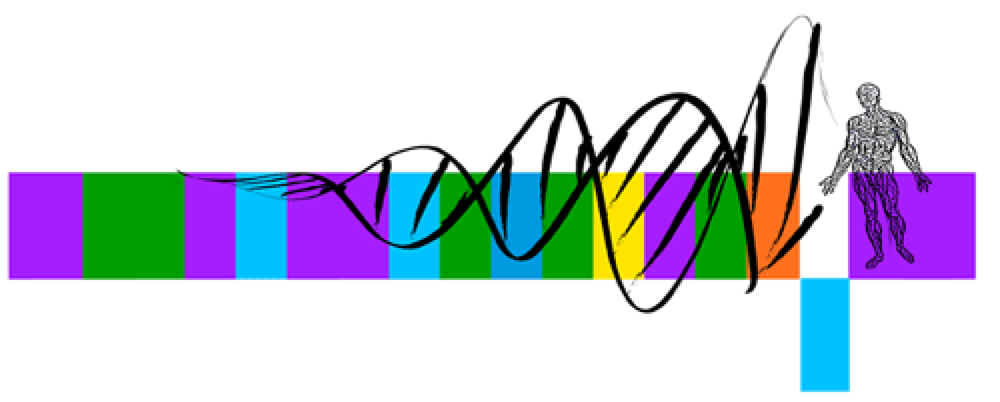

Sequence Analysis

III:

Genomics and Genome Browsers

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WIBR Bioinformatics and Research Computing

