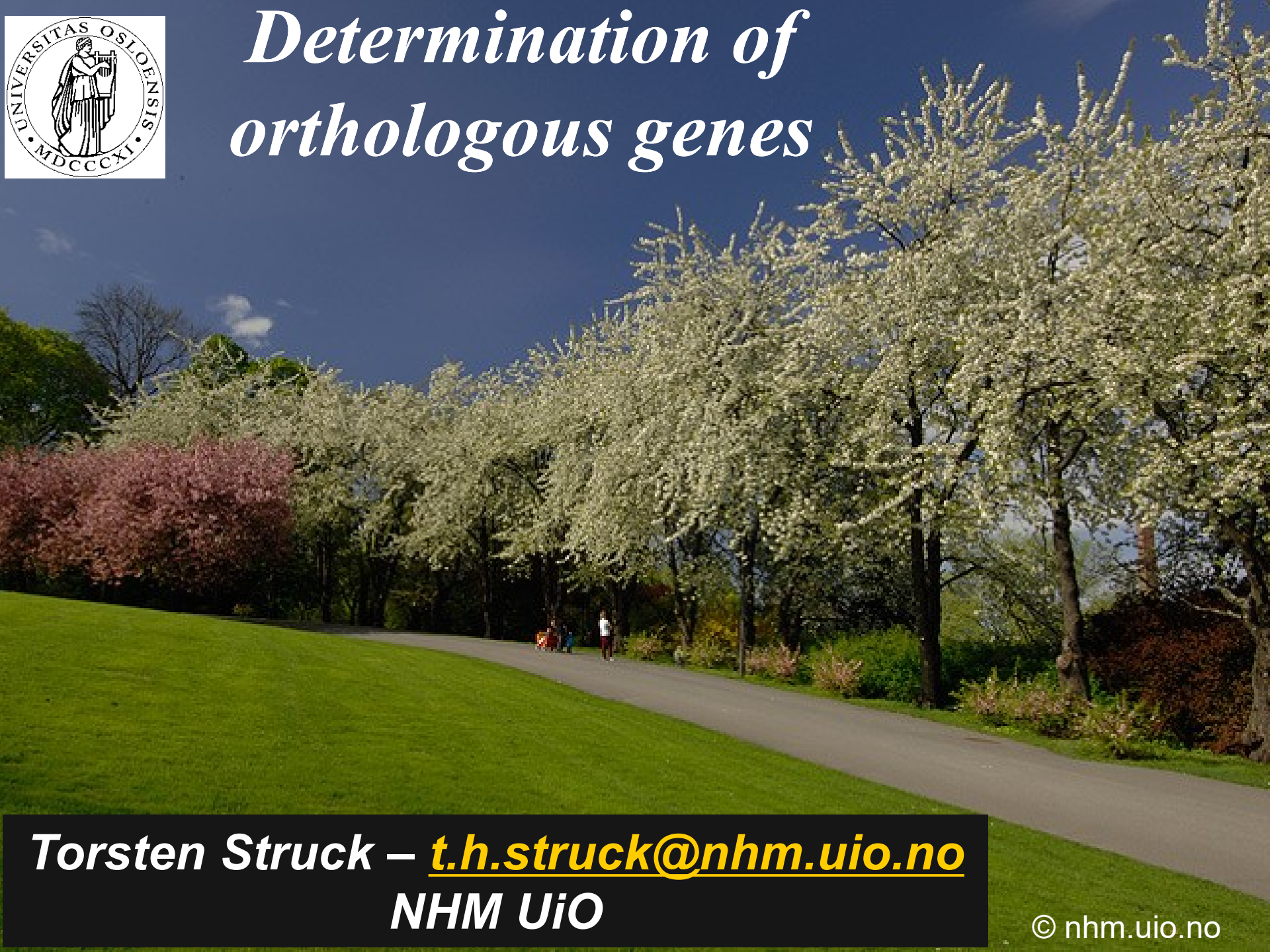

Determination of orthologous genes

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Functional Annotation – kind of

