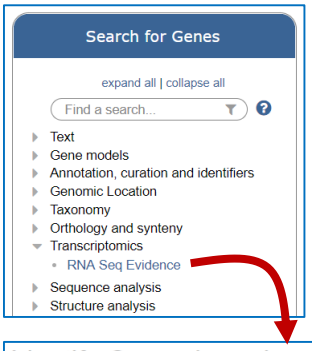
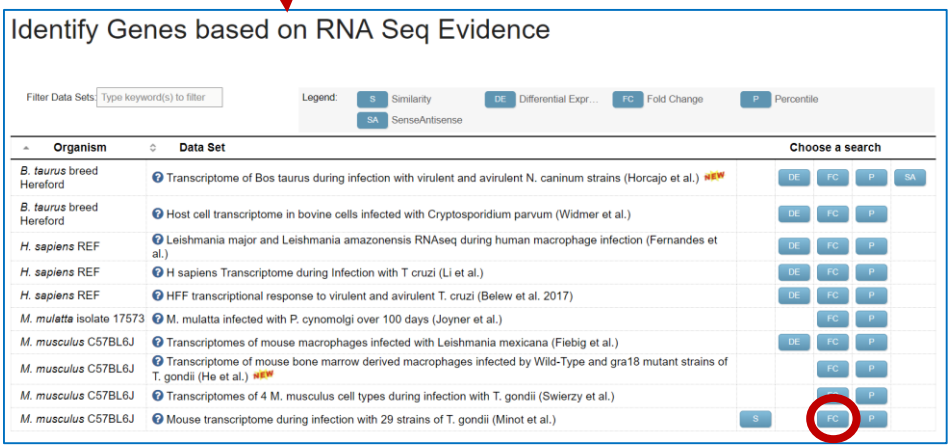
# Host Response

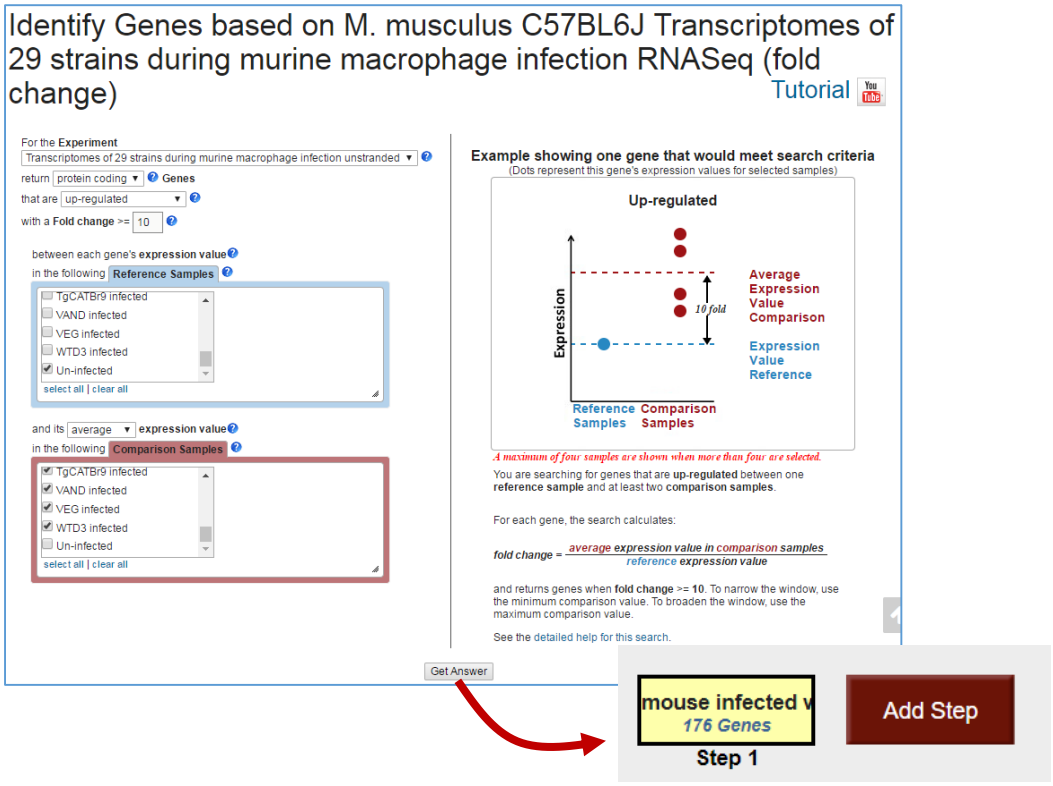
1. Find host genes that are upregulated in infected mouse cells compared to uninfected ones. For this exercise use http://hostdb.org

