

TRANSFAC Getting Started Guide Version 2016.3

TRANSFAC is a database containing published data on eukaryotic transcription factors and miRNAs, their experimentally-proven binding sites, and regulated genes. The extensive compilation of binding sites forms the basis of derived positional weight matrices which can be used with the included Match tool to search DNA sequences for putative transcription factor binding sites – a method that is crucial for filling in gaps in the published literature. Integrated transcription factor ChIP-Seq, DNase hypersensitivity and histone methylated intervals from the ENCODE project complement the curated and predicted binding site data.

This guide will walk you through some of the most common uses of TRANSFAC. For additional help, please contact our support team at mailto:support@genexplain.com

Quick links to sections covered:

Identify experimentally demonstrated regulators for a gene of interest Visualize a gene's promoter and associated regulatory features Identify predicted binding sites for a gene of interest Identify experimentally demonstrated targets for a transcription factor of interest Upload your own sequence and identify the predicted binding sites within Import your own custom matrix for use in binding site predictions Compare your own custom matrix against TRANFAC's matrix library Analyze a microarray or gene-based RNA-seq data set Analyze a transcript-based RNA-seq data set

Identify experimentally demonstrated regulators for a gene of interest

TRANSFAC Locus Reports provide information about genes, including the transcription factors which have been experimentally demonstrated to bind to the gene's sequence.

To navigate to the Locus Report for a gene of interest:

- Select the "Genes and proteins" radio button
- Select the "Name" radio button
- Enter the name of your gene in the search box (PTEN in this example)
- Click the "search" button



The list of matched genes is returned:

Genes and proteins 11 of 11 total (All species)	Select results and view as:
Save these results Export these resu	ilts 💦 Pathfinder 🛞 Ontology 🚊 Match 📈 FASTA 📲 Profiles Binding factors for gene 🔻 Search
First 📢 1 🕨 Last	
Mark all on page Filter	Hits on page 25 ▼
# Name Species/Taxon	Description
PTEN Human	Phosphatase and tensin homolog, inhibits PKB signaling, phosphatidylinositol-3, 4, 5-trisphosphate production, cytokine production, and cell proliferation, downregulated in medulloblastoma, cutaneous melanoma, bladder and lung cancer, and ganglioneuroma
Pten Mouse	Phosphatase and tensin homolog, inhibits PKB signaling, phosphatidylinositol-3, 4, 5-trisphosphate production, cytokine production, and cell proliferation; human PTEN correlates with medulloblastoma, melanoma, bladder and lung cancer, and ganglioneuroma
Pten Rat	Phosphatase and tensin homolog, inhibits PKB signaling, phosphatidylinositol-3, 4, 5-trisphosphate production, cytokine production, and cell proliferation; human PTEN correlates with medulloblastoma, melanoma, bladder and lung cancer, and ganglioneuroma

Click the human "PTEN" link to navigate to the Locus Report.

Scroll down the page or use the "Table of Contents" section to jump to the "Transcriptional Regulation" section:

A summary of all curated, experimental binding sites is given in the table. For more detailed information about a specific binding site, click the link in the "Identifier" column to navigate to the Site Report which contains detailed supporting information about the method(s) used to measure binding, the experimental system and more, including links to the abstract of the reference from which the information was curated.



Identifier 🗘	Relative Location	Genomic Location 🗘	Binding Factor(s) 🗘	DNA Binding Reaction	Effect 🗘
HS\$PTEN_10	-2200 to -2167 *	Chr10 87862269 87862302 +	Max(m.s.)	Max(m.s.)> PTEN(h)	DNA binding
HS\$PTEN_09	-2200 to -2167 *	Chr10 87862269 87862302 +	USF1(h):USF1(h)	USF1(h):USF1(h)> PTEN(h)	DNA binding
HS\$PTEN_09	-2200 to -2167 *	Chr10 87862269 87862302 +	USF2(h):USF2(h)	USF2(h):USF2(h)> PTEN(h)	DNA binding
HS\$PTEN_09	-2200 to -2167 *	Chr10 87862269 87862302 +	USF2(h):USF1(h)	USF2(h):USF1(h)> PTEN(h)	DNA binding
HS\$PTEN_09	-2200 to -2167 *	Chr10 87862269 87862302 +	(usf1(m.s.))2	(usf1(m.s.))2> PTEN(h)	DNA binding
Showing 1 to 5	of 31 entries			First Previous 1 2 3 4 5	Next Last

Visualize a gene's promoter and associated regulatory features

TRANSFAC contains promoter sequences for human, mouse, rat, Arabidopsis, rice and soybean species. Promoter sequences are 11,000 nucleotides in length, spanning from 10,000 nucleotides upstream of the transcription start site (TSS) to 1,000 nucleotides downstream of a virtual TSS that is calculated based on clusters of experimental TSSs. An individual gene may have more than one associated promoter. When this is the case one of the promoters will be selected as the best-supported, which indicates that it is the promoter with the greatest fraction of clustered TSSs.

There are two ways to graphically visualize the promoter sequence plus associated regulatory features which have been mapped to the promoter, including experimentally demonstrated factor binding sites.

The first way is to navigate to the "Transcriptional Regulation" section of the desired gene (see <u>Identify experimentally demonstrated regulators for a gene of interest</u>) and then click one of the "predicted promoter sequences" links:



The second way is to select the desired gene in the search results and perform a second search for its associated promoter(s):

Gen	es and	proteins Il species)	Select results ar	nd view as:					
Save th	nese results	Export these res	ults Pathfinder	Ontology	Antch	\varkappa _{fasta}	Profiles	Binding factors for gene Gene Regulation	Search
First		Last						Binding factors for gene Gene bound by factor	
	Mark all on p	age Filter						Promoter	Hits on page 25 🔻
#	Name	Species/Taxon	Description					TF binding matrix	
	PTEN	Human	Phosphatase and and cell proliferat	tensin homolo ion, downregu	g, inhibits P lated in me	KB signaling dulloblastom	, phosphatidyl na, cutaneous	mRNA Regulation Regulating miRNAs	e production, cytokine production, g cancer, and ganglioneuroma
	Pten	Mouse	Phosphatase and and cell proliferat	tensin homolo ion; human P	g, inhibits P FEN correla	KB signaling tes with me	, phosphatidyl dulloblastoma,	inositol-3, 4, 5-trisphospha melanoma, bladder and lu	te production, cytokine production, ing cancer, and ganglioneuroma

Once the promoter search results load, click the link in the "Accession" column to open the Promoter Report. The Promoter Report provides a graphical view of the sequence:



Use the "Displayed features" options shown above to add or remove different types of features. The red orientation point and flanking yellow region in the frequency bar below the nucleotide sequence indicates the portion of the sequence that is displayed. Drag the red orientation point along the frequency bar to inspect different regions of the promoter at the nucleotide level.

