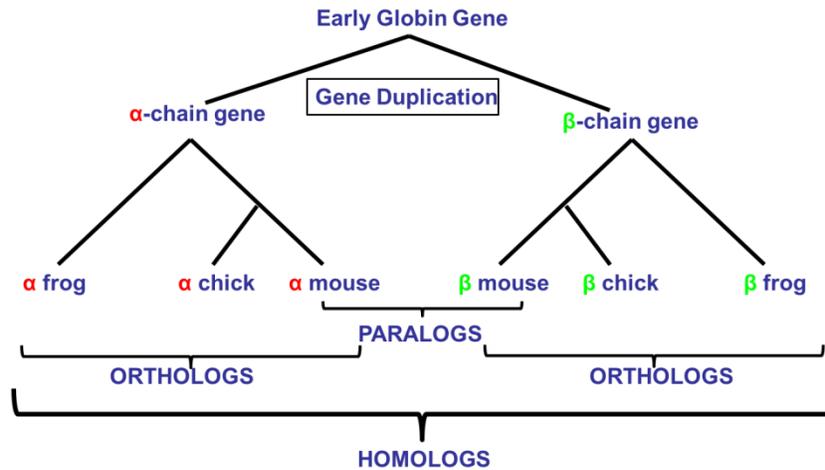


## Orthology and Phyletic Patterns

# Homology



### Learning objectives:

- Explore the orthology table on VEuPathDB gene pages
- Getting to OrthoMCL from VEuPathDB gene pages
- Run searches in OrthoMCL
- Explore the cluster graphs in OrthoMCL
- Leverage the phyletic pattern search
- Leverage the orthology transform tool

OrthoMCL is a genome-scale algorithm for grouping orthologous protein sequences. Such orthologous sequences not only share evolutionary history, but also share function. Thus, ortholog prediction is important in predicting the function of newly identified proteins. Indeed, detection of orthologs has become more widespread with the rapid progress in genome sequencing and the discovery of protein sequences (Glover et al. 2019). Importantly, proteins in OrthoMCL groups have been shown to display a high degree of functional conservation (e.g., a group's proteins have consistent EC numbers) (Li et al. 2003), highlighting that OrthoMCL is useful for functional annotation of newly sequenced genomes.

OrthoMCL not only identifies groups shared by proteins from two or more species, but also groups representing species-specific gene expansion families. To achieve this, the OrthoMCL algorithm starts with reciprocal best BLAST hits within each proteome as potential in-paralog/recent paralog pairs and reciprocal best hits across any two proteomes as potential ortholog pairs. Related proteins are interlinked in a similarity graph. Then, MCL (Markov Clustering algorithm; Dongen 2000; [www.micans.org/mcl](http://www.micans.org/mcl)) is invoked to split mega-clusters. This process is analogous to the manual review in COG construction. MCL clustering is based on weights between each pair of proteins. Thus, to account

for differences in evolutionary distance between any two organisms, the weights are normalized before running MCL.

The organism specific orthology information garnered from our OrthoMCL analysis of VEuPathDB organisms is presented on gene pages and integrated into an Orthology Phylogenetic Profile search. The OrthoMCL.org site offers a deep look into all data associated with the OrthoMCL results for orthology groups and proteins.

### 1. Getting to OrthoMCL from VEuPathDB databases

**Note:** For this exercise use <http://cryptodb.org> and <http://orthomcl.org/>

- a. Use the CryptoDB site search to visit the gene page for the *Cryptosporidium muris* gene, CMU\_034340, hypothetical protein, conserved.
- b. What information on the gene page can you use to guess a function for this gene? It is annotated as a hypothetical protein! Hint: look at the orthologs table and the domains in the protein features graph. You may also want to visit some of the external links or take a look at InterPro domains.

Transcript ID	Isoelectric Point	Molecular Weight	Has SignalP	Has TMHMM	Protein Length	Pro Bro
CMU_034340-t26_1	10.5	23784	no	no	206	Interac

- c. Go to the Orthology and Synteny section and look at the table labeled “Orthologs and Paralogs within CryptoDB”. Does this gene have orthologs in other *Cryptosporidium* species? What about other organisms? (hint: scan the organism column in the table)

## 7 Orthology and synteny

Ortholog Group: **OG6\_101337**

▼ Orthologs and Paralogs within CryptoDB [Data sets](#)

To run Clustal Omega, select genes from the table below. Then choose the sequence type and initiate the alignment with the 'Run Clustal Omega for selected genes' button.

