Bioinformatics Algorithms

(Fundamental Algorithms, module 2)

Zsuzsanna Lipták

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Scoring Matrices

More complex scoring functions

Until now:

- match, mismatch, gap (linear gap functions)
- match, mismatch, gap open, gap extend (affine gap functions)
- i.e. $f(a, b)$ depends only on $a = b$ or $a \neq b$

But:

- For protein sequences, better to differentiate between different pairs of AAs a and b, i.e. depending on how close / how different they are.
- Reason: homologous proteins often have different AAs in same position. If only match/mismatch are evaluated, then many homologous proteins are not found.

So now:

- $f(a, b)$ depends on a and b
- necessarily: $f(a, b) = f(b, a)$ (symmetry)

Scoring matrices

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  1. similarity of codon (DNA-level), e.g. $\min\{dist_{Hamming}(xyz, uvw)\ :\ xyz$ codon for a and uvw codon for $b\}$
  2. physico-chemical properties (hydrophobicity, size, basic/acidic, ...)
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  3. based on empirical data: How frequently do we observe this change?
- PAM matrices: Scoring matrices based on empirical data (Margret Dayhoff, 1978)
- PAM = Point Accepted Mutation (or: Percent Accepted Mutation)

Basic idea:

- $S_{ab} > 0$: probability that b has mutated into a at this evolutionary distance is greater than chance
- $S_{ab} = 0$: the two probabilities are equal (we cannot say anything)
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Meaning of "by chance":

- We are comparing two probabilities
- prob1: that a and b are aligned together because there has been a series of mutations changing b into a
- prob2: that a and b have been aligned together by chance (e.g. if in the database all sequences consist only of a's, then the probability that a is there in a random alignment is 1)

PAM scoring matrices

- family of matrices: PAMk (for any $k \geq 1$), common are PAM40, PAM120, PAM250
- PAMk: k is the evolutionary distance between the sequences to be scored; needs to be guessed *before* scoring
- higher k: applied to more distant / less closely related sequences / species
- the scoring matrix PAMk is not a probability matrix
- it is based on a probability matrix

Mutation probability matrix

- Dayhoff et al. generated mutation probability matrix M (PAM1 mutation matrix) based on empirical data: a large set of aligned sequences which are known to be homologous ("trusted alignments")
- M_{ab} = probability that AA b will change into AA a in one time step
- this probability is only estimated, based on observed data
- one time step = 1 PAM unit evolutionary distance = 1 mutation every 100 AAs on average
- $\sum_{a \in \Sigma} M_{ab} = 1$ (sum over each column equals 1) [1]

[1] in some areas of maths prob. matrices are defined differently: $P_{a,b}$ = prob. that a turns into b, i.e. the transpose of M; then the sum over the *rows* is 1

Mutation probability at higher distances: M^k

- How about the probability that b changes into a in 2 steps?
- possibilities are:

time step 1	time step 2
$b \to a$	a unchanged
b unchanged	$b \to a$
$c \neq a, b$: $b \to c$	$c \to a$

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- Prob(b changes into a in 2 steps) $= M_{ab} \cdot M_{aa} + M_{bb} \cdot M_{ab} + \sum_{c \neq a,b} M_{cb} M_{ac} = \sum_{c \in \Sigma} M_{ac} M_{cb} = M^2_{ab}$
- M^2_{ab} is just the entry a, b, i.e. row a and column b, of the product matrix $M^2 = M \cdot M$ (matrix multiplication)—and not the real number M_{ab} squared!
- in general: M^k contains the probabilities for k steps, i.e. M^k_{ab} = prob. that b has mutated into a after k steps

Computation of the scoring matrices

- the PAM scoring matrices are "log-odds" matrices
  - odds: compare two probabilities
  - log: take the logarithm (product → sum)
- PAMk scoring matrix:
  - take M^k
  - M^k_{ab} = Prob(b changed into a in k steps)
  - compare to: Prob(a is there by chance) = p_a
    p_a = relative frequency of a,
    e.g. if the DB is: $\{aabc, abca\}$, then $p_a = 1/2, p_b, p_c = 1/4$
- take log (base 10), multiply by 10 (for nicer numbers), round to nearest integer:

$$S_{ab} = 10 \cdot \log_{10}\left(\frac{M^k_{ab}}{p_a}\right) \qquad \text{rounded to nearest int.}$$

Computation of the scoring matrices

$$\boxed{S_{ab} = 10 \cdot \log_{10}(\tfrac{M^k_{ab}}{p_a})}$$

$$\frac{M^k_{ab}}{p_a} \quad \begin{cases} > 1 & \text{if } M^k_{ab} > p_a \\ = 1 & \text{if } M^k_{ab} = p_a \\ < 1 & \text{if } M^k_{ab} < p_a \end{cases}$$

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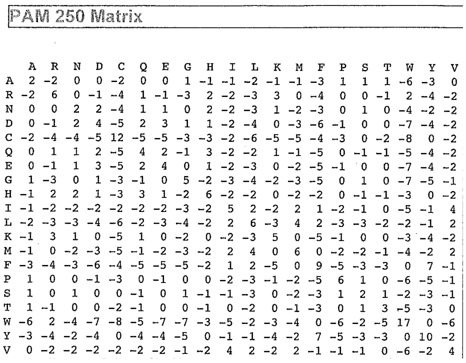
Therefore

$$S_{ab} \quad \begin{cases} > 0 & \text{if } M^k_{ab} > p_a & \text{i.e. if prob1 is greater than prob2} \\ = 0 & \text{if } M^k_{ab} = p_a & \text{i.e. if they are equal} \\ < 0 & \text{if } M^k_{ab} < p_a & \text{i.e. if prob2 is greater than prob1} \end{cases}$$

Note that scoring matrices are symmetrical (but not the prob. matrices).



