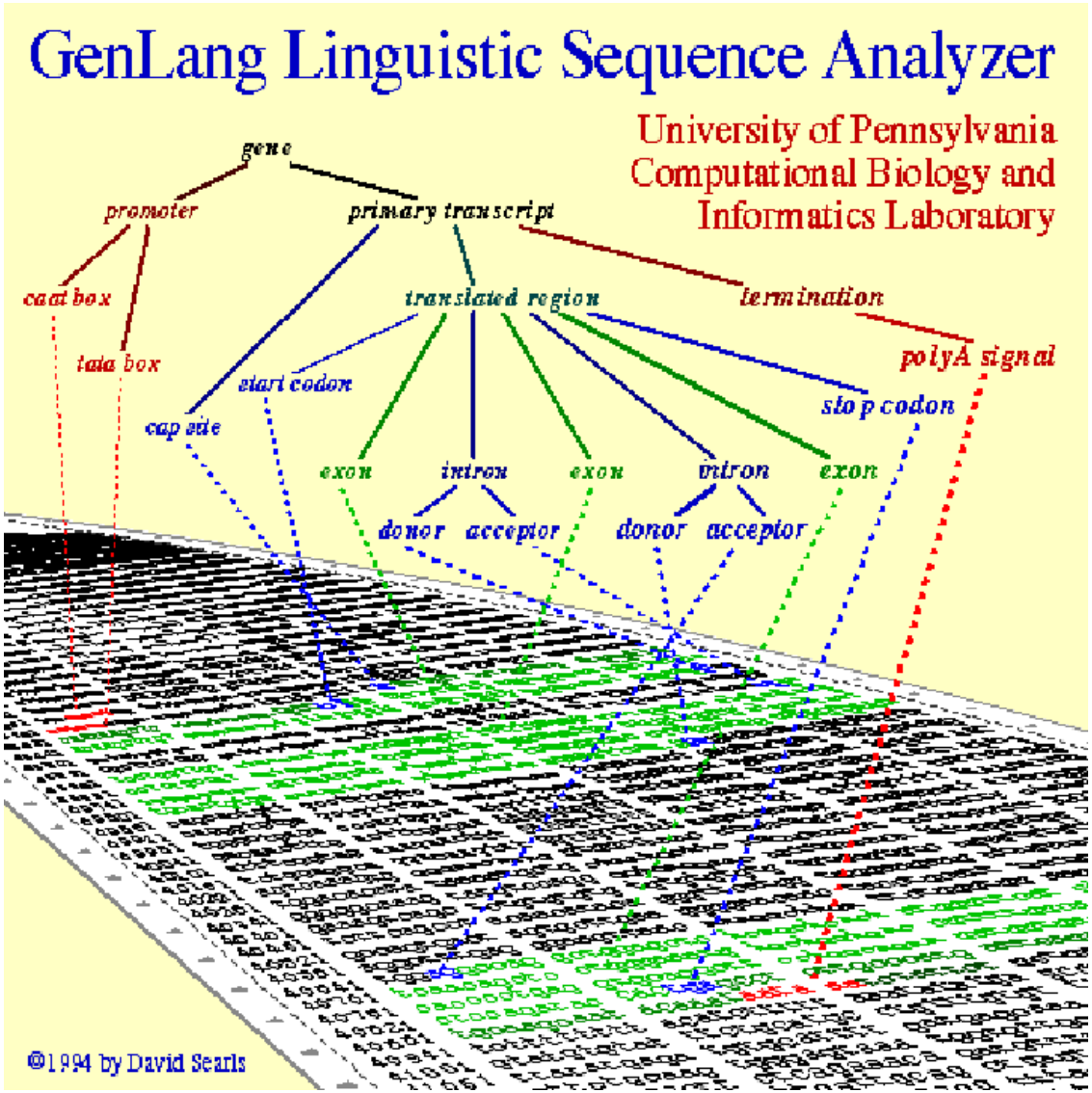
Modelling Biological Sequences by Grammatical Inference

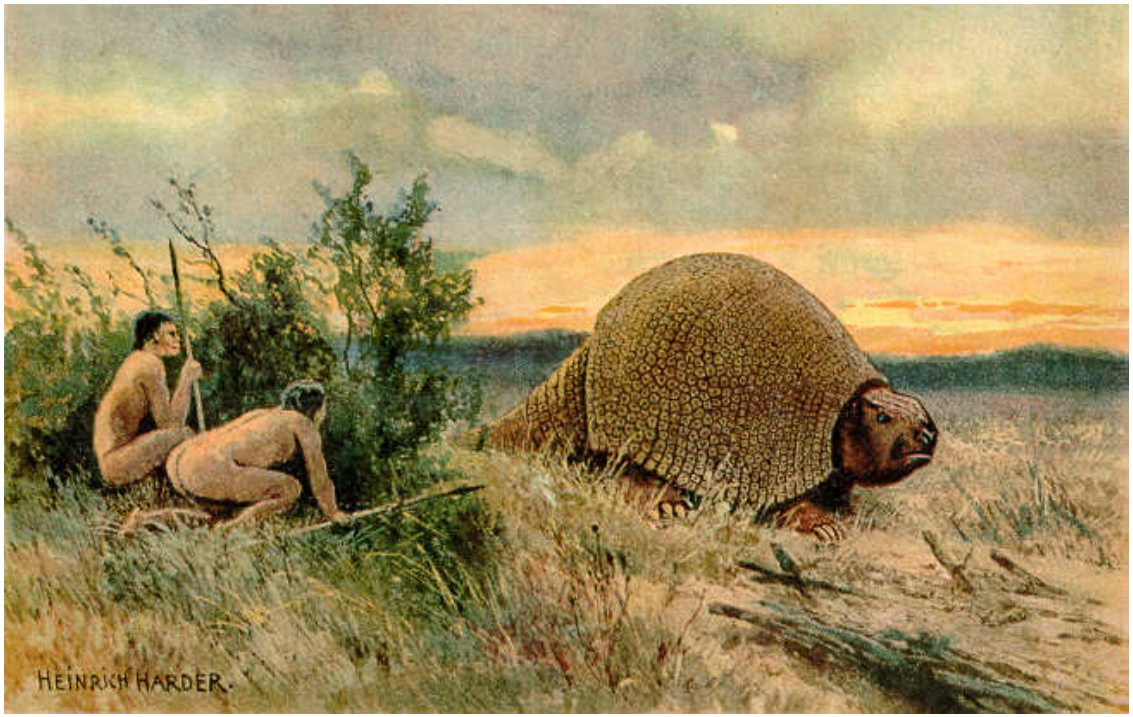
François Coste

ICGI 2010 Tutorial Day

Motivation: learning the grammar of DNA



This talk: *The Paleolithic* of Modelling Biological Sequences by Grammatical Inference



artistic view of old stone edge Glyptodon by Heinrich Harder

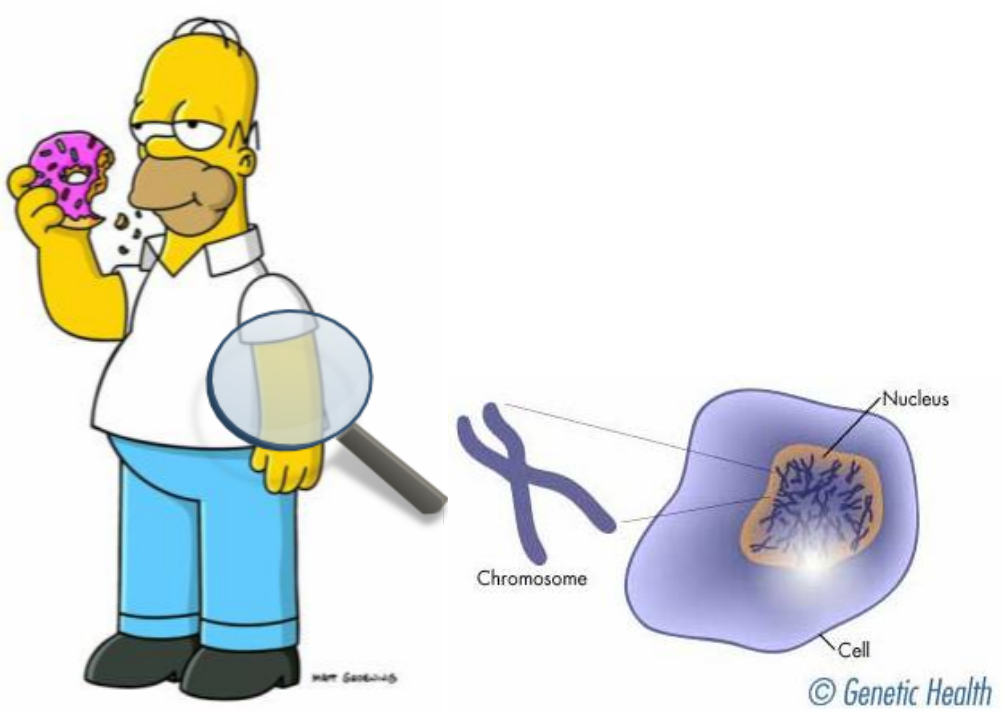
Focus on working tools and specificity of modelling biosequences

Outline

1. Molecular genetics
   - Central “dogma”
   - Technology
   - Sequences annotation
2. Modelling a set of conserved sequences
3. Inference of grammatical structure

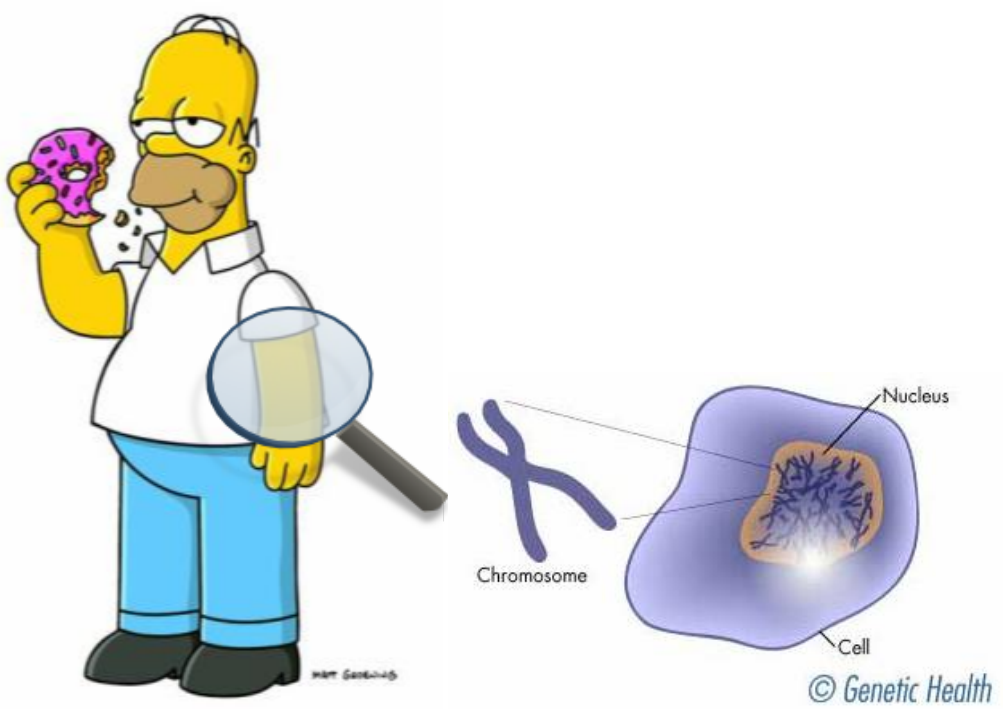
DNA

Genetic heredity



DNA

Genetic heredity

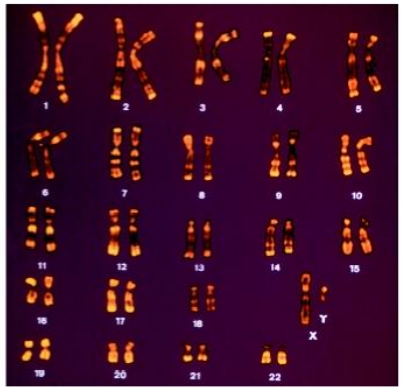


Genome

Organism's hereditary information

DNA

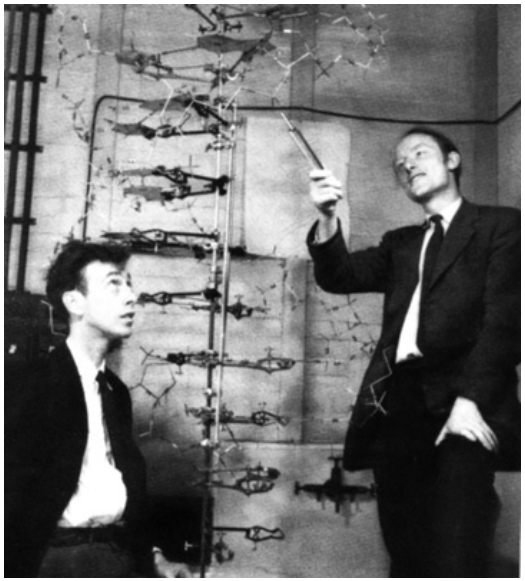
Genetic heredity



Genome

Organism's hereditary information

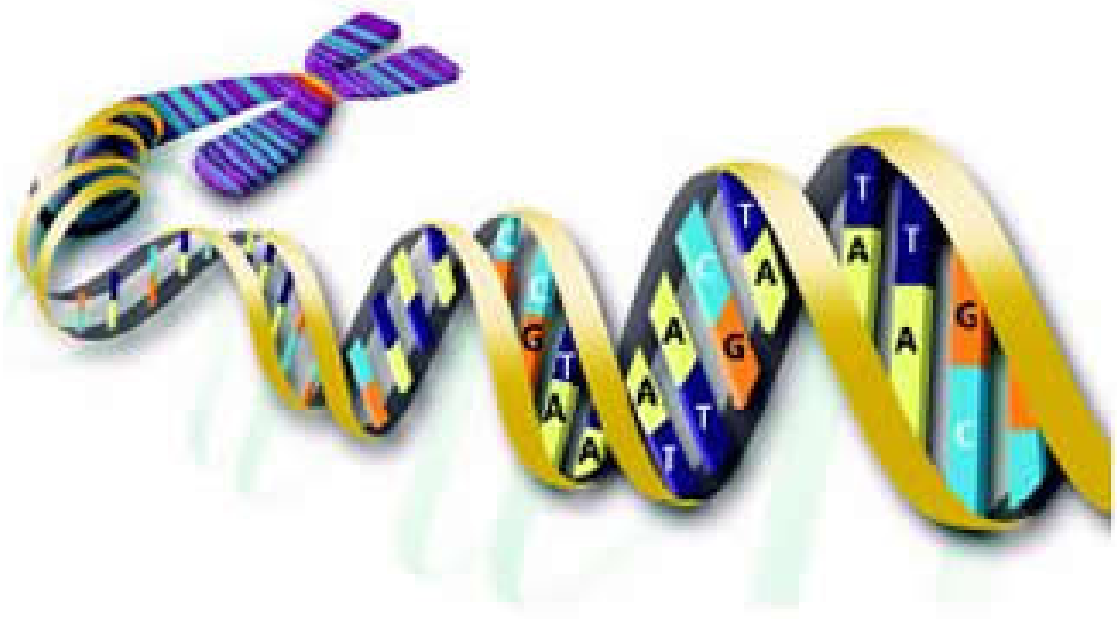
DNA structure

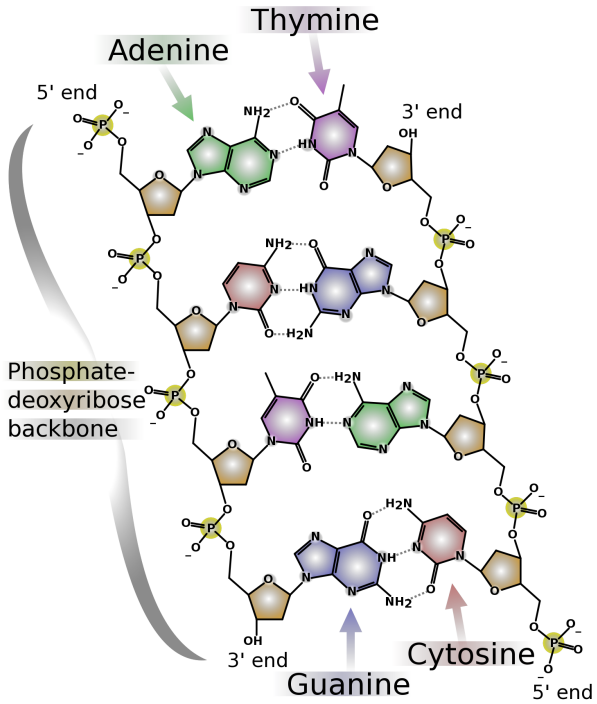


source: U.S. Department of Energy

"We wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest."
John Watson and Francis Crick in *Molecular Structure of Nucleic Acids. A structure for deoxyribose nucleic acid.* Nature No. 4356, April 25, 1953

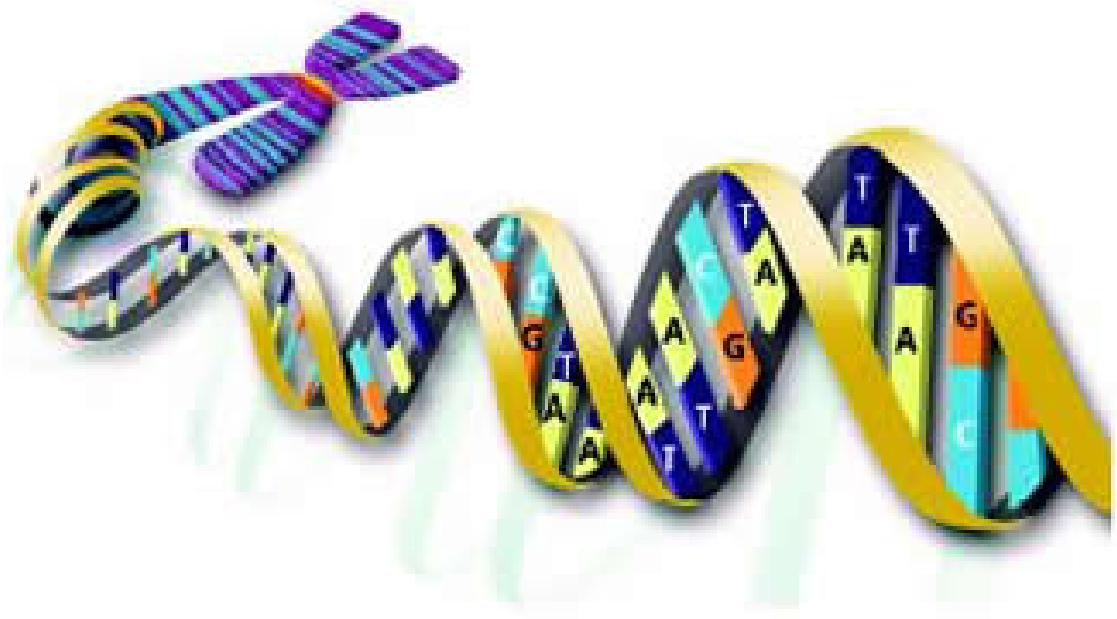
DNA sequence



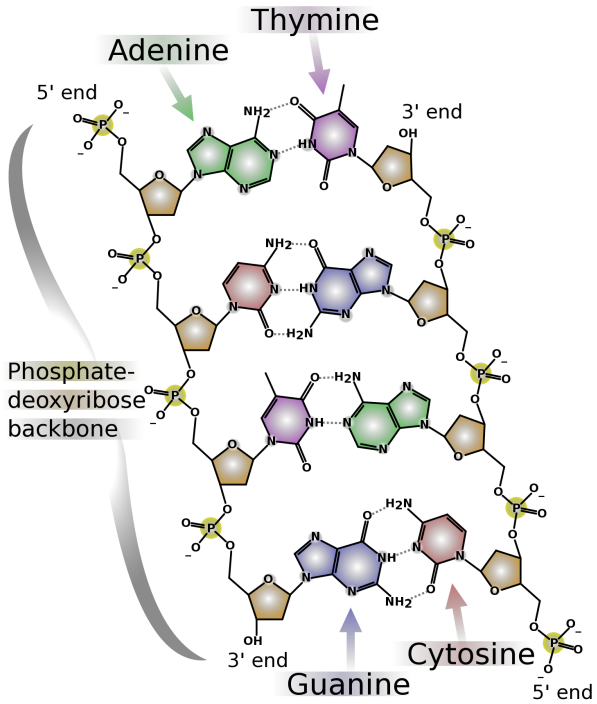


source: U.S. Department of Energy

DNA sequence



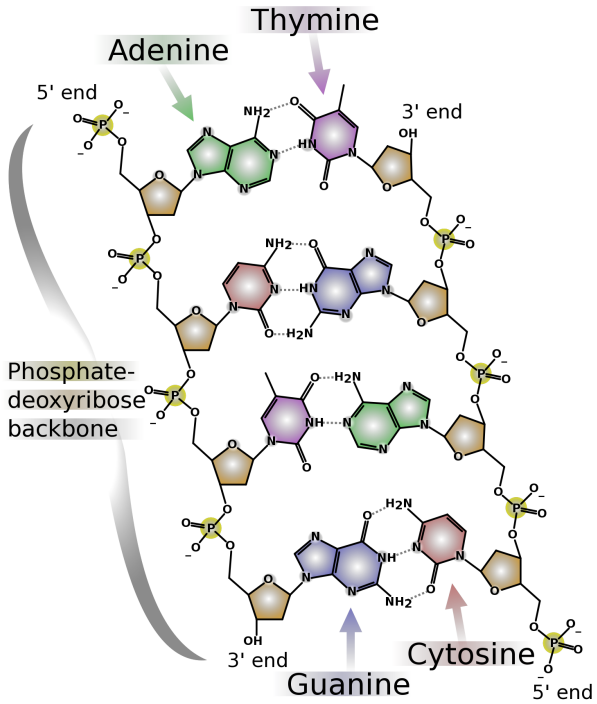
source: U.S. Department of Energy



DNA information

Sequence over a 4 letter alphabet {A,C,G,T} (nucleotide bases)

DNA sequence



source: U.S. Department of Energy

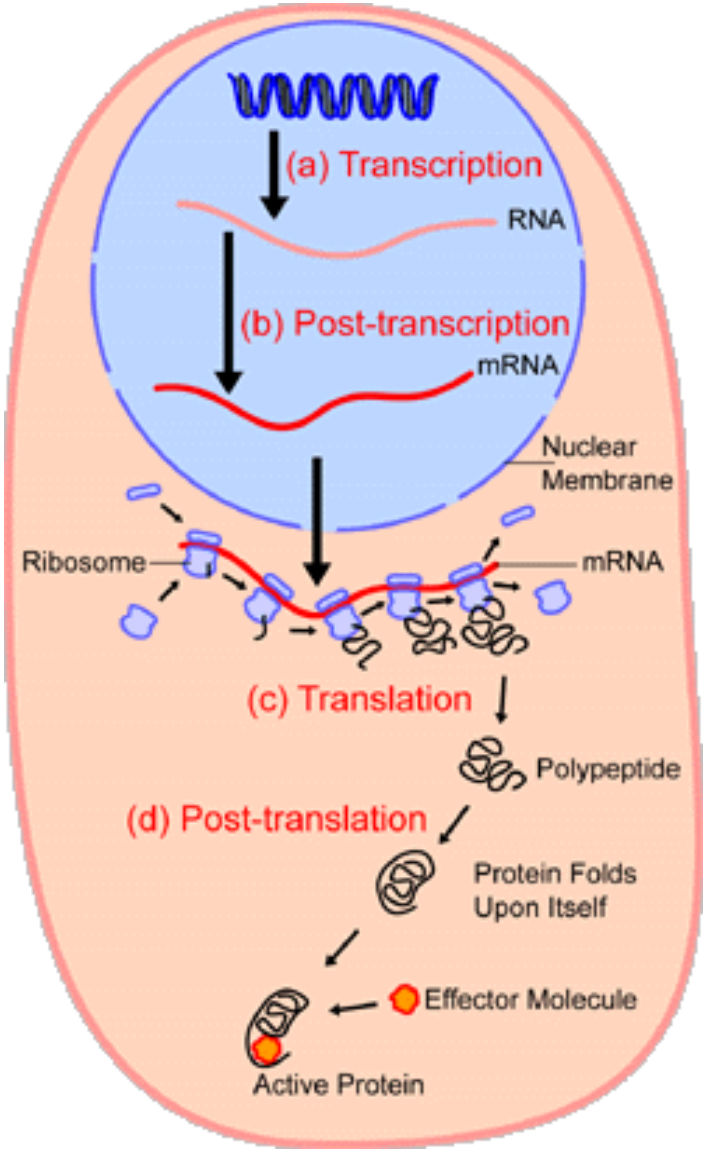
ACTGGATCACAGGTCTATCACCCTATTAACCACTCACGGGAGCT
CTCCATGCATTTGGTATTTTCGTCTGGGGGGTGTGCACGCGAT
AGCATTGCGAGACGCTG...

