

Finding Genes, Building Search Strategies and Visiting a Gene Page

1. Finding a gene using text search.
For this exercise use <http://www.plasmodb.org>

- a. Find all possible kinases in *Plasmodium*.

Hint: use the keyword “kinase” (without quotations) in the “Gene Text Search” box.



- How many genes did you get?
- Look closely at the sections of the result page. How many of those are in *P. falciparum*? How did you find this out?

Hint – the filter table is located between the strategy panel and the result table and shows the distribution of results across the organisms that you searched. Click on a number to display on that species’ portion of the results.

The screenshot shows the search results page. At the top, there is a 'My Strategies' panel with 'New', 'Opened (1)', 'All (258)', 'Basket', and 'Public Strategies'. Below this is a 'Text' strategy with '223 Genes' and 'Step 1'. An 'Add Step' button is visible. Below the strategy panel is a table showing the distribution of results across organisms. The table is circled in red.

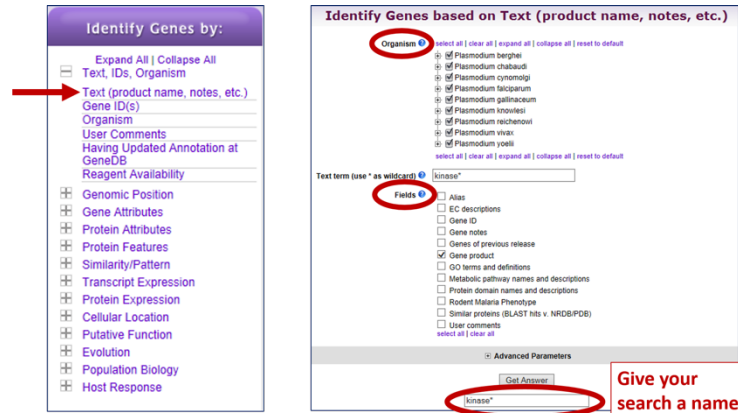
| All Results | Ortholog Groups | Plasmodium | | | | | |
|-------------|-----------------|---------------|--------------------|-----------------|---------------------------------|-----|-----------------|
| | | Pberghel ANKA | Pchabaudi chabaudi | Pcynomol strain | Pfalciparum (nr genes: 222) 3D7 | IT | Pgallinaceum 8A |
| 2037 | 243 | 173 | 174 | 171 | 223 | 196 | 0 |

- What happens if you search using the term **kinases** in the Gene Text Search box? How many results are returned?
- b. Find only the kinases that specifically have the word “kinase” in the gene product name.

The search you ran in step 1a using the Gene Text Search box initiates a preconfigured search. Initiating the search from the full text search form - **Identify Genes based on**

Text, allows you to configure the search yourself, choosing parameters that best meet your needs. Use the search form to search for genes that have the word kinase in their **gene product** name/description.

- There are several ways to navigate to the **Identify Genes based on Text** page. Notice the sections of the search page. At the top are parameters and the Get Answer button followed by a search description and a list of datasets used by the search.



- How can you make sure to find your text term in plural form or in compound words like “kinases” or “6-phosphofruktokinase”. Adding a wild card (wildcard = asterisk and means any character) in your search term will broaden your search. Use the full text search, the specific page where you can define the fields to be searched (Fields = Gene Product).

