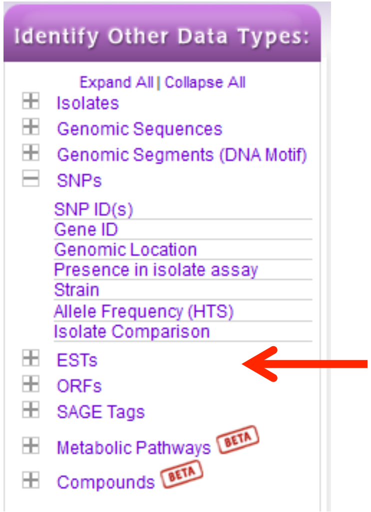
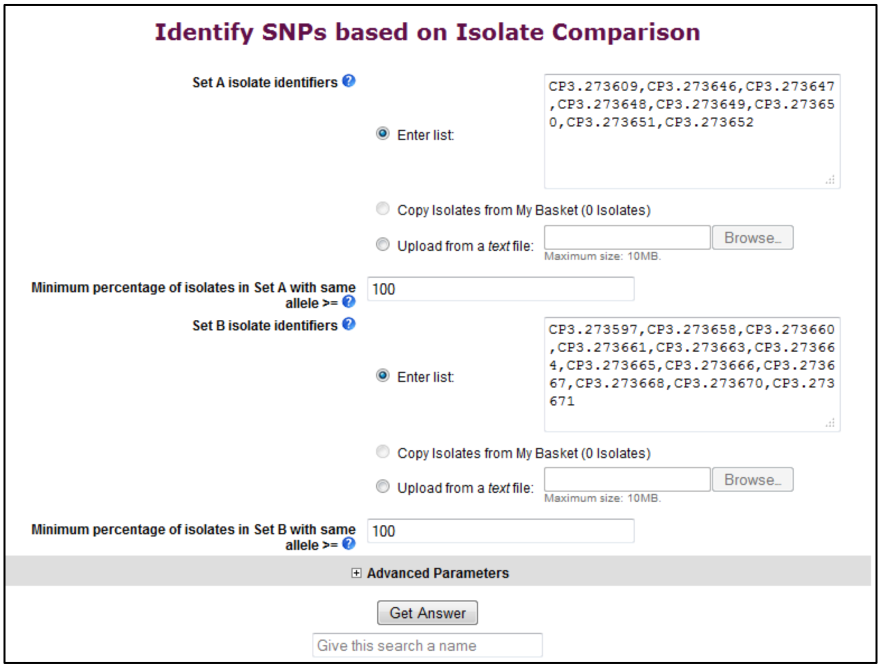
HTS and Population data
(Metadata and SNPs)
Exercise 6

1 Isolate comparison

Note: For this exercise use http://www.plasmodb.org

a. Go to the "Identify SNPs based on Isolate Comparison" search.
Hint: you can find this under "SNPs" in the "Identify Other Data Types" section.




Identify SNPs based on Isolate Comparison

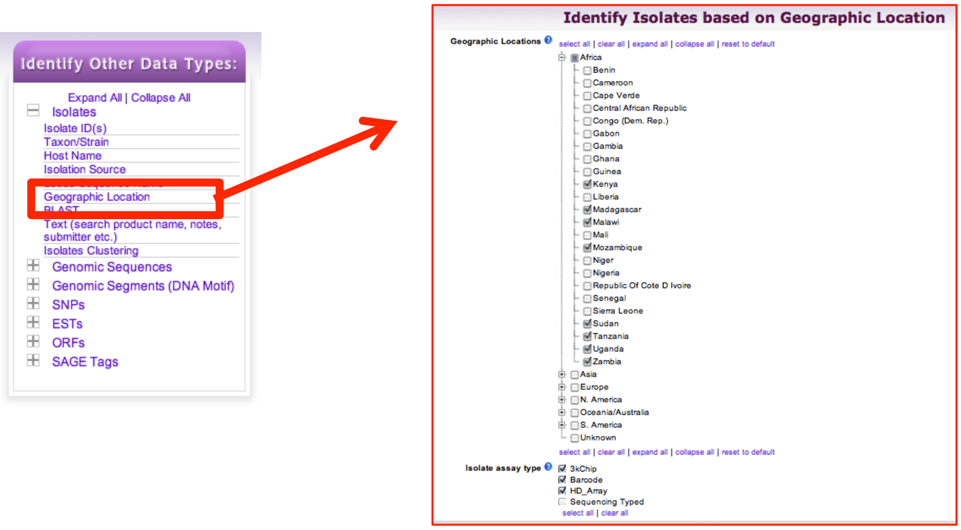
Set A isolate identifiers
● Enter list: CP3.273609,CP3.273646,CP3.273647,CP3.273648,CP3.273649,CP3.273650,CP3.273651,CP3.273652
○ Copy Isolates from My Basket (0 Isolates)
○ Upload from a *text* file:
Minimum percentage of isolates in Set A with same allele >= 100

Set B isolate identifiers
● Enter list: CP3.273597,CP3.273658,CP3.273660,CP3.273661,CP3.273663,CP3.273664,CP3.273665,CP3.273666,CP3.273667,CP3.273668,CP3.273670,CP3.273671
○ Copy Isolates from My Basket (0 Isolates)
○ Upload from a *text* file:
Minimum percentage of isolates in Set B with same allele >= 100

b. What does this search do? What is in Set A and B? Run the query and look at your results. How many SNPs were identified between isolates from Brazil and Malawi? What could you use this information for?

c. Find SNPs that differentiate isolates from East Africa and those from West Africa.

- For this exercise we are going to use the same 'Identify SNPs by Isolate Comparison' search as above. However, first we have to identify isolate IDs from West Africa and ones from East Africa. To do this use the 'Identify Isolates by Geographic Location' query under the isolates section (note that you will need to run this query twice, once for each set of countries):



Identify Isolates based on Geographic Location

Geographic Locations

- Africa
  - ☐ Benin
  - ☐ Cameroon
  - ☐ Cape Verde
  - ☐ Central African Republic
  - ☐ Congo (Dem. Rep.)
  - ☐ Gabon
  - ☐ Gambia
  - ☐ Ghana
  - ☐ Guinea
  - ☑ Kenya
  - ☐ Liberia
  - ☑ Madagascar
  - ☑ Malawi
  - ☐ Mali
  - ☑ Mozambique
  - ☐ Niger
  - ☐ Nigeria
  - ☐ Republic Of Cote D Ivoire
  - ☐ Senegal
  - ☐ Sierra Leone
  - ☑ Sudan
  - ☑ Tanzania
  - ☑ Uganda
  - ☑ Zambia
- ☐ Asia
- ☐ Europe
- ☐ N. America
- ☐ Oceania/Australia
- ☐ S. America
- ☐ Unknown

