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FACULTY OF ENGINEERING &TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

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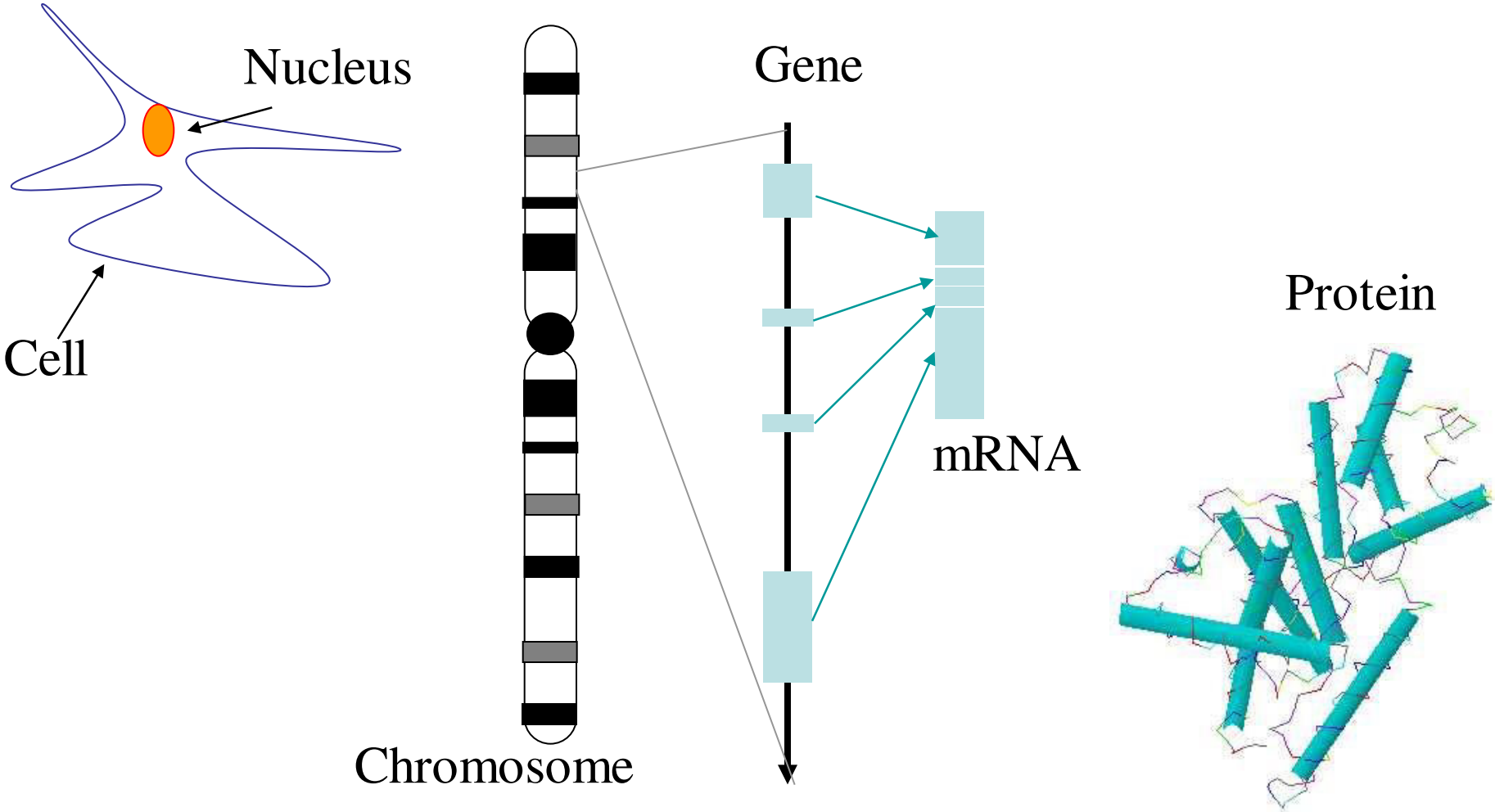
Genome Annotation

Cells, Chromosomes, DNA, and Genes



Definitions

- Unless otherwise stated, annotation refers to prediction of protein-coding genes

- Methods exist to annotate
  - tRNA, rRNA
  - Several other small RNAs
  - Repetitive elements
  - [microRNAs]

