PAM and BLAST

Ulf Leser

Klausurtermin

- Montag, 27.8.2018, 11-14 (11.30 – 13.30) Uhr
- Raum: 3.001
- Keine Hilfsmittel erlaubt

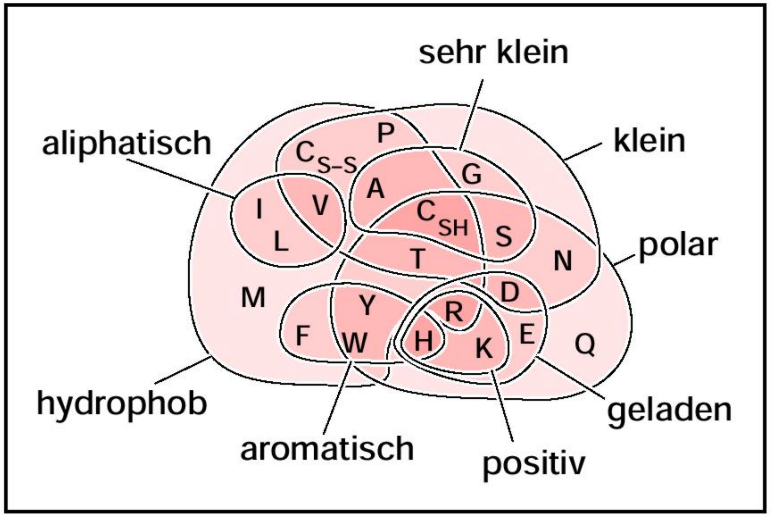
- Anmelden
- Übungsschein
- Mündliche Prüfungen

This Lecture

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

Substitution Matrices

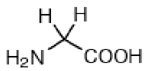
- Recall
  - A scoring function (substitution matrix) is a function s: Σ'xΣ' → N
- DNA: symmetric, simple matrices
- Protein sequences are different
  - Different AA have very different properties
  - Substitutions may change the 3D structure completely or just a little bit or not at all