Search this table...

Clustal Omega	Gene	Organism	Product	is syntenic	has comments
<input type="checkbox"/>	<a href="#">Cvel_467</a>	Chromera velia CCMP2878	rRNA-processing protein FCF1 homolog, putative	no	no
<input type="checkbox"/>	<a href="#">cand_030400</a>	Cryptosporidium andersoni isolate 30847	hypothetical protein	yes	no
<input type="checkbox"/>	<a href="#">Chro.70261</a>	Cryptosporidium hominis TU502	hypothetical protein	yes	no
<input type="checkbox"/>	<a href="#">CHUDEA7_2290</a>	Cryptosporidium hominis UdeA01	unspecified product	yes	no
<input type="checkbox"/>	<a href="#">GY17_00002025</a>	Cryptosporidium hominis isolate 30976	rRNA-processing protein Fcf1/Utp23	yes	no
<input type="checkbox"/>	<a href="#">ChTU502y2012_407q1140</a>	Cryptosporidium	Fcf1	yes	no

- d. What about orthologs in organisms not in VEuPathDB? (hint: click on the Ortholog Group link above the table to examine the orthology information for the group at OrthoMCL.org). Does it have any orthologs in bacteria or archaea?

## 1 Phyletic distribution

▼ Phyletic Distribution of Proteins [Download](#)

Numbers refer to the number of proteins in that organism or taxonomic group.

expand all | collapse all

Hide zero counts

Type a taxonomic name

▼ Eukaryota (EUKA)	555
▶ Alveolates (ALVE)	107
▶ Amoebozoa (AMOE)	13
▶ Euglenozoa (EUGL)	60
▶ Fungi (FUNG)	198
▶ Metazoa (META)	113
▶ Other Eukaryota (OEUK)	45
▶ Viridiplantae (VIRI)	19
▼ Archaea (ARCH)	26
Nitrosopumilus maritimus (strain SCM1) (nmar)	1
▶ Crenarchaeota (CREN)	13
▶ Euryarchaeota (EURY)	10
▶ Korarchaeota (KORA)	1
▶ Nanoarchaeota (NANO)	1

- e. Scroll down to the Pfam domains section. Domain architectures are found under the Pfam Architecture of Each Protein table and are described in the Pfam Legend table. Do all the proteins in this group have similar domain architecture? What is the distribution of the PF04900 domain across the 581 proteins in this ortholog group? PF00149?

▼ PFam Legend [Download](#)

Search this table...

Accession	Symbol	Description	Count	Legend
PF04900	Fcf1	Fcf1	554	
PF01850	PIN	PIN domain	3	
PF00149	Metallophos	Calcineurin-like phosphoesterase	1	
PF13638	PIN_4	PIN domain	1	
PF05811	DUF842	Eukaryotic protein of unknown function (DUF842)	1	

▼ PFam Architecture of Each Protein [Download](#)

Search this table...

Accession	Taxon	Core/Peripheral	Protein Length	
aacu ASPACDRAFT_77294	Aspergillus aculeatus ATCC 16872	Peripheral	189	
aaeg-old AAEL007697	Aedes aegypti LVP_AGWG (old build 2019-12-20)	Core	241	
aaeg AAEL007697	Aedes aegypti LVP_AGWG	Peripheral	241	
aalb LOC109404604	Aedes albopictus Foshan FPA	Peripheral	204	
aalr H312_01396	Anncalia algerae PRA339	Peripheral	171	
aals AALB001602	Anopheles albimanus STECLA	Peripheral	204	
aapi AAP_05507	Ascospaera apis ARSEF 7405	Peripheral	193	
aara AARA011876	Anopheles arabiensis Dongola	Peripheral	203	
aast-old H257_18301	Aphanomyces astaci strain APO3 (old build 2014-01-14)	Core	197	

f. Based on the orthologs and the PFam domains shared by the group, what do you think this protein might be doing? If you had to give this gene a name, what would you call it?

**2. Using the phyletic pattern tool in OrthoMCL**

**Note:** For this exercise use <http://orthomcl.org/>

a. How many orthology groups OrthoMCL do not have any orthologs in bacteria or archaea? How many protein groups do not contain orthologs from bacteria and archaea?

OrthoMCL DB VeuPathDB Project

Site search, e.g. G6L\_100861 or PF307\_1132\* or "binding protein"

My Strategies Searches Tools My Workspace Data About Help Contact Us

**Search for...**

expand all | collapse all

Filter the searches below...

