

Sequence Exercises: Motifs, Domains and Colocation

1. Using InterPro domain searches to identify unannotated kinesin motor proteins.

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

- a. Identify all genes annotated as hypothetical in all *Giardia* assemblages. Use the full text search and look for genes with the word “hypothetical” in their product names.

Identify Genes based on Text (product name, notes, etc.)

Organism select all | clear all | expand all | collapse all | reset to default
 Giardia
 Spirotrunculus
 select all | clear all | expand all | collapse all | reset to default

Text term (use * as wildcard)

Fields Alias
 Cellular localization
 Community annotation
 EC descriptions
 Gene ID
 Gene notes
 Gene product
 GO terms and definitions
 Protein domain names and descriptions
 PubMed
 Similar proteins (BLAST hits v. NRDB/PDB)
 User comments
 select all | clear all

Text 16140 Genes Step 1 [Add Step]

[Get Answer]

- b. How many of these hypothetical genes have a kinesin-motor protein PFAM domain?
 - Add a step to the strategy. Go to the “Interpro Domain” search under similarity/pattern, start typing the work kinesin and it should autocomplete.

Add Step

Run a new Search for
 Transform by Orthology
 Add contents of Basket
 Add existing Strategy
 Filter by assigned Weight
 Transform to Pathways
 Transform to Compounds

Genes
 Genomic Segments
 SNPs
 ORFs

Text, IDs, Organism
 Genomic Position
 Gene Attributes
 Protein Attributes
 Protein Features
 Similarity/Pattern
 Transcript Expression
 Protein Expression
 Cellular Location
 Putative Function
 Evolution

Protein Motif Pattern
 InterPro Domain
 BLAST

[Close]

Add Step 2 : InterPro Domain

Organism select all | clear all | expand all | collapse all | reset to default
 Giardia
 Spirotrunculus
 select all | clear all | expand all | collapse all | reset to default

Domain Database

Specific Domain(s)
 PF06920 : Ded_cyto Dedicator of cytokinesis
 PF05804 : KAP Kinesin-associated protein (KAP)
 PF00225 : Kinesin Kinesin motor domain

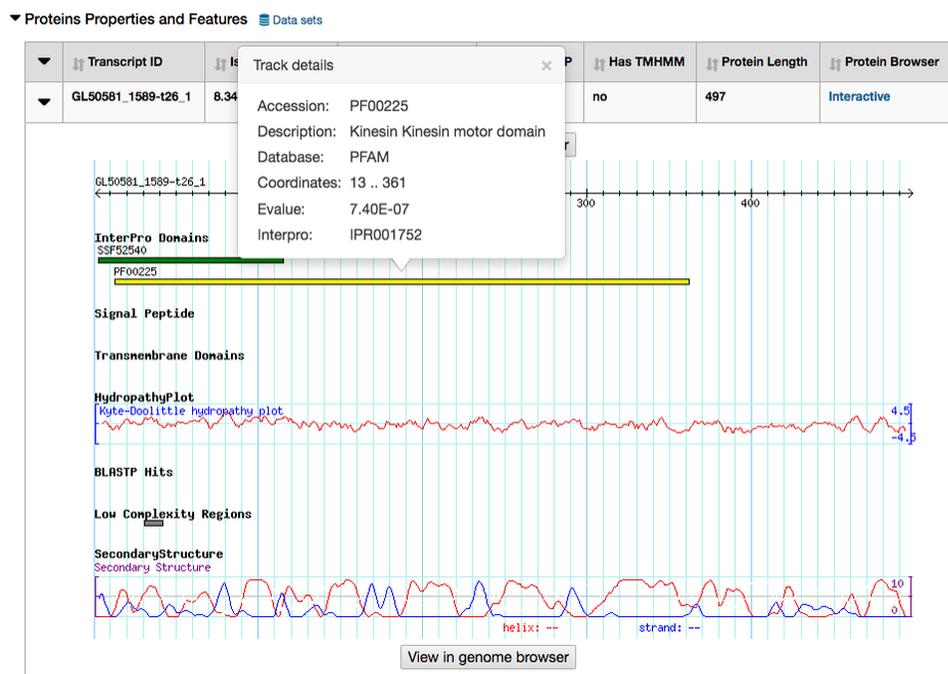
Free Text (use "" for wildcard)

