



UNIVERSITY OF  
CAMBRIDGE

## Bioinformatic Algorithms

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Computer Laboratory

12 lectures — Michaelmas 2015

start 8 October, 2015



## Topics and List of algorithms

- Basic concepts in genetics.
- Dynamic programming (Longest Common Subsequence, Needleman-Wunsch, Smith-Waterman, Hirschberg, Nussinov).
- Progressive alignment (Clustal).
- Homology database search (Blast, Patternhunter).
- Next Generation sequencing (De Bruijn graph, Burrows-Wheeler transform)
- Phylogeny - parsimony-based - (Fitch, Sankoff).
- Phylogeny - distance based - (UPGMA, Neighbor Joining; Bootstrap).
- Clustering (K-means, Markov Clustering)
- Hidden Markov Models applications in Bioinformatics (Genescan, TMHMM).
- Pattern search in sequences (Gibbs sampling).
- Biological Networks reconstruction (Wagner) and simulation (Gillespie).



- The course focuses on algorithms used in bioinformatics
- The algorithms presented in this course could be also applied in other data-rich fields.
- At the end of the course the student should be able to describe the main aspects of the algorithms.
- The student should understand how bioinformatics combines biology and computing.
- The exam papers will not contain biological questions.
- References and links to additional material at the end of the lecture notes may help the students to understand better the applications of the algorithms (this is not essential to answer exam questions).



First we provide an overview of the most important biological concepts. Then we learn how to compare 2 strings representing DNA sequences (or different parts of the same string). Searching a database for nearly exact matches is a key task in a Bioinformatics lab. Algorithms for big sequence data. We learn how to build trees to study sequences relationship and how to cluster biological data. We use hidden Markov models to predict properties of sequence parts such as exon/intron arrangements in a gene or the structure of a membrane protein. Sequence Patterns dispersed in sequences could be identified by iterated techniques. Biological networks: algorithms for reconstructing genetic regulatory networks and simulation of biochemical reaction networks. Material and figure acknowledgements at the end of this notes and during the lectures.



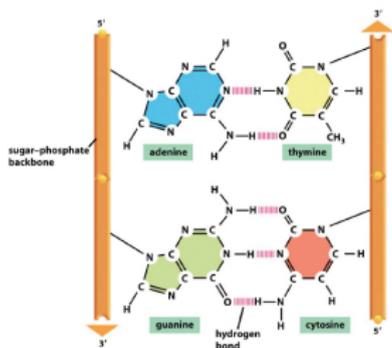
Basic concepts in Genetics-Genomics Some other concepts in the next lectures.  
Important concepts: what is a gene, what is a genome, genotype-phenotype, importance of biological networks, questions we would like to answer, algorithms we use to answer, type of data.  
At the end of the lecture notes there are links to material relevant to each chapter.



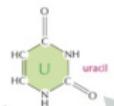
## Basic concepts in genetics and genomics

DNA could be represented as a string of symbols from a 4-letter (bases) alphabet, A (adenine), T (thymine), C (cytosine) and G (guanine). In the double helix A pairs with T, C with G (so only the sequence of one filament is vital to keep). A gene is a string of DNA that contains information for a specific cell function. The Genome is the entire DNA in a cell nucleus.

RNA is same as DNA but T is replaced by U (uracil); proteins are strings of amino acids from an alphabet of 20. The proteins have a 3D shape that could be described by a graph. The genetic code is a map between 61 triplets of DNA bases and 20 amino acids.



**A to T**  
**G to C**



In RNA (different five carbon sugar in the backbone) uracil is used instead of thymine



# Comparing CS and Biological information

from [www.nsta.org/publications/news/story.aspx?id=47561](http://www.nsta.org/publications/news/story.aspx?id=47561)

## Biology

1. Digital alphabet consists of bases A, C, T, G
2. Codons consist of three bases
3. Genes consist of codons
4. Promoters indicate gene locations
5. DNA information is transcribed into hnRNA and processed into mRNA
6. mRNA information is translated into proteins
7. Genes may be organized into operons or groups with similar promoters
8. "Old" genes are not destroyed; their promoters become nonfunctional
9. Entire chromosomes are replicated
10. Genes can diversify into a family of genes through duplication
11. DNA from a donor can be inserted into host chromosomes
12. Biological viruses disrupt genetic instructions
13. Natural selection modifies the genetic basis of organism design
14. A successful genotype in a natural population outcompetes others

## Computer science

1. Digital alphabet consists of 0, 1
2. Computer bits form bytes
3. Files consist of bytes
4. File-allocation table indicates file locations
5. Disc information is transcribed into RAM
6. RAM information is translated onto a screen or paper
7. Files are organized into folders
8. "Old" files are not destroyed; references to their location are deleted
9. Entire discs can be copied
10. Files can be modified into a family of related files
11. Digital information can be inserted into files
12. Computer viruses disrupt software instructions
13. Natural selection procedures modify the software that specifies a machine design
14. A successful website attracts more "hits" than others



Unit: DNA base (A (adenine), T (thymine), C (cytosine) and G (guanine))

Polymer: DNA molecule

Unit: RNA base (A (adenine), U (uracil), C (cytosine) and G (guanine)) Polymer: RNA molecule

Unit: amino acid (there are 20 amino acids) Polymer: the protein (a linear, unbranched chain of amino acids); it can bind to other polymers.

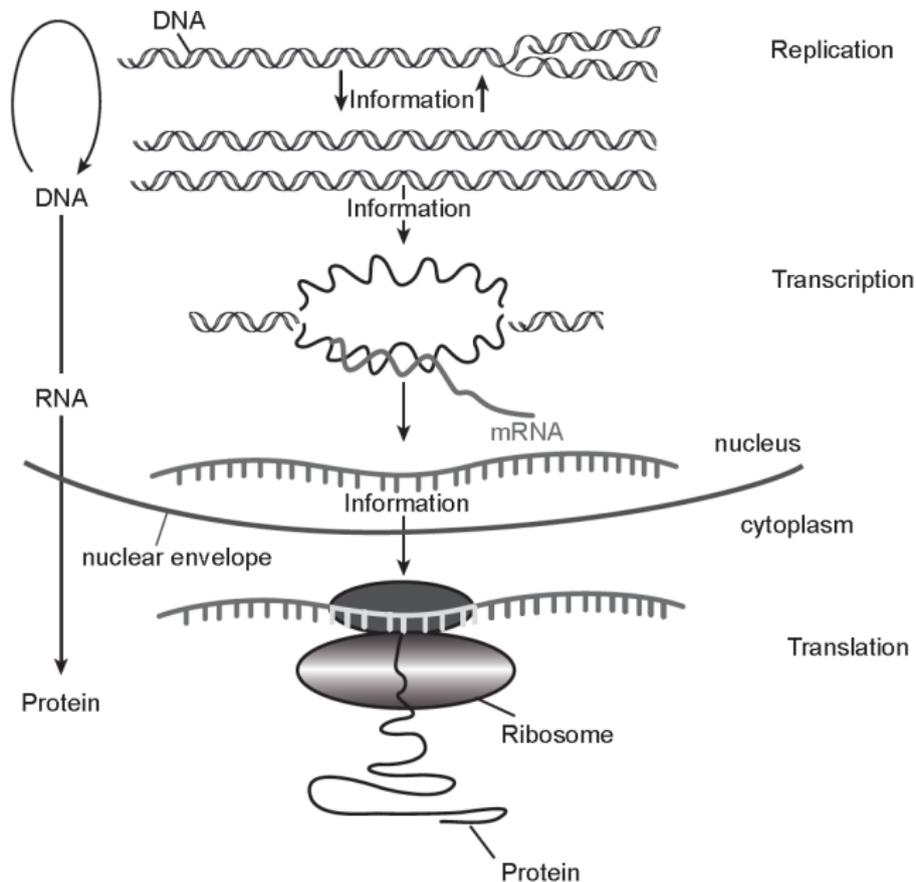
Polymers can be thought as strings (the information is the sequence of symbols) or as graph (the information is the 3 dimensional structure)

During species evolution, the strings undergo modifications of length n-units (mutations (base or amino acid replacement), insertions (adding more bases or amino acids), deletions (loss of n bases or amino acids)); large part of bioinformatics deals with sequence (string) algorithms for alignment, tree, searching for conserved motifs, etc. The study of these modifications is the core of the course.

The 3D graph topology of the protein determines the 3D connectivity with other proteins or DNA (forming functional networks). We will study networks in the last lecture.

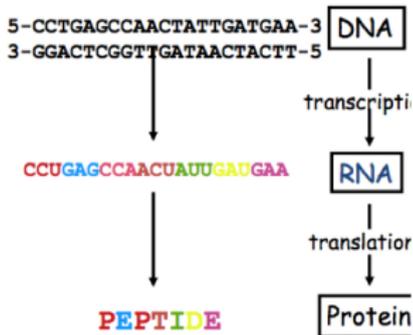


# The (almost) Dogma



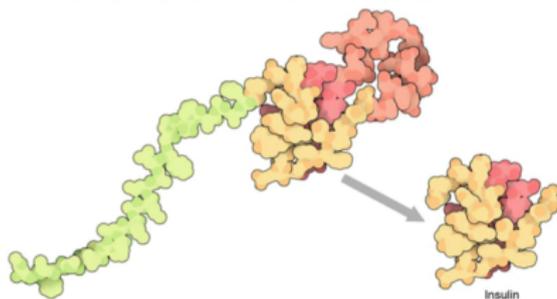
# The most Common flow of biological information

DNA makes RNA makes proteins (the 3D graph below); given the pairing rule in a DNA double strands molecule, all the information is in each single strand. The RNA is termed mRNA (messenger); triplets of bases of mRNA are copied into a chain of amino acids (the protein) according to the genetic code.

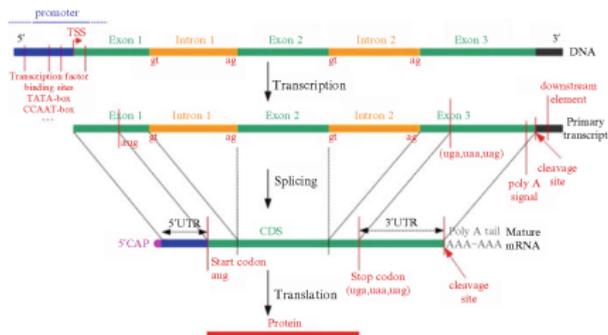


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# Transcription information process and the translation mapping, i.e. Genetic code

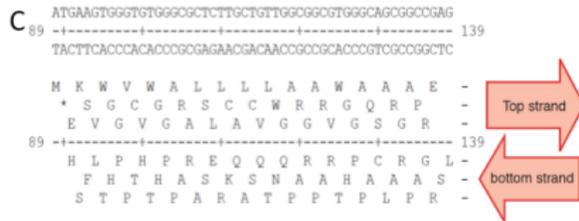
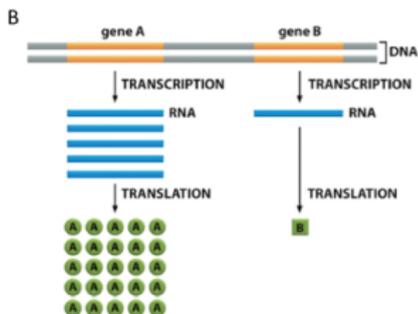
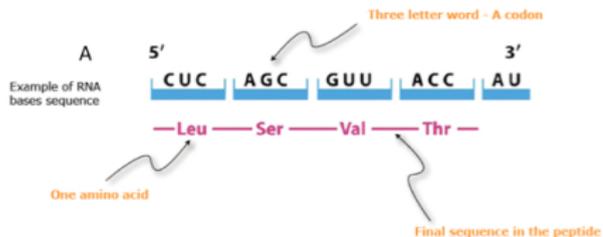


		Second Position of Codon					
		U	C	A	G		
F	i	UUU Phe [F]	UCU Ser [S]	UAU Tyr [Y]	UGU Cys [C]	U	C
		UUC Phe [F]	UCC Ser [S]	UAC Tyr [Y]	UGC Cys [C]		
		UUA Leu [L]	UCA Ser [S]	UAA Ter [end]	UGA Ter [end]		
		UUG Leu [L]	UCG Ser [S]	UAG Ter [end]	UGG Trp [W]		
s	t	CUU Leu [L]	CCU Pro [P]	CAU His [H]	CGU Arg [R]	U	C
		CUC Leu [L]	CCC Pro [P]	CAC His [H]	CGC Arg [R]		
		CUA Leu [L]	CCA Pro [P]	CAA Gln [Q]	CGA Arg [R]		
		CUG Leu [L]	CCG Pro [P]	CAG Gln [Q]	CGG Arg [R]		
o	s	AUU Ile [I]	ACU Thr [T]	AAU Asn [N]	AGU Ser [S]	U	C
		AUC Ile [I]	ACC Thr [T]	AAC Asn [N]	AGC Ser [S]		
		AUA Ile [I]	ACA Thr [T]	AAA Lys [K]	AGA Arg [R]		
		AUG Met [M]	ACG Thr [T]	AAG Lys [K]	AGG Arg [R]		
G	n	GUU Val [V]	GCU Ala [A]	GAU Asp [D]	GGU Gly [G]	U	C
		GUC Val [V]	GCC Ala [A]	GAC Asp [D]	GGC Gly [G]		
		GUA Val [V]	GCA Ala [A]	GAA Glu [E]	GGA Gly [G]		
		GUG Val [V]	GCG Ala [A]	GAG Glu [E]	GGG Gly [G]		

Part of the information is in the gene (which protein to make); other part of information is the flanking sequences (where a gene starts; extract and link exons).



# A gene (a set of bases with begin and end signals) contains information for a string of amino acids (a protein) which form a 3D graph



- Each triplet of bases codes for one amino acid.
- Genes differ for the amount of messenger RNA and protein molecules they produce (variable among cells type, position and time regulation).
- Potentially the DNA strands could code for 6 proteins.





Genes are activated or repressed by regulatory proteins which bind to gene flanking sequences and are coded by the same or other genes.

A biochemical reaction converts biochemical compounds (analogous to a production rule).

An enzyme is a protein that accelerates chemical reactions. Each enzyme is encoded by one or more genes.

A pathway is a linked set of biochemical reactions occurring in a cell (analogous to a chain of rules).

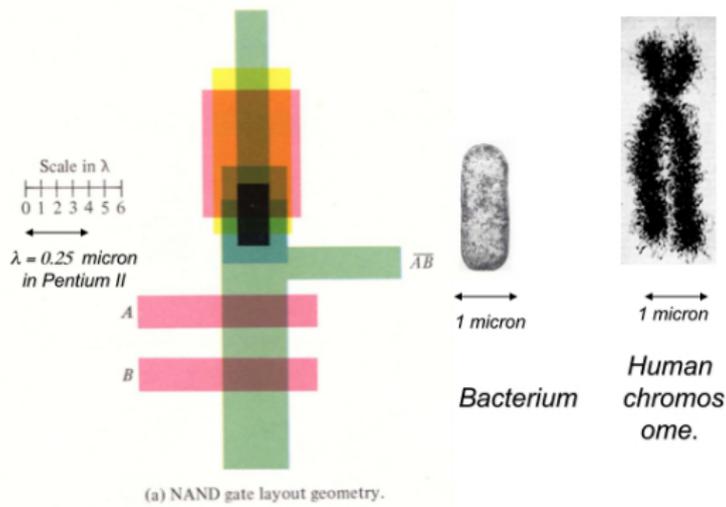
A pathway is a conceptual unit of the metabolism; it represents an ordered set of interconnected, directed biochemical reactions.

The set of metabolic pathways makes the metabolic network that makes the cell phenotype.

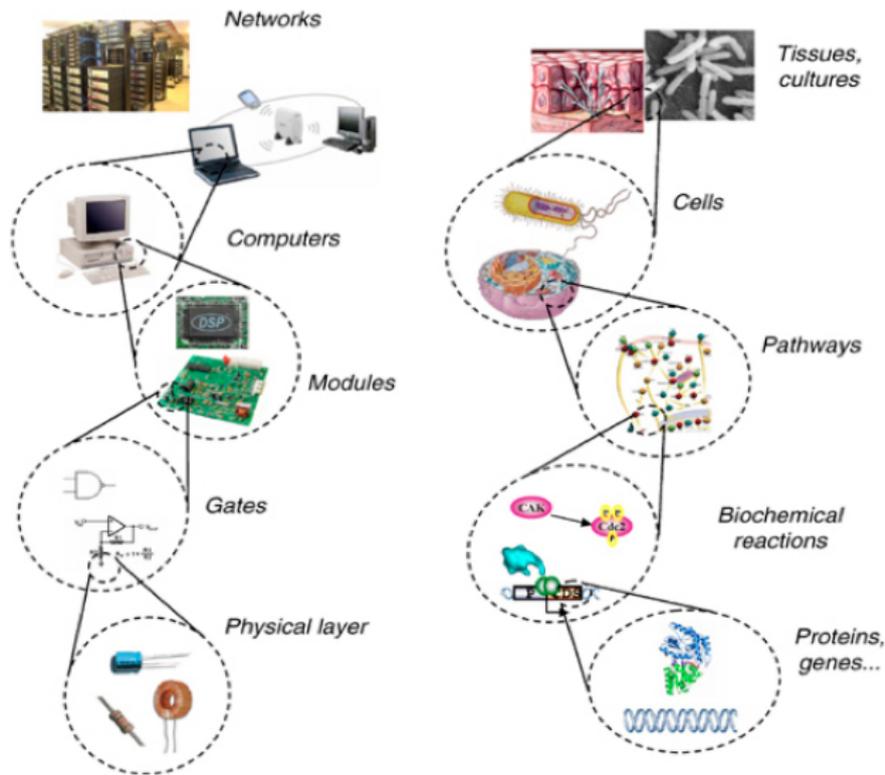


# DNA and chromosomes

In eukaryotes the genetic information is distributed over different DNA molecules. A human cell contains 24 different such chromosomes. If all DNA of a human cell would be laid out end-to-end it would reach approximately 2 meters. The nucleus however measures only  $6\mu\text{m}$ . Equivalent of packing 40 km of fine thread into a tennis ball with a compression ratio of 10000.



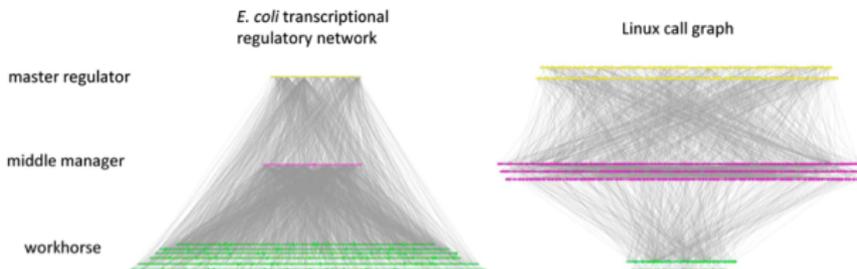
# Comparison between system networks and biological systems



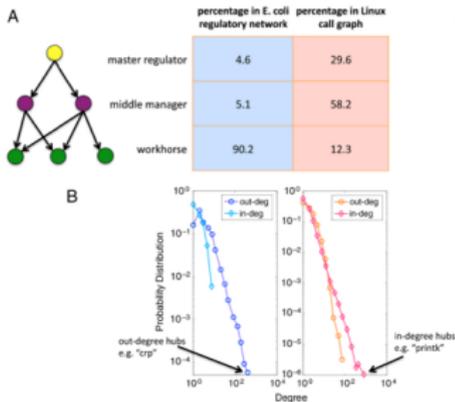
from Andrianantoandro et. al. Mol Syst Biol (2006)



# Comparing Gene networks and Operating Systems

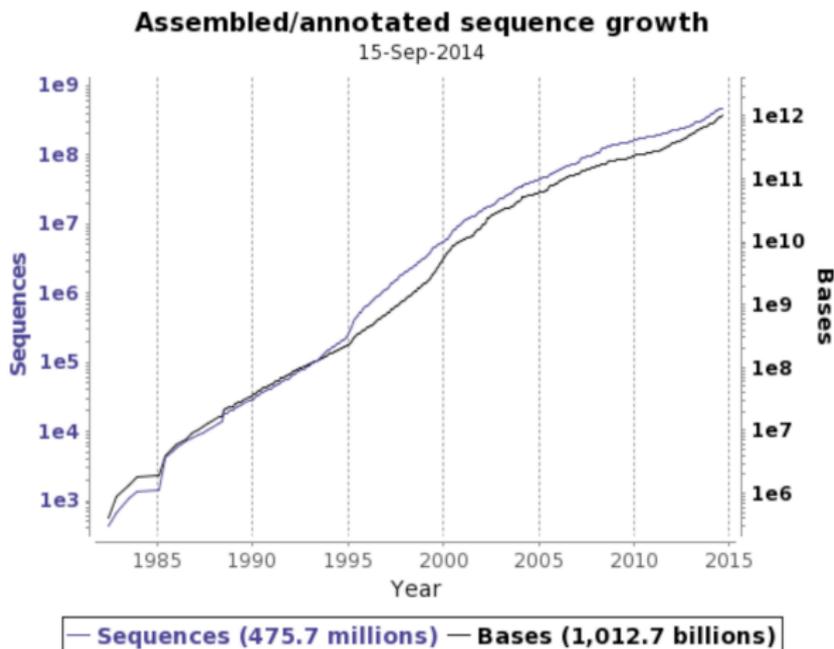


**Fig. 1.** The hierarchical layout of the *E. coli* transcriptional regulatory network and the Linux call graph. (Left) The transcriptional regulatory network of *E. coli*. (Right) The call graph of the Linux Kernel. Nodes are classified into three categories on the basis of their location in the hierarchy: master regulators (nodes with zero in-degree, Yellow), workhorses (nodes with zero out-degree, Green), and middle managers (nodes with nonzero in- and out-degree, Purple). Persistent genes and persistent functions (as defined in the main text) are shown in a larger size. The majority of persistent genes are located at the workhorse level, but persistent functions are underrepresented in the workhorse level. For easy visualization of the Linux call graph, we sampled 10% of the nodes for display. Under the sampling, the relative portion of nodes in the three levels and the ratio between persistent and nonpersistent nodes are preserved compared to the original network. The entire *E. coli* transcriptional regulatory network is displayed.



The transcriptional regulatory network (1,378 nodes) follows a conventional hierarchical picture, with a few top regulators and many workhorse proteins. The Linux call graph (12,391 nodes), on the other hand, possesses many regulators; the number of workhorse routines is much lower in proportion. The regulatory network has a broad out-degree distribution but a narrow in-degree distribution. The situation is reversed in the call graph, where we can find in-degree hubs, but the out-degree distribution is rather narrow. Yan et al. PNAS 2010, 107, 20.





## A typical DNA record at the NCBI

accession.version

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DEFINITION  Yeast CTA1 gene for catalase A.
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VERSION    X13028.1 GI:3604
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  ORGANISM  Saccharomyces cerevisiae
            Eukaryota; Fungi; Ascomycota; Saccharomycotina; Saccharomycetes;
            Saccharomycetales; Saccharomycetaceae; Saccharomyces.
REFERENCE  1 (bases 1 to 2785)
AUTHORS    Cohen,G., Rapatz,W. and Ruis,H.
TITLE      Sequence of the Saccharomyces cerevisiae CTA1 gene and amino acid
            sequence of catalase A derived from it
JOURNAL    Eur. J. Biochem. 176 (1), 159-163 (1988)
PUBMED     3046940
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CDS

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```

## ORIGIN

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//



## Same sequence in Fasta format

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Source: NCBI GQuery, <http://www.ncbi.nlm.nih.gov/gquery>



```
AAATGGTCCAATAAAAACGGAGATTGGCATTATGTGCAAGTTCATATCAAACCGGATCAAGGAATAAAGA
ATTTGACCATAGAAGAGGCTACCAAAAATTGCGGGATCCAATCCAGATTACTGCCAGCAGGATTTATTTGA
GGCTATTCAGAATGAAAACATCCTTCTCGGACAGTTTATATTCAAACAATGACCGAACGCGATGCCAAA
AAATTACCATTTTCAGTCTTTGATTTGACTAAAGTATGGCCCTCAGGGGCAATTCCCTTACGGCGTGTGG
GTAAGATTGTTTTGAACGAGAATCCACTGAACCTCTTCGCACAGGTGGAACAAGCTGCCTTCGCCCCAG
TACCACGGTTCCTTACCAAGAAGCAAGCGCTGATCCAGTATTACAGGCCCGTTTGTTTTTCATATGCGGAT
GCTCATAGATACAGGCTAGGTCTTAACCTCCATCAAATACCCGTAACCTGTCCATATGCATCTAAATTTT
TCAATCCCGCTATCAGAGATGGACCGATGAATGTTAACGGCAACTTCGGCTCAGAACCTACATATTTGGC
CAACGATAAATCGTACACGTATATCCAACAGGACAGACCCATTCAACAACACCAAGAGGTATGGAATGGG
CCAGCTATCCCTTATCATTGGGCAACATCCCCAGGTGATGTAGATTTCTGTGCAAGCAAGAAATCTCTACC
GCGTTTTGGGTAACAACCTGGACAGCAAAAAGAACTTGGCATATAACATCGGCATTTCATGTAGAAGGCGC
CTGTCCCTCAAATACAGCAGCGCGTTTATGATATGTTTGCTCGTGTGATAAGGGACTATCTGAGGCAATT
AAAAAAGTAGCTGAGGCAAAAACATGCTTCTGAGCTTTTCGAGTAACCTCAAATTTTGAACGCTCAAGTAA
CAAATGAGTGGCGTTGTTCCACGACAATTTATGATAGTGTGTATTTTTAACACATTTTATTTATTA
CAATTTATGTATTTTGTTATGAATTTATTTATATACGACTAATAGGTGATGCTCATATTTCTCGTGTAG
AAAGTTAAAAAATATCATTTTACACATAGGAAAAGCTCGTCGCGCCGGGGAAAAAGCTGAGGAATCTCT
ATTATTAGGGGTAAAGTTCACACACATTCAGTATGAGATAAGTGTGTCTTCAAGAGAGATGCAGCACTGAG
TAGGGAACCAAGAAACGATGTCTGAATTAAGATAGCTTTGAGACAGAGTTTGCGAAATTTTATACCGA
```

# Gene activity data

	A	B	C	D	E	F	G	H	I	J	K	L	M
1				PU00249	PU00250	PU00251	PU00252	PU00253	PU00254	PU00255	PU00256	PU00257	PU00258
2				0.71	0.71	0.71	0.476	0.476	0.476	0.377	0.377	0.377	0.41
3	1	b0020	nhaR	8.8939	8.94616	9.08628	8.77897	8.78882	8.63416	8.79036	8.7343	8.83552	9.32239
4	2	b0034	calI	8.98215	8.968	9.1899	9.56071	9.72042	9.9312	9.38679	9.05435	9.14938	7.9915
5	3	b0064	araC	8.40112	8.39603	8.7541	8.25553	8.28069	8.27877	8.43989	8.54276	8.26429	8.27392
6	4	b0069	sgfR	9.12415	9.27152	9.29754	9.39484	9.3061	9.44534	9.14423	9.19663	9.0907	9.20484
7	5	b0076	leuO	7.57223	7.43093	7.72137	7.66272	7.55189	7.77201	7.47209	7.55864	7.69624	7.98007
8	6	b0080	cra	9.42842	9.53777	9.54285	9.99558	9.89722	10.0349	9.7884	9.84957	9.6796	9.74028
9	7	b0113	pdhR	8.90958	8.95447	9.07071	9.21234	8.79036	8.71998	9.30762	9.35835	9.28916	9.38757
10	8	b0146	sfsA	10.1123	10.2093	10.3636	9.98242	9.76419	9.96919	10.2145	10.5028	10.3943	10.7587
11	9	b0162	cdaR	8.68579	8.73203	8.89094	8.60445	8.40199	8.51366	8.50878	8.57931	8.49583	8.63569
12	10	b0208	yafC	8.20064	8.30706	8.44073	8.3292	8.3108	8.4055	8.18764	8.23167	8.18876	8.17563
13	11	b0226	dinJ	10.6385	10.714	10.7607	10.5488	10.5288	10.5674	10.6654	10.714	10.4664	10.9027
14	12	b0240	cri	12.1271	11.9904	12.1271	10.5371	10.5437	10.6193	11.7773	11.7655	11.7543	10.8609
15	13	b0254	perR	8.21104	7.79748	8.25455	8.33924	8.22761	8.5769	7.93373	7.95502	7.70662	8.00819
16	14	b0267	yagA	9.3932	9.59107	9.53524	9.49492	9.33486	9.42119	9.38436	9.43885	9.58761	9.64772
17	15	b0272	yagI	9.30297	9.39725	9.53944	9.52848	9.32239	9.41964	9.15985	9.22367	9.41252	9.31773
18	16	b0282	yagP	7.66739	7.67364	7.94502	7.48573	7.5142	7.81485	7.5029	7.90647	7.59544	8.12235
19	17	b0294	ecpR	6.26593	5.99952	6.4083	6.40188	6.48827	6.67086	6.59526	6.70132	6.46447	6.28317
20	18	b0300	ykgA	7.47992	7.34954	7.37407	7.56208	7.42863	7.49529	7.70958	7.54484	7.29879	7.65817
21	19	b0305	ykgD	8.24772	8.37059	8.25943	8.58173	8.32828	8.38908	8.37059	8.22349	8.29864	8.30615
22	20	b0313	betI	9.67686	9.78941	9.87423	8.87532	8.79864	8.80015	9.39725	9.4421	9.56071	9.39967
23	21	b0316	yahB	8.32096	8.3154	8.47013	8.13395	7.99533	8.16683	8.05973	8.14247	8.19638	8.29203
24	22	b0318	yahD	8.79339	8.79713	8.91473	8.75105	8.742	9.02089	8.70105	8.66356	8.7949	9.12638
25	23	b0330	prpR	8.09326	8.32734	8.41515	7.98268	7.89761	7.94746	8.3117	8.37939	8.37326	7.92742
26	24	b0338	cyrR	8.67507	8.80536	8.70709	8.69722	8.63729	8.74424	8.52738	8.50796	8.57213	8.8204
27	25	b0345	lacI	9.07295	9.14645	9.22064	9.20633	9.08628	9.12266	9.02764	9.12044	9.13009	9.23203
28	26	b0346	mhpR	10.1195	10.2067	10.2448	9.36385	9.20484	9.30222	10.6263	10.745	10.7044	9.5505
29	27	b0357	frmR	10.2581	10.1839	10.3127	10.0765	10.1367	10.17	9.99121	10.0213	10.0812	10.051
30	28	b0375	yaiV	5.71158	5.59532	5.76974	5.94479	5.6065	5.92942	5.7577	5.92942	5.962	5.84119
31	29	b0378	yaiW	9.00826	9.20114	9.08921	9.25693	9.18242	9.19364	9.03063	9.07663	9.1153	9.11075
32	30	b0399	phoB	9.08032	9.11456	9.28452	9.03578	9.0223	9.06549	8.968	8.86486	8.90065	8.99999
33	31	b0413	nrdR	10.2976	10.5189	10.4261	10.3752	10.1687	10.3438	10.3352	10.3508	10.2249	10.4869
34	32	b0435	bolA	9.78257	9.95728	10.1111	9.08697	9.1123	9.05736	9.39805	9.71018	9.54285	9.94202
35	33	b0440	hupB	11.1981	11.5879	11.5559	11.6111	11.9223	11.859	11.2979	11.192	11.3796	10.8716

In rows the different genes, in the columns there are different samples (experimental replicates, temporal series, different patients ect)



www.ebi.ac.uk  
www.ensembl.org  
www.ncbi.nlm.nih.gov/  
[www.ddbj.nig.ac.jp/](http://www.ddbj.nig.ac.jp/) etc

- 1) strings for DNA and protein sequences (see
  - a. [http://www.ensembl.org/Homo\\_sapiens/Gene/Summary?db=core;g=ENSG00000260629;r=11:5244554-5245547;t=ENST00000564523](http://www.ensembl.org/Homo_sapiens/Gene/Summary?db=core;g=ENSG00000260629;r=11:5244554-5245547;t=ENST00000564523)
  - b. [http://www.ensembl.org/Homo\\_sapiens/Transcript/ProteinSummary?db=core;g=ENSG00000213934;r=11:5244554-5245547;t=ENST00000330597](http://www.ensembl.org/Homo_sapiens/Transcript/ProteinSummary?db=core;g=ENSG00000213934;r=11:5244554-5245547;t=ENST00000330597)
  - c. <http://www.ncbi.nlm.nih.gov/nuccore/U01317.1>
  - d. <http://www.ncbi.nlm.nih.gov/protein/AAA16334.1>
- 2) gene expression (intensity of gene activity) matrix (csv format) (see
  - a. <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE53655>
  - b. <http://www.genome.jp/kegg/expression/>
- 3) 3d graphs for protein structure (see
  - a. <http://www.ncbi.nlm.nih.gov/Structure/mmdb/mmdbsrv.cgi?uid=122520>
  - b. <http://prosite.expasy.org/PS01033> [http://prosite.expasy.org/cgi-bin/pdb/pdb\\_structure\\_viewer.cgi?pdb=101M&ps=PS01033](http://prosite.expasy.org/cgi-bin/pdb/pdb_structure_viewer.cgi?pdb=101M&ps=PS01033)
- 4) medical literature  
[www.ncbi.nlm.nih.gov/pubmed/?term=human+beta+globin](http://www.ncbi.nlm.nih.gov/pubmed/?term=human+beta+globin)
- 5) metabolic pathways (see <http://www.genome.jp/kegg/pathway.html>)
- 6) Browsers ([http://en.wikipedia.org/wiki/Genome\\_browser](http://en.wikipedia.org/wiki/Genome_browser);  
<http://www.ensembl.org/index.html>; <http://genome.ucsc.edu/>)



# Bioinformatics libraries (various programming languages)

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Products & Services > Bioinformatics Toolbox

### Bioinformatics Toolbox

Read, analyze, and visualize genomic and proteomic data

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### BioPerl

From Wikipedia, the free encyclopedia

BioPerl™ is a collection of Perl modules that facilitates the development of Perl scripts for bioinformatics applications. It has played an integral role in the success of [Bioinformatics Resource Project](#).

1 Background  
2 Features  
3 Usage  
4 Related Libraries in C  
5 References

### Bioconductor

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### Biopython

Introduction

Biopython is a set of freely available tools for biological computation written in Python 2.7 by an international team of developers.

It is a distributed collaborative effort to develop Python libraries and applications which address the needs of current and future work in bioinformatics. The source code is made available under the [Biopython License](#) (a), which is extremely liberal and compatible with almost every license in the world. We work closer with the [Python Bioinformatics Community](#) to also generally host our website, bug tracker, and mailing lists.

This will help you download and install Biopython.

Get Started

Main Page

The Biopython project

Biopython is an open-source project dedicated to providing a complete and consistent interface to biological databases and all other tools. They are licensed under GPL 2.1.

Please cite:

Biopython: an open-source framework for bioinformatics // [Bioinformatics](#) 2004; **19**: 308-312. doi:10.1093/bioinformatics/btt020. PMID: 15032150. Epub 2004/02/01. [http://www.ncbi.nlm.nih.gov/pubmed/15032150]

4273pi: Bioinformatics hardware

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### The NCBI C++ Toolkit

Online documentation for C++ Toolkit

- C++ Toolkit tools

NCBI C++ Toolkit provides free, portable, public domain binaries with no restrictions for use on Linux, MS Windows, and Mac OS platforms:

- Networking and Interprocess Communication (IPC) library with CDT/CDL adapters
- Multi-Threading Library
- CGI and FastCGI Library
- URL, Generation Library
- SQL Database Access Library

Comments:

- The Source Code
- Installing
- Public Releases
- Documentation
- Support

Installation Links:

- Package Files
- FTP Download

Documentation Link

- Online Documentation
- PDF Documentation

Other Links

- NCBI Home Page

### BioRuby

Open source bioinformatics library for Ruby

### Announcements

- At the 15th Annual Bioinformatics Open Source Conference (BOSC 2014) held in Boston on July 11-12, we will be presenting a talk entitled "BioRuby and distributed development" ([abstract](#)).

### BIOCAML

The OCaml Bioinformatics Library

### Source Code

is hosted on GitHub: <https://github.com/biocaml/biocaml>

### BioPHP

PHP for Bioinformatics

The aim of this site is to share knowledge by using a Wiki-like service: classes, functions, methods and minitools may be edited by registered users. More info [here](#).