**Ortholog Groups**

- Q % Pairs w/ Similarity
- Q All Groups
- Q Avg % Homology
- Q Avg % Identity
- Q Avg % Match Length
- Q Avg E-Value
- Q EC Number
- Q Group ID(s)
- Q Group or Sequence ID
- Q Number of Sequences
- Q Number of Taxa
- Q PFam ID or Keyword
- Q Phyletic Pattern
- Q Text Terms

**Proteins**

**Overview of Resources and Tools**

OrthoMCL FAQ About OrthoMCL Type of Searches in OrthoMCL Understanding Ortho Search Results Search Strategies

**About OrthoMCL**

OrthoMCL is a genome-scale algorithm for sequences. Such orthologous sequences also share function. Thus, ortholog predicts function of newly identified proteins. Indeed, more widespread with the rapid progress in discovery of protein sequences (Claverie et al, 2003), highlighting that OrthoMCL is useful sequenced genomes.

**Tutorials and Exercises**

Ortholog Phyletic

## Identify Ortholog Groups based on Phyletic Pattern

In the graphical tree display:

- Click on the icons to show or hide subtaxa and species.
- Click on the icons to specify which taxa or species to include or exclude in the profile.
- Refer to the legend below to understand other icons.

Expression: ARCH=0T AND BACT=0T

Key: = no constraints | = must be in group | = at least one subtaxon must be in group | = must not be in group

expand all | collapse all

Type a taxonomic name

- \* Root (ALL)
  - Eukaryota (EUKA)
    - Alveolates (ALVE)
    - Amoebozoa (AMOE)
    - Euglenozoa (EUGL)
    - Fungi (FUNG)
    - Metazoa (META)
    - Other Eukaryota (OEUK)
    - Viridiplantae (VIRI)
  - Archaea (ARCH)
    - Nitrosopumilus maritimus (strain SCM1) (nmar)
    - Crenarchaeota (CREN)
    - Euryarchaeota (EURY)
    - Korarchaeota (KORA)
    - Nanoarchaeota (NANO)
  - Bacteria (BACT)
    - Firmicutes (FIRM)
    - Other Bacteria (OBAC)
    - Proteobacteria (PROT)

Get Answer

## My Search Strategies

Opened (1) All (1) Public (26) Help

Unnamed Search Strategy \*

Phyletic  
777,711 Ortholog Groups  
Step 1

+ Add a step

777,711 Ortholog Groups [Revise this search](#)

Ortholog Group Results

1 2 3 ... 38,886

Rows per page: 20

Download

Add to

Ortholog Group	Total Number Proteins	Keywords	Top PFam Domains	EC Numbers
OG6_100001	14709	unknown; hypothetical protein; conserved hypothetical protein	PF13388 (4233), PF04665 (3687), PF04851 (212)	N/A
OG6_100002	6864	unknown; conserved hypothetical protein	PF12943 (5254), PF10544 (1424), PF04383 (2), PF12789 (2)	N/A

- b. Find all groups that contain orthologs from at least one species of *Cryptosporidium* and *Giardia* but not from bacteria or archaea. If you are getting frustrated trying to figure this one out, you have a right to be! You cannot answer this question by using the check boxes (we will discuss why). However, OrthoMCL has an added feature that allows you to enter an expression to define the phyletic pattern. This option provides additional flexibility. Can you figure out what expression to use to answer this question? (hint: scroll down to the bottom of the page to find additional information about expression parameters.

Before looking at the answer below, try this on your own or with the people in your breakout room.

### Identify Ortholog Groups based on Phyletic Pattern

Find Ortholog Groups that have a particular phyletic pattern, i.e., that include or exclude taxa or species that you specify.

The search is controlled by the Phyletic Pattern Expression (PPE) shown in the text box. Use either the text box or the graphical tree display, or both, to specify your pattern. The graphical tree display is a friendly way to generate an expression. You can always edit the expression directly. For PPE help see the instructions at the bottom of this page.

In the graphical tree display:

- Click on the > icons to show or hide subtaxa and species.
- Click on the ● icons to specify which taxa or species to include or exclude in the profile.
- Refer to the legend below to understand other icons.

