Probabilistic sequence modeling frequency and profiles

Genome and genes

- Genome: an organism's genetic material
- Gene: discrete units of hereditary information located on the chromosomes and consisting of DNA

Gene prediction: computational challenge

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Gene prediction: computational challenge

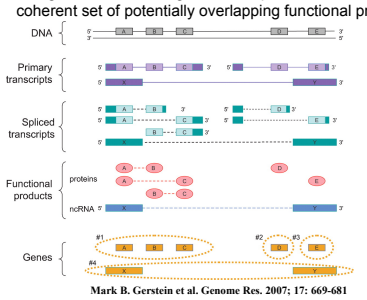
- Gene: A sequence of nucleotides coding for protein
- Gene Prediction Problem: Determine the beginning and end positions of genes in a genome

What's a gene

- What is a gene, post-ENCODE?
  - Gerstein et al. Genome Res. 2007 17: 669-681
  - ENCODE consortium: characterization of 1% of the human genome by experimental and computational techniques
- Definitions:
  - Definition 1970s–1980s: Gene as open reading frame (ORF) sequence pattern
  - Definition 1990s–2000s: Annotated genomic entity, enumerated in the databanks
  - The gene is a union of genomic sequences encoding a coherent set of potentially overlapping functional products

Post-ENCODE definition

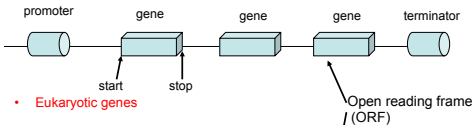
The gene is a union of genomic sequences encoding a coherent set of potentially overlapping functional products



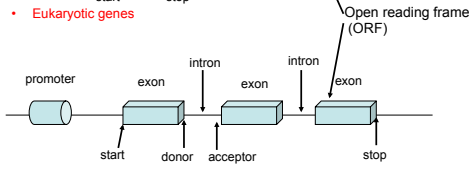
Mark B. Gerstein et al. Genome Res. 2007; 17: 669-681

Can we still do gene prediction?

- Prokaryotic genes

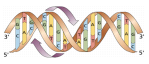


- Eukaryotic genes



A simple model for gene prediction: frequency-based DNA modeling

- DNA is a double stranded molecule
  - G-C pair → strong
  - A-T pair → weak



- Coding regions often have higher GC content than non-coding regions

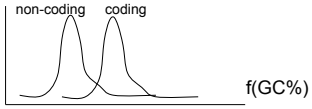
Frequency-based DNA modeling of coding vs. non-coding regions

- To predict coding regions in an organism (e.g. human), collect a set of known coding and non-coding DNA sequences from this organism (**training set**)
- Compute the frequency distribution of GC pairs in coding and non-coding regions, respectively: f(GC%|c), f(GC%|nc)

An example from zygomycete *Phycomyces blakesleeanus*

Table 1. GC content of *Phycomyces* DNA.

DNA type[a]	GC	Sample size[b]	Sample length (bp)[c]
Protein-coding DNA	48 ± 0.6	56	29 862
Total non-coding DNA	30 ± 1.0	49	13 088
Introns	29 ± 1.5	28	2837
5′-end	34 ± 2.1	10	6182
3′-end	30 ± 1.1	11	4069



Model comparison

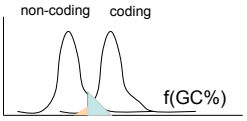
- Two models: coding vs. non-coding
- Given a DNA sequence, its likelihood of being a coding sequence, based on its GC content (GC%)

$$P(c|GC\%) = \frac{P(GC\%|c)P(c)}{P(GC\%)} = \frac{P(GC\%|c)P(c)}{P(GC\%|c)P(c) + P(GC\%|nc)P(nc)}$$

$$P(nc|GC\%) = \frac{P(GC\%|nc)P(nc)}{P(GC\%)} = \frac{P(GC\%|nc)P(nc)}{P(GC\%|c)P(c) + P(GC\%|nc)P(nc)}$$

