CS2220 Introduction to Computational Biology

# Lecture 8: Gene Finding by Computational Analysis

**Limsoon Wong**

**16 March 2007**

NUS
National University
of Singapore

# 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

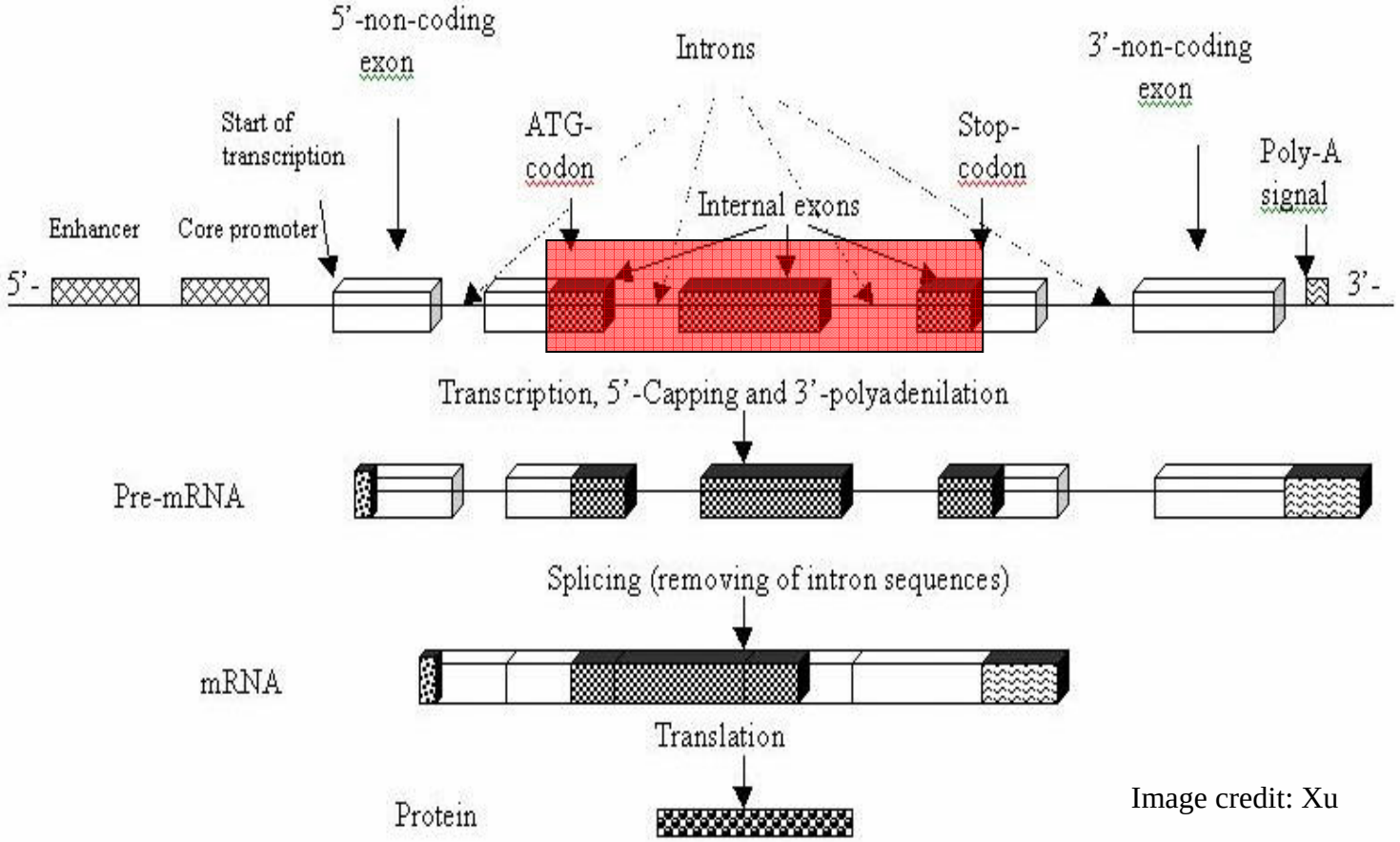



Image credit: Xu

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

# Reading Frame

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

Forward strand: 

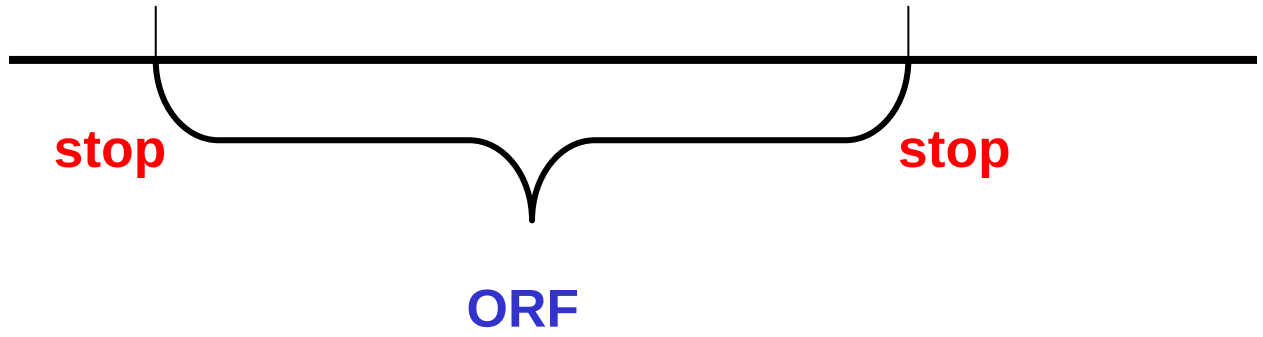

| Reading frame #1 | Reading frame #2 | Reading frame #3 |
|---|---|---|
| ATG | TGG | GGC |
| GCT | CTT | TTA |
| TAC | ACG | CGC |
| GCT | CTT | TTG |
| TGC | GA. | A.. |

Reverse strand: 


| 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**

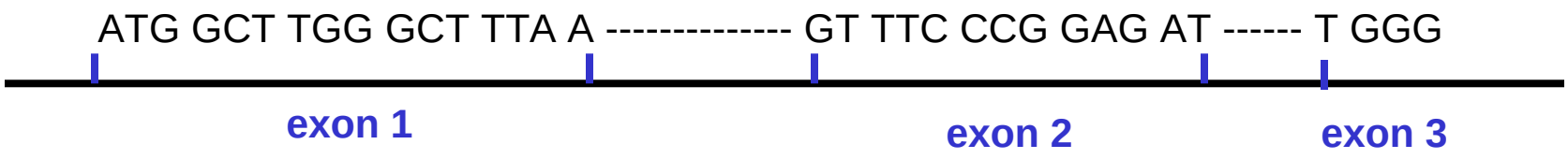
NB: Other definitions are also used. Most impt aspect is that there is no stop codon in the middle.

