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MathIOmica: Dynamic Transcriptome

The MathIOmica Dynamic Transcriptome is a brief guide to analyzing the dynamics of transcriptome data. The presentation is streamlined, without discussion of the functions used, but with links to each function provided at each step of the calculation. For more details consult MathIOmica's documentation of each funciton, and the MathIOmica Tutorial for a deeper presentation of a multiple omics analysis.

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[8]:=
```
<< MathIOmica`
```

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[9]:=
```
rnaExample = Get[FileNameJoin[{ConstantMathIOmicaExamplesDirectory, "rnaExample"}]]
```

Out[9]=
```
⟨| 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[10]:= Query[Keys]@rnaExample
Out[10]= {7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21}
```

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

```
In[11]:= 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[12]:= rnaLongitudinal = KeyMap[sampleToDays, rnaExample]
```

```
Out[12]= ⟨|186 → ⟨|{FAM138A, RNA} → {{0}, {OK}},
             {OR4F5, RNA} → {{0}, {OK}}, {LOC729737, RNA} → {{2.73998}, {OK}}, ··· 25262 ···,
             {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...**

Processing OmicsObject Transcriptome Data

We normalize the transcriptome data using the QuantileNormalization function.

```
In[13]:= rnaQuantileNormed = QuantileNormalization[rnaLongitudinal, ListIndex → 1, ComponentIndex → 1]
```

```
Out[13]= ⟨|186 → ⟨|{FAM138A, RNA} → {{0.}, {OK}}, {OR4F5, RNA} → {{0.}, {OK}},
             {LOC729737, RNA} → {{2.2946}, {OK}}, ··· 25262 ···, {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...**

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

```
In[14]:= rnaZeroTagged = LowValueTag[rnaQuantileNormed, 0]
```

```
Out[14]= ⟨|186 → ⟨|{FAM138A, RNA} → {{Missing[]}, {OK}},
             {OR4F5, RNA} → {{Missing[]}, {OK}}, {LOC729737, RNA} → {{2.2946}, {OK}}, ··· 25263 ···,
             {RNA45S5, RNA} → {{Missing[]}, {OK}}, {DUX4L, RNA} → {{Missing[]}, {OK}}|⟩,
           255 → ⟨| ··· 1 ··· |⟩, ··· 11 ···, ··· 1 ···, 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:

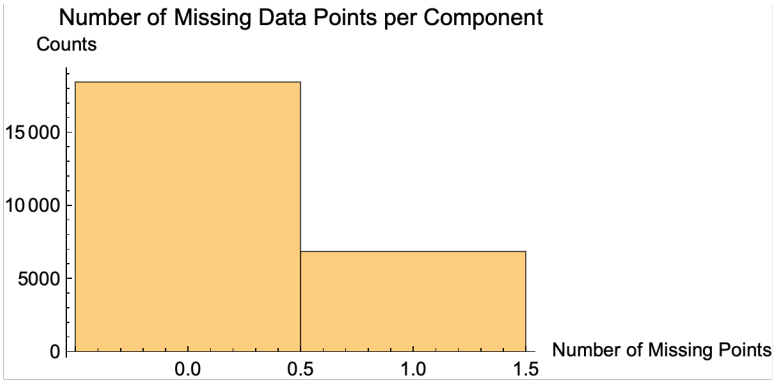
In[15]:=
```
rnaNoiseAdjusted = LowValueTag[rnaZeroTagged, 1, ValueReplacement → 1]
```

Out[15]=
```
⟨|186 → ⟨|{FAM138A, RNA} → {{Missing[]}, {OK}},
   {OR4F5, RNA} → {{Missing[]}, {OK}}, {LOC729737, RNA} → {{2.2946}, {OK}}, ··· 25 263 ···,
   {RNA45S5, RNA} → {{Missing[]}, {OK}}, {DUX4L, RNA} → {{Missing[]}, {OK}}|⟩,
 255 → ⟨| ··· 1 ··· |⟩, ··· 11 ···, ··· 1 ···, 400 → ⟨| ··· 1 ··· |⟩|⟩
```

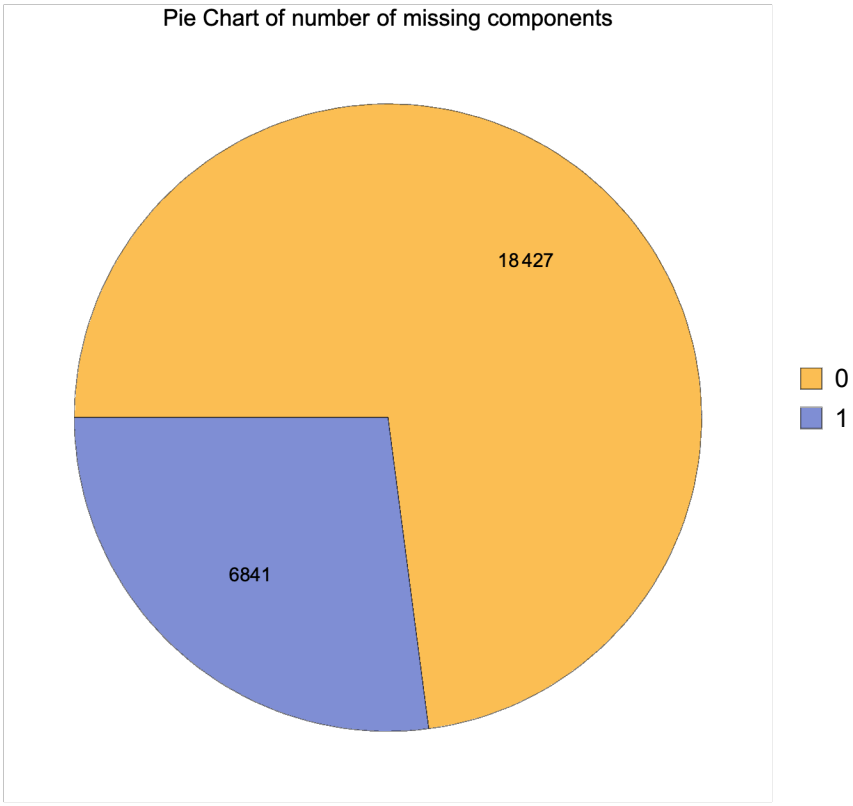
large output | **show less** | **show more** | **show all** | **set size limit...**

We filter out data using FilterMissing where the reference healthy point "255" is missing and retain data with at least 3/4 points available:

In[16]:=
```
rnaFiltered = FilterMissing[rnaNoiseAdjusted, 3 / 4, Reference → "255"]
```




```
{Missing -> Counts: , ‹|0 → 18427, 1 → 6841|›}
```



Out[16]=

```
‹|186 → ‹|{FAM138A, RNA} → {{Missing[]}, {OK}},
   {OR4F5, RNA} → {{Missing[]}, {OK}}, {LOC729737, RNA} → {{2.2946}, {OK}}, ··· 25263 ···,
   {RNA45S5, RNA} → {{Missing[]}, {OK}}, {DUX4L, RNA} → {{Missing[]}, {OK}}|›,
 255 → ‹| ··· 1 ···|›, ··· 11 ···, ··· 1 ···, 400 → ‹| ··· 1 ···|›|›
```

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

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

In[17]:=
```
timesRNA = TimeExtractor[rnaFiltered]
```

Out[17]=
```
{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 using CreateTimeSeries :

In[18]:=
```
timeSeriesRNA = CreateTimeSeries[rnaFiltered]
```

Out[18]=
```
⟨|{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},
 ··· 25260 ···, {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...**

We use SeriesApplier to implement a logarithm transformation:

In[19]:=
```
timeSeriesRNALog = SeriesApplier[Log, timeSeriesRNA]
```

Out[19]=
```
⟨|{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},
 ··· 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...**

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

In[20]:=
```
rnaCompared = SeriesInternalCompare[timeSeriesRNALog, ComparisonIndex → 2]
```

Out[20]=
```
⟨|{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...**

Next, we normalize each series, using again SeriesApplier :

In[21]:=
```
normedRNACompared = SeriesApplier[Normalize, rnaCompared]
```

Out[21]=
```
⟨| {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},
 ··· 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...**

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

In[22]:=
```
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[22]=
```
⟨| {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},
 ··· 11 782 ···, {UTY, RNA} → {-0.0324671, 0., -0.0127666, -0.256982, -0.0805343, -0.152071,
   ··· 3 ···, -0.198929, -0.280753, -0.310545, -0.339742, -0.346361, -0.542312} |⟩
```

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. We repeat the steps in the processing section above using a resampled set of measurements.

We create a resampling of 100000 sets using BootstrapGeneral :

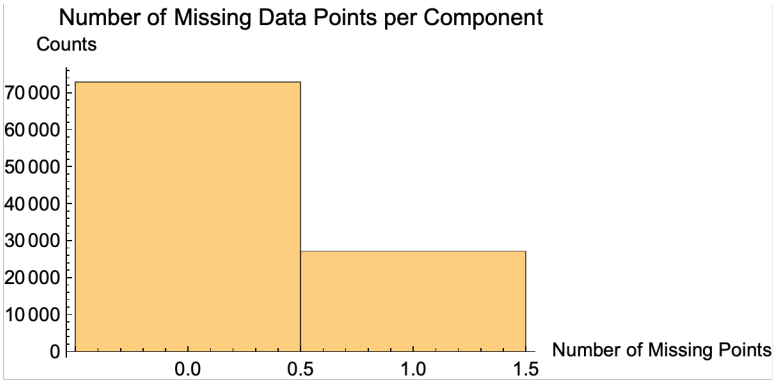
In[23]:=
```
rnaBootstrap = BootstrapGeneral[rnaLongitudinal, 100 000]
```

Out[23]=
```
⟨| 186 → ⟨| 1 → {{0}, {OK}}, 2 → {{0}, {OK}}, 3 → {{10.0429}, {OK}},
   4 → {{12.8612}, {OK}}, 5 → {{2.37963}, {OK}}, ··· 99 991 ···, 99 997 → {{43.8242}, {OK}},
   99 998 → {{2.40797}, {OK}}, 99 999 → {{4.68299}, {OK}}, 100 000 → {{3.29531}, {OK}} |⟩,
 255 → ⟨| ··· 1 ··· |⟩, ··· 11 ···, ··· 1 ···, 400 → ⟨| ··· 1 ··· |⟩ |⟩
```

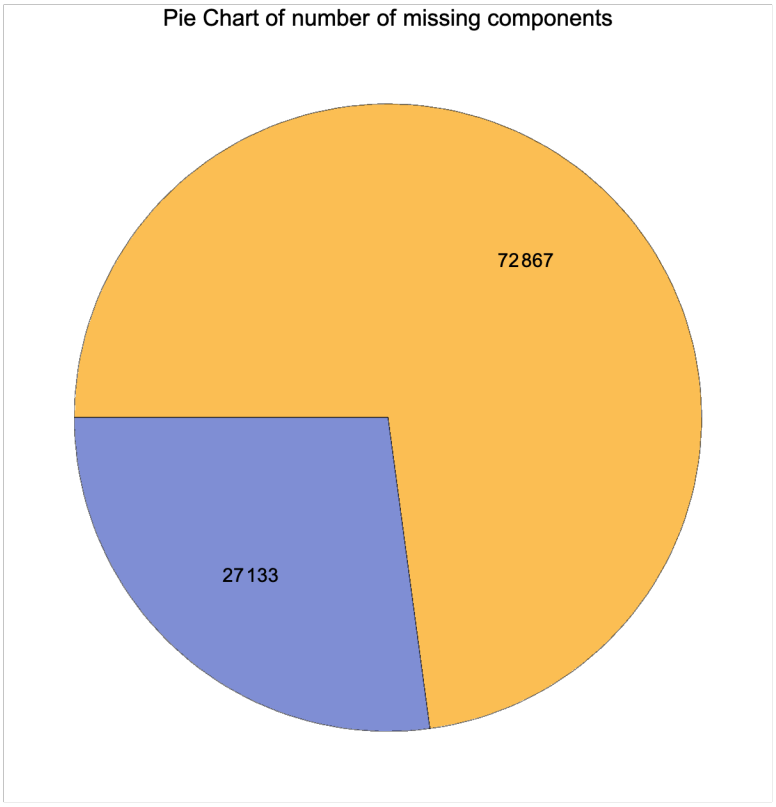
large output | **show less** | **show more** | **show all** | **set size limit...**

As with the regular data we: 1. normalize , 2. tag zero values, 3. tag values of FPKM <1, 4. filter missing data, 5. create a time series, 6. take a logarithm, 7. compare to "255" reference, 8. take the norm of each time series, 9. clean out constant series

In[24]:=
```
(*1*)rnaBootstrapQuantileNormed = QuantileNormalization[rnaBootstrap, ListIndex → 1, ComponentIndex → 1];
(*2*)rnaBootstrapZeroTagged = LowValueTag[rnaBootstrapQuantileNormed, 0];
(*3*) rnaBootstrapNoiseAdjusted = LowValueTag[rnaBootstrapZeroTagged, 1, ValueReplacement → 1];
(*4*) rnaBootstrapFiltered = FilterMissing[rnaBootstrapNoiseAdjusted, 3 / 4, Reference → "255"];
(*5*) timeSeriesBootstrapRNA = CreateTimeSeries[rnaBootstrapFiltered];
(*6*) timeSeriesBootstrapRNALog = SeriesApplier[Log, timeSeriesBootstrapRNA];
(*7*)rnaBootstrapCompared = SeriesInternalCompare[timeSeriesBootstrapRNALog, ComparisonIndex → 2];
(*8*)normedBootstrapRNACompared = SeriesApplier[Normalize, rnaBootstrapCompared];
(*9*)rnaBootstrapFinalTimeSeries = ConstantSeriesClean[normedBootstrapRNACompared];
```




