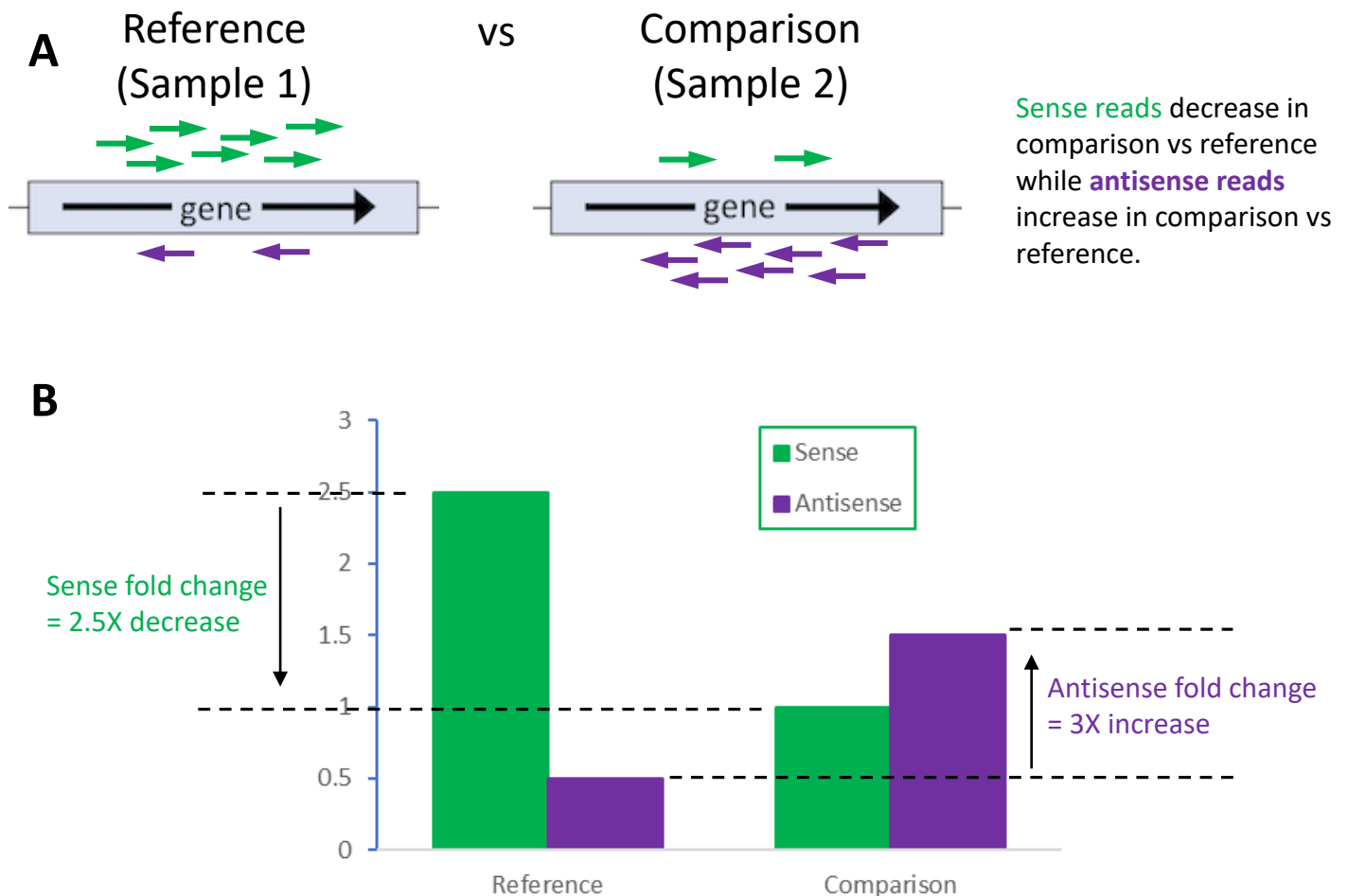


The EuPathDB Sense/Antisense search is available for any RNA sequence data set based on strand specific data. For each gene, the search compares the fold change in sense reads to the fold change in antisense reads (A) and returns genes that have simultaneous changes in sense vs antisense expression between two samples (B). Versatile search parameters allow for choosing the direction and magnitude of the change in sense and antisense transcripts. The search result page lists genes and indicates the paired Reference->Comparison samples that meet the criteria, and offers two graphs to help interpret the search results (FPKM vs sample bar graph, antisense FC vs sense FC scatter plot).

