DNA1: (Last week)

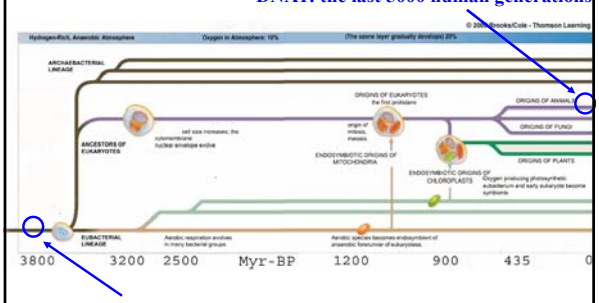
Types of mutants
Mutation, drift, selection
  Binomial for each
Association studies χ^2 statistic
Linked & causative alleles
Alleles, Haplotypes, genotypes
Computing the first genome,
  the second ...
New technologies
Random and systematic errors

DNA2: Aligning ancient diversity

Comparing types of alignments & algorithms
Dynamic programming
Multi-sequence alignment
Space-time-accuracy tradeoffs
Finding genes -- motif profiles
Hidden Markov Model for CpG Islands

DNA2: diversity & continuity

DNA1: the last 5000 human generations



Integrate1&2: ancient genetic code

http://www.colorado.edu/epob/epob1030cornwall/fall_2001/Evolution_of_Life.gif

Applications of Dynamic Programming

- To sequence analysis
  - Shotgun sequence assembly
  - Multiple alignments
  - Dispersed & tandem repeats
  - Bird song alignments
  - Gene Expression time-warping
- 3D-structure alignment
- Through HMMs
  - RNA gene search & structure prediction
  - Distant protein homologies
  - Speech recognition

Alignments & Scores

Global (e.g. haplotype)
```
ACCACACA
::xx::x:
ACACCATA
```
Score= 5(+1) + 3(-1) = 2

Local (motif)
```
ACCACACA
::::
ACACCATA
```
Score= 4(+1) = 4

Suffix (shotgun assembly)
```
ACCACACA
   :::
   ACACCATA
```
Score= 3(+1) =3

Increasingly complex (accurate) searches

Exact (StringSearch)	CGCG
Regular expression (PrositeSearch)	CGN{0-9}CG = CGAACG
Substitution matrix (BlastN)	CGCG ~= CACG
Profile matrix (PSI-blast)	CGc(g/a) ~= CACG
Gaps (Gap-Blast)	CGCG ~= CGAACG
Dynamic Programming (NW, SM)	CGCG ~= CAGACG

Hidden Markov Models (HMMER)  WU

"Hardness" of (multi-) sequence alignment

Align 2 sequences of length N allowing gaps.

```
ACCAC-ACA        ACCACACA
::x::x:x:        :xxxxxx:
AC-ACCATA   ,    A-----CACCATA  , etc.
```

2N gap positions, gap lengths of 0 to N each:
A naïve algorithm might scale by $O(N^{2N})$.
For N= $3x10^9$ this is rather large.

Now, what about k>2 sequences?
or rearrangements other than gaps?

Testing search & classification algorithms

Separate Training set and Testing sets
Need databases of non-redundant sets.
Need evaluation criteria (programs)
Sensistivity and Specificity (false negatives & positives)

sensitivity (true_predicted/true)
specificity (true_predicted/all_predicted)

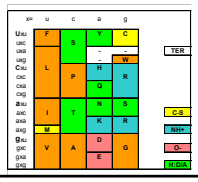
Where do training sets come from?
More expensive experiments: crystallography, genetics, biochemistry

Comparisons of homology scores

Pearson WR Protein Sci 1995 Jun;4(6):1145-60
Comparison of methods for searching protein sequence databases. Methods Enzymol 1996;266:227-58
Effective protein sequence comparison.

