Comparative Genomics

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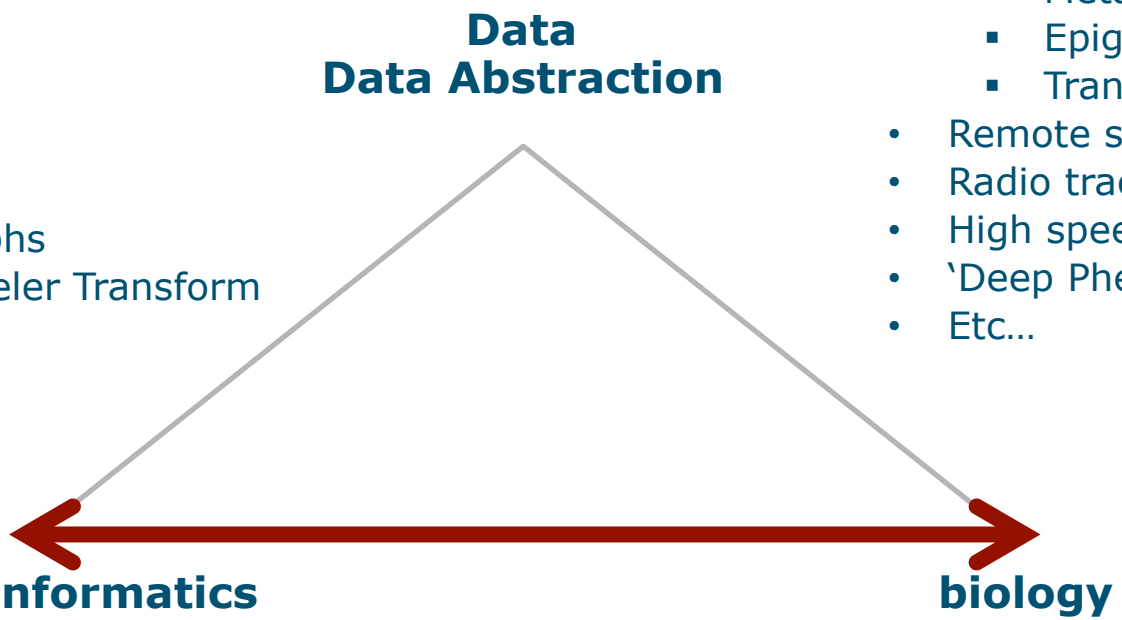
A word on ‘bioinformatics’….

Information technology
- Hardware
  - Cpu
  - RAM
  - HD/Storage
- Databases
- Internet
- Cloud
- Algorithms
  - De Bruin Graphs
  - Burrows-Wheeler Transform
  - Etc..
- Etc…

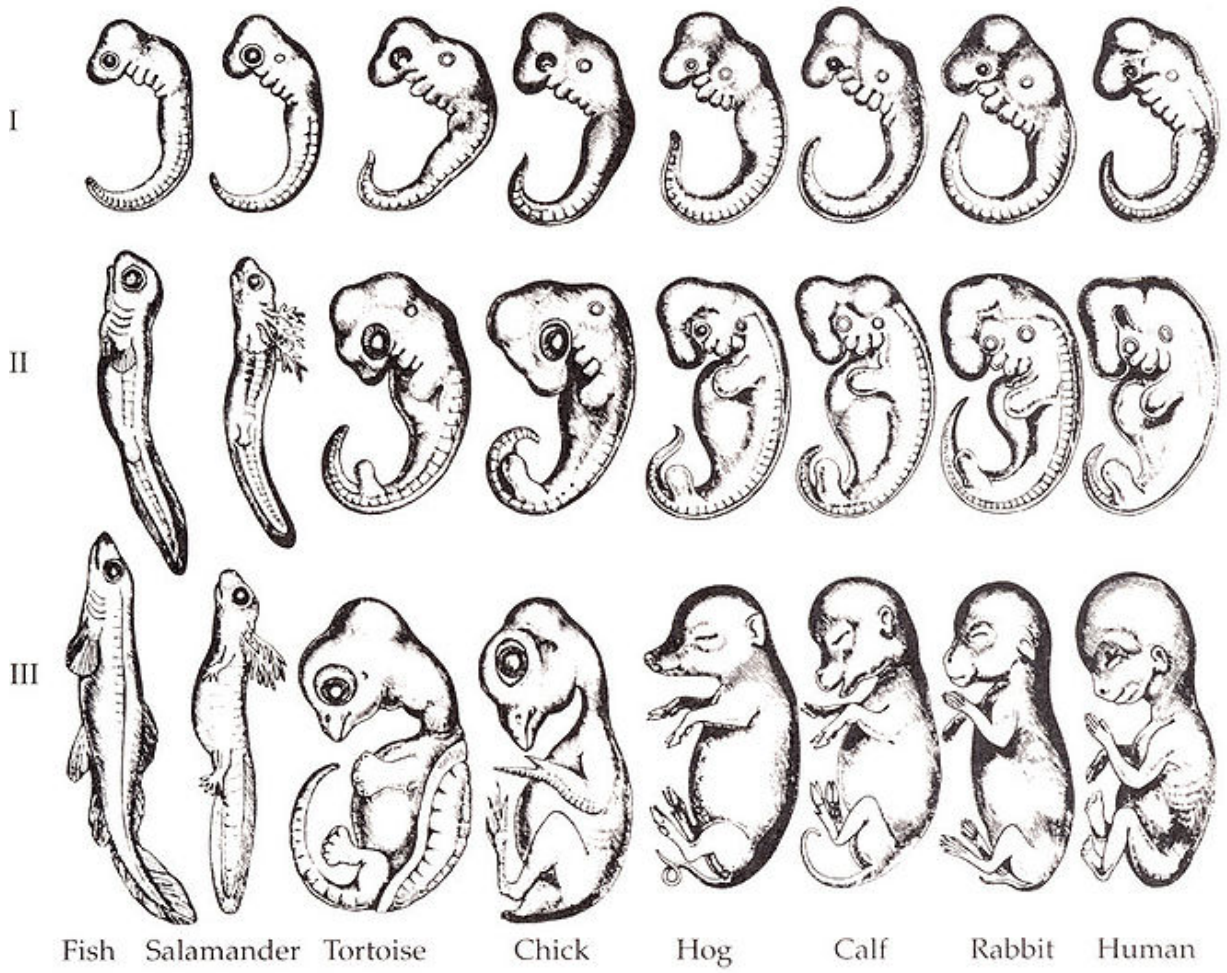


Sensor technology
- Sequencing
  - De novo assembly
  - Variant Calling
  - Metagenomics
  - Epigenetics
  - Transcriptomics
- Remote sensing
- Radio tracking
- High speed cameras
- ‘Deep Phenotyping’
- Etc…

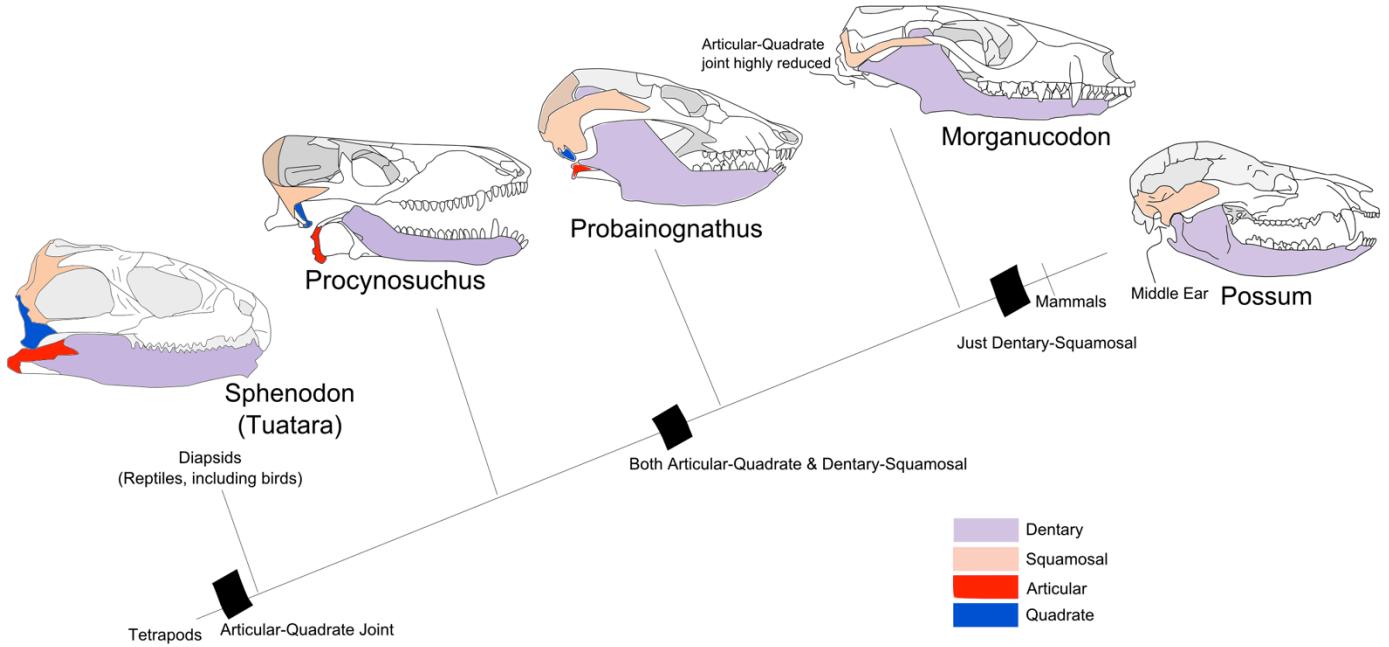
Bioinformatics MacGyverism….

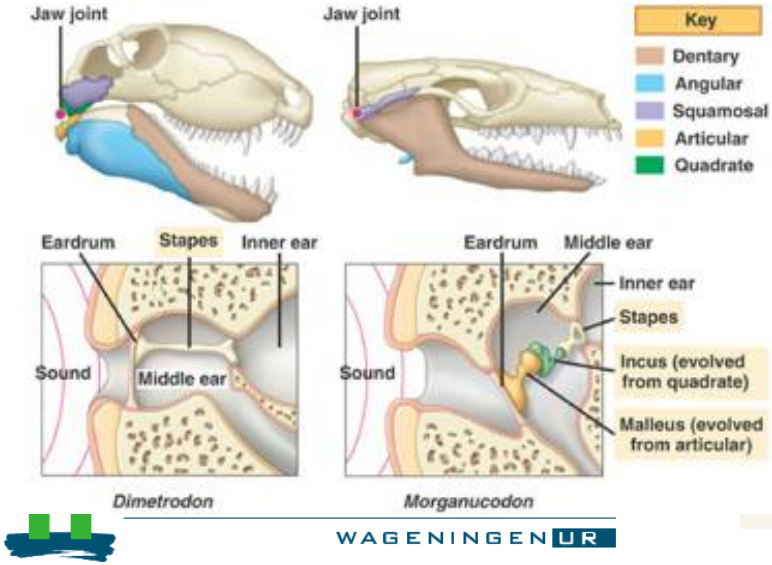
Bioinformatics MacGyverism….

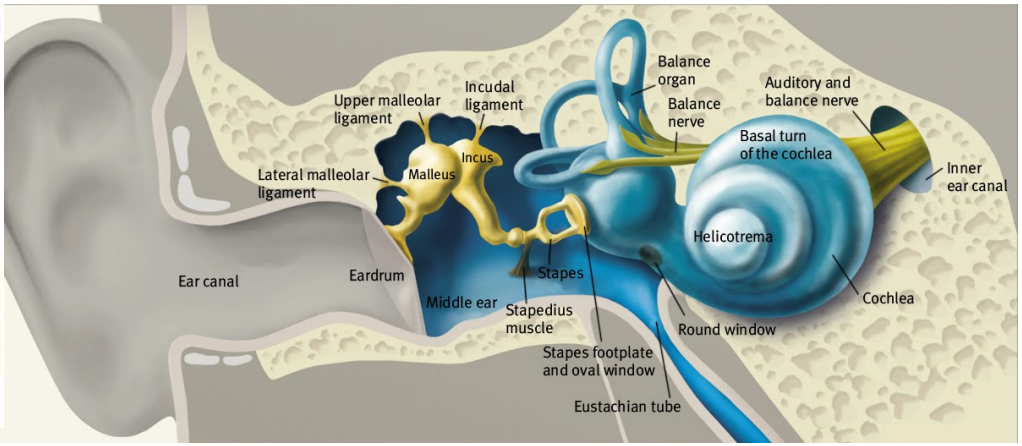
Comparative biology



Comparative biology: an earful of jaw







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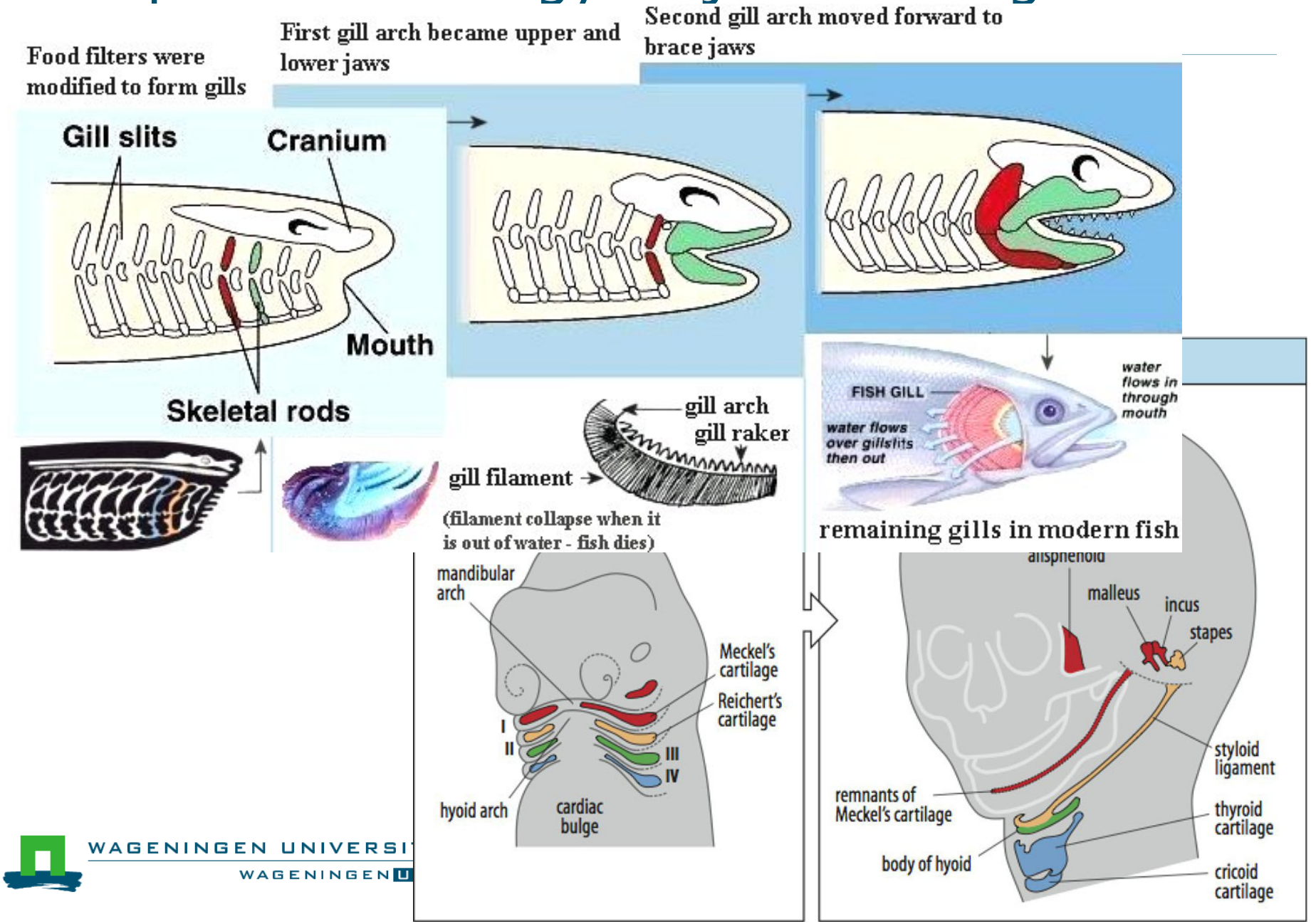
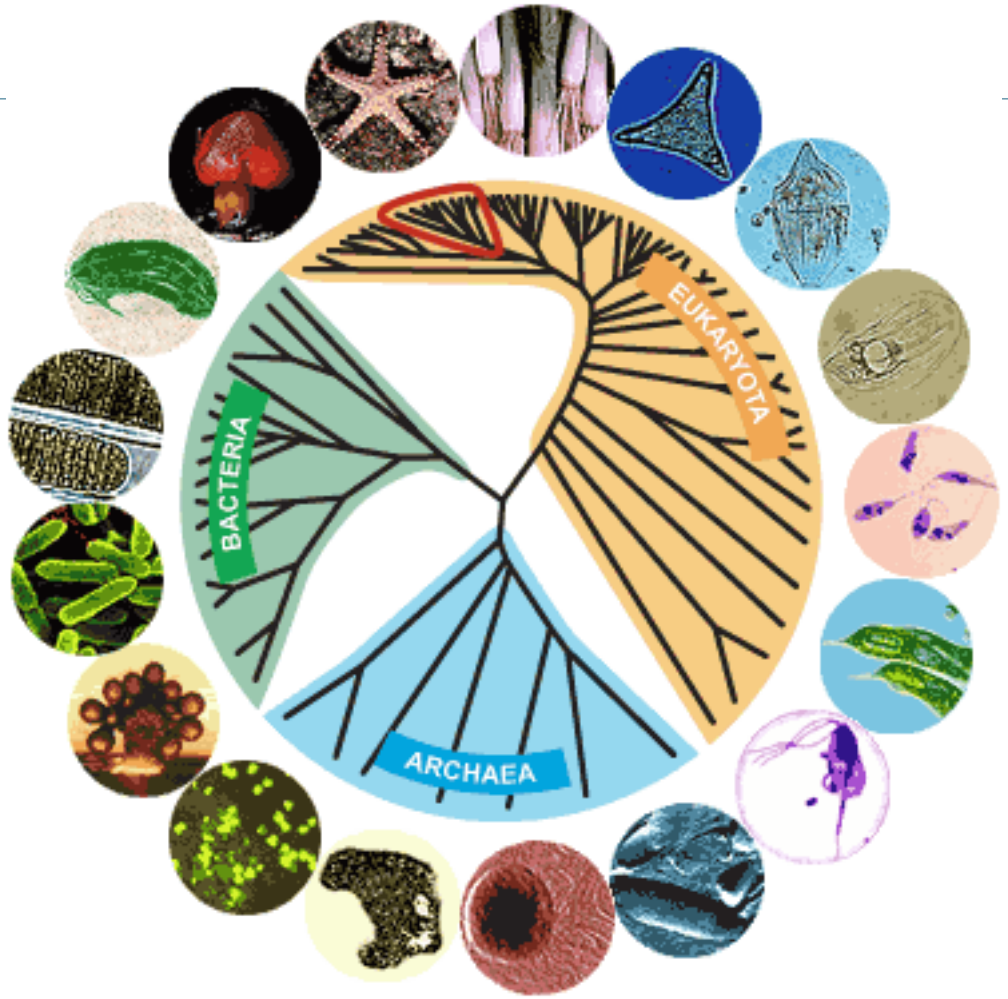
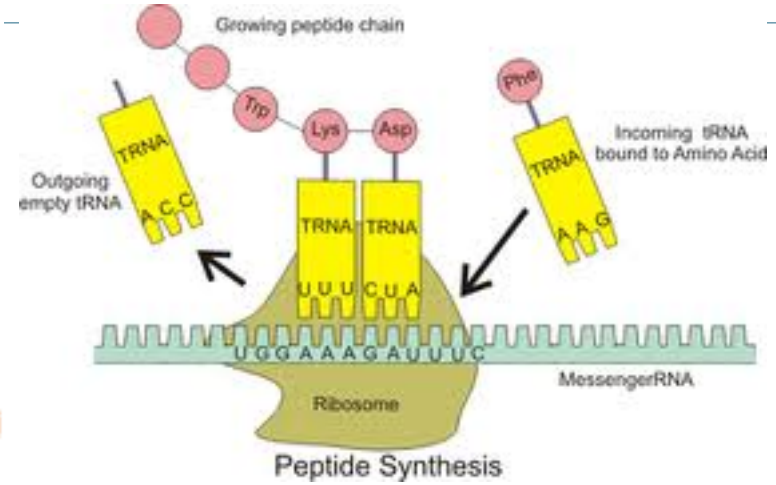
Comparative biology: a jaw full of gill