# 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

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




Exercise: Define frame consistency mathematically

# Overview of Gene Finding

**Some slides here are “borrowed” from Mark Craven**

# 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 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

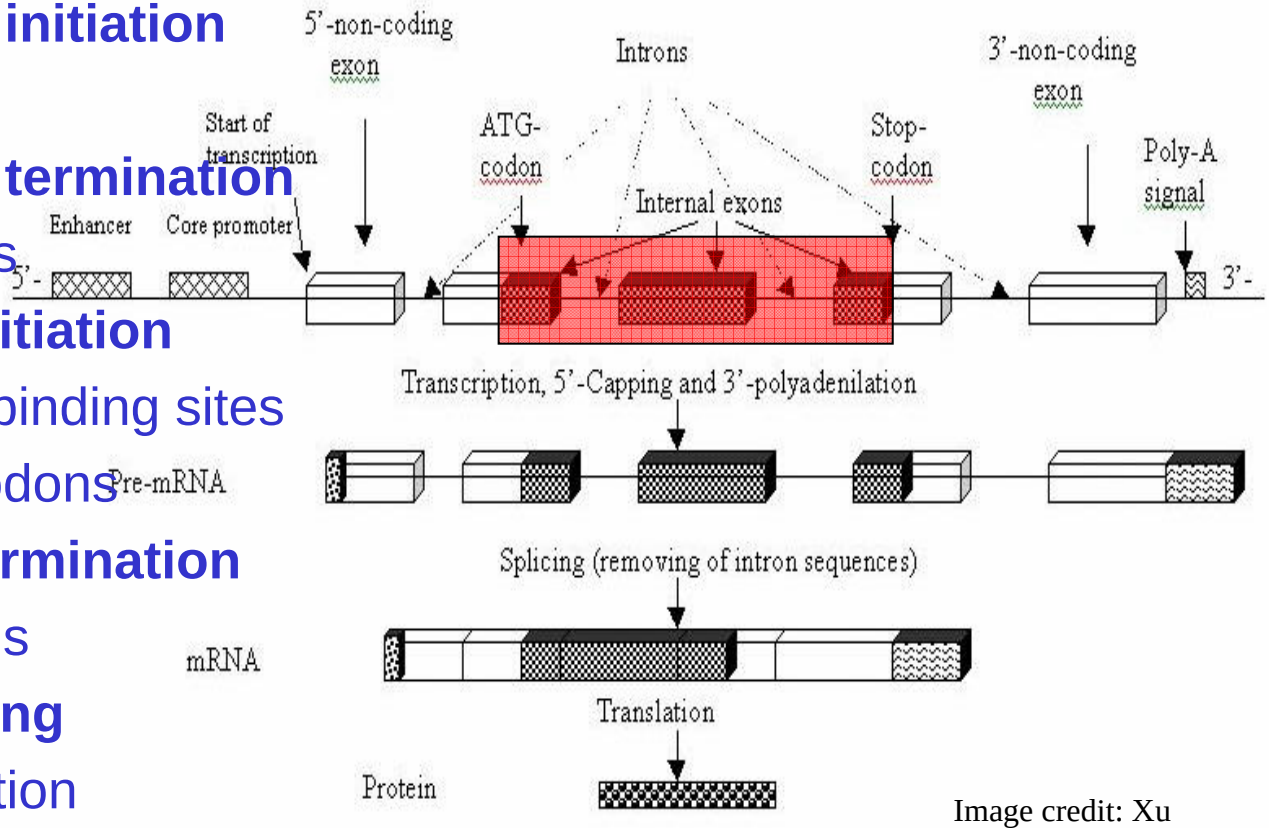



Image credit: Xu

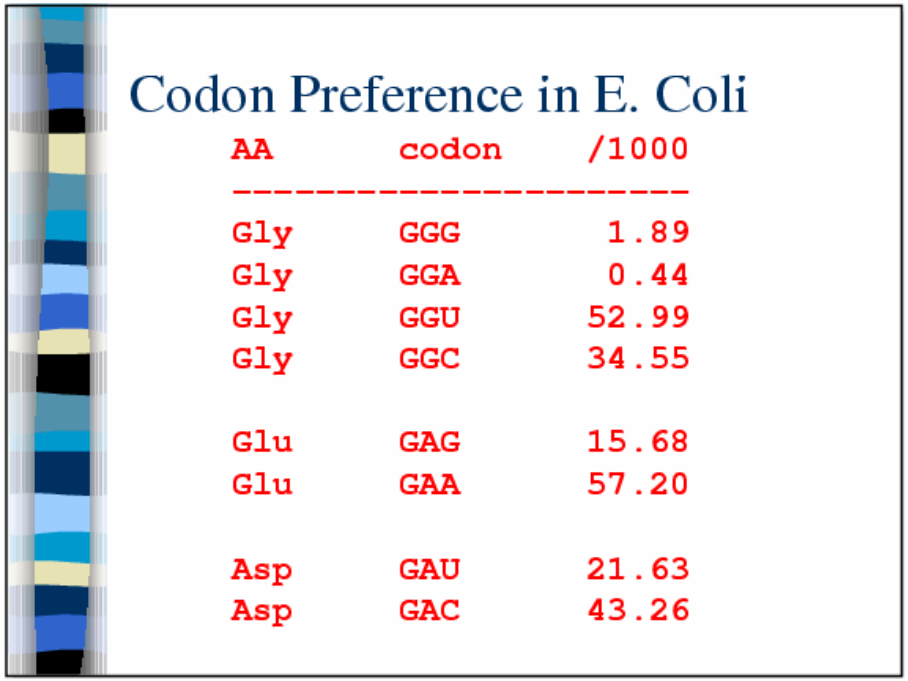
# How Search-by-Signal Works

- **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

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

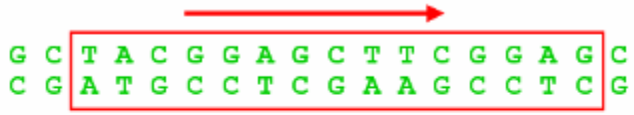
probability that sequence encodes a protein in frame i