Combine Genes in Step 1 with Genes in Step 2:
 1 Intersect 2
 1 Union 2
 1 Relative to 2, using genomic colocation
 1 Minus 2
 2 Minus 1

InterPro Dom 155 Genes
 Text 16140 Genes Step 1 [Add Step]

[Run Step]

- c. Go to the gene page for GL50581_1589 and look at the protein feature section. Does this look like a possible motor protein?
- Click on the ID for GL50581_1589 in the result table to go to the gene page. Scroll down to the protein section and mouse over the glyphs in the Protein Features graphic.



2. Using regular expressions to find motifs in TriTrypDB: finding active trans-sialidases in *T. cruzi*.

Note: for this exercise use <http://tritrypdb.org>

- T. cruzi* has an expanded family of trans-sialidases. In fact, if you run a text search for any gene with the word “trans-sialidase”, you return over 4000 genes among the strains in the database!!! Try this and see what you get.
- Not all of the genes returned in (a) are predicted to be active. It is known that active trans-sialidases have a signature tyrosine (Y) at position 342 in their amino acid sequence. Add a motif search step to the text search in ‘a’ to identify only the active trans-sialidases.

- Write a regular expression that defines a protein sequence that starts with a methionine, and is followed by 340 of any amino acids, followed by a tyrosine ‘Y’. Refer to [regular expression tutorial](#) if you need to.
- <http://tritrypdb.org/tritrypdb/im.do?s=0d7be75a64dbc2bb>

The screenshot displays the TriTrypDB workflow interface. On the left, a workflow diagram shows 'Text 4397 Genes Step 1' connected to 'Add Step', which then connects to 'Prot Motif 691 Genes Step 2'. A red arrow points from the 'Add Step' button to the main configuration window.

The main window is titled 'Add Step 2 : Protein Motif Pattern'. It contains the following fields:

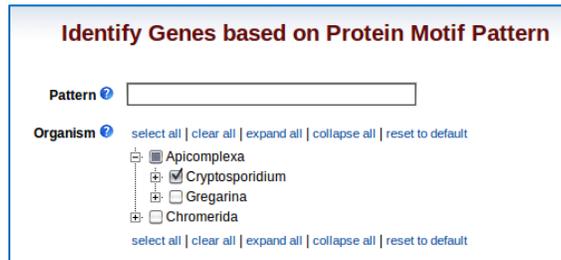
- Pattern:** `^M.{340}Y`
- Organism:** A list of organisms with checkboxes. *Trypanosoma cruzi* is selected.
- Combine Genes in Step 1 with Genes in Step 2:** Radio buttons for 'Intersect 2', 'Union 2', '1 Minus 2', and '2 Minus 1'. 'Intersect 2' is selected.
- Run Step:** A button to execute the step.

At the bottom right, the workflow diagram is updated to show 'Text 4397 Genes Step 1' and 'Prot Motif 691 Genes Step 2' both connected to a central node labeled '46 Genes Step 2'. A red arrow points from the 'Run Step' button to this updated diagram.

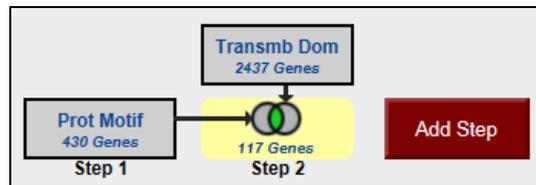
3. Find *Cryptosporidium* genes with the YXXΦ receptor signal motif. Note: for this exercise use <http://cryptodb.org>

The YXXΦ (Y=tyrosine, X=any amino acid, Φ=bulky hydrophobic [phenylalanine, tyrosine, threonine]) motif is conserved in many eukaryotic membrane proteins that are recognized by adaptor proteins for sorting in the endosomal/lysosomal pathway. This motif is typically located in the c-terminal end of the protein.