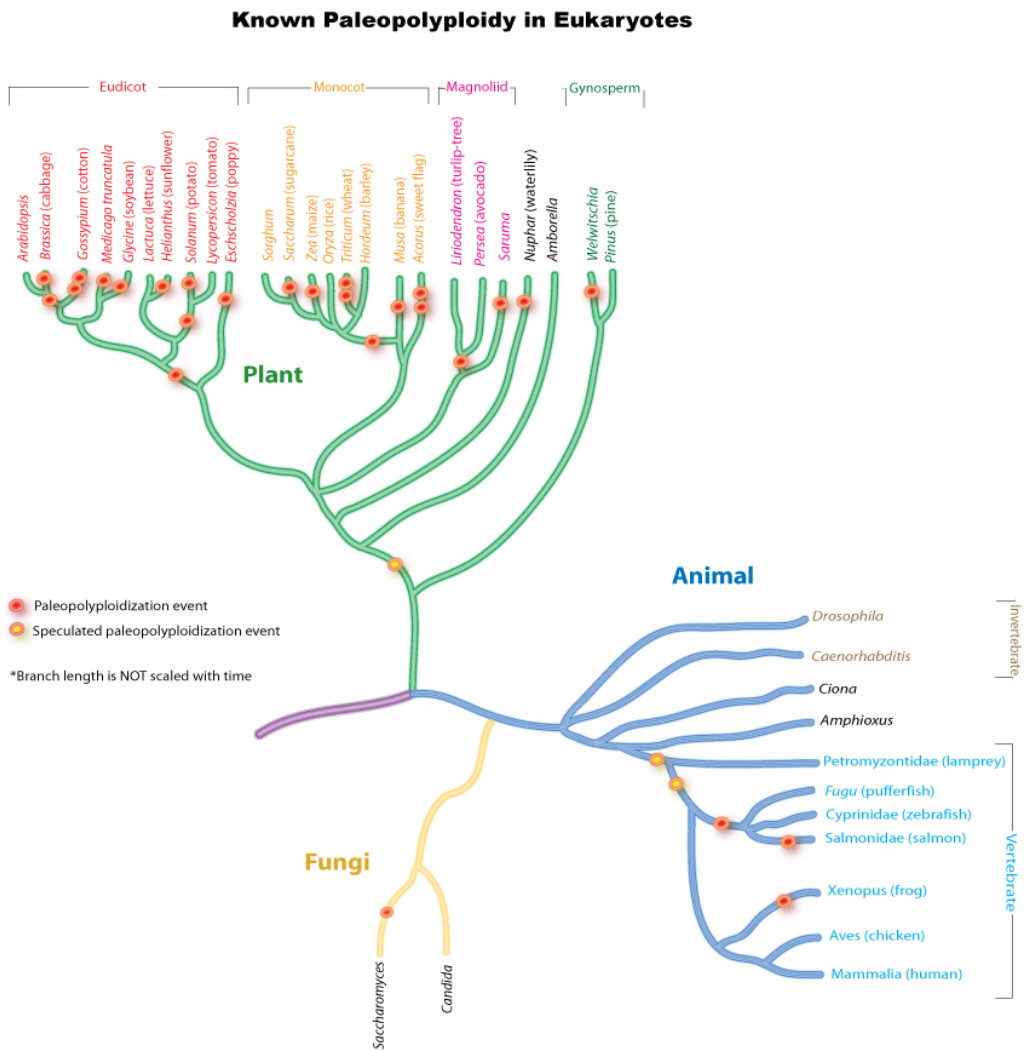


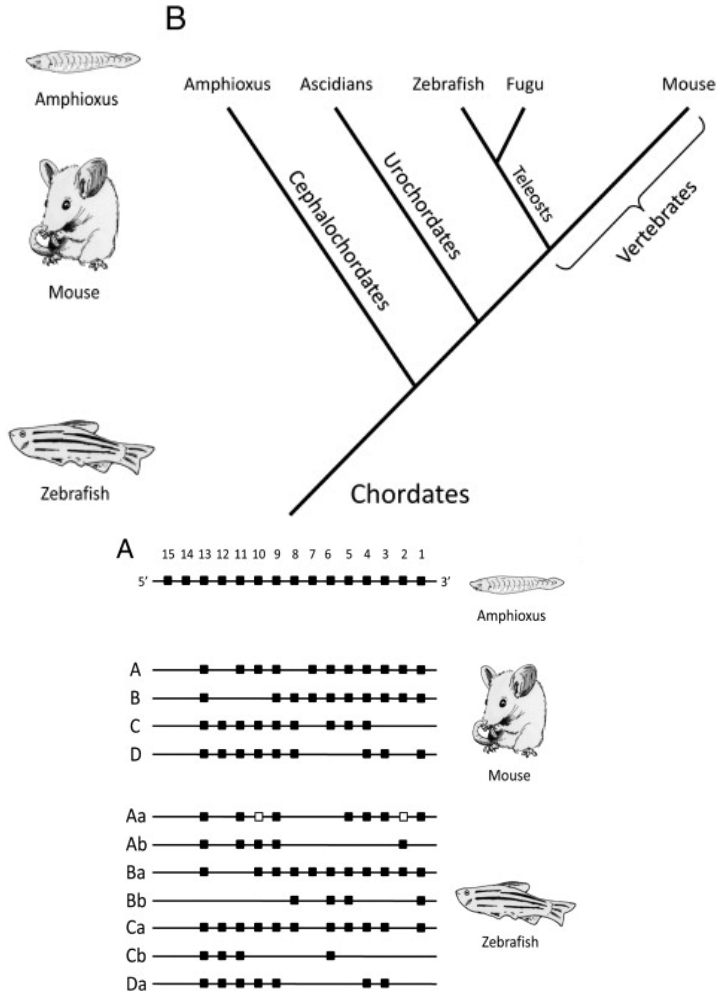
The Tree of Life: comparative biology of rDNA





Virtually all Eukaryotes show evidence of paleo-duplication





Despite re-diploidization, certain genes may retain duplicated nature (e.g. Hox genes in vertebrates).

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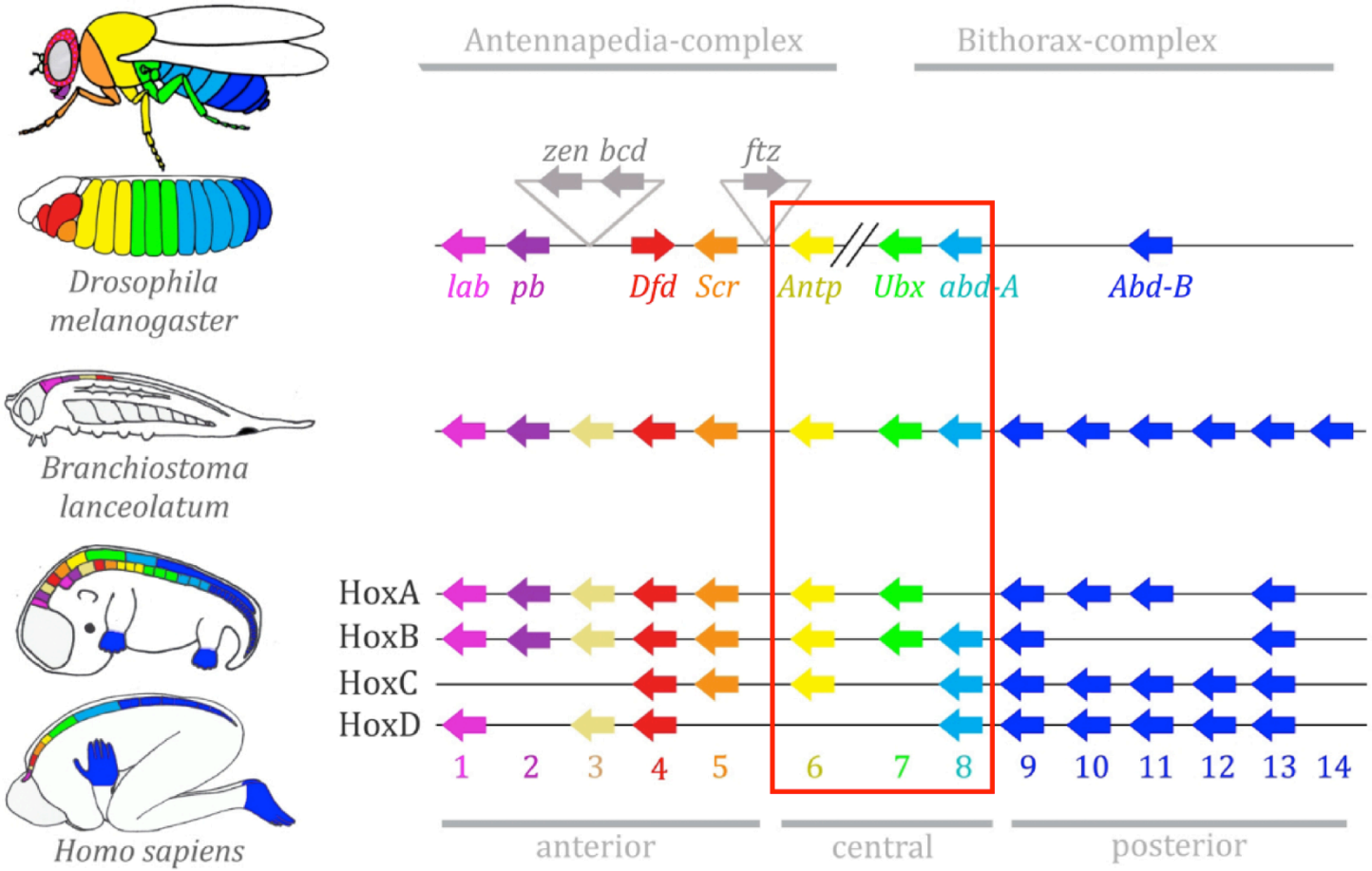
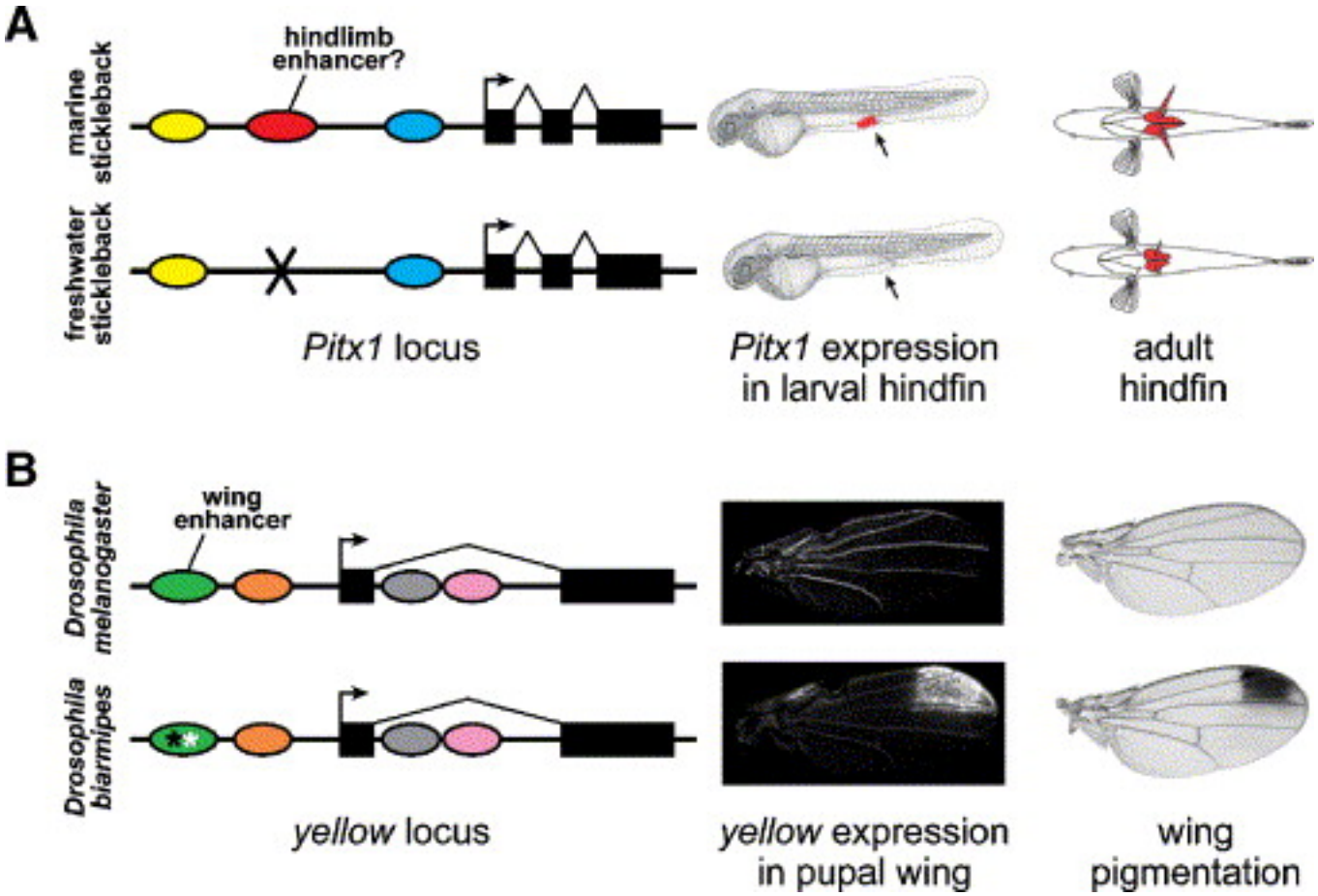
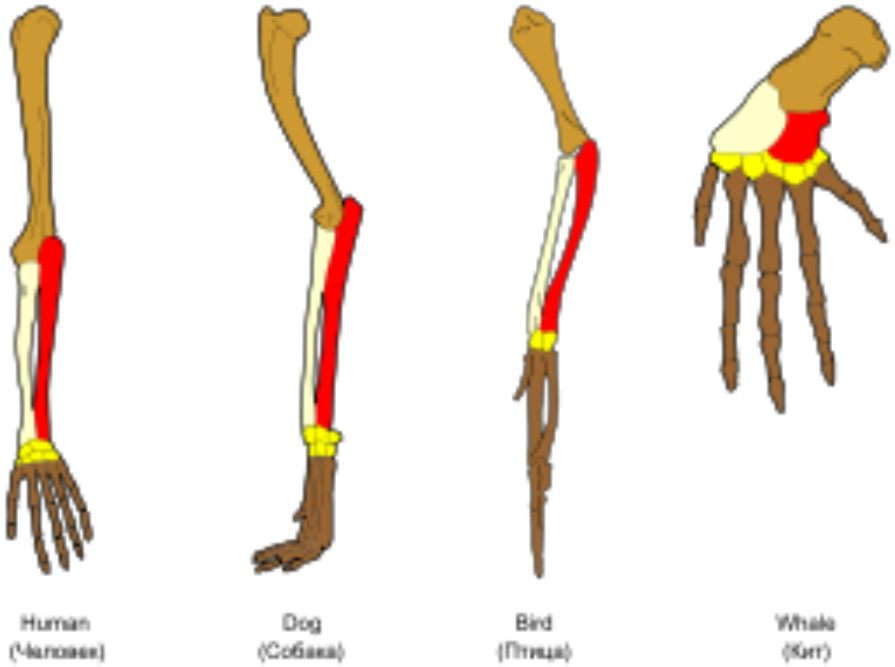
Segmentation is 'homologous' at a molecular level between arthropods and vertebrates

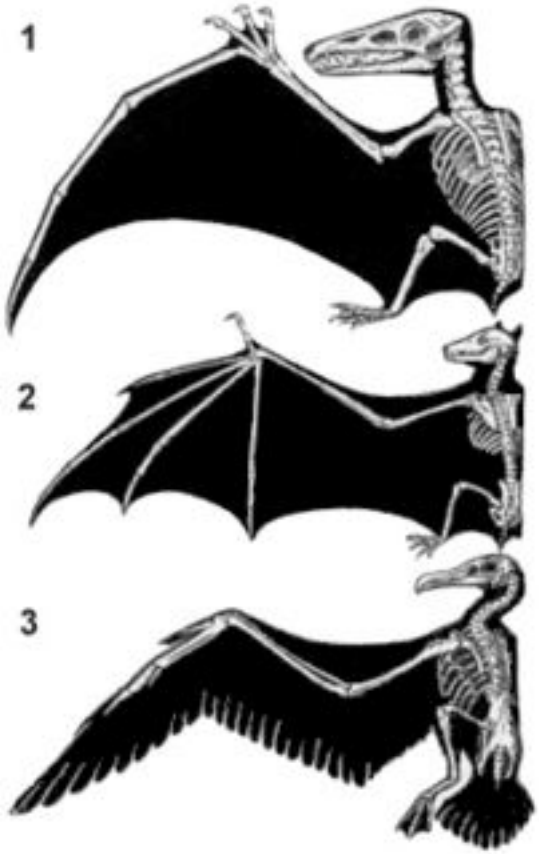


Novel 'switches' → novel functions



Comparative Biology: homology



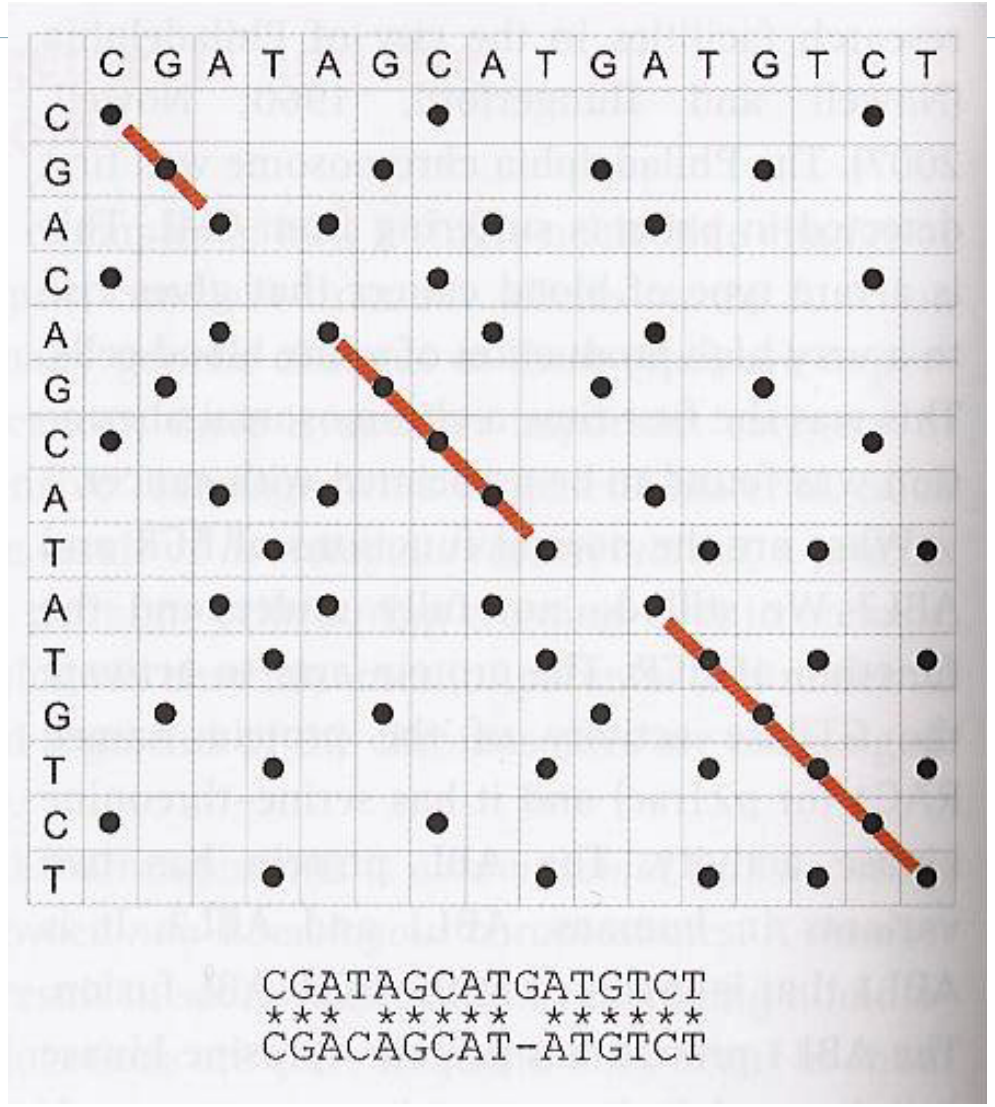


Comparative genomics: sequence alignment

- Sequence homology: entirely defined by sequence similarity.