Try kinase *kinase *kinase*

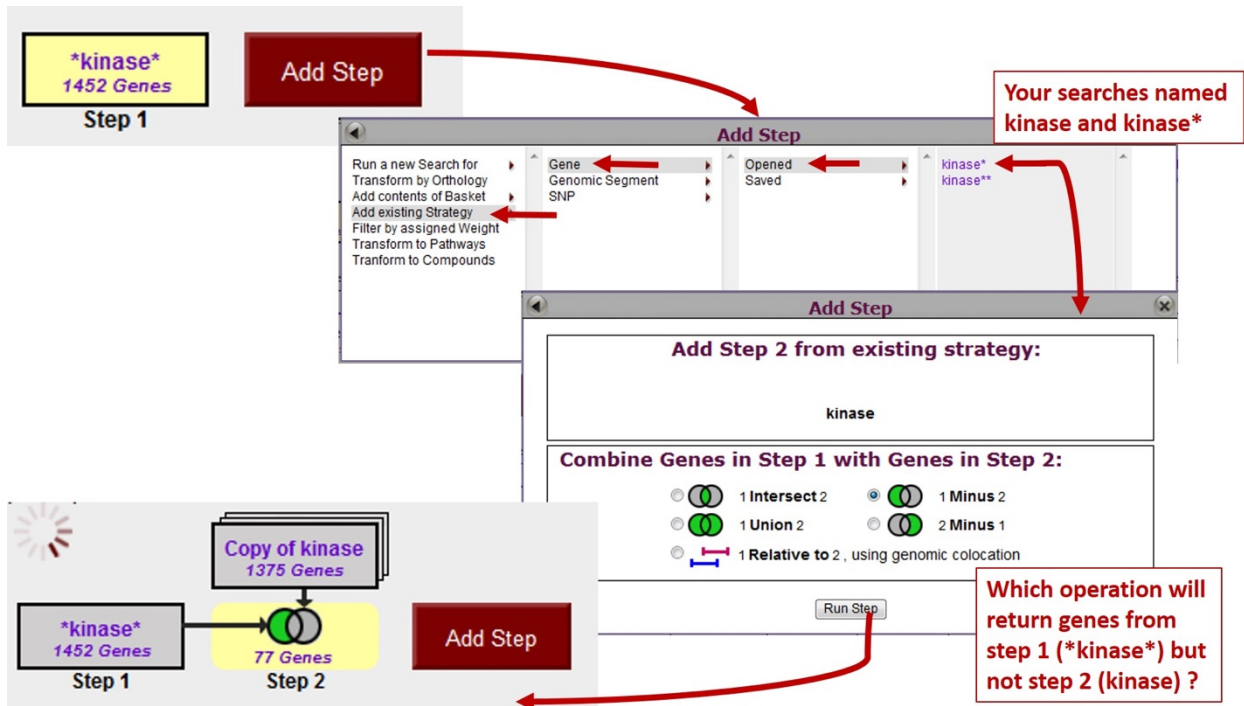
- Give each new search a name to help you keep track of the searches.
- How did you get to the Text Search page?
- How does limiting the number of fields searched affect your results?
- Did you remember to use the wild card?
- How many genes have the word kinase in their product names?

c. Combine the results of two text searches.

Find genes that were identified using the key word ***kinase*** but not the word **kinase**?

- Here we will build a search strategy that combines 2 of your searches. If you are not displaying the results of the ***kinase*** search (the strategy box will be highlighted in yellow), return to it by clicking on that step box in the strategy panel. To add your **kinase** search to this strategy, click on “Add Step” and select “existing strategy”:
- Select the right strategy from your list of Gene Strategies and combine the strategies with the correct operation. Notice that there is an extra asterisk at the end of an

unsaved strategy name. The list of available searches will have an * at the end of the name.



- Do the results make sense? Do all the product names contain the word kinase? From the result page look at the table of gene IDs returned by the search. The Product Description column contains the gene product name.

2. Combing text search results with results from other searches

a. Find kinase genes that are likely secreted.

In exercise 1b. you identified genes that have the word **kinase** somewhere in their gene product name (searching *kinase* in gene product field). Grow your search strategy by adding a step that returns genes whose protein products are predicted to have a signal peptide. In this search you are querying the results of our genome-wide analysis that used the SignalP program to predict the presence and location of signal peptide cleavage sites in amino acid sequences.

<http://www.cbs.dtu.dk/services/SignalP/>

Focus your Strategies section on the ***kinase*** search and click Add Step. For the second search choose **Identify Genes based on Protein Features, Predicted Signal Peptide**

- How did you combine the search results?

- How many kinases are predicted to have a signal peptide?

