Gene Prediction in Eukaryotes

Talk *Gene Prediction* on Feb. 22th, 2012

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Gene Finding Problem

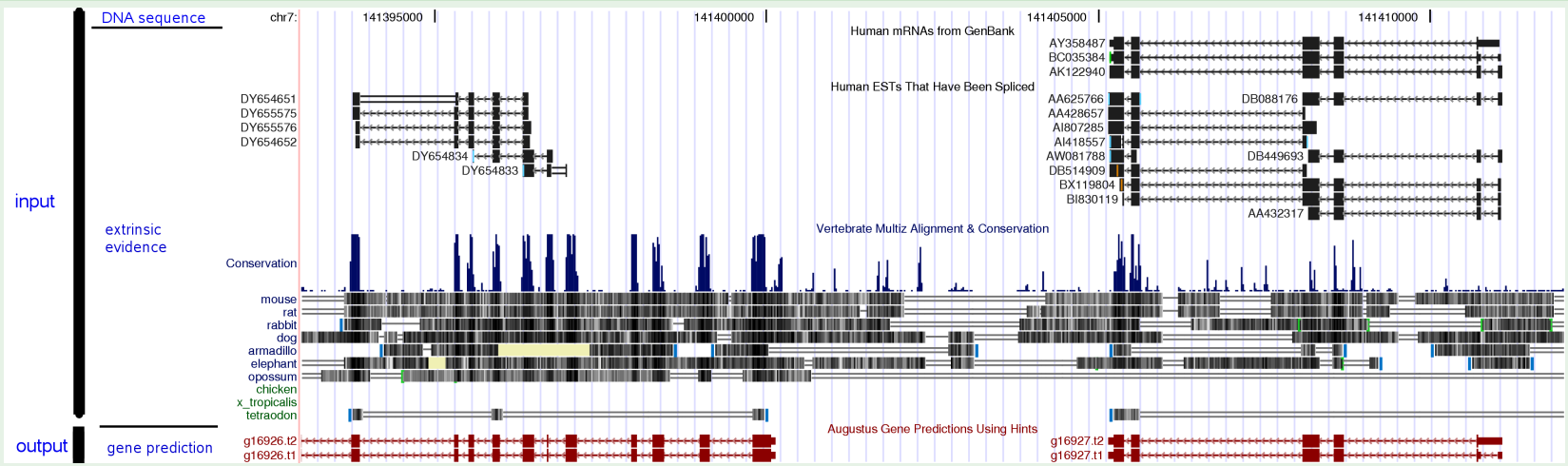
Input *y*

- target DNA sequence, e.g. a chromosome
- optional: extrinsic evidence, e.g. from RNA-Seq

Output *x*

- start- and end positions of genes, coding regions, exons and introns
- predicted amino acid sequences

Example (19000 bp of human chromosome 7)



Major Approaches to Gene Prediction

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approach	extrinsic evidence used
ab initio	-

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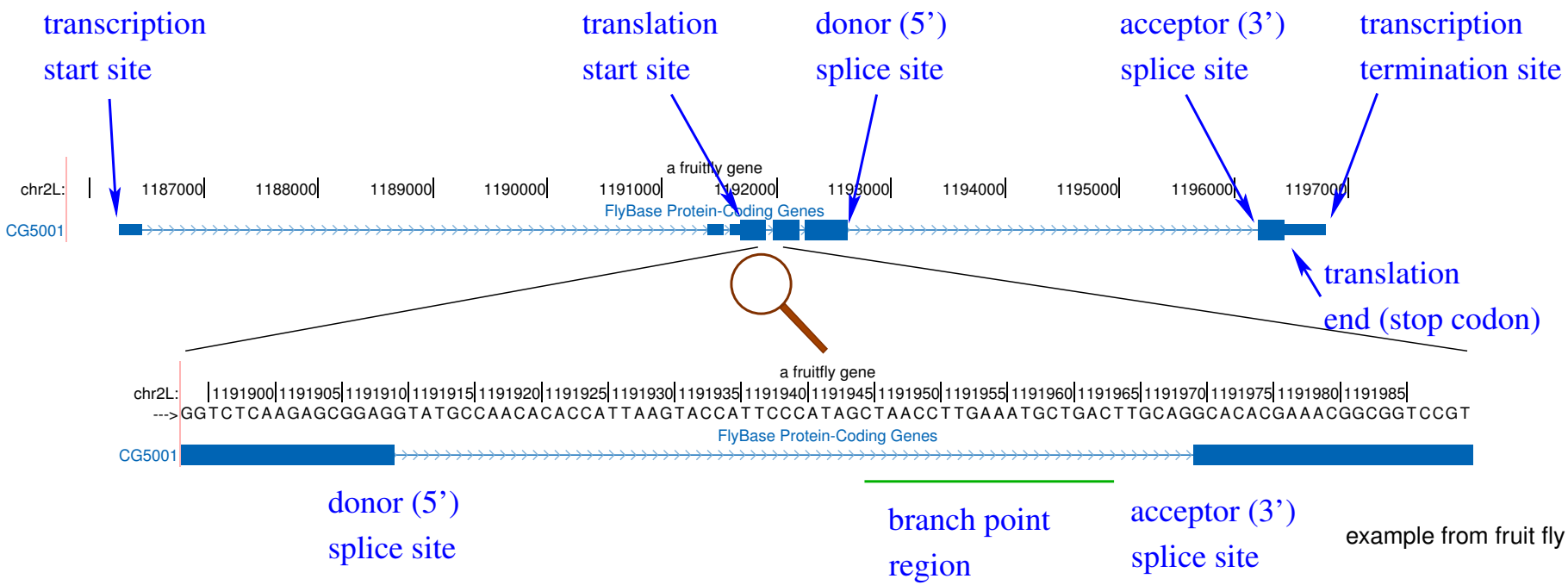
The annotation of most genomes requires a combination of approaches:

Use for every part of a gene all evidence available for that gene or region.

Signals


transcription
start site
translation
start site
donor (5')
splice site
acceptor (3')
splice site
transcription
termination site
a fruitfly gene
chr2L:
1187000
1188000
1189000
1190000
1191000
1192000
1193000
1194000
1195000
1196000
1197000
FlyBase Protein-Coding Genes
CG5001
translation
end (stop codon)
a fruitfly gene
chr2L:
1191900
1191905
1191910
1191915
1191920
1191925
1191930
1191935
1191940
1191945
1191950
1191955
1191960
1191965
1191970
1191975
1191980
1191985
--->GGTCTCAAGAGCGGAGGTATGCCAACACACCATTAAGTACCATTCCCATAGCTAACCTTGAAATGCTGACTTGCAGGCACACGAAACGGCGGTCCGT
FlyBase Protein-Coding Genes
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splice site
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example from fruit fly

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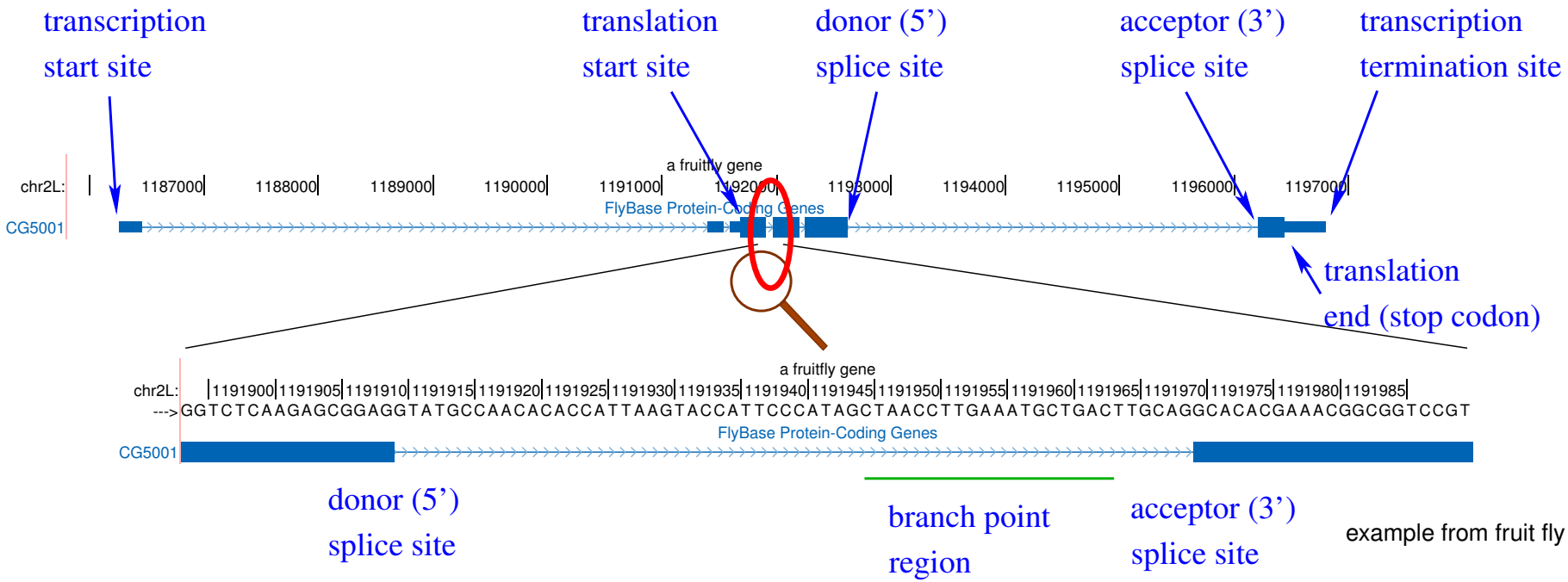

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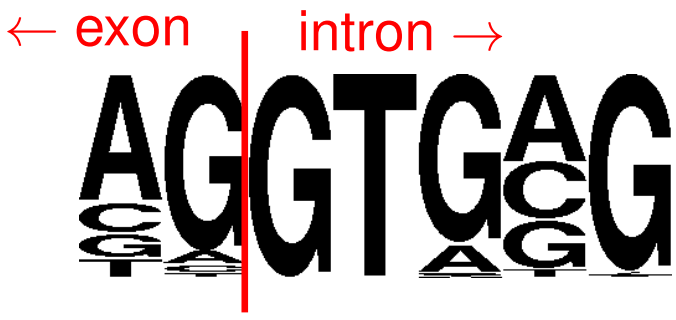
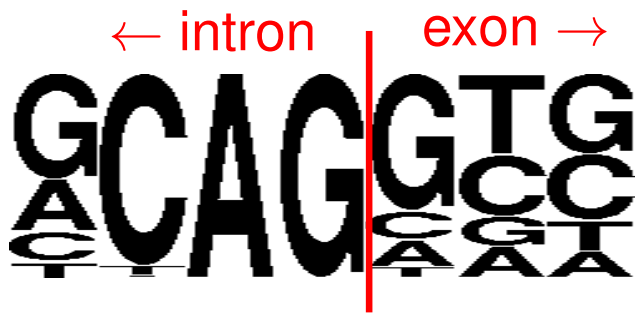


donor splice site (DSS) signal

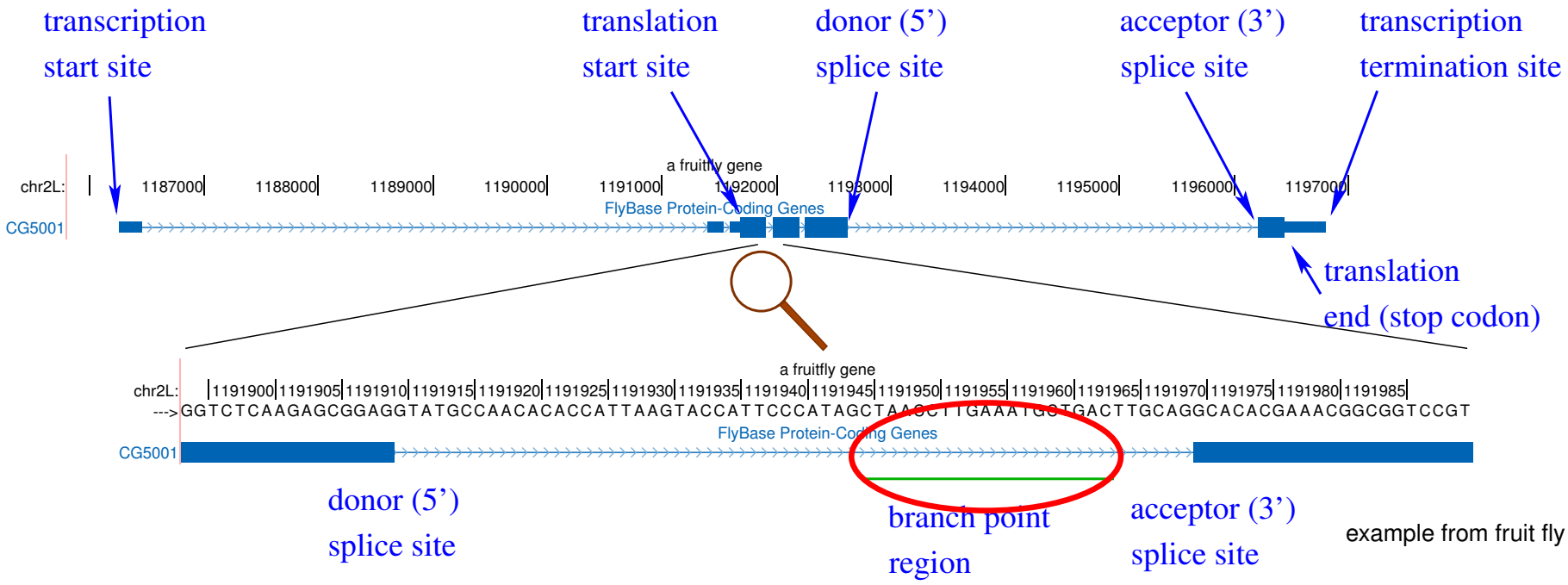


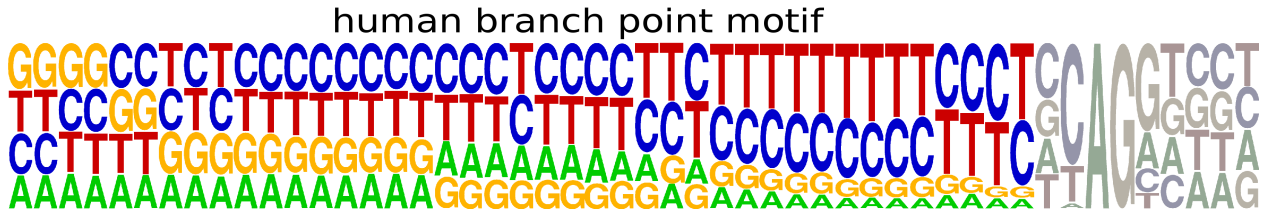
acceptor splice site (ASS) signal

Frequency of the nucleotides at positions relative to splice site.

from green algae *Chlamydomonas*

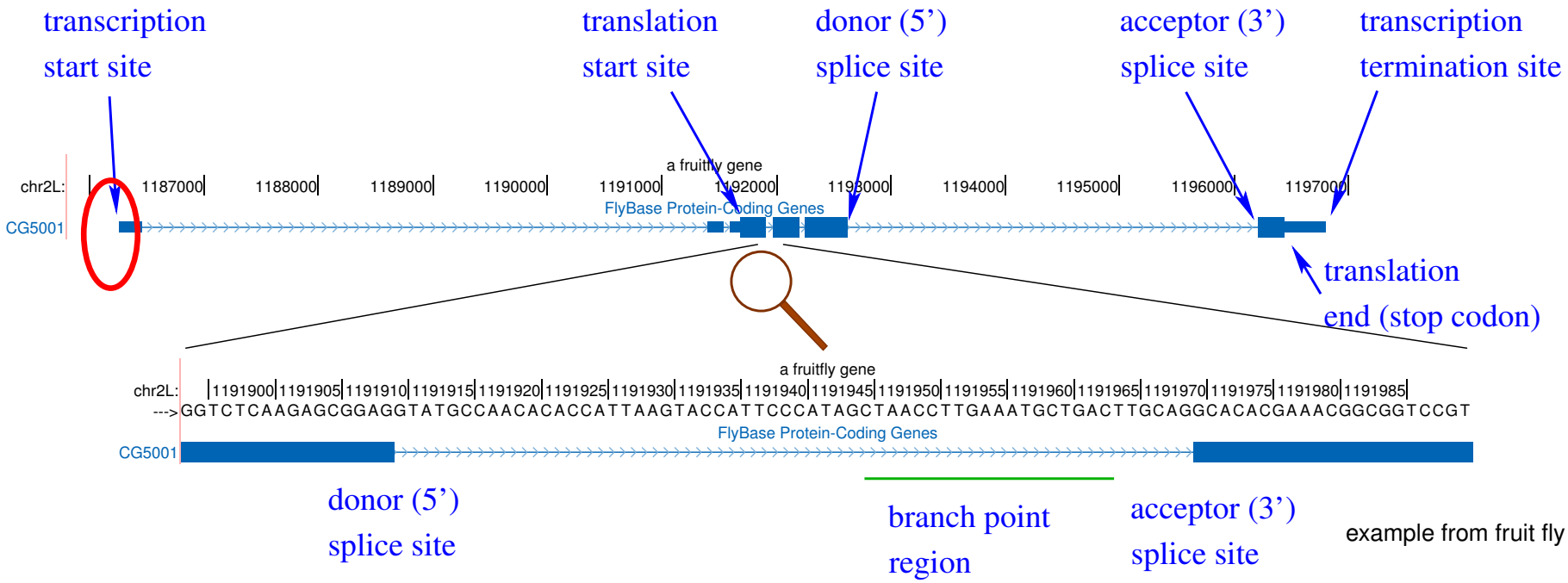
Signals





Branch point: upstream of 3' splice site, a single conserved adenine at variable distance to 3' splice site ($\approx$ -30), a splicing complex binds to it, pyrimidine (C,T) rich in human

