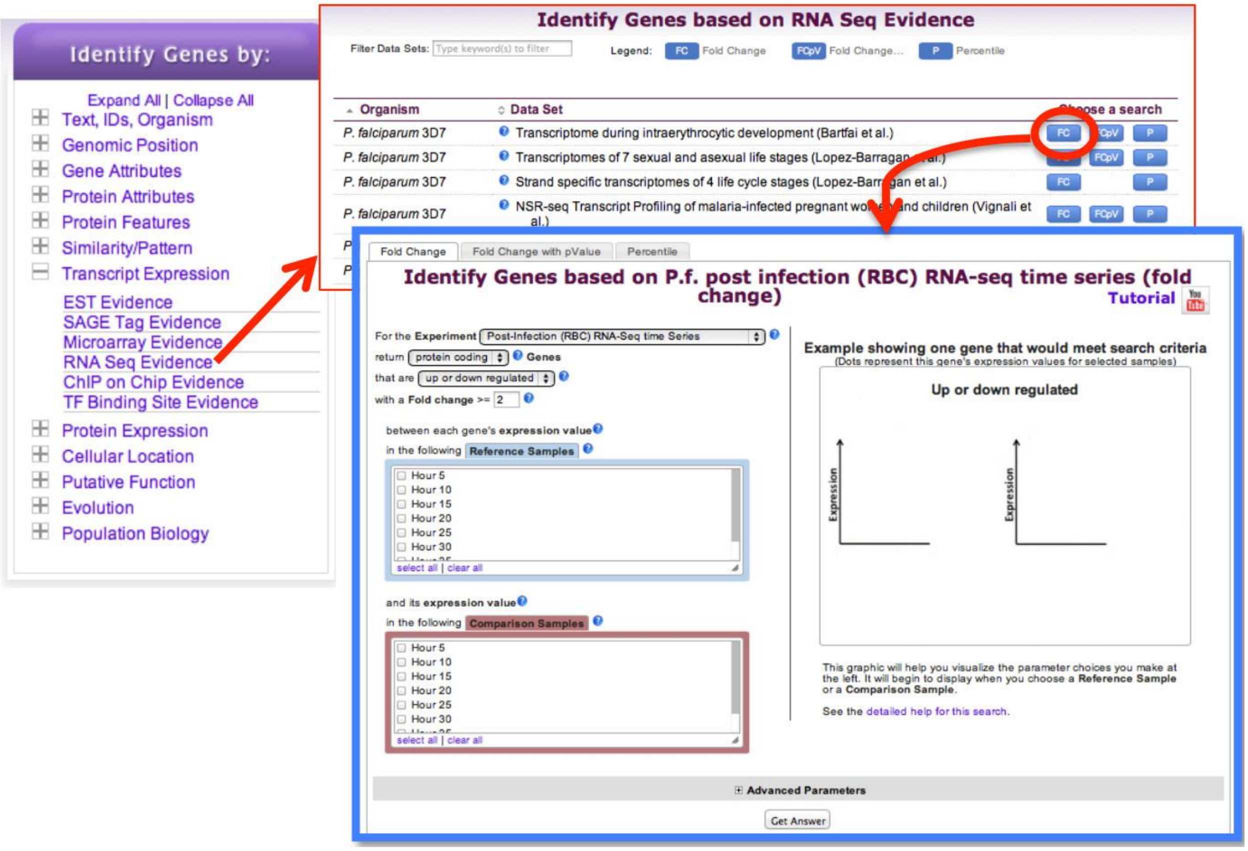
Exploring Transcriptomic data

1. Exploring RNA sequence data in *Plasmodium falciparum.*

Note: For this exercise use http://www.plasmodb.org

a. Find all genes in *P. falciparum* that are up-regulated during the later stages of the intraerythrocytic cycle.

- Hint: Use the fold change search for the data set "Transcriptome during intraerythrocytic development (Bartfai *et al.*)". For this data set, synchronized Pf3D7 parasites were assayed by RNA-seq at 8 time-points during the iRBC cycle. We want to find genes that are up-regulated in the later time points (30, 35, 40 hours) using the early time points (5, 10, 15, 20, 25 hours) as reference.



Identify Genes based on RNA Seq Evidence

Filter Data Sets: Type keyword(s) to filter

Legend: FC Fold Change FCpV Fold Change... P Percentile

Organism	Data Set	Choose a search
P. falciparum 3D7	Transcriptome during intraerythrocytic development (Bartfai et al.)	FC FCpV P
P. falciparum 3D7	Transcriptomes of 7 sexual and asexual life stages (Lopez-Barragan et al.)	FC FCpV P
P. falciparum 3D7	Strand specific transcriptomes of 4 life cycle stages (Lopez-Barragan et al.)	FC P
P. falciparum 3D7	NSR-seq Transcript Profiling of malaria-infected pregnant women and children (Vignali et al.)	FC FCpV P

Identify Genes based on P.f. post infection (RBC) RNA-seq time series (fold change)

For the **Experiment** Post-Infection (RBC) RNA-Seq time Series

return protein coding **Genes**

that are up or down regulated

with a **Fold change** >= 2

between each gene's **expression value**

in the following **Reference Samples**

□ Hour 5
□ Hour 10
□ Hour 15
□ Hour 20
□ Hour 25
□ Hour 30

select all | clear all

and its **expression value**

in the following **Comparison Samples**

□ Hour 5
□ Hour 10
□ Hour 15
□ Hour 20
□ Hour 25
□ Hour 30

select all | clear all

Example showing one gene that would meet search criteria

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

This graphic will help you visualize the parameter choices you make at the left. It will begin to display when you choose a **Reference Sample** or a **Comparison Sample**.

See the detailed help for this search.

- Hint: there are a number of parameters to manipulate in this search. As you modify parameters on the left side note the dynamic help on the right side. See screenshots.

- Direction: the direction of change in expression. Choose up-regulated.

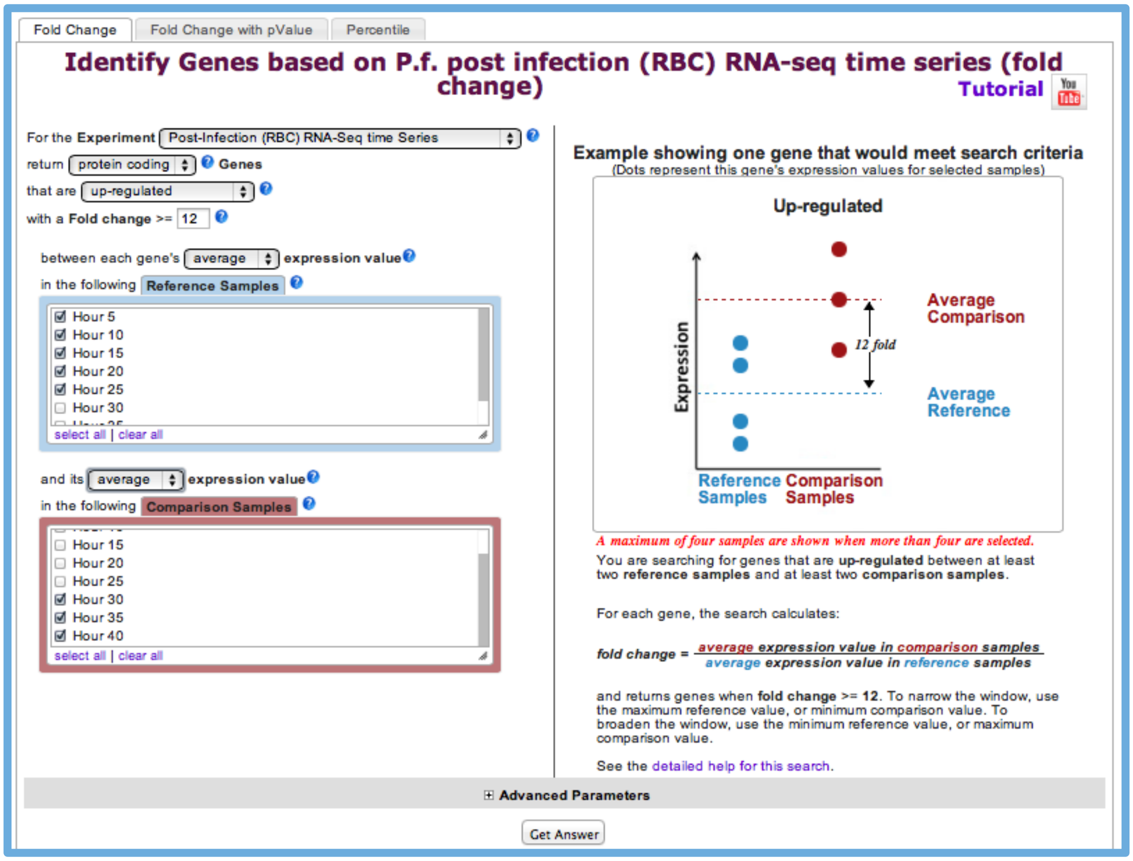
- Fold Change>=: the intensity of difference in expression needed before a gene is returned by the search. Choose 12 but feel free to modify this.