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**

## Codon Usage Method

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




## Predicted Coding Regions

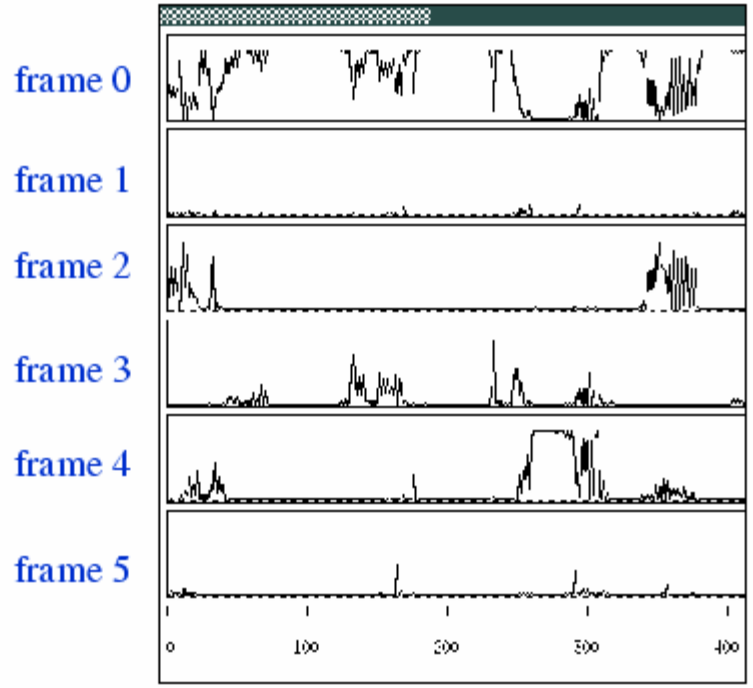


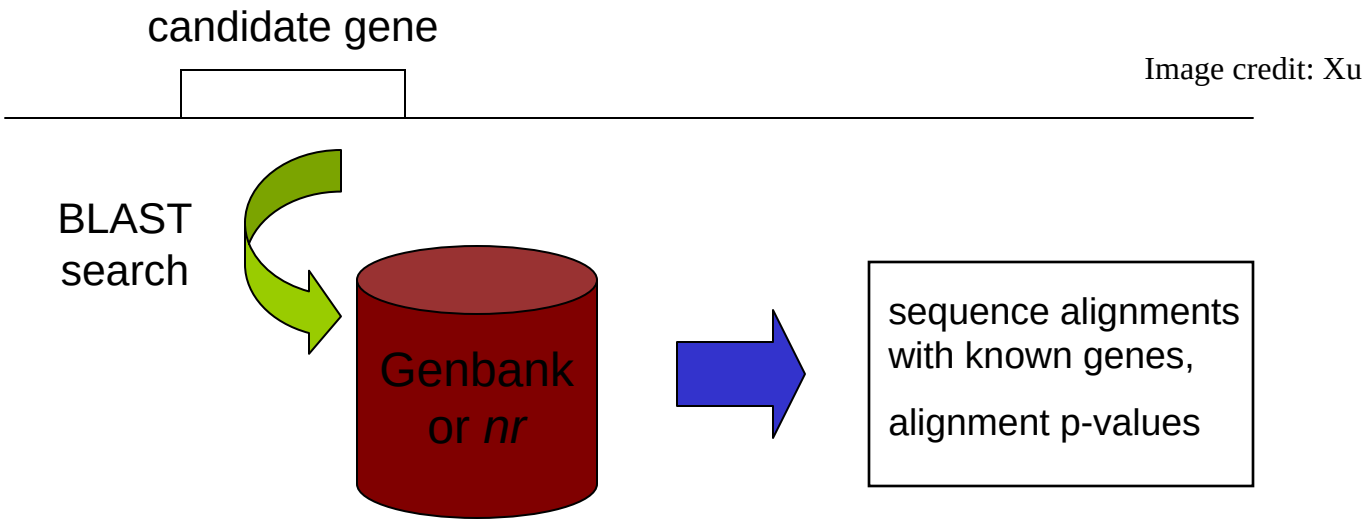

Image credit: Craven

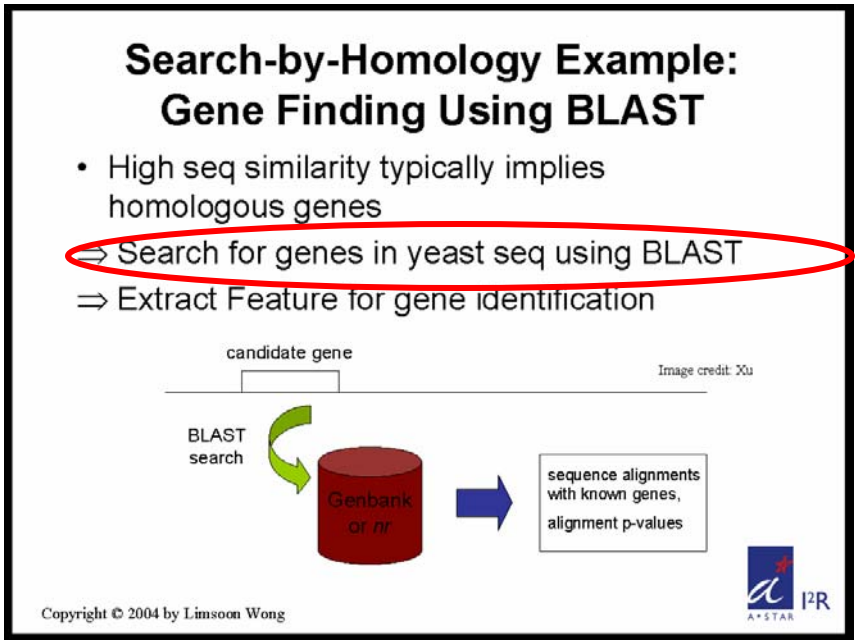
# Search-by-Homology Example: Gene Finding Using BLAST

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

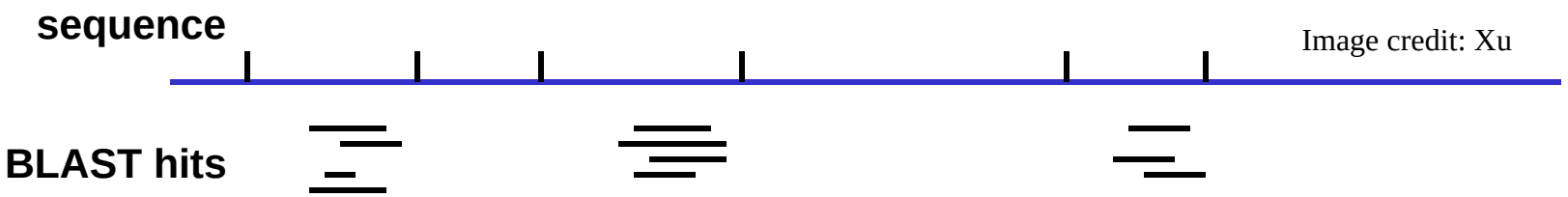
⇒ **Search for genes in yeast seq using BLAST**

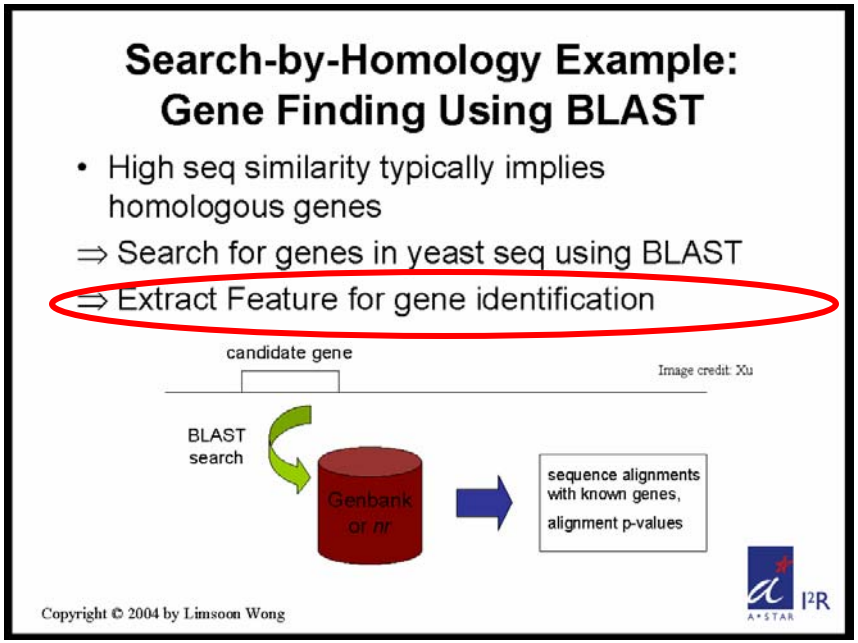
⇒ **Extract Feature for gene identification**