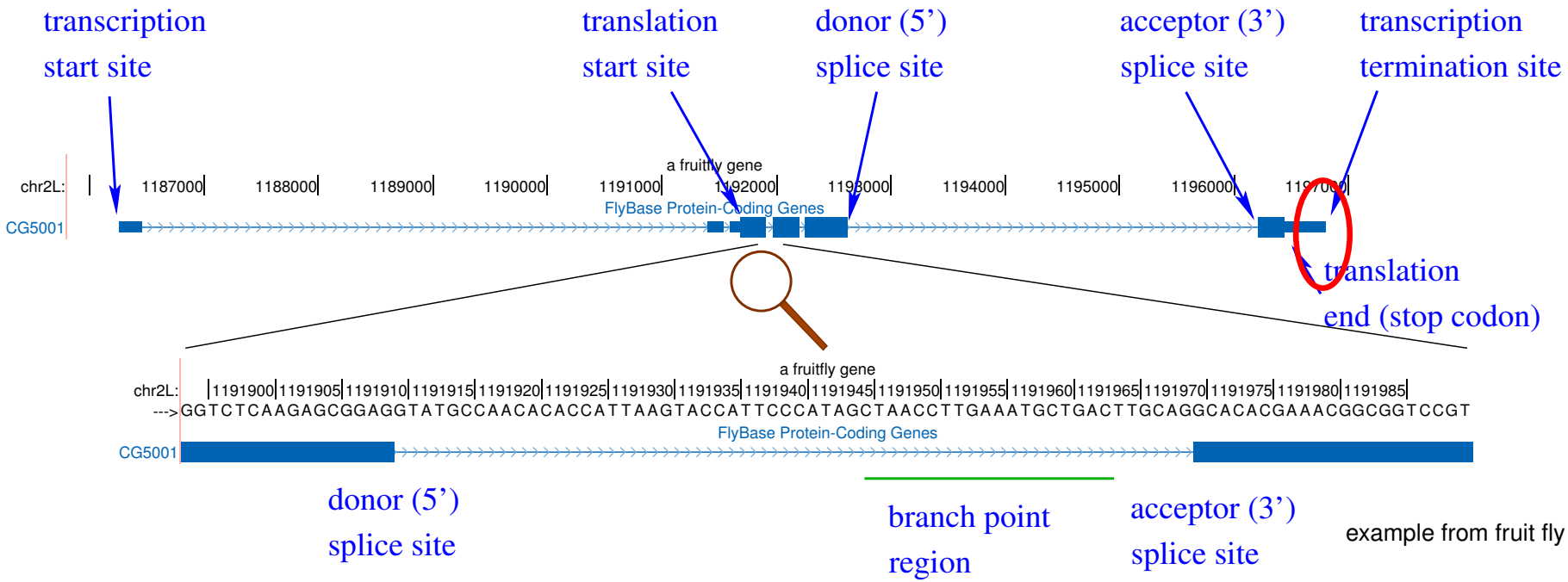
Signals



Transcription start site: Transcription from DNA to RNA by RNA polymerase starts here facilitated by promoter elements.
Promoter elements are diverse and their profiles tend to contain little info:

- diverse transcription factor binding sites at very variable positions
- sometimes TATA-box
- "CpG islands"

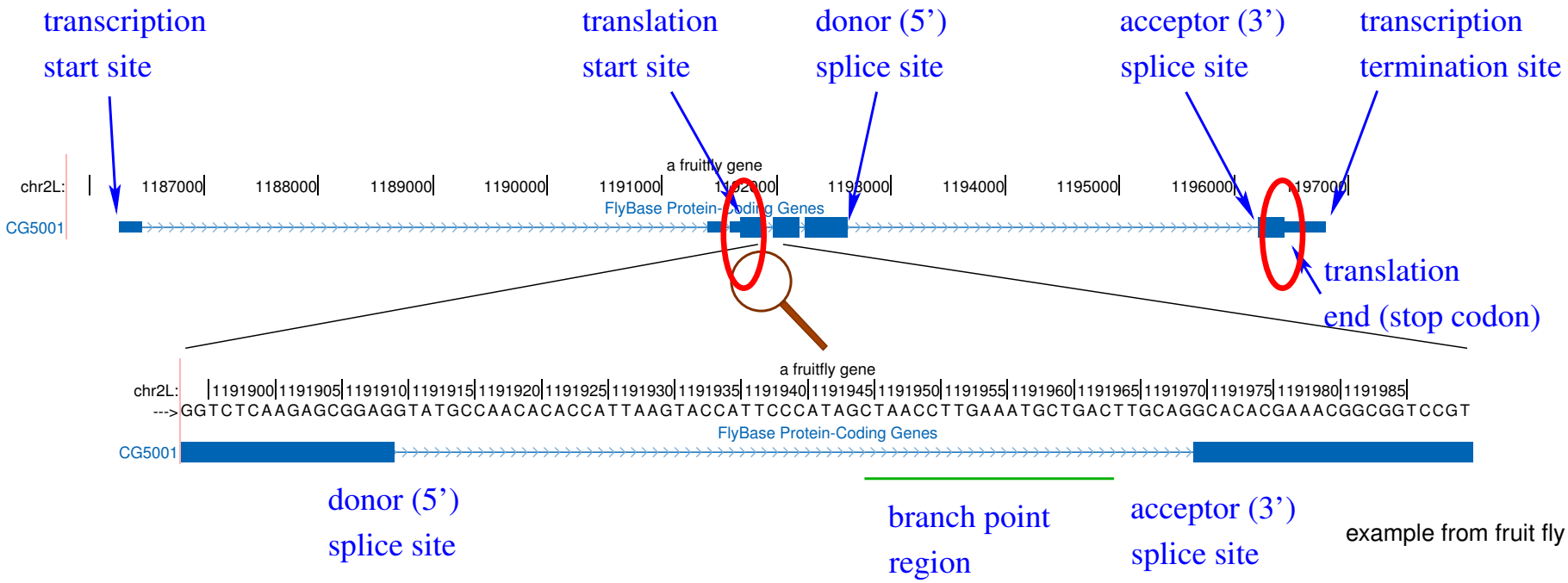
Signals



Transcription termination site (TTS):

- cleavage of the transcript.
- some non-templated A's are appended (polyadenylation).
- polyadenylation is triggered in many species in many genes by the hexamer aataaa roughly 15 bp upstream of the TTS.

Signals



Start and stop codon:

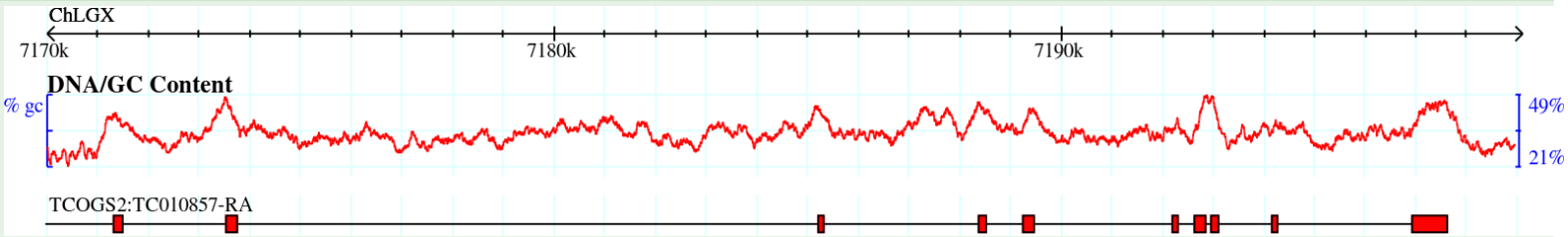
- start codon: ATG
- stop codons: TAA, TAG, TGA
  In some species the genetic code is altered and a "stop codon" is actually coding for an amino acid.

Nucleotide Composition of Coding and Noncoding Regions

Sequence Content

Besides the signals, position-unspecific frequencies of nucleotide patterns can be used to guess biological classification (e.g. CDS, non-coding, CpG-island) of longer sequence intervals.

Example (GC content in red flour beetle)



Typically, higher order patterns are examined:
E.g. reading-frame dependent k-mer frequencies ($k = 5, 6$) for protein-coding regions.

Remark

Sequence content is usually only indirect evidence.

Signal and Content Models

Scores

- usually, we cannot decide with certainty whether something is an exon, splice site, etc
- $\Rightarrow$ therefore assign a number (score s or probability) to exon candidates, splice site candidates
- aim:
  - the larger the score the more likely there is a true signal
  - the score is small for false signals

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Signal and Content Models

- Signal Model: to score whether a biological signal (e.g. splice site) is present at a certain position
- Content Model: to score whether a sequence region belongs to a given class (e.g. coding)

Example Signal Score

Example (DSS position weight matrix)

Have position specific frequency matrix for DSS

$$m(i, b) \quad (i = 1, 2, \ldots, 7, b \in \text{A,C,G,T}),$$

$$m(i, \text{A}) + m(i, \text{C}) + m(i, \text{G}) + m(i, \text{T}) = 1$$

Have "background" distribution of nucleotides $q(b)$

$$q(\text{A}) + q(\text{C}) + q(\text{G}) + q(\text{T}) = 1.$$

Define log-odds score: $s = \log \prod_{i=1}^{7} m(i, w_i)/q(w_i)$.

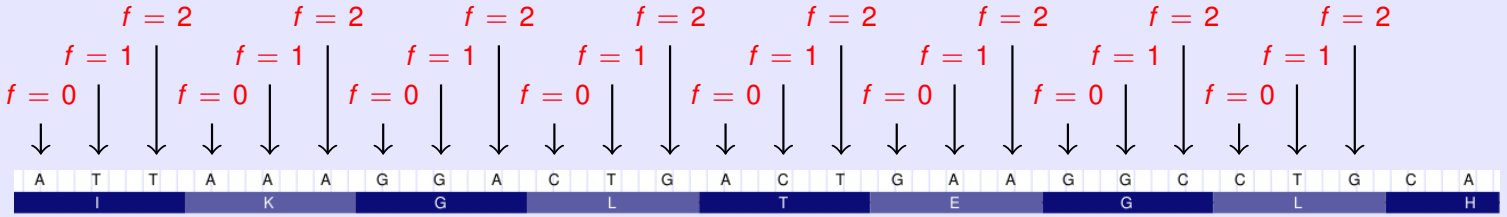
Decision rule

$$\text{predict splice site} :\Leftrightarrow s \geq \text{threshold}$$

guaranteed to have most true predictions given a limit on false positive rate under certain assumptions (Neyman-Pearson).

Example Content Score

Base composition is frame-dependent



nucleotide frequencies in human:

	coding sequence				noncoding
	$f=0$	$f=1$	$f=2$	all f	sequence
A	0.248	0.291	0.146	0.229	0.26
C	0.264	0.243	0.351	0.286	0.24
G	0.321	0.201	0.312	0.278	0.24
T	0.166	0.265	0.190	0.207	0.26

Example Content Score

Example (frame-dependent Markov chain of order k)

Let w be the DNA word of length n to be scored as CDS.
Let $f \in \{0,1,2\}$ be the frame of the first position of w.

$$P(w) := p_f(w_1,\ldots,w_k) \cdot \prod_{i=k+1}^{n} p_{f(i)}(w_i \mid w_{i-k},\ldots,w_{i-1})$$

Here:
- p_f is a start probability for the first k bases
- $f(i) \in \{0,1,2\}$ such that $f(i) \equiv f - 1 + i \mod 3$ is the frame of the i-th position of w

Define $s(w) = \log(P(w)/Q(w))$,
where $Q(w)$ is the probability of w in a "background" model (e.g. non-coding).

Remark:
Division by background $\Rightarrow$ good exon candidates get positive score

Example Content Score - Continued

Example

$w = \text{ATTCTGC}$
frame $f = 2$, i.e. with these codon breaks: A||TTC||TGC
$k = 2$

$$\begin{aligned} P(\text{ATTCTGC}) &= p_2(\text{AT})p_1(\text{T}|\text{AT})p_2(\text{C}|\text{TT}) \\ &\quad p_0(\text{T}|\text{TC})p_1(\text{G}|\text{CT})p_2(\text{C}|\text{TG}) \end{aligned}$$

Choice of order k

- probabilities $p_r(x \mid y_1, \ldots, y_k)$ can be estimated on known coding sequences
- if $k \geq 2$ above content model can reflect codon usage
- usually: the higher k the bigger the difference between coding and noncoding

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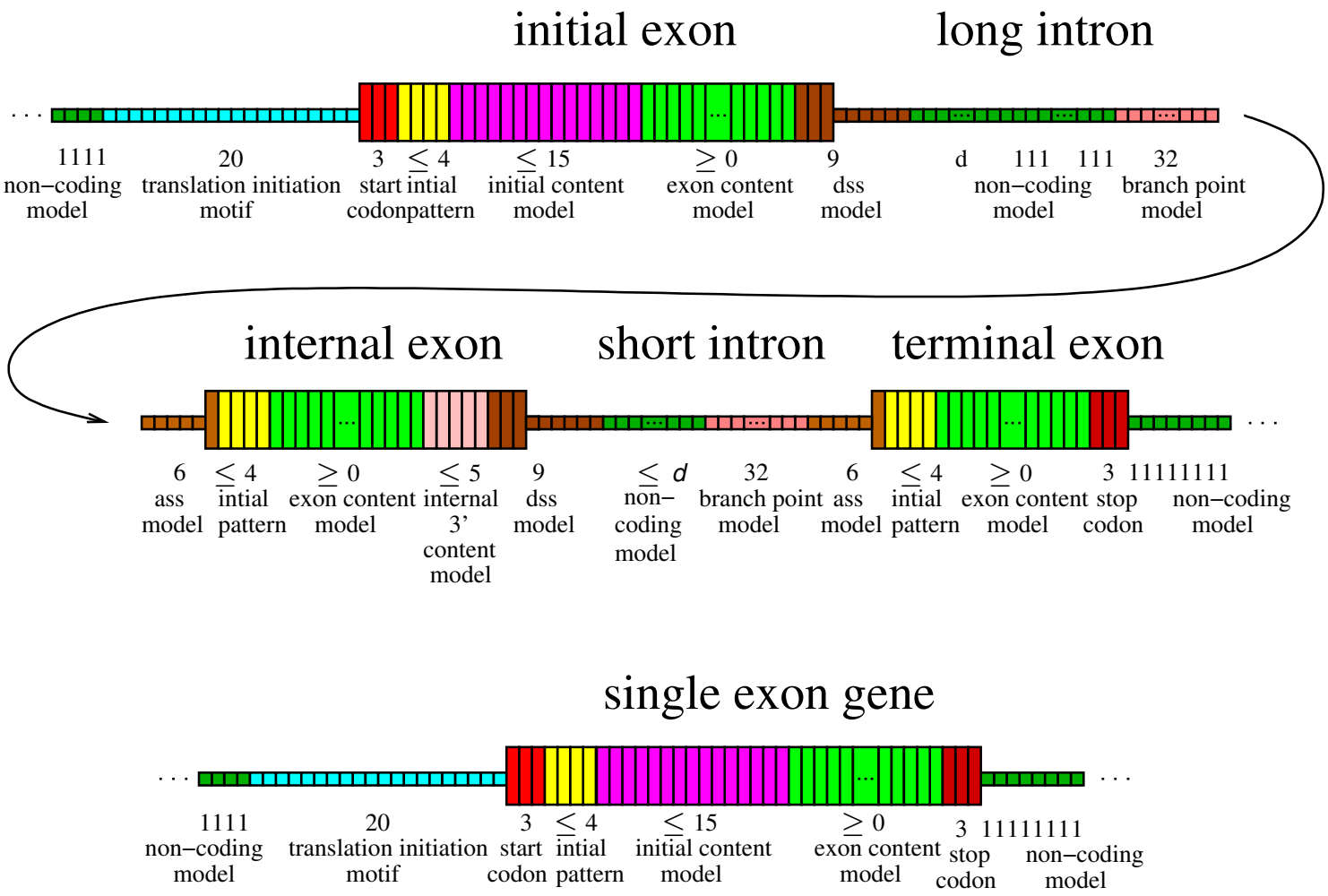
- if $k \geq 2$ above content model can reflect codon usage
- usually: the higher k the bigger the difference between coding and noncoding
- but: content model $\neq$ reality (dead genes)
- typical: $k = 4$ or $k = 5$

Signal and Content Models of AUGUSTUS (without UTR)

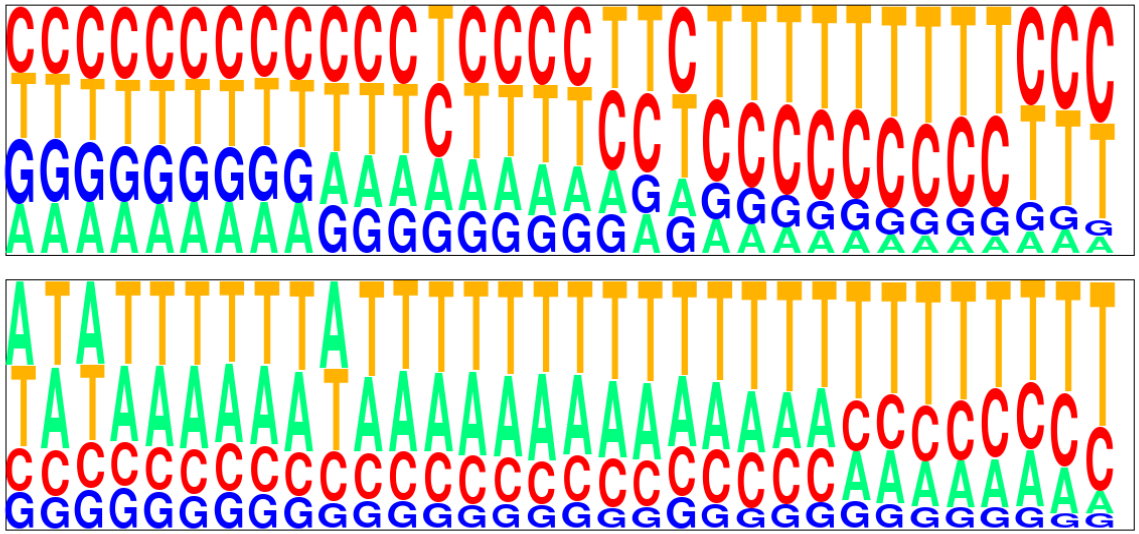


How Species-Specific Must Gene Finding Models Be?

