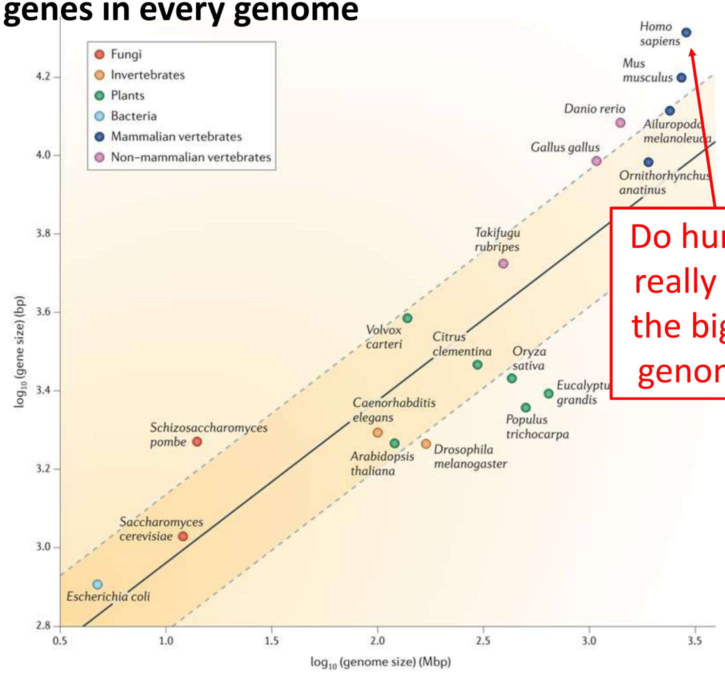
Gene Finding

BCH364C/391L Systems Biology / Bioinformatics – Spring 2015

Edward Marcotte, Univ of Texas at Austin

Lots of genes in every genome

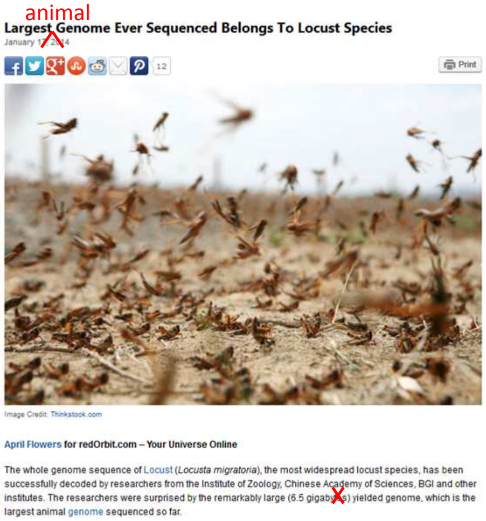


Do humans really have the biggest genomes?

Nature Reviews Genetics 13:329-342 (2012)

Lots of genes in every genome

animal



Largest Genome Ever Sequenced Belongs To Locust Species

January 17, 2014

Image Credit: Thinkstock.com

April Flowers for redOrbit.com – Your Universe Online

The whole genome sequence of Locust (*Locusta migratoria*), the most widespread locust species, has been successfully decoded by researchers from the Institute of Zoology, Chinese Academy of Sciences, BGI and other institutes. The researchers were surprised by the remarkably large (6.5 gigabytes) yielded genome, which is the largest animal genome sequenced so far.

6.5 Gb
(2X human)
17K genes

gigabases (not gigabytes)

World's largest genome belongs to slow-growing mountain flower

An unremarkable and slow-growing plant has stunned scientists after they found it had the world's largest genome – 50 times bigger than that of our own species.

The DNA contained within Paris japonica dwarves all other plant and animal genomes that have been analysed so far Photo: CLIVE NICHOLS

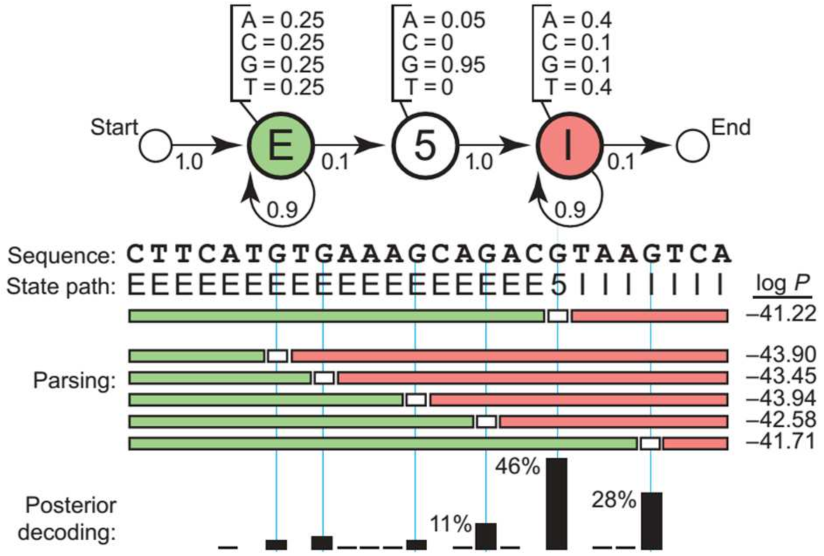
Where are the genes? How can we find them?

```
GATCACTTGATAAATGGGCTGAAGTAACTCGCCCAGATGAGGAGTGTGCTGCCTCCAGAAT
CCAAACAGGCCCACTAGGCCCGAGACACCTTGTCTCAGATGAAACTTTGGACTCGGAATT
TTGAGTTAATGCCGGAATGAGTTCAGACTTTGGGGGACTGTTGGGAAGGCATGATTGGTT
TCAAAATGTGAGAAGGACATGAGATTTGGGAGGGGCTGGGGGCAGAATGATATAGTTTG
GCTCTGCGTCCCCACCCAATCTCATGTCAAATTGTAATCCTCATGTGTCAGGGGAGAGGCCT
GGTGGGATGTGATTGGATCATGGGAGTGGATTTCCCTCTTGCAGTTCTCGTGATAGTGAGT
GAGTTCTCACGAGATCTGGTTGTTTGAAAGTGTGCAGCTCCTCCCCCTTCGCGCTCTCTCTC
TCCCCTGCTCCACCATGGTGAGACGTGCTTGCGTCCCCTTTGCCTTCTGCCATGATTGTAAG
CTTCCTCAGGCGTCCTAGCCACGCTTCCTGTACAGCCTGAGGAACTGGGAGTCAATGAAA
CCTCTTCTCTTCATAAATTACCCAGTTTCAGGTAGTTCTTTCTAGCAGTGTGATAATGGACGA
TACAAGTAGAGACTGAGATCAATAGCATTTGCACTGGGCCTGGAACACACTGTTAAGAAC
GTAAGAGCTATTGCTGTCATTAGTAATATTCTGTATTATTGGCAACATCATCACAATACACTGC
TGTGGGAGGGTCTGAGATACTTCTTTGCAGACTCCAATATTTGTCAAAACATAAAATCAGG
AGCCTCATGAATAGTGTTTAAATTTTTACATAATAATACATTGCACCATTTGGTATATGAGTCT
TTTTGAAATGGTATATGCAGGACGGTTTCCTAATATACAGAATCAGGTACACCTCCTCTTCCA
TCAGTGCGTGAGTGTGAGGGATTGAATTCCTCTGGTTAGGAGTTAGCTGGCTGGGGGTTC
TACTGCTGTTGTTACCCACAGTGCACCTCAGACTCACGTTTCTCCAGCAATGAGCTCCTGTT
CCCTGCACTTAGAGAAGTCAGCCCGGGGACCAGACGGTTCTCTCCTCTTGCCTGCTCCAG
CCTTGGCCTTCAGCAGTCTGGATGCCTATGACACAGAGGGCATCCTCCCCAAGCCCTGGTC
CTTCTGTGAGTGGTGAGTTGCTGTTAATCCAAAAGGACAGGTGAAAACATGAAAGCC…
```

A toy HMM for 5′ splice site recognition (fron

Remember this?