N bases	1/prob occuring	num times by chance in human genome
5	1024	2929687.5
7	16384	183105.4688
9	262144	11444.0918
11	4194304	715.2557373
13	67108864	44.70348358
15	1073741824	2.793967724
17	17179869184	0.174622983
19	2.74878E+11	0.010913936
21	4.39805E+12	0.000682121
23	7.03687E+13	4.26326E-05
25	1.1259E+15	2.66454E-06

DNA alignment as DotPlot



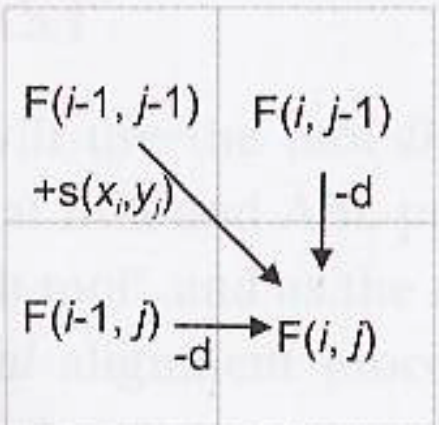
Local alignment using dynamic programming

Gap penalty = d = 2
Mismatch penalty = -1
Match = 2
Match or mismatch = s

A

$$F(i,j) = \max \begin{cases} 0, \\ F(i-1, j-1) + s(x_i, y_j), \\ F(i-1, j) - d, \\ F(i, j-1) - d \end{cases}$$

B



C

		C		A		A		C		A		A	
	0	0		0		0		0		0		0	
T	0	-1	-2	-1	-2	-1	-2	-1	-2	-1	-2	-1	-2
		-2	0	-2	0	-2	0	-2	0	-2	0	-2	0
A	0	-1	-2	2	-2	2	-2	-1	-2	2	-2	2	-2
		-2	0	-2	2	0	2	0	0	-2	2	0	2
A	0	-1	-2	2	0	4	0	1	-2	2	0	4	0
		-2	0	-2	2	0	4	2	2	0	2	0	4
A	0	-1	-2	2	0	4	2	3	0	4	0	4	2
		-2	0	-2	2	0	4	2	3	1	4	2	4
A	0	-1	-2	2	0	4	2	3	1	5	2	6	2
		-2	0	-2	2	0	4	2	3	1	5	3	6

D

```
AA-AA
|| ||
AACAA
```

BLOSUM62: **blo**cks of amino acid **su**bstitution **m**atrix

	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W
C	**9**																			
S	-1	**4**																		
T	-1	1	**4**																	
P	-3	-1	1	**7**																
A	0	1	0	-1	**4**															
G	-3	0	-2	-2	0	**6**														
N	-3	1	0	-2	-2	0	**6**													
D	-3	0	-1	-1	-2	-1	1	**6**												
E	-4	0	-1	-1	-1	-2	0	2	**5**											
Q	-3	0	-1	-1	-1	-2	0	0	2	**5**										
H	-3	-1	0	-2	-2	-2	1	-1	0	0	**8**									
R	-3	-1	-1	-2	-1	-2	0	-2	0	1	0	**5**								
K	-3	0	-1	-1	-1	-2	0	-1	1	1	-1	2	**5**							
M	-1	-1	-1	-2	-1	-3	-2	-3	-2	0	-2	-1	-1	**5**						
I	-1	-2	-1	-3	-1	-4	-3	-3	-3	-3	-3	-3	-3	1	**4**					
L	-1	-2	-1	-3	-1	-4	-3	-4	-3	-2	-3	-2	-2	2	2	**4**				
V	-1	-2	0	-2	0	-3	-3	-3	-2	-2	-3	-3	-2	1	3	1	**4**			
F	-2	-2	-2	-4	-2	-3	-3	-3	-3	-3	-1	-3	-3	0	0	0	-1	**6**		
Y	-2	-2	-2	-3	-2	-3	-2	-3	-2	-1	2	-2	-2	-1	-1	-1	-1	3	**7**	
W	-2	-3	-2	-4	-3	-2	-4	-4	-3	-2	-2	-3	-3	-1	-3	-2	-3	1	2	**1**

Approximate String Matching using dynamic programming:

example from “Mastering Perl for Bioinformatics

```
my $pattern = 'EIQADEVRL';
print "PATTERN:\n$pattern\n";

my $text = 'SVLQDRSMPHQEILAADEVLQESEMRQQDMISHDE';
print "TEXT:\n$text\n";
```

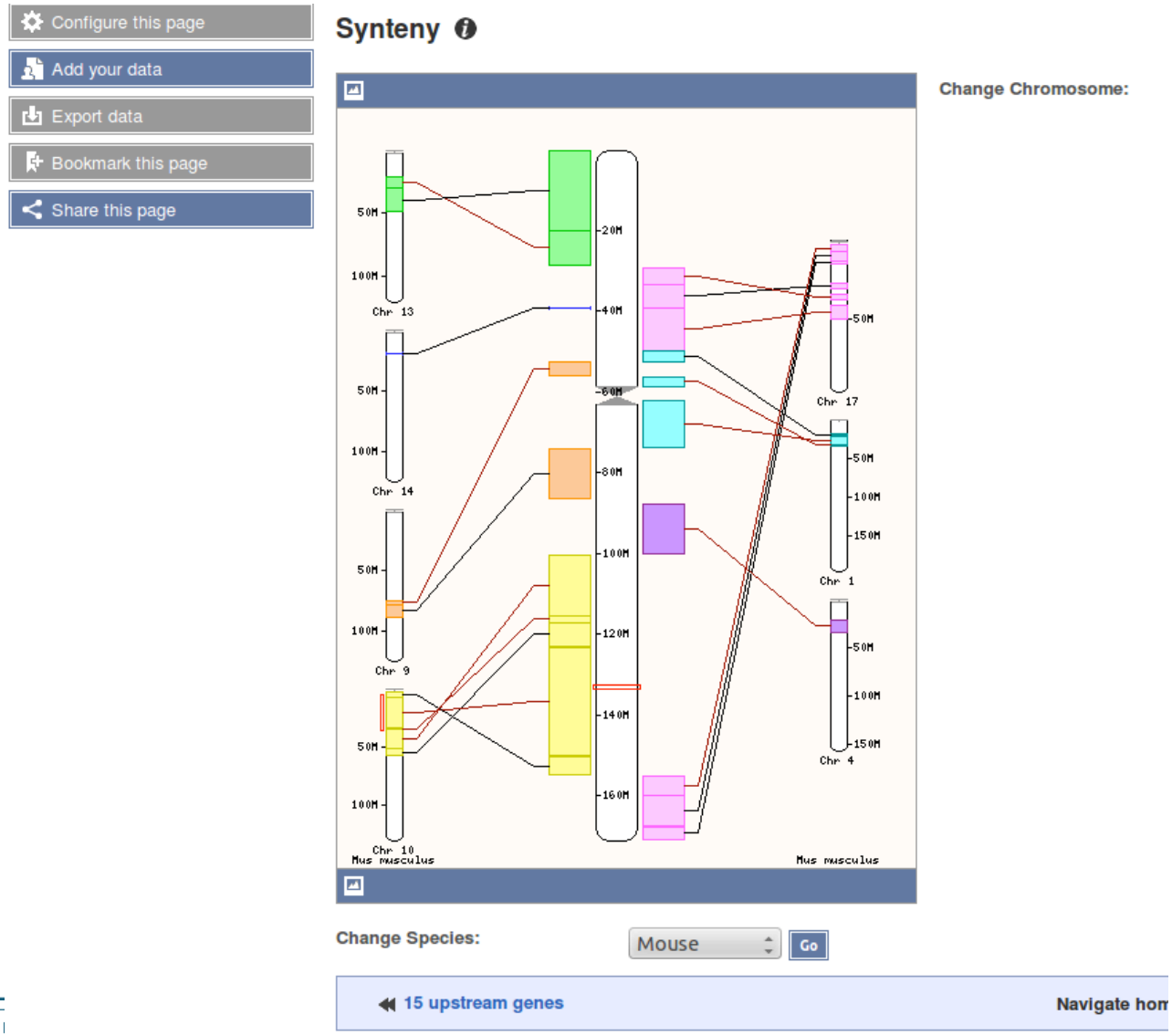
```
     S V L Q D R S M P H Q E I L A A D E V L Q E S E M R Q Q D M I S H D E
   0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
E  1 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 0 1 1 1 0 1 0 1 1 1 1 1 1 1 1 1 1 0
I  2 2 2 2 2 2 2 2 2 2 2 1 0 1 2 2 2 1 1 2 2 1 1 1 1 2 2 2 2 2 1 2 2 2 1
Q  3 3 3 2 3 3 3 3 3 3 2 2 1 1 2 3 3 2 2 2 2 2 2 2 2 2 2 3 3 2 2 3 3 2
A  4 4 4 4 3 3 4 4 4 4 4 3 3 2 2 1 2 3 3 3 3 3 3 3 3 3 3 3 4 3 3 3 4 3
D  5 5 5 4 3 4 5 5 5 5 4 4 3 3 2 2 2 3 4 4 4 4 4 4 4 4 4 3 4 4 4 4 3 4
E  6 6 6 5 4 4 5 6 6 6 5 4 4 4 3 3 3 2 3 4 5 4 5 4 5 5 5 5 4 4 5 5 4 3
V  7 7 6 7 6 5 5 5 6 7 7 6 5 5 5 4 4 4 3 2 3 4 5 5 5 5 6 6 6 5 5 5 6 5 4
R  8 8 7 7 7 6 5 6 6 7 8 7 6 6 6 5 5 5 4 3 3 4 5 6 6 6 5 6 7 6 6 6 6 5
L  9 9 8 7 8 7 6 6 7 7 8 8 7 7 6 6 6 6 5 4 3 4 5 6 7 7 6 7 7 7 7 7 7 6
```

Figure 2-1. Edit distance matrix

Alignment types and alignment software

- Pairwise alignment
  - Database searches
    - BLAST, BLAT
  - Short Read Mappers
    - BWA (Burrows-Wheeler Transform), Mosaik (Smith-Watterman)
  - Genome Aligners
    - MUMmer, (B)LastZ
- Multiple sequence alignment
  - Genes, short sequences
    - Clustal(w|x), Muscle, T-Coffee
  - Genome
    - Mauve, Mulan

Synteny: usually defined by gene order



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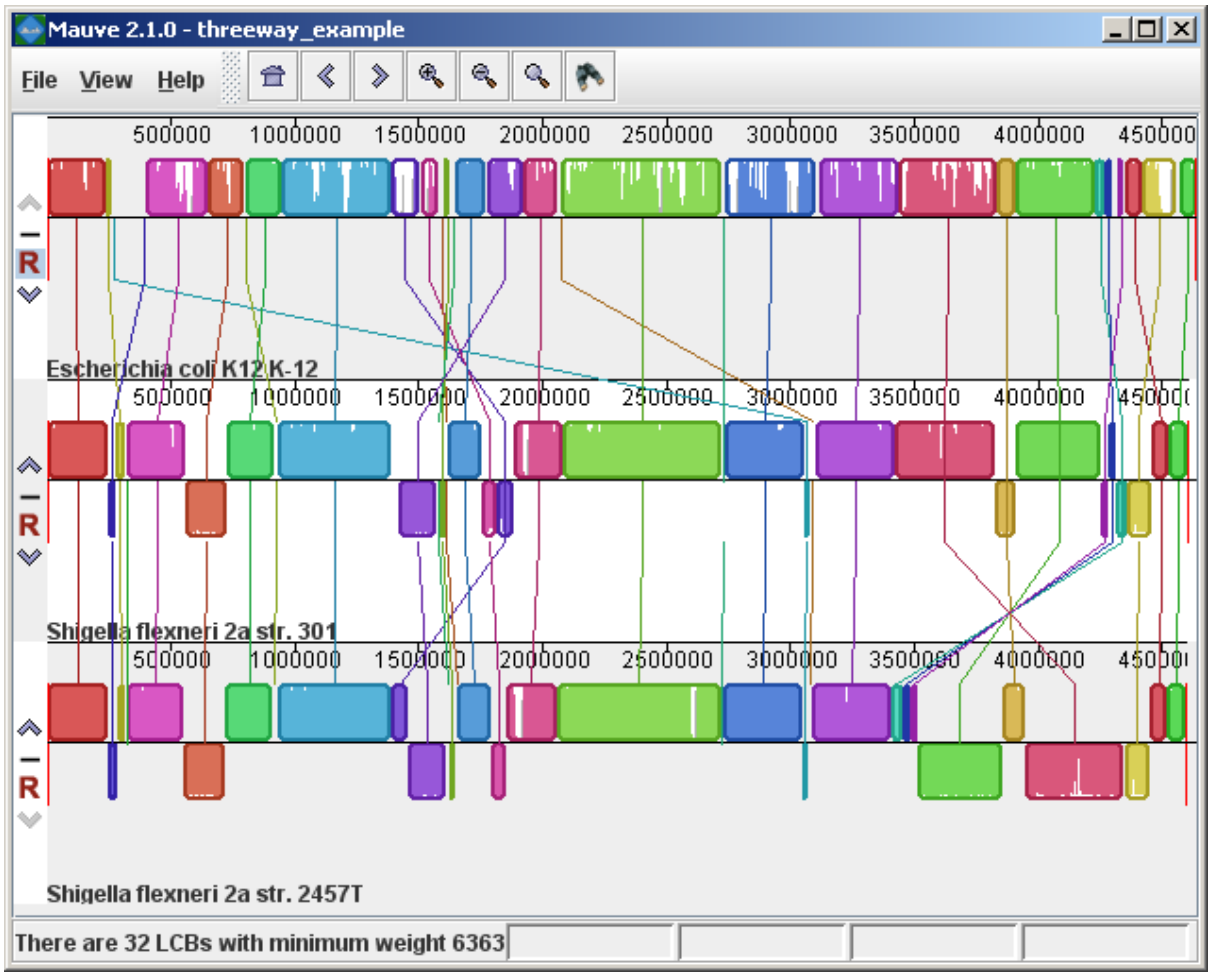
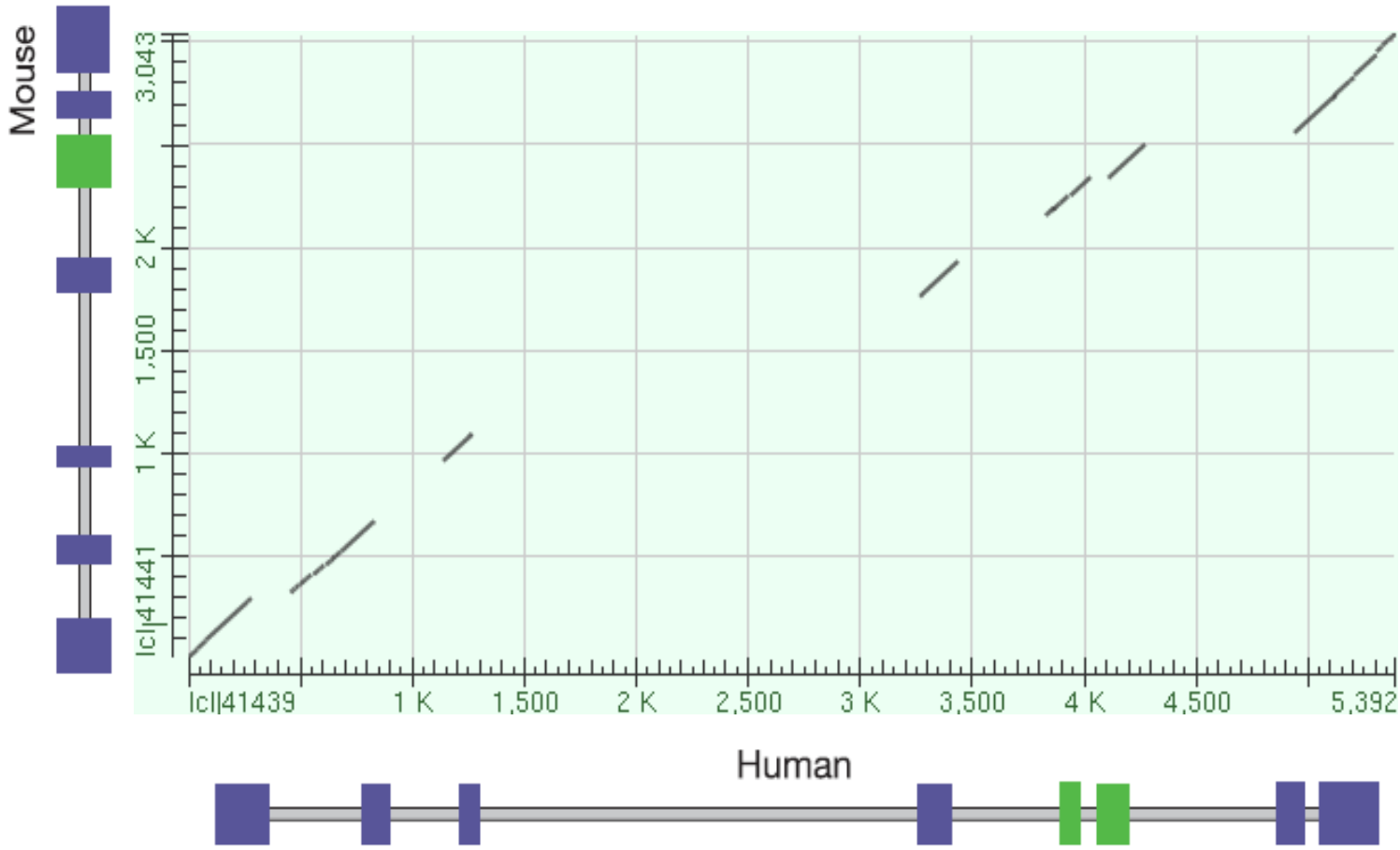
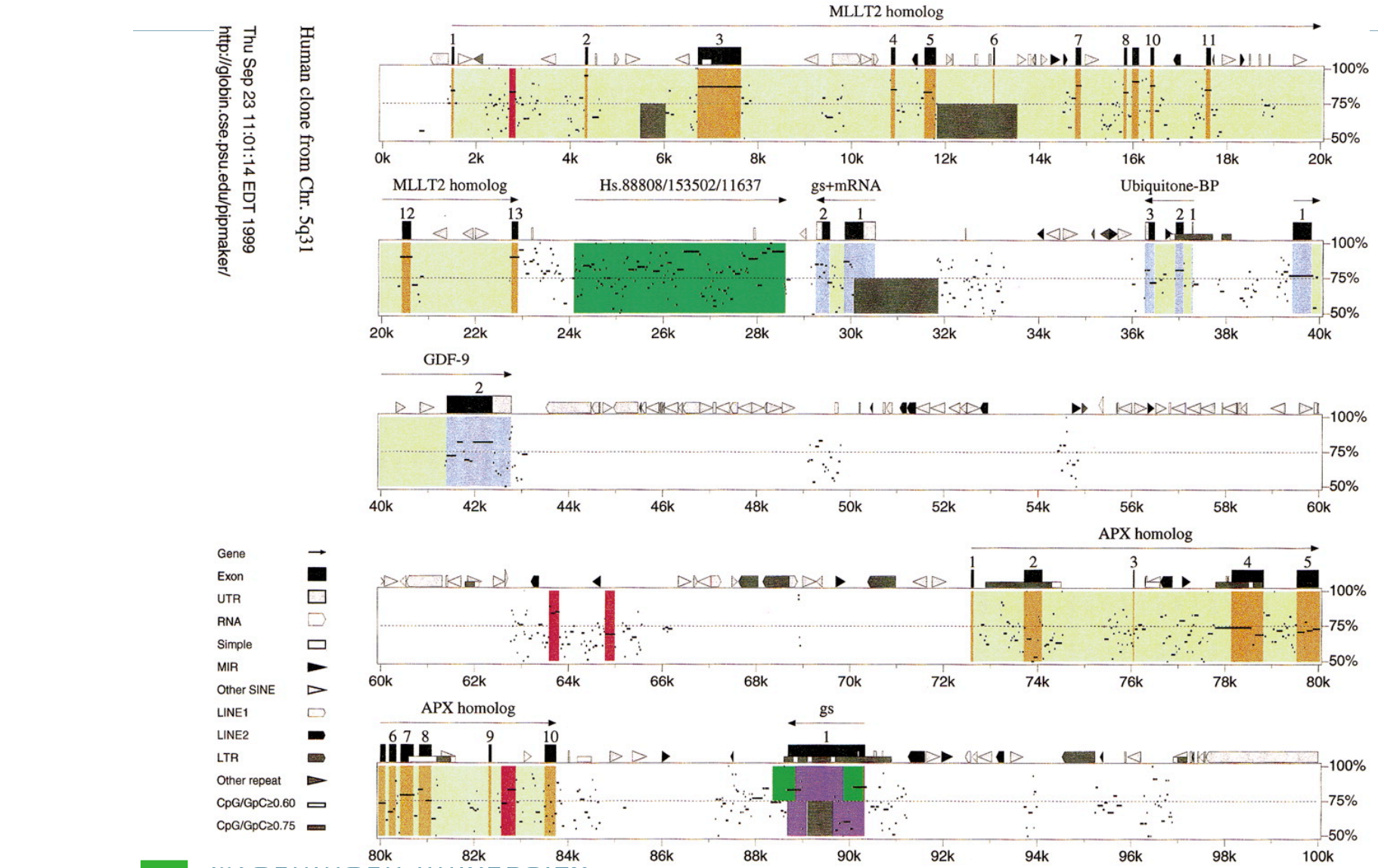
Synteny: whole genome multiple alignment – example using Mauve



