

## 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 “Transcriptomics” section then select “RNA Seq Evidence”. Select the fold change query for the “Transcriptomes of 29 strains during murine macrophage infection (Minot et al.)” experiment.

The image shows two screenshots from the HostDB website. The top screenshot is the 'Search for Genes' page, which has a search bar and a list of categories. The 'Transcriptomics' category is expanded, and 'RNA Seq Evidence' is selected. A red arrow points from this selection to the bottom screenshot. The bottom screenshot is the 'Identify Genes based on RNA Seq Evidence' page. It features a search filter, a legend with buttons for 'S', 'DE', 'FC', and 'P', and a table of data sets. The table has columns for 'Organism', 'Data Set', and 'Choose a search'. The second data set is selected, and the 'FC' button is highlighted.

**Search for Genes**

expand all | collapse all

Find a search...

- Text
- Gene models
- Annotation, curation and Identifiers
- Genomic Location
- Taxonomy
- Orthology and synteny
- Transcriptomics
  - RNA Seq Evidence
- Sequence analysis

**Identify Genes based on RNA Seq Evidence**

Filter Data Sets:

Legend:  Similarity  Differential Ex...  Fold Change  Percentile

Organism	Data Set	Choose a search
<i>M. musculus</i> C57BL6J	Transcriptomes of mouse macrophages infected with <i>Leishmania mexicana</i> (Fiebig et al.) <b>NEW</b>	<input type="button" value="DE"/> <input type="button" value="FC"/> <input type="button" value="P"/>
<i>M. musculus</i> C57BL6J	Transcriptomes of 29 strains during murine macrophage infection (Minot et al.)	<input checked="" type="button" value="S"/> <input type="button" value="FC"/> <input type="button" value="P"/>

- 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.
  - What do these genes represent?

# Identify Genes based on M. musculus C57BL6J Transcriptomes of 29 strains during murine macrophage infection RNASeq (fold change)

Tutorial 

For the Experiment  
 Transcriptomes of 29 strains during murine macrophage infection unstranded  
 return protein coding Genes  
 that are up-regulated  
 with a Fold change >= 10

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

- TgCATBr9 infected
- VAND infected
- VEG infected
- WTD3 infected
- Un-infected

select all | clear all

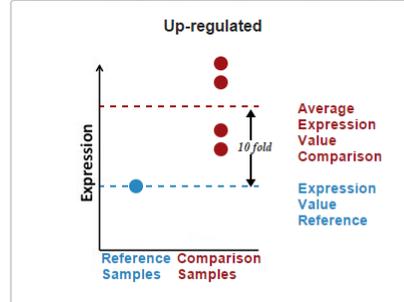
and its average expression value  
 in the following Comparison Samples

- TgCATBr9 infected
- VAND infected
- VEG infected
- WTD3 infected
- Un-infected

select all | clear all

## Example showing one gene that would meet search criteria

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



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 >= 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 \(0\)](#) [Help](#)

(Genes) Strategy: mouse infected w/ 29 Tg strains (fc) \* Rename Duplicate Save As Share Delete

mouse infected w/ 29 Tg strains (fc) 64 Genes Add Step

Step 1

## 64 Genes from Step 1

Strategy: mouse infected w/ 29 Tg strains (fc)

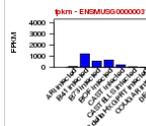
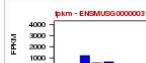
Click on a number in this table to limit/filter your results

All Results	Ortholog Groups	Homo Mus	
		H.sapiens	M.musculus
		REF	C57BL6J
64	64	0	64

Gene Results Genome View Analyze Results

Genes: 64 Transcripts: 182  Show Only One Transcript Per Gene

First 1 2 3 4 5 Next Last Advanced Paging Download Add to Basket Add Columns

Gene ID	Transcript ID	Organism	Product Description	Fold Change	Chosen Ref	Chosen Comp	mouse infected w/ 2
ENSMUSG00000031779.3	ENSMUST000000034231	M. musculus C57BL6J	chemokine (C-C motif) ligand 22	575.7	0.95	1125.32	
		M. musculus	chemokine (C-C motif)				

- c. What are the functional characteristics of the genes in this result? What kinds of GO terms are enriched? Does the host immune response appear to be turned on?  
*Hint:* click on the “Analyze Results” tab and perform a GO enrichment analysis for the biological process ontology.