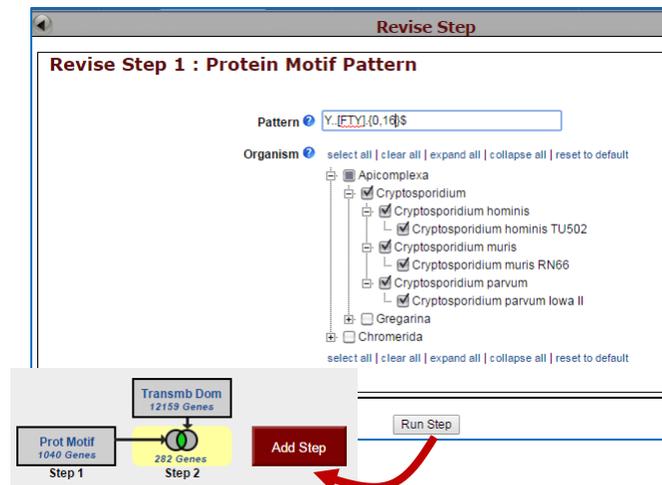
- a. Use the “protein motif pattern” search to find all *Cryptosporidium* proteins that contain this motif anywhere in the terminal 10 amino acids of proteins. (hint: for your regular expression, remember that you want the first amino acid to be a tyrosine, followed any two amino acids, followed by any bulky hydrophobic amino acid (phenylalanine, tyrosine, threonine). Refer to [regular expression tutorial](#) if you need to).



- b. How many of these proteins also contain at least one transmembrane domain.



- c. What would happen if you revise the first step (the motif pattern step) to include genes with the sorting motif in the C-terminal 20 amino acids? (hint: edit the first step and modify your regular expression) <http://cryptodb.org/cryptodb/im.do?s=37e8b03ea8087b5a>

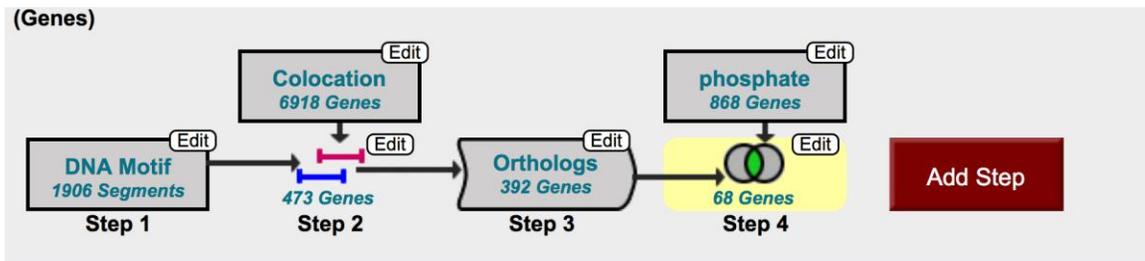


4. Find fungal genes downstream of a regulatory DNA motif.

For this exercise use: <http://fungidb.org>

Transcriptional start sites are often located within a certain distance upstream of the genes or gene clusters that they regulate. In fungi, DNA motifs are also important for regulation of processes linked to host cell invasion or production of secondary metabolites. Readily available genomic data facilitate the discovery of regulatory motifs via examination of orthologous sequences.

The goal of this exercise is to identify all genes harboring upstream CACGTG motif, known for its role in transcriptional regulation. We will start our search in an extensively studied model organism *Saccharomyces cerevisiae*, and expand our search to *Fusarium graminearum*. Here is a summary of the search strategy:



a. Find the CACGTG DNA motif in the *Saccharomyces cerevisiae* genome.

