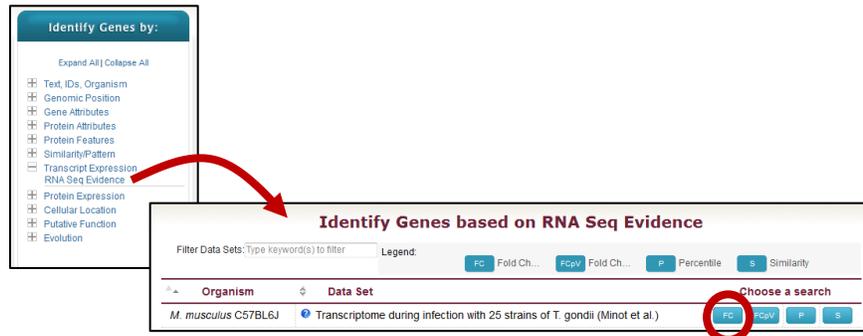


## Host Response

1. Find host genes that are upregulated in infected mouse cells compared to uninfected ones. For this exercise use <http://hostdb.org>
  - a. Navigate to the “Transcript Expression” section then select “RNA Seq Evidence”. Select the fold change query for the “Transcriptome during infection with 25 strains of *T. gondii* (Minot et al.)” experiment.



- b. Configure the search to compare all infected samples to the uninfected control. Make sure to select upregulated. In the example below a fold change of 10 was selected and the “average” operation was applied on the comparison samples.

### Identify Genes based on M.mus. Transcriptome during infection with 25 strains of *T. gondii* RNASeq (fold change)

Tutorial
YouTube

For the Experiment  
 Transcriptome during infection with 25 strains of *T. gondii*

return protein coding Genes  
 that are up-regulated  
 with a Fold change  $\geq 10$

between each gene's expression value  
 in the following Reference Samples

T. gondii infected  
 VAND infected  
 VEG infected  
 WTD3 infected  
 not infected

select all | clear all

and its average expression value  
 in the following Comparison Samples

T. gondii infected  
 VAND infected  
 VEG infected  
 WTD3 infected  
 not infected

select all | clear all

#### Example showing one gene that would meet search criteria

(Dots represent this gene's expression values for selected samples)

Up-regulated

Average Comparison

Reference

*A maximum of four samples are shown when more than four are selected.*  
 You are searching for genes that are up-regulated between one reference sample and at least two comparison samples.

For each gene, the search calculates:

$$\text{fold change} = \frac{\text{average expression value in comparison samples}}{\text{reference expression value}}$$

and returns genes when fold change  $\geq 10$ . To narrow the window, use the minimum comparison value. To broaden the window, use the maximum comparison value.

See the detailed help for this search.

[Get Answer](#)

My Strategies: [New](#) [Opened \(1\)](#) [All \(1\)](#) [Basket](#) [Public Strategies \(1\)](#) [Help](#)

(Genes) Strategy: **mouse infected with 25 Tg strains (fc) \***

mouse infected with 200 Genes Step 1 [Add Step](#)

[Rename](#) [Duplicate](#) [Save As](#) [Share](#) [Delete](#)

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**200 Genes from Step 1** Strategy: **mouse infected with 25 Tg strains (fc)** [Add 200 Genes to Basket](#) | [Download 200 Genes](#)

Filter results by species

All Results	Ortholog Groups	<i>H. sapiens</i>	<i>M. Musculus</i>
200	105	0 genes	104 genes
		0 transcripts	200 transcripts

Gene Results [Genome View](#) [Analyze Results](#) **BETA**

First 1 2 3 4 5 Next Last [Advanced Paging](#) [Add Columns](#)

Gene ID	Organism	Product Description	Fold Change	Chosen Ref (log2)	Chosen Comp (log2)	mouse infected with 25 Tg strain: fpkm Graph
ENSMUSG00000031779_1	<i>M. musculus</i> C57BL6J	chemokine (C-C motif) ligand 22 (Ccl22)	456.4	0.84	9.68	

- c. What are the functional characteristics of the genes in this result? What kinds of GO terms are enriched? *Hint:* click on the “Analyze Results” tab and select the GO enrichment analysis.