Algorithm: FASTA, Blastp, Blitz
Substitution matrix:PAM120, PAM250, BLOSUM50, BLOSUM62
Database: PIR, SWISS-PROT, GenPept

Switch to protein searches when possible



A Multiple Alignment of Immunoglobulins

```
VTISCTGSSSNIGAG-NHVKWYQQLPG
VTISCTGTSSNIGS--ITVNWYQQLPG
LRLSCSSSGFIFSS--YAMYWVRQAPG
LSLTCTVSGTSFDD--YYSTWVRQPPG
PEVTCVVVDVSHEDPQVKFNWYVDG--
ATLVCLISDFYPGA--VTVAWKADS--
AALGCLVKDYFPEP--VTVSWNSG---
VSLTCLVKGFYPSD--IAVEWESNG--
```


Scoring matrix based on large set of distantly related blocks: Blosum62

	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	
10	1	6	6	4	8	2	6	6	9	2	4	4	4	5	6	6	7	1	3	%	
A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y		
8	0	-4	-2	-4	0	-4	-2	-2	-2	-2	-4	-2	-2	-2	2	0	0	-6	-4		A
	18	-6	-8	-4	-6	-6	-2	-6	-2	-2	-6	-6	-6	-6	-2	-2	-2	-4	-4		C
		12	4	-6	-2	-2	-6	-2	-8	-6	2	-2	0	-4	0	-2	-6	-8	-6		D
			10	-6	-4	0	-6	2	-6	-4	0	-2	4	0	0	-2	-4	-6	-4		E
				12	-6	-2	0	-6	0	0	-6	-8	-6	-6	-4	-4	-2	2	6		F
					12	-4	-8	-4	-8	-6	0	-4	-4	-4	0	-4	-6	-4	-6		G
						16	-6	-2	-6	-4	2	-4	0	0	-2	-4	-6	-4	4		H
							8	-6	4	2	-6	-6	-6	-6	-4	-2	6	-6	-2		I
								10	-4	-2	0	-2	2	4	0	-2	-4	-6	-4		K
									8	4	-6	-6	-4	-4	-4	-2	2	-4	-2		L
										10	-4	-4	0	-2	-2	-2	2	-2	-2		M
											12	-4	0	0	2	0	-6	-8	-4		N
												14	-2	-4	-2	-2	-4	-8	-6		P
													10	2	0	-2	-4	-4	-2		Q
														10	-2	-2	-6	-6	-4		R
															8	2	-4	-6	-4		S
																10	0	-4	-4		T
																	8	-6	-2		V
																		22	4		W
																			14		Y

Scoring Functions and Alignments

- Scoring function:
  - ω(match) = +1;
  - ω(mismatch) = -1;
  - ω(indel) = -2;
  - ω(other) = 0.

  } substitution matrix
- Alignment score: sum of columns.
- Optimal alignment: maximum score.

Calculating Alignment Scores

(1) ATGA / :xx: / ACTA (2) A-TGA / : : : / ACT-A

$$(1)\textbf{Score} = \omega\begin{pmatrix}A\\A\end{pmatrix}+\omega\begin{pmatrix}T\\C\end{pmatrix}+\omega\begin{pmatrix}G\\T\end{pmatrix}+\omega\begin{pmatrix}A\\A\end{pmatrix}=1-1-1+1=0.$$

$$(2)\textbf{Score} = \omega\begin{pmatrix}A\\A\end{pmatrix}+\omega\begin{pmatrix}-\\C\end{pmatrix}+\omega\begin{pmatrix}T\\T\end{pmatrix}+\omega\begin{pmatrix}G\\-\end{pmatrix}+\omega\begin{pmatrix}A\\A\end{pmatrix}=1-2+1-2+1=-1.$$

if $\omega(\textbf{indel})=-1$, $\textbf{Score}=1-1+1-1+1=+1$.

DNA2: Aligning ancient diversity

Comparing types of alignments & algorithms

Dynamic programming

Multi-sequence alignment

Space-time-accuracy tradeoffs

Finding genes -- motif profiles

Hidden Markov Model for CpG Islands

What is dynamic programming?

