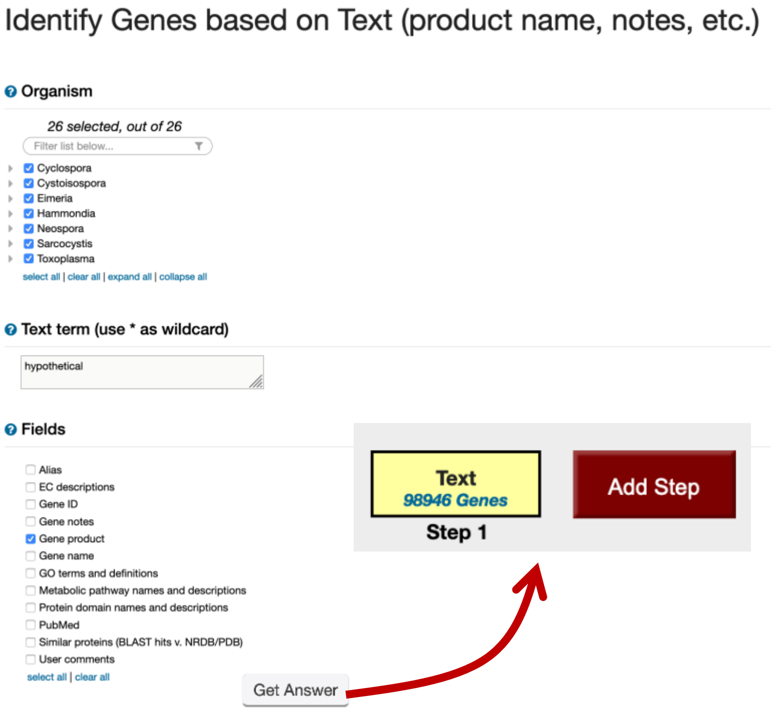
Sequence Exercises: Motifs, Domains and Colocation

1. **Using InterPro domain searches to identify unannotated kinesin motor proteins. Note: For this exercise use http://toxodb.org**

   a. Identify all genes annotated as hypothetical in organisms in ToxoDB (select the gene product field). Use the full text search and look for genes with the word *hypothetical* in their Gene products.



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

Organism

26 selected, out of 26

Filter list below...

- ☑ Cyclospora
- ☑ Cystoisospora
- ☑ Eimeria
- ☑ Hammondia
- ☑ Neospora
- ☑ Sarcocystis
- ☑ Toxoplasma

select all | clear all | expand all | collapse all

Text term (use * as wildcard)

hypothetical

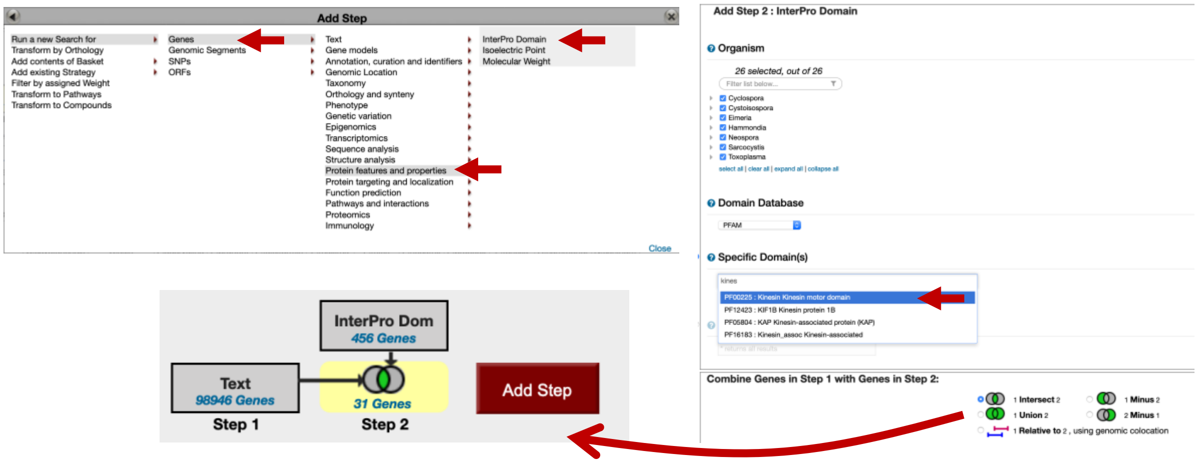
Fields

- □ Alias
- □ EC descriptions
- □ Gene ID
- □ Gene notes
- ☑ Gene product
- □ Gene name
- □ GO terms and definitions
- □ Metabolic pathway names and descriptions
- □ Protein domain names and descriptions
- □ PubMed
- □ Similar proteins (BLAST hits v. NRDB/PDB)
- □ User comments

select all | clear all

   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 ‘Protein features and properties’ similarity/pattern, start typing the work kinesin and it should autocomplete.