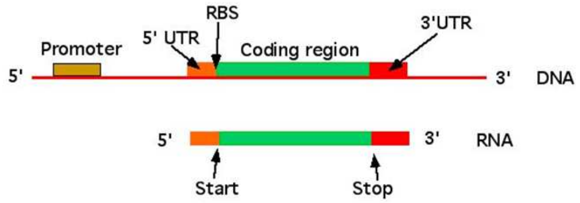
linked on the course web page)



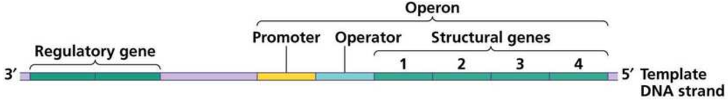
Let’s start with prokaryotic genes

What elements should we build into an HMM to find bacterial genes?

Let’s start with prokaryotic genes



Can be polycistronic:



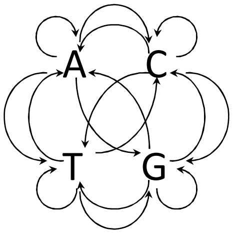
Copyright © 2006 Pearson Education, Inc., publishing as Benjamin Cummings.

http://nitro.biosci.arizona.edu/courses/EEB600A-2003/lectures/lecture24/lecture24.html

A CpG island model might look like:

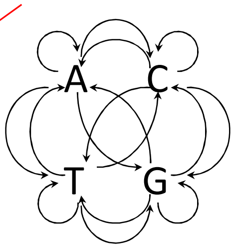
Remember this?

(of course, need the parameters, but maybe these are the most important….)



p(C→G) is higher

CpG island model

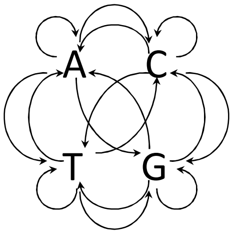


p(C→G) is lower

Not CpG island model

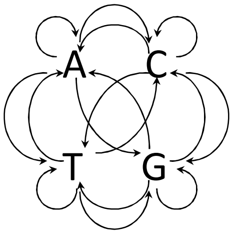
Could calculate $$\frac{P(\,X \mid \text{CpG island})}{P(\,X \mid \text{not CpG island})}$$ (or log ratio) along a sliding window, just like the fair/biased coin test

One way to build a minimal gene finding Markov model



Transition probabilities reflect codons

Coding DNA model



Transition probabilities reflect intergenic DNA

Intergenic DNA model

Could calculate $$\frac{P(\,X \mid \text{coding})}{P(\,X \mid \text{not coding})}$$ (or log ratio) along a sliding window, just like the fair/biased coin test

Really, we'll want to detect codons.
The usual trick is to use a *higher-order Markov process*.

A standard Markov process only considers the current position in calculating transition probabilities.

An *nth-order Markov process* takes into account the past *n* nucleotides, e.g. as for a 5th order:

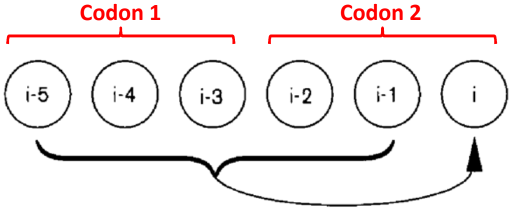
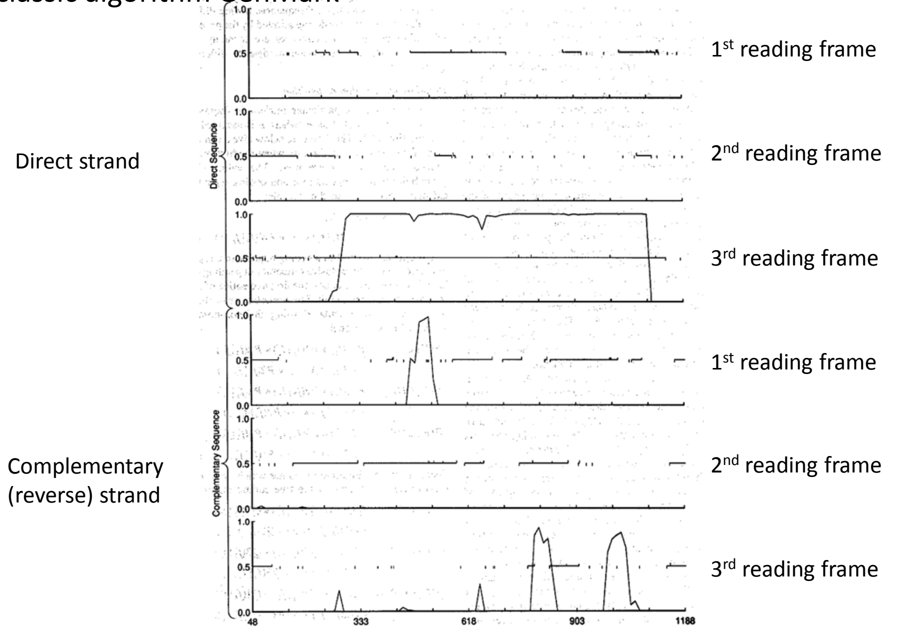


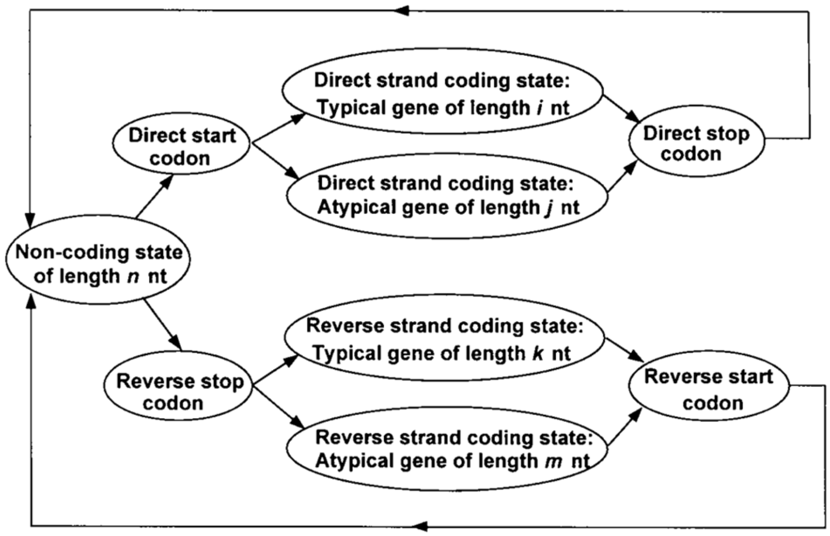
Image from *Curr Op Struct Biol* 8:346-354 (1998)