The image shows a bioinformatics workflow interface. At the top left, a box labeled "Step 1" contains the search term "*kinase*" and "1452 Genes". A red "Add Step" button is next to it. A red arrow points from this button to a larger "Add Step" dialog box. This dialog box has a tree view with categories like "Genes", "Genomic Segments", "SNPs", "ORFs", "Text, IDs, Organism", "Genomic Position", "Gene Attributes", "Protein Attributes", "Protein Features", "Similarity/Pattern", "Transcript Expression", "Protein Expression", "Cellular Location", "Putative Function", and "Predicted Signal Peptide". A red arrow points from the "Add Step" dialog box to a second "Add Step 2: Predicted Signal Peptide" dialog box. This second dialog box has a list of organisms (Plasmodium species) and "Advanced Parameters" for combining genes from Step 1 and Step 2. The options are:

- 1 Intersect 2
- 1 Minus 2
- 1 Union 2
- 2 Minus 1
- 1 Relative to 2, using genomic colocation

 A red box with the text "Which operation will return genes that are in both search result sets?" is positioned over the "Intersect 2" option. A third red arrow points from the "Add Step 2" dialog box back to the "Add Step" button in the workflow view. At the bottom, a workflow diagram shows Step 1 (1452 Genes) and Step 2 (91 Genes) connected by an arrow, with a "sig pep" box (10604 Genes) above Step 2. A red "Add Step" button is next to Step 2.

- b. Now that you have a list of possible secreted kinases, expand this strategy even further.

There is no wrong answer here!!

- From a biological standpoint what else would be interesting to know about these kinases? Add more searches to grow this strategy. Open the categories under Identify Genes By: on the home page and explore the types of searches that are available. You can reduce (or expand) your result set by adding searches that are based on many types of data.
- For example, how many of the secreted kinases also have transmembrane domains?

- c. In the above example, how can you define kinases that have either a secretory signal peptide AND/OR a transmembrane domain(s)?

Hint: to do this properly you will have to employ the "Nested Strategy" feature. Nesting a strategy allows you to control the order in which your result sets are combined. Think about the difference between two mathematical equations.

Equation without nesting: $2 \times 3 + 5 = 11$

Equation with nesting: $2 \times (3 + 5) = 16$

The screenshot shows a workflow interface with two steps: Step 1 (*kinase* 1452 Genes) and Step 2 (sig pep 10604 Genes). A red arrow points from the 'sig pep' box to a search results window. The search window title is 'Signal Pep' and it lists various Plasmodium species. Below the list are parameters: Minimum SignalP-NN Conclusion Score: 0.5, Minimum SignalP-NN D-Score: 0.5, Minimum SignalP-HMM Signal Probability: 0.5, and any or all advanced parameters: any. The results show 9366 Genes. A red arrow also points from the search window to the 'Expanded View of Step Signal Pep' section, which shows Step 1 (Signal Pep 10604 Genes) and an 'Add Step' button.

A

Diagram A shows a workflow with Step 1 (*kinase* 1452 Genes) and Step 2 (Signal Pep 23166 Genes). An 'Add Step' button is next to Step 2. Below is the 'Expanded View of Step Signal Pep', which shows Step 1 (Signal Pep 10604 Genes) and Step 2 (Transmb Dom 18524 Genes) with an 'Add Step' button. This indicates that Step 2 is a logical OR of the two criteria.

Strategy Logic:

Strategy A returns kinases that have a signal peptide OR a TM domain OR both. (SP and/or TM) (either or both)

Strategy B returns kinases that have a signal peptide AND a TM domain

B

Diagram B shows a three-step workflow: Step 1 (*kinase* 1452 Genes), Step 2 (Signal Pep 10604 Genes), and Step 3 (Transmb Dom 18524 Genes). The result of Step 2 is 91 Genes, and the result of Step 3 is 58 Genes. An 'Add Step' button is next to Step 3. This indicates that Step 3 is a logical AND of the two criteria.

3. Finding a gene by BLAST Similarity.

Note: For this exercise start with <http://www.toxodb.org>

Imagine that you generated an insertion mutant in *Toxoplasma* that is providing you with some of the most interesting results in your career! You sequence the flanking region and you are only able to get sequence from one side of the insertion (the sequence shown below). You immediately go to ToxoDB to find any information about this sequence. What do you do?