Add Step 2 : InterPro Domain

Organism

26 selected, out of 26

Filter list below...

- ☑ Cyclospora
- ☑ Cystoisospora
- ☑ Eimeria
- ☑ Hammondia
- ☑ Neospora
- ☑ Sarcocystis
- ☑ Toxoplasma

select all | clear all | expand all | collapse all

Domain Database

PFAM

Specific Domain(s)

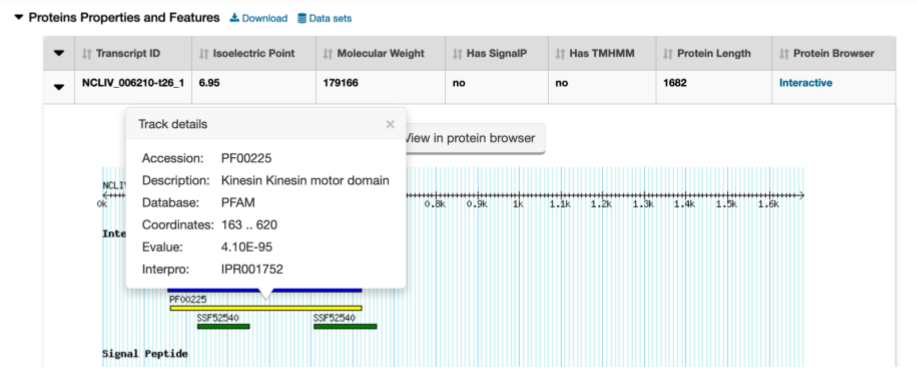
kines

- PF00225 : Kinesin Kinesin motor domain
- PF12423 : KIF1B Kinesin protein 1B
- PF05804 : KAP Kinesin-associated protein (KAP)
- PF16183 : Kinesin_assoc Kinesin-associated

Combine Genes in Step 1 with Genes in Step 2:

- ● 1 Intersect 2
- ○ 1 Minus 2
- ○ 1 Union 2
- ○ 2 Minus 1
- ○ 1 Relative to 2 , using genomic colocation

c. Go to the gene page for NCLIV_006210 and look at the protein feature section. Does this look like a possible motor protein?
   - Click on the ID for NCLIV_006210 in the result table to go to the gene page. Scroll down to the Protein Properties and Features section and mouse over the glyphs in the InterPro domain section.



▾ Proteins Properties and Features Download Data sets

	Transcript ID	Isoelectric Point	Molecular Weight	Has SignalP	Has TMHMM	Protein Length	Protein Browser
▾	NCLIV_006210-t26_1	6.95	179166	no	no	1682	Interactive

Track details

Accession:	PF00225
Description:	Kinesin Kinesin motor domain
Database:	PFAM
Coordinates:	163 .. 620
Evalue:	4.10E-95
Interpro:	IPR001752

   - What other evidence on the gene page supports your conclusion?

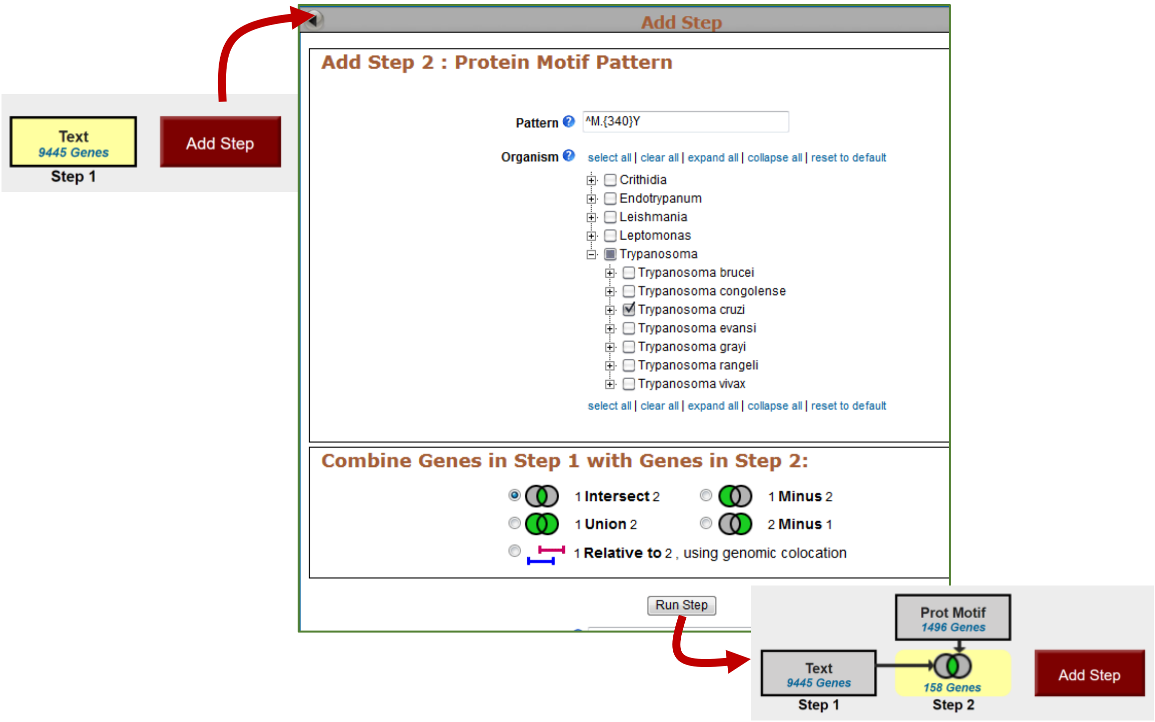
2. Using regular expressions to find motifs in TriTrypDB: finding active trans-sialidases in *T. cruzi.* Note: for this exercise use http://tritrypdb.org