Gene Results [Genome View](#) [Gene Ontology Enrichment](#) [Analyze Results](#) **BETA**

[Rename This Analysis](#) | [Copy These Parameter Values](#)

**Gene Ontology Enrichment**

Find Gene Ontology terms that are enriched in your gene result. [Read More](#)

[Parameters](#)

**Analysis Results** [Download Analysis Results](#)

Got a total of 1,263 results Filter:

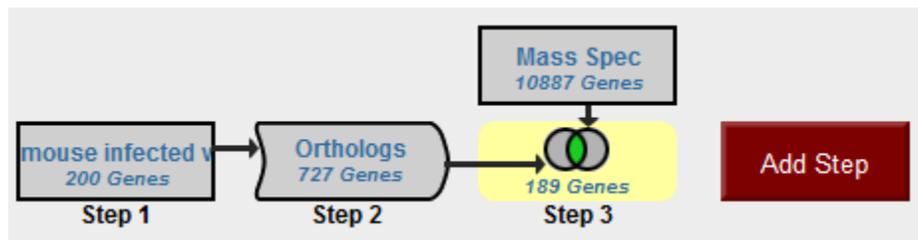
This analysis result may be lost if you change your gene result. To save this analysis result, please download.

GO ID	GO Term	Genes in the bkgd with this term	Genes in your result with this term	Percent of bkgd Genes in your result	Fold enrichment	Odds ratio	P-value	Benjamini	Bonferroni
GO:0009611	response to wounding	1017	38	3.7	8.79	10.7	3.31e-24	4.18e-21	4.18e-21
GO:001817	regulation of cytokine production	640	29	4.5	10.66	12.36	9.28e-21	4.76e-18	1.17e-17
GO:0006950	response to stress	4086	64	1.6	3.68	5.01	1.13e-20	4.76e-18	1.43e-17
GO:0010033	response to organic substance	2918	54	1.9	4.35	5.65	2.19e-20	6.92e-18	2.77e-17
GO:0006952	defense response	1184	36	3.0	7.15	8.56	4.16e-20	1.06e-17	5.28e-17
GO:0051240	positive regulation of multicellular organismal process	908	32	3.5	8.29	9.74	1.01e-19	2.12e-17	1.27e-16
GO:0070206	protein trimerization	63	14	22.2	52.26	56.27	2.45e-19	4.43e-17	3.10e-16
GO:0032496	response to lipopolysaccharide	387	23	5.9	13.98	15.73	4.27e-19	6.74e-17	5.39e-16
GO:002237	response to molecule of bacterial origin	411	23	5.6	13.16	14.81	1.50e-18	2.11e-16	1.90e-15
GO:0051704	multi-organism process	1445	37	2.6	6.02	7.21	2.85e-18	3.60e-16	3.60e-15
GO:0042127	regulation of cell proliferation	2165	44	2.0	4.78	5.9	5.39e-18	6.19e-16	6.81e-15
GO:0050710	negative regulation of cytokine secretion	44	12	27.3	64.14	68.32	1.11e-17	1.14e-15	1.41e-14
GO:0016477	cell migration	1076	32	3.0	6.99	8.19	1.17e-17	1.14e-15	1.48e-14
GO:0032879	regulation of localization	2555	47	1.8	4.33	5.4	1.44e-17	1.26e-15	1.82e-14
GO:0050707	regulation of cytokine secretion	145	16	11.0	25.95	28.2	1.50e-17	1.26e-15	1.89e-14

- d. What are the human orthologs of these mouse genes? Expand the result set to include orthologs/paralogs of these genes. *Hint:* add a “Transform by Orthology” step choosing Homo sapiens.