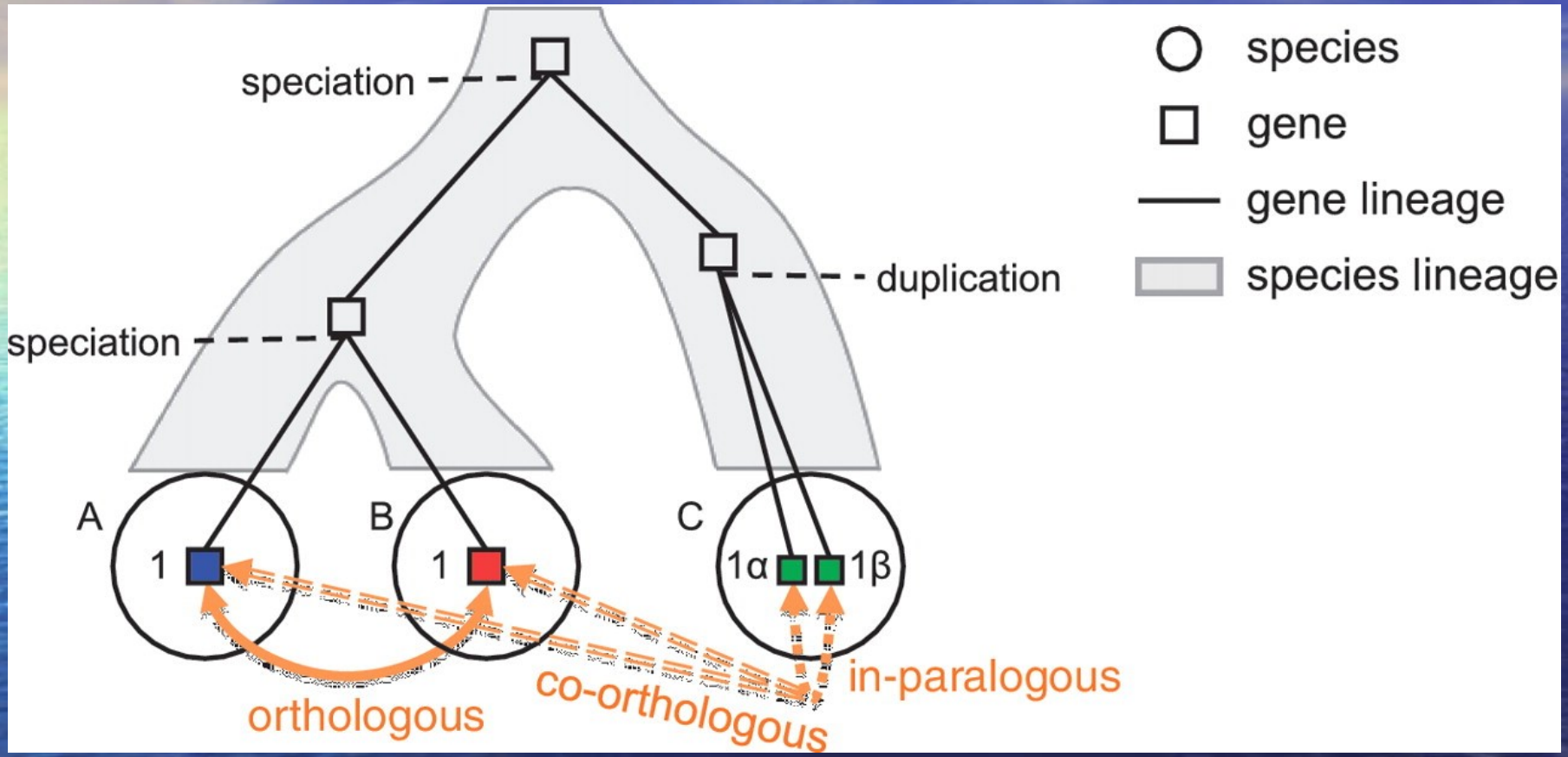
1.) Functional annotation with gene names

e.g. Trinotate → same gene name = orthology

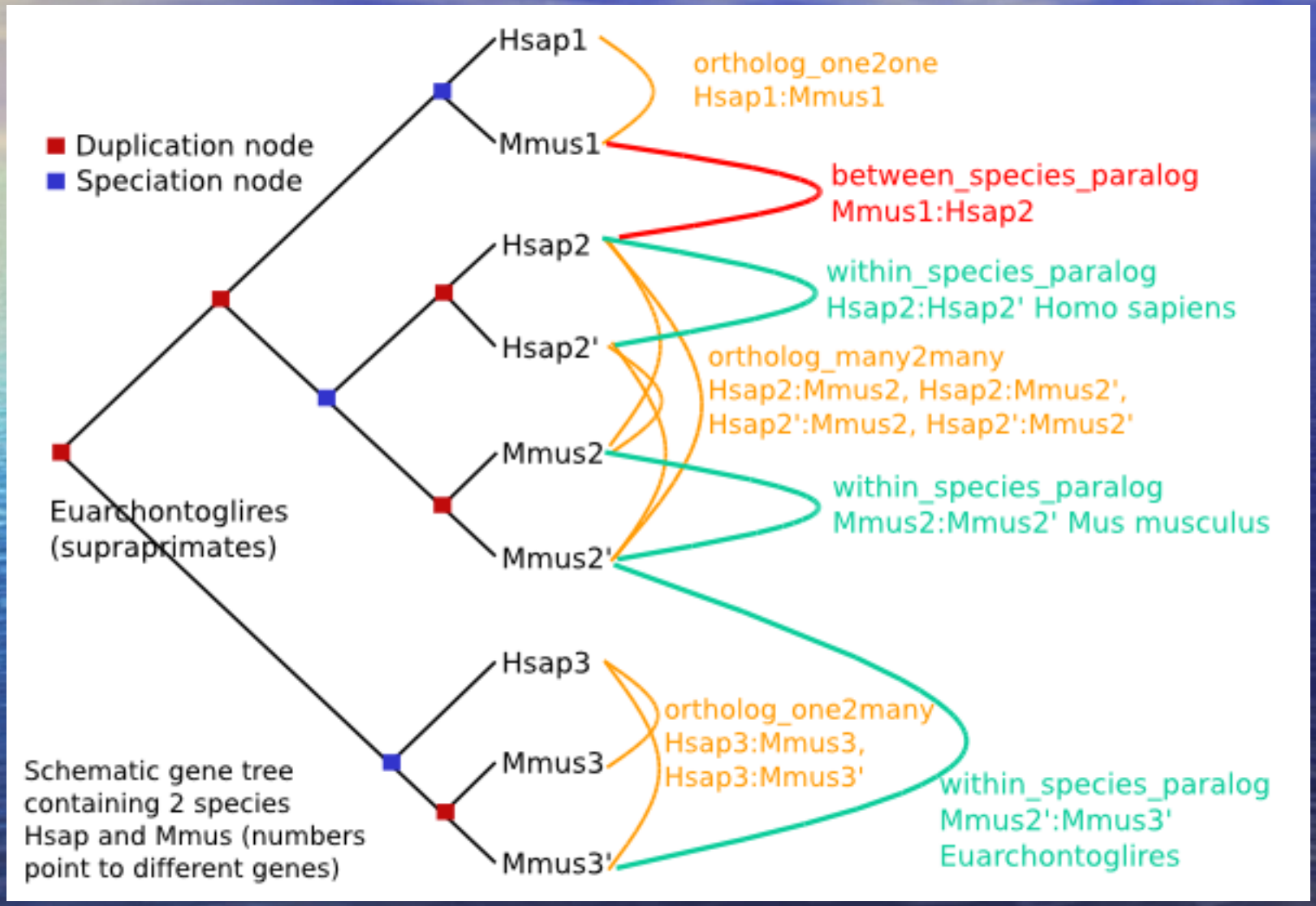
Problem:

gene name does not always imply orthology → paralogs

Orthology vs. Paralogy



Orthology vs. Paralogy



Functional Annotation – kind of

1.) Functional annotation with gene names

e.g. Trinotate → same gene name = orthology

Problem:

gene name does not always imply orthology → paralogs

→ sequence-based approach

Functional Annotation – kind of

1.) Functional annotation with gene names

e.g. Trinotate → same gene name = orthology

Problem:

gene name does not always imply orthology → paralogs

2.) All – vs. – All Blast

e.g. OrthoMCL → no prior knowledge necessary (from scratch)
→ slow & not expandable with new taxa

3.) Reciprocal All – vs. – All Blast using a core ortholog set

e.g. Orthograph → prior knowledge necessary (core set)
→ fast & easily expandable with new taxa

OrthoMCL

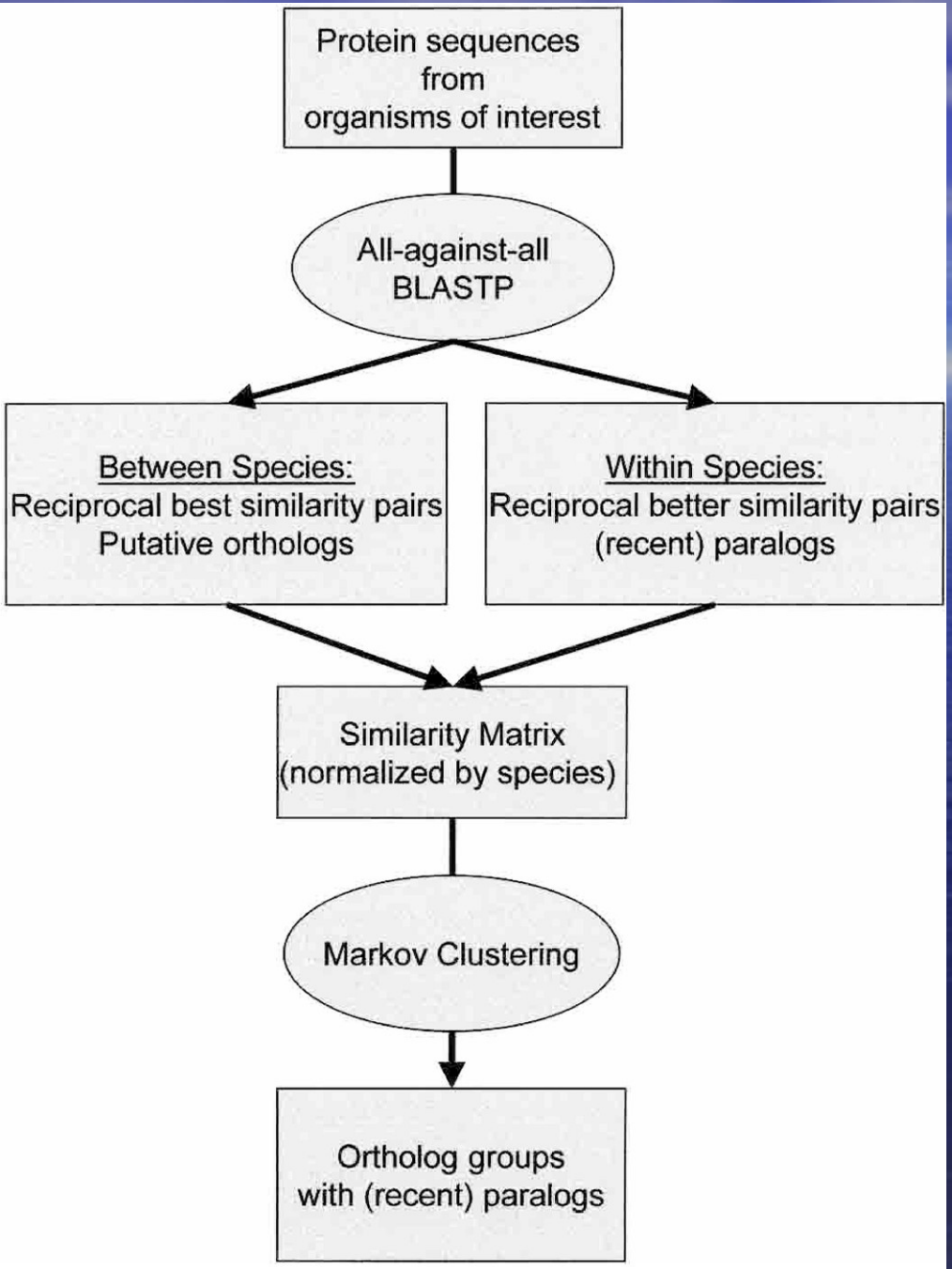


Figure 1 Flow chart of the OrthoMCL algorithm for clustering orthologous proteins.

