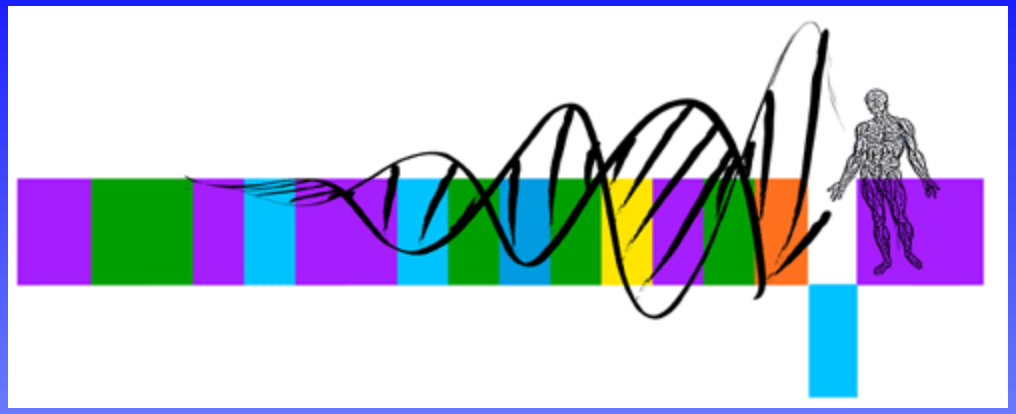

Sequence Analysis

III:

Genomics and Genome Browsers

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WIBR Bioinformatics and Research Computing

Genomics and Genome Browsers

- Introduction to genomics
- Genomics with genome browsers
- Conservation and evolution
- Introduction to comparative genomics
- Genome-wide data analysis

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Genomics: some big questions

- What is a gene?
  - one definition: a region of DNA that encodes functional RNA or protein.
- What is the sequence of the genome? SNPs?
- Where are all of the genes?
- What are the proteins they encode? What do they do?
- Where's the regulatory sequence? What does it do?
- How can one integrate all of this information?

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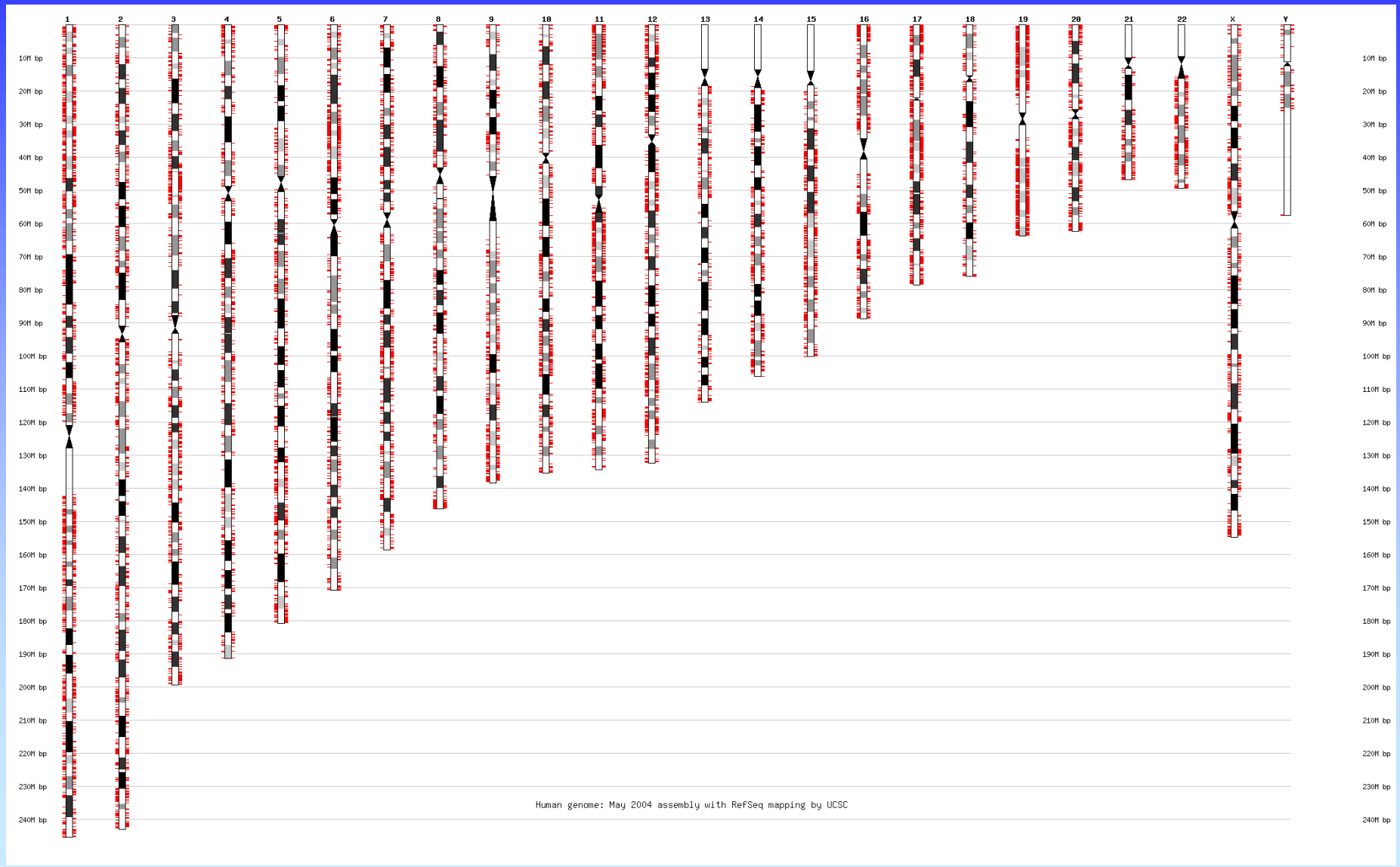
The human genome



