Automatic retrieval of orthologs and paralogs in databases of gene families

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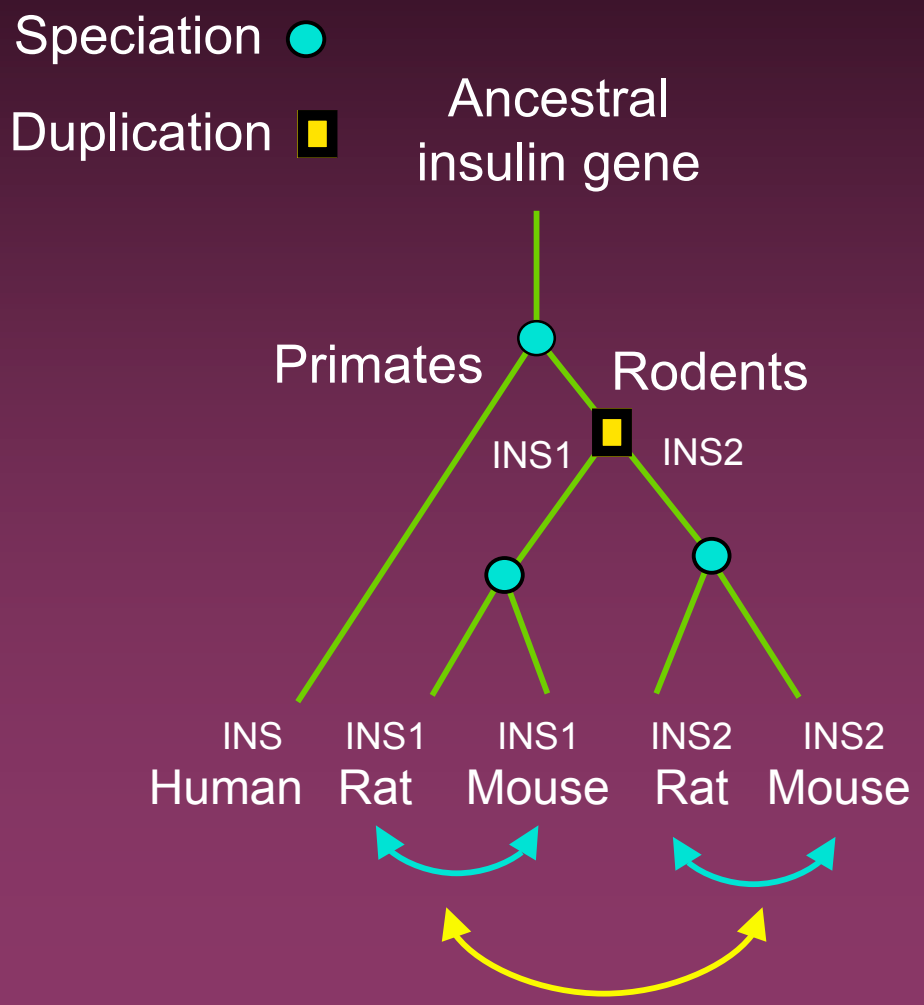
Comparative genomics

- Functional genomics:
  - Prediction of gene function, protein structure
  - Identification of functional constraints
  - Identification of regulatory elements
- Molecular evolution studies:
  - Search for horizontal transfers
  - Species-specific metabolic pathways
  - Ancestral genome content
  - Chromosomal rearrangements
  - Gene, genome duplication and acquisition of novel functions
  - ...

Orthology/Paralogy



Homology: two genes are homologous if they share a common ancestor

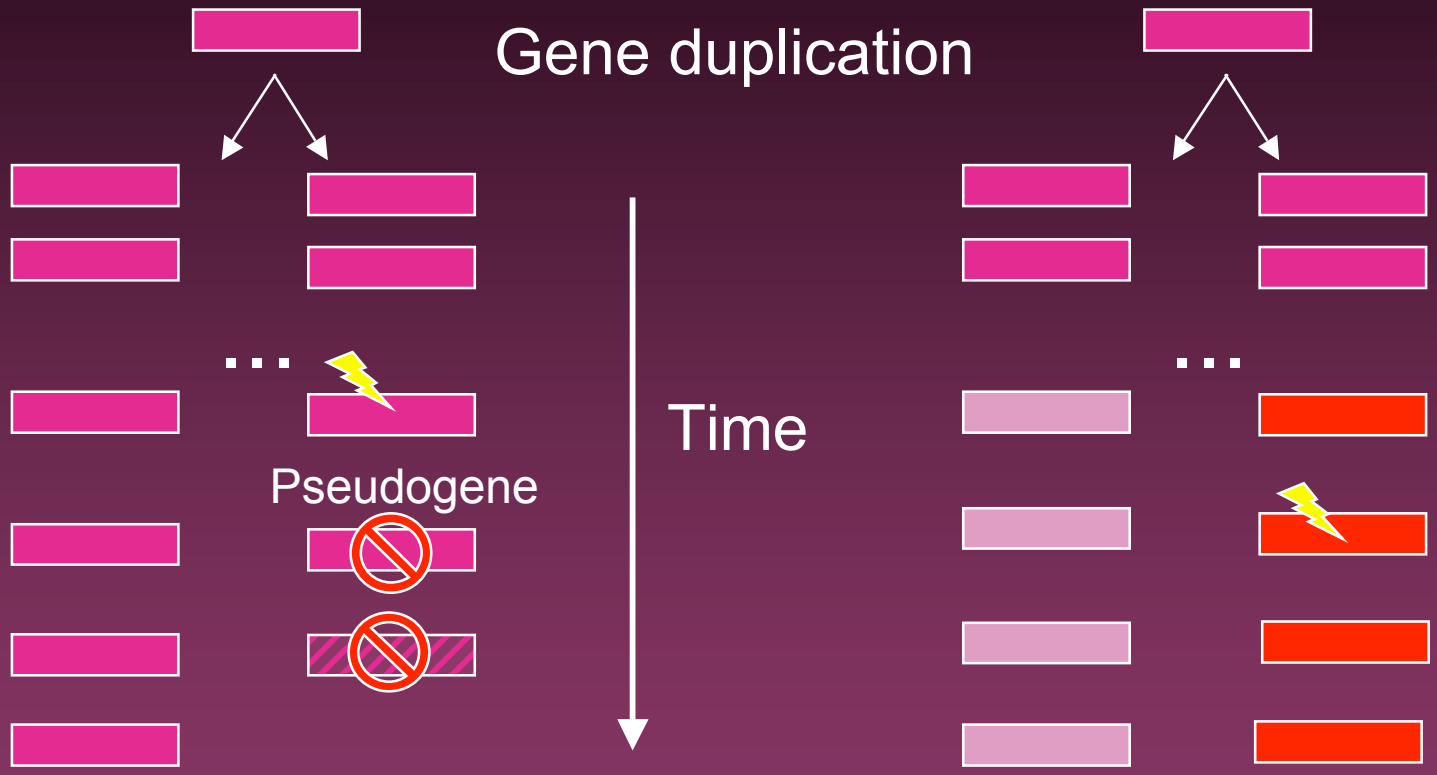
↔ Orthologs: homologs that have diverged after a speciation

↔ Paralogs: homologs that have diverged after a duplication

Why is it important ?

- Distinguishing orthologs and paralogs is essential for:
  - Phylogeny: inference of the species tree from the gene tree
  - Comparative mapping, inference of genome rearrangements
  - Prediction of function by homology: duplications promote the evolution of gene function
    - neo-functionalization
    - sub-functionalization

Gene duplication and evolution of function

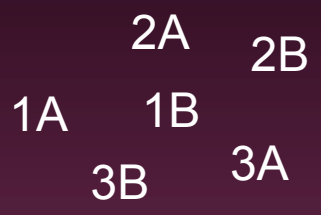


Ancient paralogs → Specific function

e.g. expression pattern, subcellular localisation, biochemical activity, ...

Phylogenomic approach for function prediction

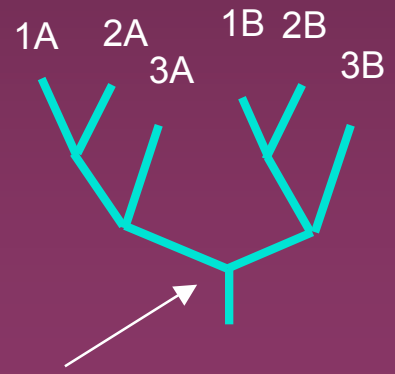
1) Identify homologs



Species: 1, 2, 3

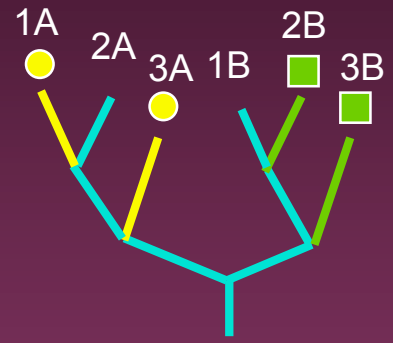
2) Align sequences

3) Compute phylogenetic tree

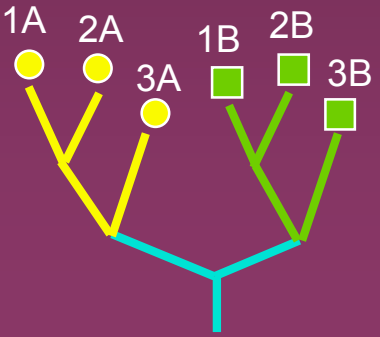


gene duplication

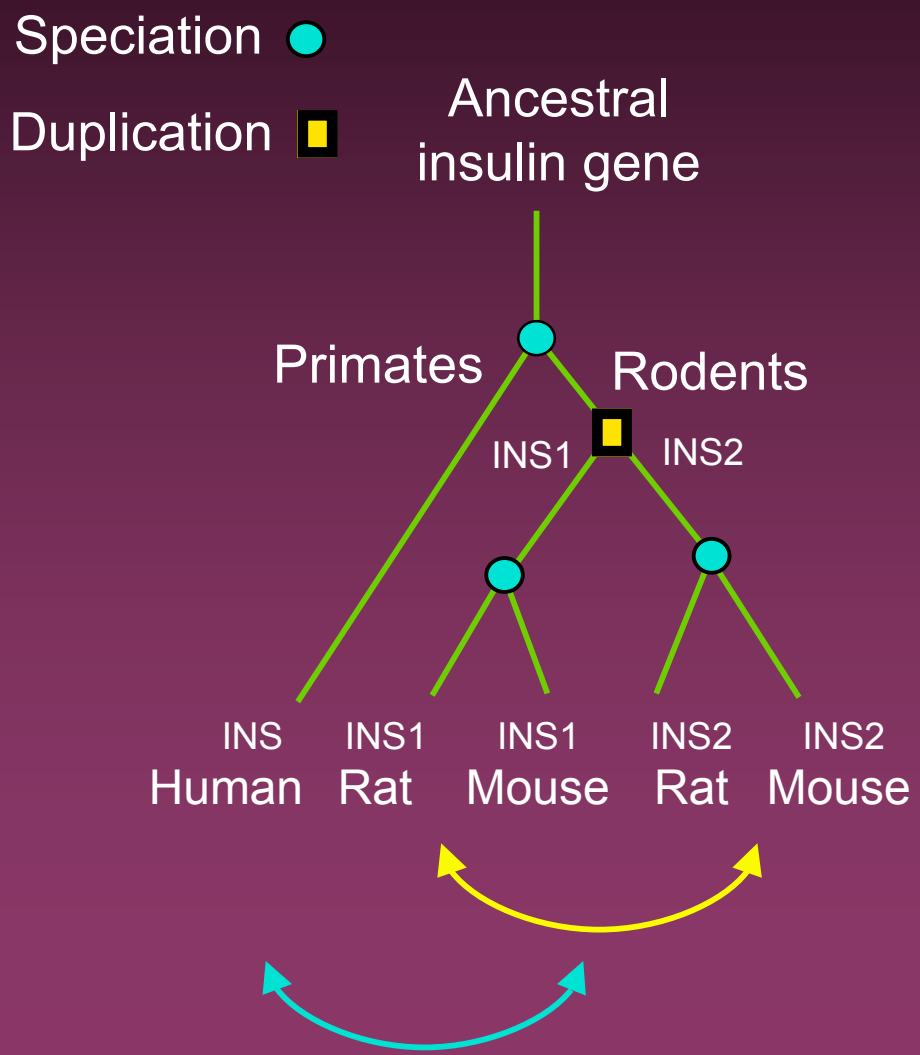
4) Place known functions in the tree



5) Infer the likely function of other genes



Ortholog ≠ Functional equivalent !!