- Select the “Search for genomic segments (DNA motif)” menu from the Search menu and look for CACGTG in *S. cerevisiae*.

Identify Genomic Segments based on DNA Motif Pattern

Search for Other Data Types

DNA

Genomic Segments

- DNA Motif Pattern

Organism

- select all | clear all | expand all | collapse all | reset to default
- Oomycetes
- Fungi
 - Agaricomycetes
 - Blastocladiomycetes
 - Chytridiomycetes
 - Eurotiomycetes
 - Leotiomycetes
 - Pneumocystidomycetes
 - Pucciniomycetes
 - Saccharomycetes
 - Candida
 - Saccharomyces
 - Saccharomyces cerevisiae S288c
 - Yarrowia
 - Schizosaccharomycetes
 - Sordariomycetes
 - Tremellomycetes
 - Ustilaginomycetes
 - Zygomycetes

- select all | clear all | expand all | collapse all | reset to default

Pattern

CACGTG

Get Answer

- Your search should return 1906 DNA segments containing GACGTG motif. Next, let’s look for putative regulatory targets of this motif by searching for genes that are located 600bp downstream of this sequence.

b. Identify genes with the CACGTG motif located 600bp upstream of an open reading frame.

EuPathDB offers a colocation function to identify genomic features within a specified distance of each other.

- Click “Add Step”. Choose “Run a new search for Genes” > “Taxonomy” > “Organism” and select “Relative to genomic location”.
- Set up the colocation using the following guidelines:

Return each gene from step 2 whose upstream region (600bp) overlaps the exact region of a Genomic Segment in Step 1 (CACGTG) and is on either strand.

Genomic Colocation  

Combine Step 1 and Step 2 using relative locations in the genome
You had 1906 Genomic Segments in your Strategy (Step 1). Your new Genes search (Step 2) returned 6918 Genes.

“Return each whose **upstream region** overlaps the **exact region** of a Genomic Segment in Step 1 and is on ”

(6918 Genes in Step)



Region
Gene

Exact

Upstream: 600 bp

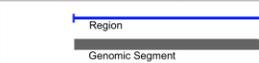
Downstream: 1000 bp

Custom:

begin at: bp

end at: bp

(1906 Genomic Segments in Step)



Region
Genomic Segment

Exact

Upstream: 1000 bp

Downstream: 1000 bp

Custom:

begin at: bp

end at: bp

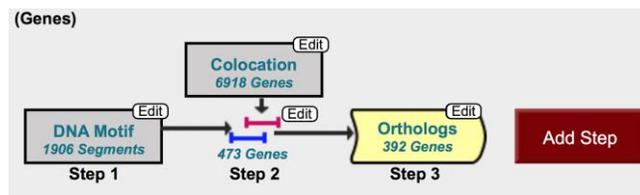
- Your search results should indicate that there are 473 genes that have CACGTG motif upstream of their reading frame.

c. Identify orthologs *S. cerevisiae* genes in *Fusarium graminearum*.

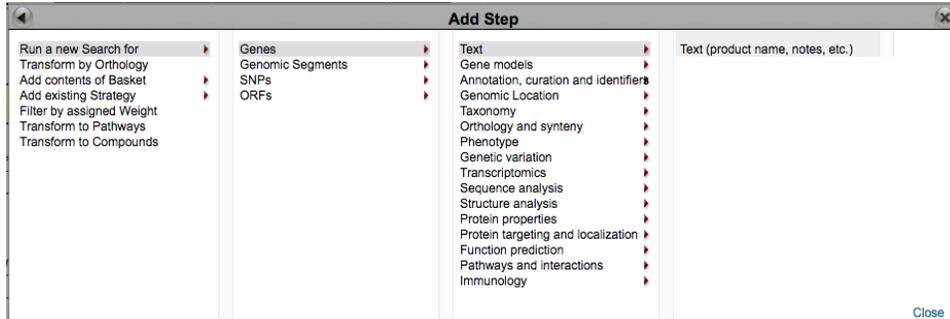
All EuPathDB sites offer “Transform by Orthology” function, which is a comparative genomics approach to identify gene orthologs.