GenePHP - Download | Minitools - Download all minitools (.zip)

Sequence Class | Sequence Database Class | Sequence Alignment Class | Restriction Enzyme Class | Miscellaneous Class

Sequence manipulation and data loads

Melting Temp | PCR Amplify | Microsatellite | Find Palindrome | Alignment

### Blip: Biomedical Logic Programming

<About Blip> <Features> <News>

### About Blip

Restriction | Blip is a collection of logic programming modules intended primarily for bioinformatics and related modules which may be of more general interest. Blip is intended to be both an application written in [SWI-Prolog](#), a fast, robust and scalable implementation of ISO Prolog.

Protein to D | Blip is [Free Software](#), available, licensed under the [LGPL](#).

c, c++, python, perl, ruby, matlab, R, ect

### LECTURE 1

- <http://bionumbers.hms.harvard.edu/>
- <http://www.thomas-schlitt.net/Bioproject.html>;
- <http://www.biostat.wisc.edu/craven/hunter.pdf>
- N. Jones, P. Pevzner An Introduction to Bioinformatics Algorithms



Algorithms in this lecture: Longest common subsequence, Needleman-Wunsch, Smith-Waterman, Affine gap, Hirschberg, Nussinov RNA folding. Typical tasks: align genome and protein sequences; we want to detect all differences at the single base to block of bases levels. In the RNA folding problem we want to align a molecule with itself.

Data: DNA or protein (amino acid) sequences considered as strings; input: two strings (Nussinov accepts one string in input and search for internal similarities). Output: a set of aligned positions that makes easy the identification of conserved patterns. Note that each string belongs to a double helix so the information could be related to one of the two strands and read in one or the opposite orientation.

Many events (mutations) could lead to sequence changes. Therefore the conservation of a substring between two strings may suggest to a crucial functional role for the cell. The dynamic programming algorithms could be used to detect similarities within a single string (last section of the lecture). This is particularly useful to find the folding of RNA molecules (in a RNA molecule the T is replaced by U).

Main question in this lecture: how similar are these two sequences?



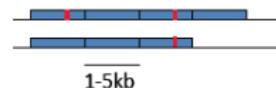
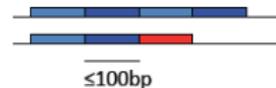
## Sequence Alignment: The Biological problem

- Single nucleotide polymorphisms (SNPs)
  - 1 every few hundred bp, mutation rate\*  $\approx 10^{-9}$
- Short indels (=insertion/deletion)
  - 1 every few kb, mutation rate v. variable
- Microsatellite (STR) repeat number
  - 1 every few kb, mutation rate  $\leq 10^{-3}$
- Minisatellites
  - 1 every few kb, mutation rate  $\leq 10^{-1}$
- Repeated genes
  - rRNA, histones
- Large deletions, duplications, inversions
  - Rare, e.g. Y chromosome

```
TGCATTGCGTAGGC
TGCATTCCGTAGGC
```

```
TGCATT---TAGGC
TGCATTCCGTAGGC
```

```
TGCTCATCATCATCAGC
TGCTCATCA-----GC
```



**Figure :** Type and frequency of mutations (replacements, insertions, deletions) in the human genome per generation; mutations change single DNA bases (SNP polymorphism) or rearrange DNA strings at different length scales. In sequence alignment we compare sequences that are different because of mutations.



Alignment is a way of arranging two DNA or protein sequences to identify regions of similarity that are conserved among species. Each aligned sequence appears as a row within a matrix. Gaps are inserted between the amino acids of each sequence so that identical or similar bases in different sequences are aligned in successive positions. Each gap spans one or more columns within the alignment matrix. Given two strings  $x = x_1, x_2, \dots, x_M$ ,  $y = y_1, y_2, \dots, y_N$ , an alignment is an assignment of gaps to positions  $0, \dots, M$  in  $x$ , and  $0, \dots, N$  in  $y$ , so as to line up each letter in one sequence with either a letter, or a gap in the other sequence.

```
AGGCTATCACCTGACCTCCAGGCCGATGCCC  
TAGCTATCACGACCGCGGTTCGATTTGCCCGAC
```

```
-AGGCTATCACCTGACCTCCAGGCCGA--TGCCC---  
TAG-CTATCAC--GACCGC--GGTCGATTTGCCCGAC
```



Hamming distance

always compares

$i^{\text{th}}$  letter of  $\mathbf{v}$  with

$i^{\text{th}}$  letter of  $\mathbf{w}$

$\mathbf{V} = \text{ATATATAT}$

$\mathbf{W} = \text{TATATATA}$

Just one shift  
Make it all line up  $\rightarrow$

Edit distance

may compare

$i^{\text{th}}$  letter of  $\mathbf{v}$  with

$j^{\text{th}}$  letter of  $\mathbf{w}$

$\mathbf{V} = -\text{ATATATAT}$

$\mathbf{W} = \text{TATATATA}$

**Hamming distance:**

$$d(\mathbf{v}, \mathbf{w})=8$$

Computing Hamming distance

is a **trivial** task

**Edit distance:**

$$d(\mathbf{v}, \mathbf{w})=2$$

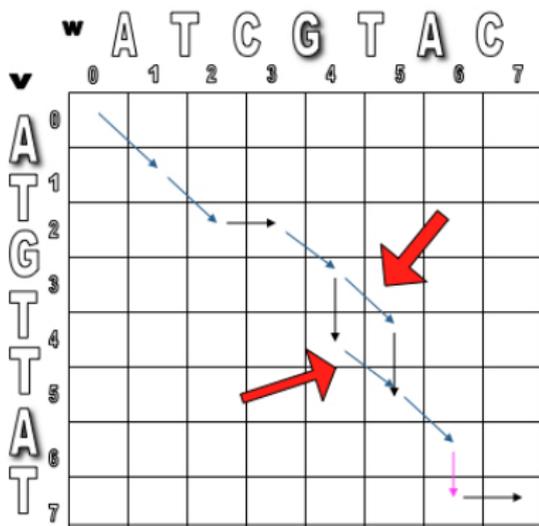
Computing edit distance

is a **non-trivial** task

**Figure :** The Hamming distance is a column by column number of mismatches; the Edit distance between two strings is the minimum number of operations (insertions, deletions, and substitutions) to transform one string into the other.



## Alignment as a Path in the Edit Graph



first alignment

0122345677

v= AT\_GTTAT\_

w= ATCGT\_A\_C

0123455667

second alignment

0122345677

v= AT\_GTTAT\_

w= ATCG\_TA\_C

0123445667

The score of the alignment paths are 5.

**Figure :** Create a matrix  $M$  with one sequence as row header and the other sequence as column header. Assign a ONE where the column and row site matches (diagonal segments), a ZERO otherwise (horizontal or vertical segments); [sequence alignment can be viewed as a Path in the Edit Graph](#). The edit graph is useful to introduce the dynamic programming technique.



DP is a method for reducing a complex problem to a set of identical sub-problems. The best solution to one sub-problem is independent from the best solution to the other sub-problems.

**Recursion is a top-down mechanism**, we take a problem, split it up, and solve the smaller problems that are created; **DP is a bottom-up mechanism**: we solve all possible small problems and then combine them to obtain solutions for bigger problems. The reason that this may be better is that, using recursion, it is possible that we may solve the same small subproblem many times. Using DP, we solve it once. Consider the Fibonacci Series:  $F(n) = F(n - 1) + F(n - 2)$  where  $F(0) = 0$  and  $F(1) = 1$ .

A recursive algorithm will take exponential time to find  $F(n)$  while a DP solution takes only  $n$  steps. A recursive algorithm is likely to be polynomial if the sum of the sizes of the subproblems is bounded by  $kn$ . If, however, the obvious division of a problem of size  $n$  results in  $n$  problems of size  $n-1$  then the recursive algorithm is likely to have exponential growth.



## The Longest Common Subsequence (LCS)

A subsequence of a string  $v$ , is a set of characters that appear in left-to-right order, but not necessarily consecutively. A common subsequence of two strings is a subsequence that appears in both strings. Substrings are consecutive parts of a string, while subsequences need not be.

A longest common subsequence is a common subsequence of maximal length.

Example:

$v_1 = \langle A, C, B, D, E, G, C, E, D, B, G \rangle$  and

$v_2 = \langle B, E, G, C, F, E, U, B, K \rangle$

the LCS is  $\langle B, E, G, C, E, B \rangle$ .

With respect to DNA sequences:

$v_1 = \text{AAACCGTGAGTTATTCGTTCTAGAA}$   $v_2 = \text{CACCCCTAAGGTACCTTTGGTTC}$

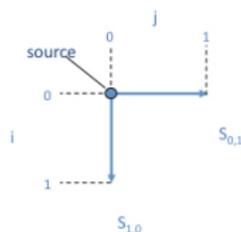
LCS is **ACCTAGTACTTTG**



- The LCS problem is the simplest form of sequence alignment; it allows only insertions and deletions (no mismatches).
- Given two sequences  $v = v_1 v_2 \dots v_m$  and  $w = w_1 w_2 \dots w_n$ . The LCS of  $v$  and  $w$  is a sequence of positions in  $v$ :  $1 < i_1 < i_2 < \dots < i_t < m$  and a sequence of positions in  $w$ :  $1 < j_1 < j_2 < \dots < j_t < n$  such that  $i_t$  letter of  $v$  equals to  $j_t$ -letter of  $w$  and  $t$  is maximal
- In the LCS problem, we score 1 for matches and 0 for indels (we will see that in DNA sequence alignment we use different scores for match, mismatch and gap).



# The Longest Common Subsequence

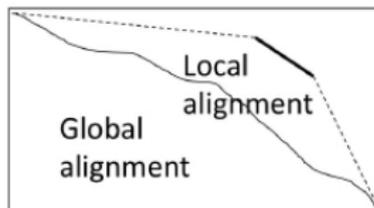


```
LCS(v,w)
for i ← 1 to n
  s_{i,0} ← 0
for j ← 1 to m
  s_{0,j} ← 0
for i ← 1 to n
  for j ← 1 to m
    s_{i,j} ← max {
      s_{i-1,j}
      s_{i,j-1}
      s_{i-1,j-1} + 1, if v_i = w_j
    }
    b_{i,j} ← {
      ↑ if s_{i,j} = s_{i-1,j}
      ← if s_{i,j} = s_{i,j-1}
      ↖ if s_{i,j} = s_{i-1,j-1} + 1
    }
return (s_{n,m}, b)
```

**Figure :** It takes  $O(nm)$  time to fill in the  $n$  by  $m$  dynamic programming matrix. The pseudocode describes two nested for loops to build up a  $n$  by  $m$  matrix.



- The Global Alignment Problem tries to find the longest path between vertices  $(0,0)$  and  $(n,m)$  in the edit graph.
- The Local Alignment Problem tries to find the longest path among paths between **arbitrary vertices**  $(i,j)$  and  $(i',j')$  in the edit graph.



- **Global Alignment**

```

--T--CC-C-AGT--TATGT-CAGGGGACACG-A-GCATGCAGA-GAC
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
AATTGCCGCC-GTCGT-T-TTCAG----CA-GTTATG-T-CAGAT--C
    
```

- **Local Alignment**—better alignment to find conserved segment

```

                tccCAGTTATGTcAGgggacacgagcatgcagagac
                | | | | | | | | | |
aattgccgccgctcgttttcagCAGTTATGTcAGatc
    
```

**Figure :** The same sequences could be used in both alignments; we need to set the match score, the mismatch and gap penalties.



1. Initialization (two sequences of length M and N).

- a.  $F(0, 0) = 0$
- b.  $F(0, j) = -j \times d$
- c.  $F(i, 0) = -i \times d$

2. Main Iteration. Filling-in partial alignments

For each  $i = 1 \dots M$

For each  $j = 1 \dots N$

$$F(i, j) = \max \begin{cases} F(i-1, j) - d & \text{[case 1]} \\ F(i, j-1) - d & \text{[case 2]} \\ F(i-1, j-1) + s(x_i, y_j) & \text{[case 3]} \end{cases}$$
$$Ptr(i, j) = \begin{cases} \text{UP,} & \text{if [case 1]} \\ \text{LEFT} & \text{if [case 2]} \\ \text{DIAG} & \text{if [case 3]} \end{cases}$$

3. Termination.  $F(M, N)$  is the optimal score, and from  $Ptr(M, N)$  can trace back optimal alignment



Example, Match= 2 (s=2); Gap= -1 (d=1); Mismatch=-1 (s=1)

Gap penalty=-1; match=+2; mismatch=-1			A	C	G	C	T	G
		0	1	2	3	4	5	6
0		0	-1	-2	-3	-4	-5	-6
C	1	-1	-1	1				
A	2	-2						
T	3	-3						
G	4	-4						
T	5	-5						

Red arrows indicate the path from (0,0) to (1,2) and (2,4). A yellow callout box points to the cell (1,2) containing the value 1, with the text "AC" and "-C" inside.



Example, Match= 2 (s=2); Gap= -1 (d=1); Mismatch=-1 (s=1)

		A	C	G	C	T	G
	0	1	2	3	4	5	6
0	0	-1	-2	-3	-4	-5	-6
C 1	-1	-1	1	0	-1	-2	-3
A 2	-2	1	0	0	-1	-2	-3
T 3	-3	0	0	-1	-1	1	0
G 4	-4	-1	-1	2	1	0	3
T 5	-5	-2	-2	1	1	3	2

Diagram illustrating a dynamic programming table for sequence alignment. The table shows scores for alignments between a sequence (rows) and a reference sequence (columns). The reference sequence is A, C, G, C, T, G. The sequence being aligned is C, A, T, G, T. The scores are calculated based on Match (2), Gap (-1), and Mismatch (-1). Red arrows indicate the path of the optimal alignment, starting from the bottom-right cell (2) and moving towards the top-left cell (0). The value 2 in the bottom-right cell is circled in red.



		A	C	G	C	T	G
	0	1	2	3	4	5	6
0	0	-1					
C 1	-1		1	0			
A 2		1		0	-1		
T 3			0			1	
G 4				2	1		3
T 5						3	2

ACGCTG-  
-C-ATGT

		A	C	G	C	T	G
	0	1	2	3	4	5	6
0	0	-1					
C 1	-1		1	0			
A 2		1		0	-1		
T 3			0			1	
G 4				2	1		3
T 5						3	2

ACGCTG-  
-CA-TGT



Match= 2; Gap= -1; Mismatch=-1

		A	C	G	C	T	G
0	0	-1					
C 1	-1		1	0			
A 2		1		0	-1		
T 3			0			1	
G 4				2	1		3
T 5						3	2

**-ACGCTG**  
**CATG-T-**



## The choice of scores (match, gap and mismatch) depends on the data

		x			
		0	A	G	C
y	0	0	-2	-4	-6
	A 1	-2	1	-1	-3
	A 2	-4	-1	0	-2
	A 3	-6	-3	-2	-1
	C 4	-8	-5	-4	-1

Match 1  
 Mismatch -1  
 Gap -2

AAAC  
 A-GC

AAAC  
 -AGC

AAAC  
 AG-C

**Figure :** Given a  $m \times n$  matrix, the overall complexity of computing all sub-values is  $O(nm)$ . The final optimal score is the value at position  $n,m$ . In this case we align the sequences AGC and AAAC.



The score of an alignment is calculated by summing the rewarding scores for match columns that contain the same bases and the penalty scores for gaps and mismatch columns that contain different bases.

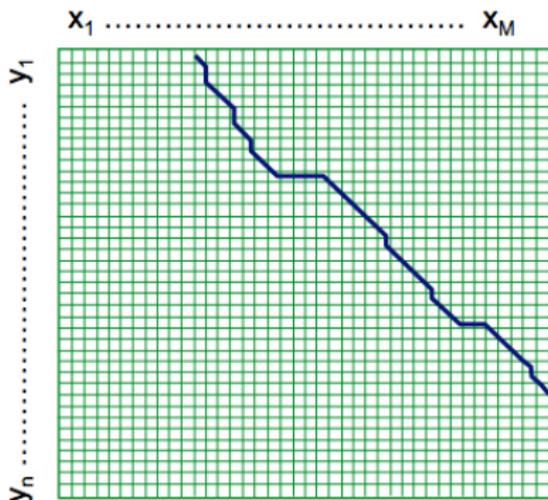
A scoring scheme specifies the scores for matches and mismatches, which form the scoring matrix, and the scores for gaps, called the gap cost. **There are two types of alignments for sequence comparison: global and local.** Given a scoring scheme, calculating a **global alignment** is a kind of global optimization process that forces the alignment to span the entire length of two query sequences, whereas **local alignments** identify regions of high similarity between two sequences.



# Alignment of sequences of different lengths

Maybe it is OK to have an unlimited # of gaps in the beginning and end:

-----CTATCACCTGACCTCCAGGCCGATGCCCTTCCGGC  
GCGAGTTCATCTATCAC--GACCGC--GGTCG-----



Changes:

## 1. Initialization

For all  $i, j$ ,

$$F(i, 0) = 0$$

$$F(0, j) = 0$$

## 2. Termination

$$F_{OPT} = \max \begin{cases} \max_i F(i, N) \\ \max_j F(M, j) \end{cases}$$



## The local alignment: the Smith-Waterman algorithm

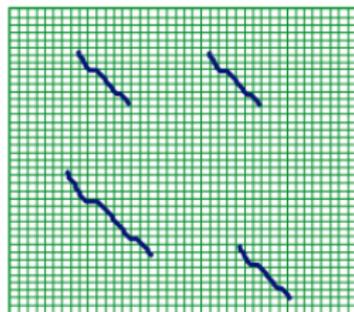
**Idea:** Ignore badly aligning regions: Modifications to Needleman-Wunsch

e.g.  $x = \text{aaaacccccgggg}$

$y = \text{cccgggaaccaacc}$

**Initialization:**  $F(0, j) = F(i, 0) = 0$

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



**Termination:**

1. If we want the **best** local alignment...

$$F_{\text{OPT}} = \max_{i,j} F(i, j)$$

2. If we want **all** local alignments **scoring > t**

For all  $i, j$  find  $F(i, j) > t$ , and trace back



# Example, local alignment TAATA vs TACTAA

$y = \text{TAATA}$   
 $x = \text{TACTAA}$

$y \backslash x$	0	T	A	C	T	A	A
0	0	0	0	0	0	0	0
T 1	0	1	0	0	1	0	0
A 2	0	0	2	0	0	2	1
A 3	0	0	1	1	0	1	3
T 4	0	0	0	0	2	0	1
A 5	0	0	1	0	0	3	-1

$y = \text{TAATA}$   
 $x = \text{TACTAA}$

$y \backslash x$	0	T	A	C	T	A	A
0	0	0	0	0	0	0	0
T 1	0	1	0	0	1	0	0
A 2	0	0	2	0	0	2	1
A 3	0	0	1	1	0	1	3
T 4	0	0	0	0	2	0	1
A 5	0	0	1	0	0	3	-1

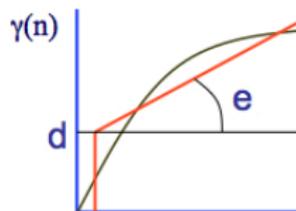


## Affine gap: two different penalties for gap insertion

Insertions and deletions often occur in blocks longer than a single nucleotide. If there are many gaps we do not want to penalise too much; a non linear function could be expensive to implement; so we may implement two gap penalties: one for the first gap (opening) and one, smaller, for the following required gaps (see the figure below).

$$\gamma(n) = d + (n - 1)e$$

|                      |  
gap                      gap  
open                      extend



To compute optimal alignment,

At position  $i, j$ , need to “remember” best score if gap is open  
best score if gap is not open

$F(i, j)$ : score of alignment  $x_1 \dots x_i$  to  $y_1 \dots y_j$   
if  $x_i$  aligns to  $y_j$

$G(i, j)$ : score if  $x_i$  aligns to a gap after  $y_j$

$H(i, j)$ : score if  $y_j$  aligns to a gap after  $x_i$

$V(i, j) =$  best score of alignment  $x_1 \dots x_i$  to  $y_1 \dots y_j$



## Affine gap: two penalties for gap insertion

Time complexity - As before  $O(nm)$ , as we only compute four matrices instead of one.

Space complexity: there's a need to save four matrices (for  $F$ ,  $G$ ,  $H$  and  $V$  respectively) during the computation. Hence,  $O(nm)$  space is needed, for the trivial implementation.

**Initialization:**  $V(i, 0) = d + (i - 1) \times e$   
 $V(0, j) = d + (j - 1) \times e$

**Iteration:**

$$V(i, j) = \max\{ F(i, j), G(i, j), H(i, j) \}$$

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

$$G(i, j) = \max \begin{cases} V(i - 1, j) - d \\ G(i - 1, j) - e \end{cases}$$

$$H(i, j) = \max \begin{cases} V(i, j - 1) - d \\ H(i, j - 1) - e \end{cases}$$

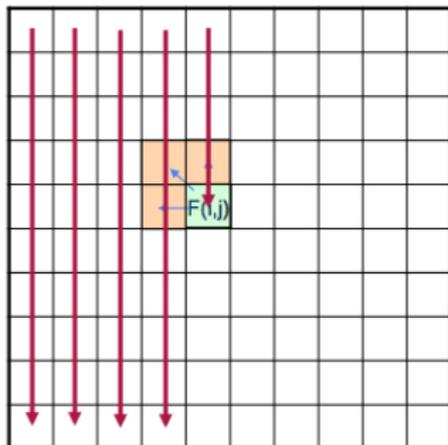
**Termination:** similar



When comparing long DNA sequences, the limited resource in sequence alignment is not time but space. Hirschberg in 1975 proposed a divide-and-conquer approach that performs alignment in linear space for the expense of just doubling the computational time. **The time complexity of the dynamic programming algorithm for sequence alignment is roughly the number of edges in the edit graph, i.e.,  $O(nm)$ . The space complexity is roughly the number of vertices in the edit graph, i.e.,  $O(nm)$ .** However, if we only want to compute the score of the alignment (rather than the alignment itself), then the space can be reduced to just twice the number of vertices in a single column of the edit graph, i.e.,  $O(n)$ .



It is easy to compute  $F(M, N)$  in linear space



```
Allocate ( column[1] )
```

```
Allocate ( column[2] )
```

```
For i = 1...M
```

```
  If i > 1, then:
```

```
    Free( column[i - 2] )
```

```
    Allocate( column[ i ] )
```

```
  For j = 1...N
```

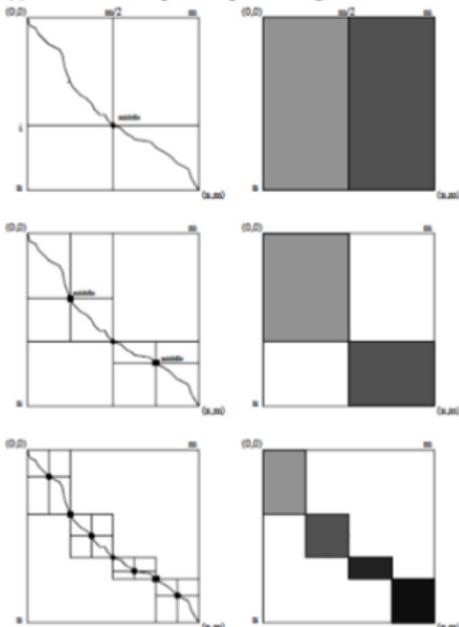
```
    F(i, j) = ...
```

**Figure :** Space complexity of computing just the score itself is  $O(n)$ ; we only need the previous column to calculate the current column, and we can then throw away that previous column once we have done using it



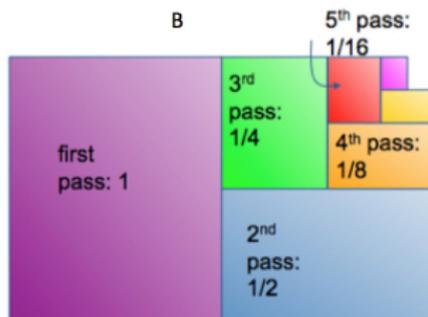
# Space-Efficient Sequence Alignment, Hirschberg algorithm

## A Linear-Space Sequence Alignment



A: space complexity

B: time complexity



The reduction comes from observation that the only values needed to compute the alignment scores  $s_{*j}$  (column  $j$ ) are the alignment scores  $s_{*,j-1}$  (column  $j - 1$ ). Therefore, the alignment scores in the columns before  $j - 1$  can be discarded while computing alignment scores for columns  $j, j + 1, \dots$ .

The longest path in the edit graph connects the start vertex  $(0,0)$  with the sink vertex  $(n, m)$  and passes through an (unknown) middle vertex  $(i, m/2)$  (assume for simplicity that  $m$  is even). Let's try to find its middle vertex instead of trying to find the entire longest path. This can be done in linear space by computing the scores  $s_{*,m/2}$  (lengths of the longest paths from  $(0,0)$  to  $(i, m/2)$  ) for  $0 < i < n$ ) and the scores of the paths from  $(i, m/2)$  to  $(n,m)$ . The latter scores can be computed as the scores of the paths  $s_*^{reverse}$  from  $(n,m)$  to  $(i, m/2)$  in the reverse edit graph (i.e., the graph with the directions of all edges reversed). The value  $S_{i,m/2} + S_{i,m/2}^{reverse}$  is the length of the longest path from  $(0,0)$  to  $(n, m)$  passing through the vertex  $(i, m/2)$ . Therefore,  $\max_i [S_{i,m/2} + S_{i,m/2}^{reverse}]$  computes the length of the longest path and identifies a middle vertex.



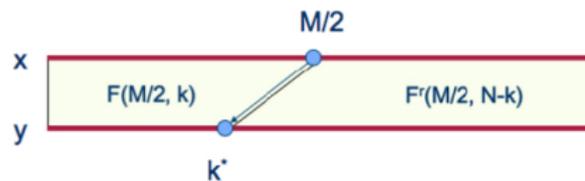
Computing these values requires the time equal to the area of the left rectangle (from column 1 to  $m/2$ ) plus the area of the right rectangle (from column  $m/2 + 1$  to  $m$ ) and the space  $O(n)$ . After the middle vertex  $(i, m/2)$  is found the problem of finding the longest path from  $(0,0)$  to  $(n, m)$  can be partitioned into two subproblems: finding the longest path from  $(0,0)$  to the middle vertex  $(i, m/2)$  and finding the longest path from the middle vertex  $(i, m/2)$  to  $(n, m)$ . Instead of trying to find these paths, we first try to find the middle vertices in the corresponding rectangles. This can be done in the time equal to the area of these rectangles, which is two times smaller than the area of the original rectangle. Computing in this way, we will find the middle vertices of all rectangles in  $time = area + area/2 + area/4 + \dots < 2 * area$  and therefore compute the longest path in time  $O(nm)$  and space  $O(n)$ .



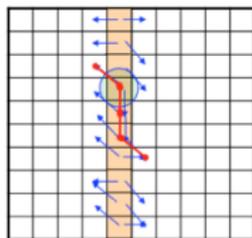
- 1 **Path** (source, sink)
- 2 **if** source and sink are in consecutive columns
- 3 output the longest path from the source to the sink
- 4 **else**
- 5 middle  $<$  middle vertex between source and sink
- 6 **Path** (source, middle)
- 7 **Path** (middle, sink)



# Hirschberg algorithm: details

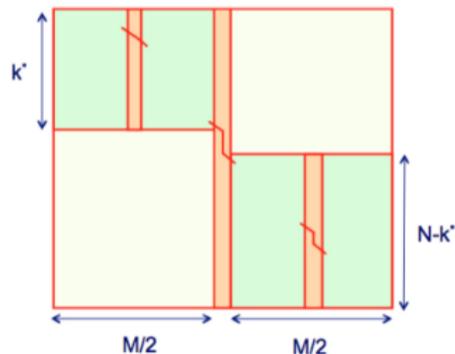


Now, we can find  $k^*$  maximizing  $F(M/2, k) + F'(M/2, k)$   
Also, we can trace the path exiting column  $M/2$  from  $k^*$

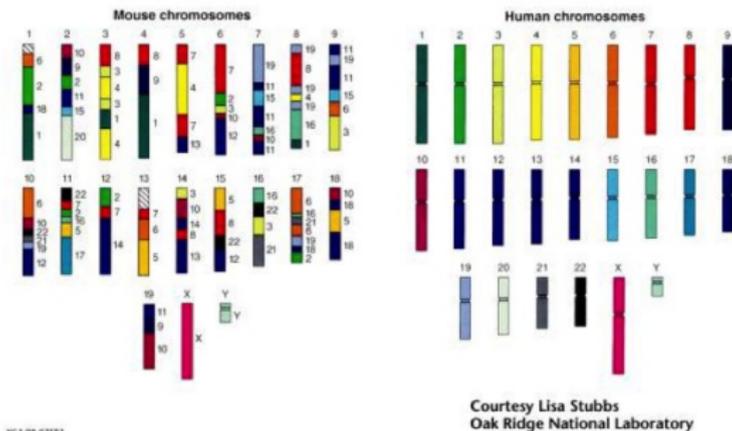


Conclusion: In  $O(NM)$  time,  $O(N)$  space,  
we found optimal alignment path at column  $M/2$

- Iterate this procedure to the left and right!

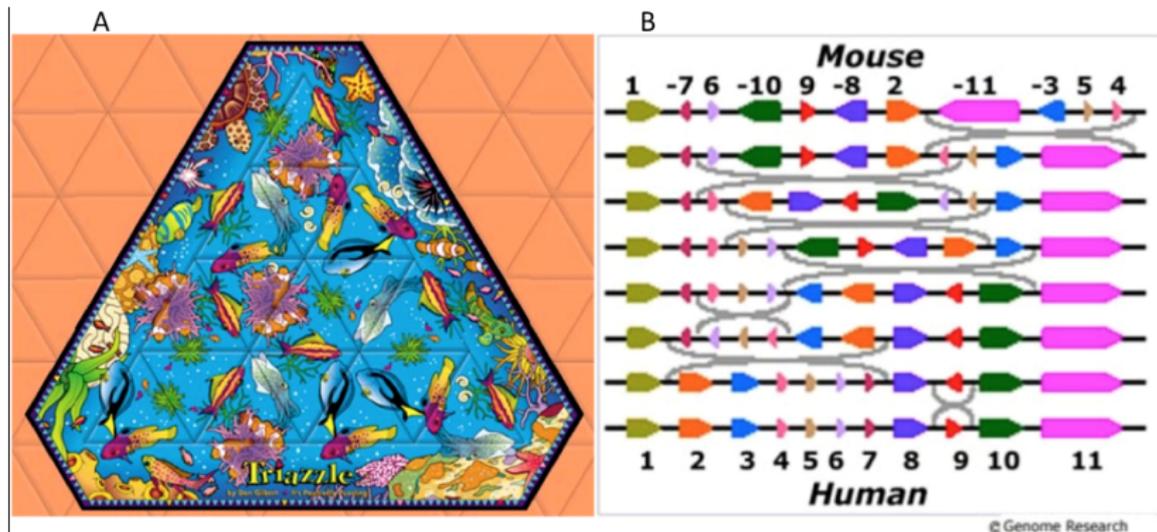


## Mouse and Human Genetic Similarities



**Figure :** Human and mouse genome sequence comparison ( each of 3 billion DNA bases). The color map reflects similar segments in the two species up to a similarity threshold

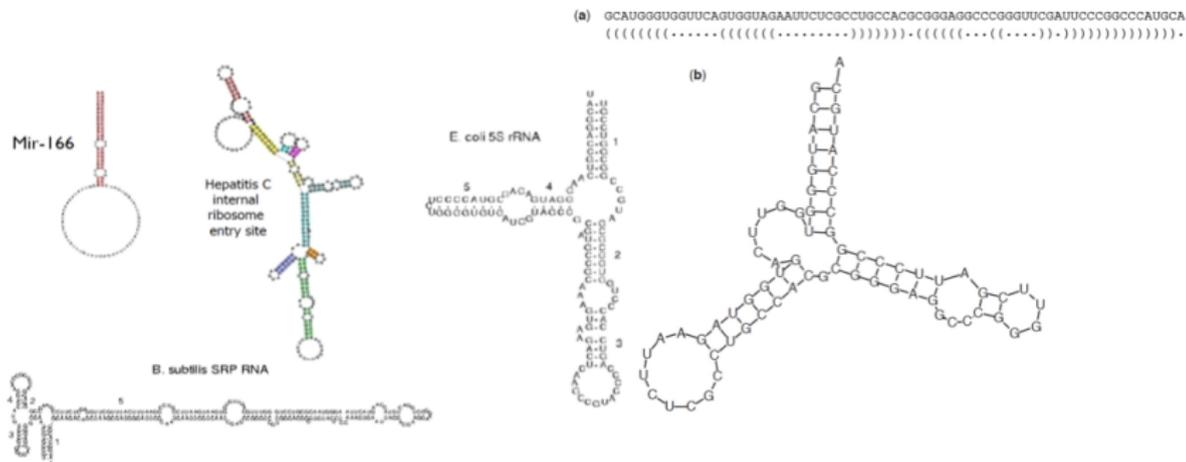
## Challenges in alignment: repeats and inversions



The comparison of sequences containing repeats of different length (as in puzzles) and inverted blocks (could be also nested) is particularly difficult.



# Single string folding: the Biological problem

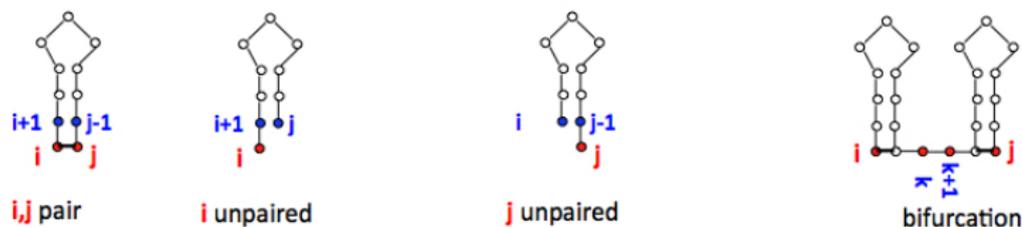


**Figure :** Examples of RNA molecules in nature; many molecules of RNA do not translate into proteins; using the pairing rule A-T, C-G, the molecule could find regions of perfect pairing so to have intrachain interactions. Therefore, the molecule folds into 2 Dimensional shape (termed secondary structure) and then into 3 Dimensional shape (tertiary structure) and regulates cell processes by interacting with proteins. On the right, in (a) the prediction of the contacts of the RNA molecule shown immediately below in (b).



The intrachain folding of RNA reveals the RNA Secondary Structure

This tells us which bases are paired in the subsequence from  $x_i$  to  $x_j$  Every optimal structure can be built by extending optimal substructures.



**Figure :** Set of paired positions on interval  $[i, j]$ . Suppose we know all optimal substructures of length less than  $j - i + 1$ . The optimal substructure for  $[i, j]$  must be formed in one of four ways:  $i, j$  paired;  $i$  unpaired;  $j$  unpaired; combining two substructures. Note that each of these consists of extending or joining substructures of length less than  $j - i + 1$ .

- 1 Let  $\beta(i, j)$  be the maximum number of base pairs in a folding of subsequence  $S[i \dots j]$ .
- 2 for  $1 \leq i \leq n$  and  $i < j \leq n$ :  
 for  $i = 1, \dots, n : \beta(i, i) = 0$ ;  
 for  $2 \leq i \leq n : \beta(i, i - 1) = 0$   

$$\beta(i, j) = \max \begin{cases} \beta(i + 1, j) \\ \beta(i, j - 1) \\ \beta(i + 1, j - 1) + \delta(i, j) \\ \max_{i < k < j} [\beta(i, k) + \beta(k + 1, j)] \end{cases}$$
- 3 Where  $\delta(i, j) = 1$  if  $x_i$  and  $x_j$  are a complementary base pair i.e. (A, U) or (C, G), and  $\delta(i, j) = 0$ , otherwise.

There are  $O(n^2)$  terms to be computed, each requiring calling of  $O(n)$  already computed terms for the case of bifurcation. Thus overall complexity is  $O(n^3)$  time and  $O(n^2)$  space.



