
# PAM and BLAST

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# Klausurtermin

- Freitag, 14.8.2017, 11-14 (11.30 – 13.30) Uhr
- Raum: 3.001
- Keine Hilfsmittel erlaubt

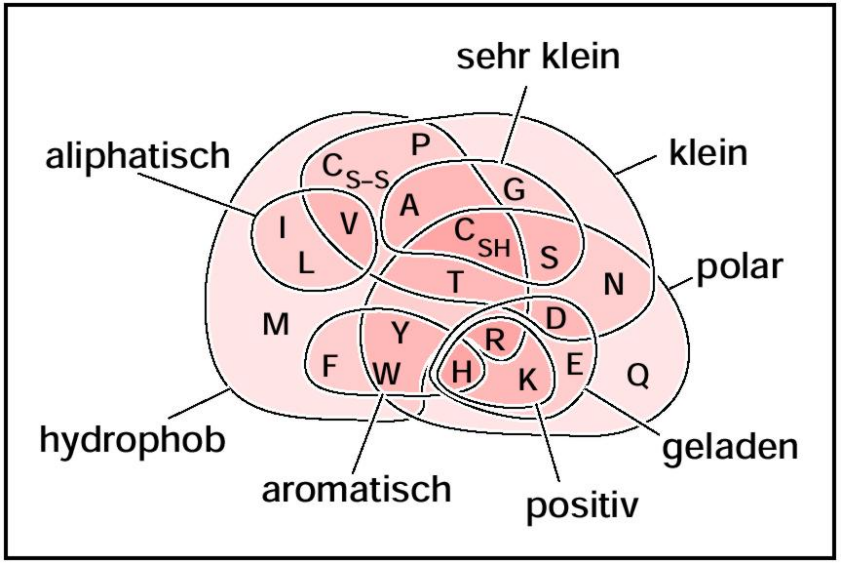
- Anmelden
- Übungsschein

# This Lecture

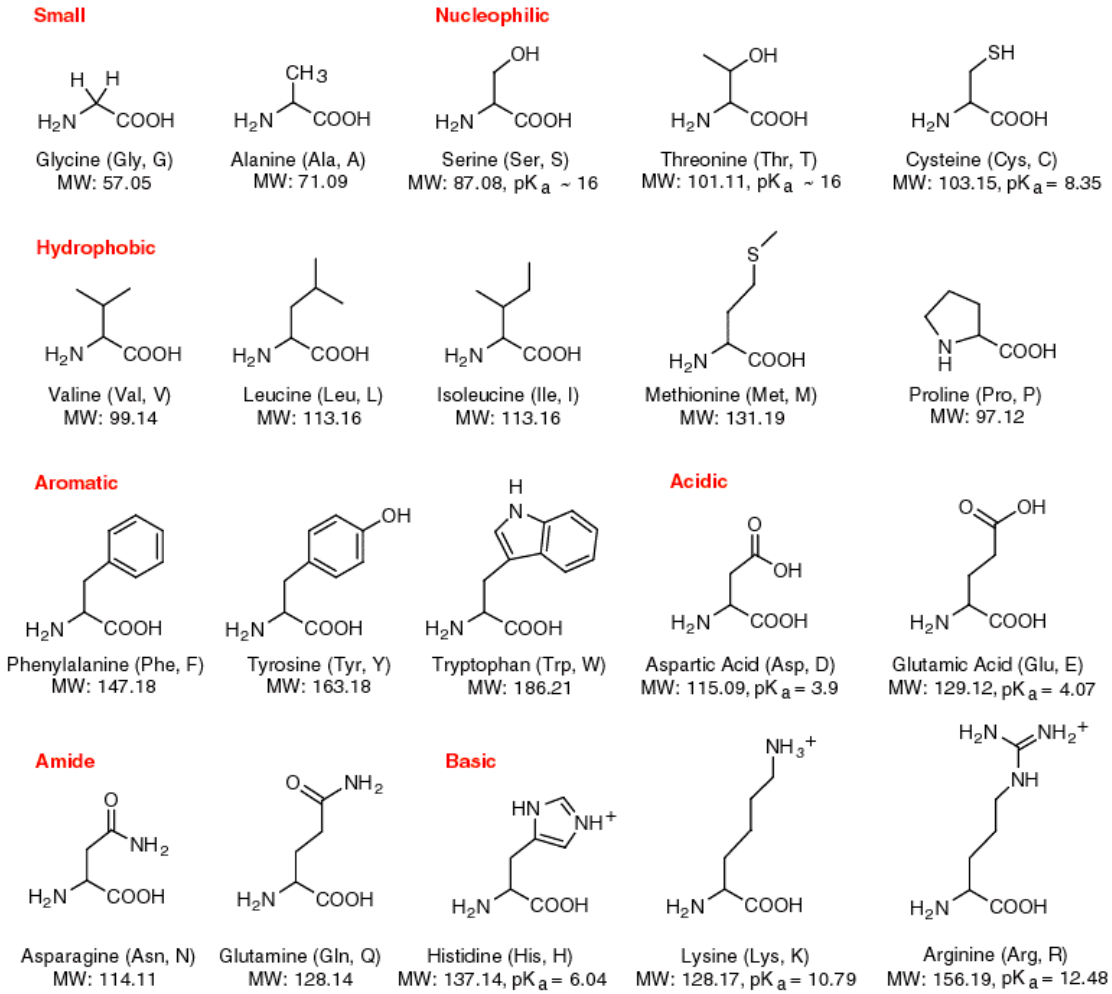
- Substitution Matrices
  - PAM distance
  - PAM matrices
- Scaling up Local Alignments
  - BLAST