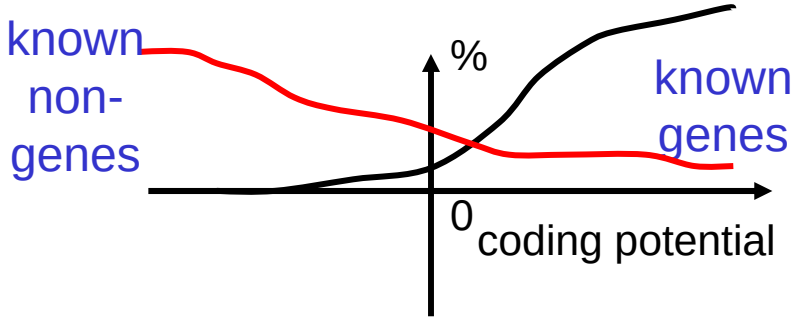


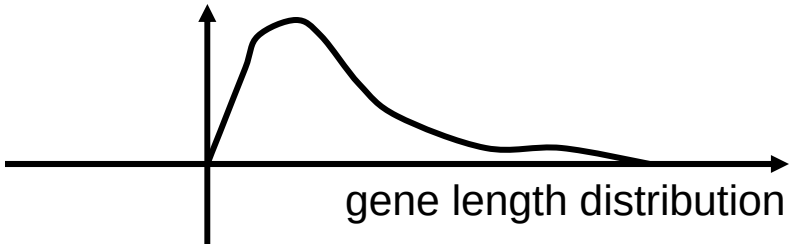



- **Searching all ORFs against known genes in nr db helps identify an initial set of (possibly incomplete) genes**






- **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,
# 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**

# 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

| 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 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?

# 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**

$\Rightarrow$ **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 toward 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 |

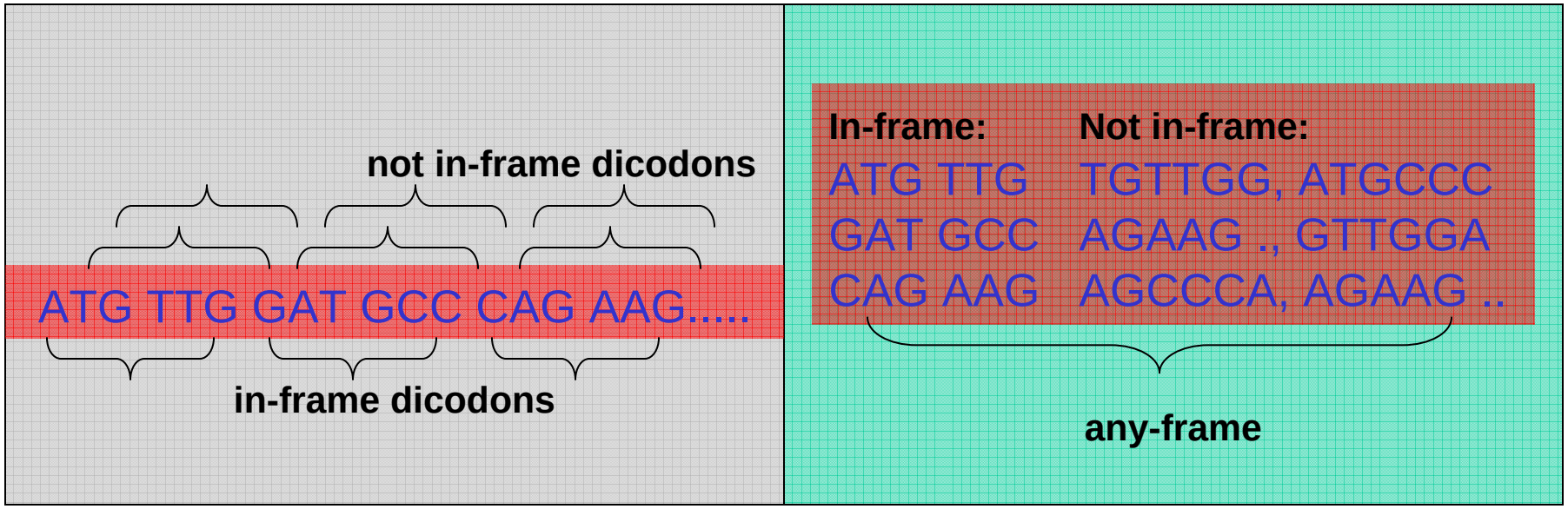
Shewanella

| 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

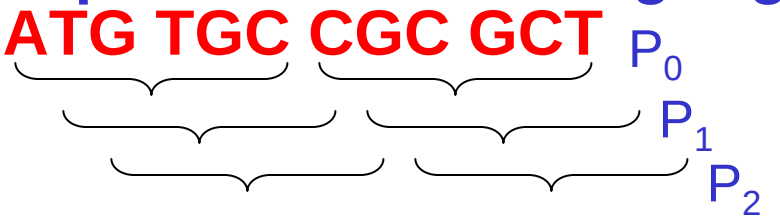
- **The in-frame + i preference value $P_i(X)$ of a dicodon X is defined as**

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

**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**

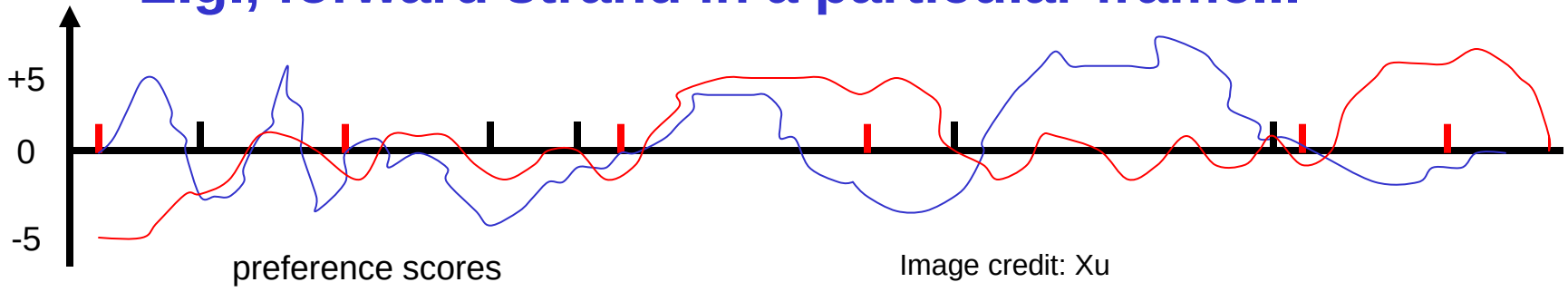
$$S_i(R) = \Sigma_{X \text{ is a dicodon at in-frame + i position in R}} P_i(X)$$

