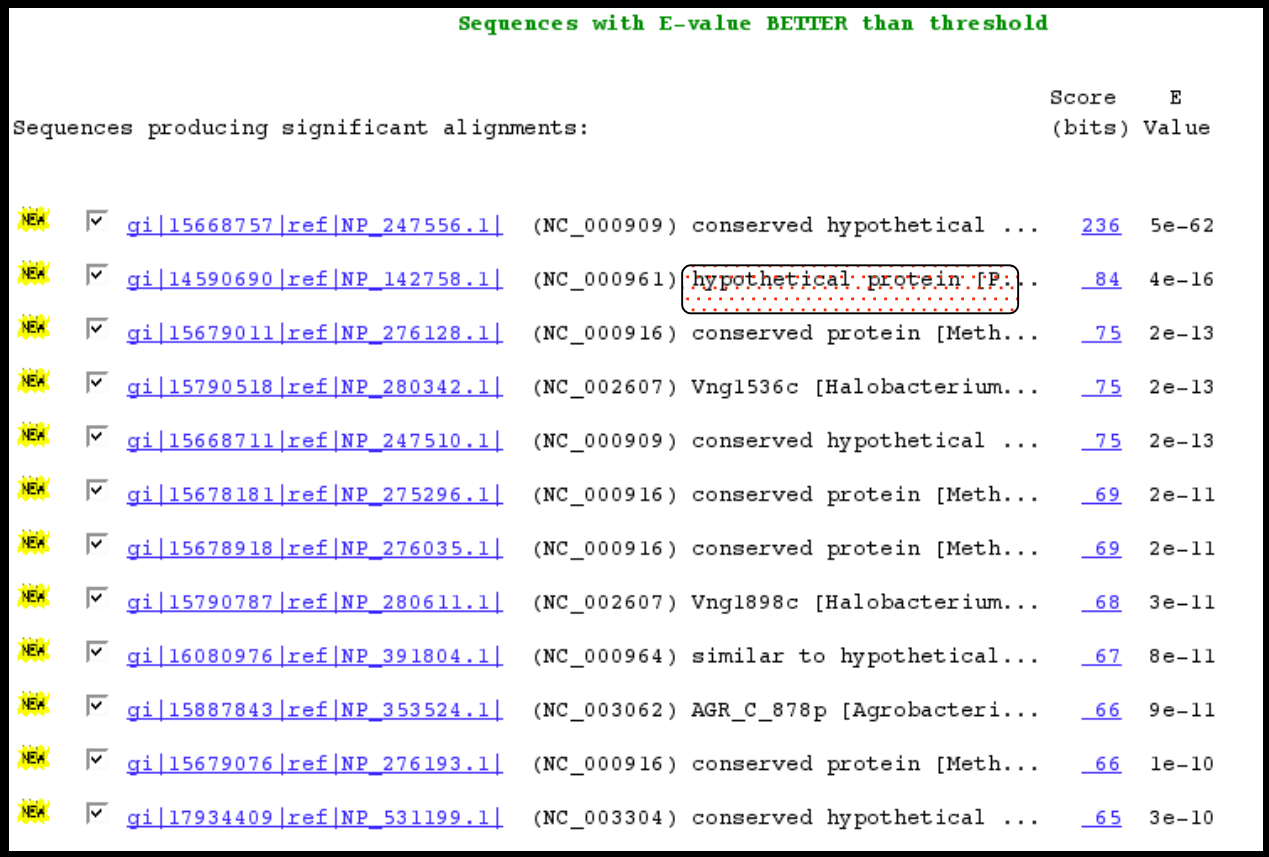
# Start with a BLASTP search



Sequences with E-value BETTER than threshold

| Sequences producing significant alignments: | | Score (bits) | E Value |
|---|---|---|---|
| gi\|15668757\|ref\|NP_247556.1\| | (NC_000909) conserved hypothetical ... | 236 | 5e-62 |
| gi\|14590690\|ref\|NP_142758.1\| | (NC_000961) hypothetical protein [P... | 84 | 4e-16 |
| gi\|15679011\|ref\|NP_276128.1\| | (NC_000916) conserved protein [Meth... | 75 | 2e-13 |
| gi\|15790518\|ref\|NP_280342.1\| | (NC_002607) Vng1536c [Halobacterium... | 75 | 2e-13 |
| gi\|15668711\|ref\|NP_247510.1\| | (NC_000909) conserved hypothetical ... | 75 | 2e-13 |
| gi\|15678181\|ref\|NP_275296.1\| | (NC_000916) conserved protein [Meth... | 69 | 2e-11 |
| gi\|15678918\|ref\|NP_276035.1\| | (NC_000916) conserved protein [Meth... | 69 | 2e-11 |
| gi\|15790787\|ref\|NP_280611.1\| | (NC_002607) Vng1898c [Halobacterium... | 68 | 3e-11 |
| gi\|16080976\|ref\|NP_391804.1\| | (NC_000964) similar to hypothetical... | 67 | 8e-11 |
| gi\|15887843\|ref\|NP_353524.1\| | (NC_003062) AGR_C_878p [Agrobacteri... | 66 | 9e-11 |
| gi\|15679076\|ref\|NP_276193.1\| | (NC_000916) conserved protein [Meth... | 66 | 1e-10 |
| gi\|17934409\|ref\|NP_531199.1\| | (NC_003304) conserved hypothetical ... | 65 | 3e-10 |