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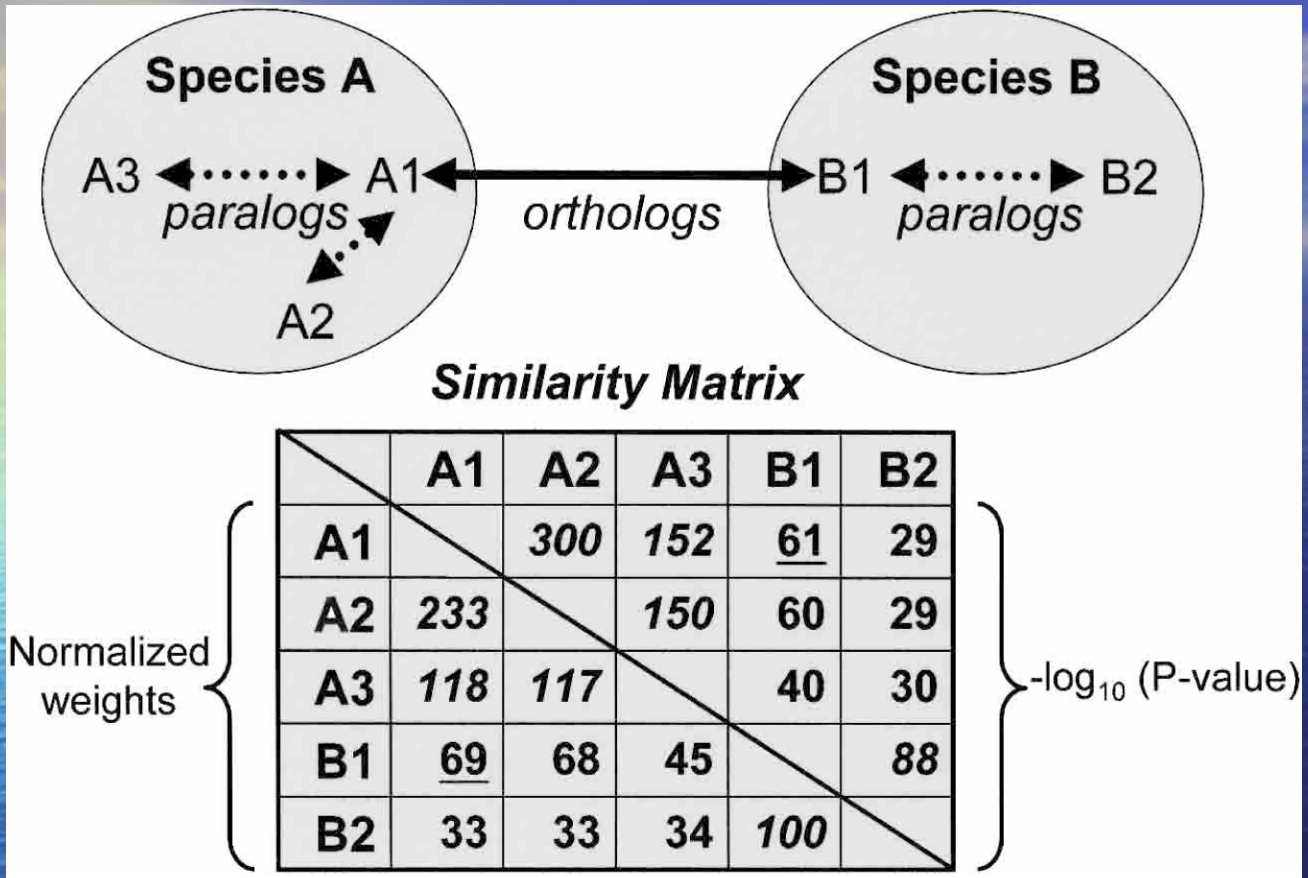
OrthoMCL – Similarity matrix



	A1	A2	A3	B1	B2
A1		*300*	*152*	61	29
A2	*233*		*150*	60	29
A3	*118*	*117*		40	30
B1	69	68	45		*88*
B2	33	33	34	*100*	

Figure 2 Illustration of sequence relationships and similarity matrix construction. Dotted arrows represent "recent" paralogy (duplication subsequent to speciation); solid arrows represent orthology. The *upper right* half of the matrix contains initial weights calculated as average $-\log_{10}$ (*P*-value) from pairwise WU-BLASTP similarities. The *lower left* half contains corrected weights supplied to the MCL algorithm; the edge weight connecting each pair of sequences w_{ij} is divided by W_{ij}/W, where W represents the average weight among all ortholog (underlined) and "recent" paralog (italicized) pairs, and W_{ij} represents the average edge weight among all ortholog pairs from species i and j. The net result of this normalization is to correct for systematic differences in comparisons between two species (e.g., differences attributable to nucleotide composition bias), and when i = j, to minimize the impact of "recent" paralogs (duplication within a given species) on the clustering of cross-species orthologs.

