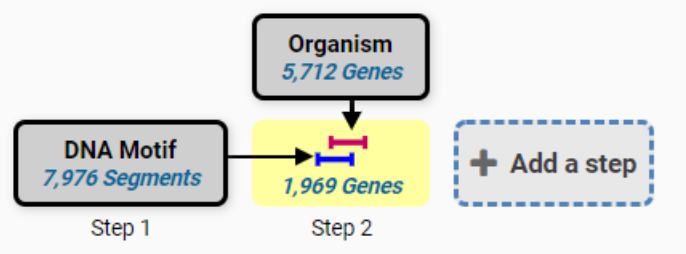
VEuPathDB Webinar 4 June 2020: Motifs and Regular expressions

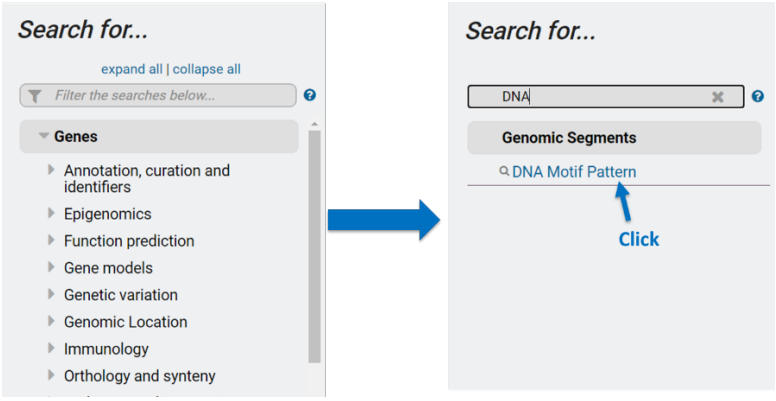
Slide deck - https://docs.google.com/presentation/d/1vvLkSxix-9S5718WB0LLe4wWZ1QD7FtsbEtkKpf5dLU/edit#slide=id.g862fa1b519_3_168

Example: Find EcoR1 restriction enzyme sites within *Plasmodium falciparum* 3D7 genes.

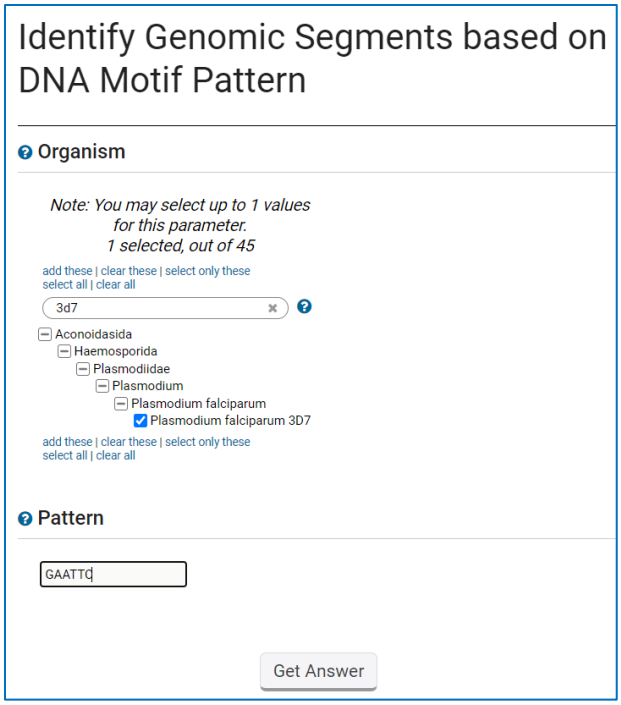
EcoR1 is a restriction enzyme that will cleave DNA whenever it finds the sequence **GAATTC**. EcoR1 can tolerate no ambiguity in the DNA sequence; it always needs GAATTC. VEuPathDB sites have a search called 'Identify Genomic Segments based on DNA Motif Pattern' that will locate and return DNA motifs as genomic segments (a VEuPathDB record type). We will use the DNA Motif Pattern search to find areas of the *Plasmodium falciparum* 3D7 genome that contain the GAATTC motif. The search returns a list of genomic segments. To correlate these genomic segments with genes, we will run a second search for all genesin Plasmodium falciparum 3D7 and use the colocation tool to reduce the list to only those genes that contain the genomic segments we found with the DNA Motif Pattern search. The final strategy will look like this. although future releases of PlasmoDB may return different numbers, since the genome and annotation for *Plasmodium falciparum* 3D7 may change.