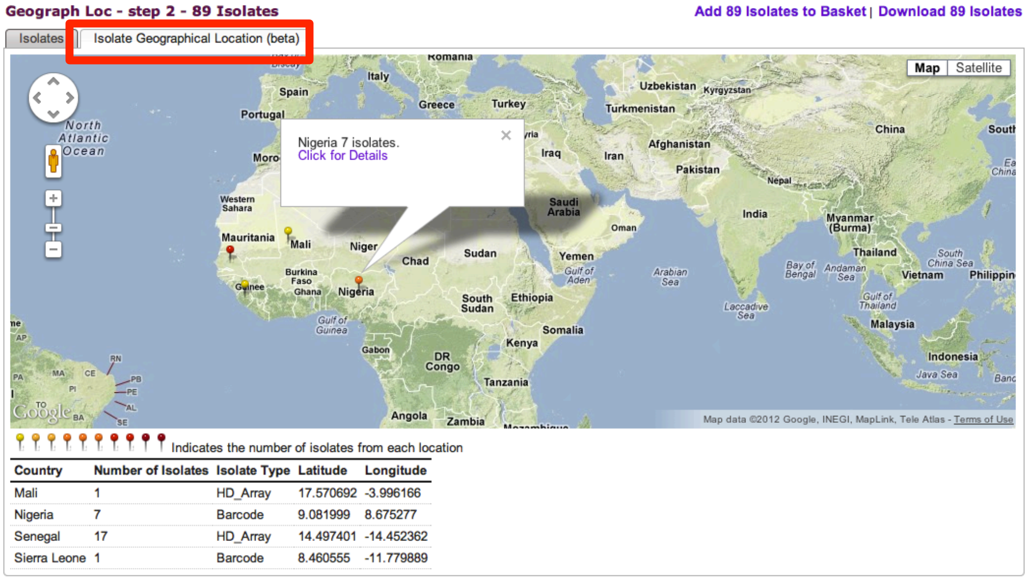
Isolate assay type

- ☑ 3kChip
- ☑ Barcode
- ☑ HD_Array
- ☐ Sequencing Typed

Some East African countries: Kenya, Madagascar, Malawi, Mozambique, Tanzania, Sudan, Uganda, Zambia

Some West African Countries: Cameroon, Gabon, Liberia, Mali, Nigeria, Senegal, Siera Leone

- For isolate assay type select HD_Array since this array has the most SNPs. You could also try the 3K_chip or even Barcode but shouldn't mix the assay types in one analysis.
- Confirm the distribution of the isolates you get by clicking on the "Geographic location" tab of the result page:

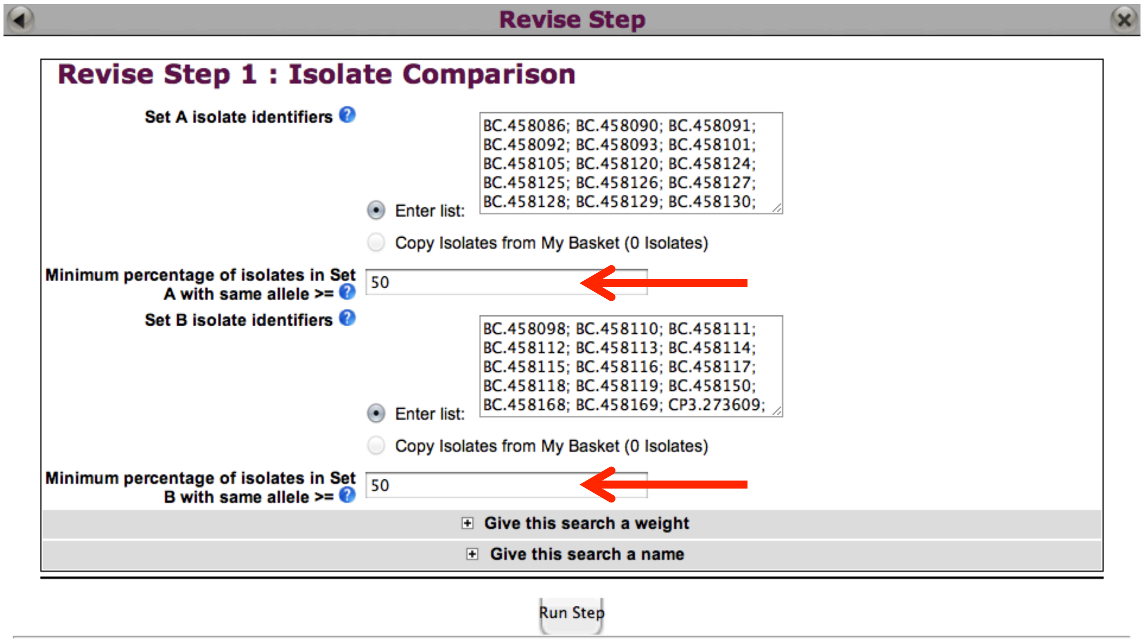


Nigeria 7 isolates.
Click for Details

Indicates the number of isolates from each location

Country	Number of Isolates	Isolate Type	Latitude	Longitude
Mali	1	HD_Array	17.570692	-3.996166
Nigeria	7	Barcode	9.081999	8.675277
Senegal	17	HD_Array	14.497401	-14.452362
Sierra Leone	1	Barcode	8.460555	-11.779889

- Once you have isolates based on geographic location you will need to copy the IDs and paste them into the SNPs by isolate comparison query (make sure you put isolates from one set of countries into the input box for set A and the other set in the input box for Set B). You might find it useful to use the NotePad on your PC or open the query in another window or tab.
    - To do this easily, click on “Download Results”, select “Tab delimited (Excel):” then unselect all the columns and click on “Get Report”. Now copy the list of IDs.
    - If the above steps are taking too long, feel free to copy the IDs from the following link: **http://goo.gl/rhRdO**
- Once you have the isolate IDs pasted in the isolate comparison query, run it and examine your results. Did you get any results? Revise the query and change the minimum percentage parameters to 70 for both set A and B:



Revise Step 1 : Isolate Comparison

Set A isolate identifiers
● Enter list: BC.458086; BC.458090; BC.458091; BC.458092; BC.458093; BC.458101; BC.458105; BC.458120; BC.458124; BC.458125; BC.458126; BC.458127; BC.458128; BC.458129; BC.458130;
○ Copy Isolates from My Basket (0 Isolates)
Minimum percentage of isolates in Set A with same allele >= 50

Set B isolate identifiers
● Enter list: BC.458098; BC.458110; BC.458111; BC.458112; BC.458113; BC.458114; BC.458115; BC.458116; BC.458117; BC.458118; BC.458119; BC.458150; BC.458168; BC.458169; CP3.273609;
○ Copy Isolates from My Basket (0 Isolates)
Minimum percentage of isolates in Set B with same allele >= 50

- What do your results look like now?
  - Which SNP differentiates more isolates (hint: look at the numbers in the columns for Set A and Set B)?
  - Do you think these SNPs are synonymous or non-synonymous? (hint: click on “select columns” and add the column called “non-synonymous”.
  - What are the genes that include these SNPs? (hint: click on the gene IDs in the “Gene ID” column).