# Substitution Matrices

- Recall
  - A scoring function is a function s: $\Sigma' x \Sigma' \rightarrow$ Integer
    - We also call s a substitution matrix
- DNA: symmetric, simple matrices
- Protein sequences are different
  - AA have very different properties
  - Substitutions may change the 3D structure completely or just a little bit or not at all



# Amino Acids


Small
Glycine (Gly, G)
MW: 57.05
Alanine (Ala, A)
MW: 71.09
Nucleophilic
Serine (Ser, S)
MW: 87.08, pKa ~ 16
Threonine (Thr, T)
MW: 101.11, pKa ~ 16
Cysteine (Cys, C)
MW: 103.15, pKa = 8.35
Hydrophobic
Valine (Val, V)
MW: 99.14
Leucine (Leu, L)
MW: 113.16
Isoleucine (Ile, I)
MW: 113.16
Methionine (Met, M)
MW: 131.19
Proline (Pro, P)
MW: 97.12
Aromatic
Phenylalanine (Phe, F)
MW: 147.18
Tyrosine (Tyr, Y)
MW: 163.18
Tryptophan (Trp, W)
MW: 186.21
Acidic
Aspartic Acid (Asp, D)
MW: 115.09, pKa = 3.9
Glutamic Acid (Glu, E)
MW: 129.12, pKa = 4.07
Amide
Asparagine (Asn, N)
MW: 114.11
Glutamine (Gln, Q)
MW: 128.14
Basic
Histidine (His, H)
MW: 137.14, pKa = 6.04
Lysine (Lys, K)
MW: 128.17, pKa = 10.79
Arginine (Arg, R)
MW: 156.19, pKa = 12.48

# Example

Where do all these numbers come from?

| | A | R | N | D | C | Q | E | G | H | I | L | K | M | F | P | S | T | W | Y | V | B | Z |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 4 | -1 | -2 | -2 | 0 | -1 | -1 | 0 | -2 | -1 | -1 | -1 | -1 | -2 | -1 | 1 | 0 | -3 | -2 | 0 | -2 | -1 |
| R | -1 | 5 | 0 | -2 | -3 | 1 | 0 | -2 | 0 | -3 | -2 | 2 | -1 | -3 | -2 | -1 | -1 | -3 | -2 | -3 | -1 | 0 |
| N | -2 | 0 | 6 | 1 | -3 | 0 | 0 | 0 | 1 | -3 | -3 | 0 | -2 | -3 | -2 | 1 | 0 | -4 | -2 | -3 | 3 | 0 |
| D | -2 | -2 | 1 | 6 | -3 | 0 | 2 | -1 | -1 | -3 | -4 | -1 | -3 | -3 | -1 | 0 | -1 | -4 | -3 | -3 | 4 | 1 |
| C | 0 | -3 | -3 | -3 | 9 | -3 | -4 | -3 | -3 | -1 | -1 | -3 | -1 | -2 | -3 | -1 | -1 | -2 | -2 | -1 | -3 | -3 |
| Q | -1 | 1 | 0 | 0 | -3 | 5 | 2 | -2 | 0 | -3 | -2 | 1 | 0 | -3 | -1 | 0 | -1 | -2 | -1 | -2 | 0 | 3 |
| E | -1 | 0 | 0 | 2 | -4 | 2 | 5 | -2 | 0 | -3 | -3 | 1 | -2 | -3 | -1 | 0 | -1 | -3 | -2 | -2 | 1 | 4 |
| G | 0 | -2 | 0 | -1 | -3 | -2 | -2 | 6 | -2 | -4 | -4 | -2 | -3 | -3 | -2 | 0 | -2 | -2 | -3 | -3 | -1 | -2 |
| H | -2 | 0 | 1 | -1 | -3 | 0 | 0 | -2 | 8 | -3 | -3 | -1 | -2 | -1 | -2 | -1 | -2 | -2 | 2 | -3 | 0 | 0 |
| I | -1 | -3 | -3 | -3 | -1 | -3 | -3 | -4 | -3 | 4 | 2 | -3 | 1 | 0 | -3 | -2 | -1 | -3 | -1 | 3 | -3 | -3 |
| L | -1 | -2 | -3 | -4 | -1 | -2 | -3 | -4 | -3 | 2 | 4 | -2 | 2 | 0 | -3 | -2 | -1 | -2 | -1 | 1 | -4 | -3 |
| K | -1 | 2 | 0 | -1 | -3 | 1 | 1 | -2 | -1 | -3 | -2 | 5 | -1 | -3 | -1 | 0 | -1 | -3 | -2 | -2 | 0 | 1 |
| M | -1 | -1 | -2 | -3 | -1 | 0 | -2 | -3 | -2 | 1 | 2 | -1 | 5 | 0 | -2 | -1 | -1 | -1 | -1 | 1 | -3 | -1 |
| F | -2 | -3 | -3 | -3 | -2 | -3 | -3 | -3 | -1 | 0 | 0 | -3 | 0 | 6 | -4 | -2 | -2 | 1 | 3 | -1 | -3 | -3 |
| P | -1 | -2 | -2 | -1 | -3 | -1 | -1 | -2 | -2 | -3 | -3 | -1 | -2 | -4 | 7 | -1 | -1 | -4 | -3 | -2 | -2 | -1 |
| S | 1 | -1 | 1 | 0 | -1 | 0 | 0 | 0 | -1 | -2 | -2 | 0 | -1 | -2 | -1 | 4 | 1 | -3 | -2 | -2 | 0 | 0 |
| T | 0 | -1 | 0 | -1 | -1 | -1 | -1 | -2 | -2 | -1 | -1 | -1 | -1 | -2 | -1 | 1 | 5 | -2 | -2 | 0 | -1 | -1 |
| W | -3 | -3 | -4 | -4 | -2 | -2 | -3 | -2 | -2 | -3 | -2 | -3 | -1 | 1 | -4 | -3 | -2 | 11 | 2 | -3 | -4 | -3 |
| Y | -2 | -2 | -2 | -3 | -2 | -1 | -2 | -3 | 2 | -1 | -1 | -2 | -1 | 3 | -3 | -2 | -2 | 2 | 7 | -1 | -3 | -2 |
| V | 0 | -3 | -3 | -3 | -1 | -2 | -2 | -3 | -3 | 3 | 1 | -2 | 1 | -1 | -2 | -2 | 0 | -3 | -1 | 4 | -3 | -2 |
| B | -2 | -1 | 3 | 4 | -3 | 0 | 1 | -1 | 0 | -3 | -4 | 0 | -3 | -3 | -2 | 0 | -1 | -4 | -3 | -3 | 4 | 1 |
| Z | -1 | 0 | 0 | 1 | -3 | 3 | 4 | -2 | 0 | -3 | -3 | 1 | -1 | -3 | -1 | 0 | -1 | -3 | -2 | -2 | 1 | 4 |