Expression:  Get Answer

**Key:** ● = no constraints | ✓ = must be in group | ✓ = at least one subtaxon must be in group | ✗ = must not be in group | \* = mixture of constraints

giard

- \* Root (ALL)
- \* Eukaryota (EUKA)
  - \* Other Eukaryota (OEUK)
    - ✓ Giardia Assemblage A isolate WB (gass)
    - ✓ Giardia Assemblage A isolate WB (old build 2013-02-08) (gass-o1d)
    - ✓ Giardia Assemblage A2 isolate DH (gadh)
    - ✓ Giardia Assemblage B isolate GS (gasb)
    - ✓ Giardia Assemblage B isolate GS\_B (gabb)
    - ✓ Giardia Assemblage E isolate P15 (gase)
    - ✓ Giardia muris strain Roberts-Thomson (gmur)

ARCH=0T AND BACT=0T AND cand+chom+chod+choi+chot+cmel+cmur+cpia+cpar+cpar-old+ctyz+cubi>=1T AND gass+gass-old+gadh+gasb+gabb+gase+gmur>=1T

- c. All VEuPathDB sites also have a phyletic pattern search that uses OrthoMCL data under Genes -> Orthology and synteny -> Orthology Phylogenetic Profile. This search is very useful to identify genes in your organism of interest that are restricted in their profile. For example, you frequently want to identify genes that are conserved among organisms in your genus but not present in the host as these genes may make good drug targets or vaccine candidates. Optional: go to your favorite VEuPathDB site and run this search to identify all genes that are not present in human or mouse.

3. Combining searches in OrthoMCL (Use <http://orthomcl.org> for this exercise).

Find all plant proteins that are likely phosphatases that do not have orthologs outside of plants.

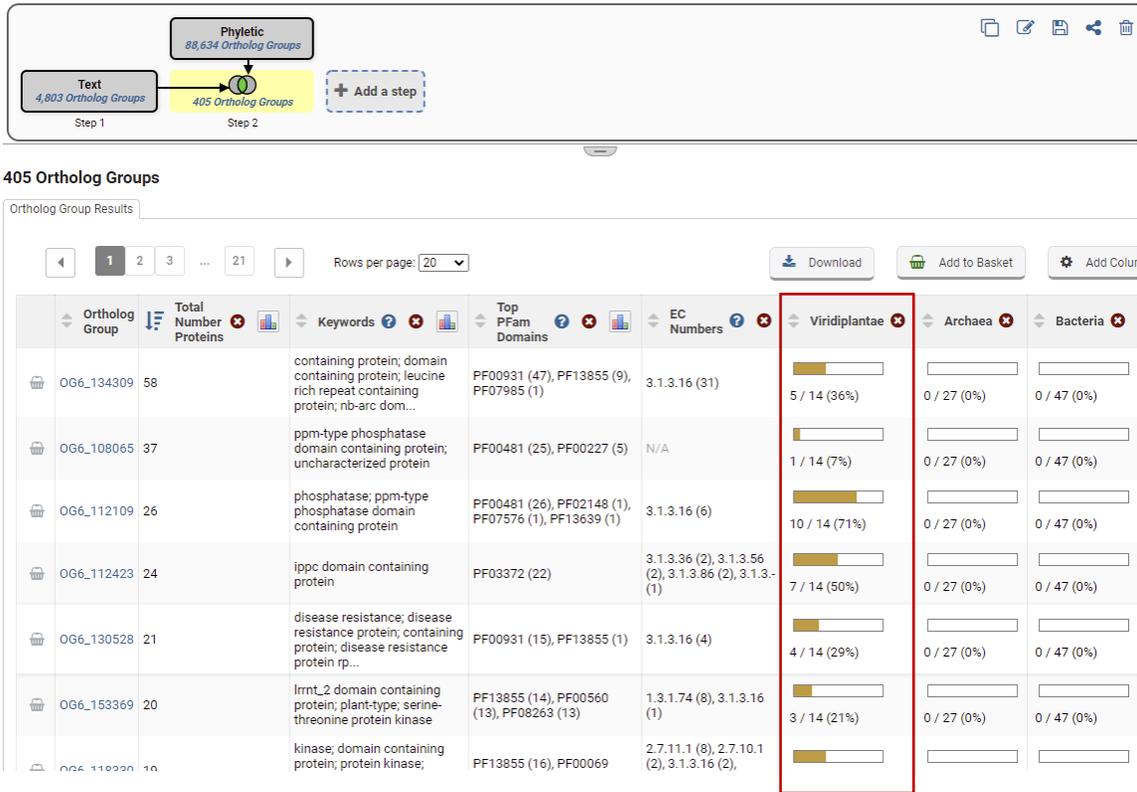
- a. Use the text search to find OrthoMCL groups that contain the word “\*phosphatase\*” (note that the search should be run without the quotation marks but with the asterisks).