# Start with a BLASTP search



Sequences with E-value BETTER than threshold

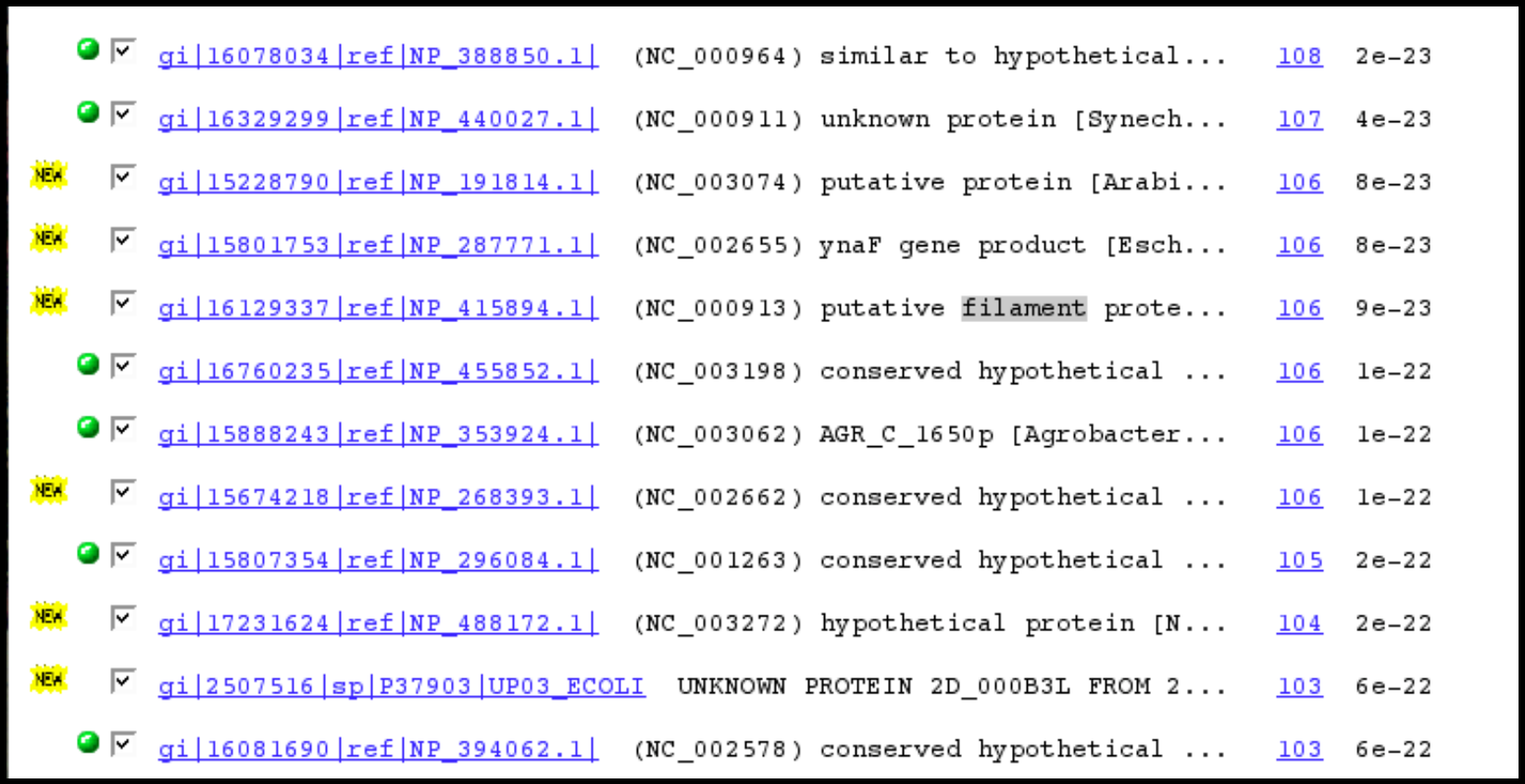
| Sequences producing significant alignments: | | Score (bits) | E Value |
|---|---|---|---|
| gi\|15668757\|ref\|NP_247556.1\| | (NC_000909) conserved hypothetical ... | 236 | 5e-62 |
| gi\|14590690\|ref\|NP_142758.1\| | (NC_000961) hypothetical protein [P:... | 84 | 4e-16 |
| gi\|15679011\|ref\|NP_276128.1\| | (NC_000916) conserved protein [Meth... | 75 | 2e-13 |
| gi\|15790518\|ref\|NP_280342.1\| | (NC_002607) Vng1536c [Halobacterium... | 75 | 2e-13 |
| gi\|15668711\|ref\|NP_247510.1\| | (NC_000909) conserved hypothetical ... | 75 | 2e-13 |
| gi\|15678181\|ref\|NP_275296.1\| | (NC_000916) conserved protein [Meth... | 69 | 2e-11 |
| gi\|15678918\|ref\|NP_276035.1\| | (NC_000916) conserved protein [Meth... | 69 | 2e-11 |
| gi\|15790787\|ref\|NP_280611.1\| | (NC_002607) Vng1898c [Halobacterium... | 68 | 3e-11 |
| gi\|16080976\|ref\|NP_391804.1\| | (NC_000964) similar to hypothetical... | 67 | 8e-11 |
| gi\|15887843\|ref\|NP_353524.1\| | (NC_003062) AGR_C_878p [Agrobacteri... | 66 | 9e-11 |
| gi\|15679076\|ref\|NP_276193.1\| | (NC_000916) conserved protein [Meth... | 66 | 1e-10 |
| gi\|17934409\|ref\|NP_531199.1\| | (NC_003304) conserved hypothetical ... | 65 | 3e-10 |