## Nussinov algorithm for RNA folding

Note that only the upper (or lower) half of the matrix needs to be filled. Therefore, after initialization the recursion runs from smaller to longer subsequences as follows:

- 1 for  $l = 1$  to  $n$  do
- 2 for  $i = 1$  to  $(n + 1 - l)$  do
- 3  $j = i + l$
- 4 compute  $\beta(i, j)$
- 5 end for
- 6 end for

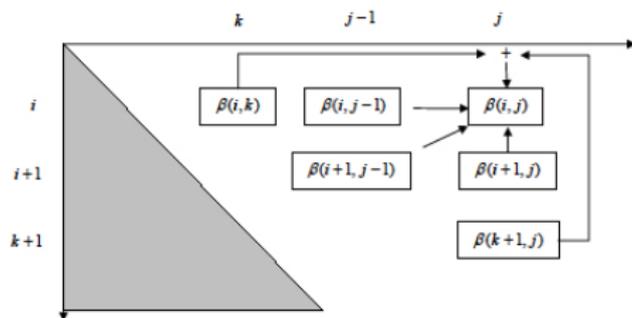


Figure : Details of matrix filling

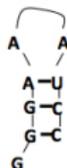


# Nussinov algorithm: example

	G	G	G	A	A	A	U	C	C
G	0								
G	0	0							
G	0	0	0						
A				0	0				
A				0	0				
A					0	0			
U						0	0		
C							0	0	
C								0	0

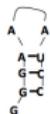
Example:

GGGAAAUCC



	G	G	G	A	A	A	U	C	C
G	0	0	0	0	0	0	1	2	3
G	0	0	0	0	0	0	1	2	3
G	0	0	0	0	0	0	1	2	2
A				0	0	0	0	1	1
A					0	0	0	1	1
A						0	0	1	1
U							0	0	0
C								0	0
C									0

Fill up the table (DP matrix) -- diagonal by diagonal

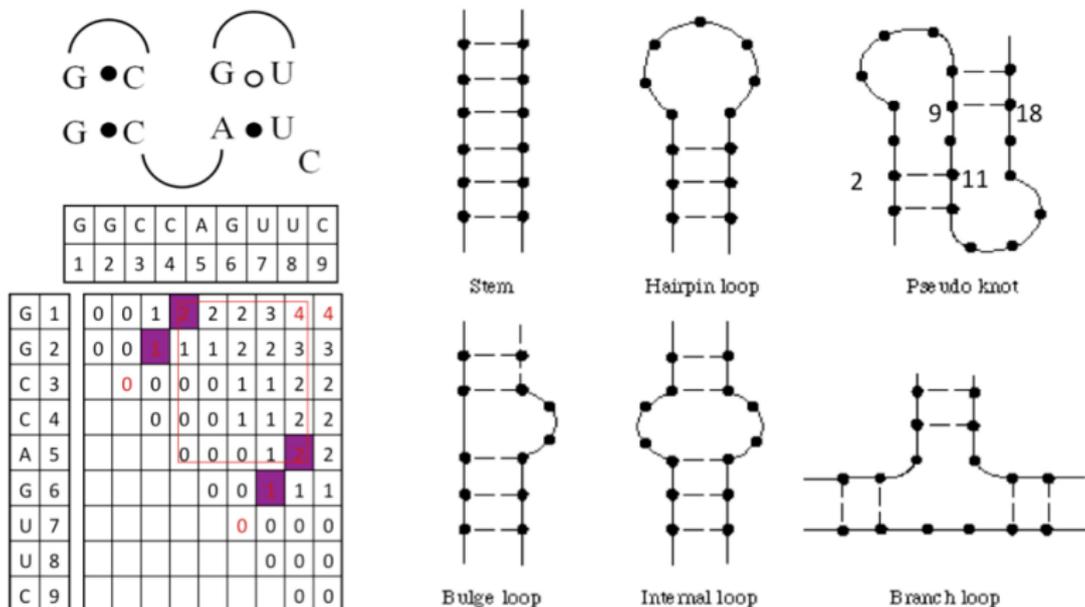


	G	G	G	A	A	A	U	C	C
G	0	0	0	0					
G	0	0	0	0					
G	0	0	0	0	0				
A				0	0	0	0	?	
A					0	0	0	1	
A						0	0	1	1
U							0	0	0
C								0	0
C									0

**Figure** : order: top left, bottom left, right: a matrix will be filled along the diagonals and the solution can be recovered through a traceback step.



# Challenges in RNA folding



**Figure :** left: the case of bifurcation; right: from easy to difficult cases

### LECTURE 2

- N. Jones, P. Pevzner An Introduction to Bioinformatics Algorithms
- T.F. Smith, M.S. Waterman, Identification of common molecular subsequences, J Mol Biol 147, 195-197, 1981.
- <https://www.youtube.com/watch?v=P-mMvhfJhu8>
- affine gaps: <http://courses.cs.washington.edu/courses/cse527/00wi/lectures/lect05.pdf> - D.S. Hirschberg, A linear space algorithm for computing longest common subsequences, Communications of the ACM 18, 341-343, 1975;  
<http://drp.id.au/align/2d/AlignDemo.shtml>.
- Nussinov, R., Pieczenik, G., Griggs, J. R. and Kleitman, D. J. (1978). Algorithms for loop matchings, SIAM J. Appl. Math



Homology database search (Blast, Patternhunter). Data: sequences; input: a short string as a query against a database; output: all the records showing a good match with the query.

In an Internet search you run a query for exact keyword search against a database with a size limit of 6 billion people x homepage size.

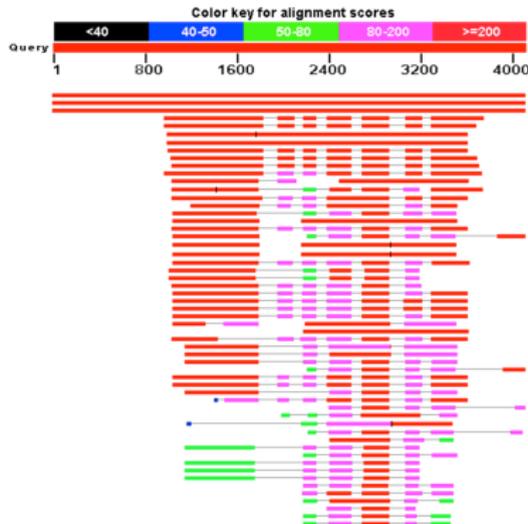
Given two DNA sequences you want to find all local similar regions, using edit distance; the size limit: 6 billion people x 3 billion basepairs + millions of species x billion bases.

Main question: find the sequences more similar to my query sequence.



## Approximate Search algorithms

The problem is to find in a database all sequences with interesting similarities. Below there is an example of output for the following task: a query (an unknown gene sequence) is compared with other sequences with known functions in a database. The figure below shows an example of output. Perfect hits are red colored. Regions that were weaker in match are pink, green, or blue; alignment details are also available. Algorithms considered: Blast, Patternhunter.



It is common to observe strong sequence similarity between a gene (or a protein) and its counterpart in another species that diverged hundreds of millions of years ago. Accordingly, the best method to identify the function of a new gene or protein is to find its sequence- related genes or proteins whose functions are already known.

**The Basic Local Alignment Search Tool (BLAST) is a computer program for finding regions of local similarity between two DNA or protein sequences.** It is designed for comparing a query sequence against a target database. It is a heuristic that finds short matches between query and database sequences and then attempts to start alignments from these seed hits. BLAST is arguably the most widely used program in bioinformatics. By sacrificing sensitivity for speed, it makes sequence comparison practical on huge sequence databases currently available.



Dynamic Programming (DP) is an effective way to construct alignments (in some applications too slow). Since the DP is  $O(n^2)$ , matching two  $9 \times 10^9$  base length sequences would take about  $9 \times 10^{18}$  operations. BLAST is an alignment algorithm which runs in  $O(n)$  time. **The key to BLAST is that we only actually care about alignments that are very close to perfect. A match of 70% is worthless; we want something that matches 95% or 99% or more.** What this means is that correct (near perfect) alignments will have long substrings of nucleotides that match perfectly. Most popular Blast-wise algorithms use a seed-and-extend approach that operates in two steps: 1. Find a set of small exact matches (called seeds) 2. Try to extend each seed match to obtain a long inexact match.



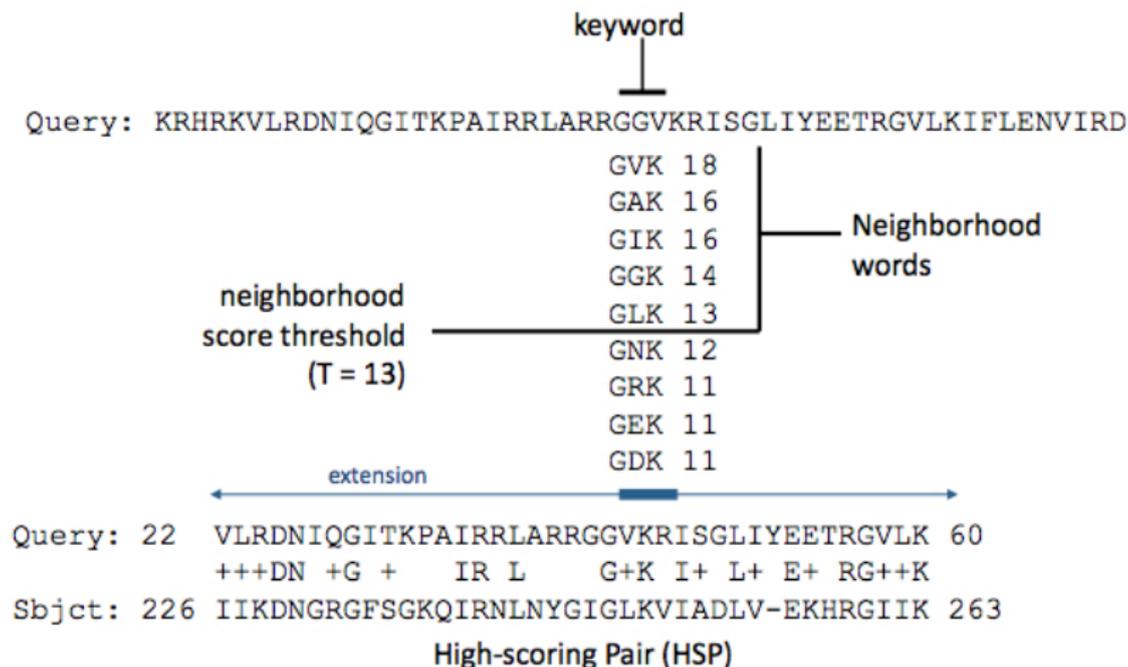
The main steps of the algorithm are the follows:

- 1 Split query into overlapping words of length  $W$  ( $W$ -mers).
- 2 Find a neighborhood of similar words for each word in the query (see the figure next slide).
- 3 Lookup each word in the neighborhood in a hash table to find where in the database each word occurs. Call these the seeds.
- 4 Extend all seed collections until the score of the alignment drops off below a threshold.
- 5 Report matches with overall highest scores.



## BLAST (Basic Local Alignment Search Tools)

BLAST provides a trade off between speed and sensitivity, by setting a "threshold" parameter T. A higher value of T yields greater speed, but also an increased probability of missing weak similarities (the figure shows an example with protein query; it shows perfect matches and nearly perfect matches, + ).



**To speed up the homology search process, BLAST employs a filtration strategy:** it first scans the database for length- $w$  word matches of alignment score at least  $T$  between the query and target sequences and then extends each match in both ends to generate local alignments (in the sequences) with score larger than a threshold  $x$ . The matches are called high-scoring segment pairs (HSPs). BLAST outputs a list of HSPs together with E-values that measure how frequent such HSPs would occur by chance.

A HSP has the property that it cannot be extended further to the left or right without the score dropping significantly below the best score achieved on part of the HSP. The original BLAST algorithm performs the extension without gaps. Variants are gapped Blast, psi-Blast, Mega Blast and others.

see <http://blast.ncbi.nlm.nih.gov/Blast.cgi>



- Assume that the length  $m$  and  $n$  of the query and database respectively are sufficiently large; a segment-pair  $(s, t)$  consists of two segments, one in  $m$  (say the amino acid string: VALLAR) and one in  $n$  (say PAMMAR), of the same length. We think of  $s$  and  $t$  as being aligned without gaps and score this alignment; the alignment score for  $(s, t)$  is denoted by  $\sigma(s, t)$ .
- Given a cutoff score  $x$ , a segment pair  $(s, t)$  is called a high-scoring segment pair (HSP), if it is locally maximal and  $\sigma(s, t) \geq x$  and the goal of BLAST is to compute all HSPs.
- The BLAST algorithm has three parameters: the word size  $W$ , the word similarity threshold  $T$  and the minimum match score  $x$  (cutoff score  $x$ ).
- BLAST outputs a list of HSPs together with E-values that measure how frequent such HSPs would occur by chance. This is calculated with respect of a database with similar size and random data. E-value close to zero means that the sequences retrieved is almost identical to the query.



The list of all words of length  $W$  that have similarity  $\geq T$  to some word in the query sequence  $m$  is generated. The database sequence  $n$  is scanned for all hits  $t$  of words  $s$  in the list. Each such seed  $(s, t)$  is extended until its score  $\sigma(s, t)$  falls a certain distance below the best score found for shorter extensions and then all best extensions are reported that have score  $\geq x$ . In practice,  $W$  is usually 4 (amino acids) for proteins. The list of all words of length  $W$  that have similarity  $\geq T$  to some word in the query sequence  $m$  can be produced in time proportional to the number of words in the list. These are placed in a keyword tree and then, for each word in the tree, all exact locations of the word in the database  $n$  are detected in time linear to the length of  $n$ . The original version of BLAST did not allow indels, making hit extension very fast. Note that the use of seeds of length  $W$  and the termination of extensions with fading scores are both steps that speed up the algorithm, but also imply that BLAST is not guaranteed to find all HSPs.



- The list of all words of length  $W$  in the query sequence  $m$  is generated. The database  $n$  is scanned for all hits of words in this list. Blast uses a two-bit encoding for DNA. This saves space and also search time, as four bases are encoded per byte. In practice,  $W$  is usually 12 for DNA.
- HSP scores are characterized by two parameters,  $W$  and  $\lambda$ . The expected number of HSPs with score at least  $Z$  is given by the E-value, which is:  $E(Z) = Wmne^{-\lambda Z}$ .
- Essentially,  $W$  and  $\lambda$  are scaling-factors for the search space and for the scoring scheme, respectively.
- As the E-value depends on the choice of the parameters  $W$  and  $\lambda$ , one cannot compare E-values from different BLAST searches.



- For a given HSP (s, t) we transform the raw score  $Z = \sigma(s, t)$  into a bit-score thus:  $Z' = \frac{\lambda Z - \ln W}{\ln 2}$ . Such bit-scores can be compared between different BLAST searches. To see this, solve for Z in the previous equation and then plug the result into the original E-value.
- E-values and bit scores are related by  $E = mn2^{-Z'}$
- The number of random HSPs (s, t) with  $\sigma(s, t) \geq x$  can be described by a Poisson distribution. Hence the probability of finding exactly k HSPs with a score  $\geq Z$  is given by  $P(k) = \frac{E^k}{k!} e^{-E}$  (see also [www.ncbi.nlm.nih.gov/blast/tutorial/Altschul-1.html](http://www.ncbi.nlm.nih.gov/blast/tutorial/Altschul-1.html))
- The probability of finding at least one HSP by chance is  $P = 1 - P(X = 0) = 1 - e^{-E}$ , called the P-value, where E is the E-value for Z.
- BLAST reports E-values rather than P-values as it is easier, for example, to interpret the difference between an E-value of 5 and 10, than to interpret the difference between a P-value of 0.993 and 0.99995. For small E-values  $< 0.01$ , the two values are nearly identical.



## Blast of human beta globin DNA against human DNA

```

                                Score   E
Sequences producing significant alignments:                      (bits) Value
gi|19849266|gb|AF487523.1| Homo sapiens gamma A hemoglobin (HBG1... 289 1e-75
gi|183868|gb|M11427.1|HUMHBG3E Human gamma-globin mRNA, 3' end 289 1e-75
gi|44887617|gb|AY534688.1| Homo sapiens A-gamma globin (HBG1) ge... 280 1e-72
gi|31726|emb|V00512.1|HSGGL1 Human messenger RNA for gamma-globin 260 1e-66
gi|38683401|ref|NR_001589.1| Homo sapiens hemoglobin, beta pseud... 151 7e-34
gi|18462073|gb|AF339400.1| Homo sapiens haplotype PB26 beta-glob... 149 3e-33

ALIGNMENTS
>gi|28380636|ref|NG_000007.3| Homo sapiens beta globin region (HBB) on chromosome 11
Length = 81706
Score = 149 bits (75), Expect = 3e-33
Identities = 183/219 (83%)
Strand = Plus / Plus

Query: 267   ttgggagatgccacaaagcacctggatgatctcaagggcacctttgccagctgagtga 326
            || ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct: 54409 ttggaaaaagctgttatgotcacggatgacctcaaggcacctttgtctacactgagtga 54468

Query: 327   ctgcactgtgacaagctgcatgtggactctgagaacttc 365
            ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct: 54469 ctgcactgtaacaagctgacgtggacctgagaacttc 54507
```

from Altschul: The expected-time computational complexity of BLAST is approximately  $aW + bN + cNW/20^W$ , where  $W$  is the number of words generated,  $N$  is the number of residues in the database and  $a$ ,  $b$  and  $c$  are constants. The  $W$  term accounts for compiling the word list, the  $N$  term covers the database scan, and the  $NW$  term is for extending the hits. Although the number of words generated,  $W$ , increases exponentially with decreasing the threshold, it increases only linearly with the length of the query, so that doubling the query length doubles the number of words.



ttgacdtagatgagatgtogttcadt tadtgagdtacagaaaa

ttg|acc|tag|atg|aga|tgt|cgt|tca|ctt|tta|ctg|agc|tac|aga|aaa  
L T x M R C R S L L L S Y R K

t|tga|cct|aga|tga|gat|gtc|gtt|cac|ttt|tac|tga|gct|aca|gaa|aa  
x P R x D V V H F Y x S T E

tt|gac|cta|gat|gag|atg|tgg|ttc|act|ttt|act|gag|cta|cag|aaa|a  
D L D E M S F T F T E L Q K

**Figure :** Blast DNA query (top) against a database of proteins will process all the potential triplets forming codons which code for amino acid (the capitol letters)



### BLAST may also miss a hit

GAGTACTCAACA CCAACAT TAGTGGGCAATGGAAAAT  
|| ||||| ||||| | ||||| |||||  
GAATACTCAACAGCAACATCAATGGGCA GCAGAAAAT

←————→  
9 matches

In this example, despite a clear homology, there is no sequence of continuous matches longer than length 9. BLAST uses a length 11 and because of this, BLAST does not recognize this as a hit!

Resolving this would require reducing the seed length to 9, which would have a damaging effect on speed



The big problem for BLAST is low sensitivity (and low speed). Massive parallel machines are built to do Smith Waterman exhaustive dynamic programming.

A spaced seed is formed by two words, one from each input sequence, that match at positions specified by a fixed pattern and one don't care symbol respectively. For example, the pattern 1101 specifies that the first, second and fourth positions must match and the third one contains a mismatch.

PatternHunter (PH) was the first method that used carefully designed spaced seeds to improve the sensitivity of DNA local alignment.

Spaced seeds have been shown to improve the efficiency of lossless filtration for approximate pattern matching, namely for the problem of detecting all matches of a string of length  $m$  with  $q$  possible substitution errors.



If you want to speed up, you have to use a longer seed. However, we now face a dilemma: increasing seed size speeds up, but loses sensitivity; decreasing seed size gains sensitivity, but loses speed. How do we increase sensitivity and speed simultaneously?

**Spaced Seed: nonconsecutive matches and optimized match positions** Represent BLAST seed by 111111111111;

Spaced seed: 111010010100110111 where 1 means a required match and 0 means don't care position.

This simple change makes a huge difference: significantly increases hit number to homologous region while reducing bad hits. Spaced seeds give PH a unique opportunity of using several optimal seeds to achieve optimal sensitivity, this was not possible by BLAST technology. PH II uses multiple optimal seeds; it approaches Smith-Waterman sensitivity while is 3000 times faster.

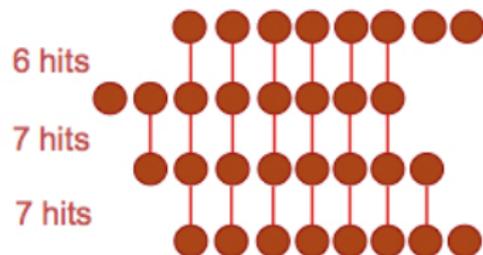
Example: Smith-Waterman (SSearch): 20 CPU-days, PatternHunter II with 4 seeds: 475 CPU-seconds: 3638 times faster than Smith-Waterman dynamic programming at the same sensitivity



# Sensitivity and Specificity

Sensitivity: The probability to find a local alignment. Specificity: In all local alignments, how many alignments are homologous

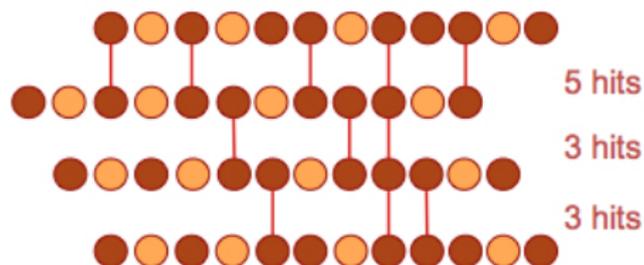
## Consecutive Positions



On a 70% conserved region:

	<u>Consecutive</u>
Expected # hits:	1.07
Prob[at least one hit]:	0.30

## Non-Consecutive Positions



Non-consecutive

0.97
0.47



- 111010010100110111 (called a model)
  - Eleven required matches (weight=11)
  - Seven "don't care" positions

```

GAGTACTCAACACCAACATAGTGGCAATGGAAAAT...
|| ||||| |||| | | |||| | |||||
GAATACTCAACAGCAACACTAATGGCAGCAGAAAAT...
      111010010100110111

```

- Hit = all the required matches are satisfied.
- BLAST seed model = 1111111111

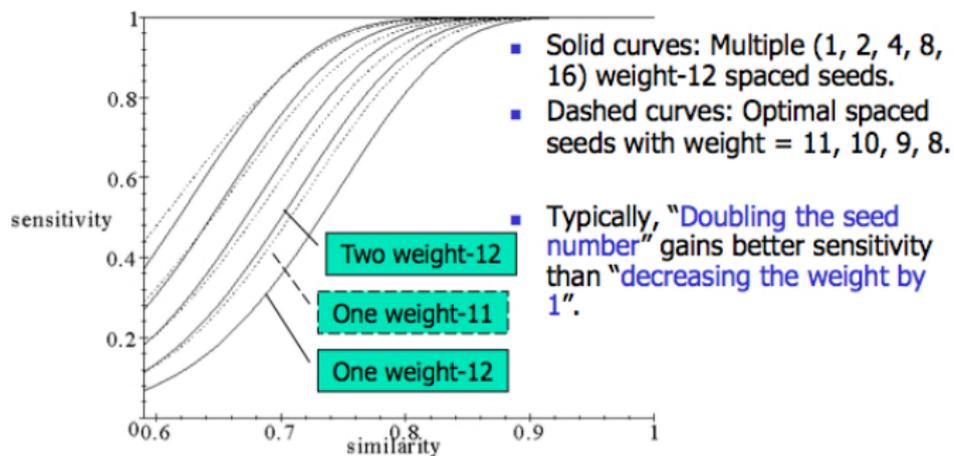
```

111010010100110111
 111010010100110111
   111010010100110111
    111010010100110111
     111010010100110111
      111010010100110111
       111010010100110111
        111010010100110111
         . . . . .

```

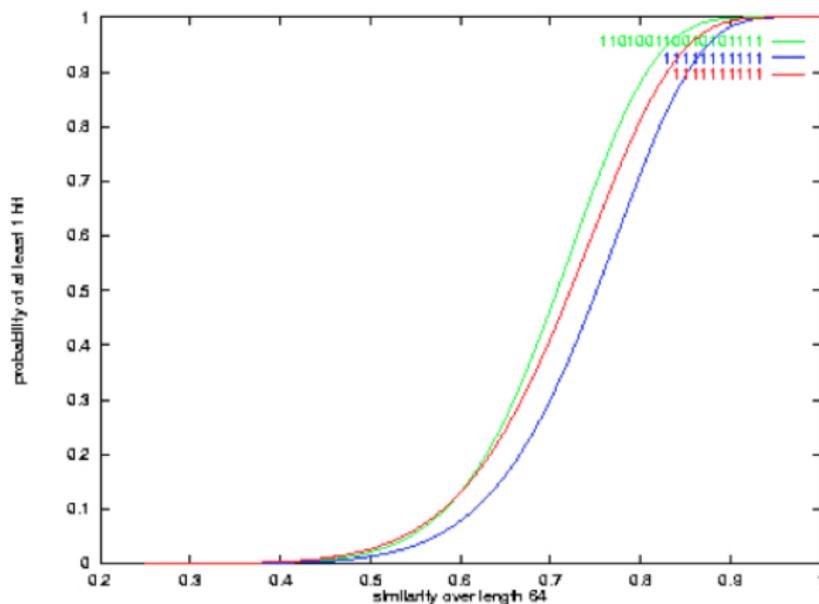


In PatternHunter, the spaced model has often weight 11 and length 18.

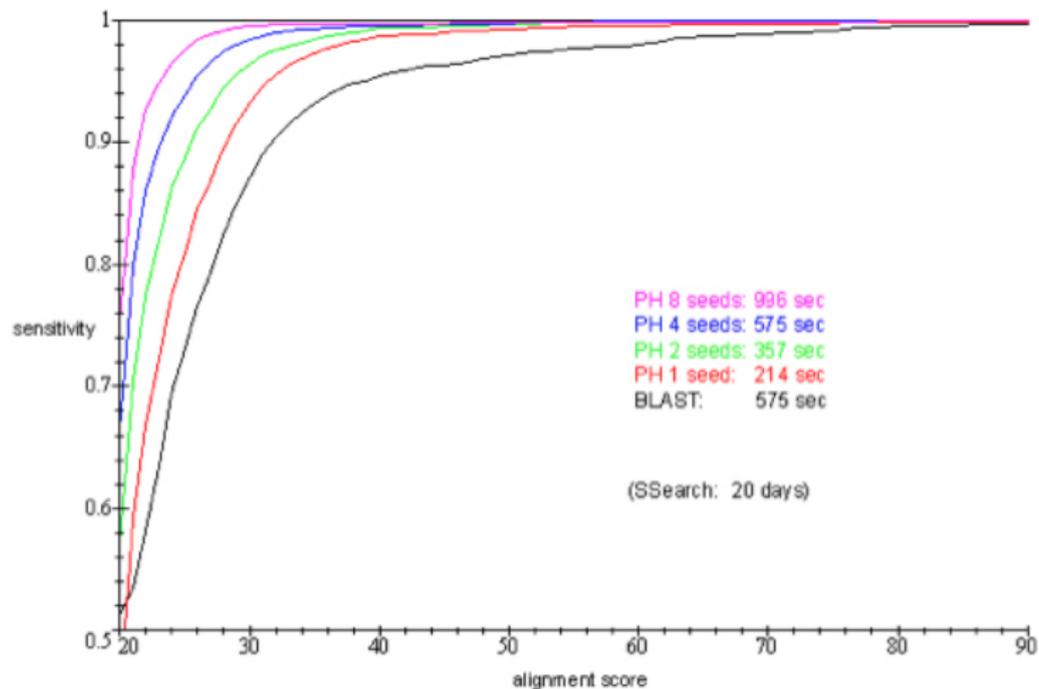


The non-consecutive seed is the primary difference and strength of Patternhunter

## Sensitivity: PH weight 11 seed vs BLAST 11 & 10



## Comparing different seeds number



**Figure :** sensitivity versus alignment score



- <http://blast.ncbi.nlm.nih.gov/Blast.cgi>
- Altschul, S.F. and Gish, W. (1996) "Local alignment statistics." *Meth. Enzymol.* 266:460-480
- Altschul, S.F., et al. (1990) "Basic local alignment search tool." *J. Mol. Biol.* 215:403-410
- <https://www.youtube.com/watch?v=LInMtl2Sg4g>
- Ma B., Tromp J., and Li M. (2002) PatternHunter: faster and more sensitive homology search, *Bioinformatics* 18 (3): 440-445. doi: 10.1093/bioinformatics/18.3.440



Progressive alignment (Clustal). Input: a set of sequences in Fasta format (also thousands).

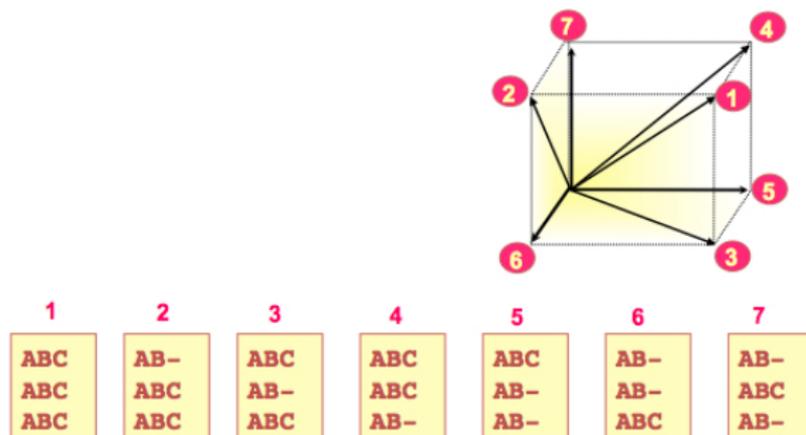
Output: alignment of the set of sequences: multi sequence alignment (MSA). Interest: find conserved patterns (across sequences, i.e. columns retaining similar patterns) may indicate functional constraints. In other words, if the same pattern is conserved in multiple sequences from different species, the substring could have an important functional role.

Main question in this lecture: how similar is this group of sequences?

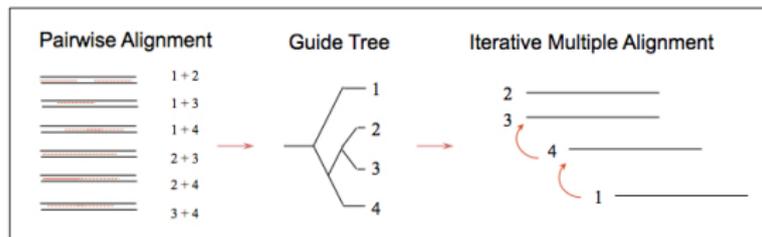


## Challenges of extending dynamic programming to n sequences

- For two sequences, there are three ways to extend an alignment
- for n sequences, a n-dimensional dynamic programming hypercube has to be computed and for each entry we have to evaluate  $(2^n - 1)$  predecessors.
- Given 3 sequences, the figure below shows a three-dimensional alignment path matrix: there are  $= (2^3 - 1) = 7$  ways to extend an alignment.

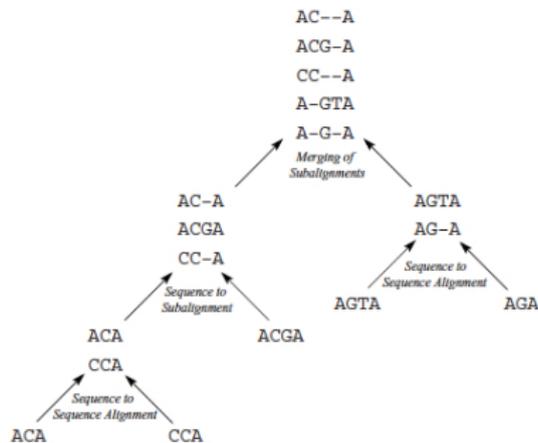
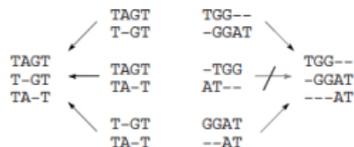


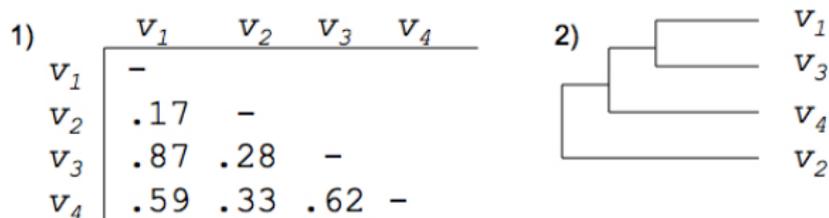
- Progressive alignment methods are heuristic in nature. They produce multiple alignments from a number of pairwise alignments.
- Perhaps the most widely used algorithm of this type is CLUSTALW.
- Given N sequences, align each sequence against each other and obtain a similarity matrix; similarity = exact matches / sequence length (percent identity)
- Create a guide tree using the similarity matrix; the tree is reconstructed using clustering methods such as UPGMA or neighbor-joining (explained later).
- Progressive Alignment guided by the tree.



# Progressive alignment

Not all the pairwise alignments build well into multiple sequence alignment (top figure); the progressive alignment builds a final alignment by merging sub-alignments with a guide tree (bottom figure).





Calculate:

- 3)  $v_{1,3}$  = alignment( $v_1, v_3$ )  
 $v_{1,3,4}$  = alignment( $(v_{1,3}), v_4$ )  
 $v_{1,2,3,4}$  = alignment( $(v_{1,3,4}), v_2$ )

**Figure :** Progressive alignment of 4 sequences ( $v_1, v_2, v_3, v_4$ ): 1) distance matrix from pairwise alignment; 2) pairwise alignment score analysis; tree showing the best order of progressive alignment, 3) building up the alignment.



Blosum is a symmetric amino acid replacement matrix used as scoring matrix in Blast search and in phylogeny. Using only the conserved regions of protein sequences in a MSA, we compute  $p_{ij}$  i.e. for each column of the MSA, the probability of two amino acids  $i$  and  $j$  replacing each other, and  $p_i$  and  $p_j$  are the background probabilities of finding the amino acids  $i$  and  $j$  in any protein sequence. Finally we compute:  $Score_{ij} = (k^{-1})\log(p_{ij}/p_i p_j)$  where the  $k$  is a scaling factor.

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4	-1	-2	-2	0	-1	-1	0	-2	-1	-1	-1	-1	-2	-1	1	0	-3	-2	0
R	-1	5	0	-2	-3	1	0	-2	0	-3	-2	2	-1	-3	-2	-1	-1	-3	-2	-3
N	-2	0	6	1	-3	0	0	0	1	-3	-3	0	-2	-3	-2	1	0	-4	-2	-3
D	-2	-2	1	6	-3	0	2	-1	-1	-3	-4	-1	-3	-3	-1	0	-1	-4	-3	-3
C	0	-3	-3	-3	9	-3	-4	-3	-3	-1	-1	-3	-1	-2	-3	-1	-1	-2	-2	-1
Q	-1	1	0	0	-3	5	2	-2	0	-3	-2	1	0	-3	-1	0	-1	-2	-1	-2
E	-1	0	0	2	4	2	5	-2	0	-3	-3	1	-2	-3	-1	0	-1	-3	-2	-2
G	0	-2	0	-1	-3	-2	-2	6	-2	-4	-4	-2	-3	-3	-2	0	-2	-2	-3	-3
H	-2	0	1	-1	-3	0	0	-2	8	-3	-3	-1	-2	-1	-2	-1	-2	-2	2	-3
I	-1	-3	-3	-1	-3	-3	-4	-3	4	2	-3	1	0	-3	-2	-1	-3	-1	3	3
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4	-2	2	0	-3	-2	-1	-2	-1	1
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5	-1	-3	-1	0	-1	-3	-2	-2
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5	0	-2	-1	-1	-1	-1	1
F	-2	-3	-3	-3	-2	-3	-3	-1	0	0	-3	0	6	-4	-2	-2	1	3	-1	1
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	4	7	-1	-1	-4	-3	-2
S	-1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4	1	-3	-2	-2
T	0	-1	0	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5	-2	-2	0	0
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11	2	-3
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	-1
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4



AAA  
AAA  
AAT  
ATC

Let's start from an alignment of four sequences (above the first three columns);  
Compute the frequencies for the occurrence of each letter in each column of multiple alignment  $p_A = 1$ ,  $p_T=p_G=p_C=0$  (1st column);  
 $p_A = 0.75$ ,  $p_T = 0.25$ ,  $p_G=p_C=0$  (2nd column);  
 $p_A = 0.50$ ,  $p_T = 0.25$ ,  $p_C=0.25$   $p_G=0$  (3rd column);  
Compute entropy of each column:  $E = - \sum_{X=A,C,G,T} p_x \log(p_x)$   
The entropy for a multiple alignment is the sum of entropies of each column of the alignment.