Orthology: not necessarily one-to-one relationship (one-to-many or many-to-many)

e.g.: the human *INS* gene has two orthologs in rodents (*Ins1* and *Ins2*)

The rodent *Ins1* gene is more closely related to its paralog *Ins2* than to its human ortholog *INS*.

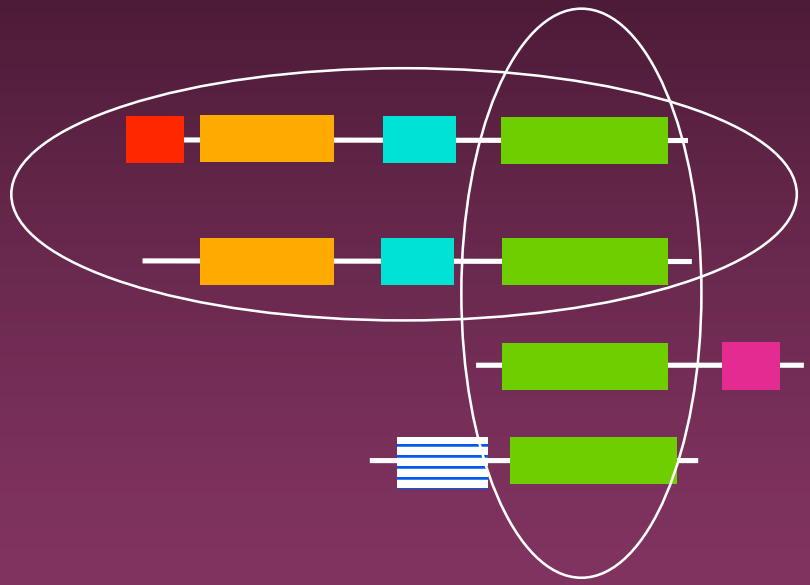
Databases of homologous genes

- Goal :
  - Provide a simple access to data required for phylogenomics (alignments, trees, taxonomic data, sequence annotations)

- Databases of homologous genes at PBIL:
  - HOVERGEN (1994): vertebrates
  - HOBACGEN (2000): prokaryotes =>
  - HOGENOM: complete genomes (EBI complete proteomes)
  - HOMOLENS: Ensembl complete genomes (animals)

Domain *vs.* gene families

- Modular evolution of protein genes



Gene families: homologous protein domains:

- Evolution by domain shuffling (by speciation, loss, translocation, duplication)
- Sequences are homologous over their entire length (or almost)

Databases for comparative genomics

- Databases of homologous protein domains
  - PROSITE
  - PFAM
  - PRODOM
  - ...
  - InterPro
- Databases of gene families
  - COG
  - HOVERGEN, HOGENOM
  - ...

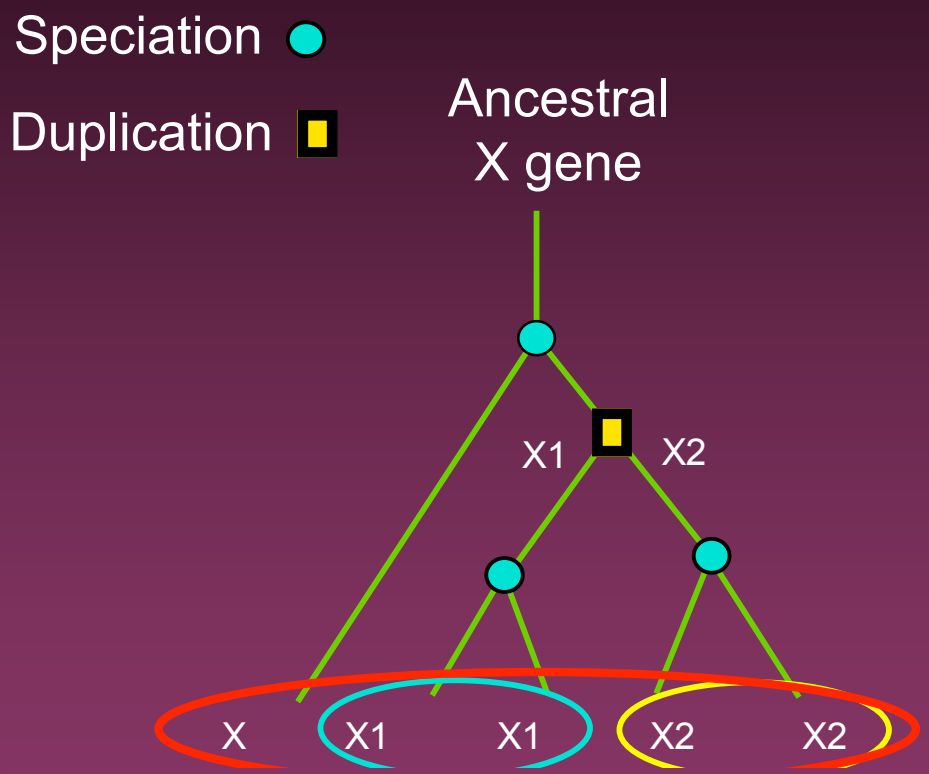
Different databases for different purposes

- Databases of protein domains (InterPro, etc.)
  - Prediction of the biochemical activity of proteins:
    - Does this protein have a kinase catalytic site ?
    - Does it contain a DNA binding domain ?
    - …
  - Prediction of protein structures
    - Does this protein contain a domain homologous to an already known 3D structure ?
  - …

Different databases for different purposes

- Databases of gene families (HOGENOM, etc.): identify **orthologues** or **paralogues** within a given set of taxa
  - Identify all orthologues between human, mouse and zebrafish
    - Prediction of gene function
    - Phylogenetics
    - Comparative mapping
  - Identify all paralogous genes originating from a duplication in the last common ancestor of vertebrates
    - Evolution of the function of duplicated genes
    - Analysis of genome duplications
  - Identify all the genes that are specific to a pathogenic strain of *E. coli*
  - …

Why not a database of *ORTHOLOGOUS* genes ?



Retrieve all orthologs between rat and mouse

Retrieve all orthologs between primates and rodents

- The clustering of homologs into groups of orthologs depends on the taxa being considered.
- No "universal" clustering of orthologous genes !

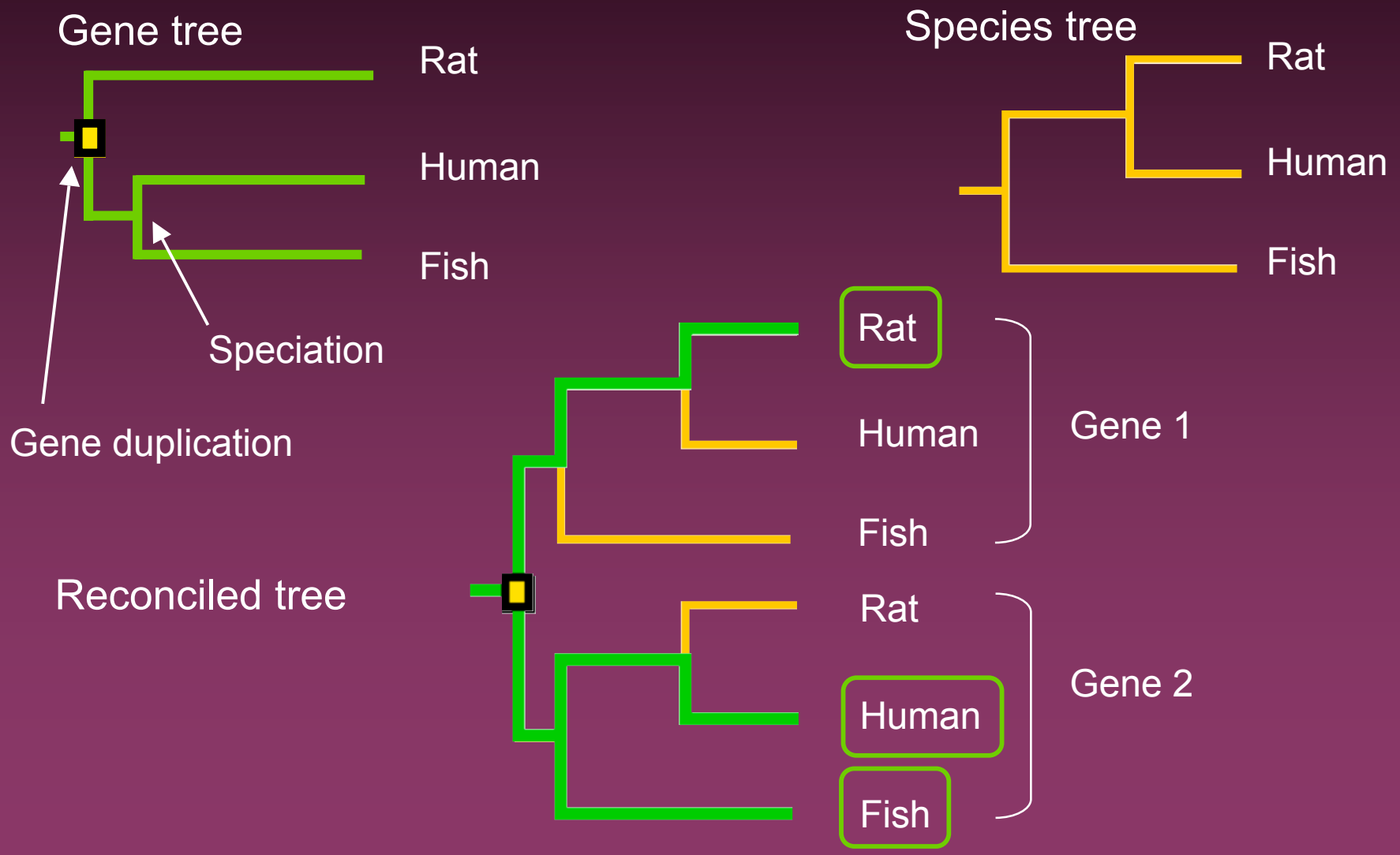
Databases of homologous genes

- Gene families include both orthologs and paralogs
- Development of tools for automatic retrieval of orthologs for a given set of taxa

Orthology / Paralogy: automatic detection

- Comparison of gene and species trees: tree reconciliation
- Identification of speciation and duplication nodes

