Strategies Tutorial

Note: This exercise uses PlasmoDB.org as an example, but the same functionality is available on a VEuPathDB resources.

Learning objectives:

- Build a multistep strategy
- Use the Text, GO Term, RNA-Seq, and SNP searches
- Combine search results using Boolean operators and the colocation tool
- Transform genes of one organism into their orthologs in another organism
- Infer expression timing from a well-studied organism onto another organism that lacks data.

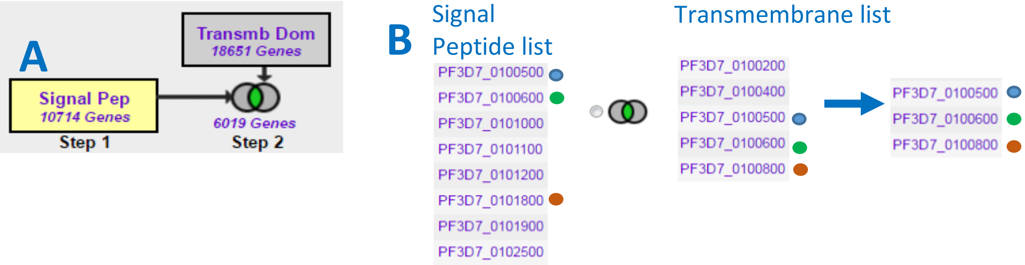
In this tutorial you will find *P. vivax* genes that are likely expressed in gametocytes, act as proteases and have variation in their upstream regions, possibly affecting promoter regions and other gene features. The strategy you build will take advantage of the data rich organism of *P. falciparum* 3D7 to perform three different searches against data from *P. falciparum*. You will take advantage of the orthology profiles to transform the *P. falciparum* genes into their *P. vivax* orthologs and then search for SNPs in the upstream regions of the *P.vivax* genes. The ortholog transform enables you make inferences about genes in *P. vivax*, an organism with limited functional data, based on existing data in the closely related and well-studied *P. falciparum*. The *P. vivax* genes returned by the search are likely to share two biological properties, proteolytic activity and expression in gametocytes, and show variation in their upstream regions, possibly affecting promoter regions or other gene features.

Before we get started… a few words about combining search results:

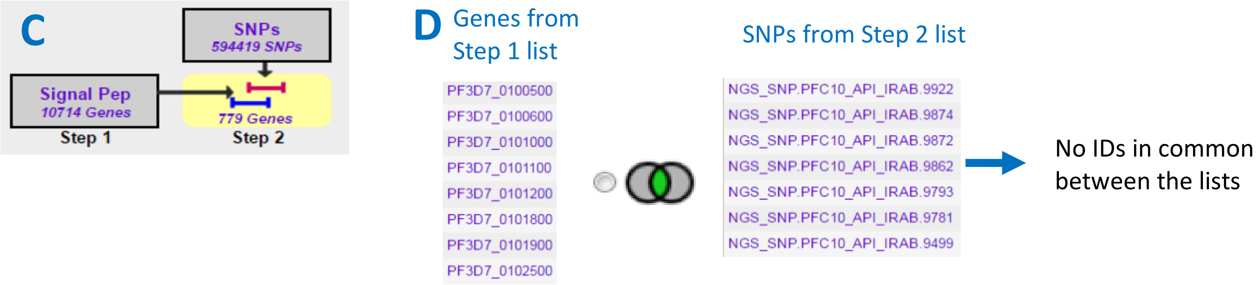
Each search returns a list of IDs. When two searches are combined, the two result sets (list of IDs) are merged. The table shows the 5 options for combining search results.

Operator	:	Combined Result will contain:
○ 1 INTERSECT 2	:	IDs in common between the two lists
◉ 1 UNION 2	:	IDs from list 1 and list 2
○ 1 MINUS 2	:	IDs unique to 1
○ 2 MINUS 1	:	IDs unique to 2
○ 1 **Relative to** 2	:	IDs whose features are near each other (collocated) in the genome

If the searches return the same type of genomic feature they can be combined using any of the 5 operators (i.e. search 1 returns genes, search 2 returns genes as in screenshot group A below).



However, searches that return different genomic features will yield no results when combined with intersect, union or minus operators. The Genomic Co-Location tool takes advantage of the genomic location of each gene and each SNP and returns features based on their relative genomic location, i.e. SNPs that are near or within genes.

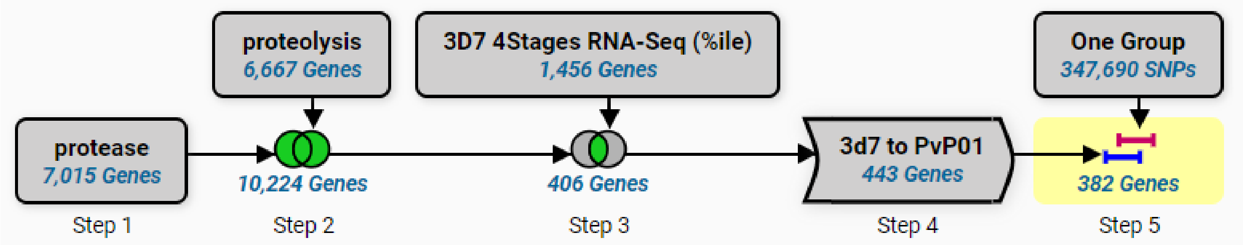


PF3D7_0100500
PF3D7_0100600
PF3D7_0101000
PF3D7_0101100
PF3D7_0101200
PF3D7_0101800
PF3D7_0101900
PF3D7_0102500

NGS_SNP.PFC10_API_IRAB.9922
NGS_SNP.PFC10_API_IRAB.9874
NGS_SNP.PFC10_API_IRAB.9872
NGS_SNP.PFC10_API_IRAB.9862
NGS_SNP.PFC10_API_IRAB.9793
NGS_SNP.PFC10_API_IRAB.9781
NGS_SNP.PFC10_API_IRAB.9499