- Between each gene's AVERAGE expression value: This parameter sets the operation applied to reference samples. Fold change is calculated as the ratio of two values (expression in reference)/(expression in comparison). When you choose multiple samples to serve as reference, we generate one number for the fold change calculation by using the minimum, maximum, or average. Choose average

- Reference Sample: the samples that will serve as the reference when comparing expression between samples. choose 5, 10, 15, 20, 25

- And it's AVERAGE expression value: This is the operation applied to comparison samples. see explanation above. Choose average

- Comparison Sample: the sample that you are comparing to the reference. In this case you are interested in genes that are up-regulated in later time points choose 30, 35,



Identify Genes based on P.f. post infection (RBC) RNA-seq time series (fold change)

Tutorial

For the Experiment Post-Infection (RBC) RNA-Seq time Series

return protein coding Genes

that are up-regulated

with a Fold change >= 12

between each gene's average expression value

in the following Reference Samples

☑ Hour 5
☑ Hour 10
☑ Hour 15
☑ Hour 20
☑ Hour 25
□ Hour 30

select all | clear all

and its average expression value

in the following Comparison Samples

□ Hour 15
□ Hour 20
□ Hour 25
☑ Hour 30
☑ Hour 35
☑ Hour 40

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 at least two **reference samples** and at least two **comparison samples**.

For each gene, the search calculates:

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

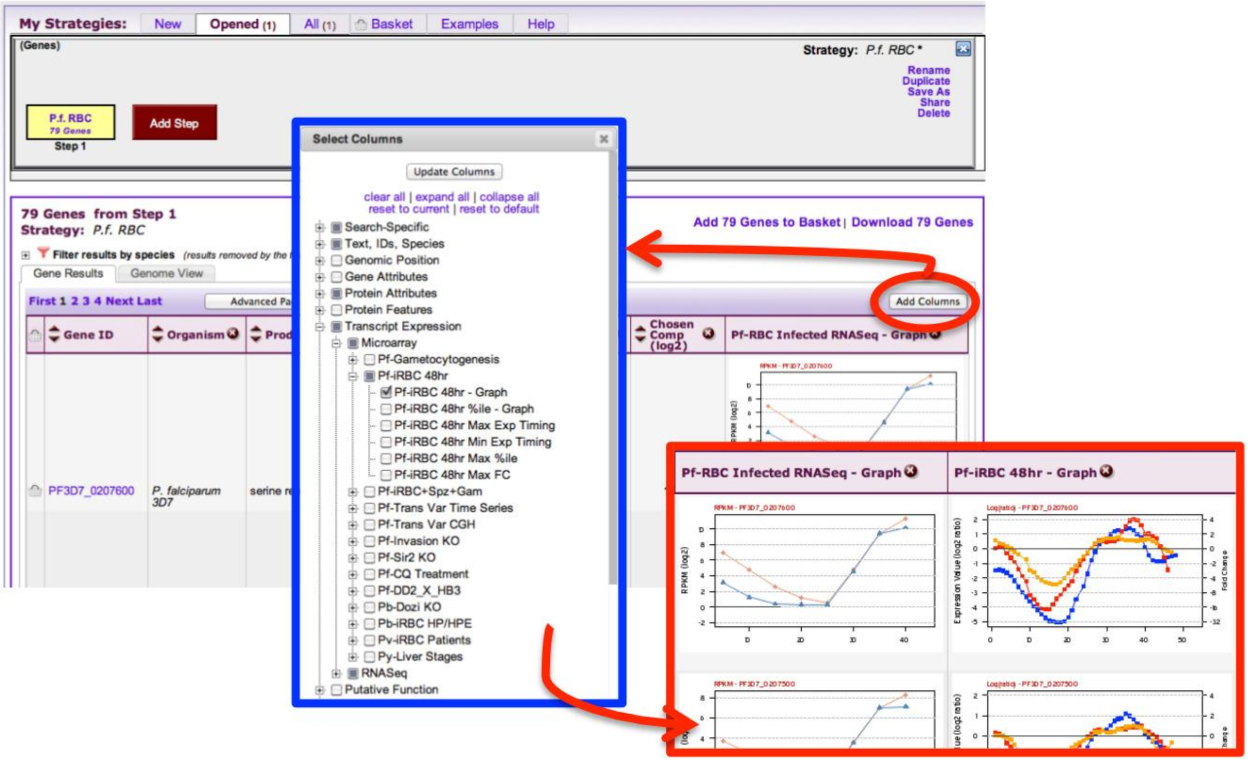
and returns genes when **fold change >= 12**. To narrow the window, use the maximum reference value, or minimum comparison value. To broaden the window, use the minimum reference value, or maximum comparison value.

See the detailed help for this search.

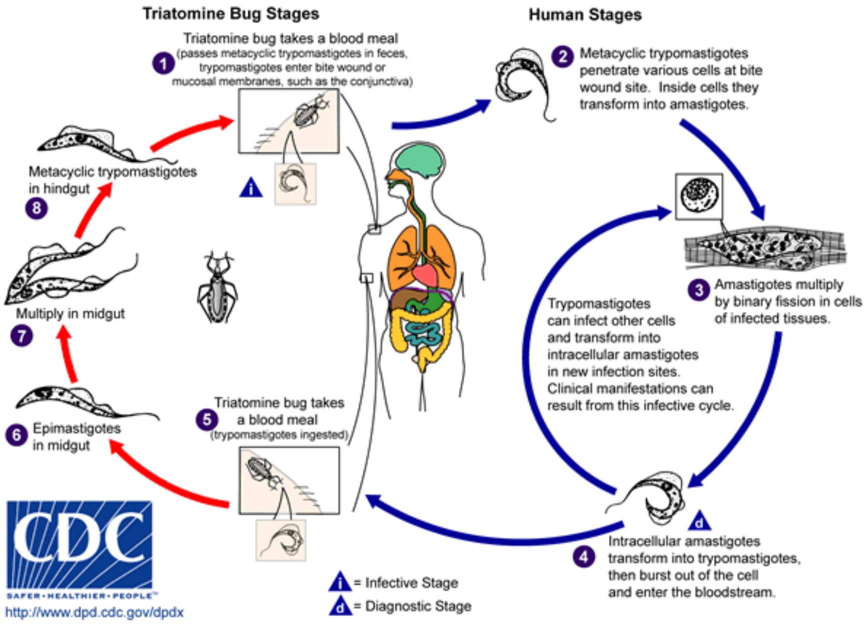
40

b. For the genes returned by the search, how does the RNA-sequence data compare to microarray data?