Sequence comparison: Dot plot

Due to purifying selection: High sequence conservation of functional regions



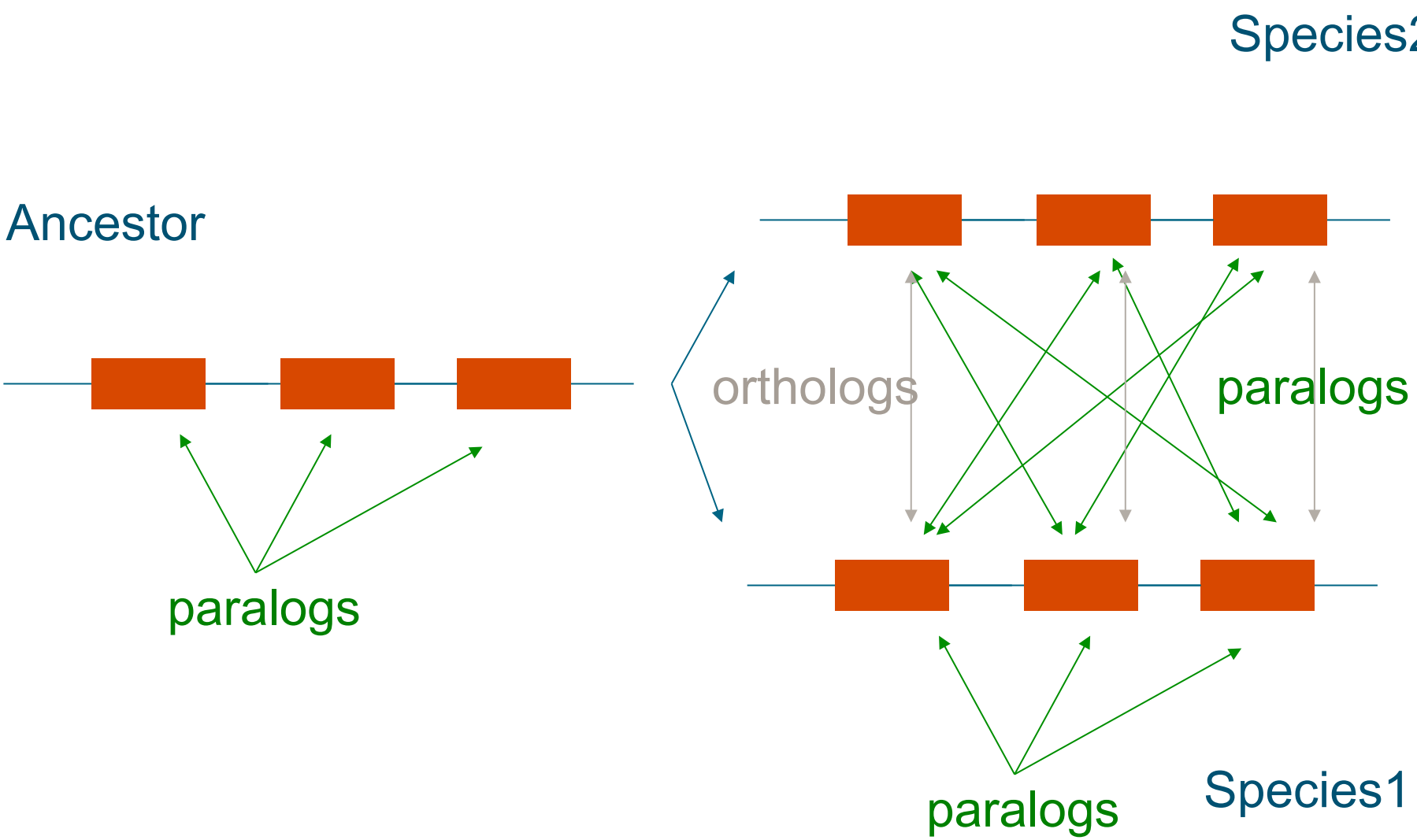


Orthology and Paralogy

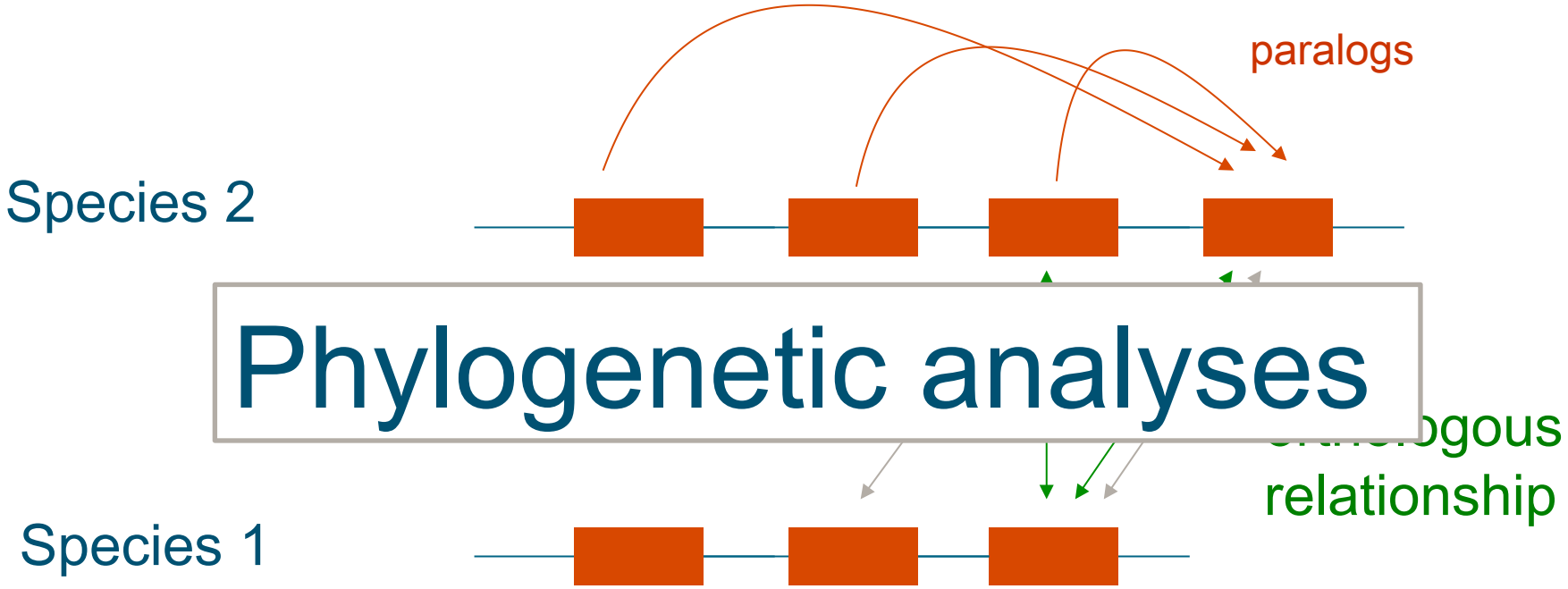
Orthology: Sequences are orthologous
if they were separated by a **speciation** event

Paralogy: Sequences are paralogous
if they were separated by a **gene duplication** event

Orthology vs Paralogy



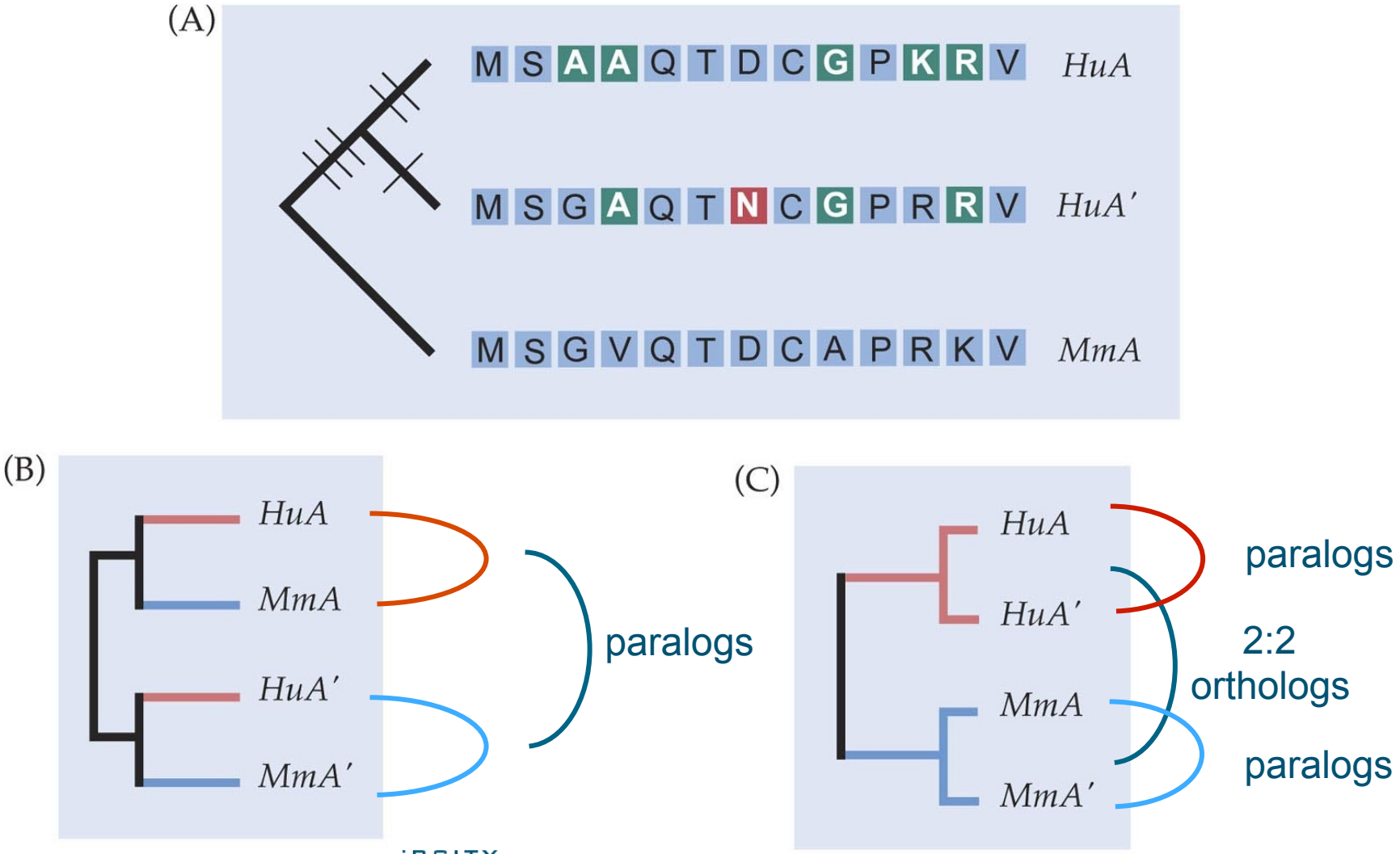
Orthology vs Paralogy complications



It is very likely that you would refer to these two combinations as being the orthologs

Clustering of sequences and building phylogenetic trees based on genes from species helps to clarify this

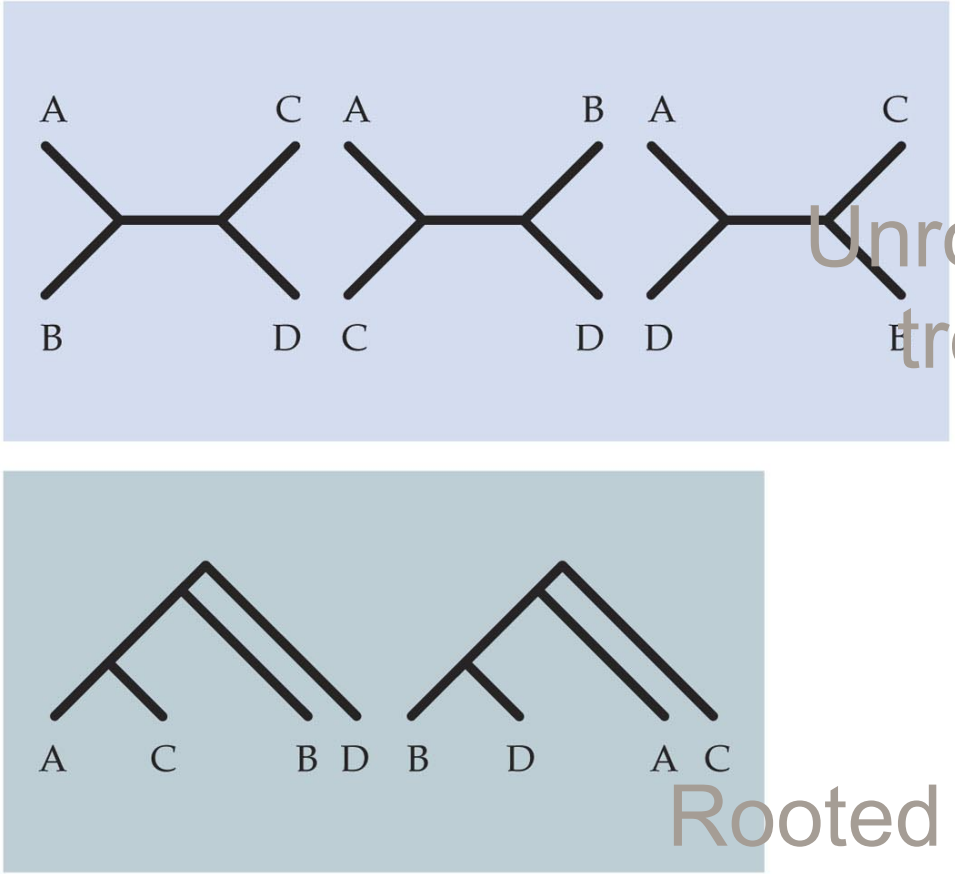
Identification of orthologs vs. paralogs



Both 1:1 orthologs

Phylogenetic trees

- Distance Methods
- Parsimony methods
- Maximum Likelihood
- Bayesian

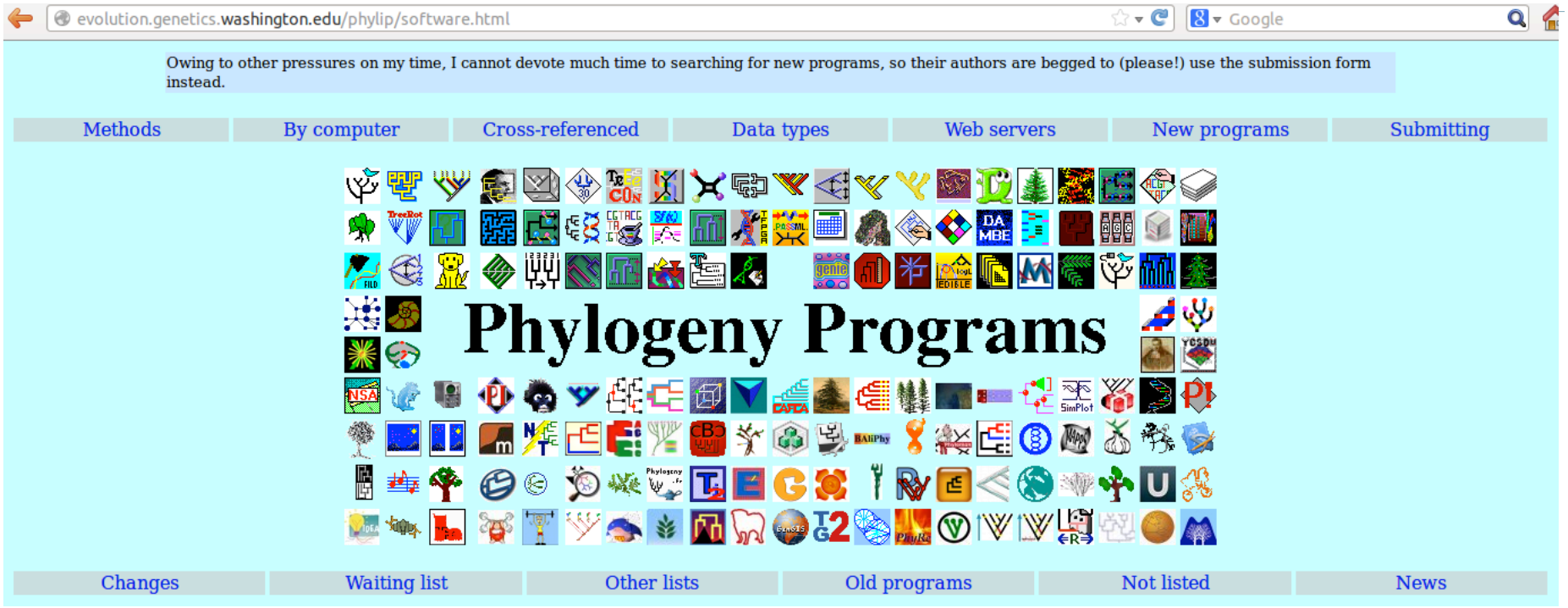


Unrooted trees

Rooted trees

A PRIMER OF GENOME SCIENCE, Second Edition, Box 2.4 (Part 1) © 2005 Sinauer Associates, Inc.

Methods in CB: Phylogeny



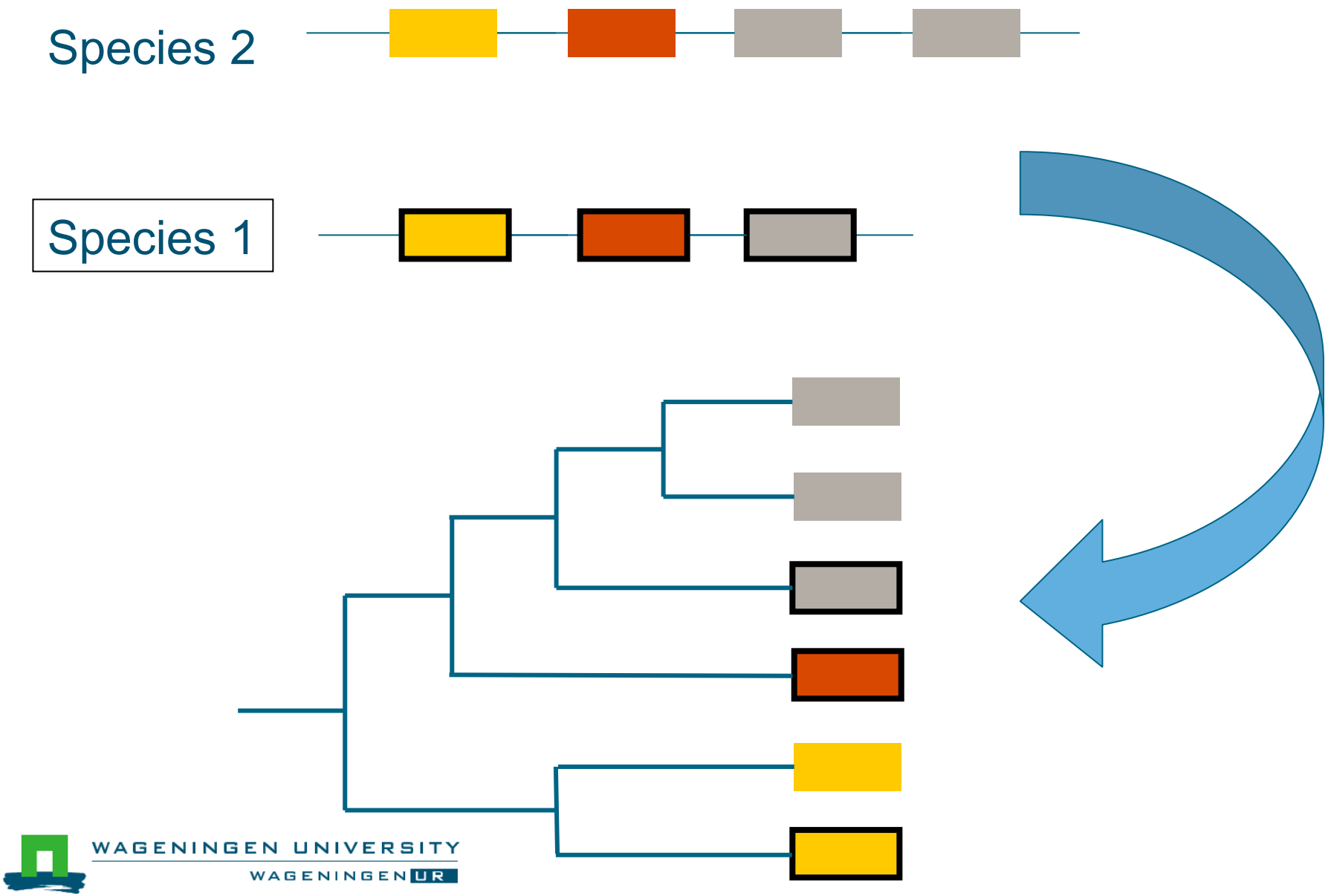
BIOINFORMATICS APPLICATIONS NOTE Vol. 22 no. 21 2006, pages 2688–2690
doi:10.1093/bioinformatics/btl446

Phylogenetics

RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models

Alexandros Stamatakis

Orthology vs Parology complications

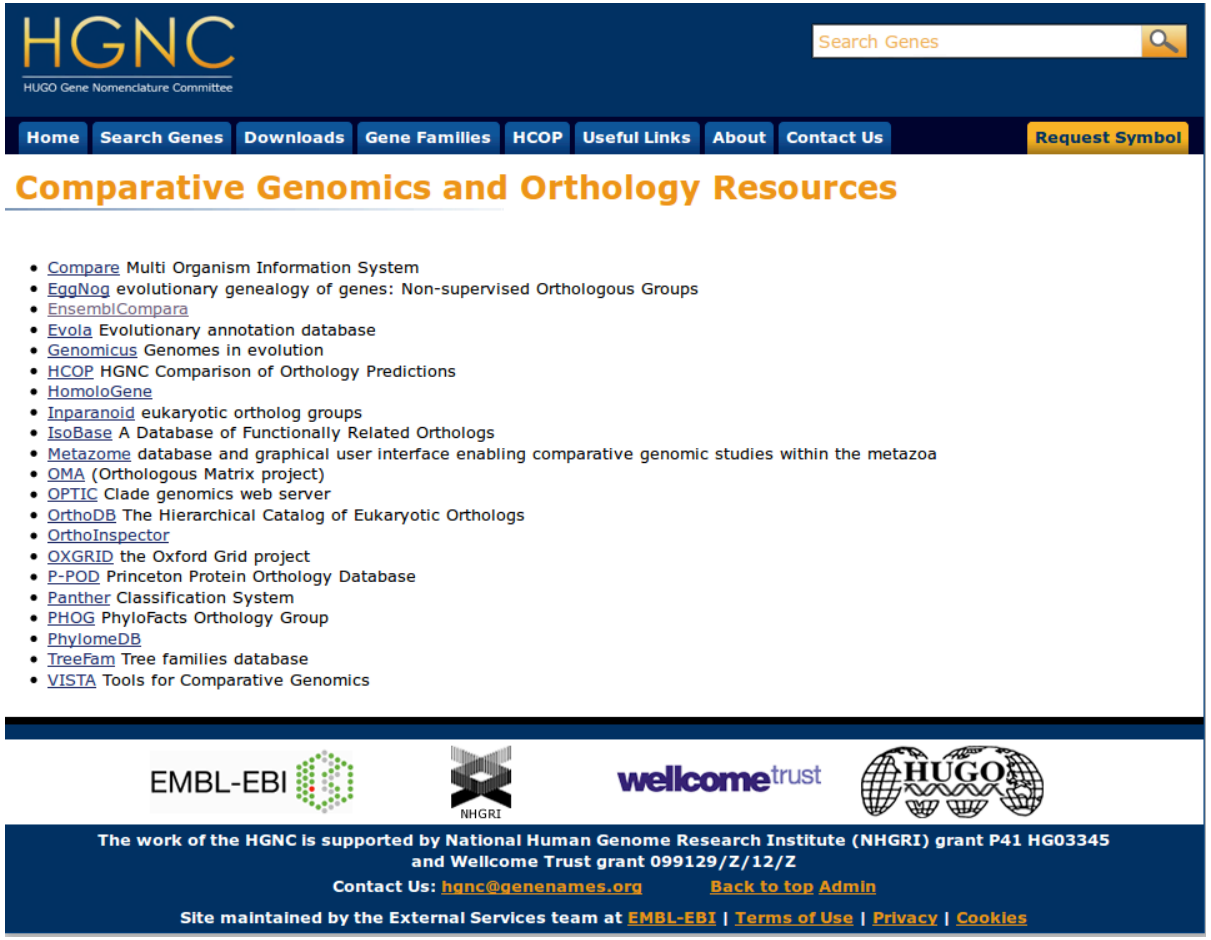


Genome Annotation and Phylogenetic Analysis

- Much of the annotation for non-model organisms is based on comparative work with model organisms.
- Transfer/optimization of gene models
- Inference of function (e.g. GO annotation)
- Becoming ever more important with the rapidly increasing number of non-model organisms being sequenced