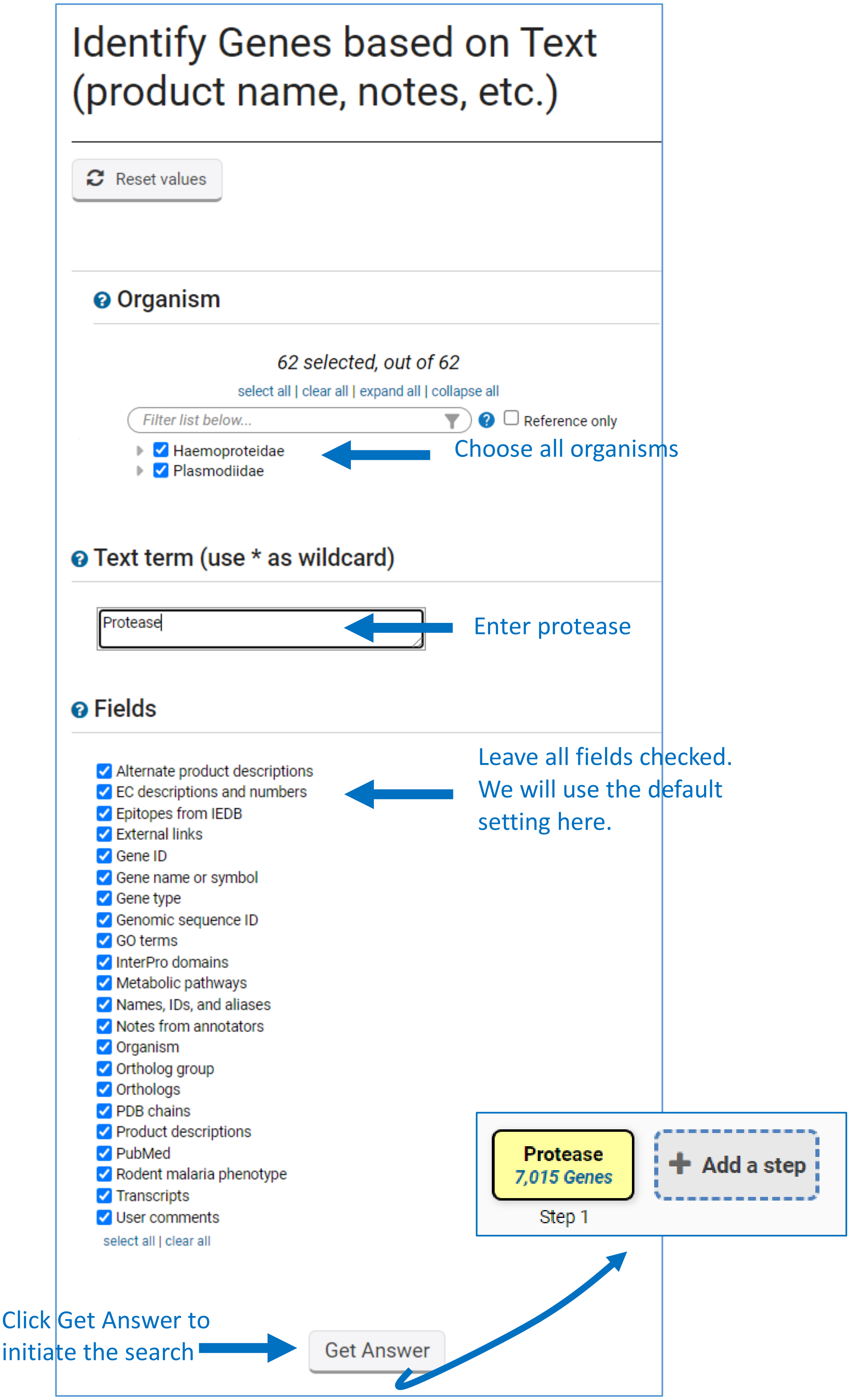
Building the Strategy:

Find *P. vivax* genes that are possible proteases, likely expressed during the gametocyte stages and contain SNPs in their upstream regions. The final strategy will look like this.



Step by Step Instructions

1. **Run a text search using protease as the text term.**
   Navigation: >PlasmoDB >Search for Genes >Text > Text (product name, notes, etc.)



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

Reset values

Organism

62 selected, out of 62

select all | clear all | expand all | collapse all

Filter list below... □ Reference only

- ☑ Haemoproteidae
- ☑ Plasmodiidae

Choose all organisms

Text term (use * as wildcard)

Protease

Enter protease

Fields

- ☑ Alternate product descriptions
- ☑ EC descriptions and numbers
- ☑ Epitopes from IEDB
- ☑ External links
- ☑ Gene ID
- ☑ Gene name or symbol
- ☑ Gene type
- ☑ Genomic sequence ID
- ☑ GO terms
- ☑ InterPro domains
- ☑ Metabolic pathways
- ☑ Names, IDs, and aliases
- ☑ Notes from annotators
- ☑ Organism
- ☑ Ortholog group
- ☑ Orthologs
- ☑ PDB chains
- ☑ Product descriptions
- ☑ PubMed
- ☑ Rodent malaria phenotype
- ☑ Transcripts
- ☑ User comments