```
{Missing -> Counts: , ‹|0 → 72 867, 1 → 27 133|›}
```




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

Classification, Clustering and Visualization of Transcriptome Time Series

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

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

```
In[33]:= q95RNA = QuantileEstimator[rnaBootstrapFinalTimeSeries, timesRNA]
Out[33]= 0.85987
```

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

```
In[34]:= q95RNASpikes = QuantileEstimator[rnaBootstrapFinalTimeSeries, timesRNA, Method → "Spikes"]
Out[34]= ⟨| 14 → {0.886757, -0.348387}, 15 → {0.861302, -0.337344} |⟩
```

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

```
In[35]:= rnaClassification = TimeSeriesClassification[rnaFinalTimeSeries,
           timesRNA, LombScargleCutoff → q95RNA, SpikeCutoffs → q95RNASpikes]
         Method → "LombScargle"
```

```
Out[35]= ⟨| SpikeMax → ⟨| ··· 1 ··· |⟩, ··· 7 ···,
           f7 → ⟨| {MIR6723, RNA} → {{0.214503, 0.000338299, 0.0390479, 0.0653206, 0.291434, 0.336712, 0.865961},
                {··· 1 ···}}, ··· 60 ···, {DNASE1L1, RNA} → ··· 1 ··· |⟩ |⟩
```

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

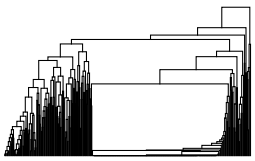
To obtain the possible frequencies we simply run LombScargle over the desired times for one of the time series and set the `FrequenciesOnly` option to True :

```
In[36]:= LombScargle[rnaFinalTimeSeries[[1]], timesRNA, FrequenciesOnly → True]
Out[36]= ⟨| f1 → 0.00500668, f2 → 0.0104306, f3 → 0.0158545,
           f4 → 0.0212784, f5 → 0.0267023, f6 → 0.0321262, f7 → 0.0375501 |⟩
```

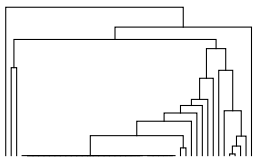
We now cluster our RNA data using TimeSeriesClusters :

```
In[39]:= rnaClusters = TimeSeriesClusters[rnaClassification, PrintDendrograms → True]
```

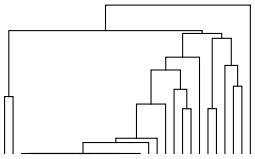
```
⟨| SpikeMax → ⟨| G1 → ⟨| {G1S1} →
```



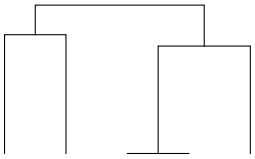
```
, {G1S2} →
```



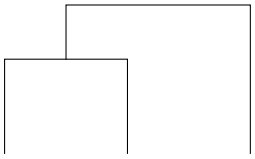
```
,
{G1S3} →
```



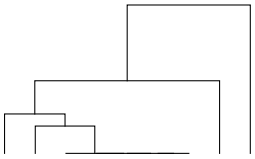
```
, {G1S4} →
```



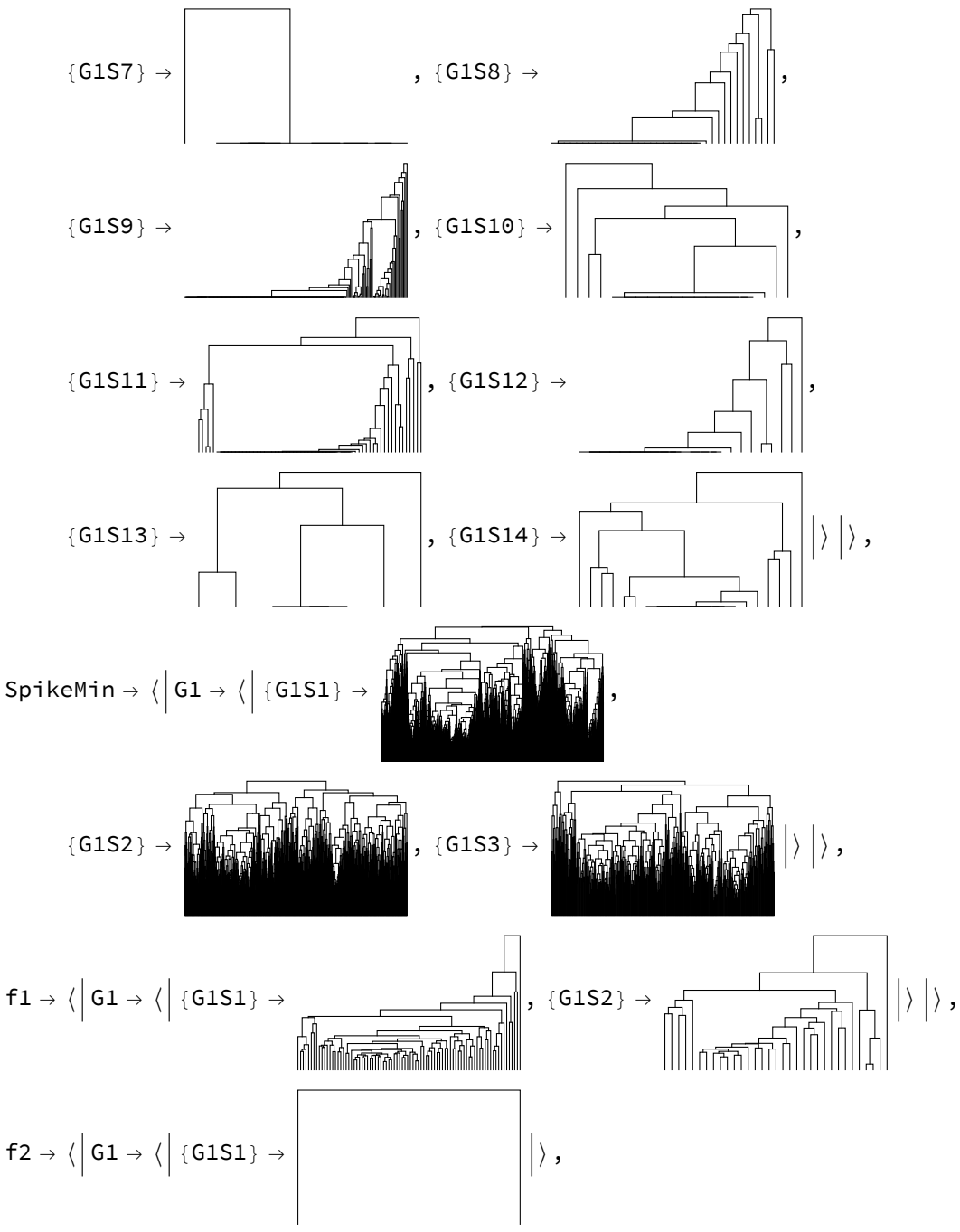
```
,
{G1S5} →
```



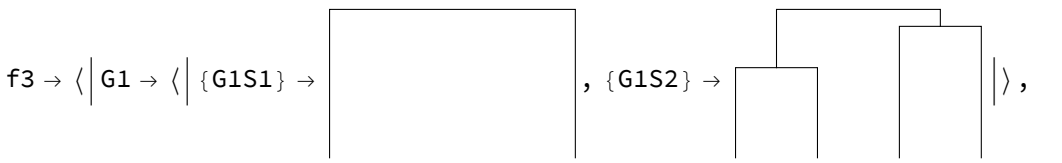
```
, {G1S6} →
```



```
,
```

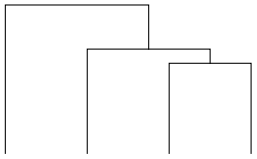



```
G2 → ‹| {G2S1} → {{0.283264, 0., 0.149932, 0.415971, 0.146512,
      0.2947, 0.267954, 0.15466, 0.432236, 0.281295, 0.151677,
      0.170078, 0.149432, 0.249221, 0.343349} → {POMP, RNA}} |› ⟩ ,
```

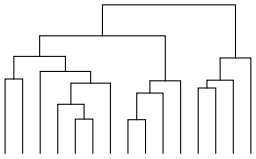



```
G2 → ⟨ | {G2S1} → {{-0.0325957, 0., 0.295712, 0.400274, 0.29765, 0.246613,
```

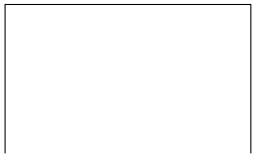
```
0.0892792, 0.27966, 0.493911, 0.0203708, 0.100827, 0.223014, 0.386987,
0.111948, 0.221638} → {DUSP23, RNA}}, {G2S2} →
```



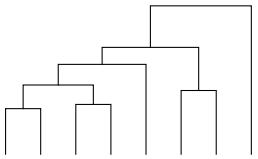
```
⟩,
G3 → ⟨| {G3S1} →
```



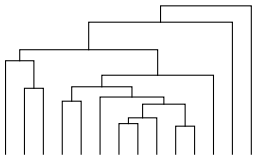
```
, {G3S2} → {{-0.0555808, 0., 0.317043,
0.41872, 0.395921, 0.283783, 0.184223, 0.30154, 0.265713, 0.167588,
0.138036, 0.110511, 0.434502, 0.0277974, 0.198492} → {PYCARD, RNA}} |⟩,
G4 → ⟨| {G4S1} →
```



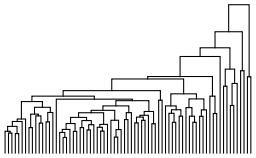
```
, {G4S2} →
{{0.0572204, 0., -0.22398, -0.11871, -0.0686182, -0.0820599,
-0.216559, -0.187636, -0.682766, 0.0415532, -0.197678,
0.14124, -0.497177, -0.0370276, 0.251877} → {ST7, RNA}} |⟩ |⟩,
f4 → ⟨| G1 → ⟨| {G1S1} →
```



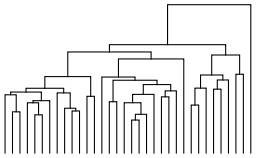
```
, {G1S2} →
```