- e. Do any of these human genes also have peptide evidence for their expression during infection? *Hint:* add a step and explore the “Mass Spec Evidence” data in the protein expression section. Run the search using the default parameters.



2. Find *Plasmodium falciparum* genes that are immunogenic in infected humans.  
For this exercise use <http://plasmodb.org>

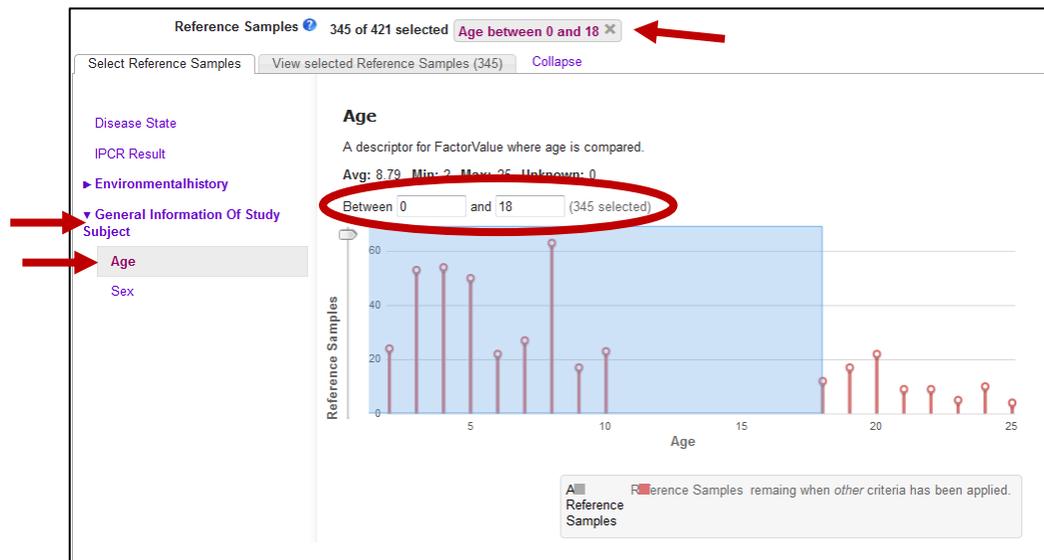
Anibodies are central to malaria immunity, which is only acquired after years of exposure to *Plasmodium falciparum* (Pf). Despite the enormous worldwide burden of malaria, the targets of protective Abs and the basis of their inefficient acquisition are unknown. PlasmoDB contains protein array data which provides a measure of a gene's 'immugenicity' by hybridizing serum (which contains antibodies) to an array spotted with peptides from 2320 *P. falciparum* gene products. Searching this data in PlasmoDB identifies genes whose protein products acted as antigens in malaria patients.

- a. Identify genes (antigens) that exhibited an increased immunogenicity in asymptomatic children (ages 0-18) compared to children with disease (infected). *Hint:* the "Protein Array" search is available in the "Host Response" menu item in the "Identify Genes By" section of the home page. Choose the experiment **Protein targets of serum antibodies in response to infection (Crompton et al.)**.

The image shows a screenshot of the PlasmoDB website. On the left, a sidebar titled "Identify Genes by:" contains a list of search criteria. A red arrow points from the "Protein Array" option in this list to the main search page. The main page is titled "Identify Genes based on Protein Array" and features a table of data sets. A red circle highlights the "TSTU" button in the "Choose a" column of the table. Below the table, a red arrow points to a detailed search page titled "Identify Genes based on P.fal. Protein targets of serum antibodies in response to infection Antibody Array (p-value)". This page has several input fields: "Reference Samples" (421 of 421 selected), "Comparison Samples" (421 of 421 selected), "Metadata category to color graph by" (set to "DiseaseState"), "Direction" (set to "increased immunogenicity"), and "P value less than or equal to" (set to "0.05"). A "Get Answer" button is at the bottom. A red text box on the right side of the detailed search page says: "This is a view of the search page with the reference and comparison sample parameters collapsed."