A dynamic programming algorithm solves every subsubproblem just once and then saves its answer in a table, avoiding the work of recomputing the answer every time the subsubproblem is encountered.

-- Cormen *et al*. "Introduction to Algorithms", The MIT Press.

Recursion of Optimal Global Alignments

$s\begin{pmatrix}u\\v\end{pmatrix}$: **optimal global alignment score of *u* and *v*.**

$$s\begin{pmatrix}\textbf{ATG}A\\\textbf{ACT}\end{pmatrix}=\max\begin{cases}s\begin{pmatrix}\textbf{ATG}A\\\textbf{ACT}\end{pmatrix}+\omega\begin{pmatrix}-\\A\end{pmatrix};\\ s\begin{pmatrix}\textbf{ATG}\\\textbf{ACT}\end{pmatrix}+\omega\begin{pmatrix}A\\A\end{pmatrix};\\ s\begin{pmatrix}\textbf{ATG}\\\textbf{ACT}A\end{pmatrix}+\omega\begin{pmatrix}A\\-\end{pmatrix}.\end{cases}$$

Recursion of Optimal Local Alignments

$s\begin{pmatrix}u\\v\end{pmatrix}$: **optimal local alignment score of *u* and *v*.**

$$s\begin{pmatrix}u_1u_2\ldots u_i\\v_1v_2\ldots v_j\end{pmatrix}=\max\begin{cases}s\begin{pmatrix}u_1u_2\ldots u_i\\v_1v_2\ldots v_{j-1}\end{pmatrix}+\omega\begin{pmatrix}-\\v_j\end{pmatrix};\\ s\begin{pmatrix}u_1u_2\ldots u_{i-1}\\v_1v_2\ldots v_{j-1}\end{pmatrix}+\omega\begin{pmatrix}u_i\\v_j\end{pmatrix};\\ s\begin{pmatrix}u_1u_2\ldots u_{i-1}\\v_1v_2\ldots v_j\end{pmatrix}+\omega\begin{pmatrix}u_i\\-\end{pmatrix};\\ 0.\end{cases}$$

Computing Row-by-Row

		A	C	T	A
	0	min	min	min	min
A	min	1	-1	-3	-5
T	min				
G	min				
A	min				

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

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

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

min = -10^{99}

Traceback Optimal Global Alignment

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

$\begin{pmatrix} A & G & T & A \\ : & \times & \times & : \\ A & T & C & A \end{pmatrix}$

Local and Global Alignments

		A	C	C	A	C	A	C	A
	0	0	0	0	0	0	0	0	0
A	0	1	0	0	1	0	1	0	1
C	0	0	2	1	0	2	0	2	0
A	0	1	0	1	2	0	3	1	3
C	0	0	2	1	0	3	1	4	2
C	0	0	0	3	2	1	2	2	3
A	0	1	0	1	4	2	2	1	3
T	0	0	0	0	2	3	1	1	1
A	0	1	0	0	1	1	4	2	2

		A	C	C	A	C	A	C	A
	0	m	m	m	m	m	m	m	m
A	m	1	-1	-3	-5	-7	-9	-11	-13
C	m	-1	2	0	-2	-4	-6	-8	-10
A	m	-3	0	1	1	-1	-3	-5	-7
C	m	-5	-2	1	0	2	0	-2	-4
C	m	-7	-4	-1	0	1	1	1	-1
A	m	-9	-6	-3	0	-1	2	0	2
T	m	-11	-8	-5	-2	-1	0	1	0
A	m	-13	-10	-7	-4	-1	0	-1	2

Time and Space Complexity of Computing Alignments

For two sequences $u=u_1u_2...u_n$ and $v=v_1v_2...v_m$, finding the optimal alignment takes $O(mn)$ time and $O(mn)$ space.