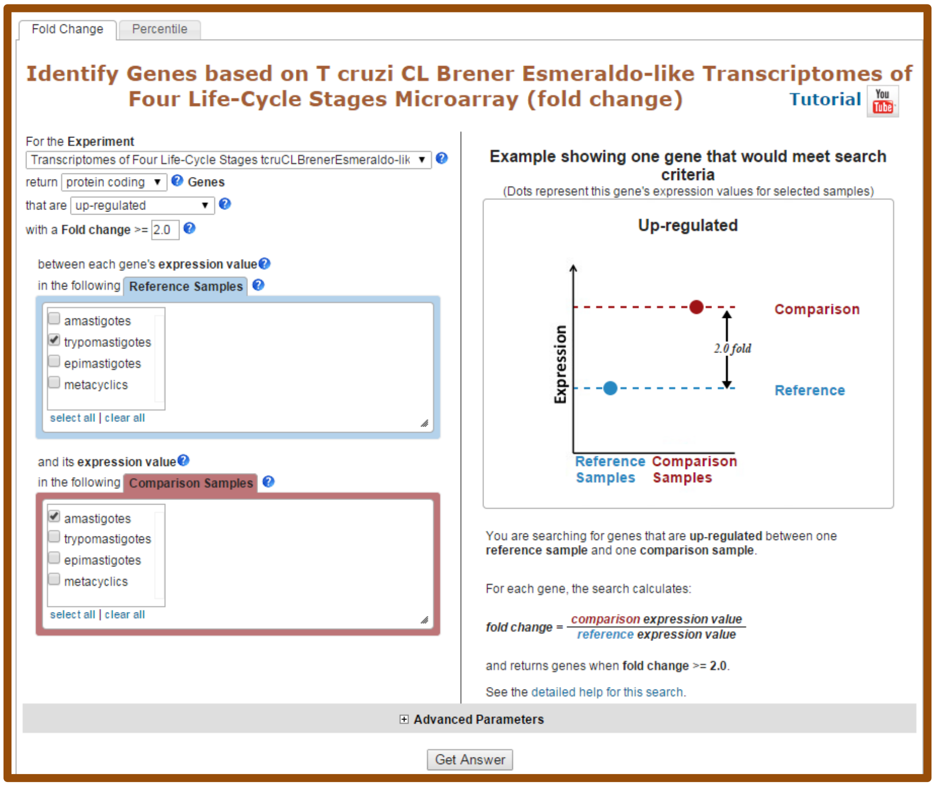
- Hint: PlasmoDB contains data from a similar experiment that was analyzed by microarray instead of RNA sequencing. This experiment is called: Erythrocytic expression time series (3D7, DD2, HB3) (Bozdech et al. and Linas et al.). To directly compare the data for genes returned by the RNA seq search that you just ran, add the column called "Pf-iRBC 48hr - Graph".



2. Exploring microarray data in TriTrypDB.
   Note: For this exercise use http://www.tritrypdb.org


Triatomine Bug Stages
Human Stages
1 Triatomine bug takes a blood meal (passes metacyclic trypomastigotes in feces, trypomastigotes enter bite wound or mucosal membranes, such as the conjunctiva)
2 Metacyclic trypomastigotes penetrate various cells at bite wound site. Inside cells they transform into amastigotes.
3 Amastigotes multiply by binary fission in cells of infected tissues.
Trypomastigotes can infect other cells and transform into intracellular amastigotes in new infection sites. Clinical manifestations can result from this infective cycle.
4 Intracellular amastigotes transform into trypomastigotes, then burst out of the cell and enter the bloodstream.
5 Triatomine bug takes a blood meal (trypomastigotes ingested)
6 Epimastigotes in midgut
7 Multiply in midgut
8 Metacyclic trypomastigotes in hindgut
i = Infective Stage
d = Diagnostic Stage
CDC
SAFER • HEALTHIER • PEOPLE™
http://www.dpd.cdc.gov/dpdx

a. Find *T. cruzi* protein coding genes that are upregulated in amastigotes compared to trypomastigotes. Go to the transcript expression section then select microarray. The experiment is called: Transcriptomes of Four Life-Cycle Stages (Minning et al.).



Identify Genes based on T cruzi CL Brener Esmeraldo-like Transcriptomes of Four Life-Cycle Stages Microarray (fold change)

For the **Experiment**
Transcriptomes of Four Life-Cycle Stages tcruCLBrenerEsmeraldo-lik ▾
return protein coding ▾ **Genes**
that are up-regulated ▾
with a **Fold change** >= 2.0

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

□ amastigotes
☑ trypomastigotes
□ epimastigotes
□ metacyclics

select all | clear all

and its **expression value**
in the following **Comparison Samples**

☑ amastigotes
□ trypomastigotes
□ epimastigotes
□ metacyclics

select all | clear all

Example showing one gene that would meet search criteria
(Dots represent this gene's expression values for selected samples)

You are searching for genes that are **up-regulated** between one **reference sample** and one **comparison sample**.

For each gene, the search calculates:

$$fold\ change = \frac{comparison\ expression\ value}{reference\ expression\ value}$$

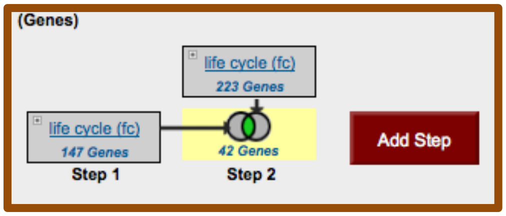
and returns genes when **fold change** >= **2.0**.

See the detailed help for this search.

- Select the direction of regulation, your reference sample and your comparison sample. For the fold change keep the default value 2.

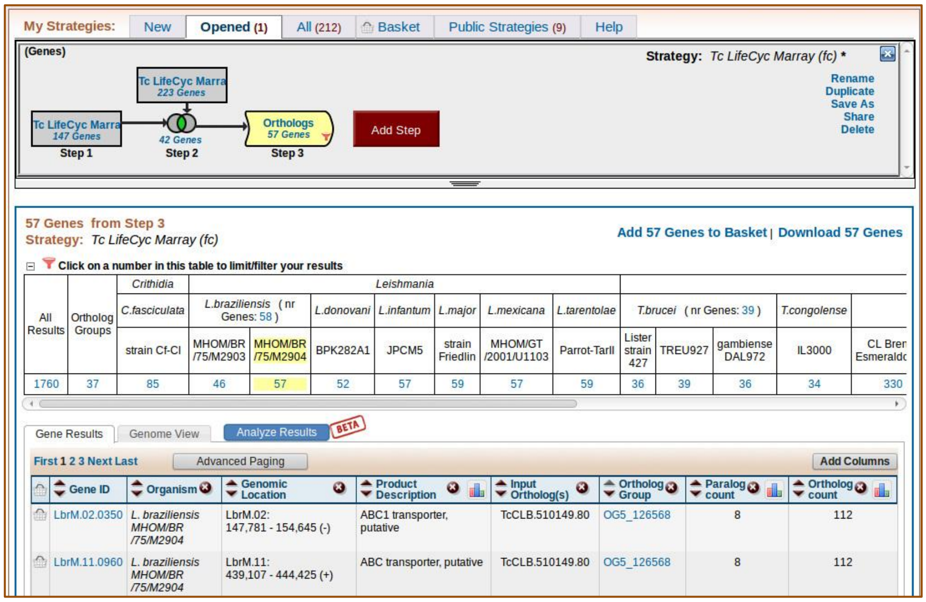
- How many genes did you find? Do the results seem plausible?

- Are any of these genes also up-regulated in the replicative insect stage (epimastigotes)? How can you find this out? (*Hint*: add a step and run a microarray search comparing expression of epimastigotes to metacyclics).



- Do these genes have orthologs in other kinetoplastids? (*Hint*: add a step and run an ortholog transform on your results).

- How many orthologs exist in *L. braziliensis*? (*Hint*: look at the filter table between the strategy panel and your result list. Click on the number in of gene to view results from a specific species). Explore your results. Did you find anything interesting?



Strategy: *Tc LifeCyc Marray (fc)* *

57 Genes from Step 3
Strategy: *Tc LifeCyc Marray (fc)*

Add 57 Genes to Basket | Download 57 Genes

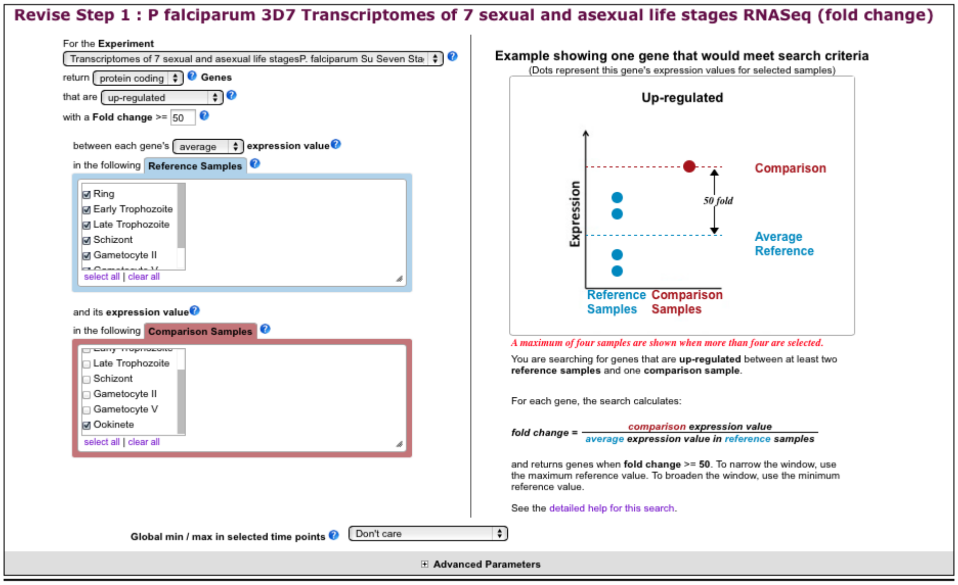
Click on a number in this table to limit/filter your results