This tutorial demonstrates the Sense/Antisense search using the PlasmoDB data set [Intraerythrocytic cycle transcriptome \(3D7\)](#), a time course strand specific RNA sequence analysis of 8 time points. We will configure the search to return protein coding genes whose sense transcripts decrease 2 fold while their antisense transcripts increase 2 fold between any time points in the Intraerythrocytic cycle transcriptome experiment.



1. Navigate to the 'Identify Genes by RNA Sequence Evidence' page and choose the Sense/Antisense (SA) search for the data set called 'Intraerythrocytic cycle transcriptome (3D7) (Hoeijmakers et al.)'.

Search for Genes

expand all | collapse all

Find a search... ?

- Text
- Gene models
- Annotation, curation and identifiers
- Genomic Location
- Taxonomy
- Orthology and synten
- Phenotype
- Genetic variation
- Epigenomics
- Transcriptomics
 - EST Evidence
 - Microarray Evidence
 - RNA Seq Evidence

Identify Genes based on RNA Seq Evidence

Filter Data Sets: <input type="text" value="Type keyword(s) to filter"/>		Legend: DE Differential E... FC Fold Change P Percentile SA SenseAntise...			
Organism	Data Set	Choose a search			
<i>P. berghei</i> ANKA	Female and male gametocyte (Yeoh et al.)	DE	FC	P	SA
<i>P. berghei</i> ANKA	5 asexual and sexual stage transcriptomes (Hoeijmakers et al.)		FC	P	
<i>P. chabaudi</i> chabaudi	Trophozoite transcriptomes after mosquito transmission or direct injection into mice (Spence et al.)	DE	FC	P	
<i>P. cynomolgi</i> strain M	Transcriptome of <i>P. cynomolgi</i> during 100-day infection in <i>M. mulatta</i> (Joyner et al.)		FC	P	
<i>P. falciparum</i> 3D7	NSR-seq Transcript Profiling of malaria-infected pregnant women and children (Vignali et al.)		FC	P	
<i>P. falciparum</i> 3D7	Polysomal and steady-state asexual stage transcriptomes (Bunnik et al.)		FC	P	
<i>P. falciparum</i> 3D7	Blood stage transcriptome (3D7) (Otto et al.)		FC	P	
<i>P. falciparum</i> 3D7	Transcriptomes of 7 sexual and asexual life stages (Lopez-Barragan et al.)		FC	P	
<i>P. falciparum</i> 3D7	Intraerythrocytic cycle transcriptome (3D7) (Hoeijmakers et al.)		FC	P	SA
<i>P. falciparum</i> 3D7	Ring, Oocyst and Sporozoite Transcriptomes (Zanghi et al.)	DE	FC	P	SA
<i>P. falciparum</i> 3D7	Strand specific transcriptomes of 4 life cycle stages (Lopez-Barragan et al.)		FC	P	SA

2. Arrange the search parameters according to the figure below to return all genes whose antisense transcripts increase by 2-fold while their sense transcripts decrease by 2-fold in all possible pairwise combinations of reference and comparison samples.

Identify Genes based on RNA Seq Evidence

Filter Data Sets: Legend: Differential Ex... Fold Change Percentile SenseAntisense

Organism	Data Set	Choose a search
<i>P. falciparum</i> 3D7	Intraerythrocytic cycle transcriptome (3D7) (Hoeijmakers et al.)	<input type="button" value="FC"/> <input type="button" value="P"/> <input type="button" value="SA"/>

[Show All Data Sets](#)

Fold Change Percentile SenseAntisense

[Learn more about this search](#)

Identify Genes based on *P. falciparum* 3D7 Intraerythrocytic cycle transcriptome (3D7) changes in sense and antisense transcripts

Find genes whose antisense transcripts

increase

with a fold change \geq

2

while the same gene's sense transcripts

decrease

with a fold change \geq

2

between expression in any of the following Reference Samples:

- ☒ 12-20 hours post-invasion
 - ☒ 17-25 hours post-invasion
 - ☒ 2-10 hours post-invasion
 - ☒ 22-30 hours post-invasion
 - ☒ 27-35 hours post-invasion
 - ☒ 32-40 hours post-invasion
 - ☒ 40-5 hours post-invasion
 - ☒ 7-15 hours post-invasion
- [select all](#) | [clear all](#)

and expression in any of the following Comparison Samples:

