Gene Finding by Computational Analysis

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Lecture Plan

- **Gene structure basics**
- **Gene finding overview**
- **GRAIL**
- **Indel & frame-shift in coding regions**
- **Histone promoters: A cautionary case study**

Gene Structure Basics

Some slides here are “borrowed” from Ken Sung

Body

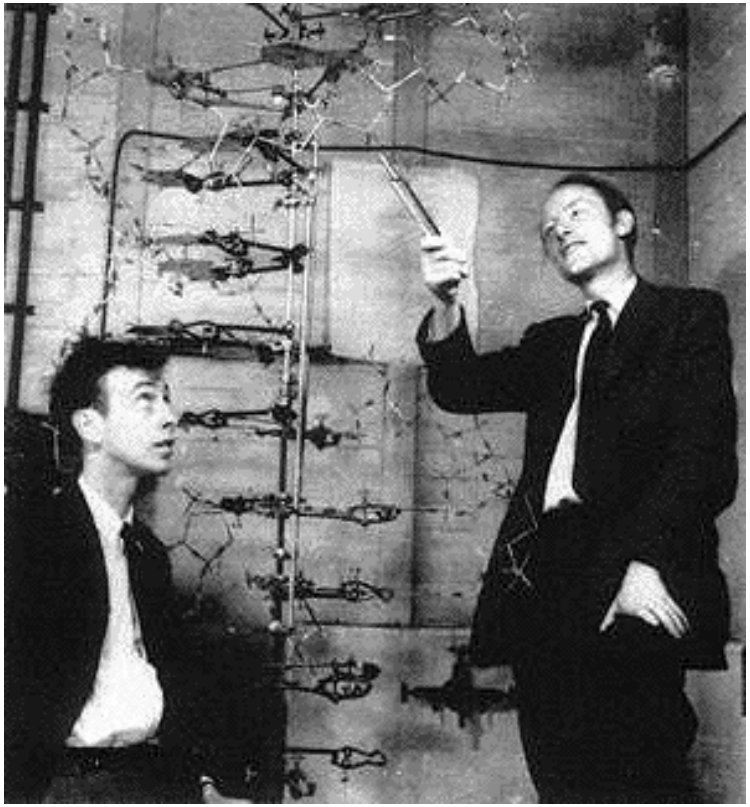
- **Our body consists of a number of organs**
- **Each organ composes of a number of tissues**
- **Each tissue composes of cells of the same type**

Cell

- **Performs two types of function**
  - Chemical reactions necessary to maintain our life
  - Pass info for maintaining life to next generation
- **In particular**
  - Protein performs chemical reactions
  - DNA stores & passes info
  - RNA is intermediate between DNA & proteins

DNA

- **DNA stores instruction needed by the cell to perform daily life function**
- **Consists of two strands interwoven together and form a double helix**
- **Each strand is a chain of some small molecules called nucleotides**



Francis Crick shows James Watson the model of DNA in their room number 103 of the Austin Wing at the Cavendish Laboratories, Cambridge

Chromosome

- **DNA is usually tightly wound around histone proteins and forms a chromosome**
- **The total info stored in all chromosomes constitutes a genome**
- **In most multi-cell organisms, every cell contains the same complete set of chromosomes**
  - May have some small different due to mutation
- **Human genome has 3G base pairs, organized in 23 pairs of chromosomes**

Gene

- **A gene is a sequence of DNA that encodes a protein or an RNA molecule**

- **About 30,000 – 35,000 (protein-coding) genes in human genome**

- **For gene that encodes protein**
  - In Prokaryotic genome, one gene corresponds to one protein
  - In Eukaryotic genome, one gene can corresponds to more than one protein because of the process "alternative splicing"

Complexity of Organism vs. Genome Size

- **Human Genome: 3G base pairs**
- ***Amoeba dubia* (a single cell organism): 600G base pairs**

$\Rightarrow$ **Genome size has no relationship with the complexity of the organism**

Number of Genes vs. Genome Size

- **Prokaryotic genome (e.g., *E. coli*)**
  - No. of base pairs: 5M
  - Number of genes: 4k
  - Average length of a gene: 1000 bp

- **Eukaryotic genome (e.g., human)**
  - No. of base pairs: 3G
  - Estimated number of genes: 30k – 35k
  - Estimated average length of a gene: 1000-2000 bp

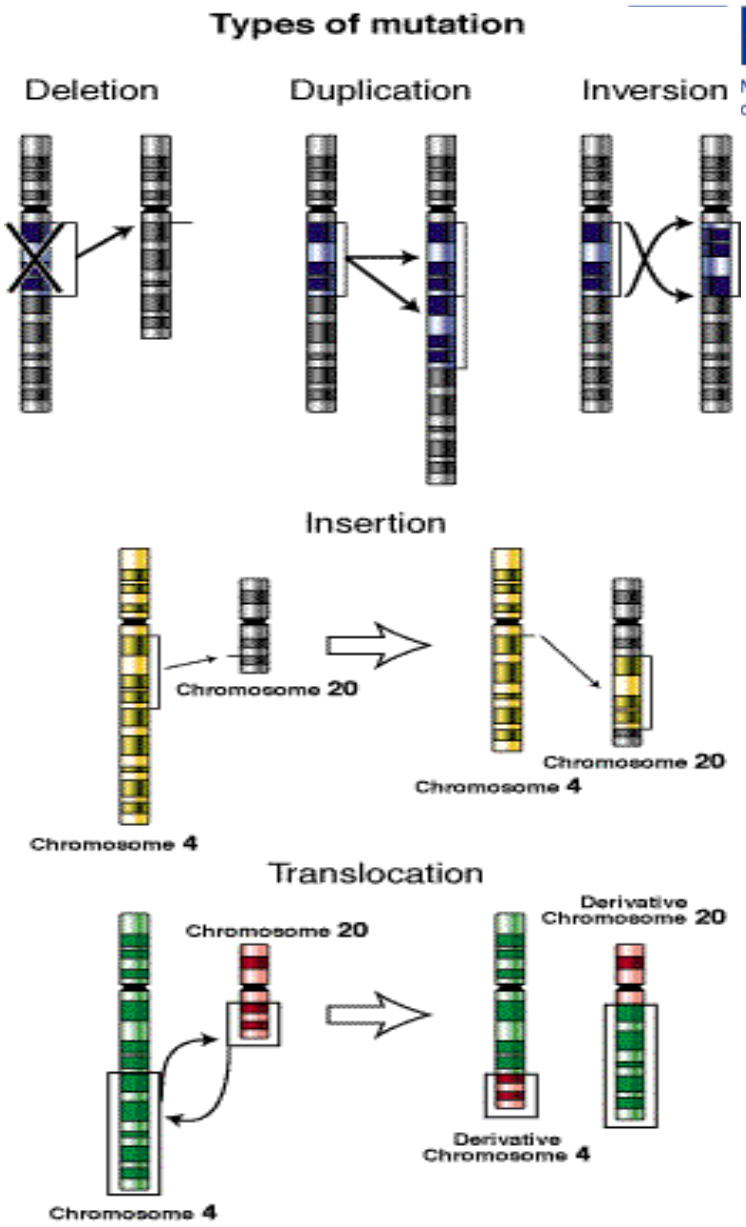
- **~ 90% of *E. coli* genome are of coding regions**

- **< 3% of human genome is believed to be coding regions**

⇒ **Genome size has no relationship with the number of genes!**

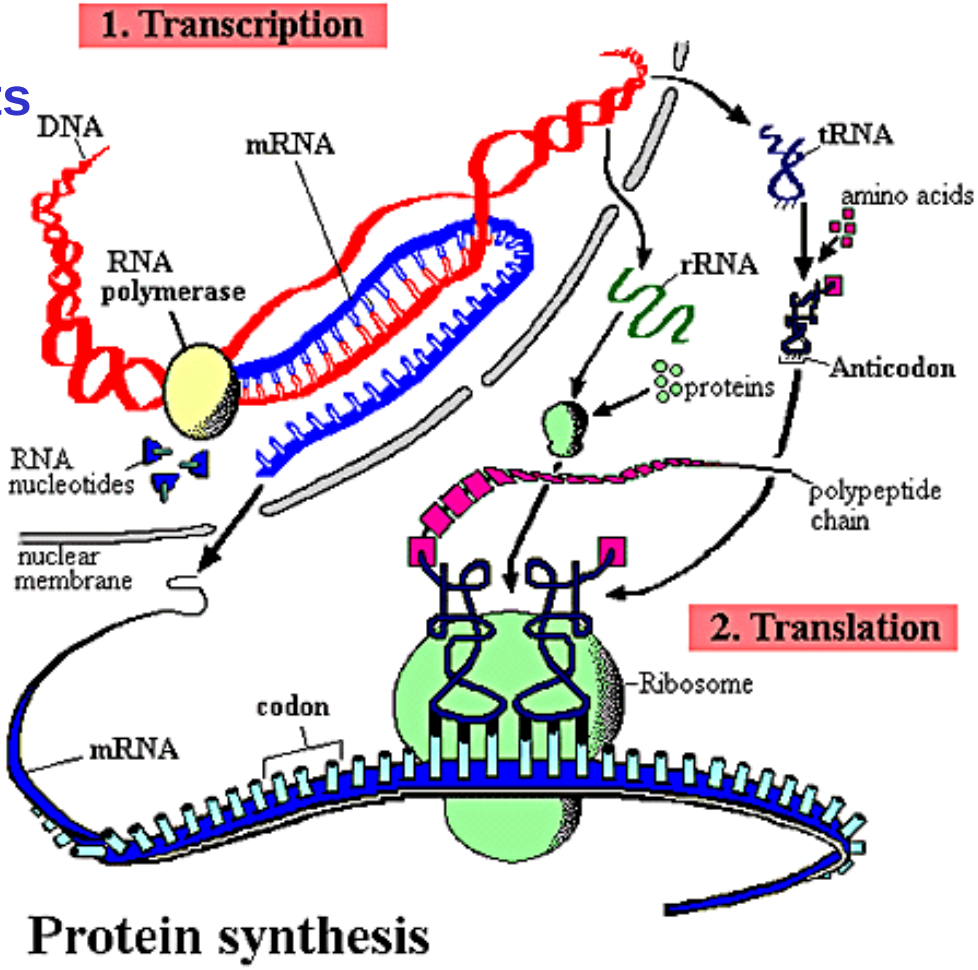
Mutation

- **Mutation is a sudden change of genome**
- **Basis of evolution**
- **Cause of cancer**
- **Can occur in DNA, RNA, & Protein**



Central Dogma

- **Gene expression consists of two steps**
  - Transcription
    DNA → mRNA
  - Translation
    mRNA → Protein



Genetic Code

- **Start codon: ATG (code for M)**
- **Stop codon: TAA, TAG, TGA**

First Position	Second Position of Codon: T	Second Position of Codon: C	Second Position of Codon: A	Second Position of Codon: G	Third Position
T	TTT Phe [F]	TCT Ser [S]	TAT Tyr [Y]	TGT Cys [C]	T
	TTC Phe [F]	TCC Ser [S]	TAC Tyr [Y]	TGC Cys [C]	C
	TTA Leu [L]	TCA Ser [S]	TAA *Ter* [end]	TGA *Ter* [end]	A
	TTG Leu [L]	TCG Ser [S]	TAG *Ter* [end]	TGG Trp [W]	G
C	CTT Leu [L]	CCT Pro [P]	CAT His [H]	CGT Arg [R]	T
	CTC Leu [L]	CCC Pro [P]	CAC His [H]	CGC Arg [R]	C
	CTA Leu [L]	CCA Pro [P]	CAA Gln [Q]	CGA Arg [R]	A
	CTG Leu [L]	CCG Pro [P]	CAG Gln [Q]	CGG Arg [R]	G
A	ATT Ile [I]	ACT Thr [T]	AAT Asn [N]	AGT Ser [S]	T
	ATC Ile [I]	ACC Thr [T]	AAC Asn [N]	AGC Ser [S]	C
	ATA Ile [I]	ACA Thr [T]	AAA Lys [K]	AGA Arg [R]	A
	ATG Met [M]	ACG Thr [T]	AAG Lys [K]	AGG Arg [R]	G
G	GTT Val [V]	GCT Ala [A]	GAT Asp [D]	GGT Gly [G]	T
	GTC Val [V]	GCC Ala [A]	GAC Asp [D]	GGC Gly [G]	C
	GTA Val [V]	GCA Ala [A]	GAA Glu [E]	GGA Gly [G]	A
	GTG Val [V]	GCG Ala [A]	GAG Glu [E]	GGG Gly [G]	G

Introns and exons

- **Eukaryotic genes contain introns & exons**
  - Introns are seq that are ultimately spliced out of mRNA
  - Introns normally satisfy GT-AG rule, viz. begin w/ GT & end w/ AG
  - Each gene can have many introns & each intron can have thousands bases
- **Introns can be very long**
- **An extreme example is a gene associated with cystic fibrosis in human:**
  - Length of 24 introns ~1Mb
  - Length of exons ~1kb

Typical Eukaryotic Gene Structure

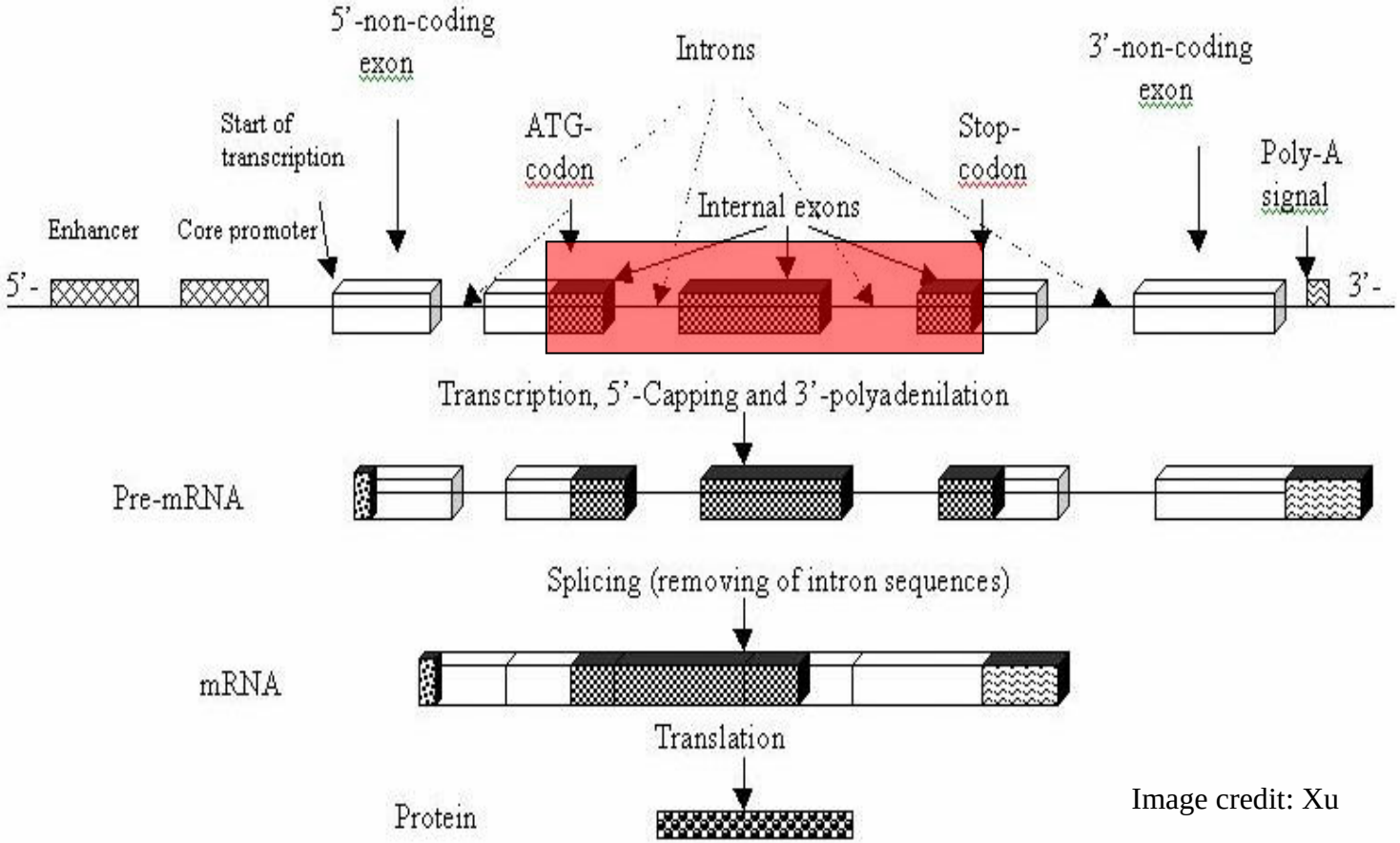


Image credit: Xu

- **Unlike eukaryotic genes, a prokaryotic gene typically consists of only one contiguous coding region**