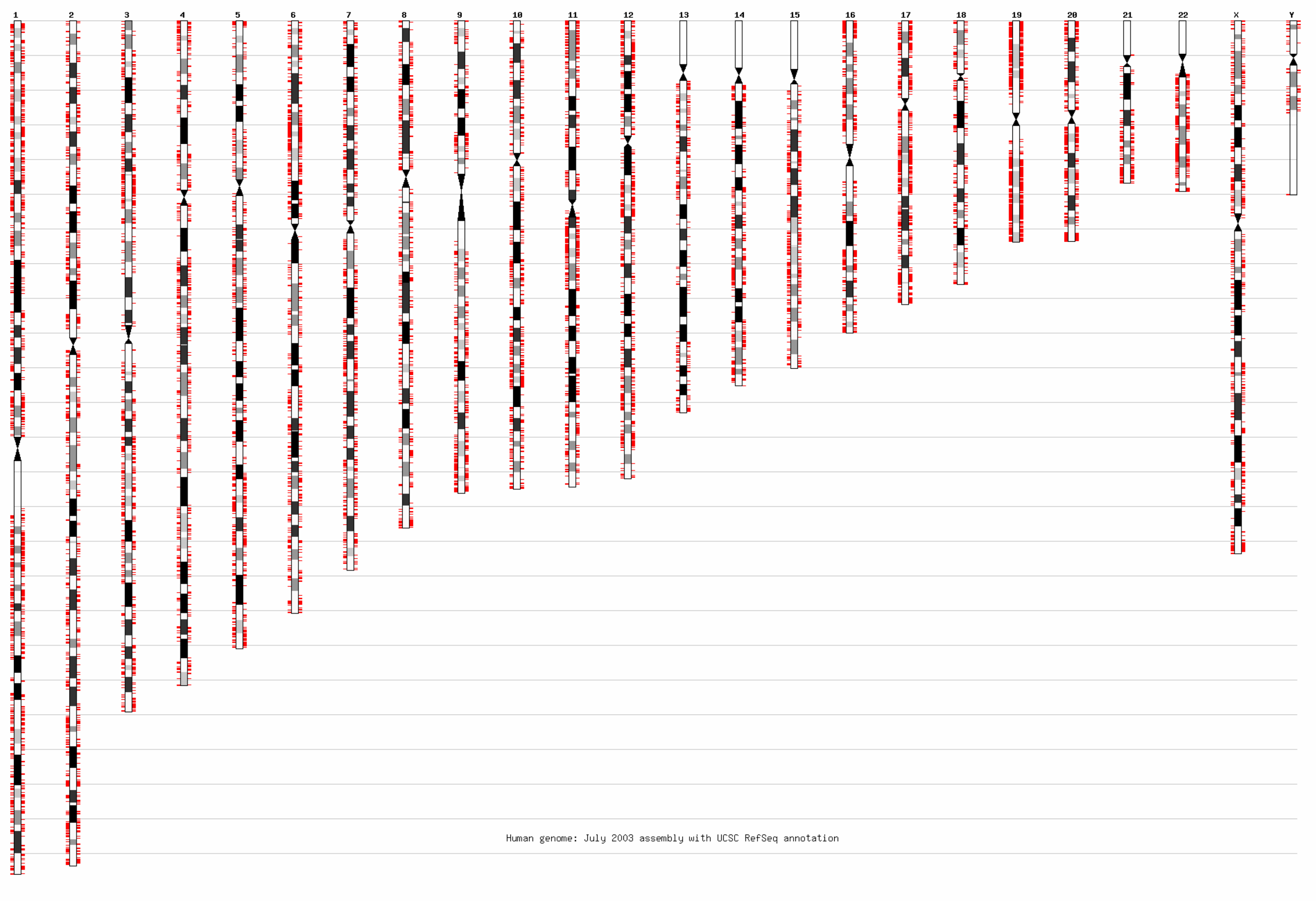
Genomics and Genome Browsers

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

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?

The human genome



Human genome: July 2003 assembly with UCSC RefSeq annotation

The human genome

- Last assembly: July 2003

  3.2 billion bases, mostly complete
  Ensembl annotation: 23,531 genes; 31,609 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.

Identifying genes

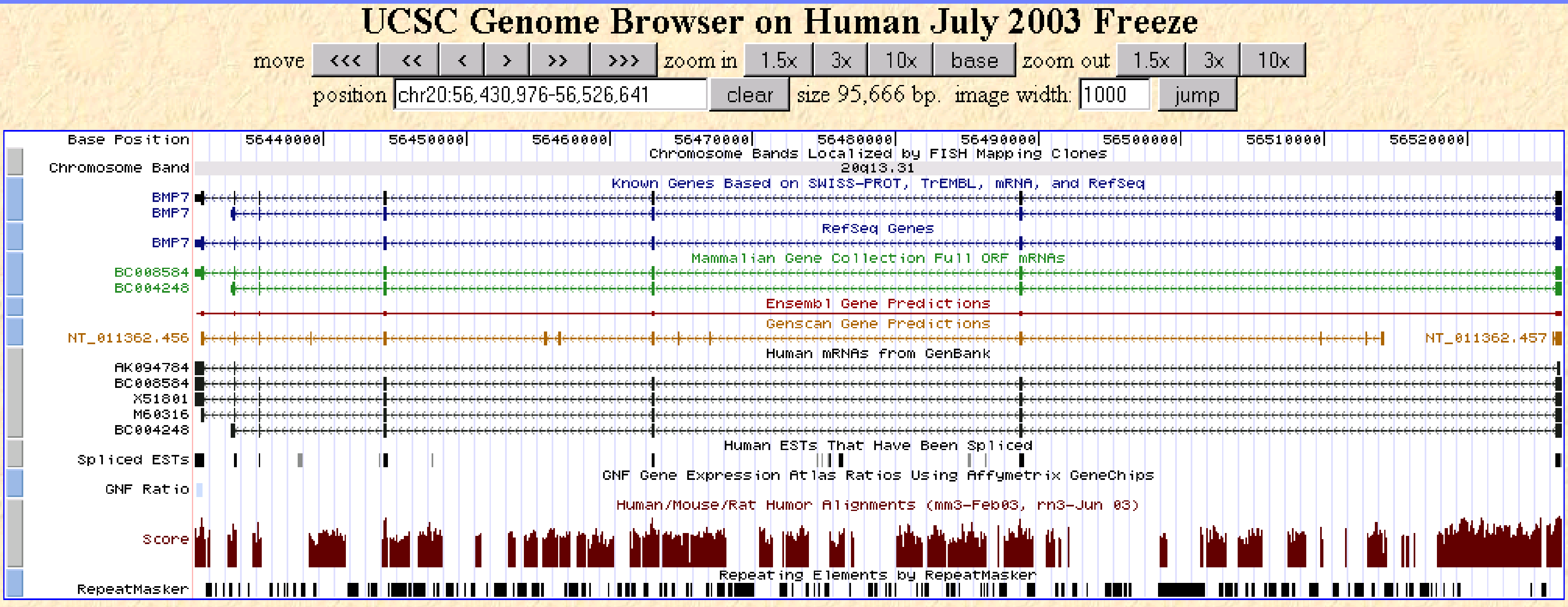
- Optimal protocol: Collect all RNA from all cell types in all conditions, sequence it and map it to the genome.
- Practical protocols:
  - predict genes de novo
  - cluster ESTs
  - sequence full-length clones
  - search with known genes in another species
  - a combination of those techniques above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

How many genes and transcripts?