Organism	Data Set	Choose a
<i>P. falciparum</i> 3D7	Protein targets of serum antibodies in response to infection (Crompton et al.)	TSTU
<i>P. falciparum</i> 3D7	International Centers of Excellence for Malaria Research (International Centers of Excellence for Malania Research (ICEMR))	TSTU
<i>P. falciparum</i> 3D7	Treatment-time to reinfection cohort from Kisumu area, Kenya collected in 2003 (Dent et al.)	TSTU

In this example, your **comparison samples will be normal children** and your **reference samples will be infected children**. So each set of samples (reference and comparison) has two parameters that need to be set, age and disease state. The age parameter should be set to 0-18 years for both the reference and the comparison samples. To set the age parameter for the reference samples, choose the 'General Information of Study Subject' and then 'Age' from the left menu to reveal choices for the parameter on the right. Set the range to 0-18 (see image below).



Now set the disease state for the reference samples to infected. Move on the comparison samples and set the age to 0-18 and the disease state to normal. The default settings for other parameters are good – increased immunogenicity and p-value = 0.05.



Reference Samples 265 selected Age is between 0 and 18 Disease State is Infected

Select Reference Samples View selection (265) Collapse

expand all | collapse all

Disease State  
 The name of the pathology diagnosed in the organism from which the biomaterial...  
 read more

select all | clear all

Infected 272 64.61%

Normal 149 35.39%

■ All Reference Samples  
 ■ % Reference Samples from other selected options

Comparison Samples 80 selected Age is between 0 and 18 Disease State is Normal

Refine selection

Metadata category to color graph by DiseaseState

Direction increased immunogenicity

P value less than or equal to 0.05

Advanced Parameters

Get Answer

You are ready to click Get Answer! What do your results look like? Could these represent potential protective antigens? (result image below)

My Strategies: New Opened (1) All (1) Basket Public Strategies (33) Help

(Genes) Strategy: Serum Ab Response (p-val)\*

Serum Ab Respo  
31 Genes  
Step 1 Add Step

Rename  
Duplicate  
Save As  
Share  
Delete

**31 Genes from Step 1**  
Strategy: Serum Ab Response (p-val) Add 31 Genes to Basket | Download 31 Genes

Click on a number in this table to limit/filter your results

Gene Results Genome View Analyze Results BETA

First 1 2 Next Last Advanced Paging Add Columns

Gene ID	Organism	Product Description	P Value	Avg Ref (arcsinh(1+50x))	Avg Comp (arcsinh(1+50x))	Expression - graph
PF3D7_0304600	<i>P. falciparum</i> 3D7	circumsporozoite (CS) protein (CSP)	1.72E-04	4.696921	5.00801	

Signal Intensity Values - arcsinh(1+50x) - PF3D7\_0304600

Samples colored based on DiseaseState

**3. Find *falciparum* antigens that may be protective from recurrence of malaria (and potentially re-infection) For this exercise use <http://plasmodb.org>**

A recently published study from Kenya ([view paper](#)) followed participants for 12 weeks after an initial screening for malaria and treatment with anti-malarial drugs. The authors collected patient serum, analyzed the serum by protein array and EuPathDB integrated the data set. On a weekly basis, the authors also collected other data associated with the serum sample (metadata) such as 'presence of parasites' and clinical symptoms of malaria. We can use the metadata to stratify the samples and compare subsets of the participants (young vs old, symptomatic vs asymptomatic) when we search for genes that act as antigens. The study concluded:

- increased antigenicity was present in children who did not show clinical symptoms of malaria and suggest that these antigens are protective in children whose malaria symptoms did not recur.
- there was no correlation between antigenicity and time to re-infection

Test both these conclusions.