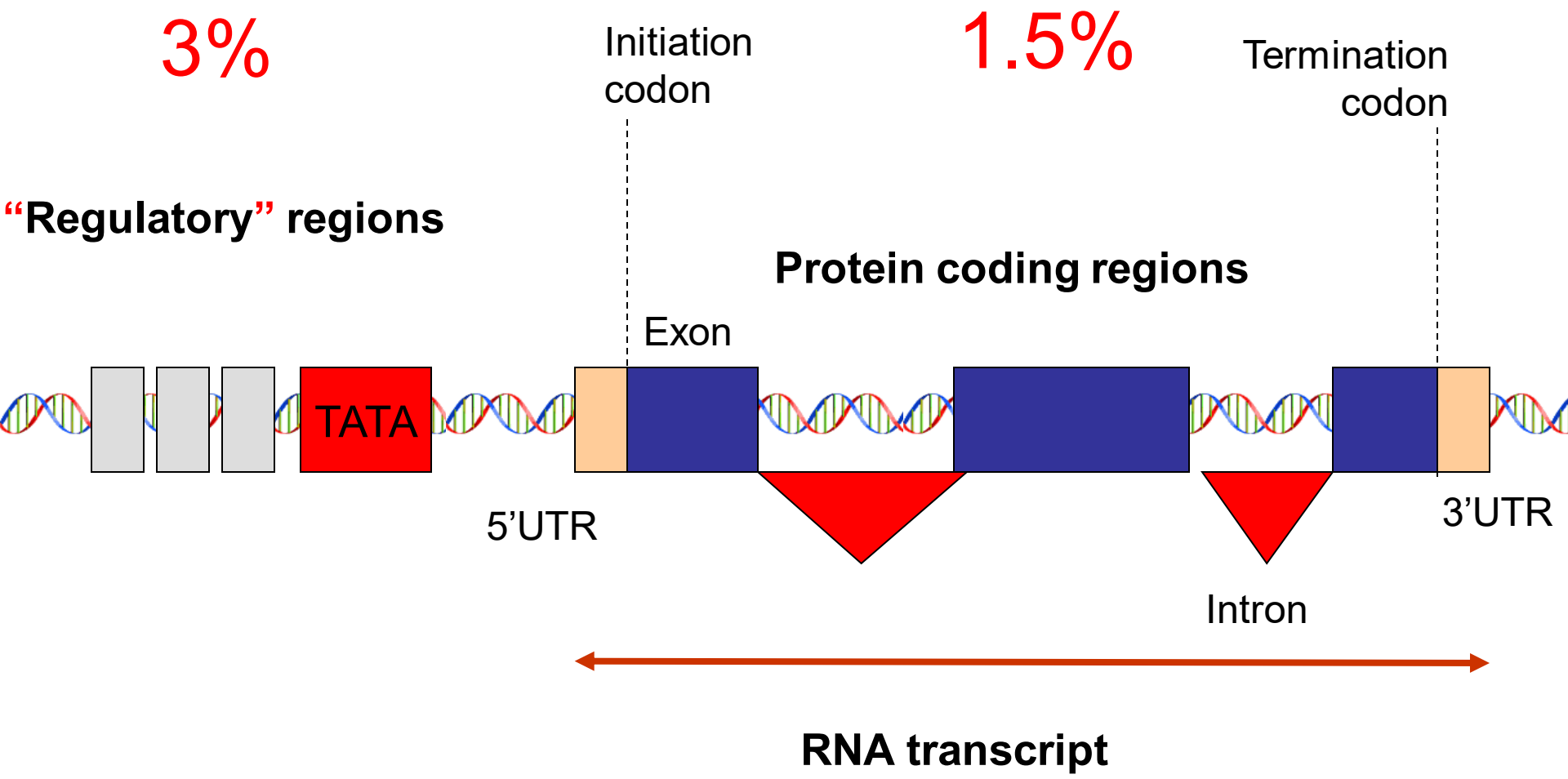
Challenges

- Signal to noise
  - 1% protein coding sequence
  - Pseudogenes
- Splicing
  - Discontinuous nature of eukaryotic genes
  - Alternative splicing
- Non-uniform genome characteristics
  - Range of G+C content
  - Range of mutation rates
  - Gene/Genome segment duplication