   a. HostDB has data from a published study that performed a comparative transcriptome analysis of 29 different strains of *Toxoplasma gondii* and the murine macrophages infected with them. We loaded the parasite component of the data in ToxoDB and the host component in HostDB. Go to HostDB.org and navigate to the "Transcriptomics" section then select "RNA Seq Evidence". Select the fold change query for the "Mouse transcriptomes during infection with 29 strains of T gondii (Minot et al.)" experiment.

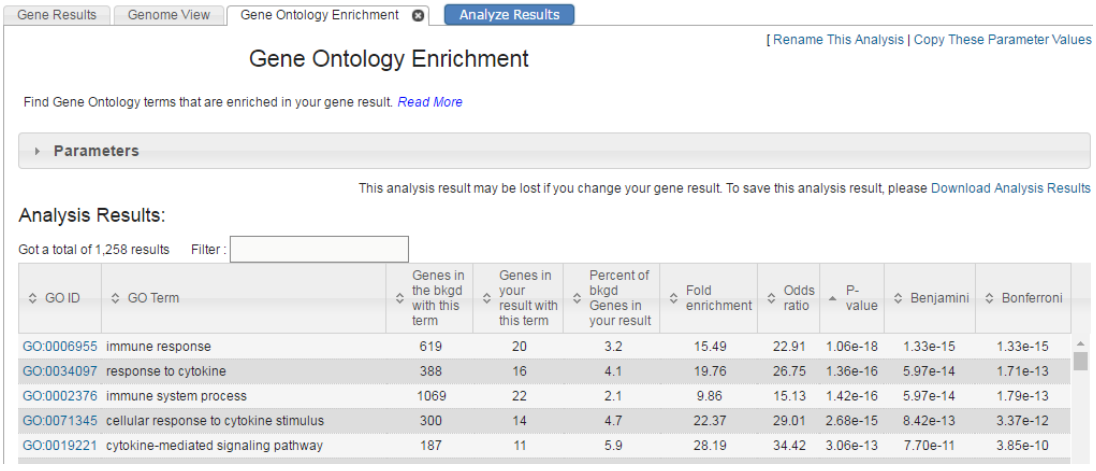



   b. Configure the search to return genes that are up-regulated at least 10-fold across all strains in the experiment compared to the uninfected control. Make sure to select upregulated. In the example below a fold change of 10 was selected and the **"average"** operation was applied on the comparison samples.

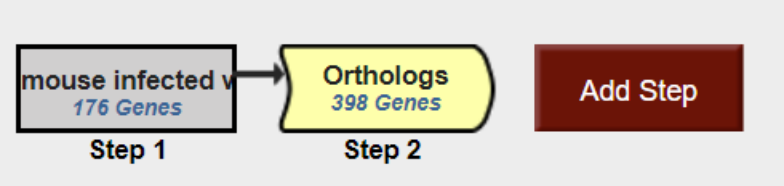



c. What are the functional characteristics of the genes in this result? What kinds of GO terms are enriched? Does the host immune response appear to be turned on? Is there a particular cellular location that is common in this group of genes?
*Hint*: click on the "Analyze Results" tab and perform a GO enrichment analysis for the biological process ontology.