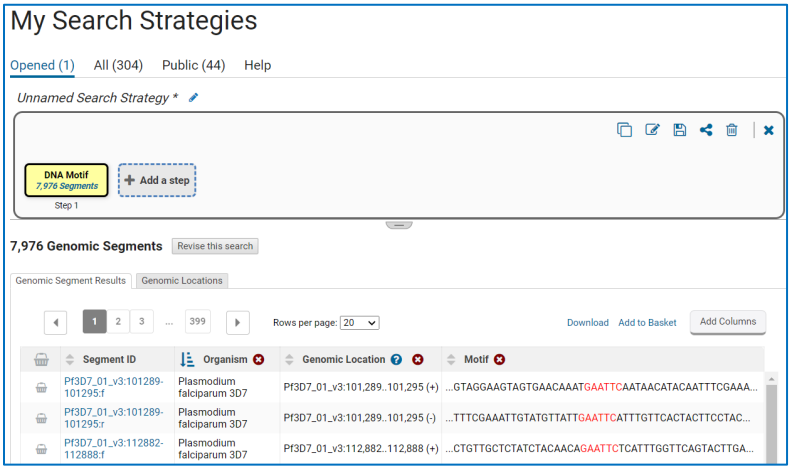
1. Navigate to the DNA motif search.
    a. Go to the home page beta.plasmodb.org https://beta.plasmodb.org/plasmo.beta/app/
    b. Begin typing DNA motif in the Search For… filter and then choose DNA Motif Pattern



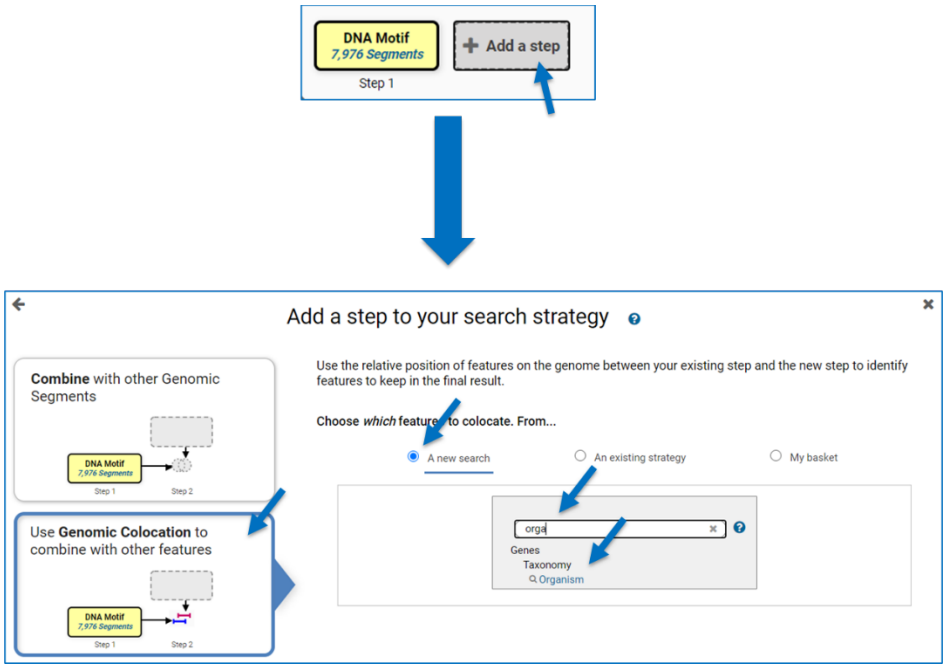
2. Initiate the DNA Motif Pattern Search
    a. Choose to search *Plasmodium falciparum* 3D7.
    b. Type the EcoR1 recognition site, GAATTC, into the **Pattern** field.
    c. Click **Get Answer**



d. Your results are 'segments' of DNA that contain the motif. Each segment has a known location within the *P. falciparum* 3D7 genome.

