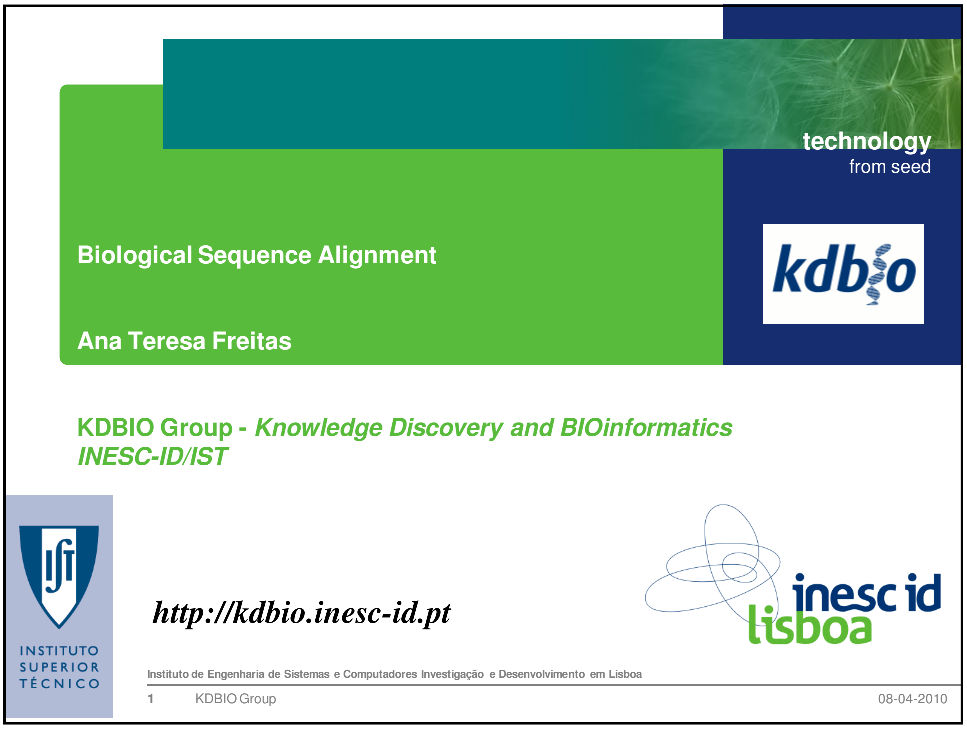

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Biological Sequence Alignment

Ana Teresa Freitas

KDBIO Group - *Knowledge Discovery and BIOinformatics*
INESC-ID/IST

http://kdbio.inesc-id.pt

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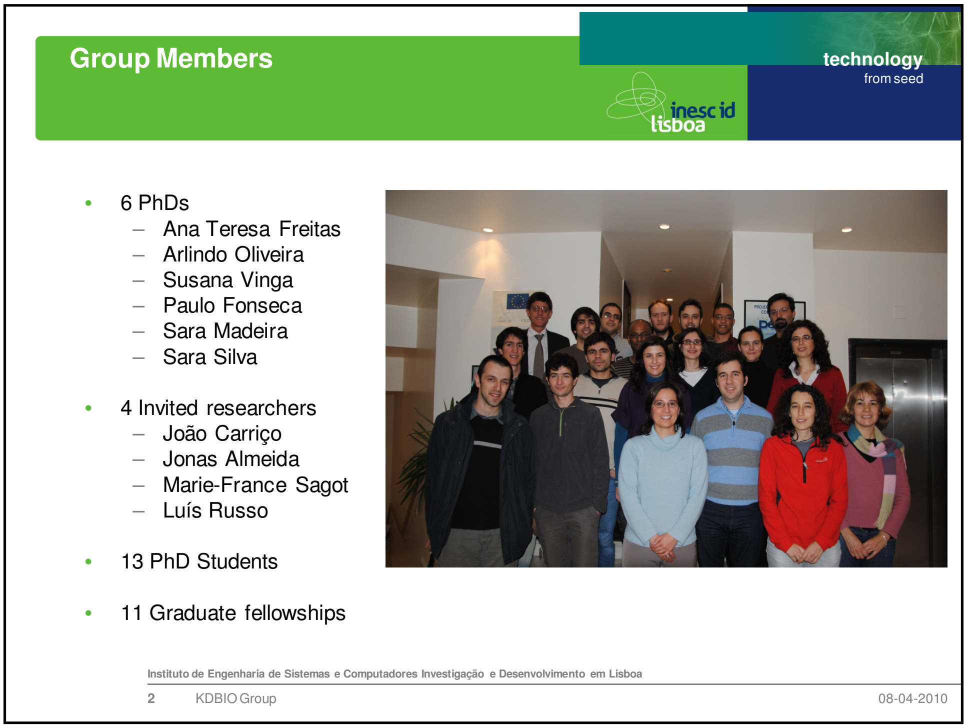
1 KDBIO Group 08-04-2010



Group Members

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- 6 PhDs
  - Ana Teresa Freitas
  - Arlindo Oliveira
  - Susana Vinga
  - Paulo Fonseca
  - Sara Madeira
  - Sara Silva
- 4 Invited researchers
  - João Carriço
  - Jonas Almeida
  - Marie-France Sagot
  - Luís Russo
- 13 PhD Students
- 11 Graduate fellowships

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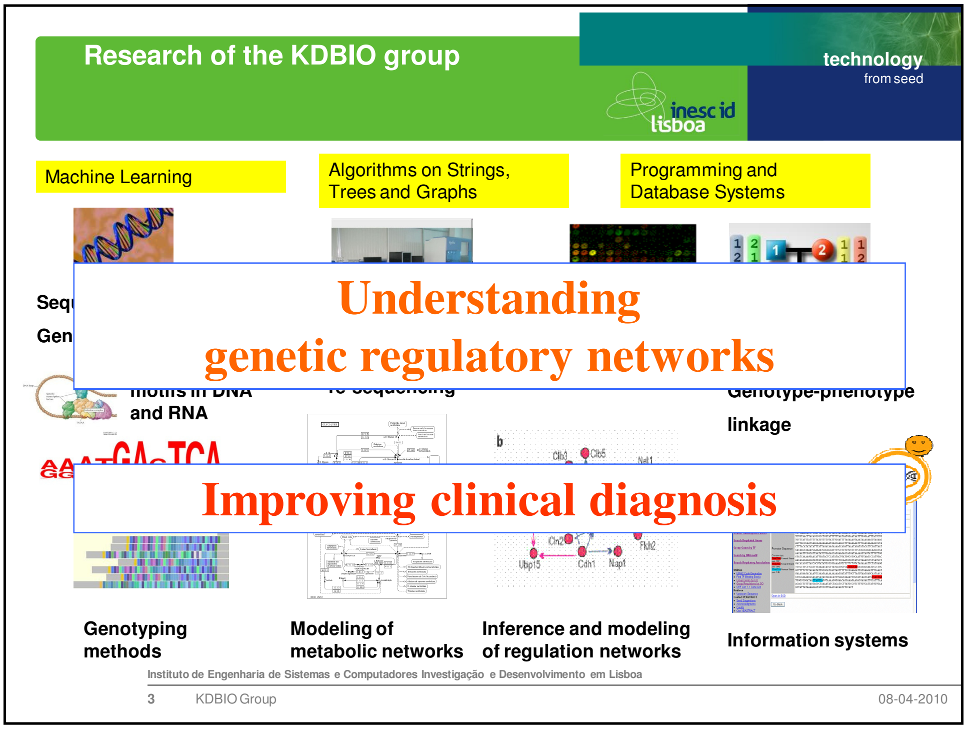
2 KDBIO Group 08-04-2010



Research of the KDBIO group

Machine Learning

Algorithms on Strings, Trees and Graphs

Programming and Database Systems

Seq

Gen

Understanding genetic regulatory networks

motifs in DNA and RNA

re-sequencing

Genotype-phenotype linkage

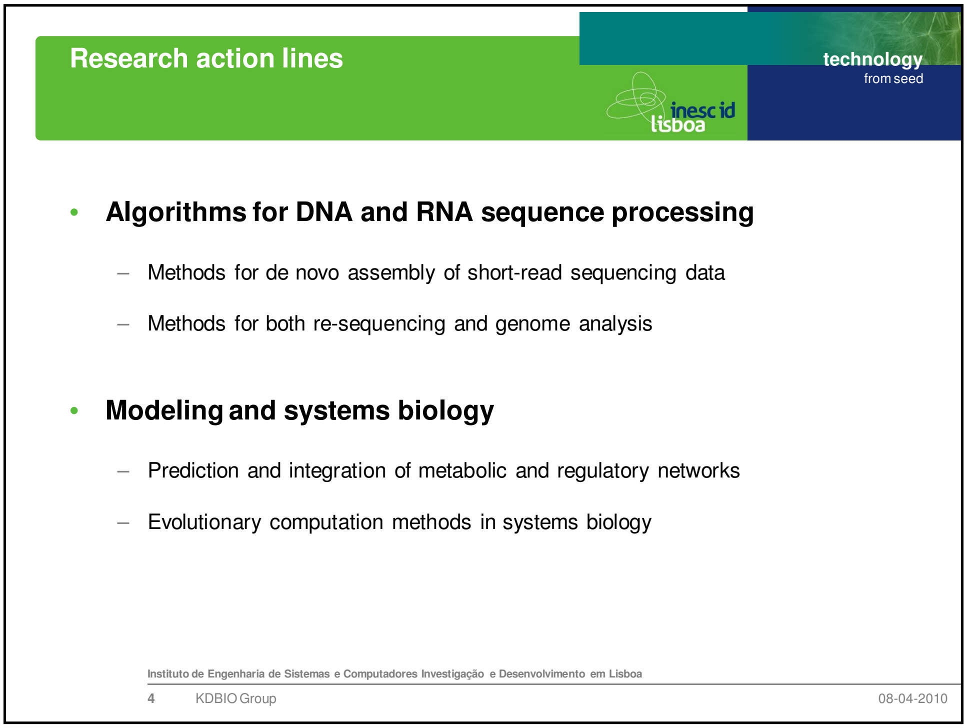
Improving clinical diagnosis

Genotyping methods

Modeling of metabolic networks

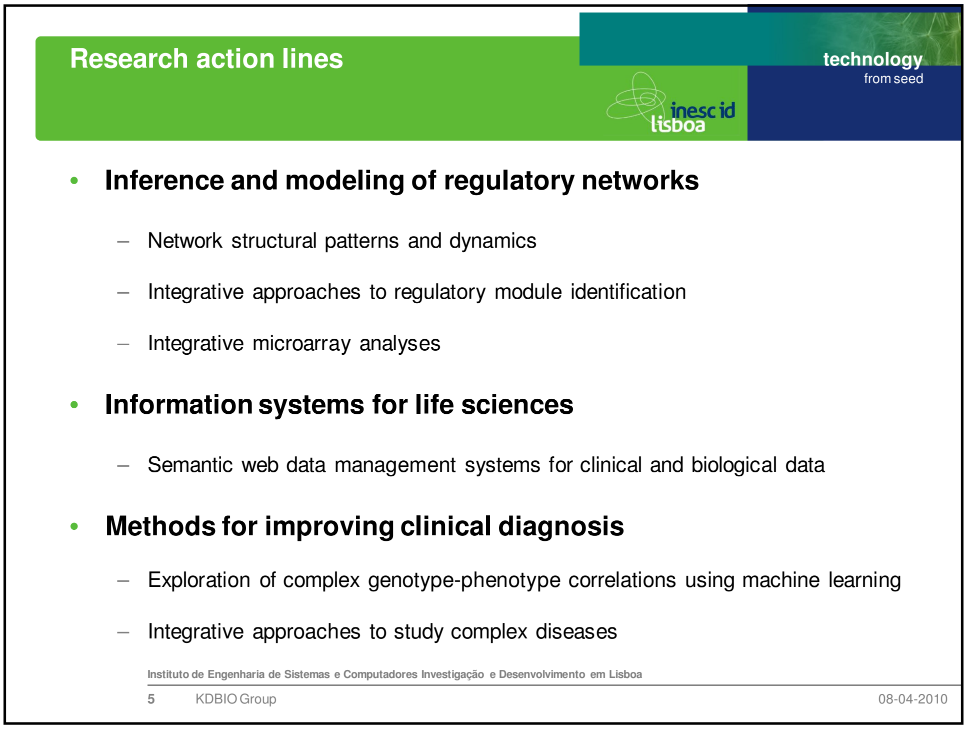
Inference and modeling of regulation networks