- a. Compare children (age 0-12) who got clinical malaria during the study (time to first malaria Dx weeks 4-9) to those who didn't (week 11+).
  - Set two parameters for the Reference sample:  
Age = 0-12  
Time to First Malaria Dx = weeks 4-9.
  - Set two parameters for the Comparison Sample:  
Age = 0-12  
Time to First Malaria Dx = Week 11+ and Week 11+.
  - Try running with increased immunogenicity then revise and change to decreased immunogenicity. See images below for help configuring the search.
  - Do these results make sense?
- b. Ask the same question (age 0-12) except compare time to re-infection weeks 3 and 4 with time to reinfection weeks 9,10,11,11+. Do you get significant results? Does this agree with the conclusions of the paper? Revise the search and remove the age limits, just keeping the times to re-infection.

P. falciparum 3D7 Treatment-time to reinfection cohort from Kisumu area, Kenya collected in 2003 (Dent et al.) TSTU

Show All Data Sets

T-Test (unequal variance)

### Identify Genes based on P.fal. Treatment-time to reinfection cohort from Kisumu area, Kenya collected in 2003 Antibody Array (p-value)

Reference Samples 19 of 172 selected Age between 0.61 and 12 Time to first malaria dx is We...

Select Reference Samples View selected Reference Samples (19) Collapse

General Information

Age

Specimen Collection Information

Laboratory Methods And Results

Parasite Detection

Blood Smear Result Summary

**Time To First Malaria Dx**

Time To Reinfection

#### Time To First Malaria Dx

select all | clear all

Time to first malaria dx	Total Reference Samples	Matching Reference Samples	Distribution
<input type="checkbox"/> Week 11+	151	64	<div style="width: 42%;"></div>
<input type="checkbox"/> Week 111+	2	2	<div style="width: 100%;"></div>
<input checked="" type="checkbox"/> Week 4	3	3	<div style="width: 100%;"></div>
<input checked="" type="checkbox"/> Week 5	2	2	<div style="width: 100%;"></div>
<input checked="" type="checkbox"/> Week 6	2	2	<div style="width: 100%;"></div>
<input checked="" type="checkbox"/> Week 7	5	5	<div style="width: 100%;"></div>
<input checked="" type="checkbox"/> Week 8	5	5	<div style="width: 100%;"></div>
<input checked="" type="checkbox"/> Week 9	2	2	<div style="width: 100%;"></div>

Legend: ■ All ■ Reference Samples

Reference Samples remaining when other criteria has been applied.

Comparison Samples 66 of 172 selected Age between 0 and 12 Time to first malaria dx is We...

Select Comparison Samples View selected Comparison Samples (66) Collapse

General Information

Age

Specimen Collection Information

Laboratory Methods And Results

Parasite Detection

Blood Smear Result Summary

**Time To First Malaria Dx**

Time To Reinfection

#### Time To First Malaria Dx

select all | clear all

Time to first malaria dx	Total Comparison Samples	Matching Comparison Samples	Distribution
<input checked="" type="checkbox"/> Week 11+	151	64	<div style="width: 42%;"></div>
<input checked="" type="checkbox"/> Week 111+	2	2	<div style="width: 100%;"></div>
<input type="checkbox"/> Week 4	3	3	<div style="width: 100%;"></div>
<input type="checkbox"/> Week 5	2	2	<div style="width: 100%;"></div>
<input type="checkbox"/> Week 6	2	2	<div style="width: 100%;"></div>
<input type="checkbox"/> Week 7	5	5	<div style="width: 100%;"></div>
<input type="checkbox"/> Week 8	5	5	<div style="width: 100%;"></div>
<input type="checkbox"/> Week 9	2	2	<div style="width: 100%;"></div>

Legend: ■ All ■ Comparison Samples

Comparison Samples remaining when other criteria has been applied.

Metadata category to color graph by age

Direction increased immunogenicity

P value less than or equal to 0.05

Treatment-time to reinfection cohort from Kisumu area, Kenya collected in 2003 Antibody Array (p-value)

39 Genes

Step 1

Add Step

Get Answer