- ☒ 12-20 hours post-invasion
 - ☒ 17-25 hours post-invasion
 - ☒ 2-10 hours post-invasion
 - ☒ 22-30 hours post-invasion
 - ☒ 27-35 hours post-invasion
 - ☒ 32-40 hours post-invasion
 - ☒ 40-5 hours post-invasion
 - ☒ 7-15 hours post-invasion
- [select all](#) | [clear all](#)

To calculate fold-changes, use a floor of:

10 reads (.2 FPKM)

Protein Coding Only:

protein coding

Advanced Parameters

Change in antisense fraction \geq

0.5

Get Answer

Find genes whose antisense transcripts **increase** with a fold change \geq **2**

The search calculates the fold change in sense mapped reads for each gene

while the same gene's sense transcripts **decrease** with a fold change \geq **2**

The search calculates the fold change in antisense mapped reads for each gene

between expression in any of the following Reference Samples: **all samples**

Choose any or all samples according to the biological comparison you want to make. The search considers all possible pairwise combinations.

and expression in any of the following Comparison Samples: **all samples**

Choose any or all samples according to the biological comparison you want to make. The search considers all possible pairwise combinations.

To calculate fold-changes, use a floor of: **10 reads (.2 FPKM)**

Choose the default setting of 10 reads or greater to avoid misleading FC values when FPKM values are close to zero.

Protein Coding Only: **protein coding**

Choose to query all genes or the subset of protein coding

Advanced Param – Change in antisense fraction \geq **0.5**

Leave as default. The fraction of total transcripts that are antisense must change (up or down) by at least this amount. Enter a number between 0 and 1. Larger fractions will select for genes with a large fraction of antisense in one sample and small fraction of antisense in the other sample.

3. Explore your results. The search returns over 500 genes (1) with at least a 2-fold increase in antisense transcripts and a 2-fold decrease in sense transcripts between samples in the iRBC experiment. Four search specific result columns appear in the result table: Reference->Comparison sample pairs that meet criteria (2), Max Sense_FC*Antisense_FC (3), expression graph (FPKM vs Sample) (4), and Strand specific fold change graph (log2 antisense vs log2 sense) (5). See the next page for details about each result column.

My Strategies: New Opened (1) All (1) Basket Public Strategies (29) Help

1

search strategy panel

PF3D7 iRBC cycle RNAseq
514 Genes

Add Step

Step 1

Strategy: PF3D7 iRBC cycle RNAseq (sense antisense) *

Rename

Duplicate

Save As

Share

Delete

514 Genes from Step 1 Revise

Strategy: PF3D7 iRBC cycle RNAseq (sense antisense)

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

		Plasmodium																												
All Results	Ortholog Groups	<i>P. adleri</i>	<i>P. berghei</i>	<i>P. billicollinsi</i>	<i>P. blacklocki</i>	<i>P. chabaudi</i>	<i>P. coatneyi</i>	<i>P. cynomolgi</i> (nr Genes: 0)	<i>P. falciparum</i> (nr Genes: 514)	<i>P. fragile</i>	<i>P. gaboni</i> (nr Genes: 0)	<i>P. gallinaceum</i>	<i>P. inui</i>	<i>P. knowlesi</i> (nr Genes: 0)	<i>P. mairiae</i>	<i>P. ovale curtisi</i>	<i>P. praefalciparum</i>	<i>P. reichenowi</i> (nr Genes: 0)	<i>P. relictum</i>	<i>P. vinckei</i> (nr Genes: 0)	<i>P. vivax</i> (nr Genes: 0)	<i>P. yoelii</i> Genes: (
		G01	ANKA	G01	G01	chabaudi	Hackeri	strain B	strain M	3D7	IT	strain nilgiri	strain G01	strain SY75	8A	San Antonio 1	strain H	strain Malayan Pk1 A	UG01	GH01	strain G01	CDC	G01	SGS1-like	petit strain CR	vinckei strain vinckei	P01	Sal-1	yoelii 17XNL	yoelii 17X
514	487	0	0	0	0	0	0	0	0	514	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Gene Results