1% codes for protein


3% conserved non-coding

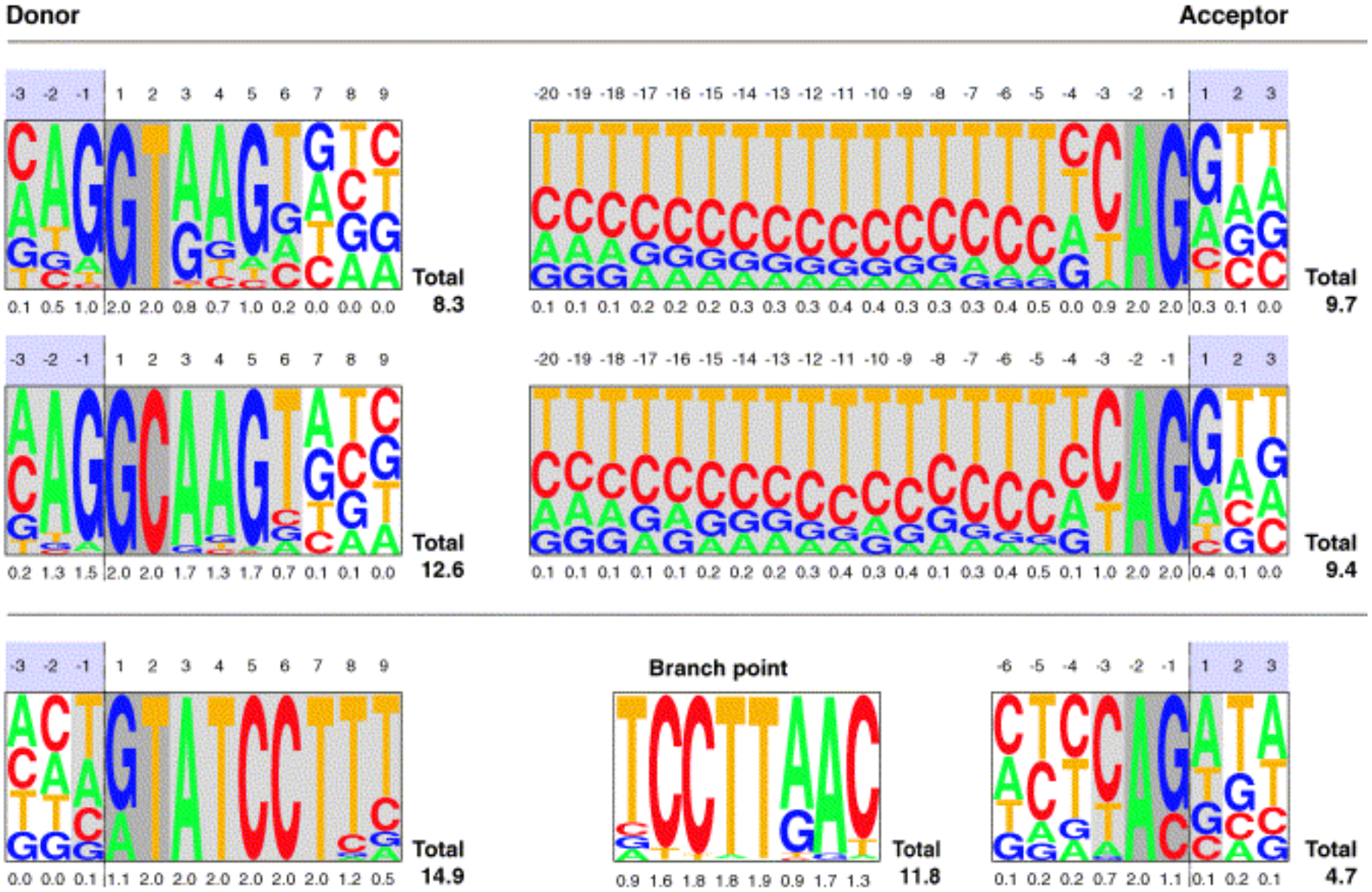
Structure of Genes



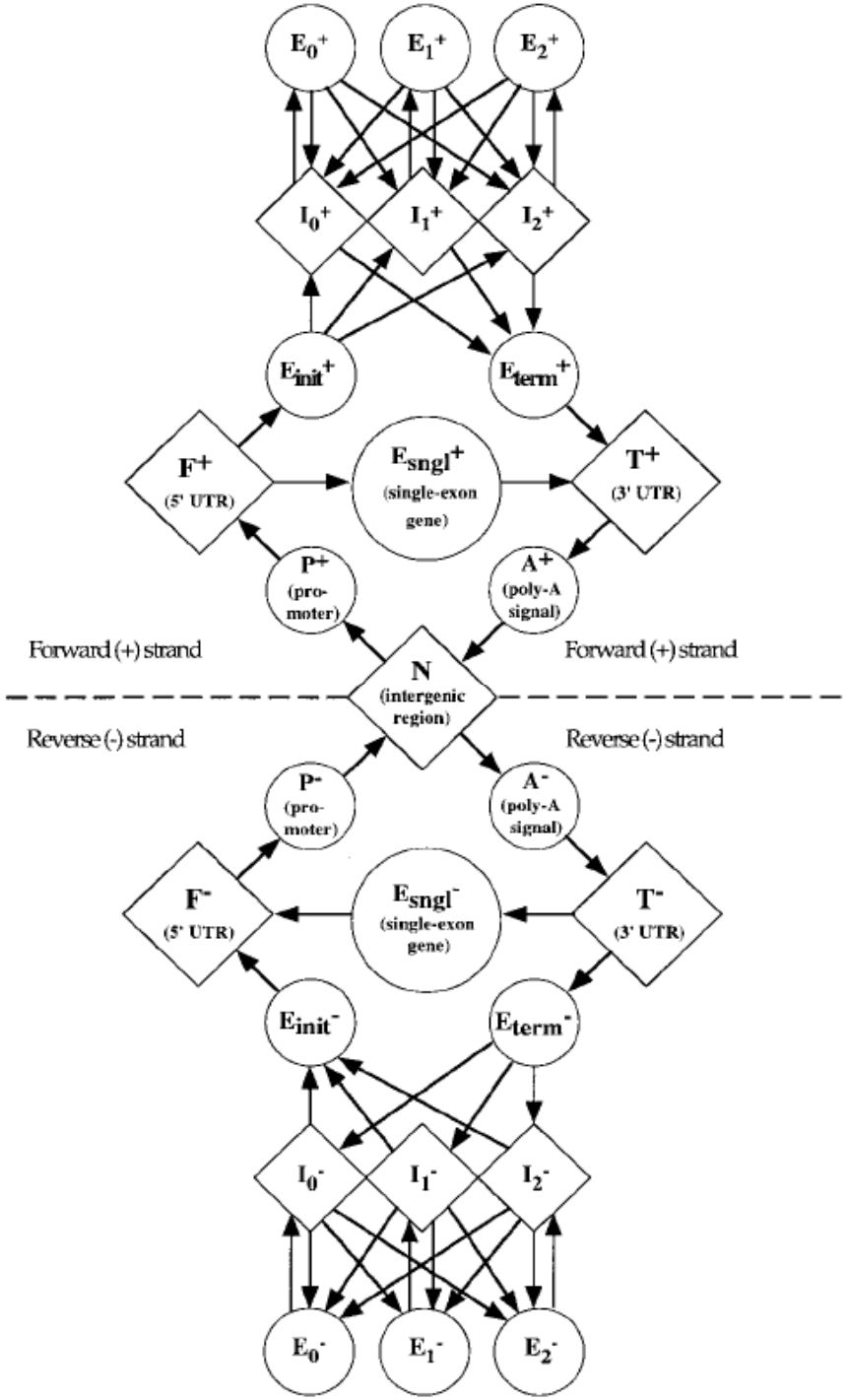
Genome Annotation Methods

- Known Genes
  - Blastn cDNA against genome
- Protein similarity
  - Blastx genome against SWISS-PROT
- Genome-Genome alignment
  - Blastz
- *De novo* prediction
  - GRAIL, Genscan, FGENESH
- Integrated methods
  - Expert review, ‘Combiner’ algorithms

Signal Detection: Splice Sites



Genscan's View of a Gene



E = Exon
I = Intron
A = polyadenylation signal
P = Promoter
F, T = UTR
N = Intergenic sequence