- **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...**




preference scores

Image credit: Xu

# Problem with Coding Region Boundaries

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

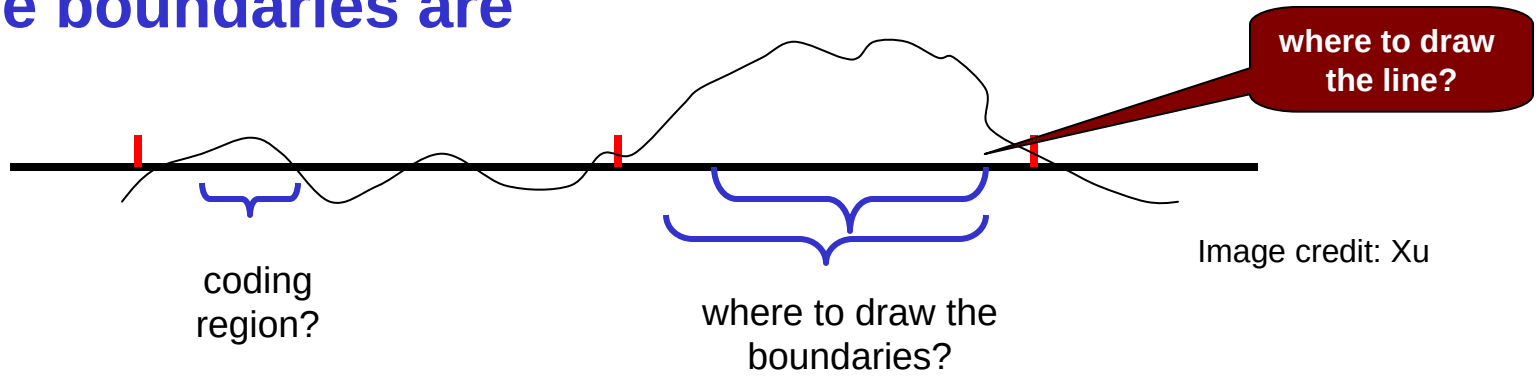



Image credit: Xu

⇒ **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" 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**

# 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 |

Nucleotide distribution around human donor sites

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

# 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

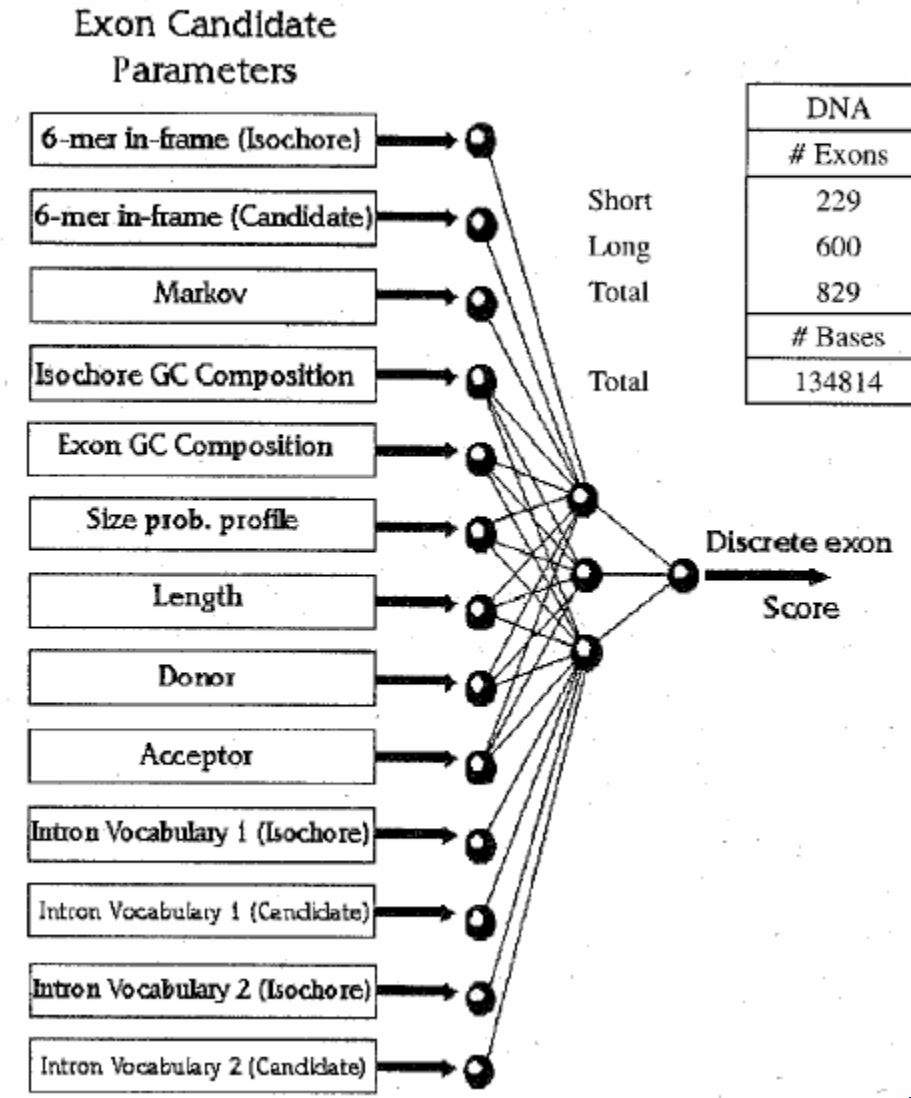
TGTGTCTCA: .11 + .12 + .03 +1.0 + 1.0 + .02 + .07 + .05 + .16 = 2.56