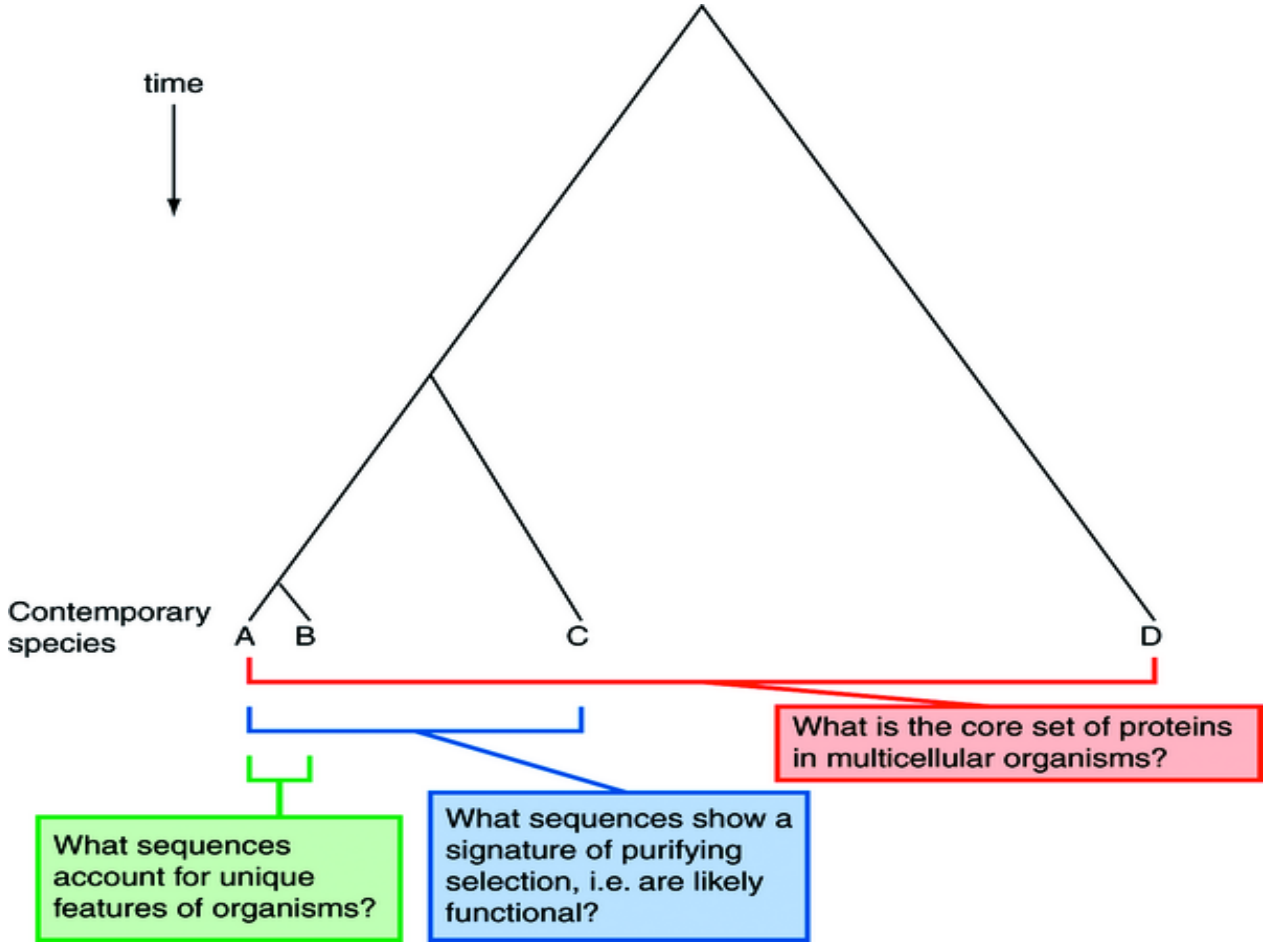
Gene Naming:

the Hugo Gene Nomenclature Committee is effectively determining gene names across the vertebrates

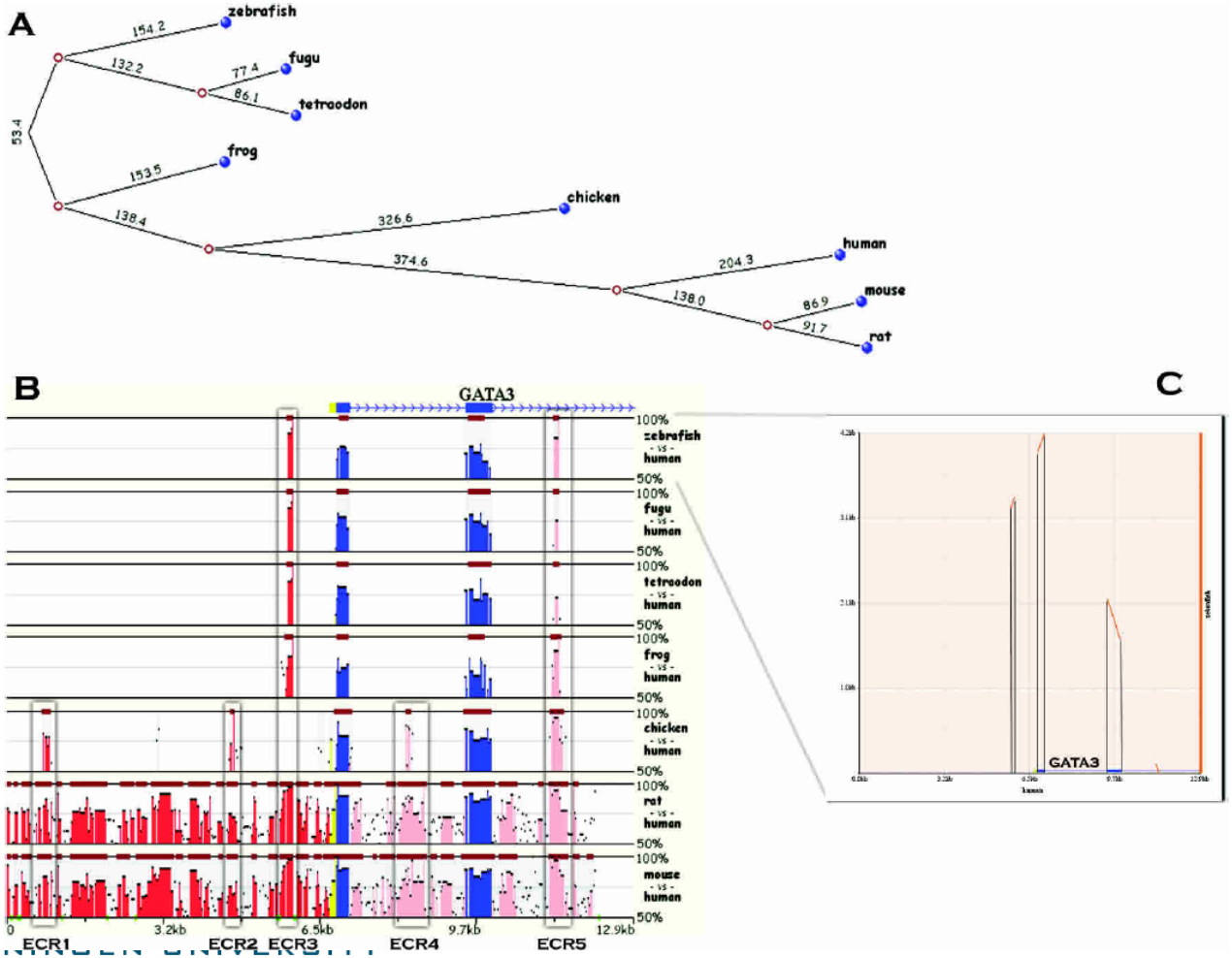


Comparative Biology

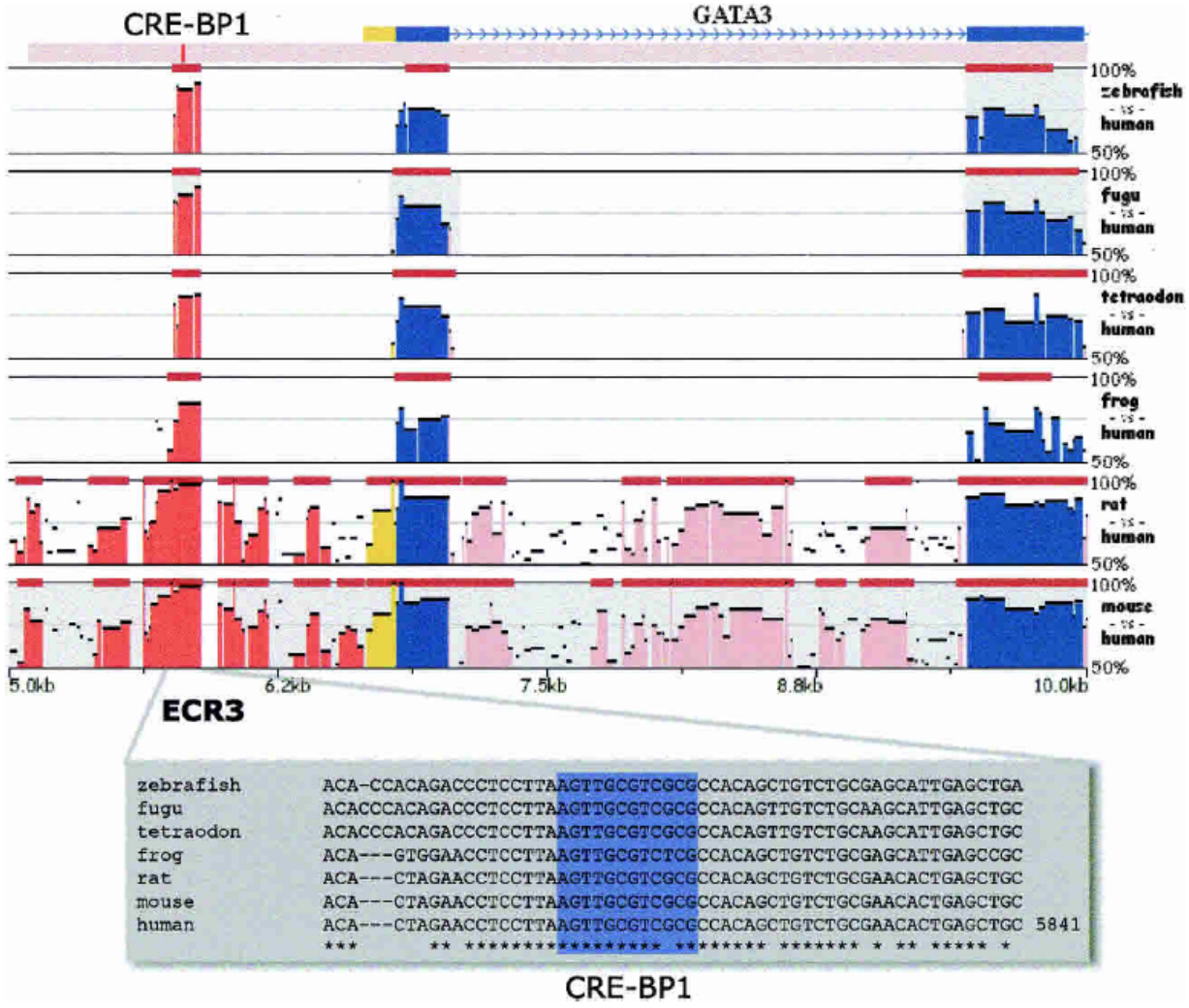
Comparisons of Genomes at Different Phylogenetic Distances Are Appropria to Address Different Questions



Predicting function: Transcription Factor Binding Site prediction (Mulan)



Predicting function: Transcription Factor Binding Site prediction (Mulan)



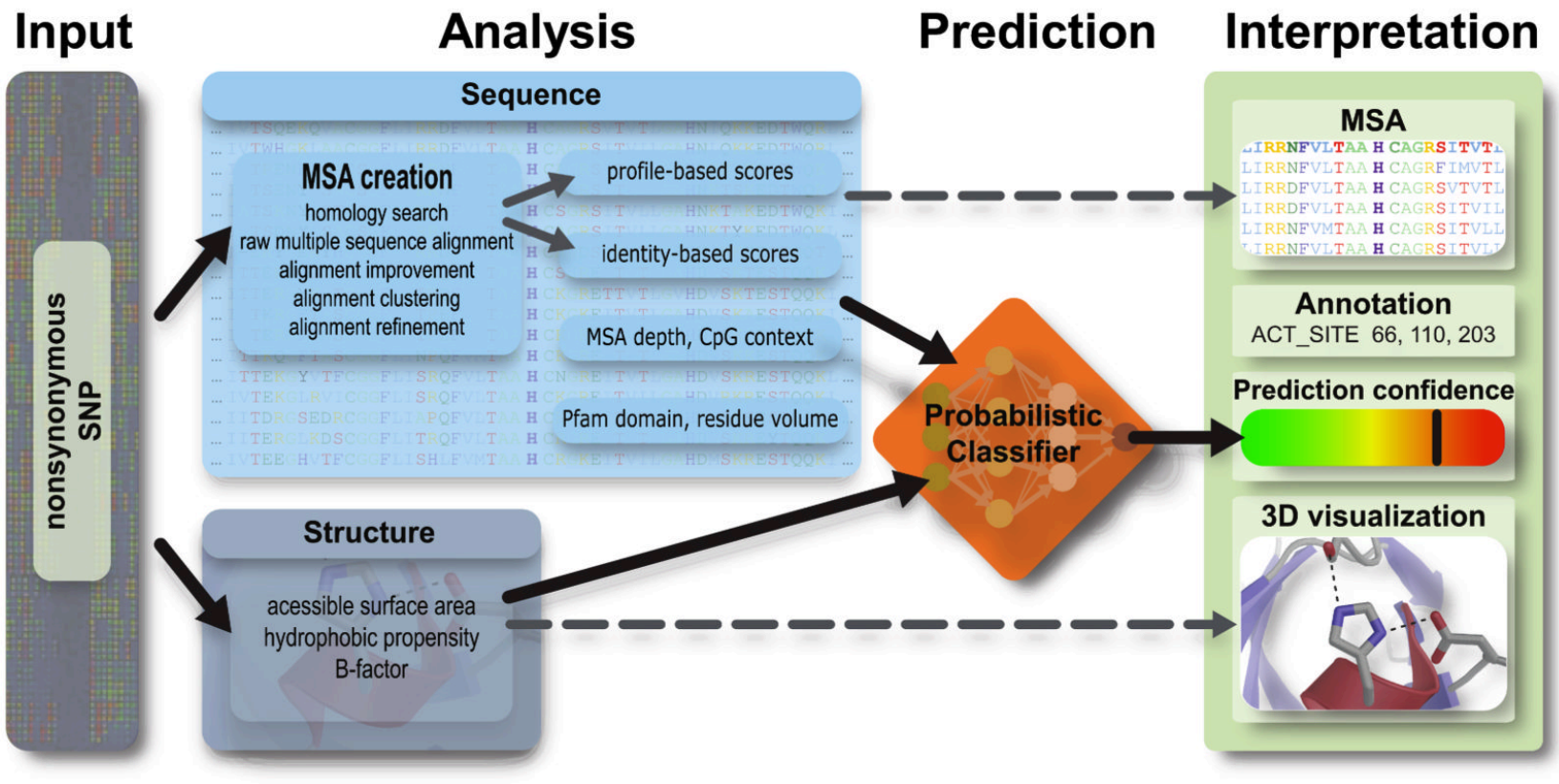
Predicting functional aspect of amino acid substitutions

- An amino-acid substitution that is rare in a certain taxonomic group is more likely to have functional consequences than an amino acid substitution that occurs often.

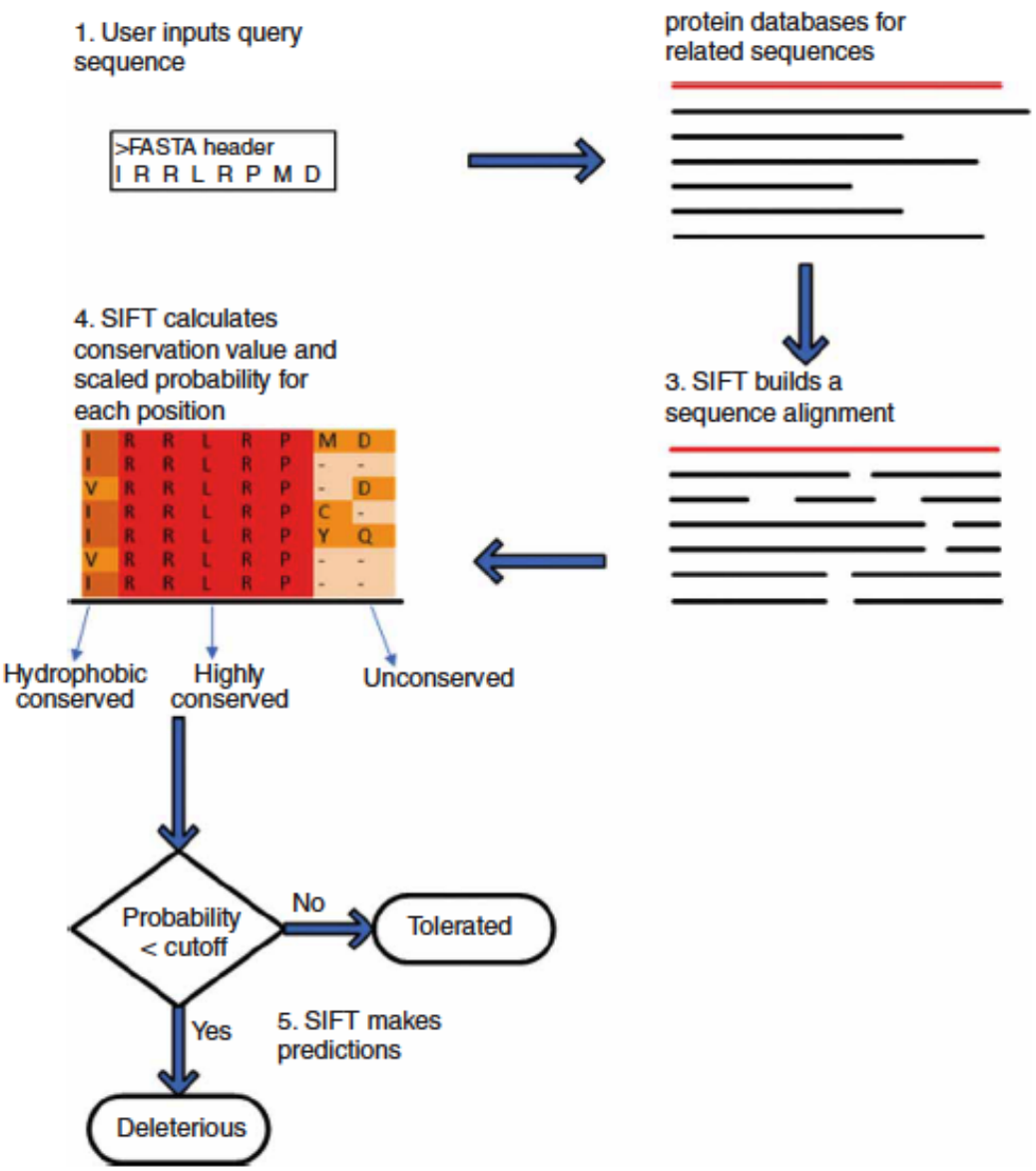
Method	Algorithm
SIFT (http://sift.jcvi.org)	SIFT uses sequence homology; scores are calculated using position-specific scoring matrices with Dirichlet priors
Polyphen[12] (http://genetics.bwh.harvard.edu/pph/)	Polyphen uses sequence conservation, structure and SWISS-PROT annotation
PMUT[13] (http://mmb2.pcb.ub.es:8080/PMut/)	PMUT provides prediction by neural networks, which use internal databases, secondary structure prediction and sequence conservation
SNPs3D[18] (http://www.snps3d.org/)	SNPs3D is based on support vector machine that uses structural or sequence conservation features
PantherPSEC[19] (http://www.pantherdb.org/tools/csnpScoreForm.jsp)	Panther PSEC uses sequence homology; scores are calculated using PANTHER Hidden Markov model families
MAPP[14] (http://mendel.stanford.edu/SidowLab/downloads/MAPP/index.html)	MAPP considers the physicochemical variation present in a column of a protein sequence alignment to predict the effect of all possible amino acid substitutions on protein function
Align-GVGD[15] (http://agvgd.iarc.fr/agvgd_input.php)	Align-GVGD combines the biophysical characteristics of amino acids and protein multiple sequence alignments

Predicting function: PolyPhen

a



Predicting function: SIFT



Predicting function: SIFT

Protein ID	Substitution	dbSNP ID	Prediction	Score	Median Info	Number of Seqs at position
NP_001008498	V352I	rs27180025	TOLERATED	1.00	1.38	99
NP_001008499	Y219C	rs33644739	DAMAGING	0.00	1.90	98
NP_001008501	C192Y	rs32419104	TOLERATED	0.25	1.46	84
82934505	Q23L	rs13484479	DAMAGING *Warning! Low confidence.	0.00	4.32	1
82934725	K52E	rs26925385	TOLERATED	1.00	4.32	2
82934691	P52R	rs26956085	TOLERATED	1.00	4.23	101

* Low confidence means that the protein alignment does not have enough sequence diversity. Because the position artifically appears to be conserved, an amino acid may incorrectly

Click here to download the following table in tab separated format. You can open it using excel with delimiter set as TAB

If you received a warning that the sequences were not diverse enough, you can have SIFT choose more diverse sequences here.