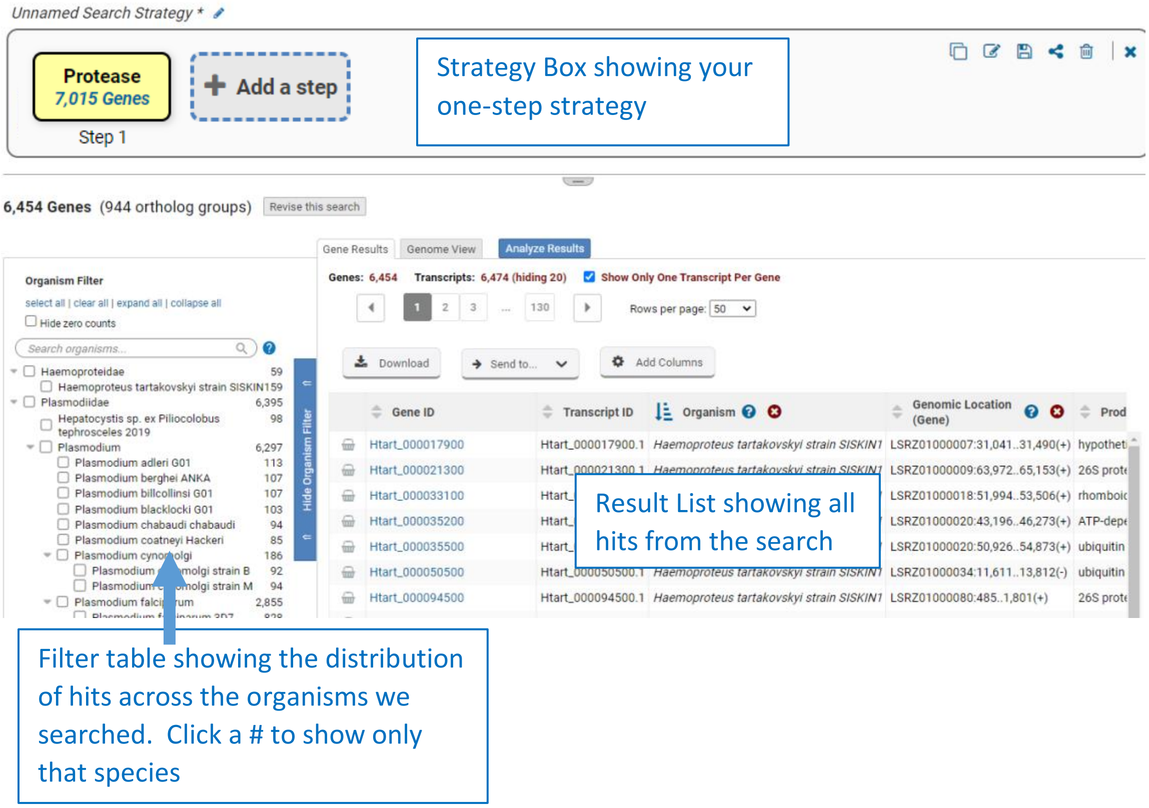
select all | clear all

Leave all fields checked. We will use the default setting here.

Protease
7,015 Genes
Step 1

Click Get Answer to initiate the search

You created a one-step strategy by running the text search. The strategy returns 7015 genes that are annotated with the word ‘protease’. This annotation could appear in any field that you searched. You can analyze this result by exploring the hits. Look at the data in the columns of the result table. You can add more data with the **Add Columns** button. Clicking a gene ID in the first column will take you to that gene’s record page. Please explore your results to see if they make sense. For example, gene product names might contain the word ‘protease’.



Strategy Box showing your one-step strategy

6,454 Genes (944 ortholog groups) Revise this search

Organism Filter

select all | clear all | expand all | collapse all

□ Hide zero counts

□ Haemoproteidae	59
□ Haemoproteus tartakovskyi strain SISKIN1	59
□ Plasmodiidae	6,395
□ Hepatocystis sp. ex Piliocolobus tephrosceles 2019	98
□ Plasmodium	6,297
□ Plasmodium adleri G01	113
□ Plasmodium berghei ANKA	107
□ Plasmodium billcollinsi G01	107
□ Plasmodium blacklocki G01	103
□ Plasmodium chabaudi chabaudi	94
□ Plasmodium coatneyi Hackeri	85
□ Plasmodium cynomolgi	186
□ Plasmodium cynomolgi strain B	92
□ Plasmodium cynomolgi strain M	94
□ Plasmodium falciparum	2,855

Genes: 6,454 **Transcripts:** 6,474 (hiding 20) ☑ Show Only One Transcript Per Gene

Rows per page: 50

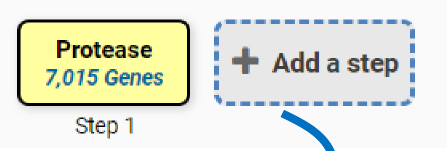
Gene ID	Transcript ID	Organism	Genomic Location (Gene)	Prod
Htart_000017900	Htart_000017900.1	*Haemoproteus tartakovskyi strain SISKIN1*	LSRZ01000007:31,041..31,490(+)	hypotheti
Htart_000021300	Htart_000021300.1	*Haemoproteus tartakovskyi strain SISKIN1*	LSRZ01000009:63,972..65,153(+)	26S prote
Htart_000033100	Htart_		LSRZ01000018:51,994..53,506(+)	rhomboic
Htart_000035200	Htart_		LSRZ01000020:43,196..46,273(+)	ATP-depe
Htart_000035500	Htart_		LSRZ01000020:50,926..54,873(+)	ubiquitin
Htart_000050500	Htart_000050500.1	*Haemoproteus tartakovskyi strain SISKIN1*	LSRZ01000034:11,611..13,812(-)	ubiquitin
Htart_000094500	Htart_000094500.1	*Haemoproteus tartakovskyi strain SISKIN1*	LSRZ01000080:485..1,801(+)	26S prote

Result List showing all hits from the search

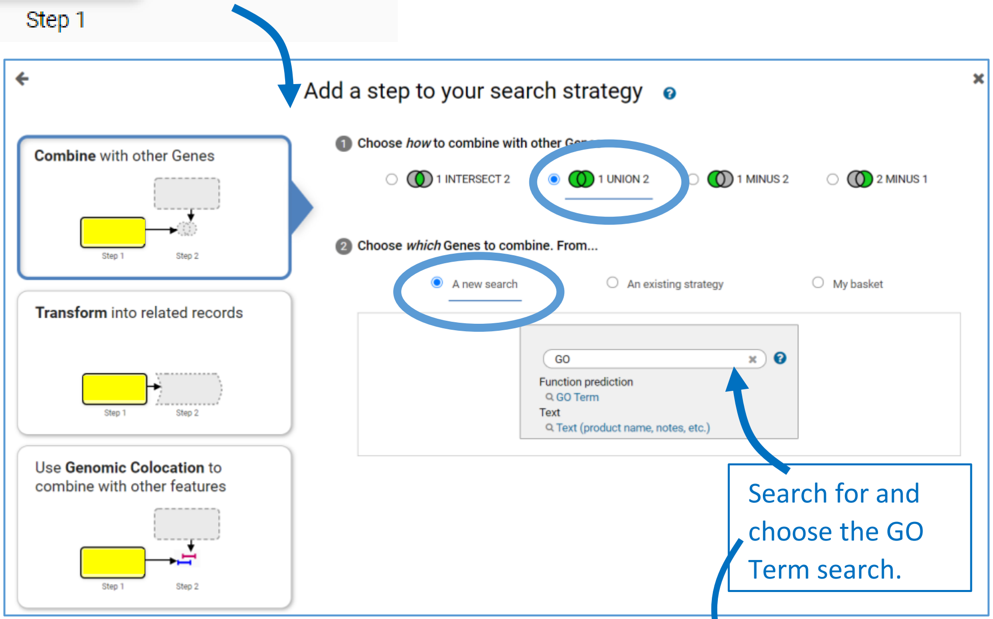
Filter table showing the distribution of hits across the organisms we searched. Click a # to show only that species

Add a step choosing to run a search for genes annotated with the biological process gene ontology term – GO:0006508: proteolysis. Gene Ontology annotations offer a second line of evidence for finding proteases.