All Results	Ortholog Groups	*Crithidia*	*Leishmania*											
		C.fasciculata	*L.braziliensis* (nr Genes: 58)		*L.donovani*	*L.infantum*	*L.major*	*L.mexicana*	*L.tarentolae*	*T.brucei* (nr Genes: 39)			*T.congolense*	
		strain Cf-Cl	MHOM/BR /75/M2903	MHOM/BR /75/M2904	BPK282A1	JPCM5	strain Friedlin	MHOM/GT /2001/U1103	Parrot-TarII	Lister strain 427	TREU927	gambiense DAL972	IL3000	CL Brer Esmeraldc
1760	37	85	46	57	52	57	59	57	59	36	39	36	34	330

Gene ID	Organism	Genomic Location	Product Description	Input Ortholog(s)	Ortholog Group	Paralog count	Ortholog count
LbrM.02.0350	*L. braziliensis MHOM/BR /75/M2904*	LbrM.02: 147,781 - 154,645 (-)	ABC1 transporter, putative	TcCLB.510149.80	OG5_126568	8	112
LbrM.11.0960	*L. braziliensis MHOM/BR /75/M2904*	LbrM.11: 439,107 - 444,425 (+)	ABC transporter, putative	TcCLB.510149.80	OG5_126568	8	112

3. Finding genes based on RNAseq evidence and inferring function of hypothetical genes.
   Note: Use http://plasmodb.org for this exercise.

a. Find all genes in *P. falciparum* that are up-regulated at least 50-fold in ookinetes compared to other stages: "Transcriptomes of 7 sexual and asexual life stages (Lopez-Barragan et al.)". For this search select "average" for the operation applied on the reference samples.



Revise Step 1 : P falciparum 3D7 Transcriptomes of 7 sexual and asexual life stages RNASeq (fold change)

For the **Experiment** Transcriptomes of 7 sexual and asexual life stagesP. falciparum Su Seven Sta

return protein coding **Genes**

that are up-regulated

with a **Fold change** >= 50

between each gene's average **expression value**

in the following **Reference Samples**

☑ Ring
☑ Early Trophozoite
☑ Late Trophozoite
☑ Schizont
☑ Gametocyte II

select all | clear all

and its **expression value**

in the following **Comparison Samples**

□ Late Trophozoite
□ Schizont
□ Gametocyte II
□ Gametocyte V
☑ Ookinete

select all | clear all

Global min / max in selected time points Don't care

⊞ **Advanced Parameters**

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 at least two **reference samples** and one **comparison sample**.

For each gene, the search calculates:

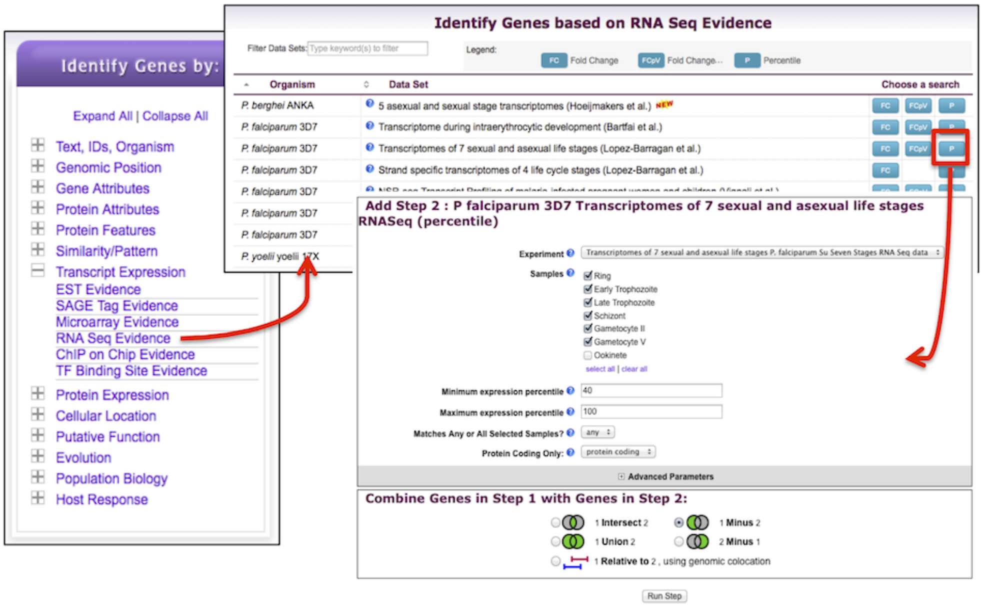
$$\textit{fold change} = \frac{\textit{comparison expression value}}{\textit{average expression value in reference samples}}$$

and returns genes when **fold change** >= **50**. To narrow the window, use the maximum reference value. To broaden the window, use the minimum reference value.

See the detailed help for this search.

b. The above search will give you all genes that are up-regulated by 50 fold in ookinetes compared to the other stages. However, this does not mean that these genes are not expressed well in the other stages. How can you remove genes from the list that are likely not expressed in the other stages?

- Hint: run a search for genes based on RNAseq evidence from the same experiment, but this time select the percentile search: P.f. seven stages - RNA Seq (percentile)). What minimal percentile values should you choose? Try different values - for example, 40 (minimum) and 100(maximum).



Identify Genes by:

Expand All | Collapse All

- Text, IDs, Organism
- Genomic Position
- Gene Attributes
- Protein Attributes
- Protein Features
- Similarity/Pattern
- Transcript Expression
  - EST Evidence
  - SAGE Tag Evidence
  - Microarray Evidence
  - RNA Seq Evidence
  - ChIP on Chip Evidence
  - TF Binding Site Evidence
- Protein Expression
- Cellular Location
- Putative Function
- Evolution
- Population Biology
- Host Response

Identify Genes based on RNA Seq Evidence

Filter Data Sets: Type keyword(s) to filter

Legend: FC Fold Change FCpV Fold Change... P Percentile

Organism	Data Set	Choose a search
P. berghei ANKA	5 asexual and sexual stage transcriptomes (Hoeijmakers et al.) NEW	FC FCpV P
P. falciparum 3D7	Transcriptome during intraerythrocytic development (Bartfai et al.)	FC FCpV P
P. falciparum 3D7	Transcriptomes of 7 sexual and asexual life stages (Lopez-Barragan et al.)	FC FCpV P
P. falciparum 3D7	Strand specific transcriptomes of 4 life cycle stages (Lopez-Barragan et al.)	FC P
P. falciparum 3D7	NSR-seq Transcript Profiling of malaria-infected pregnant women and children (Vignali et al.)	FC FCpV P
P. falciparum 3D7		
P. falciparum 3D7		
P. yoelii yoelii 17X		

Add Step 2 : P falciparum 3D7 Transcriptomes of 7 sexual and asexual life stages RNASeq (percentile)

Experiment: Transcriptomes of 7 sexual and asexual life stages P. falciparum Su Seven Stages RNA Seq data

Samples:
☑ Ring
☑ Early Trophozoite
☑ Late Trophozoite
☑ Schizont
☑ Gametocyte II
☑ Gametocyte V
☐ Ookinete
select all | clear all

Minimum expression percentile: 40

Maximum expression percentile: 100

Matches Any or All Selected Samples?: any

Protein Coding Only: protein coding

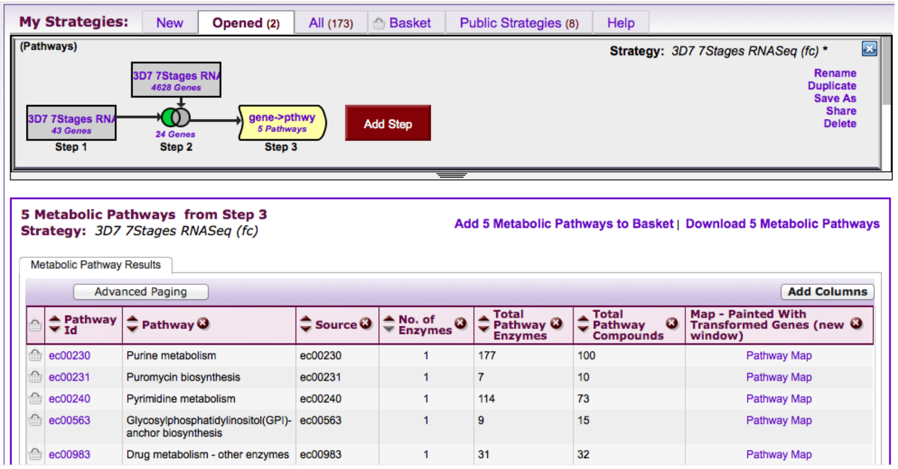
⊞ Advanced Parameters

Combine Genes in Step 1 with Genes in Step 2:

○ 1 Intersect 2   ● 1 Minus 2
○ 1 Union 2   ○ 2 Minus 1
○ 1 Relative to 2 , using genomic colocation

Run Step

c. Which metabolic pathways are represented in this gene list? (*Hint:* add a step and transform results to pathways).