Predicting function: SIFT

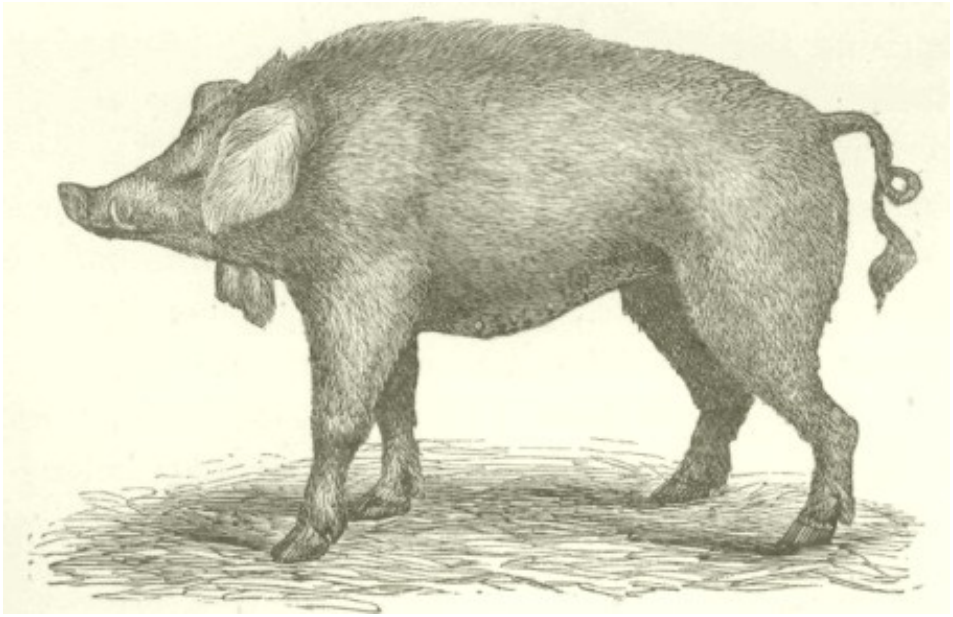
Predict not tolerated	Position	Seq Rep	Predict tolerated
d c g w h n e s p r k q y t	3M	0.60	a f v i ML
w	4A	0.60	c f m y i h v l p r q t n k s e GDA
w m h f	5C	0.80	y i q r e l k d p n v g t a S C
w c m f	6R	0.80	y i h v p l d g n q e t a k S R
w d h q p n c e r g k s	7V	0.80	y t a m F l I V
w d h g	8I	0.80	n c r q p e y k s f m t A v L I
	9N	0.80	w c h p f y Mi q r v g e d t a k s l N
c w m f i d	10R	0.80	p v y g s l t n a e q Hk R
c w f m y i v d h p g l	11R	0.80	n s t a e k Q R
c w f m y i v d h p g l	12R	0.80	n s t a e k Q R
y w v t s r q p n m l k i g f e d c a	13H	0.80	H

Figure 7 | Output prediction for all substitutions for a single protein. Shown here are amino acid positions 3–13 of a protein. Capital letters indicate amino acids appearing in the protein sequence alignment used for prediction whereas lower case letters indicate amino acids not observed in the protein alignment. The numbers in the 'Seq Rep' column represent the fraction of sequences in the alignment that have an amino acid at the corresponding position.

pos	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
1M 0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2E 0.25	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3M 0.50	0.07	0.02	0.02	0.03	0.12	0.02	0.02	0.24	0.03	1.00	0.63	0.02	0.03	0.03	0.03	0.03	0.05	0.17	0.02	0.04
4A 0.50	1.00	0.05	0.13	0.17	0.04	0.68	0.04	0.05	0.16	0.09	0.04	0.12	0.14	0.10	0.10	0.30	0.15	0.11	0.01	0.05
5C 0.75	0.59	1.00	0.17	0.15	0.09	0.33	0.08	0.11	0.17	0.15	0.06	0.19	0.18	0.12	0.12	0.93	0.44	0.21	0.03	0.11
6R 0.75	0.37	0.04	0.24	0.36	0.06	0.23	0.11	0.10	0.58	0.17	0.06	0.26	0.15	0.29	1.00	0.63	0.33	0.15	0.02	0.09
7V 0.75	0.10	0.03	0.02	0.03	0.22	0.04	0.03	0.99	0.04	0.48	0.10	0.03	0.03	0.03	0.04	0.05	0.09	1.00	0.02	0.09
8I 0.75	0.44	0.07	0.07	0.13	0.21	0.07	0.07	0.86	0.13	1.00	0.21	0.08	0.09	0.10	0.10	0.13	0.24	0.85	0.04	0.12
9N 0.75	0.90	0.15	0.73	0.85	0.41	0.60	0.37	0.54	0.94	1.00	0.36	0.95	0.36	0.59	0.66	0.93	0.84	0.64	0.10	0.46
10R 0.75	0.14	0.02	0.08	0.16	0.07	0.10	0.38	0.06	0.46	0.12	0.04	0.13	0.08	0.19	1.00	0.13	0.13	0.09	0.02	0.11
11R 0.75	0.10	0.01	0.06	0.13	0.02	0.06	0.05	0.03	0.33	0.07	0.02	0.08	0.05	0.46	1.00	0.09	0.08	0.05	0.01	0.03
12R 0.75	0.10	0.01	0.06	0.13	0.02	0.06	0.05	0.03	0.33	0.07	0.02	0.08	0.05	0.46	1.00	0.09	0.08	0.05	0.01	0.03
13H 0.75	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Figure 8 | Output scaled probability matrix for a single protein. Shown here are the results for amino acid positions 3–13 of a protein. The threshold score for intolerance of a substitution is 0.05. Rows correspond to positions in the reference protein and columns correspond to all possible 20 amino acids. The header column on the left contains the position, reference amino acid and the fraction of sequences in the alignment that have an amino acid at the corresponding position. Each element of the matrix represents the scaled probability of substitution at each position. Substitutions predicted to be intolerant are highlighted in red.

Transition to modern pig breeds



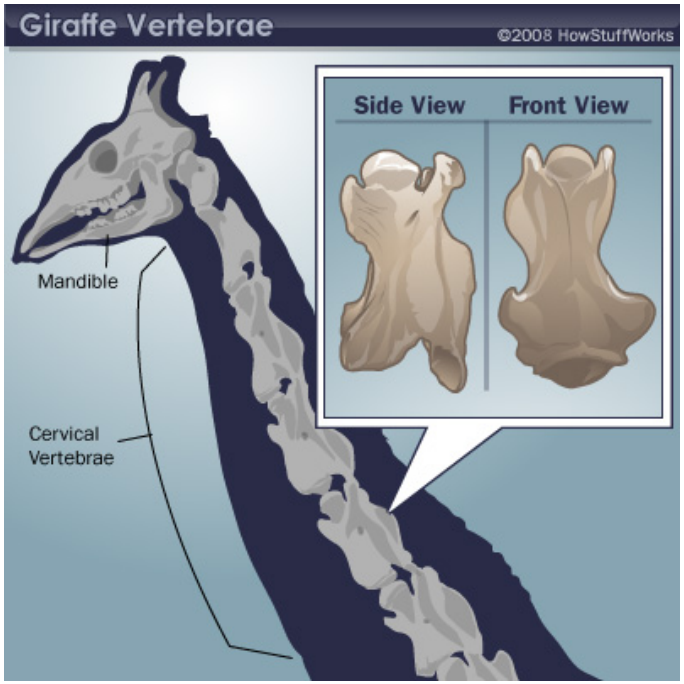
- Asian ‘genes’ have been important in shaping modern pig breeds.
- But evidently selection has been the major driver – selection on Asian (e.g. IGF2), and European variation?

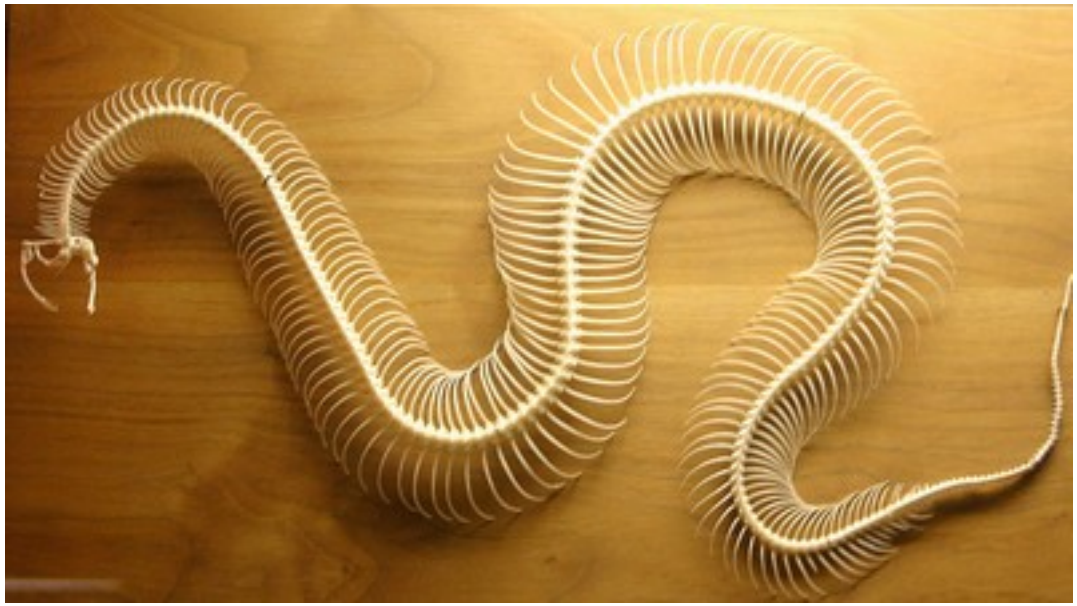
Pigs got longer in the 19th Century

	English Long-legged Male.	African Female.	Chinese Male.	Wild Boar, from Cuvier.	French Domestic Boar, from Cuvier.
Dorsal vertebræ ..	15	13	15	14	14
Lumbar	6	6	4	5	5
Dorsal and lumbar together	21	19	19	19	19
Sacral	5	5	4	4	4
Total number of vertebræ	26	24	23	23	23

Darwin, C. R. 1868. *The variation of animals and plants under domestication*. London: John Murray. First edition, first issue. Volume 1.

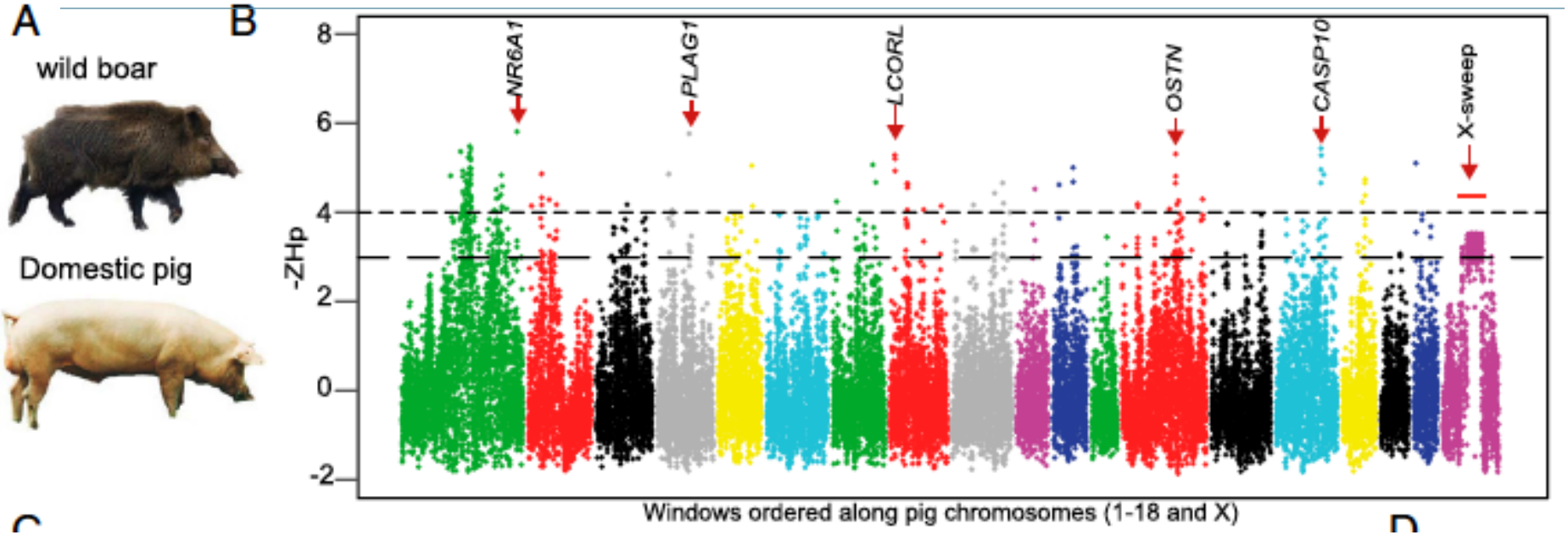
Number of vertebrae are usually very conserved in mammals



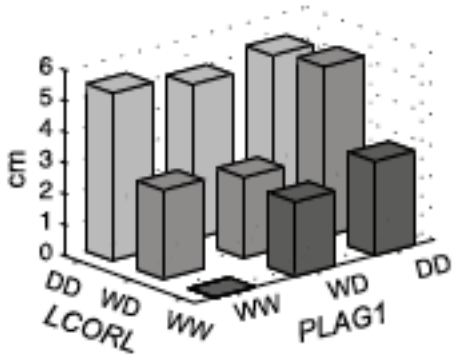


Giraffe has 7 neck vertebrae, just like us. Snakes on the other hand vary widely in number of vertebrae

Three of the major signatures of selection in modern pigs are related to body length



- NR6A1
- PLAG1
- LCORL



Rubin, Megens, et al., 2012, PNAS 109:19529

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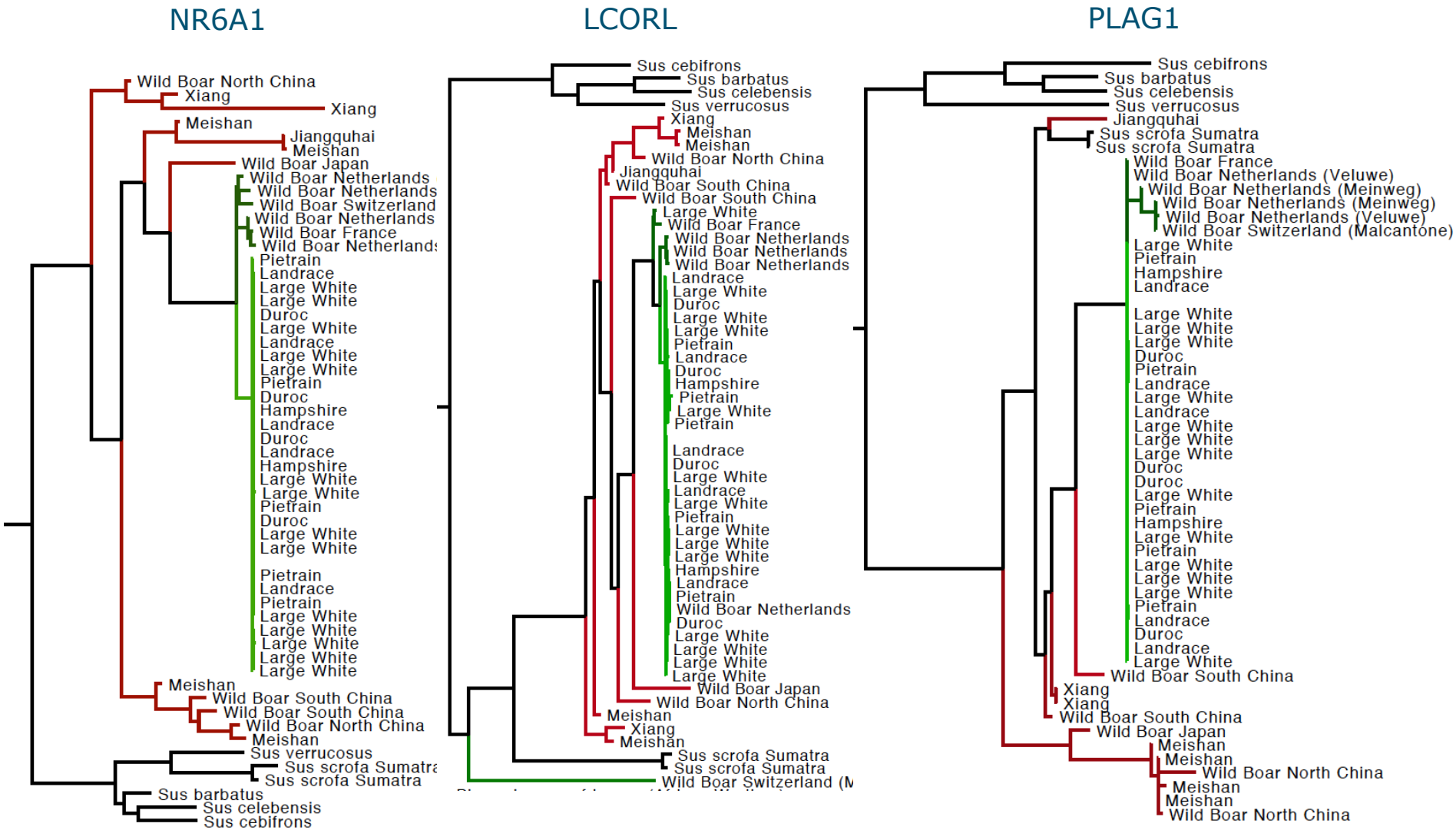
The three ‘elongation genes’ are not introduced from Asia



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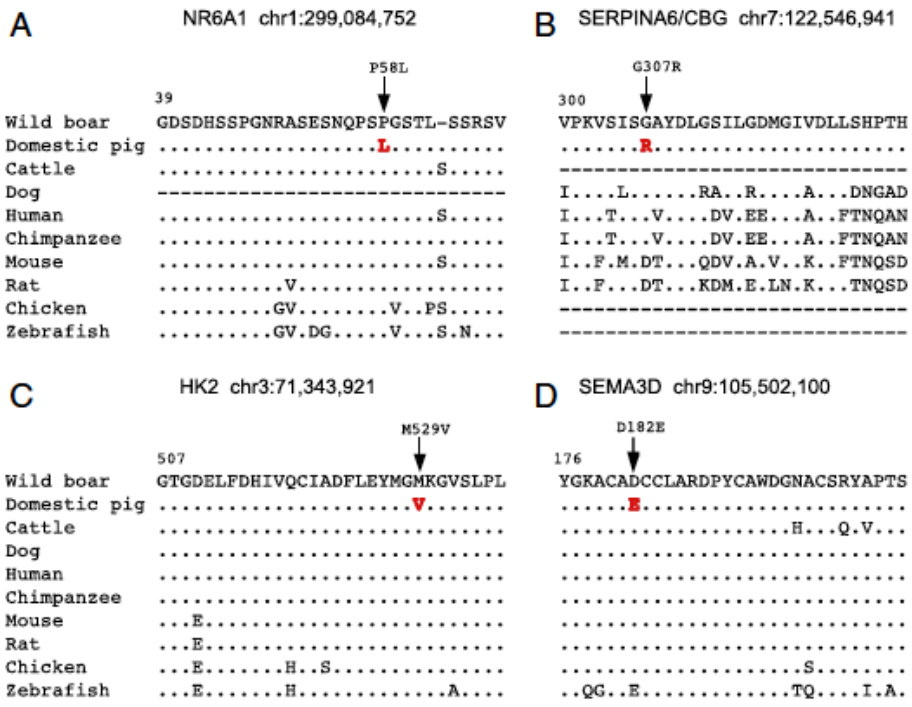
Rubin, Megens, et al., 2012, PNAS 109:19529

Derived nucleotide substitutions showing marked allele frequency differences between wild boar and domestic pigs are enriched in QTn

Substitution	Population	
	Domestic pig	Wild boar
Nonsynonymous	72	6
Synonymous	87	37

Values indicate the number of derived substitutions in which the frequency of the ancestral allele is >0.80 in the indicated population and <0.20 in the other.