## Example of a multiple sequence alignment (globin amino acid sequences)

```

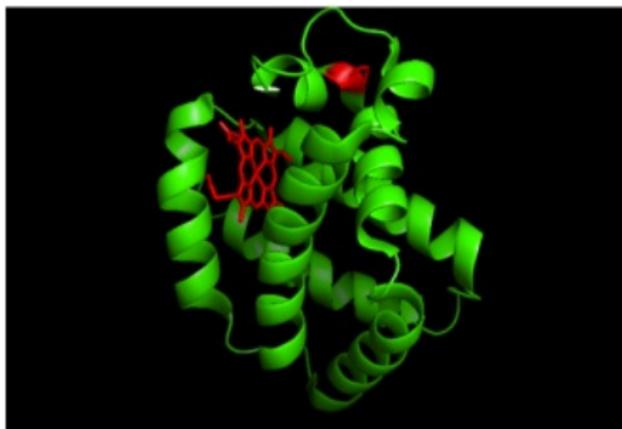
HBA_HUMAN      -----VLSPADKINVKAAWGKVGAHAGEYGA--EALERMFLSFPTTKTYFFHF-DL 48
HBA_HORSE      -----VLSAADKINVKAAWSKVGGHAGEYGA--EALERMFLGFPTTKTYFFHF-DL 48
HBB_HUMAN      -----VHLTPEEKSAVTALWGRKVN--VDEVGG--EALGRLLVVVYPTQRFDFESFGDL 48
HBB_HORSE      -----VQLSGEKEAAVALALWGRKVN--EEVGG--EALGRLLVVVYPTQRFDFESFGDL 48
GLB5_PETMA     PIVDTGSAPLSAAEKTRIRSAMAPVYSTYETSQV--DILVRFFTSTPAAQEFPPFKGL 58
MYG_PHYCA     -----VLSGEVQLVLRVWAKVEADVAGHGQ--DILIRLFKSHPETLEKDRRPHL 49
GLB1_GLYDI     -----GLSAAQRQVIAATWEDIAGADNGACVGRDCILIKFLSAHPQMAAVPFGS--- 48
GLB3_CHITH     -----LSADQISTVQASFDKVK-----GDPVGIYAVFKADPPSINAKFTQFACK 44
LGB2_LUPLU     -----GALTESQAALVKSSWEEFNANIPKHTH--RFFILVLEIAPAAKDLFSFLKGT 50
               *  :  :  :  :  .  :  :  :  *  *

HBA_HUMAN      S----HGSAQVKGHGKKVADALTNVAHVYDD----MPNALSALSDLHA--HKLRVDPV 96
HBA_HORSE      S----HGSAQVKAHGKVKGDALTLAVGHLLD----LPGALSNSLSDLHA--HKLRVDPV 96
HBB_HUMAN      STPDAVHGNPKVKAHGKVKVLAFAFDGLAHLDN----LKGTFATLSELHC--DKLRHVP 101
HBB_HORSE      SNPGAVHGNPKVKAHGKVKVLSFGEVHLDN----LKGTFATLSELHC--DKLRHVP 101
GLB5_PETMA     TTADQLKKSADVRMHAERINAVNDVAASHDDT--EKMSNKLRLDLSGKHA--KSFQVDP 114
MYG_PHYCA     KTEAENKASEDLKQHGVTVLTAIGAILKQKRGH----HEAEKPLAQSQA--TRHKPIK 102
GLB1_GLYDI     ----GASDPGVAALGAKVLAQIGVAVSHLQDE--GRHVAQMKAVGVRRHGYGNHIIKAQ 101
GLB3_CHITH     DLES-TRGTAPFEIHANRIVGFFSKIIGELFN----IEADVNTFVASHK---FRGVTHD 95
LGB2_LUPLU     SEVP--QNNPELQAHAGKVKFLVYEAALQLQVTVVVVDATLKNLGLSRRV---SKGVADA 105
               .  .  :  .  .  .  .  .  .  *  :

HBA_HUMAN      NFKLLSHCLLVTLAAHLPAEFTPVAHASLDKFLASVSTVLTSKYR----- 141
HBA_HORSE      NFKLLSHCLLSTLAVHLFNDFTPVAHASLDKFLSSVSTVLTSKYR----- 141
HBB_HUMAN      NFRLLGNVLCVLAHDFGDEFTPVQAAQKVVAVGANALAHKYH----- 146
HBB_HORSE      NFRLLGNVLVVLRARHFGDFTFPELQASYQKVVAVGANALAHKYH----- 146
GLB5_PETMA     YFKVLAAYIADTYAAG-----DAGFEKLMHSKICILLRSAY----- 149
MYG_PHYCA     YLEFISEAIIHVLHSRHPGDFGADAGGANDKALELFRKDIAAKYKELGQYG 153
GLB1_GLYDI     YFEPLGASLLSAMEHRIGGKKNAAAKDAAVAAAYADISGALISGLQS----- 147
GLB3_CHITH     QLNFRAGFVSYHKAHTD---FAGAAEAAAGATLDTFFGHIFSKH----- 136
LGB2_LUPLU     HFPVVKIATLKTIKVYVVGAKWSEEINSAVTIAYDELATVIRKEHNDAA--- 153
               :  .  :  :  :  .  .  .  .  :
    
```

**Figure :** Chemical properties of amino acids are in color code. The globin proteins from different species could be easily aligned because they have many similar substrings in common.





**Figure :** Human globin 3D structure. The small amount of changes in the globin alignment suggests that globin sequences are likely to have very similar 3D structure (figure). Columns rich of gaps (previous slide) often correspond to unstructured regions (loops); conserved regions often correspond to binding sites or regions where one protein interacts with a DNA sequence or with another protein: most important bits are more conserved.



D.G. Higgins, J.D. Thompson, and T.J. Gibson. Using CLUSTAL for multiple sequence alignments. *Methods in Enzymology*, 266:383402, 1996.  
<http://www.ebi.ac.uk/Tools/msa/>



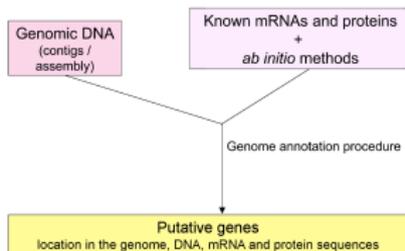
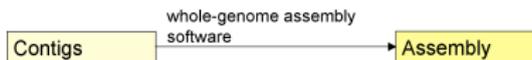
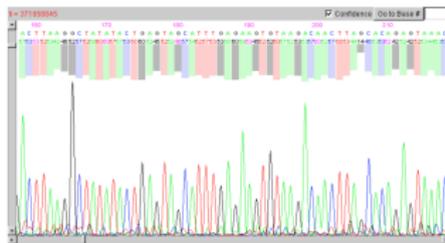
Algorithms: Genome Assembly (Burrows-Wheeler transform) and De Bruijn graph

Data: DNA sequence Input: reads (short sequences); Output: genomes (long sequences)

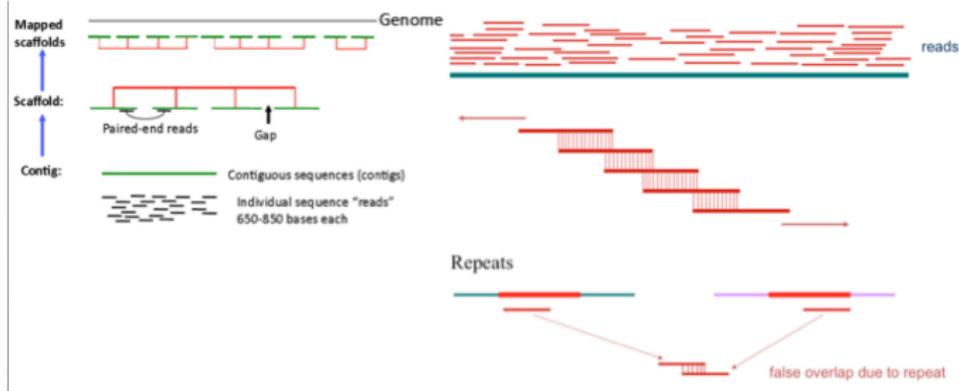
Main question: how to build a genome from little bits?



# Building genomes from short sequences (reads)



Genome scaffolding (i.e. the process of ordering and orientating contigs) of de novo assemblies usually represents the first step in most genome finishing pipelines (figure below on the right). The preferred approach to genome scaffolding is currently based on assembling the sequenced reads into contigs and then using paired end information to join them into scaffolds. The figure below show the overlapping reads used to cover the assemble of the genome and the problem with repeats. The algorithm presented here is the Burrows- Wheeler transform.



The current sequencing procedures are characterized by highly parallel operations, much lower cost per base, but they produce several millions of "reads", short stretches of DNA bases (usually 35-400 bp). In many experiments, e.g., in ChIP-Seq, the task is to align these reads to a reference genome.

The main effort is to reduce the memory requirement for sequence alignment (such as Bowtie, BWA and SOAP2); the Burrows-Wheeler transform, BWT (1994) is commonly used. The Burrows and Wheeler transform (BWT) is a block sorting lossless and reversible data transform. The BWT can permute a text into a new sequence which is usually more compressible.

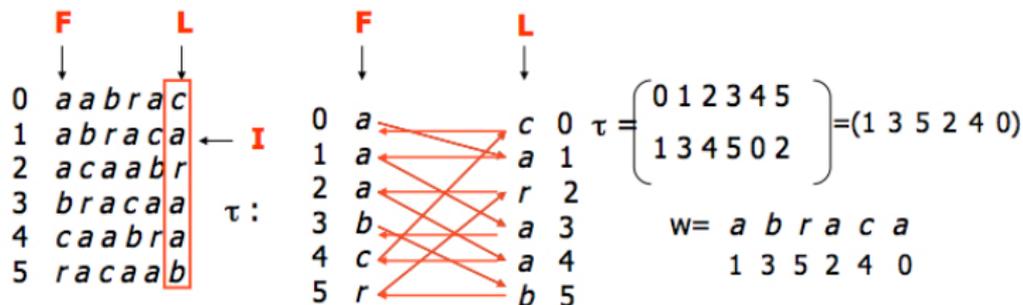
The transformed text can be better compressed with fast locally-adaptive algorithms, such as run-length-encoding (or move-to-front coding) in combination with Huffman coding (or arithmetic coding). Burrows obtained the Ph.D at the Computer Laboratory.



# Burrows-Wheeler Transform

INPUT (example):  $T = \text{"abrac"}a$ ; then we sort lexicographically all the cyclic shifts of  $T$ . For all  $i \neq I$ , the character  $L[i]$  is followed in  $T$  by  $F[i]$ ; for any character  $ch$ , the  $i$ -th occurrence of  $ch$  in  $F$  corresponds to the  $i$ -th occurrence of  $ch$  in  $L$ .

OUTPUT:  $BWT(T) = \text{"caraab"}$  and the index  $I$ , that denotes the position of the original word  $T$  after the lexicographical sorting. The Burrows-Wheeler Transform is reversible, in the sense that, given  $BWT(T)$  and an index  $I$ , it is possible to recover the original word  $T$ .

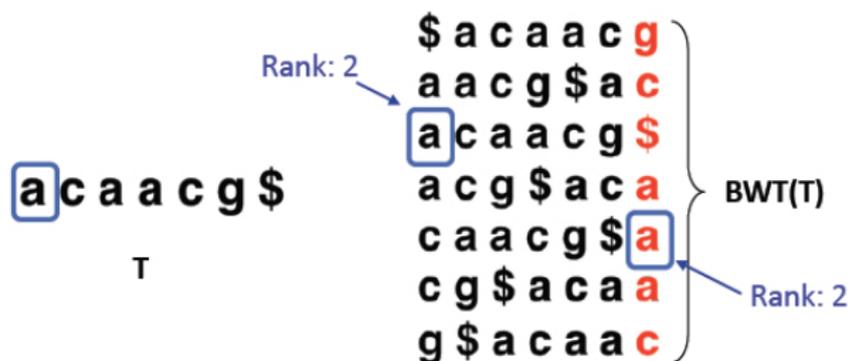


## Burrows-Wheeler Transform example

Once BWT(T) is built, all else shown here (i.e. the matrix) is discarded. Three steps: 1) Form a  $N \times N$  matrix by cyclically rotating (left) the given text to form the rows of the matrix. Here we use '\$' as a sentinel (lexicographically greatest character in the alphabet and occurs exactly once in the text but it is not a must). 2) Sort the matrix according to the alphabetic order. Note that the cycle and the sort procedures of the Burrows-Wheeler induce a partial clustering of similar characters providing the means for compression. 3) The last column of the matrix is BWT(T) (we need also the row number where the original string ends up).

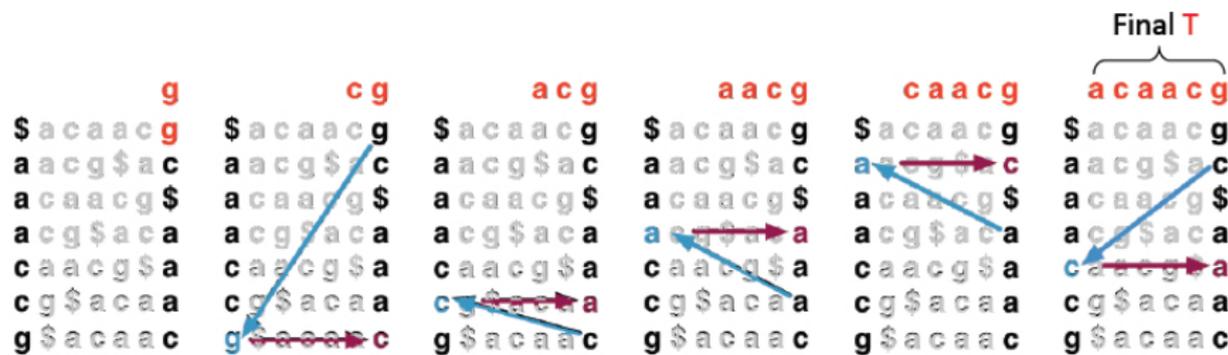


Property that makes  $BWT(T)$  reversible is LF Mapping: the  $i$ th occurrence of a character in **L**ast column is same text occurrence as the  $i$ th occurrence in the **F**irst column (i.e. the sorting strategy preserves the relative order in both last column and first column).



# LF Mapping

To recreate  $T$  from  $BWT(T)$ , repeatedly apply the rule:  $T = BWT[LF(i)] + T$ ;  $i = LF(i)$  where  $LF(i)$  maps row  $i$  to row whose first character corresponds to  $i$ 's last per LF Mapping. First step:  $S = 2$ ;  $T = \$$ . Second step:  $s = LF[2] = 6$ ;  $T = g\$$ . Third step:  $s = LF[6] = 5$ ;  $T = cg\$$ .



## The BWT(T) is more amenable to subsequent compression algorithms

t	a	t	a	t	a	t	a	t	a	\$
a	t	a	t	a	t	a	t	a	\$	t
t	a	t	a	t	a	t	a	\$	t	a
a	t	a	t	a	t	a	\$	t	a	t
t	a	t	a	t	a	\$	t	a	t	a
a	t	a	t	a	\$	t	a	t	a	t
t	a	t	a	\$	t	a	t	a	t	a
a	t	a	\$	t	a	t	a	t	a	t
t	a	\$	t	a	t	a	t	a	t	a
a	\$	t	a	t	a	t	a	t	a	t
\$	t	a	t	a	t	a	t	a	t	a

\$	t	a	t	a	t	a	t	a	t	a
a	\$	t	a	t	a	t	a	t	a	t
t	a	\$	t	a	t	a	t	a	t	a
a	t	a	\$	t	a	t	a	t	a	t
t	a	t	a	\$	t	a	t	a	t	a
a	t	a	t	a	\$	t	a	t	a	t
t	a	t	a	t	a	\$	t	a	t	a
a	t	a	t	a	t	a	\$	t	a	t
t	a	\$	t	a	t	a	t	a	t	a
a	\$	t	a	t	a	t	a	t	a	t
\$	t	a	t	a	t	a	t	a	t	a

**Figure :** in the left,the word "tatatatata\$" undergoes cyclic shift and it is sorted in the right. Note that the BWT(tatatatata\$) is a word (atnttaaaa\$) with good clustering of T's and A's and so it can be written in a more compact way. The DNA is from an alphabet of 4 symbols so the clustering happens very often.



# Next Generation sequencing (NGS): The Biological problem

Instead of considering a DNA sequence, for sake of clarity, let's consider a sentence and we trim all spaces.

Copies of the sentence are divided into fragments called reads which could be converted into k-mers. We would like to assemble the original sentence using the reads or the k-mers.

itwasthebestoftimesitwastheworstoftimesitwastheageofwisdomitwastheageoffoolishness...

Generate random 'reads'



How do we assemble?

ficredul geoffoolk iwasthebe itwasthebe geofwisdom itwastheep epochofinc linesitwas sithespocho nessitwas wastheage theepochf sithespocho hofincrodu estofimes eoffoolish lshnessk hofbelieli pochofincr itwasthwo twastheage tofimesit domitwasch ochofbelie apochofbe apochofbe astheworst chofincrod itageofwi lshfwasit sctwasthe asthepoch sffwasitw widomitwa ageoffooli wasthewor ochofbelie sdomitwas sctwasthe apochofbe foolishne eofwisdom hofestofit sithageoff twastheage eworstofit stoffimes ithepochf sctwasthe hepochof ithepochf sdomitwas astheworst rstofimes worstofim sithespocho geoffoola foolishne itmeshas lshnessk sithageoff eworstofit orstofime fwadomitw wastheage heageofwis increduft lshnessitw twasthepo wasthewors asthepoch heworstofit obelieft wastheage hepochof pochofincr heageofwis sithageofw fircrodul asthageoff widomitwa wastheage asthepoch disthnessi asthepoch itwastheep twastheage widomitwa beliefew bestofime epochofwi ithepochf sctwasthe lshnessk hofbelieli iwasthebe lshnessitw sctwasthe ageofwisdo twastheage sctwasthe twastheage shnessitwa fircrodul beliefew ithepochf meitwasitw domitwas ochofbelie heageofwis otfimesitw sithespocho bestofime twastheage foolishnas fimesitwa thebestof itwasthep ithepochf sctwasthe obelieft bestofime ritwastwa imesitwas limesitwas orstofime estofimes twastheas stoffimesitw sdomitwas widomitwa theworst astheworst sctwasthe itageoffo apochofbe etc etc.

## Convert reads into "Kmers"

Kmer: a substring of defined length

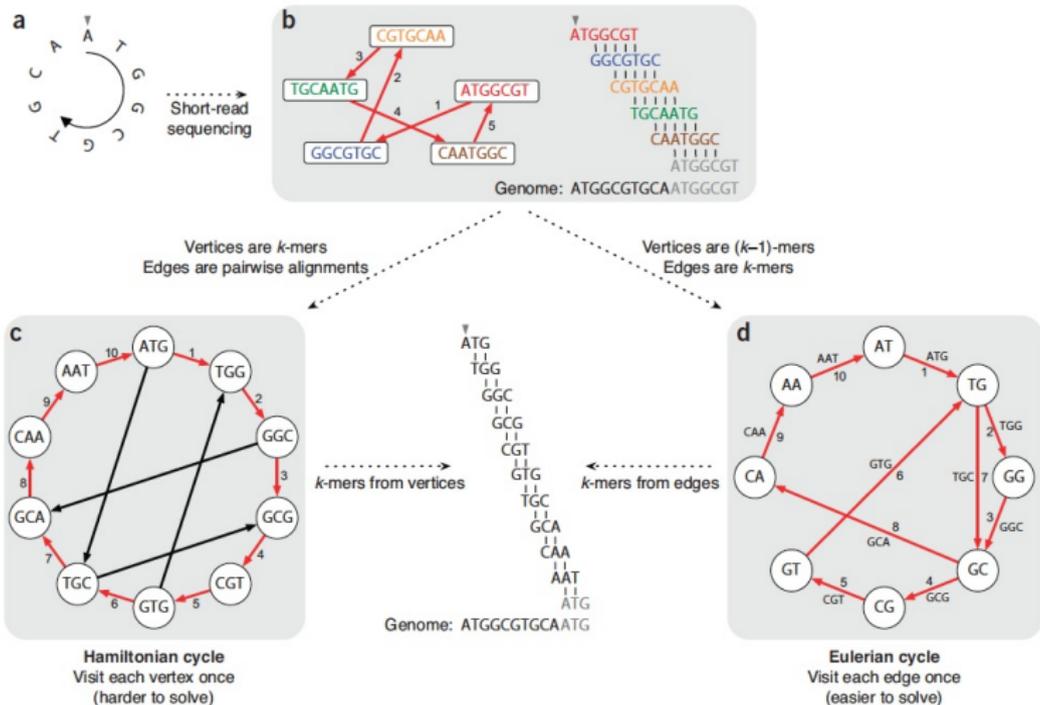
Reads: theageofwi sthebestof astheageof worstoftim imesitwas

Kmers :  
(k=3) the sth ast wor ime  
hea the sth ors mes  
eag heb the rst esi  
age ebe hea sto sit  
geo bes eag tof itw  
eof est age oft twa  
ofw sto geo fti was  
fwi tof eof tim ast



# Three methods to reconstruct the original sequence

The genome is shown in a. One method (shown in b) uses the reads, the two other methods use k-mers derived from the reads (shown in c and d).



**(a) A small circular genome.** In **(b)** reads are represented as nodes in a graph, and edges represent alignments between reads. **Following the edges in numerical order allows one to reconstruct the circular genome by combining alignments between successive reads.** In **(c)** reads are divided into all possible  $k$ -mers ( $k = 3$ ), ATGGCGT comprises ATG, TGG, GGC, GCG and CGT.

Following a Hamiltonian cycle (indicated by red edges) allows one to reconstruct the genome by forming an alignment in which each successive  $k$ -mer (from successive nodes) is shifted by one position. **(d) Modern short-read-based genome assembly algorithms construct a de Bruijn graph by representing all  $k$ -mer prefixes and suffixes as nodes and then drawing edges that represent  $k$ -mers having a particular prefix and suffix.** For example, the  $k$ -mer edge ATG has prefix AT and suffix TG.

Finding an Eulerian path allows one to reconstruct the genome by forming an alignment in which each successive  $k$ -mer (from successive edges) is shifted by one position.



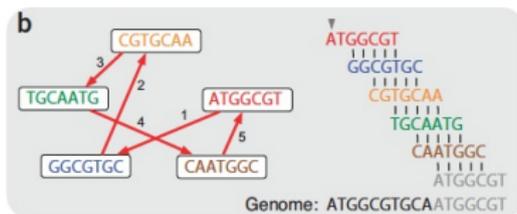


Figure b (see previous slide): **The Hamiltonian graph is a graph in which each read is represented by a node and overlap between reads is represented by an arrow (called a directed edge) joining two reads.** For instance, two nodes representing reads may be connected with a directed edge if the reads overlap by at least five nucleotides. **The Hamiltonian cycle is a path that travels to every node exactly once and ends at the starting node, meaning that each read will be included once in the assembly.**

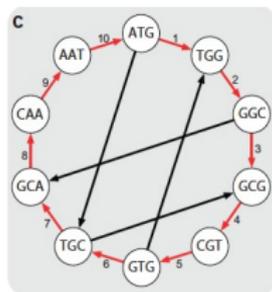


Figure c: The Hamiltonian cycle approach can be generalized to make use of k-mers by constructing a graph as follows. **First**, from a set of reads, make a node for every k-mer appearing as a consecutive substring of one of these reads. **Second**, given a k-mer, define its suffix as the string formed by all its nucleotides except the first one and its prefix as the string formed by all of its nucleotides except the last one. **k-mer to another using a directed edge if the suffix of the former equals the prefix of the latter**, that is, if the two k-mers completely overlap except for one nucleotide at each end. **Third**, look for a Hamiltonian cycle, which represents a candidate genome because it visits each detected k-mer.

Hamilton path is a graph that covers all vertex exactly once. When this path returns to its starting point than this path is called Hamilton cycle.

There is no known efficient algorithm for finding a Hamiltonian cycle in a large graph with millions (let alone billions) of nodes.

The Hamiltonian cycle approach was feasible for sequencing the first microbial genome in 1995 and the human genome in 2001.

**The computational problem of finding a Hamiltonian cycle belongs to the NP-Complete class of problems.**

Next: Euler path is a graph using every edge of the graph exactly once. Euler cycle is a Euler path that returns to it starting point after covering all edges.



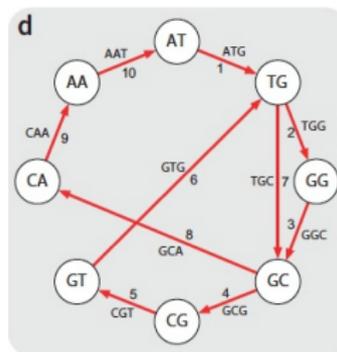


Figure d: **Instead of assigning each k-mer contained in some read to a node, we will now assign each such k-mer to an edge.** This allows the construction of a **de Bruijn** graph. First, form a node for every distinct prefix or suffix of a k-mer. Then, connect node x to node y with a directed edge if some k-mer has prefix x and suffix y, and label the edge with this k-mer.

We visit all edges of the de Bruijn graph, which represents all possible  $k$ -mers; traveling will result in spelling out a candidate genome; for each edge that is traversed, one records the first nucleotide of the  $k$ -mer assigned to that edge. Euler considered graphs for which there exists a path between every two nodes (called connected graphs). **He proved that a connected graph with undirected edges contains an Eulerian cycle exactly when every node in the graph has an even number of edges touching it.** The case of directed graphs (that is, graphs with directed edges) is similar. **For any node in a directed graph, define its indegree as the number of edges leading into it and its outdegree as the number of edges leaving it. A graph in which indegrees are equal to outdegrees for all nodes is called balanced.**

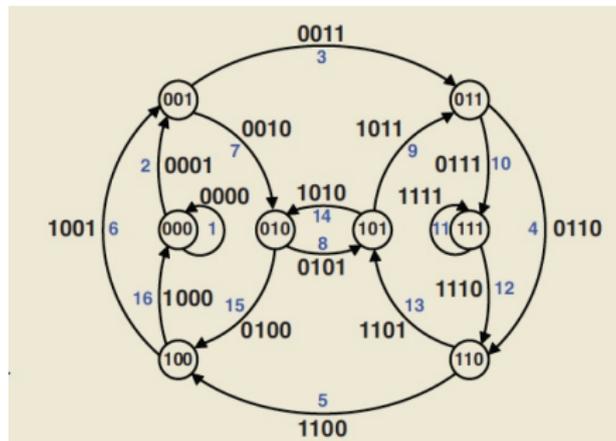


**Eulers theorem states that a connected directed graph has an Eulerian cycle if and only if it is balanced.** In particular, Eulers theorem implies that our de Bruijn graph contains an Eulerian cycle as long as we have located all  $k$ -mers present in the genome. Indeed, in this case, for any node, both its indegree and outdegree represent the number of times the  $k - 1$ -mer assigned to that node occurs in the genome. To see why Eulers theorem must be true, first note that a graph that contains an Eulerian cycle is balanced because every time we traverse an Eulerian cycle and we need to pass through a particular vertex, we enter on one edge of the cycle and exit on the next edge. **This pairs up all the edges touching each vertex, showing that half the edges touching the vertex lead into it and half lead out from it.** It is a bit harder to see the converse: that every connected balanced graph contains an Eulerian cycle.



## De Bruijn graph: representing the data as a graph

A De Bruijn graph for  $k = 4$  and a two character alphabet composed of the digits 0 and 1. This graph has an Eulerian cycle because each node has indegree and outdegree equal to 2. Following the blue numbered edges in order from 1 to 16 traces an Eulerian cycle 0000, 0001, 0011, 0110, 1100, 1001, 0010, 0101, 1011, 0111, 1111, 1110, 1101, 1010, 0100, 1000. Recording the first character of each edge label spells the cyclic superstring 0000110010111101.



The time required to run a computer implementation of Euler algorithm is roughly proportional to the number of edges in the de Bruijn graph. In the Hamiltonian approach, the time is potentially a lot larger, because of the large number of pairwise alignments needed to construct the graph and the NP-Completeness of finding a Hamiltonian cycle.



Sequencing is cheap, we generate sub-strings (reads) at random from throughout the genome. In next generation sequencing we have 10s of millions of reads. The difficult part is how we put them back together again in the right order. An intuitive way to do this may be in all versus all comparisons to search for overlaps. This is how traditional assemblers work. The solution offered by the De Bruijn approach is to represent the data as a graph.

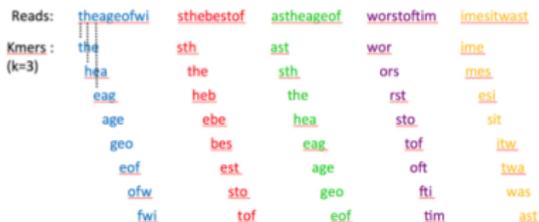
The first step of the De Bruijn assembler is to deconstruct the sequencing reads into its constitutive k-mers. As specified before a K-mer is a substring of defined length. **If we split reads in k-mers we control the size and the overlapping.** To Kmerize the dataset, we move through our read in one letter increments from the beginning to the end until we have recorded all possible 3 letter words. We then do this for all reads in the dataset. From this point on the algorithm operates on k-mers rather than on the reads.



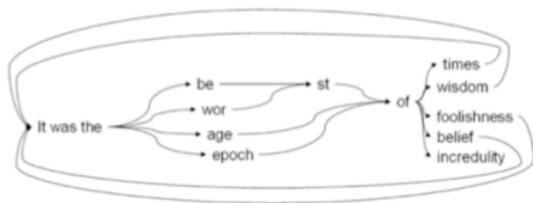
The next stage is to represent the stored  $k$ -mers in the De Bruijn graph. This is done by searching for overlaps of  $k - 1$ . The graph has all consecutive  $k$ -mers by  $k - 1$  bases. Note that: 1) Adding  $k$ -mers from a second read of an overlapping region of the genome shows how the graph can be extended. It also reveals the redundancy in the data which need not be stored by the computer. This is how memory efficiency is achieved. 2) Adding a  $k$ -mers from a third read that comes from a similar but non-overlapping part of the genome illustrates the effect of repeats, i.e. we get a branch in the graph. **Long unbranched stretches represent unique sequence in the genome, branches and loops are the result of repeats.**



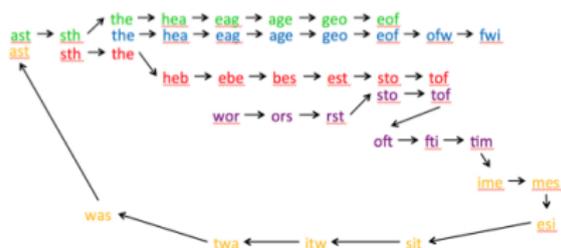
# Details of the De Bruijn graph method



A



C



B

The final assembly (k=3)

wor times itwasthe foolishness st wisdom  
incredibility age epoch be of belief



Repeat with a longer "kmer" length

A better assembly (k=10)

itwasthebestoftimesitwastheworstoftimesitwastheageofwisdomitwastheageoffools...

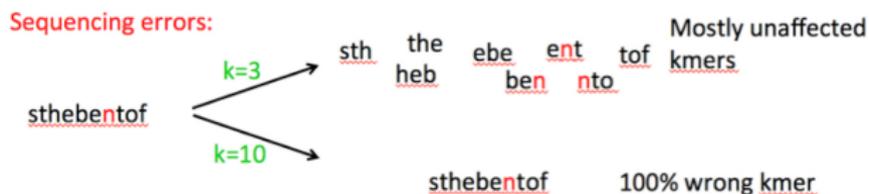
D

A, Kmerize the data; B, Build the graph; C, simplify the graph; D, get the final assembly.



The final step is to remove redundancy, result in the final De Bruijn Graph representation of the genome under study. Strengths and weaknesses of this approach: 1) **a strength is that the information from millions of reads is stored in computer memory in a graph that is proportional to the genome size.** Another strength is that the overlaps between reads are implicit in the graph, so all the millions versus millions of comparisons are not required. **On the downside, information is lost as repetitive sequences are collapsed into a single representation.** While this may be a satisfying solution to a computer scientist, it is not practically useful to a biologist who wants to annotate repeats (repeats are often not junk DNA).





De Bruijn method can only resolve  $k$  long repeat. Validation: look in your assembly for gene that should be there; N50: Weighted median such as 50% of your assembly is contained in contig of length  $\geq N50$

Given a set of contigs, each with its own length, the N50 length is defined as the length for which the collection of all contigs of that length or longer contains at least half of the sum of the lengths of all contigs, and for which the collection of all contigs of that length or shorter also contains at least half of the sum of the lengths of all contigs.

Software implementation:

**Velvet**: <http://www.ebi.ac.uk/zerbino/velvet/>;

**ABYSS**: <http://www.bcgsc.ca/platform/bioinfo/software/abyss/>;

**SOAP-denovo**: <http://soap.genomics.org.cn/soapdenovo.html>;

**ALLPATH-LG**: <http://www.broadinstitute.org/software/allpaths-lg/blog/>;

**IDBA-UD**: [http://i.cs.hku.hk/alse/hkubrg/projects/idba\\_ud](http://i.cs.hku.hk/alse/hkubrg/projects/idba_ud)



- Li, H and Durbin, R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25:1754-60.
- Compeau P, Pevzner P and Tesler G. How to apply de Bruijn graphs to genome assembly. *Nature Biotechnology* 29: 987 2011
- <http://www.homolog.us/Tutorials/index.php?p=2.1&s=1>
- <https://www.youtube.com/watch?v=4n7NPk5lwbl>
- Schatz, M., Delcher, A. and Salzberg, S. *Genome Res.* 20, 11651173 (2010).



Phylogeny - parsimony-based - (Fitch, Sankoff). Phylogeny - distance based - (UPGMA, Neighbor Joining) Bootstrap.

We reconstruct evolution (phylogeny) by studying the relationships among multiple sequences.

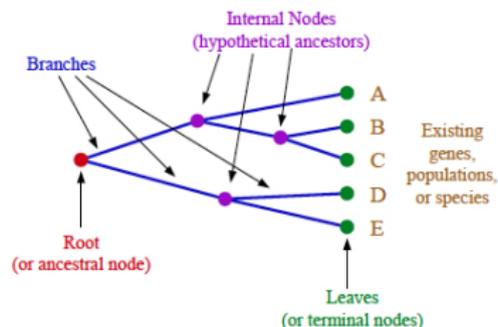
The input is a multiple alignment, the output is a tree. There are several biological applications but also applications in computer science.

Main question in this lecture: how related are the sequences I am observing?



## Phylogenetic analysis

The reconstruction of the evolutionary history of species formation could be done by comparing DNA and amino acid sequences. A phylogeny is a tree where the leaves are existing species; an internal node is node with degree greater than one. Internal nodes represent common ancestors. We typically do not have DNA data for internal nodes (except fossil). Here we use the terms species and taxa in a synonymous way. We compute the tree for each column of a multiple alignment.

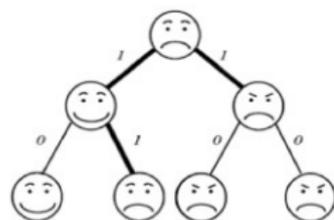


**Figure :** tree representation:  $((a, (b, c)), (d, e))$ ; trees could also be unrooted

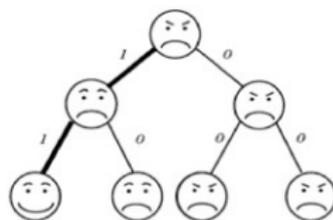


## Phylogeny using parsimony (= economy of mutations)

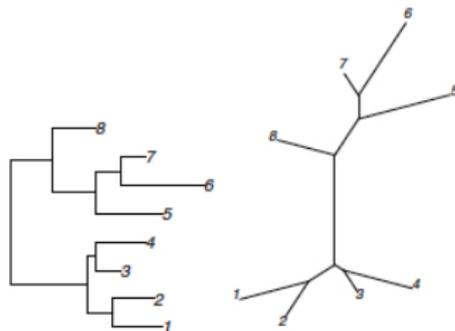
Biological aims: from sequence alignment to phylogeny (a tree) by minimising the number of changes (mutations, see figure below from [www.bioalgorithms.info](http://www.bioalgorithms.info)). Parsimony means economy; there are two main algorithms (developed by Fitch and Sankoff); the output trees are rooted (below the difference between rooted, left, and unrooted trees, right).