# Is it Really Necessary?

| Code | Häufig-keit | Mutier-barkeit |
|---|---|---|
| L | 0.091 | 54 |
| A | 0.077 | 100 |
| G | 0.074 | 50 |
| S | 0.069 | 117 |
| V | 0.066 | 98 |
| E | 0.062 | 77 |
| K | 0.059 | 72 |
| T | 0.059 | 107 |
| I | 0.053 | 103 |
| D | 0.052 | 86 |
| P | 0.051 | 58 |
| R | 0.051 | 83 |
| N | 0.043 | 104 |
| Q | 0.041 | 84 |
| F | 0.040 | 51 |
| Y | 0.032 | 50 |
| M | 0.024 | 93 |
| H | 0.023 | 91 |
| C | 0.020 | 44 |
| W | 0.014 | 25 |

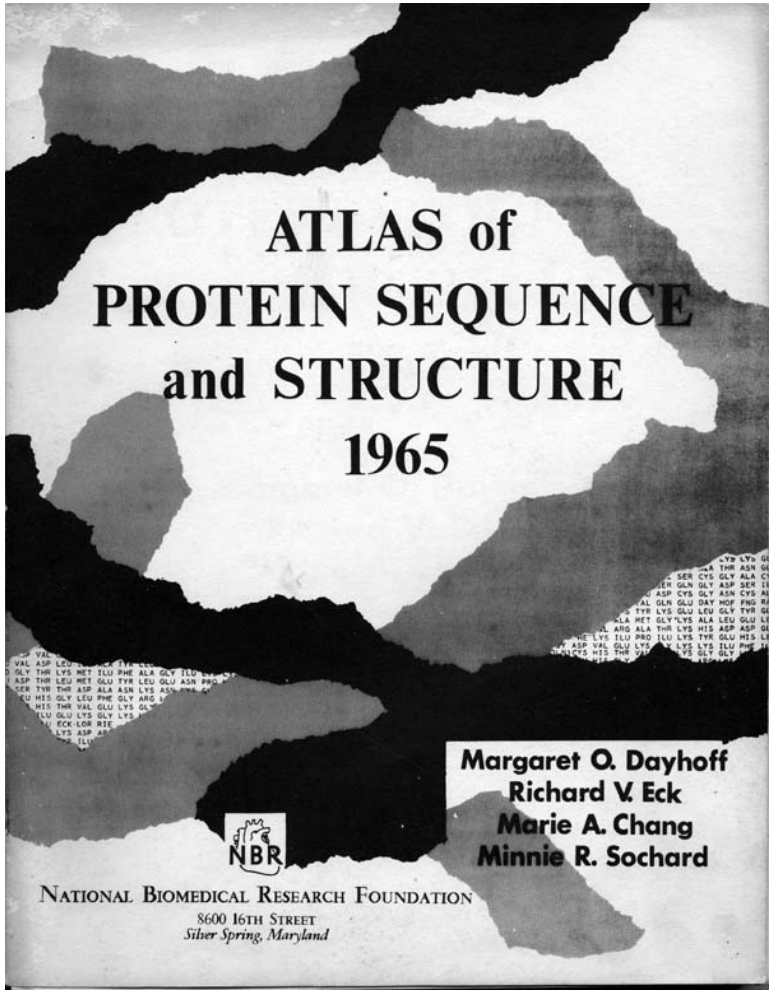
- We count how often a particular AA was replaced by any other AA
  - Using “sure” sequence alignments
- Replacement rate of Alanin (A) := 100%
- Obviously no equal distribution
- Even if we assume that mutations happen more or the less at the same rate, they obviously don’t survive at the same rate
  - Mutations are suppressed to different degrees
  - W (Tryptophan): Strong suppression
  - S (Serin): Little suppression