Genome View

Analyze Results

Genes: 514

Transcripts: 523

Show Only One Transcript Per Gene

First

1

2

3

4

5

Next

Last

Advanced Paging

2

3

4

5

Download

ret

Add Columns

Gene ID	Transcript ID	Organism	Product Description	Reference->Comparison sample pairs that meet criteria	Max Sense_FC*Antisense_FC	PF3D7 iRBC cycle RNAseq - Both_strands fpkm graph	PF3D7 iRBC cycle RNAseq - Strand_specific fold change graph
PF3D7_1133000	PF3D7_1133000.1	<i>P. falciparum</i> 3D7	conserved Plasmodium protein, unknown function	27-35 hours post-invasion->12-20 hours post-invasion, 27-35 hours post-invasion->17-25 hours post-invasion, 27-35 hours post-invasion->2-10 hours post-invasion, 27-35 hours post-invasion->22-30 hours post-invasion, 27-35 hours post-invasion->7-15 hours post-invasion, 32-40 hours post-invasion->12-20 hours post-invasion, 32-40 hours post-invasion->22-30 hours post-invasion, 32-40 hours post-invasion->7-15 hours post-invasion, 40-5 hours post-invasion->12-20 hours post-invasion, 40-5 hours post-invasion->7-15 hours post-invasion	36909.8		
PF3D7_0731400	PF3D7_0731400.1	<i>P. falciparum</i> 3D7	serine/threonine protein kinase, FIKK family, pseudogene	12-20 hours post-invasion->2-10 hours post-invasion, 12-20 hours post-invasion->40-5 hours post-invasion, 17-25 hours post-invasion->2-10 hours post-invasion, 17-25 hours post-invasion->40-5 hours post-invasion, 22-30 hours post-invasion->2-10 hours post-invasion, 22-30 hours post-invasion->40-5 hours post-invasion, 27-35 hours post-invasion->2-10 hours post-invasion, 27-35 hours post-invasion->40-5 hours post-invasion, 32-40 hours post-invasion->2-10 hours post-invasion, 32-40 hours post-invasion->40-5 hours post-invasion, 7-15 hours post-invasion->2-10 hours post-invasion	26922.8		

- **Reference->Comparison sample pairs that meet criteria** – Because we arranged the search to query all samples, the search returns all possible combinations of reference and comparison that meet the criteria (2X increase antisense, 2X decrease in sense). For the gene, PF3D7_1133000, thirteen sample combinations meet the criteria.
- **Max Sense_FC*Antisense_FC** – This column is an in-house metric derived from multiplying ‘Max sense strand fold change’ and ‘Max antisense fold change’ for that gene. This metric can be used to roughly rank the genes according to the magnitude of differential between sense and antisense transcription.
- **Pf3D7 iRBC cycle RNAseq- Both_strands fpkm graph** – This graph, FPKM vs sample, is a visual representation of the simultaneous changes detected by the search. For example, comparing the 17-25 hr sample to the 12-20 hr sample, sense transcripts decrease (green arrows) while the antisense transcripts increase (purple arrows). This graph is also available on the gene page in the transcriptomics section.
- **Pf3D7 iRBC cycle RNAseq – Strand_specific fold change graph** – Each point shows the sense vs antisense fold change between a pair of samples. Points on the diagonal (red dotted line) represent sample pairs with equivalent fold change between samples. Points within the red dotted box meet your search criteria. The blue inset shows a gene with a consistent negative correlation between sense and antisense expression, i.e. $\log_2(\text{antisenseFC}) \sim -\log_2(\text{senseFC})$; or as antisenseFC increases, the senseFC decreases. Clicking on the graph opens a new tab with an enlarged image which retrieves sample pair details on mouseover.

Reference->Comparison sample pairs that meet criteria	Max Sense_FC*Antisense_FC	Pf3D7 iRBC cycle RNAseq - Both_strands fpkm graph	Pf3D7 iRBC cycle RNAseq - Strand_specific fold change graph
27-35 hours post-invasion->12-20 hours post-invasion, 27-35 hours post-invasion->17-25 hours post-invasion, 27-35 hours post-invasion->2-10 hours post-invasion, 27-35 hours post-invasion->22-30 hours post-invasion, 27-35 hours post-invasion->7-15 hours post-invasion, 32-40 hours post-invasion->12-20 hours post-invasion, 32-40 hours post-invasion->17-25 hours post-invasion, 32-40 hours post-invasion->2-10 hours post-invasion, 32-40 hours post-invasion->22-30 hours post-invasion, 32-40 hours post-invasion->7-15 hours post-invasion, 40-5 hours post-invasion->12-20 hours post-invasion, 40-5 hours post-invasion->2-10 hours post-invasion, 40-5 hours post-invasion->7-15 hours post-invasion	36909.8		