Navigation: Add Step >Combine with other Genes >1 union 2 > A new search >GO Term



Protease
7,015 Genes
Step 1



Add a step to your search strategy

Combine with other Genes
Step 1
Step 2
Transform into related records
Step 1
Step 2
Use Genomic Colocation to combine with other features
Step 1
Step 2

1 Choose *how* to combine with other Ge

○ 1 INTERSECT 2 ● 1 UNION 2 ○ 1 MINUS 2 ○ 2 MINUS 1

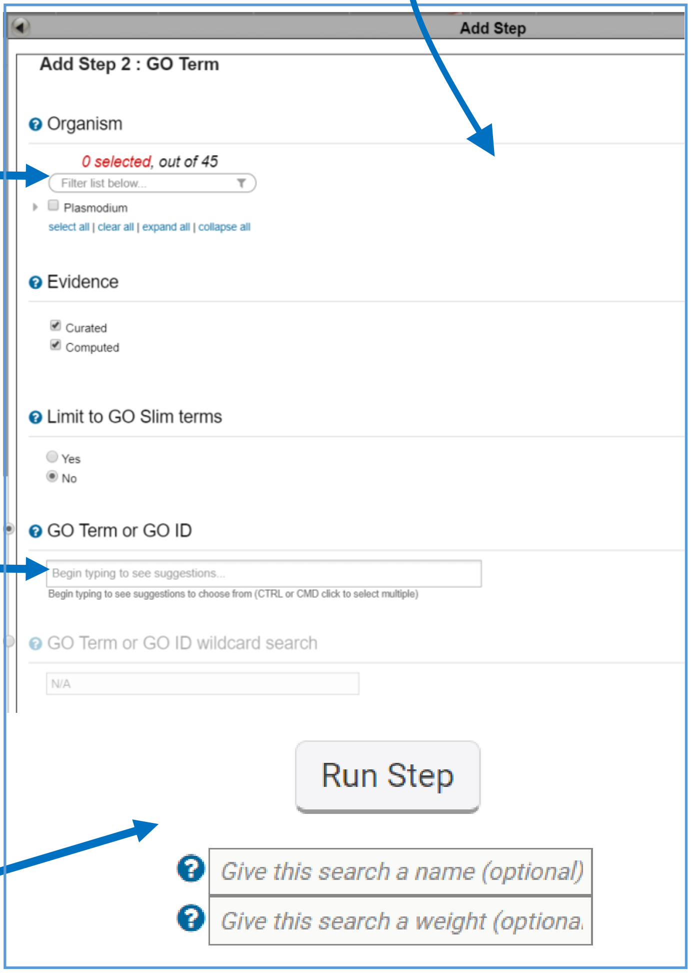
2 Choose *which* Genes to combine. From...

● A new search ○ An existing strategy ○ My basket

GO

Function prediction
GO Term
Text
Text (product name, notes, etc.)

Search for and choose the GO Term search.



Add Step

Add Step 2 : GO Term

Organism

0 selected, out of 45

Filter list below...

□ Plasmodium

select all | clear all | expand all | collapse all

Evidence

☑ Curated
☑ Computed

Limit to GO Slim terms

○ Yes
● No

GO Term or GO ID

Begin typing to see suggestions...

Begin typing to see suggestions to choose from (CTRL or CMD click to select multiple)

GO Term or GO ID wildcard search

N/A

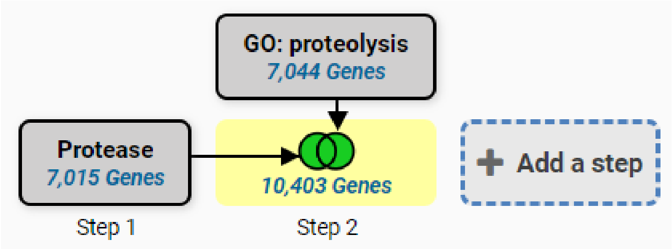
Give this search a name (optional)
Give this search a weight (optiona

Which organism is chosen by default for this search? Click 'select all' to run the search on all organisms

Begin typing Proteolysis and then choose the correct GO term from the list

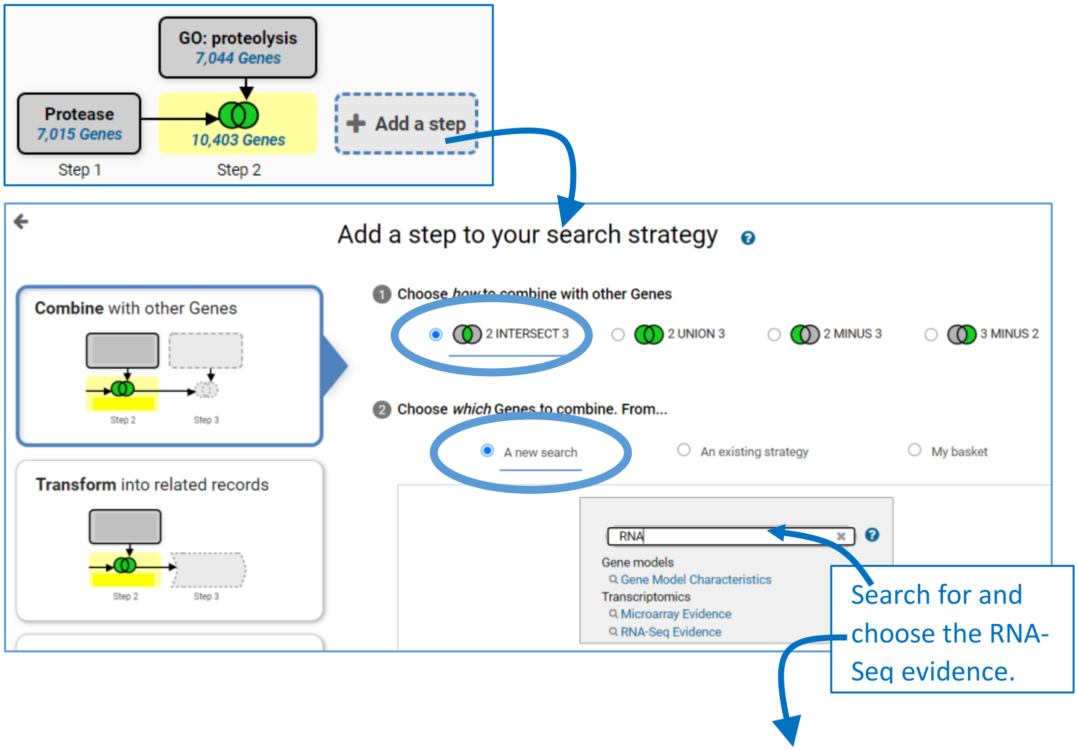
Click Run Step to initiate the search

Strategy Result: The GO term search returned 7044 genes annotated with the proteolysis GO term. The union of the text and GO search returns 10,403 genes that are suspected to have proteolytic activity.

