

Complex strategies with Genomic Colocation Exercise 14

14.1 Divergent genes with similar expression profiles.

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

Identify genes that meet these four criteria:

1. are located within 1000 bp of each other
2. are divergently transcribed,
3. are expressed maximally at day 30 of the iRBC cycle +/- 8 hrs and,
4. show at least a 3-fold increase in expression.

- Hint: first use the “Genes bases on Microarray Evidence” -> “*Intraerythrocytic Infection Cycle (DeRisi)*” -> “[P.f. Intraerythrocytic Infection Cycle \(fold change\)](#)” search.

Identify Genes based on P.f. Intraerythrocytic Infection Cycle (fold change) REVISED

Experiment ? iRBC HB3 (48 Hour scaled)
 iRBC Dd2 (48 Hour scaled)
 iRBC 3D7 (48 Hour scaled)

Direction ?

Reference Samples ? [select all](#) | [clear all](#) | [expand all](#) | [collapse all](#) | [reset to default](#)
 1-16 Hours
 17-30 Hours
 17-23 Hours
 24-30 Hours
 31-48 Hours
[select all](#) | [clear all](#) | [expand all](#) | [collapse all](#) | [reset to default](#)

Operation Applied to Reference Samples ?

Comparison Samples ? [select all](#) | [clear all](#) | [expand all](#) | [collapse all](#) | [reset to default](#)
 1-16 Hours
 17-30 Hours
 17-23 Hours
 24-30 Hours
 31-48 Hours
 31-39 Hours
 40-48 Hours
[select all](#) | [clear all](#) | [expand all](#) | [collapse all](#) | [reset to default](#)

Operation Applied to Comparison Samples ?

Fold change >= ?

Global min / max in selected time points ?

Protein Coding Only: ?

Give this search a weight
 Give this search a name

- Add a step that is the same as the first step and select the genomic colocation (1 relative to 2) operation.
- Set up the form to identify those genes that are transcribed on the opposite strand that have their starts located within 1000 bp of another genes start.
- If you are having difficulty setting this up, you can see the strategy at:

<http://plasmodb.org/plasmo/im.do?s=6b8094bdb6738e05> Cut and paste the link into your browser if the hyperlink does not work

- Turn on the “Pf-iRBC 48hr - Graph” column to assess how well the pairs of genes compare in terms of expression. The pairs of genes are located one above the other in the result table if sorted by location.
- Note that you could do similar types of experiments to look at potential co-regulation / shared enhancers / divergent promoters with other sorts of data such as:
 - Genes by ChiP-chip peaks in ToxoDB.
 - DNA motifs for transcription factor binding sites.
 - Of course other expression queries.
 - Etc ...
- The screenshot below shows one way (there are MANY) to configure the genome collocation form to identify genes that are divergently transcribed located with their start within 1000 bp of each other.

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

You had 684 Genes in your Strategy (Step 1). Your new Genes search (Step 2) returned 684 Genes.

"Return each Gene from Step 1 whose upstream region overlaps the upstream region of a Gene in Step 2 and is on the opposite strand"

(684 Genes in Step 1)

Region

Gene

Exact

Upstream: 1000 bp

Downstream: 1000 bp

Custom:

begin at: start -- 1000 bp

end at: start -- 1 bp

(684 Genes in Step 2)

Region

Gene

Exact

Upstream: 1 bp

Downstream: 1000 bp

Custom:

begin at: start -- 1 bp

end at: start -- 1 bp

Submit

Close

14.2 Finding possible oocyst expressed genes based on DNA motifs.

Note: for this exercise use <http://toxodb.org>


In exercise 13.4 you defined a number of *T. gondii* genes that are preferentially expressed in the oocyst stages. How can you use this information to expand the number of possible oocyst regulated genes? One possibility is to try and define common elements in promoter or 5'UTR regions (ie. 5' to the start of the genes). For this you will have to be able to retrieve 5' sequence from all of the genes in the oocyst list. How would you do this? (hint: click on download genes then select


14.3. Identifying conserved DNA elements upstream of genes


The goal of this exercise is to identify a DNA element in the upstream region of similarly regulated genes.

- a. Identify genes that are up-regulated in malaria sporozoites compared to blood stage parasites. Examine the list of searchable experiments on the PlasmoDB microarray search page: Identify Genes based on Microarray Evidence. Can you identify an experiment that would give you this answer? (hint: look at *Plasmodium* species other than *P. falciparum*, ie. *P. yoelii* [Parasite Liver Stages Survey (Kappe) --> P.y. Liver Stages (fold change)])

Identify Genes based on P.y. Liver Stages (fold change)