5th order Markov chain, using models of coding vs. non-coding using the classic algorithm GenMark



Computers Chem. Vol. 17, No. 2, pp. 123–133, 1993

An HMM version of GenMark

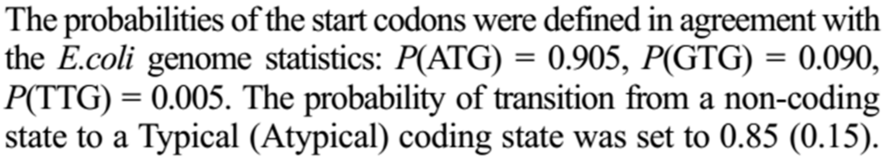


GeneMark.hmm: new solutions for gene finding

Alexander V. Lukashin and Mark Borodovsky[1,*]

Nucleic Acids Research, 1998, Vol. 26, No. 4 1107–1115

For example, accounting for variation in start codons…



The probabilities of the start codons were defined in agreement with the *E.coli* genome statistics: $P(\text{ATG}) = 0.905$, $P(\text{GTG}) = 0.090$, $P(\text{TTG}) = 0.005$. The probability of transition from a non-coding state to a Typical (Atypical) coding state was set to 0.85 (0.15).

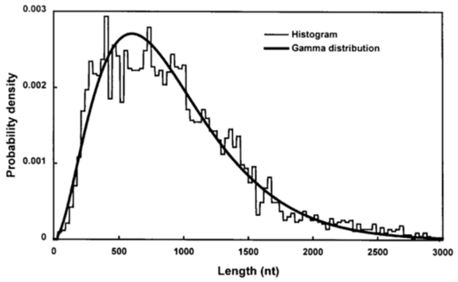
GeneMark.hmm: new solutions for gene finding

Alexander V. Lukashin and Mark Borodovsky[1,*]

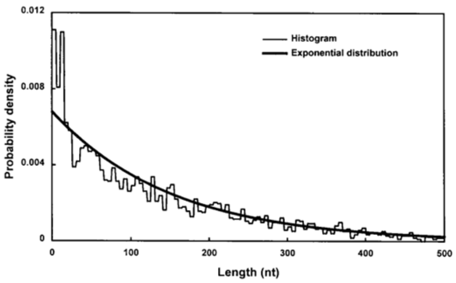
Nucleic Acids Research, 1998, Vol. 26, No. 4 1107–1115

… and variation in gene lengths

Length distributions (in # of nucleotides)



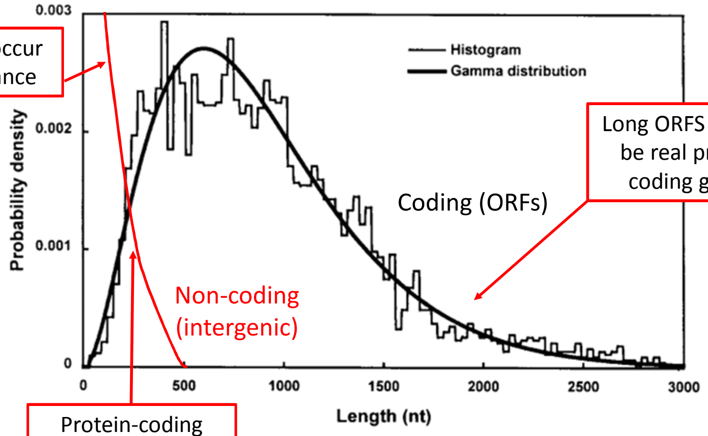
Coding (ORFs)



Non-coding (intergenic)

GeneMark.hmm: new solutions for gene finding
Alexander V. Lukashin and Mark Borodovsky[1,*]
Nucleic Acids Research, 1998, Vol. 26, No. 4 1107–1115

(Placing these curves on top of each other)



Short ORFS occur often by chance

Long ORFS tend to be real protein coding genes

Protein-coding genes <100 aa's are hard to find

GeneMark.hmm: new solutions for gene finding
Alexander V. Lukashin and Mark Borodovsky[1,*]
Nucleic Acids Research, 1998, Vol. 26, No. 4 1107–1115

Model for a ribosome binding site (based on ~300 known RBS's)

Nucleotide	Position 1	2	3	4	5
T	0.161	0.050	0.012	0.071	0.115
C	0.077	0.037	0.012	0.025	0.046
A	**0.681**	0.105	0.015	**0.861**	0.164
G	0.077	**0.808**	**0.960**	0.043	**0.659**

GeneMark.hmm: new solutions for gene finding

Alexander V. Lukashin and Mark Borodovsky[1,*]

Nucleic Acids Research, 1998, Vol. 26, No. 4 ***1107–1115***

How well does it do on well-characterized genomes?

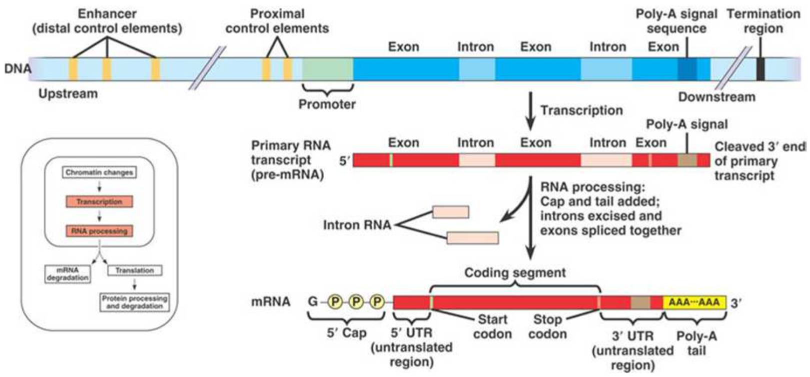
Genome	Genes annotated	Genes predicted	Exact prediction (%)	Missing genes (%)	Wrong genes (%)
A.fulgidus	2407	2530	73.1	10.8 (2.0)	15.1
B.subtilis	4101	4384	77.5	3.6 (2.8)	9.8
E.coli	4288	4440	75.4	5.0 (2.7)	8.2
H.influenzae	1718	1840	86.7	3.8 (3.2)	10.2
H.pylori	1566	1612	79.7	6.0 (4.4)	8.7
M.genitalium	467	509	78.4	9.9 (1.7)	17.3
M.jannaschii	1680	1841	72.7	4.6 (0.8)	12.9
M.pneumoniae	678	734	70.1	7.8 (4.1)	13.6
M.thermoauthotrophicum	1869	1944	70.9	5.0 (3.5)	8.6
Synechocystis	3169	3360	89.6	4.0 (1.5)	9.4
Averaged	21 943	23 194	78.1	5.4 (2.7)	10.4

But this was a long time ago!

