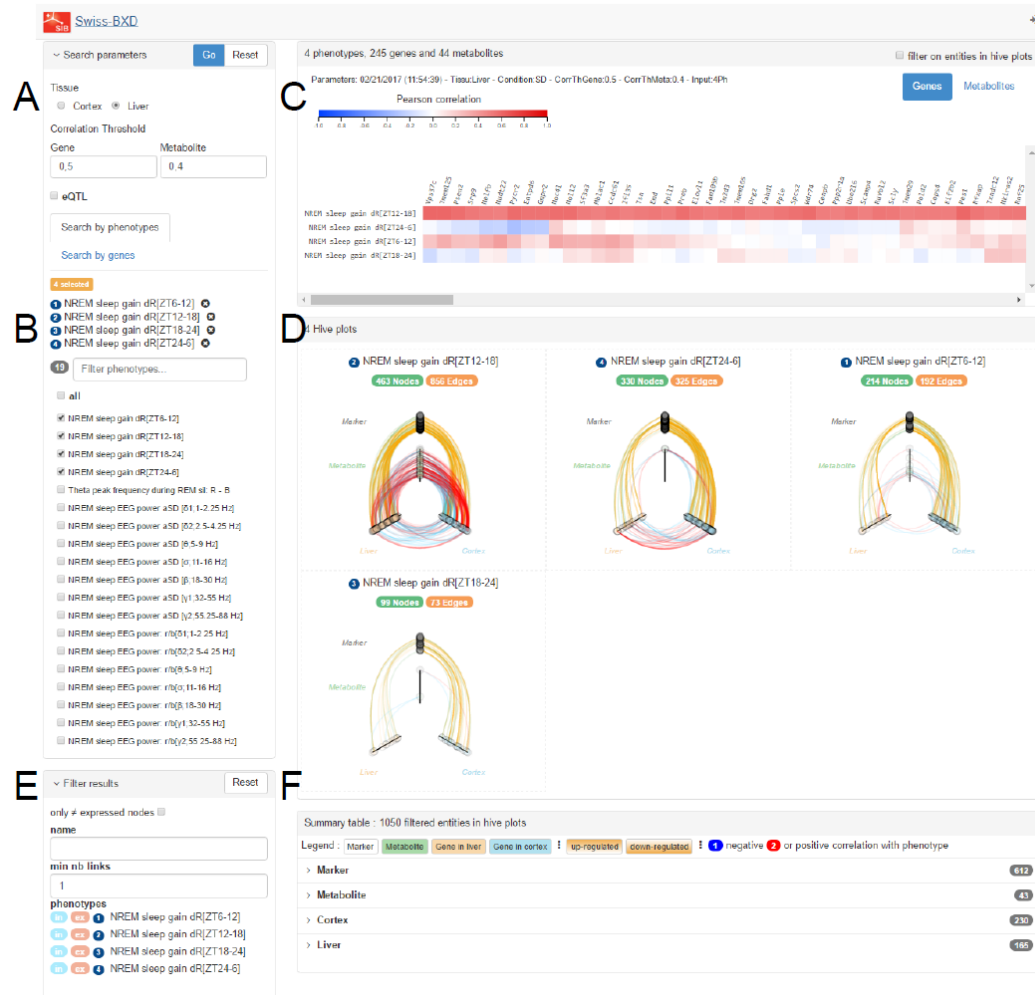


## BXD web interface informations:

Home page displays the information for the NREM sleep gain during the 24 hours (in four 6- hour intervals) after sleep deprivation.



**(A) Search parameters - Tissue & cutoff:** Sets the parameters for selection of genes in the specified tissue (cortex or liver) according to the specified cutoff for the pearson correlation coefficient and/ or a *cis*-eQTL q-value.

**(B) Search parameters - Phenotypes or genes:** One can search by phenotype(s) or by gene(s). A search by phenotype will output the genes that correlate to the submitted phenotype(s) with a correlation coefficient larger than the cutoff set in A). Search by gene(s) will output the most correlated phenotype(s) to the submitted gene(s).