$$P(GC\%|c) = \int_0^x f(x < GC\%|c)$$

$$P(GC\%|nc) = \int_x^1 f(x > GC\%|nc)$$

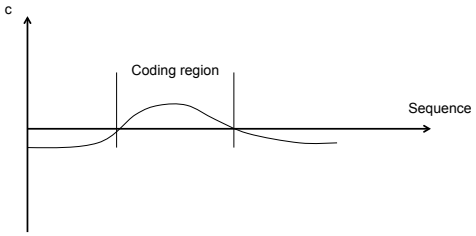


Assume P(c)=P(nc),

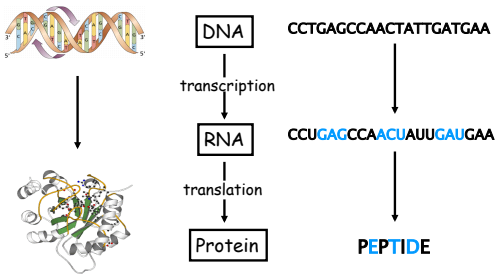
$$R = log(\frac{P(GC\%|c)}{P(GC\%|nc)})$$

Sliding window approach

Sliding window approach



A more complicated model: codon usages



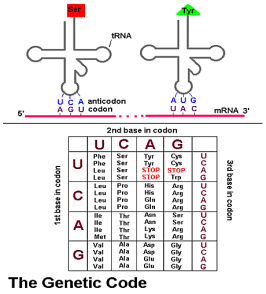
Translating nucleotides into amino acids

- Codon: 3 consecutive nucleotides
- $4^3 = 64$ possible codons
- Genetic code is degenerative and redundant
  - Includes start and stop codons
  - An amino acid may be coded by more than one codon (codon degeneracy)

Codons

- In 1961 Sydney Brenner and Francis Crick discovered frameshift mutations
- Systematically deleted nucleotides from DNA
  - Single and double deletions dramatically altered protein product
  - Effects of triple deletions were minor
  - Conclusion: every triplet of nucleotides, each ***codon***, codes for exactly one amino acid in a protein

Genetic code and stop codons



The Genetic Code

UAA, UAG and UGA correspond to 3 Stop codons that (together with Start codon ATG) delineate Open Reading Frames

Six frames in a DNA sequence

- stop codons – TAA, TAG, TGA
- start codons - ATG

Testing reading frames

- Create a 64-element hash table and count the frequencies of codons in a reading frame;
- Amino acids typically have more than one codon, but in nature certain codons are more in use;
- Uneven use of the codons may characterize a coding region;

Codon usage in Human genome

	U		C		A		G	
U	UUU Phe	57	UCU Ser	16	UAU Tyr	58	UGU Cys	45
	UUC Phe	43	UCC Ser	15	UAC Tyr	42	UGC Cys	55
	UUA Leu	13	UCA Ser	13	UAA Stp	62	UGA Stp	30
	UUG Leu	13	UCG Ser	15	UAG Stp	8	UGG Trp	100
C	CUU Leu	11	CCU Pro	17	CAU His	57	CGU Arg	37
	CUC Leu	10	CCC Pro	17	CAC His	43	CGC Arg	38
	CUA Leu	4	CCA Pro	20	CAA Gln	45	CGA Arg	7
	CUG Leu	49	CCG Pro	51	CAG Gln	66	CGG Arg	10
A	AUU Ile	50	ACU Thr	18	AAU Asn	46	AGU Ser	15
	AUC Ile	41	ACC Thr	42	AAC Asn	54	AGC Ser	26
	AUA Ile	9	ACA Thr	15	AAA Lys	75	AGA Arg	5
	AUG Met	100	ACG Thr	26	AAG Lys	25	AGG Arg	3
G	GUU Val	27	GCU Ala	17	GAU Asp	63	GGU Gly	34
	GUC Val	21	GCC Ala	27	GAC Asp	37	GGC Gly	39
	GUA Val	16	GCA Ala	22	GAA Glu	68	GGA Gly	12
	GUG Val	36	GCG Ala	34	GAG Glu	32	GGG Gly	15

Codon usage in Mouse genome

AA	codon	/1000	frac
Ser	TCG	4.31	0.05
Ser	TCA	11.44	0.14
Ser	TCT	15.70	0.19
Ser	TCC	17.92	0.22
Ser	AGT	12.25	0.15
Ser	AGC	19.54	0.24
Pro	CCG	6.33	0.11
Pro	CCA	17.10	0.28
Pro	CCT	18.31	0.30
Pro	CCC	18.42	0.31