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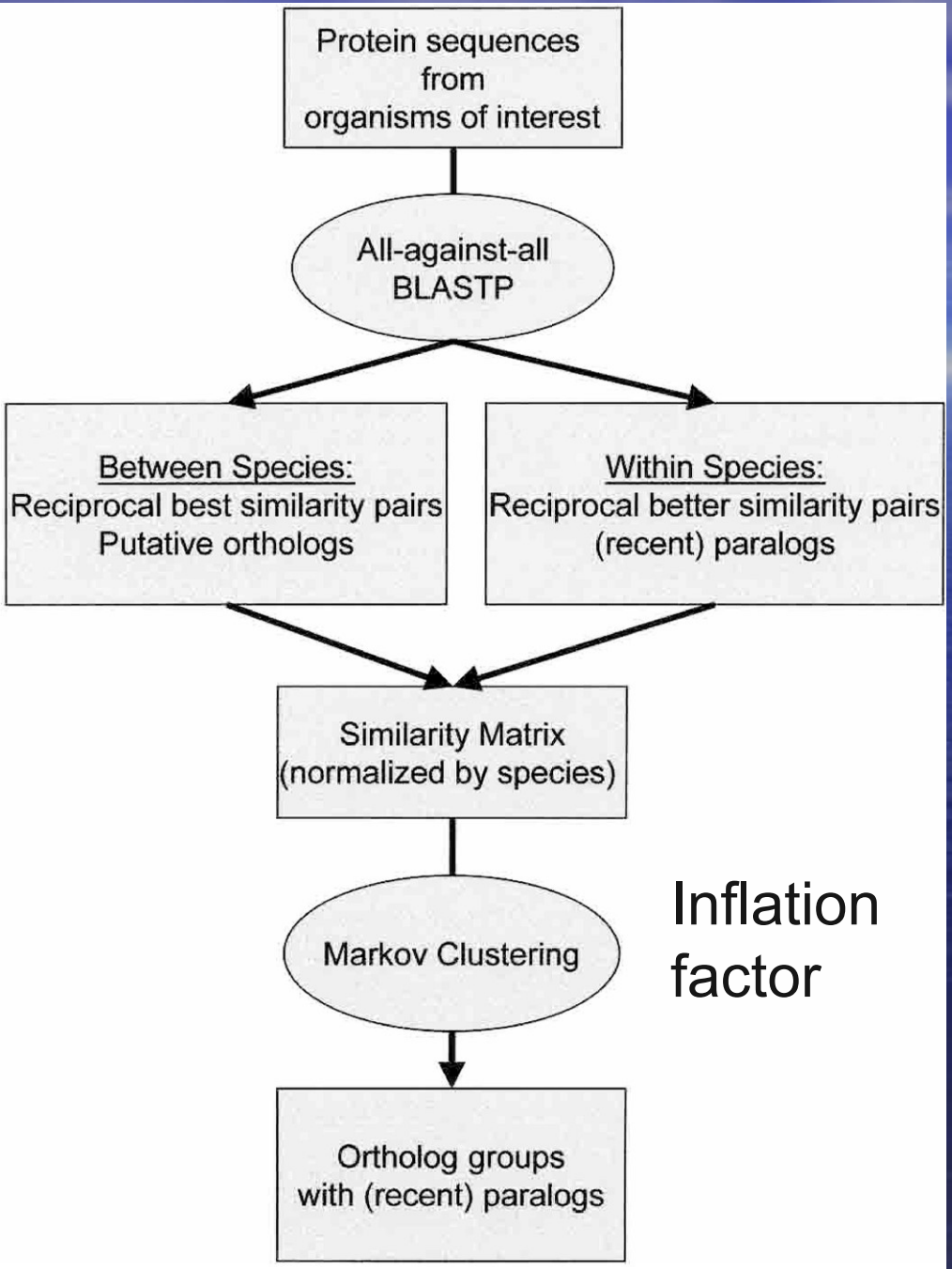

Figure 1 Flow chart of the OrthoMCL algorithm for clustering orthologous proteins.

OrthoMCL

An important parameter in the MCL algorithm is the inflation value, regulating the cluster tightness (granularity); increasing the inflation value increases cluster tightness.