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The human genome

- Last assembly: May 2004 ("NCBI 35")

  3.0 billion bases, mostly complete
  Ensembl annotation: 24,194 genes; 35,838 transcripts
  Heterochromatin (light staining) is not sequenced

Mean GC content:	41%
Repetitive DNA:	50%
Coding sequence:	1.5%
Under selection:	5%

- Reference genome sequence comprises one strand of each chromosome.

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Identifying genes

- Optimal protocol: Collect all RNA from all cell types in all conditions, sequence it and map it to the genome.
- Practical protocols:
  - predict genes de novo
  - cluster ESTs
  - sequence full-length clones
  - search with known genes in another species
  - a combination of those techniques above
- Still problems with pseudogenes

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How many genes and transcripts?

- Gene-centric databases (one entry per gene)
  - Ensembl (Hs= 24,194; Mm=28,069)
  - LocusLink (32,688; 67,653) incl. other "stuff"
- Human-curated full-length cDNA resources (one entry per transcript)
  - RefSeq (23,534; 30,462)
  - Mammalian Gene Collection (17,747; 14,639)
- EST-centric clusters (one entry per cluster)
  - UniGene (52,888; 45,719)
  - TIGR Gene Indices (227,631; 161,499)

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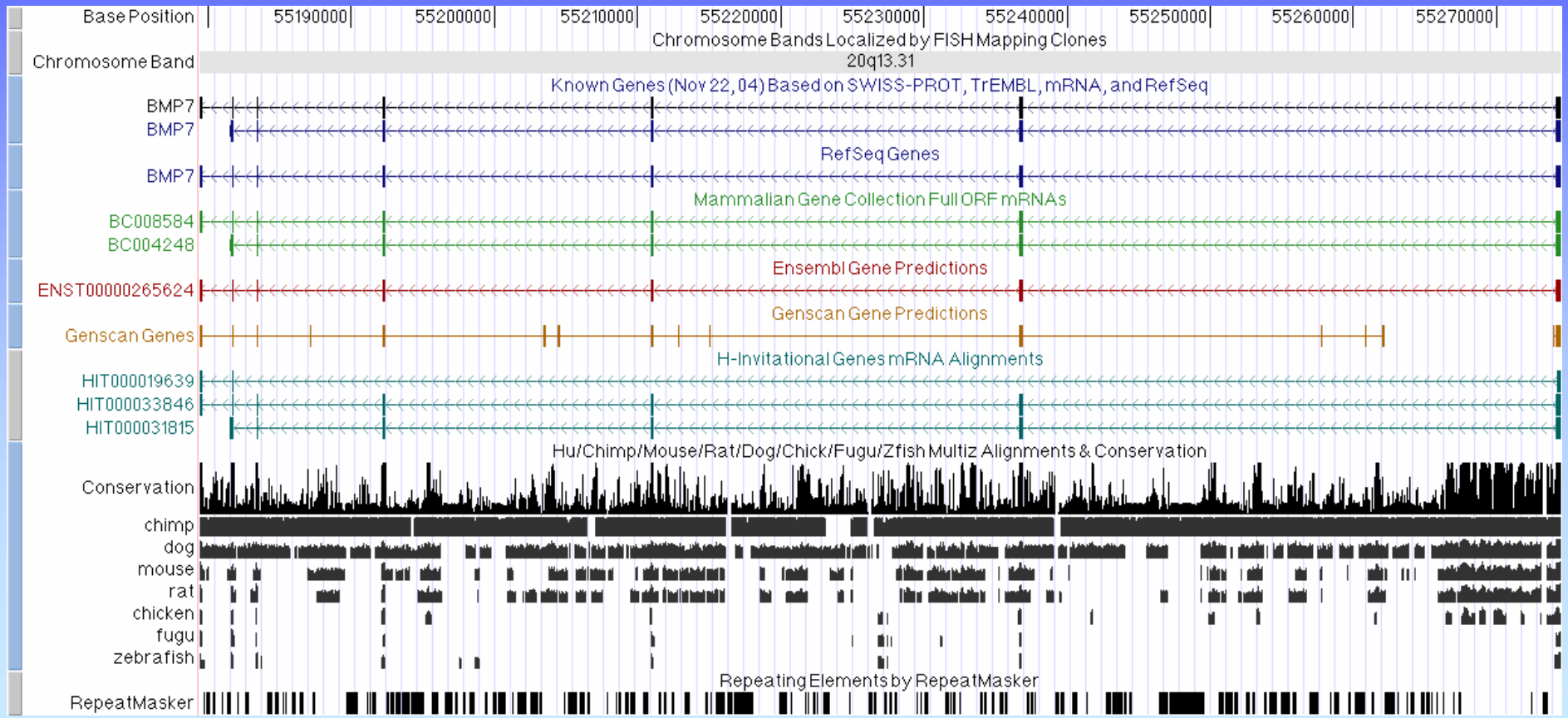
Genome Browsers

Examples: UCSC, Ensembl, NCBI, WIBR



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Genome Browser tracks

Mapping and Sequencing Tracks