Comparison 

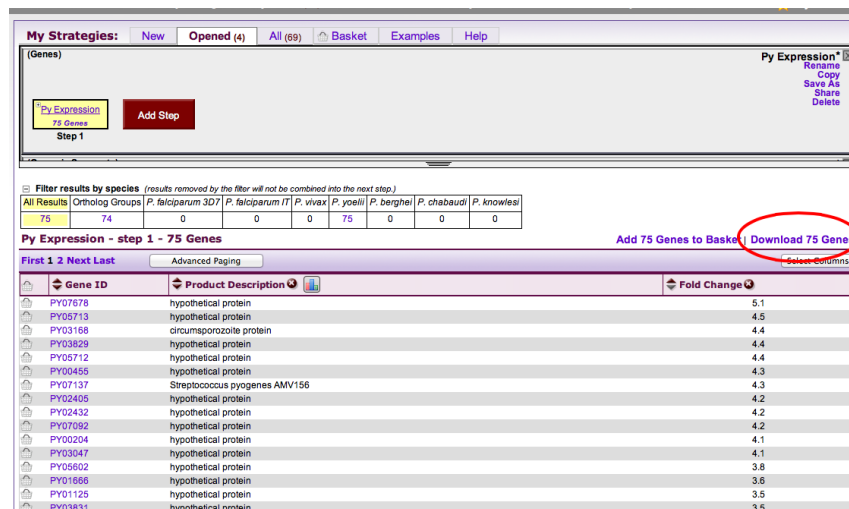
Fold change >= 

Direction 

Give this search a weight

Give this search a name

- b. How many genes did you find? What you are interested in is looking at the nucleotide sequence upstream of the start sites of these genes. How can you do this in bulk? PlasmoDB has a sequence retrieval tool that allows you to download results of your searches in bulk. This includes a tool that allows you to specify the sequence you want.



The screenshot shows the PlasmoDB interface for a search titled "Py Expression". The search results are displayed in a table with columns for Gene ID, Product Description, and Fold Change. The table lists 75 genes, all of which are "hypothetical protein". The fold change values range from 3.5 to 5.1. A red circle highlights the "Download 75 Genes" button in the top right corner of the results area.

Gene ID	Product Description	Fold Change
PY07678	hypothetical protein	5.1
PY05713	hypothetical protein	4.5
PY03168	circumsporozoite protein	4.4
PY03829	hypothetical protein	4.4
PY05712	hypothetical protein	4.4
PY00455	hypothetical protein	4.3
PY07137	Streptococcus pyogenes AMV156	4.3
PY02405	hypothetical protein	4.2
PY02432	hypothetical protein	4.2
PY07092	hypothetical protein	4.2
PY00204	hypothetical protein	4.1
PY03047	hypothetical protein	4.1
PY05602	hypothetical protein	3.8
PY01656	hypothetical protein	3.6
PY01125	hypothetical protein	3.5
PY03831	hypothetical protein	3.5



- c. After you click on “Download ### Genes”, you are offered a drop down menu of options. Explore these; which one will allow you to specify the sequence to download. (hint: Configurable FASTA)


Download 75 Genes from the search:
P.y. Liver Stages (fold change)

Please select a format from the dropdown list to create the download report.
****Note: Gene IDs will automatically be included in the report.**

▼ --- Select a format ---

- Tab delimited (Excel): choose from columns
- Text: choose from columns and/or tables
- Configurable FASTA
- GFF3: Gene models and optional sequences
- XML: choose from columns and/or tables
- json: choose from columns and/or tables

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- d. Define the sequence you want to retrieve. For this exercise retrieve 500 nucleotides up-stream of the start of translation.

Download 75 Genes from the search:
P.y. Liver Stages (fold change)

Please select a format from the dropdown list to create the download report.
****Note: Gene IDs will automatically be included in the report.**

Configurable FASTA

This reporter will retrieve the sequences of the genes in your result.

Choose the type of sequence: genomic protein CDS transcript

Choose the region of the sequence(s):

begin at Translation Start (ATG) - 500 nucleotides

end at Translation Start (ATG) + 0 nucleotides

Download Type: Save to File Show in Browser

[Get Sequences](#)

***** Note: If UTRs have not been annotated for a gene, then choosing "transcription start" may have the same effect as choosing "translation start".**

- e. The next step is to take this sequence and run it through a DNA motif finder such as MEME (<http://meme.sdsc.edu/meme/intro.html>). To speed up this process we have pre-run the motif finder and results are presented here:

g. How many times did this motif occur in the genome? How many of them are in the upstream region of genes? Can you find all *P. yoelii* genes that are within 1000 nucleotides downstream of the motif? (hint: use the genomic colocation option when combining searches).

Genomic Colocation ?

Combine Step 1 and Step 2 using relative locations in the genome
 You had **1257 Genomic Segments** in your Strategy (Step 1). Your new **Genes** search (Step 2) returned **7774 Genes**.

"Return each whose **upstream region** overlaps the **exact region** of a Genomic Segment in Step 1 and is on

(7774 Genes in Step 2)

Exact

Upstream: bp

Downstream: bp

Custom:

begin at: bp

end at: bp

(1257 Genomic Segments in Step 1)

Exact

Upstream: bp

Downstream: bp

Custom:

begin at: bp

end at: bp

h. Do these genes have orthologs in other *Plasmodium* species? (hint: add a step to your search strategy and transform the results to their orthologs).

Add Step

Run a new Search for

Transform by Orthology

Add contents of Basket

Add existing Strategy

Filter by assigned Weight

Add Step 4 : Transform by Orthology

Organism

- Plasmodium berghei
- Plasmodium chabaudi
- Plasmodium falciparum
- Plasmodium knowlesi
- Plasmodium vivax
- Plasmodium yoelii

Syntenic Orthologs Only?

Population Biology