DNA information

Sequence over a 4 letter alphabet {A,C,G,T} (nucleotide bases)

From DNA to Function

DNA sequence
↓
RNA sequence
↓
Protein sequence
↓
Protein structure
↓
Function (Phenotype)



see also `http://www.youtube.com/watch?v=ZjRCmUO_dhY`

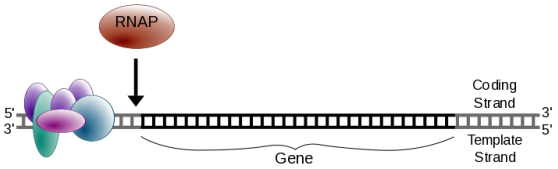
DNA to RNA

Transcription

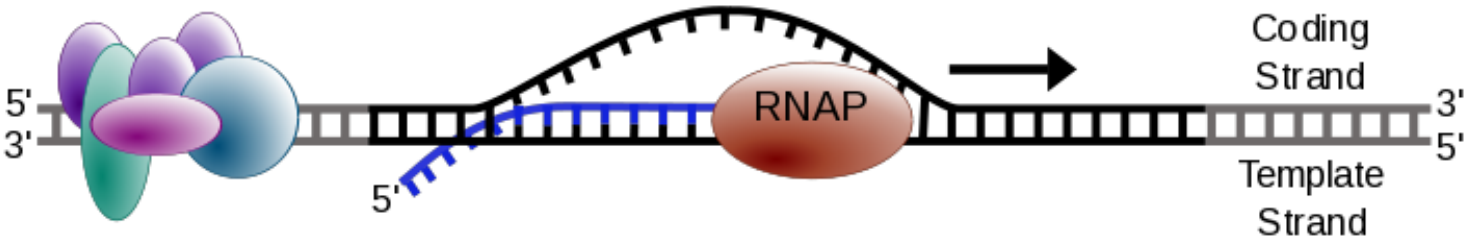
(double strand) DNA sequence {A, T, C, G}

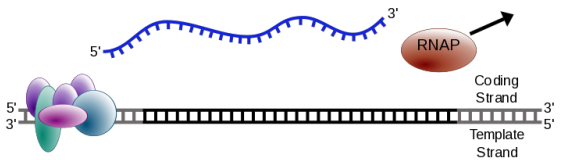
↓

(simple) RNA sequence {A, U, C, G}



RNA-Polymerase





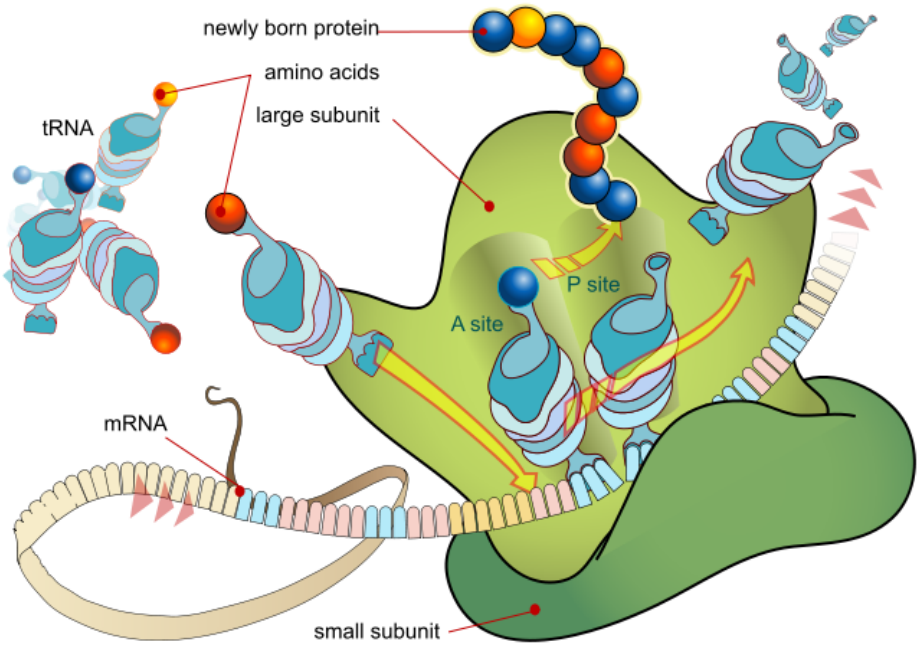
RNA to Protein

Translation

RNA sequence {A, U, C, G}

↓

Protein sequence {20 amino acids}

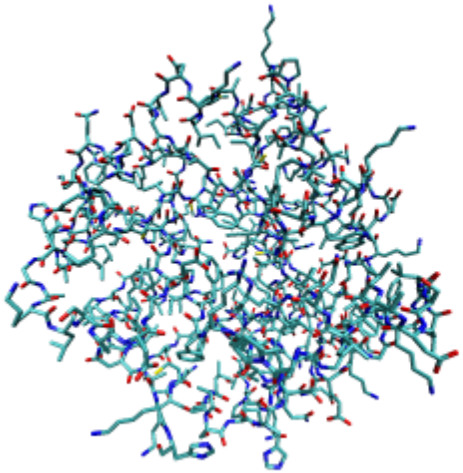


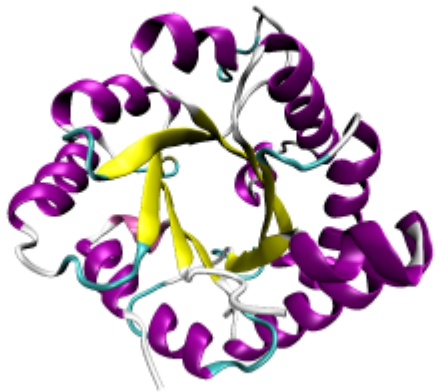
Ribosome

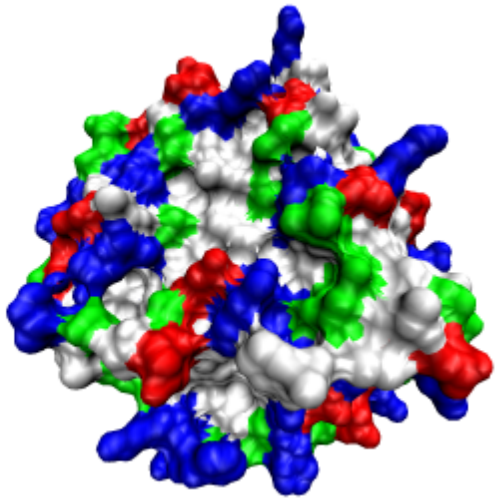
Seond letter

First letter	U	C	A	G	Third letter
U	UUU Phe, UUC Phe	UCU Ser, UCC Ser, UCA Ser, UCG Ser	UAU Tyr, UAC Tyr	UGU Cys, UGC Cys	U C A G
	UUA Leu, UUG Leu		UAA Stop, UAG Stop	UGA Stop, UGG Trp	
C	CUU Leu, CUC Leu, CUA Leu, CUG Leu	CCU Pro, CCC Pro, CCA Pro, CCG Pro	CAU His, CAC His	CGU Arg, CGC Arg, CGA Arg, CGG Arg	U C A G
			CAA Gin, CAG Gin		
A	AUU Ile, AUC Ile, AUA Ile	ACU Thr, ACC Thr, ACA Thr, ACG Thr	AAU Asn, AAC Asn	AGU Ser, AGC Ser	U C A G
	AUG Met		AAA Lys, AAG Lys	AGA Arg, AGG Arg	
G	GUU Val, GUC Val, GUA Val, GUG Val	GCU Ala, GCC Ala, GCA Ala, GCG Ala	GAU Asp, GAC Asp	GGU Gly, GGC Gly, GGA Gly, GGG Gly	U C A G
			GAA Glu, GAG Glu		

Proteins







Homer Simpson, 70kg

- Water: 42kg
- Protein: 12kg
- Fat: 12kg
- Bone Minerals: 3.5kg
- Carbohydrate: 0.5kg

Protein functions:

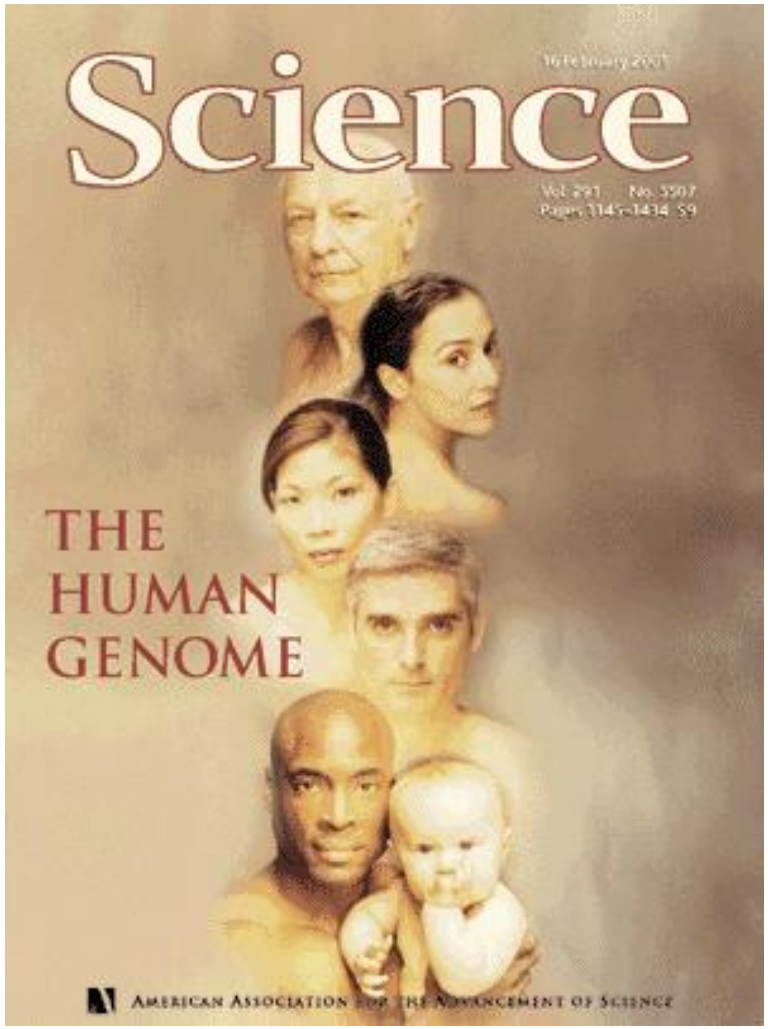
Structure, Transport, Antibodies, Muscles, Enzymes, Hormones, Brain

...

Reading the Book of Life

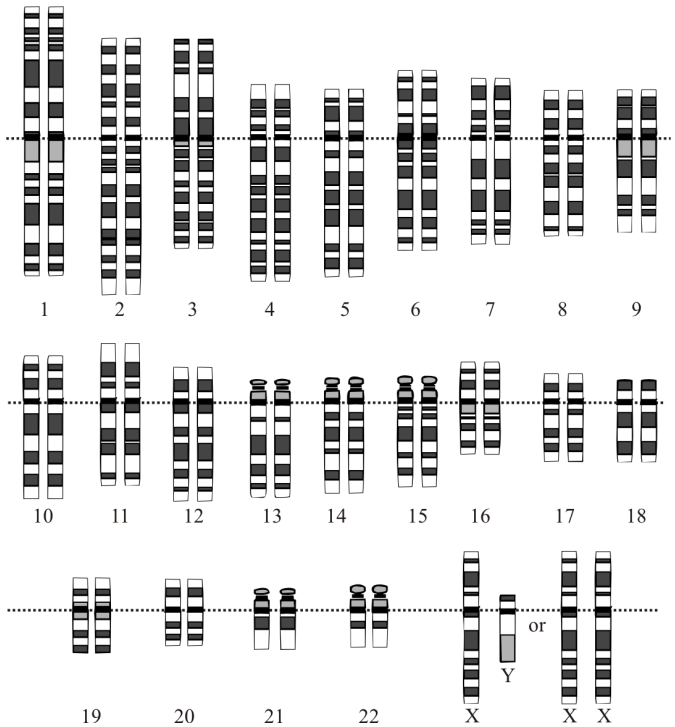
Genome Sequencing

First draft of the human genome (2001)



First draft of the human genome (2001)

Consortium 20 labs, 6 countries



3.2 billion bp, $\sim$ 25000 genes (previous estimates was $\sim$ 100,000, function unknown for 50% of predicted genes) covering about 3% genome length, >50% repeat sequences...

Genome sequencing projects

Genome Project Statistic:

Genome sequencing projects statistics

Organism	Complete	Draft assembly	In progress	total
Prokaryotes	794	580	568	1942
Archaea	75	5	33	113
Bacteria	720	575	535	1830
Eukaryotes	38	249	270	557
Animals	5	109	119	233
Mammals	2	38	41	81
Birds		2	13	15
Fishes		13	13	26
Insects	2	25	19	46
Flatworms		2	3	5
Roundworms	1	13	11	25
Amphibians		1		1
Reptiles		1		1
Other animals		16	22	38
Plants	6	23	74	103
Land plants	3	19	69	91
Green Algae	3	4	4	11
Fungi	18	82	36	136
Ascomycetes	15	63	25	103
Basidiomycetes	1	12	8	21
Other fungi	2	7	3	12
Protists	9	33	37	79
Apicomplexans	5	10	4	19
Kinetoplasts	3	2	3	8
Other protists	1	20	30	51
total:	**832**	**829**	**838**	**2499**

Revised: Sep 07, 2010

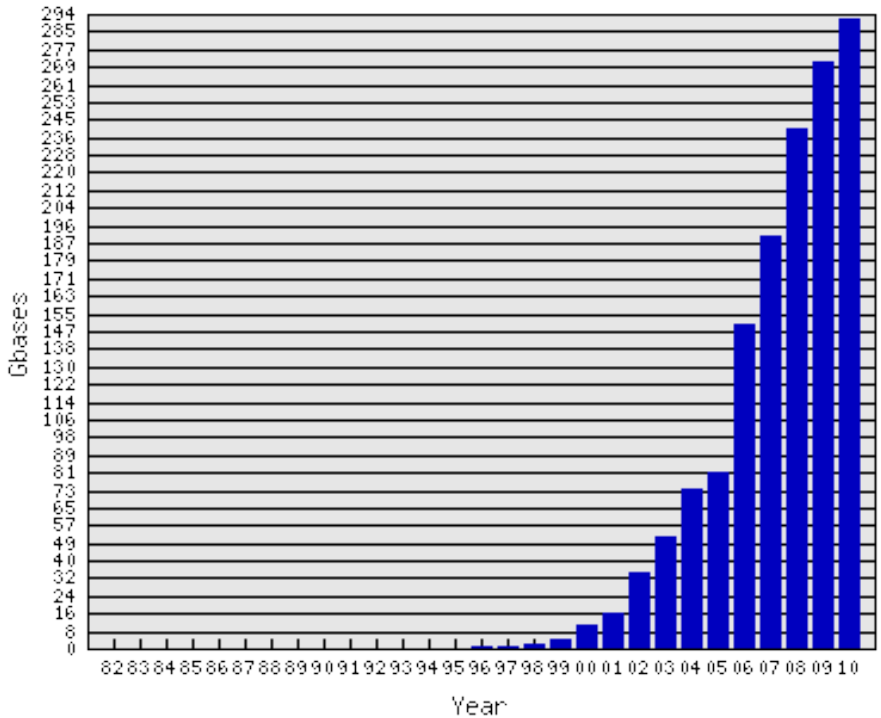
New generation sequencing

Next-generation sequencing machines at the Wellcome Trust Sanger Institute. Wellcome Library, London.

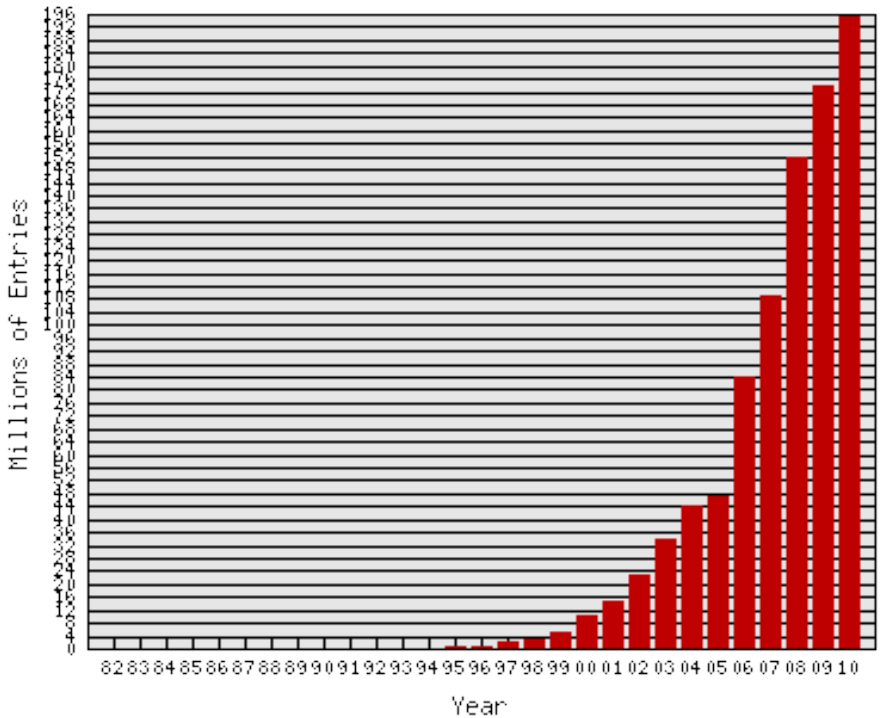
- Human Genome Project: $\sim$ 10 years, <\$3 billion USD
- 2008: 2 weeks, \$60,000 USD
- Archon X Prize for Genomics:
  <2013: 100 human genomes in ten days for \$10,000 USD each
- 1000\$ USD personal genome...

EMBL Database Growth

Total nucleotides (current 292,065,553,621)

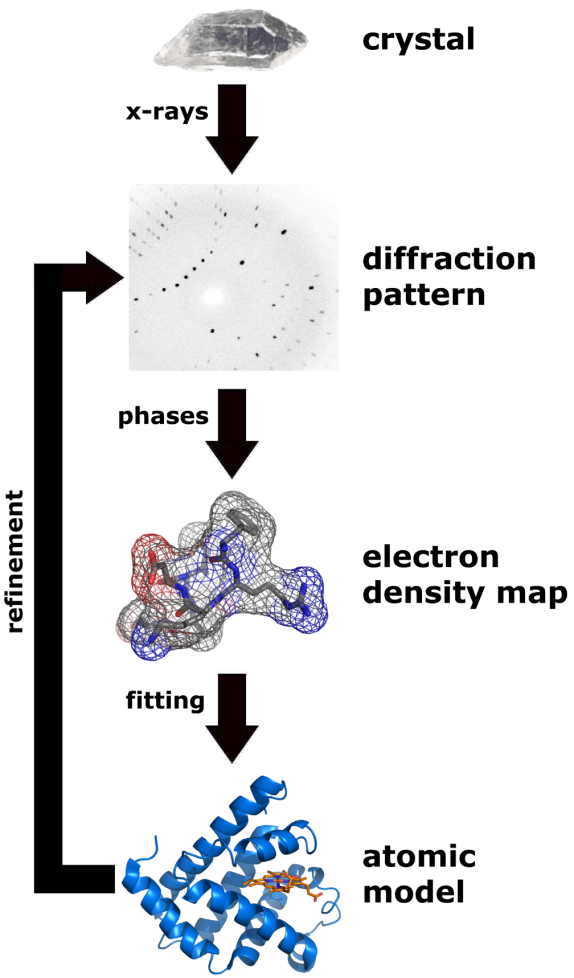


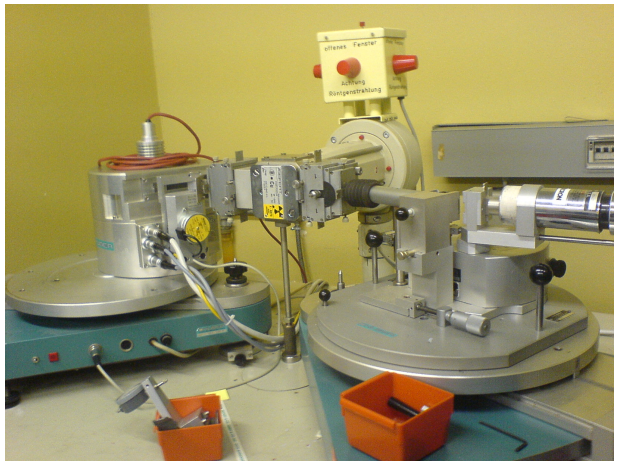
Number of entries (current 195,230,921)



source: http://www.ebi.ac.uk/embl/Services/DBStats/ Sep 6, 2010

Protein structure determination





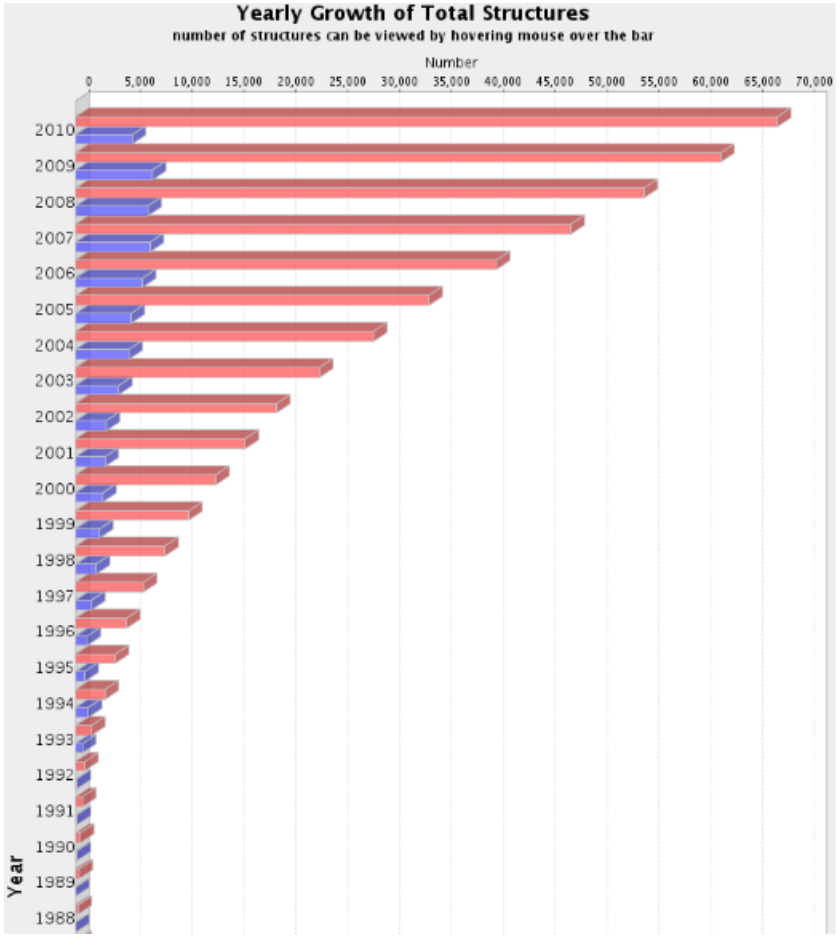
Structures in RCSP Protein Data Bank

`www.pdb.org`

- 90% by X-ray crystallography
  9% Nuclear Magnetic Resonance
- Proteins: 62773, Nucleic Acids: 2172, Protein/NA: 2811 Others: 38
  Total: 67794
- Mainly globular proteins

PDB database Growth

67,794 structures, Sep 07, 2010 ▸ embl

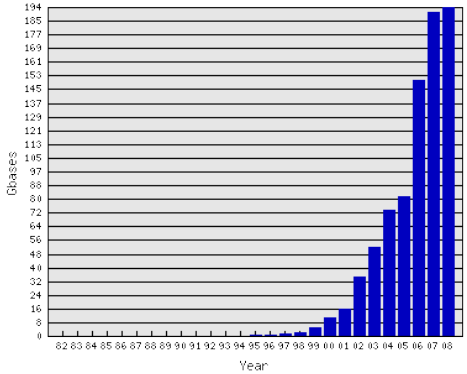


http://www.rcsb.org/pdb/statistics/holdings.do

http://www.rcsb.org/pdb/statistics/contentGrowthChart.do?content=total

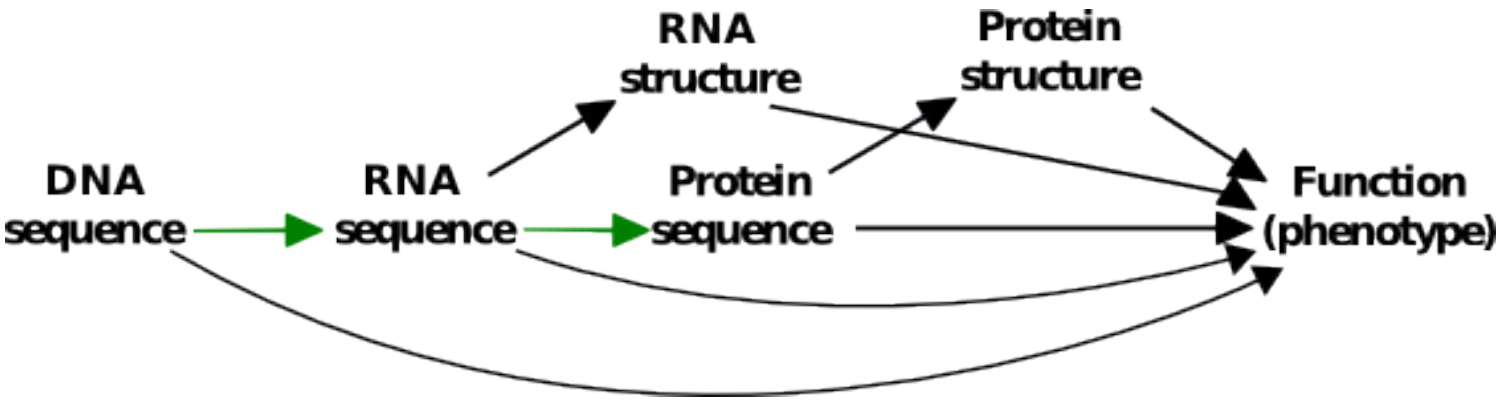
Sequences annotation

High throughput production of raw sequences



Problem

Function(s) of these sequences ?

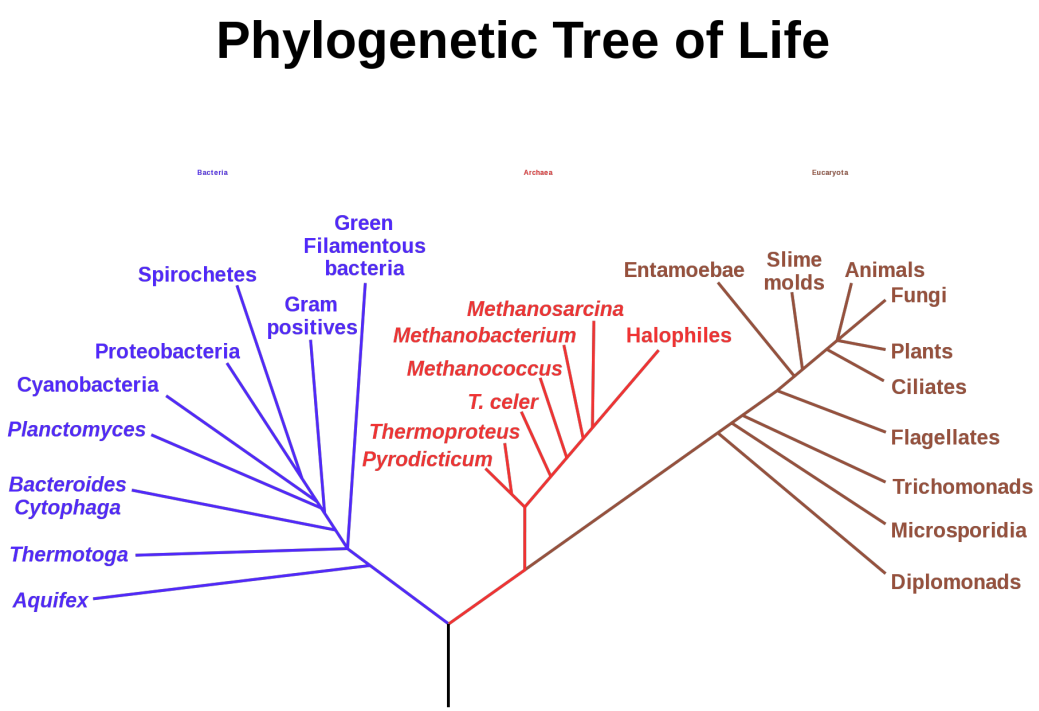


How to predict the function of a sequence ?

2 main approaches:

1. Find a similar sequence
   needs:
   - a database of annotated sequences
     GenBank (NCBI), EMBL Nucleotide Sequence Database, DNA Data Bank of Japan (DDBJ), ...
   - a tool returning similar sequences
     BLAST ...
2. Find a model accepting the sequence
   needs:
   - a database of model for each "function" of interest
     TRANSFAC,JASPAR, PROSITE, INTERPRO
   - a tool returning models recognizing the sequence
     ScanProsite, InterProScan, ...

Conservation

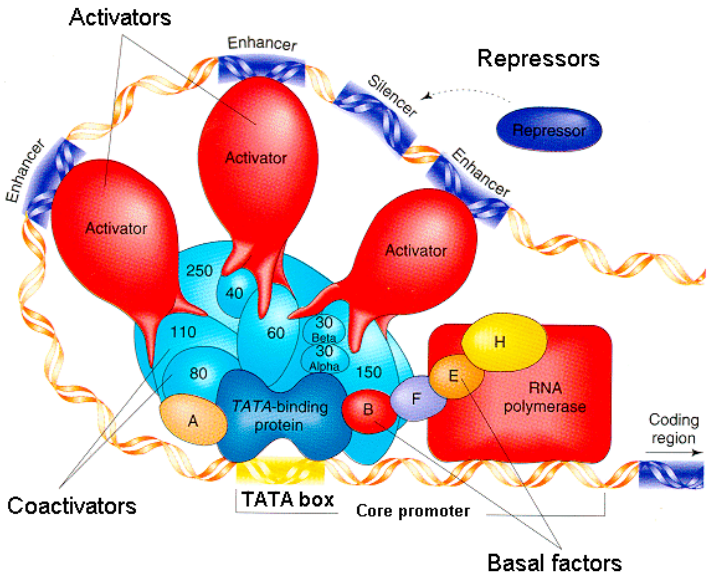


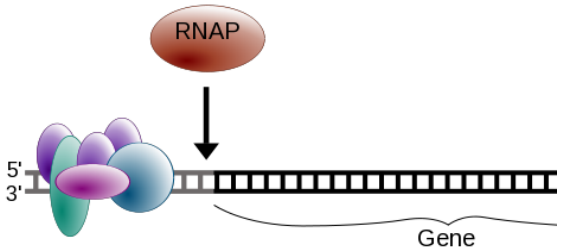
Key idea

Evolution + Natural Selection $\Rightarrow$ Conservation is meaningful

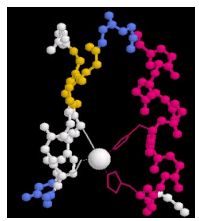
Conserved sequence

- DNA: TATA-box (5'-TATAAA-3')





- Protein: zinc finger



From identity to similarity

Blocks

From identity to similarity

Blocks

BLOSUM62 **substitution matrix** Frequently observed substitutions receive positive scores and seldom observed substitutions are given negative scores (log odds ratio)

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

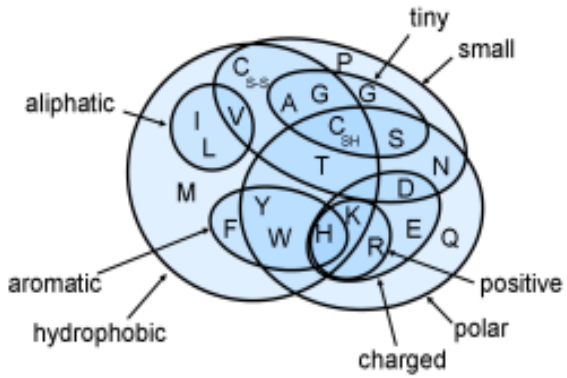
From identity to similarity

Blocks

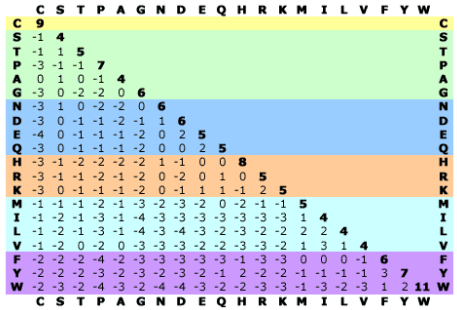
```
P41696  CPYCHRLFSQATHLEVHVRSHIGYKPFVCD
D6W2H2  CPYCHRLFSQATHLEVHVRSHIGYKPFVCD
A6ZNW1  CPYCHRLFSQATHLEVHVRSHIGYKPFVCD
C7GMM3  CPYCHRLFSQATHLEVHVRSHIGYKPFVCD
C5DSE2  CPYCHRFFTQSTHLEVHVRSHIGYKPFLCE
        ******:*:*:**********************:*:
```

BLOSUM62 **substitution matrix** Frequently observed substitutions receive positive scores and seldom observed substitutions are given negative scores (log odds ratio)

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



Aligning two proteins



Smith&Waterman, Blast, ...

