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:

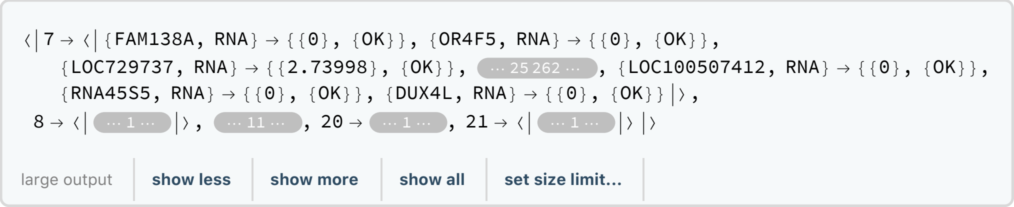
```
In[8]:= << MathIOmica`
```

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 ⋯|⟩|⟩
```

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}}, ⋯ 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...
```

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}}, ⋯ 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...
```

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}}, ⋯ 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...
```

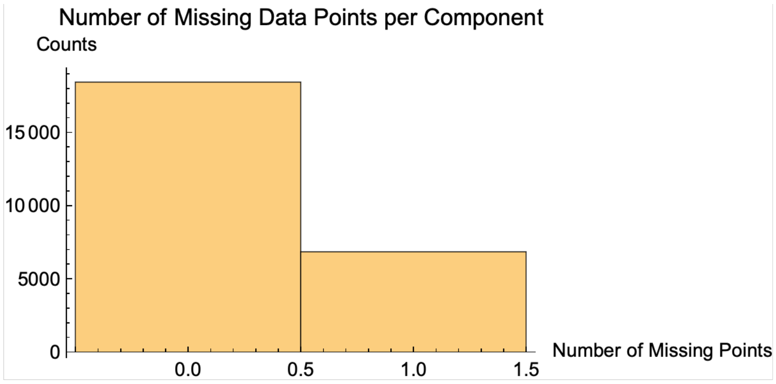
We next use LowValueTag again to set all FPKM values <1 to unity:

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

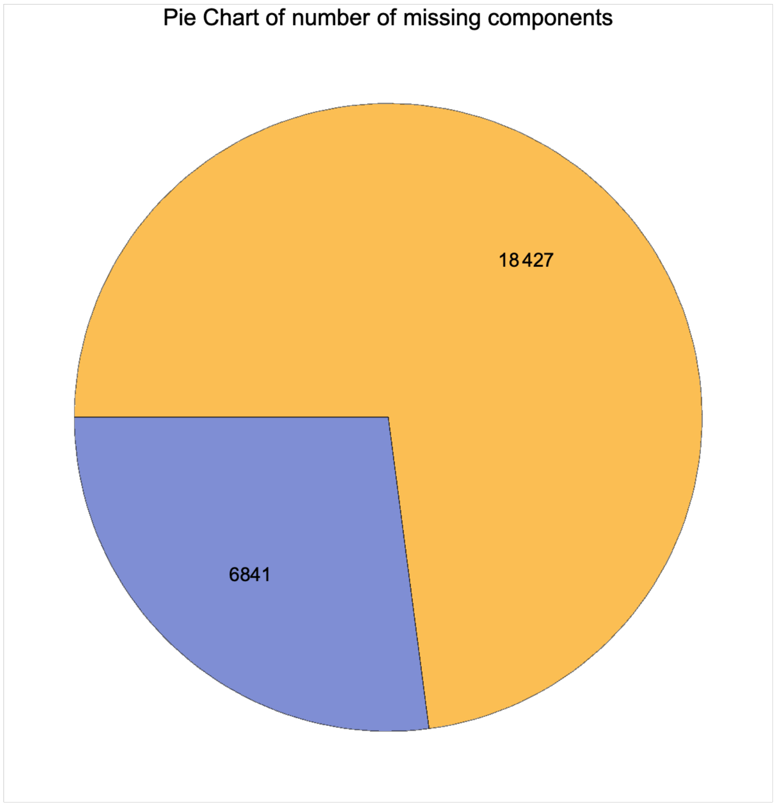
```
Out[15]=
<|186 → <|{FAM138A, RNA} → {{Missing[]}, {OK}},
    {OR4F5, RNA} → {{Missing[]}, {OK}}, {LOC729737, RNA} → {{2.2946}, {OK}}, ⋯ 25 263 ⋯,
    {RNA45S5, RNA} → {{Missing[]}, {OK}}, {DUX4L, RNA} → {{Missing[]}, {OK}}|>,
  255 → <| ⋯ 1 ⋯ |>, ⋯ 11 ⋯, ⋯ 1 ⋯, 400 → <| ⋯ 1 ⋯ |> |>

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 → 18 427, 1 → 6841|>}
```



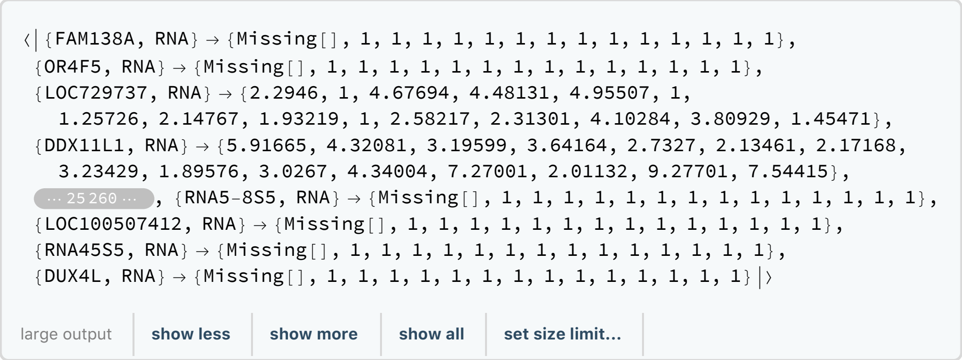
```
Out[16]= <|186 → <|{FAM138A, RNA} → {{Missing[]}, {OK}},
            {OR4F5, RNA} → {{Missing[]}, {OK}}, {LOC729737, RNA} → {{2.2946}, {OK}}, ⋯ 25 263 ⋯,
            {RNA45S5, RNA} → {{Missing[]}, {OK}}, {DUX4L, RNA} → {{Missing[]}, {OK}}|>,
          255 → <|⋯ 1 ⋯|>, ⋯ 11 ⋯, ⋯ 1 ⋯, 400 → <|⋯ 1 ⋯|>|>

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

We 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 :

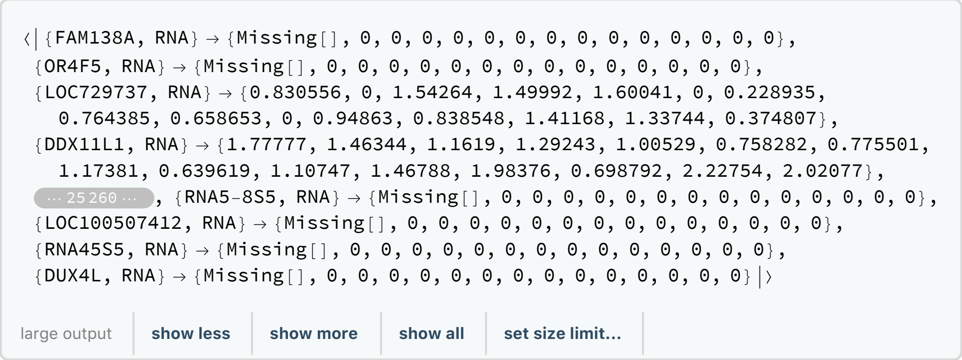
```
In[18]:= timeSeriesRNA = CreateTimeSeries[rnaFiltered]
```