Reading Frame

- **Each DNA segment has six possible reading frames**

Forward strand: ATGGCTTACGCTTGA

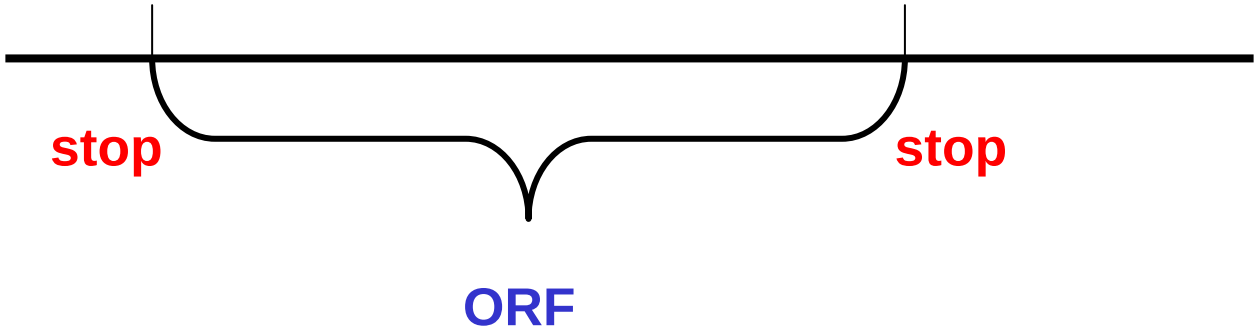
Reading frame #1	Reading frame #2	Reading frame #3
ATG	TGG	GGC
GCT	CTT	TTA
TAC	ACG	CGC
GCT	CTT	TTG
TGC	GA.	A..

Reverse strand: TCAAGCGTAAGCCAT

Reading frame #4	Reading frame #5	Reading frame #6
TCA	CAA	AAG
AGC	GCG	CGT
GTA	TAA	AAG
AGC	GCC	CCA
CAT	AT.	T..

Open Reading Frame (ORF)

- **ORF is a segment of DNA with two in-frame stop codons at the two ends and no in-frame stop codon in the middle**



- **Each ORF has a fixed reading frame**

Coding Region

- **Each coding region (exon or whole gene) has a fixed translation frame**
- **A coding region always sits inside an ORF of same reading frame**
- **All exons of a gene are on the same strand**
- **Neighboring exons of a gene could have different reading frames**

Frame Consistency

- **Neighbouring exons of a gene should be frame-consistent**

ATG GCT TGG GCT TTA A ---------- GT TTC CCG GAG AT ------ T GGG

exon 1 **exon 2** **exon 3**

Overview of Gene Finding

Some slides here are “borrowed” from Mark Craven

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What is Gene Finding?

- **Find all coding regions from a stretch of DNA sequence, and construct gene structures from the identified exons**

- **Can be decomposed into**
  - Find coding potential of a region in a frame
  - Find boundaries betw coding & non-coding regions

Image credit: Xu

Approaches

- **Search-by-signal: find genes by identifying the sequence signals involved in gene expression**
- **Search-by-content: find genes by statistical properties that distinguish protein coding DNA from non-coding DNA**
- **Search-by-homology: find genes by homology (after translation) to proteins**

- **State-of-the-art systems for gene finding usually combine these two strategies**

Relevant Signals for Search-by-Signals

- **Transcription initiation**
  - Promoter
- **Transcription termination**
  - Terminators
- **Translation initiation**
  - Ribosome binding sites
  - Initiation codons
- **Translation termination**
  - Stop codons
- **RNA processing**
  - Splice junction

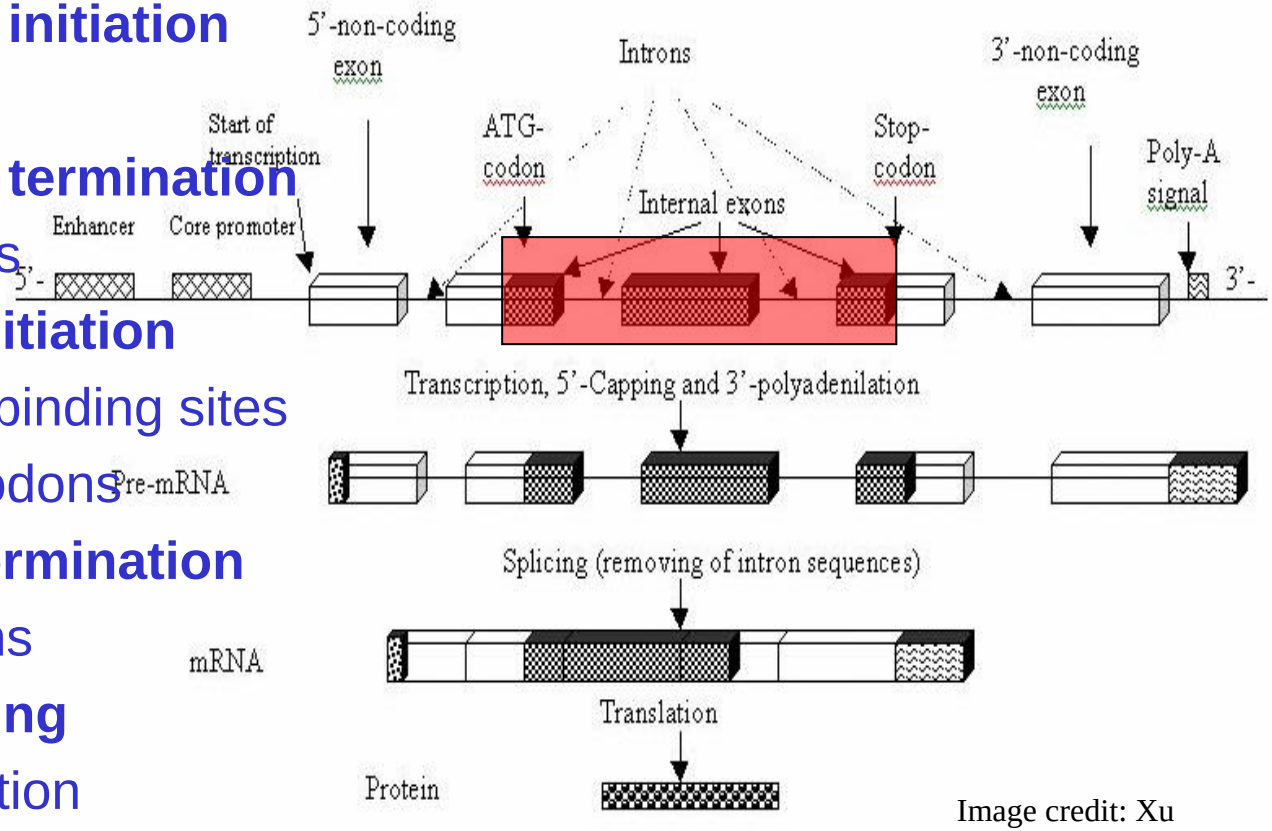


Image credit: Xu

How Search-by-Signal Works

- **There are 2 impt regions in a promoter seq**
  - –10 region, ~10bp before TSS
  - –35 region, ~35bp before TSS
- **Consensus for–10 region in *E. coli* is TATAAT, but few promoters actually have this seq**

⇒ **Recognize promoters by**
  - – weight matrices
  - – probabilistic models
  - – neural networks, …

How Search-by-Content Works

- **Encoding a protein affects stats properties of a DNA seq**
  - some amino acids used more frequently
  - diff number of codons for diff amino acids
  - for given protein, usually one codon is used more frequently than others

⇒ **Estimate prob that a given region of seq was "caused by" its being a coding seq**

Codon Preference in E. Coli

AA	codon	/1000
Gly	GGG	1.89
Gly	GGA	0.44
Gly	GGU	52.99
Gly	GGC	34.55
Glu	GAG	15.68
Glu	GAA	57.20
Asp	GAU	21.63
Asp	GAC	43.26

Image credit: Craven

How Search-by-Homology Works

- **Translate DNA seq in all reading frames**
- **Search against protein db**
- **High-scoring matches suggest presence of homologous genes in DNA**

⇒ **You can use BLASTX for this**

Search-by-Content Example: Codon Usage Method

- **Staden & McLachlan, 1982**
- **Process a seq w/ "window" of length *L***
- **Assume seq falls into one of 7 categories, viz.**
  - Coding in frame 0, frame 1, …, frame 5
  - Non-coding
- **Use Bayes' rule to determine prob of each category**
- **Assign seq to category w/ max prob**

Codon Usage Method

$$\Pr(\text{coding}_i \mid S) = \frac{\Pr(S \mid \text{coding}_i)\Pr(\text{coding}_i)}{\Pr(S)}$$

probability that sequence encodes a protein in frame i

Codon Usage Method

- make simplifying assumption that the codons in a window are independent of one another

$$\Pr(S \mid \text{coding}_i) \approx \prod_{j=1}^{n} \Pr(S_i(j) \mid \text{coding}_i)$$

probability of the jth codon in frame i given the sequence is coding

Image credit: Craven

Codon Usage Method

$$\Pr(\text{coding}_i \mid S) = \frac{\Pr(S \mid \text{coding}_i)\Pr(\text{coding}_i)}{\Pr(S)}$$

probability that sequence encodes a protein in frame i

Codon Usage Method

$$\Pr(S) = \sum_i \left[\Pr(S \mid \text{coding}_i)\Pr(\text{coding}_i)\right] + \Pr(S \mid \text{noncoding})\Pr(\text{noncoding})$$

Sometimes this term is dropped since it's difficult to estimate these statistics

Image credit: Craven

Codon Usage Method

$$\Pr(\text{coding}_i \mid S) = \frac{\Pr(S \mid \text{coding}_i)\Pr(\text{coding}_i)}{\Pr(S)}$$

probability that sequence encodes a protein in frame i

- **$\Pr(\text{coding}_i)$ is the same for each frame if window size fits same number of codons in each frame**
- **otherwise, consider relative number of codons in window in each frame**

Image credit: Craven

Codon Usage Method

- By sliding the window, we can generate predictions for the extent of our sequence