- aaaggagagaaagataaaaatatacaaaggtcccagagacacgatagtgttactgacaa
catacagaatcaggtcgagcaatggaagaaccaagcaccggcgccagagattgaactcgc
ttggattgccgtagcgtttatgagttgatagcttggctctaaaaaacaaggctgaaa
atggaaaaaatgtctccaat
- Sequence is also available from this URL:
<http://tinyurl.com/ex1blast>
- Try using the BLAST search with this sequence (hint: you can get to the BLAST tool by clicking on the BLAST link under tools on the home page).

The screenshot shows the ToxoDB website interface. At the top, there is a search bar with 'Gene ID: TGGT_230470' and 'Gene Text Search: synth'. Below the search bar, there is a navigation menu with 'Tools' selected. A dropdown menu is open under 'Tools', showing options: BLAST, Results Analysis, Sequence Retrieval, Pathogen Portal, PubMed and Entrez, Genome Browser, and Ancillary Genome Browser. A red arrow points to 'BLAST' in the dropdown menu. In the background, there is a 'Tools' section with a 'BLAST' link highlighted by a red arrow. The page also features a 'Data Summary' section, 'News and Tweets' section, and a 'Community Resources' section.

- Which blast program should you use? (hint: try different combinations, just keep in mind that you have a nucleotide sequence so you have to use an appropriate BLAST program).

Note on BLAST programs:

- blastp compares an amino acid sequence against a protein sequence database;
- blastn compares a nucleotide sequence against a nucleotide sequence database;
- blastx compares the six-frame conceptual translation products of a nucleotide sequence (both strands) against a protein sequence database;
- tblastn compares a protein sequence against a nucleotide sequence database dynamically translated in all six reading frames (both strands);
- tblastx compares the six-frame translations of a nucleotide sequence against the six-frame translations of a nucleotide sequence database.

1. Choose your target data type. What type of sequence in the database do you want to match your sequence to?

2. Choose the BLAST program to use.

3. Choose the target organism. What genome do you want to match your sequence to?

Target Data Type Transcripts
 Proteins
 Genome
 EST
 ORF
 Isolates

BLAST Program blastn
 blastp
 blastx
 tblastn
 tblastx

Target Organism
 Eimeria
 Hammondia
 Neospora
 Toxoplasma

Input Sequence

Note: only one input sequence allowed.
maximum allowed sequence length is 31K bases.

Expectation value

Maximum descriptions/alignments (V-B)

Low complexity filter

- Are you getting any results from blastx? tblastn? What about blastn?
- What is your gene? (hint: after running a blastn against *Toxoplasma* ME49 (Target organism) genomic sequence (Target Data Type), click on the “link to the genome browser”. In the genome browser zoom out to see what gene is in the area).

4. Viewing data on a gene page.

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

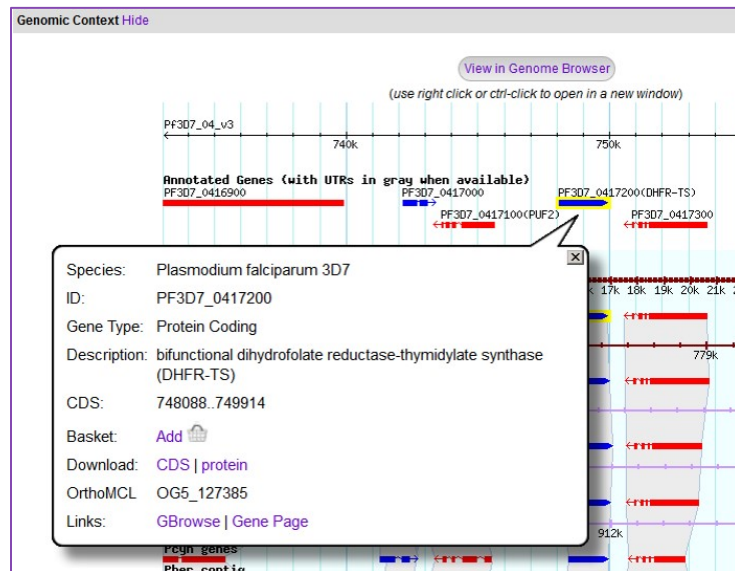
- Find the gene page for one of the following *P. falciparum* genes and explore the information there to answer these questions.
 - bifunctional dihydrofolate reductase-thymidylate synthase (DHFR-TS, PF3D7_0417200)

2. apical membrane antigen 1 gene (AMA1, PF3D7_1133400)

- How did you navigate to this gene? What other ways could you get there? I can think of 4 ways to reach the gene page.