Tree reconciliation



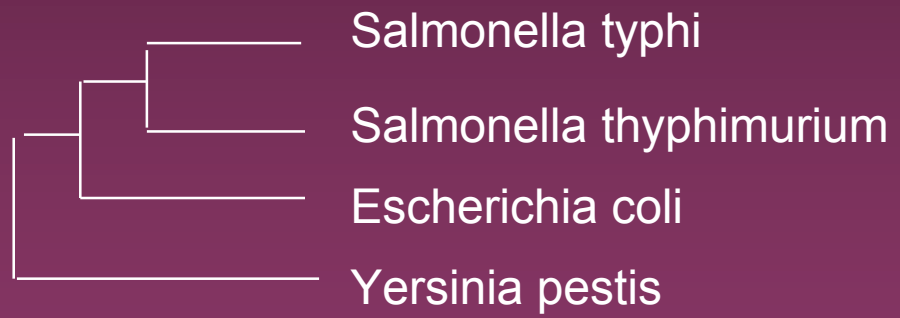
Orthology / Paralogy: automatic detection

- Improvement of existing algorithms (Dufayard et al. *Bioinformatics* 2005):
  - Unresolved nodes in species or gene trees
  - Branch lengths
  - Tree rooting
- HOVERGEN, HOGENOM, HomolEns: systematic reconciliation of all phylogenetic trees
- Limitations (bacteria !): species tree, horizontal transfer

Generalization: searching patterns in trees

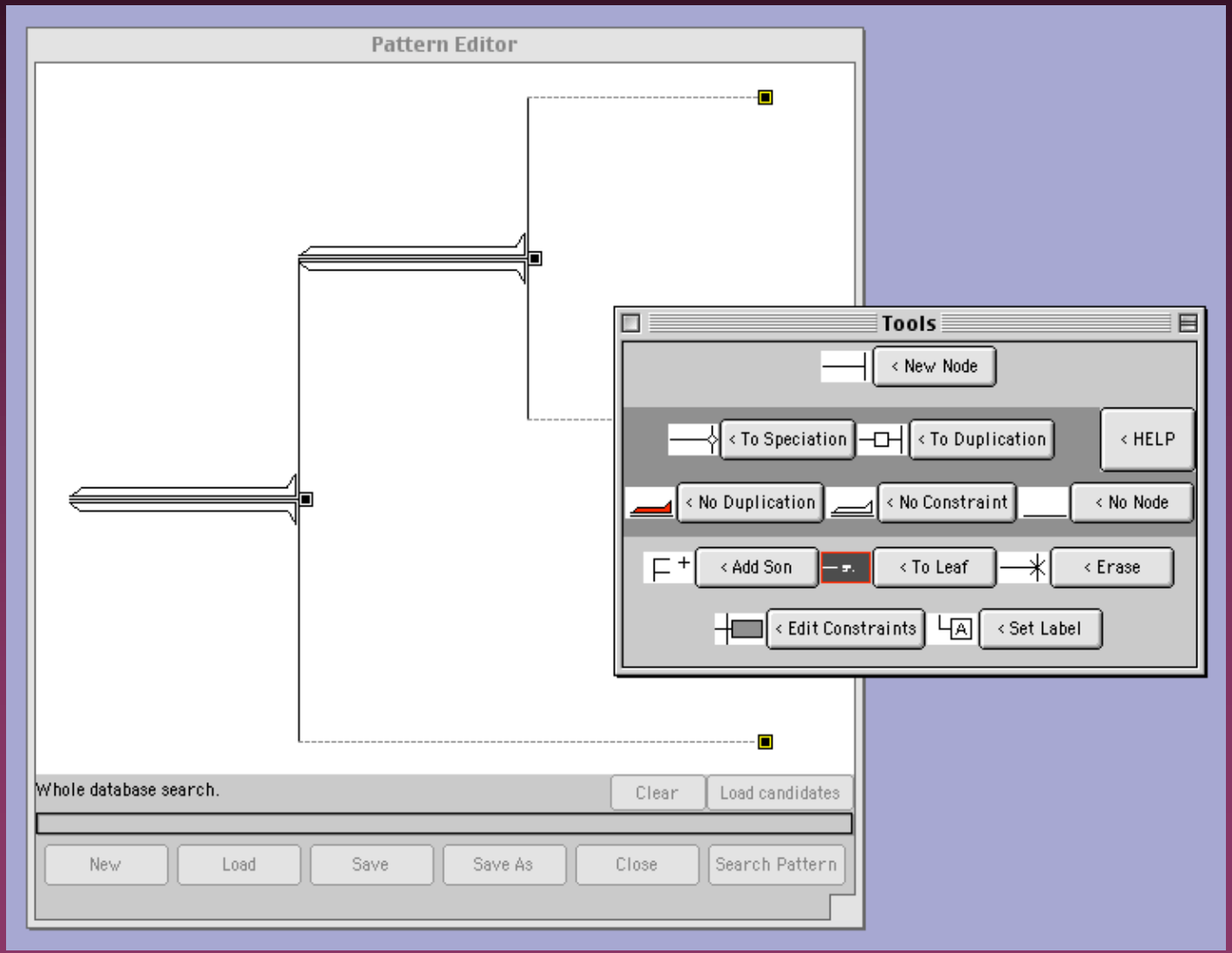
- Automatic detection of any pattern in gene trees
- Example: Search for orthologs present in completely sequenced in enterobacteriaceae

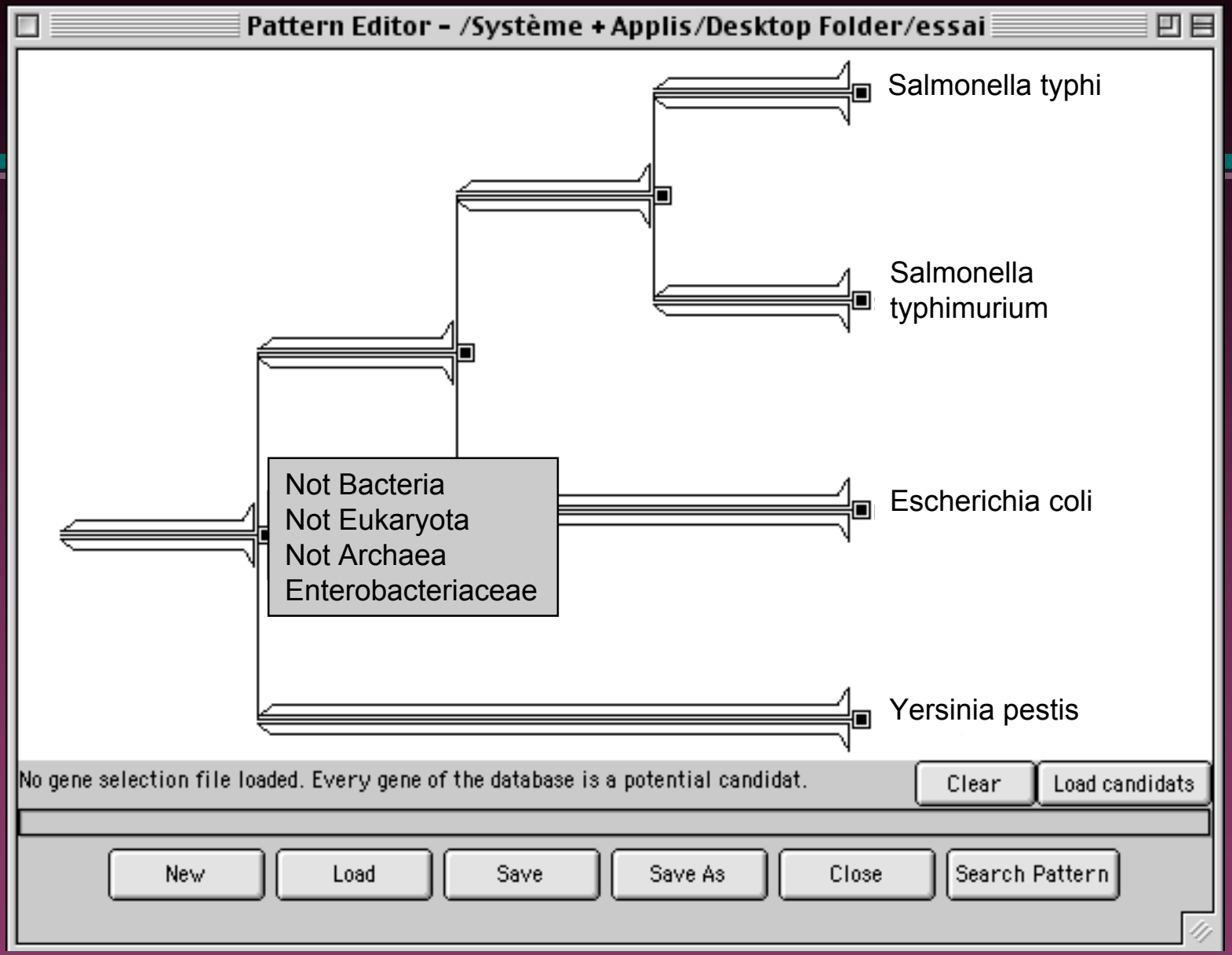
  - Known species phylogeny:



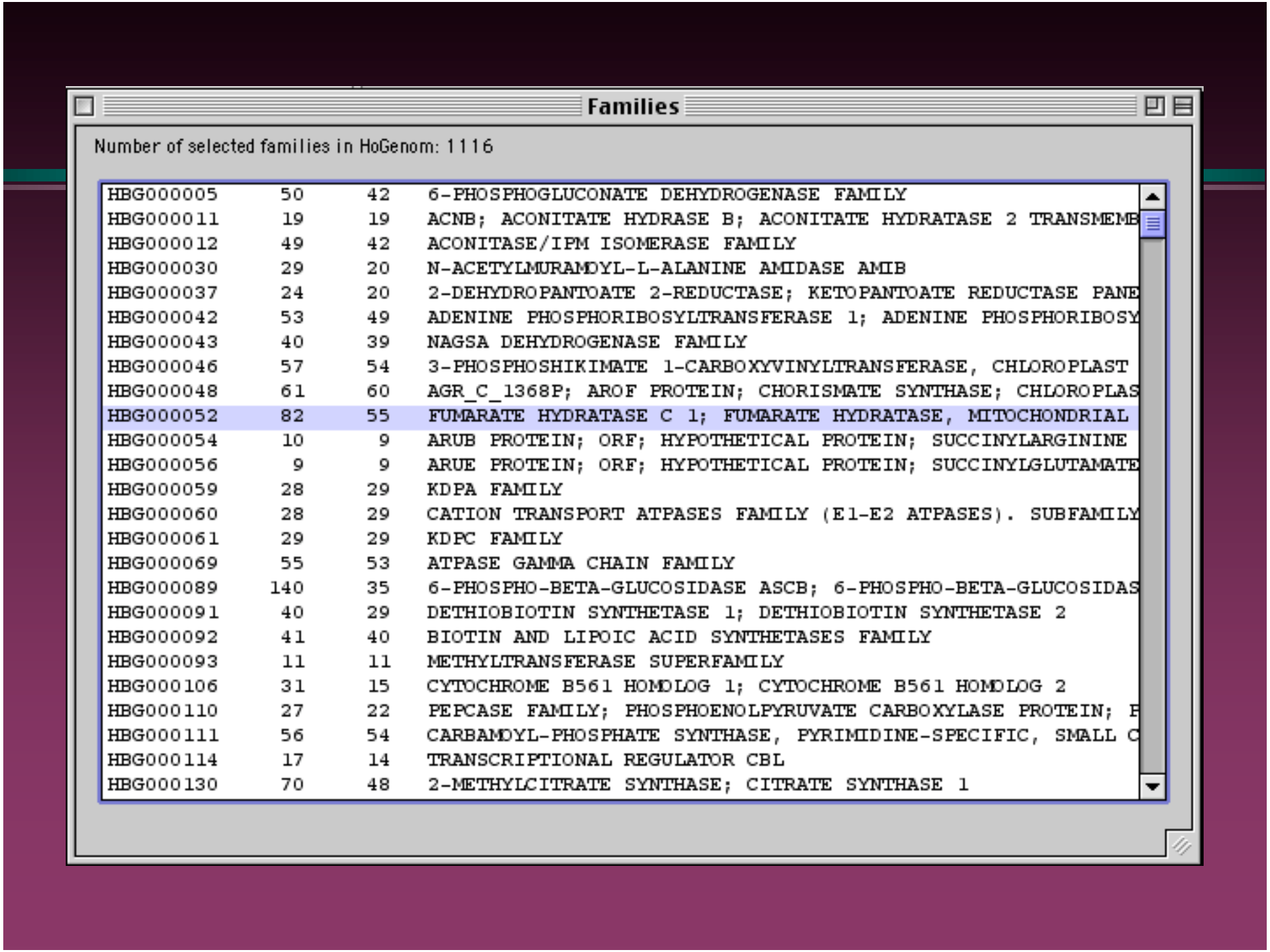
  - Select all families containing this tree pattern
  - Only enterobacteriaceae species in this subtree