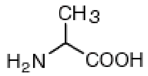
Amino Acids

Klein, leicht

Small

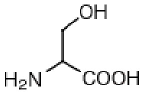


Glycine (Gly, G)
MW: 57.05

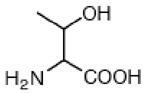


Alanine (Ala, A)
MW: 71.09

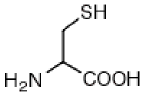
Nucleophilic



Serine (Ser, S)
MW: 87.08, pK_a ~ 16

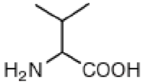


Threonine (Thr, T)
MW: 101.11, pK_a ~ 16

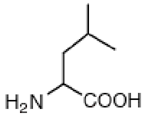


Cysteine (Cys, C)
MW: 103.15, pK_a = 8.35

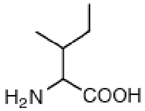
Hydrophobic



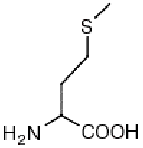
Valine (Val, V)
MW: 99.14



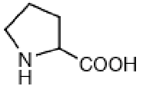
Leucine (Leu, L)
MW: 113.16



Isoleucine (Ile, I)
MW: 113.16



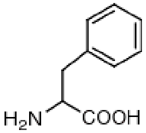
Methionine (Met, M)
MW: 131.19



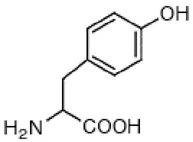
Proline (Pro, P)
MW: 97.12

Groß, schwer

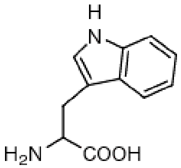
Aromatic



Phenylalanine (Phe, F)
MW: 147.18

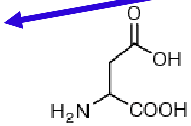


Tyrosine (Tyr, Y)
MW: 163.18

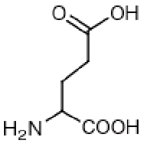


Tryptophan (Trp, W)
MW: 186.21

Acidic

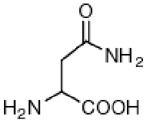


Aspartic Acid (Asp, D)
MW: 115.09, pK_a = 3.9

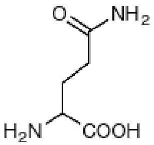


Glutamic Acid (Glu, E)
MW: 129.12, pK_a = 4.07

Amide

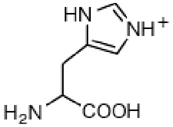


Asparagine (Asn, N)
MW: 114.11

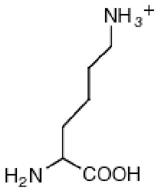


Glutamine (Gln, Q)
MW: 128.14

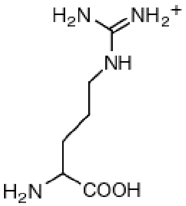
Basic



Histidine (His, H)
MW: 137.14, pK_a = 6.04



Lysine (Lys, K)
MW: 128.17, pK_a = 10.79



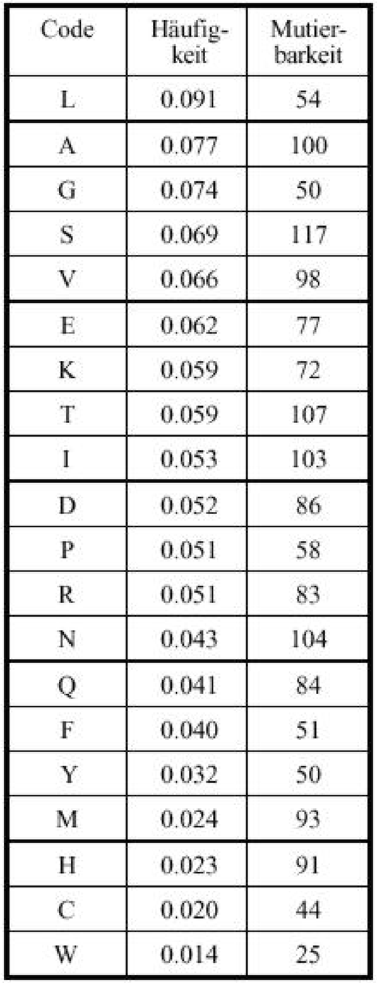
Arginine (Arg, R)
MW: 156.19, pK_a = 12.48

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

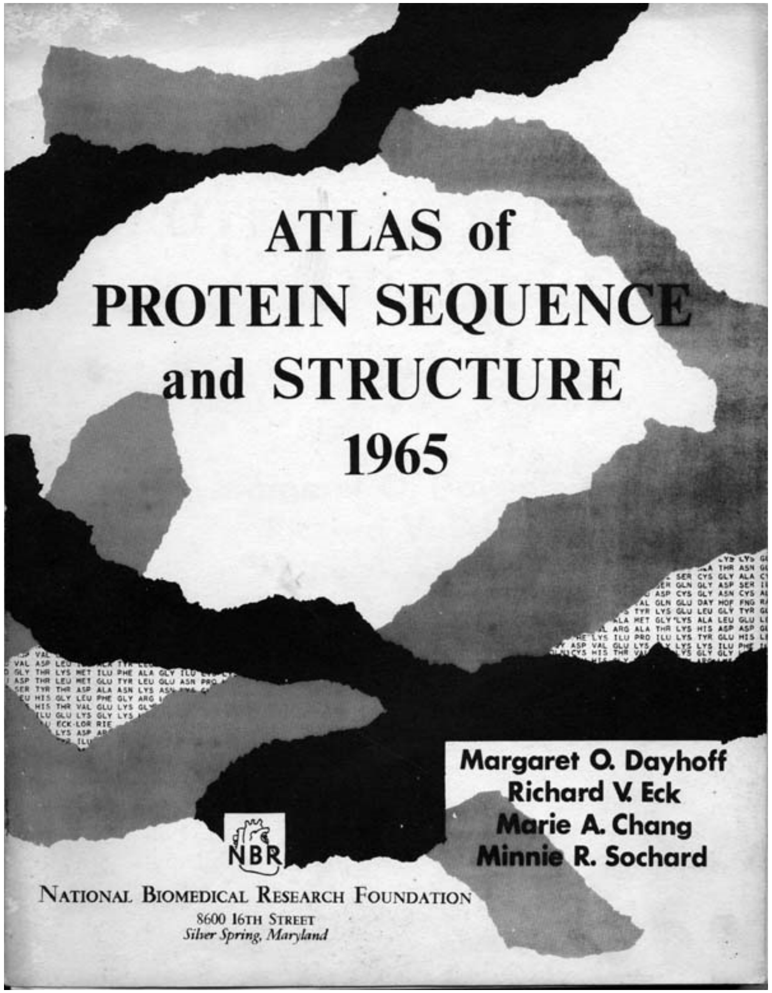
- We count how often a particular AA was replaced by any other AA
  - Using "sure" sequence alignments
- Replacement rate of Alanin (A) := 100%
- Obviously no equal distribution
- Even if we assume that mutations happen more or the less at the same rate, they obviously don't survive at the same rate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

