CS2220 Introduction to Computational Biology

Lecture 7: Gene Finding by Computational Analysis

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Outline

- **Gene structure basics**
- **Gene finding overview**
- **GRAIL**
- **Indel & frame-shift in coding regions**

Gene Structure Basics

A brief refresher

Some slides here are "borrowed" from Ken Sung

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"

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

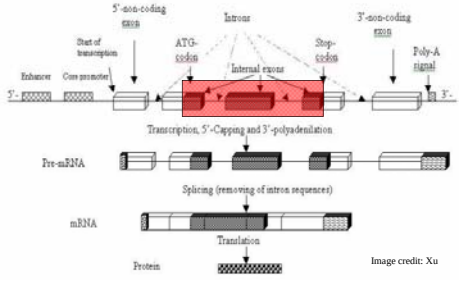

Image credit: Xu

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

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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..

How do I get this reverse strand?

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..

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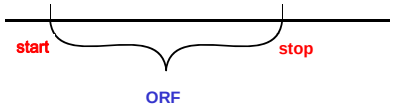
Open Reading Frame (ORF)

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



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

NB: Other definitions are also used. Most impt aspect is that there is no stop codon in the middle.

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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**

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Frame Consistency

- **Neighboring 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

Exercise: Define frame consistency mathematically

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

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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 strategies**

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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

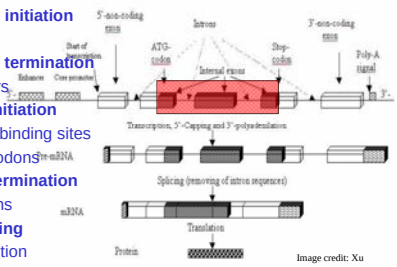

Image credit: Xu

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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, …

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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**

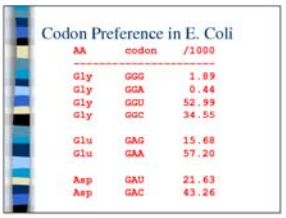

Image credit: Craven

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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**

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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**

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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

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Codon Usage Method

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

Predicted Coding Regions

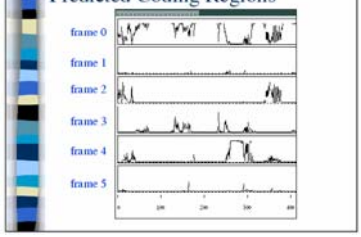

Image credit: Craven

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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

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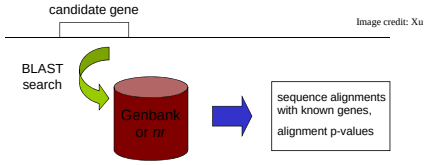
Search-by-Homology Example: Gene Finding Using BLAST

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

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

⇒ **Extract Feature for gene identification**



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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

Image credit: Craven

- **$\Pr(\text{coding}_i)$ 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**

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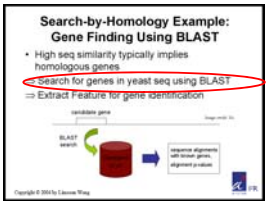
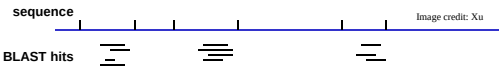


- **Searching all ORFs against known genes in nr db helps identify an initial set of (possibly incomplete) genes**



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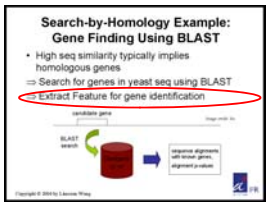

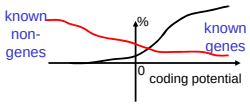

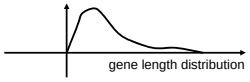

- A (yeast) gene starts w/ ATG and ends w/ a stop codon, in same reading frame of ORF
- Have "strong" coding potentials, measured by, preference models, Markov chain model, ...
- Have "strong" translation start signal, measured by weight matrix model, ...
- Have distributions wrt length, G+C composition,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

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Coding Signal

- Dimer preference implies dicodon (6-mers like AAA TTT) bias in coding vs non-coding regions
- Relative freq of a dicodon 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 dicodon 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?