```
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},
 ⋯ 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}|⟩
```

We use SeriesApplier to implement a logarithm transformation:

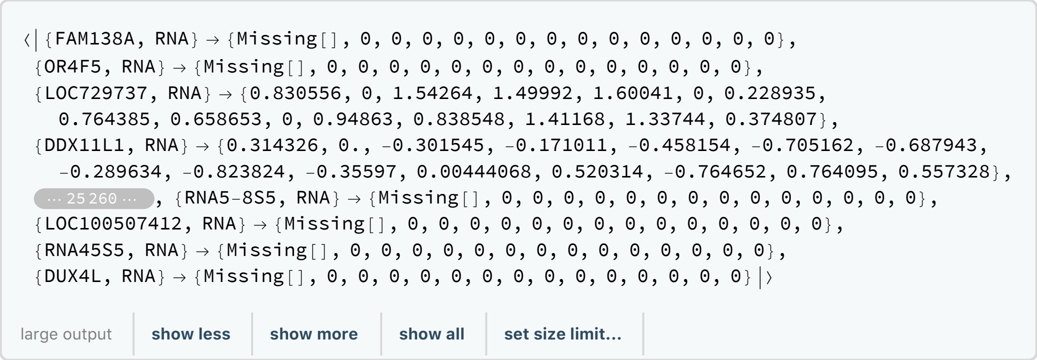
```
In[19]:= timeSeriesRNALog = SeriesApplier[Log, timeSeriesRNA]
```



```
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},
 ⋯ 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}|⟩
```

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},
 ⋯ 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}|⟩
```

Next, we normalize each series, using again SeriesApplier :

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

         ⟨|{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},
          {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,
Out[21]=    -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},
          {DUX4L, RNA} → {Missing[], 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0}|⟩

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```

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].

         ⟨|{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},
Out[22]=  … 11782 …, {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

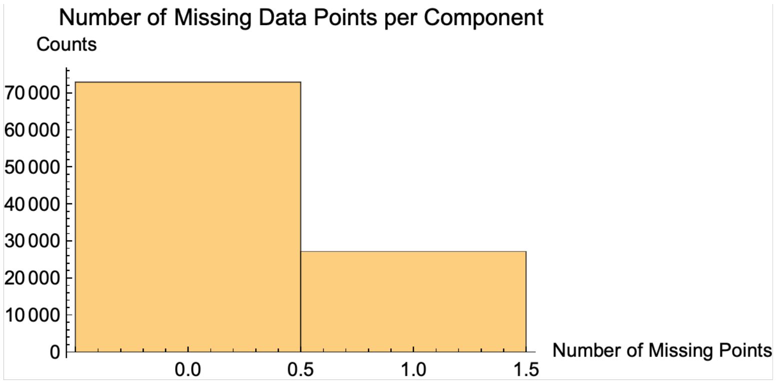
In addition to the above, we want to create a resampled distribution for the transcriptome dataset prior to classification and clustering. We repeat the steps in the processing section above using a resampled set of measurements.

We create a resampling of 100000 sets using BootstrapGeneral :

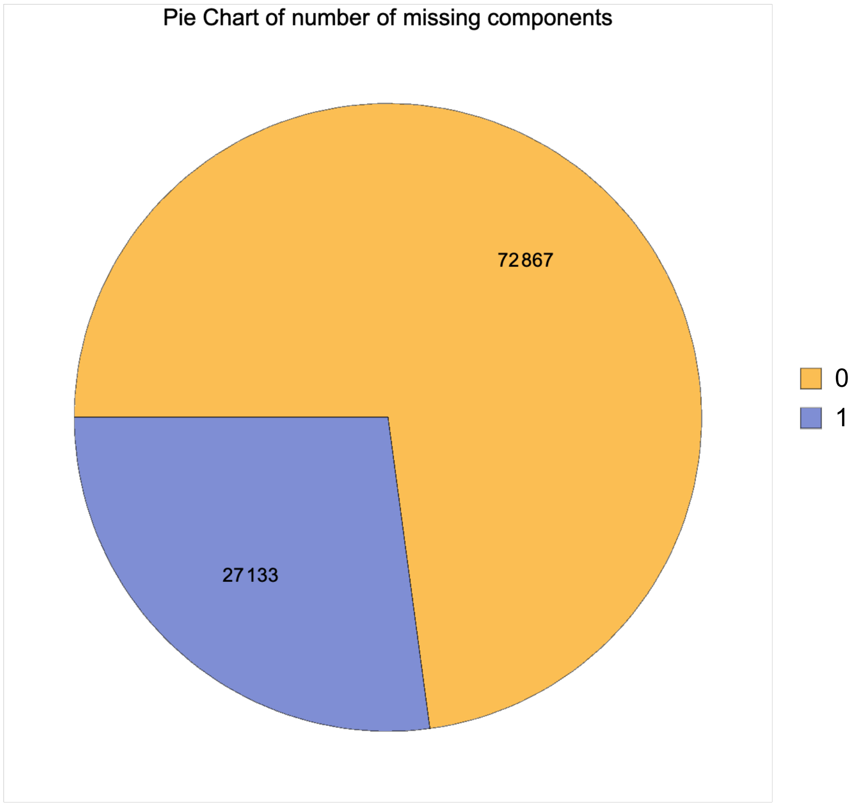
```
In[23]:= rnaBootstrap = BootstrapGeneral[rnaLongitudinal, 100 000]

         ⟨|186 → ⟨|1 → {{0}, {OK}}, 2 → {{0}, {OK}}, 3 → {{10.0429}, {OK}},
            4 → {{12.8612}, {OK}}, 5 → {{2.37963}, {OK}}, … 99991 …, 99 997 → {{43.8242}, {OK}},
Out[23]=    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[313]:= q95RNA = QuantileEstimator[rnaBootstrapFinalTimeSeries, timesRNA]

Out[313]= 0.85987
```

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

```
In[314]:= q95RNASpikes = QuantileEstimator[rnaBootstrapFinalTimeSeries, timesRNA, Method → "Spikes"]

Out[314]= <|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[315]:= rnaClassification = TimeSeriesClassification[rnaFinalTimeSeries,
             timesRNA, LombScargleCutoff → q95RNA, SpikeCutoffs → q95RNASpikes]

              Method → "LombScargle"

           <|SpikeMax → <|⋯1⋯|>, ⋯7⋯,
             f7 → <|{MIR6723, RNA} → {{0.214503, 0.000338299, 0.0390479, 0.0653206, 0.291434, 0.336712, 0.865961},
Out[315]=        {⋯1⋯}}, ⋯60⋯, {DNASE1L1, RNA} → ⋯1⋯|>|>

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```
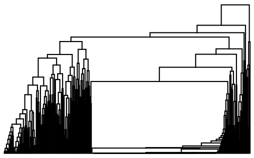

To obtain the possible frequencies we simply run LombScargle over the desired times for one of the time series and set the `FrequenciesOnly` option to True :

```
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|>
```

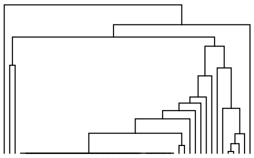
We now cluster our RNA data using TimeSeriesClusters :

```
In[316]:= rnaClusters = TimeSeriesClusters[rnaClassification, PrintDendrograms → True]

   ⋯ Agglomerate: 426 ties have been detected; reordering input may produce a different result.