Rubin, Megens, et al., 2012, PNAS 109:19529

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Comparative Genomics & Genome Databases

Hendrik-Jan Megens

WAGENINGEN UNIVERSITY
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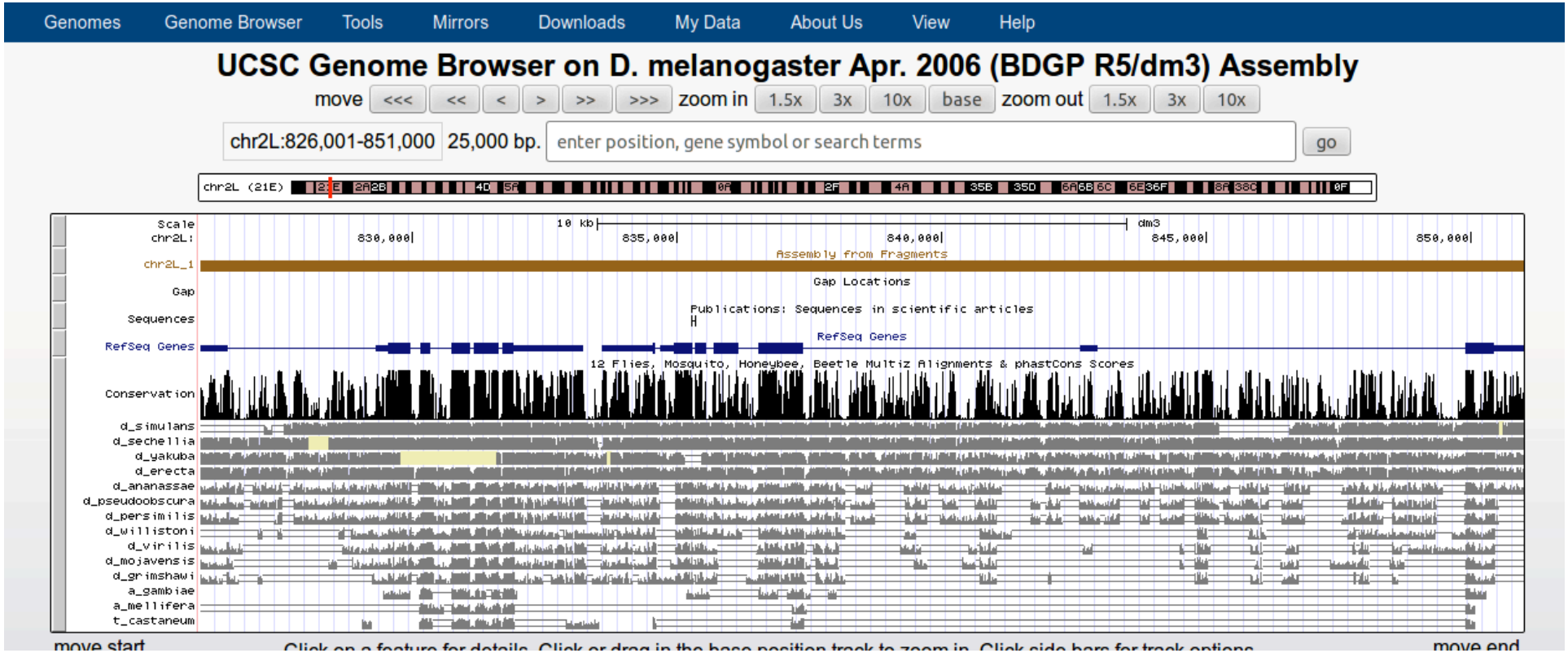
Animal Breeding & Genomics Centre
Wageningen University

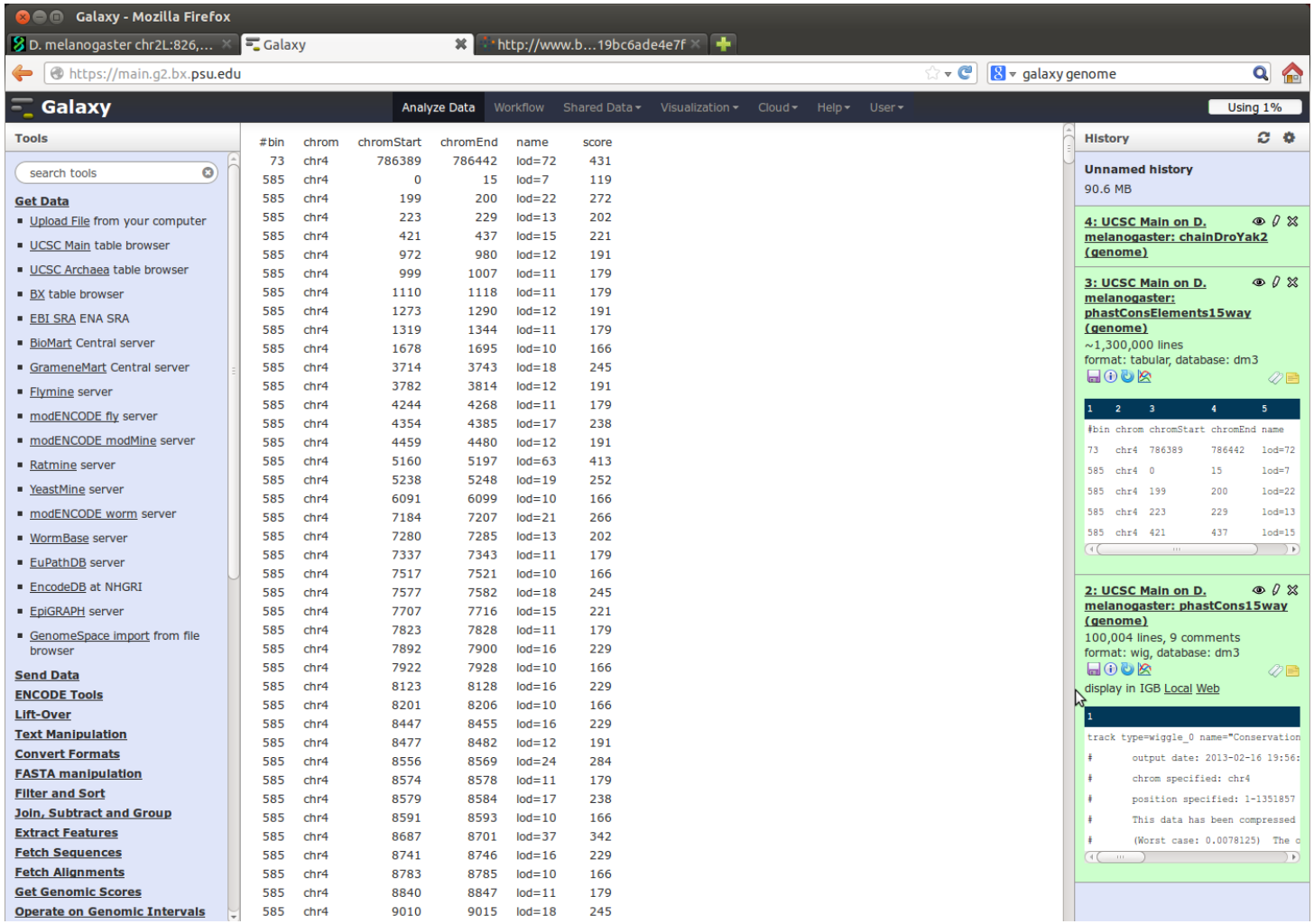
Comparative genomics ‘big bang’

- Reference assemblies are currently drafted for many species
- Various –omics data being generated for model, and non model species:
  - Expression/RNAseq
  - Epigenetics/methylomes
  - DNA-protein interactions (‘Encode-type data’)
  - Variation
- Genome browsers increasingly geared towards comparative tools
  - But buckling under the flood of new species data

Comparing Genomes: UCSC



UCSC and Galaxy



UCSC API

- No officially supported API exists
- Third-party API in Ruby

Mishima *et al. BMC Bioinformatics* 2012, **13**:240
http://www.biomedcentral.com/1471-2105/13/240

BMC Bioinformatics

SOFTWARE Open Access

The Ruby UCSC API: accessing the UCSC genome database using Ruby

Hiroyuki Mishima[1*], Jan Aerts[2,3], Toshiaki Katayama[4], Raoul J P Bonnal[5] and Koh-ichiro Yoshiura[1]

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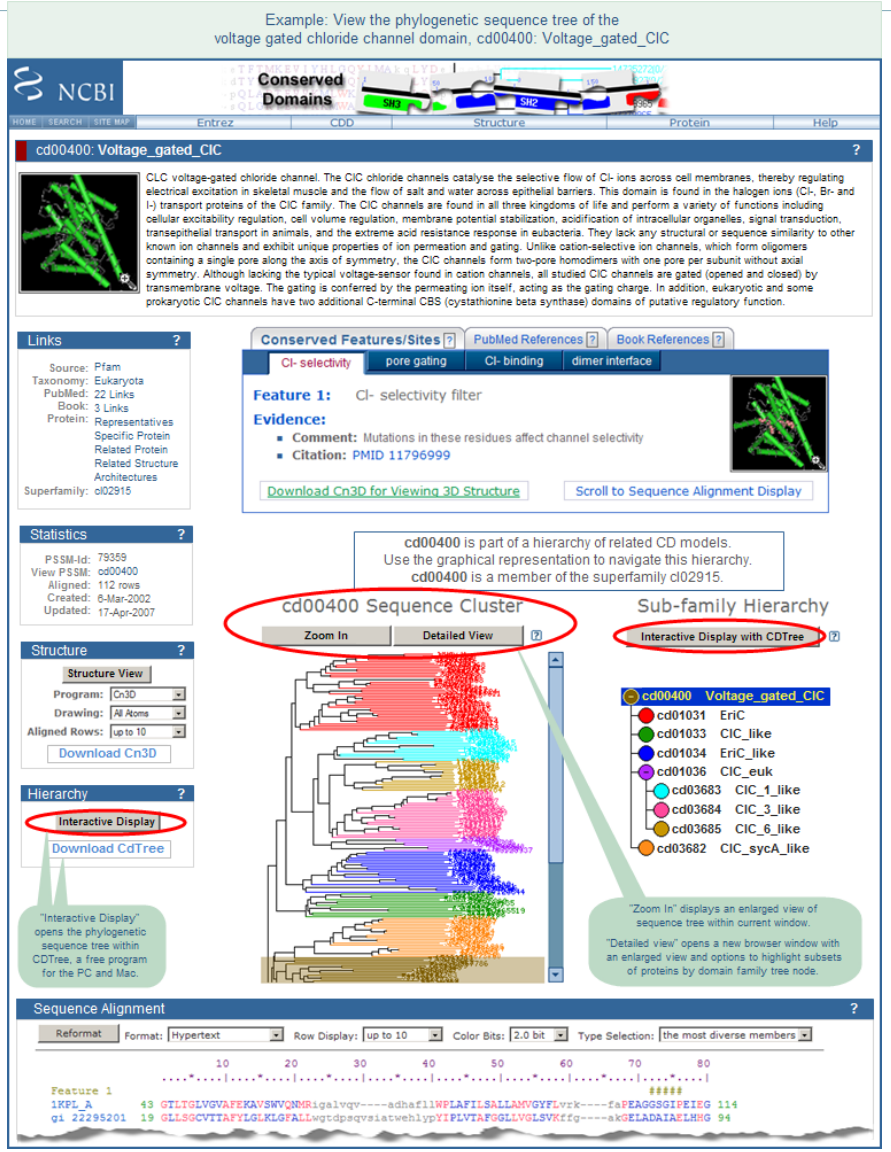
NCBI: Conserved Domains database



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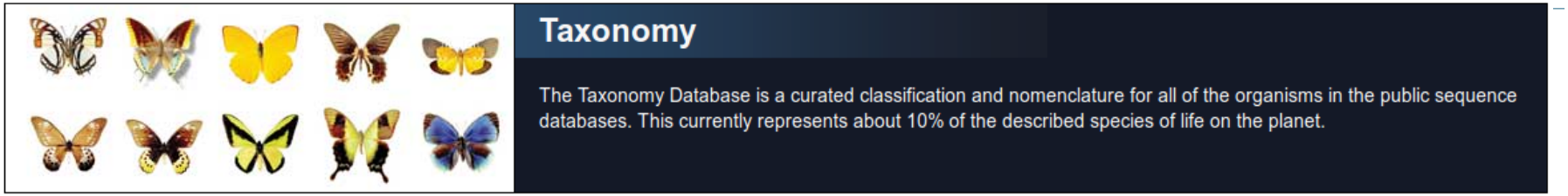
NCBI Taxonomy: one of the largest databases with organism names



NCBI Taxonomy Browser

Entrez | PubMed | Nucleotide | Protein | Genome | Structure | PMC | Taxonomy | Books

Search for [ ] as complete name ☑ lock Go Clear

Display 3 levels using filter: none

Sus scrofa

Taxonomy ID: 9823
Genbank common name: **pig**
Inherited blast name: **even-toed ungulates**
Rank: species
Genetic code: Translation table 1 (Standard)
Mitochondrial genetic code: Translation table 2 (Vertebrate Mitochondrial)
Other names:
common name: **wild boar**
common name: **swine**
common name: **pigs**
authority: **Sus scrofa Linnaeus, 1758**

Lineage(full)
cellular organisms; Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Bilateria; Deuterostomia; Chordata; Craniata; Vertebrata; Gnathostomata; Teleostomi; Euteleostomi; Sarcopterygii; Tetrapoda; Amniota; Mammalia; Theria; Eutheria; Laurasiatheria; Cetartiodactyla; Suina; Suidae; Sus

Entrez records

Database name	Subtree links	Direct links
Nucleotide	509,509	508,796
Nucleotide EST	1,669,420	1,669,337
Nucleotide GSS	1,162,428	1,162,428
Protein	42,352	41,853
Structure	606	602
Genome	1	1
Popset	384	376
SNP	566,003	566,003
GEO Datasets	6,020	5,997
UniGene	50,106	50,106
UniSTS	13,987	13,987
PubMed Central	1,250	1,125
Gene	33,533	33,507
OMIA	221	-
SRA Experiments	1,467	1,438
Probe	21,422	21,422
Assembly	6	-
Bio Project	269	266
Bio Sample	1,936	1,905
Bio Systems	1,391	1,391
dbVar	88	88
GEO Profiles	11,692	11,692
Protein Clusters	13	13
Taxonomy	12	1

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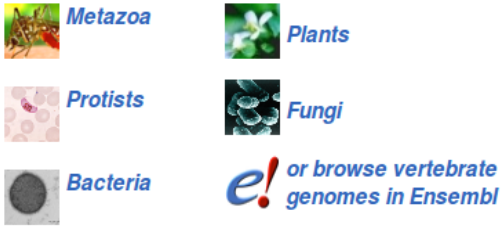
Ensembl: Main databases

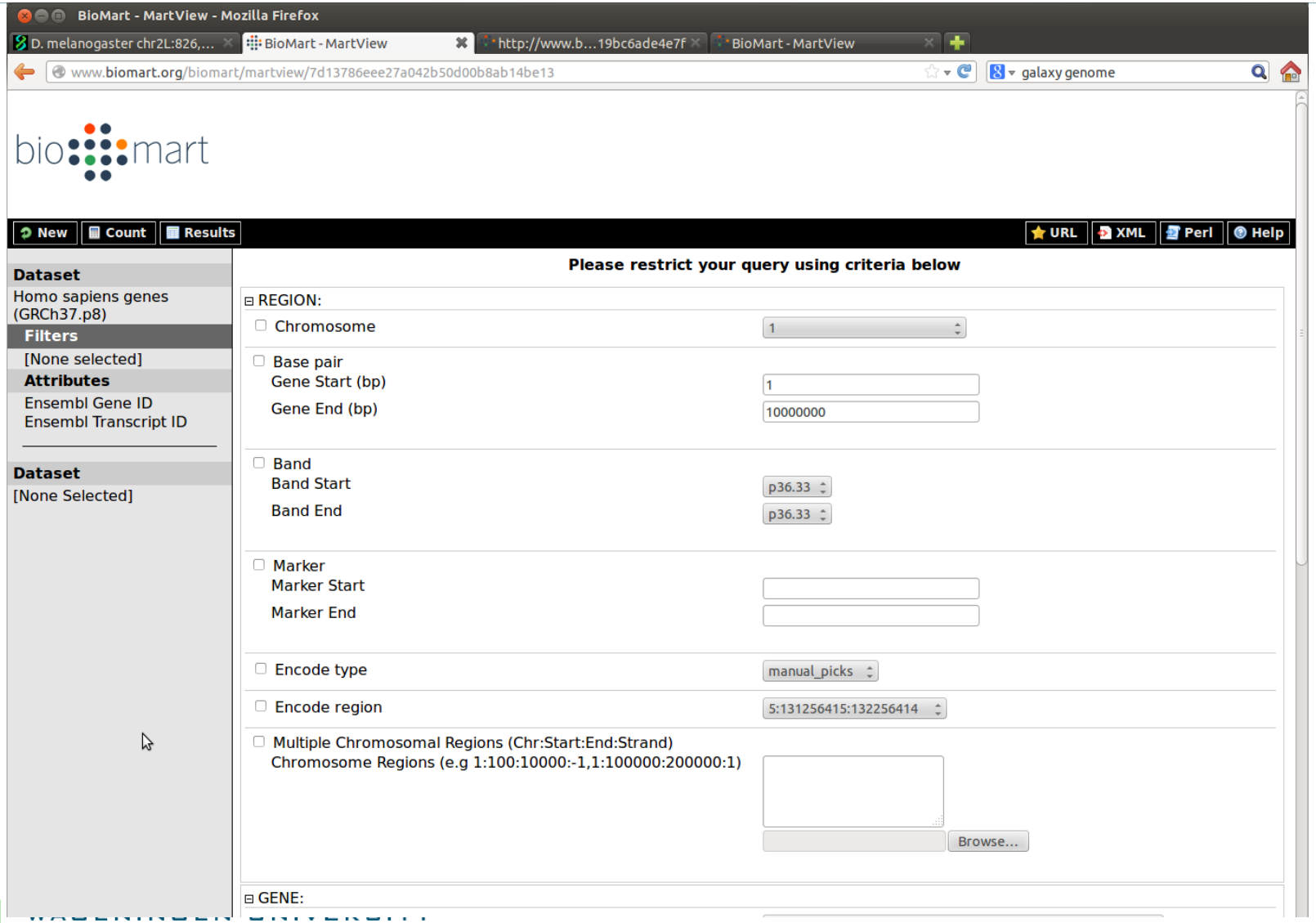


- 'Ensembl' – vertebrate-oriented
  - Human, Mouse, Pig, Chicken, Anole lizard, Zebrafish
- Ensembl genomes
  - Metazoans
    - Invertebrates: insects, other arthropods, nematodes, sea urchin, flatworm, etc.
  - Plants
    - Arabidopsis, Corn, Rice, Potato, Tomato, etc.
  - Fungi
    - Saccharomyces, Aspergillus,
  - Protists
    - Plasmodium, Leishmania, Toxoplasma, Giardia, etc.
  - Bacteria
    - 6000 bacterial genomes available

Ensembl

- Data internally stored and queried through MySQL databases
- APIs exist to connect to these dbs directly (Perl-API)
- You can download the db, and install locally
- Much of the annotation data (gtf, bed) and sequence data (fasta) can be downloaded
- Has alternative implementation: BioMart
- Various ways of retrieving data programmatically (Perl API, REST, BioMart API for Perl, R)

Biomart Web Portal



Biomart Perl API

```
# An example script demonstrating the use of BioMart API.
# This perl API representation is only available for configuration versions >=  0.5
use strict;
use BioMart::Initializer;
use BioMart::Query;
use BioMart::QueryRunner;

my $confFile = "PATH TO YOUR REGISTRY FILE UNDER biomart-perl/conf/. For Biomart Central Registry navigate to
                                                http://www.biomart.org/biomart/martservice?type=registry";
#
# NB: change action to 'clean' if you wish to start a fresh configuration
# and to 'cached' if you want to skip configuration step on subsequent runs from the same registry
#

my $action='cached';
my $initializer = BioMart::Initializer->new('registryFile'=>$confFile, 'action'=>$action);
my $registry = $initializer->getRegistry;

my $query = BioMart::Query->new('registry'=>$registry,'virtualSchemaName'=>'default');


        $query->setDataset("hsapiens_gene_ensembl");
        $query->addFilter("hgnc_symbol", ["igf1"]);
        $query->addAttribute("ensembl_gene_id");
        $query->addAttribute("ensembl_transcript_id");

$query->formatter("HTML");

my $query_runner = BioMart::QueryRunner->new();
############################## GET COUNT ############################
# $query->count(1);
# $query_runner->execute($query);
# print $query_runner->getCount();
####################################################################


############################## GET RESULTS ##########################
# to obtain unique rows only
# $query_runner->uniqueRowsOnly(1);

$query_runner->execute($query);
$query_runner->printHeader();
$query_runner->printResults();
$query_runner->printFooter();
####################################################################
```

Ensembl APIs

- Biomart
  - R
  - Perl
  - Java
  - Python
- Perl API
  - Main
  - Compara
  - Variation
  - Functional Genomics
- REST
  - Independent of programming language

Comparing Genomes: Ensembl

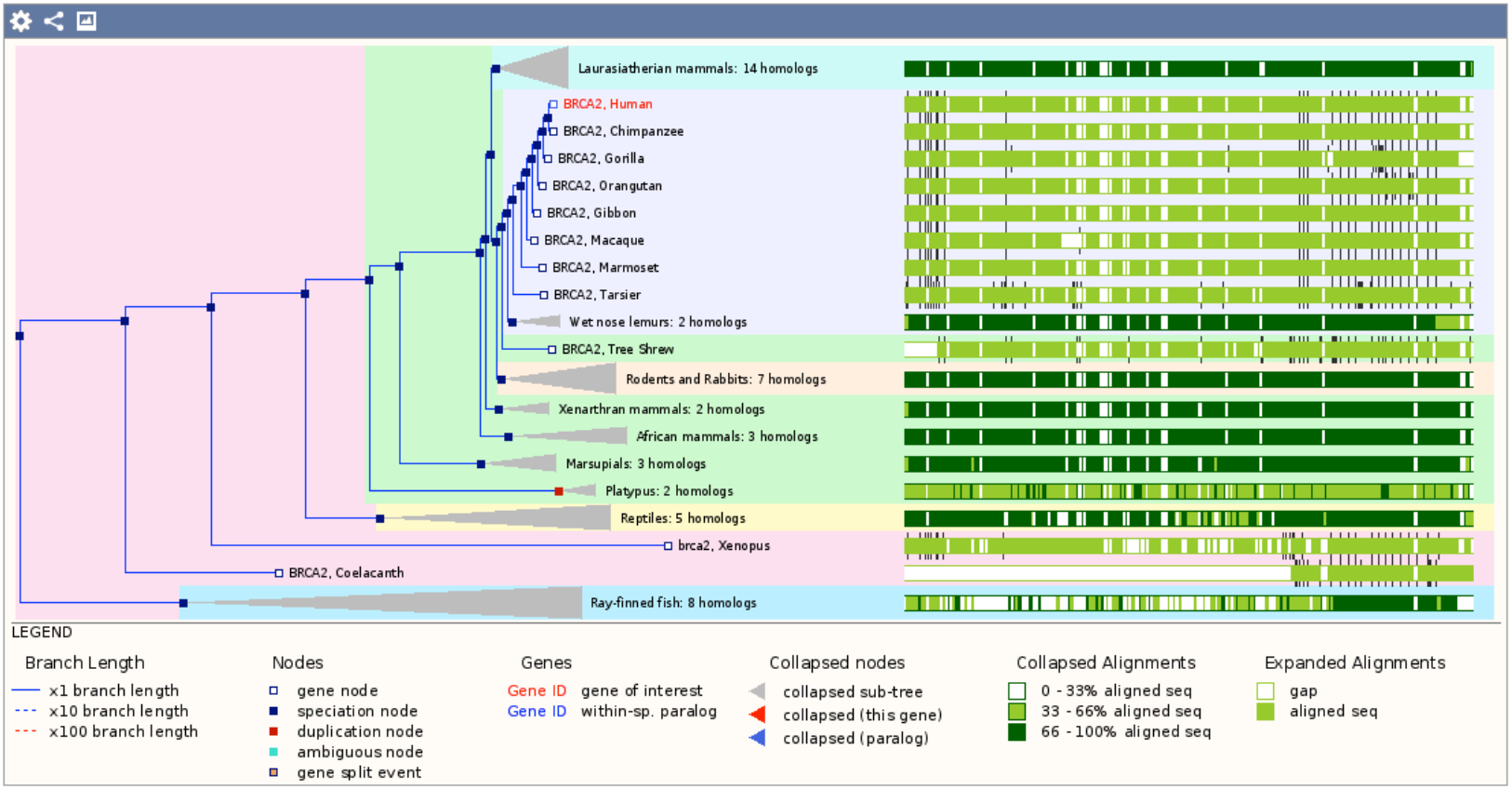
- Gene trees
  - are constructed using a representative protein for every gene in Ensembl: proteins are clustered using hcluster_sg based on WU-BLAST scores, and each cluster of proteins is aligned using M-Coffee. Finally, TreeBeST is used to produce a gene tree from each multiple alignment, reconciling it with the species tree to call duplication events. Homologues are deduced from these trees. We also determine gene gain and loss events using the CAFE software.
- ncRNA trees
- Families
  - are constructed by MCL clustering of all Ensembl proteins (potentially several per gene). Metazoan proteins from UniProtKB SwissProt and SPTREMBL are added to extend the protein set.
- Whole genome alignments
  - are performed either pairwise between two species using BlastZ-net or translated Blat analysis, or using multiple species.
- Ancestral sequences
  - are calculated from multi-species whole genome alignments.
- Conservation scores and constrained elements
  - are calculated from the whole genome multiple alignments.
- Syntenies
  - are calculated from the pairwise alignments.
- Stable IDs
  - are provided for Families and Gene Trees and relate exclusively to the content of the Family or the Gene Tree.