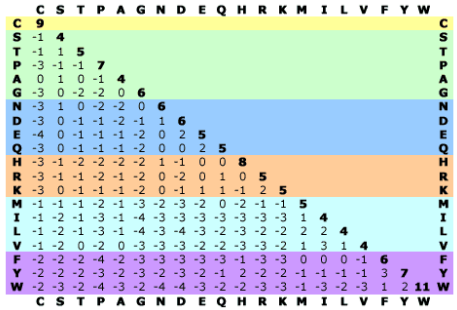
```
AAB24882        TYHMCQFHCRYVNNHSGEKLYECNERSKAFSCPSHLQCHKRRQIGEKTHEHNQCGKAFPT 60
AAB24881        --------------------YECNQCGKAFAQHSSLKCHYRTHIGEKPYECNQCGKAFSK 40
                                     ****: .***:  * *:** * :*****.:* ********..

AAB24882        PSHLQYHERTHTGEKPYECHQCGQAFKKCSLLQRHKRTHTGEKPYE-CNQCGKAFAQ- 116
AAB24881        HSHLQCHKRTHTGEKPYECNQCGKAFSQHGLLQRHKRTHTGEKPYMNVINMVKPLHNS 98
                 **** *:***********:***:**.: .**************     :  *.: :
```

Alignment score:

$$\Sigma_p(Blosum(a_{1,p}, a_{2,p})) + f(\text{gap})$$

Aligning two proteins



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                                     ****: .***:  * *:** * :*****.:* ********..

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AAB24881        HSHLQCHKRTHTGEKPYECNQCGKAFSQHGLLQRHKRTHTGEKPYMNVINMVKPLHNS 98
                 **** *:***********:***:**.: .*****************     :  *.: :
```

Alignment score:

$$\Sigma_p(Blosum(a_{1,p}, a_{2,p})) + f(\text{gap})$$

+ significativity (E-value) $\longrightarrow$ tool for approach 1.

Multiple sequence alignment

ClustalW2, . . .

```
ABC3G_LAGLA/285-305     Cfs..CaekVaeflqenpHvnl..H
ABRU_DROME/546-567      Cpk..CgkiYrsahtlrtHledk.H
ACE1_TRIRE/402-424      CrepgCtkeFkrpcdltkHekt..H
ACE2_SCHPO/445-467      ClyngCnkrIarkynvesHiqt..H
ACE2_SCHPO/475-495      Cdl..CkagFvrhhdlkrHlri..H
ACE2_YEAST/605-627      ClypnCnkvFkrrynirsHiqt..H
ACE2_YEAST/635-657      CdfpgCtkaFvrnhdlirHkis..H
ADNP2_HUMAN/772-793     Clf..CpctFhdikglseHsrnr.H
ADNP2_HUMAN/877-899     Cpf..CfgpFvtteayelHlkerhH
ADNP2_MOUSE/802-823     Clf..CpctFhdvrglveHsrtk.H
```

Multiple sequence alignment

ClustalW2, ...

```
ABC3G_LAGLA/285-305     Cfs..CaekVaeflqenpHvnl..H
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ACE2_SCHPO/475-495      Cdl..CkagFvrhhdlkrHlri..H
ACE2_YEAST/605-627      ClypnCnkvFkrrynirsHiqt..H
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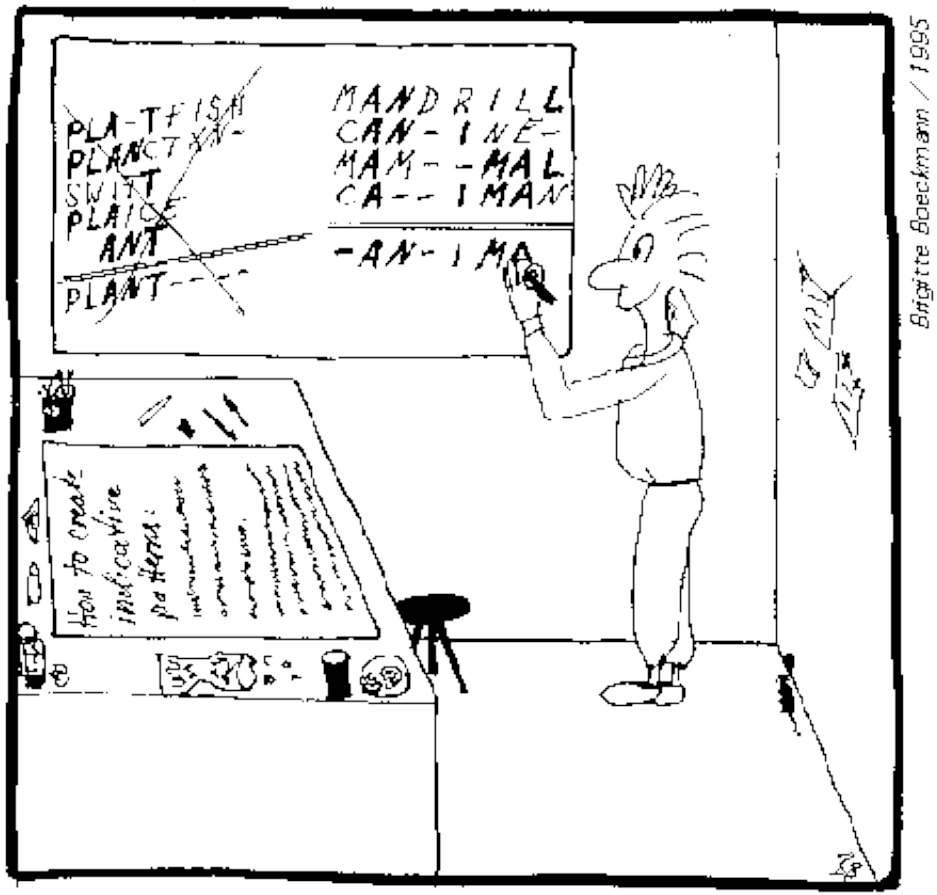
ADNP2_MOUSE/802-823     Clf..CpctFhdvrglveHsrtk.H
```

Can be used to build models of the sequence family for approach 2. (exact models and their probabilistic mate)

How we develop Prosite patterns!



http://expasy.org/prosite/

1 Molecular genetics

2 Modelling a set of conserved sequences
- Patterns
- Allowing insertion
- Allowing insertions and deletions
- With covariations

3 Inference of grammatical structure

Modelling a set of aligned sequences

Task: Model a family of (bio)sequences
(same function, structure, localization, expression, . . .)
Goals: Understand, Predict, Generate
Assumption: Characterization of family from sequence only
Available: A representative sample of the family and a multiple alignment of its conserved region (from bio experiments or a computational alignment tool)

```
ABC3G_LAGLA/285-305     Cfs..CaekVaeflqenpHvnl..H
ABRU_DROME/546-567      Cpk..CgkiYrsahtlrtHledk.H
ACE1_TRIRE/402-424      CrepgCtkeFkrpcdltkHekt..H
ACE2_SCHPO/445-467      ClyngCnkrIarkynvesHiqt..H
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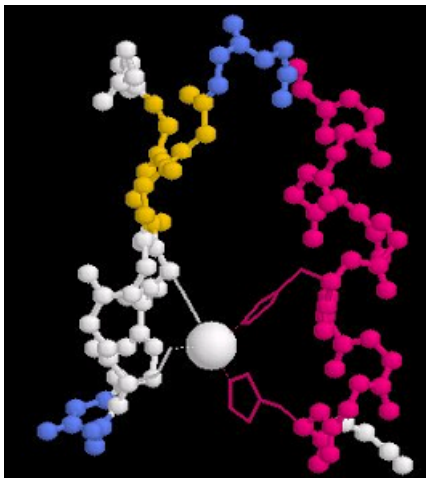
ADNP2_MOUSE/802-823     Clf..CpctFhdvrglveHsrtk.H
```



Modelling a set of aligned sequences

Task: Model a family of (bio)sequences
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ADNP2_HUMAN/772-793     Clf..CpctFhdikglseHsrnr.H
ADNP2_HUMAN/877-899     Cpf..CfgpFvtteayelHlkerhH
ADNP2_MOUSE/802-823     Clf..CpctFhdvrglveHsrtk.H
```
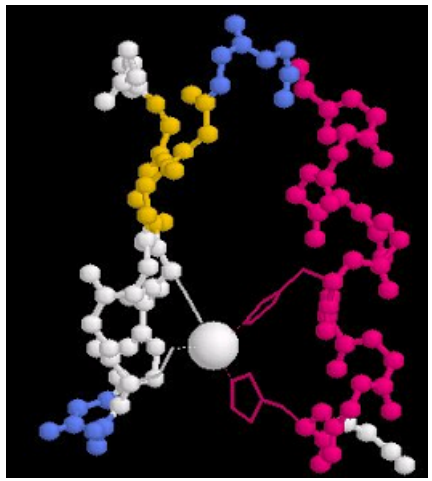
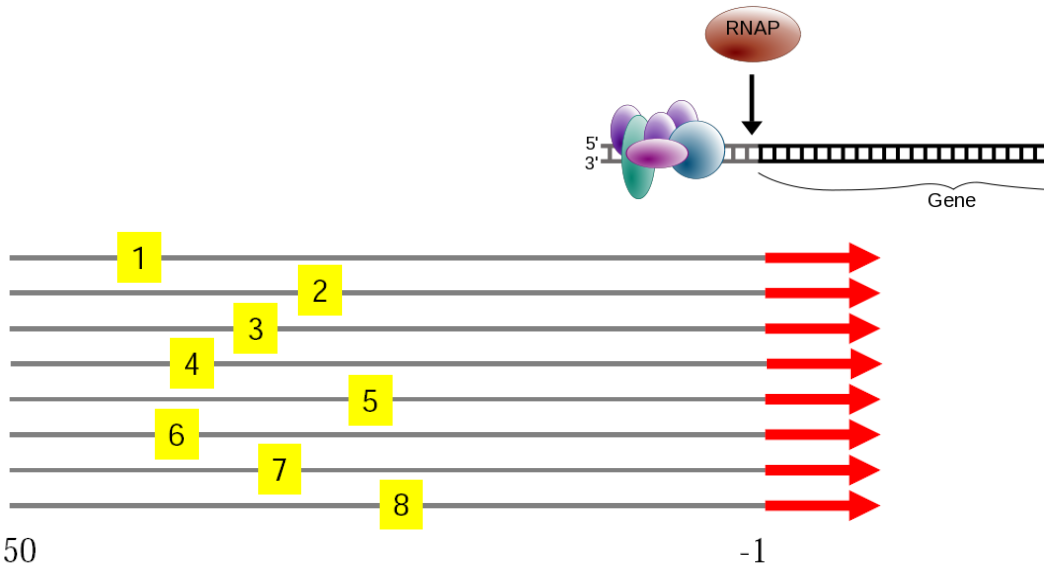



How to model this conservation ?

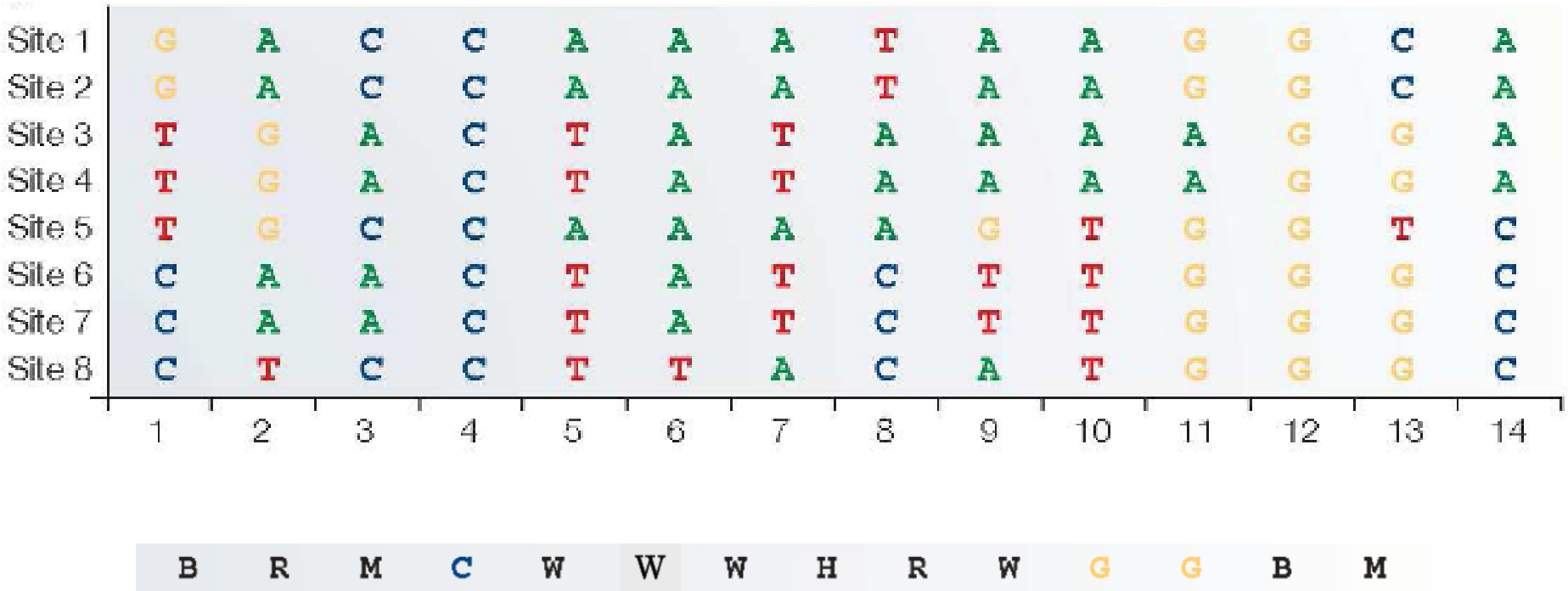
Example: Modelling binding sites



Site 1	G	A	C	C	A	A	A	T	A	A	G	G	C	A
Site 2	G	A	C	C	A	A	A	T	A	A	G	G	C	A
Site 3	T	G	A	C	T	A	T	A	A	A	A	G	G	A
Site 4	T	G	A	C	T	A	T	A	A	A	A	G	G	A
Site 5	T	G	C	C	A	A	A	A	G	T	G	G	T	C
Site 6	C	A	A	C	T	A	T	C	T	T	G	G	G	C
Site 7	C	A	A	C	T	A	T	C	T	T	G	G	G	C
Site 8	C	T	C	C	T	T	A	C	A	T	G	G	G	C
	1	2	3	4	5	6	7	8	9	10	11	12	13	14

example from Maximilian Haussler, INRIA tech. report RR-5714

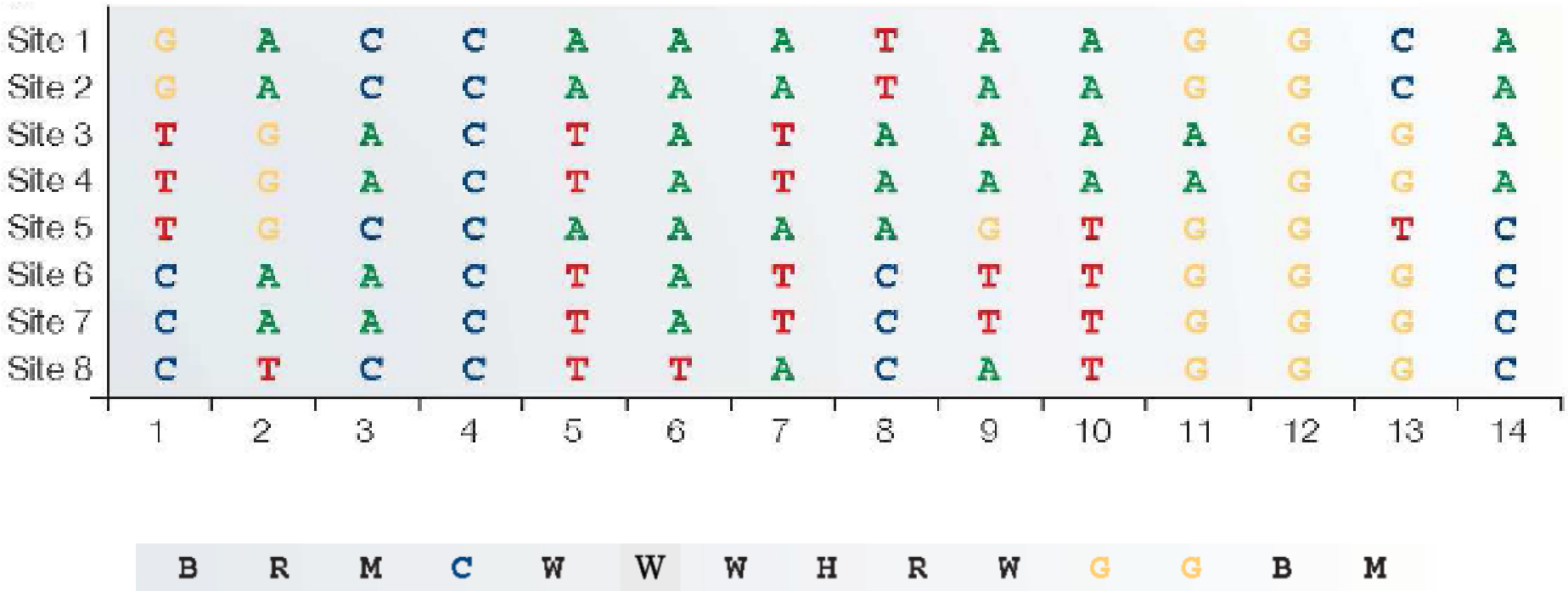
Consensus motif



Consensus motif (sequence), IUPAC code

[TCG] [ATG] [AC] C [AT] [AT] [AT] [ATC] [ATG] [AT] G G [TCG] [AC]

Consensus motif



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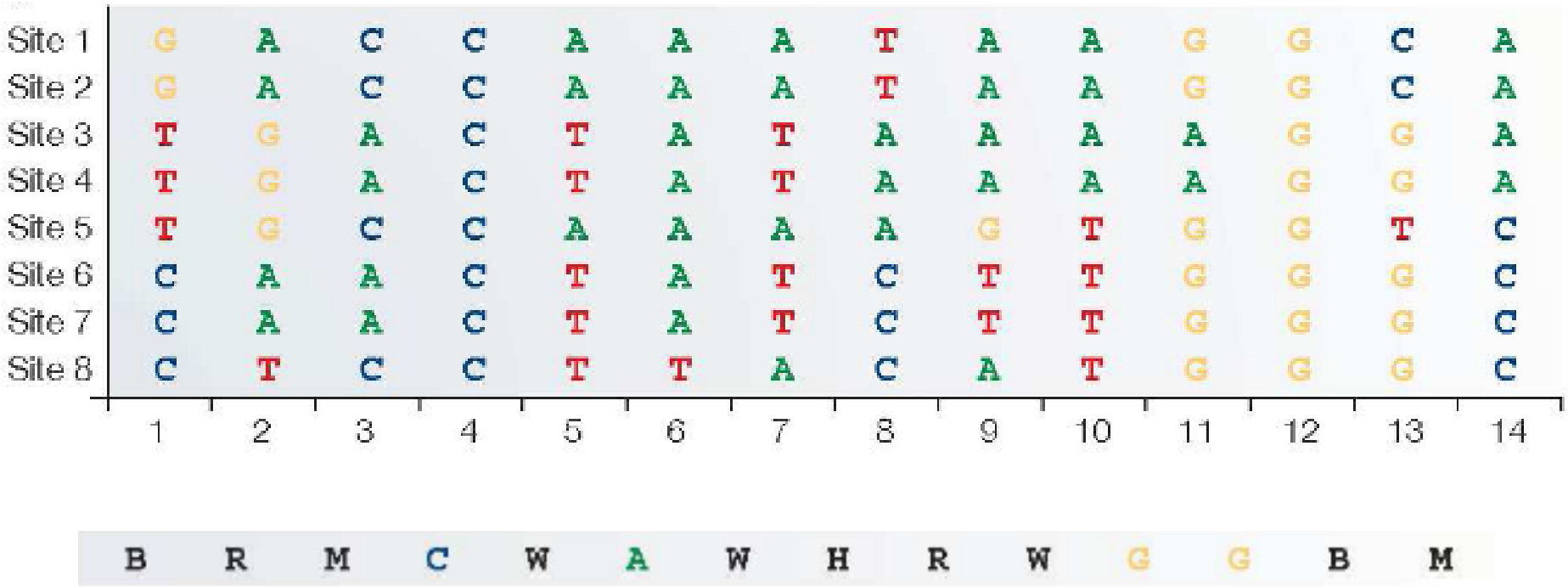
[TCG] [ATG] [AC] C [AT] [AT] [AT] [ATC] [ATG] [AT] G G [TCG] [AC]

GTACATTTGAAGTA vs TAACTATAATGGGA ?

Consensus motif



Consensus motif (sequence), IUPAC code

[TCG] [ATG] [AC] C [AT] A [AT] [ATC] [ATG] [AT] G G [TCG] [AC]

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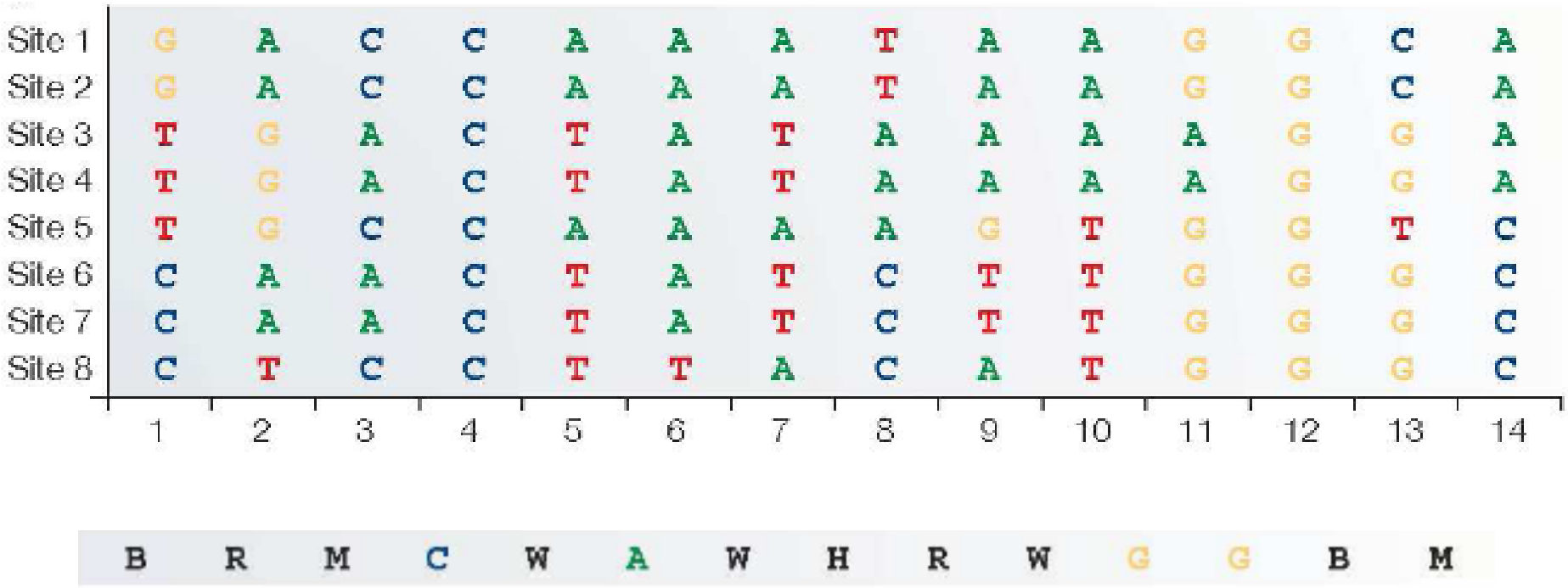
Consensus motif



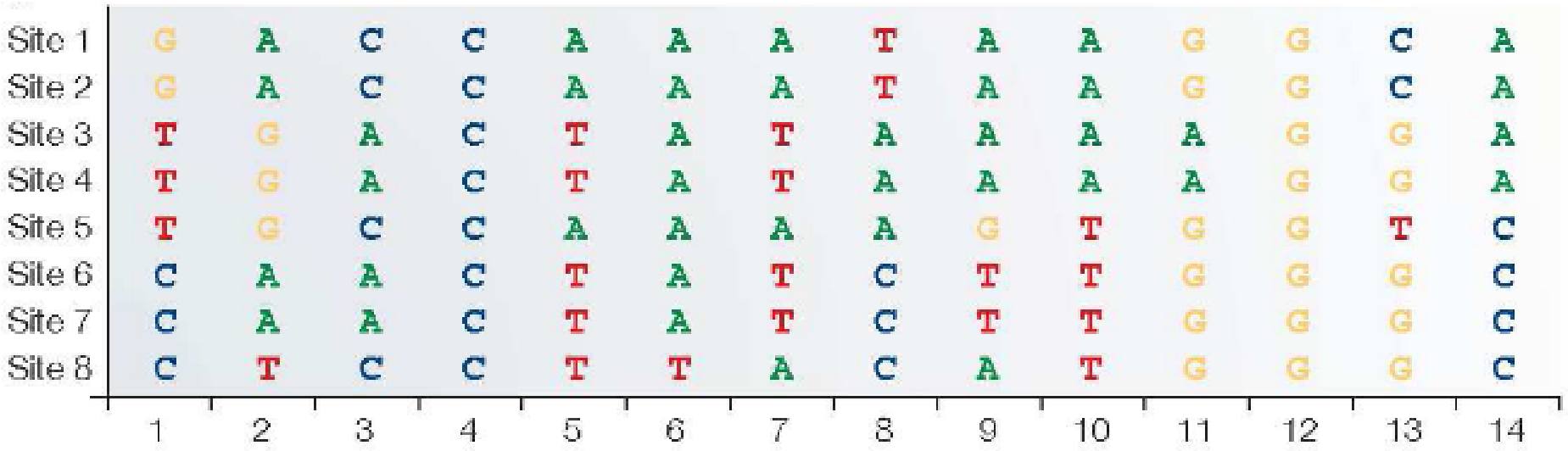
Consensus motif (sequence), IUPAC code

[TCG] [ATG] [AC] C [AT] A [AT] [ATC] [ATG] [AT] G G [TCG] [AC]

+ allow a limited number of errors

GTACATTTGAAGTA vs TAACTATAATGGGA ?