Inflation factor

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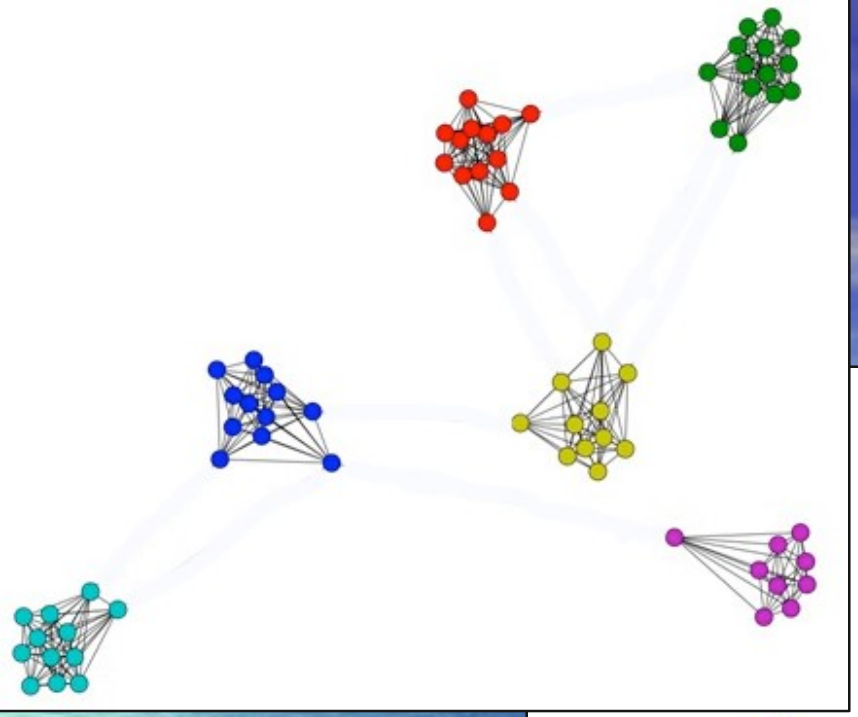
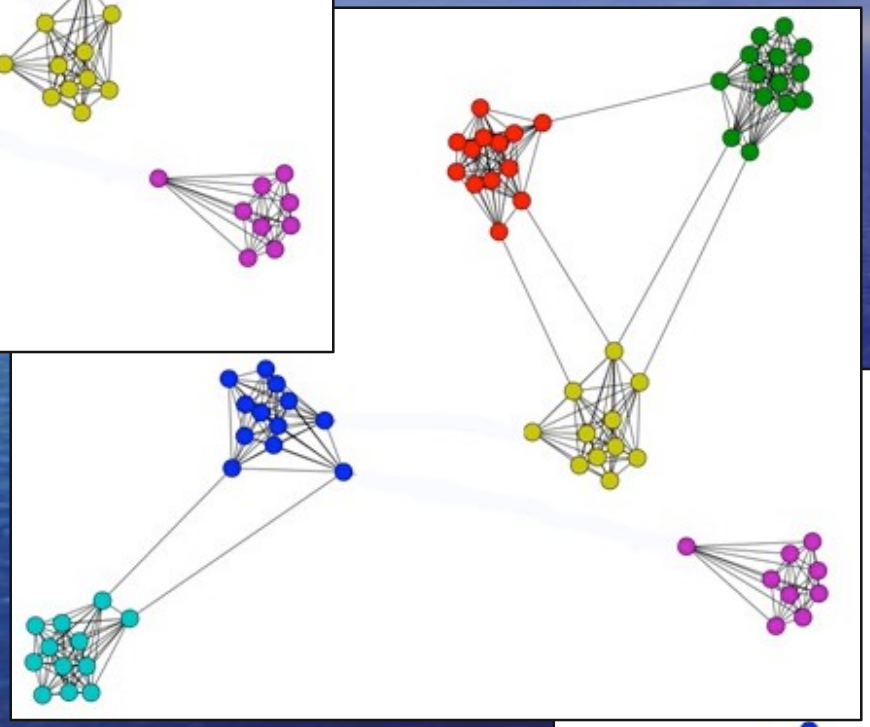
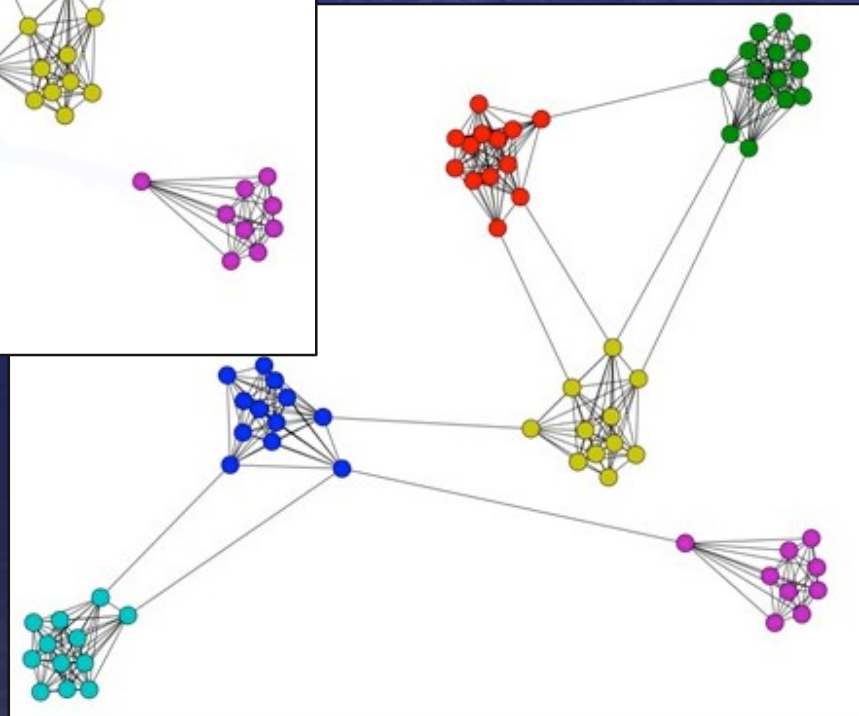
OrthoMCL – Clustering