PAM 250 Matrix

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	2	-2	0	0	-2	0	0	1	-1	-1	-2	-1	-1	-3	1	1	1	-6	-3	0
R	-2	6	0	-1	-4	1	-1	-3	2	-2	-3	3	0	-4	0	0	-1	2	-4	-2
N	0	0	2	2	-4	1	1	0	2	-2	-3	1	-2	-3	0	1	0	-4	-2	-2
D	0	-1	2	4	-5	2	3	1	1	-2	-4	0	-3	-6	-1	0	0	-7	-4	-2
C	-2	-4	-4	-5	12	-5	-5	-3	-3	-2	-6	-5	-5	-4	-3	0	-2	-8	0	-2
Q	0	1	1	2	-5	4	2	-1	3	-2	-2	1	-1	-5	0	-1	-1	-5	-4	-2
E	0	-1	1	3	-5	2	4	0	1	-2	-3	0	-2	-5	-1	0	0	-7	-4	-2
G	1	-3	0	1	-3	-1	0	5	-2	-3	-4	-2	-3	-5	0	1	0	-7	-5	-1
H	-1	2	2	1	-3	3	1	-2	6	-2	-2	0	-2	-2	0	-1	-1	-3	0	-2
I	-1	-2	-2	-2	-2	-2	-2	-3	-2	5	2	-2	2	1	-2	-1	0	-5	-1	4
L	-2	-3	-3	-4	-6	-2	-3	-4	-2	2	6	-3	4	2	-3	-3	-2	-2	-1	2
K	-1	3	1	0	-5	1	0	-2	0	-2	-3	5	0	-5	-1	0	0	-3	-4	-2
M	-1	0	-2	-3	-5	-1	-2	-3	-2	2	4	0	6	0	-2	-2	-1	-4	-2	2
F	-3	-4	-3	-6	-4	-5	-5	-5	-2	1	2	-5	0	9	-5	-3	-3	0	7	-1
P	1	0	0	-1	-3	0	-1	0	0	-2	-3	-1	-2	-5	6	1	0	-6	-5	-1
S	1	0	1	0	0	-1	0	1	-1	-1	-3	0	-2	-3	1	2	1	-2	-3	-1
T	1	-1	0	0	-2	-1	0	0	-1	0	-2	0	-1	-3	0	1	3	-5	-3	0
W	-6	2	-4	-7	-8	-5	-7	-7	-3	-5	-2	-3	-4	0	-6	-2	-5	17	0	-6
Y	-3	-4	-2	-4	0	-4	-4	-5	0	-1	-1	-4	-2	7	-5	-3	-3	0	10	-2
V	0	-2	-2	-2	-2	-2	-2	-1	-2	4	2	-2	2	-1	-1	-1	0	-6	-2	4

Why use logarithm?

We use logarithms for computational reasons:

- since log is strictly monotonically increasing, one can replace all x with $\log x$: We have $x < y$ if and only if $\log x < \log y$.
- products of probs → sums of log-of-probs
- easier to compute sums than products of very small numbers (note that all probabilities are between 0 and 1): reduce rounding errors

Two caveats

PAM matrices use two silent assumptions:

1. mutations (changes) of AAs happen independently (i.e. independent of context): scoring by individual columns
2. uses an evolutionary model: k distance $= k$ identical steps (i.e. with same probabilites)

BLOSUM matrices

BLOSUM scoring matrices (Henikoff and Henikoff, 1992)

- other family of commonly used scoring matrices
- remedies second issue: uses no underlying evolutionary model
- same principle as PAM matrices, but:
- used different sets of aligned sequences for different distances
- BLOSUM m: only used sequences that had m% identity or less
- higher number $\hat{=}$ closer related
- common: BLOSUM 45, 62, 80; BLOSUM62 $\sim$ PAM120

Summary

PAM matrices

- allow scoring different AA pairs according to evolutionary relatedness
- different PAMk acc. to evolutionary distance
- all modern AA scoring matrices are based on empirical data: observed frequencies in trusted alignment data
- the probabilities are estimated probabilites of AAs (from the data)
- mutation probability matrix M (1 step $=$ 1 PAM unit)
  $\rightsquigarrow$ M^k mutation probability matrix for k steps (k PAM units)
  $\rightsquigarrow$ PAMk scoring matrix S (log-odds matrix)
- higher number $\hat{=}$ less related $\hat{=}$ more distant
- commonly used: PAM40, PAM120, PAM160, PAM250
- k in PAMk needs to be decided before scoring
- BLOSUM: similar to PAM but higher number $\hat{=}$ more related