Introduction to Bioinformatics

Lecture 3:
Sequence alignment

Sequence alignment

- *The biological problem*
- Global alignment
- Local alignment
- Multiple alignment

Background: comparative genomics

- Basic question in biology: *what properties are shared among organisms?*
- Genome sequencing allows comparison of organisms at DNA and protein levels
- Comparisons can be used to
  - Find evolutionary relationships between organisms
  - Identify functionally conserved sequences
  - Identify corresponding genes in human and model organisms: develop models for human diseases

Homologs

- Two genes (sequences in general) g_B and g_C evolved from the same ancestor gene g_A are called *homologs*
- Homologs usually exhibit conserved functions
- Close evolutionary relationship => expect a high number of homologs

g_A = agtgtccgttaagtgcgttc

g_B = agtgccgttaaagttgtacgtc

g_C = ctgactgtttgtggttc

Sequence similarity

- We expect homologs to be "similar" to each other
- Intuitively, similarity of two sequences refers to the degree of match between corresponding positions in sequence

agtgccgttaaagttgtacgtc

ctgactgtttgtggttc

- What about sequences that differ in length?

Similarity vs homology

- Sequence similarity is not sequence homology
  - If the two sequences g_B and g_C have accumulated enough mutations, the similarity between them is likely to be low

#mutations		#mutations	
0	agtgtccgttaagtgcgttc	64	acagtccgttcgggctattg
1	agtgtccgttatagtgcgttc	128	cagagcactaccgc
2	agtgtccgcttatagtgcgttc	256	cacgagtaagatatagct
4	agtgtccgcttaagggcgttc	512	taatcgtgata
8	agtgtccgcttcaaggggcgt	1024	acccttatctacttcctggagtt
16	gggccgttcatgggggt	2048	agcgacctgcccaa
32	gcagggcgtcactgagggct	4096	caaac

Homology is more difficult to detect over greater evolutionary distances.

Similarity vs homology (2)

- Sequence similarity can occur by chance
  - *Similarity does not imply homology*
- Consider comparing two short sequences against each other

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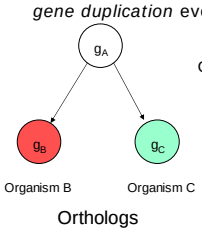
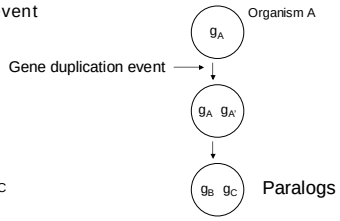
Orthologs and paralogs

- We distinguish between two types of homology
  - Orthologs: homologs from two different species, separated by a *speciation* event
  - Paralogs: homologs within a species, separated by a *gene duplication* event



Orthologs



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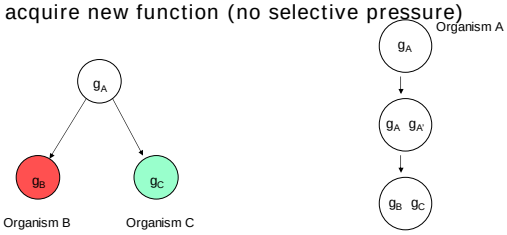
Orthologs and paralogs (2)

- Orthologs typically retain the original function
- In paralogs, one copy is free to mutate and acquire new function (no selective pressure)



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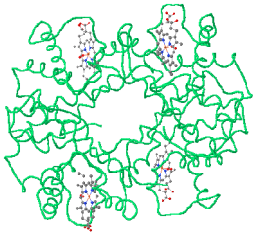
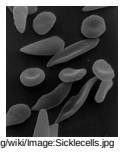
Paralogy example: hemoglobin

- Hemoglobin is a protein complex which transports oxygen
- In humans, hemoglobin consists of four protein subunits and four non-protein heme groups



Hemoglobin A, www.rcsb.org/pdb/explore.do?structureId=1GZX

Sickle cell diseases are caused by mutations in hemoglobin genes



http://en.wikipedia.org/wiki/Image:Sicklecells.jpg

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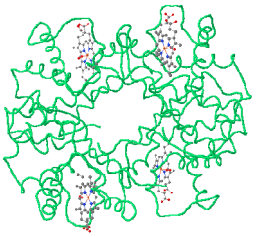
Paralogy example: hemoglobin

- In adults, three types are normally present
  - Hemoglobin A: 2 alpha and 2 beta subunits
  - Hemoglobin A2: 2 alpha and 2 delta subunits
  - Hemoglobin F: 2 alpha and 2 gamma subunits
- Each type of subunit (alpha, beta, gamma, delta) is encoded by a separate gene



Hemoglobin A, www.rcsb.org/pdb/explore.do?structureId=1GZX

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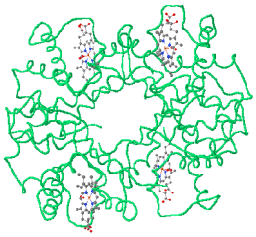
Paralogy example: hemoglobin