(a) Parsimony Score=3



(b) Parsimony Score=2



# Fitch parsimony model for DNA sequences

## Fitch downpass algorithm

Bottom-up phase: Determine set of possible states for each internal node; top-down phase: Pick states for each internal node. If the descendant state sets  $S_q$  and  $S_r$  overlap, then the state set of node p will include the states present in the intersection of  $S_q$  and  $S_r$ . If the descendant state sets do not overlap, then the state set of p will include all states that are the union of  $S_q$  and  $S_r$ . States that are absent from both descendants will never be present in the state set of p.

- 1  $S_p \leftarrow S_q \cap S_r$
- 2 if  $S_p = 0$  then
- 3  $S_p \leftarrow S_q \cup S_r$
- 4  $l \leftarrow l + 1$
- 5 end if

Initialization:  $R_i = [s_i]$ ; Do a post-order (from leaves to root) traversal of tree Determine  $R_i$  of internal node i with children j, k:

$$R_i = \begin{cases} R_j \cap R_k & \text{if } R_j \cap R_k \neq 0 \\ R_j \cup R_k & \text{otherwise} \end{cases}$$



# Fitch parsimony model for DNA sequences

## Fitch uppass algorithm

Assume that we have the final state set  $F_a$  of node a, which is the immediate ancestor of node p ( $S_p$ ) that has two children q ( $S_q$ ) and r ( $S_r$ ).

- 1  $F_p \leftarrow S_p \cap F_a$
- 2 if  $F_p \neq F_a$  then
- 3 if  $S_q \cap S_r \neq \emptyset$  then
- 4  $F_p \leftarrow ((S_q \cup S_r) \cap F_a) \cup S_p$
- 5 else
- 6  $F_p \leftarrow S_p \cup F_a$
- 7 end if
- 8 end if

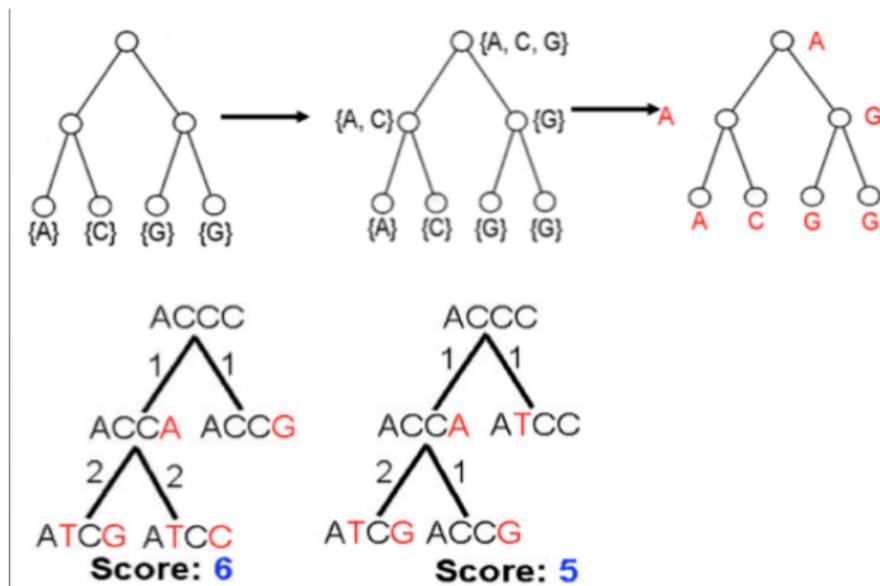
$$R_i(s) = \begin{cases} 0 & \text{if } s_i = s \\ \infty & \text{otherwise} \end{cases}$$

$$R_i(s) = \min_{s'} \{R_j(s') + S(s', s)\} + \min_{s'} \{R_k(s') + S(s', s)\}$$

If the downpass state set of p includes all of the states in the final set of a, then each optimal assignment of final state to a can be combined with the same state at p to give zero changes on the branch between a and p and the minimal number of changes in the subtree rooted at p. If the final set of a includes states that are not present in the downpass set of p, then there is a change on the branch between a and p.



## Fitch algorithm, details



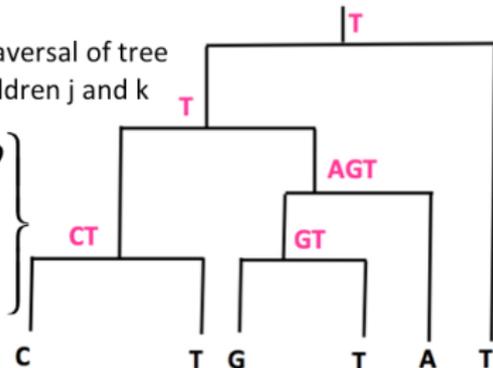
The figure in the top shows the Fitch two-step procedure. The tree is the hypothesis you are testing and you choose the tree that minimises the score. Bottom figure: you can sum the score for all the column of the alignment for each candidate tree and then you select the best tree. Choosing the candidate trees: there are algorithms for exploring the tree space.



## Example of Fitch's algorithm

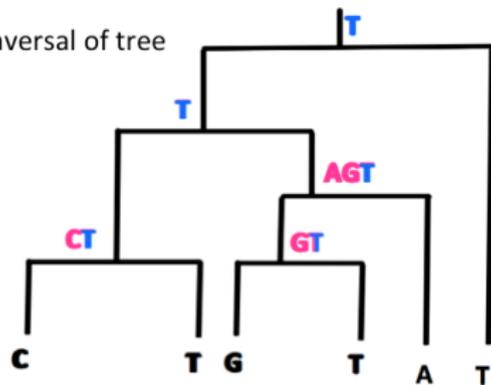
FITCH STEP1: Do a post-order (from leaves to root) traversal of tree  
Find out possible states  $R_i$  of internal node  $i$  with children  $j$  and  $k$

$$R_i = \begin{cases} R_j \cup R_k & \text{if } R_j \cap R_k = \phi \\ R_j \cap R_k & \text{otherwise} \end{cases}$$



FITCH STEP2: Do a pre-order (from root to leaves) traversal of tree  
Select state  $r_j$  of internal node  $j$  with parent  $i$

$$r_j = \begin{cases} r_i & \text{if } r_i \in R_j \\ \text{arbitrary state} \in R_j & \text{otherwise} \end{cases}$$



# Sankoff general parsimony: each mutation costs differently

## Sankoff downpass algorithm

- 1 for all i do
- 2  $h_i^{(q)} \leftarrow \min_j (c_{ij} + g_j^{(q)})$
- 3  $h_i^{(r)} \leftarrow \min_j (c_{ij} + g_j^{(r)})$
- 4 end for
- 5 for all i do
- 6  $g_i^{(p)} \leftarrow h_i^{(q)} + h_i^{(r)}$
- 7 end for

Sankoff parsimony is based on a cost matrix  $C = c_{ij}$ , the elements of which define the cost  $c_{ij}$  of moving from a state  $i$  to a state  $j$  along any branch in the tree. The cost matrix is used to find the minimum cost of a tree and the set of optimal states at the interior nodes of the tree.



- 1  $F_p \leftarrow 0$
- 2 for all  $i$  in  $F_a$  do
- 3  $m \leftarrow c_{i1} + g_1^{(p)}$
- 4 for all  $j \neq 1$  do
- 5  $m \leftarrow \min(c_{ij} + g_j^{(p)}, m)$
- 6 end for
- 7 for all  $j$  do
- 8 if  $c_{ij} + g_j^{(p)} = m$  then
- 9  $F_p \leftarrow F_p \cup j$
- 10 end if
- 11 end for
- 12 end for

- 1 for all  $j$  do
- 2  $f_j^{(p)} \leftarrow \min_i(f_i^{(a)} - h_i^{(p)} + c_{ij})$
- 3 end for

Complexity: if we want to calculate the overall length (cost) of a tree with  $m$  taxa,  $n$  characters, and  $k$  states, it is relatively easy to see that the Fitch algorithms has complexity  $O(mnk)$  and the Sankoff algorithm is of complexity  $O(mnk^2)$ .



# Example of Sankoff's algorithm

Let  $c_{ij}$  the matrix cost (on the right) and  $S_v(\mathbf{k})$  be the smallest number of steps needed to evolve the subtree at or above node  $v$ , given that node  $v$  is in state  $\mathbf{k}$ .

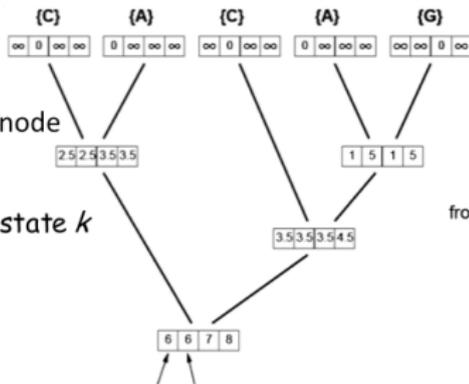
$$S_v(k) = \begin{cases} 0 & \text{if node } v \text{ has (or could have) state } k \\ +\infty & \text{otherwise} \end{cases}$$

If  $v$  is a leaf (tip)

If  $v$  is a node with descendants  $u$  and  $w$

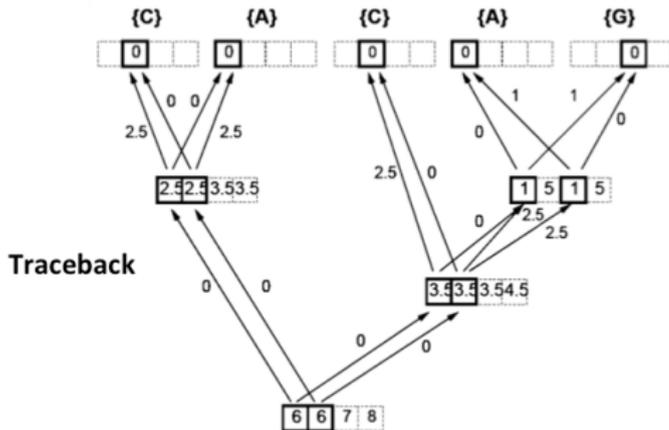
The minimum number of (weighted) steps is

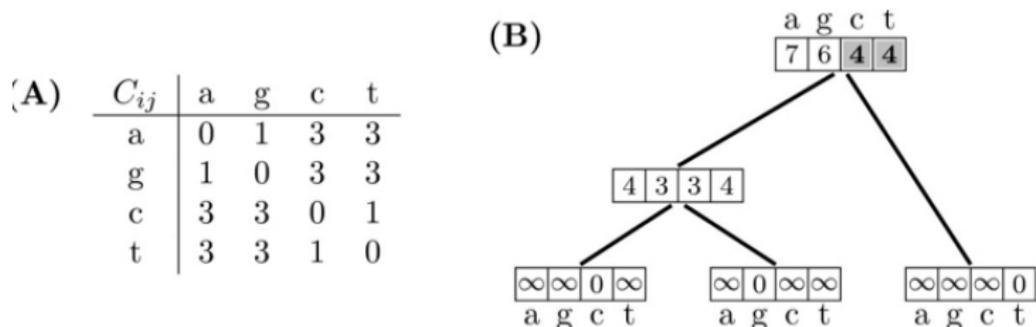
$$S^* = \min_k S_{root}(k)$$



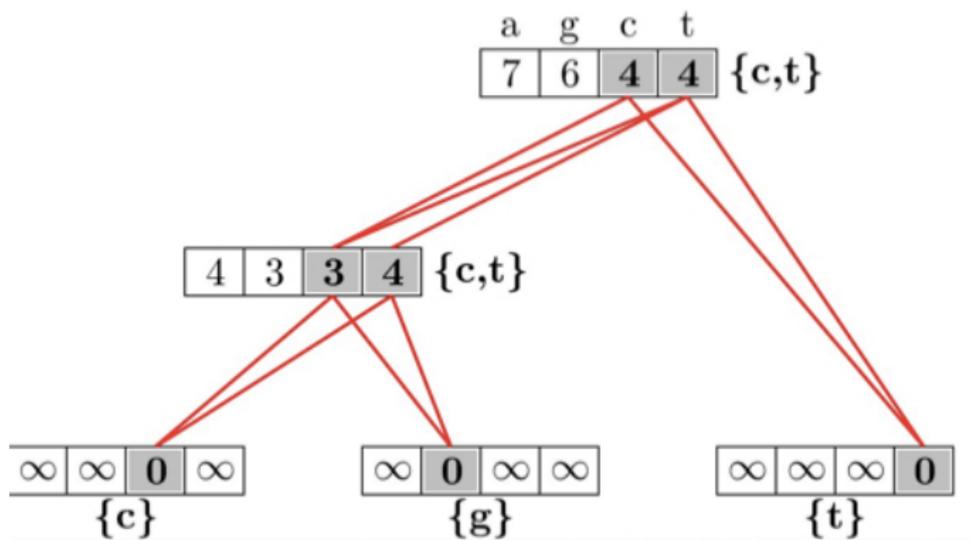
cost matrix:

from \ to	A	C	G	T
A	0	2.5	1	2.5
C	2.5	0	2.5	1
G	1	2.5	0	2.5
T	2.5	1	2.5	0





**Figure :** If the leaf has the character in question, the score is 0; else, score is  $\infty$ . Each mutation  $a \rightarrow b$  costs the same in Fitch and differently in Sankoff parsimony algorithm (weighted matrix in A). An example of a weighted matrix for Sankoff (for proteins) is the Blossum, presented before in this course



**Figure :** Example of Sankoff algorithm. Note that in the parsimony approaches (Fitch and Sankoff) the tree (i.e. the topology and leaves order) is the hypothesis you are testing. So you try different trees and select the one that is most parsimonious for each column of the alignment, then you select the tree that is the most representative.

## Phylogeny based on a matrix of distances

Distance methods convert the changes counted in each column of the alignment, top figure, into a single distance matrix, bottom figure (dissimilarity matrix= 1 - similarity) to construct a tree and are kin to clustering methods. We can use the same matrix we use for Blast search, for example the Blosum matrix or others. The UPGMA outputs a rooted tree while the neighbour joining outputs an unrooted tree.

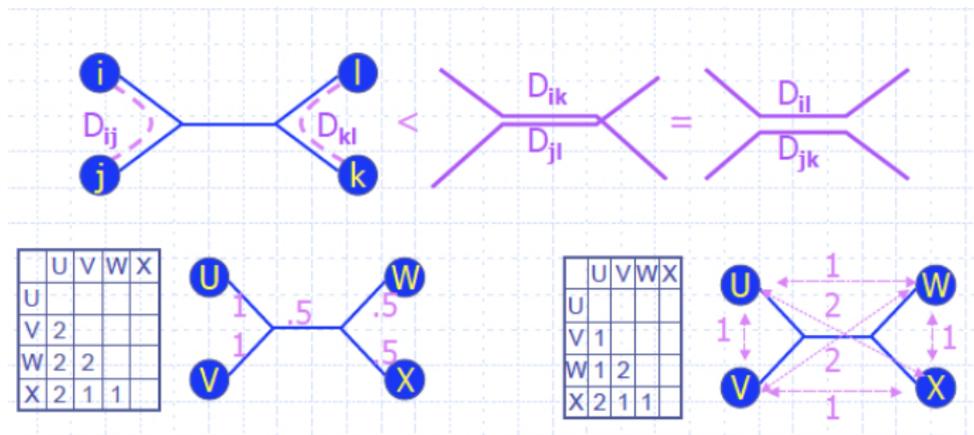
Species	Characters
A	ACTGTTCGTTCTGA
B	ACCGTTCCTTCTAG
C	CCTGTTGCTTCTGA
D	ACTGTCCTTCTAG

	A	B	C	D
A	–	0.75	0.35	0.27
B	0.75	–	0.85	0.33
C	0.35	0.85	–	0.31
D	0.27	0.33	0.31	–



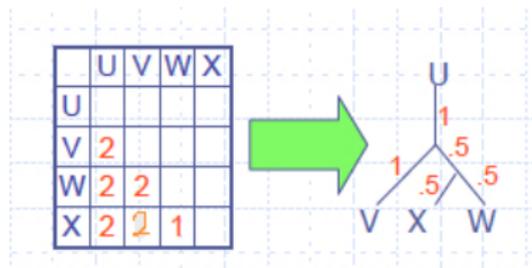
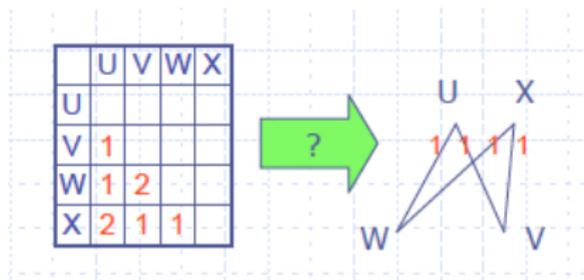
## Additivity: a distance matrix could be converted into a tree

A matrix  $D$  is additive if for every four indices  $i, j, k, l$  we can write the following:  
 $D_{ij} + D_{kl} \leq D_{ik} + D_{jl} = D_{il} + D_{jk}$ . If the distance matrix is not additive we could find the tree which best fits the distance matrix.



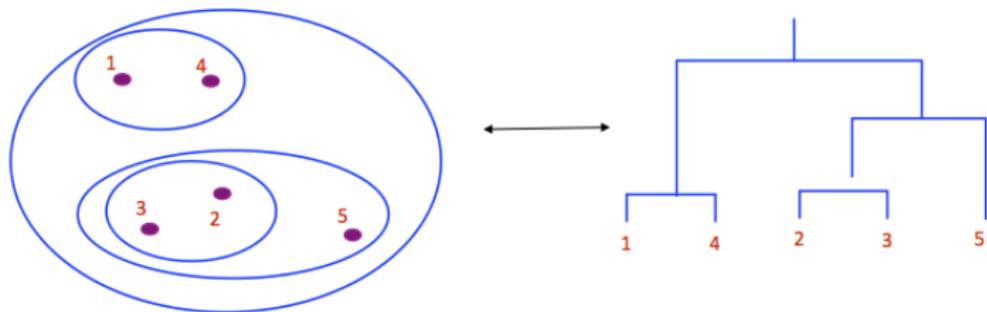
## The additivity property

Top: distance matrix does not turn into a tree; Bottom: the distance matrix turns into a tree.



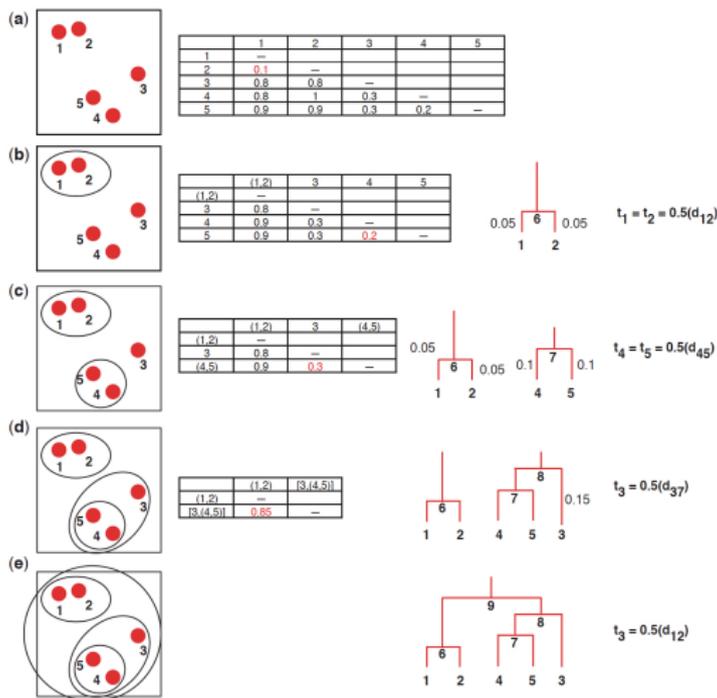
## UPGMA: Unweighted Pair Group Method with Arithmetic Mean

UPGMA is a sequential clustering algorithm that computes the distance between clusters using average pairwise distance and assigns a height to every vertex in the tree, effectively assuming the presence of a molecular clock and dating every vertex. The algorithm produces an ultrametric tree : the distance from the root to any leaf is the same (this corresponds to a constant molecular clock: the same proportion of mutations in any pathway root to leaf). Input is a distance matrix of distances between species; the iteration combines the two closest species until we reach a single cluster.



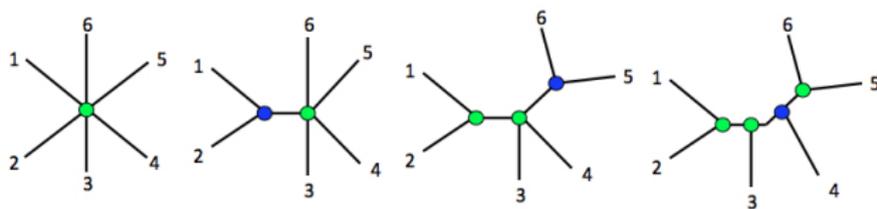
- 1 Initialization: Assign each species to its own cluster  $C_i$
- 2 Each such cluster is a tree leaf
- 3 Iteration:
- 4 Determine  $i$  and  $j$  so that  $d(C_i, C_j)$  is minimal
- 5 Define a new cluster  $C_k = C_i \cup C_j$  with a corresponding node at height  $d(C_i, C_j)/2$
- 6 Update distances to  $C_k$  using weighted average
- 7 Remove  $C_i$  and  $C_j$
- 8 Termination: stop when just a single cluster remains





In UPGMA when choosing the closest pair, we do not take into account the distance from all the other nodes (as we do in Neighbor Joining).





**Figure :** NJ starts with a star topology (i.e. no neighbors have been joined) and then uses the smallest distance in the distance matrix to find the next two pairs to move out of the multifurcation then recalculate the distance matrix that now contains a tip less.

- 1 Identify  $i, j$  as neighbor if their distance is the shortest.
- 2 Combine  $i, j$  into a new node  $u$ .
- 3 Update the distance matrix.
- 4 Distance of  $u$  from the rest of the tree is calculated
- 5 If only 3 nodes are left finish.

The distance between any taxon (=species) pair  $i$  and  $j$  is denoted as  $d(i, j)$  and can be obtained from the alignment. NJ iteratively selects a taxon pair, builds a new subtree, and agglomerates the pair of selected taxa to reduce the taxon set by one. Pair selection is based on choosing the pair  $i, j$  that minimizes the following  $Q$  (matrix) criterion:

$$Q(i, j) = (r - 2)d(i, j) - \sum_{k=1}^r d(i, k) - \sum_{k=1}^r d(j, k)$$

where  $r$  is the current number of taxa and the sums run on the taxon set. Let  $f, g$  be the selected pair. NJ estimates the length of the branch  $(f, u)$  using

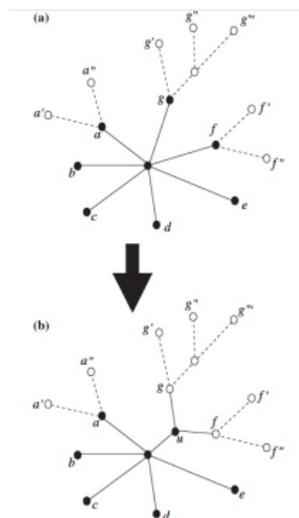
$$d(f, u) = \frac{1}{2}d(f, g) + \frac{1}{2(r-2)}[\sum_{k=1}^r d(f, k) - \sum_{k=1}^r d(g, k)]$$

and  $d(g, u)$  is obtained by symmetry. Finally, NJ replaces  $f$  and  $g$  by  $u$  in the distance matrix, using the reduction formula:

$$d(u, k) = \frac{1}{2}[d(f, k) - d(f, u)] + \frac{1}{2}[d(g, k) - d(g, u)]$$

NJ still reconstructs the correct tree when the distance matrix is perturbed by small noise and that NJ is optimal regarding tolerable noise amplitude.





One NJ agglomeration step. In the current tree (a), the taxon set contains a, b, c, d, e, f, and g; some are original taxa, whereas the others (i.e., a, f, and g) correspond to subtrees built during the previous steps. Tree (b): after selection of the (f, g) pair, a new subtree is built, and both f and g are replaced by a unique taxon denoted as u. NJ terminates when the central node is fully resolved. Neighbor joining on a set of  $r$  taxa requires  $r-3$  iterations. At each step one has to build and search a Q matrix. Initially the Q matrix is size  $r^2$ , then the next step it is  $(r-1)^2$ , etc. This leads to a time complexity of  $O(r^3)$ .



# NJ: Example

Distance matrix

	A	B	C	D	E
B	5				
C	4	7			
D	7	10	7		
E	6	9	6	5	
F	8	11	8	9	8

	U <sub>1</sub>	C	D	E
C	3			
D	6	7		
E	5	6	5	
F	7	8	9	8

	U <sub>1</sub>	C	U <sub>2</sub>
C	3		
U <sub>2</sub>	3	4	
F	7	8	6

	U <sub>2</sub>	U <sub>3</sub>
U <sub>2</sub>	2	
F	6	6

	U <sub>4</sub>
F	5

## Step 1

S calculations

$$S_A = (5+4+7+6+8)/4 = 7.5$$

$$S_B = (5+7+10+9+11)/4 = 10.5$$

$$S_C = (4+7+7+6+8)/4 = 8$$

$$S_D = (7+10+7+5+9)/4 = 9.5$$

$$S_E = (6+9+6+5+8)/4 = 8.5$$

$$S_F = (8+11+8+9+8)/4 = 11$$

$$S_{U_1} = (3+6+5+7)/3 = 7$$

$$S_C = (3+7+6+8)/3 = 8$$

$$S_D = (6+7+5+9)/3 = 9$$

$$S_E = (5+6+5+8)/3 = 8$$

$$S_F = (7+8+9+8)/3 = 10.6$$

$$S_{U_1} = (3+3+7)/2 = 6.5$$

$$S_C = (3+4+8)/2 = 7.5$$

$$S_{U_2} = (3+4+6)/2 = 6.5$$

$$S_F = (7+8+6)/2 = 10.5$$

$$S_{U_2} = (2+6)/1 = 8$$

$$S_{U_3} = (2+6)/1 = 8$$

$$S_F = (6+6)/1 = 12$$

Because  $N - 2 = 0$ , we cannot do this calculation.

## Step 2

Calculate pair with smallest  $M_{ij}$ , where  $M_{ij} = D_{ij} - S_i - S_j$ .

Smallest are

$$M_{AB} = 5 - 7.5 - 10.5 = -13$$

$$M_{BE} = 5 - 9.5 - 8.5 = -13$$

Choose one of these (AB here).

Smallest is

$$M_{CU_1} = 3 - 7 - 8 = -12$$

$$M_{DE} = 5 - 9 - 8 = -12$$

Choose one of these (DE here).

Smallest is

$$M_{CU_1} = 3 - 6.5 - 7.5 = -11$$

Smallest is

$$M_{U_2F} = 6 - 8 - 12 = -14$$

$$M_{U_3F} = 6 - 8 - 12 = -14$$

$$M_{U_2U_3} = 2 - 8 - 8 = -14$$

Choose one of these ( $M_{U_2U_3}$  here).

## Step 3

Create a node (U) that joins pair with lowest  $M_{ij}$  such that  $S_U = D_{ij}/2 + (S_i + S_j)/2$ .

U<sub>1</sub> joins A and B:

$$S_{AU_1} = D_{AB}/2 + (S_A + S_B)/2 = 1$$

$$S_{BU_1} = D_{AB}/2 + (S_B + S_A)/2 = 4$$

U<sub>2</sub> joins D and E:

$$S_{DU_2} = D_{DE}/2 + (S_D + S_E)/2 = 3$$

$$S_{EU_2} = D_{DE}/2 + (S_E + S_D)/2 = 2$$

U<sub>1</sub> joins C and U<sub>1</sub>:

$$S_{CU_1} = D_{CU_1}/2 + (S_C + S_{U_1})/2 = 2$$

$$S_{U_1U_1} = D_{CU_1}/2 + (S_{U_1} + S_C)/2 = 1$$

U<sub>3</sub> joins U<sub>2</sub> and U<sub>2</sub>:

$$S_{U_2U_4} = D_{U_2U_2}/2 + (S_{U_2} + S_{U_2})/2 = 1$$

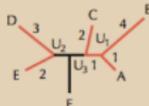
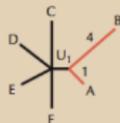
$$S_{U_3U_4} = D_{U_2U_2}/2 + (S_{U_3} + S_{U_2})/2 = 1$$

length = 5.

For last pair, connect U<sub>4</sub> and F with branch length = 5.

## Step 4

Join  $i$  and  $j$  according to S above and make all other taxa in form of a star. Branches in black are of unknown length. Branches in red are of known length.



## Step 5

Calculate new distance matrix of all other taxa to U with  $D_{iU} = D_{iA} + D_{iB} - D_{AB}$ , where  $i$  and  $j$  are those selected from above.

## Comments

Note this is the same tree we started with (drawn in unrooted form here).

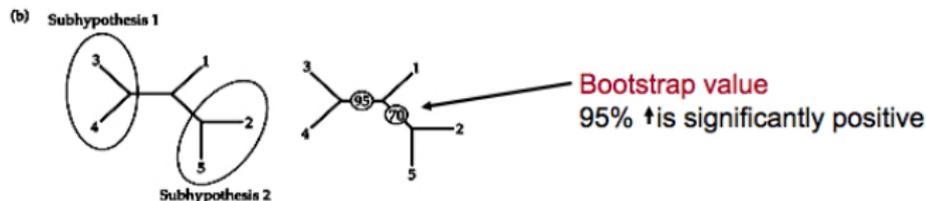
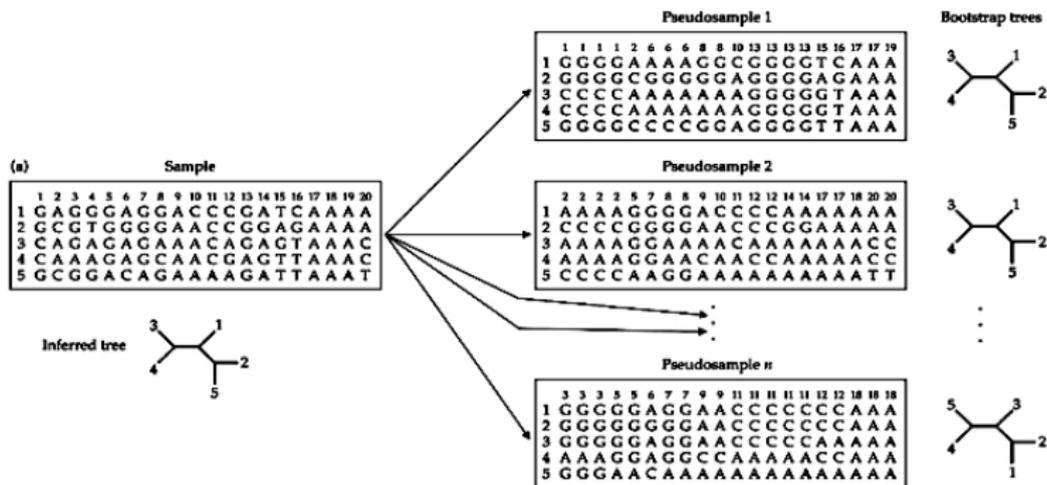


If there are  $m$  sequences, each with  $n$  nucleotides, a phylogenetic tree can be reconstructed using some tree building methods.

- 1 From each sequence,  $n$  nucleotides are randomly chosen with replacements, giving rise to  $m$  rows of  $n$  columns each. These now constitute a new set of sequences.
- 2 A tree is then reconstructed with these new sequences using the same tree building method as before.
- 3 Next the topology of this tree is compared to that of the original tree. Each interior branch of the original tree that is different from the bootstrap tree is given a score of 0; all other interior branches are given the value 1.
- 4 This procedure of resampling the sites and tree reconstruction is repeated several hundred times, and the percentage of times each interior branch is given a value of 1 is noted. This is known as the bootstrap value. As a general rule, if the bootstrap value for a given interior branch is 95% or higher, then the topology at that branch is considered "correct".



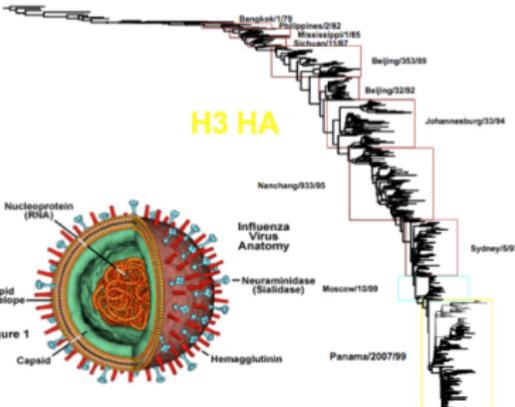
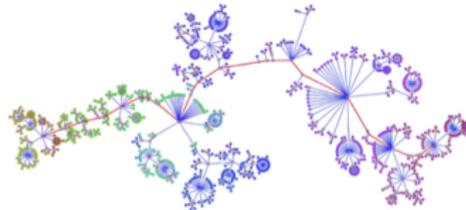
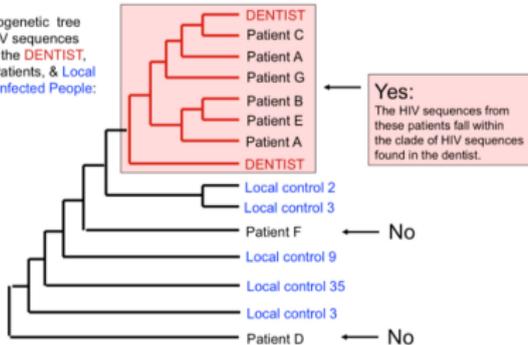
# How good is this tree? bootstrap algorithm

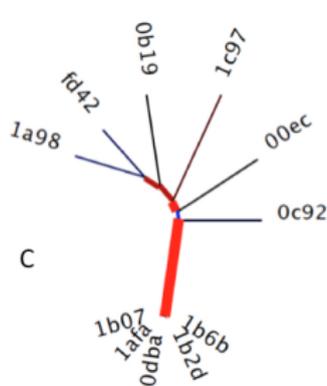
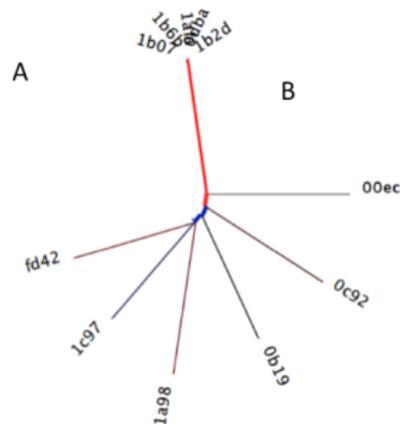
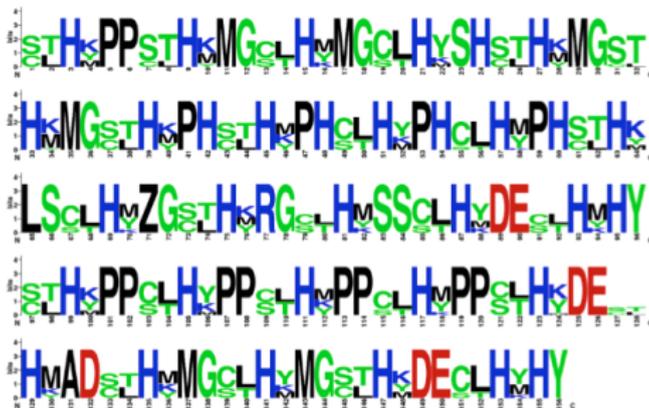


# Biological examples

## Did the *Florida Dentist* infect his patients with HIV?

Phylogenetic tree of HIV sequences from the **DENTIST**, his Patients, & Local HIV-infected People:





Distance algorithm in computer science

A) A sequence logo for the FakeAV-DO function "F1". Positions with large characters indicate invariant parts of the function; positions with small characters vary due to code metamorphism

B) A neighbour joining tree of FakeAV-DO set of procedures F1.

