MathIOmica: Omics Analysis Tutorial

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MathIOmica is an omics analysis package designed to facilitate method development for the analysis of multiple omics in Mathematica, particularly for dynamics (time series/longitudinal data). This extensive tutorial follows the analysis of multiple dynamic omics data (transcriptomics, proteomics, and metabolomics from human samples). Various MathIOmica functions are introduced in the tutorial, including additional discussion of related functionality. We should note that the approach methods are simply an illustration of MathIOmica functionality, and should not be considered as a definitive approach. Additionally, certain details are included to illustrate common complications (e.g. renaming samples, combining datasets, transforming accessions from one database to another, dealing with replicates and Missing data, etc.).

After a brief discussion of data in MathIOmica, each example data (transcriptome, proteome and metabolome) are imported and preprocessed. Next a simulation is carried out to obtain datasets for each omics used to assess statistical significance cutoffs. The datasets are combined, and classified for time series patterns, followed by clustering. The clusters are visualized, and biological annotation of Gene Ontology (GO) and pathway analysis (KEGG: Kyoto Encyclopedia of Genes and Genomes) are finally considered.

N.B.1 For a more streamlined/simple example with less discussion please check out the tutorial on MathIOmica Dynamic Transcriptome.

N.B.2 We highly recommend the saving of intermediate results whenever possible. Some functions perform lengthy intensive computations and the performance may vary from system to system. Please use Put to save expressions to a file, and equivalently Get to recover these expressions.

Loading the MathIOmica Package

The functions defined in the `MathIOmica`` context provide support for conducting analyses of omics data (See also the MathIOmica Overview).

This loads the package:

In[1]:= `<< MathIOmica``

Also we can load MathIOmica as:

In[1]:= `Needs["MathIOmica`"]`

Data in MathIOmica

In this section we will discuss the data objects in use by MathIOmica, particularly the format of an OmicsObject. The data in the tutorial will be imported as an OmicsObject which is first described in this section. Then we present the example data included with MathIOmica. The example data will be imported in subsequent sections to illustrate analysis methods available in MathIOmica.

Data Format: OmicsObject

In MathIOmica the calculations utilize what we term an omics object (OmicsObject). An OmicsObject is an association of associations with some additional characteristics. It has an external (outer) association to denote samples and an internal (inner) association for annotation.

OmicsObject Structure

In an OmicsObject the outer association has M outer labels as keys, corresponding to M samples. Across the samples there are N inner labels (e.g. identifiers for genes/proteins), and inner labels are the same across samples. For a given j^{th} outer label, $OuterLabel_j$, the kth inner label, $InnerLabel_k$ has a value of :

$$InnerLabel_k \rightarrow \{\{Measurements_{jk}\}, \{Metadata_{jk}\}\}$$

OmicsObject structure:

```
<|OuterLabel₁ → <|InnerLabel₁ → {{Measurements₁₁}, {Metadata₁₁}},
    InnerLabel₂ → {{Measurements₁₂}, {Metadata₁₂}},
    InnerLabel₃ → {{Measurements₁₃}, {Metadata₁₃}},
    ...,
    InnerLabelₖ → {{Measurements₁ₖ}, {Metadata₁ₖ}},
    ...,
    InnerLabelₙ → {{Measurements₁ₙ}, {Metadata₁ₙ}}|>,
 OuterLabel₂ → <|InnerLabel₁ → {{Measurements₂₁}, {Metadata₂₁}},
    InnerLabel₂ → {{Measurements₂₂}, {Metadata₂₂}},
    InnerLabel₃ → {{Measurements₂₃}, {Metadata₂₃}},
    ...,
    InnerLabelₖ → {{Measurements₂ₖ}, {Metadata₂ₖ}},
    ...,
    InnerLabelₙ → {{Measurements₂ₙ}, {Metadata₂ₙ}}|>,
 ...,
 OuterLabelⱼ → <|InnerLabel₁ → {{Measurementsⱼ₁}, {Metadataⱼ₁}},
    InnerLabel₂ → {{Measurementsⱼ₂}, {Metadataⱼ₂}},
    InnerLabel₃ → {{Measurementsⱼ₃}, {Metadataⱼ₃}},
    ...,
    InnerLabelₖ → {{Measurementsⱼₖ}, {Metadataⱼₖ}},
    ...,
    InnerLabelₙ → {{Measurementsⱼₙ}, {Metadataⱼₙ}}|>,
 ...,
 OuterLabelₘ → <|InnerLabel₁ → {{Measurementsₘ₁}, {Metadataₘ₁}},
    InnerLabel₂ → {{Measurementsₘ₂}, {Metadataₘ₂}},
    InnerLabel₃ → {{Measurementsₘ₃}, {Metadataₘ₃}},
    ...,
    InnerLabelₖ → {{Measurementsₘₖ}, {Metadataₘₖ}},
    ...,
    InnerLabelₙ → {{Measurementsₘₙ}, {Metadataₘₙ}}|>
|>
```

For any j^{th} outer label, $OuterLabel_j$, it is possible that the mth inner label, $InnerLabel_m$ is missing and takes a Missing[] value in the form $InnerLabel_m \rightarrow Missing[]$. This can happen if the measurement was not performed for the sample, or no value was recorded (e.g. mass spectrometry data).

For example here is a list of 3 samples using protein identifiers (specifically, these are UniProt accessions). The measurements are relative intensities in this case and the metadata is the number of peptides per sample.

```
In[2]:= omicsObjectExample = ⟨|"FirstSample" → ⟨|{"A0AVT1"} → {{0.937}, {17}}, {"A0MZ66"} → {{1.059}, {9}},
         {"A1A4S6"} → {{1.03}, {11}}, {"A1L0T0"} → {{1.268}, {4}}, {"A0FGR8"} → Missing[]|⟩,
      "SecondSample" → ⟨|{"A0AVT1"} → {{1.003}, {17}}, {"A0MZ66"} → Missing[],
         {"A1A4S6"} → {{0.779}, {11}}, {"A1L0T0"} → {{0.917}, {4}}, {"A0FGR8"} → {{0.921}, {24}}|⟩,
      "ThirdSample" → ⟨|{"A0AVT1"} → {{1.064}, {19}}, {"A0MZ66"} → Missing[],
         {"A1A4S6"} → {{0.545}, {5}}, {"A1L0T0"} → Missing[], {"A0FGR8"} → {{0.87}, {23}}|⟩|⟩;
```

The outer labels of an OmicsObject are strings, while the inner labels are typically lists of strings.

Methods to Import Data as an OmicsObject

There are multiple methods to import data as an OmicsObject using MathIOmica. Four functions assist with importing data directly from text files:

(i) **DataImporter** provides a graphical dynamic interface that utilizes file headers to assist with the creation of OmicsObject variables from multiple files.

(ii) The **OmicsObjectCreator** function provides a function to create an OmicsObject from already existing/imported data in a Mathematica notebook.

(iii) **DataImporterDirect** and (iv) **DataImporterDirectLabeled** provide additional expert mode functions that may be used to directly import data as OmicsObject variables without a graphical interface.

`DataImporter` [*associationName*]	provides a graphical interface to extract data and create an **OmicsObject** variable *associationName* for associations of information.
`OmicsObjectCreator` [*outerLabels*, *innerLabels*, *measurements,metadata*]	creates an **OmicsObject** for use with MathIOmica. It uses the following inputs:

outerLabels	Outer labels (keys) for the OmicsObject.
innerLabels	Inner labels (keys) for identifiers in the OmicsObject.
measurements	List of measurements for each inner label.
metadata	List of metadata for each label.

`DataImporterDirect` [*positionsList*, *fileList*, *headerLines*]	*Expert Usage:* The **DataImporterDirect** function is a helper function originally created for **DataImporter**. **DataImporterDirect** [*positionsList*, *fileList*, *headerLines*] creates an **OmicsObject** importing the column number in *positionsList* from the *fileList* file path list, and importing data by skipping a number of *headerLines*.
`DataImporterDirectLabeled` [*sampleRules*, *fileList*, *headerLines*, *headerColumnAssociations*]	*Expert Usage:* The **DataImporterDirectLabeled** function creates an `OmicsObject` association for *variableName*, by imporing data from the files at the paths specified in the *fileList*, using the *sampleRules* as a label to column header imported rule for each file, and the *headerColumnAssociations* list of associations to associate column headers to column positions for each file.

Functions for importing/creating OmicsObject datasets.

Working with OmicsObject Data

An **OmicsObject** is an association of associations, and so **Query** can be used directly to access and manipulate components. MathIOmica also offers multiple functions that can implement computations and manipulation of an **OmicsObject**:

Applier[*function*, *inputData*]	applies *function* to OmicsObject, association or list *inputData* components.
ApplierList[*function*, *inputData*]	applies *function* to list of lists from an association, nested association or components or a matrix *inputData*.
ConstantAssociator[*inputAssociation*, *associationAddition*]	adds multi key constant to an OmicsObject (or an association of associations) *inputAssociation*, with each addition specified in a single association *associationAddition*, of form <|addition1→ Value1,addition2→ Value2,...|>.
CreateTimeSeries[*dataIn*]	creates a time series list across an OmicsObject *dataIn* using outer Keys for points.
EnlargeInnerAssociation[*omicsObjectList*]	combines a list of OmicsObject (associations of associations) *omicsObjectList* elements by enlarging the inner associations – inner association Keys must be different.
EnlargeOuterAssociation[*omicsObjectList*]	combines a list, *omicsObjectList*, of OmicsObject (or associations of associations) elements to a combined output by enlarging the outer associations – outer association keys must be different.
FilteringFunction[*omicsObject*,*cutoff*]	filters an OmicsObject data by a chosen comparison (by default greatr or equal) to a *cutoff*.
FilterMissing[*omicsObject*, *percentage*]	filters out data from *omicsObject* if across the datasets a *percentage* of data points is missing.
LowValueTag[*omicsObject*, *valueCutoff*]	takes an *omicsObject* and tags values in specified position as Missing[] based on provided *valueCutoff*.
MeasurementApplier[*function*,*omicsObject*]	applies a *function* to the measurement list of an *omicsObject*, ignoring missing values.
OmicsObjectMerge[{$omicsObject_1$,$omicsObject_2$, …},*f*]	merges a list of OmicsObject components {$omicsObject_1$,$omicsObject_2$, …}, using the function *f* to combine values with the same inner and outer keys.
OmicsObjectPairedMerge[$omicsObject_1$,$omicsObject_2$]	merges pairwise *omicsObject1* and *omicsObject2* values that have the same inner and outer keys.
Returner[*originalAssociation*, *update*]	returns a modified *originalAssociation* updated at a specified position by the single association *update*, e.g. from Applier or ApplierList result.

Functions for manipulating OmicsObject datasets.

Example Data

MathIOmica comes with multiple example data. The data can be found in the ConstantMathIOmicaExamplesDirectory :

> We can get a listing of the current example Data by evaluating:

```
In[3]:=  FileNames[__, ConstantMathIOmicaExamplesDirectory]
```

The data contains both initial (raw) data and additionally intermediate data that have been analyzed in MathIOmica and are used in the examples (**N.B.** *these files should **not** be altered or removed*). The dynamic raw datasets are from an integrative Personal Omics Profile as described below:

integrative Personal Omics Profiling (iPOP)	Data from the first integrative Omics Profiling (iPOP) is used comprised of dynamics from proteomics transcriptomics and metabolomics. The data corresponds to a time series analysis of omics from blood componenets from a single individual.
	Different samples (from 7 to 21 included here) were obtained at different time points. The time points included here correspond to days ranging from 186th to the 400th day of the study, (this can be represented in the following sample to day association: $\langle|7{\to}186,8{\to}255,9{\to}289,10{\to}290,11{\to}292,12{\to}294,13{\to}297,14{\to}301,15{\to}307,16{\to}311,17{\to}322,18{\to}329,19{\to}369,20{\to}380,21{\to}400|\rangle$. On day 289 the subject of the study had a Respiratory syncytial virus infection. Additionally, after day 301, the subject displayed high glucose levels and was eventually diagnosed with type 2 diabetes. The analyzed mapped data are used in these examples for illustrative purposes – these and additional dynamic omics data that will become available can also be accessed MathIOmica website at https://mathiomica.org. More information regarding the iPOP dataset can also be found in the original iPOP paper: Chen*, Mias*, Li – Pook – Than*, Jiang* et al., *Personal Omics Profiling Reveals Dynamic Molecular and Medical Phenotypes*. Cell 148 (6), p1293 (2012), PMID : 22 424 236. http : // dx.doi.org / 10.1016 / j.cell .2012 .02 .009. and related review (including summary): Mias and Snyder *Personal Genomes Quantitative Dynamic Omics and Personalized Medicine*. Quantitative Biology 1 (1) (2013), PMCID : PMC4366006. http : // dx.doi.org / 10.1007 / s40484 – 013 – 0005 – 3.

Example iPOP Set Description	*File Names located in the* ***ConstantMathIOmicaExamplesDirectory****.*
iPOP Transcriptome. The transcriptomic data included was obtained from mapping of the originally RNA Sequencing raw data using the Tuxedo suite. The data corresponds to transcriptome from peripheral blood mononuclear cells (PBMCs).	iPOP_ 07_genes.fpkm_tracking iPOP_ 08_genes.fpkm_tracking iPOP_ 09_genes.fpkm_tracking iPOP_ 10_genes.fpkm_tracking iPOP_ 11_genes.fpkm_tracking iPOP_ 12_genes.fpkm_tracking iPOP_ 13_genes.fpkm_tracking iPOP_ 14_genes.fpkm_tracking iPOP_ 15_genes.fpkm_tracking iPOP_ 16_genes.fpkm_tracking iPOP_ 17_genes.fpkm_tracking iPOP_ 18_genes.fpkm_tracking iPOP_ 19_genes.fpkm_tracking iPOP_ 20_genes.fpkm_tracking iPOP_ 21_genes.fpkm_tracking
iPOP Proteome. The Proteomics data from analysis of mass spectrometry data using the Sequest algorithm implemented by ProteomeDiscoverer. The data corresponds to proteome from PBMCs. The names of the files provide a correspondence of samples to Tandem Mass Tag labels in order of increasing m/z values from 126 to 131 amu. 6 TMT labels were used in each experiment. The data has been adapted from the original to UniProt accessions.	8_7_9_10_11_14_MulticonsensusReports_3Replicates.csv 8_12_13_15_16_14_MulticonsensusReports_3Replicates.csv 8_17_19_20_21_14_MulticonsensusReports_3Replicates.csv
iPOP Metabolome. The Metabolomics data from analysis of mass spectrometry data. The data corresponds to small molecule metabolomics from plasma ran with technical triplicates. The names of the files provide a correspondence of samples ran in positive or negative mode.	metabolomics_negative_mode.csv metabolomics_positive_mode.csv

Description of Example iPOP original datasets and corresponding files in the **ConstantMathIOmicaExamplesDirectory** . N.B. this table is provided as a reference for the examples, and these files should *not* be altered or removed.

Various analyzed datasets are used in the MathIOmica documentation for examples:

Data Description	*File Name(s) located in the* **`ConstantMathIOmicaExamplesDirectory`***.*
iPOP transcriptome imported as an OmicsObject across all timepoints.	rnaExample
iPOP proteome data imported as an OmicsObject across all timepoints.	proteinExample
iPOP metabolome data imported as an OmicsObject across all timepoints and technical replicates for negative and positive mode aligned mass spectrometry features.	metabolomicsNegativeModeExample metabolomicsPositiveModeExample
Example time series from proteomics.	proteinTimeSeriesExample
Example classification results from proteomics.	proteinClassificationExample
Example classification results from proteomics.	proteinClusteringExample
Example combined clustering results from transcriptome, proteome and metabolome data.	combinedClustersExample
Example enrichment analysis results for Gene Ontology and KEGG pathway analysis for combined omics data in this tutorial.	combinedGOAnalysis combinedKEGGAnalysis
Spectra from proteomics mass spectrometry data examples.	small.pwiz.1.1.mzML exampleMS3.mzXML

Description of example analyzed datasets and corresponding files in the **ConstantMathIOmicaExamplesDirectory** . N.B. this table is provided as a reference for the examples, and these files should *not* be altered or removed.

Transcriptome Data

In this section we import the example transcriptome iPOP dataset, and illustrate a preprocessing approach for this omic dataset.

Importing OmicsObject Transcriptome Data

We first import the transcriptomics data example (for details on how to import such data please refer to DataImporter , DataImporterDirect , DataImporterDirectLabeled and OmicsObjectCreator documentation).

We import the transcriptomics OmicsObject

In[66]:= `rnaExample = Get[FileNameJoin[{ConstantMathIOmicaExamplesDirectory, "rnaExample"}]]`

Out[66]=
⟨| 7 → ⟨| {FAM138A, RNA} → {{0}, {OK}}, {OR4F5, RNA} → {{0}, {OK}},
    {LOC729737, RNA} → {{2.73998}, {OK}}, ⬤ … 25 262 … , {LOC100507412, RNA} → {{0}, {OK}},
    {RNA45S5, RNA} → {{0}, {OK}}, {DUX4L, RNA} → {{0}, {OK}} |⟩,
  8 → ⟨| … 1 … |⟩, ⬤ … 11 … , 20 → ⟨| … 1 … |⟩, 21 → ⟨| … 1 … |⟩ |⟩

large output   **show less**   **show more**   **show all**   **set size limit…**

There are multiple samples given by the outer associations. We can use Query to get any data. For example we can get the outer keys:

In[67]:= `Query[Keys] @ rnaExample`

Out[67]= `{7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21}`

Notice that we have used "@" to form a Query using a prefix function application, which is used throughout the MathIOmica tutorials and documentation. This is the same as using the [ ] form:

In[68]:= `Query[Keys][rnaExample]`

Out[68]= `{7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21}`

We can get the expression raw data from any sample and entry. For example, the 10th and 14th entries in sample 12:

In[69]:= `Query["12", {7777, 55}] @ rnaExample`

Out[69]= ⟨| {NDNL2, RNA} → {{21.1197}, {OK}}, {ATAD3C, RNA} → {{0.560212}, {OK}} |⟩

The keys correspond to "Gene Symbols" and are also tagged with an "RNA" label. The values of all the keys/IDs correspond to `{{measurements}, {metadata}}`, and in this particular example `{{"FPKM" values}, {"FPKM status"}}`. Here, FPKM stands for Fragments Per Kilobase of transcript per Million mapped reads. The example is from mapped RNA-Sequencing data. FPKM is then a relative measure of transcript (gene) expression.

We can query all timepoints for a particular gene of interest if it exists. We must use the same labels as the actual keys of the OmicsObject:

In[70]:= `Query[All, Key@{"NFKBIB", "RNA"}] @ rnaExample`

Out[70]= ⟨| 7 → {{12.7644}, {OK}}, 8 → {{14.9997}, {OK}}, 9 → {{15.8482}, {OK}},
    10 → {{17.3504}, {OK}}, 11 → {{18.5309}, {OK}}, 12 → {{16.7081}, {OK}}, 13 → {{14.6549}, {OK}},
    14 → {{17.3951}, {OK}}, 15 → {{8.93065}, {OK}}, 16 → {{16.2545}, {OK}}, 17 → {{17.9217}, {OK}},
    18 → {{16.0331}, {OK}}, 19 → {{18.7293}, {OK}}, 20 → {{10.8115}, {OK}}, 21 → {{12.9051}, {OK}} |⟩

We note that we added Key@ before the bracket to indicate that this list is used as a key for the inner associations.

We can query all timepoints for multiple genes of interest if it exists. We must use the same labels as the actual keys of the OmicsObject:

```
In[71]:= Query[All, {Key@{"NFKBIB", "RNA"}, Key@{"NDNL2", "RNA"}}]@rnaExample
```

```
Out[71]= ‹|7 → ‹|{NFKBIB, RNA} → {{12.7644}, {OK}}, {NDNL2, RNA} → {{13.6201}, {OK}}|›,
         8 → ‹|{NFKBIB, RNA} → {{14.9997}, {OK}}, {NDNL2, RNA} → {{16.3813}, {OK}}|›,
         9 → ‹|{NFKBIB, RNA} → {{15.8482}, {OK}}, {NDNL2, RNA} → {{16.2763}, {OK}}|›,
        10 → ‹|{NFKBIB, RNA} → {{17.3504}, {OK}}, {NDNL2, RNA} → {{17.2483}, {OK}}|›,
        11 → ‹|{NFKBIB, RNA} → {{18.5309}, {OK}}, {NDNL2, RNA} → {{18.3254}, {OK}}|›,
        12 → ‹|{NFKBIB, RNA} → {{16.7081}, {OK}}, {NDNL2, RNA} → {{21.1197}, {OK}}|›,
        13 → ‹|{NFKBIB, RNA} → {{14.6549}, {OK}}, {NDNL2, RNA} → {{22.0412}, {OK}}|›,
        14 → ‹|{NFKBIB, RNA} → {{17.3951}, {OK}}, {NDNL2, RNA} → {{17.1224}, {OK}}|›,
        15 → ‹|{NFKBIB, RNA} → {{8.93065}, {OK}}, {NDNL2, RNA} → {{10.4774}, {OK}}|›,
        16 → ‹|{NFKBIB, RNA} → {{16.2545}, {OK}}, {NDNL2, RNA} → {{23.6771}, {OK}}|›,
        17 → ‹|{NFKBIB, RNA} → {{17.9217}, {OK}}, {NDNL2, RNA} → {{21.8782}, {OK}}|›,
        18 → ‹|{NFKBIB, RNA} → {{16.0331}, {OK}}, {NDNL2, RNA} → {{21.4414}, {OK}}|›,
        19 → ‹|{NFKBIB, RNA} → {{18.7293}, {OK}}, {NDNL2, RNA} → {{19.9134}, {OK}}|›,
        20 → ‹|{NFKBIB, RNA} → {{10.8115}, {OK}}, {NDNL2, RNA} → {{22.5756}, {OK}}|›,
        21 → ‹|{NFKBIB, RNA} → {{12.9051}, {OK}}, {NDNL2, RNA} → {{22.55}, {OK}}|›|›
```

Or in a more concise form

```
In[72]:= Query[All, Key[#] & /@ {{"NFKBIB", "RNA"}, {"NDNL2", "RNA"}}]@rnaExample
```

```
Out[72]= ‹|7 → ‹|{NFKBIB, RNA} → {{12.7644}, {OK}}, {NDNL2, RNA} → {{13.6201}, {OK}}|›,
         8 → ‹|{NFKBIB, RNA} → {{14.9997}, {OK}}, {NDNL2, RNA} → {{16.3813}, {OK}}|›,
         9 → ‹|{NFKBIB, RNA} → {{15.8482}, {OK}}, {NDNL2, RNA} → {{16.2763}, {OK}}|›,
        10 → ‹|{NFKBIB, RNA} → {{17.3504}, {OK}}, {NDNL2, RNA} → {{17.2483}, {OK}}|›,
        11 → ‹|{NFKBIB, RNA} → {{18.5309}, {OK}}, {NDNL2, RNA} → {{18.3254}, {OK}}|›,
        12 → ‹|{NFKBIB, RNA} → {{16.7081}, {OK}}, {NDNL2, RNA} → {{21.1197}, {OK}}|›,
        13 → ‹|{NFKBIB, RNA} → {{14.6549}, {OK}}, {NDNL2, RNA} → {{22.0412}, {OK}}|›,
        14 → ‹|{NFKBIB, RNA} → {{17.3951}, {OK}}, {NDNL2, RNA} → {{17.1224}, {OK}}|›,
        15 → ‹|{NFKBIB, RNA} → {{8.93065}, {OK}}, {NDNL2, RNA} → {{10.4774}, {OK}}|›,
        16 → ‹|{NFKBIB, RNA} → {{16.2545}, {OK}}, {NDNL2, RNA} → {{23.6771}, {OK}}|›,
        17 → ‹|{NFKBIB, RNA} → {{17.9217}, {OK}}, {NDNL2, RNA} → {{21.8782}, {OK}}|›,
        18 → ‹|{NFKBIB, RNA} → {{16.0331}, {OK}}, {NDNL2, RNA} → {{21.4414}, {OK}}|›,
        19 → ‹|{NFKBIB, RNA} → {{18.7293}, {OK}}, {NDNL2, RNA} → {{19.9134}, {OK}}|›,
        20 → ‹|{NFKBIB, RNA} → {{10.8115}, {OK}}, {NDNL2, RNA} → {{22.5756}, {OK}}|›,
        21 → ‹|{NFKBIB, RNA} → {{12.9051}, {OK}}, {NDNL2, RNA} → {{22.55}, {OK}}|›|›
```

We should also note that we can take advantage of Mathematica's native direct access to Wolfram Alpha, to look up any "Gene Symbol" information by evaluating (needs a network connection):

✴ NFKBIB

Here is an image of the output:

Assuming "NFKBIB" is a gene | Use as a protein instead
Assuming NFKBIB (human gene) | Use Nfkbib (mouse gene) or Nfkbib (rat gene) or NFKBIB (chimpanzee gene) instead

Input interpretation:
  NFKBIB (human gene)

Standard name:
  nuclear factor of kappa light polypeptide gene enhancer in B−cells inhibitor, beta

Alternate names: More
  IKBB | ikB−B | TRIP9 | TRIP−9 | …

Location: genome build 37

Location...   genome build 37 | ▾ ⊕

locus	chromosome 19	q13.1
strand	plus
coordinates	39 390 615 to 39 399 534

chromosome 19

0 Mb   30 Mb   60 Mb

Reference sequence: More ⊕

CCGGCAAAGCCCAGCTACAGGCGGGCGACTGCGGGGGGCC
… CCAGTTTAATAAACAAAACCCTAGTTCTGACAACCAGA

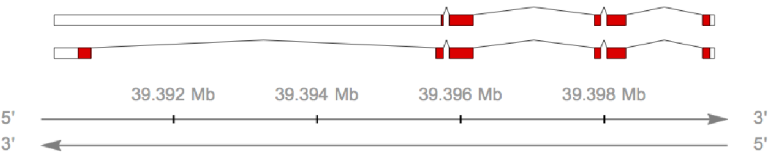**Reference sequence length:**   ⊕

8920 bp (base pairs)

Nearby genes: More | Show table ⊕

NFKBIB

(+ strand)   5' ──────────────── 3'
(− strand)   3' ──────────────── 5'
                   SIRT2
                        LOC643669

Gene splicing structures: Show legend | Show table ⊕

         39.392 Mb   39.394 Mb   39.396 Mb   39.398 Mb
5' ───────────────────────────────────────▶ 3'
3' ◀─────────────────────────────────────── 5'

Protein names: ⊕

NF−kappa−B inhibitor beta isoform 1 | NF−kappa−B inhibitor beta isoform 2

Protein molecular weight: ⊕

37.64 kDa (kilodaltons)

Gene Ontology characteristics: ⊕

Functions:
protein binding | signal transducer activity | transcription coactivator activity
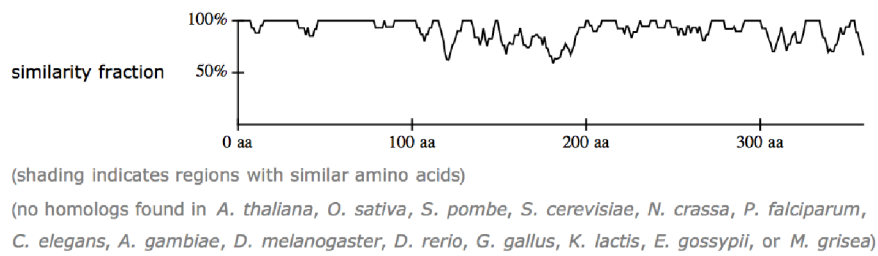
Locations:
cytosol | nucleus | cytoplasm

Processes:
transcription | signal transduction | cytoplasmic sequestering of NF−kappaB

Homologs across organisms: Show legend ⊕

NFKBIB (*H. sapiens*)
Nfkbib (*R. norvegicus*)
NFKBIB (*B. taurus*)
NFKBIB (*C. familiaris*)
NFKBIB (*P. troglodytes*)
Nfkbib (*M. musculus*)

(shading indicates regions with similar amino acids)

(no homologs found in *A. thaliana*, *O. sativa*, *S. pombe*, *S. cerevisiae*, *N. crassa*, *P. falciparum*, *C. elegans*, *A. gambiae*, *D. melanogaster*, *D. rerio*, *G. gallus*, *K. lactis*, *E. gossypii*, or *M. grisea*)

WolframAlpha

Processing Transcriptome Mapped Data

We will next preprocess the imported transcriptome data. We will first relabel the data, carry out quantile normalization and filtering and we will finally create time series.

Labeling, Normalization and Filtering

Re-labeling Samples with Times

First, we illustrate how to change the outer keys. In this example, we notice that the sample numberings do not correspond to actual days, so we may want to adjust the outer keys to correspond to real times.

We form an association between samples to actual days of the study:

```
In[73]:= sampleToDays =
    <|"7" → "186", "8" → "255", "9" → "289", "10" → "290", "11" → "292", "12" → "294", "13" → "297", "14" → "301",
     "15" → "307", "16" → "311", "17" → "322", "18" → "329", "19" → "369", "20" → "380", "21" → "400"|>;
```

We can now do a KeyMap to rename the outer keys:

```
In[74]:= rnaLongitudinal = KeyMap[sampleToDays, rnaExample]
```

```
Out[74]= ⟨| 186 → ⟨| {FAM138A, RNA} → {{0}, {OK}},
     {OR4F5, RNA} → {{0}, {OK}}, {LOC729737, RNA} → {{2.73998}, {OK}}, ⟨ ⋯ 25 262 ⋯ ⟩,
     {LOC100507412, RNA} → {{0}, {OK}}, {RNA45S5, RNA} → {{0}, {OK}}, {DUX4L, RNA} → {{0}, {OK}} |⟩,
    255 → ⟨| ⟨ ⋯ 1 ⋯ ⟩ |⟩, ⟨ ⋯ 11 ⋯ ⟩, 380 → ⟨ ⋯ 1 ⋯ ⟩, 400 → ⟨| ⟨ ⋯ 1 ⋯ ⟩ |⟩ |⟩
```

large output | show less | show more | show all | set size limit...

Quantile Normalization

QuantileNormalization[*data*]   performs quantile normalization of *data*.

QuantileNormalization can perform quantile normalization across various samples for multiple forms of data, including **OmicsObject** and matrix data.

We normalize the transcriptome data using the QuantileNormalization function. The Measurement is located in position 1, 1 in the list.

In[75]:= `rnaQuantileNormed = QuantileNormalization[rnaLongitudinal, ListIndex → 1, ComponentIndex → 1]`

Out[75]=
```
⟨|186 → ⟨|{FAM138A, RNA} → {{0.}, {OK}}, {OR4F5, RNA} → {{0.}, {OK}},
    {LOC729737, RNA} → {{2.2946}, {OK}},  ··· 25 262 ··· , {LOC100507412, RNA} → {{0.}, {OK}},
    {RNA45S5, RNA} → {{0.}, {OK}}, {DUX4L, RNA} → {{0.}, {OK}}|⟩,  ··· 13 ··· , 400 → ⟨| ··· 1 ··· |⟩|⟩
```
large output   **show less**   **show more**   **show all**   **set size limit…**

Tag Missing and Low Values

Next, we will tag values of less than 1 FPKM as Missing. Additionally, we will treat values of FPKM less than 5 as "noise" and set them all to a token value of 1.

LowValueTag[*omicsObject*, *valueCutoff*]	takes an *omicsObject* and tags values in specified position as Missing[] based on provided *valueCutoff*.

LowValueTag allows us to tag low values.

option name	default value	
ComponentIndex	1	Selection of which component of a list to use in the association or OmicsObject input values.
ListIndex	1	Selection of which list to use in the association or OmicsObject input values.
OtherReplacement	_Missing :> Missing[]	Replacement rule for any other kind of replacement in the data.
ValueReplacement	Missing[]	Value that specifies how tagged data points will be replaced.

Options for LowValueTag .

We first use LowValueTag to tag values of 0 as Missing[]:

In[76]:= `rnaZeroTagged = LowValueTag[rnaQuantileNormed, 0]`

Out[76]=
```
⟨|186 → ⟨|{FAM138A, RNA} → {{Missing[]}, {OK}}, {OR4F5, RNA} → {{Missing[]}, {OK}},
    {LOC729737, RNA} → {{2.2946}, {OK}},  ··· 25 263 ··· , {RNA45S5, RNA} → {{Missing[]}, {OK}},
    {DUX4L, RNA} → {{Missing[]}, {OK}}|⟩,  ··· 13 ··· , 400 → ⟨| ··· 1 ··· |⟩|⟩
```
large output   **show less**   **show more**   **show all**   **set size limit…**

We next use LowValueTag again to set all FPKM values <1 to unity:

In[77]:= `rnaNoiseAdjusted = LowValueTag[rnaZeroTagged, 1, ValueReplacement → 1]`

Out[77]=

⟨| 186 → ⟨| {FAM138A, RNA} → {{Missing[]}, {OK}}, {OR4F5, RNA} → {{Missing[]}, {OK}},
{LOC729737, RNA} → {{2.2946}, {OK}}, ⸱⸱⸱ 25 263 ⸱⸱⸱ , {RNA45S5, RNA} → {{Missing[]}, {OK}},
{DUX4L, RNA} → {{Missing[]}, {OK}} |⟩, ⸱⸱⸱ 13 ⸱⸱⸱ , 400 → ⟨| ⸱⸱⸱ 1 ⸱⸱⸱ |⟩ |⟩

large output   **show less**   **show more**   **show all**   **set size limit…**

Filter Data

We will next remove values that have been tagged as Missing[], retaining data that have at least 3/4 data points available across all samples. Here we use the function FilterMissing :

FilterMissing[*omicsObject*, *percentage*]	filters out data from *omicsObject*, retaining data across the datasets with a *percentage* of data points not missing.

FilterMissing allows the removal of data marked as Missing[], and retains only data with measurements available for a certain percentage of samples.

option name	default value	
MininumPoints	3	Minimum number of datapoints to keep.
Reference	{}	Select a reference outer key for which should remove dataset if the reference point has a **Missing** value.
ShowPlots	True	Whether to show summary plots.

Options for FilterMissing .

In this dataset we will use a reference point, day "255" which was a healthy measurement.