# Filling a Substitution Matrix for Protein Sequences

- We need app. 200 values
  - Assuming a symmetric matrix
- Possibility 1: Analytical
  - Capture weight, polarity, size, ...
  - Find a scoring scheme to measure the difference between two AA
  - Needs to produce a single value per AA pair
  - Not used in practice
- Possibility 2: Empirical
  - Count which substitutions survived at which frequency in reality
  - Needs true alignments: Pairs of homologues and aligned sequences

# Margaret O. Dayhoff

- Goal: “Deduce evolutionary relationships of the biological kingdoms, phyla, and other taxa from sequence evidence”
- Collection of all known protein sequences
  - First edition: 65 proteins
  - Several releases followed
  - Resulted in the Protein Information Resource (PIR)




Thanks to Antje Krause

# PAM: Point-Accepted Mutations

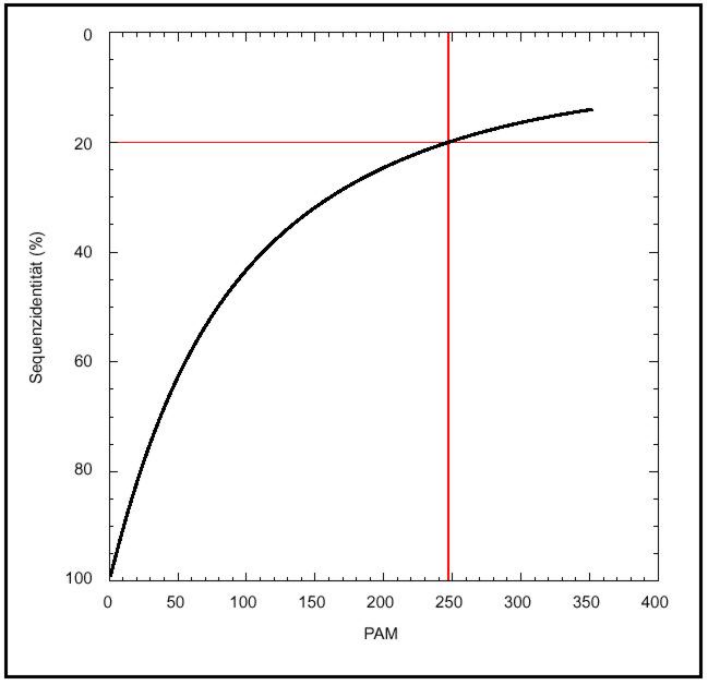
- Dayhoff, M. O., R. V. Eck, C. M. Park. (1972)
  *A model of evolutionary change in proteins.*
  in M. O. Dayhoff (ed.), Atlas of Protein Sequence and Structure Vol. 5.
- PAM has two meanings
  - 1 PAM – Unit for measuring the similarity of two AA sequences
  - PAM-X matrix – Substitution matrix to use when aligning two sequences that are X PAM distant

# PAM as Distance Measure

- Definition
  Let *$S_1$, $S_2$ be two protein sequences with $|S_1|=|S_2|$. We say $S_1$ and $S_2$ are x PAM distant, iff $S_1$ most probably was produced from $S_2$ with x mutations per 100 AAs*
- Remarks
  - PAM is motivated by evolution
  - Assumptions: Mutations happen with the same rate at every position of a sequence
  - If mutation rate is high, mutations will occur again and again at the same position
  - PAM ≠ %-sequence-identity

# PAM as Distance Measure

- No INDELS, only replacements
- The PAM distance d of two DNA sequences can be derived analytically from their %-sequence-diversity p
  - d = -3/4*ln(1-4/3*p) (Jukes-Cantor model)
  - Derivation skipped
- Pairs with PAM >250 are probably not homologues
  - %-sequence-identity < 20%
  - Twilight zone
  - Which %-sequence-identity will two random protein sequences have?



# PAM Matrices

- The PAM-X matrix contains measures for the probability that a given AA was replaced by another given AA in two sequences that are x PAM distant
- Estimated from data
  - Let $(S_{1,1}, S_{2,1})$, ..., $(S_{1,n}, S_{2,n})$ be n x-PAM distant pairs of aligned sequences
  - Compute f(i), the relative frequency of AA $A_i$ in all pairs
  - Compute f(i,j), the relative substitution frequency of $A_i$ and $A_j$
    - Number of positions k in any of the aligned pairs with $S_{1,z}$'[k]=$A_i$ and $S_{2,z}$'[k]=$A_j$ or vice versa
  - Then

$$M_x(i,j) = \log\left(\frac{f(i,j)}{f(i) * f(j)}\right)$$

# Some Explanations

- Log-likelihood ratio combining
  - Expectation: chances to generate this mutation by chance given the relative frequencies of the two involved AAs
  - Observation: observed frequency of this mutation

$$M_x(i, j) = \log\left(\frac{f(i, j)}{f(i) * f(j)}\right)$$

- Meaning
  - M(i,j) = 0: No selection
  - M(i,j) < 0: Negative selection, suppression of mutation
  - M(i,j) > 0: Positive selection, mutation is favored