Burge & Karlin, *J. Mol. Biol.* 268:78, 1997

De novo methods: Single genome

- GRAIL
  - Uberbacher & Mural *PNAS* 88:11261, 1991
  - Neural network
  - Trained using known gene structures

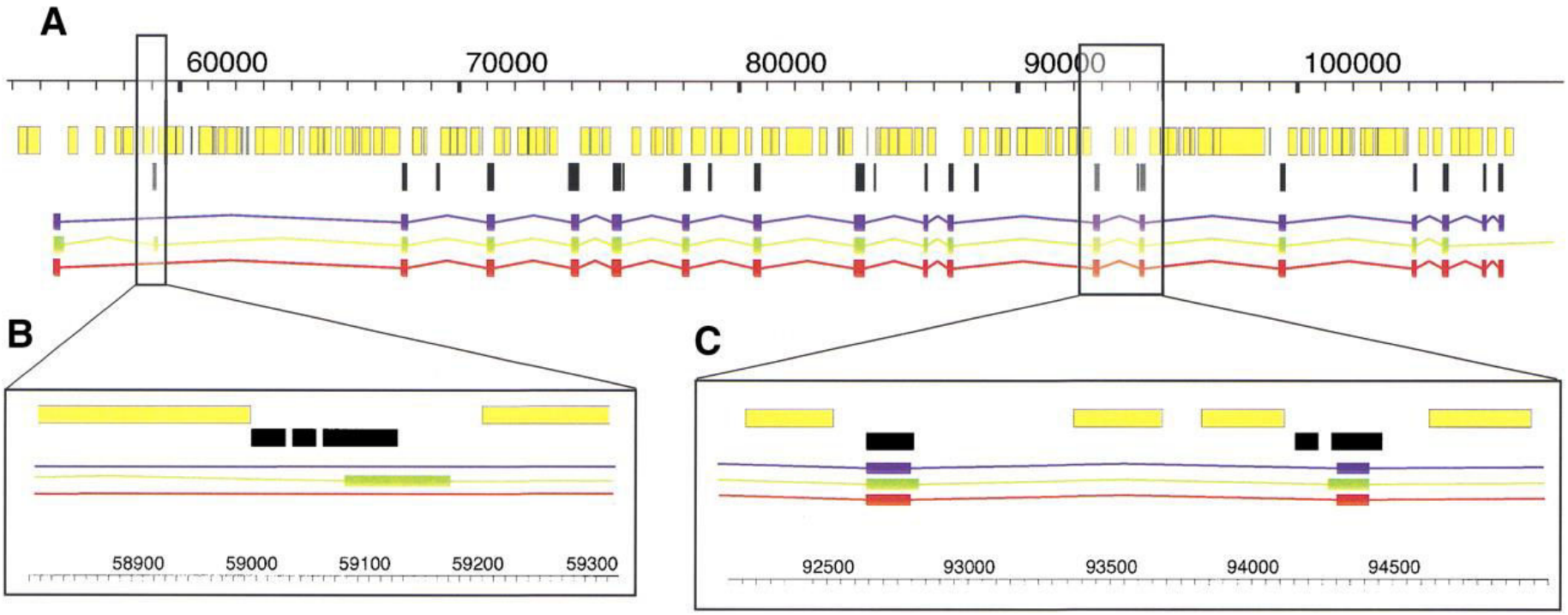
- GENSCAN
  - Burge & Karlin *J. Mol. Biol.* 268:78, 1997
  - Generalized hidden Markov model approach
  - Probabilistic model of gene structure
  - Uses descriptions of transcriptional, translational, and splicing signals
  - Distinct parameter sets for varying gene density and structure across G+C ranges
  - Allows for partial genes, multiple genes, and genes on both strands

