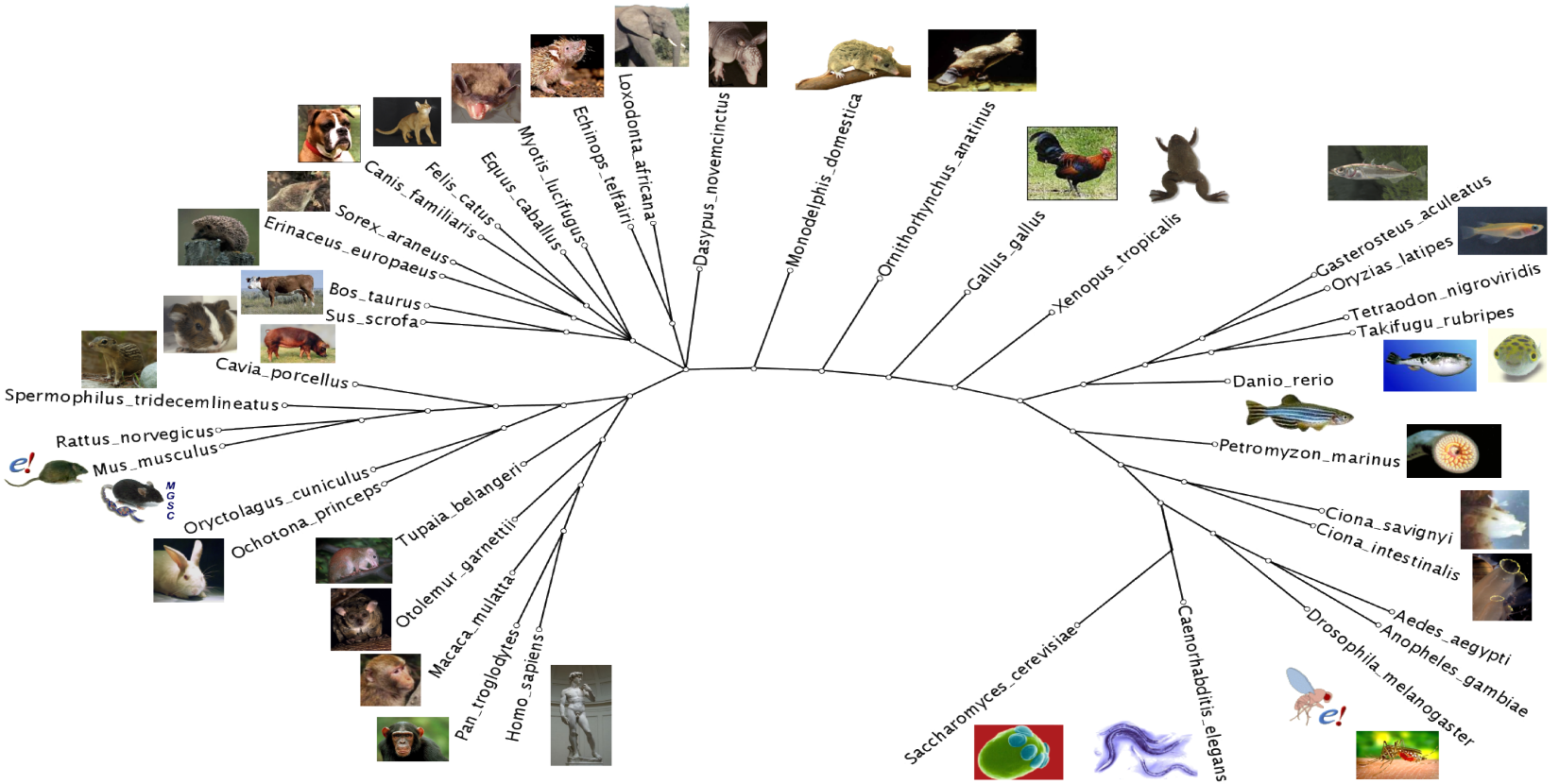
Comparative Genomics in Ensembl



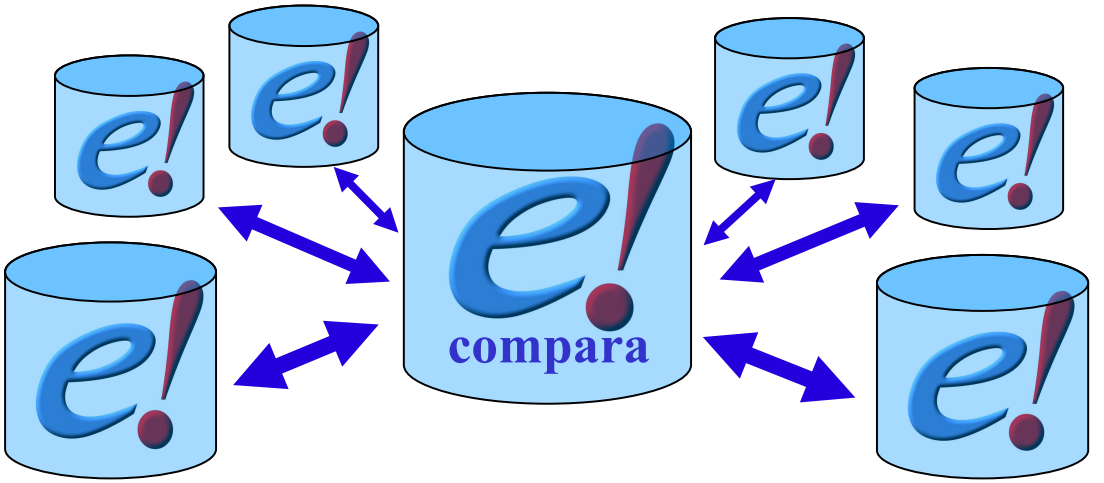
Javier Herrero

http://www.ebi.ac.uk/~jherrero/

EBI - Wellcome Trust Genome Campus, UK

Sep 2008

Ensembl Compara



A single database which contains precalculated comparative genomics data and which is linked to all the Ensembl Species databases.

Access via web interface, perl API and mysql

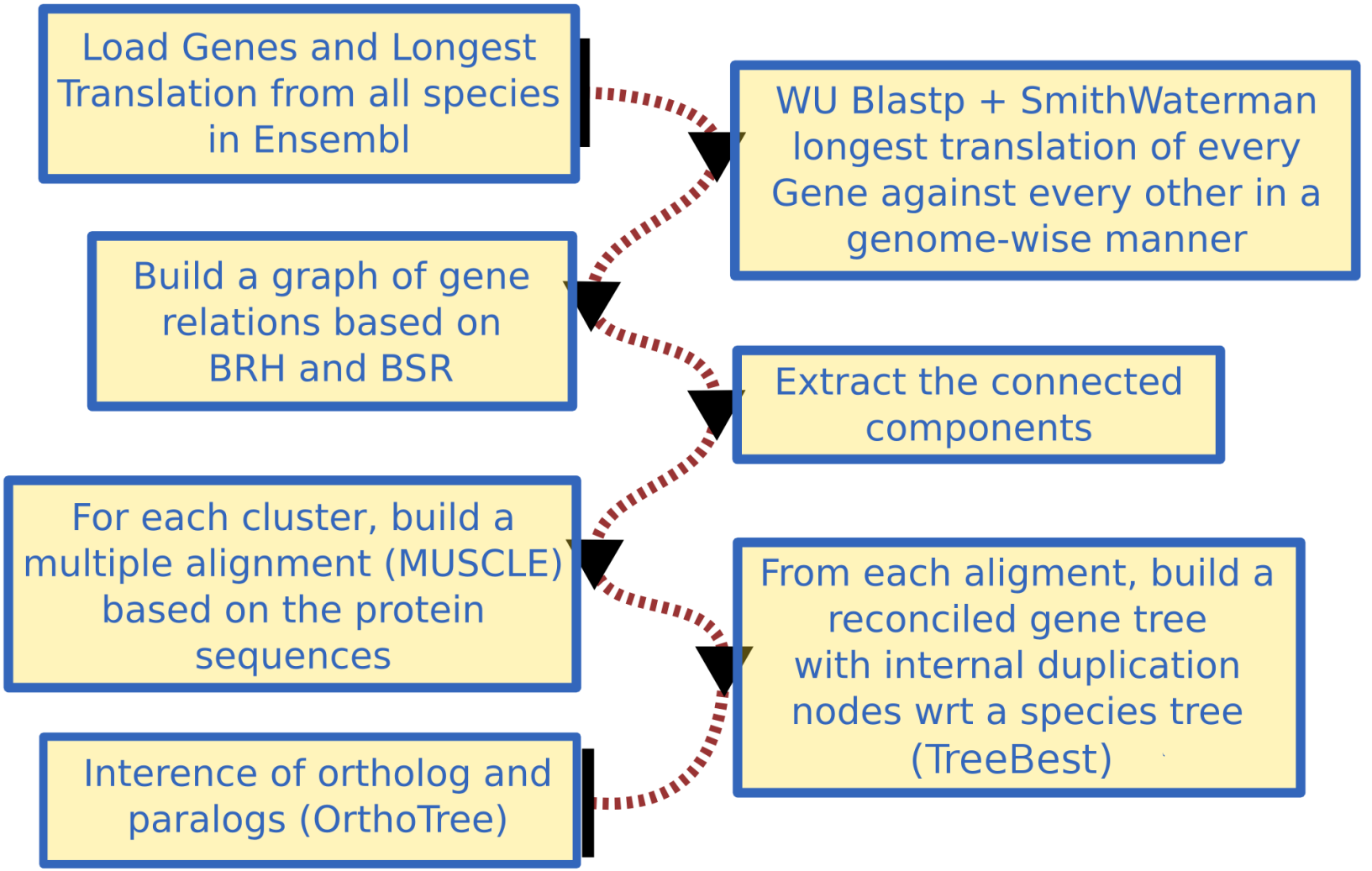
A production system for generating that database

Studying the evolution

- Comparing extant species
- Protein level
  - Multiple alignments
  - Gene Trees (protein trees)
- Genomic level
  - Pairwise alignments
  - Multiple alignments
  - Syntenies

- Conserved regions
- Non-conserved-regions
- Lineage-specific changes

Protein homology

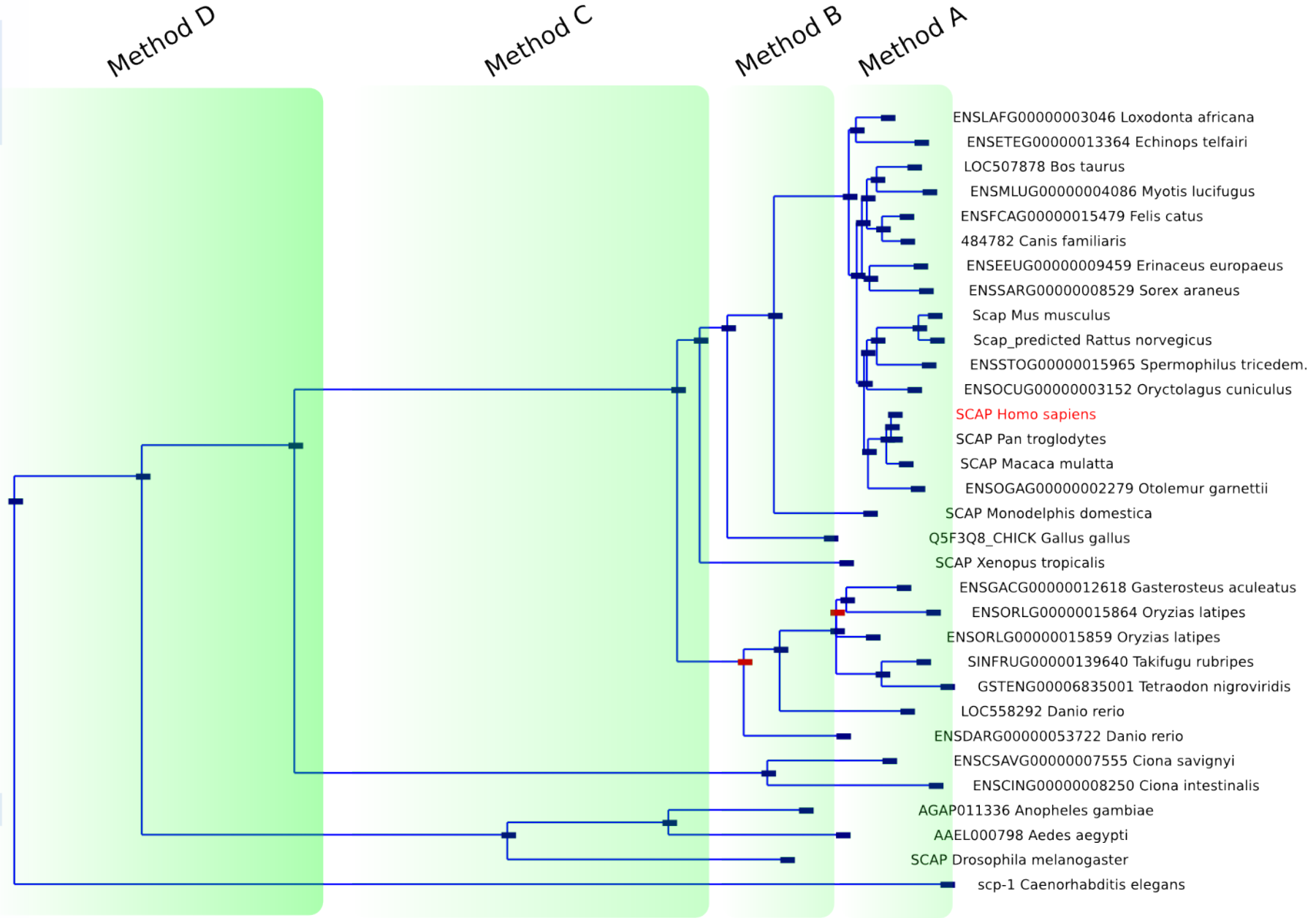


BSR: Blast Score Ratio. When 2 proteins P1 and P2 are compared, BSR=scoreP1P2/max(self-scoreP1 or self-scoreP2). The default threshold used in the initial clustering step is 0.33.