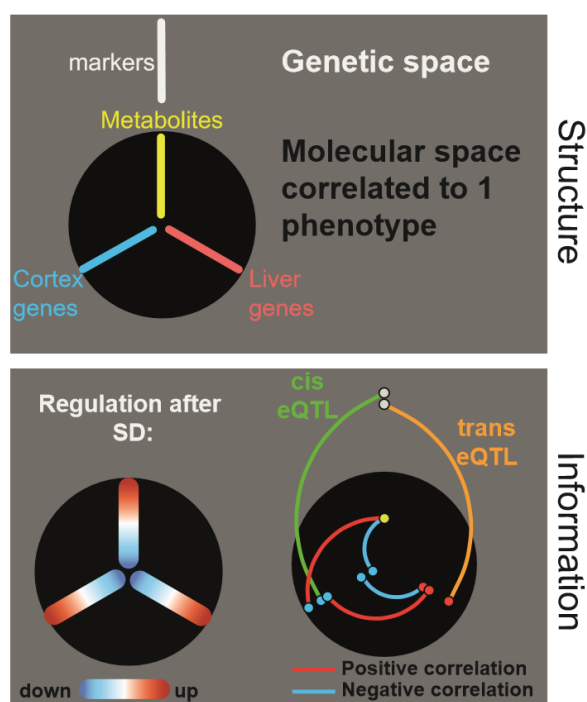
**(C) Results - Heatmap:** The results are displayed as heatmaps according to the selections made under Search parameters in A and B.

**(D) Results - Hiveplots:** For each phenotype present in the heatmap in C, the corresponding hiveplot is displayed.

**(E) Hiveplot-Filtering:** Filtering options specific for the hive plots.

**(F) Results – Table:** Tables list all genes, markers, and metabolites and their relations for each hiveplot in D.


### Hiveplots details:



Each plot represents one EEG/behavioral phenotype and its underlying associated molecular network; i.e., only the genes and metabolites strongly associated with a given phenotype are displayed. Each hiveplot is composed of 3 radial axes containing the molecular data with nodes assigned to the 2 bottom axis for genes expressed in the cortex (in blue) and liver (in red) and nodes on the vertical axis (in yellow) represent metabolites. On top, we added a separate 'genetic' axis (white) containing the genotypes. The node position on the 3 (molecular) radial axes was determined by the response to sleep deprivation; i.e., molecules closer to the center were down-regulated more strongly, while more upregulated genes/metabolites were closer to the axis' edge. Edges connecting nodes represent positive/negative correlations (red/blue, respectively) between molecules using expression values. Genetic markers linked to genes by eQTLs connect the genetic and molecular space.

### Notes:

- URLs can be easily send to share search options and results among users
- History of your search is kept during your working session


Swiss-BXD

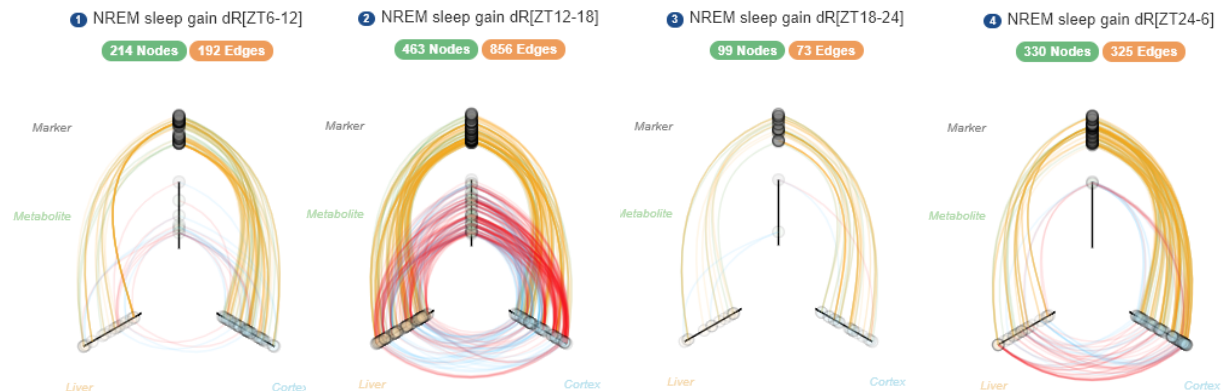
[Previous](#)
[Home](#)