Ensembl: Gene Trees

Gene tree (image)

GeneTree ENSGT00390000003602

Number of genes	57
Number of speciation nodes	53
Number of duplication	2
Number of ambiguous	1
Number of gene split events	0



biomaRt – R

```
Load library and create mart object
> library(biomaRt)
> ensembl_hs = useMart("ensembl", dataset= "hsapiens_gene_ensembl")

Retrieve the Ensembl and Entrezgene IDs based on the Hugo name:
FOXP2
> getBM(attributes= c("ensembl_gene_id", "ensembl_gene_id"),
filters= "hgnc_symbol", values=c('FOXP2'), mart=ensembl_hs)

Retrieve the protein sequence:
> seq = getSequence(id= 'ENSG00000128573', type="ensembl_gene_id",
seqType='peptide', mart=ensembl_hs)
> seq[1,]

Retrieve the FOXP2 orthologue from chimpanzee:
> getBM(attributes= c("ptroglodytes_homolog_ensembl_gene"), filters=
"ensembl_gene_id", values=c('ENSG00000128573'), mart=ensembl_hs)
```

Ensembl Compara API

e!Ensembl BLAST/BLAT | BioMart | Tools | Downloads | Help & Documentation | Blog | Mirrors

Login · Register

Search all species…

Help & Documentation > API Documentation

Perl API Documentation

Ensembl uses MySQL relational databases to store its information. A comprehensive set of Application Programme Interfaces (APIs) serve as a middle-layer between underlying database schemes and more specific application programmes. The APIs aim to encapsulate the database layout by providing efficient high-level access to data tables and isolate applications from data layout changes.

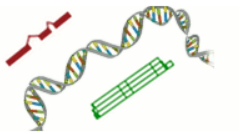
POWERED BY PERL

Ensembl's API is written in Perl:

- installation instructions
- full documentation of all modules

Core

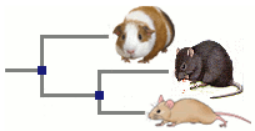
Sequence, genes and other automated annotation



- Perl API
- Database schema
- Tutorial

Comparative genomics

Homologues, paralogues and protein families



- Perl API
- Database schema
- Tutorial

Variation

SNPs, somatic mutations and structural variants

- Perl API
- Database schema
- Tutorial

Regulation

Regulatory features, motifs and oligoprobes

- Perl API
- Database schema
- Tutorial

Ensembl Registry

The Registry system allows to tell your programs where to find the EnsEMBL databases and how to connect to them. It has been implemented for the Ensembl Core and Compara APIs.

More about the Registry...

Ensembl Software Support

Ensembl is an open project and we would like to encourage correspondence and discussions on any subject on any aspect of Ensembl. Please see the Ensembl Contacts page for suitable options for getting in touch with us.

If you are interested in undertaking a short-term collaborative project, our "Geek for a Week" scheme allows developers and researchers to work alongside Ensembl team members.

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Permanent link

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Perl API

```
use Bio::EnsEMBL::Registry;
Bio::EnsEMBL::Registry->load_registry_from_db(
        -host => 'ensembldb.ensembl.org',
        -user => 'anonymous',
        -port => 5306);
```

Create adaptor objects

```
my $slice_adaptor =
        Bio::EnsEMBL::Registry->get_adaptor($ref_organism,'core','Slice');

my $gene_adaptor =
        Bio::EnsEMBL::Registry->get_adaptor($ref_organism,'core','Gene');

my $member_adaptor =
        Bio::EnsEMBL::Registry->get_adaptor('Multi','compara','Member');

my $homology_adaptor =
        Bio::EnsEMBL::Registry->get_adaptor('Multi', 'compara', 'Homology')
```

Objects have methods

example of retrieving gene objects by gene symbol

```
my $gene_adaptor =
      Bio::EnsEMBL::Registry->get_adaptor($ref_organism,'core','Gene');


@genes = @{$gene_adaptor->fetch_all_by_external_name('IGF1')};
```

Gene objects have methods too

```
my $gene = $gene_adaptor -> fetch_by_stable_id($stable_id);


        my $start = $gene->start();

        my $end = $gene->end();

        my $chromosome = $gene->seq_region_name();

        my $strand = $gene->strand();
```

Example of retrieving sequence data by coordinates

```
my $slice_adaptor =
      Bio::EnsEMBL::Registry->get_adaptor($cspecies,'core','Slice');

my $slice =
$slice_adaptor->fetch_by_region('chromosome', $chromosome, ($start-1000),
($end+1000));

my $sequpstream = $slice ->get_repeatmasked_seq()->seq();
```

creating a member object

```
my $member =
    $member_adaptor -> fetch_by_source_stable_id("ENSEMBLGENE",$gene_id);
```

```
$hum_chrom = $member->chr_name;
        $hum_chrom_start = $member->chr_start;
        $hum_chrom_end = $member->chr_end;
        $descripton = $member->description;

        my $taxon = $member->taxon;
```

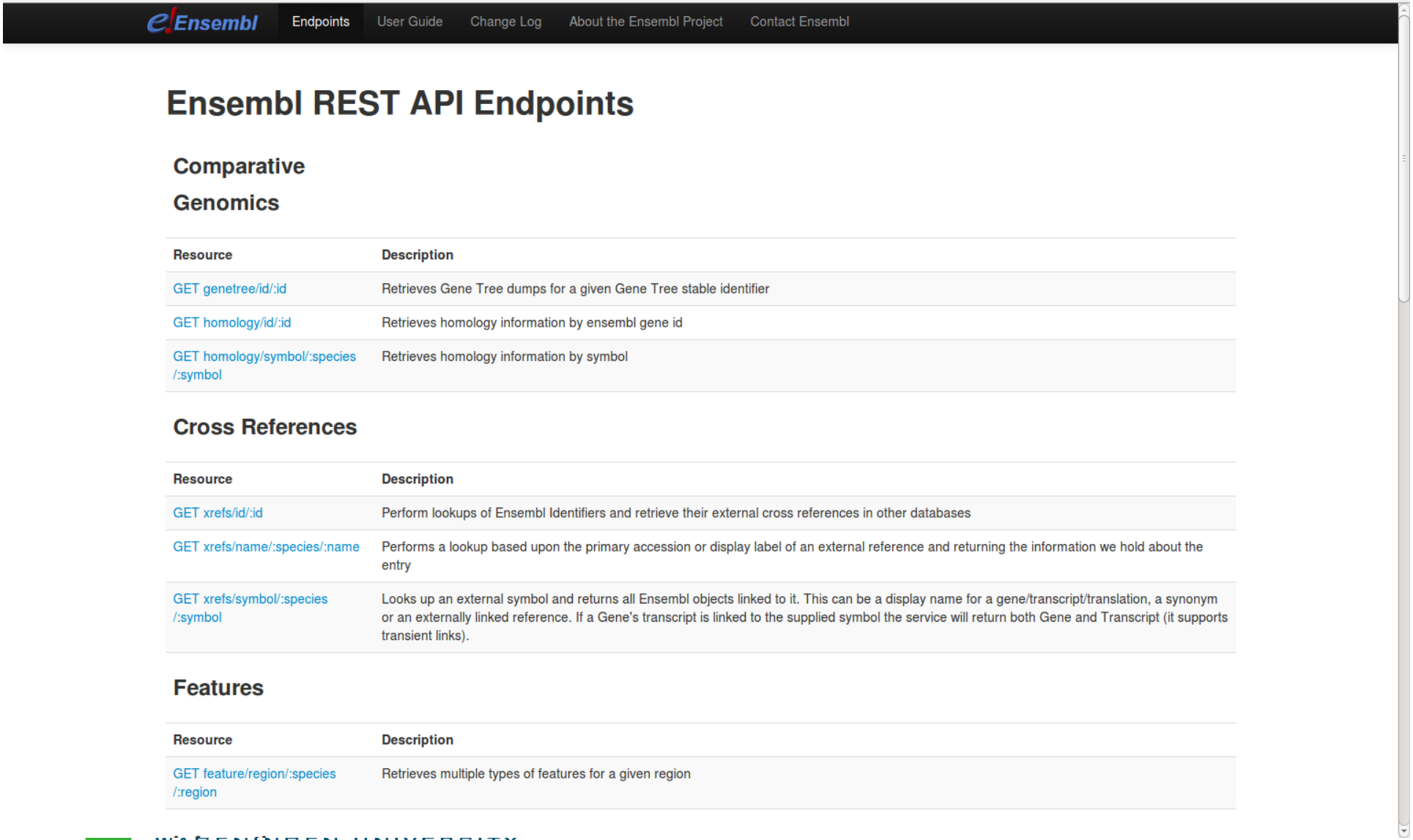
Member objects can be used to retrieve homologies

```
my $homologies = $homology_adaptor >fetch_all_by_Member($member);


foreach my $homology (@{$homologies}) {….

}
```

Ensembl – REST API



Example: retrieve the external references of an Ensembl Gene ID

Example Requests

/xrefs/id/ENSG00000157764?content-type=application/json

Example output | Perl | Python | Ruby | Curl | **Wget**

```
1.  wget -q --header='Content-type:application/json' 'http://beta.rest.ensembl.org/xrefs/id/ENSG00000157764?'  -O -
```

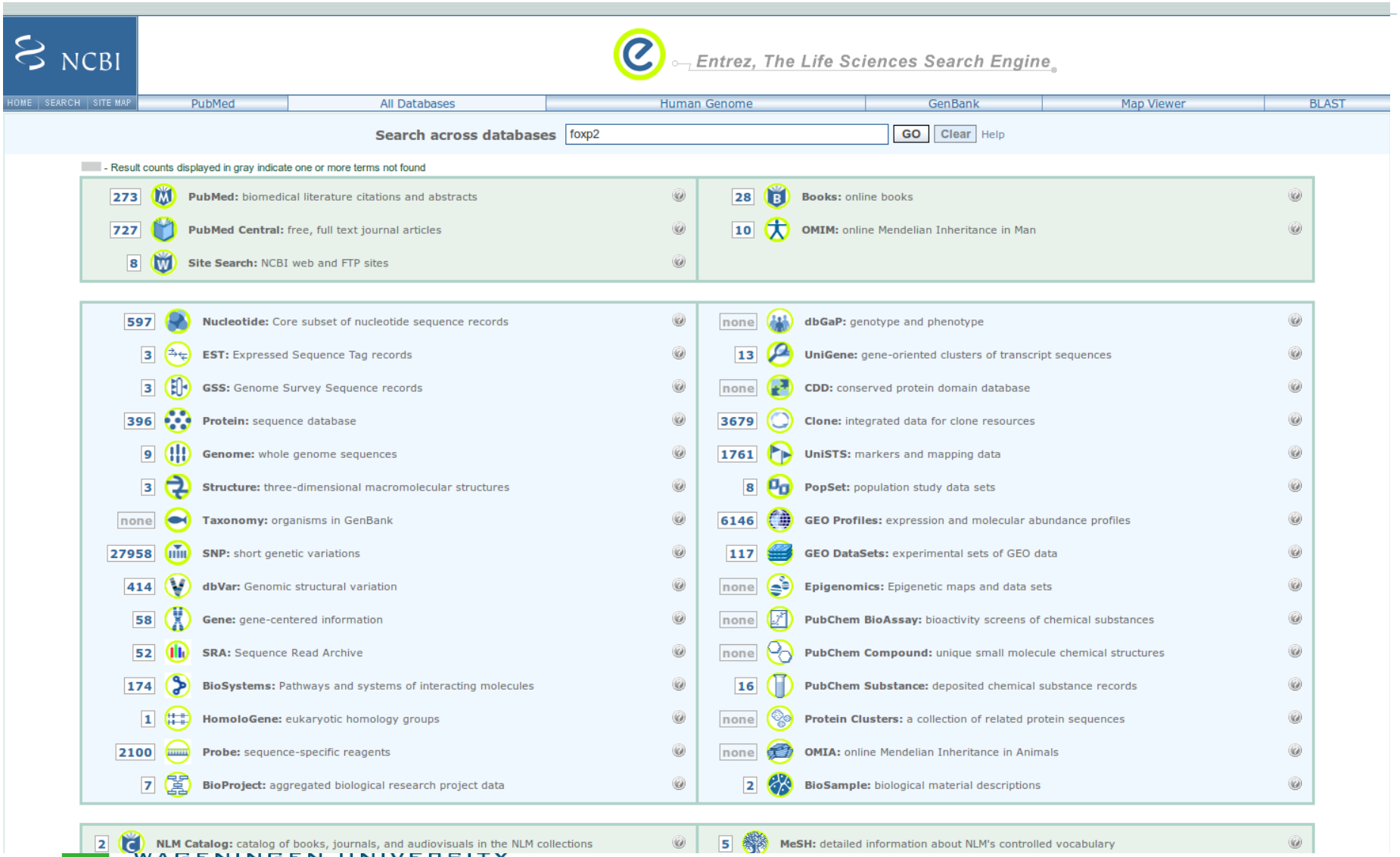

Example Requests

/xrefs/id/ENSG00000157764?content-type=application/json

Example output | Perl | Python | Ruby | Curl | Wget


```
[
   {
      "display_id" : "OTTHUMG00000157457",
      "primary_id" : "OTTHUMG00000157457",
      "version" : "1",
      "description" : null,
      "dbname" : "OTTG",
      "synonyms" : [],
      "info_type" : "NONE",
      "info_text" : "",
      "db_display_name" : "Havana gene"
   },
   {
      "display_id" : "ENSG00000157764",
      "primary_id" : "ENSG00000157764",
      "version" : "0",
      "description" : "",
```

NCBI - Genbank



NCBI

- Many databases
- Every ID (pubmed, gene, SNP, protein etc) is a number
- Implemented in SQL databases, data can be downloaded as flat text
- Relationship tables between databases (e.g.: genes annotated to pubmed articles)

NCBI – Homology databases

- **Conserved Domain Database (CDD)**
  - A collection of sequence alignments and profiles representing protein domains conserved in molecular evolution. It also includes alignments of the domains to known 3-dimensional protein structures in the MMDB database.
- **HomoloGene**
  - A gene homology tool that compares nucleotide sequences between pairs of organisms in order to identify putative orthologs. Curated orthologs are incorporated from a variety of sources via the Gene database.
- **Protein Clusters**
  - A collection of related protein sequences (clusters), consisting of Reference Sequence proteins encoded by complete prokaryotic and organelle plasmids and genomes. The database provides easy access to annotation information, publications, domains, structures, external links, and analysis tools.

Homologene provides conserved domain perspective

1: HomoloGene:41. Gene conserved in Euteleostomi

Genes

Genes identified as putative homologs of one another during the construction of HomoloGene.

BRCA2, *H.sapiens*
breast cancer 2, early onset

BRCA2, *P.troglodytes*
breast cancer 2, early onset

BRCA2, *M.mulatta*
breast cancer 2, early onset

BRCA2, *C.lupus*
breast cancer 2, early onset

BRCA2, *B.taurus*
breast cancer 2, early onset

Brca2, *M.musculus*
breast cancer 2

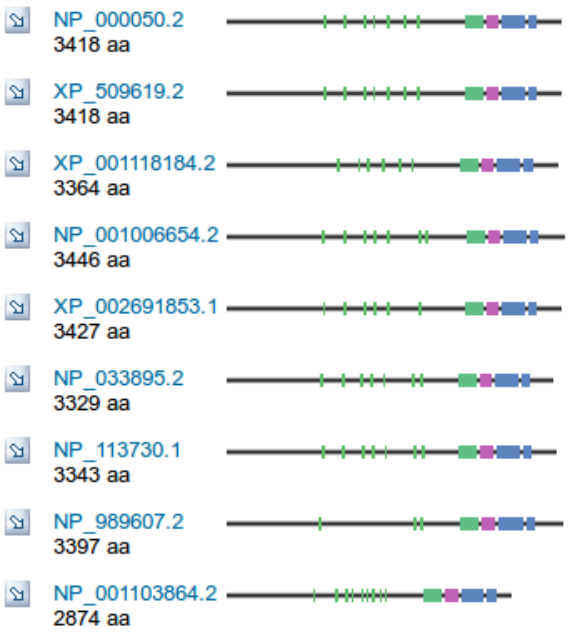
Brca2, *R.norvegicus*
breast cancer 2

BRCA2, *G.gallus*
breast cancer 2, early onset

brca2, *D.rerio*
breast cancer 2, early onset

Proteins

Proteins used in sequence comparisons and their conserved domain architectures.



NP_000050.2
3418 aa

XP_509619.2
3418 aa

XP_001118184.2
3364 aa

NP_001006654.2
3446 aa

XP_002691853.1
3427 aa

NP_033895.2
3329 aa

NP_113730.1
3343 aa

NP_989607.2
3397 aa

NP_001103864.2
2874 aa

Protein Alignments

Protein multiple alignment, pairwise similarity scores and evolutionary distances.

Show Multiple Alignment

Conserved Domains

Conserved Domains from CDD found in protein sequences by rpsblast searching.

BRCA-2_OB1 (pfam09103)
BRCA2, oligonucleotide/oligosaccharide-binding, domain

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NCBI- Eutils: esearch

wget "http://www.ncbi.nlm.nih.gov/entrez/eutils/**esearch.fcgi**?
db=pubmed&term=science[journal]+AND+breast+cancer+AND+2008[pdat]&retmax=100"
-O output.txt

Output:
<Id>19008416</Id>
<Id>18927361</Id>
<Id>18787170</Id>
<Id>18487186</Id>
<Id>18239126</Id>
<Id>18239125</Id>

NCBI Eutils: efetch

wget 'http://www.ncbi.nlm.nih.gov/entrez/eutils/**efetch.fcgi**?db=pubmed&id=19008416' -O pubmed19008416.txt

```
},
   cit {
     title {
       name "Genomic loss of microRNA-101 leads to overexpression of histone
methyltransferase EZH2 in cancer."
     },
……….
   },
   abstract "Enhancer of zeste homolog 2 (EZH2) is a mammalian histone
methyltransferase that contributes to the epigenetic silencing of target
genes and regulates the survival and metastasis of cancer cells. EZH2 is
overexpressed in aggressive solid tumors by mechanisms that remain unclear.
Here we show that the expression and function of EZH2 in cancer cell lines
are inhibited by microRNA-101 (miR-101). Analysis of human prostate tumors
revealed that miR-101 expression decreases during cancer progression,
paralleling an increase in EZH2 expression. One or both of the two genomic
loci encoding miR-101 were somatically lost in 37.5% of clinically localized
prostate cancer cells (6 of 16) and 66.7% of metastatic disease cells (22 of
33). We propose that the genomic loss of miR-101 in cancer leads to
overexpression of EZH2 and concomitant dysregulation of epigenetic pathways,
resulting in cancer progression.",
   mesh {
{
term
```

Eutils: easy to integrate in any programming language

hjm@ubuntu: ~/shared/UbuntuSharedStuff/AdvBioinf/EUtils

```perl
#!/usr/bin/perl -w

use strict;
use LWP::Simple;


#search term to find
my $search_term = 'breast cancer';

#maximum number of results to retrieve
my $retmax = 10;

my $utils = 'http://www.ncbi.nlm.nih.gov/entrez/eutils';
my $db_name = 'pubmed';

# Submit the search and retrieve the XML based results
my $esearch_result = get( $utils . '/esearch.fcgi?db=' . $db_name . '&retmax='.$retmax.'&term=' . $search_term );

# paper IDs
my @ids = ($esearch_result =~ m|.*<Id>(.*)</Id>.*|g);

#loop through all the ids
# get individual papers (if not, then abstacts)
foreach my $id (@ids) {

        #get all details for each paper - full text if available
        my $efetch = $utils . '/efetch.fcgi?db=' . $db_name . '&id=' . $id;

        #prints out to a xml file (file name generated from database name and current paper ID)
        open(OUTFILE, ">$db_name$id.xml");
        print OUTFILE get($efetch);
        close OUTFILE;
}
~
```

NCBI Eutils: esearch

"http://www.ncbi.nlm.nih.gov/entrez/eutils/esearch.fcgi?db=gene&term=FOXP2[sym]+AND+human[orgn]" -O -

NCBI Eutils: efetch

```
wget "http://www.ncbi.nlm.nih.gov/entrez/eutils/
efetch.fcgi?db=homologene&id=33482&rettype=FASTA" -
O -
```

NCBI Eutils: elink

```
wget "http://www.ncbi.nlm.nih.gov/entrez/eutils/
elink.fcgi?dbfrom=gene&db=homologens&id=93986" -O -
```