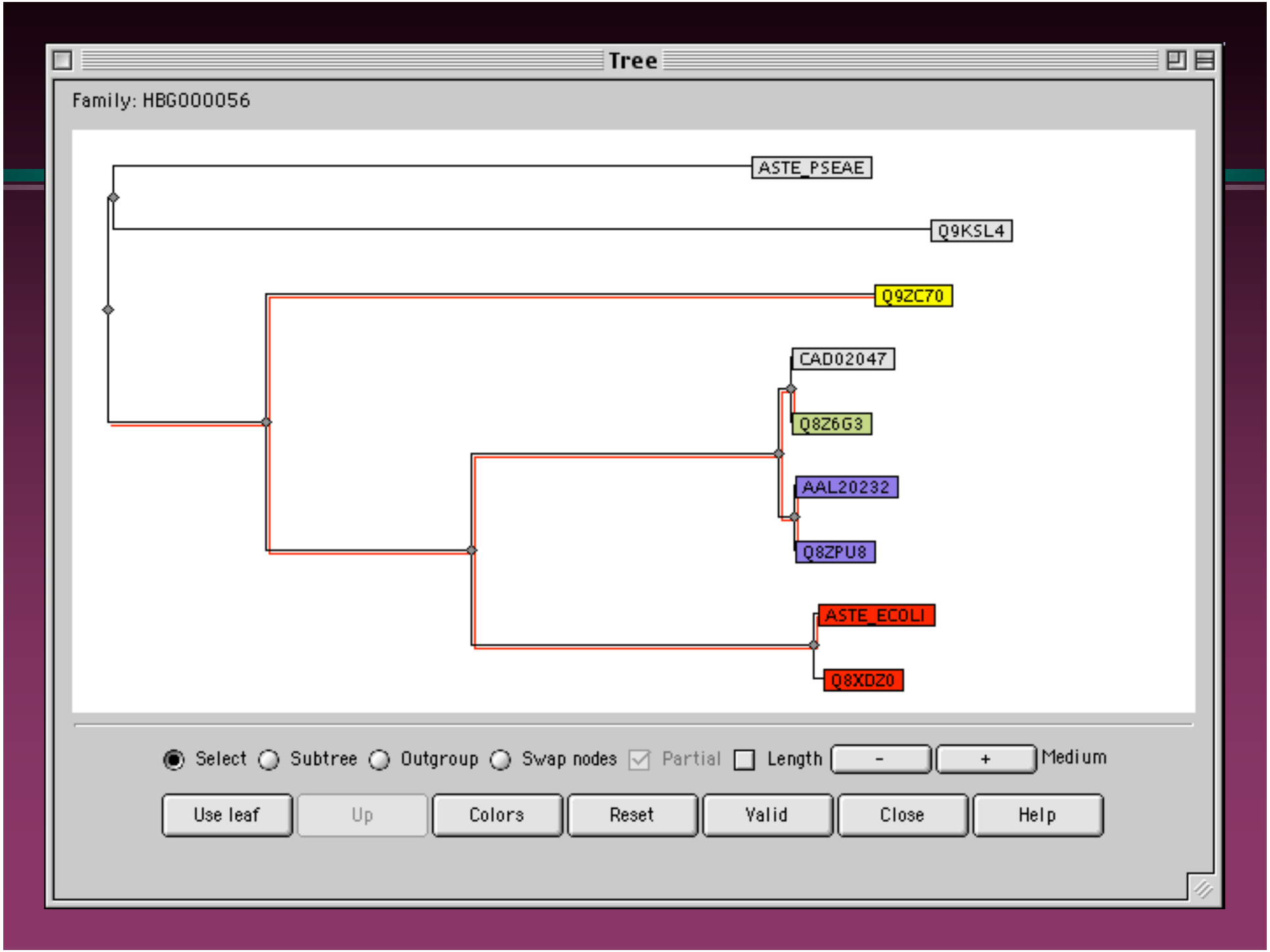
Tree pattern editor



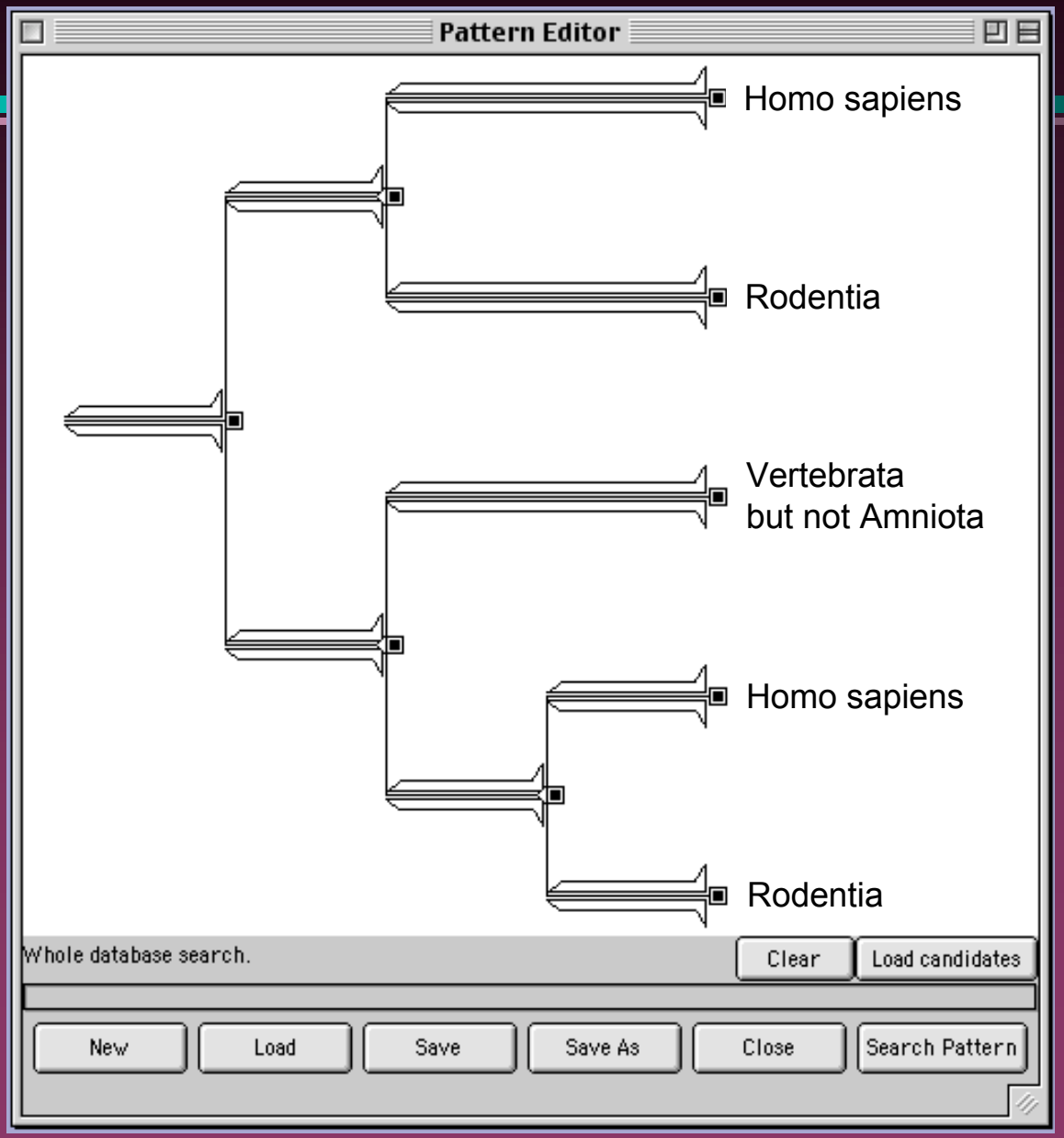


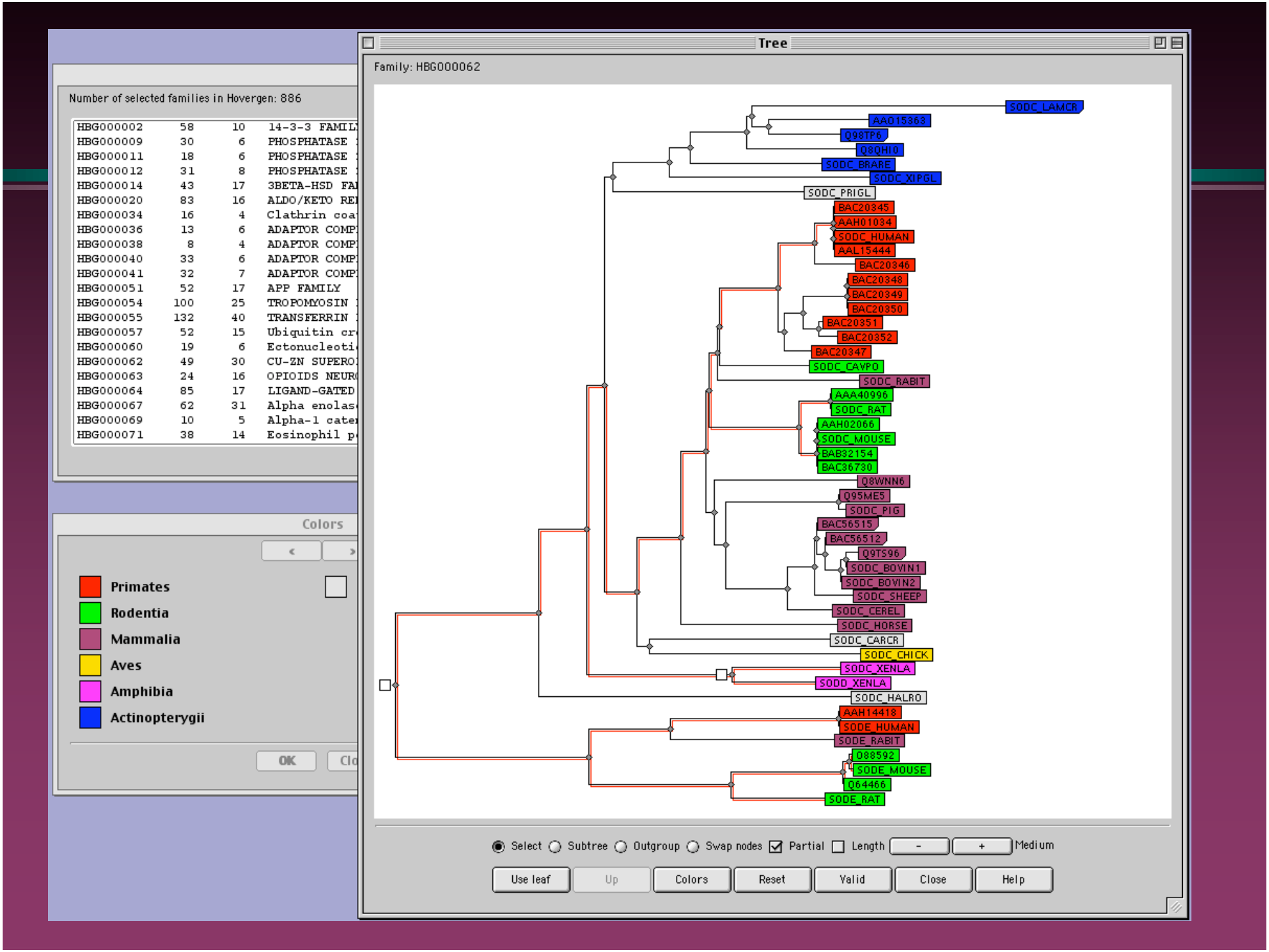
Search for orthologs in enterobacteriaceae