- The subunit genes are paralogs of each other, i.e., they have a common ancestor gene
- Exercise: hemoglobin human paralogs in NCBI sequence databases
  http://www.ncbi.nlm.nih.gov/sites/entrez?db=Nucleotide
  - Find human hemoglobin alpha, beta, gamma and delta
  - Compare sequences



Hemoglobin A, www.rcsb.org/pdb/explore.do?structureId=1GZX

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Orthology example: insulin

- The genes coding for insulin in human (*Homo sapiens*) and mouse (*Mus musculus*) are orthologs:
  - They have a common ancestor gene in the ancestor species of human and mouse
  - Exercise: find insulin orthologs from human and mouse in NCBI sequence databases

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Sequence alignment

- Alignment specifies which positions in two sequences match

```
acgtctag   acgtctag    acgtctag
||           |||||     || |||||
actctag-   -actctag    ac-tctag
```

2 matches	5 matches	7 matches
5 mismatches	2 mismatches	0 mismatches
1 not aligned	1 not aligned	1 not aligned

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Sequence alignment

- Maximum alignment length is the total length of the two sequences

```
acgtctag-------   -------acgtctag

--------actctag   actctag--------
```

0 matches	0 matches
0 mismatches	0 mismatches
15 not aligned	15 not aligned

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Mutations: Insertions, deletions and substitutions

Indel: insertion or deletion of a base with respect to the ancestor sequence

```
acgtctag
 |  |||||
-actctag
```

Mismatch: substitution (point mutation) of a single base

- Insertions and/or deletions are called *indels*
  - *We can't tell whether the ancestor sequence had a base or not at indel position!*

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Problems

- What sorts of alignments should be considered?
- How to score alignments?
- How to find optimal or good scoring alignments?
- How to evaluate the statistical significance of scores?

In this course, we discuss each of these problems briefly.

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Sequence Alignment (chapter 6)

- The biological problem
- *Global alignment*
- Local alignment
- Multiple alignment

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Global alignment

- Problem: find optimal scoring alignment between two sequences (Needleman & Wunsch 1970)
- Every position in both sequences is included in the alignment
- We give score for each position in alignment
  - Identity (match) +1
  - Substitution (mismatch) -μ
  - Indel -δ
- Total score: sum of position scores

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Scoring: Toy example

- Consider two sequences with characters drawn from the English language alphabet: WHAT, WHY

```
WHAT
||
WH-Y
```

S(WHAT/WH-Y) = 1 + 1 – δ – μ

```
WHAT
-WHY
```

S(WHAT/-WHY) = – δ – μ – μ – μ

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Dynamic programming

- How to find the optimal alignment?
- We use previous solutions for optimal alignments of smaller subsequences
- This general approach is known as dynamic programming

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Introduction to dynamic programming: the money change problem

- Suppose you buy a pen for 4.23€ and pay for with a 5€ note
- You get 77 cents in change – what coins is the cashier going to give you if he or she tries to minimise the number of coins?
- The usual algorithm: start with largest coin (denominator), proceed to smaller coins until no change is left:
  - 50, 20, 5 and 2 cents
- This greedy algorithm is *incorrect*, in the sense that it does not always give you the correct answer

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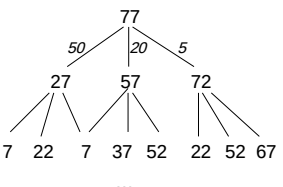
The money change problem

- How else to compute the change?
- We could consider all possible ways to reduce the amount of change
- Suppose we have 77 cents change, and the following coins: 50, 20, 5 cents
- We can compute the change with *recursion*
  - *C(n) = min { C(n – 50) + 1, C(n – 20) + 1, C(n – 5) + 1 }*
- Figure shows the recursion tree for the example



- Many values are computed more than once!
- This leads to a correct but very inefficient algorithm

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The money change problem

- We can speed the computation up by solving the change problem for all i ≤ n
  - Example: solve the problem for 9 cents with available coins being 1, 2 and 5 cents
- Solve the problem in steps, first for 1 cent, then 2 cents, and so on
- In each step, utilise the solutions from the previous steps

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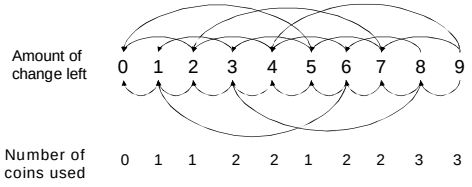
The money change problem



- Algorithm runs in time proportional to Md, where M is the amount of change and d is the number of coin types
- The same technique of storing solutions of subproblems can be utilised in aligning sequences

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Representing alignments and scores

Alignments can be represented in the following tabular form.