The graphic is followed by a tabular view of the different features

Identify predicted binding sites for a gene of interest

While the Locus Report provides an overview of all curated, experimental binding sites for a gene (see <u>Identify experimentally demonstrated regulators for a gene of interest</u>) and the Promoter Report provides a graphical view of those experimental binding site plus additional regulatory features located within the gene's promoter (see <u>Visualize a gene's</u> <u>promoter and associated regulatory features</u>), the Predict TF binding sites tool and the Match algorithm that it employs enables you to scan the gene's promoter for additional predicted binding sites using TRANSFAC's comprehensive positional weight matrix library.

There are two ways to interrogate the gene's promoter.

The first way is to navigate to the "Transcriptional Regulation" section of the Locus Report (see <u>Identify experimentally demonstrated regulators for a gene of interest</u>) and then click the Match icon to launch the Match algorithm:

BIOB	Locus Report - Human		logout	help –
BIOLOGICAL DATABASES	OPTEN	Table of Contents		↓
Transcriptional Regulation of PTE Predicted promote	Regulation what is this? EN gene expression er sequences : Match →			

The second way is to perform a search for the gene of interest (see <u>Identify experimentally</u> <u>demonstrated regulators for a gene of interest</u>), and then launch the Match algorithm directly from the search results by selecting the gene of interest and clicking the Match icon:

Genes and proteins 11 of 11 total (All species)	Select results and view as:
Save these results Export these results	Pathfinder 🕫 Ontology 🚊 Match 🛛 FASTA 🖣 Profiles Promoter 🔹 Search
First 📢 1 🐌 Last	
Mark all on page Filter	Hits on page 25 •
# Name Species/Taxon Des	scription
PTEN Human Pho and	osphatase and tensin homolog, inhibits PKB signaling, phosphatidylinositol-3, 4, 5-trisphosphate production, cytokine production, d cell proliferation, downregulated in medulloblastoma, cutaneous melanoma, bladder and lung cancer, and ganglioneuroma

In both cases an intermediate window will open where you are asked to:

- Select the analysis method (use the default "Match")
- Select the promoter to be analyzed (use the default "best supported promoter" if you want to analyze the promoter with the greatest number of clustered TSSs, or change to "all promoters" if you would like to analyze all promoters for comparison)
- Specify the region of the promoter to analyze (use the default "-10,000 bp to +1,000 bp" to analyze the entire promoter, or select a smaller region within those bounds)
- Specify the data version (use the default, current version)
- Specify the profile (group of matrices) to be used (use the default "vertebrate_non_redundant_minFP" which contains representative matrices for distinctive binding motifs unless you are working with non-vertebrate species or want to limit your analysis to factors that are active in a particular tissue)
- Specify the cut-off criteria (use the default "minimize false positives" which is automatically assigned with the "vertebrate_non_redundant_minFP" profile)

Match : Predicting transcription factor binding sites
The Match program uses positional weight matrices (PWMs) from the TRANSFAC matrix library to search the stored DNA sequences of the selected gene(s) for potential transcription factor binding sites. Learn more.
 Select analysis method: Match - search for TF binding sites
Por the selected gene(s), analyze: best supported promoter
Within a nucleotide window of: -10000 bp(max) to +1000 bp(max)
2015.3 ▼
2 Using the profile (group of matrices):
vertebrate_non_redundant_minFP
☑ Use only high quality matrices
? With cut-off criteria: minimize false positives ▼
Submit

When you click the Submit button your analysis will be forwarded to the taskbar. Once the analysis completes, the Match Analysis Report will automatically load:

Match Analysis Report						
Save this report Export this report						
Analysis summary						
Match analysis identified:						
Total sequence length : 11001 Total Total number of sites : 312 Num Frequency of sites : 0.02836 Aver	number of sequences : 1 ber of sequences with sites : 1 age number of sites per sequence: 312.00					
Show parameters used						
Matrix summary						
First 🔌 1 2 3 4 5 🕨 L	ast					
Mark all on page (selecting matrice	s will filter sequence summary) Filter Profiles Expo	t table				Hits on page 5 🔻
Showing 5 of 69 entries						
# Matrix Factor	Consensus sequence	Classification	Category	Sites	Sequences	Sites/Sequences F
V\$CPBP_Q6 CPBP	CCc	ZFC2H2	matrix compiled from individual genomic sites	28	1	28.00
V\$ZNF333_01 ZNF333	ATAAT	ZFC2H2	SELEX (CASTing, SAAB, TDA, Target detection	27	1	27.00

The "Matrix summary" section provides a tabular overview of all matrices that were found to have binding sites within the promoter. Scrolling down to the "Sequence details" section a graphical view of the results is provided:

Sequence details	
	¢
	▲NF333
CGGCTGGCACATCCAGGGACCCGGGCCGGTTTTAAACCTCCCGTGCGCCGCCGCCGCCGCGCGGCGCGGGGCCGCGGGGGG	GTTCGGAGGATTATTCGTC
4100 87864110 87864120 87864130 87864140 87864150 87864160 87864170 87864180 87864190 878642	200 87864210
La Bade - Bade - Barrow Marrow Mar Viewing nucleotides 87864100 to 87864219	<u></u>
Filter by: ChIP fragments DNase hypersensitivity sites Phastcons intervals	

A frequency bar displays the distribution of sites along the entire sequence, with the red orientation point and flanking yellow highlighting indicating the portion of the sequence that is displayed at the nucleotide level above. Slide the red orientation point along the frequency bar to view other portions of the sequence in detail.

Optionally use the ChIP fragments, DNase hypersensitivity sites and Phastcons intervals filters to apply additional biological context to your results. Activating these filters removes any sites that do not overlap with regions of sequence that are suggested to be available to the transcriptional machinery based on being bound by transcription factors (ChIP fragments), being accessible to DNase enzymes (DNase hypersensitivity) or conserved between human and mouse (Phastcons).

Please note that if "all promoters" was selected for the analysis it will be necessary to click the Sequence name in the "Sequence summary" section to display the nucleotide level view.

The table below the graphical view lists all identified binding sites with supporting details:

First M Showi	1 2 3 4 5 Mark all on page Filter Export ng 25 of 312 entries	Last table				Hits on page 25 •
#	Matrix	Factor name	Position (strand) 🛓	Core score	Matrix score	Sequence
	V\$YY1_Q6_03	YY1	87854376 (+)	1.000	1.000	CCATTtt
	V\$DRI1_01	DRI1	87854613 (-)	1.000	1.000	TTAATt
	V\$HMGIY_Q3	HMGIY	87854623 (+)	1.000	0.947	tttttAATTTttttc
	V\$DRI1_01	DRI1	87854626 (-)	1.000	1.000	TTAATt
	V\$IK_Q5_01	Ikaros	87854696 (-)	1.000	1.000	CCTCCCA

Identify experimentally demonstrated targets for a transcription factor of interest

There are two ways to identify the experimentally determined targets for a transcription factor. The first way is to navigate to the Locus Report for the factor and view the information provided in the "Transcriptional Regulation" section.