Hence, we filter out data where the reference point "255" is missing and retain data with at least 3/4 points available:

In[78]:= `rnaFiltered = FilterMissing[rnaNoiseAdjusted, 3/4, Reference → "255"]`

Number of Missing Data Points per Component
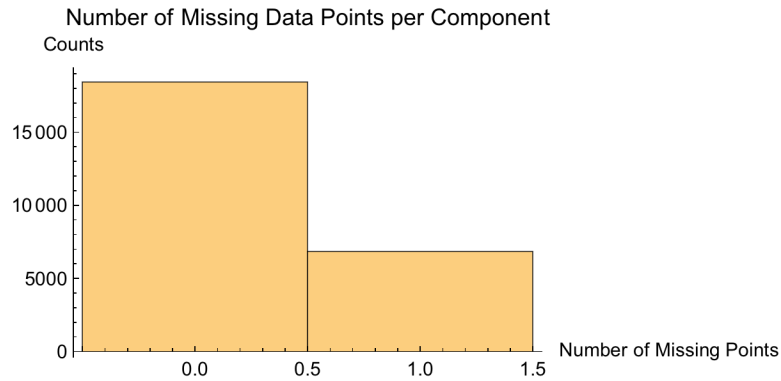
{Missing -> Counts: , ⟨|0 → 18 427, 1 → 6841|⟩ }

Pie Chart of number of missing components
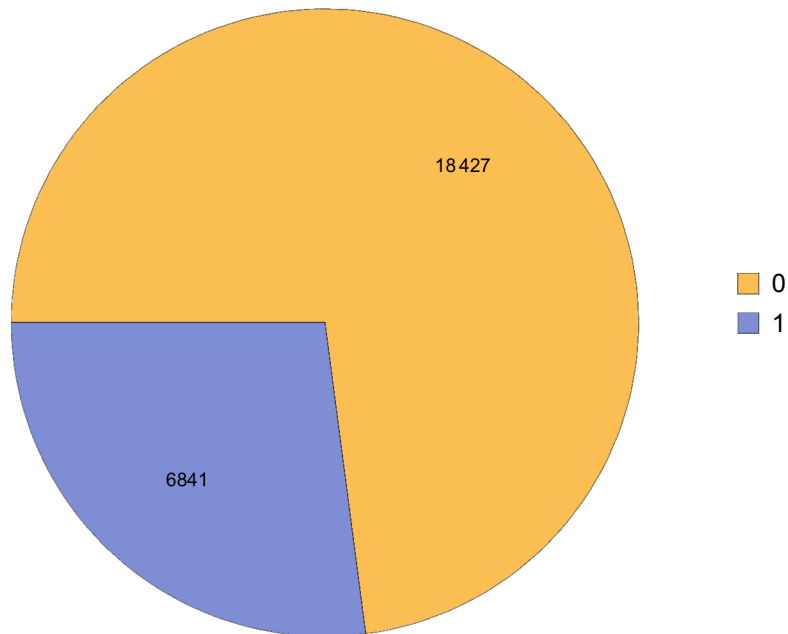
Out[78]= ⟨|186 → ⟨|{FAM138A, RNA} → {{Missing[]}, {OK}}, {OR4F5, RNA} → {{Missing[]}, {OK}},
{LOC729737, RNA} → {{2.2946}, {OK}}, ⸱⸱⸱ 25 263 ⸱⸱⸱ , {RNA45S5, RNA} → {{Missing[]}, {OK}},
{DUX4L, RNA} → {{Missing[]}, {OK}}|⟩, ⸱⸱⸱ 13 ⸱⸱⸱ , 400 → ⟨|⸱⸱⸱ 1 ⸱⸱⸱|⟩|⟩

large output   **show less**   **show more**   **show all**   **set size limit...**

Create Transcriptome Time Series

We can now create time series for each of the genes. MathIOmica provides functions to facilitate the process, such as **CreateTimeSeries** and

TimeExtractor . The functions assume an **OmicsObject** as an input for which times have been used as the sample labels (outer keys).

CreateTimeSeries[*omicsObject*]	creates a time series list across an OmicsObject using outer keys as times.
TimeExtractor[*omicsObject*]	extracts a list of sorted times from an OmicObject's outer keys.

We extract the times for the filtered RNA data using TimeExtractor:

In[79]:= `timesRNA = TimeExtractor[rnaFiltered]`

Out[79]= {186, 255, 289, 290, 292, 294, 297, 301, 307, 311, 322, 329, 369, 380, 400}

For each gene we now extract a time series (list of values) corresponding to these times:

In[80]:= `timeSeriesRNA = CreateTimeSeries[rnaFiltered]`

Out[80]=
```
⟨|{FAM138A, RNA} → {Missing[], 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1},
 {OR4F5, RNA} → {Missing[], 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1},
 {LOC729737, RNA} → {2.2946, 1, 4.67694, 4.48131, 4.95507, 1,
   1.25726, 2.14767, 1.93219, 1, 2.58217, 2.31301, 4.10284, 3.80929, 1.45471},
 {DDX11L1, RNA} → {5.91665, 4.32081, 3.19599, 3.64164, 2.7327, 2.13461, 2.17168,
   3.23429, 1.89576, 3.0267, 4.34004, 7.27001, 2.01132, 9.27701, 7.54415},
 ⋯ 25 260 ⋯ , {RNA5-8S5, RNA} → {Missing[], 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1},
 {LOC100507412, RNA} → {Missing[], 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1},
 {RNA45S5, RNA} → {Missing[], 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1},
 {DUX4L, RNA} → {Missing[], 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1}|⟩
```

large output	show less	show more	show all	set size limit…

Take Log Ratios Compared to Reference in Transcriptome Time Series

Next, we want to use log ratios of expression at any time point compared to a healthy datapoint.

SeriesApplier[*function,data*]	applies a given *function* to *data*, an association of lists, implementing masking for Missing values.

Applying a function to a series with Missing data.

We first use SeriesApplier to implement the logarithm:

In[81]:= `timeSeriesRNALog = SeriesApplier[Log, timeSeriesRNA]`

Out[81]=

```
⟨|{FAM138A, RNA} → {Missing[], 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
  {OR4F5, RNA} → {Missing[], 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
  {LOC729737, RNA} → {0.830556, 0, 1.54264, 1.49992, 1.60041, 0, 0.228935,
     0.764385, 0.658653, 0, 0.94863, 0.838548, 1.41168, 1.33744, 0.374807},
  {DDX11L1, RNA} → {1.77777, 1.46344, 1.1619, 1.29243, 1.00529, 0.758282, 0.775501,
     1.17381, 0.639619, 1.10747, 1.46788, 1.98376, 0.698792, 2.22754, 2.02077},
  ··· 25 260 ··· , {RNA5-8S5, RNA} → {Missing[], 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
  {LOC100507412, RNA} → {Missing[], 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
  {RNA45S5, RNA} → {Missing[], 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
  {DUX4L, RNA} → {Missing[], 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0}|⟩
```

large output   **show less**   **show more**   **show all**   **set size limit…**

Now we need to compare to use log ratios of expression at any time point compared to a healthy datapoint. We can use the function SeriesInternalCompare :

`SeriesInternalCompare[`*associationOfLists*`]`	compares each value in each list of *associationOfLists* to an internal reference value in the list, if the reference point itself is not **Missing**.

Comparing values in a series to an internal reference point in the series.

option name	default value	

`CompareFunction`	`(If[MatchQ[`
	`    Head[#2],`
	`    Missing],`
	`  Missing[],`
	`  (#1- #2)]&)`
		The function is used by a Query operation on non-missing input data. Namely:
		`Query[All,CompareFunction[`
		`   #,#[[ComparisonIndex]]]&]@`

`ComparisonIndex`	`1`	List position of list value that will be used as a reference data point.

`DeleteRule`	`{Head, Missing}`	`DeleteRule` allows the customization of how to select values for the reference data point for which its key should be deleted. The `DeleteRule` value takes the structure
		`deleteRuleOptionValue =   .`
		`  {MatchQ first argument,`
		`    MatchQ second argument}`
		The MatchQ function referred to here is implemented by SeriesInternalCompare internally, and uses the `deleteRuleOptionValue` as:
		`MatchQ[`
		`  deleteRuleOptionValue[[1]][`
		`    reference`
		`      comparison value],`
		`  deleteRuleOptionValue[[2]]]`
		The default removes the corresponding key if the value used for reference in the comparison is actually Missing, i.e. the comparison reference point has Head that matches Missing.

Options for **SeriesInternalCompare** .

We compare every value in each series to the healthy "255" time point, which is the second element in each series:

In[82]:= `rnaCompared = SeriesInternalCompare[timeSeriesRNALog, ComparisonIndex → 2]`

Out[82]=
```
⟨|{FAM138A, RNA} → {Missing[], 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
 {OR4F5, RNA} → {Missing[], 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
 {LOC729737, RNA} → {0.830556, 0, 1.54264, 1.49992, 1.60041, 0, 0.228935,
   0.764385, 0.658653, 0, 0.94863, 0.838548, 1.41168, 1.33744, 0.374807},
 {DDX11L1, RNA} → {0.314326, 0., -0.301545, -0.171011, -0.458154, -0.705162, -0.687943,
   -0.289634, -0.823824, -0.35597, 0.00444068, 0.520314, -0.764652, 0.764095, 0.557328},
  … 25260 … , {RNA5-8S5, RNA} → {Missing[], 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
 {LOC100507412, RNA} → {Missing[], 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
 {RNA45S5, RNA} → {Missing[], 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
 {DUX4L, RNA} → {Missing[], 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0}|⟩
```
large output   **show less**   **show more**   **show all**   **set size limit…**

Take the Norm and Remove Constant Transcriptome Time Series

Next, we normalize each series, using again SeriesApplier :

In[83]:= `normedRNACompared = SeriesApplier[Normalize, rnaCompared]`

Out[83]=
```
⟨|{FAM138A, RNA} → {Missing[], 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
 {OR4F5, RNA} → {Missing[], 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
 {LOC729737, RNA} → {0.218293, 0., 0.40545, 0.39422, 0.420632, 0., 0.0601705,
   0.200902, 0.173112, 0., 0.249326, 0.220394, 0.371029, 0.351517, 0.0985097},
 {DDX11L1, RNA} → {0.156411, 0., -0.150051, -0.0850959, -0.22798, -0.350893, -0.342324,
   -0.144124, -0.40994, -0.177133, 0.00220971, 0.258911, -0.380495, 0.380218, 0.27733},
  … 25260 … , {RNA5-8S5, RNA} → {Missing[], 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
 {LOC100507412, RNA} → {Missing[], 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
 {RNA45S5, RNA} → {Missing[], 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
 {DUX4L, RNA} → {Missing[], 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0}|⟩
```
large output   **show less**   **show more**   **show all**   **set size limit…**

ConstantSeriesClean [*dataIn*]   removes constant list series from an association of lists.

Removing constant series.

Finally, we use ConstantSeriesClean to remove constant series, as we are interested in changing time patterns:

In[85]:= `rnaFinalTimeSeries = ConstantSeriesClean[normedRNACompared]`

```
Removed series and returning filtered
  list. If you would like a list of removed keys run the
  command ConstantSeriesClean[data,ReturnDropped → True].
```

Out[85]=
```
⟨|{LOC729737, RNA} → {0.218293, 0., 0.40545, 0.39422, 0.420632, 0., … 3 … , 0.,
  0.249326, 0.220394, 0.371029, 0.351517, 0.0985097}, … 11782 … , {UTY, RNA} → { … 1 … }|⟩
```
large output   **show less**   **show more**   **show all**   **set size limit…**

Resampling Transcriptome Data

In addition to the above, we want to create a resampled distribution for the transcriptome dataset prior to classification and clustering. In this subsection we first resample the imported and labeled transcriptome dataset, Then, we carry out the full analysis in this "bootstrap" dataset, to create a set of random time series. This bootstrap distribution of time series will be used to provide the cutoffs used in the time series classification in the following subsection.

Resampling the Transcriptome Data

First, we use BootstrapGeneral :

`BootstrapGeneral[` *omicsObject*, *numberResampled*`]`	performs a resampling of the *omicsObject* data with replacement, and generates a new association structure with numbering corresponding to the *numberResampled* of new identities.

We can perform resampling of an OmicsObject to create a bootstrap dataset to be used for statistical considerations.

We create a resampling of 100000 sets:

In[86]:= `rnaBootstrap = BootstrapGeneral[rnaLongitudinal, 100 000]`

Out[86]= ⟨| 186 → ⟨| 1 → {{0}, {OK}}, 2 → {{0}, {OK}}, 3 → {{1.31474}, {OK}}, 4 → {{0}, {OK}}, 5 → {{3.2581}, {OK}},
   ⋯ 99 990 ⋯ , 99 996 → {{22.8169}, {OK}}, 99 997 → {{0}, {OK}}, 99 998 → {{4.57805}, {OK}},
   99 999 → {{0.00408523}, {OK}}, 100 000 → {{0.596732}, {OK}} |⟩, ⋯ 13 ⋯ , 400 → ⟨| ⋯ 1 ⋯ |⟩ |⟩ |⟩

large output   **show less**   **show more**   **show all**   **set size limit…**

Processing the Bootstrap Transcriptome and Creating Bootstrap Time Series

We normalize the transcriptome bootstrap data using the QuantileNormalization function:

In[87]:= `rnaBootstrapQuantileNormed = QuantileNormalization[rnaBootstrap, ListIndex → 1, ComponentIndex → 1];`

We use LowValueTag to tag zero values as Missing[]:

*In[88]:=* `rnaBootstrapZeroTagged = LowValueTag[rnaBootstrapQuantileNormed, 0];`

We next use LowValueTag again to set all FPKM values <1 to unity:

*In[89]:=* `rnaBootstrapNoiseAdjusted = LowValueTag[rnaBootstrapZeroTagged, 1, ValueReplacement → 1];`

Next, we filter out data where the reference point "255" is missing and retain data with at least 3/4 points available:

In[90]:= `rnaBootstrapFiltered = FilterMissing[rnaBootstrapNoiseAdjusted, 3 / 4, Reference → "255"]`

Number of Missing Data Points per Component

Counts

Number of Missing Points
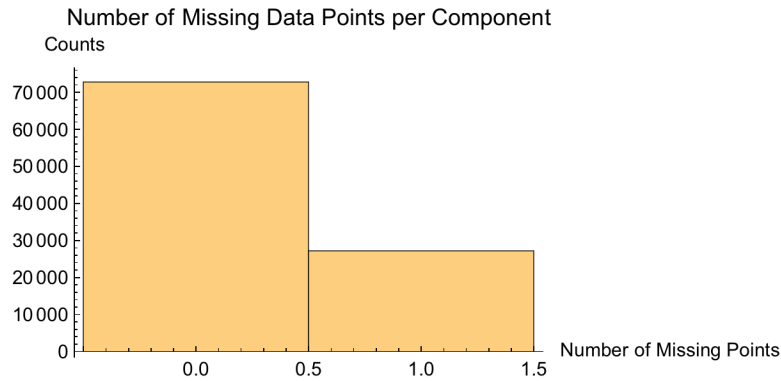
{Missing -> Counts: , ⟨|0 → 72 787, 1 → 27 213|⟩}

Pie Chart of number of missing components

72 787

27 213

☐ 0
☐ 1
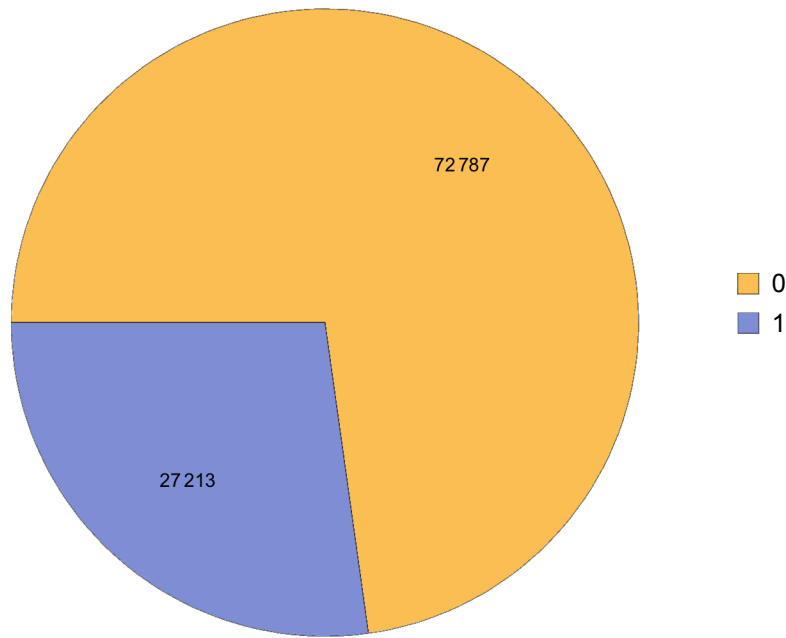
Out[90]=

⟨|186 → ⟨|1 → {{Missing[]}, {OK}}, 2 → {{Missing[]}, {OK}},
  3 → {{1.10552}, {OK}}, 4 → {{Missing[]}, {OK}}, ⋯ 99 992 ⋯ , 99 997 → {{Missing[]}, {OK}},
  99 998 → {{3.96839}, {OK}}, 99 999 → {{1}, {OK}}, 100 000 → {{1}, {OK}}|⟩,
  ⋯ 13 ⋯ , 400 → ⟨|1 → { ⋯ 1 ⋯ }, ⋯ 99 998 ⋯ , ⋯ 1 ⋯ |⟩ |⟩

large output   **show less**   **show more**   **show all**   **set size limit...**

For each bootstrap member we now extract a time series (list of values) corresponding to the series times:

In[91]:= `timeSeriesBootstrapRNA = CreateTimeSeries[rnaBootstrapFiltered]`

Out[91]=
```
⟨|1 → {Missing[], 2.81738, 1, 1, 1, 1, 28.4552, 1, 1, 1, 25.4077, 1, 17.9537, 3.95274, 1}, ⟨ ... 99998 ... ⟩,
  100000 → {1, 1.08859, 1, 1, 1, 1, 6.25135, ⟨ ... 18 ... ⟩, 6.18548, 5.81494, 1, 1, 3.91914, 1, 21.4263}|⟩
```
large output **show less** **show more** **show all** set size limit…

We use SeriesApplier to implement a logarithm:

In[92]:= `timeSeriesBootstrapRNALog = SeriesApplier[Log, timeSeriesBootstrapRNA]`

Out[92]=
```
⟨|1 → {Missing[], 1.03581, 0, 0, 0, 0, 3.34833, 0, 0, 0, 3.23505, 0, 2.8878, 1.37441, 0},
  ⟨ ... 99998 ... ⟩, 100000 → {0, 0.0848876, 0, 0, 0, 0, ⟨ ... 3 ... ⟩, 1.76043, 0, 0, 1.36587, 0, 3.06462}|⟩
```
large output **show less** **show more** **show all** set size limit…

We compare every value in each series to the healthy "255" time point, which is the second element in each series:

In[93]:= `rnaBootstrapCompared = SeriesInternalCompare[timeSeriesBootstrapRNALog, ComparisonIndex → 2]`

Out[93]=
```
⟨|1 → {Missing[], 0., -1.03581, -1.03581, -1.03581, -1.03581, 2.31252, -⟨ ... 19 ... ⟩,
  -⟨ ... 19 ... ⟩, -1.03581, 2.19925, -1.03581, 1.85199, 0.338604, -1.03581}, ⟨ ... 99999 ... ⟩|⟩
```
large output **show less** **show more** **show all** set size limit…

Next, we normalize each series, using again SeriesApplier :

In[94]:= `normedBootstrapRNACompared = SeriesApplier[Normalize, rnaBootstrapCompared]`

Out[94]=
```
⟨| ⟨ ... 1 ... ⟩ |⟩
```
large output **show less** **show more** **show all** set size limit…

Finally, we use ConstantSeriesClean to remove constant series, as we are interested in changing time patterns:

In[95]:= `rnaBootstrapFinalTimeSeries = ConstantSeriesClean[normedBootstrapRNACompared]`

```
  Removed series and returning filtered
     list. If you would like a list of removed keys run the
     command ConstantSeriesClean[data,ReturnDropped → True].
```

Out[95]=
```
⟨| ⟨ ... 1 ... ⟩ |⟩
```
large output **show less** **show more** **show all** set size limit…

Classification of Transcriptome Time Series

In this subsection we will classify the transcriptome time series based on patterns in the series. For the classification we will use TimeSeriesClassification .

TimeSeriesClassification[*data*, *setTimes*] takes a *data* association (or list of lists) of values corresponding to intensities collected over time and classifies the values into classes (groups) that show distinct similar temporal patterns. TimeSeriesClassification takes as inputs:

data	Association with series as values, or a list of series, where the series contain information regarding time intensities/observations. Each series may include Missing data points and may be entered as list of N signal intensities corresponding one–to–one to the N *setTimes* with Missing inserted appropriately if the data is absent, $\{X_1 = X(t_1),$. $X_2 = X(t_2), \ldots,$ $X_N = X(t_N)\}$ Alternatively, each series data may be a list of pairs of values $\{\{t_1, X_1\}, \{t_2, X_2\}, \ldots, \{t_N, X_N\}\}$ for only existing measurements.
setTimes	A global complete set of all possible N times during which all data series could have been collected in the window of the experiment, including times for which no values were reported or are missing, $\{t_1, t_2, \ldots, t_N\}$.

Classifying a set of time series based on temporal behavior.

option name	default value

AutocorrelationCutoffs	{0}	Cutoffs, for "Autocorrelation" and "InterpolatedAutocorrelation" methods, for different lags that will be used to filter out data series for which the lags are not within cutoffs. The list length corresponds to cuttofs at different lags, with the ith lag cutoff provided as the ith index, i.e. $\rho_c = \{\rho_{c1}, \rho_{c2}, \ldots, \rho_{ci}, \ldots, \rho_{jk}\}$ up to k, where $1 \leq k \leq n$, and typically $n = \text{Floor}[\text{Length}[\text{setTimes}] / 2]$. The classification will only consider lags up to the length of the list provided. The cutoffs are user–provided and typically calculated through simulation.
AutocorrelationLogic	False	Option to return the autocorrelation logic list for each signal, with the default set to False. If set to True, a logic vector is returned indicating whether or not at a particular lag the autocorrelation for a signal is above or below the AutocorrelationCutoffs.
AutocorrelationOptions	$\{\text{UpperFrequencyFactor} \to 1\}$	Options that are used by the internal Autocorrelation function in the case that the Method → "Autocorrelation" is set.
InterpolationDeltaT	"Auto"	Time step used to grid the time window over which calculations will be performed. If set to "Auto" the step will correspond to dividing the span of the interval into a number of equal steps equal to the number of input time points.
InterpolationOptions	{}	Options list for the internal Interpolation function used to interpolate between data points that have Missing values or uneven spacing.
LombScargleCutoff	0	Cutoff value for "LombScargle" method, for filtering the highest intensity observed in the power spectrum. The cutoff is user–provided and typically calculated through simulation.

LombScargleOptions	{PairReturn→ False, NormalizeIntens- ities→ True}	Options that are used by the internal LombScargle function if the case that the `Method → "LombScargle"` is set.
Method	"LombScargle"	Selection of which algorithm to use in the classification scheme.
ReturnAllSpikes	False	Option whether each signal may maintain unique membership to each spike class, or be allowed to belong to multiple classes. Used in "Autocorrelation" and "InterpolatedAutocorrelation" methods. If set to False, first spike maxima are classified, and only signals found not to belong to spike maxima are then considered for membership in the spike minima class.
ReturnData	True	If set to True will return input keys to data associations in the classification. If set to False will only return the keys of the input data in the classification.
ReturnModels	False	Whether to return the models as well as the classification information for the input data. The data is returned as an association with the key "TimeSeriesClasses" for classification groups and one of the following: (i) "Models" for model–based methods, (ii) "LombScargle" for periodograms in the "LombScargle" method, (iii) "Autocorrelations" for autocorrelation based methods.

| SpikeCutoffs | `<\|1 →{.99,-99},`
`  2 → {.99,-99}\|>` | Association with number, n, of data points as keys, and values corresponding to cutoffs, in the form
`<\|n → {Maximum Spike Cutoff_n,`
`        Minimum Spike Cutoff_n}\|>`
used to call spike maxima and minima for a time series with this number of datapoints. The values are provided by the user depending on data approach based on simulation. The default values are only place–holders and should be replaced by real values. The association must have corresponding keys for all lengths of input datasets, so that
`Keys[OptionValue[` , i.e. all `SpikeCutoffs]] ∈`
`  {Possible lengths of`
`        numeric data}.`
possible lengths of series constructed by excluding Missing or other non–numeric values). |

Options for **TimeSeriesClassification** .

TimeSeriesClassification uses multiple methods to classify data. The periodogram/autocorrelation methods used use cutoffs from simulation/user-provided values, to assess class membership based on statistical significance. In this tutorial we will use the "LombScargle" method, to classify data based on a Lomb-Scargle computation of a periodogram. The data is classified based into classes major (highest intensity) frequencies based on the generated periodogram for a signal, when the intensity of this frequency is above an intensity threshold cutoff. Additionally, data that displays spikey behavior in the real intensity, that is not classified into any frequency classes, is classified as a SpikeMaximum or SpikeMinimum if the spike is higher or lower respectively than what one would expect from a random signal.

Method	Description

`"LombScargle"`	Classification based on periodograms (power spectra) generated by a Lomb–Scargle computation as implemented internally by the **LombScargle** function. The data is classified into classes of major (highest intensity) frequencies and spikes (maxima or minima in real signal intensity), depending on cutoffs typically provided by simulation and passed to the function by the `LombScargleCutoffs` and `SpikeCutoffs` option values. The returned {computed classification vector} for this method is the intensity list of the periodogram for each signal.
`"Autocorrelation"`	Classification based on autocorrelations generated by a Lomb–Scargle approach using an inverser Fourier transform of spectral intensities, as implemented through the **Autocorrelation** function. The data is classified into autocorrelations at different lags and spikes (maxima or minima) classes, depending on cutoffs typically provided by simulation. The returned {computed classification vector} for this method is the autocorrelation list for each signal.
`"InterpolatedAutocorrelation"`	Classification based on autocorrelations generated directly in time, with **Missing** data handled through interpolation. The data is classified into autocorrelations at different lags and spikes (maxima or minima) classes depending on cutoffs typically provided by simulation. The returned {computed classification vector} for this method is the autocorrelation list for each signal.
`"TimeSeriesModelAggregate"`	Classification based on model fitting of time series through **TimeSeriesModelFit** and all available models therein. The data is classified into aggregate model classes. The returned {computed classification vector} for this method is the actual input signal.

`"TimeSeriesModelDetailed"`	Classification based on model fitting of time series through **TimeSeriesModelFit** and all available models therein. The data is classified into model classes based on individual model degree parameters. The returned {computed classification vector} for this method is the "BestFitParameters" for the model fit. If this list is empty an integer list is returned {token integer} – this is used in subsequent clustering applications.

Methods for **TimeSeriesClassification** .

To create the cutoffs for the classification we will first use the bootstrap time series set created in the previous subsection, and **QuantileEstimator** .

`QuantileEstimator` [*data*, *timepoints*]	obtains the quantile estimator following bootstrap for time series. It takes as inputs:	
	data	Association or list with series as values, from which to generate a distribution.
	timepoints	Timepoints over which the time series run.

Estimating the quantile value that can be used as a cutoff for classification of time series based on bootstrap simulations.

option name	default value	
AutocorrelationOptions	{}	Specific options when calculating autocorrelations for the time series.
InterpolationDeltaT	"Auto"	Time step used to grid the time window over which calculations will be performed. If set to "Auto" the step will correspond to dividing the span of the interval into a number of equal steps equal to the number of input time points.
InterpolationOptions	{}	Options list for the internal Interpolation function used to interpolate between data points that have Missing values or uneven spacing.
LombScargleOptions	{PairReturn → False, NormalizeIntens- ities→ True}	Specific options when calculating LombScargle periodograms for the time series.
Method	"LombScargle"	Method of calculation. Choices include one of the following: {"LombScargle","Autocorrelation", "InterpolatedAutocorrelation","Spikes"}
QuantileValue	0.95	Which quantile to extract.

Options for **QuantileEstimator** .

Depending on the cutoffs we would like to generate, we select the appropriate Method (also considering the Method that the downstream **TimeSeriesClassification** will use).

Method	Description
`"Autocorrelation"`	List of values corresponding to selected quantile of autocorrelations, with the ith lag quantile provided as the ith index, i.e. $\rho_c = \{\rho_{c1}, \rho_{c2}, ..., \rho_{ci}, ..., \rho_{ck}\}$ up to k lags, where $1 \leq k \leq n$, and typically n=Floor[Length[timepoints]/2]. The method utilizes the **Autocorrelation** function internally.
`"InterpolatedAutocorrelation"`	List of values corresponding to selected quantile for autocorrelations, with the ith lag quantile provided as the ith index, i.e. $\rho_c = \{\rho_{c1}, \rho_{c2}, ..., \rho_{ci}, ..., \rho_{ck}\}$ up to k lags, where $1 \leq k \leq n$, and typically n=(Length[timepoints]−1). The method utilizes an **Interpolation** followed by a **CorrelationFunction** implementation to compute autocorrelations, i.e. missing data or uneven sampling is handled by data interpolation.
`"LombScargle"`	Single value corresponding to selected quantile of maximum peak intensity of periodogram. The method utilizes the **LombScargle** function internally.
`"Spikes"`	Association with number, n, of data points as keys, and values corresponding to quantiles for maxima and minima of the series, in the form `<|n → {Maximum Spike Quantile`$_n$`, ` . The keys are `Maximum Spike Quantile`$_n$`}|>` generated automatically so that so that `Keys[output] ∈` , i.e. all `{Possible lengths of numeric data}.` possible lengths of input series constructed by excluding Missing or other non−numeric values).

Method selection and output for **QuantileEstimator** .

The default output for **TimeSeriesClassification** is an **Association** with outer keys being the classification classes, inner keys being the class members, and each class member value being a list of `{{computed classification vector}, {input data list}}`. The general output structure is for M output classes of each having m_1 members:

```
<| Class₁ → <|Member₁₁ → {{classification vector₁₁}, {input data vector₁₁}},
    Member₁₂ → {{classification vector₁₂}, {input data vector₁₂}}, ...,
    Member₁ₘ₁ → {{classification vector₁ₘ₁}, {input data vector₁ₘ₁}}|>,
  Class₂ → <|Member₂₁ -> {{classification vector₂₁}, {input data vector₂₁}},
    Member₂₂ -> {{classification vector₂₂}, {input data vector₂₂}}, ...,
    Member₂ₘ₂ → {{classification vector₂ₘ₂}, {input data vector₂ₘ₂}}|>, ...,
  Classₘ → <|Memberₘ₁ -> {{classification vectorₘ₁}, {input data vectorₘ₁}},
    Memberₘ₂ -> {{classification vectorₘ₂}, {input data vectorₘ₂}}, ...,
    Memberₘₘₘ → {{classification vectorₘₘₘ}, {input data vectorₘₘₘ}}|>|>
```

Before we classify our transcriptome data, we estimate for the "LombScargle" Method a 0.95 quantile cutoff from the bootstrap transcriptome data:

In[96]:= `q95RNA = QuantileEstimator[rnaBootstrapFinalTimeSeries, timesRNA]`

Out[96]= `0.860232`

Next, we estimate the "Spikes" 0.95 quantile cutoff from the bootstrap transcriptome data:

In[97]:= `q95RNASpikes = QuantileEstimator[rnaBootstrapFinalTimeSeries, timesRNA, Method → "Spikes"]`

Out[97]= `⟨|14 → {0.884016, -0.348069}, 15 → {0.858813, -0.337635}|⟩`

Now we can classify the transcriptome time series data based on these cutoffs:

In[98]:= `rnaClassification = TimeSeriesClassification[rnaFinalTimeSeries,`
`    timesRNA, LombScargleCutoff → q95RNA, SpikeCutoffs → q95RNASpikes]`

> `Method → "LombScargle"`

Out[98]=
`⟨|SpikeMax → ⟨| … 1 … |⟩, … 7 … ,`
`  f7 → ⟨|{MIR6723, RNA} → {{0.214503, 0.000338299, 0.0390479, 0.0653206, 0.291434, 0.336712, 0.865961},`
`    { … 1 … }}, … 59 … , {DNASE1L1, RNA} → { … 1 … }|⟩|⟩`

large output   **show less**   **show more**   **show all**   **set size limit…**

The default output for TimeSeriesClassification is an Association with outer keys being the classification classes, inner keys being the class members, and each class member value being a list of `{{computed classification vector}, {input data list}}`. The general output structure is for M output classes of each having m_i members:

`⟨| Class₁ → ⟨|Member₁₁ → {{classification vector₁₁}, {input data vector₁₁}},`
`    Member₁₂ → {{classification vector₁₂}, {input data vector₁₂}}, …,`
`    Member₁ₘ₁ → {{classification vector₁ₘ₁}, {input data vector₁ₘ₁}}|⟩,`
` Class₂ → ⟨|Member₂₁ -> {{classification vector₂₁}, {input data vector₂₁}},`
`    Member₂₂ -> {{classification vector₂₂}, {input data vector₂₂}}, …,`
`    Member₂ₘ₂ → {{classification vector₂ₘ₂}, {input data vector₂ₘ₂}}|⟩, …,`
` Classₘ → ⟨|Memberₘ₁ -> {{classification vectorₘ₁}, {input data vectorₘ₁}},`
`    Memberₘ₂ -> {{classification vectorₘ₂}, {input data vectorₘ₂}}, …,`
`    Memberₘₘₘ → {{classification vectorₘₘₘ}, {input data vectorₘₘₘ}}|⟩|⟩`

If we want the classes produced, we can query the keys:

In[99]:= `Keys[rnaClassification]`

Out[99]= `{SpikeMax, SpikeMin, f1, f2, f3, f4, f5, f6, f7}`

For the number of members in each class we have:

In[100]:= `Query[All, Length] @rnaClassification`

Out[100]= `⟨|SpikeMax → 822, SpikeMin → 5963, f1 → 116, f2 → 3, f3 → 30, f4 → 128, f5 → 35, f6 → 13, f7 → 61|⟩`

We can obtain the membership list in any class of interest:

```
In[101]:= Query["f1", Keys] @rnaClassification
```

```
Out[101]= {{PADI4, RNA}, {AHDC1, RNA}, {CCDC28B, RNA}, {AGO1, RNA}, {JAK1, RNA}, {C1orf52, RNA}, {IARS2, RNA},
          {URB2, RNA}, {HSPA14, RNA}, {WBP1L, RNA}, {PDZD8, RNA}, {LOC102288414, RNA}, {TRMT112, RNA},
          {DRAP1, RNA}, {CDK2AP2, RNA}, {FAM168A, RNA}, {FLI1, RNA}, {EFCAB4B, RNA}, {EMG1, RNA}, {NDUFA12, RNA},
          {ELK3, RNA}, {SSH1, RNA}, {C12orf49, RNA}, {IPO5, RNA}, {TMCO3, RNA}, {KIAA0586, RNA}, {JKAMP, RNA},
          {PCNX, RNA}, {EHD4, RNA}, {CLPX, RNA}, {AAGAB, RNA}, {RCCD1, RNA}, {FAM173A, RNA}, {LINC00921, RNA},
          {ZC3H7A, RNA}, {GLG1, RNA}, {MBTPS1, RNA}, {TNFRSF13B, RNA}, {ZNF207, RNA}, {ACLY, RNA}, {PSME3, RNA},
          {TEX2, RNA}, {PRKCA, RNA}, {ATP9B, RNA}, {MBP, RNA}, {ADNP2, RNA}, {HOOK2, RNA}, {EMR3, RNA},
          {SDHAF1, RNA}, {ZNF529, RNA}, {RPL18, RNA}, {CTU1, RNA}, {GEMIN6, RNA}, {GMCL1, RNA}, {COA5, RNA},
          {MRPS9, RNA}, {GTF3C3, RNA}, {NDUFS1, RNA}, {RALGAPA2, RNA}, {LOC284801, RNA}, {SAMHD1, RNA},
          {SERINC3, RNA}, {USP25, RNA}, {RUNX1, RNA}, {DSCR3, RNA}, {THAP7, RNA}, {MAPK1, RNA}, {PITPNB, RNA},
          {EWSR1, RNA}, {NPTXR, RNA}, {TCF20, RNA}, {ARPC4, RNA}, {STT3B, RNA}, {SNRK-AS1, RNA}, {CCDC12, RNA},
          {PRKAR2A, RNA}, {GSK3B, RNA}, {PTPLB, RNA}, {DNAJC13, RNA}, {LRCH3, RNA}, {KLF3, RNA}, {ANTXR2, RNA},
          {GPRIN3, RNA}, {INPP4B, RNA}, {PTGER4, RNA}, {NNT, RNA}, {CCDC125, RNA}, {POC5, RNA}, {ERAP1, RNA},
          {TBC1D22B, RNA}, {HACE1, RNA}, {SYNJ2, RNA}, {CYTH3, RNA}, {STAG3L1, RNA}, {STAG3L3, RNA},
          {MTERF, RNA}, {MBLAC1, RNA}, {TRIM56, RNA}, {AHCYL2, RNA}, {RNF122, RNA}, {ADAM9, RNA}, {PRKDC, RNA},
          {AGO2, RNA}, {ERMP1, RNA}, {KDM4C, RNA}, {FOCAD, RNA}, {CEP78, RNA}, {RC3H2, RNA}, {GTF3C4, RNA},
          {ZRSR2, RNA}, {PDK3, RNA}, {CASK, RNA}, {DDX3X, RNA}, {TIMP1, RNA}, {ARHGEF6, RNA}, {IDS, RNA}}
```

We may also want to know what these frequencies correspond to. The "LombScargle" method uses a LombScargle transformation.