# Start with a BLASTP search

Sequences with E-value BETTER than threshold

| Sequences producing significant alignments: | | Score (bits) | E Value |
|---|---|---|---|
| gi\|15668757\|ref\|NP_247556.1\| | (NC_000909) conserved hypothetical ... | 236 | 5e-62 |
| gi\|14590690\|ref\|NP_142758.1\| | (NC_000961) hypothetical protein [P... | 84 | 4e-16 |
| gi\|15679011\|ref\|NP_276128.1\| | (NC_000916) conserved protein [Meth... | 75 | 2e-13 |
| gi\|15790518\|ref\|NP_280342.1\| | (NC_002607) Vng1536c [Halobacterium... | 75 | 2e-13 |
| gi\|15668711\|ref\|NP_247510.1\| | (NC_000909) conserved hypothetical ... | 75 | 2e-13 |
| gi\|15678181\|ref\|NP_275296.1\| | (NC_000916) conserved protein [Meth... | 69 | 2e-11 |
| gi\|15678918\|ref\|NP_276035.1\| | (NC_000916) conserved protein [Meth... | 69 | 2e-11 |
| gi\|15790787\|ref\|NP_280611.1\| | (NC_002607) Vng1898c [Halobacterium... | 68 | 3e-11 |
| gi\|16080976\|ref\|NP_391804.1\| | (NC_000964) similar to hypothetical... | 67 | 8e-11 |
| gi\|15887843\|ref\|NP_353524.1\| | (NC_003062) AGR_C_878p [Agrobacteri... | 66 | 9e-11 |
| gi\|15679076\|ref\|NP_276193.1\| | (NC_000916) conserved protein [Meth... | 66 | 1e-10 |
| gi\|17934409\|ref\|NP_531199.1\| | (NC_003304) conserved hypothetical ... | 65 | 3e-10 |

# PSI-BLAST - Iteration 1



| | | | | |
|---|---|---|---|---|
| gi\|16078034\|ref\|NP_388850.1\| | (NC_000964) similar to hypothetical... | 108 | 2e-23 |
| gi\|16329299\|ref\|NP_440027.1\| | (NC_000911) unknown protein [Synech... | 107 | 4e-23 |
| NEW gi\|15228790\|ref\|NP_191814.1\| | (NC_003074) putative protein [Arabi... | 106 | 8e-23 |
| NEW gi\|15801753\|ref\|NP_287771.1\| | (NC_002655) ynaF gene product [Esch... | 106 | 8e-23 |
| NEW gi\|16129337\|ref\|NP_415894.1\| | (NC_000913) putative filament prote... | 106 | 9e-23 |
| gi\|16760235\|ref\|NP_455852.1\| | (NC_003198) conserved hypothetical ... | 106 | 1e-22 |
| gi\|15888243\|ref\|NP_353924.1\| | (NC_003062) AGR_C_1650p [Agrobacter... | 106 | 1e-22 |
| NEW gi\|15674218\|ref\|NP_268393.1\| | (NC_002662) conserved hypothetical ... | 106 | 1e-22 |
| gi\|15807354\|ref\|NP_296084.1\| | (NC_001263) conserved hypothetical ... | 105 | 2e-22 |
| NEW gi\|17231624\|ref\|NP_488172.1\| | (NC_003272) hypothetical protein [N... | 104 | 2e-22 |
| NEW gi\|2507516\|sp\|P37903\|UP03_ECOLI | UNKNOWN PROTEIN 2D_000B3L FROM 2... | 103 | 6e-22 |
| gi\|16081690\|ref\|NP_394062.1\| | (NC_002578) conserved hypothetical ... | 103 | 6e-22 |

# PSSM from PSI-BLAST

Amino acids

| | A | R | N | D | C | Q | E | G | H | I | L | K | M | F | P | S | T | W | Y | V |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 2 | 3 | 2 | 4 | 1 | 1 | 4 | 3 | 0 | 3 | 3 | 7 | 3 | 3 | 2 | 1 | 0 | 1 | 2 |
| 2 | 6 | 0 | 3 | 3 | 5 | 4 | 0 | 3 | 2 | 5 | 0 | 1 | 2 | 2 | 4 | 1 | 3 | 2 | 4 | 2 |
| 3 | 4 | 3 | 0 | 3 | 3 | 1 | 3 | 2 | 4 | 2 | 3 | 2 | 5 | 0 | 1 | 2 | 1 | 0 | 5 | 7 |
| 4 | 3 | 2 | 3 | 2 | 4 | 9 | 3 | 3 | 5 | 4 | 0 | 3 | 2 | 5 | 1 | 2 | 2 | 4 | 1 | 2 |
| 5 | 0 | 1 | 2 | 2 | 4 | 1 | 6 | 3 | 3 | 1 | 3 | 2 | 0 | 4 | 8 | 3 | 1 | 0 | 3 | 0 |
| 6 | 4 | 3 | 2 | … | | | | | | | | | | | | | | | | |
| • | … | | | | | | | | | | | | | | | | | | | |
| • | … | | | | | | | | | | | | | | | | | | | |
| N | | | | | | | | | | | | | | | | | | | | |