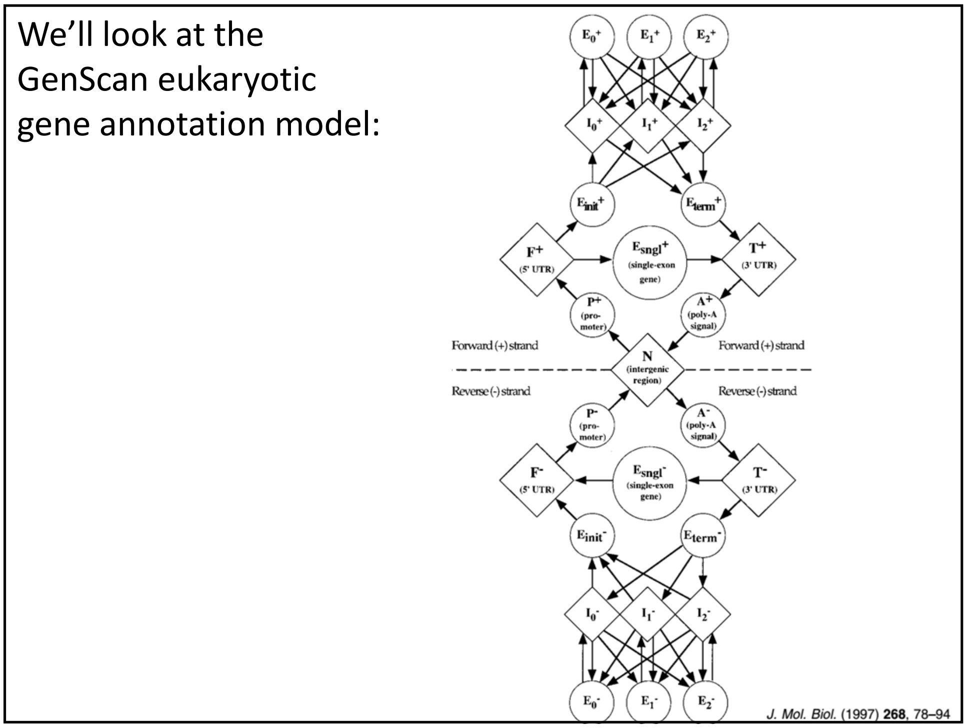
Eukaryotic genes

What elements should we build into an HMM to find eukaryotic genes?

Eukaryotic genes

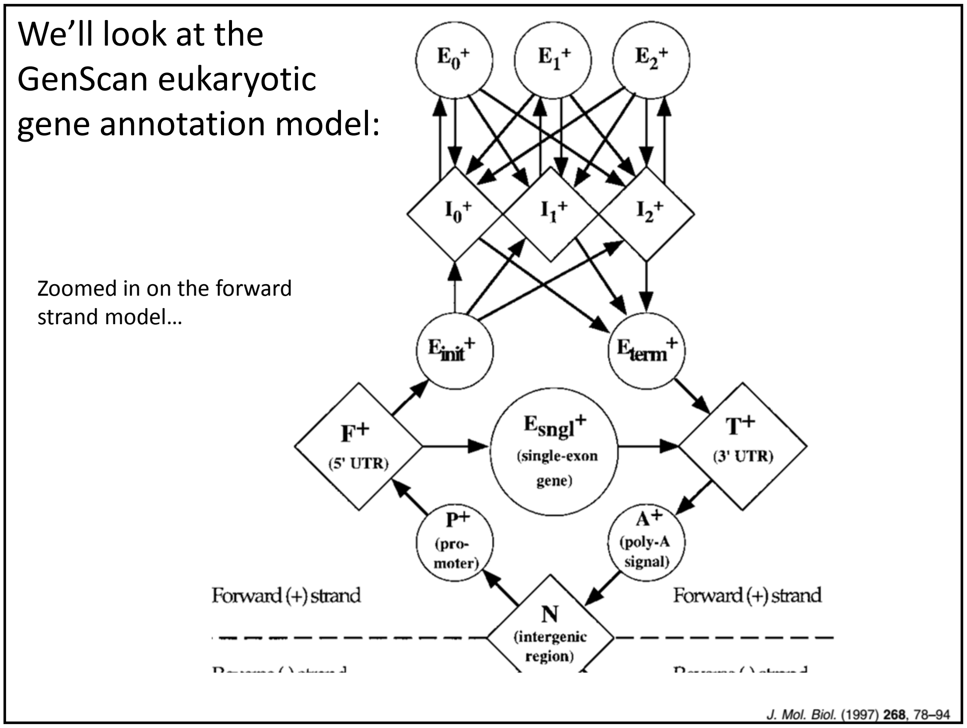


http://greatneck.k12.ny.us/GNPS/SHS/dept/science/krauz/bio_h/Biology_Handouts_Diagrams_Videos.htm



We'll look at the GenScan eukaryotic gene annotation model:

J. Mol. Biol. (1997) **268**, 78–94

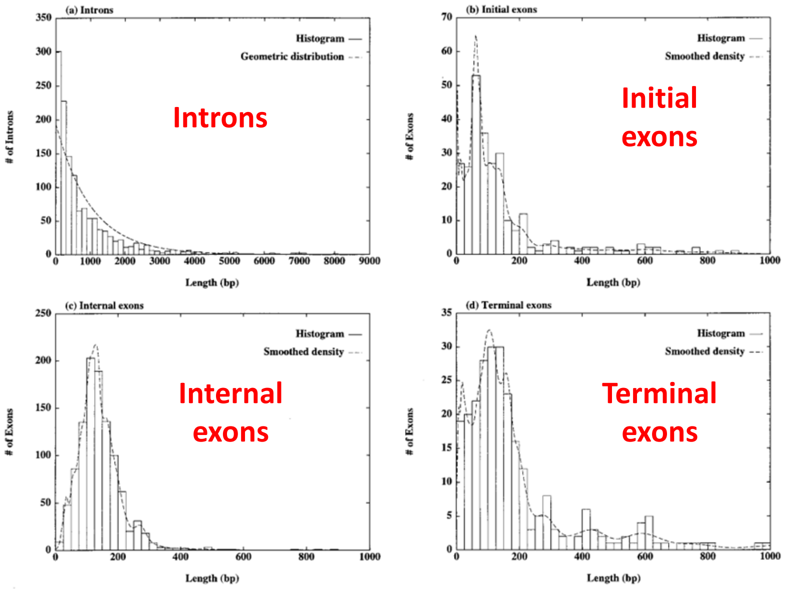


We'll look at the GenScan eukaryotic gene annotation model:

Zoomed in on the forward strand model…

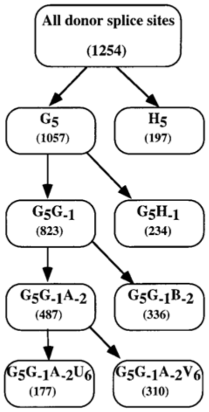
J. Mol. Biol. (1997) **268**, 78–94

Introns and different flavors of exons all have different typical lengths



J. Mol. Biol. (1997) **268**, 78–94

Taking into account donor splice sites



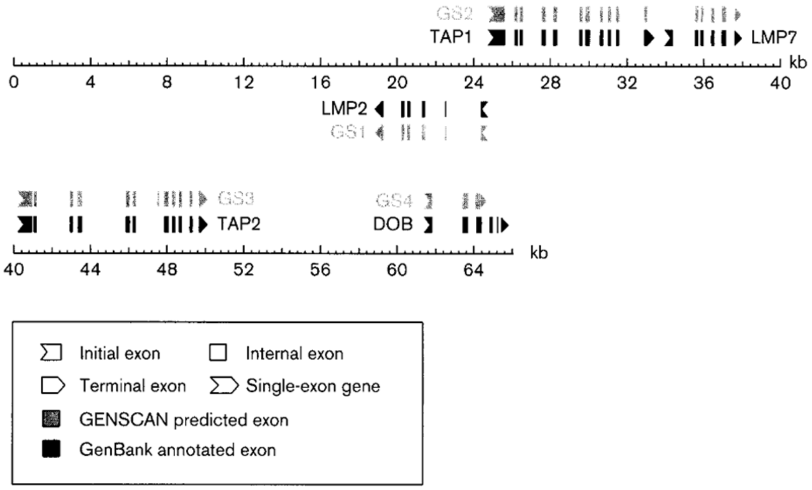
Pos	A%	C%	G%	U%
-3	33	36	19	13
-2	56	15	15	15
-1	9	4	78	9
+3	44	3	51	3
+4	75	4	13	9
+6	14	18	19	49
-3	34	37	18	11
-2	59	10	15	16
+3	40	4	53	3
+4	70	4	16	10
+6	17	21	21	42
-3	37	42	18	3
+3	39	5	51	5
+4	62	5	22	11
+6	19	20	25	36
-3	32	40	23	5
+3	27	4	59	10
+4	51	5	25	19

Pos	A%	C%	G%	U%
-3	35	44	16	6
-2	85	4	7	5
-1	2	1	97	0
+3	81	3	15	2
+4	51	28	9	12
+6	22	20	30	28
-3	29	31	21	18
-2	43	30	17	11
+3	56	0	43	0
+4	93	2	3	3
+6	5	10	10	76
-3	29	30	18	23
+3	42	1	56	1
+4	80	4	8	8
+6	14	21	16	49
-3	39	43	15	2
+3	46	6	46	3
+4	69	5	20	7

All sites:		**Position**									
	Base	**-3**	**-2**	**-1**	**+1**	**+2**	**+3**	**+4**	**+5**	**+6**	
	A%	**33**	**60**	8	0	0	**49**	**71**	6	15	
	C%	**37**	13	4	0	0	3	7	5	19	
	G%	18	14	**81**	**100**	0	**45**	12	**84**	20	
	U%	12	13	7	0	**100**	3	9	5	**46**	
U1 snRNA:	**3'**	**G**	**U**	**C**	**C**	**A**	**U**	**U**	**C**	**A**	**5'**

J. Mol. Biol. (1997) **268**, 78–94

An example of an annotated gene…



<u>Current Opinion in Structural Biology</u> 1998, 8:346–354

How well do these programs work?

We can measure how well an algorithm works using these:

True answer:

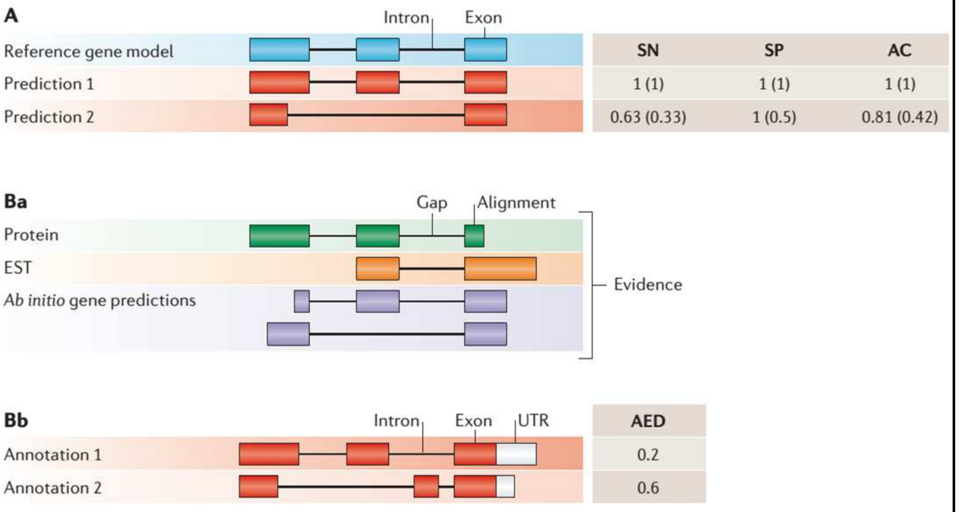
		Positive	Negative
Algorithm predicts:	Positive	True positive	False positive
	Negative	False negative	True negative

Specificity = TP / (TP + FP)

Sensitivity = TP / (TP + FN)

Nature Reviews Genetics 13:329-342 (2012)

How well do these programs work?
How good are our current gene models?

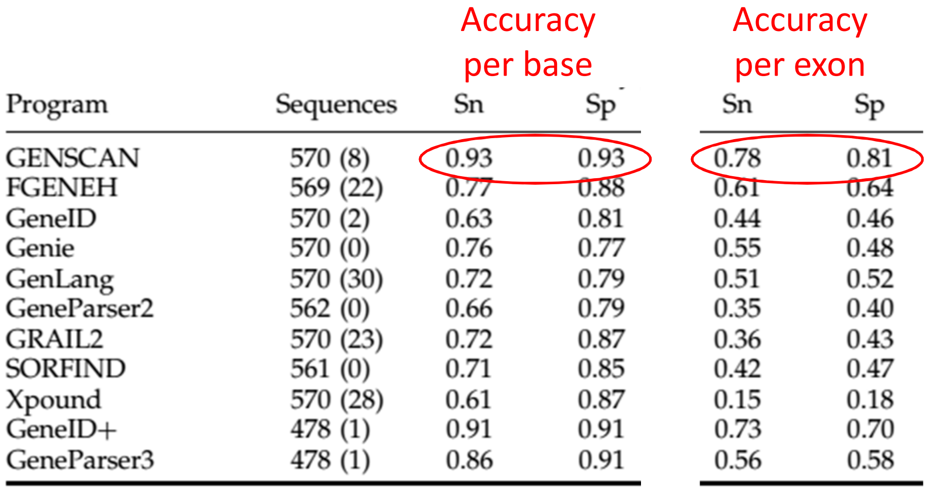


	SN	SP	AC
Prediction 1	1 (1)	1 (1)	1 (1)
Prediction 2	0.63 (0.33)	1 (0.5)	0.81 (0.42)

	AED
Annotation 1	0.2
Annotation 2	0.6

Nature Reviews Genetics 13:329-342 (2012)