Filling a Substitution Matrix for Protein Sequences

- We need app. (20*20)/2=200 values
  - Scoring functions should be symmetric
- 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)



ATLAS of PROTEIN SEQUENCE and STRUCTURE 1965

Margaret O. Dayhoff
Richard V. Eck
Marie A. Chang
Minnie R. Sochard

NBR

NATIONAL BIOMEDICAL RESEARCH FOUNDATION
8600 16TH STREET
Silver Spring, Maryland

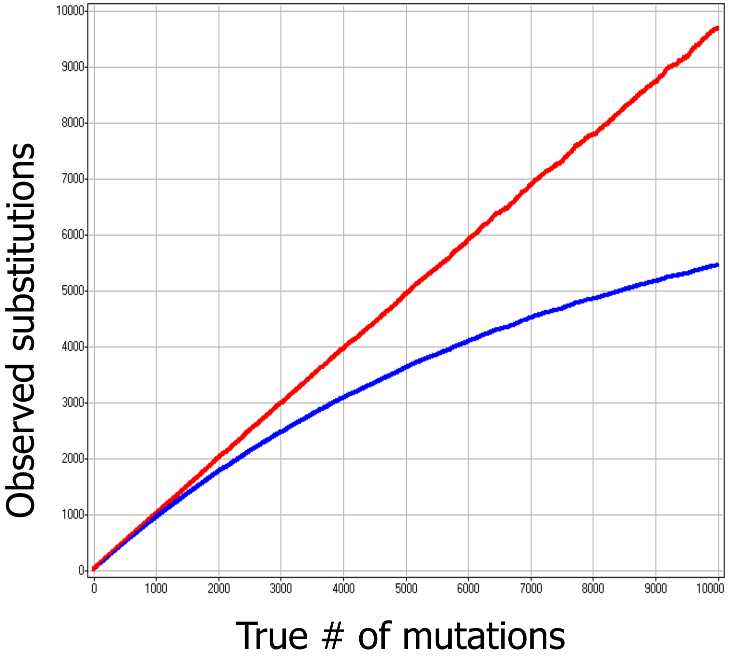
Thanks to Antje Krause

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

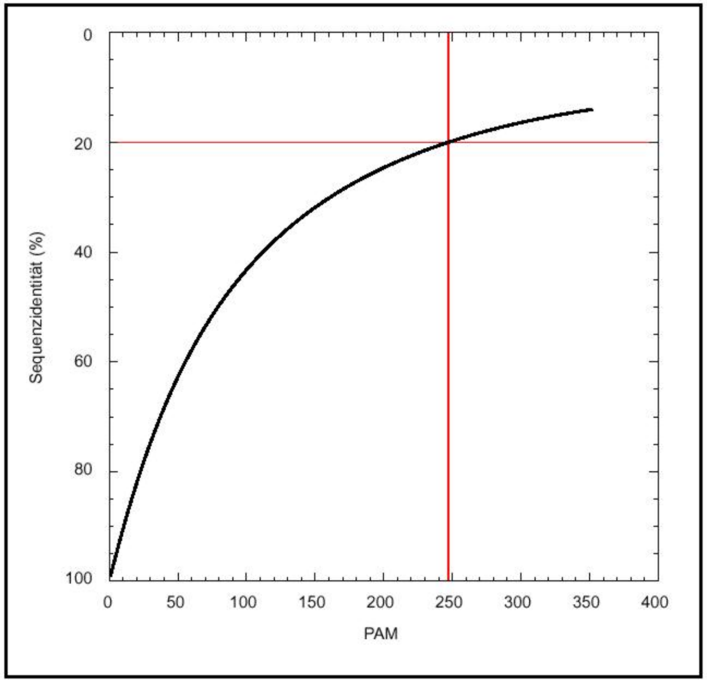
PAM as Distance Measure

- 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 or time is long, mutations will occur at the same positions
  - 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)
  - p = 1-"sequence identity"
  - 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 AA (column) was replaced by another AA (row) 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 on the frequencies of PAM-1 mutations
  - PAM-x matrices are computed by repeated matrix multiplication of PAM-1 with itself (assuming a linear relationship)
- The complete (highly heuristic) procedure
  - Choose set of n pairs with small PAM distance and align manually
  - Use these alignments to compute M_1
  - Compute $M_x = (M_1)^x$