GRAIL, An Important 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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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

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Coding Signal

- Freq distribution of dimers in protein seq
- 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

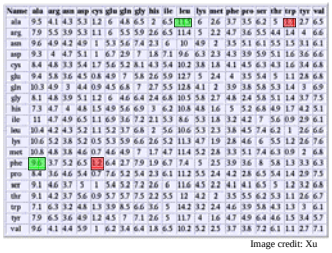

Image credit: Xu

Exercise: What is shewanella?

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Coding Signal

- Most dicodons show bias toward either coding or non-coding regions

⇒ 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

⇒ Dicodon freq are key signal used for coding region detection; all gene finding programs use this info

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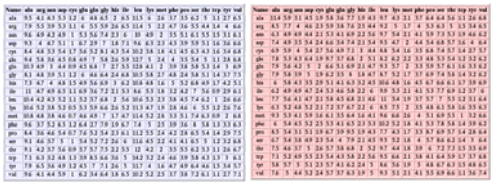
Coding Signal

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

Image credit: Xu



Shewanella Bovine

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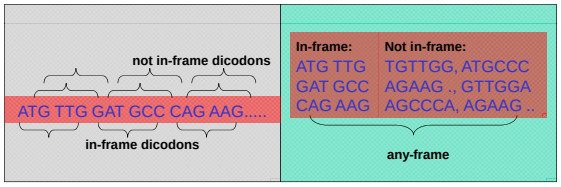
Coding Signal

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



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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

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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**

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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) = –∞,
  treating STOP codons differently

⇒ **A region consisting of only these dicodons is probably a non-coding region**

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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

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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

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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

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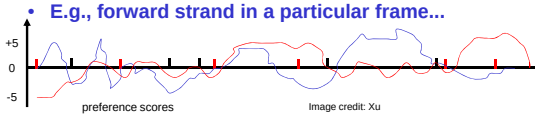
Coding Region Prediction: An Example Procedure

- 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...



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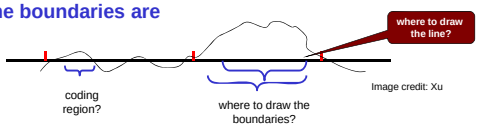
Problem with Coding Region Boundaries

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



⇒ 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

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Types of Coding Region Boundaries

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

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

What do you expect at translation stop?

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Signals for Coding Region Boundaries

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

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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**

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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**

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What Positions Have "High" Info Content?

- **For a weight matrix, information content of each column is calculated as**
  - **$-\Sigma_{X \in \{A,C,G,T\}} F(X)*\log (F(X)/0.25)$**
- **When a column has evenly distributed nucleotides, its information content is lowest**
- **Only need to look at positions having high information content**

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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

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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

Nucleotide distribution around human donor sites

Image credit: Xu

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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		Exercise: Fill in the rest of the table					
G	0/3	2/3	0/3							
T	0/3	0/3	2/3							

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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

Nucleotide distribution around human donor sites

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**

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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**
- **.....**

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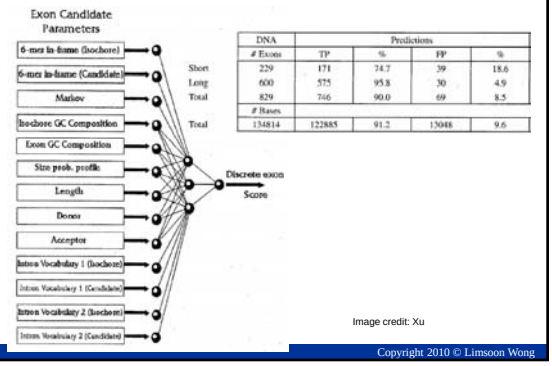
Info Fusion by ANN in GRAIL



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

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Remaining Challenges in GRAIL

- **Initial exon**
- **Final exon**
- **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

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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 .....