11/07/2017 (16:35:35) - Tissu:Liver - Condition:SD - CorrThGene:0.5 - CorrThMeta:0.4 - Input:2Ph  
[Clear history](#)

## Tutorial 1. Single phenotype mining

In this section, we assume we are interested in a single sleep phenotype only. We will approach the data by multiple filtering steps.


### 1. Visual comparison of hiveplot:



Hive-plots for NREM sleep gain in 4 consecutive 6h recovery intervals after the sleep deprivation ending at ZT6. Compared to the other 3 intervals, NREM gain was strongly associated with metabolites during the 2nd 6h interval; i.e., the 1st 6h of the recovery dark period (ZT12-18). We will focus on this phenotype. These hiveplots (and phenotypes) are set as the [default](#) on the homepage.

### 2. Remove other phenotypes

The screenshot shows the search parameters interface. The 'Tissue' section has 'Cortex' and 'Liver' radio buttons, with 'Liver' selected. The 'Correlation Threshold' section has input fields for 'Gene' (0.5) and 'Metabolite' (0.4). The 'eQTL' section has a checkbox and two buttons: 'Search by phenotypes' and 'Search by genes'. Below the search parameters, a list of 4 selected phenotypes is shown: 1) NREM sleep gain dR[ZT6-12], 2) NREM sleep gain dR[ZT12-18], 3) NREM sleep gain dR[ZT18-24], and 4) NREM sleep gain dR[ZT24-6]. A red arrow points to the second phenotype, indicating it is the one to be kept.

Only the NREM sleep gain between ZT12-18 is kept. Other phenotypes can be [removed](#) clicking 

To apply filtering selection, press



### 3. Heatmap:



Now we only have a single hiveplot that we can explore further. The heatmap **(C)** displays all genes for which the expression is correlated to this sleep phenotype (note that liver is the default tissue). You can explore genes in cortex by changing the filtering parameters (see below). You can sort genes by correlation strength by clicking on the phenotype name in the heatmap. A similar heatmap can be displayed for the metabolites.



**Notes:** More genes are displayed in the hiveplot than in the heatmap. The heatmap contains all the genes that pass the search parameters selected. The hiveplot is filtered according to the following rules: i) the genes and metabolites must pass the pearsons r cutoff, ii) the nodes (genes/metabolites/markers) that are unconnected (no edges) are removed, and iii) the eQTL threshold is not applied in order to keep cross-tissue associations.

## 5. Save and highlight potential targets:

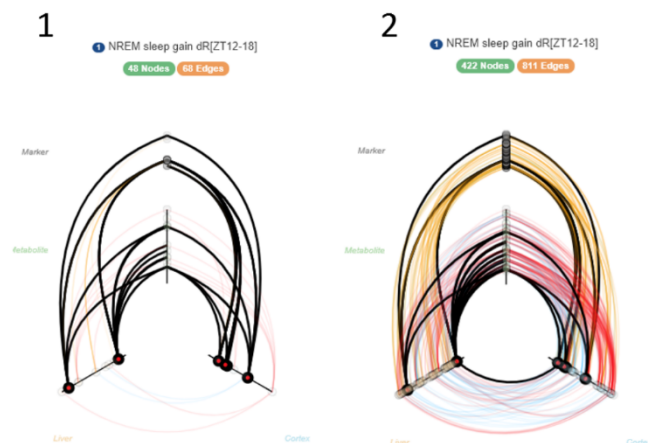
Once you applied your filtering parameters, you can save a target of interest to highlight it within the hiveplot. Double-clicking on genes within the heatmap will select the genes to highlight. A new tab will be created for each target saved. You can remove these saved targets by again double clicking the gene

within the heatmap or pressing  within the heatmap or pressing 

Here we highlight all genes that passed our filtering parameters:

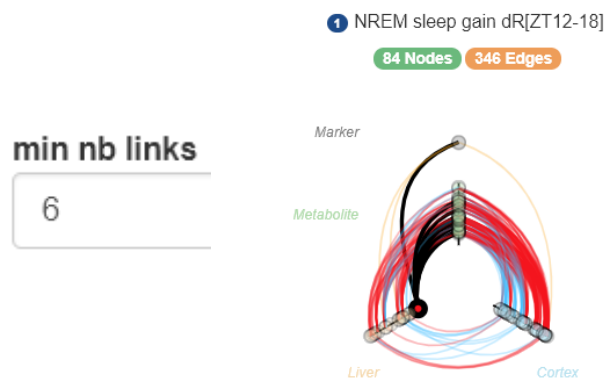


These genes can be highlighted in the filtered hiveplot **(1)** or the user can [reset](#) the filtering parameters **(2)**.



## 6. Hiveplot filtering:

Further filtering can be applied to the hiveplot. We can select for genes that have a minimum number of links (edges), e.g. 6 edges on our unfiltered hiveplot.



*Acot11* is the only highlighted gene that is kept after applying these filtering options. Other options are available, such as keeping only top differentially expressed genes in the selection:

only  $\neq$  expressed nodes ☒

## 7. Hiveplot investigation:

The hiveplots can be investigated using 'mouseover' the different edges and nodes. This will display the node name (gene name, metabolite name, or marker name), or the nodes connected to an edge. All nodes and edges within the displayed hiveplot are listed in the table (F).

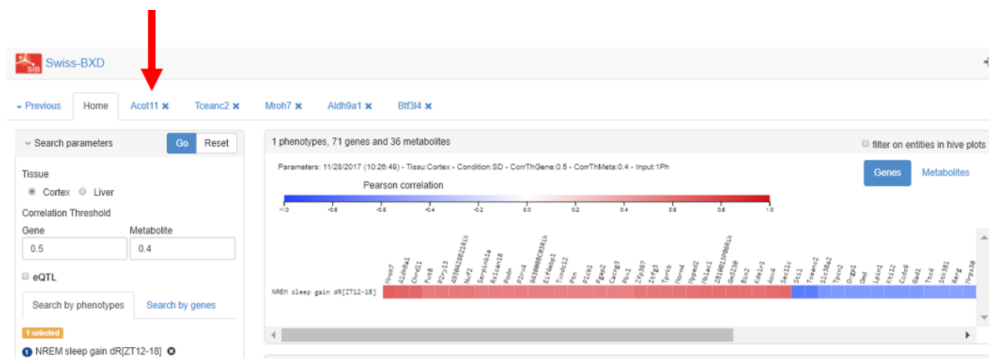
The table gives the following information: i) the type of node: genes in cortex (blue), genes in liver (orange), metabolites (green), and markers (white), ii) effects of sleep deprivation on genes and metabolites are indicated by fading color: upregulated (solid color on top, fading down to white), downregulated (solid color on bottom)

up-regulated    down-regulated

<b>Acot11</b>	Ovcp1	rs28135130	rs13477683	rs28150397	PC_aa_C36_2	PC_aa_C36_5	PC_aa_C38_0	PC_ae_C34_2
	PC_ae_C36_2	PC_ae_C36_3	PC_ae_C38_2	PC_ae_C38_3	PC_ae_C40_2	PC_ae_C40_3	PC_ae_C40_4	
	PC_ae_C40_6	PC_ae_C42_5						

Expression of *Acot11* in liver is downregulated after sleep deprivation and is highly correlated with 13 metabolites, one cortical transcript, and 3 SNPs. The correlation was positive for all (red symbol with phenotype number). Negative correlations would have been indicated in blue.

## 8. Target investigation:



The tab containing the highlighted genes shows additional information concerning gene function, the 5 genes with the highest partial-correlation, within the same tissue, across-tissue, and metabolite. The tab contains the gene name (1), the Uniprot description of the gene (2), Synonyms (3), a Uniprot and MGI access ID links (4) and related GO terms (5).