POSITIONS

# PSSM from PSI-BLAST

Amino acids

| | A | R | N | D | C | Q | E | G | H | I | L | K | M | F | P | S | T | W | Y | V |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 2 | 3 | 2 | 4 | 1 | 1 | 4 | 3 | 0 | 3 | 3 | 7 | 3 | 3 | 2 | 1 | 0 | 1 | 2 |
| 2 | 6 | 0 | 3 | 3 | 5 | 4 | 0 | 3 | 2 | 5 | 0 | 1 | 2 | 2 | 4 | 1 | 3 | 2 | 4 | 2 |
| 3 | 4 | 3 | 0 | 3 | 3 | 1 | 3 | 2 | 4 | 2 | 3 | 2 | 5 | 0 | 1 | 2 | 1 | 0 | 5 | 7 |
| 4 | 3 | 2 | 3 | 2 | 4 | 9 | 3 | 3 | 5 | 4 | 0 | 3 | 2 | 5 | 1 | 2 | 2 | 4 | 1 | 2 |
| 5 | 0 | 1 | 2 | 2 | 4 | 1 | 6 | 3 | 3 | 1 | 3 | 2 | 0 | 4 | 8 | 3 | 1 | 0 | 3 | 0 |
| 6 | 4 | 3 | 2 | … | | | | | | | | | | | | | | | | |
| • | … | | | | | | | | | | | | | | | | | | | |
| • | … | | | | | | | | | | | | | | | | | | | |
| N | | | | | | | | | | | | | | | | | | | | |