Increasing
Inflation factor

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OrthoMCL – Inflation factor

Table 4. Consistency of OrthoMCL Groups with EC Assignments

Inflation (cluster tightness)	Total data set		Groups with ≥1 protein for which complete EC annotation is available			Groups with ≥2 proteins for which complete EC annotations are available			Consistent EC assignments[c]		
	Groups	Proteins (% of proteome)[a]	Groups	Proteins	EC-annotated (% of total)[b]	Groups	Proteins	EC-annotated (% of total)	Groups (% poss.)	Proteins	EC-annotated (% possible)
1.1	6,249	50,771 (50)	999	12,032	2,921 (82)	664	9,561	2,586 (73)	528 (80)	5,476	1,958 (76)
1.5	**7,265**	**47,668 (47)**	**1,117**	**8,730**	**2,877 (81)**	**696**	**6,318**	**2,456 (69)**	**596 (86)**	**4,768**	**2,067 (84)**
2.0	7,569	46,245 (46)	1,148	8,343	2,849 (80)	701	5,916	2,402 (67)	611 (87)	4,610	2,073 (86)
2.5	7,681	45,473 (45)	1,160	8,171	2,840 (80)	705	5,789	2,385 (67)	617 (88)	4,556	2,062 (86)
3.0	7,786	44,729 (44)	1,172	7,975	2,831 (79)	706	5,553	2,365 (66)	621 (88)	4,450	2,059 (87)
3.5	7,857	44,263 (44)	1,180	7,889	2,821 (79)	707	5,506	2,348 (66)	624 (88)	4,444	2,057 (88)
4.0	7,896	43,900 (43)	1,186	7,784	2,811 (79)	704	5,414	2,329 (65)	623 (88)	4,372	2,048 (88)

[a]Total proteome size = 101,047 (*Arabidopsis thaliana,* 25,009 sequences; *Caenorhabditis elegans,* 19,774; *Drosophila melanogaster,* 13,288; *Homo sapiens,* 27,049; *Plasmodium flaciparum,* 5279; *Saccharomyces cerevisiae,* 6358; *Escherichia coli,* 4290).
[b]A total of 3562 EC-annotated proteins were obtained from the ENZYME database (*A. thaliana,* 370; C. elegans, 269; *D. melanogaster,* 210; *H. sapiens,* 1160; *S. cerevisiae,* 778; *E. coli,* 775).
[c]All EC-annotated sequences in the group were assigned the same EC number. Percentages indicate fraction of ortholog groups containing at least two complete EC assignments (the only data set for which consistency can be assessed), or percentage of EC-annotated sequences properly identified.

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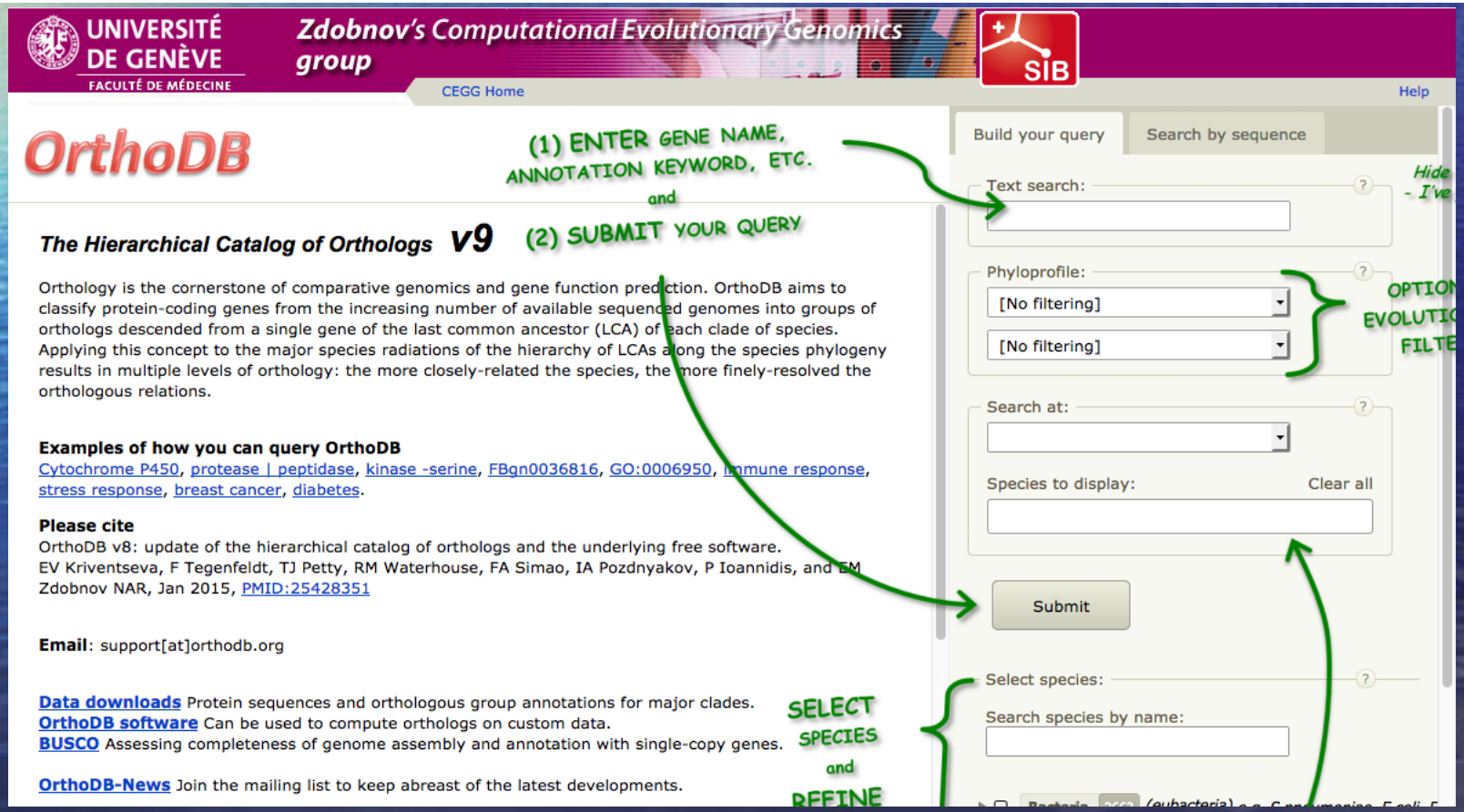
HaMStR – ancestor of Orthograph