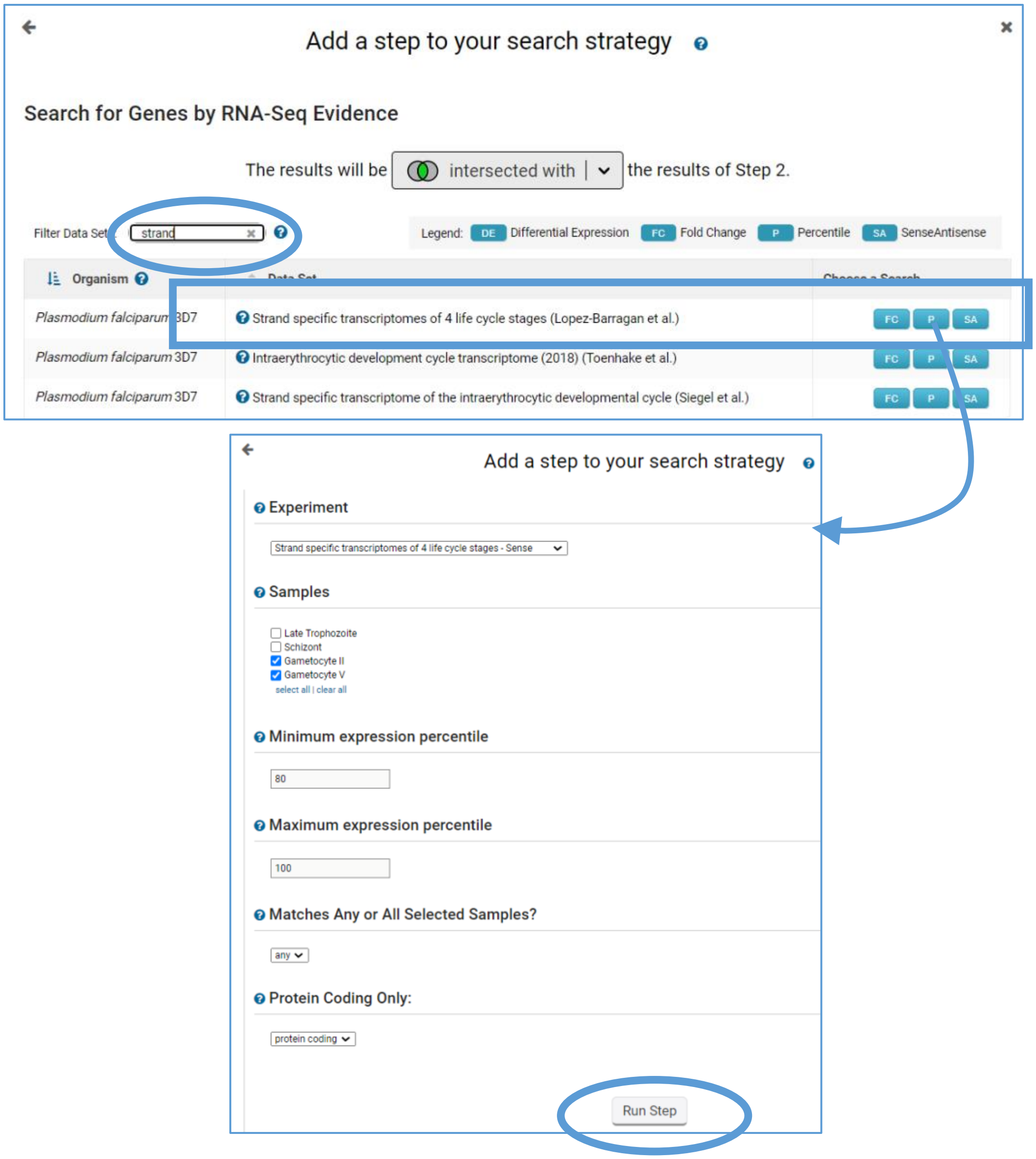


2. **Add a step choosing to run a search for genes based on Transcript Expression using RNA-Seq Evidence.** Since PlasmoDB has integrated several RNA sequencing data sets you must first choose what data set (experiment) to search before you are taken to the search form to choose parameters. Choose the experiment "**Strand specific transcriptomes of 4 life cycle stages (Lopez-Barragan et al.)**". This data contains RNA-Seq transcriptomes for trophozoites, schizonts and gametocytes. Since you want the resulting genes to be proteases AND show expression in gametocytes, choose **intersect** to combine the steps.

   Navigation: Add Step >Combine with other Genes >2 intersect 3 >A new search >RNA Seq Evidence



Search for and choose the RNA-Seq evidence.



Add a step to your search strategy

Search for Genes by RNA-Seq Evidence

The results will be [intersected with] the results of Step 2.

Filter Data Set: strand

Legend: DE Differential Expression FC Fold Change P Percentile SA SenseAntisense

Organism	Data Set	Choose a Search
Plasmodium falciparum 3D7	Strand specific transcriptomes of 4 life cycle stages (Lopez-Barragan et al.)	FC P SA
Plasmodium falciparum 3D7	Intraerythrocytic development cycle transcriptome (2018) (Toenhake et al.)	FC P SA
Plasmodium falciparum 3D7	Strand specific transcriptome of the intraerythrocytic developmental cycle (Siegel et al.)	FC P SA

Add a step to your search strategy

Experiment: Strand specific transcriptomes of 4 life cycle stages - Sense

Samples:

☐ Late Trophozoite
☐ Schizont
☑ Gametocyte II
☑ Gametocyte V

select all | clear all

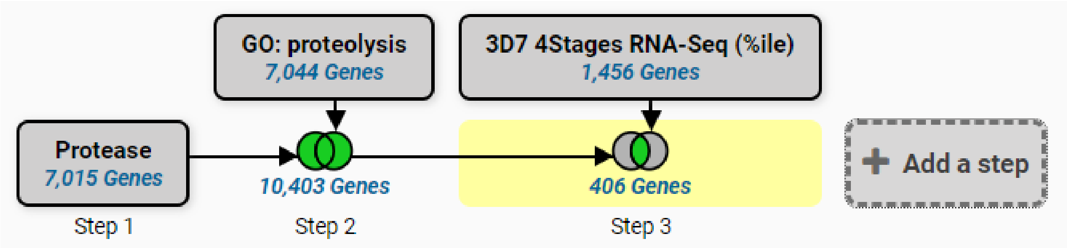
Minimum expression percentile: 80

Maximum expression percentile: 100

Matches Any or All Selected Samples?: any

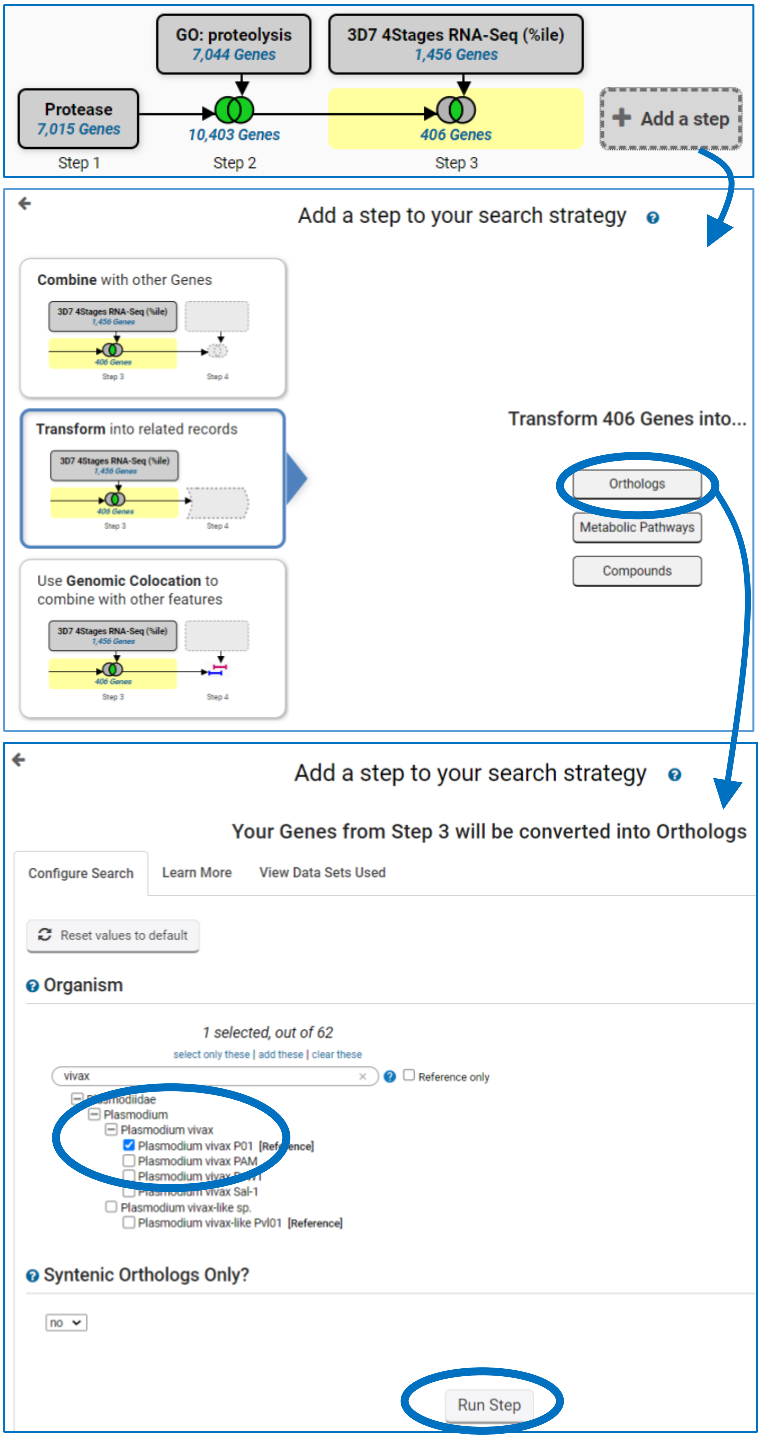
Protein Coding Only: protein coding

Strategy result: We have a three-step strategy that returns 406 *P. falciparum* genes that are suspected proteases with evidence for expression in gametocytes based on RNA Sequencing data. Explore your gene list!!



3. **Add a step to the strategy that transforms the 406 *P. falciparum* genes into *P. vivax* genes.**
P. falciparum is a well-studied organism with active curatorial efforts and large amounts of functional data. A researcher interested in *P. vivax* can take advantage of the *P. falciparum* data by creating a strategy based on *P. falciparum* data then transforming the results to their *P. vivax* orthologs.

Navigation: >Add Step >Transform into related records >Orthologs



Add a step to your search strategy

Transform 406 Genes into...

Add a step to your search strategy

Your Genes from Step 3 will be converted into Orthologs

Organism

1 selected, out of 62

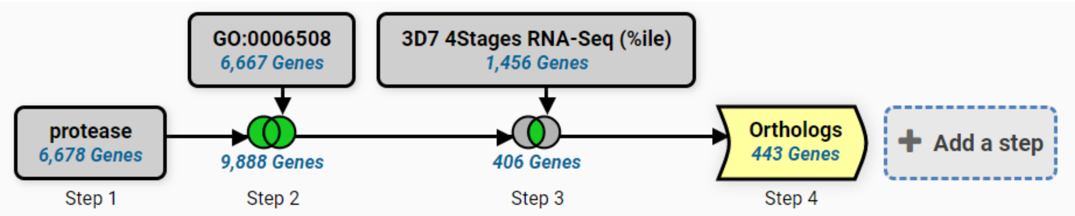
vivax ☐ Reference only

⊟ Plasmodiidae
⊟ Plasmodium
⊟ Plasmodium vivax
☑ Plasmodium vivax P01 [Reference]
☐ Plasmodium vivax PAM
☐ Plasmodium vivax PvW1
☐ Plasmodium vivax Sal-1
☐ Plasmodium vivax-like sp.
☐ Plasmodium vivax-like Pvl01 [Reference]

Syntenic Orthologs Only?