   ⋯ Agglomerate: 1 ties have been detected; reordering input may produce a different result.

   ⋯ Agglomerate: 1 ties have been detected; reordering input may produce a different result.

   ⋯ General: Further output of Agglomerate::ties will be suppressed during this calculation.
```

<|SpikeMax → <|G1 → <|{G1S1} →

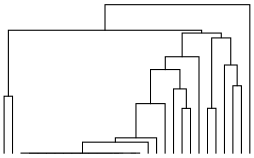


, {G1S2} →

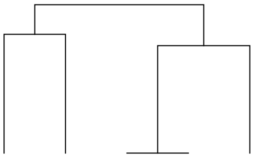


,

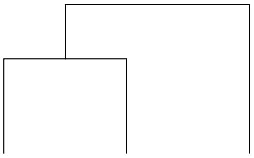
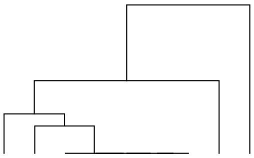
{G1S3} →

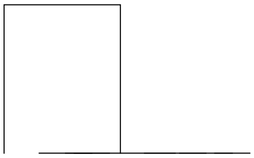
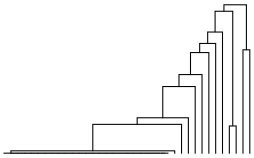


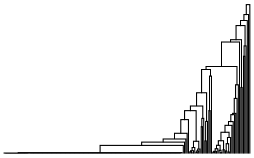
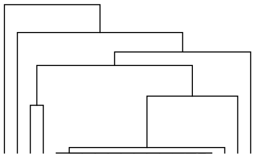
, {G1S4} →

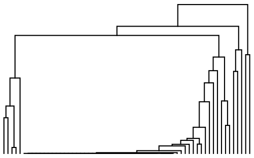
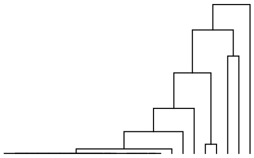


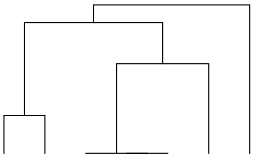
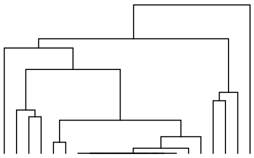
,

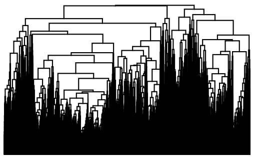
{G1S5} →  , {G1S6} →  ,

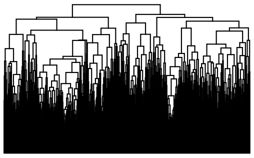
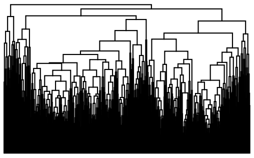
{G1S7} →  , {G1S8} →  ,

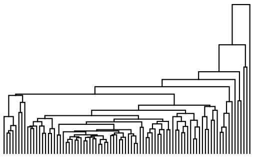
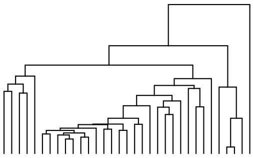
{G1S9} →  , {G1S10} →  ,

{G1S11} →  , {G1S12} →  ,

{G1S13} →  , {G1S14} →  |⟩ |⟩ ,

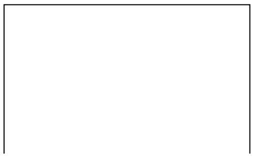
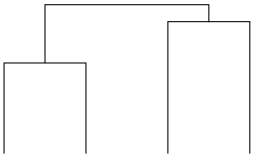
SpikeMin → ⟨| G1 → ⟨| {G1S1} →  ,

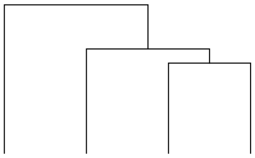
{G1S2} →  , {G1S3} →  |⟩ |⟩ ,

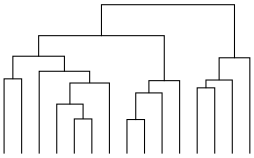