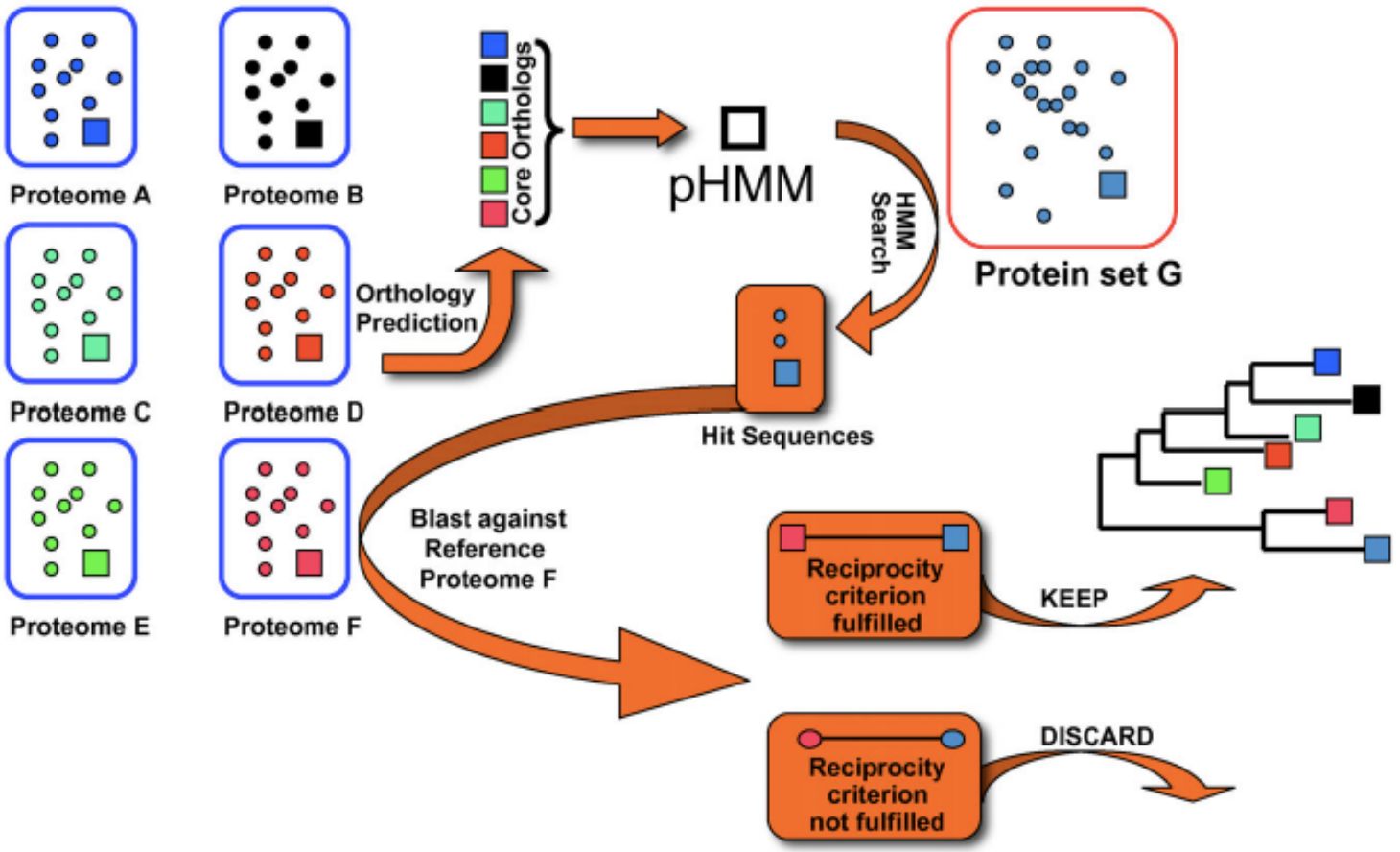
Build your own custom set of core orthologous genes

Different resources are available, which provide well curated set of orthologous genes.

e.g. EMBL UniProt, InParanoid, OrthoDB9



Reciprocal BLAST searches



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