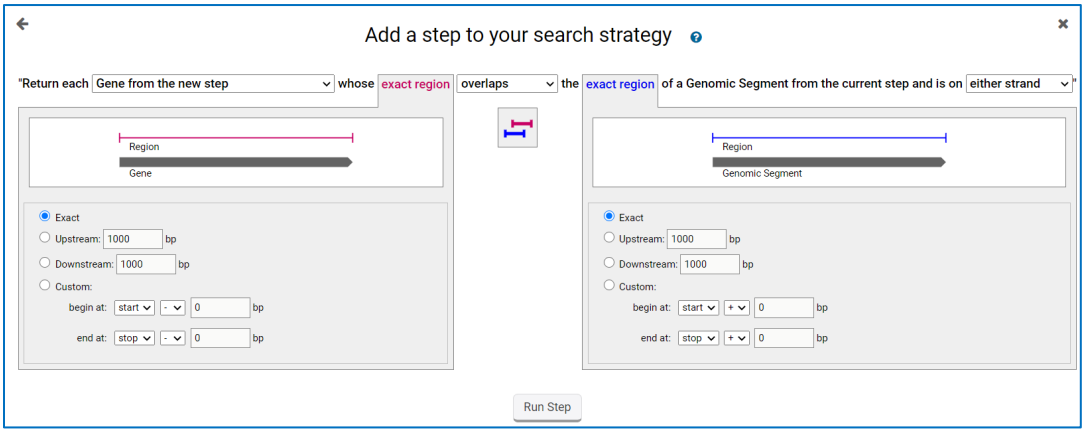


3. Colocate these EcoR1 motifs with *Plasmodium falciparum* 3D7 genes.
    a. Click **Add a step** and choose **Use Genomic Colocation** to combine with other features.
    b. Choose to run **A new search** for **Gene**s based on **Taxonomy, Organism**

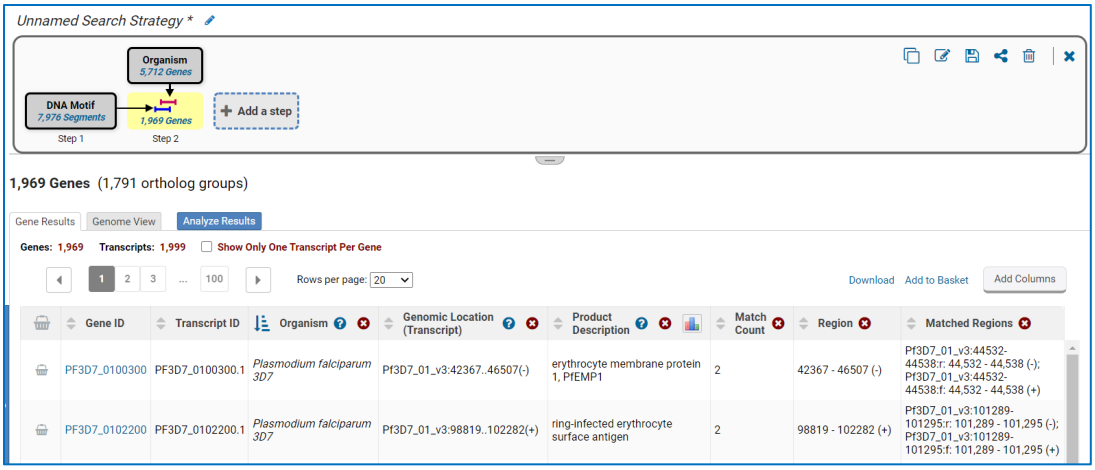


c. From the search form choose *P. falciparum* 3D7 and click Get Answer

d. Arrange the colocation tool to read “Return each gene from the new step whose exact region overlaps the exact region of the genomic segment from the current step and is on either strand”.



e. The result is a list of genes that contain the EcoR1 restriction site (the motif we searched for) and the Region column shows the location of the motif.

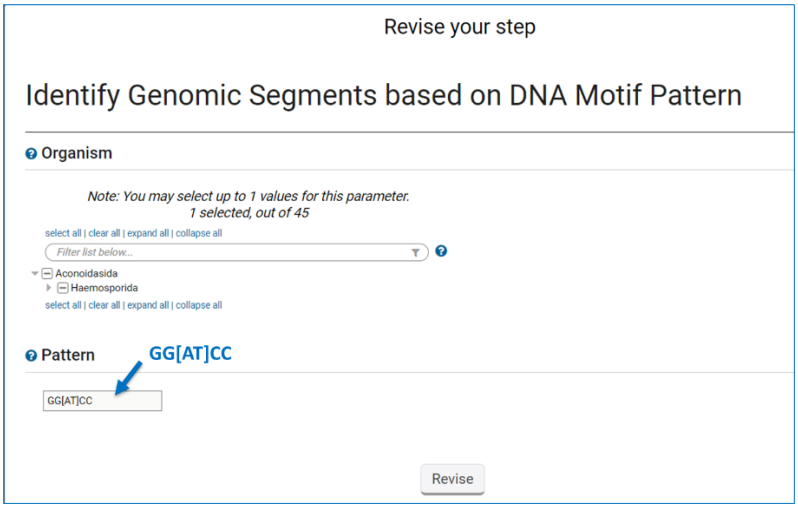


Example: Find AvaII restriction enzyme sites within *Plasmodium falciparum* 3D7 genes.