f1 → ⟨| G1 → ⟨| {G1S1} →  , {G1S2} →  |⟩ |⟩ ,

f2 → ⟨| G1 → ⟨| {G1S1} → |⟩ ,

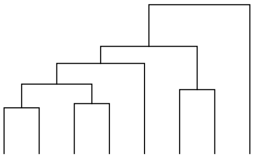
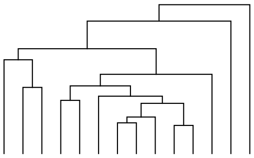
G2 → <| {G2S1} → {{0.283264, 0., 0.149932, 0.415971, 0.146512,
0.2947, 0.267954, 0.15466, 0.432236, 0.281295, 0.151677,
0.170078, 0.149432, 0.249221, 0.343349} → {POMP, RNA}} |> |⟩ ,

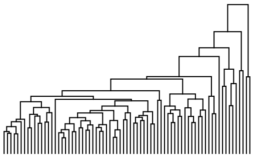
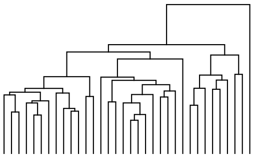
f3 → ⟨|G1 → ⟨|{G1S1} →  , {G1S2} →  |⟩,

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} →  |⟩,

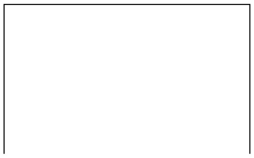
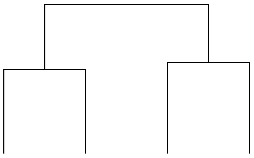
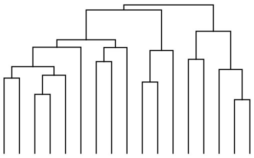
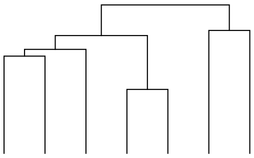
G3 → ⟨|{G3S1} →  , {G3S2} → {{-0.0555808, 0., 0.317043,
0.41872, 0.395921, 0.283783, 0.184223, 0.30154, 0.265713, 0.167588,
0.138036, 0.110511, 0.434502, 0.0277974, 0.198492} → {PYCARD, RNA}} |⟩,

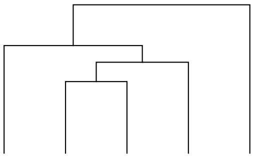
G4 → ⟨|{G4S1} → , {G4S2} →
{{0.0572204, 0., -0.22398, -0.11871, -0.0686182, -0.0820599,
-0.216559, -0.187636, -0.682766, 0.0415532, -0.197678,
0.14124, -0.497177, -0.0370276, 0.251877} → {ST7, RNA}} |⟩ |⟩,

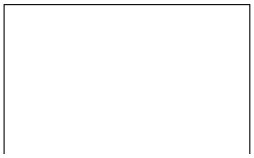
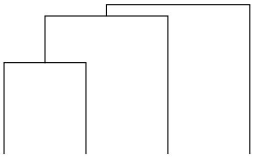
f4 → ⟨|G1 → ⟨|{G1S1} →  , {G1S2} →  |⟩,

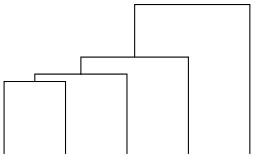
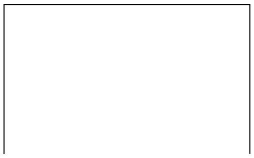
G2 → ⟨|{G2S1} →  , {G2S2} →  |⟩ |⟩,

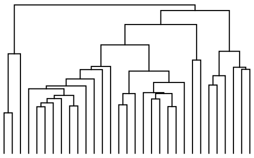
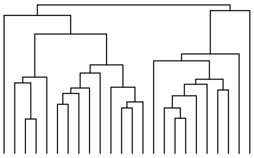