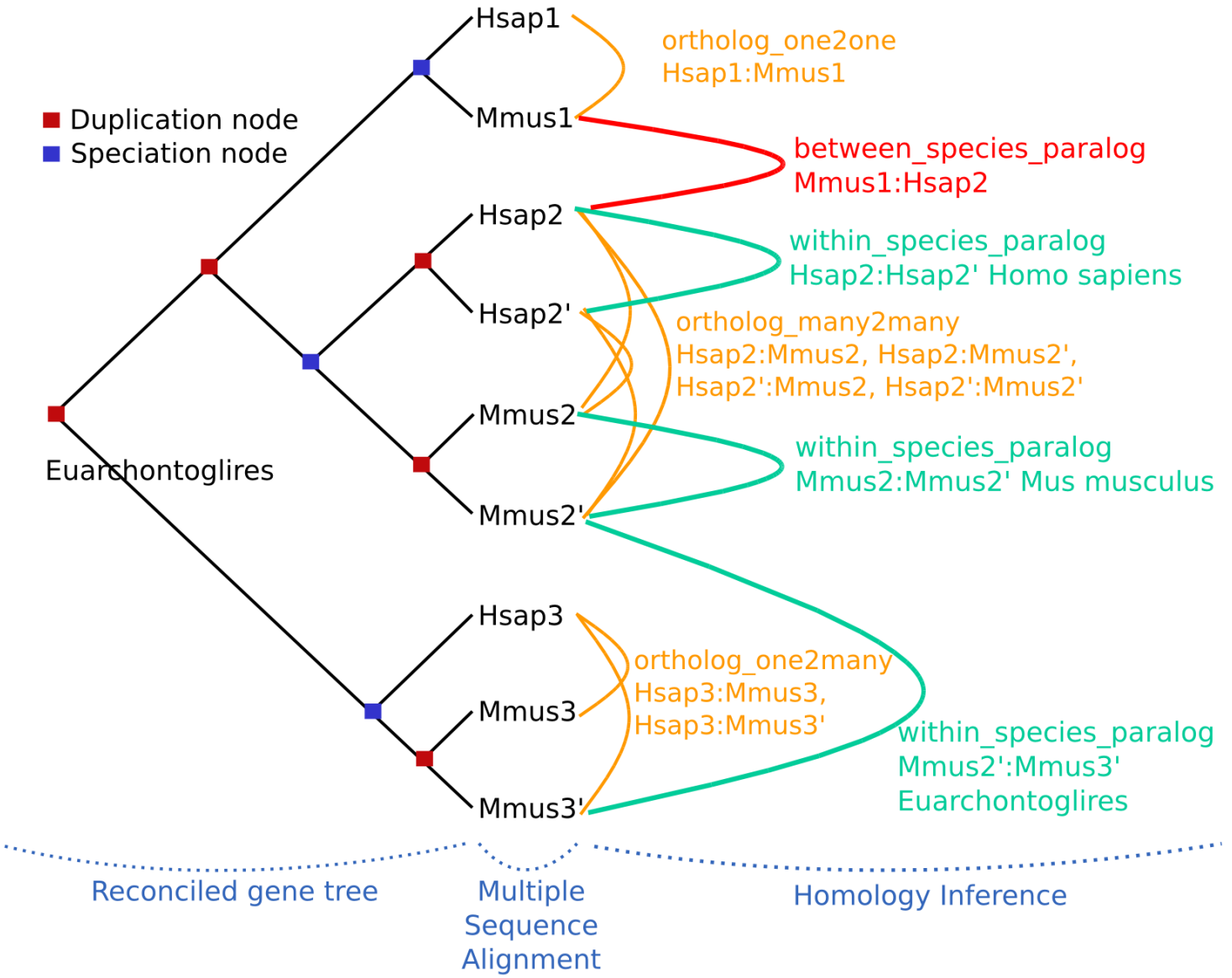
TreeBeST – treemerge algorithm

- ML-AA-WAG4 – WAG matrix aminoacidic model – Maximum Likelihood (PHYML)
- ML-NT-HKY85 – Hasegawa-Kishino-Yano nucleotidic model – Maximum Likelihood (PHYML)
- NJ-NT-p-distance – any substitutions – neighbor-joining with bootstrap
- NJ-NT-dN – non-syn substitutions – neighbor-joining with bootstrap
- NJ-NT-dS – synonymous substitutions – neighbor-joining with bootstrap
- Curated tree topology (if provided)

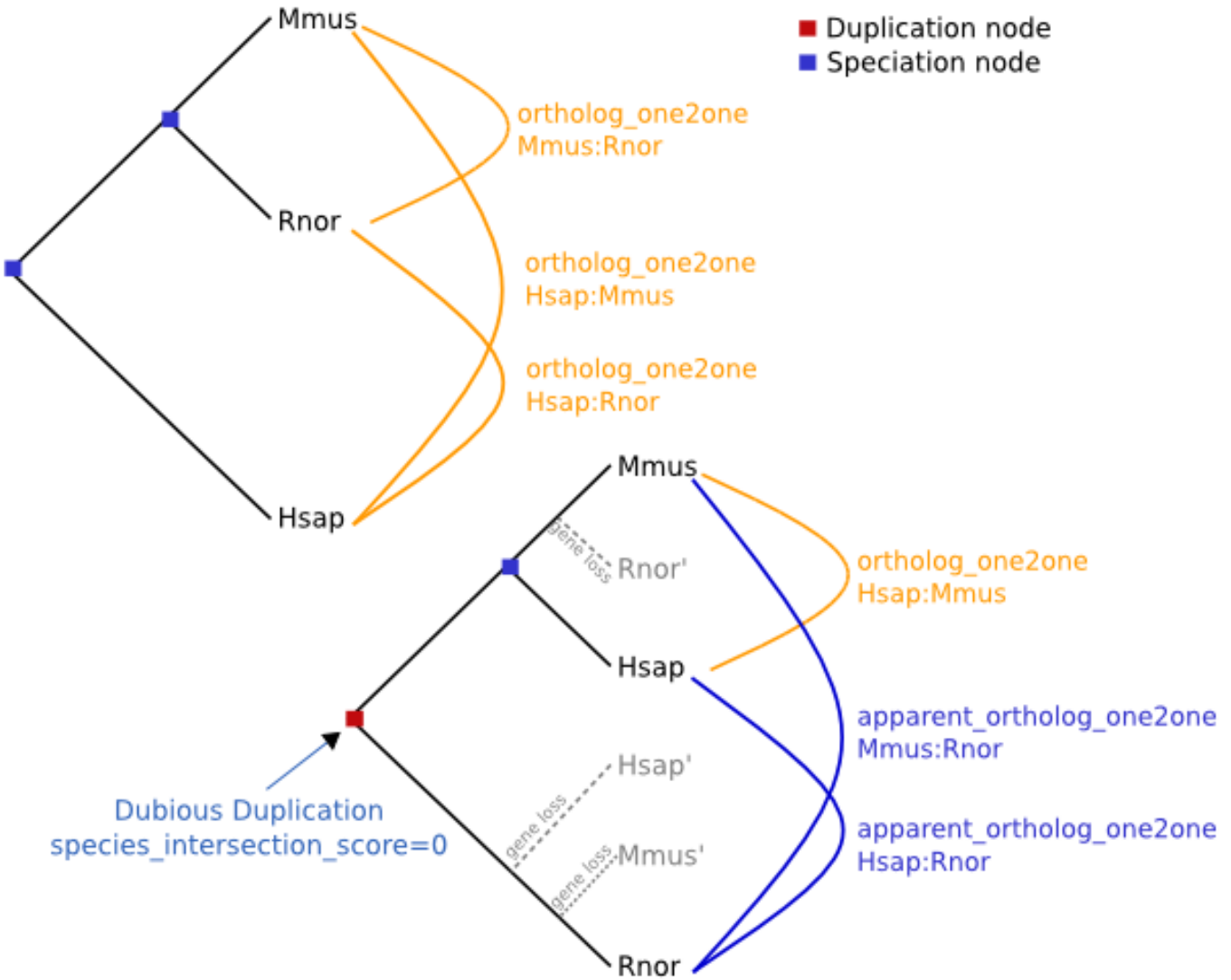
TreeBeST approach



Homology inference

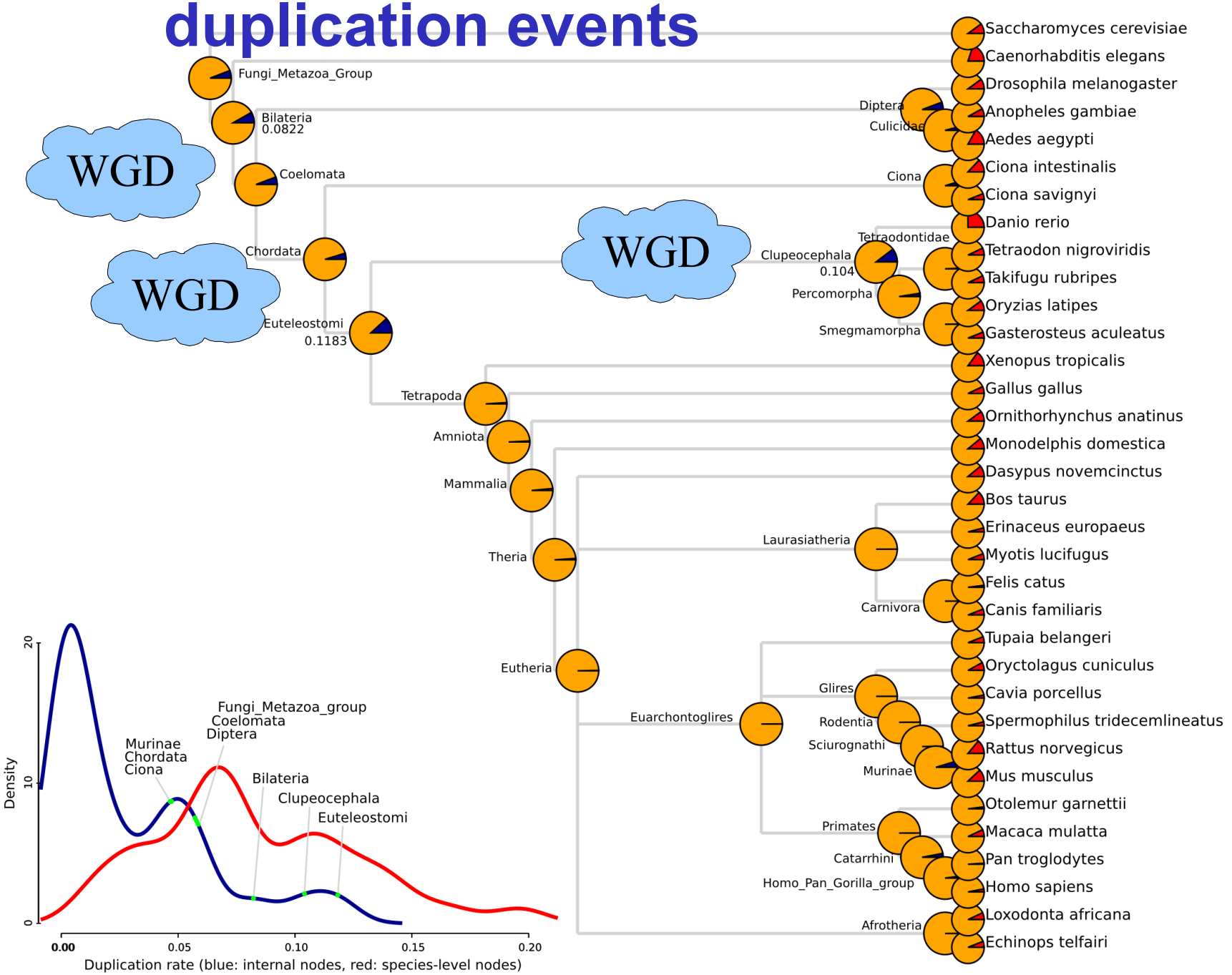


Dubious duplications



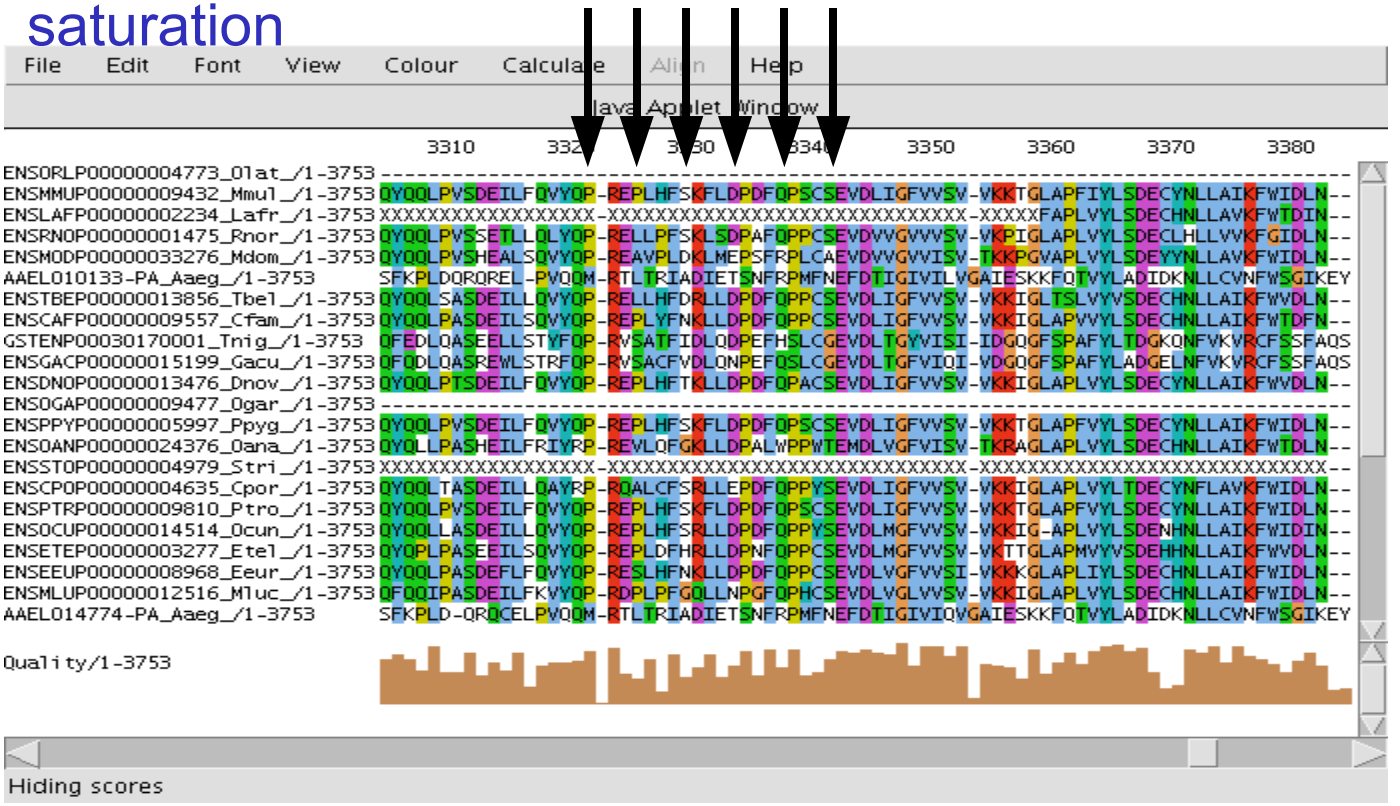
Orthologues : any gene pairwise relation where the ancestor node is a SPECIATION event.
Paralogues : any gene pairwise relation where the ancestor node is a DUPLICATION event.