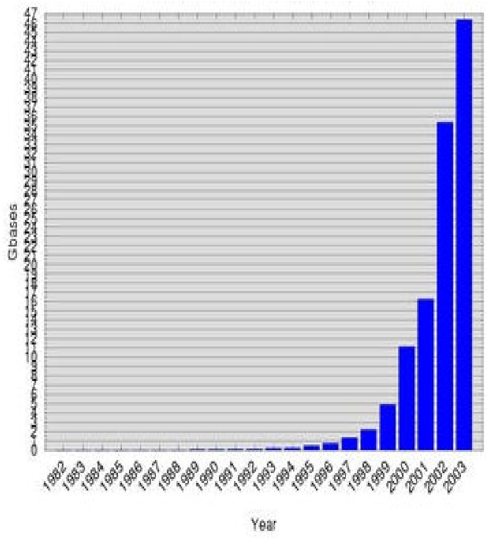
BLOSUM

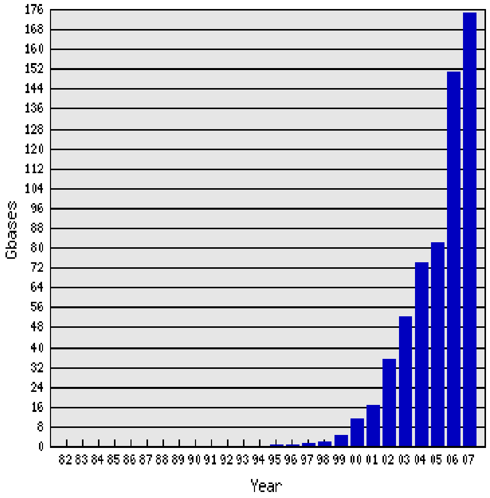
- PAM is a bit old-fashioned
- BLOSUM: BLOcks SUbstitution Matrix
  - Henikoff and Henikoff, 1993
  - Removes assumption of equal mutation rates across each sequence position by considering conserved blocks
  - Direct estimation for different PAM distances instead of error-propagating self multiplication

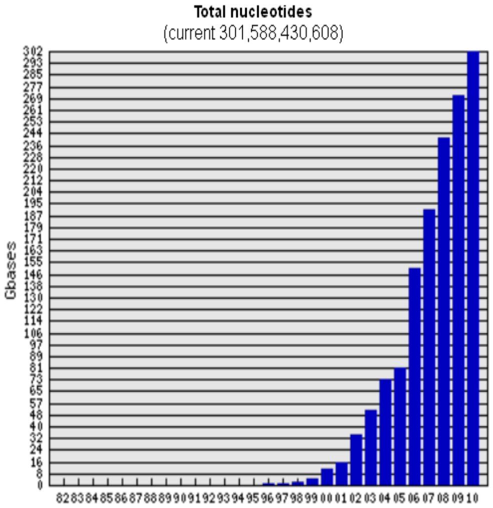
This Lecture

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

Scaling Up Local Alignment



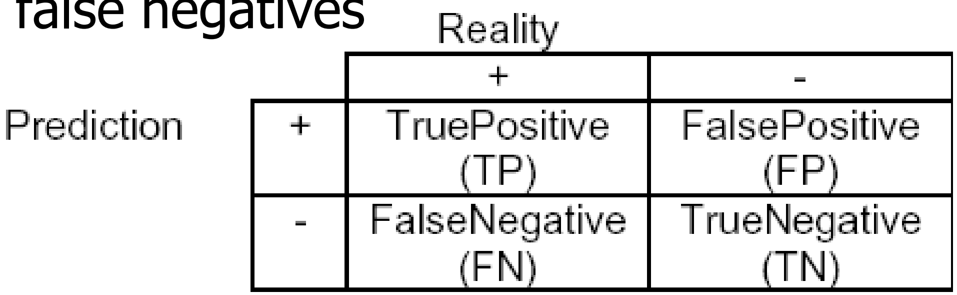



Total nucleotides
(current 301,588,430,608)