1

name

Acot11

2

description

Has acyl-CoA thioesterase activity towards medium (C12) and long-chain (C18) fatty acyl-CoA substrates.

3

Synonym

4

Uniprot

Q8VHQ9

4

MGI

MGI:1913736

5

GO functions

- acyl-CoA hydrolase activity
- carboxylic ester hydrolase activity
- lipid binding

5

GO process

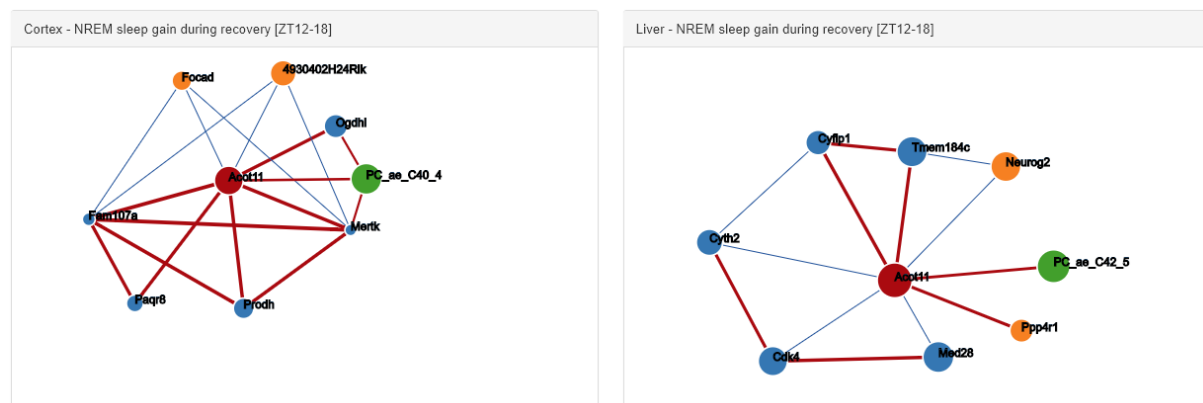
- acyl-CoA metabolic process
- fatty acid metabolic process
- intracellular signal transduction
- more...

5

GO component

- cytosol
- extracellular exosome

Here the network of the highlighted gene (*Acot11*) with the 5 top genes partially-correlated to within the same tissue (blue), 2 top genes partially-correlated to the highlighted gene within the other tissue (orange) and the top correlated metabolite (green). Red edges indicate positive correlation and blue edges indicates negative correlation. The size of the nodes indicates the correlation coefficient with the phenotype.



## Tutorial 2. Gene search:

The interface allows you also to search for specific genes associated with a sleep phenotype at a specific correlation cutoff.

If you previously made some filtering and research, you can reset your settings by pressing the RESET button.

▼ Search parameters Go Reset

Then select the relevant "search by genes" area

▼ Search parameters Go Reset

Tissue  
☐ Cortex ☒ Liver

Correlation Threshold

Gene  Metabolite

☒ eQTL

Search by phenotypes Search by genes

Select genes...

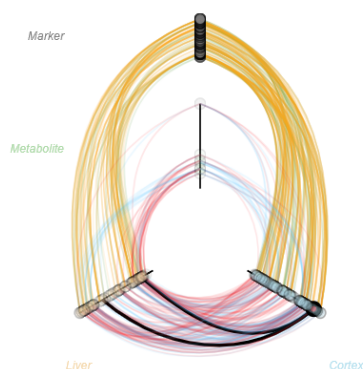
Below are 2 examples of sleep related genes:

1. [GWAS search for Per2](#): *Per2* is a well known circadian gene. It is associated in the BXD set with NREM sleep gain after SD [ZT12-18] ([Per2 BXD search](#)), and is associated with 2 metabolites: PC aa C36:3 & PC ae C40:0
2. *Sik3* KO mice were associated with the increase total of NREM sleep (minutes/24h) and a decrease in total wake in mouse by (Funato, Miyoshi et al. 2016) using random genetic screening. With our extended database, we found that: *Sik3* cortical expression in baseline was correlated ( $r=0.55$ ) with the wake amount at the beginning of light (BXDw24bsl.h1).