Topological timing of duplication events

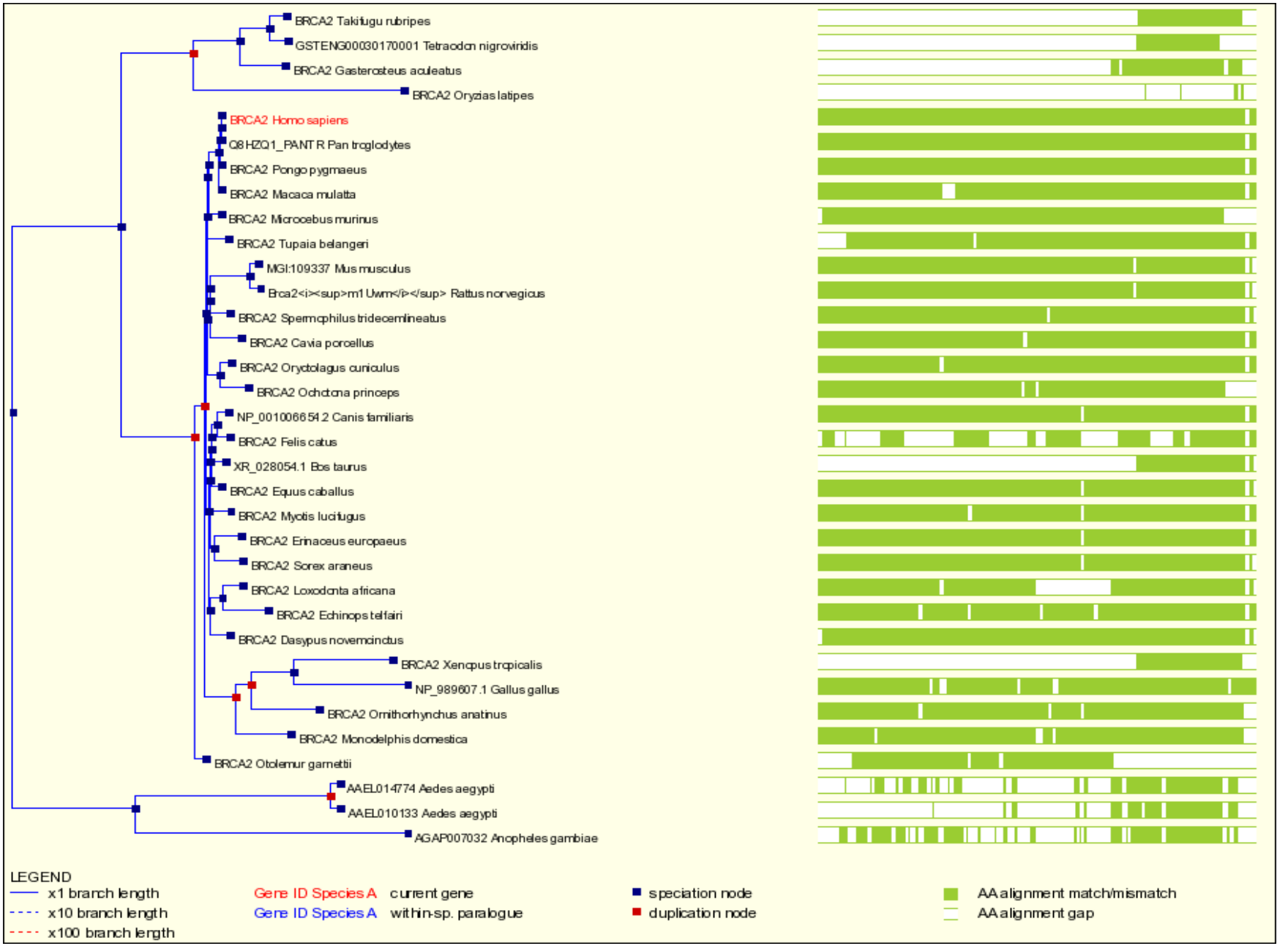


Sitewise dN/dS

- Looking for **nonneutral** evolution at **specific codons** in the alignments
- SLR by Massingham and Goldman (EBI)
  - Doable in 24hr x 400CPUs
  - SLREnsembl -- Choosing subtrees based on dS saturation

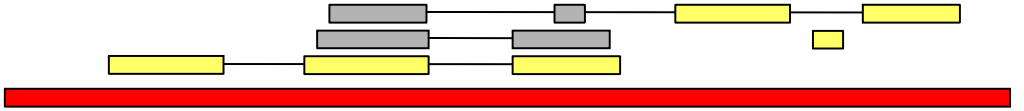


Gene Tree in Ensembl



Genomic Alignments

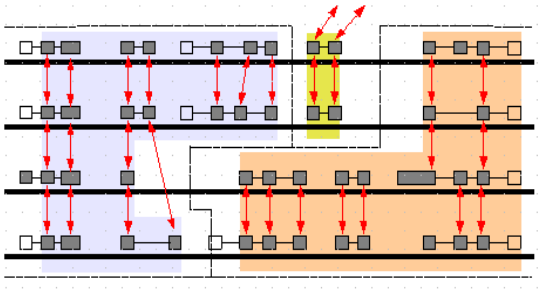
- BlastZ-Net
  - used to compare closely related pair of species
  - BlastZ-raw → BlastZ-chain → BlastZ-net



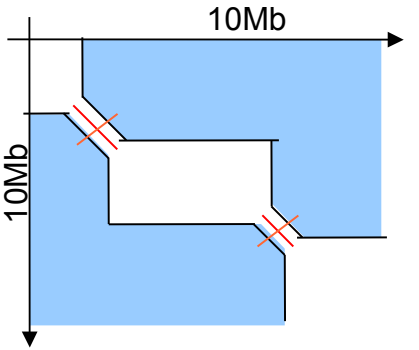
- Translated BLAT
  - used to compare more distant pair of species
  - we use the same approach (chain & net) starting from 50!
- Pecan (Mercator-Pecan)
  - multiple global alignments
  - all vs all coding exons wublastp → Mercator → Pecan on each syntenic block
- EPO (Enredo-Pecan-Ortheus)
  - Segmental duplications + multiple alignments + ancestral sequences inference
  - Anchors → Enredo → Pecan → Ortheus
- GERP (G. Cooper *et al.*, Stanford)
  - Scores the conservation of each col. in the alignment
  - Define constrained elements as stretches of high scores

Mercator-Pecan Pipeline Overview

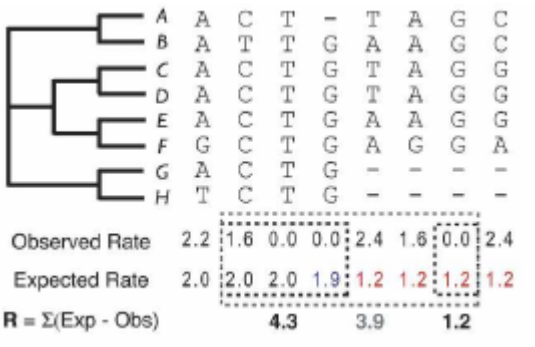
- **Mercator**
  - Defines blocks of orthologous sequences based on coding exon similarities



- **Pecan**
  - Consistency based multiple aligner
  - Optimized to cope with long genomic sequences



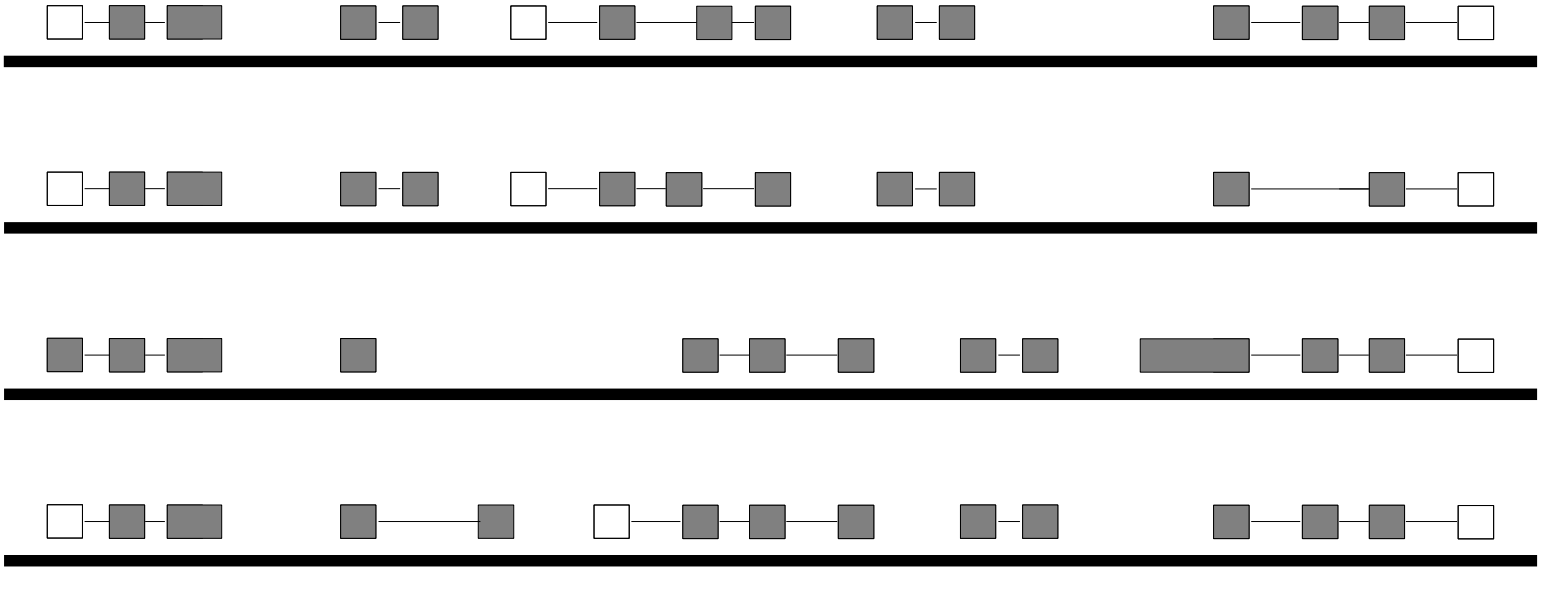
- **GERP**
  - Estimates the conservation of each position in the alignment by looking at the expected and observed number of mutations



Strategy

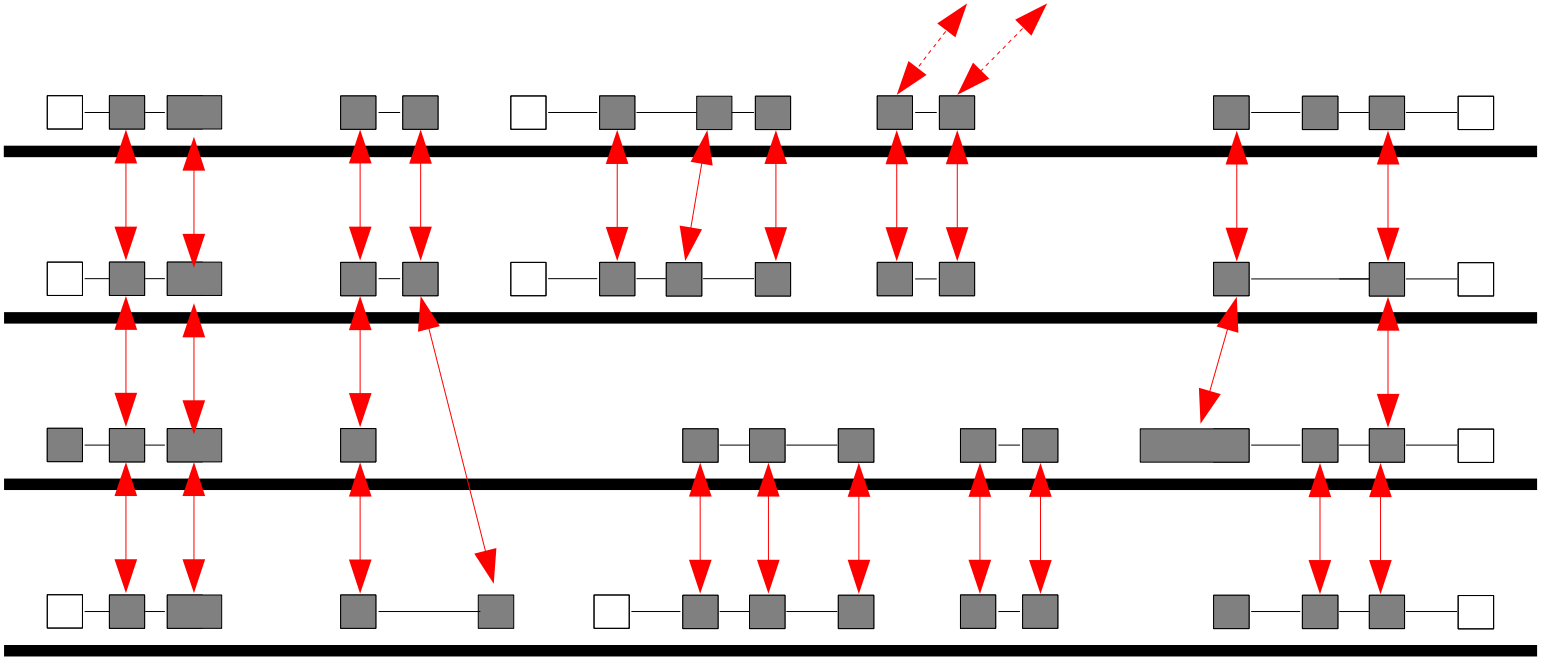
- Global aligner needs orthology maps
- Mercator-Pecan pipeline:
  - 1. Get all coding exons
  - 2. all-vs-all blastp
  - 3. Mercator => strict maps
  - 4. Pecan => multiple alignments