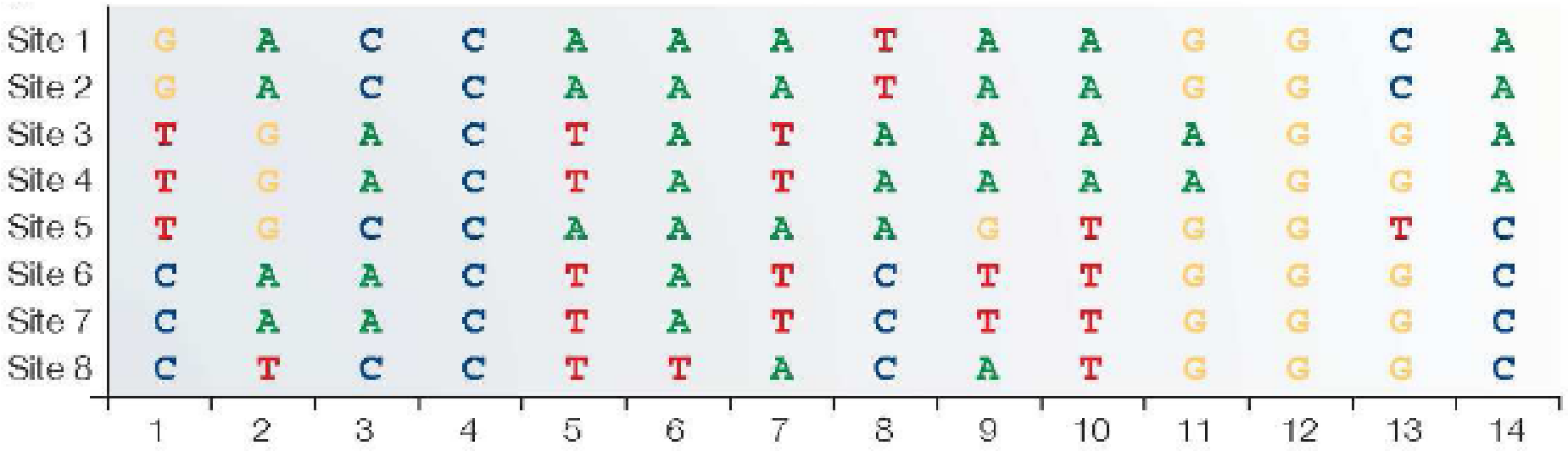
Consensus sequence



Consensus sequence

+ allow a limited number of errors

Consensus sequence

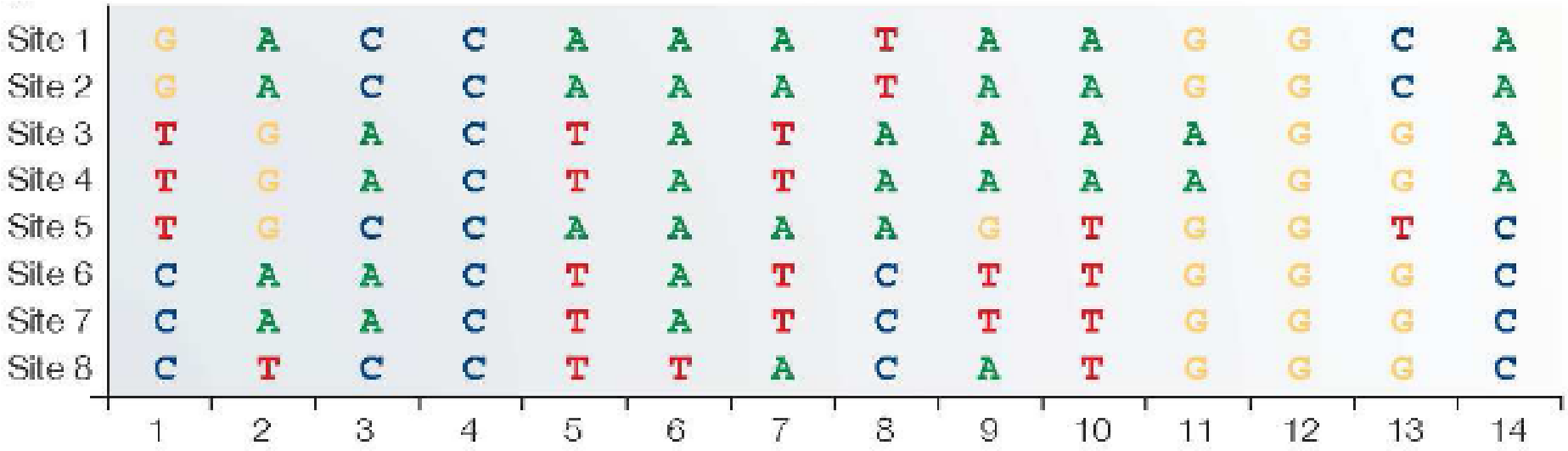


T A A C T A T A A T G G G A

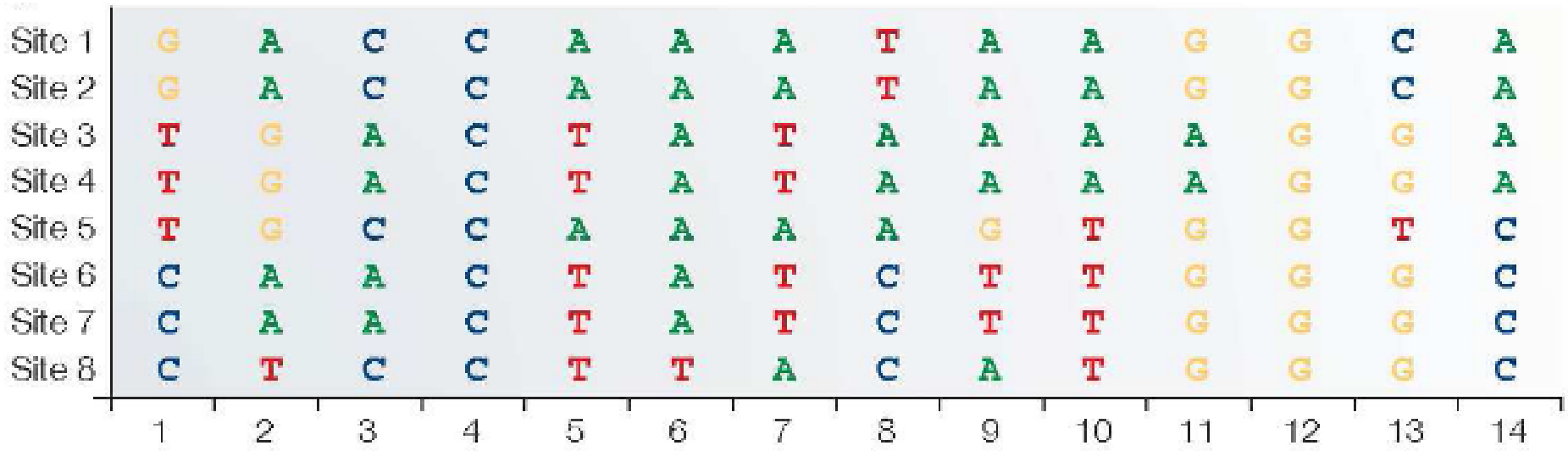
Consensus sequence
+ allow a limited number of errors

Some positions mutate more easily...
Some substitutions occur more likely...

Position frequency matrix



Position frequency matrix



$\longrightarrow$

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A	0	4	4	0	3	7	4	3	5	4	2	0	0	4
C	3	0	4	8	0	0	0	3	0	0	0	0	2	4
G	2	3	0	0	0	0	0	0	1	0	6	8	5	0
T	3	1	0	0	5	1	4	2	2	4	0	0	1	0

Position frequency matrix (PFM)

Position frequency matrix

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A	0	4	4	0	3	7	4	3	5	4	2	0	0	4
C	3	0	4	8	0	0	0	3	0	0	0	0	2	4
G	2	3	0	0	0	0	0	0	1	0	6	8	5	0
T	3	1	0	0	5	1	4	2	2	4	0	0	1	0

Position frequency matrix (PFM)

Position frequency matrix

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A	0	4	4	0	3	7	4	3	5	4	2	0	0	4
C	3	0	4	8	0	0	0	3	0	0	0	0	2	4
G	2	3	0	0	0	0	0	0	1	0	6	8	5	0
T	3	1	0	0	5	1	4	2	2	4	0	0	1	0

Position frequency matrix (PFM)

Exercises

- How to use the PFM to assign probabilities to sequences ?
  - P(GTACATTTGAAGTA) = ?
  - P(TAACTATAATGGGA) = ?
  - P(AAACTATAATGGGA) = ?
- Significativity of these probabilities ?
- What if the nucleotides composition is biased ?

Position probabilities and weights at each position/column

Notation: $O(x)$ frequency/count of nucleotide x in the column $\vec{O}$ of the PFM

Maximum likelihood estimation of probability of x:

$$\hat{P}(x|\vec{O}) = \frac{O(x)}{\Sigma_{x'} O(x')}$$

or rather use pseudo-counts:

$$\hat{P}(x|\vec{O}) = \frac{O(x)+1}{\Sigma_{x'}(O(x')+1)}$$

Weight of x (*Log odds*)

$$W(x|\vec{O}) = \log \frac{\hat{P}(x|\vec{O})}{P_0(x)}$$

where $P_0(x)$: background probability of x

Position weight matrix

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A	0	4	4	0	3	7	4	3	5	4	2	0	0	4
C	3	0	1	8	0	0	0	3	0	0	0	0	2	1
G	2	3	0	0	0	0	0	0	1	0	6	8	5	0
T	3	1	0	0	5	1	1	2	2	1	0	0	1	0

Position frequency matrix

A	−1.93	0.79	0.79	−1.93	0.45	1.50	0.79	0.45	1.07	0.79	0.00	−1.93	−1.93	0.79
C	0.45	−1.93	0.79	1.68	−1.93	−1.93	−1.93	0.45	−1.93	−1.93	−1.93	−1.93	0.00	0.79
G	0.00	0.45	−1.93	−1.93	−1.93	−1.93	−1.93	−1.93	−0.66	−1.93	1.30	1.68	1.07	−1.93
T	0.15	−0.66	−1.93	−1.93	1.07	−0.66	0.79	0.00	0.00	0.79	−1.93	−1.93	−0.66	−1.93

Position weight matrix (PWM)

(with $p(A) = p(T) = p(G) = p(C) = 1/4$)

Scoring a site

A	–1.93	0.79	0.79	–1.93	0.45	1.50	0.79	0.45	1.07	0.79	0.00	–1.93	–1.93	0.79
C	0.45	–1.93	0.79	1.68	–1.93	–1.93	–1.93	0.45	–1.93	–1.93	–1.93	–1.93	0.00	0.79
G	0.00	0.45	–1.93	–1.93	–1.93	–1.93	–1.93	–1.93	–0.66	–1.93	1.30	1.68	1.07	–1.93
T	0.15	–0.66	–1.93	–1.93	1.07	–0.66	0.79	0.00	0.00	0.79	–1.93	–1.93	–0.66	–1.93

Position weight matrix

0.45	–0.66	0.79	1.68	0.45	–0.66	0.79	0.45	–0.66	0.79	0.00	1.68	–0.66	0.79
T	**T**	**A**	**C**	**A**	**T**	**A**	**A**	**G**	**T**	**A**	**G**	**T**	**C**

$\Sigma = 5.23$, 78% of maximum

TRANSFAC

```
AC   M00231
XX
ID   V$MEF2_02
XX
DT   26.01.1996 (created); ewi.
DT   26.01.1996 (updated); ewi.
CO   Copyright (C), Biobase GmbH.
XX
NA   MEF-2
XX
DE   myogenic MADS factor MEF-2
XX
BF   T00505 MEF-2; Species: mouse, Mus musculus.
BF   T01005 MEF-2; Species: human, Homo sapiens.
XX
PO       A      C      G      T
01       5     28     25     42      N
02      16     32     31     21      N
03      18     36     27     19      N
04      19     25     33     23      N
05      22     12     43     23      N
06      33      9     21     37      N
07      20      4     43     33      K
08       3     85      3      9      C
09       3      8      0     89      T
10      85      0      0     15      A
11      57      0      0     41      W
12      91      0      1      8      A
13      96      0      0      4      A
14      93      0      1      6      A
15       0      0      0    100      T
16     100      0      0      0      A
17       9      0     90      1      G
18      34     46     11      9      M
19      36     28      8     28      N
20      20     37     15     28      N
21      30     34     13     23      N
22      23     23     22     32      N
XX
BA   104 selected sequences binding MEF-2 activity of skeletal muscle
XX
CC   sequences binding to protein that reacts with anti-MEF-2A antibodies were
CC   isolated by 9 cycles of selection and amplification from a pool of random
CC   40-mers, nucleotide distribution given in percentages
XX
RN   [1]
RX   MEDLINE; 96007455.
RA   Andres V., Cervera M., Mahdavi V.
RT   Determination of the consensus binding site for MEF2 expressed in muscle
RT   and brain reveals tissue-specific sequence constraints
RL   J. Biol. Chem. 270:23246-23249 (1995).
XX
//
```

Information content

Relative entropy (Kullback-Leibler distance) of matrix wrt background distribution

- Information content of one column/position:

$$IC_{pos} = \sum_{\alpha \in \Sigma} f_{pos,\alpha} \log_2 \frac{f_{pos,\alpha}}{f_\alpha}$$

(ADN, 1/4: between 0 and 2 bits)

- Information content of a matrix:

$$IC_{matrix} \sum_{pos=1}^{len} IC_{pos}$$

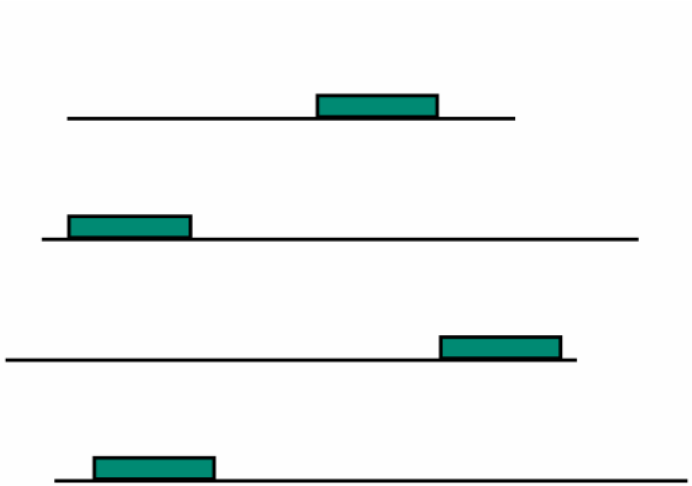
(ADN, 1/4: max = len $\times$ 2)

Most surprising set of conserved words

Information content

Examples

$$\sum_{pos=1}^{len} \sum_{\alpha \in \Sigma} f_{pos,\alpha} \log_2 \frac{f_{pos,\alpha}}{f_\alpha}$$



C	T	A	T	T
T	C	C	A	A
G	A	G	G	C
A	G	T	C	G

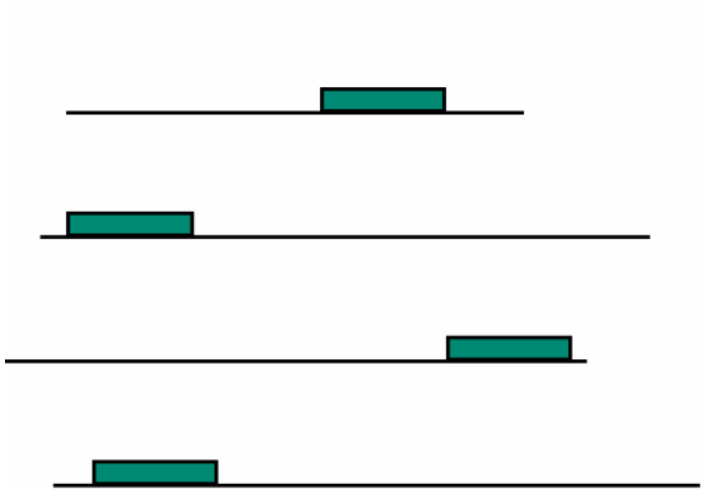
$f_\alpha = \frac{1}{4}$

0	=	0	0	0	0	0

Information content

Examples

$$\sum_{pos=1}^{len} \sum_{\alpha \in \Sigma} f_{pos,\alpha} \log_2 \frac{f_{pos,\alpha}}{f_\alpha}$$



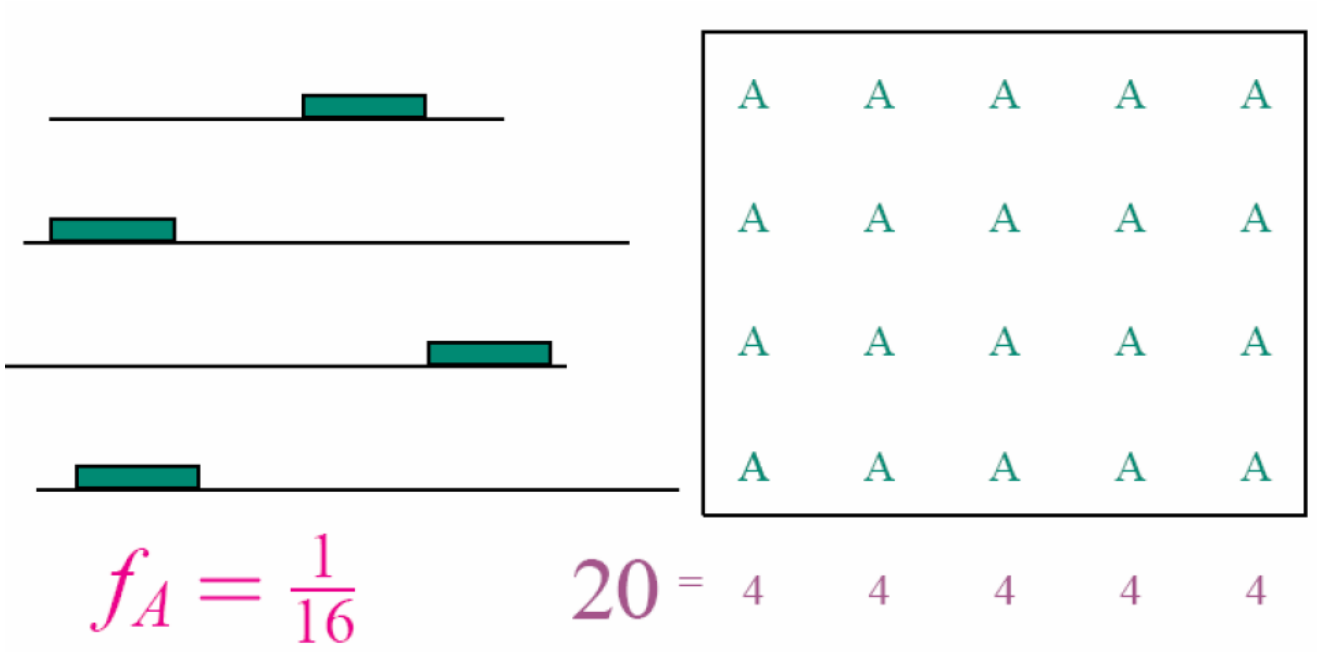
A	T	C	G	C
A	T	C	G	C
A	T	C	G	C
A	T	C	G	C
10 =	2	2	2	2	2

$f_\alpha = \frac{1}{4}$

Information content

Examples

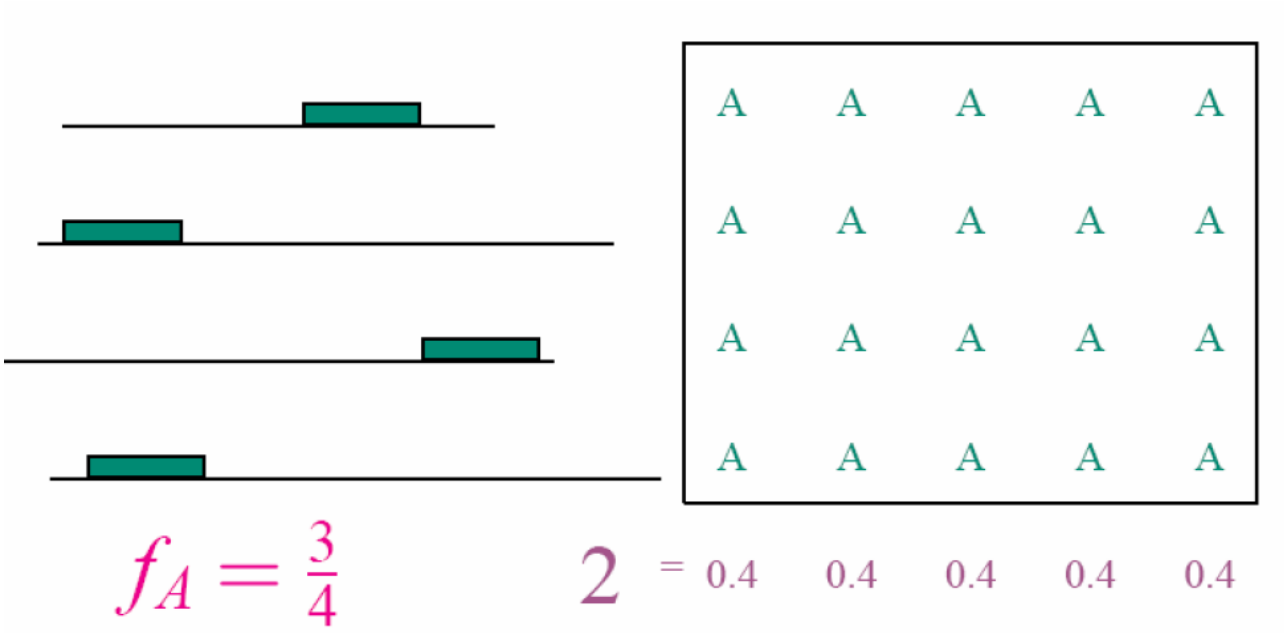
$$\sum_{pos=1}^{len} \sum_{\alpha \in \Sigma} f_{pos,\alpha} \log_2 \frac{f_{pos,\alpha}}{f_\alpha}$$



Information content

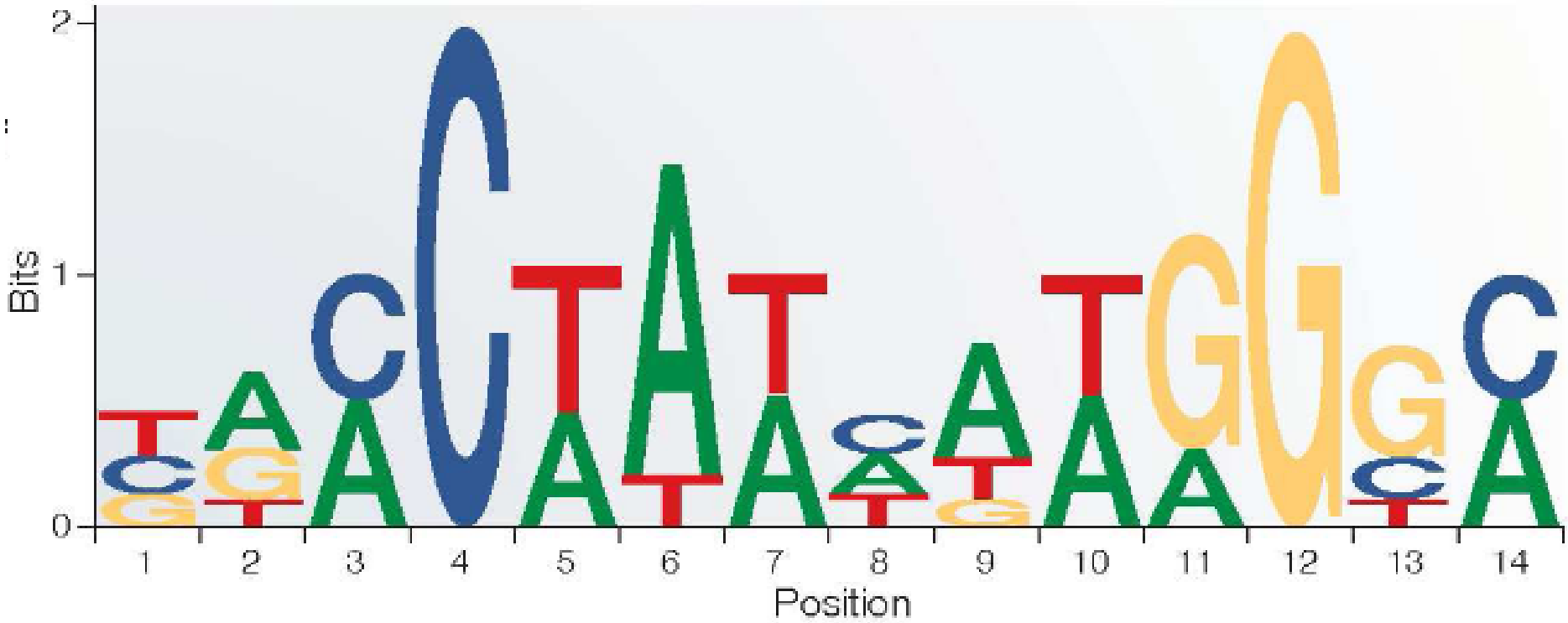
Examples

$$\sum_{pos=1}^{len} \sum_{\alpha \in \Sigma} f_{pos,\alpha} \log_2 \frac{f_{pos,\alpha}}{f_\alpha}$$



A graphical view

Sequence Logos



Motif discovery as local alignment

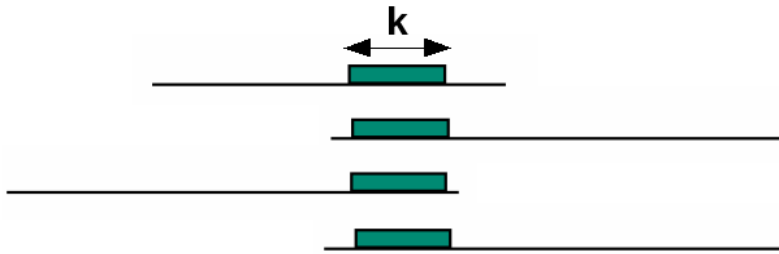
Input:

Data: A set of sequences

Parameter: k length of alignment

Output:

A set of word of length k, one in each sequence, s.t. the information content of the matrix is maximal

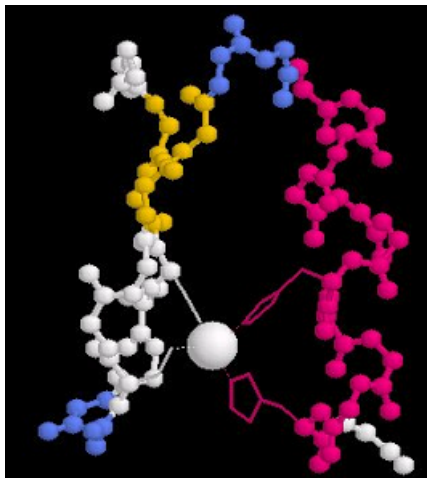


Heuristics:

- Expectation-Maximization: MEME, Bailey, 1995
- Gibbs Sampling: Lawrence et al, 1993, Thijs et al, 2001
- Greedy: (w)consensus, Hertz et al, 1999
- Projection: Buhler et al, 2000

Modelling insertions

```
ABC3G_LAGLA/285-305     Cfs..CaekVaeflqenpHvnl..H
ABRU_DROME/546-567      Cpk..CgkiYrsahtlrtHledk.H
ACE1_TRIRE/402-424      CrepgCtkeFkrpcdltkHekt..H
ACE2_SCHPO/445-467      ClyngCnkrIarkynvesHiqt..H
ACE2_SCHPO/475-495      Cdl..CkagFvrhhdlkrHlri..H
ACE2_YEAST/605-627      ClypnCnkvFkrrynirsHiqt..H
ACE2_YEAST/635-657      CdfpgCtkaFvrnhdlirHkis..H
ADNP2_HUMAN/772-793     Clf..CpctFhdikglseHsrnr.H
ADNP2_HUMAN/877-899     Cpf..CfgpFvtteayelHlkerhH
ADNP2_MOUSE/802-823     Clf..CpctFhdvrglveHsrtk.H
```



- Prosite patterns
- Prosite profiles

Prosite's pattern syntax

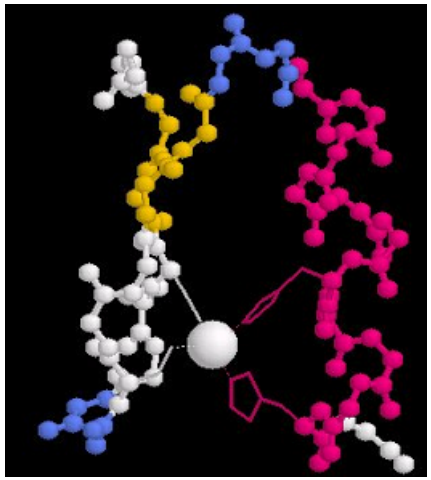
F	single-letter amino acid symbol: F
x	wildcard character: ny amino acid
[GST]	list: G, S, or T
{F}	exclusion list: not F
[ST](2)	multiplier: two S or T in a row
x(0,1)	range multiplier: between zero and times any amino acid
<G	N-terminal anchor: G at the beginning of the sequence
G>	C-terminal anchor: G at the end of the sequence
-	pattern element separator

Examples:

```
[AC]-x-V-x(4)-{ED}
<A-x-[ST](2)-x(0,1)-V
```

Exact models: Prosite patterns

```
ABC3G_LAGLA/285-305     Cfs..CaekVaeflqenpHvnl..H
ABRU_DROME/546-567      Cpk..CgkiYrsahtlrtHledk.H
ACE1_TRIRE/402-424      CrepgCtkeFkrpcdltkHekt..H
ACE2_SCHPO/445-467      ClyngCnkrIarkynvesHiqt..H
ACE2_SCHPO/475-495      Cdl..CkagFvrhhdlkrHlri..H
ACE2_YEAST/605-627      ClypnCnkvFkrrynirsHiqt..H
ACE2_YEAST/635-657      CdfpgCtkaFvrnhdlirHkis..H
ADNP2_HUMAN/772-793     Clf..CpctFhdikglseHsrnr.H
ADNP2_HUMAN/877-899     Cpf..CfgpFvtteayelHlkerhH
ADNP2_MOUSE/802-823     Clf..CpctFhdvrglveHsrtk.H
```



Prosite's C2H2 Pattern:

C-x(2,4)-C-x(3)-[LIVMFYWC]-x(8)-H-x(3,5)-H

Prosite's Pattern

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Entry: PS00028

General information about the entry	
Entry name	**ZINC_FINGER_C2H2_1**
Accession number	**PS00028**
Entry type	PATTERN
Date	APR-1990 (CREATED); JUN-1994 (DATA UPDATE); DEC-2007 (INFO UPDATE).
PROSITE Documentation	PDOC00028
Name and characterization of the entry	
Description	Zinc finger C2H2 type domain signature.
Pattern	C-x(2,4)-C-x(3)-[LIVMFYWC]-x(8)-H-x(3,5)-H.