`LombScargle`[*data, setTimes*]	calculates the Lomb–Scargle power spectrum for time series *data* that runs over specified *setTimes*. It takes as input:

data	Time series (data as a list; list may be the value of a single key in an association). The series may include Missing data points. Data may be entered as list of N signal intensities corresponding one–to–one to the N setTimes with Missing inserted appropriately if the data is absent, $\{X_1=X(t_1),$ $\quad . $ $X_2=X(t_2),...,$ $X_N=X(t_N)\}$ Alternatively, the data may be a list of pairs of values $\{\{t_1,X_1\},\{t_2,X_2\},...,\{t_N,X_N\}\}$ for only existing measurements.
setTimes	A complete set of all possible N times during which data could have been collected in the window of the experiment, including times for which no data was collected, $\{t_1, t_2, ..., t_N\}$.

Calculating the power spectrum of a (possibly unevenly sampled) time series.

option name	default value	
FrequenciesOnly	False	Whether to return only the computation frequencies. An association of frequencies "f" ordered from low to high by index i is returned in the form: $<\vert$ "f1" $\rightarrow$ frequency$_1$, "f2" $\rightarrow$ frequency$_2$, ..., "fi" $\rightarrow$ frequency$_i$,..., "fn" $\rightarrow$ frequency$_n$ $\vert>$
NormalizeIntensities	False	Whether the intensities list should be normalized or not.
OversamplingRate	1	Rate at which to oversample the time series using zero–padding.
PairReturn	False	Whether data should be returned as {frequency list,intensity list} or as pairs: {{frequency1,intensity1}, {frequency2, intensity2},...,{frequencyN,intensityN}.
UpperFrequencyFactor	1	Value ≥ 1, by which to scale the upper Nyquist cutoff frequency and increase spectral resolution.

Options for **LombScargle** .

To obtain the possible frequencies we simply run LombScargle over the desired times for one of the time series and set the FrequenciesOnly option to True :

In[102]:= `LombScargle[rnaFinalTimeSeries[[1]], timesRNA, FrequenciesOnly → True]`

Out[102]= ⟨| f1 → 0.00500668, f2 → 0.0104306, f3 → 0.0158545,
f4 → 0.0212784, f5 → 0.0267023, f6 → 0.0321262, f7 → 0.0375501 |⟩

Proteomic Data

Importing OmicsObject Proteome Data

We now import the proteomics data example (for details on how to import such data please refer to DataImporter , DataImporterDirect , DataImporterDirectLabeled and OmicsObjectCreator documentation).

We import the proteomics OmicsObject MathIOmica example:

In[103]:= `proteinExample = Get[FileNameJoin[{ConstantMathIOmicaExamplesDirectory, "proteinExample"}]]`

Out[103]=
⟨|7 → ⟨|{A0AVT1, Protein} → {{0.937}, {17}}, {A0FGR8, Protein} → {{1.073}, {24}},
    {A0MZ66, Protein} → {{1.059}, {9}}, ⟨… 5219 …⟩, {Q9Y6I4, Protein} → Missing[],
    {Q9Y6I9, Protein} → Missing[], {Q9Y6X3, Protein} → Missing[]|⟩,
  9 → ⟨|⟨… 1 …⟩|⟩, ⟨… 9 …⟩, 20 → ⟨|⟨… 1 …⟩|⟩, 21 → ⟨|⟨… 1 …⟩|⟩|⟩

large output   **show less**   **show more**   **show all**   **set size limit…**

There are multiple samples given by the outer associations. We can use Query to get any data. For example we can get the outer keys:

In[104]:= `Query[Keys]@proteinExample`

Out[104]= `{7, 9, 10, 11, 14, 12, 13, 15, 16, 17, 19, 20, 21}`

We notice that sample 8 is missing - this is because it was used as a reference in the proteomics experiment. Point 18 is missing as there was no sample for that time point. We will address this in the next section.

We can get the expression raw data from any sample and entry. For example, the 14th and 214th entries in sample 12:

In[105]:= `Query["12", {14, 22}]@proteinExample`

Out[105]= ⟨|{A5PLN9, Protein} → {{1.057}, {3}}, {A6NGU5, Protein} → Missing[]|⟩

The keys correspond to UniProt accessions, and have been tagged with a "Protein" label as well. The values of all the keys/IDs correspond to `{{measurements}, {metadata}}`, and in this particular example: `{{relative intensity compared to reference}, {number of unique peptides identified for the given protein}}`.

The measurement for each protein is a relative intensity, i.e. the ratio of the value for the protein compared to the reference timepoint that has been chosen as the healthy sample "8", day "255" (in the experiment this was TMT reporter with 126 amu). The last list, the "metadata", in the proteomics OmicsObject was chosen to be the number of unique peptides identified for the given protein.

Additional Information: Gene Translation

As an aside, let us consider the form of the protein identifiers. MathIOmica can perform basic GeneTranslation going from one kind of identifier to another, using GetGeneDictionary :

GeneTranslation [*inputIDList*, *targetIDList*, *geneDictionary*]	uses *geneDictionary* to convert *inputIDList* IDs to different annotations as indicated by *targetIDList*. It takes for inputs:

inputIDList	List of n IDs (strings) to be converted in the form $\{inputID_1, inputID_2, ..., inputID_n\}$
targetIDList	List of target identifier strings, as used in the gene geneDictionary, $\{target\ ID_1, targetID_2, ... target\ ID_k\}$, e.g. {"UniProt ID","Gene Symbol"}. Can also be provided as a single string for only one kind of IDs.
geneDictionary	Gene dictionary to base translation on in the form generated by GetGeneDictionary.

GetGeneDictionary[]	creates an ID/accession dictionary from a UCSC table search – typically of gene annotations. GetGeneDictionary uses MathIOmica data for the annotations..

Translating gene identifiers using a gene dictionary.

We use GetGeneDictionary to define a gene dictionary:

In[106]:= **geneDictionary = GetGeneDictionary[]**

Out[106]= ⟨| human → ⟨| UCSC ID → {uc001aaa.3, uc010nxr.1, uc010nxq.1, uc001aal.1, uc001aaq.2, uc001aar.2, uc001aau.3, uc021oeh.1, ⸱⸱⸱ 121 567 ⸱⸱⸱ , uc022cfk.1, uc031tkn.1, uc022cgh.1, uc022cha.1, uc022chb.1, uc022chc.1, uc022che.1, uc022cpe.1}, ⸱⸱⸱ 6 ⸱⸱⸱ , HGU13 … ix ID → ⸱⸱⸱ 1 ⸱⸱⸱ |⟩ |⟩

large output	**show less**	**show more**	**show all**	**set size limit...**

The current version of the gene dictionary has accessions for the following identifiers:

In[107]:= `Query[All, Keys] @geneDictionary`

Out[107]= ⟨| human → {UCSC ID, UniProt ID, Gene Symbol, RefSeq ID,
    NCBI Protein Accession, Ensembl ID, KEGG Gene ID, HGU133Plus2 Affymetrix ID} |⟩

We can now use GeneTranslation (setting the optional InputID to "UniProt ID") to convert our example "UniProt ID" accessions to "Gene Symbol":

In[108]:= `GeneTranslation[{"A5PLN9", "A6NGU5"}, {"Gene Symbol"}, geneDictionary, InputID → {"UniProt ID"}]`

Out[108]= ⟨| Gene Symbol → ⟨| A5PLN9 → {TRAPPC13}, A6NGU5 → Missing[] |⟩ |⟩

We note that an ID might not necessarily be annotated across all databases, as in the above example.

Processing of Proteome Data

We will next preprocess the imported proteome data. We will first perform a transformation on the data towards a normal distribution, then we will re-label the samples with real time and carry out filtering for unique peptides present in each protein identification, as well as for missing data. Finally, we will create the proteomics time series or relative intensities compared to the healthy reference point for each protein.

Power Transformation, Labeling and Filtering

Data Power Transformation

To make the data comparable across time points, and as close to a normal distribution as possible for each sample, we normalize each time point /sample by using ApplyBoxCoxTransform .

`ApplyBoxCoxTransform [`*data*`]`	for a given *data* set, computes the Box–Cox transformation at the maximum likelihood λ parameter.

Applying a power transformation (Box-Cox) for an optimized parameter for each dataset.

option name	default value	
ListIndex	Missing[]	Selection of which list to use in the **OmicsObject** input.
ComponentIndex	Missing[]	Selection of which component of a list to use in the **OmicsObject** input.
HorizontalSelection	False	Horizontal selection across components for a single level association with multi–list values.

Options for ApplyBoxCoxTransform .

We apply a Box-Cox transformation to the proteomics data measurement in the OmicsObject, which is in the first list first component for each identifier. The optimized $\hat{\lambda}$ parameter for each sample is printed out for reference:

In[109]:= `transformedProteinData = ApplyBoxCoxTransform[proteinExample, ListIndex → 1, ComponentIndex → 1]`

```
Calculated Box-Cox parameter λ̂ = -0.152638

Calculated Box-Cox parameter λ̂ = -0.177086

Calculated Box-Cox parameter λ̂ = -0.421581

Calculated Box-Cox parameter λ̂ = -0.292287

Calculated Box-Cox parameter λ̂ = -0.432042

Calculated Box-Cox parameter λ̂ = 0.346673

Calculated Box-Cox parameter λ̂ = 0.368061

Calculated Box-Cox parameter λ̂ = 0.0834074

Calculated Box-Cox parameter λ̂ = 0.13413

Calculated Box-Cox parameter λ̂ = 0.166336

Calculated Box-Cox parameter λ̂ = 0.0866284

Calculated Box-Cox parameter λ̂ = -0.199247

Calculated Box-Cox parameter λ̂ = -0.221778
```

Out[109]=

⟨| 7 → ⟨| {A0AVT1, Protein} → {{-0.0653962}, {17.}},
  {A0FGR8, Protein} → {{0.0700809}, {24.}}, {A0MZ66, Protein} → {{0.057075}, {9.}}, ⬭ ⋯5220⋯ ⬭,
  {Q9Y6I9, Protein} → Missing[], {Q9Y6X3, Protein} → Missing[] |⟩, ⬭ ⋯11⋯ ⬭, 21 → ⟨| ⬭ ⋯1⋯ ⬭ |⟩ |⟩

| large output | **show less** | **show more** | **show all** | **set size limit…** |

We can plot the data to see what the resulting distributions look like:

In[110]:= **Histogram[#] & /@ (Query[All, Values, 1, 1] @ transformedProteinData)**

Out[110]= ⟨| 7 → [histogram], 9 → [histogram], 10 → [histogram], 11 → [histogram], 14 → [histogram], 12 → [histogram], 13 → [histogram], 15 → [histogram], 16 → [histogram], 17 → [histogram], 19 → [histogram], 20 → [histogram], 21 → [histogram] |⟩
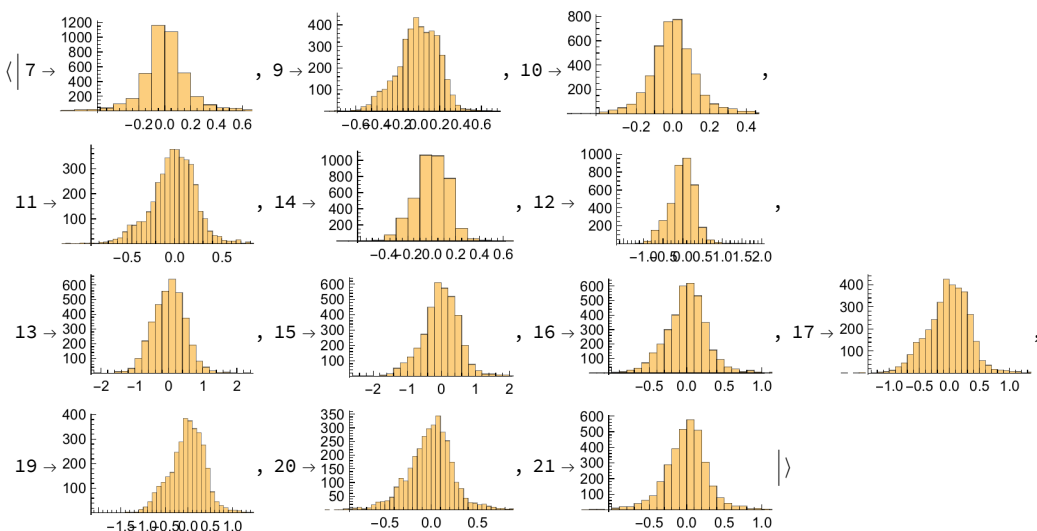
Re-labeling Samples with Times

As with the transcriptome, we notice that the sample numberings do not correspond to actual days, so we may adjust using the sampleToDays association created before and reproduced here for reference:

```
In[111]:= sampleToDays =
          <|"7" → "186", "8" → "255", "9" → "289", "10" → "290", "11" → "292", "12" → "294", "13" → "297", "14" → "301",
            "15" → "307", "16" → "311", "17" → "322", "18" → "329", "19" → "369", "20" → "380", "21" → "400"|>;
```

We can now do a KeyMap to rename the outer keys:

```
In[112]:= proteinLongitudinal = KeyMap[sampleToDays, transformedProteinData]
```

```
Out[112]=  ⟨|186 → ⟨|{A0AVT1, Protein} → {{-0.0653962}, {17.}},
             {A0FGR8, Protein} → {{0.0700809}, {24.}}, {A0MZ66, Protein} → {{0.057075}, {9.}},  ⋯ 5220 ⋯ ,
             {Q9Y6I9, Protein} → Missing[], {Q9Y6X3, Protein} → Missing[]|⟩,  ⋯ 11 ⋯ , 400 → ⟨| ⋯ 1 ⋯ |⟩ |⟩
```

large output   **show less**   **show more**   **show all**   **set size limit…**

Now let's check the timepoints in this dataset:

```
In[113]:= timesProteinRawData = TimeExtractor[proteinLongitudinal]
```

```
Out[113]= {186, 289, 290, 292, 294, 297, 301, 307, 311, 322, 369, 380, 400}
```

We notice a small complication: there are two timepoints missing, compared to the transcriptome: (i) the reference time point "255" does not appear explicitly in our computation (corresponding to a zero value about which other timepoints are computed for proteins with at least 2 unique peptides). (ii) there is no sample for day "329".

We can use the ConstantAssociator function to append these to the transformed data. Timepoints "255" (zero measurement assumed to have at least 2 unique peptides available per protein) and "329", assumed to be Missing data:

```
In[114]:= proteinLongitudinalEnlarged =
          ConstantAssociator[proteinLongitudinal, <|"255" → {{0}, {2}}, "329" → Missing[]|>]
```

```
Out[114]=  ⟨|186 → ⟨|{A0AVT1, Protein} → {{-0.0653962}, {17.}},
             {A0FGR8, Protein} → {{0.0700809}, {24.}}, {A0MZ66, Protein} → {{0.057075}, {9.}},  ⋯ 5220 ⋯ ,
             {Q9Y6I9, Protein} → Missing[], {Q9Y6X3, Protein} → Missing[]|⟩,  ⋯ 13 ⋯ , 329 → ⟨| ⋯ 1 ⋯ |⟩ |⟩
```

large output   **show less**   **show more**   **show all**   **set size limit…**

We can now check the timepoints again:

```
In[115]:= timesProtein = TimeExtractor[proteinLongitudinalEnlarged]
```

```
Out[115]= {186, 255, 289, 290, 292, 294, 297, 301, 307, 311, 322, 329, 369, 380, 400}
```

Filter Unique Peptides

Typically, proteomics data from mass spectrometry is filtered to retain only identifications of proteins that are supported by at least 2 unique peptides having been identified per protein. We can use FilteringFunction to implement the filtering:

FilteringFunction[*omicsObject*, *cutoff*]		filters OmicsObject data by a chosen comparison (by default greatr or equal) to a *cutoff*.

FilteringFunction can be used to filter data in an OmicsObject.

option name	default value	
ListIndex	Missing[]	Selection of which list to use in the **OmicsObject** input.
ComponentIndex	Missing[]	Selection of which component of a list to use in the **OmicsObject** input.
SelectionFunction	GreaterEqual	Selection of comparison to use for filtering.

Options for **FilteringFunction** .

We filter out proteomics data with less than 2 unique peptides per protein. The unique peptides is reported as the second list, first component in the OmicsObject values in this case:

In[116]:= `proteinUnique = FilteringFunction[proteinLongitudinalEnlarged, 2, ListIndex → 2, ComponentIndex → 1]`

Out[116]=
```
⟨|186 → ⟨|{A0AVT1, Protein} → {{-0.0653962}, {17.}},
    {A0FGR8, Protein} → {{0.0700809}, {24.}}, {A0MZ66, Protein} → {{0.057075}, {9.}}, ⬤ ⋯ 5220 ⋯ ,
    {Q9Y616, Protein} → Missing[], {Q9Y6X3, Protein} → Missing[]|⟩, ⬤ ⋯ 13 ⋯ , 329 → ⟨| ⬤ ⋯ 1 ⋯ |⟩|⟩|⟩
```
| large output | **show less** | **show more** | **show all** | **set size limit...** |

Filter Data

We will next remove values that have been tagged as Missing[], retaining data that have at least 3/4 data points available across all samples. Here we use the function FilterMissing :

In[117]:= `filteredProteinData = FilterMissing[proteinUnique, 3/4]`

Number of Missing Data Points per Component
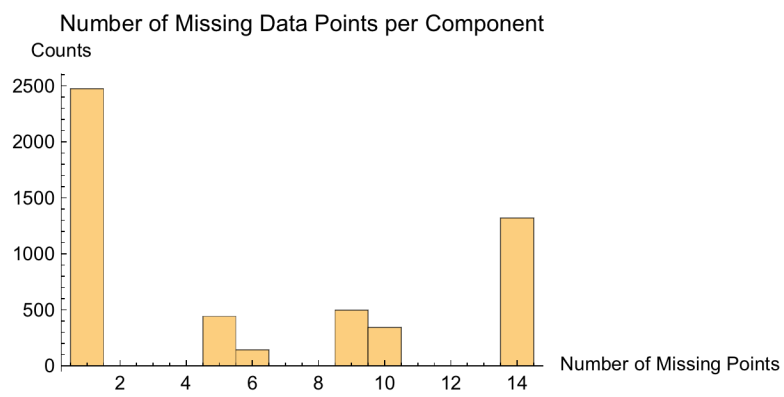
{Missing -> Counts: , ⟨|1 → 2475, 5 → 443, 6 → 143, 9 → 499, 10 → 345, 14 → 1320|⟩}

Pie Chart of number of missing components
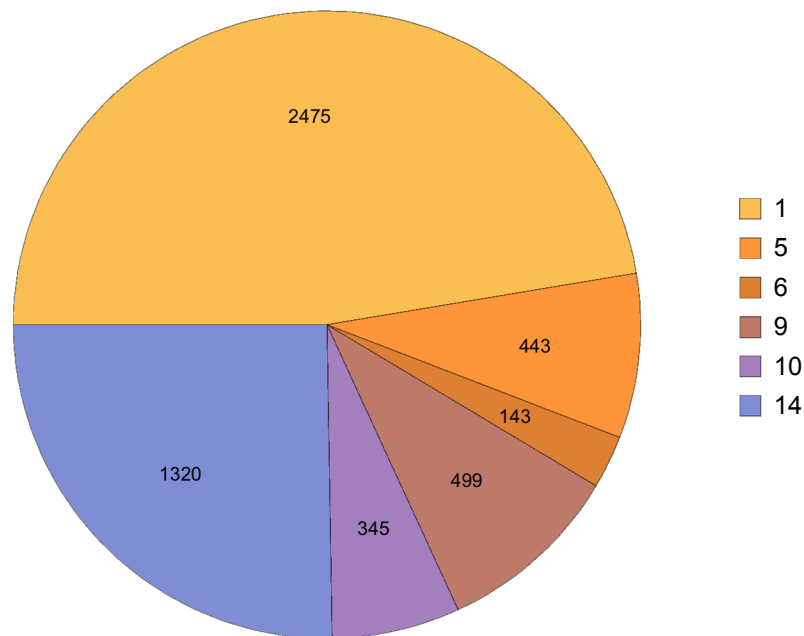
Out[117]=

⟨|186 → ⟨|{A0AVT1, Protein} → {{-0.0653962}, {17.}},
    {A0FGR8, Protein} → {{0.0700809}, {24.}}, ⸬ 2471 ⸬ , {Q9Y6W5, Protein} → {{-0.0514946}, {14.}},
    {Q9Y6Y8, Protein} → {{-0.026397}, {10.}}|⟩, ⸬ 13 ⸬ , 329 → ⟨| ⸬ 1 ⸬ |⟩|⟩

| large output | **show less** | **show more** | **show all** | **set size limit…** |

Create Proteome Time Series

We can now create time series for each of the proteins.

For each protein we now extract a time series (list of values) corresponding to these times:

In[118]:= `timeSeriesProtein = CreateTimeSeries[filteredProteinData]`

Out[118]= ⟨| {A0AVT1, Protein} → {−0.0653962, 0, 0.00299471, −0.0348449, −0.0182123, 0.0627073, ⋯ 3 ⋯ ,
0.0829594, 0.0689856, Missing[], −0.050132, −0.137674, −0.0120888}, ⋯ 2473 ⋯ , ⋯ 1 ⋯ |⟩

large output   **show less**   **show more**   **show all**   set size limit...

Take the Norm and Remove Constant Proteome Time Series

Next, we normalize each protein series, using SeriesApplier :

In[119]:= `normedProteinAll = SeriesApplier[Normalize, timeSeriesProtein]`

Out[119]= ⟨| {A0AVT1, Protein} → {−0.205122, 0., 0.00939321, −0.109294, −0.0571245, 0.196687, ⋯ 3 ⋯ ,
0.26021, 0.21638, Missing[], −0.157244, −0.431828, −0.0379175}, ⋯ 2473 ⋯ , ⋯ 1 ⋯ |⟩

large output   **show less**   **show more**   **show all**   set size limit...

Finally, we use ConstantSeriesClean to remove constant series, as we are interested in changing time patterns:

In[120]:= `proteinFinalTimeSeries = ConstantSeriesClean[normedProteinAll]`

Out[120]= ⟨| {A0AVT1, Protein} → {−0.205122, 0., 0.00939321, −0.109294, −0.0571245, 0.196687, ⋯ 3 ⋯ ,
0.26021, 0.21638, Missing[], −0.157244, −0.431828, −0.0379175}, ⋯ 2473 ⋯ , ⋯ 1 ⋯ |⟩

large output   **show less**   **show more**   **show all**   set size limit...

Resampling Proteome Data

In addition to the above, we want to create a resampled distribution for the proteome dataset prior to classification and clustering. In this subsection we first resample the imported and labeled proteome dataset, Then, we carry out the full analysis in this "bootstrap" dataset, to create a set of random proteome time series. This bootstrap distribution of time series will be used to provide the cutoffs used in the time series classification in the following subsection.

Resampling the Proteome Data

We create a resampling of 100000 sets:

In[121]:= `proteinBootstrap = BootstrapGeneral[proteinExample, 100 000]`

Out[121]= ⟨| 7 → ⟨| 1 → Missing[], 2 → {{1.123}, {8}}, 3 → {{0.87}, {2}}, 4 → {{0.49}, {4}},
5 → {{1.106}, {1}}, 6 → Missing[], 7 → Missing[], ⋯ 99 987 ⋯ , 99 995 → {{0.93}, {4}},
99 996 → {{1.048}, {3}}, 99 997 → {{0.972}, {2}}, 99 998 → {{1.212}, {3}},
99 999 → Missing[], 100 000 → {{0.838}, {3}} |⟩, ⋯ 11 ⋯ , 21 → ⟨| ⋯ 1 ⋯ |⟩ |⟩

large output   **show less**   **show more**   **show all**   set size limit...

Processing the Bootstrap Proteome and Creating Bootstrap Time Series

We apply a Box-Cox transformation to the bootstrap set proteomics data measurement in the OmicsObject, which is in the first list first component for each identifier. The optimized $\hat{\lambda}$ parameter for each sample is printed out for reference:

In[122]:= `transformedProteinBootstrapData = ApplyBoxCoxTransform[proteinBootstrap, ListIndex → 1, ComponentIndex → 1]`

Calculated Box-Cox parameter $\hat{\lambda}$ = -0.122293

Calculated Box-Cox parameter $\hat{\lambda}$ = -0.177476

Calculated Box-Cox parameter $\hat{\lambda}$ = -0.479732

Calculated Box-Cox parameter $\hat{\lambda}$ = -0.28628

Calculated Box-Cox parameter $\hat{\lambda}$ = -0.442429

Calculated Box-Cox parameter $\hat{\lambda}$ = 0.340028

Calculated Box-Cox parameter $\hat{\lambda}$ = 0.375712

Calculated Box-Cox parameter $\hat{\lambda}$ = 0.0953692

Calculated Box-Cox parameter $\hat{\lambda}$ = 0.146456

Calculated Box-Cox parameter $\hat{\lambda}$ = 0.176869

Calculated Box-Cox parameter $\hat{\lambda}$ = 0.107587

Calculated Box-Cox parameter $\hat{\lambda}$ = -0.178747

Calculated Box-Cox parameter $\hat{\lambda}$ = -0.210116

Out[122]=
```
⟨|7 → ⟨|1 → Missing[], 2 → {{0.115185}, {8.}}, 3 → {{-0.140455}, {2.}}, 4 → {{-0.74539}, {4.}},
    ⋯ 99 993 ⋯ , 99 998 → {{0.190029}, {3.}}, 99 999 → Missing[], 100 000 → {{-0.178661}, {3.}}|⟩,
  9 → ⟨| ⋯ 1 ⋯ |⟩, ⋯ 9 ⋯ , 20 → ⋯ 1 ⋯ , 21 → ⟨| ⋯ 1 ⋯ |⟩ |⟩
```
large output | **show less** | **show more** | **show all** | **set size limit...**

We can now do a KeyMap to rename the outer keys to actual days:

In[123]:= `proteinBootstrapLongitudinal = KeyMap[sampleToDays, transformedProteinBootstrapData];`

Now let's check the timepoints in this dataset:

In[124]:= `timesProteinBootstrapData = TimeExtractor[proteinBootstrapLongitudinal]`

Out[124]= {186, 289, 290, 292, 294, 297, 301, 307, 311, 322, 369, 380, 400}

As with the regular protein data above use the ConstantAssociator function to append these to the transformed bootstrap data. Timepoints "255" (zero measurement assumed to have at least 2 unique peptides available per protein) and "329", assumed to be Missing data:

In[125]:= `proteinBootstrapLongitudinalEnlarged =`
`    ConstantAssociator[proteinBootstrapLongitudinal, <|"255" → {{0}, {2}}, "329" → Missing[]|>];`

We can now check the timepoints again:

In[126]:= `timesProteinBootstrap = TimeExtractor[proteinBootstrapLongitudinalEnlarged]`

Out[126]= {186, 255, 289, 290, 292, 294, 297, 301, 307, 311, 322, 329, 369, 380, 400}

We filter out proteomics bootstrap data with less than 2 unique peptides per protein. The unique peptides is reported as the second list, first component in the OmicsObject values in this case:

```
In[127]:=  proteinBootstrapUnique =
             FilteringFunction[proteinBootstrapLongitudinalEnlarged, 2, ListIndex → 2, ComponentIndex → 1]
```

```
Out[127]=  ⟨| 186 → ⟨| 2 → {{0.115185}, {8.}}, 3 → {{-0.140455}, {2.}}, 4 → {{-0.74539}, {4.}}, 8 → {{0.0655238}, {8.}},
              ··· 99 992 ··· , 66 056 → Missing[], 46 250 → Missing[], 27 909 → Missing[], 76 569 → Missing[] |⟩,
              ··· 13 ··· , 329 → ⟨| 2 → Missing[], ··· 99 998 ··· , 76 569 → ··· 1 ··· |⟩ |⟩
```

large output | **show less** | **show more** | **show all** | **set size limit…**

We will next remove values that have been tagged as Missing[], retaining data that have at least 3/4 data points available across all bootstrap samples. Here we use the function FilterMissing :

```
In[128]:=  filteredProteinBootstrapData = FilterMissing[proteinBootstrapUnique, 3/4]
```

Number of Missing Data Points per Component
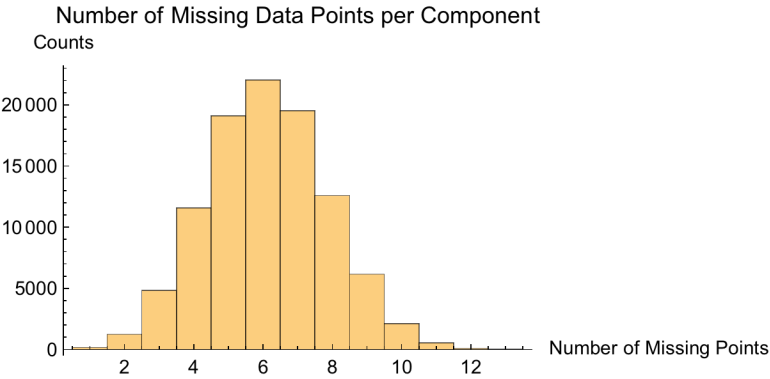
{Missing -> Counts: , ⟨|1 → 163, 2 → 1249, 3 → 4829, 4 → 11 579, 5 → 19 112,
6 → 22 028, 7 → 19 507, 8 → 12 591, 9 → 6166, 10 → 2129, 11 → 538, 12 → 100, 13 → 9|⟩}

Pie Chart of number of missing components
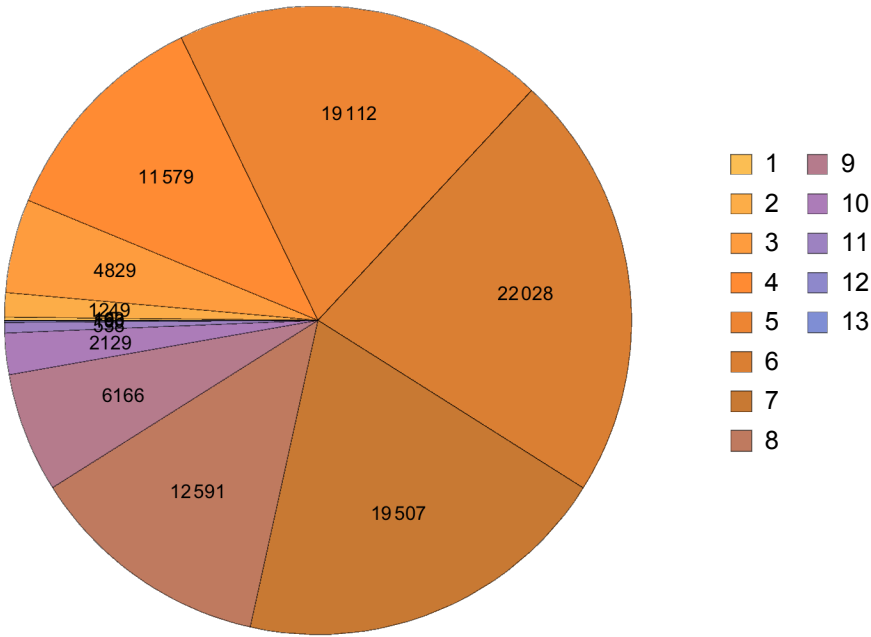
Out[128]=

⟨|186 → ⟨|36 → {{0.0227079}, {4.}}, 44 → {{-0.294185}, {5.}},
55 → {{-0.0451214}, {2.}}, 64 → {{0.0580618}, {20.}}, ⸱⸱⸱ 6233 ⸱⸱⸱ ,
94 811 → Missing[], 96 867 → Missing[], 97 975 → Missing[], 98 740 → Missing[]|⟩,
⸱⸱⸱ 13 ⸱⸱⸱ , 329 → ⟨|36 → Missing[], ⸱⸱⸱ 6239 ⸱⸱⸱ , 98 740 → ⸱⸱⸱ 1 ⸱⸱⸱ |⟩|⟩

large output   **show less**   **show more**   **show all**   **set size limit...**

For each bootstrap protein we now extract a time series (list of values):

In[129]:= `timeSeriesProteinBootstrap = CreateTimeSeries[filteredProteinBootstrapData]`

Out[129]=
⟨| 36 → {0.0227079, 0, -0.101835, -0.0628029, Missing[], 0.115541, -0.698119, Missing[], 0.606335, -0.282985, 0.0441887, Missing[], 0.527867, -0.00200236, 0.0745179}, ⬤··· 6239 ···, 98 740 → ⬤··· 1 ··· |⟩

large output   **show less**   **show more**   **show all**   set size limit...

Next, we normalize each protein series, using SeriesApplier :

In[130]:= `normedProteinBootstrapAll = SeriesApplier[Normalize, timeSeriesProteinBootstrap]`

Out[130]=
⟨| 36 → {0.0203152, 0., -0.0911051, -0.0561855, Missing[], 0.103366, ⬤··· 3 ···, -0.253168, 0.0395326, Missing[], 0.472247, -0.00179138, 0.0666662}, 44 → {⬤··· 1 ···}, ⬤··· 6237 ···, ⬤··· 1 ···, 98 740 → {⬤··· 1 ···} |⟩

large output   **show less**   **show more**   **show all**   set size limit...