2 Analyzing SNPs on a defined list of genes.
Note: For this exercise use http://www.plasmodb.org

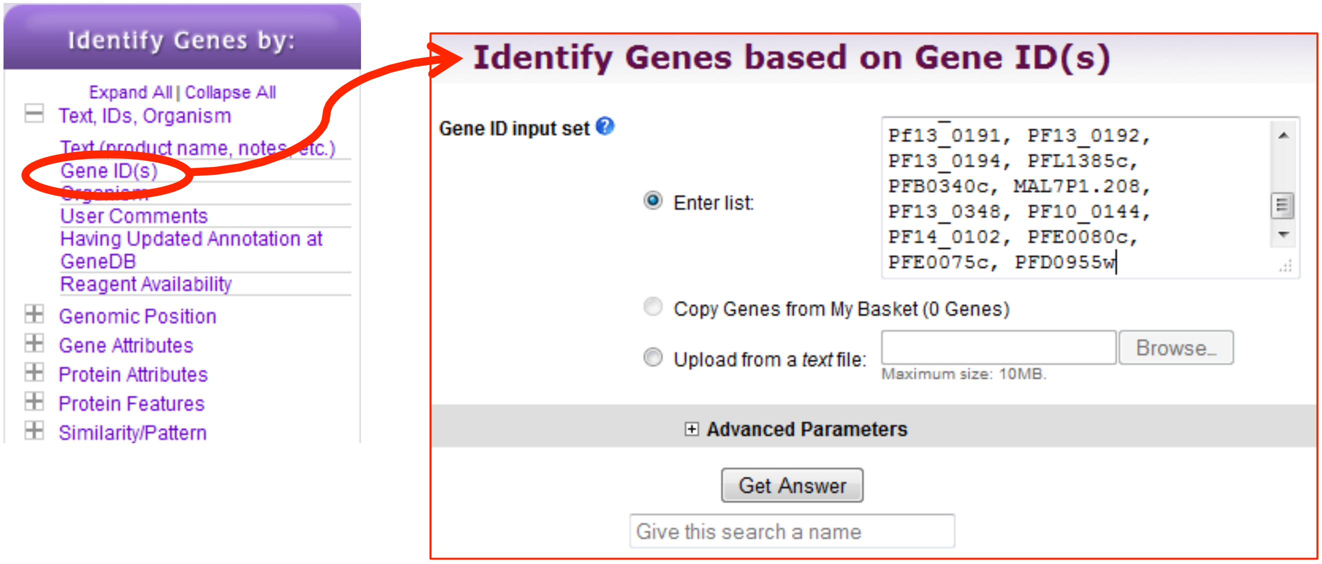
You just read the recent paper by Tetteh *et.al.* (http://www.ncbi.nlm.nih.gov/pubmed/19440377) where they perform an analysis of SNPs on a set of *P. falciparum* genes. Their conclusion is that these genes are under “balancing” selection – under diversifying selection due to their exposure to the host’s immune pressure. You decide you would like to analyze their list of genes in PlasmoDB.

Here is the list of gene IDs from their paper:

PFF0615c, Pf13_0338, PFE0395c, PF14_0201, PFF0995c, PF10_0346, PF10_0347, PF10_0348, PF10_0352, PF13_0197, PF13_0196, MAL13P1.174, PF13_0193, MAL13P1.173, Pf13_0191, PF13_0192, PF13_0194, PFL1385c, PFB0340c, MAL7P1.208, PF13_0348, PF10_0144, PF14_0102, PFE0080c, PFE0075c, PFD0955w

- **Create a strategy step with the gene IDs from the paper.**

  Hint: enter the above list into the ‘Identify Genes based on Gene ID(s)’ search option.



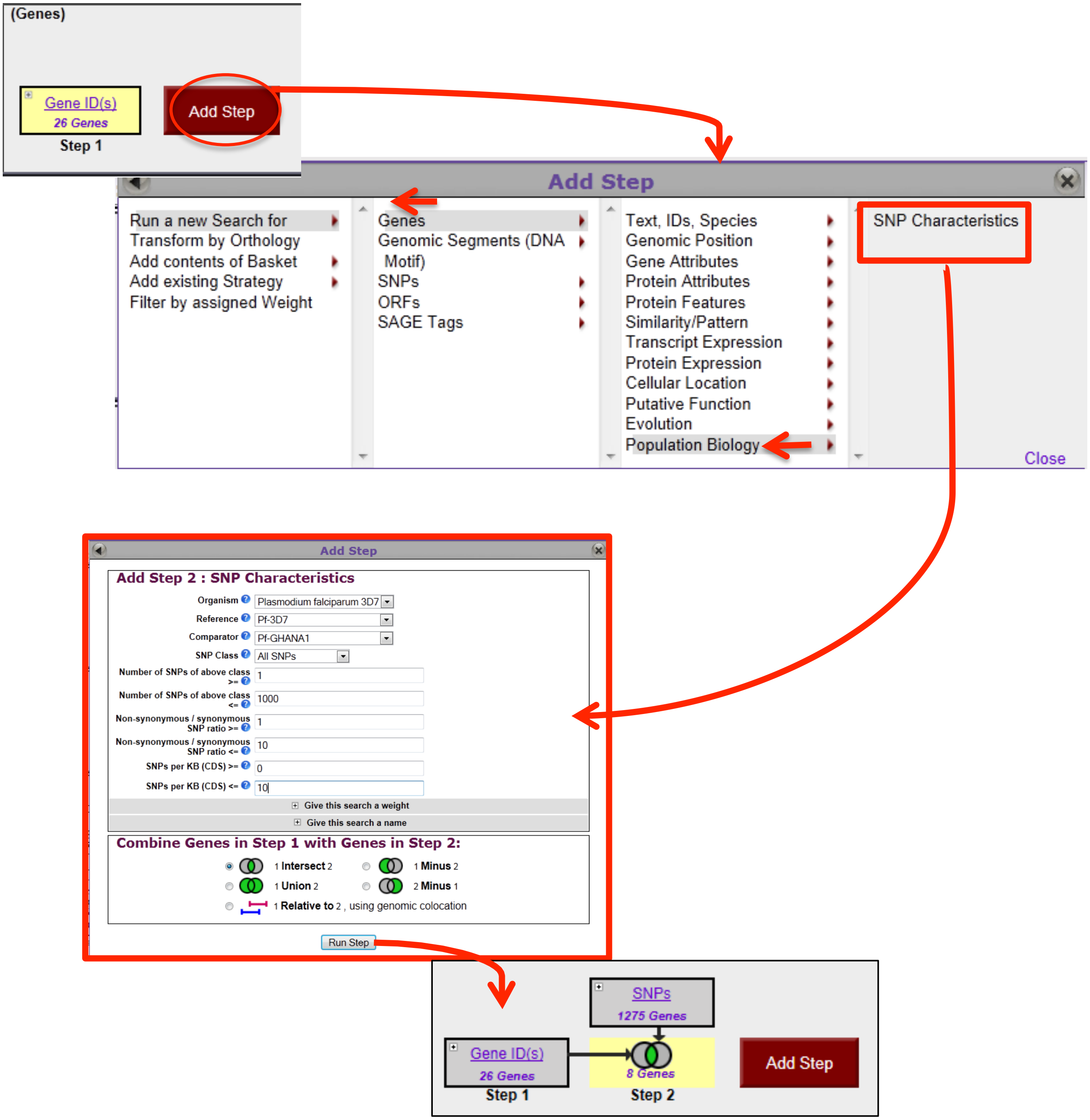
● Enter list:

```
Pf13_0191, PF13_0192,
PF13_0194, PFL1385c,
PFB0340c, MAL7P1.208,
PF13_0348, PF10_0144,
PF14_0102, PFE0080c,
PFE0075c, PFD0955w
```

