Efektywne algorytmy do porównań sekwencji

Bartek Wilczyński

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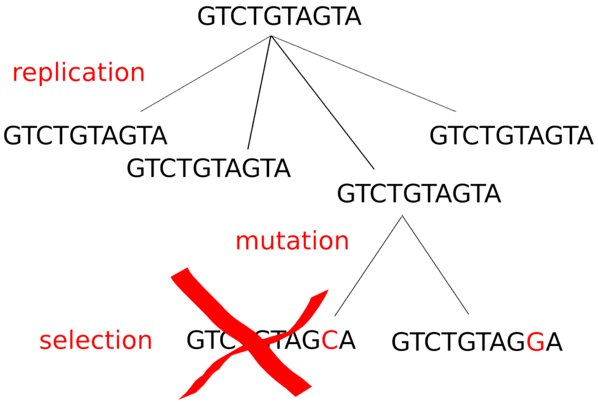
How sequences evolve?



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Fundamental problem

- How far in evolution are sequences we can observe in different living species?
- More formally: Can we define a measure of sequence similarity

$$d : \Sigma^* \times \Sigma^* \to \mathcal{R}^+$$

  approximating the true evolutionary distance?
- Hint: We should count the number of mutations leading to the observed divergence.

Problems with DNA evolution models

- Mutations occur on DNA level, but selection acts much higher: on the phenotype level.
- This makes the assumption of base independence invalid
- Long evolutionary times violate time-reversibility
- Multiplicative measure not too convenient in practice
- We can only account for substitutions, not for insertions or deletions

Suggested solutions:

- Use protein sequences for comparisons
- Define additive substitution matrices

Protein substitution matrices

- We are still assuming time-reversible Markov chain, but now in space of protein sequences.
- Matrix entries contain log-probabilities, leading to additive measures of similarity
- PAM (Point accepted mutations) matrices (Dayhoff, 1978) describe observed probabilities of occurence of point mutations for a given average divergence (PAM1 = one mutation/100 bases, mostly used PAM250)
- BLOSUM (BLOcks Substitution Matrix) (Henikoff, Henikoff 1992) were constructed using short protein alignments (Blocks) of given sequence identity. e.g.BLOSUM80 was derived from sequences of $\geqslant 80\%$ identity

Blosum50 matrix

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	**5**	−2	−1	−2	−1	−1	−1	0	−2	−1	−2	−1	−1	−3	−1	1	0	−3	−2	0
R	−2	**7**	−1	−2	−4	1	0	−3	0	−4	−3	3	−2	−3	−3	−1	−1	−3	−1	−3
N	−1	−1	**7**	2	−2	0	0	0	1	−3	−4	0	−2	−4	−2	1	0	−4	−2	−3
D	−2	−2	2	**8**	−4	0	2	−1	−1	−4	−4	−1	−4	−5	−1	0	−1	−5	−3	−4
C	−1	−4	−2	−4	**13**	−3	−3	−3	−3	−2	−2	−3	−2	−2	−4	−1	−1	−5	−3	−1
Q	−1	1	0	0	−3	**7**	2	−2	1	−3	−2	2	0	−4	−1	0	−1	−1	−1	−3
E	−1	0	0	2	−3	2	**6**	−3	0	−4	−3	1	−2	−3	−1	−1	−1	−3	−2	−3
G	0	−3	0	−1	−3	−2	−3	**8**	−2	−4	−4	−2	−3	−4	−2	0	−2	−3	−3	−4
H	−2	0	1	−1	−3	1	0	−2	**10**	−4	−3	0	−1	−1	−2	−1	−2	−3	2	−4
I	−1	−4	−3	−4	−2	−3	−4	−4	−4	**5**	2	−3	2	0	−3	−3	−1	−3	−1	4
L	−2	−3	−4	−4	−2	−2	−3	−4	−3	2	**5**	−3	3	1	−4	−3	−1	−2	−1	1
K	−1	3	0	−1	−3	2	1	−2	0	−3	−3	**6**	−2	−4	−1	0	−1	−3	−2	−3
M	−1	−2	−2	−4	−2	0	−2	−3	−1	2	3	−2	**7**	0	−3	−2	−1	−1	0	1
F	−3	−3	−4	−5	−2	−4	−3	−4	−1	0	1	−4	0	**8**	−4	−3	−2	1	4	−1
P	−1	−3	−2	−1	−4	−1	−1	−2	−2	−3	−4	−1	−3	−4	**10**	−1	−1	−4	−3	−3
S	1	−1	1	0	−1	0	−1	0	−1	−3	−3	0	−2	−3	−1	**5**	2	−4	−2	−2
T	0	−1	0	−1	−1	−1	−1	−2	−2	−1	−1	−1	−1	−2	−1	2	**5**	−3	−2	0
W	−3	−3	−4	−5	−5	−1	−3	−3	−3	−3	−2	−3	−1	1	−4	−4	−3	**15**	2	−3
Y	−2	−1	−2	−3	−3	−1	−2	−3	2	−1	−1	−2	0	4	−3	−2	−2	2	**8**	−1
V	0	−3	−3	−4	−1	−3	−3	−4	−4	4	1	−3	1	−1	−3	−2	0	−3	−1	**5**