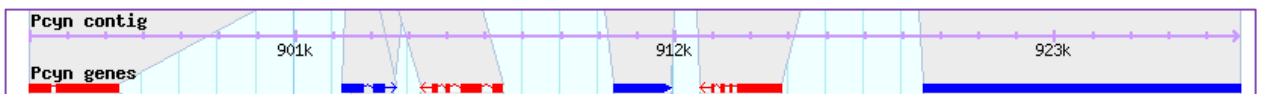
Look at the information on the gene page.

- What chromosome is this gene on?
- How many exons does this gene have? Hint: look at the graphic in the Genomic Context data track and mouse over the glyph representing the gene.
- What direction is the gene relative to the chromosome?
- How many nucleotides of coding sequence?
- Do you see a way to quickly download the coding and protein sequences?
- Does this gene have a user comment?



b. What genes are located upstream & downstream of DHFR-TS (AMA1) in *P. falciparum*?

- Is synteny (chromosome organization) in this region maintained in other species? Hint: look in the genomic context section of the gene page – what does the shading mean?
- How complete is the genome assembly for other species? Each genome is displayed as two tracks – the genomic sequence (chromosome or contig) on top and the gene models underneath. Do the contigs contain gaps or truncations?



- What does synteny look like across the entire chromosome? To do this:

- Click on the “**View in Genome Browser**” button in the genomic context section.
 - Zoom out to the entire chromosome. There are a few ways to do this. For example, drag your cursor across the entire chromosome in the Overview panel and then select “zoom” from the popup menu.
 - Click on the tab called “Select tracks”. Select the track called “Syntenic Sequences and Genes (Shaded by Orthology)”. Go back to the Browser tab (this may take a minute to load).
 - Which genome is composed of the most fragments? Are there any other interesting observations you can support by looking at synteny over large genomic regions?
- c. Does the *P. falciparum* DHFR-TS (or AMA1) gene contain Single Nucleotide Polymorphisms (SNPs)?

SNPs are represented in a table called “SNP Overview” and using the “Isolate Alignments in this Gene Region” track you can view an alignment showing SNPs between specific strains/isolates.

- Examine the SNP Overview table.
- What is the total number of SNPs in the gene?
- How many impact the predicted protein sequence?
- Is this likely to define the full spectrum of sequence variation in these particular strains?
- Compare the SNP characteristics of this gene to upstream and downstream genes. How do these results compare with SNP distribution in other genes?
- Open the Isolate Alignments in this Gene Region data track and run an alignment between several isolates: 303.1, 383.1, 7G8_2, GB4, N011-A, O222-A, PS097, PS206_E11, RV_3635, RV_3675

Isolate Alignments in this Gene Region Hide

- 303.1
- 383.1
- 7G8_2
- GB4
- N011-A
- O222-A
- PS097
- PS206_E11
- RV_3635
- RV_3675
- RV_3714
- RV_3737
- SantaLucia_Salvadori
- SenT021.09
- SenT042.09
- SenT068.08
- SenT092.08
- SenT110.09
- SenT135.09
- SenT149.09
- SenT197.08
- TRIPS_303
- TRIPS_331
- TRIPS_340
- TRIPS_347
- TRIPS_350
- TRIPS_355
- TRIPS_364
- TRIPS_373
- TRIPS_410
- TRIPS_440
- TRIPS_456
- TRIPS_461
- TRIPS_467
- TRIPS_470
- TRIPS_474
- TRIPS_487
- TRIPS_490
- TRIPS_499
- TRIPS_501
- TRIPS_504
- TRIPS_700
- UGK_396.1
- UGK_408.2
- UGK_432.4
- UGK_443.2
- UGK_659.1
- UGK_707.3
- UGK_730.2
- UGK_815.1
- vt1_s