- Gene-centric databases (one entry per gene)
  - Ensembl (Hs=23,531; Mm=26,762)
  - LocusLink (37,497; 69,612) incl. other "stuff"
- Human-curated full-length cDNA resources (one entry per transcript)
  - RefSeq (21,150; 17,017)
  - Mammalian Gene Collection (11,196; 10,216)
- EST-centric clusters (one entry per cluster)
  - UniGene (118,517; 82,482)
  - TIGR Gene Indices (201,258; 145,559)

Genome Browsers

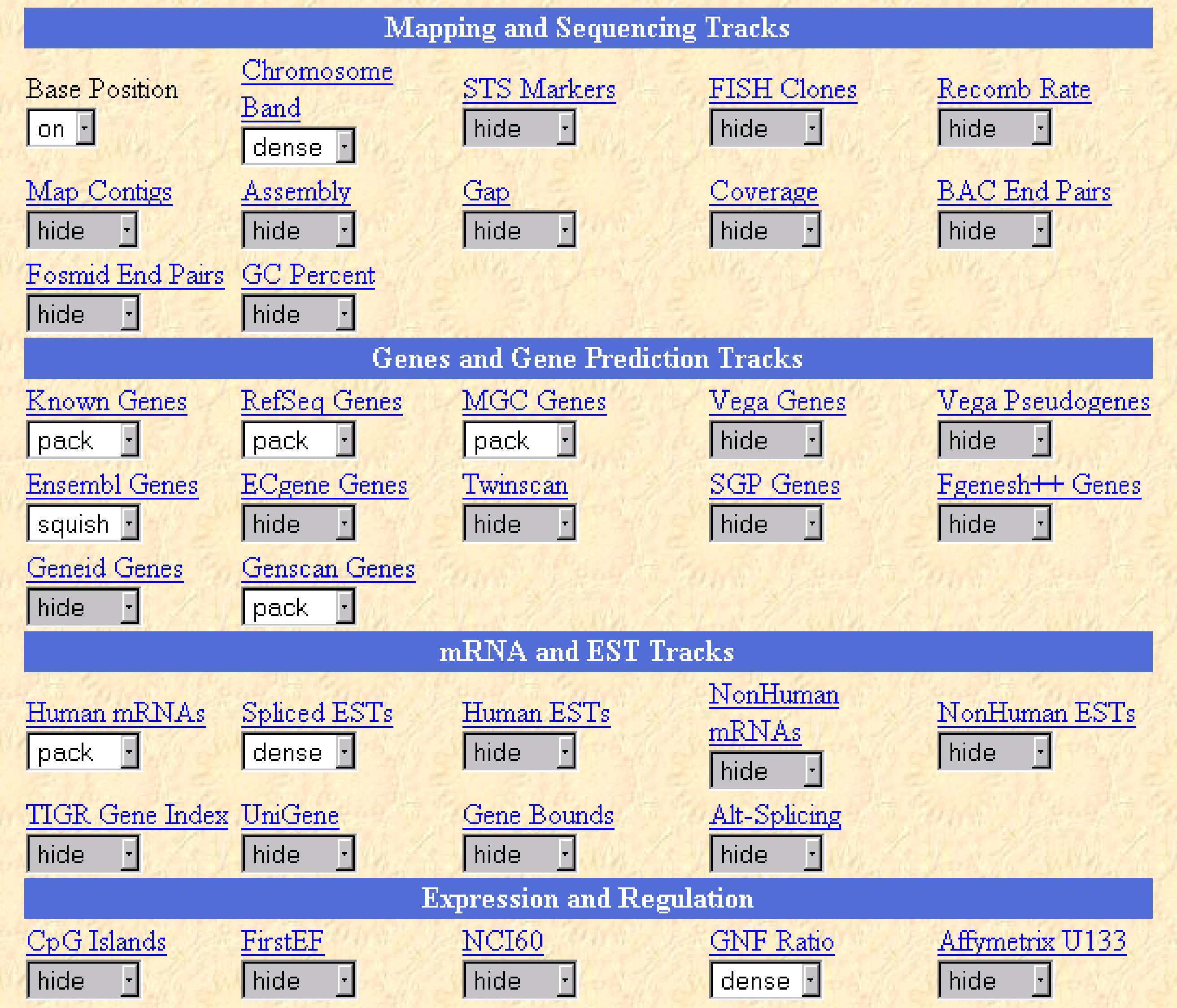
Examples: UCSC, Ensembl, NCBI, WIBR



UCSC Genome Browser on Human July 2003 Freeze

position chr20:56,430,976-56,526,641 size 95,666 bp. image width: 1000

Genome Browser tracks



Mapping and Sequencing Tracks

Base Position on	Chromosome Band dense	STS Markers 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			