POSITIONS

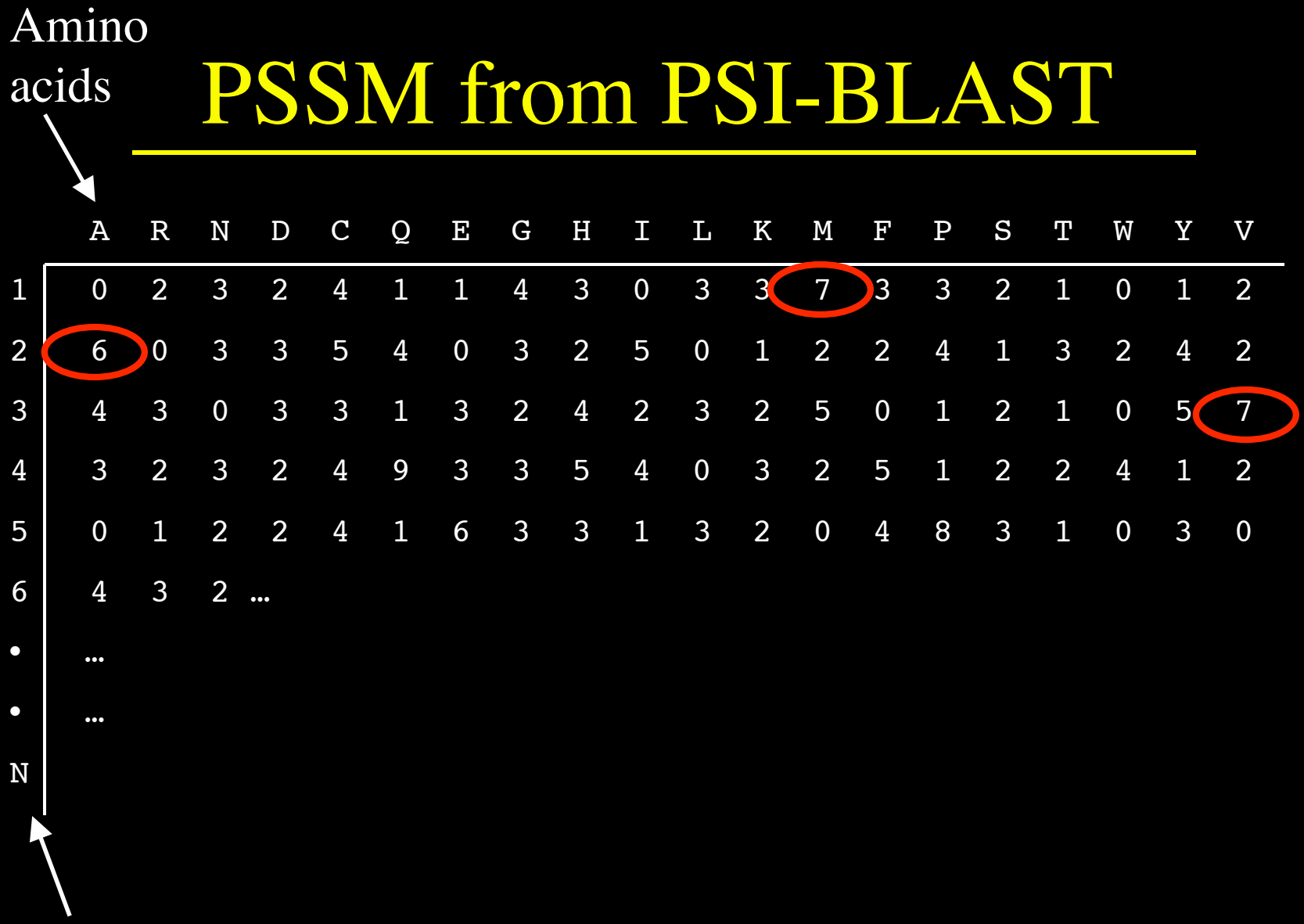
# PSSM from PSI-BLAST

Amino acids

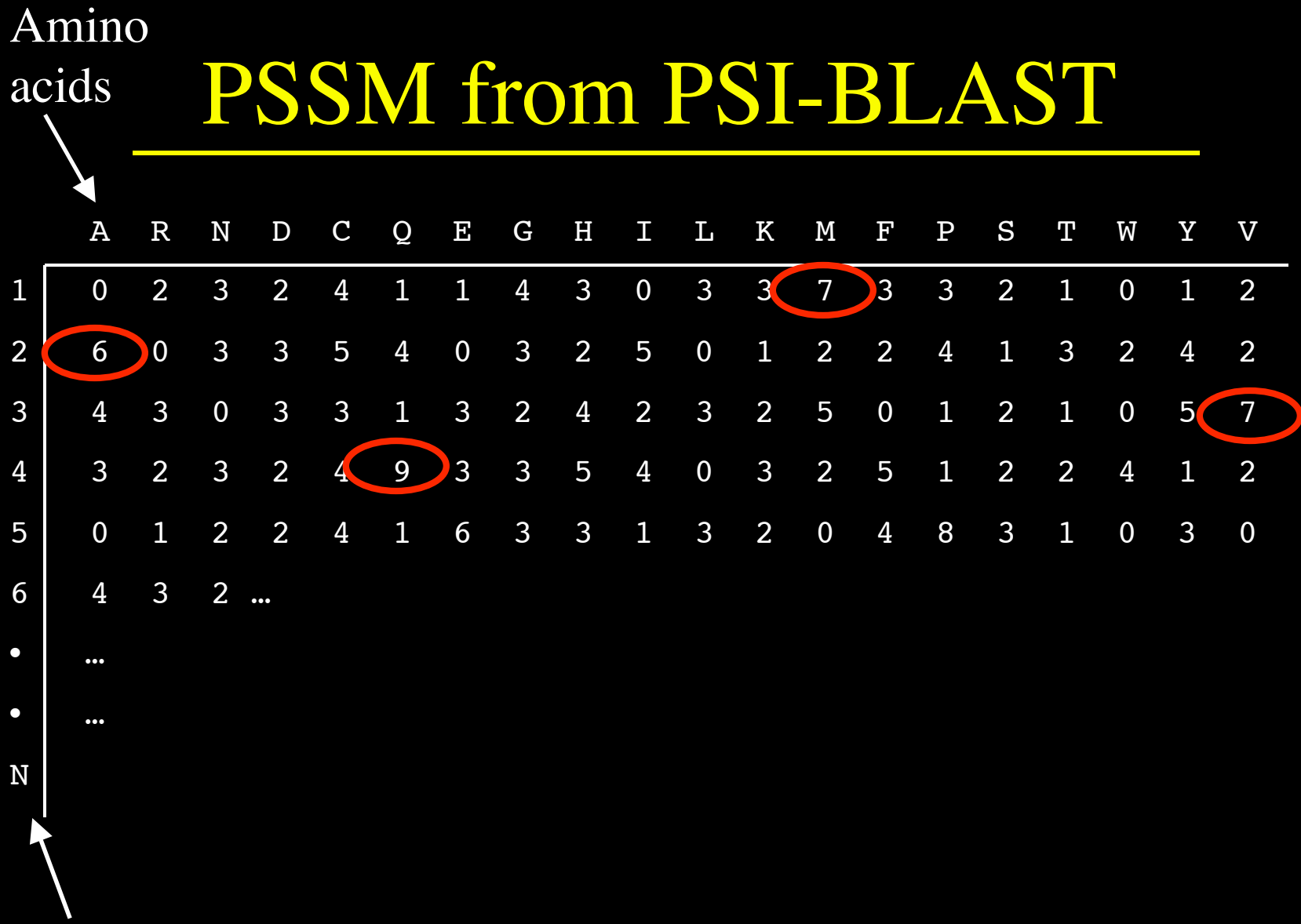
| | A | R | N | D | C | Q | E | G | H | I | L | K | M | F | P | S | T | W | Y | V |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 2 | 3 | 2 | 4 | 1 | 1 | 4 | 3 | 0 | 3 | 3 | 7 | 3 | 3 | 2 | 1 | 0 | 1 | 2 |
| 2 | 6 | 0 | 3 | 3 | 5 | 4 | 0 | 3 | 2 | 5 | 0 | 1 | 2 | 2 | 4 | 1 | 3 | 2 | 4 | 2 |
| 3 | 4 | 3 | 0 | 3 | 3 | 1 | 3 | 2 | 4 | 2 | 3 | 2 | 5 | 0 | 1 | 2 | 1 | 0 | 5 | 7 |
| 4 | 3 | 2 | 3 | 2 | 4 | 9 | 3 | 3 | 5 | 4 | 0 | 3 | 2 | 5 | 1 | 2 | 2 | 4 | 1 | 2 |
| 5 | 0 | 1 | 2 | 2 | 4 | 1 | 6 | 3 | 3 | 1 | 3 | 2 | 0 | 4 | 8 | 3 | 1 | 0 | 3 | 0 |
| 6 | 4 | 3 | 2 | … | | | | | | | | | | | | | | | | |
| • | … | | | | | | | | | | | | | | | | | | | |
| • | … | | | | | | | | | | | | | | | | | | | |
| N | | | | | | | | | | | | | | | | | | | | |