Information systems



Research action lines

- **Algorithms for DNA and RNA sequence processing**
  - Methods for de novo assembly of short-read sequencing data
  - Methods for both re-sequencing and genome analysis
- **Modeling and systems biology**
  - Prediction and integration of metabolic and regulatory networks
  - Evolutionary computation methods in systems biology



Research action lines

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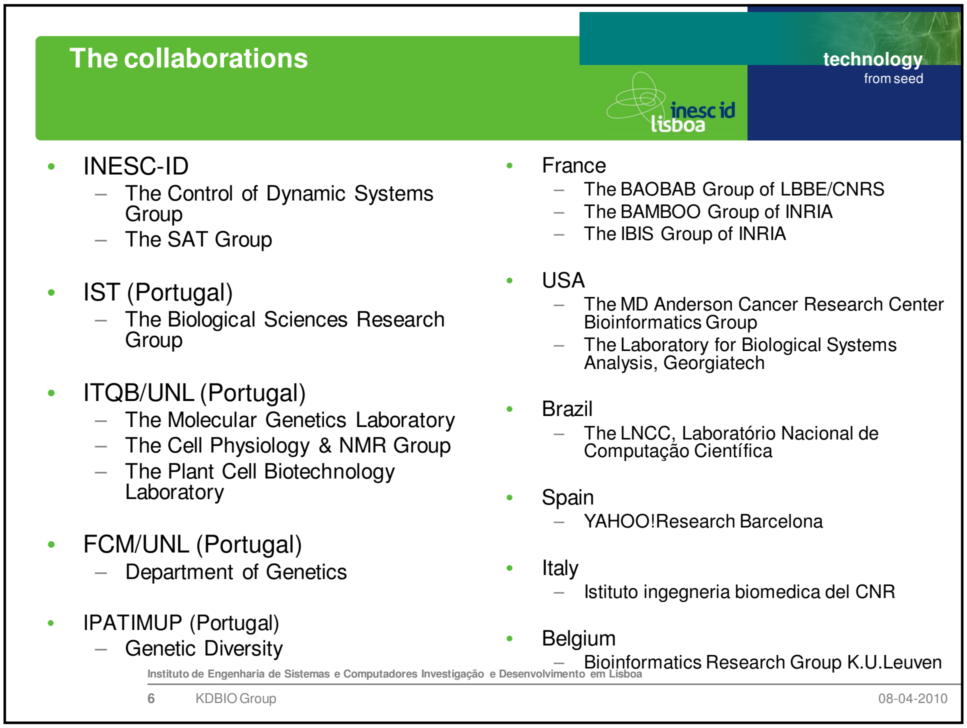
- **Inference and modeling of regulatory networks**
  - Network structural patterns and dynamics
  - Integrative approaches to regulatory module identification
  - Integrative microarray analyses
- **Information systems for life sciences**
  - Semantic web data management systems for clinical and biological data
- **Methods for improving clinical diagnosis**
  - Exploration of complex genotype-phenotype correlations using machine learning
  - Integrative approaches to study complex diseases



The collaborations

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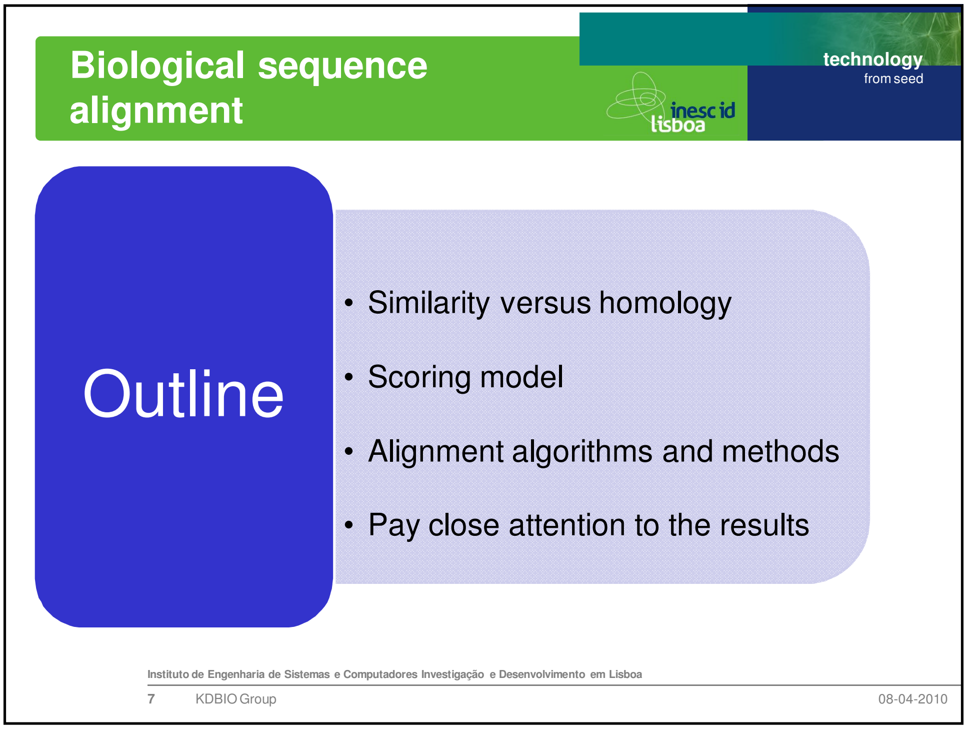
- INESC-ID
  - The Control of Dynamic Systems Group
  - The SAT Group
- IST (Portugal)
  - The Biological Sciences Research Group
- ITQB/UNL (Portugal)
  - The Molecular Genetics Laboratory
  - The Cell Physiology & NMR Group
  - The Plant Cell Biotechnology Laboratory
- FCM/UNL (Portugal)
  - Department of Genetics
- IPATIMUP (Portugal)
  - Genetic Diversity
- France
  - The BAOBAB Group of LBBE/CNRS
  - The BAMBOO Group of INRIA
  - The IBIS Group of INRIA
- USA
  - The MD Anderson Cancer Research Center Bioinformatics Group
  - The Laboratory for Biological Systems Analysis, Georgiatech
- Brazil
  - The LNCC, Laboratório Nacional de Computação Científica
- Spain
  - YAHOO!Research Barcelona
- Italy
  - Istituto ingegneria biomedica del CNR
- Belgium
  - Bioinformatics Research Group K.U.Leuven



Biological sequence alignment

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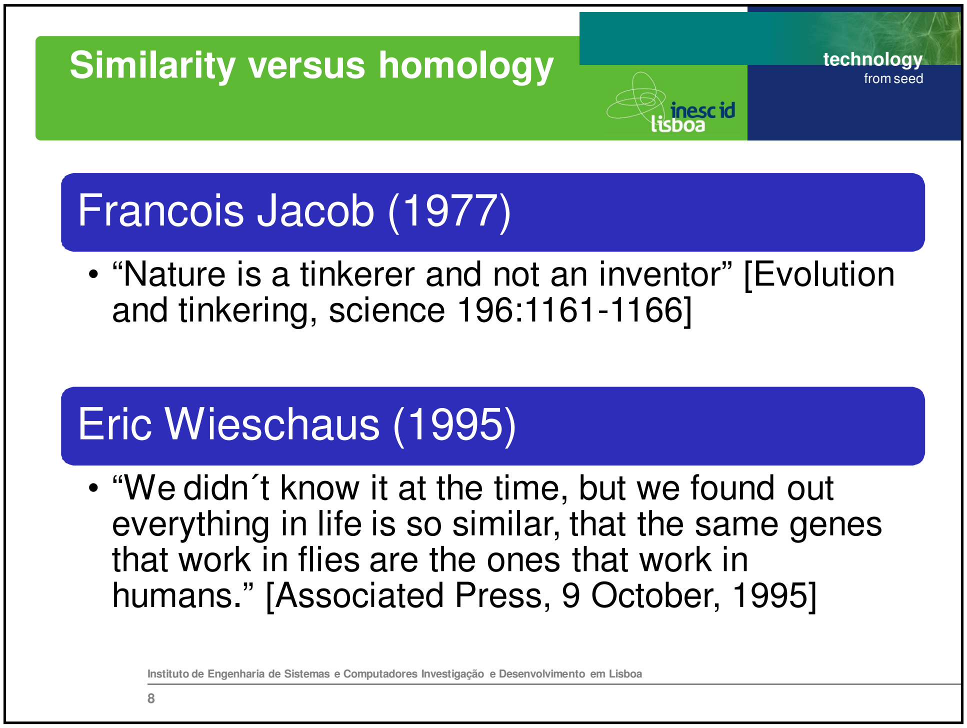
Outline

- Similarity versus homology
- Scoring model
- Alignment algorithms and methods
- Pay close attention to the results



Similarity versus homology

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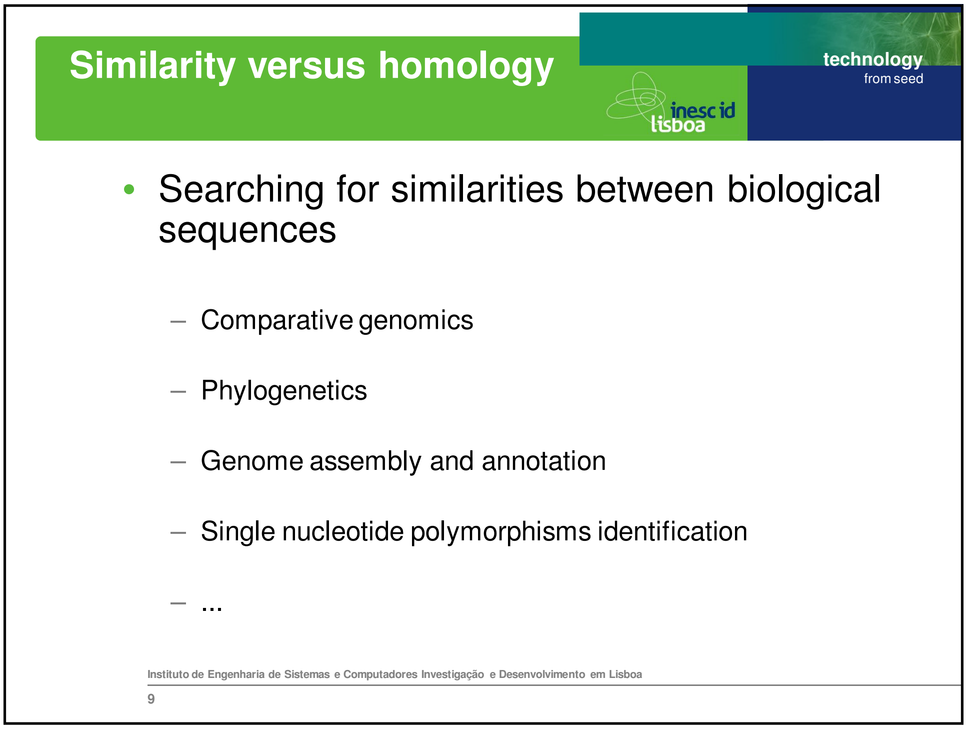
Francois Jacob (1977)