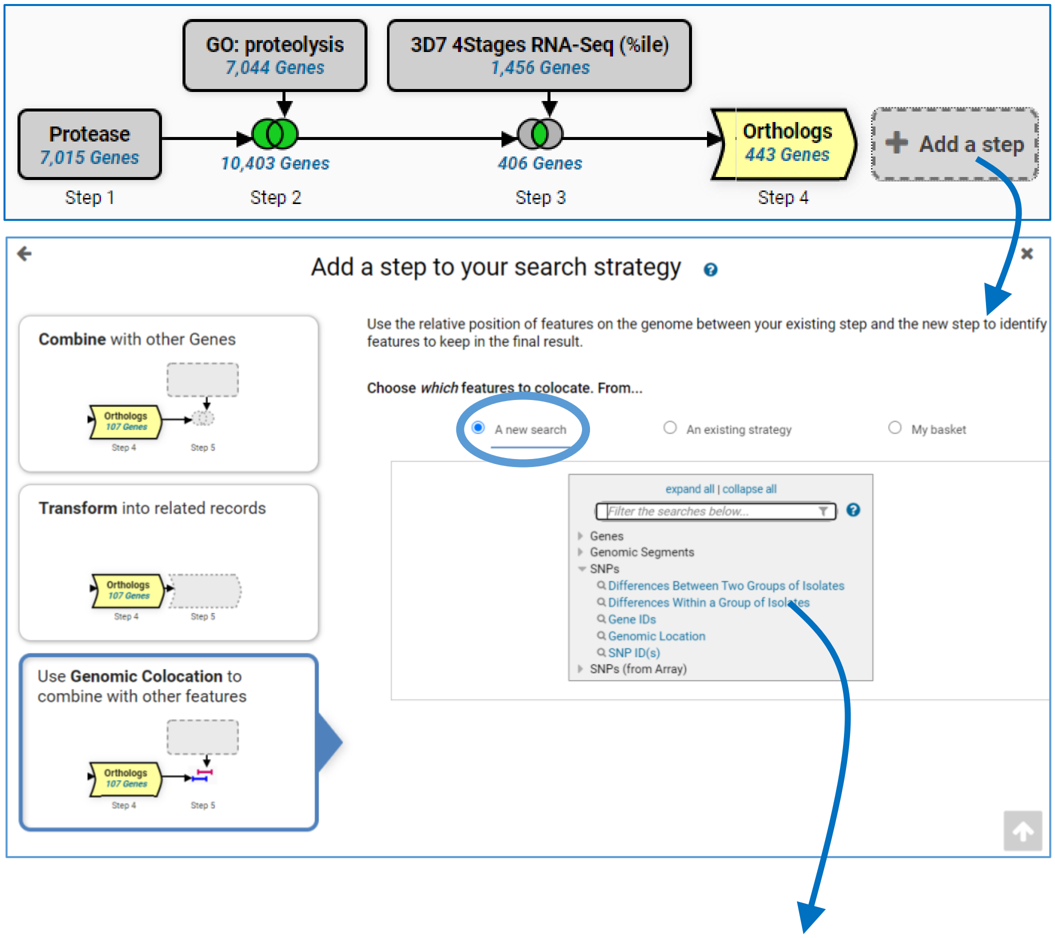
no

Strategy Result: We have a four-step strategy that returns 443 *P. vivax* genes that are suspected proteases with evidence for expression in gametocytes based on RNA Sequencing data.



4. **Add a step to the strategy that returns *P vivax* SNPs and collocate those SNPs to the upstream 1000bp of the *P. vivax* genes in step 4.** We can look for variation (SNPs) associated with the genes from Step 4. PlasmoDB integrates whole genome resequencing data from many isolates, and PlasmoDB contains 236 datasets from whole-genome sequencing of *P. vivax* isolates. The search we will use analyzes whole genome resequencing data from all *P. vivax* isolates to find SNPs shared between all isolates. You will notice that initiating the search does not immediately bring up the result, but instead leads you to the colocation tool.

Navigation: >Add Step >Use Genomic Colocation >A new search >Differences Within a Group of Isolates