f5 → ⟨|G1 → ⟨|{G1S1} → {{-0.335157, 0., 0.107286, 0.337104, -0.0121183,
0.243878, 0.043649, -0.0358548, 0.410512, 0.305297, 0.354136,
0.00802356, 0.0854209, 0.461454, 0.303753} → {ALG14, RNA}},

{G1S2} →  , {G1S3} →  |⟩, G2 → ⟨| {G2S1} → , {G2S2} → ,

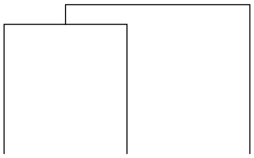
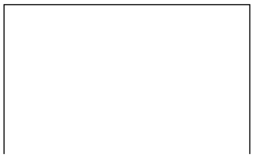
{G2S3} →  |⟩ |⟩,

f6 → ⟨| G1 → ⟨| {G1S1} → , {G1S2} →  |⟩,

G2 → ⟨| {G2S1} → , {G2S2} →  |⟩ |⟩,

f7 → ⟨| G1 → ⟨| {G1S1} → , {G1S2} →  |⟩,

G2 → ⟨| {G2S1} → ,

{G2S2} → , {G2S3} →  |⟩ |⟩ |⟩

Out[316]=

⟨| ⋯ 1 ⋯ |⟩

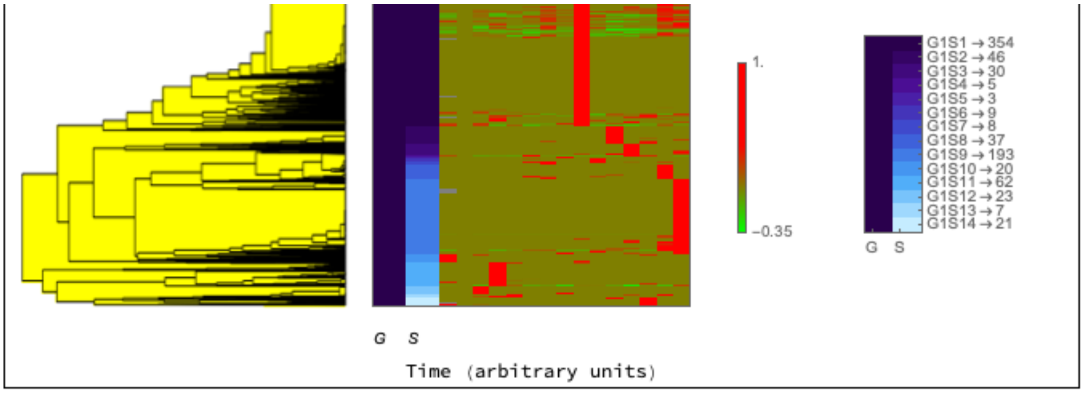
large output | show less | show more | show all | set size limit...

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.

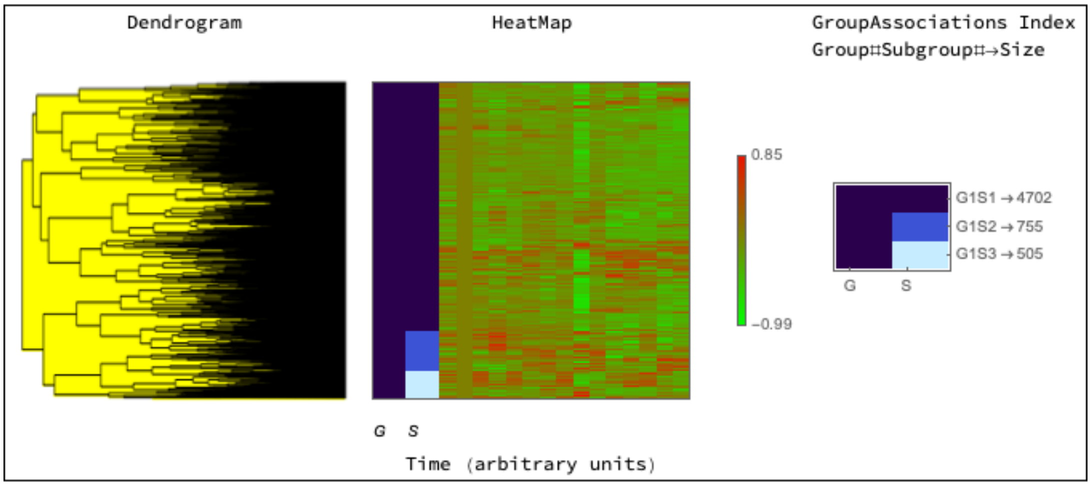
In[317]:=
```
TimeSeriesDendrogramsHeatmaps[rnaClusters]
```

SpikeMax

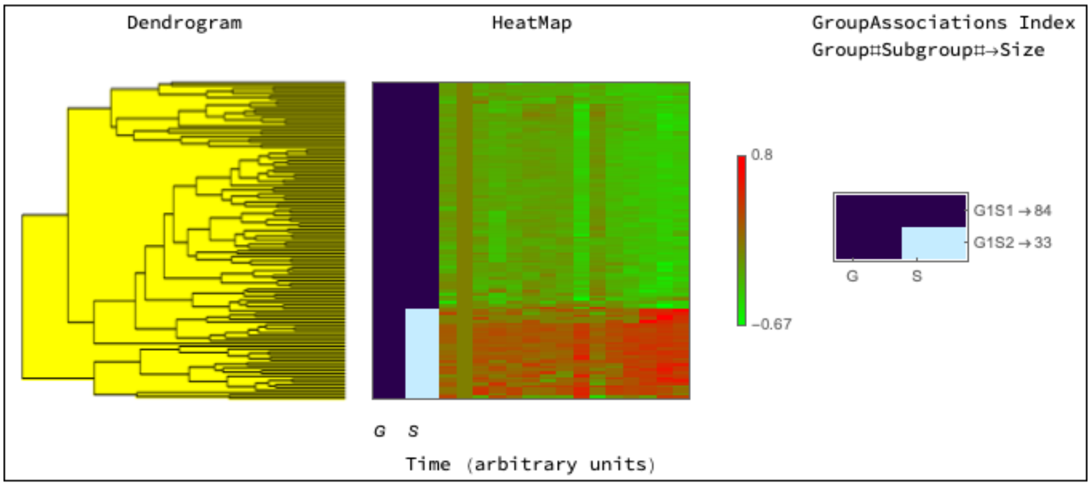
Dendrogram HeatMap GroupAssociations
Index
Group⌗Subgroup⌗→Size



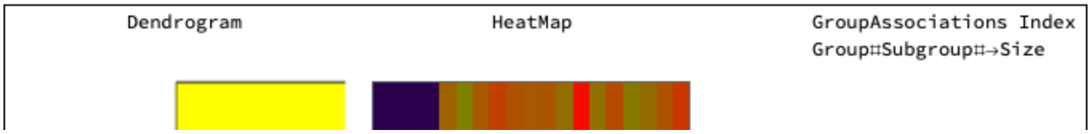
SpikeMin

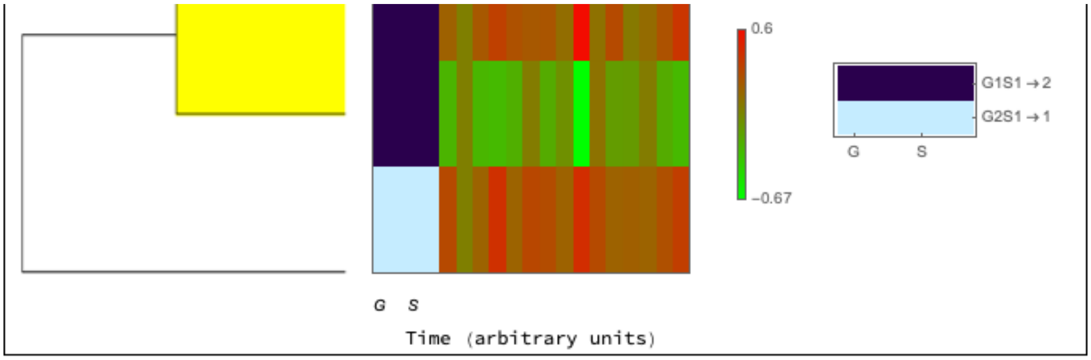


f1



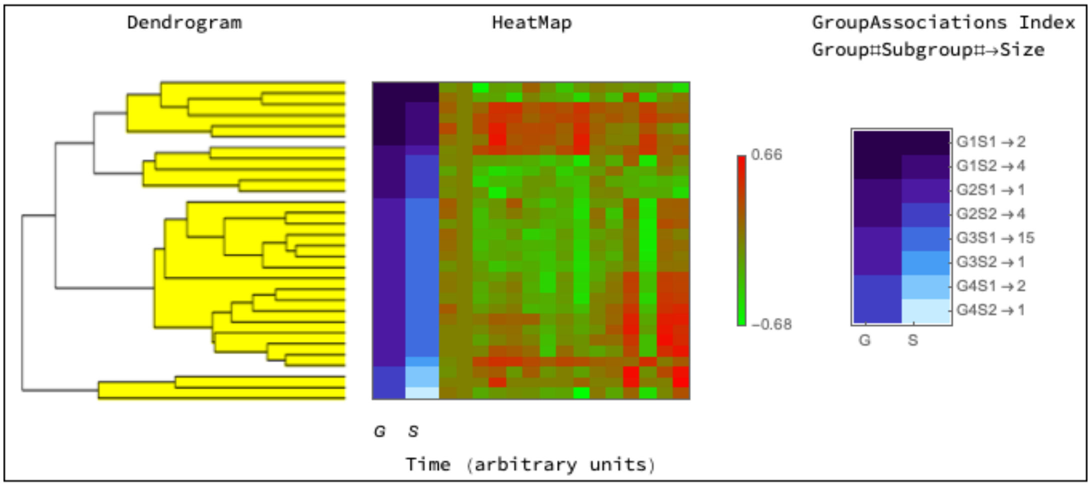
f2



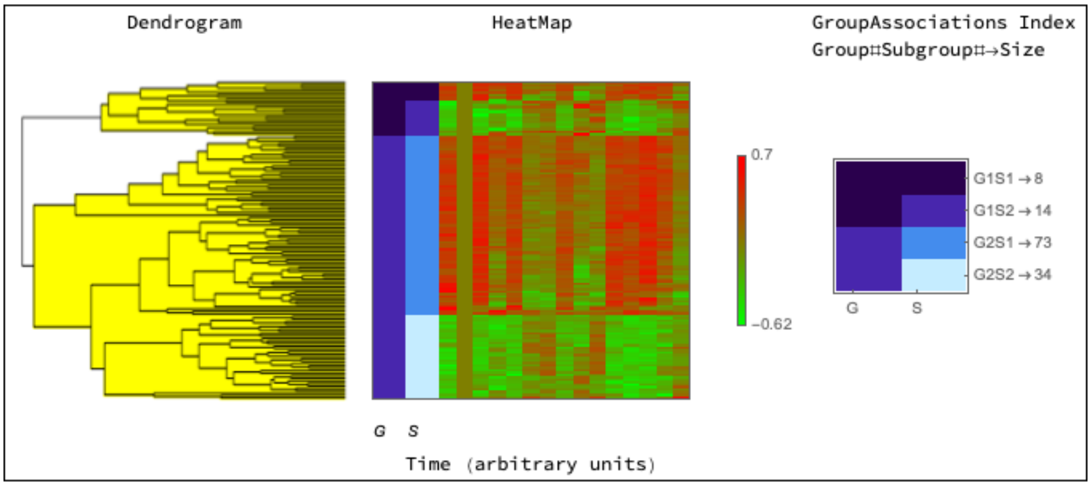


f3

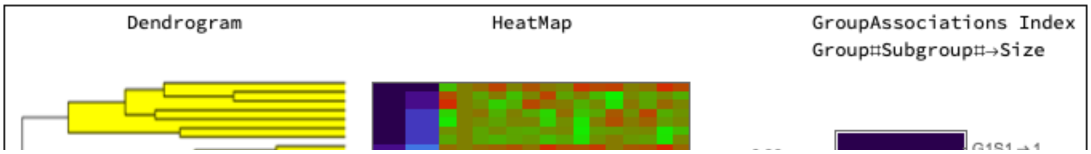
Out[317]=

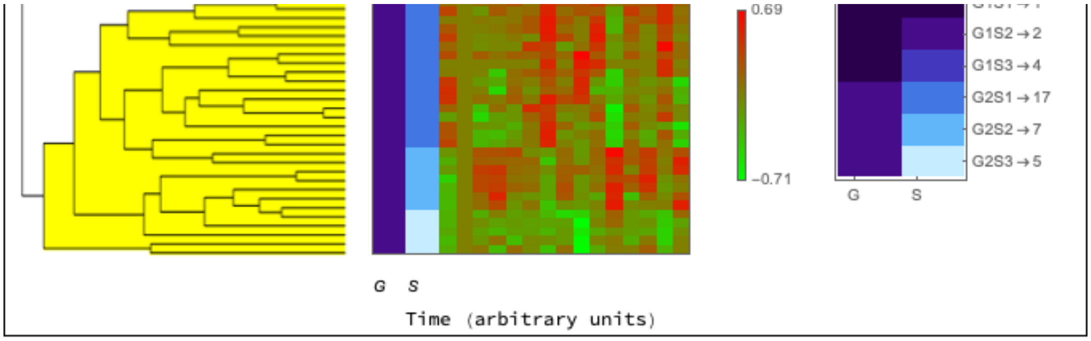


f4

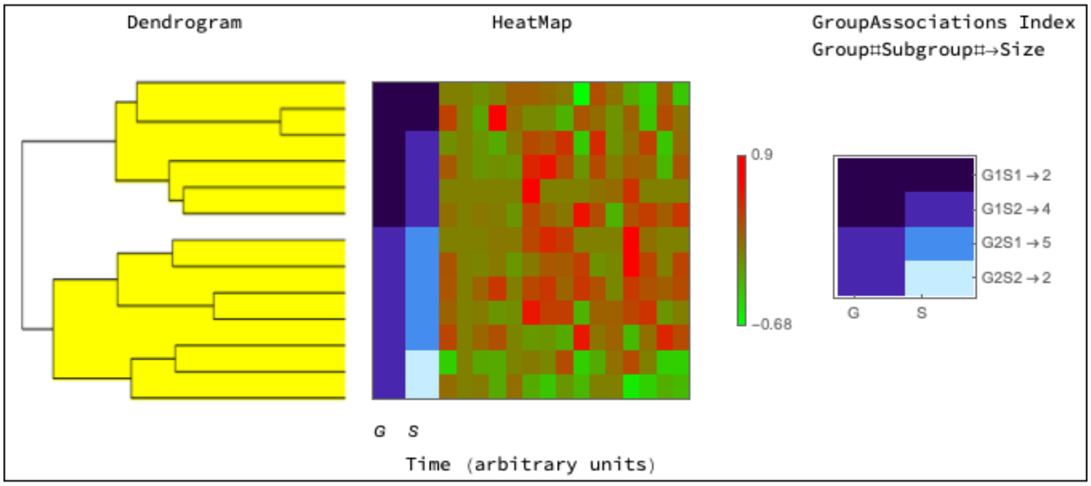


f5

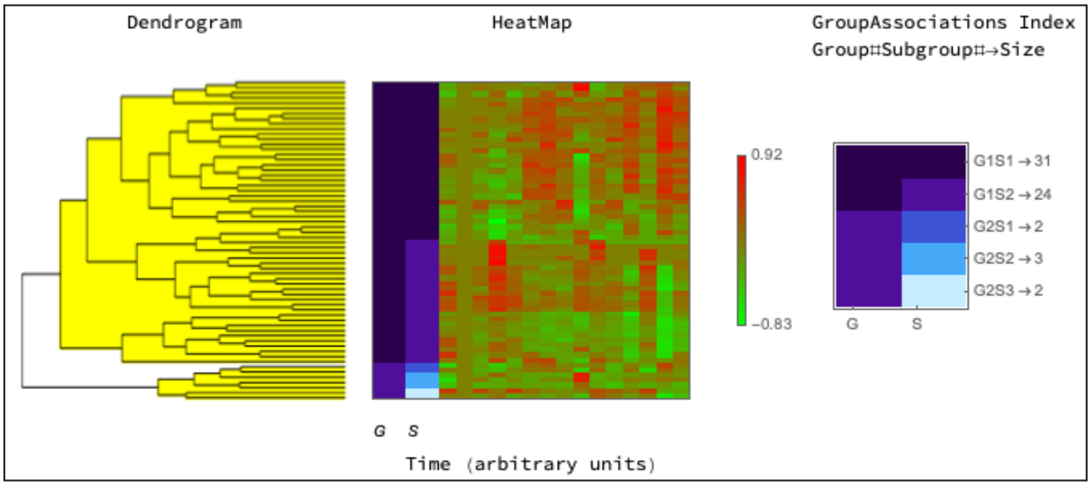




f6



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

The output of GOAnalysis has enrichments for each class and group