http://plasmodb.org/cgi-bin/isolateClustalw?project_id=PlasmoDB;type=hts;SNP;si

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PF3DT_04_v3 748098 ATGATGGAAC AAGTCTGCGA CGTITTCGAT ATTATGCCA TATGTCATG TTGTAAGTT GAAACAAAA ATGAGGGGAA
303.1 748098 TGATGGAC AAGTCTGCGA CGTITTCGAT ATTATGCCA TATGTCATG TTGTAAGTT GAAACAAAA ATGAGGGGAA
383.1 748098 TGATGGAC AAGTCTGCGA CGTITTCGAT ATTATGCCA TATGTCATG TTGTAAGTT GAAACAAAA ATGAGGGGAA
7G8_2 748098 TGATGGAC AAGTCTGCGA CGTITTCGAT ATTATGCCA TATGTCATG TTGTAAGTT GAAACAAAA ATGAGGGGAA
GB4 748098 TGATGGAC AAGTCTGCGA CGTITTCGAT ATTATGCCA TATGTCATG TTGTAAGTT GAAACAAAA ATGAGGGGAA
N011-A 748098 TGATGGAC AAGTCTGCGA CGTITTCGAT ATTATGCCA TATGTCATG TTGTAAGTT GAAACAAAA ATGAGGGGAA
O222-A 748098 TGATGGAC AAGTCTGCGA CGTITTCGAT ATTATGCCA TATGTCATG TTGTAAGTT GAAACAAAA ATGAGGGGAA
PS097 748098 TGATGGAC AAGTCTGCGA CGTITTCGAT ATTATGCCA TATGTCATG TTGTAAGTT GAAACAAAA ATGAGGGGAA
PS206_E11 748098 TGATGGAC AAGTCTGCGA CGTITTCGAT ATTATGCCA TATGTCATG TTGTAAGTT GAAACAAAA ATGAGGGGAA
RV_3635 748098 TGATGGAC AAGTCTGCGA CGTITTCGAT ATTATGCCA TATGTCATG TTGTAAGTT GAAACAAAA ATGAGGGGAA
RV_3675 748098 TGATGGAC AAGTCTGCGA CGTITTCGAT ATTATGCCA TATGTCATG TTGTAAGTT GAAACAAAA ATGAGGGGAA
RV_3714 748098 TGATGGAC AAGTCTGCGA CGTITTCGAT ATTATGCCA TATGTCATG TTGTAAGTT GAAACAAAA ATGAGGGGAA
RV_3737 748098 TGATGGAC AAGTCTGCGA CGTITTCGAT ATTATGCCA TATGTCATG TTGTAAGTT GAAACAAAA ATGAGGGGAA
PF3DT_04_v3 748168 AAAAAATGAG GTTTTAATA ACTACACAT TAGAGGTCTA GGAATAAAG GAGTATTACC ATGGAATGT ATTCCCTAG
303.1 748167 AAAAAATGAG GTTTTAATA ACTACACAT TAGAGGTCTA GGAATAAAG GAGTATTACC ATGGAATGT ATTCCCTAG
383.1 748167 AAAAAATGAG GTTTTAATA ACTACACAT TAGAGGTCTA GGAATAAAG GAGTATTACC ATGGAATGT ATTCCCTAG
7G8_2 748167 AAAAAATGAG GTTTTAATA ACTACACAT TAGAGGTCTA GGAATAAAG GAGTATTACC ATGGAATGT ATTCCCTAG
GB4 748167 AAAAAATGAG GTTTTAATA ACTACACAT TAGAGGTCTA GGAATAAAG GAGTATTACC ATGGAATGT ATTCCCTAG
N011-A 748167 AAAAAATGAG GTTTTAATA ACTACACAT TAGAGGTCTA GGAATAAAG GAGTATTACC ATGGAATGT ATTCCCTAG
O222-A 748167 AAAAAATGAG GTTTTAATA ACTACACAT TAGAGGTCTA GGAATAAAG GAGTATTACC ATGGAATGT ATTCCCTAG
PS097 748167 AAAAAATGAG GTTTTAATA ACTACACAT TAGAGGTCTA GGAATAAAG GAGTATTACC ATGGAATGT ATTCCCTAG
PS206_E11 748167 AAAAAATGAG GTTTTAATA ACTACACAT TAGAGGTCTA GGAATAAAG GAGTATTACC ATGGAATGT ATTCCCTAG
RV_3635 748167 AAAAAATGAG GTTTTAATA ACTACACAT TAGAGGTCTA GGAATAAAG GAGTATTACC ATGGAATGT ATTCCCTAG
RV_3675 748167 AAAAAATGAG GTTTTAATA ACTACACAT TAGAGGTCTA GGAATAAAG GAGTATTACC ATGGAATGT ATTCCCTAG
RV_3714 748167 AAAAAATGAG GTTTTAATA ACTACACAT TAGAGGTCTA GGAATAAAG GAGTATTACC ATGGAATGT ATTCCCTAG
RV_3737 748167 AAAAAATGAG GTTTTAATA ACTACACAT TAGAGGTCTA GGAATAAAG GAGTATTACC ATGGAATGT ATTCCCTAG
  