- **Add a step to your strategy to identify how many of these genes are under diversifying selection.**

  Hint: the “Identify Genes based on SNP Characteristics” is found under the population biology menu (see figure on the next page).

  - What parameters would you chose?
  - Would you expect genes under balancing selection to have a high or low non-synonymous/synonymous SNP ratio?
  - How many genes were returned by your search? Of these, how many intersect with the set of genes from the paper?
  - Click on the result for your ID search in the first step (26 genes) and add columns for SNP characteristics (under population biology). Do all these genes appear to be under balancing selection? Is this consistent with the results of your strategy?



Add Step 2 : SNP Characteristics

Organism: Plasmodium falciparum 3D7
Reference: Pf-3D7
Comparator: Pf-GHANA1
SNP Class: All SNPs
Number of SNPs of above class >=: 1
Number of SNPs of above class <=: 1000
Non-synonymous / synonymous SNP ratio >=: 1
Non-synonymous / synonymous SNP ratio <=: 10
SNPs per KB (CDS) >=: 0
SNPs per KB (CDS) <=: 10

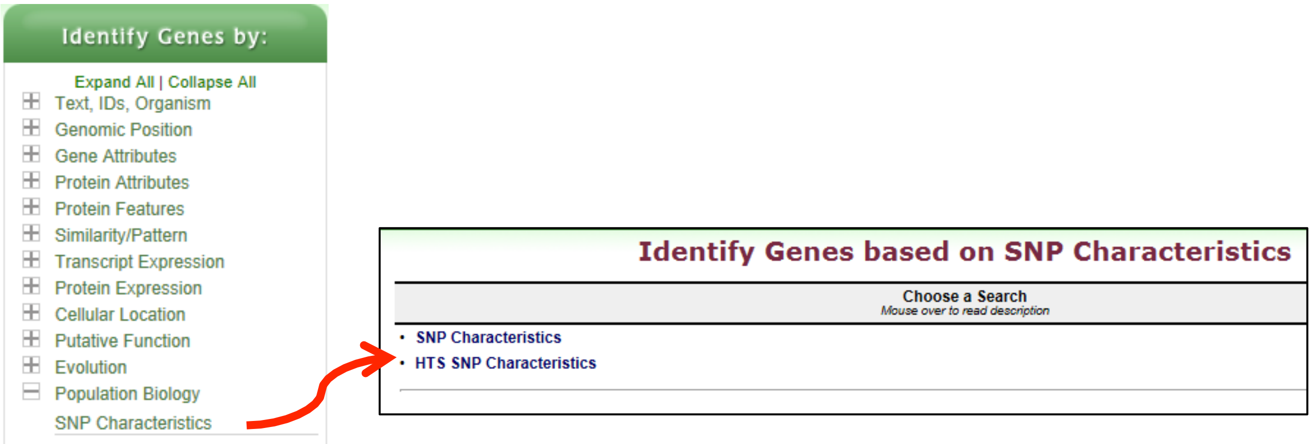
Combine Genes in Step 1 with Genes in Step 2:

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

3 Find genes with at least 30 non-synonymous SNPs that appear to be under diversifying selection when comparing all human isolates to a bear isolate.

For this exercise use http://toxodb.org/toxo/

a. Navigate to the search ‘Identify Genes based on HTS SNP Characteristics’
   - Use this search to find genes that contain SNPs that were identified by comparing high throughput sequencing data of parasite isolates.