- “Nature is a tinkerer and not an inventor” [Evolution and tinkering, science 196:1161-1166]

Eric Wieschaus (1995)

- “We didn´t know it at the time, but we found out everything in life is so similar, that the same genes that work in flies are the ones that work in humans.” [Associated Press, 9 October, 1995]



Similarity versus homology

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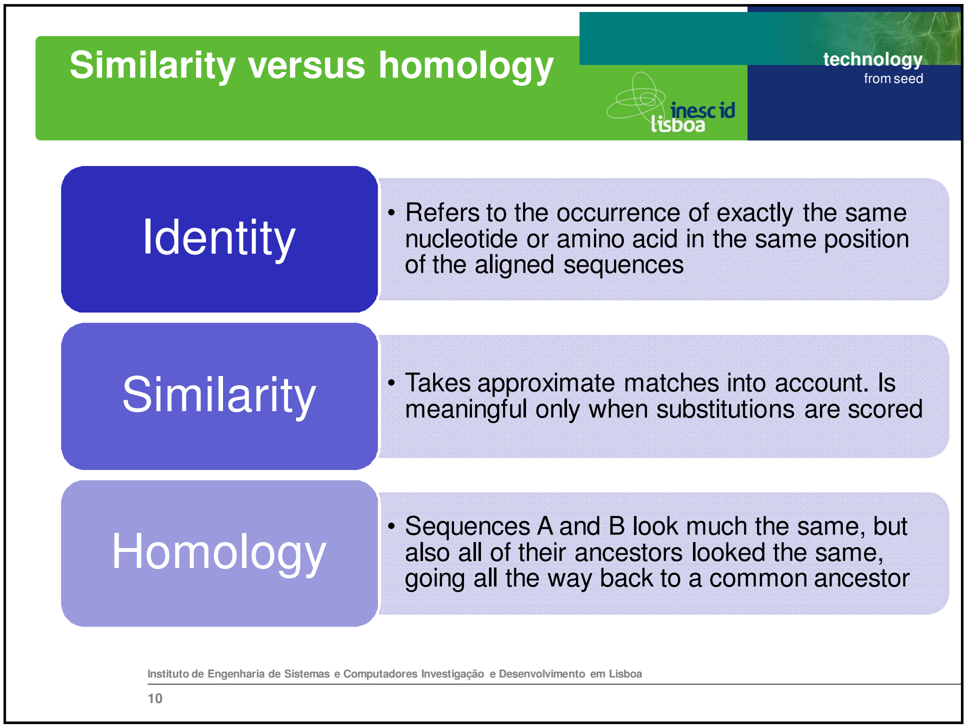
- Searching for similarities between biological sequences
  - Comparative genomics
  - Phylogenetics
  - Genome assembly and annotation
  - Single nucleotide polymorphisms identification
  - ...



Similarity versus homology

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Identity

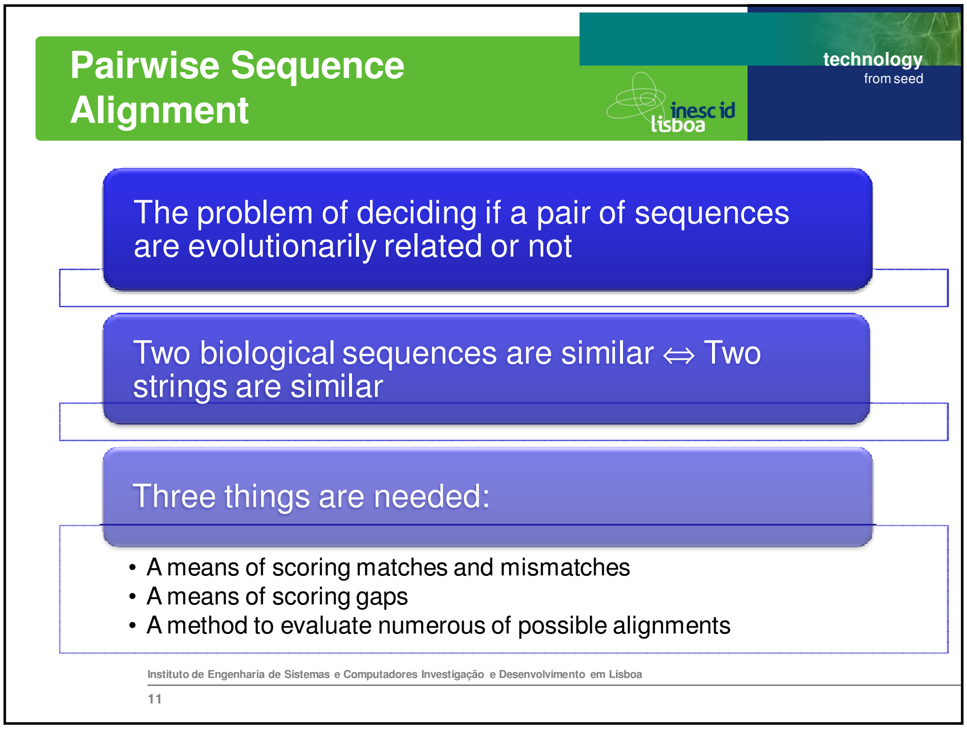
- Refers to the occurrence of exactly the same nucleotide or amino acid in the same position of the aligned sequences

Similarity

- Takes approximate matches into account. Is meaningful only when substitutions are scored

Homology

- Sequences A and B look much the same, but also all of their ancestors looked the same, going all the way back to a common ancestor



Pairwise Sequence Alignment

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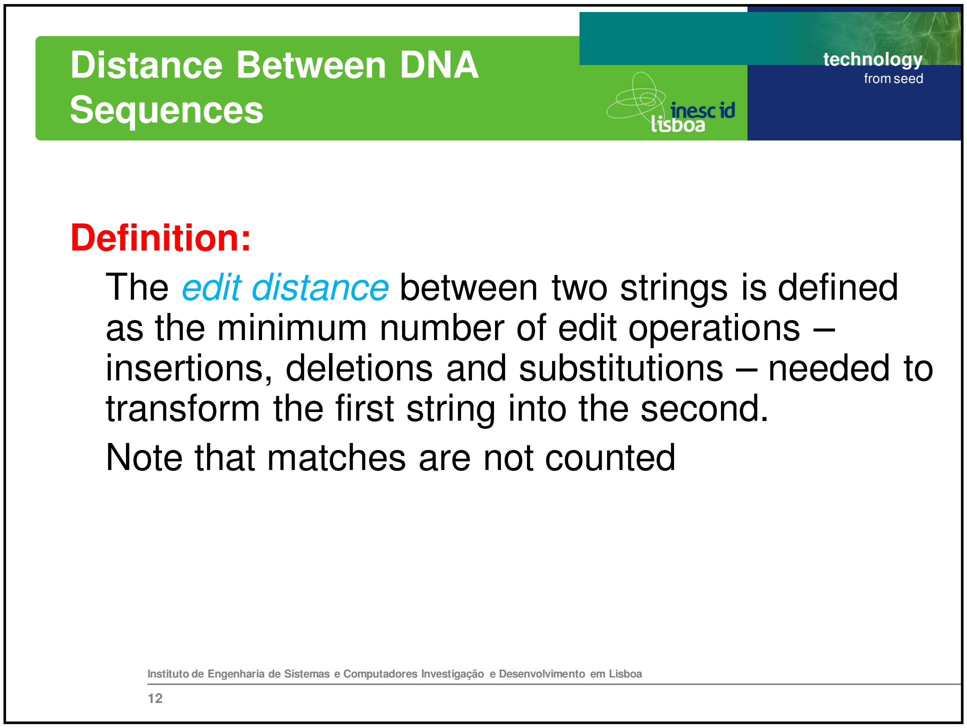
The problem of deciding if a pair of sequences are evolutionarily related or not

Two biological sequences are similar ⇔ Two strings are similar

Three things are needed:

- A means of scoring matches and mismatches
- A means of scoring gaps
- A method to evaluate numerous of possible alignments



Distance Between DNA Sequences

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Definition:

The *edit distance* between two strings is defined as the minimum number of edit operations – insertions, deletions and substitutions – needed to transform the first string into the second.

Note that matches are not counted

Gaps

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- Gaps help create alignments that better conform to underlying biological models
- Mechanisms that make long insertions or deletions in DNA include: unequal crossing-over in meiosis; DNA slippage during replication; insertion of transposable elements into DNA string; insertions of DNA by retro-viruses; etc...