The image shows two screenshots from the OrthoMCL web interface. The left screenshot displays a search filter menu with 'Text Terms' selected. A blue arrow points from this menu to the right screenshot. The right screenshot is titled 'Identify Ortholog Groups based on Text Terms'. It has a text input field containing '\*phosphatase\*'. Below the input field, there is a 'Fields' section with several checkboxes: EC Number, Keywords, Ortholog group, Pfam Domains, Protein Description, Protein ID, Protein Previous Groups, Protein Taxon Abbreviation, and Protein Taxon Name. A 'Get Answer' button is circled in blue. To the right of the 'Get Answer' button, a yellow box displays 'Text 4,803 Ortholog Groups' and 'Step 1'.

- b. Add a step and run a phyletic pattern search for groups that contain any plant protein but do not contain any other organism outside plants. (hint: make sure everything has a red x on it except for plants (Viridiplantae (VIRI)), which should be a grey circle).

The image shows a phyletic pattern search result. It is a tree-like structure of taxonomic groups. The root is labeled '\* Root (ALL)'. The groups are listed as follows: Eukaryota (EUKA) with a red 'x' icon; Alveolates (ALVE) with a red 'x' icon; Amoebozoa (AMOE) with a red 'x' icon; Euglenozoa (EUGL) with a red 'x' icon; Fungi (FUNG) with a red 'x' icon; Metazoa (META) with a red 'x' icon; Other Eukaryota (OEUK) with a red 'x' icon; Viridiplantae (VIRI) with a grey circle icon; Bacillariophyta (BACI) with a grey circle icon; Chlorophyta (CHLO) with a grey circle icon; Cryptophyta (CRYP) with a grey circle icon; Rhodophyta (RHOD) with a grey circle icon; Streptophyta (STRE) with a grey circle icon; Archaea (ARCH) with a red 'x' icon; Nitrosopumilus maritimus (strain SCM1) (nmar) with a red 'x' icon; Crenarchaeota (CREN) with a red 'x' icon; Euryarchaeota (EURY) with a red 'x' icon; Korarchaeota (KORA) with a red 'x' icon; Nanoarchaeota (NANO) with a red 'x' icon; Bacteria (BACT) with a red 'x' icon; Firmicutes (FIRM) with a red 'x' icon; Other Bacteria (OBAC) with a red 'x' icon; Proteobacteria (PROT) with a red 'x' icon.

- c. Examine your results. How many groups were returned by the search? What is the distribution of plant proteins in each orthology group?



- d. Run a multiple sequence alignment for OG6\_112109. Click on the group ID in your result table and navigate to the List of Proteins section of the group page. The Clustal Omega tool is integrated into the table. There are several formats available for the Clustal output, making it easy to take these results to other visualization programs.

OrthoMCL DB

Site search, e.g. OG6\_105861 or PF3D7\_11331 or "binding protein"

My Strategies Searches Tools My Workspace Data About Help Contact Us

### 3 List of proteins

OG6\_112109

expand all | collapse all

Search section names...

- 1 Phyletic distribution
- 2 Group summary
- 3 List of proteins
- 4 Pfam domains
- 5 Cluster graph

expand all | collapse all

▼ List of All Proteins Download

To align sequences, select proteins from the table below. Then choose the 'Output format' and click the 'Run Clustal Omega for selected genes' button.

Search this table...

Clustal Omega	Accession	Description	Organism	Taxon	Core/Peripheral	Length
<input checked="" type="checkbox"/>	vcariD8UBL1	PPM-type phosphatase domain-containing protein	Volvox carteri f. nagariensis	Viridiplantae	Peripheral	1309
<input checked="" type="checkbox"/>	crei A0A2K3DZC7	PPM-type phosphatase domain-containing protein	Chlamydomonas reinhardtii (Chlamydomonas smithii)	Viridiplantae	Core	1237
<input checked="" type="checkbox"/>	vcariD8TYP9	Uncharacterized protein	Volvox carteri f. nagariensis	Viridiplantae	Peripheral	988
<input checked="" type="checkbox"/>	apro A0A087SRW5	PPM-type phosphatase domain-containing protein	Auxenochlorella protothecoides (Green microalga) (Chlorella protothecoides)	Viridiplantae	Core	708
<input checked="" type="checkbox"/>	cbra A0A388JMB4	PPM-type phosphatase domain-containing protein	Chara braunii (Braun's stonewort)	Viridiplantae	Core	704
<input checked="" type="checkbox"/>	apro A0A087SJZ6	PPM-type phosphatase domain-containing protein	Auxenochlorella protothecoides (Green microalga) (Chlorella protothecoides)	Viridiplantae	Core	543
<input checked="" type="checkbox"/>	crei A0A2K3DBF3	PPM-type phosphatase domain-containing protein	Chlamydomonas reinhardtii (Chlamydomonas smithii)	Viridiplantae	Core	491
<input checked="" type="checkbox"/>	osat Q0JMD4	Probable protein phosphatase 2C.3	Oryza sativa subsp. japonica (Rice)	Viridiplantae	Core	485