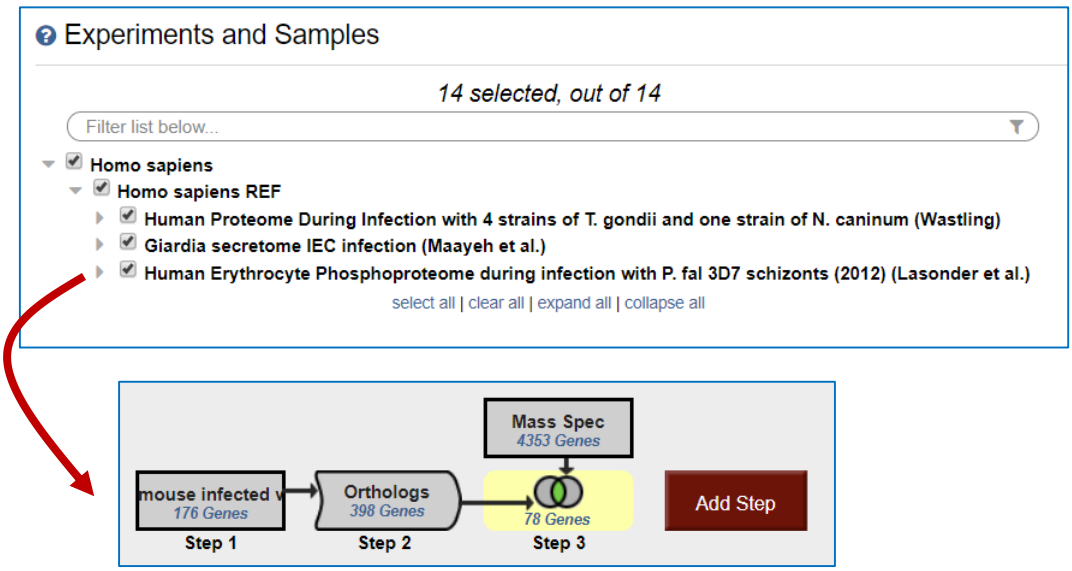

| GO ID | GO Term | Genes in the bkgd with this term | Genes in your result with this term | Percent of bkgd Genes in your result | Fold enrichment | Odds ratio | P-value | Benjamini | Bonferroni |
|---|---|---|---|---|---|---|---|---|---|
| GO:0006955 | immune response | 619 | 20 | 3.2 | 15.49 | 22.91 | 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 |