Genes and Gene Prediction Tracks

Known Genes pack	RefSeq Genes pack	MGC Genes pack	Vega Genes hide	Vega Pseudogenes hide
Ensembl Genes squish	ECgene Genes hide	Twinscan hide	SGP Genes hide	Fgenesh++ Genes hide
Geneid Genes hide	Genscan Genes pack			

mRNA and EST Tracks

Human mRNAs pack	Spliced ESTs dense	Human ESTs hide	NonHuman mRNAs hide	NonHuman ESTs hide
TIGR Gene Index hide	UniGene hide	Gene Bounds hide	Alt-Splicing hide	

Expression and Regulation

CpG Islands hide	FirstEF hide	NCI60 hide	GNF Ratio dense	Affymetrix U133 hide

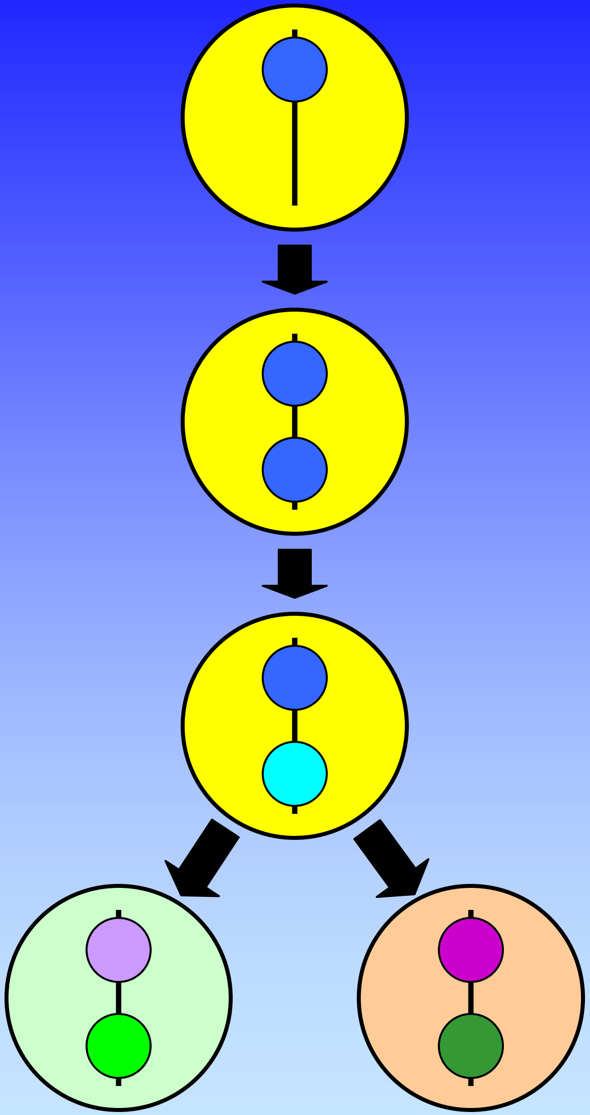
Genome Browser data

- 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.

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.