C) Neighbor joining tree of FakeAV-DO set of procedures F2 from the same samples of B.

(W.M. Khoo Unity in diversity: Phylogenetic-inspired techniques for reverse engineering and detection of malware families)



- UPGMA: <http://www.southampton.ac.uk/re1u06/teaching/upgma/>
- Gascuel O. and Steel M. Neighbor-Joining Revealed Molecular Biology and Evolution 2006 - <http://mbe.oxfordjournals.org/content/23/11/1997.full.pdf>
- Atteson K. 1999. The performance of the neighbor-joining methods of phylogenetic reconstruction. *Algorithmica* 25:25178.
- <http://www.cs.princeton.edu/mona/Lecture/phylogeny.pdf>



Algorithms for Clustering biological data (K-means, Markov Clustering)

Input: a table of gene activity measurements (usually an excel file) which is estimated by measuring the amount of mRNA for a set of genes

More mRNA usually indicates more gene activity.

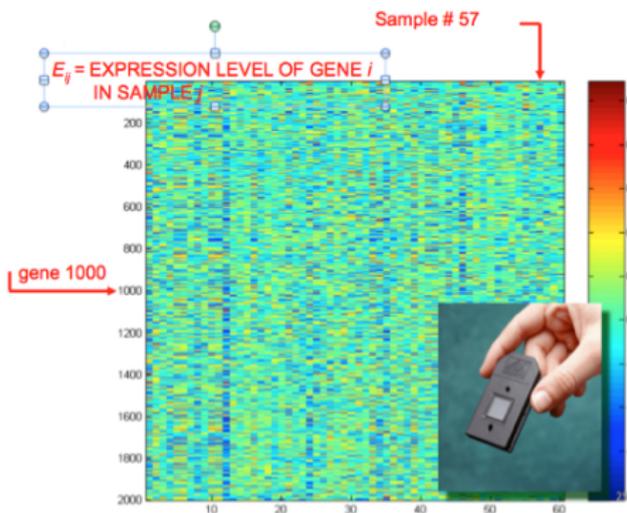
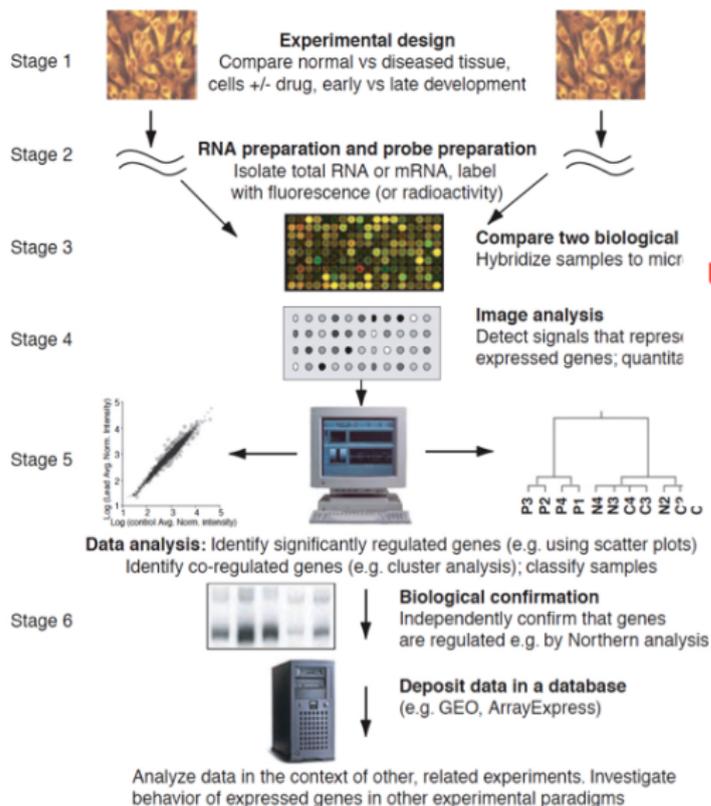
Technology: Microarray (gene chips).

Output: The clustering analysis allows scientists to identify the genes that change in disease with respect to control.

Main question: How many groups of sequences are there?



# The Biological problem



From P. Pevzner

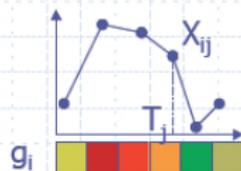
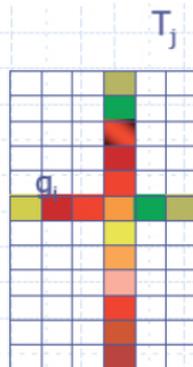
There are two typical experiments:

## ■ Differentiation

- Compare expression levels under different conditions
- A test  $T_j$  represents expression levels of a condition
- E.g., cancer or drug-treated cell vs. normal cell

## ■ Temporal expression

- Explore temporal evolution of expression levels
- A test  $T_j$  represents expression levels at a given time
- E.g., study cell response to heat-shock, starvation



Gene expression profile

**Figure :** The color of the spot indicates activation with respect to control (red) or repression with respect to the control (green) or absence of regulation (yellow) of a gene, or error in the technological process (black). The sample can be all the genes of an organism (example the 6000 genes of yeast), or a selection of genes of interest (+ control genes).

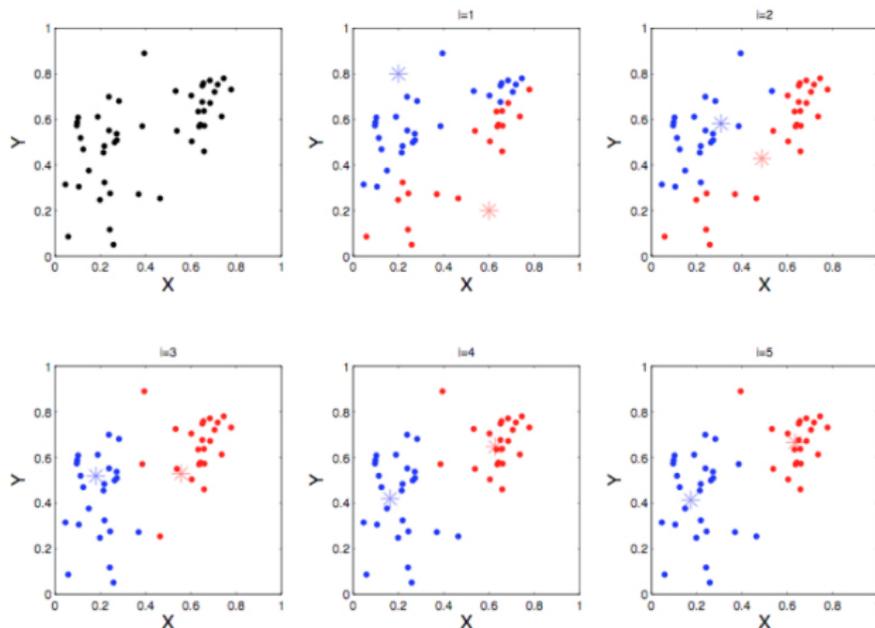
- 1 Arbitrarily assign the  $k$  cluster centers
- 2 while the cluster centers keep changing
- 3 Assign each data point to the cluster  $C_i$  corresponding to the closest cluster representative (center) ( $1 \leq i \leq k$ )
- 4 After the assignment of all data points, compute new cluster representatives according to the center of gravity of each cluster, that is, the new cluster representative is  $\frac{\sum_{v \in C} v}{|C|}$  for all  $v$  in  $C$  for every cluster  $C$ .



- 1 Select an arbitrary partition  $P$  into  $k$  clusters
- 2 while forever
- 3  $\text{bestChange} \leftarrow 0$
- 4 for every cluster  $C$
- 5 for every element  $i$  not in  $C$
- 6 if moving  $i$  to cluster  $C$  reduces its clustering cost
- 7 if  $\text{cost}(P) - \text{cost}(P_{i \rightarrow C}) > \text{bestChange}$
- 8  $\text{bestChange} \leftarrow \text{cost}(P) - \text{cost}(P_{i \rightarrow C})$
- 9  $i' \leftarrow i$
- 10  $C' \leftarrow C$
- 11 if  $\text{bestChange} > 0$
- 12 Change partition  $P$  by moving  $i'$  to  $C'$
- 13 else
- 14 return  $P$



# Progressive greedy K-means Algorithm

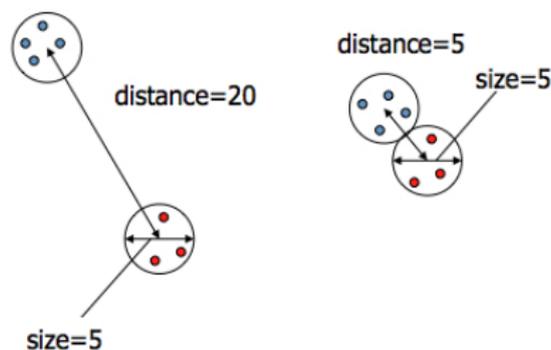


**Figure :** K-means progression from left to right and top to bottom; stars are center points (the centers of the cluster).



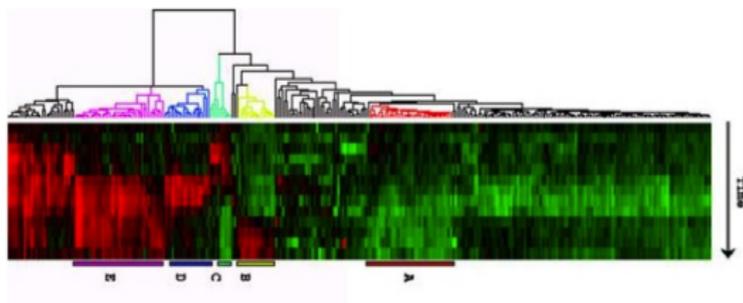
## Progressive greedy K-means Algorithm

The quality of the cluster results could be assessed by ratio of the distance to nearest cluster and cluster diameter. A cluster can be formed even when there is no similarity between clustered patterns. This occurs because the algorithm forces  $k$  clusters to be created. Linear relationship with the number of data points; the complexity is  $O(nKI)$  where  $n$  = number of points,  $K$  = number of clusters,  $I$  = number of iterations.



## Results of clustering on microarray data

The aim is clustering gene expression data: it is easy to interpret the data if they are partitioned into clusters combining similar data points.



**Figure :** Clustering analysis obtained using Hierarchical clustering (UPGMA). The clusters are coloured differently.



Unlike most clustering algorithms, the MCL ([micans.org/mcl](http://micans.org/mcl)) does not require the number of expected clusters to be specified beforehand. The basic idea underlying the algorithm is that dense clusters correspond to regions with a larger number of paths.

ANALOGY: We take a random walk on the graph described by the similarity matrix, but after each step we weaken the links between distant nodes and strengthen the links between nearby nodes. A random walk has a higher probability to stay inside the cluster than to leave it soon. The crucial point lies in boosting this effect by an iterative alternation of expansion and inflation steps.

An **inflation parameter** is responsible for both strengthening and weakening of current.

(Strengthens strong currents, and weakens already weak currents). An **expansion parameter**,  $r$ , controls the extent of this strengthening / weakening. In the end, this influences the granularity of clusters.





- 1 Input is an un-directed graph, with power parameter  $e$  (usually =2), and inflation parameter  $r$  (usually =2).
- 2 Create the associated adjacency matrix
- 3 Normalize the matrix;  $M'_{pq} = \frac{M_{pq}}{\sum_i M_{iq}}$
- 4 Expand by taking the  $e$ -th power of the matrix; for example, if  $e = 2$  just multiply the matrix by itself.
- 5 Inflate by taking inflation of the resulting matrix with parameter  $r$  :  $M_{pq} = \frac{(M'_{pq})^r}{\sum_i (M'_{iq})^r}$
- 6 Repeat steps 4 and 5 until a steady state is reached (convergence).

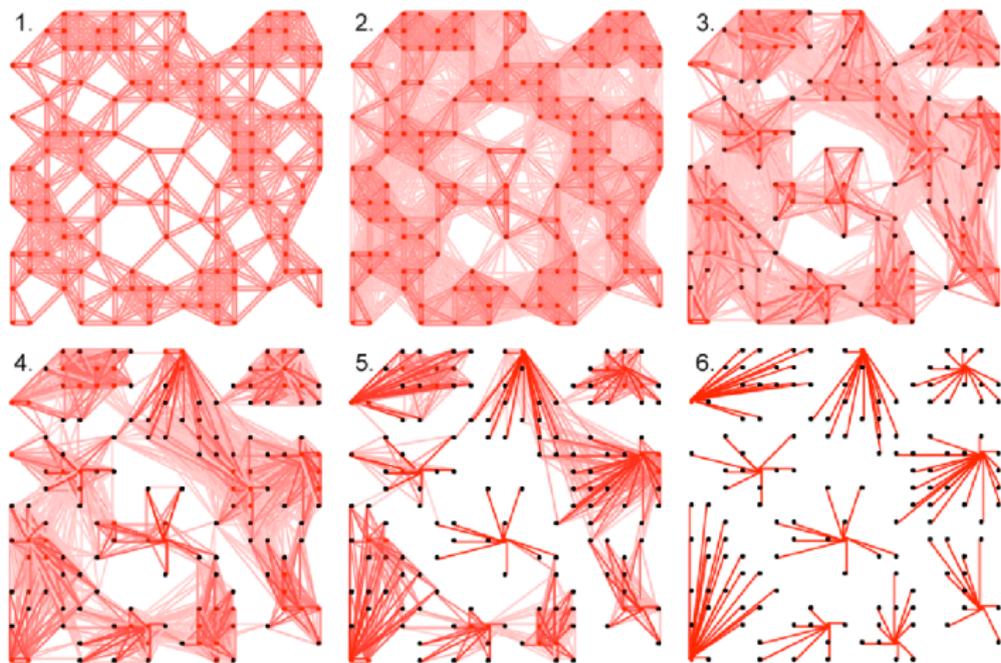


The number of steps to converge is not proven, but experimentally shown to be 10 to 100 steps, and mostly consist of sparse matrices after the first few steps. There are several distinct measures informing on the clustering and its stability such as the following clustering entropy:

$S = -1/L \sum_{ij} (P_{ij} \log_2 P_{ij} + (1 - P_{ij}) \log_2 (1 - P_{ij}))$  where the sum is over all edges and the entropy is normalized by the total number of edges. This might be used to detect the best clustering obtained after a long series of clusterings with different granularity parameters each time.

The expansion step of MCL has time complexity  $O(n^3)$ . The inflation has complexity  $O(n^2)$ . However, the matrices are generally very sparse, or at least the vast majority of the entries are near zero. Pruning in MCL involves setting near-zero matrix entries to zero, and can allow sparse matrix operations to improve the speed of the algorithm vastly.





**Figure :** Example of various steps 1-6

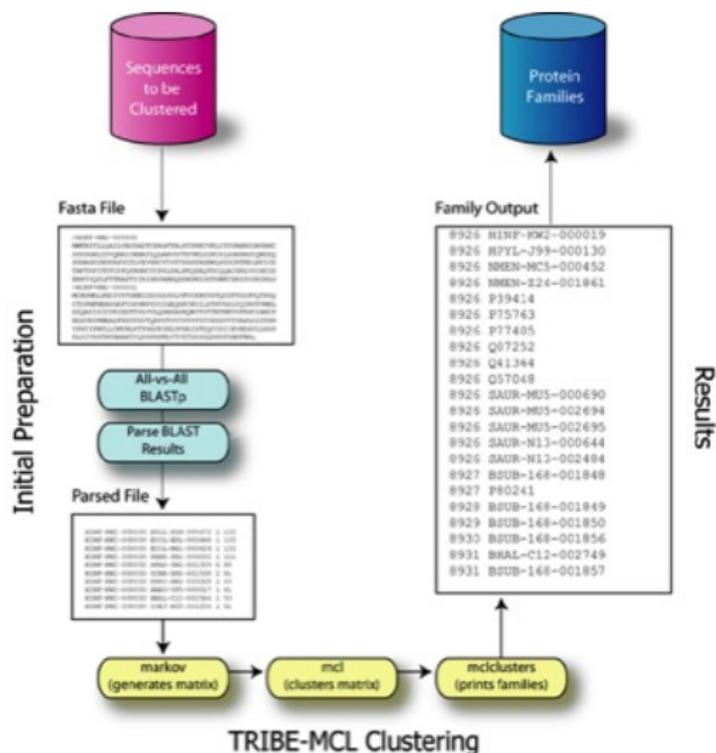


Figure : example of mcl application from ([www.ncbi.nlm.nih.gov/pubmed/11917018](http://www.ncbi.nlm.nih.gov/pubmed/11917018))





Hidden Markov Models applications in Bioinformatics (Genescan, TMHMM). Input: Sequence data.

Output: the prediction of the pattern.



- MCL: <http://micans.org/mcl/ani/mcl-animation.html>
- Enright AJ, Van Dongen S, Ouzounis CA. An efficient algorithm for large-scale detection of protein families. *Nucleic Acids Res.* 2002 30:1575-84.



HMMs form a useful class of probabilistic graphical models used to find genes, predict protein structure and classify protein families.

Definition: A hidden Markov model (HMM) has an Alphabet =  $b_1, b_2, \dots, b_M$ , set of states  $Q = 1, \dots, K$ , and transition probabilities between any two states

$a_{ij}$  = transition prob from state  $i$  to state  $j$

$a_{i1} + \dots + a_{iK} = 1$ , for all states  $i = 1, \dots, K$

Start probabilities  $a_{0i}$

$a_{01} + \dots + a_{0K} = 1$

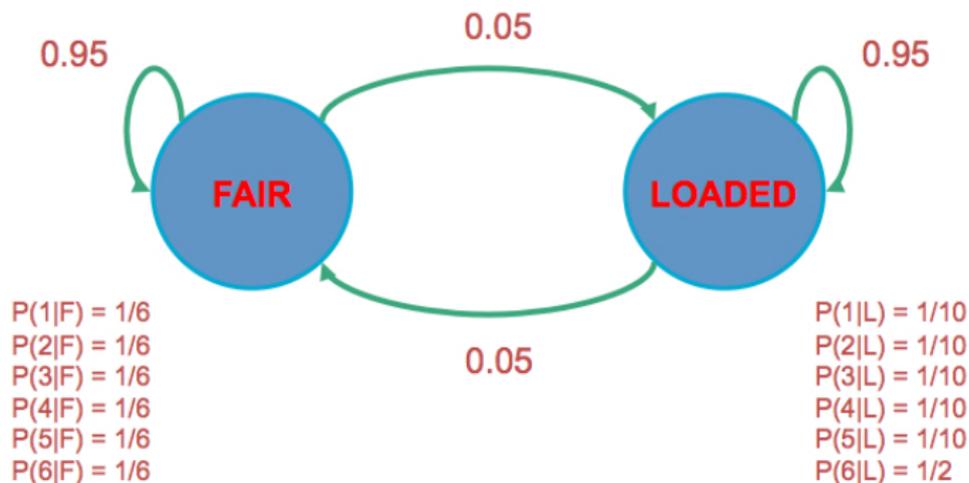
Emission probabilities within each state  $e_i(b) = P(x_i = b | \pi_i = k)$

$e_i(b_1) + \dots + e_i(b_M) = 1$ , for all states  $i = 1, \dots, K$

A Hidden Markov model is memoryless:  $P(\pi_{t+1} = k | \text{whatever happened so far}) = P(\pi_{t+1} = k | \pi_1, \pi_2, \dots, \pi_t, x_1, x_2, \dots, x_t) = P(\pi_{t+1} = k | \pi_t)$  at each time step  $t$ , only matters the current state  $\pi_t$ .



## The dishonest casino model



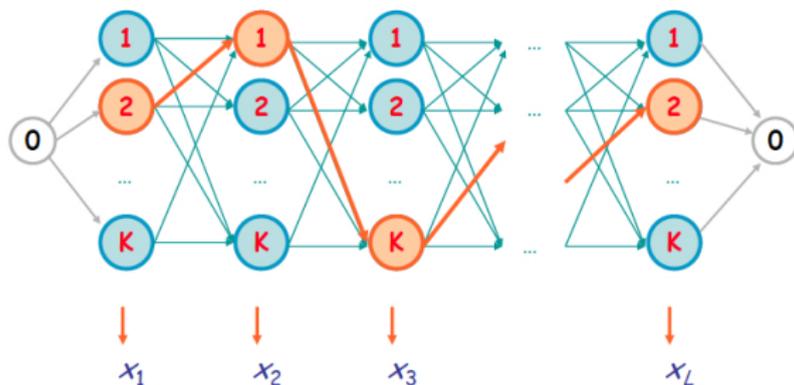
<http://ai.stanford.edu/serafim/>



- Known: The structure of the model
- The transition probabilities
- Hidden: What the casino did (ex FFFFFLLLLLLLLFFFF)
- Observable: The series of die tosses, ex 3415256664666153...
- What we must infer:
- When was a fair die used?
- When was a loaded one used?



## A “parse” of a sequence

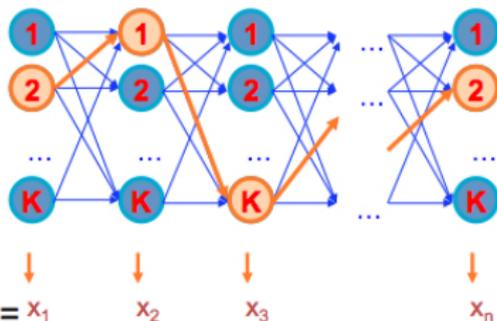


$$\Pr(x, \pi) = a_{0\pi_1} \prod_{i=1}^L e_{\pi_i}(x_i) \cdot a_{\pi_i\pi_{i+1}}$$

Given a sequence  $x = x_1 x_2 \dots x_N$ , a parse of  $x$  is a sequence of states  $\pi = \pi_1, \pi_2, \dots, \pi_N$ .

Given a sequence  $\mathbf{x} = x_1, \dots, x_N$   
 and a parse  $\pi = \pi_1, \dots, \pi_N$ ,

To find how likely is the parse:  
 (given our HMM)



$$P(\mathbf{x}, \pi) = P(x_1, \dots, x_N, \pi_1, \dots, \pi_N) = x_1 \quad x_2 \quad x_3 \quad x_n$$

$$P(x_N, \pi_N \mid \pi_{N-1}) P(x_{N-1}, \pi_{N-1} \mid \pi_{N-2}) \dots P(x_2, \pi_2 \mid \pi_1) P(x_1, \pi_1) =$$

$$P(x_N \mid \pi_N) P(\pi_N \mid \pi_{N-1}) \dots P(x_2 \mid \pi_2) P(\pi_2 \mid \pi_1) P(x_1 \mid \pi_1) P(\pi_1) =$$

$$a_{0\pi_1} a_{\pi_1\pi_2} \dots a_{\pi_{N-1}\pi_N} e_{\pi_1}(x_1) \dots e_{\pi_N}(x_N)$$



## 1. Evaluation

GIVEN a HMM  $M$ , and a sequence  $x$ ,  
FIND  $\text{Prob}[x | M]$

## 2. Decoding

GIVEN a HMM  $M$ , and a sequence  $x$ ,  
FIND the sequence  $\pi$  of states that maximizes  $P[x, \pi | M]$

## 3. Learning

GIVEN a HMM  $M$ , with unspecified transition/emission probs.,  
and a sequence  $x$ ,  
FIND parameters  $\theta = (e_i(\cdot), a_{ij})$  that maximize  $P[x | \theta]$

Evaluation: forward algorithm or the backwards algorithm; decoding: Viterbi; Learning: Baum Welch = forward-backward algorithm (not in this course).



$P[x | M]$ : The probability that sequence  $x$  was generated by the model; The model is: architecture (#states, etc)  
+ parameters  $\theta = a_{ij}, e_i(\cdot)$

So,  $P[x | \theta]$ , and  $P[x]$  are the same, when the architecture, and the entire model, respectively, are implied

Similarly,  $P[x, \pi | M]$  and  $P[x, \pi]$  are the same

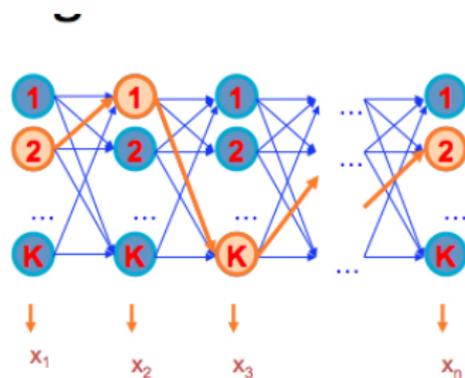
In the **LEARNING** problem we always write  $P[x | \theta]$  to emphasize that we are seeking the  $\theta$  that maximizes  $P[x | \theta]$



GIVEN  $x = x_1 x_2 \dots x_N$

We want to find  $\pi = \pi_1, \dots, \pi_N$ ,  
such that  $P[x, \pi]$  is maximized

$\pi^* = \operatorname{argmax}_{\pi} P[x, \pi]$



We can use dynamic programming!

Let  $V_k(i) = \max_{\{\pi_1, \dots, i-1\}} P[x_1 \dots x_{i-1}, \pi_1, \dots, \pi_{i-1}, x_i, \pi_i = k]$   
= Probability of most likely sequence of states  
ending at state  $\pi_i = k$



Given that for all states  $k$ , and for a fixed position  $i$ ,

$$V_k(i) = \max_{\{\pi_1, \dots, i-1\}} P[x_1 \dots x_{i-1}, \pi_1, \dots, \pi_{i-1}, x_i, \pi_i = k]$$

What is  $V_k(i+1)$ ?

From definition,

$$\begin{aligned} V_l(i+1) &= \max_{\{\pi_1, \dots, i\}} P[x_1 \dots x_i, \pi_1, \dots, \pi_i, x_{i+1}, \pi_{i+1} = l] \\ &= \max_{\{\pi_1, \dots, i\}} P(x_{i+1}, \pi_{i+1} = l \mid x_1 \dots x_i, \pi_1, \dots, \pi_i) P[x_1 \dots x_i, \pi_1, \dots, \pi_i] \\ &= \max_{\{\pi_1, \dots, i\}} P(x_{i+1}, \pi_{i+1} = l \mid \pi_i) P[x_1 \dots x_{i-1}, \pi_1, \dots, \pi_{i-1}, x_i, \pi_i] \\ &= \max_k P(x_{i+1}, \pi_{i+1} = l \mid \pi_i = k) \max_{\{\pi_1, \dots, i-1\}} P[x_1 \dots x_{i-1}, \pi_1, \dots, \pi_{i-1}, x_i, \pi_i = k] = \\ & e_l(x_{i+1}) \max_k a_{kl} V_k(i) \end{aligned}$$



Input:  $x = x_1 \dots x_N$

**Initialization:**

$$V_0(0) = 1 \quad (0 \text{ is the imaginary first position})$$

$$V_k(0) = 0, \text{ for all } k > 0$$

**Iteration:**

$$V_j(i) = e_j(x_i) \times \max_k a_{kj} V_k(i-1)$$

$$\text{Ptr}_j(i) = \operatorname{argmax}_k a_{kj} V_k(i-1)$$

**Termination:**

$$P(x, \pi^*) = \max_k V_k(N)$$

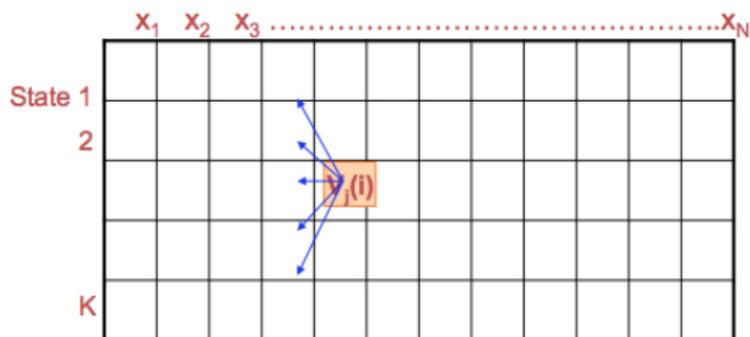
**Traceback:**

$$\pi_N^* = \operatorname{argmax}_k V_k(N)$$

$$\pi_{i-1}^* = \text{Ptr}_{\pi_i^*}(i)$$



## Complexity of the Viterbi Algorithm



Similar to “aligning” a set of states to a sequence

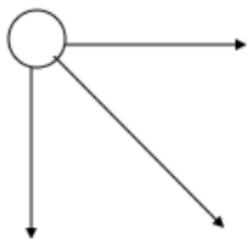
**Time:**

$$O(K^2N)$$

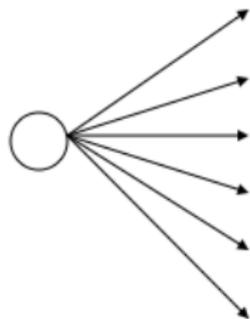
**Space:**

$$O(KN)$$





Valid directions in the  
*alignment problem.*

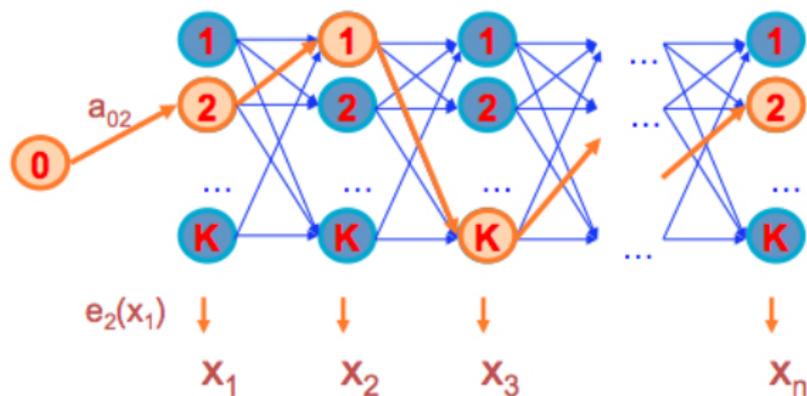


Valid directions in the  
*decoding problem.*

## Generating a sequence by the model

Given a HMM, we can generate a sequence of length  $n$  as follows:

- 1 Start at state  $\pi_1$  according to prob  $a_{0\pi_1}$
- 2 Emit letter  $x_1$  according to prob  $e_{\pi_1}(x_1)$
- 3 Go to state  $\pi_2$  according to prob  $a_{\pi_1\pi_2}$
- 4 until emitting  $x_n$



$P(x)$  Probability of  $x$  given the model

$P(x_i \dots x_j)$  Probability of a substring of  $x$  given the model

$P(\pi_i = k \mid x)$  Probability that the  $i^{\text{th}}$  state is  $k$ , given  $x$

A more refined measure of which states  $x$  may be in



We want to calculate

$P(\mathbf{x})$  = probability of  $\mathbf{x}$ , given the HMM

Sum over all possible ways of generating  $\mathbf{x}$ :

$$P(\mathbf{x}) = \sum_{\pi} P(\mathbf{x}, \pi) = \sum_{\pi} P(\mathbf{x} \mid \pi) P(\pi)$$

To avoid summing over an exponential number of paths  $\pi$ , define

$$f_k(i) = P(x_1 \dots x_i, \pi_i = k) \quad (\text{the forward probability})$$



Define the forward probability:

$$\begin{aligned}f_i(i) &= P(x_1 \dots x_i, \pi_i = l) \\&= \sum_{\pi_1 \dots \pi_{i-1}} P(x_1 \dots x_{i-1}, \pi_1, \dots, \pi_{i-1}, \pi_i = l) e_l(x_i) \\&= \sum_k \sum_{\pi_1 \dots \pi_{i-2}} P(x_1 \dots x_{i-1}, \pi_1, \dots, \pi_{i-2}, \pi_{i-1} = k) a_{kl} e_l(x_i) \\&= e_l(x_i) \sum_k f_k(i-1) a_{kl}\end{aligned}$$



We can compute  $f_k(i)$  for all  $k, i$ , using dynamic programming!

### Initialization:

$$f_0(0) = 1$$

$$f_k(0) = 0, \text{ for all } k > 0$$

### Iteration:

$$f_i(i) = e_i(x_i) \sum_k f_k(i-1) a_{ki}$$

### Termination:

$$P(x) = \sum_k f_k(N) a_{k0}$$

Where,  $a_{k0}$  is the probability that the terminating state is  $k$   
(usually =  $a_{0k}$ )



## VITERBI

### Initialization:

$$V_0(0) = 1$$

$$V_k(0) = 0, \text{ for all } k > 0$$

### Iteration:

$$V_j(i) = e_j(x_i) \max_k V_k(i-1) a_{kj}$$

### Termination:

$$P(x, \pi^*) = \max_k V_k(N)$$

## FORWARD

### Initialization:

$$f_0(0) = 1$$

$$f_k(0) = 0, \text{ for all } k > 0$$

### Iteration:

$$f_l(i) = e_l(x_i) \sum_k f_k(i-1) a_{kl}$$

### Termination:

$$P(x) = \sum_k f_k(N) a_{k0}$$



We want to compute

$$P(\pi_i = k \mid x),$$

the probability distribution on the  $i^{\text{th}}$  position, given  $x$

We start by computing

$$\begin{aligned} P(\pi_i = k, x) &= P(x_1 \dots x_i, \pi_i = k, x_{i+1} \dots x_N) \\ &= P(x_1 \dots x_i, \pi_i = k) P(x_{i+1} \dots x_N \mid x_1 \dots x_i, \pi_i = k) \\ &= P(x_1 \dots x_i, \pi_i = k) P(x_{i+1} \dots x_N \mid \pi_i = k) \end{aligned}$$

Forward,  $f_k(i)$       Backward,  $b_k(i)$



Define the backward probability:

$$\begin{aligned}b_k(i) &= P(x_{i+1} \dots x_N \mid \pi_i = k) \\&= \sum_{\pi_{i+1} \dots \pi_N} P(x_{i+1}, x_{i+2}, \dots, x_N, \pi_{i+1}, \dots, \pi_N \mid \pi_i = k) \\&= \sum_l \sum_{\pi_{i+1} \dots \pi_N} P(x_{i+1}, x_{i+2}, \dots, x_N, \pi_{i+1} = l, \pi_{i+2}, \dots, \pi_N \mid \pi_i = k) \\&= \sum_l e_l(x_{i+1}) a_{kl} \sum_{\pi_{i+1} \dots \pi_N} P(x_{i+2}, \dots, x_N, \pi_{i+2}, \dots, \pi_N \mid \pi_{i+1} = l) \\&= \sum_l e_l(x_{i+1}) a_{kl} b_l(i+1)\end{aligned}$$



We can compute  $b_k(i)$  for all  $k, i$ , using dynamic programming

**Initialization:**

$$b_k(N) = a_{k0}, \text{ for all } k$$

**Iteration:**

$$b_k(i) = \sum_l e_l(x_{i+1}) a_{kl} b_l(i+1)$$

**Termination:**

$$P(x) = \sum_l a_{0l} e_l(x_1) b_l(1)$$



What is the running time, and space required, for Forward and Backward?