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



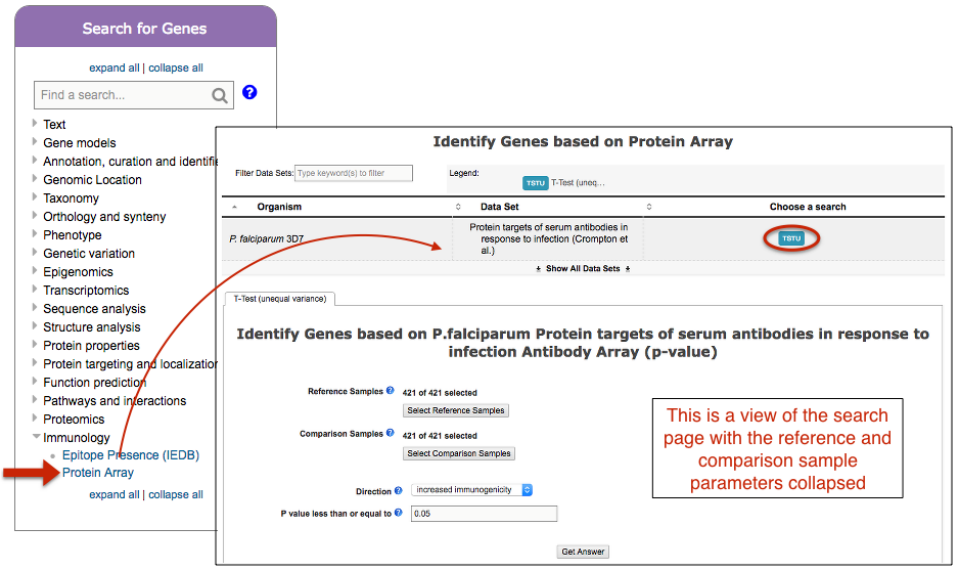

e. Does this set of human genes also show enriched GO terms? What, if any, are the enriched GO terms?

f. Do any of these human genes also have peptide evidence for their expression during infection? *Hint:* add a step and explore the proteomics data "Human Proteome During T. gondii infection"

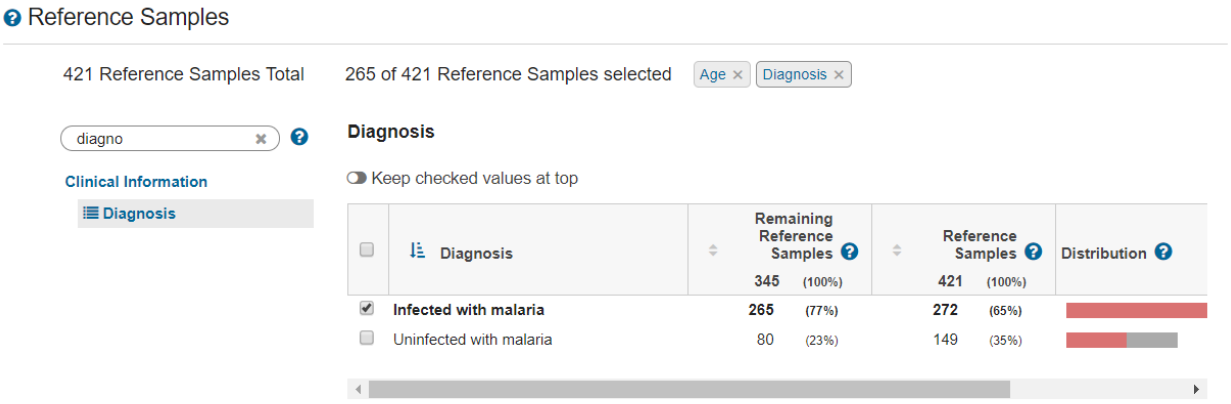


2. Find *Plasmodium falciparum* antigens that are immunogenic.
   For this exercise use http://plasmodb.org

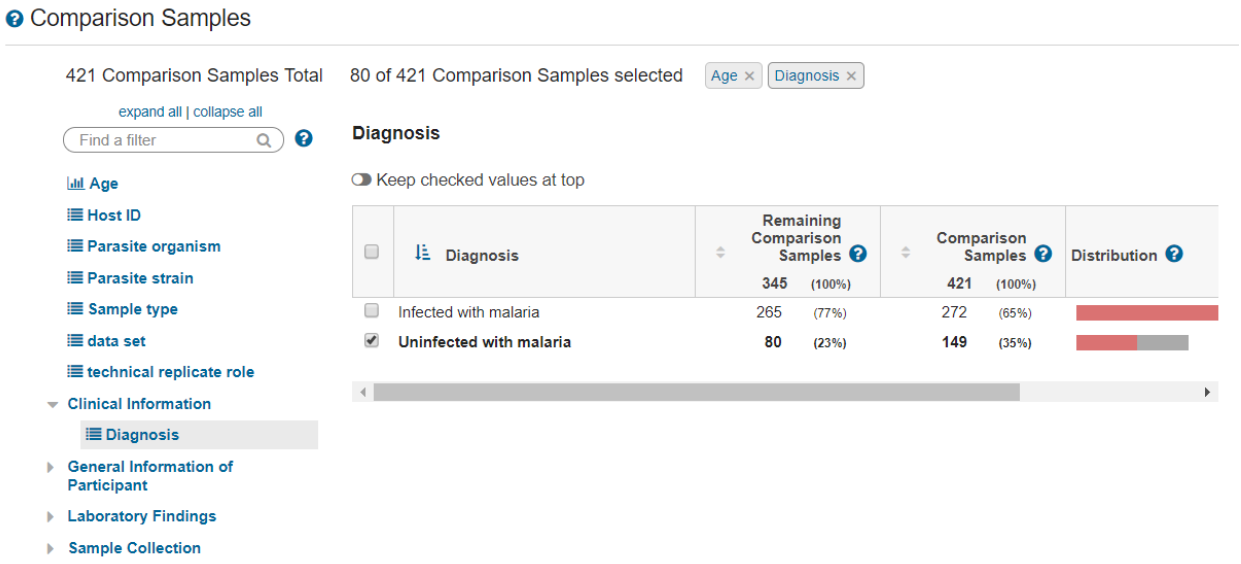
   a. Identify antigens (genes) that exhibited an increased immunogenicity in children (ages 0-18) with no disease (normal) compared to children with disease (malaria). *Hint*: navigate through the "Identify Genes By", "Immunology", "Protein Array". Choose the experiment "**Protein targets of serum antibodies in response to infection (Crompton et al.)**".