```
|⟩,
G2 → ⟨| {G2S1} →
```

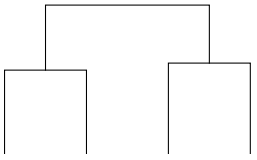


```
, {G2S2} →
```

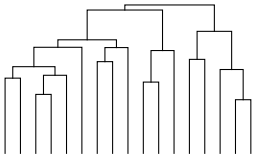


```
|⟩ |⟩,
f5 → ⟨| G1 → ⟨| {G1S1} → {{-0.335157, 0., 0.107286, 0.337104, -0.0121183,
0.243878, 0.043649, -0.0358548, 0.410512, 0.305297, 0.354136,
0.00802356, 0.0854209, 0.461454, 0.303753} → {ALG14, RNA}},
{G1S2} →
```

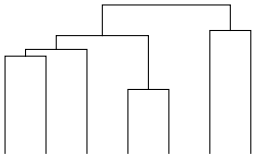
```
, {G1S3} →
```



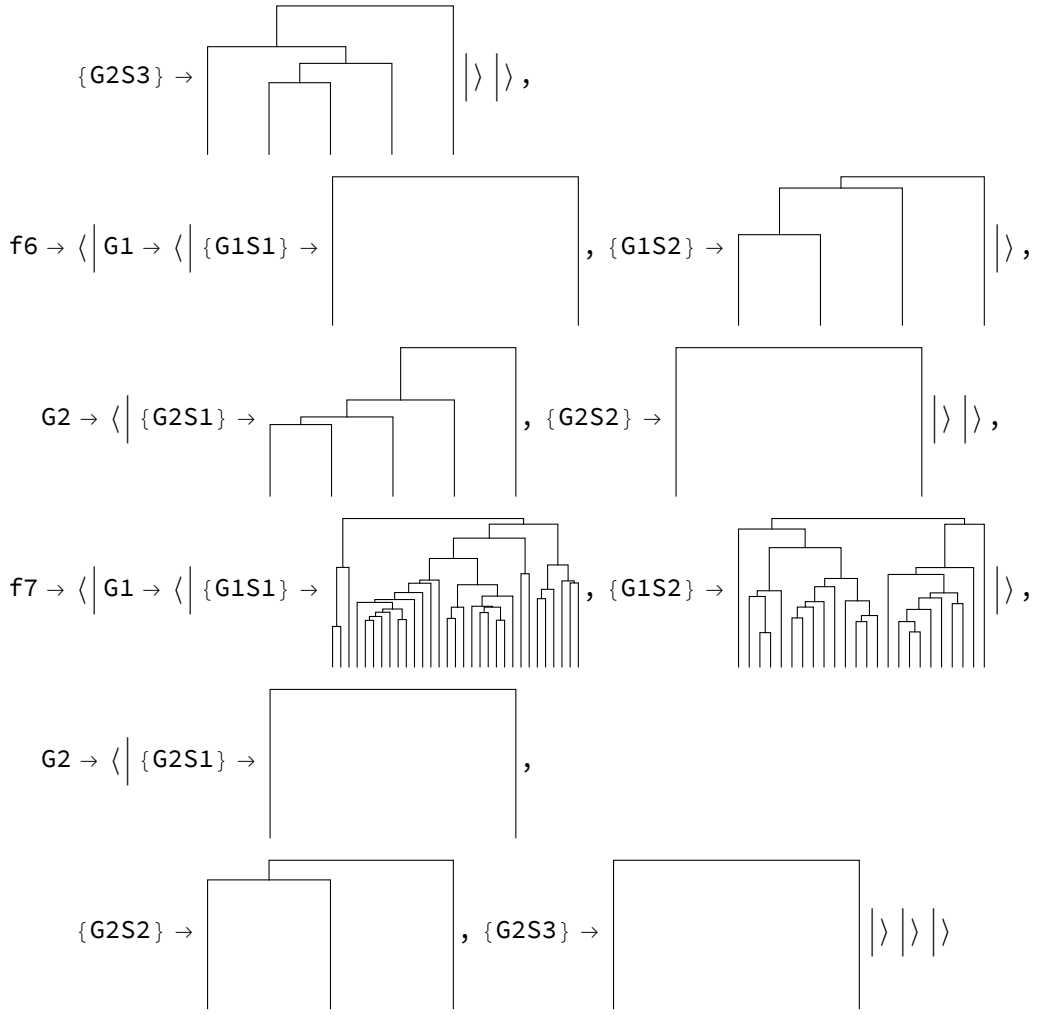
```
|⟩,
G2 → ⟨| {G2S1} →
```