Check All Uncheck All

Please note: selecting a large number of proteins will take several minutes to align.

Output format: **Mismatches highlighted**

Run Clustal Omega for selected proteins

#### 4. Explore a specific OrthoMCL group - examining the cluster graph. Use <http://orthomcl.org>

- Visit the Orthomcl group OG6\_131670. Type the ID into the site search to navigate to the OG6\_131670.
- Examine the Phyletic Distribution. What is the phylogenetic distribution of the members of this group? The distribution is presented as a tree. Expand the tree to view the distribution.

#### 1 Phyletic distribution

##### ▼ Phyletic Distribution of Proteins Download

Numbers refer to the number of proteins in that organism or taxonomic group.

expand all | collapse all

Hide zero counts

Type a taxonomic name

▼ Eukaryota (EUKA) 97

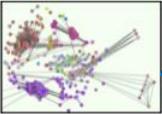
- ▶ Alveolates (ALVE) 94
- ▶ Metazoa (META) 3

- c. Navigate to the Cluster graph tab. Modify the E-value cutoff slider. What happens when you increase or decrease the E-value? Can you identify subclusters of orthologs? The view of the graph can be changed using the Edge type options and the Node options.

5 Cluster graph

Click to open the Cluster graph in a new tab

Cluster graph of all proteins



Cluster Graph: OG6\_131670 (97 proteins)

**Edge Options**

Edge Type

Ortholog  Coortholog

Inparalog  Peripheral-Core

Peripheral-Peripheral  Other Similarities

E-Value Cutoff

Max E-Value: 1E-22

**Node Options**

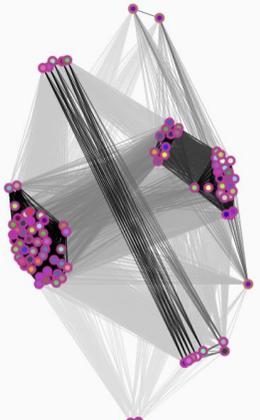
Show Nodes By

Taxa  ED Numbers  PFam Domains

Core/Peripheral

Mouse over a taxon legend to highlight sequences of that taxon.

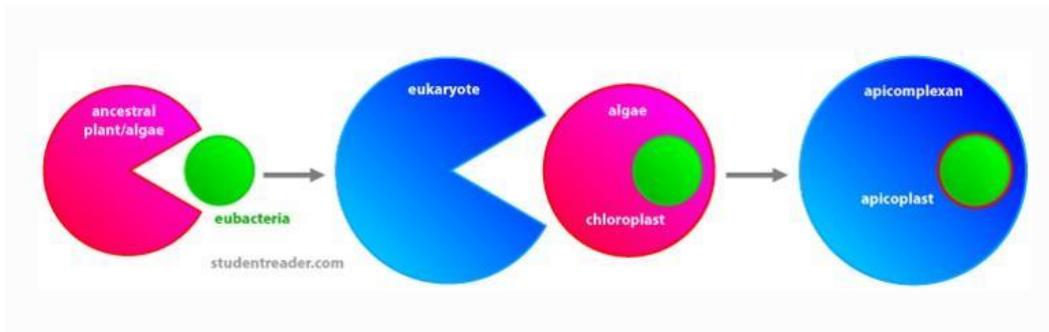
<input checked="" type="radio"/> gnid (1)	<input checked="" type="radio"/> gnip-old (1)	<input checked="" type="radio"/> hpl (1)
<input checked="" type="radio"/> padl (1)	<input checked="" type="radio"/> ober (1)	<input checked="" type="radio"/> pbl (1)
<input checked="" type="radio"/> pbg (1)	<input checked="" type="radio"/> pshe (1)	<input checked="" type="radio"/> psca (1)
<input checked="" type="radio"/> psym (1)	<input checked="" type="radio"/> psym (1)	<input checked="" type="radio"/> pfa (1)
<input checked="" type="radio"/> pfa-old (1)	<input checked="" type="radio"/> pfag (1)	<input checked="" type="radio"/> pfac (1)
<input checked="" type="radio"/> pfad (1)	<input checked="" type="radio"/> pfga (1)	<input checked="" type="radio"/> pfpb (1)
<input checked="" type="radio"/> pfgn (1)	<input checked="" type="radio"/> pfho (1)	<input checked="" type="radio"/> pfa (1)
<input checked="" type="radio"/> pfke (1)	<input checked="" type="radio"/> pfkn (1)	<input checked="" type="radio"/> prft (1)
<input checked="" type="radio"/> pfml (1)	<input checked="" type="radio"/> pfnd (1)	<input checked="" type="radio"/> pfan (1)