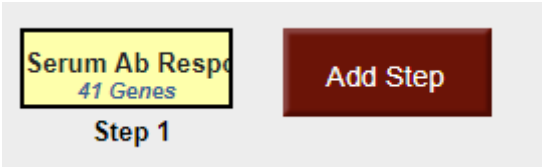

- We want to learn which genes were protective against malaria. Our strategy is to look for genes with increased immunogenicity in children without malaria (comparison samples) using children with malaria as reference (reference sample). In this example, your **reference samples will be children** with **a diagnosis of 'Infected with malaria',** and your **comparison samples will be children** with **'Uninfected with malaria'**. Each set of samples (reference and comparison) has two parameters that need to be set, age and diagnosis. For the reference, set the age parameter **to 0-18** years and the Diagnosis (under Clinical Information) to **Infected with malaria'**. You should be left with 265 samples in the Reference group of children with malaria.




- Move on the comparison samples and set the age to **0-18** and the diagnosis to **uninfected with malaria**. Your comparison group should contain 80 samples.




- The default settings for other parameters are good – increased immunogenicity and p-value = 0.05.
- You are ready to click Get Answer! What do your results look like? Could these represent potential protective antigens? (result image below)



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

   For this exercise use http://plasmodb.org

   PlasmoDB contains a data from a published study from Kenya (view paper) where participants were followed for 12 weeks after an initial screening for malaria and treatment with anti-malarials. Each week patients were assessed for the 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:

   1. increased antigenicity was present in children who did not show clinical symptoms of malaria. The authors suggested that these antigens are protective in children who did not get a recurrence of symptomatic malaria.
   2. There was no correlation between antigenicity and time to re-infection (could be asymptomatic).

   This study collected two clinical parameters: Time to First Malaria Diagnosis and Time to Reinfection. Explore the protein array data and take advantage of the sample filter capabilities to compare children whose clinical symptoms were delayed to those who got sick quickly.

   *Hint #1: compare children (age at time of visit 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?