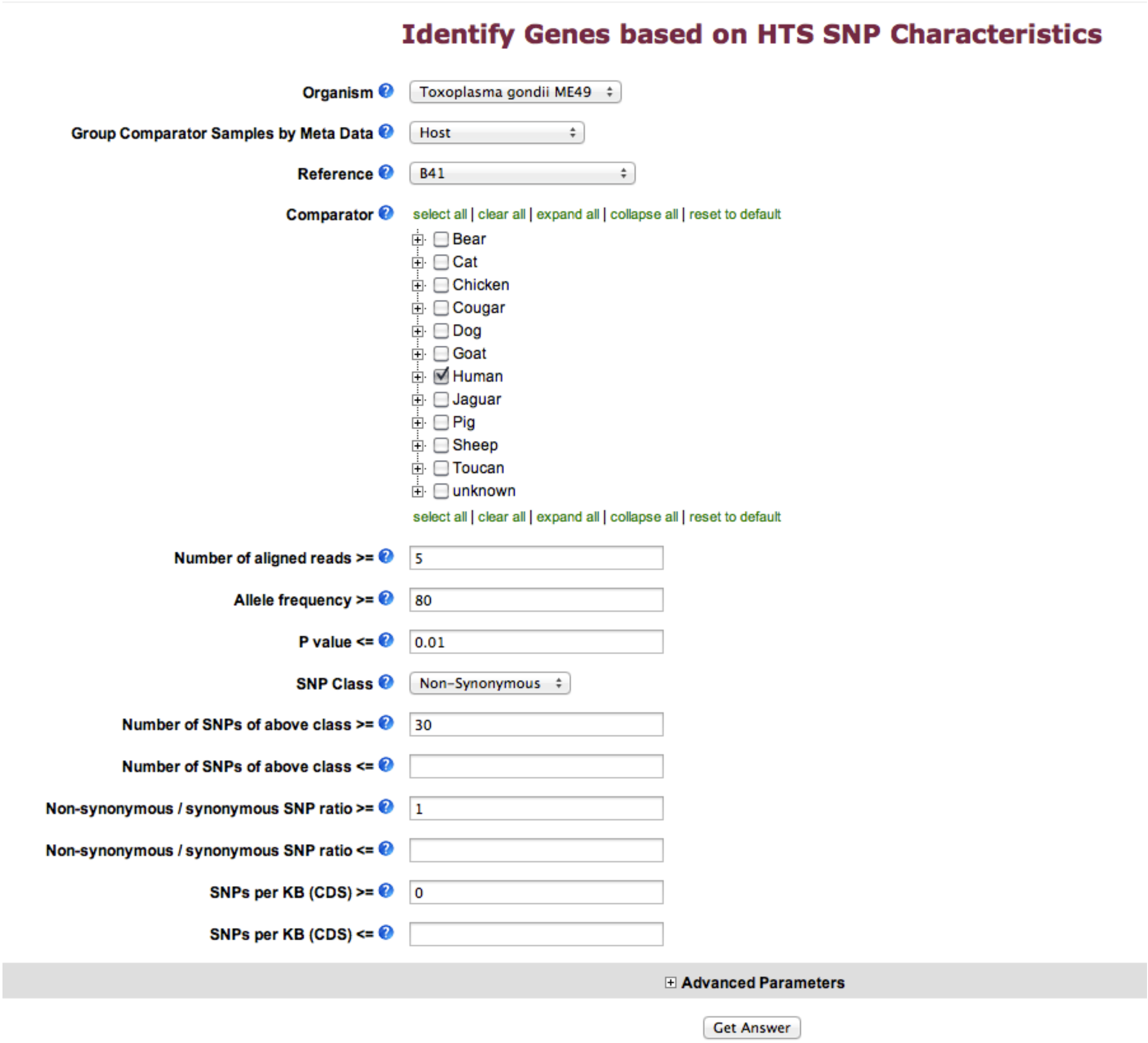


b. Group isolates by host.
   - The datasets used in this search are high throughput sequencing data obtained on isolates. Meta data associated with these isolates include year collected, host, haplogroup, geographic location, ATCC#, strain or line name. In order to compare a bear isolate with all human isolates, we need to group the samples according to their host.

c. Choose reference and comparison samples.
   - We want to use a bear isolate as reference and all human as comparator. Expanding the comparator Bear category shows that the B41 and B73 isolates are from a bear, so we want to choose either of these for our reference. Expand the comparotor Human categor shows about 20 samples sequenced from humans. Choose all samples by clicking the box next to Human.

d. Set other paramters
   - SNP class = non-synonymous
   - Number of SNPs of above class>= 30

- Non-synonymous / synonymous SNP ratio > 1



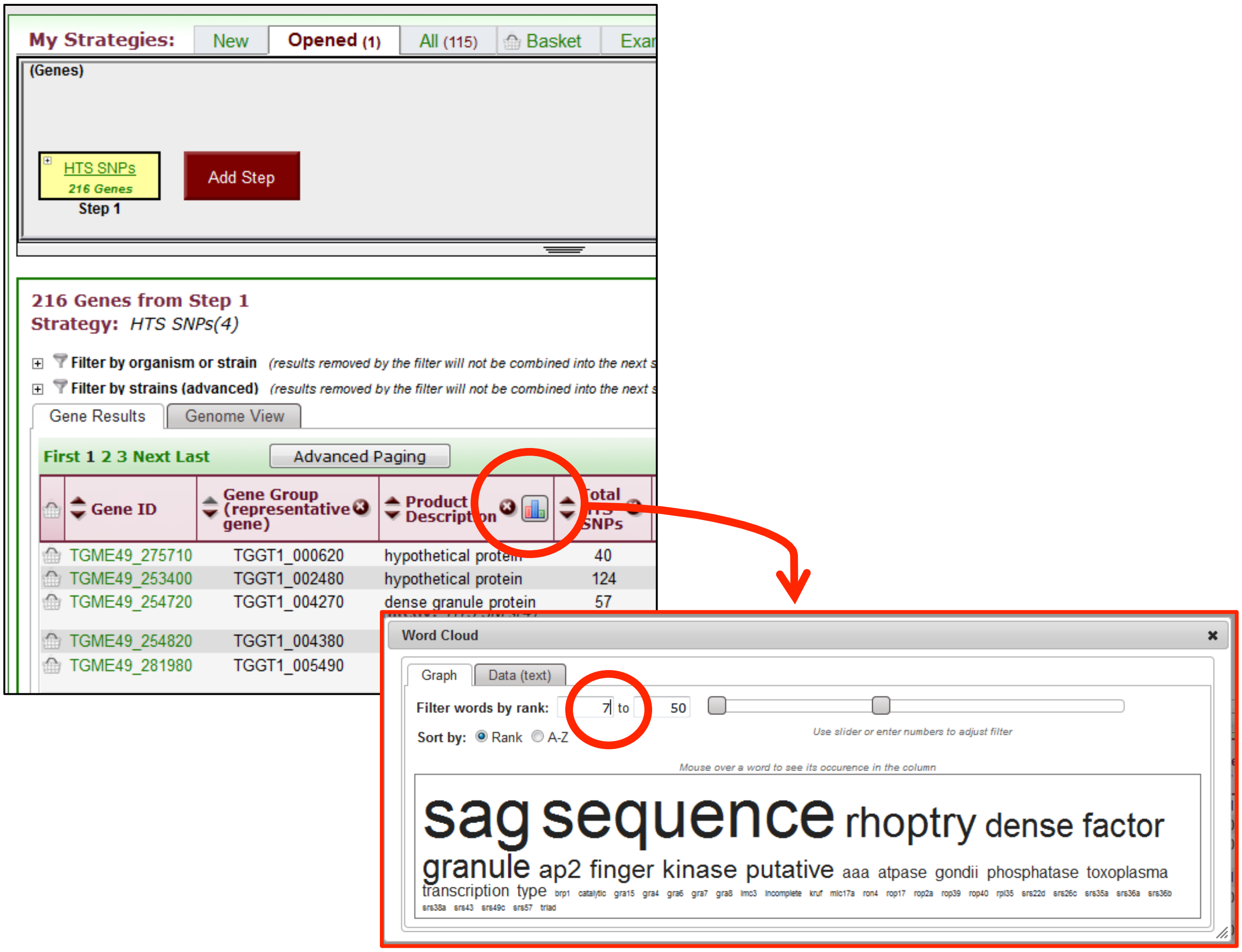
Identify Genes based on HTS SNP Characteristics

Organism	Toxoplasma gondii ME49
Group Comparator Samples by Meta Data	Host
Reference	B41
Comparator	select all | clear all | expand all | collapse all | reset to default ☐ Bear ☐ Cat ☐ Chicken ☐ Cougar ☐ Dog ☐ Goat ☑ Human ☐ Jaguar ☐ Pig ☐ Sheep ☐ Toucan ☐ unknown select all | clear all | expand all | collapse all | reset to default
Number of aligned reads >=	5
Allele frequency >=	80
P value <=	0.01
SNP Class	Non-Synonymous
Number of SNPs of above class >=	30
Number of SNPs of above class <=	
Non-synonymous / synonymous SNP ratio >=	1
Non-synonymous / synonymous SNP ratio <=	
SNPs per KB (CDS) >=	0
SNPs per KB (CDS) <=	

e. How many genes did you get?

f. How can you quickly get an idea of the function of the genes in your result set?