- 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

		Reality	
		+	-
Prediction	+	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, $|X \cap T|=9$

	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, $|X \cap T|=7$

	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
```

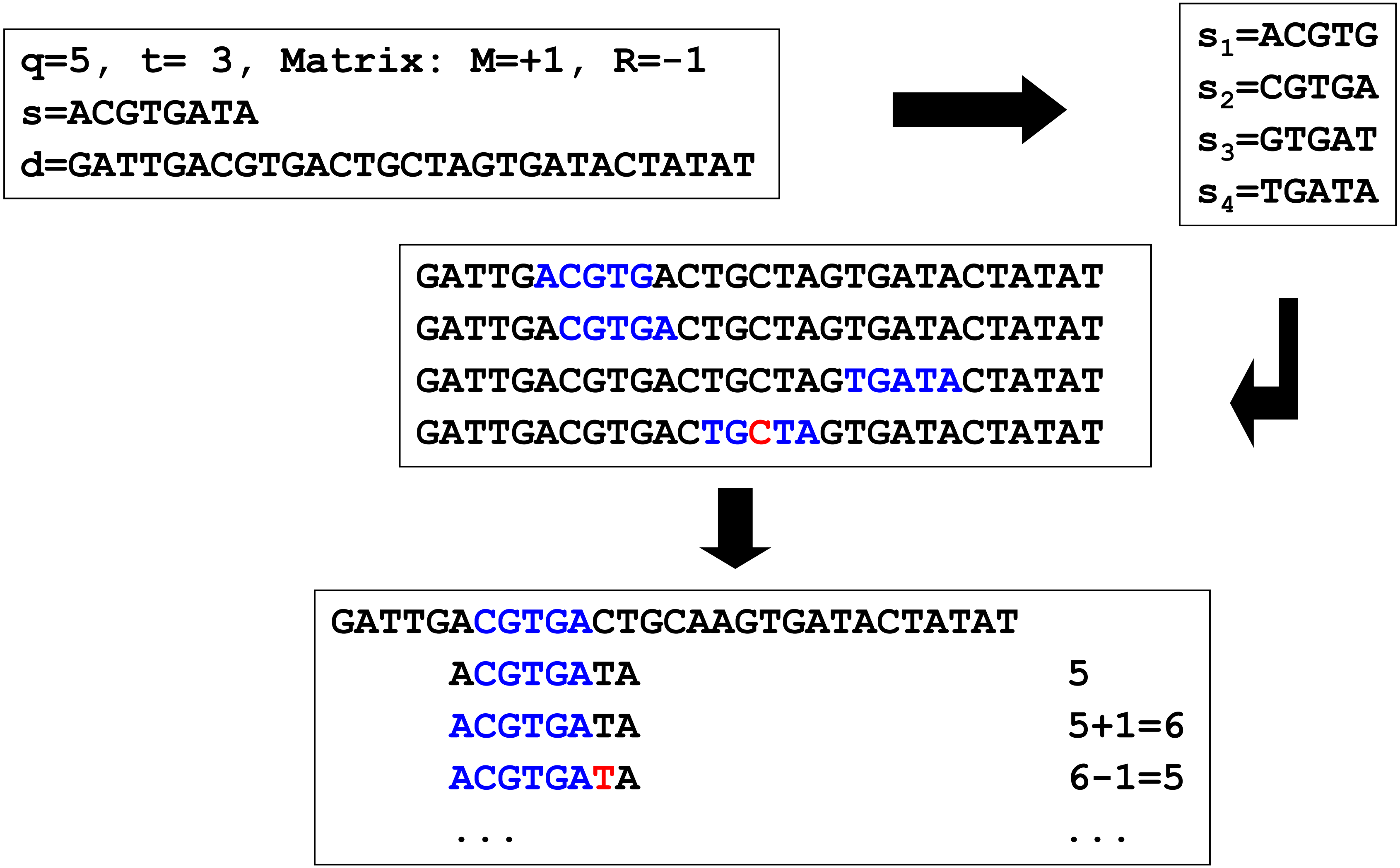
```
   TTGACTC GATTATAGTCGCGGAT ATACTATCG
CCTATCACAA GAATATAGTCCCTGAT CCAGC
```