# Identify Genes based on P. falciparum 3D7 Treatment-time to reinfection cohort from Kisumu area, Kenya collected in 2003 Antibody Array (p-value)

## Reference Samples

172 Reference Samples Total

19 of 172 Reference Samples selected

Time to First Malaria Diagnosis ×

expand all | collapse all

Find a filter

- Age
- Parasite organism
- Parasite strain
- Sample type
- data set
- Clinical Information
  - Time to First Malaria Diagnosis
  - Time to Reinfection
- General Information of Participant
- Laboratory Findings
- Sample Collection

expand all | collapse all

**Time to First Malaria Diagnosis**

Keep checked values at top

| Time to First Malaria Diagnosis | Remaining Reference Samples 172 (100%) | Reference Samples 172 (100%) | Distribution | % |
|---|---|---|---|---|
| **Week 4** | **3 (2%)** | **3 (2%)** | | **(100%)** |
| **Week 5** | **2 (1%)** | **2 (1%)** | | **(100%)** |
| **Week 6** | **2 (1%)** | **2 (1%)** | | **(100%)** |
| **Week 7** | **5 (3%)** | **5 (3%)** | | **(100%)** |
| **Week 8** | **5 (3%)** | **5 (3%)** | | **(100%)** |
| **Week 9** | **2 (1%)** | **2 (1%)** | | **(100%)** |
| Week 11+ | 151 (88%) | 151 (88%) | | (100%) |
| Week 111+ | 2 (1%) | 2 (1%) | | (100%) |

## Comparison Samples

172 Comparison Samples Total

153 of 172 Comparison Samples selected

Time to First Malaria Diagnosis ×

expand all | collapse all

Find a filter

- Age
- Parasite organism
- Parasite strain
- Sample type
- data set
- Clinical Information
  - Time to First Malaria Diagnosis
  - Time to Reinfection
- General Information of Participant
- Laboratory Findings
- Sample Collection

expand all | collapse all

**Time to First Malaria Diagnosis**

Keep checked values at top

| Time to First Malaria Diagnosis | Remaining Comparison Samples 172 (100%) | Comparison Samples 172 (100%) | Distribution | % |
|---|---|---|---|---|
| Week 4 | 3 (2%) | 3 (2%) | | (100%) |
| Week 5 | 2 (1%) | 2 (1%) | | (100%) |
| Week 6 | 2 (1%) | 2 (1%) | | (100%) |
| Week 7 | 5 (3%) | 5 (3%) | | (100%) |
| Week 8 | 5 (3%) | 5 (3%) | | (100%) |
| Week 9 | 2 (1%) | 2 (1%) | | (100%) |
| **Week 11+** | **151 (88%)** | **151 (88%)** | | **(100%)** |
| **Week 111+** | **2 (1%)** | **2 (1%)** | | **(100%)** |

Edit

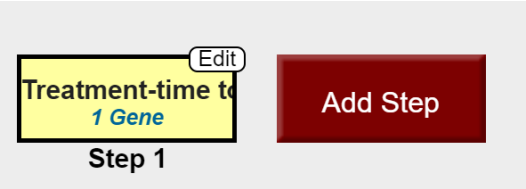
Treatment-time t

*65 Genes*

Add Step

**Step 1**

*Hint #2: 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****.*

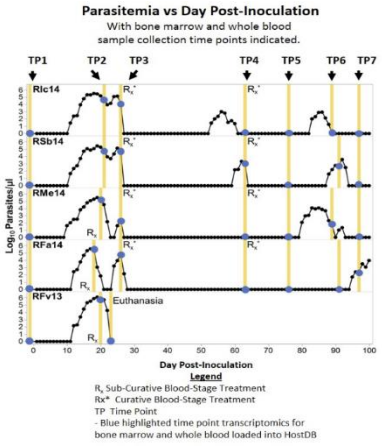



Do you get significant results? Does this agree with the conclusions of the paper? Revise the search and remove the age limits, just keeping the times to re-infection.