Log-odds $\log \frac{P_{x,y}}{Q_x Q_y}$ instead of probabilities or substitution rates.

image (c) Durbin et al.

Quiz – using silent mutations

We know two types of mutations in DNA *silent* and *coding*

- Which of them are more interesting for calculating divergence between species?
- And which are more interesting for paternity testing?

Counting mutations – Hamming distance

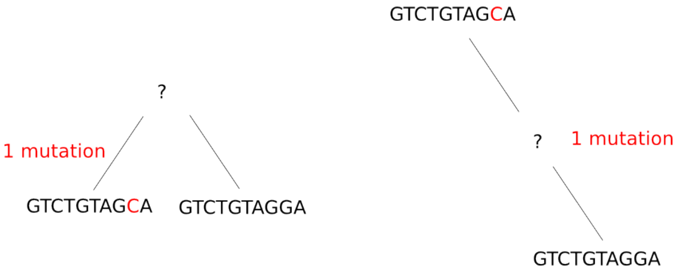
- Hamming distance: a metric originating from Information theory
- Given two vectors of the same length, it returns the number of positions where they differ.
- $$D_H(s_1, s_2) = \Sigma_{i=1}^{n}\{1 : s_1[i] \neq s_2[i]; 0 : otherwise\}$$
- A proper distance (satisfies triangle inequality)

Insertions/deletions – small and large

- DNA polymerase can (rarely) slide over nucleotides
- especially over stretches of low complexity
- this leads to short deletions of DNA after replication
- Transposable elements lead to insertions of larger segments
- Chromosome recombination leads to duplications and deletions on different chromosomes at the same time

Insertions/deletions – symmetric cases

Number of mutations needed to *evolve* two sequences from a common ancestor is the same (under parsimony assumption) as the number of mutations needed to *evolve* one into the other



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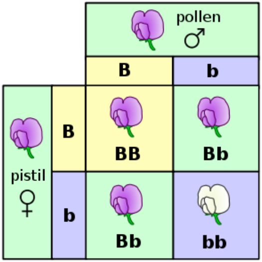
Genes – units of evolutionary information

- Classically, genes are the **basic units of heritability**
- Gregor Mendel (1822-1884) laid foundations of genetics with his experiments on peas
- He also introduced the term allele and formulated laws of inheritance (segregation and independence)
- He knew nothing about DNA!



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Genes – modern view

- Currently, we know that genes are carried by DNA
- Current definition of a gene is substantially more complex:
- *a locatable region of genomic sequence, corresponding to a unit of inheritance, which is associated with regulatory regions, transcribed regions, and or other functional sequence regions* (Pearson, Nature, 2006)
- This is overly complex for our purposes, so
- We will be most concerned with *protein coding genes*, i.e. DNA sequences encoding proteins

Edit distance – solution or a problem?