Finally, we use ConstantSeriesClean to remove constant series, as we are interested in changing time patterns:

In[131]:= `proteinBootstrapFinalTimeSeries = ConstantSeriesClean[normedProteinBootstrapAll]`

Out[131]=
⟨| 36 → {0.0203152, 0., -0.0911051, -0.0561855, Missing[], 0.103366, ⬤··· 3 ···, -0.253168, 0.0395326, Missing[], 0.472247, -0.00179138, 0.0666662}, 44 → {⬤··· 1 ···}, ⬤··· 6237 ···, ⬤··· 1 ···, 98 740 → {⬤··· 1 ···} |⟩

large output   **show less**   **show more**   **show all**   set size limit...

Classification of Proteome Time Series

In this subsection we will classify the proteome time series based on patterns in the series. For the classification we will use TimeSeriesClassification .

We will use QuantileEstimator for the "LombScargle" method to provide a cutoff for the TimeSeriesClassification inputs.

First, we estimate for the "LombScargle" Method, 0.95 quantile cutoff from the bootstrap proteome data:

In[132]:= `q95Protein = QuantileEstimator[proteinBootstrapFinalTimeSeries, timesProteinBootstrap]`

Out[132]= `0.835064`

Next, we estimate the "Spikes" 0.95 quantile cutoff from the bootstrap proteome data:

In[133]:=
`q95ProteinSpikes =`
`  QuantileEstimator[proteinBootstrapFinalTimeSeries, timesProteinBootstrap, Method → "Spikes"]`

Out[133]= ⟨| 12 → {0.804265, -0.838055}, 13 → {0.802793, -0.81749}, 14 → {0.787772, -0.821609} |⟩

Now we can classify the proteome time series data based on these cutoffs:

```
In[134]:= proteinClassification = TimeSeriesClassification[proteinFinalTimeSeries,
              timesProtein, LombScargleCutoff → q95Protein, SpikeCutoffs → q95ProteinSpikes]

           Method → "LombScargle"
```

Out[134]=

⟨| ⋯ 1 ⋯ |⟩

large output | **show less** | **show more** | **show all** | **set size limit…**

As discussed above, the default output for TimeSeriesClassification is an Association with outer keys being the classification classes, inner keys being the class members, and each class member value being a list of {{computed classification vector}, {input data list}}.

If we want the classes produced, we can query the keys:

```
In[138]:= Keys[proteinClassification]
```
```
Out[138]= {SpikeMax, SpikeMin, f1, f5, f6, f7}
```

For the number of members in each class we have:

```
In[139]:= Query[All, Length] @ proteinClassification
```
```
Out[139]= ⟨| SpikeMax → 124, SpikeMin → 48, f1 → 77, f5 → 7, f6 → 36, f7 → 18 |⟩
```

We can obtain the membership list in any class of interest:

```
In[140]:= Query["f1", Keys] @ proteinClassification
```
```
Out[140]= {{O00160, Protein}, {O00267, Protein}, {O00273, Protein}, {O00571, Protein},
          {O15031, Protein}, {O43143, Protein}, {O43175, Protein}, {O43312, Protein},
          {O43516, Protein}, {O60271, Protein}, {O60879, Protein}, {O75643, Protein},
          {O75792, Protein}, {O95498, Protein}, {P00488, Protein}, {P00915, Protein}, {P02042, Protein},
          {P02671, Protein}, {P04844, Protein}, {P08174, Protein}, {P09326, Protein}, {P09496, Protein},
          {P11021, Protein}, {P12956, Protein}, {P13501, Protein}, {P13611, Protein}, {P13667, Protein},
          {P19387, Protein}, {P23141, Protein}, {P23368, Protein}, {P32119, Protein}, {P32189, Protein},
          {P33176, Protein}, {P40306, Protein}, {P42892, Protein}, {P50225, Protein}, {P51531, Protein},
          {P52888, Protein}, {P54920, Protein}, {P55036, Protein}, {P60660, Protein}, {P84095, Protein},
          {Q01518, Protein}, {Q07021, Protein}, {Q08722, Protein}, {Q09666, Protein}, {Q13151, Protein},
          {Q13217, Protein}, {Q13488, Protein}, {Q14165, Protein}, {Q14643, Protein}, {Q14653, Protein},
          {Q15084, Protein}, {Q5H9R7, Protein}, {Q6NYC8, Protein}, {Q709C8, Protein}, {Q86YP4, Protein},
          {Q92499, Protein}, {Q96AT9, Protein}, {Q96L92, Protein}, {Q96RT1, Protein}, {Q99439, Protein},
          {Q9BTE3, Protein}, {Q9BTV4, Protein}, {Q9BWS9, Protein}, {Q9C0I1, Protein}, {Q9H0D6, Protein},
          {Q9H2U2, Protein}, {Q9H444, Protein}, {Q9H4Z3, Protein}, {Q9NS69, Protein}, {Q9NUP9, Protein},
          {Q9NVJ2, Protein}, {Q9NYB0, Protein}, {Q9UQ35, Protein}, {Q9Y277, Protein}, {Q9Y2Q0, Protein}}
```

To obtain the possible frequencies we simply run LombScargle over the desired times for one of the time series and set the FrequenciesOnly option to True:

```
In[141]:= LombScargle[proteinFinalTimeSeries[[1]], timesRNA, FrequenciesOnly → True]
```
```
Out[141]= ⟨| f1 → 0.00500668, f2 → 0.0104306, f3 → 0.0158545,
          f4 → 0.0212784, f5 → 0.0267023, f6 → 0.0321262, f7 → 0.0375501 |⟩
```

Metabolomic Data

Importing OmicsObject Metabolome Data

We now import the metabolomics data example (for details on how to import such data please refer to DataImporter , DataImporterDirect ,

DataImporterDirectLabeled and OmicsObjectCreator documentation).

We import the metabolomics OmicsObject MathIOmica examples for each of positive and negative mass spectrometry aligned mass features:

In[142]:= **metabolitesNegativeModeExample =**
  Get[FileNameJoin[{ConstantMathIOmicaExamplesDirectory, "metabolomicsNegativeModeExample"}]]

Out[142]=
⟨| 8 → ⟨| {457.002, 0.34764, Meta} →
    {{23 444, 16 317, 1}, { [C16 H11 N9 S4, db=0.00, overall=47.55, mfg=95.11], }},
    … 2289 … , {421.948, 0.392875, Meta} → {{1, 115 528, 130 042},
    { [C11 H12 Cl2 O11 S, db=0.00, overall=48.58, mfg=97.17], }}|⟩, … 10 … , 20 → ⟨| … 1 … |⟩ |⟩

large output | **show less** | **show more** | **show all** | **set size limit…**

In[143]:= **metabolitesPositiveModeExample =**
  Get[FileNameJoin[{ConstantMathIOmicaExamplesDirectory, "metabolomicsPositiveModeExample"}]]

Out[143]=
⟨| 8 → ⟨| {202.033, 0.332607, Meta} → {{263 741, 276 622, 337 241}, {, }},
    {174.038, 0.334514, Meta} → {{78 435, 88 529, 121 073}, {, }},
    … 3670 … , {422.34, 14.7601, Meta} → {{1, 36 919, 102 737}, {, }}|⟩,
  9 → ⟨| … 1 … |⟩ , … 8 … , 19 → ⟨| … 1 … |⟩ , 20 → ⟨| … 1 … |⟩ |⟩

large output | **show less** | **show more** | **show all** | **set size limit…**

There are multiple samples given by the outer associations. We can use Query to get any data. For example we can get the outer keys:

In[144]:= **Query[Keys] @metabolitesNegativeModeExample**

Out[144]= {8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 19, 20}

In[145]:= **Query[Keys] @metabolitesPositiveModeExample**

Out[145]= {8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 19, 20}

We notice that sample 7, 18 and 21 are missing as there was no sample for these time points. This will be addressed further below.

We can get the intensity data from any sample and entry. For example, the 77th and 155th entries in sample 14:

In[146]:= **Query["14", {77, 155}] @metabolitesNegativeModeExample**

Out[146]= ⟨| {322.089, 0.440241, Meta} →
    {{31 950, 29 801, 27 440}, {Isosorbide-2-glucuronide [C12 H18 O10, db=60.03, overall=60.67, mfg=61.31,
      KEGG ID=, CAS ID=29542-01-6], 29542-01-6}}, {146.059, 0.742692, Meta} → {{62 667, 1, 60 382},
    {Adipic acid [C6 H10 O4, db=45.74, overall=46.59, mfg=47.44, KEGG ID=, CAS ID=124-04-9], 124-04-9}}|⟩

The outer keys correspond to the identified features in the form {mass to charge ratio (m/z), retention time, "Meta"}, i.e. each m/z and retention time has been tagged with a "Meta" label as well to indicate these are metabolomics data. The values of all the keys/IDs correspond to {{measurements}, {metadata}}, and in this particular example: {{intensity technical replicate 1, intensity technical replicate 2, intensity technical replicate 3},. {Annotations, CAS Number}}

We would like to combine the positive and negative mode metabolomics data. We will use EnlargeInnerAssociation :

In[147]:=
```
metabolitesExample =
  EnlargeInnerAssociation[{metabolitesNegativeModeExample, metabolitesPositiveModeExample}]
```

Out[147]=
⟨|8 → ⟨|{457.002, 0.34764, Meta} →
  {{23 444, 16 317, 1}, { [C16 H11 N9 S4, db=0.00, overall=47.55, mfg=95.11], }},
  ⬤ ⋯ 5962 ⋯ , {422.34, 14.7601, Meta} → {{1, 36 919, 102 737}, {, }}|⟩, ⬤ ⋯ 10 ⋯ ,
  20 → ⟨|{457.002, 0.34764, Meta} → {{20 788, 12 425, 1}, ⬤ ⋯ 1 ⋯ }, ⬤ ⋯ 5962 ⋯ , ⬤ ⋯ 1 ⋯ |⟩|⟩

large output   **show less**   **show more**   **show all**   **set size limit...**

Processing of Metabolome Data

We will next preprocess the imported metabolome data. We will first perform calculate the median of the technical replicates, transform the data towards a normal distribution, then we will re-label the samples with real time and carry out filtering for missing data. Finally, we will create the metabolomics time series or relative intensities compared to the healthy reference point for each mass feature identified.

Medians of Technical Triplicates, Data Transformation, Labeling, Filtering, Matching Mass

Median of Technical Triplicates

The metabolomics intensities have three measurements, corresponding to technical triplicates. Typically we would like to use the median of these values. An additional complication is that some of the triplicates have intensity values of 1, which should be taken as a Missing value. We can use MeasurementApplier to perform the calculation:

MeasurementApplier [*function* , *omicsObject*]	applies a *function* to the measurement list of an *omicsObject*, ignoring missing values.

Applying a function to the measurements in an OmicsObject.

option name	default value	
ComponentIndex	All	ComponentIndex is an option for MathIOmica functions, such as Applier, that allows selection of which component of a list to use in an association or OmicsObject input or output values.
IgnorePattern	_Missing	IgnorePattern is an option for MeasurementApplier specifying a pattern of values to delete prior to applying the function to the measurement list.
ListIndex	1	ListIndex is an option for MathIOmica functions, such as Applier that allows selection of which list to use in the association or OmicsObject input or output values.

Options for **MeasurementApplier** .

We implement a Median calculation, and ignoring entries with missing and values of 1:

In[148]:= `metaboliteMedians = MeasurementApplier[Median, metabolitesExample, IgnorePattern → (_Missing | 1 | 1.)]`

Out[148]=
```
⟨| 8 →
   ⟨| {457.002, 0.34764, Meta} → {{19 880.5}, { [ C16 H11 N9 S4, db=0.00, overall=47.55, mfg=95.11 ], }},
      ⋯ 5962 ⋯ , {422.34, 14.7601, Meta} → {{69 828.}, {, }}|⟩,  ⋯ 10 ⋯ , 20 → ⟨| ⋯ 1 ⋯ |⟩ |⟩
```
large output   **show less**   **show more**   **show all**   **set size limit…**

Data Power Transformation

We apply a Box-Cox transformation to the metabolite median data in the OmicsObject, which is now the first list first component for each identifier. The optimized $\hat{\lambda}$ parameter for each sample is printed out for reference:

In[149]:= `transformedMetaboliteData = ApplyBoxCoxTransform[metaboliteMedians, ListIndex → 1, ComponentIndex → 1]`

```
Calculated Box-Cox parameter λ̂ = -0.288857

Calculated Box-Cox parameter λ̂ = -0.282374

Calculated Box-Cox parameter λ̂ = -0.276202

Calculated Box-Cox parameter λ̂ = -0.262075

Calculated Box-Cox parameter λ̂ = -0.271308

Calculated Box-Cox parameter λ̂ = -0.27703

Calculated Box-Cox parameter λ̂ = -0.295395

Calculated Box-Cox parameter λ̂ = -0.264833

Calculated Box-Cox parameter λ̂ = -0.278556

Calculated Box-Cox parameter λ̂ = -0.269513

Calculated Box-Cox parameter λ̂ = -0.265784

Calculated Box-Cox parameter λ̂ = -0.262769
```

Out[149]=

⟨| 8 →
⟨| {457.002, 0.34764, Meta} → {{3.26345}, { [C16 H11 N9 S4, db=0.00, overall=47.55, mfg=95.11], }},
… 5962 … , {422.34, 14.7601, Meta} → {{3.32386}, {, }} |⟩,
… 10 … , 20 → ⟨| {457.002, 0.34764, Meta} → { … 1 … }, … 5962 … , … 1 … |⟩ |⟩

large output | **show less** | **show more** | **show all** | **set size limit...**

We can plot the data to see what the resulting distributions look like:

In[150]:= `Histogram[#] & /@ (Query[All, Values, 1, 1]@transformedMetaboliteData)`
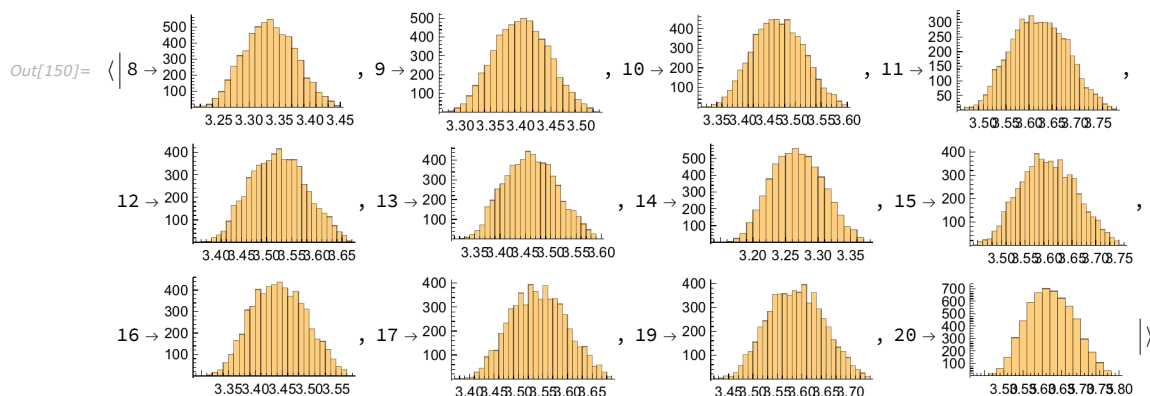
We may also wish to standardize the distributions:

In[151]:= `metabolitesStandardized =`
`  Returner[transformedMetaboliteData, Applier[StandardizeExtended[#, Mean, StandardDeviation] &,`
`    transformedMetaboliteData, ListIndex → 1, ComponentIndex → 1], ListIndex → 1, ComponentIndex → 1]`

Out[151]=
⟨| 8 →
  ⟨| {457.002, 0.34764, Meta} → {{-1.71178}, { [C16 H11 N9 S4, db=0.00, overall=47.55, mfg=95.11], }},
   ⸺ 5962 ⸺ , {422.34, 14.7601, Meta} → {{-0.247328}, {, }} |⟩,
  9 → ⟨| ⸺ 1 ⸺ |⟩ , ⸺ 8 ⸺ , 19 → ⟨| ⸺ 1 ⸺ |⟩ , 20 → ⟨| ⸺ 1 ⸺ |⟩ |⟩

large output   **show less**   **show more**   **show all**   **set size limit...**

We can again plot the data to see what the standardized distributions look like:

In[152]:= `Histogram[#] & /@ (Query[All, Values, 1, 1] @metabolitesStandardized)`
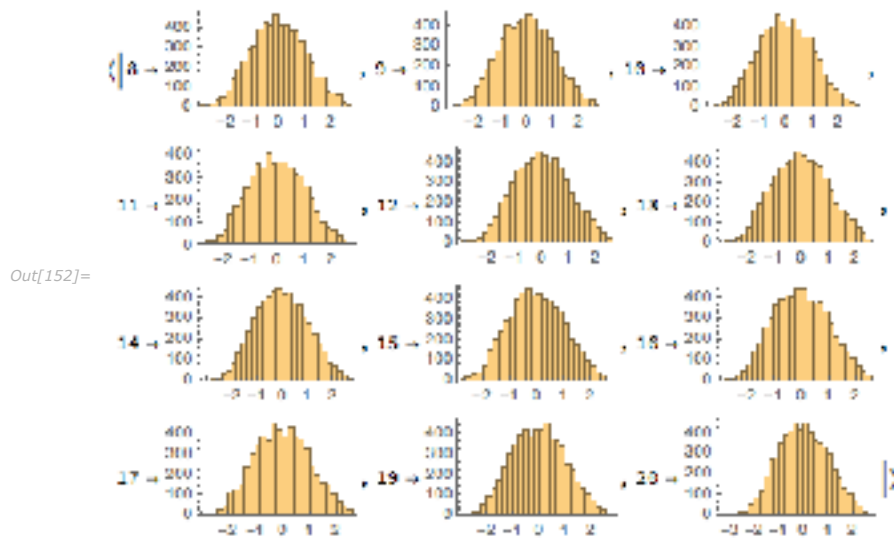
Out[152]=

Re-labeling Samples with Times

As with the transcriptome, we notice that the sample numberings do not correspond to actual days, so we may adjust using the sampleToDays association created above:

In[153]:= `sampleToDays =`
`  <|"7" → "186", "8" → "255", "9" → "289", "10" → "290", "11" → "292", "12" → "294", "13" → "297", "14" → "301",`
`   "15" → "307", "16" → "311", "17" → "322", "18" → "329", "19" → "369", "20" → "380", "21" → "400"|>;`

We can now do a KeyMap to rename the outer keys:

In[154]:= `metabolitesLongitudinal = KeyMap[sampleToDays, metabolitesStandardized]`

Out[154]=
⟨| 255 →
  ⟨| {457.002, 0.34764, Meta} → {{-1.71178}, { [C16 H11 N9 S4, db=0.00, overall=47.55, mfg=95.11], }},
   ⸺ 5962 ⸺ , {422.34, 14.7601, Meta} → {{-0.247328}, {, }} |⟩,
  289 → ⟨| ⸺ 1 ⸺ |⟩ , ⸺ 8 ⸺ , 369 → ⟨| ⸺ 1 ⸺ |⟩ , 380 → ⟨| ⸺ 1 ⸺ |⟩ |⟩

large output   **show less**   **show more**   **show all**   **set size limit...**

Now let's check the timepoints in this dataset:

In[155]:= **timesMetaboliteRawData = TimeExtractor[metabolitesLongitudinal]**

Out[155]= {255, 289, 290, 292, 294, 297, 301, 307, 311, 322, 369, 380}

We notice a complication: there are three timepoints missing, corresponding to the three samples for which we had indicated above that there were no measurements (compared to the transcriptome samples). These are samples on days "186", "329" and "400".

We can use the ConstantAssociator function to append these to the transformed data, tagging these data as Missing data:

In[156]:= **metabolitesLongitudinalEnlarged =**
　　ConstantAssociator[metabolitesLongitudinal, <|"186" → Missing[], "329" → Missing[], "400" → Missing[]|>]

Out[156]=
⟨| 255 →
　　⟨| {457.002, 0.34764, Meta} → {{-1.71178}, { [C16 H11 N9 S4, db=0.00, overall=47.55, mfg=95.11], }},
　　　… 5962 … , {422.34, 14.7601, Meta} → {{-0.247328}, {, }} |⟩,
　289 → ⟨| … 1 … |⟩ , … 11 … , 329 → ⟨| … 1 … |⟩ , 400 → ⟨| … 1 … |⟩ |⟩

large output　　**show less**　　**show more**　　**show all**　　**set size limit…**

We can now check the timepoints again:

In[157]:= **timesMetabolites = TimeExtractor[metabolitesLongitudinalEnlarged]**

*Out[157]=* {186, 255, 289, 290, 292, 294, 297, 301, 307, 311, 322, 329, 369, 380, 400}

Filter Data

We will next remove values that have been tagged overall as Missing[], retaining data that have at least 3/4 data points available across all samples. Additionally we remove data where the reference healthy sample "255" was missing. We use the function FilterMissing for this implementation:

In[158]:= **filteredMetaboliteData = FilterMissing[metabolitesLongitudinalEnlarged, 3/4, Reference → "255"]**

Number of Missing Data Points per Component
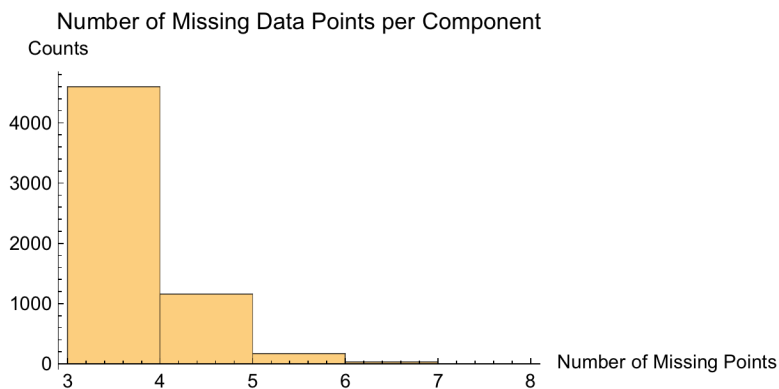
Counts

{Missing -> Counts: , ⟨|3 → 4601, 4 → 1158, 5 → 172, 6 → 31, 7 → 2|⟩}

Pie Chart of number of missing components
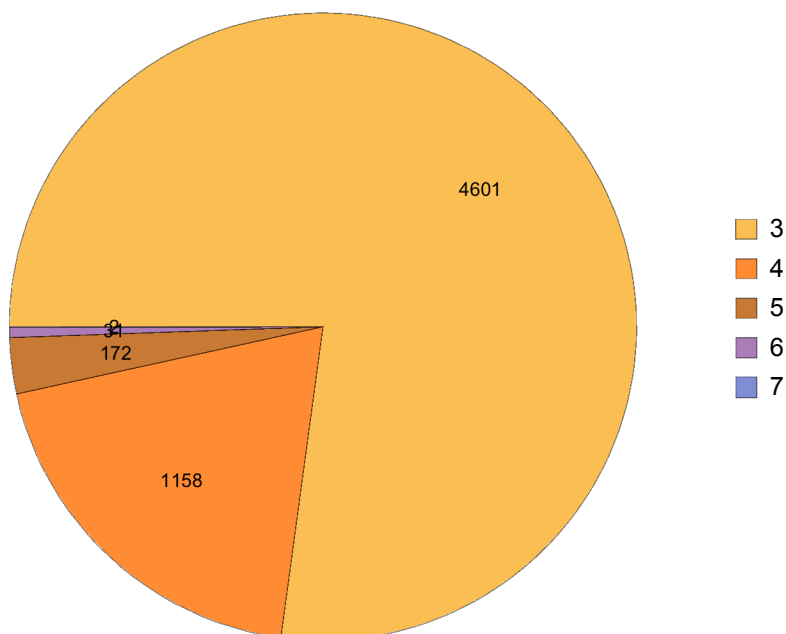
Out[158]=

⟨|255 →
 ⟨|{457.002, 0.34764, Meta} → {{-1.71178}, { [C16 H11 N9 S4, db=0.00, overall=47.55, mfg=95.11], }},
 ⟨ ... 4599 ... ⟩, {406.381, 14.5609, Meta} → {{-1.34842}, {2,4,6-trimethyl-2, ... id ID=, KEGG ID=], }}|⟩,
 ⟨ ... 13 ... ⟩, 400 → ⟨| ... 1 ... |⟩|⟩|⟩

| large output | show less | show more | show all | set size limit... |

Matching Unique Mass

We may want to match a unique mass to the metabolites. This is a putative mass identification based on the uniqueness of the mass feature. If matched, a KEGG compound identity can be prepended to the identifier using **OmicsObjectUniqueMassConverter** .

| OmicsObjectUniqueMassConverter [*omicsObject* , *massAccuracy*] | assigns a unique putative mass identification to each of *omicsObject*'s inner association keys, using the *massAccuracy* in parts per million. |

Matching putative mass identifications to mass features in an **OmicsObject** of metabolites.

We match our identities to KEGG compound identifiers, using a 2ppm accuracy (this may take some time depending on the number of matching data):

In[159]:= `massMatchedFilteredMetabolites = OmicsObjectUniqueMassConverter[filteredMetaboliteData, 2]`

Out[159]=
```
⟨|255 →
  ⟨|{457.002, 0.34764, Meta} → {{-1.71178}, { [ C16 H11 N9 S4, db=0.00, overall=47.55, mfg=95.11 ], }},
   ⋯ 4599 ⋯ , {406.381, 14.5609, Meta} → {{-1.34842}, {2,4,6-trimethyl-2, … id ID=, KEGG ID= ], }}|⟩,
  ⋯ 13 ⋯ , 400 → ⟨| ⋯ 1 ⋯ |⟩ |⟩
```
large output   **show less**   **show more**   **show all**   **set size limit…**

Create Metabolome Time Series

We can now create time series for each of the proteins.

For each metabolite feature we now extract a time series (list of values) corresponding to the set of times:

In[160]:= `timeSeriesMetabolites = CreateTimeSeries[massMatchedFilteredMetabolites]`

Out[160]=
```
⟨| ⋯ 1 ⋯ |⟩
```
large output   **show less**   **show more**   **show all**   **set size limit…**

Take Difference Compared to Reference in Metabolome Time Series.

Now we need to compare to compare the difference of each intensity for a given metabolite's time series to the intensity of the ratios of expression at any time point compared to a healthy datapoint. We can use the function SeriesInternalCompare :

We compare every value in each series to the healthy "255" time point, which is the second element in each series:

In[161]:= `metabolitesCompared = SeriesInternalCompare[timeSeriesMetabolites, ComparisonIndex → 2]`

Out[161]=
```
⟨|{457.002, 0.34764, Meta} → {Missing[], 0., -0.326659, -0.244843, 0.0307746, -0.112847, ⋯ 3 ⋯ ,
   -0.640794, -0.165613, Missing[], -0.340455, -0.143904, Missing[]}, ⋯ 4599 ⋯ , { ⋯ 1 ⋯ } → ⋯ 1 ⋯ |⟩
```
large output   **show less**   **show more**   **show all**   **set size limit…**

Take the Norm and Remove Constant Metabolome Time Series

Next, we normalize each series, using again SeriesApplier :

In[162]:= `normedMetabolitesCompared = SeriesApplier[Normalize, metabolitesCompared]`

Out[162]=

⟨| {457.002, 0.34764, Meta} → {Missing[], 0., -0.343784, -0.25768, 0.032388, -0.118763, ⸺ 3 ⸺, -0.674389,
  -0.174295, Missing[], -0.358304, -0.151448, Missing[]}, ⸺ 4599 ⸺, { ⸺ 1 ⸺ } → ⸺ 1 ⸺ |⟩

large output ⸺ **show less** ⸺ **show more** ⸺ **show all** ⸺ **set size limit...**

Finally, we use ConstantSeriesClean to remove constant series, as we are interested in changing time patterns:

In[163]:= `metabolomeFinalTimeSeries = ConstantSeriesClean[normedMetabolitesCompared]`

Out[163]=

⟨| {457.002, 0.34764, Meta} → {Missing[], 0., -0.343784, -0.25768, 0.032388, -0.118763, ⸺ 3 ⸺, -0.674389,
  -0.174295, Missing[], -0.358304, -0.151448, Missing[]}, ⸺ 4599 ⸺, { ⸺ 1 ⸺ } → ⸺ 1 ⸺ |⟩

large output ⸺ **show less** ⸺ **show more** ⸺ **show all** ⸺ **set size limit...**

Resampling Metabolome Data

We also would like to create a resampled distribution for the metabolome dataset prior to classification and clustering. In this subsection we first resample the imported metabolome dataset. Then, we carry out the full analysis in this "bootstrap" dataset, to create a set of random metabolome time series. This bootstrap distribution of time series will be used to provide the cutoffs used in the time series classification in the following subsection.

Resampling the Proteome Data

We create a resampling of 100000 sets:

In[164]:= `metabolitesBootstrap = BootstrapGeneral[metabolitesExample, 100 000]`

Out[164]=

⟨| 8 → ⟨| 1 → {{88 478, 100 725, 59 680},
  {2-pentadecenoic acid [C15 H28 O2, db=82.32, overall=82.52, mfg=46.15, Lipid ID=, KEGG ID=],
  }}, ⸺ 99 998 ⸺ ,
  100 000 → {{44 327, 153 862, 33 442}, {5alpha-Cholan-24-oic Acid ... .87, Lipid ID=, KEGG ID=], }} |⟩,
  ⸺ 10 ⸺ , 20 → ⟨| ⸺ 1 ⸺ |⟩ |⟩

large output ⸺ **show less** ⸺ **show more** ⸺ **show all** ⸺ **set size limit...**

Processing the Bootstrap Metabolome and Creating Bootstrap Time Series

We implement a Median calculation, and ignoring entries with missing and values of 1 for the bootstrap set:

In[165]:= `metaboliteBootstrapMedians =`
`    MeasurementApplier[Median, metabolitesBootstrap, IgnorePattern → (_Missing | 1 | 1.)];`

We apply a Box-Cox transformation to the bootstrap metabolite median data in the OmicsObject, which is now the first list first component for each identifier. The optimized $\hat{\lambda}$ parameter for each sample is printed out for reference:

In[166]:= `transformedBootstrapMetaboliteData =`
`    ApplyBoxCoxTransform[metaboliteBootstrapMedians, ListIndex → 1, ComponentIndex → 1]`

```
Calculated Box-Cox parameter λ̂ = -0.287152
Calculated Box-Cox parameter λ̂ = -0.280376
Calculated Box-Cox parameter λ̂ = -0.276347
Calculated Box-Cox parameter λ̂ = -0.260243
Calculated Box-Cox parameter λ̂ = -0.270257
Calculated Box-Cox parameter λ̂ = -0.273974
Calculated Box-Cox parameter λ̂ = -0.294708
Calculated Box-Cox parameter λ̂ = -0.265066
Calculated Box-Cox parameter λ̂ = -0.280128
Calculated Box-Cox parameter λ̂ = -0.269042
Calculated Box-Cox parameter λ̂ = -0.265108
Calculated Box-Cox parameter λ̂ = -0.262923
```

Out[166]=

⟨|8 → ⟨|1 → {{3.35022},
  {2-pentadecenoic acid [C15 H28 O2, db=82.32, overall=82.52, mfg=46.15, Lipid ID=, KEGG ID=],
  }}, 2 → { ... 1 ... }, ... 99 996 ... , 99 999 → ... 1 ... , 100 000 →
  {{3.32119}, {5alpha-Cholan-24-oic Acid [... 96.87, Lipid ID=, KEGG ID=], }}|⟩, ... 10 ... , ... 1 ... |⟩

large output   **show less**   **show more**   **show all**   **set size limit...**

We may also wish to standardize the distributions:

In[167]:=
```
metabolitesBootstrapStandardized = Returner[transformedBootstrapMetaboliteData,
  Applier[StandardizeExtended[#, Mean, StandardDeviation] &, transformedBootstrapMetaboliteData,
  ListIndex → 1, ComponentIndex → 1], ListIndex → 1, ComponentIndex → 1]
```

Out[167]=

⟨|8 → ⟨|1 → {{-0.0237496},
  {2-pentadecenoic acid [C15 H28 O2, db=82.32, overall=82.52, mfg=46.15, Lipid ID=, KEGG ID=],
  }}, 2 → { ... 1 ... }, ... 99 996 ... , 99 999 → ... 1 ... , 100 000 →
  {{-0.714262}, {5alpha-Cholan-24-oic Acid ... .87, Lipid ID=, KEGG ID=], }}|⟩, ... 10 ... , ... 1 ... |⟩

large output   **show less**   **show more**   **show all**   **set size limit...**

We can now do a KeyMap to rename the outer keys with labels corresponding to days:

In[168]:=
```
metabolitesBootstrapLongitudinal = KeyMap[sampleToDays, metabolitesBootstrapStandardized];
```

Now let's check the timepoints in this dataset:

In[169]:=
```
timesMetaboliteBootstrapData = TimeExtractor[metabolitesBootstrapLongitudinal]
```

Out[169]= {255, 289, 290, 292, 294, 297, 301, 307, 311, 322, 369, 380}

We can use the ConstantAssociator function to append the "186", "329" and "400" missing days to the transformed bootstrap data:

In[170]:=
```
metabolitesBootstrapLongitudinalEnlarged = ConstantAssociator[
  metabolitesBootstrapLongitudinal, <|"186" → Missing[], "329" → Missing[], "400" → Missing[]|>];
```

We can now check the timepoints again:

In[171]:= **timesMetabolitesBootstrap = TimeExtractor[metabolitesBootstrapLongitudinalEnlarged]**

*Out[171]=* {186, 255, 289, 290, 292, 294, 297, 301, 307, 311, 322, 329, 369, 380, 400}

We next remove values that have been tagged overall as Missing[], retaining data that have at least 3/4 data points available across all samples.
Additionally we remove data where the reference healthy sample "255" was missing. We use the function FilterMissing for this implementation:

In[172]:= **filteredMetaboliteBootstrapData =
    FilterMissing[metabolitesBootstrapLongitudinalEnlarged, 3/4, Reference → "255"];**

Number of Missing Data Points per Component
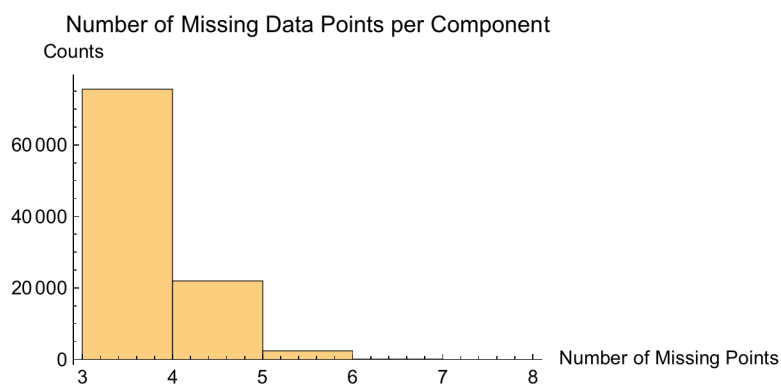
{Missing -> Counts: , ⟨|3 → 75 579, 4 → 21 924, 5 → 2352, 6 → 142, 7 → 3|⟩}

Pie Chart of number of missing components
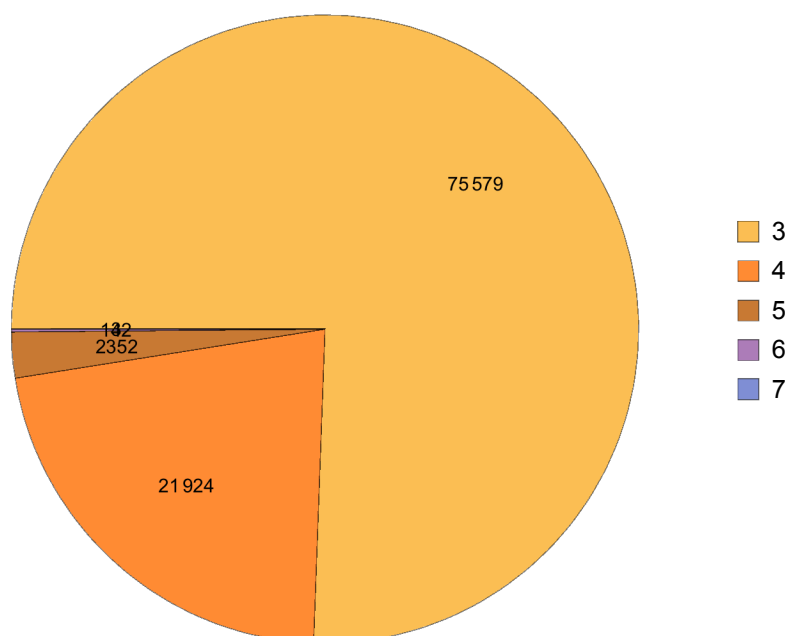
For each bootstrap metabolite feature we now extract a time series (list of values) corresponding to the set of times:

In[173]:= **timeSeriesMetabolitesBootstrap = CreateTimeSeries[filteredMetaboliteBootstrapData];**

We compare every value in each bootstrap series to the healthy "255" time point, which is the second element in each series:

In[174]:= `metabolitesBootstrapCompared = SeriesInternalCompare[timeSeriesMetabolitesBootstrap, ComparisonIndex → 2];`

Next, we normalize each series, using again SeriesApplier :

In[175]:= `normedMetabolitesBootstrapCompared = SeriesApplier[Normalize, metabolitesBootstrapCompared];`

Finally, we use ConstantSeriesClean to remove constant series, as we are interested in changing time patterns:

In[176]:= `metabolomeBootstrapFinalTimeSeries = ConstantSeriesClean[normedMetabolitesBootstrapCompared];`

Classification of Metabolome Time Series

In this subsection we will classify the metabolome time series based on patterns in the series. For the classification we will use TimeSeriesClassification . We will use QuantileEstimator for the "LombScargle" method to provide a cutoff for the TimeSeriesClassification inputs.