POSITIONS

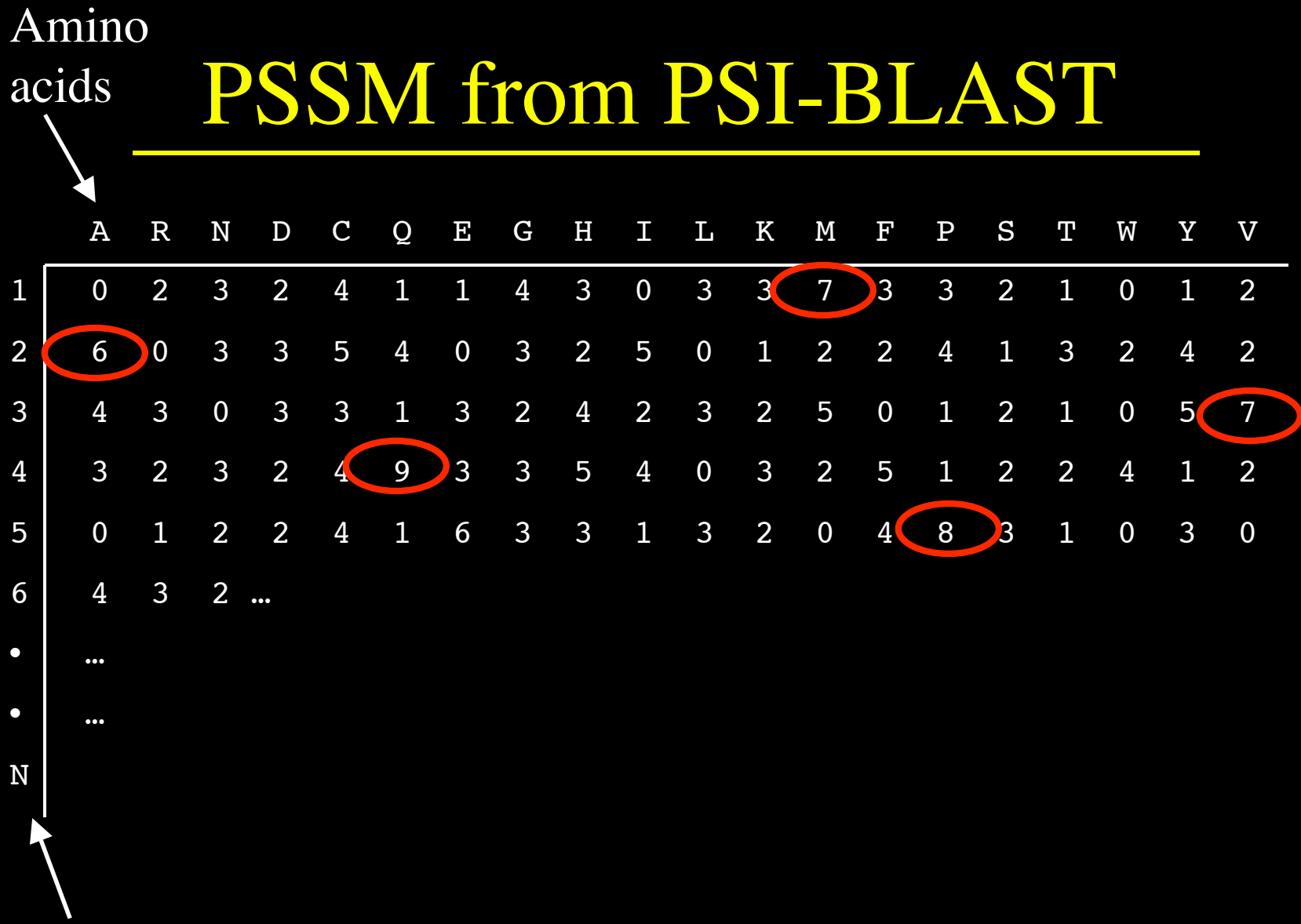
# PSSM from PSI-BLAST



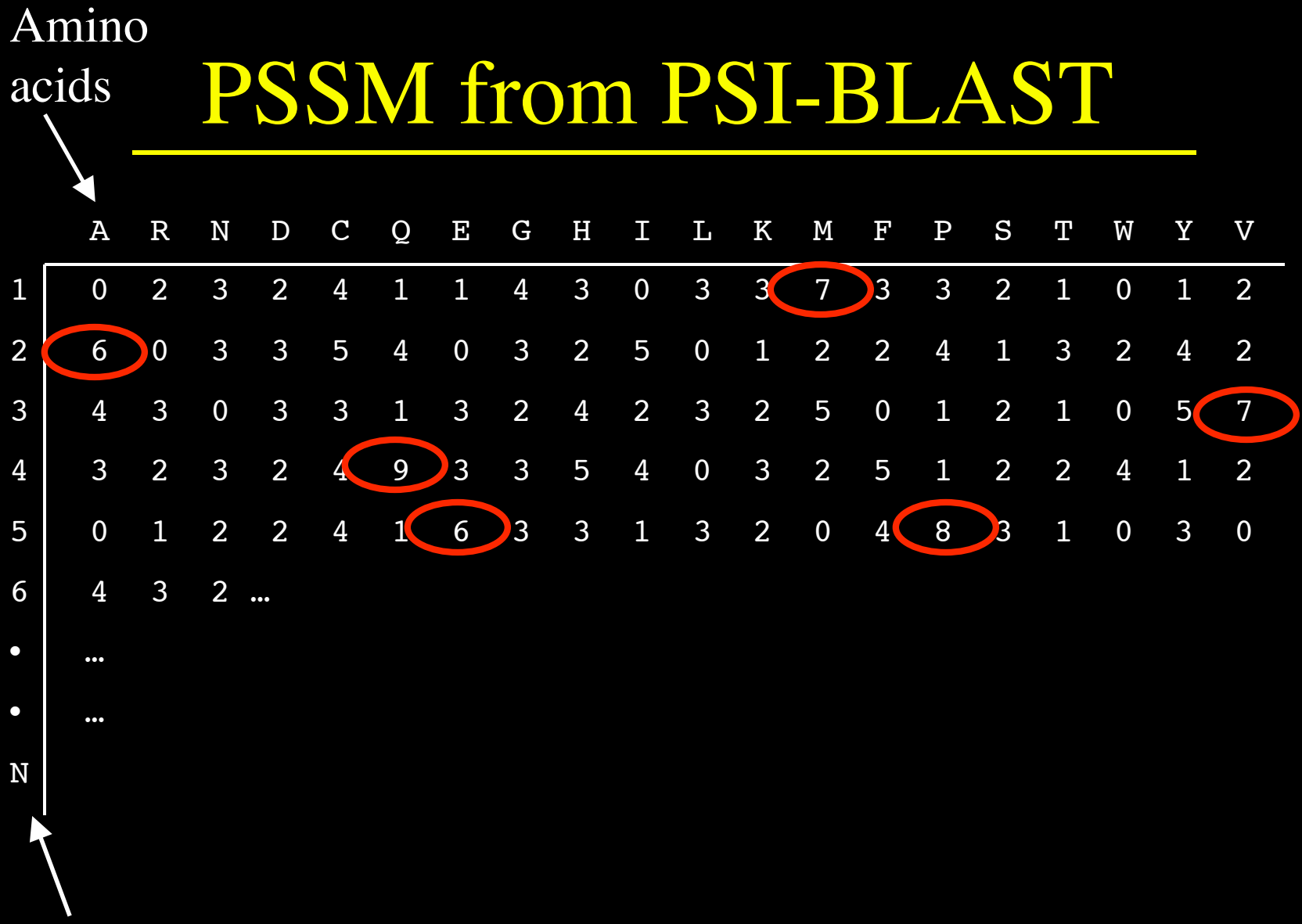
# PSSM from PSI-BLAST



# PSSM from PSI-BLAST



# PSSM from PSI-BLAST



# Pattern Hit Initiated (PHI)-BLAST

>HUMAN MSH2

MAVQPKETLQLESAAEVGFVRFFQGMPEKPTTTVRLFDRGDFYTAHGEDALLAAREVFKTQGVIKYMGPA
GAKNLQSVVLSKMNFESFVKDLLLVRQYRVEVYKNRAGNKASKENDWYLAYKASPGNLSQFEDILFGNND
MSASIGVVGVKMSAVDGQRQVGVGYVDSIQRKLGLCEFPDNDQFSNLEALLIQIGPKECVLPGGETAGDM
GKLRQIIQRGGILITERKKADFSTKDIYQDLNRLLKGKKGEQMNSAVLPEMENQVAVSSLSAVIKFLELL
SDDSNFGQFELTTFDFSQYMKLDIAAVRALNLFQGSVEDTTGSQSLAALLNKCKTPQGQRLVNQWIKQPL