Strategy



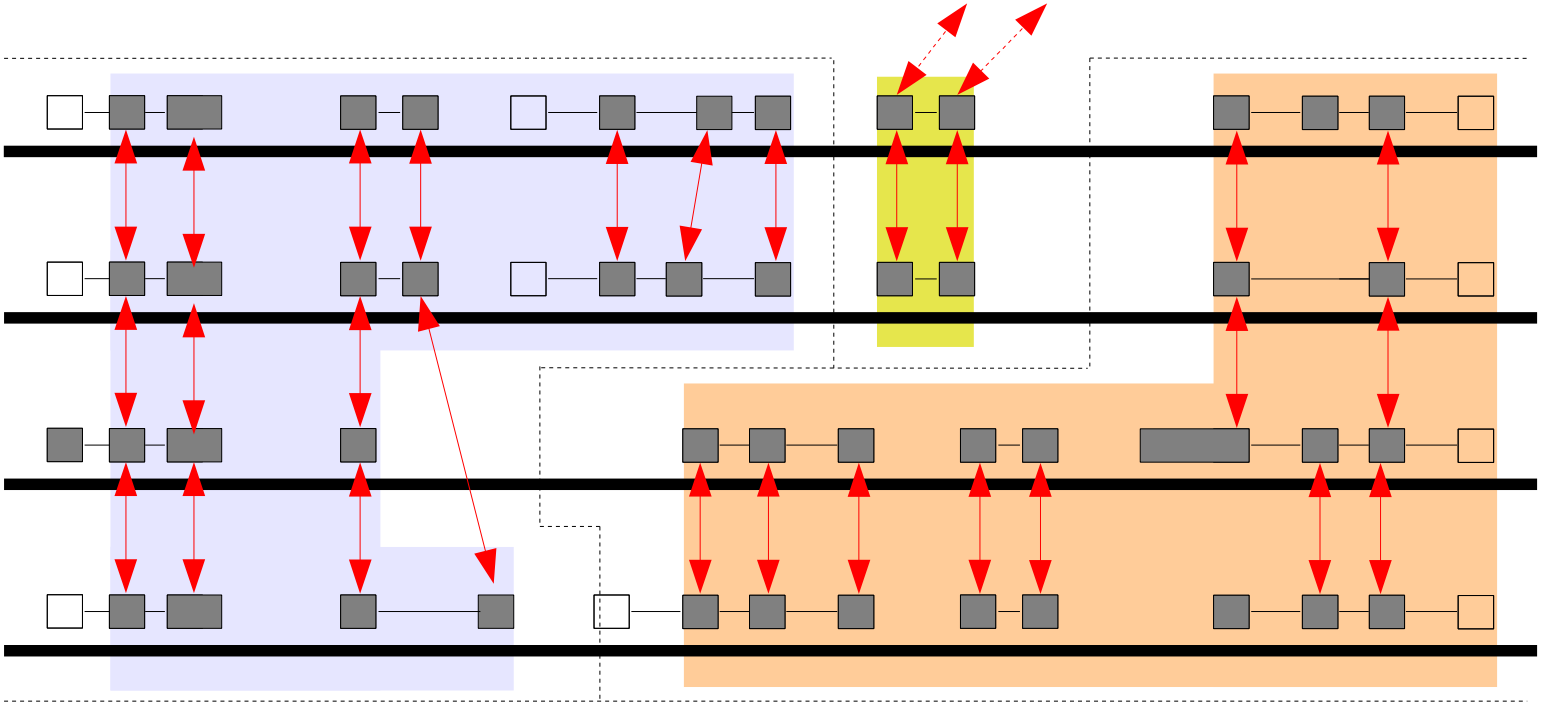
- Use all coding exons

Strategy



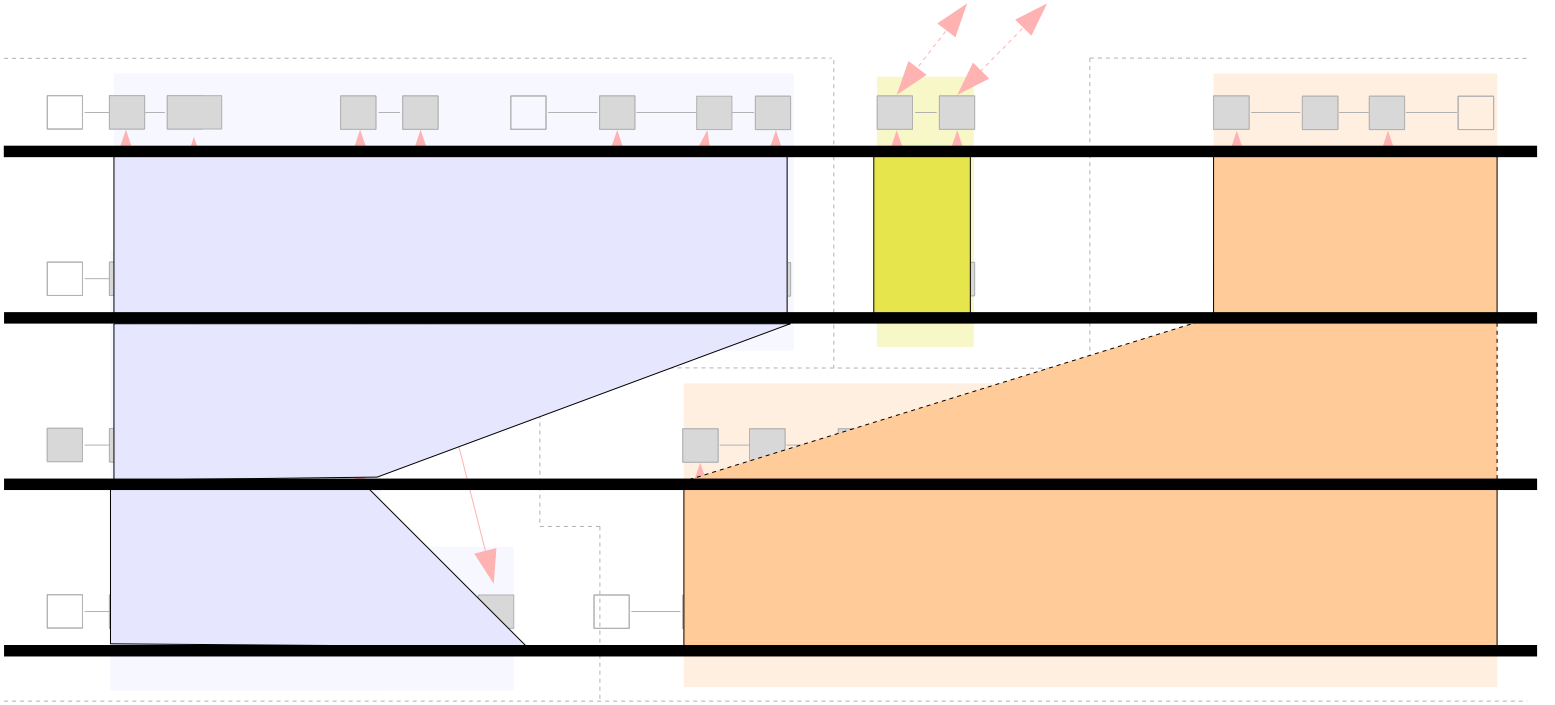
- Use all coding exons
- Get sets of best reciprocal hits

Strategy



- Use all coding exons
- Get sets of best reciprocal hits
- Create orthology maps

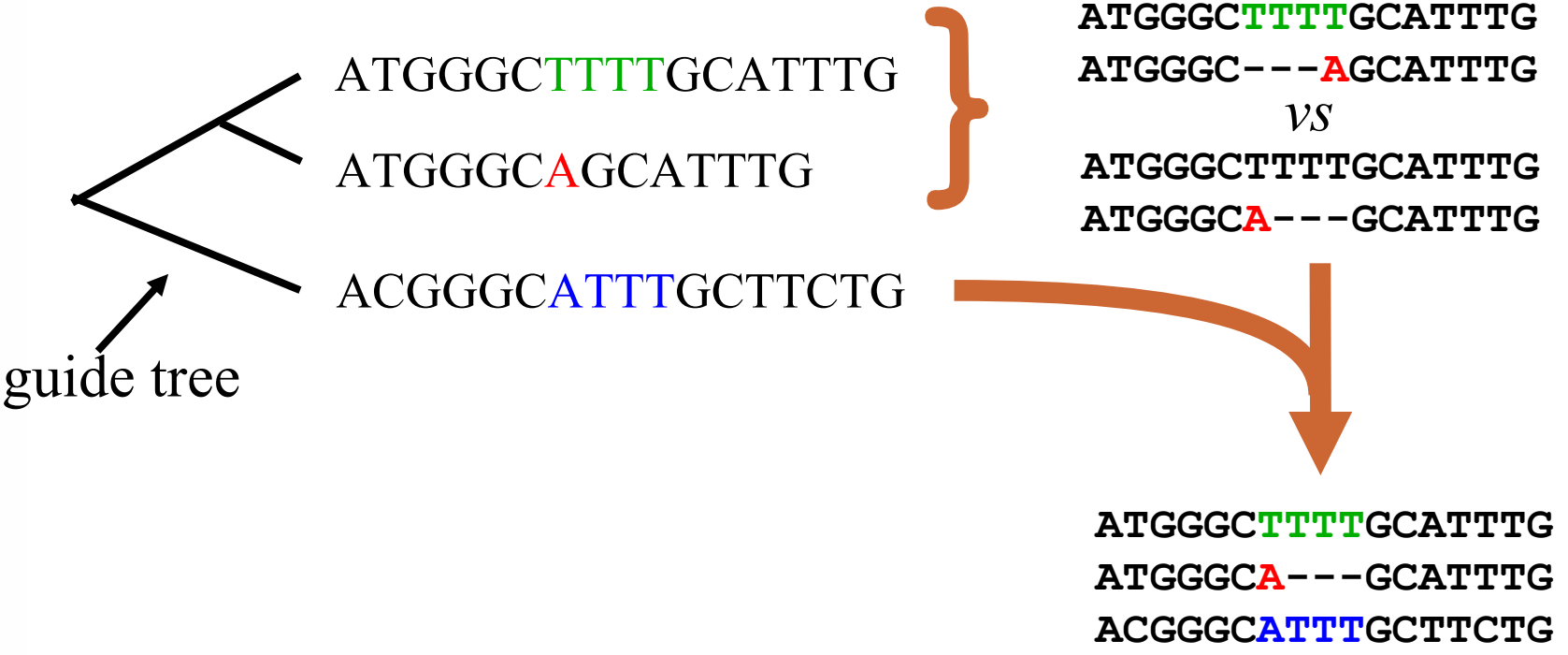
Strategy



- Use all coding exons
- Get sets of best reciprocal hits
- Create orthology maps
- Build multiple global alignments

Pecan

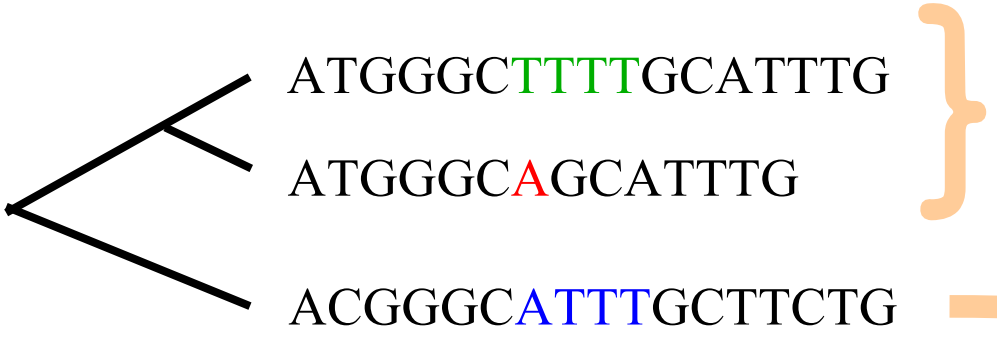
a consistency based multiple-alignment program



Progressive aligner

Pecan

a consistency based multiple-alignment program




```
ATGGGCTTTTGCATTTG
ATGGGC---AGCATTTG
   vs
ATGGGCTTTTGCATTTG
ATGGGCA---GCATTTG
```

```
ATGGGCTTTTGCATTTG
ACGGGCATTTGCTTCTG
```

```
ATGGGCA---GCATTTG
ACGGGCATTTGCTTCTG
```

```
ATGGGCTTTTGCATTTG
ATGGGCA---GCATTTG
```