### Cortical Sik3 in baseline

1 BXDw24bsl quant out h1

573 Nodes 734 Edges





## Tutorial 3. Edges filtering:

Hiveplot filtering parameters allow to recover common edges among hiveplots or to exclude common edges.

The first example shows how to exclude common edges:

1. First we [select](#) phenotypes of EEG power after SD for all the bands.

7 selected

- 1 NREM sleep EEG power aSD [ $\delta$ 1;1-2.25 Hz] ✕
- 2 NREM sleep EEG power aSD [ $\delta$ 2;2.5-4.25 Hz] ✕
- 3 NREM sleep EEG power aSD [ $\theta$ ;5-9 Hz] ✕
- 4 NREM sleep EEG power aSD [ $\sigma$ ;11-16 Hz] ✕
- 5 NREM sleep EEG power aSD [ $\beta$ ;18-30 Hz] ✕
- 6 NREM sleep EEG power aSD [ $\gamma$ 1;32-55 Hz] ✕
- 7 NREM sleep EEG power aSD [ $\gamma$ 2;55.25-88 Hz] ✕

2. Here we are interested in slow delta power after sleep deprivation ( $\delta$ 1: 1.0-2.25Hz) during NREM sleep, and we want to remove all genes, metabolites that are present also within other bands. Therefore, we exclude other phenotypes using the “x” button in the hiveplot filtering options (E):

phenotypes

in	ex	1	NREM sleep EEG power aSD [ $\delta$ 1;1-2.25 Hz]
in	ex	2	NREM sleep EEG power aSD [ $\delta$ 2;2.5-4.25 Hz]
in	ex	3	NREM sleep EEG power aSD [ $\theta$ ;5-9 Hz]
in	ex	4	NREM sleep EEG power aSD [ $\sigma$ ;11-16 Hz]
in	ex	5	NREM sleep EEG power aSD [ $\beta$ ;18-30 Hz]
in	ex	6	NREM sleep EEG power aSD [ $\gamma$ 1;32-55 Hz]
in	ex	7	NREM sleep EEG power aSD [ $\gamma$ 2;55.25-88 Hz]

3. Finally, we keep only genes differentially expressed and genes connected to 3 other elements.

1 NREM sleep EEG power aSD [ $\delta$ 1;1-2.25 Hz]

6 Nodes 1 Edges

Marker

Metabolite

Liver

Cortex

only ≠ expressed nodes ☒

name

min nb links

3

phenotypes

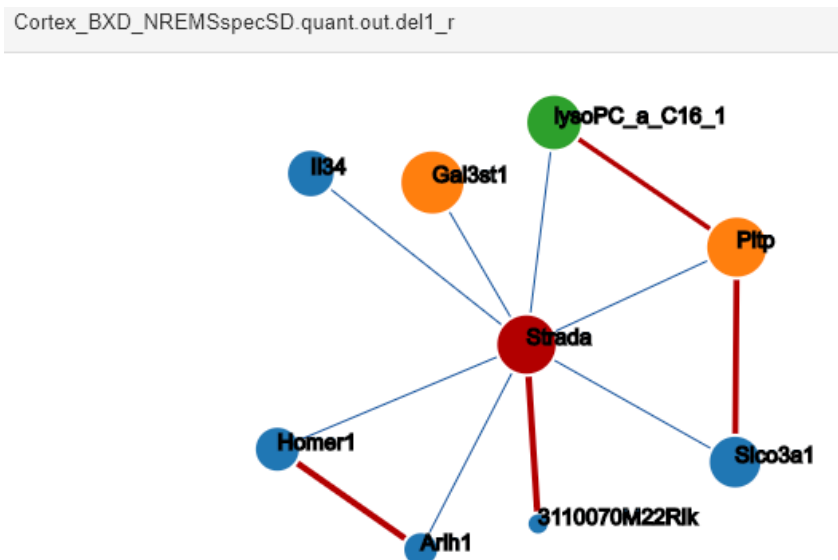
in	ex	1	NREM sleep EEG power aSD [ $\delta$ 1;1-2.25 Hz]
in	ex	2	NREM sleep EEG power aSD [ $\delta$ 2;2.5-4.25 Hz]
in	ex	3	NREM sleep EEG power aSD [ $\theta$ ;5-9 Hz]
in	ex	4	NREM sleep EEG power aSD [ $\sigma$ ;11-16 Hz]
in	ex	5	NREM sleep EEG power aSD [ $\beta$ ;18-30 Hz]
in	ex	6	NREM sleep EEG power aSD [ $\gamma$ 1;32-55 Hz]
in	ex	7	NREM sleep EEG power aSD [ $\gamma$ 2;55.25-88 Hz]