Differences:

- distribution at signals, e.g. branch point region

top: human / bottom: fly



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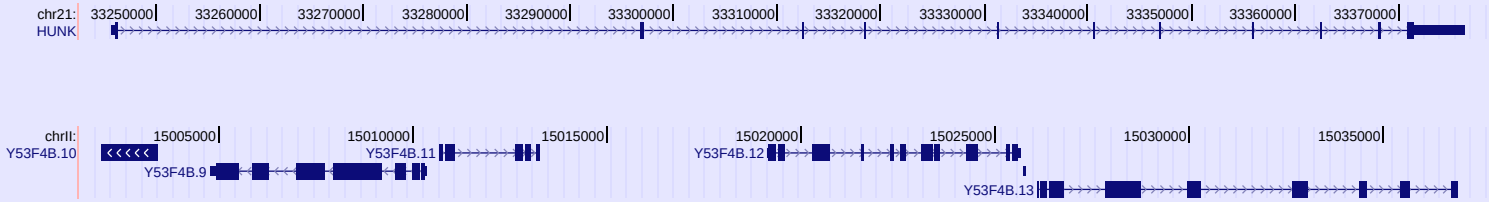
- number and length distribution of introns

top: human / bottom: *C. elegans*



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- GC content highly variable
- number and length distribution of introns
- length distribution of UTRs
- gene density

Training: Estimate Species-Specific Parameters

"Training Set"

- input: set of annotated sequences

$$(x^{(k)}, y^{(k)})_{k=1,\ldots,N},$$

  such that the parse $x^{(k)}$ represents the gene structure of DNA sequence $y^{(k)}$.
- at least a few hundred genes (≥ 200, more better up to ≈ 1000)
- used to estimate species-specific parameters
- self-training programs: use their own preliminary prediction as training set and iterate prediction and training (GeneMark-ES, Borodovsky et al.)
  (does not work in all species)

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Some Options to Construct a Training Set

Option 1: PASA

-

Some Options to Construct a Training Set

Option 2: SCIPIO

-

Some Options to Construct a Training Set

Option 3: Predict and iterate

-

Some Options to Construct a Training Set

Option 4: No retraining

-

Problems and General Ansatz

Problems

- known signal models do not carry much information
- false positive signals because of low number of true positives
- sequence content can be misleading (pseudogenes, repeats)

Ansatz

- combine all individual weak info to boost discriminatory power
- enforce standard gene structure:
  - reading frame consistency between exons
  - minimal splice site consensus (GT/AG, maybe GC/AG)
  - no in-frame stop codons
  - minimal intron length ($\approx$ 40 bp)

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Gene Structure Formally

observed variables:

DNA
S

region of Fugu DNA

CTTCCTAACGGTCAAAGTCCTGGAGGACAATCTGAACACTCTGACTGGGGTAAGAAAGCACCAGCCTCCACGCCACTTTGGTATATTTGAAGGTTATAACAAGTTCTGGTCTTTTCCAGGTGTCTGAACAGCCTCACAAAGTGCCAGGGACCCCCTCCGTGGGGGAGATGACCACCACGGATGTGTCCAA

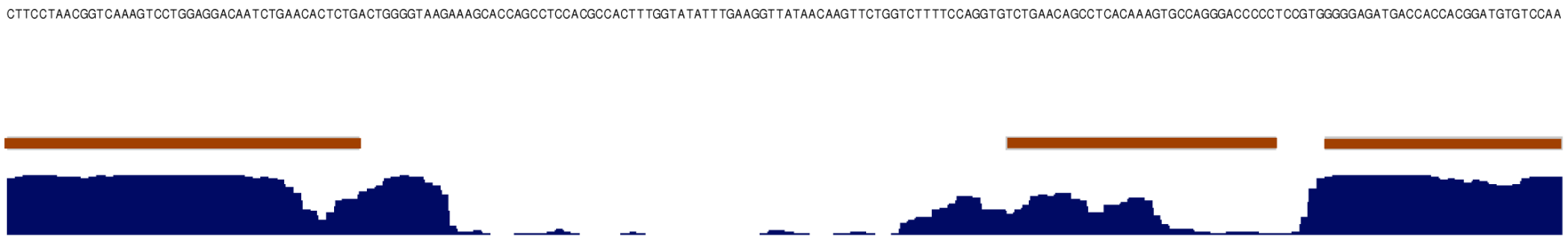
hints
H

regions with similarity to human protein

Gene Structure Formally

unobserved variables:

DNA
S



hints
H

exon

Gene Structure Formally

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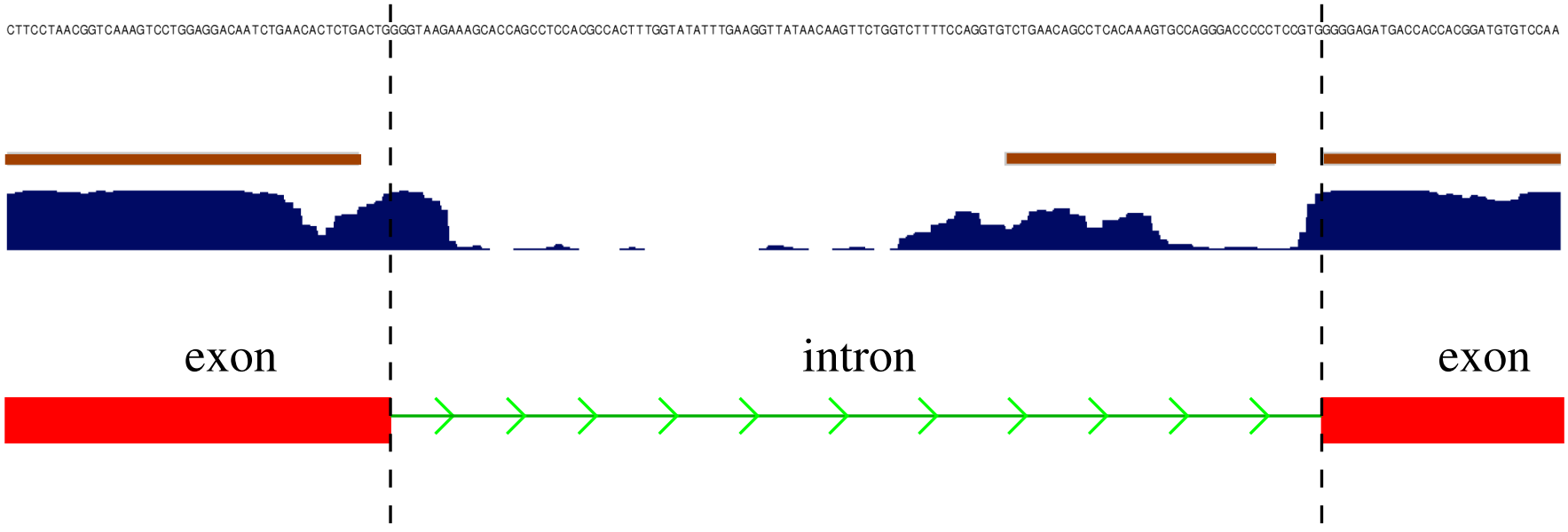

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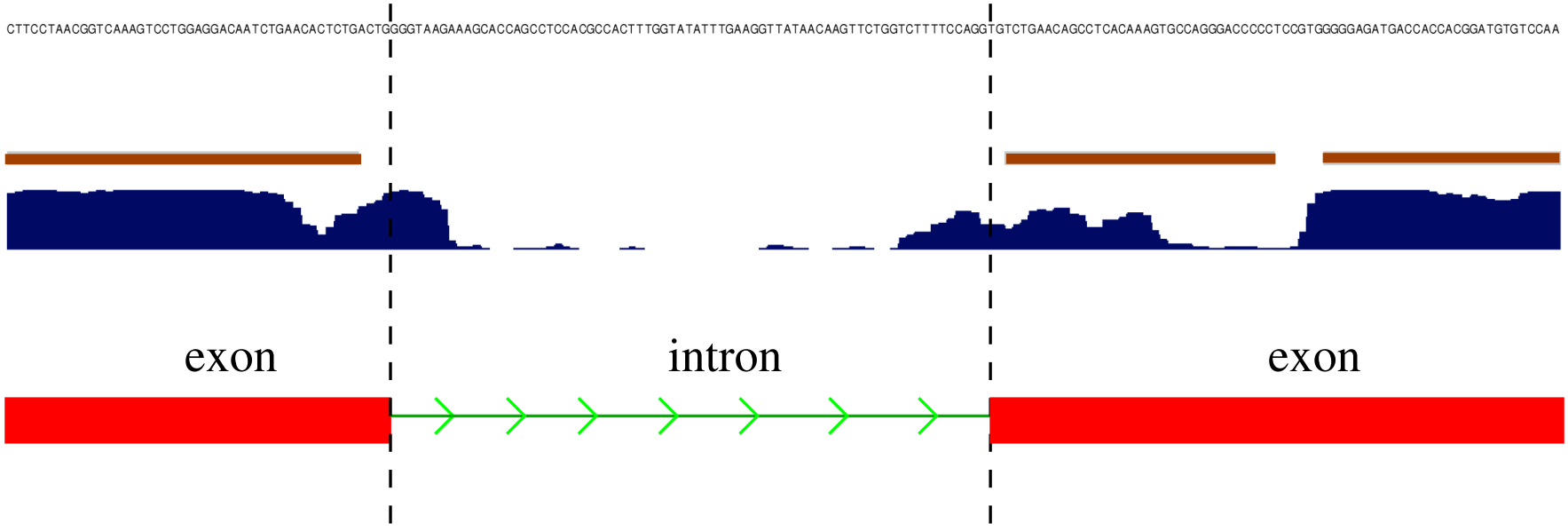

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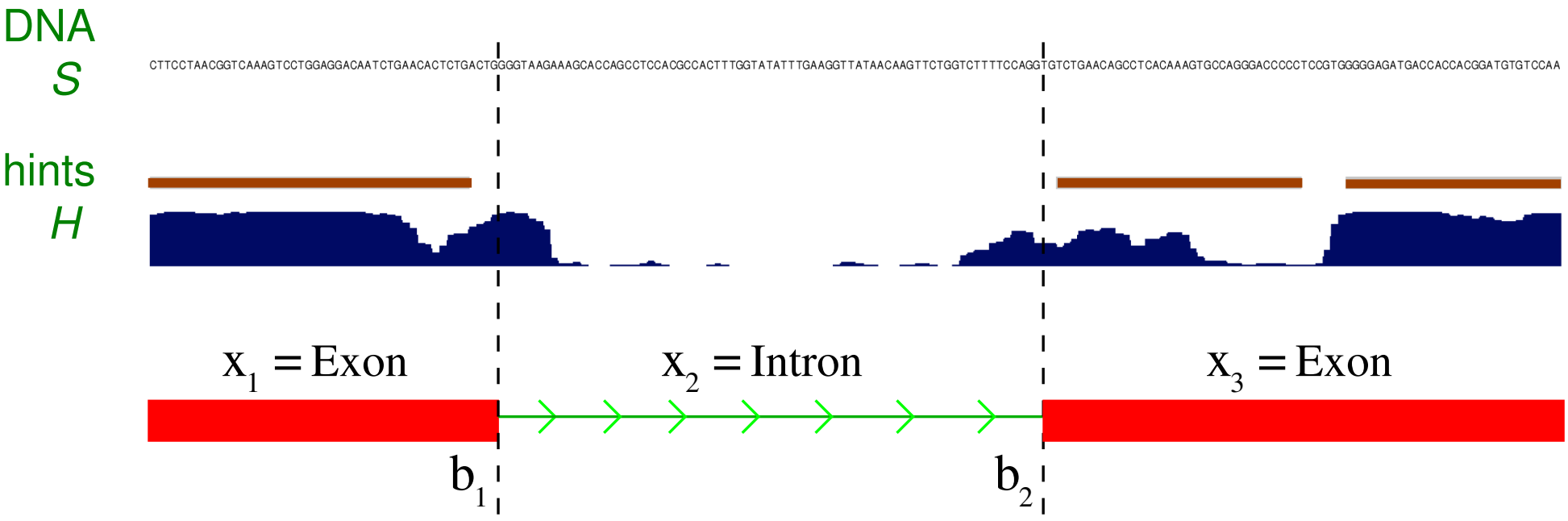


gene structure $x = ((x_1, b_1), (x_2, b_2), \ldots, (x_n, b_n))$ is a labelled segmentation of DNA

$x_1, x_2, \ldots$ biological labels, e.g. "exon", "intron", "intergenic region"
$b_1, b_2, \ldots$ segment boundaries

Generic Approach

Input

sequence: DNA sequence to find genes in
evidence: optinal additional evidence, e.g. conservation

Output

gene structure x: start end end positions of exons, genes

Gene finding as optimization problem

maximize

$$s(\text{gene structure } x, \text{sequence } S, \text{evidence } H)$$

subject to constaint

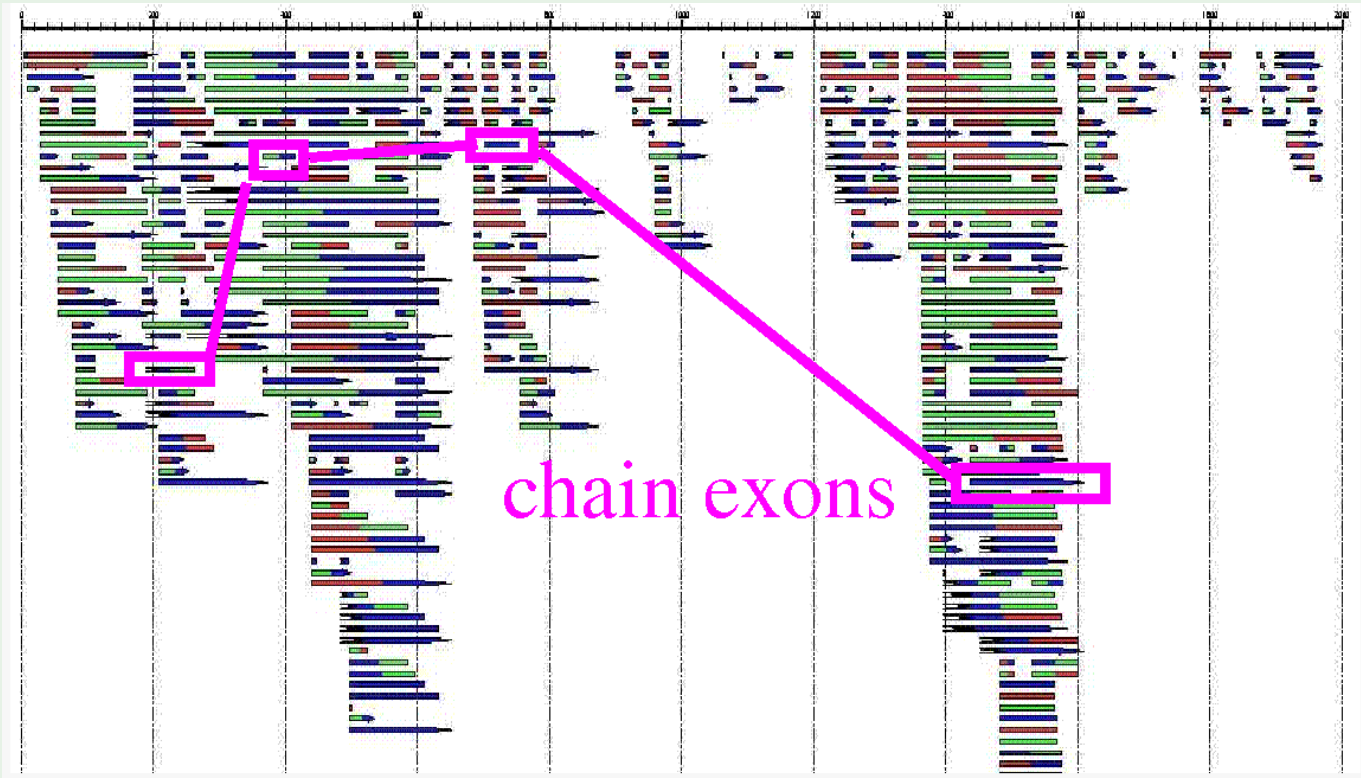
gene structure x makes biologically sense

A Hierarchical Approach Using Graphs

Exon Chaining

- find nonoverlapping compatible exon candidates with highest sum of scores
- e.g. done by GENEID (Parra et al., 2000)

Example (exon candidates in a DNA of length 2000)



http://monstrel.imim.es/courses

exon candidates of the program GENEID

Explicit Probabilistic Models of DNA and Genes

Hidden Markov Model (HMM)

GENSCAN (Burge and Karlin, 1996),
GENIE (Kulp et al., 1996),
GENEMARK (Lukashin and Borodovsky, 1998),
FGENESH (Salamov and Solovyev, 2000),
AUGUSTUS (Stanke and Waack, 2003)

First publication on our gene finder AUGUSTUS

BIOINFORMATICS

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Gene prediction with a hidden Markov model and a new intron submodel

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Some Genome Annotation Projects using AUGUSTUS

Aedes aegypti	yellow fewer mosquito: dengue fever	*Science*, 2007
Brugia malayi	parasitic worm, causes elephantiasis	*Science*, 2007
Tribolium castaneum	red flour beetle, pest and model organism	*Nature*, 2008
Schistosoma mansoni	parasite causing bilharziosis	*Nature*, 2009
Acyrthosiphon pisum	pea aphid, agricultural pest	*PLOS Biology*, 2010
Coprinus cinereus	fungus	*PNAS*, 2010
Nasonia vitripennis	wasp	*Science*, 2010
Amphimedon queenslandica	sponge	*Nature*, 2010
Culex pipiens	common mosquito	*Science*, 2010
Ricinus communis	castor bean	*Nature Biotechnology*, 2010
Chlamydomonas reinhardtii	green algae	*Proteomics*, 2011
Galdieria sulphuraria	red algae	*Planta*, 2007
Arabidopsis thaliana	plant model organism	*PNAS*, 2008
Leishmania tarentolae	lizard parasite	*Nuc. Acids Res.*, 2011
Verticillium longisporum	fungus infecting oilseed rape	
Tetrahymena thermophilia	model organisms in biomedical research	
Nicotiana tabaccum	tobacco	
Toxoplasma gondii	causes toxoplasmosis	
Zea mays	maize	
Heliconius melpomene	butterfly	
Apis mellifera	honey bee	

AUGUSTUS is open source.