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

Predicted Coding Regions

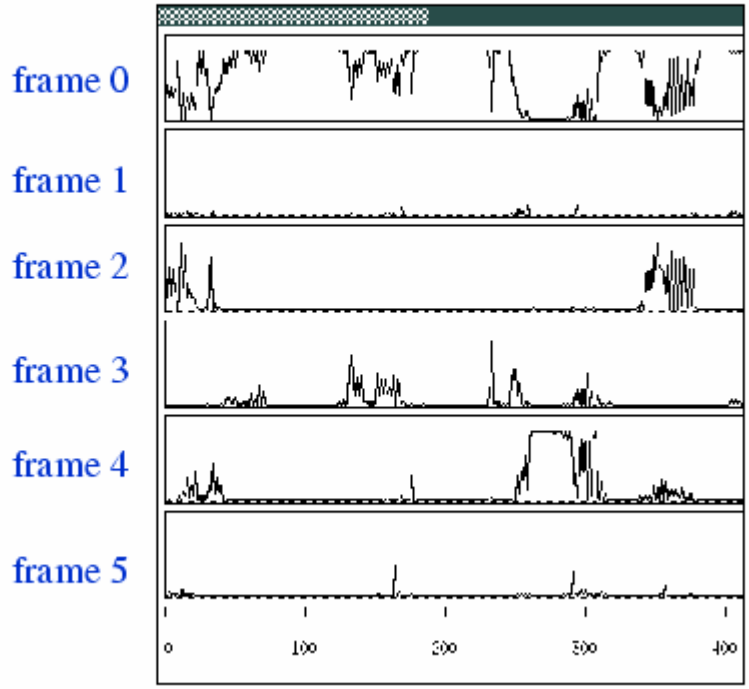


Image credit: Craven

Search-by-Homology Example: Gene Finding Using BLAST

- **High seq similarity typically implies homologous genes**

⇒ **Search for genes in yeast seq using BLAST**

⇒ **Extract Feature for gene identification**

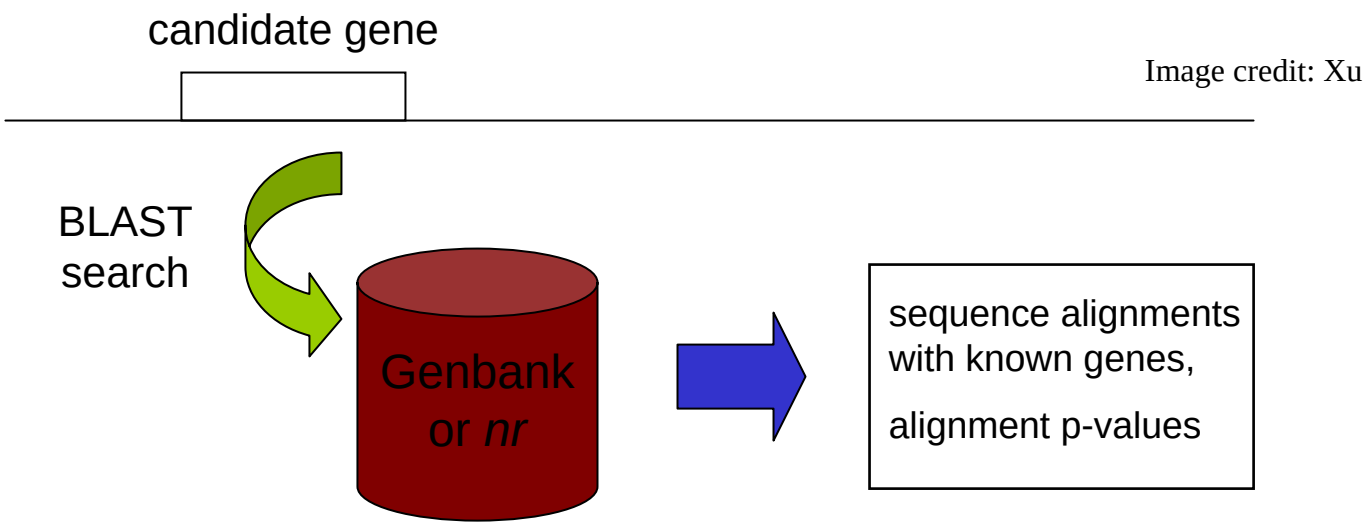
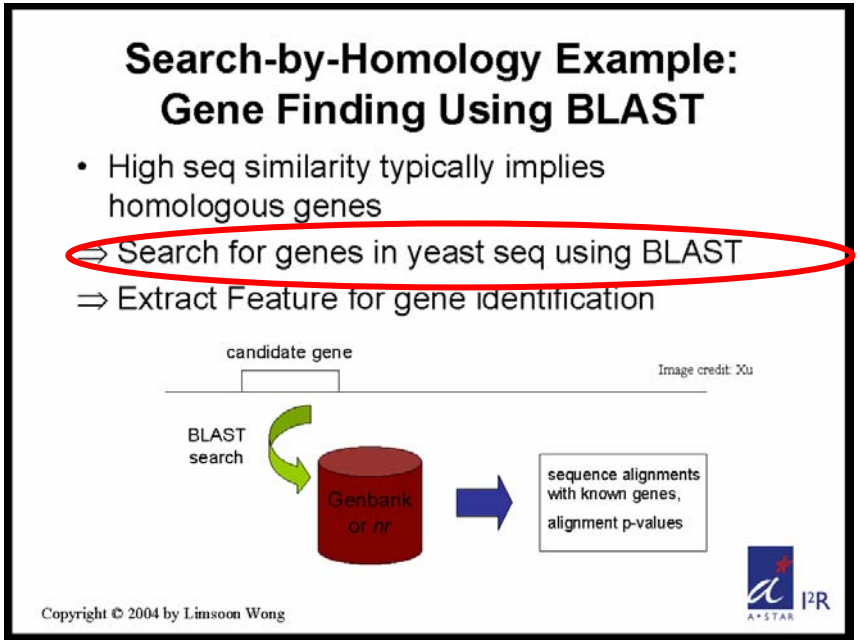
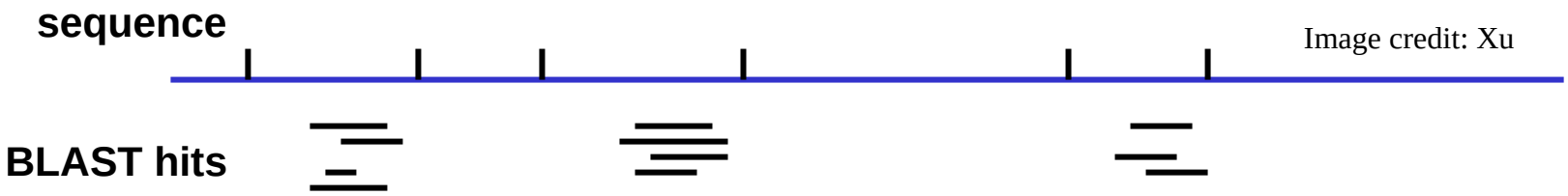
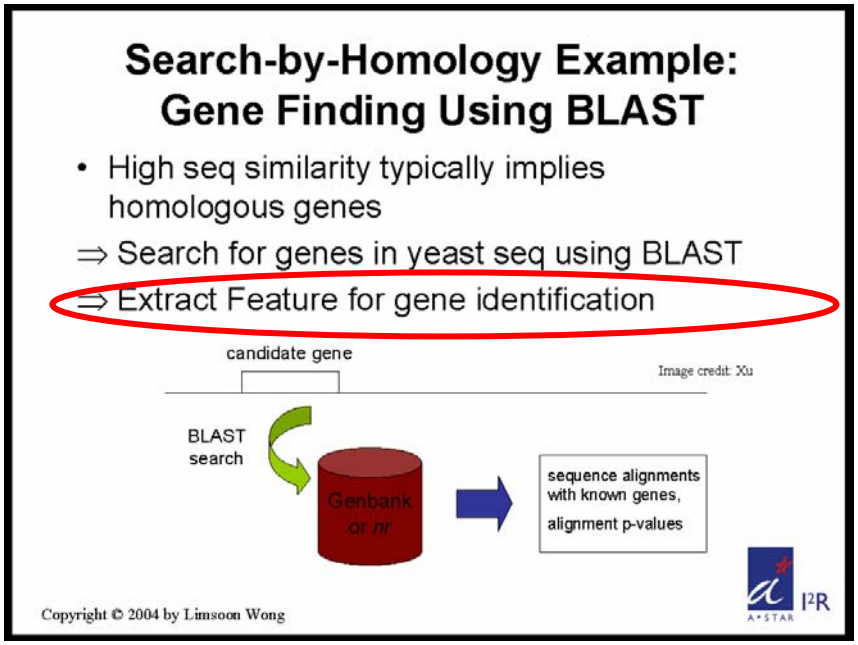


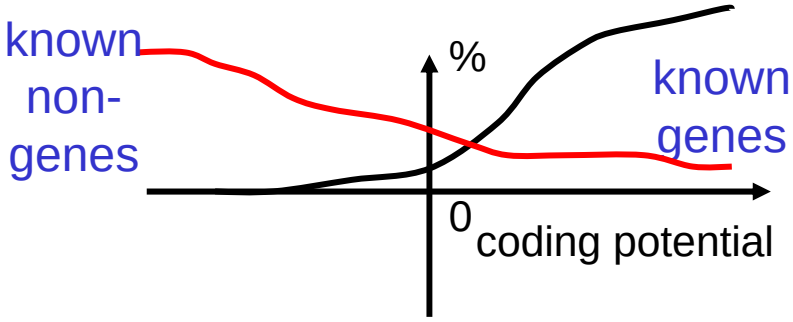
Image credit: Xu

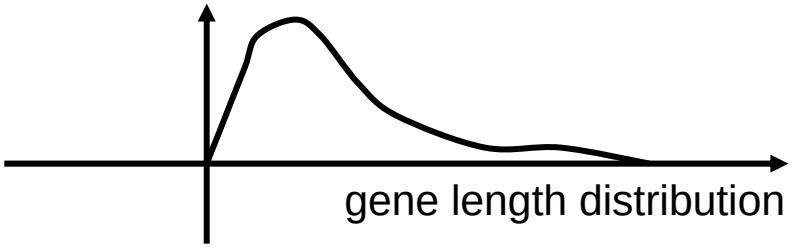


- **Searching all ORFs against known genes in nr db helps identify an initial set of (possibly incomplete) genes**









- **A (yeast) gene starts w/ ATG and ends w/ a stop codon, in same reading frame of ORF**
- **Have "strong" coding potentials, measured by, preference models, Markov chain model, ...**
- **Have "strong" translation start signal, measured by weight matrix model, ...**
- **Have distributions wrt length, G+C composition, ...**
- **Have special seq signals in flanking regions, ...**

GRAIL,
A Pioneer Gene Finding Program

Signals assoc w/ coding regions
Models for coding regions
Signals assoc w/ boundaries
Models for boundaries
Other factors & information fusion

Some slides here are "borrowed" from Ying Xu

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Coding Signal

- **Freq distribution of dimers in protein sequence**
- **E.g., *Shewanella***
  - Ave freq is 5%
  - Some amino acids prefer to be next to each other
  - Some amino acids prefer to be not next to each other

Name	ala	arg	asn	asp	cys	glu	gln	gly	his	ile	leu	lys	met	phe	pro	ser	thr	trp	tyr	val
ala	9.5	4.1	4.3	5.3	1.2	6	4.8	6.5	2	6.5	11.5	6	2.6	3.7	3.5	6.2	5	1.1	2.7	6.5
arg	7.9	5.5	3.9	5.3	1.1	6	5.5	5.9	2.6	6.5	11.4	5	2.2	4.7	3.6	5.5	4.4	1.4	4	6.6
asn	9.6	4.9	4.2	4.9	1	5.3	5.6	7.4	2.3	6	10	4.9	2	3.5	5.1	6.1	5.5	1.5	3.1	6.1
asp	9.3	4	4.7	5.1	1	6.7	2.9	7	1.8	7.1	9.6	6.3	2.3	4.3	3.9	5.9	5.1	1.6	3.6	6.6
cys	8.4	4.8	3.3	5.4	1.7	5.6	5.2	8.1	4.3	5.4	10.2	3.8	1.8	4.1	4.5	6.3	4.3	1.6	3.4	6.8
glu	9.4	5.8	3.6	4.5	0.8	4.9	7	5.8	2.6	5.9	12.7	5	2.4	4	3.5	5.4	5	1.1	2.8	6.8
gln	10.3	4.9	3	4.4	0.9	4.5	6.8	7	2.7	5.5	12.8	4.1	2	3.9	3.8	5.8	5.3	1.4	3	6.9
gly	8.1	4.8	3.9	5.1	1.2	6	4.6	6.4	2.4	6.8	10.5	5.8	2.7	4.8	2.4	5.8	5.1	1.4	3.7	7.5
his	7.3	4.7	4	4.8	1.5	4.9	5.6	6.9	3	6.2	10.8	4.8	1.6	5	5.2	6.8	4.9	1.7	4.2	5.1
ile	11	4.7	4.9	6.5	1.1	6.9	3.6	7.2	2.1	5.3	8.6	5.3	1.8	3.2	4.2	7	5.6	0.9	2.9	6.1
leu	10.4	4.2	4.3	5.2	1.1	5.2	3.7	6.8	2	5.6	10.6	5.3	2.3	3.8	4.5	7.4	6.2	1	2.6	6.6
lys	10.6	5.2	3.8	5.2	0.5	5.3	5.9	6.6	2.6	5.2	11.3	4.7	1.9	2.8	4.6	6	5.5	1.2	2.6	7.6
met	10.8	4.8	3.8	4.6	0.7	4.6	4.9	7	1.7	4.7	11.4	5.2	2.8	3.3	5.1	7.4	6.3	0.9	2	6.8
phe	9.6	3.7	5.2	6.5	1.2	6.4	2.7	7.9	1.9	6.7	7.4	5	2.5	3.9	3.6	8	5.8	1.3	3.3	6.3
pro	8.4	3.6	4.6	5.4	0.7	7.6	5.2	5.4	2.3	6.1	11.2	5.5	2.4	4.2	2.8	6.5	5.4	1.4	2.9	7.5
ser	9.1	4.6	3.7	5	1	5.4	5.2	7.2	2.6	6	11.6	4.5	2.2	4.1	4.1	6.5	5	1.2	3.2	6.8
thr	9.1	4.2	3.7	5.6	0.9	5.7	5.7	7.5	2.2	5.5	12	4.2	2	3.5	5.5	6.2	5.3	1.1	2.6	6.7
trp	7.1	6.3	3.2	4.8	1.3	3.9	8.5	6.6	3.6	5	14.2	3.2	2.4	4.6	3.9	5.8	4.3	1.3	3	6.1
tyr	7.9	6.5	3.6	4.9	1.2	4.5	7	7.1	2.6	5	11.7	4	1.6	4.7	4.9	6.4	4.6	1.5	3.4	5.7
val	9.6	4.1	4.4	5.9	1	6.2	3.4	6.4	1.8	6.5	10.2	5.2	2.5	3.7	3.8	7.2	6.1	1.1	2.7	7.1

Image credit: Xu

Exercise: What is shewanella?

Coding Signal

- **Dimer preference implies dicodon (6-mers like AAA TTT) bias in coding vs non-coding regions**

- **Relative freq of a di-codon in coding vs non-coding**
  - Freq of dicodon X (e.g, AAA AAA) in coding region, total number of occurrences of X divided by total number of dicocon occurrences
  - Freq of dicodon X (e.g, AAA AAA) in noncoding region, total number of occurrences of X divided by total number of dicodon occurrences

- **Exercise: In human genome, freq of dicodon "AAA AAA" is ~1% in coding region vs ~5% in non-coding region. If you see a region with many "AAA AAA", would you guess it is a coding or non-coding region?**

Why Dicodon (6-mer)?

- **Codon (3-mer)-based models are not as info rich as dicodon-based models**
- **Tricodon (9-mer)-based models need too many data points**

There are

4^3 = 64 codons

4^6 = 4096 dicodons

4^9 = 262144 tricodons

- **To make stats reliable, need ~15 occurrences of each X-mer**

⇒ **For tricodon-based models, need at least 15*262144 = 3932160 coding bases in our training data, which is probably not going to be available for most genomes**

Coding Signal

- **Most dicodons show bias towards either coding or non-coding regions**

$\Rightarrow$ **Foundation for coding region identification**

Regions consisting of dicodons that mostly tend to be in coding regions are probably coding regions; otherwise non-coding regions

$\Rightarrow$ **Dicodon freq are key signal used for coding region detection; all gene finding programs use this info**

Coding Signal

- **Dicodon freq in coding vs non-coding are genome-dependent**

Image credit: Xu

Name	ala	arg	asn	asp	cys	glu	gln	gly	his	ile	leu	lys	met	phe	pro	ser	thr	trp	tyr	val
ala	9.5	4.1	4.3	5.3	1.2	6	4.8	6.5	2	6.5	11.5	6	2.6	3.7	3.5	6.2	5	1.1	2.7	6.5
arg	7.9	5.5	3.9	5.3	1.1	6	5.5	5.9	2.6	6.5	11.4	5	2.2	4.7	3.6	5.5	4.4	1.4	4	6.6
asn	9.6	4.9	4.2	4.9	1	5.3	5.6	7.4	2.3	6	10	4.9	2	3.5	5.1	6.1	5.5	1.5	3.1	6.1
asp	9.3	4	4.7	5.1	1	6.7	2.9	7	1.8	7.1	9.6	6.3	2.3	4.3	3.9	5.9	5.1	1.6	3.6	6.6
cys	8.4	4.8	3.3	5.4	1.7	5.6	5.2	8.1	4.3	5.4	10.2	3.8	1.8	4.1	4.5	6.3	4.3	1.6	3.4	6.8
glu	9.4	5.8	3.6	4.5	0.8	4.9	7	5.8	2.6	5.9	12.7	5	2.4	4	3.5	5.4	5	1.1	2.8	6.8
gln	10.3	4.9	3	4.4	0.9	4.5	6.8	7	2.7	5.5	12.8	4.1	2	3.9	3.8	5.8	5.3	1.4	3	6.9
gly	8.1	4.8	3.9	5.1	1.2	6	4.6	6.4	2.4	6.8	10.5	5.8	2.7	4.8	2.4	5.8	5.1	1.4	3.7	7.5
his	7.3	4.7	4	4.8	1.5	4.9	5.6	6.9	3	6.2	10.8	4.8	1.6	5	5.2	6.8	4.9	1.7	4.2	5.1
ile	11	4.7	4.9	6.5	1.1	6.9	3.6	7.2	2.1	5.3	8.6	5.3	1.8	3.2	4.2	7	5.6	0.9	2.9	6.1
leu	10.4	4.2	4.3	5.2	1.1	5.2	3.7	6.8	2	5.6	10.6	5.3	2.3	3.8	4.5	7.4	6.2	1	2.6	6.6
lys	10.6	5.2	3.8	5.2	0.5	5.3	5.9	6.6	2.6	5.2	11.3	4.7	1.9	2.8	4.6	6	5.5	1.2	2.6	7.6
met	10.8	4.8	3.8	4.6	0.7	4.6	4.9	7	1.7	4.7	11.4	5.2	2.8	3.3	5.1	7.4	6.3	0.9	2	6.8
phe	9.6	3.7	5.2	6.5	1.2	6.4	2.7	7.9	1.9	6.7	7.4	5	2.5	3.9	3.6	8	5.8	1.3	3.3	6.3
pro	8.4	3.6	4.6	5.4	0.7	7.6	5.2	5.4	2.3	6.1	11.2	5.5	2.4	4.2	2.8	6.5	5.4	1.4	2.9	7.5
ser	9.1	4.6	3.7	5	1	5.4	5.2	7.2	2.6	6	11.6	4.5	2.2	4.1	4.1	6.5	5	1.2	3.2	6.8
thr	9.1	4.2	3.7	5.6	0.9	5.7	5.7	7.5	2.2	5.5	12	4.2	2	3.5	5.5	6.2	5.3	1.1	2.6	6.7
trp	7.1	6.3	3.2	4.8	1.3	3.9	8.5	6.6	3.6	5	14.2	3.2	2.4	4.6	3.9	5.8	4.3	1.3	3	6.1
tyr	7.9	6.5	3.6	4.9	1.2	4.5	7	7.1	2.6	5	11.7	4	1.6	4.7	4.9	6.4	4.6	1.5	3.4	5.7
val	9.6	4.1	4.4	5.9	1	6.2	3.4	6.4	1.8	6.5	10.2	5.2	2.5	3.7	3.8	7.2	6.1	1.1	2.7	7.1