Homology



- Genes are *homologous* if they arose from the same ancestor.
- Orthologs: homologs (in different species)

  that arose from a speciation event
- Paralogs: homologs (in the same species)

  that arose from a duplication event

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 $K_a/K_s = 0.12$

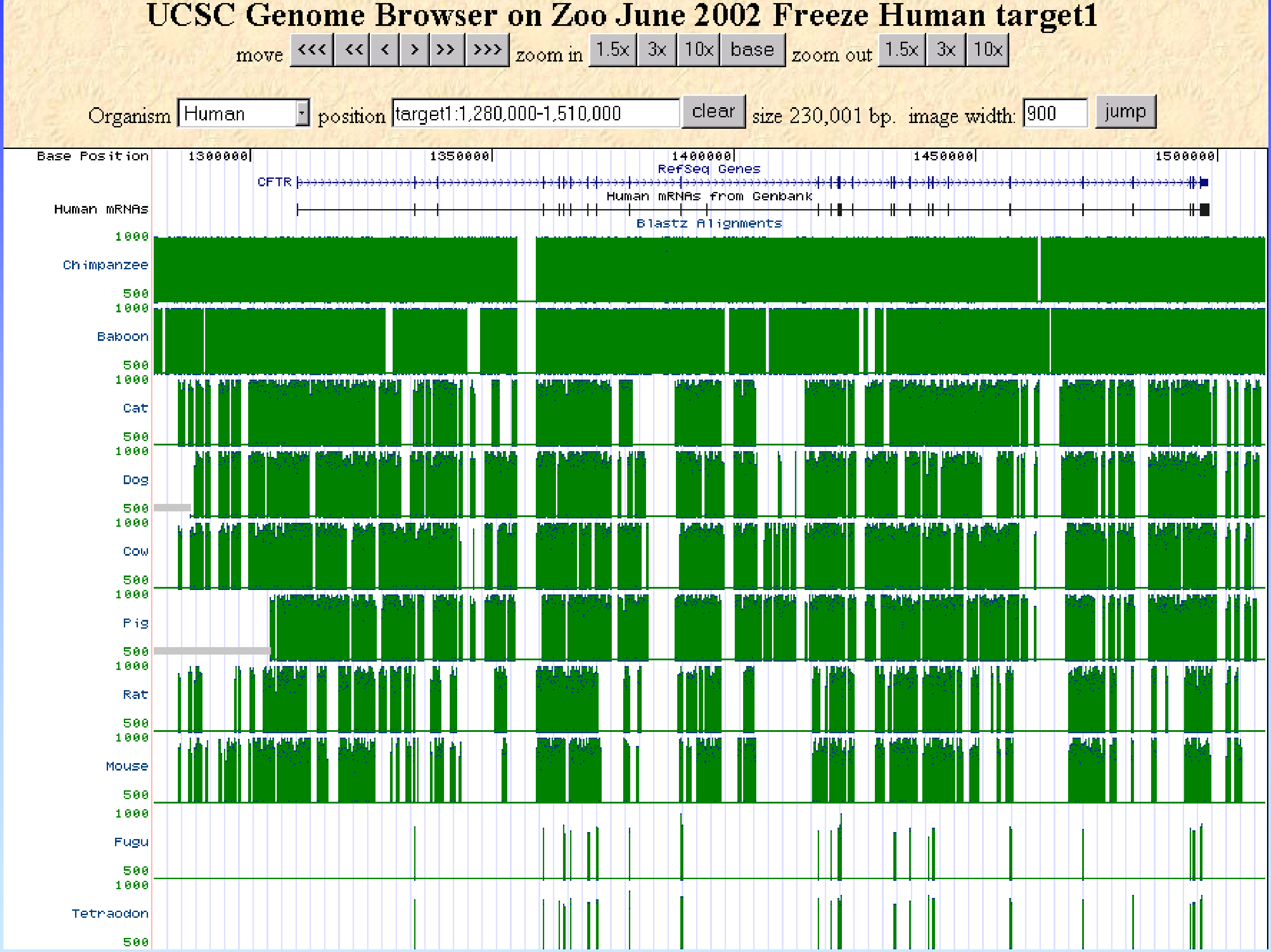
   => 88% of AA-changing mutations are deleterious

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

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.