This function uses known classifications, OrthoMCL algorithm, and BLAST similarity search to order protein-coding genes from available sequenced genomes into groups of orthologs based on their similarity across multiple species.

Use “Add step” to initiate transformation by orthology. Your search in *F. graminearum* should return 392 hits.



Lipid biosynthesis plays an important role during the invasion of the plant cell by this fungal pathogen. Use the text search to look for genes associated with the lipid biosynthesis pathway in *F. graminearum*.



Revise Step 4 : Text (product name, notes, etc.)

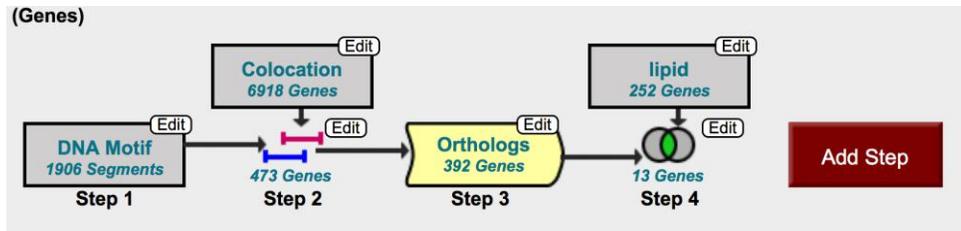
Organism [select all](#) | [clear all](#) | [expand all](#) | [collapse all](#) | [reset to default](#)

- Oomycetes
- Fungi
 - Agaricomycetes
 - Blastocladiomycetes
 - Chytridiomycetes
 - Eurotiomycetes
 - Leotiomycetes
 - Pneumocystidomycetes
 - Pucciniomycetes
 - Saccharomycetes
 - Schizosaccharomycetes
 - Sordariomycetes
 - Fusarium
 - Fusarium graminearum
 - Fusarium graminearum PH-1
 - Fusarium oxysporum
 - Fusarium verticillioides
 - Magnaporthe
 - Neurospora
 - Sordaria
 - Trichoderma
 - Tremellomycetes
 - Ustilaginomycetes
 - Zygomycetes

[select all](#) | [clear all](#) | [expand all](#) | [collapse all](#) | [reset to default](#)

Text term (use * as wildcard)

Examine returned results.



- Do all genes display word “lipid” in the gene product name?
- Click on the first gene listed: FGSG_15852. Look through the gene information page and determine the reason for which this gene is being displayed in your results.

d. Investigate GO enrichment and Metabolic pathways records via Analyze results tab.

Gene Ontology features three structured ontologies that describe gene products in terms of their (1) associated biological processes, (2) cellular components role, and (2) molecular functions in a *species-independent* manner.

Biological Process includes processes like the cell cycle, DNA replication, limb formation, etc. Cellular Component assigns gene function to location (for example, a gene product can function in an organelle and/or be a functional component of an enzyme complex). Molecular Function deals with the function/s carried out by a gene product.

It is not uncommon for the same gene product, especially if it belongs to multi-protein enzyme complex, to carry out multiple functions. In this case the same gene product may be identified in several molecular function that make up a biological process.

- Explore GO enrichment for cellular component via **Analyze Results** tab.
- How many GO terms were enriched in the Biological Process search?
- Sort results by the number of genes present in your search. Examine p-values for the results. Are these numbers consistent with your results?

Metabolic Enrichment function: Visualization of enzymatic and chemical flows within biosynthetic pathways.

How many metabolic processes were identified in your search?

Click on ec00564 - Glycerophospholipid metabolism