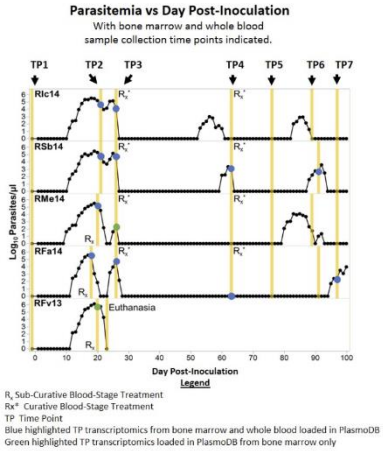
4. **Host vs parasite expression changes in dual RNA seq samples with clinical data. -** The Malaria Host-Pathogen Interacting Center (MaHPIC) aims to characterize host-pathogen interactions during malaria infections. Dual RNA seq data (host and pathogen) from MaHPIC Experiment 4, a 100 day infection of *M. mulatta* with *P. cynomolgi,* is integrated into HostDB and PlasmoDB, respectively. Five monkeys were inoculated with *P. cynomolgi* sporozoites on day 1 and followed for 100 days with daily blood collections, drug treatments and 7 pathology-driven time point collections of bone marrow and blood. Supporting information including parasitemia charts and disease progression definitions is available in the data set records (links below). Clinical data is available in PlasmoDB Downloads.

    - Data record page in HostDB - *M mulatta* infected with *P cynomolgi* over 100 days http://hostdb.org/hostdb/app/record/dataset/DS_4585d065bf
    - Data record page in PlasmoDB - Transcriptome of *P cynomolgi* during 100-day infection in *M mulatta* http://plasmodb.org/plasmo/app/record/dataset/DS_40a06f276b
    - MaHPIC Data Record page http://plasmodb.org/plasmo/mahpic.jsp
    - MaHPIC data files in PlasmoDB http://plasmodb.org/common/downloads/MaHPIC/

**HostDB**




**PlasmoDB**




| Primate ID | Baseline (Pre-infection) | Acute Primary | Post-peak | Inter-relapse | Relapse |
|---|---|---|---|---|---|
| Rlc14 | TP1 | TP2 | TP3 | TP5, TP7 | TP4, TP6 |
| RSb14 | TP1 | TP2 | TP3 | TP5, TP7 | TP4, TP6 |
| RMe14 | TP1 | TP2 | TP3 | TP4, TP5, TP7 | TP6 |
| RFa14 | TP1 | TP2 | TP3 | TP4, TP5, TP6 | TP7 |
| RFv13 | TP1 | TP2 | TP3 | N/A | N/A |

a. Find genes that are upregulated at least 2-fold in the bone marrow of monkeys during the acute primary infection (Time point 2 – TP2) vs the post-peak infection (Time point 3 – TP3).

- Navigate to http://hostdb.org/hostdb/ and open the RNA sequence fold change search for the experiment called "*M mulatta* infected with *P cynomolgi* over 100 days (Joyner et al.)".
- *Hint – use the information in the data records (links and small screen shots above) to create a fold change search comparing time point 2 (Acute primary infection) and time point 3 (post peak infection). There are 4 monkeys with data for each time points because monkey RFv13 was too sick to complete the study.*
- Give it a try on your own ☺. Then compare your results with the strategy below or have a peak at the screenshots

  Search - http://hostdb.org/hostdb/im.do?s=ee8250c6640668c1

  Screenshots - https://docs.google.com/presentation/d/1WnzsBPEQv8ruWgEynL-iR0qKRPD12ySdY_itbcyNB3Y/edit?usp=sharing

- What functional Characteristics are shared by these *M. mulatta* genes?
  Hint – run a GO enrichment for Biological process)
- What human genes are likely associated with acute primary vs post peak infections? Hint – transform to human
- What functional characteristics are shared by the human genes?

Hint – run a GO enrichment for biological process.

- Are there differences between the GO enrichment results between the human and *M. mulatta* gene lists? If so, what underlying data contributes to the differences? What can you say about the difference between *M mulatta* genome annotation and the human genome annotation?

b. OPTIONAL: Create the same strategy in PlasmoDB and explore the results (minus the human transformation, of course). Do you find similar functional enrichment profiles for the plasmodium genes that are differentially expressed between acute and post-peak disease state? (Hint - Transform to an organism with mature annotation such as *P. falciparu*m before functional enrichment).