An $O(1)$-time operation: one comparison, three multiplication steps, computing an entry in the alignment table…

An $O(1)$-space memory: one byte, a data structure of two floating points, an entry in the alignment table…

Time and Space Problems

- Comparing two one-megabase genomes.
- Space:
  - An entry: 4 bytes;
  - Table: 4 * 10^6 * 10^6 = 4 G bytes memory.
- Time:
  - 1000 MHz CPU: 1M entries/second;
  - 10^12 entries: 1M seconds = 10 days.

Time & Space Improvement for w-band Global Alignments

- Two sequences differ by at most w bps (w<<n).
- w-band algorithm: O(wn) time and space.
- Example: w=3.

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

Summary

Dynamic programming
Statistical interpretation of alignments
Computing optimal global alignment
Computing optimal local alignment
Time and space complexity
Improvement of time and space
Scoring functions

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A Multiple Alignment of Immunoglobulins

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VTISCTGSSSNIGAG-NHVKWYQQLPG
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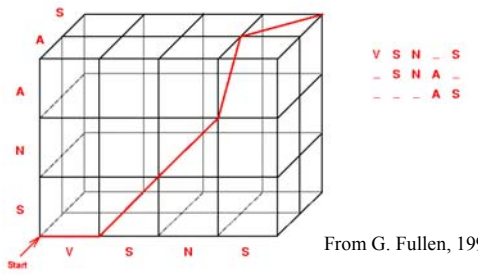
LSLTCTVSGTSFDD--YYSTWVRQPPG
PEVTCVVVDVSHEDPQVKFNWYVDG--
ATLVCLISDFYPGA--VTVAWKADS--
AALGCLVKDYFPEP--VTVSWNSG---
VSLTCLVKGFYPSD--IAVEWESNG--
```


A multiple alignment <=> Dynamic programming on a hyperlattice



From G. Fullen, 1996.

Multiple Alignment vs Pairwise Alignment

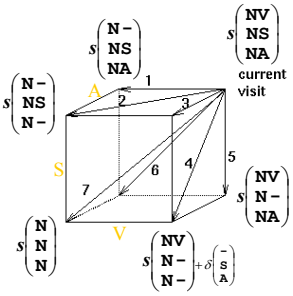
```
AT
AT
AT
A-
-T
AT
AT
```

```
A-
-T
```

Optimal Multiple Alignment

Non-Optimal Pairwise Alignment

Computing a Node on Hyperlattice



$k=3 \quad 2^k-1=7$

$$s\begin{pmatrix}NV\\NS\\NA\end{pmatrix} = \max\left\{\begin{array}{l} s\begin{pmatrix}N\\N\\N\end{pmatrix}+\delta\begin{pmatrix}V\\S\\A\end{pmatrix} \\ s\begin{pmatrix}NV\\N-\\N-\end{pmatrix}+\delta\begin{pmatrix}-\\S\\A\end{pmatrix} \\ s\begin{pmatrix}N-\\NS\\N-\end{pmatrix}+\delta\begin{pmatrix}V\\-\\A\end{pmatrix} \\ s\begin{pmatrix}N-\\N-\\NA\end{pmatrix}+\delta\begin{pmatrix}V\\S\\-\end{pmatrix} \\ s\begin{pmatrix}N-\\NS\\NA\end{pmatrix}+\delta\begin{pmatrix}V\\-\\-\end{pmatrix} \\ s\begin{pmatrix}NV\\N-\\NA\end{pmatrix}+\delta\begin{pmatrix}-\\S\\-\end{pmatrix} \\ s\begin{pmatrix}NV\\NS\\N-\end{pmatrix}+\delta\begin{pmatrix}-\\-\\A\end{pmatrix} \end{array}\right.$$

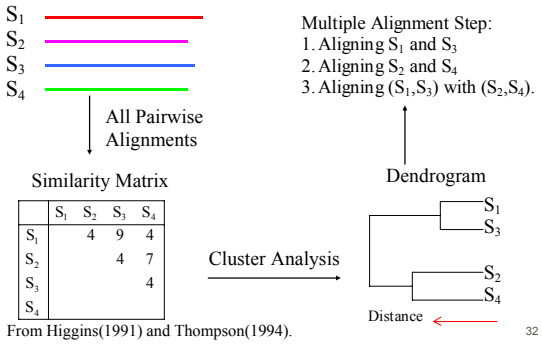
Challenges of Optimal Multiple Alignments

- Space complexity (hyperlattice size): $O(n^k)$ for k sequences each n long.
- Computing a hyperlattice node: $O(2^k)$.
- Time complexity: $O(2^k n^k)$.
- Find the optimal solution is exponential in k (non-polynomial, NP-hard).

Methods and Heuristics for Optimal Multiple Alignments

- Optimal: dynamic programming
  - Pruning the hyperlattice (MSA)
- Heuristics:
  - tree alignments(ClustalW)
  - star alignments
  - sampling (Gibbs) (discussed in RNA2)
  - local profiling with iteration (PSI-Blast, ...)

ClustalW: Progressive Multiple Alignment



From Higgins(1991) and Thompson(1994).

Star Alignments

s_1 = **ATTGCCATT**
s_2 = **ATGGCCATT**
s_3 = **ATCCAATTTT**
s_4 = **ATCTTCTT**
s_5 = **ACTGACC**

Pairwise Alignment

Similarity Matrix

	s_2	s_3	s_4	s_5
s_1	7	−2	0	−3
s_2		−2	0	−4
s_3			0	−7
s_4				−3

Find the Central Sequence s_1

Multiple Alignment