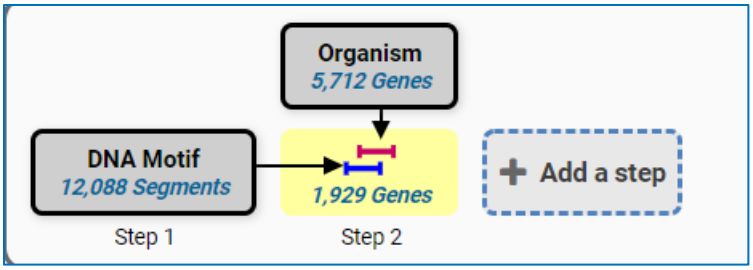
We will use the workflow shown above to find AvaII sites within *Plasmodium falciparum* 3D7 genes. AvaII is a restriction enzyme that will cleave DNA whenever it finds the sequence **GGACC** or **GGTCC**. So AvaII can tolerate some ambiguity in the DNA binding sequence; the middle base can either be an A or a T. To inform the 'Identify Genomic Segments based on DNA Motif Pattern' search about the ambiguous motif that we want to find, we must use a regular expression to communicate the motif sequence.

GGACC or GGTCC = GG[AT]CC (regular expression)

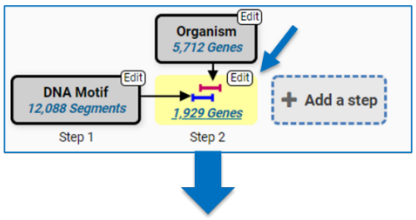
The workflow above will find genes that contain the DNA motif in Step 1. The only change needed in the workflow is to enter the AvaII regular expression for the Pattern in Step 1.

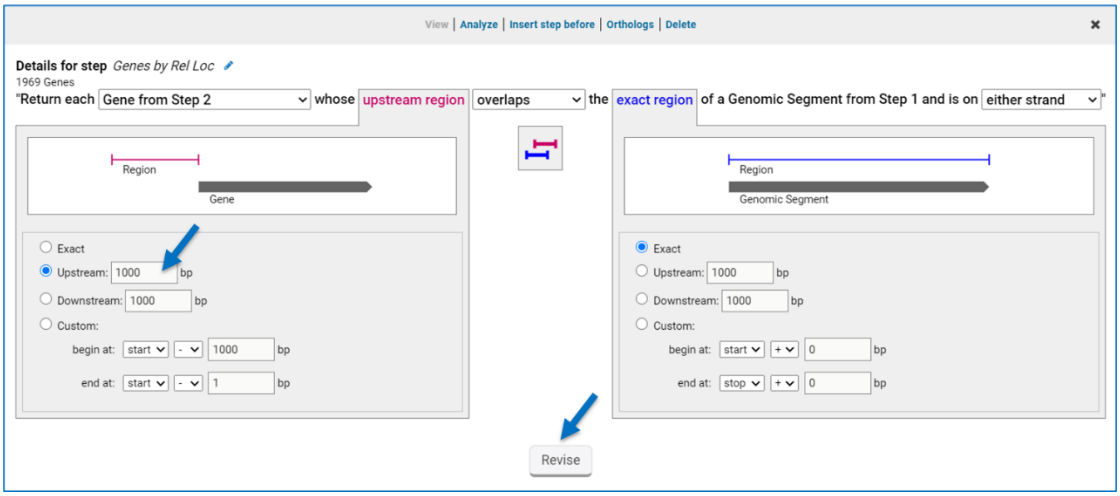


The final strategy will look like this:

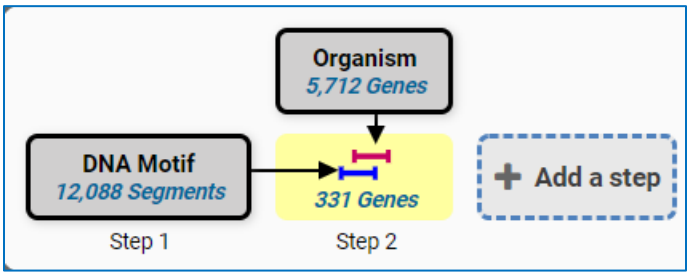


Optional: Find AvaII sites upstream of Pf3D7 genes. The above workflow finds genes that contain restriction sites. To find genes with upstream restriction sites, change the colocation tool to read. "Return each Gene from Step 2 whose upstream region (Upstream 1000bp) overlap with the genomic segments in Step 1 and are on either strand."