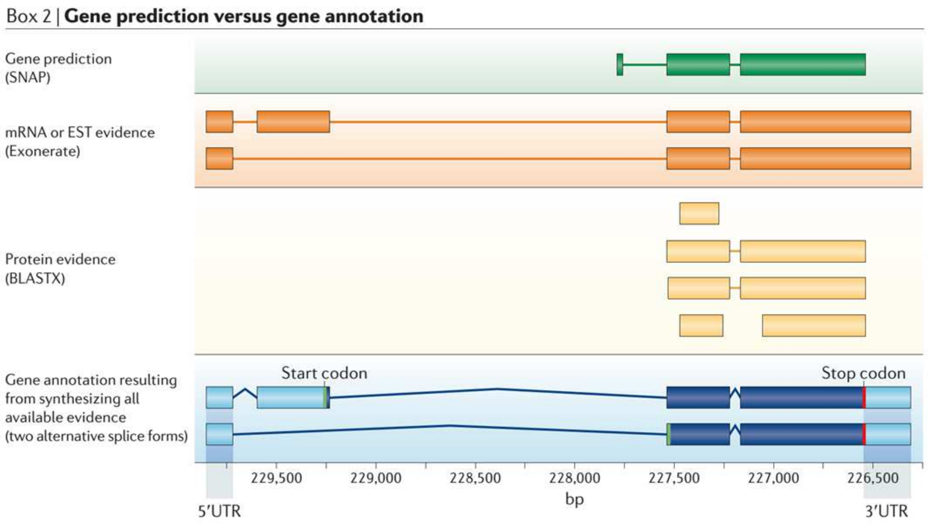
GENSCAN, when it was first developed….



		Accuracy per base		Accuracy per exon	
Program	Sequences	Sn	Sp	Sn	Sp
GENSCAN	570 (8)	0.93	0.93	0.78	0.81
FGENEH	569 (22)	0.77	0.88	0.61	0.64
GeneID	570 (2)	0.63	0.81	0.44	0.46
Genie	570 (0)	0.76	0.77	0.55	0.48
GenLang	570 (30)	0.72	0.79	0.51	0.52
GeneParser2	562 (0)	0.66	0.79	0.35	0.40
GRAIL2	570 (23)	0.72	0.87	0.36	0.43
SORFIND	561 (0)	0.71	0.85	0.42	0.47
Xpound	570 (28)	0.61	0.87	0.15	0.18
GeneID+	478 (1)	0.91	0.91	0.73	0.70
GeneParser3	478 (1)	0.86	0.91	0.56	0.58

J. Mol. Biol. (1997) **268**, 78–94

In general, we can do better with more data, such as mRNA and conservation



Nature Reviews Genetics 13:329-342 (2012)

How well do we know the genes now?

In the year 2000

Genome Annotation Assessment in *Drosophila melanogaster*

= scientists from around the world held a contest ("GASP") to predict genes in part of the fly genome, then compare them to experimentally determined "truth"

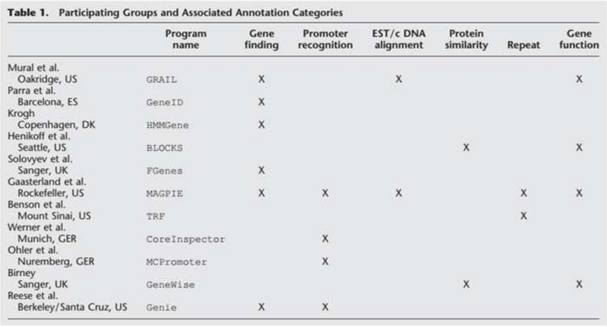


Table 1. Participating Groups and Associated Annotation Categories

	Program name	Gene finding	Promoter recognition	EST/c DNA alignment	Protein similarity	Repeat	Gene function
Mural et al. Oakridge, US	GRAIL	X		X			X
Parra et al. Barcelona, ES	GeneID	X					
Krogh Copenhagen, DK	HMMGene	X					
Henikoff et al. Seattle, US	BLOCKS				X		X
Solovyev et al. Sanger, UK	FGenes	X					
Gaasterland et al. Rockefeller, US	MAGPIE	X	X	X		X	X
Benson et al. Mount Sinai, US	TRF					X	
Werner et al. Munich, GER	CoreInspector		X				
Ohler et al. Nuremberg, GER	MCPromoter		X				
Birney Sanger, UK	GeneWise				X		X
Reese et al. Berkeley/Santa Cruz, US	Genie	X	X				

Genome Research 10:483–501 (2000)

How well do we know the genes now?

In the year 2000

“Over <u>95%</u> of the coding nucleotides … were correctly identified by the majority of the gene finders.”

“…the correct intron/exon structures were predicted for <u>>40%</u> of the genes.”

Most promoters were missed; many were wrong.

“Integrating gene finding and cDNA/EST alignments with promoter predictions decreases the number of false-positive classifications but <u>discovers less than one-third of the promoters in the region</u>.”

Genome Research 10:483–501 (2000)

How well do we know the genes now?

In the year *2006*

EGASP: the **Assessment**
Project

= scientists f SP”) to
predict gene are them to
experimenta

18 groups
36 programs

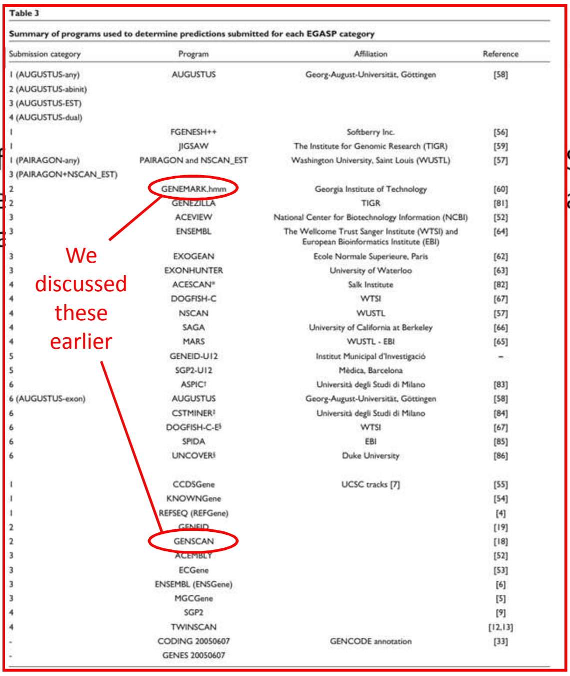


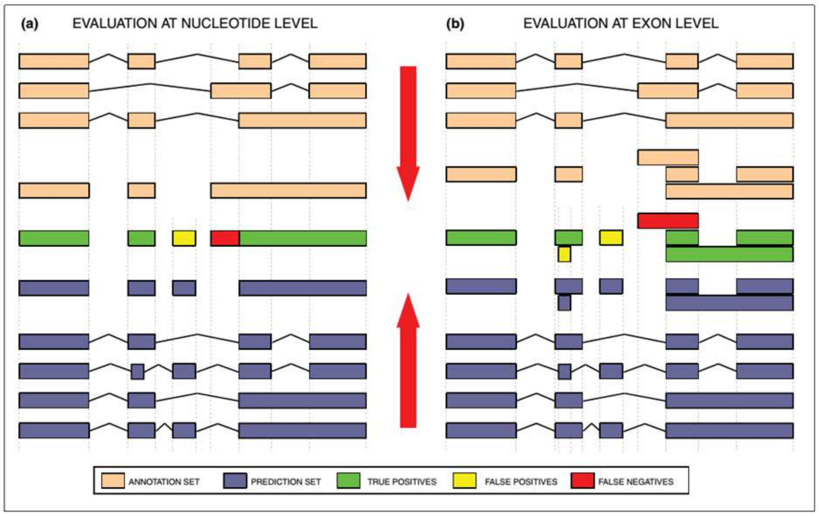
Table 3

Summary of programs used to determine predictions submitted for each EGASP category