```
ATTGCCATT--
ATGGCCATT--
ATC-CAATTTT
ATCTTC-TT--
ACTGACC----
AT*GCCATTTT
```

Combine into Multiple Alignment

Pairwise Alignment

```
ATTGCCATT
ATGGCCATT

ATTGCCATT--
ATC-CAATTTT

ATTGCCATT
ATCTTC-TT

ATTGCCATT
ACTGACC
```


DNA2: Aligning ancient diversity

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Accurately finding genes & their edges

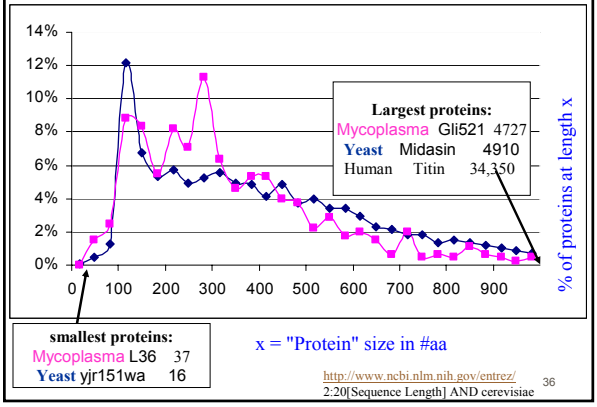
What is distinctive ?

0. Promoters & CGs islands
1. Preferred codons
2. RNA splice signals
3. Frame across splices
4. Inter-species conservation
5. cDNA for splice edges

Failure to find edges?

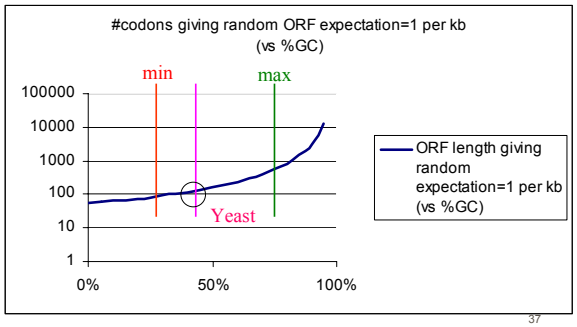
Variety & combinations
Tiny proteins (& RNAs)
Alternatives & weak motifs
Alternatives
Gene too close or distant
Rare transcript

Annotated "Protein" Sizes



http://www.ncbi.nlm.nih.gov/entrez/
2:20[Sequence Length] AND cerevisiae

Predicting small proteins (ORFs)



Small coding regions

Mutations in domain II of 23 S rRNA facilitate translation of a 23 S rRNA-encoded **pentapeptide** conferring erythromycin resistance. Dam et al. 1996 J Mol Biol 259:1-6

Trp (W) leader peptide, 14 codons:
MKAIFVLKGWWRTS STOP

Phe (F) leader peptide, 15 codons:
MKHIPFFFAFFFTFP STOP

His (H) leader peptide, 16 codons:
MTRVQFKHHHHHHHHPD STOP

http://www.andrew.cmu.edu/~berget/Education/attenuation/atten.html

Other examples in proteomics lectures

Motif Matrices

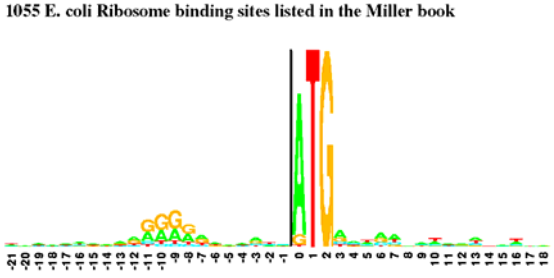
```
    a a t g
    c a t g
    g a t g
    t g t g