First, we estimate for the "LombScargle" Method, 0.95 quantile cutoff from the bootstrap metabolome data:

In[177]:= `q95Metabolites = QuantileEstimator[metabolomeBootstrapFinalTimeSeries, timesMetabolitesBootstrap]`

Out[177]= `0.846125`

Next, we estimate the "Spikes" 0.95 quantile cutoff from the bootstrap proteome data:

In[178]:= `q95MetabolitesSpikes =`
`  QuantileEstimator[metabolomeBootstrapFinalTimeSeries, timesMetabolitesBootstrap, Method → "Spikes"]`

Out[178]= `⟨| 12 → {0.67052, -0.651833} |⟩`

Now we can classify the proteome time series data based on these cutoffs:

In[179]:= `metaboliteClassification = TimeSeriesClassification[metabolomeFinalTimeSeries,`
`    timesMetabolites, LombScargleCutoff → q95Metabolites, SpikeCutoffs → q95MetabolitesSpikes]`

`   Method → "LombScargle"`

Out[179]=
```
⟨| SpikeMax →
  ⟨| {1514.1, 0.366235, Meta} → {{0.150094, 0.150759, 0.336515, 0.197558, 0.430385, 0.667846, 0.41379},
     {Missing[], 0.,  … 12 … , Missing[]}},  … 134 … , { … 1 … } →  … 1 … |⟩,  … 6 … |⟩
```

| large output | show less | show more | show all | set size limit... |

As discussed above, the default output for TimeSeriesClassification is an Association with outer keys being the classification classes, inner keys being the class members, and each class member value being a list of {{computed classification vector}, {input data list}}.

If we want the classes produced, we can query the keys:

In[180]:= `Keys[metaboliteClassification]`

Out[180]= `{SpikeMax, SpikeMin, f1, f2, f5, f6, f7}`

For the number of members in each class we have:

In[181]:= `Query[All, Length] @metaboliteClassification`

Out[181]= `⟨| SpikeMax → 136, SpikeMin → 713, f1 → 63, f2 → 38, f5 → 43, f6 → 15, f7 → 33 |⟩`

We can obtain the membership list in any class of interest:

In[182]:= `Query["f1", Keys]@metaboliteClassification`

Out[182]= {{373.859, 0.411324, Meta}, {cpd:C11821, 184.024, 0.653444, Meta}, {221.109, 10.3062, Meta},
{cpd:C18218, 272.235, 12.7737, Meta}, {294.166, 13.0495, Meta}, {631.385, 13.5221, Meta},
{563.32, 13.7008, Meta}, {779.604, 13.9622, Meta}, {362.266, 14.001, Meta},
{cpd:C17873, 384.36, 14.2982, Meta}, {390.297, 14.3592, Meta}, {420.361, 14.6658, Meta},
{434.376, 14.7796, Meta}, {392.366, 15.0173, Meta}, {394.381, 15.1519, Meta}, {1599.15, 15.281, Meta},
{693.628, 15.6921, Meta}, {874.715, 15.9118, Meta}, {281.986, 0.390455, Meta}, {504.309, 14.3911, Meta},
{416.313, 14.4627, Meta}, {735.521, 15.1792, Meta}, {571.961, 0.388167, Meta}, {489.958, 0.388912, Meta},
{325.95, 0.392472, Meta}, {465.913, 0.393056, Meta}, {383.909, 0.397722, Meta}, {301.906, 0.407861, Meta},
{219.903, 0.412111, Meta}, {161.944, 0.413086, Meta}, {139.061, 0.458472, Meta},
{115.064, 0.463972, Meta}, {71.074, 0.482559, Meta}, {253.165, 9.12729, Meta}, {298.132, 9.30967, Meta},
{cpd:C20605, 411.179, 9.3167, Meta}, {440.201, 11.2909, Meta}, {355.218, 12.7443, Meta},
{338.244, 12.8545, Meta}, {1061.15, 13.0612, Meta}, {210.198, 13.1613, Meta}, {501.367, 13.296, Meta},
{594.375, 13.3701, Meta}, {1538.03, 13.3796, Meta}, {404.314, 13.6028, Meta}, {692.323, 13.7652, Meta},
{670.265, 13.8732, Meta}, {814.584, 14.1513, Meta}, {366.349, 14.3015, Meta}, {442.402, 14.3568, Meta},
{406.381, 14.3581, Meta}, {278.152, 14.364, Meta}, {cpd:C19658, 344.271, 14.4331, Meta},
{420.358, 14.4446, Meta}, {311.319, 14.6119, Meta}, {791.583, 15.4236, Meta}, {1553.18, 15.4429, Meta},
{1545.17, 15.5017, Meta}, {352.052, 0.53368, Meta}, {cpd:C17237, 254.073, 12.2926, Meta},
{336.228, 12.5103, Meta}, {638.402, 13.4139, Meta}, {668.324, 13.988, Meta}}

To obtain the possible frequencies we simply run LombScargle over the desired times for one of the time series and set the `FrequenciesOnly` option to True:

In[183]:= `LombScargle[metabolomeFinalTimeSeries[[1]], timesMetabolites, FrequenciesOnly → True]`

Out[183]= ⟨| f1 → 0.00500668, f2 → 0.0104306, f3 → 0.0158545,
f4 → 0.0212784, f5 → 0.0267023, f6 → 0.0321262, f7 → 0.0375501 |⟩

Combined Data Clustering

In this section we will combine the omics data classes from the individual classifications above using JoinNestedAssociations and hierarchically cluster the information to obtain a second level of classification using TimeSeriesClusters . We will visualize the results in the following section.

Combining Multi-omics Classified Data

`JoinNestedAssociations[`*associationList*`]` merges the nested **associationList** (an association of associations) by joining the inner associations for each matching key.

Joining classification data.

We combine the classification data using JoinNestedAssociations:

In[184]:= `combinedClassification =`
`JoinNestedAssociations[{rnaClassification, proteinClassification, metaboliteClassification }]`

Out[184]= ⟨| SpikeMax → ⟨| ... 1 ... |⟩, ... 7 ... ,
f7 → ⟨| {MIR6723, RNA} → {{0.214503, 0.000338299, 0.0390479, 0.0653206, 0.291434, 0.336712, 0.865961},
{ ... 1 ... }}, ... 110 ... , {632.536, 15.9029, Meta} → ... 1 ... |⟩ |⟩

large output   **show less**   **show more**   **show all**   **set size limit...**

We can check the keys before and after the combination:

In[185]:= **Keys[#] & /@ {rnaClassification, proteinClassification, metaboliteClassification }**

Out[185]= {{SpikeMax, SpikeMin, f1, f2, f3, f4, f5, f6, f7},
{SpikeMax, SpikeMin, f1, f5, f6, f7}, {SpikeMax, SpikeMin, f1, f2, f5, f6, f7}}

In[186]:= **Keys @ combinedClassification**

Out[186]= {SpikeMax, SpikeMin, f1, f2, f3, f4, f5, f6, f7}

We can also check the membership counts before and after the combination:

In[187]:= **Query[All, Length] @# & /@ {rnaClassification, proteinClassification, metaboliteClassification }**

Out[187]= { ⟨| SpikeMax → 822, SpikeMin → 5963, f1 → 116, f2 → 3, f3 → 30, f4 → 128, f5 → 35, f6 → 13, f7 → 61 |⟩,
⟨| SpikeMax → 124, SpikeMin → 48, f1 → 77, f5 → 7, f6 → 36, f7 → 18 |⟩,
⟨| SpikeMax → 136, SpikeMin → 713, f1 → 63, f2 → 38, f5 → 43, f6 → 15, f7 → 33 |⟩ }

In[188]:= **Query[All, Length] @ combinedClassification**

Out[188]= ⟨| SpikeMax → 1082, SpikeMin → 6724, f1 → 256, f2 → 41, f3 → 30, f4 → 128, f5 → 85, f6 → 64, f7 → 112 |⟩

Clustering of Classified Data

Now that we have combined the classes for the various omics, we can cluster them together to obtain the various trends using TimeSeriesClusters . A two-tier hierarchical clustering of the data is performed, using a set of two classification vectors, {{classification vector$_1$}, {classification vector$_2$}} for each time series to cluster the data pairwise. The vectors are typically the output from TimeSeriesClassification . Similarities at each clustering tier are then computed using in succession from each time series first {classification vector$_1$}, and subsequently {classification vector$_2$} (which corresponds to the {input data time series} if the input is from TimeSeriesClassification).

The number of groups and subgroups for each tier of clustering is automatically determined by using internally the "Silhouette" (default) or "Gap" as "SignificanceTest" methods (see also Partitioning Data into Clusters).

`TimeSeriesClusters`[*data*]   performs clustering of time series data using two tiers of hierarchical clustering to identify groups and subgroups in the data. TimeSeriesClusters takes as input series data, where each data is comprised of two lists and performs clustering of the data to identify groups and subgroups based on similarities between the input series. The form of the input data is either an association of classes and members, where each member must have a list of two components, typically two vectors used in classification: `{{classification vector`$_1$`},` `.` `{classification vector`$_2$`}}` in the most common case of using as input data that came from performing a TimeSeriesClassification, the `{classification vector`$_2$`}` will correspond to input original data for the corresponding time series.

Clustering of classified time series.

option name	default value	
ClusterLabeling	""	Additional label to append to each cluster being computed to prepend to the inbuilt G♯S♯ labeling.
DendrogramPlotOptions	{}	Options passed to the DendrogramPlot function used internally to generate the dendrograms.
DistanceFunction	EuclideanDistance	Distance function to be used in calculating the similarities between different time series in the first tier of clustering.
LinkageMeasure	"Average"	Which linkage measure to use in computing fusion coefficients.
PrintDendrograms	False	Option to print dendrograms for the clustering computed.
ReturnDendrograms	False	Option to return the dendrograms as output.
SignificanceCriterion	"Silhouette"	Method used in determining the number of groups and subgroups at each tier of clustering.
SingleAssociationLabel	"1"	Label to use in case a list is provided to name the class of data produced.
SubclusteringDistanceFunction	EuclideanDistance	Distance function to be used in calculating the similarities between different time series in the second tier of clustering.

Options for **TimeSeriesClusters** .

The output of TimeSeriesClusters is always an association of associations, providing a summary of the two tier clustering results for each class provided in the input. The output has the form:

```
output =
 <| Class₁ → <|"Cluster" → cluster object₁,
      "InitialSplitCluster" → {InitialSplitCluster₁₁, InitialSplitCluster₁₂ ...},
      "IntermediateClusters" → {IntermediateCluster₁₁, IntermediateCluster₁₂ ...},
      "SubsplitClusters" → {{SubsplitClusters₁₁} {SubsplitClusters₁₂}},
      "Data" → {{input data vector₁₁} → Member₁₁, ...,},
      "GroupAssociations" → <|"G1S1" → {member list G1S1},
         "G1S2" → {member list for G1S2},
         ...,
         "G2S1" → { ...}|>|>,
   Class₂ → <|"Cluster" → cluster object₂,
      "InitialSplitCluster" → {InitialSplitCluster₂₁, InitialSplitCluster₂₂ ...},
      "IntermediateClusters" → {IntermediateCluster₂₁, IntermediateCluster₂₂ ...},
      "SubsplitClusters" → {{SubsplitClusters₂₁} {SubsplitClusters₂₂}},
      "Data" → {{input data vector₂₁} → Member₂₁, ...,},
      "GroupAssociations" → <|"G1S1" → {member list G1S1},
         "G1S2" → {member list for G1S2},
         ...,
         "G2S1" → { ...}|>|>,
   ...,
   Classₘ → <|"Cluster" → cluster objectₘ,
      "InitialSplitCluster" → {InitialSplitClusterₘ₁, InitialSplitClusterₘ₂ ...},
      "IntermediateClusters" → {IntermediateCluster ₘ₁, IntermediateClusterₘ₂ ...},
      "SubsplitClusters" → {{subsplitClustersₘ₁} {subsplitClustersₘ₂}},
      "Data" → {{input data vectorₘ₁} → Memberₘ₁, ...,},
      "GroupAssociations" → <|"G1S1" → {member list G1S1},
         "G1S2" → {member list for G1S2},
         ...,
         "G2S1" → { ...}|>|>
 |>
```

Method	Description
`"Cluster"`	Cluster generated using the input $\{\texttt{classification vector}_1\}$ for similarity calculations.
`"InitialSplitCluster"`	Clusters resulting from splitting the initial cluster (reported by key "Cluster") into groups using the SignificanceCriterion to determine the number of clusters.
`"IntermediateClusters"`	Aglomerative clustering result of hierarchical clustering of each of the initial split clusters (reported by "InitialSplitCluster")
`"SubsplitClusters"`	Custers generated from splitting the clusters following the second tier clustering (reported by "IntermediateClusters") into subgroups using the SignificanceCriterion to determine the number of clusters.
`"Data"`	Data reported in the order of clustering results as rules of $\{\texttt{classification vector}_2\} \rightarrow \texttt{label}$ for each time series, sorted in order of the clustering results.
`"GroupAssociations"`	Association denoting membership of each initial data label to groups and subgroups generated by the two tier clustering.

Output keys for **TimeSeriesClusters** provide clustering information.

We now cluster our combined data (a printout of the clusters is included as a default option):

In[189]:= `combinedClusters = TimeSeriesClusters[combinedClassification]`

Out[189]=
⟨| SpikeMax → ⟨| ⋯ 1 ⋯ |⟩, SpikeMin → ⟨| ⋯ 1 ⋯ |⟩, ⋯ 5 ⋯, f6 → ⋯ 1 ⋯,
  f7 → ⟨| Cluster → Cluster[⋯ 1 ⋯], ⋯ 4 ⋯, GroupAssociations → ⟨| G1S1 → {{MIR6723, RNA}, {ZNF324, RNA},
         {CBX6, RNA}, ⋯ 106 ⋯, {O60884, Protein}, {RTFDC1, RNA}, {246.121, 0.940379, Meta}}|⟩ |⟩ |⟩

large output   **show less**   **show more**   **show all**   **set size limit...**

Visualization

After our data have been clustered, we would like to visualise the results in heatmaps and dendrograms. For the two-tier clustering we have performed MathIOmica can output all the clusterings in labeled dendrograms and heatmaps using TimeSeriesDendrogramsHeatmaps, which iteratively calls TimeSeriesDendrogramHeatmap on each class.

TimeSeriesDendrogramsHeatmaps [*data*]	generates dendrograms and associated heatmap plots for clustered time series data, typically the output of all classes generated by implementing TimeSeriesClusters.
TimeSeriesDendrogramHeatmap [*data*]	generates a dendrogram and heatmap plot for one set of time series *data* clusters, typically the output of a single class of TimeSeriesClusters.

Visualizing the results of classification.

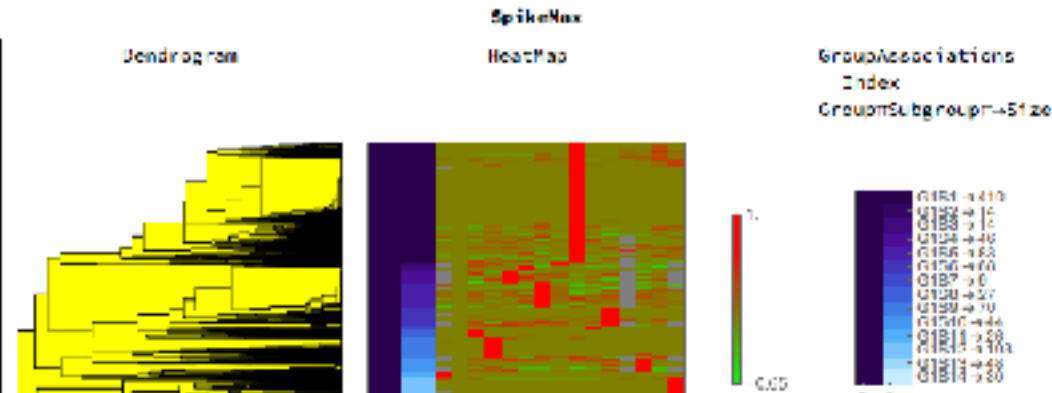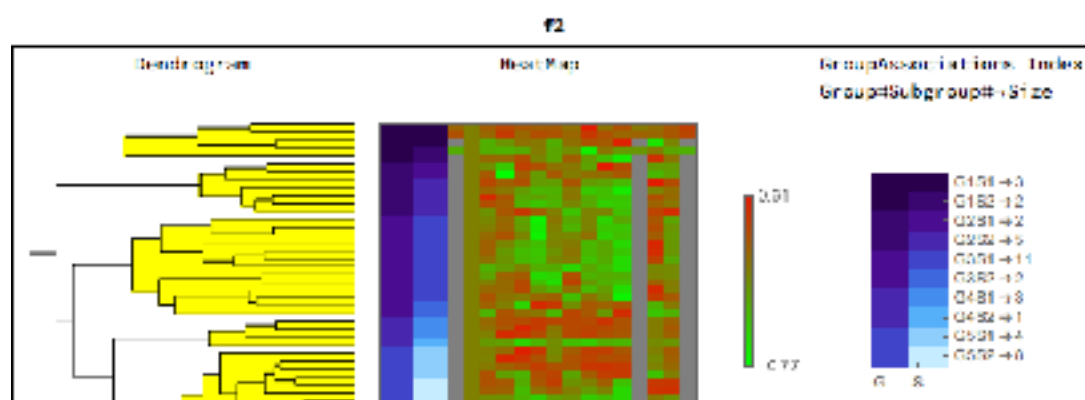| option name	default value	
FunctionOptions	{ImageSize -> 200}	Options list passed to the internal TimeSeriesDendrogramHeatmap function.

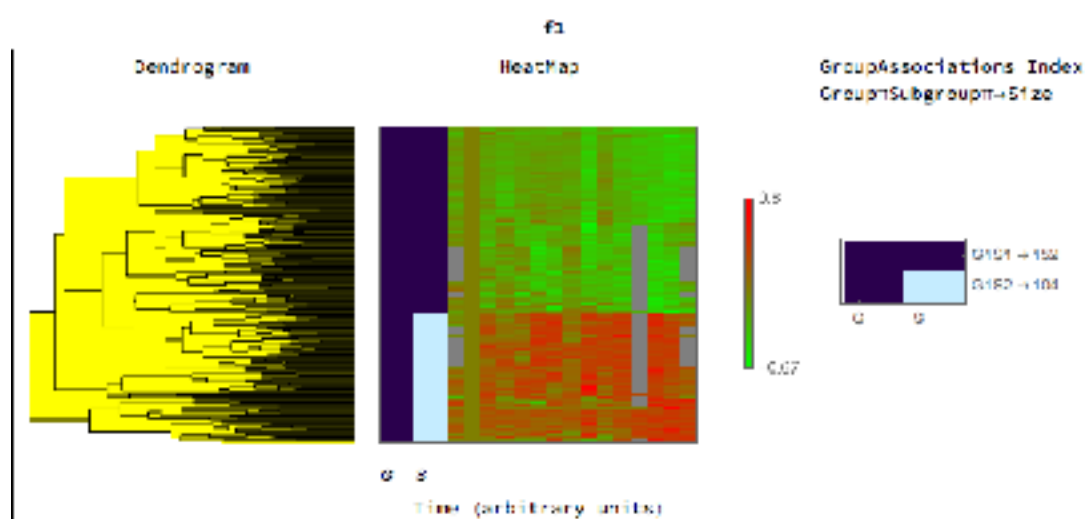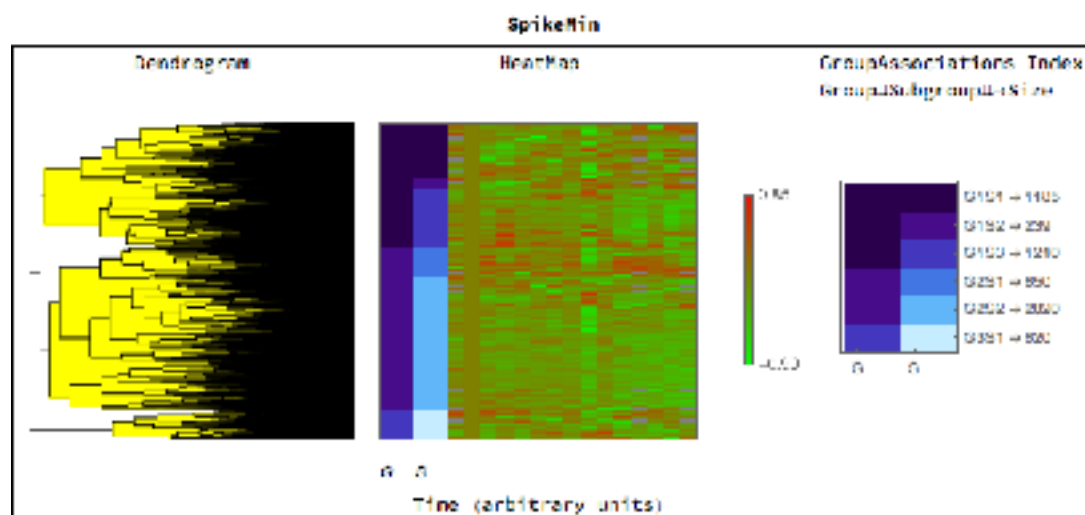Options for TimeSeriesDendrogramsHeatmaps .

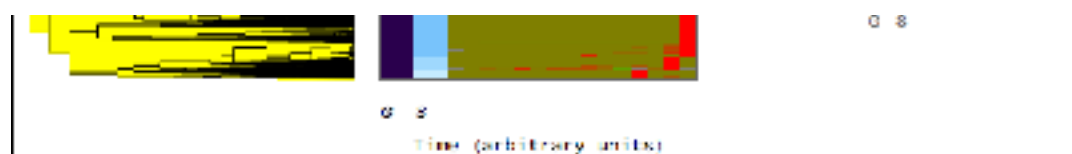| option name	default value	
ColorBlending	`{CMYKColor[1, 0, 1, 0], CMYKColor[0, 1, 1, 0]}`	Color scheme for the plot. The color list is passed to an internal **Blend** function to create a **ColorFunction** for an internal **ArrayPlot** function.
DendrogramColor	`RGBColor[1, 1, 0]`	Color to highlight the dendrograms.
FrameName	`"Dendrogram and Heatmap"`	Label for plot frame.
GroupSubSize	`{0.1, 0.1}`	Relative size of group and subgroup reference column in plot.
HorizontalAxisName	`"Time (arbitrary units)"`	Label for the horizontal heatmap axis.
HorizontalLabels	`None`	Labels for horizontal axis for each column.
IndexColor	`"DeepSeaColors"`	Choice of color for labeling the group/subgroup index.
ImageSize	`200`	**ImageSize** is an option that specifies the overall size of an image to display for an object.
ScaleShift	`None`	Option to reset the blend of the colors used overall. The option is a real positive number, and is used as a multiplier for an internal **Blend** function's second argument.
VerticalLabels	`None`	Labels for vertical axis for each row.

Options for TimeSeriesDendrogramHeatmap .

For each class a separate plot is generated: dendrograms are represented on the left, and are highlighted to represent the grouping level. The G, S, columns represent the groupings and subgroupings generated by the clustering. The legend shows the corresponding groupings and subgrouping, and the number of elements in each group subgroup.

In[190]:= **TimeSeriesDendrogramsHeatmaps[combinedClusters]**

SpikeMin

f1

f2

f3

Dendrogram HeatMap GroupAssociations Index
GroupuSubgroupu→Size

Out[190]=

Time (arbitrary units)

f4

Dendrogram HeatMap GroupAssociations Index
GroupuSubgroupu→Size

Time (arbitrary units)

f5

Dendrogram HeatMap GroupAssociations Index
GroupuSubgroupu→Size

f6

f7
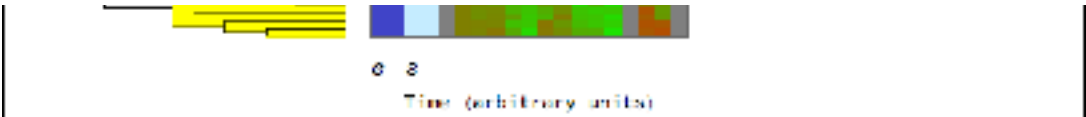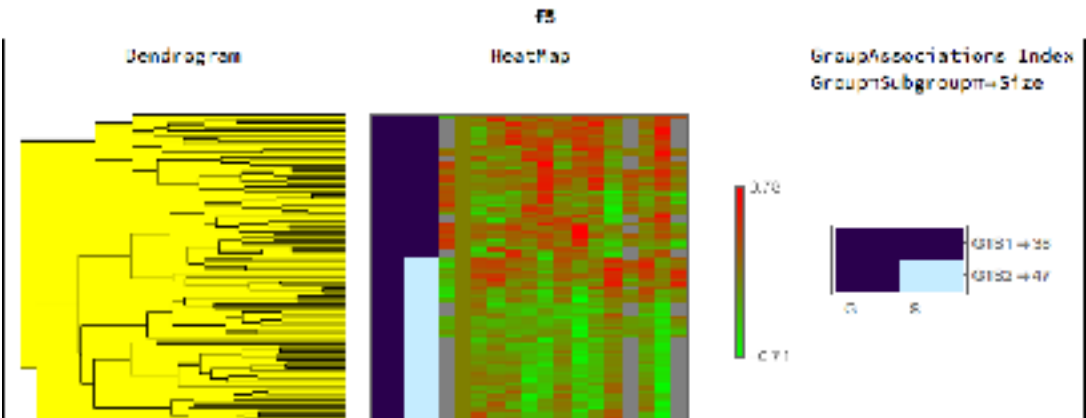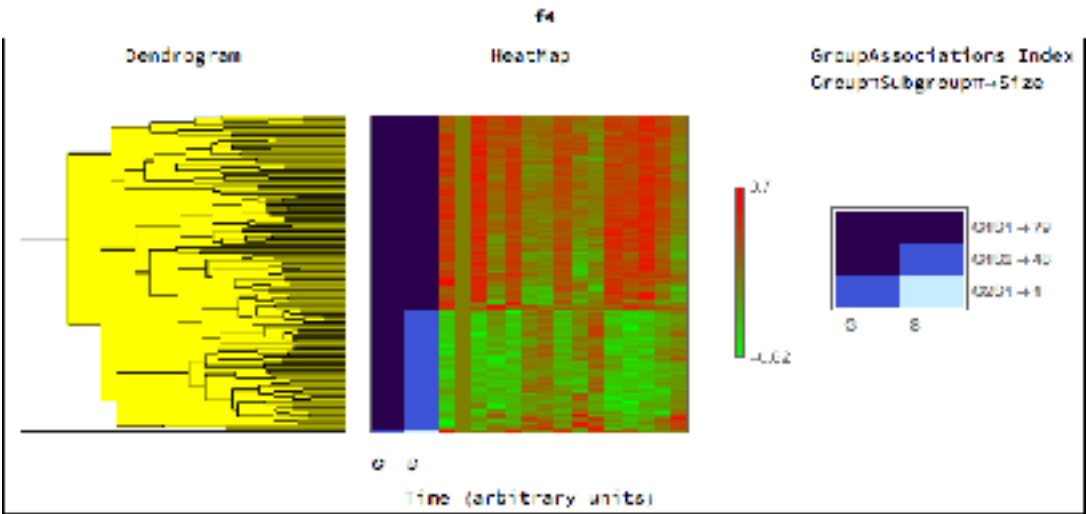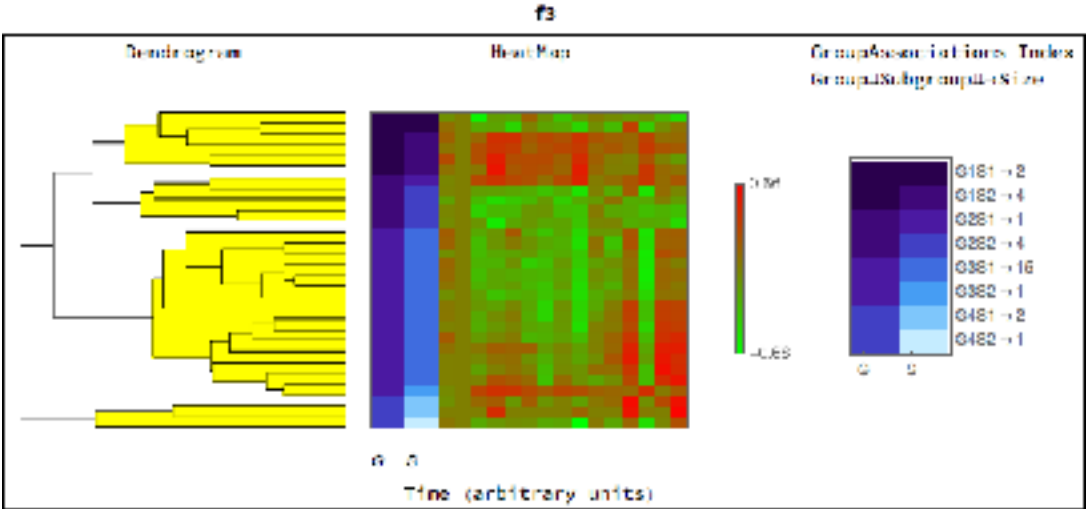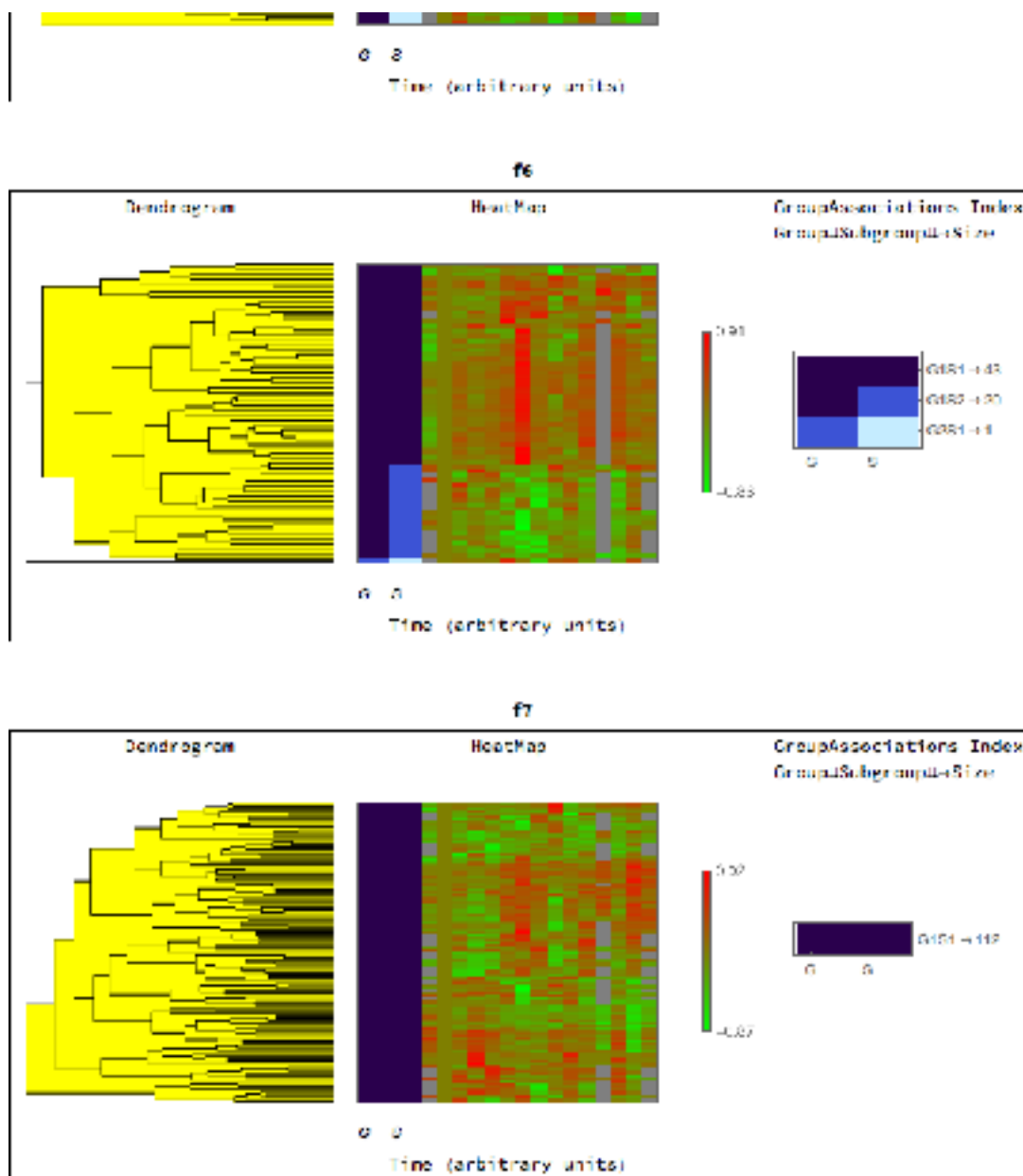
Annotation and Enrichment

Having carried out the classification and clustering of data base on its temporal pattern, we would like to perform annotation of these data for gene ontology (GO) and pathways from KEGG: Kyoto Encyclopedia of Genes and Genomes.