```
, {G2S2} →
```



```
,
```



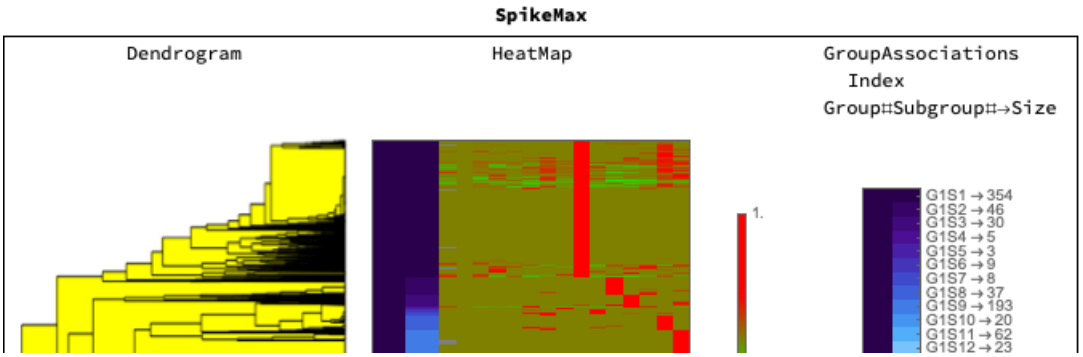
Out[39]=

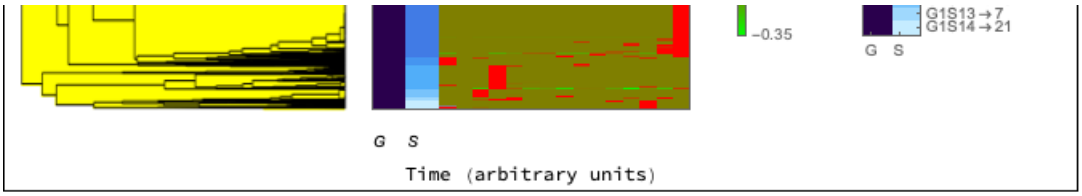
⟨| ··· 1 ··· |⟩

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

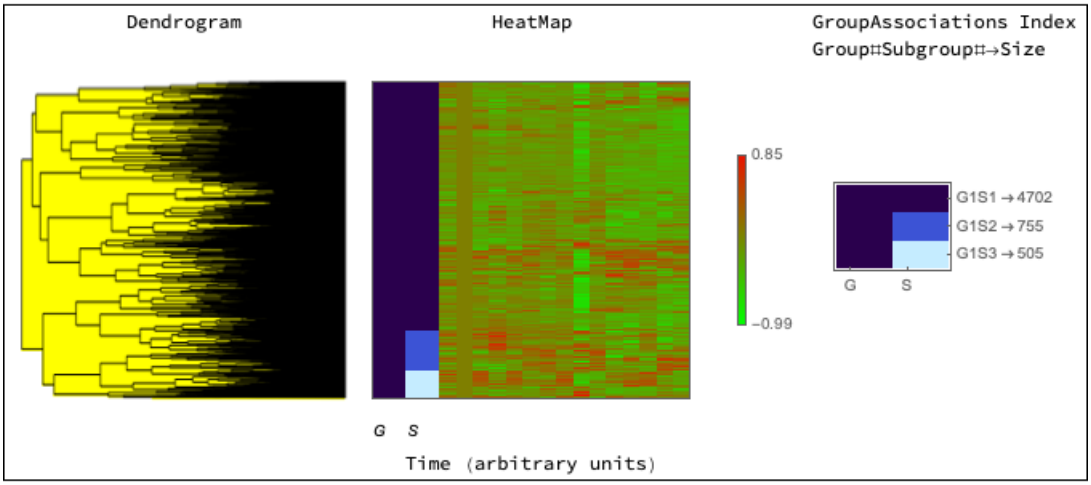
For each class we can generate a dendrogram/heatmap plot using TimeSeriesDendrogramsHeatmaps , with groupings represented on the left, and highlighted to represent the grouping level. The G, S, columns represent the groupings and subgroupings generated by the clustering. The legend shows the corresponding groupings and subgrouping, and the number of elements in each group subgroup.

In[40]:=
```
TimeSeriesDendrogramsHeatmaps[rnaClusters]
```

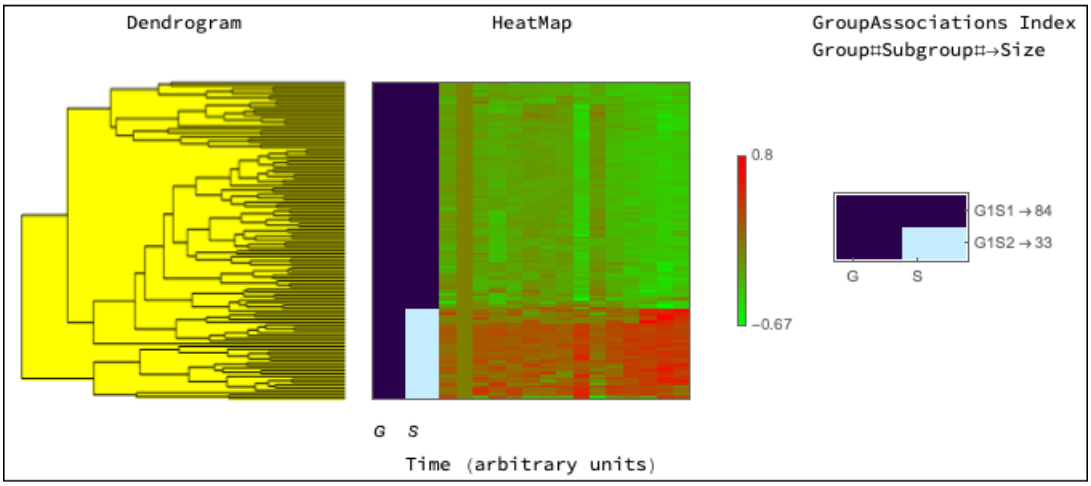




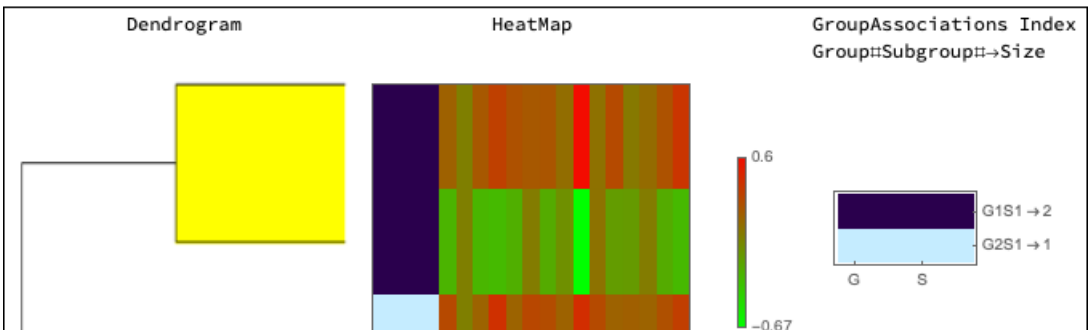
SpikeMin



f1

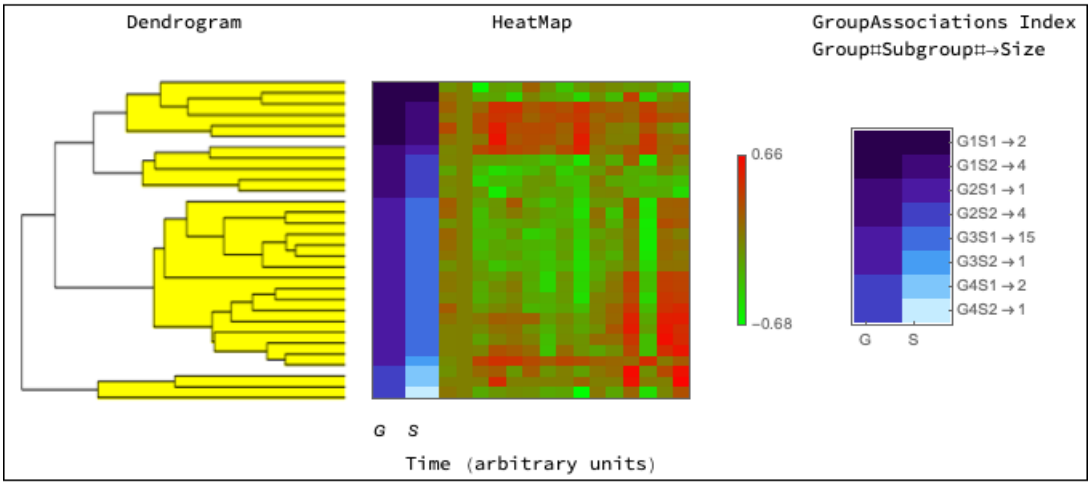


f2

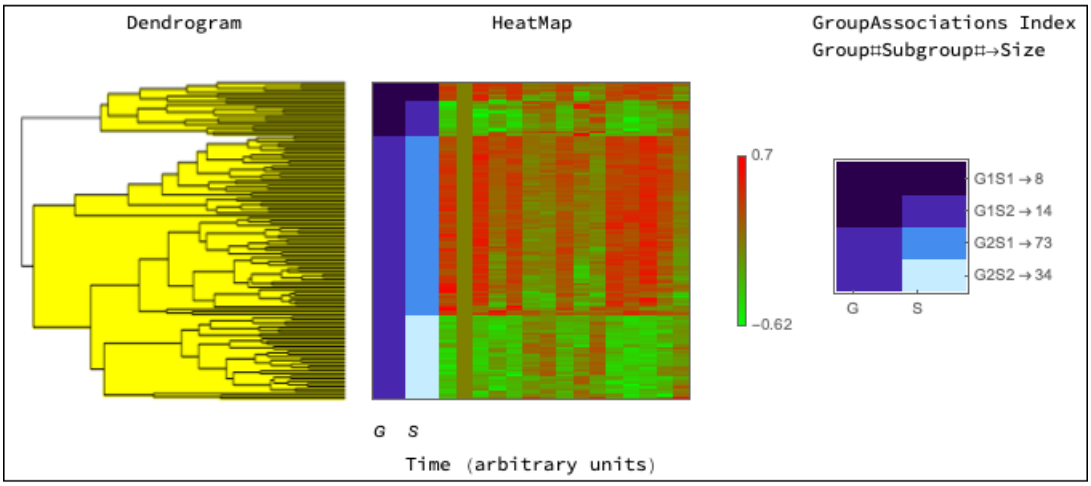


Out[40]=

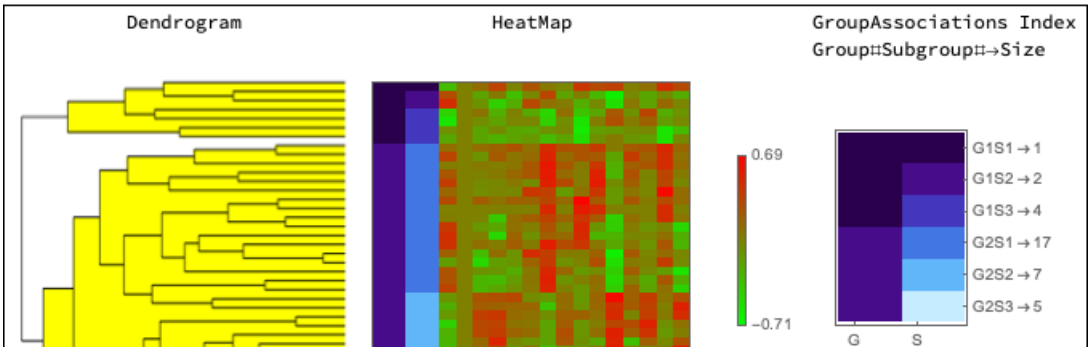
f3

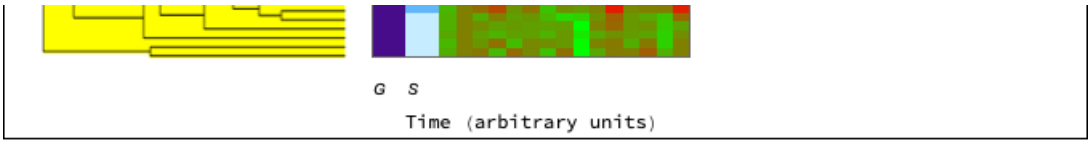


f4

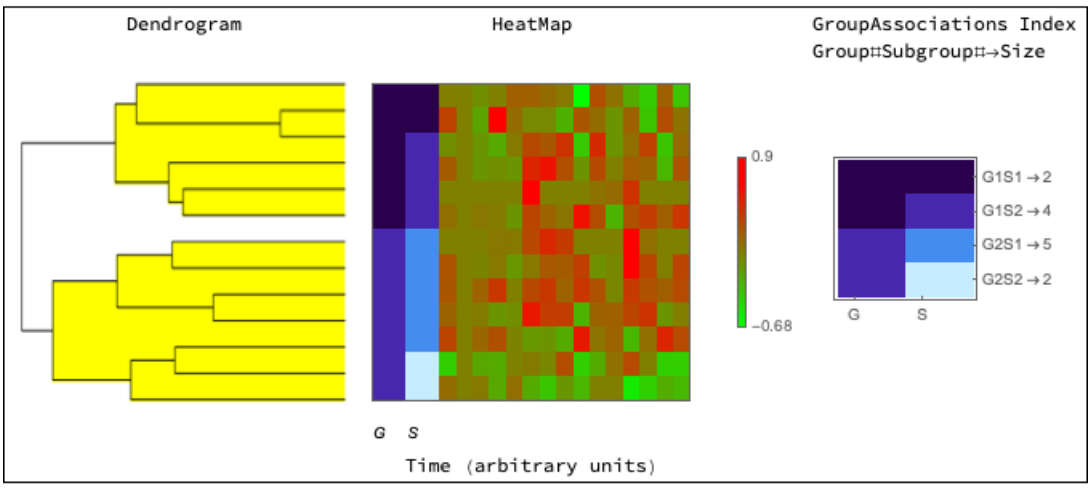


f5

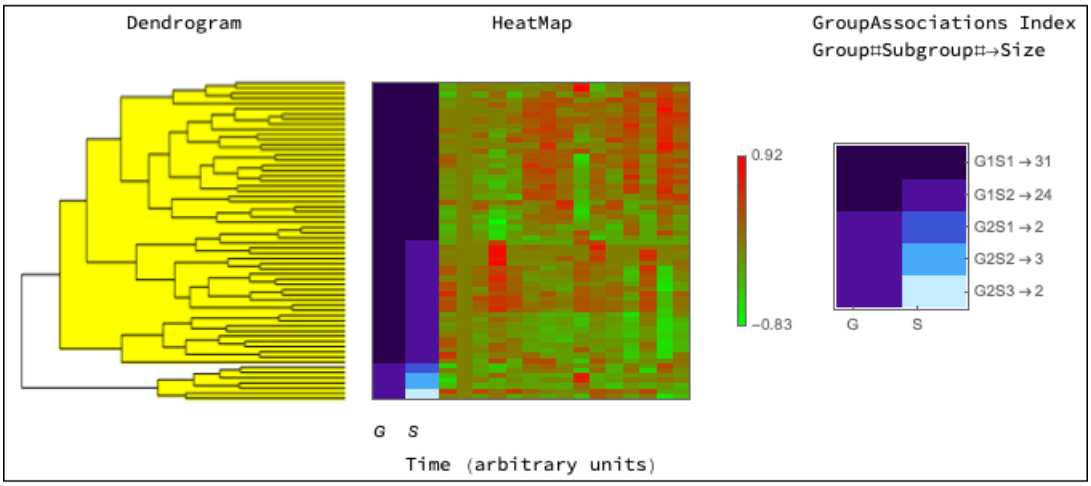




f6



f7



Annotation and Enrichment

We can carry out Gene Ontology analysis using GOAnalysis for all the classes and groups/subgroups. We only report terms for which there are at least 3 members (2 sets of GO terms, one each for proteomics and transcriptomics). Please note that this may be a time consuming computation.

In[41]:=
```
goAnalysisRNA = GOAnalysis[rnaClusters, OntologyLengthFilter → 3, ReportFilter → 3];
```

The output of GOAnalysis has enrichments for each class and group

In[42]:=
```
Query[Keys]@goAnalysisRNA
```

Out[42]=
```
{SpikeMax, SpikeMin, f1, f2, f3, f4, f5, f6, f7}
```

In[43]:=
```
Query[All, Keys]@goAnalysisRNA
```

Out[43]=
```
⟨|SpikeMax → {G1S1, G1S2, G1S3, G1S4, G1S5, G1S6, G1S7, G1S8, G1S9, G1S10, G1S11, G1S12, G1S13, G1S14},
 SpikeMin → {G1S1, G1S2, G1S3}, f1 → {G1S1, G1S2}, f2 → {G1S1, G2S1},
 f3 → {G1S1, G1S2, G2S1, G2S2, G3S1, G3S2, G4S1, G4S2}, f4 → {G1S1, G1S2, G2S1, G2S2},
 f5 → {G1S1, G1S2, G1S3, G2S1, G2S2, G2S3}, f6 → {G1S1, G1S2, G2S1, G2S2}, f7 → {G1S1, G1S2, G2S1, G2S2, G2S3}|⟩
```

We can view results for any of the groups (and also check out the behavior using the heatmaps generated in the previous section

In[44]:=
```
Query["SpikeMax", "G1S1"]@goAnalysisRNA
```

Out[44]=
```
⟨|GO:0005515 →
  {{1.22292×10^-16, 1.40636×10^-13, True}, {193, 8801, 47241, 86}, {{protein binding, molecular_function},
    {{AHI1, RNA}}, {{FAM153C, RNA}}, {{SASS6, RNA}}, {{AP1S3, RNA}}, {{ERO1LB, RNA}}, {{ABL2, RNA}},
     {{KIAA0020, RNA}}, {{EPM2AIP1, RNA}}, {{TUBGCP4, RNA}}, {{SNTB2, RNA}}, {{SRRM1, RNA}},
     {{PIAS2, RNA}}, {{CUL5, RNA}}, {{CEP135, RNA}}, {{RAD50, RNA}}, {{USP8, RNA}}, {{RB1CC1, RNA}},
     {{NEK1, RNA}}, {{KPNA5, RNA}}, {{PWWP2A, RNA}}, {{ARL13B, RNA}}, {{STRN3, RNA}}, {{CHD9, RNA}},
     {{PAG1, RNA}}, {{RAB3IP, RNA}}, {{TAOK3, RNA}}, {{TLK2, RNA}}, {{PRPF4B, RNA}}, {{TRIP11, RNA}},
     {{MLLT4, RNA}}, {{HELLS, RNA}}, {{ITSN1, RNA}}, {{SPRY1, RNA}}, {{PSMD11, RNA}}, {{AZI2, RNA}},
     {{SYNJ2BP, RNA}}, {{RAB11FIP2, RNA}}, {{ZFHX3, RNA}}, {{SLC4A1AP, RNA}}, {{PLCB1, RNA}},
     {{UGGT1, RNA}}, {{ATXN2, RNA}}, {{LCOR, RNA}}, {{KLHL42, RNA}}, {{ITSN2, RNA}}, {{CD84, RNA}},
     {{LGALS8, RNA}}, {{HMBOX1, RNA}}, {{KIF3B, RNA}}, {{EEA1, RNA}}, {{DCUN1D5, 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}}, {{HCST, RNA}},
     {{NTNG2, RNA}}, {{PRKCDBP, RNA}}, {{HES1, RNA}}, {{EVI5, RNA}}, {{INSR, RNA}}, {{ZFC3H1, RNA}}}}},
 GO:0005737 → {{3.78446×10^-10, 2.17606×10^-7, True}, {193, 6648, 47241, 61},
   {{cytoplasm, cellular_component}, {{{GDPD1, RNA}}, {{KIAA1731, RNA}}, {{AK9, RNA}}, {{DGKE, RNA}},
     {{CELF2, RNA}}, {{NAPEPLD, RNA}}, {{TYW5, RNA}}, {{LPIN1, RNA}}, {{EPM2AIP1, RNA}}, {{SNTB2, RNA}},
     {{BDP1, RNA}}, {{USP8, RNA}}, {{RB1CC1, RNA}}, {{NEK1, RNA}}, {{KPNA5, RNA}}, {{STRN3, RNA}},
     {{CHD9, RNA}}, {{TAOK3, RNA}}, {{MLLT4, RNA}}, {{BGLAP, RNA}}, {{PRR11, RNA}}, {{SPATS2L, RNA}},
     {{SPRY1, RNA}}, {{AZI2, RNA}}, {{MAP7D3, RNA}}, {{ZFHX3, RNA}}, {{SLC4A1AP, RNA}}, {{PLCB1, RNA}},
     {{DHX33, RNA}}, {{ATXN2, RNA}}, {{KLHL42, RNA}}, {{ITSN2, RNA}}, {{HMBOX1, RNA}}, {{EEA1, RNA}},
     {{PPIAL4D, RNA}, {PPIAL4E, RNA}}, {{AS3MT, RNA}}, {{HRASLS2, RNA}}, {{KIAA1377, RNA}}, {{FGD6, RNA}},
     {{ANKS1B, RNA}}, {{CEP128, RNA}}, {{BRCA1, RNA}}, {{RNF165, RNA}}, {{MAP4K3, RNA}}, {{BARD1, RNA}},
     {{KIAA1524, RNA}}, {{GK5, RNA}}, {{SPATA5, RNA}}, {{CCRN4L, RNA}}, {{SMAD1, RNA}}, {{GDAP1, RNA}},
     {{SCAI, RNA}}, {{CPEB3, RNA}}, {{RNASEK-C17orf49, RNA}}, {{ALS2CR12, RNA}}, {{NME9, RNA}},
     {{HSP90AB4P, RNA}}, {{CNTLN, RNA}}, {{PRKCDBP, RNA}}, {{HES1, RNA}}, {{SAPCD2, RNA}}}}},
 GO:0005814 → {{2.31616×10^-9, 8.1084×10^-7, True}, {193, 123, 47241, 9},
   {{centriole, cellular_component}, {{{AHI1, RNA}}, {{KIAA1731, RNA}}, {{SASS6, RNA}}, {{CEP135, RNA}},
     {{SCLT1, RNA}}, {{CEP128, RNA}}, {{CEP152, RNA}}, {{CCDC146, RNA}}, {{CNTLN, RNA}}}}},
 GO:0005813 → {{2.82031×10^-9, 8.1084×10^-7, True}, {193, 581, 47241, 16},
   {{centrosome, cellular_component}, {{{AHI1, RNA}}, {{KIAA1731, RNA}}, {{SASS6, RNA}}, {{ANKRD26, RNA}},
     {{TUBGCP4, RNA}}, {{CEP135, RNA}}, {{NEK1, RNA}}, {{RAB3IP, RNA}}, {{SCLT1, RNA}}, {{MAP7D3, RNA}},
     {{ITSN2, RNA}}, {{KIF3B, RNA}}, {{KIAA1377, RNA}}, {{CCDC15, RNA}}, {{IKZF4, RNA}}, {{CEP152, RNA}}}}},
 GO:0005634 → {{8.50629×10^-9, 1.95645×10^-6, True}, {193, 7197, 47241, 61},
   {{nucleus, cellular_component}, {{{AK9, RNA}}, {{ZNF286B, RNA}}, {{CELF2, RNA}}, {{ZFP14, RNA}},
     {{LPIN1, RNA}}, {{SRRM1, RNA}}, {{PIAS2, RNA}}, {{NEK1, RNA}}, {{STRN3, RNA}}, {{CHD9, RNA}},
     {{ZNF292, RNA}}, {{RAB3IP, RNA}}, {{TLK2, RNA}}, {{TRIP11, RNA}}, {{HELLS, RNA}}, {{PRR11, RNA}},
     {{PSMD11, RNA}}, {{MAP7D3, RNA}}, {{DHX29, RNA}}, {{ZFHX3, RNA}}, {{SLC4A1AP, RNA}},
     {{MITF, RNA}}, {{PLCB1, RNA}}, {{LCOR, RNA}}, {{HELZ, RNA}}, {{HMBOX1, RNA}}, {{SP110, RNA}},
     {{NFIA, RNA}}, {{IKZF4, RNA}}, {{ZNF605, RNA}}, {{BRCA1, RNA}}, {{RNF165, RNA}}, {{ZNF763, RNA}},
     {{KIAA1683, RNA}}, {{ZNF682, RNA}}, {{ZNF829, RNA}}, {{DNAJC27, RNA}}, {{BARD1, RNA}},