To navigate to the Locus Report for a transcription factor of interest:

- Select the "Transcription factors" radio button
- Select the "Name" radio button
- Enter the name of your gene in the search box (HNF1A in this example)
- Click the "search" button



The list of matched factors is returned:

Tran 7 of 7 f	scription fa	ctors s	elect results and view as:
Save the	se results Expor	t these results	🕻 Pathfinder 🍄 Ontology 🚊 Match 🏹 FASTA 📕 Profiles 🛛 Binding factors for gene 🔹 Search
First	📢 1 🍺 Las	st	
Mi Mi	ark all on page Filt	er	Hits on page 25 🔻
#	Name	Species/Taxo	Description
		Human	HNF1 homeobox A, a transcriptional activator that functions in insulin secretion and fatty acid transport, aberrantly expressed in kidney and liver cancers; gene mutation causes diabetes, mouse <mark>Hnf1a</mark> is associated with phenylketonuria and Fanconi syndrome gene
	Inter	Mouse	HNF1 homeobox A, a transcriptional activator that functions in insulin secretion and fatty acid transport; genetic knockout is associated with phenylketonuria and Fanconi syndrome, human HNF1A is associated with diabetes and kidney and liver cancers gene
	Hnf1a	Rat	Transcription factor 1, a transcriptional activator that functions in insulin secretion and fatty acid transport; human HNF1A is associated with diabetes and kidney and liver cancers, mouse Hnf1a is associated with phenylketonuria and Fanconi syndrome gene

Click the human "HNF1A" link to navigate to the Locus Report.

Scroll down the page or use the "Table of Contents" section to jump to the "Transcriptional Regulation" section and scroll to the "Regulation of gene expression by HNF1A" subsection:

	Locus Report - Human HNF1A (HNF-1alpha)				logout	help 🗕
				Table of Contents		1
egulation of g	gene expression by HNF1A	A.		Gene Ontology Molecular function Biological process		^
Transcription f	actor classification :			Cellular component		
HNF-1alpha (HNF- POU domain factor them probably cor Genes bound b	1A, LF-B1); 3.1.10.7.1 rs; 3.1.10 (The POU domain consists of nprising an alpha-helix and enriched in y HNF1A (77 entries)	a POU-specific box and an approp basic or hydroxyl amino acids, res	riately spaced homeod p. The POU-specific boy	Expression Tissue expression Transcriptional Regulation		
Chave E	tui			Regulation of HNF1A gene expression		
snow 5 • en	unes			Regulation of gene expression by HNF1A		
Gene	 Location within Gene \$ 	Binding Site Identifier 🗘	DNA Binding Read	Regulation of HNF1A mRNA expression		
ABCC2(h)	-2065 to -2037	HS\$ABCC2_03	HNF-1alpha(h)>	Hanschpton factors which interact with the IA		
ABCC2(h)	-168 to -140	HS\$ABCC2_04	HNF-1alpha(h)>	RNA Features		
ACE2(h)	-333 to -307	HS\$ACE2_09	HNF-1alpha(h)>	Overview of RNA sequence		
ACE2(h)	-362 to -236	HS\$ACE2_10	HNF-1alpha(h)>	Protein Features		
Afm(m)	-139 to -120	MOUSE\$AFM_01	HNF-1alpha(h)>	Overview of protein sequence and structure		
Showing 1 to 5	of 77 entries			view complexes containing HNPIA protein		-
Artificial sites	bound by HNF1A (1 entry)					
Show 5 • en	tries			Search:		
Description	· 1	Binding Site Identifier		Quality Score		3

A summary of all curated, experimental binding sites for human HNF1A is given in the table. For more detailed information about a specific binding site, click the link in the "Identifier" column to navigate to the Site Report which contains detailed supporting information about the method(s) used to measure binding, the experimental system and more, including links to the abstract of the reference from which the information was curated.

It is important to note that because curation is species specific, this Locus Report will only provide information for results of experiments where human HNF1A was the focus. Results

from experiments using closely related mouse and rat Hnf1a factors will only be covered on the respective mouse and rate Locus Reports.

The second way to identify the experimentally determined binding sites for a transcription factor of interest avoids this separation of information by species. In this case perform the same "Transcription factors" search for HNF1A. Then when the results are returned, select the human, mouse and rat entries, select the "Gene bound by factor" option in the pulldown menu in the search results header and click the Search button next to the pulldown menu:

Trar 7 of 7	scription fa	ctors s	elect results and view as:
Save th First	ese results Expo	rt these results st ter Species/Taxo	Pathfinder Ontology Antch RASTA Profiles Binding factors for gene Gene Regulation Binding factors for gene Gene Bound by factor Interacting factors Promoter TF binding matrix
۲	HNF1A	Human	HNF1 homeobox A, a transcriptional activator that functions expressed in kidney and liver cancers; gene mutation causes and Fanconi syndrome gene
	Hnf1a	Mouse	HNF1 homeobox A, a transcriptional activator that functions in mound secretion and rate action dansport; genetic knockout i associated with phenylketonuria and Fanconi syndrome, human HNF1A is associated with diabetes and kidney and liver cancers gene
	Hnf1a	Rat	Transcription factor 1, a transcriptional activator that functions in insulin secretion and fatty acid transport; human HNF1A is associated with diabetes and kidney and liver cancers, mouse Hnf1a is associated with phenylketonuria and Fanconi syndrome gene

All three factors are used as input to the search. The results are a combined list of the genes that each have been shown to bind experimentally:

Gene bound by factor 25 of 129 total			Select results and	d view as:
Save thes	e results Export	these results	Pathfinder	Ontology 🚊 Match 🕅 FASTA 📲 Profiles Binding factors for gene 🔹 Search
First	1 2 3 4	5 🐌 Las	t	
🔲 Ma	rk all on page Filter			Hits on page 25 ▼
#	Search Term	Name	Species/Taxon	Description
	GN000004288 - HNF1A GN000002682 - Hnf1a	SERPINA1	Human	Serpin peptidase inhibitor clade A member 1, acts in acute-phase response, aberrantly expressed in rheumatoid arthritis, asthma, pancreatitis, Crohn disease, and Alzheimer disease; gene mutation causes alpha 1-Antitrypsin deficiency and cystic fibrosis
	GN000004288 - HNF1A GN000009226 - Hnf1a GN000002682 - Hnf1a	Fgb	Rat	Fibrinogen beta chain, a structural molecule that acts in cell adhesion and acute-phase response, may act in blood coagulation; human FGB is associated with afibrinogenemia, venous thrombosis, coronary artery disease, and recurrent pregnancy loss
	GN000004288 - HNF1A GN000009226 - Hnf1a GN000002682 - Hnf1a	HNF4A	Human	Hepatocyte nuclear factor 4 alpha, a transcriptional coactivator that acts in hepatocyte differentiation and glucose metabolism, downregulated in Crohn disease; mRNA is overexpressed in hepatocellular carcinoma and SNP correlates with type 2 diabetes
	GN000004288 - HNF1A	SLCO1B1	Human	Solute carrier organic anion transporter family member 1B1, a transmembrane transporter that acts in transport of drug, thyroid hormone, prostaglandin, and bile acid and bile salt

Following the link to the Locus Report of the transcription factor or the gene and navigating to the "Transcriptional Regulation" section will allow you to access the same experimental details described above.