```
In[319]:= Query[Keys]@goAnalysisRNA

Out[319]= {SpikeMax, SpikeMin, f1, f2, f3, f4, f5, f6, f7}
```

```
In[320]:= Query[All, Keys]@goAnalysisRNA

Out[320]= <|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[321]:= Query["SpikeMax", "G1S1"]@goAnalysisRNA

Out[321]= <|GO:0005814 → {{0.0000103087, 0.0133394, True}, {193, 141, 19 772, 9},
              {{centriole, cellular_component}, {{{AHI1, RNA}}, {{KIAA1731, RNA}}, {{SASS6, RNA}}, {{CEP135, RNA}},
                {{SCLT1, RNA}}, {{CEP128, RNA}}, {{CEP152, RNA}}, {{CCDC146, RNA}}, {{CNTLN, RNA}}}}}|>
```

```
In[322]:= Query["f1", "G1S1"]@goAnalysisRNA

Out[322]= <|GO:0010501 → {{3.52017×10^-6, 0.00325263, True},
              {80, 8, 19 772, 3}, {{RNA secondary structure unwinding, biological_process},
               {{{AGO2, RNA}}, {{DDX3X, RNA}}, {{AGO1, RNA}}}}},
           GO:0035196 → {{0.0000280223, 0.0129463, True}, {80, 15, 19 772, 3},
              {{production of miRNAs involved in gene silencing by miRNA, biological_process},
               {{{AGO2, RNA}}, {{ZC3H7A, RNA}}, {{AGO1, RNA}}}}},
           GO:0005515 → {{0.000220804, 0.0277165, True}, {80, 9629, 19 772, 55},
              {{protein binding, molecular_function}, {{{PADI4, RNA}}, {{USP25, RNA}}, {{ZNF207, RNA}},
                {{ADAM9, RNA}}, {{AGO2, RNA}}, {{HACE1, RNA}}, {{WBP1L, RNA}}, {{ADNP2, RNA}}, {{PSME3, RNA}},
                {{C12orf49, RNA}}, {{PTPLB, RNA}}, {{PRKAR2A, RNA}}, {{CCR4, RNA}}, {{CLPX, RNA}}, {{ACLY, RNA}},
                {{SSH1, RNA}}, {{ANTXR2, RNA}}, {{ARHGEF6, RNA}}, {{GTF3C4, RNA}}, {{MAPK1, RNA}}, {{AHCYL2, RNA}},
                {{GSK3B, RNA}}, {{ERAP1, RNA}}, {{SAMHD1, RNA}}, {{DDX3X, RNA}}, {{STT3B, RNA}}, {{EFCAB4B, RNA}},
                {{ELK3, RNA}}, {{AGO1, RNA}}, {{NDUFS1, RNA}}, {{DNAJC13, RNA}}, {{FAM168A, RNA}},
                {{MBP, RNA}}, {{JAK1, RNA}}, {{KLF3, RNA}}, {{FLI1, RNA}}, {{PRKDC, RNA}}, {{GMCL1, RNA}},
                {{FOCAD, RNA}}, {{IPO5, RNA}}, {{TCF20, RNA}}, {{CASK, RNA}}, {{INPP4B, RNA}}, {{PRKCA, RNA}},
                {{SYNJ2, RNA}}, {{EHD4, RNA}}, {{RUNX1, RNA}}, {{PTGER4, RNA}}, {{DSCR3, RNA}}, {{AAGAB, RNA}},
                {{PDK3, RNA}}, {{GTF3C3, RNA}}, {{HSPA14, RNA}}, {{TBC1D22B, RNA}}, {{ARPC4, RNA}}}}},
           GO:0035198 → {{0.000239341, 0.0277165, True}, {80, 30, 19 772, 3},
              {{miRNA binding, molecular_function}, {{{AGO2, RNA}}, {{ZC3H7A, RNA}}, {{AGO1, RNA}}}}},
           GO:0018105 → {{0.000480577, 0.0370045, True}, {80, 160, 19 772, 5},
              {{peptidyl-serine phosphorylation, biological_process},
               {{{MAPK1, RNA}}, {{GSK3B, RNA}}, {{PRKDC, RNA}}, {{PRKCA, RNA}}, {{PDK3, RNA}}}}},
           GO:0005844 → {{0.000700504, 0.0438824, True}, {80, 43, 19 772, 3},
              {{polysome, cellular_component}, {{{AGO2, RNA}}, {{AGO1, RNA}}, {{HSPA14, RNA}}}}},
           GO:0016020 → {{0.000760622, 0.043918, True}, {80, 1974, 19 772, 18}, {{membrane, cellular_component},
               {{{AGO2, RNA}}, {{PSME3, RNA}}, {{PRKAR2A, RNA}}, {{ACLY, RNA}}, {{PDZD8, RNA}}, {{ERAP1, RNA}},
                {{NNT, RNA}}, {{STT3B, RNA}}, {{EFCAB4B, RNA}}, {{RC3H2, RNA}}, {{DNAJC13, RNA}}, {{JAK1, RNA}},
                {{PRKDC, RNA}}, {{IPO5, RNA}}, {{GLG1, RNA}}, {{EHD4, RNA}}, {{ERMP1, RNA}}, {{HSPA14, RNA}}}}},
           GO:0035556 → {{0.000886844, 0.043918, True}, {80, 381, 19 772, 7},
              {{intracellular signal transduction, biological_process}, {{{PRKAR2A, RNA}}, {{PDZD8, RNA}},
                {{MAPK1, RNA}}, {{GSK3B, RNA}}, {{DDX3X, RNA}}, {{JAK1, RNA}}, {{PRKCA, RNA}}}}},