De novo methods: Dual genome

- Pair HMM approach (SLAM)
  - Joint probability model for sequence alignment and gene structure definition
  - Dynamic programming algorithm combines classic alignment algorithms and HMM decoding

- 'Informant genome' approach (SGP-2, TWINSCAN)
  - Alignments performed first (BLASTN, TBLASTX)
  - Alignments 'inform' prediction algorithms based on single-genome predictors (e.g. GENSCAN)

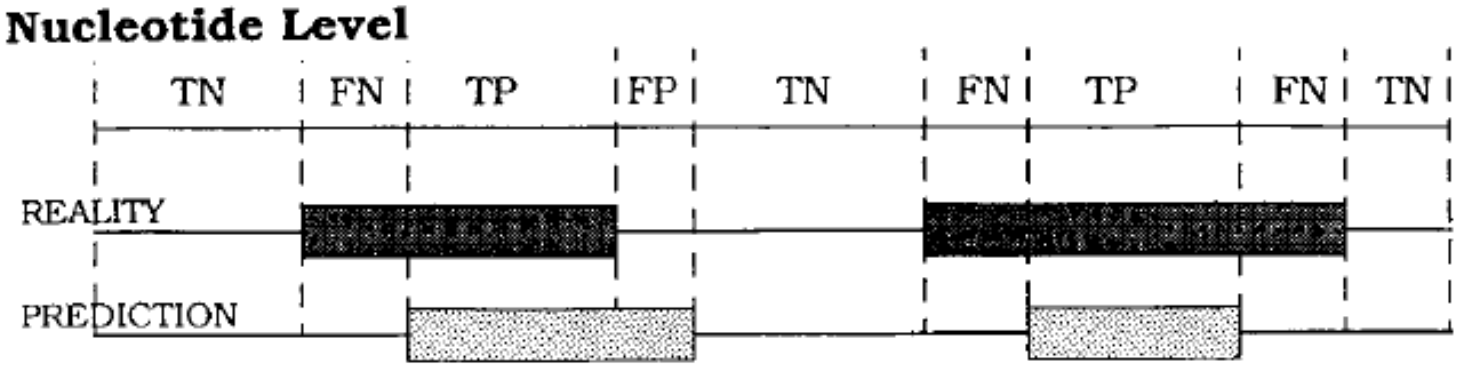
Genscan vs Twinscan



A detailed view of a TWINSCAN prediction (red), a GENSCAN prediction (green), and an aligned RefSeq transcript (blue). Masked repetitive and low-complexity regions (yellow) and mouse alignments (black) are indicated. (*A*) Complete gene prediction at the *KIAA1630* gene (NM_018706) from *Homo sapiens* 10p14. Note that the presence of conservation is neither a necessary (e.g., the first exon), nor a sufficient (e.g., the first alignment block condition) for TWINSCAN to predict an exon. (*B*) A magnified region around the second exon predicted by GENSCAN. TWINSCAN correctly omits this exon because the conserved region ends within it. (*C*) A magnified region around the 11th and 12th RefSeq exons. TWINSCAN correctly predicts both splice sites because they are within the aligned regions.