Upload your own sequence and identify the predicted binding sites within

TRANSFAC provides preloaded promoter sequences for human, mouse, rat, Arabidopsis, rice and soybean genes but you may upload your own sequence or set of sequences for these or any other species and then analyze the sequence(s) for predicted transcription factor binding sites.

Select the "Predict TF binding sites" option from the tools menu:



When the tool loads, click the "I am analyzing DNA sequences" radio button followed by the "Upload a new sequence" radio button:

Predict Transcription Factor Binding Sites what is this?
 I am analyzing DNA sequences I am analyzing a gene or miRNA set Example sequence Select a previously uploaded sequence: 3000_seqs_250_bp Upload a new sequence
Name: I am uploading DNA sequences Genomic intervals for automatic sequence retrieval Copy and paste DNA sequences up to 10,000,000 nucleotides in total length in FASTA, EMBL, Genbank or RAW format
or browse for file reset form

You will be presented with the option to upload your data as a set of DNA sequences in FASTA, EMBL, Genbank or RAW format or as a set of genomic intervals in .bed format (note that the genomic intervals option is only supported for human, mouse and rat species).

Once you have specified the sequence(s) to be uploaded, accept or adapt in the remaining parameters:

- Select the analysis method (use the default "Match")
- Specify the profile (group of matrices) to be used (use the default "vertebrate_non_redundant_minFP" which contains representative matrices for distinctive binding motifs unless you are working with non-vertebrate species or want to limit your analysis to factors that are active in a particular tissue)
- Keep the "Use default parameters" option enabled

Select analysis method:	
Match - search for TF binding sites	•
Select a profile (group of matrices):	
vertebrate_non_redundant_minFP	
✓ Use default parameters	
Data version: 2015.3 🔻	
Use only high-quality matrices	
Set cut-offs: Minimize false positives	
start search reset form	
	_

When you click the start search button your analysis will be forwarded to the taskbar. Once the analysis completes, the Match Analysis Report will automatically load:

Match Analysis Report Return to Predict TF binding sites page					
Save this report Export this report					
Analysis summary					
Match analysis identified:					
Total sequence length : 11001 Total number of sequences : 1 Total number of sites : 312 Number of sequences with sites : 1 Frequency of sites : 0.02336 Average number of sites per sequence: 312.00					
Show parameters used					
Matrix summary					
					Hits on page 5
Mark all on page (selecting matrices will filter sequence summary) Filter Profiles Exponent	ort table				This of page 5
Showing 5 of 69 entries					
# Matrix Factor Consensus sequence	Classification	Category	Sites	Sequences	Sites/Sequences F
V\$CPBP_Q6 CPBP	ZFC2H2	matrix compiled from individual genomic sites	28	1	28.00
V\$ZNF333_01 ZNF333 ATAAT	ZFC2H2	SELEX (CASTing, SAAB, TDA, Target detection	27	1	27.00

The "Matrix summary" section provides a tabular overview of all matrices that were found to have binding sites within the uploaded sequence. Scrolling down to the "Sequence details" section a graphical view of the results is provided:

Sequence details				
				ø
MECP2	CPBP Churchii			NF333
CGGCTGGCACATCCAGGGACCCGGGCCGGTTTTAAACCTCCCG	IGCGCCGCCGCCGCACCCCCC	GTGGCCCGGGCTCCGGAGGC	CGCCGGCGGAGGCAGCCGT	TCGGAGGATTATTCGTC
4100 87864110 87864120 87864130 87864140	87864150 87864160	87864170 87864180	87864190 87864200	87864210
Viewing nucleotides 87864100 to 87864219	land had no d. t	mush sum h	16.118_11. <mark>.</mark>	
Filter by: ChIP fragments DNase hypersensitivity sites	Phastcons intervals			

A frequency bar displays the distribution of sites along the entire sequence, with the red orientation point and flanking yellow highlighting indicating the portion of the sequence that is displayed at the nucleotide level above. Slide the red orientation point along the frequency bar to view other portions of the sequence in detail.

If you uploaded your sequence in genomic coordinates, you may optionally use the ChIP fragments, DNase hypersensitivity sites and Phastcons intervals filters to apply additional biological context to your results. Activating these filters removes any sites that do not overlap with regions of sequence that are suggested to be available to the transcriptional machinery based on being bound by transcription factors (ChIP fragments), being accessible to DNase enzymes (DNase hypersensitivity) or conserved between human and mouse (Phastcons).

Please note that if more than one sequences was submitted for analysis it will be necessary to click the Sequence name in the "Sequence summary" section to display the nucleotide level view.

The table below the graphical view lists all identified binding sites with supporting details:

First	▲ 1 2 3 4 5)	Last				
. N	lark all on page Filter Expor	t table				Hits on page 25 🔻
Showi	ing 25 of 312 entries					
#	Matrix	Factor name	Position (strand) =	Core score	Matrix score	Sequence
	V\$YY1_Q6_03	YY1	87854376 (+)	1.000	1.000	CCATTtt
	V\$DRI1_01	DRI1	87854613 (-)	1.000	1.000	TTAATt
	V\$HMGIY_Q3	HMGIY	87854623 (+)	1.000	0.947	tttttAATTTttttc
	V\$DRI1_01	DRI1	87854626 (-)	1.000	1.000	TTAATt
	V\$IK_Q5_01	Ikaros	87854696 (-)	1.000	1.000	CCTCCCA

Your analysis will be preserved in the taskbar for 7 days. To preserve it indefinitely, click the "Save this report" link at the top of the report.

Import your own custom matrix for use in binding site predictions

TRANSFAC's matrix library is based on the published literature and published data sets. If

you have your own matrix, or set of sequences that you would like to compile into a matrix, you can upload it into TRANSFAC which will make it available to use in all analyses.

Select the "Create and compare matrices" option from the tools menu:



When the tool loads, click the "Create matrices" option to refresh the view if needed:

Create and compare matrices
Create matrices Compare matrices against TF library
Create a new matrix what is this?
Upload matrix : browse for file No file selected
O Compile matrix from unaligned sequences
O Compile matrix from aligned sequences
create matrix reset form

You have the option to:

- Upload a matrix in TRANSFAC file format
- Compile a matrix from unaligned sequences
- Compile a matrix from aligned sequences

To compile a matrix from unaligned sequences, you need to provide a set of positive sequences (those that are expected to contain the motif) and a set of negative sequences (those that are not expected to contain the motif).

To compile a matrix from aligned sequences, you need to provide a minimum of five sequences that are expected to contain the motif and are of the exact same length.