4. Remaining known genes are *Strada*, *Mapk4* and *Fhit* in cortex and *Susd4* in liver.

▼ Cortex	
A630072M18Rik	rs47567435  Arg  Lys
Strada	Gal3st1  Nqo1  rs31249441  rs31983887  rs32366561
Fhit	rs13482054  rs13474970  rs13480802  rs32031084  rs3658886  rs50922039
Mapk4	rs36351914  rs31550539  rs6161409
▼ Liver	
Susd4	rs13460889  rs13476092  rs217087290  rs33203145  rs46196875

These genes thus correlate only to slow delta and not to other EEG frequency bands of the EEG spectra during NREM sleep after sleep deprivation.

Inspection of *Strada* in cortex informed us that *Homer1* is among the 5 closest genes of *Strada*. *Homer1* has been implicated in the homeostatic regulation of EEG delta power.



The second example shows how to inspect common edges:

1. In this [case](#) we are interested in genes common to slow delta gain (r/b) during NREM sleep correlated within other delta bands.

- 1 NREM sleep EEG power aSD [ $\delta$ 1;1-2.25 Hz] ✖
- 2 NREM sleep EEG power aSD [ $\delta$ 2;2.5-4.25 Hz] ✖
- 3 NREM sleep EEG power: r/b[ $\delta$ 1;1-2.25 Hz] ✖
- 4 NREM sleep EEG power: r/b[ $\delta$ 2;2.5-4.25 Hz] ✖

2. Using the “in” button in the hiveplot filtering option (E) for slow delta gain after SD, the common edges and nodes within other bands will be kept. We also filter for genes differentially expressed after SD.

phenotypes

- in

ex

1

NREM sleep EEG power aSD [δ1;1-2.25 Hz]
- in

ex

2

NREM sleep EEG power aSD [δ2;2.5-4.25 Hz]
- in

ex

3

NREM sleep EEG power: r/b[δ1;1-2 25 Hz]
- in

ex

4

NREM sleep EEG power: r/b[δ2;2 5-4 25 Hz]

3. Inspecting the table (F), we see that only 1 gene is connected to 3 other bands. *Wrn* is present in the hiveplot 1, 2, and 3 [NREM sleep EEG power aSD δ1, NREM sleep power aSD δ2 and NREM sleep EEG power gain (r/b) δ1].

▼ Cortex

Prosc	rs32818308 1 6
Wtn	rs51740715 1 2 3
Tspan11	rs30384178 2 3   rs30065833 2 3   rs32857022 2 3   rs38187574 2 3   rs50472734 2 3
Lrp8	novel_0_133057853 3   rs51891312 3
EIF4ebp1	rs13404209 3
Chrm3	rs13479873 3   rs32257788 3
Fut10	rs38851632 3   rs32818574 3
Kif11	PC_wg_C30_2 3
Fam211a	rs13481062 3   Afl_10352113 3   rs13474399 3   rs13478287 3   rs29843540 3   rs8280784 3
Tbcd	novel_1_173258231 3 3   rs8288220 3 3
Cenpf	PC_wg_C30_2 3