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

# 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**
- **Final exon**
- **Indels & frame shifts**

# Indel & Frame-Shift in Coding Regions

**Problem definition**

**Indel & frameshift identification**

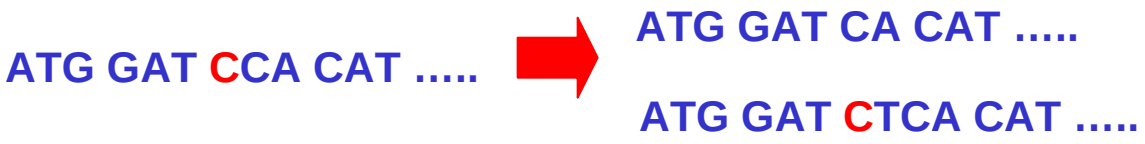
**Indel correction**

**An iterative strategy**

**Some slides here are "borrowed" from Ying Xu**

# Indels in Coding Regions

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



# Effects of Indels on Exon Prediction

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

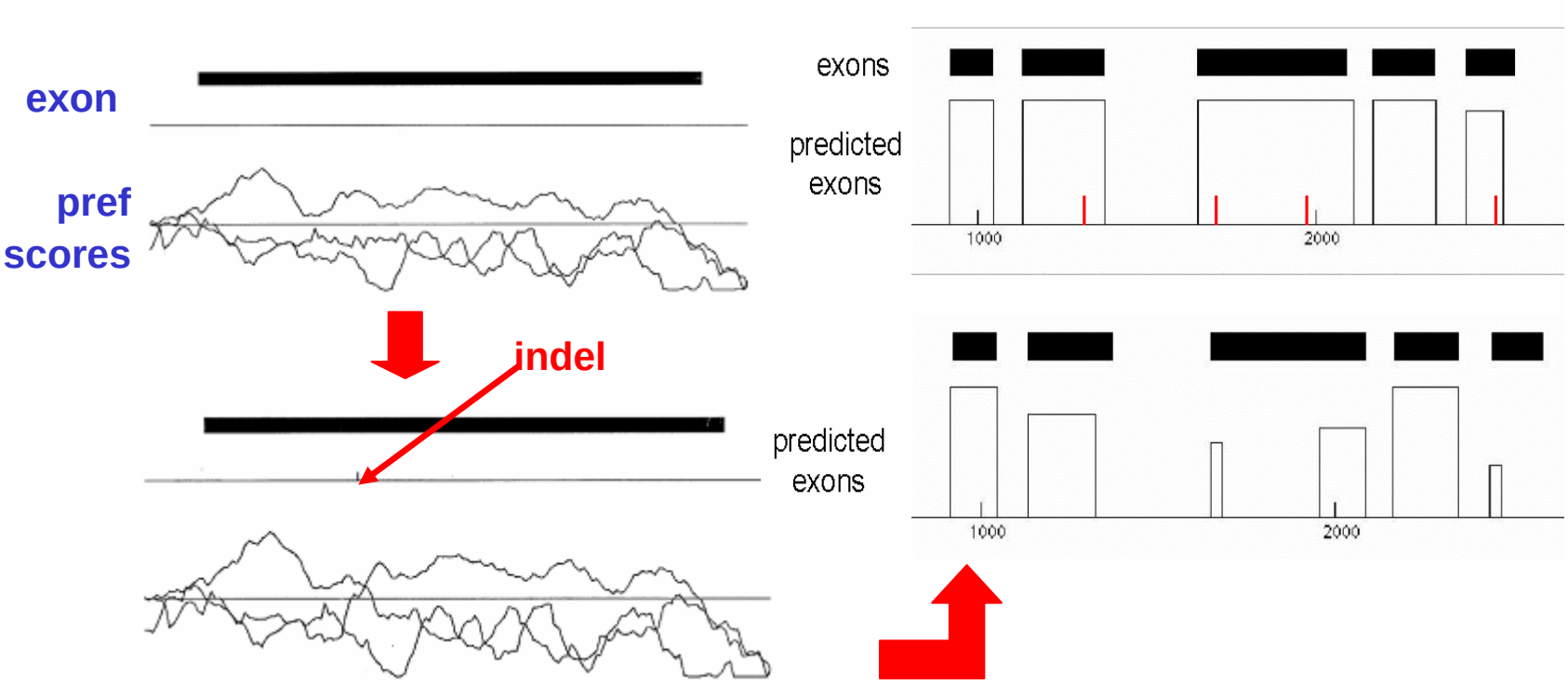



Image credit: Xu

# 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**

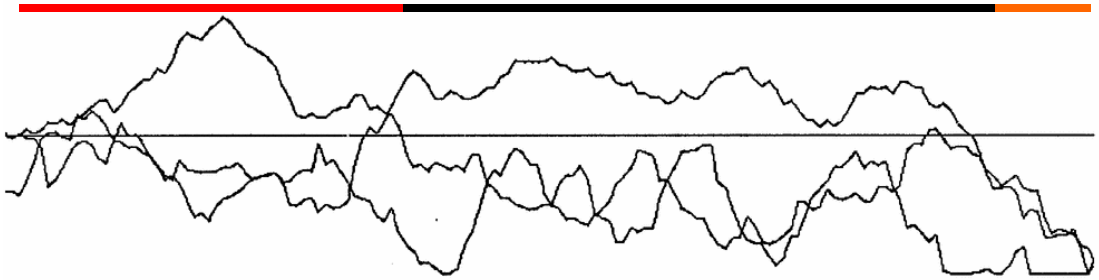


Image credit: Xu

⇒ **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 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

# 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)*

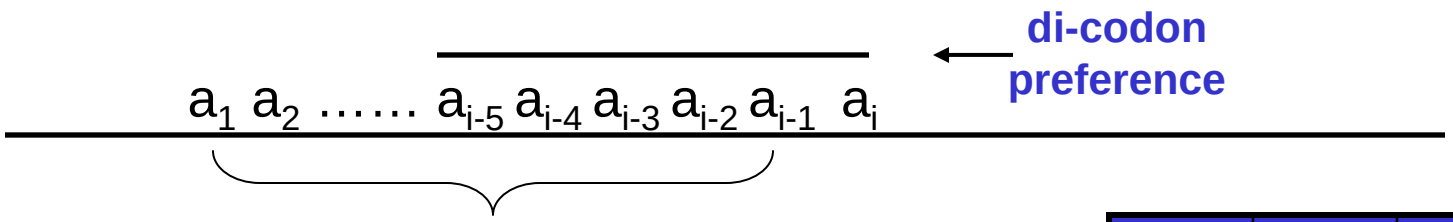
- **To calculate *C(i,r)*, there are 3 possible cases for each position *i*:**
  - Case 1: no indel occurred at position *i*
  - Case 2: $a_i$ is an inserted base
  - Case 3: a base has been deleted in front of $a_i$