Your result will change to:



Example: Explore *Anopheles gambiae* str. PEST genes that have zinc finger domains and the genes that they may regulate.

A zinc finger is a small protein structural motif that is characterized by the coordination of one or more zinc ions (Zn2+) to stabilize the fold. Proteins that contain zinc fingers (zinc finger proteins) are classified into several different structural families. In spite of the large variety of these proteins, the vast majority of zinc finger proteins function as interaction modules that bind DNA, RNA, proteins. Variations in structure serve primarily to alter the binding specificity. (https://en.wikipedia.org/wiki/Zinc_finger)

A zinc finger protein will carry some version of a zinc finger structural motif. Once such motif is described by the PROSITE entry PS00344 GATA-ZN_FINGER_1 https://prosite.expasy.org/PS00344. The pattern described for this zinc finger is:

C-x-[DNEHQSTI]-C-x(4,6)-[ST]-x(2)-[WM]-[HR]-[RKENAMSLPGQT]-x(3,4)-[GNEP]-x(3,6)-C-[NES]-[ASNR]-C

Written as a regular expression, the motif is:

C.[DNEHQSTI]C.{4,6}[ST].{2}[WM][HR][RKENAMSLPGQT].{3,4}[GNEP].{3,6}C[NES][ASNR]C

The genes that zinc finger proteins regulate will contain a 'GATA box' DNA binding motif in their regulatory regions. The motif is short and relatively general:

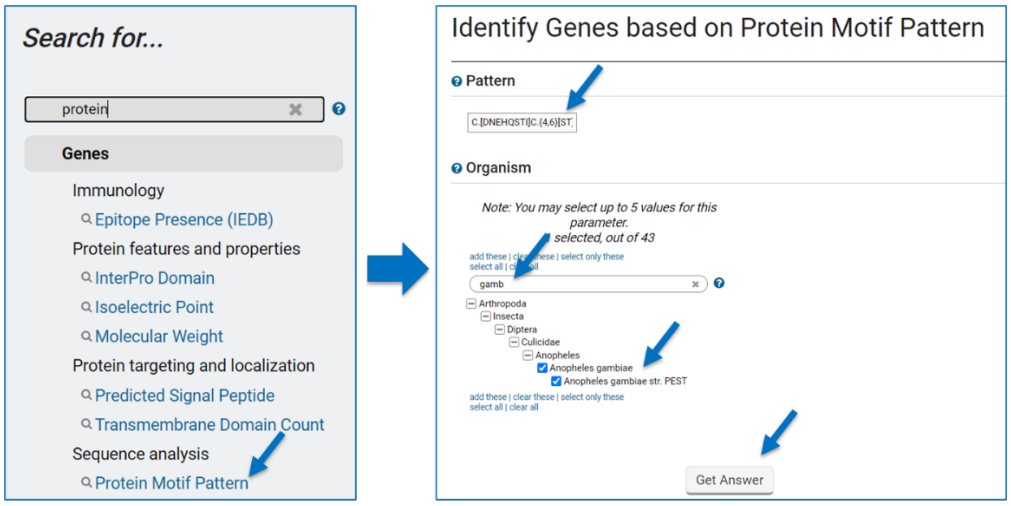
(A/T)GATA(A/G)

and can be written as a regular expression:

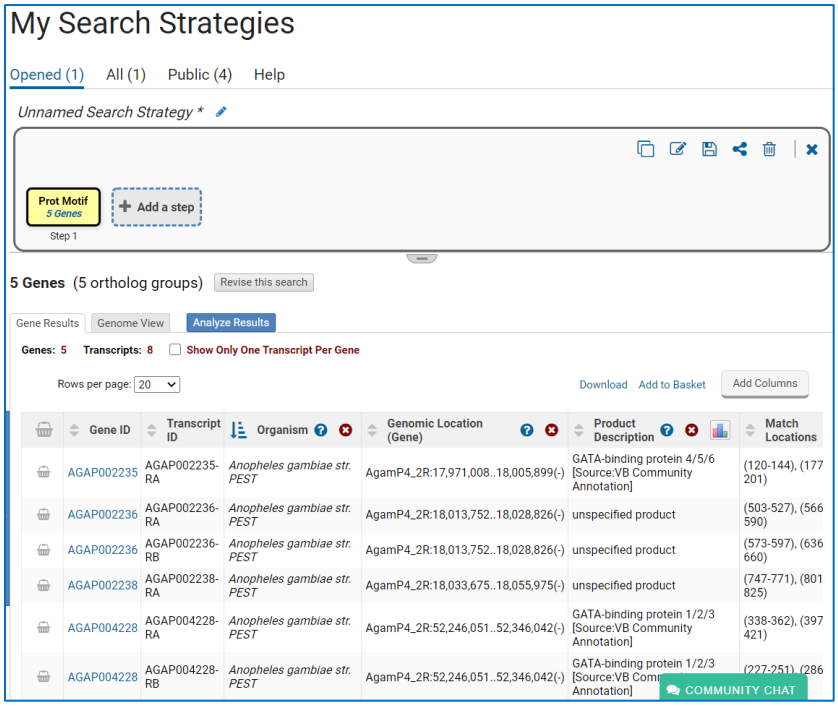
[AT]GATA[AG].

In this exercise we will use beta.vectorbase.org to find *Anopheles gambiae* str. PEST genes whose proteins contain our zinc finger domain and we will also search for possible binding partners by searching for genes that contain the GATA zinc finger domain upstream of their start sites.

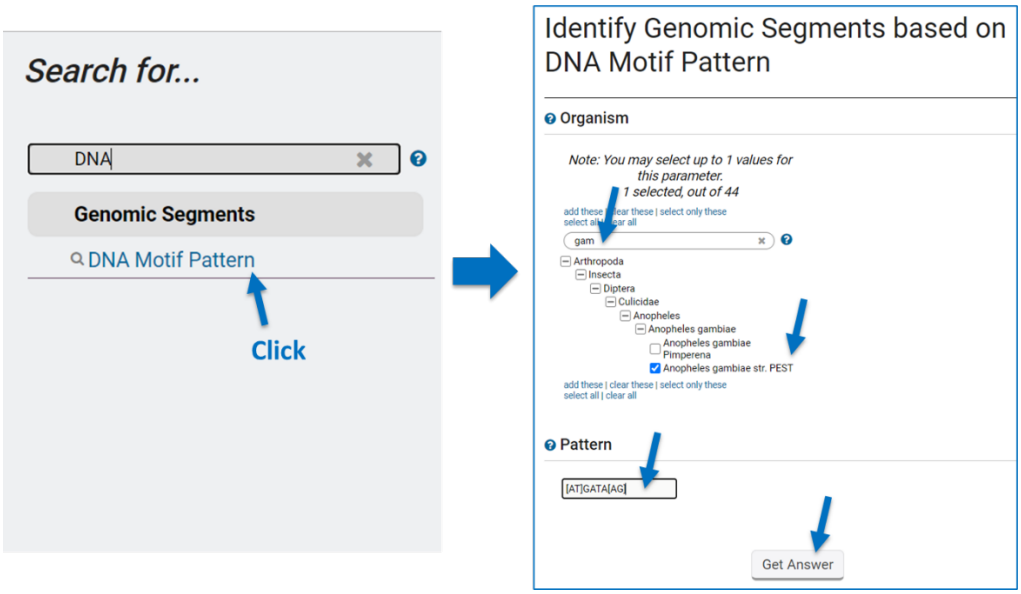
1. Navigate to the Protein Motif Pattern search.
    a. Go to the home page beta.vectorbase.org https://beta.vectorbase.org/vectorbase.beta/app/
    b. Begin typing Protein motif in the Search For… filter and then choose Protein Motif Pattern.
    c. At the search form, enter the protein regular expression, choose *Anopheles gambiae* str. PEST as the organism and click Get Answer.
    Protein regular expression=
    C.[DNEHQSTI]C.{4,6}[ST].{2}[WM][HR][RKENAMSLPGQT].{3,4}[GNEP].{3,6}C[NES][ASNR]C



d. Your results are 5 genes (7 transcripts) whose protein products contain the motif. While three of these genes are already annotated as GATA-binding proteins, two genes (3 transcripts) returned by the search are annotated as unspecified product. In one search we identified functions for two genes with previously unknown functions!