Hidden Markov Model

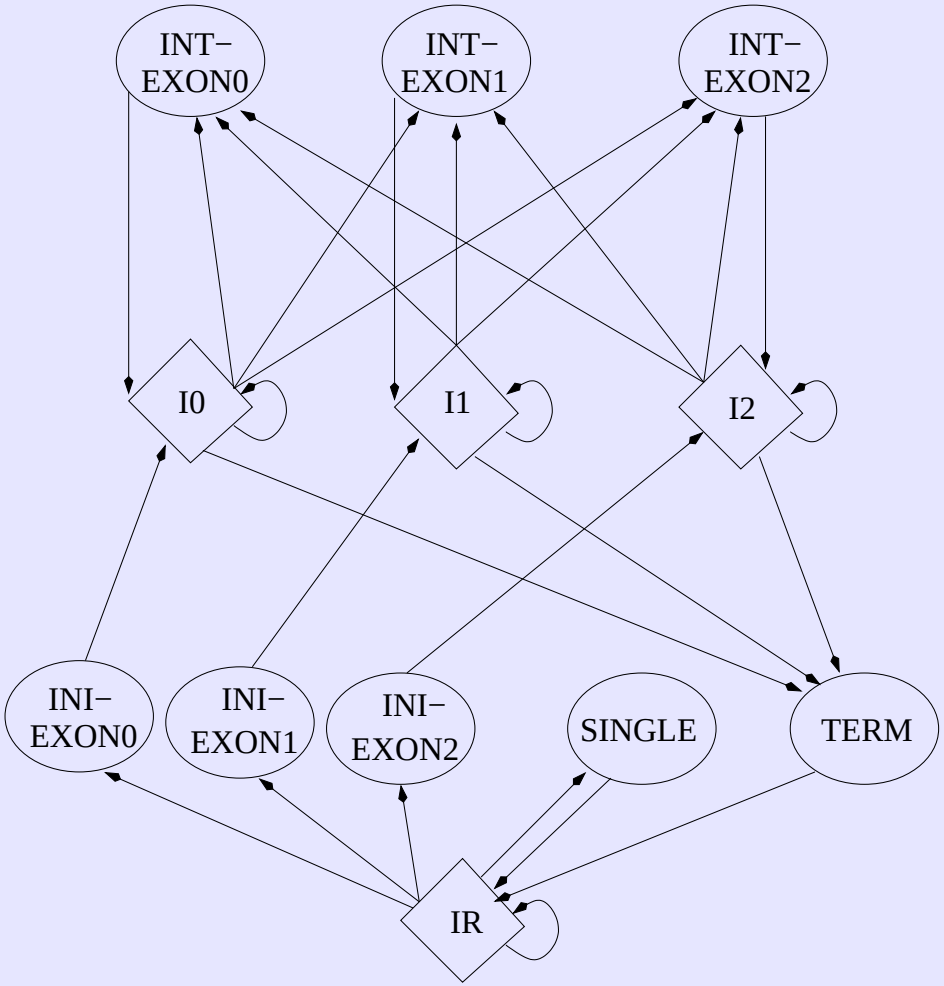
HMM

$$P(x, y) = \prod_{i=1}^{n} P(x_i|x_{i-1}) \cdot P(y(b_{i-1}, b_i] \mid x_i)$$

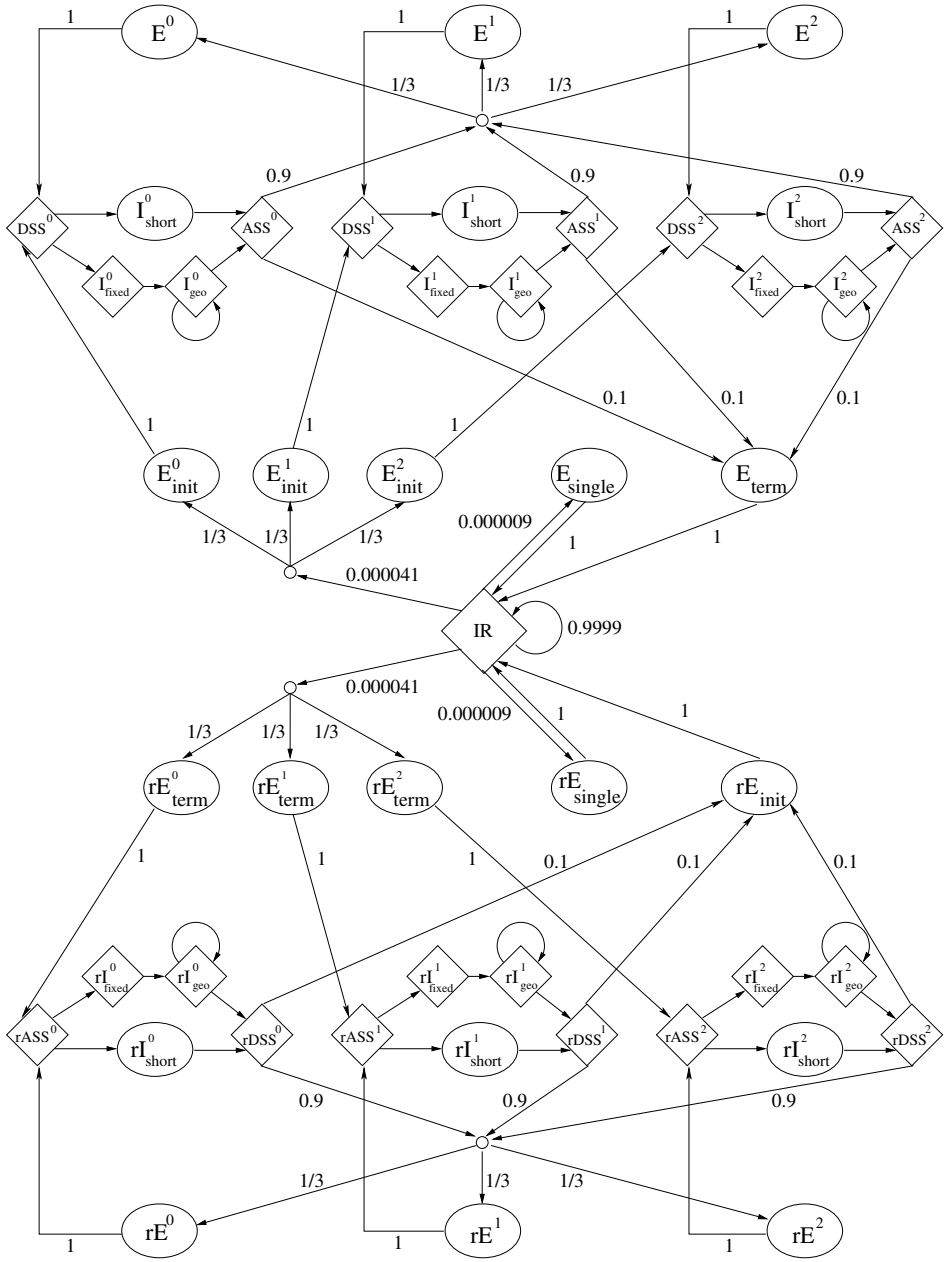
is a generative model: everything that is used in prediction (y), must have a distribution.
E.g. in comparative gene finding: Must define probability of any observable alignment column.

A Simple (Semi-Markov) HMM for Gene Finding: Model Topology

Model for (multiple) eukaryotic genes on forward strand



Transition Matrix of AUGUSTUS (without UTRs)



Discriminative Models of Genes given DNA

Conditional Random Field (CRF)

CRAIG (Bernal et al., 2007),
CONTRAST (Gross et al., 2007),
AUGUSTUS (unpublished)

Conditional Random Field for Gene Prediction

Definition (CRF)

A (Semi-)Conditional-Random-Field is a conditional distribution of gene structures x given the observation y of the following form

$$P(x \mid y) = \frac{1}{Z(y)} \exp \sum_{i=1}^{n} f(x_{i-1}, x_i, b_{i-1}, b_i, y).$$

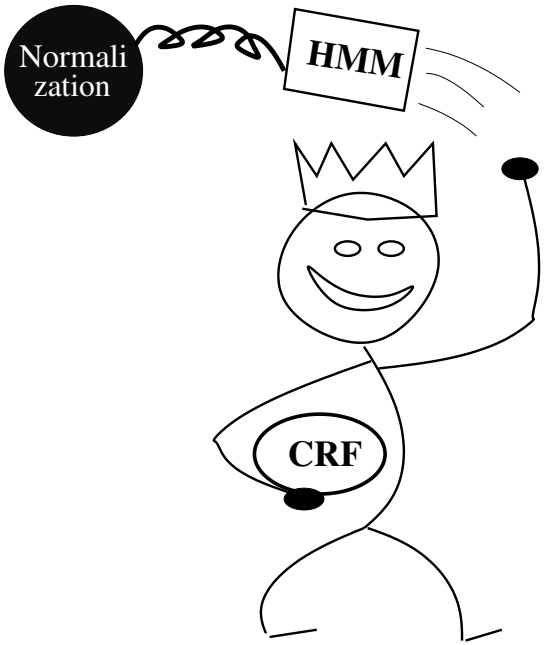
CRF

- gene structure is scored locally:
  score may only locally depend on one labelled segment
- not scorable, e.g.: gene should have a total exon length of 1000bp

Conditional Random Field versus Hidden Markov Model

CRF

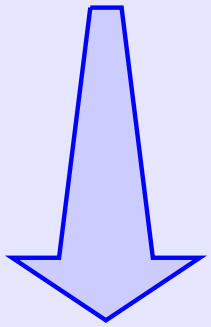
- for every HMM there is a CRF with same $P(x|y)$
- only *globally* normalizaion necessary
- can easier be dicriminatively trained



Motivation for Model Change

Signal models: disadvantage of HMM parameter estimation

Call splice site pattern strong when relative frequency in real genes large.

relative frequency in range	strength	counts of correct DSS	HMM: counts of predicted DSS
$[0, 7.72 \cdot 10^{-6})$		0	108
$[7.72 \cdot 10^{-6}, 0.000139)$		57	83
$[0.000139, 0.000275)$		175	183
$[0.000275, 0.00071)$		532	465
$[0.00071, 1]$		3053	2186
sum		3817	3025

$\Rightarrow$ Predicted gene structures have fewer strong splice sites than correct gene structures and more weak splice sites.

One should be able to improve predictions by putting more weight on the splice site model than classical HMMs do.

Conditional Random Field

Online large-margin training

Iteratively update parameters θ (human: 8272 parameters) using a training set of gene structures g_{train} (200-2000 genes) so that

$$P_\theta(g_{\text{train}} \mid \text{DNA}) \quad > \quad P_\theta(g \mid DNA) \quad + \quad \underbrace{L(g_{\text{train}}, g)}_{\geq 0}$$

punishes important errors

for all false gene structures $g \neq g_{\text{train}}$.

Conditional Random Field versus GHMM training

Accuracy improvement due to Semi-CRF training

ab initio accuracy	human		*Drosophila*		*Chlamydomonas*	
	GHMM	Semi-CRF	GHMM	Semi-CRF	GHMM	Semi-CRF
base sn	.754	.817	.907	.960	.889	.986
base sp	.659	.782	.870	.886	.994	.977
exon sn	.601	.731	.807	.811	.875	.958
exon sp	.567	.712	.743	.771	.918	.952

(sn = sensitivity = true positives / actual positives, sp = specificity = true positives / predicted positives)

Conservation of Gene Structure and Sequence

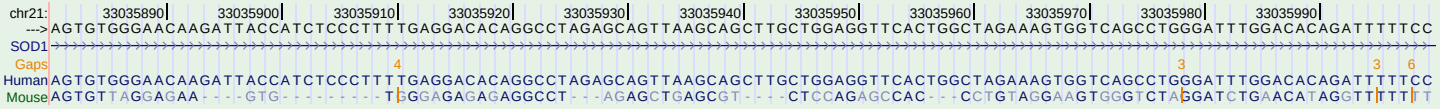
Observation

Protein sequences and rough structure of genes are often conserved between species that are tens of millions of years separated.

Example (Human-Mouse: 75 million years)

- 95% of orthologous gene pairs have same number of exons in human and mouse
- coding sequence to ≈ 85% identical

- noncoding sequence to ≈ 35% identical



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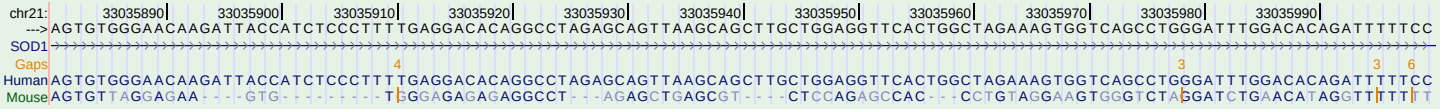

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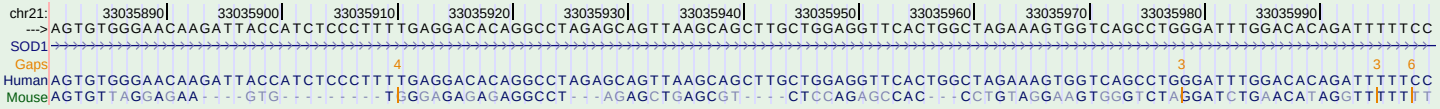
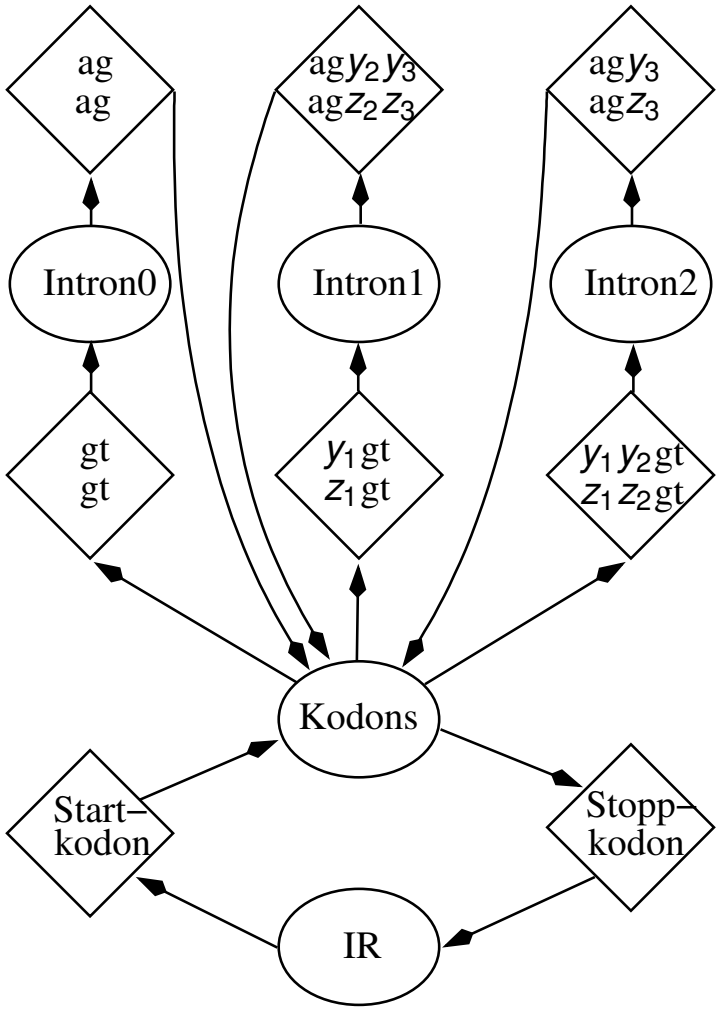


Pair HMMs for Eukaryotic Gene Finding



- DOUBLESCAN (Meyer and Durbin, 2002)
- does alignment of two genomics sequences and gene prediction in both at same time
- running time quadratic in sequence length
- does not generalize efficiently to >2 species
- not popular

CONTRAST (Gross et al., 2007)

CONTRAST

- still best *de novo* predictor for human (?)
- discriminatory model (SVM+CRF)
- uses 11 other vertebrate genomes as informants
- roughly for 50% of human genes at least one splice form is correctly predicted

```
human       ACAGGTGAGGAGGCG
macaque     ...............
mouse       ...............
rat         ACAGGTGAGAAAG..
rabbit      ...............
dog         ACAGGTGAGGAGTCG
cow         ACAGGTGAGCAGTCG
armadillo   ACAGGTGAGGAG_CA
elephant    ...............
tenrec      ...............
opossum     CCAGGGAAG......
chicken     CCAGGTGA.......
EST         SSSSIIIIIIIIIII
```

Using Extrinsic Evidence to Improve the Accuracy of AUGUSTUS

Extrinsic evidence

additional information to aid gene prediction, not contained in the sequence

AUGUSTUS currently can use

- mRNA/EST/454 alignments, RNA-Seq
- genomic conservation
- annotation of related species using a syntenic alignment
- protein alignments, peptide mass spectrometry

Hint

piece of additional local information about gene structure, in general uncertain

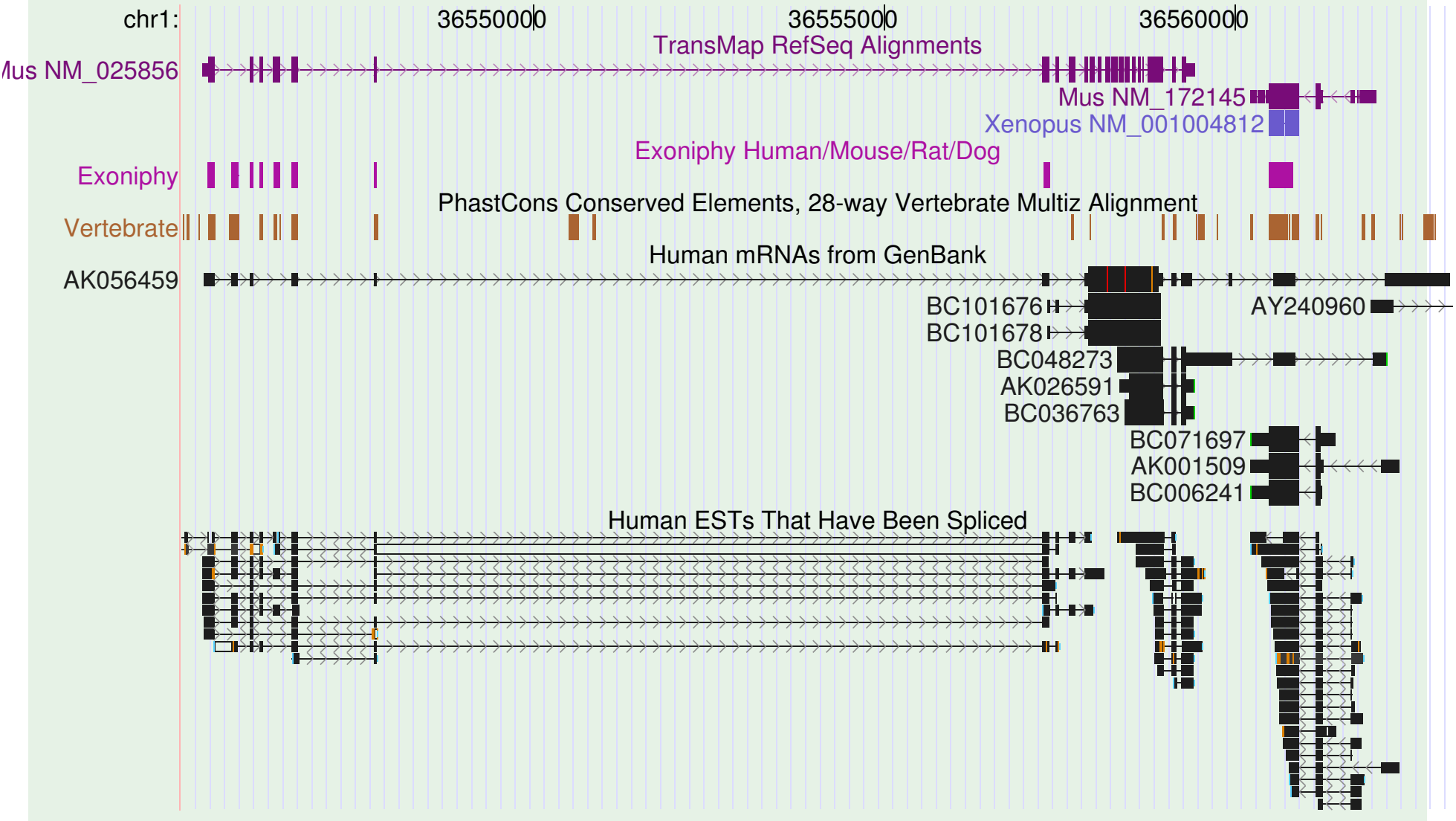
Types of Hints

AUGUSTUS understands these hints...

type	meaning	source of information
start	start codon	protein, TransMap
stop	stop codon	protein, TransMap
tss	transcription start site	TransMap, CpG islands, promoter predictor
tts	transcription termination site	TransMap, 3' ESTs
ass	acceptor splice site	cDNA, proteins, TransMap, conservation
dss	donor splice site	cDNA, proteins, TransMap, conservation
exonpart	part of biological exon	cDNA
exon	biological exon	cDNA
intronpart	part of intron	TransMap
intron	intron	cDNA, proteins, TransMap
CDSpart	part of coding part of exon	protein, TransMap, conservation
CDS	coding part of exon	protein, conservation
UTRpart	part of non-coding part of exon	TransMap
UTR	non-coding part of exon	
irpart	part of intergenic region	mRNA, TransMap, cDNA
nonexonpart	intron or intergenic region	retro genes, repeats

Sources of Hints - Example

Example



Integration of Hints into the Model

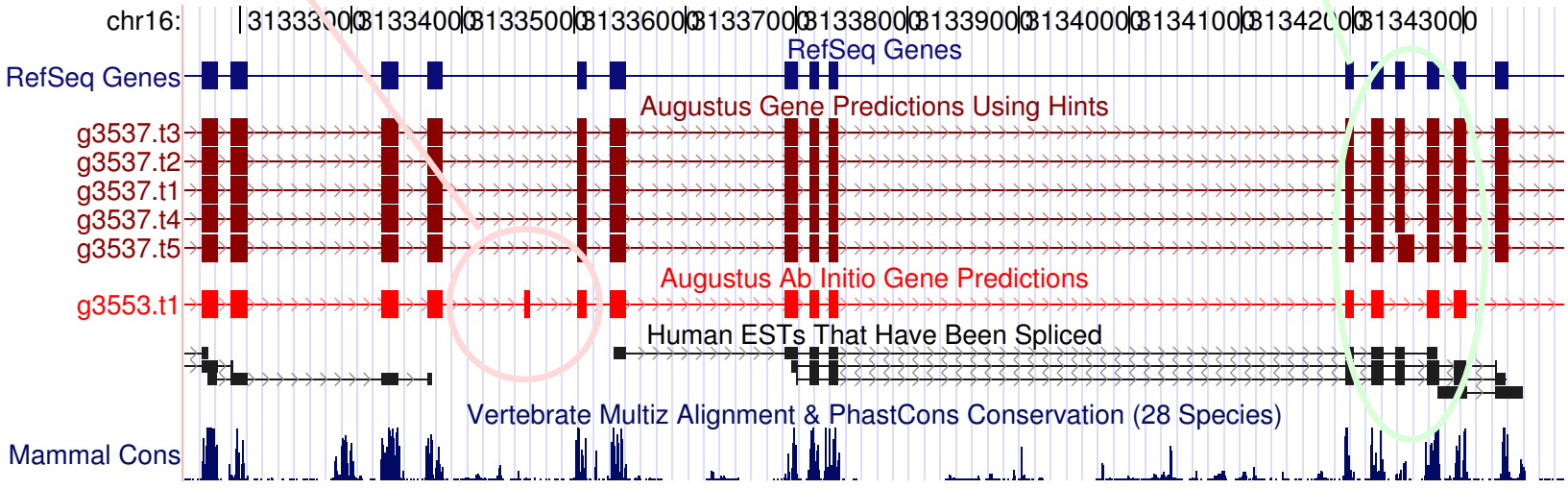
Hints modify the joint probability

$$P(\text{gene structure}, \text{DNA}, \text{hints})$$

by a factor that depends on the compatibility of the gene structure with the hints.