```
   TTGACTC GATTATAGTCGCGGAT ATACTATCG
CCTATCACAA GAATATAGTCCCTGAT CCAGC
```

Algorithm

- Given query sequence s and sequence database $D=\{d_i\}$
- 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 d_j
- 3. Extend seeds to left and right in s_i and d_j until
  - [Constantly update the similarity score]
  - … the score drops sharply
  - … s_i 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
     ...                         ...
```

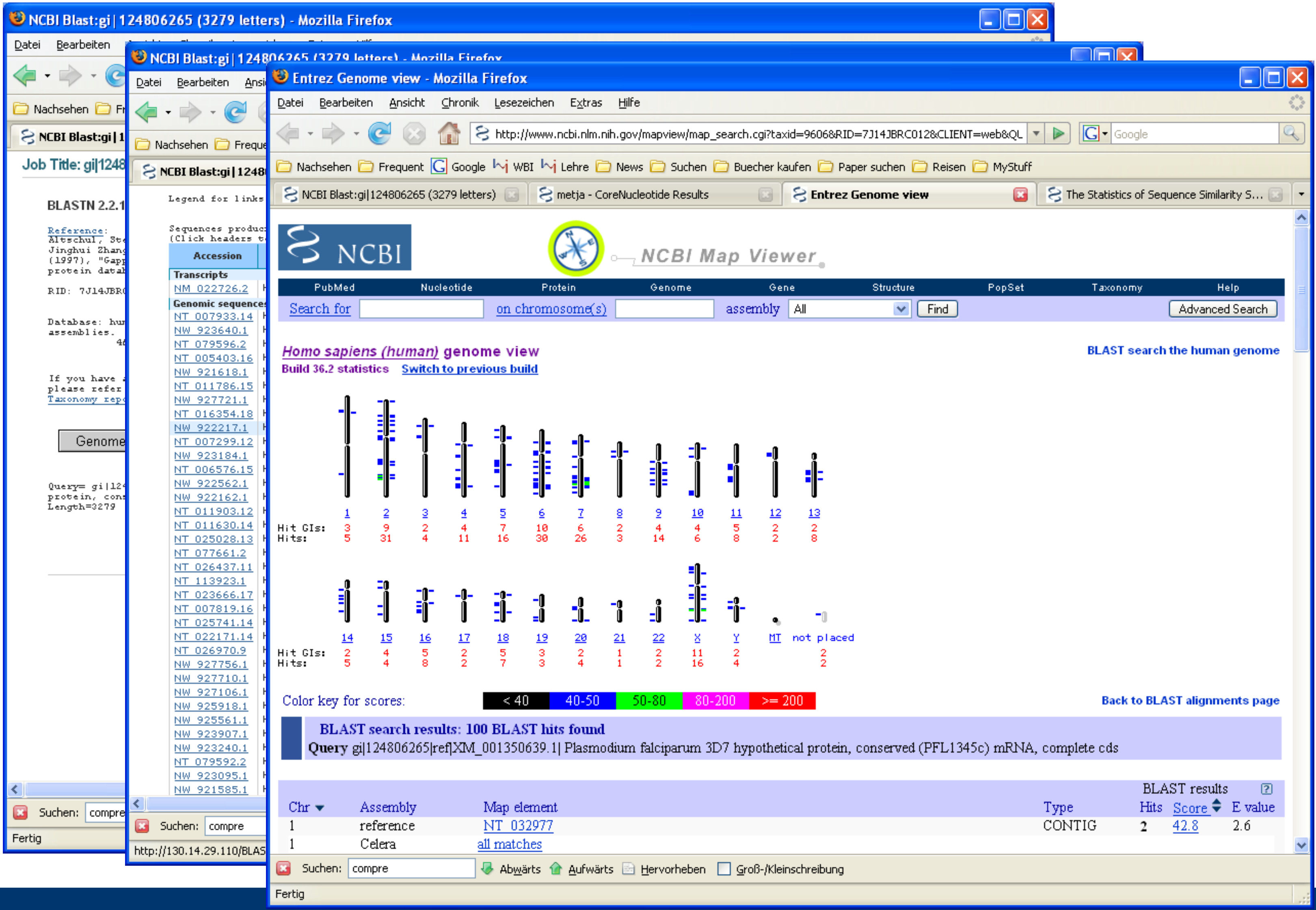
Properties

- Finding seeds efficiently requires more work
  - Pre-compute all q-grams of all d_i
  - 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