Sequence List

ID	Accession	Taxon	Length
bbes	BESB_010830	bbes	536
cand	cand_009760	cand	318
ccay	ccay_07054	ccay	553
ccmf	CCML0C_042890	ccmf	553
chod	CHOLU0E4L_5030	chod	325
chot	CHY17_0000954	chot	325
chom	Chro_80575	chom	325
chot	CHTUS02912_400fg2000	chot	325
cmel	OmniK0MEL_07710	cmel	325
cmur	CMU_011520	cmur	318
cpar-old	cpo8_5030	cpar-old	324
cpar	cpo8_5030	cpar	324
cpia	CPATCC_004380	cpia	324
csu	CSU_001537	csu	577
ctyz	CTYZ_0000545	ctyz	325
cubi	cubl_03730	cubi	325
eace	EAH_00023650	eace	550
ebru	EBH_0061790	ebru	551
efaf	EFAB_MINUS_13398.g1198	efaf	547
emax	EMWV1_00029900	emax	652
emh	EMH_0005590	emh	551
enec	EMH_00050470	enec	553
epra	EPH_0014470	epra	551
eten	ETH_00031620	eten	553
gnip-old	GNL_098990	gnip-old	387

5. Using the orthology transform tool to identify apicoplast targeted genes in *Toxoplasma* and *Neospora*. Note: For this exercise use <http://veupathdb.org>



The apicoplast likely became encased in four membranes via a double endosymbiotic event. The chloroplast arose by engulfment of a cyanobacteria by a plant/algae ancestor. An algae was then engulfed by the ancestor of all apicomplexans. Thus, an apicoplast organelle arose with four membranes.

- a. Start by finding genes in *Plasmodium* that are predicted to target to the apicoplast. Hint: Navigate to the P.f. Subcellular Localization search. You can further expand your list of potentially Apicoplast targeted proteins by running a GO terms search for the term “apicoplast” or the GO ID: GO:0020011 in *P. falciparum* 3D7 (hint, click on add step the go to the function prediction category and select the GO term search). Which Boolean operation did you use? Union or intersect?

**Search for...**  
expand all | collapse all  
Filter the searches below...  
▶ Pathways and interactions  
▶ Phenotype  
▶ Protein features and properties  
▼ Protein targeting and localization  
    ↳ Exported Protein  
    ↳ **P.f. Subcellular Localization**  
    ↳ Predicted Signal Peptide  
    ↳ Transmembrane Domain Count  
▶ Proteomics

**Identify Genes based on P.f. Subcellular Localization**  
Localization  
Apicoplast  
Get Answer

**Subcell Loc**  
513 Genes  
Step 1

+ Add a step

**Organism**

1 selected, out of 439

add these | clear these | select only these  
select all | clear all

3d7

- Apicomplexa
  - Aconoidasida
    - Haemosporida
      - Plasmodium
        - Plasmodium falciparum
          - Plasmodium falciparum 3D7 [Reference]

add these | clear these | select only these  
select all | clear all

**Evidence**

Curated  
 Computed  
select all | clear all

**Limit to GO Slim terms**

Yes  
 No

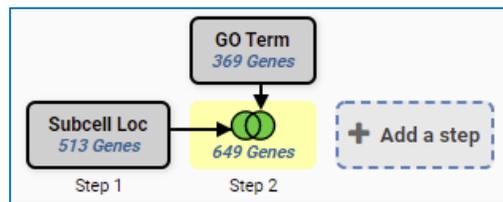
**GO Term or GO ID**

GO:0020111:apicoplast:7

**GO Term or GO ID wildcard search**

N/A

Run Step



- b. Transform the results into their *Toxoplasma* and *Neospora* orthologs. Add a step to your strategy that transforms the results into *Toxoplasma* and *Neospora*.