# Example

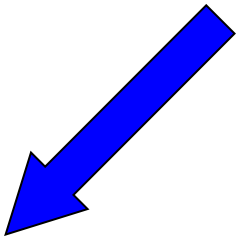
$S_{1,1}$: **ACGTGAC**
$S_{2,1}$: **AGGTGCC**

$S_{1,2}$: **GTTAGTA**
$S_{2,2}$: **TTTAGTA**

$S_{1,3}$: **GGTCA**
$S_{2,3}$: **AGTCA**

## Relative frequencies

| A: 10/38 | C: 6/38 | G: 11/38 | T: 11/38 |
|---|---|---|---|



## Mutation rates

| | A | C | G | T |
|---|---|---|---|---|
| A | 4/19 | 1/19 | 1/19 | 0/19 |
| C | | 2/19 | 1/19 | 0/19 |
| G | | | 4/19 | 1/19 |
| T | | | | 5/19 |

## Matrix

| | A | C | G | T |
|---|---|---|---|---|
| A | 0,48 | 0,10 | -0,16 | - |
| C | | 0,63 | 0,06 | - |
| G | | | 0,40 | -0,20 |
| T | | | | 0,50 |

# Problems

- Depends on predefined alignments
- We need a substitution matrix to find optimal alignments
  - A hen-egg problem
  - Alternative: Do it manually using experience, 3D-structure, ..
- Makes several assumptions
  - Mutations are equally likely at every position in a sequence
  - Mutations are equally likely independent from AA neighbors
  - ...

# Real Substitution Matrices

- PAM requires large n for each x to adequately capture rare mutations
- Dirty trick: Molecular clock assumption
  - Assume that mutations appear with equal rate over time
  - Then the frequencies of PAM-x mutations depend linearly on the frequencies of PAM-1 mutations
  - PAM-x matrices are computed by repeated matrix multiplication of PAM-1 with itself
- Complete, highly heuristic procedure
  - Choose set of n pairs with small distance and align manually
  - Use these alignments to compute $M_1$
  - Compute $M_x = (M_1)^x$

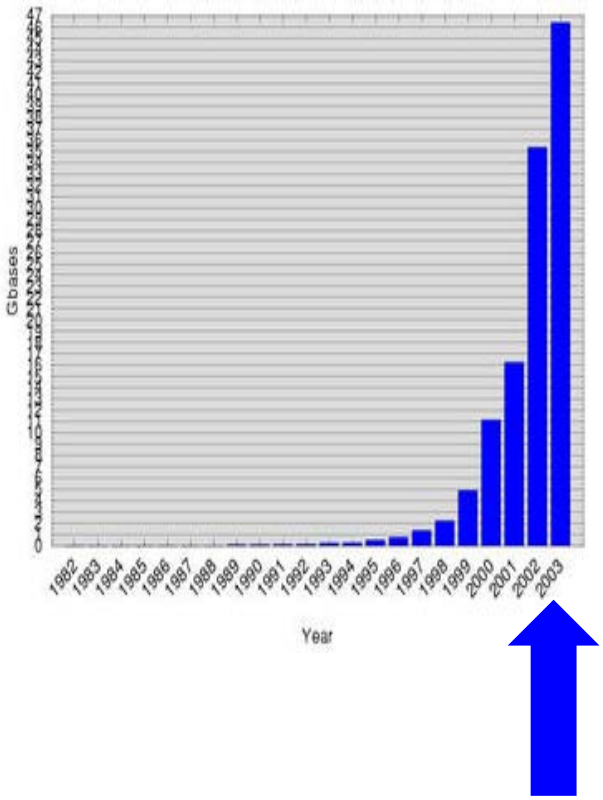
# BLOSUM

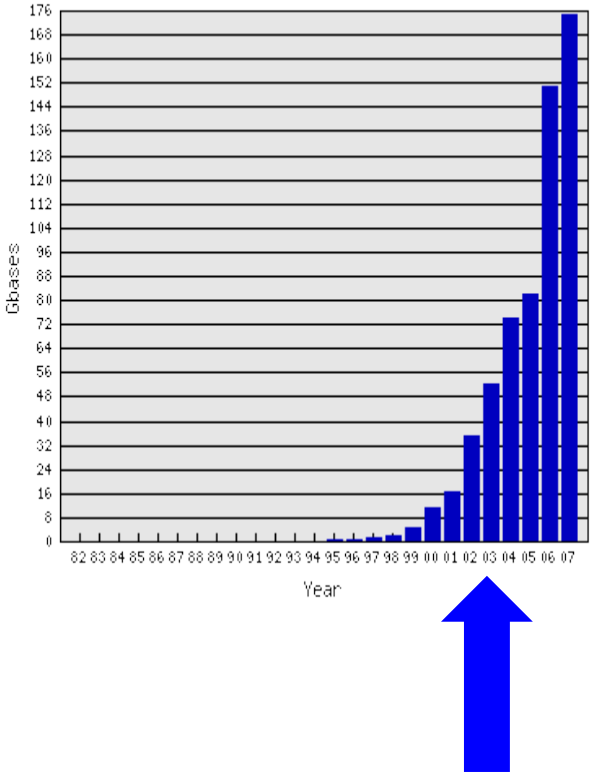
- PAM is a bit old-fashioned
- BLOSUM: BLOcks SUbstitution Matrix
  - Henikoff and Henikoff, 1993
  - Removes assumption of equal mutation rates across each sequence position by considering conserved blocks
  - Direct estimation for different PAM distances instead of error-propagating self multiplication