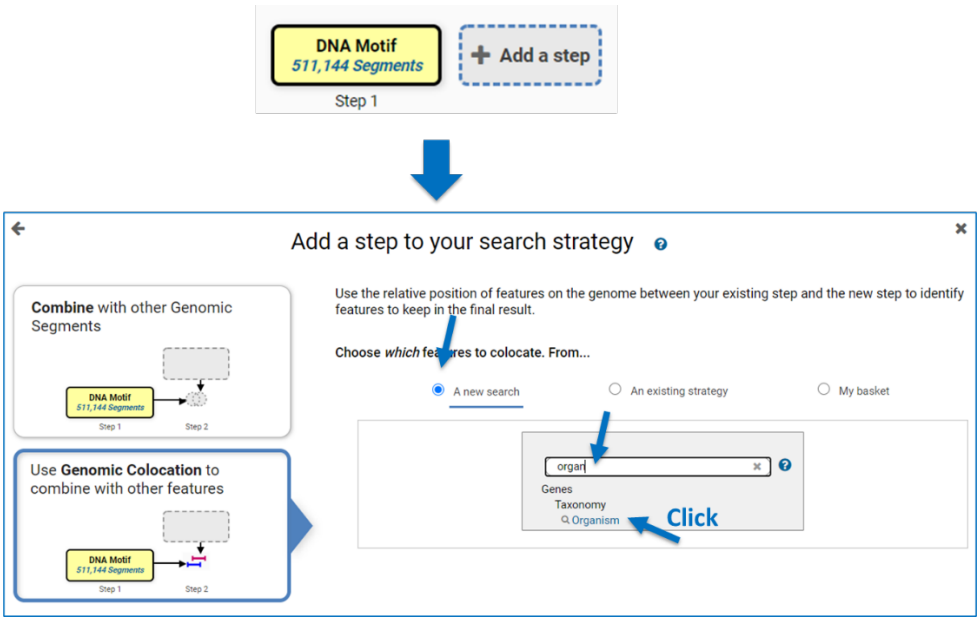


2. Start a new strategy by initiating a DNA Motif Pattern search.
   a. Choose to search *Anopheles gambiae* str. PEST.
   b. Enter the regular expression that represents the GATA site consensus sequence [AT]GATA[AG] into the **Pattern** field.
   c. Click **Get Answer**

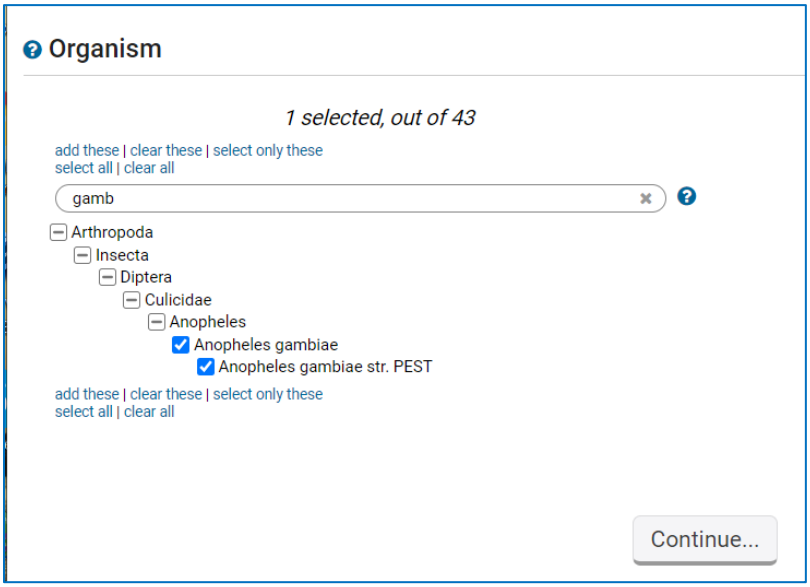


d. Your results are 'segments' of DNA that contain the motif. Each segment has a known location within the *Anopheles gambiae* str. PEST genome. The search returned over 500,000 motifs!