Definition: A *gap* is any maximal, consecutive run of spaces in a single string of a given alignment

Pairwise Sequence Alignment

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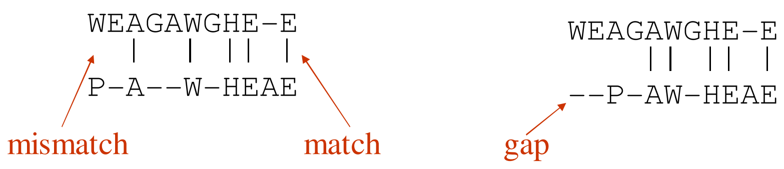
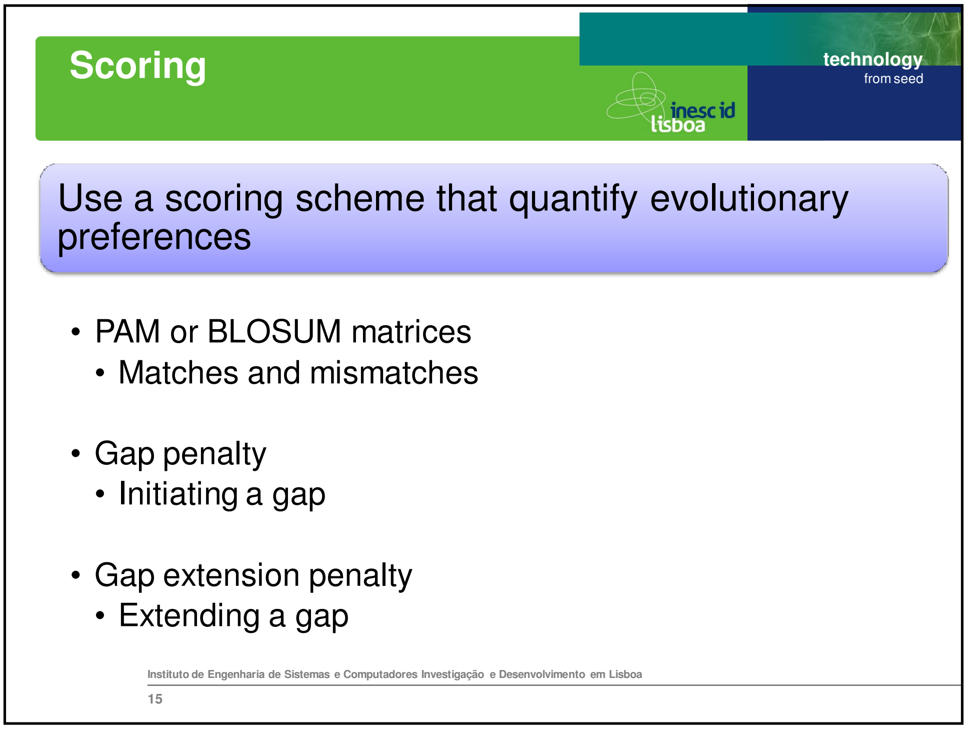
- Example:

```
WEAGAWGHEE
PAWHEAE
```



```
WEAGAWGHE-E              WEAGAWGHE-E
  |  | || |                  || || |
P-A--W-HEAE              --P-AW-HEAE
```

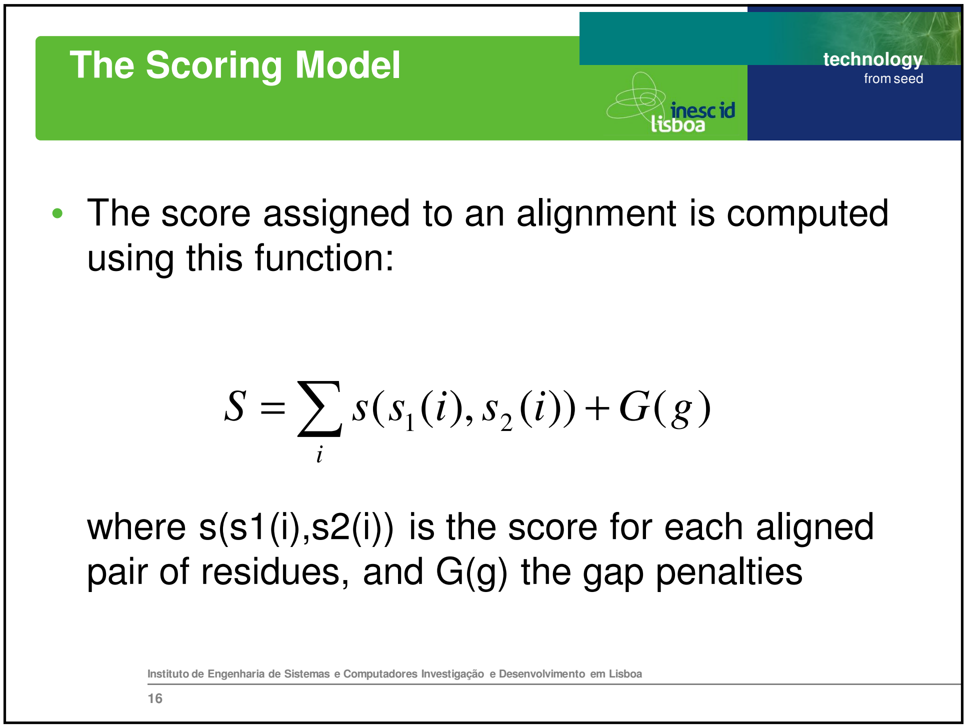
  - Which one is better?
  - Is it a true or a spurious alignment?



Scoring

Use a scoring scheme that quantify evolutionary preferences

- PAM or BLOSUM matrices
  - Matches and mismatches
- Gap penalty
  - Initiating a gap
- Gap extension penalty
  - Extending a gap

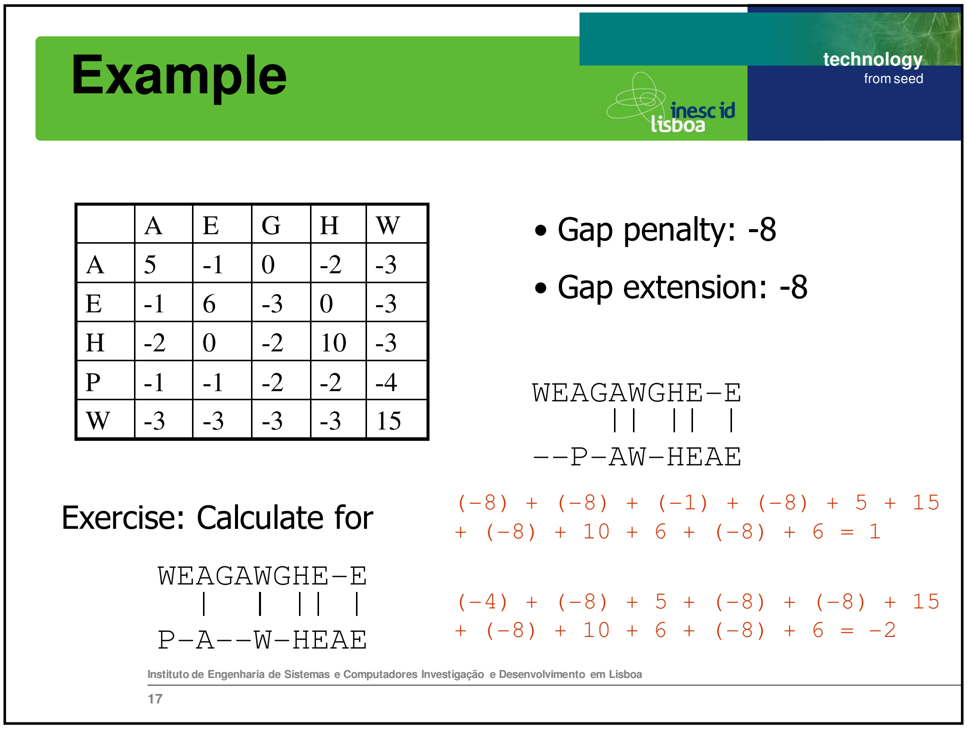


The Scoring Model

- The score assigned to an alignment is computed using this function:

$$S = \sum_i s(s_1(i), s_2(i)) + G(g)$$

where s(s1(i),s2(i)) is the score for each aligned pair of residues, and G(g) the gap penalties



Example

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

- Gap penalty: -8
- Gap extension: -8

```
WEAGAWGHE-E
    ||  ||  |
--P-AW-HEAE
```

(-8) + (-8) + (-1) + (-8) + 5 + 15 + (-8) + 10 + 6 + (-8) + 6 = 1

Exercise: Calculate for

```
WEAGAWGHE-E
  |   |  ||  |
P-A--W-HEAE
```

(-4) + (-8) + 5 + (-8) + (-8) + 15 + (-8) + 10 + 6 + (-8) + 6 = -2

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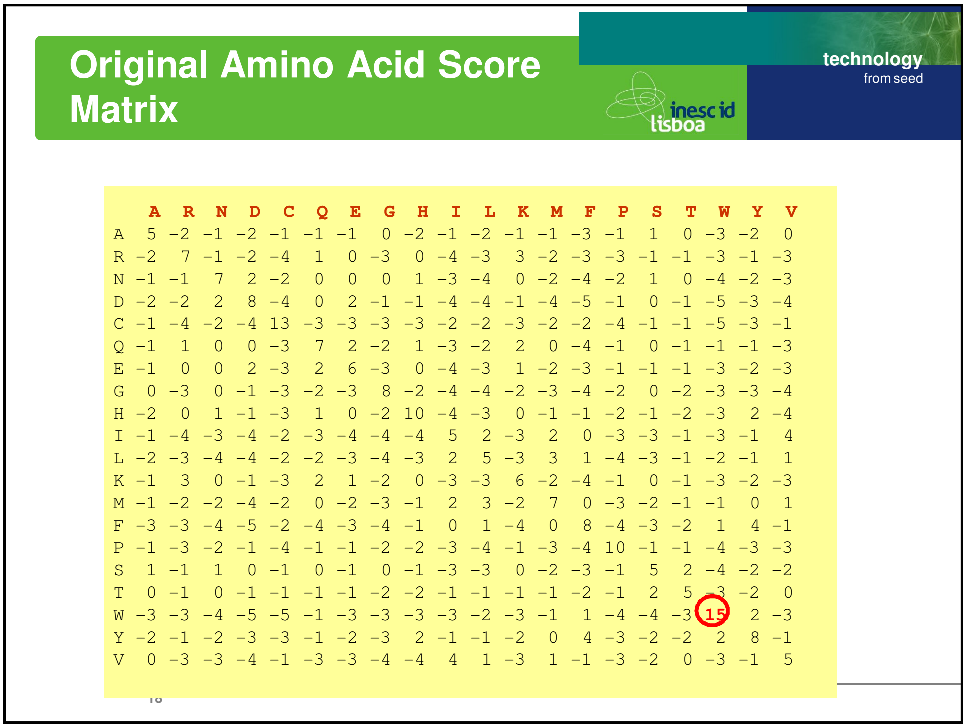

Original Amino Acid Score Matrix

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	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