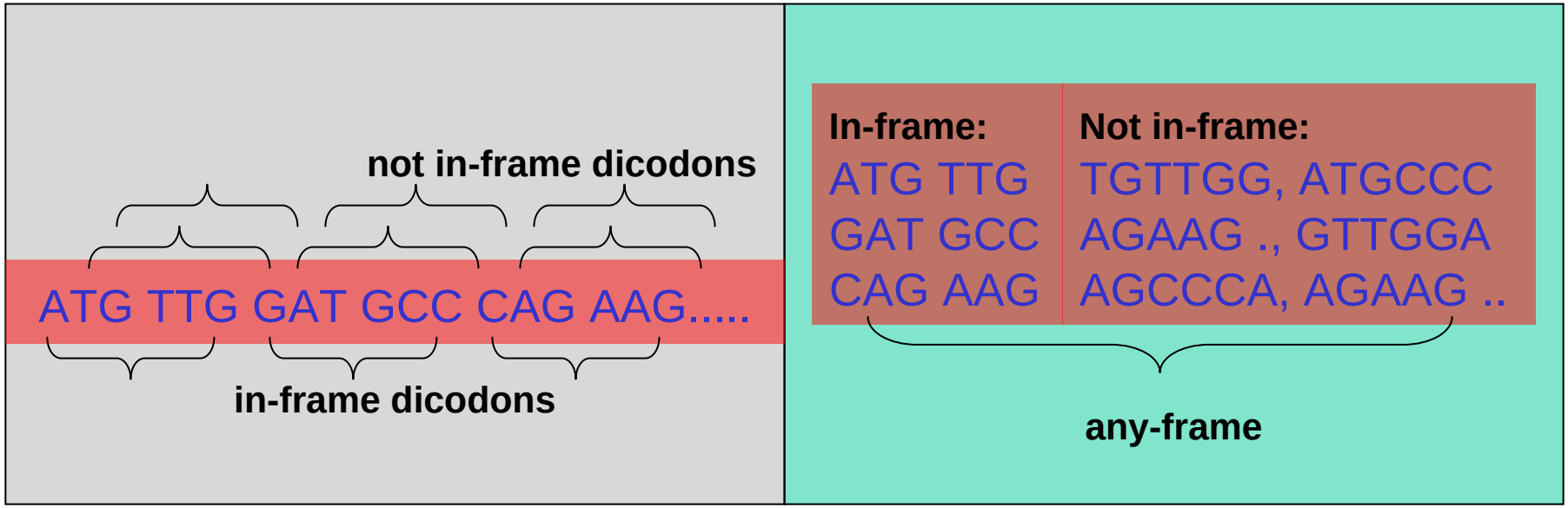
Shewanella

Name	ala	arg	asn	asp	cys	glu	gln	gly	his	ile	leu	lys	met	phe	pro	ser	thr	trp	tyr	val
ala	11.4	5.9	3.1	4.5	1.9	5.8	3.6	7.7	1.9	4.3	9.7	4.3	2.1	3.7	6.4	6.4	5.6	1.1	2.6	6.8
arg	8.5	7.7	4	4.6	2.3	5.9	3.8	7.6	2.5	4.4	9.2	5	1.7	4	5.3	6.3	5	1.5	3.4	6.5
asn	6.3	4.9	4.9	4.4	2.1	5.3	4.1	6.9	2.2	5.6	9.7	5.4	2.1	4.1	5.9	7.3	5.3	1.9	4.6	6.2
asp	7.4	4.9	3.5	5.4	2.4	6.6	3.4	7.4	2.1	5.4	9.5	4.7	2	4.4	5.4	6.8	5.7	1.6	4	6.4
cys	6.9	5.9	4	5.4	2.7	5.6	4.9	7.1	3	4.4	8.8	5.4	1.6	3.5	6.8	7.4	5.7	1.4	2.7	5.7
glu	7.8	5.3	4.3	6.4	1.9	9.7	3.7	6.8	2	5.1	8.2	6.2	2.2	3.3	4.8	5.3	5.4	1.2	3.2	6.2
gln	7.9	5.6	4.2	5	2	6.6	5.1	6.9	2.1	4.7	9.3	5.7	2	3.3	5.9	5.7	6.1	1.6	3.3	6.2
gly	7.9	5.8	3.9	5	1.9	6.2	3.5	8	1.8	4.7	8.7	5.2	1.7	3.7	6.9	7.4	5.8	1.4	3.2	6.2
his	6	5.8	4.3	3.5	2.9	5.1	4.1	6.3	3.2	4.5	10.6	4.8	1.6	4.5	6.7	6.6	6.1	1.7	3.9	6.9
ile	6.2	4.9	4.9	4.7	2.4	5.3	4.6	5.8	2.2	6	9.9	5.3	2.1	4.1	5.3	7.7	6.9	1.2	3.7	6
leu	7.7	5.6	4.1	4.7	2.1	5.8	4.5	6.8	2.1	4.6	11	5.4	1.9	3.7	5.7	7	5.5	1.2	3.1	6.4
lys	6.3	5.2	4.8	5.2	2.1	7.2	3.7	6.7	2.2	6	8.5	7.5	2	3.5	4.8	6.1	5.8	1.6	3.5	6.3
met	9.3	5.3	4.1	5.9	1.6	6.1	3.5	6.4	1.6	4.1	9.6	6.6	2.6	4	5.1	6.9	5.5	1	3.2	6.6
phe	6	5.4	4.5	5.2	2.5	5.5	4.1	6.5	2.3	5.3	10.2	5.2	1.8	4.1	5.3	7.8	5.8	1.4	3.9	6.2
pro	8.5	5.4	3.1	5.1	1.9	6.7	3.9	9.5	1.9	4.3	7.7	4.3	1.7	3.3	8.7	6.9	5.7	1.4	2.8	6.4
ser	6.7	5.4	3.8	4.9	2.3	5.4	4	7.9	2.1	4.5	9.5	5.2	1.8	4	5.7	8.6	6.2	1.4	3	6.4
thr	7.5	4.6	3.7	5	2.6	5.7	3.8	6.8	2	5.2	9.7	4.4	1.8	3.9	6	7.2	7.3	1.5	3.5	6.9
trp	7.1	5.2	4.9	5.5	2.3	5.4	4.3	5.8	2.2	5.6	9.5	6.6	2.1	3.8	4.1	6.4	5.9	1.7	3.7	6.8
tyr	5.8	5.7	5	5.1	2.3	5.7	4.1	6.2	2.4	5	8.6	5.6	1.9	5	4.8	6.7	6.3	1.5	4.8	6.5
val	7.6	5	4.4	5.2	2.4	5.7	3.7	6.3	1.9	5	9.3	5.1	2.1	4.1	5.5	6.9	6.6	1.1	3.6	7.4

Bovine

Coding Signal

- **In-frame vs any-frame dicodons**
- **In-frame dicodon freq provide a more sensitive measure than any-frame dicodon freq**



Dicodon Preference Model

- **The preference value *P(X)* of a dicodon *X* is defined as**

  $$P(X) = \log FC(X)/FN(X)$$

  where

  ***FC(X)* is freq of *X* in coding regions**

  ***FN(X)* is freq of *X* in non-coding regions**

Dicodon Preference Model's Properties

- ***P(X) = 0*** **if** ***X*** **has same freq in coding and non-coding regions**
- ***P(X) > 0*** **if** ***X*** **has higher freq in coding than in non-coding region; the larger the diff, the more positive the score is**
- ***P(X) < 0*** **if** ***X*** **has higher freq in non-coding than in coding region; the larger the diff, the more negative the score is**

Dicodon Preference Model Example

- **Suppose AAA ATT, AAA GAC, AAA TAG have the following freq:**

  FC(AAA ATT) = 1.4%
  FN(AAA ATT) = 5.2%

  FC(AAA GAC) = 1.9%
  FN(AAA GAC) = 4.8%

  FC(AAA TAG) = 0.0%
  FN(AAA TAG) = 6.3%

- **Then**

  P(AAA ATT) = –0.57
  P(AAA GAC) = –0.40
  P(AAA TAG) = $-\infty$,
  treating STOP codons differently

$\Rightarrow$ **A region consisting of only these dicodons is probably a non-coding region**

Frame-Insensitive Coding Region Preference Model

- **A frame-insensitive coding preference $S_{is}(R)$ of a region R can be defined as**

$$S_{is}(R) = \Sigma_{X \text{ is a dicodon in } R} P(X)$$

- **R is predicted as coding region if $S_{is}(R) > 0$**

- ***NB. This model is not commonly used***

In-Frame Dicodon Preference Model

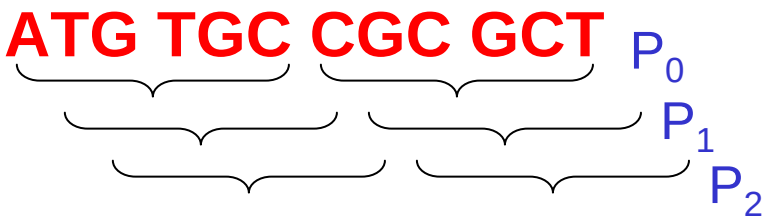
- **The *in-frame* + *i* preference value $P_i(X)$ of a dicodon *X* is defined as**

$$P_i(X) = \log FC_i(X)/FN(X)$$

- **where**

  $FC_i(X)$ is freq of *X* in coding regions at *in-frame* + *i* positions

  $FN(X)$ is freq of *X* in non-coding regions



In-Frame Coding Region Preference Model

- **The *in-frame + i* preference $S_i(R)$ of a region *R* can be defined as**

$$S_i(R) = \Sigma_{X \text{ is a dicodon at in-frame + i position in } R} P_i(X)$$

- ***R* is predicted as coding if $\Sigma_{i=0,1,2} S_i(R)/|R| > 0$**

- ***NB. This coding preference model is commonly used***

Coding Region Prediction: An Example Procedure

- **Calculate all ORFs of a DNA segment**
- **For each ORF**
  - Slide thru ORF w/ increment of 10bp
  - Calculate in-frame coding region preference score, in same frame as ORF, within window of 60bp
  - Assign score to center of window
- **E.g., forward strand in a particular frame...**

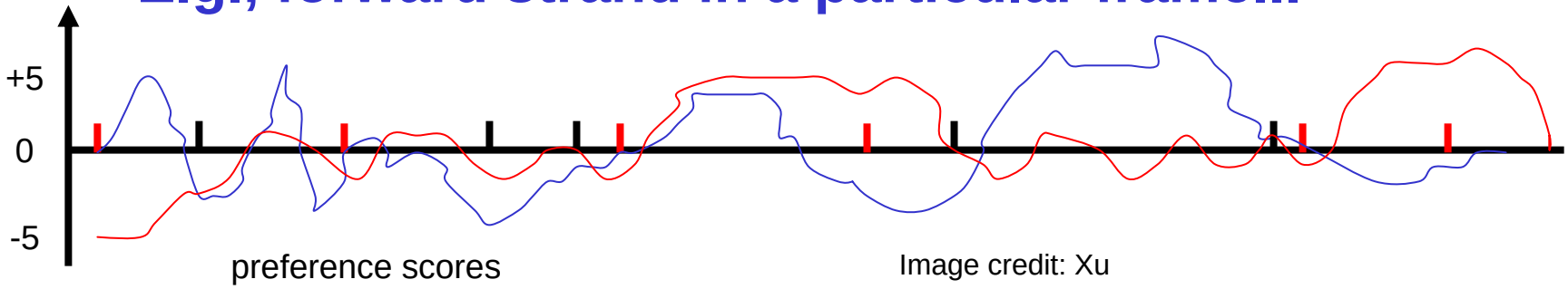


Image credit: Xu

Problem with Coding Region Boundaries

- **Making the call: coding or non-coding and where the boundaries are**

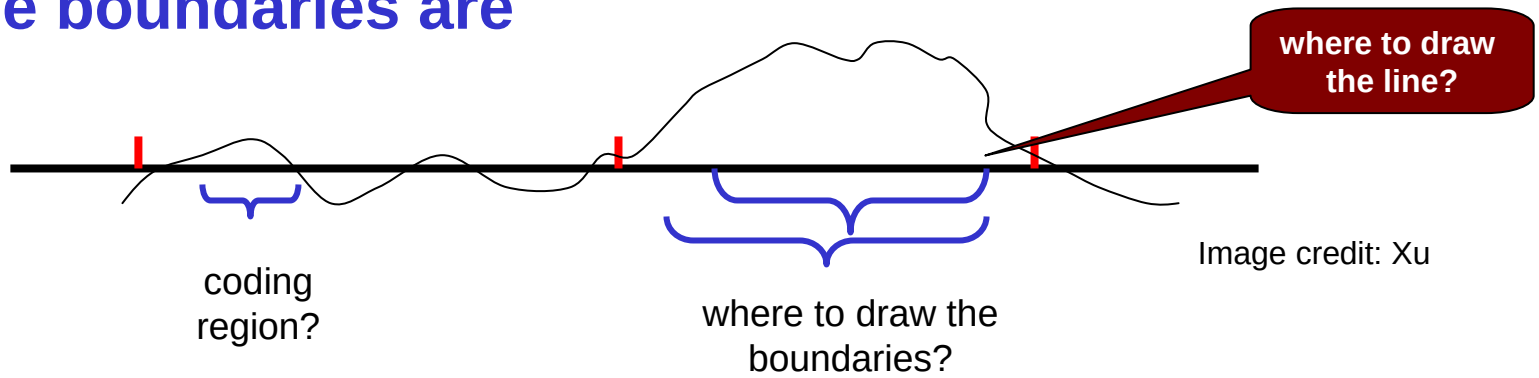


Image credit: Xu

⇒ **Need training set with known coding and non-coding regions to select threshold that includes as many known coding regions as possible, and at the same time excludes as many known non-coding regions as possible**

Types of Coding Region Boundaries

- **Knowing boundaries of coding regions helps identify them more accurately**
- **Possible boundaries of an exon**

{ translation start, acceptor site } **{ translation stop, donor site }**

Image credit: Xu

- **Splice junctions:**
  - Donor site: coding region | GT
  - Acceptor site: CAG | TAG | coding region
- **Translation start**
  - in-frame ATG

Signals for Coding Region Boundaries

- **Splice junction sites and translation starts have certain distribution profiles**
- **For example, ...**

Acceptor Site (Human Genome)

- **If we align all known acceptor sites (with their splice junction site aligned), we have the following nucleotide distribution**

	-14	-13	-12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	1
A	11.1	12.7	3.2	4.8	12.7	8.7	16.7	16.7	12.7	9.5	26.2	6.3	100	0.0	21.4
C	36.5	30.9	19.1	23.0	34.9	39.7	34.9	40.5	40.5	36.5	33.3	68.2	0.0	0.0	7.9
G	9.5	10.3	15.1	12.7	8.7	9.5	16.7	4.8	2.4	6.3	13.5	0.0	0.0	100	62.7
U	38.9	41.3	58.7	55.6	42.1	40.5	30.9	37.3	44.4	47.6	27.0	25.4	0.0	0.0	7.9

Image credit: Xu

- **Acceptor site: CAG | TAG | coding region**

Donor Site (Human Genome)

- **If we align all known donor sites (with their splice junction site aligned), we have the following nucleotide distribution**

	-3	-2	-1	1	2	3	4	5	6
A	34.0	60.4	9.2	0.0	0.0	52.6	71.3	7.1	16.0
C	36.3	12.9	3.3	0.0	0.0	2.8	7.6	5.5	16.5
G	18.3	12.5	80.3	100	0.0	41.9	11.8	81.4	20.9
U	11.4	14.2	7.3	0.0	100	2.5	9.3	5.9	46.2

Image credit: Xu

- **Donor site: coding region | GT**

What Positions Have "High" Information Content?