Takes into account all pairwise alignments, across the entire tree

```
ATGGGCTTTTGCATTTG
ATGGGCA---GCATTTG
ACGGGCATTTGCTTCTG
```

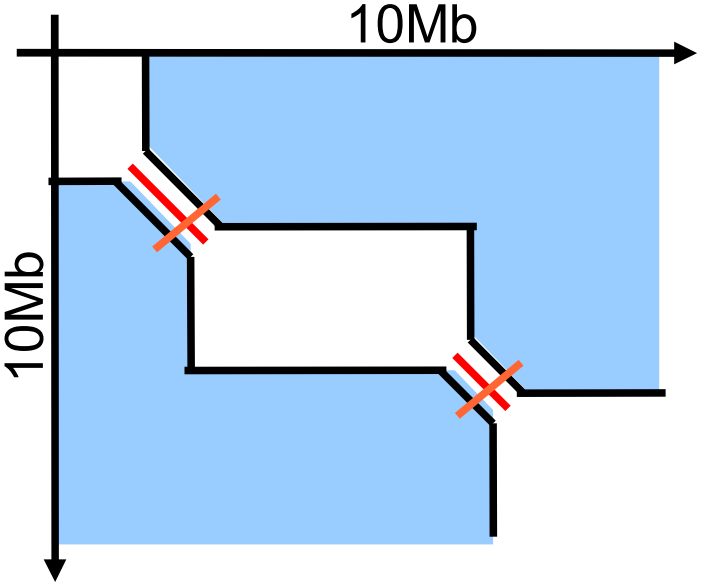
Consistency based aligner

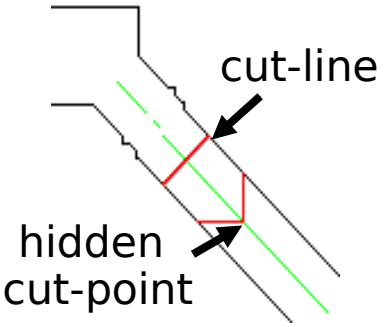
```
ATGGGCTTTTGCATTTG
ATGGGCA---GCATTTG
ACGGGCATTTGCTTCTG
```

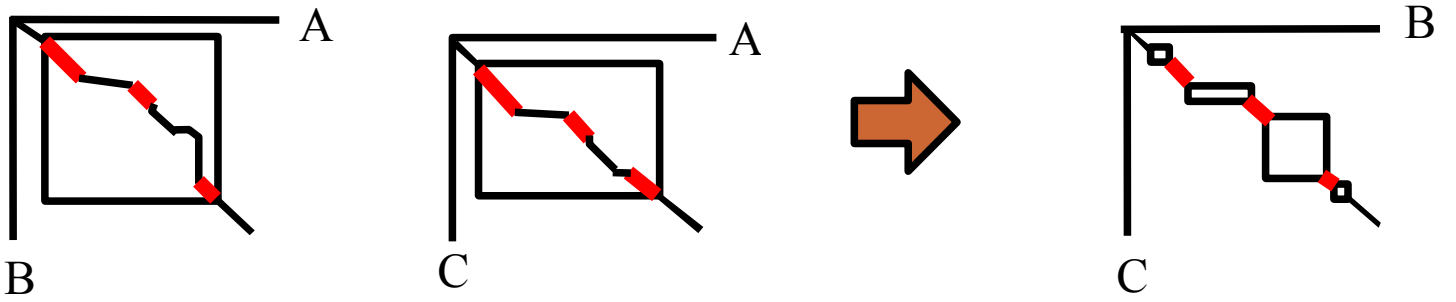
Progressive aligner

Pecan optimizations

- Look for anchors (regions of high similarity)
- perform a banded posterior alignment
- Use cut lines and points to generate effective sub problems for each pairwise alignment simultaneously
- Much redundancy between pairwise alignments: use transitive anchors

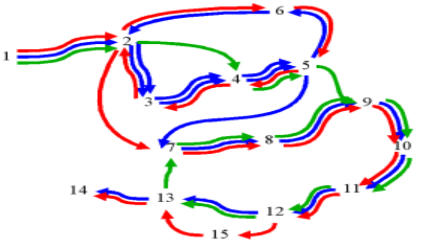


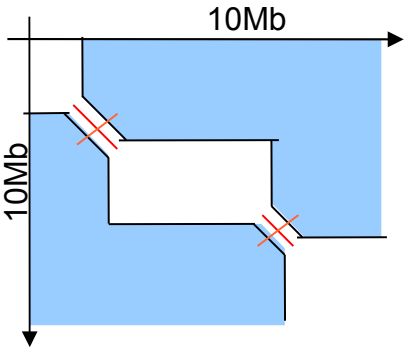


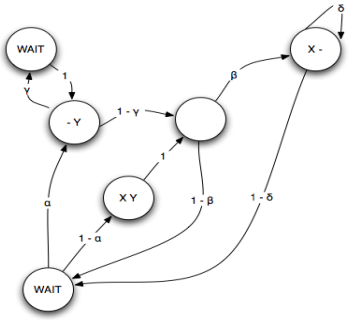


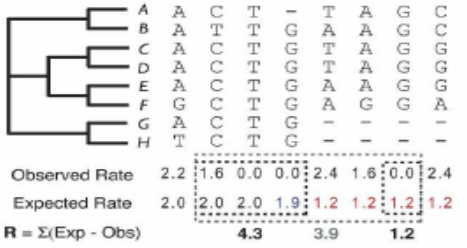
EPO Pipeline Overview

- **Enredo**
  - Defines blocks of collinear sequences
  - Supports segmental duplications
- **Pecan**
  - Consistency based multiple aligner
  - Optimized to cope with long genomic sequences
- **Ortheus**
  - Ancestral sequences reconstructor (Tree Aligner)
  - Infers the history of insertion and deletions
- **GERP**
  - Estimates the conservation of each position in the alignment







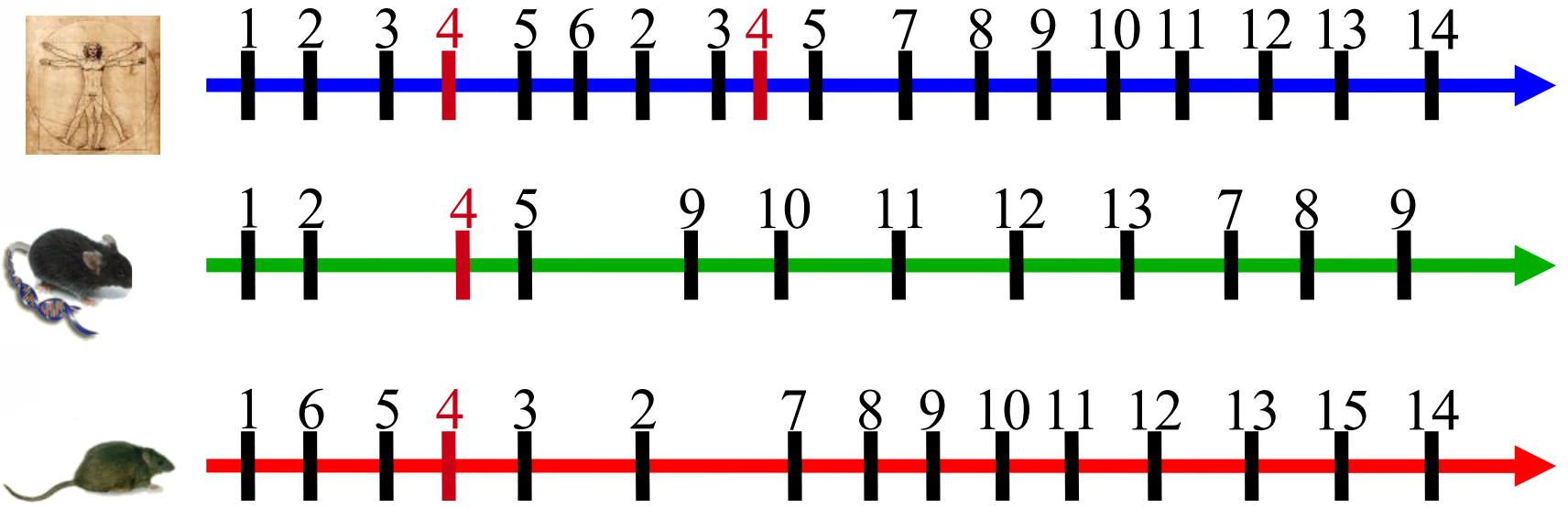


ENREDO graph

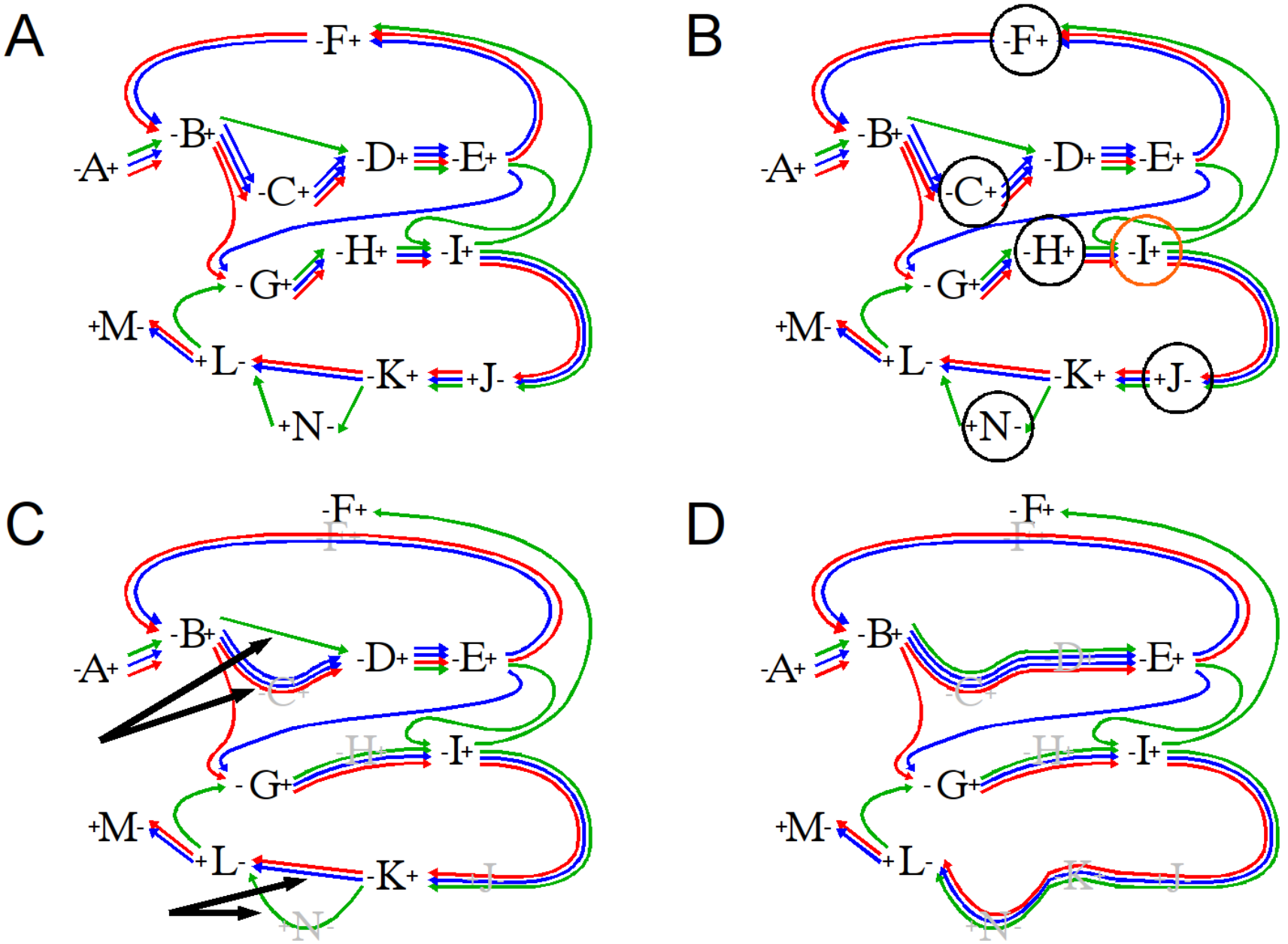
- Similar in spirit to a De Bruijn graph of sequences used for assembly
  - homologous regions between genomes will be represented as one edge
- Formed by creating a set of non-redundant anchors (short regions) which are present 0, 1 or multiple times in each extant genome
- Anchors could be all coding exons, made non-redundant to handle duplications
- In our case, a series of pairwise alignments defines short regions of high homology between genomes

ENREDO: Mapping the anchors

- Mapping the anchors
- Cleaning up the anchor set
  - Removal of overlapping anchors
  - Removal of anchors mapping too many times