Families
Number of selected families in HoGenom: 1116
HBG000005 50 42 6-PHOSPHOGLUCONATE DEHYDROGENASE FAMILY
HBG000011 19 19 ACNB; ACONITATE HYDRASE B; ACONITATE HYDRATASE 2 TRANSMEMB
HBG000012 49 42 ACONITASE/IPM ISOMERASE FAMILY
HBG000030 29 20 N-ACETYLMURAMOYL-L-ALANINE AMIDASE AMIB
HBG000037 24 20 2-DEHYDROPANTOATE 2-REDUCTASE; KETOPANTOATE REDUCTASE PANE
HBG000042 53 49 ADENINE PHOSPHORIBOSYLTRANSFERASE 1; ADENINE PHOSPHORIBOSY
HBG000043 40 39 NAGSA DEHYDROGENASE FAMILY
HBG000046 57 54 3-PHOSPHOSHIKIMATE 1-CARBOXYVINYLTRANSFERASE, CHLOROPLAST
HBG000048 61 60 AGR_C_1368P; AROF PROTEIN; CHORISMATE SYNTHASE; CHLOROPLAS
HBG000052 82 55 FUMARATE HYDRATASE C 1; FUMARATE HYDRATASE, MITOCHONDRIAL
HBG000054 10 9 ARUB PROTEIN; ORF; HYPOTHETICAL PROTEIN; SUCCINYLARGININE
HBG000056 9 9 ARUE PROTEIN; ORF; HYPOTHETICAL PROTEIN; SUCCINYLGLUTAMATE
HBG000059 28 29 KDPA FAMILY
HBG000060 28 29 CATION TRANSPORT ATPASES FAMILY (E1-E2 ATPASES). SUBFAMILY
HBG000061 29 29 KDPC FAMILY
HBG000069 55 53 ATPASE GAMMA CHAIN FAMILY
HBG000089 140 35 6-PHOSPHO-BETA-GLUCOSIDASE ASCB; 6-PHOSPHO-BETA-GLUCOSIDAS
HBG000091 40 29 DETHIOBIOTIN SYNTHETASE 1; DETHIOBIOTIN SYNTHETASE 2
HBG000092 41 40 BIOTIN AND LIPOIC ACID SYNTHETASES FAMILY
HBG000093 11 11 METHYLTRANSFERASE SUPERFAMILY
HBG000106 31 15 CYTOCHROME B561 HOMOLOG 1; CYTOCHROME B561 HOMOLOG 2
HBG000110 27 22 PEPCASE FAMILY; PHOSPHOENOLPYRUVATE CARBOXYLASE PROTEIN; P
HBG000111 56 54 CARBAMOYL-PHOSPHATE SYNTHASE, PYRIMIDINE-SPECIFIC, SMALL C
HBG000114 17 14 TRANSCRIPTIONAL REGULATOR CBL
HBG000130 70 48 2-METHYLCITRATE SYNTHASE; CITRATE SYNTHASE 1


Tree
Family: HBG000056
ASTE_PSEAE
Q9KSL4
Q9ZC70
CAD02047
Q8Z6G3
AAL20232
Q8ZPU8
ASTE_ECOLI
Q8XDZ0
Select
Subtree
Outgroup
Swap nodes
Partial
Length
-
+
Medium
Use leaf
Up
Colors
Reset
Valid
Close
Help

Search for ancient gene duplications in vertebrates

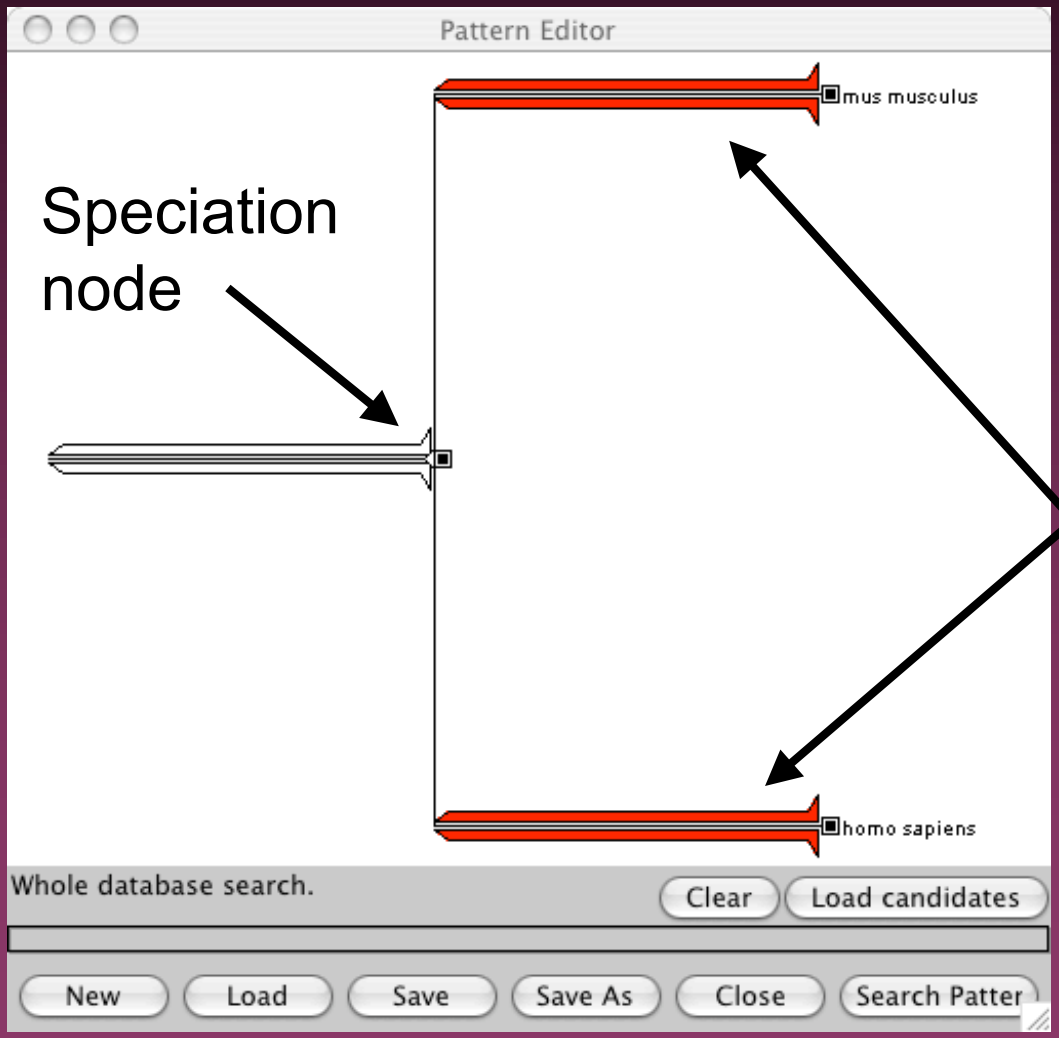



Number of selected families in Hovergen: 886
HBG000002 58 10 14-3-3 FAMIL
HBG000009 30 6 PHOSPHATASE
HBG000011 18 6 PHOSPHATASE
HBG000012 31 8 PHOSPHATASE
HBG000014 43 17 3BETA-HSD FA
HBG000020 83 16 ALDO/KETO RE
HBG000034 16 4 Clathrin coa
HBG000036 13 6 ADAPTOR COMP
HBG000038 8 4 ADAPTOR COMP
HBG000040 33 6 ADAPTOR COMP
HBG000041 32 7 ADAPTOR COMP
HBG000051 52 17 APP FAMILY
HBG000054 100 25 TROPOMYOSIN
HBG000055 132 40 TRANSFERRIN
HBG000057 52 15 Ubiquitin cr
HBG000060 19 6 Ectonucleoti
HBG000062 49 30 CU-ZN SUPERO
HBG000063 24 16 OPIOIDS NEUR
HBG000064 85 17 LIGAND-GATED
HBG000067 62 31 Alpha enolas
HBG000069 10 5 Alpha-1 cate
HBG000071 38 14 Eosinophil p
Colors
Primates
Rodentia
Mammalia
Aves
Amphibia
Actinopterygii
OK
Tree
Family: HBG000062
SODC_LAMCR
AAO15363
Q98TP6
Q8QHI0
SODC_BRARE
SODC_XIPGL
SODC_PRIGL
BAC20345
AAH01034
SODC_HUMAN
AAL15444
BAC20346
BAC20348
BAC20349
BAC20350
BAC20351
BAC20352
BAC20347
SODC_CAVPO
SODC_RABIT
AAA40996
SODC_RAT
AAH02066
SODC_MOUSE
BAB32154
BAC36730
Q8WNN6
Q95ME5
SODC_PIG
BAC56515
BAC56512
Q9TS96
SODC_BOVIN1
SODC_BOVIN2
SODC_SHEEP
SODC_CEREL
SODC_HORSE
SODC_CARCR
SODC_CHICK
SODC_XENLA
SODD_XENLA
SODC_HALRO
AAH14418
SODE_HUMAN
SODE_RABIT
Q88592
SODE_MOUSE
Q64466
SODE_RAT
Select
Subtree
Outgroup
Swap nodes
Partial
Length
-
+
Medium
Use leaf
Up
Colors
Reset
Valid
Close
Help

Tree Pattern vs. Reciprocal Best Hits

- Search all 1:1 orthologs between human and mouse
- Gene set: Ensembl Rel. 24
  - 22,077 human protein genes
  - 24,132 mouse protein genes
  - longest CDS of each gene
- Reciprocal Best Hits:
  - NCBI BLASTP, default filtering parameters, E<e-04