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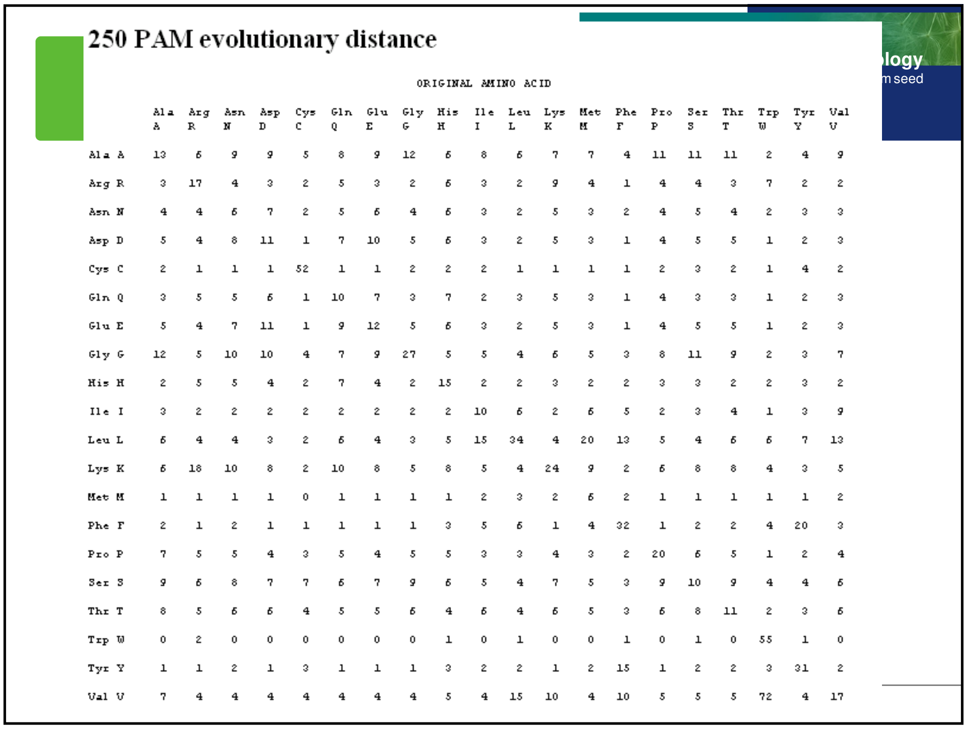
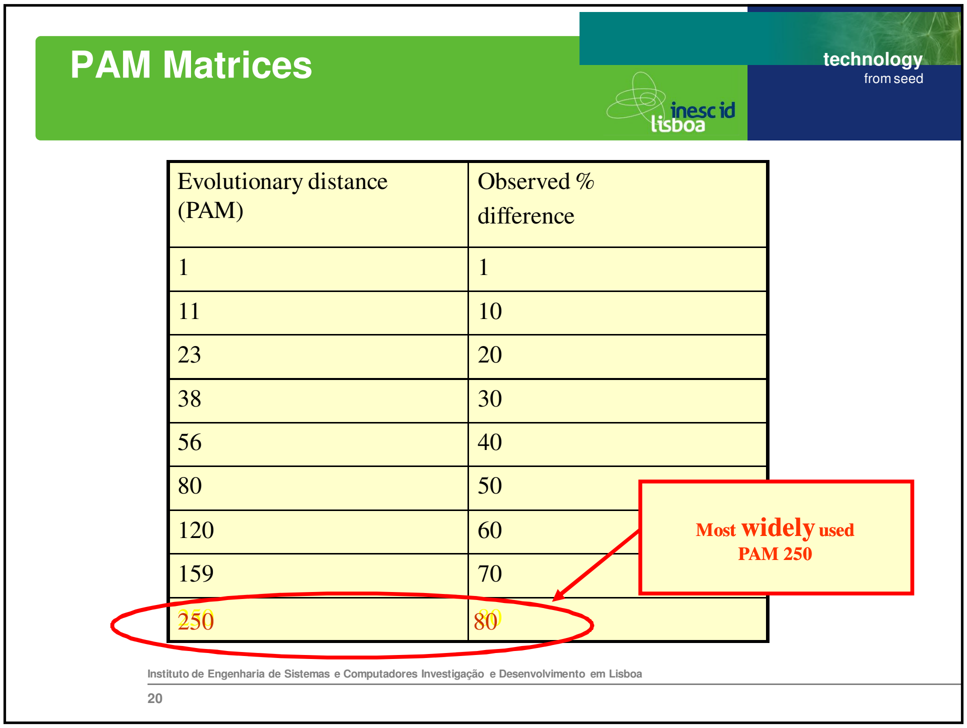


250 PAM evolutionary distance

ORIGINAL AMINO ACID

	Ala A	Arg R	Asn N	Asp D	Cys C	Gln Q	Glu E	Gly G	His H	Ile I	Leu L	Lys K	Met M	Phe F	Pro P	Ser S	Thr T	Trp W	Tyr Y	Val V
Ala A	13	6	9	9	5	8	9	12	6	8	6	7	7	4	11	11	11	2	4	9
Arg R	3	17	4	3	2	5	3	2	6	3	2	9	4	1	4	4	3	7	2	2
Asn N	4	4	6	7	2	5	6	4	6	3	2	5	3	2	4	5	4	2	3	3
Asp D	5	4	8	11	1	7	10	5	6	3	2	5	3	1	4	5	5	1	2	3
Cys C	2	1	1	1	52	1	1	2	2	2	1	1	1	1	2	3	2	1	4	2
Gln Q	3	5	5	6	1	10	7	3	7	2	3	5	3	1	4	3	3	1	2	3
Glu E	5	4	7	11	1	9	12	5	6	3	2	5	3	1	4	5	5	1	2	3
Gly G	12	5	10	10	4	7	9	27	5	5	4	6	5	3	8	11	9	2	3	7
His H	2	5	5	4	2	7	4	2	15	2	2	3	2	2	3	3	2	2	3	2
Ile I	3	2	2	2	2	2	2	2	2	10	6	2	6	5	2	3	4	1	3	9
Leu L	6	4	4	3	2	6	4	3	5	15	34	4	20	13	5	4	6	6	7	13
Lys K	6	18	10	8	2	10	8	5	8	5	4	24	9	2	6	8	8	4	3	5
Met M	1	1	1	1	0	1	1	1	1	2	3	2	6	2	1	1	1	1	1	2
Phe F	2	1	2	1	1	1	1	1	3	5	6	1	4	32	1	2	2	4	20	3
Pro P	7	5	5	4	3	5	4	5	5	3	3	4	3	2	20	6	5	1	2	4
Ser S	9	6	8	7	7	6	7	9	6	5	4	7	5	3	9	10	9	4	4	6
Thr T	8	5	6	6	4	5	5	6	4	6	4	6	5	3	6	8	11	2	3	6
Trp W	0	2	0	0	0	0	0	0	1	0	1	0	0	1	0	1	0	55	1	0
Tyr Y	1	1	2	1	3	1	1	1	3	2	2	1	2	15	1	2	2	3	31	2
Val V	7	4	4	4	4	4	4	4	5	4	15	10	4	10	5	5	5	72	4	17



PAM Matrices

Evolutionary distance (PAM)	Observed % difference
1	1
11	10
23	20
38	30
56	40
80	50
120	60
159	70
250	80

Most widely used PAM 250

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PAM vs. BLOSUM

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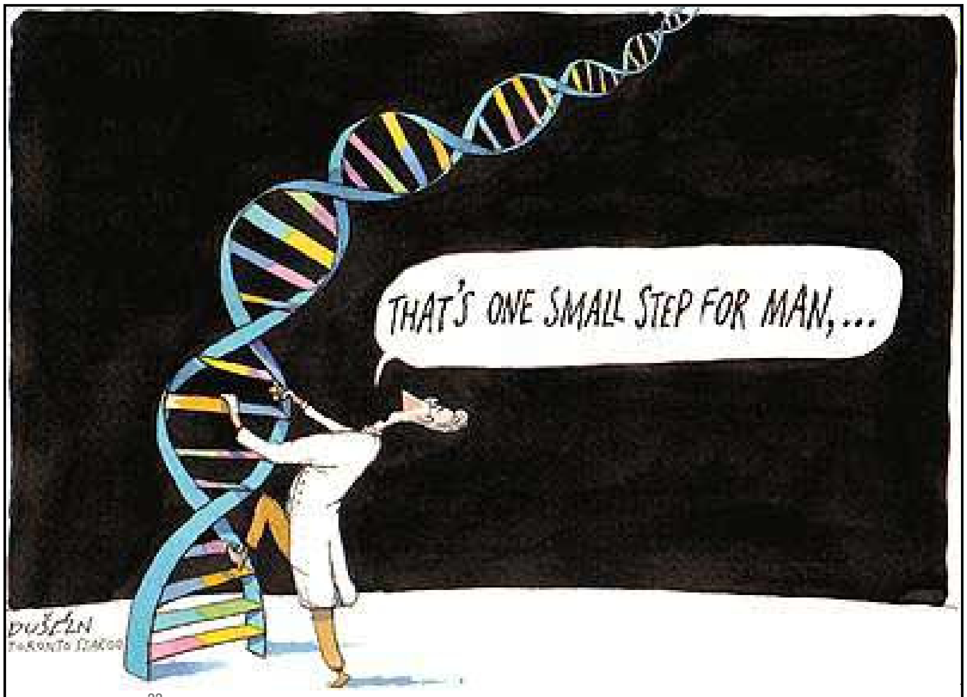
- PAM model is designed to track evolutionary origin of proteins
- Blosum model is designed to find conserved domains of proteins

Thumb rules

- Lower PAMs and higher Blosums find short local alignment of highly similar sequences
- Higher PAMs and lower Blosums find longer weaker local alignment



Alignment Algorithms
How difficult is this?

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- Consider two sequences of length *n*
- There are

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

possible global alignments, and we need to find an optimal one from amongst those

So what?

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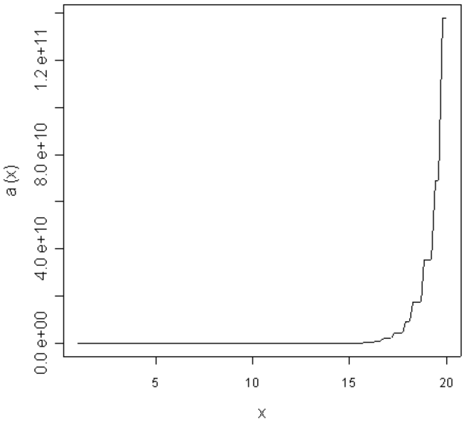
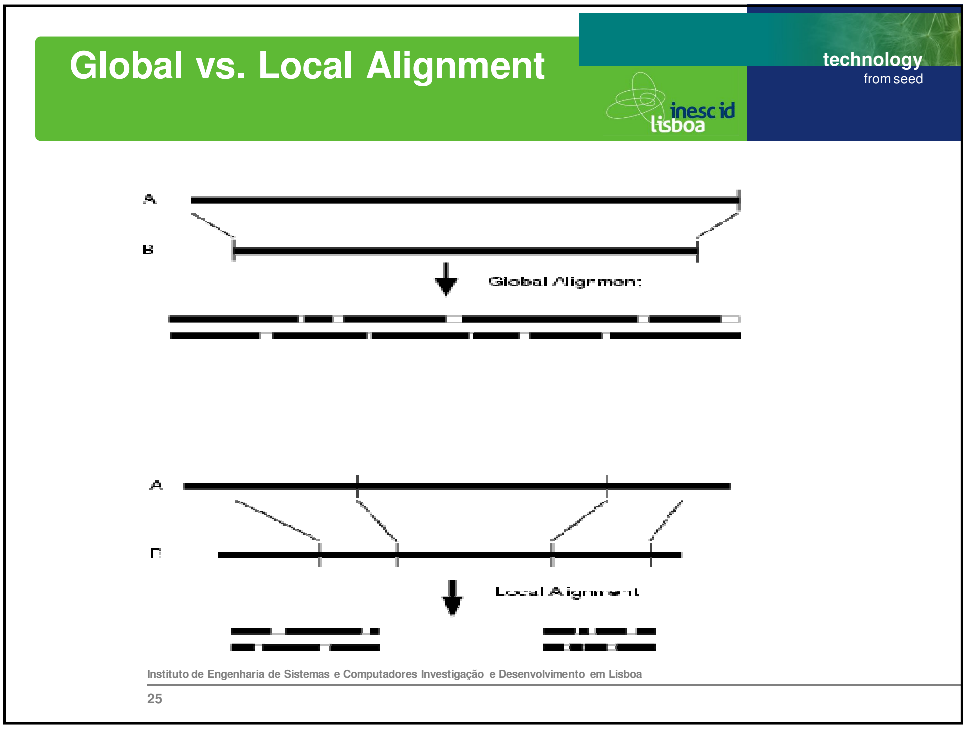
- So at *n* = 20, we have over 120 billion possible alignments
- We want to be able to align much, much longer sequences
  - some proteins have 1000 amino acids
  - genes can have several thousand base pairs