- **For a weight matrix, information content of each column is calculated as**

  $$- \Sigma_{X \in \{A,C,G,T\}} F(X)*\log (F(X)/0.25)$$

$\Rightarrow$ **When a column has evenly distributed nucleotides, its information content is lowest**

$\Rightarrow$ **Only need to look at positions having high information content**

Information Content Around Donor Sites in Human Genome

	-3	-2	-1	1	2	3	4	5	6
A	34.0	60.4	9.2	0.0	0.0	52.6	71.3	7.1	16.0
C	36.3	12.9	3.3	0.0	0.0	2.8	7.6	5.5	16.5
G	18.3	12.5	80.3	100	0.0	41.9	11.8	81.4	20.9
U	11.4	14.2	7.3	0.0	100	2.5	9.3	5.9	46.2

Image credit: Xu

- **Information content**

column –3 = – .34*log (.34/.25) – .363*log (.363/.25) – .183* log (.183/.25) – .114* log (.114/.25) = 0.04

column –1 = – .092*log (.92/.25) – .03*log (.033/.25) – .803* log (.803/.25) – .073* log (.73/.25) = 0.30

Weight Matrix Model for Splice Sites

- **Weight matrix model**
  - build a weight matrix for donor, acceptor, translation start site, respectively
  - use positions of high information content

	-3	-2	-1	1	2	3	4	5	6
A	34.0	60.4	9.2	0.0	0.0	52.6	71.3	7.1	16.0
C	36.3	12.9	3.3	0.0	0.0	2.8	7.6	5.5	16.5
G	18.3	12.5	80.3	100	0.0	41.9	11.8	81.4	20.9
U	11.4	14.2	7.3	0.0	100	2.5	9.3	5.9	46.2

Image credit: Xu

Just to make sure you know what I mean …

- **Give me 3 DNA seq of length 10:**
  - Seq_1 = ACCGAGTTCT
  - Seq_2 = AGTGTACCTG
  - Seq_3 = AGTTCGTATG
- **Then the weight matrix is …**

1-mer	pos1	pos2	pos3	pos4	pos5	pos6	pos7	pos8	pos9	pos10
A	3/3	0/3	0/3							
C	0/3	1/3	1/3							
G	0/3	2/3	0/3							
T	0/3	0/3	2/3							

Exercise: Fill in the rest of the table

Splice Site Prediction: A Procedure

	-3	-2	-1	1	2	3	4	5	6
A	34.0	60.4	9.2	0.0	0.0	52.6	71.3	7.1	16.0
C	36.3	12.9	3.3	0.0	0.0	2.8	7.6	5.5	16.5
G	18.3	12.5	80.3	100	0.0	41.9	11.8	81.4	20.9
U	11.4	14.2	7.3	0.0	100	2.5	9.3	5.9	46.2

Image credit: Xu

- **Add up freq of corr letter in corr positions:**

AAGGTAAGT: .34 + .60 + .80 +1.0 + 1.0 + .52 + .71 + .81 + .46 = 6.24

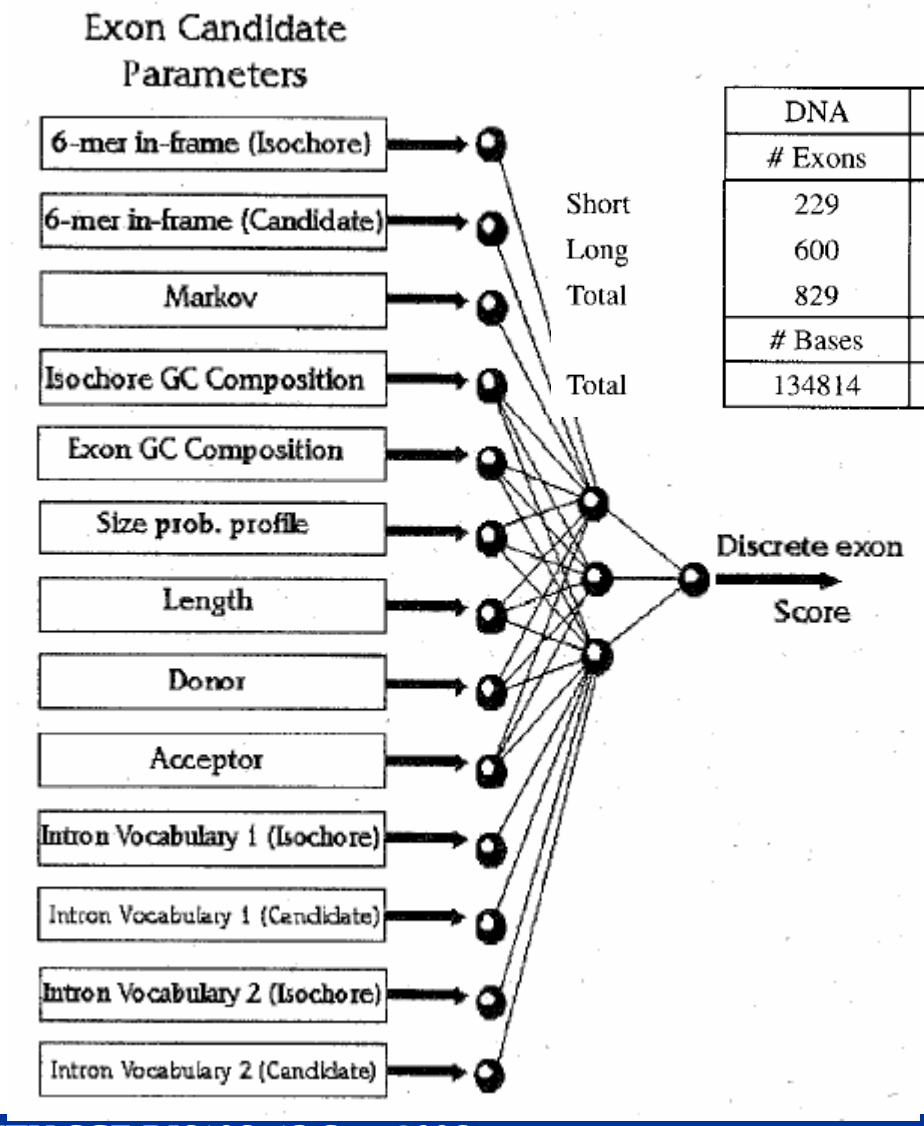
TGTGTCTCA: .11 + .12 + .03 +1.0 + 1.0 + .02 + .07 + .05 + .16 = 2.56

- **Make prediction on splice site based on some threshold**

Other Factors Considered by GRAIL

- **G+C composition affects dicodon distributions**
- **Length of exons follows certain distribution**
- **Other signals associated with coding regions**
  - periodicity
  - structure information
  - .....
- **Pseudo genes**
- **.....**

Info Fusion by ANN in GRAIL



DNA	Predictions			
# Exons	TP	%	FP	%
229	171	74.7	39	18.6
600	575	95.8	30	4.9
829	746	90.0	69	8.5
# Bases				
134814	122885	91.2	13048	9.6

Image credit: Xu

Remaining Challenges in GRAIL

- **Initial exon**
  - R. V. Davuluri et al., "Computational identification of promoters and first exons in the human genome", *Nat. Genet.*, 29:412--417, 2001
  - H. Liu et al., "Data Mining Tools for Biological Sequences", *JBCB*, 1:139--168, 2003
  - V. B. Bajic et al., "Dragon Gene Start Finder: An advanced system for finding approximate locations of the start of gene transcriptional units", *Genome Research*, 13:1923--1929, 2003
- **Final exon**
  - J. E. Tabaska et al., "Identifying the 3'-terminal exon in human DNA", *Bioinformatics*, 17:602--607, 2001
  - J. E. Tabaska et al., "Detection of polyadenylation signals in human DNA sequences", *Gene*, 23:77--86, 1999
  - H. Liu et al., "An in-silico method for prediction of polyadenylation signals in human sequences", *GIW*, 14:84--93, 2003
- **Indels & frame shifts**

Indel & Frame-Shift in Coding Regions

Problem definition
Indel & frameshift identification
Indel correction
An iterative strategy

Some slides here are "borrowed" from Ying Xu

NUS
National University of Singapore

Indels in Coding Regions

- **Indel = insertion or deletion in coding region**
- **Indels are usually caused by seq errors**

ATG GAT CCA CAT ..... → **ATG GAT CA CAT .....**

ATG GAT CTCA CAT .....

Effects of Indels on Exon Prediction

- **Indels may cause shifts in reading frames & affect prediction algos for coding regions**

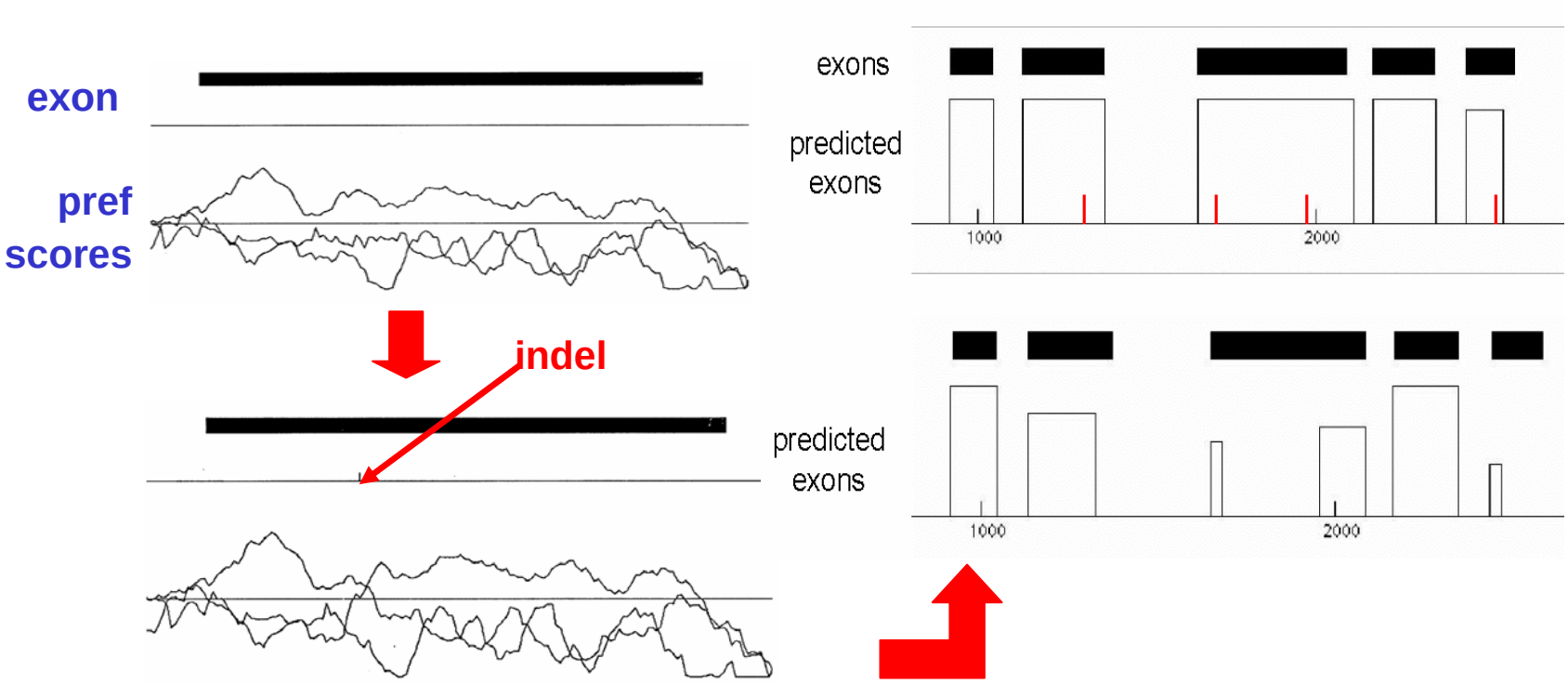


Image credit: Xu

Key Idea for Detecting Frame-Shift

- **Preferred reading frame is reading frame w/ highest coding score**
- **Diff DNA segments may have diff preferred reading frames**

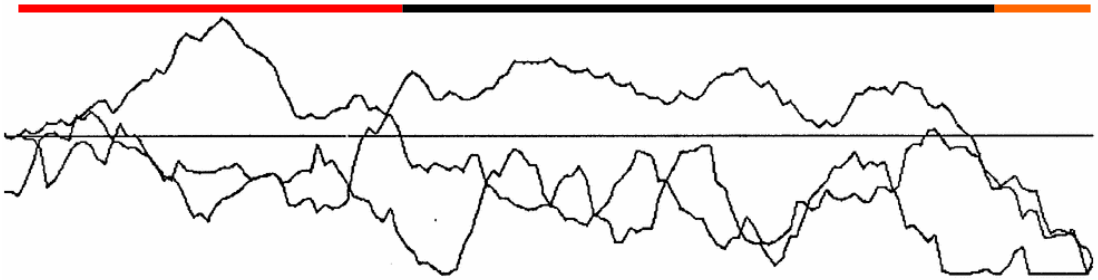


Image credit: Xu

⇒ **Segment a coding sequence into regions w/ *consistent* preferred reading frames corr well w/ indel positions**

⇒ **Indel identification problem can be solved as a sequence segmentation problem!**

Frame-Shift Detection by Sequence Segmentation

- **Partition seq into segs so that**
  - Chosen frames of adjacent segs are diff
  - Each segment has >30 bps to avoid small fluctuations
  - Sum of coding scores in the chosen frames over all segments is maximized

This can be solved as a dynamic programming problem …

Frame-Shift Detection: A Simplified Treatment

- **Given DNA sequence $a_1 \ldots a_n$**
- **Define key quantities**

$$C(i, r) = \text{max score on } a_1 \ldots a_i, \text{ w/ the last segment in frame } r$$