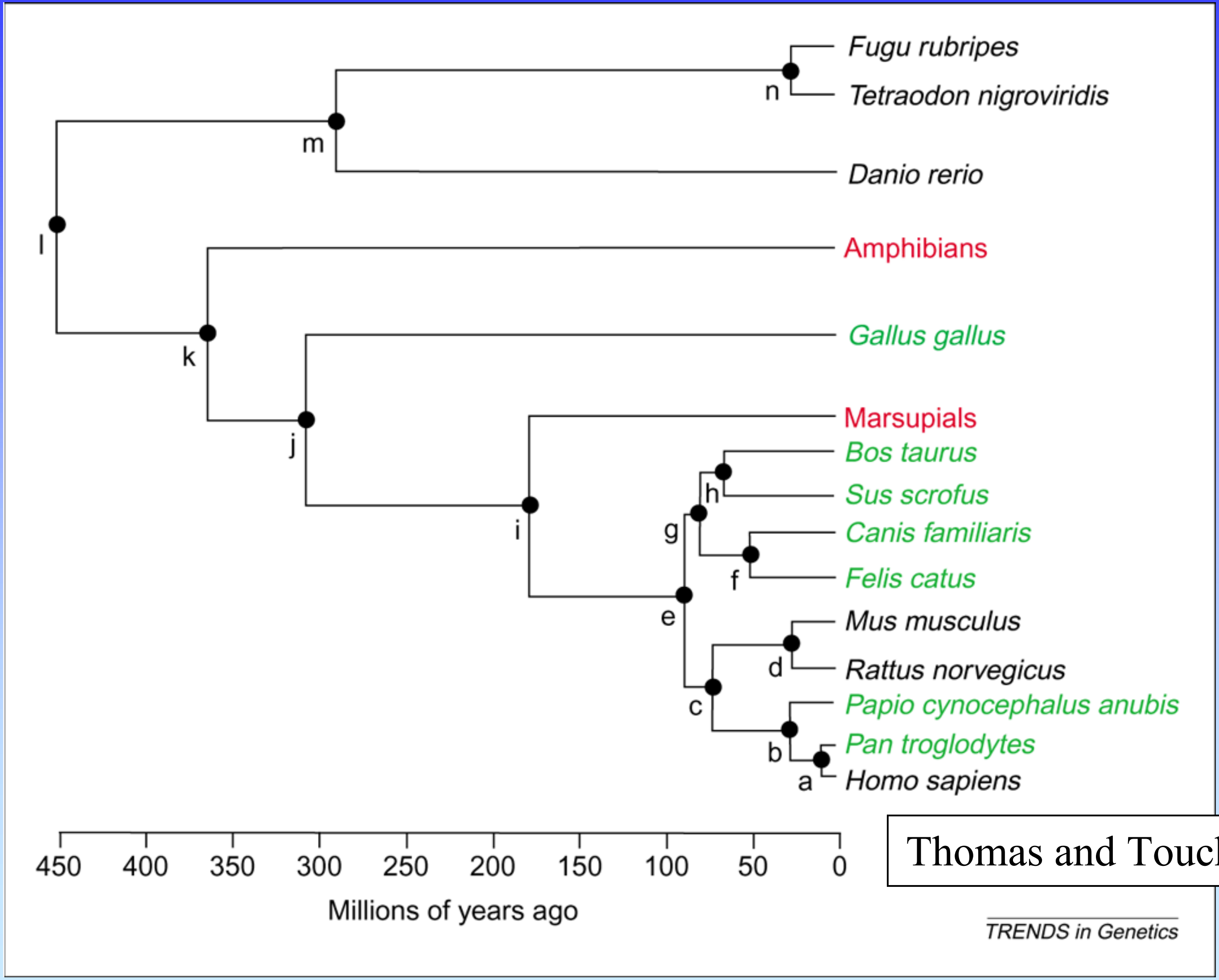
Multiple-species comparisons



UCSC Genome Browser on Zoo June 2002 Freeze Human target1

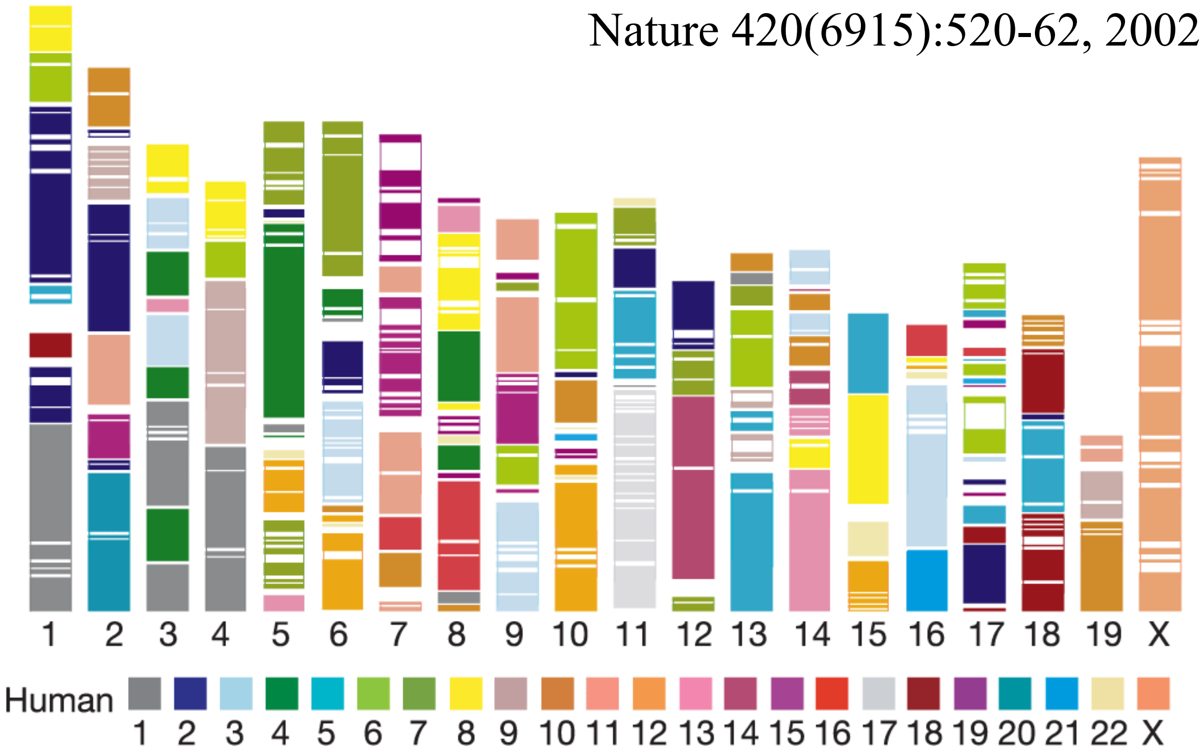
Organism Human position target1:1,280,000-1,510,000 size 230,001 bp. image width: 900

Vertebrate sequencing projects



Thomas and Touchman, 2002

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.

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:

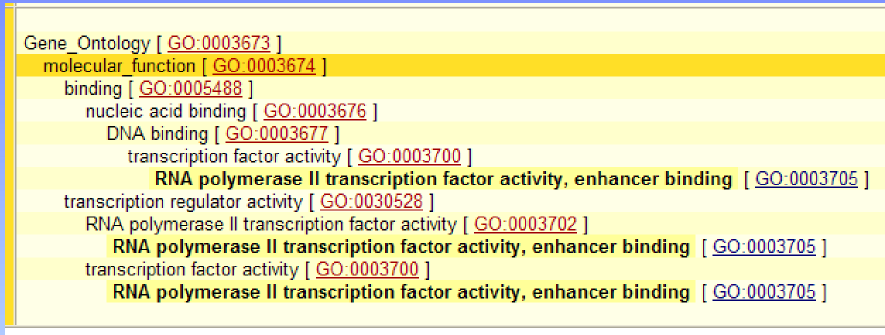
  Ensembl, NCBI, genome browsers
- Complicated by paralogous genes

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



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Gene_Ontology [ GO:0003673 ]
  molecular_function [ GO:0003674 ]
    binding [ GO:0005488 ]
      nucleic acid binding [ GO:0003676 ]
        DNA binding [ GO:0003677 ]
          transcription factor activity [ GO:0003700 ]
            RNA polymerase II transcription factor activity, enhancer binding  [ GO:0003705 ]
    transcription regulator activity [ GO:0030528 ]
      RNA polymerase II transcription factor activity [ GO:0003702 ]
        RNA polymerase II transcription factor activity, enhancer binding  [ GO:0003705 ]
      transcription factor activity [ GO:0003700 ]
        RNA polymerase II transcription factor activity, enhancer binding  [ GO:0003705 ]
```

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

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

Summary

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

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")

Exercises

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