My Strategies: New | Opened (2) | All (173) | Basket | Public Strategies (8) | Help

(Pathways)

Strategy: *3D7 7Stages RNASeq (fc)* *

5 Metabolic Pathways from Step 3
Strategy: *3D7 7Stages RNASeq (fc)*

Add 5 Metabolic Pathways to Basket | Download 5 Metabolic Pathways

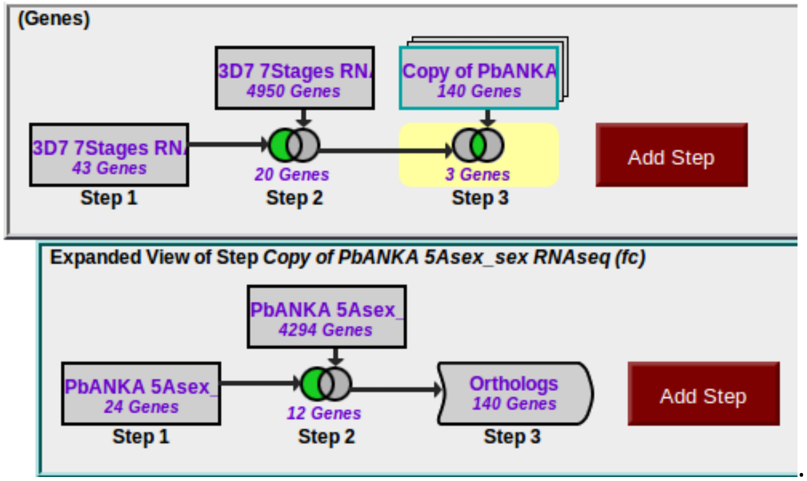
Metabolic Pathway Results

Advanced Paging   Add Columns

Pathway Id	Pathway	Source	No. of Enzymes	Total Pathway Enzymes	Total Pathway Compounds	Map - Painted With Transformed Genes (new window)
ec00230	Purine metabolism	ec00230	1	177	100	Pathway Map
ec00231	Puromycin biosynthesis	ec00231	1	7	10	Pathway Map
ec00240	Pyrimidine metabolism	ec00240	1	114	73	Pathway Map
ec00563	Glycosylphosphatidylinositol(GPI)-anchor biosynthesis	ec00563	1	9	15	Pathway Map
ec00983	Drug metabolism - other enzymes	ec00983	1	31	32	Pathway Map

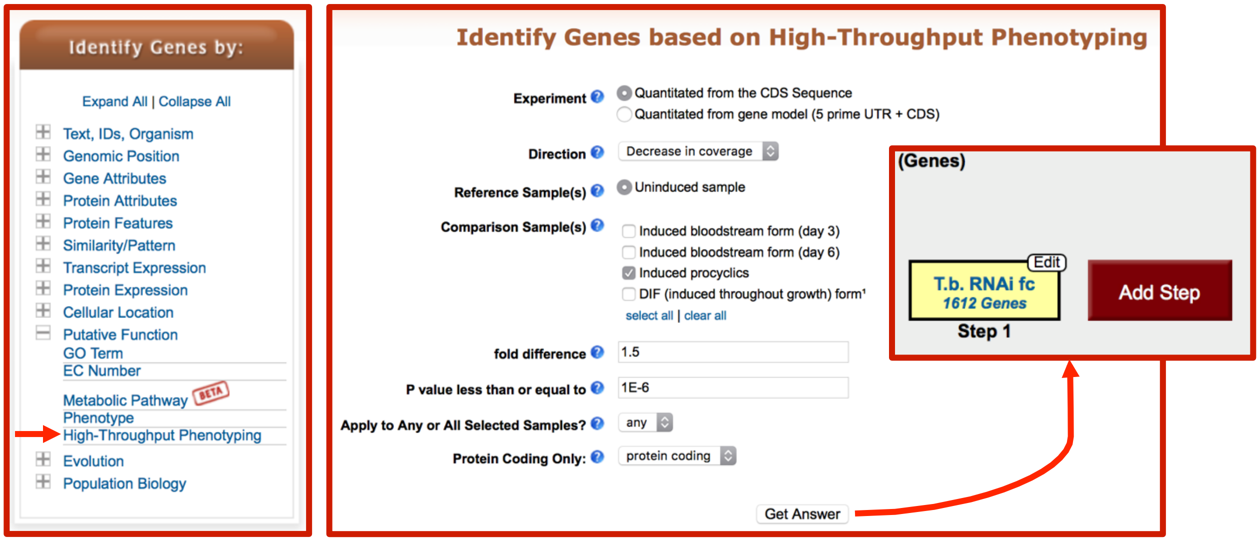
d. What happens is you revise the first step and modify the fold difference to a lower value - 10 for example?

e. PlasmoDB also has an experiment examining gene expression during sexual development in *Plasmodium berghei* (rodent malaria). Can you determine if there are genes that are up-regulated in both human and rodent ookinetes (compared to all other stages)? *Hint:* start by deleting the last step you added in this exercise (transform to pathways). To do this click on edit then delete in the popup. Next add steps for the *P. berghei* experiments "P berghei ANKA 5 asexual and sexual stage transcriptomes RNASeq". Note that you will have to use a nested strategy or by running a separate strategy then combining both strategies.



4. Find genes that are essential in procyclics but not in blood form *T. brucei*. Note: for this exercise use http://TriTrypDB.org.

- Find the query for High Throughput Phenotyping. Think about how to set up this query (*Hint*: you will have to set up a two-step strategy). Remember you can play around with the parameters but there is no one correct way of setting them up – try the default parameters first and select the "induced procyclics" as the comparison sample.



Identify Genes based on High-Throughput Phenotyping

Experiment: ● Quantitated from the CDS Sequence ○ Quantitated from gene model (5 prime UTR + CDS)

Direction: Decrease in coverage

Reference Sample(s): ● Uninduced sample

Comparison Sample(s):
□ Induced bloodstream form (day 3)
□ Induced bloodstream form (day 6)
☑ Induced procyclics
□ DIF (induced throughout growth) form¹
select all | clear all

fold difference: 1.5

P value less than or equal to: 1E-6

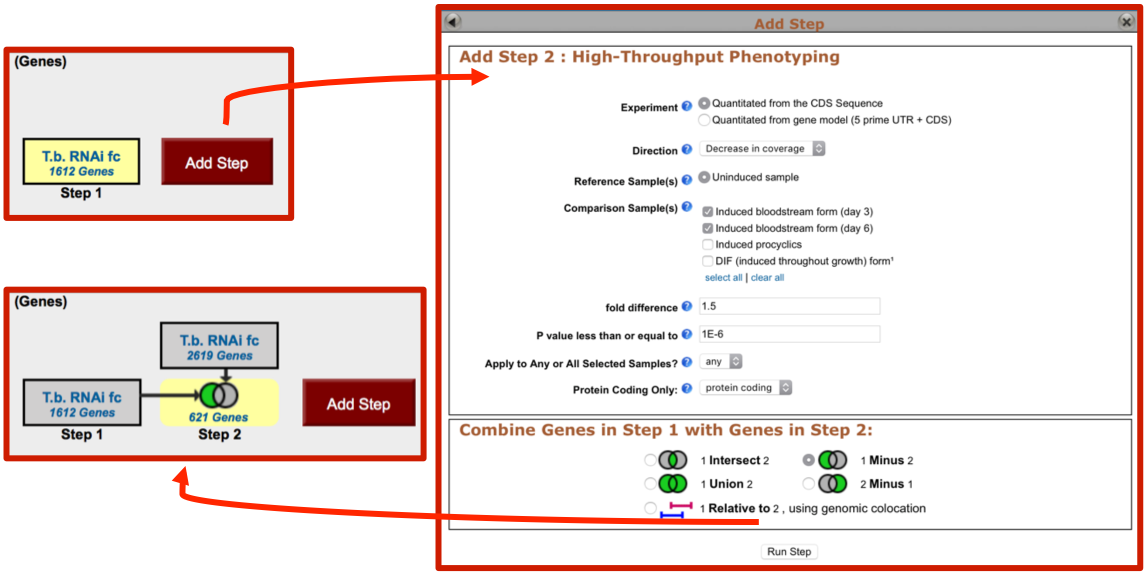
Apply to Any or All Selected Samples?: any

Protein Coding Only: protein coding

Get Answer

- Next add a step and run the same search except this time select the "induced bloodstream form" samples.