- **Then**

$$\max_{r \in \{0, 1, 2\}} C(n, r) \text{ is optimal solution}$$

Frame-Shift Detection: *C(i,r)*

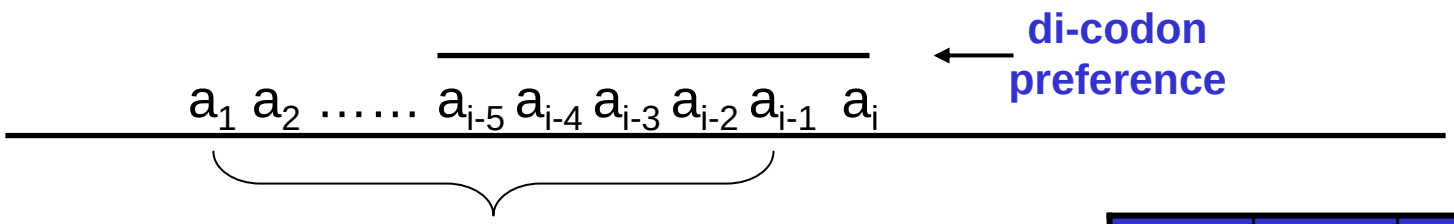
- **To calculate *C(i,r)*, there are 3 possible cases for each position *i*:**
  - Case 1: no indel occurred at position *i*
  - Case 2: a_i is an inserted base
  - Case 3: a base has been deleted in front of a_i

$\Rightarrow$ ***C(i, r) = max { Case 1, Case 2, Case 3 }***

Frame-Shift Detection: Case 1

- **No indel occurs at position *i*. Then**

$$C(i,r) = C(i-1, r') + P_r(a_{i-5} \ldots a_i)$$



r''	r'	r
1	2	0
2	0	1
0	1	2

Frame-Shift Detection: Case 2

- a_{i-1} is an inserted base. Then

$$C(i,r) = C(i-2, r') + P_r(a_{i-6} \ldots a_{i-2} a_i)$$

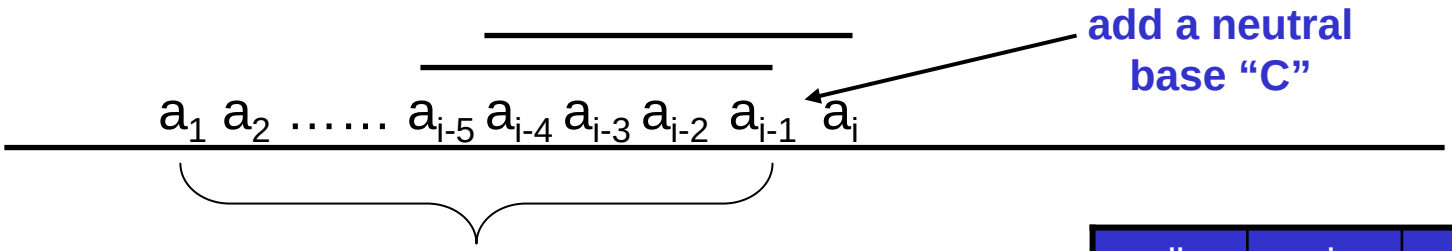
$a_1\ a_2\ \ldots\ldots\ a_{i-6}\ a_{i-5}\ a_{i-4}\ a_{i-3}\ a_{i-2}\ a_{i-1}\ a_i$ ← di-codon preference

r''	r'	r
1	2	0
2	0	1
0	1	2

Frame-Shift Detection: Case 3

- **A base has been deleted in front of a_i. Then**

$$C(i, r) = C(i-1, r'') + P_{r'}(a_{i-5} \ldots a_{i-1}C) + P_r(a_{i-4} \ldots a_{i-1}Ca_i)$$



r''	r'	r
1	2	0
2	0	1
0	1	2

Exercise: why is "C" is best choice for the purpose above?

Frame-Shift Detection: Initiation

- **Initial conditions,**

$$C(k, r) = -\infty,\ k < 6$$

$$C(6, r) = P_r(a_1 \ldots a_6)$$

- **This is a dynamic programming (DP) algorithm; the equations are DP recurrences**

Exercise: How to modified the recurrence so that each fragment is at least 30bp?

Frame-Shift Detection: Step 3

- **Calculation of $max_{r \in \{0, 1, 2\}} C(i, r)$ gives an optimal segmentation of a DNA sequence**
- **Tracing back the transition points---viz. case 2 & case 3---gives the segmentation results**

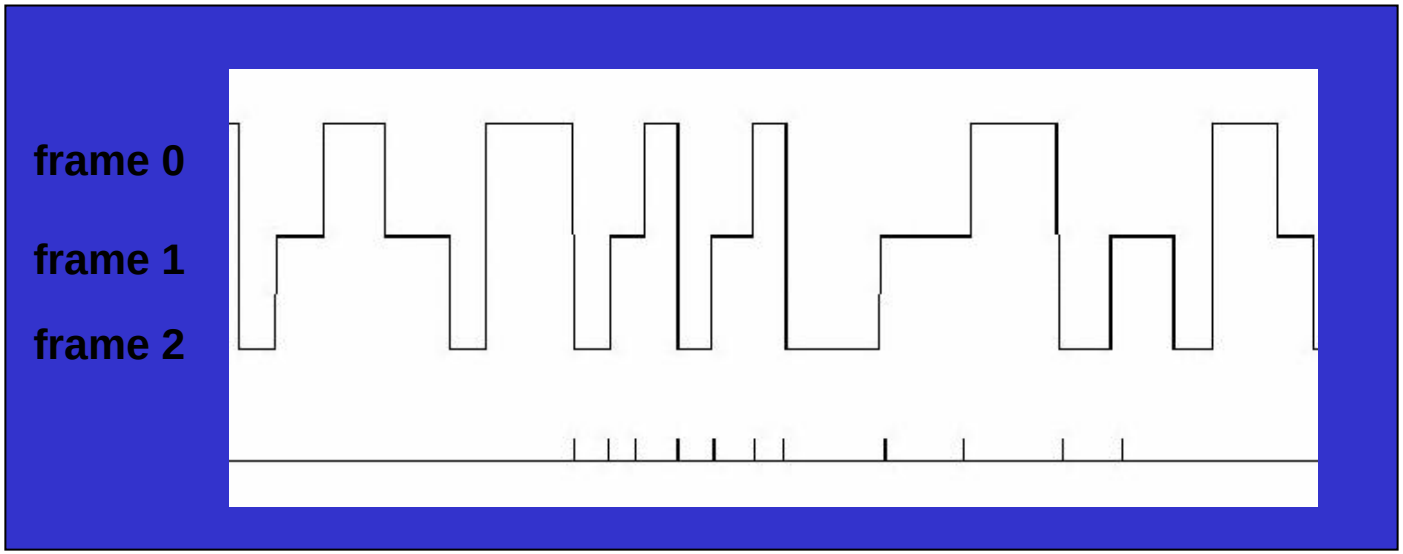


Image credit: Xu

Frame-Shift Detection: Determine Coding Regions

- **For given H_1 and H_2 (e.g., = 0.25 for noncoding and 0.75 for coding), partition a DNA seq into segs so that each seg has >30 bases & coding values of each seg are consistently closer to one of H_1 or H_2 than the other**

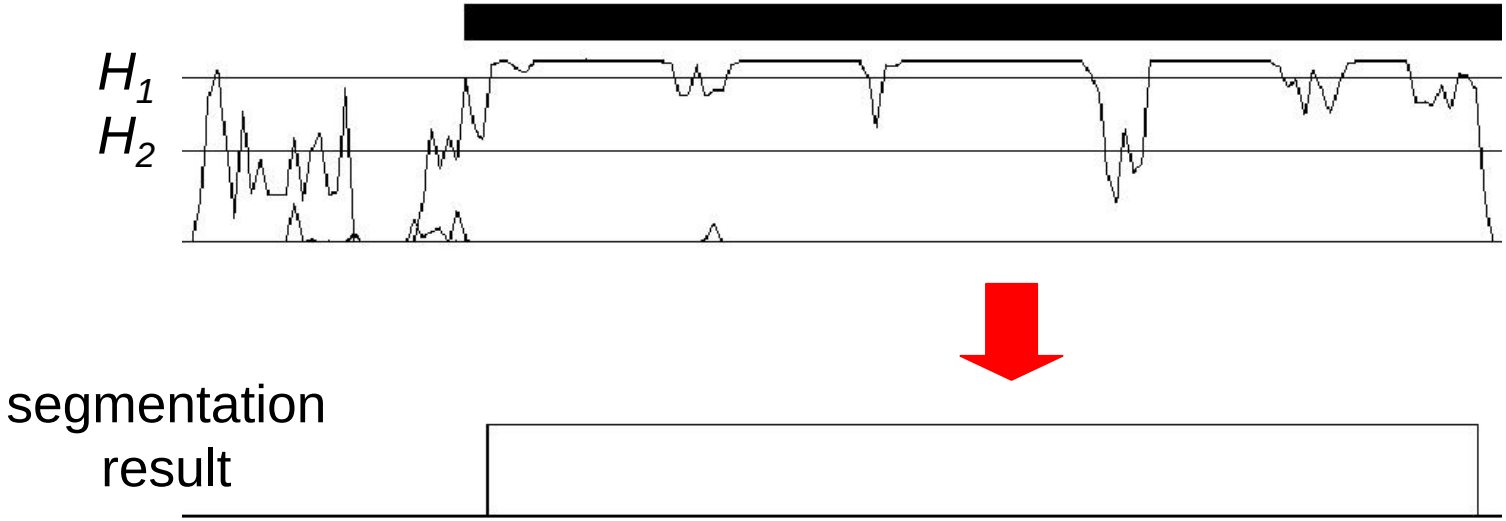


Image credit: Xu

Frame-Shift Detection: Step 5

- **Overlay "preferred reading-frame segs" & "coding segs" gives coding region predictions regions w/ indels**

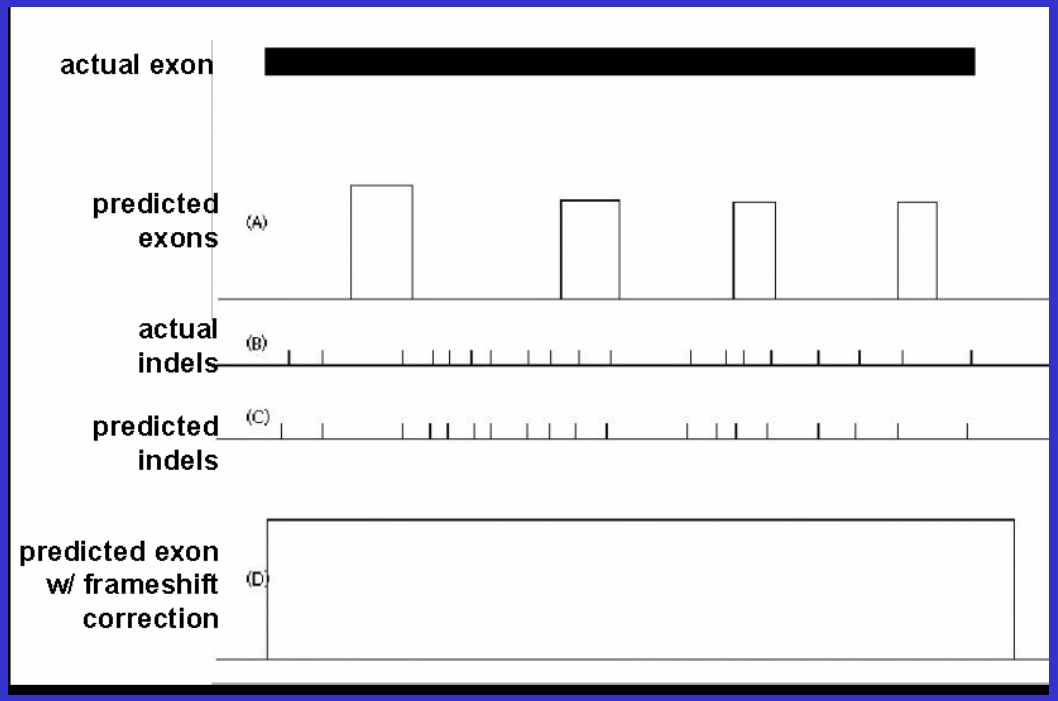


Image credit: Xu

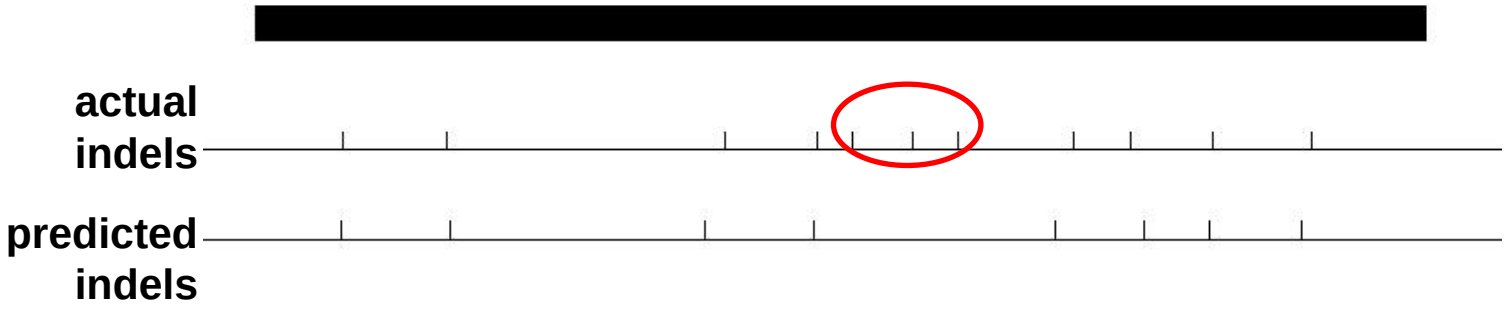
Frame-Shift Detection: Step 6

- **We still need to correct the identified indels...**

- If an “insertion” is detected, delete the base at the transition point
- If a “deletion” is detected, add a neutral base “C” at transition point

What Happens When Indels Are Close Together?