Each alignment corresponds to a path through the table.

```
WHAT
||
WH-Y
```

	-	W	H	A	T
-					
W					
H					
Y					

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Representing alignments and scores

```
WH-AT
||
WHY--

WHAT---

----WHY
```

	-	W	H	A	T
-					
W					
H					
Y					

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Representing alignments and scores

```
WHAT
||
WH-Y
```

Global alignment score $S_{3,4} = 2\text{-}\delta\text{-}\mu$

	-	W	H	A	T
-	0				
W		1			
H			2	2-δ	
Y					2-δ-μ

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Filling the alignment matrix

	-	W	H	A	T
-					
W		Case 1		Case 2	
H		Case 3			
Y					

Consider the alignment process at shaded square.

Case 1. Align H against H (match)

Case 2. Align H in WHY against – (indel) in WHAT

Case 3. Align H in WHAT against – (indel) in WHY

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Filling the alignment matrix (2)

	-	W	H	A	T
-					
W		Case 1		Case 2	
H		Case 3			
Y					

Scoring the alternatives.

Case 1. $S_{2,2} = S_{1,1} + s(2, 2)$

Case 2. $S_{2,2} = S_{1,2} - \delta$

Case 3. $S_{2,2} = S_{2,1} - \delta$

$s(i, j) = 1$ for matching positions,

$s(i, j) = -\mu$ for substitutions.

Choose the case (path) that yields the maximum score.

Keep track of path choices.

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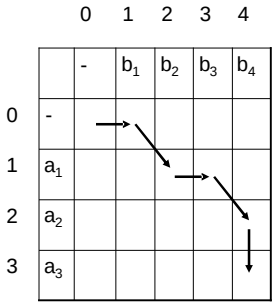
Global alignment: formal development

$A = a_1a_2a_3\ldots a_n$,
$B = b_1b_2b_3\ldots b_m$

b_1	b_2	b_3	b_4	-
-	a_1	-	a_2	a_3

- Any alignment can be written as a unique path through the matrix
- Score for aligning A and B up to positions i and j:

$S_{i,j} = S(a_1a_2a_3\ldots a_i, b_1b_2b_3\ldots b_j)$



Scoring partial alignments

- Alignment of $A = a_1a_2a_3\ldots a_i$ with $B = b_1b_2b_3\ldots b_j$ can be end in three possible ways
  - Case 1: $(a_1a_2\ldots a_{i-1})\ a_i$ / $(b_1b_2\ldots b_{j-1})\ b_j$
  - Case 2: $(a_1a_2\ldots a_{i-1})\ a_i$ / $(b_1b_2\ldots b_j)$ -
  - Case 3: $(a_1a_2\ldots a_i)$ – / $(b_1b_2\ldots b_{j-1})\ b_j$

Scoring alignments

- Scores for each case:
  - Case 1: $(a_1a_2\ldots a_{i-1})\ a_i$ / $(b_1b_2\ldots b_{j-1})\ b_j$
  - Case 2: $(a_1a_2\ldots a_{i-1})\ a_i$ / $(b_1b_2\ldots b_j)$ –
  - Case 3: $(a_1a_2\ldots a_i)$ – / $(b_1b_2\ldots b_{j-1})\ b_j$

$$s(a_i, b_j) = \begin{cases} +1 \text{ if } a_i = b_j \\ -\mu \text{ otherwise} \end{cases}$$

$s(a_i, -) = s(-, b_j) = -\delta$

Scoring alignments (2)

- First row and first column correspond to initial alignment against indels:
  - $S(i, 0) = -i\ \delta$
  - $S(0, j) = -j\ \delta$
- Optimal global alignment score $S(A, B) = S_{n,m}$

		0	1	2	3	4
		-	b_1	b_2	b_3	b_4
0	-	0	-δ	-2δ	-3δ	-4δ
1	a_1	-δ				
2	a_2	-2δ				
3	a_3	-3δ				

Algorithm for global alignment

```
Input sequences A, B, n = |A|, m = |B|
Set S_{i,0} := -δi for all i
Set S_{0,j} := -δj for all j
for i := 1 to n
  for j := 1 to m
    S_{i,j} := max{S_{i-1,j} – δ, S_{i-1,j-1} + s(a_i,b_j), S_{i,j-1} – δ}
  end
end
```

Algorithm takes O(nm) time

Global alignment: example

μ = 1
δ = 2

		-	T	G	G	T	G
	-	0	-2	-4	-6	-8	-10
	A	-2					
	T	-4					
	C	-6					
	G	-8					
	T	-10					?

Global alignment: example

μ = 1

δ = 2

	-	T	G	G	T	G
-	0	-2	-4	-6	-8	-10
A	-2	-1	-3			
T	-4					
C	-6					
G	-8					
T	-10					?

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Global alignment: example (2)

μ = 1

δ = 2

	-	T	G	G	T	G
-	0	-2	-4	-6	-8	-10
A	-2	-1	-3	-5	-7	-9
T	-4	-1	-2	-4	-4	-6
C	-6	-3	-2	-3	-5	-5
G	-8	-5	-2	-1	-3	-4
T	-10	-7	-4	-3	0	-2

```
ATCGT-
|  ||
-TGGTG
```

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