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

# Frame-Shift Detection: Case 1

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

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

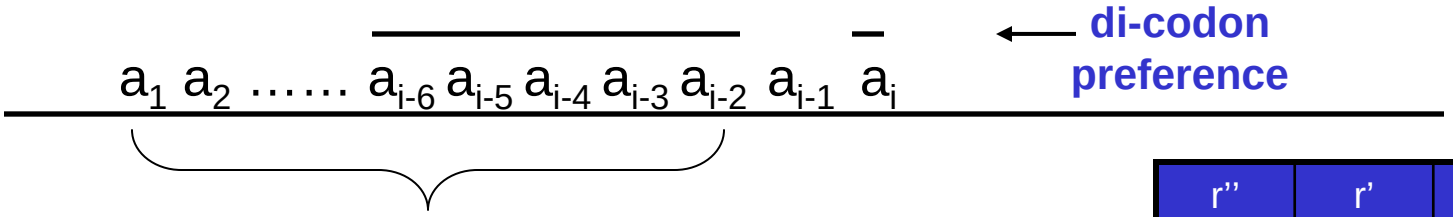



| r'' | r' | r |
|---|---|---|
| 1 | 2 | 0 |
| 2 | 0 | 1 |
| 0 | 1 | 2 |

# Frame-Shift Detection: Case 2

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

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

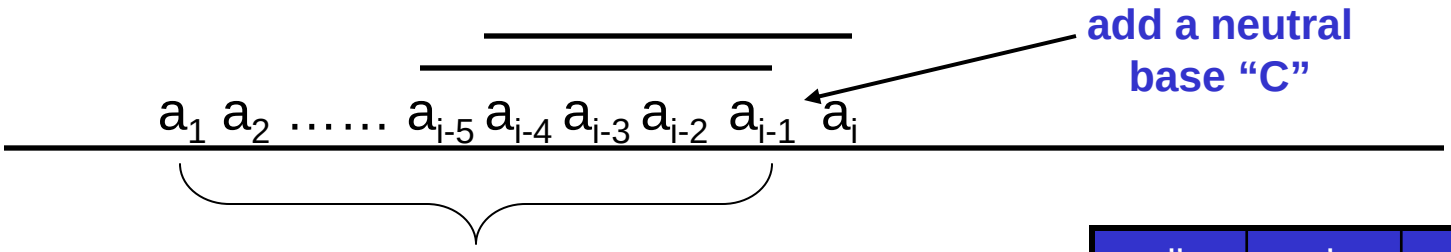



| r'' | r' | r |
|---|---|---|
| 1 | 2 | 0 |
| 2 | 0 | 1 |
| 0 | 1 | 2 |

# 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: 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**

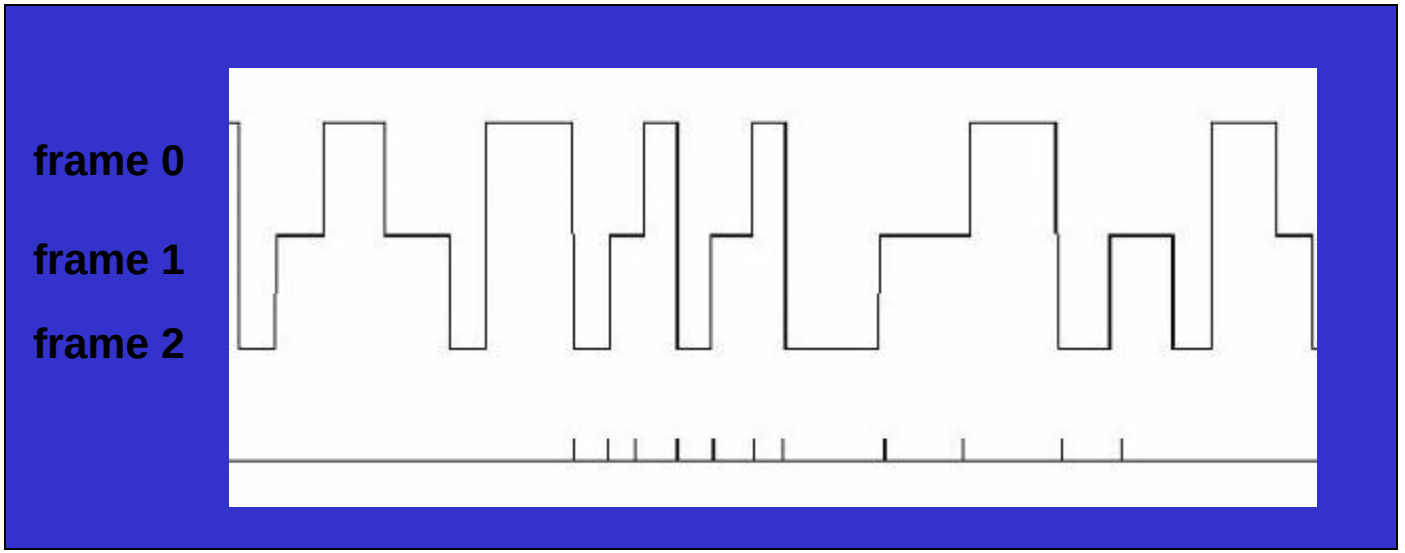



Image credit: Xu

# 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**

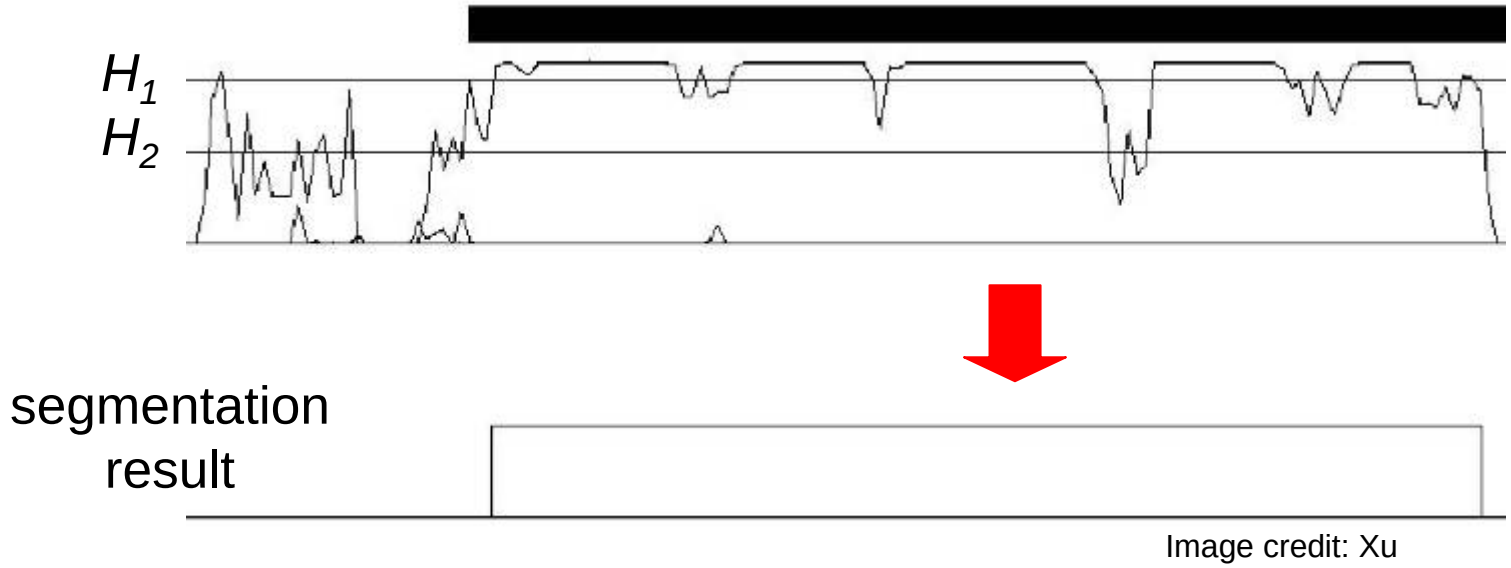



Image credit: Xu

# Frame-Shift Detection: Finally…

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

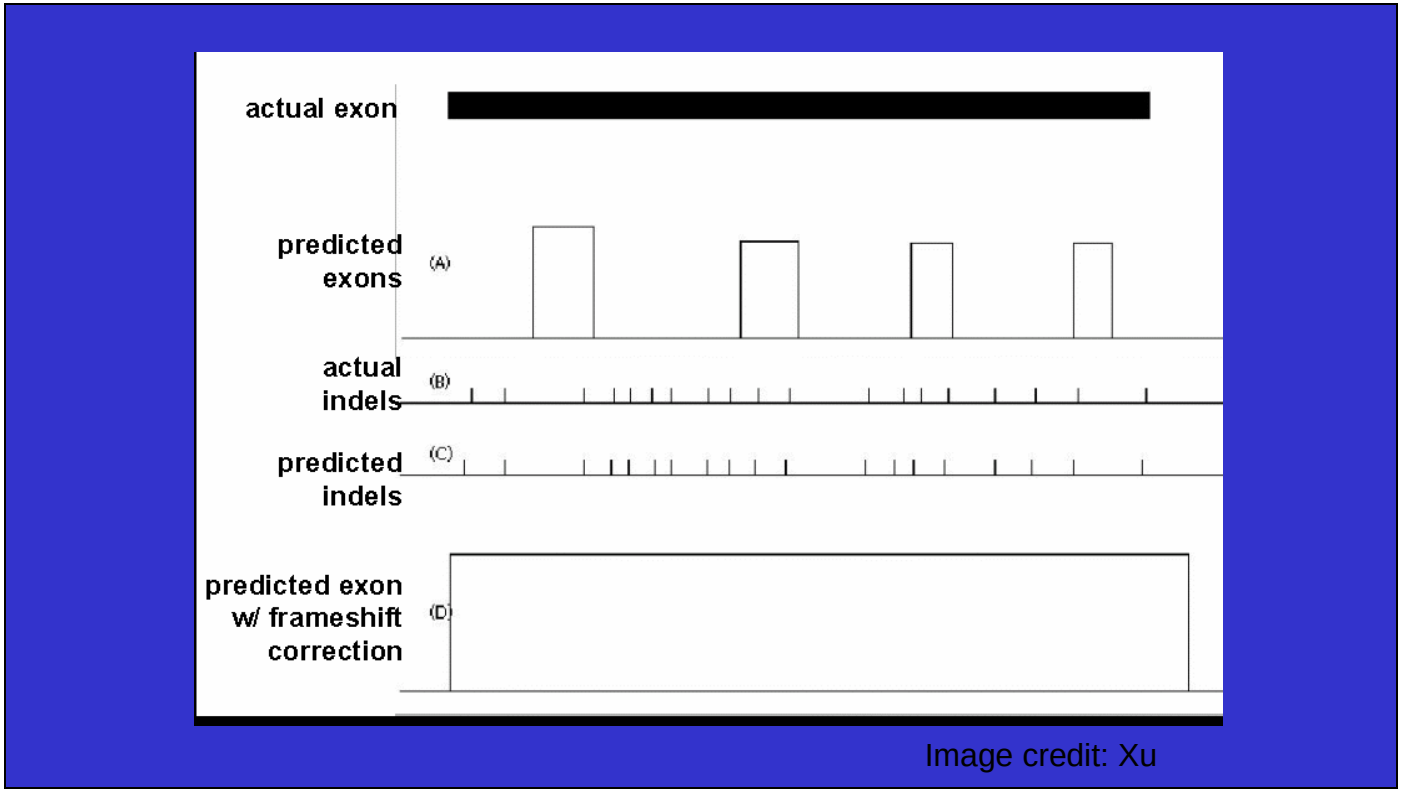
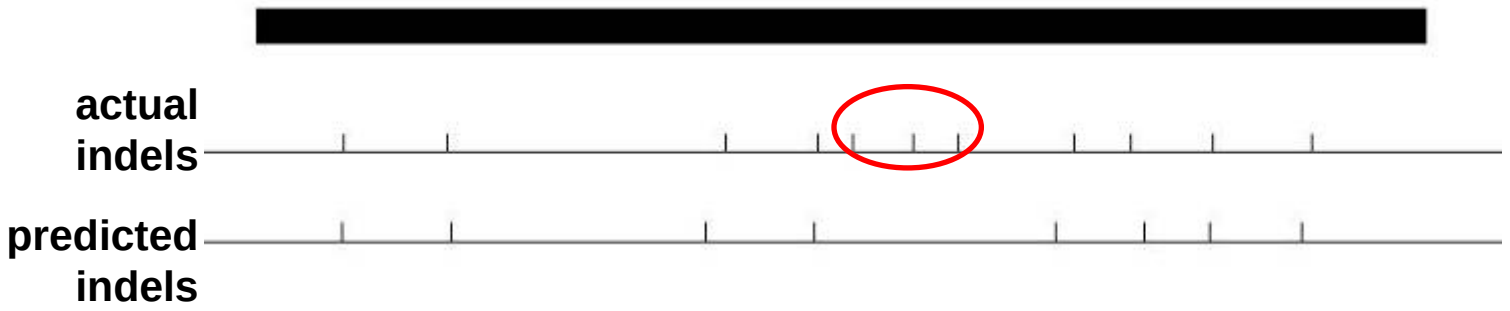



Image credit: Xu

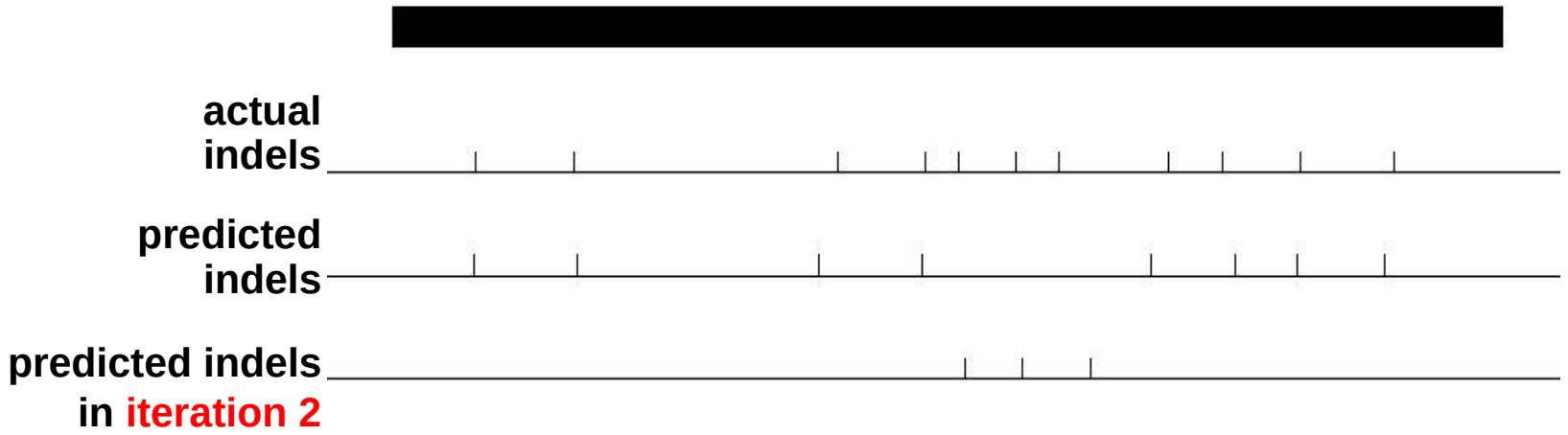
# What Happens When Indels Are Close Together?

- **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
  - Correct them
  - Iterate until no more indels can be found