AA	codon	/1000	frac
Leu	CTG	39.95	0.40
Leu	CTA	7.89	0.08
Leu	CTT	12.97	0.13
Leu	CTC	20.04	0.20
Ala	GCG	6.72	0.10
Ala	GCA	15.80	0.23
Ala	GCT	20.12	0.29
Ala	GCC	26.51	0.38
Gln	CAG	34.18	0.75
Gln	CAA	11.51	0.25

Using codon frequency to find correct reading frame

Consider sequence $x_1 x_2 x_3 x_4 x_5 x_6 x_7 x_8 x_9 \ldots$
where x_i is a nucleotide

let $p_1 = p_{x1\,x2\,x3}\, p_{x4\,x5\,x6} \cdots$
$p_2 = p_{x2\,x3\,x4}\, p_{x5\,x6\,x7} \cdots$
$p_3 = p_{x3\,x4\,x5}\, p_{x6\,x7\,x8} \cdots$

then probability that ith reading frame is the coding frame is:

$$P_i = \frac{p_i}{p_1 + p_2 + p_3}$$

slide a window along the sequence and compute P_i

Adding the background model: gene finding

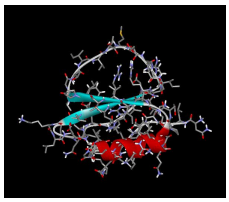
- In the previous model, we assume at least one reading frame is the codon sequence → testing reading frames, not gene finding
- Adding a background model
  - $p_0 = p_{x1}p_{x2}p_{x3}p_{x4}\cdots$
  - Based on the nucleotide frequency in the non-coding sequence
  - $P_i = p_i / (p_0+p_1+p_2+p_3)$
- In practice, this model should be extended to all six reading frames.

Protein secondary structure prediction

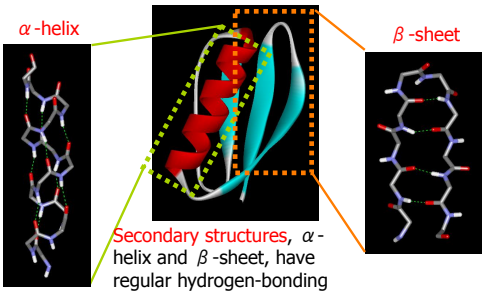
Amino acid sequence

NLKTEWPELVGKSVEE
AKKVILQDKPEAQIIVL
PVGTIVTMEYRIDRVR
LFVDKLDNIAEVPRVG

folding →



Basic structural units of proteins: Secondary structure



Secondary structures, α-helix and β-sheet, have regular hydrogen-bonding patterns.

Secondary structure prediction

- Given a protein sequence, secondary structure prediction aims at predicting the state of each amino acid as being either H (helix), E (extended=strand), or O (other).
- The quality of secondary structure prediction is measured with a "3-state accuracy" score, or Q_3. Q_3 is the percent of residues that match "reality" (X-ray structure).

Chou and Fasman: a frequency model

- $P(\alpha|S)=\Pi_s p(\alpha|s)= \Pi_s p(\alpha|f(s))$
  - $p(\alpha|f(s))\sim p(f(s)|\alpha)/p(f(s))$
  - Similarly for β and turn structures

Chou and Fasman: a frequency model

Amino Acid	α-Helix	β-Sheet	Turn	
Ala	1.29	0.90	0.78	Favors α-Helix
Cys	1.11	0.74	0.80	
Leu	1.30	1.02	0.59	
Met	1.47	0.97	0.39	
Glu	1.44	0.75	1.00	
Gln	1.27	0.80	0.97	
His	1.22	1.08	0.69	
Lys	1.23	0.77	0.96	
Val	0.91	1.49	0.47	Favors β-strand
Ile	0.97	1.45	0.51	
Phe	1.07	1.32	0.58	
Tyr	0.72	1.25	1.05	
Trp	0.99	1.14	0.75	
Thr	0.82	1.21	1.03	
Gly	0.56	0.92	1.64	Favors turn
Ser	0.82	0.95	1.33	
Asp	1.04	0.72	1.41	
Asn	0.90	0.76	1.23	
Pro	0.52	0.64	1.91	
Arg	0.96	0.99	0.88	

Profile model

- The frequency model does not consider the **order** of the training sequences
  - Permuting the training sequences will not change the model
- In some cases, the order is of important biological meaning, e.g. sequence motifs
- Profile model fully constrains the order of the training sequences