Time:  $O(K^2N)$

Space:  $O(KN)$

Useful implementation technique to avoid underflows

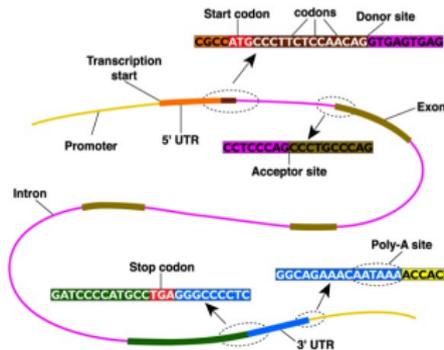
Viterbi:        sum of logs

Forward/Backward: rescaling at each position by multiplying by a constant



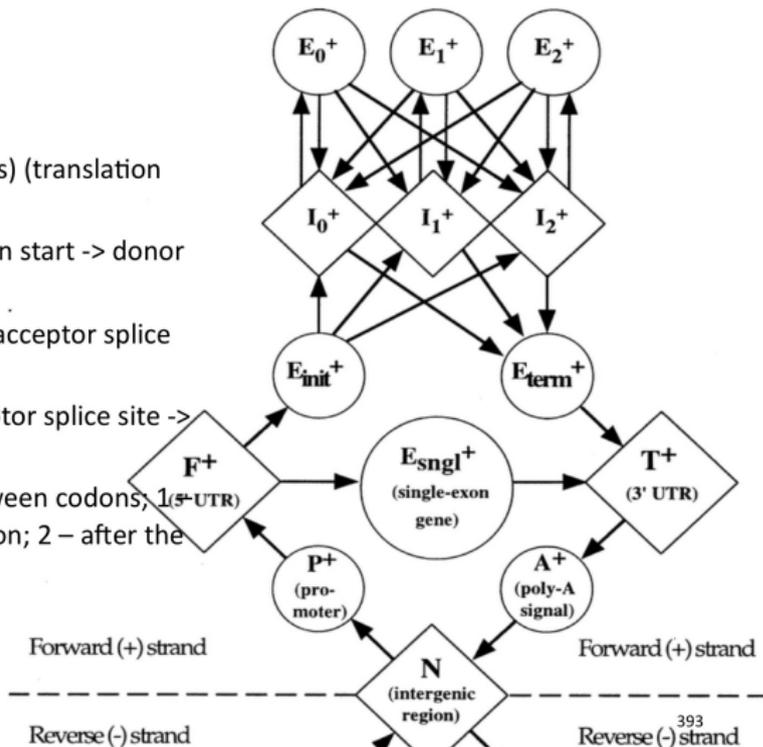
# Applications of Hidden Markov models (HMM): recognition of a human gene in sequence data

The gene information starts with the promoter, which is followed by a transcribed (i.e. RNA) but non-coding (i.e. not translated) region called 5' untranslated region (5' UTR). The initial exon contains the start codon which is usually ATG. There is an alternating series of introns and exons, followed by the terminating exon, which contains the stop codon. It is followed by another non-coding region called the 3' UTR; at the end there is a polyadenylation (polyA) signal, i.e. a repetition of Adenine. The intron/exon and exon/intron boundaries are conserved short sequences and called the acceptor and donor sites. For all these different parts we need to know their probability of occurrence in a large database.



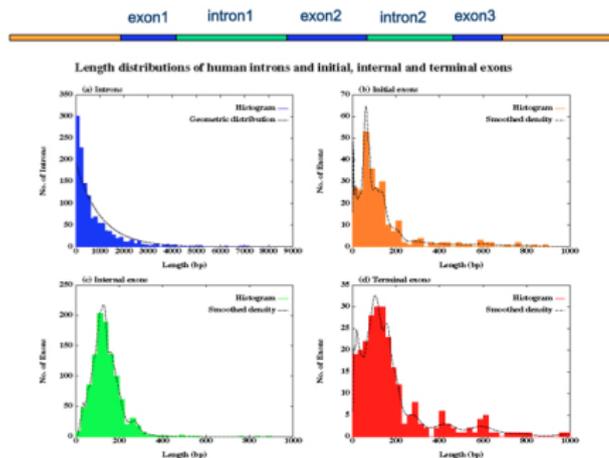
# GenScan

- N - intergenic region
- P - promoter
- F - 5' untranslated region
- $E_{sngl}$  - single exon (intronless) (translation start -> stop codon)
- $E_{init}$  - initial exon (translation start -> donor splice site)
- $E_k$  - phase k internal exon (acceptor splice site -> donor splice site)
- $E_{term}$  - terminal exon (acceptor splice site -> stop codon)
- $I_k$  - phase k intron: 0 - between codons, 1 (5' UTR) after the first base of a codon; 2 - after the second base of a codon



# Identifying genes and their parts (exons and introns)

In order to identify genes and their parts (exons and introns) we need to know their length distribution (see example in figures below). Human genes comprise about 3% of the human genome; average length:  $\sim 8,000$  DNA base pairs (bp); 5-6 exons/gene; average exon length:  $\sim 200$  bp; average intron length:  $\sim 2,000$  bp;  $\sim 8\%$  genes have a single exon and some exons can be as small as 1 or 3 bp. Below the statistics we could implement into a HMM.

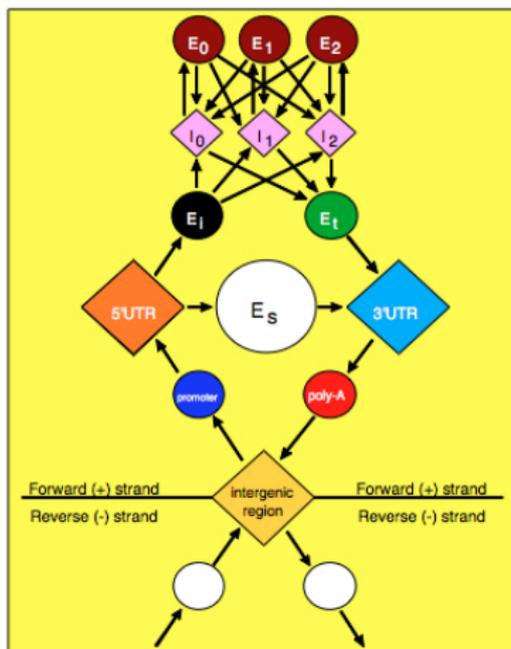


2



# Identifying genes and their parts (exons and introns)

## GENSCAN (Burge & Karlin)



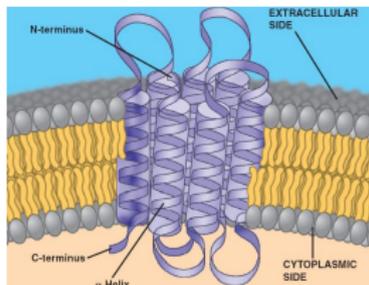
62001	AGACAGSTA CCGCTGTCAT CACTTADACC TCACCCTGTG GAGCCACACC
62051	CTAGGTTGG CCAATCTACT CCGAGAGCA GGGAGGACAG GAGCCAGGG
62101	TGGCATAAA AGTCAGGCA GAGCCACTCA TGGCTACAT TTGCTCTCAG
62151	CACAACTGG TTCACTAGCA ACCTCAANCA GACAC
62201	
62251	CTT TGGTATCAAG GTTACAGAC
62301	AGGTTTAAAG AGACCAATAG AAACGTGGCA TGGGAGACCA GAGAGACTC
62351	TTGGGTTTT GATAGGCAT GACTCTCTCT GCTATTGGT CTATTTCCG
62401	ACCTTAACT GCGGAGGCT GACACTTGGT AGCAAGGAG TGTGAGGCT
62451	CTTGGAGAT CTGAGCACT CTGAGTCTGT TATGGCAAG GCAAGAGGCT
62501	AGGTCATGG CAGAAAGAG CTGGGAGGCT TATGTAGGG CTGGGCTCA
62551	CTGACAACT TAAAGGAGC CTTGGACCA CTGAGGAGG TACACTGTA
62601	CAAGCTCAC CTGAGAGGCT AGAACTTCA TGGAGGCTA TGGAGCCCT
62651	GATGTTTTCT TTGCTCTCT TTCTATGGT TAAGTCTAG TATAGGAAG
62701	GGAGAGATA ACAGGATACA GTTTAGAGT GGAACAGAC GAATGATGC
62751	ATCAGTGGG AAGTCTCAGG ATCGTTTTAG TTCTTTTAT TTGCTGTCA
62801	TAAACAATGT TTCTTTTGT TTAATCTTGT CTCTCTTCT TTCTCTCTC
62851	CGCAATTTT ACTATTATC TTAATGCTCT AACATTTGG ATACAAAGG
62901	GAAATATCT TGAGATACAT TAACTACTT AAAAAAATC TTACACAGT
62951	CTGGTAGTA CATTACTAT TGGAAATAT GTGTGCTTAT TTGCAATTC
63001	ATAAATCCG TACTTATTT TCTTTTATTT TAAATGATA CATAATCAT
63051	ATACATATT ATGGGTTAAA GTGTAAATGT TAAATAGTG TACACATAT
63101	GACCAATAT GGTAAATTTT CAAATTTGAA TTTTAAAAA TGTCTTTCT
63151	TTTTAATAA CTTTTTGTG TACTTATTT CTAATACTT CCAAAAGCTC
63201	TTCTTTCAG GGCATAAATG ATACAAGTA TCACTGCTCT TTGACACTC
63251	CTAAAGATA ACAGTGATA TTTTGGGTT AGGCAGTAG CAATATCT
63301	GCATATAAT ATTTCTGAT ATAAATTTGA ACTGATGTA GAGGTTTAT
63351	ATTCATATA GCAGCTACA TCCAGCTACC ATTCGCTTCT TATTTATGG
63401	TTGGGTAAG CTGGGATAT TCGAGTCCA AGCTAGGCC TTTCGCTAAT
63451	CATGTCATA CCGCTTACT TCTTCCACA CTGDTGGG AACGTGCTG
63501	TGTGGTGTG GGGGATCAC TTGGCAGAG AATTCACCC ACAGTGGCT
63551	GCTGCTATC AGAAAGTGG GCGTGGTGG GCTAATGCC TGGCCACAA
63601	GTATCACTAA GCGGCTTCT TTGAGTCCA ATTTATATA AAGGATATG
63651	TGTTCCTAA GTCAATCAC TAACTGGGG GATATTAGA MGGCTTTA
63701	GCATCTGAT CTGACCAAT AAAAAAGAT TATTTTCAAT CAATGATGT

**Figure :** The model (left) and the output (right) of Genscan prediction of a genomic region; the result is a segmentation of a genome sequence, i.e. the colours map the HMM states with the predicted functional genomic segments

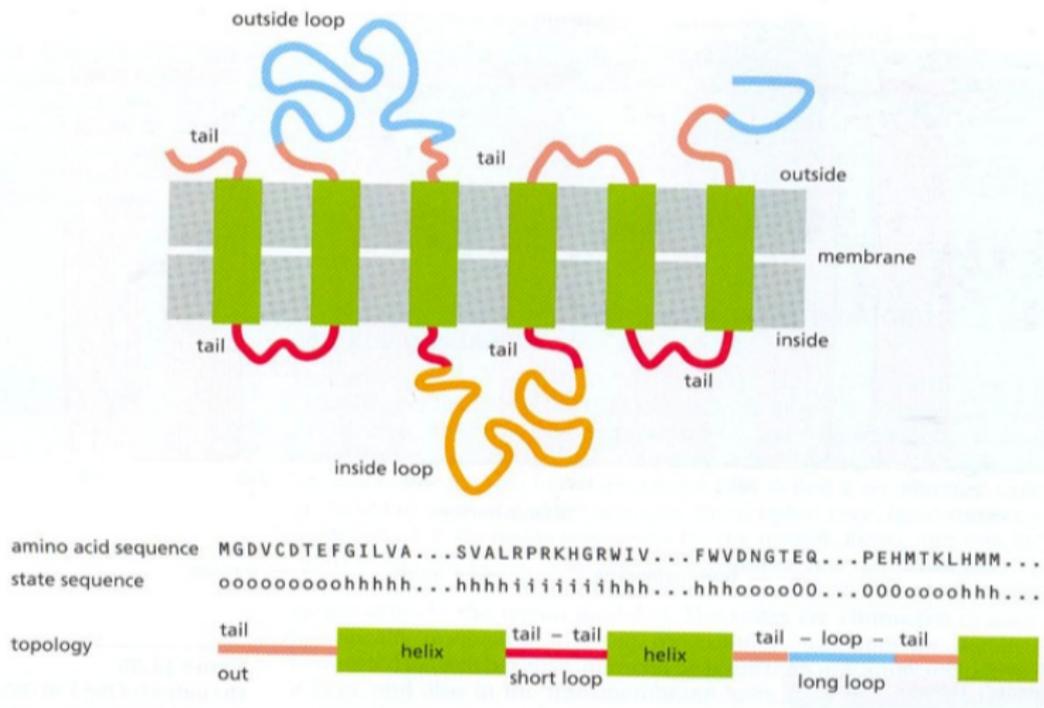


## Applications of HMM: recognition of the protein structure

Membrane proteins that are important for cell import/export. We would like to predict the position in the amino acids with respect to the membrane. The prediction of gene parts and of the membrane protein topology (i.e. which parts are outside, inside and buried in the membrane) will require to train the model with a dataset of experimentally determined genes / transmembrane helices and to validate the model with another dataset. The figure below describes a 7 helix membrane protein forming a sort of a cylinder (porus) across the cell membrane.

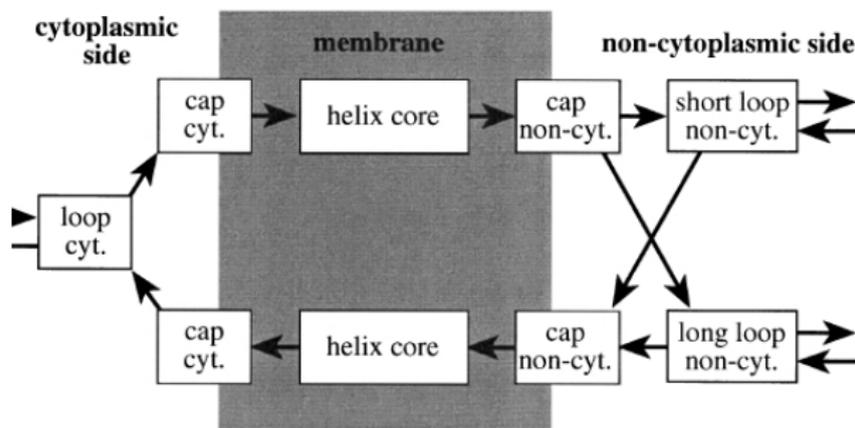


# Prediction of amino acid segments included in membrane proteins



**Figure :** top: the 3D graph previous figure could be represented as a 2D graph; bottom, 3 state prediction: each amino acid could be in the membrane (h), outside the cell (o) or inside the cell (i)

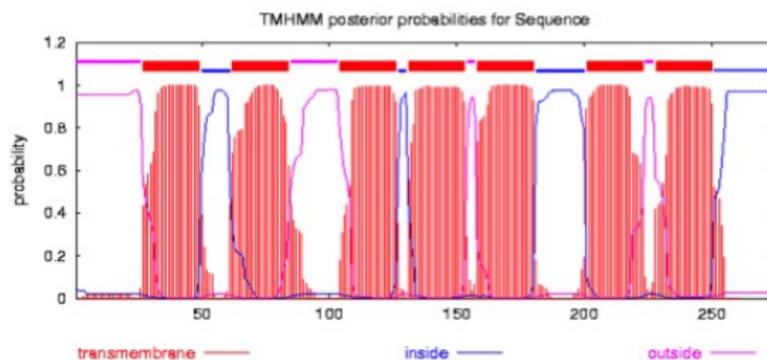




**Figure :** The THMM model: a three state prediction model (h,o,i) could be then refined adding more states, for example caps, i.e. the boundary between outside and membrane and inside and membrane. This refinement improves the prediction of the topology of the protein.

```

# Sequence Length: 274
# Sequence Number of predicted TMs: 7
# Sequence Exp number of AAs in TMs: 153.74681
# Sequence Exp number, first 60 AAs: 22.08833
# Sequence Total prob of N-in: 0.04171
# Sequence POSSIBLE N-term signal sequence
Sequence TMHMM2.0 outside 1 26
Sequence TMHMM2.0 TMhelix 27 49
Sequence TMHMM2.0 inside 50 61
Sequence TMHMM2.0 TMhelix 62 84
Sequence TMHMM2.0 outside 85 103
Sequence TMHMM2.0 TMhelix 104 126
Sequence TMHMM2.0 inside 127 130
Sequence TMHMM2.0 TMhelix 131 153
Sequence TMHMM2.0 outside 154 157
Sequence TMHMM2.0 TMhelix 158 180
Sequence TMHMM2.0 inside 181 200
Sequence TMHMM2.0 TMhelix 201 223
Sequence TMHMM2.0 outside 224 227
Sequence TMHMM2.0 TMhelix 228 250
Sequence TMHMM2.0 inside 251 274
    
```



Other important and related application: Use of HMM in sequence alignment (PFAM: <http://pfam.xfam.org/>)



- 1 be predicted to occur: Predicted Positive (PP)
- 2 be predicted not to occur: Predicted Negative (PN)
- 3 actually occur: Actual Positive (AP)
- 4 actually not occur: Actual Negative (AN)
- 5 True Positive  $TP = PP \cap AP$
- 6 True Negative  $TN = PN \cap AN$
- 7 False Negative  $FN = PN \cap AP$
- 8 False Positive  $FP = PP \cap AN$
- 9 Sensitivity: probability of correctly predicting a positive example  $S_n = TP / (TP + FN)$
- 10 Specificity: probability of correctly predicting a negative example  $S_p = TN / (TN + FP)$  or
- 11 Probability that positive prediction is correct  $S_p = TP / (TP + FP)$ .



- Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids Richard Durbin, Sean R. Eddy, Anders Krogh, Graeme Mitchison  
[http://books.google.co.uk/books/about/Biological\\_Sequence\\_Analysis.html?id=R5P2GJjvigQC](http://books.google.co.uk/books/about/Biological_Sequence_Analysis.html?id=R5P2GJjvigQC)
- Viterbi: <http://www.cs.umb.edu/~sreuilak/viterbi/>
- Lenwood S. Heath Naren Ramakrishnan (Eds) Problem Solving Handbook in Computational Biology and Bioinformatics
- <http://web.mit.edu/seven/doc/genscan/genscan.txt>



Patterns in sequence alignment (Gibbs sampling).

Input: a multi sequence alignment. Output: a previously unknown pattern occurring with some variation, in all the sequences. Main question: is there a sequence pattern common to the group of sequences I am analysing? This is a qualitative (non mathematical) introduction.



# Gibbs sampling: the string searching problem

```
atgaccgggatactgatAgAagAAAGGtTGGGggcgtacacattagataaacgtatgaagtacgttagactcggcgccg
accctatTTTTTgagcagatttagtgacctggaaaaaaatttgagtacaaaactttccgaataCAATAAACGGCGGA
tgagtatccctgggatgacttAAAAAATGGAGtGGtgctctcccgattttgaatatgtaggatcattcgcagggtccga
gctgagaattggatgCAAAAAAGGGattTGTccacgcaatcgcaaccaacgcggacccaaggcaagaccgataaaaggaga
tcccttttgcgtaatgtgccgggaggctggttacgtaggaagccctaacggacttaataATAATAAGGaaGGGcttatag
gtcaatcatgttcttgtgaatggattAACAAATAAGGGctGGgaccgcttggcgcacccaaattcagtggtggcgagcgcaa
cggttttggccctttagaggccccgtATAAAcAAGGaGGGc caattatgagagagctaatctatcgcgtgctgttcat
aacttgagttAAAAATAGGGaGcCctggggcacatacaagaggagtcttcttatcagttaatgctgtatgacactatgta
ttggccattggctaaaagcccaacttgacaatggaagatagaatccttgcatActAAAAAGGaGCGGaccgaaaggaag
ctggtgagcaacgacagattcttacgtgcattagctcgcttccgggatctaatagcaggaagcttActAAAAAGGaGCGGa
```

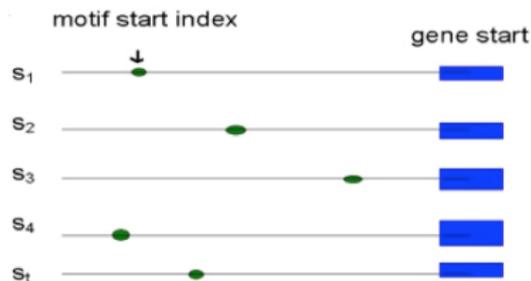
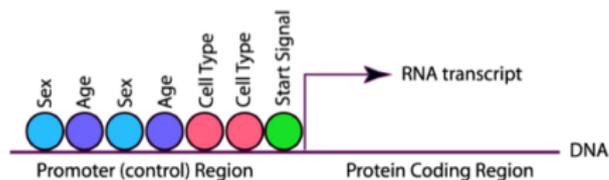
**AgAagAAAGGtTGGG**  
..|..|||..|..|||  
**CAATAAACGGCGGA**

**Figure :** Inserting a 15-bases motif with 4 mutations: why finding the motif is difficult? Reason to search for motifs: we know from microarray analysis that n genes are activated together so there may be a protein that binds somewhere before the start of each of them.



## Gibbs sampling: the Biological problem

Given a set of sequences, find the motif shared by all or most sequences; while its starting position in each sequence is unknown, each motif appears exactly once in one sequence and it has fixed length.



**Figure :** The regulation of a gene could be very complex with several binding proteins (transcription factor) involved (left). Right: several genes are co-regulated (activated or repressed) by same protein that binds before the gene start (co-regulated genes could be identified with microarray).



Gibbs Sampling is an example of a Markov chain Monte Carlo algorithm; it is an iterative procedure that discards one l-mer after each iteration and replaces it with a new one. Gibbs Sampling proceeds slowly and chooses new l-mers at random increasing the odds that it will converge to the correct solution. It could be used to identify short strings, motifs, common to all co-regulated genes which are not co-aligned. The algorithm in brief:

- 1 Randomly choose starting positions  $s = (s_1, \dots, s_t)$  and form the set of l-mers associated with these starting positions.
- 2 Randomly choose one of the t sequences
- 3 Create a profile p from the other t - 1 sequences (or you can also use all the t sequences).
- 4 For each position in the removed sequence, calculate the probability that the l-mer starting at that position was generated by p.
- 5 Choose a new starting position for the removed sequence at random based on the probabilities calculated in step 4.
- 6 Repeat steps 2-5 until there is no improvement.



Considering a set of unaligned sequences, we choose initial guess of motifs

1. Select a **random** position in each sequence

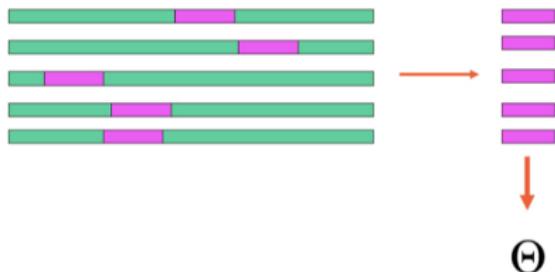


**Figure :** motifs in purple, the rest of the sequences in green; next figures: theta is the weight matrix i.e. the frequency of each base in the aligned set of motifs; red the best fitting motif; in y axis the likelihood of each motif with respect to the current weight matrix.

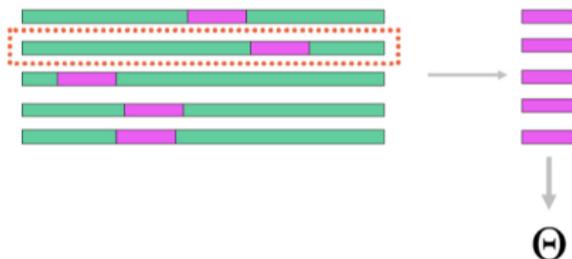
First Gibbs Sampling implementations: AlignACE ([arep.med.harvard.edu/mrnadata/mrnasoft.html](http://arep.med.harvard.edu/mrnadata/mrnasoft.html)) and BioProspector ([ai.stanford.edu/~xslui/BioProspector/](http://ai.stanford.edu/~xslui/BioProspector/)). See [ccmbweb.ccv.brown.edu](http://ccmbweb.ccv.brown.edu)



## 2. Build a weight matrix



## 3. Select a sequence at random



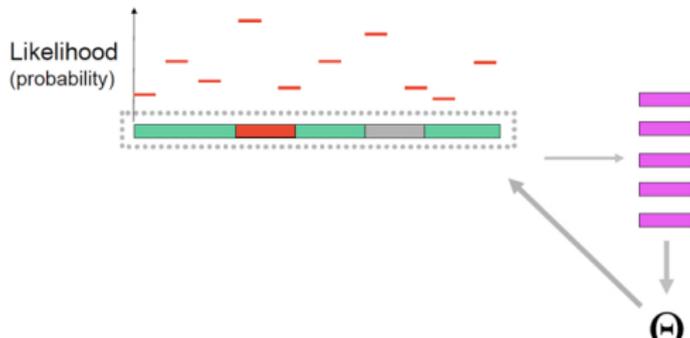
A weight matrix  $\theta$  has one row for each symbol of the alphabet and one column for each position in the pattern. It is a position probability matrix computed from the frequency of each symbol in each position.

## 4. Score possible sites in seq using weight matrix

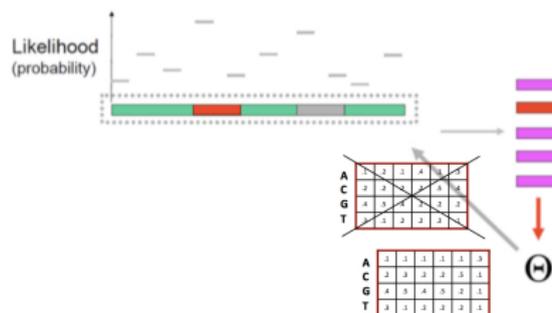


$$\text{Score} = 0.2 + 0.5 + 0.2 + 0.2 + 0.2 + 0.1 = 1.4$$

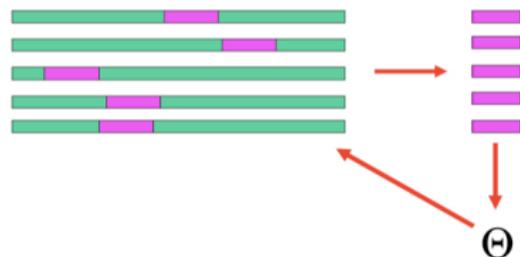
## 5. Sample a new site proportional to likelihood



## 6. Update weight matrix



## 7. Iterate until convergence (no change in sites/ $\theta$ )



Doesn't do reinitializations in the middle to get out of local maxima. Doesn't optimize the width (you have to specify width explicitly).

- <http://www.bio.davidson.edu/courses/genomics/chip/chip.html>
- Lawrence, Altschul, Boguski, Liu, Neuwald, Wootton, Detecting Subtle Sequence Signals: a Gibbs Sampling Strategy for Multiple Alignment Science, 1993
- <http://ccmbweb.ccv.brown.edu/cgi-bin/gibbs.12.pl>
- <https://github.com/mitbal/gibbs-sampler-motif-finding>

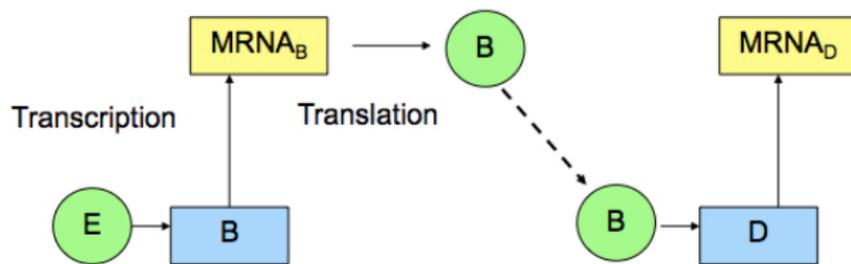


Biological Networks reconstruction (Wagner) and simulation (Gillespie). Input: a table (say an excel table) from a microarray experiment (say gene in y axis and conditions or time in x axis); output is a graph showing the quantitative relationships among all genes (nodes). Usually the experiments are replicated so averages and distances could be computed. Main question: find the direct dependencies among genes.



## The biological problem

A biological network is a group of genes in which individual genes can influence the activity of other genes. Let us assume that there are two related genes, B and D, neither of which is expressed initially, but E causes B to be expressed and this in turn causes D to be expressed. The addition of CX by itself may not affect expression of either B or D, but both CX and E will have elevated levels of  $mRNA_B$  and low levels of  $mRNA_D$ .

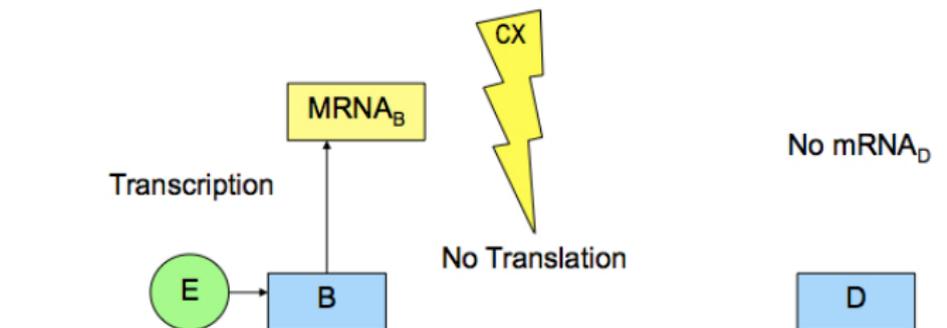


**Figure :** We have E only; B is a Primary Target of E; Production of  $mRNA_B$  is enhanced by E; D is a Secondary Target of E; Production of  $mRNA_D$  is enhanced by B;  $mRNA_B$  and  $mRNA_D$  quantified by microarray.



## A genetic perturbation is an experimental manipulation of gene activity by manipulating either a gene itself or its product

Such perturbations include point mutations, gene deletions, overexpression, inhibition of translation, or any other interference with the activity of the product.



**Figure :** E and CX both present; B is a Primary Target; Production of  $RNA_B$  is enhanced by E; Production of  $RNA_D$  is decreased (prevented)

When manipulating a gene and finding that this manipulation affects the activity of other genes, the question often arises as to whether this is caused by a direct or indirect interaction?

An algorithm to reconstruct a genetic network from perturbation data should be able to distinguish direct from indirect regulatory effects.

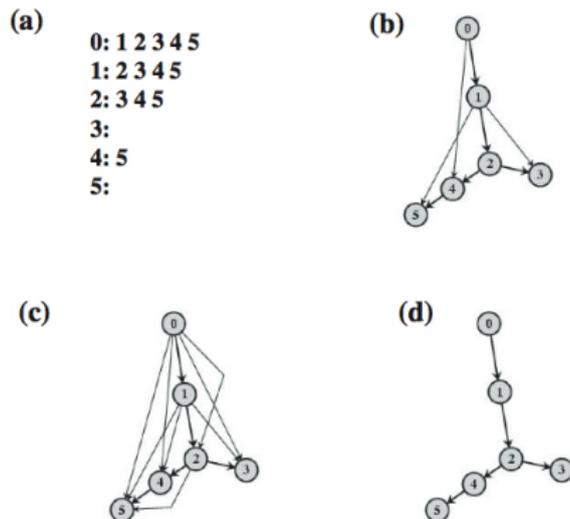
Consider a series of experiments in which the activity of every single gene in an organism is manipulated. (for instance, non-essential genes can be deleted, and for essential genes one might construct conditional mutants). The effect on mRNA expression of all other genes is measured separately for each mutant.



- How to reconstruct a large genetic network from  $n$  gene perturbations in fewer than  $n^2$  steps?
- Motivation: perturb a gene network one gene at a time and use the effected genes in order to discriminate direct vs. indirect gene-gene relationships
- Perturbations: gene knockouts, over-expression, etc.
- Method: For each gene  $g_i$ , compare the control experiment to perturbed experiment and identify the differentially expressed genes Use the most parsimonious graph that yields the graph as its reachable graph.







**Figure :** The figure illustrates three graphs (Figs. B,C,D) with the same accessibility list Acc (Fig. A). There is one graph (Fig. D) that has Acc as its accessibility list and is simpler than all other graphs, in the sense that it has fewer edges. Lets call Gpars the most parsimonious network compatible with Acc.

Figure A shows a graph representation of a hypothetical genetic network of 21 genes. Figure B shows an alternative representation of the network shown in A. For each gene  $i$ , it simply shows which genes activity state the gene influences directly. In graph theory, a list like that shown in Fig. B is called the adjacency list of the graph. We will denote it as  $Adj(G)$ , and will refer to  $Adj(i)$  as the set of nodes (genes) adjacent to (directly influenced by) node  $i$ . One might also call it the list of nearest neighbors in the gene network, or the list of direct regulatory interactions.

When perturbing each gene in the network shown in Figure A, one would get the list of influences on the activities of other genes shown in Figure C.

Starting from a graph representation of the network in Figure A, one arrives at the list of direct and indirect causal interactions in Figure C by following all paths leaving a gene. That is, one follows all arrows emanating from the gene until one can go no further.



In graph theory, the list  $\text{Acc}(G)$  is called the accessibility list of the graph  $G$ , because it shows all nodes (genes) that can be accessed (influenced in their activity state) from a given gene by following paths of direct interactions.

In the context of a genetic network one might also call it the list of perturbation effects or the list of regulatory effects.

$\text{Acc}(i)$  is the set of nodes that can be reached from node  $i$  by following all paths of directed edges leaving  $i$ .  $\text{Acc}(G)$  then simply consists of the accessibility list for all nodes  $i$



The adjacency matrix of a graph  $G$ ,  $A(G) = (a_{ij})$  is an  $n$  by  $n$  square matrix, where  $n$  is the number of nodes (genes) in the graph. An element  $(a_{ij})$  of this matrix is equal to one if and only if a directed edge exists from node  $i$  to node  $j$ . All other elements of the adjacency matrix are zero.

The accessibility matrix  $P(G) = p_{ij}$  is also an  $n$  by  $n$  square matrix. An element  $p_{ij}$  is equal to one if and only if a path following directed edges exists from node  $i$  to node  $j$ . otherwise  $p_{ij}$  equals zero.

Adjacency and accessibility matrices are the matrix equivalents of adjacency and accessibility lists.

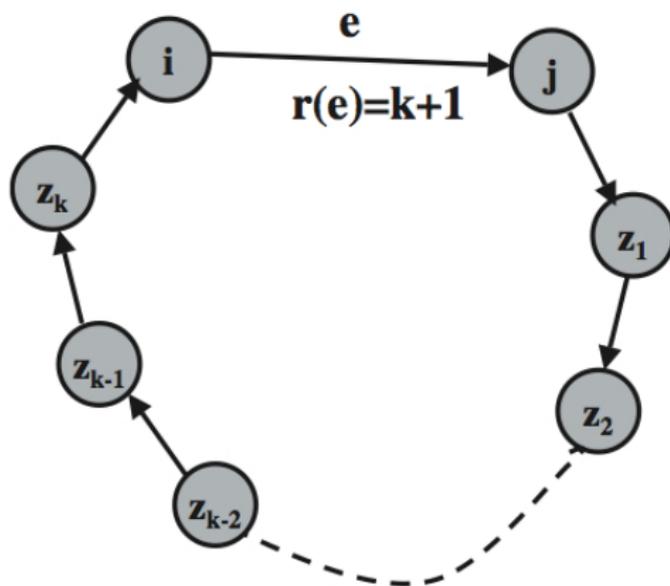
Lets first consider only graphs without cycles, where cycles are paths starting at a node and leading back to the same node. Graphs without cycles are called acyclic graphs.

Later generalize to graphs with cycles.

An acyclic directed graph defines its accessibility list, but the converse is not true.

In general, if  $Acc$  is the accessibility list of a graph, there is more than one graph  $G$  with the same accessibility list.

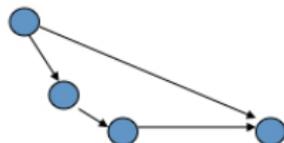




**Figure :** A shortcut is an edge connecting two nodes,  $i$  and  $j$  that are also connected via a longer path of edges. The shortcut  $e$  is a shortcut range  $k+1$ . That is, when eliminating  $e$ ,  $i$  and  $j$  are still connected by a path of length  $k+1$ .

- Step1: Graphs without cycles only (acyclic directed graph)
- Step2: Graphs with cycles

- Step 1: Shortcut:



- A **shortcut-free graph** compatible with an accessibility list is a **unique** graph with the fewest edges among all graphs compatible with the accessibility list, i.e, a shortcut-free graph is the most parsimonious graph.

- Let  $Acc$  be the accessibility list of an acyclic digraph. Then there exists exactly one graph  $G_{pars}$  that has  $Acc$  as its accessibility list and that has fewer edges than any other graph  $G$  with  $Acc$  as its accessibility list.
- This means that for any list of perturbation effects there exists exactly one genetic network  $G$  with fewer edges than any other network with the same list of perturbation effects.
- Definition: An accessibility list  $Acc$  and a digraph  $G$  are compatible if  $G$  has  $Acc$  as its accessibility list.  $Acc$  is the accessibility list induced by  $G$ .
- Definition: Consider two nodes  $i$  and  $j$  of a digraph that are connected by an edge  $e$ . The range  $r$  of the edge  $e$  is the length of the shortest path between  $i$  and  $j$  in the absence of  $e$ . If there is no other path connecting  $i$  and  $j$ , then  $r := \infty$ .