Gene Ontology Analysis

MathIOmica provides a **GOAnalysis** function using annotations (default is for human data) obtained from the Gene Ontology consortium, and by default uses human data annotated with UniProt IDs. The **GOAnalysis** function performs an over-representation (ORA) analysis, providing a "significance" cutoff based on a p-value assessed by a hypergeometric function.

GOAnalysis[*data*]   calculates input data over–representation analysis (ORA) for Gene Ontology (GO) categories. We note that the function utilizes ontologies obtained from the GO Consortium, and by default uses human data annotated with UniProt IDs.

Performing an over representation analysis for Gene Ontology (GO) terms, using clustered data in MathIOmica.

option name	default value	
AdditionalFilter	None	AdditionalFilter provides additional filtering that may be applied to the standard output structure to be returned.
AugmentDictionary	True	AugmentDictionary provides a choice whether or not to augment the current ConstantGeneDictionary variable or create a new one.
BackgroundSet	All	BackgroundSet provides a list of IDs (e.g. gene accessions) that should be considered as the background for the calculation.
FilterSignificant	True	FilterSignificant can be set to True to filter data based on whether the enrichment analysis is statistically significant, or if set to False to return all membership computations.
GeneDictionary	None	GeneDictionary points to an existing variable to use as a gene dictionary in annotations. If set to None the default ConstantGeneDictionary will be used.
GetGeneDictionaryOptions	{}	The GetGeneDictionaryOptions option specifies a list of options that will be passed to the internal GetGeneDictionary function.
GOAnalysisAssignerOptions	{}	The GOAnalysisAssignerOptions option specifies a list of options that will be passed to the internal GOAnalysisAssigner function.

HypothesisFunction	`(Query["Results"][`
	`    BenjaminiHo-`
	`       chbergFDR[`
	`       ♯1,`
	`       Significa-`
	`       nceLevel`
	`       ->`
	`       ♯2]] &)`
		The HypothesisFunction option allows us to chose a function to implement multiple hypothesis testing. The default is using the BenjaminiHochbergFDR function.
		
		The user can use any function f with three inputs, of the form f[♯1,♯2,♯3] where the inputs refer to:
		♯1 is the p–value list,
		♯2 is a significance cutoff,
		♯3 is the number of GO associations overall being tested.
		The function f must output a list of 3 values: {original p–value, adjusted p–value, True or False based on whether this value is considered statistically significant or not respectively}.

InputID	`{"UniProt ID",` `  "Gene Symbol"}`	The InputID option specifies the kind of identifiers/accessions used as input.
MultipleList	`False`	MultipleList option specifies whether the input accessions list constituted a multi–omics list input that is annotated so. If this is the case, MultipleList is set to True and each input list ID should have the form {ID,"Omics Type Label"}, e.g. {"NFKB1","Protein"}, and the different omics type are treated as different for each ID. If MultipleList is set to False, and labeled IDs are provided, labels corresponding to the same ID are treated as equivalent to avoid overcounting.
MultipleListCorrection	`None`	MultipleListCorrection is an option whether or not to correct for multi–omics analysis. The choices are None, Automatic, or a custom number. This essentially enlarges the population by this factor to account for additional IDs being considered as the result of a multi–omics cluster analysis. If the value is set to Automatic the number of unique ID labels is used to make the correction.

`OBOGODictionaryOptions`	`{}`	OBOGODictionaryOptions specifies a list of options to be passed to the internal **OBOGODictionary** function that provides the GO annotations.
`OBODictionaryVariable`	`None`	OBODictionaryVariable can provide a GO annotation variable. If set to **None**, **OBOGODictionary** will be used internally to automatically generate the default GO annotation.
`OntologyLengthFilter`	`2`	OntologyLengthFilter can be used to set the value for which terms to consider in the computation, by excluding GO terms that have fewer items compared to the OntologyLengthFilter value. It is used by the internal **GOAnalysisAssigner** function.
`OutputID`	`"UniProt ID"`	The OutputID option takes a string value that specifies what kind of IDs/accessions to convert the input IDs to compute the GO enrichment.
`pValueCutoff`	`0.05`	pValueCutoff provides a cutoff p–value for adjusted p–values to assess statistical significance.
`ReportFilter`	`1`	ReportFilter provides a cutoff for membership in ontologies in selecting which terms/categories to return. It is used in conjunction with ReportFilterFunction.
`ReportFilterFunction`	`GreaterEqualThan`	ReportFilterFunction specifies what operator form will be used to compare against ReportFilter option value in selecting which terms/categories to return. The default is to use GreaterEqualThan.
`Species`	`"human"`	The Species option specifies the species considered in the calculation.

| TestFunction | `N[1 - CDF[` `Hypergeom-` `etricDist-` `ribution[` `#1, #2,` `#3], #4 -` `1]] &` | The TestFunction option provides a function used to calculate the p–values for the enrichment of each term. It can be a function of four inputs, f[#1,#2,#3,#4] (e.g. the default is using a hypergeometric distribution CDF, N[1–CDF[**HypergeometricDistribution**[#1,#2,#3],#4–1]]]. The four inputs refer to: #1 is number of draws (members in group being tested), #2 is number of successes for category in population, #3 is total number of members in population, #4 is number of successes (or more) in current group being tested for specific category. The output is a p–value (real positive number ≤ 1). |

Options for **GOAnalysis** .

The input data for **GOAnalysis** be a single list of n genes in the form:

$data = \{ID_1, ID_2, ..., ID_n\}$

The IDs may be provided as ID strings, or as labeled strings in the case of multiple omics being considered. Labeled IDs are provided as $\{\{ID_1, label_1\}, \{ID_2, label_2\}, ... \{ID_3, label_2\}\}$. The labels are typically a string, e.g. typically "RNA" or "Protein".

The default output contains each GO:term that was considered and found to be statistically significant. For each GO term we schematically have an association with keys $GO : Term \rightarrow \{\{testing\ outcomes\}, \{statistics\}, \{\{GO\ term\}, \{Membership\}\}$. The output has the following structures: for a single list input:

$listOutput = <|$
$\quad GO : Term_1 \rightarrow \{\{p - value_1, multiple\ hypothesis\ adjusted\ p - value_1, True / False\ for\ statistical\ significance\},$
$\quad\quad \{\{number\ of\ members\ in\ group\ being\ tested, number\ of\ successes\ for\ term_1\ in\ population, total\ number\ of$
$\quad\quad\quad members\ in\ population, number\ of\ members\ (or\ more)\ in\ current\ group\ being\ tested\ associated\ to\ term_1\},$
$\quad\quad \{\{GO\ term_1\ description,\ ontology\ category\ for\ term_1\}, \{input\ IDs\ associated\ to\ Term_1\}\}\}\},$
$\quad GO : Term_2 \rightarrow \{\{p - value_2, multiple\ hypothesis\ adjusted\ p - value_2, True / False\ for\ statistical\ significance\},$
$\quad\quad \{\{number\ of\ members\ in\ group\ being\ tested, number\ of\ successes\ for\ term_2\ in\ population, total\ number\ of$
$\quad\quad\quad members\ in\ population, number\ of\ members\ (or\ more)\ in\ current\ group\ being\ tested\ associated\ to\ term_2\},$
$\quad\quad \{\{GO\ term_2\ description,\ ontology\ category\ for\ term_2\}, \{input\ IDs\ associated\ to\ Term_2\}\}\}\}, ...,$
$\quad GO : Term_n \rightarrow \{\{p - value_n, multiple\ hypothesis\ adjusted\ p - value_n, True / False\ for\ statistical\ significance\},$
$\quad\quad \{\{number\ of\ members\ in\ group\ being\ tested, number\ of\ successes\ for\ term_n\ in\ population, total\ number\ of$
$\quad\quad\quad members\ in\ population, number\ of\ members\ (or\ more)\ in\ current\ group\ being\ tested\ associated\ to\ term_n\},$
$\quad\quad \{\{GO\ term_n\ description,\ ontology\ category\ for\ term_n\}, \{input\ IDs\ associated\ to\ term_n\}\}\}\}$
$\quad |>$

GOAnalysis can also take as input the output of clustering of time series classification data, e.g. **TimeSeriesClusters** or **TimeSeriesSingleClusters** association of associations. The groups for each class will then have keys labeled "GroupAssociations", that include the labels used in the

clustering. The labels must correspond to protein or gene accessions/IDs. For each class and group the corresponding GOAnalysis enrichment is computed and returned.

We also note that GOAnalysis provides a multiple-hypothesis adjusted p-value. By default, it utilizes a Benjamini-Hochberg false discovery rate (FDR) using BenjaminiHochbergFDR .

BenjaminiHochbergFDR [*pValues*]	calculates for a list of *pValues*, $\{p_1, p_2, \ldots p_N\}$, the Benjamini Hochberg approach false discovery rates (FDR).

Calculating a false discovery rate (FDR).

We carry out our GOAnalysis for all the classes and groups/subgroups. We only report terms for which there are at least 3 members, and additionally correct for multiple omics (2 sets of GO terms, one each for proteomics and transcriptomics). Please note that this is a time consuming computation.

In[191]:= `goAnalysisCombined = GOAnalysis[combinedClusters, OntologyLengthFilter → 3,`
`    ReportFilter → 3, MultipleList → True, MultipleListCorrection → 2 ];`

We see that the classification is maintained:

In[192]:= `Keys @ goAnalysisCombined`

Out[192]= $\{$SpikeMax, SpikeMin, f1, f2, f3, f4, f5, f6, f7$\}$

Let us extract the top 3 results from all the "SpikeMax" data:

In[197]:= `Query["SpikeMax", All, 1 ;; 3] @ goAnalysisCombined`

Out[197]= ⟨| G1S1 → ⟨| GO:0005515 →
    $\{\{1.89513 \times 10^{-21}, 2.68729 \times 10^{-18}, \text{True}\}, \{243, 17\,602, 94\,482, 110\}, \{\{\text{protein binding, molecular\_function}\},$
    {{{AHI1, RNA}}, {{FAM153C, RNA}}, {{SASS6, RNA}}, {{AP1S3, RNA}}, {{ERO1LB, RNA}}, {{ABL2, RNA}},
    {{RAB3IP, RNA}}, {{TAOK3, RNA}}, {{PAG1, RNA}}, {{CHD9, RNA}}, {{STRN3, RNA}}, {{ARL13B, RNA}},
    {{TLK2, RNA}}, {{SRRM1, RNA}}, {{KIAA0020, RNA}}, {{EPM2AIP1, RNA}}, {{TUBGCP4, RNA}},
    {{SNTB2, RNA}}, {{MLLT4, RNA}}, {{HELLS, RNA}}, {{ITSN1, RNA}}, {{SPRY1, RNA}}, {{PSMD11, RNA}},
    {{NEGR1, RNA}}, {{LYSMD1, RNA}}, {{PPP1R12B, RNA}}, {{KIAA1377, RNA}}, {{CCDC153, RNA}},
    {{PDE1B, RNA}}, {{CEP152, RNA}}, {{SLC24A1, RNA}}, {{BRCA1, RNA}}, {{KIAA1683, RNA}},
    {{DNAJC27, RNA}}, {{MAP4K3, RNA}}, {{ICA1L, RNA}}, {{BARD1, RNA}}, {{KIAA1524, RNA}},
    {{CXCL2, RNA}}, {{SMAD1, RNA}}, {{HOMER1, RNA}}, {{CCDC170, RNA}}, {{CCDC146, RNA}},
    {{RFX3, RNA}}, {{ZNF169, RNA}}, {{SCAI, RNA}}, {{CPEB3, RNA}}, {{CCL4L2, RNA}}, {{SGOL2, RNA}},
    {{ALS2CR12, RNA}}, {{JAKMIP2, RNA}}, {{DCUN1D5, RNA}}, {{TRIP11, RNA}}, {{PRPF4B, RNA}},
    {{SLC4A1AP, RNA}}, {{AZI2, RNA}}, {{PRPF40A, RNA}}, {{RB1CC1, RNA}}, {{USP8, RNA}},
    {{RAD50, RNA}}, {{CUL5, RNA}}, {{PIAS2, RNA}}, {{CEP135, RNA}}, {{KPNA5, RNA}}, {{PWWP2A, RNA}},
    {{O75323, Protein}}, {{P06576, Protein}}, {{EVI5, RNA}}, {{INSR, RNA}}, {{ZFHX3, RNA}},
    {{RAB11FIP2, RNA}}, {{SYNJ2BP, RNA}}, {{P10809, Protein}}, {{P38646, Protein}}, {{Q969V3, Protein}},
    {{P55084, Protein}}, {{P49411, Protein}}, {{Q96A33, Protein}}, {{Q16822, Protein}},
    {{P20674, Protein}}, {{HCST, RNA}}, {{NTNG2, RNA}}, {{PRKCDBP, RNA}}, {{HES1, RNA}},
    {{P30519, Protein}}, {{P10515, Protein}}, {{ZFC3H1, RNA}}, {{P22695, Protein}}, {{P84243, Protein}},
    {{P40939, Protein}}, {{P22307, Protein}}, {{Q9HAN9, Protein}}, {{P10606, Protein}}, {{UGGT1, RNA}},
    {{PLCB1, RNA}}, {{LCOR, RNA}}, {{ATXN2, RNA}}, {{KLHL42, RNA}}, {{FOXO3, RNA}}, {{NEK1, RNA}},
    {{EEA1, RNA}}, {{KIF3B, RNA}}, {{ITSN2, RNA}}, {{CD84, RNA}}, {{LGALS8, RNA}}, {{HMBOX1, RNA}},
    {{O75396, Protein}}, {{O75489, Protein}}, {{P28288, Protein}}, {{Q8NF91, Protein}}}}},
    GO:0005739 → $\{\{1.59686 \times 10^{-16}, 1.13217 \times 10^{-13}, \text{True}\}, \{243, 3200, 94\,482, 40\},$
    {{mitochondrion, cellular_component}, {{{C5orf63, RNA}}, {{AS3MT, RNA}}, {{KIAA1683, RNA}},
    {{DNAJC27, RNA}}, {{SPATA5, RNA}}, {{PPARGC1B, RNA}}, {{PRSS35, RNA}}, {{GDAP1, RNA}},
    {{CXorf23, RNA}}, {{Q9NSE4, Protein}}, {{O75323, Protein}}, {{P06576, Protein}},
    {{DHX29, RNA}}, {{SYNJ2BP, RNA}}, {{P10809, Protein}}, {{Q99798, Protein}},
    {{P38646, Protein}}, {{Q9H9B4, Protein}}, {{P55084, Protein}}, {{Q9NUJ1, Protein}},
    {{P49411, Protein}}, {{P13804, Protein}}, {{P17568, Protein}}, {{P22033, Protein}},
    {{Q16822, Protein}}, {{P83111, Protein}}, {{O95571, Protein}}, {{Q8N4H5, Protein}},
    {{O96008, Protein}}, {{P10515, Protein}}, {{Q96I99, Protein}}, {{P42126, Protein}},
    {{P51970, Protein}}, {{P22695, Protein}}, {{P40939, Protein}}, {{O75947, Protein}},

`` `` `` `` `` `` `` `` `` `` `` `` `` ``
{{Q02218, Protein}}, {{P22307, Protein}}, {{P10606, Protein}}, {{O75489, Protein}}}}},
GO:0005759 → {{3.96966×10⁻¹³, 1.87632×10⁻¹⁰, True}, {243, 686, 94 482, 18},
{{mitochondrial matrix, cellular_component}, {{{MMAA, RNA}}, {{PDP2, RNA}},
{{IBA57, RNA}}, {{BRCA1, RNA}}, {{Q9NSE4, Protein}}, {{P06576, Protein}},
{{P10809, Protein}}, {{Q99798, Protein}}, {{Q9NUJ1, Protein}}, {{P13804, Protein}},
{{P22033, Protein}}, {{Q16822, Protein}}, {{O95571, Protein}}, {{P10515, Protein}},
{{Q96I99, Protein}}, {{P42126, Protein}}, {{Q02218, Protein}}, {{O75489, Protein}}}}}|⟩,
G1S2 → ⟨| |⟩, G1S3 → ⟨|GO:0005634 → {{0.0125255, 0.0296058, True}, {4, 14 394, 94 482, 3},
{{nucleus, cellular_component}, {{{RAB6C, RNA}}, {{MEOX1, RNA}}, {{ZNF613, RNA}}}}}}|⟩,
G1S4 → ⟨|GO:0005737 → {{0.0223137, 0.0308136, True}, {5, 13 296, 94 482, 3},
{{cytoplasm, cellular_component}, {{{SMTN, RNA}}, {{AIFM2, RNA}}, {{NOS3, RNA}}}}}}|⟩,
G1S5 → ⟨|GO:0005515 → {{4.76574×10⁻³³, 3.73157×10⁻³⁰, True}, {75, 17 602, 94 482, 62},
{{protein binding, molecular_function},
{{{P60900, Protein}}, {{P13612, Protein}}, {{Q8IUZ5, Protein}}, {{Q9Y285, Protein}},
{{P13861, Protein}}, {{O94979, Protein}}, {{O14933, Protein}}, {{Q9Y6Y8, Protein}},
{{Q7L2H7, Protein}}, {{P01732, Protein}}, {{Q13439, Protein}}, {{Q15819, Protein}},
{{P19784, Protein}}, {{O14745, Protein}}, {{Q07812, Protein}}, {{Q86UP2, Protein}},
{{Q8N1G4, Protein}}, {{Q01082, Protein}}, {{Q9UEU0, Protein}}, {{Q8N8A2, Protein}},
{{Q13043, Protein}}, {{O14732, Protein}}, {{Q7Z4H3, Protein}}, {{O60826, Protein}},
{{Q9UBE0, Protein}}, {{P30520, Protein}}, {{P54136, Protein}}, {{Q13596, Protein}},
{{P25098, Protein}}, {{P41227, Protein}}, {{Q9HC16, Protein}}, {{P61457, Protein}},
{{Q9Y3L3, Protein}}, {{Q92888, Protein}}, {{P62263, Protein}}, {{P85037, Protein}},
{{O00487, Protein}}, {{P54725, Protein}}, {{Q2TAY7, Protein}}, {{P52756, Protein}},
{{O94776, Protein}}, {{Q13148, Protein}}, {{P06127, Protein}}, {{Q02818, Protein}},
{{P19474, Protein}}, {{P07766, Protein}}, {{Q9Y333, Protein}}, {{ZNF624, RNA}}, {{BLM, RNA}},
{{ZNF772, RNA}}, {{P25788, Protein}}, {{P35998, Protein}}, {{Q9Y3D0, Protein}}, {{O43813, Protein}},
{{Q9Y2V2, Protein}}, {{Q13347, Protein}}, {{Q5JSL3, Protein}}, {{BRMS1L, RNA}},
{{O60841, Protein}}, {{O75534, Protein}}, {{O95218, Protein}}, {{Q99623, Protein}}}}},
GO:0005829 → {{2.69551×10⁻²¹, 1.05529×10⁻¹⁸, True}, {75, 6952, 94 482, 36}, {{cytosol, cellular_component},
{{{P60900, Protein}}, {{O43252, Protein}}, {{Q9Y285, Protein}}, {{P13861, Protein}},
{{O94979, Protein}}, {{O14933, Protein}}, {{Q9Y6Y8, Protein}}, {{Q7L2H7, Protein}},
{{Q13439, Protein}}, {{P19784, Protein}}, {{Q07812, Protein}}, {{P56192, Protein}},
{{Q01082, Protein}}, {{Q9UEU0, Protein}}, {{Q13043, Protein}}, {{O14732, Protein}},
{{Q9UBE0, Protein}}, {{P30520, Protein}}, {{P55263, Protein}}, {{P54136, Protein}},
{{Q13596, Protein}}, {{P25098, Protein}}, {{Q9HC16, Protein}}, {{P61457, Protein}},
{{Q92888, Protein}}, {{P62263, Protein}}, {{P63220, Protein}}, {{O00487, Protein}},
{{P19474, Protein}}, {{Q9Y333, Protein}}, {{P25788, Protein}}, {{P35998, Protein}},
{{Q9Y2V2, Protein}}, {{Q13347, Protein}}, {{Q5JSL3, Protein}}, {{O60841, Protein}}}}},
GO:0005737 → {{9.62342×10⁻¹⁵, 2.51171×10⁻¹², True}, {75, 13 296, 94 482, 39},
{{cytoplasm, cellular_component}, {{{P60900, Protein}}, {{Q96DE0, Protein}}, {{Q9Y285, Protein}},
{{P13861, Protein}}, {{O94979, Protein}}, {{Q9Y6Y8, Protein}}, {{Q13439, Protein}},
{{Q15819, Protein}}, {{O14745, Protein}}, {{P56192, Protein}}, {{Q01082, Protein}},
{{Q9UEU0, Protein}}, {{Q13043, Protein}}, {{O14732, Protein}}, {{P30520, Protein}},
{{P55263, Protein}}, {{P54136, Protein}}, {{Q13596, Protein}}, {{P25098, Protein}},
{{P41227, Protein}}, {{Q9HC16, Protein}}, {{P61457, Protein}}, {{Q9Y3L3, Protein}},
{{Q92888, Protein}}, {{P62263, Protein}}, {{P63220, Protein}}, {{Q32P44, Protein}},
{{Q2TAY7, Protein}}, {{Q13148, Protein}}, {{P19474, Protein}}, {{BLM, RNA}},
{{P25788, Protein}}, {{P35998, Protein}}, {{Q9Y3D0, Protein}}, {{O43813, Protein}},
{{O60841, Protein}}, {{O75534, Protein}}, {{O43402, Protein}}, {{Q99623, Protein}}}}}|⟩,
G1S6 → ⟨|GO:0030424 → {{0.000330195, 0.0291532, True}, {28, 454, 94 482, 3},
{{axon, cellular_component}, {{{NRN1L, RNA}}, {{ADC, RNA}}, {{LMTK3, RNA}}}}},
GO:0005102 → {{0.00194357, 0.0291532, True}, {28, 840, 94 482, 3},
{{receptor binding, molecular_function}, {{{EDA, RNA}}, {{IGFBP6, RNA}}, {{TNK1, RNA}}}}},
GO:0004674 → {{0.00509068, 0.0320462, True}, {28, 1184, 94 482, 3},
{{protein serine/threonine kinase activity, molecular_function},
{{{MAPK11, RNA}}, {{PKN3, RNA}}, {{LMTK3, RNA}}}}}|⟩,
G1S7 → ⟨| |⟩, G1S8 → ⟨|GO:0005615 → {{0.00552693, 0.0281821, True}, {12, 2972, 94 482, 3},
{{extracellular space, cellular_component}, {{{CTSK, RNA}}, {{MDGA1, RNA}}, {{SEMA4F, RNA}}}}}}|⟩,
G1S9 → ⟨|GO:0005887 → {{7.04316×10⁻⁸, 0.0000290263, True}, {50, 3156, 94 482, 12},
{{integral component of plasma membrane, cellular_component},
{{{MSR1, RNA}}, {{GPRC5C, RNA}}, {{EPHB2, RNA}}, {{P2RY2, RNA}}, {{SLC6A12, RNA}}, {{GPR84, RNA}},
{{KCNJ15, RNA}}, {{SLC22A4, RNA}}, {{SGMS2, RNA}}, {{CD1A, RNA}}, {{MMP14, RNA}}, {{CCRL2, RNA}}}}},
GO:0005886 → {{1.32238×10⁻⁷, 0.0000290263, True}, {50, 9422, 94 482, 19},

```
      {{plasma membrane, cellular_component}, {{{MSR1, RNA}}, {{CLEC4G, RNA}}, {{EPHB2, RNA}},
         {{SDC3, RNA}}, {{HTRA1, RNA}}, {{P2RY2, RNA}}, {{SLC6A12, RNA}}, {{CLEC6A, RNA}}, {{GPR84, RNA}},
         {{DSC2, RNA}}, {{SERPINB2, RNA}}, {{SIGLEC1, RNA}}, {{KCNJ15, RNA}}, {{SLC22A4, RNA}},
         {{SGMS2, RNA}}, {{CD1A, RNA}}, {{MRAS, RNA}}, {{MMP14, RNA}}, {{CCRL2, RNA}}}}},
   GO:0007200 → {{0.0000289161, 0.00364219, True}, {50, 110, 94482, 3},
      {{phospholipase C-activating G-protein coupled receptor signaling pathway, biological_process},
         {{{P2RY2, RNA}}, {{GPR84, RNA}}, {{TGM2, RNA}}}}}⟩,
G1S10 → ⟨|GO:0005737 → {{0.00748941, 0.0203967, True}, {10, 13296, 94482, 5},
      {{cytoplasm, cellular_component},
         {{{ELMO3, RNA}}, {{HIC1, RNA}}, {{HSD17B1, RNA}}, {{ENO3, RNA}}, {{NEIL1, RNA}}}}}|▷,
G1S11 → ⟨|GO:0005515 → {{0.00704381, 0.0284705, True}, {17, 17602, 94482, 8},
      {{protein binding, molecular_function}, {{{Q16864, Protein}}, {{O75175, Protein}}, {{AGPAT4, RNA}},
         {{CYSLTR2, RNA}}, {{STK36, RNA}}, {{PFKFB2, RNA}}, {{FANCD2, RNA}}, {{Q9Y376, Protein}}}},
   GO:0006351 → {{0.00783482, 0.0284705, True}, {17, 4570, 94482, 4},
      {{transcription, DNA-templated, biological_process},
         {{{O75175, Protein}}, {{ZNF577, RNA}}, {{TBX19, RNA}}, {{ZNF841, RNA}}}}},
   GO:0003700 → {{0.0192051, 0.039221, True}, {17, 3246, 94482, 3},
      {{transcription factor activity, sequence-specific DNA binding, molecular_function},
         {{{ZNF577, RNA}}, {{TBX19, RNA}}, {{ZNF841, RNA}}}}}|▷,
G1S12 → ⟨|GO:0005515 → {{1.17633×10⁻²⁹, 1.88096×10⁻²⁶, True}, {167, 17602, 94482, 97},
      {{protein binding, molecular_function}, {{{PTPRS, RNA}}, {{SLC7A8, RNA}}, {{PPAP2C, RNA}},
         {{SPRY2, RNA}}, {{CDCA3, RNA}}, {{NQO1, RNA}}, {{GINS2, RNA}}, {{CDT1, RNA}}, {{TUBB3, RNA}},
         {{PLK1, RNA}}, {{CCNB2, RNA}}, {{CD276, RNA}}, {{ELOVL6, RNA}}, {{FGFR1, RNA}}, {{CRMP1, RNA}},
         {{FBLN1, RNA}}, {{SFRP1, RNA}}, {{SEMA6A, RNA}}, {{GPC3, RNA}}, {{GFPT2, RNA}}, {{AURKA, RNA}},
         {{TSPAN6, RNA}}, {{NTS, RNA}}, {{SOX2, RNA}}, {{SPRY4, RNA}}, {{CRABP1, RNA}}, {{PTPRZ1, RNA}},
         {{KRT19, RNA}}, {{IGF2BP1, RNA}}, {{RRM2, RNA}}, {{BUB1, RNA}}, {{CDK1, RNA}}, {{DPYSL3, RNA}},
         {{HMGB3, RNA}}, {{RAC3, RNA}}, {{FST, RNA}}, {{AURKB, RNA}}, {{CRABP2, RNA}}, {{EPHB4, RNA}},
         {{PEG10, RNA}}, {{CDC20, RNA}}, {{MYH10, RNA}}, {{CBS, RNA}}, {{CSRP2, RNA}}, {{DNAJB5, RNA}},
         {{PMEL, RNA}}, {{HMGA2, RNA}}, {{ATP1A2, RNA}}, {{RBPMS, RNA}}, {{PAWR, RNA}}, {{DMKN, RNA}},
         {{ETV4, RNA}}, {{BIRC5, RNA}}, {{PYCR1, RNA}}, {{GINS4, RNA}}, {{KRT8, RNA}}, {{MAP1B, RNA}},
         {{CDCA5, RNA}}, {{COL4A2, RNA}}, {{ALPL, RNA}}, {{FOXM1, RNA}}, {{CDH2, RNA}}, {{TDGF1, RNA}},
         {{YES1, RNA}}, {{TPX2, RNA}}, {{FZD7, RNA}}, {{NANOG, RNA}}, {{MYCN, RNA}}, {{FAM64A, RNA}},
         {{SPATC1L, RNA}}, {{CCNB1, RNA}}, {{DNMT3B, RNA}}, {{PODXL, RNA}}, {{TNFRSF12A, RNA}},
         {{THY1, RNA}}, {{WASF1, RNA}}, {{NUSAP1, RNA}}, {{USP44, RNA}}, {{CIB2, RNA}}, {{PTK7, RNA}},
         {{FANCL, RNA}}, {{UBE2C, RNA}}, {{DPPA4, RNA}}, {{RARRES2, RNA}}, {{GJA1, RNA}}, {{LIN28A, RNA}},
         {{UCHL1, RNA}}, {{TEAD2, RNA}}, {{APOE, RNA}}, {{TUBB2B, RNA}}, {{PCGF2, RNA}}, {{IGF2BP3, RNA}},
         {{BEX1, RNA}}, {{SEPHS1, RNA}}, {{STAP2, RNA}}, {{TIPIN, RNA}}, {{ZNF404, RNA}}}},
   GO:0005829 → {{2.77974×10⁻¹⁵, 2.09006×10⁻¹², True}, {167, 6952, 94482, 46},
      {{cytosol, cellular_component}, {{{SPRY2, RNA}}, {{CDCA3, RNA}}, {{NQO1, RNA}}, {{POLR3G, RNA}},
         {{CDT1, RNA}}, {{PLK1, RNA}}, {{CCNB2, RNA}}, {{FGFR1, RNA}}, {{CRMP1, RNA}}, {{SFRP1, RNA}},
         {{GFPT2, RNA}}, {{AURKA, RNA}}, {{SOX2, RNA}}, {{CRABP1, RNA}}, {{IGF2BP1, RNA}}, {{FABP7, RNA}},
         {{RRM2, RNA}}, {{BUB1, RNA}}, {{CDK1, RNA}}, {{DPYSL3, RNA}}, {{RAC3, RNA}}, {{AURKB, RNA}},
         {{CRABP2, RNA}}, {{EPHB4, RNA}}, {{CDC20, RNA}}, {{MYH10, RNA}}, {{CBS, RNA}}, {{DNAJB5, RNA}},
         {{BCAT1, RNA}}, {{ATP1A2, RNA}}, {{BIRC5, RNA}}, {{MAP1B, RNA}}, {{CDCA5, RNA}}, {{YES1, RNA}},
         {{TPX2, RNA}}, {{NXN, RNA}}, {{QPRT, RNA}}, {{GGH, RNA}}, {{CCNB1, RNA}}, {{THY1, RNA}},
         {{UBE2C, RNA}}, {{GJA1, RNA}}, {{LIN28A, RNA}}, {{UCHL1, RNA}}, {{IGF2BP3, RNA}}, {{STAP2, RNA}}}},
   GO:0007067 → {{3.92132×10⁻¹⁵, 2.09006×10⁻¹², True}, {167, 540, 94482, 16},
      {{mitotic nuclear division, biological_process},
         {{{CDCA3, RNA}}, {{TUBB3, RNA}}, {{PLK1, RNA}}, {{CCNB2, RNA}}, {{AURKA, RNA}}, {{BUB1, RNA}},
         {{CDK1, RNA}}, {{CDC20, RNA}}, {{HMGA2, RNA}}, {{BIRC5, RNA}}, {{CDCA5, RNA}},
         {{FSD1, RNA}}, {{TPX2, RNA}}, {{FAM64A, RNA}}, {{USP44, RNA}}, {{TIPIN, RNA}}}}}|▷,
G1S13 → ⟨|GO:0070062 → {{0.000600024, 0.0270011, True}, {26, 5572, 94482, 7},
      {{extracellular exosome, cellular_component}, {{{CA6, RNA}}, {{C20orf195, RNA}},
         {{PLOD2, RNA}}, {{IGLL1, RNA}}, {{ECM1, RNA}}, {{GSTA1, RNA}}, {{PBLD, RNA}}}}}|▷,
G1S14 → ⟨|GO:0003677 → {{0.00388716, 0.0234702, True}, {22, 4688, 94482, 5},
      {{DNA binding, molecular_function},
         {{{ZNF835, RNA}}, {{ZNF2, RNA}}, {{E2F2, RNA}}, {{ZNF771, RNA}}, {{ZNF485, RNA}}}}},
   GO:0006355 → {{0.0162542, 0.0413128, True}, {22, 6622, 94482, 5},
      {{regulation of transcription, DNA-templated, biological_process},
         {{{ZNF835, RNA}}, {{ZNF2, RNA}}, {{E2F2, RNA}}, {{ZNF771, RNA}}, {{ZNF485, RNA}}}}},
   GO:0006351 → {{0.0198775, 0.0450152, True}, {22, 4570, 94482, 4},
      {{transcription, DNA-templated, biological_process},
         {{{ZNF835, RNA}}, {{ZNF2, RNA}}, {{ZNF771, RNA}}, {{ZNF485, RNA}}}}}|⟩|⟩
```

Let us extract the names of the top 10 ontology group results from all the "f1" Group1 subgroup 1 data (G1S1). These are in the 3rd list, first component for GOAnalysis outputs (see above and documentation:

In[198]:= `Query["f1", "G1S1", All, 3, 1] @goAnalysisCombined`

Out[198]= ⟨|GO:0005515 → {protein binding, molecular_function}, GO:0016020 → {membrane, cellular_component},
GO:0070062 → {extracellular exosome, cellular_component}, GO:0005829 → {cytosol, cellular_component},
GO:0005783 → {endoplasmic reticulum, cellular_component}, GO:0005737 → {cytoplasm, cellular_component},
GO:0005886 → {plasma membrane, cellular_component}, GO:0005925 → {focal adhesion, cellular_component},
GO:0045785 → {positive regulation of cell adhesion, biological_process},
GO:0005739 → {mitochondrion, cellular_component},
GO:0005788 → {endoplasmic reticulum lumen, cellular_component},
GO:0045087 → {innate immune response, biological_process},
GO:0018105 → {peptidyl-serine phosphorylation, biological_process},
GO:0019899 → {enzyme binding, molecular_function}, GO:0005759 → {mitochondrial matrix, cellular_component},
GO:0005789 → {endoplasmic reticulum membrane, cellular_component},
GO:0005741 → {mitochondrial outer membrane, cellular_component},
GO:0031625 → {ubiquitin protein ligase binding, molecular_function},
GO:0005654 → {nucleoplasm, cellular_component}, GO:0000139 → {Golgi membrane, cellular_component},
GO:1901998 → {toxin transport, biological_process},
GO:0030335 → {positive regulation of cell migration, biological_process},
GO:0046872 → {metal ion binding, molecular_function}, GO:0009986 → {cell surface, cellular_component},
GO:0042493 → {response to drug, biological_process}, GO:0031982 → {vesicle, cellular_component},
GO:0045944 → {positive regulation of transcription from RNA polymerase II promoter, biological_process},
GO:0010501 → {RNA secondary structure unwinding, biological_process},
GO:0045766 → {positive regulation of angiogenesis, biological_process},
GO:0006464 → {cellular protein modification process, biological_process},
GO:0014823 → {response to activity, biological_process},
GO:0036498 → {IRE1-mediated unfolded protein response, biological_process},
GO:0005634 → {nucleus, cellular_component}, GO:0044822 → {poly(A) RNA binding, molecular_function},
GO:0001047 → {core promoter binding, molecular_function},
GO:0030433 → {ER-associated ubiquitin-dependent protein catabolic process, biological_process},
GO:0000932 → {cytoplasmic mRNA processing body, cellular_component},
GO:0050729 → {positive regulation of inflammatory response, biological_process},
GO:0034976 → {response to endoplasmic reticulum stress, biological_process},
GO:0008134 → {transcription factor binding, molecular_function},
GO:0005524 → {ATP binding, molecular_function}, GO:0007568 → {aging, biological_process},
GO:0048471 → {perinuclear region of cytoplasm, cellular_component},
GO:0071260 → {cellular response to mechanical stimulus, biological_process},
GO:0009636 → {response to toxic substance, biological_process},
GO:0042470 → {melanosome, cellular_component}, GO:0016032 → {viral process, biological_process},
GO:0005178 → {integrin binding, molecular_function},
GO:0004674 → {protein serine/threonine kinase activity, molecular_function},
GO:0045454 → {cell redox homeostasis, biological_process},
GO:0005102 → {receptor binding, molecular_function}, GO:0007155 → {cell adhesion, biological_process},
GO:0006351 → {transcription, DNA-templated, biological_process},
GO:0007049 → {cell cycle, biological_process}, GO:0030496 → {midbody, cellular_component},
GO:0030529 → {intracellular ribonucleoprotein complex, cellular_component},
GO:0002250 → {adaptive immune response, biological_process},
GO:0043231 → {intracellular membrane-bounded organelle, cellular_component},
GO:0003729 → {mRNA binding, molecular_function}, GO:0003677 → {DNA binding, molecular_function}|⟩

Let us extract the corresponding p-values/test results of the top 10 ontology group results from all the "SpikeMin" Group1 subgroup 1 data (G1S1). These are in the 1st list for GOAnalysis outputs (see above and documentation:

In[199]:= `Query["f1", "G1S1", All, 1]@goAnalysisCombined`

Out[199]= $\langle|$ GO:0005515 $\rightarrow \{5.79761\times10^{-27}, 6.97452\times10^{-24}, \text{True}\}$, GO:0016020 $\rightarrow \{1.90489\times10^{-15}, 1.14579\times10^{-12}, \text{True}\}$,

GO:0070062 $\rightarrow \{9.29517\times10^{-14}, 3.72736\times10^{-11}, \text{True}\}$, GO:0005829 $\rightarrow \{3.22699\times10^{-11}, 9.70517\times10^{-9}, \text{True}\}$,

GO:0005783 $\rightarrow \{2.31735\times10^{-7}, 0.0000557553, \text{True}\}$, GO:0005737 $\rightarrow \{6.18555\times10^{-7}, 0.00011456, \text{True}\}$,

GO:0005886 $\rightarrow \{6.66598\times10^{-7}, 0.00011456, \text{True}\}$, GO:0005925 $\rightarrow \{1.27235\times10^{-6}, 0.00019133, \text{True}\}$,

GO:0045785 $\rightarrow \{5.35662\times10^{-6}, 0.000716002, \text{True}\}$, GO:0005739 $\rightarrow \{8.46014\times10^{-6}, 0.000973828, \text{True}\}$,

GO:0005788 $\rightarrow \{8.9045\times10^{-6}, 0.000973828, \text{True}\}$, GO:0045087 $\rightarrow \{0.0000105328, 0.00105592, \text{True}\}$,

GO:0018105 $\rightarrow \{0.0000129024, 0.00119397, \text{True}\}$, GO:0019899 $\rightarrow \{0.0000193821, 0.00166548, \text{True}\}$,

GO:0005759 $\rightarrow \{0.0000229516, 0.0017892, \text{True}\}$, GO:0005789 $\rightarrow \{0.0000237966, 0.0017892, \text{True}\}$,

GO:0005741 $\rightarrow \{0.0000706044, 0.00423336, \text{True}\}$, GO:0031625 $\rightarrow \{0.000072913, 0.00423336, \text{True}\}$,

GO:0005654 $\rightarrow \{0.0000952723, 0.00423336, \text{True}\}$, GO:0000139 $\rightarrow \{0.0000977363, 0.00423336, \text{True}\}$,

GO:1901998 $\rightarrow \{0.000098532, 0.00423336, \text{True}\}$, GO:0030335 $\rightarrow \{0.000106804, 0.00443051, \text{True}\}$,

GO:0046872 $\rightarrow \{0.000120781, 0.00482555, \text{True}\}$, GO:0009986 $\rightarrow \{0.000142886, 0.00505565, \text{True}\}$,

GO:0042493 $\rightarrow \{0.000159739, 0.00537574, \text{True}\}$, GO:0031982 $\rightarrow \{0.000161505, 0.00537574, \text{True}\}$,

GO:0045944 $\rightarrow \{0.000169177, 0.00537574, \text{True}\}$, GO:0010501 $\rightarrow \{0.000178744, 0.00537574, \text{True}\}$,

GO:0045766 $\rightarrow \{0.000197788, 0.00580338, \text{True}\}$, GO:0006464 $\rightarrow \{0.000262741, 0.00718358, \text{True}\}$,

GO:0014823 $\rightarrow \{0.00027623, 0.00738455, \text{True}\}$, GO:0036498 $\rightarrow \{0.000363447, 0.00930269, \text{True}\}$,

GO:0005634 $\rightarrow \{0.000388808, 0.00958297, \text{True}\}$, GO:0044822 $\rightarrow \{0.00039573, 0.00958297, \text{True}\}$,

GO:0001047 $\rightarrow \{0.000466652, 0.0102421, \text{True}\}$, GO:0030433 $\rightarrow \{0.000561486, 0.0120619, \text{True}\}$,

GO:0000932 $\rightarrow \{0.000755461, 0.015147, \text{True}\}$, GO:0050729 $\rightarrow \{0.000786215, 0.0153365, \text{True}\}$,

GO:0034976 $\rightarrow \{0.000817755, 0.0153712, \text{True}\}$, GO:0008134 $\rightarrow \{0.000972558, 0.0170703, \text{True}\}$,

GO:0005524 $\rightarrow \{0.000999704, 0.0170703, \text{True}\}$, GO:0007568 $\rightarrow \{0.00100643, 0.0170703, \text{True}\}$,

GO:0048471 $\rightarrow \{0.00100747, 0.0170703, \text{True}\}$, GO:0071260 $\rightarrow \{0.00106117, 0.0177304, \text{True}\}$,

GO:0009636 $\rightarrow \{0.00143542, 0.0226364, \text{True}\}$, GO:0042470 $\rightarrow \{0.0019934, 0.0285484, \text{True}\}$,

GO:0016032 $\rightarrow \{0.00294008, 0.0388672, \text{True}\}$, GO:0005178 $\rightarrow \{0.00301894, 0.0390515, \text{True}\}$,

GO:0004674 $\rightarrow \{0.00338566, 0.0419891, \text{True}\}$, GO:0045454 $\rightarrow \{0.00371487, 0.0440661, \text{True}\}$,

GO:0005102 $\rightarrow \{0.0037719, 0.0440661, \text{True}\}$, GO:0007155 $\rightarrow \{0.00379185, 0.0440661, \text{True}\}$,

GO:0006351 $\rightarrow \{0.00380954, 0.0440661, \text{True}\}$, GO:0007049 $\rightarrow \{0.00392833, 0.0443698, \text{True}\}$,

GO:0030496 $\rightarrow \{0.00414031, 0.0458212, \text{True}\}$, GO:0030529 $\rightarrow \{0.0042288, 0.0462477, \text{True}\}$,

GO:0002250 $\rightarrow \{0.00459421, 0.0482646, \text{True}\}$, GO:0043231 $\rightarrow \{0.00461382, 0.0482646, \text{True}\}$,

GO:0003729 $\rightarrow \{0.00468845, 0.0484272, \text{True}\}$, GO:0003677 $\rightarrow \{0.00470988, 0.0484272, \text{True}\}|\rangle$

Pathway Analysis

Enrichment of Genomic KEGG Pathways (KEGG: Kyoto Encyclopedia of Genes and Genomes)

MathIOmica provides a KEGGAnalysis function using annotations (default is for human data) obtained from KEGG: Kyoto Encyclopedia of Genes and Genomes, and by default uses human data annotated with KEGG Gene IDs. The KEGGAnalysis function performs an over-representation (ORA) analysis, providing a "significance" cutoff based on a p-value assessed by a hypergeometric function.

KEGGAnalysis [*data*]	calculates input data over–representation analysis for KEGG: Kyoto Encyclopedia of Genes and Genomes pathways. We note that the function utilizes data obtained from the KEGG databases, and by default uses human data annotated by "KEGG Gene ID".

Performing an over representation analysis for KEGG:Kyoto Encyclopedia of Genes and Genomes pathways, using clustered data in MathIOmica.

option name	default value

AdditionalFilter	None	AdditionalFilter provides additional filtering that may be applied to the standard output structure to be returned.
AnalysisType	"Genomic"	AnalysisType provides a selection for the type of analysis to perform. "Genomic" analysis (default) uses gene identifier based analysis. "Molecular" analysis uses molecular analysis. Setting the option to All carries out all possible analysis types for the input data.
AugmentDictionary	True	AugmentDictionary provides a choice whether or not to augment the current ConstantGeneDictionary variable or create a new one.
BacgroundSet	All	BackgroundSet provides a list of IDs (e.g. gene accessions) that should be considered as the background for the calculation.
FilterSignificant	True	FilterSignificant can be set to True to filter data based on whether the enrichment analysis is statistically significant, or if set to False to return all membership computations.
GeneDictionary	None	GeneDictionary points to an existing variable to use as a gene dictionary in annotations. If set to None the default ConstantGeneDictionary will be used.
GetGeneDictionaryOptions	{}	The GetGeneDictionaryOptions option specifies a list of options that will be passed to the internal GetGeneDictionary function.

HypothesisFunction	`(Query["Results"][`
	`  Benjamini-`
	`  HochbergF-`
	`  DR[`
	`  ♯1,`
	`  Significa-`
	`  nceLevel`
	`  ->`
	`  ♯2]] &) &`
		The HypothesisFunction option allows us to chose a function to implement multiple hypothesis testing. The default is using the BenjaminiHochbergFDR function.
		
		The user can use any function f with three inputs, of the form f[♯1,♯2,♯3] where the inputs refer to:
		♯1 is the p–value list,
		♯2 is a significance cutoff,
		♯3 is the number of GO associations overall being tested.
		The function f must output a list of 3 values: {original p–value, adjusted p–value, True or False based on whether this value is considered statistically significant or not respectively}.

InputID	`{"UniProt ID",` `  "Gene Symbol"}`	The InputID option specifies the kind of identifiers/accessions used as input.
KEGGAnalysisAssignerOptions	`{}`	The KEGGAnalysisAssignerOptions option specifies a list of options that will be passed to the internal KEGGAnalysisAssigner function.
KEGGDatabase	`"pathway"`	KEGGDatabase value indicates which KEGG database to use as the target database.
KEGGDictionaryOptions	`{}`	KEGGDictionaryOptions specifies a list of options to be passed to the internal KEGGDictionary function that provides the KEGG annotations.
KEGGDictionaryVariable	None	KEGGDictionaryVariable can provide a KEGG annotation variable. If set to None, KEGGDictionary will be used internally to automatically generate the default KEGG annotation.
KEGGMolecular	`"cpd"`	KEGGMolecular specifies which database to use for molecular analysis. The default is the compound database ("cpd").
KEGGOrganism	`"hsa"`	KEGGOrganism indicates which organism (org) to use for "Genomic" type of analysis. The default is human analysis org="hsa".

`MathIOmicaDataDirectory`	`ConstantMathIOmic-aDataDirectory`	MathIOmicaDataDirectory option specifies the directory where the default MathIOmica package data are stored. By default the option is set to create the standard directory if it does not exist already.
`MolecularInputID`	`{"cpd"}`	MolecularInputID is a string list to indicate the kind of ID to use for the input molecule entries.
`MolecularOutputID`	`"cpd"`	MolecularOutputID is a string to indicate the kind of ID to convert input molecule entries. The default is "cpd" consistently with use of the "cpd" database as the default molecular analysis.
`MolecularSpecies`	`"compound"`	MolecularSpecies specifies the kind of molecular input.
`MultipleList`	`False`	MultipleList option specifies whether the input accessions list constituted a multi–omics list input that is annotated so. Each ID j input must be a list form, i.e. enclosed as $\{ID_j\}$. If this is the case, MultipleList is set to True and each input list ID should have the form {ID,"Omics Type Label"}, e.g. {"NFKB1","Protein"}, and the different omics type are treated as different for each ID. If MultipleList is set to False, and labeled IDs are provided, labels corresponding to the same ID are treated as equivalent to avoid overcounting.
`MultipleListCorrection`	`None`	MultipleListCorrection is an option whether or not to correct for multi–omics analysis. The choices are None, Automatic, or a custom number. This essentially enlarges the population by this factor to account for additional IDs being considered as the result of a multi–omics cluster analysis. If the value is set to Automatic the number of unique ID labels is used to make the correction.

NonUCSC	False	NonUCSC option set to False assumes UCSC browser was used in determining an internal GeneDictionary used in ID translations where the KEGG identifiers for genes are number strings (e.g. 4790). The NonUCSC option can be set to True if standard KEGG accessions are used in a user provided GeneDictionary variable, in the form OptionValue[KEGGOrganism] <>":"<>"number string", e.g. "hsa:4790"
OutputID	"KEGG Gene ID"	OutputID is a string to indicate the kind of ID to convert input genomic analysis entries. The default is "KEGG Gene ID" consistently with use of the "pathway" database as the default genomic analysis.
PathwayLengthFilter	2	PathwayLengthFilter can be used to set the value for which terms to consider in the computation, by excluding KEGG pathways that have fewer items compared to the PathwayLengthFilter value. It is used by the internal KEGGAnalysisAssigner function.
pValueCutoff	0.05	pValueCutoff provides a cutoff p–value for adjusted p–values to assess statistical significance.
ReportFilter	1	ReportFilter provides a cutoff for membership in pathways in selecting which terms/pathways to return. It is used in conjunction with ReportFilterFunction.
ReportFilterFunction	GreaterEqualThan	ReportFilterFunction specifies what operator form will be used to compare against ReportFilter option value in selecting which terms/pathways to return. The default is to use GreaterEqualThan
Species	"human"	The Species option specifies the species considered in the calculation.

TestFunction	`N[1 - CDF[ Hypergeom- etricDist- ribution[ #1, #2, #3], #4 - 1]] &`	The TestFunction option calculates the p–values for the enrichment of each term. It can be a function of four inputs, f[#1,#2,#3,#4] (e.g. the default is using a hypergeometric distribution CDF, N[1–CDF[**HypergeometricDistribution**[#1,#2,#3],#4–1]]]. The four inputs refer to: #1 is number of draws (members in group being tested), #2 is number of successes for category in population, #3 is total number of members in population, #4 is number of successes (or more) in current group being tested for specific category. The output is a p–value (real positive number ≤ 1).

Options for **KEGGAnalysis** .

The input data can be a single list of n genes in the form:

$\text{data} = \{ID_1, ID_2, ..., ID_n\}$

The IDs may be provided as ID strings, ID_j (e.g. "NFKB1") as strings enclosed in list brackets $\{ID_j\}$, (e.g. {"NFKB1"} or as labeled strings in the case of multiple omics being considered. Labeled IDs are typically provided as:

$\{\{ID_1, ... \text{optional label items}_1, \text{label}_1\},$
$\{ID_2, ... \text{optional label items}_2, ..., \text{label}_2\}, ... \{ID_n, ..., \text{optional label items}_n, ..., \text{label}_n\}\}.$

The ID labels are typically a string, e.g. typically "RNA" or "Protein", (e.g. {"NFKB1","Protein"}) or for a molecular ID obtained from metabolomics experiments, can also contain other optional label items such as mass and retention time {"cpd:C00449", 276.133, 11.0041, "Meta"}. The main label must always be the last element in the list.

The output has the following structures: for a single list input:

```
listOutput = <|KEGG : pathway₁ →
    {{p − value₁, multiple hypothesis adjusted p − value₁, True / False for statistical significance},
      {{number of members in group being tested, number of successes for term₁ in population,
        total number of members in population, number of members (or more) in current group being tested
          associated to pathway₁}, {KEGG pathway₁ description, {input IDs associated to pathway₁}}}},
  KEGG : pathway₂ → {{p − value₂, multiple hypothesis adjusted p − value₂,
      True / False for statistical significance}, {{number of members in group being tested,
        number of successes for term₂ in population, total number of members in population,
        number of members (or more) in current group being tested associated to pathway₂},
      {KEGG pathway₁ description, {input IDs associated to pathway₂}}}}, ..., KEGG : pathwayₙ →
    {{p − value ₙ, multiple hypothesis adjusted p − value ₙ, True / False for statistical significance},
      {{number of members in group being tested, number of successes for term ₙ in population,
        total number of members in population, number of members (or more) in current group being tested
          associated to pathway ₙ}, {KEGG pathway ₙ description, {input IDs associated to pathway ₙ}}}}
  |>
```

The input data can also be an association of multiple L groups to be tested:

```
data = <|Group₁ → {ID₁₁, ID₁₂, ..., ID₁ n₁},
    Group₂ → {ID₂₁, ID₂₂, ..., ID₂ n₂}, ...,
    Group_L → {ID₁₁, ID₁₂, ..., ID₁ n_L}|>.
```

In this case the output for each group has the `listOutput` format described above:

```
associationOutput = <|Group₁ → listOutput₁,
  Group₂ → listOutput₂, ...,
  Group_L → listOutput_L|>
```

`KEGGAnalysis` can also take as input the output of clustering of time series classification data, e.g. TimeSeriesClusters or TimeSeriesSingleClusters association of associations. The groups for each class will then have keys labeled "GroupAssociations", that include the labels used in the clustering. The labels must correspond to protein or gene accessions/IDs. For each class and group the corresponding KEGGAnalysis enrichment is computed and returned.

There are two types of analyses that are carried out, which can be set by the AnalysisType option value. The default "Genomic" analysis is based on input gene symbols. The "Molecular" analysis is based on molecular input accessions (e.g. compounds "cpd" databases). For multi-omic input the user may select to do All analyses. In this case an additional outer association is created with labels indicating each of "Genomic" or "Molecular" analysis carried out.

The enrichment analysis is an over-representation calculation, using a hypergeometric test. For a given a given group (e.g. members of a cluster after classification), we try to identify which KEGG pathway terms are over-represented by membership of IDs to that cluster. The KEGGAnalysis function allows us to select the background, and hence address selection bias. Additionally a Benjamini-Hochberg procedure false discovery rate (FDR) may be calculated for each representation.

We carry out our KEGGAnalysis for all the classes and groups/subgroups. We only report terms for which there are at least 2 members, and additionally correct for multiple omics (2 sets of KEGG terms, one each for proteomics and transcriptomics). Please note that this is a time consuming computation.

In[200]:=
```
keggAnalysisCombined = KEGGAnalysis[combinedClusters,
    ReportFilter → 2, MultipleList → True, MultipleListCorrection → 2 , AnalysisType → All];
```
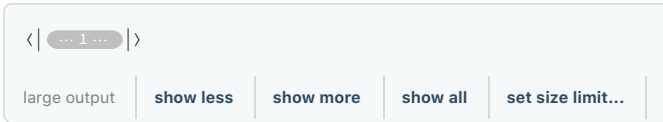
We see that both "Molecular" and "Genomic" analysis is performed:

In[201]:=
```
Keys@keggAnalysisCombined
```

Out[201]= {Molecular, Genomic}

We can extract both Genomic and molecular analysis:

In[202]:= **keggAnalysisCombined["Genomic"]**

Out[202]=

⟨| ⚬⚬⚬ 1 ⚬⚬⚬ |⟩

large output   **show less**   **show more**   **show all**   **set size limit…**

In[203]:= **keggAnalysisCombined["Molecular"]**

Out[203]= ⟨| SpikeMax → ⟨|G1S1 → ⟨| |⟩, G1S2 → ⟨| |⟩, G1S3 → ⟨| |⟩, G1S4 → ⟨| |⟩, G1S5 → ⟨| |⟩, G1S6 → ⟨| |⟩, G1S7 → ⟨| |⟩,
     G1S8 → ⟨| |⟩, G1S9 → ⟨| |⟩, G1S10 → ⟨| |⟩, G1S11 → ⟨| |⟩, G1S12 → ⟨| |⟩, G1S13 → ⟨| |⟩, G1S14 → ⟨| |⟩ |⟩,
   SpikeMin → ⟨|G1S1 → ⟨| |⟩, G1S2 → ⟨| |⟩, G1S3 → ⟨| |⟩, G2S1 → ⟨| |⟩,
     G2S2 → ⟨|path:map01100 → {{0.0248138, 0.0413563, True}, {5, 1654, 5841, 4},
         {Metabolic pathways, {{{cpd:C06124, 379.249, 12.6871, Meta}}, {{cpd:C20199, 238.12, 9.70221, Meta}},
           {{cpd:C19614, 270.22, 12.7198, Meta}}, {{cpd:C05446, 436.355, 14.3015, Meta}}}}} |⟩,
     G3S1 → ⟨|path:map04976 → {{0.000826861, 0.00496117, True}, {3, 98, 5841, 2},
         {Bile secretion, {{{cpd:C04555, 368.165, 12.0826, Meta}, {cpd:C04555, 368.166, 12.3718, Meta}},
           {{cpd:C01921, 465.309, 11.8056, Meta}}}}} |⟩ |⟩, f1 → ⟨|G1S1 → ⟨| |⟩, G1S2 → ⟨| |⟩ |⟩,
   f2 → ⟨|G1S1 → ⟨| |⟩, G1S2 → ⟨| |⟩, G2S1 → ⟨| |⟩, G2S2 → ⟨| |⟩, G3S1 → ⟨| |⟩, G3S2 → ⟨| |⟩, G4S1 → ⟨| |⟩,
     G4S2 → ⟨| |⟩, G5S1 → ⟨| |⟩, G5S2 → ⟨| |⟩ |⟩,
   f3 → ⟨|G1S1 → ⟨| |⟩, G1S2 → ⟨| |⟩, G2S1 → ⟨| |⟩, G2S2 → ⟨| |⟩, G3S1 → ⟨| |⟩,
     G3S2 → ⟨| |⟩, G4S1 → ⟨| |⟩, G4S2 → ⟨| |⟩ |⟩,
   f4 → ⟨|G1S1 → ⟨| |⟩, G1S2 → ⟨| |⟩, G2S1 → ⟨| |⟩ |⟩,
   f5 → ⟨|G1S1 → ⟨| |⟩, G1S2 → ⟨| |⟩ |⟩,
   f6 → ⟨|G1S1 → ⟨| |⟩, G1S2 → ⟨| |⟩, G2S1 → ⟨| |⟩ |⟩,
   f7 → ⟨|G1S1 → ⟨| |⟩ |⟩ |⟩

Let us extract the names of the pathways found for the "SpikeMin" data:

In[204]:= **Query["SpikeMin", All, All, 3, 1] @ keggAnalysisCombined["Genomic"]**

Out[204]= ⟨| G1S1 → ⟨|path:hsa03030 → DNA replication - Homo sapiens (human), path:hsa01100 →
       Metabolic pathways - Homo sapiens (human), path:hsa03010 → Ribosome - Homo sapiens (human),
     path:hsa00280 → Valine, leucine and isoleucine degradation - Homo sapiens (human),
     path:hsa01212 → Fatty acid metabolism - Homo sapiens (human),
     path:hsa03430 → Mismatch repair - Homo sapiens (human),
     path:hsa04640 → Hematopoietic cell lineage - Homo sapiens (human) |⟩,
   G1S2 → ⟨|path:hsa00471 → D-Glutamine and D-glutamate metabolism - Homo sapiens (human),
     path:hsa01100 → Metabolic pathways - Homo sapiens (human),
     path:hsa00250 → Alanine, aspartate and glutamate metabolism - Homo sapiens (human),
     path:hsa00533 → Glycosaminoglycan biosynthesis - keratan sulfate - Homo sapiens (human),
     path:hsa00220 → Arginine biosynthesis - Homo sapiens (human),
     path:hsa04964 → Proximal tubule bicarbonate reclamation - Homo sapiens (human) |⟩, G1S3 →
     ⟨|path:hsa04142 → Lysosome - Homo sapiens (human), path:hsa05152 → Tuberculosis - Homo sapiens (human),
     path:hsa04210 → Apoptosis - Homo sapiens (human), path:hsa05140 → Leishmaniasis - Homo sapiens (human),
     path:hsa04145 → Phagosome - Homo sapiens (human), path:hsa05134 → Legionellosis - Homo sapiens (human),
     path:hsa04650 → Natural killer cell mediated cytotoxicity - Homo sapiens (human),
     path:hsa05150 → Staphylococcus aureus infection - Homo sapiens (human),
     path:hsa05142 → Chagas disease (American trypanosomiasis) - Homo sapiens (human),
     path:hsa05133 → Pertussis - Homo sapiens (human),
     path:hsa04722 → Neurotrophin signaling pathway - Homo sapiens (human),
     path:hsa04380 → Osteoclast differentiation - Homo sapiens (human),
     path:hsa05323 → Rheumatoid arthritis - Homo sapiens (human),
     path:hsa04620 → Toll-like receptor signaling pathway - Homo sapiens (human),
     path:hsa04062 → Chemokine signaling pathway - Homo sapiens (human),
     path:hsa05120 → Epithelial cell signaling in Helicobacter pylori infection - Homo sapiens (human),
     path:hsa04640 → Hematopoietic cell lineage - Homo sapiens (human),
     path:hsa04668 → TNF signaling pathway - Homo sapiens (human),
     path:hsa00531 → Glycosaminoglycan degradation - Homo sapiens (human),
     path:hsa04064 → NF-kappa B signaling pathway - Homo sapiens (human),
     path:hsa04670 → Leukocyte transendothelial migration - Homo sapiens (human),
     path:hsa04010 → MAPK signaling pathway - Homo sapiens (human), path:hsa05161 →

   Hepatitis B – Homo sapiens (human), path:hsa01200→Carbon metabolism – Homo sapiens (human),
   path:hsa04920→Adipocytokine signaling pathway – Homo sapiens (human),
   path:hsa05110→Vibrio cholerae infection – Homo sapiens (human),
   path:hsa00051→Fructose and mannose metabolism – Homo sapiens (human),
   path:hsa05164→Influenza A – Homo sapiens (human),
   path:hsa04662→B cell receptor signaling pathway – Homo sapiens (human),
   path:hsa04130→SNARE interactions in vesicular transport – Homo sapiens (human),
   path:hsa00071→Fatty acid degradation – Homo sapiens (human),
   path:hsa05132→Salmonella infection – Homo sapiens (human),
   path:hsa04931→Insulin resistance – Homo sapiens (human),
   path:hsa01100→Metabolic pathways – Homo sapiens (human), path:hsa00532→
    Glycosaminoglycan biosynthesis – chondroitin sulfate / dermatan sulfate – Homo sapiens (human),
   path:hsa04621→NOD-like receptor signaling pathway – Homo sapiens (human),
   path:hsa04330→Notch signaling pathway – Homo sapiens (human),
   path:hsa04115→p53 signaling pathway – Homo sapiens (human),
   path:hsa05144→Malaria – Homo sapiens (human)|⟩,
G2S1→⟨|path:hsa03010→Ribosome – Homo sapiens (human),
   path:hsa04066→HIF-1 signaling pathway – Homo sapiens (human),
   path:hsa05210→Colorectal cancer – Homo sapiens (human),
   path:hsa04660→T cell receptor signaling pathway – Homo sapiens (human)|⟩,
G2S2→⟨|path:hsa04120→Ubiquitin mediated proteolysis – Homo sapiens (human),
   path:hsa04662→B cell receptor signaling pathway – Homo sapiens (human),
   path:hsa05220→Chronic myeloid leukemia – Homo sapiens (human),
   path:hsa04660→T cell receptor signaling pathway – Homo sapiens (human),
   path:hsa05169→Epstein-Barr virus infection – Homo sapiens (human),
   path:hsa04722→Neurotrophin signaling pathway – Homo sapiens (human),
   path:hsa05161→Hepatitis B – Homo sapiens (human),
   path:hsa04910→Insulin signaling pathway – Homo sapiens (human),
   path:hsa04012→ErbB signaling pathway – Homo sapiens (human),
   path:hsa04810→Regulation of actin cytoskeleton – Homo sapiens (human),
   path:hsa04062→Chemokine signaling pathway – Homo sapiens (human),
   path:hsa05205→Proteoglycans in cancer – Homo sapiens (human),
   path:hsa01521→EGFR tyrosine kinase inhibitor resistance – Homo sapiens (human),
   path:hsa01522→Endocrine resistance – Homo sapiens (human),
   path:hsa01200→Carbon metabolism – Homo sapiens (human), path:hsa04380→
    Osteoclast differentiation – Homo sapiens (human), path:hsa05214→Glioma – Homo sapiens (human),
   path:hsa04650→Natural killer cell mediated cytotoxicity – Homo sapiens (human),
   path:hsa05213→Endometrial cancer – Homo sapiens (human),
   path:hsa04010→MAPK signaling pathway – Homo sapiens (human),
   path:hsa05221→Acute myeloid leukemia – Homo sapiens (human),
   path:hsa05211→Renal cell carcinoma – Homo sapiens (human),
   path:hsa04070→Phosphatidylinositol signaling system – Homo sapiens (human),
   path:hsa04664→Fc epsilon RI signaling pathway – Homo sapiens (human),
   path:hsa04211→Longevity regulating pathway – Homo sapiens (human),
   path:hsa05215→Prostate cancer – Homo sapiens (human),
   path:hsa04620→Toll-like receptor signaling pathway – Homo sapiens (human),
   path:hsa05016→Huntington's disease – Homo sapiens (human),
   path:hsa04210→Apoptosis – Homo sapiens (human),
   path:hsa04919→Thyroid hormone signaling pathway – Homo sapiens (human),
   path:hsa01100→Metabolic pathways – Homo sapiens (human),
   path:hsa04370→VEGF signaling pathway – Homo sapiens (human),
   path:hsa00562→Inositol phosphate metabolism – Homo sapiens (human),
   path:hsa05212→Pancreatic cancer – Homo sapiens (human),
   path:hsa04066→HIF-1 signaling pathway – Homo sapiens (human),
   path:hsa04666→Fc gamma R-mediated phagocytosis – Homo sapiens (human),
   path:hsa05210→Colorectal cancer – Homo sapiens (human),
   path:hsa04071→Sphingolipid signaling pathway – Homo sapiens (human),
   path:hsa04915→Estrogen signaling pathway – Homo sapiens (human),
   path:hsa04213→Longevity regulating pathway – multiple species – Homo sapiens (human),
   path:hsa05162→Measles – Homo sapiens (human), path:hsa04144→Endocytosis – Homo sapiens (human),
   path:hsa04510→Focal adhesion – Homo sapiens (human),
   path:hsa05223→Non-small cell lung cancer – Homo sapiens (human),
   path:hsa04611→Platelet activation – Homo sapiens (human),
   path:hsa00240→Pyrimidine metabolism – Homo sapiens (human),
   path:hsa05230→Central carbon metabolism in cancer – Homo sapiens (human),

path:hsa05120 → Epithelial cell signaling in Helicobacter pylori infection – Homo sapiens (human),
path:hsa04933 → AGE-RAGE signaling pathway in diabetic complications – Homo sapiens (human),
path:hsa04150 → mTOR signaling pathway – Homo sapiens (human),
path:hsa04520 → Adherens junction – Homo sapiens (human),
path:hsa04912 → GnRH signaling pathway – Homo sapiens (human),
path:hsa04962 → Vasopressin-regulated water reabsorption – Homo sapiens (human),
path:hsa05166 → HTLV-I infection – Homo sapiens (human),
path:hsa00230 → Purine metabolism – Homo sapiens (human),
path:hsa04668 → TNF signaling pathway – Homo sapiens (human),
path:hsa04064 → NF-kappa B signaling pathway – Homo sapiens (human),
path:hsa03040 → Spliceosome – Homo sapiens (human),
path:hsa04068 → FoxO signaling pathway – Homo sapiens (human),
path:hsa04720 → Long-term potentiation – Homo sapiens (human),
path:hsa04917 → Prolactin signaling pathway – Homo sapiens (human),
path:hsa04072 → Phospholipase D signaling pathway – Homo sapiens (human),
path:hsa05200 → Pathways in cancer – Homo sapiens (human),
path:hsa04914 → Progesterone-mediated oocyte maturation – Homo sapiens (human),
path:hsa05131 → Shigellosis – Homo sapiens (human),
path:hsa04114 → Oocyte meiosis – Homo sapiens (human),
path:hsa04142 → Lysosome – Homo sapiens (human), path:hsa04110 → Cell cycle – Homo sapiens (human),
path:hsa04670 → Leukocyte transendothelial migration – Homo sapiens (human),
path:hsa04922 → Glucagon signaling pathway – Homo sapiens (human),
path:hsa04921 → Oxytocin signaling pathway – Homo sapiens (human),
path:hsa04621 → NOD-like receptor signaling pathway – Homo sapiens (human),
path:hsa00640 → Propanoate metabolism – Homo sapiens (human),
path:hsa04725 → Cholinergic synapse – Homo sapiens (human),
path:hsa05010 → Alzheimer's disease – Homo sapiens (human),
path:hsa00630 → Glyoxylate and dicarboxylate metabolism – Homo sapiens (human),
path:hsa04730 → Long-term depression – Homo sapiens (human),
path:hsa05231 → Choline metabolism in cancer – Homo sapiens (human),
path:hsa05100 → Bacterial invasion of epithelial cells – Homo sapiens (human),
path:hsa00020 → Citrate cycle (TCA cycle) – Homo sapiens (human),
path:hsa05020 → Prion diseases – Homo sapiens (human),
path:hsa05110 → Vibrio cholerae infection – Homo sapiens (human),
path:hsa04750 → Inflammatory mediator regulation of TRP channels – Homo sapiens (human),
path:hsa05164 → Influenza A – Homo sapiens (human),
path:hsa04623 → Cytosolic DNA-sensing pathway – Homo sapiens (human),
path:hsa04540 → Gap junction – Homo sapiens (human), path:hsa03020 →
 RNA polymerase – Homo sapiens (human), path:hsa05152 → Tuberculosis – Homo sapiens (human),
path:hsa00280 → Valine, leucine and isoleucine degradation – Homo sapiens (human),
path:hsa04022 → cGMP-PKG signaling pathway – Homo sapiens (human),
path:hsa04350 → TGF-beta signaling pathway – Homo sapiens (human),
path:hsa03060 → Protein export – Homo sapiens (human),
path:hsa04960 → Aldosterone-regulated sodium reabsorption – Homo sapiens (human),
path:hsa05140 → Leishmaniasis – Homo sapiens (human),
path:hsa00030 → Pentose phosphate pathway – Homo sapiens (human),
path:hsa05145 → Toxoplasmosis – Homo sapiens (human), path:hsa05160 → Hepatitis C – Homo sapiens (human),
path:hsa00310 → Lysine degradation – Homo sapiens (human),
path:hsa04146 → Peroxisome – Homo sapiens (human),
path:hsa04961 → Endocrine and other factor-regulated calcium reabsorption – Homo sapiens (human),
path:hsa05142 → Chagas disease (American trypanosomiasis) – Homo sapiens (human),
path:hsa00010 → Glycolysis / Gluconeogenesis – Homo sapiens (human),
path:hsa05130 → Pathogenic Escherichia coli infection – Homo sapiens (human),
path:hsa04530 → Tight junction – Homo sapiens (human),
path:hsa03410 → Base excision repair – Homo sapiens (human),
path:hsa04320 → Dorso-ventral axis formation – Homo sapiens (human),
path:hsa00061 → Fatty acid biosynthesis – Homo sapiens (human),
path:hsa04622 → RIG-I-like receptor signaling pathway – Homo sapiens (human),
path:hsa04931 → Insulin resistance – Homo sapiens (human),
path:hsa00620 → Pyruvate metabolism – Homo sapiens (human),
path:hsa04014 → Ras signaling pathway – Homo sapiens (human),
path:hsa05216 → Thyroid cancer – Homo sapiens (human),
path:hsa04932 → Non-alcoholic fatty liver disease (NAFLD) – Homo sapiens (human),
path:hsa05218 → Melanoma – Homo sapiens (human) |⟩,
G3S1 → ⟨|path:hsa04510 → Focal adhesion – Homo sapiens (human),

```
.
path:hsa04622 → RIG-I-like receptor signaling pathway - Homo sapiens (human),
path:hsa05161 → Hepatitis B - Homo sapiens (human),
path:hsa04114 → Oocyte meiosis - Homo sapiens (human), path:hsa04110 → Cell cycle - Homo sapiens (human),
path:hsa05231 → Choline metabolism in cancer - Homo sapiens (human),
path:hsa04810 → Regulation of actin cytoskeleton - Homo sapiens (human),
path:hsa05210 → Colorectal cancer - Homo sapiens (human)|⟩|⟩
```

The results from a MathIOmica time series clustering enrichment analysis can be exported to spreadsheets using EnrichmentReportExport.