NCBI Blast:gi|124806265 (3279 letters) - Mozilla Firefox

Job Title: gi|1248

BLASTN 2.2.1

Reference: Altschul, Ste Jinghui Zhang (1997), "Gapp protein datab

RID: 7J14JBR

Database: hu assemblies. 46

If you have please refer Taxonomy repo

Query= gi|12 protein, cons Length=3279

Legend for links

Sequences produc (Click headers t

Accession
Transcripts
NM_022726.2
Genomic sequence
NT_007933.14
NW_923640.1
NT_079596.2
NT_005403.16
NW_921618.1
NT_011786.15
NW_927721.1
NT_016354.18
NW_922217.1
NT_007299.12
NW_923184.1
NT_006576.15
NW_922562.1
NW_922162.1
NT_011903.12
NT_011630.14
NT_025028.13
NT_077661.2
NT_026437.11
NT_113923.1
NT_023666.17
NT_007819.16
NT_025741.14
NT_022171.14
NT_026970.9
NW_927756.1
NW_927710.1
NW_927106.1
NW_925918.1
NW_925561.1
NW_923907.1
NW_923240.1
NT_079592.2
NW_923095.1
NW_921585.1

http://130.14.29.110/BLAS

Entrez Genome view - Mozilla Firefox

http://www.ncbi.nlm.nih.gov/mapview/map_search.cgi?taxid=9606&RID=7J14JBRC012&CLIENT=web&QL

NCBI Map Viewer

Search for [ ] on chromosome(s) [ ] assembly All Find Advanced Search

Homo sapiens (human) genome view

Build 36.2 statistics Switch to previous build

BLAST search the human genome

	1	2	3	4	5	6	7	8	9	10	11	12	13
Hit GIs:	3	9	2	4	7	10	6	2	4	4	5	2	2
Hits:	5	31	4	11	16	30	26	3	14	6	8	2	8

	14	15	16	17	18	19	20	21	22	X	Y	MT	not placed
Hit GIs:	2	4	5	2	5	3	2	1	2	11	2		2
Hits:	5	4	8	2	7	3	4	1	2	16	4		2

Color key for scores: < 40 | 40-50 | 50-80 | 80-200 | >= 200

Back to BLAST alignments page

BLAST search results: 100 BLAST hits found

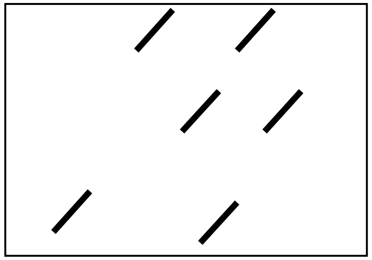
Query gi|124806265|ref|XM_001350639.1| Plasmodium falciparum 3D7 hypothetical protein, conserved (PFL1345c) mRNA, complete cds

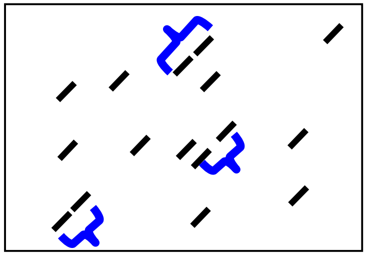
Chr	Assembly	Map element	Type	Hits	Score	E value
1	reference	NT_032977	CONTIG	2	42.8	2.6
1	Celera	all matches				

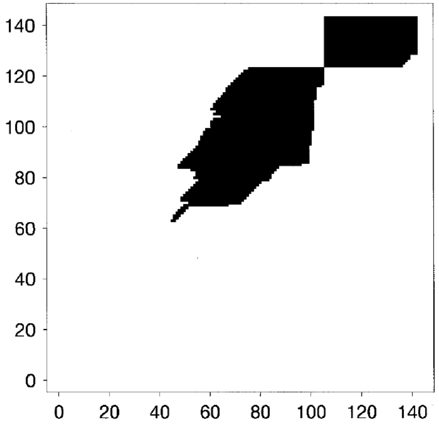
Suchen: compre

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 seeds
    - Allows an increase of t







Further Reading

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