a   1 3 0 0
c   1 0 0 0
g   1 1 0 4
t   1 0 4 0

Align and calculate frequencies.
Note: Higher order correlations lost.
```


Protein starts

GeneMark



Motif Matrices

```
    a a t g   1+3+4+4 = 12
    c a t g   1+3+4+4 = 12
    g a t g   1+3+4+4 = 12
    t g t g   1+1+4+4 = 10

a   1 3 0 0
c   1 0 0 0
g   1 1 0 4
t   1 0 4 0

Align and calculate frequencies.
Note: Higher order correlations lost.
Score test sets:
a c c c   1+0+0+0 = 1
```


DNA2: Aligning ancient diversity

Comparing types of alignments & algorithms
Dynamic programming
Multi- sequence alignment
Space time accuracy tradeoffs
Finding genes-- motif profiles
Hidden Markov Model for CpG Islands

Why probabilistic models in sequence analysis?

- **Recognition -** Is this sequence a protein start?
- **Discrimination -** Is this protein more like a hemoglobin or a myoglobin?
- **Database search -** What are all of sequences in SwissProt that look like a serine protease?

A Basic idea

Assign a number to every possible sequence such that

$$\sum_s P(s|M) = 1$$

P(s|M) is a probability of sequence s given a model M.

Sequence recognition

***Recognition question* - What is the probability that the sequence s is from the start site model M ?**

P(M|s) = P(M)* P(s|M) / P(s)

(Bayes' theorem)

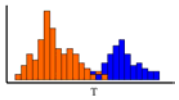
P(M) and P(s) are prior probabilities and P(M|s) is posterior probability.

Database search

- **N = null model (random bases or AAs)**
- **Report all sequences with**

  logP(s|M) - logP(s|N) > logP(N) - logP(M)

- **Example, say α/β hydrolase fold is rare in the database, about 10 in 10,000,000. The threshold is 20 bits. If considering 0.05 as a significant level, then the threshold is 20+4.4 = 24.4 bits.**



Plausible sources of mono, di, tri, & tetra- nucleotide biases

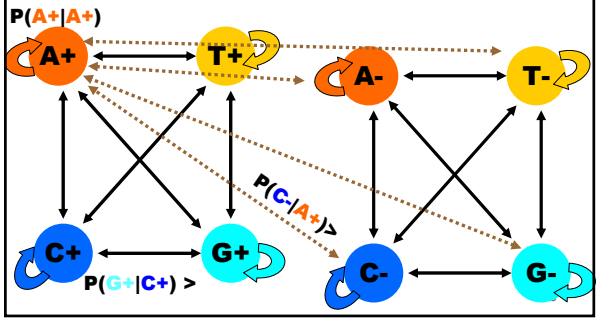
C rare due to lack of uracil glycosylase (cytidine deamination)
TT rare due to lack of UV repair enzymes.
CG rare due to 5methylCG to TG transitions (cytidine deamination)
AGG rare due to low abundance of the corresponding Arg-tRNA.
CTAG rare in bacteria due to error-prone "repair" of CTAGG to C*CAGG.