Base Position: full	Chromosome Band: dense	STS Markers: hide	RGD QTL: hide	FISH Clones: hide
Recomb Rate: hide	Map Contigs: hide	Assembly: hide	Gap: hide	Coverage: hide
BAC End Pairs: hide	Fosmid End Pairs: hide	GC Percent: hide	WSSD Duplication: hide	Short Match: hide
Blat Sequence: hide				

Genes and Gene Prediction Tracks

Known Genes: pack	RefSeq Genes: full	MGC Genes: full	Ensembl Genes: full	Acembly Genes: hide
ECgene Genes: hide	Twinscan: hide	SGP Genes: hide	Geneid Genes: hide	Genscan Genes: dense
Retroposed Genes: hide	Superfamily: hide	sno/miRNA: hide		

mRNA and EST Tracks

Human mRNAs: hide	Spliced ESTs: hide	Human ESTs: hide	Non-Human mRNAs: hide	Non-Human ESTs: hide
H-Inv: full	TIGR Gene Index: hide	UniGene: hide	Gene Bounds: hide	

Other groups:

- Expression and Regulation
- Comparative Genomics
- ENCODE Tracks
- Variation and Repeats

Genome Browser data

- Potential to show any data that can be mapped to a genome.
- Visual examination can be more powerful than any automated analysis tool.
- Positive strand of reference chromosome is shown.
- Conventions: gene “start” < “end”
- Coordinates change with each assembly.
- Sequence is often soft- or hard-masked for repetitive DNA.

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Conservation and evolution

- Functional regions of a genome can be difficult to find in a large, repetitive sequence.
- During evolution, pressure for selection leads to greater conservation of some regions of a genome.
- Searching for regions of purifying selection is hoped to lead to elements of functional significance.