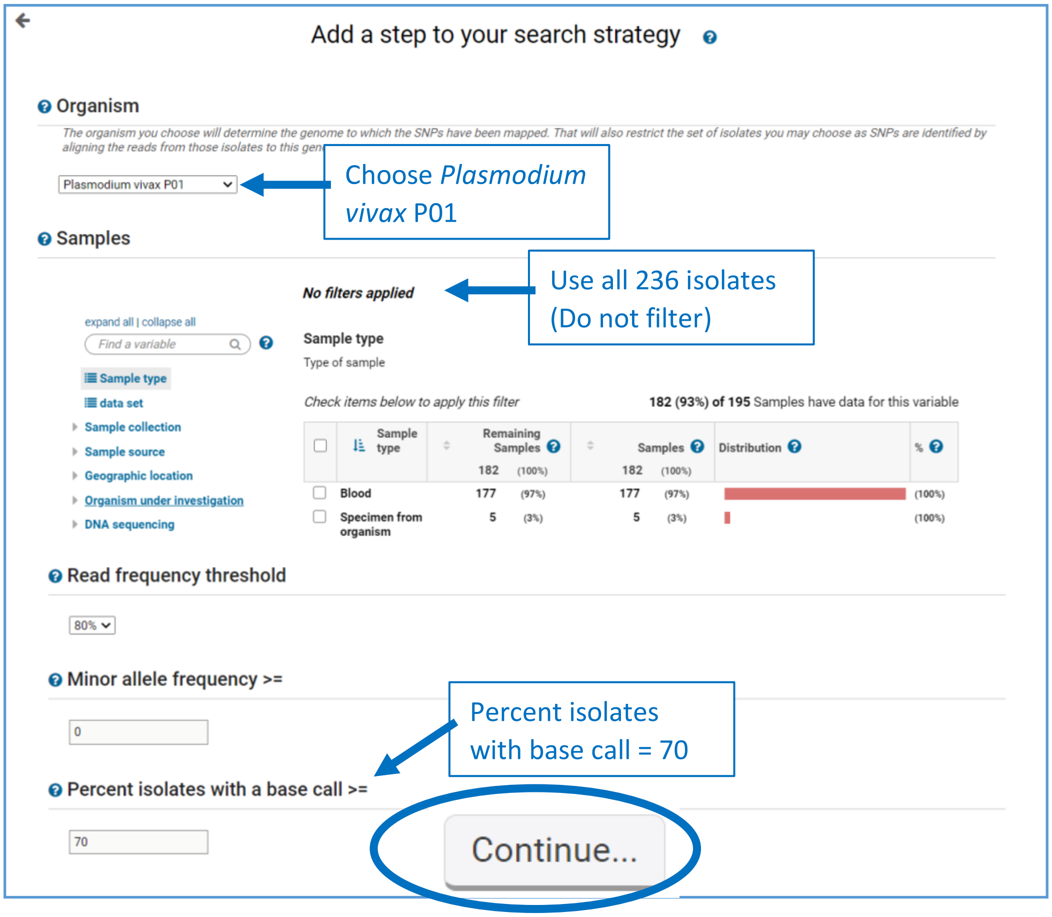
Add a step to your search strategy

Use the relative position of features on the genome between your existing step and the new step to identify features to keep in the final result.

Choose *which* features to colocate. From...

● A new search ○ An existing strategy ○ My basket

- Genes
- Genomic Segments
- SNPs
  - Differences Between Two Groups of Isolates
  - Differences Within a Group of Isolates
  - Gene IDs
  - Genomic Location
  - SNP ID(s)
- SNPs (from Array)



Add a step to your search strategy

Organism

The organism you choose will determine the genome to which the SNPs have been mapped. That will also restrict the set of isolates you may choose as SNPs are identified by aligning the reads from those isolates to this gen

Plasmodium vivax P01

Choose *Plasmodium vivax* P01

Samples

No filters applied

Use all 236 isolates (Do not filter)

Sample type

Type of sample

Check items below to apply this filter

182 (93%) of 195 Samples have data for this variable

□	Sample type	Remaining Samples	Samples	Distribution	%
		182 (100%)	182 (100%)		
□	Blood	177 (97%)	177 (97%)		(100%)
□	Specimen from organism	5 (3%)	5 (3%)		(100%)

Read frequency threshold

80%

Minor allele frequency >=

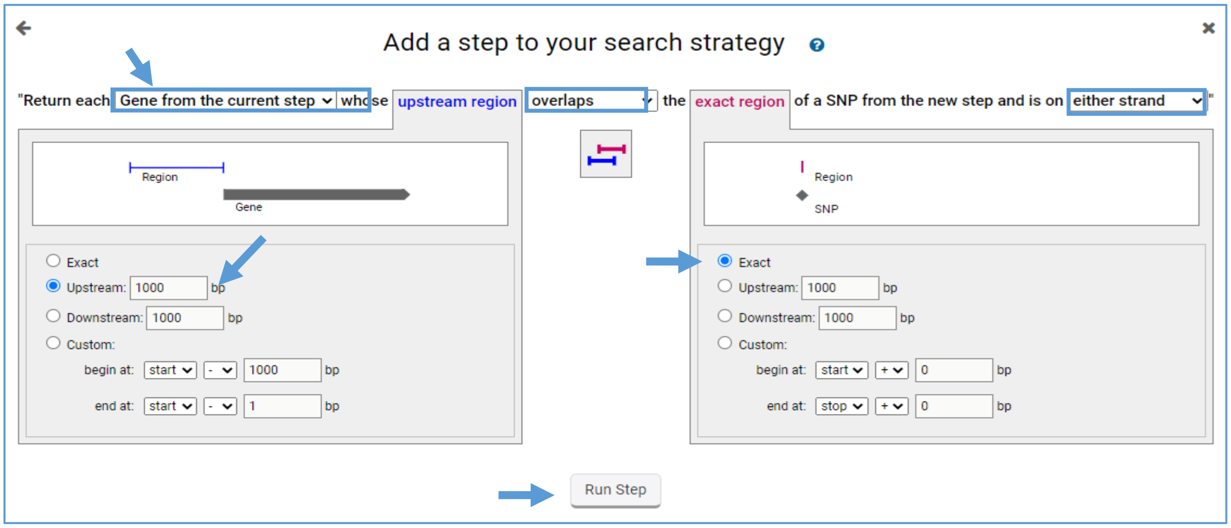
0

Percent isolates with a base call >=

70

Percent isolates with base call = 70

Colocation: Because this search returns SNPs and not genes, the only option for combining the two result lists is by relative genomic location. Arrange the statement in the Colocation popup to: **Return Genes from the current step whose upstream 1000bp region overlaps the exact region of a SNP in Step 5 and is on either strand**. Remember to indicate that you want to locate the SNPs in the upstream region of the gene.



Add a step to your search strategy

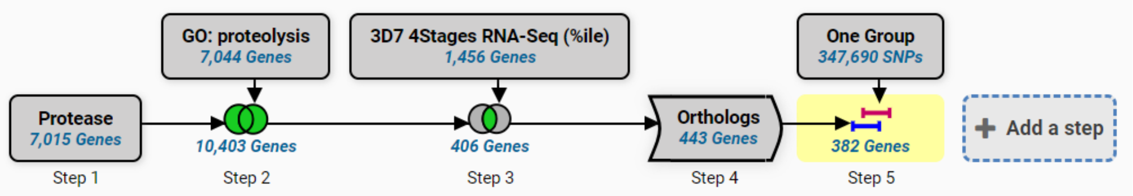
"Return each Gene from the current step whose upstream region overlaps the exact region of a SNP from the new step and is on either strand"

○ Exact
● Upstream: 1000 bp
○ Downstream: 1000 bp
○ Custom:
begin at: start - 1000 bp
end at: start - 1 bp

● Exact
○ Upstream: 1000 bp
○ Downstream: 1000 bp
○ Custom:
begin at: start + 0 bp
end at: stop + 0 bp

Strategy: Congratulations! You have completed the strategy and have a list of 234 *P. vivax* genes that are possible proteases, are likely expressed in gametocytes and have upstream SNPs.

This link will retrieve the completed strategy:
https://plasmodb.org/plasmo/app/workspace/strategies/import/d67d74edca408d0b


GO: proteolysis
7,044 Genes
3D7 4Stages RNA-Seq (%ile)
1,456 Genes
One Group
347,690 SNPs
Protease
7,015 Genes
10,403 Genes
406 Genes
Orthologs
443 Genes
382 Genes
Add a step
Step 1
Step 2
Step 3
Step 4
Step 5