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We have 204 Isolate strains for alignment.
[Show Alignment on Checked Strains](#) [Check All](#) [Uncheck All](#)

d. Is the DHFR-TS (or AMA1) gene expressed?

Look at the gene page sections entitled “Protein” and “Expression”. You may have to click on the **show** link to reveal the data associated with that data track.

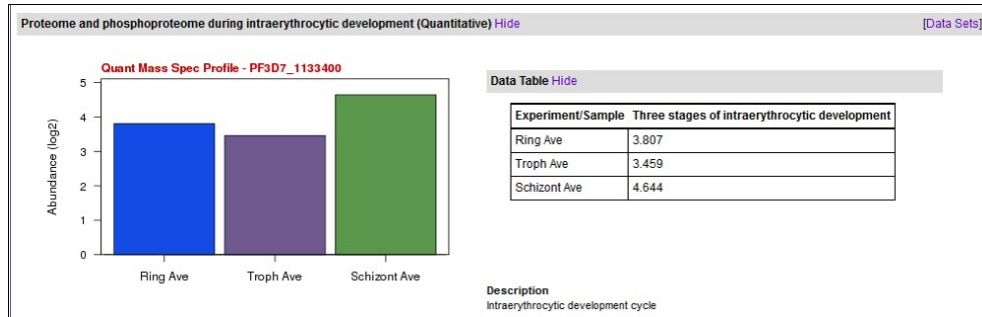
- What kinds of data in PlasmoDB provide evidence for expression? Hint: open the Protein Features graphic which is the first data track in the Protein section.
- Is this gene expressed at the protein level in salivary gland sporozoites? – in the blood stage phosphoproteome? Look at the Protein context graphic and the table of Mass Spec.-based Expression Evidence.
- Can you quickly link to the data set record for proteomics experiments?

Mass Spec.-based Expression Evidence Hide

| Experiment | Sample | Sequences | Spectra | Data Set |
|--|----------------------------------|-----------|---------|----------------------|
| Blood stage phospho- and total proteome (3D7) | schizont phosphopeptide-depleted | 6 | 16 | View |
| Cytoplasmic and nuclear fractions from rings, trophozoites and schizonts (3D7) | Ring stage nuclear fraction 1 | 5 | 5 | View |
| Cytoplasmic and nuclear fractions from rings, trophozoites and schizonts (3D7) | Schizont nuclear fraction 1 | 3 | 3 | View |

[Data Sets]

- How abundant is DHFR-TS (AMA1) protein? How confident are you of this analysis? Abundance can be estimated by counting the number of spectra supporting a peptide spectra that maps to the protein. Where do you find information about the number of spectra?
- Is the protein more abundant in the ring or schizont life cycle stage? Hint: open the quantitative proteomics track called **Proteome and phosphoproteome during intraerythrocytic development (Quantitative)**.



- Look at the Expression data track labeled **Life cycle expression data (3D7)**. Based on this data, at what life cycle stage is DHFR-TS (AMA1) most abundant? Does this make sense?
- Do the life cycle microarray expression profiles from different data tracks (and thus different experiments/data sets) give the same results? What tracks did you use?
- What about RNA-sequence data, does it agree with microarray data? See these two data tracks – **Strand specific transcriptomes of 4 life cycle stages; Transcriptomes of 7 sexual and asexual life stages.**