Hints have two effects

1. gene structure candidates that obey a hint get a relative bonus.
2. gene structure candidates with unsupported exons, introns or signals get a relative malus (penalty)



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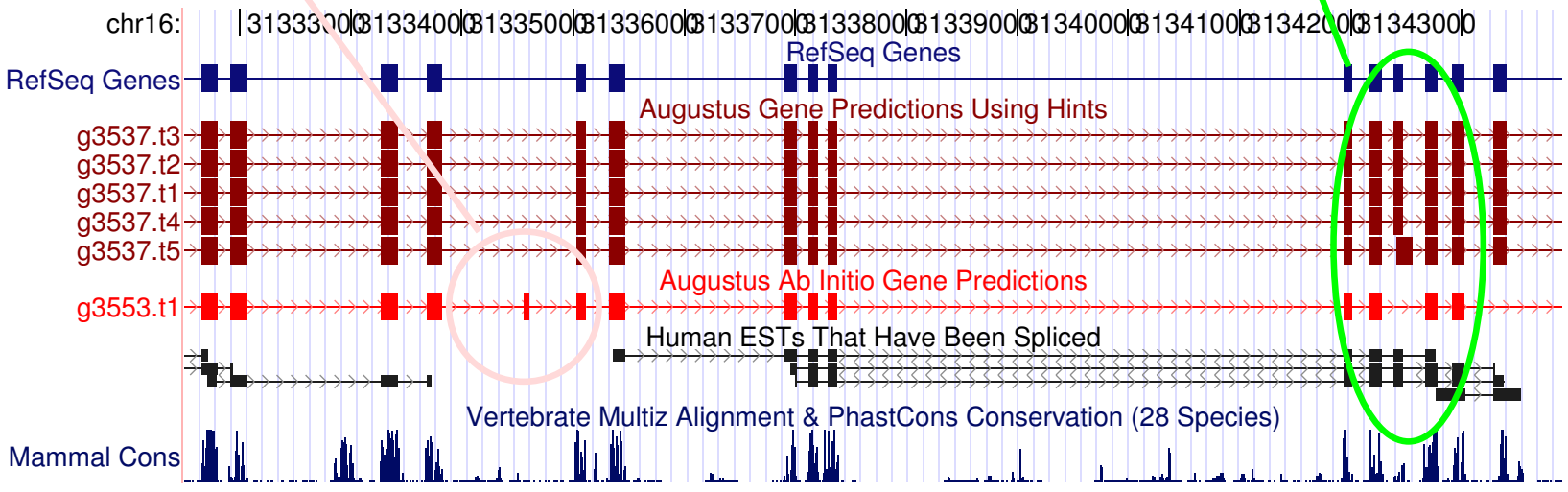

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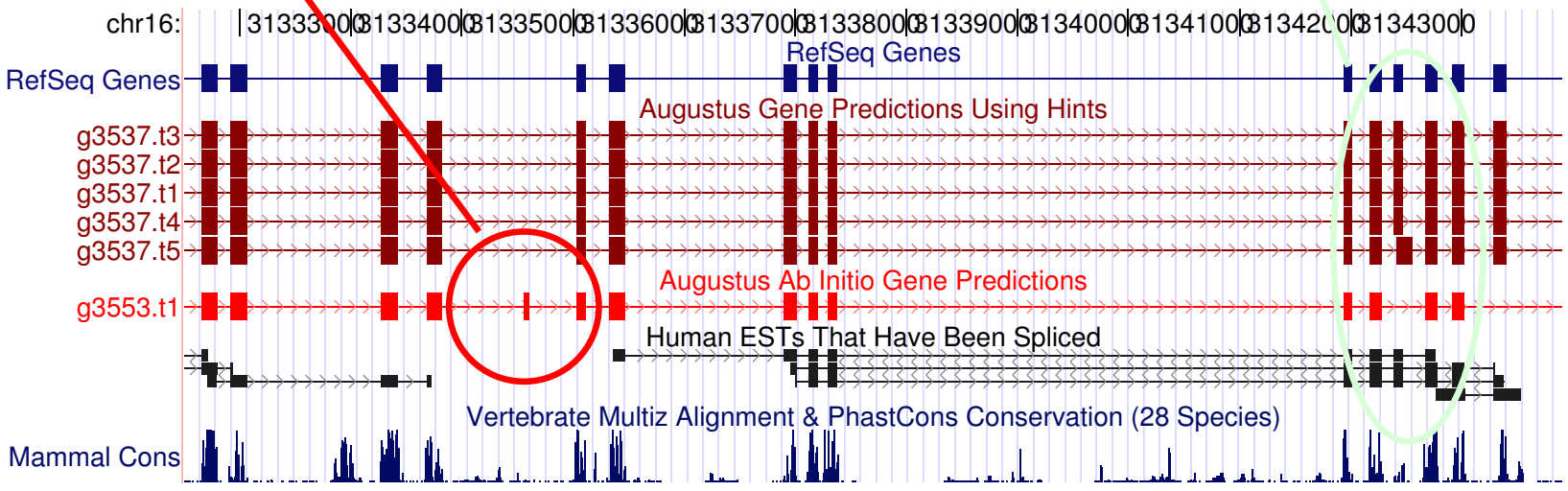
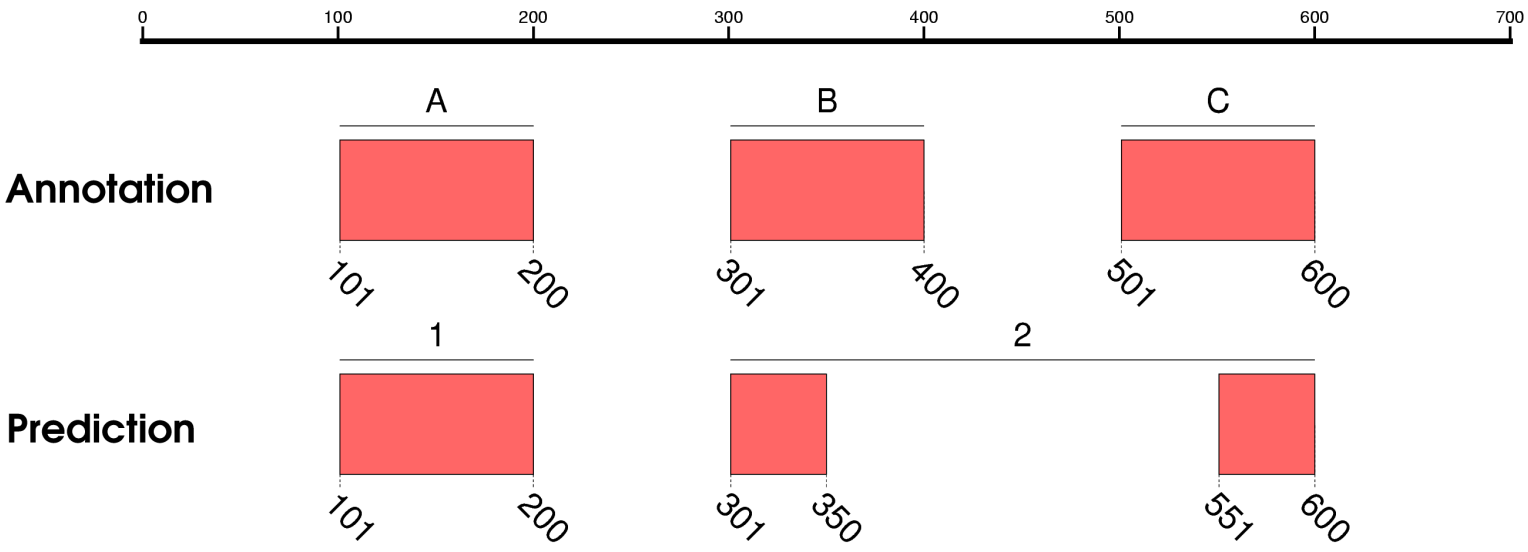


Prediction Evaluation Measures



$$\text{Sensitivity sn} = \frac{\text{true positives}}{\text{annotated positives}}$$

$$\text{Specificity sp} = \frac{\text{true positives}}{\text{predicted positives}}$$

Gene sn	33.3%
Gene sp	50%
Transcript sn	33.3%
Transcript sp	50%
Exon sn	33.3%
Exon sp	33.3%
Base sn	66.7%
Base sp	100%

Accuracy of Genome Annotation

Two independent published gene prediction assessments since 2000:

- EGASP on human ENCODE regions, 2005:

  genes correctly predicted:

ab initio	24% (AUGUSTUS)
using any available info	73% (JIGSAW)

  (Guigo et al., *Genome Biology*, 2006)

- nGASP on *C. elegans*, 2007:

  genes correctly predicted:

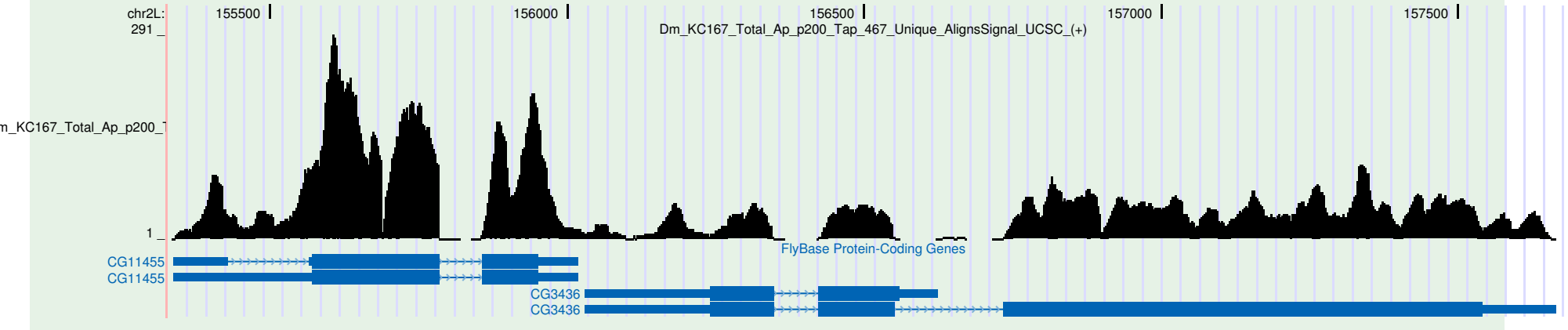
ab initio	61% (AUGUSTUS, mGENE, FGENESH)
using ESTs & proteins	80% (AUGUSTUS, FGENESH, mGENE)
best-performing combiner	80% (JIGSAW)
for comparison:	65% (GLEAN)

  (Coghlan et al., *BMC Bioinformatics*, 2008)

Note: Most species have less available evidence than these two!

Next Generation Sequencing of Transcriptomes (RNA-Seq)

Example (coverage per position in genome)



Chances and Challenges

- genes expressed in sample have usually good coverage
- intuitively, should give boost to gene prediction accuracy
- but how?

RGASP: RNA-Seq Genome Annotation Assessment Project

RGASP

- assess the current progress of automatic gene building using RNAseq
- part of ENCODE project, http://www.gencodegenes.org/rgasp/
- 17 participating groups submitting 2 years ago, all on same data:
  - on human (41Gb), fly (13Gb), worm (21Gb)
  - Illumina, SOLiD, Helicos
  - paired, unpaired reads
  - unstranded, stranded reads
- accuracy results on site `https://compgen.bio.ub.es` (Josep Abril)

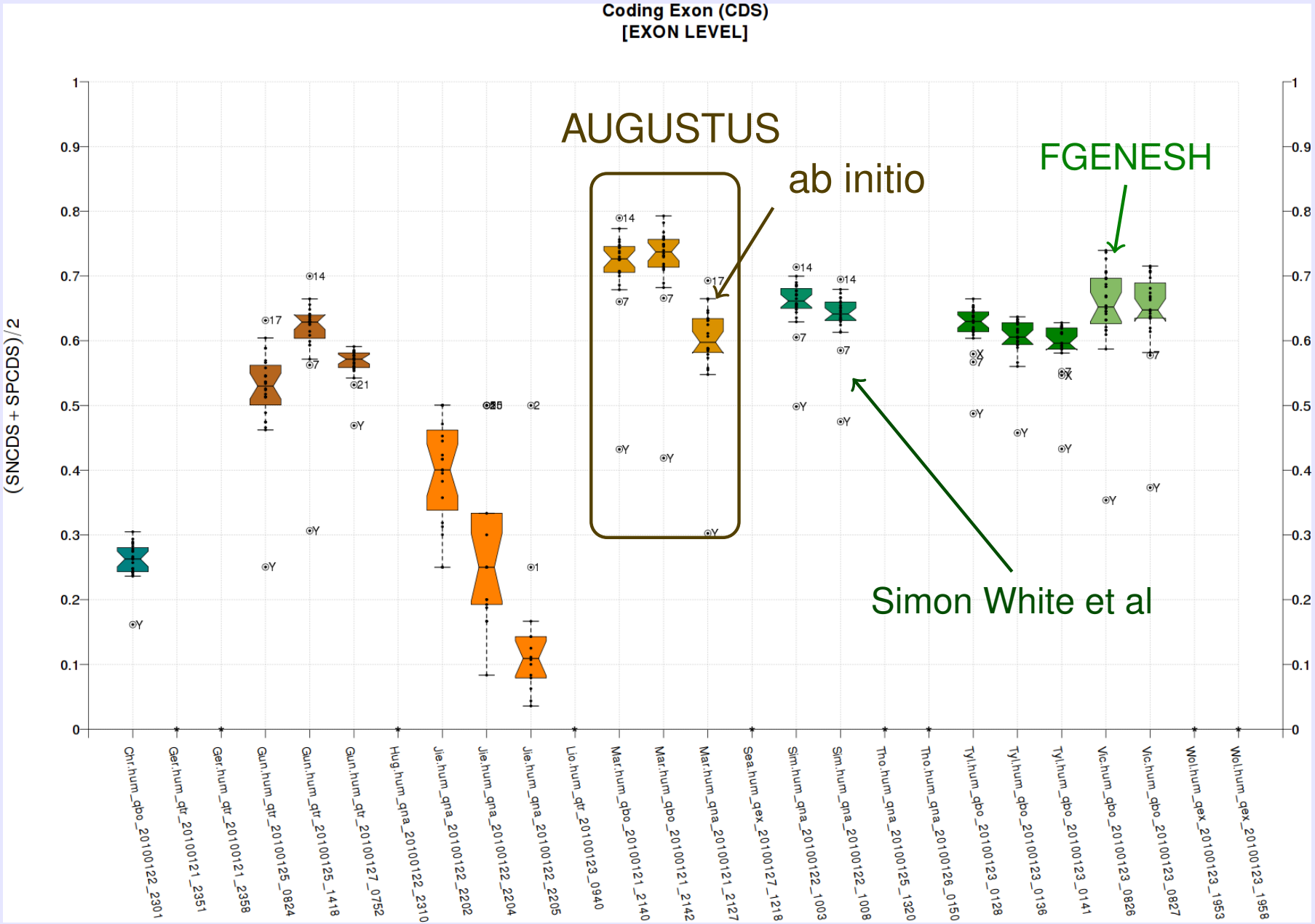
RGASP: RNA-Seq Genome Annotation Assessment Project

Three major approaches to transcript identification with RNA-Seq

- evidence integration into gene finder (e.g. AUGUSTUS, FGENESH, mGENE, GENEID)
  1. align reads to genome first
  2. integrate evidence from coverage and spliced alignments into gene finder
- purely alignment-based (e.g. Cufflinks)
  1. align reads to genome first
  2. construct transcripts from spliced alignments (no gene finding)
- de novo assembly of reads
  1. assemble transcriptome reads into transcript contigs
  2. use contigs for gene finding or just align them