Global vs. Local Alignment

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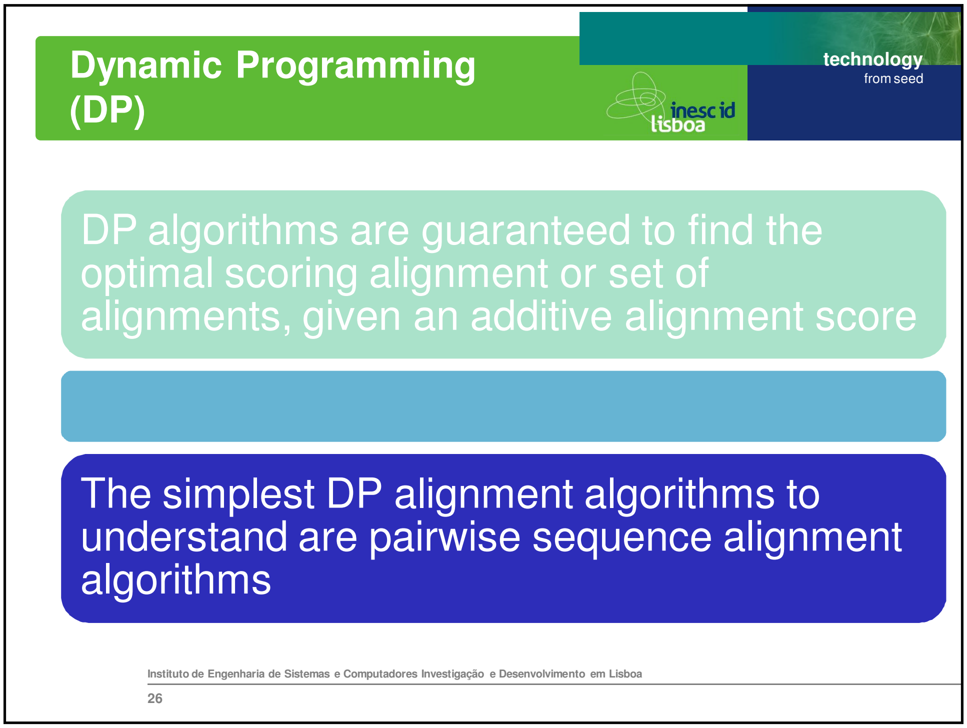

Dynamic Programming (DP)

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DP algorithms are guaranteed to find the optimal scoring alignment or set of alignments, given an additive alignment score

The simplest DP alignment algorithms to understand are pairwise sequence alignment algorithms

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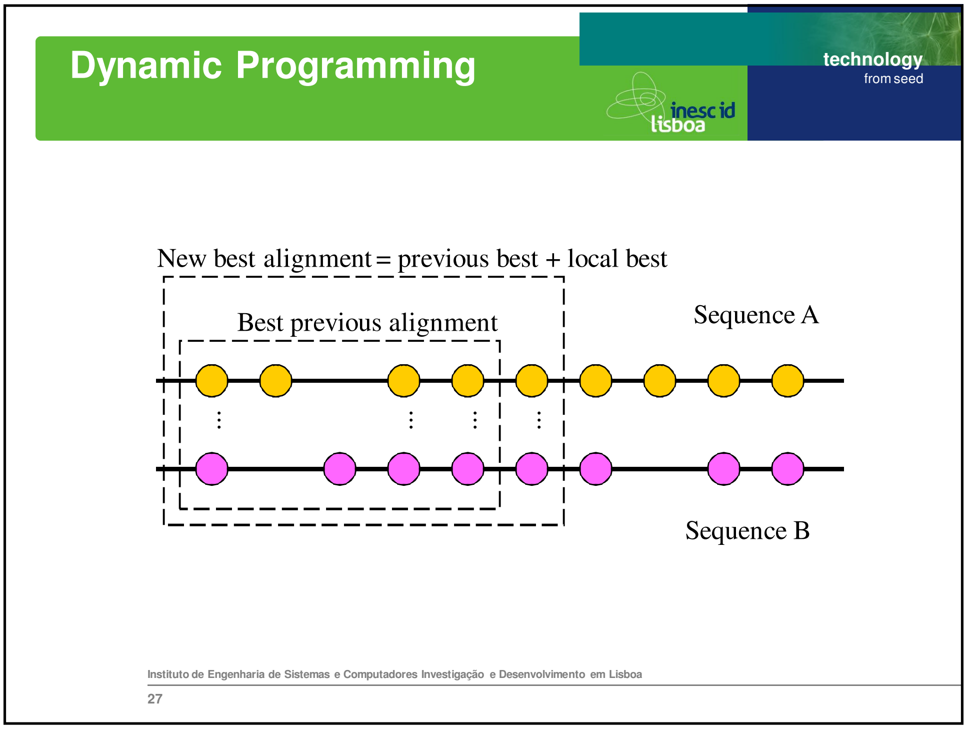


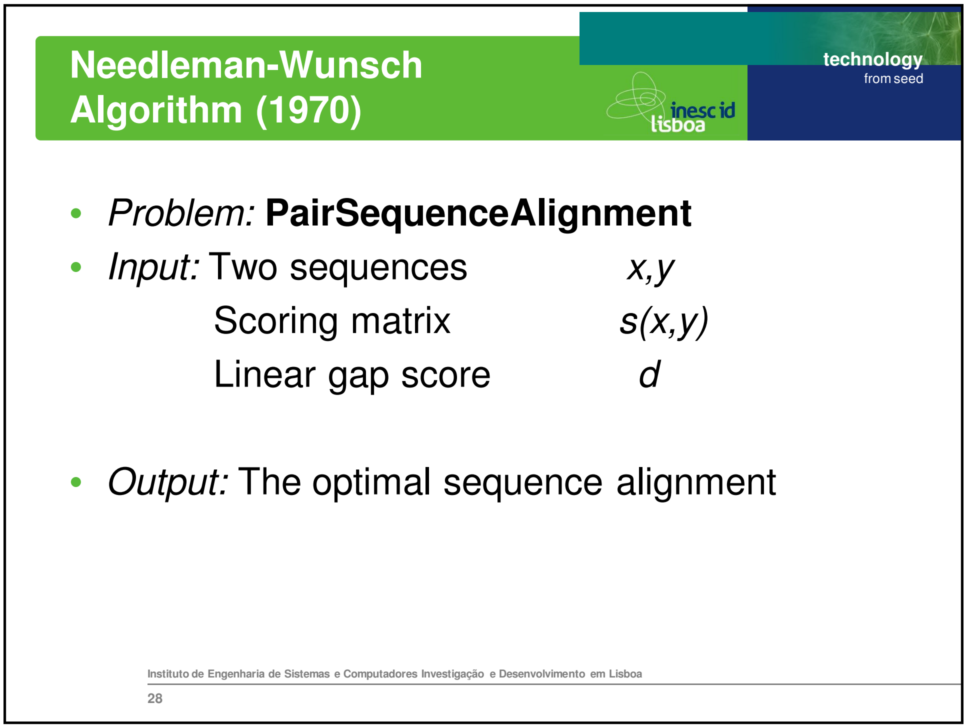
Dynamic Programming

New best alignment = previous best + local best

Best previous alignment

Sequence A

Sequence B



Needleman-Wunsch Algorithm (1970)

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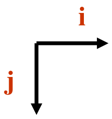
- *Problem:* **PairSequenceAlignment**
- *Input:* Two sequences x,y
  Scoring matrix $s(x,y)$
  Linear gap score d
- *Output:* The optimal sequence alignment

F matrix



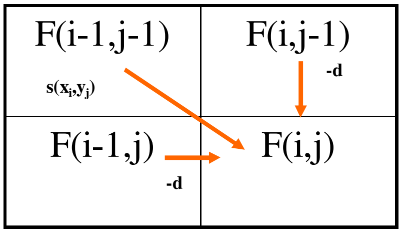
		H	E	A	G	A	W	G	H	E	E
P											
A											
W					X						
H											
E											
A											
E											

Three ways to obtain the best score F(i,j)

- x_i is aligned to y_j
- x_i is aligned to a gap
- y_j is aligned to a gap

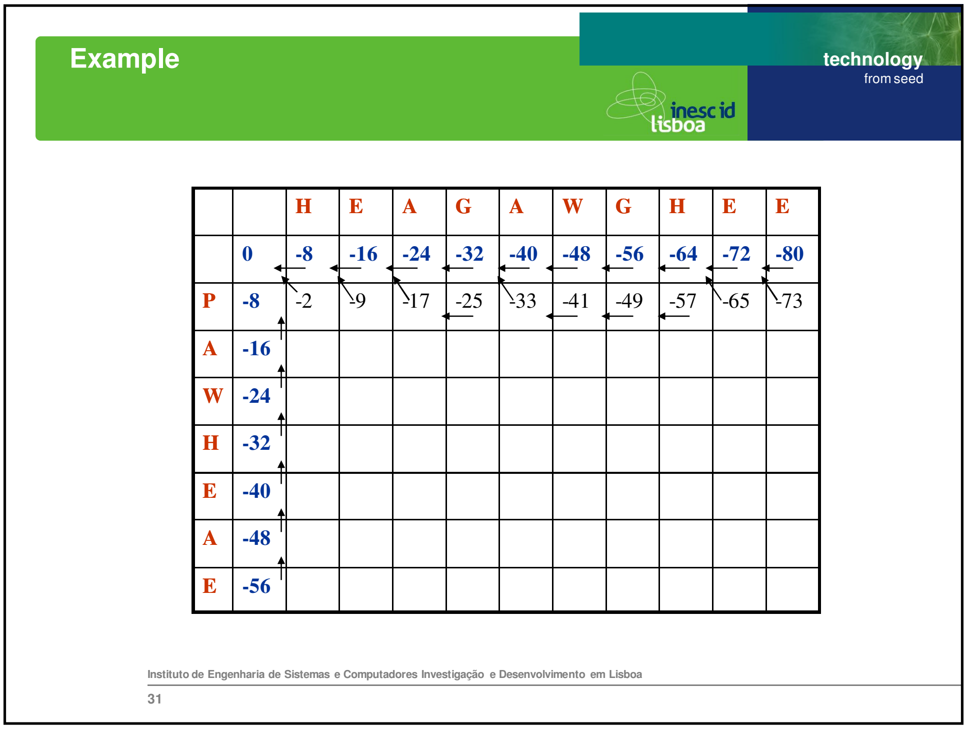
HEA x_i
-PA y_i

$$F(i,j)=F(i-1,j-1)+s(x_i,y_i)$$



- While building the table, keep track of where optimal score came from
- Initialize: F(0,0) = 0, F(i,0) = -id, F(0,j)=-jd
- Fill from top left to bottom right using the recursive relation