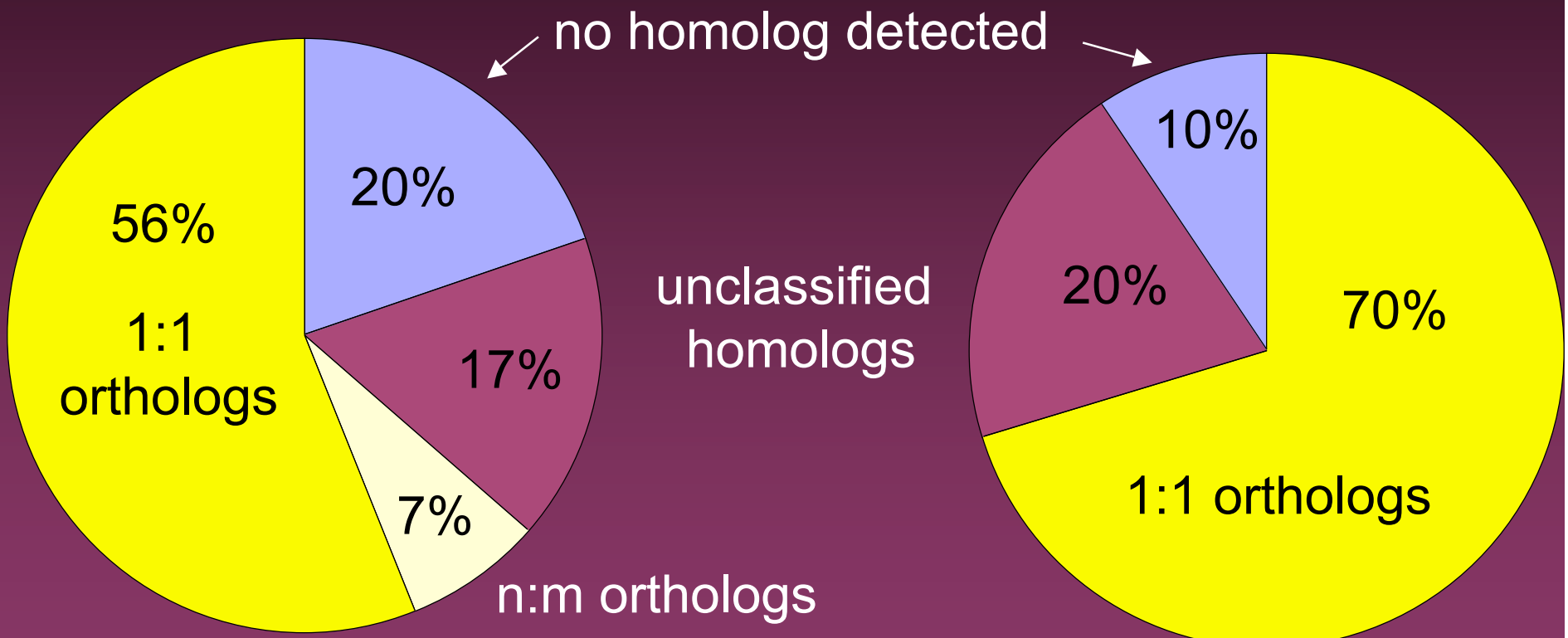
Tree Pattern



No duplication

Tree Pattern

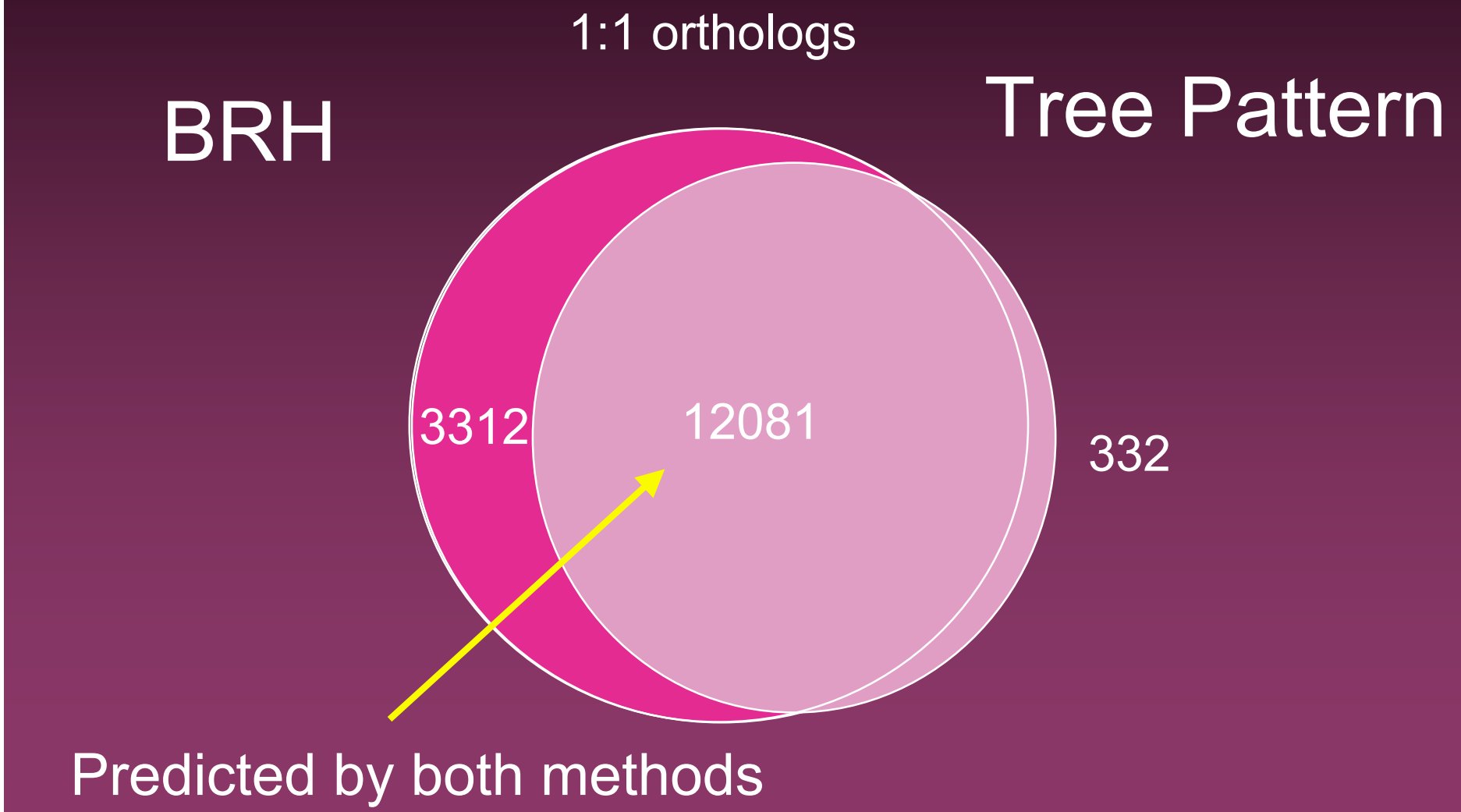
BRH



Total orthologs : 63%

Total orthologs : 70%

Tree Pattern vs. Reciprocal Best Hits

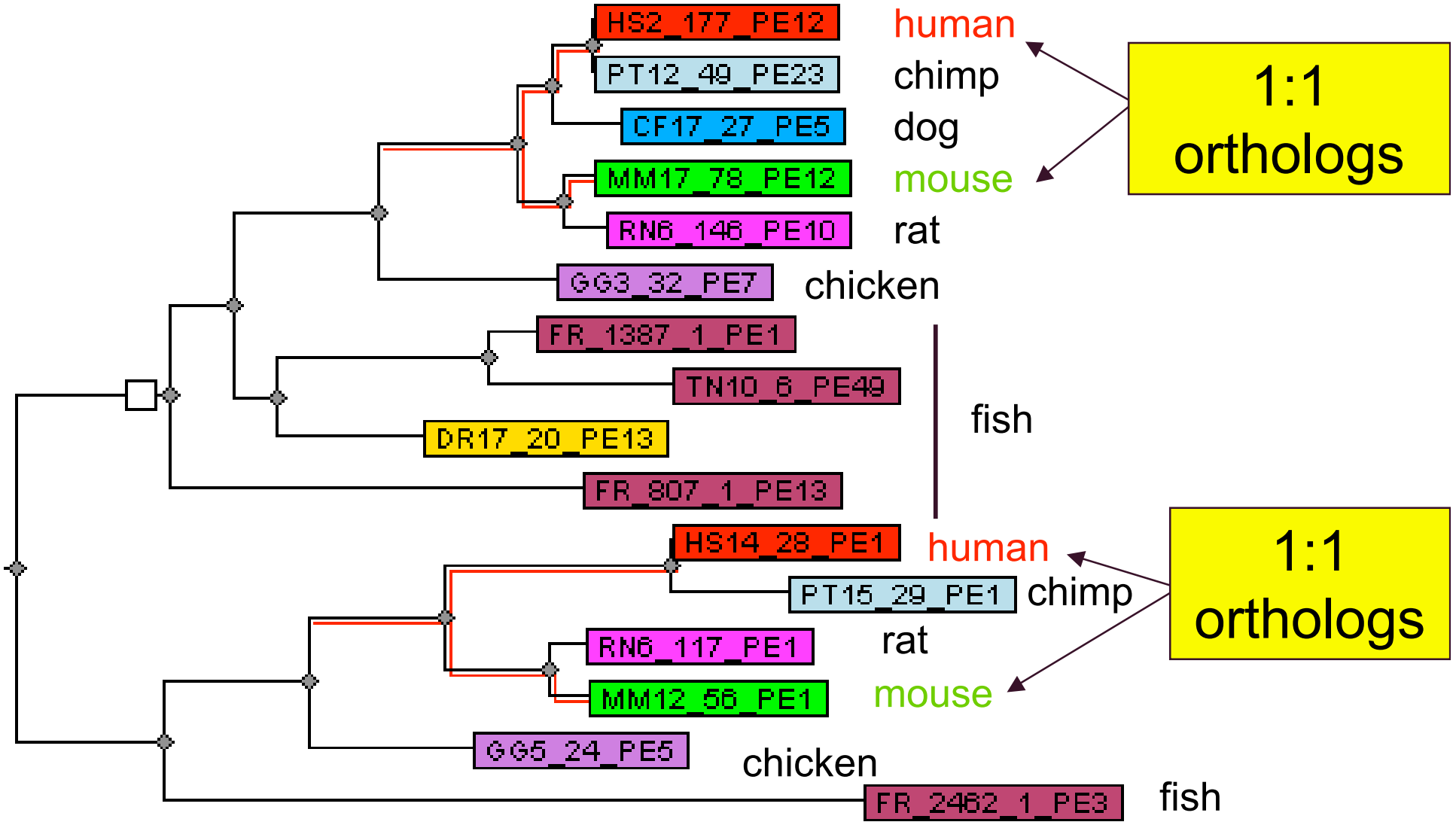


Predicted by Tree Pattern + BRH

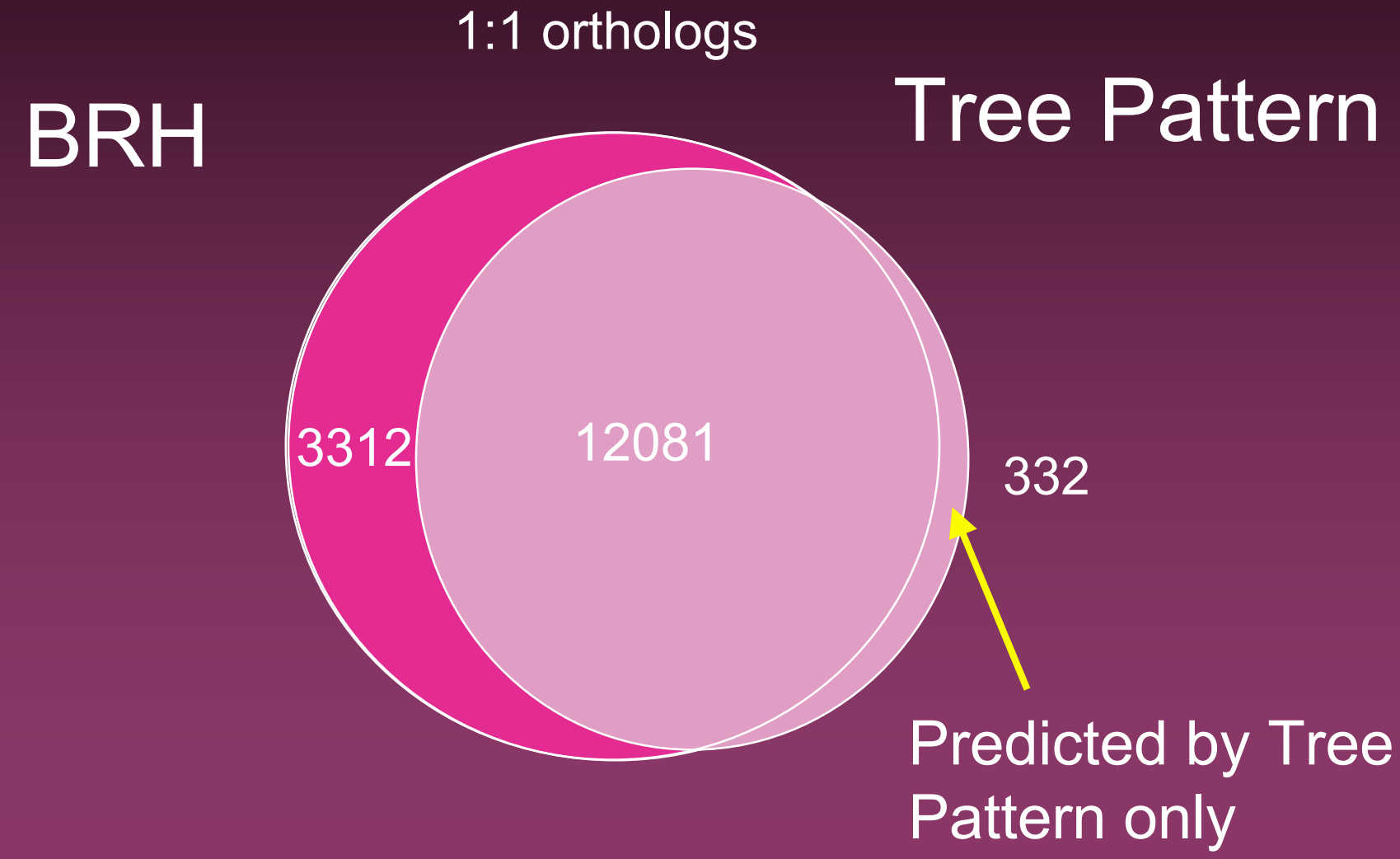
- N=12081 predicted 1:1 orthologs
- Manual expertise of 50 genes (randomly sampled):
  - 98% true positive
  - 0% false positive
  - 2% unsure (need more expertise)

- True positive :
  - Gene tree is consistent with the species tree
  - Orthologs are also found in non-mammalian vertebrates

Predicted by Tree Pattern + BRH

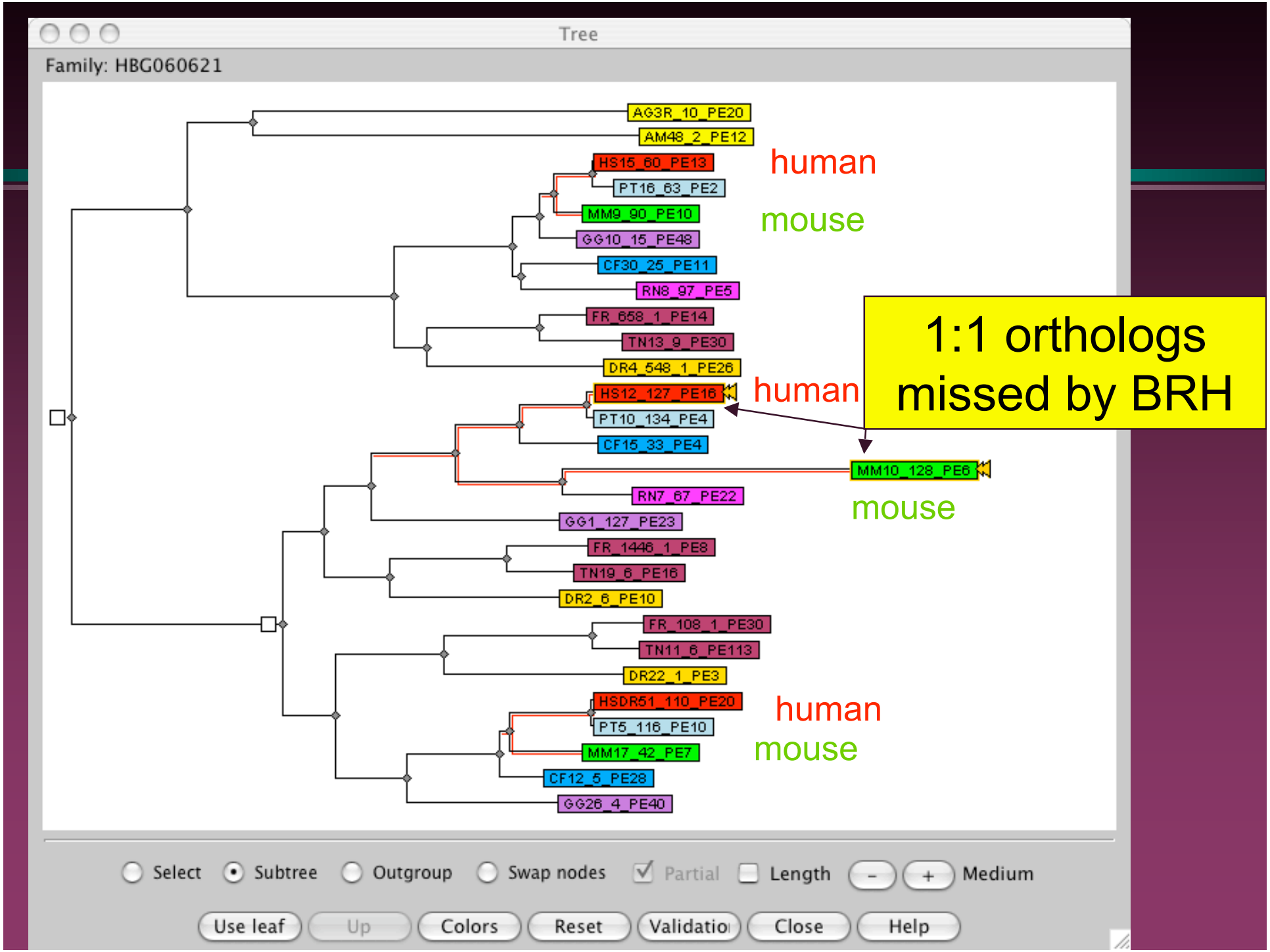


Tree Pattern vs. Reciprocal Best Hits



Predicted by Tree Pattern but not BRH