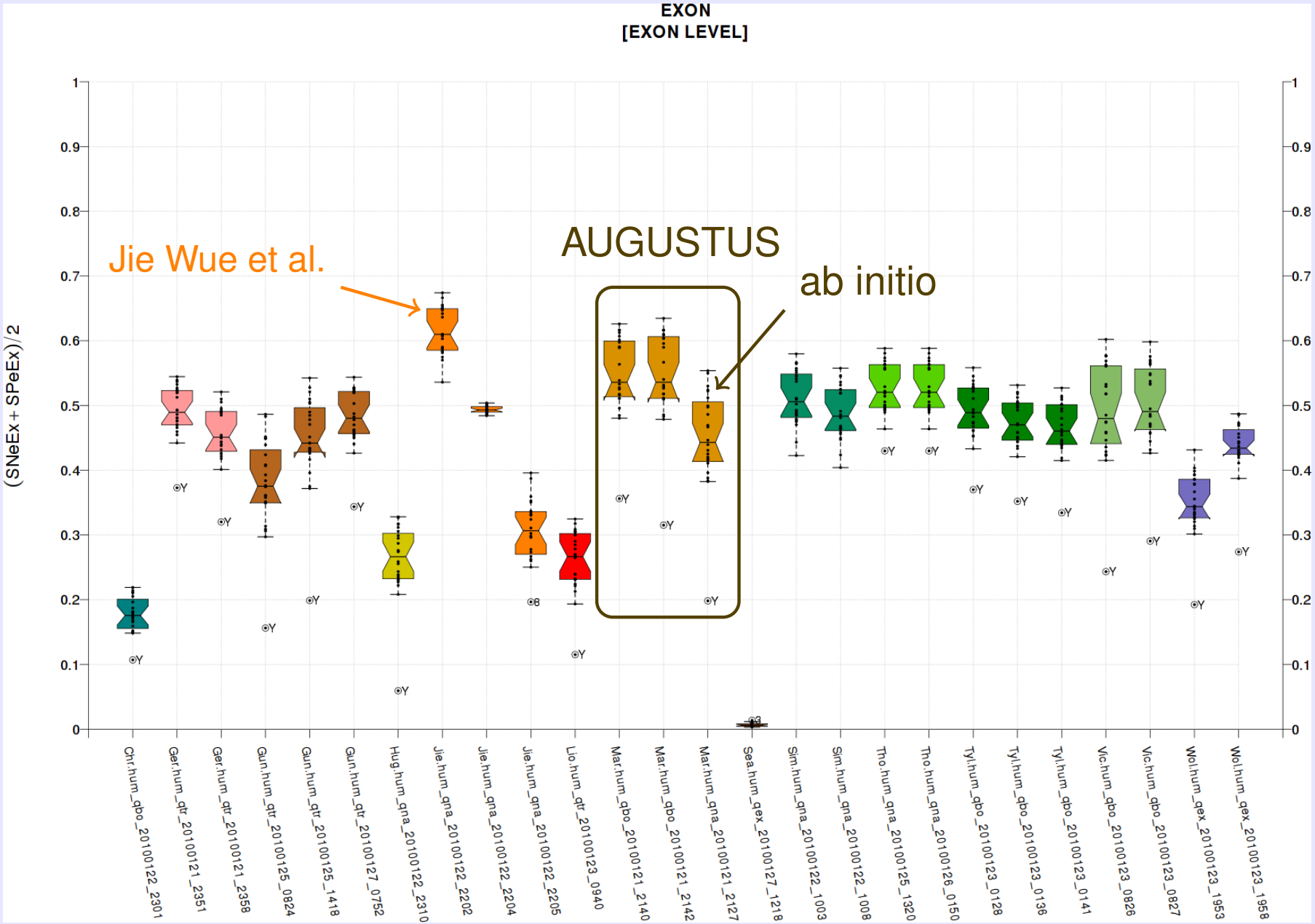
RGASP: results by submissions

human, coding exon level



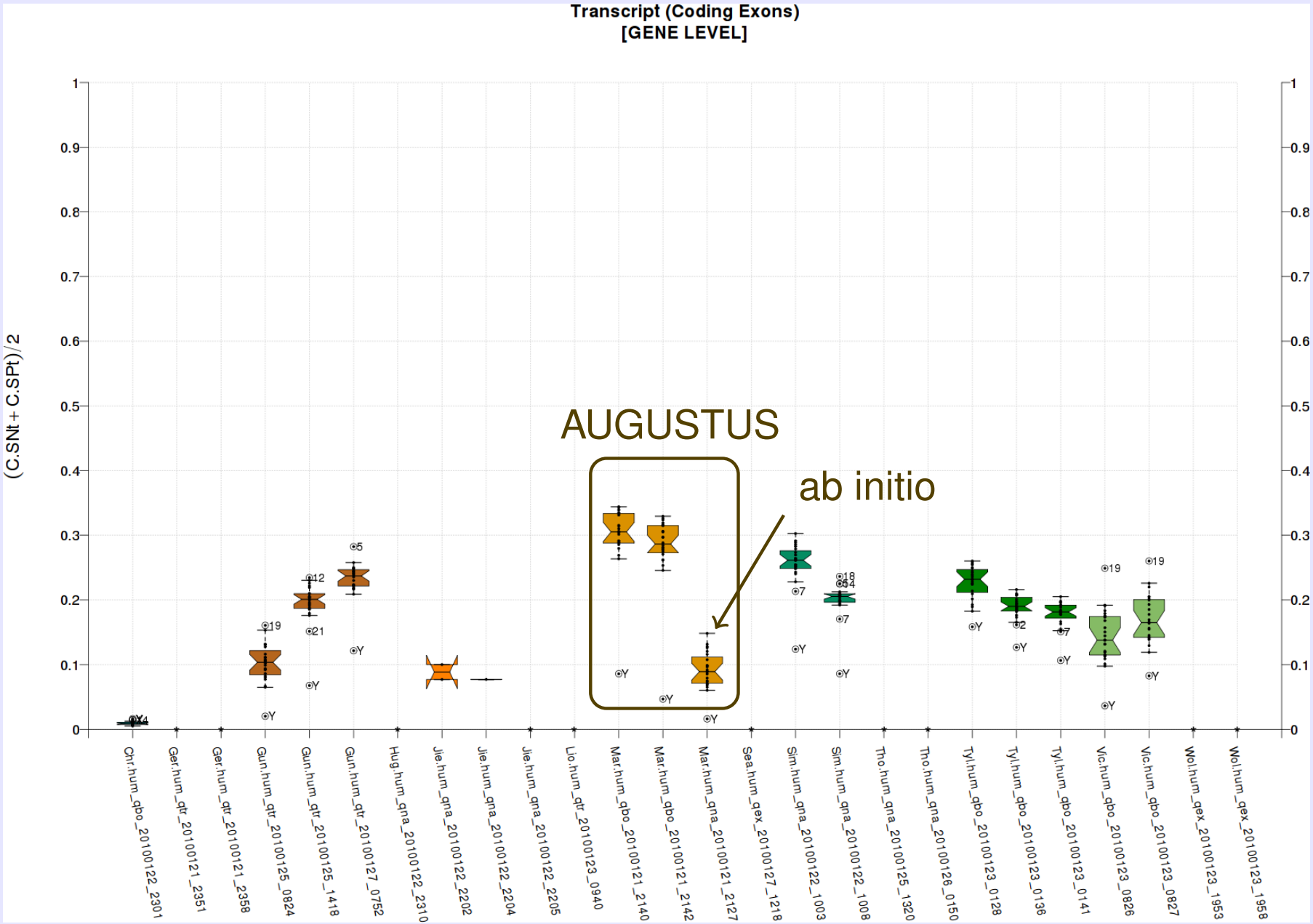
RGASP: results by submissions

human, exon level (no coding sequence predicted)



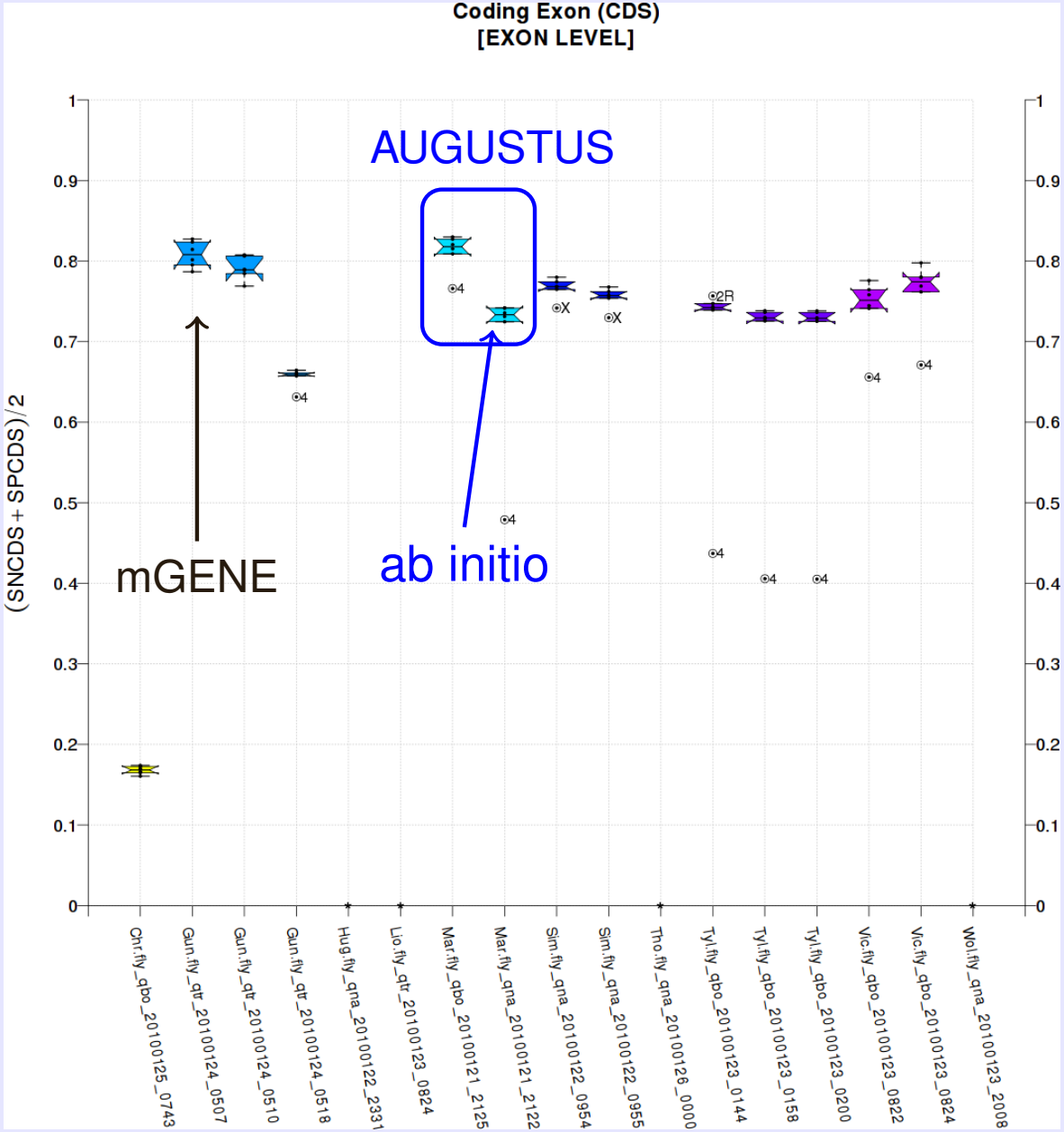
RGASP: results by submissions

human, transcript level, coding sequence



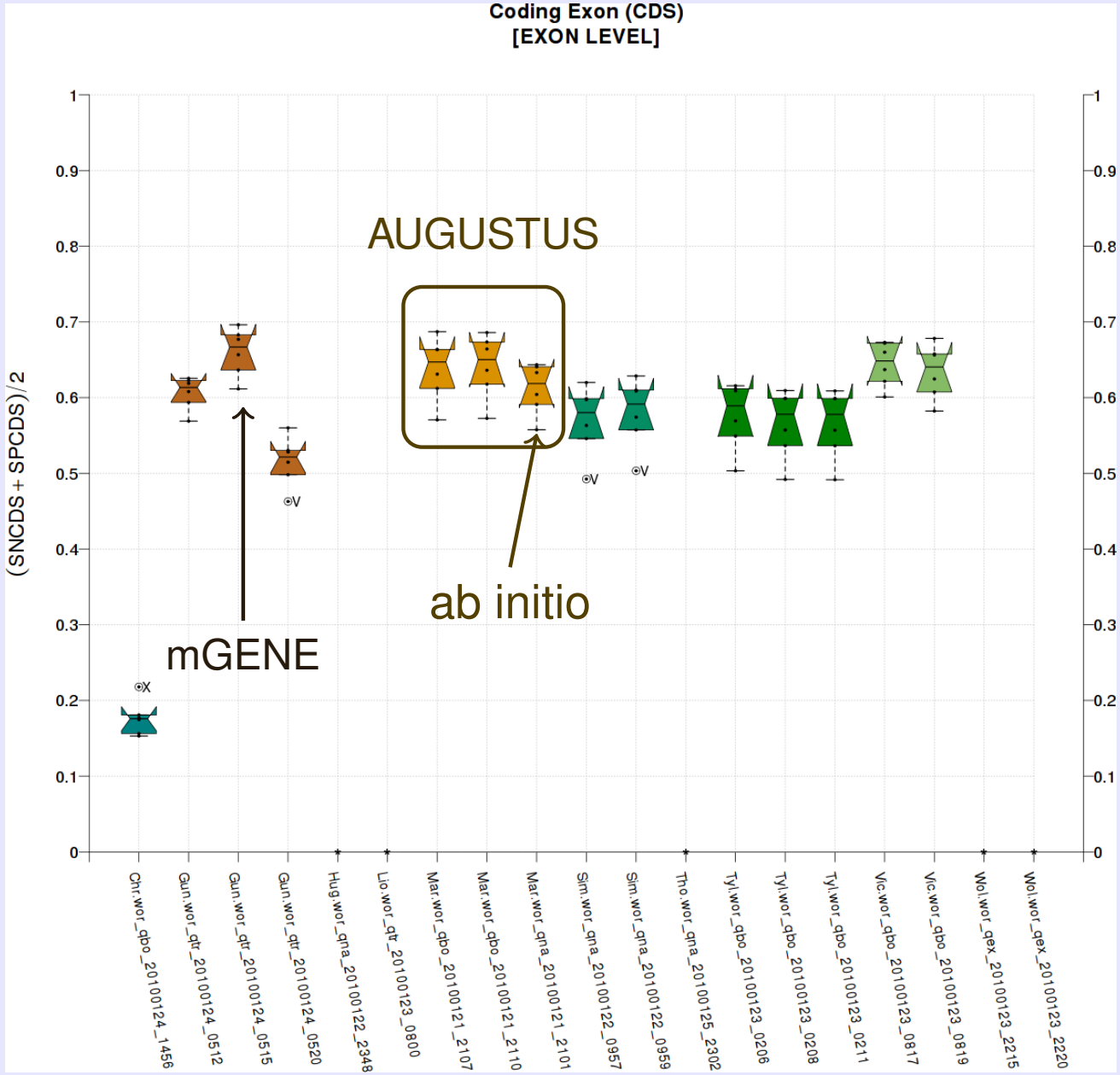
RGASP: results by submissions

Drosophila, coding exon level



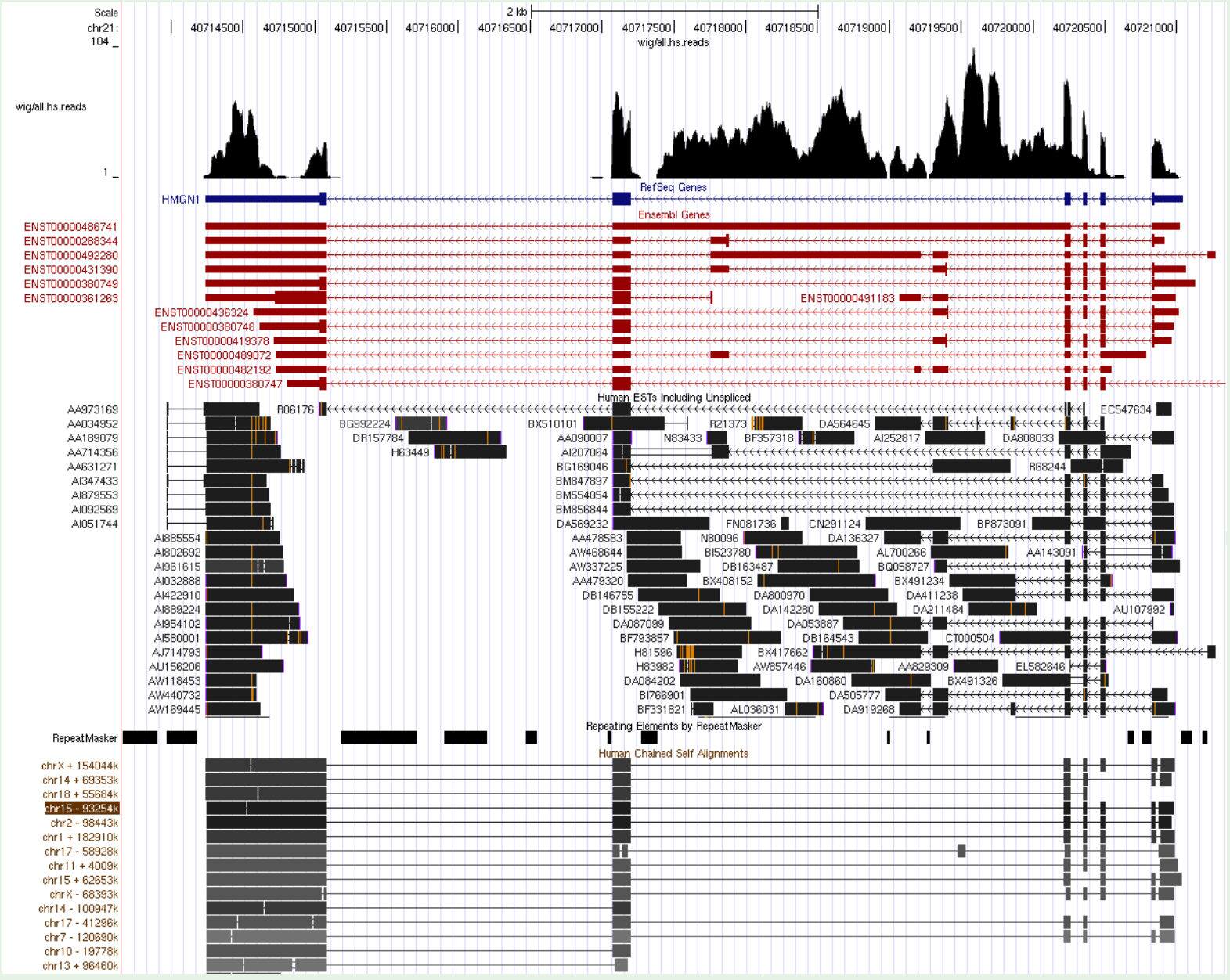
RGASP: results by submissions

C. elegans, coding exon level



Why was the accuracy not better?

Problems 1 and 2: intronic transcription, self-similarity of genome



Why was the accuracy not better?