a. *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 9000 genes among the strains in the database!!! Try this and see what you get.

b. 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.

   - Use this link only if you give up: http://tritrypdb.org/tritrypdb/im.do?s=0d7be75a64dbc2bb



Add Step 2 : Protein Motif Pattern

Pattern ^M.{340}Y

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

- □ Crithidia
- □ Endotrypanum
- □ Leishmania
- □ Leptomonas
- ▣ Trypanosoma
  - □ Trypanosoma brucei
  - □ Trypanosoma congolense
  - ☑ Trypanosoma cruzi
  - □ Trypanosoma evansi
  - □ Trypanosoma grayi
  - □ Trypanosoma rangeli
  - □ Trypanosoma vivax

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

Combine Genes in Step 1 with Genes in Step 2:

● 1 **Intersect** 2 ○ 1 **Minus** 2

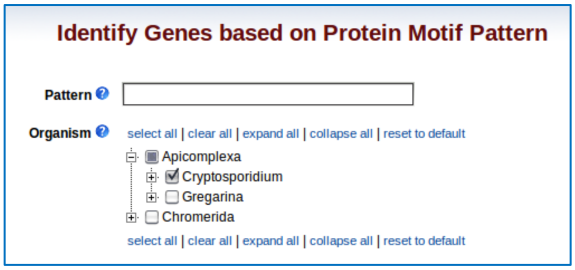
○ 1 **Union** 2 ○ 2 **Minus** 1

○ 1 **Relative to** 2 , using genomic colocation

3. **Find Cryptosporidium genes with the YXXΦ receptor signal motif. Note: for this exercise use** [**http://cryptodb.org**](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. ****Note**: do not look for the Φ symbol on your keyboard – this will not work. Rather you should use the amino acid symbols.*

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).



Identify Genes based on Protein Motif Pattern

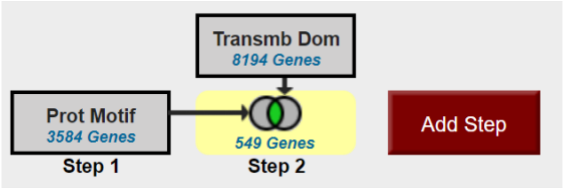
Pattern

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

- ▣ Apicomplexa
  - ☑ Cryptosporidium
  - □ Gregarina
- □ Chromerida

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

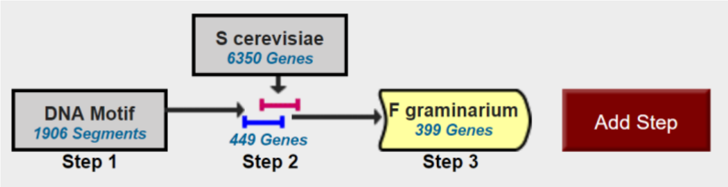
b. How many of these proteins also contain at least one transmembrane domain.