Numerical results

- UniProtKB/Swiss-Prot release number: **54.7**, total number of sequence entries in that release: **333445**.
- Total number of hits in UniProtKB/Swiss-Prot: **11060 hits in 1794 different sequences**
- Number of hits on proteins that are known to belong to the set under consideration: **10810 hits in 1609 different sequences**
- Number of hits on proteins that could potentially belong to the set under consideration: **26 hits in 12 different sequences**
- Number of false hits (on unrelated proteins): **224 hits in 173 different sequences**
- Number of known missed hits: **60**
- Number of partial sequences which belong to the set under consideration, but which are not hit by the pattern or profile because they are partial (fragment) sequences: **1**
- Precision (true hits / (true hits + false positives)): **97.97 %**
- Recall (true hits / (true hits + false negatives)): **99.45 %**

Probabilistic version: Prosite's Profile

Home ScanProsite ProRule Documents Downloads Links

Entry: PS50157

General information about the entry	
Entry name	**ZINC_FINGER_C2H2_2**
Accession number	**PS50157**
Entry type	MATRIX
Date	DEC-2001 (CREATED); DEC-2001 (DATA UPDATE); DEC-2007 (INFO UPDATE).
PROSITE Documentation	PDOC00028
Associated ProRule	PRU00042
Name and characterization of the entry	
Description	Zinc finger C2H2 type domain profile.

Matrix / Profile

```
/GENERAL_SPEC: ALPHABET='ABCDEFGHIKLMNPQRSTVWYZ'; LENGTH=28;
/DISJOINT: DEFINITION=PROTECT; N1=3; N2=26;
/NORMALIZATION: MODE=1; FUNCTION=LINEAR; E1=-0.6689; E2=0.02078310; TEXT='-LogE';
/CUT_OFF: LEVEL=0; SCORE=441; N_SCORE=8.5; MODE=1; TEXT='!';
/CUT_OFF: LEVEL=-1; SCORE=344; N_SCORE=6.5; MODE=1; TEXT='?';
/DEFAULT: D=-20; I=-20; B1=-50; E1=-50; BI=-105; BD=-105; ID=-105; DI=-105;

                A   B   C   D   E   F   G   H   I   K   L   M   N   P   Q   R   S   T   V   W   Y   Z
/I:         B1=0; BI=-105; BD=-105;
/M: SY='Y'; M=-19, -21, -24, -25, -21,  39, -28,  10,  -2, -17,   2,   0, -17, -28, -19, -13, -18, -10,  -7,  12,  48, -21;
/M: SY='E'; M= -4,  -5, -23,  -5,   6, -20, -18,  -9, -14,   7, -15,  -7,  -5,  -9,   3,   2,  -2,  -2,  -9, -25, -12,   4;
/M: SY='C'; M=-10, -20, 118, -30, -30, -20, -30, -30, -30, -30, -20, -20, -20, -40, -30, -30, -10, -10, -10, -50, -30, -30;
/M: SY='R'; M= -5,   3, -24,   3,   6, -22, -11,  -6, -20,   1, -21, -14,   4,  -1,   1,  -3,   5,   2, -18, -29, -15,   3;
/I:         I=-12; MI=0; MD=-30; IM=0; DM=-30;
/M: SY='R'; M= -9,  -2, -26,   1,  14, -18, -17,  -4, -13,  -1, -11,  -8,  -5, -12,   4,  -5,  -5,  -8, -12, -24,  -9,   8;
/M: SY='C'; M=-10, -20, 119, -30, -30, -20, -30, -30, -30, -30, -20, -20, -20, -40, -30, -30, -10, -10, -10, -50, -29, -30;
/M: SY='G'; M= -3,  -1, -28,  -1,  -7, -28,  36, -11, -33, -11, -27, -18,   4, -15, -10, -12,   1, -13, -27, -24, -23,  -9;
/M: SY='E'; M=-10,  -2, -28,  -3,   8, -25, -19,  -7, -26,  36, -24,  -8,  -1, -12,  10,  27,  -9,  -9, -18, -19,  -8,   8;
/M: SY='A'; M=  8,  -7,  -9, -11,  -7, -17,  -7, -14, -16,  -6, -16, -11,  -4, -15,  -6,  -5,   8,   4,  -7, -27, -15,  -7;
/M: SY='F'; M=-19, -29, -19, -37, -28,  71, -29, -17,   0, -28,   9,   0, -20, -30, -36, -19, -19,  -9,  -1,   9,  31, -28;
/M: SY='S'; M=  0,  -5, -17,  -9,  -6, -16, -11, -10, -14,  -3, -16, -10,   0, -12,  -4,   0,   8,   7,  -8, -27, -12,  -6;
/M: SY='E'; M=-10,  -3, -20,  -4,   0, -18, -17,   2, -19,   3, -16,  -8,   0, -17,   8,   9,  -1,  -3, -17, -19,  -5,   3;
/M: SY='E'; M= -4,  -4, -22,  -6,   0, -19, -13,  -5, -18,   7, -18,  -9,   1, -10,   2,   8,   2,  -2, -14, -25, -11,   0;
/M: SY='S'; M=  2,  -1, -16,  -1,  -1, -18,  -4,  -6, -19,  -7, -22, -14,   4, -12,  -2,  -7,  16,   7, -13, -29, -12,  -2;
/M: SY='H'; M= -5,   5, -20,   1,   0, -18, -10,   8, -18,  -5, -18, -11,   9, -16,   1,  -4,   4,  -1, -17, -27,  -7,  -1;
/M: SY='L'; M=-11, -29, -20, -30, -20,  12, -29, -19,  17, -27,  43,  18, -28, -29, -20, -18, -28, -10,   9, -18,   2, -20;
/M: SY='E'; M= -6,  -6, -22, -10,  -4, -15, -20,  -8,  -7,   2,  -9,  -3,  -2, -16,   0,   3,  -3,   0,  -6, -24,  -8,  -3;
/M: SY='E'; M= -6,  -6, -23,  -7,   0, -19, -17,  -7, -14,   7, -13,  -6,  -2, -16,   4,  12,  -3,  -3, -10, -24, -10,   0;
/M: SY='R'; M=-20,   0, -30,   0,   0, -20, -20,  99, -30, -10, -20,   0,  10, -20,  10,   0, -10, -20, -30, -30,  20,   0;
/M: SY='Q'; M=-10, -10, -25, -12,   1, -16, -22,  -2,  -6,   1,  -3,   6,  -9, -17,  13,   3,  -9,  -8,  -9, -19,  -4,   6;
/M: SY='E'; M=-13,  -8, -26,  -9,   0, -19, -19,  -4, -21,  20, -16,  -6,  -2, -17,   6,  35,  -8,  -7, -14, -21,  -9,   0;
/I:         I=-12; MI=0; MD=-29; IM=0; DM=-29;
/M: SY='V'; M= -3, -16, -17, -21, -17,  -6, -25, -20,  11, -15,   2,   3, -12, -18, -14, -14,  -2,   9,  13, -25,  -7, -17;
/M: SY='R'; M=-20,   0, -30,   0,   0, -20, -20,  97, -30, -10, -20,   0,  10, -20,  10,   0, -10, -20, -30, -30,  19,   0;
/M: SY='T'; M=  1,  -2, -13,  -8,  -6, -14, -15, -15, -12,  -7, -13,  -9,   0, -12,  -6,  -8,  14,  25,  -5, -29, -12,  -6;
/M: SY='G'; M= -3,  -4, -27,  -4,  -8, -27,  34, -14, -32,  -9, -25, -16,   2, -15, -10,  -9,   0, -13, -24, -23, -23, -10;
/M: SY='R'; M= -9,   6, -27,  12,  33, -26, -17,  -3, -24,   7, -18, -15,  -1,  -6,  12,   0,  -2,  -8, -22, -28, -16,  22;
/M: SY='E'; M=-11,  -1, -28,  -2,   6, -25, -17,  -6, -27,  32, -25, -10,   1, -11,   8,  28,  -7,  -8, -19, -22, -11,   6;
/M: SY='P'; M= -7, -14, -32,  -9,  -1, -24, -17, -15, -18,  -7, -23, -15, -13,  51,  -7, -13,  -6,  -6, -23, -28, -22,  -7;
/I:         E1=0;
```

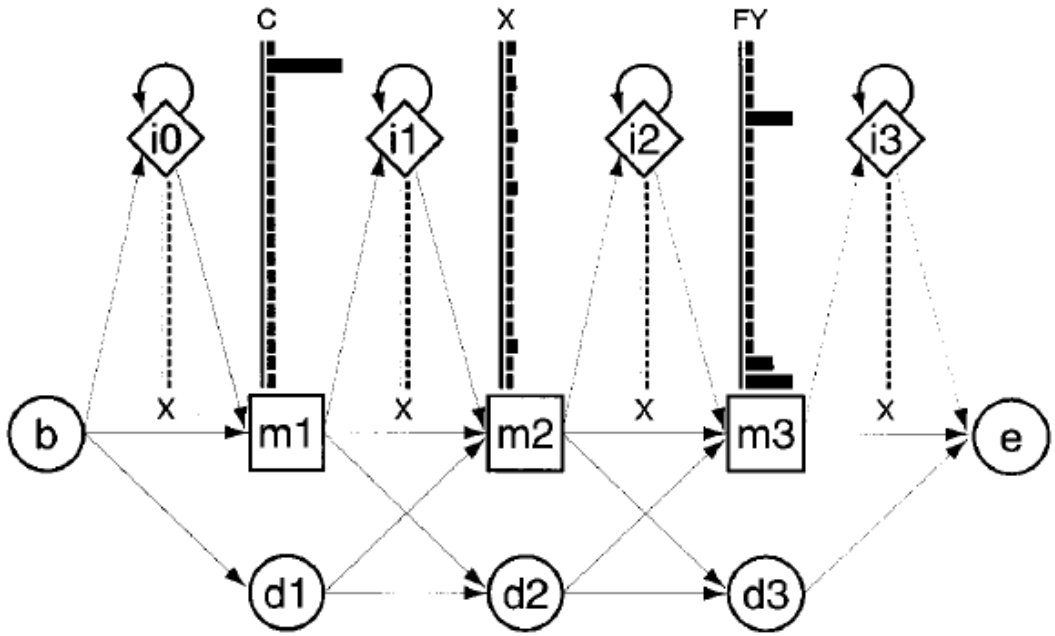
Numerical results

- UniProtKB/Swiss-Prot release number: **54.7**, total number of sequence entries in that release: **333445**.
- Total number of hits in UniProtKB/Swiss-Prot: **11272 hits in 1555 different sequences**
- Number of hits on proteins that are known to belong to the set under consideration: **11257 hits in 1540 different sequences**

Profile HMM

Simple left-to-right topology: PWM + insertions and deletion state

1	2	3
C	A	F
C	G	W
C	D	Y
C	V	F
C	K	Y



m: Match state, i: Insertion state, d: Deletion (silent) state

picture from Sean Eddy

- Each match state has its own emission probability distribution
- Handles deletions and insertions, enabling different costs to enter, leave or continue in a mode

Parameter estimation

- From aligned sequences
- (Simplified) Baum-Welsh, ...

Simple topology but still an important number of free parameters.
One needs big training sample. Prone to overspecialization.

Example: SH3 domain

source: An Introduction to Hidden Markov Models for Biological Sequences, Anders Krogh

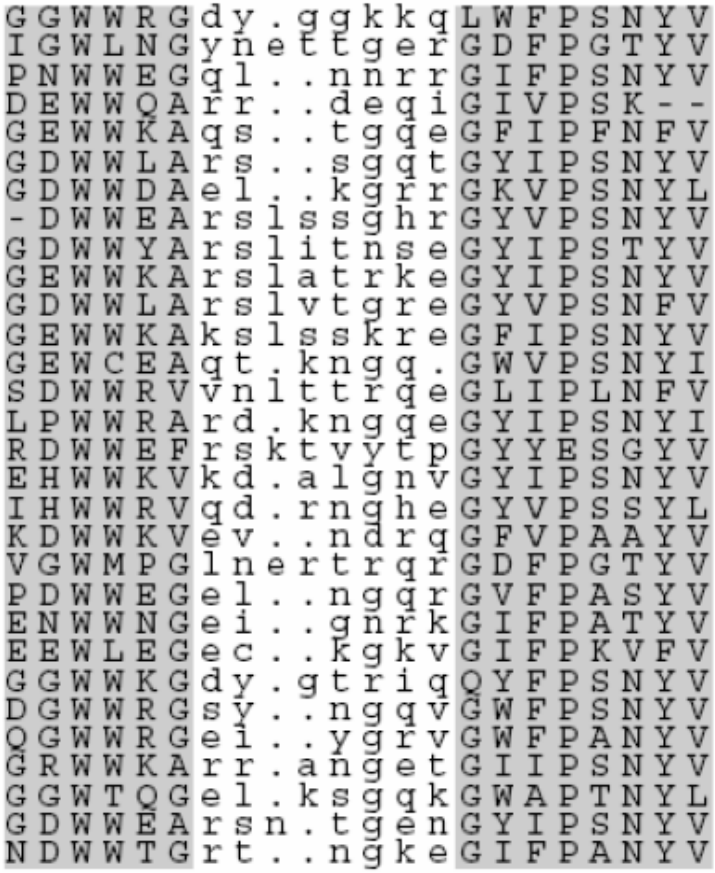


Figure 4.4: An alignment of 30 short amino acid sequences chopped out of a alignment of the SH3 domain. The shaded areas are the most conserved and were chosen to be represented by the main states in the HMM. The unshaded area with

Example: SH3 domain

source: An Introduction to Hidden Markov Models for Biological Sequences, Anders Krogh

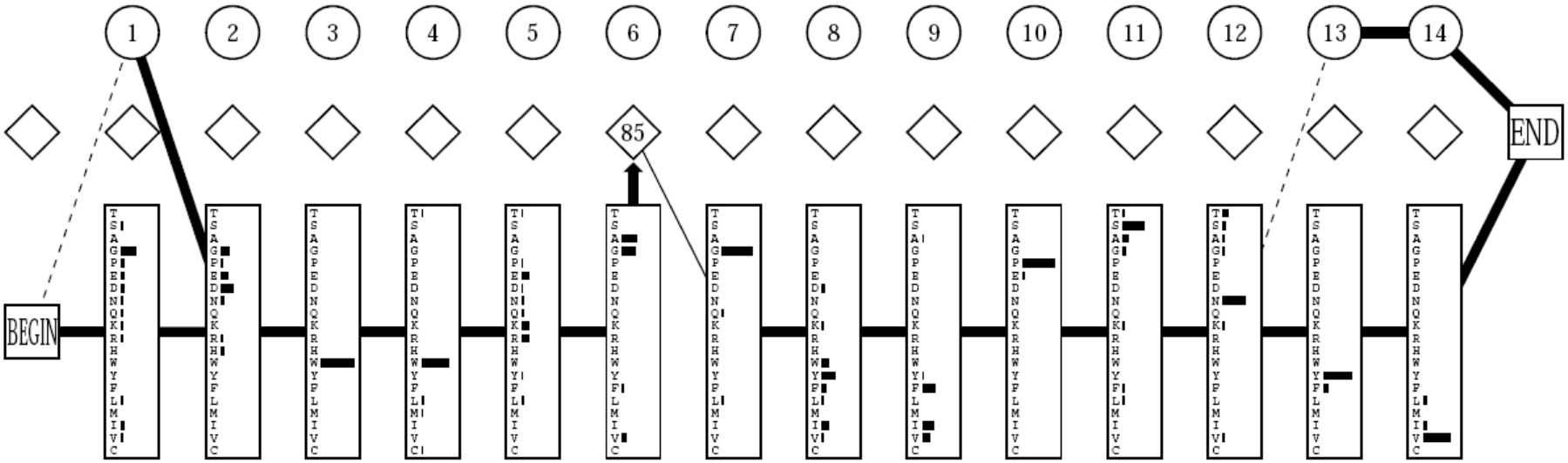


Figure 4.5: A profile HMM made from the alignment shown in Fig. 4.4. Transition lines with no arrow head are transitions from left to right. Transitions with probability zero are not shown, and those with very small probability are shown as dashed lines. Transitions from an insert state to itself is not shown; instead the probability times 100 is shown in the diamond. The numbers in the circular delete states are just position numbers. (This figure and Fig. 4.6 were generated by a program in the SAM package of programs.)

Example: SH3 domain

source: An Introduction to Hidden Markov Models for Biological Sequences, Anders Krogh

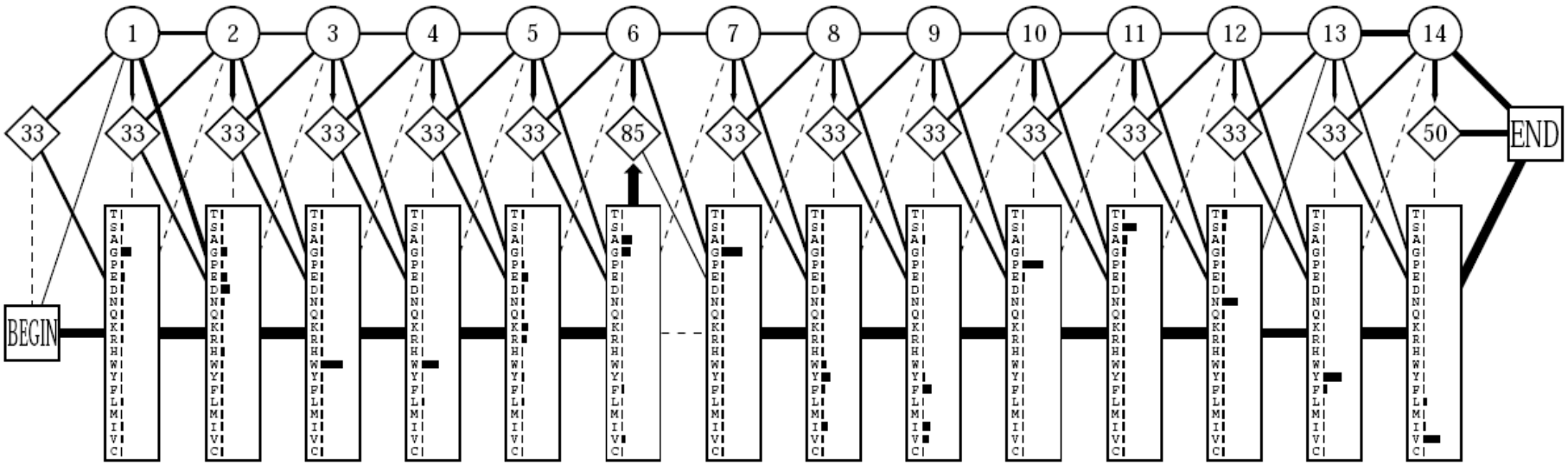


Figure 4.6: Model obtained in the same way as Fig. 4.5, but using a pseudocount of one.

Example: 2crd Charybdotoxin

source: tutorial ISMB, SAM-T98

With maximum likelihood weighting scheme:

```
2crd   XFTNVSCTTSKECWSVCQRLHNTSRGKCMNKKCRCYS
1cmr   ------CTTSKECWSVCQRLHNTSKGWCDHRGCICES
2bmt   XFTNVSCSASSQCWPVCKKLFGTYRGKCMNSKCRCYS
```

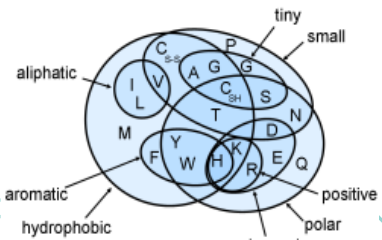
Example: 2crd Charybdotoxin

source: tutorial ISMB, SAM-T98

With maximum likelihood weighting scheme:

```
2crd   XFTNVSCTTSKECWSVCQRLHNTSRGKCMNKKCRCYS
1cmr   ------CTTSKECWSVCQRLHNTSKGWCDHRGCICES
2bmt   XFTNVSCSASSQCWPVCKKLFGTYRGKCMNSKCRCYS
```

Can't we replace a L by a I ?



Adding pseudocounts

source: tutorial ISMB, SAM-T98

Add-one pseudocount estimation (Laplace rule):

$$\hat{P}(a|\vec{O}) = \frac{O(a)+1}{\Sigma_{\text{amino acids } j}\, O(j)+1}$$

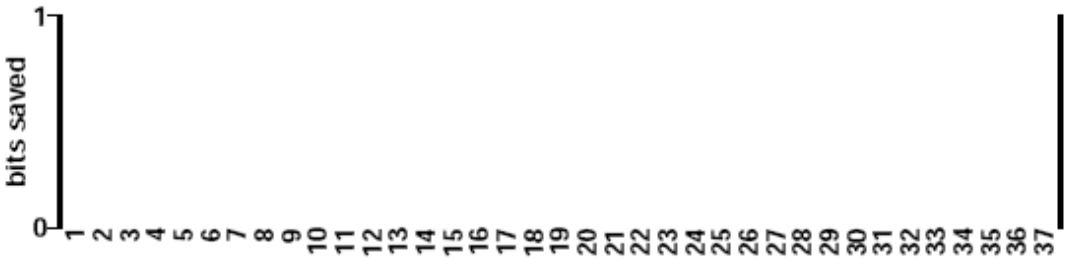
Adding pseudocounts

source: tutorial ISMB, SAM-T98

Add-one pseudocount estimation (Laplace rule):

$$\hat{P}(a|\vec{O}) = \frac{O(a)+1}{\Sigma_{\text{amino acids } j}\, O(j)+1}$$

```
2crd   XFTNVSCTTSKECWSVCQRLHNTSRGKCMNKKCRCYS
1cmr   ------CTTSKECWSVCQRLHNTSKGWCDHRGCICES
2bmt   XFTNVSCSASSQCWPVCKKLFGTYRGKCMNSKCRCYS
```



Adding pseudocounts

source: tutorial ISMB, SAM-T98

Add-one pseudocount estimation (Laplace rule):

$$\hat{P}(a|\vec{O}) = \frac{O(a)+1}{\Sigma_{\text{amino acids } j}\, O(j)+1}$$

More sophisticated pseudocounts:

$$\hat{P}(a|\vec{O}) = \frac{O(a) + A\ P_0(a)}{\Sigma_{a'}\, O(a') \quad + \quad A}$$

where A = weight on pseudocounts
and $P_0(a)$ = background probability of a

Using substitution matrix

source: tutorial ISMB, SAM-T98

Gribskov Average Score:

$$\hat{P}(a|\vec{O}) \leftarrow P_0(a)e^{\frac{\sum_b M_{a,b}O(b)}{|\vec{O}|}}$$

```
2crd   XFTNVSCTTSKECWSVCQRLHNTSRGKCMNKKCRCYS
1cmr   ------CTTSKECWSVCQRLHNTSKGWCDHRGCICES
2bmt   XFTNVSCSASSQCWPVCKKLFGTYRGKCMNSKCRCYS
```

Using substitution matrix

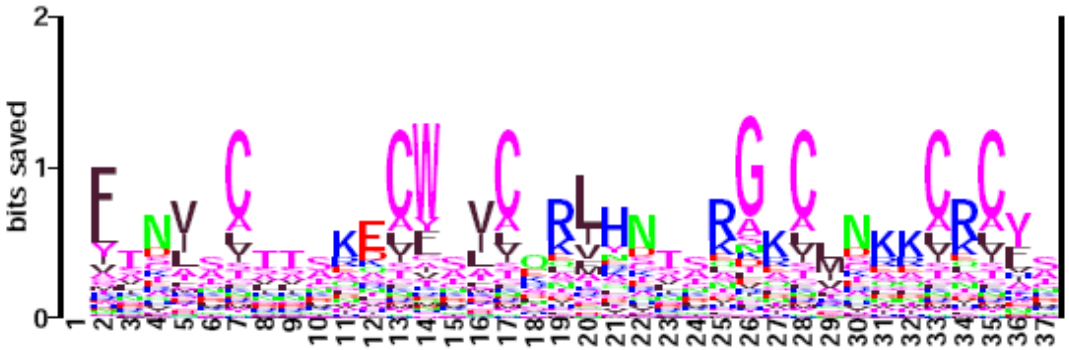
source: tutorial ISMB, SAM-T98

Gribskov Average Score:

$$\hat{P}(a|\vec{O}) \leftarrow P_0(a)e^{\frac{\sum_b M_{a,b}O(b)}{|\vec{O}|}}$$

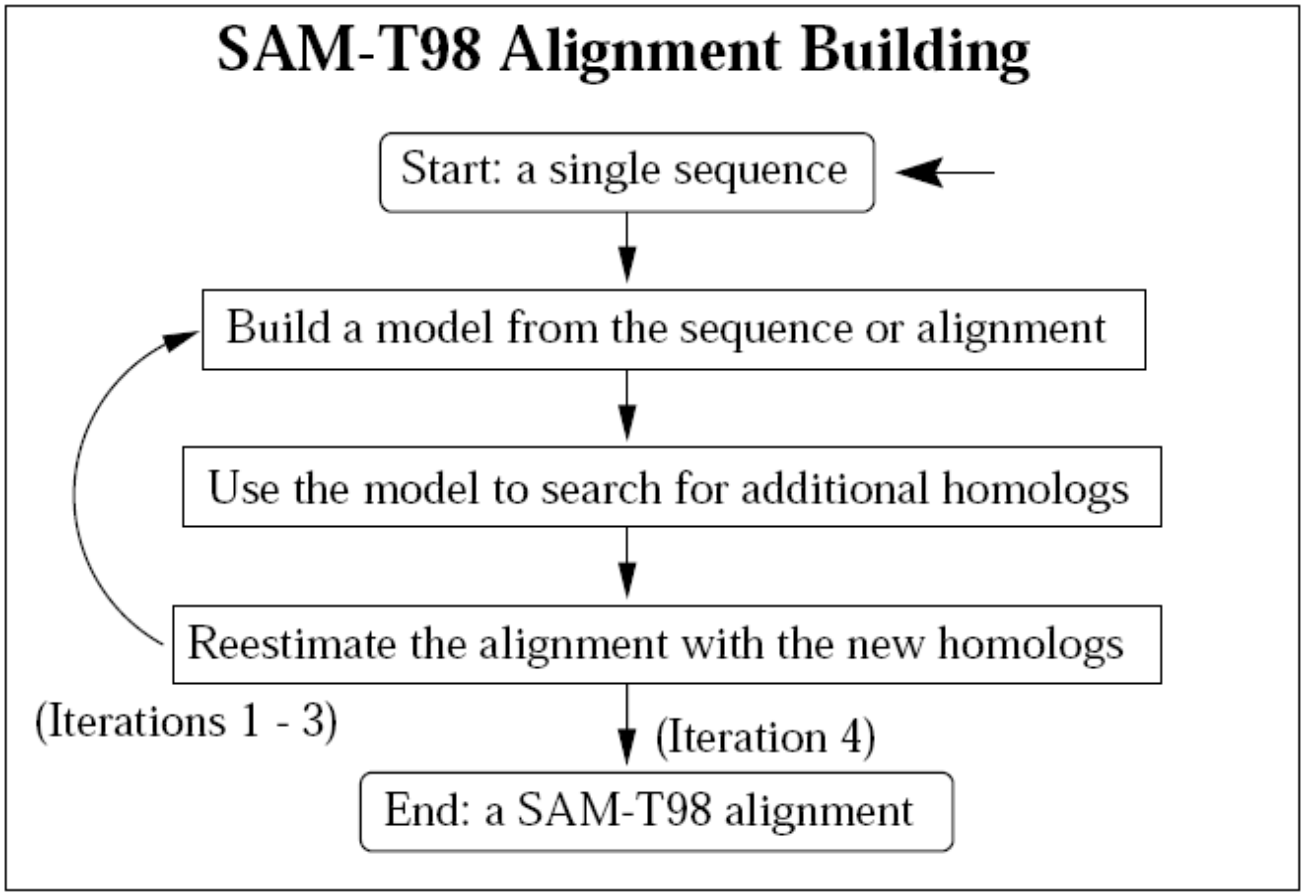
Only one sequence: $\sim$ signal searched by Blast

```
2crd  XFTNVSCTTSKECWSVCQRLHNTSRGKCMNKKCRCYS
```



SAM-T98 workflow

Starts from a single sequence !