     {{SYCP2, RNA}}, {{CCRN4L, RNA}}, {{SMAD1, RNA}}, {{PPARGC1B, RNA}}, {{SUMO4, RNA}},
     {{GDAP1, RNA}}, {{RFX3, RNA}}, {{ZNF169, RNA}}, {{FKTN, RNA}}, {{SCAI, RNA}}, {{RPA4, RNA}},
     {{CPEB3, RNA}}, {{ZNF563, RNA}}, {{ZNF573, RNA}}, {{ZNF618, RNA}}, {{ZNF514, RNA}}, {{UBN2, RNA}},
     {{HES1, RNA}}, {{ZNF503, RNA}}, {{SAPCD2, RNA}}, {{EVI5, RNA}}, {{INSR, RNA}}, {{USP36, RNA}}}}},
 GO:0005654 → {{5.3418×10^-8, 0.0000102384, True}, {193, 3749, 47241, 39},
   {{nucleoplasm, cellular_component}, {{{AK9, RNA}}, {{LPIN1, RNA}}, {{KIAA0020, RNA}}, {{EPM2AIP1, RNA}},
     {{SRRM1, RNA}}, {{CHD6, RNA}}, {{BDP1, RNA}}, {{PIAS2, RNA}}, {{RAD50, RNA}}, {{USP8, RNA}},
     {{KPNA5, RNA}}, {{STRN3, RNA}}, {{CHD9, RNA}}, {{PRPF4B, RNA}}, {{MLLT4, RNA}}, {{SPRY1, RNA}},
     {{PSMD11, RNA}}, {{MAP7D3, RNA}}, {{RAB11FIP2, RNA}}, {{ZFHX3, RNA}}, {{SLC4A1AP, RNA}}, {{DHX33, RNA}},
```

{{ATXN2, RNA}}, {{NFIA, RNA}}, {{PPP1R12B, RNA}}, {{IKZF4, RNA}}, {{CEP152, RNA}}, {{BRCA1, RNA}},
{{BARD1, RNA}}, {{CCRN4L, RNA}}, {{SMAD1, RNA}}, {{PPARGC1B, RNA}}, {{C8orf44, RNA}}, {{SCAI, RNA}},
{{RPA4, RNA}}, {{RNASEK-C17orf49, RNA}}, {{SGOL2, RNA}}, {{UBN2, RNA}}, {{HES1, RNA}}}}},
GO:0042384 → {{2.50214×10^-7, 0.0000411066, True}, {193, 153, 47241, 8},
{{cilium assembly, biological_process}, {{{AHI1, RNA}}, {{NEK1, RNA}}, {{ARL13B, RNA}},
{{RAB3IP, RNA}}, {{SCLT1, RNA}}, {{KIAA1377, RNA}}, {{RFX3, RNA}}, {{WDPCP, RNA}}}}},
GO:0003677 → {{3.68453×10^-7, 0.000052965, True}, {193, 2344, 47241, 28},
{{DNA binding, molecular_function},
{{{ZNF286B, RNA}}, {{ZFP14, RNA}}, {{KIAA0020, RNA}}, {{EPM2AIP1, RNA}}, {{SRRM1, RNA}}, {{CHD6, RNA}},
{{BDP1, RNA}}, {{PIAS2, RNA}}, {{RAD50, RNA}}, {{CHD9, RNA}}, {{ZNF292, RNA}}, {{LCOR, RNA}},
{{HMBOX1, RNA}}, {{SP110, RNA}}, {{ZNF605, RNA}}, {{BRCA1, RNA}}, {{ZNF763, RNA}}, {{ZNF829, RNA}},
{{SYCP2, RNA}}, {{ZBED3, RNA}}, {{RFX3, RNA}}, {{ZNF169, RNA}}, {{RNASEK-C17orf49, RNA}},
{{ZNF563, RNA}}, {{ZNF573, RNA}}, {{ZNF618, RNA}}, {{ZNF514, RNA}}, {{HES1, RNA}}}}},
GO:0005829 → {{1.78908×10^-6, 0.000228605, True}, {193, 3476, 47241, 34},
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{{LPIN1, RNA}}, {{TUBGCP4, RNA}}, {{SRRM1, RNA}}, {{CUL5, RNA}}, {{CEP135, RNA}}, {{USP8, RNA}},
{{RB1CC1, RNA}}, {{KPNA5, RNA}}, {{RAB3IP, RNA}}, {{MLLT4, RNA}}, {{ITSN1, RNA}}, {{SPRY1, RNA}},
{{PSMD11, RNA}}, {{SCLT1, RNA}}, {{PLCB1, RNA}}, {{LGALS8, RNA}}, {{KIF3B, RNA}}, {{EEA1, RNA}},
{{PPP1R12B, RNA}}, {{AS3MT, RNA}}, {{PDE1B, RNA}}, {{ARHGAP11B, RNA}}, {{CEP152, RNA}}, {{SMAD1, RNA}},
{{ZBED3, RNA}}, {{SGOL2, RNA}}, {{EVI5, RNA}}, {{INSR, RNA}}, {{UTS2, RNA}}, {{ATG10, RNA}}}}},
GO:0006351 → {{2.45157×10^-6, 0.000281931, True}, {193, 2285, 47241, 26},
{{transcription, DNA-templated, biological_process}, {{{ZNF286B, RNA}}, {{ZFP14, RNA}}, {{LPIN1, RNA}},
{{CHD6, RNA}}, {{BDP1, RNA}}, {{PIAS2, RNA}}, {{RB1CC1, RNA}}, {{CHD9, RNA}}, {{HELLS, RNA}},
{{HMBOX1, RNA}}, {{SP110, RNA}}, {{IKZF4, RNA}}, {{ZNF605, RNA}}, {{BRCA1, RNA}}, {{ZNF763, RNA}},
{{ZNF682, RNA}}, {{ZNF829, RNA}}, {{RFX3, RNA}}, {{ZNF169, RNA}}, {{SCAI, RNA}}, {{ZNF563, RNA}},
{{ZNF573, RNA}}, {{ZNF618, RNA}}, {{ZNF514, RNA}}, {{HES1, RNA}}, {{ZNF503, RNA}}}}},
GO:0043234 → {{0.0000174221, 0.0018214, True}, {193, 355, 47241, 9},
{{protein complex, cellular_component}, {{{SNTB2, RNA}}, {{STRN3, RNA}}, {{SPATS2L, RNA}},
{{BRCA1, RNA}}, {{RNF165, RNA}}, {{SMAD1, RNA}}, {{MREG, RNA}}, {{PRKCDBP, RNA}}, {{SASH1, RNA}}}}},
GO:0000729 → {{0.0000294608, 0.00282333, True}, {193, 15, 47241, 3},
{{DNA double-strand break processing, biological_process},
{{{RAD50, RNA}}, {{BRCA1, RNA}}, {{BARD1, RNA}}}}},
GO:0008022 → {{0.000147416, 0.0118384, True}, {193, 192, 47241, 6},
{{protein C-terminus binding, molecular_function},
{{{CEP135, RNA}}, {{MLLT4, RNA}}, {{SCLT1, RNA}}, {{SYNJ2BP, RNA}}, {{ATXN2, RNA}}, {{SASH1, RNA}}}}},
GO:0000732 → {{0.000162862, 0.0118384, True}, {193, 26, 47241, 3},
{{strand displacement, biological_process}, {{{RAD50, RNA}}, {{BRCA1, RNA}}, {{BARD1, RNA}}}}},
GO:0007099 → {{0.000182669, 0.012357, True}, {193, 27, 47241, 3},
{{centriole replication, biological_process}, {{{SASS6, RNA}}, {{CEP135, RNA}}, {{CEP152, RNA}}}}},
GO:0051298 → {{0.000203975, 0.0123936, True}, {193, 28, 47241, 3},
{{centrosome duplication, biological_process}, {{{SASS6, RNA}}, {{TUBGCP4, RNA}}, {{CEP152, RNA}}}}},
GO:0044822 → {{0.000204764, 0.0123936, True}, {193, 1108, 47241, 14},
{{poly(A) RNA binding, molecular_function}, {{{CELF2, RNA}}, {{IBA57, RNA}}, {{KIAA0020, RNA}},
{{SNTB2, RNA}}, {{SRRM1, RNA}}, {{PRPF4B, RNA}}, {{SPATS2L, RNA}}, {{DHX29, RNA}}, {{DHX33, RNA}},
{{ATXN2, RNA}}, {{HELZ, RNA}}, {{CPEB3, RNA}}, {{ZFC3H1, RNA}}, {{USP36, RNA}}}}},
GO:0045944 → {{0.000251161, 0.0126549, True}, {193, 995, 47241, 13},
{{positive regulation of transcription from RNA polymerase II promoter, biological_process},
{{{AHI1, RNA}}, {{LPIN1, RNA}}, {{PIAS2, RNA}}, {{STRN3, RNA}},
{{ZNF292, RNA}}, {{NFIA, RNA}}, {{IKZF4, RNA}}, {{BRCA1, RNA}}, {{SMAD1, RNA}},
{{ZBED3, RNA}}, {{PPARGC1B, RNA}}, {{RFX3, RNA}}, {{HES1, RNA}}}}},
GO:0046872 → {{0.000253098, 0.0126549, True}, {193, 3010, 47241, 26},
{{metal ion binding, molecular_function}, {{{GDPD1, RNA}}, {{DGKE, RNA}}, {{ZNF286B, RNA}},
{{PDP2, RNA}}, {{ZFP14, RNA}}, {{RAD50, RNA}}, {{NEK1, RNA}}, {{ZNF292, RNA}}, {{HELZ, RNA}},
{{PDE1B, RNA}}, {{IKZF4, RNA}}, {{FGD6, RNA}}, {{ZNF605, RNA}}, {{ZNF763, RNA}}, {{ZNF682, RNA}},
{{ZNF829, RNA}}, {{CCRN4L, RNA}}, {{SMAD1, RNA}}, {{ZBED3, RNA}}, {{ZNF169, RNA}}, {{ZNF563, RNA}},
{{ZNF573, RNA}}, {{ZNF618, RNA}}, {{ZNF514, RNA}}, {{ZNF503, RNA}}, {{ZFC3H1, RNA}}}}},
GO:0006974 → {{0.000337653, 0.0158252, True}, {193, 224, 47241, 6},
{{cellular response to DNA damage stimulus, biological_process},
{{{RAD50, RNA}}, {{TAOK3, RNA}}, {{TLK2, RNA}}, {{BRCA1, RNA}}, {{BARD1, RNA}}, {{NUGGC, RNA}}}}},
GO:0033574 → {{0.000365926, 0.0161852, True}, {193, 34, 47241, 3},
{{response to testosterone, biological_process}, {{{BGLAP, RNA}}, {{INSR, RNA}}, {{UTS2, RNA}}}}},
GO:0000731 → {{0.000399031, 0.0169958, True}, {193, 35, 47241, 3},
{{DNA synthesis involved in DNA repair, biological_process},