EnrichmentReportExport[*results*]	exports results from enrichment analyses to Excel spreadsheets, particularly suited for exporting multi-omics TimeSeriesClusters enrichment analysis results (via KEGGAnalysis or GOAnalysis). An excel spreadsheet is generated for each Class, named after the Class key, with sheets created for and named after each Group in that Class containing the enrichment output for that Group.

Exporting the enrichment analysis results to spreadsheets.

option name	default value	
AppendString	""	String that will be appended to the file name after the class name. If a string is not provided the current Date is appended.
OutputDirectory	None	OutputDirectory specifies the location of a directory to output the Excel spreadsheets generated by the function. If it is set to None the NotebookDirectory[] will be used as a default output directory.

Options for EnrichmentReportExport.

We can export the reports, for example to the $UserDocumentDirectory :

```
In[205]:= EnrichmentReportExport[keggAnalysisCombined["Genomic"],
    OutputDirectory → $UserDocumentsDirectory, AppendString → "KEGGAnalysisCombined"];
```

We can export the GO analysis results as well, for example to the $UserDocumentDirectory :

```
In[206]:= EnrichmentReportExport[goAnalysisCombined,
    OutputDirectory → $UserDocumentsDirectory, AppendString → "GOAnalysisCombined"];
```

Visualization of Pathways from KEGG

MathIOmica allows visualization and coloring of KEGG pathways using KEGGPathwayVisual.

KEGGPathwayVisual[*pathway*]	generates a visual representation for a KEGG: Kyoto Encyclopedia of Genes and Genomes *pathway*.

Visualizing KEGG pathways.

option name	default value	
AnalysisType	"Genomic"	AnalysisType provides a selection for the type of analysis to perform. "Genomic" analysis (default) uses gene identifier based pathway visualization. "Molecular" analysis uses molecular analysis map visualization.
AugmentDictionary	True	AugmentDictionary provides a choice whether or not to augment the current ConstantGeneDictionary variable or create a new one.
BlendColors	{RGBColor[0, 0, 1], RGBColor[0, 0, 1], RGBColor[0.5, 0.5, 0.5], RGBColor[1, 0, 0], RGBColor[1, 0, 0]}	BlendColors provides a list of colors to be used in coloring intensities provided and is used by the IntensityFunction as its first argument. The colors must be provided as RGBColor[] specification.
ColorSelection	<|"RNA" → "bg", "Protein" → "fg"|>	ColorSelection assigns foreground and background colors in the KEGG pathway through an association. The Keys point to labels for multi–omics data, and the values "bg" and "fg" can point to background and foreground representations respectively for each key.
DefaultColors	{"fg" -> RGBColor[0, 0, 0], "bg" -> RGBColor[0, 1, 0]}	DefaultColors provides a list of rules for setting the colors to be used as default values for the foreground "fg" and background "bg" respectively in the generated pathways. The colors must be provided as RGBColor[] specification.

ExportMovieOptions	{"VideoEncoding"→ "MPEG-4 Video", "FrameRate"→1}	ExportMovieOptions provides options for the Export function used internally to export the pathway list when Intensities have been provided for a time series representation of data.
FileExtend	".mov"	FileExtend provides a string to be appended to the file name if the ResultsFormat is set to "Movie".
GeneDictionary	None	GeneDictionary points to an existing variable to use as a gene dictionary in annotations. The gene dictionary is used to convert MemberSet identities provided to corresponding KEGG identifiers. If GeneDictionary is set to None the default ConstantGeneDictionary will be created or augmented through the use of GetGeneDictionary.
GetGeneDicitonaryOptions	{}	The GetGeneDictionaryOptions option specifies a list of options that will be passed to the internal GetGeneDictionary function.
InputID	{"UniProt ID", "Gene Symbol"}	The InputID option specifies the kind of identifiers/accessions used as input when identifiers are provided through setting the MemberSet values.

`Intensities`	`None`	Intensities may be used to provide a set of intensities that will be used for coloring components of the pathway. The intensities are provided as an association for each ID as single values, or as a list of values in the case of series data: $<\|ID_1 \rightarrow$ $\{intensity\ list\ for\ ID_1\},$ $ID_2 \rightarrow \{intensity\ list$ $for\ ID_2\},\ ...,$ $ID_N \rightarrow \{intensity\ list$ $for\ ID_N\}\|>.$ Intensities must be scaled from –1 to 1, or selected such that the IntensityFunction can convert them to a number between 0 to 1.
`IntensityFunction`	`(Blend[#1,` `(#2+1)/2]&)`	IntensityFunction is a function of two arguments that allows customization of the coloring for the intensities. The IntensityFunction value can be any function which outputs a color, I(#1,#2), (∗where#1 is the BlendColors option value, and #2 is an intensity vector, that has values typically ranging from [–1,1].
`KEGGAnalysisAssignerOptions`	`{}`	The KEGGAnalysisAssignerOptions option specifies a list of options that will be passed to the internal KEGGAnalysisAssigner function.
`KEGGDatabase`	`"pathway"`	KEGGDatabase value indicates which KEGG database to use as the target database.
`KEGGMolecular`	`"cpd"`	KEGGMolecular specifies which database to use for molecular analysis. The default is the compound database ("cpd").
`KEGGOrganism`	`"hsa"`	KEGGOrganism indicates which organism (org) to use for "Genomic" type of analysis. The default is human analysis org="hsa".

MathIOmicaDataDirectory	`ConstantMathIOmicaDataDirectory`	MathIOmicaDataDirectory option specifies the directory where the default MathIOmica package data are stored. By default the option is set to create the standard directory if it does not exist already.
MemberSet	`All`	MemberSet selects which members of the pathway are to be considered. The choices are: All: return the pathway only. {list of identifiers}: a list of identifiers that will be highlighted. If ORA is set to True the list must be the output from an over representation analysis, and the identifiers will be selected from the last list, second sublist. Only IDs that are found to match in the pathway are colored. An internal gene dictionary (see **GetGeneDictionary**) is used to convert IDs to KEGG IDs.
MissingValueColor	`RGBColor[0.4, 0.4, 0.4]`	MissingValueColor provides a color to be used when Intensities are provided to represent values that are tagged as Missing[]. The color must be provided as RGBColor[] specification.
MolecularInputID	`{"cpd"}`	MolecularInputID is a string list to indicate the kind of ID to use for the input molecule entries.
MolecularOutputID	`"cpd"`	MolecularOutputID is a string to indicate the kind of ID to convert input molecule entries. The default is "cpd" consistently with use of the "cpd" database as the default molecular analysis.
MolecularSpecies	`"compound"`	MolecularSpecies specifies the kind of molecular input.

MovieFilePath	None	MovieFilePath indicates the path (including file name) where if ResultsFormat is set to "Movie" the movie generated will be saved. The default value None will generate a file named after the pathway with extension set by the FileExtend option in the current directory.
NonUCSC	False	NonUCSC option set to False assumes UCSC browser was used in determining an internal GeneDictionary used in ID translations where the KEGG identifiers for genes are number strings (e.g. 4790). The NonUCSC option can be set to True if standard KEGG accessions are used in a user provided GeneDictionary variable, in the form OptionValue[KEGGOrganism]<>":"<>"number string", e.g. "hsa:4790"
ORA	False	ORA can be set to True or False depending on whether the input is from an over representation analysis (e.g. output from KEGGAnalysis), or not respectively.
OutputID	"KEGG Gene ID"	OutputID is a string to indicate the kind of ID to convert input genomic analysis entries. The default is "KEGG Gene ID" consistently with use of the "pathway" database as the default genomic analysis.
ResultsFormat	"URL"	ResultsFormat provides a choice of output format, the choices are: "URL": returns a URL of the pathway, "Figure": returns figure output(s) for the pathway, "Movie": in the case of series data returns a movie/animation of the series pathway snapshots.
SingleColorPlace	"bg"	SingleColorPlace selects in the case of a single identifier input whether to place the color to the foreground, ("fg") or background ("bg" set by default).
Species	"human"	The Species option specifies the species considered in the calculation.

| StandardHighlight | {"fg" -> RGBColor[1, 0, 0], "bg" -> RGBColor[0.5, 0.7, 1]} | StandardHighlight provides a list of rules for setting the highlight colors for the IDs represented in the pathway (when no intensities are provided). The list specifies color rules for foregroung, "fg", and background, "bg", respectively. The colors must be provided as RGBColor[] specification. |

Options for KEGGPathwayVisual .

ResultsFormat option setting	"Results" value for returned data
"URL"	Browser URL pointing to pathway on KEGG database, or if a list of Intensities was provided a series of URLs corresponding to each time point or sequential data in the series.
"Figure"	Pathway figure downloaded from the KEGG database, or if a list of Intensities was provided a series of figures corresponding to each time point or sequential data in the series.
"Movie"	Name of the output file that contains the generated movie/animation that is based on the list of Intensities provided.

ResultsFormat option output for KEGGPathwayVisual

For example, we can look at the B-cell receptor pathway:

```
In[207]:=  exampleBCellReceptor = KEGGPathwayVisual["path:hsa04662"]
```

```
Out[207]=  <|Pathway → path:hsa04662, Results → {http://www.kegg.jp/kegg-bin/show_pathway?map=hsa04662}|>
```

We can open this in a browser:

```
In[208]:=  SystemOpen[exampleBCellReceptor["Results"][[1]]]
```

We can import directly the pathway:

```
In[209]:=  exampleBCellReceptorFigure = KEGGPathwayVisual["path:hsa04662", ResultsFormat → "Figure"]
```
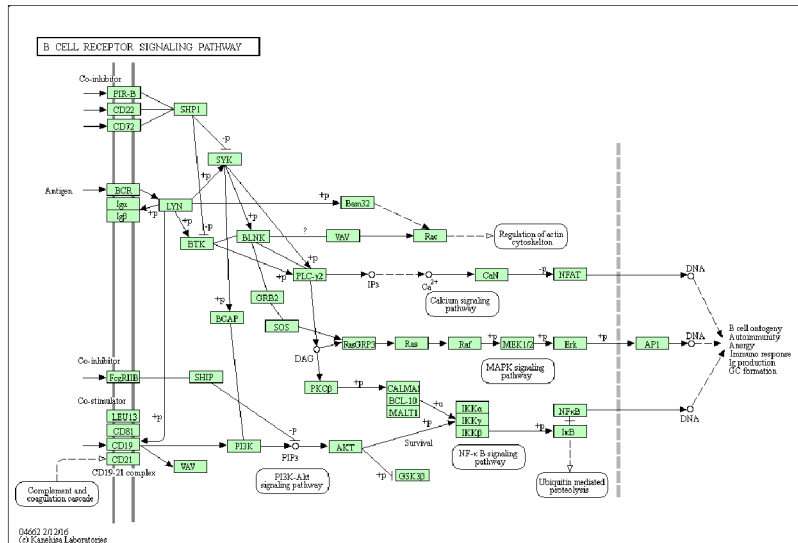


```
Out[209]=  <|Pathway → path:hsa04662, Results → |>
```

We can zoom in:

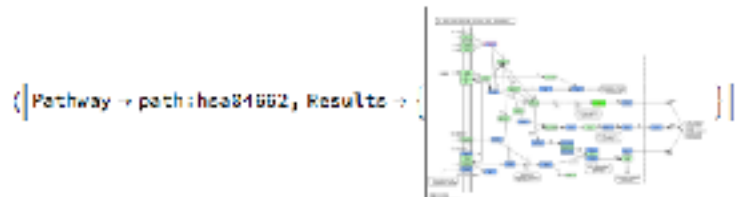*In[210]:=* `Show[exampleBCellReceptorFigure["Results"][[1]], ImageSize → 500]`

Out[210]=

We can highlight the components:

In[224]:= `exampleBCellReceptorFigureHighlight = KEGGPathwayVisual["path:hsa04662", ResultsFormat → "Figure", MemberSet → Query["SpikeMin", "G2S2", "path:hsa04662"]@keggAnalysisCombined["Genomic"], ORA → True]`
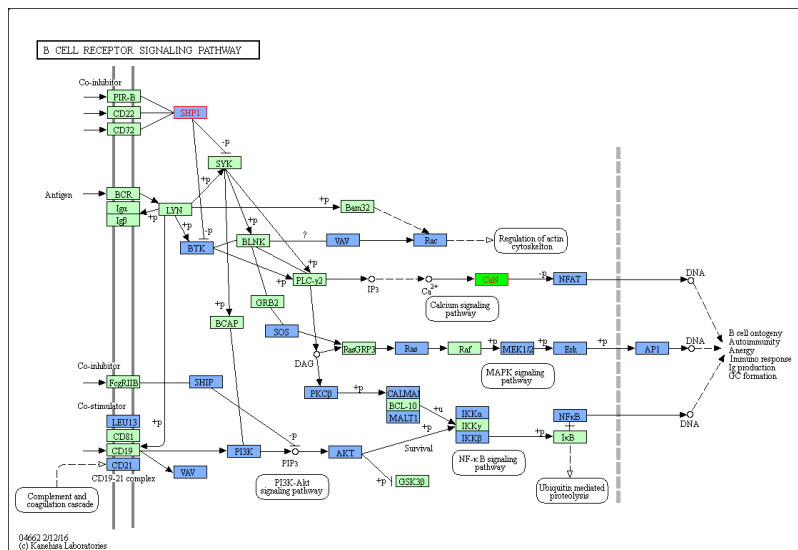
Out[224]=

We can zoom in:

In[225]:= `Show[exampleBCellReceptorFigureHighlight["Results"][[1]], ImageSize → 500]`

Out[225]=

We can also create snapshots and an animation of this data.

First, let's extract the members of the pathway in the analysis:

```
In[226]:= membersBCellReceptor =
    (Query["SpikeMin", "G2S2", "path:hsa04662", 3, 2]@keggAnalysisCombined["Genomic"])[[All, 1]]
```

```
Out[226]= {{PTPN6, RNA}, {IKBKB, RNA}, {PIK3R5, RNA}, {INPPL1, RNA}, {NFATC3, RNA}, {Q08209, Protein},
    {JUN, RNA}, {PPP3R1, RNA}, {CARD11, RNA}, {VAV1, RNA}, {MAPK3, RNA}, {AKT2, RNA}, {INPP5D, RNA},
    {RELA, RNA}, {IFITM1, RNA}, {P29350, Protein}, {NFATC1, RNA}, {KRAS, RNA}, {PRKCB, RNA},
    {CHUK, RNA}, {SOS2, RNA}, {NRAS, RNA}, {RAC2, RNA}, {PIK3R1, RNA}, {PPP3CB, RNA}, {MAP2K1, RNA},
    {PIK3CB, RNA}, {PIK3CD, RNA}, {SOS1, RNA}, {PIK3CA, RNA}, {MALT1, RNA}, {CR2, RNA}, {BTK, RNA}}
```

First, let's extract the members of the pathway in the analysis:

```
In[227]:= intensitiesRNABCellReceptor = DeleteMissing[Query[Key[#] & /@ membersBCellReceptor]@rnaFinalTimeSeries];
    intensitiesproteinBCellReceptor =
        DeleteMissing[Query[Key[#] & /@ membersBCellReceptor]@proteinFinalTimeSeries];
    intensitiesAll = Join[intensitiesRNABCellReceptor, intensitiesproteinBCellReceptor]
```

```
Out[229]= ⟨|{PTPN6, RNA} → {0.0387787, 0., 0.330891, 0.22067, 0.28749, -0.0437275, -0.0980776,
        0.122511, -0.324151, -0.333178, -0.375027, -0.219102, 0.397171, 0.0462055, -0.408056},
    {IKBKB, RNA} → {0.418249, 0., 0.434628, -0.0971056, 0.159125, -0.162659, -0.195735,
        0.105537, -0.207119, -0.282028, -0.105363, -0.175181, 0.126955, -0.255261, -0.529153},
    {PIK3R5, RNA} → {0.229787, 0., 0.120616, -0.0759332, 0.0914169, -0.133715, -0.118217,
        0.0286412, -0.532713, -0.286592, -0.188971, -0.191828, -0.0412379, -0.446288, -0.49654},
    {INPPL1, RNA} → {0.10616, 0., 0.188406, 0.0480471, 0.166196, -0.211755, -0.124324, 0.0384117,
        -0.502481, -0.166036, -0.196146, -0.307538, -0.0171249, -0.308092, -0.594314},
    {NFATC3, RNA} → {0.0504122, 0., -0.151596, -0.341818, -0.101656, -0.130359, 0.267591,
        -0.0967033, 0.768389, 0.0553952, 0.160331, 0.158179, 0.0784528, 0.20758, 0.236414},
    {JUN, RNA} → {-0.476684, 0., -0.214994, 0.179413, 0.21055, -0.0432207, -0.27211, -0.19091,
        0.256887, -0.0859087, 0.20595, -0.394457, -0.30324, -0.225096, -0.351661},
    {PPP3R1, RNA} → {-0.0166293, 0., -0.469365, -0.134715, -0.268084, 0.0407812, 0.273464,
        -0.145734, -0.134715, 0.263563, -0.480814, -0.0874638, -0.505594, 0.0341164, 0.0921134},
    {CARD11, RNA} → {-0.111217, 0., 0.216264, -0.381699, 0.12295, -0.112672, -0.241875, 0.0902658,
        -0.681623, -0.199501, -0.00531363, -0.0727515, -0.0269626, -0.247732, -0.35899},
    {VAV1, RNA} → {-0.167868, 0., 0.0851155, -0.0958852, 0.0546416, -0.0792823, -0.19252,
        -0.00932615, -0.641909, -0.203594, -0.200131, -0.308351, -0.076712, -0.365806, -0.425006},
    {MAPK3, RNA} → {-0.181382, 0., -0.0897729, -0.16923, -0.0868179, -0.181637, -0.219791,
        -0.164712, -0.685976, -0.111924, -0.0535102, -0.323499, -0.186045, -0.32823, -0.285451},
    {AKT2, RNA} → {-0.181665, 0., -0.0308197, -0.272944, -0.11343, -0.195227, -0.288764,
        -0.0677041, -0.527355, -0.156887, -0.0753422, -0.260169, -0.203779, -0.424779, -0.393137},
    {INPP5D, RNA} → {-0.0946381, 0., -0.0499842, -0.209521, -0.0834031, -0.206053, -0.194235,
        -0.138577, -0.445556, -0.18886, -0.191841, -0.264879, -0.211622, -0.443452, -0.505684},
    {RELA, RNA} → {-0.182184, 0., -0.102832, -0.192087, -0.107091, -0.107886, -0.209808,
        -0.161115, -0.732229, -0.128305, -0.179304, -0.0954536, -0.186766, -0.321845, -0.306438},
    {IFITM1, RNA} → {-0.0762908, 0., -0.0762908, -0.328786, -0.103862, -0.14067, -0.436521,
        -0.031433, -0.59476, -0.358704, -0.167038, -0.153718, -0.290912, -0.14067, -0.14067},
    {NFATC1, RNA} → {-0.207392, 0., 0.0243293, -0.206792, -0.0454187, -0.210496, -0.164606,
        0.029871, -0.73394, -0.23687, -0.0276469, -0.136814, -0.229538, -0.212809, -0.35643},
    {KRAS, RNA} → {-0.295759, 0., -0.270522, -0.19744, -0.195045, -0.0274281, 0.0647688,
        -0.15653, -0.337896, 0.0696863, -0.415278, -0.372864, -0.371336, -0.315801, -0.254436},
    {PRKCB, RNA} → {-0.134799, 0., -0.202564, -0.36102, -0.194269, -0.368006, -0.234408,
        -0.278034, 0.0129622, -0.22718, -0.30994, -0.336047, -0.400156, -0.132084, -0.258268},
    {CHUK, RNA} → {-0.221426, 0., -0.243572, -0.110392, -0.163651, -0.016607, -0.0265009,
        -0.143153, -0.0850638, 0.0363063, -0.410573, -0.443598, -0.350686, -0.483875, -0.316522},
    {SOS2, RNA} → {-0.109612, 0., -0.21095, -0.180964, -0.218469, -0.222684, -0.134366, -0.214812,
        -0.142651, -0.119655, -0.334894, -0.511564, -0.336646, -0.363223, -0.309248},
    {NRAS, RNA} → {-0.295624, 0., -0.327573, -0.246054, -0.26694, 0.00690398, 0.0789832,
        -0.283173, -0.204311, 0.130486, -0.27171, -0.37123, -0.338956, -0.354779, -0.275433},
    {RAC2, RNA} → {-0.27057, 0., -0.200258, -0.253468, -0.206899, -0.200258, -0.188386,
        -0.240359, -0.492962, -0.154825, -0.194383, -0.27057, -0.240359, -0.29196, -0.353953},
    {PIK3R1, RNA} → {-0.134125, 0., -0.221849, -0.39922, -0.206738, -0.142619, -0.0985213,
        -0.194229, -0.353593, -0.0867095, -0.316644, -0.281426, -0.383254, -0.315641, -0.32308},
```

```
{PPP3CB, RNA} → {-0.134308, 0., -0.324192, -0.236684, -0.227212, -0.0392361, 0.000598265,
   -0.254447, -0.389098, 0.0704552, -0.232981, -0.289371, -0.296473, -0.43436, -0.363307},
{MAP2K1, RNA} → {-0.290602, 0., -0.263418, -0.097213, -0.162076, 0.0113004, 0.0584464,
   -0.163838, -0.551688, 0.0989885, -0.215994, -0.337159, -0.323867, -0.348424, -0.281565},
{PIK3CB, RNA} → {-0.0578027, 0., -0.318755, -0.101769, -0.142235, -0.0286284, -0.0888864,
   -0.245651, -0.337763, -0.014764, -0.494598, -0.24891, -0.524684, -0.132151, -0.285936},
{PIK3CD, RNA} → {0.0425586, 0., 0.0819898, -0.245952, 0.0546317, -0.122236, -0.0207547,
   0.106307, -0.504487, -0.273122, -0.293284, -0.120979, -0.160252, -0.312786, -0.589954},
{SOS1, RNA} → {-0.0843912, 0., -0.140657, -0.199758, -0.155036, -0.225141, -0.118111,
   -0.179889, -0.387049, -0.112787, -0.294319, -0.383107, -0.385321, -0.338174, -0.391661},
{PIK3CA, RNA} → {-0.109393, 0., -0.161505, -0.217789, -0.141512, -0.0218752, -0.00510719,
   -0.115095, -0.344167, -0.0684767, -0.367387, -0.263293, -0.370436, -0.402421, -0.504122},
{MALT1, RNA} → {-0.176556, 0., -0.115214, -0.270208, -0.153426, -0.15263, -0.143132,
   -0.135963, -0.436426, -0.0337053, -0.235971, -0.372782, -0.408248, -0.331674, -0.365796},
{CR2, RNA} → {-0.137687, 0., -0.0350415, -0.23329, -0.305072, -0.0614728, 0.00616466,
   0.0884987, -0.701171, -0.109768, -0.228735, -0.404247, -0.300084, 0.105746, -0.00376155},
{BTK, RNA} → {0.0774535, 0., -0.157636, -0.0652611, -0.130991, -0.163068, -0.12647,
   -0.256728, -0.354206, -0.169189, -0.440392, -0.42477, -0.377294, -0.26322, -0.314868},
{Q08209, Protein} → {-0.0216864, 0., -0.266155, 0.0661018, -0.0537324, -0.182569, -0.825726,
   -0.0314044, -0.429717, -0.099843, 0.00134231, Missing[], -0.0659157, 0.0159995, -0.0778852},
{P29350, Protein} → {0.101145, 0., -0.0464515, -0.021366, 0.0211927, -0.0857754, -0.339972,
   -0.15356, -0.876384, -0.107782, -0.166325, Missing[], -0.162937, -0.0754141, -0.0249273}|⟩
```

We can now extract and plot the sequence of figures:

In[230]:= **exampleBCellReceptorFigureTimeSet = KEGGPathwayVisual["path:hsa04662",**
   ResultsFormat → "Figure", MemberSet → membersBCellReceptor, Intensities → intensitiesAll]

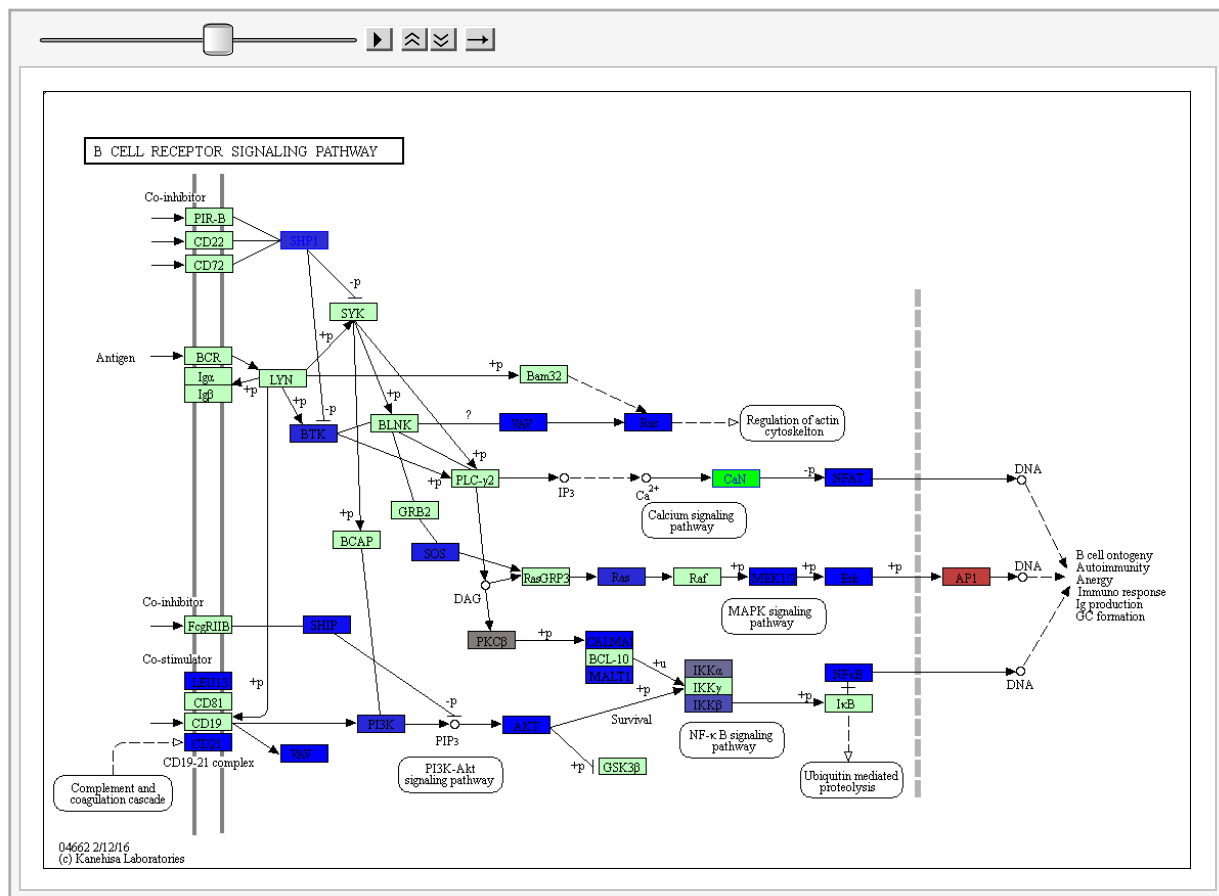{| Pathway → path:hsa04652,
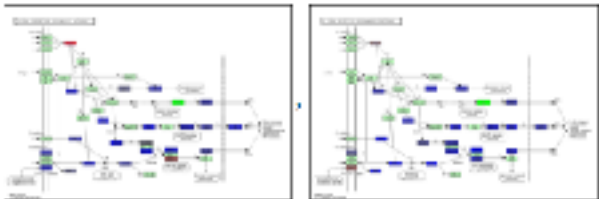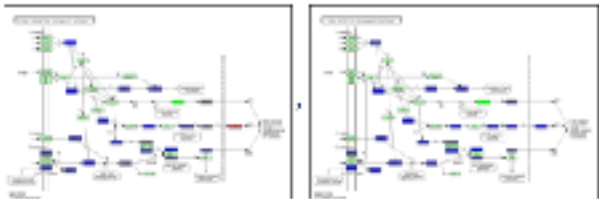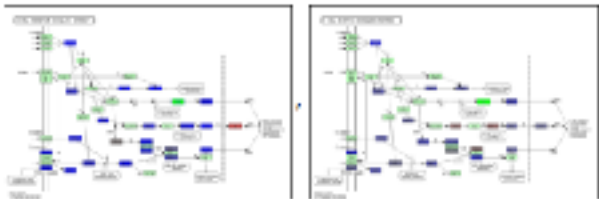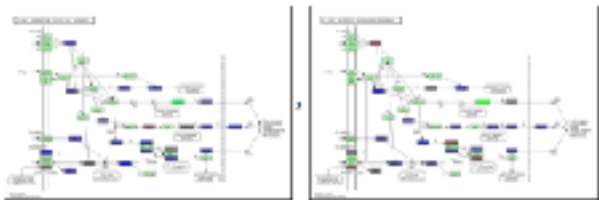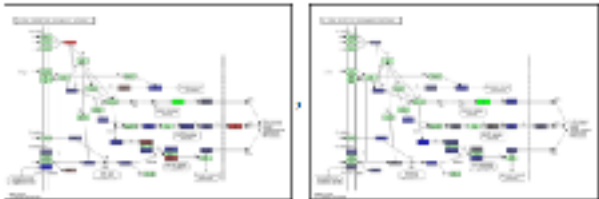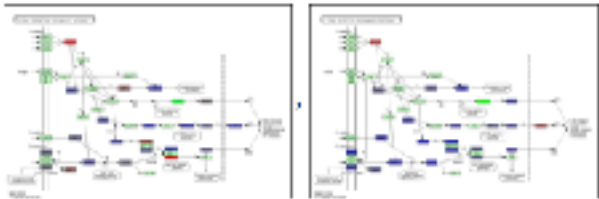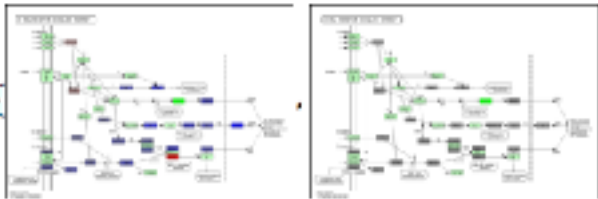
Results →

Out[230]=

We can use ListAnimate to generate a movie/animation of the results

In[231]:= `ListAnimate[exampleBCellReceptorFigureTimeSet["Results"], ImageSize → Automatic]`

Out[231]=

We can set the ResultsFormat to "Movie" to output a movie version:

In[232]:= `KEGGPathwayVisual["path:hsa04662", ResultsFormat → "Movie",`
`MemberSet → membersBCellReceptor, Intensities → intensitiesAll]`

```
File path_hsa04662.mov already exists. Please choose a
   different file name or rename/move existing file and retry.
```

Related Tutorials

- MathIOmica Dynamic Transcriptome

- MathIOmica Overview

- MathIOmica Guide