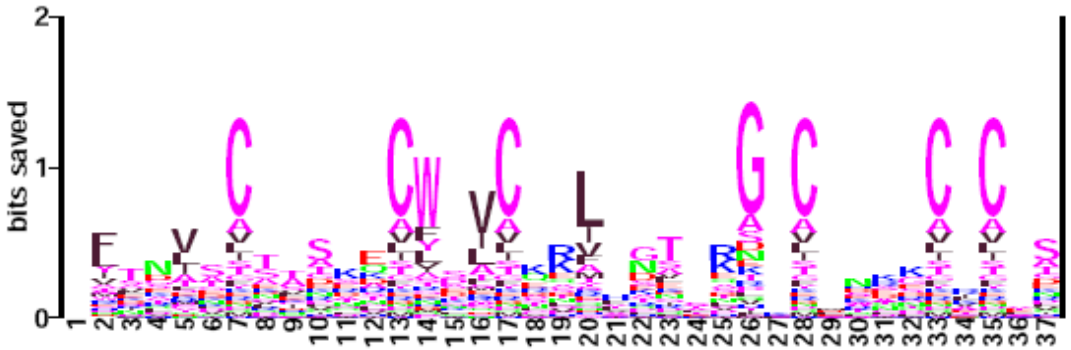


Dirichlet mixtures

source: tutorial ISMB, SAM-T98

$$\hat{P}(a|\vec{O}) = \sum_c P(c|\vec{O}) \frac{O(a) + \alpha_{c,a}}{\Sigma_{\text{amino acids } b} O(b) + \alpha_{c,b}}$$

```
2crd   XFTNVSCTTSKECWSVCQRLHNTSRGKCMNKKCRCYS
1cmr   ------CTTSKECWSVCQRLHNTSKGWCDHRGCICES
2bmt   XFTNVSCSASSQCWPVCKKLFGTYRGKCMNSKCRCYS
```



Dirichlet mixtures

uprior.9comp									
	uprior9.0	uprior9.1	uprior9.2	uprior9.3	uprior9.4	uprior9.5	uprior9.6	uprior9.7	uprior9.8
c	0.182962	0.057607	0.089823	0.079297	0.083183	0.091122	0.115962	0.06604	0.234006
$\|\theta\|$	1.18065	1.35583	6.66436	2.08141	2.08101	2.56819	1.76606	4.98468	0.0995
A	0.270671	0.021465	0.561459	0.070143	0.041103	0.115607	0.093461	0.452171	0.005193
C	0.039848	0.0103	0.045448	0.01114	0.014794	0.037381	0.004737	0.114613	0.004039
D	0.017576	0.011741	0.438366	0.019479	0.00561	0.012414	0.387252	0.06246	0.006722
E	0.016415	0.010883	0.764167	0.094657	0.010216	0.018179	0.347841	0.115702	0.006121
F	0.014268	0.385651	0.087364	0.013162	0.153602	0.051778	0.010822	0.284246	0.003468
G	0.131916	0.016416	0.259114	0.048038	0.007797	0.017255	0.105877	0.140204	0.016931
H	0.012391	0.076196	0.21494	0.077	0.007175	0.004911	0.049776	0.100358	0.003647
I	0.022599	0.035329	0.145928	0.032939	0.299635	0.796882	0.014963	0.55023	0.002184
K	0.020358	0.013921	0.762204	0.576639	0.010849	0.017074	0.094276	0.143995	0.005019
L	0.030727	0.093517	0.24732	0.072293	0.999446	0.285858	0.027761	0.700649	0.00599
M	0.015315	0.022034	0.118662	0.02824	0.210189	0.075811	0.01004	0.27658	0.001473
N	0.048298	0.028593	0.441564	0.080372	0.006127	0.014548	0.187869	0.118569	0.004158
P	0.053803	0.013086	0.174822	0.037661	0.013021	0.015092	0.050018	0.09747	0.009055
Q	0.020662	0.023011	0.53084	0.185037	0.019798	0.011382	0.110039	0.126673	0.00363
R	0.023612	0.018866	0.465529	0.506783	0.014509	0.012696	0.038668	0.143634	0.006583
S	0.216147	0.029156	0.583402	0.073732	0.012049	0.027535	0.119471	0.278983	0.003712
T	0.147226	0.018153	0.445586	0.071587	0.035799	0.088333	0.065802	0.358482	0.00369
V	0.65438	0.0361	0.22705	0.042532	0.180085	0.94434	0.02543	0.66175	0.002967
W	0.003758	0.07177	0.02951	0.011254	0.012744	0.004373	0.003215	0.061533	0.002772
Y	0.009621	0.419641	0.12109	0.028723	0.026466	0.016741	0.018742	0.199373	0.002686

small aromatic polar + charge large I/V hydrophilic hydro- highly

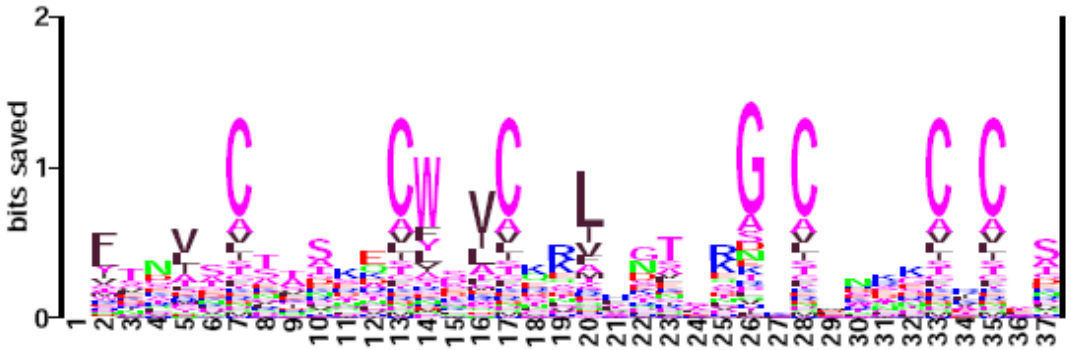
NP phobic conserved

Dirichlet mixtures

source: tutorial ISMB, SAM-T98

$$\hat{P}(a|\vec{O}) = \sum_c P(c|\vec{O}) \frac{O(a) + \alpha_{c,a}}{\Sigma_{\text{amino acids } b} O(b) + \alpha_{c,b}}$$

```
2crd   XFTNVSCTTSKECWSVCQRLHNTSRGKCMNKKCRCYS
1cmr   ------CTTSKECWSVCQRLHNTSKGWCDHRGCICES
2bmt   XFTNVSCSASSQCWPVCKKLFGTYRGKCMNSKCRCYS
```

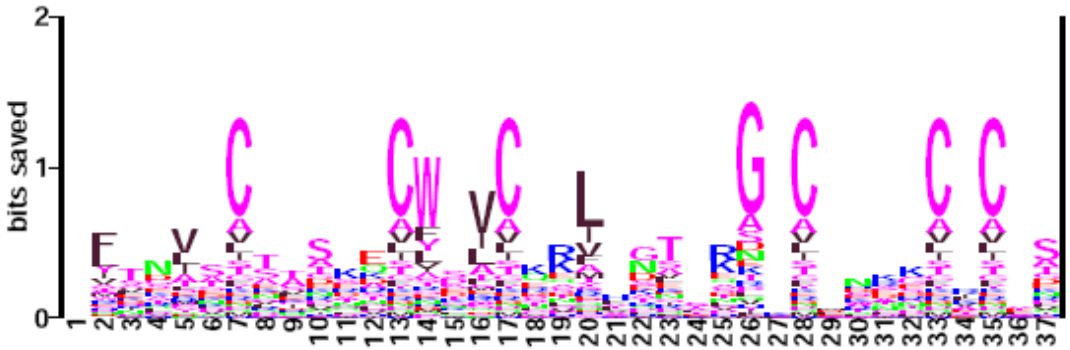


Dirichlet mixtures

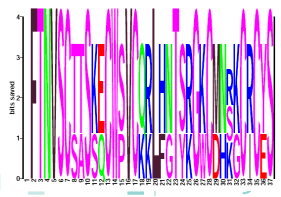
source: tutorial ISMB, SAM-T98

$$\hat{P}(a|\vec{O}) = \sum_c P(c|\vec{O}) \frac{O(a) + \alpha_{c,a}}{\Sigma_{\text{amino acids } b} O(b) + \alpha_{c,b}}$$

```
2crd   XFTNVSCTTSKECWSVCQRLHNTSRGKCMNKKCRCYS
1cmr   ------CTTSKECWSVCQRLHNTSKGWCDHRGCICES
2bmt   XFTNVSCSASSQCWPVCKKLFGTYRGKCMNSKCRCYS
```



The state-of-the-art!



PHMM: a success story on proteins

- Tools
  - HMMR
  - SAM
- PHMM Databases
  - PFAM
  - TIGRFAM
  - SUPERFAMILY
  - CATH
  - ...

And we could add the closely related tool PSI-Blast, based on profiles, and the databases relying on it ...

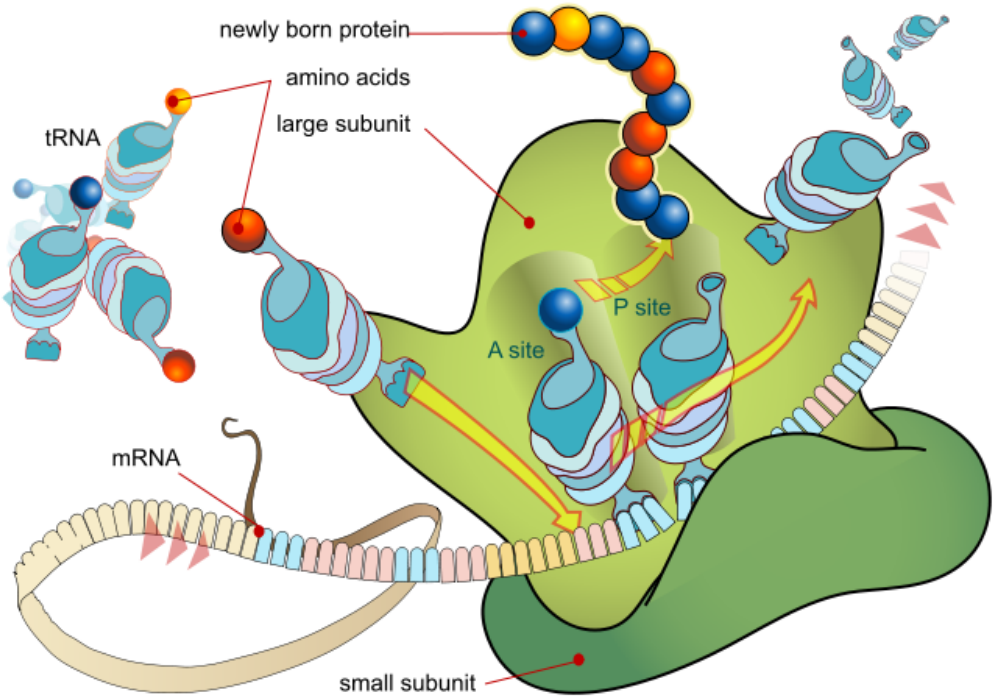
What about RNA ?

What about RNA ?

Functional (*i.e.* non-coding) RNA

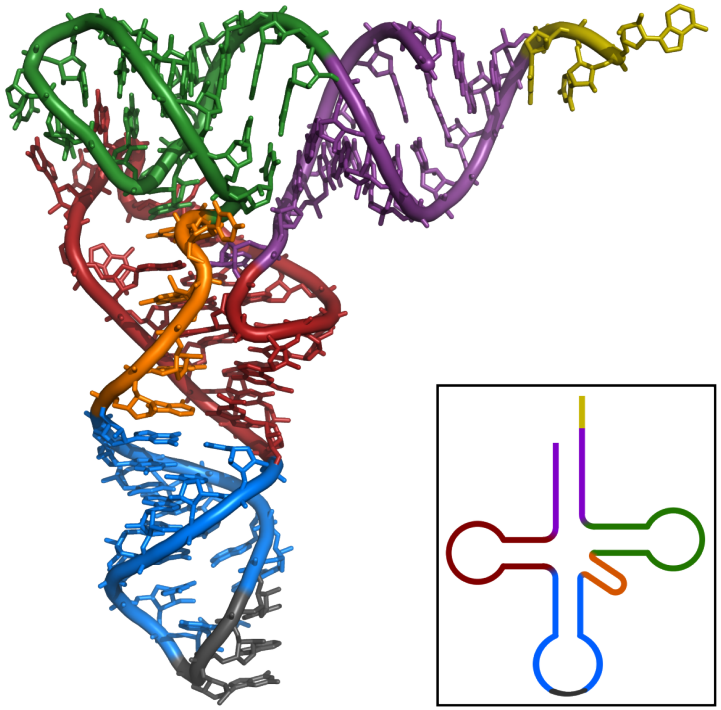
What about RNA ?

For instance, transfer RNA (many other ncRNA):



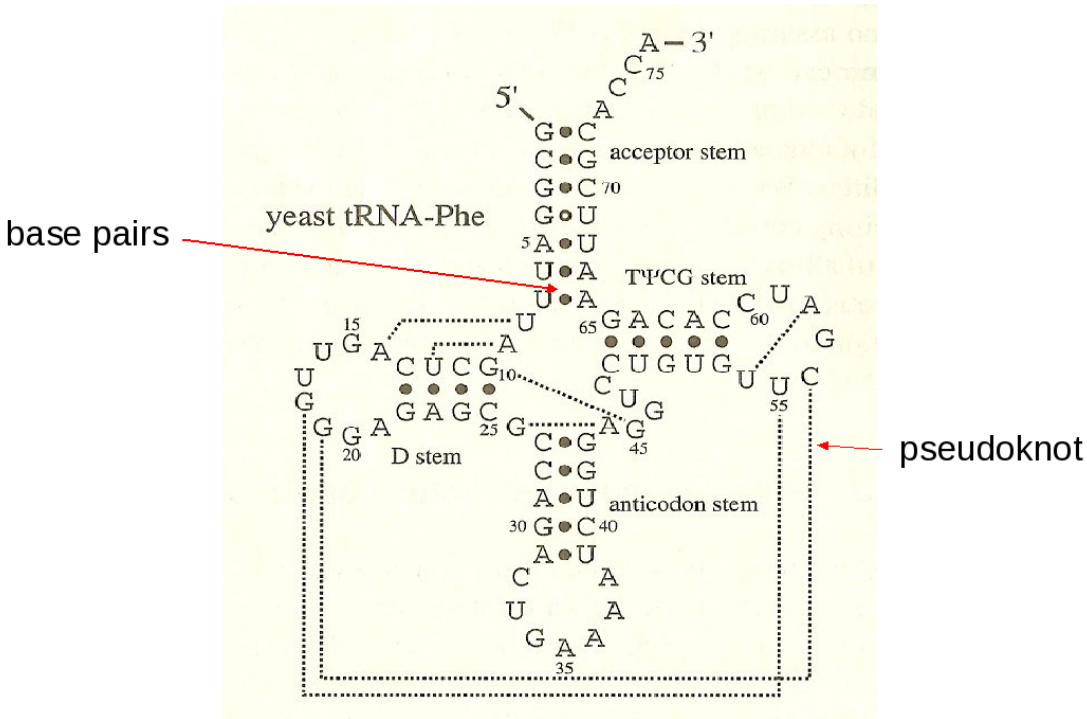
What about RNA ?

For instance, transfer RNA (many other ncRNA):



What about RNA ?

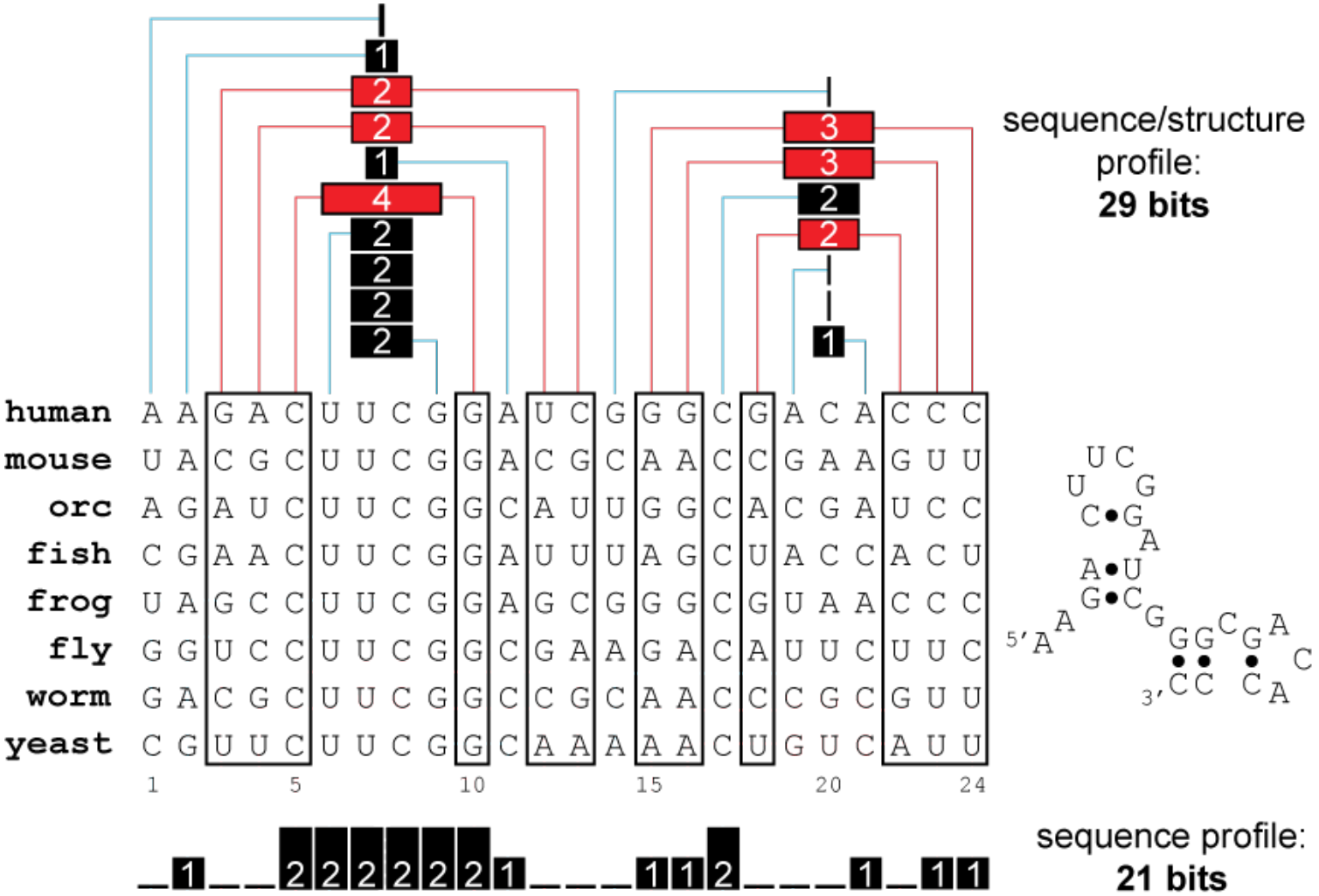
For instance, transfer RNA (many other ncRNA):



source Y. Sakakibara

RNA: conservation of secondary structure and sequence

Pairwise correlation



source S. Eddy

Profile SCFG

1. Terminal alphabet is $\{\mathtt{A}, \mathtt{C}, \mathtt{G}, \mathtt{U}\}$.

2. Production rules are of the following forms :
   (where, X, Y, Z are non-terminals, and $a, b \in \{\mathtt{A}, \mathtt{C}, \mathtt{G}, \mathtt{U}\}$).

   - To represent Watson-Crick complementary pair : $X \to aYb$
     For examples, $X \to \mathtt{A}Y\mathtt{U}$, $X \to \mathtt{U}Y\mathtt{A}$, $X \to \mathtt{C}Y\mathtt{G}$, $X \to \mathtt{G}Y\mathtt{C}$.
   - To represent single base (loops) : $X \to aY$ and $X \to a$.
   - To insert gaps in alignments (deletion states) : $X \to Y$.
   - To represent branching structures : $X \to YZ$.

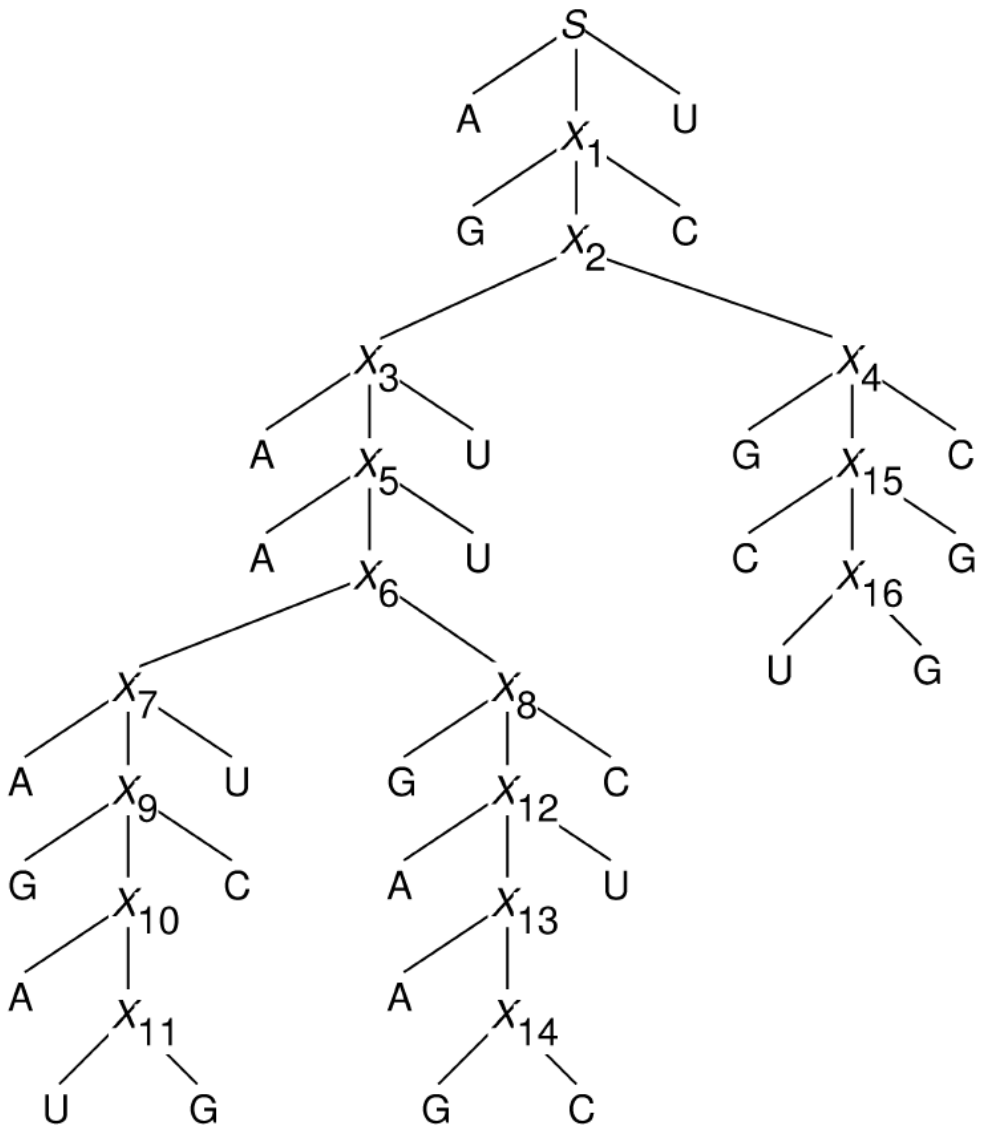
source Y. Sakakibara

Example

$$
P = \left\{
\begin{array}{lll}
S \to \mathtt{A}X_1\mathtt{U}, & & \\
X_1 \to \mathtt{G}X_2\mathtt{C}, & X_2 \to X_3X_4, & X_3 \to \mathtt{A}X_5\mathtt{U}, \\
X_5 \to \mathtt{A}X_6\mathtt{U}, & X_6 \to X_7X_8, & X_7 \to \mathtt{A}X_9\mathtt{U}, \\
X_9 \to \mathtt{G}X_{10}\mathtt{C}, & X_{10} \to \mathtt{A}X_{11}, & X_{11} \to \mathtt{UG}, \\
X_8 \to \mathtt{G}X_{12}\mathtt{C}, & X_{12} \to \mathtt{A}X_{13}\mathtt{U}, & X_{13} \to \mathtt{A}X_{14}, \\
X_{14} \to \mathtt{GC}, & X_4 \to \mathtt{G}X_{15}\mathtt{C}, & X_{15} \to \mathtt{C}X_{16}\mathtt{G}, \\
X_{16} \to \mathtt{UG} & &
\end{array}
\right\}
$$