- Hint: use the analysis function on the Product Description column. Click the graph icon to analyse the data in the column. Since Product Description is text, the analysis performed is a word cloud. You can exclude common words using the Filter Words By Rank



216 Genes from Step 1
Strategy: *HTS SNPs(4)*

Filter by organism or strain *(results removed by the filter will not be combined into the next s*

Filter by strains (advanced) *(results removed by the filter will not be combined into the next s*

Gene ID	Gene Group (representative gene)	Product Description	otal HTS SNPs
TGME49_275710	TGGT1_000620	hypothetical protein	40
TGME49_253400	TGGT1_002480	hypothetical protein	124
TGME49_254720	TGGT1_004270	dense granule protein	57
TGME49_254820	TGGT1_004380		
TGME49_281980	TGGT1_005490		

Filter words by rank: 7 to 50

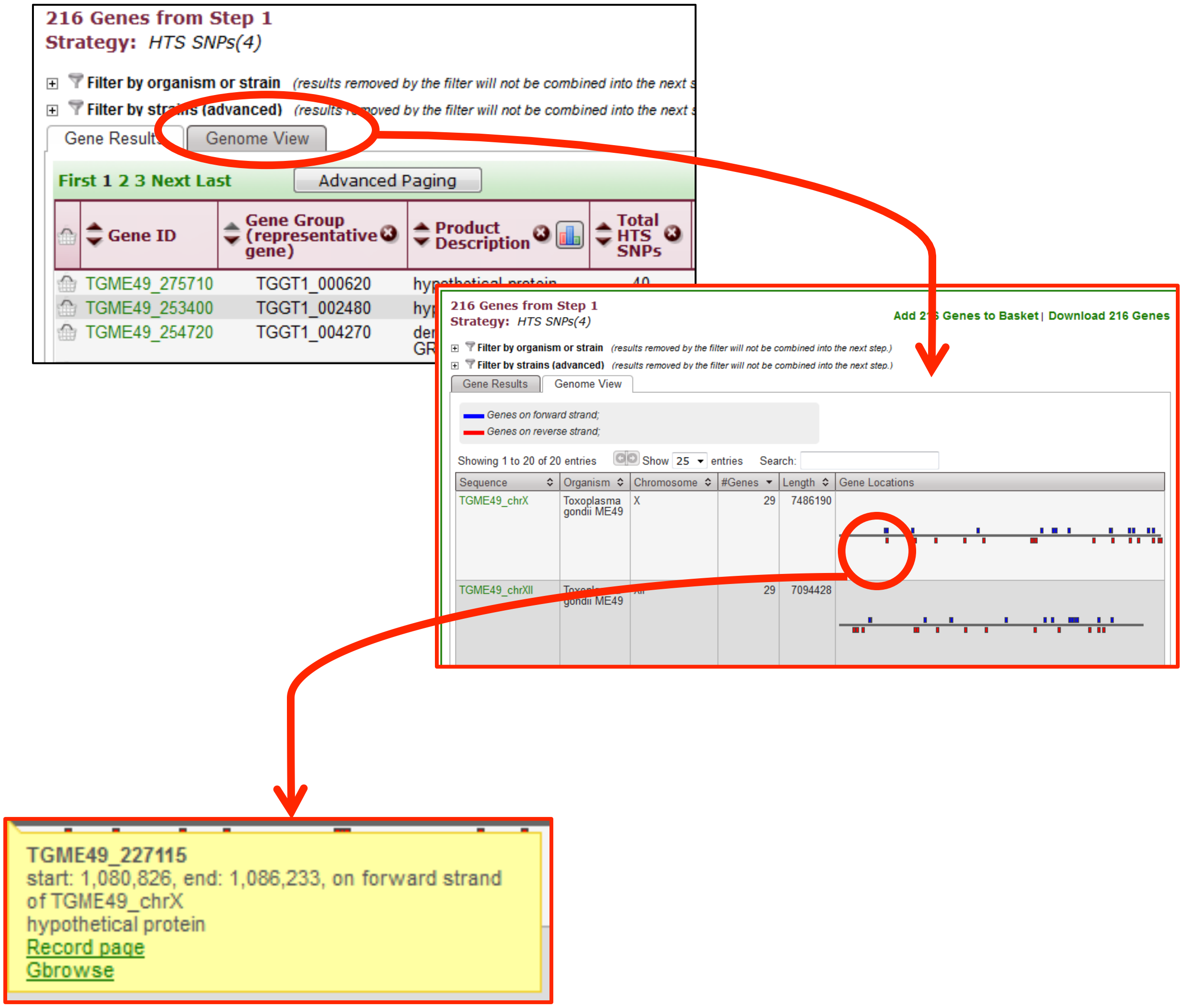
Sort by: ● Rank ○ A-Z

Use slider or enter numbers to adjust filter

sag sequence rhoptry dense factor granule ap2 finger kinase putative aaa atpase gondii phosphatase toxoplasma transcription type brp1 catalytic gra15 gra4 gra6 gra7 gra8 imc3 incomplete kruf mic17a ron4 rop17 rop2a rop39 rop40 rpl35 srs22d srs26c srs35a srs36a srs36b srs38a srs43 srs49c srs57 triad

g. How can you quickly get an idea of the function of the genes in your result set?

- The genome view tab displays your gene results mapped onto the chromosome (or corresponding genomic sequence).
- Hover over the glyphs to see information about the gene and to link to GBrowse at the location of the gene.



216 Genes from Step 1
Strategy: HTS SNPs(4)

⊞ Filter by organism or strain (results removed by the filter will not be combined into the next s
⊞ Filter by strains (advanced) (results removed by the filter will not be combined into the next s

Gene ID	Gene Group (representative gene)	Product Description	Total HTS SNPs
TGME49_275710	TGGT1_000620	hypothetical protein	40
TGME49_253400	TGGT1_002480	hyp	
TGME49_254720	TGGT1_004270	der GR	

216 Genes from Step 1
Strategy: HTS SNPs(4)

⊞ Filter by organism or strain (results removed by the filter will not be combined into the next step.)
⊞ Filter by strains (advanced) (results removed by the filter will not be combined into the next step.)