Once you have chosen the relevant method and supplied the requested information, click the "create matrix" button. The consensus sequence and nucleotide position frequency is displayed:

Create matrices Matrix name : Example Date created : Sat Sep 19 15:59:15 2015 Identifier : V\$Example_30313 **ϫΑτC**GAC Nucleotide position frequency А С G Т Consensus 3 0 0 2 w 5 0 0 0 А 0 0 1 4 т 5 0 С 0 0 G 0 1 4 0 5 0 0 0 А С 0 5 0 0 2

Save matrix and specify cut-off values (Note that this operation may take a few minutes)

М

0

1

2

Click the "Save matrix and specify cut-off values" button to complete the process of calculating the cut-offs. It may take a few minutes, but once the calculation is complete the cut-offs will be displayed and you will be notified that your matrix has been saved:

Cut-offs :					
		Core similarity	Matrix similari	ty	
Minimize false negative r	matches	0.748	0.840		
Minimize false positive m	natches	0.748	0.964		
Minimize sum of both err	or-rates	0.748	0.908		
Aatrix quality : high					
alse positive frequency whe	en tolerating a false r	negative rate :			
FN	10%	30%	50%	70%	90%
MSS	(0.840)	(0.923)	(0.977)	(0.987)	(0.996)
FP frequency	1.455	0.118	0.014	0.007	0.006

In order to use the matrix in an analysis you will need to take the additional step of creating a profile that contains the matrix. From the help menu on the right hand side of the screen, click the "Create a profile" link:

Creating and comparing matrices	
A positional weight matrix is a motif which describes the nucleotide frequency at each position of a set of sequences bound by a transcription factor. The Mato tool uses matrices to search DNA sequences for predicted transcription factor binding sites. The TRANSFAC matrix library provides a large collection of matrices which have been compiled from individual genomic binding sites as well as ChIP Seq, SELEX ar other types of experiments. Alternatively, you may us the tools provided here to create your own matrix an compare against the TRANSFAC matrix library.	ch of nd se d
Creating matrices: Use the Create matrices tool to create a custom matrix. Start by providing five or me aligned sequences in FASTA, ClustalW or Gibbs form After specifying the cutroff values and saving the matrix it can be used to Create a profile for use in Match analysis.	ore lat.
Comparing matrices: Use the Compare matrices to	ol

to compare a created matrix against the TRANSFAC matrix library to identify similar, characterized motifs.

When the Create profile tool loads you can quickly find your matrix by clicking the "Show only user matrices" option:

Select matrice Exclude low Show 5 •	s to be in quality ma entries	cluded in a profile from atri es	matrices			Search:		Select all	Desel	ect all
Accession Number	Matrix Quality	Matrix Identifier	Matrix Name	Matrix Category	Matrix Classification	Sequence Logo				
U00002	high	V\$CREB_1105_01	CREB_1105_01			TGACGT SA				
U00003	high	V\$Example_30313	Example	A		<mark>∻A⊤C⊴AC</mark> ∽	First Dra	vious 1	Nevt	Last
Select matrice	es Res	set	5,505 total entries	;)			Thist Pre	1005 1	HEAL	Lust

Click on your matrix to select it and then click the "Select matrices" button. It will be

displayed below with the calculated cut-offs:

Select matrices to include in the profile.					Proc	ceed to cut-off selection
		FP frequency	(per 1kb nucleotides	s) when false negat	ive rate of:	
Matrix	Quality	10%	30%	50%	70%	90%
Example V\$Example_30313 U00003	high	1.455	0.118	0.014	0.007	0.006

Click the "Proceed to cut-off selection" button. A new window loads where you are able to specify a name for your profile as well as set the default cut-off to be used in analyses:

ease enter a name for yo	ur profile:				Save profil	e			
elect the following cut-of	f for all matr	ices: minFP	▼ wh	at is this?					
Unmark low quality ma	trices								
		Core / Mat	rix similarity (and FP freque	ncy) at the se	lected cut-off	s		
		FN10 /	ENDO	EN50	EN70	FN90	minFP	minSUM	Core / Matrix similarity
Matrix	Quality	minFN	FNSU	11030					

The suggested default is minFP. Click the "Save profile" button to save the profile and make it available in the matrices pull-down menu of all analyses.

Compare your own custom matrix against TRANFAC's matrix library

If you have uploaded a custom matrix (see <u>Import your own custom matrix for use in</u> <u>binding site predictions</u>) you can use the Compare matrices tool to investigate whether your matrix is similar to other matrices within the TRANSFAC matrix library.

Select the "Create and compare matrices" option from the tools menu:



When the tool loads, click the "Compare matrices against TF library" option to refresh the view if needed:

Create and compare matrices						
Create matrices Compare matrices against TF library						
Compare matrices against the TRANSFAC matrix library what is this?						
Select a matrix -						
Use our example: (U00001;V\$CREB)						
Select a previously uploaded matrix: Select one						
O Upload a file: browse for file No file selected						
Data version: 2015.3 V						
Limit comparison to:						
Vertebrate Plant Insect Fungi						
compare matrices reset form						

Select your previously uploaded matrix from the pull-down menu, select the data version (use the default current version) and select whether you want to limit your comparison to vertebrate, plant, insect or fungal matrices. Click the "compare matrices" button. The screen will be refreshed and the results of the comparison will be provided in a table:

Result what is this?

Create and compare matrices

Create matrices | Compare matrices against TF library

1000001 V/CREP 01 M000000 P71P V/cm 1 10105 10	
000001 V\$CREB_01 M00039 BZIP VIEW 1.1913E-10	5.2096E-7
U00001 V\$ATF_B M00338 BZIP View 6.6155E-8	1.2175E-4
U00001 V\$CREBP1_Q2 M00179 BZIP View 8.3561E-8	1.2175E-4
U00001 V\$ATF2_Q6 M07312 BZIP View 1.5165E-7	1.6576E-4
U00001 V\$CREB1_01 M02279 BZIP View 4.8909E-7	3.8012E-4
U00001 V\$CREB_Q4 M00178 BZIP View 5.2214E-7	3.8012E-4
U00001 V\$CREB1_Q6 M03544 BZIP View 1.1678E-6	5.0606E-4
U00001 V\$CREB_Q4_01 M00917 BZIP View 1.2472E-6	5.0606E-4
U00001 V\$ATF2_Q5 M01862 BZIP View 1.2598E-6	5.0606E-4
U00001 V\$ATF1_03 M02738 BZIP View 1.3240E-6	5.0606E-4

Up to ten best matching matrices will be listed. Click the "View" link in the Alignment column to see a graphical alignment of the motifs:



Analyze a microarray or gene-based RNA-seq data set

Use TRANSFAC's step-by-step analysis workflow for gene-level microarray and RNA-seq data sets to identify the transcription factors that are most likely to be responsible for the differential expression pattern observed in your experimental data as compared to background data.

Select the "Step-by-step data analysis" options from the tools menu:



Then select the Gene-level microarray or RNA-seq data set option:



Specify whether your data is in the form of a pre-filtered list (only the subset of genes that were identified as up- or down-regulated is included) or whether you are uploading the complete data set including genes that showed no change in expression:



Upload your data set (in this example we are uploading the complete data set):

Step-By-Step Data Analysis what is this?
You've selected to analyze a gene-level microarray or RNA-seq data set
Now select the complete gene-level microarray or RNA-seq data set to be analyzed:
Upload a new data set browse for file
Enter gene set name
 Names Identifiers Which species? Homo sapiens
Synonym handling? Match synonyms if necessary 🔻
File contains column headers : 🔍 Yes 💿 No
Next

Note that once you've run at least one analysis you will have the additional option to select a previously uploaded data set instead of uploading a new data set.