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Effects of Indels on Exon Prediction

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

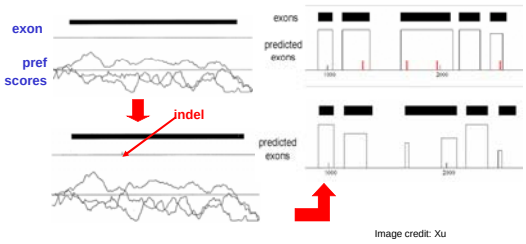

Image credit: Xu

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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**

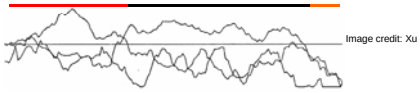

Image credit: Xu

⇒ **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!**

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Frame-Shift Detection by Seq 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

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Frame-Shift Detection: A Simplified Treatment

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

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

- **Then**

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

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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

⇒ ***C(i, r) = max { Case 1, Case 2, Case 3 }***

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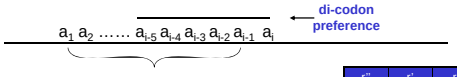
Frame-Shift Detection: Case 1

- **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

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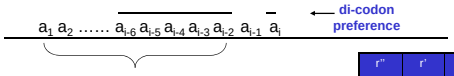
Frame-Shift Detection: Case 2

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

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



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

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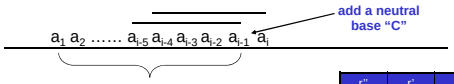
Frame-Shift Detection: Case 3

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

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



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

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

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Frame-Shift Detection: Initiation

- Initial conditions,

$$C(k, r) = -\infty, k < 6$$
$$C(6, r) = P_r(a_1 ... a_6)$$

- 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?

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Frame-Shift Detection: Determining Indel Positions

- 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

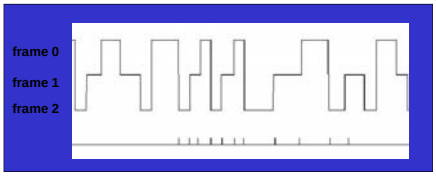

Image credit: Xu

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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

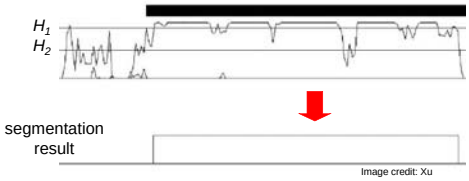

Image credit: Xu

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Frame-Shift Detection: Finally…

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

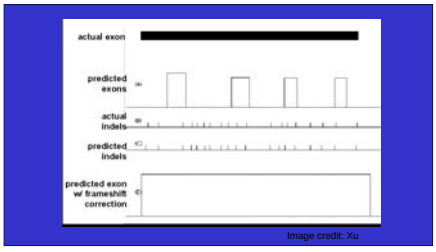

Image credit: Xu

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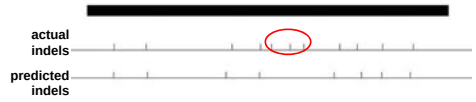
What Happens When Indels Are Close Together?

- **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...**



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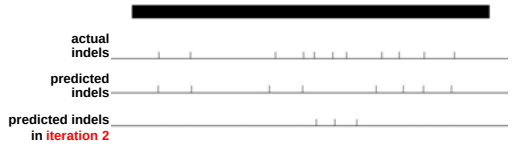
Handling Indels That Are Close Together

- **Employ an iterative process, viz**
  - Find one set of indels
  - Correct them
  - Iterate until no more indels can be found



Any Question?

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