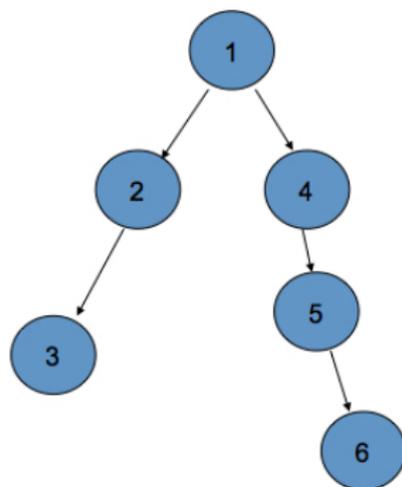


Let  $Acc(G)$  be the accessibility list of an acyclic directed graph,  $G_{pars}$  its most parsimonious graph, and  $V(G_{pars})$  the set of all nodes of  $G_{pars}$ . We have the following equation (1):

$$\forall i \in V(G_{pars}) \dots Adj(i) = Acc(i) \setminus \cup_{j \in Acc(i)} Acc(j)$$

In words, for each node  $i$  the adjacency list  $Adj(i)$  of the most parsimonious genetic network is equal to the accessibility list  $Acc(i)$  after removal of all nodes that are accessible from any node in  $Acc(i)$ .





**Figure :**  $Adj(1) = Acc(1) - (Acc(2) + Acc(3) + Acc(4) + Acc(5) + Acc(6)) = (2, 3, 4, 5, 6) - (3 \cup (5, 6) \cup 6) = (2, 4)$

Proof: I will first prove that every node in  $\text{Adj}(i)$  is also contained in the set defined by the right hand side of (1).

Let  $x$  be a node in  $\text{Adj}(i)$ . This node is also in  $\text{Acc}(i)$ . Now take, without loss of generality any node  $j \in \text{Acc}(i)$ . Could  $x$  be in  $\text{Acc}(j)$ ? If  $x$  could be in  $\text{Acc}(j)$  then we could construct a path from  $i$  to  $j$  to  $x$ . But because  $x$  is also in  $\text{Adj}(i)$ , there is also an edge from  $i$  to  $x$ . This is a contradiction to  $G_{\text{pars}}$  being shortcut-free. Thus, for no  $j \in \text{Acc}(i)$  can  $x$  be in  $\text{Acc}(j)$ .  $x$  is therefore also not an element of the union of all  $\text{Acc}(j)$  shown on the right-hand side of (1). Thus, subtracting this union from  $\text{Acc}(i)$  will not lead to the difference operator in (1) eliminating  $x$  from  $\text{Acc}(i)$ . Thus  $x$  is contained in the set defined by the right-hand side of (1).



Next to prove: Every node in the set of the right-hand side of (1) is also in  $\text{Adj}(i)$ .  
Let  $x$  be a node in the set of the right-hand side of (1). Because  $x$  is in the right hand side of (1),  $x$  must a fortiori also be in  $\text{Acc}(i)$ . That is,  $x$  is accessible from  $i$ . But  $x$  can not be accessible from any  $j$  that is accessible from  $i$ .  
For if it were, then  $x$  would also be in the union of all  $\text{Acc}(j)$ . Then taking the complement of  $\text{Acc}(i)$  and this union would eliminate  $x$  from the set in the right hand side of (1). In sum,  $x$  is accessible from  $i$  but not from any  $j$  accessible from  $i$ . Thus  $x$  must be adjacent to  $i$ .



Let  $i$ ,  $j$ , and  $k$  be any three pairwise different nodes of an acyclic directed shortcut-free graph  $G$ . If  $j$  is accessible from  $i$ , then no node  $k$  accessible from  $j$  is adjacent to  $i$ .

Proof: Let  $j$  be a node accessible from node  $i$ . Assume that there is a node  $k$  accessible from  $j$ , such that  $k$  is adjacent to  $i$ . That is,  $j \in \text{Acc}(i)$ ,  $k \in \text{Acc}(j)$  and  $k \in \text{Adj}(i)$ . That  $k$  is accessible from  $j$  implies that there is a path of length at least one from  $j$  to  $k$ . For the same reason, there exists a path of length at least one connecting  $i$  to  $j$ . In sum, there must exist a path of length at least two from  $i$  to  $k$ . However, by assumption, there also exists a directed edge from  $i$  to  $k$ . Thus, the graph  $G$  can not be short-cut free.



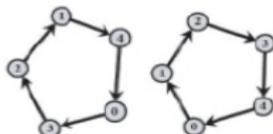
## Step 2: How about graphs with cycles?

Two different cycles have the same accessibility list

Perturbations of any gene in the cycle influences the activity of all other genes in the same cycle

Cant decide a unique graph if cycle happens

Not an algorithmic but an experimental limitation



0: 3

1: 4

2: 1

3: 2

4: 0

0: 1

1: 2

2: 3

3: 4

4: 0

0: 1 2 3 4

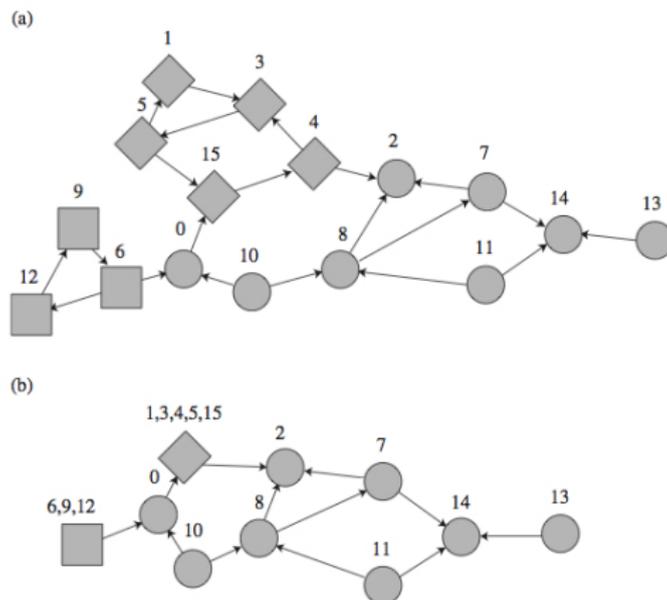
1: 0 2 3 4

2: 0 1 3 4

3: 0 1 2 4

4: 0 1 2 3





**Figure :** Basic idea: Shrink each cycles (strongly connected components) into one node and apply the algorithm of step 1. A graph after shrinking all the cycles into nodes is called a condensation graph



- 1 Unable to resolve cycled graphs
- 2 Require more data than conventional methods using gene expression correlations.
- 3 There are many networks consistent with the given accessibility list. The algorithm construct the most parsimonious one.
- 4 The same problem was proposed around 1980 which is called transitive reduction.
- 5 The transitive reduction of a directed graph  $G$  is the directed graph  $G'$  with the smallest number of edges such for every path between vertices in  $G$ ,  $G'$  has a path between those vertices.
- 6 An  $O(V)$  algorithm for computing transitive reduction of a planar acyclic digraph was proposed by Sukhamay Kundu. ( $V$  is the number of nodes in  $G$ )



- Measures of algorithmic complexity are influenced by the average number of entries in a nodes accessibility list. Let  $k < n - 1$  be that number.
- For all practical purposes, there will be many fewer entries than that, not only because accessibility lists with nearly  $n$  entries are not accessibility lists of acyclic digraphs, but also because most real-world graphs are sparse.
- During execution, each node accessible from a node  $j$  induces one recursive call of PRUNEACC, after which the node accessed from  $j$  is declared as visited. Thus, each entry of the accessibility list of a node is explored no more than once.
- However, line 15 of the algorithm loops over all nodes  $k$  adjacent to  $j$ . If  $a = |Adj(j)|$ , on average, then overall computational complexity becomes  $O(nka)$ .
- For practical matters, large scale experimental gene perturbations in the yeast *Saccharomyces cerevisiae* ( $n = 6300$ ) suggests that  $k < 50$ ,  $a < 1$  and thus that  $nka < n^2$  in that case.



The algorithm itself takes the accessibility list of a graph and eliminates entries inconsistent with Theorem 2 and Corollary 2.

It does so recursively until only the adjacency list of the shortcut-free graph is left.

The algorithm is shown as pseudocode. Because it operates on lists, programming languages such as perl or library extensions of other languages permitting list operations will facilitate its implementation.

(In Appendix a perl implementation of the algorithm, where accessibility and adjacency list are represented by a two-dimensional hashing array.)

```
1   for all nodes  $i$  of  $G$ 
2      $Adj(i) = Acc(i)$ 
3   for all nodes  $i$  of  $G$ 
4     if node  $i$  has not been visited
5       call PRUNE_ACC( $i$ )
6     end if
7   PRUNE_ACC( $i$ )
8     for all nodes  $j \in Acc(i)$ 
9       if  $Acc(j) = \emptyset$ 
10        declare  $j$  as visited.
11      else
12        call PRUNE_ACC( $j$ )
13      end if
14    for all nodes  $j \in Acc(i)$ 
15      for all nodes  $k \in Adj(j)$ 
16        if  $k \in Acc(i)$ 
17          delete  $k$  from  $Adj(j)$ 
18        end if
19    declare node  $i$  as visited
20  end PRUNE_ACC( $i$ )
```



The algorithm needs an accessibility list for each node  $i$ ,  $Acc(i)$ , which would be obtained from gene perturbation data and subsequent gene activity measurements for a genetic network.

In lines one and two, for each node  $i$  the adjacency list  $Adj(i)$  is initialized as equal to the accessibility list.

The algorithm will delete elements from this  $Adj(i)$  until the adjacency list of the most parsimonious network of  $Acc(G)$  is obtained.

```
1  for all nodes  $i$  of  $G$ 
2       $Adj(i) = Acc(i)$ 
3
4  for all nodes  $i$  of  $G$ 
5      if node  $i$  has not been visited
6          call PRUNE_ACC( $i$ )
7          end if
8
9  PRUNE_ACC( $i$ )
10     for all nodes  $j \in Acc(i)$ 
11         if  $Acc(j) = \emptyset$ 
12             declare  $j$  as visited.
13         else
14             call PRUNE_ACC( $j$ )
15         end if
16
17     for all nodes  $j \in Acc(i)$ 
18         for all nodes  $k \in Adj(j)$ 
19             if  $k \in Acc(i)$ 
20                 delete  $k$  from  $Adj(j)$ 
21             end if
22     end if
23     declare node  $i$  as visited
24 end PRUNE_ACC( $i$ )
```



The master loop in lines 3-6 cycles over all nodes of  $G$ , and calls the routine PRUNE\_ACC for each node  $i$ .

In the last statement of this routine (line 19) the calling node is declared as visited.

A visited node is a node whose adjacency list  $Adj(i)$  needs not be modified any further.

This is the purpose of the conditional statement in the master loop (line 4), which skips over nodes that have already been visited.

```
1  for all nodes  $i$  of  $G$ 
2       $Adj(i) = Acc(i)$ 
3  for all nodes  $i$  of  $G$ 
4      if node  $i$  has not been visited
5          call PRUNE_ACC( $i$ )
6      end if
7  PRUNE_ACC( $i$ )
8      for all nodes  $j \in Acc(i)$ 
9          if  $Acc(j) = \emptyset$ 
10             declare  $j$  as visited.
11          else
12             call PRUNE_ACC( $j$ )
13          end if
14      for all nodes  $j \in Acc(i)$ 
15          for all nodes  $k \in Adj(j)$ 
16             if  $k \in Acc(i)$ 
17                 delete  $k$  from  $Adj(i)$ 
18             end if
19  declare node  $i$  as visited
20  end PRUNE_ACC( $i$ )
```



Aside from storing *Acc* and *Adj*, the algorithm thus also needs to keep track of all visited nodes.

In an actual implementation, *Acc*, *Adj*, and any data structure that keeps track of visited nodes would need to be either global variables or passed into the routine PRUNE\_ACC, preferably by reference.

In contrast, the calling node *i* needs to be a local variable because of the recursivity of PRUNE\_ACC.

```
1  for all nodes i of G
2      Adj(i)=Acc(i)
3
4  for all nodes i of G
5      if node i has not been visited
6          call PRUNE_ACC(i)
7          end if
8
9  PRUNE_ACC(i)
10     for all nodes j ∈ Acc(i)
11         if Acc(j)=∅
12             declare j as visited.
13         else
14             call PRUNE_ACC(j)
15         end if
16
17     for all nodes j ∈ Acc(i)
18         for all nodes k ∈ Adj(j)
19             if k ∈ Acc(i)
20                 delete k from Adj(i)
21             end if
22     end for
23     declare node i as visited
24 end PRUNE_ACC(i)
```



**Function PRUNE\_ACC**

It contains of two loops. The first loop (lines 8-13) cycles over all nodes  $j$  accessible from the calling node  $i$ . If there exists a node accessible from  $j$ , then PRUNE\_ACC is called from  $j$ . If no node is accessible from  $j$ , that is, if  $Acc(j) = \emptyset$ , then  $j$  is declared as visited.

Because its accessibility list is empty, its adjacency list must be empty as well ( $Adj(i) \subseteq Acc(i)$ ), and needs no further modification.

```

1  for all nodes  $i$  of  $G$ 
2       $Adj(i) = Acc(i)$ 
3  for all nodes  $i$  of  $G$ 
4      if node  $i$  has not been visited
5          call PRUNE_ACC( $i$ )
6      end if
7  PRUNE_ACC( $i$ )
8      for all nodes  $j \in Acc(i)$ 
9          if  $Acc(j) = \emptyset$ 
10             declare  $j$  as visited.
11          else
12             call PRUNE_ACC( $j$ )
13          end if
14
15      for all nodes  $j \in Acc(i)$ 
16          for all nodes  $k \in Adj(j)$ 
17             if  $k \in Acc(i)$ 
18                 delete  $k$  from  $Adj(i)$ 
19             end if
20      declare node  $i$  as visited
21  end PRUNE_ACC( $i$ )

```



Thus, through the first loop PRUNE\_ACC calls itself recursively until a node is reached whose accessibility list is empty.

There always exists such a node, otherwise the graph would not be acyclic.

This also means that infinite recursion is not possible for an acyclic graph. Thus, the algorithm always terminates.

More precisely, the longest possible chain of nested calls of PRUNE\_ACC is  $(n-1)$  if  $G$  has  $n$  nodes.

For any node  $i$  calling PRUNE\_ACC, the number of nested calls is at most equal to the length of the longest path starting at  $i$ .

```
1  for all nodes  $i$  of  $G$ 
2       $Adj(i) = Acc(i)$ 
3
4  for all nodes  $i$  of  $G$ 
5      if node  $i$  has not been visited
6          call PRUNE_ACC( $i$ )
7          end if
8
9  PRUNE_ACC( $i$ )
10     for all nodes  $j \in Acc(i)$ 
11         if  $Acc(j) = \emptyset$ 
12             declare  $j$  as visited.
13         else
14             call PRUNE_ACC( $j$ )
15         end if
16
17     for all nodes  $j \in Acc(i)$ 
18         for all nodes  $k \in Adj(j)$ 
19             if  $k \in Acc(i)$ 
20                 delete  $k$  from  $Adj(i)$ 
21             end if
22     end for
23     declare node  $i$  as visited
24 end PRUNE_ACC( $i$ )
```



The second loop of PRUNE\_ACC (lines 14-18) only starts once the algorithm has explored all nodes accessible from the calling node  $i$ , that is, as the function calls made during the first loop return.

In the second loop the principle of Corollary 2 is applied.

Specifically, the second loop cycles over all nodes  $j$  accessible from  $i$  in line 14.

```

1  for all nodes  $i$  of  $G$ 
2       $Adj(i) = Acc(i)$ 
3  for all nodes  $i$  of  $G$ 
4      if node  $i$  has not been visited
5          call PRUNE_ACC( $i$ )
6      end if
7  PRUNE_ACC( $i$ )
8      for all nodes  $j \in Acc(i)$ 
9          if  $Acc(j) = \emptyset$ 
10             declare  $j$  as visited.
11          else
12             call PRUNE_ACC( $j$ )
13          end if
14          for all nodes  $j \in Acc(i)$ 
15             for all nodes  $k \in Adj(j)$ 
16                 if  $k \in Acc(i)$ 
17                     delete  $k$  from  $Adj(i)$ 
18                 end if
19          declare node  $i$  as visited
20      end PRUNE_ACC( $i$ )
    
```



In a slight deviation from what Corollary 2 suggests, line 15 cycles not over all nodes  $k \in Acc(j)$ , but only over  $k \in Adj(j)$ .

All nodes  $k \in Adj(j)$  are deleted from  $Adj(i)$  in lines 16-18. Cycling only over  $k \in Adj(j)$  saves time, but does not compromise the requirement that all nodes  $k \in Adj(i)$  be removed, because line 14 covers all nodes  $j$  accessible from  $i$ .

Because of the equality proven in Theorem 2, once this has been done, the adjacency list need not be modified further. This is why upon leaving this routine, the calling node is declared as visited.

Notice also that if a node  $j$  with  $Acc(j) = \epsilon$  is encountered, the loop in line 15 is not executed.

```

1   for all nodes  $i$  of  $G$ 
2      $Adj(i) = Acc(i)$ 
3
3   for all nodes  $i$  of  $G$ 
4     if node  $i$  has not been visited
5       call PRUNE_ACC( $i$ )
6     end if
7
7   PRUNE_ACC( $i$ )
8     for all nodes  $j \in Acc(i)$ 
9       if  $Acc(j) = \emptyset$ 
10        declare  $j$  as visited.
11      else
12        call PRUNE_ACC( $j$ )
13      end if
14
14    for all nodes  $j \in Acc(i)$ 
15      for all nodes  $k \in Adj(j)$ 
16        if  $k \in Acc(i)$ 
17          delete  $k$  from  $Adj(i)$ 
18        end if
19    declare node  $i$  as visited
20  end PRUNE_ACC( $i$ )

```



```
1   for all nodes  $i$  of  $G$ 
2       if  $component[i]$  has not been defined
3           create new node  $x$  of  $G^*$ 
4            $component[i]=x$ 
5           for all nodes  $j \in Acc(i)$ 
6               if  $i \in Acc(j)$ 
7                    $component[j]=x$ 
8               end if
9           end if

10  for all nodes  $i$  of  $G^*$ 
11       $Acc_{G^*}(i)=\emptyset$ 
12  for all nodes  $i$  of  $G$ 
13      for all nodes  $j \in Acc(i)$ 
14          if  $component[i] \neq component[j]$ 
15              if  $component[j] \notin Acc_{G^*}(component[i])$ 
16                  add  $component[j]$  to  $Acc_{G^*}(component[i])$ 
17              end if
18          end if
```

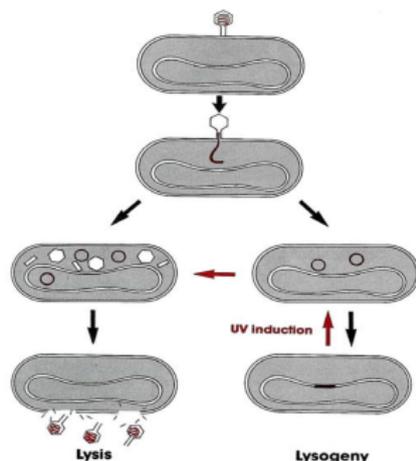


- A. Wagner Bioinformatics 17, 2001  
(a different method: ARACNE: an algorithm for the reconstruction of gene regulatory networks in a mammalian cellular context <http://www.ncbi.nlm.nih.gov/pubmed/16723010>)



## Gillespie algorithm: The Biological problem

Many studies have reported occurrence of stochastic fluctuations and noise in living systems. Observation of gene expression in individual cells has clearly established the stochastic nature of transcription and translation. When using deterministic modeling approaches, for examples differential equations, we assume that the biological system evolves along a fixed path from its initial state. Such an approach cannot be taken for modeling stochastic processes such as gene networks. Using deterministic methods, it is not possible to capture emergent phenomena that arise from inherent randomness. Example below: virus decision (hide in the genome vs kill the host and exit) is dictated by noise.



Consider a system of  $N$  molecular species  $S_1, \dots, S_N$  interacting through  $M$  elemental chemical reactions  $R_1, \dots, R_M$ .

We assume that the system is confined to a constant volume  $W$  and is well stirred and at a constant temperature. Under these assumptions, the state of the system can be represented by the populations of the species involved.

We denote these populations by  $X(t) = X_1(t), \dots, X_N(t)$ , where  $X_i(t)$  is the number of molecules of species  $S_i$  in the system at time  $t$ . The well stirred condition is crucial. For each reaction  $R_j$ , we define a propensity function  $a_j$ , such that  $a_j(x)dt$  is the probability, given  $X(t) = x$ , that one  $R_j$  reaction will occur in time interval  $[t, t + dt)$ . State change vector  $v_j$ , whose  $i$ th component is defined by  $v_{j,i}$  the change in the number of  $S_i$  molecules produced by one  $R_j$  reaction.



The most important method to simulate a network of biochemical reactions is the Gillespie's stochastic simulation algorithm (SSA)

- The Gillespie algorithm is widely used to simulate the behavior of a system of chemical reactions in a well stirred container
- The key aspects of the algorithm is the drawing of two random numbers at each time step, one to determine after how much time the next reaction will take place, the second one to choose which one of the reactions will occur.
- Each execution of the Gillespie algorithm will produce a calculation of the evolution of the system. However, any one execution is only a probabilistic simulation, and the chances of being the same as a particular reaction is vanishingly small.
- Therefore it should be run many times in order to calculate a stochastic mean and variance that tells us about the behaviour of the system.
- the complexity of the Gillespie algorithm is  $O(M)$  where  $M$  is the number of reactions.



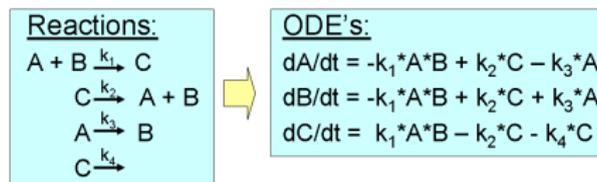
- 1 Initialise: set the initial molecule numbers, set time  $t = 0$ .
- 2 Calculate the propensity function  $a_j$  for each reaction, and the total propensity according to equation  $a_0(x) \equiv \sum_{j=1}^M a_j(x)$ ,  $j = 1, \dots, M$ .
- 3 Generate two uniformly distributed random numbers  $rand_1$  and  $rand_2$  from the range (0, 1).
- 4 Compute the time  $\tau$  to the next reaction using equation  $\tau = \frac{1}{a_0(x)} \ln\left(\frac{1}{rand_1}\right)$ .
- 5 Decide which reaction  $R_\mu$  occurs at the new time using equation  $rand_2 > \sum_{k=1}^{\mu-1} a_k \dots \text{and} \dots rand_2 < \frac{1}{a_0} \sum_{k=1}^{\mu} a_k$ .
- 6 Update the state vector  $v$  (molecules quantity) by adding the update vector :  
 $v(t + \tau) = v(t) + (\nu)_\mu$
- 7 Set  $t = t + \tau$ . Return to step 2 until  $t$  reaches some specified limit  $t_{MAX}$ .



In each step, the SSA starts from a current state  $x(t) = x$  and asks two questions: When will the next reaction occur? We denote this time interval by  $t$ . When the next reaction occurs, which reaction will it be? We denote the chosen reaction by the index  $j$ . To answer the above questions, one needs to study the joint probability density function  $p(\tau, j | x, t)$  that is the probability, given  $X(t) = x$ , that the next reaction will occur in the infinitesimal time interval  $[t + \tau, t + \tau + dt]$ . The theoretical foundation of SSA is given by  $p(\tau, j | x, t) = a_j(x) \exp(-a_0(x)\tau)$ , where  $a_0(x) \equiv \sum_{j=1}^M a_j(x)$ . It implies that the time  $t$  to the next occurring reaction is an exponentially distributed random variable with mean  $1/a_0(x)$ , and that the index  $j$  of that reaction is the integer random variable with point probability  $a_j(x)/a_0(x)$ . The  $\tau$  is given by  $\tau = \frac{1}{a_0(x)} \ln\left(\frac{1}{r_1}\right)$ .

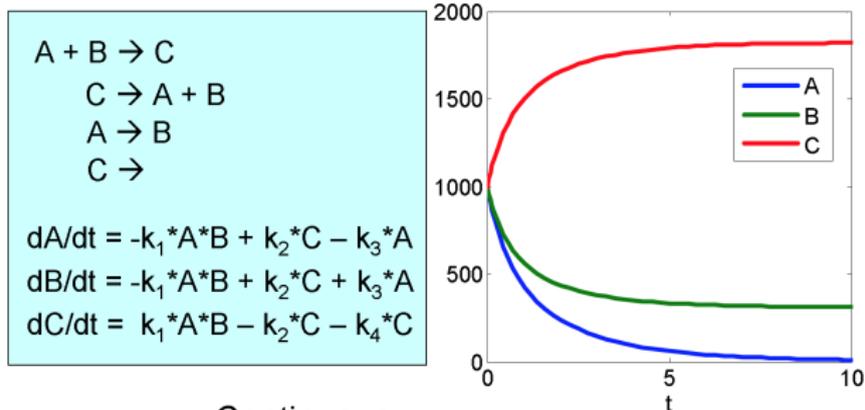
The system state is then updated according to  $X(t + \tau) = x + \nu_j$  and this process is repeated until the simulation final time or until some other terminating condition is reached.





In a deterministic modeling approach, given a set of reactions and the initial conditions, we integrate the coupled equations for some period of time.

## Deterministic Solution

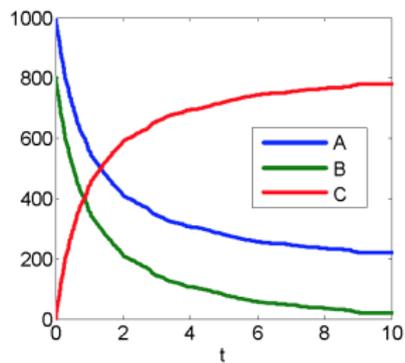
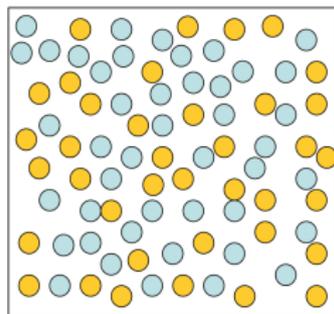


- Continuous
- Average kinetic rates represent reaction probabilities

Solving the systems of ODEs we obtain the concentrations/numbers of species A,B,C in time.



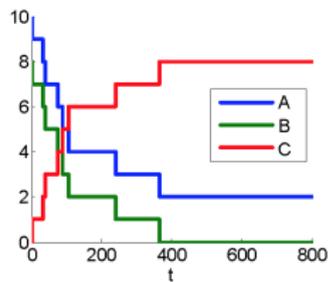
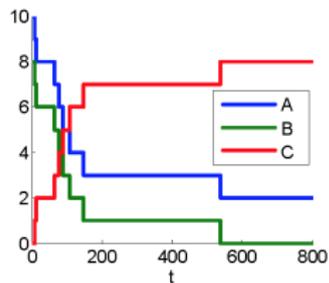
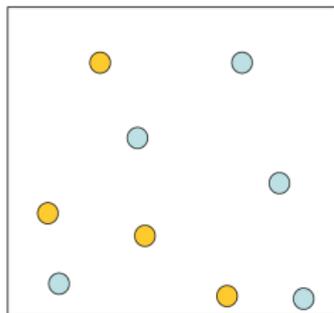
## Validity of Deterministic Solution



The deterministic approach works well with large numbers.



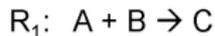
# Validity of Deterministic Solution



With small number of molecules the approach shows limitations.



- $c_{\mu} dt$  = average probability that a *particular* combination of reactants will react according to  $R_{\mu}$  in the next time interval  $dt$
- $h_{\mu}$  = number of reactant combinations
- $h_{\mu} c_{\mu} dt = a_{\mu} dt$  = average probability that an  $R_{\mu}$  reaction will occur somewhere inside  $V$  in the next time interval  $dt$



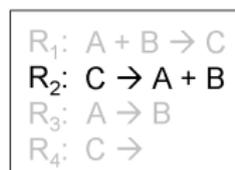
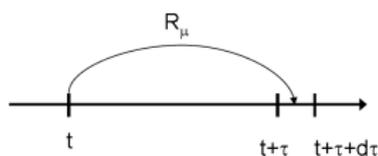
X molecules of A  
Y molecules of B }  $h_1 = XY$  reactant combinations

$XYc_1 dt$  = probability that an  $R_1$  reaction will occur somewhere inside  $V$  in the next time interval  $dt$



- Avoid averaging assumptions
- Probabilistic formulation
  - *When* does next reaction occur?
  - *Which* reaction occurs next?
- Reaction probability density function:
 

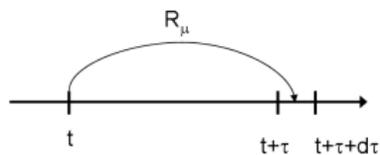
$P(\tau, \mu)d\tau =$  probability at time  $t$  that the next reaction is  $R_\mu$  and occurs in interval  $(t+\tau, t+\tau+d\tau)$



System State:

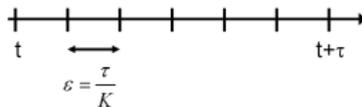
	$t$	$t+\tau$
A	1	2
B	1	2
C	8	7





$$P(\tau, \mu) d\tau = \underbrace{P_0(\tau)}_{\text{probability that no reaction occurs during } (t, t+\tau)} \underbrace{h_\mu c_\mu d\tau}_{\text{probability that reaction } \mu \text{ occurs during } (t+\tau, t+\tau+d\tau)}$$

- Divide  $(t, t+\tau)$  into  $K$  subintervals of width  $\varepsilon$



- Probability that none of the reactions occur in any of the  $K$  subintervals:

$$P_0(\tau) = \left[ 1 - \sum_{i=1}^M h_i c_i \varepsilon \right]^K = \lim_{K \rightarrow \infty} \left[ 1 - \frac{\sum_{i=1}^M h_i c_i \tau}{K} \right]^K = e^{-\sum_{i=1}^M h_i c_i \tau}$$



# Deriving $P_0(\tau)$ for Reaction PDF

$$\begin{aligned}
 P(\tau, \mu) &= h_\mu c_\mu P_0(\tau) = h_\mu c_\mu e^{-\sum_{i=1}^M h_i c_i \tau} \\
 &= a_\mu e^{-a_0 \tau} \\
 &= \left( a_0 e^{-a_0 \tau} \right) \left( \frac{a_\mu}{a_0} \right) = \underbrace{P(\tau)}_{\text{when next reaction occurs}} \cdot \underbrace{P(\mu|\tau)}_{\text{which reaction occurs}}
 \end{aligned}$$

$R_M$	$a_M$
	...
$R_3$	$a_3$
$R_2$	$a_2$
$R_1$	$a_1$

}

$a_0 = \sum_{i=1}^M a_i$

$\leftarrow \text{rand}_2 a_0$

$$\tau = (1/a_0) \ln(1/\text{rand}_1)$$

$\mu$  is the integer for which  $\sum_{i=1}^{\mu-1} a_i < \text{rand}_2 a_0 < \sum_{i=1}^{\mu} a_i$



1. Initialization
  - Set values of  $c_\mu$  for the M reactions.
  - Set initial population sizes
2. Calculate the M values  $a_\mu$  and  $a_0 = \sum a_\mu$ .
3. Generate  $(\tau, \mu)$  based on  $P(\tau, \mu)$
4. Adjust population levels according to the reaction  $R_{\mu'}$  and increase  $t$  by  $\tau$
5. Return to Step 2



- Advantages
  - continuous time, discrete population changes
  - captures effects of noise
  - simple implementation
  - small memory requirements
- Disadvantages
  - CPU intensive
  - typically must simulate many runs
  - must use good random number generator
    - periodicity affects size of simulation
    - resolution limits range of probabilities



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- Progressive alignment: <http://www.ebi.ac.uk/Tools/msa/clustalw2/>;
- Phylogenetic software: <http://evolution.genetics.washington.edu/phylip/software.html>
- HMM: <http://www.cbs.dtu.dk/services/TMHMM/>
- <http://genes.mit.edu/GENSCAN.html>
- Gibbs sampling [http://bayesweb.wadsworth.org/cgi-bin/gibbs.8.pl?data\\_type=DNA](http://bayesweb.wadsworth.org/cgi-bin/gibbs.8.pl?data_type=DNA)
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## Figures acknowledgement (also orally during the lecture; it refers to the numbers in the copies distributed during the lectures, i.e. 4 slides/pages)

- pag 3, Church et al, Next-Generation Digital Information Storage in DNA, 28 SEPTEMBER 2012 VOL 337 - pag 4 from Bower and Boulori Computational modeling of genetic and biochemical networks, MIT Press, from Shalini Venkataraman and Vidhya Gunaseelan CS 594: An Introduction to Computational Molecular Biology, Nir Friedmans and Shlomo Moran lectures. - pag 5 H Koepl's Lecture at ETH. - pag 11 from Pevzner P Computational Molecular Biology: An Algorithmic Approach, MIT Press. - pag 14 from Volker Sperschneider Bioinformatics: Problem Solving Paradigms, springer. - pag 18,19 from Ming Li, University of Waterloo. - pag 21,22 from Wall lab website, Harvard. - pag 23,24 from Compeau Nature Biotechnology 29, 987991, (2011). - pag 22,26 from Alicia Clum, DOE Joint Genome Institute. - pag 33 from G Caldarelli at the European Complex Systems Conference. - pag 34 from paper on TribeMCL (see previous slide). - pag 36,37,38 from Nir Friedmans and Shlomo Moran lectures at [www.cs.huji.ac.il](http://www.cs.huji.ac.il). - pag 40, 41 from C. Burge and S. Karlin Prediction of Complete Gene Structures in Human Genomic DNA.J. Mol. Biol. (1997) 268, 78-94. - pag 42,43 Chris Burge's lecture on DNA Motif Modeling and Discovery at MIT. - pag 52 from Wilkinson Stochastic Modeling, CRC Press.



- Align the two strings: ACGCTG and CATGT, with match score =1 and mismatch, gap penalty equal -1
- Describe with one example the difference between Hamming and Edit distances
- Discuss the complexity of an algorithm to reconstruct a genetic network from microarray perturbation data
- Discuss the properties of the Markov clustering algorithm and the difference with respect to the k-means and hierarchical clustering algorithms



## Examples of Answers

Align the two strings: ACGCTG and CATGT, with match score =1 and mismatch, gap penalty equal -1

		A	C	G	C	T	G
	0	1	2	3	4	5	6
0	0	-1	-2	-3	-4	-5	-6
C 1	-1	-1	1	0	-1	-2	-3
A 2	-2	1	0	0	-1	-2	-3
T 3	-3	0	0	-1	-1	1	0
G 4	-4	-1	-1	2	1	0	3
T 5	-5	-2	-2	1	1	3	2

Describe with one example the difference between Hamming and Edit distances  
*TGCATAT* → *ATCCGAT* in 4 steps; *TGCATAT* (insert A at front); *ATGCATAT* (delete 6th T); *ATGCATA* (substitute G for 5th A); *ATGCGTA* (substitute C for 3rd G); *ATCCGAT* (Done).



## Examples of Answers

Discuss the complexity of an algorithm to reconstruct a genetic network from microarray perturbation data

Reconstruction:  $O(nka)$  where  $n$  is the number of genes,  $k$  is the average number of entries in the accession list;  $a$  is the average number of entries in adjacency list. Large scale experimental gene perturbations in the yeast *Saccharomyces cerevisiae* ( $n=6300$ ) suggests that  $k < 50$ ,  $a < 1$ , and thus that  $nka \ll n^2$ .



Discuss the properties of the Markov clustering algorithm and the difference with respect to the k-means and hierarchical clustering algorithms

MCL algorithm: We take a random walk on the graph described by the similarity matrix and after each step we weaken the links between distant nodes and strengthen the links between nearby nodes.

The k-means algorithm is composed of the following steps: 1) Place  $K$  points into the space represented by the objects that are being clustered. These points represent initial group centroids. 2) Assign each object to the group that has the closest centroid. 3) When all objects have been assigned, recalculate the positions of the  $K$  centroids. 4) Repeat Steps 2 and 3 until the centroids no longer move. This produces a separation of the objects into groups from which the metric to be minimized can be calculated.

Hierarchical clustering: Start with each point its own cluster. At each iteration, merge the two clusters; with the smallest distance. Eventually all points will be linked into a single cluster. The sequence of mergers can be represented with a rooted tree.