Flicek, *Genome Res.* 13:46, 2003

Evaluation of Predictions



	REALITY: coding	REALITY: no coding	
PREDICTION: coding	TP	FP	TP+FP
PREDICTION: no coding	FN	TN	FN+TN
	TP+FN	TF+TN	

$$Sn = \frac{TP}{TP + FN}$$

Sensitivity

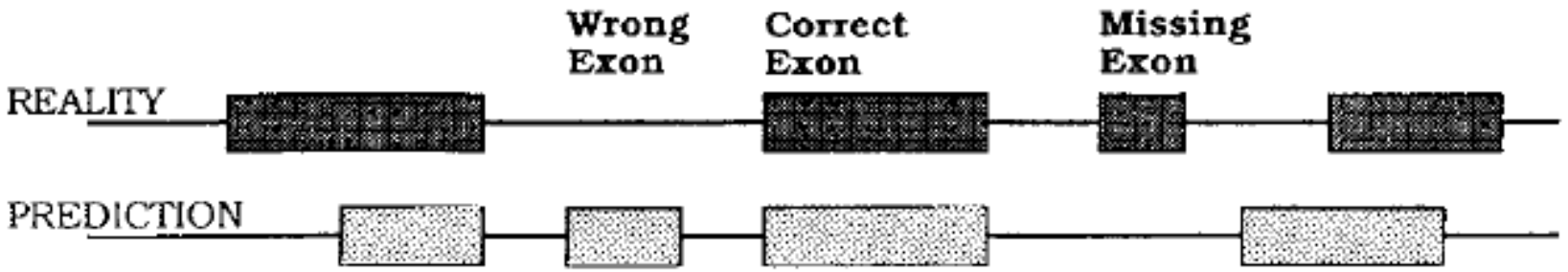
$$Sp = \frac{TP}{TP + FP}$$

Specificity

Burset & Guigo, *Genomics* 15:353, 1996

Evaluation of Predictions

Exon Level



$$Sn = \frac{\text{number of Correct Exons}}{\text{number of Actual Exons}}$$ **Sensitivity**

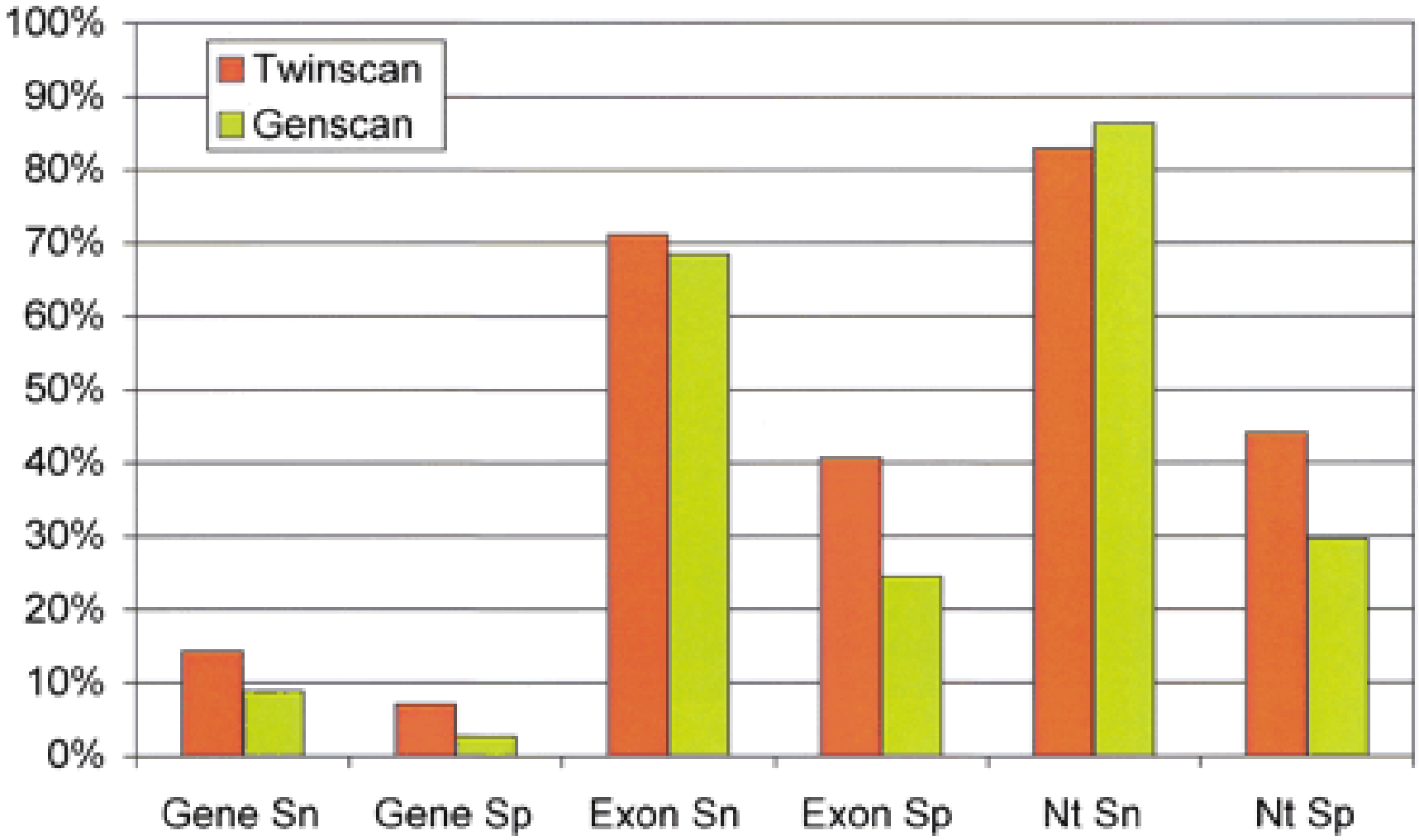
$$Sn = \frac{\text{number of Correct Exons}}{\text{number of Predicted Exons}}$$ **Specificity**

$$ME = \frac{\text{number of Missing Exons}}{\text{number of Actual Exons}}$$ **(Sensitivity)**