Other problems with RNA-Seq

Problem 3 repeats

Problem 4 alignment errors

Problem 5 mRNA does not determine protein sequence (see Natalie's talk)

Conclusions from RGASP

1. even with RNA-Seq the best methods still make a lot of errors or miss genes
2. improving an underlying ab initio model also improves RNA-Seq-based performance

RGASP: preliminary results

My Conclusions

- RNA-Seq did not help as much as expected:
  - substantial intronic transcription
  - mapping ambiguity due to self-similarity of genomes
- still a lot of potential for improvement

Proteomics and Genomics: Proteogenomics

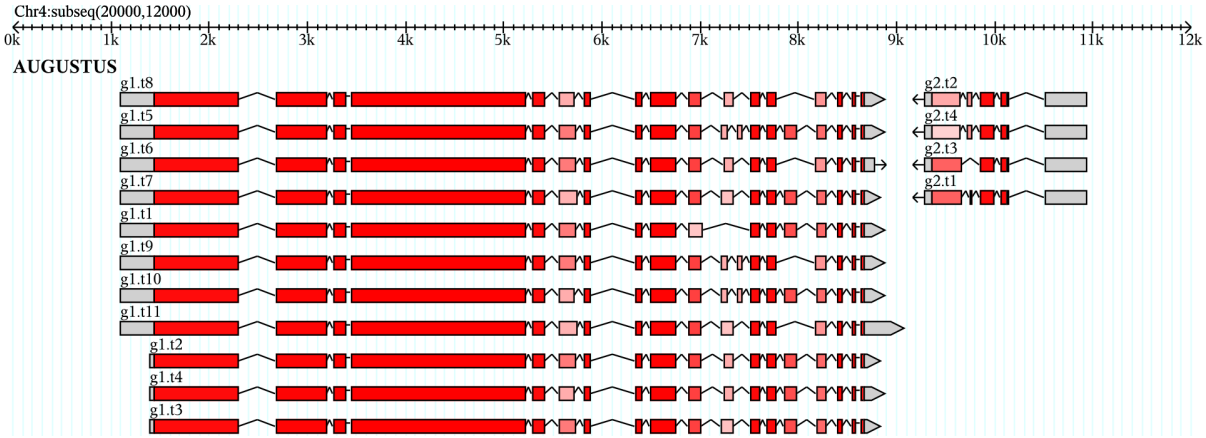
Peptide from Tandem Mass Spectrometry (MS/MS) for Gene Prediction

- unknown proteins of interest cleaved into smaller peptides
- masses of peptides measured with a mass spectrometer
- masses compared *in silico* with peptides from a candidate protein database
- result: set of peptides that is actually translated

Candidate Protein Database

should be very inclusive

1. six-frame translation of genome
2. known genes
3. predicted genes:
   - sample many gene structures with AUGUSTUS

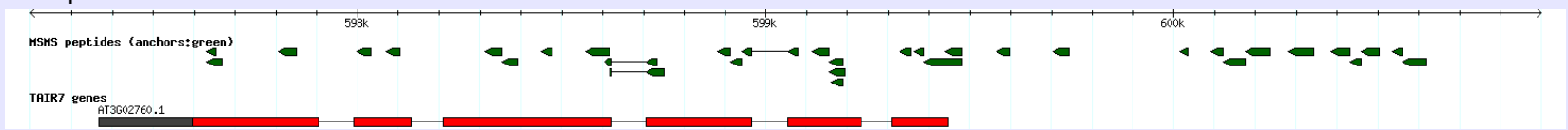


Map Peptides to Genome

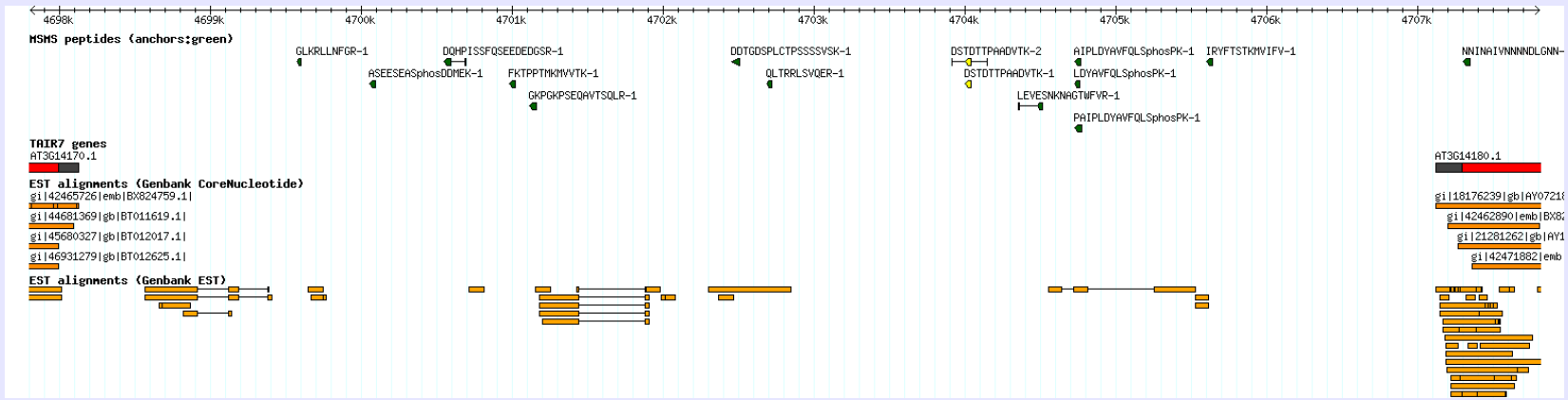
Peptides can

- match protein from set of “known genes”: Partially confirm gene structure, existence of gene
- not match any known gene: What then?
  Extend known gene? Join two known genes? New splice form?
  Replace splice form of “known” gene?

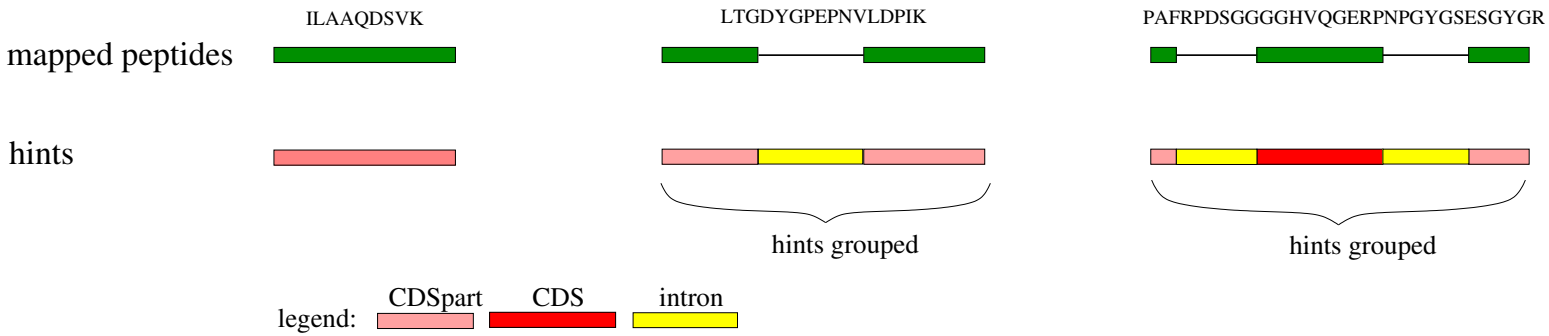
example 1:



example 2:



Peptides to "Hints"



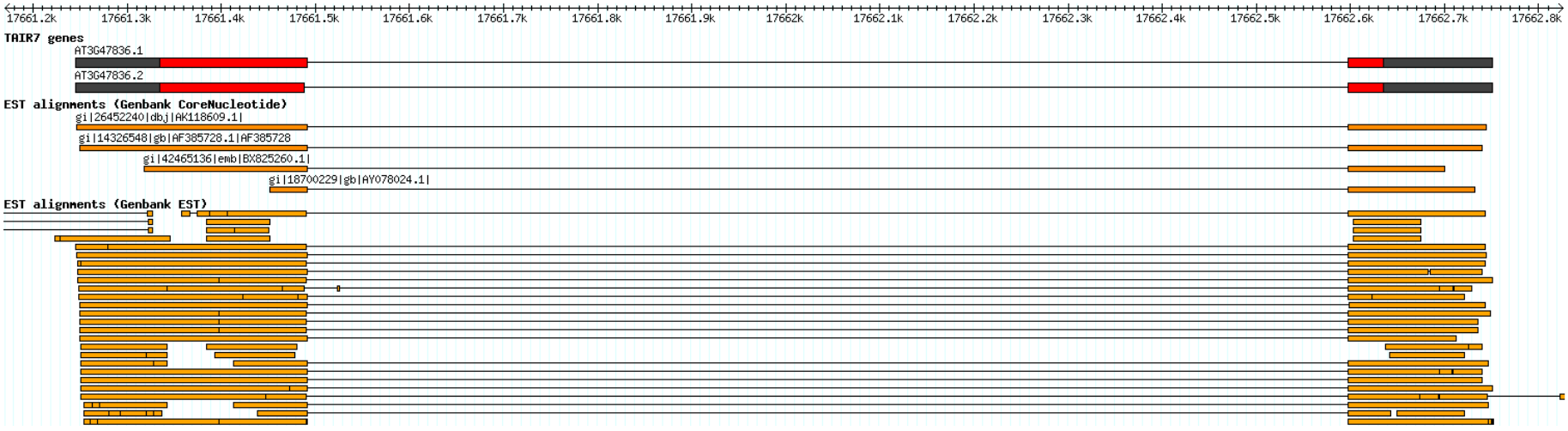
(average peptide length: 15.2)

CDSpart hint:	region is likely protein-coding
CDS hint:	region is exact protein-coding exon
intron hint:	region is likely an intron with exact boundaries

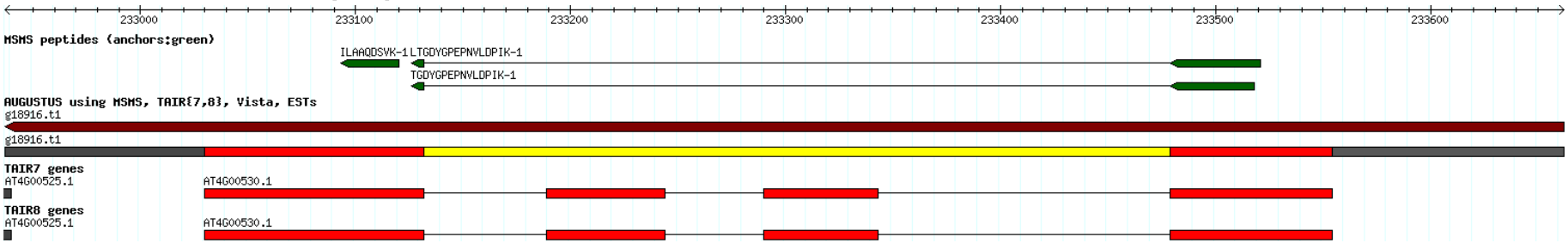
Hints can include strand and frame information.

Examples for Gene Corrections

Who would have doubted this gene?



precious *spliced* peptides



Conclusions on Proteogenomic Evidence

MS/MS

- peptides not only good for “verification” of given gene structures but also valuable, additional orthogonal source of evidence for predicting gene structures
- new translation-level info (reading frame)
- not enough coverage for identification of alternative splicing based on MS/MS alone

Incorporating Protein Homology: "The Pipeline Way"

Use known proteins from other species

1. align proteins to genome
   (e.g. with BLASTX, TBLASTN, Exonerate)
2. use matches from step 1 as evidence for coding regions in gene finding
   (many gene finders)

Disadvantages

- step 1 misses info: no weak homology or many false positives
- gene finders as subject to split gene errors
- protein family MSAs hold more info than individual proteins

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Protein Homology in One Step

Genewise (Birney 2004)

- marry (multiply) two HMMs:
  - simple (species-unspecific) HMM for gene prediction with
  - profile HMM for protein family (like HMMer)
- one big (product) HMM that does both:
  - gene finding and
  - protein to protein-family alignment + membership decision

Disadvantages

- slow
- poor performance for weak homology
  cannot cope with missing homology
- no integration with other evidence (RNA-Seq, ESTs)

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- poor performance for weak homology
  cannot cope with missing homology
- no integration with other evidence (RNA-Seq, ESTs)

Protein Profile Extension of AUGUSTUS

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A novel hybrid gene prediction method employing protein multiple sequence alignments

Oliver Keller [1,*], Martin Kollmar [2], Mario Stanke [3,*] and Stephan Waack [1]

AUGUSTUS-PPX: Protein Profile eXtension

- find members of given protein family (MSA) in DNA
- seamless extension of AUGUSTUS:
  - same program, same species parameters, etc.
  - UTR prediction possible
  - additional hints integration possible, e.g. RNA-Seq
  - defaults to normal AUGUSTUS for non-member genes and gene parts without homology

AUGUSTUS-PPX

Block

Ungapped and highly conserved section of a MSA

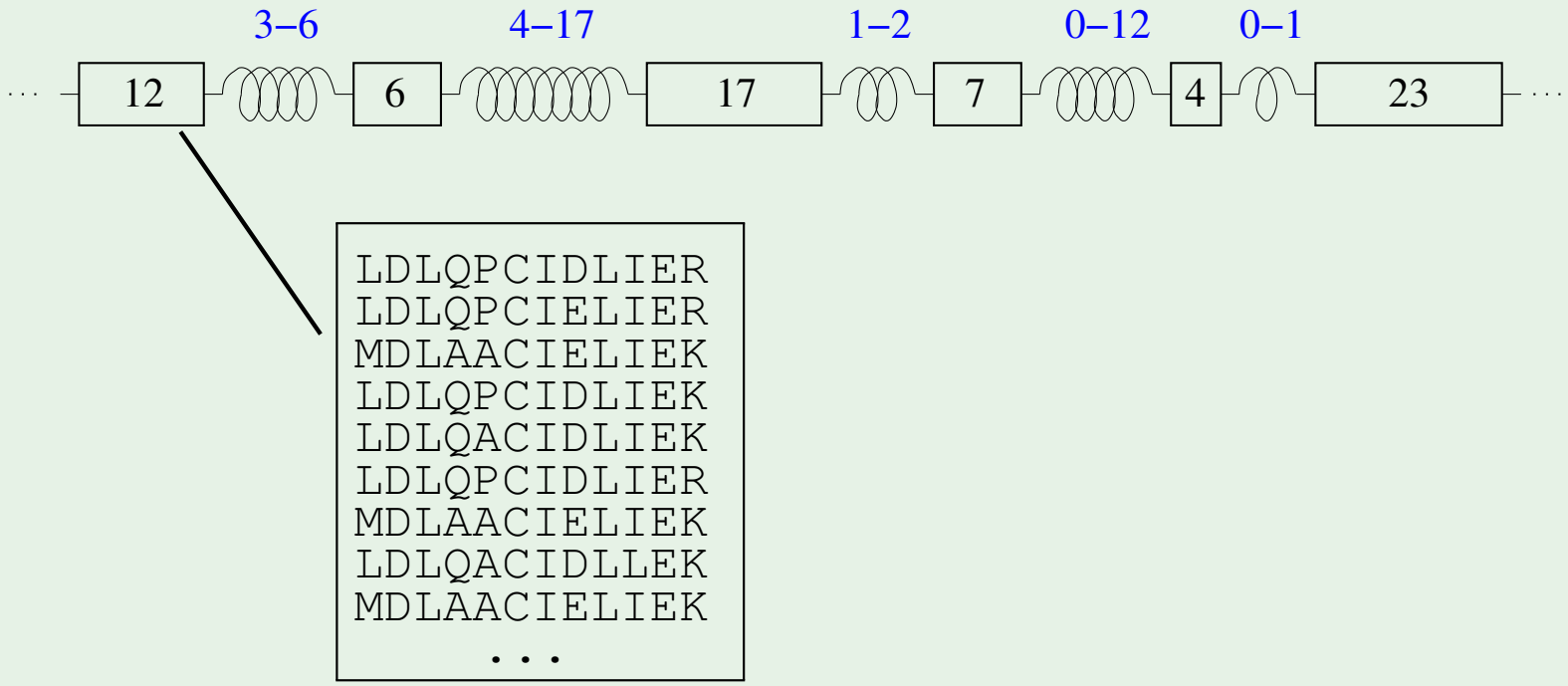
Example

AUGUSTUS-PPX

Block Profile

- frequency table for each block column
- distances between blocks constrained by intervals $[d_{\text{min}}, d_{\text{max}}]$
- no insertions or deletions
- order of blocks preserved

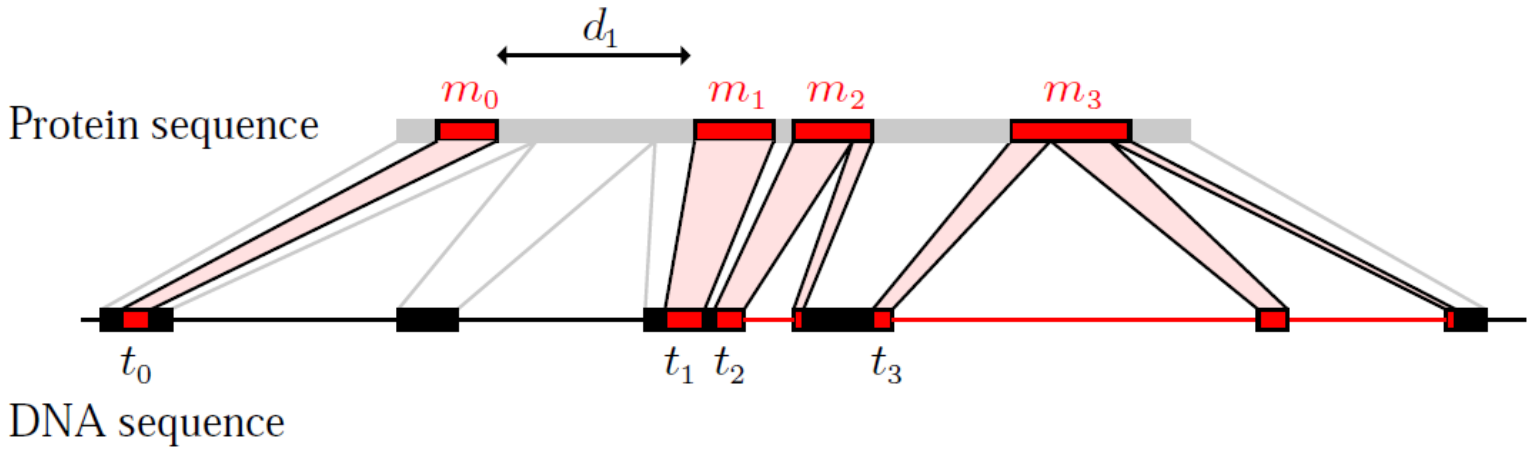
Example (Myosins)



AUGUSTUS-PPX

Problem:

Find gene structure in genome under hypothesis, that predicted protein sequence fits into family.