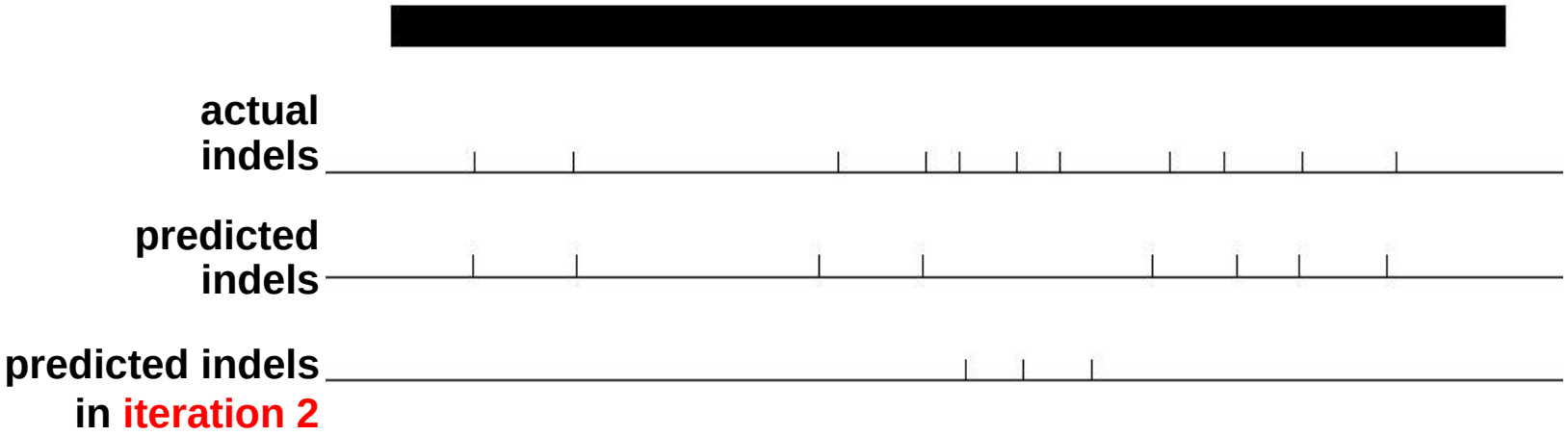
- **Our procedure works well when indels are not too close together (i.e., >30 bases apart)**
- **When indels are too close together, they will be missed...**



Handling Indels That Are Close Together

- **Employ an iterative process, viz**

  Find one set of indels and correct them & then iterate until no more indels can be found



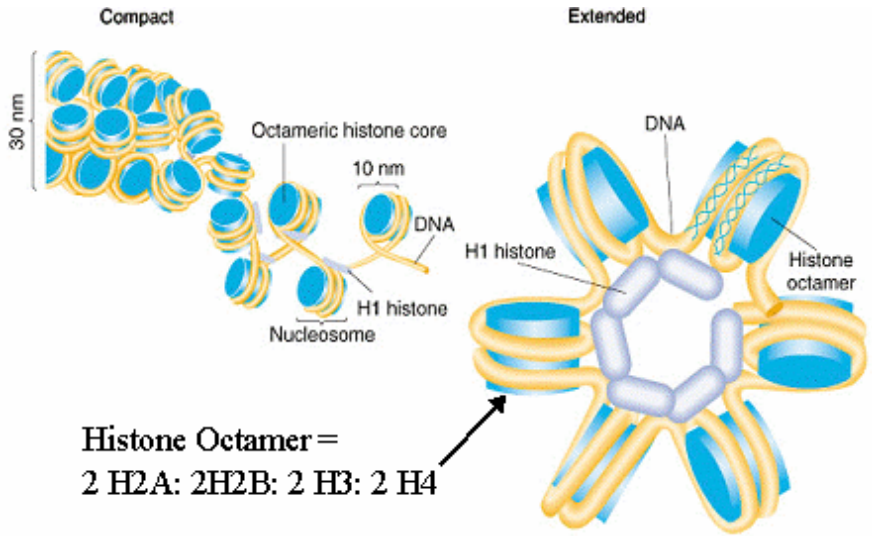
Modeling & Recognition of Histone Promoters

Some slides here are "borrowed" from Rajesh Chowdhary

NUS National University of Singapore

Histone

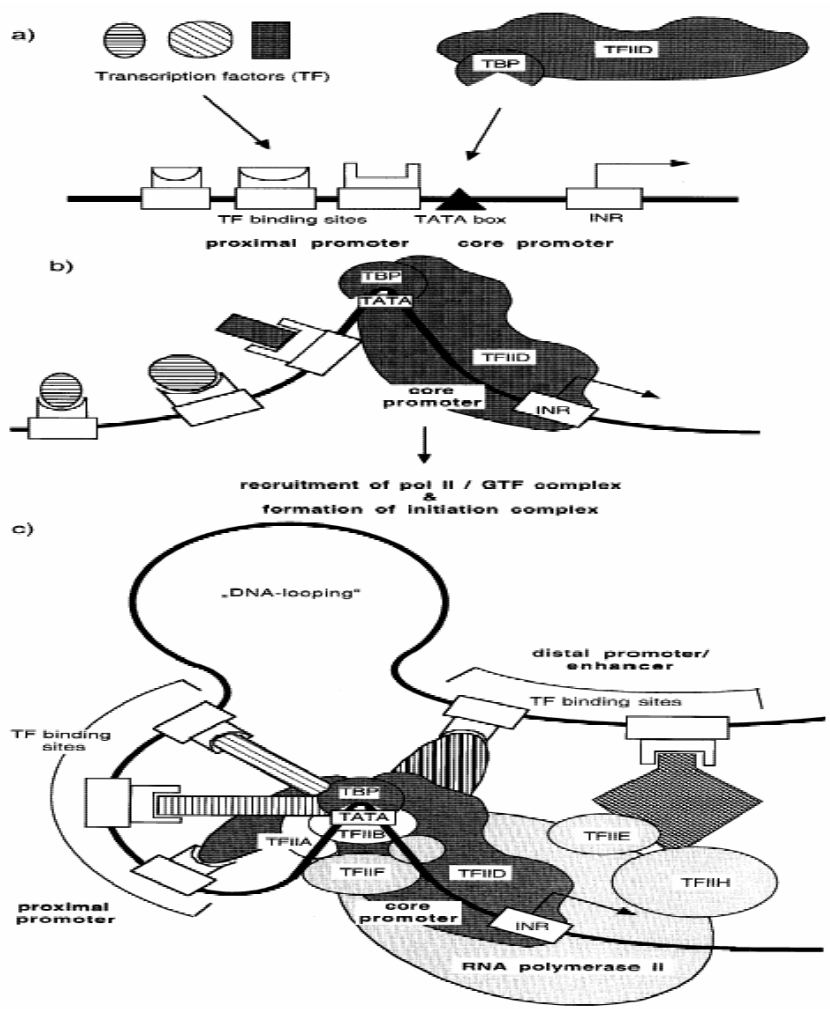
- **Basic proteins of eukaryotic cell nucleus**
- **Form a major part of chromosomal proteins**
- **Help in packaging DNA in the chromatin complex**
- **Five types, namely H1, H2A, H2B, H3 and H4**
- **Highly conserved across species**
  - H1 least conserved, H3 & H4 most conserved



chromosomal processes

- gene transcription, regulation,
- chromosome condensation, recombination & replication

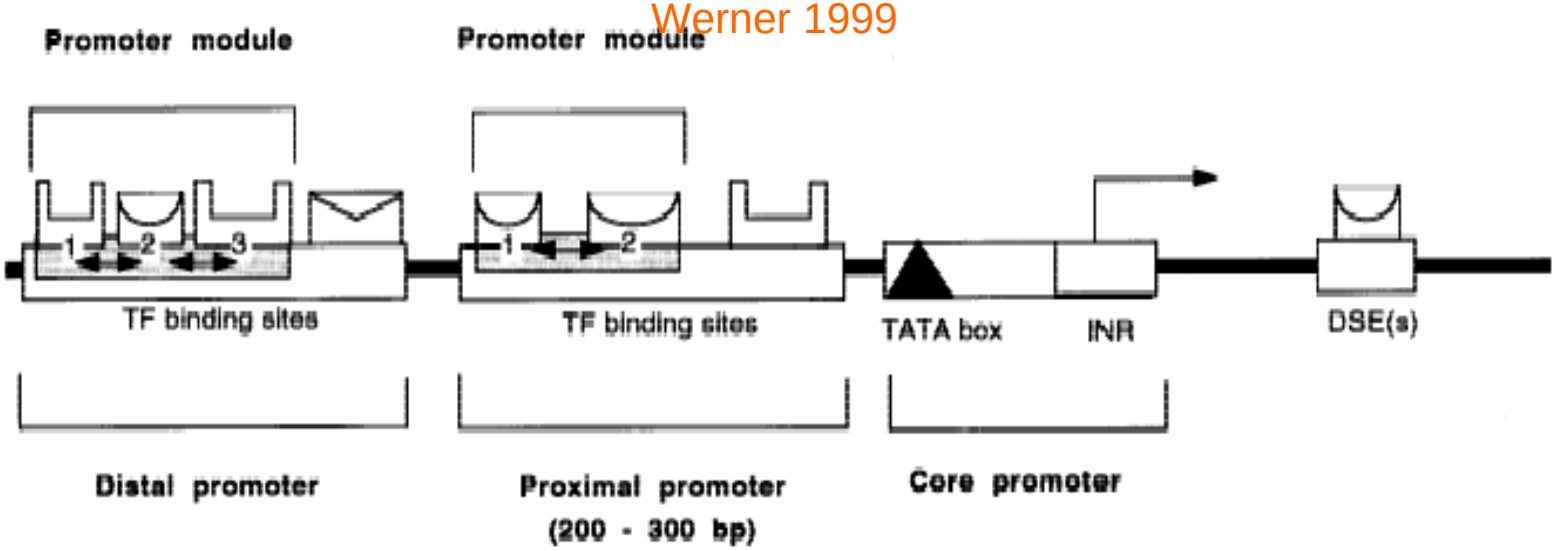
Histone Transcription



- **TFs bound in core, proximal, distal promoter & enhancer regions**
- **TFIID binds to TATA box & identifies TSS with help of TAFs & TBP**
- **RNA Pol-II supplemented by GTFs (A,B,D,E,F,H) recruited to core promoter to form Pre-initiation complex**
- **Transcription initiated**
  - Basal/Activated, depending on space & time

Histone Promoter Modeling

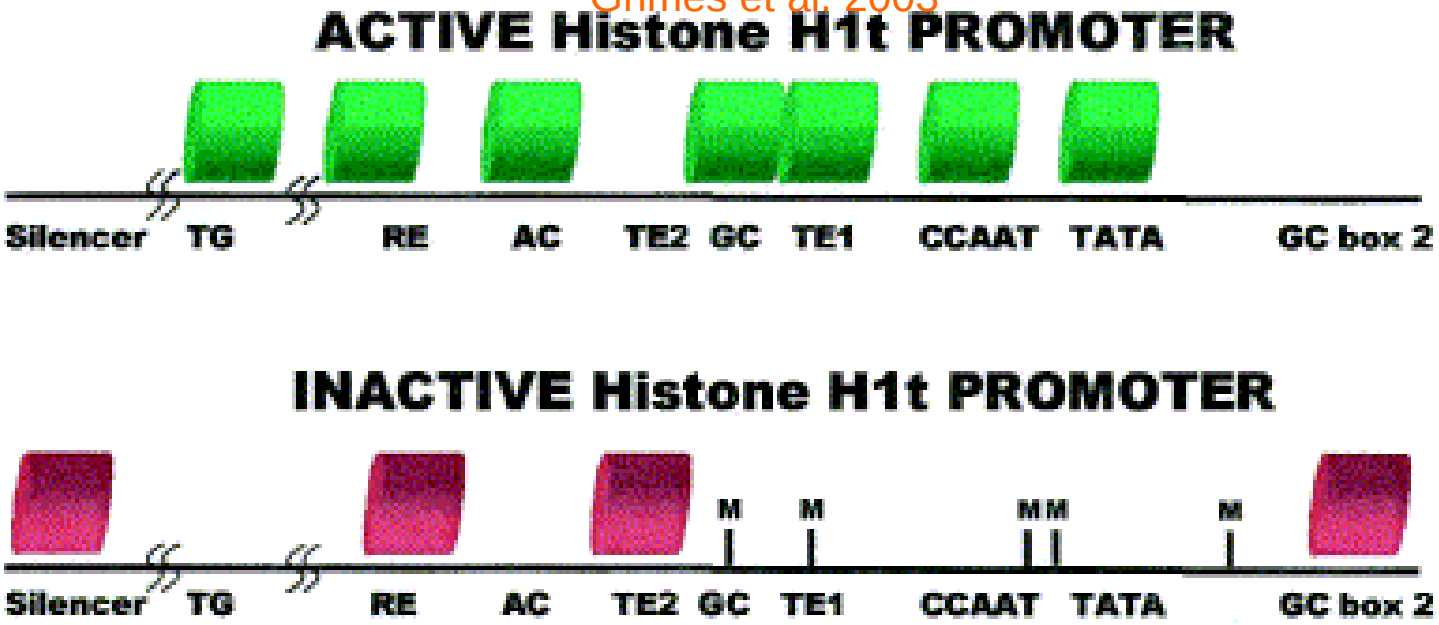
Werner 1999

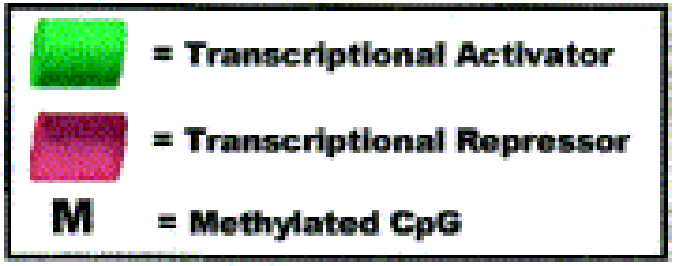


- **Three promoter types: Core, proximal and distal**
- **Characterised by the presence of specific TFBSs**
  - CAAT box, TATA Box, Inr, & DPE
  - Order and mutual distance of TFBS modules is specific & determine function

Histone H1t Gene Regulation

Grimes et al. 2003





- **One gene can express in diff ways in diff cells**
- **Same binding site can have diff functions in diff cells**

Why Model Histone promoters

- **To understand histone's regulatory mechanism**
  - To characterise regulatory features from known promoters
  - To identify promoter from uncharacterised genomic sequence (promoter recognition)
  - To find other genes with similar regulatory behaviour and gene-products
  - To define potential gene regulatory networks

Difficulties of Histone Promoter Modeling

- **Not a plain sequence alignment problem**
- **Not all features are common among different groups**
- **Not only TFBSs' presence, but their location, order, mutual distance and orientation are critical to promoter function**
- **Not all TFs & TFBSs have been characterized yet**

Tools for Promoter Modeling

- **Genomic signals in promoter v/s non-promoter**
  - Core promoter (TATA Box, Inr, DPE) and/or few TFBS outside core promoter
  - Entire promoter (core, proximal & distal) with whole ensemble of TFBS
- **Genomic content in promoter v/s non-promoter**
  - CpG islands, GC content
- **2D-3D DNA structural features**
- **Model with a scoring system based on training data (good data not always available)**
  - Input seq scanned for desired patterns & those whose scores above certain threshold are reported

Promoter Recognition Programs

"GENERAL PROMOTERS"			
First Generation			
Name	**Scoring Technique used**	**Search by content/signal**	**Features used**
NNPP	Time delay NN	Signal	TATA box, Inr
Promoter 2	NN	Signal	TATA box, Inr, CAAT box, GC Box
PromFind	Discriminative count	Content	Hexamer frequency
PromoterScan	Discriminative count	Signal	TATA box, TFBS
TSSG/TSSW	Linear discriminant analysis	Content + signal	TATA box, TSS, hexamer frequency upstream TSS, TFBS
Second Generation			
DGSF	NN	Content + signal	CpG island, TSS, DPF output
DPF	NN	Content + signal	Promoter, exon, intron, TSS
Eponine	SVM variant	Content + signal	TATA box, GC rich content, TSS
FirstEF	Quadratic discriminant analysis	Content + signal	First exon, CpG islands
Mcpromoter	NN & Interpolated markov models	Content + signal	TATA box, CAAT box, GC box, nucleosome position
PromoterInspector	Discriminative counts	Content	Oligonuleotides, Exon, Intron, 3'UTR, Promoter genomic context
CpG Promoter	Quadratic discriminant analysis	Content + signal	CpG island, TSS
CpGProD	Generalised linear model	Content	CpG island, AT/GC content
"SUB-CLASS OF PROMOTERS"			
Muscle family	Discriminative counts	Signal	TFBS, relative distance
Globin family	Logical operators AND, OR and NOT	Signal	TFBS, relative distance

- **Programs have different objectives**
- **Use various combinations of genomic signals and content**
- **Typically analyse 5' region [-1000,+500]**
- **Due to low accuracy, programs developed for sub-classes of promoters**

Image credit: Rajesh

Steps for Building Histone Promoter Recognizer

- **Exercise: What do you think these steps are?**

MEME

- **MEME is a powerful and good method for finding motifs from biological sequences**
- **T. L. Bailey & C. Elkan, "Fitting a mixture model by expectation maximization to discover motifs in biopolymers", *ISMB*, 2:28--36, 1994**

Motifs Discovered by MEME in Histone Gene 5' Region [-1000,+500]

Image credit: Rajesh

H2A

Motifs Discovered by MEME in Histone Gene 5' Region [-1000,+500]