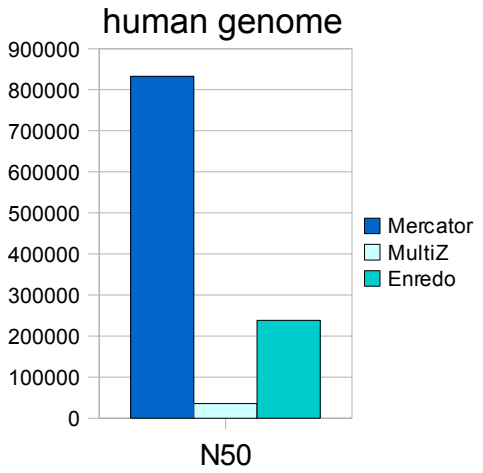


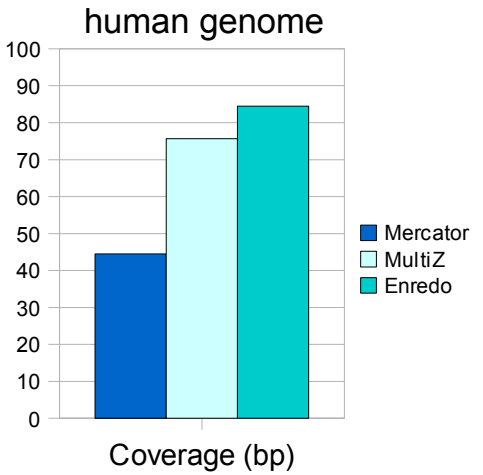
ENREDO Graph

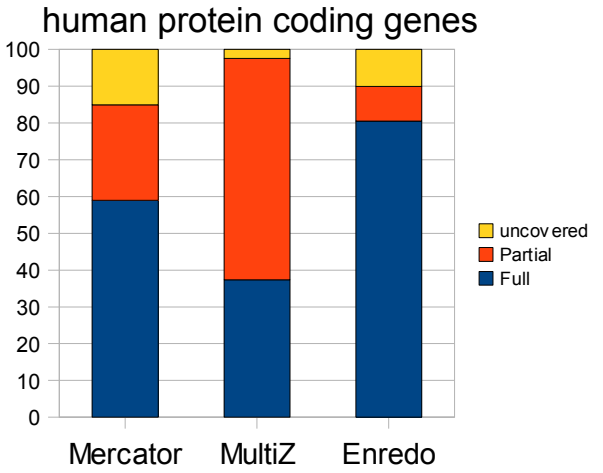


Enredo assessment

- Human, Mouse, Rat, Dog and Cow
- Mercator, MultiZ and Enredo coverage





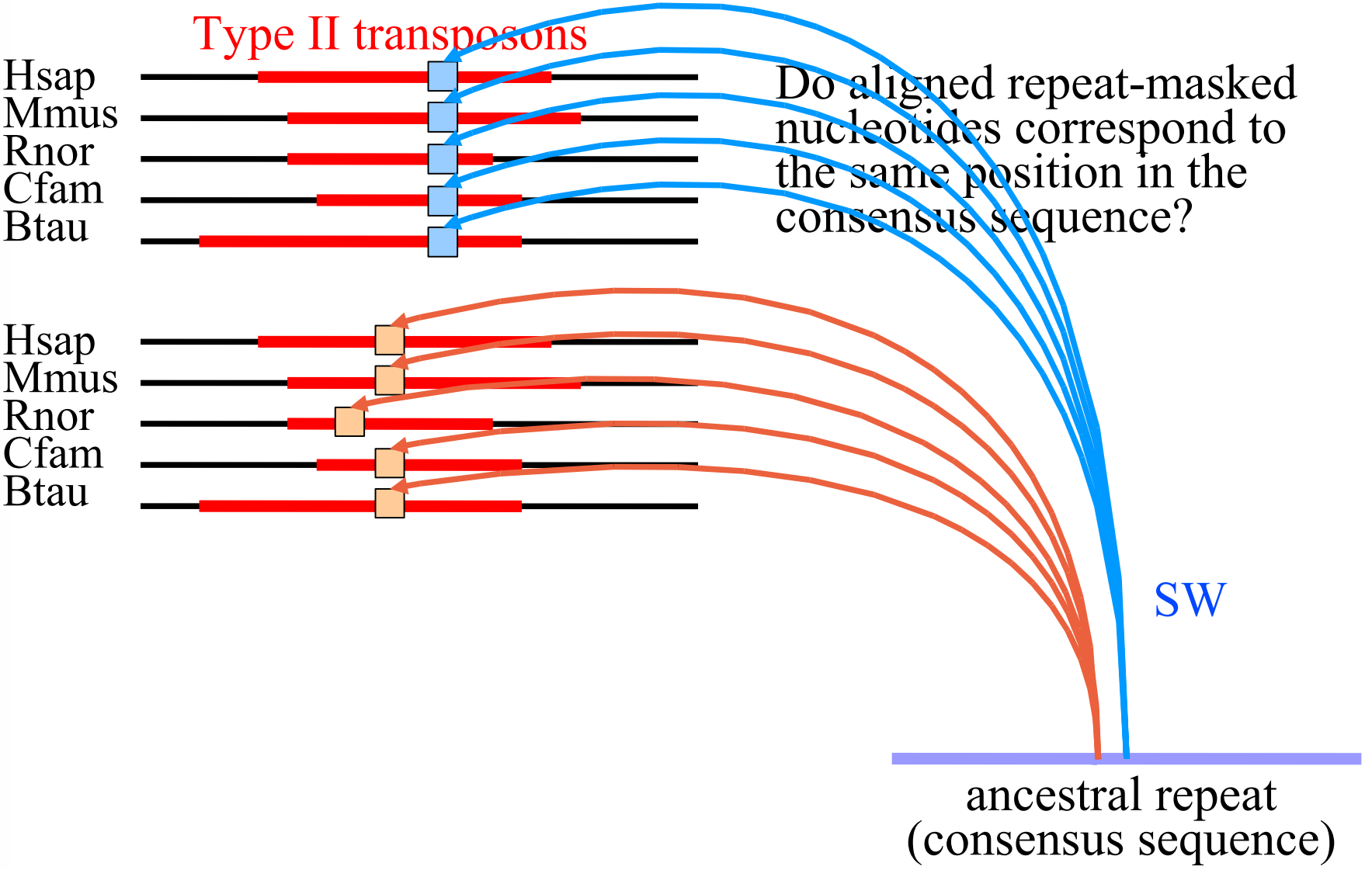


- Putative rearrangements between human chromosome X and any autosome in another species

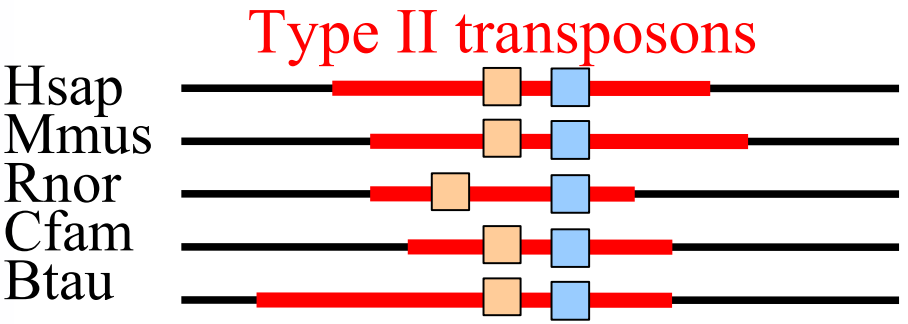
Method	blocks		length	
Mercator	15	6.7%	2750241	4.0%
MultiZ*	211117	28.0%	25785059	19.0%
Enredo	19	1.3%	1168017	1.0%

* from UCSC 17 way MultiZ

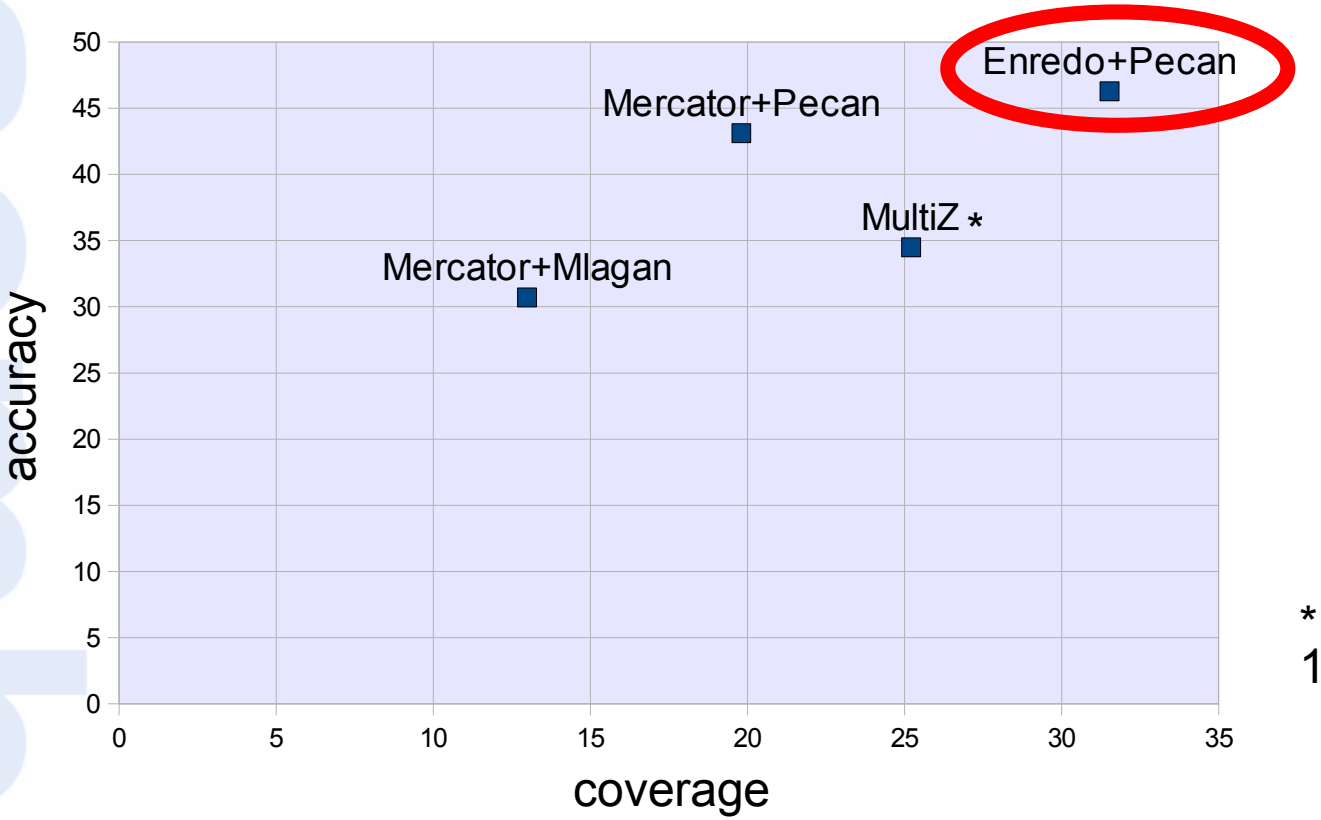
Multiple aligner assessment: ancestral repeats



Multiple aligner assessment: ancestral repeats



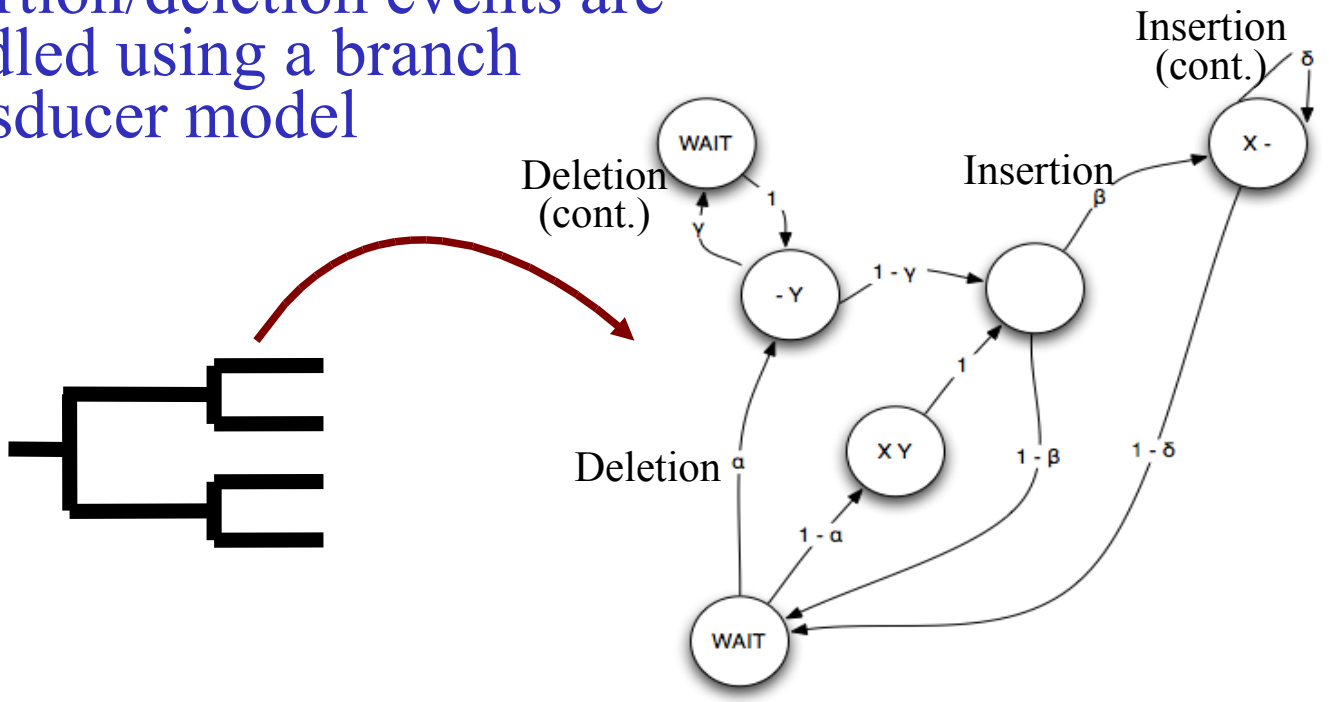
Do aligned repeat-masked nucleotides correspond to the same position in the consensus sequence?