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



Example

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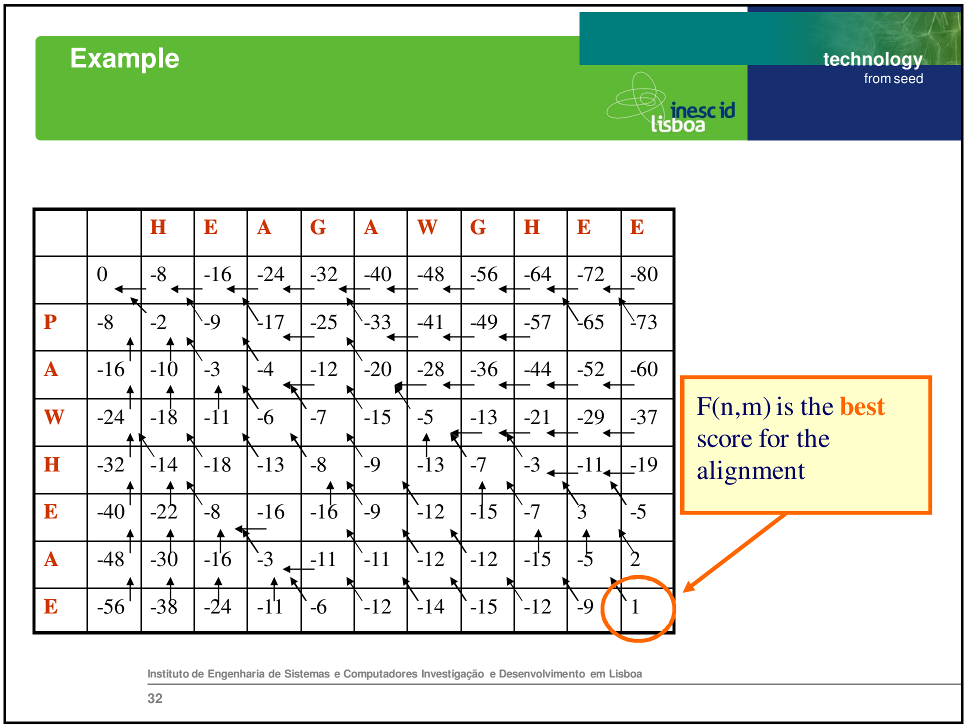
		H	E	A	G	A	W	G	H	E	E
	0	-8	-16	-24	-32	-40	-48	-56	-64	-72	-80
P	-8	-2	-9	-17	-25	-33	-41	-49	-57	-65	-73
A	-16										
W	-24										
H	-32										
E	-40										
A	-48										
E	-56										



Example

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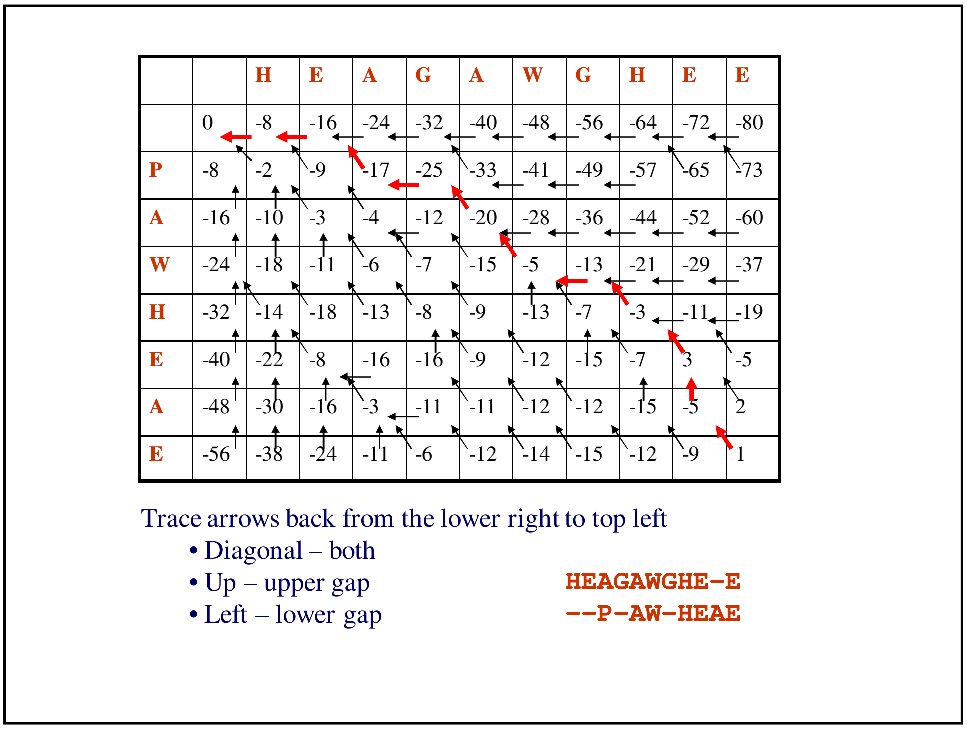
		H	E	A	G	A	W	G	H	E	E
	0	-8	-16	-24	-32	-40	-48	-56	-64	-72	-80
P	-8	-2	-9	-17	-25	-33	-41	-49	-57	-65	-73
A	-16	-10	-3	-4	-12	-20	-28	-36	-44	-52	-60
W	-24	-18	-11	-6	-7	-15	-5	-13	-21	-29	-37
H	-32	-14	-18	-13	-8	-9	-13	-7	-3	-11	-19
E	-40	-22	-8	-16	-16	-9	-12	-15	-7	3	-5
A	-48	-30	-16	-3	-11	-11	-12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

F(n,m) is the **best** score for the alignment

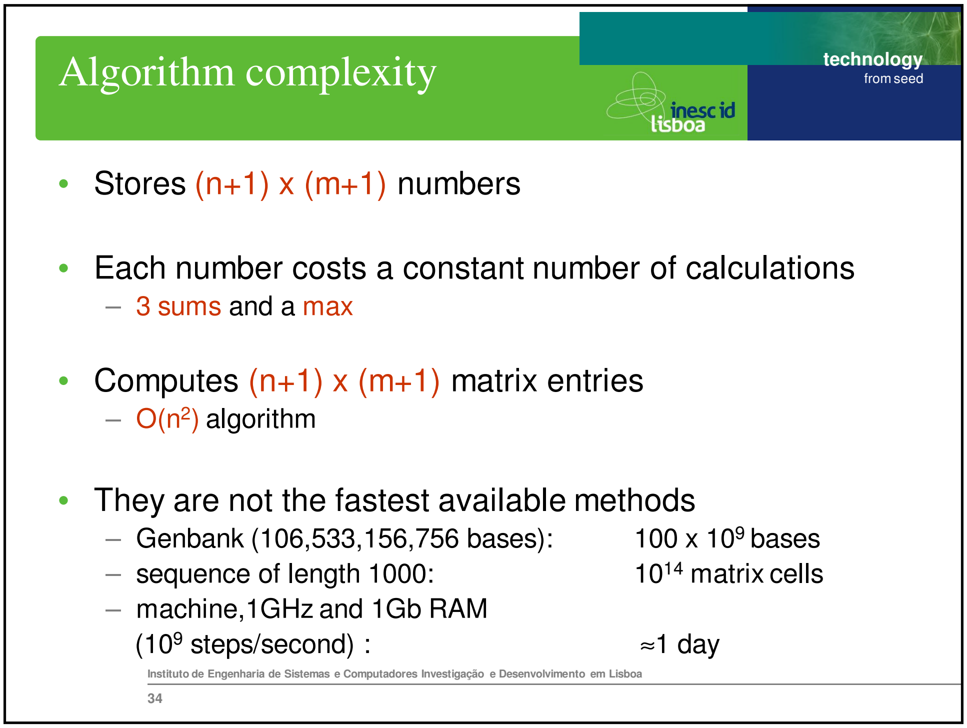


		H	E	A	G	A	W	G	H	E	E
	0	-8	-16	-24	-32	-40	-48	-56	-64	-72	-80
P	-8	-2	-9	-17	-25	-33	-41	-49	-57	-65	-73
A	-16	-10	-3	-4	-12	-20	-28	-36	-44	-52	-60
W	-24	-18	-11	-6	-7	-15	-5	-13	-21	-29	-37
H	-32	-14	-18	-13	-8	-9	-13	-7	-3	-11	-19
E	-40	-22	-8	-16	-16	-9	-12	-15	-7	3	-5
A	-48	-30	-16	-3	-11	-11	-12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

Trace arrows back from the lower right to top left

- Diagonal – both
- Up – upper gap
- Left – lower gap

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



Algorithm complexity

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- Stores (n+1) x (m+1) numbers
- Each number costs a constant number of calculations
  - 3 sums and a max
- Computes (n+1) x (m+1) matrix entries
  - O(n^2) algorithm
- They are not the fastest available methods
  - Genbank (106,533,156,756 bases): 100 x 10^9 bases
  - sequence of length 1000: 10^{14} matrix cells
  - machine,1GHz and 1Gb RAM
    (10^9 steps/second) : ≈1 day

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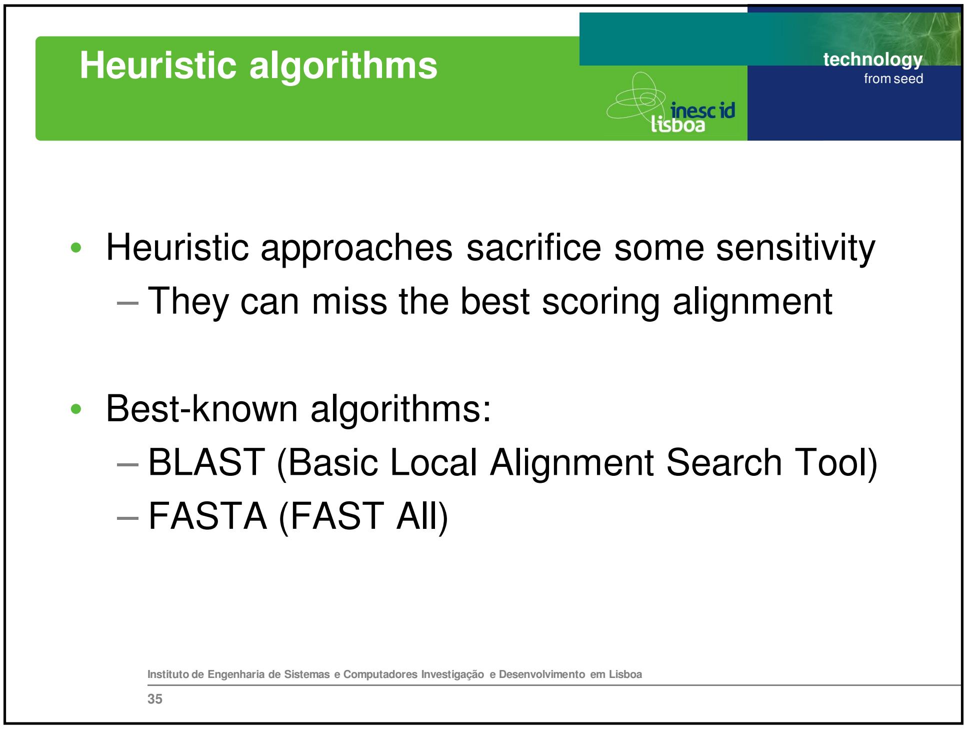
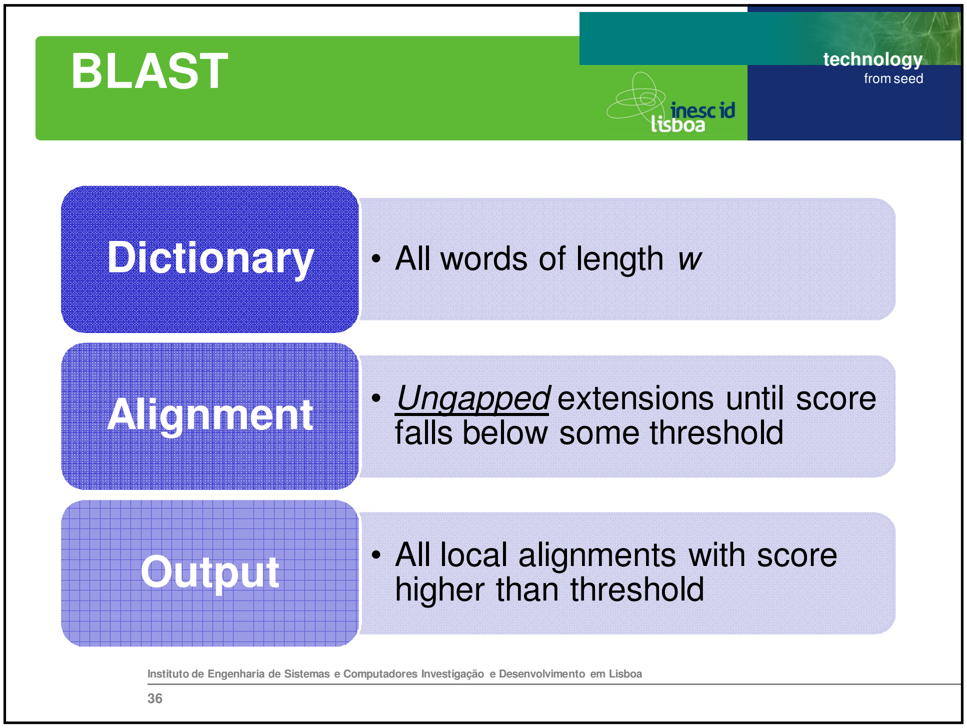