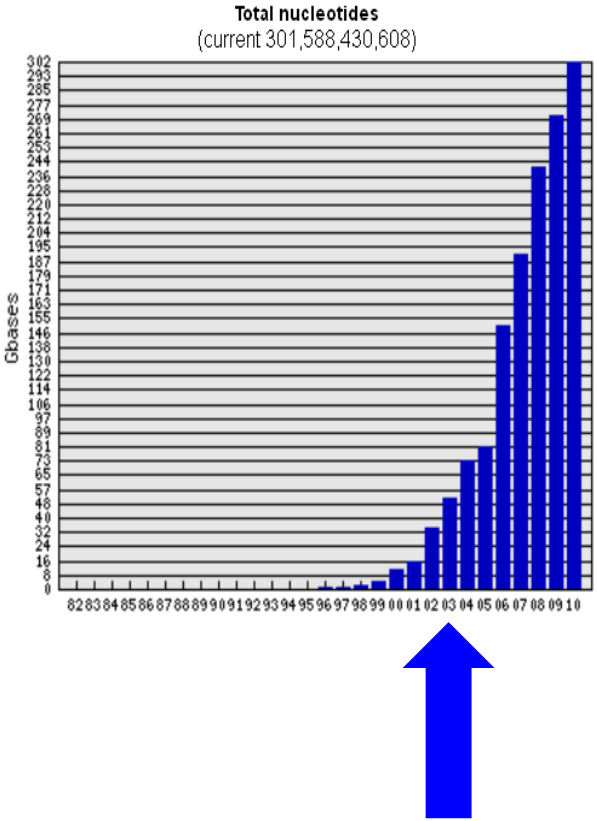
# This Lecture

- Substitution Matrices
  - PAM distance
  - PAM matrices
- Scaling up Local Alignments
  - BLAST

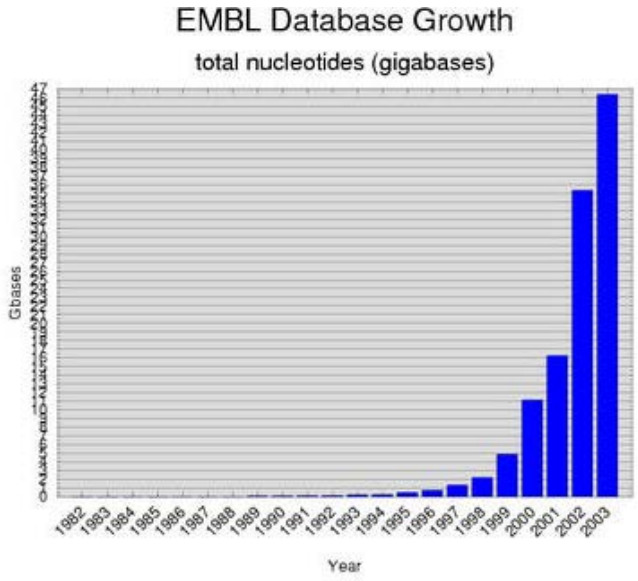
# Growth of EMBL

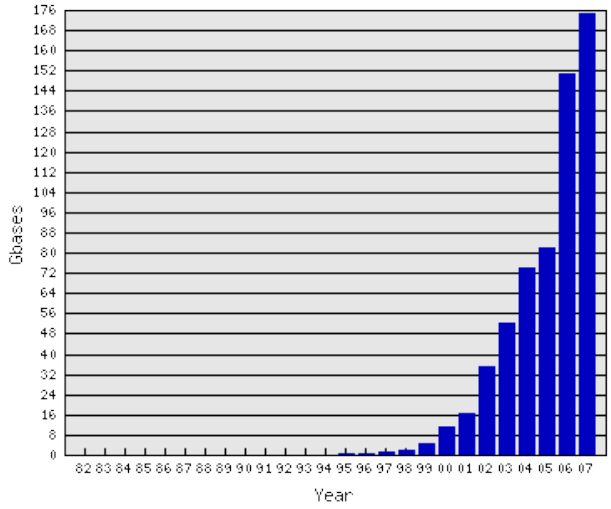


# Scaling Up Local Alignment




- Searching similar sequences (with a high local alignment score) is a fundamental operation in Bioinformatics
- Sequence databases grow exponentially
- We need faster algorithms, even if they sometimes fail

# Similarity Search Problems and their Accuracy

- Task: Given a sequence s and a database D, find all sequences T in D that are sufficiently local-similar to s
  - Often, exactly computing T is not feasible and not necessary (think of the WWW and search engines)
- Assume a method that finds a set X of answers for s
- How good is this method?
  - Some sequences will be in X and T – true positives
  - Some will be in X but not T – false positives
    - Also called Type I error
  - Some will be in T but not X – false negatives
    - Also called Type II error
  - Some will be neither in X nor T – true negatives

| | | Reality | |
|---|---|---|---|
| | | + | - |
| Prediction | + | TruePositive (TP) | FalsePositive (FP) |
| | - | FalseNegative (FN) | TrueNegative (TN) |

# Precision and Recall

- Precision = TP/(TP+FP)
  - What is the fraction of correct answers in X?
  - Related to specificity

| Prediction \ Reality | + | - |
|---|---|---|
| + | TruePositive (TP) | FalsePositive (FP) |
| - | FalseNegative (FN) | TrueNegative (TN) |

- Recall = TP/(TP+FN)
  - Which fraction of correct answers from T are also in X?
  - Also called sensitivity
- Trade-Offs
  - Usual methods compute a score per element of D
  - All sequences with a score above a threshold t are returned as X
  - Increasing t : higher precision, lower recall
  - Lowering t: lower precision, higher recall
  - ... if the score correlates with correctness ...