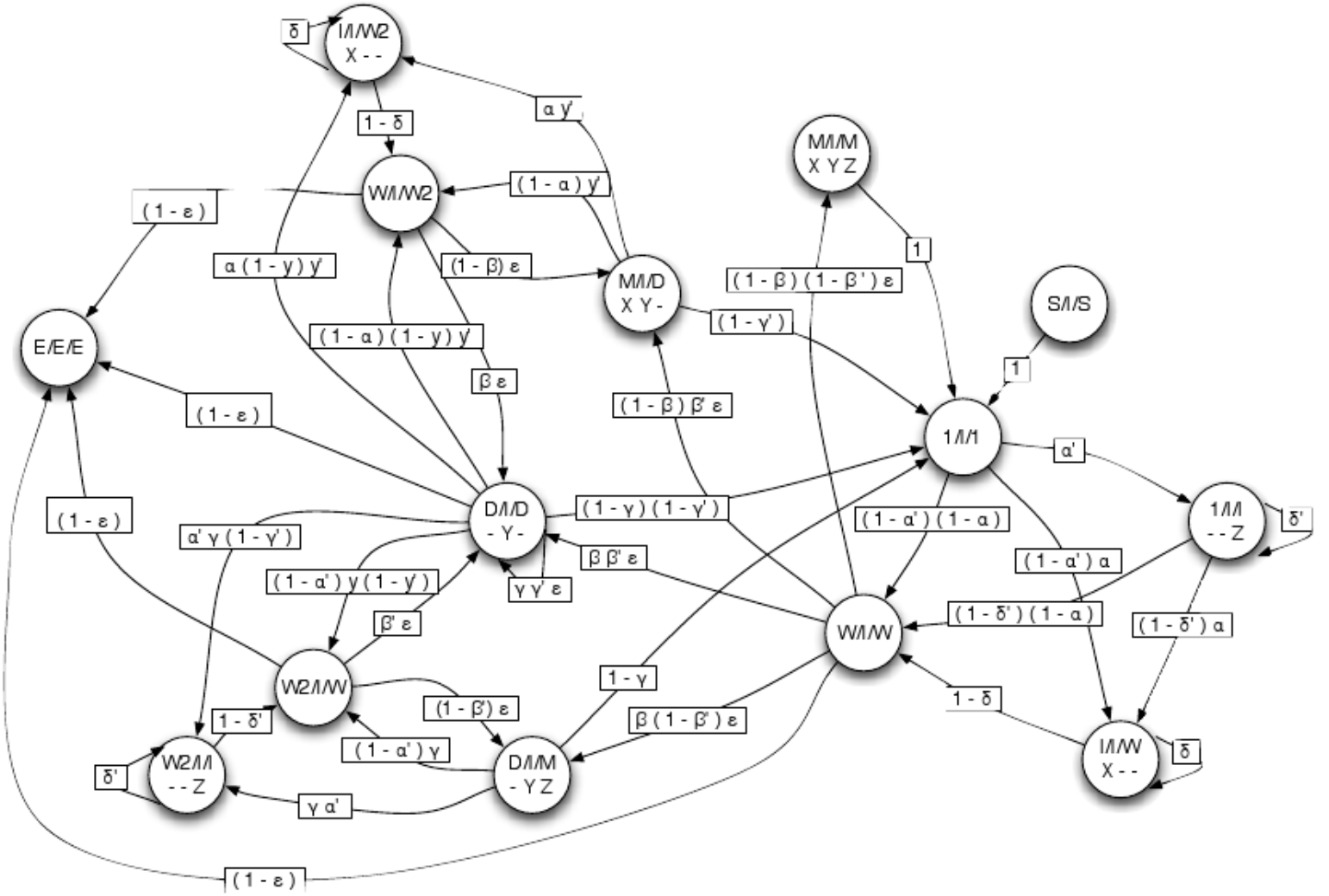
* from UCSC 17way multiZ

Ortheus

- Addresses the inference of insertion-deletion histories and substitution events
- Uses a multiple alignment as guiding input
- Reconstructs the ancestral sequences in the tree and refines the input alignment
- Insertion/deletion events are handled using a branch transducer model

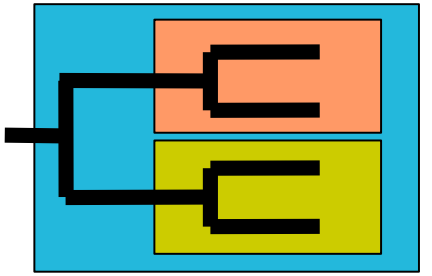


Ortheus transducer model for 2 descendants and 1 ancestor

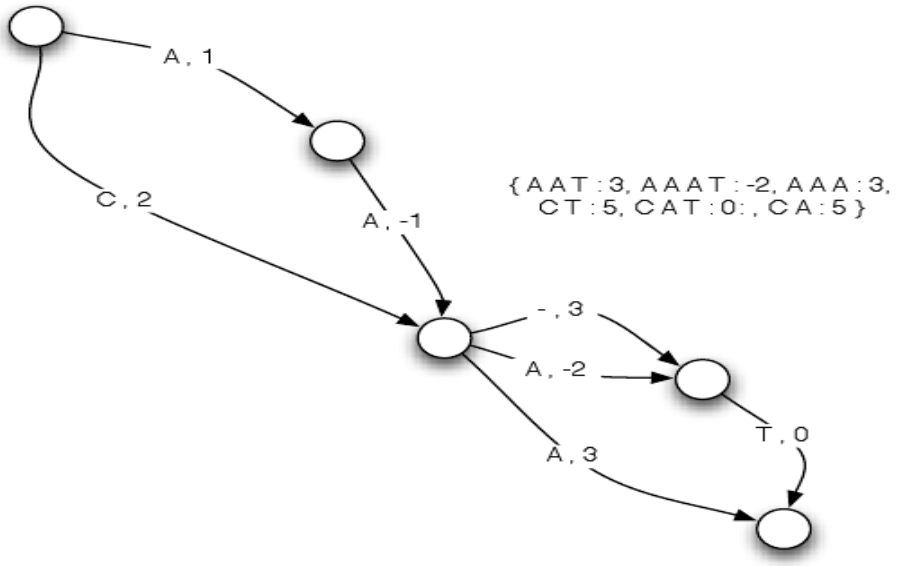


Ortheus: inference of the ancestral sequence

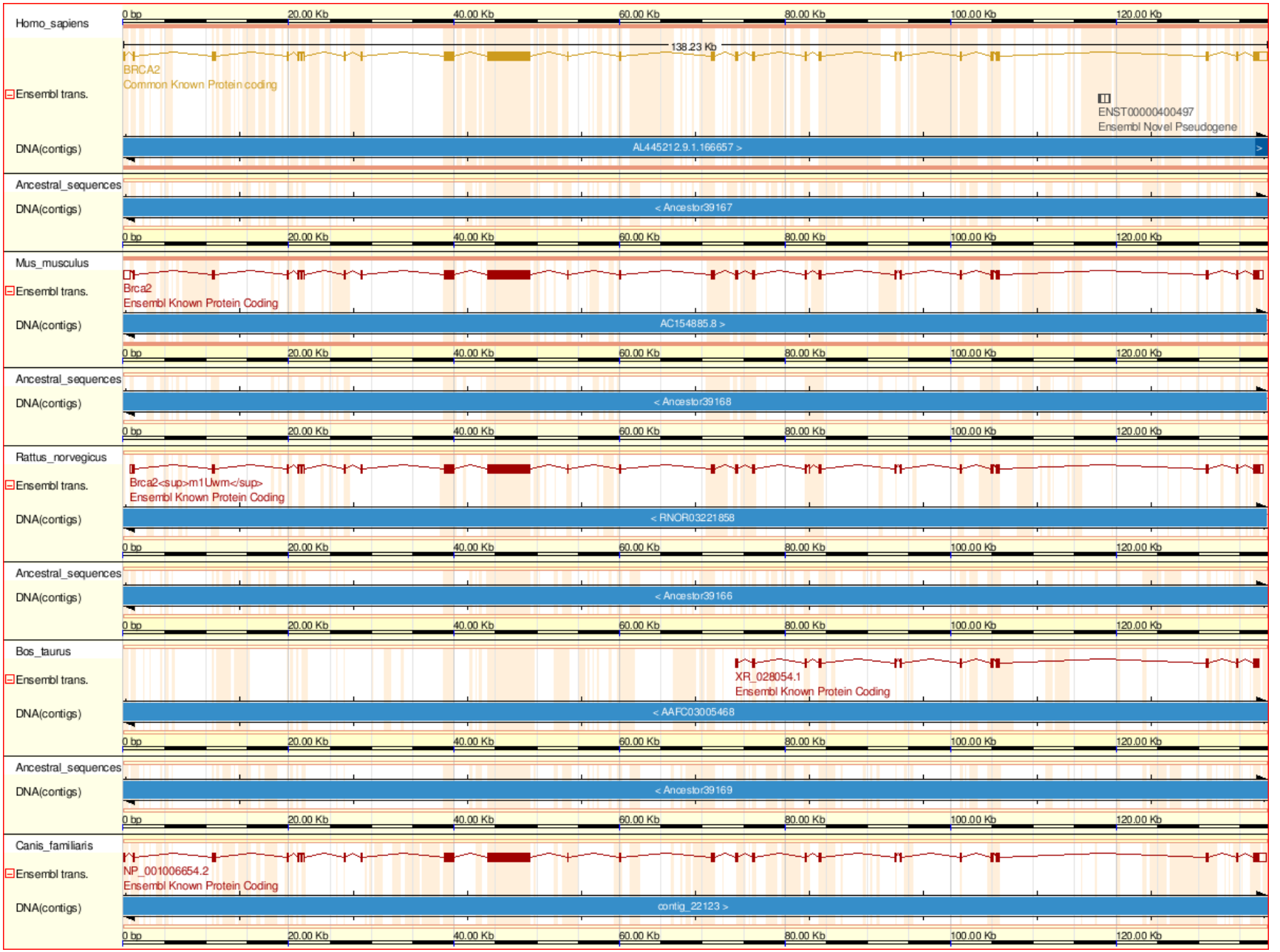
- Substitution are handled using Tamura-Nei nucleotide substitution model.
- Works in a progressive manner:



- Ancestral sequences are represented using weighted sequence graphs



Display on AlignSliceView

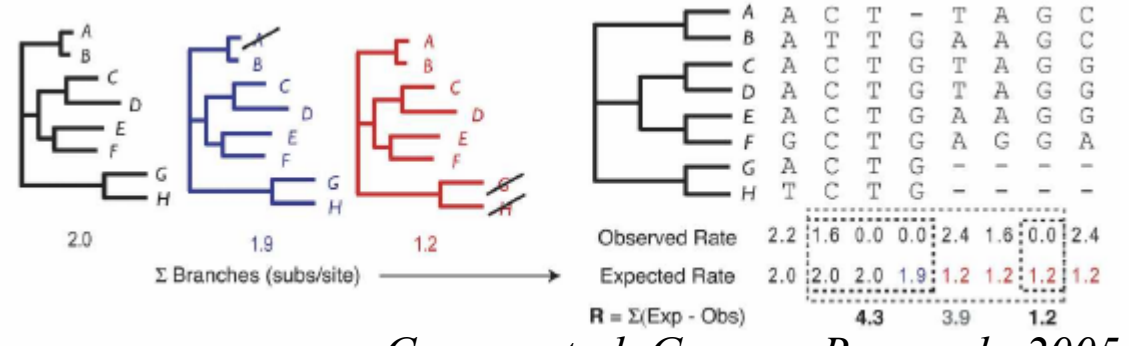


Current sets of alignments

- Primates:
  - 4-way EPO alignments (high-coverage genomes only)
- Mammals
  - 9-way EPO alignments (high-coverage genomes only)
  - 23-way EPO alignments (including 2X genomes)
- Amniota
  - 12-way Mercator-Pecan alignments (high-cov. Only)
- *Fish*
  - *Planning a 5-way EPO alignments set for 2009*

Gerp Constrained Elements

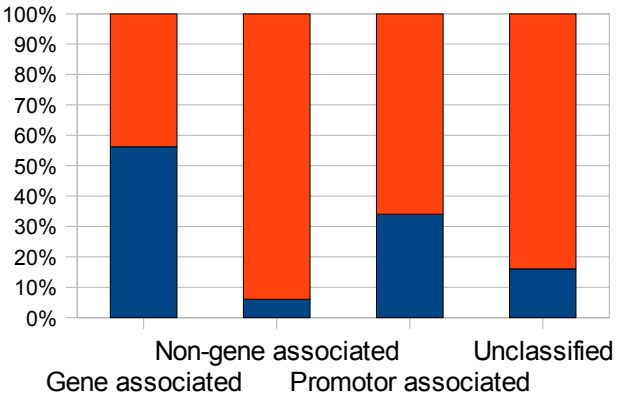
- Stretches of the alignment with a high conservation