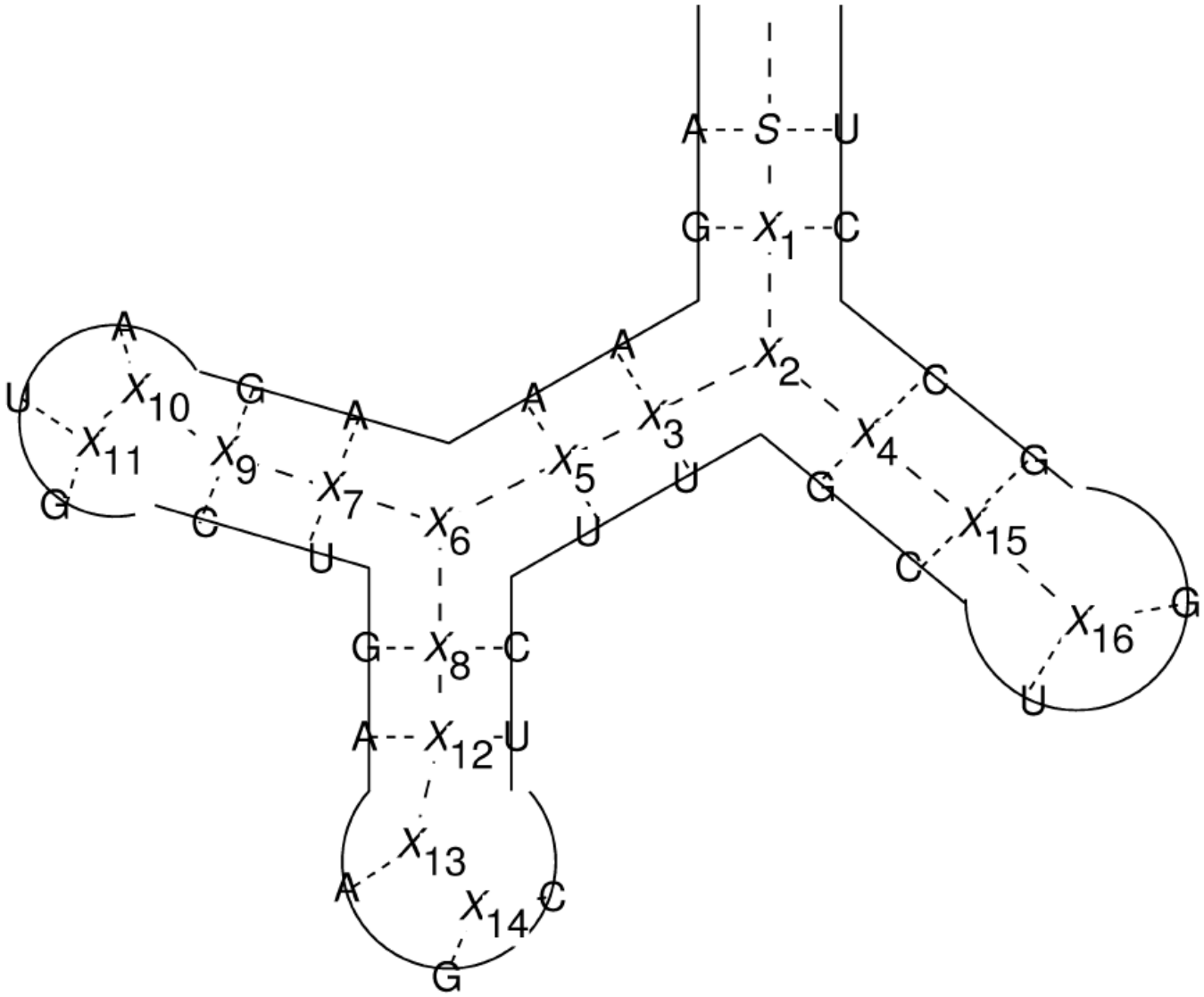
source Y. Sakakibara

A derivation tree



source Y. Sakakibara

The corresponding secondary structure



source Y. Sakakibara

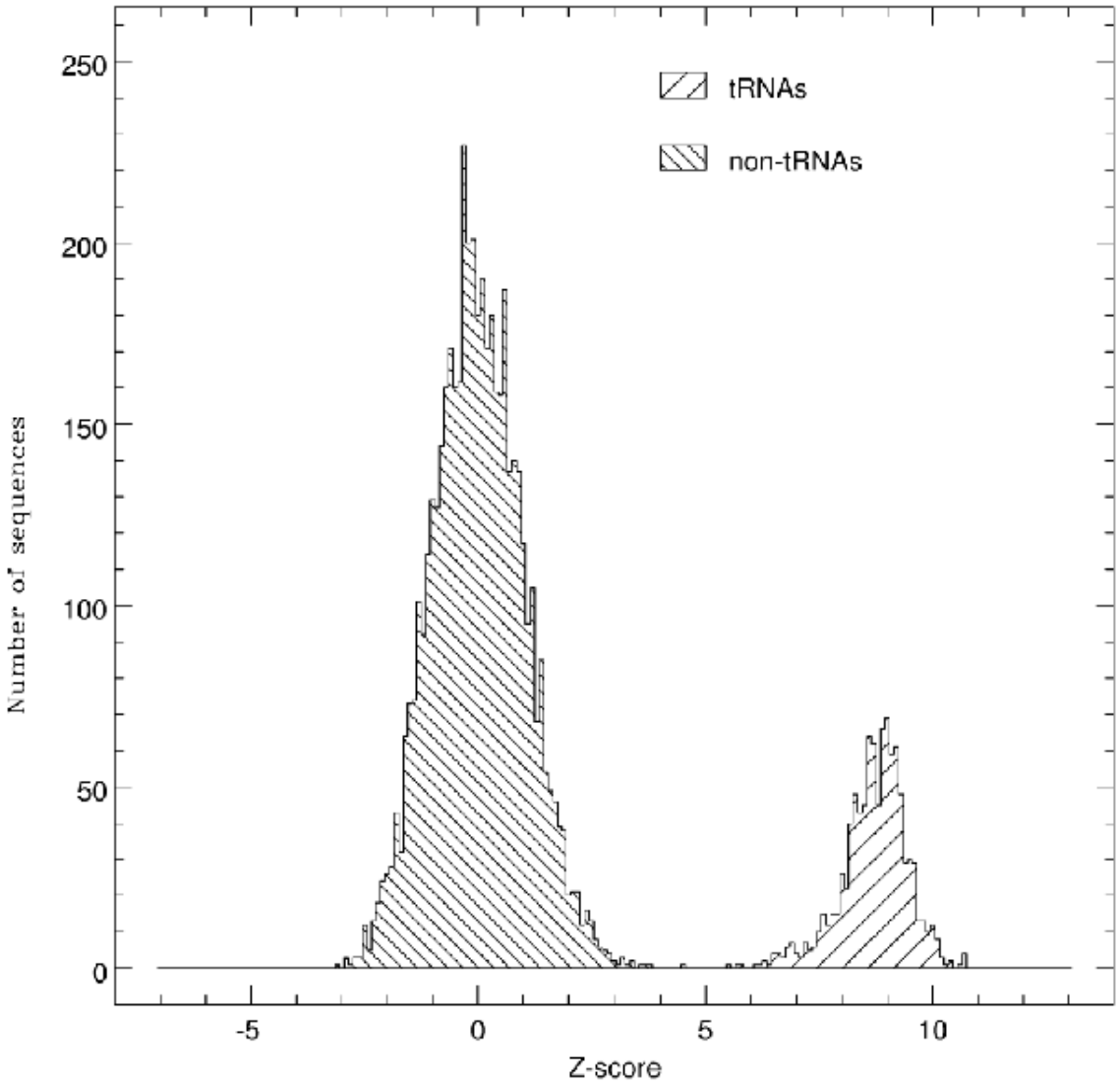
Multiple alignment

```
    [     ]  <     D-domain    > <   Anticodon   >< Extra ><    T-domain   >[     ]
    (((((((  ((((           )))) (((((  ===  )))))         (((((       ))))))))))))

1   -GCCAAGGUGGCAG.AGUUcGGccUAACGCGGCGGCCUGCAGAGCCGCUC---AUCGCCGGUUCAAAUCCGGCCCUUGGCU---
2   -GGGCGUGUGGCGU.AGUC.GG..UAGCGCGCUCCCUUAGCAUGGGAGAGG---UCUCCGGUUCGAUUCCGGACUCGUCCA---
3   -GCCCC-AUCGUCU.AGAG.GCc.UAGGACACCUCCCUUUCACGGAGGCG----ACGGGGAUUCGAAUUCCCCU-GGGGGU--A
4   -GGCGGCAUAGCCA.AGC-.GG..UAAGGCCGUGGAUUGCAAAUCCUCUA---UUCCCCAGUUCAAAUCUGGGUGCCGCCU---
5   -GUCUGAUUAGCGC.AACU.GG..CAGAGCAACUGACUCUUAAUCAGUGGG---UUGUGGGUUCGAUUCCCACAUCAGGCACCA
6   -GGGCGAAUAGUGUcAGCG.GG..-AGCACACCAGACUUGCAAUCUGGUA----GGGAGGGUUCGAGUCCCUCUUUGUCCACCA
7   -GGGGCUAUAGUUU.AACU.GG..UAAAACGGCGAUUUUGCAUAUCGUUA----UUUCAGGAUCGAGUCCUGAUAACUCCA---
8   -AGCUUUGUAGUUU.A--U.GU..GAAAAUGCUUGUUUGUGAUAUGAGUGA--AAU----------------UGGAGCUU---
```

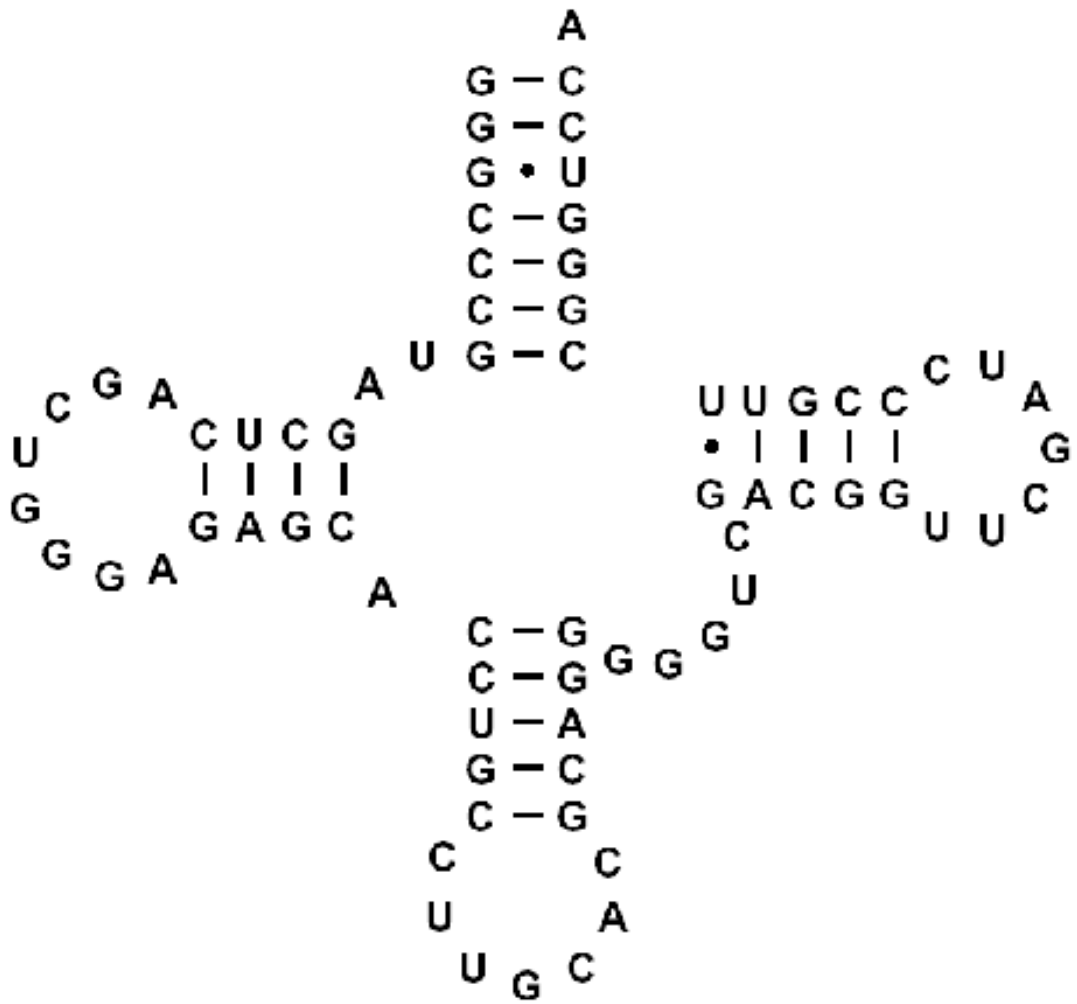
source Y. Sakakibara

Discrimination ability :



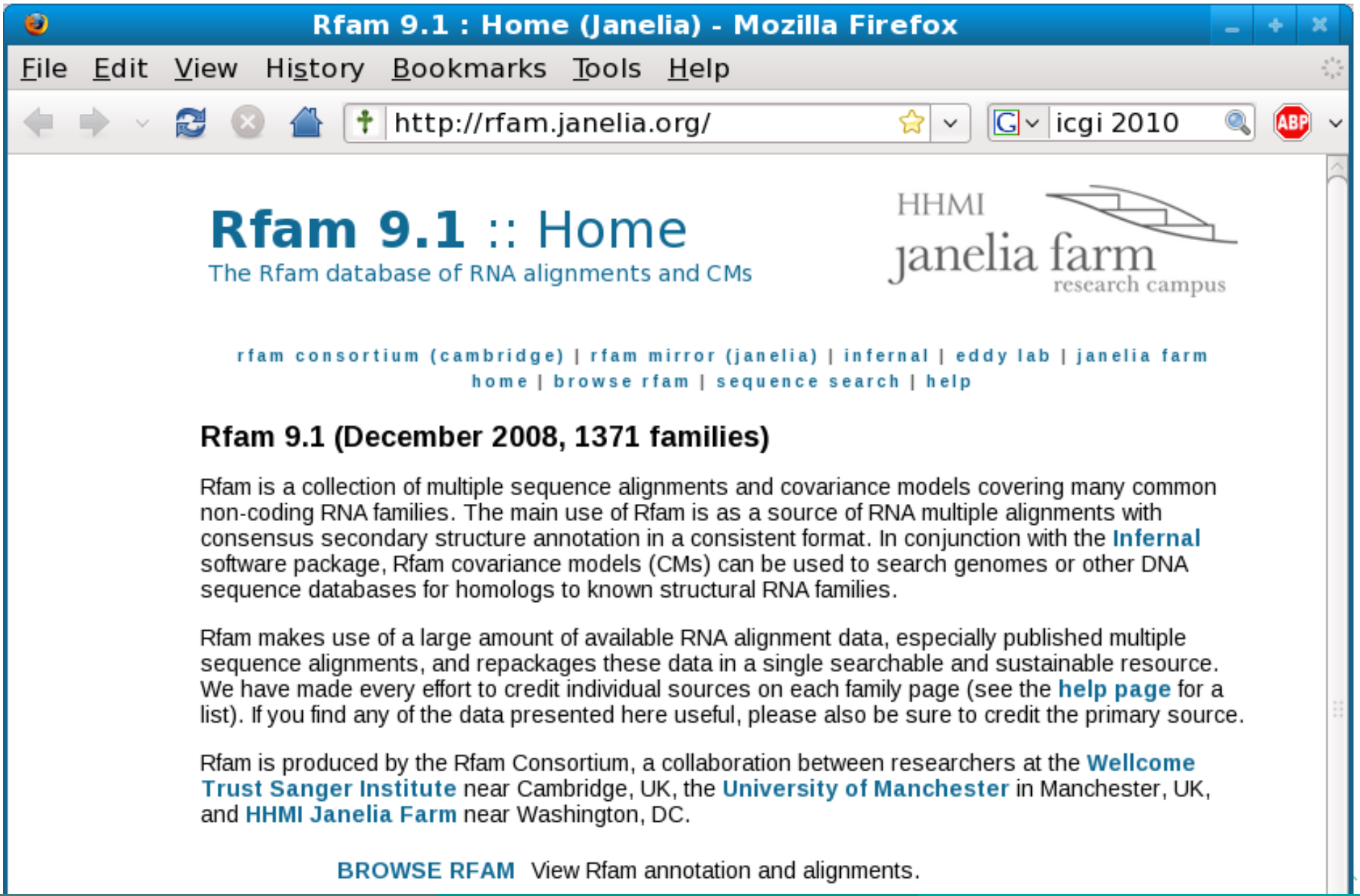
source Y. Sakakibara

Predicted secondary structure :



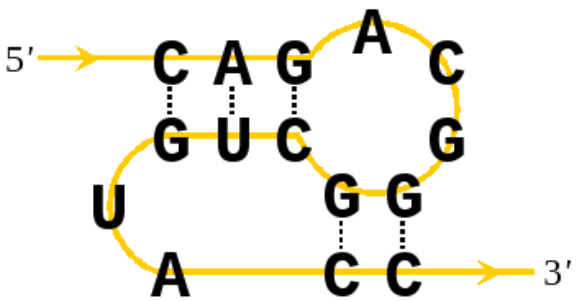
source Y. Sakakibara

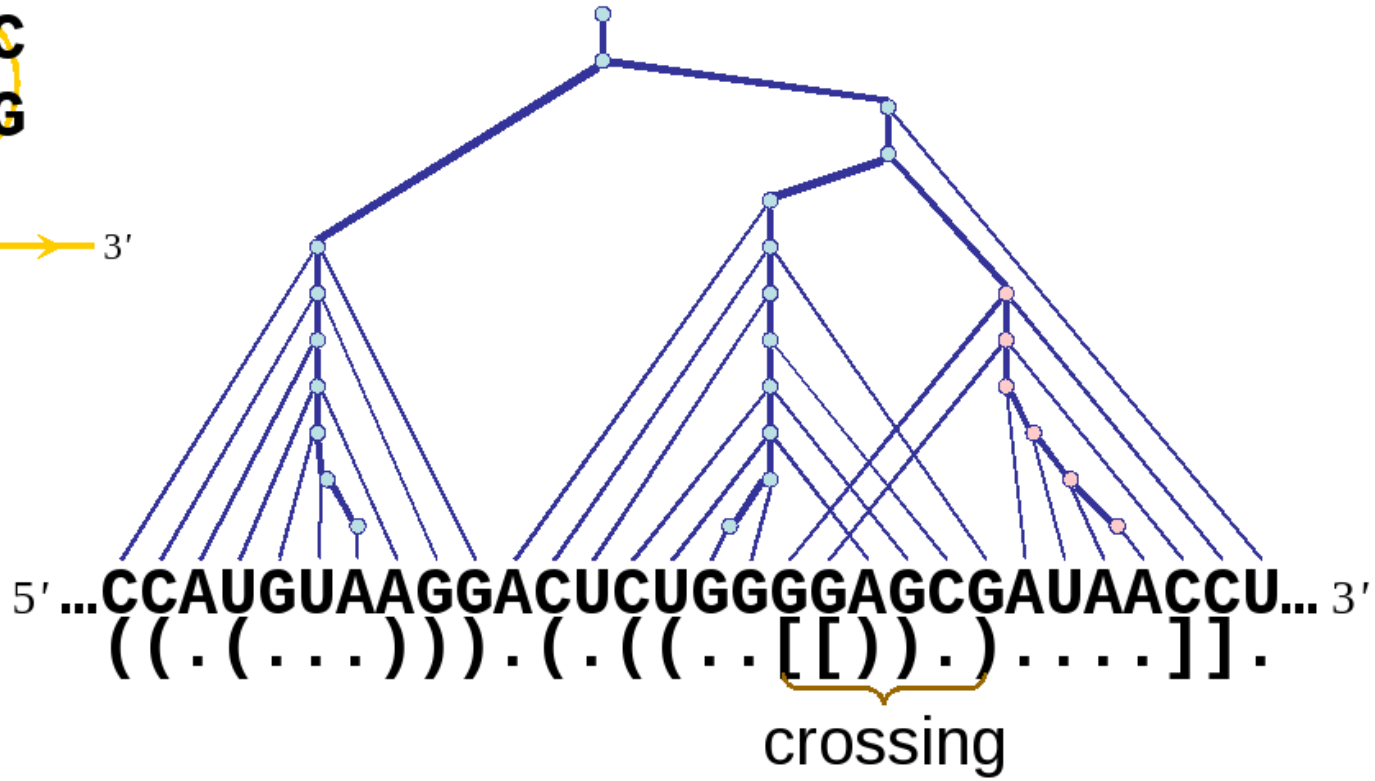
Rfam


Rfam 9.1 : Home (Janelia) - Mozilla Firefox
File Edit View History Bookmarks Tools Help
http://rfam.janelia.org/
icgi 2010
Rfam 9.1 :: Home
The Rfam database of RNA alignments and CMs
HHMI janelia farm research campus
rfam consortium (cambridge) | rfam mirror (janelia) | infernal | eddy lab | janelia farm
home | browse rfam | sequence search | help
Rfam 9.1 (December 2008, 1371 families)
Rfam is a collection of multiple sequence alignments and covariance models covering many common non-coding RNA families. The main use of Rfam is as a source of RNA multiple alignments with consensus secondary structure annotation in a consistent format. In conjunction with the Infernal software package, Rfam covariance models (CMs) can be used to search genomes or other DNA sequence databases for homologs to known structural RNA families.
Rfam makes use of a large amount of available RNA alignment data, especially published multiple sequence alignments, and repackages these data in a single searchable and sustainable resource. We have made every effort to credit individual sources on each family page (see the help page for a list). If you find any of the data presented here useful, please also be sure to credit the primary source.
Rfam is produced by the Rfam Consortium, a collaboration between researchers at the Wellcome Trust Sanger Institute near Cambridge, UK, the University of Manchester in Manchester, UK, and HHMI Janelia Farm near Washington, DC.
BROWSE RFAM View Rfam annotation and alignments.

Profile STAG . . .

- Branches (parentheses) cross in pseudoknots





Conclusion of this part

So far:

- Ad-hoc simple topologies designed wrt domain knowledge
- Modelling conserved regions
- Efficient parameter weighting schemes
- It works well!

We would like also:

- More expressive topologies, learnt automatically
- Better understand chaining of the conserved regions (grammar of the DNA ?)
- Efficient parameter weighting schemes (why not, but maybe less important if the topology is good)
- and it should still work well!

1. Molecular genetics
2. Modelling a set of conserved sequences
3. Inference of grammatical structure
   - Pattern (motif) Discovery
   - Learning Automata

Pattern (motif) Discovery

- DNA: A lot of work on the search of over/under-represented words (topology :-( )
- Proteins: not so much tools (20 letters, substitutions)
  Teiresias, Splash, Emotif, Pratt...
  No need anymore of aligned sequences. Topology ? Maximum level of expressiveness: Prosite motifs (Pratt), still unable to

  accept:
  ...R...E...
  ...E...R...
  and reject:
  ...E...E...
  ...R...R...

  (position specific pattern: [ER]x(l,k)[ER])

Learning local languages and application to DNA

Yokomori et al, 1994, 1998

- Splicing systems: model of recombination of DNA [Head 1987] (crossover operation)
- *Persistent* splicing languages are equivalent to *strictly locally testable* (regular) languages

$$\{L \mid \exists k, \exists A, B, C \subseteq \Sigma^k, \forall w, |w| \geq k : \\ w \in L \text{ iff } L_k(w) \in A, I(w) \in B, R(w) \in C\}$$

- Class of **k**-testable language is learnable in the limit using DFA

Sequence Encoding

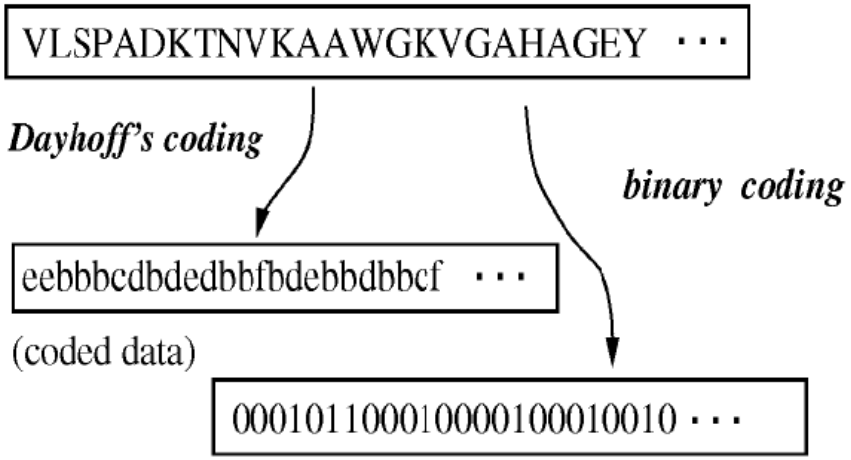


Fig. 5. Raw data of amino acid sequence from human hemoglobin is coded into new data over D_6 and B.

TRANSLATION TABLES
DAYHOFF'S CODING [9]

Amino Acids	Properties	New Symbols
C	sulfur polymerization	a
S, T, P, A, G	small	b
N, D, E, Q	acid & amide	c
H, R, K	basic	d
M, I, L, V	hydrophobic	e
F, Y, W	aromaticity	f

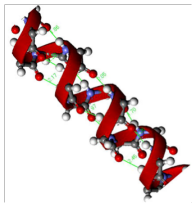
(a)

BINARY CODING [25]

Amino Acids	Hydropathy Index	New Symbols
A, C, F, G, I, L, M, N, S, T, V, W, Y	High	0
D, E, H, K, P, Q, R	Low	1

(b)

Experiments



Protein α-chain identification [Yokomori et al 1994, 1998]

$\sim$ 95% classification success (hemoglobin ?)

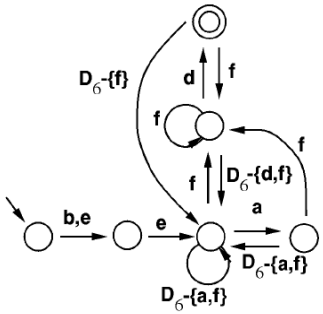


Fig. 8. A DFA $M_{2,30}$ most frequently obtained in experiments Exp(2, 30) over D_6. This DFA was obtained more than 70 among 100 times of random experiments.

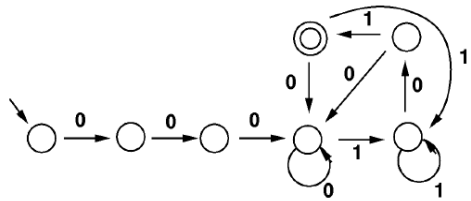
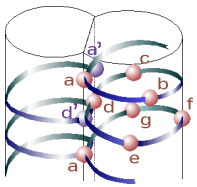
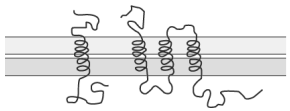


Fig. 9. A DFA $M_{3,6}$ most frequently obtained in experiments Exp(3, 6) over B. This DFA was obtained more than 10 among 100 times of random experiments.



Coiled-coil proteins [Peris et al ICGI 2006]



Transmembrane domain [Peris et al BMC Bioinf. 2008]

Learning automata on proteins

It is easy to represent a set of protein sequences by an automaton!

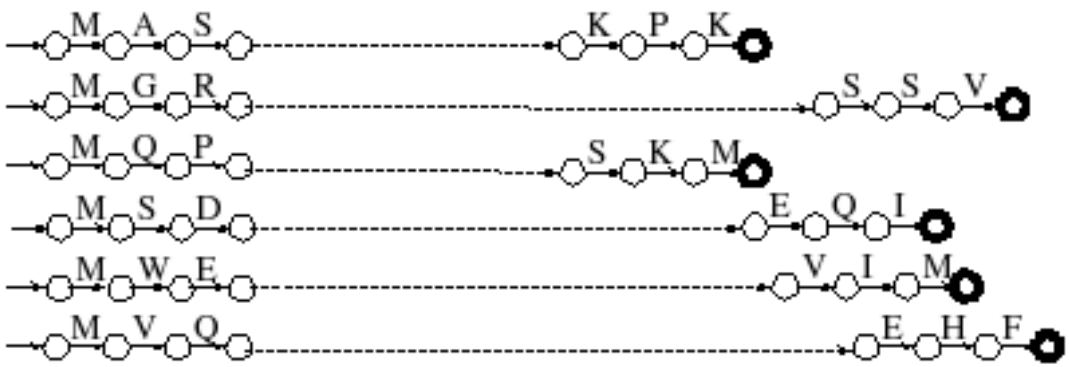
>AQP1_BOVIN
MASEFKKKLFWRAVVAEFL...KPK
>AQP3_MOUSE
MGRQKELMNRCGE...SSV
>AQP9_HUMAN
MQPEGAEKGKSFKQRLVLKSSLA...SKM
>AQP4_BOVIN
MSDRPAATRWGKCGPLCTRES..EQI
>AQP2_RAT
MWELRSIAFSRAVLAEFLAT...VIM
>AQP7_HUMAN
MVQASGHRRSTRGSKMVSWSVP...EHF

Learning automata on proteins

It is easy to represent a set of protein sequences by an automaton!

>AQP1_BOVIN
MASEFKKKLFWRAVVAEFL...KPK
>AQP3_MOUSE
MGRQKELMNRCGE...SSV
>AQP9_HUMAN
MQPEGAEKGKSFKQRLVLKSSLA...SKM
>AQP4_BOVIN
MSDRPAATRWGKCGPLCTRES..EQI
>AQP2_RAT
MWELRSIAFSRAVLAEFLAT...VIM
>AQP7_HUMAN
MVQASGHRRSTRGSKMVSWSVP...EHF

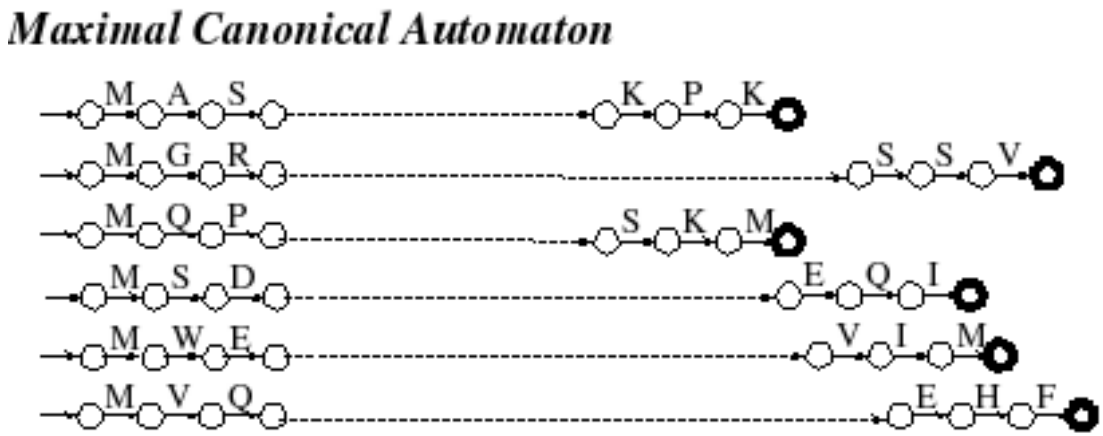
Maximal Canonical Automaton



Learning automata on proteins

It is easy to represent a set of protein sequences by an automaton!

>*AQP1_BOVIN*
MASEFKKKLFWRAVVAEFL...KPK
>*AQP3_MOUSE*
MGRQKELMNRCGE...SSV
>*AQP9_HUMAN*
MQPEGAEKGKSFKQRLVLKSSLA...SKM
>*AQP4_BOVIN*
MSDRPAATRWGKCGPLCTRES..EQI
>*AQP2_RAT*
MWELRSIAFSRAVLAEFLAT...VIM
>*AQP7_HUMAN*
MVQASGHRRSTRGSKMVSWSVP...EHF



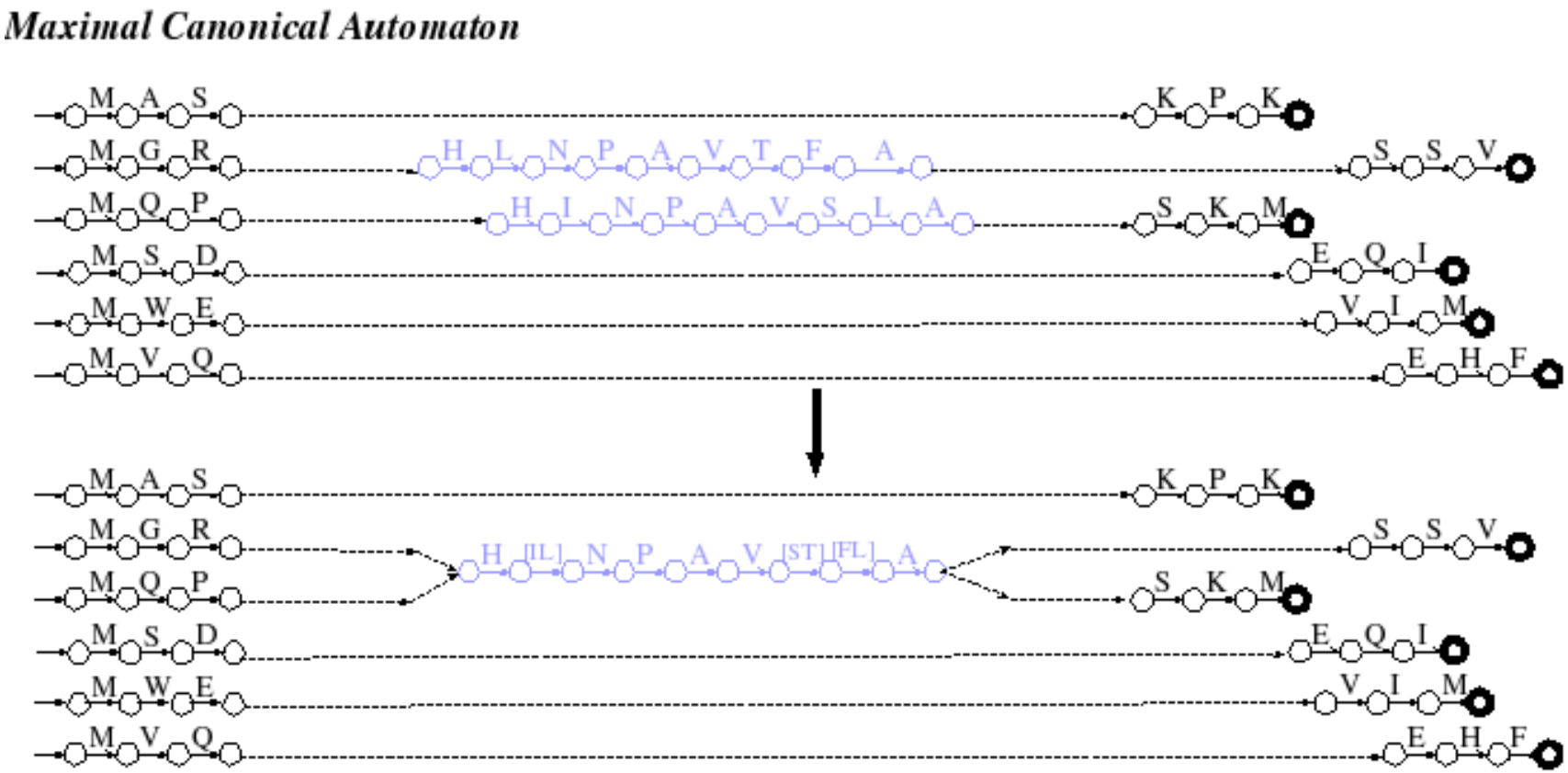
Rote learning, an inductive leap is needed...