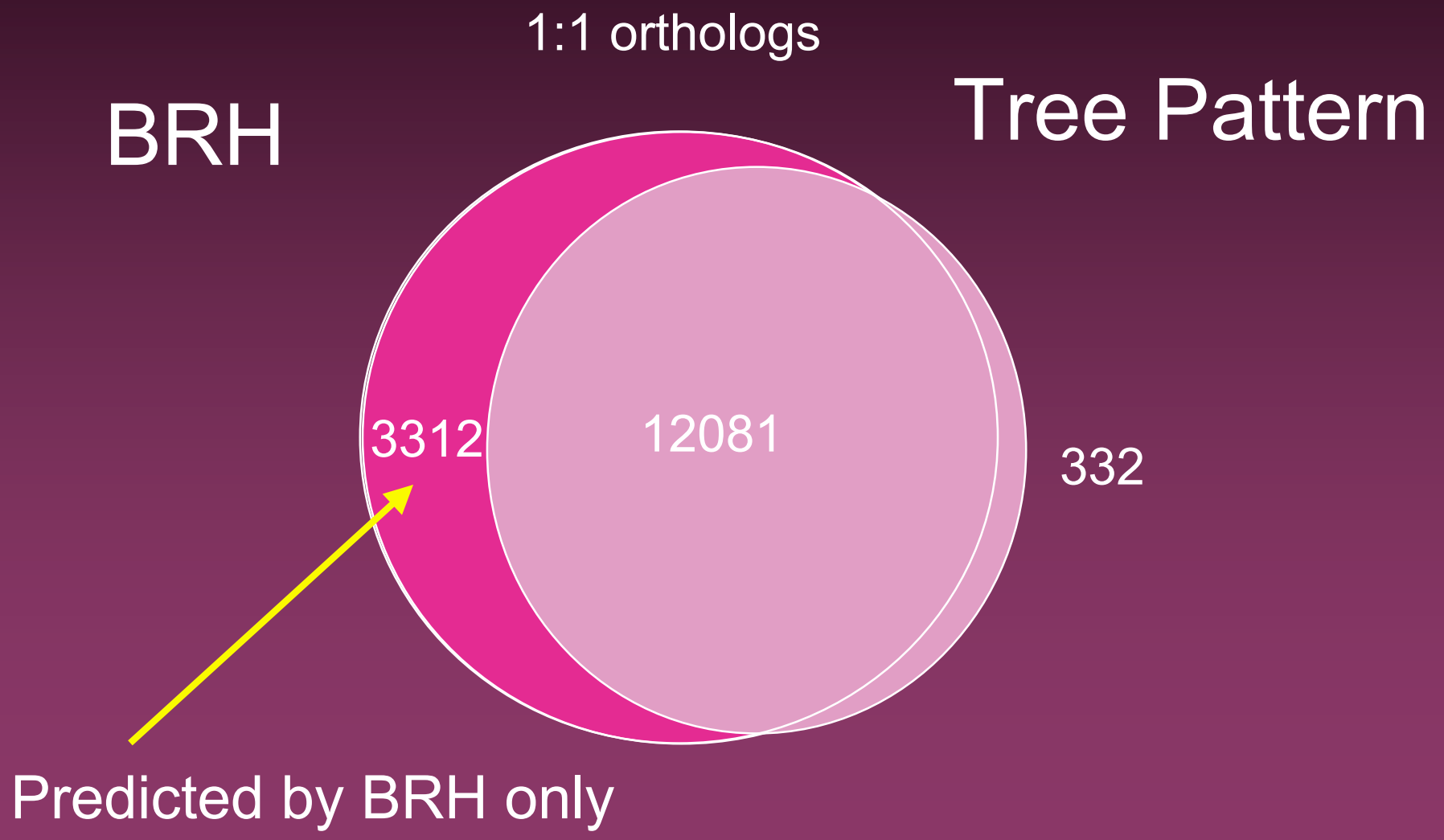
- N=332 predicted 1:1 orthologs
- Manual expertise of 47 genes:
  - 64% true positive
  - 23% false positive
  - 13% unsure (need more expertise)
- True positive = orthologs missed by BRH:
  - fast evolving gene in one lineage


Tree
Family: HBG060621
AG3R_10_PE20
AM48_2_PE12
HS15_60_PE13
PT16_63_PE2
MM9_90_PE10
GG10_15_PE48
CF30_25_PE11
RN8_97_PE5
FR_658_1_PE14
TN13_9_PE30
DR4_548_1_PE26
HS12_127_PE16
PT10_134_PE4
CF15_33_PE4
MM10_128_PE6
RN7_67_PE22
GG1_127_PE23
FR_1446_1_PE8
TN19_6_PE16
DR2_6_PE10
FR_108_1_PE30
TN11_6_PE113
DR22_1_PE3
HSDR51_110_PE20
PT5_116_PE10
MM17_42_PE7
CF12_5_PE28
GG26_4_PE40
human
mouse
human
mouse
human
mouse
1:1 orthologs missed by BRH
Select
Subtree
Outgroup
Swap nodes
Partial
Length
-
+
Medium
Use leaf
Up
Colors
Reset
Validatio
Close
Help