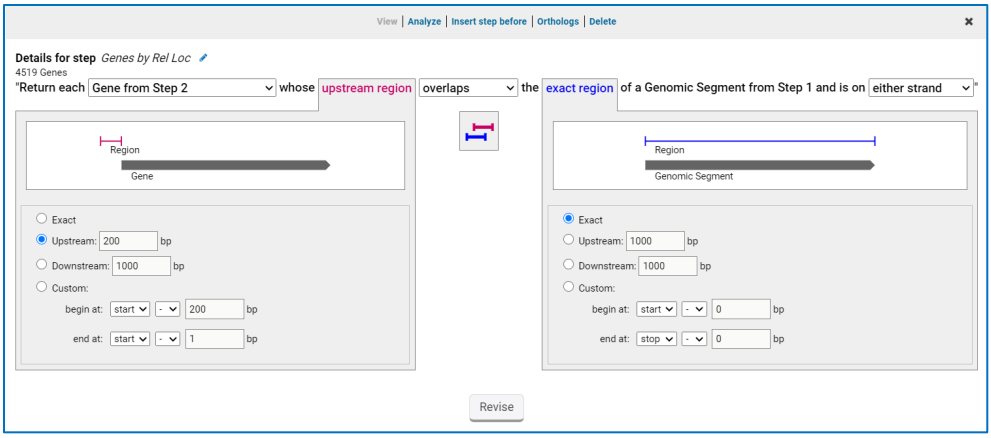
3. Colocate these GATA motifs with *Anopheles gambiae* str. PEST genes.
    a. Click **Add a step** and choose **Use Genomic Colocation** to combine with other features.
    b. Choose to run **A new search** for **Gene**s based on **Taxonomy, Organism**



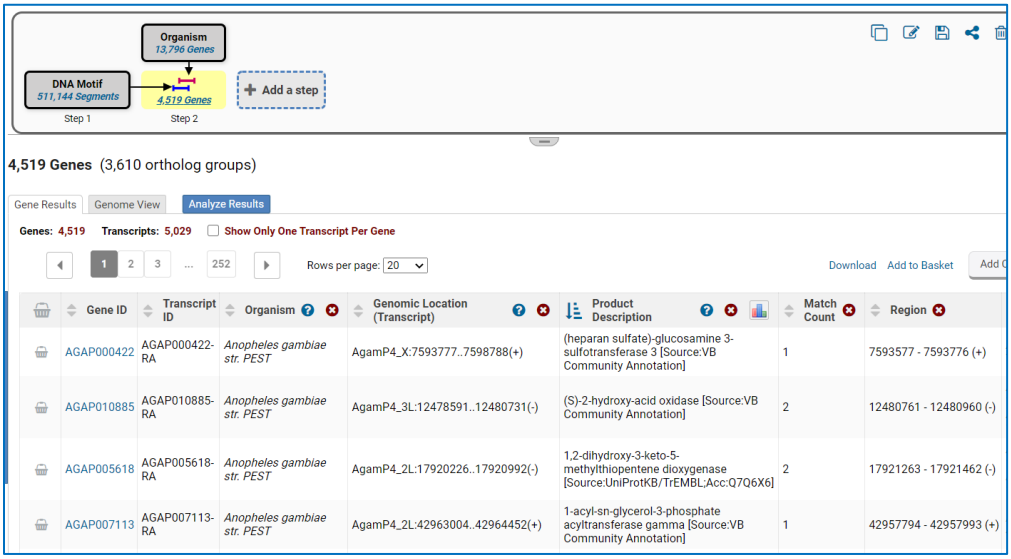
c. At the search form choose *Anopheles gambiae* str. PEST for the **Organism** parameter.



d. Arrange the colocation tool to read “Return each **gene from the new step** whose **upstream 200bp** region **overlaps** the **exact** region of the genomic segment from the current step and is on **either** strand”.



e. The result is a list of genes whose upstream 200bp regions contain the GATA binding motif. The Region column shows the location of the motif.



Gene ID	Transcript ID	Organism	Genomic Location (Transcript)	Product Description	Match Count	Region
AGAP000422	AGAP000422-RA	*Anopheles gambiae str. PEST*	AgamP4_X:7593777..7598788(+)	(heparan sulfate)-glucosamine 3-sulfotransferase 3 [Source:VB Community Annotation]	1	7593577 - 7593776 (+)
AGAP010885	AGAP010885-RA	*Anopheles gambiae str. PEST*	AgamP4_3L:12478591..12480731(-)	(S)-2-hydroxy-acid oxidase [Source:VB Community Annotation]	2	12480761 - 12480960 (-)
AGAP005618	AGAP005618-RA	*Anopheles gambiae str. PEST*	AgamP4_2L:17920226..17920992(-)	1,2-dihydroxy-3-keto-5-methylthiopentene dioxygenase [Source:UniProtKB/TrEMBL;Acc:Q7Q6X6]	2	17921263 - 17921462 (-)
AGAP007113	AGAP007113-RA	*Anopheles gambiae str. PEST*	AgamP4_2L:42963004..42964452(+)	1-acyl-sn-glycerol-3-phosphate acyltransferase gamma [Source:VB Community Annotation]	1	42957794 - 42957993 (+)