Gene Results | Genome View | Gene Ontology Enrichment | **Analyze Results** [Rename This Analysis | Copy These Parameter Values]

### Gene Ontology Enrichment

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

Parameters

This analysis result may be lost if you change your gene result. To save this analysis result, please [Download Analysis Results](#)

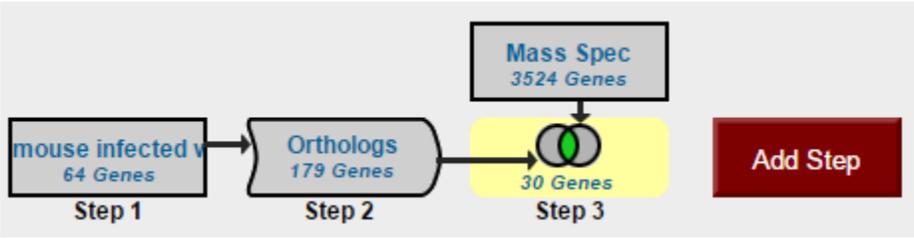
**Analysis Results:**  
 Got a total of 1,258 results Filter:

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:0006955	immune response	619	20	3.2	15.49	22.91	1.06e-18	1.33e-15	1.33e-15
GO:0034097	response to cytokine	388	16	4.1	19.76	26.75	1.36e-16	5.97e-14	1.71e-13
GO:0002376	immune system process	1069	22	2.1	9.86	15.13	1.42e-16	5.97e-14	1.79e-13
GO:0071345	cellular response to cytokine stimulus	300	14	4.7	22.37	29.01	2.68e-15	8.42e-13	3.37e-12
GO:0019221	cytokine-mediated signaling pathway	187	11	5.9	28.19	34.42	3.06e-13	7.70e-11	3.85e-10
GO:0071310	cellular response to organic substance	968	18	1.9	8.91	12.39	8.09e-13	1.70e-10	1.02e-9
GO:0006952	defense response	714	16	2.2	10.74	14.36	1.25e-12	2.04e-10	1.58e-9
GO:0051239	regulation of multicellular organismal process	1497	21	1.4	6.72	9.89	1.30e-12	2.04e-10	1.63e-9
GO:0010033	response to organic substance	1526	21	1.4	6.6	9.69	1.86e-12	2.60e-10	2.34e-9
GO:0001817	regulation of cytokine production	309	12	3.9	18.61	23.11	2.53e-12	3.18e-10	3.18e-9
GO:0006954	inflammatory response	234	11	4.7	22.53	27.46	3.13e-12	3.58e-10	3.94e-9

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



- e. Does this set of human genes also show enriched GO terms? What, if any, are the enriched GO terms?  
 f. 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* antigens that are immunogenic.

For this exercise use <http://plasmodb.org>

- a. Identify antigens (genes) that exhibited an increased immunogenicity in children (ages 0-18) with no disease (normal) compared to children with disease (malaria). *Hint:* the “Protein Array” search is available in the “Immunology” 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.org search interface. On the left is a 'Search for Genes' sidebar with a search bar and a list of categories. The 'Immunology' category is expanded, and 'Protein Array' is selected. A red arrow points from this menu item to the main search area. The main area is titled 'Identify Genes based on Protein Array'. It features a table with columns for 'Organism', 'Data Set', and 'Choose a search'. The 'Organism' column contains 'P. falciparum 3D7'. The 'Data Set' column contains 'Protein targets of serum antibodies in response to infection (Crompton et al.)'. The 'Choose a search' column has a 'T-Test' button circled in red. Below the table, there are options for 'T-test (unequal variance)', 'Reference Samples' (421 of 421 selected), 'Comparison Samples' (421 of 421 selected), 'Direction' (increased immunogenicity), and 'P value less than or equal to' (0.05). A red box on the right contains the text: 'This is a view of the search page with the reference and comparison sample parameters collapsed'. A 'Get Answer' button is at the bottom.