🟦 Genes on forward strand;
🟥 Genes on reverse strand;

Showing 1 to 20 of 20 entries Show 25 entries Search:

Sequence	Organism	Chromosome	#Genes	Length	Gene Locations
TGME49_chrX	Toxoplasma gondii ME49	X	29	7486190	
TGME49_chrXII	Toxoplasma gondii ME49	XII	29	7094428	

TGME49_227115
start: 1,080,826, end: 1,086,233, on forward strand of TGME49_chrX
hypothetical protein
Record page
Gbrowse

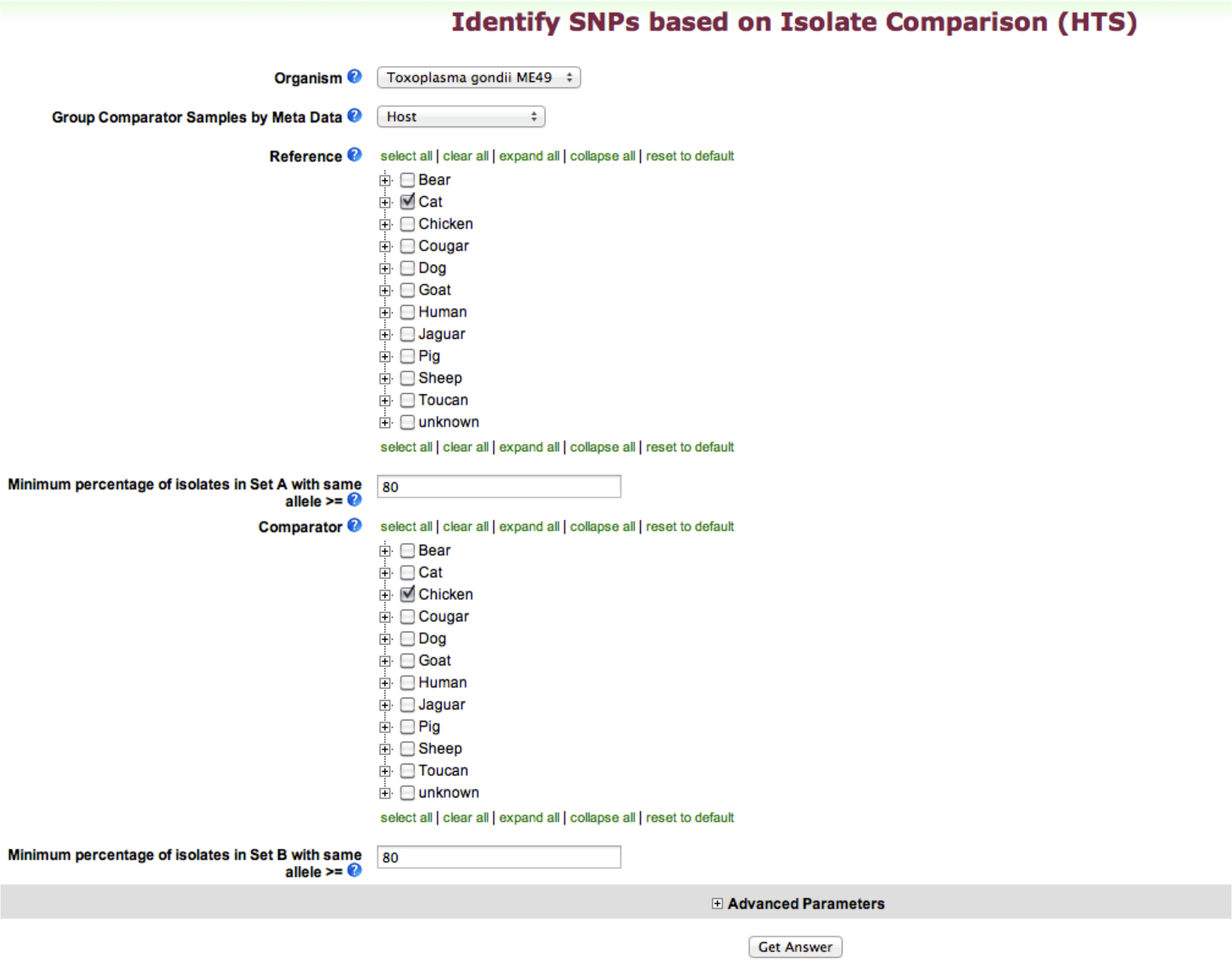
4 Optional Exercise: Find genes that contain SNPs that distinguish *Toxoplasma gondii* strains isolated from chickens as compared to those isolated from cats.

For this exercise use http://ToxoDB.org

Note that this exercise combines three different data types (Isolated, SNPs and Genes). Lets start by doing a SNP search to identify SNPs that distinguish these two populations. Navigate to “Identify SNPs based on Isolate Comparison (HTS)”.

a. Choose Host from the “Group Comparator Samples by Meta Data” parameter. This will generate the reference and comparator menus using the meta data associated with these samples. In this case the isolates are organized the host from which they were isolated. These isolates have a fairly rich set of meta data so there are other options such as haplogroup or geographic location.

b. Choose all Cat samples from the reference list. Note that you should “clear all” or check off the initial samples that are checked.
c. Choose all Chicken samples from the comparator list.
d. We let the percentages stay at 80 but you can play with these parameters to get more or fewer SNPs back. For this exercise 80 works well.



Identify SNPs based on Isolate Comparison (HTS)

Organism: Toxoplasma gondii ME49

Group Comparator Samples by Meta Data: Host

Reference:
select all | clear all | expand all | collapse all | reset to default
☐ Bear
☑ Cat
☐ Chicken
☐ Cougar
☐ Dog
☐ Goat
☐ Human
☐ Jaguar
☐ Pig
☐ Sheep
☐ Toucan
☐ unknown
select all | clear all | expand all | collapse all | reset to default

Minimum percentage of isolates in Set A with same allele >=: 80

Comparator:
select all | clear all | expand all | collapse all | reset to default
☐ Bear
☐ Cat
☑ Chicken
☐ Cougar
☐ Dog
☐ Goat
☐ Human
☐ Jaguar
☐ Pig
☐ Sheep
☐ Toucan
☐ unknown
select all | clear all | expand all | collapse all | reset to default

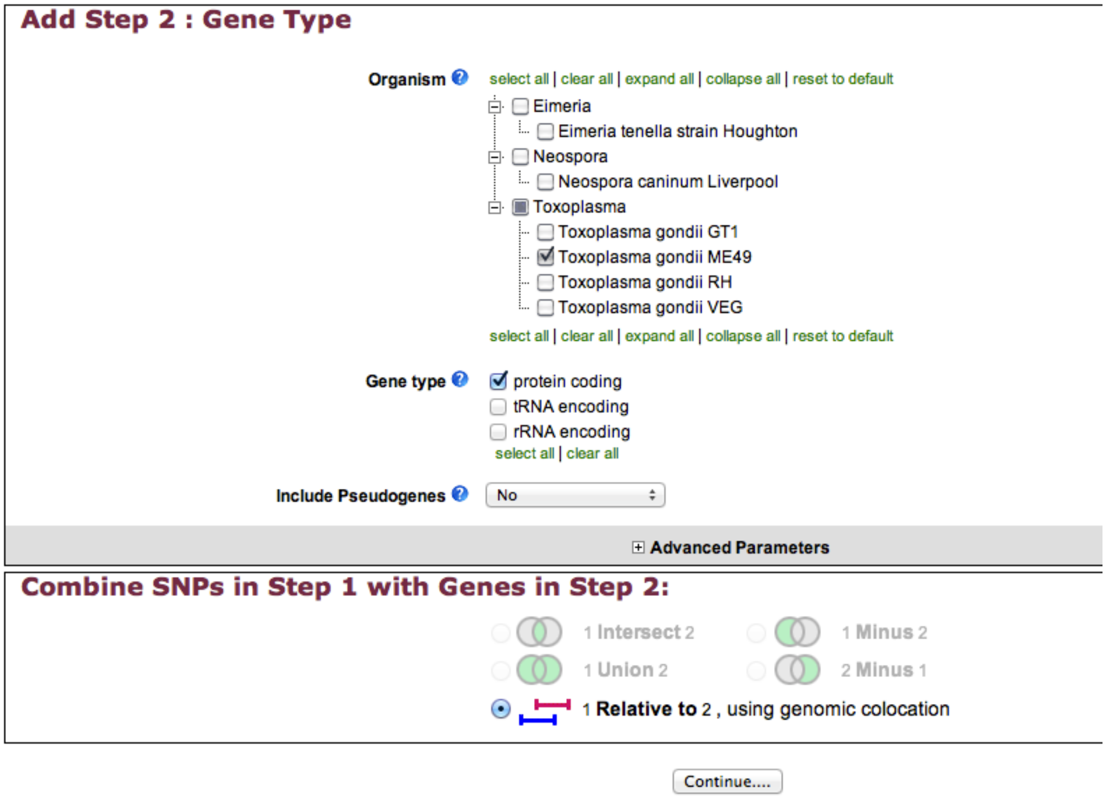
Minimum percentage of isolates in Set B with same allele >=: 80