You will be given a preview of the list of matched genes and then will be forwarded to a screen where you will specify the criteria for selecting the up-regulated, down-regulated and non-changed gene sets:



In this example, we have chosen to select all up-regulated genes which show a log2 fold change of >1 as our experimental set and to select all genes which show a log2 fold change of 0 as the non-changed set.

Note that if your data is uploaded in the form of a pre-filtered list this step will automatically be skipped.

Next you will specify the background set to use:

Step-By-Step Data Analysis what is this?
You've selected to analyze a gene-level microarray or RNA-seq data set
You've selected the experimental data set: HUVEC_GSE2639_all_8764_log2FC
Now select the background data set to be used in the analysis :
A non-change set from the experiment set
Randomly generated gene set
Previously uploaded gene set : Housekeeping_human_90
Upload a new background data set
Next

You have multiple options for the background set including:

- Using the non-change genes from the data set that you just uploaded, (the default, recommended option)
- Using a randomly generated gene set
- Using a previously uploaded gene set
- Uploading a new gene set

Note that if your data is in the form of a pre-filtered list you will not have the option to directly use the non-change genes from the data set as they were not included, you will only have the latter three options.

Next you will accept or change the default analysis parameters:

Step-By-Step Data Analysis what is this?
You've selected to analyze a gene-level microarray or RNA-seq data set
You've selected the experimental data set: HUVEC_GSE2639_all_8764_log2FC You've selected the background data set : non-change set
Now select :
A profile (group of matrices): vertebrate_non_redundant_minFP
Data version: 2015.3 🔻
P-value threshold: 0.01
Specify the nucleotide region of the gene's promoter to be analyzed : -500 to +100 A range of -10,000 to +1,000 bp is supported. Learn more
Run FMatch

You will:

- Specify the profile (group of matrices) to be used (use the default "vertebrate_non_redundant_minFP" which contains representative matrices for distinctive binding motifs unless you are working with non-vertebrate species or want to limit your analysis to factors that are active in a particular tissue)
- Specify the data version (use the default, current version)
- Specify the P-value threshold (0.01 is the default)
- Specify the region of the gene's promoter to be considered in the analysis (a range of -10,000 bp to +1,000 bp relative to the TSS is supported, but the default range of -500 bp to +100 bp relative to the TSS is recommended for most analyses)

When you click the "Run FMatch" button your analysis will be forwarded to the taskbar. Once the analysis completes, the FMatch Analysis Report will automatically load.

The "Matrix summary" section provides a tabular overview of all matrices that were found to have significantly more binding sites within your experimental data set than were found in the background data set:

Mati	rix summary								
First	📣 1 🍺 Las	t							
	Mark all on page (sele	ecting matrices will f	filter sequence summary) Filter	Profiles	Export tabl	e View factors		Hits on pag	je 5 🔻
Show	ing 3 of 3 entries								
#	Matrix	Factor name	Graph	Yes	No	Yes/No F	Matched promoters in Yes	Matched promoters in No	>>
#	Matrix V\$BBX_03	Factor name Bbx	Graph	Yes 0.0115	No 0.0023	Yes/No F 5.0000	Matched promoters in Yes 0.0077	Matched promoters in No 0.0023	>>
#	Matrix V\$BBX_03 V\$GLI_Q3	Factor name Bbx GLI	Graph	Yes 0.0115 0.0107	No 0.0023 0.0023	Yes/No F 5.0000 4.6667	Matched promoters in Yes 0.0077 0.0092	Matched promoters in No 0.0023 0.0023	>>

Scrolling down to the "Sequence details" section a graphical view of the results is provided:

equen	nce summary					
perimen	ntal set Background set					
First	📢 1 2 3 4 5 🐌 Last					
м	lark all on page Eilter Export table	logy View genes Eurotional analysis Netwo	rk analysis			Hits on page 5
Showi	ng 5 of 185 entries	ogy view genes i anedonar anarysis neewo				
#	Sequence	Promoter report	Matrices count 🗐	Sites count	Strand	Observation
	CXCL11_PM000589149 (chr4)	View promoter PM000589149	4	15	REVERSE	2.74
	1			_		
	HIVEP2_PM000636912 (chr6)	View promoter PM000636912	5	14	REVERSE	1.98
	ب					
					_	_

Click the Sequence name to display the nucleotide level view for a specific promoter:



A frequency bar displays the distribution of sites along the entire sequence, with the red orientation point and flanking yellow highlighting indicating the portion of the sequence that is displayed at the nucleotide level above. Slide the red orientation point along the frequency bar to view other portions of the sequence in detail.

Optionally use the ChIP fragments, DNase hypersensitivity sites and Phastcons intervals filters to apply additional biological context to your results. Activating these filters removes any sites that do not overlap with regions of sequence that are suggested to be available

to the transcriptional machinery based on being bound by transcription factors (ChIP fragments), being accessible to DNase enzymes (DNase hypersensitivity) or conserved between human and mouse (Phastcons).

Your analysis will be preserved in the taskbar for 7 days. To preserve it indefinitely, click the "Save this report" link at the top of the report.

Analyze a ChIP-seq data set

Use TRANSFAC's step-by-step analysis workflow for ChIP-seq data sets to identify the transcription factors that are most likely to be responsible for the differential expression pattern observed in your experimental data as compared to background data.

Select the "Step-by-step data analysis" options from the tools menu:

BIOB ASE Welcome to TRANSFAC	® logout		
BIOLOGICAL DATABASES Search -	tools -		Home taskbar my account help 🗸
	Gene regulation analysis tools		
✔ BIOBASE search ♥Hide search options Limit search to ● Genes and proteins ● Tran ● miRNAs ● Matr	Step-by-step data analysis Use this easy, step-by-step workflow for guided analysis of microarray and ChIP-seq data Predict TF binding sites Use the Match algorithm and TRANSFAC matrices to predict transcription factor	C eate and compare matrices C ate matrices from aligned sequences al compare to the TRANSFAC matrix ii rary Identify miRNA regulators Use the miRNA analysis too! to analyze genes for_the reservence of shared miRNA	example searches: name BRCA1 (optionally specify Human) identifier 672 (specify EntrezGene) BLASTp SKAVGDLGT YVSCVAHFAN DRVDIANDQ GNRTTPSFVA FTDTERLIGD AAKNQAA TF site R24252 or H5\$TLR2_02
Pathways Search genes and proteins by		binding sites	When you search Genes and proteins, you have the option to search by Name, Identifier, BLASTp or TF binding site.
Name Identifier BLASTP .	Functional analysis tools		synonyms of genes, proteins, and complexes.
Search which species? Search all species	View pathways and build networks View canonical signaling and metabolic pathways or build customized networks	Identify shared attributes Use the Functional analysis tool to analyze sets of genes or miRNAs for shared	Choose Identifier to limit your search to BIOBASE and 3rd party identifiers for genes and proteins. Specify the identifier type for a faster and more accurate search.
Upload a list of genes or proteins in I	Identify shared networks	Predict protein attributes	Choose BLASTp to search using up to five fasta- formatted sequences.
Genes and proteins search help	Use the Network analysis tool to analyze sets of genes for clusters of highly connected nodes	Use the BioKnowledge Transfer tool to assign predictive functions to uncharacterized protein sequences	Choose TF binding site to search for genes linked to TRANSFAC binding sites.
View statistics for: TRANSFAC 2014.3		anena becenzea protem sequences	Choose Upload a list of genes or proteins in bulk to search by multiple names or identifiers.