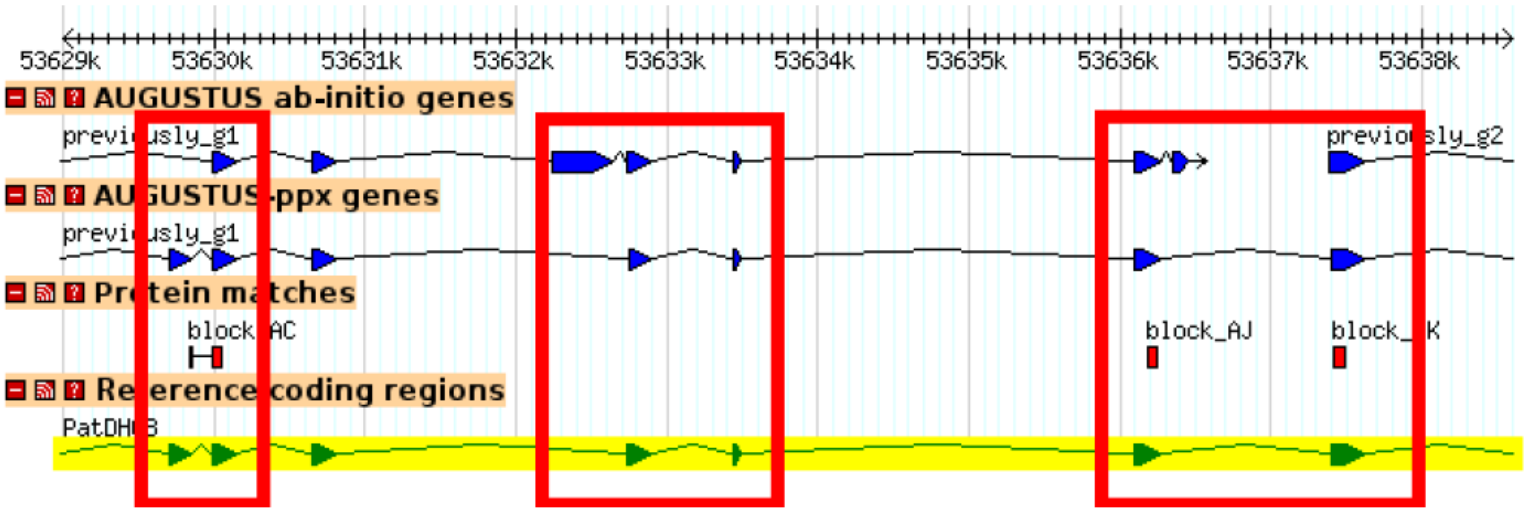


Scoring

$$\begin{aligned} & \text{score}(\text{gene structure}, \text{DNA}, \text{block profile}) \\ = \; & \text{score}(\text{gene structure}, \text{DNA}) \\ & \cdot \text{score}(\text{block profile} \mid \underbrace{\text{gene structure}, \text{DNA}}_{\rightarrow \text{protein sequence cand.}}) \end{aligned}$$

AUGUSTUS-PPX: How it helps

Example



Corrections w.r.t. ab initio

- add exons containing blocks
- remove false positive exons (constraint on inter-block lengths)
- join split genes

AUGUSTUS-PPX: Results

Test on: Dynein Heavy Chain (DHC) family

- > 4000 amino acids, up to 100 exons, several 100 000 bp genomic range
- 42 Blocks with 1214 sites

Accuracy Results

species	AUGUSTUS-PPX		AUG.	Genewise	
	full	ex-ortho	ab-initio	full	ex-ortho
highly accurate genes (%)					
human	62.5	62.5	31.3	93.8	6.3
mouse	72.2	61.1	22.2	55.6	0.0
chicken	58.3	50.0	8.3	16.7	0.0
frog	44.4	38.9	0.0	11.1	0.0
zebrafish	57.1	35.7	57.1	14.3	0.0
snail	44.4	44.4	5.6	11.1	0.0
total	56.3	49.0	11.5	34.4	1.0

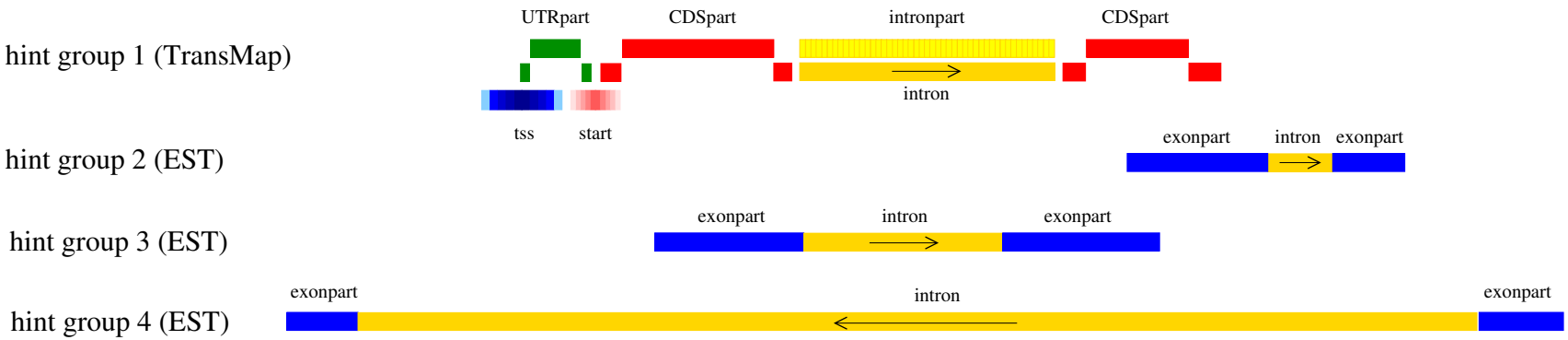
Evidence Suggests Alternative Splicing

AUGUSTUS: Alternative Transcripts Based on Evidence

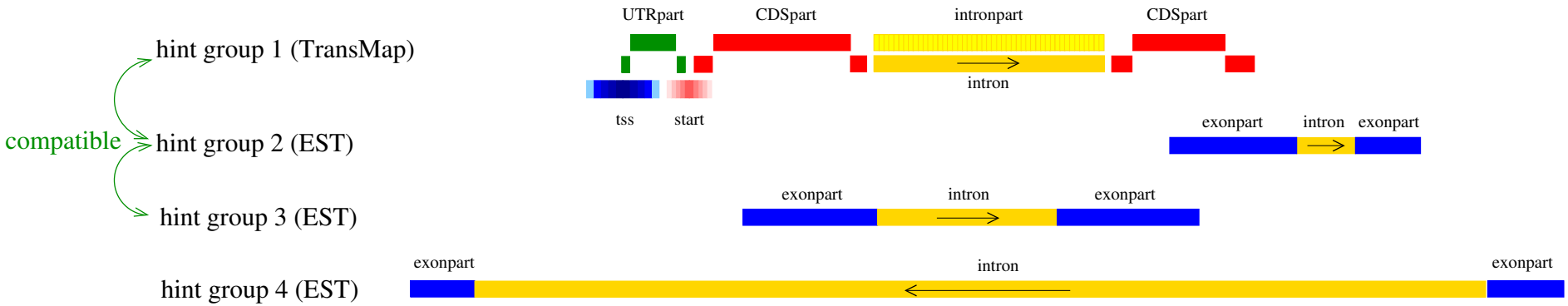
Hints can be grouped to tell AUGUSTUS they are coming from the same transcript.
For example: All hints from one cDNA alignment belong to one group. Based on evidence predict alternative splice forms.

1. Split input sequence between genes based on hint groups and preliminary gene prediction. For each piece, do:
2. Determine compatibility between hint groups.
3. Filter out outlier hint groups.
4. For each hint group g, do:
    1. Deactivate all hint groups h that are incompatible with g.
    2. Run Viterbi algorithm to predict single-transcript genes.
5. Join transcripts to genes

Alternative Transcripts Based on Evidence

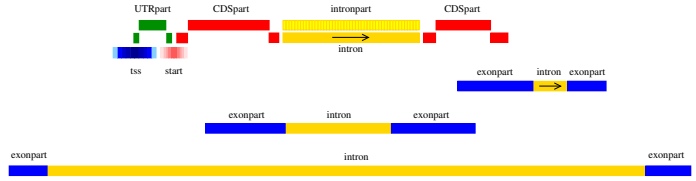


Alternative Transcripts Based on Evidence



Alternative Transcripts Based on Evidence

prediction run 1



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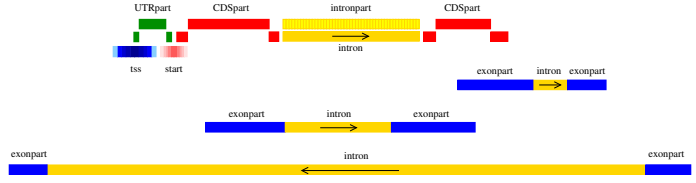
prediction run 2



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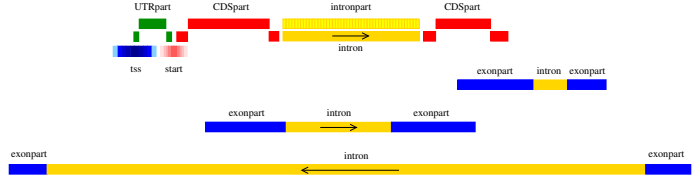
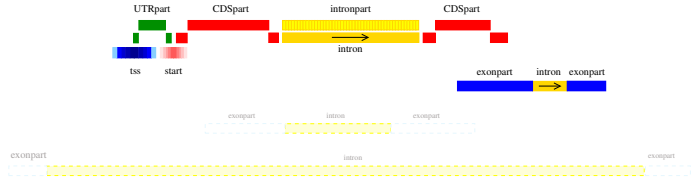
prediction run 3



Alternative Transcripts Based on Evidence

prediction
run 1



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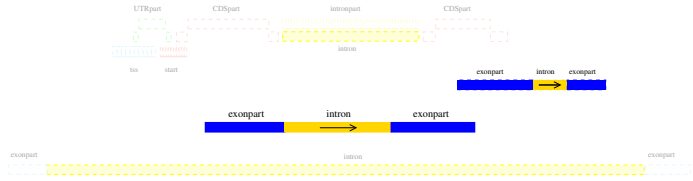
prediction
run 2



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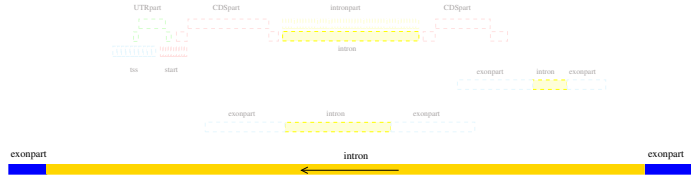
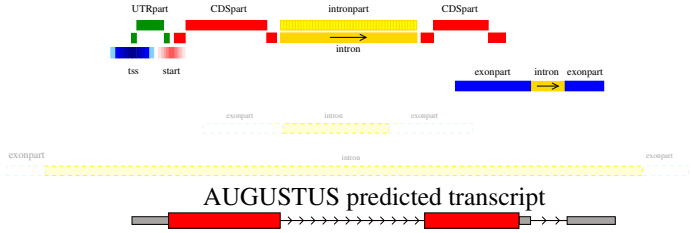
prediction
run 3

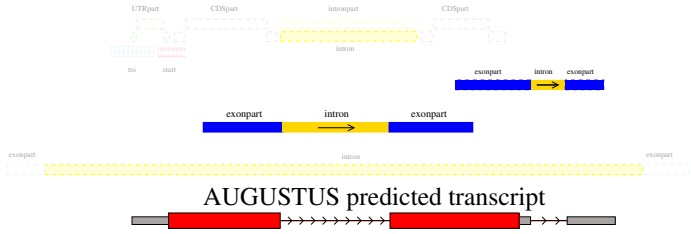


Alternative Transcripts Based on Evidence

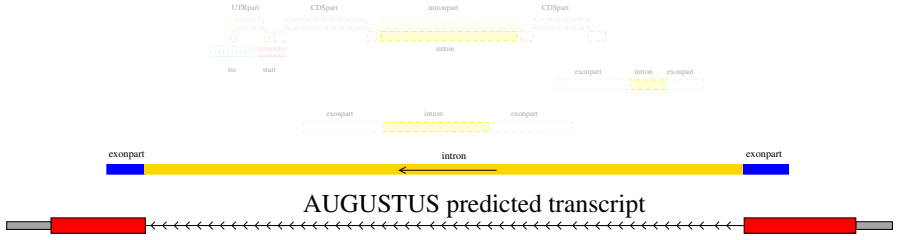
prediction run 1



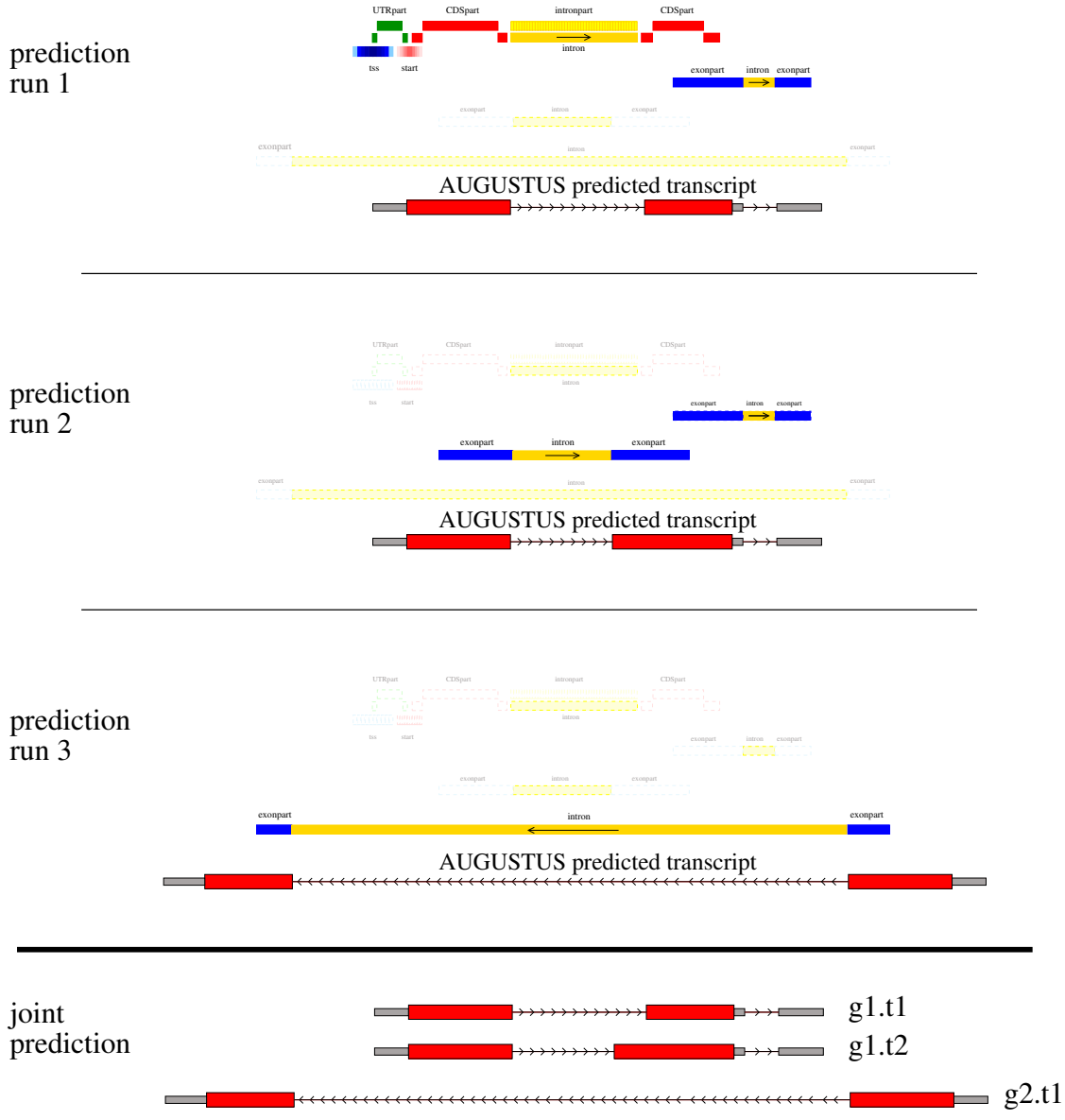
prediction run 2



prediction run 3

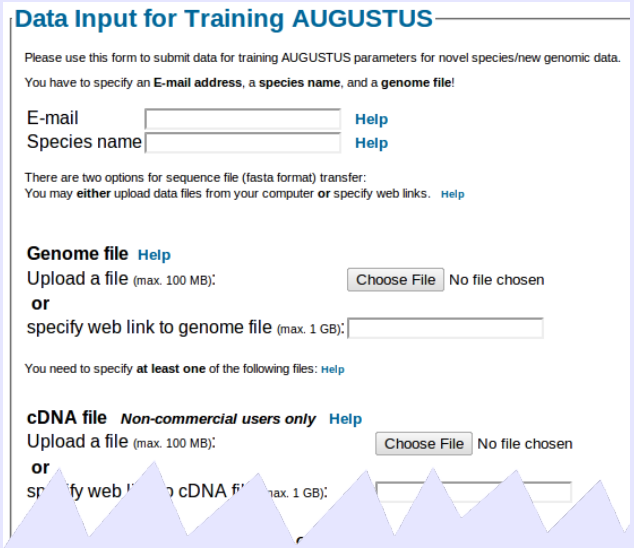


Alternative Transcripts Based on Evidence

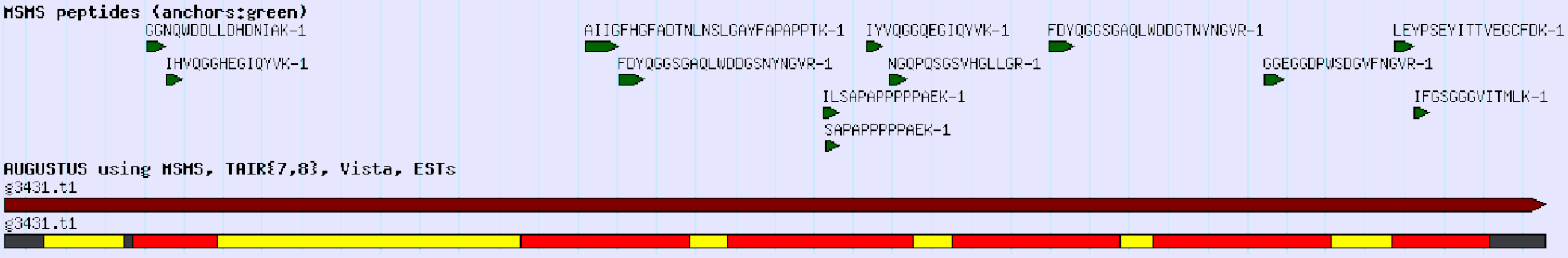


What else?

- new training web server:
  `http://bioinf.uni-greifswald.de`
  - upload genome & transcriptome (currently, ESTs & 454 only)
  - get annotation & trained AUGUSTUS parameter set



- use other evidence
  - e.g. peptides from MS/MS (Natalie's talk)



  - AUGUSTUS-PPX extension (predicts protein family members in genome)

Acknowledgements

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