```
    {{{RAD50, RNA}}, {{BRCA1, RNA}}, {{BARD1, RNA}}}}},
  GO:0045892 → {{0.000503096, 0.0199504, True}, {193, 555, 47241, 9},
    {{negative regulation of transcription, DNA-templated, biological_process},
     {{{STRN3, RNA}}, {{ZFHX3, RNA}}, {{PLCB1, RNA}}, {{HMBOX1, RNA}}, {{IKZF4, RNA}},
      {{BRCA1, RNA}}, {{PPARGC1B, RNA}}, {{RFX3, RNA}}, {{HES1, RNA}}}}},
  GO:0090307 → {{0.000550529, 0.0210806, True}, {193, 39, 47241, 3},
    {{mitotic spindle assembly, biological_process}, {{{MAP9, RNA}}, {{TUBGCP4, RNA}}, {{KIF3B, RNA}}}}},
  GO:0004386 → {{0.000695554, 0.024239, True}, {193, 97, 47241, 4}, {{helicase activity, molecular_function},
     {{{CHD9, RNA}}, {{HELLS, RNA}}, {{DHX33, RNA}}, {{HELZ, RNA}}}}},
  GO:0051301 → {{0.000887049, 0.0292877, True}, {193, 371, 47241, 7},
    {{cell division, biological_process}, {{{NEK1, RNA}}, {{HELLS, RNA}},
      {{KLHL42, RNA}}, {{SGK494, RNA}}, {{SYCP2, RNA}}, {{SGOL2, RNA}}, {{EVI5, RNA}}}}},
  GO:0003700 → {{0.00109863, 0.0341467, True}, {193, 1623, 47241, 16},
    {{transcription factor activity, sequence-specific DNA binding, molecular_function},
     {{{ZNF286B, RNA}}, {{ZFP14, RNA}}, {{STRN3, RNA}}, {{ZFHX3, RNA}}, {{MITF, RNA}}, {{LCOR, RNA}},
      {{NFIA, RNA}}, {{IKZF4, RNA}}, {{ZNF605, RNA}}, {{ZNF829, RNA}}, {{CCRN4L, RNA}},
      {{SMAD1, RNA}}, {{RFX3, RNA}}, {{ZNF573, RNA}}, {{ZNF514, RNA}}, {{HES1, RNA}}}}},
  GO:0006303 → {{0.00114239, 0.0345724, True}, {193, 50, 47241, 3},
    {{double-strand break repair via nonhomologous end joining, biological_process},
     {{{RAD50, RNA}}, {{BRCA1, RNA}}, {{BARD1, RNA}}}}},
  GO:0045202 → {{0.0011758, 0.0346711, True}, {193, 191, 47241, 5}, {{synapse, cellular_component},
     {{{SLC4A7, RNA}}, {{SNTB2, RNA}}, {{ITSN1, RNA}}, {{CPEB3, RNA}}, {{INSR, RNA}}}}},
  GO:0016607 → {{0.00134798, 0.0370593, True}, {193, 197, 47241, 5}, {{nuclear speck, cellular_component},
     {{{GPATCH2, RNA}}, {{SRRM1, RNA}}, {{PIAS2, RNA}}, {{PLCB1, RNA}}, {{NUGGC, RNA}}}}},
  GO:0045766 → {{0.00135347, 0.0370593, True}, {193, 116, 47241, 4},
    {{positive regulation of angiogenesis, biological_process},
     {{{RLN2, RNA}}, {{BRCA1, RNA}}, {{SASH1, RNA}}, {{UTS2, RNA}}}}},
  GO:0019901 → {{0.0014672, 0.0383472, True}, {193, 405, 47241, 7},
    {{protein kinase binding, molecular_function}, {{{RB1CC1, RNA}}, {{RAB11FIP2, RNA}},
      {{PPP1R12B, RNA}}, {{CEP152, RNA}}, {{SMAD1, RNA}}, {{CNTLN, RNA}}, {{SASH1, RNA}}}}},
  GO:0016925 → {{0.00153325, 0.0391831, True}, {193, 120, 47241, 4},
    {{protein sumoylation, biological_process},
     {{{PIAS2, RNA}}, {{KIAA1586, RNA}}, {{BRCA1, RNA}}, {{SUMO4, RNA}}}}},
  GO:0016568 → {{0.00162909, 0.0395104, True}, {193, 122, 47241, 4},
    {{chromatin modification, biological_process},
     {{{CHD6, RNA}}, {{CHD9, RNA}}, {{TLK2, RNA}}, {{RNASEK-C17orf49, RNA}}}}},
  GO:0032922 → {{0.0022263, 0.0483066, True}, {193, 63, 47241, 3},
    {{circadian regulation of gene expression, biological_process},
     {{{ZFHX3, RNA}}, {{CCRN4L, RNA}}, {{PRKCDBP, RNA}}}}},
  GO:0000086 → {{0.00229308, 0.0488342, True}, {193, 134, 47241, 4},
    {{G2/M transition of mitotic cell cycle, biological_process},
     {{{CEP135, RNA}}, {{PLCB1, RNA}}, {{PPP1R12B, RNA}}, {{CEP152, RNA}}}}},
  GO:0005516 → {{0.00245274, 0.0494852, True}, {193, 226, 47241, 5},
    {{calmodulin binding, molecular_function},
     {{{SNTB2, RNA}}, {{STRN3, RNA}}, {{PLCB1, RNA}}, {{EEA1, RNA}}, {{PDE1B, RNA}}}}}|>
```


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

In[45]:=
```
EnrichmentReportExport[goAnalysisRNA,
  OutputDirectory → $UserDocumentsDirectory, AppendString → "GOAnalysisRNA"];
```

We carry out our KEGG: Kyoto Encyclopedia of Genes and Genomes pathway analysis using KEGGAnalysis for all the classes and groups/subgroups. We only report terms for which there are at least 2 members. Please note that this is a time consuming computation.

In[46]:=
```
keggAnalysisRNA = KEGGAnalysis[rnaClusters, ReportFilter → 2];
```

The output of KEGGAnalysis has enrichments for each class and group

In[47]:=
```
Query[Keys]@keggAnalysisRNA
```

Out[47]=
```
{SpikeMax, SpikeMin, f1, f2, f3, f4, f5, f6, f7}
```

In[48]:=
```
Query[All, Keys]@keggAnalysisRNA
```

Out[48]=
```
⟨|SpikeMax → {G1S1, G1S2, G1S3, G1S4, G1S5, G1S6, G1S7, G1S8, G1S9, G1S10, G1S11, G1S12, G1S13, G1S14},
 SpikeMin → {G1S1, G1S2, G1S3}, f1 → {G1S1, G1S2}, f2 → {G1S1, G2S1},
 f3 → {G1S1, G1S2, G2S1, G2S2, G3S1, G3S2, G4S1, G4S2}, f4 → {G1S1, G1S2, G2S1, G2S2},
 f5 → {G1S1, G1S2, G1S3, G2S1, G2S2, G2S3}, f6 → {G1S1, G1S2, G2S1, G2S2}, f7 → {G1S1, G1S2, G2S1, G2S2, G2S3}|⟩
```

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

In[49]:=
```
EnrichmentReportExport[keggAnalysisRNA,
 OutputDirectory → $UserDocumentsDirectory, AppendString → "KEGGAnalysisRNA"]
```

We can view results for any of the groups (and also check out the behavior using the heatmaps generated in the previous section

In[52]:=
```
Query["SpikeMax"]@keggAnalysisRNA
```

Out[52]=
```
⟨|G1S1 → ‹| |›, G1S2 → ‹| |›, G1S3 → ‹| |›, G1S4 → ‹| |›, G1S5 → ‹| |›, G1S6 → ‹| |›,
 G1S7 → ‹| |›, G1S8 → ‹|path:hsa00910 → {{0.0000808101, 0.00137377, True}, {6, 17, 7086, 2},
      {Nitrogen metabolism - Homo sapiens (human), {{{CA6, RNA}}, {{CA13, RNA}}}}}|›,
 G1S9 → ⟨|path:hsa04520 → {{2.82404×10^-6, 0.000364301, True}, {61, 74, 7086, 7},
      {Adherens junction - Homo sapiens (human), {{{YES1, RNA}}, {{PTPRF, RNA}},
        {{WASF3, RNA}}, {{RAC3, RNA}}, {{TCF7L1, RNA}}, {{FGFR1, RNA}}, {{WASF1, RNA}}}}},
   path:hsa04114 → {{0.0000802526, 0.00517629, True}, {61, 123, 7086, 7},
      {Oocyte meiosis - Homo sapiens (human), {{{PLK1, RNA}}, {{CCNB2, RNA}}, {{BUB1, RNA}},
        {{CDK1, RNA}}, {{AURKA, RNA}}, {{CDC20, RNA}}, {{CCNB1, RNA}}}}}, path:hsa04115 →
    {{0.000294854, 0.0126787, True}, {61, 69, 7086, 5}, {p53 signaling pathway - Homo sapiens (human),
      {{{PERP, RNA}}, {{RRM2, RNA}}, {{CCNB2, RNA}}, {{CDK1, RNA}}, {{CCNB1, RNA}}}}},
   path:hsa04110 → {{0.000643206, 0.0207434, True}, {61, 124, 7086, 6}, {Cell cycle - Homo sapiens (human),
      {{{PLK1, RNA}}, {{CCNB2, RNA}}, {{BUB1, RNA}}, {{CDK1, RNA}}, {{CDC20, RNA}}, {{CCNB1, RNA}}}}},
   path:hsa04914 → {{0.00147265, 0.0379943, True}, {61, 98, 7086, 5},
      {Progesterone-mediated oocyte maturation - Homo sapiens (human),
       {{{PLK1, RNA}}, {{CCNB2, RNA}}, {{BUB1, RNA}}, {{CDK1, RNA}}, {{CCNB1, RNA}}}}}|⟩,
 G1S10 → ‹| |›, G1S11 → ‹| |›, G1S12 → ‹| |›, G1S13 → ‹| |›,
 G1S14 → ‹| |›|⟩
```

In[53]:=
```
Query["SpikeMin", "G1S1"]@keggAnalysisRNA
```

Out[53]=
```
⟨|path:hsa04660 → {{5.67782×10^-15, 1.67496×10^-12, True},
   {1521, 105, 7086, 59}, {T cell receptor signaling pathway - Homo sapiens (human),
    {{{GRAP2, RNA}}, {{NCK2, RNA}}, ··· 55 ···, {{GRB2, RNA}}, {{VAV2, RNA}}}}},
 ··· 139 ···, path:hsa04918 → { ··· 1 ··· }|⟩
```

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

In[56]:=
```
nfkbPathwayRNAExample = Query["SpikeMin", "G1S1", {"path:hsa04064"}]@keggAnalysisRNA
```

Out[56]=
```
⟨|path:hsa04064 → {{2.25958×10^-8, 3.50829×10^-7, True},
   {1521, 93, 7086, 44}, {NF-kappa B signaling pathway - Homo sapiens (human),
    {{{PRKCB, RNA}}, {{BCL2L1, RNA}}, {{PRKCQ, RNA}}, {{MAP3K7, RNA}}, {{PLCG1, RNA}},
    {{TAB2, RNA}}, {{TRAF6, RNA}}, {{CFLAR, RNA}}, {{MAP3K14, RNA}}, {{IKBKB, RNA}},
    {{PARP1, RNA}}, {{BCL2, RNA}}, {{RIPK1, RNA}}, {{MALT1, RNA}}, {{ICAM1, RNA}}, {{TRAF3, RNA}},
    {{IRAK1, RNA}}, {{TIRAP, RNA}}, {{CSNK2A1, RNA}}, {{BTK, RNA}}, {{TAB3, RNA}}, {{PIAS4, RNA}},
    {{CD40LG, RNA}}, {{DDX58, RNA}}, {{TICAM2, RNA}}, {{CHUK, RNA}}, {{BIRC2, RNA}}, {{TRAF2, RNA}},
    {{ZAP70, RNA}}, {{BLNK, RNA}}, {{CCL4, RNA}}, {{RELB, RNA}}, {{TRADD, RNA}}, {{CSNK2A2, RNA}},
    {{TAB1, RNA}}, {{CARD11, RNA}}, {{LCK, RNA}}, {{PLCG2, RNA}}, {{RELA, RNA}},
    {{TNFAIP3, RNA}}, {{TLR4, RNA}}, {{SYK, RNA}}, {{LYN, RNA}}, {{MYD88, RNA}}}}}|⟩
```