How many SNPs were returned? Were you surprised at this fairly large number? Why might there be so many? It turns out that the *T. gondii* genome is quite divergent … there are on the order of 2.3 million SNPs between all the strains that have been re-sequenced in the 65 MB genome.

If the Gene ID column is not present please add it. How many of the SNPs seem to be in genes (estimate, please don't count). Are some of the genes enriched for SNPs? Ie, does it seem that some of the genes may be involved in the differential host preference?

How might you ask this question directly? What we really want is to identify the genes that contain these SNPs that we've found. We can do this directly … can you figure out how to do it without looking further at the help?

e. Click the "Add Step" button and add a search for "Genes by gene type" and set the parameters to return all T. gondii ME49 protein coding genes. Notice that the ONLY option available to Combine the steps is the last one …. Why is this??
f. In the Genomic Colocation window "Return each gene from step 2 whose exact region overlaps with the exact region of a SNP in step 1 on either strand". NOTE: we will cover genomic colocation in more detail later so don't worry if you are confused!!



Add Step 2 : Gene Type

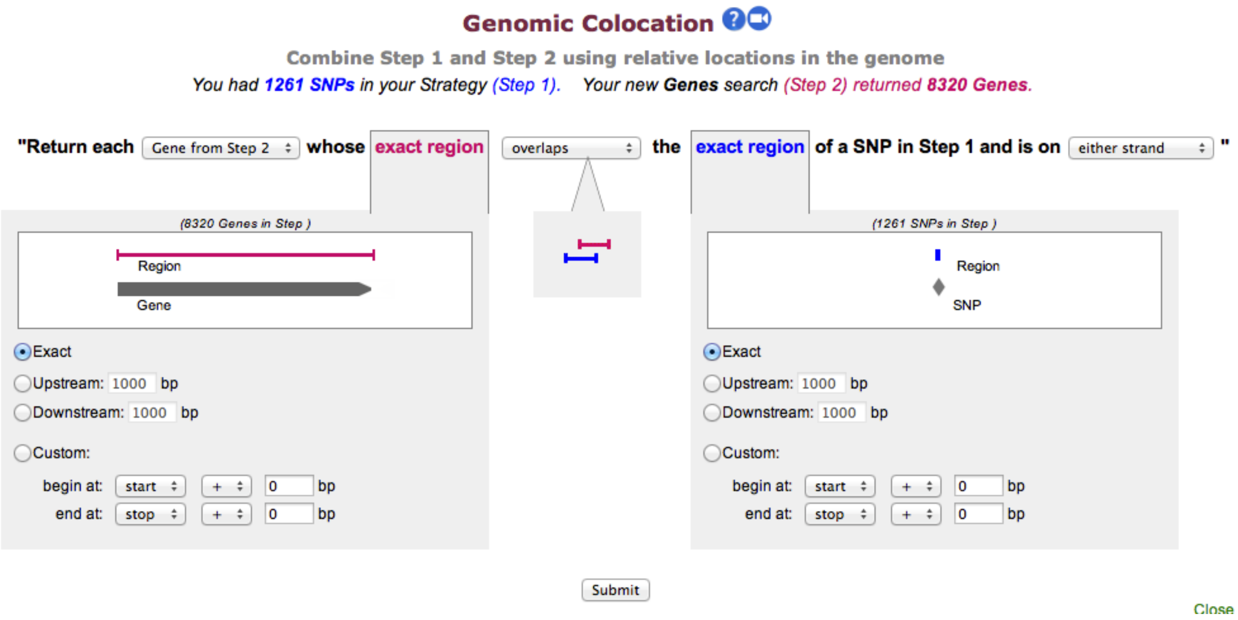
Organism
- ☐ Eimeria
  - ☐ Eimeria tenella strain Houghton
- ☐ Neospora
  - ☐ Neospora caninum Liverpool
- ▨ Toxoplasma
  - ☐ Toxoplasma gondii GT1
  - ☑ Toxoplasma gondii ME49
  - ☐ Toxoplasma gondii RH
  - ☐ Toxoplasma gondii VEG

Gene type
- ☑ protein coding
- ☐ tRNA encoding
- ☐ rRNA encoding

Include Pseudogenes No

Combine SNPs 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



Genomic Colocation

Combine Step 1 and Step 2 using relative locations in the genome

*You had **1261 SNPs** in your Strategy (Step 1). Your new **Genes** search (Step 2) returned **8320 Genes**.*

"Return each [Gene from Step 2] whose exact region [overlaps] the exact region of a SNP in Step 1 and is on [either strand] "

(8320 Genes in Step)

● Exact

○ Upstream: 1000 bp

○ Downstream: 1000 bp

○ Custom:

begin at: start + 0 bp

end at: stop + 0 bp

(1261 SNPs in Step)

● Exact

○ Upstream: 1000 bp

○ Downstream: 1000 bp

○ Custom:

begin at: start + 0 bp

end at: stop + 0 bp

How many genes were returned? Note that some of the genes have many matched regions … ie SNPs from step 1 that are contained within (overlap since SNPs are 1 bp long) the gene. In order to make this easier to look at, remove the "region" and "matched regions" columns. What do you think it means that there are so many SNPs in some of these genes? What kinds of genes are these (look at the product description column). Might these genes be involved in host preference?

Is there anything interesting about how these genes are distributed in the genome? *Hint: click the Genome View tab*. What do you think this means?

Are there other searches that you might want to add to this strategy in order to better understand this result and help refine your hypotheses?