MDKNRIEERLNLVEAFVEDAELRQTLQEDLLRRFPDLNRLAKKFQRQAANLQDCYRLYQGINQLPNVIQA
LEKHEGKHQKLLLAVFVTPLTDLRSDFSKFQEMIETTLDMDQVENHEFLVKPSFDPNLSELREIMNDLEK
KMQSTLISAARDLGLDPGKQIKLDSSAQFGYYFRVTCKEEKVLRNNKNFSTVDIQKNGVKFTNSKLTSLN
EEYTKNKTEYEEAQDAIVKEIVNISSGYVEPMQTLNDVLAQLDAVVSFAHVSNGAPVPYVRPAILEKGQG
RIILKASRHACVEVQDEIAFIPNDVYFEKDKQMFHIITGPNMGGKSTYIRQTGVIVLMAQIGCFVPCESA
EVSIVDCILARVGAGDSQLKGVSTFMAEMLETASILRSATKDSLIIIDELGRGTSTYDGFGLAWAISEYI
ATKIGAFCMFATHFHELTALANQIPTVNNLHVTALTTEETLTMLYQVKKGVCDQSFGIHVAELANFPKHV
IECAKQKALELEEFQYIGESQGYDIMEPAAKKCYLEREQGEKIIQEFLSKVKQMPFTEMSEENITIKLKQ
LKAEVIAKNNSFVNEIISRIKVTT