Predicted by Tree Pattern but not BRH

- N=332 predicted 1:1 orthologs
- Manual expertise of 47 genes:
  - 64% true positive
  - 23% false positive
  - 13% unsure (need more expertise)
- True positive = orthologs missed by BRH:
  - fast evolving gene in one lineage
  - incomplete or incorrect BLAST alignment => wrong evolutionary distance
- False positive :
  - n:m orthologs

Tree Pattern vs. Reciprocal Best Hits

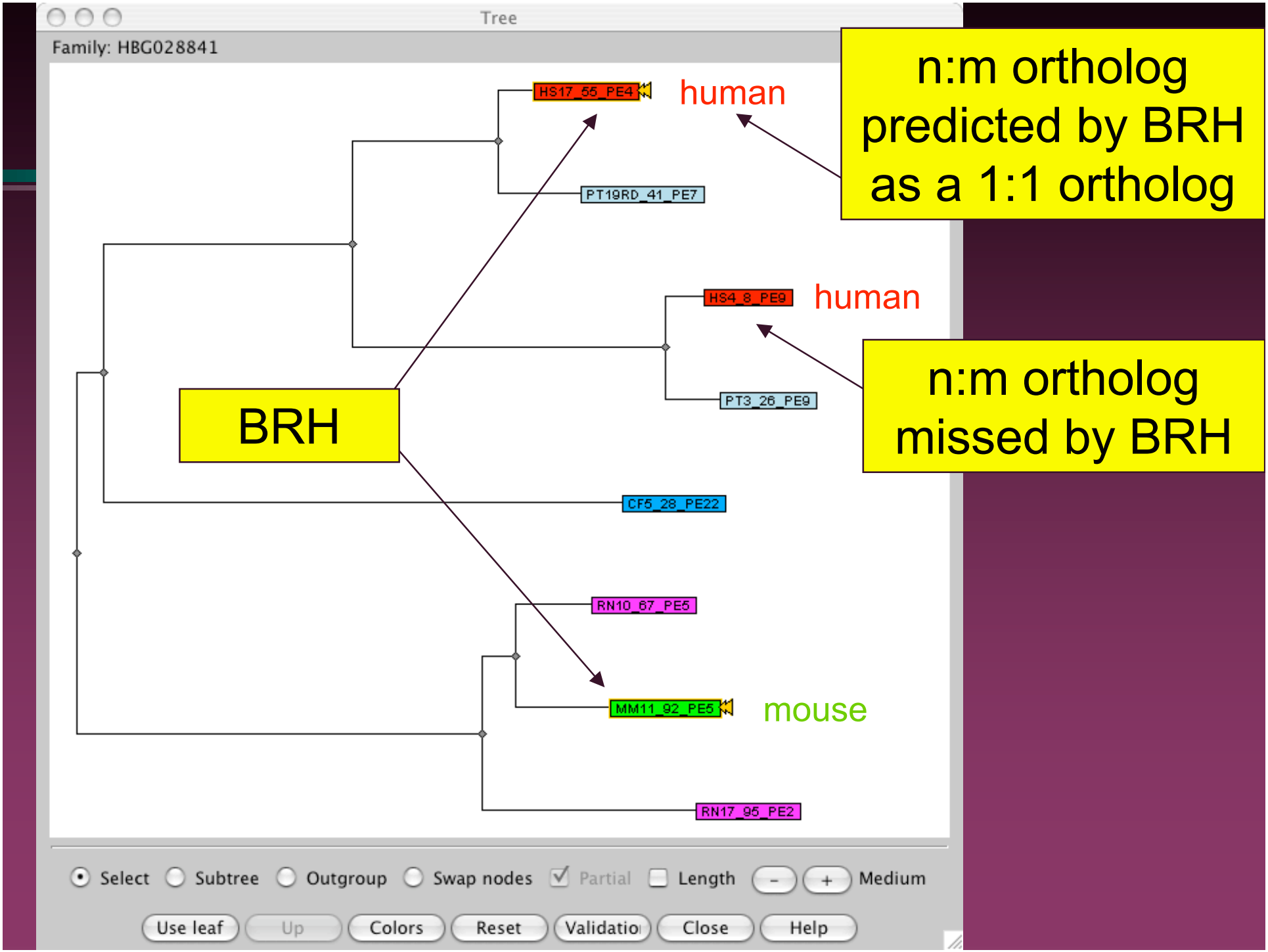


Predicted by BRH but not by Tree Pattern

- N=3,312 predicted 1:1 orthologs
- 1,099 (33%) do not fit the clustering criteria (alignment cover 80% of protein length)
  - real changes in gene structure ?
  - incorrect/incomplete gene annotation ?
- 1,059 (32%) are in families with > 500 genes
  - no tree computed in HOGENOM => missed by Tree Pattern

Predicted by BRH but not by Tree Pattern

- 1,154 (35%)
  - alignment ≥ 80% protein length
  - families with less than 500 genes => tree
- Manual expertise of 25 gene families:
  - 6/25 (24%) True positive
  - 19/25 (76%) False positive = n:m orthologs


Tree
Family: HBG028841
HS17_55_PE4
human
PT19RD_41_PE7
HS4_8_PE9
human
PT3_26_PE9
BRH
CF5_28_PE22
RN10_67_PE5
MM11_92_PE5
mouse
RN17_95_PE2
Select
Subtree
Outgroup
Swap nodes
Partial
Length
Medium
Use leaf
Up
Colors
Reset
Validatio
Close
Help
n:m ortholog predicted by BRH as a 1:1 ortholog
n:m ortholog missed by BRH

Tree pattern advantages: good specificity

- Explicitely based on phylogenetic trees
  - better estimates of evolutionary relationships than simple BLAST scores
- Does not require an exhaustive gene set
  - incomplete genome sequences, missing annotations, gene losses
  - NB: human and mouse gene sets are the most complete and accurate ones
- Distinguish 1:1 and n:m orthologs
  - essential for the comparison of duplicated genomes (e.g. fish vs. tetrapodes, vertebrates vs. invertebrates)
- Possibility to search for complex tree patterns
  - more than two species
  - search for gene duplications, gene losses, horizontal transfers, ...

Tree pattern searches: Limitations

- Large gene families: presently, phylogenetic trees are not computed for families with > 500 genes
  - possible improvements
- Classification criteria (alignment ≥ 80% length)
  - highly divergent orthologs, or orthologs with important differences in gene length (possibly annotation problems) are missed
- Quality of phylogenetic trees
  - quality of multiple alignments
  - possible improvements: GBlocks, PHYML
- => problems of sensitivity for distantly related species

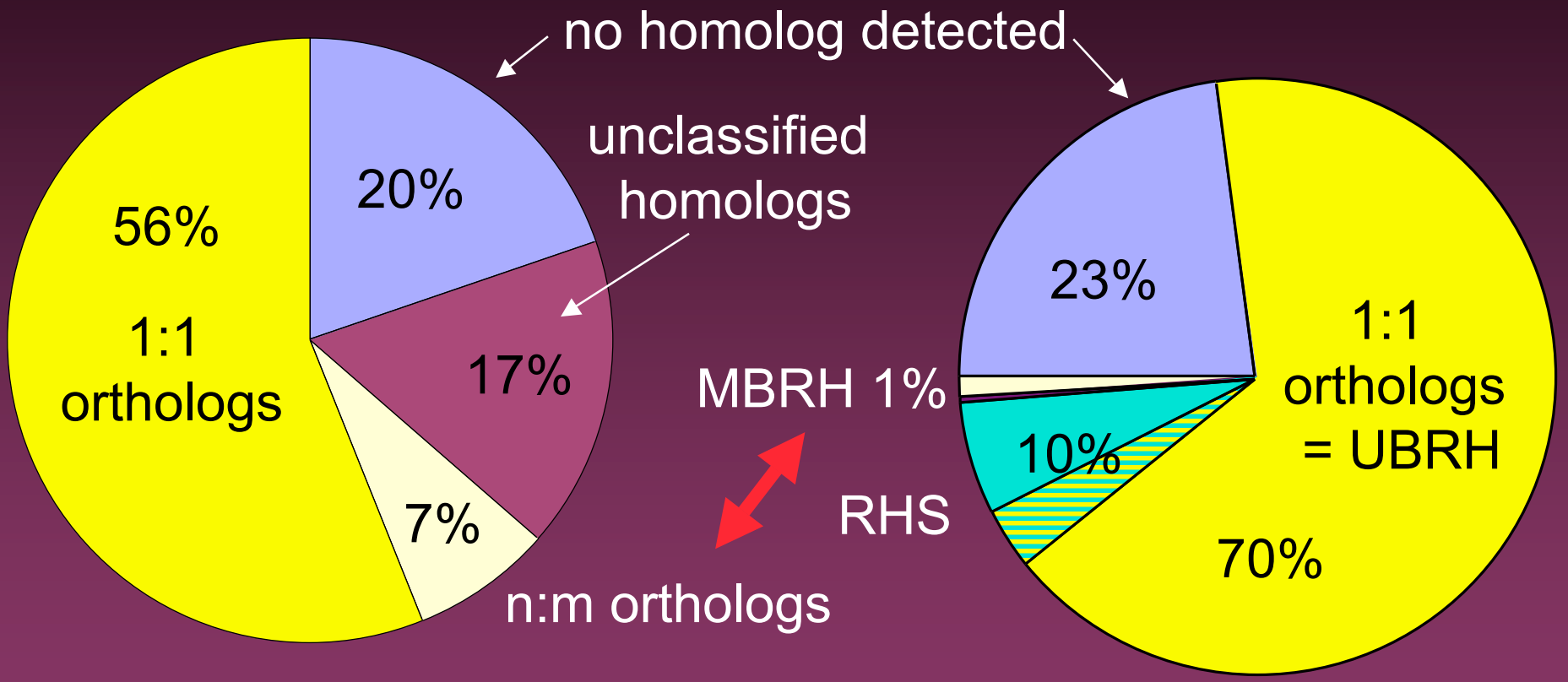
Reciprocal Best Hits

- Easy to implement
- Good sensitivity for 1:1 orthologs (but systematically miss n:m orthologs)

- Miss some 1:1 orthologs (not many)
  - differences in evolutionary rates
  - incorrect alignment (possible improvement)
- Requires a complete gene set
  - problems with incomplete or not fully annotated genomes
- Problems of specificity
  - many n:m orthologs predicted as 1:1
- Difficult to extend to more than 2 species

Tree Pattern

Ensembl orthologs



Total orthologs : 63%

Total orthologs : 77%

UBRH: unique reciprocal best hit

MBRH: multiple BRH

RHS: reciprocal hit based on synteny

Perspectives

- Tree Pattern : possible improvements
  - multiple alignment (muscle, Gblocks)
  - phylogenetic tree (phyml)
  - tree reconciliation: combine tree reconstruction and tree reconciliation
- Combine Tree Pattern with informations based on synteny

People

- PBIL (Lyon)
  - S. Penel
  - G. Perrière
  - M. Gouy
  - J. Grassot
  - L. Duret
- INRIA (Grenoble)
  - J.F. Dufayard
- IN2P3 (Lyon)
  - P. Calvat