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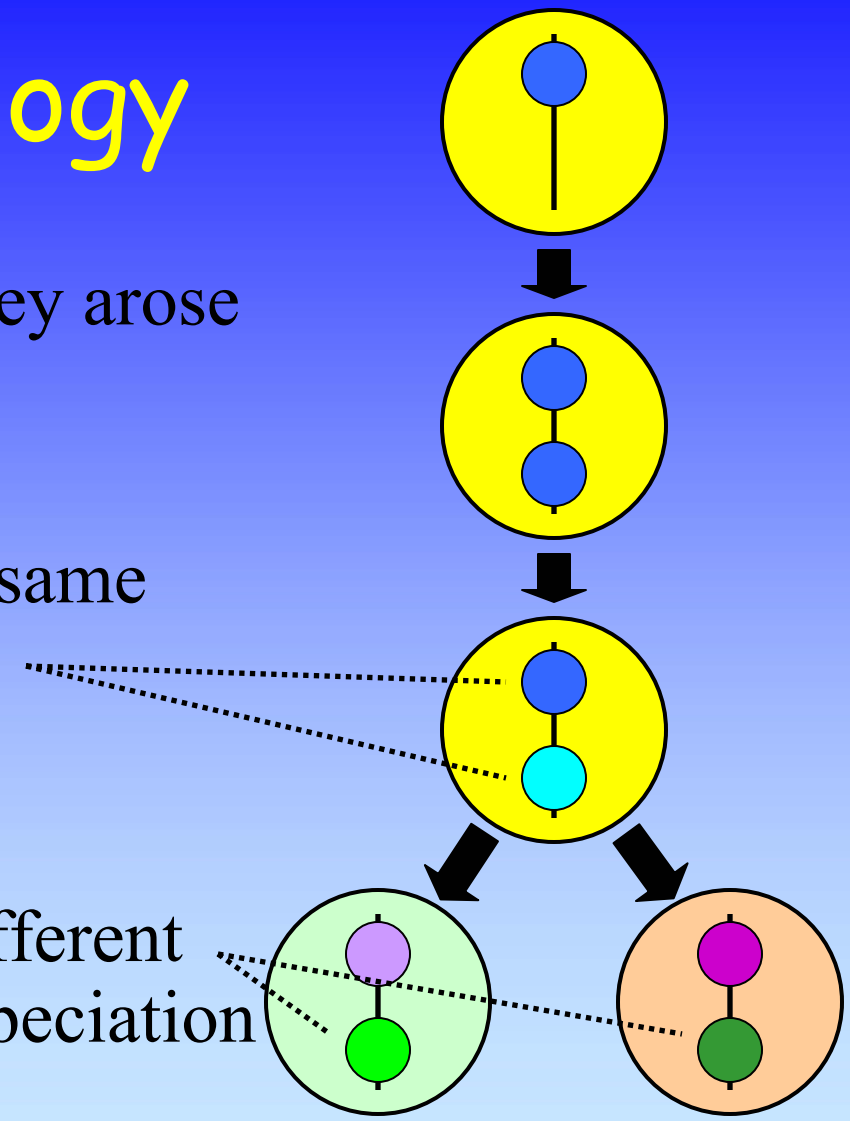
Homology

- Genes are *homologous* if they arose from the same ancestor.
- Paralogs: homologs (in the same species) that arose from a duplication event
- Orthologs: homologs (in different species) that arose from a speciation event



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Quantifying evolution of coding regions

1. Percentage of AA identity or similarity

   For human-mouse orthologs, median identity = 79%

2. The K_a/K_s ratio

$$\frac{\text{AA substitution rate}}{\text{Neutral substitution rate}} = \frac{\text{Non-synonymous substitution rate}}{\text{Synonymous substitution rate}}$$

   For human-mouse orthologs, median $K_a/K_s = 0.12$

   => 88% of AA-changing mutations are deleterious

- Domain-containing regions have evolved less.
- Pseudogenes have a K_a/K_s ratio close to 1.

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Comparative genomics

- Conservation between genomes is a very effective way to identify genes and regulatory regions.
- Comparison of multiple genomes can identify functional elements without any previous understanding of their function.
- With increasing conservation of a region of interest, comparisons between more distant species becomes more informative.
- Comparison of two species is rarely as effective as that of multiple species.