Then select the ChIP-seq data set option:



Specify whether your data is in the format of sequences (FASTA, EMBL, Genbank and RAW formats are supported) or .bed formatted genomic intervals:

Step-By-Ste	p Data Analysis 🐭	'hat is this?
You've selected t	to analyze a ChIP-seq data :	set
My data is in th	ne following format (cho	ose one):
	Sequences	Genomic intervals

Upload your data set (in this example we are uploading a set of genomic intervals):

Step-By-Step Data Analysis what is this?
You've selected to analyze a ChIP-seq data set
Now upload the intervals in BED format :
Opload a new interval set:
browse for file reset form
Name:
Genome: Human hg38/GRCh38 🔻
Next

Note that once you've run at least one analysis you will have the additional option to select a previously uploaded data set instead of uploading a new data set.

Next you will specify the background set to use:

Step-By-Step Data Analysis what is this?
You've selected to analyze a ChIP-seq data set You've selected the experimental genomic intervals: ChIP_JUN_exp595_K562_chr1
Now select the background sequence set to be used in the analysis :
Experimental genomic intervals shifted by 1000 bp
Randomly generated sequences
Use a previously uploaded interval set: ChIP_JUN_exp595_K562_chr1
O Upload a new interval set:
Next

You have multiple options for the background set including:

- Using the data set that you just uploaded, shifted by 1,000 bp (the default, recommended option)
- Using a randomly generated sequence set
- Using a previously uploaded interval set
- Uploading a new interval set

Note that if your data is uploaded as sequences you will not have the option to shift the data set by 1,000 bp, you will only the latter three options.

Next you will accept or change the default analysis parameters:

Step-By-Step Data Analysis what is this?
You've selected to analyze a ChIP-seq data set
You've selected the experimental genomic intervals: ChIP_ETS1_exp405_K562_hg19_chr1 You've selected the background sequence set : shifted genomic intervals
Now select :
A profile (group of matrices): vertebrate_non_redundant_minFP
Data version: 2015.2 🔻
P-value threshold: 0.01
Run FMatch

You will:

- Specify the profile (group of matrices) to be used (use the default "vertebrate_non_redundant_minFP" which contains representative matrices for distinctive binding motifs unless you are working with non-vertebrate species or want to limit your analysis to factors that are active in a particular tissue)
- Specify the data version (use the default, current version)
- Specify the P-value threshold (0.01 is the default)

When you click the "Run analysis" button your analysis will be forwarded to the taskbar. Once the analysis completes, the FMatch Analysis Report will automatically load.

The "Matrix summary" section provides a tabular overview of all matrices that were found to have significantly more binding sites within your uploaded data set than were found in the background data set:

Matrix summary										
First 📢 1 🕨 Last										
Mark all on page (selecting matrices will filter sequence summary) Filter Profiles Export table View factors Hits on page 5										
#	Matrix	Factor name	Graph	Yes	No	Yes/No 루	Matched promoters in Yes	Matched promoters in No	>>	
	V\$BBX_03	Bbx		0.0115	0.0023	5.0000	0.0077	0.0023		
	V\$GLI_Q3	GLI	.	0.0107	0.0023	4.6667	0.0092	0.0023		
	V\$CEBPA_Q6	C/EBPalpha		0.0667	0.0437	1.5263	0.0636	0.0429		

Scrolling down to the "Sequence details" section a graphical view of the results is provided:

eque	ence summary ental set Background set			
First	t 41 2 3 4 5 Last Mark all on page Filter Export table Ontolog wing 5 of 1305 entries	JY View genes Functional analysis Network analysis		Hits on page 5
#	Sequence	Promoter report	Matrices count =	Sites count
	FR036964578 (chr1)	View promoter PM000628922	2	3
		L		
	FR036963796 (chr1)		1	2

Click the Sequence name to display the nucleotide level view for a specific sequence:

Sequence detai Full sequence view	ls									ø
TAGCAGGAACTTG1 4712590 2247126	AATGTAGCCAGGA 00 224712610	GTTGAAAGGA 224712620	<u>GGAAATTCAA</u> 224712630	<u>GCAGAAAGA</u> 224712640	AGAGTGTCTAG 224712650	TATCAATATT 224712660	C/EBPalpha TTGTGCAAGA 224712670	TGCAGGTGAT 224712680	AATCAGACCCC 224712690	TGGGGACT 224712700
								_		
Viewing nucleotide	s 224712588 to 2: transcription factor (fi	24712705	DNase hypers	ensitivity sites	(filter)	Phastcons interva	ls (filter)			

A frequency bar displays the distribution of sites along the entire sequence, with the red orientation point and flanking yellow highlighting indicating the portion of the sequence that is displayed at the nucleotide level above. Slide the red orientation point along the frequency bar to view other portions of the sequence in detail.

Optionally use the ChIP fragments, DNase hypersensitivity sites and Phastcons intervals filters to apply additional biological context to your results. Activating these filters removes any sites that do not overlap with regions of sequence that are suggested to be available to the transcriptional machinery based on being bound by transcription factors (ChIP fragments), being accessible to DNase enzymes (DNase hypersensitivity) or conserved between human and mouse (Phastcons). Note that this option will not be available if you uploaded your data as a set of FASTA or other formatted sequences.

Your analysis will be preserved in the taskbar for 7 days. To preserve it indefinitely, click the "Save this report" link at the top of the report.

Analyze a transcript-based RNA-seq data set

Use TRANSFAC's step-by-step analysis workflow for transcript-level RNA-seq data sets to

identify the transcription factors that are most likely to be responsible for the differential expression pattern observed in your experimental data as compared to background data.

Select the "Step-by-step data analysis" options from the tools menu:



Then select the Transcript-level RNA-seq data set option:

I want to analyze (choose one): Gene-level microarray or RNA- seq data set	Step-By-Step Data Analysis what is this?									
	I want to analyze (choose one Gene-level microarray or RNA- seq data set): ChIP-seq data set	Transcript-level RNA-seq data set							

Specify whether your data is in the form of a pre-filtered list (only the subset of transcripts that were identified as up- or down-regulated is included) or whether you are uploading the complete data set including transcripts that showed no change in expression:

Step-By-Step Data Analysis what is this?									
You've selected to analyze a transcript-level RNA-seq data set									
Now upload the data set to be analyzed (choose the appropriate format) :									
	Pre-filtered transcript-level data set		Complete transcript-level data set						

Upload your data set (in this example we are uploading the complete data set):

Step-By-Step Data Analysis what is this?
You've selected to analyze a transcript-level RNA-seq data set
Now select the complete transcript-level RNA-seq data set to be analyzed:
Upload a new data set
browse for file
Enter gene set name
Identifiers
Ensembl v
File contains column headers : 🔘 Yes 💿 No
Next

Note that once you've run at least one analysis you will have the additional option to select a previously uploaded data set instead of uploading a new data set.