Profile / PSSM

- DNA / protein segments of the same length L
- Often represented as positional frequency matrix

```
LTMTRGDIGNYLGLTVETISRLLGRFQKSGML
LTMTRGDIGNYLGLTIETISRLLGRFQKSGMI
LTMTRGDIGNYLGLTVETISRLLGRFQKSEIL
LTMTRGDIGNYLGLTVETISRLLGRLQKMGIL
LAMSRNEIGNYLGLAVETVSRVFSRFQQNELI
LAMSRNEIGNYLGLAVETVSRVFTRFQQNGLI
LPMSRNEIGNYLGLAVETVSRVFTRFQQNGLL
VRMSREEIGNYLGLTLETVSRLFSRFGREGLI
LRMSREEIGSYLGLKLETVSRTLSKFHQEGLI
LPMCRRDIGDYLGLTLETVSRALSQLHTQGIL
LPMSRRDIADYLGLTVETVSRAVSQLHTDGVL
LPMSRQDIADYLGLTIETVSRTFTKLERHGAI
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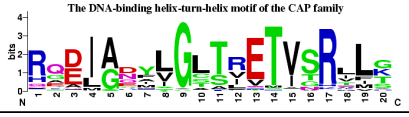


A DNA profile (matrix)

TATAAA
TATAAT
TATAAA
TATAAA
TATAAA
TATTAA
TTAAAA
TAGAAA

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

Sparse data → pseudo-counts

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

Testing a motif

```
TATAAA    TCGAAT
TATAAT    GCATTT
TATAAA    ACTTAA
TATAAA    CGCTGC
TATAAA    AAACCG
TATTAA    CGATAC
TTAAAA    CCAAGT
TAGAAA    GACCTA
```

- Equivalent to computing the significance of a sequence motif

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

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

Model comparison: relative entropy

- $H(x) = \sum_i \sum_j P(x_{ij}) \log (P(x_{ij})/P_0(x_j)) = \sum_{i=1}^{L} \sum_{j=1}^{K} (n_{ij}/N) \log\left(\frac{n_{ij}/N}{b_j}\right)$
  - b_j → the random background distribution
  - *N* sequences of length *L*
  - *K* → 4

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

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

Probability distribution

- What is the probability P(H|B) of getting a matrix with a relative entropy H from the background model B = $\{b_j\}$?
- p(h) → the probability distribution of relative entropy score for the frequency of a *single* column (can be pre-calculated)
- $P(H) = \sum_{\substack{(s_1,\ldots,s_L) \\ s_1+\ldots+s_L=H}} p(s_1) \cdot p(s_2) \cdot \ldots \cdot p(s_L)$
  - convolution of function p(s), can be calculated only approximately by Fast Fourier Transformation (FFT)

Searching profiles: inference

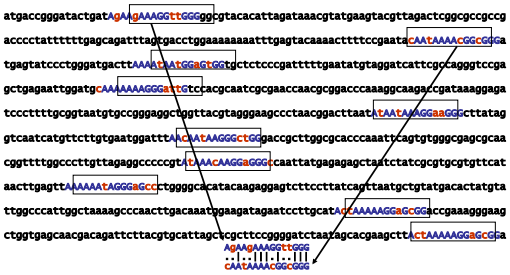
- Give a sequence *S* of length *L*, compute the likelihood ratio of being generated from a profile vs. from background model:

$$R(S|P) = \prod_{i=1}^{L} \frac{(n_{is_i}/N)}{b_{s_i}}$$

  - Searching motifs in a sequence: sliding window approach

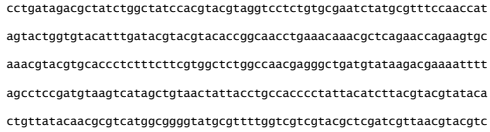
Finding a motif

Motif finding is difficult



The motif finding problem

- Given a set of DNA sequences:



- Find the motif in each of the individual sequences

The motif finding problem

- If starting positions **s**=(s_1, s_2,… s_t) are given, finding consensus is easy because we can simply construct (and evaluate) the profile to find the motif.
- But… the starting positions **s** are usually not given. How can we find the "best" profile matrix?
  - Gibbs sampling
  - Expectation-Maximization (EM) algorithm

Conclusion

- Frequency and profile are two basic models for sequence analysis
- They represent two extreme models in terms of incorporating order information in the sequences
- Model selection should be based on biological ideas