- How did you combine the results? Remember you want to find genes that are essential in procyclics and not in blood form.



Add Step

Add Step 2 : High-Throughput Phenotyping

Experiment: ● Quantitated from the CDS Sequence ○ Quantitated from gene model (5 prime UTR + CDS)

Direction: Decrease in coverage

Reference Sample(s): ● Uninduced sample

Comparison Sample(s):
☑ Induced bloodstream form (day 3)
☑ Induced bloodstream form (day 6)
☐ Induced procyclics
☐ DIF (induced throughout growth) form[1]
select all | clear all

fold difference: 1.5

P value less than or equal to: 1E-6

Apply to Any or All Selected Samples?: any

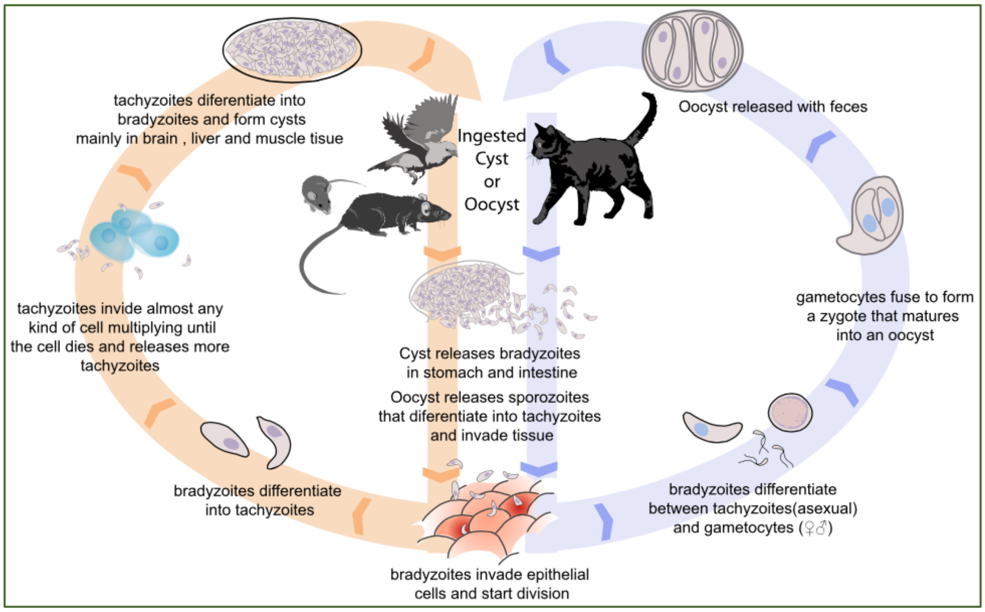
Protein Coding Only: protein coding

Combine Genes in Step 1 with Genes in Step 2:

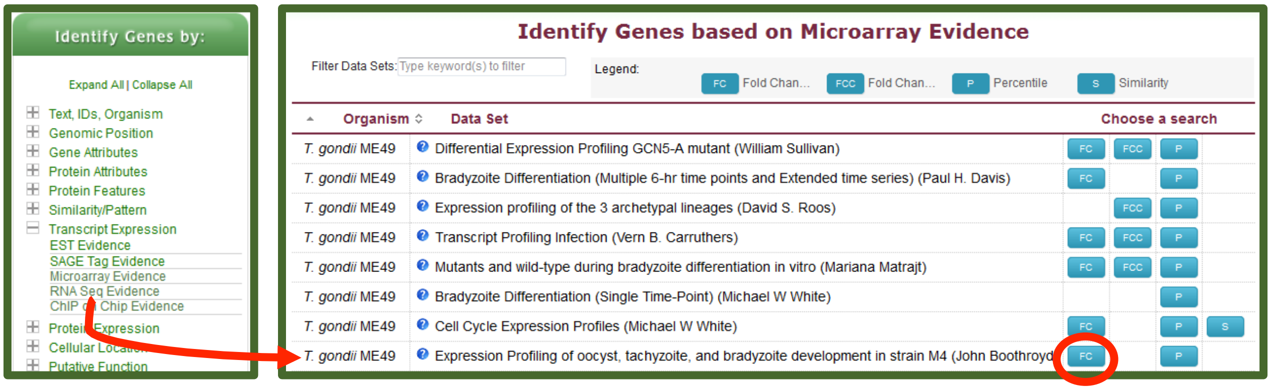
○ 1 **Intersect** 2 ● 1 **Minus** 2
○ 1 **Union** 2 ○ 2 **Minus** 1
○ 1 **Relative to** 2 , using genomic colocation

Run Step

5. Finding oocyst expressed genes in *T. gondii* based on microarray evidence. Note: For this exercise use http://toxodb.org


tachyzoites diferentiate into
bradyzoites and form cysts
mainly in brain , liver and muscle tissue
Ingested
Cyst
or
Oocyst
Oocyst released with feces
tachyzoites invide almost any
kind of cell multiplying until
the cell dies and releases more
tachyzoites
gametocytes fuse to form
a zygote that matures
into an oocyst
Cyst releases bradyzoites
in stomach and intestine
Oocyst releases sporozoites
that diferentiate into tachyzoites
and invade tissue
bradyzoites differentiate
into tachyzoites
bradyzoites differentiate
between tachyzoites(asexual)
and gametocytes (♀♂)
bradyzoites invade epithelial
cells and start division

a. Find genes that are expressed at 10 fold higher levels in one of the oocyst stages than in any other stage in the “Oocyst, tachyzoite, and bradyzoite developmental expression profiles (M4) (John Boothroyd)” microarray experiment.



Identify Genes by:

Expand All | Collapse All

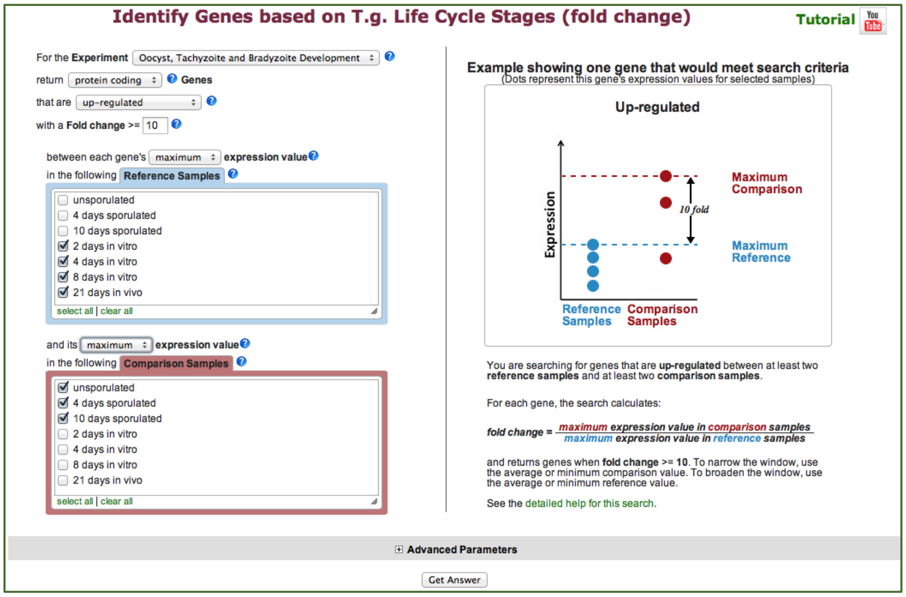
- ⊞ Text, IDs, Organism
- ⊞ Genomic Position
- ⊞ Gene Attributes
- ⊞ Protein Attributes
- ⊞ Protein Features
- ⊞ Similarity/Pattern
- ⊟ Transcript Expression
  - EST Evidence
  - SAGE Tag Evidence
  - Microarray Evidence
  - RNA Seq Evidence
  - ChIP on Chip Evidence
- ⊞ Protein Expression
- ⊞ Cellular Location
- ⊞ Putative Function