           GO:0005524 → {{0.000903076, 0.043918, True}, {80, 1501, 19 772, 15},
              {{ATP binding, molecular_function}, {{{ATP9B, RNA}}, {{IARS2, RNA}}, {{CLPX, RNA}},
                {{ACLY, RNA}}, {{MAPK1, RNA}}, {{GSK3B, RNA}}, {{DDX3X, RNA}}, {{JAK1, RNA}}, {{PRKDC, RNA}},
                {{CASK, RNA}}, {{PRKCA, RNA}}, {{EHD4, RNA}}, {{RUNX1, RNA}}, {{PDK3, RNA}}, {{HSPA14, RNA}}}}},
           GO:0006986 → {{0.00109014, 0.0492168, True}, {80, 50, 19 772, 3}, {{response to unfolded protein,
                biological_process}, {{{STT3B, RNA}}, {{JKAMP, RNA}}, {{HSPA14, 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[323]:= keggAnalysisRNA = KEGGAnalysis[rnaClusters, ReportFilter → 2];
```

The output of KEGGAnalysis has enrichments for each class and group

```
In[324]:= Query[Keys]@keggAnalysisRNA

Out[324]= {SpikeMax, SpikeMin, f1, f2, f3, f4, f5, f6, f7}
```

```
In[325]:= Query[All, Keys]@keggAnalysisRNA

Out[325]= ⟨|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[327]:= Query["SpikeMax"]@keggAnalysisRNA

Out[327]= ⟨|G1S1 → ⟨||⟩, G1S2 → ⟨||⟩, G1S3 → ⟨||⟩, G1S4 → ⟨||⟩, G1S5 → ⟨||⟩, G1S6 → ⟨||⟩,
           G1S7 → ⟨||⟩, G1S8 → ⟨|path:hsa00910 → {{0.0000654978, 0.00157195, True}, {6, 17, 7873, 2},
               {Nitrogen metabolism - Homo sapiens (human), {{{CA6, RNA}}, {{CA13, RNA}}}}}|⟩,
           G1S9 → ⟨|path:hsa04520 → {{1.31015×10^-6, 0.000193902, True}, {62, 72, 7873, 7},
               {Adherens junction - Homo sapiens (human), {{{YES1, RNA}}, {{PTPRF, RNA}},
                 {{WASF3, RNA}}, {{RAC3, RNA}}, {{TCF7L1, RNA}}, {{FGFR1, RNA}}, {{WASF1, RNA}}}}},
             path:hsa04114 → {{0.0000596029, 0.00441062, True}, {62, 128, 7873, 7},
               {Oocyte meiosis - Homo sapiens (human), {{{PLK1, RNA}}, {{CCNB2, RNA}},
                 {{BUB1, RNA}}, {{CDK1, RNA}}, {{AURKA, RNA}}, {{CDC20, RNA}}, {{CCNB1, RNA}}}}},
             path:hsa04914 → {{0.000118171, 0.00582977, True}, {62, 99, 7873, 6},
               {Progesterone-mediated oocyte maturation - Homo sapiens (human),
                {{{PLK1, RNA}}, {{CCNB2, RNA}}, {{BUB1, RNA}}, {{CDK1, RNA}}, {{AURKA, RNA}}, {{CCNB1, RNA}}}}},
             path:hsa04115 → {{0.00023993, 0.00887741, True}, {62, 72, 7873, 5},
               {p53 signaling pathway - Homo sapiens (human),
                {{{PERP, RNA}}, {{RRM2, RNA}}, {{CCNB2, RNA}}, {{CDK1, RNA}}, {{CCNB1, RNA}}}}},
             path:hsa04110 → {{0.000404297, 0.0119672, True}, {62, 124, 7873, 6}, {Cell cycle - Homo sapiens (human),
                {{{PLK1, RNA}}, {{CCNB2, RNA}}, {{BUB1, RNA}}, {{CDK1, RNA}}, {{CDC20, RNA}}, {{CCNB1, RNA}}}}}|⟩,
           G1S10 → ⟨||⟩, G1S11 → ⟨||⟩, G1S12 → ⟨||⟩, G1S13 → ⟨||⟩,
           G1S14 → ⟨||⟩|⟩
```

In[328]:=
```
Query["SpikeMin", "G1S1"]@keggAnalysisRNA
```

Out[328]=
```
<|path:hsa04660 → {{4.68108×10^-17, 1.01928×10^-14, True},
   {1514, 103, 7873, 58}, {T cell receptor signaling pathway - Homo sapiens (human),
    {{{GRAP2, RNA}}, {{NCK2, RNA}}, ⋯ 54 ⋯ , {{GRB2, RNA}}, {{VAV2, RNA}}}}},
 ⋯ 177 ⋯ , path:hsa05014 → { ⋯ 1 ⋯ }|>
```

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

In[329]:=
```
nfkbPathwayRNAExample = Query["SpikeMin", "G1S1", {"path:hsa04064"}]@keggAnalysisRNA
```

Out[329]=
```
<|path:hsa04064 → {{8.5145×10^-10, 1.02174×10^-8, True},
   {1514, 100, 7873, 46}, {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}}, {{CYLD, RNA}}, {{PIAS4, RNA}},
     {{EDAR, 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[330]:=
```
pathwaymembers = Query["SpikeMin", "G1S1", "path:hsa04064", 3, 2, All, 1]@keggAnalysisRNA
```