Submission category	Program	Affiliation	Reference
1 (AUGUSTUS-any)	AUGUSTUS	Georg-August-Universität, Göttingen	[58]
2 (AUGUSTUS-abinit)			
3 (AUGUSTUS-EST)			
4 (AUGUSTUS-dual)			
1	FGENESH++	Softberry Inc.	[56]
1	JIGSAW	The Institute for Genomic Research (TIGR)	[59]
1 (PAIRAGON-any)	PAIRAGON and NSCAN_EST	Washington University, Saint Louis (WUSTL)	[57]
3 (PAIRAGON+NSCAN_EST)			
2	GENEMARK.hmm	Georgia Institute of Technology	[60]
2	GENEZILLA	TIGR	[81]
3	ACEVIEW	National Center for Biotechnology Information (NCBI)	[52]
3	ENSEMBL	The Wellcome Trust Sanger Institute (WTSI) and European Bioinformatics Institute (EBI)	[64]
3	EXOGEAN	Ecole Normale Superieure, Paris	[62]
3	EXONHUNTER	University of Waterloo	[63]
4	ACESCAN*	Salk Institute	[82]
4	DOGFISH-C	WTSI	[67]
4	NSCAN	WUSTL	[57]
4	SAGA	University of California at Berkeley	[66]
4	MARS	WUSTL - EBI	[65]
5	GENEID-U12	Institut Municipal d'Investigació	–
5	SGP2-U12	Mèdica, Barcelona	
6	ASPIC†	Università degli Studi di Milano	[83]
6 (AUGUSTUS-exon)	AUGUSTUS	Georg-August-Universität, Göttingen	[58]
6	CSTMINER‡	Università degli Studi di Milano	[84]
6	DOGFISH-C-E§	WTSI	[67]
6	SPIDA	EBI	[85]
6	UNCOVER§	Duke University	[86]
1	CCDSGene	UCSC tracks [7]	[55]
1	KNOWNGene		[54]
1	REFSEQ (REFGene)		[4]
2	GENEID		[19]
2	GENSCAN		[18]
3	ACEMBLY		[52]
3	ECGene		[53]
3	ENSEMBL (ENSGene)		[6]
3	MGCGene		[5]
4	SGP2		[9]
4	TWINSCAN		[12,13]
-	CODING 20050607	GENCODE annotation	[33]
-	GENES 20050607		

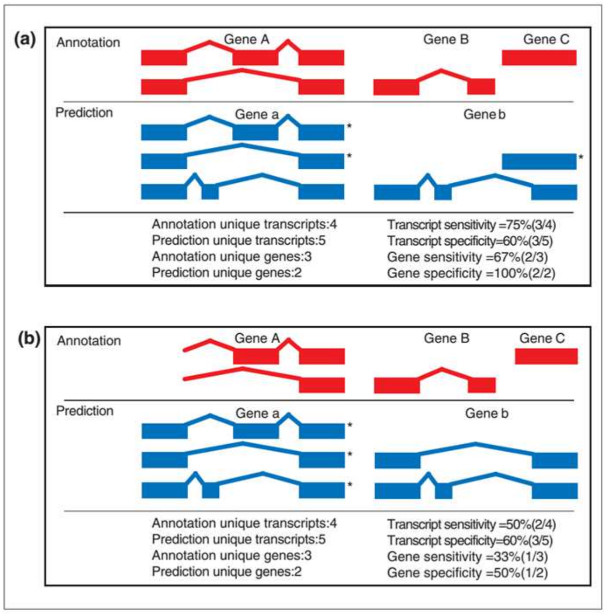
We discussed these earlier

*Genome **Biology*** 2006, **7(Suppl 1):**S2



Genome **Biology** 2006, **7(Suppl 1):**S2

Transcripts vs. genes



(a)

Annotation unique transcripts:4
Prediction unique transcripts:5
Annotation unique genes:3
Prediction unique genes:2

Transcript sensitivity =75%(3/4)
Transcript specificity=60%(3/5)
Gene sensitivity =67%(2/3)
Gene specificity =100%(2/2)

(b)

Annotation unique transcripts:4
Prediction unique transcripts:5
Annotation unique genes:3
Prediction unique genes:2

Transcript sensitivity =50%(2/4)
Transcript specificity=60%(3/5)
Gene sensitivity =33%(1/3)
Gene specificity =50%(1/2)

Genome **Biology** 2006, **7(Suppl 1):**S2

In the year *2006*

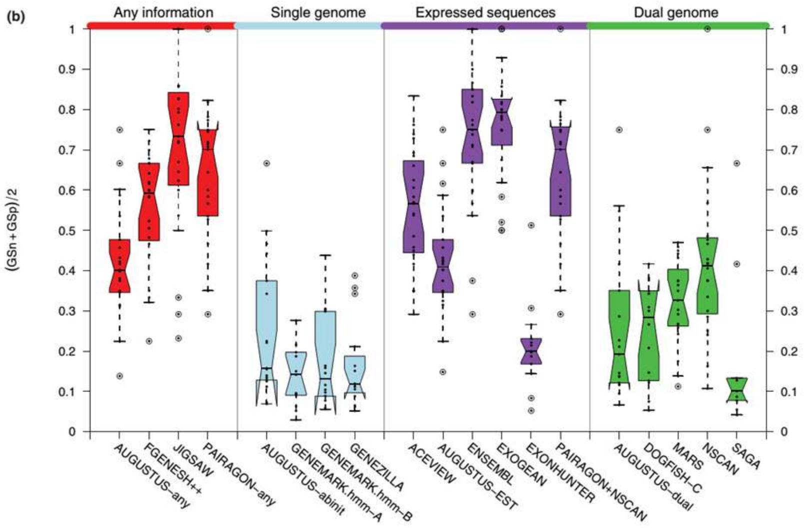
So how did they do?

- “The best methods had <u>at least one gene transcript</u> correctly predicted for close to **70%** of the annotated genes.”
- “…<u>taking into account alternative splicing</u>, … only approximately **40%** to **50%** accuracy.
- At the coding <u>nucleotide</u> level, the best programs reached an accuracy of **90%** in both sensitivity and specificity.”

Genome **Biology** 2006, **7(Suppl 1):**S2

In the year *2006*

At the gene level, most genes have errors



Genome **Biology** 2006, **7(Suppl 1):**S2

How well do we know the genes now?