Note that with the initial release only Ensembl transcript identifiers are supported. You will be given a preview of the list of matched transcripts and then will be forwarded to a screen where you will specify the criteria for selecting the up-regulated, down-regulated and non-changed gene sets:

Step-By-Step Data Analysis what is this?								
You've selected to analyze a transcript-level RNA-seq data set								
You've selected the experimental data set: HEK gkq041								
Extract Up/Down/Non-change								
29.4955								
796 Rows: 852 Up: 23 Down: 0 NC: 439								
Select observation column: normalized expression (Fold change)								
 Select all upregulated elements: expression > 2.512575 Select all downregulated elements: expression < 0 								
Select non-changed elements: expression = $0.0 + - 0.15$								
Analyze Up and Down regulated sets in parallel								
Select								

In this example, we have chosen to select all up-regulated transcripts which show a normalized expression value of >2.51 as our experimental set and the set of transcripts which show a normalized expression value of 0 + - 0.15 as our non-changed transcript set.

Note that if your data is uploaded in the form of a pre-filtered list this step will automatically be skipped.

Next you will specify the background set to use:

Step-By-Step Data Analysis what is this?
You've selected to analyze a transcript-level RNA-seq data set
You've selected the experimental data set: HEK gkq041
Now select the background data set to be used in the analysis :
A non-change set from the experiment set
Randomly generated gene set
Previously uploaded gene set : Housekeeping_human_90
Upload a new background data set
Next

You have multiple options for the background set including:

- Using the non-change transcripts from the data set that you just uploaded, (the default, recommended option)
- Using a randomly generated transcript set
- Using a previously uploaded transcript set
- Uploading a new transcript set

Note that if your data is in the form of a pre-filtered list you will not have the option to directly use the non-change transcripts from the data set as they were not included, you will only have the latter three options.

Next you will accept or change the default analysis parameters:

You've selected to analyze a transcript-level RNA-seq data set
You've selected the experimental data set: HEK gkq041 You've selected the background data set : non-change set
Now select :
A profile (group of matrices): vertebrate_non_redundant_minFP
Data version: 2015.3 🔻
P-value threshold: 0.01
Specify the nucleotide region around the start of the transcript to be analyzed: -500 to +100 A range of -10,000 to +1,000 bp is supported. Learn more
Run FMatch

You will:

- Specify the profile (group of matrices) to be used (use the default "vertebrate_non_redundant_minFP" which contains representative matrices for distinctive binding motifs unless you are working with non-vertebrate species or want to limit your analysis to factors that are active in a particular tissue)
- Specify the data version (use the default, current version)
- Specify the P-value threshold (0.01 is the default)
- Specify the region surrounding the TSSs (determined by the Ensembl transcripts your data was mapped to) to be considered in the analysis (a range of -10,000 bp to +1,000 bp relative to the TSS is supported, but the default range of -500 bp to +100 bp relative to the TSS is recommended for most analyses)

When you click the "Run FMatch" button your analysis will be forwarded to the taskbar. Once the analysis completes, the FMatch Analysis Report will automatically load.

The "Matrix summary" section provides a tabular overview of all matrices that were found to have significantly more binding sites within your experimental data set than were found in the background data set:

Mati	rix summary								
First	📢 1 🍺 Las	t							
	Mark all on page (sele	ecting matrices will f	ilter sequence summary) Filter	Profiles	Export tabl	e View factors		Hits on pag	je <mark>5 ▼</mark>
Show	ving 3 of 3 entries								
#	Matrix								
	The state of the s	Factor name	Graph	Yes	No	Yes/No F	Matched promoters in Yes	Matched promoters in No	>>
	V\$BBX_03	Factor name Bbx	Graph	Yes 0.0115	No 0.0023	Yes/No F 5.0000	Matched promoters in Yes 0.0077	Matched promoters in No 0.0023	>>
	V\$BBX_03 V\$GLI_Q3	Factor name Bbx GLI	Graph	Yes 0.0115 0.0107	No 0.0023 0.0023	Yes/No F 5.0000 4.6667	Matched promoters in Yes 0.0077 0.0092	Matched promoters in No 0.0023 0.0023	>>

Scrolling down to the "Sequence details" section a graphical view of the results is provided, along with a summary of promoters and genes that are linked to the transcript:

eque perim	ence summary						
First	t 4 1 2 3 4 5 b La Mark all on page Filter Export table wing 5 of 852 entries	st Ontology View genes Functiona	ıl analysis Netw	rork analysis			Hits on page 5 v
#	Sequence	Promoter report	Gene	Matrices count F	Sites count	Strand	normalized expression (Fold change)
	ENST00000216122 (chr1)	View promoter PM000590233	Clorf110	6	9	REVERSE	0.743883
	ENST00000361874 (chr19)	View promoter PM000611093 View promoter PM000611094 View promoter PM000611092	BBC3	3	9	REVERSE	0.636014

Click the Sequence name to display the nucleotide level view for a specific transcript:

Sequenc Full sequence	e details										
											ø
											Sp1
BEN										Sp1	Sp1
GATCGCTG	erecceccec	cecceccecc	AGGCGCCCGC	TCGCATGTGG	CTCGCGCCGC	GTCTCAGGCC	GCCCGGCGGA	TCCCGGGCCC	GCTCCAAAGC	aeaaaaaaaa	aeaaaaaa
47231140	47231150	47231160	47231170	47231180	47231190	47231200	47231210	47231220	47231230	47231240	47231250
		<u>ج</u>	al a								_
Viewing nu	cleotides 472	31137 to 4723	31254								
Filter by:											
🗆 📒 ChIF	e: normal transcr	ription factor (filt	er)	DNase hyperse	nsitivity sites (fil	ter) 🔲 🗌 Pł	astcons intervals	s (filter)			

A frequency bar displays the distribution of sites along the entire sequence, with the red orientation point and flanking yellow highlighting indicating the portion of the sequence that is displayed at the nucleotide level above. Slide the red orientation point along the frequency bar to view other portions of the sequence in detail.

Optionally use the ChIP fragments, DNase hypersensitivity sites and Phastcons intervals filters to apply additional biological context to your results. Activating these filters removes

any sites that do not overlap with regions of sequence that are suggested to be available to the transcriptional machinery based on being bound by transcription factors (ChIP fragments), being accessible to DNase enzymes (DNase hypersensitivity) or conserved between human and mouse (Phastcons).

Your analysis will be preserved in the taskbar for 7 days. To preserve it indefinitely, click the "Save this report" link at the top of the report.