Identify Genes based on Microarray Evidence

Filter Data Sets: Type keyword(s) to filter

Legend: FC Fold Chan... FCC Fold Chan... P Percentile S Similarity

Organism	Data Set	Choose a search			
T. gondii ME49	Differential Expression Profiling GCN5-A mutant (William Sullivan)	FC	FCC	P	
T. gondii ME49	Bradyzoite Differentiation (Multiple 6-hr time points and Extended time series) (Paul H. Davis)	FC		P	
T. gondii ME49	Expression profiling of the 3 archetypal lineages (David S. Roos)		FCC	P	
T. gondii ME49	Transcript Profiling Infection (Vern B. Carruthers)	FC	FCC	P	
T. gondii ME49	Mutants and wild-type during bradyzoite differentiation in vitro (Mariana Matrajt)	FC	FCC	P	
T. gondii ME49	Bradyzoite Differentiation (Single Time-Point) (Michael W White)			P	
T. gondii ME49	Cell Cycle Expression Profiles (Michael W White)	FC		P	S
T. gondii ME49	Expression Profiling of oocyst, tachyzoite, and bradyzoite development in strain M4 (John Boothroyd	FC		P	



Identify Genes based on T.g. Life Cycle Stages (fold change)

Tutorial

For the **Experiment** Oocyst, Tachyzoite and Bradyzoite Development

return protein coding **Genes**

that are up-regulated

with a **Fold change** >= 10

between each gene's maximum **expression value**

in the following **Reference Samples**

- □ unsporulated
- □ 4 days sporulated
- □ 10 days sporulated
- ☑ 2 days in vitro
- ☑ 4 days in vitro
- ☑ 8 days in vitro
- ☑ 21 days in vivo

select all | clear all

and its maximum **expression value**

in the following **Comparison Samples**

- ☑ unsporulated
- ☑ 4 days sporulated
- ☑ 10 days sporulated
- □ 2 days in vitro
- □ 4 days in vitro
- □ 8 days in vitro
- □ 21 days in vivo

select all | clear all

Example showing one gene that would meet search criteria
(Dots represent this gene's expression values for selected samples)

You are searching for genes that are **up-regulated** between at least two **reference samples** and at least two **comparison samples**.

For each gene, the search calculates:

$$\textit{fold change} = \frac{\textit{maximum expression value in comparison samples}}{\textit{maximum expression value in reference samples}}$$

and returns genes when **fold change >= 10**. To narrow the window, use the average or minimum comparison value. To broaden the window, use the average or minimum reference value.

See the detailed help for this search.

⊞ **Advanced Parameters**

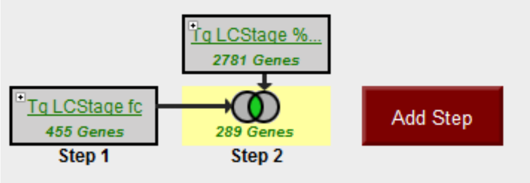
Get Answer

In this example the maximum expression value between genes in the reference and comparison groups was used to determine the fold difference.

b. Add a step to limit this set of genes to only those for which all the non-oocyst stages are expressed below 50th percentile … ie likely not expressed at those stages. (*Hint*: after you click on add step find the same experiment under microarray expression and chose the percentile search).

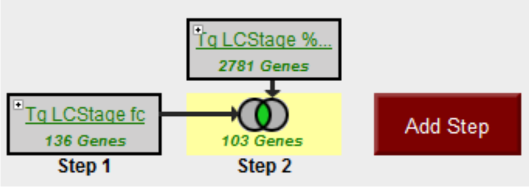
- Select the 4 **non-oocyst** samples.
- We want all to have less than 50th percentile so set ***minimum percentile*** to 0 and ***maximum percentile*** to 50.

- Since we want all of them to be in this range, choose ALL in the "*Matches Any or All Selected Samples*".
- Note: you can turn on the columns called "Tg-M4 Life Cycle Stages – graph" and "Tg-M4 Life Cycle Stage %ile- graph" (inside the "Tg-Life Cycle" Microarray) to view the graphs in the final result table.



c. Revise the first step of this strategy and compare the maximum expression of the reference samples to the minimum of the comparison samples.

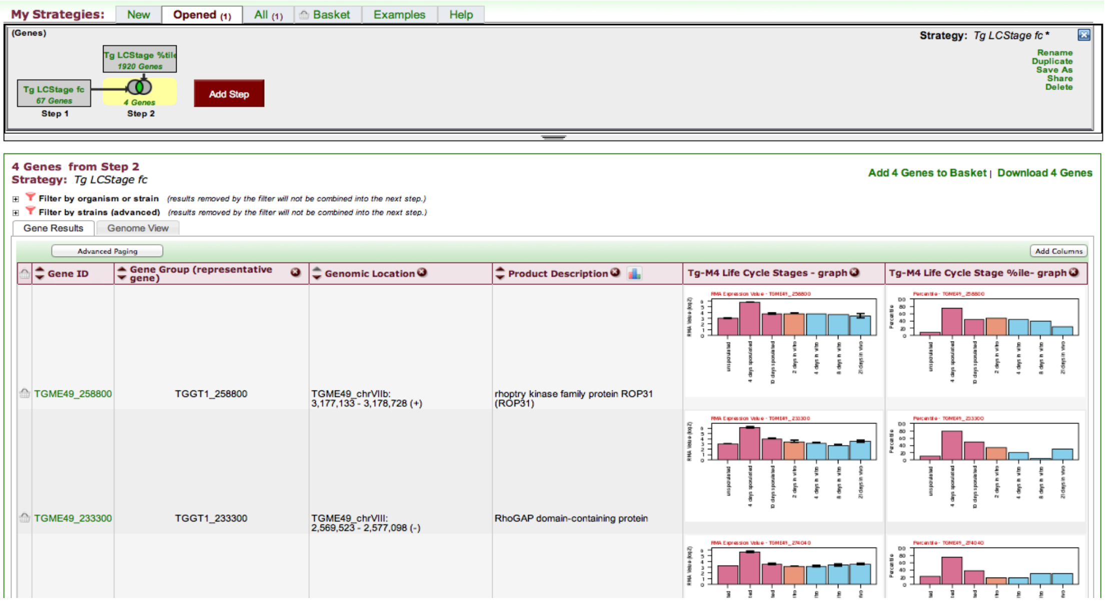
- Does this result look cleaner/more convincing? Why?
- Would you consider these genes to be oocyst specific?



Save this strategy so that you can use it for an exercise we are doing later during the course.

d. Revise the first step of this strategy to find genes that are 3 fold higher in day 4 oocysts than any other life cycle stage in this experiment.

- Do all these genes have day 4 oocysts as the global maximum time point?
- Note that we still have the step to limit the percentile of non-oocyst samples to <= 50th percentile. What happens if you revise this step to also include the unsporulated and day 10 oocyst samples in this percentile range? Do you get more of fewer results back? Why?



Strategy: *Tg LCStage fc* *

4 Genes from Step 2

Strategy: *Tg LCStage fc*

Add 4 Genes to Basket | Download 4 Genes

⊞ Filter by organism or strain *(results removed by the filter will not be combined into the next step.)*

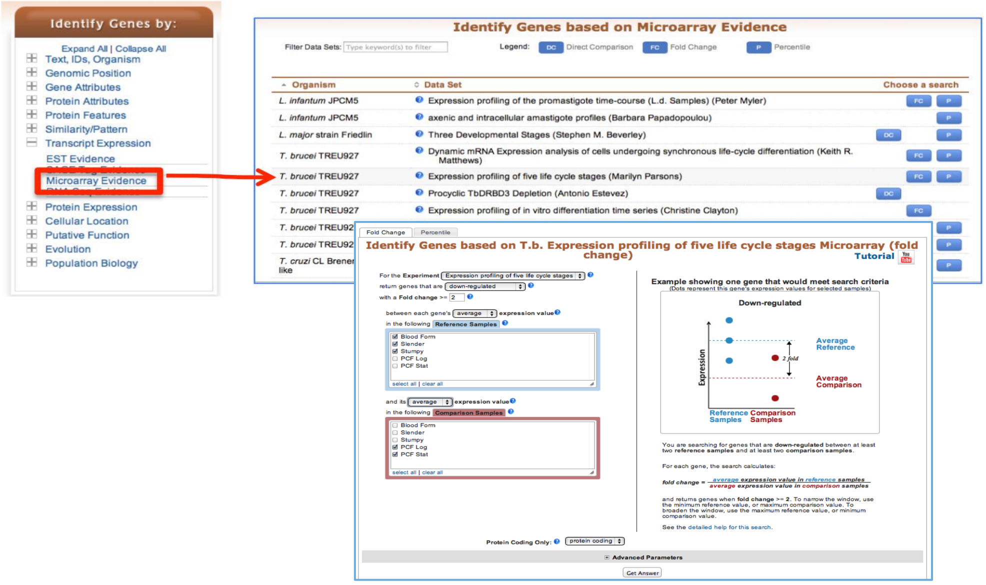
⊞ Filter by strains (advanced) *(results removed by the filter will not be combined into the next step.)*