- We can introduce *edit distance*: the number of editing operations needed to transform one sequence into the other. These operations are:
  - Substitutions
  - Insertions
  - Deletions
- The *procedural* definition of the distance makes it difficult to work with
- Does it matter in what order I make the operations (*If i delete a character, I cannot substitute it anymore...*)
- It turns out the *optimal* edit distances are simpler and can be described in a formal way as sequence *alignments*

Sequence alignment – problem statement

For a given sequences s, t over an alphabet Σ, their alignment is a pair of words s', t' over the extended alphabet $\Sigma' = \Sigma \cup \{-\}$. Sequences s', t' need to satisfy the following:

- $|s'| = |t'|$
- $s'_{|\Sigma} = s$ and $t'_{|\Sigma} = t$
- for no position i, $s'[i] = t'[i] = -$

For example, one of the words `HEAGAWGHEE` and `PAWHEAE` is

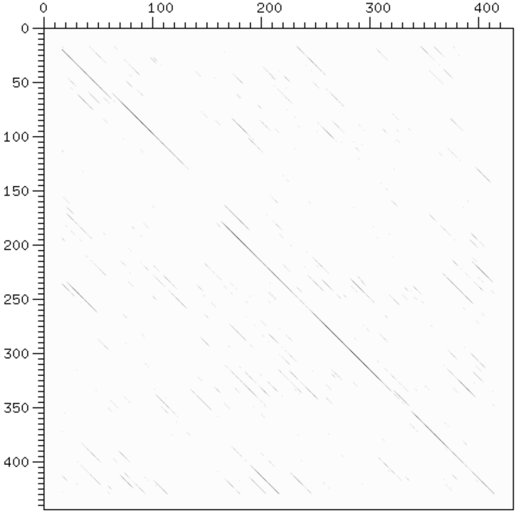
```
HEAGAWGHE-E
--P-AW-HEAE
```

Number of possible alignments for words of length n

$$\binom{2n}{n} = \frac{(2n)!}{(n!)^2} \simeq \frac{2^{2n}}{\sqrt{\pi n}}$$

Scoring alignments: binary dotplots

Dotplot of the alignment of human haemoglobin α vs β chains



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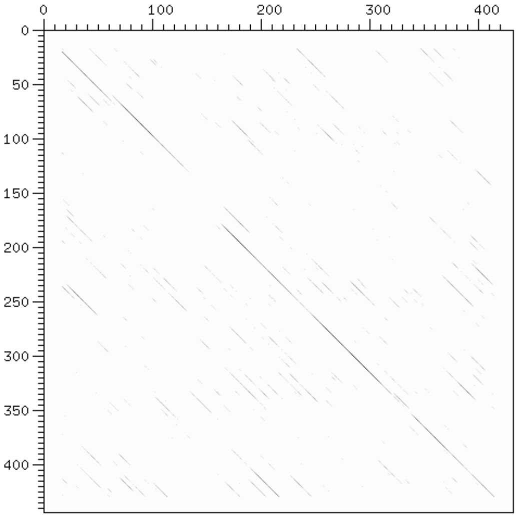
Scoring alignments: BLOSUM score matrix



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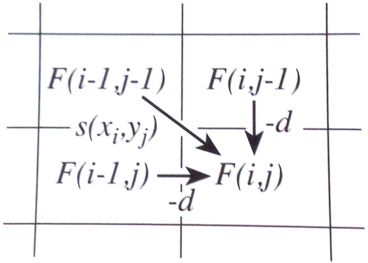
Recursive equation for sequence alignment

	H	E	A	G	A	W	G	H	E	E
P	−2	−1	−1	−2	−1	−4	−2	−2	−1	−1
A	−2	−1	**5**	0	**5**	−3	0	−2	−1	−1
W	−3	−3	−3	−3	−3	**15**	−3	−3	−3	−3
H	**10**	0	−2	−2	−2	−3	−2	**10**	0	0
E	0	**6**	−1	−3	−1	−3	−3	0	**6**	**6**
A	−2	−1	**5**	0	**5**	−3	0	−2	−1	−1
E	0	**6**	−1	−3	−1	−3	−3	0	**6**	**6**

image (c) Durbin et al.