Protomata Learner

[Coste et al 2005,2006]

State (fragment) merging approach, EDSM inspiration

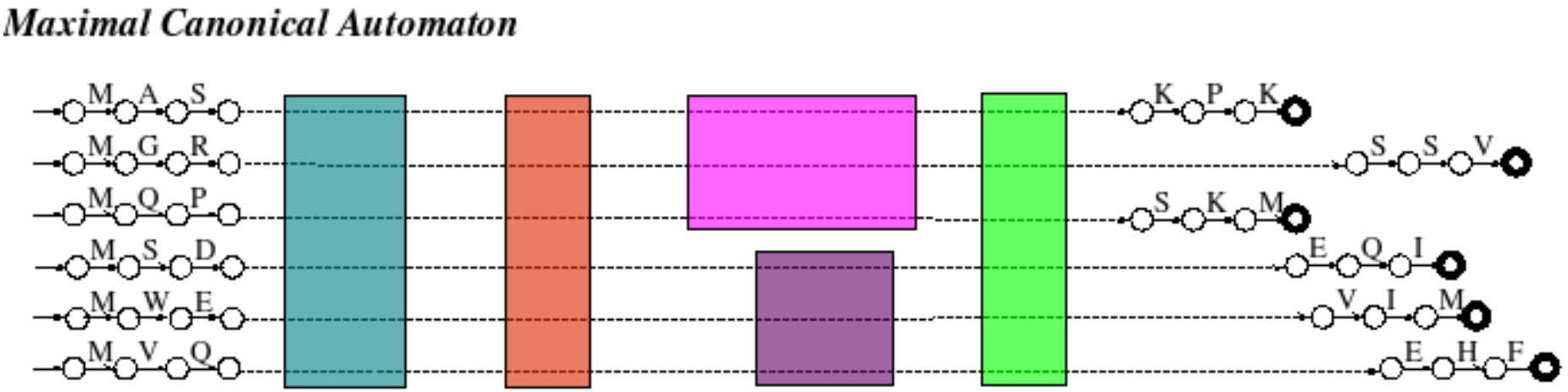
- Merging similar fragment pairs



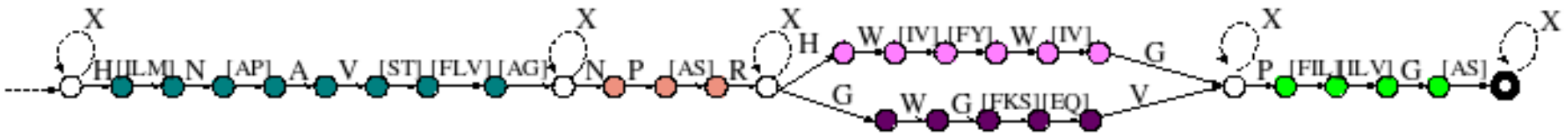
Protomata Learner

[Coste et al 2005,2006]

A new kind of alignment: Partial Local Multiple Alignment

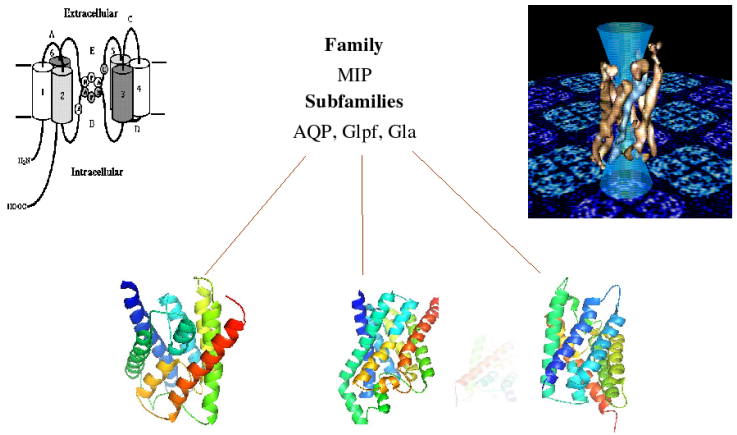


Merge blocks, detect exceptions, merge gaps:

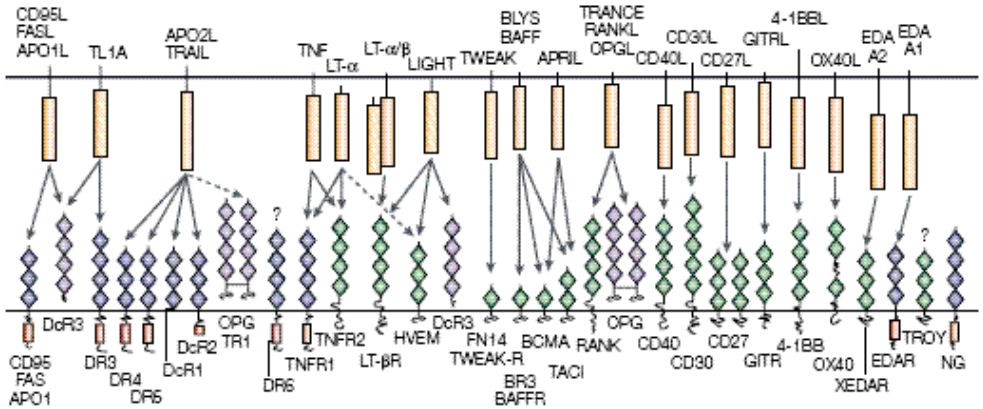


Experiments

- Discrimination of subfamilies (Major Intrinsic Proteins)

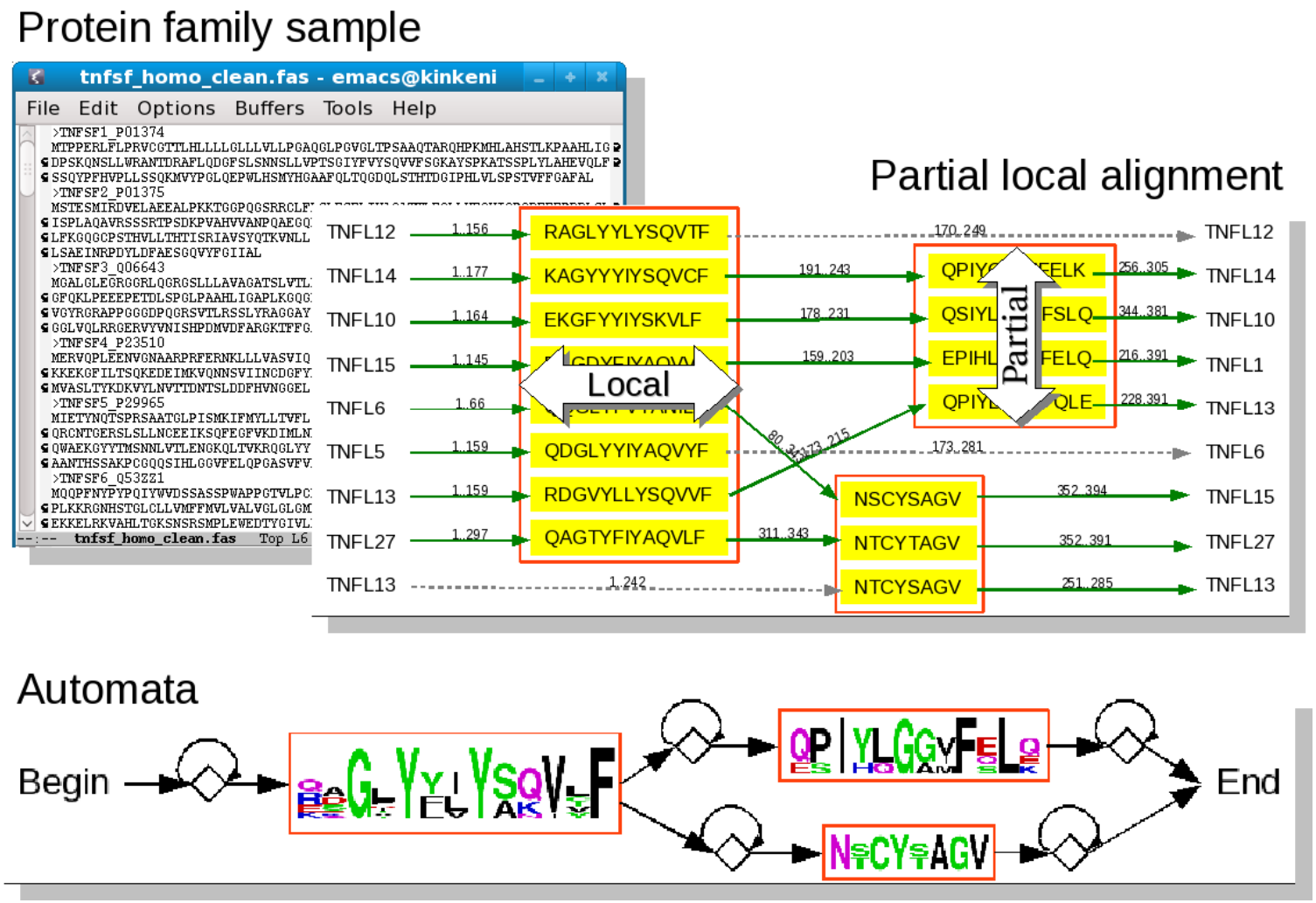


- Modelling low similarity family (Tumor Necrosis Factor)

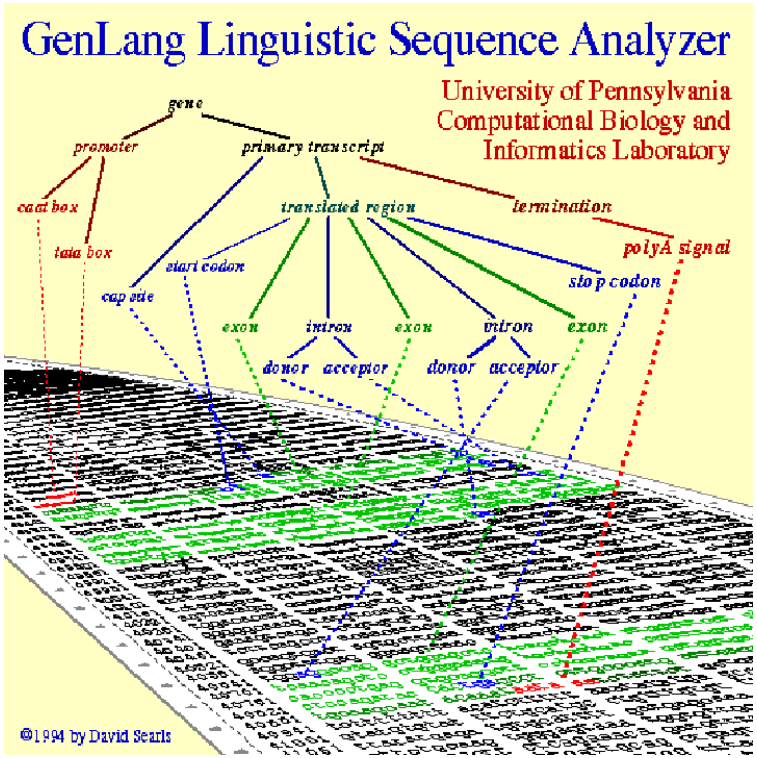


Web Server `http://protomata-learner.genouest.org/`

A new *weighted* version soon. . .



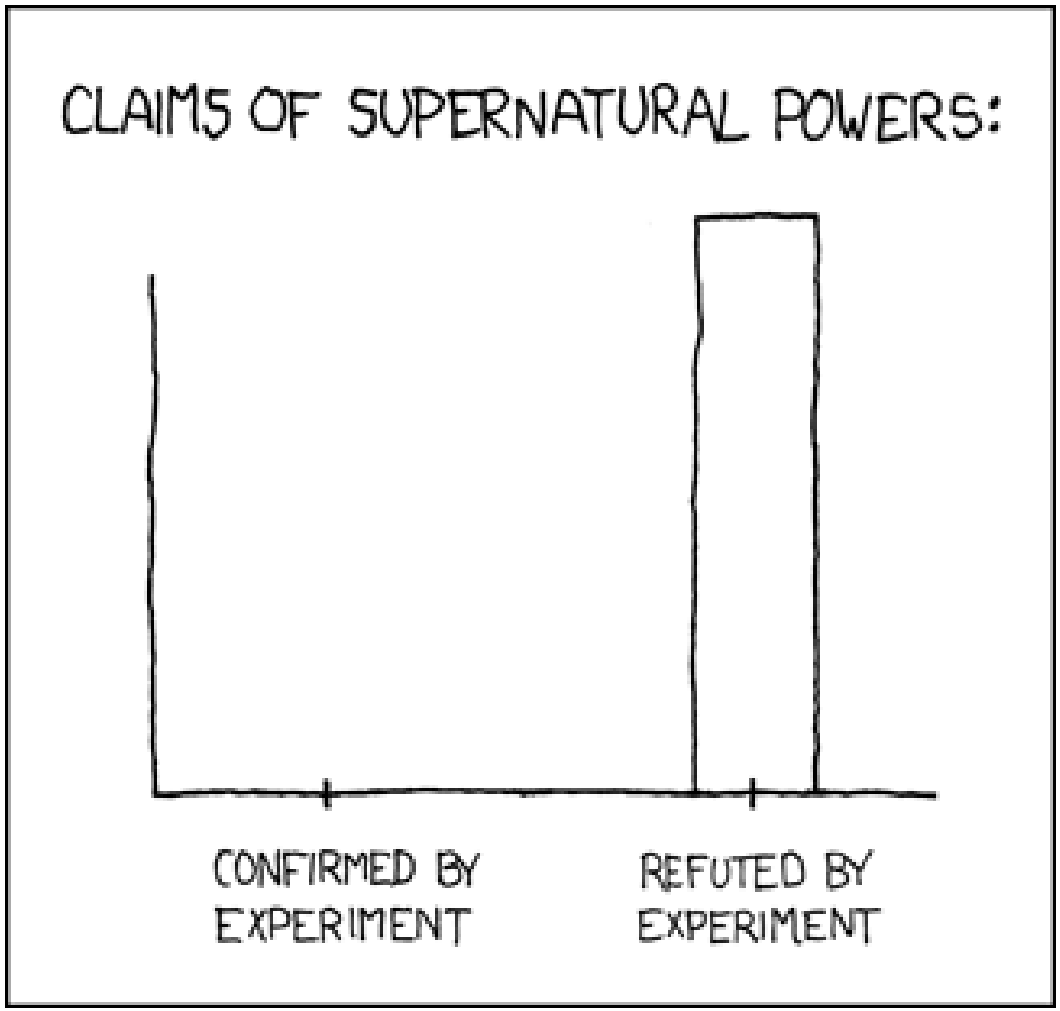
Learning the grammar of DNA sequences ?



More expressive topologies ?

- Adios, ABL, Distributional learning ?
- Hierarchical (context-free) structure inference (Sequitur . . .)
- Invited talk of D. Searls on Wednesday . . .

In-silico predictions. . .

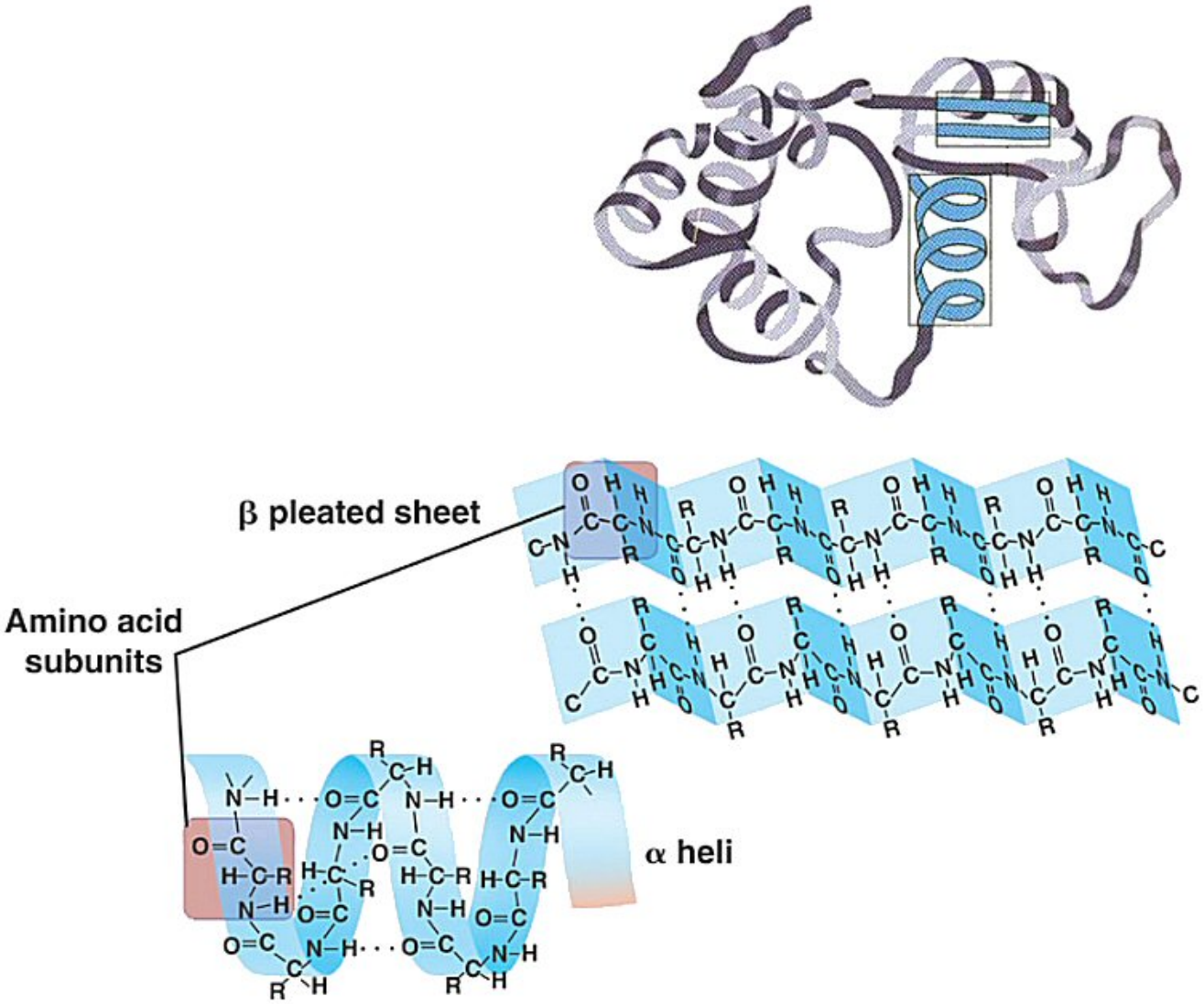


Bibliography

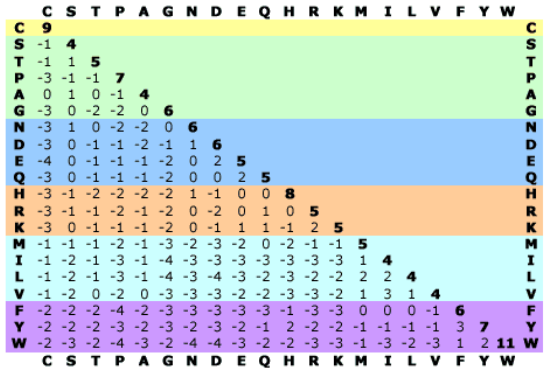
"Tutorial on Modelling Biological Sequences by Grammatical Inference: Bibliography", F. Coste, ICGI'2010 Tutorial Day

Appendix

Protein secondary structure



Dayhoff encoding



a	C	sulfhydryl
b	G, S, T, A, P	small hydrophilic
c	D, E, N, Q	acid, acid-amide and hydrophilic
d	R, H, K	basic
e	L, V, M, I	small hydrophobic
f	Y, F, W	aromatic

Prosite's Pattern

Pattern	C-x(2,4)-C-x(3)-[LIVMFYWC]-x(8)-H-x(3,5)-H.

Numerical results

- UniProtKB/Swiss-Prot release number: **54.7**, total number of sequence entries in that release: **333445**.
- Total number of hits in UniProtKB/Swiss-Prot: **11060 hits in 1794 different sequences**
- Number of hits on proteins that are known to belong to the set under consideration: **10810 hits in 1609 different sequences**
- Number of hits on proteins that could potentially belong to the set under consideration: **26 hits in 12 different sequences**
- Number of false hits (on unrelated proteins): **224 hits in 173 different sequences**
- Number of known missed hits: **60**
- Number of partial sequences which belong to the set under consideration, but which are not hit by the pattern or profile because they are partial (fragment) sequences: **1**
- Precision (true hits / (true hits + false positives)): **97.97 %**
- Recall (true hits / (true hits + false negatives)): **99.45 %**

Probabilistic version: Prosite's Profile

Matrix / Profile

```
                A   B   C   D   E   F   G   H   I   K   L   M   N   P   Q   R   S   T   V   W   Y   Z
/I:         Bl=0; BI=-105; BD=-105;
/M: SY='Y'; M=-19,-21,-24,-25,-21, 39,-28, 10, -2,-17,  2,  0,-17,-28,-19,-13,-18,-10, -7, 12, 48,-21;
/M: SY='K'; M= -4, -5,-23, -5,  6,-20,-18, -9,-14,  7,-15, -7, -5, -9,  3,  2, -2, -2, -9,-25,-12,  4;
/M: SY='C'; M=-10,-20,118,-30,-30,-20,-30,-30,-30,-30,-20,-20,-20,-40,-30,-30,-10,-10,-10,-50,-30,-30;
/M: SY='R'; M= -5,  3,-24,  3,  6,-22,-11, -6,-20,  1,-21,-14,  4, -1,  1, -3,  5,  2,-18,-29,-15,  3;
/I:         I=-12; MI=0; MD=-30; IM=0; DM=-30;
/M: SY='E'; M= -9, -2,-26,  1, 14,-18,-17, -4,-13, -1,-11, -8, -5,-12,  4, -5, -5, -8,-12,-24, -9,  8;
/M: SY='C'; M=-10,-20,119,-30,-30,-20,-30,-30,-30,-30,-20,-20,-20,-40,-30,-30,-10,-10,-10,-50,-29,-30;
/M: SY='G'; M= -3, -1,-28, -1, -7,-28, 36,-11,-33,-11,-27,-18,  4,-15,-10,-12,  1,-13,-27,-24,-23, -9;
/M: SY='E'; M=-10, -2,-28, -3,  8,-25,-19, -7,-26, 36,-24, -8, -1,-12, 10, 27, -9, -9,-18,-19, -8,  8;
/M: SY='A'; M=  8, -7, -9,-11, -7,-17, -7,-14,-16, -6,-16,-11, -4,-15, -6, -5,  8,  4, -7,-27,-15, -7;
/M: SY='F'; M=-19,-29,-19,-37,-28, 71,-29,-17,  0,-28,  9,  0,-20,-30,-36,-19,-19, -9, -1,  9, 31,-28;
/M: SY='S'; M=  0, -5,-17, -9, -6,-16,-11,-10,-14, -3,-16,-10,  0,-12, -4,  0,  8,  7, -8,-27,-12, -6;
/M: SY='R'; M=-10, -3,-20, -4,  0,-18,-17,  2,-19,  3,-16, -8,  0,-17,  8,  9, -1, -3,-17,-19, -5,  3;
/M: SY='R'; M= -4, -4,-22, -6,  0,-19,-13, -5,-18,  7,-18, -9,  1,-10,  2,  8,  2, -2,-14,-25,-11,  0;
/M: SY='S'; M=  2, -1,-16, -1, -1,-18, -4, -6,-19, -7,-22,-14,  4,-12, -2, -7, 16,  7,-13,-29,-12, -2;
/M: SY='H'; M= -5,  5,-20,  1,  0,-18,-10,  8,-18, -5,-18,-11,  9,-16,  1, -4,  4, -1,-17,-27, -7, -1;
/M: SY='L'; M=-11,-29,-20,-30,-20, 12,-29,-19, 17,-27, 43, 18,-28,-29,-20,-18,-28,-10,  9,-18,  2,-20;
/M: SY='R'; M= -6, -6,-22,-10, -4,-15,-20, -8, -7,  2, -9, -3, -2,-16,  0,  3, -3,  0, -6,-24, -8, -3;
/M: SY='R'; M= -6, -6,-23, -7,  0,-19,-17, -7,-14,  7,-13, -6, -2,-16,  4, 12, -3, -3,-10,-24,-10,  0;
/M: SY='H'; M=-20,  0,-30,  0,  0,-20,-20, 99,-30,-10,-20,  0, 10,-20, 10,  0,-10,-20,-30,-30, 20,  0;
/M: SY='Q'; M=-10,-10,-25,-12,  1,-16,-22, -2, -6,  1, -3,  6, -9,-17, 13,  3, -9, -8, -9,-19, -4,  6;
/M: SY='R'; M=-13, -8,-26, -9,  0,-19,-19, -4,-21, 20,-16, -6, -2,-17,  6, 35, -8, -7,-14,-21, -9,  0;
/I:         I=-12; MI=0; MD=-29; IM=0; DM=-29;
/M: SY='V'; M= -3,-16,-17,-21,-17, -6,-25,-20, 11,-15,  2,  3,-12,-18,-14,-14, -2,  9, 13,-25, -7,-17;
/M: SY='H'; M=-20,  0,-30,  0,  0,-20,-20, 97,-30,-10,-20,  0, 10,-20, 10,  0,-10,-20,-30,-30, 19,  0;
/M: SY='T'; M=  1, -2,-13, -8, -6,-14,-15,-15,-12, -7,-13, -9,  0,-12, -6, -8, 14, 25, -5,-29,-12, -6;
/M: SY='G'; M= -3, -4,-27, -4, -8,-27, 34,-14,-32, -9,-25,-16,  2,-15,-10, -9,  0,-13,-24,-23,-23,-10;
/M: SY='R'; M= -9,  6,-27, 12, 33,-26,-17, -3,-24,  7,-18,-15, -1, -6, 12,  0, -2, -8,-22,-28,-16, 22;
/M: SY='E'; M=-11, -1,-28, -2,  6,-25,-17, -6,-27, 32,-25,-10,  1,-11,  8, 28, -7, -8,-19,-22,-11,  6;
/M: SY='P'; M= -7,-14,-32, -9, -1,-24,-17,-15,-18, -7,-23,-15,-13, 51, -7,-13, -6, -6,-23,-28,-22, -7;
/I:         Kl=0;
```

Numerical results

- UniProtKB/Swiss-Prot release number: **54.7**, total number of sequence entries in that release: **333445**.
- Total number of hits in UniProtKB/Swiss-Prot: **11272 hits in 1555 different sequences**
- Number of hits on proteins that are known to belong to the set under consideration: **11257 hits in 1540 different sequences**
- Number of hits on proteins that could potentially belong to the set under consideration: **6 hits in 6 different sequences**
- Number of false hits (on unrelated proteins): **9 hits in 9 different sequences**
- Number of known missed hits: **126**
- Number of partial sequences which belong to the set under consideration, but which are not hit by the pattern or profile because