Heuristic algorithms

- Heuristic approaches sacrifice some sensitivity
  - They can miss the best scoring alignment
- Best-known algorithms:
  - BLAST (Basic Local Alignment Search Tool)
  - FASTA (FAST All)



BLAST

Dictionary
- All words of length *w*

Alignment
- *Ungapped* extensions until score falls below some threshold

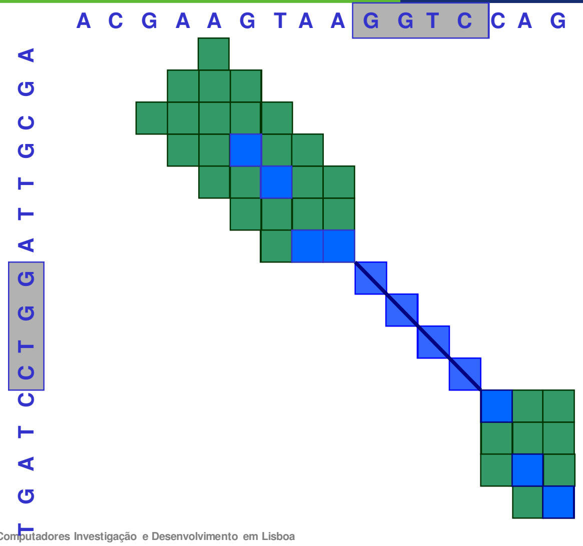
Output
- All local alignments with score higher than threshold

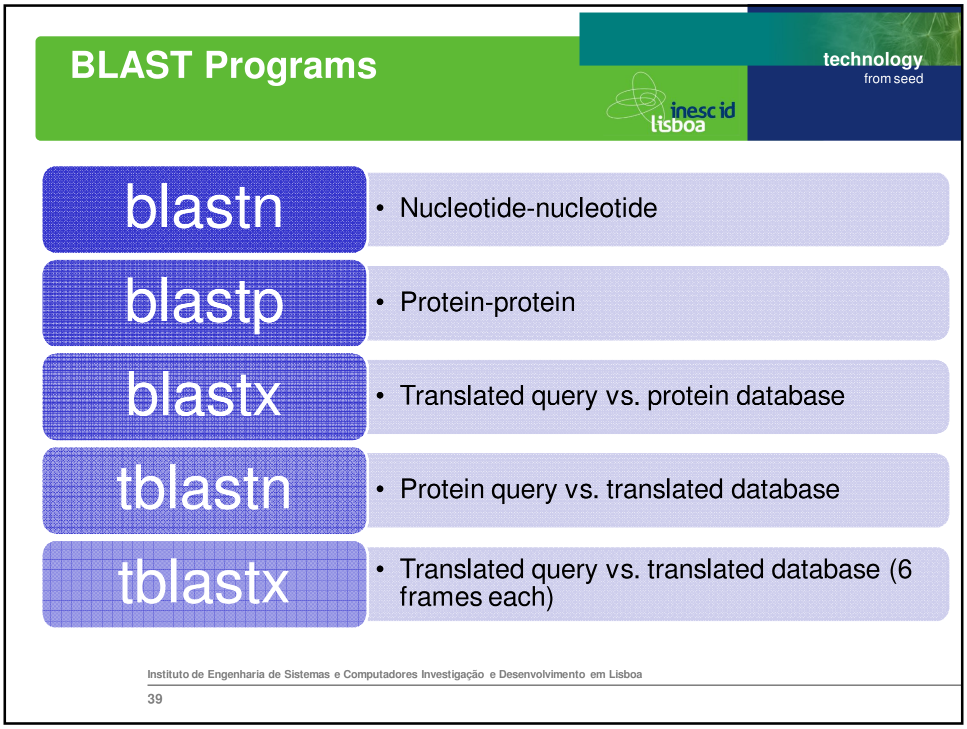
BLAST

- Make a list of *neighborhood words*
  - length 3 for proteins, 11 for nucleic acids
- Match query with score higher than some threshold
  - usually 2 bits per residue
- Scans database for words
- When a hit is obtained, extends the match in both direction as ungapped alignment

BLAST

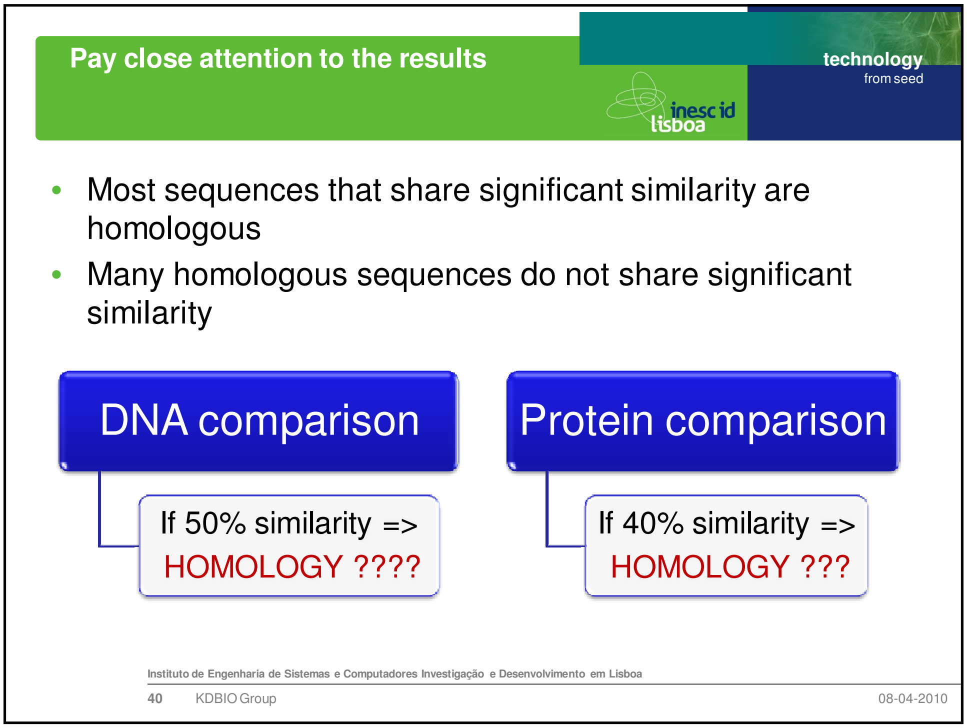
- Original BLAST exact keyword search
- Extend with gaps in a zone around ends of exact match





BLAST Programs

blastn	Nucleotide-nucleotide
blastp	Protein-protein
blastx	Translated query vs. protein database
tblastn	Protein query vs. translated database
tblastx	Translated query vs. translated database (6 frames each)



Pay close attention to the results

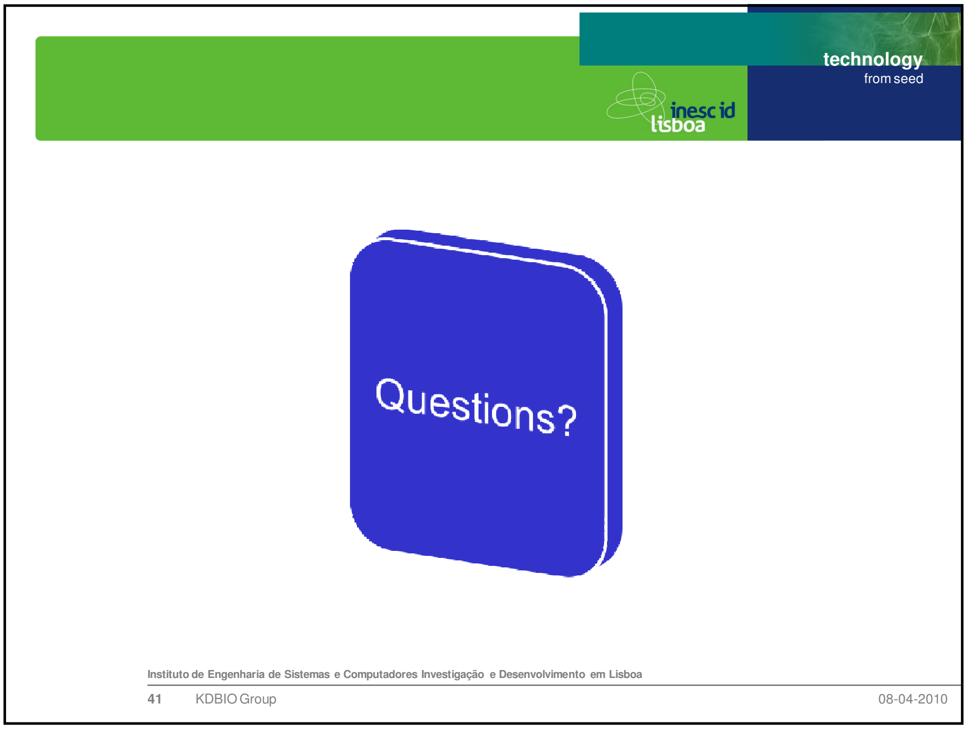
- Most sequences that share significant similarity are homologous
- Many homologous sequences do not share significant similarity

DNA comparison

If 50% similarity => HOMOLOGY ????

Protein comparison

If 40% similarity => HOMOLOGY ???



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Questions?

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