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). Use this link only if you give up: 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

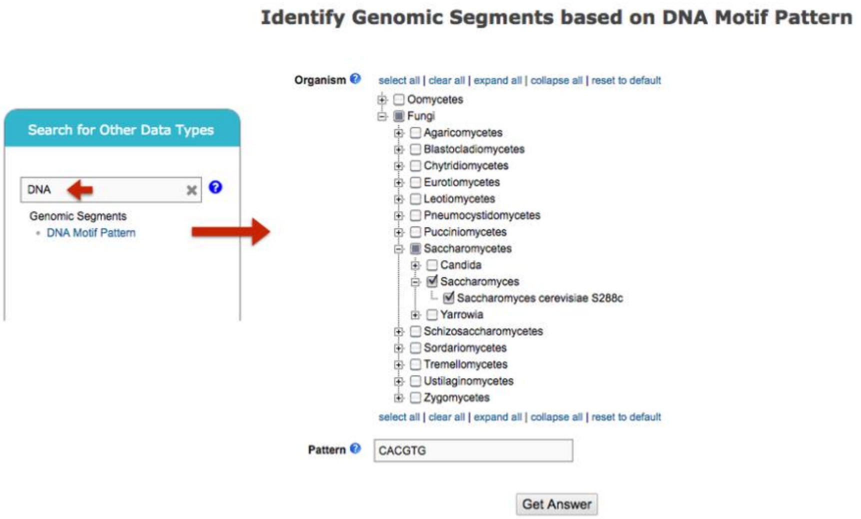
Transcriptional start sites are often located within a certain distance upstream of the genes or gene clusters that they regulate. In fungi, DNA motifs are also important for regulation of processes linked to host cell invasion or production of secondary metabolites. Readily available genomic data facilitate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

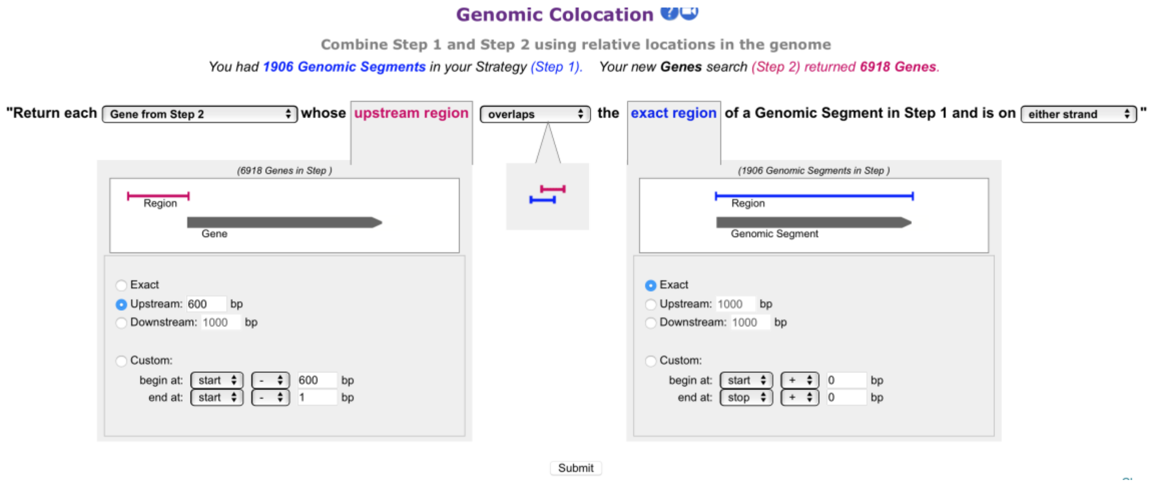
Get Answer

- Your search returns over 1900 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. Run a search for all genes in Saccharomyces cerevisiae and use the colocation tool to identify genes that contain the CACGTG motif in their upstream regions

- 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:



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 [Gene from Step 2] whose upstream region [overlaps] the exact region of a Genomic Segment in Step 1 and is on [either strand]"

(6918 Genes in Step)

○ Exact
● Upstream: 600 bp
○ Downstream: 1000 bp

○ Custom:
begin at: [start] [-] 600 bp
end at: [start] [-] 1 bp

(1906 Genomic Segments in Step)

● Exact
○ Upstream: 1000 bp
○ Downstream: 1000 bp

○ Custom:
begin at: [start] [+] 0 bp
end at: [stop] [+] 0 bp

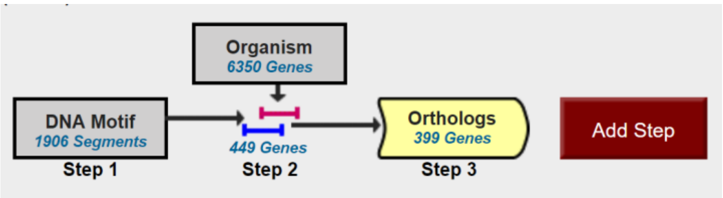
Return each gene from step 2 whose upstream region (600bp) overlaps the exact region of a Genomic Segment in Step1 (CACGTG) and is on either strand.

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 into *F. graminearum*.


Organism
6350 Genes
DNA Motif
1906 Segments
Step 1
449 Genes
Step 2
Orthologs
399 Genes
Step 3
Add Step