In the year *2008*

nGASP – the nematode genome annotation assessment project

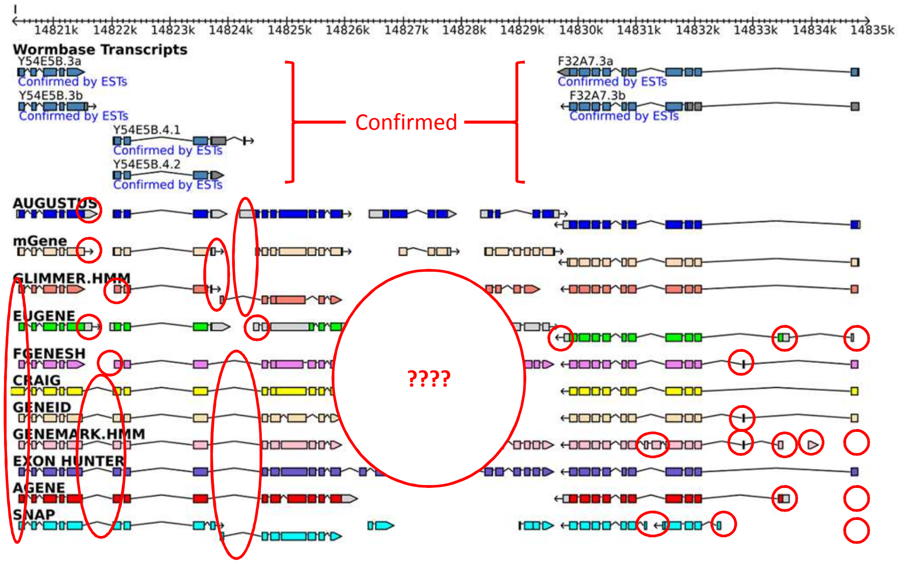
= scientists from around the world held a contest (“NGASP”) to predict genes in part of the worm genome, then compare them to experimentally determined “truth”

- 17 groups from around the world competed
- “Median gene level sensitivity … was **78%**”
- “their specificity was **42%**”, comparable to human

BMC Bioinformatics 2008, **9**:549

For example:

In the year *2008*



BMC Bioinformatics 2008, **9**:549

How well do we know the genes now?

In the year *2012*

GENCODE: The reference human genome annotation for The ENCODE Project

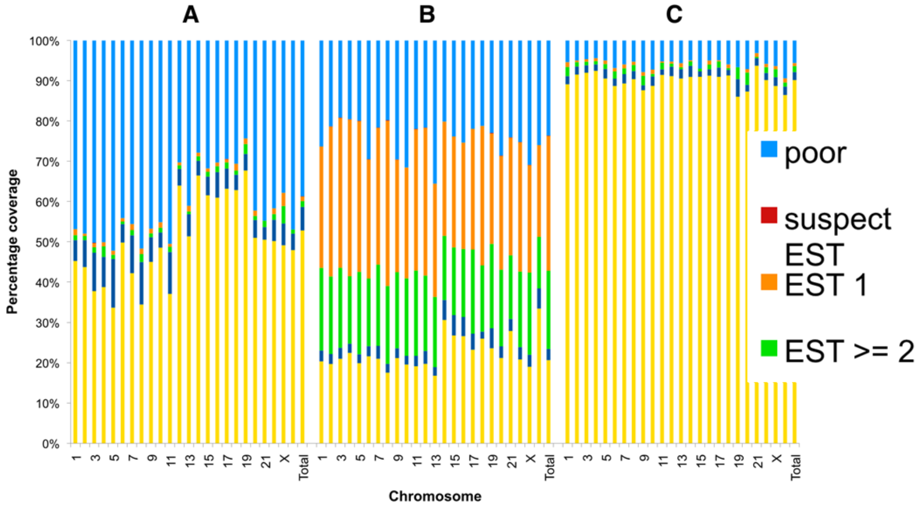
= a large consortium of scientists trying to annotate the human genome using a combination of experiment and prediction.
Best estimate of the current state of human genes.

Genome Res. 2012 22: 1760-1774

How well do we know the genes now?

In the year *2012*

Quality of evidence used to support automatic, manually, and merged annotated transcripts (probably reflective of transcript quality)



23,855 transcripts 89,669 transcripts 22,535 transcripts

Genome Res. 2012 22: 1760-1774

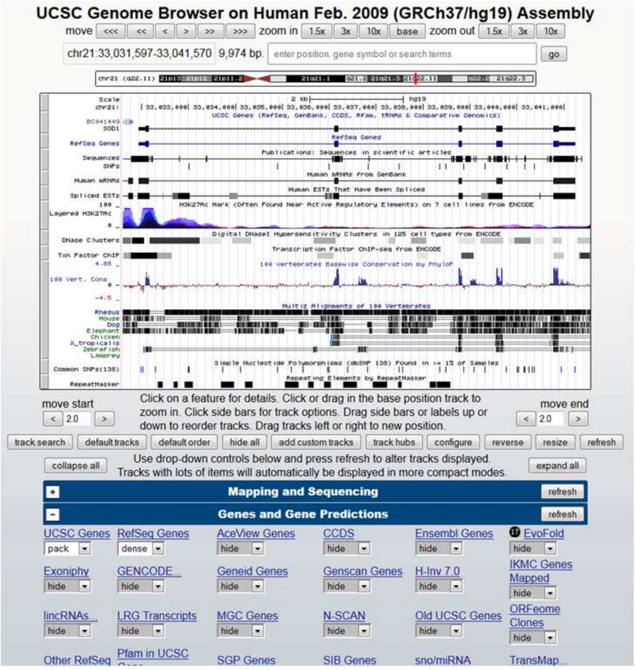
How well do we know the genes now?

In the year *2015*

The bottom line:

- **Gene prediction and annotation are hard**
- **Annotations for all organisms are still buggy**
- **Few genes are 100% correct; expect multiple errors per gene**
- **Most organisms' gene annotations are probably much worse than for humans**

The Univ of California Santa Cruz genome browser



UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly

chr21:33,031,597-33,041,570 9,974 bp.

move start: 2.0

move end: 2.0

Click on a feature for details. Click or drag in the base position track to zoom in. Click side bars for track options. Drag side bars or labels up or down to reorder tracks. Drag tracks left or right to new position.

Use drop-down controls below and press refresh to alter tracks displayed. Tracks with lots of items will automatically be displayed in more compact modes.

UCSC Genes pack	RefSeq Genes dense	AceView Genes hide	CCDS hide	Ensembl Genes hide	EvoFold hide
Exoniphy hide	GENCODE... hide	Geneid Genes hide	Genscan Genes hide	H-Inv 7.0 hide	IKMC Genes Mapped hide
lincRNAs... hide	LRG Transcripts hide	MGC Genes hide	N-SCAN hide	Old UCSC Genes hide	ORFeome Clones hide
Other RefSeq	Pfam in UCSC	SGP Genes	SIB Genes	sno/miRNA	TransMap...

The Univ of California Santa Cruz genome browser