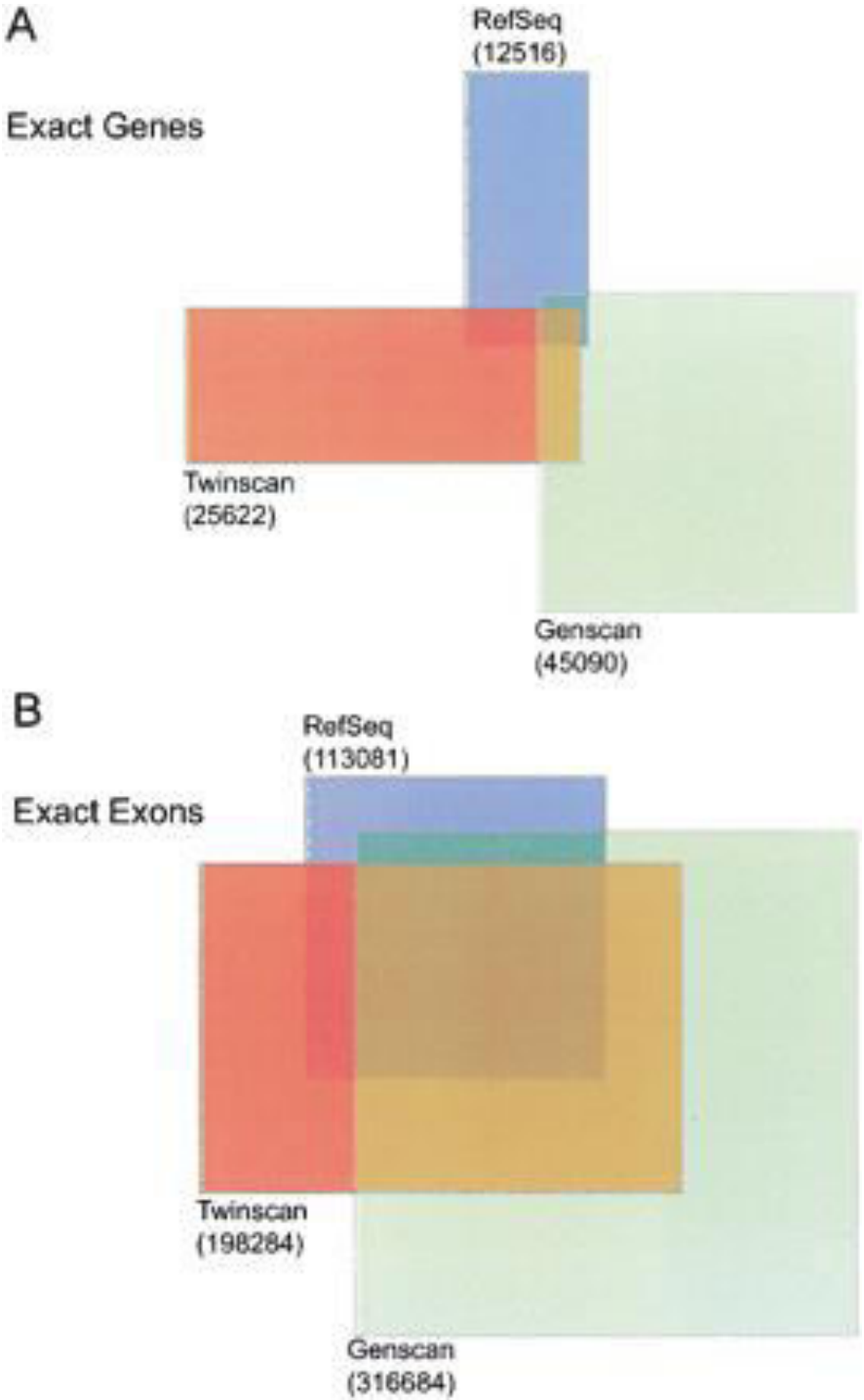
$$WE = \frac{\text{number of Wrong Exons}}{\text{number of Predicted Exons}}$$ **(Specificity)**