```
In[59]:= pathwaymembers = Query["SpikeMin", "G1S1", "path:hsa04064", 3, 2, All, 1]@keggAnalysisRNA
```

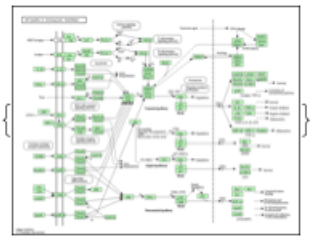
```
Out[59]= {{PRKCB, RNA}, {BCL2L1, RNA}, {PRKCQ, RNA}, {MAP3K7, RNA}, {PLCG1, RNA}, {TAB2, RNA},
  {TRAF6, RNA}, {CFLAR, RNA}, {MAP3K14, RNA}, {IKBKB, RNA}, {PARP1, RNA}, {BCL2, RNA},
  {RIPK1, RNA}, {MALT1, RNA}, {ICAM1, RNA}, {TRAF3, RNA}, {IRAK1, RNA}, {TIRAP, RNA},
  {CSNK2A1, RNA}, {BTK, RNA}, {TAB3, RNA}, {PIAS4, RNA}, {CD40LG, RNA}, {DDX58, RNA},
  {TICAM2, RNA}, {CHUK, RNA}, {BIRC2, RNA}, {TRAF2, RNA}, {ZAP70, RNA}, {BLNK, RNA},
  {CCL4, RNA}, {RELB, RNA}, {TRADD, RNA}, {CSNK2A2, RNA}, {TAB1, RNA}, {CARD11, RNA}, {LCK, RNA},
  {PLCG2, RNA}, {RELA, RNA}, {TNFAIP3, RNA}, {TLR4, RNA}, {SYK, RNA}, {LYN, RNA}, {MYD88, RNA}}
```

We can visualize any KEGG pathway using KEGGPathwayVisual , getting (1) a link to the website, (2) importing the figure (3) importing the figure with highlighted annotations, (4) importing a series of figures with intensities corresponding to each time point, (5) export a series of figures with time intensities as a movie (animation).

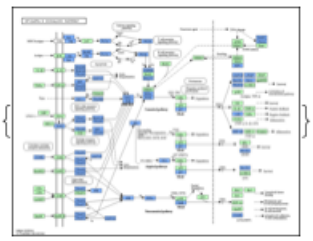
```
In[20]:= (*1*)KEGGPathwayVisual["path:hsa04064"]
```

```
Out[20]= ‹| Pathway → path:hsa04064, Results → {https://www.kegg.jp/kegg-bin/show_pathway?map=hsa04064} |›
```

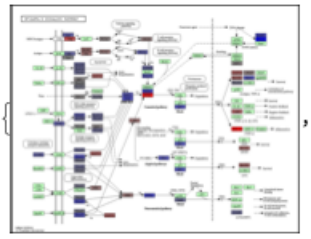
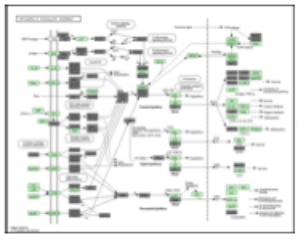
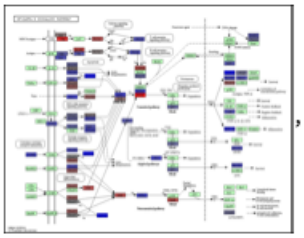
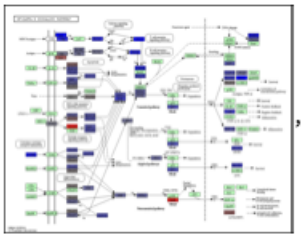
```
In[21]:= (*2*)KEGGPathwayVisual["path:hsa04064", ResultsFormat → "Figure"]
```