AAAA excess due to polyA pseudogenes and/or polymerase slippage.

AmAcid	Codon	Number	/1000	Fraction
Arg	AGG	3363.00	1.93	0.03
Arg	AGA	5345.00	3.07	0.06
Arg	CGG	10558.00	6.06	0.11
Arg	CGA	6853.00	3.94	0.07
Arg	CGT	34601.00	19.87	0.36
Arg	CGC	36362.00	20.88	0.37

ftp://sanger.otago.ac.nz/pub/Transterm/Data/codons/bct/Esccol.cod

CpG Island + in a ocean of - First order Hidden Markov Model

MM=16, HMM= 64 transition probabilities (adjacent bp)



Estimate transistion probabilities -- an example

Training set

S= aaacagcctgacatggttc**CGAACAGCCTCGACATGGCGTT**

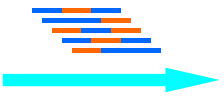
Isle	A+	C+	G+	T+	A-	C-	G-	T-	
A+	.20	.40	.20	.20	.00	.00	.00	.00	1.00
C+	.29	.14	.43	.14	.00	.00	.00	.00	1.00
G+	.33	.33	.17	.17	.00	.00	.00	.00	1.00
T+	.00	.25	.25	.25	.25	.00	.00	.00	1.00
	.82	1.13	1.05	.76	.25	.00	.00	.00	Sums
Ocean	A-	C-	G-	T-	A+	C+	G+	T+	
A-	.33	.33	.17	.17	.00	.00	.00	.00	1.00
C-	.40	.20	.00	.20	.00	.20	.00	.00	1.00
G-	.25	.25	.25	.25	.00	.00	.00	.00	1.00
T-	.00	.25	.50	.25	.00	.00	.00	.00	1.00
	.98	1.03	.92	.87	.00	.20	.00	.00	Sums

P(G|C) = 3/7 =
#(CG) / $\sum_N$ #(CN)

Laplace pseudocount: Add +1 count to each observed.
(p.9,108,321 Dirichlet)

Estimated transistion probabilities from 48 "known" islands

Training set



P(G|C) =
#(CG) / $\sum_N$ #(CN)

(+)	A	C	G	T	
A	.18	.27	.43	.12	1.00
C	.17	.37	.27	.19	1.00
G	.16	.34	.38	.13	1.00
T	.08	.36	.38	.18	1.00

(-)	A	C	G	T	
A	.30	.21	.29	.21	1.00
C	.32	.30	.08	.30	1.00
G	.25	.25	.30	.21	1.00
T	.18	.24	.29	.29	1.00
	1.05	.99	.95	1.01	Sums

Viterbi: dynamic programming for HMM

1/8*.27

v	S_i =	C	G	C	G
begin	1	0	0	0	0
A+	0	0	0	0	0
C+	0	**0.125**	0	**0.012**	0
G+	0	0	**0.034**	0	**0.0032**
T+	0	0	0	0	0
A-	0	0	0	0	0
C-	0	0.125	0	0.0026	0
G-	0	0	0.01	0	0.0002
T-	0	0	0	0	0

Most probable path

l,k=2 states

Recursion:

$v_l(i+1)$ =

$e_l(x_i+1)$ max$(v_k(i)a_{kl})$

a= table in slide 50

e= emit S_i in state l (Durbin p.56)

DNA2: Aligning ancient diversity

Comparing types of alignments & algorithms
Dynamic programming
Multi- sequence alignment
Space time accuracy tradeoffs
Finding genes-- motif profiles
Hidden Markov Model for CpG Islands