Burset & Guigo, *Genomics* 15:353, 1996

Annotation of the Mouse Genome



Flicek, *Genome Res.* 13:46, 2003



Assessment of Genscan and Twinscan

Flicek, *Genome Res.* 13:46, 2003

Addition of Evidence

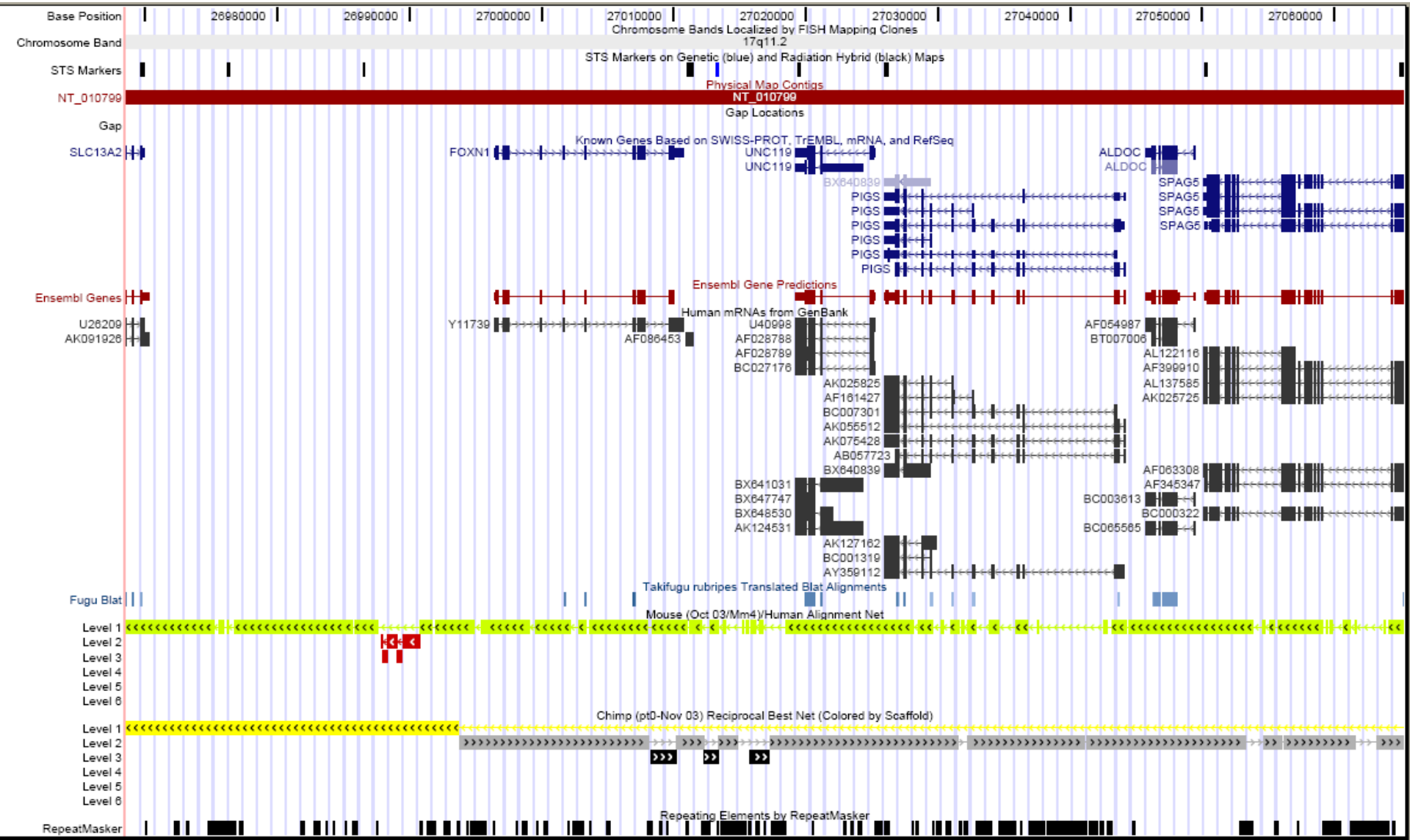
- Known cDNAs
- ESTs (partial cDNA sequence)
- Known genes
- [Predicted genes from other species]
- Genome comparison

- Repeat-masking


repeat
CpG
Genscan
BlastN
Bn:GBCDS
Bn:h_EST
Bn:CHGI
Bn:ensembl
Bn:refSeq
Bn:Mouse
Bn:r_EST
Bn:CRGI
Bn:CMGI
S4:r_EST
S4:CMGI
S4:GBCDS
S4:h_EST
S4:CHGI
BlastX:nraa
LAP:nraa
Grail
FgenesH
TigrCombiner
S4:ensembl
S4:refSeq
Otto
Promoted
Workspace
Axis
0
200k
400k
600k
800k
1m

http://genome.ucsc.edu

Genome Browser



Additional Reading

- Brent & Guigo, Recent advances in gene structure prediction.
  Curr. Op. Struct. Biol. 14(3) 264-272, 2004
- Fickett, JW. The gene identification problem: an overview for developers.
  Comput. Chem. 20:103-118, 1996