# Example

- Let |DB| = 1000, |X|=15, |T|=20, |X∩T|=9

| | Real: Positive | Real: Negative |
|---|---|---|
| Alg: Positive | TP = 9 | FP = 6 |
| Alg: Negative | FN = 11 | TN= 974 |

  - Precision = TP/(TP+FP) = 9/15 = 60%
  - Recall = TP/(TP+FN) = 9/20 = 45%

- Assume we increase t: |X|=10, |X∩T|=7

| | Real: Positive | Real: Negative |
|---|---|---|
| Alg: Positive | TP = 7 | FP = 3 |
| Alg: Negative | FN = 13 | |

  - Precision: 70%, recall = 35%

# BLAST

- Altschul, Gish, Miller, Myers, Lipman: „Basic Local Alignment Search Tool“, J Mol Bio, 1990
  - A heuristic algorithm for sequence similarity search
  - Very fast, high recall, not perfect
  - Very successful: You “blast” a sequence
  - NCBI runs thousands of BLAST searches every day
- A family of tools
  - Gapped-BLAST, PSI-BLAST, MegaBlast, BLAST-ALL, PATHBLAST, Name-BLAST, ...
  - BLAST for DNA, protein, DNA-protein, protein-DNA, ...
  - We only look at the simple DNA-DNA version
  - We skip several heuristic and domain-specific tricks

# Fundamental Idea

- Fundamental idea : If two sequences have a good local alignment, then the matching area contains, with very high probability, a sub-area where the match is even better (or even exact)
- These sub-areas are called seeds

```
TTGACTCGATTATAGTCGCGGATATACTATCG
CCTATCACAAGAATATAGTCCCTGATCCAGC

   TTGACTC GATTATAGTCGCGGAT ATACTATCG
CCTATCACAA GAATATAGTCCCTGAT CCAGC

   TTGACTC GATTATAGTCGCGGAT ATACTATCG
CCTATCACAA GAATATAGTCCCTGAT CCAGC
```

# Algorithm

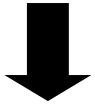
- Given query sequence s and sequence database $D=\{d_j\}$
- 1. Compute all substrings $s_i$ of s of length q
  - Also called q-grams
  - How many?
- 2. Find all approximate occurrences of all $s_i$ in all $d_j$
  - Gap-free alignment with matrix; score must be above threshold t
  - Hits are called seeds –approx. occurrences of some $s_i$ in some $d_j$
- 3. Extend seeds to left and right in $s_i$ and $d_j$ until
  - [Constantly updating the similarity score]
  - ... the score drops sharply
  - ... s or $d_j$ ends
  - ... the score gets too bad compared to other hits found earlier

# Example

```
q=5, t=3, Matrix: M=+1, R=-1
s=ACGTGATA
d=GATTGACGTGACTGCTAGTGATACTATAT
```

$s_1$=ACGTG
$s_2$=CGTGA
$s_3$=GTGAT
$s_4$=TGATA

```
GATTGACGTGACTGCTAGTGATACTATAT
GATTGACGTGACTGCTAGTGATACTATAT
GATTGACGTGACTGCTAGTGATACTATAT
GATTGACGTGACTGCTAGTGATACTATAT
```



```
GATTGACGTGACTGCAAGTGATACTATAT
      ACGTGATA                  5
      ACGTGATA                  5+1=6
      ACGTGATA                  6-1=5
      ...                       ...
```