# Pattern Hit Initiated (PHI)-BLAST

>HUMAN MSH2

MAVQPKETLQLESAAEVGFVRFFQGMPEKPTTTVRLFDRGDFYTAHGEDALLAAREVFKTQGVIKYMGPA
GAKNLQSVVLSKMNFESFVKDLLLVRQYRVEVYKNRAGNKASKENDWYLAYKASPGNLSQFEDILFGNND
MSASIGVVGVKMSAVDGQRQVGVGYVDSIQRKLGLCEFPDNDQFSNLEALLIQIGPKECVLPGGETAGDM
GKLRQIIQRGGILITERKKADFSTKDIYQDLNRLLKGKKGEQMNSAVLPEMENQVAVSSLSAVIKFLELL
SDDSNFGQFELTTFDFSQYMKLDIAAVRALNLFQGSVEDTTGSQSLAALLNKCKTPQGQRLVNQWIKQPL
MDKNRIEERLNLVEAFVEDAELRQTLQEDLLRRFPDLNRLAKKFQRQAANLQDCYRLYQGINQLPNVIQA
LEKHEGKHQKLLLAVFVTPLTDLRSDFSKFQEMIETTLDMDQVENHEFLVKPSFDPNLSELREIMNDLEK
KMQSTLISAARDLGLDPGKQIKLDSSAQFGYYFRVTCKEEKVLRNNKNFSTVDIQKNGVKFTNSKLTSLN
EEYTKNKTEYEEAQDAIVKEIVNISSGYVEPMQTLNDVLAQLDAVVSFAHVSNGAPVPYVRPAILEKGQG
RIILKASRHACVEVQDEIAFIPNDVYFEKDKQMFHIITGPNMGGKSTYIRQTGVIVLMAQIGCFVPCESA
EVSIVDCILARVGAGDSQLKGVSTFMAEMLETASILRSATKDSLIIIDELGRGTSTYDGFGLAWAISEYI
ATKIGAFCMFATHFHELTALANQIPTVNNLHVTALTTEETLTMLYQVKKGVCDQSFGIHVAELANFPKHV
IECAKQKALELEEFQYIGESQGYDIMEPAAKKCYLEREQGEKIIQEFLSKVKQMPFTEMSEENITIKLKQ
LKAEVIAKNNSFVNEIISRIKVTT

DNA mismatch repair proteins mutS family signature

# PHI-BLAST

```
>gi|4099512|gb|AAD00647.1| (U87911) MutS homolog 2 [Arabidopsis thaliana]
          Length = 117

 Score =  136 bits (364), Expect = 1e-40
 Identities = 88/117 (75%), Positives = 98/117 (83%)

Query:  668 TGPNMGGKSTYIRQTGVIVLMAQIGCFVPCESAEVSIVDCILARVGAGDSQLKGVSTFMA 727
            TGPNMGGKST+IRQ GVIVLMAQ+G FVPC+ A +SI DCI ARVGAGD QL+GVSTFM
Sbjct:  1   TGPNMGGKSTFIRQVGVIVLMAQVGSFVPCDKASISIRDCIFARVGAGDCQLRGVSTFMQ 60

Query:  728 EMLETASILRSATKDSLIIIDELGRGTSTYDGFGLAWAISEYIATKIGAFCMFATHF 784
pattern 743                 *****************
            EMLETASIL+ AT  SLIIIDELGRGTSTYDGFGLAWAI E++     A  +FATH+
Sbjct:  61  EMLETASILKGATDKSLIIIDELGRGTSTYDGFGLAWAICEHLVQVKRAPTLFATHY 117
```

# PHI-BLAST

```
>gi|4099512|gb|AAD00647.1| (U87911) MutS homolog 2 [Arabidopsis thaliana]
          Length = 117

 Score =  136 bits (364), Expect = 1e-40
 Identities = 88/117 (75%), Positives = 98/117 (83%)

Query:  668 TGPNMGGKSTYIRQTGVIVLMAQIGCFVPCESAEVSIVDCILARVGAGDSQLKGVSTFMA 727
           TGPNMGGKST+IRQ GVIVLMAQ+G FVPC+ A +SI DCI ARVGAGD QL+GVSTFM
Sbjct:  1   TGPNMGGKSTFIRQVGVIVLMAQVGSFVPCDKASISIRDCIFARVGAGDCQLRGVSTFMQ 60

Query:  728 EMLETASILRSATKDSLIIIDELGRGTSTYDGFGLAWAISEYIATKIGAFCMFATHF 784
pattern 743                 *****************
           EMLETASIL+ AT  SLIIIDELGRGTSTYDGFGLAWAI E++     A  +FATH+
Sbjct:  61  EMLETASILKGATDKSLIIIDELGRGTSTYDGFGLAWAICEHLVQVKRAPTLFATHY 117
```

# Basic Searching Strategies

- Search early and often
- Use specialized databases
- Use multiple matrices
- Use filters
- Consider Biology

# Demo

- Readseq
- Entrez
- NCBI
  - BLAST2.0
  - PSI-BLAST
  - PHI-BLAST
- WU-BLAST2
- FASTA
- Smith-Waterman

# BLAST and FASTA Citations

- **PNAS, 1988, 85: 2444-2448.**
- **Journal of Molecular Biology, 1990, 215: 403-410.**
- **Nature Genetics, 1994, 6: 119-129.**
- **Nucleic Acids Research, 1997, 25(17):3389-3402.**
- **Nucleic Acids Research, 1998, 26(17):3986-3990.**
- **TIBS, 1998, 23:444-447.**
- **Nucleic Acids Research, 2001, 29(2):351-361.**
- **Nucleic Acids Research, 2001, 29(14):2994-3005.**

**http://www.ncbi.nlm.nih.gov/blast**
**http://www.ebi.ac.uk/blast2/**
**http://www2.ebi.ac.uk/fasta33/**
**http://www2.ebi.ac.uk/bic_sw/**