Filling in the alignment matrix

$$F(i,j) = \max \begin{cases} F(i-1, j-1) + s(x_i, y_j), \\ F(i-1, j) - d, \\ F(i, j-1) - d. \end{cases}$$



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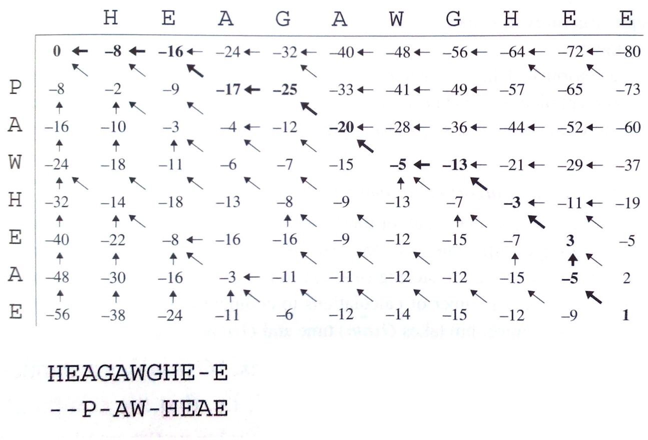
Tracing back alignments



	H	E	A	G	A	W	G	H	E	E
0	**–8**	**–16**	–24	–32	–40	–48	–56	–64	–72	–80
P	–8	–2	–9	**–17**	**–25**	–33	–41	–49	–57	–65	–73
A	–16	–10	–3	–4	–12	**–20**	–28	–36	–44	–52	–60
W	–24	–18	–11	–6	–7	–15	**–5**	**–13**	–21	–29	–37
H	–32	–14	–18	–13	–8	–9	–13	–7	**–3**	–11	–19
E	–40	–22	–8	–16	–16	–9	–12	–15	–7	**3**	–5
A	–48	–30	–16	–3	–11	–11	–12	–12	–15	**–5**	2
E	–56	–38	–24	–11	–6	–12	–14	–15	–12	–9	**1**

HEAGAWGHE-E

--P-AW-HEAE

image (c) Durbin et al.

Finding local alignments - Smith,Waterman '82

$$F(i,j) = \max \begin{cases} 0, \\ F(i-1,j-1)+s(x_i,y_j), \\ F(i-1,j)-d, \\ F(i,j-1)-d. \end{cases}$$

		H	E	A	G	A	W	G	H	E	E
	0	0	0	0	0	0	0	0	0	0	0
P	0	0	0	0	0	0	0	0	0	0	0
A	0	0	0	5	0	**5**	0	0	0	0	0
W	0	0	0	0	2	0	**20**	**12**	4	0	0
H	0	10	2	0	0	0	12	18	**22**	14	6
E	0	2	16	8	0	0	4	10	18	**28**	20
A	0	0	8	21	13	5	0	4	10	20	27
E	0	0	6	13	18	12	4	0	4	16	26

```
AWGHE
AW-HE
```

image (c) Durbin et al.

Scoring alignments: general gap penalty

General gap penalty

$$F(i,j) = \max \begin{cases} F(i-1,j-1) + s(x_i,y_j), & \\ F(k,j) + \gamma(i-k), & k = 0,\dots,i-1, \\ F(i,k) + \gamma(j-k), & k = 0,\dots,j-1. \end{cases}$$

Affine gap penalty (caching)

$$M(i,j) = \max \begin{cases} M(i-1,j-1) + s(x_i,y_j), \\ I_x(i-1,j-1) + s(x_i,y_j), \\ I_y(i-1,j-1) + s(x_i,y_j); \end{cases}$$

$$I_x(i,j) = \max \begin{cases} M(i-1,j) - d, \\ I_x(i-1,j) - e; \end{cases}$$

$$I_y(i,j) = \max \begin{cases} M(i,j-1) - d, \\ I_y(i,j-1) - e. \end{cases}$$

image (c) Durbin et al.