In this example, your **comparison samples will be normal children** and your **reference samples will be infected children**. Each set of samples (reference and comparison) has two parameters that need to be set, age and health status. Set the age parameter to 0-18 years for both the reference and the comparison samples.

Reference Samples 345 of 421 selected Age is between 0 and 18



Now set the **health status** for the reference samples to **malaria**. 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 of 421 selected Age is between 0 and 18 Health\_Status is malaria



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 (91) Basket Public Strategies (26) Help

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

Serum Ab Respo  
41 Genes Add Step

Step 1

Rename  
Duplicate  
Save As  
Share  
Delete

41 Genes from Step 1  
Strategy: Serum Ab Response (p-val)

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

Gene Results Genome View Analyze Results

Genes: 41 Transcripts: 42  Show Only One Transcript Per Gene

Advanced Paging Download Add to Basket Add Column

Gene ID	Transcript ID	Organism	Product Description	P Value	Avg Ref (arcsinh(1+50x))	Avg Comp (arcsinh(1+50x))	Expression - graph
PF3D7_1334200	PF3D7_1334200.1	<i>P. falciparum</i> 3D7	chaperone binding protein, putative	1.77E-04	5.730435	6.167731	

### 3. Find *falciparum* antigens that may be protective from reoccurrence of malaria (and potentially reinfection)

For this exercise use <http://plasmodb.org>

A recently published study from Kenya ([view paper](#)) where participants were followed for 12 weeks following an initial screening for malaria and treatment with anti-malarials is available in PlasmoDB. Each week patients were assessed for presence of parasites and clinical symptoms of malaria. Select the **“Treatment-time to reinfection cohort from Kisumu area, Kenya collected in 2003 (Dent et al.)”** experiment from the protein array searches and configure the parameters to see if you can reproduce the results of the paper. They concluded that increased antigenicity was present in children who did not show clinical symptoms of malaria, and suggest that these antigens are protective in children who did not get a recurrence of symptomatic malaria (compared to those children who did exhibit malaria symptoms). They also concluded that there did not appear to be a correlation between antigenicity and time to re-infection (could be asymptomatic). Test both these conclusions.

*Hint: compare children (age 0-12.5) who got clinical malaria during the study (time to first malaria Dx weeks 4-9) compared to those who didn't (week 11+). Try running with increased immunogenicity then revise and change to decreased immunogenicity. See image below for help configuring the search. Do these results make sense?*

Ask the same question (age 0-12.5) 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.

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 12.64](#) [Time to first malaria dx is We...](#)

Select Reference Samples View selected Reference Samples (19) Collapse

▼ General Information

Age

▼ Specimen Collection Information

▶ Sample Collection Location

Date Sample Collected

End Of Observation Period

Sample Id

▼ 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: ■ Reference Samples

Reference Samples remaining when other criteria has been applied.

Comparison Samples 66 of 172 selected Age between 0.61 and 12.55 Time to first malaria dx is We...

Select Comparison Samples View selected Comparison Samples (66) Collapse

**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	
<input checked="" type="checkbox"/> Week 11+	2	2	
<input type="checkbox"/> Week 4	3	3	
<input type="checkbox"/> Week 5	2	2	
<input type="checkbox"/> Week 6	2	2	
<input type="checkbox"/> Week 7	5	5	
<input type="checkbox"/> Week 8	5	5	
<input type="checkbox"/> Week 9	2	2	

All 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

Get Answer

**Treatment-time to 39 Genes**  
Step 1 Add Step