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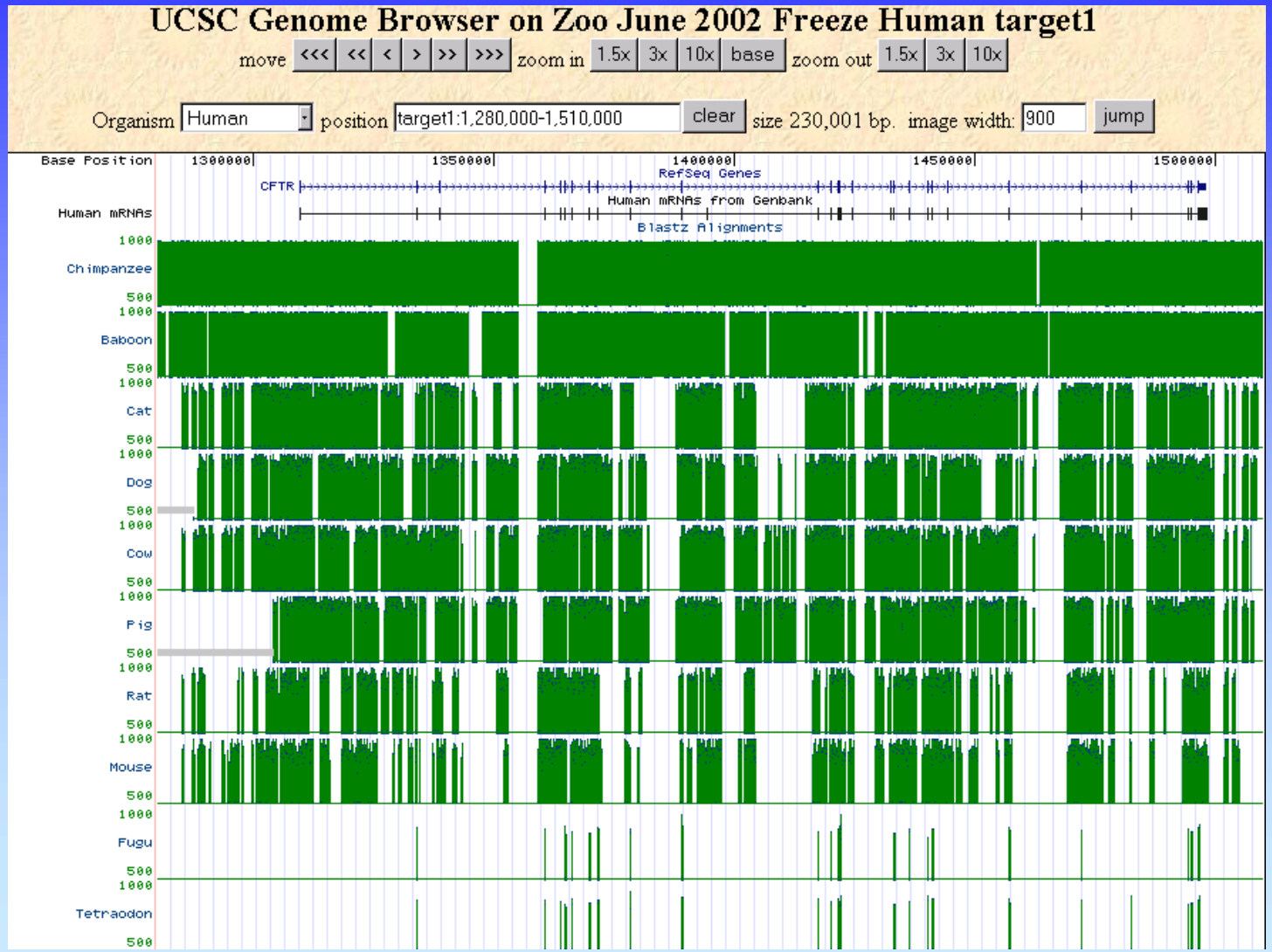
Multiple-species comparisons