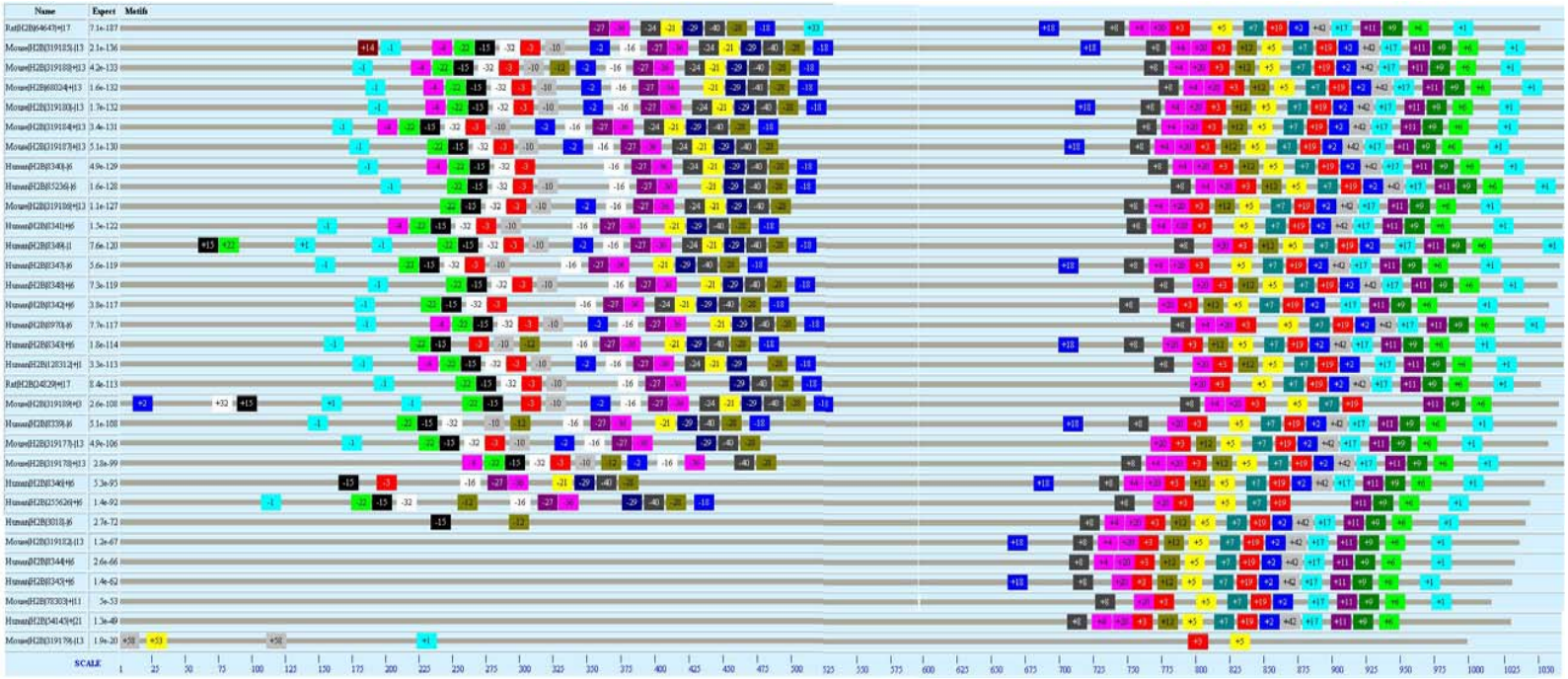


Image credit: Rajesh

H2B

Are These Really Motifs of H2A and H2B Promoters?

- **One could use the motifs discovered by MEME to detect H2A & H2B promoters**
- **But….it is strange that the motifs for H2A and H2B are generally the same, but in opposite orientation**
- **Exercise: Suggest a possible explanation**

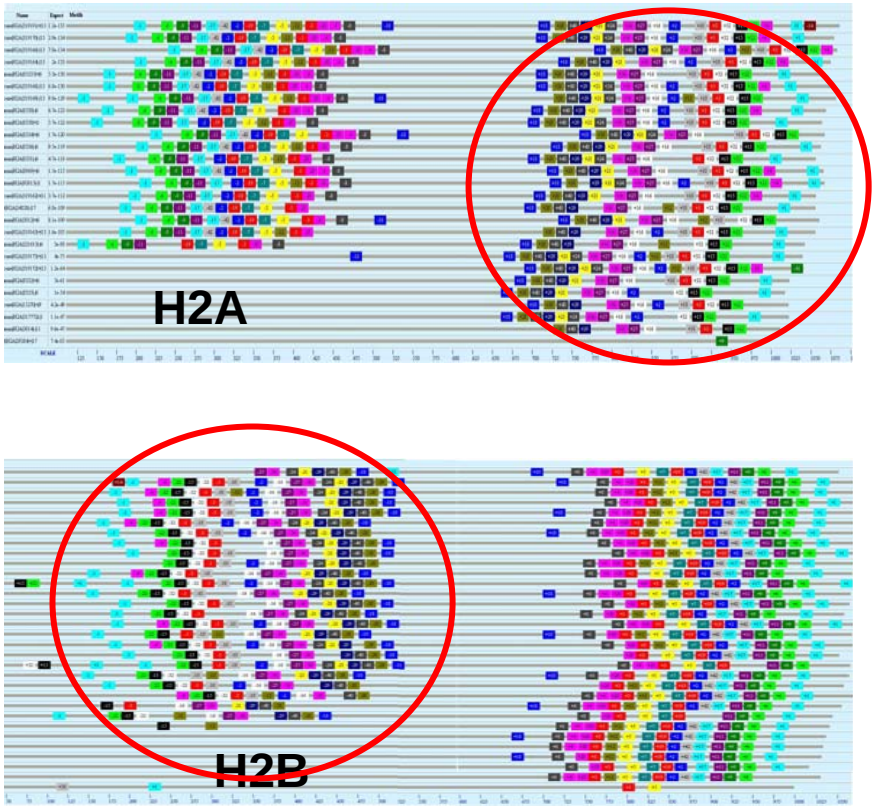
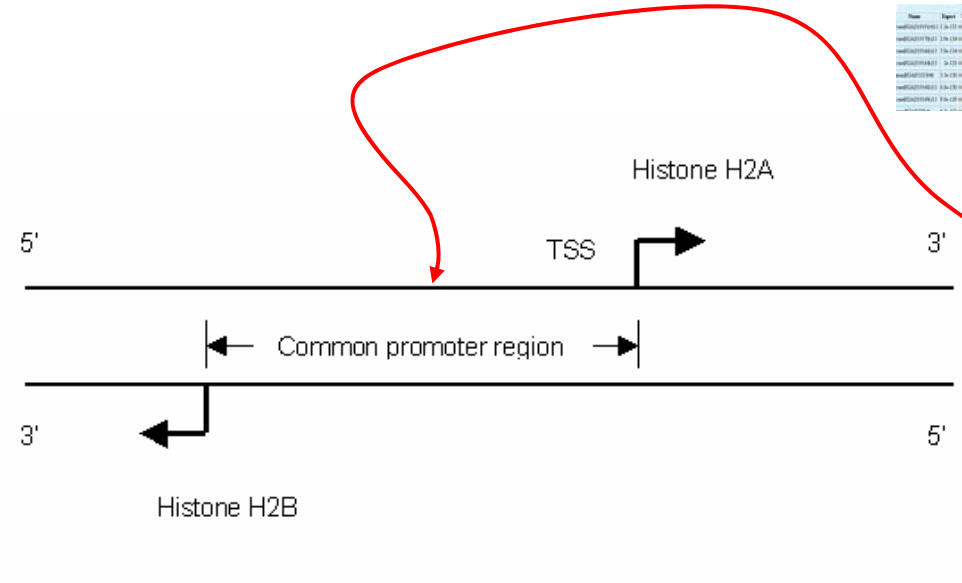


Image credit: Rajesh

The Real Common Promoter Region of H2A & H2B is at [-250,-1]!



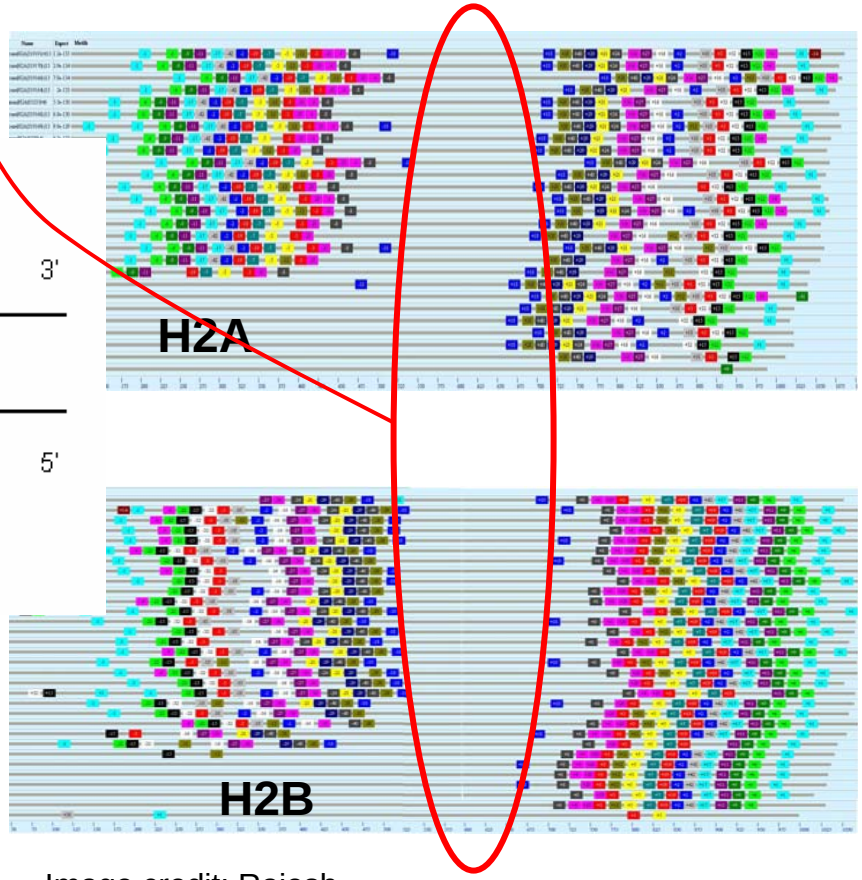


Image credit: Rajesh

- **MEME was overwhelmed by coding region & did not find the right motifs!**

Motifs Discovered by MEME in Histone Promoter 5' Region [-250,-1]

MOTIF NO.	MOTIF DEFINITION	TFBS AND ASSOCIATED FACTORS	TRANSFAC SITE NUMBER
1	TCTGATTGGTTA	CCAAT-box: H1TF2 (La Bella et al. 1989; Martinelli and Heintz 1994; Gallinari et al. 1989), HiNF-B (van Wijnen et al. 1988a,b), NF-Y (Mantovani 1999), HiNF-D (van Wijnen et al 1996; Grimes et al. 2003)	R00660
2	ATGCAAATGAGG	Oct-1: Octamer transcription factor 1 (OTF-1) (Fletcher et al. 1987)	R00662
3	CTATAAAAACC	TATA-box: TBP, TFIID (Nakajima et al. 1988)	R00770
4	TTTTCGCGCCCA	E2F-binding site: E2F-1 factor (Oswald et al. 1996)	R09798
5	CAATCAGGTCCG	H4TF2 binding site: H4TF2 (La Bella and Heintz 1991)	R00681
6	AACAAACACAA	AC-box: H1TF1 (La Bella et al. 1989), HiNF-A (van Wijnen et al. 1988b), HiNF-D (van Wijnen et al. 1996; Grimes et al. 2003)	R00658
7	CAGCCAATCAGA	CCAAT-box: H1TF1 (La Bella et al. 1989), HiNF-B (van Wijnen et al. 1988a,b), NF-Y (Mantovani 1999), HiNF-D (van Wijnen et al. 1996; Grimes et al. 2003), H1TF2 (La Bella et al. 1989; Martinelli and Heintz 1994; Gallinari et al. 1989)	R00659, R00660
8	CCATTGGTTAAA	CCAAT-box: H1TF2 (La Bella et al. 1989; Martinelli and Heintz 1994; Gallinari et al. 1989), HiNF-B (van Wijnen et al. 1988a,b), NF-Y (Mantovani 1999), HiNF-D (van Wijnen et al. 1996; Grimes et al. 2003)	R00660
9	CCCCGCCCCCCG	GC-box: HiNF-C (van Wijnen et al. 1989), Sp1 (Courey and Tjian 1988), Sp3 (Bimbaum et al. 1995; Hagen et al. 1994)	R00684

- **Discovered 9 motifs among all 127 histone promoters**
- **All 9 motifs are experimentally proven TFBSs (TRANSFAC)**

Image credit: Rajesh

Deriving Histone Promoter Models

Histone H1 genes	Name	Motifs					
Model 1: H1.1-H1.5 (cell cycle dependent)							
Human|H1|3024|-|6	H1.1	-	-	-6	-9	-1	+3
Mouse|H1|80838|+|13	H1.1	-	-	-	-9	-1	+3
Human|H1|3006|-|6	H1.2	-	-	+6	-	-1	+3
Mouse|H1|50708|+|13	H1.2	-	-	+6	-	-1	+3
Human|H1|3007|-|6	H1.3	+4	-	+6	-	-1	+3
Mouse|H1|14957|+|13	H1.3	+4	-	+6	-	-1	+3
Human|H1|3008|+|6	H1.4	-	-	+6	-	-1	+3
Mouse|H1|50709|-|13	H1.4	-	-	+6	-	-1	+3
Human|H1|3009|-|6	H1.5	+4	+8	+6	-	-1	-3
Mouse|H1|56702|-|13	H1.5	-	-	+6	-	-1	+3
Consensus Model 1		+4	+8	+6	-9	-1	+3
Model 2: H1f0/H1(zero) (cell cycle independent/replacement)							
Rat|H1|24437|+|7	H1F0	-	-	-	-	-	-
Human|H1|3005|+|22	H1F0	-4	-9	+6	+5	-	-
Mouse|H1|14958|+|15	H1F0	-	-	+6	+5	-	-
Consensus Model 2		-4	-9	+6	+5	-	-
Model 3: H1X (alike cell cycle independent histone genes)							
Human|H1|8971|-|3	H1X	-	-	+6	-5	-	+3
Consensus Model 3		-	-	+6	-5	-	+3
Model 4: H1fo (ovary-specific)							
Human|H1|132243|+|3	H1FO	-	-	+7	-	+1	+3
Mouse|H1|171506|+|6	H1FO	-	-	-7	-	+8	+3
Consensus Model 4		-	-	+7/-7	-	+1/+8	+3
Model 5: H1t (testis-specific)							
Mouse|H1|107970|+|13	H1T	-	-	+6	-9	-1	+3
Rat|H1|24438|+|17	H1T	-	-	+6	-9	-1	+3
Human|H1|3010|-|6	H1T	-	-	+6	-9	-1	+3
Consensus Model 5		-	-	+6	-9	-1	+3
Overall Consensus model for H1 histone group		+4	-	+6	-9	-1	+3

- **Divide H1 seqs into 5 subgroups**
- **Aligned seqs within each subgroup**
- **Consensus alignment matches biologically known H1 subgroup models**

⇒ **Can apply same approach to find promoter models for H2A, H2B, H3, H4...**

Image credit: Rajesh

Acknowledgements

I "borrowed" a lot of materials in this lecture from

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