Gene ID	Gene Group (representative gene)	Genomic Location	Product Description	Tg-M4 Life Cycle Stages - graph	Tg-M4 Life Cycle Stage %ile- graph
TGME49_258800	TGGT1_258800	TGME49_chrVIIb: 3,177,133 - 3,178,728 (+)	rhoptry kinase family protein ROP31 (ROP31)		
TGME49_233300	TGGT1_233300	TGME49_chrVIII: 2,569,523 - 2,577,098 (-)	RhoGAP domain-containing protein		

6. Comparing RNA abundance and Protein abundance data.
   Note: for this exercise use http://TriTrypDB.org.

In this exercise we will compare the list of genes that show differential RNA abundance levels between procyclic and blood form stages in *T. brucei* with the list of genes that show differential protein abundance in these same stages.

a. Find genes that are down-regulated 2-fold in procyclic form cells. Go to the search page for Genes by Microarray Evidence and select the fold change search for the "Expression profiling of five life cycle stages (Marilyn Parsons)" experiment and configure the search to return protein-coding genes that are down-regulated 2 fold in procyclic form (PCF) relative to the Blood Form reference sample. Since there are two PCF samples, it is reasonable to choose both and average them.



Identify Genes based on Microarray Evidence

Filter Data Sets: Type keyword(s) to filter

Legend: DC Direct Comparison FC Fold Change P Percentile

Organism	Data Set	Choose a search
L. infantum JPCM5	Expression profiling of the promastigote time-course (L.d. Samples) (Peter Myler)	FC P
L. infantum JPCM5	axenic and intracellular amastigote profiles (Barbara Papadopoulou)	P
L. major strain Friedlin	Three Developmental Stages (Stephen M. Beverley)	DC P
T. brucei TREU927	Dynamic mRNA Expression analysis of cells undergoing synchronous life-cycle differentiation (Keith R. Matthews)	FC P
T. brucei TREU927	Expression profiling of five life cycle stages (Marilyn Parsons)	FC P
T. brucei TREU927	Procyclic TbDRBD3 Depletion (Antonio Estevez)	DC
T. brucei TREU927	Expression profiling of in vitro differentiation time series (Christine Clayton)	FC
T. brucei TREU92		P
T. brucei TREU92		P
T. cruzi CL Brener like		P

Fold Change | Percentile

Identify Genes based on T.b. Expression profiling of five life cycle stages Microarray (fold change)

Tutorial

For the Experiment: Expression profiling of five life cycle stages
return genes that are: down-regulated
with a Fold change >= 2

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

☑ Blood Form
☑ Slender
☑ Stumpy
☐ PCF Log
☐ PCF Stat

select all | clear all

and its average expression value
in the following Comparison Samples

☐ Blood Form
☐ Slender
☐ Stumpy
☑ PCF Log
☑ PCF Stat

select all | clear all

Example showing one gene that would meet search criteria
(Dots represent this gene's expression values for selected samples)

You are searching for genes that are **down-regulated** between at least two **reference samples** and at least two **comparison samples**.

For each gene, the search calculates:

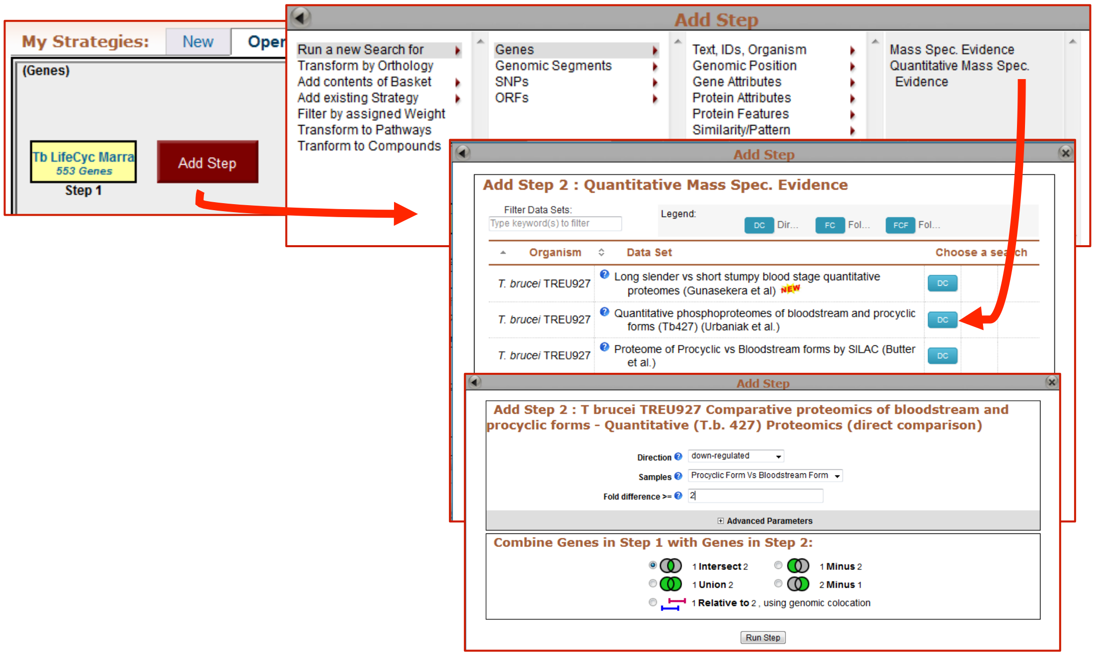
$$\textit{fold change} = \frac{\textit{average expression value in reference samples}}{\textit{average expression value in comparison samples}}$$

and returns genes when **fold change >= 2**. To narrow the window, use the minimum reference value, or maximum comparison value. To broaden the window, use the maximum reference value, or minimum comparison value.

See the detailed help for this search.

Protein Coding Only: protein coding

b. Add a step to compare with quantitative protein expression. Select protein expression then "Quantitative Mass Spec Evidence" and the "Quantitative phosphoproteomes of bloodstream and procyclic forms (Tb427) (Urbaniak et al.)" experiment. Configure this search to return genes that are down-regulated in procyclic form relative to blood form.



Add Step 2 : Quantitative Mass Spec. Evidence

Organism	Data Set	Choose a search
T. brucei TREU927	Long slender vs short stumpy blood stage quantitative proteomes (Gunasekera et al) NEW	DC
T. brucei TREU927	Quantitative phosphoproteomes of bloodstream and procyclic forms (Tb427) (Urbaniak et al.)	DC
T. brucei TREU927	Proteome of Procyclic vs Bloodstream forms by SILAC (Butter et al.)	DC

Add Step 2 : T brucei TREU927 Comparative proteomics of bloodstream and procyclic forms - Quantitative (T.b. 427) Proteomics (direct comparison)

Direction: down-regulated

Samples: Procyclic Form Vs Bloodstream Form

Fold difference >=: 2

⊞ Advanced Parameters

Combine Genes in Step 1 with Genes in Step 2:

● 1 Intersect 2 ○ 1 Minus 2
○ 1 Union 2 ○ 2 Minus 1
○ 1 Relative to 2 , using genomic colocation

Run Step

c. How many genes are in the intersection? Does this make sense? Make certain that you set the directions correctly.

d. Try changing directions and compare up-regulated genes/proteins. (*Hint:* revise the existing strategy … you might want to duplicate it so you can keep both). When you change one of the steps but not the other do you have any genes in the intersection? Why might this be?

e. Can you think of ways to provide more confidence (or cast a broader net) in the microarray step? (*Hint:* you could insert steps to restrict based on percentile or add a RNA Sequencing step that has the same samples).