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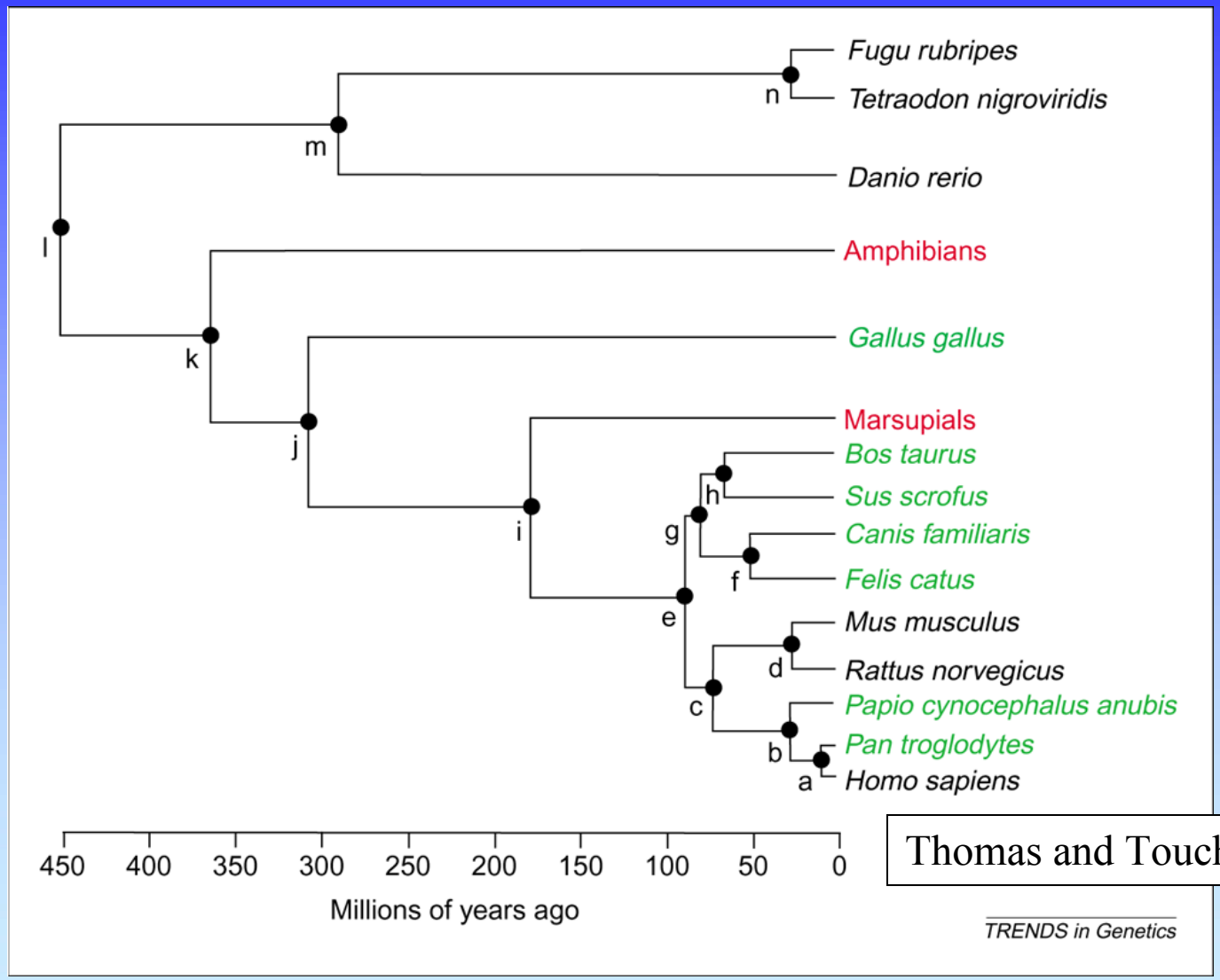
Vertebrate sequencing projects