```
Out[21]= ⟨| Pathway → path:hsa04064, Results → {  } |⟩
```

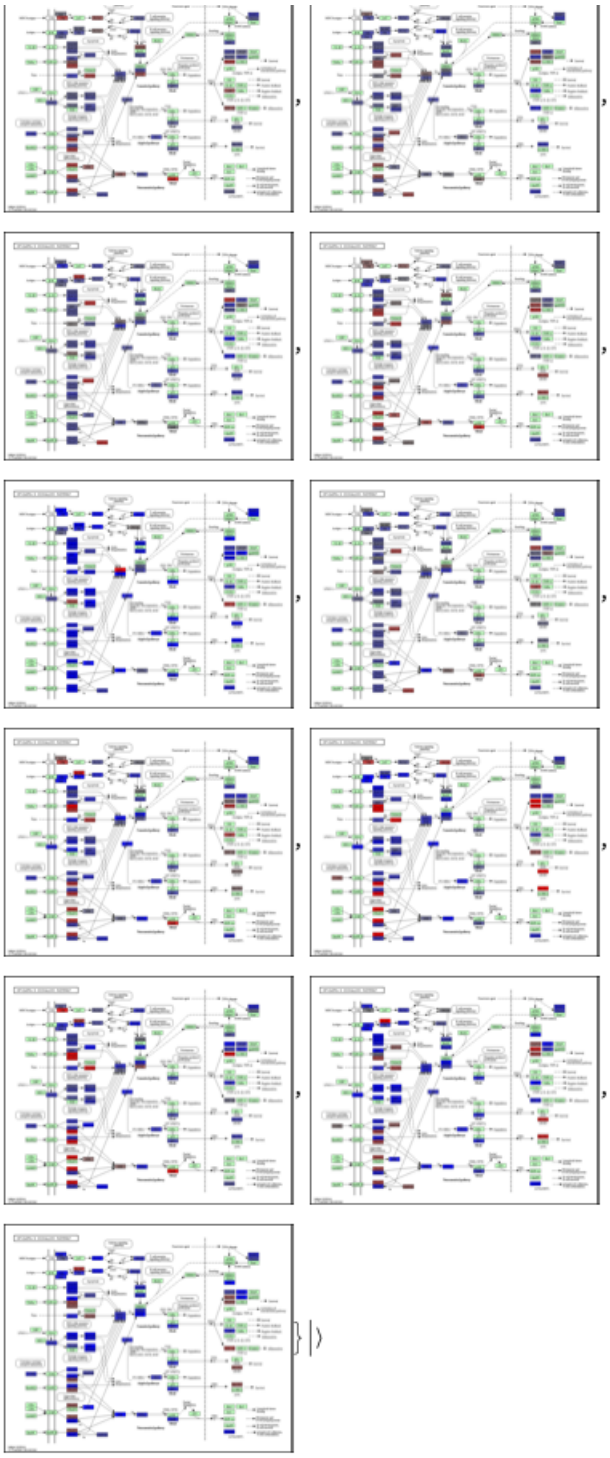
```
In[62]:= (*3*)KEGGPathwayVisual["path:hsa04064", ResultsFormat → "Figure", MemberSet → pathwaymembers]
```

```
Out[62]= ⟨| Pathway → path:hsa04064, Results → {  } |⟩
```

```
In[63]:= (*4*)nfkbPathwayFigureList = KEGGPathwayVisual["path:hsa04064", ResultsFormat → "Figure",
    MemberSet → pathwaymembers, Intensities → Query[Key[#] & /@ pathwaymembers]@rnaFinalTimeSeries]
```

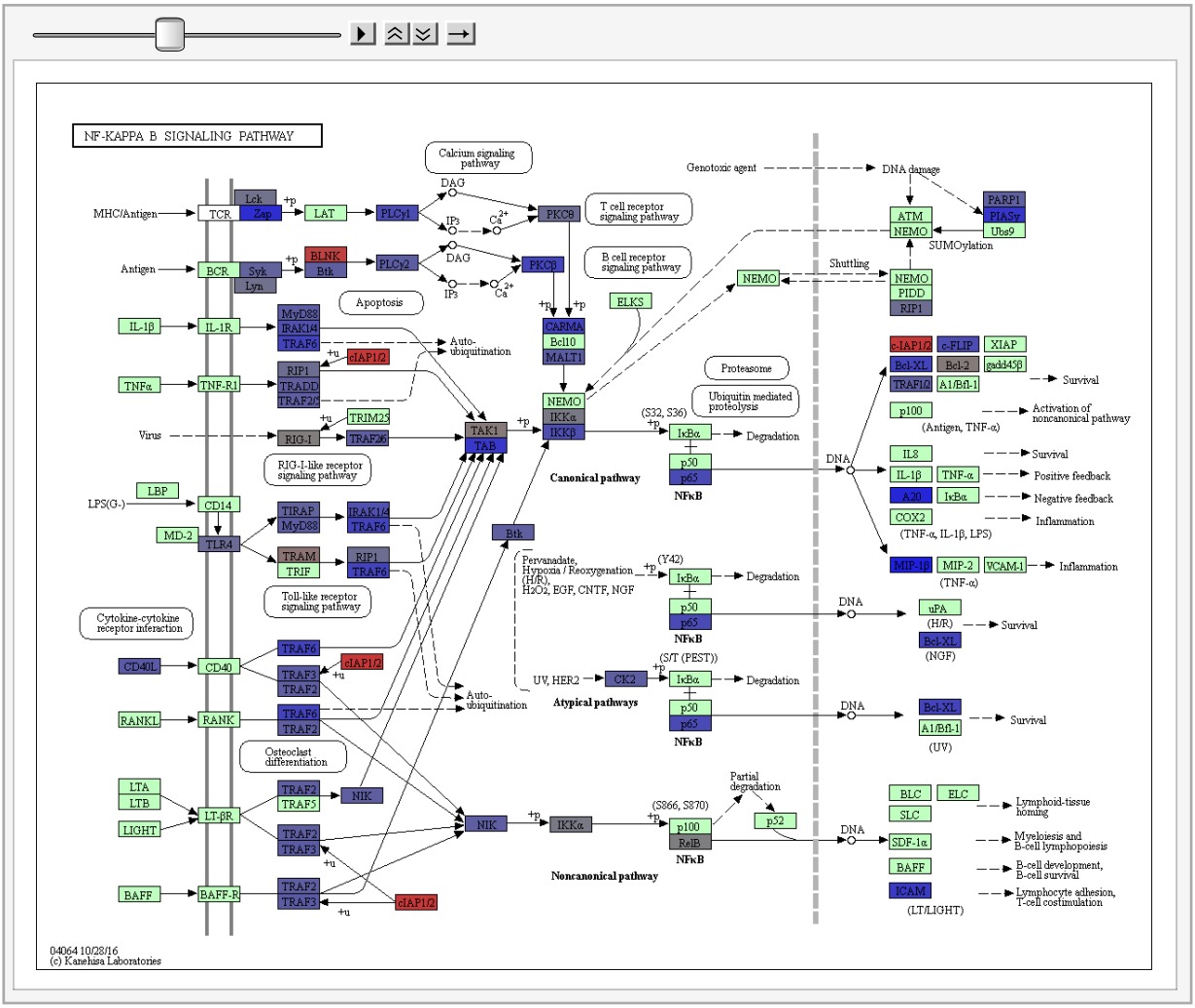
```
⟨| Pathway → path:hsa04064,
Results → {  ,  ,
 ,  ,
 
```

Out[63]=



In[64]:=
```
ListAnimate[nfkbPathwayFigureList["Results"], ImageSize → Automatic]
```

Out[64]=




```
In[65]:= (*5*)KEGGPathwayVisual["path:hsa04064", ResultsFormat → "Movie",
          MemberSet → pathwaymembers, Intensities → Query[Key[#] & /@ pathwaymembers]@rnaFinalTimeSeries]
Out[65]= ‹| Pathway → path:hsa04064, Results → path_hsa04064.mov |›
```

Appendix: All Commands Up to Enrichment Analysis in One Step

As a summary, we list here all the commands up to the enrichment analysis is one step:

```
In[1]:= << MathIOmica`;
rnaExample = Get[FileNameJoin[{ConstantMathIOmicaExamplesDirectory, "rnaExample"}]];
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"|>;
rnaLongitudinal = KeyMap[sampleToDays, rnaExample];
rnaQuantileNormed = QuantileNormalization[rnaLongitudinal, ListIndex → 1, ComponentIndex → 1];
rnaZeroTagged = LowValueTag[rnaQuantileNormed, 0];
rnaNoiseAdjusted = LowValueTag[rnaZeroTagged, 1, ValueReplacement → 1];
rnaFiltered = FilterMissing[rnaNoiseAdjusted, 3 / 4, Reference → "255", ShowPlots → False];
timesRNA = TimeExtractor[rnaFiltered];
timeSeriesRNA = CreateTimeSeries[rnaFiltered];
timeSeriesRNALog = SeriesApplier[Log, timeSeriesRNA];
rnaCompared = SeriesInternalCompare[timeSeriesRNALog, ComparisonIndex → 2];
normedRNACompared = SeriesApplier[Normalize, rnaCompared];
rnaFinalTimeSeries = ConstantSeriesClean[normedRNACompared];
(*Bootstrap*)
rnaBootstrap = BootstrapGeneral[rnaLongitudinal, 100 000];
(*1*) rnaBootstrapQuantileNormed = QuantileNormalization[rnaBootstrap, ListIndex → 1, ComponentIndex → 1];
(*2*) rnaBootstrapZeroTagged = LowValueTag[rnaBootstrapQuantileNormed, 0];
(*3*) rnaBootstrapNoiseAdjusted = LowValueTag[rnaBootstrapZeroTagged, 1, ValueReplacement → 1];
(*4*)
rnaBootstrapFiltered = FilterMissing[rnaBootstrapNoiseAdjusted, 3 / 4, Reference → "255", ShowPlots → False];
(*5*) timeSeriesBootstrapRNA = CreateTimeSeries[rnaBootstrapFiltered];
(*6*) timeSeriesBootstrapRNALog = SeriesApplier[Log, timeSeriesBootstrapRNA];
(*7*) rnaBootstrapCompared = SeriesInternalCompare[timeSeriesBootstrapRNALog, ComparisonIndex → 2];
(*8*) normedBootstrapRNACompared = SeriesApplier[Normalize, rnaBootstrapCompared];
(*9*) rnaBootstrapFinalTimeSeries = ConstantSeriesClean[normedBootstrapRNACompared];
q95RNA = QuantileEstimator[rnaBootstrapFinalTimeSeries, timesRNA];
q95RNASpikes = QuantileEstimator[rnaBootstrapFinalTimeSeries, timesRNA, Method → "Spikes"];
rnaClassification = TimeSeriesClassification[rnaFinalTimeSeries,
   timesRNA, LombScargleCutoff → q95RNA, SpikeCutoffs → q95RNASpikes];
rnaClusters = TimeSeriesClusters[rnaClassification, PrintDendrograms → True];
goAnalysisRNA = GOAnalysis[rnaClusters, OntologyLengthFilter → 3, ReportFilter → 3 ];
keggAnalysisRNA = KEGGAnalysis[rnaClusters, ReportFilter → 2];
EnrichmentReportExport[goAnalysisRNA,
  OutputDirectory → $UserDocumentsDirectory, AppendString → "GOAnalysisRNA"];
EnrichmentReportExport[keggAnalysisRNA, OutputDirectory → $UserDocumentsDirectory,
 AppendString → "KEGGAnalysisRNA"]
TimeSeriesDendrogramsHeatmaps[rnaClusters]
```

Related Tutorials

- MathIOmica Overview
- MathIOmica Tutorial
- MathIOmica Guide