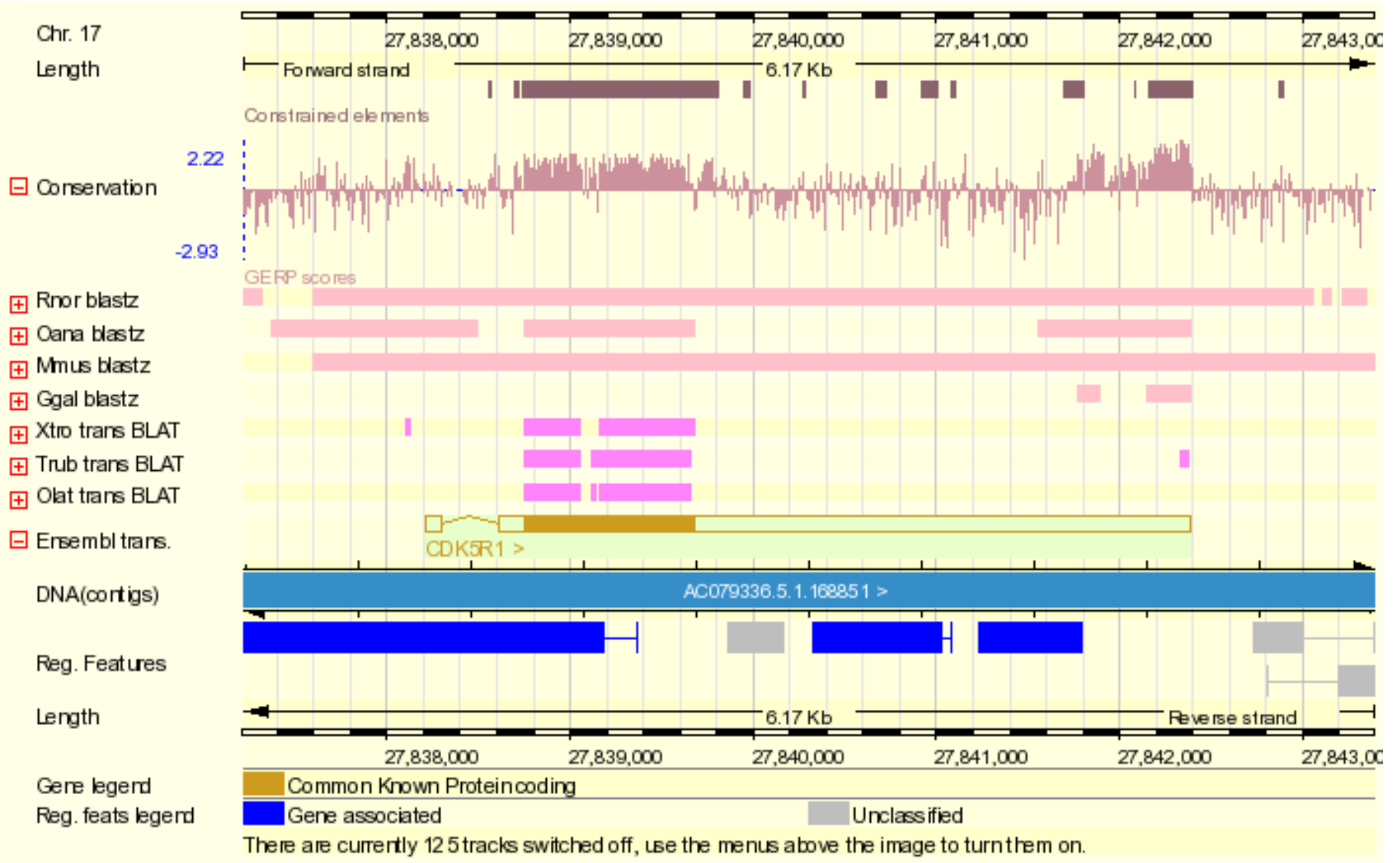
Cooper et al. Genome Research, 2005

- Constrained elements and coding exons
  - 74% of coding exons are associated with constr. elem.
  - 22% of constr. elem. are associated with coding exons

- Co-occurrence of features
  - Annotation of constr. elements
    - genes, TSS, Reg. features...
  - Annotation of SNPs
    - in constrained elements or not



ContigView: p23



GeneSeqAlingView: p23

THIS STYLE: Location of conserved regions (where >50% of bases in alignments match)

THIS STYLE: Location of START/STOP codons

THIS STYLE: Location of selected exons

THIS STYLE: Location of SNPs

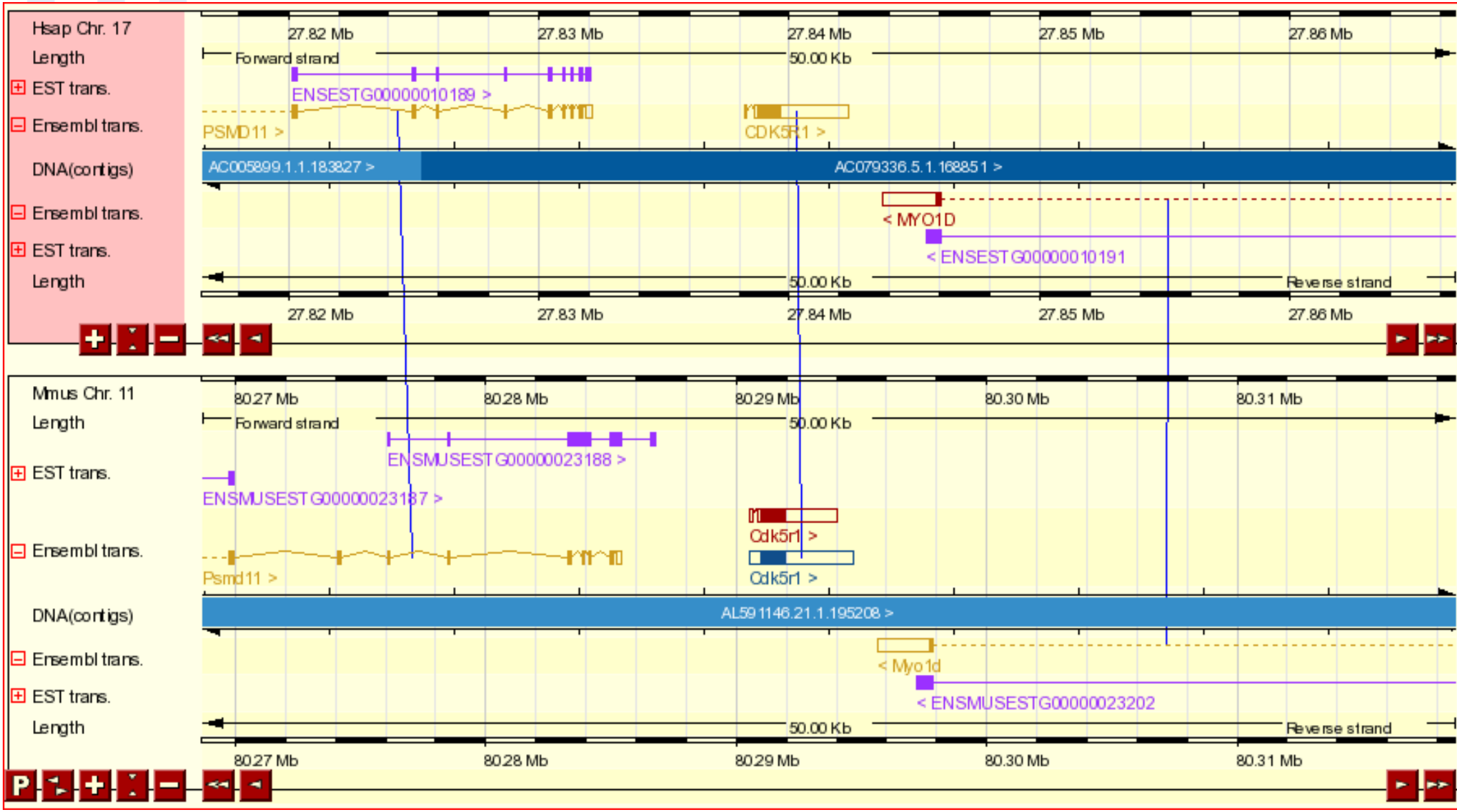
THIS STYLE: Location of deletions

Homo_sapiens > chromosome:NCBI36:17:27838014:27842583:1

Macaca_mulatta > chromosome:MMUL_1:16:27819375:27823565:1

```
Homo_sapiens        481 AGGGCCCGGGACTGGGGCGGCGGGGTGCGCCGAGGCGCGGGGCGGAGGGGCGCAGGGGCG 540
Macaca_mulatta      481 AGGGCCCGGGACTGGGGCCGCGGGGTCCGCGGAGGCGCGGGGCGGAGGGGCGC------- 540

Homo_sapiens        541 CAGGGGCGCGGCGCGGAGCCCAGCCTGGCGCTAAGAACCATCTTGTTTTCCAGGCAGATC 600
Macaca_mulatta      541 ---------GGCGCGGAGCCCAGCTTGGCGCTAAGAACCATCTTGTTTTCCAGGCAGATC 600

Homo_sapiens        601 CAAGGGGGCAGCACGCTTCCCGGGAGCGCCCCCGCCTCCTCTCCGGGGCCGCCGCAGGCT 660
Macaca_mulatta      601 CAAGGGGGCAGCACGCTTCCCGGGAGCGCCCCCGCCTCCTCCCCGGGGCCACCGCAGGCT 660

Homo_sapiens        661 CGGTGAGCGGTTTTATCCYTCCGGCCGGCAGGCTGGGCGCGCAGGGGCGCGAGCCCCCGC 720
Macaca_mulatta      661 CCGTGAGTGGTTTTATCCCTCCGGCCGGCAGGCTGGGCGCGCAGGGGCGCGAGCCCCCGC 720

Homo_sapiens        721 CCGGCGCGCAGCAGCACCATGGGCACGGTGCTGTCCCTGTCTCCCAGCTACCGGAAGGCC 780
Macaca_mulatta      721 CCGGCGCGCAGCGGCACCATGGGCACGGTGCTGTCCCTGTCCCCCAGCTACCGGAAGGCC 780

Homo_sapiens        781 ACGCTGTTTGAGGATGGCGCGGCCACCGTGGGCCACTATACGGCCGTACAGAACAGCAAG 840
Macaca_mulatta      781 ACGCTGTTTGAGGATGGCGCGGCCACCGTGGGCCACTATACGGCCGTACAGAACAGCAAG 840

Homo_sapiens        841 AACGCCAAGGACAAGAACCTGAAGCGCCACTCCATCATCTCCGTGCTGCCTTGGAAGAGA 900
Macaca_mulatta      841 AACGCCAAGGACAAGAACCTGAAGCGCCACTCCATCATCTCCGTGCTGCCTTGGAAGAGA 900

Homo_sapiens        901 ATCGTGGCCGTGTCGGCCAAGAAGAAGAACTCMAAGAAGGTGCAGCCYAACAGCAGCTAC 960
Macaca_mulatta      901 ATCGTGGCCGTGTCGGCCAAGAAGAAGAACTCCAAGAAGGTGCAGCCCAACAGCAGCTAC 960

Homo_sapiens        961 CAGAACAACATCACGCACCTCAACAATGAGAACCTGAAGAAGTCGCTGTCRTGYGCCAAC 1020
Macaca_mulatta      961 CAGAACAACATCACGCACCTCAACAATGAGAACCTGAAGAAGTCGCTGTCGTGCGCCAAC 1020
```

MultiContigView

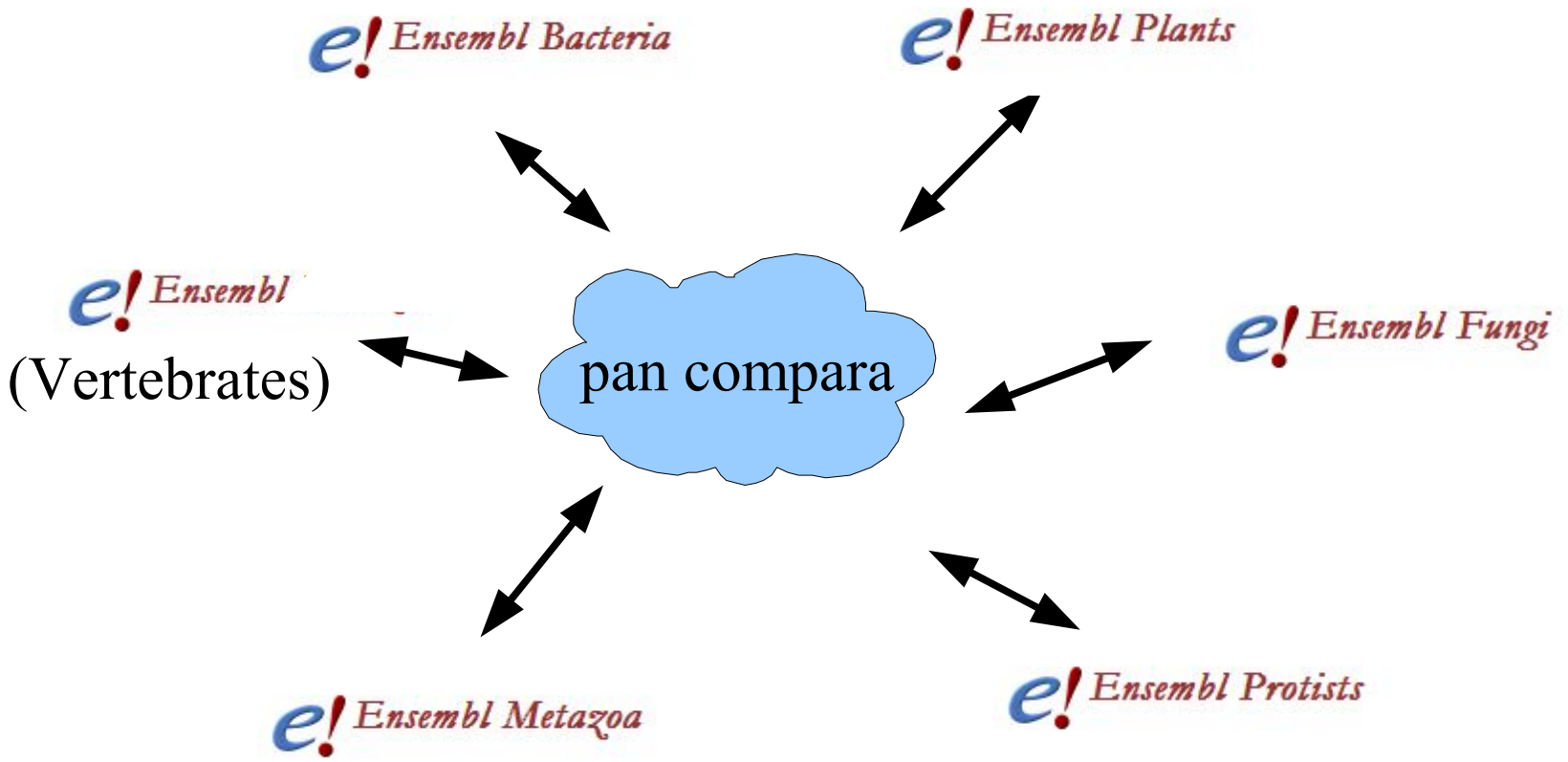


Summary

- Ensembl is a system created for the study and analysis of the genomes
- Comparative genomics
  - Protein tree and inference of homologues
  - Genomic alignments, conserved regions
- Many views to match different usages
  - ContigView: genomic region
  - MultiContigView: side-by-side comparison
  - AlignSliceView: alignment in genomic context
  - GeneSeqAlignView: alignment of genomic regions
  - GeneTreeView: protein trees, homologues
  - many other views...
- All data accessible through the web and the Perl API

Pan-Ensembl compara

- Take advantage of the whole new span of Ensembl Genomes
- Link the projects together
- Breakout session after the coffee/tea break!!



EMBL-EBI

wellcome trust sanger institute

Ensembl

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