← Add a step to your search strategy

Combine with other Genes

Transform into related records

Use Genomic Colocation to combine with other features

Transform 649 Genes into... Orthologs

**Organism**

16 selected, out of 439

select all | clear all | expand all | collapse all

Filter list below...

- Amoebozoa
- Apicomplexa
  - Aconoidasida
  - Conoidasida
    - Coccidia
      - Cryptosporidiidae
      - Eimeriidae
      - Sarcocystidae
        - Besnoitia besnoiti strain Bb-Ger1 [Reference]
        - Cystoisospora suis strain Wien I [Reference]
        - Hammondia hammondi strain H.H.34 [Reference]
        - Neospora caninum Liverpool [Reference]
        - Sarcocystis
        - Toxoplasma
- Eugregarinorida
  - Chromeraceae

- Euglenozoa
- Fornicata
- Fungi
- Heterolobosea
- Metazoa
- Oomycota
- Parabasalia
- Preaxostyla
- Vitrellaceae

select all | clear all | expand all | collapse all

Run Step

- c. Although *Cryptosporidium* is an apicomplexan parasite it has lost its apicoplast! Can you use this fact to refine your results from the above search? Hint: try subtracting out any orthologs present in *Cryptosporidium*. You will need to use a nested strategy and use the ortholog transform back to *Toxoplasma* and *Neospora* genes for the subtraction to complete.

### Add a step to your search strategy

**Combine with other Genes**

**Transform into related records**

**Use Genomic Colocation to combine with other features**

**1 Choose how to combine with other Genes**

3 INTERSECT 4  
  3 UNION 4  
  3 MINUS 4

**2 Choose which Genes to combine. From...**

A new search  
  An existing strategy

- Phenotype
  - Q, CRISPR Phenotype
  - Q, Phenotype Evidence
- Proteomics
  - Q, Mass Spec. Evidence
  - Q, Quantitative Mass Spec. Evidence
- Taxonomy
  - Q, Organism

### Add a step to your search strategy

**Search for Genes by Organism**

The results will be  subtracted from |  the results of Step 3.

**Organism**

11 selected, out of 439

add these | clear these | select only these  
select all | clear all

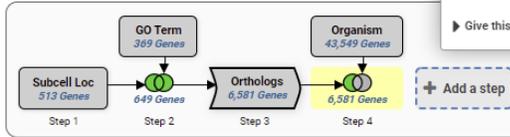
- Apicomplexa
  - Conoidasida
    - Coccidia
      - Cryptosporidiidae
        - Cryptosporidium andersoni isolate 30847 [Reference]
        - Cryptosporidium hominis
          - Cryptosporidium hominis TU502 [Reference]
          - Cryptosporidium hominis UdeA01
          - Cryptosporidium hominis isolate 30976
          - Cryptosporidium hominis isolate TU502\_2012
        - Cryptosporidium meleagridis strain UKMEL1 [Reference]
        - Cryptosporidium muris RN66 [Reference]
        - Cryptosporidium parvum
          - Cryptosporidium parvum IOWA-ATCC
          - Cryptosporidium parvum Iowa II [Reference]
        - Cryptosporidium tyzzeri isolate UGAS5 [Reference]
        - Cryptosporidium ubiquitum isolate 39726 [Reference]

add these | clear these | select only these  
select all | clear all

Unnamed Search Strategy \*



Unnamed Search Strategy \*



View | Analyze | Revise | Make nested strategy | Insert step before | Orthologs | Delete

**Details for step** Organism

43549 Genes

**Organism** Cryptosporidium andersoni isolate 30847, Cryptosporidium hominis TU502, Cryptosporidium hominis UdeA01, Cryptosporidium hominis isolate 30976, Cryptosporidium hominis isolate TU502\_2012, Cryptosporidium meleagridis strain UKMEL1, Cryptosporidium muris RN66, Cryptosporidium parvum IOWA-ATCC, Cryptosporidium parvum Iowa II, Cryptosporidium tyzzeri isolate UGA55, Cryptosporidium ubiquitum isolate 39726

▶ Give this search a weight