Thomas and Touchman, 2002

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Conserved synteny

Nature 420(6915):520-62, 2002

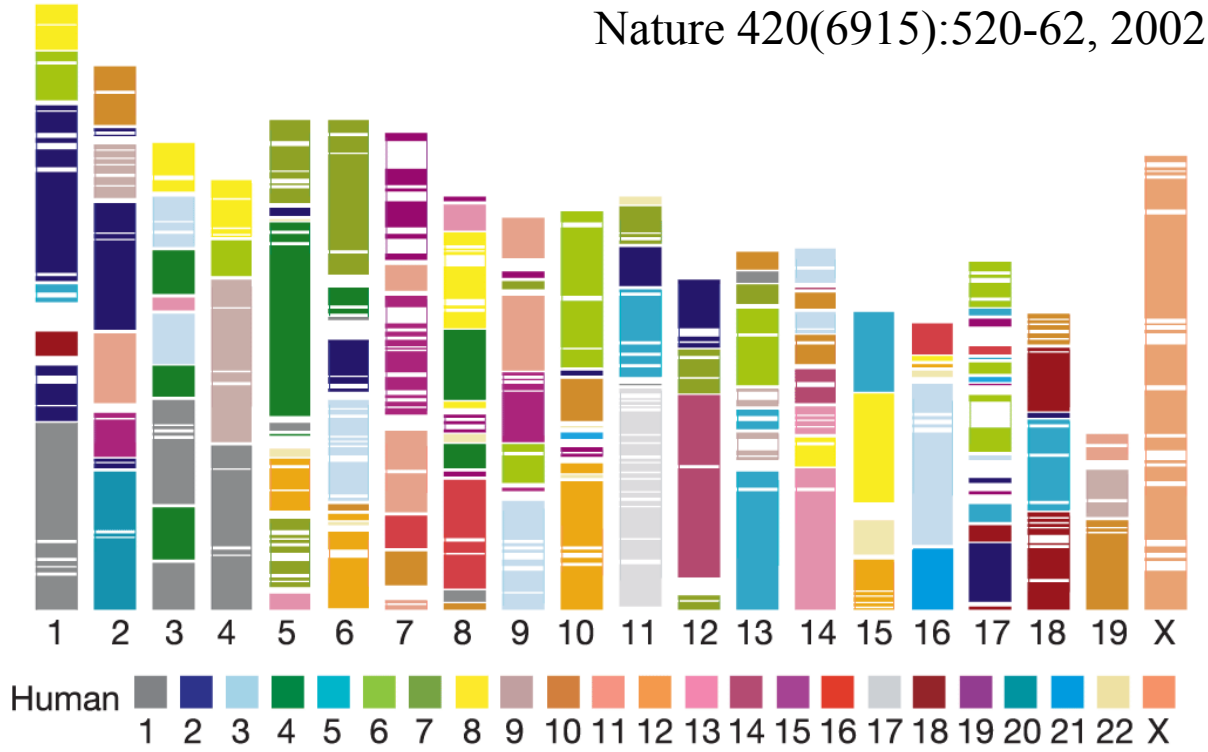

Figure 3 Segments and blocks >300 kb in size with conserved synteny in human are superimposed on the mouse genome. Each colour corresponds to a particular human chromosome. The 342 segments are separated from each other by thin, white lines within the 217 blocks of consistent colour.

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Finding orthologous genes

- Traditional method 1: reciprocal best BLASTP hits in all vs. all searches
- Traditional method 2: synteny maps
- Current methods: sequence analysis and conserved synteny
- Resources:

  Ensembl, NCBI, genome browsers
- Complicated by paralogous genes

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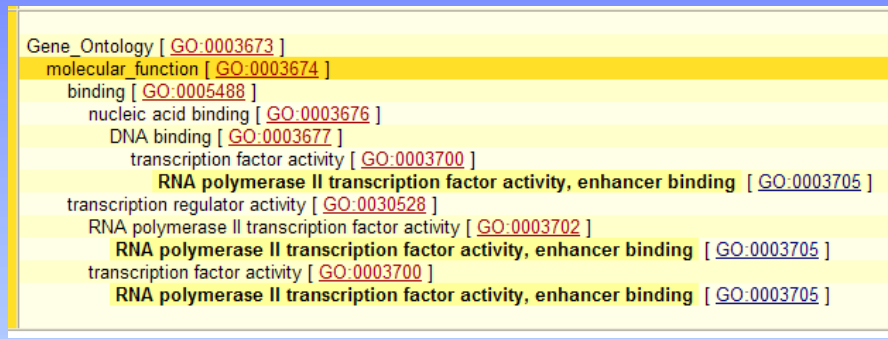
What do all the genes do?

Q: How can every molecular function and biological process be systematically organized?

A: The Gene Ontology Consortium

- The three GO ontologies:
  - Molecular function
  - Biological Process
  - Cellular Component



- Components of the ontologies are like hierarchies except that a “child” can have more than one “parent”.
- Evidence for annotation varies.

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Genome-wide data analysis

- Ensembl and UCSC genome downloads
- NCBI flat file downloads
- EnsMart for genome-wide queries on the web
- Ensembl and WIBR LocusLink for SQL queries
- Analyzing sequence vs. annotations
- Transitivity of sequences and annotations?
- Check with BaRC about data on their servers

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Summary

- Introduction to genomics
- Genomics with genome browsers
- Conservation and evolution
- Introduction to comparative genomics
- Genome-wide data analysis

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Selected references

- Initial sequencing and analysis of the human genome. Nature. 409:860-921, 2001.
- Initial sequencing and comparative analysis of the mouse genome. Nature. 420:520-62, 2002.
- A User's Guide to the Human Genome II. Nature Genetics. 35 Suppl 1:4, 2003. (“web special”)

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Exercises

- Browsing for genomic information
- Extracting annotated genomic sequence
- Gene-finding with comparative mammalian genomics
- Gene and genome analysis through annotation
- Command-line applications

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