Out[330]=
```
{{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}, {CYLD, RNA}, {PIAS4, RNA}, {EDAR, 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}}
```

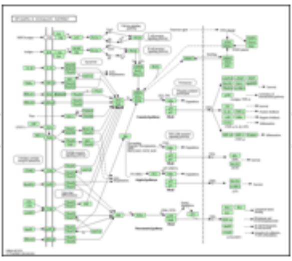
We can visualize any KEGG pathway using KEGGPathwayVisual , getting (1) a link to the website, (2) importing the figure (3) importing the figure with highlighted annotations, (4) importing a series of figures with intensities corresponding to each time point, (5) export a series of figures with time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

In[331]:=
```
(*1*)KEGGPathwayVisual["path:hsa04064"]
```

Out[331]=
```
<|Pathway → path:hsa04064, Results → {https://www.kegg.jp/kegg-bin/show_pathway?map=hsa04064}|>
```

In[332]:=
```
(*2*)KEGGPathwayVisual["path:hsa04064", ResultsFormat → "Figure"]
```

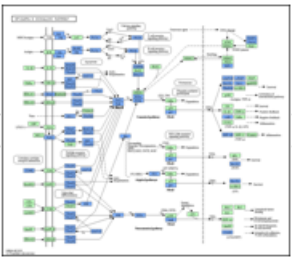
Out[332]=
```
<|Pathway → path:hsa04064, Results → {
```



```
}|>
```

```
In[333]:= (*3*)KEGGPathwayVisual["path:hsa04064", ResultsFormat → "Figure", MemberSet → pathwaymembers]
```

```
Out[333]= ⟨|Pathway → path:hsa04064, Results → {
```

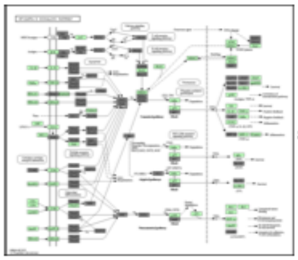


```
}|⟩
```

```
In[334]:= (*4*)nfkbPathwayFigureList = KEGGPathwayVisual["path:hsa04064", ResultsFormat → "Figure",
            MemberSet → pathwaymembers, Intensities → Query[Key[#] & /@ pathwaymembers]@rnaFinalTimeSeries]
```

```
Out[334]= ⟨|Pathway → path:hsa04064,
            Results → {
```

,



,

,

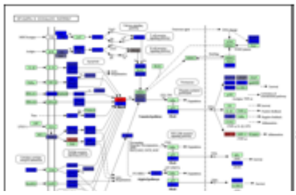
,

,

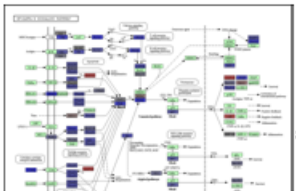
,

,

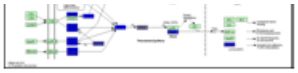
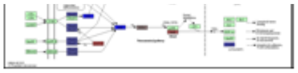
,



,



,

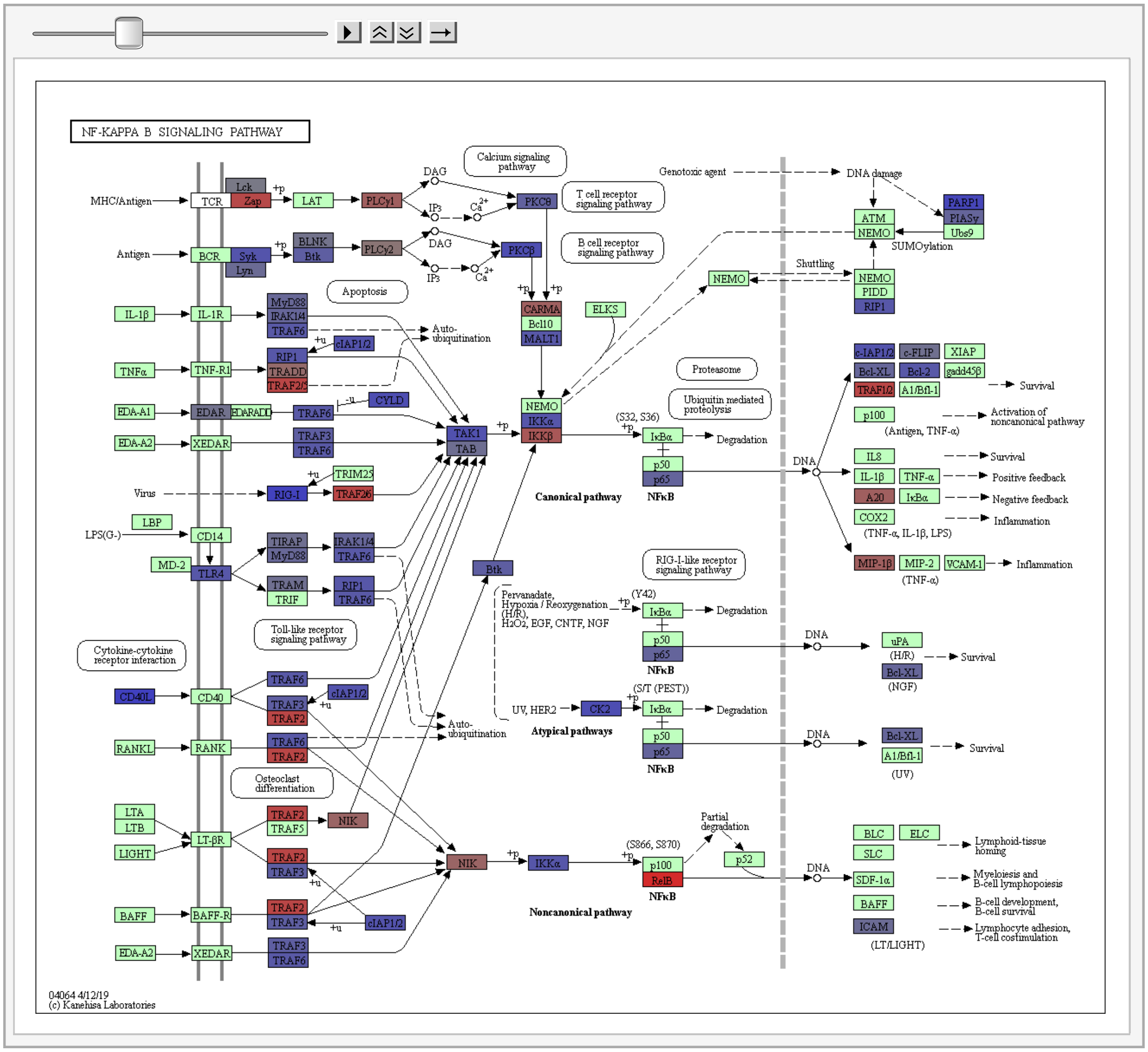
, ,

, ,

}|⟩

```
In[335]:=  ListAnimate[nfkbPathwayFigureList["Results"], ImageSize → Automatic]
```

Out[335]=



```
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, 100000];
        (*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