Any Question?

# Acknowledgements


- **I "borrowed" a lot of materials in this lecture from Xu Ying (Univ of Georgia) and Mark Craven (Univ of Wisconsin)**

# References


- Y. Xu et al. “GRAIL: A Multi-agent neural network system for gene identification”, Proc. IEEE, 84:1544--1552, 1996
- R. Staden & A. McLachlan, “Codon preference and its use in identifying protein coding regions in long DNA sequences”, NAR, 10:141--156, 1982
- Y. Xu, et al., "Correcting Sequencing Errors in DNA Coding Regions Using Dynamic Programming", Bioinformatics, 11:117--124, 1995
- Y. Xu,  et al., "An Iterative Algorithm for Correcting DNA Sequencing Errors in Coding Regions", JCB, 3:333--344, 1996
- D. J. States, W. Gish, “Combined use of sequence similarity and codon bias for coding region identification”, JCB, 1:39--50, 1994

# References


- C. Burge & S. Karlin. “Prediction of Complete Gene Structures in Human Genomic DNA”, JMB, 268:78--94, 1997
- V. Solovyev et al. "Predicting internal exons by oligonucleotide composition and discriminant analysis of spliceable open reading frames", NAR, 22:5156--5163, 1994
- V. Solovyev & A. Salamov. “The Gene-Finder computer tools for analysis of human and model organisms genome sequences", ISMB, 5:294--302, 1997