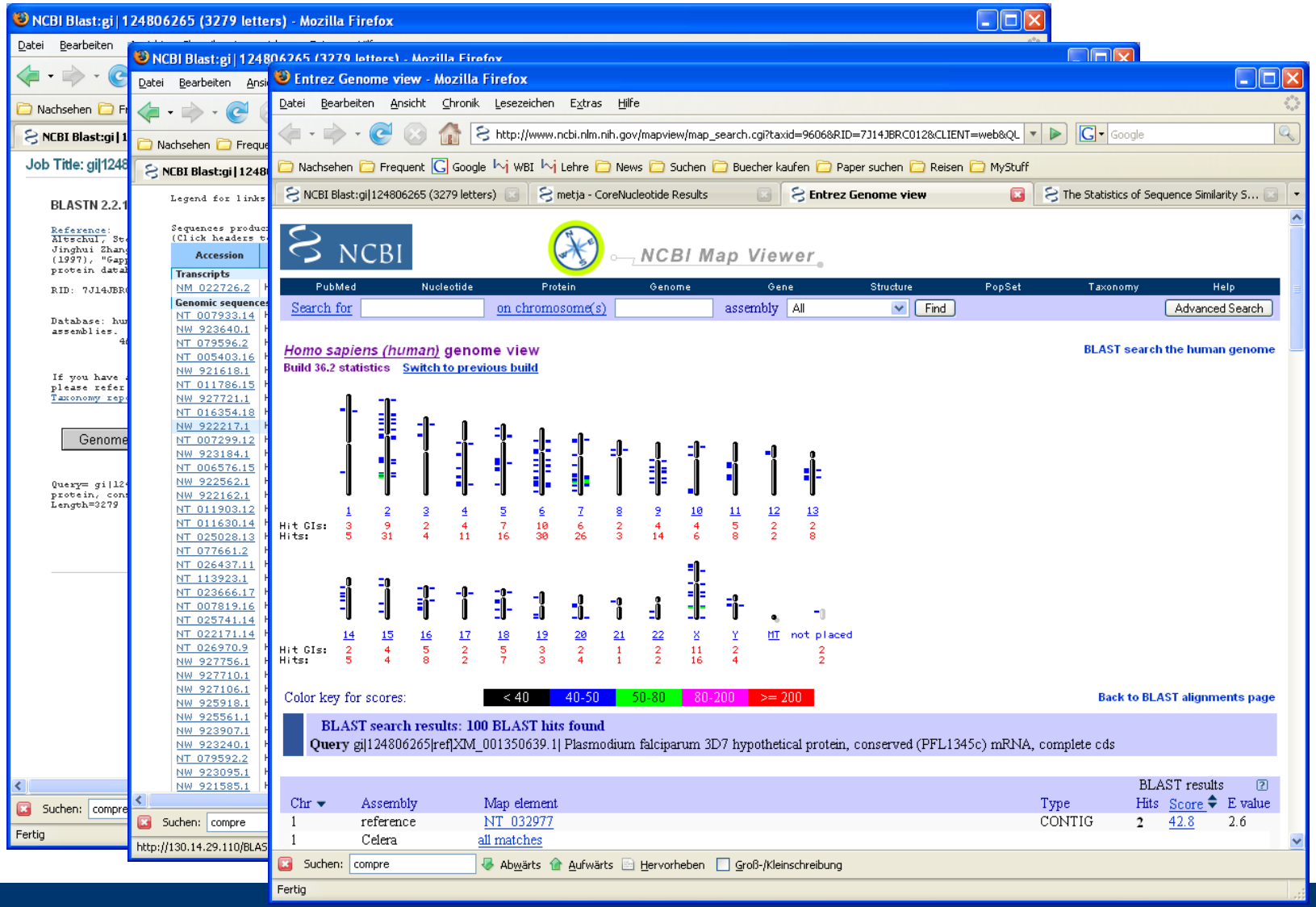
# Properties

- Finding seeds efficiently requires more work
  - Pre-compute all q-grams of all $d_j$
  - Group by q-gram
  - Called a hash-index (should be kept in main memory)
  - Lookup: Given s, find all matching q-grams (as seeds)
- Exclusion method
  - Vast majority of all sequences in DB are never looked at because they do not contain a seed
  - The “seed” idea is the basis of nearly all fast alignment methods
- Where it fails
  - Sensitive to t: Too high – missing hits; too low – slow
  - Does not consider gaps

# Speed – Precision - Recall

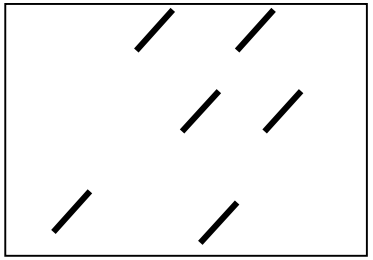
- Increasing t
  - Higher requirements for any seed
  - Less seeds, less extensions
  - Lower recall, higher speed, precision stays
- Increasing q (and adapting t)
  - Higher requirements for any seed
  - Less seeds, less extensions
  - Lower recall, higher speed, precision stays

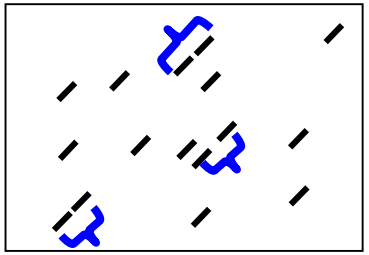
# BLAST Screenshots

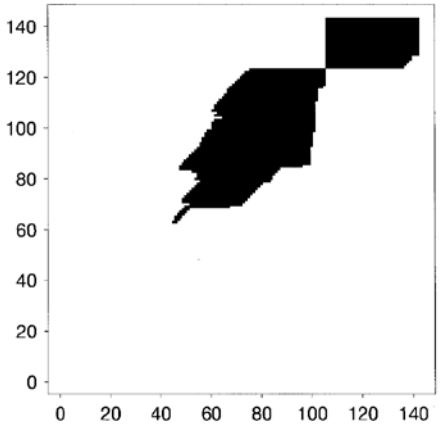


# BLAST-2

- Altschul, Madden, Schaffer, Zhang, Zhang, Miller, Lipman: „Gapped BLAST and PSI-BLAST: a new generation of protein database search programs“, NAR, 1997
- Faster
  - BLAST: 90% of time spend in extensions
  - BLAST2: Two seeds in short distance
    - Needs a decrease in t
- Higher recall
  - BLAST didn’t even consider gaps in the extension phase
  - BLAST2: Full local alignment starting from a central position between the two seeds
    - Allows an increase of t







# Further Reading

- Substitution matrixes: Krane & Raymer, Chapter 3
- BLAST, BLAST2: Merkl & Waack, Chapter 12